Comments on the "Integrated Science Assessment for Particulate Matter (First External Review Draft, December 2008)"

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Executive Summary

Based on our review of the United States Environmental Protection Agency's (US EPA's) discussion of the human clinical data for diesel exhaust (DE) exposures in the December 2008 first external review draft of the "Integrated Science Assessment for Particulate Matter" (Draft PM ISA), we have identified the following five main criticisms:

1. The Draft PM ISA relies in part on human clinical studies of exposure to whole diesel exhaust (DE) or diesel exhaust particulate (DEP) to support the causal determinations between ambient PM exposure and health outcomes. The relevance of those studies to ambient PM must be questioned since they generally involved levels of DEP exposure orders of magnitude higher than ambient PM, let alone ambient DEP (*e.g.*, 100-300 $\mu g/m^3$ *vs*. <1 to ~20 $\mu g/m^3$), and further exposed subjects to diesel exhaust gases as well. Thus, such studies should not be viewed as key evidence in support of US EPA's causal conclusions regarding health effects of ambient PM levels.
2. The human chamber studies cited in the Draft PM ISA regarding exposure to diesel exhaust were primarily conducted with older diesel technology from the early 1990s that is no longer representative of diesel-exhaust emissions from the current vehicle fleet. The relevance of such studies to addressing current and future ambient PM levels is questionable given the dramatic quantitative and qualitative changes in diesel engine emissions that have occurred over the last two decades.
3. The Draft PM ISA leaves out many findings from studies of DE and DEP that reported inconclusive or negative results, and thus leaves the reader with a false impression that all of the diesel exposure studies demonstrated adverse health effects. The literature includes many examples of negative results, and when viewed in their totality, demonstrates mixed and diverse results, even at the elevated exposure levels used.
4. The Draft PM ISA fails to mention that some human diesel exposure studies provide evidence of a threshold of effects. Also, while referring to some findings from US EPA's Diesel Health Assessment Document (Diesel HAD), the Draft PM ISA does not point out that the Diesel HAD concluded that the laboratory animal studies provide a no observed adverse effect level (NOAEL) for diesel exhaust PM of 460 $\mu g/m^3$.
5. Based on a review of the literature regarding human and animal exposure studies of DE and DEP, the Draft PM ISA largely mischaracterizes this literature as supporting a causal association between ambient PM and significantly adverse human health effects in the range of the current standard of 15 $\mu g/m^3$.

Introduction

Gradient Corporation was asked to review and prepare an independent analysis of US Environmental Protection Agency's (US EPA's) discussion of the human clinical data for diesel exhaust (DE) exposures in the December 2008 first external review draft of the "Integrated Science Assessment for Particulate Matter" (hereafter referred to as the "Draft PM ISA"). As summarized in the Chapter 2, Integrative Health Effects Overview to the Draft PM ISA, US EPA heavily relied upon the controlled human exposure studies for DE, which have typically employed exposures with 100 to 300 $\mu g/m^3$ diesel exhaust particulate (DEP), in reaching its determination that there exists a "causal" relationship between short-term exposures to ambient $PM_{2.5}$ and cardiovascular morbidity. Specifically, on p. 2-16 of the Draft PM ISA, US EPA concluded that the human clinical data for both DE and concentrated ambient particles (CAPs) "provide strong evidence of $PM_{2.5}$-induced decreases in HRV [heart rate variability] and vasomotor function, as well as increases in markers for systemic oxidative stress." In addition, US EPA also states in Chapter 2 that human clinical data for DE "provide strong evidence" in support of a "likely causal" relationship between short-term exposure to $PM_{2.5}$ and respiratory morbidity, referring specifically to DE human clinical studies reporting increased markers of pulmonary inflammation, oxidative responses, and allergic responses and allergic sensitization.

