

**Comparative Toxicity of Combined Particle and
Semi-Volatile Organic Fractions of Gasoline and Diesel Emissions**

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Introduction

Little is known about the relative health hazards presented by emissions from in-use gasoline and diesel engines. Adverse health effects have been ascribed to engine emissions on the basis of: 1) the presence of known toxic agents in emissions; 2) high-dose animal and bacterial mutagenicity tests; and 3) studies indicating gradients of health effects with proximity to roadways. Most attention has been given to the particulate fraction of emissions; little attention has been given to the semi-volatile organic fraction. However, the semi-volatile fraction overlaps the particulate fraction in composition and is always present in the vicinity of fresh emissions. Although the potential health effects of diesel emissions have been frequently studied and debated during the past 20 years (EPA, 2002), relatively little attention has been given to the toxicity of emissions from gasoline engines. In view of the considerable progress in cleaning up diesel emissions, it would be useful to compare the toxicity of emissions from contemporary on-road diesel technology with that of emissions from the in-use gasoline fleet that is well-accepted by the public. It would also be useful to have a set of validated tests for rapid, cost-effective comparisons of the toxicity of emission samples, both for comparisons among competing technologies (e.g., diesel, gasoline, natural gas) and for determining the impacts of new fuel, engine, and after-treatment strategies on toxicity.

The Office of Heavy Vehicle Technologies has sponsored research aimed at developing and applying rapid-response toxicity tests for collected emission samples (Seagrave et al., 2000). This report presents selected results from that work, which is being published in much greater detail in the peer-reviewed literature (Seagrave et al., 2002).

Methods

Particle ("PM", mass collected by filter) and semi-volatile organic compound ("SVOC", mass

collected by polyurethane foam-XAD resin trap) fractions of emissions from normal-emitting and high-emitting in-use light- and medium-duty vehicles operated on chassis dynamometers on the Unified Driving Cycle were collected by Southwest Research Institute (San Antonio, TX). The vehicle groups included three current technology diesels ("D"; 1998 Mercedes-Benz E300, 1999 Dodge Ram 2500, 2000 Volkswagen Beetle), one high-emitter diesel ("HD"; 1991 Dodge Ram 2500), five average gasoline ("G"; 1982 Nissan Maxima, 1993 Mercury Sable, 1994 GMC 1500, 1995 Ford Explorer, 1996 Mazda Millenia), one white smoker gasoline ("WG"; 1990 Mitsubishi Montero), and one black smoker gasoline ("BG"; 1976 Ford F-150). The current technology diesel and average gasoline groups were sampled while operating at both 72°F ("D", "G") and 30°F ("D₃₀", "G₃₀"). Samples from the multiple vehicles in each group were pooled for testing.

The PM and SVOC materials were extracted from collection media by Desert Research Institute (Reno, NV) and analyzed chemically. A portion of each sample was concentrated into a small volume of acetone and sent to the Lovelace Respiratory Research Institute (Albuquerque, NM) for toxicity testing. The PM and SVOC fractions of each sample were re-combined at Lovelace in their original collection ratios and tested as a combined PM/SVOC sample.

This report discusses the comparative toxicity of the seven PM/SVOC samples in two biological test systems, rat lungs dosed by intratracheal instillation, and strain TA100 Salmonella bacterial (Ames) mutagenicity. Each sample was tested at three doses by intratracheal instillation of a saline suspension of the material into young adult male F344 rats, five at each dose. The rats were killed 24 hours later, and responses were evaluated by histopathology and bronchoalveolar lavage (lung washing). Recovered lavage fluid was

analyzed for cells (total leukocytes, polymorphonuclear neutrophils [PMN], macrophages), the inflammatory mediator MIP-2, the cytoplasmic enzyme LDH, and protein. Tissues were examined for histopathology indicating inflammation or tissue damage.

Strain TA100 Salmonella was cultured in medium into which one of several doses of sample were mixed, and the culture plates were examined after incubation for colonies indicating mutational changes. Strain TA100 is sensitive to mutations from both frame-shift and base pair substitution. Mutagenicity was assessed both with and without metabolic activation by inclusion of S9 rat liver microsomal fraction in the medium.

The potency of each sample was estimated by fitting the coefficients defining the slope of log-linear (rat lung toxicity) or linear (mutagenicity) functions to the data. These potency estimates (i.e., fitted slopes of the dose response curves) were used to compare toxicity among the samples.

