Final Technical Report

Project No. DE-FC26-05NT42304

Lovelace Biomedical and Environmental Research Institute
Albuquerque, NM

Health Effects of Subchronic Inhalation of Simulated Downwind Coal Combustion Emissions

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Principal Author: Joe L. Mauderly, DVM
Lovelace Biomedical and Environmental Research Institute
2425 Ridgecrest Dr. SE
Albuquerque, NM 87111
505-348-9432
jmauderl@lrri.org

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Abstract

This Report summarizes accomplishments resulting from project DE-FC26-05NT42304 "Health Effects of Subchronic Inhalation of Simulated Downwind Coal Combustion Emissions". The project was initiated on February 3, 2005, and funding from NETL was terminated early due to internal programmatic funding constraints. This report describes accomplishments through March 2008. The project was co-funded by NETL and other sponsors, and is being completed on a somewhat delayed schedule using funds from the other sponsors.

The purpose of this project was to conduct a comprehensive laboratory-based evaluation of selected respiratory and cardiac health hazards of subchronic (up to 6 months) inhalation of simulated key components of "downwind plume" emissions of coal combustion. This project was performed as an integral part of a joint government-industry program termed the "National Environmental Respiratory Center" (NERC), which is aimed at disentangling the roles of different physical-chemical air pollutants and their sources in the health effects associated statistically with air pollution. The characterization of the exposure atmosphere and the health assays were identical to those employed in the NERC protocols used to evaluate other pollution source emissions, such as diesel, gasoline, and wood combustion.

The project had two phases, each encompassing multiple tasks. Guidelines for the composition of the exposure atmosphere were set by consensus of an expert workshop. Development of the capability to generate the exposure atmosphere and pilot studies of the comparative exposure composition using two coal types were accomplished in Phase 1. In Phase 2, the toxicological study was conducted using Powder River Basin Sub-bituminous coal. NETL provided 50 % support for the work in Phase 1 and had intended to provide 20% support for the work in Phase 2. Phase 1 is completed and Phase 2 is in the final stages. All animal exposures were completed without incident, and the composition of the exposure atmospheres met the targets. All of the health sample collections are completed, but some samples remain to be analyzed. Data summaries and final statistical analysis of results remain to be completed. The goal is to submit all publications before the end of FY-08.

Repeated exposure to simulated downwind coal emissions caused some significant health effects, but the number of effects tended to be fewer than those caused by the other NERC exposures (diesel and gasoline emissions and hardwood smoke). The lowest concentration, a dilution containing approximately 100 μg particulate matter (PM)/m^3, was a no-effects level for nearly all measured variables. One of the most interesting findings was that few, if indeed any, health outcomes appeared to be caused by the PM component of the exposure. This finding strongly suggests that PM simulating the major contributions of coal combustion to environmental PM is of very low toxicity.

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1. Results of Work During the Reporting Period

a. Approach and Status

1) Approach

The general approach taken in this project did not change from that described in the application. The project had two phases. In Phase 1, a system capable of generating exposure atmospheres simulating a "downwind" coal combustion emissions mixture was constructed, generation trials were conducted using two coal types, and results were summarized in a report which served as a basis for a decision on which coal type to use for animal exposures. It was found to be feasible to use either type of coal, and Powder River Basin low sulfur sub-bituminous coal was selected for the main study.

Phase 2 consisted of setting up the animal exposure system, adjusting the system to achieve the desired exposure atmosphere using the selected coal type, conducting the subchronic animal inhalation study, and making detailed measurements of the exposure atmospheres and health responses.

2) Status

Phase 2, Task 2 (conduct animal exposures) is completed. Phase 2, Task 3 (conduct health measurements and analyze data), and Phase 2, Task 4 (characterize the exposure atmospheres and analyze data) are nearly completed, but will necessarily still be underway until the last data are analyzed and results are summarized for publication.

