



# STATEMENT

Synopsis of Research Report 184, Parts 1–4

HEALTH  
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## Effects of Lifetime Exposure to Inhaled New-Technology Diesel Exhaust in Rats

### INTRODUCTION

This Statement summarizes HEI's independent evaluation, conducted by a specially convened Review Panel, of four studies conducted as a single phase (Phase 3B) of the Advanced Collaborative Emissions Study (ACES) program. The ACES Phase 3B studies investigated the health effects of chronic, lifetime exposures of rats (up to 30 months) and subchronic exposures (3 months) of mice to "new-technology diesel exhaust" (NTDE) — emissions from a heavy-duty diesel engine system compliant with 2007 U.S. Environmental Protection Agency (EPA) regulations. The studies were led by Drs. Jacob D. McDonald of the Lovelace Respiratory Research Institute (LRRI), Albuquerque, New Mexico, Jeffrey C. Bemis of Litron Laboratories, Rochester, New York, Lance M. Hallberg of the University of Texas Medical Branch, Galveston, Texas, and Daniel J. Conklin of the University of Louisville, Louisville, Kentucky.

### BACKGROUND

In light of concerns identified over many decades about the potential health effects of diesel emissions, the U.S. EPA and the California Air Resources Board adopted stringent regulations for heavy-duty highway diesel engines, which were required to meet a new standard for particulate matter (PM) by 2007. A tighter standard for nitrogen oxides (primarily nitric oxide [NO] and nitrogen dioxide [NO<sub>2</sub>]) came into effect in 2010. The regulatory agencies also mandated that sulfur in fuel be reduced substantially. To address these regulations and standards, motor vehicle and engine manufacturers introduced new technologies. These developments were expected to substantially reduce emissions from diesel engines.

To characterize the exhaust emissions from heavy-duty diesel engines that met the new standards and to assess the possible adverse health effects of exposure to these emissions, HEI, working in collaboration with the Coordinating Research

### What This Study Adds

- This is the first study to conduct a comprehensive evaluation of lifetime inhalation exposure to emissions from heavy-duty 2007-compliant engines (referred to as "new-technology diesel exhaust," or NTDE).
- The study evaluated the long-term effects of multiple concentrations of inhaled NTDE, which has greatly reduced particle emissions compared with "traditional-technology diesel exhaust" (TDE) in male and female rats on more than 100 different biologic endpoints, including tumor development, and compared the results with biologic effects seen in earlier studies in rats after exposure to TDE.
- Lifetime inhalation exposure of rats exposed to one of three levels of NTDE from a 2007-compliant engine, for 16 hours per day, 5 days a week, with use of a strenuous operating cycle that more accurately reflected the real-world operation of a modern engine than cycles used in previous studies, did not induce tumors or pre-cancerous changes in the lung and did not increase tumors that were considered to be related to NTDE in any other tissue. A few mild changes were seen in the lungs, consistent with long-term exposure to NO<sub>2</sub>, a major component of NTDE, which is being further substantially reduced in 2010-compliant engines.

This Statement, prepared by the Health Effects Institute, summarizes a research project funded by HEI and conducted by Drs. Jacob D. McDonald of the Lovelace Respiratory Research Institute, Albuquerque, New Mexico, Jeffrey C. Bemis of Litron Laboratories, Rochester, New York, and Lance M. Hallberg of the University of Texas Medical Branch, Galveston, Texas, and their colleagues, and Daniel J. Conklin and Maiying Kong of the University of Louisville, Louisville, Kentucky. The complete report, *Advanced Collaborative Emissions Study (ACES): Lifetime Cancer and Non-Cancer Assessment in Rats Exposed to New-Technology Diesel Exhaust* (© 2015 Health Effects Institute), can be obtained from HEI or our Web site (see last page).

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Council, a nonprofit organization with expertise in emissions characterization, launched the multiphase ACES program. Phases 1 and 2 focused on emissions characterization, and Phase 3A established conditions for animal exposure. Phase 3B was designed to evaluate health outcomes in rats exposed to NTDE for up to 24 months, with the possibility of extension to 30 months, and in mice exposed for up to 3 months.

Through competitive processes, HEI funded several investigator teams in Phase 3B: a core study at LRRRI, led by McDonald (who became principal investigator after the retirement of Dr. Joe L. Mauderly), and ancillary studies to evaluate endpoints not assessed in the core study. The overall hypothesis for ACES Phase 3B was that NTDE would *not* increase tumor formation or have substantial toxic health effects in rats and mice, although some biologic effects might occur.

