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Investigation of NOx Artifacts in Diesel Emission Tests

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APPENDIXES

ABSTRACT

It has been suggested by some researchers that there is a potential for the diesel particulate sampling technique to alter the characteristics of the samples. A test program was undertaken to investigate the effect of diesel NOx emissions on diesel particulate samples collected in a manner similar to standard EPA procedures. The specific purpose of the program was to determine whether the NOx gases flowing across the particulates trapped on the filtering media would alter the biological characteristics of these samples. The test program was conducted from December 1978 through October 1979. Steady-state tests were run using a small, single-cylinder diesel engine and the Ames bioassay technique was used on the particulate samples to test for changes in biological activity.

The engine was tested using both No. 2 diesel fuel and a nitrogen-free fuel (decane) using both air and a simulated nitrogen-free "air". These tests provided an evaluation of the potential for diesel exhaust NOx emissions to alter the characteristics of the particulate samples. The Ames tests of these samples showed that the presence or absence of NOx caused no significant difference in the bioassay activity of the particulate samples.

BACKGROUND

The Environmental Protection Agency has an ongoing program of studies to investigate vehicle emissions and their related health effects. As part of this effort, EPA's Emission Control Technology Division has for several years been focusing a large effort on the detailed study of diesel exhaust emissions.

The emissions of a diesel vehicle differ from the emissions of a gasoline vehicle in several aspects. One of these is that there is a much greater amount of carbonaceous particulate matter in the exhaust of a diesel vehicle than in the exhaust of a gasoline fueled vehicle. Typically, diesel vehicles emit 30 to 100 times more particulate per mile than a catalyst-equipped gasoline vehicle. Since diesel vehicles are expected to become an increasing percentage of the total vehicle population, the contribution of the diesel emissions to the ambient total suspended particulate (TSP) could be significant. Therefore, diesel particulate levels are now regulated[1].

Diesel particulate consists of both solid carbonaceous particulate matter and organics bound to these solids. The study of the health effects of these particulate-bound organics is the subject of considerable effort by government, industry and educational institutions. A commonly used method to investigate the biological activity, which may be health related, of these organics is the Ames Test[2].

Published work indicates that these particulate-bound organics may be artificially modified by the sampling process due to the unusual chemistry that can occur on the surface of the filter[3]. Because of the

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[1]Standard for Emission of Particulate Regulations for Diesel Fueled Light Duty Vehicles and Light Duty Trucks. 40 CRF Part 86 Published March 5, 1980.

[2]Ames Test - bacteriological test, developed by Dr. Bruce Ames and colleagues at the University of California at Berkley. The test is used to evaluate the mutagenetic potential of compounds.

[3]Benzo[a]pyrene (BaP) is a known particulate-bound exhaust carcinogen that is not directly active (mutagenic) in the Ames test. In order for it to be an active mutagen in a body system, the Bap must be activated by body enzymes. However, work by Dr. James N. Pitts of the University of California at Riverside has shown "that when a filter was preloaded with BaP and then a stream of 1 ppm NO_2 (nitrogen dioxide) and a carrier gas was drawn through it the BaP nitrosated to nitrobenzo[a]pyrenes (the 1, 3 and 6 isomers). These nitrosated BaP compounds are direct acting mutagens in the Ames test and as such do not require metabolic activation." Reference: Memorandum dated April 5, 1978 from Thomas M. Baines, EPA, to Charles L. Gray, EPA, subject "Carcinogenesis, Dr. Bruce Ames, PNA's, and Characterization".

potential impact of this effect on the accuracy of the test procedures for unregulated emissions, the sampling methods needed to be further studied. Therefore, EPA initiated a small in-house test program to investigate whether NOx artificially influences the Ames test results.

The conclusions to be drawn from this EPA test effort are, necessarily, of limited applicability to diesel vehicles. The Ames bacteria tester strains used were those normally used in light-duty diesel vehicle exhaust studies. The test engine was a small displacement, stationary, diesel generator set and all testing was done at a constant speed and load. Therefore, the conclusions of this EPA study can be considered to be quantitatively valid only for the specific stationary diesel engine tested and for only the specific Ames bacteria tester strains. However, it is reasonable to suggest that similar trends may be observed in vehicle testing for similar test conditions.

PROGRAM DESIGN

1. Methodology

During the engine combustion process, some of the nitrogen in the combustion air is oxidized to nitrogen oxides (NOx). To determine if NOx artifacts are introduced into the particulate samples gathered for the Ames test, a diesel engine was tested both with and without nitrogen in the fuel/"air" mixture. Diesel particulate samples were obtained using both normal air and a nitrogen-free "air". The engine was tested with both standard diesel fuel and a nitrogen-free fuel.

The nitrogen-free fuel, decane, was produced by reducing linear alpha olefins. The fuels obtained by the process, (decane, duodecane, and tetradecane) are nitrogen-free and readily separated. Since decane is a light end component of diesel fuel, EPA anticipated no special problems in starting or running the engine at the nominal test temperature of 70°F.

To minimize the combustion air requirements, a small displacement diesel engine was needed for the test program. To simplify the application of the output shaft loads, a small-displacement diesel generator set was selected.

Existing test procedures and equipment used to test diesel vehicles were modified to permit their use in testing this single-cylinder engine. A description of the procedures and equipment used is given on page 8.

2. Ames Test

The Ames test is a bacteriological technique which is used to evaluate the mutagenic potential of compounds. For diesel particulate emissions, test samples are gathered on a filter medium and the soluble organic fraction (SOF) is later chemically separated by Soxhlet extraction using either methyl or dimethyl chloride. The initial extraction solvent is then removed and the SOF is then dissolved in dimethyl sulfoxide (DMSO) for the bioassay tests. This solvent-extract is placed in Petri dishes with various standardized Salmonella Typhimurium bacteria tester strains to test the mutagenicity of the chemical compound. A broader and more detailed description of Ames bioassay testing and its applicability to diesels is given in Appendix A.

For Ames tests of vehicle exhaust emissions, the cooled (less than 125° F) diluted exhaust is passed through a large teflon-coated glass fiber filter to trap the vehicle exhaust particulate. Typically, a 20x20 inch filter is installed in the diluted exhaust stream to trap as much particulate matter as possible.

3. Artificial Combustion "Air"

Engines are designed to run on an air/fuel mixture which has very specific properties. To determine the properties for the best artificial "air" blend, researchers who were known to have had experience with using various blends of artificial combustion "air" were contacted to solicit their ideas as to the proper blend of gases to be used when there is to be no nitrogen in the combustion "air". These included individuals from the Air Force Propulsion Lab, Air Force Environmental Activities Group, Amoco, Bureau of Mines, Cummins Engine Company, GM Research*, GM Truck & Coach, Gulf Research*, Essex*, University of California*, and the University of Michigan. None of these individuals had done work under the particular test conditions required in this project.

As a result of these discussions, a limited literature search, and some preliminary calculations, criteria were developed for the blend of gases used to replace nitrogen in the artificial "air":

a. The gases had to be chemically inert during the combustion process.

b. The gases should not aid or detract from the normal combustion process.

c. The gases should be readily available.

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*Individuals from these organizations had actually conducted some testing with artificial "air" blends.

d. The blend of gases should have the same physical properties as air in terms of density, molecular weight, specific heat at constant pressure, specific heat at constant volume, specific heat ratio, thermal capacity, and viscosity.

