LIBRARY - AIR RESOURCES BOARD

CONTRACT NUMBER A5-130-33

FINAL REPORT

GENOTOXICITY OF DIESEL EXHAUST PARTICLES AND VAPORS COLLECTED FROM ENGINES WITH AND WITHOUT PARTICULATE TRAP OXIDIZERS

REGENTS OF THE UNIVERSITY OF CALIFORNIA

UNIVERSITY OF CALIFORNIA, IRVINE

JULY 31, 1988

PREPARED FOR CALIFORNIA AIR RESOURCES BOARD

BY

RONALD E. RASMUSSEN, PH.D., PRINCIPAL INVESTIGATOR

RA
577
A9
R377
1988

CALIFORNIA AIR RESOURCES BOARD CONTRACT NUMBER A5-130-33

GENOTOXICITY OF DIESEL EXHAUST PARTICLES AND VAPORS COLLECTED FROM ENGINES WITH AND WITHOUT PARTICULATE TRAP OXIDIZERS

PRINCIPAL INVESTIGATOR:

RONALD E. RASMUSSEN, PH.D.
ASSOCIATE ADJUNCT PROFESSOR
COMMUNITY AND ENVIRONMENTAL MEDICINE
UNIVERSITY OF CALIFORNIA, IRVINE

RA
577
A9
R377
1988

Rasumssen, Ronald E.
Genotoxicity of diesel exhaust particles and vapors collected from engines with and without particle trap oxidizers

GENOTOXICITY OF DIESEL EXHAUST PARTICLES AND VAPORS COLLECTED FROM ENGINES WITH AND WITHOUT PARTICULATE TRAP OXIDIZERS

ABSTRACT

Diesel emissions are known to contain mutagenic and carcinogenic chemicals. Reduction of diesel emissions may be accomplished through installation of various kinds of catalytic traps or oxidizers in the exhaust train. This project examined two relevant aspects of this problem. The first objective was to determine whether, and to what extent such traps may reduce the emission of mutagenic chemicals into the environment. The second objective was to examine the effect of fuel formulation on mutagenic activity associated with the particulate phase of diesel exhaust. In the studies of the effectiveness of particulate trap oxidizers, exhaust particles were collected from diesel automobiles, with and without traps, at the Southwest Research Institute (SwRI) at San Antonio, TX. Mutagenesis tests were conducted using the Ames Salmonella bacterial test system on extracts of the particles. The mutation rate was calculated in terms of mutants per mile of travel during a set of standard test cycles. The results indicated a two to twenty-fold reduction in particle-associated mutagenic activity present in the exhaust, which could be attributed to a reduction by the traps of particulate emission. There was no change in the specific mutagenic activity (revertants/microgram of soluble organic fraction, SOF) of the material extracted from particles collected with or without the oxidizer traps. Since most of the mutagenic and carcinogenic activity in diesel exhaust has been shown to be associated with the particulate phase, these studies indicate that installation of exhaust traps on diesel powered vehicles may substantially reduce the emission of carcinogenic materials into the environment. For the studies of the effect of fuel formulation on emission of mutagenic material by diesel engines, particles were collected from a heavy-duty engine (Cummins NTCC 400) on a test stand at the SwRI. Fuel variables were percent aromatic content, weight-percent sulfur, and 90% boiling point. The results indicated a positive correlation between the aromatic content of the fuel and the emission of particle-associated mutagenic material, calculated as mutants/hp-hr, but no such association between either sulfur content or 90% boiling point of the fuels tested. A limited number of samples were also collected from the automobiles while burning high or low aromatic fuels but the data were not sufficient to draw any conclusions.

ACKNOWLEDGEMENTS

Dr. Lawrence R. Smith of the Southwest Research Institute (SwRI) provided the particle samples collected from the exhaust of a Mercedes Benz and a Volkwagen automobile. That work was performed under ARB contract #A5-159-32, "Characterization of Diesel Exhaust Emissions from Trap-Equipped Light-Duty Diesels".

Terry Ullman of SwRI provided the toluene/ethanol extracts of exhaust particles collected as part of the CRC VE-1 project. This project was supported in part by ARB Contract A4-132-32, "Emissions Evaluation of Two Heavy-Duty Diesel Engines on Two Low-Sulfur Fuels with Variation in Aromatics".

Principal technician at UC Irvine was Gary Devillez who performed most of the particle extractions and mutagenesis tests of the extracts.

Contract Manager for the California Air Resources Board was Elizabeth Ota.

This report was submitted in fulfillment of ARB Contract Number A5-130-33, "Genotoxicity of Diesel Exhaust Particles and Vapors Collected from Engines With and Without Particulate Trap Oxidizers", by the Regents of the University of California, University of California, Irvine, under the sponsorship of the California Air Resources Board. Work was completed as of June 1, 1988.

"The statements and conclusions in this report are those of the contractor and not necessarily those of the California Air Resources Board. The mention of commercial products, their source, or their use in connection with material reported herein is not to be construed as either an actual or implied endorsement of such products."

TABLE OF CONTENTS

PAGE

ABSTRACT.....3

ACKNOWLEDGEMENTS.....4

LIST OF TABLES.....6

PROJECT SUMMARY AND CONCLUSIONS.....9

RECOMMENDATIONS.....12

INTRODUCTION AND BACKGROUND.....14

EXPERIMENTAL.....16

Vehicles and Fuels.....16

Collection of Exhaust Particles and Vapor.....17

Collection and Handling of Samples from CRC VE-1 Project...18

Extraction of Filters.....21

Mutagenesis testing with the Ames Salmonella System.....22

Data Analysis.....24

RESULTS.....26

Preliminary Studies.....26

Assay of Material Collected from Mercedes Benz Diesel.....33

Assay of Material Collected from Volkswagen Diesel.....41

Assay of CRC Project VE-1 Samples.....62

DISCUSSION AND CONCLUSIONS.....62

REFERENCES.....73

APPENDIX: STUDIES WITH DIESEL BUS.....77

LIST OF TABLES

TABLE		PAGE
1.	Fuel Formulations Used in CRC Project VE-1	19
2.	Soluble Organic Fractions (SOF) of Exhaust Particles Collected During Fuel Tests with Cummins NTCC400. (CRC Project VE-1)	20
3.	Mutagenic Activity of a DCM Extract of Heavy Duty Diesel Exhaust Particles as Determined by the Standard Ames Test	27
4.	Mutagenic Activity of a DCM Extract of Heavy-Duty Diesel Exhaust Particles as Determined by the Micro Ames Test	29
5.	Mutagenic Activity of a DCM Extract of SRM-1650 Reference Diesel Exhaust Particles Determined by the Standard Ames Test	30
6.	Mutagenic Activity of Aromatic and Aliphatic Subfractions of a DCM Extract of SRM 1650 Reference Diesel Exhaust Particles as Determined by the Standard Ames Test	31
7.	Mutagenic Activity of a DCM Extract of SRM-1650 Reference Diesel Exhaust Particles as Determined by the Micro Ames Test	31
8.	Mutagenic Activity of HPLC Subfractions of the Aromatic Fraction of a DCM Extract of SRM-1650 Reference Diesel Exhaust Particles	32
9.	Extraction Dates, Test Dates and Particle Yields for Mercedes Benz Diesel Automobile	34
10.	Yields of Extractable Material from Particles Collected from Mercedes Benz Automobile	35
11.	Percent Reduction of Particle Emission by Mercedes Benz Oxidizer Trap	35
12.	Positive Mutagenesis Controls for Tests of Extracts of Mercedes Benz Exhaust Particles	36
13.	Negative Controls for Mutagenesis Tests of Extracts of Mercedes Benz Exhaust Particles	36
14.	Mutagenic Activity in DCM Extracts of Exhaust Particles Collected from Mercedes Benz Auto With an Oxidizer Trap Expressed as Revertants per Microgram of Extracted Material	37

15. Mutagenic Activity in DCM Extracts of Exhaust Particles Collected from a Mercedes Benz Auto Without an Oxidizer Trap Expressed as Revertants per Microgram of Extracted Material.....38

16. Mutagenic Activity in DCM Extracts of Exhaust Particles Collected from a Mercedes Benz Auto With and Without an Oxidizer Trap Expressed as Revertants per Mile of Travel...39

17. Percent Reduction by Mercedes Benz Oxidizer Trap of the Emission of Particle-Associated Mutagenic Material.....40

18. Extraction Dates, Test Dates and Particle Yields for Volkswagen Diesel Automobile.....42

19. Yields of Extractable Material from Volkswagen Diesel Exhaust Particles.....43

20. Percent Reduction by Oxidizer Trap of Emission of Exhaust Particles by Volkswagen Auto.....43

21. Positive Controls for Mutagenesis Tests of DCM Extracts of Volkswagen Diesel Exhaust Particles.....44

22. Negative Controls for Mutagenesis Tests of DCM Extracts of Volkswagen Diesel Exhaust Particles.....44

23. Mutagenic Activity of DCM Extracts of Volkswagen Diesel Exhaust Particles Expressed as Revertants per Microgram of Extracted Material.....45

24. Mutagenic Activity of DCM Extracts of Volkswagen Diesel Exhaust Particles Expressed as Revertants per Mile of Travel.....46

25. Percent Reduction by Volkswagen Oxidizer Trap of Emission of Particle-Associated Mutagenic Activity.....47

26. Particle Collection and Extraction Data for Mercedes Benz Loaded Trap and Regeneration Tests.....50

27. Particle Collection and Extraction Data for Volkswagen Loaded Trap and Regeneration Tests.....51

28. Mutagenic Activity of a DCM Extract of Exhaust Particles Collected from a Mercedes Benz Auto Operating with a Loaded Oxidizer Trap.....52

29. Mutagenic Activity of DCM Extracts of Exhaust Particles Collected from a Mercedes Benz Auto During Oxidizer Trap Regeneration.....53

30. Mutagenic Activity of DCM Extracts of Exhaust Particles Collected from a Mercedes Benz Auto During Baseline Tests With Low Aromatic Fuel.....54

31. Mutagenic Activity of a DCM Extract of Diesel Exhaust Particles Collected from a Volkswagen Auto Operating With a Loaded Oxidizer Trap.....56

32. Mutagenic Activity of DCM Extracts of Diesel Exhaust Particles Collected from a Volkswagen Auto During Oxidizer Trap Regeneration.....57

33. Mutagenic Activity of DCM Extracts of Diesel Exhaust Particles Collected from a Volkswagen Auto Equipped With an Oxidizer Trap During Baseline Tests with Low Aromatic Fuel.....58

34. Mutagenic Activity of DCM Extracts of Diesel Exhaust Particles Collected from a Volkswagen Auto Without An Oxidizer Trap During Baseline Tests with Low Aromatic Fuel.....60

35. Mutagenic Activity of SOF of Diesel Exhaust Particles Collected During Hot-Start Transient Portion of CRC Project VE-1 Expressed as Revertants per Microgram of Extracted Material.....63

36. Mutagenic Activity of SOF of Diesel Exhaust Particles Collected During Hot-Start Transient Portion of CRC Project VE-1 Expressed as Revertants per Hp-Hr.....65

37. Mutagenic Activity of SOF of Diesel Exhaust Particles Collected During Cold-Start Transient and Staedy-State (Rated Speed, 25% Load) Portions of CRC Project VE-1 Expressed as Revertants per Microgram of Extract.....66

38. Mutagenic Activity of SOF of Diesel Exhaust Particles Collected During Cold-Start Transient and Steady-State (Rated Speed, 25% Load) Portions of CRC Project VE-1 Expressed as Revertants per Hp-Hr.....68

39. Regression Coefficients (Slopes) of the Relationships Between Fuel Characteristics and Mutagenic Activity of Exhaust Particle Extracts.....69

PROJECT SUMMARY AND CONCLUSIONS

Diesel exhaust particles are acknowledged carriers of mutagenic and carcinogenic chemicals. Their submicron size range allows them to be inhaled into the deep lung, and there is concern that some level of risk for lung cancer or other lung disease may result from the presently uncontrolled emission of these particles from vehicles and stationary sources in the urban environment. Reduction of the emission of these particles might be accomplished by the use of oxidizer traps in the exhaust stream of the diesel engines. However, practical traps are presently available only on some light duty automobile engines, which actually represent a small minority of diesel-powered vehicles. The decision to design and manufacture large capacity oxidizer traps, and then to equip all vehicles with such traps would entail a formidable investment of resources, especially for governmental agencies which are responsible for thousands of trucks and busses. Therefore, before taking such a decision, it is important to have some measure of the expected health benefit to be obtained. Epidemiologic studies of workers exposed to diesel exhaust have yielded equivocal results regarding an association between exposure and lung diseases, including cancer, however, the power of these studies is weakened by confounding factors such as smoking. The present studies have attempted a more objective approach by directly measuring the biological activity, as mutagenic potential, associated with the diesel particles collected with and without exhaust particle oxidizers. In this way it was hoped that a more useful estimate of the benefit of diesel exhaust traps could be obtained.

To study the effect of particulate trap oxidizers on exhaust emissions, two diesel automobiles, a Mercedes Benz and a Volkwagen, were run on a dynamometer at the Southwest Research Institute at San Antonio, TX, and exhaust particles were collected on Pallflex teflon-coated glass fiber filters. Vapor phase material was not collected from the automobiles for mutagenesis testing. The Mercedes Benz automobile was equipped with the manufacturer's porous silver alloy catalyzed ceramic trap which operates somewhat in the nature of an "afterburner". Particles from the engine are collected by the trap which allows the vapor phase of the exhaust to pass through. When the trap has reached the combustion temperature of the collected particles they are oxidized, and the resulting, presumably less hazardous, gases are released. The Volkwagen trap, also provided by the manufacturer, is basically similar, except that a proprietary metal catalyst mixture

is added to the fuel mixture just before combustion. The metal catalysts lower the combustion temperature of the particles collected by the trap and therefore, more effective oxidation of the particles is obtained.

The collected particles and filters were transferred to UC Irvine where they were Soxhlet-extracted with dichloromethane (DCM) to recover soluble organic compounds which include the bulk of the mutagens and carcinogens. The extracts were quantified by gravimetric methods, and tested for mutagenic activity using the Ames Salmonella typhimurium bacterial test system.

With the Mercedes Benz, the presence of the manufacturer's trap reduced the total emission of carbon particles and extractables by about 80-90%, depending on the test cycle. When the auto was intentionally operated in a manner that would overload the trap with particulate material, and not permit regeneration (i.e., operation on the New York City driving cycle) the emission of particulate material was increased by about 172% over that observed when the trap was operating normally. Also, during trap regeneration at highway speed (i.e., highway fuel economy, HFET, cycle), the emission of particulate material was subtantially increased by about 430% over the normal particulate level. However, the number of mutants per mg of extractable material was not significantly different between particles collected with and without the trap. Specific mutagenicity, calculated as mutants/mile traveled, was also substantially reduced in the presence of the trap by up to 95%, depending on the particular test cycle and the bacterial strain tested. Therefore, the reduction in emission of mutagenic material can be attributed to a reduction in the mass of particles emitted. No significant differences were seen in particle emission or mutagenic activity when burning high (36.2%) or low (16.2%) aromatic fuels.