We address some of the key caveats regarding the interpretation of the DE/DEP studies as relevant to ambient $PM_{2.5}$. We also note DE/DEP studies that are either not mentioned by US EPA in the Draft PM ISA, or that are mentioned but are not fully accounted for by US EPA in their assessment of the literature. Drawing upon information presented in our forthcoming *Critical Reviews in Toxicology* publication "Non-cancer health effects of diesel exhaust (DE): A critical assessment of recent human and animal toxicological literature,"[1] we first comment on the relevance of experimental DEP exposures to typical ambient PM exposures. Next, we highlight the fact that most of the cited human clinical studies of DE exposures have been conducted using older diesel technology (*e.g.*, a 1991 4-cylinder Volvo TD45 diesel engine) that is not representative of present-day, modern diesel technology. This is not discussed at all by US EPA, despite significant implications regarding the relevance of these study findings in projecting the potential health impacts from present-day (2009) and future $PM_{2.5}$ mixtures. Lastly, we discuss how human clinical studies have generally reported very mixed findings, including some findings

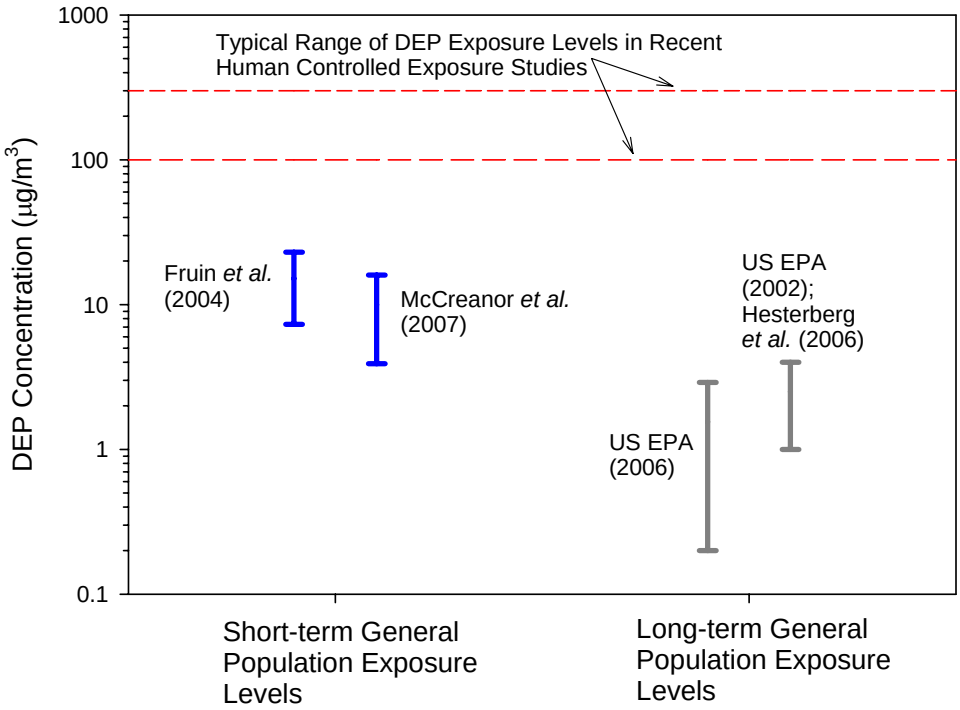
[1] Hesterberg, TW; Long; CM; Bunn, WB; Lapin, CA; Sax, SN; Valberg, PA. 2009. "Non-cancer health effects of diesel exhaust (DE): A critical assessment of recent human and animal toxicological literature." *Crit. Rev. Toxicol.* (In Press).

suggestive of a possible threshold, despite the use of high exposure concentrations from outdated diesel engine technologies.