Based on the preceding considerations, a blend of 15% argon, 35% carbon dioxide, 29% helium, and 21% oxygen was selected for the nitrogen-free combustion "air". The physical properties of air and this blend of gases are tabulated in Appendix B.

4. Test Engine - Selection Criteria and Description

The basic objective of this test program was to obtain particulate samples for Ames analysis from a diesel engine operating on both normal air and nitrogen-free "air". Since the testing would require large amounts of this artificial combustion "air", the primary criterion for selection of the engine was that it have a small displacement. Similarity to passenger vehicle diesel combustion chamber design, ease of testing, and availablility were secondary requirements.

The engine selected was a small Onan diesel which is part of a generator set model DJA. It is a 4 cycle, single-cylinder, air-cooled 30 CID unit. A more detailed description is given in Appendix C.

This engine had the following similarities to those used in a diesel passenger vehicle:

a. compression ratio

b. displacement similar to that of individual cylinders on multicylinder passenger car diesel engine

c. 4 stroke cycle

d. indirect injection

e. precombustion chamber in head

f. governed rpm of 1800 which is reasonably representative of that of an engine in a passenger vehicle that is cruising at 50 mph.

This engine differed from those used in a passenger vehicle in that it was air-cooled and restricted to steady-state operation.

Since such a small engine cannot be tested accurately on EPA's large dynamometers, it was decided that it would be tested in its designed application using resistive loads. This engine was tested at 65% of

the maximum rated continuous load of the engine. This would provide a sufficient amount of sample without requiring an excessive amount of artificial "air". Since Ames samples are generally obtained during a 50-55 mph cruise or the HFET and the principal difference in using different driving cycles is the rate of particulate generation (the amount of particulates generated per unit time usually increases with engine load and the Ames response is not appreciably altered by these load changes), these were not thought to be serious differences.

TEST PROCEDURES

The procedures and equipment used to gather Ames samples are a modification of the procedures normally used to obtain particulate samples of diesel vehicles using either the Federal Test Procedure (FTP) or Highway Fuel Economy Test (HFET)[4]. The main difference is the addition of a large filter to collect as large a sample of the particulate as possible for the bioassay testing. The test procedures and equipment used to obtain the diesel NOx artifact samples were patterned on the test procedures used to obtain vehicle particulate samples for Ames tests.

1. Test System

The particulate generation and collection system consisted of the artificial "air" gas cylinders, the diesel generator set, a resistive load bank, an 8-inch dilution tunnel, 47mm particulate sampling unit, a bulk stream filter for the Ames sample, a positive displacement pump (PDP) Constant Volume Sampler (CVS), auxiliary analyzers, instrumentation, plumbing, and ductwork. A schematic of the test set-up is given in Figure 1.

The particulate sampling system used an 8-inch dilution tunnel. The exhaust gases and dilution air are mixed by the turbulent flow created by the orifice plate in the tunnel. The exhaust enters the tunnel at the plane of the orifice plate. The total particulate mass was obtained by taking particulate samples on 47mm filters downstream of the orfice plate. The filter probe had a knife edge and the flow rate through the filter was adjusted to permit isokinetic sampling*. The total particulate mass was then calculated by relating the flow through the 47mm filter to the total flow through the tunnel. This calculation is described in Reference 4. A drawing of the tunnel is given in Figure 2.

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[4]Environmental Protection Agency 40 CFR Part 86 Federal Register Vol.45, No. 45, March 5, 1980 "Standard for Emission of Particulate Regulation for Diesel-Fueled Light-Duty Vehicles and Light-Duty Trucks".

*For isokinetic sampling, the velocity of the flow at the sample probe is equal to the velocity of the free stream flow.