The last progress report summarized the exposures and the health results available at that time. This report updates currently available health response data. Some of the last data are yet to be summarized, and final statistical comparisons will be needed before the results are ready for publication.

b. Activities and Progress

The principal activities occurring since the last quarterly report have been completion of the analysis of samples collected from animals, and processing of data through quality assurance audit.

c. Results and Discussion

The results must be considered preliminary and incomplete until the final statistical analyses are performed; however, the key outcomes of the study are largely evident. It should be recalled that a key purpose of the program is to compare results among studies of different source-based pollution mixtures. The information presented below compares the exposures and responses between this study (coal emissions) and the previous studies (diesel emissions, wood smoke, and gasoline emissions

1) Comparative Compositions of Exposure Atmospheres

The cumulative average concentrations of components of the exposure atmospheres that are measured daily were reported last quarter. The exposures were very consistent throughout the study.

The following figures compare the exposure atmosphere in this study to those of the preceding three NERC studies. Figure 1 illustrates the mass concentrations of particulate matter (PM) in the four studies (Fig. 1A) and the mass proportions of the major components of PM. This (coal) study employed the same PM mass concentrations as the diesel and wood smoke studies, with the exception that the lowest of four levels was eliminated in order to include a filtered (no PM) atmosphere at the highest concentration (that was also done in the gasoline study). The gasoline study employed lower PM concentrations because PM is a much lesser constituent of gasoline emissions. Instead of matching PM concentrations, the gasoline study employed the same approximate dilution ratios as the diesel study. There were clear differences among the exposures in PM composition (Fig. 1B). The diesel, wood smoke, and gasoline PM was largely carbonaceous, while the coal PM was largely sulfate. This was planned, and modeled the predominant contribution of coal combustion to ambient PM by downwind oxidation of SO_2 and formation of sulfate components of the ambient aerosol.

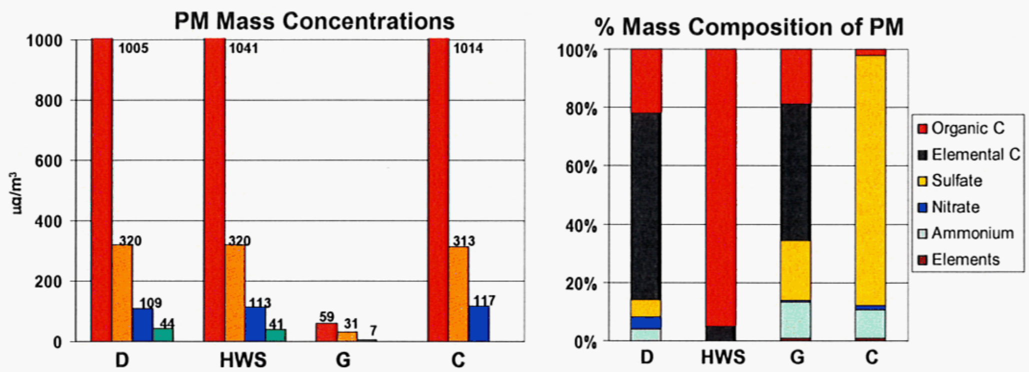

Figure 1. Concentrations and Composition of PM Mass in Diesel (D), hardwood smoke (HWS), Gasoline (G), and Simulated Downwind Coal Emissions (C) Exposure Atmospheres.

Although the PM mass concentrations in the coal study were similar to those in the first two studies, PM only contributes a minor portion of the total mass of emissions. Gases and vapors contribute much larger portions of mass to all combustion emissions, although that point is often overlooked in debates about the relative importance of different air pollutants. The relative amounts of total "exposure" mass (mass contributed by the test atmospheres) are illustrated in Figure 2. When total mass except for CO_2 and H_2O is compared among the exposures, it is apparent that the coal exposure involved the least total mass and gasoline involved the most mass (Fig. 2A). It is also apparent that PM is a very small portion of the total mass. When CO_2 is also considered (Fig. 2B), it is apparent that CO_2 contributes more mass than all of the other components combined (O_2, N_2, etc. are not considered in this display).

