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For the Commentary on Research Report 166

Advanced Collaborative Emissions Study (ACES) Subchronic Exposure Results: Biologic Responses in Rats and Mice and Assessment of Genotoxicity

HEI ACES Review Panel

Commentary Appendix: Summary and Evaluation of ACES Phase 3B Chamber Diesel Exhaust Generation and Characterization

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COMMENTARY APPENDIX: SUMMARY AND EVALUATION OF ACES PHASE 3B CHAMBER DIESEL EXHAUST GENERATION AND CHARACTERIZATION

The key features of the exposure system and atmosphere characterization for Phase 3B of the Advanced Collaborative Emissions Study (ACES) are summarized below.

GENERATION

McDonald and colleagues generated diesel exhaust (DE) from a 2007-compliant heavy-duty diesel engine equipped with emission controls (selected from four candidate engines in Phase 1 of the ACES Program; see Khalek et al. 2009; 2011). The engine was operated on a dynamometer and fueled with ultra low-sulfur diesel fuel that met current on-road specifications. The engine and associated systems were maintained as recommended by the engine manufacturer. The crankcase lubricating oil, which was changed every 250 hours, was a proprietary blend provided by Lubrizol that had also been used in Phase 1 of the ACES program. The engine was run on a 16-hour cycle that was specifically designed for the ACES study (as described in the Preface) to represent a combination of highway and urban driving conditions.

Engine exhaust that had passed through the aftertreatment system and gases from the crankcase entered a primary dilution tunnel where they were mixed and diluted with filtered air. The dilution ratio was approximately 5:1. The exhaust mixture then passed into transit lines connected to each exposure chamber, where it was further diluted before entering each chamber. Three exposure levels were targeted based on the nitrogen dioxide (NO_2) concentrations. Additional dilution air was added in the chambers as needed to maintain stable NO_2 concentrations and to remain close to the concentration targets: namely, 4.2 ppm (high-DE exposure), 0.8 ppm (mid-DE exposure), and 0.1 ppm (low-DE exposure) NO_2. The overall total dilution ratios at these concentrations were approximately 25:1, 115:1, and 840:1, respectively. The residence time of DE in the dilution tunnel and transit lines was less than 5 seconds. After the exhaust reached the exposure chamber, the residence time was approximately 4 minutes. The control exposure consisted of filtered air.

CHARACTERIZATION

The chamber air was characterized by sampling from a port located inside the exposure chambers. For some PM characterization, air was also sampled from the transit line, just before the chamber.

Continuous concentrations of nitrogen oxides (NO_x), made up of nitric oxide (NO) and nitrogen dioxide NO_2, were measured daily at each exposure level throughout each exposure day. NO_x concentrations were also measured from the primary dilution tunnel for the purpose of calculating the dilution ratio between the primary tunnel and the chambers. Continuous concentrations of carbon monoxide (CO), carbon dioxide (CO_2), nonmethane hydrocarbons, particle mass (using a Dekati Mass Monitor) and size distribution (using an aerodynamic particle sizer), and black carbon (using a photoacoustic spectrometer) were measured daily in the high-DE exposure chamber. During periodic intensive characterizations, these measurements were taken at the other exposure levels, and on those days, the measurements were not made at the high-DE exposure level.

A more detailed characterization of particle mass and size was conducted once per week at each exposure level. Integrated PM mass concentration was measured by gravimetric analysis of Teflon-membrane filters at both the inlet of the chamber and inside the exposure chamber. These two measurements allowed determination of how much PM the animals in the exposure chamber contributed to the total PM in the chamber. A fast-mobility particle sizer (FMPS) was used to measure particle volume (mass), number, and number-based particle size distribution for particles between 5 and 500 nm in diameter, and an aerodynamic particle sizer was used to measure the mass-based size distribution (data not provided in the report by McDonald et al.).