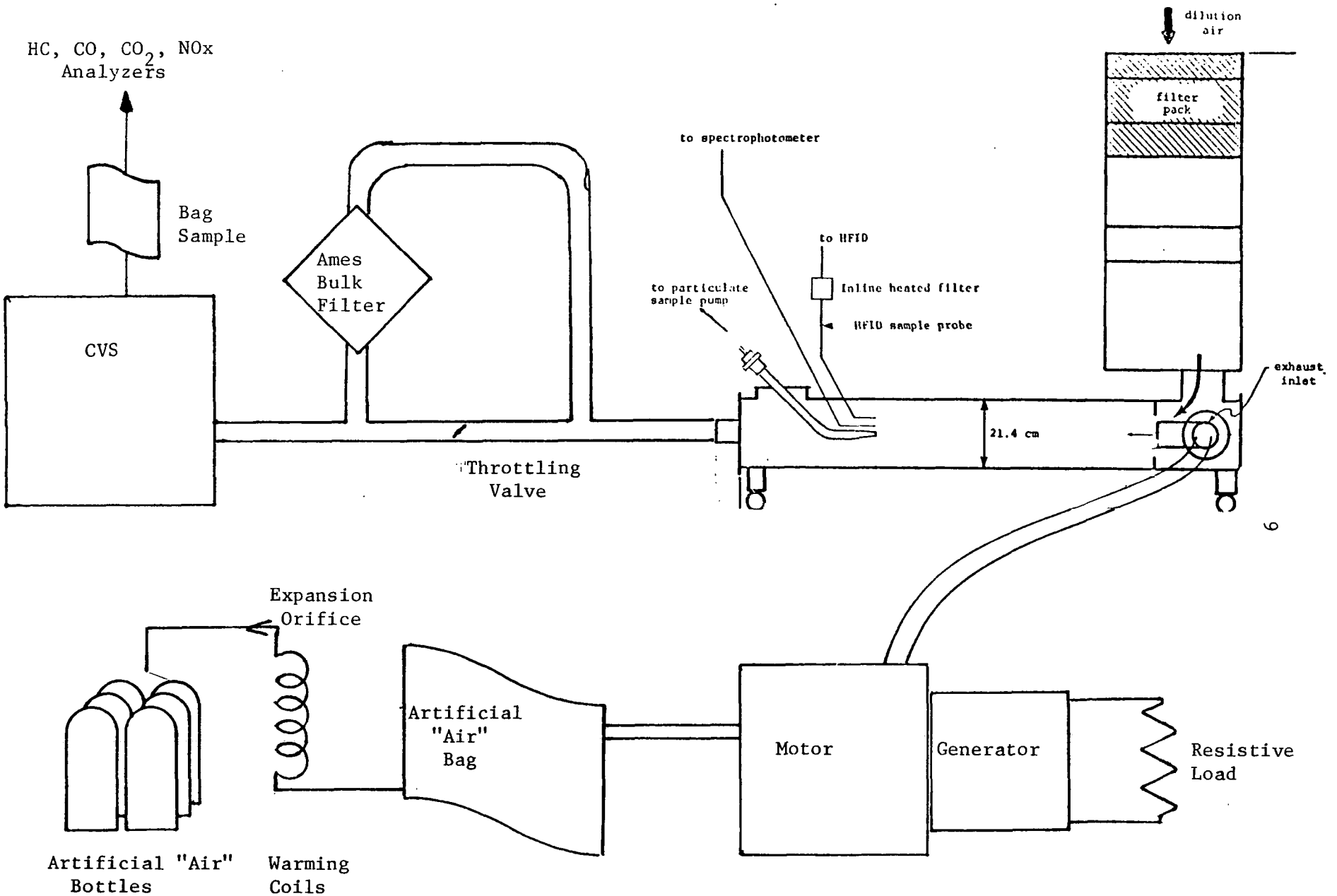

Figure 1 Schematic of Test Set-Up

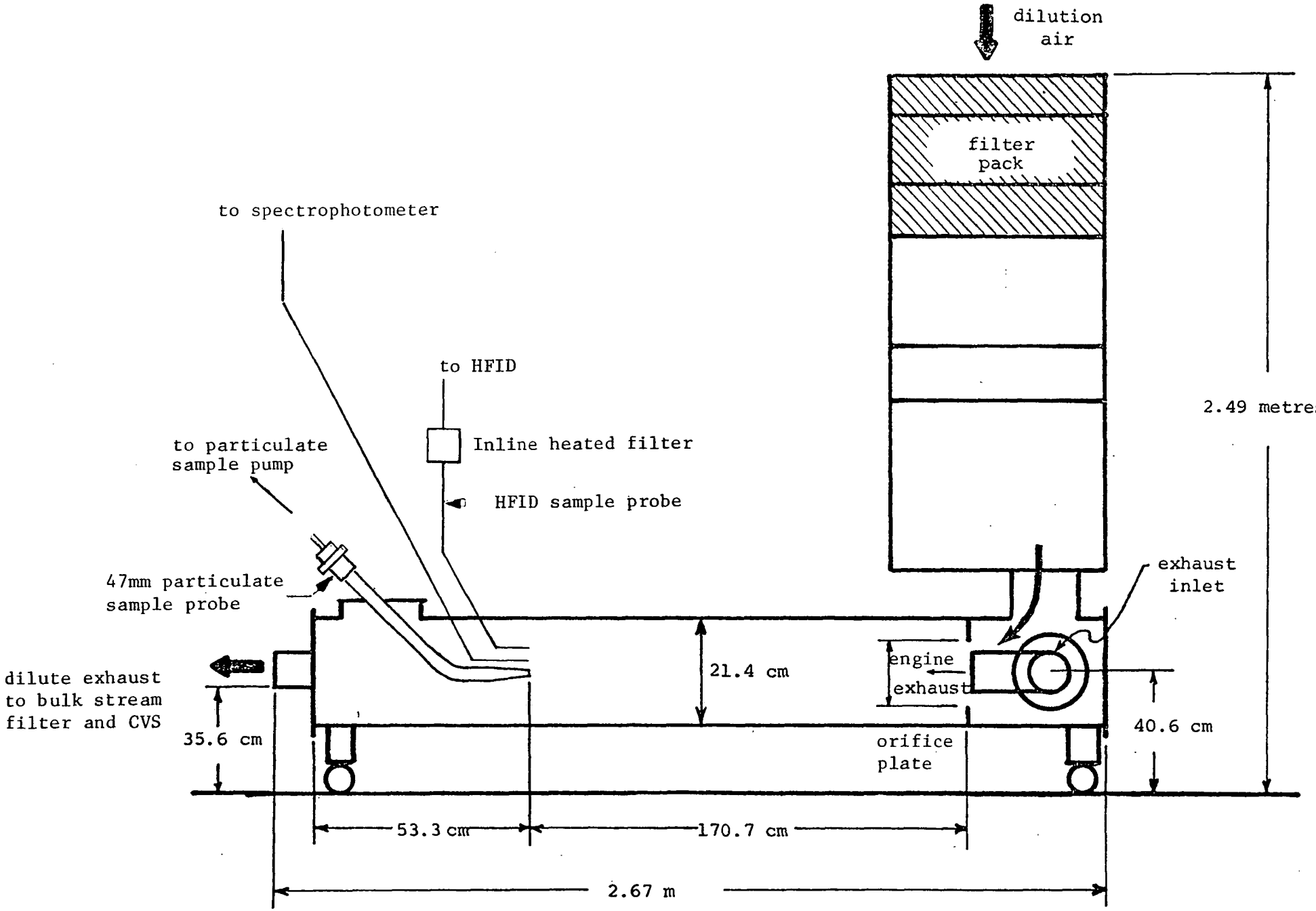

Figure 2. Schematic of Particulate Sample Dilution Tunnel

Six A-size cylinders (200 cubic feet each) were manifolded together to supply the artificial combustion "air". A pressure regulator controlled the flow of this high pressure "air" to the flow control orifice. Since the expansion through the orifice would cool the "air" it was routed though 50 feet of copper tubing. This allowed the "air" to warm to the ambient test temperature. The "air" then passed into a large sealed bag that was attached to the air intake of the engine. This system ensured that artificial "air" was at ambient pressure, eliminated waste, permitted adjustment of the flow rate to operating flow needs, allowed the engine to be readily switched between air and artificial "air", and permitted the operator to readily monitor and control the "air" flow.

2. Sample Size

For Ames bioassay testing, the particulate-extractable organics are tested at several logarithmically spaced doses. Typically, a 2 mg/µl solution of extractables in DMSO (2 milligrams of extractable organic material per microliter of dimethylsulfoxide) is applied to the Ames tester strain at six doses (500µl, 300µl, 100µl, 50µl, 30µl, and 10µl). Each dose is tested in triplicate, both with and without metabolic activation for each of the five tester strains (See Appendix A). The entire test is replicated. Thus, 600mg of organic extractables are needed for a complete Ames test of a diesel vehicle. With typical extraction efficiencies of 20%, a total of 3 to 5 grams of particulate must be collected on the filters used for five tester strains.

Since the TSP generated by a diesel vehicle in any one FTP or HFET provides insufficient sample for analysis, multiple HFET or 50-60 mph steady-state tests on each of several filters are required to obtain sufficient sample. Similarily, for these tests to investigate NOx artifacts, the diesel generator set was operated at 65% load until the filter was fully loaded with particulate matter. The limiting factors were the reduction in flow and the tendency of the particulates to flake off the filter as the particulates accumulate on the filter. The 20x20 inch filters were able to be loaded with 1 to 3 grams of particulates.

3. Equipment Checkout

The test hardware for this testing was integrated into a system. After the initial leak and functional checks of the total system, the performance of the individual components of the system was verified.

The 47mm particulate sampling system (probe, filter, flowmeter, pump, and plumbing) calibration was checked by using it concurrently with a calibrated system installed in an 18-inch tunnel that was being used

to test a vehicle. The artifact sampling system and the calibrated vehicle system agreed to within better than 1/2% for each of the three bags of the FTP test cycle.

The ability of the 8-inch tunnel to mix the exhaust and dilution air was checked by traversing the tunnel at the gaseous and particulate sampling plane. Good mixing was noted. There was less than five percent variation across the tunnel.

Due to the potential effect that even a small amount of nitrogen dioxide could have on the organic-bound particulates[3], it was necessary to insure that the nitrogen-free "air" and the two fuels did not contain trace amounts of nitrogen. The commercial methods used to manufacture and fill the nitrogen-free "air" bottles could allow trace amounts of nitrogen to exist as a contaminant--possibly even as high as 1%. After combustion, some of this nitrogen would be in the form of NO and NO_2, and therefore, negate the efforts of this study. Since the bottles had been filled in batches, a sample of each batch was analyzed for trace nitrogen by a commercial lab using a gas chromatograph--mass spectrometer technique. The results of this analysis, Appendix D, showed that there was no nitrogen detected in the artificial "air" blend.

Similarily the two fuels were analyzed for trace nitrogen. Due to the method used to manufacture the decane, it was unlikely to contain a trace of nitrogen. However, diesel fuels may contain traces of nitrogen compounds. Therefore, duplicate samples of the two fuels, decane and diesel fuel No. 2, were analyzed for trace nitrogen. The results of this analysis, Appendix E, showed that there was no nitrogen detected in the fuels.