The mutagenic activity in the exhaust of the Volkswagen diesel was similar to that of the Mercedes, and was also reduced when the trap was in operation, to about the same extent, i.e., by 80-90%. With a loaded trap on the NYCC cycle, particulate emission was increased by 411% over the level with a normally operating trap. Particulate emission was also increased during trap regeneration at highway speed, to the extent of 357% over normal. However, there was a difference between the vehicles which may be of significance. The Mercedes trap appeared to decrease the output of carbon particles to about the same extent as the mutagenic activity, based on Kodak gray scale comparisons of the color of the filters before extraction.

However, the Volkswagen trap appeared to eliminate carbon emission almost entirely, while mutagenic activity was still present. This observation suggests that noncarbon particles which may act as carriers for mutagenic compounds are emitted by the Volkswagen when the exhaust trap is in operation. The nature of these particles was not investigated. There were only small differences between particle emission observed when burning high (36.2%) or low (16.2%) aromatic content fuels. When burning low aromatic fuel without the trap, particle emission was 44% lower during the cold-start FTP cycle and 36% lower during the hot-start FTP cycle. With the trap in place, the differences were not significant.

In addition to the use of trap oxidizers, the modification of fuel formulations may be a method to reduce emission of mutagenic compounds by diesel engines. In past work, high aromatic content has been correlated with increased emission of polynuclear hydrocarbons and mutagenic compounds (e.g., Candeli et al., 1982; Lewtas, 1982). In the present study, mutagenic activity was measured in extracts of exhaust particles collected from a heavy-duty engine burning fuels with a range of aromatic content. The extracts were obtained as part of CRC project VE-1, carried out at the SwRI. A Cummins NTCC 400 engine on a test stand was run with 9 specific fuel formulations under specified load conditions. Details of the fuel formulations and test conditions may be found in the Final Report for CARB Contract No. A4-132-32, submitted by the SwRI in April, 1988. Exhaust particles were collected on teflon-coated glass fiber filters (Pallflex), and extracted at the SwRI with a mixture of toluene and ethanol (30:70, v:v). The extracts were shipped to UC Irvine, where Ames mutagenicity testing was done. The results indicated a positive correlation between aromatic content and mutagenic activity expressed as Ames test revertants/hp-hr. No correlation, either positive or negative, was found with fuel sulfur content or 90% boiling point. These results confirm previous reports and indicate that emission of potentially mutagenic compounds by diesel engines can be reduced by lowering the aromatic content of diesel fuels.

The major conclusion from this study is that, in the case of light duty diesel engines in automobiles, either catalytic- or additive-type exhaust traps can substantially reduce the output of particle-associated mutagenic substances by reducing the particulate emission. Although the effectiveness of the traps varied under different driving conditions, the observed reduction was at least 60% and was as great as 95%, calculated on a mutants/mile basis. This finding supports the

position that exhaust traps on light duty diesel engines are a practical way to reduce the emission of potentially hazardous substances.

With respect to fuel formulations, the results with the heavy-duty engine indicated that lowering the aromatic content of the fuel was associated with reduced emission of mutagenic material in the particulate phase of the exhaust. However, the data obtained with the automobiles were inadequate to make any conclusions.

RECOMMENDATIONS

1. Since heavy duty engines continue to comprise the largest source of environmental diesel exhaust pollution, further work should be done to develop and test the efficiency of reliable exhaust traps for vehicles using such engines.

2. The tests of mutagenic activity in the exhaust of diesel automobiles were done on samples obtained with the engines operating under a limited range of conditions, with the engines in good operating order. Further work may be needed to determine the effects of degraded (e.g., inactive or poisoned catalyst) traps, and well-worn engines.

3. The results with the Volkswagen diesel automobile suggest that the exhaust emissions with the trap in place are qualitatively different from those without the trap. Particles collected with the trap in place should be examined further to determine their physical and chemical nature. For example, are these non-carbon particles of a size that can readily be inhaled? Are they soluble or insoluble? Organic or Inorganic?

4. The presence of exhaust traps may lead to formation of new chemical species which will not be indicated by non-specific tests for mutagenic activity of the kind performed in this project. Analyses of the profiles of exhaust hydrocarbons with and without traps are needed, in combination with further tests for mutagenic activity, to determine whether new, more mutagenic, species may be produced by the traps.

5. Should diesel exhaust traps be mandated, it will be necessary to have methods of ensuring proper operation. The development of economical methods of sample

collection for bioassay and chemical analysis should be pursued.

6. In this project, an attempt was made to examine mutagenic activity in that fraction of the "vapor phase" of heavy duty diesel exhaust that can be collected using XAD-2 adsorbent resin after removal of the particulate phase. No mutagenic activity was detected. The vapor phase was not studied in the case of the diesel automobiles. Since the operating conditions of the automobile traps differ from those of the heavy duty trap, it is not possible to predict that no mutagenic activity will be present in the resin-collectable vapor phase of the former. Further work is therefore required to characterize the biological properties of this fraction of the vapor phase of the diesel automobile exhausts, with and without the traps in place.

7. It is known that a substantial portion (up to 40% or more) of the total hydrocarbons in diesel exhaust are not collected as particles or by passing the exhaust through adsorbent resin. Although available evidence suggests that little mutagenic activity is present in this uncollected material, the effect of oxidizer traps on vapor phase chemistry needs to be examined and biological activity studied in appropriate test systems that will permit exposure of mammalian cells and bacteria to the low molecular weight vapor phase.

8. Data presented in this report indicate that fuel formulation can have a significant effect on mutagenic activity present in the particulate phase of heavy-duty diesel exhaust, with high aromatic fuels in general leading to greater emission of mutagenic compounds. Further studies are needed to determine whether this observation applies to light-duty diesels, and whether an optimum fuel formulation can be defined which preserves desired combustion characteristics while minimizing emission of potentially harmful compounds.

INTRODUCTION AND BACKGROUND

In the 1970's the reduced supply of gasoline temporarily stimulated the demand for diesel-powered passenger automobiles. This, in turn, caused concern for the possible health effects of diesel exhaust, if the use of diesel cars became widespread. The 1978 report (Huisingh et al., 1978) of mutagens and carcinogens in diesel particles further increased this concern. Since that time a number of epidemiological studies have produced equivocal findings regarding the health risks to humans of diesel exhaust. Although animal studies have shown induction of lung cancer after high exposures to whole diesel exhaust (See; Ishinishi et al., 1986, for reviews of several studies) human data have shown no strong linkage. However, human data are always contaminated by confounding factors such as smoking. In a recent review McClellan (1987) used the available data to estimate that in the U.S. between 40 and 3,500 excess lung cancer cases per year may have diesel exhaust exposure as a major contributing factor, although the possibility of no effect could not be ruled out. Even though proof of an association between exposure and disease is lacking in humans, the fact of the presence of known carcinogens on an inhalable, insoluble particle (a sine qua non for experimental lung cancer induction by polynuclear aromatic hydrocarbons; see: Saffiotti et al., 1968; Feron et al., 1980; Rasmussen et al., 1984) is considered sufficient cause by many to push for control of diesel emissions.

The success of catalytic converters in reducing the emissions from gasoline-powered vehicles has not been matched by attempts at control of diesel exhaust emissions. The major hurdle has been that diesel engines may produce up to two orders of magnitude more unburned hydrocarbons or products of incomplete combustion (PICs) than do gasoline engines of equivalent power (Lewtas, 1983; McClellan, 1987). Also, the higher combustion temperature of diesels leads to more formation of oxides of nitrogen, which, in turn, leads to more nitrated polycyclic aromatic hydrocarbons (nitro-PAH) in the exhaust stream (Scheutzle, 1983). Under these conditions, catalytic traps of the type used on gasoline-powered vehicles do not have the capacity to control exhaust emissions. The approach taken by most manufacturers has been to provide a method of secondary combustion in the exhaust stream which will trap and burn hydrocarbons and PICs that escape from the engine. Although some success has been achieved with passenger cars, notably by Mercedes Benz and Volkswagen, a practical, efficient trap for heavy duty diesels is not yet

commercially available, and the development of such traps is still in the experimental stage.

In a study of the effect of a non-catalytic ceramic trap on the emission of materials by a heavy-duty diesel engine, Bagley et al. (1987) found a reduction of emission of total particulate materials (TPM), but an increase in the specific mutagenic activity (mutants/microgram) of a DCM extract (soluble organic fraction, SOF) of particles collected post-trap. Overall, the total mutagenic activity in the SOF, when calculated as mutants/kilowatt-hr, was reduced from 25% to 45%, depending on the engine load. These results indicated that an exhaust trap could indeed reduce the emission of mutagenic materials by diesel engines, at least under bench-test conditions.

The present project was concerned with determining the effectiveness of exhaust particle trap oxidizers in reducing the emission of mutagenic chemicals when the traps were installed on operating vehicles. The approach was to collect particles while the vehicles were undergoing dynamometer testing, to extract the particles, and test the extracts in established mutagenesis test systems. The general methods had been established in other laboratories, but required some modification for the present work.

A major part of the project has included analysis of exhaust particles collected from diesel automobiles at the Southwest Research Institute, under the direction of Dr. Lawrence R. Smith. (CARB contract #A5-159-32; "Characterization of Exhaust Emissions from Trap-Equipped Light-Duty Diesels"). A Mercedes Benz and a Volkswagen automobile, equipped with the manufacturers' exhaust traps, have been run under specified conditions and exhaust particles collected with and without the trap in place. The filters bearing the particles were then shipped to UC Irvine for extraction and mutagenicity testing. These studies were carried out as originally planned.

An additional portion of this project involved analysis of extracts of particles collected during bench tests of a Cummins diesel engine at the Southwest Research Institute (CRC Project No. VE-1). These tests were concerned with the influence of fuel formulation on the presence of mutagenic activity in the exhaust particles.

EXPERIMENTAL

Vehicles and Fuels.

The automobile tests were conducted at the Southwest Research Institute, San Antonio, TX, under the direction of Dr. L. R. Smith.

Vehicles:

1986 Mercedes Benz Model 300 SDL equipped with the manufacturer's ceramic trap oxidizer with incorporated silver metal catalyst.

1987 Volkswagen Golf sedan equipped with the manufacturer's ceramic trap oxidizer and using a proprietary metallic catalyst added to the fuel just before combustion.

Baseline high aromatic fuel for the automobiles was #2 diesel from a single lot (SwRI #EM-619-F) having the following major characteristics:

90% B.P.: 598°F
Sulfur: 0.05 wt.%
Aromatics: 36.2 vol.%
Saturated Hydrocarbons: 63.8 vol.%
Cetane No.: 45

Low aromatic fuel contained 16.2% aromatics.

Test Cycles. Both automobiles were run, with and without their oxidizer traps, through the same set of test cycles which consisted of the following:

NYCC. New York City Cycle; A series of stop and start maneuvers, lasting 599 sec and equivalent to 1.19 miles in distance.

HFET. Highway Fuel Economy Test cycle, consisting of 765 sec and equivalent to 10.25 miles in distance.

CFTP. A cold-start FTP cycle consisting of a 505 sec transient phase and an 867 sec stabilized phase, with an equivalent mileage of 7.45 miles.

HFTP. A hot-start FTP cycle consisting of a 505 sec transient phase and an 867 sec stabilized phase for a total mileage of 7.45 miles.

Loaded Trap. The automobiles were run several times through the NYCC cycle which does not allow the trap to reach the temperature necessary for regeneration. Particles were collected during a final NYCC cycle run.

Regeneration. After loading the trap on several NYCC runs, the vehicle was run on the HFET cycle, which allowed the trap to reach regeneration temperature. Particles were collected during this HFET run.

Quadruplicate particle samples were collected on teflon-coated glass fiber filters for each combination of vehicle and driving cycle, with and without the oxidizer traps. Of these, one filter was sent to UC Irvine for extraction and Ames testing. Two of the remaining filters were used for chemical analysis at the SwRI, and the last filter was held in reserve. Each test was repeated at least twice, and two filters from each test cycle were made available for Ames testing at UC Irvine. A total of 23 filters from the Mercedes Benz tests and 27 filters from the Volkswagen tests were extracted and the extracts tested for mutagenic activity, as described below.

Collection of Exhaust Particles and Vapor. The methods were based on published procedures which have been shown to be efficient for particles of the size range in diesel exhaust (Pierson et al., 1983; Manabe et al., 1985; Salmeen et al., 1984). The raw exhaust was diluted approximately 1:10 with air in a stainless steel dilution tunnel. Using a constant volume sampler a known volume was pulled through a preweighed 508 x 508 mm Pallflex T60A20 teflon-coated fiberglass filter (Pallflex Corp., Putnam, CT) held in a specially contructed apparatus designed to accept this size filter. The filters were not precleaned, but were used as received. Upon extraction with dichloromethane (see below) the filters released between 2-4 mg of material which was not mutagenic in the Ames test. Maximum sampling time for individual test runs was 23 min. This short sampling time was assumed not to introduce significant artifacts into the mutagenesis tests through the production of

mutagenic nitro compounds by the interaction of NO_2 and PAH collected on the filters (Pitts et al., 1978; Tokiwa et al., 1981; Scheutzle, 1983). Following collection of the particles, the filters were carefully folded and sealed in Tedlar bags in a nitrogen atmosphere, and stored in the dark at -20°C (automobile samples) until transfer to UC Irvine. The sample filters were shipped on dry ice by air freight to UC Irvine. At UC Irvine, all samples were stored at -40°C in the dark until extraction.

Vapor phase material was collected from the automobile exhausts on polyurethane foam (PUF) cartridges approximately 1 x 3 inches. While these PUF cartridges collected adequate material for GC analysis, they did not provide enough material for the Ames mutagenesis tests, and consequently no studies of the mutagenic activity in the vapor phase of the automobile exhausts were done.

Collection and Handling of Samples from CRC VE-1 Project. The samples tested in this study originated from CRC VE-1 Project which was intended to examine the effects on diesel emissions of varying the composition of the diesel fuel. A Cummins NTCC 400 on a test stand was run with 9 specific fuel formulations under specified load conditions. Further details of the fuel formulations and engine test conditions may be found in the Final Report for CARB Contract no. A4-132-32, submitted by the SwRI, April 1988. Exhaust particles were collected on teflon-coated fiberglass filters (Pallflex T60A20), and extracted at SwRI using a mixture of toluene and ethanol (30:70, v:v). The extracts (50 ml total volume) were shipped to UC Irvine where Ames mutagenicity testing was done. Table 1 summarizes the fuel formulations used in this study. Samples tested at UCI included the soluble organic fractions (SOF) from the hot-start, cold-start, and steady-state test modes. The hot-start and cold-start cycles consist of a 20 min FTP-defined transient test following either a hot or cold engine start. The steady state test mode for the particles used in this study was a 20 min run at rated speed and 25% load. The pertinent data on these samples, provided by SwRI, are shown in Table 2.

The toluene:ethanol extracts were shipped from SwRI on dry ice by air freight to UCI. On arrival at UCI, the samples were stored at -40°C until assay. To prepare a sample for mutagenesis testing, a 5-ml aliquot of the original 50 ml sample was dried over 0.1 g of anhydrous sodium sulfate and the clear supernatant carefully removed. This procedure was followed because of the likelihood that the alcoholic

TABLE 1. Fuel Formulations Used in CRC Project VE-1.