A detailed (referred to as “intensive” in the IR) characterization of the gaseous and particulate phase compounds was, and continues to be, conducted periodically as indicated in the protocol: at 1.5 months for the mouse exposures, and at 2.5 and 11.5 months and approximately one week before

the end of the exposures of the rats. Results of the first of these characterizations (in the mouse chambers) are provided in Appendix B of the report by McDonald and colleagues. Results of the subsequent analyses will be provided when the study is completed.

The temperature inside the chambers ranged between 18°C and 26°C.

RESULTS AND INTERPRETATION

ROUTINE EXPOSURE CHARACTERIZATION

Results of the daily chamber atmosphere characterization and weekly gravimetric determination of PM mass concentrations at the inlet and inside the chambers are provided in Tables 3 (mouse exposures) and 4 (rat exposures) of the report by McDonald and colleagues. The 16-hour average high, medium, and low NO_2 exposure concentrations summarized over the entire 3-month exposure duration were 4.3, 0.8, and 0.1 ppm for the mouse exposures and 3.6, 0.95, and 0.11 ppm for the rat exposures.

Representative real-time particle mass measurements and particle number-based size distributions from the high-DE exposure chamber are presented in Figures 1 and 2 of the report by McDonald and colleagues. They show that PM mass concentrations and number-based size distributions increased only during periods of trap regeneration, as was observed in Phase 3A of ACES (Mauderly and McDonald 2012). In that report the authors noted that regeneration occurred once or twice during one 16-hour cycle. Each regeneration event lasted approximately 90 minutes.

Concentrations of PM mass collected on filters in the clean-air, low-, mid-, and high-exposure chambers ranged between 32 and 38 $\mu g/m^3$ in the mouse chambers and 27 and 37 $\mu g/m^3$ in the rat chambers, regardless of the dilution ratio. PM mass concentrations at the chamber inlet were always substantially lower (by 60% or more) than PM mass concentrations in the chamber, indicating that much of PM mass in the chamber was contributed by the animals (likely from both dander and fine food dust). The average PM mass concentrations at the chamber inlet for high-DE, mid-DE, and low-DE exposure were, respectively, 9, 3, and 2 $\mu g/m^3$ in the mouse chambers and 13, 4, and 2 $\mu g/m^3$ in the rat chambers. The investigators note that, in contrast with PM mass concentrations in the chambers, the PM mass concentrations at the inlet were "dilution-dependent."

In contrast to the gravimetric PM mass, PM mass and number concentrations measured with the FMPS showed a dilution-dependent change. The analyses of the number-based and mass-based particle size distributions conducted with the FMPS during the detailed intensive characterization period (see below) showed that the median number-based size in the mid- and high-exposure chambers was approximately 20 nm, but the median mass-based size was 40 nm at the mid- and high-DE exposures. These values are consistent with measurements made in Phase 3A (Mauderly and McDonald 2012) and also in Phase 1 (Khalek et al. 2011). In Phase 1 the investigators determined that the geometric number mean diameter for the four engines in the chamber during the same cycle used in Phase 3 was 25 nm.

The investigators noted that the particles in the low-DE and control exposure chambers were "much larger" but that the mass concentrations (measured with the FMPS) were very low (<1 $\mu g/m^3$).

The ACES Review Panel noted that the number-based size distribution in the low-DE exposure chamber did not appear to differ from that at the higher exposures, but the number concentrations were much lower. Overall, these results indicate that the particles generated by the engine are in the ultrafine range (that is, smaller than 0.1 µm in aerodynamic diameter) and can be differentiated from those generated by the animals by comparing the particle measurements taken both at the chamber inlet and inside the chamber using different instruments and particle metrics.

DETAILED EXPOSURE CHARACTERIZATION

Gaseous Species

Figure 3 of the report by McDonald and colleagues summarizes the relative contributions of CO, NO_x, NO_2, sulfur dioxide (SO_2), and volatile organic compounds (VOCs) (as the percentage of total mass of these species) at the different exposure levels. CO_2 was also measured, but was not included in the calculation because of its abundance. Results of the first detailed chamber exposure characterizations (during the mouse study) are presented in Table B.2 of Appendix B.