4. System Operating Checks and Adjustments

Because the test engine was new, it was necessary to break-in the engine. During the 225 engine operating hours required for break-in, the test setup and procedures were adjusted and modified to optimize the sampling process. A list of the test conditions monitored and/or controlled while operating the engine are given in Appendix F.

The engine was cyclically operated at 0,5,16, and 25 (the rated full load) Amp loads for break-in. Emissions and fuel consumption were monitored in order to determine when they had sufficiently stabilized so that the test samples could be taken. The CVS flow rate was adjusted to the minimum flowrate that would still maintain the diluted exhaust temperature below 125°F. The particulate mass flowrate (47mm filter) was then adjusted for isokinetic sampling at the test load, 16 Amps, (65% of full load) and the minimum CVS flow rate (100 CFM).

Operating procedures were developed for the bulk stream filter used to obtain the Ames samples. Due to the operating constraints of dilute exhaust temperature, available CVS flow rates, bulk filter size, and allowable pressure drop across the filter, it was necessary to have a portion of the flow bypass the bulk filter. Also, due to the reduction in flow across the filter as it loaded, it was necessary to periodically readjust the bypass damper to maintain adequate flow through the filter. Typically, at the test conditions, the pressure drop across the filter was 5 inches water when new and 13 inches when fully loaded. Initially, an 8x10-inch bulk stream filter was used and the diluted exhaust was drawn through the filter by a small pump. This filtered exhaust did not pass through the CVS. After the 20X20-inch filter holder had been fabricated and checked out, it was possible to pass most of the diluted exhaust through the filter and the entire flow, both filtered and bypass, passed through the CVS unit.

Engine operating performance was checked with artificial "air" and with decane. Several artificial "air blends" with different CO_2 concentrations were tried. It was found that with the blend of 35% CO_2, 21% O_2, 15% argon, and 29% helium, the engine operating conditons were nearly the same as they were with air (see next paragraph). With decane, a slight lowering of the exhaust gas temperature (EGT) was noted.

Engine performance was characterized by gaseous emissions, particulate emissions, fuel consumption, EGT, and generator amperage and voltage. Except for the slight decrease in EGT noted when operating with decane, engine operation (load and temperature) was essentially unchanged for all test conditions.

The NOx in the normal engine exhaust consists principally of NO (nitric oxide) and NO_2 (nitrogen dioxide) with most of it being NO. Since the concentration of NO_2 was of specific interest, additional techniques and equipment were employed to monitor NO_2. Although most of the NOx in an exhaust sample is NO, the concentration is not stable. The NO oxidizes to NO_2 until an equilibrium condition is reached. The chemiluminescence analyzer used for NOx analysis can be readily operated in a bypass mode to detect only NO. During testing, some of the samples of the exhaust were checked by this method and the NOx was found to be over 90% NO. This implies that the NO_2 was less than 10% of the NOx for all tests using air. A second derivative spectrophotometer, a Lear Siegler SM400, was also used to check the nitrogen oxides. This instrument is able to directly measure the dilute exhaust concentrations in real time. This instrument showed the same NO/NO_2 exhaust relation. A bag sample of the dilute exhaust was also crosschecked with both instruments over a 50 minute period. As expected, the concentration of NO continually decreased. Both instruments tracked this change and were in good agreement throughout this check.

TEST RESULTS-AMES MUTAGENICITY

Due to scheduling priorities, the Ames analysis of the test samples was to be done a considerable time after the samples were generated. Therefore, as an initial screening control, the particulate SOF was tested for BaP* by HPLC ** to insure meaningful samples were being generated. The results of this extraction and screening are given in Appendix G[5].

The particulate samples for the Ames analysis were all taken at 65% load and 1800 rpm. The Ames results are given in Appendixes I, J, and K and are more fully discussed by our Characterization and Technology Assessment Branch in the analysis given in Appendix H. The results of the diesel artifact testing are expressed by both the linear regression (Appendix I) and curve fitting (Appendix K) models. The results of the individual tests are also summarized as an average dose/response relationship (Appendix J) for the linear regression model.

Overall, for the four test fuel/"air" combinations, there were no significant differences in Ames activity when comparing like samples, i.e. samples with (metabolic) activation to samples with activation and samples without activation to samples without activation for the five Ames tester strains used.

There were differences in Ames activity between samples with activation and without activation for the decane and artificial air tests. Differences were also observed to a lesser degree in the diesel fuel and artificial air experiments. These results indicate that it is possible that the lack of nitrogen may have caused a decrease in direct acting mutagens. However, as noted in Appendix H, these observed differences are within the acceptable limits for Ames test variability for the test configuration.

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*BaP - Benzo[a]pyrene - is a known carcinogen, gives a positive Ames response, and is a normal component of diesel exhaust.

**HPLC-High Performance Liquid Chromatography - a fractionation technique used to separate the compounds in the sample. The amount of BaP was then determined by using UV with flourescence detection at specific wave lengths.

[5]Fuel and lubricants are inactive in the Ames test. Most of the organic extract is inactive fuel and lubricant derived material which serves only to dilute the sample and obscure the analysis. Reference: Pre-test discussions with EPA researchers who were conducting Ames tests on diesel particulates.

Therefore, the overall conclusion is that, for these test conditions, the Ames tests showed that the presence or absence of NOx caused no significant difference in the bioassay activity of the particulate samples. That is, there were no NOx artifacts observed in the Ames tests.

TEST RESULTS-GASEOUS AND PARTICULATE EMISSIONS

The gaseous and particulate emission results are given in Appendix L. The emission levels are given in grams per kilowatt hour and the fuel consumption is in kilowatt hours per gallon. These values are expressed for the generator output rather than the engine output since the actual efficiency* of the generator was unknown. These data were all taken at 65% load and 1800 rpm.

Although it appears there are significant differences in gaseous and particulate emissions for the four test conditions, some of these differences are not as significant as they might otherwise appear due to changes in engine emission characteristics that were observed throughout the test program. The following factors affecting these results were noted:

1. There were long term trends of continually lower gaseous and particulate emission levels. This new engine was operated for over 225 hours before official testing began. Emissions were periodically checked throughout this break-in period and at 225 hours these downward trends, although not stopped, were judged to have sufficiently stabilized for the purposes of this test program. Post test checks confirmed that these downward trends had continued and that the changes were still acceptable.

2. The testing occurred sequentially in the order given in Appendix L. Since it took several hours at each test condition to obtain a sufficient particulate sample for Ames analysis, over 50 hours elapsed between the start and end of testing.

3. The engine periodically exhibited changes in one or more pollutants that would last throughout the sample period, i.e., for decane-normal air testing the hydrocarbon levels changed.

However the differences of the following items in Appendix L are probably significant:

1. With normal air, the NOx emissions are lower with decane than with No. 2 diesel fuel. This may be due to lower peak combustion temperatures and/or reduction of the NOx that is formed.

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*At the test conditions of 60% load at 1800 rpm, the generator efficiency was probably about 90%.

2. The very low levels of NOx with artificial "air" are due principally to the minimal amount of nitrogen in the combustion oxidizer.