Results

All samples were toxic and mutagenic at some dose, but the strength of the response varied from sample to sample such that the potency of the samples could be differentiated and ranked. The results for lung toxicity are summarized as bargraphs in Figures 1 and 2, and the mutagenicity results are summarized as a bargraph in Figure 3.

The ranking of the samples for inflammatory (irritant) activity is shown in Figure 1. Five different parameters indicative of inflammatory response included histopathology and four lavage parameters (total leukocytes, PMNs, macrophages, and MIP-2). For this display (and for Figures 2 and 3), the highest response (greatest toxicity) for each of the five parameters was set at a value of 1.0, and the responses of the five parameters for each sample were averaged. The bars, therefore, compare the average inflammatory response of each sample with that of the most toxic sample (WG) set at 1.0. The resulting toxicity ranking was $WG \gg HD, BG \gg D, D_{30} > G > G_{30}$. The WG sample was obviously the most toxic,

followed by the other two high-emitters. The toxicities of all the normal-emitter samples were similar.

The ranking of the samples for cytotoxic responses (tissue damage) is shown in Figure 2, which was constructed using the same approach as for Figure 1. There were three cytotoxicity parameters: histopathology and two lavage parameters (LDH, protein). The rankings for cytotoxicity were $WG \gg BG, HD > D, D_{30}, G, G_{30}$. Again, WG was clearly the most toxic, followed by the other two high-emitters, and the toxicities of all the normal-emitter samples were similar.

The ranking of the samples for mutagenicity in Strain TA100 Salmonella is shown in Figure 3. This figure was constructed like Figures 1 and 2, except that the individual data points for each sample represent mutagenicity with and without metabolic activation, and the bars represent the ranking of the average of mutagenicity under the two conditions. The rankings for mutagenicity were $D_{30} > WG, HD, D > BG, G_{30} > G$. Although D_{30} was the most mutagenic and G the least, there was little difference in mutagenicity among the other samples. Metabolic activation with S9 changed mutagenicity slightly in different samples, but had little effect on the ranking of the samples.

Discussion

The lung toxicity tests provided good discrimination in demonstrating a 4–5-fold range of toxicity among the samples. The lung tests encompassed types of effects that would be expected to contribute to non-cancer health hazards, such as irritation of asthma and other non-cancer lung diseases. There was generally good agreement among the five inflammation parameters and among the three cytotoxicity parameters, and the evidence from multiple parameters lends confidence to the ranking. The WG sample was clearly the most toxic, and the toxicity of the three high-emitter samples was greater than that of the normal-emitter samples. Among these responses, operating temperature appeared to make little difference in toxicity.

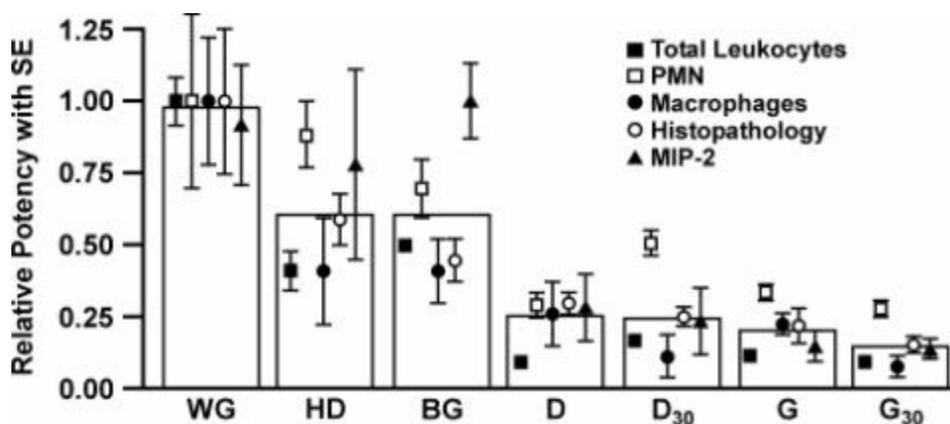


Figure 1. The ranking of the inflammatory potential of the samples is shown. For each inflammation parameter, the potencies of the samples were adjusted to a scale of 0 to 1.0, by dividing each value by the value of the potency of the most toxic sample (data points are shown as mean \pm SE). The bars indicate the average of the potency rankings of the five parameters for each sample.