The investigators reported that CO, NO, and NO_2 accounted for most of the mass of the measured components in the DE exposure atmosphere in the chambers. They stated that the contribution of NO and NO_2 as a fraction of total gaseous mass increased proportionally with exposure level, "as expected." They reported that, although VOCs were present in low concentrations in all exposure chambers, their percentage of the total gaseous mass was higher (up to 10%) in the control and low-DE exposure chambers, and they concluded that the animals contributed a significant amount of VOCs.

The Committee noted that if all the components of the exhaust were diluted to the same extent, their relative proportion would not vary with the exposure level. Therefore, these results suggest that chemical reactions may occur and affect the level of various compounds differently. There could be measurement errors, given that many of the concentrations are very low and near the limit of detection of the instruments.

Total VOC concentrations were higher in the chambers with DE, but the increase was not proportional to the dilution: the highest concentration was measured in the low-DE exposure chamber. The concentrations were 19.1 $\mu g/m^3$ in the clean-air chamber, 92.5 $\mu g/m^3$ in the low-DE chamber, 33.1 $\mu g/m^3$ in the mid-DE chamber, and 74.9 $\mu g/m^3$ in the high-DE chamber. While the investigators indicate that the high VOC concentration in the low-DE chamber is an exception, results of the other intensive characterizations are not available to verify this statement. These data suggest that the mice contribute some VOCs, but do not entirely support the authors' conclusions (see above) that the animals make a "significant" contribution. In Phase 3A the sum of the VOCs ranged between 44.3 and 90.9 $\mu g/m^3$ during four separate tests at the high DE chamber, indicating a high variability in the concentrations; VOCs were not measured in the clean-air chamber. Of particular interest are the VOCs listed by the EPA as mobile source air toxics: benzene, toluene, ethyl benzene, and xylene (the four together are referred to as BTEX), and 1,3-butadiene. The concentrations of BTEX were very low and showed a trend toward increasing with the DE exposure level, especially benzene and toluene. However, for toluene, an unusually high concentration was measured in the low-DE exposure chamber (21 $\mu g/m^3$, versus 2.1 $\mu g/m^3$ in the high-DE exposure). While the level of toluene in the high-exposure chamber was similar to that measured (without the animals present) in Phase 3A (2.3 $\mu g/m^3$), the levels of benzene were about 10 times higher in Phase 3B (4.1 $\mu g/m^3$) than in Phase 3A (0.34 $\mu g/m^3$). These discrepancies may be in part due to the instrument sensitivity and are difficult to interpret at this time. 1,3-Butadiene was reported as having a concentration of zero.

Carbonyl concentrations were detected in the clean-air exposure chamber (39.6 $\mu g/m^3$) and were highest in the low-DE exposure chambers (129 $\mu g/m^3$) and lowest in the high-DE exposure chamber (0.3 $\mu g/m^3$). The dilution-dependent decrease was interpreted as being due to reactions of carbonyls with NO_x during sampling. The investigators noted that they used a cobalt oxide denuder to remove NO_x before sampling, but discovered — after the fact — that the denuder was not removing these species. For the subsequent detailed characterizations they set up a protocol to regenerate the denuder after every sampling period and were thus able to scrub NO_x with high efficiency.

The ACES Review Panel agreed that these findings are likely related to measurement artifacts of the cartridge impregnated with 2,4-dinitrophenylhydrazine (DNPH). The cartridge typically is outfitted with an ozone scrubber (e.g., potassium iodide) to remove negative interference (due to the reaction of ozone with the DNPH substrate). While ozone is not an issue in this study, NO_2 — which

is an oxidant as well — is likely overwhelming the oxidant scrubber and reacting with the DNPH or its carbonyl derivatives. Regarding the cobalt oxide denuder, the panel noted that it can introduce another variable and cause loss of carbonyls by itself. In this case, the cobalt oxide could provide a catalytic surface to enhance the reaction of NO_2 with carbonyls.