3. The levels of NOx with decane and artificial "air" are extremely low, even lower than the No. 2 diesel fuel and artificial "air" tests. The NOx gaseous samples were considerably less than 1 ppm and close to the background NOx levels. This could be expected based on items 1 and 2. However the difference in NOx levels for the two artificial "air" tests could also occur if the artificial "air" blend used for the No. 2 diesel fuel tests possibly had a higher level of nitrogen as an unmeasured contaminant gas than the blend used for the decane tests.*

   Another source of differences in these NOx levels could occur if the No. 2 diesel fuel had a small amount of nitrogen as an unmeasured contaminant (below the detection limit). No. 2 diesel fuel usually contains some nitrogen but the decane, because of the manner in which it was manufactured, is nitrogen free. However, since the minimum detectable level for the nitrogen in the fuel was one-third of that for the nitrogen-free "air" and the ratio of air to fuel is about 20 to 1, the fuel is a less likely source of nitrogen for these low levels of NOx than the combustion air.

4. The high levels of CO for the decane-artificial "air" tests is probably real.

CONCLUSIONS

A 30 cubic inch, single-cylinder diesel engine was successfully operated using two fuels and two combustion gases. The fuel and combustion air combinations tested were:

1. No. 2 diesel fuel and standard air

2. decane fuel and standard air

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*The absolute level of nitrogen in the artificial "air" was not determined. In all cases it was not detectable. That is, the nitrogen content was below the minimal detectable level of 100 ppm nitrogen.

**Similarly the absolute level of nitrogen in the fuels was not determined. For both fuels it was not dectable. That is, the nitrogen content was below the minimal detectable level of 30 ppm nitrogen.

3. No. 2 diesel fuel and an artificial, nitrogen-free, "air" (21% oxygen, 35% carbon dioxide, 15% argon, and 29% helium)

4. decane fuel and artificial "air" (same blend).

Gaseous emission and particulate samples were obtained at 60% load for the preceding fuel/"air" combinations. The same test equipment and procedures that are used to test diesel vehicles were used to the maximum extent possible. High volume particulate samples were collected using either 8x10 inch or 20x20 inch filters for extraction and subsequent bioassay analysis by the Ames Test. There were no difficulties encountered when operating the engine on decane or artificial "air".

Except for NOx, the gaseous and particulate emission data were similar for all test conditions. As expected, the NOx values were very low for the tests using nitrogen-free "air".

Overall, for the four test fuel/"air" combinations, there were no significant differences in Ames activity when comparing like samples, i.e., samples with (metabolic) activation to samples with activation and samples without activation to samples without activation for the five Ames tester strains used.

There were differences in Ames activity between samples with activation and without activation for the decane and artificial air tests. Differences were also observed to a lesser degree in the diesel fuel and artificial air experiments. These results indicate that it is possible that the lack of nitrogen may have caused a decrease in direct acting mutagens. However, these observed differences are within the acceptable limits for Ames test variability for the test configuration.

Therefore, the overall conclusion is that, for these test conditions, there were no NOx artifacts observed in the Ames tests.

APPENDIX A

Ames Bioassay Testing

1. Purpose of the Ames Test

The Ames bioassay was developed by Dr. Bruce Ames of University of California as a screening test for potential carcinogens. The advantage of this test is that it can be conducted at a small fraction of the cost and time required for whole animal tests. According to Dr. Ames, when known carcinogens have been tested with the Ames bioassay, up to 80-90% of them have yielded positive mutagenic responses. On substances that have been shown not to be carcinogenic in whole animal tests, only about 10% yield positive mutagenic responses on the Ames test[6]. Therefore, Dr. Ames feels that mutagenicity, as determined by the Ames bioassay, correlates reasonably well with carcinogenicity and can be used as an indicator of potential carcinogenicity. Other researchers feel that the positives are not as high a percentage.

2. Description of the Ames Test

The Ames test is a bioassay utilizing various strains of a certain bacteria, Salmonella Typhimurium, to test for the mutagenicity of chemical compounds. In the testing of diesel exhaust particulate, the organic bound fraction of the particulates collected on the glass fiber filter are extracted with methylene chloride. The methylene chloride is then evaporated and the residue is compared to the original filter particulate loading to determine the "percent extractable". An organic solvent, such as dimethysulfoxide (DMSO) is used to dissolve this fraction of the particulate for subsequent bioassay tests. A measured quantity of this diluted extract is then placed in a Petri dish with a strain of the Salmonella bacteria. Tests are conducted with various tester strains because different types of mutagens are detected by different strains of the bacteria.

The strains of Salmonella used in the test are histidine-requiring strains, but they are mutant strains which are unable to produce their own histidine. Therefore, unless histidine is supplied to them or something causes them to revert to their original, histidine-producing form, they will die. For the Ames test itself, the mutant strain is placed in a Petri dish with the chemical being tested and with a minimal amount of histidine (enough for a few cell divisions). If the chemical mutates the bacteria (thus correcting the genetic defect), the Salmonella returns to normal, produces histidine, and is able to survive. Those that do not revert, die upon using up the small amount of histidine available. By counting the number of colonies of surviving bacteria that have thus "reverted", an indication of the mutagenic potential of the chemical can be obtained.

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[6]Memorandum dated April 5, 1978 from Thomas M. Baines, EPA, to Charles L. Gray, EPA, subject "Carcinogenesis, Dr. Bruce Ames, PNA's, and Characterization."

A chemical that causes a statistically significant increase in the number of revertants is said to have given a positive Ames response. Conversely, a chemical that does not cause a statistically significant increase in the number of revertents is said to have given a negative Ames response.

Dr. Ames and his colleagues have developed several tester strains of Salmonella that are able to differentiate between various types of mutation. For example, one mutation type would be a point mutation where a specific sector of the DNA molecule would be disrupted, thus yielding a mutation. Another type of mutation would be the frame shift mutation which occurs in the repetitive sequence areas of the DNA. This slippage occurs only in these repetitive sequence areas and involves a much larger portion of the DNA.

In order to make the tests more valid, Dr. Ames eliminated the DNA repair enzyme from the tester strains. Therefore, in the event that a mutagenic compound affects the DNA, the DNA repair mechanism of the cell will not be activated, thus repairing the damage done by the chemical and thereby masking the mutagenicity of the chemical.

They have also developed a process by which they strip the lipopolysacchride sheath from the exterior of the bacterial membrane wall. This lipopolysacchride membrane serves to resist the entrance of certain chemical species. With this barrier stripped off the cells, they will take up a wider variety of chemicals.

3. Metabolic Activation of the Ames Samples

Many chemicals cause cancer by mutating. Certain polynuclear aromatics (PNA) such as BaP (a diesel exhaust component) have been proven to be carcinogenic but they are not by themselves mutagens. However, BaP is one of a group of chemicals that are transformed in the body to a form that can be carcinogenic. For example, liver contains enzymes that are very effective at transforming these compounds into mutagenic/carcinogenic compounds. In order for the Ames test to make a truly effective analysis of this class of compounds, the compounds must be converted into the chemically active form. This is done by metabolically "activating" them with compounds such as a liver microsomal extract obtained from ground up rat liver. The samples are therefore tested for Ames response:

a. without metabolic activation - i.e., directly

b. with metabolic activation

4. Application of the Ames Test to Diesel Vehicles

Most of the organic diesel exhaust products that are Ames reactive condense on the exhaust solid particulates. The diluted cooled exhaust is filtered to trap these particulates and then the organic fraction is chemically extracted. This extract is then tested for Ames response, both with and without activation. The following Salmonella tester strains have proven useful for diesel studies.

a. TA 98 is a frame shift detection strain; the particulate extract from diesel vehicles usually gives a positive response to this tester strain.

b. TA 100 is a point mutation detection strain; the particulate extract from diesel vehicles usually gives a strong positive response to this strain.

c. TA 1535 is a point mutation detection strain; the particulate extract from diesel vehicles usually gives a negative response to this tester strain. It therefore serves as both a control and detector of unusual activity.

d. TA 1537 is a frame shift detection strain; the particulate extract from diesel vehicles usually gives a positive response to this tester strain.

e. TA 1538 is a frame shift detection strain; the particulate extract from diesel vehicles usually gives a positive response to this tester strain.

