RELATIONSHIP BETWEEN COMPOSITION AND TOXICITY OF ENGINE EMISSION SAMPLES

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ABSTRACT

Differences in the lung toxicity and bacterial mutagenicity of seven samples from gasoline and diesel vehicle emissions were reported previously [1]. Filter and vapor-phase semivolatile organic samples were collected from normal and high-emitter gasoline and diesel vehicles operated on chassis dynamometers on the Unified Driving Cycle, and the compositions of the samples were measured in detail. The two fractions of each sample were combined in their original mass collection ratios, and the toxicity of the seven samples was compared by measuring inflammation and tissue damage in rat lungs and mutagenicity in bacteria. There was good agreement among the toxicity response variables in ranking the samples and demonstrating a five-fold range of toxicity. The relationship between chemical composition and toxicity was analyzed by a combination of principal component analysis (PCA) and partial least squares regression (PLS, also known as projection to latent surfaces). The PCA/PLS analysis revealed the chemical constituents co-varying most strongly with toxicity and produced models predicting the relative toxicity of the samples with good accuracy. The results demonstrated the utility of the PCA/PLS approach, which is now being applied to additional samples, and it also provided a starting point for confirming the compounds that actually cause the effects.

INTRODUCTION

Two interrelated issues pertaining to the health hazards of engine emissions continue to present serious challenges to manufacturers, regulatory decision makers, toxicologists, and risk assessors. First, it is important to identify the most important contributors to health risk among the myriad physical-chemical species contained in emissions. Second, it is important to be able to estimate changes in health risks, for better or worse, that will result from changes in the composition of emissions. Both issues are important for ensuring that the most health-relevant components are controlled and that technological strategies for meeting emissions regulations reduce, rather than increase, hazards. The current knowledge base does little to support such judgments because there have been few direct comparisons of the health effects of different types of emissions. Moreover, with the exception of bio-directed fractionation schemes that have identified nitro-aromatic compounds as major drivers of bacterial mutagenicity, there has been no data set suitable for the types of statistical analyses that can determine the chemical species driving the health hazards of complex emissions.

As reported recently [1-3], the DOE Office of Heavy Vehicle Technologies supported the collection, chemical analysis, and toxicological evaluation of a set of seven samples of emissions from normal- and high-emitting gasoline and diesel vehicles. Because the samples had a five-fold range of toxicity, and because their composition was determined in detail, an opportunity existed to explore the relationship between chemical composition and toxicity. A statistical approach was selected [4] that had been successfully used to determine the key components of complex petroleum mixtures causing mutations in bacteria.

METHODS

Emission Samples

Particle and vapor-phase semivolatile organic (SVOC) fractions were collected at the Southwest Research Institute using filters and polyurethane foam/XAD resin traps, respectively, from diluted, fresh emissions from vehicles operated on chassis dynamometers on the Unified Driving Cycle as described [2,5]. Five vehicles or groups of vehicles were sampled: a group of five normal-emitting gasoline vehicles (G); a group of three normal-emitting diesel vehicles (D); two single gasoline vehicles emitting white (WG) and black (BG) smoke; and a single high-emitting diesel vehicle (HD). All vehicles were in-use light- or medium-duty passenger cars, pickup trucks, or vans, ranging from 1976 to 2000 models and were tested with fuel and crankcase oil as received. The normal-emitting groups were sampled while operating both at room temperature and at 30°F (G_{30}, D_{30}), and samples from the two groups of normal-emitting vehicles were pooled, making a total of seven samples.

Chemical and Toxicological Evaluations

The chemical composition of the particle and SVOC fractions of each of the seven samples were analyzed separately in detail at the Desert Research Institute. Analyses included temperature fractions of organic and elemental carbon, elements, ions, and full speciation of resolvable organic compounds. A total of 176 composition variables was measured. Parallel aliquots were provided to the Lovelace Respiratory Research Institute for toxicological evaluation.

For toxicological evaluation, the particle and SVOC fractions were combined in their original collection ratios and mixed with lung cell culture media [3], Salmonella culture media (Ames bacterial reverse mutation assay) [2], or instilled into lungs of rats [2] over a range of doses of combined particle + SVOC mass. Because the ranking of toxicity among the seven samples by cultured lung cells differed from the ranking by lungs [3], composition-response relationships were only tested statistically using lung and bacterial mutagenicity responses.

