Inhalation Toxicology, 17:255–261, 2005

ISSN: 0895-8378 print / 1091-7691 online
DOI: 10.1080/08958370590912941

Taylor&Francis
healthsciences

Effects of Subchronic Exposures to Concentrated Ambient Particles in Mice: IX. Integral Assessment and Human Health Implications of Subchronic Exposures of Mice to CAPs

Morton Lippmann, Terry Gordon, and Lung Chi Chen

Nelson Institute of Environmental Medicine, Department of Environmental Medicine, New York University School of Medicine, Tuxedo, New York, USA

In order to examine the biologic plausibility of adverse chronic cardiopulmonary effects in humans associated with ambient particulate matter (PM) exposure, we exposed groups of normal mice (C57) and knockout mice that develop atherosclerotic plaques (ApoE$^{-/-}$ and ApoE$^{-/-}$ LDLr$^{-/-}$) for 6 h/day, 5 days/wk for 5 or 6 mo during the spring/summer of 2003 to either filtered air or 10-fold concentrated ambient particles (CAPs) in Tuxedo, NY (average $PM_{2.5}$ concentration during exposure = 110 $\mu g/m^3$). Some of the mice had implanted electocardiographic monitors. We demonstrated that: (1) this complex interdisciplinary study was technically feasible in terms of daily exposures, collection of air quality monitoring data, the collection, analysis, and interpretation of continuous data on cardiac function, and the collection and analyses of tissues of the animals sacrificed at the end of the study; (2) the daily variations in CAPs were significantly associated, in ApoE$^{-/-}$ mice, with daily variations in cardiac function; (3) there were significant differences between CAPs and sham-exposed ApoE$^{-/-}$ mice in terms of cardiac function after the end of the exposure period, as well as small differences in atherosclerotic plaque density, coronary artery disease, and cell density in the substantia nigra in the brain in the ApoE$^{-/-}$ mice; and (4) there are suggestive indications of gene expression changes for genes associated with the control of circadian rhythm in the ApoE$^{-/-}$ LDLr$^{-/-}$ double knockout (DK) mice. These various CAPs-related effects on cardiac function and the development of histological evidence of increased risk of clinically significant disease at the end of the exposures in animal models of atherosclerosis provide biological plausibility for the premature mortality associated with $PM_{2.5}$ exposure in human subjects and provide suggestive evidence for neurogenic disease as well.

A large and rapidly growing body of epidemiological evidence implicates elevated daily levels of ambient air particulate matter (PM) with excess daily mortality, morbidity, and lost time from work or school. Also, differences in annual average PM concentrations have been associated with differences in annual mortality rates and lung function growth in children (U.S. EPA, 2004). However, some toxicologists and clinicians remain skeptical about the existence of a causal relationship between exposure to ambient air PM and adverse health effects because controlled exposures to laboratory-generated PM, even at relatively high concentrations, have generally failed to produce health-related responses in acute exposure studies that correspond to the effects reported in observational studies of human populations. The only acute PM exposure studies that have produced responses with some relevance to the effects seen in the epidemiological studies involved one or several days of exposures to concentrated ambient air particles (CAPs) of laboratory animals or human volunteers, and the results of the various studies have often been inconsistent with respect to reporting significant exposure-related effects and/or in the direction of the

Received 30 September 2004; accepted 8 November 2004.

This article has not been published elsewhere, nor has it been submitted for publication elsewhere. This research was performed as part of a Center Grant from the U.S. Environmental Protection Agency (R827351), and utilized facility core services supported by a Center Grant from the National Institute of Environmental Health Sciences (ES 00260).

Address correspondence to Morton Lippmann, Nelson Institute of Environmental Medicine, Department of Environmental Medicine, New York University School of Medicine, 57 Old Forge Road, Tuxedo, NY 10987, USA. E-mail: lippmann@env.med.nyu.edu

effects that have been seen. To date, no chronic or subchronic CAPs exposure studies have been reported.

The hypothesis underlying this study was that repeated daily CAPs exposures in an animal model for humans with chronic atherosclerotic disease would produce biological responses that could account for the progression of cardiopulmonary diseases in susceptible human populations. The animal models selected were the ApoE$^{-/-}$ mouse, which develops aortic plaque as it ages, and a double knockout (DK) mouse (ApoE$^{-/-}$–LDLr$^{-/-}$), which develops plaque in both the aorta and coronary artery. The literature supporting this hypothesis was reviewed in some detail by Lippmann et al. (2005). In order to be sure that the murine knockout model was indeed susceptible to CAPs exposures, a comparable cohort of normal animals was included in the study. In addition, we decided to have groups of both susceptible and normal animals undergo sham (clean air) exposures following the same protocols used to expose animals to CAPs.