The relative proportions of the major components of total mass (excluding CO_2 and H_2O) in the four atmospheres are illustrated in Figure 3. In this display, it is readily seen that carbon monoxide and nitrogen oxides comprise the largest portions of mass in the engine emission atmospheres, carbon monoxide and volatile organic carbon (VOC) comprise the greatest portion of mass in the wood smoke atmosphere, and VOC comprises the greatest portion of total mass in the coal atmosphere. In considering these differences, it is important to remember that the total mass concentration varies among the atmospheres, as shown above. PM contributed a very minor portion of total mass in all four atmospheres. Although this fact is counterintuitive to those engrossed by the currently overwhelming focus of attention on PM, it is a very important perspective when comparing hazards among different source emissions and/or attempting to resolve which pollutants cause the different health effects.

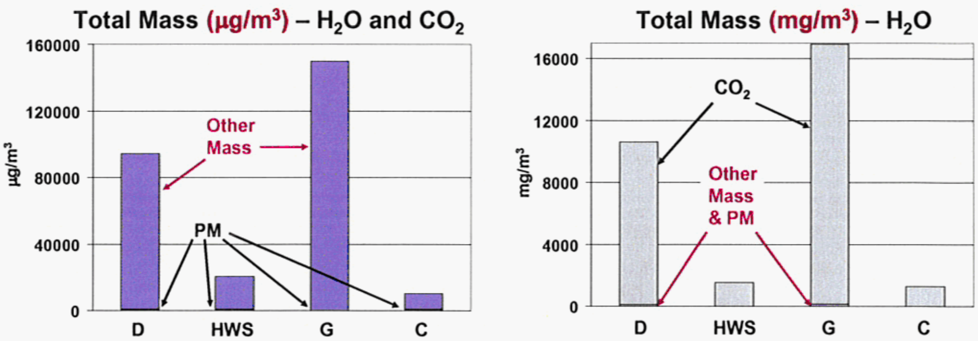

Figure 2. Comparisons of Total Mass Among Exposures, Excluding CO_2 and H_2O (A) and including CO_2 but excluding H_2O (B).

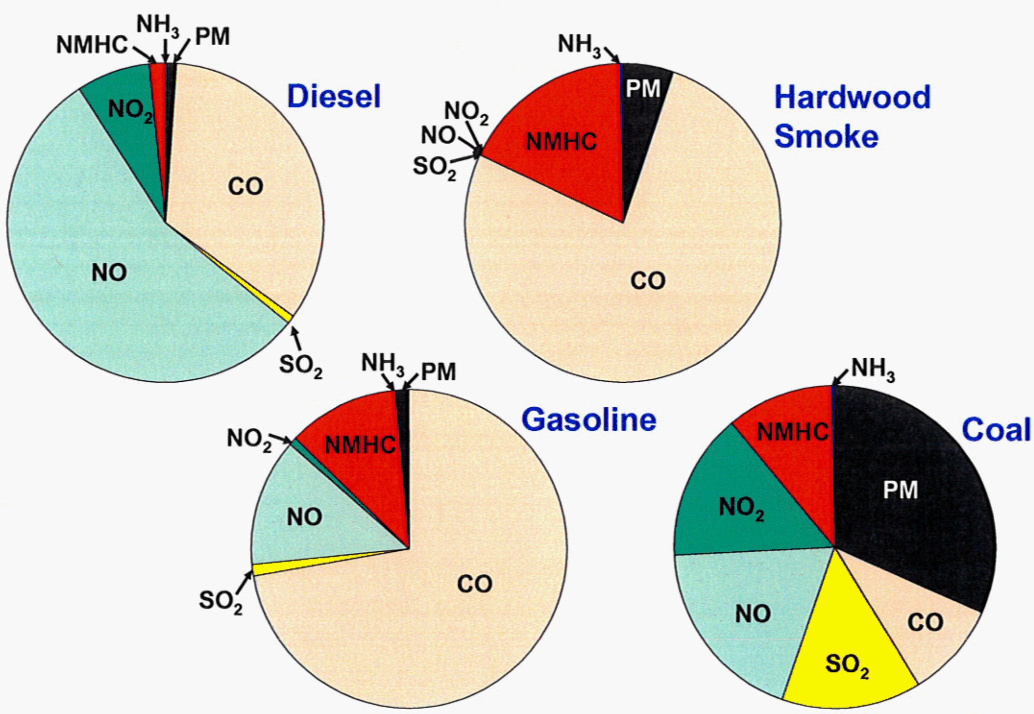

Figure 3. Composition of Total Exposure Mass of Different NERC Exposure Atmospheres (minus CO_2, CH_4, and H_2O).