Responses in rat lungs were evaluated at 4 (the cytokine MIP-2) or 24 (all other responses) hours after dosing as described [2]. Lungs were removed and weighed, and then cells, protein, enzymes, and chemical mediators of inflammation were sampled by washing (bronchoalveolar lavage). Lung tissue was then fixed and examined by light microscopy for histological evidence of inflammation and tissue damage. In all, 11 lung response variables were measured. Dose-response slopes were calculated for each variable for each emission sample, and the slopes were used to rank the toxicity of the samples for each response variable.

Bacterial mutagenicity was evaluated in Ames tester strains TA98 and TA100, both with and without metabolic activation by a liver microsome preparation (S9) [2]. Mutagenicity was determined over a range of doses, response slopes were constructed for each of the four test cases (two strains, each ± S9), and the mutagenicity of the samples was ranked as done for the lung response variables.

Statistical Analysis

A combination of principal component analysis (PCA) and partial least squares regression, also known as projection to latent surfaces, (PLS) was used to assess relationships between composition and response variables with the aim of determining the composition variables most closely associated with differences in toxicity among the seven samples. The PCA/PLS analysis was conducted using Simca 10.0 software (Umetrics, Umea, Sweden) using an iterative exploratory process described previously [4,6]. PCA/PLS analysis determines groupings among composition (predictor) variables and response (dependent) variables by projection to planes or hyperplanes in multiple dimensions, and tends to overcome difficulties caused by inter-correlations among variables and a larger number of variables than of samples (both types of difficulties were presented by this data set). The PLS analysis also allows construction, testing, and optimizing equations for predicting health responses from groupings of composition variables.

RESULTS

Ranking of Sample Toxicity

The seven samples had a five-fold range of relative toxicity in rat lungs, as illustrated by the ranking for lung inflammation shown in Figure 1. The mean ranking by five different inflammation variables is shown in the figure, with the magnitude of response for the most toxic sample set at a value of 1.0. The most toxic sample (WG) caused approximately five times greater lung inflammation as the least toxic (G_{30}). Confidence in the ranking is gained from the fact that, with few exceptions, the five inflammation variables yielded the same ranking. The ranking for the tissue damage variables was identical to that for inflammation; thus, the 11 "lung toxicity" variables could be grouped together in the PCA/PLS analysis.

The seven samples also had a five-fold range of relative mutagenicity in the Ames test, although the ranking order differed from that for lung inflammation. The mean ranking for strain TA100 with and without S9 activation is shown in Figure 2, with the magnitude of response for the most mutagenic sample set at a value of 1.0. The most mutagenic sample (D_{30}) was approximately five times more mutagenic than the least mutagenic sample (G).

Relationship Between Composition and Toxicity

The PCA/PLS analysis yielded a ranking of the relative association of each of the 176 composition variables to the response outcomes; that is, the extent to which the differences among samples in the amount of each composition variable tracked with the differences among samples in toxicity. Relationships were tested between each composition and response variable and between groupings of composition and response variables that were determined to co-vary (and thus could be lumped together). An example is illustrated in Figure 3, which shows the relative strength of association of each of the top 18 composition variables with total lung lavage inflammatory cells (total leukocytes, or white blood cells). In this example, "O2TC", a temperature fraction of organic carbon, bore the strongest relationship to differences in inflammation, followed by total nitrate and other individual compounds and physical-chemical classes. Interestingly, the nine individual compounds bearing the strongest relationship to inflammation were all two- to four-ring polycyclic aromatic hydrocarbons (PAHs) ranging from Bibenzyl (MW 182) to stearane (MW 372), and including both SVOC and particle-bound species.