This Statement summarizes results reported from the core study and the ancillary studies led by Bemis and Hallberg, which assessed genotoxic endpoints in the exposed animals, and by Conklin, which assessed inflammatory and thrombotic endpoints. Reports from the investigator teams were reviewed by a specially convened ACES Review Panel, comprising members of HEI's Health Review Committee and outside experts. The current report focuses on findings in rats over the entire study; findings from subchronic exposures of mice and rats (up to 3 months of exposure) have already been published in HEI Research Report 166.

### APPROACH

McDonald and colleagues generated exhaust from a 2007-compliant heavy heavy-duty diesel engine (defined as an engine installed in a vehicle with gross vehicle weight rating above 33,000 lb; hereafter called "heavy-duty") equipped with emission controls. The engine was fueled with ultra-low-sulfur diesel fuel meeting current on-road specifications and was operated with a dynamometer.

The investigators exposed male and female 6-week-old Wistar Han rats (140 animals of each sex per exposure level) to one of three target dilutions of whole diesel exhaust — 4.2 (high), 0.8 (mid), or 0.1 (low) ppm NO<sub>2</sub> — or to filtered air as a control. Exposure levels were set based on NO<sub>2</sub> rather than PM, which had been used in previous studies of TDE, because the PM level in NTDE, identified in earlier phases of ACES, was so substantially reduced compared with TDE. Thus, calibrating exposures based on PM would have been problematic. In addition, the

highest NO<sub>2</sub> exposure level was chosen to provide a comparison with the same cumulative exposure to NO<sub>2</sub> (the product of concentration and exposure duration) used in prior HEI-funded long-term inhalation studies in rats conducted by Mauderly and colleagues, in which minor biologic changes — but no cancer or pre-cancerous changes — were observed in the respiratory tract.

Exposures were conducted for 16 hours per day from approximately 1600 to 0800 hours for 5 days per week. The engine was run on a unique and strenuous operating cycle that represented more closely the behavior of modern engines than operating cycles used in older long-term studies of TDE. The emissions were characterized before they reached the animal exposure chambers as well as inside the chambers; in this way, the investigators could assess how the presence of the animals affected the composition of the exposure atmospheres.

Groups of male and female rats were euthanized at LRRRI after 1, 3, 12, and 24 months of exposure, as well as at the terminal sacrifice — 28 months for males, 30 months for females. The LRRRI investigators harvested blood and tissues for their analyses at these time points (10 animals of each sex per exposure group) and also sent aliquots of blood and appropriate tissue samples from 5 to 10 animals of each sex per exposure group to the ACES Phase 3B ancillary studies investigators. McDonald and colleagues evaluated animals histologically throughout the study for the presence of tumors and other types of lesions in the airways and in multiple tissues. In addition, they examined a vast array of biologic endpoints: hematologic (several cell types, plus coagulation), serum chemistry (including triglyceride and protein components), lung lavage (including numbers of cells and levels of multiple cytokines and markers of oxidative stress and tissue injury), and pulmonary function.

For the assessments of genotoxicity, Bemis and colleagues measured the number of reticulocytes — immature red blood cells — containing micronuclei in peripheral blood. Micronuclei can form as a result of a break in deoxyribonucleic acid (DNA) or from the disruption of chromosome segregation during cell division. Hallberg and colleagues assessed several markers of oxidative damage to cell components, which is believed to be involved in the induction of carcinogenesis. To detect damage to DNA, the Hallberg team used a comet assay on lung cells and measured 8-hydroxydeoxyguanosine levels in blood. As a measure of damage to lipids, they assessed levels of thiobarbituric acid

reactive substances in brain tissue. Conklin and Kong measured multiple plasma markers of inflammation and thrombosis, and whether chronic exposure had an effect on cardiac fibrosis or the remodeling of the aorta.

### RESULTS AND CONCLUSIONS

In its independent review of the core ACES Phase 3B report by McDonald and colleagues, the HEI ACES Review Panel concluded that their study is the first to conduct a careful, comprehensive, and well-executed evaluation in rodents of lifetime inhalation exposure to NTDE from a 2007-compliant engine. Using appropriate statistical approaches to analyze the data from more than 100 endpoints in the broad areas of histology, serum chemistry, systemic and lung inflammation, and respiratory function, the investigators confirmed the *a priori* hypothesis, namely, that NTDE would *not* cause an increase in tumor formation or substantial toxic health effects in rats, although some biologic effects might occur.

Over the entire exposure period, the investigators attained NTDE exposure atmospheres within 20% of the target NO<sub>2</sub> levels. In their extensive analysis of the physical and chemical composition of the emissions, McDonald and colleagues found that the most abundant pollutants were carbon dioxide, carbon monoxide, NO, and NO<sub>2</sub>. Concentrations of engine-generated PM were very low (< 11 µg/m<sup>3</sup>) at all exposure levels (in the ultrafine range of 20–40 nm in diameter), as were concentrations of sulfur dioxide and semivolatile and volatile organic species. These findings confirm that the concentrations of components of NTDE differ strikingly from those of older engines, in which the concentrations of PM, as well as volatile and PM-associated organic species, are much higher.