5. Ames Results - presentation of data

The Ames test result is a dose/response result. Each tester strain is tested at several logarithmically spaced dose levels. The response (number of revertants), is a measure of the mutagenic potential of the compound. The results are corrected for spontaneous revertants and then expressed by a dose/response relationship as the slope of the curve.

A major problem with the Ames test is toxicity. As the dose is increased, the potential of the bioassay systems to produce revertants is hampered by a concurrent increase in toxicity. Therefore, there is typically a dose level at which the results are not meaningful. To account for these difficulties, various techniques are used to screen the data and to present the results. Two methods of data presentation were employed here since the modeling/methods of presentation are still being developed.

The linear regression model expresses the results as a slope -- revertants per microgram of diluted extract. The other model, the curve fitting model, corrects the results for the rate at which the Ames assay becomes toxic. The results are again expressed as a slope -- revertants per microgram of diluted extract. Therefore, because of this correction factor, the mutagenic rate predicted by the curve fitting model is higher than that for the linear regression model.

Since the methods of presentation of data were still being developed, the results of the diesel artifact testing are expressed by both methods. The results of the individual tests are also summarized as an average dose response relationship.

6. Handling and storage of particulate filters.

In the absence of completed studies on the handling, storage, and shipping of diesel particulate samples for chemical and biological analysis, the following procedures were developed with the help of ORD to preserve the sample integrity;

a. Polyethylene disposable gloves were worn by the technicians handling the filters. This protected the technician from contact with the filter particulates. The gloves also protected the clean and particulate laden filters from biological contamination by the technicians.

b. The loaded filters were handled in the dark or under yellow light (Eastman Kodak Kodachrome Yellow II filter) to minimize the possibility of ultraviolet (UV) light altering this biological sample. BaP, a typical particulate bound component of diesel exhaust, is altered by UV light.

c. The loaded filters were double folded to prevent sample loss in handling.

d. The folded, loaded filters were placed in a glassine envelope and then in a manila envelope. The manila envelope was placed in a Ziplock plastic bag which was sealed inside a polyethylene plastic bag.

e. The sealed, loaded filters were stored in the dark at -30° C (-22°F) to preserve the biological sample.

f. For shipment the samples were packed with dry ice in an insulated shipping container. The samples were sent to EPA at Research Triangle Park (RTP) for chemical extraction and Ames testing.

APPENDIX B

Physical Properties of the Combustion Gases*

Gas	Density gm/l	Molecular Weight	Specific heat ** Cp	Specific heat ratio Cp/Cv	Thermal Capacity ***	Viscosity Poises x 10^6
Argon	1.78	39.94	.12	1.67	.22	224
Carbon Dioxide	1.98	44.00	.20	1.30	.39	146
Helium	.18	4.00	1.25	1.66	.22	197
Nitrogen	1.25	28.01	.24	1.40	.30	171
Oxygen	1.43	32.00	.22	1.40	.31	196
Air	1.29	28.95	.24	1.40	.31	181
Nitrogen-free "air"	1.31	29.27	.49	1.48	.30	183

* Properties of the nitrogen-free artificial "air" were calculated by ssuming that they are the percentage weighted sums of the properties of the individual gases. These are based on a blend of 15% argon, 35% carbon dioxide, 29% helium, and 21% oxygen by volume.

** Cp is gram-cal per gram (Btu per pound) at constant pressure.

***Thermal capacity is the density x Cp.

APPENDIX C

Test Engine Description

3000 Watt Onan Diesel Generator Series DJA

Engine

mfg	Onan
model	DJA
type	4 cycle diesel, single cylinder overhead valve
bore and stroke	3.25 x 3.625 in/82.6 x 92.0 mm
displacement	30 CID/491.6 cc
compression ratio	19 : 1
maximum continuous power @rpm	5.7 horsepower/4.25 kW @1800 rpm
combustion chamber	precombustion chamber in head
governor	gear driven, mechanical flyball
governed speed	1800 rpm, stable within $\pm$.3%
cooling	aircooled by centrifugal flywheel blower
fuel system	American Bosch injection pump with pintle injection nozzle
fuel	Diesel no. 2, tested with Diesel no. 2 and decane
starting aids	intake air preheater, glow plug, and a decompression solenoid

Generator

mfg	Onan
type	revolving armature, 4 pole, self excited, mounted to engine shaft
volts/amps	120/240 volts 25/12.5 amps
phase	1
power	3000 Watts
frequency	60 Hertz
frequency regulation	3 Hertz no load to full load
cooling	direct drive, centrifugal blower

APPENDIX D

Artificial Air Analysis by Gas Chromatography Mass Spectrometer

Bottle No.	Nominal Blend[1] gas percentages	Analysis				
		Nitrogen	Carbon Dioxide	Oxygen	Argon	Helium[3]
1	0, 35, 21, 15, 29	ND(2)	35.4%	16.9%	14.6%	NA
2	0, 35, 21, 15, 29	ND	32.9%	21%	12.6%	NA
3	0, 35, 21, 15, 29	ND	34.8%	23.3%	13.5%	NA
4	0, 35, 21, 15, 29	ND	38.8%	16.5%	13.5%	NA
5	0, 30, 21, 20, 29	ND	28.4%	19.3%	17.6%	NA

(1) Percentages of nitrogen, carbon dioxide, oxygen, argon, and helium respectively.

(2) ND - not detectable, below minimal detectable level of 100 ppm nitrogen.

(3) Helium content was not measured as this gas is used as the carrier gas in the analysis.

APPENDIX E

Fuel Analysis for Trace Nitrogen by Gas Chromatography with Thermal Conductivity

No. 2 diesel Fuel	ND	(duplicate analysis)
Decane	ND	(duplicate analysis)

ND - Not detectable below minimal detectable level of 30 ppm nitrogen. Blank samples also had no nitrogen response.

Note: Analysis by gas chromatograph analysis with thermal conductivity. Procedure used combustion with a hot catalyst to eliminate oxygen present and break up NOx.