Mathematical models were then constructed, tested, and refined iteratively to determine the combination of composition variables giving the best prediction of the ranking of sample toxicity for each response variable. Because the 11 lung toxicity variables ranked the samples similarly, a single best model for predicting lung toxicity was derived. A model that included 63 composition variables proved optimal, and the correlation between the resulting prediction and the actual sample ranking ("goodness of fit") was calculated for each response variable. The model yielded correlation coefficients (R^2) ranging from 0.68 to 0.96 for the 11 responses, with the best fit for histological evidence of inflammation (Figure 4). Figure 4 illustrates that the model predicted the toxicity of samples from high-emitters (BG, HD, and WG) very well, but it did not discriminate very well among the toxicities of the samples from normal vehicles (G, G_{30}, D, D_{30}). This reflected the fact that the toxicities of samples from normal vehicles were similar and illustrated the point that the strength of the PCA/PLS analysis in identifying composition-response relationships is related to the diversity of sample composition and toxicity.

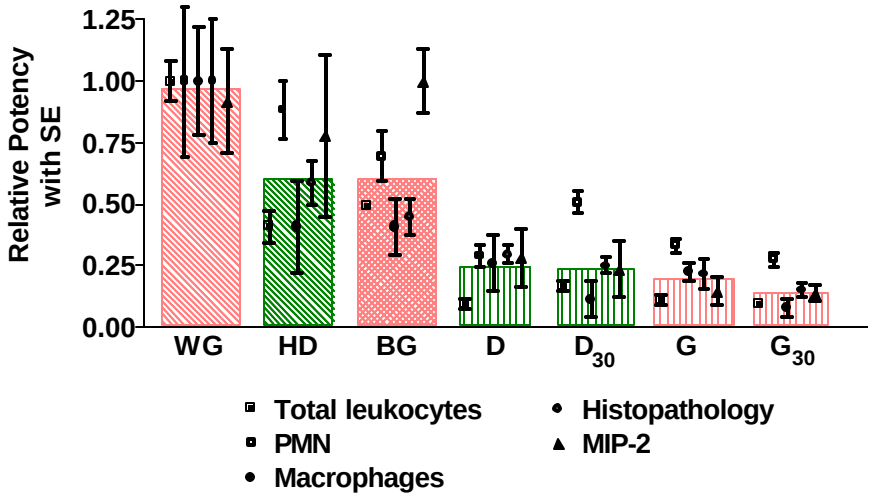

Figure 1. The relative ranking of the seven samples for lung inflammation is shown. The magnitude of the greatest response for each of five response variables, and also for the mean of the five responses, was set at a value of 1.0.

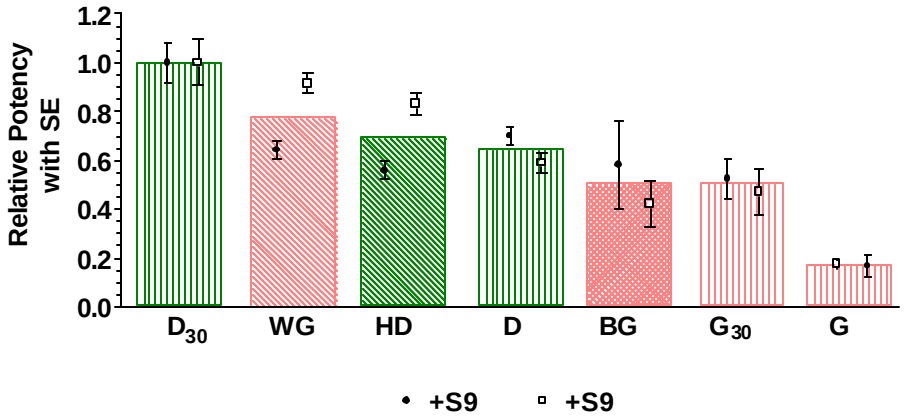

Figure 2. The relative ranking of the seven samples for mutagenicity in strain TA100 is shown. The magnitude of the greatest response for mutagenicity with and without activation by S9 and also for the mean of the two test conditions was set at a value of 1.0.