In launching a study focused on the chronic effects of CAPs on cardiac function, we did not want to miss the opportunity to examine, in the exposed animals, evidence for other adverse health effects associated with acute and chronic $PM_{2.5}$ exposures in the epidemiological studies. Thus, we examined the exposed animals for other biological effects that have been reported in human populations. Our coordinate objectives were to look for other $PM_{2.5}$-related responses, including:

- Short-term changes in cardiac function associated with daily peak $PM_{2.5}$ concentrations and/or specific air trajectories associated with major air pollution sources and/or specific elemental tracers.
- Lung lavage parameters at the end of exposure that were related to lung inflammation.
- Histopathological changes in the lung and heart at the end of exposure.
- Aortic plaque formation and/or increased plaque size at the end of exposure.
- Gene activation at the end of the exposure.
- Histopathological changes in the brains at the end of the exposures.

To complement the evidence for acute cardiac function changes related to daily variations in $PM_{2.5}$ exposure, we collected concentrated PM each day in an air sampler that operated in parallel with the particle concentrators that fed aerosol into the exposure chambers, in order to perform *in vitro* assays on the toxicity of the particles that were sampled. The design of the CAPs exposure system was described by Maciejczyk et al. (2005), and details on the results obtained were described by Hwang et al. (2005), Chen and Hwang (2005), Chen and Nadziejko (2005), Gunnison and Chen (2005), Veronesi et al. (2004), and Maciejczyk and Chen (2005), elsewhere in this special issue of *Inhalation Toxicology*.

In performing this first CAPs inhalation exposure study lasting over many months we learned that: (1) Such a complex interdisciplinary study is technically feasible in terms of daily exposures within target goals, the collection of continuous and daily average air quality monitoring data, the collection, analysis, and interpretation of continuous data on cardiac function, core body temperature, and physical activity, and the planned collection and analyses of blood and tissues of the animals sacrificed at the end of the study; (2) the daily variations in CAPs were significantly associated, in ApoE$^{-/-}$ mice, with daily variations in cardiac function and was CAPs exposure concentration dependent; (3) there were significant differences between CAPs and sham-exposed ApoE$^{-/-}$ mice in terms of cardiac function after the end of the exposure period, as well as differences in atherosclerotic plaque density, coronary artery disease, and cell density in the substantia nigra in the brain in the knockout mice; (4) the upwind air pollution source profile has a profound influence on the ability of CAPs to produce a biological response (i.e., an increased level of nuclear factor-kappa B, NF-κB); (5) alterations in heart-rate variability (HRV) were found to be correlated with NF-κB activation, supporting the role of oxidative stress in adverse cardiac effects; and (6) there were suggestive indications of gene expression changes for genes associated with the control of circadian rhythm in the knockout mice.

In this article, we seek to provide an overall framework for interpreting these findings and their coherence, and their ability to provide support for the biological plausibility of epidemiological studies of excess mortality in human cohorts and other human health effects.

CARDIOVASCULAR DISEASE

The design of this subchronic CAPs inhalation study was primarily focused on an examination of the cardiovascular system's response to $PM_{2.5}$ in ambient air using an animal model of atherosclerosis. The rationale derived from the growing literature in which premature mortality and increased morbidity in human populations have been linked to cardiovascular endpoints for both acute and chronic exposures to $PM_{2.5}$. Our study design, involving semicontinuous electrocardiogram (EKG) recordings, enabled us to look for both transient changes to peak daily exposures as well as shifts in cardiac function attributable to cumulative CAPs exposures over 5 mo. For the cumulative effects, the morphometric analyses of the samples of aorta, heart, lung, and brain that were collected after the last exposure provided additional information on the exposure-related cardiac system responses and their interrelationships.

The evidence for significant CAPs exposure-related changes in EKG patterns (Hwang et al., 2005; Chen & Hwang, 2005) and morphometric indices in the aorta and heart (Chen & Nadziejko, 2005) in the ApoE$^{-/-}$ and DK mice, and their absence in the C57 mice, demonstrates that the knockout mice used were indeed especially sensitive to the exposures, and that they can serve as a useful model for humans with atherosclerosis. The results of the exploratory studies of the effects of subchronic CAPs exposures on gene expression (Gunnison et al., 2005) and brain cell distribution (Veronesi et al., 2005), discussed in the next

section of this article, may have implications for cardiac function as well.

