Ministerio del Medio Ambiente

Gobierno de Chile

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MAT.: Remite expediente público del proceso de Revisión de la Norma de Calidad Primaria de MP10 (D.S N°59/98 de MINSEGPRES)

SANTIAGO, 02 DIC. 2011

DE : MARÍA IGNACIA BENÍTEZ PEREIRA
MINISTRA DEL MEDIO AMBIENTE

A : SRES. INTEGRANTES DEL CONSEJO CONSULTIVO

Por Resolución Nº1309 de fecha 2 de noviembre de 2011, del Ministerio de Medio Ambiente, se aprobó el Anteproyecto de Revisión de la **Norma de Calidad Primaria para Material Particulado Respirable MP10** establecida por el D.S Nº59 de 1998, del Ministerio Secretaría General de la República, la cual ordenó someterlo a consulta pública, periodo que abarca desde el día 21 de noviembre al día 13 de febrero del año 2012.

De acuerdo al Reglamento para la Dictación de Normas de Calidad Ambiental y de Emisión, D.S. Nº 93 de 1995 del Ministerio Secretaría General de la Presidencia, en su artículo 18, una vez publicada la resolución que aprueba el anteproyecto, se debe remitir copia del expediente al Consejo Consultivo, para que emita su opinión fundada sobre el anteproyecto.

En virtud de lo indicado, informo que el expediente digital podrá ser revisado a través del link: http://www.sinia.cl/1292/w3-article-51242.html de la página web del Ministerio de Medio Ambiente.

Sin otro particular, saluda atentamente a Ud.,

MINISTERIO DEL MEDIO AMBIENTE

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Nota Aclaratoria

El siguiente documento se adjunta con el fin de respaldar los fundamentos de obtuvo la Agencia de Protección Ambiental (EPA) de Estados Unidos, en cuyo caso ha revocado la norma anual de MP10, manteniendo la norma diaria para MP10.

Debido a la cantidad de páginas que contiene el documento (1.071 páginas) se ha incorporado la información completa en formato digital (CD adjunto) y se incorpora al expediente el indice del documento para visualizar su contenido y el capítulo 7 sobre los efectos a largo plazo del material particulado, en el cual se puede constatar que la evidencia sobre los efectos perjudiciales a la salud es más reciente, y no sugiere una asociación entre la exposición de largo plazo del material particulado grueso y los efectos sobre la salud de las personas.

EPA United States Environmental Protection Agency

December 2009
EPA/600/R-08/139F

Integrated Science Assessment for Particulate Matter

Includes Errata Sheet created on 2/10/2010

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Chapter 7. Integrated Health Effects of Long-Term PM Exposure

7.1. Introduction

This chapter reviews, summarizes, and integrates the evidence on relationships between health effects and long-term exposures to various size fractions and sources of PM. Cardiopulmonary health effects of long-term exposure to PM have been examined in an extensive body of epidemiologic and toxicological studies. Both epidemiologic and toxicological studies provide a basis for examining reproductive and developmental and cancer health outcomes with regard to long-term exposure to PM. In addition, there is a large body of epidemiologic literature evaluating the relationship between mortality and long-term exposure to PM.

Conclusions from the 2004 PM AQCD are summarized briefly at the beginning of each section, and the evaluation of evidence from recent studies builds upon what was available during the previous review. For each health outcome (e.g., respiratory infections, lung function), results are summarized for studies from the specific scientific discipline, i.e., epidemiologic and toxicological studies. The major sections (i.e., cardiovascular, respiratory, reproductive/developmental, cancer) conclude with summaries of the evidence for the various health outcomes within that category and integration of the findings that lead to conclusions regarding causality based upon the framework described in Chapter 1. Determination of causality is made for the overall health effect category, such as cardiovascular effects, with coherence and plausibility being based upon the evidence from across disciplines and also across the suite of related health outcomes including cause-specific mortality. Section 7.6 provides detailed discussions on the epidemiologic literature for long-term exposure to PM and mortality. In each summary section (7.2.11, 7.3.9, 7.4.3, 7.5.4, and 7.6.5), the evidence is briefly reviewed and independent conclusions drawn for relationships with $PM_{2.5}$, $PM_{10-2.5}$, and UF particles (UFPs).

7.2. Cardiovascular and Systemic Effects

Studies examining associations between long-term exposure to ambient PM (over months to years) and CVD morbidity had not been conducted and thus were not included in the 1996 or 2004 PM Air Quality Criteria Documents (U.S. EPA, 1996, 079380; U.S. EPA, 2004, 056905). A number of studies were included in the 2004 PM AQCD that evaluated the effect of long-term $PM_{2.5}$ exposure on cardiovascular mortality and found consistent associations. No toxicological studies examined chronic atherosclerotic effects of PM exposure in animal models. However, a subchronic study that evaluated atherosclerosis progression in hyperlipidemic rabbits was discussed and this study provided the foundation for the subsequent work that has been conducted in this area (Suwa et al., 2002, 028588). No previous toxicological studies evaluated effects of subchronic or chronic PM exposure on diabetes measures, or HR or HRV changes, nor were there animal toxicological studies included in the 2004 PM AQCD that evaluated systemic inflammatory or blood coagulation markers following subchronic or chronic PM exposure.

Several new epidemiologic studies have examined the long-term PM-CVD association among U.S. and European populations. The studies investigate the association of both $PM_{2.5}$ and PM_{10} exposures with a variety of clinical and subclinical CVD outcomes. Epidemiologic and toxicological studies have provided evidence of the adverse effects of long-term exposure to $PM_{2.5}$ on

Note: Hyperlinks to the reference citations throughout this document will take you to the NCEA HERO database (Health and Environmental Research Online) at http://epa.gov/hero. HERO is a database of scientific literature used by U.S. EPA in the process of developing science assessments such as the Integrated Science Assessments (ISA) and the Integrated Risk Information System (IRIS).

cardiovascular outcomes, including atherosclerosis, clinical and subclinical markers of cardiovascular morbidity, and cardiovascular mortality. The evidence of these effects from long-term exposure to $PM_{10-2.5}$ is weaker.

7.2.1. Atherosclerosis

Atherosclerosis is a progressive disease that contributes to several adverse outcomes, including acute coronary syndromes such as myocardial infarction, sudden cardiac death, stroke and vascular aneurysms. It is multifaceted, beginning with an early injury or inflammation that promotes the extravasation of inflammatory cells. Under conditions of oxidative or nitrosative stress and high lipid or cholesterol concentrations, the vessel wall undergoes a chronic remodeling that is characterized by the presence of foam cells, migrated and differentiated smooth muscle cells, and ultimately a fibrous cap. The advanced lesion that develops from this process can occlude perfusion to distal tissue, causing ischemia, and erode, degrade, or even rupture, revealing coagulant initiators (tissue factor) that promote thrombosis, stenosis, and infarction or stroke. Several detailed reviews of atherosclerosis pathology have been published elsewhere (Ross, 1999, 156926; Stocker and Keaney, 2004, 157013).

7.2.1.1. Epidemiologic Studies

Measures of Atherosclerosis

Although no study has examined the association between long-term PM exposure and longitudinal change in subclinical markers of atherosclerosis, several cross sectional studies have been conducted. Markers of atherosclerosis used in these studies include coronary artery calcium (CAC), carotid intima-media thickness (CIMT), ankle-brachial index (ABI), and abdominal aortic calcium (AAC). These measures are descried briefly below.

CAC represents the accumulation of calcium in coronary artery macrophages and represents an advanced stage of atherosclerosis. As such CAC is a measure of atherosclerosis assessed by non-contrast, cardiac-gated electron beam computed tomography (EBCT) or multidetector computed tomography (MDCT) of the coronary arteries in the heart (Greenland and Kizilbash, 2005, 156496; Hoffmann et al., 2005, 156556; Mollet et al., 2005, 155988). The prevalence of CAC is strongly related to age. Few people have detectable CAC in their second decade of life but the prevalence of CAC rises to approximately 100% by age 80 (Ardehali et al., 2007, 155662). Previous studies suggest that while the absence of CAC does not rule out atherosclerosis, it does imply a very low likelihood of significant arterial obstruction (Achenbach and Daniel, 2001, 156189; Arad et al., 1996, 155661; Shaw et al., 2003, 156083; Shemesh et al., 1996, 156085). Conversely, the presence of CAC confirms the existence of atherosclerotic plaque and the amount of calcification varies directly with the likelihood of obstructive disease (Ardehali et al., 2007, 155662). CAC is a quantified using the Agatston method (Agatston et al., 1990, 156197). Its repeatability depends on the laboratory and the method of calculation (O'Rourke et al., 2000, 192159). Agatston scores are frequently used to classify individuals into one of five groups (zero; mild; moderate; severe; extensive) or according to age- and sex-specific percentiles of the CAC distribution (Erbel et al., 2007, 155768).

CIMT is a measure of atherosclerosis assessed by high-resolution, B-mode ultrasonography of the carotid arteries in the neck, the walls of which have inner (intimal), middle (medial) and outer (adventitial) layers (Craven et al., 1990, 155740; O'Leary et al., 1999, 156826; Wendelhag et al., 1993, 157136). CIMT estimates the distance in mm or μm between the innermost (blood-intima) and outermost (media-adventitia) interfaces, often by averaging over three arterial segments in the common carotid, carotid bulb, and internal carotid artery (Amato et al., 2007, 155656). CIMT has been associated with atherosclerosis risk factors (Heiss et al., 1991, 156535; O'Leary et al., 1992, 156825; Salonen and Salonen, 1991, 156938), prevalent coronary heart disease (Chambless et al., 1997, 156329; Geroulakos et al., 1994, 155788), and incident coronary and cerebral events (O'Leary et al., 1999, 156826; van der Meer et al., 2004, 156129). Several studies have indicated that CIMT measurements are accurate (Girerd et al., 1994, 156474; Pignoli et al., 1986, 156026; Wendelhag et

al., 1991, 157135) and reproducible (Montauban et al., 1999, 156777; Smilde et al., 1997, 156988; Willekes et al., 1999, 157147), especially for the common carotid artery (Montauban et al., 1999, 156777).

ABI, which is also known as the ankle-arm or resting (blood) pressure index, is a measure of lower extremity arterial occlusive disease commonly caused by advanced atherosclerosis (Weitz et al., 1996, 156150). It is assessed by continuous wave Doppler and manual or automated oscillometric sphygmomanometry, the latter having been shown to have higher repeatability and validity (Weitz et al., 1996, 156150). ABI is defined as the unitless ratio of ankle to brachial systolic blood pressures measured in mmHg. As ankle pressure is normally equal to or slightly higher than arm pressure (resulting in an ABI $\geq$ 1.0), epidemiologic studies typically define the normal ABI range as 0.90 to 1.50 (Resnick et al., 2004, 156048). Low ABI has been associated with all-cause and CVD mortality (Newman et al., 1993, 156805; Vogt et al., 1993, 157100), as well as myocardial infarction and stroke (Karthikeyan and Lip, 2007, 156626).

AAC is a measure of atherosclerosis assessed by non-contrast, EBCT or MDCT of the abdominal aorta. It is scored much like CAC (Agatston et al., 1990, 156197), but the age-specific prevalence and extent of AAC is greater, particularly among women and at ages >50 yr. Although AAC has not been studied as extensively as CAC, it is associated with carotid and coronary atherosclerosis as well as cardiovascular morbidity and mortality (Allison et al., 2004, 156210; Allison et al., 2006, 155653; Hollander et al., 2003, 156562; Khoury et al., 1997, 156636; Oei et al., 2002, 156820; Walsh et al., 2002, 157103; Wilson et al., 2001, 156159; Witteman et al., 1986, 156161) and measurements are sufficiently reproducible to allow serial investigations over time (Budoff et al., 2005, 192105).

Study Findings

Diez Roux et al. (2008, 156401) conducted cross-sectional analyses of the association of three of these subclinical markers of atherosclerosis (CAC, CIMT and ABI), collected from 2000 to 2003 during baseline examinations of participants enrolled in the Multi-Ethnic Study of Atherosclerosis (MESA), with long-term exposure to $PM_{2.5}$ and PM_{10}. The study population included 5,172 ethnically diverse people (53% female) residing in Baltimore, MD; Chicago, IL; Forsyth County, NC; Los Angeles, CA; New York, NY; and St. Paul, MN ranging in age from 45 to 84 yr old. Authors used spatio-temporal modeling of pollutant concentrations, weather and demographic data to impute 20-yr avg exposures to $PM_{2.5}$ and PM_{10}. They reported small increases in CIMT of 1% (95% CI: 0-1.4) and 0.5% (95% CI: 0-1), which correspond to absolute changes of 8 (95% CI: 0-12) and 7 (95% CI: 0-14) µm, per 10 µg/m^3 increase in 20-yr avg PM_{10} and $PM_{2.5}$ concentration, respectively. Evidence of age-, gender-, lipid- and smoking-related susceptibility was lacking. They also reported weak, non-significant increases in the relative prevalence of CAC of 1% (95% CI: -2 to 4) and 0.5% (95% CI: -2 to 3) per 10 µg/m^3 increase in PM_{10} and $PM_{2.5}$, respectively. Among the subset of 2,586 participants with EBCT-identified calcification, similarly weak associations were observed. There was little evidence of modification of the CAC associations by demographic, socioeconomic or clinical characteristics. Finally, the authors report no differences in mean ABI with PM_{10} or $PM_{2.5}$ concentrations. The null findings for ABI exhibited little heterogeneity among participant subgroups and were similarly null when ABI was modeled as a dichotomous outcome using a cutpoint of 0.9 units.

MESA investigators also examined the chronic $PM_{2.5}$-AAC association in a residentially stable subset of 1,147 participants (mean age = 66 yr; 50% female) randomly selected from all MESA centers, except Baltimore, MD for enrollment in its Aortic Calcium Ancillary Study(Allen et al., 2009, 156209). The authors used kriging and inverse residence-to-monitor distance-weighted averaging of EPA AQS data to estimate 2-yr mean exposures to $PM_{2.5}$. In cross-sectional analyses, the authors found a 6% (95% CI: -4 to 16) excess risk of a non-zero Agatston score and an 8% (95% CI: -30 to 46) increase in AAC, i.e., approximately 50 (95% CI: -251 to 385) Agatston units, per 10 µg/m^3 increase in $PM_{2.5}$ concentration. These associations were stronger among users than non-users of lipid lowering drugs.

Kunzli et al. (2005, 087387) used baseline data collected between 1998-2003 from two randomized placebo-controlled clinical trials, the Vitamin E Atherosclerosis Progression Study (VEAPS) and the B-Vitamin Atherosclerosis Intervention Trial (BVAIT), for their ancillary cross-sectional analyses of the effect of long-term $PM_{2.5}$ exposure on CIMT. The study population included 798 residents of the greater Los Angeles, CA area who were more than 40 yr old at baseline and 44% were female. The authors used universal kriging of $PM_{2.5}$ data from 23 state and local monitors

operating in 2000 to estimate 1-yr avg exposure to $PM_{2.5}$ at each participant's geocoded U.S. Postal Service ZIP code. They found a 4.2% (95% CI: -0.2 to 8.9) or approximately 32 (95% CI: -2 to 68) μm increase in CIMT per 10 $\mu g/m^3$ increase in $PM_{2.5}$ concentration. In contrast to findings from the relatively large, ethnically diverse, yet geographically overlapping MESA ancillary study described above, PM-related increases in CIMT were two- to three-fold larger among older and female participants taking lipid lowering drugs in this study. PM-related increases in CIMT were also higher in never smokers when compared with current or former smokers.

Hoffmann et al. (2007, 091163) conducted a cross-sectional analysis of data collected at baseline (2000-2003) for 4,494 residents of Essen, Mülheim and Bochum, Germany enrolled in the Heinz Nixdorf Recall Study from 2000 to 2003. The age of participants ranged from 45-74 yr and 51% were female. In this cross-sectional study the authors used dispersion and chemistry transport modeling of emissions, climate and topography data to estimate 1-yr avg exposure to $PM_{2.5}$ in 2002 (the midpoint of the baseline exam.) They reported an imprecise 43% (95% CI: -15 to 115) or 102 (95% CI: -77 to 273) Agatston unit increase in CAC per 10 $\mu g/m^3$ increase in $PM_{2.5}$. Differences in strength of association between subgroups defined by demographic and clinical characteristics were small. The authors reported a more consistent association of CAC with traffic exposure (distance from a major roadway) than with $PM_{2.5}$ in this study.

In a subsequent analysis of these data, Hoffmann et al. (2009, 190376) examined the PM-ABI association in this population. In this cross-sectional study, no changes in ABI were observed in association with $PM_{2.5}$ concentration nor was evidence of effect modification by demographic and clinical characteristics apparent. As in the previous study (Hoffmann et al., 2007, 091163), residing near a major roadway was a stronger predictor of atherosclerotic changes. Absolute changes in ABI of -0.024 (95% CI: -0.047 to -0.001) were associated with living within 50 m of a major roadway compared to living more than 200 m away.

Each of the studies described above relied on cross-sectional analyses examining differences in long-term average $PM_{2.5}$ concentrations across space (as well as time to the extent baseline examinations were conducted over time). Such associations may reflect the effect of compositional differences in $PM_{2.5}$ as well as the effect of higher $PM_{2.5}$ concentrations. Most associations of $PM_{2.5}$ with CAC (Diez et al., 2008, 156401; Hoffmann et al., 2007, 091163), CIMT (Diez et al., 2008, 156401; Kunzli et al., 2005, 087387), ABI (Diez et al., 2008, 156401; Hoffmann et al., 2009, 190376) and AAC (Allen et al., 2009, 156209) reviewed in this section were weak and/or imprecise. However, several factors including exposure measurement error, variation in baseline measures atherosclerosis, as well as limited power may contribute to the insensitivity of these cross-sectional studies to detect small differences in CAC, CIMT, ABI and AAC. The study by Hoffmann et al. (2007, 091163), which reported large, imprecise and non-significant increases in CAC in association with $PM_{2.5}$, is not distinguished from the other studies reviewed by a superior study design or larger sample size. The several fold difference in the magnitude of CIMT associations reported by Kunzli et al. (2005, 087387) and Diez Roux et al. (2008, 156401) may be related to differences between the study populations. The ambient PM concentrations from these studies are characterized in Table 7-1.

7.2.1.2. Toxicological Studies

In the only study of this kind described in the 2004 PM AQCD, Suwa et al. (2002, 028588) demonstrated more advanced atherosclerotic lesions based on phenotype and volume fraction in the left main and right coronary arteries of rabbits exposed to PM_{10} (5 mg/kg, 2 times/wk×4 wk). Although this study was conducted using IT exposure methodology at a relatively high dose, it provided the first experimental evidence that PM exposure may result in progression of atherosclerosis. Recent toxicological studies conducted using inhalation exposures have replicated these findings at relevant concentrations and are discussed below.

CAPs

New studies have demonstrated increased atherosclerotic plaque area in aortas of $ApoE^{-/-}$ mice exposed to $PM_{2.5}$ CAPs for 4-6 mo (6 h/day×5 days/wk). Average CAPs concentrations ranged from 85 to 138 $\mu g/m^3$ and all of the studies were conducted in Tuxedo or Manhattan, NY. Chen and Nadziejko (2005, 087219) reported that the percentage of aortic intimal surface covered by atherosclerotic lesions in $ApoE^{-/-}$ mice was increased. In male $ApoE^{-/-}/LDLR^{-/-}$ mice, both lesion area

and cellularity in the aortic root were enhanced by Tuxedo, NY CAPs exposure, although there was no change in lipid content. Genetic profiles within plaques recovered from ApoE$^{-/-}$ mice included many of the molecular pathways known to contribute to atherosclerosis, including inflammation (Floyd et al., 2009, 190350). Sun (2005, 087952) similarly demonstrated an enhancement of atherosclerosis in ApoE$^{-/-}$ mice exposed Tuxedo, NY CAPs. Plaque area in the aortic arch and abdominal aorta was significantly increased in the PM-exposed, high fat-chow group compared to air-exposed, high fat-chow group. Macrophage infiltration in the abdominal aorta was also observed in the groups exposed to CAPs. A study conducted in Manhattan for 4 mo (May- September 2007) showed that $PM_{2.5}$ CAPs exposure increased atherosclerotic plaque area and led to higher levels of macrophage infiltration, collagen deposition, and lipid composition in thoracic aortas of ApoE$^{-/-}$ mice (Ying et al., 2009, 190111), which is consistent with the previous two studies described that were conducted in Tuxedo, NY.

Alteration of vasomotor function has been observed in aortic rings of ApoE$^{-/-}$ mice on a high fat diet with long-term exposure to CAPs (Sun et al., 2005, 087952; Ying et al., 2009, 190111). Sun (2005, 087952) reported that. $PM_{2.5}$-exposed animals exhibited increased vasoconstrictor responsiveness to serotonin and PE. Increased ROS and elevated iNOS protein expression in aortic sections of CAPs-exposed mice may have resulted alterations in the NO pathway and generation of peroxynitrite that could have affected vascular reactivity. In contrast, Ying, et al. (2009, 190111) demonstrated decreased maximum constriction induced by PE following Manhattan CAPs exposure. Pretreatment with the soluble guanylate cyclase (sGC) inhibitor ODQ attenuated the response, indicating that CAPs exposure resulted in abnormal NO/sGC signaling. Expression of iNOS mRNA and protein was increased in aortas of CAPs-exposed mice, further supporting a role for NO production. In conjunction with increased NO, aortic superoxide production was demonstrated that appeared to be partially driven by increased NADPH oxidase activity. The difference in vasoconstrictor responses between these two studies may be attributable to varying durations (6 versus 4 mo, respectively) or CAPs compositions.

Sun (2005, 087952) and Ying et al. (2009, 190111) reported similar relaxation responses to ACh for air- and CAPs-exposed mice. However, Manhattan CAPs-exposed mice had a markedly decreased response to A23187, indicating that NO release occurred via Ca^{2+}-dependent mechanisms (Ying et al., 2009, 190111). Abnormal eNOS function is likely responsible for the decreased relaxation response, as activation of eNOS (but not iNOS) is Ca^{2-}-dependent.

A recent study (Sun et al., 2008, 157033) that was part of the research described above (Sun et al., 2005, 087952) investigated tissue factor (TF) expression in aortas, which is a major regulator of hemostasis and thrombosis following vascular injury or plaque erosion. In $PM_{2.5}$-exposed ApoE$^{-/-}$ mice on a high-fat diet, TF was significantly elevated in the plaques of aortic sections compared to air-exposed mice on the high-fat diet. TF expression was generally detected in (1) the extracellular matrix surrounding macrophages and foam cell-rich areas; and (2) around smooth muscle cells.

One new study of CAPs $PM_{2.5}$ or UFPs derived from traffic was conducted. Araujo et al. (2008, 156222) compared the relative impact of UF (0.01-0.18 μm) and fine (0.01-2.5 μm) PM inhalation on aortic lesion development in ApoE$^{-/-}$ mice following a 40-day exposure (5 h/day×3 days/wk for 75 total h). Animals were on a normal chow diet and exposed to CAPs in a mobile inhalation laboratory parked 300 m from a freeway in downtown Los Angeles. Exposure concentrations were ~440 μg/m^3 for $PM_{2.5}$ and ~110 μg/m^3 for UFPs, and the number concentrations were roughly equivalent (4.56×10^5 and 5.59×10^5 particles/cm^3 for $PM_{2.5}$ and UFPs, respectively). Significant increases in plaque size (estimated by lesions at the aortic root) were reported for mice exposed to UFPs only. The lesions were largely comprised of macrophages with intracellular lipid accumulation. Increased total cholesterol measured at the end of the exposure protocol was observed only in the $PM_{2.5}$ group. HDL isolated from the UF PM-exposed mice demonstrated decreased anti-inflammatory protective capacity against LDL-induced monocyte chemotactic activity in an in vitro assay. The livers from the UFP-exposed mice demonstrated significant increases in lipid peroxidation and several stress-related gene products (catalase, glutathione S-transferase Y_a, NADPH-quinone oxidoreductase1, superoxide dismutase 2). Thus, UFPs in these exposures had a substantially greater impact on the systemic response than did $PM_{2.5}$.

Ambient Air

A study employing young BALB/c mice examined the effects of a 4-month exposure (24 h/day×7 days/wk) to ambient air on arterial histopathology (Lemos et al., 2006, 088594). Outdoor exposure chambers were located in downtown Sao Paulo, Brazil next to streets of high traffic density. In the control chamber, PM_{10} and NO_2 were filtered with 50% and 75% efficiency, respectively. The average pollutant concentrations were 2.06 ppm for CO (8-h mean), 104.75 $\mu g/m^3$ for NO_2 (24-h mean), 11.07 $\mu g/m^3$ for SO_2 (24-h mean), and 35.52 $\mu g/m^3$ for PM_{10} (24-h mean) at a monitoring site within 100 m of the inhalation chambers. The pulmonary and coronary arteries demonstrated significant decreases in L/W ratio for animals exposed to the entire ambient mixture compared to controls, indicating thicker walls in these vessels. There was no difference reported for the L/W ratio in renal arteries. Morphologic examination suggested that the increases in L/W ratio were due to muscular hypertrophy rather than fibrosis. The results of this study indicate vascular remodeling of the pulmonary and coronary arteries, as opposed to changes in tone.

To examine the role of systemic inflammation and recruitment of monocytes into plaque tissue as a possible pathway for accelerated atherosclerosis, Yatera et al. (2008, 157162) exposed female Watanabe heritable hyperlipidemic rabbits (42 week old) to Ottawa PM_{10} (EHC-93) via IT instillation (5 mg/rabbit; approximately 1.56 mg/kg) twice a week for 4 wk. Transfusion of whole blood harvested to from exposed and non-exposed animals to donor rabbits supplied labeled monocytes for assessment of monocyte recruitment from the blood to the aortic wall. The fraction of aortic surface and volume of aortic wall taken up by atherosclerotic plaque was increased and the number of labeled monocytes in the atherosclerotic plaques was elevated in rabbits exposed to PM_{10}. In addition, labeled monocytes were attached onto the endothelium overlying atherosclerotic plaques and the number that migrated into the smooth muscle underneath plaques in aortic vessel walls was greater with PM_{10} exposure compared to control. These responses were not observed in normal vessel walls. ICAM-1 and VCAM-1 expression was elevated in atherosclerotic lesions, likely indicating enhanced monocyte adhesion to endothelium and migration into plaques. Monocytes in plaque tissue stained with immunogold demonstrated foam cell characteristics, which were more numerous in the rabbits exposed to PM_{10}.

Gasoline Exhaust

Lund and colleagues (2007, 125741) used whole emissions from gasoline exhaust to investigate changes in the transcriptional regulation of several gene products with known roles in both the chronic promotion and acute degradation/destabilization of atheromatous plaques. These 50-day exposures (6 h/day×7 days/wk) employed $ApoE^{-/-}$ mice on high-fat chow and the concentrations of the high exposure group were 61 $\mu g/m^3$ for PM, 19 ppm for NO_X, 80 ppm for CO, and 12.0 ppm for total hydrocarbons. The average particle number median diameter was approximately 15 nm (McDonald et al., 2007, 156746). Dilutions of gasoline engine emissions induced a concentration-dependent increase in transcription of matrix metalloproteinase (MMP) isoform 9, ET-1, and HO-1 in aortas; MMP-3 and -9 mRNA levels were only increased in animals in the highest exposure group. Strong increases in oxidative stress markers (nitrotyrosine and TBARS) in the aortas were also observed. However, using a high-efficiency particle trap, they established that most of the effects were caused by the gaseous portion of the emissions and not the particles. This study did not directly address lesion area.

7.2.2. Venous Thromboembolism

One epidemiologic study examined the relationship between long term PM_{10} concentration, venous thromboembolism, and laboratory measures of hemostasis (prothrombin and activated partial thomboplastin times [PT; PTT]). PT and PTT measure the extrinsic and intrinsic blood coagulation pathways, the former activated in response to blood vessel injury, the latter, key to subsequent amplification of the coagulation cascade and propagation of thrombus (Mackman et al., 2007, 156723). Decreases in PT and PTT are consistent with a hypercoagulable, prothrombotic state.

7.2.2.1. Epidemiologic Studies

Baccarelli et al. (2008, 157984) studied 2,081 residents (56% female) of the Lombardy region of Italy whose ages ranged from 18 to 84 yr old. In this case-control study of 871 patients with ultrasonographically or venographically diagnosed lower extremity deep vein thrombosis (DVT) and 1,210 of their healthy friends or relatives (1995-2005), the authors used arithmetic averaging of PM_{10} data available at 53 monitors in nine geographic areas to estimate 1-yr avg residence-specific exposures. They found -0.06 (95% CI: -0.11 to 0) and -0.12 (95% CI: -0.23 to 0) decreases in standardized correlation coefficients for PT as well as 0.01 (95% CI: -0.03 to 0.04) and -0.09 (95% CI: -0.19 to 0.01) decreases in standardized correlation coefficients for PTT among cases and controls, respectively, per 10 $\mu g/m^3$ increase in PM_{10}. Patients with DVT who were taking heparin or coumarin anticoagulants were not asked to stop taking them before measurement of PT and aPTT. Of additional note, PT was neither adjusted for differences in reagents used to determine it nor conventionally reported as the International Normalized Ratio (INR). The ambient PM concentrations from this study are characterized in Table 7-1.

7.2.3. Metabolic Syndromes

7.2.3.1. Epidemiologic Studies

Chen and Schwartz (2008, 190106) studied 2,978 residentially stable participants in 33 U.S. communities (age range = 20-89 yr; 49% female) who were examined during phase 1 of the National Health and Nutrition Examination Survey III (1989-1991). In this cross-sectional study, the authors used inverse-distance weighted averaging of U.S. EPA AQS monitored data from participant and adjacent counties of residence to estimate 1-yr avg exposures to PM_{10}. They found that after adjustment, residents of communities with lower PM_{10} concentrations had fewer white blood cells than residents of communities with higher PM_{10} concentrations. This difference increased with increasing number of metabolic abnormalities (insulin resistance; hypertension; hypertriglyceridemia; low high-density lipoprotein cholesterol; abdominal obesity) reported by the participant. This observed difference across individuals with different degrees of metabolic abnormalities supports the concept that the presence of a metabolic syndrome may impart greater susceptibility to PM-associated long-term CVD effects.

7.2.3.2. Toxicological Studies

Diabetics as a potentially susceptible subpopulation have only recently been evaluated. A toxicological study of a diet-induced obesity mouse model (C57BL/6 fed high-fat chow for 10 wk) examined the effects of a 128-day $PM_{2.5}$ CAPs exposure (mean mass concentration 72.7 $\mu g/m^3$; Tuxedo, NY) on insulin resistance, adipose inflammation, and visceral adiposity (Sun et al., 2009, 190487). Elevated fasting glucose and insulin levels were observed in CAPs-exposed mice compared to air-exposed during the glucose tolerance test. Aortic rings of mice exposed to CAPs demonstrated decreased peak relaxation to ACh or insulin, which was associated with reduced NO bioavailability. Additionally, insulin signaling was impaired in aortic tissue via lowered endothelial Akt phosphorylation. Increases in adipokines and systemic inflammatory markers (i.e., TNF-α, IL-6, E-selectin, ICAM-1, PAI-1, resistin, leptin) were reported for CAPs–exposed mice. CAPs resulted in increased visceral and mesenteric fat mass, as well as greater adipose tissue macrophages in epididymal fat pads and larger adipocyte size compared to mice in the filtered air group. The results of this study demonstrate that $PM_{2.5}$ exposure can exaggerate insulin resistance, visceral adiposity, and inflammation in mice fed high-fat chow.

7.2.4. Systemic Inflammation, Immune Function, and Blood Coagulation

7.2.4.1. Epidemiologic Studies

As discussed in Section 7.2.3.1, Chen and Schwartz (2008, 190106) conducted a cross-sectional study in 33 U.S. communities and used inverse-distance weighted averaging of U.S. EPA AQS monitored data from participant and adjacent counties of residence to estimate 1-yr avg exposures to PM_{10} (median concentration within quartiles = 23.1, 31.2, 38.8 and 53.7 $\mu g/m^3$). They found that after adjustment, residents of communities in quartile 1 had 138 (95% CI: 2-273) fewer white blood cells ($\times 10^6$/L) than residents of communities in quartiles 2-4. This difference increased with increasing number of metabolic abnormalities.

Forbes et al. (2009, 190351) studied approximately 25,000 adults (age ≥ 16 yr; 53% female) who were representatively sampled from 720 English postcode sectors and participated in the Health Survey for England (1994, 1998 and 2003). In this fixed-effects meta-analysis of year-specific cross-sectional findings, the authors used dispersion modeling of emissions and weather data to estimate 2-yr avg exposures to PM_{10} at participant postcode sector centroids (median in 1994, 1998 and 2003 = 19.5, 17.9 and 16.2 $\mu g/m^3$, respectively). They found little evidence of a PM_{10}-inflammatory marker association, i.e., only a -0.08% (95% CI: -0.25 to 0.10) decrease in fibrinogen concentration and a 0.14% (95% CI: -1.00 to 1.30) increase in CRP concentration per 1 $\mu g/m^3$ increase in PM_{10}.

Calderon-Garciduenas et al. (2007, 091252) compared residentially stable, non-smoking healthy children (age range: 6-13 yr) living and attending school between 2003-2004 in Mexico City (historically high PM; altitude 2,250 m) and Polotitlán (historically low PM; altitude 2,380 m). In this ecologic study, residents of Mexico City (n = 59; 93% female) had fewer white blood cells and neutrophils ($\times 10^9$/L) than residents of Polotitlán (n = 22; 69% female): unadjusted mean 6.2 (95% CI: 5.7-6.6) versus 6.9 (95% CI: 6.3-7.5) and 2.9 (95% CI: 2.3-3.5) versus 3.8 (95% CI: 3.2-4.4), respectively.

Calderon-Garciduenas et al. (2009, 192107) subsequently compared 37 unadjusted mean measures of immune function and inflammation among an expanded number of these participants. They found that under a two-sided type I error rate (α) = 0.05, 16 (43%) of the measures were significantly different in residents of southwest Mexico City (n = 66; 48% female) than those in Polotitlán (n = 93; 57% female). However, only 8 measures were significantly different after Bon Ferroni-correction (α = 0.05 / 37 = 0.001) and even fewer would be after adjustment for reported correlation between the measures of immune function and inflammation, e.g., CRP and lipopolysaccharide binding protein (Pearson's r = 0.71).

Two cross-sectional analyses of PM_{10} concentration and markers of immune function or inflammation have been conducted with significant changes observed in the NHANES population (stronger effects among those with metabolic disorders) (Chen and Schwartz, 2008, 190106) but not in a relative large survey of adults, which was conducted in England (Forbes et al., 2009, 190351). Ecological analyses comparing children in high versus low pollution regions in Mexico show differences in unadjusted blood markers that may be related to PM concentration or other unmeasured risk factors that differs across the communities studied (Calderon-Garciduenas et al., 2007, 091252; Calderón-Garcidueñas et al., 2009, 192107).

7.2.4.2. Toxicological Studies

In addition to the $PM_{2.5}$ study mentioned previously that showed increased TF expression (an important initiator of thrombosis) in aortas of $ApoE^{-/-}$ mice following subchronic CAPs exposure (Sun et al., 2008, 157033), three recent studies examined hematology and clotting parameters in rats and mice exposed to DE, gasoline exhaust, or hardwood smoke for 1 week or 6 mo (Reed et al., 2004, 055625; Reed et al., 2006, 156043; Reed et al., 2008, 156903). In all studies, male and female F344 rats were exposed to the mixtures by whole-body inhalation for 6 h/day, 7 day/wk. Respiratory effects for these studies are presented in Section 7.3.3.

Diesel Exhaust

The target PM concentrations in the DE study was 30, 100, 300, and 1,000 μg/m^3 and the MMAD was 0.10–0.15 μm (Reed et al., 2004, 055625). Male and female rats exposed to DE at the highest concentration (NO concentration 45.3 ppm; NO_2 concentration 4.0 ppm; CO concentration 29.8 ppm; SO_2 concentration 365 ppb) for 6 mo demonstrated decreased serum Factor VII, but no change in plasma fibrinogen or thrombin anti-thrombin complex (TAT) (Reed et al., 2004, 055625). White blood cells were decreased only in female rats in the highest exposure group. Another DE study of shorter duration (4 wk, 4 h/day, 5day/wk; PM mass concentration 507 or 2201 μg/m^3, CO 1.3 and 4.8 ppm, NO <2.5 and 5.9 ppm, NO_2 <0.25 and 1.2 ppm, SO_2 0.2 and 0.3 ppm for low and high PM exposures, respectively) did not demonstrate changes in hematologic parameters or those related to coagulation (i.e., PT, PPT, plasma fibrinogen, D-dimer) or inflammation (i.e., CRP) in SH or WKY rats (Gottipolu et al., 2009, 190360). Together, these findings do not support a DE-related stimulation of blood coagulation following 1 or 6 mo of exposure.

Hardwood Smoke

The target PM concentrations in the hardwood smoke study was 30, 100, 300, and 1,000 μg/m^3 and the MMAD was 0.25-0.36 μm (Reed et al., 2006, 156043). In male rats exposed to hardwood smoke, the mid-low group (PM concentration 113 μg/m^3; NO, NO_2, SO_2 concentrations 0 ppm; CO concentration 1,832.3 ppm) had the greatest responses in hematology parameters, including increased hematocrit, hemoglobin, lymphocytes, and decreased segmented neutrophils (Reed et al., 2006, 156043). Platelets were elevated in male and female rats after 1 week of exposure, but this response returned to control values following the 6-month exposure. No changes were observed for any coagulation markers at 6 mo.

Gasoline Exhaust

PM mass in the gasoline exhaust study ranged from 6.6 to 59.1 μg/m^3, with the corresponding number concentration between 2.6×10^4 and 5.0×10^5 particles/cm^3; the dilutions for the gasoline exhaust were 1:10, 1:15 or 1:90 and filtered PM at the 1:10 dilution (Reed et al., 2008, 156903). Similar to the responses observed with hardwood smoke, male and female rats in the mid- and high-gasoline exhaust exposure groups (NO concentrations 11.9 and 18.4 ppm; NO_2 concentrations 0.5 and 0.9 ppm; CO concentration 73.2 and 107.3 ppm; SO_2 concentration 0.38 and 0.62 ppm, respectively) demonstrated elevated hematocrit and hemoglobin; RBC count was also elevated in these groups (Reed et al., 2008, 156903). The only response that appeared somewhat dependent on the presence of particles was increased RBC in female rats at 6 mo, although the authors attributed the observed increases to the high concentration of CO.

Collectively, these studies do not indicate robust systemic inflammation or coagulation responses in F344 rats following 6-month exposures to diesel, hardwood smoke, or gasoline exhaust. The limited effects that were observed could possibly be due to the varying gas concentrations in the exposure mixtures.

7.2.5. Renal and Vascular Function

Two recent epidemiologic studies have tested associations between PM exposure and indicators of renal and vascular function (urinary albumin to creatinine ratio [UACR] and blood pressure). UACR is a measure of urinary albumin excretion (National Kidney Foundation, 2008, 156796). When calculated as the ratio of albumin to creatinine concentrations in untimed ("spot") urine samples, UACR approximates 24-h urinary albumin excretion and can be used to identify albuminuria, a marker of generalized vascular endothelial damage (Xu et al., 2008, 157157). Values ≥ 30 mg/g (3.5 mg/mmol) and ≥ 300 mg/g (34 mg/mmol) usually define micro- and macroalbuminuria, both of which are associated with increases in CVD incidence and mortality (Bigazzi et al., 1998, 156272; Deckert et al., 1996, 156389; Dinneen and Gerstein, 1997, 156403; Gerstein et al., 2001, 156466; Mogensen, 1984, 156769). Several researchers have called the

dichotomization of albuminuria into question, observing that there is no threshold below which risk of cardiovascular and end-stage kidney disease disappears (Forman and Brenner, 2006, 156439; Knight and Curhan, 2003, 179900; Ruggenenti and Remuzzi, 2006, 156933).

Systolic, diastolic, pulse, and mean arterial blood pressures (SBP; DBP; PP; MAP) in mmHg have also been used as measures of cardiovascular disease. Franklin et al. (1997, 156446) suggested that SBP and PP were the only two measures predictive of carotid stenosis in a multivariable analysis considering all 4 measures, whereas Khattar et al. (2001, 155896) suggested that their prognostic significance in hypertensive populations may differ by age, with SBP and PP being most predictive among those ≥ 60 yr and DBP among those <60 yr old (Khattar et al., 2001, 155896).

7.2.5.1. Epidemiologic Studies

O'Neill et al. (2007, 156006) examined the association of UACR with $PM_{2.5}$ and PM_{10} among members of the MESA population described previously (Diez et al., 2008, 156401). For this study of UACR, which included cross-sectional and longitudinal analyses, the study population was restricted to a subset of 3,901 participants (mean age = 63 yr; 52% female) with complete covariate, outcome and exposure data at their first through third exams (2000-2004). In cross-sectional analyses, the authors found that after adjustment for demographic and clinical characteristics, 10 $\mu g/m^3$ increases in 20-yr imputed exposures to $PM_{2.5}$ and PM_{10} were associated with negligible 0.002 (95% CI: -0.048 to 0.052) and -0.002 (95% CI: -0.038 to 0.035) mean differences in baseline log UACR, respectively. Similarly, small statistically non-significant decreases in the prevalence of microalbuminuria (defined in this setting as ≥ 25 mg/g) provided little evidence of an effect on renal function. These largely null cross-sectional findings mirrored those based on the study's shorter-term (30- and 60-day) $PM_{2.5}$ and PM_{10} exposures. Moreover, longitudinal analyses revealed only a weak association between 3-yr change in log UACR and 20-yr PM_{10} exposure. Evidence of effect modification by demographic and geographic characteristics was not apparent in either the cross-sectional or longitudinal analyses.

Auchincloss et al. (2008, 156234) focused on automated, oscillometric, sphygmomanometric measures of blood pressures in mmHg (SBP; DBP; PP; MAP). Like O'Neill (2007, 156006), Diez et al. (2008, 156401) and Allen et al. (2007, 156006), Auchincloss et al. (2008, 156234) based their examination on the previously described MESA population. The authors included 5,112 study participants (age range = 45-84 yr; 52% female) who were free of clinically manifested CVD at their baseline exam in one of six primarily urban U.S. locations (2000-2002). In this cross-sectional study, they used arithmetic averaging of EPA AQS $PM_{2.5}$ data available at the monitor nearest to each participant's geocoded U.S. Postal Service ZIP code centroid to estimate 30- and 60-day avg exposures to $PM_{2.5}$. They found small nonsignificant increases of 1.5 (95% CI: -0.2 to 3.2), 0.2 (95% CI: -0.7 to 1.0), 1.3 (95% CI: 0.1 to 2.6), and 0.6 (95% CI: -0.4 to 1.7) mmHg increases in SBP, DBP, PP and MAP, respectively, per 10 $\mu g/m^3$ increase in 30-day avg $PM_{2.5}$ exposure. Associations were slightly weaker for 60-day avg $PM_{2.5}$ exposure and among participants without hypertension, during cooler weather, in the presence of low NO_2, residing >300 m from a highway, or surrounded by lower road density.

Finally, the Calderon-Garciduenas et al. (2007, 091252) ecologic study introduced in Section 7.2.3.1 also found that children residing in Mexico City had higher mean pulmonary artery pressure as assessed by Doppler echocardiography and fasting plasma endothelin-1 (ET-1) than residents in Polotitlán: unadjusted mean 17.5 (95% CI: 15.7-19.4) versus 14.6 (95% CI: 13.8-15.4) mmHg and 2.23 (95% CI: 1.93-2.53) versus 1.23 (95% CI: 1.11-1.35) pg/mL, respectively. Within Mexico City, ET-1 was higher in residents of the Northeast (historically higher $PM_{2.5}$) than those of the Southwest (historically lower $PM_{2.5}$).

The MESA analyses of UACR (O'Neill et al., 2007, 156006) and the ecologic study of children living in a highly polluted area of Mexico (Calderon-Garciduenas et al., 2007, 091252) provide little evidence that long-term exposure to $PM_{2.5}$ had an effect on renal and vascular function, respectively. Auchincloss et al. (2008, 156234) reports small nonsignificant associations of blood pressure with 30- and 60-day avg $PM_{2.5}$ concentrations. PM concentrations from the analyses are characterized in Table 7-1.

Table 7-1. Characterization of ambient PM concentrations from studies of subclinical measures of cardiovascular diseases and long-term exposure.

Study	Location	Mean Concentration ($\mu g/m^3$)	Upper Percentile Concentrations ($\mu g/m^3$)
PM_{10}			
Diez Roux et al. (2008, 156401)	MESA: 6 Cities U.S.	20 yr imputed mean: 34	NR
O'Neill et al. (2007, 156005)	MESA: 6 Cities U.S.	Long-Term Exposure: 1982-2002: 34.7 1982-1987: 40.5 1988-1992: 36 1993-1997: 30.6 1998-2002: 29.7 Previous Month: 27.5	NR
Baccarelli et al. (2008, 157984)	Lombardy Region Italy	NR	NR
Rosenlund et al. (2006, 089796)	Stockholm, Sweden	30-y avg PM_{10} (traffic) Cases: 2.6 Controls: 2.4	5th-95th %: 0.5-6 0.6-5.9
Chen and Swartz (2008, 190106)	US Population (NHANES)	Annual avg: 36.8	NR
Forbes et al. (2009, 190351)	British Population	1994: 19.5 (median) 1998: 17.9 (median) 2003: 16.2 (median)	1994, Min-Max: 12.5-36.1 1998, Min-Max: 12.6-27.0 2003, Min-Max: 11.0-22.7
$PM_{2.5}$			
Hoffmann et al.(2007, 091163)	HNRS, 3 Cities Germany	Annual avg: 22.8	NR
Allen et al. (2009, 156209)	MESA: 5 Cities	Annual avg: 15.8	Min-Max: 10.8-24.7
Kunzli et al. (2006, 087387)	VEAPS BVAIT	Annual avg: 20.3	Min-Max: 5.2-26.9
Auchincloss et al. (2008, 156234)	MESA: 6 Cities	Prior 30 days: 16.8 Prior 60 days: 16.7	NR
O'Neill (2007, 156006)	MESA: 6 Cities U.S.	Previous Month: 16.5	NR
Diez Roux et al. (2008, 156401)	MESA: 6 Cities U.S.	20-y imputed mean: 21.7	NR
Hoffmann et al. (2009, 190376)	HNRS: 3 Cities Germany	Annual avg: 22.8	Min-max: 19.8-26.8
Calderon-Garcidueñas et al. (2009, 192107)	Southwest Mexico (high pollution)	Annual avg: 25	NR
	Potitlan (low pollution)	Annual avg: <15	NR
Calderon-Garcidueñas et al. (2007, 091252)	Southwest Mexico (high pollution)	NR	NR
	Potitlan (low pollution)	NR	NR

MESA: Multi-Ethnic Study of Atherosclerosis
HNRS: Heinz Nixdorf Recall Study
VEAPS: Vitamin E Atherosclerosis Progression Study
BVAIT: B-Vitamin Atherosclerosis Intervention Trial

7.2.5.2. Toxicological Studies

In a $PM_{2.5}$ CAPs study of 10 wk (6 h/day×5 days/wk) in Tuxedo, NY (mean mass concentration 79.1 $\mu g/m^3$), there was no difference in mean arterial pressure (MAP) in SD rats between groups (Sun et al., 2008, 157032). When angiotensin II (Ang II) was infused during the last week of exposure to induce systemic hypertension, the MAP slope was consistently greater in the CAPs-exposed rats compared to the filtered air group. Furthermore, thoracic aortic rings were more responsive to phenylephrine-induced constriction and less responsive to ACh-induced relaxation in the PM+Ang II vessels. In contrast to the latter findings, the relaxation response was exaggerated in the PM+Ang II aortic segments with a Rho-kinase (ROCK) inhibitor. Superoxide production in aortic rings increased in the PM+Ang II group compared to the filtered air group and the addition of

NAD(P)H oxidase inhibitor (apocynin) or a NOS inhibitor (L-NAME) attenuated the superoxide generation. The levels of tetrahydrobiopterin (BH_4) were decreased in mesenteric vasculature and the heart by 46% and 41% in the PM+Ang II group compared to controls, respectively; furthermore, levels of BH_4 in the liver were similarly reduced, which is consistent with a systemic effect of CAPs. Together, these findings indicate that CAPs potentiate Ang II-induced hypertension and alter vascular reactivity, perhaps through activated NADPH oxidase and eNOS uncoupling that result in oxidative stress generation and triggering of the Rho/ROCK signaling pathway.