Fuel #	Sulfur, wt. %	Aromatics, Vol. %	90% B.P., °F
1	0.30	15	543
2	0.30	41	551
3	0.30	41	630
4	0.30	16	635
5	0.30	31	600
6	0.05	31	600
7	0.17	31	600
8	0.04	10	598
9	0.06	41	602

solutions would have absorbed moisture from the air. A solution of toluene/ethanol (30/70, v/v) was similarly treated for use as a control in the mutagenesis tests. The weight of material in the dried extract (soluble organic fraction, SOF) was determined by evaporation of duplicate 50 microliter aliquots on preweighed aluminum planchets, and weighing them in a Cahn microbalance to the nearest microgram. The average of the 2 values was used in preparing the extracts for subsequent mutagenesis testing. The total SOF, as determined at UCI, are given in Table 2. The values for most samples were slightly higher than the SwRI values, but the relative differences among the weights were similar. For calculation of specific mutagenic activity (revertants/hp-hr), the SOF weights determined at UC Irvine and the extractable SOF of the particles (mg/hp-hr) determined at SwRI were used. The amount of toluene:ethanol extract required for a mutagenesis test was reduced in volume under N_2. An amount of dimethylsulfoxide (DMSO) was added such that 5 microliters would contain 40 micrograms of the extracted material. The remaining toluene:ethanol was then evaporated, as indicated by reduction in volume to the added volume of DMSO. For lower concentrations of the extract, aliquots of this solution were diluted in DMSO, as appropriate, so that each mutagenesis test plate received a total volume of 5 microliters of DMSO solution. The DMSO solutions were not stored, but made up immediately before use.

Control diesel exhaust particles were obtained from the National Bureau of Standards (Stock no. SRM 1650). This material represents a pool of exhaust particles from several heavy-duty engines, and is considered by the NBS to be typical of diesel particles in general.

TABLE 2. Soluble Organic Fractions (SOF) of Exhaust Particles Collected During Fuel Tests with Cummins NTCC400 (CRC Project VE-1). Data was provided by SwRI.

Sample #	Fuel #	Test Date	Mg SOF in 50ml		SOF of Particles.	
			SwRI	UCI	mg/hp-hr	mg/lb fuel
Hot-Start Transient:						
53	1	12-16-86	14.0	12.6	68	160
69	2	12-29-86	25.0	20.4	110	260
86	3	1-30-87	25.5	20.4	110	260
96	4	2-11-87	26.0	22.8	110	280
77	5	1-6-87	25.5	19.2	110	270
114	6	4-8-87	14.0	5.4	68	160
130	7	4-15-87	13.5	16.8	63	150
143	8	4-22-87	12.0	13.8	56	130
161	9	4-30-87	13.0	22.2	61	140
141	5	4-21-87	18.5	21.6	84	210
Cold-Start Transient						
52	1	12-16-86	17.0	21.9	78	170
68	2	12-29-86	48.0	53.7	200	430
85	3	1-30-87	46.0	50.7	210	450
95	4	2-11-87	33.0	39.3	140	320
76	5	1-6-87	33.0	36.9	130	280
113	6	4-8-87	21.0	27.3	97	210
129	7	4-14-87	21.5	27.9	96	220
142	8	4-22-87	17.0	26.1	74	170
160	9	4-30-87	19.0	26.7	87	190
180	5HS*	2-19-87	23.5	26.7	100	250
Steady-State						
55	1	12-16-86	34.0	38.1	120	240
71	2	12-29-86	48.0	32.1	170	310
88	3	1-30-87	68.0	80.7	230	430
98	4	2-11-87	79.0	98.5	270	530
79	5	1-5-87	53.0	56.7	170	350
116	6	4-8-87	27.0	35.7	97	190
132	7	4-15-87	34.0	47.1	120	230
145	8	4-22-87	29.5	40.5	100	200
158	9	4-30-87	25.5	41.7	97	190
168	5HS*	5-5-87	19.0	26.1	86	200

*Extract from hot-start transient included as internal reference.

Extraction of Filters. All extractions and subsequent mutagenesis testing were done under subdued lighting or in a special room equipped with "gold" fluorescent lights which do not emit near UV light which would be photochemically active. Prior to extraction, the filters in sealed bags were removed from the freezer and allowed to come to room temperature before opening. As a method to compare the relative amounts of particles on the filters, the color density was estimated using a Kodak photographic gray scale. Total particulate weights were determined by weighing the filters to the nearest mg and subtracting the initial weight of the filters as measured at the SwRI. The filters were then placed in the thimble of a one-liter Soxhlet extractor, and extracted for 12-14 hr with approximately 700 ml of HPLC grade DCM. This time represented about 30 cycles of the extractor. During the extraction a continuous trickle of dry nitrogen was introduced into the apparatus to exclude oxygen. Also, the extraction was conducted in a darkened fume cabinet to avoid possible photochemical effects. The extract was reduced approximately 100-fold in volume using a Kuderna-Danish evaporator, again under nitrogen and reduced illumination. As a control for the extraction and mutagenicity testing, a weighed portion of the SRM 1650 reference diesel particles was placed in the center of a clean filter, which was then carefully folded, and extracted as above. The reduced DCM extracts were stored in glass tubes with teflon-lined caps at -40°C until use. On some initial test filters, the extraction was repeated with absolute methanol. However, the methanol extracts did not show significant mutagenic activity in the Ames test (see Results), and therefore this second extraction was discontinued, as a routine activity. After extraction, the filters were dried at 100°C, and reweighed until a constant weight was attained to determine the approximate extracted fraction. Some very fine carbon particles were eluted during the extraction, which were removed by centrifugation. The extractable fraction was determined by gently drying duplicate 50-microliter aliquots of the clarified extract on preweighed aluminum planchets, and weighing to the nearest microgram in a Cahn electrobalance. The total volume of extract was measured and the total weight extracted was calculated.

To obtain information on the chemical nature of the mutagens extracted from the diesel particles, DCM extracts of SRM 1650 reference particles and of particles collected during a preliminary test run of the bus engine were fractionated into "aromatic" and "aliphatic" subfractions using the method described by Henderson et al. (1980). In this procedure, the DCM solvent is exchanged for dimethylsulfoxide

(DMSO) and the DMSO extracted with an equal volume of hexane to remove the aliphatic compounds. The DMSO is then diluted with 2 volumes of water, and extracted a second time with hexane to recover the aromatic compounds. These subfractions were then tested for mutagenic activity as described below. In some cases, the aromatic subfraction was further fractionated by HPLC into "nonpolar", "moderately polar", and "highly polar" subfractions as described by Scheutzle et al. (1985), and mutagenic activity measured as described below. It was not possible to carry out this analysis on all samples because of limitations in the amount of material.

Mutagenesis Testing with the Ames Salmonella System. The general procedures have been described in detail by Maron and Ames (1983). Because the mutagenic activity in extracts of diesel particles has been shown by others (and confirmed in this study) to be rather low, the micro modifications suggested by Kado and coworkers (1983, 1986) have been used to increase the sensitivity of the tests, as indicated in the Results section. The two Salmonella tester strains used in this project have been TA98 and TA100, which were obtained from Dr. B. N. Ames of the University of California, Berkeley. TA98 is sensitive to mutagens such as the PAH which cause shifts in the reading frame of bacterial DNA; TA100 is sensitive to alkylating or arylating mutagens which cause base pair changes in DNA. The endpoint for both is the reversion from histidine dependence to independence, as indicated by the ability to grow on histidine- deficient agar plates. Upon receipt from Dr. Ames, the bacteria were grown to stationary phase in Oxoid nutrient broth #2 (Oxoid Ltd., Hants, Great Britain). Aliquots of 1.5 ml were dispensed into screw-cap plastic tubes and stored frozen in the vapor phase over liquid nitrogen. This one lot of frozen cells was used throughout the experiments described here. To initiate a culture for testing, a small amount of a frozen culture was scraped into approximately 10 ml nutrient broth, and incubated overnight at 37^{o}. This liquid culture was then used to inoculate a larger nutrient broth culture of the volume required for the experiment. After overnight incubation, the bacteria were harvested by centrifugation, suspended in the same volume of phosphate-buffered saline (PBS, 0.15 M, pH 7.2-7.4), recentrifuged and resuspended in PBS at a density of approximately 2×10^{10} cells/ml.

Because many chemical mutagens (e.g., PAH) require some kind of metabolic processing in order to become active mutagens, the test system is supplemented with a enzyme preparation containing rat liver homogenate and cofactors (S9 mix; Maron and Ames,

1983) to accomplish oxidative metabolism of compounds like PAH. Male Sprague-Dawley rats (Charles River Labs, Wilmington, MA) were pretreated for 5 days with phenobarbital (1 mg/ml in drinking water) and beta- naphthoflavone (80 mg/kg b.w., i.p., 3 days prior to sacrifice), which increase the activity of the required liver enzymes. This pretreatment is equivalent to treatment of the rats with the PCB Aroclor 1242 and avoids the problems of contamination of the S9 with Aroclor and the hazards of handling PCBs (Matsushima et al., 1980). The rats were killed by CO_2 asphyxiation, the livers removed aseptically, and rat liver S9 was prepared as descibed by Maron and Ames (1983). In all of the studies described here, the "S9 mix" contained 8 mM $MgCl_2$, 33 mM KCl, 5 mM glucose-6-phosphate, 4 mM NADP, 100 mM sodium phosphate, pH 7.4, and 0.04 ml S9 per ml of mix. Protein concentration of the rat liver S9 preparation was 20.0 mg/ml as determined by the method of Lowry et al. (1951).

To prepare a particle extract for testing, an aliquot of the concentrated DCM extract sufficient for the test to be done was mixed with a volume of dimethylsulfoxide (DMSO) such that the final concentration of DMSO in the test samples would be 2.5% (i.e., 5 microliters in a total incubation volume of 0.20 ml) and would contain the desired amount of extract. The mixture was evaporated at room temperature under nitrogen to remove the more volatile DCM, as indicated by reduction in volume to that of the added DMSO. Aliquots of this DMSO solution, or dilutions appropriate to the test at hand, were then added to bacterial suspensions, as indicated below. Final concentrations of the extracts are indicated in the text where appropriate. The DMSO solutions were not stored, but were made up for immediate use, as required.

The basic procedure for mutagenicity testing for compounds that do not require metabolic activation is to add an aliquot of the test material in solution to a suspension of about 2×10^9 bacteria in warm, molten 0.6% agar ("top agar"; 0.6% agar, 0.5% NaCl, 0.05 mM histidine, 0.05 mM biotin), and then to spread this suspension over a minimal agar base (Vogel and Bonner, 1956). After allowing the agar to harden, the plates are incubated for 48 hr at 37°C to permit colony development. Incubation for longer than 48 hr does not increase the number of colonies eventually formed, but may increase the chance of contamination by other organisms. Colonies are scored, and mutagenic activity calculated as revertants/weight of material added to the suspended bacteria. If the toxicity of

the test compound is limited, then the mutant yield is not greatly affected by moderately toxic effects. This is because the top agar contains a trace of histidine which permits surviving bacteria to multiply until the histidine is exhausted. The final number of viable bacteria in the top agar is thus more a function of the concentration of histidine, than of the number of viable bacteria present initially. If the histidine concentration is held constant over all experiments, then the bacterial population at risk for mutation will also be the about the same in all cases (Green and Muriel, 1976). Those bacteria that have reverted to histidine independence will continue to grow and form colonies.

To test for compounds in the particle extracts that required metabolic activation, the microincubation procedure of Kado et al. (1983) was followed in all tests, except where otherwise indicated. Triplicate plates were made for each test concentration. Prior to plating, as described above, the bacteria were preincubated for 90 min in a mixture that contained 2×10^9 bacteria, the test substance dissolved in 5 microliters of DMSO, and S9 mix in a total volume of 0.2 ml. Control cultures were incubated without the test material but with S9 mix. At the end of preincubation, 2 ml of top agar was added and the mixture poured onto minimal agar plates. Tests for mutagenicity without S9 were conducted in a similar manner.

Compounds used as positive controls for mutation response were, for strain TA98, 2-aminofluorene (with S9) and 2-nitrofluorene (without S9), and for TA100, methyl methanesulfonate (without S9). Negative controls received DMSO only.

Using the above methods, dose-response experiments were done using concentrations of the particle extracts which did not produce excessive toxicity to the bacteria. In some cases, the dose range was limited by the amount of material extracted from the particles. However, adequate material was available for at least one experiment with all samples. Three test plates were prepared for each test concentration. The actual concentrations used in each experiment are indicated at the appropriate point in the text.

Data Analysis. Specific mutagenic activity (revertants/microgram of particle extract) was calculated from the linear portion of the dose-response curves using regression analysis and a modification of the method described by Bernstein et al. (1982). The precision of determination of the true mutagenic activity was limited

by two major factors, (1) the relatively low specific mutagenic activity of the extracts and (2) the cytotoxicity of the extracts to the bacteria. These two factors combined to limit the concentration range of the extracts over which a linear dose-response could be defined. In most cases, as the concentration of extract was increased, the specific mutagenic activity appeared to decrease, and, with some samples, the response curve became concave downwards. In order to compare mutagenic activity among the various samples, a simplistic modification of the more complex procedure of Bernstein et al. (1982) was made. The values for mutagenic activity obtained with the lowest concentrations of particle extracts were assumed to lie on the linear portion of the response curve. A regression line was calculated using these values and those for the spontaneous background mutation rate. The projected value for the next higher concentration was then calculated and compared with the actual observed value. If the observed value fell within 2 standard deviations (assuming the mean = S^2) of the projected value, the regression line was recalculated using the observed value. The process was repeated until the next higher mutation value fell outside the specified range, at which point the slope of the regression line was taken as the specific mutagenic activity of that sample. The major shortcoming of this method is probably that it introduces a downward bias in the calculated mutagenic activity (i.e., the calculated mutagenic activity is less than the actual activity). However, in actual practice this bias is likely to be relatively small since, in most cases, the toxicity was a steep function of concentration, and the spacing of concentration values was such that values of mutagenic activity departing from linearity were readily apparent. In some early tests the actual toxicity of the extracts was measured by dilution and plating of the bacteria immediately following preincubation with the extract. The use of this procedure is probably inappropriate because it does not account for further multiplication of the bacteria plated on the mutation test plates, and also the fact that the latter will continue to be exposed to the test material. Determination of the specific mutagenic activity using the values for bacterial toxicity obtained in this manner yielded response curves which were concave upwards, an anomalous result. Once the specific mutation rate had been determined, the mutagenic activity was related to vehicle or engine energy output as indicated in the individual tables.

RESULTS

Preliminary Studies. The methods for extraction of diesel particles and adsorbent resin were evaluated using particulate samples collected during preliminary test runs at the Mobile Source Division. The particles were extracted as described and the extracts tested for mutagenic activity using the standard Ames test. A portion of the extract was fractionated into aromatic and aliphatic subfractions as described, and assayed in the standard test and using the micro modification of Kado et al. (1983). The results of these tests are shown in Tables 3 and 4. The standard Ames test (Table 3) indicated a low level of mutagenic activity, in general agreement with expectations. The tests with subfractions clearly showed that the mutagenic activity was largely confined to the "aromatic" subfraction. The results with the micro modification (Table 4) indicated a greater sensitivity than the standard test, with both the unfractionated extract as well as the subfractions. A further advantage of the microtest was that 10 times less material was required to perform the tests.