Most biologic endpoints evaluated showed no NTDE-associated changes after exposure of rats for up to 28 months in males and 30 months in females. In particular, chronic exposure to NTDE did not induce tumors or pre-cancerous changes in the lung and did not increase tumors that were considered to be related to NTDE in any other tissue. Some mild histologic changes were found in the lung; however, these were not pre-cancerous lesions, previously described in long-term exposure studies of rats to TDE. Rather, the histologic changes — periacinar epithelial hyperplasia, bronchiolization, accumulation of macrophages, and periacinar interstitial fibrosis — were confined to a small region, the centriacinus, which is involved in gas exchange.

HEI convened a separate panel of expert pathologists, the Pathology Working Group (PWG), to evaluate the histopathology data collected. The PWG findings confirmed the major histopathologic observations reported by the investigators. Also, the PWG, by evaluating the findings of this study side by side with findings from prior long-term exposure studies, provided a context with which to compare and contrast the current study findings with those of other relevant long-term studies of exposure to TDE and oxidant gases. The overall conclusion was that chronic exposure of rats to NTDE did not produce tumors in the lung, in marked contrast to the effects of chronic exposure to TDE observed in multiple previous rat studies, in which lung tumors, as well as inflammation and the deposition of soot in the lung, were observed. Rather, the effects of NTDE in the lung more closely resembled changes noted after long-term exposures to gaseous oxidant pollutants, in particular NO<sub>2</sub>, and to TDE from which particles have been filtered out. It is possible that components of NTDE other than NO<sub>2</sub> may have contributed to the effects reported, but the low levels of other components suggest that they would not be primarily responsible.

The ACES Review Panel concluded that the multiple toxicity endpoints evaluated — including lung and serum chemistry and respiratory function — were appropriate for evaluating a wide range of possible biologic effects. There were small decreases in some respiratory endpoints, in particular those concerned with expiratory flow, predominantly at the highest exposure level and more in females than males. The diffusing capacity of carbon monoxide (DL<sub>CO</sub>, a measure of alveolar–capillary gas exchange) showed a small effect of exposure to NTDE. The Panel considered the small reductions in DL<sub>CO</sub> to be consistent with the histopathologic findings of mild changes in the gas-exchange regions of the lung, indicating that the histologic changes might have had functional effects. In addition, some small changes in a few markers of oxidative stress and inflammation were detected in lung tissue, bronchoalveolar lavage fluid, and blood. The Panel identified a minor limitation to the study: some biochemical assays lacked positive controls (to determine that each was sensitive enough to detect any changes).

The Panel considered that the ancillary studies by Bemis et al., Hallberg et al., and Conklin and Kong were valuable extensions to the ACES core investigation. These generally well implemented studies took advantage of samples collected by McDonald

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and colleagues at several exposure time points up to 24 months to assess multiple endpoints that are not normally part of chronic inhalation bioassays. The genotoxicity studies assessed well-accepted endpoints — the frequency of micronucleated reticulocytes (immature red blood cells) in blood in the report by Bemis et al., and DNA damage and lipid peroxidation in the report by Hallberg et al. Conklin and Kong assessed a wide range of plasma markers associated with systemic inflammation and thrombosis, as well as markers of more chronic effects, to identify possible cardiovascular effects of NTDE. The Panel agreed with the conclusions of Bemis and colleagues and of Hallberg and colleagues that no genotoxic effects could be detected that were associated with exposure for up to 24 months to NTDE. However, the Panel noted that the assays measured relatively short-term effects (lasting 1 month or less), which somewhat reduced confidence in the utility of these negative findings. In Conklin and Kong's study, NTDE had no effect on cardiac fibrosis or aortic remodeling and few effects, predominantly in females and of uncertain pathophysiologic significance, on the inflammatory

and thrombotic pathway endpoints measured in plasma over 24 months of exposure.

Overall, these results indicate that rats exposed to one of three levels of NTDE from a 2007-compliant engine for up to 30 months, for 16 hours per day, 5 days a week, with use of a strenuous operating cycle that more accurately reflected the real-world operation of a modern engine than cycles used in previous studies, showed few exposure-related biologic effects. In contrast to the findings in rats chronically exposed to TDE, there was no induction of tumors or pre-cancerous changes in the lung and no increase in tumors that were considered to be related to NTDE in any other tissue. The effects that were observed with NTDE were limited to the respiratory tract and were mild and generally seen only at the highest exposure level. The histologic changes in the lungs were consistent with previous findings in rats after long-term exposure to NO<sub>2</sub> — a major component of the exposure atmosphere, which is being substantially further reduced in 2010-compliant engines.

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