7.2.6. Autonomic Function

7.2.6.1. Toxicological Studies

Hwang et al. (2005, 087957) and Chen and Hwang (2005, 087218) used radiotelemetry to examine the chronic changes in HR and HRV resulting from the same CAPs exposures described previously (Chen and Nadziejko, 2005, 087219). The overall average CAPs exposure concentration was 133 $\mu g/m^3$ and results indicate differing responses to CAPs between $ApoE^{-/-}$ mice and their genetic background strain, C57BL/6J mice (Hwang et al., 2005, 087957). Using the time period of 1:30-4:30 a.m., C57BL/6J mice showed a HR increase only over the last month of exposure. In contrast, $ApoE^{-/-}$ mice had chronic decreases of 33.8 beat/min for HR. Changes in HRV (SDNN and rMSSD) were somewhat more complicated, with biphasic responses in $ApoE^{-/-}$ mice over the 5-month period (initial increase over first 6 wk, decrease over next 12 wk, and slight upward turn for remainder of the study)(Chen and Hwang, 2005, 087218). Increasing linear trends were observed in C57BL/6J mice for SDNN and rMSSD. The average CAPs concentration for the HRV study was 110 $\mu g/m^3$. However, only three C57BL/6J mice in the exposure group were included in the analysis compared to ten $ApoE^{-/-}$ animals, thus making it difficult to interpret the C57BL/6J mice responses (Chen and Hwang, 2005, 087218; Hwang et al., 2005, 087957).

7.2.7. Cardiac changes

7.2.7.1. Toxicological studies

Two recent toxicological studies have evaluated the effects of PM on cardiac effects including pathology and gene expression. Cardiac mitochondrial function has also been evaluated following PM exposure in rats.

Diesel Exhaust

A recent study of DE exposure (PM mass concentration 507 or 2,201 $\mu g/m^3$, CO 1.3 or 4.8 ppm, NO <2.5 or 5.9 ppm, NO_2 <0.25 or 1.2 ppm, SO_2 0.2 or 0.3 ppm for low and high PM exposures, respectively; geometric median number diameter 85 nm) indicated a hypertensive-like cardiac gene expression in WKY rats that mimicked baseline patterns in air-exposed SH rats (Gottipolu et al., 2009, 190360). Exposure to the high concentration of DE for 4 wk (4 h/day, 5 day/wk) led to downregulation of genes involved in stress, antioxidant compensatory response, growth and extracellular matrix regulation, membrane transport of molecules, mitochondrial function, thrombosis regulation, and immune function. No genes were affected by DE in SH rats. A dose-dependent inhibition of mitochondrial aconitase activity in both rat strains was observed, indicating a DE effect on oxidative stress. It should be noted that while DE-related cardiovascular effects were found in WKY rats only, pulmonary inflammation and injury were observed in both strains (Sections 7.3.3.2 and 7.3.5.1).

Model Particles

Wallenborn et al. (2008, 191171) examined the subchronic (5 h/day, 3 day/wk, 16 wk) pulmonary, cardiac, and systemic effects of nose-only exposure to particulate $ZnSO_4$ (9, 35, or 120 $\mu g/m^3$) in WKY rats. Particle size was reported to be 31-44 nm measured as number median diameter. Although changes in pulmonary inflammation or injury and cardiac pathology were not observed, effects on cardiac mitochondrial protein and enzyme levels were noted (i.e., increased ferritin levels, decrease in succinate dehydrogenase activity), possibly indicating a small degree of mitochondrial dysfunction. Glutathione peroxidase, an antioxidant enzyme, was also decreased in the cardiac cytosol. Gene expression analysis identified alterations in cardiac genes involved in cell signaling events, ion channels regulation, and coagulation in animals exposed to the highest $ZnSO_4$ concentration only. This study demonstrates a possible direct effect of $ZnSO_4$ on extrapulmonary systems, as suggested by the lack of pulmonary effects (Section 7.3.3.2).

7.2.8. Left Ventricular Mass and Function

Van Hee et al. (2009, 192110) studied 3,827 participants (age range = 45-84 yr; 53% female) who underwent magnetic resonance imaging (MRI) of the heart at the baseline examination of the MESA cohort (2000-2002). This cross-sectional study focused on two MRI-based outcome measures: left ventricular mass index (LVMI, g/m^2) and ejection fraction (EF, %), the former estimated using the DuBois formula for body surface area, the latter as the ratio of stroke volume to end diastolic volume. The study also estimated annual mean exposures to $PM_{2.5}$ at participants' geocoded residential addresses in 2000 using ordinary kriging of U.S. EPA AQS concentration data. In fully adjusted models, it found 3.8 (95% CI: -6.1 to 13.7) g/m^2 and -3.0% (-8.0 to 2.0) differences in LVMI and EF per 10 $\mu g/m^3$ increment in $PM_{2.5}$. The findings were small and imprecise, albeit suggestive of a slight, PM-associated increase in the mass and decrease in the function of the left ventricle. The effect of living within 50 m of a major roadway on LVMI was greater than the effect of $PM_{2.5}$ (i.e., 1.4 g/m^2 [95% CI: 0.3-2.5] per 10 $\mu g/m^3$.)

7.2.9. Clinical Outcomes in Epidemiologic Studies

Several epidemiologic studies of U.S. and European populations have examined associations between long-term PM exposures and clinical CVD events (Baccarelli et al., 2008, 157984; Hoffmann et al., 2006, 091162; Hoffmann et al., 2009, 190376; Maheswaran et al., 2005, 088683; Maheswaran et al., 2005, 090769; Miller et al., 2007, 090130; Rosenlund et al., 2006, 089796; Solomon et al., 2003, 156994; Zanobetti and Schwartz, 2007, 091247). Results from these studies are summarized in Figure 7-1. The ambient PM concentrations from these studies are characterized in Table 7-2.

Coronary Heart Disease

Epidemiologic studies examining the association of coronary heart disease (CHD) with long-term PM exposure are discussed below (Hoffmann et al., 2006, 091162; Maheswaran et al., 2005, 090769; Miller et al., 2007, 090130; Puett et al., 2008, 156891; Rosenlund et al., 2006, 089796; Rosenlund et al., 2009, 190309; Zanobetti and Schwartz, 2007, 091247). Cases of CHD were variably defined in these studies to include history of angina pectoris, MI, coronary artery revascularization (bypass graft; angioplasty; stent; atherectomy), and congestive heart failure (CHF). Results pertaining to death from CHD are described in Section 7.6.

Miller et al. (2007, 090130) studied incident, validated MI, revascularization, and CHD death, both separately and collectively, among 58,610 post-menopausal female residents of 36 U.S. metropolitan areas (age range = 50-79 yr) enrolled in the Women's Health Initiative Observational Study (WHI OS, 1994-1998). In this prospective cohort study of participants free of CVD at baseline (median duration of follow-up = 6 yr), the authors used arithmetic averaging of year 2000 EPA AQS $PM_{2.5}$ data available at the monitor nearest to each participant's geocoded U.S. Postal Service five-digit ZIP code centroid to estimate 1-yr avg exposures. They found 6% (95% CI: -15 to 34), 20% (95% CI: 0-43) and 21% (95% CI: 4-42) increases in the overall risk of MI, revascularization, and

their combination with CHD death per 10 μg/m^3 increase in $PM_{2.5}$, respectively. Hazards were higher within than between cities and in the obese. For the combined CVD outcome (MI, revascularization, stroke, CHD death, cerebrovascular disease), authors reported a 24% (95% CI: 9-41) increase in risk that was higher among participants at higher than lower quintiles of body mass index, waist-to-hip ratio, and waist circumference. The $PM_{2.5}$-CVD association was stronger among non-diabetic than diabetic participants.

Table 7-2. Characterization of ambient PM concentrations from studies of clinical cardiovascular diseases and long-term exposure.

Study	Location	Mean Annual Concentration (μg/m^3)	Upper Percentile Concentrations (μg/m^3)
PM_{10}			
Puett et al. (2008, 156891)	13 U.S. States	21.6	
Zanobetti and Schwartz (2007, 091247)	21 U.S. Cities	28.8	Overall range NR
Rosenlund et al. (2006, 089796)	Stockholm, Sweden	30 y avg PM_{10} (traffic) Cases: 2.6 Controls: 2.4	5th-95th Percentile 0.5-6.0 0.6-5.9
Rosenlund et al. (2009, 190309)	Stockholm, Sweden	5-yr avg PM_{10} from traffic: Cases: 2.4 (median) Controls: 2.2 (median)	
Maheswaran et al. (2005, 090769)	Sheffield, U.K.	Range of means in each quintile: 16-23.3	NR
Baccarelli et al. (2008, 157984)	Lombardia Region, Italy	NR	NR
$PM_{2.5}$			
Miller et al. (2007, 090130)	WHI: 36 Metropolitan areas	Citywide avg (yr 2000): 13.5	Min-max: 4-19.3
Hoffmann et al. (2006, 091162)	HNRS: 2 Cities Germany	23.3	NR
Hoffman et al. (2009, 190376)	HRNS: 2 Cities German	22.8	NR

WHI: Women's Health Initiative
HNRS: Heinz Nixdorf Recall Study

Puett et al. (2008, 156891) studied incident, validated CHD, CHD death, and non-fatal MI among 66,250 female residents (mean age = 62 yr) of metropolitan statistical areas in thirteen northeastern U.S. states who were enrolled in the Nurses' Health Study (NHS, 1992-2002). In this prospective cohort study of women without a history of non-fatal MI at baseline (maximum duration of follow-up = 4 yr), the authors used two-stage, spatially smoothed, land use regression to estimate residence-specific, 1-yr ma PM_{10} exposures from U.S. EPA AQS and emissions, IMPROVE, and Harvard University monitor data. They found a 10% (95% CI: -6 to 29) increase in risk of first CHD event per 10 μ/m^3 increase in 1-yr avg PM_{10} exposure, while the association with MI was close to the null value. The association with fatal CHD event of 30% (95% CI: 0-71) was stronger. Furthermore, associations with CHD death were higher in the obese and in the never smokers.

Rosenlund et al. (2006, 089796) studied 2,938 residents of Stockholm County, Sweden (age range = 45-70 yr; 34% female). In this case-control study of 1,085 patients with their first, validated non-fatal MI and an age-, gender- and catchment-stratified random sample of 1,853 controls without MI (1992-1994), the authors used street canyon-adjusted dispersion modeling of emissions data to estimate 30-yr avg exposure to PM_{10} (median = 2.4 μg/m^3). They found that the OR for prevalent MI per 10 μg/m^3 increase in PM_{10} was 0.85 (95% CI: 0.50-1.42). The OR for fatal MI was elevated, but not statistically significant.

In a more recent study, Rosenlund et al. (2009, 190309) evaluated 554,340 residents (age range = 15-79 yr; 49% female) of Stockholm County, Sweden (1984-1996). In this population-based, case-control study of 43,275 cases of incident, validated MI, the authors used dispersion modeling of traffic emissions and land use data to estimate 5-yr avg exposure to PM_{10}. They found that after

adjustment for demographic, temporal, and socioeconomic characteristics, the OR for MI per 5 $\mu g/m^3$ increase in PM_{10} was 1.04 (95% CI: 1.00-1.09). ORs were higher after restriction to fatal cases, in- or out-of-hospital deaths, and participants who did not move between population censuses. Authors state that control for confounding was superior in their previous study (Rosenlund et al., 2006, 089796) although the size of the population was larger in this recent study (Rosenlund et al., 2009, 190309).

Zanobetti and Schwartz (2007, 091247) studied ICD-coded recurrent MI (ICD 9 410) and post-infarction CHF (ICD 9 428) among 196,131 Medicare recipients (age ≥ 65 yr; 50% female) discharged alive following MI hospitalization in 21 cities from 12 U.S. states (1985-1999). In this ecologic, open cohort study of re-hospitalization among MI survivors (mean duration of follow-up = 3.6 and 3.7 yr for MI and CHF, respectively), the authors used arithmetic averaging of EPA AQS PM_{10} data available in the county of hospitalization to estimate 1-yr avg exposures. They found 17% (95% CI: 5-31) and 11% (95% CI: 3-21) increases in the risk of recurrent MI and post-infarction CHF, respectively, per 10 $\mu g/m^3$ increase in PM_{10} exposure. Hazards were somewhat higher among persons aged >75 yr.

Hoffmann et al. (2006, 091162) studied self-reported CHD (MI or revascularization) among 3,399 residents of Essen and Mülheim, Germany (age range = 45-75 yr; 51% female) at the baseline exam of the Heinz Nixdorf Recall Study (2000-2003) introduced previously. In this cross-sectional ancillary study, the authors used dispersion modeling of emissions, climate and topography data to estimate 1-yr avg exposure to $PM_{2.5}$ (mean = 23.3 $\mu g/m^3$). They found little evidence of an association between $PM_{2.5}$ and CHD in these data. After adjustment for geographic, demographic and clinical characteristics, the OR for prevalent CHD per 10 $\mu g/m^3$ increase in exposure was 0.55 (95% CI: 0.14-2.11).

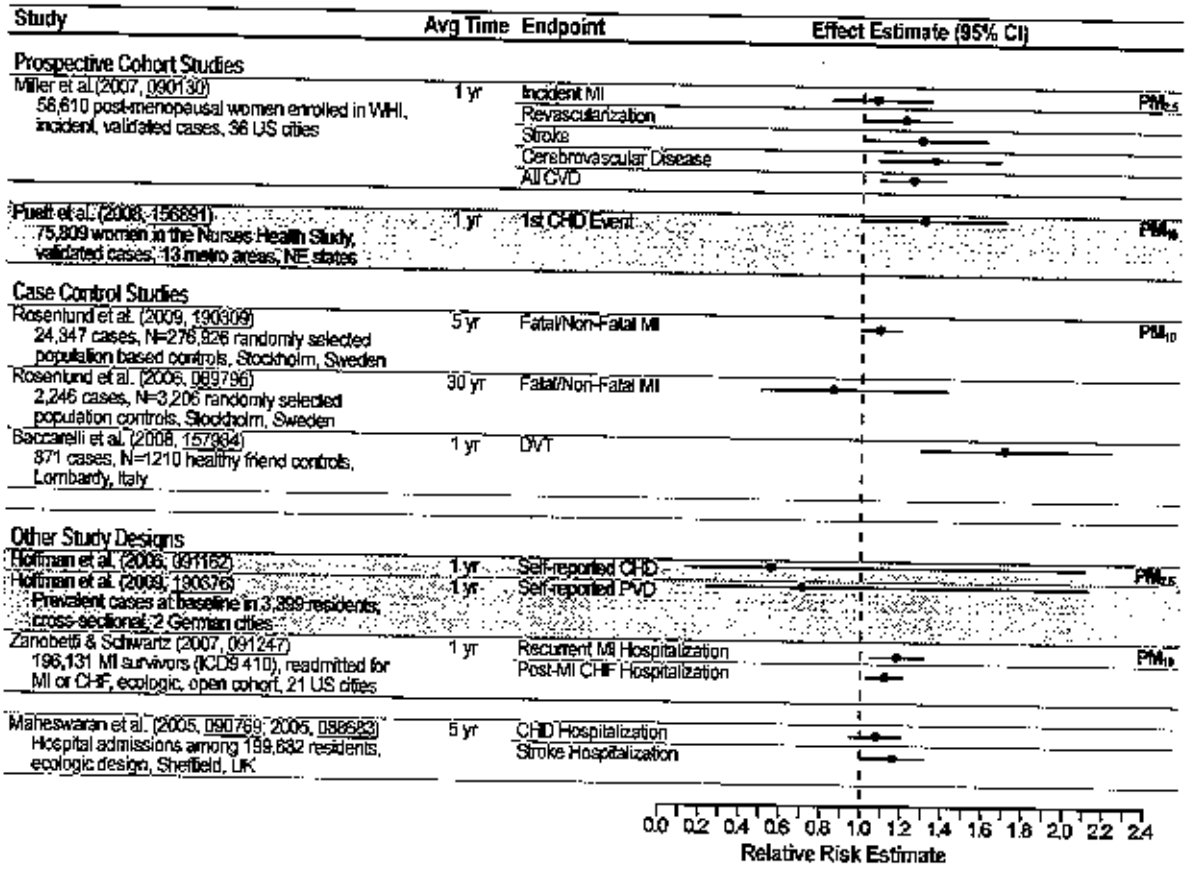


Figure 7-1. Risk estimates for the associations of clinical outcomes with long-term exposure to ambient $PM_{2.5}$ and PM_{10}.

In the study of 1,030 census enumeration districts in Sheffield, U.K. described previously, Maheswaran et al. (2005, 090769) studied 11,407 ICD-10-coded emergency hospitalizations for CHD (ICD10 I20-25) among 199,682 residents (age ≥ 45 yr; 45% female). In this ecologic study, the authors used dispersion modeling of emissions and climate data to estimate 5-yr avg exposure to PM_{10}. They found that after adjusting for smoking prevalence, controlling for socioeconomic factors, and smoothing, the age- and gender-standardized rate ratios for CHD admission were 1.01 (95% CI: 0.92-1.11), 1.04 (95% CI: 0.93-1.15), 0.97 (95% CI: 0.87-1.08), and 1.07 (95% CI: 0.95-1.20) across PM_{10} quintiles. The linear trend was somewhat stronger for CHD mortality (Section 7.3).

The study of post-menopausal women enrolled in the WHI OS by Miller et al. (2007, 090130) was the only U.S. study to examine the effect of $PM_{2.5}$ rather than PM_{10}. This study, which provides strong evidence of an association, was distinguished by its prospective cohort design, validation of incident cases and large population. Puett et al. (2008, 156891), the other U.S. study with comparable design features, provides evidence of an association of incident CHD with long- term PM_{10} exposure. Findings from Swedish case control studies of incident validated cases of MI were not consistent. A cross-sectional study of self-reported CHD did not provide evidence of an association with $PM_{2.5}$, while findings from two ecologic studies of PM_{10} indicated positive associations of CHD hospitalizations with PM_{10} (Maheswaran et al., 2005, 088683; Zanobetti and Schwartz, 2007, 091247).

Stroke

Miller et al. (2007, 090130) found 28% (95% CI: 2-61) and 35% (95% CI: 8-68) increases in the overall risk of validated stroke and cerebrovascular disease, respectively, per 10 $\mu g/m^3$ increase in 1-yr avg $PM_{2.5}$ exposure. Risks were higher within than between cities. In the study of 1030 Census of enumeration districts in Sheffield, U.K. described previously, Maheswaran et al. (2005, 088683) studied 5,122 ICD-10-coded emergency hospital admissions for stroke (I60-69) among 199,682 residents (age ≥ 45 yr; 45% female) of 1,030 census enumeration districts in Sheffield, U.K. (1994-1999). In this ecologic study, the authors used dispersion modeling of emissions and climate data to estimate 5-yr avg exposure to PM_{10}. They found that the age- and gender-standardized rate ratios for stroke admission were 1.05 (95% CI: 0.94-1.17), 1.07 (95% CI: 0.95-1.20), 1.06 (95% CI: 0.94-1.20), and 1.15 (95% CI: 1.01-1.31) across PM_{10} quintiles. Linear trend was somewhat stronger for stroke mortality (Section 7.6).

These studies examining the long-term PM-stroke relationship provide evidence of association. Maheswaran et al. (2005, 088683) examined emergency room hospital admissions in Sheffield, U.K. using an ecologic design while results reported by Miller et al. (2007, 090130) are based on the prospective cohort study of the WHI OS population (both introduced previously).

Peripheral Arterial Disease

The German Heinz Nixdorf Recall cross-sectional study described in Section 7.2.1.1 (Hoffmann et al., 2009, 190376) also evaluated the association between 1-yr avg exposure to $PM_{2.5}$ and peripheral arterial disease (self-reported history of a surgical or procedural intervention or an ABI <0.9 in one or both legs). The authors found no evidence of an increase in risk. The OR for peripheral arterial disease was 0.87 (95% CI: 0.57-1.34) per 3.9 $\mu g/m^3$ increase in $PM_{2.5}$. However, evidence of an association with traffic exposure was present in these data. ORs of 1.77 (95% CI: 1.01-3.10), 1.02 (95% CI: 0.58-1.80), and 1.07 (95% CI: 0.68-1.68) for residing ≤ 50, 50-100, and 100-200 m of a major road (reference category: >200 m), respectively were observed. ORs were higher among participants with CAC scores ≤ 75th percentile, women, and smokers.

Deep Vein Thrombosis

The Italian case-control study (introduced in Section 7.2.1.2) also examined the chronic PM_{10}-DVT association (Baccarelli et al., 2008, 157984). The authors found a 70% (95% CI: 30-223) increase in the odds of DVT per 10 $\mu g/m^3$ increase in 1-yr avg PM_{10} exposure. This finding was consistent with the decreases in PT and PTT also observed among controls in this context as well as

the 47% (95% CI: 11-96) increase in the odds of DVT per inter-decile range (242 m) increase in the residence-to-major-roadway distance observed among a subset of cases and controls (Baccarelli et al., 2009, 188183). The PM_{10}-DVT and distance-DVT associations were both weaker among women and among users of oral contraceptives or hormone therapy.

7.2.10. Cardiovascular Mortality

New epidemiologic evidence reports a consistent association between long-term exposure to $PM_{2.5}$ and increased risk of cardiovascular mortality. There is little evidence for the long-term effects of $PM_{10-2.5}$ on cardiovascular mortality. This section focuses on cardiovascular mortality outcomes in response to long-term exposure to PM. The studies that investigate long-term exposure and mortality due to any specific or all (nonaccidental) causes are evaluated in Section 7.6. A summary of the mean PM concentrations reported for the studies characterized in this section is presented in Table 7-8, and the effect estimates are presented in Figure 7-7 and Figure 7-8.

A number of large, U.S. cohort studies have found consistent associations between long-term exposure to $PM_{2.5}$ and cardiovascular mortality. The American Cancer Society (ACS) (Pope et al. (2004, 055880) reported positive associations with deaths from specific cardiovascular diseases, particularly ischemic heart disease, and a group of cardiac conditions including dysrhythmia, heart failure and cardiac arrest (RR for cardiovascular mortality = 1.12 [95% CI: 1.08-1.15] per 10 $\mu g/m^3$ $PM_{2.5}$). In an additional reanalysis that extended the follow-up period for the ACS cohort to 18 yr (1982-2000) (Krewski et al., 2009, 191193), investigators found effect estimates that were similar, though generally higher, than those reported in previous ACS analyses.

A follow-up to the Harvard Six Cities study (Laden et al., 2006, 087605) used updated air pollution and mortality data and found positive associations between long-term exposure to $PM_{2.5}$ and mortality. Of special note is a statistically significant reduction in mortality risk reported with reduced long-term fine particle concentrations. This reduced mortality risk was observed for deaths due to cardiovascular and respiratory causes, but not for lung cancer deaths.

The WHI cohort study (Miller et al., 2007, 090130) (described previously) found that each 10 $\mu g/m^3$ increase of $PM_{2.5}$ was associated with a 76% increase in the risk of death from cardiovascular disease (hazard ratio, 1.76 [95% CI: 1.25-2.47]). The WHI study not only confirms the ACS and Six City Study associations with cardiovascular mortality in yet another well characterized cohort with detailed individual-level information, it also has been able to consider the individual medical records of the thousands of WHI subjects over the period of the study. This has allowed the researchers to examine not only mortality, but also related morbidity in the form of heart problems (cardiovascular events) experienced by the subjects during the study. These morbidity co-associations with $PM_{2.5}$ in the same population lend even greater support to the biological plausibility of the air pollution-mortality associations found in this study.

In an analysis for the Seventh-Day Adventist cohort in California (AHSMOG), a positive association with coronary heart disease mortality was reported among females (92 deaths; RR = 1.42 [95% CI: 1.06-1.90] per 10 $\mu g/m^3$ $PM_{2.5}$), but not among males (53 deaths; RR = 0.90 [95% CI: 0.76-1.05] per 10 $\mu g/m^3$ $PM_{2.5}$) (Chen et al., 2005, 087942). Associations were strongest in the subset of postmenopausal women (80 deaths; RR = 1.49 [95% CI: 1.17-1.89] per 10 $\mu g/m^3$ $PM_{2.5}$). The authors speculated that females may be more sensitive to air pollution-related effects, based on differences between males and females in dosimetry and exposure. As was found with $PM_{2.5}$, a positive association with coronary heart disease mortality was reported for $PM_{10-2.5}$ and PM_{10} among females (RR = 1.38 [95% CI: 0.97-1.95] per 10 $\mu g/m^3$ $PM_{10-2.5}$; RR = 1.22 [95% CI: 1.01-1.47] per 10 $\mu g/m^3$ PM_{10}), but not for males (RR = 0.92 [95% CI: 0.66-1.29] per 10 $\mu g/m^3$ $PM_{10-2.5}$; RR = 0.94 [95% CI: 0.82-1.08] per 10 $\mu g/m^3$ PM_{10}); associations were strongest in the subset of postmenopausal women (80 deaths) (Chen et al., 2005, 087942).

Two additional studies explored the effects of PM_{10} on cardiovascular mortality. The Nurses' Health Study (Puett et al., 2008, 156891) is an ongoing prospective cohort study examining the relation of chronic PM_{10} exposures with all-cause mortality and incident and fatal coronary heart disease consisting of 66,250 female nurses in MSAs in the northeastern region of the U.S. The association with fatal CHD occurred with the greatest magnitude when compared with other specified causes of death (hazard ratio 1.42 [95% CI: 1.11-1.81]). The North Rhine-Westphalia State Environment Agency (LUA NRW) initiated a cohort of approximately 4,800 women, and assessed whether long-term exposure to air pollution originating from motorized traffic and industrial sources was associated with total and cause-specific mortality (Gehring et al., 2006, 089797). They found

that cardiopulmonary mortality was associated with PM_{10} (RR = 1.52 [95% CI: 1.09-2.15] per 10 $\mu g/m^3$ PM_{10}).

In summary, the 2004 PM AQCD concluded that there was strong evidence that long-term exposure to $PM_{2.5}$ was associated with increased cardiopulmonary mortality. Recent studies investigating cardiovascular mortality provide some of the strongest evidence for a cardiovascular effect of PM. A number of large cohort studies have been conducted throughout the U.S. and reported consistent increases in cardiovascular mortality related to $PM_{2.5}$ concentrations. The results of two of these studies have been replicated in independent reanalyses. These effects are coherent with short-term epidemiologic studies of CVD morbidity and mortality and with long-term epidemiologic studies of CVD morbidity. In addition, biological plausibility and coherence are provided by toxicological studies demonstrating short-term cardiovascular effects as well as $PM_{2.5}$-related plaque progression in chronically exposed mice.

7.2.11.Summary and Causal Determinations

7.2.11.1. $PM_{2.5}$

Epidemiologic studies examining associations between long-term exposure to ambient PM (over months to years) and CVD morbidity had not been conducted and thus were not included in the 1996 or 2004 PM AQCDs (U.S. EPA, 1996, 079380; U.S. EPA, 2006, 157071). A number of studies were included in the 2004 AQCD that evaluated the effect of long-term $PM_{2.5}$ exposure on cardiovascular mortality and found strong and consistent associations. No toxicological studies had evaluated the effects of subchronic or chronic PM exposure on CVD effects in the 2004 PM AQCD. Recently, epidemiologic and toxicological studies have provided evidence of the adverse effects of long-term exposure to $PM_{2.5}$ on cardiovascular outcomes and endpoints, including atherosclerosis and clinical and subclinical markers of cardiovascular morbidity.

The strongest evidence for a CVD health effect related to long-term $PM_{2.5}$ exposure comes from epidemiologic studies of cardiovascular mortality. A number of large, multicity U.S. studies (the ACS, Six Cities Study, WHI, and AHSMOG) provide consistent evidence of an effect between long-term exposure to $PM_{2.5}$ and cardiovascular mortality (Section 7.2.10). These studies were conducted in urban areas across the U.S. where mean concentrations ranged from 10.2-29.0 $\mu g/m^3$ (Table 7-8). An epidemiologic study investigating the relationship between $PM_{2.5}$ and clinical CVD morbidity among post-menopausal women (Miller et al., 2007, 090130) provides evidence of an effect that is coherent with the cardiovascular mortality studies. This large, prospective cohort study of incident, validated cases found large increases in the adjusted risk of MI, revascularization, and stroke using a 1-yr avg $PM_{2.5}$ concentration (mean = 13.5 $\mu g/m^3$). A cross-sectional analyses of self-reported prevalence of CHD and peripheral arterial disease found no such increase in the odds of CVD morbidity (Hoffmann et al., 2006, 091162); the inconsistency of these findings with Miller et al. (2007, 090130) may be explained by differences in study design or location.

The effect of long-term $PM_{2.5}$ exposure on pre-clinical measures of atherosclerosis (CIMT, CAC, AAC or ABI) has been studied in several populations using a cross-sectional study design. The magnitude of the $PM_{2.5}$ effects and their consistency across different measures of atherosclerosis in these studies varies widely, and they may be limited in their ability to discern small changes in these measures. Kunzli et al. (2005, 087387) observed a non-significant 4.2% increase in CIMT associated with long-term $PM_{2.5}$ exposure among participants of a clinical trial in greater Los Angeles, which was several fold higher than the 0.5% increase observed by Diez-Roux et al. (2008, 156401) in their analyses of MESA baseline data. The associations in MESA of CAC and ABI with long-term $PM_{2.5}$ exposure were largely null (Diez et al., 2008, 156401), while an increase in AAC with long-term $PM_{2.5}$ exposure was reported (Chang et al., 2008, 180393). By contrast, a 43% increase in CAC was associated with long-term $PM_{2.5}$ exposure in a German study, but no similar association with ABI was observed (Hoffmann et al., 2009, 190376). Although the number of studies examining these relationships is limited, effect modification by use of lipid lowering drugs and smoking status was reported in more than one study of long-term $PM_{2.5}$ and PM_{10} exposure.

Evidence of enhanced atherosclerosis development was demonstrated in new toxicological studies that report increased plaque and lesion areas, lipid deposition, and TF in aortas of $ApoE^{-/-}$ mice exposed to CAPs (Section7.2.1.2). In addition, alterations in vasoreactivity were observed,

suggesting an impaired NO pathway. Additional toxicological studies of PM_{10} are consistent with these results. Further support is provided by a study that reported decreased L/W ratio in the pulmonary and coronary arteries of mice exposed to ambient air. However, $PM_{2.5}$ CAPs derived from traffic in Los Angeles did not affect plaque size (Araujo et al., 2008, 156222). Collectively, these toxicological studies provide biological plausibility for the associations reported in epidemiologic studies.

There is limited evidence for the effects of $PM_{2.5}$ on renal or vascular function. Cross-sectional and longitudinal epidemiologic analyses of $PM_{2.5}$ and UACR revealed no evidence of an effect (O'Neill et al., 2007, 156006), while small non-statistically significant increases in BP with 30- and 60-day avg $PM_{2.5}$ concentrations were reported (Auchincloss et al., 2008, 156234). A toxicological study did not show changes in MAP with CAPs, but indicated a CAPs-related potentiation of experimentally-induced hypertension (Sun et al., 2008, 157032). In addition, CAPs has induced changes in insulin resistance, visceral adiposity, and inflammation in a diet-induced obesity mouse model (Sun et al., 2009, 190487), indicating that diabetics may be a potentially susceptible population to PM exposure.

In summary, a number of large U.S. cohort studies report associations of long-term $PM_{2.5}$ concentration with cardiovascular mortality. These studies provide the strongest evidence for an effect of long-term $PM_{2.5}$ exposure on CVD effects. Additional evidence comes from a methodologically rigorous epidemiology study that demonstrates coherent associations between long-term $PM_{2.5}$ exposure and CVD morbidity among post-menopausal women. Toxicological studies demonstrate that this effect is biologically plausible and the effect is coherent with studies of short-term $PM_{2.5}$ exposure and CVD morbidity and mortality, and with long-term exposure to $PM_{2.5}$ and CVD mortality. Associations between $PM_{2.5}$ and subclinical measures of atherosclerosis are inconsistent, but cross-sectional studies may be limited in their ability to discern small changes in these measures. In addition, potential modification of the $PM_{2.5}$-CVD association by smoking status and the use of lipid lowering drugs has been demonstrated in epidemiologic studies that used individual-level data. Toxicological studies provide evidence for accelerated development of atherosclerosis in $ApoE^{-/-}$ mice exposed to CAPs and show effects on coagulation factors, experimentally-induced hypertension, and vascular reactivity. Available studies of clinical cardiovascular disease outcomes report inconsistent results. Based on the above findings, the epidemiologic and toxicological evidence is **sufficient to infer a causal relationship between long-term $PM_{2.5}$ exposures and cardiovascular effects.**

7.2.11.2. $PM_{10-2.5}$

One epidemiologic study evaluated the relationship between long-term exposure to $PM_{10-2.5}$ and cardiovascular mortality and found a positive association with coronary heart disease mortality among females, but not for males; associations were strongest in the subset of post-menopausal women (Chen et al., 2005, 087942). No toxicological studies of long-term exposure to ambient $PM_{10-2.5}$ and cardiovascular effects have been conducted to date. Evidence is **inadequate to infer the presence or absence of a causal relationship.**

7.2.11.3. UFPs

A few toxicological studies of long-term exposure to UFPs have been conducted. Increased plaque size was reported in mice exposed to UF CAPs derived from traffic (Araujo et al., 2008, 156222). Studies of diesel and gasoline exhaust reported relatively few changes in hematologic or coagulation parameters (Section 7.2.4.2) and one DE study demonstrated altered cardiac gene expression in normotensive rats that reflected the development of hypertension (Gottipolu et al., 2009, 190360). Whole and filtered gasoline exhaust induced increases in gene products involved in atheromatous plaque formation and/or degradation, but these effects were largely due to the gaseous emissions (Lund et al., 2007, 125741). Evidence from these studies alone is **inadequate to infer the presence or absence of a causal relationship**.

7.3. Respiratory Effects

Several cohort studies reviewed in the 2004 PM AQCD provided evidence for relationships between long-term PM exposure and effects on the respiratory system, though it did not rule out the possibility that the observed respiratory effects may have been confounded by other pollutants. In 12 southern California communities in the Children's Health Study (CHS), Gauderman et al. (2000, 012531; 2002, 026013) found that decreases in lung function growth among schoolchildren were associated with long-term exposure to PM. Declines in pulmonary function were reported with all three major PM size classes – PM_{10}, $PM_{10-2.5}$ and $PM_{2.5}$ – though the three PM measures were highly correlated. In another analysis of data from the CHS cohort, McConnell et al. (1999, 007028), reported an increased risk of bronchitis symptoms in children living in communities with higher PM_{10} and $PM_{2.5}$ concentrations. These results were found to be consistent with results of cross-sectional analyses of the 24-city study by Dockery et al. (1996, 046219) and Raizenne et al. (1996, 077268), that were assessed in the 1996 PM AQCD. These studies reported associations between increased bronchitis rates and decreased peak flow with fine particle sulfate and fine particle acidity. However, the high correlation of PM_{10}, acid vapor and NO_2 precluded clear attribution of the bronchitis effects reported by McConnell et al. (1999, 007028) to PM alone. In a prospective cohort study among a subset of children in the CHS (n = 110) who moved to other locations during the study period, Avol et al. (2001, 020552) reported that those subjects who moved to areas of lower PM_{10} showed increased growth in lung function compared with subjects who moved to communities with higher PM_{10} concentrations. Finally, the 2004 PM AQCD concluded that there was strong epidemiologic evidence for associations between long-term exposures to $PM_{2.5}$ and cardiopulmonary mortality, though the respiratory effects were not separated from the cardiovascular effects in this conclusion.

The 2004 PM AQCD (U.S. EPA, 2004, 056905) concluded that the evidence for an association between long-term exposure to PM and respiratory effects may be confounded by other pollutants. Gauderman et al. (2002, 026013) reported declines for FEV_1 and McConnell et al. (1999, 007028) reported increased ORs for bronchitic symptoms in asthmatics for PM_{10} and $PM_{2.5}$. Recent epidemiologic literature includes results from several prospective cohort studies, which found consistent, positive associations between long-term exposure to PM and respiratory morbidity. Associations were reported with $PM_{2.5}$ and PM_{10}, and the studies showing associations only with PM_{10} were conducted in locations where the PM consisted predominantly of fine particles, providing support for associations with long-term exposure to fine particles. These results are summarized below; further details of these studies are summarized in Annex E.

Very few subchronic and chronic toxicological studies investigating respiratory effects were available in the 2004 PM AQCD. However, the 2002 EPA Health Assessment Document for DE reported that chronic exposure to DE was associated with histopathology including alveolar histiocytosis, aggregation of alveolar macrophages, tissue inflammation, increased polymorphonuclear leukocytes, hyperplasia of bronchiolar and Type 2 epithelial cells, thickened alveolar septa, edema, fibrosis, emphysema and lesions of the trachea and bronchi. Since then a number of animal toxicological studies have been conducted involving inhalation exposure to CAPs, urban air, DE, gasoline exhaust, and wood smoke. These subchronic and chronic studies provide evidence of altered pulmonary function, inflammation, histopathological changes and oxidative and allergic responses following $PM_{2.5}$ exposures. These results are summarized below; further details of these studies are summarized in Annex D.

7.3.1. Respiratory Symptoms and Disease Incidence

7.3.1.1. Epidemiologic Studies

New longitudinal cohort studies provide the best evidence to evaluate the relationship between long-term exposure to ambient PM and increased incidence of respiratory symptoms or disease. A summary of the mean PM concentrations reported for the long-term exposure studies characterized in this section is presented in Table 7-3.

Bayer-Oglesby et al. (2005, 086245) examined the decline of ambient pollution levels and improved respiratory health demonstrated by a reduction in respiratory symptoms and diseases in school children (n = 9,591) in Switzerland. Reduced air pollution exposure resulted in improved respiratory health of children. Further, the average reduction of symptom prevalence was more pronounced in areas with stronger reduction of air pollution levels. The average decline of PM_{10} between 1993 and 2000 across the nine study regions was 9.8 $\mu g/m^3$ (29%). Declining levels of PM_{10} were associated with declining prevalence of chronic cough, bronchitis, common cold, nocturnal dry cough, and conjunctivitis symptoms, but no significant associations were reported for wheezing, sneezing, asthma, and hay fever, as shown in Figure 7-2. In Figure 7-2, Panel (B) illustrates that on an aggregate level across regions, the mean change in adjusted prevalence of chronic cough is associated with the mean change in PM_{10} levels ($r = 0.78$; $p = 0.02$). Similar associations were seen for nocturnal dry cough and conjunctivitis symptoms and PM_{10} levels. Rösli et al. (2000, 010296; 2001, 108738; 2005, 156923) have demonstrated that PM_{10} levels are homogeneously distributed within regions of Basel, Switzerland and are not substantially affected by local traffic, justifying the single-monitor approach for assignment of PM_{10} exposures. Based on parallel measurements of $PM_{2.5}$ and PM_{10} at seven sites in Switzerland, $PM_{2.5}$ and PM_{10} at all sites are generally highly correlated (r^2 ranging from 0.85 to 0.98) (Gehrig and Buchmann, 2003, 139678), indicating that PM_{10} consists predominantly of fine particles in these locations.

Schindler et al. (2009, 191950) reported that sustained reduction in ambient PM_{10} concentrations can lead to decreases in respiratory symptoms among Swiss adults in the SAPALDIA study. They compared baseline data in 1991 to a follow-up interview in 2002 after a substantial decline in PM_{10} concentrations served as a natural experiment. Each subject was assigned model-based estimates of PM_{10} concentrations averaged over the 12 mo preceding each health assessment with mean decline in PM_{10} levels of 6.2 $\mu g/m^3$ (SD = 3.9 $\mu g/m^3$). When the authors tested the joint hypothesis of no association between the PM_{10} difference and symptom incidence or persistence, positive results were obtained for regular cough, chronic cough or phlegm and wheezing but not regular phlegm or wheezing without a cold.

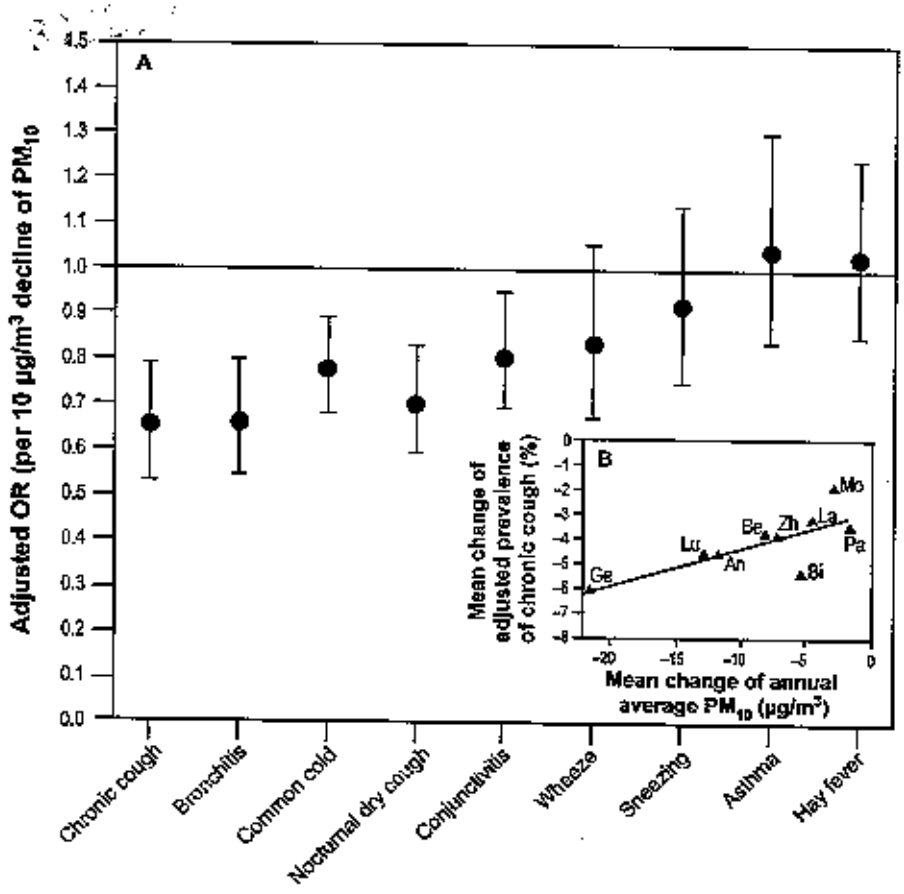
Pierse et al. (2006, 088757) studied the association between primary PM_{10} (particles directly emitted from local sources/traffic) and the prevalence and incidence of respiratory symptoms in a randomly sampled cohort of 4,400 children (aged 1-5 yr) in Leicestershire, England surveyed in 1998 and again in 2001. Annual exposure to primary PM_{10} was calculated for the home address using the Airviro statistical dispersion model. After adjusting for confounders, mean annual exposure to locally generated PM_{10} was associated with an increased prevalence of cough without a cold in both the 1998 (OR 1.21 [95% CI: 1.07-1.38], n = 2,164) and 2001 surveys (OR 1.56 [95% CI: 1.32-1.84], n = 1,756).

Nordling et al. (2008, 097998) examined the relationship between estimated PM exposure levels and respiratory health effects in a Swedish birth cohort of preschool children (n = 4,089). The spatial distributions of PM from traffic in the study area were estimated with emission databases and statistical dispersion modeling. Children were examined at 2 mo and 1, 2, and 4 yr of age. Using GIS methods, the average contribution of traffic-generated PM_{10} above regional background to the children's residential outdoor air pollution levels was determined. To evaluate the exposure assessment, the authors compared the estimated levels of traffic-generated PM_{10} with $PM_{2.5}$ measurements from 42 locations (Hoek et al., 2002, 042364) and reported modeled traffic-generated PM_{10} correlated reasonably well with measured $PM_{2.5}$ ($r = 0.61$). Persistent wheezing (cumulative incidence up to age 4 yr) was associated with exposure to traffic-generated PM_{10} (OR 2.28 [95% CI: 0.84-6.24] per 10 $\mu g/m^3$ increase) while transient and late onset wheezing was not associated. This study demonstrates that respiratory effects may be present in preschool children.

Table 7-3. Characterization of ambient PM concentrations from studies of respiratory symptoms/disease and long-term exposures.

Study	Location	Mean Annual Concentration ($\mu g/m^3$)	Upper Percentile Concentrations ($\mu g/m^3$)
$PM_{2.5}$			
Annesi-Maesano et al. (2007, 093180)	6 French Cities	Range of means across sites: 8.7-23.0 Avg of means across sites: 15.5	
Brauer et al. (2007, 090691)	The Netherlands	16.9	75th: 18.1 90th: 19.0 Max: 25.2
Goss et al. (2004, 055624)	U.S.	13.7	75th: 15.9
Islam et al. (2007, 090697)	12 CHS/CA communities		Max: 29.5
Janssen et al. (2003, 133555)	The Netherlands	20.5	75th: 22.1 Max: 24.4
Kim et al. (2004, 087383)	San Francisco, CA	Range of means across sites: 11-15 Avg of means across sites: 12	
McConnell et al. (2003, 049490)	12 CHS/CA communities	13.6	Max: 28.5
Morgenstern et al. (2008, 156782)	Munich, Germany	11.1	
PM_{10}			
Beyer-Oglesby et al. (2005, 086245)	Nine study regions in Switzerland		Max: 46
Kunzli et al. (2009, 191949)	Switzerland	21.5	
Nordling et al. (2008, 097998)	Sweden	4*	
Schindler et al. (2009, 191950)	Switzerland	**	
McConnell et al. (2003, 049490)	12 CHS/CA communities	30.8	Max: 63.5
Pierse et al. (2006, 088757)	Leicestershire, U.K.	1.33	75th: 1.84

*Source specific; PM_{10} from traffic
**Only reported change in PM concentration



Source: Bayer-Oglesby et al. (2005, 086245)

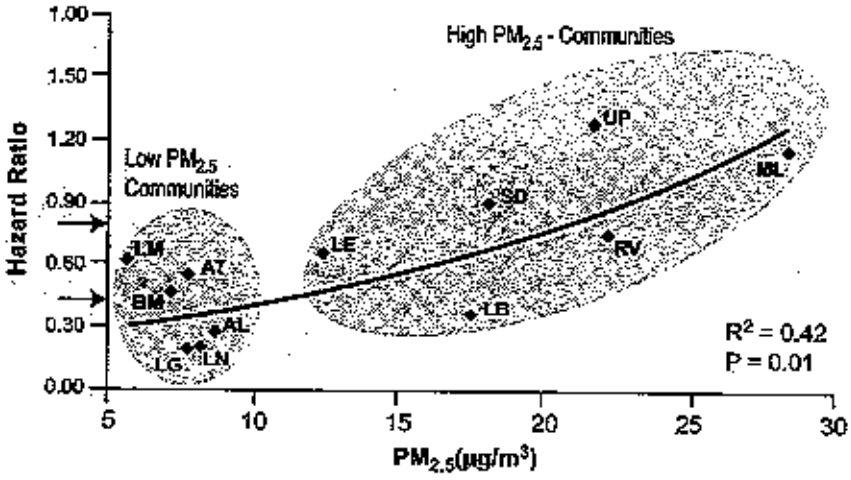
Figure 7-2. Adjusted ORs and 95% CIs of symptoms and respiratory diseases associated with a decline of 10 µg/m³ PM_{10} levels in Swiss Surveillance Program of Childhood Allergy and Respiratory Symptoms[1]. Inset: Mean change in adjusted prevalence (1998-2001 to 1992-1993) versus mean change in regional annual averages of PM_{10} (1997-2000 to 1993) for chronic cough, across nine SCARPOL regions (An: Anières. Be: Bern. Bi: Biel. Ge: Geneva. La:, Langnau. Lu: Lugano. Mo: Montana. Pa: Payerne. Zh: Zürich).

McConnell et al. (2003, 049490) conducted a prospective study examining the association between air pollution and bronchitic symptoms in 475 school children with asthma in 12 Southern California communities as part of the CHS from 1996 to 1999. They investigated both the differences between- communities with 4-yr avg and within-communities yearly variation in PM (i.e., PM_{10}, $PM_{2.5}$, $PM_{10-2.5}$, EC, and OC). Based on a 10 µg/m³ change in $PM_{2.5}$, within-communities effects were larger (OR 1.90 [95% CI: 1.10-2.70]) than those for between-communities (OR 1.30 [95% CI: 1.10-1.50]). The OR for the 10 µg/m³ range in 4-yr avg $PM_{2.5}$ concentrations across the 12 communities was 1.29 (95% CI: 1.06-1.58). Similar results were reported for PM_{10} and $PM_{10-2.5}$ but the effect estimates were smaller in magnitude and generally not statistically significant. Within-community associations were not confounded by any time-fixed personal covariates. In two-

[1] Adjusted for age, sex, nationality, parental education, number of siblings; farming status, low birth weight, breastfeeding, child who smokes, family history of asthma, bronchitis, and/or atopy, mother who smokes, indoor humidity, mode of heating and cooking, carpeting, pets allowed in bedroom, removal of carpet and/or pets for health reasons, person who completed questionnaire, month when questionnaire was completed, number of days with the maximum temperature <0°C, and belief of mother that there is an association between environmental exposures and children's respiratory health

pollutant models, the within-community effect estimates for $PM_{2.5}$ and OC were significant in the presence of several other pollutants. While the within-community single-pollutant effect of $PM_{2.5}$ ($\beta = 0.085/\mu g/m^3$) was only modestly attenuated after adjusting for some pollutants, it was markedly reduced after adjusting for NO_2 or OC. The between-community effect estimates generally were not significant in the presence of other pollutants in copollutant models.