To provide a reference during the series of tests to be conducted, samples of a collection of heavy-duty diesel particles, designated SRM-1650, were obtained from the National Bureau of Standards. Extracts of these particles, together with subfractions, were tested in the standard Ames test and the micro test. The results of these tests are shown in Tables 5 through 8. Table 5 shows that more than 90% of the mutagenic activity was present in the DCM extract, compared to that in the methanol extract. The mutagenic activity of the aromatic and aliphatic subfractions of the DCM extract are shown in Table 6. As expected, relatively little activity was found in the aliphatic fraction, when tested with Strain TA98. However, there appeared to be significant activity in the aliphatic fraction which was mutagenic to Strain TA100. This has not been explored further. Tests for mutagenic activity of the unfractionated extract using the micromodification are shown in Table 7. Again, these results showed the greater sensitivity of the modified test and the reduction in need for test material. Further fractionation of the aromatic subfraction by HPLC indicated that the mutagenic activity was concentrated in the moderately polar subfraction, in agreement with the reports of others (e.g., Schuetzle et al., 1985). These results are shown in Table 8. These preliminary studies confirmed the efficacy of the extraction procedures and Ames test methods.

TABLE 3. Mutagenic Activity of a DCM Extract of Heavy Duty Diesel Exhaust Particles as Determined by the Standard Ames Test.

The particles were extracted and a portion of the extract was fractionated into "aromatic" and "aliphatic" subfractions as described in the Experimental section. Values are revertants/microgram of whole extract or subfraction material ± 1 S.D. and are the slopes of dose-response curves obtained as described in the Experimental section. The numbers in parentheses are the concentrations of test material, in micrograms per test plate, which gave revertant data which was used in the calculation of the linear portion of the dose-response curve. Three plates were prepared at each concentration. The negative control values are the mean ± S.D. of spontaneous revertant colonies on triplicate plates. The positive control values are the slopes of dose-response curves determined as for the diesel particle extracts. 2AF = 2-aminofluorene; 2NF = 2-nitrofluorene; MMS = methyl methanesulfonate.

Whole Unfractionated Particle Extract

Tester Strain	Revertants per Microgram
TA98 + S9	1.20 ± 0.290 (0-10-25-50-100)
TA98 - S9	1.12 ± 0.251 (")
TA100 + S9	2.56 ± 0.606 (")
TA100 - S9	0.933 ± 0.362 (")

Positive Controls for Unfractionated Extract:

TA98 + S9 + 2AF: 186 ± 20.7 (0-0.1-1-5-10)
TA98 - S9 + 2NF: 176 ± 17.1 (")

TA100 - S9 + MMS: 7.52 ± 4.81 (0-0.1-1-5-10)

Negative Controls for Unfractionated Extract:

TA98 + S9: 25.0 ± 2.65 TA100 + S9: 101 ± 6.03
TA98 - S9: 15.7 ± 2.08 TA100 - S9: 101 ± 11.0

Aromatic and Aliphatic Subfractions

	Revertants per Microgram	
Test Strain	Aromatic Fraction	Aliphatic Fraction
TA98 + S9	24.2 ± 2.93 (0-5)	0.0316 ± 0.139 (0-10-25-50-100)
TA98 - S9	22.0 ± 4.87 (0-5)	0.0149 ± 0.161 (")
TA100 + S9	34.8 ± 6.64 (0-5)	0.101 ± 0.234 (0-10-25-50-100)
TA100 - S9	33.0 ± 4.79 (0-5)	0.0528 ± 0.0547 (")

Table 3, Continued.

Positive Controls for Aromatic and Aliphatic Fraction Tests.

TA98 + S9 + 2AF: 315 ± 49.4 (0-0.1-1-5-10)
TA98 - S9 + 2NF: 157 ± 26.6 (0-0.1-1-5)

TA100 - S9 + MMS: 3.13 ± 1.50 (0-1-5-10-20)

Negative Controls for Aromatic and Aliphatic Fraction Tests.

TA98 + S9 + DMSO: 44.0 ± 2.00
TA98 - S9 + DMSO: 19.7 ± 4.93
TA100 + S9 + DMSO: 117 ± 1.53
TA100 - S9 + DMSO: 116 ± 8.02

TABLE 4. Mutagenic Activity of a DCM Extract of Heavy-Duty Diesel Exhaust Particles as Determined by the Micro Ames Test.

Values are as in Table 3.

Whole Unfractionated Particle Extract

Tester Strain	Revertants per Microgram
TA98 + S9	4.93 ± 2.05 (0-2.5-5-10)
TA98 - S9	4.76 ± 1.95 (")
TA100 + S9	0.316 ± 5.06 (0-2.5-5-10)
TA100 - S9	6.72 ± 3.47 (")

Positive Controls for Unfractionated Extract.

TA98 + S9 + 2AF: 153 ± 66.6 (0-0.005-0.05-0.25-0.5)
TA98 - S9 + 2NF: 3533 ± 544 (")

TA100 - S9 + MMS: 24.3 ± 33.9 (0-1)

Negative Controls for Unfractionated Extract.

TA98 + S9: 30.7 ± 5.86 TA100 + S9: 118 ± 7.51
TA98 - S9: 30.7 ± 2.52 TA100 - S9: 91.7 ± 8.33

Aromatic and Aliphatic Subfractions

Test Strains	Revertants per Microgram: Aromatic Fraction	Aliphatic Fraction
TA98 + S9	99.0 ± 10.4 (0-0.5-1)	0.0876 ± 0.953 (0-2.5-5-10)
TA98 - S9	171 ± 45.3 (")	-0.610 ± 1.21 (")
TA100 + S9	67.0 ± 35.4 (0-0.5-1)	3.07 ± 1.94 (0-2.5-5-10)
TA100 - S9	45.0 ± 38.4 (")	0.533 ± 1.99 (")

Positive Controls for Aromatic and Aliphatic Micro Tests

TA98 + S9 + 2AF: 858 ± 100 (0-0.005-0.05-0.25)
TA98 - S9 + 2NF: 18011 ± 159 (0-0.005-0.05)

TA100 - S9 + MMS: 6.37 ± 3.77 (0-0.1-1-5-10)

Negative Controls for Aromatic and Aliphatic Micro Tests.

TA98 + S9: 25.3 ± 1.53 TA100 + S9: 69.7 ± 9.50
TA98 - S9: 25.7 ± 2.52 TA100 - S9: 69.0 ± 3.61

TABLE 5. Mutagenic Activity of a DCM Extract of SRM-1650 Reference Diesel Exhaust Particles Determined by the Standard Ames Test.

Values are revertants/microgram of extract, calculated on the basis of dose response curves obtained as described in the Experimental section. The numbers in parentheses associated with each value are the concentrations, in micrograms per plate, of the material used in the test which gave revertant values which were used to define the linear portion of the dose-response curve, as described in the Experimental section. Three test plates were prepared at each concentration. 2AF = 2-aminofluorene, 2NF = 2-nitrofluorene, MMS = methyl methanesulfonate.

	Revertants per Microgram	
Strain	DCM Extract	Methanol Extract
TA98 + S9	14.1 ± 2.50 (0-10-25-50)	1.08 ± 0.192 (0-10-25-50-100)
TA98 - S9	14.8 ± 2.10 (0-10-25-50)	0.960 ± 0.200 (0-10-25-50-100)
TA100 + S9	37.6 ± 3.25 (0-10)	1.28 ± 1.00 (0-10-25-50-100)
TA100 - S9	32.9 ± 7.80 (0-10)	1.02 ± 0.400 (0-10-25-50-100)

Negative Controls: Values are the mean ± S.D. of spontaneous revertants on triplicate plates.

TA98 + DMSO + S9: 25.0 ± 2.65
TA98 + DMSO - S9: 15.7 ± 2.08

TA100 + DMSO + S9: 101 ± 6.03
TA100 + DMSO - S9: 101 ± 11.0

Positive Controls:

TA98 + S9 + 2AF: 186 ± 17.7 (0-0.1-1-5-10)
TA98 - S9 + 2NF: 176 ± 17.2 (0-0.1-1-5-10)

TA100 - S9 + MMS: 7.52 ± 4.82 (0-0.1-1-5-10)

TABLE 6. Mutagenic Activity of Aromatic and Aliphatic Subfractions of a DCM Extract of SRM-1650 Reference Diesel Exhaust Particles as Determined by the Standard Ames Test.

Values are as in Table 5.

	Revertants per Microgram	
Strain	Aromatic Fraction	Aliphatic Fraction
TA98 + S9	31.2 ± 3.92 (0-6-10)	1.14 ± 0.838 (0-10-25)
TA98 - S9	45.0 ± 4.00 (0-6-10)	0.368 ± 0.296 (0-10-25)
TA100 + S9	72.6 ± 13.2 (0-6-10)	9.23 ± 3.32 (0-10)
TA100 - S9	80.7 ± 22.2 (0-6-10)	2.77 ± 3.05 (0-10)

Negative Controls: (As in Part 2A).

TA98 + DMSO + S9: 32.0 ± 12.5 TA100 + DMSO + S9: 111 ± 12.1
TA98 + DMSO - S9: 24.3 ± 4.16 TA100 + DMSO - S9: 116 ± 4.36

Positive Controls:

TA98 + S9 + 2AF: 401 ± 70.7 (0-0.1-1)
TA98 - S9 + 2NF: 155 ± 4.08 (0-0.1-1-5)

TA100 - S9 + MMS: 4.13 ± 3.73 (0-0.1-1-5-10)

TABLE 7. Mutagenic Activity of a DCM Extract of SRM-1650 Reference Diesel Exhaust Particles as Determined by the Micro Ames Test.

Values are as in Table 5.

Strain	Revertants per Microgram
TA98 + S9	32.5 ± 11.7 (0-1-1.25-2.5-5)
TA98 - S9	20.6 ± 6.52 (")
TA100 + S9	18.2 ± 12.5 (0-1-1.25-2.5-5)
TA100 - S9	30.2 ± 10.9 (0-1-1.25-2.5)

Negative Controls: (As in Table 5).

TA98 + DMSO + S9: 30.7 ± 5.86 TA100 + DMSO + S9: 118 ± 7.51
TA98 + DMSO - S9: 30.7 ± 2.52 TA100 + DMSO - S9: 91.7 ± 8.33

Positive Controls:

TA98 + S9 + 2AF: 153 ± 66.6 (0-0.005-0.05-0.25-0.5)
TA98 - S9 + 2NF: 3533 ± 544 (")

TA100 - S9 + MMS: 24.3 ± 33.9 (0-0.01-0.1)

TABLE 8. Mutagenic Activity of HPLC Subfractions of the Aromatic Fraction of a DCM Extract of SRM-1650 Reference Diesel Exhaust Particles.

Values are as in Table 5.

Strain	Revertants per Microgram
Nonpolar Fraction	
TA98 + S9	93.6 ± 18.0 (0-1-1.25)
TA98 - S9	21.8 ± 6.27 (0-1-1.25-2.5)
TA100 + S9	17.6 ± 11.6 (0-1-1.25-2.5-5)
TA100 - S9	1.01 ± 9.89 (")
Moderately Polar Fraction	
TA98 + S9	973 ± 176 (0-1-1.25)
TA98 - S9	370 ± 81.8 (0-1-1.25-2.5)
TA100 + S9	420 ± 54.2 (0-1-1.25)
TA100 - S9	173 ± 40.0 (0-1-1.25)
Highly Polar Fraction	
TA98 + S9	197 ± 42.0 (0-1-1.25-2.5-5)
TA98 - S9	296 ± 119 (")
TA100 + S9	64.9 ± 11.8 (0-1-1.25-2.5-5)
TA100 - S9	58.5 ± 13.7 (")

Negative Controls:

TA98 + DMSO + S9: 31.7 ± 3.21 TA100 + DMSO + S9: 115 ± 4.58
TA98 + DMSO - S9: 40.0 ± 3.00 TA100 + DMSO - S9: 137 ± 10.1

Positive Controls:

TA98 + S9 + 2AF: 402 ± 130 (0-0.005-0.05-0.25)
TA98 - S9 + 2NF: 8469 ± 936 (0-0.005-0.05-0.25)

TA100 -S9 + MMS: 47.3 ± 69.8 (0-0.015-0.15-0.75)

Assay of Material Collected from Mercedes Benz Diesel.

The procedures for extraction and testing of the Mercedes Benz diesel exhaust particles are described in the Experimental section. Tables 9 and 10 provide the extraction and testing dates, Kodak gray scale readings, and particle and extractable fraction weights. Table 9 shows that, in terms of particulate weights per mile of travel, the trap reduced particle emission by about 90%. With the trap in place, the Kodak gray scale values correlate reasonably well with the weights of extractable material. However, without the trap the filters were solid black. Table 10 shows the yields of extractable material from the collected particles. The percent extractable material was essentially the same for particles collected with or without the trap. Therefore, since the weight of particles was reduced by the trap by about 10-fold, the net effect was a reduction in emission of soluble material emitted by the vehicle by as much as 10-fold. The major effect of the trap was to decrease the emission of particulate material. Table 11 summarizes the data indicating a reduction in particle emission by between 88% and 92%.

The Ames test data for the extractable material are given in Tables 12 through 17. In all cases the micro modification of the Ames test was used. Table 12 provides data for the positive controls for the Ames test. Although there was some variability between individual test dates, the relative response of the tester strains was consistent. Because the experimental procedures used in the present study were not exactly the same as others have reported in the literature it is not possible to quantitatively compare the positive control values. However, the relative responses of the strains to the positive controls was the same as reported by others. The negative controls for these tests are shown in Table 13. The expected spontaneous revertants for strain TA98 are 30-50 and for strain TA100, 120-200 (Maron and Ames, 1983). The observed values for TA98 are consistent with this expected range, while those for TA100 are slightly low. Tables 14 and 15 give the data in the form of revertants per microgram of extracted material. There were no significant differences between the specific activities of the extracts of particles collected with or without the trap. When converted to revertants per mile, as in Table 16, it was clear that the reduction in emission of mutagenic material correlated with the reduction in emission of total particulate material. Table 17 summarizes the results as the percent reduction by the oxidizer trap of the emission of mutagenic material in the exhaust. On average, the trap reduced the emission of

TABLE 9. Extraction Dates, Test Dates and Particle Yields for Mercedes Benz Automobile*

Sample No.	Extr. Date	Kodak Scale	Mg Particles** Total	Per Mile	Test Date
With Trap					
6/HFTP	5-26-87	7-8	66	34.1	6-5-87
22/HFTP	6-2-87	10-11	73	37.7	6-30-87
10/HFET	5-27-87	7-8	35	13.1	6-30-87
26/HFET	6-3-87	8	56	21.0	7-10-87
14/NYCC	5-28-87	4-5	14	45.2	6-30-87
30/NYCC	6-4-87	4-5	22	71.1	7-10-87
18/CFTP	6-1-87	10-11	65	33.6	6-30-87
34/CFTP	6-29-87	11-12	85	43.9	7-10-87
Without Trap					
42/HFTP	6-30-87	19+	601	310	7-17-87
58/HFTP	7-8-87	19+	612	316	7-24-87
46/HFET	7-6-87	19+	547	205	7-17-87
62/HFET	7-8-87	19+	528	198	7-24-87
50/NYCC	7-6-87	19+	215	695	7-17-87
66/NYCC	7-9-87	19+	183	591	7-24-87
38/CFTP	6-30-87	19+	745	385	7-10-87
54/CFTP	7-7-87	19+	752	388	7-17-87
Blank Filter	7-9-87	0	0		7-24-87

*HFTP: Hot start, 505 sec transient phase, 867 sec stabilized phase, 7.45 miles in distance.
HFET: Highway fuel economy test, 765 sec, 10.25 miles.
NYCC: New York City Cycle, 599 sec, 1.19 miles.
CFTP: Cold start, 505 sec transient phase, 867 sec stabilized phase, 7.45 miles.
Each Sample represents 26 $\pm$ 0.7% of the total particulate material generated during each driving cycle.
**Weight in mg of particles collected on filters as determined from weights of filters before and after collection of particles.