2) Comparative Health Responses to NERC Exposures

The responses of the different health assays to the four exposures are summarized in the following tables. In each table, significant effects of exposure are indicated by arrows (🡅 = increase, 🡇 = decrease), and the color of the arrows indicate whether the response was significant in females (red), males (blue) or both genders (green). Variables for which data are not yet complete are indicated by a question mark (?). This includes the cardiovascular effects in hypertensive rats for the coal study, and the vascular effects in $ApoE^{-/-}$ mice and effect on development of respiratory allergic responses in BALBc mice for diesel and wood smoke. The latter assays were added to the protocol after the original diesel and wood smoke studies were completed, and are now being done for those atmospheres but are not yet complete. In this display, as in all preceding NERC publications, a "significant effect of exposure" is declared only if the results meet two criteria. First, the results of a trend test among the control and exposed groups must be significant; second, the responses of one or both of the groups exposed to the two highest concentrations must differ significantly from controls by pair-wise tests (adjusted for multiple comparisons, of course). Parametric or nonparametric tests are used, depending on the nature of the data distribution, and the criterion for significance is set at $p<0.05$ for all comparisons.

None of the exposures had significant effects on mortality or body weight. None of the exposures cause visible signs of illness.

The following tables compare other responses among the diesel (D), hardwood smoke (HWS), gasoline (G) and coal (C) exposures. The findings are summarized following the tables.

Table 1. Effects of Exposure on Organ Weights of Rats

Response Variable	Time	D	HWS	G	C
Organ Weight in Male and Female F344 Rats					
Heart	1 wk	–	–	–	↓
	6 mo	–	–	↑	–
Lung	1 wk	↑	–	–	–
	6 mo	↑	–	–	–
Kidney	1 wk	–	–	–	–
	6 mo	↑	–	–	↑
Liver	1 wk	↓	↓	–	–
	6 mo	–	↓	–	–
Spleen	1 wk	–	↑	–	–
	6 mo	–	–	–	–
Thymus	1 wk	↓	↓	–	–
	6 mo	–	–	–	–
Seminal vesicle	1 wk	–	–	–	–
	6 mo	–	–	↓	–

Table 2. Effects of Exposure on Organ Weights of Mice

Response Variable	Time	D	HWS	G	C
Organ Weight in Male and Female A/J Mice					
Heart	1 wk	–	–	–	–
	6 mo	–	–	–	–
Lung	1 wk	↑	–	–	–
	6 mo	↑	↓	–	–
Kidney	1 wk	–	–	–	–
	6 mo	↑	–	↓	–
Liver	1 wk	↓	–	–	↓
	6 mo	↑	–	–	–
Spleen	1 wk	–	↑	–	–
	6 mo	↓	–	–	–
Thymus	1 wk	–	–	–	–
	6 mo	–	–	–	–

Table 3. Effect of Exposure on Hematology of Rats

Response Variable	Time	D	HWS	G	C
Hematology & Serum Clotting Factors in Male and Female F344 Rats					
Total WBC	1 wk	–	↑	–	–
	6 mo	↓	–	–	–
Neutrophils	1 wk	–	–	–	–
	6 mo	–	↓	–	–
Lymphocytes	1 wk	–	↑	–	–
	6 mo	–	↑	–	–
Eosinophils	1 wk	–	–	–	–
	6 mo	–	–	–	–
Platelets	1 wk	–	↑	–	–
	6 mo	–	–	–	–
Total RBC	1 wk	–	–	↑	–
	6 mo	–	–	↑	–
Hematocrit	1 wk	–	–	↑	–
	6 mo	–	↑	↑	–
Hemoglobin	1 wk	–	–	↑	–
	6 mo	–	↑	↑	–
Factor VII	1 wk	↓	–	–	–
	6 mo	↓	–	–	–
TAT Complex	1 wk	↓	–	–	–
	6 mo	–	–	–	–
Fibrinogen	1 wk	–	–	↑	–
	6 mo	–	–	–	–