In the CHS, Islam et al. (2007, 090697) examined the hypothesis that ambient air pollution attenuates the reduced risk for childhood asthma that is associated with higher lung function (n = 2,057). At each age a distribution of pulmonary functions exists. Haland et al. (2006, 156511) found evidence that children with high lung function have a reduced risk for asthma. Islam et al. (2007, 090697) used the CHS data to study how the association of asthma incidence with lung function is modified by long-term PM exposure. The incidence rate (IR) of newly diagnosed asthma increased from 9.5/1,000 person-years for children with percent-predicted FEF_{25-75} values ≥ 120% to 20.4/1,000 person-years for children with FEF_{25-75} value ≤ 100%. Over the 10th-90th percentile range for FEF_{25-75} (57.1%), the hazard ratio of new onset asthma was 0.50 (95% CI: 0.35-0.71). The IR of asthma for $FEF_{25-75} \geq 120\%$ in the "high" $PM_{2.5}$ (13.7-29.5 $\mu g/m^3$) communities was 15.9/1,000 person-years compared to 6.4/1,000 person-years in "low" $PM_{2.5}$ (5.7-8.5 $\mu g/m^3$) communities. Loss of protection by high lung function against new onset asthma in the "high" $PM_{2.5}$ communities was observed for all the lung function measures. Figure 7-3 shows the effect of $PM_{2.5}$ on the association of lung function with asthma. Of all the pollutants examined (NO_2, PM_{10}, $PM_{2.5}$, acid vapor, O_3, EC, and OC), $PM_{2.5}$ appeared to have the strongest modifying effect on the association between lung function with asthma as it had the highest R^2 value (0.42). Over the 10th-90th percentile range of FEF_{25-75}, the hazard ratio of new onset asthma was 0.34 (95% CI: 0.21-0.56) in a community with low $PM_{2.5}$ (<13.7 $\mu g/m^3$) and 0.76 (95% CI: 0.45-1.26) in a community with high $PM_{2.5}$ (≥ 13.7 $\mu g/m^3$). The data do not indicate that PM exposure increased rates of incident asthma among children with poor lung function at study entry because rates among those with poor lung function were similar in both low and high pollution communities.



Source: Reprinted with Permission of BMJ Publishing Group Ltd & British Thoracic Society from Islam et al. (2007, 090697)

Figure 7-3. Effect of $PM_{2.5}$ on the association of lung function with asthma. Community-specific hazard ratio of newly diagnosed asthma over 10-90th percentile range (57.1%) of $FEF_{25-75\%}$ by level of ambient $PM_{2.5}$ ($\mu g/m^3$). The 12 CHS communities are shown.

In a prospective birth cohort study (n = 4,000) in The Netherlands, Brauer et al. (2007, 090691) assessed the development of asthma, allergic symptoms, and respiratory infection during the first 4 yr of life in relation to long-term $PM_{2.5}$ concentration at the home address with a validated model using GIS. $PM_{2.5}$ was associated with doctor-diagnosed asthma (OR = 1.32 [95% CI:

1.04-1.69]) for a cumulative lifetime indicator. These findings extend observations made at 2 yr of age in the same cohort (Brauer et al., 2002, 035192) providing greater confidence in the association. No associations were observed for bronchitis.

Kunzli et al. (2009, 191949) used the SAPALDIA cohort study discussed previously in this section to evaluate the relationship between the 11-yr change (1991-2002) in traffic-related PM_{10} and asthma incidence-adult onset asthma. In a cohort of 2,725 never-smokers without asthma at baseline (age: 18-60 yr in 1991), subjects reporting doctor-diagnosed asthma at follow-up were considered incident cases. Modeled traffic-related PM_{10} levels were used. Cox proportional hazard models for time to asthma onset were used with adjustments for cofounders. The study findings suggest that PM contributes to asthma development and that reductions in PM decrease asthma risk. A strong feature of SAPALDIA is the ability to assign space, time, and source-specific pollution to each subject. Further, Kunzli et al. (2008, 129258) discusses the impact of attributable health risk models for exposures that are assumed to cause both chronic disease and its exacerbations. The added impact of causing disease increases the risk compared to only exacerbations.

A matched case-control study of infant bronchiolitis (ICD 9 code 466.1) hospitalization and two measures of long-term exposure – the month prior to hospitalization (subchronic) and the lifetime average (chronic) – to $PM_{2.5}$ and gaseous air pollutants in the South Coast Air Basin of southern California was conducted by Karr et al. (2007, 090719) among 18,595 infants born between 1995-2000. For each case, 10 controls matched on date were randomly selected from birth records. Exposure was based on $PM_{2.5}$ measurements collected every third day. The mean distance between the subjects' residential ZIP code and the assigned monitor was generally 4-6 mi with a maximum distance of 30 mi. For 10 $\mu g/m^3$ increases in both sub-chronic and chronic $PM_{2.5}$ exposure, an adjusted OR of 1.09 (95% CI: 1.04-1.14) was observed. In multipollutant model analyses, the association with $PM_{2.5}$ was robust to the inclusion of gaseous pollutants. Also, in a cohort of children in Germany, Morgenstern et al. (2008, 156782) modeled $PM_{2.5}$ data at birth addresses found statistically significant effects for asthmatic bronchitis, hay fever, and allergic sensitization to pollen.

Goss et al. (2004, 055624) conducted a national study examining the relationship between air pollutants and health effects in a cohort of cystic fibrosis (CF) patients (n = 11,484) over the age of 6 yr (mean age = 18.4, SD = 10) enrolled in the Cystic Fibrosis Foundation National Patient Registry in 1999 and 2000. Exposure was assessed by linking air pollution values from the closest population monitor from the Air Quality System (AQS) with the centroid of the patient's home ZIP code that was within 30 mi. The mean distance from the patient's ZIP code to monitors for $PM_{2.5}$ and PM_{10} was 10.8 mi (SD 7.8) and 11.5 mi (SD 7.9), respectively. $PM_{2.5}$ and PM_{10} 24-h avg were collected every 1 to 12 days. CF diagnosis involves genetic screening panels and a common severe mutation used is the loss of phenylalanine at the 508th position. Genotyping was available in 74% of the population and of those genotyped, 66% carried one or more delta F508 deletions. After adjusting for confounders, a 10 $\mu g/m^3$ increase in $PM_{2.5}$ or PM_{10} was associated with a 21% (95% CI: 7-33) or 8% (95% CI: 2-15) increase in the odds of two or more exacerbations, respectively. The exacerbations were defined as a CF-related pulmonary condition requiring admission to the hospital or use of home intravenous antibiotics. The estimate for the associations between pulmonary exacerbations and $PM_{2.5}$ and PM_{10} were attenuated when the models were adjusted for lung function. Brown et al. (2001, 012307) found that particle deposition was increased in CF and that particle distribution in the lungs was enhanced in poorly ventilated tracheobronchial regions in CF patients. Such focal deposition may partially explain the association of PM and CF exacerbation.

Annesi-Maesano (2007, 093180) relate individual data on asthma and allergy from 5,338 school children (10.4 ± 0.7 yr) attending 108 randomly chosen schools in 6 French cities to the concentration of $PM_{2.5}$ monitored in school yards. Atopic asthma was related to $PM_{2.5}$ (OR 1.43 [95% CI: 1.07-1.91]) when high $PM_{2.5}$ concentrations (20.7 $\mu g/m^3$) were compared to low $PM_{2.5}$ concentrations (8.7 $\mu g/m^3$). The report is consistent with the results in an earlier paper (Penard-Morand et al., 2005, 087951) in the same sample of children that related the findings to PM_{10}.

Kim et al. (2004, 087383) conducted a school-based cross-sectional study in the San Francisco metropolitan area in 2001 comprised of 10 neighborhoods to examine the relationship between traffic-related pollutants and current bronchitic symptoms and asthma obtained by parental questionnaire (n = 1,109). They related traffic-related pollutants (PM) and bronchitic and asthma symptoms in the past 12 mo. No multipollutant models were evaluated because of the high interpollutant correlations. $PM_{2.5}$ levels ranged across the school sites from 11 to 15 $\mu g/m^3$.

Schikowski et al. (2005, 088637) examined the relationship between both long-term air pollution exposure and living close to busy roads and COPD in the Rhine-Ruhr Basin of Germany

from 1985 to 1994 using consecutive cross-sectional studies. Seven monitoring stations that were <8 km to a woman's home address provided TSP data that PM_{10} was estimated from using a conversion factor (obtained from parallel measurement of TSP and PM_{10} conducted at 7 sites in the Ruhr area). Distance to a major road was determined using GIS. The results of the study suggest that long-term exposure to air pollution from PM_{10} and living near a major road might increase the risk of developing COPD and can have a detrimental effect on lung function. All ORs for 5-yr exposures were stronger than those for 1-yr exposures.

In summary, the 2004 PM AQCD evaluated the available studies which primarily related effects to bronchitic symptoms in school-age children. New studies are using several different methods to include individual estimates of exposure to ambient PM that may reduce the impact of exposure error. The strength and consistency of the outcomes is enhanced by results being reported by several different researchers in different countries using different designs. Most recent studies have focused on children, but a few studies have also reported associations in adults.

The CHS (McConnell et al., 2003, 049490) provides evidence in a prospective longitudinal cohort study that relates $PM_{2.5}$ and bronchitic symptoms and reports larger associations for within-community effects that are less subject to confounding than between-community effects. Several new studies report similar findings with long-term exposure to PM_{10} in areas where fine particles are the predominant fraction of PM_{10}. In England, in a cohort of 4,400 children (aged 1-5 yr), an association is seen with an increased prevalence of cough without a cold. Further evidence includes a reduction of respiratory symptoms corresponding to decreasing PM levels in "natural experiments" in both a cohort of Swiss school children (Bayer-Oglesby et al., 2005, 086245) and adults (Schindler et al., 2009, 191950).

In a separate analysis of the CHS, Islam et al. (2007, 090697) showed that $PM_{2.5}$ had the strongest modifying effect on the association between lung function with asthma such that loss of protection by high lung function against new onset asthma in high $PM_{2.5}$ communities was observed for all the lung function measures from 10 to 18 yr of age. This relates new onset asthma to long-term PM exposure. In the Netherlands, Brauer et al. (2007, 090691) augments the literature with data examining the first 4 yr of life in a birth cohort showing an association with doctor-diagnosed asthma. Further, in an adult cohort in the SALPALDIA study, Kunzli et al. (2009, 191949) relate PM to asthma incidence.

7.3.2. Pulmonary Function

Several cohort studies reviewed in the 2004 PM AQCD provided evidence for relationships between long-term PM exposure and effects on the respiratory system. In 12 southern California communities in the Children's Health Study (CHS), Gauderman et al. (2000, 012531; 2002, 026013) found that decreases in lung function growth among school children were associated with long-term exposure to PM. Declines in pulmonary function were reported with all three major PM size classes – PM_{10}, $PM_{10-2.5}$ and $PM_{2.5}$ – though the three PM measures were highly correlated. These results were found to be consistent with results of cross-sectional analyses of Raizenne et al. (1996, 077268), that was assessed in the 1996 PM AQCD. That study reported associations between decreased peak-flow with fine particle sulfate and fine particle acidity. Finally, in a prospective cohort study among a subset of children in the CHS (n = 110) who moved to other locations during the study period, Avol et al. (2001, 020552) reported that those subjects who moved to areas of lower PM_{10} showed increased growth in lung function compared with subjects who moved to communities with higher PM_{10} concentrations who showed decrease growth in lung function.

7.3.2.1. Epidemiologic Studies

New longitudinal cohort studies have evaluated the relationship between long-term exposure to PM and changes in measures of pulmonary function (FVC, FEV_1, and measures of expiratory flow). Cross-sectional studies also offer supportive information (Annex E) and may provide insights derived from within community analysis. Lung function increases continue through early adulthood with growth and development, then declines with aging (Stanojevic et al., 2008, 157007; Thurlbeck, 1982, 093260; Zeman and Bennett, 2006, 157178). A summary of the mean PM concentrations reported for the long-term exposure studies characterized in this section is presented in Table 7-4.

Table 7-4. Characterization of ambient PM concentrations from studies of FEV_1 and long-term exposures.

Study	Location	Mean Annual Concentration ($\mu g/m^3$)	Upper Percentile Concentrations ($\mu g/m^3$)
$PM_{2.5}$			
Gauderman et al. (2002, 026013)	12 CHS/CA communities	5-30	
Gauderman et al. (2004, 056569)	12 CHS/CA communities	6-27	
Goss et al. (2004, 055624)	U.S.	13.7	75th: 15.9
Gotschi et al. (2008, 180364)	21 European cities	Range of means across sites: 3.7-44.7 Avg of mean across sites: 16.8	
PM_{10}			
Downs et al. (2007, 092853)	8 cities in Switzerland	Range of means across sites: 9-46 Avg of mean across sites: 21.6	
Gauderman et al. (2002, 026013)	12 CHS/CA communities	Range of means across sites: 13-78 Avg of mean across sites: NR	
Gauderman et al. (2004, 056569)	12 CHS/CA communities	Range of means across sites: 18-68 Avg of mean across sites: NR	
Nordling et al. (2008, 097998)	Sweden	Modeled exposure	
Avol et al. (2001, 020562)	Southern CA/CHS	Range of means across sites: 15.0-66.2	
Rojas-Martinez et al. (2007, 091064)	Mexico City, Mexico	75.6	75th: 92.2 90th: 112.7

The CHS prospectively examined the relationship between air pollutants and lung function (FVC, FEV_1, MMEF) in a cohort (n = 1,759) of children between the ages of 10 and 18 yr, a period of rapid lung development (Gauderman et al., 2004, 056569). Air pollution monitoring stations provided data in each of the 12 study communities from 1994-2000. The results for O_3, PM_{10}, NO_2, $PM_{2.5}$, acid vapor, and EC are depicted in Figure 7-4. In general, copollutant models for any pair of pollutants did not provide a substantially better fit to the data than the corresponding single-pollutant models due to the strong correlation between most pollutants. The pollution-related deficits in the average growth in lung function over the 8-yr period resulted in clinically important deficits in attained lung function at the age of 18.

Downs et al. (2007, 092853) prospectively examined 9,651 randomly selected adults (18-60 yr of age) in eight cities in Switzerland (see alsoAckermann-Liebrich et al., 1997, 077537) to ascertain the relationship between reduced exposure to PM_{10} and age-related decline in lung function (FVC, FEV_1, and FEF_{25-50}). An evaluated statistical dispersion model (Liu et al., 2007, 093093) provided spatially resolved concentrations of PM_{10} that enabled assignment to residential addresses for the participant examinations in 1991 and 2002 that yielded a median decline of 5.3 $\mu g/m^3$ (IQR 4.1-7.5). Decreasing PM_{10} concentrations attenuated the decline in lung function. Effects were greater in tests reflecting small airway function. No other pollutant relationships were evaluated, though a related study indicated that levels of NO_2 also declined over the same period (Ackermann-Liebrich et al., 2005, 087826). Generalized cross-validation essentially chose a linear fit for the concentration-response curve for age-related decline in lung function.

These data show that improvement in air quality may slow the annual rate of decline in lung function in adulthood indicating positive consequences for public health. Further evidence on improvement in respiratory health with reduction in air pollution levels is provided from studies conducted in East Germany related to dramatic emissions reductions after the reunification in 1990 (Fryer and Collins, 2003, 156454; Heinrich et al., 2002, 034825; Sugiri et al., 2006, 088760). This type of "natural experiment" provides additional support for epidemiologic findings that relatively low levels of airborne particles have respiratory effects.

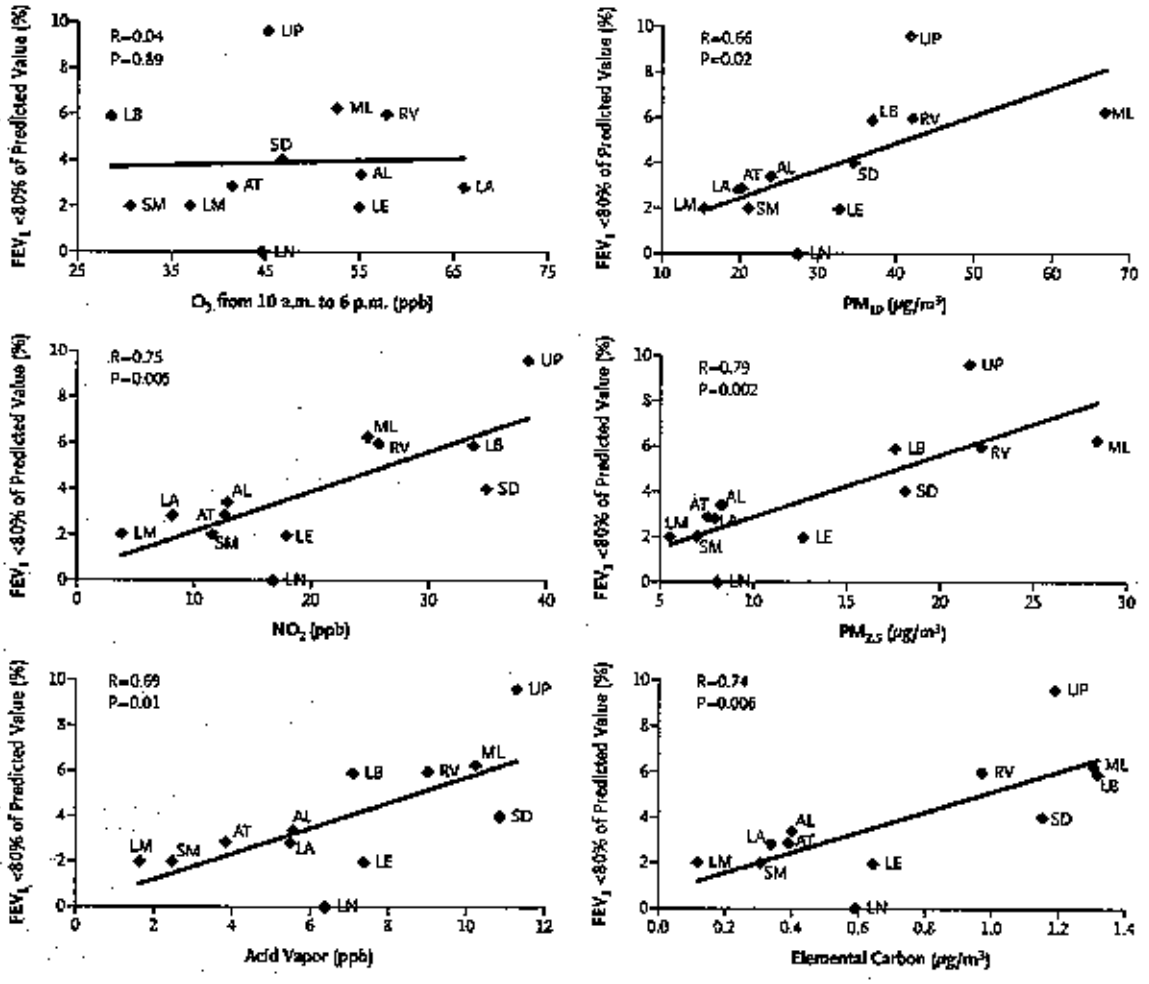


Figure 7-4. Proportion of 18-yr olds with an FEV_1 below 80% of the predicted value plotted against the average levels of pollutants from 1994 through 2000 in the 12 southern California communities of the Children's Health Study. AL = Alpine; AT = Atascadero; LA = Lake Arrowhead; LB = Long Beach; LE = Lake Elsinore; LM = Lompoc; LN = Lancaster; ML = Mira Loma; RV = Riverside; SD = San Dimas; SM = Santa Maria; UP = Upland.

In a prospective cohort study consisting of school-age children (n = 3,170) who were 8 yr of age at the beginning of the study, had not been diagnosed with asthma, and were located in Mexico City, Rojas-Martinez et al. (2007, 091064) evaluated the association between long-term exposure to PM_{10}, O_3 and NO_2 and lung function growth every 6 mo from April 1996 through May 1999. Exposure data were provided by 10 air quality monitor stations located within 2 km of each child's school. The multipollutant model effect of PM_{10} over the age of 8-10 yr of life in this cohort on FVC, FEV_1, and FEF_{25-75} showed an association. Single pollutant models showed an association between ambient pollutants (O_3, PM_{10} and NO_2) and deficits in lung function growth. The association between PM_{10} and FEF_{25-75} was not statistically significant. While the estimates from copollutant models were not substantially different than single pollutant models, independent effects for pollutants could not be estimated accurately because the traffic-related pollutants were correlated.

Although no $PM_{2.5}$ data were presented in this study, in a separate study Chow et al. (2002) report that during the winter of 1997 approximately 50% of PM_{10} was in the $PM_{2.5}$ fraction in Mexico City.

Gotschi et al. (2008, 156485) examined the relationship between air pollution and lung function in adults in the European Community Respiratory Health Survey (ECRHS). FEV_1 and FVC were assessed at baseline and after 9 yr of follow-up from 21 European centers (followed-up sample n = 5,610). No statistically significant associations were found between city-specific annual mean $PM_{2.5}$ and average lung function levels which is in contrast to the results seen by Ackermann-Liebrich (1997, 077537) (SAPALDIA) and Schikowski et al. (2005, 088637) (SALIA) which compared across far more homogenous populations than for the population assessed in the ECRHS. Misclassification and confounding may partially explain the discrepancy in findings.

In a birth cohort (n = 2,170) in Oslo, Norway, Oftedal et al. (2008, 093202) examined effects of exposure to $PM_{2.5}$ and PM_{10} on lung function (FVC, FEV_1, $FEF_{50\%}$). Spirometry was performed in 2,307 children aged 9-10 yr in 2001-2002. Residential air pollution levels over the time period 1992-2002 were calculated using EPISODE dispersion models to provide three time scales of exposure: (1) first year of life; (2) lifetime exposure; and (3) just before the lung function test. Only single pollutant models were evaluated because air pollutants were highly correlated (r = 0.83-0.95). PM exposure was associated with changes in adjusted peak respiratory flow, especially in girls. No effect was found for forced volumes. Adjusting for contextual socioeconomic factors diminished associations. Results for PM_{10} were similar to those for $PM_{2.5}$.

In an exploratory study, Mortimer et al. (2008, 187280) examined the association of prenatal and lifetime exposure to air pollutants using geocoded monthly average PM_{10} levels with pulmonary function in a San Joaquin Valley, California cohort of 232 children (ages 6-11 yr) with asthma. First and second trimester PM_{10} exposures (based on monthly average concentrations) had a negative effect on pulmonary function and may relate to prenatal exposures affecting the lungs as they begin to develop at 6-wk gestation.

Dales et al. (2008, 156378) in a cross-sectional prevalence study examined the relationship of pulmonary function and PM measures, other pollutants, and indicators of motor vehicle emissions in Windsor, Ontario, in a cohort of 2,402 school children. $PM_{2.5}$ and PM_{10} concentrations were estimated for each child's residence at the postal code level. Each 10 $\mu g/m^3$ increase in $PM_{2.5}$ was associated with a 7.0% decrease in FVC expressed in a percentage of predicted.

In Leicester, England, investigators examined the carbon content of airway macrophages in induced sputum in 64 of 114 healthy 8-15 year-old children (Grigg et al., 2008, 156499; Kulkarni et al., 2006, 089257). The carbon content of airway macrophages (Finch et al., 2002, 054603; Strom et al., 1990, 157020) was used as a marker of individual exposure to PM_{10}. Near each child's home, exposure to PM_{10} was estimated using a statistical dispersion model (Pierse et al., 2006, 088757). The authors reported a dose-dependent inverse association between the carbon content of airway macrophages and lung function in children and found no evidence that reduced lung function itself causes an increase in carbon content. Consistent results were obtained for both FVC and FEF_{25-75}. Caution should be used when interpreting these results as the accuracy of the estimates on individual PM_{10} exposures were not validated; there is potential for confounding by ethnic origin; and there is concern that the magnitude of the changes in pulmonary function associated with increased particle area appear large (Boushey et al., 2008, 192162).

Nordling et al. (2008, 097998) discussed above in the respiratory symptoms section, also reported that lower PEF at age 4 was associated with exposure to traffic-related PM_{10} (-8.93 L/min [95% CI: -17.78 to -0.088]). Goss et al. (2004, 055624), discussed in Section 7.3.1.1, found strong inverse relationships between FEV_1 and $PM_{2.5}$ concentrations in both cross-sectional and longitudinal analyses.

In summary, recent studies have greatly expanded the evidence available for the 2004 PM AQCD. The earlier CHS studies followed young children for 2-4 yr. New analyses have been conducted that include longer follow-up periods of this cohort through 18 yr of age (considered early adulthood for lung development (Stanojevic et al., 2008, 157007) and provide evidence that effects from exposure to $PM_{2.5}$ persist into early adulthood. Longitudinal studies follow effects over time and are considered to provide the best evidence as opposed to studies across communities as in cross-sectional studies. The longitudinal cohort studies in the 2004 PM AQCD provided data for children in one location in one study and new longitudinal studies have been conducted in other locations.

Gauderman et al. (2004, 056569) reported that $PM_{2.5}$ exposure was associated with clinically and statistically significant deficits in FEV_1 attained at the age of 18 yr. Clinical significance was

defined as a FEV_1 below 80% of the predicted value, a criterion commonly used in clinical settings to identify persons at increased risk for adverse respiratory conditions. This clinical aspect is an important enhancement over the earlier results reported in the 2004 PM AQCD. Further, the association reported in this study that evaluated the 8-yr time period into early adulthood not only provided evidence for the persistence of the effect, but in addition the strength and robustness of the outcomes were more positive, larger, and more certain than previous CHS studies of shorter follow-up.

Supporting this result are new longitudinal cohort studies conducted by other researchers in other locations with different methods. Though these studies report results for PM_{10}, available data discussed above indicate that the majority of PM_{10} is composed of $PM_{2.5}$ in these areas. New studies provide positive results from Mexico City, Sweden, and a national cystic fibrosis cohort in the U.S. One study reported null results in a European cohort described as having potential misclassification and confounding concerns as well as lacking a homogenous population potentially rendering the outcome as non-informative. A natural experiment in Switzerland, where PM levels had decreased, reported that improvement in air quality may slow the annual rate of decline in lung function in adulthood, indicating positive consequences for public health. These natural experiments are considered especially supportive.

The relationship between long-term PM exposure and decreased lung function is thus seen during lung growth and lung development in school-age children into adulthood. At adult ages studies continue to show a relationship between decreased lung function and long-term PM exposure. Some newer studies attempting to study the relationship of long-term PM exposure from birth through preschool are reporting a relationship. Thus, the impact of long-term PM exposure is seen over the time period of lung function growth and development and the decline of lung function with aging.

Overall, effect estimates from these studies are negative (i.e., indicating decreasing lung function) and the pattern of effects are similar between the studies for FVC and FEV_1. Thus, the data are consistent and coherent across several designs, locations, and researchers. With cautions noted, the results relating carbon content of airway macrophages to decreased measures of pulmonary function add plausibility to the epidemiologic findings. Some new studies are using individual estimates of exposure to ambient PM to reduce the impact of exposure error (Downs et al., 2007, 092853; Jerrett et al., 2005, 087381).

As was found in the 2004 PM AQCD, the studies report associations with $PM_{2.5}$ and PM_{10}, while most did not evaluate $PM_{10-2.5}$. Associations have been reported with fine particle components, particularly EC and OC. Source apportionment methods generally have not been used in these long-term exposure studies. However, numerous studies have evaluated exposures to PM related to traffic or motor vehicle sources. For example, Meng et al. (2007, 093275) investigated the associations between traffic and outdoor pollution levels and poorly controlled asthma among adults who were respondents to the California Health Interview Survey and found associations for traffic density and PM_{10}, but not $PM_{2.5}$.

7.3.2.2. Toxicological Studies

Urban Air

One new study evaluated the effects of chronic exposure to ambient levels of urban particles on lung development in the mouse (Mauad et al., 2008, 156743). Both functional and anatomical indices of lung development were measured. Male and female BALB/c mice were continuously exposed to ambient or filtered Sao Paolo air for 8 mo. Concentrations in the "polluted chamber" versus "clean chamber" were 16.8 versus 2.9 $\mu g/m^3$ $PM_{2.5}$. Thus PM levels were reduced by filtration but not entirely eliminated. Ambient concentrations of CO, NO_2 and SO_2 were 1.7 ppm, 89.4 $\mu g/m^3$ and 8.1 $\mu g/m^3$, respectively. Concentrations of gaseous pollutants were assumed to be similar to ambient levels in both chambers. After 4 mo, the animals were mated and the offspring were divided into 4 groups to provide for a prenatal exposure group, a postnatal exposure group, a pre and postnatal exposure group and a control group. Animals were sacrificed at 15 and 90 days of age for histological analysis of lungs. Pulmonary pressure-volume measurements were also conducted in the 90-day-old offspring. Statistically significant reductions in inspiratory and

expiratory volumes were found in the group receiving both prenatal and postnatal exposure, but not in the groups receiving only prenatal exposure or only postnatal exposure, compared with controls. These changes in pulmonary function correlated with anatomical changes which are discussed in Section 7.3.5.1.

Diesel Exhaust

Li et al. (2007, 155929) exposed BALB/c and C56BL/6 mice to clean air or to low-dose DE (at a PM concentration of 100 $\mu g/m^3$) for 7 h/day and 5 days/week for 1, 4 and 8 wk. Average gas concentrations were reported to be 3.5 ppm CO, 2.2 ppm NO_2, and less than 0.01 ppm SO_2. Airway hyperresponsiveness (AHR) was evaluated by whole-body plethysmography at Day 0 and after 1, 4 and 8 wk of exposure. Short-term exposure responses are discussed in Section 6.3.2.3, 6.3.3.3 and 6.3.4.2. The increased sensitivity of airways to methacholine (measured as Penh) seen in C57BL/6 but not BALB/c mice at 1 week was also seen at 4 wk but not at 8 wk. This study suggests that adaptation occurs during prolonged DE exposure. Influx of inflammatory cells, markers of oxidative stress and effects of antioxidant intervention were also evaluated (Sections 7.3.3.2 and 7.3.4.1). Although no attempt was made in this study to determine the effects of gaseous components of DE on the measured responses, concentrations of gases were very low suggesting that PM may have been responsible for the observed effects.

In many animal studies changes in ventilatory patterns are assessed using whole-body plethysmography, for which measurements are reported as enhanced pause (Penh). Some investigators report increased Penh as an indicator of AHR, but these are inconsistently correlated and many investigators consider Penh solely an indicator of altered ventilatory timing in the absence of other measurements to confirm AHR. Therefore use of the terms AHR or airway responsiveness has been limited to instances in which the terminology has been similarly applied by the study investigators.

Gottipolu et al. (2009, 190360) exposed WKY and SH rats to filtered air or DE (particulate concentration 500 and 2,000 $\mu g/m^3$) for 4 h/day and 5 days/wk over a 4-wk period. Concentrations of gases were 1.3 and 4.8 ppm CO, NO <2.5 and 5.9 ppm NO, <0.25 and 1.2 ppm NO_2, 0.2 and 0.3 ppm SO_2 for low and high PM exposures, respectively. Particle size, measured as geometric median number and volume diameters, was 85 and 220 nm, respectively. No DE-related effects were found for breathing parameters measured by whole-body plethysmography. Other pulmonary effects are described in Sections 7.3.3.2 and 7.3.5.1.

Woodsmoke

One study evaluated the effects of subchronic woodsmoke exposure on pulmonary function in Brown Norway rats. Rats were exposed 3 h/day and 5 days/week for 4 and 12 wk to air or to concentrated wood smoke from the pinyon pine which is native to the U.S. Southwest (Tesfaigzi et al., 2002, 025575). PM concentrations in the woodsmoke were 1,000 and 10,000 $\mu g/m^3$. The particles in this woodsmoke had a bimodal size distribution with the smaller size fraction (74%) characterized by a MMAD of 0.405 µm and the larger size fraction (26%) characterized by a MMAD of 6.7-11.7 µm. Many of these larger particles would not be inhalable by the rat since 8 µm MMAD particles are about 50% inhalable (Ménache et al., 1995, 006533). Concentrations of gases were reported to be 15-106.4 ppm CO, 2.2-18.9 ppm NO, 2.4-19.7 ppm NO_X and 3.5-13.8 ppm total hydrocarbon in these exposures. Respiratory function measured by whole-body plethysmography demonstrated a statistically significant increase in total pulmonary resistance in rats exposed to 1000 $\mu g/m^3$ woodsmoke. Additional effects were found at 10,000 $\mu g/m^3$. Inflammatory and histopathological responses were also evaluated (Sections 7.3.3.2 and 7.3.5.1).

7.3.3. Pulmonary Inflammation

7.3.3.1. Epidemiologic Studies

One epidemiologic study examined the relationship of airway inflammation (eNO) and PM measures, other pollutants, and indicators of motor vehicle emissions in Windsor, Ontario (Dales et al., 2008, 156378). This cohort of 2,402 school children estimated $PM_{2.5}$ and $PM_{10-2.5}$ for each child's residence at the postal code level with an evaluated statistical model (Wheeler et al., 2006, 103905). Each 10 $\mu g/m^3$ increase in 1-yr $PM_{2.5}$ was associated with a 39% increase in eNO ($p = 0.058$). Associations between eNO and $PM_{10-2.5}$ were positive but not statistically significant.

7.3.3.2. Toxicological Studies

CAPs Studies

A set of subchronic studies involved exposure of normal (C57BL1/6) mice, $ApoE^{-/-}$ and the double-knockout $ApoE^{-/-}/LDLR^{-/-}$ mice to Tuxedo, NY CAPs for 5-6 month (March, April or May through September 2003 (Lippmann et al., 2005, 087452). The average $PM_{2.5}$ exposure concentration was 110 $\mu g/m^3$. Animals were fed a normal chow diet during the CAPs exposure period. No pulmonary inflammation was observed in response to CAPs exposure as measured by BALF cell counts and histology. The lack of a persistent pulmonary response may have been due to adaptation of the lung following repeated exposures. In fact, a parallel study examined CAPs-related gene expression in the double-knockout animals and found upregulation of numerous genes in lung tissue (Gunnison and Chen, 2005, 087956). An in vitro study conducted simultaneously found daily variations in CAPs-mediated NF-κB activation in cultured human bronchial epithelial cells, suggesting that transcription factor-mediated gene upregulation could occur in response to CAPs (Maciejczyk and Chen, 2005, 087456). It should be noted that significant cardiovascular effects were observed in these subchronic studies which are discussed in Section 7.2.1.2.

Araujo et al. (2008, 156222) compared the relative impact of UF (0.01-0.18 μm) versus fine (0.01-2.5 μm) PM inhalation in $ApoE^{-/-}$ mice following a 40 day exposure (5 h/day×3 days/wk for 75 total hours). Animals were fed a normal chow diet and exposed to PM from November 3 -December 12, 2005 in a mobile inhalation laboratory that was parked 300 m from the 110 Freeway in downtown Los Angeles. Particles were concentrated to ~440 $\mu g/m^3$ for $PM_{2.5}$ exposures and ~110 $\mu g/m^3$ for the UF exposures, representing a roughly 15-fold increase in concentration from ambient levels; the number concentration of PM in the fine and UF chambers were roughly equivalent (4.56×10^5 and 5.59×10^5 particles/cm^3, respectively). Over 50% of the UFPs were comprised of OC compared to only 25% for $PM_{2.5}$. No major increase in BALF inflammatory cells was found in response to PM. However UFP exposure resulted in significant cardiovascular and systemic effects (Section 7.2.1.2).

Diesel Exhaust

Gottipolu et al. (2009, 190360) exposed WKY and SH rats to filtered air or DE for 4 wk as described in Section 7.3.2.2. Previous studies from this laboratory have shown enhanced effects of PM in SH compared with WKY rats. Although the main focus of this recent study was on DE-induced mitochondrial oxidative stress and hypertensive gene expression in the heart (Section 7.2.7.1), some pulmonary effects were also found. Subchronic exposure to DE resulted in a dose-dependent increase in BALF neutrophils in both rat strains although levels of measured cytokines were not altered. Histological analysis of lung tissue from rats exposed to the higher concentration of DE demonstrated accumulation of particle-laden macrophages as well as focal alveolar hyperplasia and inflammation. Effect on indices of injury are discussed in Section 7.3.5.1.

Ishihara and Kagawa (2003, 096404) exposed Wistar rats to filtered air and DE containing 200, 1,000 and 3,000 $\mu g/m^3$ PM for 16 h/day and 6 days/wk for 6, 12, 18 or 24 mo. The mass median particle diameter was reported to be between 0.3 and 0.5 μm. Concentrations of gases ranged from

2.93-35.67 ppm NO_x, 0.23-4.57 ppm SO_2, 1.8-21.9 ppm CO in the DE exposures. Statistically significant increases in total numbers of inflammatory cells and neutrophils in BALF were observed beginning at 6-12 mo of exposure to DE containing 1,000 and 3,000 $\mu g/m^3$ PM. When rats were exposed to DE containing 1,000 $\mu g/m^3$ PM, which was filtered to remove PM, the inflammatory cell response was significantly diminished. These results implicate the PM fraction of DE as a key determinant of the inflammation. The PM fraction was also found to mediate the increase in protein levels, the decrease in PGE_2 levels and alterations in mucus and surfactant components observed in BALF (Section 7.3.5.1).

Li et al. (2007, 155929) exposed BALB/c and C56BL/6 mice to low dose DE as described in Section 7.3.2.2. for 1, 4 and 8 wk. Increases in numbers of BALF macrophages and total inflammatory cells were observed in BALB/c mice at 8 wk but not 4 wk of DE exposure. Persistent increases in numbers of BALF neutrophils and lymphocytes were observed in both strains at 4 and 8 wk of DE exposure. Corresponding increases in BALF cytokines differed between the two strains. These results should be interpreted with caution since comparisons were made with Day 0 controls rather than age-matched controls. No histopathological changes in the lungs were seen at any time point after DE exposure. This study demonstrated differences in pulmonary responses to low dose DE between two mouse strains. AHR, pulmonary inflammation, markers of oxidative stress and effects of antioxidant intervention were also evaluated (Sections 7.3.2.2 and 7.3.4.1). Although no attempt was made in this study to determine the effects of gaseous components of DE on the measured responses, concentrations of gases were very low suggesting that PM may have been responsible for the observed effects.

In a study by Hiramatsu et al. (2003, 155846), BALB/c and C57BL/6 mice were exposed to DE (PM concentrations 100 and 3,000 $\mu g/m^3$) for 1 or 3 mo. Concentrations of gases were reported to be 3.5-9.5 ppm CO, 2.2-14.8 ppm NO_x, and less than 0.01 ppm SO_2. Modest increases in BALF neutrophils and lymphocytes were observed in response to DE in both mouse strains at 1 and 3 mo. Histological analysis demonstrated diesel exposure particle-laden alveolar macrophages in alveoli and peribronchial tissues at both time points. Bronchus-associated lymphoid tissue developed after 3-month exposure to the higher concentration of DE in both mouse strains. Mac-1 positive cells (a marker of phagocytic activation of alveolar macrophages) were also increased in BALF of BALB/c mice exposed to the higher concentration of DE for 1 and 3 mo. Increased expression of several cytokines and decreased expression of iNOS mRNA was observed in DE-exposed mice at 1 and 3 mo. NF-κB activation was also noted following 1-month exposure to the lower concentration of DE. No attempt was made in this study to determine the responses to gaseous components of the DE.

In a study by Reed et al. (2004, 055625), healthy Fisher 344 rats and A/J mice were exposed to DE (PM concentration = 30, 100, 300 and 1,000 $\mu g/m^3$) by whole body inhalation for 6 h/day, 7 days/wk for either 1 week or 6 mo. Concentrations of gases were reported to be 2.0-45.3 ppm NO, 0.2-4.0 ppm NO_2, 1.5-29.8 ppm CO and 8-365 ppb SO_2. Short-term responses are discussed in Section 6.3.3.3 and 6.3.7.2, and sub-chronic systemic effects are presented in Section 7.2.4.1. Six months of exposure resulted in no measurable effects on pulmonary inflammation. However numerous black particles were observed within alveolar macrophages after 6 mo of exposure.

Seagrave et al. (2005, 088000) evaluated pulmonary responses in male and female CDF (F-344)/CrlBR rats exposed 6 h/day for 6 mo to filtered air or DE at concentrations ranging from 30-1000 $\mu g/m^3$ PM. Concentrations of gases were reported for the highest exposure as 45.5 ppm NO, 4.0 ppm NO_2, 29.8 ppm CO and 2.2 ppm total vapor hydrocarbon. No changes in BALF cells were noted. A small decrease in TNF-α was seen in BALF of female rats exposed to the highest concentration of DE for 6 mo. Pulmonary injury also was evaluated (Section 7.3.5.1). Thus changes in BALF markers were modest and gender-specific.

Woodsmoke

Seagrave et al. (2005, 088000) also evaluated pulmonary responses in male and female CDF (F344)/CrlBR rats exposed 6 h/day for 6 mo to filtered air or hardwood smoke concentrations ranging from 30-1,000 $\mu g/m^3$ PM. Concentrations of gases were reported for the highest exposure as 3.0 ppm CO and 3.1 ppm total vapor hydrocarbon. A small increase in BALF neutrophils was observed in male rats exposed to the lowest concentration of hardwood smoke. Female rats exhibited a decrease in BALF macrophage inflammatory protein-2 (MIP-2) at the highest concentration of hardwood smoke. Pulmonary injury also was evaluated (Section 7.3.5.1). In general, responses to

hardwood smoke were more remarkable than responses to DE seen in a parallel study. However these gender-specific responses were modest and difficult to interpret.

In a study by Reed et al. (2006, 156043), Fisher 344 rats, SHR rats, A/J mice and C57BL/6 mice were exposed to clean air or hardwood smoke (PM concentrations 30, 100, 300 and 1,000 $\mu g/m^3$) by whole body inhalation for 6 h/day, 7 days/wk for either 1 week or 6 mo. Concentrations of gases ranged from 229.0-14887.6 mg/m^3 for CO, 54.9-139.3 $\mu g/m^3$ for ammonia, and 177.6- 3455.0 $\mu g/m^3$ nonmethane VOC in these exposures. Short-term responses are discussed in Section 6.3.7.2 and sub-chronic effects are presented in Section 7.2.4.1. Histological analysis of lung tissue showed minimal increases in alveolar macrophages. The effects of hardwood smoke on bacterial clearance are discussed below (Section 7.3.7.2).

Another study evaluated the effects of subchronic woodsmoke exposure in Brown Norway rats and is described in detail in Section 7.3.2.2 (Tesfaigzi et al., 2002, 025575). Numbers of alveolar macrophages in BALF were significantly increased in rats exposed to 1,000 $\mu g/m^3$ woodsmoke for 12 wk, but no changes were seen in numbers of other inflammatory cells. A large percent of BALF macrophages contained carbonaceous material. Histological analysis of lung tissue showed minimal to mild inflammation in the epiglottis of the larynx in rats exposed to both concentrations of woodsmoke.

Ramos et al. (2009, 190116) examined the effects of subchronic woodsmoke exposure on the development of emphysema in guinea pigs. Inflammation is thought to be involved in the pathogenesis of this form of COPD. Statistically significant increases in total numbers of BALF cells were observed in guinea pigs exposed to smoke for 1-7 mo, with numbers of macrophages increased at 1-4 mo and numbers of neutrophils increased at 4-7 mo. At 4 mo, alveolar mononuclear phagocytic and lymphocytic peribronchiolar inflammation were observed by histological analysis of lung tissue. This study is discussed in depth in Section 7.2.5.1.

Model Particles

Wallenborn et al. (2008, 191171) examined the pulmonary, cardiac and systemic effects of subchronic exposure to particulate $ZnSO_4$. WKY rats were exposed nose-only to 10, 30, or 100 $\mu g/m^3$ UFP of $ZnSO_4$ for 5 h/day and 3 day/wk over a 16-wk period. Particle size was reported to be 31-44 nm measured as number median diameter. No changes in pulmonary inflammation or injury were observed although cardiac effects were noted (Section 7.2.7.1). This study possibly demonstrates a direct effect of $ZnSO_4$ on extrapulmonary systems, as suggested by the lack of pulmonary effects.

7.3.4. Pulmonary Oxidative Response

7.3.4.1. Toxicological Studies

Urban Air

One new study evaluated the effects of subchronic exposure to ambient levels of urban particles on the development of emphysema in papain-treated mice (Lopes et al., 2009, 190430). Since oxidative stress is thought to contribute to the development of emphysema, 8-isoprostane levels were measured in lung tissue from the four groups of mice used in this study. A statistically significant increase in 8-isoprostane, a marker of oxidative stress, was observed in lungs from mice treated with papain and exposed to ambient air compared with the other groups of mice. This study is described in greater depth in Section 7.3.5.1.

Diesel Exhaust

Li et al. (2007, 155929) exposed mice to low dose DE for 1, 4 and 8 wk as described in Section 7.3.2.2. Markers of oxidative stress and effects of antioxidant intervention were evaluated in this model. While HO-1 mRNA and protein were increased in lung tissues of both mouse strains after 1 week of DE exposure (Section 6.3.4.2), at 8 wk of DE exposure, HO-1 protein levels remained high in C57BL/6 mice but returned to control values in BALB/c mice. This study demonstrates differences in pulmonary responses to low dose DE between two mouse strains. Furthermore, this study suggests that adaptation occurs in BALB/c mice during prolonged DE exposure since the increase in HO-1 protein seen in both strains at 1 week of exposure was only seen in C57BL/6 mice at 8 wk. AHR (Section 7.3.2.2) and pulmonary inflammation (Section 7.3.3.2) were also evaluated. Although no attempt was made in this study to determine the effects of gaseous components of DE on the measured responses, concentrations of gases were very low. This suggests that PM may have been responsible for the observed effects.

7.3.5. Pulmonary Injury

7.3.5.1. Toxicological Studies

Urban Air

One new study evaluated the effects of chronic exposure to ambient levels of urban particles on lung development in the mouse (Mauad et al., 2008, 156743). Both functional and anatomical indices of lung development were measured in mice exposed prenatally and/or postnatally as described in Section 7.3.2.2. Animals were sacrificed at 15 and 90 days of age for histological analysis of lungs. Histological analysis demonstrated the presence of mild foci of macrophages containing black dots of carbon pigment in the prenatal and postnatal exposure group at 90 days. In addition, the alveolar spaces of 15-day old mice in the prenatal and postnatal exposure group were enlarged compared with controls. Morphometric analysis demonstrated statistically significant decreases in surface to volume ratio at 15 and 90 days in the prenatal and postnatal exposure group compared with controls. Since alveolarization is normally complete by 15 days of age, these results suggest incomplete alveolarization in the 15-day-old group and an enlargement of air spaces in the 90-day-old group. These anatomical changes correlated with decrements in pulmonary function which are discussed in Section 7.3.2.2.

Prolonged exposure to low levels of ambient air pollution beginning in early life has been linked to secretory changes in the nasal cavity of mice, specifically increased production of acidic mucosubstances (Pires-Neto et al., 2006, 096734). Six-day-old Swiss mice were continuously chamber exposed to ambient or filtered São Paulo air for 5 mo. Concentrations in the "polluted chamber" versus "clean chamber" were (in $\mu g/m^3$) 59.52 versus 37.08 for NO_2, 12.52 versus 0 for BC, and 46.49 versus 18.62 for $PM_{2.5}$. Thus, pollutant levels were reduced by filtration but not entirely eliminated. Compared to filtered air, exposure to ambient air resulted in increased total mucus and acidic mucus in the epithelium lining the nasal septum, but no statistically significant differences in other parameters (amount of neutral mucus, volume proportions of neutral mucus, total mucus, or nonsecretory epithelium, epithelial thickness, or ratio between neutral and acidic mucus). The physicochemical properties of mucus glycoproteins are critical to the protective function of the airway mucus layer. Acidified mucus is more viscous, and is associated with a decrease in mucociliary transport. Thus acidic mucosubstances may represent impaired defense mechanisms in the respiratory tract.