TABLE 10. Yields of Extractable Material from Particles Collected from Mercedes Benz Automobile.

Sample No.	Mg of Particles	Mg Extract Recovered	% Extracted
With Trap			
6/HFTP	66	5.2	7.8
22/HFTP	73	6.0	8.2
10/HFET	35	6.1	17
26/HFET	56	6.5	12
14/NYCC	14	2.5	18
30/NYCC	22	2.8	12
18/CFTP	65	5.2	8.0
34/CFTP	85	10.4	12
Without Trap			
42/HFTP	601	50.5	8.4
58/HFTP	612	52.1	8.5
46/HFET	547	57.7	10
62/HFET	528	67.3	13
50/NYCC	215	15.6	7.2
66/NYCC	183	15.2	8.3
38/CFTP	745	57.8	7.8
54/CFTP	752	65.2	8.7

TABLE 11. Percent Reduction of Particle Emission by Mercedes Benz Oxidizer Trap.

Test Cycle	Mg Particles per Mile Ave. with Trap	Ave. Without Trap	With Trap as % of Without
HFTP	35.9	313	11.5
HFET	17.1	202	8.46
NYCC	58.2	643	9.04
CFTP	75.0	386	10.0

TABLE 12. Positive Mutagenesis Controls for Tests of Extracts of Mercedes Benz Exhaust Particles.

Values are revertants per microgram of test compound ± S.D., based on a dose response curve obtained as described in the Experimental section. The Sample Nos. are those samples tested together with the positive controls.

Sample Nos.	Test Date	TA98 ± S9 ± 2AF	TA98 − S9 ± 2NF	TA100 − S9 ± MMS
6	6-5-87	363 ± 60.7	4147 ± 570	56.2 ± 19.4
10,14,18,22	6-30-87	549 ± 183	1389 ± 103	64.1 ± 0.45
26,30,34,38	7-10-87	862 ± 44.6	2240 ± 171	80.3 ± 31.6
42,46,50,54	7-17-87	581 ± 120	1683 ± 83.9	24.9 ± 6.46
58,62,66, Blank Filter	7-24-87	449 ± 55.0	1196 ± 123	55.9 ± 7.77

TABLE 13. Negative Controls for Mutagenesis Tests of Extracts of Mercedes Benz Exhaust Particles.

Values are the mean number of spontaneous revertants per plate ± S.D. observed when the tester strains were incubated with the DMSO vehicle used for dissolving the particle extracts or positive control mutagens. The sample numbers refer to the samples tested together with the particular negative control. Each value is based on triplicate plates. Test dates are as in Table 9.

Sample Nos.	TA98 ± S9	TA98 − S9	TA100 ± S9	TA100 − S9
6	41.3 ± 3.51	46.5 ± 0.58	97.0 ± 22.5	115 ± 9.81
10,14,18,22	56.3 ± 3.51	47.3 ± 4.73	111 ± 5.86	113 ± 2.00
26,30,34,38	29.3 ± 4.04	21.7 ± 1.53	73.3 ± 13.6	55.0 ± 4.58
42,46,50,54	43.0 ± 5.57	35.0 ± 6.08	162 ± 10.6	143 ± 17.5
58,62,66, Blank Filter	40.7 ± 5.69	34.3 ± 10.4	183 ± 10.3	131 ± 5.51

TABLE 14. Mutagenic Activity in DCM Extracts of Exhaust Particles Collected from Mercedes Benz Auto With an Oxidizer Exhaust Trap Expressed as Revertants per Microgram of Extracted Material.

The values for mutagenic activity are revertants/microgram of extract ± one standard deviation, determined from dose-response curves as described in the Experimental section. The concentrations used for determination of the dose-response were 0, 10, 25, 50, and 75 micrograms/plate, with 3 plates at each concentration. The values for "n" represent the number of plates used to define the linear portion of the dose-response curves, and also indicate the concentration range involved. Thus, an "n" of 6 indicates 0-10 micrograms; "n" of 9 indicates 0-25 micrograms; "n" of 12 indicates 0-50 micrograms; and "n" of 15 indicates 0-75 micrograms.

	Revertants per Microgram*	
Sample No.	TA98 ± S9 (n)	TA98 - S9 (n)
With Trap		
6/HFTP	18.9 ± 2.57 (6)	17.9 ± 1.75 (6)
22/ "	4.53 ± 0.95 (6)	7.32 ± 1.37 (9)
10/HFET	9.03 ± 1.16 (9)	13.1 ± 3.58 (6)
26/ "	16.6 ± 1.35 (9)	64.8 ± 2.36 (6)
14/NYCC	4.43 ± 1.65 (6)	3.77 ± 0.99 (6)
30/ "	15.4 ± 0.71 (9)	32.3 ± 0.70 (6)
18/CFTP	5.55 ± 1.31 (9)	5.97 ± 1.05 (9)
34/ "	14.9 ± 2.41 (12)	37.5 ± 2.42 (6)
	TA100 ± S9 (n)	TA100 - S9 (n)
6/HFTP	16.1 ± 8.16 (6)	15.9 ± 3.28 (6)
22/ "	2.25 ± 2.62 (9)	3.53 ± 5.60 (6)
10/HFET	13.0 ± 2.90 (6)	12.4 ± 1.19 (6)
26/ "	15.0 ± 2.19 (6)	15.0 ± 4.26 (6)
14/NYCC	4.10 ± 2.97 (6)	3.13 ± 3.55 (6)
30/ "	12.7 ± 3.02 (6)	11.2 ± 3.74 (6)
18/CFTP	5.44 ± 1.12 (9)	6.03 ± 2.33 (6)
34/ "	11.6 ± 2.16 (6)	4.84 ± 2.56 (9)

TABLE 15. Mutagenic Activity in DCM Extracts of Exhaust Particles Collected from a Mercedes Benz Auto Without an Oxidizer Trap, Expressed as Revertants per Microgram of Extracted Material.

	Revertants per Microgram*					
	TA98 + S9		(n)	TA98 - S9		(n)
42/HFTP	10.2	± 1.44	(6)	8.80	± 1.18	(9)
58/ "	9.46	± 0.88	(9)	12.6	± 2.29	(6)
46/HFET	22.2	± 4.49	(9)	23.2	± 3.85	(9)
62/ "	10.3	± 1.91	(15)	16.1	± 5.17	(6)
50/NYCC	9.20	± 1.38	(9)	9.97	± 2.28	(9)
66/ "	7.57	± 1.13	(6)	10.4	± 2.00	(6)
38/CFTP	34.8	± 4.51	(6)	36.7	± 6.42	(6)
54/ "	13.5	± 1.91	(9)	12.8	± 4.63	(6)
Blank Filter	0.13	± 0.23	(15)	-0.30	± 0.26	(15)
	TA100 + S9		(n)	TA100 - S9		(n)
42/HFTP	12.9	± 3.97	(6)	4.00	± 1.14	(15)
58/ "	17.2	± 3.34	(6)	4.01	± 0.57	(12)
46/HFET	9.97	± 5.19	(9)	7.76	± 4.31	(9)
62/ "	14.1	± 3.67	(6)	14.5	± 3.04	(6)
50/NYCC	3.98	± 1.86	(12)	No Data		
66/ "	7.57	± 3.66	(6)	12.0	± 2.91	(6)
38/CFTP	21.1	± 2.97	(6)	20.7	± 1.29	(6)
54/ "	13.1	± 8.59	(9)	5.63	± 3.14	(12)
Blank Filter	-1.44	± 0.86	(15)	-0.57	± 0.62	(15)

*There were no statistically significant differences between the average mutagenic activities when samples collected during the same test cycle, with and without trap, were compared.

TABLE 16. Mutagenic Activity in DCM Extracts of Exhaust Particles Collected from a Mercedes Benz Auto With and Without an Oxidizer Trap Expressed as Revertants/mile of Travel.

Values were calculated using data for miles traveled supplied by SwRI and determinations of extractable material and mutagenic activity at UCI.

	Revertants/Mile x 10^{-3} ± S.D.			
Sample No.	TA98 ± S9	TA98 - S9	TA100 ± S9	TA100 - S9
With Trap*				
6/HFTP	50.3 ± 6.84	47.7 ± 4.66	42.9 ± 21.7	42.3 ± 8.73
22/ "	14.0 ± 2.94	22.7 ± 4.25	6.97 ± 8.12	10.9 ± 17.3
10/HFET	20.7 ± 2.66	30.1 ± 8.23	29.7 ± 6.64	28.4 ± 2.73
26/ "	40.4 ± 3.29	157 ± 5.72	36.4 ± 5.31	36.4 ± 12.2
14/NYCC	36.1 ± 13.4	30.6 ± 8.07	33.2 ± 24.0	25.4 ± 28.8
30/ "	137 ± 6.32	287 ± 6.24	113 ± 26.9	99.7 ± 33.3
18/CFTP	14.8 ± 3.49	15.9 ± 2.80	14.6 ± 3.01	16.1 ± 6.22
34/ "	80.0 ± 12.9	201 ± 13.0	62.5 ± 11.6	26.0 ± 13.8
Without Trap*				
42/HFTP	323 ± 45.6	229 ± 30.7	336 ± 103	104 ± 29.6
58/ "	255 ± 23.8	338 ± 61.6	464 ± 89.9	108 ± 15.4
46/HFET	480 ± 97.1	501 ± 83.1	215 ± 112	168 ± 93.3
62/ "	260 ± 48.1	406 ± 130	355 ± 92.4	366 ± 76.7
50/NYCC	464 ± 69.6	503 ± 115	202 ± 94.4	No Data
66/ "	371 ± 55.4	510 ± 98.4	371 ± 179	587 ± 142
38/CFTP	1038 ± 135	1095 ± 192	630 ± 88.7	617 ± 38.4
54/ "	454 ± 64.2	431 ± 156	441 ± 289	189 ± 105

*All values without trap are significantly greater than corresponding values with trap; $p<0.0005$.

TABLE 17. Percent Reduction by Mercedes Benz Oxidizer Trap of the Emission of Particle-Associated Mutagenic Material.

Values are the average percent reduction in emission of mutagenic compounds, calculated using the average of the values for revertants per mile of travel in Table 16. Values in parentheses are the range of values, based on the extremes of the experimental results, i.e., the lower range limit was calculated using the lowest value without the trap and the highest value with the trap; the upper range limit was calculated using the highest value without the trap and the lowest value with the trap.

Test Cycle	TA98+S9	TA98-S9	TA100+S9	TA100-S9
HFTP	89 (80-96)	87 (79-93)	94 (87-98)	75 (59-90)
HFET	92 (84-96)	79 (61-94)	88 (83-92)	88 (78-92)
NYCC	79 (63-90)	69 (43-94)	74 (44-91)	89 (83-96)
CFTP	94 (82-99)	86 (53-98)	93 (86-98)	95 (86-97)

particle-associated mutagenic activity between 69% and 95%, depending on the test cycle examined.

Assay of Material Collected from Volkswagen Diesel.

Tables 18 and 19 provide the extraction data and test dates, Kodak gray scale values and weights of extracted material for the particulates collected from the Volkswagen diesel. As shown in Table 18, with the trap in place, the filters showed only a faint coloring, but there was a substantial amount of extractable material as well as unidentified, apparently noncarbonaceous, material present on the filter. Identification of this material was beyond the scope of this project. The actual weights of particulate were comparable to those collected from the Mercedes Benz automobile (cf. Table 9). Without the trap, the filters were solid black, except for those used to collect particles during the NYCC test, which involved a shorter collection time and less total mileage. The extractable fraction of the particle weights was different for particles collected with and without the trap (Table 19). About 50% of the weight of the particles collected with the trap could be extracted with DCM, while about 23% could be extracted from particles collected without the trap. By comparison, the extractable fraction of the particles collected from the Mercedes Benz auto was about 12% with the trap and about 9% without (Table 10). As with the Mercedes Benz, the trap substantially reduced both total particulate collected and amount of extractable material. Table 20 summarizes the effect of the trap on particle emission. Depending on the driving cycle, particle emission was reduced by 86% to 95% by the trap.

Tables 21 and 22 present the positive and negative control data for the mutagenesis testing of the Volkwagen exhaust particle extracts. Although there are differences in response between test dates, the relative response of the strains is consistent. The numbers of spontaneous revertants (Table 22) was also within the expected range for the series of tests.

Tables 23 and 24 present the Ames test data for the Volkwagen diesel particle extracts. In Table 23 is shown the specific mutagenic activity of the extracts in terms of revertants per microgram of extract. Although there are some marginally significant differences between values obtained with extracts of particles collected with and without the trap, overall the values are similar in the two groups.

TABLE 18. Extraction Dates, Particle Yields, and Test Dates for Volkswagen Diesel Automobile.

Test cycle designations are as in Table 9.

Sample No.	Extr. Date	Kodak Scale	Mg Particles Total	Mg Particles Per Mile	Test Date.
With Trap					
98/CFTP	10-26-87	<1	46	23.6	11-9-87
114/ "	11-4-87	<1	14	7.17	12-8-87
102/HFTP	10-26-87	<1	39	7.2	11-6-87
118/ "	11-4-87	<1	18	9.2	12-2-87
106/HFET	10-28-87	<1	32	11.9	11-11-87
122/ "	11-12-87	<1	28	10.4	12-2-87
110/NYCC	10-28-87	<1	10	32.1	11-18-87
126/ "	11-12-87	<1	12	38.5	12-4-87
Without Trap					
+30/CFTP	11-30-87	19+	379	194	12-4-87
146/ "	12-10-87	19+	422	216	12-16-87
134/HFTP	11-30-87	19+	323	165	12-8-87
150/ "	12-10-87	19+	325	166	12-16-87
138/HFET	12-7-87	19+	218	81.2	12-11-87
154/ "	12-14-87	19+	230	85.6	12-21-87
142/NYCC	12-7-87	13	92	295	12-11-87
158/ "	12-14-87	12-13	79	253	12-21-87

TABLE 19. Yields of Extractable Material from Volkswagen Diesel Exhaust Particles.

Sample No.	Mg of Particles	Mg Extract Recovered	% Extracted
With Trap			
98/CFTP	46	18.3	40
114/ "	14	10.7	76
102/HFTP	39	19.7	51
118/ "	18	13.6	76
106/HFET	32	17.8	56
122/ "	28	12.5	45
110/NYCC	10	4.3	43
126/ "	12	3.8	32
Without Trap			
130/CFTP	379	72.2	19
146/ "	422	83.1	20
134/HFTP	323	78.6	24
150/ "	325	77.6	24
138/HFET	218	59.4	27
154/ "	230	55.5	24
142/NYCC	92	18.4	20
158/ "	79	18.5	23

TABLE 20. Percent Reduction by Oxidizer Trap of Emission of Exhaust Particles by Volkswagen Auto.

