



# STATEMENT

Synopsis of Research Report 165

HEALTH  
EFFECTS  
INSTITUTE

## Effects of Controlled Exposure to Diesel Exhaust in Allergic Asthmatic Individuals

### INTRODUCTION

Increasingly stringent emission standards and advances in engine technology have resulted in decreasing particulate matter concentrations and gaseous emissions from modern diesel engines. At the same time, diesel engine exhaust (DE) from older engines in the current fleet continues to contribute significantly to traffic-derived ambient particles, an important component of urban air pollution. Epidemiologic and experimental studies published in the 1990s suggested that short-term exposure to either whole diesel emissions or to the particulate fraction (diesel exhaust particles; DEP) may be associated with adverse respiratory and immune system effects in humans. It had been suggested that these effects could be more severe in persons with asthma and other allergic diseases.

Dr. David Diaz-Sanchez, of the University of California–Los Angeles (UCLA), and his colleagues at the University of California–Riverside and Los Amigos Research and Education Institute (LAREI) proposed to evaluate the effects of inhaled DEP on the lower airways and blood of healthy subjects and of allergic asthmatic individuals. The investigators hypothesized that inhalation of DEP would enhance inflammatory or allergic immunologic responses to allergens. HEI's Health Research Committee considered the proposed study to be a logical follow-up to the principal investigator's previous studies of the effects of DEP administered into the upper airways; but it recommended several changes in study design — most importantly, to evaluate the effects of inhalation of whole DE, thus providing the opportunity to study a more realistic concentration and route of exposure to DEP. After discussions with Diaz-Sanchez, the Committee recommended funding a revised proposal to study the effects of DE inhalation.

In the course of the study, Diaz-Sanchez moved to the U.S. Environmental Protection Agency, ending his association with the project; and Dr. Henry Gong, who had been leading the study at LAREI, died. Ultimately, Dr. Marc Riedl at UCLA assumed the role of principal investigator.

### APPROACH

For the source of DE, the investigators purchased a 1999 medium-duty diesel pickup truck with a 250-hp turbocharged V-8 engine that used ultra-low-sulfur diesel fuel and had been driven for approximately 60,000 miles. The truck was equipped with a 3-way catalyst, which the investigators decided to remove in order to generate and evaluate the effects of raw diesel exhaust.

In a pilot study, the investigators built a diesel exposure facility at LAREI with a human exposure chamber, characterized the DE generated by the truck, and exposed a small group of healthy (i.e., non-allergic, non-asthmatic) participants to DE and to filtered air. After discussing the pilot data with the Research Committee, the investigators eliminated exposures of healthy non-asthmatic participants from the main study and increased the number of participants who had both allergy and asthma. They also added an exposure to nitrogen dioxide (NO<sub>2</sub>), a gaseous component of DE that has the potential to affect some of the same health endpoints.

Thus, the goal of the main study became to evaluate the effects of DE and NO<sub>2</sub> exposure on multiple airway and systemic inflammatory responses in allergic asthmatic individuals in the *absence of* (Phase 1) or *after* (Phase 2) an inhalation challenge with an allergen to which they were sensitive. All participants were non-smokers between the ages of 18 and 50. Phase 1 included 15 subjects with either

mild-intermittent or mild-persistent asthma who were also allergic to at least one of a panel of common aero-allergens. The 15 participants in Phase 2 had the same asthma diagnosis and were sensitive to cat allergen.

Participants in both phases were exposed in random order to DE (at a particle mass concentration of  $\sim 100 \mu\text{g}/\text{m}^3$ ),  $0.35 \text{ ppm NO}_2$ , or filtered air (control) for 2 hours while intermittently exercising on a stationary bicycle. To minimize carry-over effects, exposure sessions were separated by at least 4 weeks.

The investigators measured multiple physiologic and pulmonary function endpoints that included specific airway resistance, oxygen saturation, and bronchial reactivity. Bronchial reactivity was measured 1.5 hours after exposure — in Phase 1 subjects were challenged with the bronchoconstrictor methacholine, and in Phase 2 they were challenged with cat allergen. The investigators also assessed several endpoints associated with inflammatory and immunologic responses in the airways (via sputum induction) and blood.

### RESULTS

The DE exposure atmospheres were close to the target particle concentration of  $100 \mu\text{g}/\text{m}^3$  and contained about  $0.35 \text{ ppm NO}_2$ , similar to the concentration used in the  $\text{NO}_2$ -only exposures.

Exposure to DE or  $\text{NO}_2$  affected only a few inflammatory, immunologic, or physiologic endpoints, and few of the changes occurred in both phases. Several outcome measures were below the limit of detection, and some changes were not in the direction expected for adverse effects; in particular, this was true of some sputum and blood endpoints. Levels in sputum and blood of immunoglobulin E, a key mediator of the allergic immune response, were not affected by either DE or  $\text{NO}_2$  exposure in either phase.

DE exposure slightly increased airway resistance in Phase 1 and the numbers of polymorphonuclear cells and eosinophils in sputum in Phase 2. These findings suggested to the investigators that this exposure concentration and duration may be near the lowest levels that might cause airway inflammatory effects in mildly asthmatic individuals. Exposure to  $\text{NO}_2$  resulted in only a few changes in Phase 1 (e.g., immunoglobulin M levels in blood increased) and none in Phase 2.