One new study evaluated the effects of subchronic exposure to ambient levels of urban particles on the development of emphysema in papain-treated mice (Lopes et al., 2009, 190430). Emphysema is a form of COPD caused by the destruction of extracellular matrix in the alveolar region of the lung which results in airspace enlargement, airflow limitation and a reduction of the gas-exchange area of the lung. Inflammation, oxidative stress, protease imbalance and apoptosis are thought to contribute to the development of emphysema. In this study, male BALB/c mice were

continuously exposed to ambient or filtered Sao Paulo air for 2 mo. Concentrations of $PM_{2.5}$ in the "polluted chamber" versus "clean chamber" were 33.86 ± 2.09 versus 2.68 ± 0.38 μg/m^3. Thus filtration reduced PM levels considerably. Ambient concentrations of CO and SO_2 were 1.7 ppm and 16.2 μg/m^3 respectively. No significant difference was observed in the concentrations of NO_2 in the "polluted chamber" versus "clean chamber" (60-80 μg/m^3). Half of the mice were pre-treated with papain by intranasal instillation in order to induce emphysema. Morphometric analysis of lung tissue demonstrated a statistically significant increase in mean linear intercept, a measure of airspace enlargement, in papain-treated mice compared with saline-treated controls exposed to filtered air. While exposure to ambient air failed to increase mean linear intercept values in saline-treated mice, mean linear intercept values were significantly increased in papain-treated mice exposed to ambient air compared with papain-treated mice exposed to filtered air. A similar pattern of responses was observed for the volume proportion of collagen and elastin fibers in alveolar tissue, which are markers of alveolar wall remodeling. Lung immunohistochemical analysis demonstrated an effect of papain, but not ambient air, on macrophage cell density and matrix metalloproteinase 12-positive cell density. No differences in caspase-3 positive cells, a marker of apoptosis, were observed between the four groups of mice. Oxidative stress was evaluated in this model as described in Section 7.3.4.1. Taken together, results of this study demonstrate that urban levels of PM, mainly from traffic sources, worsen protease-induced emphysema in an animal model.

Pulmonary vascular remodeling, measured by a decrease in the lumen to wall ratio, was observed in mice exposed to ambient São Paulo air for 4 mo (Lemos et al., 2006, 088594). This study is described in greater detail in Section 7.2.1.2.

Kato and Kagawa (2003, 089563) exposed Wistar rats to roadside air contaminated mainly with automobile emissions (55.7-65.2 ppb NO_2 and 63-65 μg/m^3 suspended PM [SPM]) and examined the effects on respiratory tissue after 24, 48, or 60 wk of exposure. The surface of the lungs was light gray in color after all durations of exposure, and BC particle deposits accumulated with prolonged exposure. These characteristics were not evident in filtered air-exposed control animals, although filtered air contained low levels of air pollutants (≤ 6.2 ppb NO_2 and 15 μg/m^3 SPM). The most common change observed using transmission electron microscopy was the presence of particle laden (anthracotic) alveolar macrophages, or anthracosis, in a wide range of pulmonary tissues, including the submucosa, tracheal- and bronchiole-associated lymph nodes, alveolar wall and space, pleura, and perivascular connective tissue. These changes were evident after 24 wk and increased with duration of exposure. Other changes included increases in the number of mucus granules in goblet cells, mast cell infiltration (but no degranulation) after 24 wk, increased lysosomes in ciliated cells, some altered morphology of Clara cells, and hypertrophy of the alveolar walls after 48 wk. No goblet cell proliferation was observed, but slight, variable acidification of mucus granules appeared after 24 and 48 wk and disappeared after 60 wk. Anthracotic macrophages were seen in contact with plasma cells and lymphocytes in the lymphoid tissue, suggesting immune cell interaction in the immediate vicinity of particles. Even after 60 wk, no lymph node anthracosis was observed in the filtered air group.

In a post-mortem study of lung tissues from 20 female lifelong residents of Mexico City, a high PM locale, histology demonstrated significantly greater amounts of fibrous tissue and muscle in the airway walls compared to subjects from Vancouver (Churg et al., 2003, 087899), a city with relatively low PM levels. Electron microscopy showed carbonaceous aggregates of UFPs, which the authors conclude penetrate into and are retained in the walls of small airways. The study shows an association between retained particles and airway remodeling in the form of excess muscle and fibrotic walls. The subjects were deemed suitable for examination based on never-smoker status, no use of biomass fuels for cooking, no known occupational particle/dust exposure, death by cause other than respiratory disease, and extended residence in each locale (lifelong for Mexico City and >20 yr for Vancouver). However, subjects from the two locales were not matched with respect to ethnicity, sex (20 females from Mexico City versus 13 females and 7 males from Vancouver), or mean age at death (66 ± 9 versus 76 ± 11), and other possibly influential factors such as exercise or diet were not considered.

Diesel Exhaust

Gottipolu et al. (2009, 190360) exposed WKY and SH rats to filtered air or DE as described in Section 7.3.2.2. Previous studies from this laboratory have shown enhanced effects of PM in SH

compared with WKY rats. Although the main focus of this recent study was on DE-induced mitochondrial oxidative stress and hypertensive gene expression in the heart (Section 7.2.7.1), some pulmonary effects were found. Inflammatory effects are described in Section 7.3.3.2. GGT activity in BALF was increased in both strains in response to the higher concentration of DE. No DE-related changes were observed in BALF protein or albumin. Histological analysis of lung tissue from rats exposed to the higher concentration of DE demonstrated accumulation of particle-laden macrophages as well as focal alveolar hyperplasia and inflammation. No effects on indices of pulmonary function were observed (Section 7.3.2.2.)

Ishihara and Kagawa (2003, 096404) exposed rats to DE for up to 24 mo as described in Section 7.3.3.2. A statistically significant increase in BALF protein was observed at 12 mo of exposure to DE containing 1,000 $\mu g/m^3$ PM. This response was attenuated when the DE was filtered to remove PM. Pulmonary inflammation was noted and is described in Section 7.3.3.2.

Seagrave et al. (2005, 088000) evaluated pulmonary responses in rats exposed to DE for up to 6 mo as described in Section 7.3.3.2. A small increase in LDH was seen in BALF of female rats exposed to the highest concentration of DE for 6 mo. Pulmonary inflammation was also evaluated (Section 7.3.3.2). The changes in BALF markers in this study were modest and gender-specific.

Gasoline Exhaust

Reed et al. (2008, 156903) examined a variety of health effects following subchronic inhalation exposure to gasoline engine exhaust. Male and female CDF (F344)/CrlBR rats, SHR rats and male C57BL/6 mice were exposed for 6 h/day and 7 days/wk for a period of 3 days-6 mo. The dilutions for the gasoline exhaust were 1:10, 1:15 and 1:90; filtered PM was at the 1:10 dilution. PM mass ranged from 6.6 to 59.1 $\mu g/m^3$, with the corresponding number concentration between 2.6×10^4 and 5.0×10^5 particles/cm^3. Concentrations of gases ranged from 12.8-107.3 ppm CO, 2.0-17.9 ppm NO, 0.1-0.9 ppm NO_2, 0.09-0.62 ppm SO_2 and 0.38-3.37 ppm NH_3. Other effects are described in Sections 7.2.4.1 and 7.3.6.1. No pulmonary inflammation or histopathological changes were noted in the F344 rats and A/J mice, except for a time-dependent increase in the number of macrophages containing PM. However statistically significant increases of 47% and 29% in BALF LDH were observed in female and male F344 rats, respectively, after 6 mo of exposure to the highest concentration of engine exhaust. This response was absent when gasoline exhaust was filtered, implicating PM as a key determinant of this response. In addition, exposure to the highest concentration of gasoline exhaust resulted in statistically significant decreases in hydrogen peroxide and superoxide production in unstimulated and stimulated BALF macrophages. Hypermethylation of lung DNA was observed in male F344 rats following 6 mo of exposure to gasoline exhaust containing 30 $\mu g/m^3$ PM. This response was PM-dependent since it was absent in mice exposed to filtered gasoline exhaust. The significance of this epigenetic change in terms of respiratory health effects is not known. However, altered patterns of DNA methylation can affect gene expression and are sometimes associated with altered immune responses and/or the development of cancer.

Woodsmoke

Seagrave et al. (2005, 088000) also evaluated pulmonary responses in rats exposed to hardwood smoke for 6 mo as described in Section 7.3.3.2. Increases in BALF LDH and protein were seen in male but not female rats. Female rats exhibited a decrease in BALF glutathione at the highest concentration of hardwood smoke. Decreases in BALF alkaline phosphatase were found in both males and females exposed to 1,000 $\mu g/m^3$ hardwood smoke. Male rats exposed to 100 and 300 $\mu g/m^3$ hardwood smoke exhibited a decrease in BALF β-glucuronidase activity. Pulmonary inflammation was also evaluated (Section 7.3.3.2). These changes in BALF markers in this study were modest and gender-specific.

Another study evaluated the effects of subchronic woodsmoke exposure in Brown Norway rats as described in Section 7.3.2.2. (Tesfaigzi et al., 2002, 025575). Exposure to 1,000 $\mu g/m^3$ woodsmoke for12 wk resulted in a statistically significant increase in Alcian Blue- (AB) and Periodic Acid Schiff- (PAS) positive airway epithelial cells compared to controls, indicating an increase in mucous secretory cells containing neutral and acid mucus, respectively. More significant histopathological responses were found following exposure to 10,000 $\mu g/m^3$ of DE. Pulmonary

function and inflammation were evaluated also but are not discussed here due to the extremely high exposure level (Sections 7.3.2.2. and 7.3.3.2).

Ramos et al. (2009, 190116) examined the effects of subchronic woodsmoke exposure on the development of emphysema in guinea pigs. In particular, the involvement of macrophages and macrophage-derived MMP in woodsmoke-related responses was investigated. Guinea pigs were exposed to ambient air or to whole smoke from pine wood for 3 h/day and 5 days/wk over a 7-month period. PM_{10} and $PM_{2.5}$ concentrations in the exposure chambers were reported to be 502 ± 34 and 363 ± 23 $\mu g/m^3$, respectively, while the concentration of CO was less than 80 ppm. COHb levels were reported to be 6% in controls and 15-20% in smoke-exposed guinea pigs. Statistically significant decreases in body weight were observed in guinea pigs exposed to smoke for 4 or more months compared with controls. Statistically significant increases in total numbers of BALF cells were observed in guinea pigs exposed to smoke for 1-7 mo, with numbers of macrophages increased at 1-4 month and numbers of neutrophils increased at 4-7 mo. At 4 mo, alveolar mononuclear phagocytic and lymphocytic peribronchiolar inflammation, as well as bronchiolar epithelial and smooth muscle hyperplasia, were observed by histological analysis of lung tissue. Emphysematous lesions, smooth muscle hyperplasia and pulmonary arterial hypertension were noted at 7 mo. Morphometric analysis of lung tissue demonstrated statistically significant increases in mean linear intercept values, a measure of airspace enlargement, in guinea pigs at 6 and 7 mo of exposure. Statistically significant increases in elastolytic activity was observed in BALF macrophages and lung tissue homogenates at 1-7 mo of exposure. Lung collagenolytic activity was also increased at 4-7 mo of exposure and corresponded in time with the presence of active forms of MMP-2 and MMP-9 in lung tissue homogenates and BALF. Furthermore, MMP-1 and MMP-9 immunoreactivity was detected in macrophages, epithelial and interstitial cells in smoke-exposed animals at 7 mo. Increased levels of MMP-2 and MMP-9 mRNA were also found in smoke-exposed guinea pigs after 3-7 mo. Apoptosis was found in BALF macrophages (TUNEL assay) from guinea pigs exposed to smoke for 3-7 mo and in alveolar epithelial cells (caspase-3 immunoreactivity) after 7 mo. Taken together, these results provide evidence that subchronic exposure to woodsmoke leads to the development of emphysematous lesions accompanied by the accumulation of alveolar macrophages, increased levels and activation of MMPs, connective tissue remodeling and apoptosis. However, the high levels of CO and COHb reported in this study make it difficult to conclude that woodsmoke PM alone is responsible for these dramatic effects.

7.3.6. Allergic Responses

7.3.6.1. Epidemiologic Studies

A number of epidemiologic studies have found associations between PM and allergic (or atopic) indicators. Allergy is a major driver of asthma, which has been associated with PM in studies discussed in previous sections. In a study by Annesi-Maesano (2007, 093180) (described in Section 7.3.1.1) atopic asthma was related to $PM_{2.5}$ (OR 1.43 [95% CI: 1.07-1.91]) and positive skin prick test to common allergens was also increased with higher PM levels. This report is consistent with the results from an earlier study (Penard-Morand et al., 2005, 087951) in the same sample of children that associated allergic rhinitis and atopic dermatitis with PM_{10}. Also, Morgenstern et al. (2008, 156782) found statistically significant effects for asthmatic bronchitis, hay fever, and allergic sensitization to pollen in a cohort of children in Germany examining modeled $PM_{2.5}$ data at birth addresses. Distance to a main road had a dose-response relationship with sensitization to outdoor allergens. Nordling et al. (2008, 097998) (discussed above in Section 7.3.2.1) reported a positive association of PM_{10} exposure during the first year of life with allergenic sensitization (IgE antibodies) to inhaled allergens, especially pollen. In a study by Brauer et al. (2007, 090691) (discussed above in Section 7.3.1.1) an interquartile range increase in $PM_{2.5}$ was associated with an increased risk of sensitization to food allergens (OR 1.75 [95% CI 1.23-2.47]). A significant association was found for sensitization to any allergen, but none was found for sensitization to specific indoor or outdoor aeroallergens or atopic dermatitis (eczema). In a study by Janssen et al. (2003, 133555), $PM_{2.5}$ was associated with allergic indicators such as hay fever (ever), skin prick test reactivity to outdoor allergens, current itchy rash, and conjunctivitis in Dutch children. These same outcomes were also associated with proximity of the school to truck traffic but not car traffic,

suggesting a role for diesel-related pollution. Consistent with the aforementioned Dutch study by Brauer et al. (2007, 090691), $PM_{2.5}$ was not associated with eczema.

Mortimer et al. (2008, 187280) examined the association between prenatal and early-life exposures to air pollutants with allergic sensitization in a cohort of 170 children with asthma, ages 6-11 yr, living in central California. Sensitization to at least one allergen was associated with higher levels of PM_{10} and CO during the entire pregnancy and 2nd trimester and higher PM_{10} during the first 2 yr of life. Sensitization to at least one indoor allergen was associated with higher exposures to PM_{10} and CO in during the entire pregnancy and during the 2nd trimester. However, no significant associations remained for PM_{10} after adjustment for copollutants, effect modifiers, or potential cofounders in addition to year of birth. The authors advise that the large number of comparisons may be of concern and this study should be viewed as an exploratory, hypothesis-generating undertaking. In examining the National Health Interview Survey for the years 1997-2006, Bhattacharyya et al. (2009, 180154) found relationships between air quality and the prevalence of hay fever and sinusitis. However, the air quality data were not clearly defined and as such caution is required in interpretation of these results. In contrast, Bayer-Oglesby et al. (2005, 086245) found no significant association between declining levels of PM_{10} and hay fever in Switzerland. In a study by Oftedal et al. (2007, 191948) conducted in Oslo, Norway, early-life exposure to PM_{10} or $PM_{2.5}$ was generally not associated with sensitization to allergens in 9- to 10-yr-old children; lifetime exposures to PM_{10} and $PM_{2.5}$ were associated with dust mite allergy, but the association was diminished by adjustment for socioeconomic factors . In Norway, wood burning in the wintertime is thought to account for about half of the $PM_{2.5}$ levels. Although associations between PM and reactivity to specific allergens have been reported in long-term studies, there is a consistent lack of correlation between PM and total IgE levels, indicating a selective enhancement of allergic responses.

7.3.6.2. Toxicological Studies

Diesel Exhaust

Exposure to relatively low doses of DE has been shown to exacerbate asthmatic responses in ovalbumin (OVA) sensitized and challenged BALB/c mice (Matsumoto et al., 2006, 098017). Mice were intraperitoneally sensitized and intranasally challenged 1 day prior to inhalation exposure to DE (PM concentration 100 $\mu g/m^3$; CO, 3.5 ppm; NO_2, 2.2 ppm; SO_2 <0.01 ppm) for 1 day or 1, 4, or 8 wk (7/h/day, 5 days/wk, endpoints 12 h post DE exposure). Results from the 1- and 4-wk exposures are described in Section 6.3.6.3. It should be noted that control mice were left in a clean room as opposed to undergoing chamber exposure to filtered air. The significant increases in AHR and airway sensitivity observed following shorter exposure periods did not persist at 8 wk. BALF cytokines were altered by DE exposure with only RANTES significantly elevated after 8 wk. DE had no effect on OVA challenge-induced peribronchial inflammatory or mucin positive cells. These results suggest that adaptive processes may have occurred during prolonged exposure to DE.

Gasoline Exhaust

In a study by Reed et al. (2008, 156903), BALB/c mice were exposed to whole gasoline exhaust diluted 1:10 (H), 1:15 (M), or 1:90 (L), filtered exhaust at the 1:10 (HF), or clean air for 6 h/day (atmospheric characterization described in Section 6.3.6.3). GEE exposure from conception through 4 wk of age induced slight but non-significant increases in OVA-specific IgG1 in offspring but had no significant effect on airway reactivity, BALF cytokine or cell concentrations, although there were non-significant increases in lung neutrophils and eosinophils. Significant increases in total serum IgE were observed, but this effect persisted after filtration of particles and was thus attributed to gas phase components.

Woodsmoke

In a study by Tesfaigzi et al. (2005, 156116), Brown Norway rats were sensitized and challenged with OVA. Rats were exposed for 70 days to filtered air or to 1,000 μg/m^3 hardwood smoke. Particles were characterized by a MMAD of 0.36 μm. Concentrations of gases were reported to be 13.0 ppm CO and 3.1 ppm total vapor hydrocarbon with negligible NO_X. Respiratory function was measured in anesthetized animals by whole-body plethysmography and demonstrated a significant increase in functional residual capacity as well as a significant increase in dynamic lung compliance in hardwood smoke-exposed animals compared to controls. No change in total pulmonary resistance or airway responsiveness to methacholine was observed. BALF inflammatory cells were not increased, although histological analysis demonstrated focal inflammation including granulomatous lesion and eosinophilic infiltrations in hardwood smoke-exposed rats. Alterations of several cytokines in BALF and plasma were noted. Changes in airway epithelial mucus cells and intraepithelial stored mucosubstances were modest and did not achieve statistical significance. Results of this study demonstrate that subchronic exposure to hardwood smoke had minimal effects on pulmonary responses in a rat model of allergen sensitization and challenge.

7.3.7. Host Defense

7.3.7.1. Epidemiologic Studies

Epidemiologic studies of respiratory infections indicate an association with PM. This is more evident when considering short-term exposures (Chapter 6), but studies of long-term exposures have observed associations with general respiratory symptoms often caused by infection, such as bronchitis. In a birth cohort study of approximately 4,000 Dutch children, Brauer et al. (2007, 090691)(described in Section 7.3.1.1) found significant positive associations for $PM_{2.5}$ with ear/nose/throat infections and doctor-diagnosed flu/serious cold in the first 4 yr of life. These results are consistent with an earlier study by Brauer et al. (2006, 090757), which found that an increase of 10 μg/m^3 $PM_{2.5}$ was associated with increased risk for ear infections in the Netherlands [OR 1.50 (95% CI, 1.00-2.22)]. A Swiss study by Bayer-Oglesby et al. (2005, 086245), discussed in Section 7.3.1.1 above, demonstrated that declining levels of PM_{10} were associated with declining prevalence of common cold and conjunctivitis. Because traffic-related pollutants such as UFPs are high near major roadways and then decay exponentially over a short distance, Williams, et al. (2009, 191945) assessed exposure according to residential proximity to major roads in a Seattle area study of postmenopausal women. Proximity to major roads was associated with a 21% decrease in natural killer cell function, which is an important defense against viral infection and tumors. This finding was limited to women who reported exercising near traffic; other markers of inflammation and lymphocyte proliferation did not consistently differ according to proximity to major roads. In the Puget Sound region of Washington, Karr et al. (2009, 191946) reported that there may be a modest increased risk of bronchiolitis related to $PM_{2.5}$ exposure for infants born just before the peak respiratory syncytial virus (RSV) season. Risk estimates were stronger when restricted to cases specifically attributed to RSV and for infants residing closer to highways. Emerging evidence suggests that respiratory infections, particularly infection by viruses such as RSV, can cause asthma or trigger asthma attacks.

7.3.7.2. Toxicological Studies

Diesel Exhaust

DE may affect systemic immunity. The proliferative response of A/J mouse spleen cells following stimulation with T cell mitogens was suppressed by 6 mo of daily exposure to DE at concentrations at or above 300 μg/m^3 PM (Burchiel et al., 2004, 055557). B cell proliferation was increased at 300 μg/m^3 but unaffected at higher concentrations (up to 1,000 μg/m^3). Concentrations of gases and were reported in the parallel study by Reed et al. (2004, 055625), described in

Section 7.3.3.2. The Reed study reported a decrease in spleen weight in male mice (27% reduction in the 300 $\mu g/m^3$ exposure group). The immunosuppressive effects of DE were not due to PAHs or benzo(a)pyrene (BaP)-quinones (BPQs) since there were little, if any, of these compounds present in the chamber atmosphere. It should be noted that sentinel animals were negative for mouse parvovirus at the start of the study, but seroconverted by the end of the study, indicating possible infection. Parvovirus can interfere with the modulation of lymphocyte mitogenic responses (Baker, 1998, 156245). A 6-month exposure (6h/day, 7d/wk) to 30, 100, 300 or 1,000 $\mu g/m^3$ of PM in DE did not significantly affect bacterial clearance in C57BL/6 mice infected with *Pseudomonas aeruginosa*, although all levels reduced bacterial clearance when the exposure only lasted a week (Harrod et al., 2005, 088144). Characterization of the exposure atmosphere was given by Reed et al. (2004, 055625) (Section 7.3.3.2.).

Gasoline Exhaust

In a study by Reed et al. (2008, 156903) (described in Section 6.3.7.2) long-term exposure to fresh gasoline exhaust (6h/day, 7d/wk for 6 mo) did not affect clearance of *P. aeruginosa* from the lungs of C57BL/6 mice.

Hardwood Smoke

One study demonstrated immunosuppressive effects of hardwood smoke (Burchiel et al., 2005, 088090). Exposure to hardwood smoke increased proliferation of T cells from A/J mice exposed daily to 100 $\mu g/m^3$ PM for 6 mo, but produced a concentration-dependent suppression of proliferation at PM concentrations >300 $\mu g/m^3$. No effects on B cell proliferation were observed. Concentrations of NO and NO_2 were not detectable or <40 ppb for all exposure levels. CO was reported to be 2, 4, and 13 ppm for the 100, 300 and 1,000 $\mu g/m^3$ PM concentrations, respectively. Exposure atmospheres contained significant levels of naphthalene and methylated napthalenes, fluorene, phenanthrene, and anthracene, as well as low concentrations of several metals (K, Ca, and Fe) (Burchiel et al., 2005, 088090). It should be noted that serologic analysis of study sentinel animals indicated infection with parvovirus , which can interfere with the modulation of lymphocyte mitogenic responses (Baker, 1998, 156245). In another study by Reed et al. (2006, 156043) C57BL/6 mice were exposed to 30-1,000 $\mu g/m^3$ hardwood smoke by whole-body inhalation for 6 mo prior to instillation of *P. aeruginosa*. Exposure characterizations are described in Section 7.3.3.2. Although there was a trend toward increased clearance with increasing exposure concentrations, there was no statistically significant effect of hardwood smoke exposure on bacterial clearance.

7.3.8. Respiratory Mortality

Two large U.S. cohort studies examined the effect of long-term exposure to $PM_{2.5}$ on respiratory mortality with mixed results. In the ACS study, Pope et al. (2004, 055880) reported positive associations with deaths from specific cardiovascular diseases, but no $PM_{2.5}$ associations were found with respiratory mortality. A follow-up to the Harvard Six Cities study (Laden et al., 2006, 087605) used updated air pollution and mortality data and found positive associations between long-term exposure to $PM_{2.5}$ and mortality. Of special note is a statistically significant reduction in mortality risk reported with reduced long-term fine particle concentrations observed for deaths due to cardiovascular and respiratory causes, but not for lung cancer deaths. There is some evidence for an association between $PM_{2.5}$ and respiratory mortality among post-neonatal infants (ages 1 month-1 year) (Section 7.4.1). In summary, when deaths due to respiratory causes are separated from all-cause (nonaccidental) and cardiopulmonary deaths, there is limited and inconsistent evidence for an effect of $PM_{2.5}$ on respiratory mortality, with one large cohort study finding a reduction in deaths due to respiratory causes associated with reduced $PM_{2.5}$ concentrations, and another large cohort study finding no $PM_{2.5}$ associations with respiratory mortality.

7.3.9. Summary and Causal Determinations

7.3.9.1. $PM_{2.5}$

The epidemiologic studies reviewed in the 2004 PM AQCD suggested relationships between long-term PM_{10} and $PM_{2.5}$ (or $PM_{2.1}$) exposures and increased incidence of respiratory symptoms and disease. One of these studies indicated associations with bronchitis in the 24-city cohort (Dockery et al., 1996, 046219). They also suggested relationships between long-term exposure to $PM_{2.5}$ and pulmonary function decrements in the CHS (Gauderman et al., 2000, 012531; Gauderman et al., 2002, 026013). These findings added to the database of the earlier 22-city study of $PM_{2.1}$ (Raizenne et al., 1996, 077268) that found an association between exposure to ambient particle strong acidity and impairment of lung function in children. No long-term exposure toxicological studies were reported in the 2004 PM AQCD.

Recent studies have greatly expanded the evidence available since the 2004 PM AQCD. New analyses have been conducted that include longer follow-up periods of the CHS cohort through 18 yr of age and provide evidence that effects from exposure to $PM_{2.5}$ persist into early adulthood. Gauderman et al. (2004, 056569) reported that $PM_{2.5}$ exposure was associated with clinically and statistically significant deficits in FEV_1 attained at the age of 18 yr. In addition, the strength and robustness of the outcomes were larger in magnitude, and more precise than previous CHS studies with shorter follow-up periods. Supporting this result are new longitudinal cohort studies conducted by other researchers in other locations with different methods. These studies report results for PM_{10} that is dominated by $PM_{2.5}$. New studies provide positive associations from Mexico City, Sweden, and a national cystic fibrosis cohort in the U.S. A natural experiment in Switzerland, where PM levels had decreased, reported that improvement in air quality may slow the annual rate of decline in lung function in adulthood, indicating positive consequences for public health. Thus, the data are consistent and coherent across several study designs, locations and researchers. As was found in the 2004 PM AQCD, the studies report associations with $PM_{2.5}$ and PM_{10}, while most did not evaluate $PM_{10-2.5}$. Associations have been reported with fine particle components, particularly EC and OC. Source apportionment methods generally have not been used in these long-term exposure studies.

Coherence and biological plausibility for the observed associations with lung function decrements is provided by toxicological studies (Section7.3.2.2). A recent study demonstrated that pre- and postnatal exposure to ambient levels of urban particles affected mouse lung development, as measured by anatomical and functional indices (Mauad et al., 2008, 156743). Another study suggested that the developing lung may be susceptible to PM since acute exposure to UF iron-soot decreased cell proliferation in the proximal alveolar region of neonatal rats (Pinkerton et al., 2004, 087465) (Section 6.3.5.3). Impaired lung development is a viable mechanism by which PM may reduce lung function growth in children. Other animal toxicological studies have demonstrated alterations in pulmonary function following exposure to DE and wood smoke (Section 7.3.2.2).

An expanded body of epidemiologic evidence for the effect of $PM_{2.5}$ on respiratory symptoms and asthma incidence now includes prospective cohort studies conducted by different researchers in different locations, both within and outside the U.S. with different methods. The CHS provides evidence in a prospective longitudinal cohort study that relates $PM_{2.5}$ and bronchitic symptoms and reports larger associations for within-community effects that are less subject to confounding than between-community effects (McConnell et al., 2003, 049490). Several new studies report similar findings with long-term exposure to PM_{10} in areas where fine particles predominate. In England, an association was seen with an increased prevalence of cough without a cold. Further evidence includes a reduction of respiratory symptoms corresponding to decreasing PM levels in natural experiments in cohorts of Swiss school children (Bayer-Oglesby et al., 2005, 086245) and adults (Schindler et al., 2009, 191950).

New studies examined the relationship between long-term $PM_{2.5}$ exposure and asthma incidence. $PM_{2.5}$ had the strongest modifying effect on the association between lung function with asthma in an analysis of the CHS (Islam et al., 2007, 090697). The loss of protection by high lung function against new onset asthma in high $PM_{2.5}$ communities was observed for all the lung function measures. In the Netherlands, an association with doctor-diagnosed asthma was found in a birth cohort examining the first 4 yr of life (Brauer et al., 2007, 090691) Further, findings from an adult cohort suggest that traffic-related PM_{10} contributes to asthma development and that reductions in PM decrease asthma risk (Kunzli et al., 2009, 191949).

A large proportion of asthma is driven by allergy, and the majority of recent epidemiologic studies examining allergic (or atopic) indicators found positive associations with $PM_{2.5}$ or PM_{10} (Section 7.3.6.1). Limited evidence for PM-mediated allergic responses is provided by toxicological studies of DE and woodsmoke, while effects of gasoline exhaust were attributed to gaseous components (Section 7.3.6.2).

Long-term $PM_{2.5}$ exposure is associated with pulmonary inflammation and oxidative responses. An epidemiologic study found a relationship between $PM_{2.5}$ and increased inflammatory marker eNO among school children (Dales et al., 2008, 156378). Toxicological studies of pulmonary inflammation have demonstrated mixed results, with subchronic DE exposures generating increases and CAPs and wood smoke inducing little or no response (Section 7.3.3.2). The pulmonary inflammation observed with DE was attributable to the particle fraction. Toxicological studies also reported evidence of oxidative responses (Section 7.3.4.1). Adaptation to prolonged DE was observed for some oxidative responses in addition to some allergic and pulmonary function responses (Section 7.3.2.2 and 7.3.6.2).

Additional support for the relationship between long-term $PM_{2.5}$ exposures and respiratory outcomes is provided by pulmonary injury responses observed in toxicological studies (Section 7.3.5.1). Markers of pulmonary injury were increased in rats exposed to DE and gasoline exhaust; and these changes were attributable to PM. Further, lung DNA methylation was observed in the gasoline exhaust study. Histopathological changes have also been reported following exposure to heavily-trafficked urban air and woodsmoke. Findings include nasal and airway mucous cell hyperplasia accompanied by alterations in mucus production which can lead to a loss of mucus–mediated protective functions; exacerbation of protease-induced emphysema; and mast cell infiltration and hypertrophy of alveolar walls. These results provide biological plausibility for adverse respiratory outcomes following long-term PM exposure.

Limited information is available on host defense responses (Section 7.3.7) and respiratory mortality (Section 7.3.8) resulting from $PM_{2.5}$ exposure. Several recent epidemiologic studies suggest a relationship between long-term exposure to $PM_{2.5}$ or PM_{10} and infection in children and infants (Section 7.3.7.1). A few toxicological studies suggest that DE exposure affects systemic immunity, and although impaired bacterial clearance is associated with short-term exposures to DE, neither DE or gasoline exhaust seems to have this effect after longer exposures (Section 7.3.7.2).

In summary, the strongest evidence for a relationship between long-term exposure to $PM_{2.5}$ and respiratory morbidity is provided by epidemiologic studies demonstrating associations with decrements in lung function growth in children and with respiratory symptoms and disease incidence in adults. Mean $PM_{2.5}$ concentrations in these study locations ranged from 13.8 to 30 $\mu g/m^3$ during the study periods. These studies provide evidence for associations in areas where PM is predominantly fine particles. A major challenge to interpreting the results of these studies is that the PM size fractions and concentrations of other air pollutants are often correlated; however, the consistency of findings across different locations supports an independent effect of $PM_{2.5}$. Recent toxicological studies provide support for the associations with $PM_{2.5}$ and decreases in lung function growth in children. Pre- and postnatal exposure to ambient levels of urban particles was found to affect mouse lung development, which provides biological plausibility for the epidemiologic findings. Recent subchronic and chronic toxicological studies also demonstrate altered pulmonary function, mild inflammation, oxidative responses, histopathological changes including mucus cell hyperplasia and enhanced allergic responses in response to CAPs, DE, urban air and woodsmoke and provide further coherence and biological plausibility. Exacerbation of emphysematous lesions was noted in one study involving exposure to urban air in a heavily-trafficked area. **Collectively, the evidence is sufficient to conclude that the relationship between long-term $PM_{2.5}$ exposure and respiratory effects is likely to be causal.**

7.3.9.2. $PM_{10-2.5}$

The 2004 PM AQCD did not report long-term exposure studies for $PM_{10-2.5}$. The only recent study to evaluate long-term exposure to $PM_{10-2.5}$ found positive, but not statistically significant associations with eNO (Dales et al., 2008, 156378). The evidence is **inadequate to determine if a causal relationship exists between long-term $PM_{10-2.5}$ exposures and respiratory effects.**

7.3.9.3. UFPs

The 2004 PM AQCD did not report long-term exposure studies for UFPs. The current evidence for long-term UFP effects is limited to toxicological studies. Generally, subchronic exposure to DE induced pulmonary inflammation, which was in contrast to UF CAPs exposure (Section 7.3.3.2) It appeared that the PM fraction was responsible for the inflammatory response with DE exposure. Long-term exposure to DE also resulted in oxidative and allergic responses, although lung injury was not remarkable (Sections 7.3.4.1 and 7.3.6.2). The evidence is **inadequate to determine if a causal relationship exists between long-term UFP exposures and respiratory effects.**

7.4. Reproductive, Developmental, Prenatal and Neonatal Outcomes

7.4.1. Epidemiologic Studies

This section evaluates and summarizes the scientific evidence on PM and developmental and pregnancy outcomes and infant mortality. Infants and fetal development processes may be particularly vulnerable to PM exposure, and although the physical mechanisms are not fully understood, several hypotheses have been proposed involving direct effects on fetal health, altered placenta function, or indirect effects on the mother's health (Bracken et al., 2003, 156288; Clifton et al., 2001, 156360; Maisonet et al., 2004, 156725; Schatz et al., 1990, 156073; Sram et al., 2005, 087442). Study of these outcomes can be difficult given the need for detailed data and potential residential movement of mothers during pregnancy. Two recent articles have reviewed methodological issues relating to the study of outdoor air pollution and adverse birth outcomes (Ritz and Wilhelm, 2008, 156914; Slama et al., 2008, 156985). Some of the key challenges to interpretation of these study results include the difficulty in assessing exposure as most studies use existing monitoring networks to estimate individual exposure to ambient PM; the inability to control for potential confounders such as other risk factors that affect birth outcomes (e.g., smoking); evaluating the exposure window (e.g., trimester) of importance; and limited evidence on the physiological mechanism of these effects (Ritz and Wilhelm, 2008, 156914; Slama et al., 2008, 156985). Another uncertainty is whether PM effects differ by the child's sex. A review of preterm birth and low birth weight studies found limited indication that effects may differ by gender, however sample size was limited (Ghosh et al., 2007, 091233).

Previous summaries of the association between PM concentrations and pregnancy outcomes and infant mortality were presented in previous PM AQCDs. The 1996 PM AQCD concluded that although few studies had been conducted on the link between PM and infant mortality, the research "suggested an association," particularly for post-neonates (U.S. EPA, 1996, 079380). In the 2004 PM AQCD, additional evidence was available on PM's effect on fetal and early postnatal development and mortality (U.S. EPA, 2004, 056905) and although some studies indicated a relationship between PM and pregnancy outcomes, others did not. Studies identifying associations found that exposure to PM_{10} early during pregnancy (first month of pregnancy) or late in the pregnancy (6 wk prior to birth) were linked with higher risk of preterm birth, including models adjusted for other pollutants, and that $PM_{2.5}$ during the first month of pregnancy was associated with intrauterine growth restriction. However, other work did not identify relationships between PM_{10} exposure and low birth weight. The state of the science at that time, as indicated in the 2004 PM AQCD, was that the research provided mixed results based on studies from multiple countries, and that additional research was required to better understand the impact of PM on pregnancy outcomes and infant mortality. Considering evidence from recent studies discussed below, along with previous AQCD conclusions, epidemiologic studies consistently report associations between PM_{10} and $PM_{2.5}$ exposure and low birth weight and infant mortality, especially during the post-neonatal period. Animal toxicological evidence supports these associations with $PM_{2.5}$, but provides little mechanistic information or biological plausibility. Information on the ambient concentrations of PM_{10} and $PM_{2.5}$ in these study locations can be found in Table 7-5.

7.4.1.1. Low Birth Weight

A large number of studies have investigated exposure to ambient PM and low birth weight at term, including a U.S. national study, as well as two studies in the northeast U.S., and four in California. Parker and Woodruff (2008, 156846) linked U.S. birth records for singletons delivered at 40-wk gestation in 2001-2003 during the months of March, June, September and December to quarterly estimates of PM exposure by county of residence and month of birth. They found an association between $PM_{10-2.5}$ and birthweight (-13 g [95% CI: -18.3 to -7.6]) per 10 $\mu g/m^3$ increase), but no such association for $PM_{2.5}$.

Maisonet et al. (2001, 016624) analyzed 89,557 births (1994-96) in six northeastern cities (Boston and Springfield MA; Hartford CT; Philadelphia and Pittsburgh PA; and Washington DC). Each city had three PM_{10} monitors measuring every sixth day. Results from multiple monitors were averaged in each city. Exposure was determined for each trimester of pregnancy and categorized by quartiles (<25, 25-30, 31-35, 36-43 $\mu g/m^3$) and 95th percentile (>43$\mu g/m^3$). There was no increased risk for low birth weight at term associated with PM_{10} exposure during any trimester of pregnancy. When birth weight was considered as a continuous outcome, exposure to PM_{10} was not associated with a reduction in mean birth weight.

In contrast, Bell et al. (2007, 093256) reported positive associations for both $PM_{2.5}$ and PM_{10} with birth weight in a study of births (n = 358,504) in Connecticut and Massachusetts (1999-2002). Birth data indicated county, not street address or ZIP code, so women were assigned exposure based on county residence at delivery. The difference in birth weight per 10 $\mu g/m^3$ associated with $PM_{2.5}$ was -66.8 (95% CI: -77.7 to -55.9) g. For PM_{10} it was -11.1 (95% CI: -15.0 to -7.2) g. The increased risk for low birth weight was OR = 1.054 (95% CI: 1.022-1.087) for $PM_{2.5}$ and OR = 1.027 (95% CI: 0.991-1.064) for PM_{10}, based on average exposure during pregnancy. Reductions in birth weight were also associated with third trimester exposure to PM_{10} and second and third trimester exposure to $PM_{2.5}$. Comparing this study to Maisonet et al. (2001, 016624), a larger sample size was able to detect a small increase in risk. In addition, birth weight was reduced more by exposure to $PM_{2.5}$ than by exposure to PM_{10}. Measured $PM_{2.5}$ concentrations were not available in the earlier study.

The Children's Health Study is a population based cohort of children living in 12 southern California communities, selected on the basis of differing levels of air pollution (Salam et al., 2005, 087885), as previously discussed in Section 7.3. The children in grades 4, 7 and 10 were recruited through schools. A subset of this cohort (n = 6,259) were born in California from 1975-1987. Of these, birth certificates were located for 4,842, including 3,901 infants born at term and 72 cases of low birth weight at term. Using the mother's ZIP code at the time of birth, exposure was determined by inverse distance weighting of up to three PM_{10} monitors within 50 km of the ZIP code centroid. If there was a PM_{10} monitor within 5 km of the ZIP code centroid (40% of data), exposure from that monitor was used. Exposure was calculated for the entire pregnancy, and for each trimester of pregnancy. A 10 $\mu g/m^3$ increase in PM_{10} during the third trimester reduced mean birth weight -10.9 g (95% CI: -21.1 to -0.6) in single pollutant models, but became non-significant in copollutant models controlling for the effects of O_3. Increased risks of low birth weight (<2,500 g) were not statistically significant (OR = 1.3 [95% CI: 0.9-1.9]). A strength of this study was the cohort data available included information on SES and smoking during pregnancy. A limitation is the assignment of exposure based on monitoring stations up to 50 km distant; this may have introduced substantial exposure misclassification obscuring some associations.

Table 7-5. Characterization of ambient PM concentrations from studies of reproductive, developmental, prenatal and neonatal outcomes and long-term exposure.

Study	Location	Mean Annual Concentration (μg/m³)	Upper Percentile Concentrations (μg/m³)
$PM_{2.5}$			
Basu et al. (2004, 087895)	CA	Range of means across sites: 14.5-18.2 Avg of means across sites: 16.2	Max: 26.3-34.1
Bell et al. (2007, 091059)	CT & MA	22.3	
Brauer et al. (2008, 156292)	Vancouver, Canada	5.3	Max: 37.0
Huynh et al. (2006, 091240)	CA	Range of means across trimesters: 17.5-18.8 Avg of means across trimesters: 18.2	
Jalaludin et al. (2007, 156601)	Sydney, Australia	9.0	
Liu (2007, 090428)	Multicity, Canada	12.2	75th: 15
Loomis et al. (1999, 087288)	Mexico City	27.4	Max: 85
Mannes et al. (2005, 087895)	Sydney, Australia	9.4	75th: 11.2; Max: 82.1
Parker et al. (2005, 087462)	CA	15.4	
Ritz et al. (2007, 096146)	Los Angeles, CA	20.0	
Wilhelm and Ritz (2005, 088868)	Los Angeles, CA	21.0	Max: 38.9-46.5
Woodruff et al. (2006, 088758)	CA	19.2[a]	75th: 22.7
Woodruff et al. (2008, 096386)	U.S.	Range of means across effects: 14.5-14.9[a] Avg of means across effects: 14.8[a]	75th: 18.5-18.7
$PM_{10-2.5}$			
Parker et al. (2008, 156013)	U.S.	13.2	75th: 17.5
PM_{10}			
Bell et al. (2007, 093256)	CT & MA	22.3	
Brauer et al. (2008, 156292)	Vancouver, Canada	12.7	Max: 35.4
Chen et al. (2002, 024945)[b]	Washoe County, NV	31.53	75th: 39.35; Max: 157.32
Gilboa et al. (2005, 087892)	TX	23.6[a]	75th: 29
Ha et al. (2003, 042552)	Seoul, South Korea	69.2	75th: 87.7; Max: 245.4
Hansen et al. (2006, 089818)	Brisbane, Australia	19.5	Max: 171.7
Hansen et al. (2007, 090703)	Brisbane, Australia	19.6	75th: 22.7; Max: 171.7
Jalaludin et al. (2007, 156601)	Sydney, Australia	16.3	
Kim et al. (2007, 156542)	Seoul, Korea	Range of means across time: 88.7-89.7 Avg of means across time: 89.2	
Lee et al. (2003, 043202)	Seoul, Korea	71.1	75th: 89.3; Max: 236.9
Leem et al. (2006, 089826)	Incheon, Korea	53.8[a]	75th: 64.6; Max: 106.39
Lipfert et al. (2003, 094103)	U.S.	33.1	Max: 59
Maisonet et al. (2001, 016624)	NE U.S.	31.0[a]	75th: 36.1; Max: 46.5
Mannes et al. (2005, 087895)	Sydney, Australia	16.8	75th: 19.9; Max: 104.0
Pereira et al. (1998, 007264)	Sao Paulo, Brazil	65.04	Max: 192.8
Ritz et al. (2000, 012058)	CA	49.3	Max: 178.8
Ritz et al. (2006, 088813)	CA	46.3	Max: 83.5

Study	Location	Mean Annual Concentration (μg/m³)	Upper Percentile Concentrations (μg/m³)
Rogers and Dunlop (2006, 091232)	GA	3.75	75th: 15.07
Romieu et al. (2004, 093074)	Ciudad Juarez, Mexico	33.0-45.9	
Sagiv et al. (2005, 087468)	PA	Range of means across time: 25.3-27.1 Avg of means across time: 26.2	Max: 88.9-156.3
Salam et al. (2005, 087885)	CA	Range of means across trimesters: 45.4-46.6 Avg of means across trimesters: 45.8	
Suh et al. (2008, 192977)	Seoul, Korea	Range of means across trimesters: 54.6-61.1 Avg of means across trimesters: 58.27	75th: 62.8-67.8 Max: 85.1-107.86
Tsai et al. (2006, 090709)	Kaohsiung, Taiwan	81.5	75th: 111.5; Max: 232.0
Wilhelm and Ritz (2005, 088668)	Los Angeles, CA	38.1	Max: 74.8-103.7
Woodruff et al. (2008, 096386)	U.S.	Range of means across effects: 28.6-29.6[a] Avg of means across effects: 29.1[a]	75th: 33.8-36.5
Yang et al. (2006, 090760)	Taipei, Taiwan	53.2	75th: 64.9; Max: 234.9

[a]Median concentration

Parker et al. (2005, 087462) examined births in California within 5 miles of a monitoring station (n = 18,247). Only infants born at 40 wk gestation were included. Thus all infants were the same gestational age, and had been exposed in the same year. Exposure to $PM_{2.5}$ in quartiles (<11.9, 11.9-13.9, 14.0-18.4, >18.4) was associated with decrements in birth weight. Infants exposed to >13.9 μg/m³ experienced reductions in birth weight (third quartile -13.7 g (95% CI: -34.2 to 6.9), fourth quartile -36.1 g (95% CI: -55.8 to -16.5). These are larger reductions than have been seen in some other studies. However, this study reduced misclassification by including only women living within 5 miles of a monitoring station, and only included births at 40 wk gestation. Reducing misclassification should lead to a stronger association, if the association is causal.

The effects of spatial variation in exposure were also investigated by Wilhelm and Ritz (2005, 088668). Their study included all women living in ZIP codes where 60% of the ZIP code was within two miles of a monitoring station in the Southern California Basin, and women with known addresses in Los Angeles County within 4 miles of a monitoring station. Exposure to average PM_{10} in the third trimester was analyzed for increased risk of low birth weight at term (≥ 37-wk gestation). Analysis at the ZIP code level did not detect increased risk (per 10 μg/m³ PM_{10}, OR = 1.03 [95% CI: 0.97-1.09]). However the analysis based on geocoded addresses indicated that increasing exposure to PM_{10} was associated with increased risk of low birth weight for women living within 1 mile of the station where PM_{10} was measured. For these women (n = 247 cases, 10,981 non-cases), each 10 μg/m³ increase in PM_{10} was associated with a 22% increase in risk of term low birth weight (OR = 1.22 [95% CI: 1.05-1.41]). In the categorical analysis, exposure to PM_{10} >44.4 μg/m³ was associated with a 48% increase in risk (OR = 1.48 [95% CI: 1.00-2.19]). Increased risk of low birth weight also was associated with exposure to CO in single pollutant models. However, when multipollutant models were considered, the effects of CO were attenuated but the effects of PM_{10} increased. Controlling for CO, NO_2, and O_3, each 10 μg/m³ increase in exposure to PM_{10} increased risk of low birth weight 36% (OR = 1.36 [95% CI: 1.12-1.65]).

Spatial variation in $PM_{2.5}$ exposure was investigated by Basu et al. (2004, 087896). They included only mothers who lived within 5 miles of a $PM_{2.5}$ monitor and within a California county with at least 1 monitor. To minimize potential confounding, they included only white (n = 8,597) or Hispanic (n = 8,114) women, who were married, between 20 and 30 yr of age, completed at least high school and were having their first child. Consistently, $PM_{2.5}$ exposure measured by the county monitor was more strongly associated with reductions in birth weight than exposure measured by the neighborhood monitor. The results were replicated in both the white and the Hispanic samples. Reductions in birth weight ranged from 15.2 to 43.5 g per 10 μg/m³ increase in $PM_{2.5}$.

In the remaining U.S. study, Chen et al. (2002, 024945) analyzed 33,859 birth certificates of residents of Washoe County in northern Nevada (1991-1999). There were four sites monitoring PM_{10} during the study period, it appears (not stated) that exposure was averaged over the county. A

10 μg/m^3 increase in exposure to PM_{10} during the third trimester of pregnancy was associated with an 11 g reduction in birth weight (95% CI: -2.3 to -19.8). Effects on risk of low birth weight were not statistically significant. For exposure in the third trimester of 19.77 to 44.74 μg/m^3 compared to <19.74 μg/m^3 the odds ratio for low birth weight was 1.05 (95% CI: 0.81-1.36). Comparing exposure >44.74 to the same reference category, the odds ratio was 1.10 (95% CI: 0.71-1.71). Misclassification of exposure may have occurred when exposure was averaged over a large geographic area (16,968 km^2).

Recent international studies investigating effects of particles on low birth weight include one in Munich (Slama et al., 2007, 093216), two in Canada (Brauer et al., 2008, 156292; Dugandzic et al., 2006, 088681), two in Australia (Hansen et al., 2007, 090703; Mannes et al., 2005, 087895), two in Taiwan (Lin et al., 2004, 089827; Yang et al., 2003, 087886) one in Korea (Ha et al., 2003, 042552) and two in Sao Paulo, Brazil (Gouveia et al., 2004, 055613; Medeiros and Gouveia, 2005, 089824). The majority of these studies found that PM concentrations were associated with low birth weight, though two studies (Hansen et al., 2007, 090703; Lin et al., 2004, 089827) found no associations. The effect estimates were similar in magnitude to those reported in the U.S. studies.