Test Cycle	Mg Particles Emitted per Mile Ave. With Trap	Ave. Without Trap	With Trap as % of Without
CFTP	15.4	205	7.5
HFTP	8.2	165	5.0
HFET	11.2	83.4	13.4
NYCC	35.3	274	12.9

TABLE 21. Positive Controls for Mutagenesis Tests of DCM Extracts of Volkswagen Diesel Exhaust Particles.

The values are revertants per microgram ± S.D. calculated from a dose-response curve obtained as described in the Experimental section. The Sample Nos. refer to the particular sample tested together with the positive control compounds on the indicated date.

Sample Nos.	Test Date	TA98 + S9 ± 2AF	TA98 - S9 ± 2NF	TA100 - S9 ± MMS
98	11-9-87	268 ± 26.7	1960 ± 295	7.35 ± 2.62
102	11-6-87	191 ± 13.2	3632 ± 129	7.52 ± 1.86
106	11-11-87	188 ± 68.3	3080 ± 396	6.42 ± 1.64
110	11-18-87	No data	No data	9.42 ± 2.53
118,122	12-2-87	145 ± 110	3167 ± 768	2.37 ± 2.74
126,130	12-4-87	397 ± 40.4	5015 ± 810	5.05 ± 1.36
114,134	12-8-87	375 ± 54.1	4349 ± 860	3.82 ± 0.902
138,142	12-11-87	566 ± 194	4547 ± 667	8.22 ± 2.34
146,150	12-16-87	487 ± 207	3087 ± 600	No data
154,158	12-21-87	529 ± 35.8	1620 ± 231	5.70 ± 2.71

TABLE 22. Negative Controls for Mutagenesis Tests of DCM Extracts of Volkswagen Diesel Exhaust Particles.

Values are the mean number of spontaneous revertants per plate ± S.D. observed when the tester strains were incubated with the DMSO vehicle used for dissolving the particle extracts or positive control mutagens. The sample numbers refer to the samples tested together with the particular negative control. Each value is based on triplicate plates. Test dates are as in Table 21.

Sample	TA98 + S9	TA98 - S9	TA100 + S9	TA100 - S9
98	55.0 ± 2.00	49.7 ± 4.04	134 ± 13.5	156 ± 10.0
102	60.3 ± 2.08	55.0 ± 4.58	180 ± 11.9	168 ± 12.0
106	91.3 ± 6.35	77.3 ± 5.13	202 ± 20.7	191 ± 20.7
110	69.0 ± 3.61	59.7 ± 5.03	210 ± 16.8	207 ± 12.8
114,134	54.7 ± 1.53	51.3 ± 1.15	190 ± 13.8	212 ± 9.16
118,122	67.3 ± 16.2	60.3 ± 11.5	196 ± 27.1	208 ± 18.0
126,130	97.0 ± 15.6	85.0 ± 5.00	204 ± 15.9	215 ± 10.6
138,142	63.3 ± 4.73	54.0 ± 6.24	179 ± 5.86	169 ± 6.56
146,150	56.3 ± 8.39	47.0 ± 8.54	175 ± 20.0	170 ± 8.74
154,158	50.3 ± 6.43	44.3 ± 10.4	160 ± 18.2	186 ± 20.5

TABLE 23. Mutagenic Activity of DCM Extracts of Volkswagen Diesel Exhaust Particles Expressed as Revertants per Microgram of Extracted Material ± 1 S.D.

	Revertants per Microgram			
Sample No.	TA98 + S9	TA98 - S9	TA100 + S9	TA100 - S9
With Trap				
98/CFTP	10.5 ± 1.82	14.7 ± 1.20	8.71 ± 2.27	8.49 ± 3.00
114/ "	5.33 ± 1.52	7.07 ± 1.19	4.49 ± 1.87	5.22 ± 1.87
102/HFTP	8.24 ± 1.57	15.4 ± 1.62	7.70 ± 4.07	13.9 ± 4.05
118/ "	9.88 ± 2.22	12.7 ± 2.69	8.62 ± 3.68	9.67 ± 2.99
106/HFET	19.3 ± 4.20	15.9 ± 3.08	11.2 ± 4.37	14.1 ± 5.10
122/ "	13.3 ± 1.44	18.5 ± 2.54	8.91 ± 4.38	11.8 ± 4.20
110/NYCC	3.76 ± 1.39	4.08 ± 1.87	9.35 ± 6.67	5.33 ± 2.80
126/ "	2.01 ± 1.40	3.21 ± 1.34	4.35 ± 4.48	1.96 ± 2.71
Without Trap				
130/CFTP	19.3 ± 5.81	18.1 ± 2.30	16.8 ± 8.74	14.2 ± 2.19
146/ "	9.36 ± 2.01	14.1 ± 3.17	12.9 ± 2.93	11.5 ± 4.57
134/HFTP	7.30 ± 2.08	8.02 ± 1.91	9.89 ± 2.75	7.91 ± 3.46
150/ "	8.46 ± 2.14	13.4 ± 2.69	11.1 ± 3.43	9.74 ± 3.87
138/HFET	11.3 ± 1.54	19.0 ± 3.34	14.2 ± 3.25	14.8 ± 3.77
154/ "	16.7 ± 2.08	17.6 ± 2.31	13.7 ± 3.87	11.7 ± 3.27
142/NYCC	11.0 ± 3.62	33.4 ± 4.59	13.4 ± 4.69	27.5 ± 3.25
158/ "	6.05 ± 1.78	11.8 ± 2.14	13.1 ± 4.45	7.32 ± 2.96

TABLE 24. Mutagenic Activity of Volkswagen Diesel Exhaust Particle DCM Extracts Expressed as Revertants/mile of travel.

Sample No.	Revertants/Mile x 10^{-3} ± S.D.			
	TA98 ± S9	TA98 - S9	TA100 ± S9	TA100 - S9
With Trap*				
98/CFTP	99.1 ± 17.2	139 ± 11.3	82.1 ± 21.4	80.0 ± 28.3
114/ "	29.4 ± 8.38	39.0 ± 6.56	24.7 ± 10.3	28.9 ± 10.4
102/HFTP	83.6 ± 15.9	156 ± 16.4	78.5 ± 41.5	141 ± 41.1
118/ "	69.2 ± 15.5	89.3 ± 18.9	60.4 ± 25.8	68.1 ± 21.1
106/HFET	129 ± 28.1	106 ± 20.5	75.0 ± 29.3	94.2 ± 33.4
122/ "	62.3 ± 6.74	86.7 ± 11.9	41.6 ± 20.4	55.2 ± 19.6
110/NYCC	52.4 ± 19.4	56.9 ± 26.1	131 ± 93.4	74.3 ± 39.0
126/ "	25.0 ± 17.4	40.1 ± 16.7	54.0 ± 55.6	24.3 ± 33.6
Without Trap*				
130/CFTP	721 ± 217	676 ± 193	627 ± 326	528 ± 81.4
146/ "	401 ± 86.1	604 ± 136	553 ± 126	493 ± 196
134/HFTP	296 ± 84.3	325 ± 77.4	402 ± 167	321 ± 115
150/ "	338 ± 85.5	537 ± 108	444 ± 137	391 ± 155
138/HFET	252 ± 34.3	422 ± 74.2	316 ± 72.3	330 ± 84.1
154/ "	348 ± 43.3	368 ± 48.3	284 ± 80.2	244 ± 68.2
142/NYCC	656 ± 216	1988 ± 273	798 ± 279	1635 ± 193
158/ "	359 ± 106	705 ± 128	782 ± 266	436 ± 176

*All values without trap greater than corresponding values with trap; $p<0.0005$.

TABLE 25. Percent Reduction by Volkswagen Oxidizer Trap of Emission of Particle-Associated Mutagenic Activity.

Values are the average percent reduction in emission of mutagenic compounds, calculated using the average of the values for revertants per mile of travel in Table 24. Values in parentheses are the range of values, based on the extremes of the experimental results, i.e., the lower range limit was calculated using the lowest value without the trap and the highest value with the trap; the upper range limit was calculated using the highest value without the trap and the lowest value with the trap.

Test Cycle	TA98+S9	TA98-S9	TA100+S9	TA100-S9
CFTP	88 (75-96)	86 (77-94)	91 (85-96)	89 (84-94)
HFTP	76 (72-80)	72 (52-83)	84 (80-86)	71 (56-83)
HFET	68 (49-82)	76 ((71-79)	81 (74-87)	74 (61-83)
NYCC	92 (85-96)	96 (92-98)	88 (83-93)	95 (83-98)

However, when the results are calculated as revertants per mile of travel (Table 24), the effectiveness of the trap in reducing emission of mutagens becomes apparent. Table 25 summarizes the results showing the extent of reduction by the oxidizer trap of emission of mutagenic material. The presence of the oxidizer trap reduced the emission of particle-associated mutagenic activity by between 68% and 96%, depending on the test cycle.

Tables 26 and 27 show the test conditions during collection of the particles, and the yields of extractable material for the Mercedes Benz and Volkswagen autos, respectively, when tests were done with low aromatic fuel, and during loaded trap and trap regeneration conditions. Comparison with data obtained with the trap operating normally indicated some significant differences. Particulate emission by the Mercedes with a loaded trap and driving the NYCC cycle was 172% of that seen with the trap operating normally (cf. Table 9). During trap regeneration on the HFET cycle, particulate emission was 430% of that when the trap was operating normally. When burning low aromatic fuel without the trap, the particle emission was slightly (7-11%) lower than with high aromatic fuel, but because of the few samples, the difference was not statistically significant. There were small differences in the fraction of material extractable from the particles collected under normal operating conditions and those collected with the loaded trap or during regeneration, but these, too, were not significant.

The results with particles collected from the Volkswagen were similar to those seen with the Mercedes (Table 27). With a loaded trap on the NYCC cycle, particulate emission was increased by 411% over the value found with normal trap operation (cf. Table 18). As noted above, the particulate material collected from the Volkswagen was much lighter in color than that from the Mercedes, and this was true also for the particles collected with a loaded trap. The Kodak reading was 1-2, which indicates a barely detectable color, while an equivalent weight of particles from the Mercedes would give a reading of 7-8, which is a definite medium dark gray. During regeneration on the HFET cycle, the particle emission by the Volkwagen was 357% of that seen during normal trap operation. Low aromatic fuel had some small effects on particle emission. With the trap in place on the CFTP and HFTP cycles, particle emission was slightly, but not significantly increased (132% and 112%, respectively). Without the trap, particle emission, compared to burning high aromatic fuel, was reduced during the CFTP cycle by 44%, and during the HFTP cycle

by 36%. Statistically, these latter differences were significant ($p<0.05$, 2-tailed "t" test), however, given the small numbers of samples, confirmation with further work is necessary. There were small differences in the extractable fractions of the particles collected with the loaded trap, during regeneration, and with low aromatic fuel, but these were not significant (cf. Table 19).

Table 28 shows the data for mutagenic activity extracted from particles collected from a Mercedes Benz diesel with a loaded trap and run under the NYCC driving cycle. Compared to data obtained with particles collected with the trap operating normally (cf. Table 16), there was no significant difference with respect to revertants per mile of travel.

Table 29 shows the results obtained with extracts of particles collected during the trap regeneration when operated over the HFET driving cycle. Comparison with results obtained with the trap operating normally (cf. Table 16) indicated no difference in the specific mutagenic activity of the particle extracts (revertants per microgram). However, when calculated as revertants per mile of travel there was approximately a 2-fold increase in emission of revertants during the regeneration cycle. This increase can be attributed to increased particulate emission.

Table 30 presents data obtained with extracts of particles collected when the Mercedes automobile was burning low aromatic fuel with no oxidizer trap. Compared to previous data obtained with particles collected during burning of high aromatic fuels, there were some slight differences (cf. Table 15). During the CFTP driving cycle, the specific activity (revertants per microgram of extract) of the material extracted from particles collected when burning high aromatic (baseline) fuel was up to 2-fold greater than the material obtained from particles collected when burning low aromatic fuel. A similar comparison of mutagenic activity of extracts collected during the HFTP driving cycle did not show a consistent pattern. When comparisons were made on the basis of revertants per mile of travel (cf. Table 16), then there appeared to be about a 2-fold greater emission of mutagens for both the HFTP and CFTP driving cycles when burning high aromatic (i.e., baseline) fuel. However, because of the variability in values for mutagenic activity, these differences were not statistically significant.

Table 31 shows the mutagenic activity associated with exhaust particles collected

TABLE 26. Particle Collection and Extraction Data for Mercedes Benz Loaded Trap And Trap Regeneration Tests.

The data for each sample number are for a single filter which was used to collect 26.3 ± 1.8% of the total particulate generated by the vehicles during that driving cycle. NYCC = New York City Cycle, 599 sec in duration and 1.19 miles in distance. HFET = Highway Fuel Ecomony Cycle, 765 sec duration and 10.25 miles in distance. CFTP = Cold Start, 505 sec transient phase, 867 sec stabilized phase, and a total of 7.45 miles in distance. HFTP = Hot Start, 505 sec transient phase, 867 sec stabilized phase, and a total of 7.45 miles in distance. Kodak values are an indicator of the relative density of carbon particles collected on the filters as judged by comparison with a Kodak photographic gray scale over a range of 0 (white) to 19 (black).

Test Conditions and Particle Yields.

#/Cycle	Trap	Fuel	Kodak	Mg Particles Total	Mg Particles Per Mile
70/NYCC	Loaded	Baseline	7-8	29.9	100
74/HFET	Regen.	"	16-17	240	93.7
78/HFET	Regen.	"	13-14	136	53.1
82/CFTP	No Trap	Low Arom.	18-19	706	379
90/CFTP	"	"	17-18	573	308
86/HFTP	"	"	18-19	582	312
94/HFTP	"	"	16-17	501	269

Extraction Data for Mercedes Benz Exhaust Particles.

#/Cycle	Extr. Date	Mg. Particles	Mg. Extracted	% Extracted
70/NYCC	2-22-88	29.9	5.95	19.9
74/HFET	2-22-88	240	15.5	6.46
78/HFET	2-29-88	136	10.2	7.50
82/CFTP	2-29-88	706	53.6	7.59
90/CFTP	3-7-88	573	53.7	9.23
86/HFTP	3-7-88	582	42.1	7.35
94/HFTP	3-16-88	501	41.6	8.30

TABLE 27. Particle Collection and Extraction Data for Volkswagen Loaded Trap and Trap Regeneration Tests.

Driving cycles and particle collection methods were the same as for the Mercedes Benz auto.

Test Conditions and Particle Collection Data.

#/Cycle	Trap Cond.	Fuel	Kodak	Mg Particles Total	Mg Particles Per Mile
162/NYCC	Loaded	Baseline	1-2	43.1	145
166/HFET	Regen.	"	5-6	128	50.0
174/HFET	"	"	2-3	77.1	30.1
178/CFTP	Baseline	Low Arom.	2-3	54.8	29.4
186/CFTP	"	"	1-2	21.0	11.3
182/HFTP	"	"	0-1	17.6	9.45
190/HFTP	"	"	0-1	16.7	8.97
194/CFTP	No Trap	"	18-19	203	109
202/CFTP	"	"	17-18	230	123
198/HFTP	"	"	18-19	200	107
206/HFTP	"	"	17-18	198	106

Yields of Extractable Material from Particles Collected from Volkswagen Auto.