The Uncertain Relevance of Highly Elevated Experimental DEP Exposures to Ambient $PM_{2.5}$ Exposures

Some human clinical studies of highly-elevated, whole DE exposures have reported findings suggestive of potential cardiovascular and respiratory health effects from short-term inhalation of DE (*e.g.*, the recent findings of Mills *et al*. [2007] and Tornqvist *et al*. [2007] suggesting ischemic and thrombogenic effects). The dramatic difference in concentration levels between ambient $PM_{2.5}$ and the high DE levels required to elicit these effects cannot be overlooked. In other words, the findings from the DE studies cannot be interpreted without considering the unrealistic exposure conditions under which they were observed. In Chapter 2, US EPA highlights DE studies as providing key PM health effects evidence, but fails to note the magnitude of the experimental exposure levels and the fact that they are well-above typical ambient PM levels. All of the recent human controlled exposure studies of whole DE have employed elevated DE exposure levels, with DEP concentrations in the range of 100 to 300 $\mu g/m^3$. As shown in Figure 1 below, those experimental DEP concentrations are approximately one to two orders of magnitude higher than both typical short-term and long-term average DEP and PM exposure levels representative of the general population. Importantly, DEP concentrations in the human clinical studies are substantially higher than even estimates of short-term in-vehicle DEP concentrations (*e.g.*, 7.3 to 23 $\mu g/m^3$ from Fruin *et al*., 2004) and of roadway elemental carbon measurements (*e.g.*, 3.9 to 16 $\mu g/m^3$ from McCreanor *et al*., 2007).

Figure 1
Range of Typical DEP Concentrations Employed in Recent Controlled Human Exposure Studies *versus* Ranges of Mean Short-term (*e.g.*, Hourly) and Long-term (*e.g.*, Annual Average) DEP Concentrations Representative of General Population Exposures



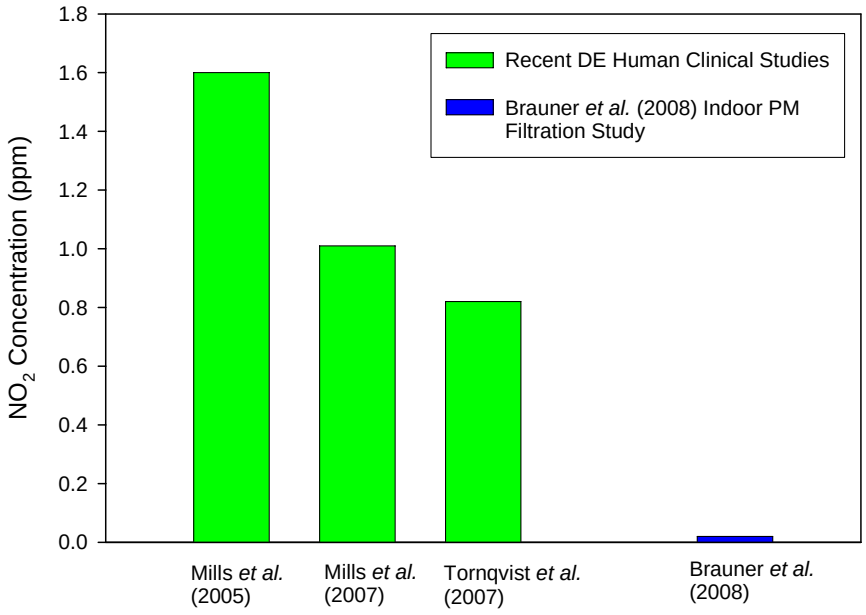
Notes:
1) Concentrations are on a logarithmic scale.
2) Source: Hesterberg et al., 2009.

In addition to the elevated DEP concentrations, it is important to note that human volunteers in clinical studies of whole DE exposures are also typically exposed to highly elevated levels of gaseous DE constituents, including nitrogen dioxide (NO_2), nitric oxide (NO), and carbon monoxide (CO). For example, Mills *et al*. (2005) measured an NO_2 concentration of 1.6 ppm for their human controlled exposure study of vascular dysfunction in healthy male volunteers, which is over 20 times higher than the median NO_2 concentration measured by McCreanor *et al*. (2007) along a heavily-trafficked London Street. As a result of the elevated exposures to gaseous DE constituents, there remains considerable uncertainty regarding whether the effects observed in the human clinical studies of whole DE can be interpreted as attributable to DEP, or instead, attributable to DE gases.