In both Phases 1 and 2, the investigators found that many endpoints, particularly those measured in sputum, showed “period” effects, indicating that a pattern of responses was related to the progression of exposure periods (1st, 2nd, 3rd) rather than to the exposure atmospheres themselves. Such effects have not been reported in other similar studies.

### INTERPRETATION AND CONCLUSIONS

The HEI Health Review Committee, which conducted an independent review of the study, noted that the study addressed an important issue — the effects of inhalation exposure to DE, a major component of ambient urban air pollution, on the lower airways and blood of allergic asthmatic individuals. The study had several strong points, including the use of a more realistic concentration and route of exposure than those used in earlier experiments by the same research team; inclusion of an additional control atmosphere ( $\text{NO}_2$ ); evaluation of responses both in the absence of (Phase 1) and after (Phase 2) a post-exposure challenge with cat allergen; and use of minimally invasive techniques for assessing multiple endpoints in lungs and blood — sites that are central to the asthmatic response.

The Committee thought the relative limitations of the study included the evaluation of effects of exposure at only one time point during the late phase of the allergic response (22 hours after exposure) and the use of only one allergen concentration in the challenge protocol in Phase 2. The detection of period effects, particularly for sputum endpoints, may suggest some sort of learning process over the course of the three exposure periods. Whatever their physiologic basis, the period effects complicated assessment of responses to pollutant atmospheres.

The Committee agreed with the authors’ conclusions that the findings were predominantly negative and did not support the original hypothesis that exposure to DE would enhance inflammatory or allergic type responses. The Committee also agreed that caution is needed in extrapolating from these findings. For example, it is possible that individuals with more severe asthma, who are exposed for longer time periods or to higher DE concentrations, or who are exposed to other DE mixtures from other types of diesel engines, fuels, or both, may have different responses.

The lack of effects of DE inhalation in Phase 2 contrasts with the results of previous studies of the

effects of DEP administered into the upper airways of allergic individuals by the same team of investigators. Explanations may include differences in the exposures (with much higher concentrations and less realistic routes of exposure in the earlier studies) and likely differences in the composition of the DEP. The times at which tissues were evaluated after challenge and the health status and allergen sensitivity of participants also differed. Furthermore, variations in study design — such as the timing of allergen challenge and the use of exercise versus rest — may also explain why this study did not find changes, which had been found in some earlier studies, in immunologic or inflammatory endpoints after exposure to 0.35 ppm NO<sub>2</sub>.

The current study evaluated effects of DE from a 1999 engine with no catalytic converter, which was likely to be representative of a fraction of the vehicle fleet in use at the time. Since 1999, however, tighter emission standards for NO<sub>x</sub> and particulate matter have significantly reduced the overall emissions of

DE and its major components from new vehicles. In particular, particle emissions from light- and heavy-duty trucks have been reduced by more than 10-fold through the use of particulate filters (starting with the 2006 and 2007 model years, respectively) and low-sulfur fuels. The concentration of DEP used in the current study (100 µg/m<sup>3</sup>) may be considered high in the United States and Europe. However, even at this comparatively high level, there appear to be very few biologic effects of exposure to this level of DEP in people with asthma.

For future studies to be relevant to the improvements in engines and fuels, they will need to target much lower concentrations of DEP while also evaluating the gaseous components that will start to dominate the exhaust mixture. HEI's ongoing Advanced Collaborative Emissions Study is among the first to comprehensively study emissions from the new diesel engine and aftertreatment technology, starting with the health effects of exhaust in animals.

## **Allergic Inflammation in the Human Lower Respiratory Tract Affected by Exposure to Diesel Exhaust**

Marc A. Riedl, David Diaz-Sanchez, William S. Linn, Henry Gong Jr., Kenneth W. Clark, Richard M. Effros, J. Wayne Miller, David R. Cocker, and Kiros T. Berhane

### **INVESTIGATORS' REPORT**

#### **Specific Aims**

#### **Study Design and Methods**

Diesel Exhaust Delivery System and Exposure Chamber  
Exposure Atmosphere Monitoring  
Subject Recruitment and Screening  
Experimental Protocol  
Response Assessment

#### **Statistical Methods and Data Analysis**

#### **Results**

Exposures

Sputum Assay Responses in Each Phase

Blood Assay Responses in Each Phase

Physiologic and Symptomatologic Responses in Each Phase

#### **Discussion and Conclusions**

Responses to Diesel Exhaust and Inhaled Allergen

Diesel Exhaust Characteristics in This Study

Compared with Others

Responses in This Study Compared with Others

#### **Implications of the Findings**

### **COMMENTARY Health Review Committee**

#### **Scientific Background**

Asthma and the Allergic Response  
Components and Health Effects of Diesel Exhaust

#### **Technical Evaluation of the Study**

Objectives and Specific Aims  
Design of the Main Study  
Generation and Characterization of Atmospheres  
Study Population  
Health Endpoint Measurements  
Statistical Methods  
Key Findings

#### **The Health Review Committee's Evaluation of the Study**

General Comments  
Strengths and Limitations of the Study  
Comparison of This and Previous Studies by These Investigators  
Other Key Considerations  
Future Studies of the Effects of Diesel Exhaust Exposure  
Summary and Conclusions