Finding statistically significant exposure-related changes in electrocardiograph (EKG) patterns and morphometric changes in the aorta and heart of knockout mice, while noteworthy in and of themselves, are not direct evidence of premature mortality or predictive of clinical disease in humans. They do, however, suggest that such outcomes might well have occurred in the mice with continued exposure over a longer fraction of their lifespan, and, in fact, some limited mortality did occur during the CAPs exposure study in both CAPs and air sham-exposed double knockout (DK) mice, and although the mortality in the two groups was not statistically significant at the end of the study, the deaths in the CAPs-exposed DK mice occurred sooner than those in the filtered air-exposed animals.

In humans, little is known about the early changes in the cardiac system that lead to a progression of clinically relevant disease states. As noted in our introductory paper (Lippmann et al., 2005) and in the U.S. EPA Criteria Document for PM (U.S. EPA, 2005), the literature on studies of ambient $PM_{2.5}$ exposures and CAPs inhalation in humans with EKG monitors has shown inconsistent responses in terms of the direction of the changes in heart-rate (HR) and heart-rate variability (HRV). It is possible that an initial or acute stimulus to an exogenous challenge perturbs the balance of a physiological process, and that a greater challenge, in terms of exposure level or duration, can produce an opposite directional change that is clearly indicative of an adverse response. We have seen this kind of bidirectional response in both humans and animals in terms of responses to sulfuric acid aerosol and cigarette smoke for the clearance of iron oxide particles from the lung conductive airways in human subjects (Lippmann & Schlesinger, 1984; Lippmann, 2000) and in experimental animals (Chen & Schlesinger, 1983).

In this subchronic CAPs exposure study, we had a wealth of EKG data that allowed us to examine exposure-related changes in HR and HRV not only during the daily exposure interval, but also throughout the balance of the day, over the weekend days without daily exposure, and over the 20 wk of exposure. As shown in Figure 1 (Figure 10 of Hwang et al., 2005), when the $ApoE^{-/-}$ mice were being exposed to CAPs, there was a significant depression in HR on the weekdays during the hours 11:00 through 13:00, which was absent on the nonexposure weekend days, as well as a downward trend of the HR during the course of the exposure series for both weekday and weekend days. By contrast, Figure 2A shows that for $ApoE^{-/-}$ mice during the hours 1:30 to 4:30, many hours after the end of the daily exposure period and when the mice were in their normal activity mode, there was a transient rise in HR during the first month, a substantial decline in the second and third month, and a maintenance of that reduced HR through the balance of the exposure period, with no significant difference between weekday and weekend days. At the same time, there was a quite different temporal pattern of change for HRV, as indexed by the standard deviation of normal RR intervals (SDNN) and by the root-mean square of successive difference (RMSSD). As shown in Figure 2 (B and C), there was a prolonged elevation, peaking at about 2 mo into the study, a decline to below the initial levels by 4 mo, and a relatively modest change in the last month of the exposure series. Also, for all three panels (A, B, and C), there was no apparent difference in HR and HRV between exposure days and weekend nonexposure days.

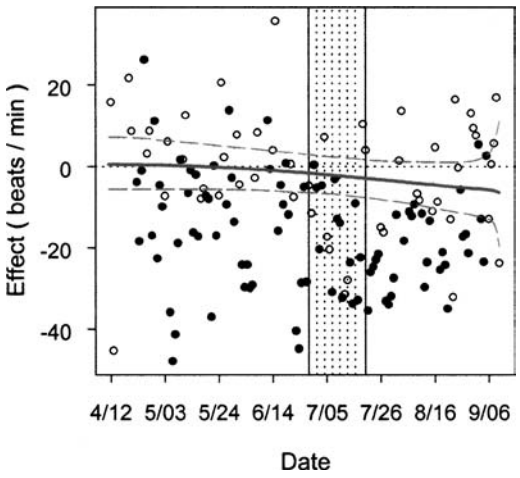

FIG. 1. Posterior means (solid) and 95% equal-tail credible intervals (dotted) of chronic effect changes for $ApoE^{-/-}$ mice heart rate during 11:00–13:00. The circles in the plots are daily crude effects estimated in the first stage (full for exposure day and empty for nonexposure day). The light failure dates determined in the model are marked with vertical bars.