In the Draft PM ISA, US EPA acknowledges the uncertainty regarding the role of DEP *versus* DE gaseous constituents, but they also suggest on page 6-40 that the DEP constituents are "the more likely

causal agents" underlying the observed effects on vascular function, pointing to findings from a recent study by Bräuner *et al*. (2008). Using periphery artery tone in fingers after arm ischemia as a measure of microvascular function (MVF), Bräuner *et al*. (2008) reported significantly improved MVF (8.1%, 95% confidence interval: 0.4-16.3%) in a panel of 21 nonsmoking couples following reduction of particle exposures by filtration of recirculated indoor air. Based on the fact that NO_2 levels (the only gas monitored by the study investigators) were unchanged as a result of filtration, US EPA (2008) highlighted the Bräuner *et al*. (2008) study findings as supporting the likely causal role for DEP, rather than gaseous DE constituents, in the effects on vascular function reported by recent DE human clinical studies (*e.g.*, Mills *et al*., 2005, 2007; Tornqvist *et al*., 2007). However, as shown in Figure 2 below, it is important to note that the NO_2 exposure levels in the Bräuner *et al*. (2008) study were 40 to 80 times less than those in most of the DE human clinical studies of vascular dysfunction (*e.g.*, Mills *et al*., 2005, 2007; Tornqvist *et al*., 2007). Furthermore, Bräuner *et al*. (2008) was a study of particles in indoor air, with the authors reporting that peaks were not due to rush hour traffic or DE, but were instead due to indoor source events (*e.g.*, cooking, burning candles). Given that indoor source events rather than DE were the likely dominant source of particle exposures to the study participants, the extrapolation of this study to predicting the role of DEP *vs*. DE gases in the human clinical studies of whole DE exposures is unjustified.

Figure 2
NO_2 Concentrations in Recent DE Human Clinical Studies
***versus* the Bräuner *et al*. (2008) Indoor PM Filtration Study**



In short, due to the use of high levels of DEP and DE gases, findings from the DE human clinical studies have uncertain relevance to the potential health impacts of low-level ambient PM exposures. These caveats need to be better conveyed in the Draft PM ISA, in particular in Chapter 2 where there is no mention of the elevated exposure levels employed in the human clinical studies of DE. Quite simply, while recent DE human clinical studies may provide insights regarding the potential mechanisms underlying respiratory and cardiovascular health responses at high PM exposure levels, the unrealistically high DE concentrations cloud the relevance of the biologic mechanisms elucidated in those studies to lower exposure levels.

US EPA fails to acknowledge other major limitations and uncertainties in the DE human clinical studies that undermine their ability to provide support for the ambient-PM epidemiology.

In addition to the use of highly elevated DE/DEP exposure levels, it is also important to note that most DE human clinical studies have used older diesel engine technologies operated with high-sulfur fuels to generate their DE-exposure atmospheres. However, due to the dramatic improvements in diesel