Considerations in Interpreting Results of Low Birth Weight Studies

Studies included subjects at distances from monitoring stations varying from as close as 1 mile or 2 km, to as far as 50 km or the size of the county. Studies that only included subjects living within a short distance (1 mile, 2 km) of the monitoring station (thus likely reducing exposure measurement error) were more likely to find that PM exposure was associated with increased risk of low birth weight. However, Basu et al. (2004, 087896) reported a stronger association between $PM_{2.5}$ exposure and birth weight when exposure was estimated based on the county monitor, rather than the monitor within 5 miles of the residence. They suggest that county level exposure may be more representative of where women spend their time, including not only home, but also other time spent away from home. Other pollutants also appeared to influence the risk associated with particle exposure. In one study, exposure to PM_{10} in a single pollutant model reduced birth weight by 11 g, but became non-significant in copollutant models with O_3 (Salam et al., 2005, 087885). In another study the risk associated with PM_{10} exposure increased from 22% to 36% when other pollutants were included in the model (Wilhelm and Ritz, 2005, 088668). All but one study in the U.S. found some association between particle exposure and reduced birth weight (Maisonet et al., 2001, 016624). The results of international studies were inconsistent. This might be related to the chemical composition of particles in the U.S., or to differences in the pollutant mixture. Studies with null results must be interpreted with caution when the comparison groups have significant exposure. This was certainly the situation in studies in Taiwan and Korea (Lee et al., 2003, 043202; Lin et al., 2004, 089827; Yang et al., 2003, 087886). Differences in geographical locations, study samples and linkage decisions may contribute to the diverse findings in the literature on the association between PM and birthweight, even within the U.S. (Parker and Woodruff, 2008, 156846).

7.4.1.2. Preterm Birth

A potential association of exposure to airborne particles and preterm birth has been investigated in numerous epidemiologic studies, including some conducted in the U.S. and others in foreign countries. Three U.S. studies have been carried out by the same group of investigators in California.

A natural experiment occurred when an open-hearth steel mill in Utah Valley was closed from August 1986 through September 1987. Parker et al. (2008, 156013) compared birth outcomes for Utah mothers within and outside of the Utah Valley, before, during, and after the mill closure. They report that mothers who were pregnant around the time of the closure of the mill were less likely to deliver prematurely than mothers who were pregnant before or after. The strongest effect estimates were observed for exposure during the second trimester (14% decrease in risk of preterm birth during mill closure). Preterm birth outside of the Utah Valley did not change during the time of the mill closure.

In 2000, Ritz et al. (2000, 012068) published the first study investigating the association of preterm birth with PM in the U.S. The study population was women living in the southern California Basin. There were eight monitoring stations measuring PM_{10} every 6th day during the study period.

Birth certificates (1989-1993) were analyzed for women living in ZIP codes within 2 miles of a monitoring station. Women with multiple gestations, chronic disease prior to pregnancy and women who delivered by cesarean section were excluded resulting in a study population of 48,904 women. The risk of preterm birth increased by 4% (RR = 1.04 [95% CI: 1.02-1.6]) per 10 $\mu g/m^3$ increase in PM_{10} averaged in the 6 wk before birth. Exposure to PM_{10} in the first month of pregnancy resulted in a 3% increase in risk (RR = 1.03 [95% CI: 1.01-1.05]). These results were robust in multipollutant models.

Wilhelm and Ritz (2005, 088668) reinvestigated this association among women in the same area in 2005, when air pollution had declined from a mean level near 50 $\mu g/m^3$ to a mean level near 40 $\mu g/m^3$. Birth certificate data from 1994-2000 was analyzed for women living in ZIP codes within 2 miles of a monitoring station, or with addresses within 5 miles of a monitoring station. No significant effects of exposure to PM_{10} were reported. Exposure to $PM_{2.5}$ 6 wk before birth resulted in an increase in preterm birth (RR = 1.19 [95% CI: 1.02-1.40]) for the highest quartile of exposure ($PM_{2.5}$ >24.3 $\mu g/m^3$). Using a continuous measure of $PM_{2.5}$, there was a 10% increase in risk for each 10 $\mu g/m^3$ increase in $PM_{2.5}$ (RR = 1.10 [95% CI: 1.00-1.21]).

There have been two major criticisms of air pollution studies using birth certificate data. First, that birth certificates only indicate the address at birth and the exposure of women who moved during pregnancy may be misclassified; second, that information about some important confounders may not be available (e.g., smoking). To obtain more precise information about these variables, Ritz et al. (2007, 096146) conducted a case-control study nested within a cohort of birth certificates (Jan 2003-Dec 2003) in Los Angeles County. Births to women residing in ZIP codes (n = 24) close to monitoring stations or major population centers or roadways (n = 87) were eligible (n = 58,316 births). All cases of low birth weight or preterm birth and an equal number of randomly sampled controls in the 24 ZIP codes close to monitors were selected. In the other 87 ZIP codes, 30% of cases and an equal number of controls were randomly sampled. Of 6,374 women selected for the case control study, 2,543 (40%) were interviewed. The association of preterm birth with exposure to $PM_{2.5}$ differed between women responding to the survey and women who did not respond. Among responders, exposure to each 10 $\mu g/m^3$ increase in $PM_{2.5}$ concentration in the first trimester increased risk to preterm birth by 23% (RR = 1.23 [95% CI: 1.02-1.48]). There was no increase in risk among non-responders (RR = 0.95 [95% CI: 0.82-1.10]), or in the entire birth cohort (RR = 1.00 [95% CI: 0.94-1.07]).

An additional case control study of preterm birth and $PM_{2.5}$ exposure (Huynh et al., 2006, 091240) used California birth certificate data. Singleton preterm infants (24-36-wk gestation) born in California (1999-2000) whose mothers lived within 5 miles of a $PM_{2.5}$ monitor were eligible. Each of these 10,673 preterm infants were matched to three term (39- to 44-wk gestation) controls (having a last menstrual period within 2 wk of the case infant), resulting in a study population of 42,692. Controlling for maternal race/ethnicity, education, marital status, parity and CO exposure, exposure to $PM_{2.5}$ >17.7 $\mu g/m^3$ increased the risk of preterm birth by 14% (OR = 1.14 [95% CI: 1.07-1.23]). Averaging $PM_{2.5}$ exposure over the first month of pregnancy, the last 2 wk before birth, or the entire pregnancy did not substantially change the risk estimate.

Two additional studies of preterm birth and exposure to particulate air pollution have been conducted in the U.S. Each has used a unique methodology. Sagiv et al. (2005, 087468) used time series to analyze births in four Pennsylvania counties between January 1997 and December 2001. In this analysis, exposure to PM_{10} is compared to the rate of preterm births each day. Both acute exposure (on the day of birth) and longer term exposure (average exposure for the preceding 6 wk) were considered in the analysis. An advantage of this analysis is that days, rather than individuals are compared, so confounding by individual risk factors is minimized. For exposure averaged over the 6 wk prior to birth, there was an increase in risk (RR = 1.07 [95% CI: 0.98-1.18]), which persisted for acute exposure with a 2-day lag (RR = 1.10 [95% CI: 1.00-1.21]) and 5-day lag (RR = 1.07 95% CI: 0.98-1.18]).

Rogers and Dunlop (2006, 091232) examined exposure to particles and risk of delivery of an infant weighing less than 1,500 g (all of which were preterm) from 24 counties in Georgia. The study included 69 preterm, small for gestational age (SGA) infants, 59 preterm appropriate for gestational age (AGA) infants and 197 term AGA controls. Exposure was estimated using an environmental transport model that considered PM_{10} emissions from 32 geographically located industrial point sources, meteorological factors, and geographic location of the birth home. Exposure was categorized by quartiles. Comparing women who delivered a preterm AGA infant to those who

delivered a term AGA infant, exposure to PM_{10}>15.07 $\mu g/m^3$ tripled the risk (OR = 3.68 [95% CI: 1.44-9.44]).

Brauer et al. (2008, 156292) evaluated the impacts of $PM_{2.5}$ on preterm birth using spatiotemporal exposure metrics in Vancouver, Canada. The authors found similar results when they used a land-use regression model or inverse distance weighting as the exposure metric. For preterm births <37 wk, they reported an OR of 1.06 (95% CI: 1.01-1.11), and for preterm births <35 wk the OR increased to 1.12 (95% CI: 1.02-1.24). There were no consistent trends for early or late gestational period to be more strongly associated with preterm births.

Suh et al. (2008, 192077) conducted a study to determine if the effects of exposure to PM_{10} during pregnancy on preterm delivery are modified by maternal polymorphisms in metabolic genes. They analyzed the effects of the gene-environment interaction between the GSTM1, GSTT1, CYP1a1-T6235C and -1462V polymorphisms and exposure to PM_{10} during pregnancy on preterm birth in a case-control study in Seoul, Korea. PM_{10} concentration ≥ 75th percentile alone was significant in the third trimester of pregnancy (OR = 2.33 [95% CI: 1.33-4.80]), but not in the first or second trimester. The risk of preterm delivery conferred by the GSTM1 null genotype was increased, and the highest risk was found during the third trimester of pregnancy (OR = 2.58 [95% CI: 1.34-4.97]). There were no statistical associations with the GSTT1 or CYP1A1 genotypes. When the gene-environment interaction was analyzed, the risk for preterm birth was substantially higher for women who carried the GSTM1 null genotype and were exposed to high levels of PM_{10} (≥ 75th percentile) than for those who carried the GSTM1 positive genotype but were only exposed to low levels of PM_{10} (<75th percentile) during the third trimester of pregnancy (OR = 6.22, 95% CI: 2.14-18.08).

In Incheon, Korea, Leem et al. (2006, 089828) estimated PM_{10} exposure spatially as well as temporally. Exposure was based on 26 monitors and kriging was used to determine exposure for 120 dongs (administrative districts, mean area 7.82 km^2, median area 1.42 km^3). The sample included 52,113 births, from 2001-2002. PM_{10} was very weakly correlated with other pollutants. Exposure was compared in quartiles for the first and third trimester of pregnancy. In the first trimester, relative risks for the second, third and fourth quartiles were RR = 1.14 (95% CI: 0.97-1.34), RR = 1.07 (95% CI: 0.94-1.37), and RR = 1.24 (95% CI: 1.09-1.41), respectively. Exposure to PM_{10} in quartile one (reference group) was 26.9-45.9 $\mu g/m^3$; fourth quartile exposure equaled 64.6-106.4 $\mu g/m^3$. The p-value for trend was 0.02. Exposure in the third trimester was not related to preterm birth, however no information was provided to determine how exposure in the third trimester was adjusted for women who delivered preterm.

Two studies investigating risks of preterm birth related to particle exposure have been reported from Australia. In Brisbane, Hansen et al. (2006, 089818) studied 28,200 births (2000-2003) in an area of low PM_{10} concentrations. Exposure to an interquartile range increase in PM_{10} exposure in the first trimester resulted in a 15% increased risk of preterm birth (OR = 1.15 [95% CI: 1.06-1.25]). This result was strongly influenced by the effect of PM_{10} exposure in the first month of pregnancy (OR = 1.19 [95% CI: 1.13-1.26]). PM_{10} was correlated with O_3 (r = 0.77) in this study and O_3 also increased risk in the first trimester. No effects were associated with exposure to PM_{10} in the third trimester.

In Sydney, associations between exposure to particles and preterm birth varied by season. Jalaludin et al. (2007, 156601) obtained information on all births in metropolitan Sydney (1998-2000). Exposure to $PM_{2.5}$ in the 3 mo preceding birth was associated with an increased risk of preterm birth (OR = 1.11 [95% CI: 1.04-1.19]). Additional effects were dependent on season of conception. Both PM_{10} (OR = 1.3 [95% CI: 1.2-1.5]) and $PM_{2.5}$ (OR = 1.4 [95% CI: 1.3-1.6]) were associated with increased risk for conceptions in the winter. Conceptions in summer were associated with reductions in risk (PM_{10} OR = 0.91 [95% CI: 0.88-0.93]) ($PM_{2.5}$ OR = 0.87 [95% CI: 0.84-0.92]). Due to both positive and negative findings, the authors recommend caution in interpreting their results.

Considerations in Analyzing Environmental Exposures and Preterm Birth

A major issue in studying environmental exposures and preterm birth is selecting the relevant exposure period, since the biological mechanisms leading to preterm birth and the critical periods of vulnerability are poorly understood (Bobak, 2000, 011448). Exposures proximate to the birth may be most relevant if exposure causes an acute effect. However, exposure occurring in early gestation

birth weight for gestational age. In this study there was a statistically significant effect of exposure to both PM_{10} (OR = 1.10 [95% CI: 1.00-1.48], per 10 $\mu g/m^3$ increase) and $PM_{2.5}$ (OR = 1.34 [95% CI: 1.10-1.63], per 10 $\mu g/m^3$ increase) for exposure during the second trimester. When analysis was restricted to births within 5 km of the monitoring station, the association for PM_{10} became slightly stronger (OR = 1.22 [95% CI: 1.10-1.34]). Exposure during other trimesters of pregnancy was not associated with IUGR.

In Brisbane, Hansen et al. (2007, 090703) examined head circumference (HC), crown heel length (CHL) and risk of SGA, defined as less than the tenth percentile of weight for gestational age and gender based on an Australian national standard. There was no consistent relationship between PM_{10} exposure and SGA, HC or CHL in any trimester of pregnancy. PM_{10} exposure was determined by averaging values from the five monitoring stations. Due to the sample size and limited number of monitoring stations, it was not possible to analyze the data for women living within 5 km of a monitoring station, as was done in Sydney.

In Canada, Liu et al. (2007, 090429) investigated the effect of $PM_{2.5}$ exposure on fetal growth in three cities, Calgary, Edmonton and Montreal. IUGR was defined as birth weight below the tenth percentile, by sex and gestational week (37-42) for all singleton live births in Canada between 1986 and 2000. Models were adjusted for maternal age, parity, infant sex, season of birth, city of residence, and year of birth. A 10 $\mu g/m^3$ increase in $PM_{2.5}$ was associated with an increased risk for IUGR (OR = 1.07 [95% CI: 1.03-1.10]) in the first trimester, and similar risks were associated with exposure in the second or third trimesters. The effect of $PM_{2.5}$ was reduced in multipollutant models including CO and NO_2.

Brauer et al. (2008, 156292) observed consistent increased risks of SGA for $PM_{2.5}$, PM_{10}, NO_2, NO and CO in Vancouver, Canada (20% increase in risk in $PM_{2.5}$ and PM_{10} per 10 $\mu g/m^3$ increase). The effects were similar for exposure estimates based on nearest monitor, inverse distance weighting, and land-use regression modeling. ORs for early or late pregnancy exposure windows were remarkably similar to those for the full duration of pregnancy.

7.4.1.4. Birth Defects

Four recent studies examined PM and birth defects. The Seoul, Korea study discussed above also considered congenital anomalies, defined as a defect in the child's body structure (Kim et al., 2007, 156642). PM_{10} levels were associated with higher risk of birth defects for the second trimester, with a 16% (95% CI: 0-34) increase in risk per 10 $\mu g/m^3$ in PM_{10}.

Two U.S. studies examined air pollution and risk of birth defects. Data were collected from the California Birth Defects Monitoring Program for four counties in Southern California (Los Angeles, Riverside, San Bernardino, and Orange) for the period 1987-1993, although each county included a subset of this period (Ritz et al., 2002, 023227). Cases (i.e., infants with birth defects) were identified as live birth infants and fetal deaths from 20-wk gestation to 1 yr post-birth, with isolated, multiple, syndrome, or chromosomal cardiac or orofacial cleft defects. Cases were restricted to those with registry data for gestational age and residence ZIP code, and those with residences <10 miles from an air pollution monitor. Six types of categories were included: aortic defects; atrium and atrium septum defects; endocrinal and mitral value defects; pulmonary artery and valve defects; conotruncal defects; and ventricular septal defects not part of the conotruncal category. PM_{10} measurements were available every 6 days. While results indicated increased risk of birth defects for higher levels of CO or O_3, the authors determined that results for PM_{10} were inconclusive, finding no consistent trend of effect after adjustment for CO and O_3.

The other U.S. study examined birth defects through a case-control design in seven Texas counties for the period 1997-2000 (Gilboa et al., 2005, 087892). Births were excluded for parents <18 yr and several non-air pollution risk factors known to be associated with birth defects (e.g., maternal diabetes, holoprosencephaly in addition to oral clef). Comparison of the highest ($\geq$ 29.0 $\mu g/m^3$) and lowest (<19.521 $\mu g/m^3$) quartiles of PM_{10} for exposure defined as the third to eighth week of pregnancy generated an OR of 2.27 (95% CI: 1.43-3.60) for risk of isolated atrial septal defects and 1.26 (95% CI: 1.03-1.55) for individual atrial septal defects. Including other pollutants (CO, NO_2, O_3, SO_2) in the model did not greatly alter results; numerical results for copollutant analysis were not provided. Strong evidence was not observed for a relationship between PM_{10} and the other birth defect categories. Review articles have concluded that the scientific literature is not sufficient to conclude a relationship between air pollution and birth defects (Sram et al., 2005, 087442).

birth weight for gestational age. In this study there was a statistically significant effect of exposure to both PM_{10} (OR = 1.10 [95% CI: 1.00-1.48], per 10 $\mu g/m^3$ increase) and $PM_{2.5}$ (OR = 1.34 [95% CI: 1.10-1.63], per 10 $\mu g/m^3$ increase) for exposure during the second trimester. When analysis was restricted to births within 5 km of the monitoring station, the association for PM_{10} became slightly stronger (OR = 1.22 [95% CI: 1.10-1.34]). Exposure during other trimesters of pregnancy was not associated with IUGR.

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A recent study of oral clefts conducted in Taiwan found no association between this birth defect and concentrations of PM_{10} during the first or second gestational month (Hwang and Jaakkola, 2008, 193794). This population-based case-control study included 653 cases and a random sample of 6,530 controls born in Taiwan between 2001 and 2003.

7.4.1.5. Infant Mortality

Many studies have identified strong associations between exposure to particles and increased risk of mortality in adults or the general population, including for short- and long-term exposure (Sections 6.5 and 7.6). Less evidence is available for the potential impact on infant mortality, although studies have been conducted in several countries. The results of these infant mortality studies are presented here with the other reproductive and developmental outcomes because it is likely that in vitro exposures contribute to this outcome. Both long-term and short-term exposure studies of infant mortality are included in this section. Results on PM and infant mortality includes a range of findings, with some studies finding associations and many statistically non-significant or null effects. Yet, more consistency is observed when results are divided into the type of health outcome based on the age of infant and cause of death.

An important question regarding the association between PM and infant mortality is the critical window of exposure during development for which infants are susceptible. Several age intervals have been explored: neonatal (<1 mo); infants (<1 yr); and postneonatal (1 mo-1 yr). Within these various age categories, multiple causes of deaths have been investigated, particularly total deaths and respiratory-related deaths. The studies reflect a variety of study designs, particle size ranges, exposure periods, regions, and adjustment for confounders.

Stillbirth

Only one study of stillbirths and PM was identified. A prospective cohort of pregnant women in Seoul, Korea from 2001 to 2004 was examined with respect to exposure to PM_{10} (Kim et al., 2007, 156642). Gestational age was estimated by the last menstrual period or by ultrasound. Whereas many of the previously discussed studies of PM and pregnancy outcomes were based on national registries, this study examined medical records and gathered individual information through interviews on socioeconomic condition, medical history, pregnancy complications, smoking, second-hand smoke exposure, and alcohol use. Mother's exposure to PM_{10} was based on residence for each month of pregnancy, each trimester defined as a three month period, and the 6 wk prior to death. Exposure was assigned by the nearest monitor. A 10 $\mu g/m^3$ increase in PM_{10} in the third trimester was associated with an 8% (95% CI: 2-14) increase in risk of stillbirth.

In São Paulo, Brazil, Poisson regression of stillbirth counts for the period 1991-1992 found that a 10 $\mu g/m^3$ increase in PM_{10} was associated with a 0.8% increase in stillbirth rates (Pereira et al., 1998, 007264). When other pollutants (NO_2, SO_2, CO, O_3) were included simultaneously in the model, the association did not remain. Stillbirths were defined as fetal loss at >28 wk of pregnancy age, weight >1,000 g, or length of fetus >35 cm.

Neonatal Mortality and Neonatal Respiratory Mortality, <1 Month

Studies on PM and neonatal mortality (<1 month) included a time-series analysis of PM_{10} for 4 yr of data (1998-2000) for São Paulo, Brazil (Lin et al., 2004, 095787). The analysis used daily counts of deaths from government registries and adjusted for temporal trend, day of the week, weather, and holidays. Findings indicated that a 10 $\mu g/m^3$ increase in PM_{10} was associated with a 1.71% (95% CI: 0.31-3.32) increase in risk of neonatal death.

A case-crossover study of 11 yr (1989-2000) in Southern California did not find an association between PM_{10} and neonatal deaths (Ritz et al., 2006, 089819). Quantitative results were not provided. The authors considered adjustment for season, county, parity, gender, prenatal care, and maternal age, education, and race/ethnicity.

These results add to previous work on PM and neonatal death, including studies identifying higher risk of neonatal mortality with higher TSP in the Czech Republic in an ecological analysis (Bobak and Leon, 1992, 044415) and case-crossover study (Bobak and Leon, 1999, 007678), and a

Poisson model study in Kagoshima City, Japan (Shinkura et al., 1999, 090050). An ecological study evaluated U.S. PM_{10} data for the year 1990 using long-term pollution levels in 180 U.S. counties (Lipfert et al., 2000, 004103). Analysis considered birth weight, sex, month of birth, location by state and county, prenatal care, and mother's race, age, educational level, marital status, and smoking status. County-level variables were included for socioeconomic status, altitude, and climate. Results indicate a 13.1% increase in neonatal mortality (95% CI: 4.4-22.6) per 10 $\mu g/m^3$ PM_{10} for non-low birth weight infants. Statistically significant associations were also observed considering all infants or low birth weight infants. However, higher levels of SO_2 were associated with lower risk of infant mortality. When sulfate and an estimate of non-sulfate particles were included in the regression simultaneously, associations were observed with non-sulfate particles and an inverse relationship with sulfate particles. Respiratory neonatal mortality was not associated with higher TSP in the Czech Republic case-control study (Bobak and Leon, 1999, 007678).

Infant Mortality and Infant Respiratory Mortality, <1Year

A literature search did not reveal new studies on PM and infant mortality (<1 year) since the previous PM AQCD. Previously conducted studies include a case-control study that reported associations between infant mortality and TSP levels over the period between birth and death for infants in the Czech Republic (Bobak and Leon, 1999, 007678). An ecological study evaluated U.S. PM_{10} data for the year 1990 using long-term pollution levels in 180 U.S. counties (Lipfert et al., 2000, 004103). The authors found a 9.64% (95% CI: 4.60-14.9) increase in risk of infant mortality for non-low birth weight infants per 10 $\mu g/m^3$ increase in PM_{10}, a 13.4% (95% CI: -10.3 to 43.5) increase in non-low birth weight respiratory-disease related deaths (ICD 9 460-519) and a 19.5% (95% CI: 0.07-42.8) increase in all non-low birth weight respiratory-related infant deaths (ICD 9 460-519, 769, 770).

Postneonatal Mortality and Postneonatal Respiratory Mortality, 1 Month–1 Year

Several studies have been conducted on PM and postneonatal mortality since the previous PM AQCD, including three from the U.S., one from Mexico, and three from Asia. Two case-control studies examined the risk of PM to postneonatal death in California. Research focused on Southern California for the period 1989-2000 linked birth and death certificates and considered PM_{10} 2 mo prior to death with adjustment for prenatal care, gender, parity, county, season, and mother's age, race/ethnicity, and education (Ritz et al., 2006, 089819). As previously noted, this study did not find an association between PM_{10} and neonatal mortality (<1 month), however an association was observed for post-neonatal mortality, with a 10 $\mu g/m^3$ increase in PM_{10} associated with a 4% (95% CI: 1-6) increase in risk. The exposure period of 2 wk before death was also considered, producing effect estimates of 5% (95% CI: 1-10) for the same PM_{10} increment. Even larger effect estimates were observed for those who died at ages 4-12 mo. When CO, NO_2, and O_3 were simultaneously included with PM_{10} in the model, the central estimate reduced to 2% for the 2-wk exposure period and 4% for the 2-mo exposure period, and both estimates lost statistical significance. The other case-control study of California considered $PM_{2.5}$ from 1999 to 2000 for infants born to mothers within five miles of a $PM_{2.5}$ monitoring station (Woodruff et al., 2006, 088758). Infants who died during the postneonatal period were matched to infants with date of birth within 2 wk and birth weight category. Exposure was estimated from the time of birth to death. Models considered parity and maternal race, education, age, and martial status. A 10 $\mu g/m^3$ increase in $PM_{2.5}$ was associated with a 7% (95% CI: -7 to 24) increase in postneonatal death

County-level PM_{10} and $PM_{2.5}$ for the first 2 mo of life for births in urban U.S. counties ($\geq$ 250,000 residents) from 1999 to 2002 were evaluated in relation to postneonatal mortality with GEE models (Woodruff et al., 2008, 098386). Births were restricted to singleton births with gestational age $\leq$ 44 wk, same county of residence at birth and death, and non-missing data on birth order, birth weight, and maternal race, education, and martial status. Higher levels of either PM metric were associated with higher risk of postneonatal mortality, with 4% (95% CI: -1 to 10) increase in mortality risk per 10 $\mu g/m^3$ in PM_{10} and 4% (95% CI: -2 to 11) increase in mortality risk for the same increment of $PM_{2.5}$. This work builds on a previous study of 86 U.S. urban areas from

1989 to 1991, finding a 4% (95% CI: 2-7) increase in postneonatal mortality per 10 $\mu g/m^3$ county-level PM_{10} over the first 2 mo of life (Woodruff et al., 1997, 084271).

In Ciudad Juarez, Mexico, a case-crossover approach was applied to data from 1997 to 2001 based on death certificates and the cumulative PM_{10} for the day of death and previous two days (Romieu et al., 2004, 093074). A case-crossover study of Kaohsiung, Taiwan from 1994 to 2000 compared the average of PM_{10} on the day of death and two previous days to PM_{10} in control periods a week before and week after death (Tsai et al., 2006, 090709). A similar approach was also applied to 1994-2000 data from Taipei, Taiwan, also using case-crossover methods for the lag 0-2 PM_{10} with referent periods the week before and after death (Yang et al., 2006, 090760). In these case-crossover studies, season was addressed through matching in the study design. A 10 $\mu g/m^3$ increase in PM_{10} was associated with a 2.0% (95% CI: -2.8 to 7.0) increase in the Mexico study, a 0.59 (95% CI: -15.0 to 18.8) increase in postneonatal death in the Kaohsiung study, and a 1.02% (95% CI: -13.2 to 17.6) increase in the Taipei study. A study in Seoul, South Korea from 1995 to 1999 used time-series approaches adjusted for temporal trend and weather, based on national death registries excluding accidental deaths (Ha et al., 2003, 042552). A 10 $\mu g/m^3$ increase in PM_{10} was associated with a 3.14% (95% CI: 2.16-4.14) increase in risk of death for postneonates.

A subset of the studies examining postneonatal mortality also considered the subset of postneonatal deaths from respiratory causes. These include the time-series study in South Korea, finding a 17.8% (95% CI: 14.4-21.2) increase in respiratory-mortality per 10 $\mu g/m^3$ increase in PM_{10} (Ha et al., 2003, 042552) and the case-crossover study in Mexico, for which the same increment in PM_{10} was associated with a 1.5% (95% CI: -14.1 to 13.0) decrease in risk (Romieu et al., 2004, 093074). Both California case-control studies identified associations, with a 5% (95% CI: 1-10) increase in risk in Southern California (Ritz et al., 2006, 089819) and 57.4% (95% CI: 7.0-132) increase in California per 10 $\mu g/m^3$ PM_{10} (Woodruff et al., 2006, 088758). The U.S. study found this increment in PM_{10} to be linked with a 16% (95% CI: 6.0-28.0) increase in respiratory postneonatal mortality, although effect estimates for $PM_{2.5}$ were not statistically significant (Woodruff et al., 2008, 098386). Earlier studies on respiratory-related postneonatal mortality include the study of 86 U.S. urban areas, finding statistically significant effects (Woodruff et al., 1997, 084271).

Sudden Infant Death Syndrome

Three studies examining the relationship between PM and sudden infant death syndrome (SIDS) have been published from 2002 onward. These studies examined infant mortality and were thereby discussed in this section previously. A case-control study over a 12-year period (1989 to 2000) matched 10 controls to deaths (cases) in Southern California (Ritz et al., 2006, 089819). A 10 $\mu g/m^3$ increase in PM_{10} the 2 mo prior to death was associated with a 3% (95% CI: -1 to 8) increase in SIDS. Adjusted for other pollutants (CO, NO_2, and O_3), the effect estimate reduced to 1% (95% CI: -5 to 7).

A case-control study, also based in California, found an OR of 1.008 (95% CI: 1.006-1.012) per 10 $\mu g/m^3$ increase in $PM_{2.5}$, considering a SIDS definition of ICD 10 R95 (Woodruff et al., 2006, 088758). Due to changes in SIDS diagnosis, another SIDS definition was explored for ICD 10 R99 in addition to ICD 10 R95. Under this SIDS definition, the effect estimate changed to 1.03 (95% CI: 0.79-1.35). The authors also examined whether the relationship between $PM_{2.5}$ and SIDS differed by season, finding no significant difference. PM_{10} and $PM_{10-2.5}$ were not associated with risk of SIDS; numerical results were not provided for these PM metrics. The third recent study of PM and SIDS examined U.S. urban counties from 1999 to 2002 (Woodruff et al., 2008, 098386). Statistically non-significant relationships were observed between SIDS and PM_{10} or $PM_{2.5}$ in the first 2 mo of life.

These studies add to earlier work, such as a U.S. study that found higher risk of SIDS with higher annual $PM_{2.5}$ levels, including in a separate analysis of normal birth weight infants (Lipfert et al., 2000, 004103), and a U.S. study identifying a 12% (95% CI: 7-17) increase in SIDS risk per 10 $\mu g/m^3$ in PM_{10} for the first 2 mo of life for normal weight births (Woodruff et al., 1997, 084271). A study based on Taiwan found higher SIDS risk with lower visibility (Knöbel et al., 1995, 155905), whereas a 12-city Canadian time-series study identified no significant associations (Dales et al., 2004, 087342).

Deaths by SIDS were identified by different methods in the studies, partly due to transition from ICD 9 to ICD 10 codes, but also due to different choices within the research design. Two studies examined multiple approaches (ICD 10 R95, ICD 10 R95 and R99) (Woodruff et al., 2006,

088758; Woodruff et al., 2008, 098386), and other studies investigated ICD 9 798.0 and ICD 10 R95 (Ritz and Wilhelm, 2008, 156914), ICD 9 798.0 (Woodruff et al., 1997, 084271), ICD 9 798.0 and 799.0 (Knöbel et al., 1995, 155905), as well as a sudden unexplained death of infant <1 year for which an autopsy did not identify a specific cause of death (Dales et al., 2004, 087342). These variations in the definition of health outcomes add to differences in populations and study designs.

Although some findings indicate a potential effect of PM on risk of SIDS, with the strongest evidence perhaps from the case-control study in California (Woodruff et al., 2006, 088758), others do not find an effect or observe an uncertain association. For the relationship between PM and SIDS, a 2004 review article concluded consistent evidence exists compared to evidence for other infant mortality effects (Glinianaia et al., 2004, 087898), whereas other reviews found weaker or insufficient evidence (Heinrich and Slama, 2007, 156534). Another review concluded that the scientific literature on air pollution and SIDS suggests an effect, but that further research is needed to draw a conclusion (Tong and Colditz, 2004, 087883).

Considerations for Comparisons across Studies

Comparison of results across studies can be challenging due to several issues, including differences in methodologies, populations and study areas, pollution levels, and the exposure timeframes used. Given the large variation in study designs, the methods to address potential confounders vary. For example, weather and season were addressed in the case-control studies by matching, in the time-series study through non-linear functions of temperature and temporal trend, and in the ecological study through county-level variables. All studies included consideration of seasonality and weather. Researchers used different definitions of respiratory-related deaths, including ICD 9 460-519 (Bobak and Leon, 1999, 007678; Lipfert et al., 2000, 004103); ICD 9 460-519, 769-770 (Lipfert et al., 2000, 004103); ICD 9 460-519, 769, 770.4, 770.7, 770.8, 770.9, and ICD 10 J00-J98, P22.0, P22.9, P27.1, P27.9, P28.0, P28.4, P28.5, and P28.9 (Ritz et al., 2006, 089819); and ICD 9 460-519 and ICD 10 J00-J99 for any cause on death certificate (Romieu et al., 2004, 093074); ICD 10 J00-99 and P27.1 excluding J69.0 (Woodruff et al., 2006, 088758; Woodruff et al., 2008, 098386); and ICD 9 460-519 (Woodruff et al., 1997, 084271).

Socioeconomic conditions were included at the individual level, typically maternal education, in many studies (e.g., Bobak and Leon, 1999, 007678; Ritz and Wilhelm, 2008, 156914; Ritz et al., 2006, 089819; Woodruff et al., 1997, 084271; Woodruff et al., 2006, 088758) and at the community-level in others (e.g., Bobak and Leon, 1992, 044415; Penna and Duchiade, 1991, 073325) or for both individual and community-level data (e.g., Lipfert et al., 2000, 004103). The time-series approach is unlikely to be confounded by socioeconomic and other variables that do not exhibit day-to-day variation. Similarly, case-crossover methods use each case as his/her own control, thereby negating the need for individual-level confounders such as socioeconomic status (e.g., Romieu et al., 2004, 093074; Tsai et al., 2006, 090709; Yang et al., 2006, 090760). All studies published after 2001 incorporated individual-level socioeconomic data or were of case-crossover or time-series design. One study specifically examined whether socioeconomic status modified the PM and mortality relationship, dividing subjects into three socioeconomic strata based on the ZIP code of residence at death (Romieu et al., 2004, 093074). This work, based in Mexico, found that at lower socio-economic levels the association between PM_{10} and postneonatal mortality increased. Although the overall association showed higher risk of death with higher PM_{10} with statistical uncertainty, for the lowest socio-economic group, a 10 $\mu g/m^3$ increment in cumulative PM_{10} over the 2 days before death was associated with a 60% (95% CI: 3–149) increase in postneonatal death. A trend of higher effect for lower socio-economic condition is observed in all 3 lag structures.

Studies differ in terms of the time frame of pregnancy that was used to estimate exposure. Exposure to PM for infant mortality (<1 yr) was estimated as the levels between birth and death (Bobak and Leon, 1999, 007678), annual community levels (Lipfert et al., 2000, 004103; Penna and Duchiade, 1991, 073325) and the 3-5 days prior to death (Loomis et al., 1999, 087288). For neonatal deaths, exposure timeframes considered were the time between birth and death (Bobak and Leon, 1992, 044415; Bobak and Leon, 1999, 007678), annual levels (Bobak and Leon, 1999, 007678; Lipfert et al., 2000, 004103), monthly levels (Shinkura et al., 1999, 090050), the same day concentrations (Lin et al., 2004, 095787), and the 2 mo or 2 wk prior to death (Ritz et al., 2006, 089819). Postneonatal mortality was associated with PM concentrations based on annual levels (Bobak and Leon, 1992, 044415; Lipfert et al., 2000, 004103), between birth and death (Bobak and

Leon, 1999, 007678; Woodruff et al., 2006, 088758), 2 mo before death (Ritz et al., 2006, 089819), the first 2 mo of life (Woodruff et al., 1997, 084271; Woodruff et al., 2006, 088758), the day of death (Ha et al., 2003, 042552), and the average of the same day as death and previous 2 days (Romieu et al., 2004, 093074; Tsai et al., 2006, 090709; Yang et al., 2006, 090760). Thus, no consistent window of exposure was identified across the studies.

PM_{10} concentrations were highest in South Korea (69.2 $\mu g/m^3$) (Ha et al., 2003, 042552) and Taiwan (81.45 $\mu g/m^3$) (Tsai et al., 2006, 090709), and lowest in the U.S. (29.1 $\mu g/m^3$) (Woodruff et al., 2008, 098386) and Japan (21.6 $\mu g/m^3$) (Shinkura et al., 1999, 090050). All studies used community-level exposure information based on ambient monitors, as opposed to exposure measured at the individual level (e.g., subject's home) or personal monitoring.

Given similar sources for multiple pollutants (e.g., traffic), disentangling the health responses of copollutants is a challenge in the study of ambient air pollution. Several studies examined multiple pollutants, most by estimating the effect of different pollutants through several univariate models. Some studies noted the difficulty of separating PM effects from those of other pollutants, but noted stronger evidence for particles than other pollutants (Bobak and Leon, 1999, 007678). A few studies applied copollutant models by including multiple pollutants simultaneously in the same model. Effect estimates for the relationship between PM_{10} and neonatal deaths in São Paulo were reduced to a null effect when SO_2 was incorporated (Lin et al., 2004, 095787). Associations between PM_{10} and postneonatal mortality or respiratory postneonatal mortality remained but lost statistical significance in a multiple pollutant model with CO, NO_2, and O_3 (Ritz et al., 2006, 089819).

Several review articles in recent years have examined whether exposure to PM affects risk of infant mortality, generally concluding that more consistent evidence has been observed for postneonatal mortality, particularly from respiratory causes (Bobak and Leon, 1999, 007678; Heinrich and Slama, 2007, 156534; Lacasaña et al., 2005, 155914; Sram et al., 2005, 087442). In one review authors identified 14 studies on infant mortality and air pollution and determined that studies on PM and infant mortality do not provide consistent results, although more evidence was present for an association for some subsets of infant mortality such as postneonatal respiratory-related mortality (Bobak and Leon, 1999, 007678). The relationship between PM and postneonatal respiratory mortality was concluded to be causal in one review (Sram et al., 2005, 087442), and strong and consistent in another (Heinrich and Slama, 2007, 156534). Meta-analysis using inverse-variance weighting of PM_{10} studies found that a 10 $\mu g/m^3$ increase in acute PM_{10} exposure was associated with 3.3% (95% CI: 2.4-4.3) increase in risk of postneonatal mortality, whereas the same increment of chronic PM_{10} exposure was linked with a 4.8% (95% CI: 2.2-7.2) increase in postneonatal mortality and a 21.6% (95% CI: 10.2-34.2) increase for respiratory postneonatal mortality (Lacasaña et al., 2005, 155914).

Studies that examined multiple outcomes and ages of death allow a direct comparison based on the same study population and methodologies, thereby negating the concern that inconsistent results are due to underlying variation in population, approaches, etc. In this review, one study, based in Southern California identified no association for neonatal effects (numerical results not provided) but statistically significant results for postneonatal mortality (Ritz et al., 2006, 089819). Figure 7-5compares risk for the postneonatal period for respiratory and total mortality. In six of the seven studies, higher effect estimates were observed for respiratory-related mortality. Results from the neonatal period found higher effects for total mortality compared to respiratory mortality (Bobak and Leon, 1999, 007678) and the reverse for a study examining infant mortality (Lipfert et al., 2000, 004103). Thus, there exists evidence for a stronger effect at the postneonatal period and for respiratory-related mortality, although this trend is not consistent across all studies.

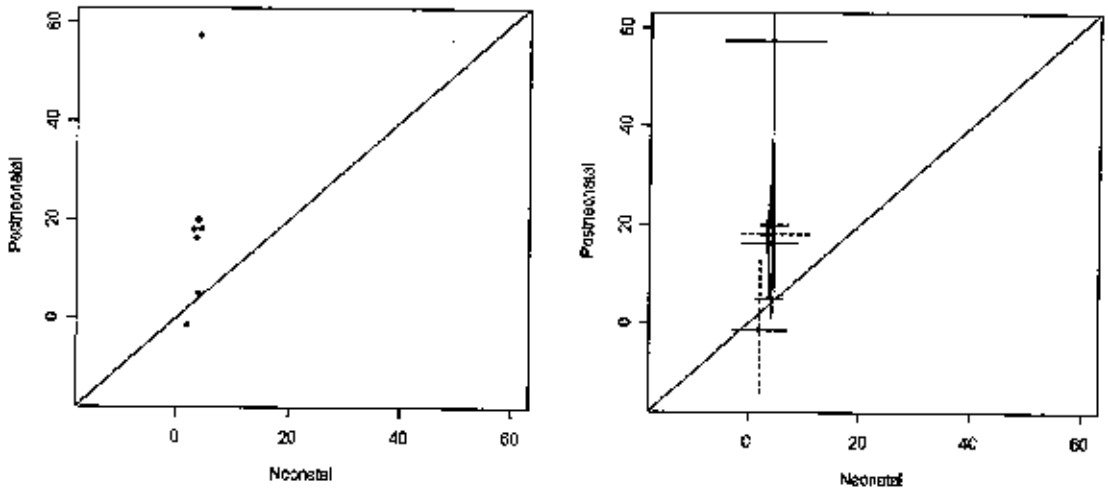

Figure 7-5. Percent increase in postneonatal mortality per 10 μg/m³ in PM_{10}, comparing risk for total and respiratory mortality. Panel a (left) provides central estimates; panel b (right) also adds the 95% intervals. The points reflect central estimates and the lines the 95% intervals. Solid lines represent statistically significant effect estimates; dashed lines represent non-statistically significant estimates.[1]

7.4.1.6. Decrements in Sperm Quality

Limited research conducted in the Czech Republic on the effect of ambient air pollution on sperm production has found associations between elevated air pollution and decrements in proportionately fewer motile sperm, proportionately fewer sperm with normal morphology or normal head shape, proportionately more sperm with abnormal chromatin (Selevan et al., 2000, 012578), and an increase in the percentage of sperm with DNA fragmentation (Rubes et al., 2005, 078091). These results were not specific to PM, but for exposure to a high-, medium- or low-polluted air mixture. Similarly, in Salt Lake City, Utah, $PM_{2.5}$ was associated with decreased sperm motility and morphology (Hammoud et al., 2009, 192156). Research in Los Angeles, California examined 5,134 semen samples from 48 donors in relation to ambient air pollution measured 0-9, 10-14, 70-90 days before semen collection over a 2-yr period (1996-1998). Ambient O_3 during all exposure periods had a significant negative correlation with average sperm concentration, and no other pollutant measures were significantly associated with sperm quality parameters, or presented quantitatively (Sokol et al., 2006, 098539).

7.4.2. Toxicological Studies

This section summarizes recent evidence on reproductive health effects reported with exposure to ambient PM; no evidence was presented in this area in the 2004 PM AQCD. Studies from different toxicological rodent models allow for investigation of specific mechanisms and modes of

[1] Studies included are Bobak and Leon (1999, 007678), Ha et al. (2003, 042552), Ritz et al. (2006, 089819), Romieu et al. (2004, 093074), Romieu et al. (2008, 156922), Woodruff et al. (1997, 084271), Woodruff et al. (2006, 088758). Findings from Bobak and Leon (1999, 007678) were based on TSP and were converted to PM_{10} estimates assuming $PM_{10}/TSP = 0.8$ as per summary data in the original article (Bobak and Leon, 1999, 007678). Findings from Woodruff et al. (1997, 084271) for respiratory-related mortality were based on non-low birth weight infants. Results for Woodruff et al. (2006, 088758) were based on $PM_{2.5}$ and were converted to PM_{10} assuming $PM_{2.5}/PM_{10} = 0.6$.

action for reproductive changes. Emphasis is placed here on results from different windows of development, i.e., exposure in utero, neonatally or as an adult can affect reproductive outcomes as an adult. In addition, studies evaluating whether fertility is affected in female and/or male animals by a similar exposure, and how exposures are transmitted to the fertility of the F_1 offspring, are summarized. Hormonal changes which can lead to decreased sperm count or changes in the estrous cycle are also of interest. Studies of pregnancy losses and placental sufficiency are also reported. Most recently, the role of environmental chemicals in shifting sex ratios (also seen in epidemiologic studies) and in affecting heritable DNA changes have become outcomes of interest.

7.4.2.1. Female Reproductive Effects

Urban Air

Windows of exposure are important in determining reproductive success as an adult. Exposure as a neonate may have a drastically different impact than does a similar adult exposure. To test this, female BALB/C mice were exposed to ambient air in Sao Paulo as neonates or as adults and then were bred to non-exposed males (Mohallem et al., 2005, 088657). Ambient concentrations of the pollutants CO, NO_2, PM_{10}, and SO_2 were 2.2 ± 1.0 ppm, 107.8 ± 42.3 μg/m^3, 35.5 ± 12.8 μg/m^3, and 11.2 ± 5.3 μg/m^3, respectively. They reported decreased fertility in animals exposed as newborns, but not in adult-exposed female BALB/c mice. There were a significantly higher number of liveborn pups from dams housed in filtered chambers (PM and gaseous components removed) versus animals exposed to ambient air as newborns. There was also a higher incidence of implantation failures in dams reared as newborns in polluted chambers. Sex ratio, number of pregnancies per group, resorptions, fetal deaths, and fetal placental weights did not differ significantly by exposure group. Thus, in these studies, exposure to ambient air pollution affected future reproductive success of females if they were exposed as neonates and not if exposed as adults.

Diesel Exhaust

Significant work has been done in male rodent models to determine the effect of PM exposure on reproductive outcomes, with fewer studies conducted using female rodents. Tsukue et al. (2004, 096643) exposed pregnant C57-BL mice to DE (0.1 mg/m^3) or to clean air (controls) for 8 h/day from GD2-13. The concentration of the gaseous materials including NO, NO_X, NO_2, CO and SO_2 are 2.2 ± 0.34 ppm, 2.5 ± 0.34 ppm, 0.0 ppm, 9.8 ± 0.69 ppm, and <0.1 ppm (not detectable), respectively. At GD14 female fetuses were collected for analysis of mRNA for two genes involved in sexual differentiation (Ad4BP-1/SF-1 and MIS), and found no significant changes. Work by Yoshida et al. (2006, 097015) showed changes in these two transcripts in male ICR fetuses exposed to similar concentrations of DE, albeit with different daily durations of exposure. Further work by Yoshida et al. (2006, 097015) showed that of three mouse strains tested, ICR male fetuses were the most sensitive to DE-dependent changes in these two genes. Nonetheless, strain sensitivity to DE particles may also differ by sex. Thus, it appears that female mice exposed in utero to DE show a lack of response at the mRNA level of MIS or Ad4bP-1/SF-1, important genes in male sexual differentiation that showed DE-dependent changes in male pups from dams exposed in utero. Female fetuses have shown a decrease in BMP-15, which is related to oocyte development (Tsukue et al., 2004, 096643).

A sensitive measure of androgenic activity in male rodents is anogenital distance (AGD), i.e., decreased AGD is seen with exposure to anti-androgenic environmental chemicals, the phthalates (Foster et al., 1980, 094701; Foster et al., 2001, 156442). To assess the role of DE exposure on reproductive success and anti-androgenic effects on offspring, Tsukue et al. (2002, 030593) exposed 6 week-old female C57-Bl mice to 4 mo of DE (0.3, 1.0, or 3.0 mg/m^3; PM MMAD of 0.4 μm) or filtered air. DE-exposed estrous females had significantly decreased uterine weight (1.0 mg/m^3). Some of the DE-exposed females were bred to unexposed males and DE–exposure led to increased, albeit not significantly increased, rates of pregnancy loss in mated females (up to 25%). Offspring were weighed after birth and decreases in body weight were observed at 6 and 8 wk (males and females, 1.0 and 3.0 mg/m^3) and 9 wk (females, 1.0 and 3.0 mg/m^3). Anogenital distance was decreased in 70-day old DE-exposed male offspring (0.3 mg/m^3). In female offspring at 70 days of

age, lower organ weights (adrenals, liver, and thymus) were observed (1.0 mg/m^3) compared to controls; thymus weight of the 0.3 mg/m^3 females was also lower at 70 days. Crown to rump length in females from dams exposed to DE (1.0 and 3.0 mg/m^3) was less than the control group. In conclusion, adult exposure to DE led to maternal-dependent reproductive changes that affected outcomes in offspring that manifested as decreased pup body weight, anti-androgenic effects like decreased AGD and decreased organ weight (which may have been confounded by changes in body weight because weights were not reported as relative organ weights).