By contrast, panels D, E, and F of Figure 2 show that there was little, if any, HR or HRV response to the CAPs exposure series in C57 mice in the 1:30 to 4:30 a.m. time interval, as indicated by the lack of consistent temporal patterns of change over the 5 mo of the exposure series. There was, however, more scatter in these data, that is, more day-to-day and apparently random variability as compared to the data for the $ApoE^{-/-}$ mice in the upper panels. This was almost certainly due to the small number (three of the original six) of the CAPs-exposed C57 mice that had functional EKG transmitters by the end of the study. Various problems such as a malfunction in a transmitter or a dead battery are possible, and even likely, occurrences in such a subchronic study, and future studies will utilize extra animals fitted with EKG monitors and procedures to conserve battery power.

A different picture of cardiac function changes emerged when the HR fluctuation (HRF) was examined using, as a reference, the data from the week between the end of the exposures and the sacrifice of the animals for histopathological examinations. As shown in Figure 3, the HRF at the end decreased by a factor of 0.7 for 15-min intervals, while over longer intervals (3–6 h), the HRF at the end increased by a factor of 1.35. For both time intervals, the changes were markedly progressing at the end of the exposure period, suggesting that more clinically significant changes could have been seen if the exposure period had been greater.

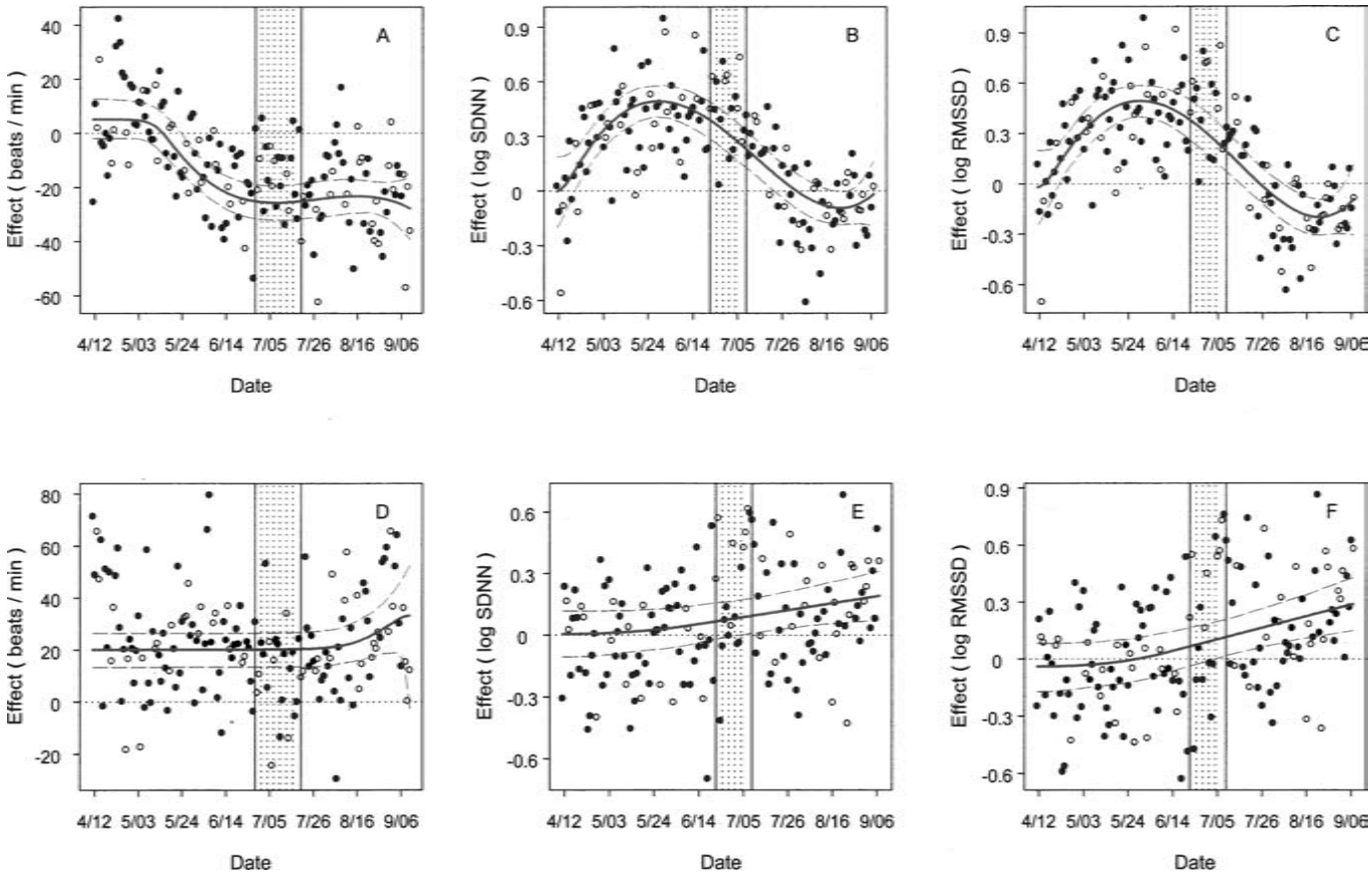

FIG. 2. Posterior means (solid) and 95% equal-tail credible intervals (dotted) of chronic effect changes for mice during the hours 1:30–4:30 a.m. obtained from the Bayesian (second stage) model: (A) and (D), for heart rate (beats/min); (B) and (E) heart-rate variability (HRV), expressed as standard deviation of normal RR intervals (SDNN); and (C) and (F), HRV expressed as root-mean-square successive differences (RMSSD). (A), (B), and (C), ApoE$^{-/-}$ mice; (D), (E), and (F), C57 mice.

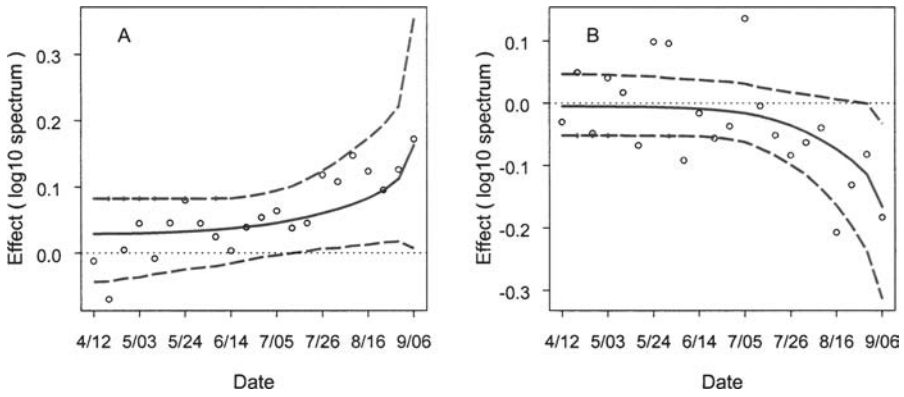