Table 4. Effect of Exposure on Serum Chemistry of Rats

Response Variable	Time	D	HWS	G	C
Serum Chemistry in Male and Female F344 Rats					
Alanine aminotransferase	1 wk	–	–	–	–
	6 mo	–	–	↓	–
Albumin	1 wk	–	–	–	–
	6 mo	↑	–	–	–
Albumin/globin	1 wk	–	–	–	–
	6 mo	–	–	–	–
Alkaline phosphatase	1 wk	↑	↓	–	–
	6 mo	–	↓	–	–
Aspartate aminotrans.	1 wk	–	↓	–	–
	6 mo	↓	↓	↓	–
Bilirubin	1 wk	–	–	–	–
	6 mo	–	↑	–	–
Blood urea nitrogen	1 wk	↑	↓	–	–
	6 mo	↓	↓	–	–
BUN/Creatinine	1 wk	–	↓	–	–
	6 mo	↓	↓	–	–
Calcium	1 wk	–	–	–	–
	6 mo	↑	–	–	–
Chloride	1 wk	–	–	–	–
	6 mo	↑	–	–	–
Cholesterol	1 wk	↓	↓	–	–
	6 mo	↓	↑	–	–
Creatinine	1 wk	–	↓	–	–
	6 mo	–	–	–	–
Gamma–glutamyl transp.	1 wk	–	–	–	–
	6 mo	↑	–	–	–
Globulin	1 wk	–	–	–	–
	6 mo	–	–	–	–
Glucose	1 wk	–	–	–	–
	6 mo	↑	↑	–	–
Phosphorous	1 wk	–	–	–	–
	6 mo	–	↑	↓	–
Protein	1 wk	–	↓	–	–
	6 mo	↓	–	–	–
Potassium	1 wk	–	–	–	–
	6 mo	–	–	–	–
Sodium	1 wk	–	↓	–	–
	6 mo	↑	↓	–	–

Table 5. Effects of Exposure on Bronchoalveolar Lavage of Rats

Response Variable	Time	D	HWS	G	C
Bronchoalveolar Lavage of Male and Female F344 Rats					
Total Nucleated Cells	1 wk	–	–	–	–
	6 mo	–	–	–	–
Macrophages	1 wk	–	–	–	–
	6 mo	–	–	–	–
Neutrophils	1 wk	–	–	–	–
	6 mo	–	–	–	–
Alkaline phosphatase	1 wk	–	–	–	–
	6 mo	↑	↓	–	–
β–Glucuronidase	1 wk	–	–	–	↑
	6 mo	↓	↓	–	–
Total Glutathione	1 wk	–	–	–	–
	6 mo	–	↓	–	–
Reduced/total glutathione	1 wk	–	–	–	–
	6 mo	–	↓	–	–
Lactate dehydrogenase	1 wk	–	–	–	–
	6 mo	↑	↓	↑	–
Protein	1 wk	–	–	↓	–
	6 mo	–	–	–	–
Macrophage inhib. Prot.–2	1 wk	–	–	–	↑
	6 mo	↓	↓	↑	↓
Tumor necrosis factor–α	1 wk	–	–	–	↓
	6 mo	↓	↑	–	–
Protein	1 wk	–	–	–	–
	6 mo	–	–	–	–
Macrophage O_2^- (stim.)	1 wk	–	–	↓	–
	6 mo	–	–	–	–
Macrophage O_2^- (unstim.)	1 wk	–	–	–	–
	6 mo	–	–	↓	–
Macrophage $O_2^=$ (stim.)	1 wk	–	–	–	–
	6 mo	–	–	–	–
Macrophage $O_2^=$ (unstim.)	1 wk	–	–	↓	↓
	6 mo	↓	–	↓	↓