7.4.2.2. Male Reproductive Effects

Diesel Exhaust

Studies were performed to determine PM-dependent strain sensitivity of the male reproductive tract using male steroidogenic enzymes as the model pathway. Three strains of pregnant mice (ICR, C57Bl/6J or ddY mice) were continuously exposed to DE at 0.1 mg/m^3 via inhalation or clean air over gestational days 2-13 (Yoshida et al., 2006, 156170). At GD14, dams were euthanized and fetuses were collected. Male fetuses were collected from each dam for mRNA analysis of genes related to male gonad development including Mullerian inhibiting substance (MIS; crucial for sexual differentiation including Mullerian duct regression in males), steroid transgenic factor (Ad4BP/SF-1, an enzyme in the testosterone synthesis pathway), cytochrome P450 cholesterol side chain cleavage enzyme (P450scc), and other steroidogenic enzymes [17β-hydroxysteroid dehydrogenase (HSD), cytochrome P450 17-α-hydroxylase (P450c17), and 3-βhydroxysteroid dehydrogenase (3βHSD)]. There were significant decreases in MIS (ICR and C57BL/6 mice) and Ad4BP/SF-1 (ICR mice) compared to the control groups. The ddY strain demonstrated no changes in Ad4BP/SF-1 or MIS, which may be due to marked changes in 3β-hD expression compared to non-DE exposed controls. From these studies, it appears that mouse strains with in utero exposure to DE show differential sensitivity in gonadal differentiation genes (mRNA) expression in male offspring; ICR are the most sensitive, followed by C57BL/6, with ddY mice being the least sensitive.

Yoshida et al. (2006, 097015) also monitored changes in the male reproductive tract after in utero exposure to DE. Timed-pregnant ICR dams were exposed during gestation (2 days post-coitus [dpc]-16 dpc) to continuous DE (0.3, 1.0 or 3.0 mg/m^3) or clean air. The reproductive tracts of male offspring were monitored at 4 wk postnatally. These pups received possible continued exposure through lactation as dams were exposed to DE during gestation and nursed pups. Exposure to 0.3 mg/m^3 of DE had no effect on male reproductive organ weight or serum testosterone. The intermediate concentration of 1.0 mg/m^3 induced increases in serum testosterone. Exposure to the higher concentration (1.0 and 3.0 mg/m^3) of DE led to significant increases in reproductive gland weight (testis, prostate, and coagulating gland). The organ weights are presented as absolute numbers and not adjusted for body weight, which is sometimes problematic for complete representation of hormonal changes, as body weight may confound absolute organ weight changes. Transcripts relating to male sexual differentiation (MIS and AD4BP/SF-1, 1.0 and 3.0 mg/m^3) were also significantly decreased. Sexual differentiation is a tightly regulated process and these changes in transcription may lead to changes that can affect genitalia development.

The effects of DE exposure on male spermatogenesis have also been demonstrated. Exposure of pregnant ICR mice to DE (2-16 dpc continuous inhalation exposure to 1.0 mg/m^3 or filtered clean air) led to impaired spermatogenesis in offspring (Ono et al., 2007, 156007). Male offspring were followed at PND 8, 16, 21 (3 wk), 35 (5 wk) and 84 (12 wk). After 16 dpc, but before termination of the study, all of the animals were transferred to a regular animal care facility and received clean air exposure until the termination of the study. No cross fostering was performed in this experiment, so pups that were born to DE-exposed dams were also nursed on these dams and may have received lactational exposure to DE. The gaseous components of the diluted DE included NO, NO_2, SO_2, and CO_2 at concentrations of 11.75 ± 1.18, 4.62 ± 0.36, 0.21 ± 0.01, and 4922 ± 244 ppm, respectively. Body weight was significantly depressed at PNDs 8 and 35. Accessory gland relative weight was significantly increased at PND8 and PND16 only. Serum testosterone was significantly decreased at 3 wk and was significantly increased at 12 wk. At 5 and 12 wk, daily sperm production (DSP) was significantly decreased. FSH receptor and StAR mRNA levels were significantly increased at 5 and 12 wk, respectively. Relative testis weight and relative epididymal weight were unchanged at all

time points. Histological changes showed sertoli cells with partial vacuolization and a significant increase in testicular multinucleated giant cells in the seminiferous tubules of DE-exposed animals compared to control. This study indicates that in utero exposure to DE had effects on spermatogenesis in offspring at the histological, hormonal and functional levels.

In utero exposure to DE and its effect on adult body weight, sex ratio, and male reproductive gland weight was measured by Yoshida et al. (2006, 097015). Pregnant ICR mice were exposed by inhalation to DE (0.3, 1.0 or 3.0 mg/m^3) or clean air from 2 dpc to 16 dpc. Pups were allowed to nurse in clean air on exposed dams until weaning and at PND28, male pups were sacrificed. At this time, serum testosterone and pup reproductive gland weight was determined. Significant increases in relative reproductive organ weights were reported at 1.0 and 3.0 mg/m^3 for the seminal vesicle, testis, epididymis, coagulating gland, prostate and liver. Male pup serum testosterone was significantly increased at 1.0 mg/m^3. Mean testosterone positively correlated with testis weight, DSP, aromatase and steroidogenic enzyme message levels (P450cc, c17 lyase, and P450 aromatase). Sex ratio did not differ in DE-exposed animals versus control. Male pup body weight of DE-exposed animals was significantly increased at PND28 (1.0 and 3.0 mg/m^3). These studies show that in utero DE-exposure led to increased serum testosterone and increased reproductive gland weight in male offspring early in life.

The effects of DE on murine adult male reproductive function were studied by exposing ICR male mice (6 wk of age) to DE (clean air control, 0.3, 1.0 or 3.0 mg/m^3) for 12 h/day for 6 mo with another group receiving a 1-mo recovery of clean air post-exposure (Yoshida and Takedab, 2004, 097760). After 6 mo of DE exposure, there was a concentration-dependent increase in degeneration of seminiferous tubules and a decrease in DSP/g of testis tissue. After 6 mo exposure to DE particles plus 1 mo of recovery in clean air, significant decreases remained in DSP at the two highest concentrations. The effect of ingestion of deposited PM on the fur with grooming cannot be ruled out as a possible exposure pathway in this experiment.

To expand on PM-dependent changes in spermatogenesis, an eloquent DE-exposure model was designed to determine if PM or the gaseous phase of DE was responsible for changes in sperm production in rodents (Watanabe, 2005, 087985). Pregnant dams (F344/DuCrj rats) exposed to DE (6 h/day exposure to 0.17 or 1.71 mg/m^3; <90% of PM less than 0.5 μm; NO_2 concentrations 0.10 and 0.79 ppm, respectively) or filtered air (removing PM only, low concentration filtered air and high concentration filtered air) from GD7 to parturition produced adult male offspring with a decreased number of sertoli cells and decreased DSP (PND 96) when compared to control mice exposed to clean air. The concentrations of NO_2 for the low and high filtered exposure groups were 0.1 and 0.8 ppm, respectively. Because both PM-filtered and DE-exposure groups showed the same outcomes, the effects are likely due to gaseous components of DE.

Motorcycle Exhaust

Adult male (8-wk old) Wistar rats were exposed to motorcycle exhaust (ME) for 1 h in the morning and 1 h in the afternoon (5 day/wk) at 1:50 dilution for 4 wk (group A), 1:10 dilution for 2 wk (group B) or 4 wk (group C), or to clean air (Huang et al., 2008, 156574). After 4 wk of exposure, both exposed groups had significantly decreased body weight compared to the control group. All three ME exposure groups showed a decreased number of spermatids in the testis. Both 1:10 exposure groups also demonstrated decreased caudal epididymal sperm counts. Group C had significant decreased testicular weight, decreased mRNA expression for the cytochrome P450 substrate 7-ehtoxycoumarin O-de-ethylase, and increased IL-6, IL-1β, and COX-2 mRNA levels. Decreased protein levels of the antioxidant, superoxide dismutase, and increased IL-6 protein were reported for group C when compared to control. In addition, serum testosterone was significantly decreased in group C. Co-treatment with the antioxidant vitamin E resulted in partial attenuation of serum testosterone levels and caudal epididymal sperm counts, and returned IL-6, IL-1β, and COX-2 ME exposure-dependent message levels to baseline. The glutathione antioxidant system and lipid peroxidation were unchanged. In conclusion, male animals exposed to ME showed significant decrements in body weight, spermatid number, and serum testosterone with an increase in inflammatory cytokines. Vitamin E co-treatment with ME-exposure led to an attenuation of inflammation and a partial rescue of testosterone levels and sperm numbers.

Summary of Toxicological Study Findings for Male Reproductive Effects

In summary, laboratory animals exposed to DE in utero or as adults manifest with abnormal effects on the male reproductive system. In utero exposure to DE induced increased reproductive gland weight and increased serum testosterone in early life (PND28), which may lead to early puberty (albeit not measured in this study). With similar in utero DE exposures, later life outcomes include decreased DSP, aberrant sperm morphology, and hormonal changes (testosterone and FSHr decrements). Chronic exposure of adult mice to DE also induced decreased DSP and seminiferous tubule degeneration. DE-dependent effects on male reproductive function have been reported in multiple animal models, with only one model separating exposure based on particulate versus gaseous components. DE and filtered air (gaseous phase only) exposure in utero induced sertoli cell and DSP decrements in both groups, indicating that the gaseous phase of DE was causative. Adult male rats exposed to ME manifested with decreased spermatid number, serum testosterone, and an increase in inflammatory cytokines. Significant effects on the male reproductive system have been demonstrated after exposure to ambient PM sources (DE or ME). Nonetheless, these models often include a complex mixture of gaseous component and PM exposure, which makes interpreting the contribution from PM alone difficult.

7.4.2.3. Multiple Generation Effects

Urban Air

Veras et al. (2009, 190496) investigated pregnancy and female reproductive outcomes in BALB/c female mice exposed to ambient air or PM-filtered ambient air at one of two different time periods (before conception and during pregnancy) near an area of high traffic density in Sao Paulo, Brazil. Exposures were 27.5 and 6.5 $\mu g/m^3$ $PM_{2.5}$ for ambient and PM-filtered air chambers, respectively, with 101 $\mu g/m^3$ NO_2, 1.81 $\mu g/m^3$ CO, and 7.66 ppm SO_2 in both chambers. Two groups of 2nd generation (G2) nulliparous female mice were continuously exposed from birth. Estrous cyclicity and ovarian follicle classification were followed at PND60 (reproductive maturation) in one group. A further group was subdivided into four groups by exposures during pregnancy following reproductive capability and pregnancy outcomes of the G2 mice. Animals exposed to ambient air versus PM-filtered air had an extended time in estrous and thus, a reduction in the number of cycles during the study period. The number of antral follicles was significantly decreased in the ambient air versus the PM-filtered air animals. Other follicular quantification (number of small, growing or preovulatory follicles) showed no differences between the two chambers. There was an increase in the time necessary for mating, a decrease in the fertility index, and an increase in the pregnancy index in the ambient air group versus the PM-filtered group. Specifically, in the ambient air groups, there was a significant increase in rate of the post-implantation loss in G1 and G2 groups. However, there was no statistically significant change in number of pups in the litter. Fetal weight was decreased in all treatment groups (ambient air groups G1 and G2, and PM-filtered G2) when compared to the PM-filtered G1 group or animals raised entirely in filtered air, showing that fetal weight was affected by both pre-gestational and gestational PM exposure.

PM exposure prior to conception is associated with increased time in estrous, which in other animal models can be related to ovarian hormone dysfunction and ovulatory problems. These estrous alterations can contribute to fecundity issues. There was no significant difference in number of preovulatory follicles in the above model, but there was a statistically significant decrease in the number of antral follicles (Veras et al., 2009, 190496). Antral follicles are the last stage in follicle development prior to ovulation, and a decrease in antral follicle number can be related to premature reproductive senescence, premature ovarian failure, or early menopause, which were not followed in this study.

In this study (Veras et al., 2009, 190496), the males that were used to generate the G1 and G2 groups were also exposed to ambient air or PM-filtered ambient air, and thus the reproductive contribution of these males to the overall fertility and mating changes in the females cannot be totally eliminated as a possible confounder to the observed effects. Thus, these effects are hard to differentiate as male- or female-dependent and likely indicate a general loss of reproductive fitness. Interestingly, both pre- and gestational exposure to ambient air induced a significant loss in post-

implantation of fetuses and this may be related to placental insufficiency as has been described in other work by this lab (Veras et al., 2008, 190493).

7.4.2.4. Receptor Mediated Effects

Arylhydrocarbon Receptor (AhR)

Diesel Exhaust Particles

The AhR is often activated by chemicals classified as endocrine disrupting compounds (EDCs), exogenous chemicals that behave as hormonally active agents, disrupting the physiological function of endogenous hormones. DE particles are known to activate the AhR. A recent study by Izawa et al. (2007, 190387) showed that certain polyphenols (quercetin from the onion) and food extracts (Ginkgo biloba extract) are able to attenuate DE particle-dependent AhR activation when measured with the Ah-Immunoassay, thus possibly attenuating the EDC activity of DE particles.

7.4.2.5. Developmental Effects

Sex Ratio

Urban Air

A correlation between PM_{10} exposure and a decrease in standardized sex ratios (SSRs) has been reported in humans exposed to air pollution (Lichtenfels et al., 2007, 097041; Wilson et al., 2000, 010288), with fewer numbers of male births reported. To understand this shift, two groups (control and exposed) of male Swiss mice were housed concurrently in Sao Paulo and received either ambient air exposure or filtered air (chemical and particulate filtering) from PND10 for 4 mo (Lichtenfels et al., 2007, 097041). Filtration efficiency for $PM_{2.5}$, CB, and NO_2 inside the chamber was found to be 55%, 100%, and 35%, respectively. After this exposure, non-exposed females were placed in either chamber to mate. After mating, the males were sacrificed and testes collected; males exposed to ambient air showed decreased testicular and epididymal sperm counts, decreased total number of germ cells, and decreased elongated spermatids, but no significant change in litter size. Females were housed in the chambers and sacrificed on GD19 when the number of pups born alive and the sex ratio were obtained. There was a significant decrease in the SSR for pups born after living in the ambient air-exposed chamber compared to the filtered chamber. In this study, a shift in SSR has been shown for both humans and rodents exposed to air pollution, but other studies with DE exposure (Yoshida et al., 2006, 156170) or ambient air in Sao Paulo (Mohallem et al., 2005, 088657) showed no changes in rodent sex ratio. Possible exposure to PM and other components of ambient air via ingestion during grooming cannot be ruled out in this rodent model.

Immunological Effects: Placenta

Diesel Exhaust

Placental insufficiency can lead to the loss of a pregnancy or to adverse fetal outcomes. DE-exposure has been shown to induce inflammation in various models. Fujimoto et al. (2005, 096556) assessed cytokine/immunological changes of DE-dependent inhalation exposure on the placenta during pregnancy. Pregnant Slc:CR mice were exposed to DE (0.3, 1.0, or 3.0 mg/m^3; PM MMAD of 0.4 μm) or clean air from 2 to 13 dpc and dams, placenta, and pups were collected at 14 dpc. There was a significant increase in the number of absorbed placentas in DE-exposed animals (0.3

and 3.0 mg/m^3) with a significant decrease in the number of absorbed placentas in DE-exposed animals at the middle concentration (1.0 mg/m^3). Absorbed placentas from DE exposed mice had undetectable levels of CYP1A1 and twofold increases in TNF-α; CYP1A1 placental mRNA from healthy placentas of DE-exposed mice was unchanged versus control. IL-2, IL-5, IL-12α, IL-12β and GM-CSF mRNA significantly increased in placentas of DE-exposed animals (0.3 and 3.0 mg/m^3). Fujimoto et al. (2005, 096556) reported DE-induced significant increases in multiple inflammatory markers in the placenta with significant increases in the number of absorbed placentas.

Immunological Effects: Asthma

Model Particles

In utero exposure may confer susceptibility to PM-induced asthmatic responses in offspring. Exposure of pregnant BALB/c mice to aerosolized ROFA leachate by inhalation or to DE particles intranasally increases asthma susceptibility to their offspring (Fedulov et al., 2008, 097482; Hamada et al., 2007, 091235). The offspring from dams exposed for 30 min to 50 mg/mL ROFA 1, 3, or 5 days prior to delivery responded to OVA immunization and aerosol challenge with airway hyperreactivity and increased antigen-specific IgE and IgG1 antibodies (Hamada et al., 2007, 091235). Airway hyperreactivity was also observed in the offspring of dams intranasally instilled with 50 μg of DE particles or TiO_2, or 250 μg CB, indicating that the same effect could be demonstrated using relatively "inert" particles (Fedulov et al., 2008, 097482). Pregnant mice were particularly sensitive to exposure to DE or TiO_2 particles, and genetic analysis indicated differential expression of 80 genes in response to TiO_2 in pregnant dams. Thus pregnancy and in utero exposure may enhance responses to PM, and exposure to even relatively inert particles may result in offspring predisposed to asthma.

Placental Morphology

Urban Air

Exposure to ambient air pollution during pregnancy is associated with reduced fetal weight in both human and animal models. The effect of particulate urban air pollution on the functional morphology of the mouse placenta was explored by exposing second generation mice in one of four groups to urban Sao Paulo air (PM was 67% $PM_{2.5}$, mainly of vehicular origin) or filtered air (Veras et al., 2008, 190493). Experimental design was: group F-F comprised of mice that were raised in filtered air chambers and completed pregnancy in filtered air chambers; group F-nF raised in filtered air and pregnant in ambient air; group nF-nF raised and completed pregnancy in non-filtered air chambers; and group nF-F mice raised in ambient air and received filtered air during pregnancy. Mean $PM_{2.5}$ concentrations in the F and nF chambers were 6.5 and 27.5 μg/m^3, respectively. Exposure was from PND20-PND60. After this exposure, the animals were mated and then maintained in their respective chambers during pregnancy. Pregnancy was terminated at GD8 (near term) with placentas and fetuses collected for analysis.

Exposure to ambient PM pre-gestationally or gestationally led to significantly smaller fetal weight (total litter weight). Pregestational exposure to ambient air induced significant increases in fetal capillary surface area and total mass-specific conductance, but this may be explained by reduced maternal/dam blood space and diameters. Gestational exposure to non-filtered air was associated with reduced volume, diameter (caliber) and surface area of maternal blood space with compensatory greater fetal capillary surface and oxygen diffusion conduction rates. Intravascular barrier thickness, a quantitative relationship between trophoblast volume and the combined surfaces of maternal blood spaces and fetal capillaries, was not reduced with ambient air exposure. This study provides evidence that fetal/placental circulatory adaptation to maternal blood deficits after ambient PM exposure may not be sufficient to overcome PM-dependent birth weight deficits in mice exposed to ambient air, with the magnitude of this effect greater in the gestationally-exposed groups.

Placental Weights and Birth Outcomes

Urban Air

Pregnant female Swiss mice were exposed to ambient air (Sao Paulo) or filtered air over various portions of gestation to determine if there was an association between fetal or placental weight or birth outcomes with exposure to air pollution (Rocha et al., 2008, 096685). The reported ambient concentrations of PM_{10} (42 ± 17 $\mu g/m^3$), NO_2 (97 ± 39 $\mu g/m^3$), and SO_2 (9 ± 4 $\mu g/m^3$) were measured 100 m away from the rodent exposure chambers. By using six time windows of exposure that covered 1-3 wk of gestation (the entire gestation period in a mouse), a significant decrease in near-term fetal weight (GD19) was induced by ambient air-exposure during the first week of gestation. Decreased placental weight could be induced by ambient air exposure during any of the 3 wk of gestation. This study points to possible windows of exposure that may be important in evaluating epidemiologic study results.

Neurodevelopmental Effects

Diesel Exhaust

The diagnosis of autism is on the rise in the Western world with its etiology mostly unknown. Autism-associated cell loss is brain region-specific and hypothesized to be developmental in origin. Sugamata et al. (2006, 097166) exposed pregnant ICR mice to DE (0.3 mg/m^3) continuously from 2 dpc to 16 dpc. Pups with in utero exposure to DE were nursed in clean air chambers, but may have received gastro-intestinal exposure via lactational transfer of various components of DE. At 11 wk of age, cerebellar brain tissue was collected. Earlier work has shown that DE particles (<0.1 μm) have been detected in the brains (cerebral cortex and hippocampus) of newborn pups who were born to dams exposed to DE during pregnancy (Sugamata et al., 2006, 097166). Histological analysis of DE-exposed pup cerebella revealed significant increases in caspase-3 (c-3) positive cells compared to control and significant decreases in cerebella Purkinje cell numbers in DE-exposed animals versus control. The ratio of cells positive for apoptosis (c-3 positive) showed a nearly significant sex difference with males displaying increased apoptosis versus females ($p = 0.09$). In humans with autism, the cerebellum has a decreased number of Purkinje cells, which is thought to be fetal and developmental in origin; further, these authors speculate that humans may be more sensitive to DE-dependent neuronal brain changes, as the human placenta is two-layers thick compared to the mouse placenta that is four layers thick.

Behavioral Effects

Diesel Exhaust Particles

Body weight decrements at birth have recently been associated through the Barker hypothesis with adverse adult outcomes. Thus, many publications have begun to focus on decreased birth weight for gestational age and associated adult changes. Hougaard et al. (2008, 156570) exposed 40 timed-pregnant C57BL/6 dams to DE particles reference materials (SRM 2975) via inhalation over GD7-GD19 of pregnancy. They found significantly decreased pup weight at weaning, albeit not at birth. PM-dependent liver changes were monitored by following various inflammatory and genotoxicity-related mRNA transcripts and there were no significant differences in pups at PND2. The comet assay from PND2 pup livers showed no significant differences in DNA damage between DE particle-exposed and control animals. The prohormone, thyroxine, was unchanged in control and DE particle-exposed dams and offspring at weaning. At 2 mo, female DE particle-exposed pups required less time than controls to locate the platform in its new location during the first trial of the spatial reversal learning task in the Morris water maze. Thus, DE particle exposure during in utero development led to behavioral changes without body weight at weaning or changes in inflammatory markers or thyroid hormone levels.

Diesel Exhaust

The effect of in utero DE exposure on CNS motor function was evaluated in male pups (ICR mice) after dams received DE exposure (8h/d×5d/wk) from GD2-GD17 (Yokota et al., 2009, 190518). The exposure atmosphere contained concentrations of 1.0 mg/m^3 for particle mass, 2.67 ppm CO, 0.23 ppm NO_2, and <0.01 ppm SO_2. Spontaneous motor activity was significantly decreased in pups (PND35), as was the dopamine metabolite homovanillic acid measured in the striatum and nucleus accumbens, indicating decreased dopamine (DA) turnover. However, DA levels were unchanged in the same areas of the brain. The authors conclude that these data demonstrate that maternal exposure to DE induced hypolocomotion, similar to earlier studies with adult and neonatal DE particle exposure (Peters et al., 2000, 001756), with decreased extracellular DA release.

Lactation

Diesel Exhaust

Tozuka et al. (2004, 090864) monitored the transfer of PAHs to fetuses and breast milk of F344 rats exposed to DE (6h/day) for 2 wk from GD7-GD 20 (minus 4 days for the weekend when no exposure occurred) with PM_{10} concentration of 1.73 mg/m^3. At PND 14, breast milk was collected. Fifteen PAHs were monitored in the DE exposure chamber and seven were quantified in dam blood with levels of phenanthrene (Phe), anthracene (Ant) and benz[a]anthracene (BaA) in the DE group being significantly higher than the control group. In breast milk, Ant, fluoranthene (Flu), pyrene (Pyr), and chrysene (Chr) showed significant increases in the DE group compared to the control group. BaA tended to be about fourfold higher than the control group in breast milk, but the increase was not significant. PAHs in dam livers of DE versus control were not significantly different. The results of this study demonstrate that PAHs derived from DE are transferred across the placenta from the DE-exposed dam to the fetus. Lactational transfer through the breast milk is also likely as PAHs were detected in dam breast milk, but this should be confirmed in future studies that cross foster control and exposed dams and pups. The lipophilicity of the PAH based on its structure likely affected its uptake in the dam, as PAHs with 3 or 4 rings were found in maternal blood and PAHs with 5 or 6 rings were not detected.

Heritable DNA Changes and Epigenetic Changes

Ambient Air

To address the role of ambient air exposure on heritable changes, Somers et al. (2004, 078098) exposed mice to ambient air in at a rural Canadian site or at an urban site near a steel mill. They showed that offspring of mice exposed to ambient air in urban regions inherited paternal-origin expanded simple tandem repeat (ESTR) mutations 1.9-2.1 times more frequently than offspring of mice exposed to HEPA filtered air or those exposed to rural ambient air. Mouse expanded simple tandem repeat (ESTR) DNA is composed of short base pair repeats which are unstable in the germline and tend to mutate by insertion or deletion of repeat units. In vivo and in situ studies have shown that murine ESTR loci are susceptible to ionizing radiation, and other environmental mutatgen-dependent germline mutations, and are thus good markers of exposure to environmental contaminants.

Expanding upon the above work and to determine if PM or the gaseous phase of the urban air was responsible for heritable mutations, Yauk et al. (2008, 157164) exposed mature male C57Bl×CBA F1 hybrid mice to either HEPA-filtered air or to ambient air in Hamilton, Ontario, Canada for 3 or 10 wk, or 10 wk plus 6 wk of clean air exposure (16 wk). Sperm DNA was monitored for expanded simple tandem repeat (ESTR) mutations. In addition, male-germ line (spermatogonial stem cell) DNA methylation was monitored post-exposure. This area in Hamilton is near two steel mills and a major highway. Air quality data provided by the Ontario Ministry of the Environment showed the highest concentrations of TSP and metals at week 4 (93.8 ± 17 and 3.6 ± 0.7 μg/m^3, respectively) and PAH at week 3 (8.3 ± 1.7 ng/m^3). Mutation frequency at ESTR

Ms6-hm locus in sperm DNA from mice exposed 3 or 10 wk did not show elevated ESTR mutation frequencies, but there was a significant increase in ESTR mutation frequency at 16 wk in ambient air-exposed males versus HEPA filter-exposed animals, pointing to a PM-dependent mechanism of action. When compared to HEPA filter air-exposed males, ambient air-exposed males manifested with hypermethylation of germ-line DNA at 10 and 16 wk. These PM-dependent epigenetic modifications (hypermethylation) were not seen in the halploid stage (3 wk) of spermatogenesis, but were nonetheless seen in early stages of spermatogenesis (10 wk) and remained significantly elevated in mature sperm even after removal of the mouse from the environmental exposure (16 wk). Thus, these studies indicate that the ambient PM phase and not the gaseous phase is responsible for the increased frequency of heritable DNA mutations and epigenetic modifications.

7.4.3. Summary and Causal Determinations

7.4.3.1. $PM_{2.5}$

The 1996 PM AQCD concluded that while few studies had been conducted on the link between PM and infant mortality, the research "suggested an association," particularly for post-neonates (U.S. EPA, 1996, 079380). In the 2004 PM AQCD, additional evidence was available on PM's effect on fetal and early postnatal development and mortality and while some studies indicated a relationship between PM and pregnancy outcomes, others did not (U.S. EPA, 2004, 056905). Studies identifying associations found that exposure to PM_{10} early during pregnancy (first month of pregnancy) or late in the pregnancy (6 wk prior to birth) were linked with higher risk of preterm birth, including models adjusted for other pollutants, and that $PM_{2.5}$ during the first month of pregnancy was associated with IUGR. However, other work did not identify relationships between PM_{10} exposure and low birth weight. The state of the science at that time, as indicated in the 2004 PM AQCD, was that the research provided mixed results based on studies from multiple countries.

Building on the evidence characterized in the previous AQCDs, recent epidemiologic studies conducted in the U.S. and Europe were able to examine the effects of $PM_{2.5}$, and all found an increased risk of low birth weight (Section 7.4.1). Exposure to $PM_{2.5}$ was usually associated with greater reductions in birth weight than exposure to PM_{10}. All of the studies that examined the relationship between $PM_{2.5}$ and preterm birth report positive associations, and most were statistically significant. The studies evaluating the association between $PM_{2.5}$ and growth restriction all found positive associations, with the strongest evidence coming when exposure was assessed during the first or second trimester (Section 7.4.1). For infant mortality (<1 yr), several studies examined $PM_{2.5}$ and found positive associations (Section 7.4.1).

Animal toxicological studies reported effects including decreased uterine weight, limited evidence of male reproductive effects, and conflicting reports of reproductive outcomes in male offspring, particularly in studies of DE (Section 7.4.2). Toxicological studies also reported effects for several development outcomes, including immunological effects (placental and related to asthma), neurodevelopmental and behavioral effects (Section 7.4.2).

In summary evidence is accumulating from epidemiologic studies for effects on low birth weight and infant mortality, especially due to respiratory causes during the post-neonatal period. The mean $PM_{2.5}$ concentrations during the study periods ranged from 5.3-27.4 $\mu g/m^3$. Exposure to $PM_{2.5}$ was usually associated with greater reductions in birth weight than exposure to PM_{10}. Several U.S. studies of PM_{10} investigating fetal growth reported 11-g decrements in birth weight associated with PM_{10} exposure. Most of these studies were conducted in California, where $PM_{2.5}$ and $PM_{10-2.5}$ contribute almost equally to the PM_{10} mass concentration. So while these results can not be attributed to one size fraction or the other, the consistency of the results strengthens the interpretation that particle exposure may be causally related to reductions in birth weight. Similarly, animal evidence supported an association between $PM_{2.5}$ and PM_{10} exposure and adverse reproductive and developmental outcomes, but provided little mechanistic information or biological plausibility for an association between long-term PM exposure and adverse birth outcomes, including low birth weight, or infant mortality. Epidemiologic studies do not consistently report associations between PM exposure and preterm birth, growth restriction, birth defects or decreased sperm quality. New evidence from animal toxicological studies on heritable mutations is of great interest, and warrants further investigation. Overall, the epidemiologic and toxicological evidence is **suggestive of a**

causal relationship between long-term exposures to $PM_{2.5}$ and reproductive and developmental outcomes.

7.4.3.2. $PM_{10-2.5}$

Evidence is **inadequate to determine if a causal relationship exists between long-term exposure to $PM_{10-2.5}$ and developmental and reproductive outcomes** because studies have not been conducted in sufficient quantity or quality to draw any conclusion. A single study found an association between $PM_{10-2.5}$ and birthweight (-13 g [95% CI: -18.3 to -7.6] per 10 $\mu g/m^3$ increase), but no such association for $PM_{2.5}$ (Parker et al., 2008, 156013).

7.4.3.3. UFPs

The 2004 PM AQCD did not report long-term exposure studies for UFPs. No epidemiologic or animal toxicology studies have been conducted to evaluate the effects of long-term UFP exposure and reproductive and developmental effects. Ambient air exposures, which likely include UFPs, are reported in this ISA but there is no delineation of the separate contribution from UFPs. The evidence is **inadequate to determine if a causal relationship exists between long-term UFP exposures and reproductive and developmental effects.**

7.5. Cancer, Mutagenicity, and Genotoxicity

Evidence from epidemiologic and animal toxicological studies has been accumulating for more than three decades regarding the mutagenicity and carcinogenicity of PM in the ambient air. DE has been identified as one source of PM in ambient air, and has been extensively studied for its carcinogenic potential. In 1989, the International Agency for Research on Cancer (IARC) found that there was sufficient evidence that extracts of DE particles were carcinogenic in experimental animals and that there was limited evidence for the carcinogenic effect of DE in humans (IARC, 1989, 002958). This conclusion was based on studies in which organic extracts of DE particles were used to evaluate the effects of concentrates of the organic compounds associated with carbonaceous soot particles. These extracts were applied to the skin or administered by IT instillation or intrapulmonary implantation to mice, rats, or Syrian hamsters and an excess of tumors on the skin, lung or at the site of injection were observed.

In 2002, the U.S. EPA reviewed over 30 epidemiologic studies that investigated the potential carcinogenicity of DE. These studies, on average, found that long-term occupational exposures to DE were associated with a 40% increase in the relative risk of lung cancer (U.S. EPA, 2002, 042866). In the same report the U.S. EPA concluded that extensive studies with salmonella had unequivocally demonstrated mutagenic activity in both particulate and gaseous fractions of DE. They further concluded that DE may present a lung cancer hazard to humans (U.S. EPA, 2002, 042866). The particulate phase appeared to have the greatest contribution to the carcinogenic effect. Both the particle core and the associated organic compounds demonstrated carcinogenic properties, although a role for the gas-phase components of DE could not be ruled out. Almost the entire diesel particle mass is ≤ 10 µm in diameter (PM_{10}), with approximately 94% of the mass of these particles <2.5 µm in diameter ($PM_{2.5}$), including a subgroup with a large number of UFPs (U.S. EPA, 2002, 042866). U.S. EPA considered the weight of evidence for potential human carcinogenicity for DE to be strong, even though inferences were involved in the overall assessment, and concluded that DE is "likely to be carcinogenic to humans by inhalation" and that this hazard applies to environmental exposures (U.S. EPA, 2002, 042866).

Two recent reviews of the mutagenicity (Claxton et al., 2004, 089008) and carcinogenicity (Claxton and Woodall, 2007, 180391) of ambient air have characterized the animal toxicological literature on ambient air pollution and cancer. The majority of these toxicological studies have been conducted using IT instillation or dermal routes of exposure. Generally, the toxicological evidence reviewed in this ISA has been limited to inhalation studies conducted with lower concentrations of

PM (<2 mg/m^3), relevant to current ambient concentrations and the current regulatory standard (Section 1.3). Because this ISA focuses on toxicological studies which use the inhalation route of exposure, it is possible that important evidence for the role of PM in mutagenicity, tumorigenicity, and/or carcinogenicity may be missed. In order to accurately characterize the relationship between PM and cancer and be consistent with the EPA Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005, 086237), these reviews (that include studies that employ IT instillation and dermal routes of exposure) are summarized briefly.

Claxton et al. (2004, 089008) reviewed the mutagenicity of air in the Salmonella (Ames) assay, and showed that hundreds of compounds identified in ambient air from varying chemical classes are mutagenic and that the commonly monitored PAHs could not account for the majority of mutagenicity associated with most airborne particles. They concluded that the smallest particles have the highest toxicity per particulate mass, with the $PM_{2.5}$ size fraction having greater mutagenic and cytotoxic potential than the PM_{10} size fraction, which had a higher mutagenic potential than the TSP size fraction. One study reviewed by Claxton et al. (2004, 089008) found that the cytotoxic potential of $PM_{2.5}$ was higher in wintertime samples than in summertime samples. A series of studies on source apportionment for ambient particle mutagenic activity reviewed by Claxton et al. (2004, 089008) indicate that mobile sources (cars and diesel trucks) account for most of the mutagenic activity.

Claxton and Woodall (2007, 180391) reviewed many studies that examined the rodent carcinogenicity of extracts of ambient PM samples; the PM was of various size classes, often from TSP samples. Among a variety of mouse and rat strains, application methods, and samples employed, the authors found no pattern that would suggest the routine use of a particular strain or protocol would be more informative than another. The primary conclusion that comes from the analysis of rodent carcinogenicity studies is that the most polluted urban air samples tested to date are carcinogenic; the contribution of PM and different size classes of PM to the carcinogenic effects of ambient air has not been delineated. The differences in response by the various strains of inbred mice indicate that the genetic background of an individual can influence tumorigenic response. Studies examining different components of ambient PM (e.g., PAHs) confirm that ambient air contains multiple carcinogens, and that the carcinogenic potential of particles from different airsheds can be quite different. Therefore, one would expect the incidence of cancers related to ambient air exposure in different metropolitan areas to differ.

Numerous epidemiologic and animal toxicological studies of ambient PM and their contributing sources have been conducted to assess the relative mutagenic or genotoxic potential. Studies previously reviewed in the 2004 PM AQCD (U.S. EPA, 2004, 056905) provide evidence that ambient PM as well as PM from specific combustion sources (e.g., fossil fuels) is mutagenic in vivo and in vitro. Building on these results, data from recent epidemiologic and animal toxicological studies that evaluated the carcinogenic, mutagenic and/or genotoxic effects of PM, PM-constituents, and combustion emission source particles are reviewed in this section.

7.5.1. Epidemiologic Studies

The 2004 PM AQCD reported on original and follow-up analyses for three prospective cohort studies that examined the relationship between PM and lung cancer incidence and mortality. Based on these findings, as well as on the results from case-control and ecologic studies, the 2004 PM AQCD concluded that long-term PM exposure may increase the risk of lung cancer incidence and mortality. The largest of the three prospective cohort studies included in the 2004 PM AQCD was the ACS study (Pope et al., 2002, 024689). This study was the follow-up to the original ACS study (Pope et al., 1995, 045159), and included a longer follow-up period and reported a statistically significant association between $PM_{2.5}$ exposure and lung cancer mortality.

A 14- to 16-yr prospective study conducted using the Six Cities Study cohort reported a slightly elevated risk of lung cancer mortality for individuals living in the most polluted city (mean PM_{10}: 46.5 μg/m^3; mean $PM_{2.5}$ 29.6 μg/m^3) as compared to the least polluted city (mean PM_{10}: 18.2 μg/m^3; mean $PM_{2.5}$ 11.0 μg/m^3) but the association was not statistically significant (Dockery et al., 1993, 044457).

Re-analysis of the AHSMOG cohort, a study of non-smoking whites living in California, concluded that elevated long-term exposure to PM_{10} was associated with lung cancer incidence among both men and women (Beeson et al., 1998, 048890). The original study had reported an excess of incident lung cancers only among women (Abbey et al., 1991, 042668). Further reanalysis

of this cohort revealed an association between PM_{10} and lung cancer mortality among men but no association among women (Abbey et al., 1999, 047559). In addition, McDonnell et al. (2000, 010319) reported increases in lung cancer mortality with long-term exposure to $PM_{2.5}$ in the AHSMOG cohort; no association was seen for $PM_{10-2.5}$.

7.5.1.1. Lung Cancer Mortality and Incidence

The following sections will examine extensions of the above mentioned cohort studies and new studies published since the 2004 PM AQCD. The section includes discussion of both lung cancer incidence and mortality, as well as markers of exposure/susceptibility. A summary of the mean PM concentrations reported for the new studies is presented in Table 7-6. In addition, a summary of the associations for lung cancer mortality and incidence are presented in Table 7-7 and Figure 7-7 (Section 7.6) Further discussion of all-cause and cause-specific mortality is presented in Section 7.6.

Table 7-6. Characterization of ambient PM concentrations from recent studies of cancer and long-term exposures to PM.

Study	Location	Pollutant	Mean Annual Concentration ($\mu g/m^3$)	Upper Percentile Concentrations ($\mu g/m^3$)
Brunekreef et al. (2009, 191947)	The Netherlands	$PM_{2.5}$	28.3	Max: 36.8
Bonner et al. (2005, 088993)	Western NY State	TSP	44	
Jerrett et al. (2005, 087600)	Los Angeles, California	$PM_{2.5}$		Max:27.1
Laden et al. (2006, 087605)	6 U.S. cities	$PM_{2.5}$	Range of means across sites: 10.2-29.0 Avg of means across sites: 16.4	
Naess et al. (2007, 090736)	Oslo, Norway	$PM_{2.5}$	15	Max: 22
		PM_{10}	19	Max: 30
Palli et al. (2006, 156837)	Florence, Italy	PM_{10}	NR	
Pedersen et al. (2006, 156848)	Czech Republic	$PM_{2.5}$		Max: 46-120
		PM_{10}		Max: 120-238.6
Sorensen et al. (2005, 083053)	Copenhagen, Denmark	$PM_{2.5}$	Range of means across sites: 12.6-20.7 Avg of means across sites: 16.7	75th: 24.9-27.7
Sram et al. (2007, 188457)	Czech Republic	PM_{10}		Max: 55
		$PM_{2.5}$		Max: 38
Sram et al. (2007, 192084)	Czech Republic	PM_{10}	Range of means across sites: 36.4-55.6 Avg of means across sites: 46.0	
		$PM_{2.5}$	Range of means across sites: 24.8-44.4 Avg of means across sites: 34.6	
Vineis et al. (2006, 192089)	Multi-city, Europe	PM_{10}	Range of means across sites: 19.9-73.4 Avg of means across sites: 35.4	
Vinzents et al. (2005, 087482)	Copenhagen, Denmark	PM_{10}	Range of means across sites: 16.9-23.5 Avg of means across sites: 20.2	

A subset of the ACS cohort study from 1982 to 2000 that included only residents of Los Angeles, California was used to examine the association between $PM_{2.5}$ and lung cancer mortality while adjusting for both individual and neighborhood covariates (Jerrett et al., 2005, 087600). There was a positive association between $PM_{2.5}$ and lung cancer mortality when adjusting for 44 individual covariates (RR 1.44 [95% CI: 0.98-2.11] per 10 $\mu g/m^3$ increase in $PM_{2.5}$). However, including all potential individual and neighborhood covariates associated with mortality reduced the association

(RR 1.20 [95% CI: 0.79-1.82] per 10 $\mu g/m^3$ increase in $PM_{2.5}$). A recent re-analysis of the full ACS cohort also demonstrated a positive association between $PM_{2.5}$ and lung cancer mortality (RR 1.11 [95% CI: 1.04-1.18]) (Krewski et al., 2009, 191193). The authors observed modification of this risk by educational attainment, with those completing a high school degree or less having greater risk. In addition to utilizing the ACS cohort for a nationwide analysis, this same study conducted two regional assessments, one in the New York City area and the other in the Los Angeles area. No association was detected between $PM_{2.5}$ and lung cancer mortality in the analysis of the region included in the New York City analysis. A positive association was observed in the Los Angeles-area analysis using an unadjusted model, but this association did not persist after control for individual, ecologic, and copollutant covariates.

The Six Cities Study was extended to include data from 1990-1998, a period including 1,368 deaths and 54,735 person-years (Laden et al., 2006, 087605). An elevated risk ratio for lung cancer mortality was reported when the entire follow-up period (1974–1998) was included in the analysis (RR 1.27 [95% CI 0.96-1.69] per 10 $\mu g/m^3$ increase in average annual $PM_{2.5}$). However, estimated decreases in $PM_{2.5}$ were not associated with reduced lung cancer mortality (RR 1.06 (95% CI: 0.43-2.62] for every 10 $\mu g/m^3$ reduction in $PM_{2.5}$).

Naess et al. (2007, 090736) studied individuals aged 51-90 yr living in Oslo, Norway in 1992. Death certificate data were obtained for 1992-1998 and information on PM was collected from 1992-1995. Women had a larger association of lung cancer mortality with $PM_{2.5}$ compared to men. Similar results were reported for PM_{10}.

Most recently, Brunekreef et al. (2009, 191947) used the Netherlands cohort study (NLCS) on diet and cancer to conduct a re-analysis of the research performed by Beelen et al. (2008, 156263) examining the association between PM and both lung cancer mortality and incidence. After 10 yr of follow-up, there was no association between $PM_{2.5}$ and lung cancer mortality for either the analysis of the full cohort (n = 105,296) (RR 1.06 [95% CI: 0.82-1.38] per 10 $\mu g/m^3$ increase in $PM_{2.5}$) or the case-cohort (n = 4,075) (RR 0.87 [95% CI: 0.52-1.47]). There was also no association with black smoke or traffic density variables, although living near a major roadway was associated with an elevated relative risk for lung cancer in the full cohort analysis (RR 1.20 [95% CI: 0.98-1.47]). The association was not present in the case-cohort analysis (RR 1.07 [95% CI: 0.70-1.64]).

In addition to lung cancer mortality, Brunekreef et al. (2009, 191947) also examined the association with lung cancer incidence using 11.3 yr of follow-up data. In both the full cohort and the case-cohort analyses no association was reported between $PM_{2.5}$ and lung cancer incidence (full cohort: RR 0.81 [95% CI: 0.63-1.04]; case-cohort: RR 0.67 [95% CI: 0.41-1.10] per 10 $\mu g/m^3$ increase in $PM_{2.5}$). The same was true for analyses of BS and traffic density variables.

The association between PM and incident lung cancers was examined in the European Prospective Investigation into Cancer and Nutrition study (EPIC) (Vineis et al., 2006, 192089). Within this cohort, a nested case-control study, the GenAir study, included cases of incident cancer and controls matched on age, gender, smoking status, country of recruitment, and time between recruitment and diagnosis. Only non-smokers and former smokers who had quit smoking at least 10 yr prior were included. The study included 113 cases and 312 controls. No association was seen between PM_{10} and lung cancer (OR 0.91 [95% CI: 0.70-1.18] per 10 $\mu g/m^3$). The OR was elevated when cotinine, a marker for cigarette exposure, was included in the model but the authors state that this is probably due to small study size (OR 2.85 [95% CI: 0.97-8.33] comparing $\geq$ 11 $\mu g/m^3$ to <11 $\mu g/m^3$). Control for other potential confounders, such as BMI, education level, and intake of fruit and vegetables, did not have a large impact on the estimate.

Table 7-7. Associations* between ambient PM concentrations from select studies of lung cancer mortality and incidence.

Study	Cohort	Location	Years	Analysis subgroup	Effect Estimate (95% CI)
MORTALITY - $PM_{2.5}$					
Dockery et al. (1993, 044457)‡	Six-Cities	Six cities across the U.S.	1974-1991		1.18 (0.89-1.57)
Krewski et al. (2000, 012261)‡	Six-Cities-Re-analysis	Six cities across the U.S.	1974-1991		1.16 (0.86-1.23)
Laden et al. (2006, 087606)	Six-Cities	Six cities across the U.S.	1974-1998		1.27 (0.96-1.69)
Beelen et al. (2008, 156263)	NLCS	Netherlands	1987-1996	Full Cohort	1.06 (0.82-1.38)
Beelen et al. (2008, 156263)	NLCS	Netherlands	1987-1996	Case Cohort	0.87 (0.52-1.47)
Brunekreef et al. (2009, 191947)	NLCS-Re-analysis	Netherlands	1987-1996	Full Cohort	1.06 (0.82-1.38)
Brunekreef et al. (2009, 191947)	NLCS-Re-analysis	Netherlands	1987-1996	Case Cohort	0.87 (0.52-1.47)
Pope et al. (1995, 045159)‡	ACS	U.S.	1982-1989		1.01 (0.91-1.12)
Pope et al. (2002, 024689)‡	ACS	U.S.	1982-2000		1.13 (1.04-1.22)
Jerret et al. (2005, 087600)	ACS-LA	Los Angeles	1982-2000	Intra-metro Los Angeles	1.44 (0.98-2.11)
Krewski et al. (2009, 191193)	ACS-Re-analysis	U.S.	1982-2000		1.11 (1.04-1.18)
Krewski et al. (2009, 191193)	ACS-Re-analysis	New York City	1982-2000	Intra-metro New York City	0.90 (0.29-2.78)
Krewski et al. (2009, 191193)	ACS-Re-analysis	Los Angeles	1982-2000	Intra-metro Los Angeles	1.31 (0.90-1.92)
McDonnell et al. (2000, 010319)†	AHSMOG	California	1973-1977	Men	1.39 (0.79-2.46)
Naess et al. (2007, 090736)		Oslo, Norway	1992-1998	Men, 51-70 yrs	1.18 (0.93-1.52)
Naess et al. (2007, 090736)		Oslo, Norway	1992-1998	Men, 71-90 yrs	1.16 (0.93-1.52)
Naess et al. (2007, 090736)		Oslo, Norway	1992-1998	Women, 51-70 yrs	1.63 (1.36-2.47)
Naess et al. (2007, 090736)		Oslo, Norway	1992-1998	Women, 71-90 yrs	1.45 (1.05-2.02)
MORTALITY - PM_{10}					
McDonnell et al. (2000, 010319)†	AHSMOG	California	1973-1977	Men	1.23 (0.84-1.80)
Naess et al. (2007, 090736)†		Oslo, Norway	1992-1998	Men, 51-70 yrs	1.12 (0.95-1.33)
Naess et al. (2007, 090736)		Oslo, Norway	1992-1998	Men, 71-90 yrs	1.14 (0.97-1.36)
Naess et al. (2007, 090736)†		Oslo, Norway	1992-1998	Women, 51-70 yrs	1.50 (1.23-1.84)
Naess et al. (2007, 090736)†		Oslo, Norway	1992-1998	Women, 71-90 yrs	1.29 (1.03-1.60)
INCIDENCE - $PM_{2.5}$					
Beelen et al. (2008, 156681)	NLCS	Netherlands	1987-1996	Full Cohort	0.81 (0.63-1.04)
Beelen et al. (2008, 156681)	NLCS	Netherlands	1987-1996	Case Cohort	0.65 (0.41-1.04)
Brunekreef et al. (2009, 191947)	NLCS-Re-analysis	Netherlands	1987-1996	Full Cohort	0.81 (0.63-1.04)
Brunekreef et al. (2009, 191947)	NLCS-Re-analysis	Netherlands	1987-1996	Case Cohort	0.67 (0.41-1.10)
INCIDENCE – PM_{10}					
Beeson et al. (1998, 048890)	AHSMOG	California	1977-1992	Men	1.99 (1.32-3.00)
Vineis et al. (2006, 192089)	GenAir	Europe	1993-1999	Case-Control	0.91 (0.70-1.18)

*per 10 $\mu g/m^3$ increase

†Results from the paper were standardized to10 $\mu g/m^3$ [For McDonnell et al. (2000, 010319) the non-standardized results were reported based on IQR increments (24.3 $\mu g/m^3$ for $PM_{2.5}$ and 29.5 $\mu g/m^3$ for PM_{10}). For Naess et al. (2007, 090736) the original hazard ratios were calculated based on quartiles of PM exposure. The results were converted to10 $\mu g/m^3$ using the mean range of the four quartiles (3.96 $\mu g/m^3$ for $PM_{2.5}$ and 5.88 $\mu g/m^3$ for PM_{10})].