FIG. 3. Posterior means (solid) and 95% equal-tail credible intervals (dotted) of chronic effect changes of heart rate fluctuation (HRF), expressed in terms of $\log_{10}$ spectrum powers over (A) the low-frequency band ($\sim$15 min) and (B) the high-frequency band (3–6 h) for ApoE$^{-/-}$ mice heart rate obtained from the Bayesian model in the second stage. The circles in the plots are daily crude effects estimated in the first stage.

The HR and HRV changes in the CAPs exposed ApoE$^{-/-}$ mice, but not in the C57 control mice (Hwang et al., 2004; Chen & Hwang, 2005), provide evidence of both early favorable functional adaptation to the exposure challenge in terms of increased HR and HRV, and a later movement into a depression of HR and HRV for an animal model of atherosclerosis. However, it is noteworthy that the transition of HRV occurred about 2 mo later than the change in HR, and at a time when there were indications of the start of progressive changes in HRF. Thus, this subchronic study indicates that a longer period of daily exposures in this animal model could produce premature mortality.

For the morphometric analyses of aorta and heart, we have data only for the cumulative effects, and not on the progression of changes during the exposures. It is clear that by the end of the exposures, both the CAPs- and air sham-exposed ApoE$^{-/-}$ and DK mice had quite advanced atherosclerotic plaque deposits, reducing the opportunity to characterize the CAPs exposure related difference.

Similarly, the coronary artery disease (CAD) lesions in the hearts were extensive for both the CAPs- and air sham-exposed DK mice, but only the CAPs-exposed mice had lesions that were invasive (Chen et al., 2005). While these observations are strongly suggestive of an influence of CAPs exposure on the expression of coronary disease at a later stage, it is also clear that this animal model, as used in this study, was not ideal. Future studies will use younger animals to limit the baseline plaque density in the aorta, and will extend the CAPs exposure period in order to determine the differential progression of plaque between CAPs exposed and air-sham exposed controls.