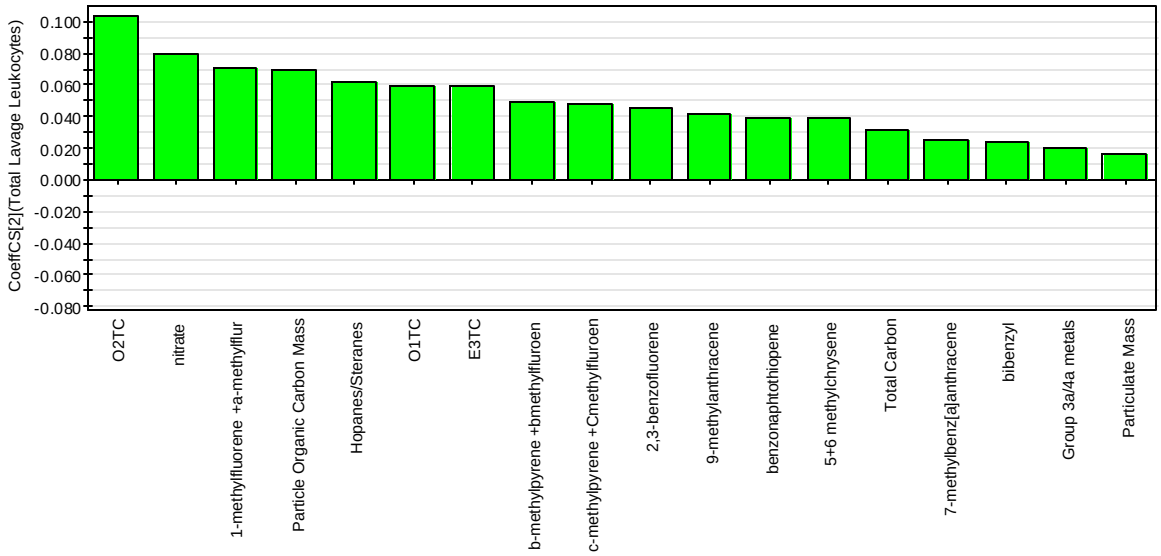

Figure 3. The relative strength of association between inter-sample differences in composition variables and differences in lung lavage inflammatory cells (total leukocytes) is shown for the top 18 physical-chemical composition variables.

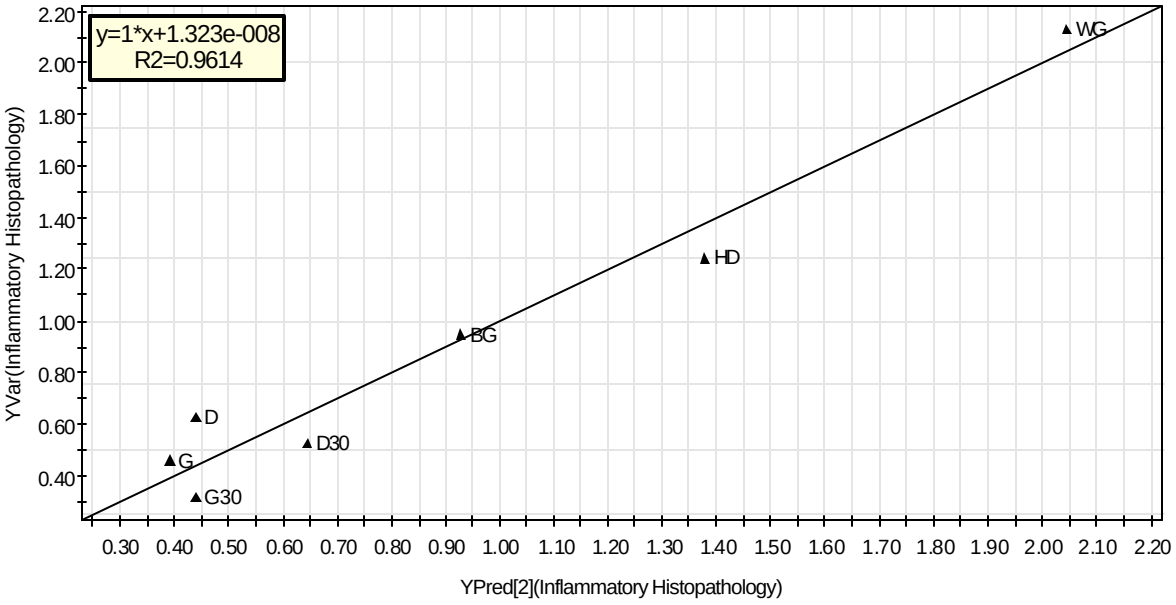

Figure 4. The fit of a model containing 63 composition variables to the ranking of samples for histological evidence of inflammation is shown.