Table 6. Effects of Exposure on Respiratory Allergy of Mice

Response Variable	Time	D	HWS	G	C
Exacerbation of Respiratory Allergic Response in BALBc Mice					
Exposed before OVA challenge					
BAL total cells		–	–	–	–
BAL eosinophils		↓	–	–	↑
IL–2, 4, & 13		–	–	–	–
IL–5		↓	–	–	–
INFγ		–	–	–	–
Serum total IgE		–	–	–	–
Serum OVA–specific IgE		↓	–	–	–
Serum OVA–specific IgG		–	–	–	–
Airway reactivity		–	–	–	–
Histopathology		–	–	–	–
Exposed after OVA challenge					
BAL total cells		↑	–	–	–
BAL eosinophils		↑	↑	–	↑
IL–2, 4, & 13		–	–	–	–
IL–5		–	–	–	–
INFγ		–	–	↓	–
Serum total IgE		–	–	–	–
Serum OVA–specific IgE		–	↑	↑	–
Serum OVA–specific IgG		–	–	–	–
Airway reactivity		–	–	–	–
Histopathology		–	–	–	–
Development of Respiratory Allergic Response in BALBc Mice Exposed *In Utero*					
BAL total cells		?	?	–	?
BAL eosinophils		?	?	–	?
IL–2, 4, & 13		?	?	–	?
IL–5		?	?	–	?
INFγ		?	?	–	?
Serum total IgE		?	?	↑	?
Serum OVA–specific IgE		?	?	–	?
Serum OVA–specific IgG		?	?	–	?
Airway reactivity		?	?	–	?
Histopathology		?	?	–	?

Table 7. Effects of Exposure on Micronuclei in Mice

Response Variable	Time	D	HWS	G	C
Micronuclei in Circulating Blood of Male and Female A/J Mice					
	6 mo	–	–	–	?

Table 8. Effects of Exposure on Clearance of Pseudomonas Bacteria from lungs of Mice

Response Variable	Time	D	HWS	G	C
***Pseudomonas* remaining in lungs of Male C57/BL6 Mice 18 hr after instillation following 1 wk exposure**					
CFU	1 wk	↑	–	–	–

Table 9. Effects of Exposure on Vascular Injury in Mice

Response Variable	Time	D	HWS	G	C
Vascular Injury in $ApoE^{-/-}$ Mice Exposed 7 Weeks					
MMP–3		?	?	↑	–
MMP–7		?	?	↑	–
MMP–9		?	?	↑	–
MMP–12		?	?	–	–
TIMP–1		?	?	–	–
TIMP–2		?	?	↑	–
HO–1		?	?	↑	–
ET–1		?	?	↑	–
TBARS		?	?	↑	–
Nitrotyrosine		?	?	↑	–

Table 10. Effects of Exposure on Cardiac Function in Rats

Response Variable	Time	D	HWS	G	C
Cardiac Effects in Male and Female SHR Rats Exposed 7 Days					
Heart rate		↑	–	–	?
ECG		↑PQ	–	–	?

Table 11. Effect of Exposure on DNA Damage in Rats and Mice

Response Variable	Time	D	HWS	G	C
DNA Damage in Male and Female F344 Rats					
Methylation	1 wk	↑	–	–	?
	6 mo	↑	–	↑	?
Oxidation	1 wk	↑	↓	nd	?
	6 mo	–	–	–	?
DNA Damage in Male and Female A/J Mice					
Methylation	1 wk	–	↓	↓	?
	6 mo	↓	↑	–	?
Oxidation	1 wk	↓	–	nd	?
	6 mo	–	–	–	

In evaluating the above results, it must be borne in mind that the results for coal emissions are not yet final. Indications are given for responses upon first examination of results, but indicators of significant effects could changes somewhat as the results are processed through the final statistical analysis.

All exposures caused significant effects, but the pattern of effects varied among exposures. Although nearly all variables demonstrated significant responses to one or more exposures, a few did not demonstrate significant effects of any exposure. Non-responsive variables constitute useful information for placing the hazards of the different exposures into context, but those variables are not useful for analysis of composition-effect relationships across all studies. Only one response variable (macrophage inhibitory protein 2, "MIP-2", after 6 mo exposure of F344 rats) demonstrated significant effects of all exposures. MIP-2 was increased by gasoline and decreased by the other exposures. It should be remembered that the database is yet incomplete – additional variables may yet prove to be affected by all exposures.