engine technology that have progressively occurred over the last two decades, DE human clinical studies of outdated diesel engine technologies likely have limited relevance to projecting the health impacts for present-day (and future) $PM_{2.5}$, particularly since an increasing fraction of diesel engine engines are running on ultra-low-sulfur diesel (ULSD) fuel formulations. For example, most of the human clinical studies of DE exposures cited in the Draft PM ISA (*e.g.*, Behndig *et al*., 2006; Bosson *et al*., 2007, 2008; Cruts *et al*., 2008; Mills *et al*., 2005, 2007; Nordenhall *et al*., 2001; Pourazar *et al*., 2005, 2008; Salvi *et al*., 1999; Stenfors *et al*., 2004; Tornqvist *et al*., 2007) were conducted using elevated DE exposure levels generated from the same early 1990s-era diesel engine source, namely a 1991 4-cylinder Volvo (TD45) diesel engine.[2] Furthermore, in those studies, this 1991 Volvo engine was operated with a high-sulfur diesel fuel well-exceeding the 2006 US on-road diesel standard (600 ppm vs. 15 ppm sulfur), *i.e.*, a fuel that has not been sold for on-road use in the US since 1993. Although the Peretz *et al*. (2007, 2008a, 2008b) and Carlsten *et al*. (2007, 2008) studies cited by US EPA were conducted using a more recent vintage diesel engine (*e.g.*, 2002 model turbocharged direct-injection 5.9-L Cummins B-series engine) operated with ULSD fuel, even for that more recent engine, the DE is not considered to be representative of "New Technology Diesel Exhaust (NTDE)" – *i.e.*, DE from post-2006 diesel engine technologies.

As discussed in a number of recent publications (*e.g.*, Hesterberg *et al*., 2005, 2006, 2008, 2009), diesel-engine emissions data reflect marked chemical and physical changes between "traditional" (*i.e.*, pre-1988) and "transitional" (1988-2006) DE and NTDE (post-2006). For example, for transit buses, Hesterberg *et al*. (2008) demonstrated significant reductions between "transitional" DE and NTDE regarding emissions of particulate matter, carbon monoxide, total hydrocarbons, non-methane hydrocarbons, formaldehyde, ethylene, benzene, acetaldehyde, and total polycyclic aromatic hydrocarbons (PAHs). Post-2006 NTDE has been shown to contain near-zero levels of diesel particulate mass (Hesterberg *et al*., 2006), as well as near-background levels of DEP particle numbers (Kittelson *et al*., 2006). Importantly, since 2007, all on-road diesel engines manufactured in the US have used ULSD fuel and have employed particle trap after-treatment, markedly reducing the emissions of PM and other air toxics (Hesterberg *et al.*, 2006, 2008, 2009).

While there are few toxicology studies of NTDE, one published study of newer diesel engine technology, namely the McDonald *et al*. (2004) laboratory study of mice inhaling DE, illustrated the potentially large differences in the toxicity of NTDE *versus* non-NTDE. Surprisingly, US EPA does not

[2] Note that this issue is more extensively discussed in our review paper that has been accepted for publication in *Critical Reviews in Toxicology*: Hesterberg, TW; Long; CM; Bunn, WB; Lapin, CA; Sax, SN; Valberg, PA. 2009. "Non-cancer health effects of diesel exhaust (DE): A critical assessment of recent human and animal toxicological literature." *Crit. Rev. Toxicol.* (In Press).

cite this study in the Draft PM ISA. As described in our forthcoming CRT article (Hesterberg *et al*., 2009), findings from this study illustrate reductions in acute lung toxicity that arise from changes in DE composition between NTDE *versus* non-NTDE. Specifically, McDonald *et al*. (2004) investigated the relative toxicity of acute inhalation exposures (6 hrs per day over 7 days) for a baseline uncontrolled, non-NTDE emissions case (approximately 200 $\mu g/m^3$ DEP) *versus* a NTDE, emissions reduction (ER) case (low sulfur fuel, catalyzed ceramic trap, near background levels for all emissions but NO_x) on a suite of sensitive measures of acute lung toxicity in mice, including lung inflammation, RSV resistance, and oxidative stress. For the baseline, non-NTDE case, McDonald *et al*. (2004) observed statistically significant DE-induced effects at 200 $\mu g/m^3$ DEP for each class of responses, while those effects were either nearly or completely eliminated for the NTDE, ER case. Despite the need to confirm these findings for a broader range of ER technologies and operating conditions and for other classes of health endpoints (*e.g.*, cardiovascular effects, allergenic effects), McDonald *et al*. (2004) concluded that their findings suggest that ER technologies can effectively reduce potential health hazards of DE exposures.