#/Cycle	Extr. Date	Mg Particles	Mg Extract	% Extracted
162/NYCC	3-16-88	43.1	14.4	33.4
166/HFET	3-21-88	128	58.5	45.7
174/HFET	3-21-88	77.1	28.0	36.3
178/CFTP	3-29-88	54.8	10.5	19.2
186/CFTP	3-30-88	21.0	9.18	43.7
182/HFTP	3-29-88	17.6	8.10	46.0
190/HFTP	3-30-88	16.7	6.89	41.3
194/CFTP	3-31-88	203	50.2	24.7
202/CFTP	4-4-88	230	59.2	25.7
198/HFTP	3-31-88	200	46.3	23.2
206/HFTP	4-4-88	198	36.0	18.2

TABLE 28. Mutagenic Activity of a DCM Extract of Exhaust Particles Collected from a Mercedes Benz Diesel Auto Operating with a Loaded Oxidizer Trap.

Particles were collected on teflon-coated glass fiber filters during the NYCC driving cycle, using baseline fuel (high aromatic). This is sample number 1280-70 from the SwRI. Extraction and mutagenesis testing were as previously described. The values for revertants per microgram of extract and for revertants per mile were calculated based on the linear portion of a dose response curve obtained with concentrations of 0,5,10,20 or 40 micrograms of extract per plate, with 3 replicate plates at each concentration. The number in parentheses following the values for revertants per microgram is the number of plates used to define the linear portion of the dose-response curve, and also indicates the concentration range involved. Thus, "15" indicates a range of 0-40 micrograms, "12" a range of 0-20 micrograms, "9" a range of 0-10 micrograms, etc.

Revertants per Microgram ± S.D.

Strain TA98 + S9: 5.97 ± 1.05 (15) TA98 - S9: 6.96 ± 1.21 (15)

Strain TA100 + S9: 5.29 ± 1.30 (15) TA100 - S9: 3.71 ± 1.62 (12)

Revertants per Mile Traveled x 10^{-3} ± S.D.

Strain TA98 + S9: 122 ± 21.5 TA98 - S9: 151 ± 26.2

Strain TA100 + S9: 139 ± 24.6 TA100 - S9: 72.0 ± 31.4

Negative Controls. Values are the mean ± S.D. of the number of spontaneous revertants on triplicate plates which received the DMSO solvent only.

TA98+S9: 44.7 ± 3.21 TA100+S9: 153 ± 17.5
TA98-S9: 36.3 ± 6.66 TA100-S9: 190 ± 5.86

Positive Controls. Values are revertants per microgram of compound as in Table 12.

Strain TA98 + S9 + 2AF: 64.6 ± 25.3
Strain TA98 - S9 + 2NF: 853 ± 138

Strain TA100 - S9 + MMS: 17.5 ± 2.06

TABLE 29. Mutagenic Activity of DCM Extracts of Exhaust Particles Collected from a Mercedes Benz Auto During Oxidizer Trap Regeneration.

Particles were collected during the HFET driving cycle while using baseline (high aromatic) fuel. These are samples #1280-74 and 1280-78 from the SwRI. Values are as in Table 28.

Revertants per Microgram of Extract ± S.D.

#	TA98 ± S9	TA98 − S9
74	15.2 ± 2.45 (15)	30.6 ± 4.16 (12)
78	7.60 ± 1.27 (15)	14.3 ± 2.25 (12)
	TA100 ± S9	**TA100 − S9**
74	19.7 ± 2.67 (9)	13.0 ± 1.82 (15)
78	12.4 ± 3.73 (9)	11.1 ± 4.03 (9)

Revertants per Mile of Travel x 10^{-3} ± S.D.

#	TA98 ± S9	TA98 − S9
74	106 ± 17.1	176 ± 24.0
78	29.0 ± 4.85	54.0 ± 8.50
	TA100 ± S9	**TA100 − S9**
74	114 ± 15.4	75.0 ± 10.5
78	47.0 ± 14.2	42.0 ± 15.3

Negative Controls and Positive Controls are the same as in Table 28.

TABLE 30. Mutagenic Activity of DCM Extracts of Exhaust Particles Collected from a Mercedes Benz Auto During Baseline Tests with Low Aromatic Fuel.

Particles were collected during test cycles CFTP and HFTP without an oxidizer trap. These are samples #1280-82, 1280-90, 1280-86, and 1280-94 from the SwRI. Values are as in Table 28.

Revertants per Microgram of Extract ± S.D.

#	TA98 ± S9	TA98 - S9
82(CFTP)	11.1 ± 4.03 (15)	15.2 ± 2.39 (12)
90(CFTP)	8.94 ± 1.52 (15)	15.4 ± 2.45 (12)
86(HFTP)	8.48 ± 0.980 (15)	10.7 ± 1.34 (12)
94(HFTP)	4.46 ± 1.12 (15)	10.9 ± 1.80 (12)
	TA100 ± S9	TA100 - S9
82(CFTP)	14.2 ± 1.90 (15)	7.93 ± 1.54 (12)
90(CFTP)	11.6 ± 2.79 (15)	9.00 ± 1.31 (12)
86(HFTP)	5.90 ± 1.75 (15)	7.71 ± 2.91 (12)
94(HFTP)	5.02 ± 1.79 (15)	10.9 ± 1.80 (12)

Revertants per Mile of Travel ± S.D. x 10^{-3}.

	TA98 ± S9	TA98 - S9
82(CFTP)	306 ± 43.9	418 ± 65.6
90(CFTP)	246 ± 41.8	424 ± 67.4
86(HFTP)	183 ± 21.1	230 ± 18.9
94(HFTP)	95.0 ± 23.9	233 ± 38.4
	TA100 ± S9	TA100 - S9
82(CFTP)	390 ± 52.0	218 ± 42.3
90(CFTP)	320 ± 76.8	248 ± 36.1
86(HFTP)	127 ± 37.7	166 ± 62.6
94(HFTP)	107 ± 38.2	94.0 ± 46.0

Negative Controls. Values are the mean ± S.D. of the number of spontaneous revertants on triplicate plates which received the DMSO solvent only.

TA98+S9:	57.7 ± 8.50	TA100+S9:	153 ± 21.7
TA98-S9:	43.3 ± 8.20	TA100-S9:	159 ± 9.24

Positive Controls. Values are revertants per microgram of compound ± 1 S.D., as in Table 12.

Strain TA98 + S9 + 2AF: 58.7 ± 5.51
Strain TA98 - S9 + 2NF: 1236 ± 110

Strain TA100 - S9 + MMS: 17.5 ± 1.02

from a Volkwagen auto operated with a loaded trap on the NYCC driving cycle. Comparison of these data with previous results (cf. Tables 23 and 24) indicated a substantial increase in the emission of mutagenic material when the trap was loaded. In terms of revertants per microgram of extracted material, there was about a 3-6 fold increase in the specific mutagenicity of the extracts. When calculated as revertants per mile of travel, this translated to an 8-25 fold increase in the emission of mutagens, depending on the tester strain used for the assay. This increase is of the same order of magnitude as the decrease produced when the trap is operating properly. These data suggest that when the Volkwagen trap becomes loaded its ability to function is essentially abolished.

Table 32 shows data obtained with particles collected during the regeneration of the Volkwagen trap during the HFET driving cycle. Comparison with previous data (cf. Tables 23 and 24) on mutagenic activity emitted during the HFET cycle indicated that specific mutagenic activity (revertants per microgram of extract) was increased from 1.5 to 4-fold and emission of mutagens per mile was increased 4 to 14-fold ($p<0.05$, 2-tailed "t" test) during the regeneration period for the Volkwagen trap.

Table 33 presents the results obtained with extracts of particles collected during tests with low aromatic fuel and driving cycles CFTP and HFTP with the oxidizer trap in operation. Only small nonsignificant differences were apparent when these data were compared with results obtained previously with particles collected during use of high aromatic fuel (cf. Tables 23 and 24). With particles collected during the CFTP driving cycle, the revertants per microgram of extract were <u>increased</u> 2 to 3-fold over the values found with high aromatic fuel, but on the basis of revertants per mile of travel, the increase was less than 2-fold. The results with extracts of particles collected during the HFTP cycle were slightly different. There was no significant difference between the specific mutagenic activity (revertants per microgram) of extracts of particles collected when burning high or low aromatic fuel. However, on the basis of revertants per mile of travel, the activity of the extracts from low aromatic fuel particles was 2 to 4-fold <u>lower</u> than the activity of extracts of the high aromatic fuel particles. Statistically, these differences were not significant.

Table 34 presents data obtained with extracts of particles collected during baseline tests with low aromatic fuel, but without the trap in operation. Comparison with

TABLE 31. Mutagenic Activity of a DCM Extract of Diesel Exhaust Particles Collected from a Volkwagen Auto Operating with a Loaded Oxidizer Trap.

Particles were collected during a NYCC driving cycle using baseline high aromatic fuel. Extraction and mutagenesis testing were as previously described. This is sample #1280-162 from the SwRI. The values are as in Table 28.

Revertants per Microgram of Extract ± S.D.

Strain TA98 + S9: 18.4 ± 2.02 (15) TA98 - S9: 25.8 ± 4.15 (12)

Strain TA100 + S9: 15.5 ± 3.05 (12) TA100 - S9: 18.5 ± 7.94 (9)

Revertants per Mile of Travel ± S.D. x 10^{-3}.

Strain TA98 + S9: 845 ± 93.0 TA98 - S9: 1190 ± 191

Strain TA100 + S9: 716 ± 140 TA100 - S9: 854 ± 366

Negative Controls. Values are the mean ± S.D. of the number of spontaneous revertants on triplicate plates which received the DMSO solvent only.

TA98+S9:	42.0 ± 6.08	TA100+S9:	133 ± 24.2
TA98-S9:	36.3 ± 4.93	TA100-S9:	127 ± 18.6

Positive Controls. Values are revertants per microgram of compound as in Table 12.

Strain TA98 + S9 + 2AF: 85.3 ± 14.3
Strain TA98 - S9 + 2NF: 1320 ± 172

Strain TA100 - S9 + MMS: 8.42 ± 3.33

TABLE 32. Mutagenic Activity of DCM Extracts of Diesel Exhaust Particles Collected from a Volkswagen Auto During Oxidizer Trap Regeneration.

Particles were collected during the HFET driving cycle using baseline (high aromatic) fuel. Extraction and mutagenesis testing were as previously described. These are samples #1280-166 and 1280-174 from the SwRI. Values in the Table are as in Table 28.

Revertants per Microgram of Extract ± S.D.

#	TA98 ± S9	TA98 - S9
166	36.2 ± 5.13 (15)	60.8 ± 10.3 (12)
174	53.2 ± 10.9 (12)	71.5 ± 14.4 (12)
	TA100 ± S9	**TA100 - S9**
166	21.0 ± 5.68 (12)	15.1 ± 4.19 (12)
174	31.9 ± 5.04 (12)	25.5 ± 10.5 (9)

Revertants per Mile of Travel ± S.D. x 10^{-3}.

#	TA98 ± S9	TA98 - S9
166	788 ± 112	1320 ± 223
174	554 ± 113	745 ± 150
	TA100 ± S9	**TA100 - S9**
166	457 ± 124	329 ± 91.0
174	332 ± 52.5	266 ± 109

Negative Controls and Positive Controls are as in Table 31.

TABLE 33. Mutagenic Activity of DCM Extracts of Diesel Exhaust Particles Collected from a Volkswagen Auto Equipped with an Oxidizer Trap During Baseline Tests with Low Aromatic Fuel.

Particles were collected during CFTP and HFTP driving cycles using a low aromatic fuel (16.2% Aromatics). Extraction of particles and mutagenesis testing were as previously described. These are samples #1280-178, 1280-186, 1280-182, and 1280-190 from the SwRI. Values in the Table are as in Table 28.

Revertants per Microgram of Extract ± S.D.

#	TA98 + S9	TA98 - S9
178(CFTP)	30.1 ± 2.52 (15)	40.9 ± 6.31 (12)
186(CFTP)	12.6 ± 2.42 (12)	20.5 ± 5.43 (12)
182(HFTP)	12.2 ± 1.52 (15)	16.2 ± 2.48 (15)
190(HFTP)	8.19 ± 1.24 (15)	8.88 ± 1.75 (15)

	TA100 + S9	TA100 - S9
178(CFTP)	18.6 ± 2.56 (15)	10.6 ± 3.12 (12)
186(CFTP)	9.15 ± 1.83 (12)	9.97 ± 3.36 (12)
182(HFTP)	8.02 ± 1.15 (15)	6.97 ± 3.62 (9)
190(HFTP)	4.94 ± 1.72 (15)	5.96 ± 2.29 (12)

Revertants per Mile of Travel ± S.D. x 10^{-3}

#	TA98 + S9	TA98 - S9
178(CFTP)	162 ± 13.6	221 ± 34.1
186(CFTP)	59.0 ± 11.3	97.0 ± 25.6
182(HFTP)	51.0 ± 6.36	67.0 ± 10.2
190(HFTP)	29.0 ± 4.39	31.0 ± 6.11

	TA100 + S9	TA100 - S9
178(CFTP)	100 ± 13.8	57.0 ± 2.32
186(CFTP)	43.0 ± 8.60	47.0 ± 15.8
182(HFTP)	33.0 ± 4.73	29.0 ± 15.1
190(HFTP)	17.0 ± 5.42	21.0 ± 8.07

Table 33., Continued.

Negative Controls.

Values are the mean ± S.D. of the number of spontaneous revertants on triplicate plates which received the DMSO solvent only.

For Sample #178:

TA98+S9:	42.0 ± 6.08	TA100+S9:	133 ± 24.4
TA98-S9:	36.3 ± 4.93	TA100-S9:	127 ± 18.6

For Samples #182,186,190:

TA98+S9:	61.7 ± 3.21	TA100+S9:	154 ± 6.66
TA98-S9:	59.3 ± 5.03	TA100-S9:	144 ± 10.3

Positive Controls

Values are revertants per microgram of compound as in Table 12.

For Sample #178:

Strain TA98 + S9 + 2AF: 85.3 ± 14.3
Strain TA98 - S9 + 2NF: 1320 ± 172

Strain TA100 - S9 + MMS: 8.42 ± 3.33

For Samples #182, 186, & 190:

Strain TA98 + S9 + 2AF: 76.7 ± 4.10
Strain TA98 - S9 + 2NF: 2948 ± 148

Strain TA100 - S9 + MMS: 18.5 ± 5.61

TABLE 34. Mutagenic Activity of DCM Extracts of Diesel Exhaust Particles Collected from a Volkwagen Auto Without an Oxidizer Trap During Baseline Tests with Low Aromatic Fuel.

Particles were collected during CFTP and HFTP driving cycles. Extraction of particles and mutagenesis testing were as previously described. These are samples 1280-194, 1280-202, 1280-198 and 1280-206 from the SwRI. Values in the Table are as in Table 28.

Revertants per Microgram of Extract ± S.D.

