A Tale of Two Diesels

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Two different samples of diesel exhaust particles (DEP) have been used by toxicologists interested primarily in cancer/genotoxicity or noncancer—such as pulmonary inflammation and asthma exacerbation—health end points. These are, respectively, a standard reference material, SRM 2975, from a heavy-duty diesel engine, and a sample collected by researchers at the Japanese National Institute for Environmental Studies from an automobile diesel engine. In this issue of *Environmental Health Perspectives* companion papers appear, by David DeMarini and co-workers and by Pramila Singh and co-workers, characterizing these samples and contrasting their *Salmonella* mutagenicity and pulmonary toxicity in mice. This commentary is a plea from an atmospheric chemist for more cooperation among toxicologists, analytical chemists, atmospheric chemists, and automotive and combustion engineers to provide a comprehensive assessment of health risks to humans exposed to contemporary diesel emissions and for greater quantities and more diverse types of DEP and ambient samples (i.e., SRMs) that can be shared and exhaustively characterized. This needs to be a continuing process as diesel engines, fuels, and exhaust components evolve in response to control regulations. *Key words:* automobile-derived particles, diesel exhaust particles, forklift-derived particles, pulmonary toxicity, *Salmonella* mutagenicity, SRM 2975. *Environ Health Perspect* 112:812–813 (2004). doi:10.1289/ehp.7031 available via *http://dx.doi.org/* [Online 13 April 2004]

In 2002 the U.S. Environmental Protection Agency (EPA) completed a health assessment document for diesel engine exhaust that concluded that "long-term (i.e., chronic) exposure to diesel engine exhaust is likely to pose a lung cancer hazard as well as damage the lung in other ways depending on exposure" and that "short-term (i.e., acute) exposures can cause transient irritation and inflammatory symptoms" (U.S. EPA 2002). The assessment also stated that "evidence is emerging that diesel exhaust exacerbates existing allergies and asthma symptoms" and included the caveat that "while EPA believes that the assessment's conclusions apply to the general use of diesel engines today, as cleaner diesel engines replace a substantial number of existing engines, the general applicability of the conclusions in this health assessment document will need to be reevaluated" (U.S. EPA 2002).

Paraphrasing the U.S. EPA: *a*) There are chronic and acute health hazards from diesel engine exhaust; *b*) we need to learn more, especially about enhanced allergic immune responses; and *c*) we will need to reevaluate the health consequences as the sources of diesel exhaust, i.e. diesel engines and exhaust systems, change over time. Thus, the companion papers in this issue by DeMarini et al. (2004) and Singh et al. (2004) come at a time when their message calling for collaborative, multidisciplinary studies on a variety of representative diesel exhaust particles (DEP) samples should be understood and acted upon.

The authors have compared two widely reported DEP samples using biologic end points relevant to both the chronic and acute health effects of DEP. One DEP sample—National Institute of Standards and Technology standard reference material, SRM 2975—was derived from a forklift with a heavy-duty diesel engine and developed in part for mutagenicity assays (Hughes et al. 1997). The other DEP sample came from a light-duty automobile engine (Kobayashi and Ito 1995; Sagai et al. 1993) and apparently has graciously been provided by scientists at the Japanese National Institute for Environmental Studies to many researchers studying inflammatory effects (frustratingly, many papers—for example, several in *The Journal of Immunology*—do not identify the source of the DEP used). Before the work reported here by DeMarini et al. (2004) and Singh et al. (2004), SRM 2975 had not been used in many pulmonary toxicity studies, and the automobile-generated DEP (A-DEP) had not been tested for genotoxicity.

Both DEP samples showed mutagenic activity (DeMarini et al. 2004) and lung injury on aspiration (Singh et al. 2004). However, the authors have shown that the two DEP samples are very different in their percent extractable organic material, amounts of polar versus nonpolar material, and the polarity of fractions in which the mutagenic activities measured in different *Salmonella* strains were distributed (DeMarini et al. 2004). Also, the two DEP samples differed in their elemental carbon content, ratios of 3- and 4-ring polycyclic aromatic hydrocarbons (PAHs), the kinds of cell inflammatory responses they produced in CD-1 mice, and in the biochemical responses of the mice bronchoalveolar lavage fluids (Singh et al. 2004).

It is surprising that this is the first time these two DEP samples have been compared in a wide range of bioassays and physical and chemical analyses. However, considering the different engines and conditions under which the samples were collected, it is not surprising that the SRM 2975 and the A-DEP samples have distinct chemical compositions and biologic effects. These results certainly reinforce the need for additional reference materials and especially for materials from newer diesel engines as the engine design, fuel, and exhaust technology change.