Furthermore, the Draft PM ISA cites two intranasal-instillation challenge studies by Diaz-Sanchez *et al*. as providing human clinical data in support of DEP-enhanced allergic inflammatory responses (Diaz-Sanchez *et al*., 1997) and potential DEP-induced *de novo* sensitization to a neoantigen (Diaz-Sanchez *et al*., 1999). However, US EPA does not provide any details on the DEP used in these experimental studies. Importantly, as discussed in several publications (Singh *et al.*, 2004; DeMarini *et al*., 2004; Arey, 2004), the DEP used in studies such as those of Diaz-Sanchez *et al.* (1997, 1999) was collected "cold" from the exhaust of a light-duty (2,740 cc), 4-cylinder, 4JB1-type Isuzu diesel engine (*i.e.*, a 1980s- or 1990s-era light-duty diesel passenger car), with the collection of the DEP on cooled surfaces promoting the condensation of PAHs and other semi-volatiles on both the particle and collection surfaces. Thus, as a result of the instillation procedure, DEP collection techniques, and the age of the diesel engine source, this DEP exposure should not be viewed as typical or representative of either modern-day DEP or ambient PM.

As we discuss in our recent review of the non-cancer health effects of DE (Hesterberg *et al*., 2009), "In evaluating results from recent DE health effect studies, it is thus important to consider their degree of relevance to the present-day mix of diesel-engine technology." This is not done in the Draft PM ISA, where US EPA does not identify that most of the cited DE human clinical studies were conducted using an 18-year-old diesel engine, operated with a higher sulfur fuel that has not been sold for on-road use in the US since 1993. As we concluded in Hesterberg *et al*. (2009), "Studies employing non-

NTDE emissions will have progressively diminishing relevance to projecting the potential human health risks of DE, as NTDE engines continue to replace an increasing fraction of the current diesel-engine fleet." The PM ISA should provide additional perspective on the diesel engines and fuels employed in the cited DE studies, and discuss how the quantitative and qualitative changes in diesel engine emissions that have occurred over the last two decades, and particularly in the last few years, have implications for relying on studies of "historical" DE to project effects attributable to present-day (and future) ambient $PM_{2.5}$.

Despite the high DEP (and gaseous) concentrations, human clinical studies of whole DE exposures have largely reported mixed findings, including findings suggestive of possible thresholds.

As discussed in portions of Chapter 6 of the Draft PM ISA, despite their basis being from highly elevated DE/DEP exposure levels from older diesel engine technology, the findings from recent DE human clinical studies remain largely mixed. For example, while Mills *et al*. (2005) reported evidence of vascular dysfunction (*i.e.*, statistically significant reductions in both endothelium-dependent and endothelium-independent vasodilation) in 30 healthy male (20-38 years old) volunteers two to six hours after 300 $\mu g/m^3$ DEP exposures, Mills *et al*. (2007) did not observe these effects six hours after similar exposures of older male volunteers with a history of previous MI. In their study of 15 healthy male (18-38 years old) volunteers exposed to 300 $\mu g/m^3$ DEP, Tornqvist *et al*. (2007) reported effects on endothelium-dependent (acetylcholine) vasodilation, but no effects on endothelium-independent vasodilation (sodium nitroprusside). Importantly, each of these studies was for whole DE exposures from the same 1991 Volvo engine, which is outdated by today's standards. Furthermore, while both the Mills *et al*. (2005, 2007) studies observed DE-induced decreases in the release of t-PA at 6-hours post-exposure, no such decrease was observed 24-hours post-exposure in the Tornqvist *et al*. (2007) study. While the Draft PM ISA discusses the Peretz *et al*. (2008a) finding of increased plasma levels of endothelin-1 (ET-1) 1-hour after exposure to DEP at 200 $\mu g/m^3$, it does not mention that no change in ET-1 was observed for exposure to DEP at 100 $\mu g/m^3$ in the Peretz *et al*. (2008a) study, nor does it mention that Mills *et al*. (2005) observed no change in ET-1 for a 300 $\mu g/m^3$ DEP exposure.