Among the data summarized to date for the four exposure atmospheres, the simulated downwind coal emissions caused the smallest number of apparently significant effects. None of the organ weight effects appeared to present a coherent pattern of response (Tables 1 and 2). Coal was the only exposure that caused no significant effect on hematology, serum clotting factors, or serum chemistry (Tables 3 and 4). The bronchoalveolar lavage alterations suggested a slight inflammatory effect and a slight depression of the production of oxidant species by alveolar macrophages, one of the means by which those cells carry out their protective functions (Table 5). The only indication that coal exposure affected respiratory allergy was an increase in eosinophils (allergic blood cells) in lavage fluid (Table 6). However, there was no accompanying increase in allergic antibodies. Exposure to coal emissions did not retard the clearance of bacteria from the lungs of mice (Table 8), nor did it cause vascular injury in mice predisposed to forming atherosclerosis (Table 9). Other data have not been summarized to the point that preliminary conclusions can be drawn.

A useful aspect of this exposure-response program is its potential for identifying exposure levels that cause no significant effect. Of course, the use of only three or four exposure levels provides only a crude evaluation of no-effects levels. A larger number of exposure concentrations would be needed to assign a "no-effects" level with confidence. Aside from this limitation however, it is clear that the lowest exposure level was a no-effects level for nearly all response variables for the four exposures to date. Among the approximately 400 variable-time-species-gender response data "cells" in the database, there have been only seven significant responses at the lowest exposure level to date (listed in text box). There was at least one significant response at the lowest exposure level of all atmospheres, but the number and type of responses varied among the atmospheres. The coal atmosphere caused only one significant response at the lowest exposure level.

D	**↓ spleen wt in m mice at 6 mo**
	↑ liver wt in f mice at 6 mo
	↑ serum Na in f rats at 6 mo
HWS	**↓ circulating neutrophils in m and f rats at 6 mo**
	↑ total serum protein in m rats at 6 mo
G	**↑ TBARS in aorta of ApoE mice at 7 wk**
C	**↓ peroxide in unstimulated macrophages on m rats at 1 wk**

It is necessary to consider the possibility of both "false positives" and "false negatives" occurring by chance when using $p<0.05$ as the criterion for significance. One might expect a few misclassifications among the hundreds of measured variables, and a formal estimate of the range of potential misclassification will be a conducted once the database is complete. Until then, indications of the presence or absence of significant effects are taken at face value. Different approaches can be taken to judging the significance of responses, including determining which responses remain significant using different criteria ($p<0.01$, $p<0.005$, etc.). Because many variables were measured in two genders, in two species, and/or at two times, the coherency of effects among those measurements can also be evaluated. Many of the effects indicated in the above tables occurred in only one of two genders, in only one of two species, or at only one of two times. Although an effect in a single gender or species, or at one time, can certainly be meaningful, greater weight might be given to effects that are coherent across the matrix of measurements. Moreover, greater weight would be given to clusters of related responses among the different health response models.

The results for comparisons between filtered and intact mixtures are not yet complete for all four atmospheres. This comparison was incorporated into the protocols for the gasoline and coal studies, but not in the earlier diesel and wood smoke studies. Animals were recently exposed to diesel emissions and wood smoke with and without particles, but those data have not yet been summarized. Among the available data for gasoline and coal, it appears questionable whether PM was solely responsible for any effect. It appears as though PM might have been responsible for a substantive portion of 8 of 33 effects of gasoline emissions, but none of 11 effects of coal emissions.

3) Adequacy of the Database for Multivariate Analysis of Composition-Response Relationships within the Combined Database from All Studies

The greatest impediment to the analysis is the very small number of exposure contrasts; only four to date. Because of the time and resources necessary to conduct the study protocol it is doubtful that a key requirement will be met - *there must be a sufficient number of exposure contrasts.* "Sufficient" is not a fixed number, but four is not likely to be sufficient for a robust analysis. Two additional exposures are recommended by the Center's External Scientific Advisory Committee, given sufficient funding; residual oil emissions and paved road dust. The addition of these two atmospheres will expand the database by 50%, and the residual oil atmosphere would provide a composition that overlaps, but contrasts with, the first four exposures. Although road dust is an important contributor to PM exposure, that atmosphere would not provide contrasts in the compositions of gases and vapors. At this time, the residual oil emissions study is given priority. If that study can be accomplished, there will be time to re-evaluate how road dust fits into the program, or if resources would be better directed toward other exposure contrasts.