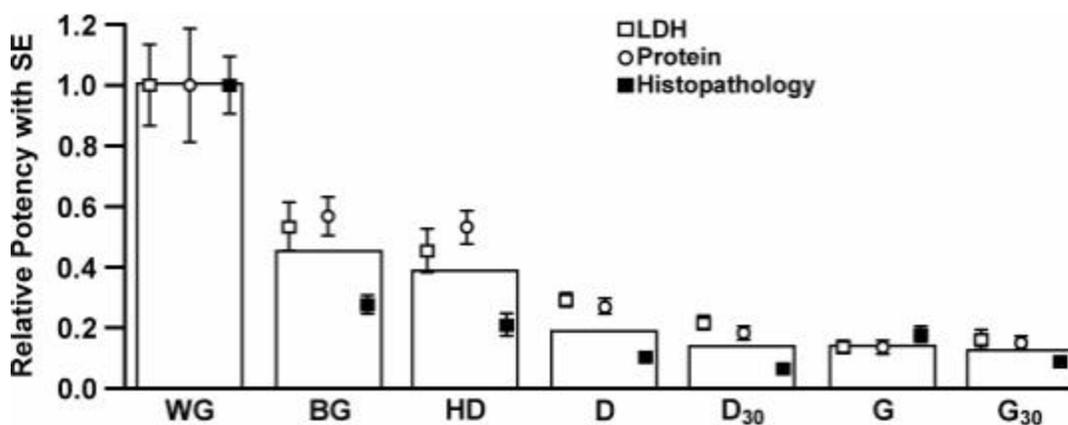


Figure 2. The ranking of the cytotoxic potential of the samples is shown. For each parameter, the potencies were scaled relative to the most potent, which was given a value of 1.0 as described for Figure 1 (data points are shown as mean \pm SE). The bars indicate the average of the potency rankings of the three parameters for each sample.

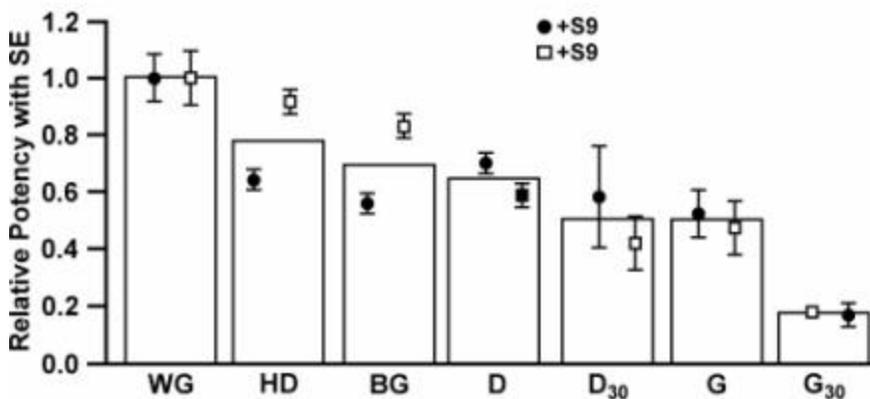


Figure 3. The ranking of the mutagenicity of the samples in strain TA100 Salmonella is shown. The data points show the ranking for each sample either with or without metabolic activation by S9 microsomal fraction (mean \pm SE) relative to the response of the most potent sample, which was set to a value of 1.0. The bars indicate the averages of the potency rankings under the two conditions.

The bacterial mutagenicity test also demonstrated differences in toxicity among the samples. Although the differences were more graded from most to least toxic than the lung responses, there was still approximately a 4-fold range from the most to least mutagenic sample. Bacterial mutagenicity is an index of the ability of material to interact with cellular DNA, which for certain chemicals, roughly corresponds to cancer potential. It appeared that operating under cold conditions tended to increase the mutagenicity of emission samples.

These results indicate that rapid toxicity tests have the ability to discriminate among different emission samples, suggesting their utility for comparing among different fuel technologies and screening for effects of new fuel, engine, and after-treatment strategies within fuel technologies. The finding of similar toxicity among normal-emitting gasoline and diesel engine emissions per unit of mass suggests that the relative health hazards of the technologies might be adequately compared on the basis of differences in mass emissions. Perhaps the most important finding was the clearly greater toxicity in the lung tests of high-emitter emissions than of emissions from normal-emitting vehicles. The implication is that high-emitters not only emit more mass, but the mass is also more toxic than that from normal-emitters. Thus, high-emitters likely contribute a disproportionate share of the health hazard from in-use, on-road vehicles.

References

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3. Seagrave, J., J. McDonald, A. Gigliotti, K. Nikula, S. Seilkop, M. Gurevich, and J. Mauderly. Mutagenicity and In Vivo Toxicity of Combined Particulate and Semi-Volatile Organic Fractions of Gasoline and Diesel Engine Emissions. *Toxicol. Sci.* (in press).