While the Draft PM ISA notes some of these inconsistent findings in Chapter 6 (but not in the Chapter 2 integration), what is not adequately discussed in the Draft PM ISA is the large number of negative findings in many of these studies, and the implications of these negative findings for the overall assessment of the literature. For example, in addition to not finding effects on either endothelium-

dependent and endothelium-independent vasodilation, the Mills *et al*. (2007) study of patients with coronary heart disease also reported no changes in resting heart rate, blood pressure, baseline blood flow, and a suite of sensitive fibrinolytic and inflammatory markers. As noted in the Draft PM ISA (p. 6-58), several DE human clinical studies (Carlsten *et al.*, 2007; Mills *et al.*, 2005, 2007; Tornqvist *et al.*, 2007) have failed to find any DE-induced changes in C-reactive protein (CRP), a commonly measured marker of inflammation that has been associated with ambient $PM_{2.5}$ in epidemiological studies. Also not cited by the Draft PM ISA is the Blomberg *et al*. (2005) human clinical study of 15 older ex-smokers with mild to moderate COPD, which did not observe any statistically significant changes in a suite of primary inflammatory and pro-thrombotic plasma or serum markers following one hour exposures to whole DE with a DEP concentration of 300 $\mu g/m^3$ during alternated moderate exercise and rest. Similarly negative findings were reported in the Carlsten *et al*. study (2007) of 13 healthy adults at rest and the Carlsten *et al*. (2008) study of 16 adults with metabolic syndrome, which are both cited in the Draft PM ISA.

Although US EPA primarily relies upon epidemiological studies when addressing the evidence relevant to the question of a threshold for PM health effects, it is important to note that DE human clinical studies also provide information relevant to a possible PM threshold. Importantly, some DE human clinical studies have been conducted at a lower DE exposure level corresponding to a DEP concentration of approximately 100 $\mu g/m^3$, and have generally reported equivocal findings. For example, both the Mudway *et al*. (2004) study (which is not cited in the Draft PM ISA) and the Behndig *et al*. (2006) study (which is cited) reported a general absence of lung inflammatory responses. Both of those studies reported findings indicating that DEP concentrations on the order of 100 $\mu g/m^3$ are well-tolerated by the lung. In fact, Behndig *et al*. (2006) concluded that their findings "provide mechanistic data supporting the existence of a threshold for acute airway responses to diesel exhaust in humans." In addition, as discussed in the Draft PM ISA, Stenfors *et al*. (2004) reported equivocal findings for DE-induced lung inflammation in their study with a DEP concentration of 108 $\mu g/m^3$, observing evidence of a lung inflammatory response in healthy volunteers but <u>not</u> in asthmatic volunteers.

With respect to cardiovascular health responses, the recent Peretz *et al*. (2008a, 2008b) studies provide results suggestive of a possible threshold for DEP effects on cardiovascular function in healthy and susceptible subjects that is between 100-200 $\mu g/m^3$. Importantly, the Peretz *et al*. (2008a, 2008b) studies are among the few DE human clinical studies that examined more than just a single DEP exposure concentration, hence providing some information on an exposure-response relationship that is not available from most of the published studies. Specifically, Peretz *et al*. (2008a, 2008b) examined DE-