‡Study was included in the 2004 PM AQCD

7.5.1.2. Other Cancers

Bonner et al. (2005, 088993) conducted a population-based, case-control study of the association between ambient exposure to PAHs in early life and breast cancer incidence among women living in Erie and Niagara counties in the state of New York. Cases (n = 1,166 of which 841 were post-menopausal) were women with primary breast cancer, and controls (n = 2,105 of which 1,495 were post-menopausal) were frequency matched to the cases by age, race, and county of residence. TSP was used as a proxy for PAH exposure. Annual average TSP concentrations (1959-1997) were obtained from the New York State Department of Environmental Conservation for Erie and Niagara Counties. Among postmenopausal women, exposure to high concentrations of TSP (>140 $\mu g/m^3$) at birth was associated with an OR of 2.42 for breast cancer (95% CI: 0.97-6.09) relative to low concentrations of TSP (<84 $\mu g/m^3$). ORs were elevated for pollution exposures at age of menarche (OR: 1.45 [95% CI: 0.74-2.87]) and age at first birth (OR: 1.33 [95% CI: 0.87-2.06]) among postmenopausal women. Among premenopausal women, exposure to high concentrations of TSP at birth was associated with an OR for breast cancer incidence of 1.79 (95% CI: 0.62-5.10) relative to low exposure levels, exposure at age of menarche was associated with an OR of 0.66 (95% CI: 0.38-1.16), and exposure at age of first birth was associated with an OR of 0.52 (95% CI: 0.22-1.20).

7.5.1.3. Markers of Exposure or Susceptibility

Several studies looked at markers of exposure or susceptibility as the outcome associated with short-term exposure. These studies are included here because they may be relevant to the mechanism that leads to cancer associated with long-term exposures. For example, inflammation can contribute to carcinogenesis by inducing genomic instability, which can then lead to altered gene expression, enhanced proliferation, and resistance to apoptotic signals. Reactive oxygen and nitrogen species, provided by PM components or inflammation pathways, can cause molecular damage leading to cellular transformation. Elevated inflammatory cytokines, chemokines, and prostaglandins promote tumor growth and angiogenesis, which in turn promotes metastasis and malignant invasion. In particular, IL-6, IL-8, IL-1β, COX-2, and TNF-α have been implicated in these processes (Kundu and Surh, 2008, 198840). Several lines of evidence support the involvement of COX-2 in the pathogenesis of lung cancer (Lee et al., 2008, 198811). Both short- and long-term exposure studies demonstrate relationships between various forms of PM and increased production of these inflammatory mediators, both in the lungs and circulation. Additionally, limited evidence suggests that exposure to PM (Chen and Schwartz, 2008, 190106), or traffic (Williams et al., 2009, 191945), or residence in a polluted airshed (Calderon-Garciduenas et al., 2007, 091252; Calderón-Garcidueñas et al., 2009, 192107) are associated with decreases in the number or function of natural killer cells or other white blood cells, indicating suppression of anti-tumor defenses.

A study performed in the Czech Republic compared 53 male policemen working at least 8 hours per day outdoors in urban air with age- and sex-matched controls who spent at least 90% of their day indoors (n = 52) (Sram et al., 2007, 188457). During the sampling period, two monitors from downtown and suburban areas detected levels of air pollutants in the following ranges: PM_{10} 32-55 $\mu g/m^3$, $PM_{2.5}$ 27-38 $\mu g/m^3$, c-PAHs 18-22 ng/m^3, and B[a]P 2.5-3.1 ng/m^3 using a VAPS monitor (measurements taken with a HiVol monitor, which has a lower flow rate, had a mean for PM_{10} of 62.6 $\mu g/m^3$). c-PAHs detected on personal monitors during sampling days had a mean of 12.04 ng/m^3 among the policemen and 6.17 ng/m^3 among the controls. No difference in percent of chromosomal aberrations was observed between the policemen and control group using conventional cytogenetic analysis. However, using fluorescent in situ hybridization (FISH), a difference in chromosomal aberrations between the policemen and control group was reported. For example, the percentage of aberrant cells, as well as the genomic frequency of translocations per 100 cells, was about 1.4-fold greater in the policemen. This was largely driven by a difference in chromosomal aberrations between nonsmoking policemen and nonsmoking controls. A similar study that included only the policemen (n = 60), reported that the mean exposure to c-PAHs and B[a]P for 40-50 days before sampling was associated with chromosomal aberrations when analyzed with FISH (Sram et al., 2007, 192084). However, when included in a model with other covariates, the association with these variables was null. No association was present with use of conventional cytogenetic analysis.

Palli et al. (2008, 156837) investigated the correlation between ambient PM_{10} concentrations and individual levels of DNA bulky adducts. Study participants were 214 healthy adults aged

35-64 yr at enrollment who resided in the city of Florence, Italy. This study was conducted between 1993 and 1998. PM_{10} exposure levels were based on daily environmental measures provided by two types of urban monitoring stations (high-traffic and low-traffic). The researchers assessed correlation between DNA bulky adducts measured in blood samples and PM_{10} concentrations prior to blood sample collection. Time windows of PM_{10} exposure evaluated in this study were 0-5 days, 0-10 days, 0-15 days, 0-30 days, 0-60 days, and 0-90 days prior to blood sample collection. Overall, average PM_{10} concentrations decreased during the study period, with some fluctuations. Quantitative values were not reported, but PM_{10} appeared to range between approximately 30 and 100 $\mu g/m^3$ for high-traffic stations, and between approximately 20 and 50 $\mu g/m^3$ for low-traffic stations. This study found that levels of DNA bulky adducts among non-smoking workers with occupational traffic exposure were positively correlated with cumulative PM_{10} levels from high-traffic stations during approximately 2 wk preceding blood sample collection (0-5 days: $r = 0.55$, $p = 0.03$; 0-10 days: $r = 0.58$, $p = 0.02$; 0-15 days: $r = 0.56$, $p = 0.02$). DNA bulky adducts were not associated with PM_{10} levels among Florence residents with no occupational exposure to vehicle emissions or among smokers. DNA bulky adducts were not associated with PM_{10} levels assessed by low-traffic urban monitoring stations.

The association between personal exposure to water-soluble transition metals in $PM_{2.5}$ and oxidative stress-induced DNA damage was investigated among 49 students from Central Copenhagen, Denmark (Sorensen et al., 2005, 083053). Researchers assessed $PM_{2.5}$ exposure by personal sampling over two weekday periods twice in one year (November 1999 and August 2000), and determined the concentration of water-soluble transition metals (V, Cr, Fe, Ni, Cu and Pt) in these samples. In addition, lymphocyte and 24-h urine samples were analyzed for DNA damage by measuring 7-hydro-8-oxo-2'-deoxyguanosine (8-oxodG). Mean concentrations and corresponding IQR of these metals differed between months of sample collection. This study found that 8-oxodG concentration in lymphocytes was significantly associated with V and Cr concentrations, with a 1.9% increase in 8-oxodG per 1 µg/L increase in V concentration and a 2.2% increase in 8-oxodG per 1 µg/L increase in Cr concentration; these associations were independent of the $PM_{2.5}$ mass concentration. The other transition metals were not significantly associated with the 8-oxodG concentration in lymphocytes, and none of the six measured transition metals was associated with the 8-oxodG concentration in urine.

Vinzents et al. (2005, 087482) investigated the association between UFP and PM_{10} concentrations with levels of purine oxidation and strand breaks in DNA using a crossover design in Copenhagen, Denmark. Study participants were 15 healthy nonsmoking individuals with a mean age of 25 yr. UFP exposure was evaluated using number concentration obtained in the breathing zone by portable instruments in six 18-h weekday periods from March to June 2003. Ambient concentrations for PM_{10} and UFP were also measured on all exposure days at curbside street stations and at one urban background station. Oxidative DNA damage was assessed by evaluating strand breaks and oxidized purines in mononuclear cells isolated from venous blood the morning after exposure measurement. Mean number concentration of UFPs (street station) was 30.4×10^3 UFPs/mL (standard deviation [SD]: 1.38), mean mass concentration of PM_{10} at a background monitoring station was 16.9 $\mu g/m^3$ (SD: 1.53), and mean mass concentration of PM_{10} at a street station was 23.5 $\mu g/m^3$ (SD: 1.48). Mean personal exposure to UFPs was 32.4×10^3 UFPs/mL (SD: 1.49) while bicycling (5 occasions), 19.6×10^3 UFPs/mL (SD: 1.78) during other outdoor activities (6 occasions), and 15.4×10^3 UFPs/mL (SD: 1.96) while indoors (6 occasions). The regression coefficients of the mixed-effects models looking at level of purine oxidation were estimated as 1.50×10^{-3} (95% CI: 0.59×10^{-3} to 2.42×10^{-3}; $p = 0.002$) for cumulative outdoor exposure and 1.07×10^{-3} (95% CI: 0.37×10^{-3} to 1.77×10^{-3}; $p = 0.003$) for cumulative indoor exposure. Neither cumulative outdoor nor cumulative indoor exposures to UFPs were associated with strand breaks. Neither ambient air concentrations of PM_{10} nor number concentrations of UFPs at monitoring stations were significant predictors of DNA damage.

Additionally, a number of studies employed ecologic study designs, comparing the prevalence of biomarkers in populations from more polluted locations to those in less polluted locations. In a pilot study conducted in the Czech Republic (Pedersen et al., 2006, 156848), children age 5-11 yr provided 5 mL blood samples and the frequency of micronuclei (MN) in peripheral blood lymphocytes was analyzed as a measure of cytogenetic effects. Significantly higher frequencies of MN were found in younger children living in Teplice ($PM_{2.5}$ concentration = 120 $\mu g/m^3$) than in Prachatice ($PM_{2.5}$ concentration = 46 $\mu g/m^3$). The levels of c-PAHs were also much higher in Teplice (nearly 30 ng/m^3 in Teplice and about 15 ng/m^3 in Prachatice). The difference in MN frequencies

observed in the children from the two locations may be attributable to differences in exposure to air pollution, but could also be due to differences in diet or other environmental exposures. This finding is noteworthy considering MN formation in peripheral blood lymphocytes is thought to be biologically relevant for carcinogenesis.

Avogbe et al. (2005, 087811) showed a correlation between the level of oxidative DNA damage in individuals and exposure to ambient UFPs. Formamidopyrimidine DNA glycosylase sensitive sites and the presence of DNA strand breaks were assessed in blood and urine samples obtained from healthy, non-smoking male volunteers that lived and worked in different areas of Cotonou, Benin. Exposure to benzene was assessed by urinary excretion of S-phenylmercapturic acid. There was a high degree of correlation between exposure to benzene and UFPs and the presence of DNA strand breaks and formamidopyrimidine DNA glycosylase sensitive sites (rural subjects < suburban subjects < residents living near high traffic roads < taxi drivers). Genotyping studies showed that the magnitude of the effects of benzene and UFPs may be modified by polymorphisms in GSTP1 and NQO1 genes.

Tovalin et al. (2006, 091322) evaluated the association between exposure to air pollutants and the level of DNA damage using the single cell gel electrophoresis (comet) assay. Mononuclear lymphocytes from outdoor and indoor workers from two areas in Mexico, Mexico City (large city) and Puebla (medium size city), were evaluated. The outcomes showed that the outdoor workers in Mexico City exhibited greater DNA damage than indoor workers in the same region. Similar levels of DNA damage were observed between indoor and outdoor workers in Puebla. The level of observed DNA damage was correlated with exposure to O_3 and $PM_{2.5}$.

In summary, several recent studies have reported an association between lung cancer mortality and long-term $PM_{2.5}$ exposure. Although many of the estimates include the null in the confidence interval, overall the results have shown a positive relationship. The two recent studies that looked at lung cancer incidence did not report an association with $PM_{2.5}$ (Brunekreef et al., 2009, 191947) or PM_{10} (Vineis et al., 2006, 192089). Studies of exposure/susceptibility markers have reported inconsistent outcomes, with some markers being associated with PM and others not.

7.5.2. Toxicological Studies

Over the past 30 yr numerous mutagenicity and genotoxicity studies of ambient PM and their contributing sources have been conducted to assess the relative mutagenic or genotoxic potential. Studies previously reviewed in the 2004 PM AQCD (U.S. EPA, 2004, 056905) provide compelling evidence that ambient PM and PM from specific combustion sources (e.g., fossil fuels) are mutagenic in vivo and in vitro. Research cited in the 2004 AQCD demonstrated mutagenic activity of ambient PM from urban centers in California, Germany and the Netherlands. These studies suggested that ubiquitous emission sources, particularly motor vehicle emissions, rather than isolated point sources were largely responsible for the mutagenic effects. In addition, the mutagenicity was dependent upon the chemical composition of the PM with unsubstituted polyaromatic compounds and semi-polar compounds being highly mutagenic. Mutagenicity was also dependent on size, with the fine fraction of urban PM having greater effects than the coarse fraction. Genotoxic activity was demonstrated for ambient PM from two high traffic areas (one upwind and one downwind) and a rural site. In addition, the 2004 AQCD reported that exhausts from gasoline and diesel engines were mutagenic and that DE was more potent. More mutagenicity was observed for exhaust from cold starts than starts at room temperature. Both gaseous and particulate fractions of DE were found to be mutagenic. Sequential fractionation of extracts from gasoline and DE implicated the polar fractions, especially nitrated polynuclear aromatic compounds, as contributing greatly to mutagenicity. Among some of the other mutagenically active compounds found in the gas phase of DE are ethylene, benzene, 1,3-butadiene, acrolein and several PAHs, all of which are also present in gasoline exhaust. Also cited in the 2004 AQCD were studies demonstrating mutagenic effects of emissions from wood/biomass burning, which were primarily attributable to the organic fraction and not the condensate. It was noted that wood smoke induced both frameshift mutations and base pair substitution but not DNA adducts. Further, emissions from coal combustion in China were found to be mutagenic, with both polar and aromatic fractions contributing to effects. Little data were available on the mutagenicity of coal fly ash emissions from U.S. conventional combustion plants. In conclusion, these studies provide evidence that ambient PM and combustion-derived PM are mutagenic/genotoxic. The 2004 AQCD noted that there is not a simple relationship between

mutagenic potential and carcinogenic potential in animals or humans. No studies evaluating carcinogenic effects of PM were reported in the 2004 AQCD.

Building on results of earlier studies in the 2004 PM AQCD, data from newly published studies that evaluated the mutagenic, genotoxic and carcinogenic effects of PM, PM-constituents, and combustion emission source particles are reviewed. Pertinent studies are described briefly in the following paragraphs. A summary table is provided in Annex D, Tables D7 and D8).

7.5.2.1. Mutagenesis and Genotoxicity

In Vitro Studies

In general, studies have focused on PM and PM extracts for mutagenicity testing using bacteria and mammalian cell lines. PM and/or PM extracts from ambient air samples, wood smoke, and coal, diesel, or gasoline combustion have all been reported to induce mutation in *S. typhimurium* and in cultured human cells (Abou et al., 2007, 098819; Gabelová et al., 2007, 156457; Gabelová et al., 2007, 156458; Hannigan et al., 1997, 083598; Hornberg et al., 1998, 095741). In addition, effects associated with PM and PM-associated constituents include induction of MN formation, DNA adduct formation, SCE, DNA strand breaks, frameshifts and inhibition of gap-junction intercellular communication (Alink et al., 1998, 087159; Arlt et al., 2007, 097257; Avogbe et al., 2005, 087811; Gabelová et al., 2007, 156457; Gabelová et al., 2007, 156458; Healey et al., 2006, 156532; Hornberg et al., 1996, 087164; Hornberg et al., 1998, 095741; Sevastyanova et al., 2007, 156969).

Constituents adsorbed onto individual particles play a large role in the genotoxic potential of PM. Poma et al. (2006, 096903) showed that fine CB particles were consistently less genotoxic than similar concentrations of $PM_{2.5}$ extracts, suggesting that the adsorbed components play a role in the genotoxic potential of PM. Total PAH and carcinogenic PAH content were correlated with the genotoxic effects of PM (De Kok et al., 2005, 088656; Sevastyanova et al., 2007, 156969). Comparison of different extracts (water-soluble versus organic) by Gutierrez-Castillo et al. (2006, 089030) indicated that water-soluble extracts were more genotoxic than the corresponding organic extracts. Sharma et al. (2007, 156975) reported that mutagenic activity of extracted PM samples collected in and around a waste incineration plant was attributed to the moderately polar and polar fractions. The polar and crude fractions were mutagenic without metabolic activation, suggesting a direct mutagenic effect. No mutagenic activity was observed from any of the nonpolar samples evaluated. Arlt and colleagues (2007, 097257) have shown that the PM constituents 2-nitrobenzanthrone (2-NB) and 3-nitrobenzanthrone were genotoxic in a variety of bacterial and mammalian cell systems.

Conflicting data have been reported on the role of metabolic enzymes in the genotoxicity of PM and their adsorbed constituents. Arlt et al. (2007, 097257) reported that the PM constituent 2-NB was genotoxic in bacterial and mammalian cells. However, metabolic activation with the human N-acetyltransferase 2 or sulfotransferase (SULT1A1) enzyme was needed for the effect to be observed in human cells. Erdinger et al. (2005, 156423) demonstrated that mutagenic activity was not affected when metabolism was induced. de Kok et al. (2005, 088656) evaluated the relationship between the physical, chemical, and genotoxic effects of ambient PM. TSP, PM_{10}, and $PM_{2.5}$ were sampled at different locations and the extracts were assessed for mutagenicity and induction of DNA adducts in cells. Overall, induction of rat liver S9 metabolism generally reduced the mutagenic potential via the Ames assay of the particle fractions and DNA reactivity (induction of DNA adducts) was generally higher after metabolic activation. Binková et al. (2003, 156274) found that the addition of S9 increased PM_{10}-dependent DNA adduct formation.

Ambient Air

A limited number of studies evaluated the impact of the season on the genotoxic effects of ambient PM. A few studies have indicated that greater genotoxic effects were associated with samples collected during the winter months compared to those collected in the summer (Abou et al., 2007, 098819; Gabelová et al., 2007, 156457; Gabelová et al., 2007, 156458). In contrast, Hannigan et al. (1997, 083598) indicated that no seasonal variation was observed. Studies have also shown that greater genotoxic effects were associated with smaller particle size extracts (e.g., $PM_{2.5}$>PM_{10}) and

from samples collected in urban areas or closer to higher trafficked areas (Abou et al., 2007, 098819; Hornberg et al., 1998, 095741).

de Kok et al. (2005, 088656) found the direct mutagenicity (Ames assay) and the direct DNA reactivity (DNA adduct formation) of the $PM_{2.5}$ size fraction was significantly higher than that of the larger size fractions (TSP, PM_{10}) at most locations.

DNA damage was assessed by the Comet assay in A549 cells exposed to PM collected from a high traffic area in Copenhagen, Denmark (TSP approximately 30 $\mu g/m^3$) and compared to the results from exposure of A549 cells to standard reference materials (SRM1650 or SRM2975) at the same concentrations (2.5-250 µg/ml) (Danielsen et al., 2008, 192092). All three particles induced strand breaks and oxidized purines in a dose-dependent manner and there were no obvious differences in potency. In contrast, only the ambient PM formed 8-oxodG when incubated with calf thymus DNA , which may be due to the concentration of transition metals.

Diesel and Gasoline Exhaust

Automobile DE particles (A-DE particles) was tested in *S. typhimurium* strains TA98, TA100, and its derivatives (e.g., TA98NR and YG1021) and found to be more mutagenic than forklift DE particles (f-DE particles, derivative SRM2975), based on PM mass. A-DE particles had 227 times more PAH-type mutagenic activity and 8-45 times more nitroarene-type mutagenic activity (DeMarini et al., 2004, 066329). Using a diesel engine without an oxidation catalytic converter (OCC), the diesel engine exhaust particle extract produced the highest number of revertant colonies in strains TA98 and TA100 with and without S9 at several tested loads when compared to extracts from low-sulfur diesel fuel (LSDF), rapeseed oil methyl ester (RME), and soybean oil methyl ester (SME). When an OCC was installed in the exhaust pipe of the engine, all extracts reduced the number of revertant colonies in both strains with and without S9 at partial loads but increased the number of revertant colonies without S9 at rated power. At idling, DE particles extracts increased the number of revertant colonies with and without S9 (Bunger et al., 2006, 156303). In a separate study, engine emissions (particle extracts and condensates) from rapeseed (canola) oil were found to produce greater mutagenic effects in *S typhimurium* strains TA98 and TA100 than DE particles (Bunger et al., 2007, 156304). Additionally, DE extract (DEE) from diesel fuel containing various percentages of ethanol was also observed to induce mutational response in two Salmonella strains. Base diesel fuel DEE and DEE from fuel with 20% ethanol caused more significant DNA damage in rat fibrocytes L-929 cells than extracts containing 5, 10, or 15% ethanol (Song et al., 2007, 155306).

DE and gasoline engine exhaust particles, as well as their semi-volatile organic compound (SVOC) extracts, induced mutations in the two *S. typhimurium* strains YG1024 and YG1029 in the absence and presence of S9; the PM extracts were more mutagenic than the SVOC extracts. Additionally, all extracts except the DE extract induced DNA damage and MN formation in Chinese hamster lung V79 cells (Liu et al., 2005, 097019). Another study demonstrated that gasoline engine exhaust significantly increased colony formation in TA98 with and without S9 (Zhang et al., 2007, 157186).

Jacobsen et al. (2008, 156597) used the FE1-Muta™ Mouse lung epithelial cell line to investigate putative mechanisms of DE particle-induced mutagenicity. Mutation ion frequencies and ROS were determined after cells were incubated with 37.5 or 75 µg/ml DE particles (SRM1650) for 72-h (n = 8). The mutation frequency at the 75 µg/ml dose was significantly increased (1.55-fold; $p<0.001$) in contrast to cells treated with 37.5 µg/ml DE particles. DE particles-induced ROS generation 1.6- to 1.9-fold in the epithelial cell cultures after 3 h of exposure compared with the 3- to 10-fold increase in ROS production previously reported for CB. The authors concluded that the mutagenic activity of DE particles is likely attributable to activity from the organic fraction that both contains reactive species and can generate ROS.

In human A549 and CHO-K1 cells, the organic fraction of DE particles significantly increased the amount of Comet and MN formation, respectively, in the presence and absence of SKF-525A (a CYP450 inhibitor) and S9, respectively (Oh and Chung, 2006, 088296). The organic base and neutral fractions of DE particles also significantly induced DNA damage but only without SKF-525A, and all fractions but the moderately polar fraction (phthalates and PAH oxyderivatives) induced MN formation with and without S9 (Bao et al., 2007, 097258). Gasoline engine exhaust significantly induced DNA damage as measured in the Comet assay and increased the frequency of MN in human A549 cells (Zhang et al., 2007, 157186). In human-hamster hybrid (A_L) cells, DE particles (SRM 2975) dose-dependently increased the mutation yield at the *CD59* locus; this was

significantly reduced by simultaneous treatment with phagocytosis inhibitors (Bao et al., 2007, 097258).

Wood Smoke

The mutagenicity of wood smoke and cigarette smoke (CS) extracts was assayed in *S. typhimurium* strains TA98 and TA100 (Ames assay) using the pre-incubation assay with exogenous metabolic activation (rat liver S-9). Extracts of both samples (62.5 or 125 µg total PM equivalent/ml) were equally mutagenic to strain TA98 but the wood smoke extract was less mutagenic than the CS extracts in strain TA100 (Iba et al., 2006, 156582).

In Vivo studies

Ambient Air

The contribution of ambient urban roadside air exposure (4, 12, 24, 48 or 60 wk) to DNA damage was examined in the lungs, nasal mucosa, and livers of adult male Wistar rats in Kawasaki, Japan (Sato et al., 2003, 096615). Messenger RNA levels of CYP450 enzymes that catalyze the transformation of PAHs to reactive metabolites were also evaluated. Concentrations of gases were reported to be 12-182 ppb NO and 0-9 ppb NO_2 in the filtered air chamber and 33-280 ppb NO and 42-81 ppb NO_2 in the experimental group chamber. Suspended PM concentrations were 11-19 µg/m^3 in the filtered air chamber and 42-100 µg/m^3 (average 63 µg/m^3) in the experimental group chamber. Body weight significantly decreased in exposed animals at 24, 48 and 60 wk. A 4-wk exposure to urban roadside air resulted in significant increases in multiple DNA adducts (lung, nasal, and liver DNA adducts). With longer exposures, there were significant increases in lung (48 wk), nasal (60 wk), and liver DNA adducts (60 wk). Changes were seen in CYP1A2 mRNA at 4 wk with a 2.3-fold increase in exposed animals compared to the control group with no change observed at 60 wk; CYP1A1 mRNA was unchanged. These results indicate that exposure to ambient air in this roadside area could induce DNA adduct formation, which may be important for carcinogenicity. Earlier studies (Ichinose et al., 1997, 053264) have shown that 8-oxodG, a DNA adduct, is elevated along with tumor formation in a dose-dependent manner in mice administered DE particles. The finding of adducts in the liver indicated that deposition of PM and its associated PAHs in the lung can have indirect effects on extrapulmonary organs. It should be noted that PM deposition on the fur and ingestion during grooming cannot be ruled out as a possible exposure route.

Another animal toxicological study employed "non-carcinogenic" particles obtained from pooled non-cancerous lung tissue collected during surgical lung resection from three non-smoking male patients diagnosed with lung adenocarcinomas (Tokiwa et al., 2005, 191952). Particles were partially purified to remove organic compounds. Morphologically the particles were similar to DE or ambient air PM and the organic extracts from the particles were directly mutagenic in S. typhimurium tester strains TA98, YG1021 and YG1024. BALB/c and ICR mice were intratracheally instilled with particles at doses of 0.25, 0.5, 1.0, or 2.0 mg/mouse. After 24 h, 8-oxodG was measured in lung DNA and found to be increased in ICR mice in a dose-dependent manner, reaching a maximum of ~2.75 8-oxodG/10^5 dG at the 2.0 mg dose. The response was statistically significant at doses of 0.5, 1.0, and 2.0 mg. The increased 8-oxodG levels observed in vivo was reported to be likely due to hydroxyl radicals presumed to be involved in phagocytosis of non-mutagenic particles by inflammatory cells that could induce hydroxylation of guanine residue on DNA.

Diesel Exhaust

An in vivo study employed *gtp* delta transgenic mice carrying the lambda EG10 on each Chromosome 17 from a C57BL/6J background to investigate the effects of DE particles on mutation frequency (Hashimoto et al., 2007, 097261). Mice were exposed via inhalation to DE particles or via IT instillation to DE particles or DE particle extract and lambda EG10 phages were rescued; *E. coli YG6020* was infected with the phage and screened for 6-thioguanine resistance. The mutagenic potency (mutation frequency per mg) caused by DE particle extract was twice that of DE particles, suggesting that the mutagenicity of DE particles is attributed primarily to compounds in the extract,

since ≈50% of the weight of DE particles was provided by the extract. There was no difference in mutation frequency between the 1 and 3 mg/m^3 DE particle groups after 12 wk of exposure.

Wood Smoke

One recent study measured the effect of freshly generated hardwood smoke on CYP1A1 activity based on ethoxyresorufin O-deethylase in pulmonary microsomes recovered from male Sprague-Dawley rats exposed to hardwood smoke by nose-only inhalation exposure (Iba et al., 2006, 156582). CYP1A1 activity in rat lung explants treated with extracts of the total PM (TPM) from hardwood smoke samples and from freshly generated cigarette smoke (CS) was also evaluated. Unlike CS, hardwood smoke did not induce pulmonary CYP1A1 activity or mRNA (assessed by northern blot analysis) nor did extracts of hardwood smoke TPM induce CYP1A1 protein (assessed by western blot analysis) in cultured rat lung explants. The results suggest that unique constituents that are activated by CYP1A1 may be present in CS but not hardwood smoke.

7.5.2.2. Carcinogenesis

Studies published prior to the 2004 AQCD that evaluated the carcinogenicity of ambient air were reviewed by Claxton and Woodall (2007, 180391). Five studies involved chronic inhalation exposures in rodents. No statistically significant increase in tumorigenesis was observed following chronic exposure to urban air pollution in Los Angeles (Gardner, 1966, 015129; Gardner et al., 1969, 015130; Wayne and Chambers, 1968, 038537). However in a study conducted in Brazil, urban air pollution was found to enhance the formation of urethane-induced lung tumors in mice (Cury et al., 2000, 192100; Reymao et al., 1997, 084653).

Two recent studies evaluated the carcinogenic potential of chronic inhalation exposures to DE (Reed et al., 2004, 055625) and hardwood smoke (Reed et al., 2006, 156043). Two indicators of carcinogenic potential, formation of MN and tumorigenesis were measured in strain A/J mice, which is a mouse model that spontaneously develops lung tumors. Exposure to DE or hardwood smoke at concentrations of 1,000 μg/m^3 and below did not cause increased formation of MN or an increased rate of lung tumors in this cancer-prone rodent model. These studies are described below.

Diesel Exhaust

A/J mice were exposed to 30, 100, 300 and 1000 μg/m^3 DE for 6 h/day and 7 days/wk for 6 mo (Reed et al., 2004, 055625). The concentration of gases in this including NO_x, NO_2, CO, SO_2, NH_3, methane, non-methane VOC, and FID total hydrocarbon ranged from control to high dose group values of 0 to 50.4 ± 0.6 ppm, 0.2 ± 0.2 to 6.9 ± 3.3 ppm, 0.3 ± 0.1 to 30.9 ± 4.5 ppm, not detectable to 955.2 ± 58.4 ppb, 176.5 ± 8.8 to 9.1 ± 0.2 μg/m^3, 1406.5 ± 253.2 to 2642.1 ± 455.9 μg/m^3, 134.0 ± 52.1 to 1578.6 ± 256.2 μg/m^3, 0.1 ± 0.1 to 2.2 ± 0.2 ppm, respectively. Particle sizes in the four exposure groups ranged from 0.10-0.15 μm MMAD with geometric standard deviations of 1.4–1.8. Following the 6-mo exposure and a 6-mo recovery period, mice were collected and MN formation in blood and tumor multiplicity and tumor incidence were measured in lungs. No increases in formation of MN or numbers of lung adenomas were observed in DE-exposed mice compared with controls.

Wood Smoke

A/J mice were exposed to 30, 100, 300 and 1,000 μg/m^3 hardwood smoke or to 30, 100, 300 and 1,000 μg/m^3 DE for 6 h/day and 7 days/wk for 6 mo (Reed et al., 2006, 156043). Gaseous components of the hardwood smoke included CO, NH_3, and non-methane VOC with concentrations from control levels to high dose hardwood smoke exposure ranging from 229 ± 31 to 14887.6 ± 832.3 ppm, 139.3 ± 2.3 to 54.9 ± 1.2 μg/m^3 and 177.6 ± 10.4 to 3455.0 ± 557.2 μg/m^3, respectively. Concentrations of NO_x, NO_2 and SO_2 were reported to be null. Particle sizes in the four exposure groups ranged from 0.25-0.36 μm MMAD with geometric standard deviations of 2.0-3.3. Following the 6-mo exposure and a 6-mo recovery period, mice were collected and MN formation in blood and tumor multiplicity and tumor incidence were measured in lungs. No increases in formation of MN or

numbers of lung adenomas were observed in hardwood smoke-exposed mice compared with controls. However, hardwood smoke from this study was mutagenic in the Ames reverse mutation assay.

7.5.2.3. Summary of Toxicological Studies

In summary, numerous new in vitro studies confirm and extend findings reported in the 2004 AQCD that ambient PM from urban sites and combustion-derived PM are mutagenic and genotoxic. A small number of new studies were conducted in vivo. One of these studies demonstrated increased mutagenic potency in mice exposed to DE particles and DE particle extract. Another study found increased formation of 8-oxodG, a DNA adduct, following IT instillation of PM in mice. A chronic inhalation study of rats exposed to urban roadside air reported increased formation of DNA adducts in nose, lung, and liver and induction of CYP1A2. Inhalation exposure of rats to hardwood smoke failed to induce CYP1A1 in another study. Finally, two chronic inhalation studies found no evidence of carcinogenic potential for DE and hardwood smoke in a cancer-prone mouse model. Collectively, these results provide some evidence, mainly from in vitro studies, to support the biological plausibility of ambient PM-lung cancer relationships observed in epidemiology studies.

7.5.3. Epigenetic Studies and Other Heritable DNA mutations

Two epidemiologic epigenetic studies examined the effect of PM on DNA methylation. Both studies examined methylation of Alu and long interspersed nuclear element-1 (LINE-1) sequences, which are located in repetitive elements. In previous studies, methylation of these sequences has been linked to global genomic DNA methylation content (Weisenberger et al., 2005, 192101; Yang et al., 2004, 192102).

The first study included men age 55 and older who were part of the Normative Aging Study in the Boston area (Baccarelli et al., 2009, 192155). A stationary monitoring site located 1 km from the examination site was used to estimate ambient $PM_{2.5}$ exposure for the duration of the study (1999-2007). During the study period, the median level of $PM_{2.5}$, averaged over 7-day periods, was 9.8 $\mu g/m^3$ (interquartile range 8.0-12.0 $\mu g/m^3$). There was no association between $PM_{2.5}$ and Alu methylation. LINE-1 methylation was associated with $PM_{2.5}$ measured over the 7 days before the examinations.

The second study included 63 healthy men aged 27-55 yr working at an electric furnace steel plant (Tarantini et al., 2009, 192010). Blood samples were taken twice, once in the morning after 2 days of not working and once in the morning after 3 full days of work. PM_{10} was measured in 11 work areas and individuals completed daily logs about the amount of time spent in each area. On average, individuals had an estimated exposure of 233.4 $\mu g/m^3$ PM_{10} (range 73.4-1220.2 $\mu g/m^3$). Short-term exposure did not alter the methylation of Alu and LINE-1. To examine effects of long-term exposure, both blood samples were considered independent of time, and Alu and LINE-1 were examined with respect to overall estimated PM_{10} exposure using mixed effects models. There was a negative association between increasing levels of PM_{10} exposure and Alu and LINE-1 methylation, indicating that PM_{10} causes epigenetic changes to occur with long-term exposure. This study also looked at levels of iNOS gene, which is a gene suppressed by DNA methylation. iNOS expression was not associated with long term exposure to PM_{10} but was affected by methylation in the short term.

Animal toxicology studies evaluating the effect of PM exposure on changes in the epigenome and other non-epigenetic heritable DNA changes have only recently been conducted. After earlier work showed increased germline mutation rates in herring gulls nesting near steel mills on Lake Ontario (Yauk and Quinn, 1996, 089093) further work was conducted to address air-dependent contribution to germline mutations by housing male and female Swiss Webster mice in the same area and comparing mutation rates in those animals with mutation rates of animals housed in a rural setting with less air pollution (Somers et al., 2002, 078100). To determine if PM or the gaseous phase of the urban air was responsible for heritable mutations, Yauk et al. (2008, 157164) exposed mature male C57Bl×CBA F1 hybrid mice to either HEPA-filtered air or to ambient air in Hamilton, Ontario, Canada for 3 or 10 wk, or 10 wk plus 6 wk of clean air exposure (16 wk) (also discussed in Section 7.4.2.5). Sperm DNA was monitored for ESTR mutations, testicular sample bulky DNA adducts, and DNA single or double strand breaks. In addition, male-germ line (spermatogonial stem

7.5.4.2. $PM_{10-2.5}$

The 2004 PM AQCD did not report long-term exposure studies for $PM_{10-2.5}$. No epidemiologic studies have been conducted to evaluate the effects of long-term $PM_{10-2.5}$ exposure and cancer. The evidence is **inadequate to assess the association between $PM_{10-2.5}$ and UFP exposures and cancer.**

7.5.4.3. UFPs

The 2004 PM AQCD did not report long-term exposure studies for UFPs. No epidemiologic studies have been conducted to evaluate the effects of long-term UFP and cancer. The evidence is **inadequate to determine if a causal relationship exists between long-term UFP exposures and cancer**.

7.6. Mortality

In the 1996 PM AQCD, results were presented for three prospective cohort studies of adult populations: the Six Cities Study (Dockery et al., 1993, 044457); the ACS Study (Pope et al., 1995, 045159); and the AHSMOG Study (Abbey et al., 1995, 000669). The 1996 AQCD concluded that the chronic exposure studies, taken together, suggested associations between increases in mortality and long-term exposure to $PM_{2.5}$, though there was no evidence to support an association with $PM_{10-2.5}$ (U.S. EPA, 1996, 079380).

Discussions of mortality and long-term exposure to PM in the 2004 PM AQCD emphasized the results of four U.S. prospective cohort studies, but the greatest weight was placed on the findings of the ACS and the Harvard Six Cities studies, which had each undergone extensive independent reanalysis, and which were based on cohorts that were broadly representative of the U.S. population. The 2004 PM AQCD concluded that the results from the Seventh-Day Adventist (AHSMOG) cohort provided some suggestive (but less conclusive) evidence for associations, while results from the Veterans Cohort provided inconsistent evidence for associations between long-term exposures to $PM_{2.5}$ and mortality. Collectively, the 2004 PM AQCD found that these studies provided strong evidence that long-term exposure to $PM_{2.5}$ was associated with increased risk of human mortality. Effect estimates for all-cause mortality ranged from 6 to 13% increased risk per 10 $\mu g/m^3$ $PM_{2.5}$, while effect estimates for cardiopulmonary mortality ranged from 6 to 19% per 10 $\mu g/m^3$ $PM_{2.5}$. For lung cancer mortality, the effect estimate was a 13% increase per 10 $\mu g/m^3$ $PM_{2.5}$, based upon the results of the extended analysis from the ACS cohort (Pope et al., 2002, 024689). With regard to $PM_{10-2.5}$, the 2004 PM AQCD reported that no association was observed between mortality and long-term exposure to $PM_{10-2.5}$ in the ACS study (Pope et al., 2002, 024689), while a positive but statistically non-significant association was reported in males in the AHSMOG cohort (McDonnell et al., 2000, 010319). Thus, the 2004 PM AQCD concluded that there was insufficient evidence for associations between long-term exposure to $PM_{10-2.5}$ and mortality. Overall, the 2004 PM AQCD concluded that there was strong epidemiologic evidence for associations between long-term exposures to $PM_{2.5}$ and excess all-cause and cardiopulmonary mortality.

At the time of the 2004 PM AQCD, only a limited number of the chronic-exposure cohort studies had considered direct measurements of constituents of PM, other than sulfates. With regard to source-oriented evaluations of mortality associations with long-term exposure, the 2004 PM AQCD noted only the study by Hoek et al. (2002, 042364), in which the authors concluded that long-term exposure to traffic-related air pollution may shorten life expectancy. However, Hoek et al. (2002, 042364) also noted that living near a major road might include other factors that contribute to mortality associations. There was not sufficient evidence at the time of the 2004 PM AQCD to draw conclusions on effects associated with specific components or sources of PM.

New epidemiologic evidence reports a consistent association between long-term exposure to $PM_{2.5}$ and increased risk of mortality. There is little evidence for the long-term effects of $PM_{10-2.5}$ on mortality. Although this section focuses on mortality outcomes in response to long-term exposure to PM, it does not evaluate studies that examine the association between PM and infant mortality.

7.5.4.2. $PM_{10-2.5}$

The 2004 PM AQCD did not report long-term exposure studies for $PM_{10-2.5}$. No epidemiologic studies have been conducted to evaluate the effects of long-term $PM_{10-2.5}$ exposure and cancer. The evidence is **inadequate to assess the association between $PM_{10-2.5}$ and UFP exposures and cancer.**

7.5.4.3. UFPs

The 2004 PM AQCD did not report long-term exposure studies for UFPs. No epidemiologic studies have been conducted to evaluate the effects of long-term UFP and cancer. The evidence is **inadequate to determine if a causal relationship exists between long-term UFP exposures and cancer.**

7.6. Mortality

In the 1996 PM AQCD, results were presented for three prospective cohort studies of adult populations: the Six Cities Study (Dockery et al., 1993, 044457); the ACS Study (Pope et al., 1995, 045159); and the AHSMOG Study (Abbey et al., 1995, 000669). The 1996 AQCD concluded that the chronic exposure studies, taken together, suggested associations between increases in mortality and long-term exposure to $PM_{2.5}$, though there was no evidence to support an association with $PM_{10-2.5}$ (U.S. EPA, 1996, 079380).

Discussions of mortality and long-term exposure to PM in the 2004 PM AQCD emphasized the results of four U.S. prospective cohort studies, but the greatest weight was placed on the findings of the ACS and the Harvard Six Cities studies, which had each undergone extensive independent reanalysis, and which were based on cohorts that were broadly representative of the U.S. population. The 2004 PM AQCD concluded that the results from the Seventh-Day Adventist (AHSMOG) cohort provided some suggestive (but less conclusive) evidence for associations, while results from the Veterans Cohort provided inconsistent evidence for associations between long-term exposures to $PM_{2.5}$ and mortality. Collectively, the 2004 PM AQCD found that these studies provided strong evidence that long-term exposure to $PM_{2.5}$ was associated with increased risk of human mortality. Effect estimates for all-cause mortality ranged from 6 to 13% increased risk per 10 $\mu g/m^3$ $PM_{2.5}$, while effect estimates for cardiopulmonary mortality ranged from 6 to 19% per 10 $\mu g/m^3$ $PM_{2.5}$. For lung cancer mortality, the effect estimate was a 13% increase per 10 $\mu g/m^3$ $PM_{2.5}$, based upon the results of the extended analysis from the ACS cohort (Pope et al., 2002, 024689). With regard to $PM_{10-2.5}$, the 2004 PM AQCD reported that no association was observed between mortality and long-term exposure to $PM_{10-2.5}$ in the ACS study (Pope et al., 2002, 024689), while a positive but statistically non-significant association was reported in males in the AHSMOG cohort (McDonnell et al., 2000, 010319). Thus, the 2004 PM AQCD concluded that there was insufficient evidence for associations between long-term exposure to $PM_{10-2.5}$ and mortality. Overall, the 2004 PM AQCD concluded that there was strong epidemiologic evidence for associations between long-term exposures to $PM_{2.5}$ and excess all-cause and cardiopulmonary mortality.

At the time of the 2004 PM AQCD, only a limited number of the chronic-exposure cohort studies had considered direct measurements of constituents of PM, other than sulfates. With regard to source-oriented evaluations of mortality associations with long-term exposure, the 2004 PM AQCD noted only the study by Hoek et al. (2002, 042364), in which the authors concluded that long-term exposure to traffic-related air pollution may shorten life expectancy. However, Hoek et al. (2002, 042364) also noted that living near a major road might include other factors that contribute to mortality associations. There was not sufficient evidence at the time of the 2004 PM AQCD to draw conclusions on effects associated with specific components or sources of PM.

New epidemiologic evidence reports a consistent association between long-term exposure to $PM_{2.5}$ and increased risk of mortality. There is little evidence for the long-term effects of $PM_{10-2.5}$ on mortality. Although this section focuses on mortality outcomes in response to long-term exposure to PM, it does not evaluate studies that examine the association between PM and infant mortality.

These studies are evaluated in Section 7.5 because it is possible that in utero exposures contribute to infant mortality. A summary of the mean PM concentrations reported for the studies characterized in this section is presented in Table 7-8.

Table 7-8. Characterization of ambient PM concentrations from studies of mortality and long-term exposures to PM.

Study	Location	Mean Concentration ($\mu g/m^3$)	Upper Percentile Concentrations ($\mu g/m^3$)
$PM_{2.5}$			
Brunekreef et al. (2009, 191947)	The Netherlands	28	95th: 32 99th: 33 Max: 37
Chen et al. (2005, 087942)	Multicity, CA	29.0	
Eftim et al. (2008, 099104)	U.S.	13.6-14.1	Max: 19.1-25.1
Enstrom (2005, 087358)	CA	23.4	Max: 36.1
Goss et al. (2004, 059624)	U.S.	13.7	75th: 15.9
Janes et al. (2007, 090827)	U.S.	14.0	
Jerrett et al. (2005, 087600)	Los Angeles, CA		Max: 27.1
Krewski et al. (2009, 191193)	U.S.	14.02	75th: 16.00 90th: 26.75 95th: 27.89 Max: 30.01
Laden et al. (2006, 087605)	Multicity, U.S.	10.2-29.0	
Lipfert et al. (2006, 088218)	U.S.	14.3	
Miller et al. (2007, 090130)	U.S.	13.5	75th: 18.3 Max: 28.3
Pope et al. (2004, 055380)	U.S.	17.1	
Schwartz et al. (2008, 156963)	Multicity, U.S.	17.5	Max: 40
Zeger et al. (2007, 157176)	U.S.		17.0
Zeger et al. (2008, 191951)	U.S.	13.2	75th: 14.9
$PM_{10-2.5}$			
Chen et al. (2005, 087942)	Multicity, CA	25.4	
Lipfert et al. (2006, 088218)	U.S.	16.0	
PM_{10}			
Chen et al. (2005, 087942)	Multicity, CA	52.6	
Gehring et al. (2006, 089797)	North Rhine, Germany	43.7-46.0	Max: 52.5-56.1
Goss et al. (2004, 059624)	U.S.	24.8	75th: 28.9
Puett et al. (2008, 156891)	NE U.S.	21.6	
Zanobetti et al. (2008, 156177)	U.S.	29.4	

7.6.1. Recent Studies of Long-Term Exposure to PM and Mortality

Studies since the last PM AQCD include results of new analyses and insights for the ACS and Harvard Six Cities studies, further analyses from the AHSMOG and Veterans study cohorts, as well as analyses of a Cystic Fibrosis cohort and subsets of the ACS from Los Angeles and New York City. In the original analyses of the Six Cities and ACS cohort studies, no associations were found between long-term exposure to $PM_{10-2.5}$ and mortality, and the extended and follow-up analyses did not evaluate associations with $PM_{10-2.5}$. The historical and more recent results for $PM_{2.5}$ of both the ACS and the Harvard Six Cities studies are compiled in Figure 7-6. Moreover, since the last PM AQCD, there is a major new cohort that investigates the effects of $PM_{2.5}$ on cardiovascular mortality in the literature: the WHI study (Miller et al., 2007, 090130). Most recently, an ecologic cohort study of the nation's Medicare population has been completed (Eftim et al., 2008, 099104). These new findings further strengthen the evidence linking long-term exposure to $PM_{2.5}$ and mortality, while providing indications that the magnitude of the $PM_{2.5}$-mortality association is larger than previously estimated (Figure 7-7). Two recent reports from the AHSMOG and Veterans study cohorts have provided some limited evidence for associations between long-term exposure to $PM_{10-2.5}$ and mortality. The original analyses of the AHSMOG cohort study found positive associations between long-term concentrations of PM_{10} and 15-yr mortality due to natural causes and lung cancer (Abbey et al., 1999, 047559). McDonnell et al. (2000, 010319) reanalyzed these data and concluded that previously observed association of long-term ambient PM_{10} concentrations with mortality for males were best explained by a relationship of mortality with the fine fraction of PM_{10} rather than the thoracic coarse fraction of PM_{10}. Recent reports from the AHSMOG study cohort, as well as the Nurses' Health Study and a cohort of women in Germany have provided some evidence for associations between long-term exposure to PM_{10} and mortality among women.

Harvard Six Cities: A follow-up study has used updated air pollution and mortality data; an additional 1,368 deaths occurred during the follow-up period (1990-1998) versus 1,364 deaths in the original study period (1974-1989) (Laden et al., 2006, 087605). Statistically significant associations are reported between long-term exposure to $PM_{2.5}$ and mortality for data for the two periods (RR = 1.16 [95% CI: 1.07-1.26] per 10 $\mu g/m^3$ $PM_{2.5}$). Of special note is a statistically significant reduction in mortality risk reported with reduced long-term $PM_{2.5}$ concentrations (RR = 0.73 [95% CI: 0.57-0.95] per 10 $\mu g/m^3$ $PM_{2.5}$). This is equivalent to an RR of 1.27 for reduced mortality risks with reduced long-term $PM_{2.5}$ concentrations. This reduced mortality risk was observed for deaths due to cardiovascular and respiratory causes, but not for lung cancer deaths. The $PM_{2.5}$ concentrations for recent years were estimated from visibility data, which introduces some uncertainty in the interpretation of the results from this study. Coupled with the results of the original analysis (Dockery et al., 1993, 044457), this study strongly suggests that a reduction in $PM_{2.5}$ pollution yields positive health benefits.

ACS Extended Analyses/Reanalysis II: Two new analyses further evaluated the associations of long-term $PM_{2.5}$ exposures with risk of mortality in 50 U.S. cities reported by Pope and colleagues (2002, 024689), adding new details about deaths from specific cardiovascular and respiratory causes (Krewski, 2009, 190075; Pope et al., 2004, 055880). Pope et al. (2004, 055880) reported positive associations with deaths from specific cardiovascular diseases, particularly ischemic heart disease (IHD), and a group of cardiac conditions including dysrhythmia, heart failure and cardiac arrest (RR for cardiovascular mortality = 1.12, 95% CI 1.08-1.15 per 10 $\mu g/m^3$ $PM_{2.5}$), but no PM associations were found with respiratory mortality.