The same iterative approach was used to determine the physical-chemical components most closely associated with bacterial mutagenicity, and to develop predictive models. The best model included 23 nitro- and oxy-PAHs. Figure 5 shows the relative strength of association between the 23 compounds and mutagenicity in TA100, and Figure 6 illustrates the extremely good fit (R^2 = 0.99) of the predicted to actual mutagenicity of the seven samples. In contrast to lung toxicity, the model for mutagenicity discriminated very well among all the samples.

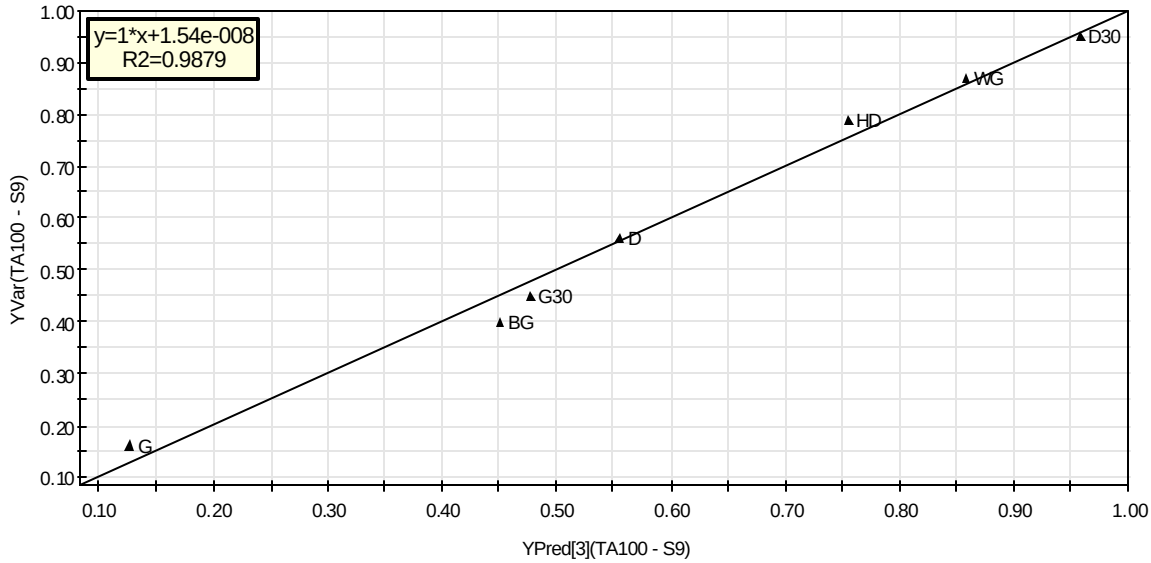

Figure 6. The fit of a model containing 23 composition variables (oxy- and nitro-PAHs) to the ranking of samples for mutagenicity in strain TA100 is shown.

CONCLUSIONS

The results demonstrated the utility of the PCA/PLS analysis for examining composition-response relationships among a large and diverse range of both composition and response variables. The approach had been used previously for bacterial mutagenicity, but it has now been shown to apply as well to other parameters of toxicity. The high predictive power for mutagenicity, although not unexpected, was especially encouraging. Decades of "bio-directed fractionation," an iterative process of testing the mutagenicity of chemically isolated fractions of organic material from combustion particles, had shown that certain nitro- and oxy-PAHs were primarily responsible for the mutagenicity of combustion products [7]. The fact that the PCA/PLS analysis of only seven samples yielded the same information lends confidence to the use of the analysis to identify emissions components responsible for other forms of toxicity and encourages the continuation of the effort.

Two strategies are needed to follow up on the present findings. First, more, and more diverse, samples need to be added to the analysis to determine whether or not the same composition-response relationships hold for other types of emissions (e.g., natural gas). Second, the associations demonstrated by the analysis suggest, but do not prove, that the compounds most highly associated with differences in toxicity actually cause the effect. One approach to determining causality would be to re-test the toxicity of low-toxicity samples "doped" with the suspect compounds and confirm not only an increase in toxicity but also that the model accurately predicts the increase. Work following up on both of these strategies is underway.

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