



# STATEMENT

Synopsis of Research Report 159

HEALTH  
EFFECTS  
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## Role of Nephilysin in Airway Inflammation Induced by Diesel Exhaust Emissions

### BACKGROUND

Nephilysin—also known as neutral endopeptidase (NEP)—is an enzyme that degrades multiple peptides that affect airway blood vessels. The expression of NEP on the surface of cells varies in a number of airway injury conditions and in several types of cancers. HEI periodically issues a Request for Preliminary Applications for novel research on the health effects of air pollutants derived from motor vehicle emissions. In response to Request for Preliminary Applications 05-3, “Health Effects of Air Pollution,” issued in 2005, Dr. Simon Wong at the University of Arizona and colleagues submitted an application to study whether exposure to diesel exhaust affected the airway expression or function of NEP. Some prior studies had shown that exposure of humans and laboratory animals to diesel exhaust particles (DEP) or whole diesel exhaust emissions (DEE) affected the airways. Dr. Wong and colleagues hypothesized that components of diesel exhaust downregulate the function or expression of NEP in the airways and that this may lead to disorders in airway function. They also hypothesized that in conditions in which NEP expression was decreased, responses to diesel exhaust would be increased.

### APPROACH

Wong and colleagues evaluated airway inflammatory responses and NEP expression and activity in the fluid and cells obtained from bronchoalveolar lavage and the lung tissue of mice genetically deficient in NEP (*Nep*-knockout mice) or wild-type mice (control). The mice were instilled with 10 or 100 µg resuspended DEP, and their BAL fluid was analyzed 7 days later; the investigators used

National Institute of Standards and Technology SRM 2975 particles, which were originally generated by a diesel-powered industrial forklift. The investigators also measured airway inflammatory responses and NEP activity in the induced sputum of 11 healthy human volunteers (ages 19–33 yr) 1 hour after exposure to DEE in a staged mining environment. Individual exposure concentrations ranged from 0.09 to 1.80 mg/m<sup>3</sup> elemental carbon (a component of DEE), and duration ranged from 56 to 134 minutes. A baseline measurement of NEP activity was taken at least 1 week before volunteers were exposed to DEE.

In addition, the investigators used a transformed human airway epithelial cell line, BEAS-2B, to evaluate whether exposure to DEP decreased the expression of NEP messenger RNA (mRNA). They used 1 or 10 µg/cm<sup>2</sup> untreated DEP (SRM 2975); DEP treated with chelating agents to remove divalent cations, particularly transition metals; DEP treated with dichloromethane to remove all but the carbonaceous core; or a control particle (standard urban dust SRM 1649a). Using microarray and real-time polymerase chain reaction approaches on extracts of these cells, the investigators also evaluated the expression of genes affected by exposure to DEP. Similar gene expression studies were conducted in DEP-exposed BEAS-2B cells with depleted levels of NEP by incubating the cells with a small interfering RNA specific for NEP.

### RESULTS

The investigators found airway inflammatory effects in response to DEP instillation—specifically, increases, found in bronchoalveolar lavage fluid,

of the numbers of macrophage and epithelial cells and levels of cytokines that these cells synthesize—that were greater in *Nep*-knockout mice than in wild-type mice that express NEP. They also found a 50% decrease in NEP protein expression in lung tissue and decreased NEP protein expression in airway epithelial cells and macrophages after DEP instillation into wild-type mice.

In human volunteers, the investigators observed that baseline levels of NEP activity in induced sputum varied considerably among the volunteers. Exposure to a high concentration of DEE via inhalation resulted in airway inflammatory effects, measured as increased numbers of macrophages and epithelial cells in the induced sputum, and, in some participants at least, increased NEP activity.

In addition, the investigators observed changes (either up or down) in the expression of several genes in the airway epithelial cell line in response to DEP in normal control cells as well as in cells in which NEP expression was decreased.

NEP mRNA expression in BEAS-2B cells was decreased by approximately 45% after exposure to untreated DEP or to DEP with transition metals removed and by approximately 60% after exposure to the control standard urban dust particle. DEP that was stripped down to its carbonaceous core did not affect NEP mRNA levels significantly.

### CONCLUSIONS

In its independent review of the study, the HEI Health Review Committee considered that Wong and colleagues had made a comprehensive attempt to explore the role of NEP in response to exposure to diesel exhaust components, using an appropriate animal model (an *Nep*-knockout mouse), human studies, and in vitro models using a human cell line. For most of the experiments performed, the study design was strong and the statistical analyses appropriate. The human exposure study, however, had the limitation that the investigators used samples and data that had already been collected in an earlier study, and so only a limited number of assays could be performed.

The investigators found that DEP instillation into the airways of mice resulted in stronger airway inflammatory effects in mice genetically deficient in *Nep* than in mice expressing *Nep* and was accompanied by a significant decrease in NEP expression in lung tissue. The Committee concluded that these findings plausibly suggested that expression of NEP

in some way damps down inflammatory responses in the airways—supporting one of the hypotheses for the study—but noted that the mechanism by which this might occur was not addressed in this study. The Committee thought that other important study findings were that exposure of human volunteers to a high concentration of DEE via inhalation resulted in airway inflammatory effects and, in some participants at least, *increased* NEP activity. However, this was at odds with the investigators' other hypothesis for the study, that exposure to DEP would *decrease* NEP levels in the airways.

The Committee noted that it was challenging to compare the responses in the mouse and human airways because the exposures were of different levels, durations, and routes, and the effects were measured at different times after the exposures. Nonetheless, the Committee thought that the study's human and mouse diesel-exhaust-associated findings could be interpreted in a consistent fashion—that is, that the observed changes in NEP levels result from a response to injury in the airways, measured as the shedding of airway epithelial cells and an increase in macrophage numbers in induced sputum (human) or bronchoalveolar lavage fluid (mouse). Since both epithelial cells and macrophages express NEP, this could explain the increase in NEP activity found in the induced sputum of some of the human volunteers.

The in vitro studies in the transformed human airway epithelial cell line provided some useful information about DEP constituents that affect NEP expression—namely, removing metals did not change NEP expression, but removing organic components did. The studies also provided information about which genes' expression in a transformed airway epithelial cell line may be affected by exposure to DEP. These data may help in determining the pathways involved in airway response to DEP and any possible NEP role in that response.

The investigators speculated in the report that changes in NEP in sputum might be a useful early marker of DEE-induced injury in humans. However, the Committee thought that changes in NEP activity or levels in the airways are unlikely to be useful biomarkers of exposure to diesel components, because the observed effects in airway cells were not specific to diesel exposures, and because baseline levels of airway NEP activity differed markedly in different people. Thus, although changes in NEP function and activity have been noted in airway conditions, particularly after injury, the role of NEP is still not resolved.