Letter to the Editor
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Comment on "Long-Term Effects of Diesel Exhaust Particles on Airway Inflammation and Remodeling in a Mouse Model" by Kim *et al*.

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We recently read with interest a paper by Kim *et al*.[1] describing the inflammatory, functional, and structural effects of long-term exposure to diesel exhaust particles in BALB/c mice. Their key findings were that exposure to nebulized diesel exhaust particles (DEP) for 1 hour a day, 5 days a week for 3 months resulted in increased responsiveness to methacholine, and increased neutrophils and lymphocytes in bronchoalevolar lavage fluid, altered levels of common cytokines, and increased collagen and the lung fibrosis in lung tissue. The authors should be commended first for expanding the body of knowledge on DEP health effects, a topic where there is still a great paucity of data and need for detailed investigation. However, we believe it is essential that experimental models of DEP exposure attempt to best reflect 'real-world' exposure and that methods used are interpreted and limited to the context of the experimental setting. This paper raises several issues that the authors may wish to address, primarily relating to the unusual choice of exposure method (nebulized DEP suspended in saline), and the use of the widely discredited enhanced-pause technique, meaning that one of the main conclusions, "that chronic exposure to DEP can cause AHR" may be an over-interpretation of the data.

First, the authors exposed mice to DEP that were sterilized by autoclaving and suspended in serum-free medium after coating with bovine serum albumin (BSA). This was done to "minimize particle aggregation and hydrophobicity," although the reference cited to support this only suspends particles in phosphate-buffered saline (PBS) and 0.05% TWEEN-80.[2] It is not clear why serum-free medium and BSA were used over PBS/TWEEN-80, nor is it clear if the mice were inhaling an aerosol of particles in medium, BSA, and PBS, or particles in PBS alone. This is important given that BSA inhalation can result in an asthmatic phenotype, including increasing airway and hyperresponsiveness.[3,4] The authors also exposed mice to processed particles via nebulization, rather than by the more common routes of inhalation,[5,6] or instillation.[7-9] This route of exposure is likely to influence results via increased deposition of particles within the nares/upper respiratory tract and may alter health outcomes via increased ingestion of particles.[10] We have previously shown that inhalation of diluted diesel exhaust, while methodologically difficult, results in more realistic functional and inflammatory outcomes in mice.[6] Deposition of DEP in PBS within the nares will also influence measurement of Penh as described below. Suspension of particles in solution also effectively eliminates the likelihood of experimental animals receiving a realistic size distribution of particles. The authors state that their ultrasonic nebulizer produced particles in the size ranging of 1 to 5 μm. This is considerably larger than the vast majority of "real-world" DEP which are at least an order of magnitude smaller in the nuclei mode, and generally smaller than 1 μm in the accumulation mode.[11]

Secondly, we feel that the authors have to appropriately justify their use of enhanced-pause (or Penh). Penh is a dimension-

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less value which reflects changes in peak inspiratory and expiratory pressures combined with a "pause" measurement derived from the expiratory breath.[12] Almost immediately after the original description of Penh was published, and many times in subsequent years, leading respiratory researchers and clinicians have raised concerns regarding its use in assessing airway hyper-responsiveness (AHR).[13-15] While Penh is an attractive option for easily and repeatedly assessing some aspects of lung function in conscious animals, it is not a measure of lung mechanics or AHR and it cannot be interpreted as such. Importantly, as mice are obligate nose breathers, in a study such as that performed by Kim *et al.*[1] where animals are subjected to an inhalational insult, Penh is further influenced by the structure of the nasal passages and/or the repeated deposition of DEP-laden saline in the nose. It is feasible that the increase in Penh measured by the authors is at least partially a result of deposition of DEP in the nose rather than in the respiratory tract. This relates to the method of particle delivery and size of particles as discussed above. Further, it is impossible to quantitatively interpret Penh data.[15] For example, in Fig. 2 of Kim *et al.*,[1] what does a Penh measurement of ~3 for the high dose group mean in relation to a Penh measurement of ~1 for control animals? It does not mean a tripling of lung resistance, and it does not necessarily mean that any increase in lung/airway resistance is occurring.

In summary, while the work of Kim *et al.*[1] should be commended for expanding the body of knowledge on DEP health effects, limitations in the methodologies used mean that caution should be used in interpreting the results and ultimately the conclusions drawn.

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