OTHER BIOLOGICAL RESPONSES

The exploratory analyses of gene expression in heart and lung tissues (Gunnison & Chen, 2005) and changes in brain cell type (Veronesi et al., 2005) of the CAPs- and air sham-exposed mice produced evidence of other effects related to chronic CAPs exposure. Of particular interest, in relation to an overall assessment of the health significance of chronic $PM_{2.5}$ exposure, was the indication that the expression of a gene (dpb) affecting circadian rhythm was upregulated in CAPs-exposed DK mice. The change in the expression of the dpb gene was an unanticipated observation of this exploratory study, and further research will be needed before the effect can be judged to be causally related to the exposures. At this point, we can only speculate that this response may have had some relation to the CAPs-related decreases in activity patterns late at night in the CAPs-exposed ApoE$^{-/-}$ mice. Several other genes that might be involved in PM-related heart or lung pathology, and/or the circadian rhythm of physiological processes, were also identified. The largest functional category contained heat-shock and other stress-response genes. A more comprehensive study is required, however, to more definitively assess differences in gene expression in heart and lung resulting from exposure to CAPs.

The unanticipated neurodegeneration exhibited in the substantia nigra nucleus compacta of the brains of the CAPs-exposed ApoE$^{-/-}$mice may also be related to control of HR and HRV in the heart, but this possibility remains speculative at this time, and warrants further research on the mechanisms involved and the implications of such $PM_{2.5}$ exposure-related changes to Parkinson's and Alzheimer's diseases. Oberdorster et al. (2004) demonstrated that ultrafine particles can be transported from airway surfaces to the brain via the olfactory bulb, and from these there are transynaptic routes to central nervous system (CNS) targets such as hypothalamus, substantia nigra, and olfactory cortex. The CAPs exposures included ultrafine particles, although at a lesser concentration enhancement ratio than for accumulation mode particles (Maciejczyk et al., 2005). In this study, therefore, it is not clear what role the accumulation-mode particles may have played in the changes observed in the substantia nigra.

The only organ system that was examined that did not exhibit CAPs-exposure-related responses in both the single and double knockout mice was the pulmonary system, where we

did not observe inflammation of the lung as indicated by lavage parameters (cell counts and protein levels) and histopathology. Since the lung is the portal of entry for the CAPs, we had anticipated that some changes would be observed in this organ system. However, tolerance or adaptation to inflammation after repeated exposure to particles or gases has been reported earlier in this lab and elsewhere in humans (Horvath et al., 1981; Superko et al., 1984; Devlin et al., 1996; Gong et al., 1997; Fine et al., 2000; Jorres et al., 2000) and animals (Plopper et al., 1994; McKinney et al., 1998; Iwasaki et al., 1998; Wesselkamper et al., 2001). In this sense, it was not surprising that no persistent inflammatory change was observed after repeated exposure to low levels of CAPs in our study. However, absence of persistent inflammation does not imply that there was no exposure-reated reaction to CAPs in the lung. Subclinical change could have occurred that could not be detected by the measurement methods that were used in this study; that is, transient changes in the lung could have occurred and been resolved by the end of our exposure period. In addition, lung cells (epithelial cells and macrophages) could have been actively secreting cytokines or chemokines without inflammatory changes. Future studies should address this issue.

In fact, analyses of *in vitro* responses of BEAS-2B lung cells to a parallel CAPs stream of particles that were collected by a BioSampler indicated exposure-related changes in NF-κB release. NF-κB can be activated by a variety of stimuli, and its activation regulates genes encoding for inflammatory cytokines, adhesion molecules, and chemokines. The observation of CAPs activation of NF-κB in airway epithelial cells is consistent with the potential for an acute inflammatory response in the lung and cardiovascular system after exposure to ambient PM. Furthermore, the response was significantly correlated with the source component that is characteristic of residual oil combustion. The fact that the oil combustion source contributed only $\sim$2% of the $PM_{2.5}$ mass is notable, as is the lesser degree of correlation with other, much larger sources of the $PM_{2.5}$ mass. If the inflammatory response influences cardiac as well as pulmonary system responses related to indices of health, it may account for the greater mortality coefficient in the northeastern United States, where residual oil combustion is concentrated, than in the rest of the country in the NMMAPS time-series study (Samet et al., 2000).