Another key requirement was fulfilled – *the compositions of the exposures must overlap, but differ.* However, an issue is whether or not the exposures presented sufficient compositional contrasts to reveal composition-response relationships. An ideal set of exposures would include more systematic variations of the proportions of different major components.

All exposures caused significant effects, and the pattern of effects varied among exposures. This fulfills two other requirements enabling multivariate analysis of the combined data from all studies to determine components of the exposure that co-vary most closely with each category of health response – *1) there must be significant effects; and 2) the effects must vary among exposures.* At issue, however, is whether there were a sufficient number of significant responses to support an analysis of causal relationships for each category of response (i.e., each response model). Ideally, most variables would demonstrate responses to all exposures so that the degree of response could be compared among exposures, and not just the presence or absence of response. Only one variable to date (MIP-2) demonstrated significant responses to all four exposures. Although nearly all variables demonstrated responses to one or more exposures, a few did not demonstrate significant effects of any exposure; thus, those variables are not useful for multivariate analysis of composition-effect relationships

d. Conclusions

The results of the study funded under this project, simulated downwind coal combustion emissions, are nearly complete. Findings to date suggest that: a) the health effects of exposure were mild even at the highest exposure level; 2) fewer effects were caused by coal than by the preceding atmospheres at the same PM or total mass levels; and 3) significant effects do not seem to be attributable to the particulate component of exposure.

2. Milestones

All project milestones have been completed except for the final analysis of exposure composition and biological response data, and submission of publications reporting results.

3. Cost Status

All NETL funds were expended prior to the beginning of the last quarter; thus, the cost status of the NETL-funded portion of the study was final as reported previously. The official DOE notification of the final project cost and funding split was received on 7 April 08. That notification matched the following figures, which had been included in previous quarterly reports:

DOE/NETL share of approved total budget	$ 781,077
Recipient share of approved total budget	$ 781,077
Total approved budget	$ 1,562,154

NETL funds were never intended to cover the full cost of the study; moreover, the study continued beyond the period of NETL funding, using a combination of funds from LRRI and other sponsors of the NERC program. As of the end of May 2008, the total expenditures for the project were approximately $2,200,000. The difference between this value and the total approved NETL budget indicated was comprised of $28,628 of EPA funding, and the remainder from non-federal funding.

DOE/NETL may be assured that the project will be completed. DOE/NETL will be made fully aware of progress and results through project completion, and will receive publications of results as originally agreed. It is assumed that although DOE funding was terminated short of the original budget (for reasons not specifically related to this project), there is still active interest within DOE in the results.

4. Significant Accomplishments

The most significant accomplishments of this project can be summarized as follows:

- Conduct of the first ever subchronic repeated inhalation study of the health effects of coal combustion emissions;
- Conduct of the first ever study of a mixture explicitly aimed at modeling the major components contributed by coal combustion to "downwind" exposures;
- Demonstration (in animals) that the adverse health effects of coal emissions were generally fewer than those of diesel emissions or hardwood smoke at equivalent particle mass concentrations;
- Demonstration that the lowest exposure level in this study caused only one significant effect, among the hundreds of measured response variables;
- Discovery that, among this mixture of pollutants, the non-particle components caused the majority of effects.

5. Problems, Delays, and Corrective Actions

The length of the total project period was extended for multiple logistical and funding reasons, as described in this and previous reports. The early termination of DOE/NETL funding slowed progress significantly. Fortunately however, the project was co-funded by multiple other sponsors and could be completed. At the time of this report, the project was nearing completion. Despite termination of DOE funding, NETL will receive all publications that result from this work. No other major technical problems were encountered.

6. Technology Transfer Activities

No technology transfer activities or issues were associated with this project. It was not anticipated that this project would generate intellectual property or technical advances that would raise technology transfer issues. The product of this project was explicitly information on the health effects of exposure to simulated downwind coal emissions, and that information is being communicated to the scientific community, public, and other stakeholders through peer-reviewed, open literature publications and presentations at various meetings.