induced cardiovascular effects in healthy subjects and a population at risk for cardiovascular disease, namely, subjects having metabolic syndrome, for two DE exposure concentrations: 100 and 200 μg/m^3 DEP. Peretz *et al*. (2008a) observed a statistically significant DE-induced decrease in forearm brachial artery diameter and an increase in plasma levels of ET-1 at the 200 μg/m^3 exposure level, but not at the lower exposure level of 100 μg/m^3 DEP. Interestingly, the authors found that the significant changes in brachial artery diameter and ET-1 levels were not correlated, and they also reported a lack of changes in flow-mediated vasodilation and plasma catecholamine. Therefore, while the authors observed responses related to endothelial function and vasomotor function, the mechanisms for these changes were not elucidated. Peretz *et al*. (2008b) assessed DE-induced effects on several indices of heart rate control, finding a statistically significant association between the 200 μg/m^3 DEP exposure (but not at the 100 μg/m^3 DEP exposure) and increased high frequency power of heart rate variability (HF). Given that elevated HF is associated with increased vagal tone, a desirable byproduct of exercise conditioning, this finding is likely not adverse in nature. In support of a possible threshold for DEP-induced CV effects, the Carlsten *et al*. (2007, 2008) studies of the same 2002 Cummins diesel engine also reported a general absence of prothrombotic effects among both healthy volunteers and subjects with metabolic syndrome for DEP exposures levels corresponding to both 100 and 200 μg/m^3.

In summary, even for highly elevated DE/DEP exposure levels from older diesel engine technologies, DE human clinical studies have in general reported mixed findings for both cardiovascular and respiratory health endpoints. The DE human clinical studies are thus not as cohesive or scientifically supportive as the Chapter 2 integration would lead one to believe, as their interpretation is hindered by inconsistencies and variability in outcomes, use of "older" engines and fuels, limitations in exposure protocols and pathways, and uncertainties in extrapolation and generalization. DE human clinical findings cannot be considered in isolation from their major limitations and uncertainties, which is the case in the Draft PM ISA, where it is not acknowledged that most of the cited DE human clinical studies were conducted on whole DE emissions from an 18-year-old diesel engine operated using higher-sulfur fuel. In support of the apparent discordance between the DE human clinical studies and the epidemiologic associations at low ambient PM levels, Peretz *et al*. (2008b) recently concluded:

> We did not observe a consistent DE effect on the autonomic control of the heart in a controlled-exposure in young participants. Efforts are warranted to understand discrepancies between epidemiological and experimental studies of air pollution's impact on HRV. (Peretz *et al*., 2008b)

Importantly, this conclusion would appear to contradict the US EPA statement in Chapter 2 of the Draft PM ISA (p. 2-16) that the human clinical data for DE "provide strong evidence of $PM_{2.5}$-induced decreases in HRV and vasomotor function, as well as increases in markers for systemic oxidative stress."

Furthermore, while the Draft PM ISA refers to the US EPA Health Assessment Document for Diesel Exhaust (US EPA, 2002) and its review of laboratory animal toxicology studies of DE, it does not mention the documentation of a no observable adverse effect level (NOAEL) for chronic DE exposures of 460 $\mu g/m^3$ DEP in the Health Assessment Document (HAD). This NOAEL is based on US EPA's comprehensive review of the large numbers of laboratory-animal studies with exposures to high levels of diesel exhaust (100 to 7,800 $\mu g/m^3$). Thus, laboratory-animal evidence for chronic DE exposure does not provide support for the PM-ISA determination that ambient $PM_{2.5}$ exposures are "likely causal" for cardiovascular morbidity, respiratory morbidity, and mortality.

Overall, considering the mixed nature of the study findings and their questionable relevance to DEP, let alone to lower ambient $PM_{2.5}$ levels, DE human clinical studies do not provide strong support for the epidemiologic associations indicating serious adverse health effects, including hospitalization and death, at low, ambient levels of PM exposure below the current NAAQS. As we concluded in our forthcoming CRT publication (Hesterberg *et al*., 2009):

> There is a clear need for additional studies of NTDE emissions to understand the relevance of findings from the available body of studies of traditional and transitional DE to the new low-emission diesel technologies. Recent controlled human exposure studies have provided useful human evidence of the potential respiratory and cardiovascular health hazards of short-term exposures to elevated DE exposures, but there is a need to better understand the human clinical significance of these findings and their relevance to lower exposure levels and a wider range of engine types, operating conditions, and population subgroups. (Hesterberg *et al*., 2009)

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