In an additional reanalysis that extended the follow-up period for the ACS cohort to 18 yr (1982-2000) (Krewski et al., 2009, 191193), investigators found effect estimates that were similar, though generally higher, than those reported in previous ACS analyses. This reanalysis also included data for seven ecologic (neighborhood-level) contextual (i.e., not individual-level) covariates, each of which represents local factors known or suspected to influence mortality, such as poverty level, educational attainment, and unemployment. The effect estimate for all cause mortality, based on $PM_{2.5}$ concentrations measured in 1999-2000 was 1.03 (95% CI: 1.01-1.05). The corresponding effect estimates for deaths due to IHD and lung cancer were 1.15 (95% CI: 1.04-1.18) and 1.11 (95% CI: 1.04-1.18), respectively. In earlier analyses of this cohort, investigators found that increasing education levels appeared to reduce the effect of $PM_{2.5}$ exposure on mortality. Results from this reanalysis show a similar pattern, although with somewhat less certainty, for all causes of death except IHD, for which the pattern was reversed. Overall, although the addition of random effects modeling and contextual covariates to the ACS model made most effect estimates higher (but

some lower), they were not statistically different from the earlier ACS effect estimates. Thus, these new analyses, with their more extensive consideration of potentially confounding factors, confirm the published ACS $PM_{2.5}$-mortality results to be robust.

California Cancer Prevention Study: In a cohort of elderly people in 11 California counties (mean age 73 yr in 1983), an association was reported for long-term $PM_{2.5}$ exposure with all-cause deaths from 1973-1982 (RR = 1.04 [95% CI: 1.01-1.07] per 10 $\mu g/m^3$ $PM_{2.5}$) (Enstrom, 2005, 087356). However, no significant associations were reported with deaths in later time periods when $PM_{2.5}$ levels had decreased in the most polluted counties (1983-2002) (RR = 1.00 [95% CI: 0.98-1.02] per 10 $\mu g/m^3$ $PM_{2.5}$). The $PM_{2.5}$ data were obtained from the EPA's Inhalation Particle Network (collected 1979-1983), and the locations represented a subset of data used in the 50-city ACS study (Pope et al., 1995, 045159). However, the use of average values for California counties as exposure surrogates likely leads to significant exposure error, as many California counties are large and quite topographically variable.

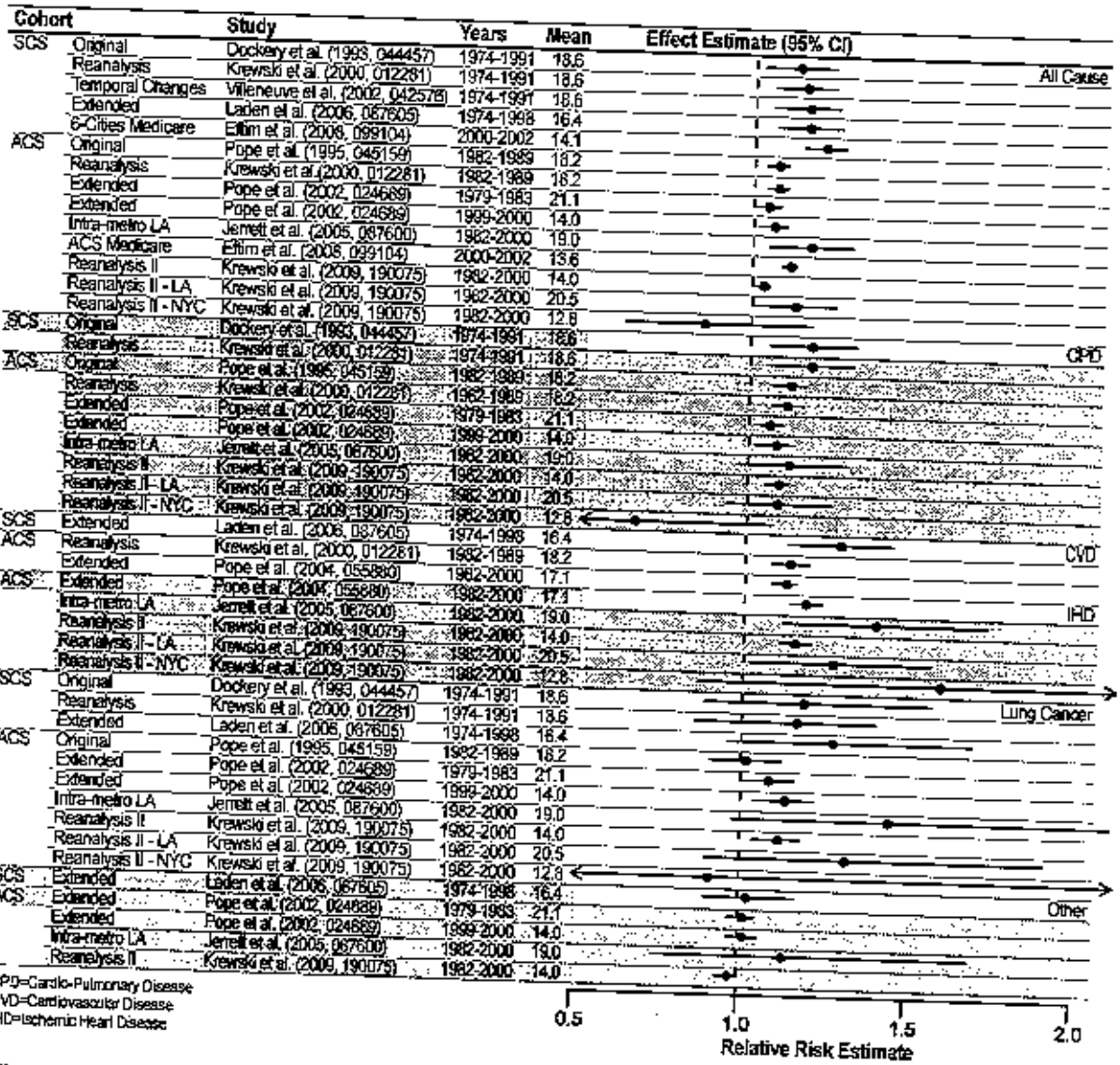

Figure 7-6. **Mortality risk estimates associated with long-term exposure to $PM_{2.5}$ from the Harvard Six Cities Study (SCS) and the American Cancer Society Study (ACS).**

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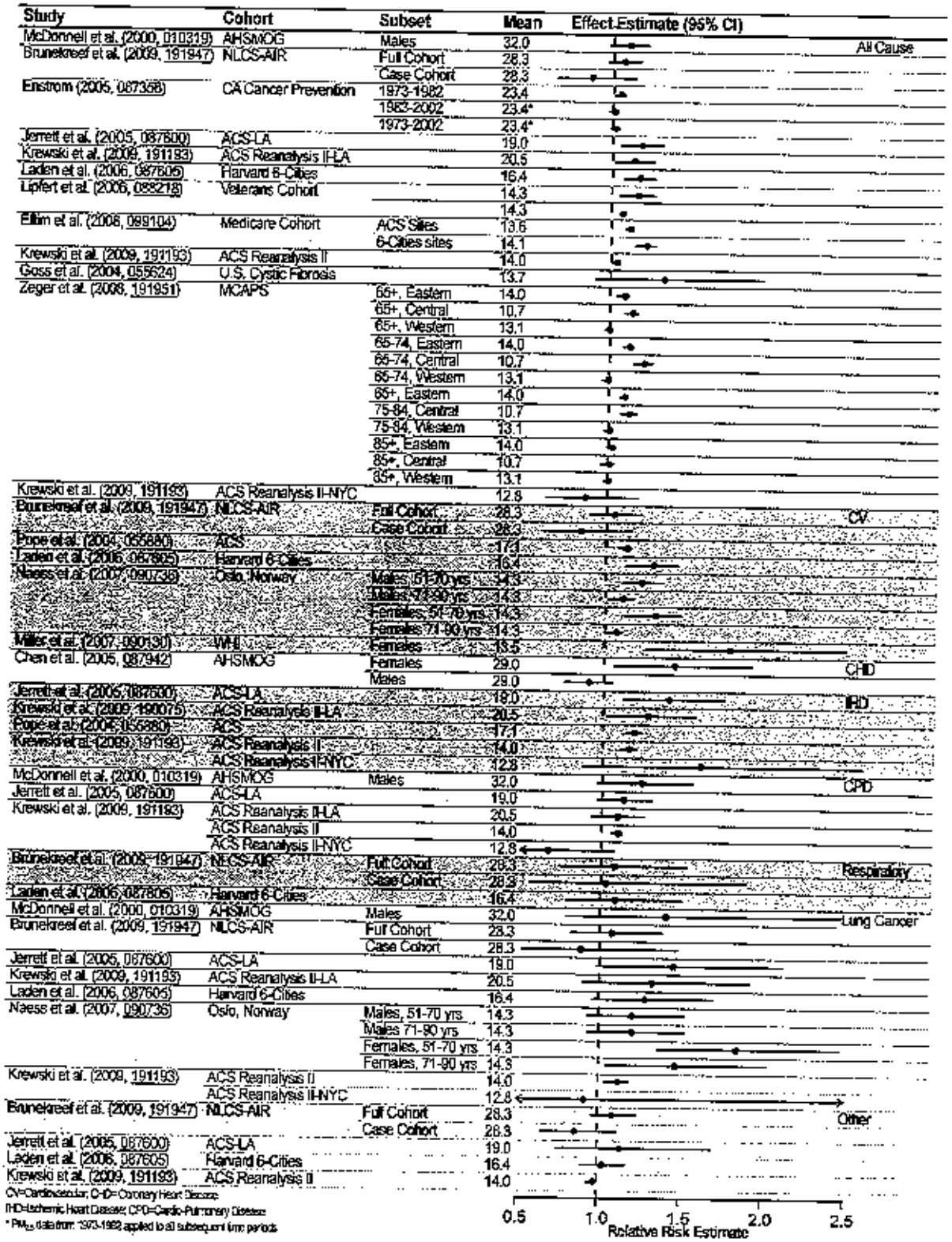


Figure 7-7. Mortality risk estimates, long-term exposure to $PM_{2.5}$ in recent cohort studies.

AHSMOG: In this analysis for the Seventh-Day Adventist cohort in California, a positive, statistically significant, association with coronary heart disease mortality was reported among females (92 deaths; RR = 1.42 [95% CI: 1.06-1.90] per 10 $\mu g/m^3$ $PM_{2.5}$), but not among males (53 deaths; RR = 0.90 [95% CI: 0.76-1.05] per 10 $\mu g/m^3$ $PM_{2.5}$) (Chen et al., 2005, 087942). Associations were strongest in the subset of postmenopausal women (80 deaths; RR = 1.49 [95% CI: 1.17-1.89] per 10 $\mu g/m^3$ $PM_{2.5}$). The authors speculated that females may be more sensitive to air pollution-related effects, based on differences between males and females in dosimetry and exposure. As was found with $PM_{2.5}$, a positive association with coronary heart disease mortality was reported for $PM_{10-2.5}$ and PM_{10} among females (RR = 1.38 [95% CI: 0.97-1.95] per 10 $\mu g/m^3$ $PM_{10-2.5}$; RR = 1.22 [95% CI: 1.01-1.47] per 10 $\mu g/m^3$ PM_{10}), but not for males (RR = 0.92 [95% CI: 0.66-1.29] per 10 $\mu g/m^3$ $PM_{10-2.5}$; RR = 0.94 [95% CI: 0.82-1.08] per 10 $\mu g/m^3$ PM_{10}); associations were strongest in the subset of postmenopausal women (80 deaths) (Chen et al., 2005, 087942).

U.S. Cystic Fibrosis cohort: A positive, but not statistically significant, association was reported for $PM_{2.5}$ in this study (RR = 1.32 [95% CI: 0.91-1.93] per 10 $\mu g/m^3$ $PM_{2.5}$) that primarily focused on evidence of exacerbation of respiratory symptoms (Goss et al., 2004, 055624). No clear association was reported for PM_{10}. However, only 200 deaths had occurred in the cohort of over 11,000 people (average age in cohort was 18.4 yr), so the power of this study to detect associations was relatively low.

Women's Health Initiative (WHI) Study: This nationwide cohort study considered 65,893 post-menopausal women with no history of cardiovascular disease who lived in 36 U.S. metropolitan areas from 1994 to 1998 (Miller et al., 2007, 090130). The study had a median subject follow-up time of 6 years. Miller and colleagues assessed each woman's exposure to air pollutants using the monitor located nearest to their residence. Hazard ratios were estimated for the first cardiovascular event, adjusting for age, race or ethnic group, smoking status, educational level, household income, body-mass index, and presence or absence of diabetes, hypertension, or hypercholesterolemia. Overall, this study concludes that "long-term exposure to fine particulate air pollution is associated with the incidence of cardiovascular disease and death among postmenopausal women." In terms of effect size, the study found that each increase of 10 $\mu g/m^3$ of $PM_{2.5}$ was associated with a 24% increase in the risk of a cardiovascular event (hazard ratio, 1.24 [95% CI: 1.09-1.41]) and a 76% increase in the risk of death from cardiovascular disease (hazard ratio, 1.76 [95% CI: 1.25-2.47]). While this study found results confirmatory to the ACS and Six Cities Study, it reports much larger relative risk estimates per $\mu g/m^3$ $PM_{2.5}$. In addition, since the study included only women without pre-existing cardiovascular disease, it could potentially be a healthier cohort population than that considered by the ACS and Six Cities Study. Indeed, the WHI Study reported only 216 cardiovascular deaths in 349,643 women-yr of follow-up, or a rate of 0.075% deaths per year (Miller et al., 2007, 090130), while the ACS Study reported that 10% of subjects died of cardiovascular disease over a 16-yr follow-up period, yielding a rate of 0.625% per year, or approximately 8 times the cardiovascular mortality rate of the WHI population (Pope et al., 2004, 055880). Thus, $PM_{2.5}$ impacts may yield higher relative risk estimates in the WHI population because the $PM_{2.5}$ risk is being compared to a much lower prevailing risk of cardiovascular death in this select study population.

The WHI study not only confirms the ACS and Six City Study associations with mortality in yet another well characterized cohort with detailed individual-level information, it also has been able to consider the individual medical records of the thousands of WHI subjects over the period of the study. This has allowed the researchers to examine not only mortality, but also related morbidity in the form of heart problems (cardiovascular events) experienced by the subjects during the study. As reported in this paper, this examination confirmed that there is an increased risk of cardiovascular morbidity, as well (Section 7.2.9). These morbidity co-associations with $PM_{2.5}$ in the same population lend even greater support to the biological plausibility of the air pollution-mortality associations found in this study.

Medicare Cohort Studies: Using Medicare data, Eftim and co-authors (2008, 099104) assessed the association of $PM_{2.5}$ with mortality for the same locations included in the ACS and Six City Study. For these locations, they estimated the chronic effects of $PM_{2.5}$ on mortality for the period 2000-2002 using mortality data for cohorts of Medicare participants and average $PM_{2.5}$ levels from monitors in the same counties included in the two studies. Using aggregate counts of mortality by county for three age groups, they estimated mortality risk associated with air pollution adjusting for age and sex and area-level covariates (education, income level, poverty, and employment), and controlled for potential confounding by cigarette smoking by including standardized mortality ratios

for lung cancer and COPD. This study is, therefore, an ecological analysis, similar to past published cross-sectional analyses, in that area-level covariates (education, income level, poverty, and employment) are employed as controlling variables, since individual level information is not available from the Medicare database (other than age and sex), which includes virtually all Americans aged 65 or greater. Exposures are also ecological in nature, as central site data are used as indices of exposure. These results indicated that a 10 $\mu g/m^3$ increase in the yearly average $PM_{2.5}$ concentration is associated with 10.9% (95% CI: 9.0-12.8) and with 20.8% (95% CI: 14.8-27.1) increases in all-cause mortality for the ACS and Six Cities Study counties, respectively. The estimates are somewhat higher than those reported by the original investigators, and there may be several possible explanations for this apparent increase, especially that this is an older population than the ACS cohort. Perhaps the most likely explanation is that the lack of personal confounder information (e.g., past personal smoking information) led to an insufficient control for the effects of these other variables' effects on mortality, inflating the pollution effect estimates somewhat, similar to what has been found in the ACS analyses when only ecological-level control variables were included. The ability of the Eftim et al. (2008, 099104) study results to qualitatively replicate the original individual-level cohort study (e.g., ACS and Six Cities Study) results suggests that past ecological cross-sectional mortality study results may also provide useful insights into the nature of the association, especially when used for consideration of time trends, or for comparisons of the relative (rather than absolute) sizes of risks between different pollutants or PM components in health effects associations.

Janes et al. (2007, 090927) used the same nationwide Medicare mortality data to examine the association between monthly averages of $PM_{2.5}$ over the preceding 12 mo and monthly mortality rates in 113 U.S. counties from 2000 to 2002. They decomposed the association between $PM_{2.5}$ and mortality into two components: (1) the association between "national trends" in $PM_{2.5}$ and mortality; and (2) the association between "local trends," defined as county-specific deviations from national trends. This second component is posited to provide evidence as to whether counties having steeper declines in $PM_{2.5}$ also have steeper declines in mortality relative to the national trend. They report that the exposure effect estimates are different at these two spatiotemporal scales, raising concerns about confounding bias in these analyses. The authors assert that the association between trends in $PM_{2.5}$ and mortality at the national scale is more likely to be confounded than is the association between trends in $PM_{2.5}$ and mortality at the local scale and, if the association at the national scale is set aside, that there is little evidence of an association between 12-month exposure to $PM_{2.5}$ and mortality in this analysis. However, in response, Pope and Burnett (2007, 090928) point out that such use of long-term time trends as the primary source of exposure variability has been avoided in most other air pollution epidemiology studies because of such concerns about potential confounding of such time-trend associations.

By linking monitoring data to the U.S. Medicare system by county of residence, Zeger et al. (2007, 157176) analyzed Medicare mortality records, comprising over 20 million enrollees in the 250 largest counties during 2000-2002. The authors estimated log-linear regression models having age-specific county level mortality rates as the outcome and, as the main predictor, the average $PM_{2.5}$ pollution level in each county during 2000. Area-level covariates were used to adjust for socio-economic status and smoking. The authors reported results under several degrees of adjustment for spatial confounding and with stratification into eastern, central and western U.S. counties. A 10 $\mu g/m^3$ increase in $PM_{2.5}$ was associated with a 7.6% increase in mortality (95% CI: 4.4-10.8). When adjusted for spatial confounding, the estimated log-relative risks dropped by 50%. Zeger et al. (2007, 157176) found a stronger association in the eastern counties than nationally, with no evidence of an association in western counties.

In a subsequent report, Zeger et al. (2008, 191951) created a new retrospective cohort, the Medicare Cohort Air Pollution Study (MCAPS), consisting of 13.2 million persons residing in 4,568 ZIP codes in urban areas having geographic centroids within 6 miles of a $PM_{2.5}$ monitor. Using this cohort, they investigated the relationship between 6-yr avg exposure to $PM_{2.5}$ and mortality risk over the period 2000-2005. When divided by region, the associations between long-term exposure to $PM_{2.5}$ and mortality for the eastern and central ZIP codes were qualitatively similar to those reported in the ACS and Six Cities Study, with 11.4% (95% CI: 8.8-14.1) and 20.4% (95% CI: 15.0-25.8) increases per 10 $\mu g/m^3$ increase in $PM_{2.5}$ in the eastern and central regions, respectively. The MCAPS results included evidence of differing $PM_{2.5}$ relative risks by age and geographic location, where risk declines with increasing age category until there is no evidence of an association among persons

≥ 85 yr of age, and there is no evidence of a positive association for the 640 urban ZIP codes in the western region of the U.S.

Using hospital discharge data, Zanobetti et al. (2008, 156177) constructed a cohort of persons discharged with COPD using Medicare data between 1985 and 1999. Positive associations in the survival analyses were reported for single year and multiple-year lag exposures, with a hazard ratio for total mortality of 1.22 (95% CI: 1.17-1.27) per 10 $\mu g/m^3$ increase in PM_{10} over the previous 4 years.

Veterans Cohort: A recent reanalysis of the Veterans cohort data focused on exposure to traffic-related air pollution (traffic density based on traffic flow rate data and road segment length) reported a stronger relationship between mortality with long-term exposure to traffic than with $PM_{2.5}$ mass (Lipfert et al., 2006, 088218). A significant association was reported between total mortality and $PM_{2.5}$ in single-pollutant models (RR = 1.12 [95% CI: 1.04-1.20] per 10 $\mu g/m^3$ $PM_{2.5}$). This risk estimate is larger than results reported in a previous study of this cohort. In multipollutant models including traffic density, the association with $PM_{2.5}$ was reduced and lost statistical significance. Traffic emissions contribute to $PM_{2.5}$ so it would be expected that the two would be highly correlated, and, thus, these multipollutant model results should be interpreted with caution. In a companion study, Lipfert et al. (2006, 088218) used data from EPA's fine particle speciation network, and reported findings for $PM_{2.5}$ which were similar to those reported by Lipfert et al. (2006, 088218). In this study (Lipfert et al., 2006, 088218), a significant association was reported between long-term exposure to $PM_{10-2.5}$ and total mortality in a single-pollutant model (RR = 1.07, 95% CI: 1.01-1.12 per 10 $\mu g/m^3$ $PM_{10-2.5}$). However, the association became negative and not statistically significant in a model that included traffic density. As it would be expected that traffic would contribute to the $PM_{10-2.5}$ concentrations, it is difficult to interpret the results of these multipollutant analyses.

Nurses' Health Study Cohort: The Nurses' Health Study (Puett et al., 2008, 156891) is an ongoing prospective cohort study examining the relation of chronic PM_{10} exposures with all-cause mortality and incident and fatal CHD consisting of 66,250 female nurses in MSAs in the northeastern region of the U.S. All cause mortality was statistically significantly associated with average PM_{10} exposures in the time period 3-48 mo preceding death. The association was strongest with average PM_{10} exposure in the 24 mo prior to death (hazard ratio 1.16 [95% CI: 1.05-1.28]) and weakest with exposure in the month prior to death (hazard ratio 1.04 [95% CI: 0.98-1.11]). The association with fatal CHD occurred with the greatest magnitude with mean exposure in the 24 mo prior to death (hazard ratio 1.42 [95% CI: 1.11-1.81]).

Netherlands Cohort Study (NLCS): The Netherlands Cohort Study (Brunekreef et al., 2009, 191947) estimates the effects of traffic-related air pollution on cause specific mortality in a cohort of approximately 120,000 subjects aged 55-69 yr at enrollment. For a 10 $\mu g/m^3$ increase in $PM_{2.5}$ concentration, the relative risk for natural-cause mortality in the full cohort was 1.06 (95% CI: 0.97-1.16), similar in magnitude to the results reported by the ACS. In a case-cohort analysis adjusted for additional potential confounders, there were no associations between air pollution and mortality.

German Cohort: The North Rhine-Westphalia State Environment Agency (LUA NRW) initiated a cohort of approximately 4,800 women, and assessed whether long-term exposure to air pollution originating from motorized traffic and industrial sources was associated with total and cause-specific mortality (Gehring et al., 2006, 089797). They found that cardiopulmonary mortality was associated with PM_{10} (RR = 1.52 [95% CI: 1.09-2.15] per 10 $\mu g/m^3$ PM_{10}).

7.6.2. Composition and Source-Oriented Analyses of PM

As discussed in the 2004 PM AQCD, only a very limited number of the chronic exposure cohort studies have included direct measurements of chemical-specific PM constituents other than sulfates, or assessments of source-oriented effects, in their analyses. One exception is the Veterans Cohort Study, which looked at associations with some constituents, and traffic.

Veterans Cohort: Using data from EPA's fine particle speciation network, Lipfert et al. (2006, 088756) reported a positive association for mortality with sulfates. Using 2002 data from the fine particle speciation network, positive associations were found between mortality and long-term exposures to nitrates, EC, Ni and V, as well as traffic density and peak O_3 concentrations. In

multipollutant models, associations with traffic density remained significant, as did nitrates, Ni and V in some models.

Netherlands Cohort Study: Beelen et al. (2008, 156263) studied the association between long-term exposure to traffic-related air pollution and mortality in a Dutch cohort. They used data from an ongoing cohort study on diet and cancer with 120,852 subjects who were followed from 1987 to 1996. Exposure to BS, NO_2, SO_2, and $PM_{2.5}$, as well as various exposure variables related to traffic, were estimated at the home address. Traffic intensity on the nearest road was independently associated with mortality. Relative risks (CI) for a 10 $\mu g/m^3$ increase in BS concentrations (difference between 5th and 95th percentile) were 1.05 (95% CI: 1.00-1.11) for natural cause, 1.04 (95% CI: 0.95-1.13) for cardiovascular, 1.22 (95% CI: 0.99-1.50) for respiratory, 1.03 (95% CI: 0.88-1.20) for lung cancer, and 1.04 (95% CI: 0.97-1.12) for mortality other than cardiovascular, respiratory, or lung cancer. Results were similar for NO_2 and $PM_{2.5}$, but no associations were found for SO_2. Traffic-related air pollution and several traffic exposure variables were associated with mortality in the full cohort, although the relative risks were generally small. Associations between natural-cause and respiratory mortality were statistically significant for NO_2 and BS. These results add to the evidence that long-term exposure to traffic-related particulate air pollution is associated with increased mortality.

Given the general dearth of published source-oriented studies of the mortality impacts of long-term PM exposure components, and given that the recent Medicare Cohort study now indicates that such ecological cross-sectional studies can be useful for evaluating time trends and/or comparisons across pollution components, it may well be that examining past cross-sectional studies comparing source-oriented components of PM may be informative. In particular, Ozkaynak and Thurston (1987, 072960), utilized the chemical speciation conducted in the Inhalable Particle (IP) Network to conduct a chemical constituent and source-oriented evaluation on long-term PM exposure and mortality in the U.S. They analyzed the 1980 U.S. vital statistics and available ambient air pollution data bases for sulfates and fine, inhalable, and TSP mass. Using multiple regression analyses, they conducted a cross-sectional analysis of the association between various particle measures and total mortality. Results from the various analyses indicated the importance of considering particle size, composition, and source information in modeling of particle pollution health effects. Of the independent mortality predictors considered, particle exposure measures most related to the respirable fraction of the aerosols, such as fine particles and sulfates, were most consistently and significantly associated with the reported SMSA-specific total annual mortality rates. On the other hand, particle mass measures that included $PM_{10-2.5}$ (e.g., total suspended particles and inhalable particles) were often found to be non-significant predictors of total mortality. Furthermore, based on the application of $PM_{2.5}$ source apportionment, particles from industrial sources and from coal combustion were indicated to be more significant contributors to human mortality than fine soil-derived particles.

7.6.3. Within-City Effects of PM Exposure

Much of the exposure gradient in the national-scale cohort studies was due to city-to-city differences in regional air pollution, raising the possibility that some or all of the original PM-survival associations may have been driven instead by city-to-city differences in some unknown (non-pollution) confounder variable. This has been evaluated by three recent studies.

ACS, Los Angeles: To investigate this issue, two new analyses using ACS data focused on neighborhood-to-neighborhood differences in urban air pollutants, using data from 23 $PM_{2.5}$ monitoring stations in the Los Angeles area, and applying interpolation methods (Jerrett et al., 2005, 087600) or land use regression methods (Krewski et al., 2009, 191193) to assign exposure levels to study individuals. This resulted in both improved exposure assessment and an increased focus on local sources of $PM_{2.5}$. Significant associations between $PM_{2.5}$ and mortality from all causes and cardiopulmonary diseases were reported with the magnitude of the relative risks being greater than those reported in previous assessments. In general, the associations for $PM_{2.5}$ and mortality using these two methods for exposure assessment were similar, though the use of land use regression resulted in somewhat smaller hazard ratios and tighter CIs (see Table 7-9). This indicates that city-to-city confounding was not the cause of the associations found in the earlier ACS Cohort studies. This provides evidence that reducing exposure error can result in stronger associations between $PM_{2.5}$ and mortality than generally observed in broader studies having less exposure detail.

Table 7-9. Comparison of results from ACS intra-urban analysis of Los Angeles and New York City using kriging or land use regression to estimate exposure.

Cause of Death	Los Angeles: Hazard Ratio[1] and 95% Confidence Interval Using Kriging[2] (Jerrett et al., 2005, 087600)	Los Angeles: Hazard Ratio[1] and 95% Confidence Interval Using Land Use Regression[3] (Krewski et al., 2009, 191193)	New York City: Hazard Ratio[1] and 95% Confidence Interval Using Land Use Regression[4] (Krewski et al., 2009, 191193)
All Cause	1.11 (0.99-1.25)	1.13 (1.01-1.25)	0.66 (0.63-1.18)
IHD	1.25 (0.99-1.59)	1.26 (1.02-1.56)	1.56 (0.67-2.88)
CPD	1.07 (0.91-1.26)	1.09 (0.94-1.26)	0.66 (0.41-1.08)
Lung Cancer	1.20 (0.79-1.82)	1.31 (0.90-1.92)	0.90 (0.29-2.78)

[1]Hazard ratios presented per 10 μg/m³ increase in $PM_{2.5}$
[2]Model included parsimonious contextual covariates
[3]Model included parsimonious individual level (23) and ecologic (4) covariates
[4]Model included all 44 individual level and 7 ecologic covariates.

ACS, New York: Krewski et al. (2009, 191193) applied the same techniques used in the land use regression analysis of Los Angeles to an investigation conducted in New York City. Annual average concentrations were calculated for each of 62 monitors from 3 yr of daily monitoring data for 1999-2001. Those data were combined with land-use data collected from traffic counting systems, roadway network maps, satellite photos of the study area, and local government planning and tax-assessment maps to assign estimated exposures to the ACS participants. The investigators did not observe elevated effect estimates for all cause, CPD or lung cancer deaths, but IHD did show a positive association with $PM_{2.5}$ concentration. The difference between the 90th and 10th percentiles of the 3-yr avg $PM_{2.5}$ concentration was 1.5 μg/m^3 and the difference between the minimum and maximum values of the 3-yr avg $PM_{2.5}$ concentration was 7.8 μg/m^3. This narrow range in $PM_{2.5}$ exposure contrasts across the New York City metropolitan area and may well account for the inconclusive results in this city-specific analysis. Relatively uniform exposures would reduce the power of the statistical models to detect patterns of mortality relative to exposure and estimate the association with precision.

WHI Study: This study also investigated the within- versus between-city effects in its cities. As shown in Figure 7-8, similar effects for both the within and between-city analyses demonstrate that this association is not due to some other (non-pollution) confounder differing between the various cities, strengthening confidence in the overall pollution-effect estimates.

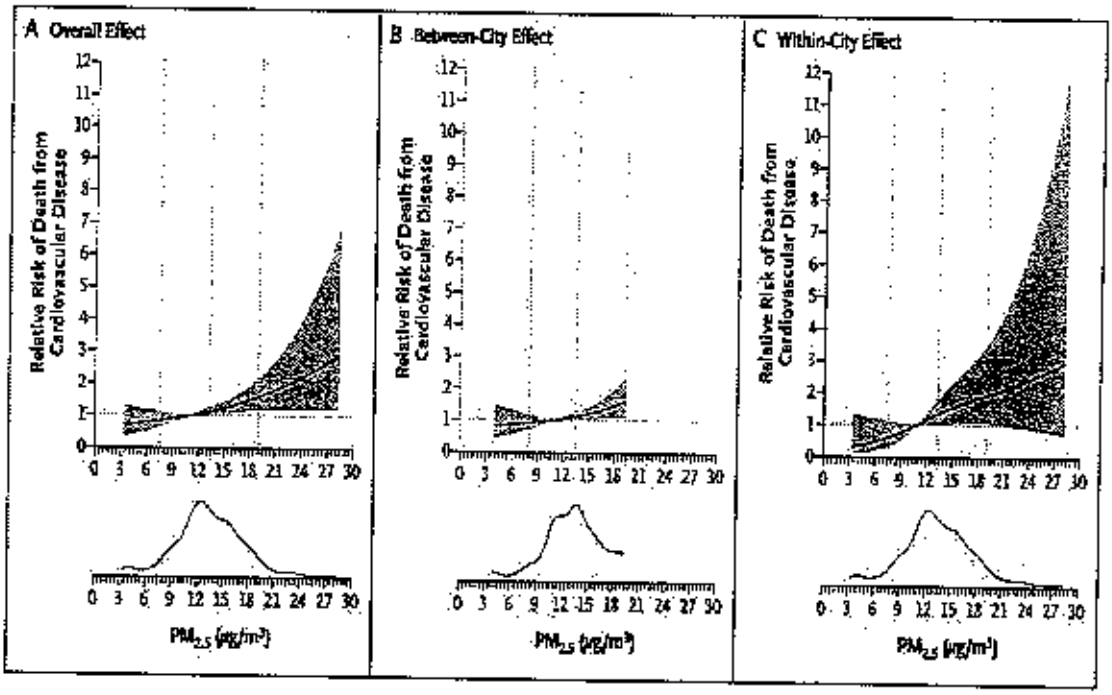
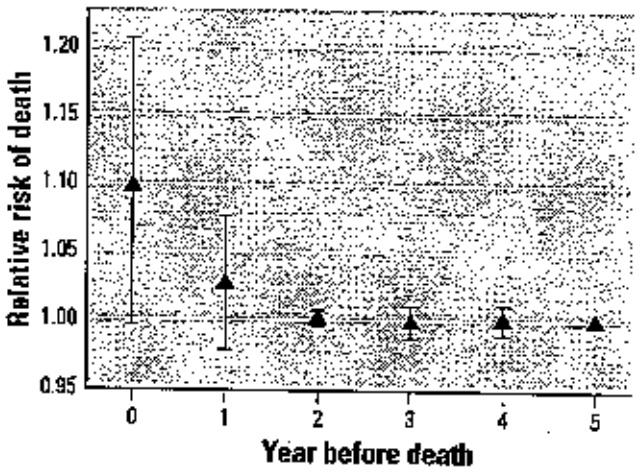


Figure 7-8. Plots of the relative risk of death from cardiovascular disease from the Women's Health Initiative study displaying the between-city and within-city contributions to the overall association between $PM_{2.5}$ and cardiovascular mortality windows of exposure-effects.

7.6.4. Effects of Different Long-term Exposure Windows

The delay between changes in exposure and changes in health has important policy implications. Schwartz et al. (2008, 156963) investigated this issue using an extended follow-up of the Harvard Six Cities Study. Cox proportional hazards models were fit to control for smoking, body mass index, and other covariates. Penalized splines were fit in a flexible functional form to the concentration response to examine its shape, and the degrees of freedom for the curve were selected based on Akaike's information criterion (AIC). The researchers also used model averaging as an alternative approach, where multiple models are fit explicitly and averaged, weighted by their probability of being correct given the data. The lag relationship by model was averaged across a range of unconstrained distributed lag models (i.e., same year, 1 yr prior, 2 yr prior, etc.). Results of the lag comparison are shown in Figure 7-9 indicating that the effects of changes in exposure on mortality are seen within 2 yr. The authors also noted that the concentration-response curve was linear, clearly continuing below the level of the current U.S. air quality standard of 15 $\mu g/m^3$.

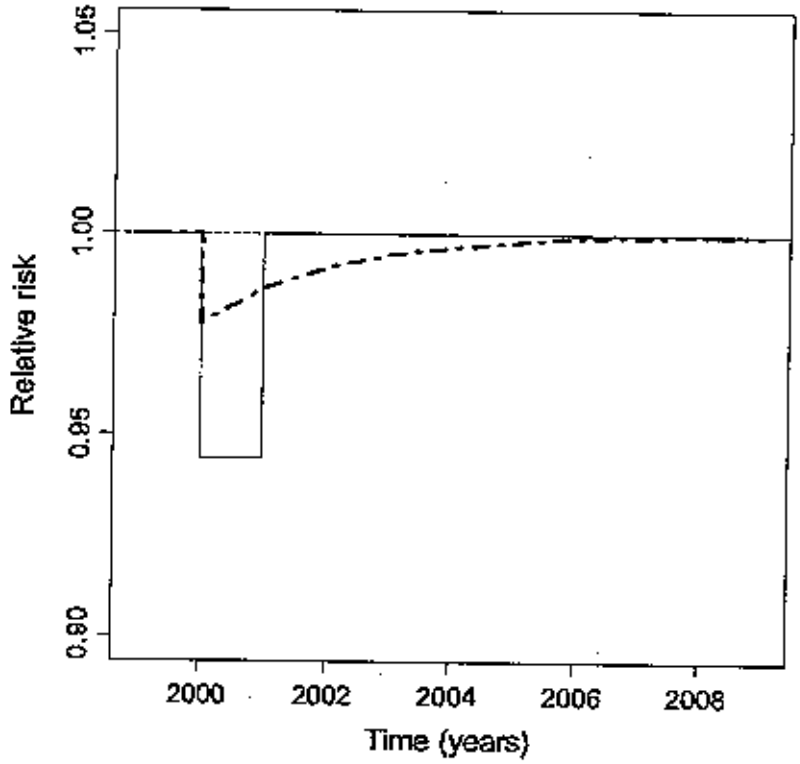


Source: Schwartz et al. (2008, 156963)

Figure 7-9. The model-averaged estimated effect of a 10-μg/m^3 increase in $PM_{2.5}$ on all-cause mortality at different lags (in years) between exposure and death. Each lag is estimated independently of the others. Also shown are the pointwise 95% CIs for each lag, based on jackknife estimates.

Similarly, the effect of long-term exposure to PM_{10} on the risk of death in a large multicity study of elderly subjects discharged alive following an admission for COPD found the effect was not limited to the exposure in each year of follow-up, and had larger cumulative effects spread over the follow-up year and three preceding years (Zanobetti et al., 2008, 156177).

Rõõsli et al. (2005, 156923) took an alternative approach to determining the window over which the mortality effects of long-term pollution exposures occurred. They fit the model shown in Figure 7-10 using k = 0.5 based on the Utah Steel Strike (Pope, 1989, 044461) and the Ireland coal ban study (Clancy et al., 2002, 035270). They found that roughly 75% of health benefits are observed in the first 5 years, as shown in Table 7-10. These results are consistent with the findings of Schwartz et al. (2008, 156963). Puett et al. (2008, 156891) also compared different long-term exposure lags, with exposure periods ranging from 1 month to 48 mo prior to death. They found statistically significant associations with average PM_{10} exposures in the time period 3–48 mo prior to death, with the strongest associations in the 24 mo prior to death and the weakest with exposure in the 1 mo prior to death.



Source: Reprinted with Permission from Oxford University Press & the International Epidemiological Society from Rösli et al. (2005, 156923)

Figure 7-10. Time course of relative risk of death after a sudden decrease in air pollution exposure during the year 2000, assuming a steady state model (solid line) and a dynamic model (bold dashed line). The thin dashed line refers to the reference scenario.

Table 7-10. Distribution of the effect of a hypothetical reduction of 10 µg/m³ PM_{10} in 2000 on all-cause mortality 2000-2009 in Switzerland.

Year	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
Proportion of total effect (%)	-	39.3	23.9	14.5	8.8	5.3	3.2	2.0	1.2	0.7	0.4
Relative risk (per 10 µg/m³ reduction in PM_{10})	1.0	0.9775	0.9863	0.9917	0.9950	0.9969	0.9981	0.9989	0.9993	0.9996	0.9997

Relative risk and proportion of total effect in each year are shown, assuming a time constant k of 0.5

Source: Rösli et al. (2005, 156923)

In the reanalysis of the ACS cohort, the investigators calculated time windows of exposure as average concentrations during successive 5-yr periods preceding the date of death (Krewski et al., 2009, 191193). The investigators considered the time window with the best-fitting model (judged by the AIC statistic) to be the period during which pollution had the strongest influence on mortality. Overall, the differences between the time periods were small and demonstrated no definitive patterns. High correlations between exposure levels in the three periods may have reduced the ability of this analysis to detect any differences in the relative importance of the time windows. The investigators did not analyze any time periods smaller than 5 yr, so the results are not directly comparable to those reported by Schwartz et al. (2008, 156963), Rösli et al. (2005, 156923), and Puett et al. (2008, 156891).

Generally, these results indicate a developing coherence of the air pollution mortality literature, suggesting that the health benefits from reducing air pollution do not require a long latency period and would be expected within a few years of intervention.

7.6.5. Summary and Causal Determinations

7.6.5.1. $PM_{2.5}$

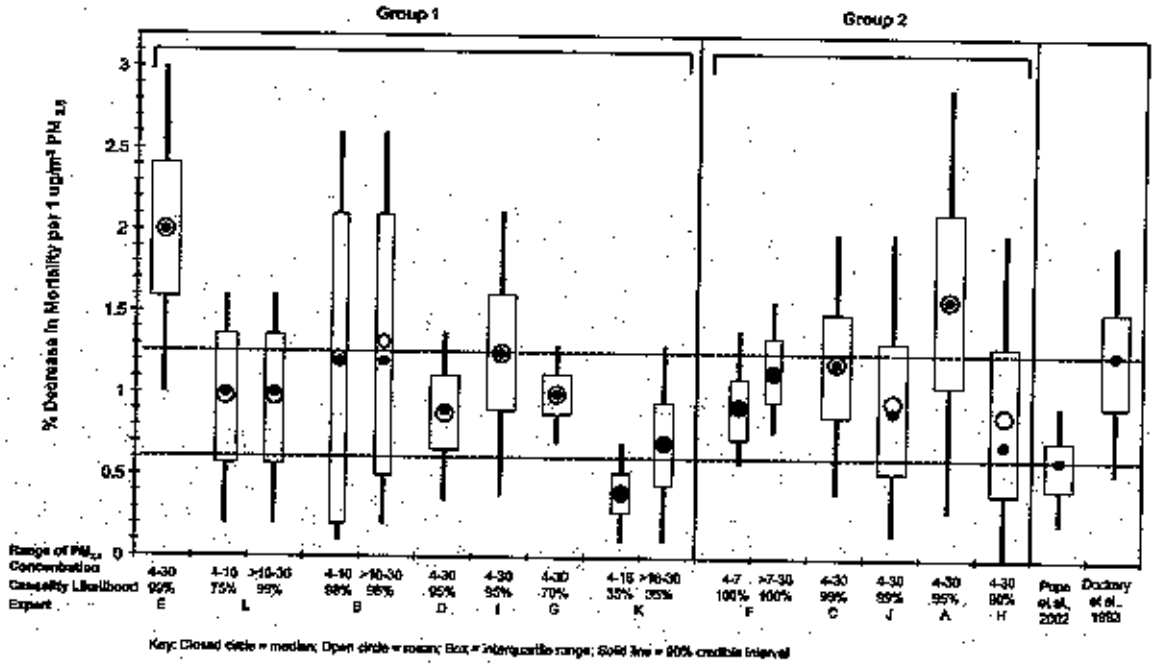
In the 1996 PM AQCD (U.S. EPA, 1996, 079380), results were presented for three prospective cohort studies of adult populations: the Six Cities Study (Dockery et al., 1993, 044457); the ACS Study (Pope et al., 1995, 045159); and the AHSMOG Study (Abbey et al., 1995, 000669). The 1996 AQCD concluded that the chronic exposure studies, taken together, suggested associations between increases in mortality and long-term exposure to $PM_{2.5}$, though there was no evidence to support an association with $PM_{10-2.5}$ (U.S. EPA, 1996, 079380). Discussions of mortality and long-term exposure to PM in the 2004 PM AQCD emphasized the results of four U.S. prospective cohort studies, but the greatest weight was placed on the findings of the ACS and the Harvard Six Cities studies, which had undergone extensive independent reanalysis, and which were based on cohorts that were broadly representative of the U.S. population. Collectively, the 2004 PM AQCD found that these studies provided strong evidence that long-term exposure to $PM_{2.5}$ was associated with increased risk of human mortality.

The recent evidence is largely consistent with past studies, further supporting the evidence of associations between long-term $PM_{2.5}$ exposure and increased risk of human mortality (Section 7.6) in areas with mean concentrations from 13.2 to 29 $\mu g/m^3$ (Figure 7-7). New evidence from the Six Cities cohort study shows a relatively large risk estimate for reduced mortality risk with decreases in $PM_{2.5}$ (Laden et al., 2006, 087605). The results of new analyses from the Six Cities cohort and the ACS study in Los Angeles suggest that previous and current studies may have underestimated the magnitude of the association (Jerrett et al., 2005, 087600). With regard to mortality by cause-of-death, recent ACS analyses indicate that cardiovascular mortality primarily accounts for the total mortality association with $PM_{2.5}$ among adults, and not respiratory mortality. The recent WHI cohort study shows even higher cardiovascular risks per $\mu g/m^3$ than found in the ACS study, but this is likely due to the fact that the study included only post-menopausal women without pre-existing cardiovascular disease (Miller et al., 2007, 090130). There is additional evidence for an association between $PM_{2.5}$ exposure and lung cancer mortality (Section 7.5.1.1). The WHI study also considered within versus between city mortality, as well as morbidity co-associations with $PM_{2.5}$ in the same population. The first showed that the results are not due to between city confounding, and the morbidity analyses show the coherence of the mortality association across health endpoints, supporting the biological plausibility of the air pollution-mortality associations found in these studies.

Results from a new study examining the relationship between life expectancy and $PM_{2.5}$ and the findings from a multiyear expert judgment study that comprehensively characterizes the size and uncertainty in estimates of mortality reductions associated with decreases in $PM_{2.5}$ in the U.S draw conclusions that are consistent with an association between long-term exposure to $PM_{2.5}$ and mortality (Pope et al., 2009, 190107; Roman et al., 2008, 156921). Pope et al. (2009, 190107) report that a decrease of 10 $\mu g/m^3$ in the concentration of $PM_{2.5}$ is associated with an estimated increase in mean (± SE) life expectancy of 0.61 ± 0.20 year. For the approximate period of 1980-2000, the average increase in life expectancy was 2.72 yr among the 211 counties in the analysis. The authors note that reduced air pollution was only one factor contributing to increased life expectancies, with its effects overlapping with those of other factors.

Roman et al. (2008, 156921) applied state-of-the-art expert judgment elicitation techniques to develop probabilistic uncertainty distributions that reflect the broader array of uncertainties in the concentration-response relationship. This study followed best standard practices for expert elicitations based on the body of literature accumulated over the past two decades. The resulting $PM_{2.5}$ effect estimate distributions, elicited from 12 of the world's leading experts on this issue, are shown in Figure 7-11. They indicate both larger central estimates of mortality reductions for decreases in long-term $PM_{2.5}$ exposure in the U.S. (averaging almost 1% per $\mu g/m^3$ $PM_{2.5}$) than reported (for example) by the ACS Study (i.e., 0.6% per $\mu g/m^3$ $PM_{2.5}$ in Pope et al. (2002, 024689),

and a wider distribution of uncertainty by each expert than provided by any one of the $PM_{2.5}$ epidemiologic studies. However, a composite uncertainty range of the overall mean effect estimate (i.e., based upon all 12 experts' estimates, but not provided in Figure 7-11) would be much narrower, and closer to that derived from the ACS study than indicated for any one expert shown in Figure 7-11.



Source: Reprinted with Permission of ACS from Roman et al. (2008, 156921)

Figure 7-11. Experts' mean effect estimates and uncertainty distributions for the $PM_{2.5}$ mortality concentration-response coefficient for a 1 µg/m³ change in annual average $PM_{2.5}$.

Overall, recent evidence supports the strong evidence reported in the 2004 PM AQCD (U.S. EPA, 2004, 056905) that long-term exposure to $PM_{2.5}$ is associated with an increased risk of human mortality. When looking at the cause of death, the strongest evidence comes from mortality due to cardiovascular disease, with additional evidence supporting an association between $PM_{2.5}$ and lung cancer mortality (Figure 7-7). Fewer studies evaluate the respiratory component of cardiopulmonary mortality, and the evidence to support an association with long-term exposure to $PM_{2.5}$ and respiratory mortality is weak (Figure 7-7). Together these findings are consistent and coherent with the evidence from epidemiologic, controlled human exposure, and animal toxicological studies for the effects of short- and long-term exposure to PM on cardiovascular effects presented in Sections 6.2 and 7.2, respectively. Evidence of short- and long-term exposure to $PM_{2.5}$ and respiratory effects (Sections 6.3 and 7.3, respectively) and infant mortality (Section 7.4) are coherent with the weak respiratory mortality effects. Additionally, the evidence for short- and long-term cardiovascular and respiratory morbidity provides biological plausibility for mortality due to cardiovascular or respiratory disease. The most recent evidence for the association between long-term exposure to $PM_{2.5}$ and mortality is particularly strong for women. Collectively, the evidence is **sufficient to conclude that the relationship between long-term $PM_{2.5}$ exposures and mortality is causal.**