The finding of carbonyls in the control chamber led the investigators to conclude that the animals contribute to their background concentrations. The most abundant carbonyls in the low-DE exposure chamber were, in order of abundance, 2-butanone, acetaldehyde, and acetone. In the control chamber, the most abundant was acetone (present at a higher concentration than in the DE exposure chambers); acetone is a product of the body's metabolism. Results of the additional intensive characterization should provide a more robust database of the levels of carbonyls in the chamber.

PM Composition

The detailed characterization of PM focused on a large number of compounds; however, since the mass collected on the filter was very small, the concentrations of many compounds were at or below the detection limit and are listed as having a concentration of zero. The most abundant PM-associated elements are reported to be zinc, manganese, copper, iron, potassium, and calcium. Sulfur, chlorine, phosphorus, and silicon were also abundant. Sulfur increased with increasing exposure.

Organic carbon (OC) did not appear to change with dilution, and the ratio of elemental carbon (EC) to OC increased with exposure level, suggesting a contribution of EC from the exhaust. Inorganic ions (ammonium, sulfate, and nitrate) were very low or undetectable in the control chamber and increased with decreasing exhaust dilution.

Based on the PM compositional data summarized in Figure 3 of the report by McDonald and colleagues, the investigators concluded that the high-DE exposure level had the largest contribution of PM that was derived from the engine (rather than from the animals), presumably because the proportions of EC, sulfate, nitrate, and elements to the total mass were the largest. They also concluded that (total) carbon accounted for about 50% of the PM mass in the chamber, and the remainder was a combination of the inorganic ions and elements. Some variability was noted in the proportion of OC relative to the total PM mass, with the higher proportion in the clean-air and the low-exposure chambers. The likely explanation, provided by the authors, was that the animals may contribute some organic species. The authors also suggested the possibility of sampling artifacts.

The investigators explained that the small changes in PM composition observed across exposure levels were likely a result of reactions between exhaust gases and ammonia (NH_3) as previously shown in the 2004 study by McDonald and colleagues. In that study, which is described in more detail in later sections, McDonald and colleagues noted that the highest concentration of NH_3 was in the clean-air group and that it decreased with increasing DE exposures. They concluded that the primary source of NH_3 in the chamber was the rodents and hypothesized that the decrease in NH_3 was related to its conversion, in the presence of exhaust gases, to secondary aerosols (such as nitrate and sulfate) and ammonium (NH_4^+). The concentrations of NH_4^+ in the current study were lower than those measured in the 2004 McDonald study, but increased with the DE exposure, as observed in that study. However, the concentrations of NH_3 were not measured in the current study.

COMPARISON WITH OTHER STUDIES

To put the results of the chamber atmosphere characterization in the current study into context, we compare them with results obtained in other studies — for example those obtained in ACES Phase 3A in the chambers without the animals present (Mauderly and McDonald 2012) and in ACES Phase 1 (Khalek et al. 2009; 2011). As part of Phase 1, the engines were operated on short test cycles and also on the 16-hour cycle, during which an intensive exhaust characterization of the diluted exhaust in a chamber (without animals) was conducted that was similar to those used in the current report. Although the emissions were reported as gram per horsepower • hour (g/hp • hour) and thus

cannot directly be compared with the chamber concentrations measured in this study, the measurements can be useful for comparing ratios of different compounds. The dilution ratio of filtered air to exhaust in Phase 1 (40:1) did not exactly match any of the dilution ratios in Phase 3B (the closest dilution ratio was 25:1); in addition, investigators in Phase 1 extensively tested engine B (with only minor testing of a duplicate engine, B′) while Phase 3B used engine B′ because this slightly updated engine had a larger share of the market for this model. Another interesting comparison is with the concentrations measured in previous inhalation studies of DE (which used older engine technologies without any emission controls). As shown in the Commentary Appendix Table, the studies are as follows:

- a chronic bioassay conducted by Mauderly and colleagues (1994) at the same facility as for Phase 3A and 3B, using a 1988 6.2 L light-duty diesel engine (16 hours/day);

- a chronic bioassay conducted by Heinrich and colleagues (1995) at the same time as the Mauderly and colleagues (1994) study, using a 1.6 L light-duty diesel engine in both rats and mice (18 hours/day); and

- a semichronic bioassay (from a few days to up to 6 months of exposure) conducted by McDonald and colleagues using a 2000 5.9 L light-duty engine (6 hours/day; see results of chamber characterization in McDonald et al. 2004). ‘

The engine cycles and sampling instruments varied across the studies. The comparison is limited to the regulated pollutants and EC/OC. Data on unregulated pollutants are limited and difficult to compare across studies.

PM, NO_x, and NO_2

The Commentary Appendix Table includes the concentrations of PM mass, NO_2, and NO_x, and their relative proportions, as well as the EC/OC ratios from the current studies and the studies described above.

NO_x and NO_2 In Phase 1, NO_2 was 65% of NO_x, and in Phase 3A, it was 58%, which is higher than the 40% to 42% (in the rat chambers) and 46% to 47% (in the mice chambers) in Phase 3B. In addition, the concentrations of NO_x in Phase 3A, which targeted a NO_2 concentration similar to that in Phase 3B, were lower (6.9 versus 8.6 [rats] and 9.3 [mice]) than NO_x concentrations at the high-exposure level in Phase 3B, suggesting that the equilibrium between NO_2 and NO_x changed in the presence of the animals.

With regard to the comparisons with two studies using older diesel engine technologies (Heinrich et al. 1995; Mauderly et al. 1994), the Commentary Appendix Table shows that the $NO_2$to NO_x ratio and the PM mass to NO_2 ratio are substantially different between old and new diesel engine technologies. In these two studies with pre-2007 engines, the NO_2 concentration at the highest exposure level was 3.8, which is similar to that in the ACES study. However, the percentage of NO_2 relative to NO_x was much lower in the chamber atmosphere from the older engines (11% to 16%) than in the chamber atmosphere from the 2007 engine (40% to 42% with rats) at the mid- to high-exposure levels. At the same time, the NO_x concentration was higher for the older engines (approximately 23 to 33 ppm) than for the 2007 engine (8.6 to 9.3 ppm with animals), showing the progress made in reducing NO_x emissions in the 2007 engines. Comparison with the measurements in the 2004 study by McDonald and colleagues using a more modern engine (a model-year 2000 light-duty engine operating with high-sulfur fuel) can be made between the mid-exposure concentrations in the current study and the mid-low concentrations in the MacDonald et al. 2004 study, which had comparable NO_2 concentrations (0.8–0.95 vs. 0.7). The results indicate that the percentage of NO_x that was NO_2 was substantially lower in the 2000 engine (11% vs. 40%–47%), as also observed in the comparison with the 1980 engines.

PM In Phase 3B, the gravimetric measurement of PM mass in the chambers ranged between 30 and 38 µg/m^3. These concentrations were higher than that measured in Phase 3A because they include the animals' contribution. The EC/OC ratio in Phase 3B was 0.44 at the high exposure level, compared with 1.1 for engine B in Phase 1 (Khalek et al. 2009), suggesting a higher proportion of organic material in the particles collected in the chambers with the animals present. (It should be noted that the EC/OC ratios varied depending on the operating cycle and across engines.) The EC/OC ratios were not available for Phase 3A. In the 2000 engine exhaust characterized by McDonald and colleagues (2004), the EC/OC ratio was around 2.3 at the mid-exposure concentration versus 0.23 in the ACES Phase 3B study (with comparable NO_2 concentrations), indicating a much large amount of soot from the older engine.