SUMMARY AND CONCLUSIONS

We have observed a range of anticipated (HR and HRV) and unanticipated (brain cell distribution and expression of clock gene-related genes) changes in $ApoE^{-/-}$ mice exposed subchronically to CAPs.

A number of important conclusions can be made from these observations, including:

- The results provide enhanced biologic plausibility for some of the epidemiological observations.
- Clear-cut acute and chronic changes occurred in the cardiovascular system in the absence of observable chronic pulmonary system changes.
- The $ApoE^{-/-}$ and $ApoE^{-/-}LDLr^{-/-}$ mouse models are useful as susceptible animal models for studying the health effects of ambient air PM on populations with preexisting atherosclerosis.
- Parallel daily CAPs exposures of BEAS-2B cells *in vitro* caused an inflammatory response that was most closely correlated with the residual oil combustion source category, indicating that such parallel studies with these and other cell lines can provide opportunities to identify PM components of particular concern.
- Serial observations of heart and brain pathology, and of gene expression throughout a chronic exposure period, can provide important information on the mechanisms by which PM produces acute and chronic adverse health-related responses.
- These various health-related changes occurred in Sterling Forest, a large state park remote from large local PM sources. After concentration, the average PM concentration was 110 $\mu g/m^3$during the exposure, and the long-term average was only 19.7 $\mu g/m^3$. These ambient particles, which were largely formed by chemical transformations from gaseous precursors, arrived at the Sterling Forest laboratory by long-range transport, and the effects observed may therefore be relevant to the very large regional population that is also exposed to long-range transported secondary ambient aerosol.

Since this was the first CAPs inhalation exposure study that extended over many months, it was, of necessity, an exploratory study. Further investigations are ongoing in this laboratory to extend this line of research to wintertime and summertime CAPs in Tuxedo, NY, and plans have been made to conduct such studies in other venues with different CAPs compositions. While no single further study can replicate the exposure pattern and composition of the first study, the similarities and differences in the biological responses can provide a source of the important new information needed to clarify the role of PM composition on both acute and persistent health-related responses to ambient air pollutant exposures.

REFERENCES

Chen, L. C., and Hwang, J.-S. 2005. Effects of subchronic exposures to concentrated ambient particles (CAPs) in mice: IV. Characterization of acute and chronic effects of ambient air fine particulate matter exposures on heart rate variability. *Inhal. Toxicol.* 17(4–5):209–216.

Chen, L. C., and Nadziejko, C. 2005. Effects of subchronic exposures to CAPs in mice: V. Exacerbation of aortic plaque development in hyperlipidemic mice after subchronic exposures to CAPs. *Inhal. Toxicol.* 17(4–5):217–224.

Chen, L. C., and Schlesinger, R. B. 1983. Response of the bronchial mucociliary clearance system in rabbits to inhaled sulfite and sulfuric acid aerosols. *Toxicol. Appl. Pharmacol*. 71:123–131.

Devlin, R. B., McDonnell, W. F., Becker, S., Madden, M. C., McGee, M. P., Perez, R., Hatch, G., House, D. E., and Koren, H. S. 1996. Time-dependent changes of inflammatory mediators in the lungs of humans exposed to 0.4 ppm ozone for 2 hr: A comparison of mediators found in bronchoalveolar lavage fluid 1 and 18 hr after exposure. *Toxicol. Appl. Pharmacol.* 138:176–185.

Fine, J. M., Gordon, T., Chen, L. C., Kinney, P., Falcone, G., Sparer, J., and Beckett, W. S. 2000. Characterization of clinical tolerance to inhaled zinc oxide in naive subjects and sheet metal workers. *J. Occup. Environ. Med.* 42:1085–1091.

Gong, H., Jr., McManus, M. S., and Linn, W. S. 1997. Attenuated response to repeated daily ozone exposures in asthmatic subjects. *Arch. Environ. Health* 52:34–41.

Gunnison, A., and Chen, L. C. 2005. Effects of subchronic exposures to concentrated ambient particles in mice: VI. Measurement of gene expression in heart and lung tissue following exposure. *Inhal. Toxicol.* 17(4–5):225–233.

Horvath, S. M., Gliner, J. A., and Folinsbee, L. J. 1981. Adaptation to ozone: Duration of effect. *Am. Rev. Respir. Dis.* 123:496–499.