APPENDIX F

Operating Test Conditions Measured and Controlled

CVS	dilute exhaust volume, time
Emissions	HC, CO, CO_2, and NOx with standard gas analysis system HC by HFID (Heated Flame Ionization Detector) for total hydrocarbons NO with standard gas analysis system in bypass mode NO directly with second derrivative spectrophotometer
Generator	amperage, frequency, voltage
Filter weights	bulk stream filter before and after test 47mm filter before and after test
47mm particulate sampling unit	volume through 47mm filter
Pressures	barometric, pressure drop across bulk stream filter
Temperatures	ambient dry bulb ambient wet bulb artificial air at engine inlet dilute exhaust temperature exhaust gas temperature (EGT) 47mm sampling system at filter probe and gas flow meter HFID at sample probe, inline filter, sample line, and oven
Time	sample time, total engine operating time

APPENDIX G

Test Filter Extraction Solubles

Ames[1] Sample No. TAEB-79-	Solubles[2] Extracted	Extraction[3] Efficiency	Bap[4] Extracted
Diesel Fuel No. 2 - Normal Air			
0001	.458 gm	30%	-
0002	.275 gm	22%	-
0003	.544 gm	39%	-
0004	.476 gm	36%	-
0005	.336 gm	-	-
Decane - Normal Air			
0012	.972 gm	-	2.35 µgm
0013	.481 gm	-	2.03 µgm
0014	.682 gm	-	1.52 µgm
0015	.800 gm	-	1.31 µgm
0016	.375 gm	-	4.71 µgm
Diesel Fuel No. 2 - Artificial Air			
0047	.507 gm	44%(5)	1.31 µgm
0048	.405 gm	53%(5)	.92 µgm
0049	.770 gm	61%(5)	1.39 µgm
0050	.208 gm	59%(5)	.53 µgm
Decane - Artificial Air			
0059	.139 gm	28%	12.66 µgm
0060	.221 gm	29%	22.06 µgm
0061	.221 gm	30%	13.76 µgm
0062	.157 gm	31%	11.03 µgm
0063	.227 gm	30%	11.00 µgm
0064	.275 gm	32%	22.96 µgm
0065	.250 gm	30%	18.43 µgm
0066	.244 gm	29%	16.58 µgm
0067	.256 gm	29%	12.16 µgm

(1) All four digit numbers in first column have the prefix TAEB-79-.

(2) Methylene chloride soluble organics extracted from large particulate filter (8X10 inch or 20X20 inch).

(3) Soluble organics extracted as a percentage of the total particulate loading.

(4) Amount of Bap contained in soluble organics.

(5) Relatively high extraction efficiency due to oil residue in the sample.

APPENDIX H

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

DATE: September 12, 1980

SUBJECT: ORD's Analysis of Ames Test Results for the Artifact Samples

FROM: Karl H. Hellman, Chief, CTAB KHH

TO: Ralph Stahman, Chief, TEB

In my memo of 6 June 1980 entitled "Preliminary Ames Test Results for TAEB Artifact Samples", I explained the situation with ORD concerning the analysis of Ames test data. At that time, ORD had not fully developed their computer programs for analyzing Ames data, so we analyzed the data ourselves. Since my memo, ORD has completed its development of the data analysis program for the Ames test and has reduced the data from the artifact experiments. These data are presented in this memo.

The analyzed artifact data represents two types of reduction methodologies. One method is based on a linear regression model. This is the basic method we used in our analysis of the artifact data (our memo is attached). However, the ORD method for linear regression analysis is different in at least one way; that they used the net number of revertants (number of revertants at a given dose minus the spontaneous revertants) while we used the total number of revertants (number of revertants at a given dose). There may well be other differences between our method of analysis and ORD's of which we are currently unaware. When we receive a written copy of ORD's present linear regression method (which is currently being written) a more complete list of differences will be presented to you.

The data presented in Table 1 has been analyzed by ORD's linear regression method. The data from Table 1 is summarized in Table 2.

After analyzing the data in Table 1, Larry Claxton concludes that within the four major divisions of this experiment, (Diesel Fuel (DF) 2 - Normal Air, Decane - Normal Air, DF2- Artificial Air, and Decane - Artificial Air), there are no significant differences when comparing like samples, i.e. samples with activation to samples with activation, samples without activation to samples without activation. The only exception is sample TAEB-79-0012. Larry contends that the results for samples TAEB-79-0013 through 0016 are in good agreement with one another and that the results for 0012 is an anomaly. Because of this observation, I did not include TAEB-79-0012 in the average of the Decane-Normal Air experiment.

Another observation which Larry notes is the difference between samples with activation and without activation in the Decane-Artificial Air experiment. In this experiment, the samples with activation gave con-

sistently higher revertants per plate per ug extract values than those without activation in all the strains which gave positive results. This trend can also be seen in the DF2-Artificial Air experiment, but to a lesser degree. Since the results from samples tested without activation are indicative of the presence or absence of direct acting mutagens, and since the trend of higher values for samples with activation versus without is seen only in Artificial Air experiments and not Normal Air experiments, one might assume a decrease in direct acting mutagens due to the lack of nitrogen in the Artificial Air. The decrease in direct acting mutagens can be seen if one uses Table 2 to compare the results of strain TA100 for all four experiments, and strain TA98 for the Decane-Artificial Air versus the Decane-Normal Air experiment. In each case, the samples produced with Artifical Air and tested without activation are less than those samples produced with Normal Air and tested without activation. With the Ames test there are large variations in results and these differences may fall within acceptable limits of variation, but there is a consistent trend of lower values for samples produced with Artificial Air and tested without activation.

The results shown in Table 3 are the data analyzed by Larry Claxton's and ORD's model based on curve fitting rather than linear regression. Attached is a draft report entitled "Modelling the Ames Test" which explains the basis for the curve fitting mode. This draft is being revised and we will pass on a copy of the revised draft as soon as we receive one.

The curve fitting data from Table 3 show the same general trend which is mentioned above for the linear regression mode. Table 3 does not contain any averaged values since numerous samples were considered statistically unacceptable for within-experiment comparison by Larry Claxton. The reasons for the unacceptable sample data are listed at the bottom of Table 3. I have also included Larry Claxton's summary sheets as an attachment.

Attachments

NOTE TABLE 1 is APPENDIX I, Page 28
TABLE 2 is APPENDIX J, Page 29
Table 3 is APPENDIX K, Page 30

APPENDIX I

Ames Test Bioactivity (linear regression model)

Slopes (revertants per plate/ug extract)

with activation/without activation

Sample No.* TAEB-79-	TA100	TA1535	TA1537	TA1538	TA98
DF2-Normal Air					
0001	.51/.82	-/-°	.16/.09	.39/.22	.61/.42
0002	.44/.59			.29/.17	
0003	.46/.56			.35/.22	
0004	/.71			.35/.20	
Avg.	.47/.67			.34/.20	
Decane-Normal Air					
0012	-/-	-/-	-/-	.12/.06	.14/.05
0013	.32/.42	-/-	.07/.04	.21/.15	.26/.18
0014	.45/1.14	-/-	.09/.07	.30/.16	.32/.22
0015	.59/1.29	-/-	.11/.11	.44/.22	.50/.48
0016	.62/.81	-/-	.17/.14	.54/.31	.67/.46
Avg.	.50/.92	-/-	.11/.09	.37/.21	.44/.34
DF2-Artificial Air					
0047	.70/.61	.03(?)/.03(?)	.26/.13	.84/.54	1.69/.70
0048	.43/.22	-/-	.10/.03	.30/.15	.45/.12
0049	.30/.18	-/-	.09/.01	.13/.12	.26/.06
0050	.16/.14	-/-	.04/.01	.20/.07	.19/.07
Avg.	.40/.29	-/-	.12/.04	.37/.22	.65/.24
Decane-Artifical Air					
0591	1.06/.16	-/-	.64/.03	.20/.05	.24/.07
0601	.61/.20	-/-	.20/.03	.27/.08	.30/.09
0621	.27/.07	-/-	.13/.09	.18/.05	.25/.08
Avg.	.65/.14	-/-	.32/.05	.22/.06	.26/.08