In Phase 3B, the PM count was 10,000 particle/cm^3 in the high-level exposure chamber versus 14,000 particles/cm^3 in Phase 3A. The count was 42,600 particles/cm^3 at the mid-exposure concentration, substantially lower than that reported in the 2004 study by McDonald and colleagues (190,000) but with comparable NO_2 concentrations, indicating that for DE with a similar NO_2 concentration, PM number also decreased in the 2007 engines relative to older engines. The PM number-based size distribution showed a median size of 20 nm, which is consistent with the mean diameter of 25 nm in Phase 1 across the four engines (Khalek et al. 2011). In Phase 3A, the investigators did not provide the mean or median particle size during the 16-hour cycle; however, the size distributions were very similar to those of Phase 3B (Mauderly and McDonald 2012). These types of measurements were not made in the earlier diesel bioassays, which relied exclusively on the gravimetric method to measure PM mass concentrations.

The most abundant PM elements measured in Phase 1 were, in order of abundance: sulfur, phosphorous, iron, and calcium. These same elements were reported as being present in the current characterization, but the relative levels differed somewhat between the two phases.

SUMMARY

This appendix summarizes the results of the characterization of the chamber during the animal exposures in Phase 3B of the ACES program. Comparisons are also made to results of exhaust characterization from previous ACES phases and earlier studies of DE. The ACES Review Panel commended the investigators for carefully developing and implementing the sampling and analytic approaches for characterizing the composition of the chamber atmospheres, which involved routine daily and weekly measurements and periodic intensive characterizations.

The detailed exhaust characterization results were obtained from only the first detailed characterization (during the 3-month mouse exposures; see Table B.2 of McDonald et al. in this report) and should not be considered conclusive. Given the low pollutant concentrations in the exhaust and possible sampling artifacts, there are some uncertainties in the data. Unexpected trends across the exposure groups will need to be confirmed by the additional detailed characterizations before any attempts to understand the reasons for the patterns. This is a very rich database that will be enhanced by additional characterizations over the course of the bioassay.

An important result is that the concentrations of PM and semivolatile and volatile organic compounds were generally very low. This confirms the original expectations during the planning of ACES and the results in Phase 1. The most abundant pollutants were CO_2, CO, NO, and NO_2. In comparison to earlier DE inhalation studies, the ratio of PM to NO_2 was at least 3 times lower. While NO_2 represented a higher percentage of NO_x in this study (almost 50%), it is apparent that the overall NO_x levels are substantially lower than those measured in the earlier DE inhalation studies. It should be noted that NO_x emissions have been further reduced starting with the 2010-compliant heavy-duty diesel engines. Three representative 2010-compliant engines are currently being tested as part of ACES Phase 2.

Because the investigators used instruments that measured different types of particles and added sampling at the chamber inlet, they were able to determine that the animals made a significant

contribution to the PM gravimetric mass, which is a metric of fine and coarse particles, but contributed very little to the particle number, which is driven primarily by particles less than 0.1 µm in mobility diameter. Data from the intensive characterization suggest that the animals contributed particle-bound organic compounds and VOCs. The particle mass and number-based size distributions (determined with the FMPS, which measures particles up to only 500 nm) confirm the presence in the chamber of very small particles with a median diameter of 20 to 40 nm, which were considered to be derived almost exclusively from the engine.

Comparisons with previous ACES studies (with an engine of the same model and make as that used in Phase 3B) indicate minor differences that could be attributed to the presence of the animals or to between-engine variability. Comparisons with older studies, although limited because of differences in study designs, illustrate trends in emission reduction over time.

Overall, the results of the detailed exhaust characterization in the four exposure-level chambers indicate that several factors may come into play when sampling organic species for 16 hours, such as chemical reactions in the chambers in the presence of the animals as well as in the sampling systems, which are not well understood. The study confirms that the animals — or other, yet-to-be-identified factors in the chambers — make an important contribution to the background concentrations of several pollutants.