Hwang, J.-S., Nadziejko, C., and Chen, L. C. 2005. Effects of subchronic exposures to concentrated ambient particles in mice: III. Acute and chronic effects of CAPs on heart rate, heart rate variance, and body temperature. *Inhal. Toxicol.* 17(4–5):199–207.

Iwasaki, T., Takahashi, M., Saito, H., and Arito, H. 1998. Adaptation of extrapulmonary responses to ozone exposure in conscious rats. *Ind. Health* 36:57–60.

Jorres, R. A., Holz, O., Zachgo, W., Timm, P., Koschyk, S., Muller, B., Grimminger, F., Seeger, W., Kelly, F. J., Dunster, C., Frischer, T., Lubec, G., Waschewski, M., Niendorf, A., and Magnussen, H. 2000. The effect of repeated ozone exposures on inflammatory markers in bronchoalveolar lavage fluid and mucosal biopsies. *Am. J. Respir. Crit. Care Med.* 161:1855–1861.

Lippmann, M. 2000. Sulfur oxides: Acidic aerosols and SO_2. In *Environmental toxicants: Human exposures and their health effects*, 2nd ed. (ed. M. Lippmann, pp. 771–809), New York: Wiley-Interscience.

Lippmann, M., and Schlesinger, R. B. 1984. Interspecies comparisons of particle deposition and mucociliary clearance in tracheobronchial airways. *J. Toxicol. Environ. Health* 13:441–469.

Lippmann, M., Gordon, T., and Chen, L. C. 2005. Effects of subchronic exposures to concentrated ambient particles (CAPs) in mice. I. Introduction, objectives and experimental plan. *Inhal. Toxicol.* 17(4–5):177–187.

Maciejczyk, P., and Chen, L. C. 2005. Effects of subchronic exposures to concentrated ambient particles in mice: VIII. Source-related daily variations in In Vitro responses to CAPs. *Inhal. Toxicol.* 17(4–5):243–253.

Maciejczyk, P., Zhong, M., Li, Q., Xiong, J., Nadziejko, C., and Chen, L. C. 2005. Effects of subchronic exposures to concentrated ambient particles (CAPs) in mice: II. The design of a CAPs exposure system for biometric telemetry monitoring. *Inhal. Toxicol.* 17(4–5):189–197.

McKinney, W. J., Jaskot, R. H., Richards, J. H., Costa, D. L., and Dreher, K. L. 1998. Cytokine mediation of ozone-induced pulmonary adaptation. *Am. J. Respir. Cell Mol. Biol.* 18:696–705.

Oberdorster, G., Sharp, Z., Atudorei, V., Elder, A., Gelein, R., Kreyling, W., and Cox, C. 2004. Translocation of inhaled ultrafinne particles to the brain. *Inhal. Toxicol.* 16:437–445.

Plopper, C. G., Chu, F. P., Haselton, C. J., Peake, J., Wu, J., and Pinkerton, K. E. 1994. Dose dependent tolerance to ozone. I. Tracheobronchial epithelial reorganization in rats after 20 months' exposure. *Am. J. Pathol.* 144:404–420.

Samet, J. M., Zeger, S. L., Dominici, F., Curriero, F., Coursac, I., Dockery, D. W., Schwartz, J., and Zanobetti, A. L. 2000. *The national morbidity, mortality, and air pollution study. Part II: Morbidity, mortality, and air pollution in the United States*. Cambridge, MA: Health Effects Institute, Research Report 94.

Superko, H. R., Adams, W. C., and Daly, P. W. 1984. Effects of ozone inhalation during exercise in selected patients with heart disease. *Am. J. Med.* 77:463–470.

U.S. Environmental Protection Agency. 2004. *Air quality criteria for particulate matter*. Research Triangle Park, NC: National Center for Environmental Assessment-RTP Office, Report EPA/600/P-99/002aF and EPA/600/p-99/0026F, October.

Veronesi, B., Makwana, O., Pooler, M., Weir, L., and Chen, L. C. 2005. Effects of subchronic exposures to concentrated ambient particles in mice: VII. Degeneration of dopaminergic neurons in $ApoE^{-/-}$ mice. *Inhal. Toxicol.* 17(4–5):235–241.

Wesselkamper, S. C., Chen, L. C., and Gordon, T. 2001. Development of pulmonary tolerance in mice exposed to zinc oxide fumes. *Toxicol. Sci.* 60:144–151.