Both DEP samples contain 1-nitropyrene, and a recent, comprehensive analysis of the nitro-PAHs in SRM 2975 showed that it also contains the potent mutagens dinitropyrenes (Bamford et al. 2003). How much of the observed mutagenic activity can be attributed to the nitro-PAHs and PAHs present in SRM 2975 is presently unknown, but additional bioassay-directed chemical analyses to answer this question for the SRM and also for the A-DEP are warranted. Cho and coworkers (in press), after developing a new chemical analysis procedure, quantified in A-DEP four PAH-quinones, compounds thought to be active in producing reactive oxygen species that may play a role, among others, in asthma exacerbation (Li et al. 2003). In light of the differences in inflammatory responses found by Singh et al. (2004) for the two DEP tested, knowledge of the PAH-quinone content of SRM 2975 would be of interest.

There is clearly a need for additional large DEP samples that are made available to all researchers so that comparative analyses can be conducted. It must be remembered, however, that ambient particles have been implicated in lung cancer and cardiopulmonary mortality (Pope et al. 2002), and DEP represents only a portion of the ambient particles. Furthermore, investigators have now recognized that the semivolatile organic compounds (SVOCs) in combustion emissions may also have toxicologic effects (Seagrave et al. 2002). Additionally, as noted by

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Mauderly (2001), diesel exhaust will age in the atmosphere, and this may affect its toxicity.

One obvious example of the potential influence of atmospheric reactions on the toxicity of ambient particles is seen by comparing several classes of mutagenic nitro-PAHs and nitro-oxy-PAHs in diesel exhaust with those found in ambient air [International Programme on Chemical Safety (IPCS) 2003 and references therein]. For example, although 1-nitropyrene is generally the most abundant nitro-PAH in DEP, the isomeric 2-nitrofluoranthene is generally more abundant in ambient particles. 2-Nitrofluoranthene is formed by radical-initiated atmospheric reactions of gas-phase fluoranthene (Arey and Atkinson 2003; Atkinson and Arey 1994). Furthermore, radical-initiated reactions of naphthalene and alkylnaphthalenes, which are also emitted in vehicle exhaust, produce what generally are the most abundant nitro-PAHs in ambient air, the semivolatile nitronaphthalenes and methylnitronaphthalenes (Cecinato 2003; Gupta et al. 1996; IPCS 2003). Recently dimethyl-/ethylnitronaphthalenes, formed from atmospheric reactions of the ethyl- and dimethylnaphthalenes present in vehicle exhaust, including diesel exhaust, have been identified in southern California's atmosphere (Reisen et al. 2003).

Bioassay-directed chemical analyses of DEP samples led to the initial discoveries of the presence of nitro-PAHs, dinitropyrenes (Schuetzle and Lewtas 1986), and, more recently, 3-nitrobenzanthrone (Enya et al. 1997). Bioassay-directed chemical analysis of ambient samples and environmental chamber reactions of PAHs led to the identification of mutagenic nitrophenanthrene lactones (Helmig et al. 1992) and nitropyrene lactones (Sasaki et al. 1995) in ambient particles. Thus, mutagenic nitro-PAHs, nitro-PAH lactones, and even nitro-PAH ketones (Phousongphouang and Arey 2003) can all be produced by atmospheric reactions of PAHs emitted from vehicle and combustion sources. Often the isomers produced by radical-initiated atmospheric reactions are distinct from those present in vehicle emissions and, therefore, impart new health effects to ambient particles. Also PAH-quinones, redox reactive compounds mentioned above and implicated in a variety of toxic mechanisms (Bolton et al. 2000; Henry and Wallace 1996; Hiura et al. 1999), are likely to be products of atmospheric reactions of PAHs (Sasaki et al. 1997) and photolysis of nitro-PAHs (Atkinson et al. 1989). Thus, there is a need to understand the atmospheric chemistry of diesel and other vehicle exhaust to know whether controls will decrease not only emissions of toxic compounds but also the toxicity of reaction products inhaled by the populace at receptor sites.

Much remains to be discovered about the chemical constituents and physical characteristics that produce the biologic activity of DEP and ambient particles. Clearly, more cooperation is needed among toxicologists, analytical chemists, atmospheric chemists, and automotive and combustion engineers to provide a comprehensive assessment of health risks to humans exposed to contemporary diesel emissions. There is a vital role to be played by standard materials that can be shared and exhaustively characterized. The papers of DeMarini et al. (2004) and Singh et al. (2004) have made an important contribution by noting a lack of shared information and the urgent need for communication and interdisciplinary collaborations among health science researchers. More than two decades ago, the importance of understanding the health risks from diesel exhaust was recognized (Lewtas 1982). Good research has been done and much has been learned, but it is important to plan cooperative, interdisciplinary future efforts to maximize information on the health effects of contemporary diesel exhaust. We need more SRMs, including more of ambient particles, for example, from highly polluted atmospheres where the "signals" may be strong, as well as for DEP from newer technology vehicles.

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