More in-depth analyses of possible artifacts would be helpful in future reports. Results of repeated characterizations over the full two-year duration of the chronic bioassay should provide a more complete data set that will help explain trends in pollutant concentrations as a function of dilution and determine whether there are any changes due to engine aging.

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ABBREVIATIONS AND OTHER TERMS

BTEX	benzene, toluene, ethyl benzene, and xylene
CO	carbon monoxide
CO_2	carbon dioxide
DE	diesel exhaust
DNPH	2,4-dinitrophenylhydrazine
EC	elemental carbon
FMPS	fast-mobility particle sizer
NH_3	ammonia
NH_4^+	ammonium
NO	nitrogen monoxide
NO_2	nitrogen dioxide
NO_x	nitrogen oxides
OC	organic carbon
SO_2	sulfur dioxide
VOC	volatile organic compound

Commentary Appendix Table. Concentrations of PM, NO_2, and NO_x in diesel chamber studies. All data were collected in inhalation chambers with rats or mice present, with the exception of data from Phase 3A[a,b].

Studies **Exposure level** **Dilution**	**PM mass gravimetric** (**mg/m^3**)	**PM/NO_2** (ratio)	**NO_2** (ppm)	**NO_x** (ppm)	**NO_2/NO_x** (%)	**EC/OC** (ratio)
ACES Phase 3B (2007 heavy-duty diesel engine B' and ultra-low-sulfur fuel, rats)						
High Exposure 25:1 dilution	0.032	0.09	**3.6**	8.6	42	NA
Mid Exposure 115:1 dilution	0.030	0.03	*0.95*	2.4	40	NA
ACES Phase 3B (2007 heavy-duty diesel engine B' and ultra-low-sulfur fuel, mice)						
High Exposure 25:1 dilution	0.035	0.008	**4.3**	9.3	46	0.44
Mid Exposure 115:1 dilution	0.038	0.05	*0.8*	1.7	47	0.23
ACES Phase 3A[c] (2007 heavy-duty diesel engine B and ultra-low-sulfur fuel, without animals)						
High Exposure 25:1 dilution	0.010	0.003	**4.0**	6.9	58	NA
ACES Phase 1[d] (2007 heavy-duty diesel engine B and ultra-low-sulfur fuel, without animals)						
20:1 dilution, CVS 40:1 dilution, chamber	NA	NA	NA	NA	65 (measured in the CVS)	1.1 (measured in the chamber)
McDonald et al. 2004 (2000 5.9 L light-duty diesel engine and high-sulfur fuel, rats)						
High Exposure 10:1 dilution	1.01	0.15	6.9	57.3	12	2.9
Mid-Low Exposure 100:1 dilution	0.11	0.16	*0.7*	6.5	11	2.3
Mauderly et al. 1994 (1988 6.2 L light-duty diesel engine and high-sulfur fuel, rats)						
High Exposure Dilution ratio NR	6.33	1.7	**3.8**	23.5	16	NA
Mid Exposure Dilution ratio NR	2.44	3.5	0.7	8.8	8	NA
Heinrich et al. 1995 (1.6 L light-duty diesel engine and high-sulfur fuel, rats)						
High Exposure 1:9 dilution	6.98	1.8	**3.8**	33.1	11	NA
Medium High Exposure 1:15 dilution	4.50	2.0	2.3	23.9	9.6	NA
Filtered exhaust 1:15 dilution	0.010	0.003	2.9	23.9	12	NA

[a] Data were averaged over the exposure period except for EC/OC, which was measured during the intensive characterization.[b] CVS indicates constant volume sampling; NA indicates data not available; boldface and italics indicate NO_2 concentration comparability across studies. [c] Mauderly and McDonald 2012
[d] Khalek et al. 2009, 2011