* All 4 digit numbers in first column have the prefix TAEB-79-

° -Indicates a negative result

APPENDIX J

Summary of Table 1

Averaged Slopes (revertants per plate/ug extract)

with activation/without activation

	TA100	TA1535	TA1537	TA1538	TA98
DF2-Normal Air	.47/.67			.34/.20	
Decane-Normal Air	.50/.92	-/-	.11/.09	.37/.21	.44/.34
DF2-Artificial Air	.40/.29	-/-	.12/.04	.37/.22	.65/.24
Decane-Artificial Air	.65/.14	-/-	.32/.05	.22/.06	.26/.08

APPENDIX K

Ames Test Bioactivity (Curve fitting model)

Slope (revertants per plate/ug extract)

with activation/without activation

TAEB-79- DF2-Normal Air	TA100	TA1535	TA1537	TA1538	TA98
0001	1.20/2.58	-/-	0.27/0.26	1.21/0.56	1.02[b]/1.09[b]
0002	.85[a]/2.78[a]			3.19[a]/0.29[a]	
0003	1.32[a]/1.40[a]			1.85[a]/1.27[a]	
0004	1.27[a]/1.63[a]			1.30[a]/0.43[a]	
Decane-Normal Air					
0012	0.30[+b]/-	-/-	[+e]/-	0.12/0.06	0.29/0.27
0013	1.07/1.30	-/-	0.16/0.22	0.41/0.46	0.26[c]/0.47[b]
0014	1.49/2.64	-/-	0.26/0.38	0.90/0.49	0.67/0.56
0015	1.49/3.38	-/-	0.19/0.13	1.33/0.51	0.89/107[c]
0016	2.25/2.94[c]	-/-	0.42/0.23	1.30/0.71	1.40/1.41
DF2-Artificial Air					
0047	+[d]/+[d]	0.4/.08	.66/.14	2.06/1.18	+[d]/+[d]
0048	+[e]/+	-/-	.21/.06	.70/.26	1.20/.24
0049	+[d]/+[d]	-/-	.20/.02	.45/.15	.57/.12
0050	.40[b]/.35	.17[g]/-	.04[g]/.02[g]	.04/.07[g]	.19/.07
Decane-Artificial Air					
0591	2.25[h]/.16[b]	-/-	1.47/.03[g]	1.28/0.11[g]	1.28/0.22[g]
0601	1.71/.43	-/-	1.54/.03[g]	1.40[g]/0.08[h]	1.41/0.27[g]
0621	+[d]/.08[g]	-/-	.96/.51	0.50/0.06[g]	1.88/0.22

a - only 4 dose levels and the degrees for freedom for the adequacy test is zero

b - low p valve for adequacy of fit, results not comparable to other samples

c - data does not fit model adequately

d - samples are positive, slope unattainable for statistical reasons

e - questionable positive

f - chi-square of Pousson low, result not comparable to other samples

g - low response when confidence limits considered

h - low p valve for mode.

APPENDIX L

Diesel Artifact Gaseous and Particulate Emissions at 60% Load

Ames Sample No.(1) TAEB-79-	Test No.	Emissions gms/kW-hr HFID	CO	CO2	NOx	NOx ppm	Particulates(2) gms/kw-hr	Particulate filter efficiency	Fuel consumption kW-hr/gal(3)
Diesel Fuel No. 2 - Normal Air (4)									
0001	79-6855	.65	3.19	1508	10.94	66.4	14.1	99%	6.7
	"	.50	3.27	1499	10.67	64.2	-	-	6.8
	"	.52	3.33	1552	11.11	67.1	-	-	6.5
0002	79-6856	.65	3.27	1631	13.48	68.9	28.3	93%	6.2
	"	.64	3.47	1617	12.24	62.6	-	-	6.3
	"	.60	3.09	1562	11.63	59.1	-	-	6.5
0003	79-6857	.33	2.23	1589	12.39	71.9	8.3	97%	6.4
	"	.56	2.45	1493	12.37	71.6	-	-	6.8
	"	.44	2.41	1484	12.66	73.4	-	-	6.8
0004	79-6858	.51	2.18	1337	11.84	67.7	7.9	91%	7.6
	"	.63	2.39	1367	12.67	72.5	-	-	7.4
	"	.46	2.36	1374	12.40	70.1	-	-	7.4
0005	79-6859	.45	2.48	1356	11.85	69.7	12.1	91%	7.5
	"	.47	2.30	1443	12.44	73.1	-	-	7.0
	"	.46	2.21	1425	12.59	74.0	-	-	7.1
Decane-Normal Air (5)									
0012	79-6493	1.25	3.24	1313	7.89	46.7	3.3	97%	7.7
	"	1.44	3.98	1356	8.29	49.0	-	-	7.5
	"	2.05	4.14	1428	8.96	52.9	-	-	7.1
0013	79-7457	-	3.63	1314	7.99	47.8	3.3	94%	7.7
	"	-	3.52	1360	8.23	49.1	-	-	7.4
0014	79-7465	-	3.08	1315	7.91	45.9	2.1	91%	7.7
	"	-	3.33	1399	8.27	48.0	-	-	7.2
0015	79-7458	-	3.17	1290	8.02	50.9	1.8	91%	7.8
	"	-	2.75	1357	8.51	54.7	-	-	7.5
0016	79-7464	.65	3.27	1308	7.98	50.9	2.2	95%	7.7
	"	.71	2.67	1212	7.45	47.4	-	-	8.4

Ames Sample No.(1) TAEB-79-	Test No.	Emissions gms/kW-hr HFID	CO	CO2	NOx	NOx ppm	Particulates(2) gms/kw-hr	Particulate filter efficiency	Fuel consumption kW-hr/gal(3)
Diesel Fuel No.-2 Artifical "Air" (5)									
0047	79-9399	.67	3.29	(6)	.21	1.1	1.4	82%	(6)
0048	"	.06	3.29	(6)	.19	1.0	-	-	(6)
0049& 0050	"	.06	3.20	(6)	.20	1.1	-	-	(6)
Decane - Artificial "Air" (4)									
0059	79-7757	1.71	19.57	(6)	.07	.4	2.8	88%	(6)
thru 0067	79-9401	-	19.56	(6)	.05	.3	-	-	(6)

(1) All four digit numbers in first column have the prefix TAEB-79-. Ames samples were taken using either an 8X10 inch filter or 20X20 inch filter.

(2) Particulate emission rate and efficiency were obtained using a 47mm filter. These filter samples were taken at the same time as the gaseous samples. However only one particulate sample was obtained for each group since the time required to obtain a sufficient loading on the 47mm filter was considerably longer than the time required to obtain a gaseous sample.

(3) Fuel consumption was calculated by the carbon balance technique. Fuel consumption of decane is expressed as the equivalent quanity of diesel fuel No. 2.

(4) Nine 8 X 10 inch filters were used in sequence for each Ames sample. Gaseous samples were obtained before and after the Ames samples.

(5) One 20 X 20 inch filter was taken for the Ames sample. Gaseous samples were obtained before and after the Ames sample.

(6) Carbon Dioxide and fuel economy were not calculated for the artificial "air" sample since the artificial "air" contained approximately 35% CO_2 and, therefore, the dilute exhaust CO_2 levels were outside the range of the highest instrument calibrations normally used. Also the exact CO_2 concentration of each bottle of the blended "air" was unknown. Thus, fuel consumption could not be calculated by the carbon balance technique.