#	TA98 ± S9	TA98 - S9
194(CFTP)	8.05 ± 0.936 (15)	9.67 ± 1.48 (15)
202(CFTP)	10.8 ± 1.41 (15)	15.2 ± 3.42 (12)
198(HFTP)	9.04 ± 1.45 (15)	12.8 ± 1.73 (15)
206(HFTP)	10.2 ± 1.32 (15)	21.5 ± 3.41 (12)

	TA100 ± S9	TA100 - S9
194(CFTP)	7.89 ± 1.40 (15)	8.11 ± 1.42 (15)
202(CFTP)	8.26 ± 2.35 (15)	8.84 ± 2.07 (15)
198(HFTP)	13.7 ± 5.41 (9)	8.69 ± 1.11 (15)
206(HFTP)	11.1 ± 3.94 (12)	12.8 ± 2.13 (15)

Revertants per Mile of Travel ± S.D. x 10^{-3}

#	TA98 ± S9	TA98 - S9
194(CFTP)	208 ± 24.2	250 ± 38.3
202(CFTP)	327 ± 42.8	461 ± 104
198(HFTP)	215 ± 34.5	305 ± 41.1
206(HFTP)	188 ± 24.2	397 ± 63.0

	TA100 ± S9	TA100 - S9
194(CFTP)	204 ± 36.2	209 ± 36.6
202(CFTP)	251 ± 71.4	268 ± 62.8
198(HFTP)	325 ± 129	206 ± 26.3
206(HFTP)	205 ± 72.8	199 ± 39.3

TABLE 34, Continued.

Negative Controls.

Values are the mean ± S.D. of the number of spontaneous revertants on triplicate plates which received the DMSO solvent only.

For Sample #194:

TA98+S9:	61.7 ± 3.21	TA100+S9:	154 ± 6.66
TA98-S9:	59.3 ± 5.03	TA100-S9:	144 ± 10.3

For Samples #198,202,206:

TA98+S9:	53.7 ± 5.13	TA100+S9:	177 ± 8.89
TA98-S9:	50.0 ± 7.00	TA100-S9:	176 ± 7.00

Positive Controls.

Values are revertants per microgram of compound, as in Table 12.

For Sample #194:

Strain TA98 + S9 + 2AF: 76.7 ± 4.10
Strain TA98 - S9 + 2NF: 2948 ± 148

Strain TA100 - S9 + MMS: 18.5 ± 5.61

For Samples # 198, 202, 206:

Strain TA98 + S9 + 2AF: 121 ± 6.68
Strain TA98 - S9 + 2NF: 3548 ± 179

Strain TA100 - S9 + MMS: 17.0 ± 2.48

previous data (cf. Tables 23 and 24) indicated slight differences between results obtained with extracts of particles collected during the HFTP cycle when burning high and low aromatic fuels, but most differences were less than 2-fold, and not statistically significant. The results with the extracts of particles collected during the CFTP cycle showed a consistently higher mutagenic activity with the extracts from the high aromatic fuel particles, which was of borderline significance.

Assay of CRC Project VE-1 Samples.

The results obtained with samples collected during the hot-start transient portions of the tests are shown in Tables 35 and 36. The results of the first test suggested a relationship between aromatic content of the fuels and mutagenic activity of the particle extracts. In order to confirm this result, a second test was done. The results of the two tests were in good qualitative agreement. Tables 37 and 38 shows the results obtained with the samples collected during the cold-start and steady-state portions of the tests. A cursory examination of the data indicated that there were substantial differences in mutagenic activity among the samples.

Using the values for revertants/hp-hr, regression lines were calculated using the three fuel characteristics, (aromatic content, sulfur content, and 90% boiling point) as the independent variables. The regression coefficients (slopes) of these lines are given in Table 39. Seven of the regression coefficients of the lines calculated with the percent aromatic content were statistically different from a slope of zero at the 5% level of significance or better, when a 2-tailed test was applied. However, with a one-tailed test (i.e., whether the slope was positive with respect to zero), 12 out of the 16 regression coefficients could be considered as significant at the 5% level. Neither the 90% boiling point nor the percent sulfur content of the test fuels showed such an association with the mutagenic activity of the particle extracts.

DISCUSSION

Studies with the Mercedes Benz Auto. The presence of the oxidizer trap did not materially affect the specific mutagenicity (revertants/microgram) of the particle extracts. Both particulates and extractable material were significantly reduced by

TABLE 35. Mutagenic Activity of SOF of Diesel Exhaust Particles Collected During Hot-Start Transient Portion of CRC Project VE-1.

Values are revertants per microgram of extract ± S.D. and are the slopes of the linear portion of dose-response curves determined over the range 0-1-5-10-20 micrograms (First Test) or 0-5-10-20-40 micrograms (Second Test) per test plate with 3 plates at each concentration.

#	FUEL	Revertants per Microgram TA98+S9	TA98-S9	TA100+S9	TA100-S9
First Test					
53	0.3/15/543	2.79±1.01	2.90±1.79	7.41±3.88	5.02±3.69
69	0.3/41/551	4.40±1.12	7.03±2.99	10.3±3.12	8.40±2.19
77	0.3/31/600	2.73±1.50	5.47±1.77	11.9±10.3	12.4±12.2
86	0.3/41/630	6.80±1.61	8.65±2.97	16.4±12.8	5.35±2.09
96	0.3/16/635	3.05±1.30	2.47±1.26	3.22±1.46	4.34±2.47
114	0.05/31/600	7.23±1.60	19.2±3.14	11.8±3.94	7.62±3.73
130	0.17/31/600	3.51±1.91	11.9±1.95	4.45±3.15	8.99±7.12
143	0.04/10/598	2.60±1.94	7.43±1.50	4.45±3.16	7.24±1.16
141	0.3/31/600	2.50±1.14	3.80±1.94	2.59±1.58	5.88±3.92
161	0.06/41/602	5.00±1.89	12.4±4.03	6.53±2.94	7.49±2.12
Blank Filter		0.677±1.50	1.18±1.04	0.681±2.26	2.58±2.52
Toluene/ethanol		0.00±1.36	0.150±0.547	2.38±3.04	3.68±2.16
Second Test					
53	0.3/15/543	1.79±0.587	3.22±0.824	2.28±1.43	3.20±1.35
69	0.3/41/551	3.71±1.01	5.51±1.47	4.73±2.78	3.74±3.06
77	0.3/31/600	1.98±0.658	2.98±0.980	3.34±1.32	1.80±1.10
86	0.3/41/630	3.65±0.978	6.01±1.08	5.36±1.78	6.85±3.50
96	0.3/16/635	2.00±0.629	2.32±0.723	2.79±1.72	2.44±1.83
114	0.05/31/600	2.15±0.521	7.12±1.13	4.62±1.14	4.72±2.02
130	0.17/31/600	4.14±0.644	10.5±2.17	4.39±2.46	6.48±1.30
143	0.04/10/598	2.28±0.698	4.97±0.819	2.75±2.77	7.23±2.65
141	0.3/31/600	2.87±0.505	5.33±0.886	4.20±1.93	5.83±1.44
161	0.06/41/602	4.06±0.825	8.68±1.73	3.22±2.72	8.53±2.70

TABLE 35, Continued.

Negative Controls for Mutagenesis Tests of Hot Start Extracts.

Values are the mean ± S.D. of the numbers of spontaneous revertants on triplicate plates which received the DMSO solvent only. The Sample Numbers refer to those samples which were tested along with the negative controls.

Sample Numbers	TA98 + S9	TA98 - S9	TA100 + S9	TA100 - S9
First Test:				
53,69,86,96	35.7±3.79	52.7±5.51	155±2.65	163±18.4
77,114,130,143	42.3±4.93	44.3±5.86	178±14.0	159±7.02
141,161,Blank, Toluene/ethanol	104±12.9	98.0±8.89	123±10.5	112±15.0
Second Test:				
53,69,86,96	76.3±7.37	64.7±0.577	309±30.1	285±20.8
77,114	59.0±2.65	57.3±7.09	140±16.1	148±12.7
130,141.143,161	57.0±13.2	52.0±7.00	236±45.2	193±17.6

Positive Controls for Mutagenesis Tests of Hot Start Extracts.

Values are revertants per microgram of test compound, and are the slope ± S.D. of the linear portion of a dose-response curve determined as described above. 2AF = 2-aminofluorene; 2NF = 2-nitrofluorene; MMS = methyl methanesulfonate. Sample Numbers refer to those particle extracts that were tested along with the indicated positive controls.

Sample Numbers	TA98+S9+2AF	TA98-S9+2NF	TA100-S9+MMS
First Test:			
53,69,86,96	284±32.3	3188±929	6.60±1.64
77,114,130,143	326±31.6	5242±414	6.68±1.14
141,161,Blank, Toluene/ethanol	323±86.8	4962±1248	3.40±1.47
Second Test:			
53,69,86,96	214±52.0	4982±307	7.63±5.43
77,114	297±50.7	5755±868	2.87±1.87
130,141,143,161	179±27.7	3730±522	5.67±1.64

TABLE 36. Mutagenic Activity of SOF of Diesel Exhaust Particles Collected During Hot Start Transient Portion of CRC Project VE-1 Expressed as Revertants per Hp-Hr.

Values for mutagenic activity are expressed as the slope (revertants per hp-hr x 10^{-3}) calculated from the results of a dose-response curve obtained over the range of 0-1-5-10-20 micrograms (First test) or 0-5-10-20-40 micrograms (Second test) of nonvolatile extract per test plate, with 3 plates at each concentration. Tests were done with Salmonella strains TA98 and TA100, using the micro modification, with and without the presence of rat liver S9 mix. Fuel composition (FUEL) is expressed as wt% sulfur/vol% aromatics/90% B.P. in °F.

FIRST TEST:

		REVERTANTS PER HP-HR X 10^{-3}			
#	FUEL	TA98+S9	TA98-S9	TA100+S9	TA100-S9
53	0.3/15/543	190±68.8	197±122	504±264	341±251
69	0.3/41/551	484±123	773±329	1130±343	924±241
77	0.3/31/600	300±165	602±195	1313±1137	1361±1346
86	0.3/41/630	748±177	952±327	1804±1408	590±230
96	0.3/16/635	336±143	272±139	354±161	477±271
114	0.05/31/600	492±109	1303±214	802±268	518±254
130	0.17/31/600	221±120	748±123	280±198	392±310
143	0.04/10/598	146±109	416±84.0	249±177	405±64.9
141	0.3/31/600	210±95.8	319±163	218±133	494±329
161	0.06/41/602	305±115	755±246	398±179	457±129
SECOND TEST					
53	0.3/15/543	122±40.0	219±56.0	155±97.2	218±92.0
69	0.3/41/551	408±111	606±162	520±306	411±336
77	0.3/31/600	218±72.4	328±108	367±145	198±121
86	0.3/41/630	402±108	661±119	590±196	754±385
96	0.3/16/635	220±69.2	255±79.5	307±189	268±201
114	0.05/31/600	146±35.4	484±76.8	314±77.5	321±137
130	0.17/31/600	261±40.6	660±137	277±155	408±81.9
143	0.04/10/598	129±39.5	278±45.8	154±155	405±148
141	0.3/31/600	241±42.4	448±74.5	353±162	490±121
161	0.06/41/602	248±50.4	529±105	196±166	520±165

TABLE 37. Mutagenic Activity of SOF of Diesel Exhaust Particles Collected During Cold-Start Transient and Steady-State (Rated Speed, 25% Load) Portions of CRC Project VE-1 Expressed as Revertants per Microgram of Extract.

Values are as in Table 35.

COLD-START TEST:

#	FUEL	Revertants per Microgram ± S.D. TA98+S9	TA98-S9	TA100+S9	TA100-S9
52	0.3/15/543	3.07±0.775	2.67±0.457	2.89±1.51	2.36±1.42
68	0.3/41/551	6.20±0.803	10.1±1.28	13.3±3.15	8.66±1.79
85	0.3/41/630	7.50±1.20	10.3±1.59	13.3±3.94	3.26±1.66
95	0.3/16/635	2.42±0.857	2.49±0.347	1.81±1.07	2.13±1.14
76	0.3/31/600	4.45±0.562	6.68±0.654	7.57±4.00	4.65±2.49
113	0.05/31/600	5.62±0.718	10.1±1.49	7.03±1.72	8.36±3.39
129	0.17/31/600	5.30±1.68	8.07±2.42	7.05±3.57	4.74±3.17
142	0.04/10/598	1.36±0.426	3.23±0.464	2.61±1.01	3.19±0.963
160	0.06/41/602	6.38±1.52	9.26±1.18	8.01±1.82	7.12±2.01
180	0.3/31/600*	2.74±0.689	2.59±0.417	2.46±1.35	4.10±1.50
Blank Filter		0.125±0.291	0.375±0.337	0.00±0.842	0.042±0.271

STEADY-STATE TEST:

55	0.3/15/543	1.65±0.433	2.82±0.799	1.49±1.08	1.00±2.06
71	0.3/41/551	5.34±1.42	9.20±2.11	5.58±1.61	6.04±1.90
88	0.3/41/630	5.27±1.60	5.43±0.974	4.16±2.17	1.48±0.836
98	0.3/16/635	1.93±0.555	2.89±1.13	3.95±1.40	3.94±2.62
79	0.3/31/600	2.49±0.557	3.77±0.852	2.92±1.87	2.30±2.03
116	0.05/31/600	2.21±1.05	5.47±0.945	2.49±2.09	4.88±1.22
132	0.17/31/600	1.65±0.767	1.98±0.555	1.62±1.62	2.16±1.69
145	0.04/10/598	1.04±0.407	1.74±0.379	1.27±1.13	1.08±1.27
158	0.06/41/602	3.24±0.495	6.88±1.55	7.42±1.49	8.27±2.52
168	0.3/31/600*	2.88±1.03	4.67±1.82	7.27±1.16	8.40±2.05
Blank Filter		0.050±0.273	0.225±0.337	0.217±0.666	0.358±0.305

*Samples #168 and #180 are extracts of particles collected during Hot-Start tests, and were included in this series for comparison.

TABLE 37, Continued.

Negative Controls for Mutagenesis Tests of Extracts of Cold Start and Steady State Particles.

Values are the mean ± S.D. of spontaneous revertants on triplicate plates which received the DMSO solvent only. The Sample Numbers refer to those sample extracts tested along with the indicated negative controls.

Sample Numbers	TA98+S9	TA98-S9	TA100+S9	TA100-S9
52,55,68,71	46.0±2.65	32.0±7.94	152±18.4	199±18.2
76,79,85,88	39.7±4.04	30.7±6.43	127±13.2	162±6.51
95,98,108,113	36.3±6.51	41.0±2.00	188±36.6	178±21.4
116,124,132	63.7±9.87	52.7±8.39	200±16.5	164±14.3
142,145,158,160	41.0±7.94	32.0±3.60	115±15.1	137±6.03
168	59.7±7.64	46.7±9.29	175±14.6	182±6.81

Positive Controls for Mutagenesis Tests of Extracts of Cold Start and Steady State Particles.

Values are revertants per microgram of test compound, and are the slope ± S.D. of the linear portion of a dose-response curve determined as described in the Final Report. 2AF = 2-aminofluorene; 2NF = 2-nitrofluorene; MMS = methyl methanesulfonate. Sample Numbers refer to those particle extracts that were tested along with the indicated positive controls.

Sample Numbers	TA98+S9+2AF	TA98-S9+2NF	TA100-S9+MMS
52,55,68,71	274±37.1	5357±651	No Data
76,79,85,88	283±44.9	5106±956	4.70±1.03
95,98,108,113	197±23.4	2886±583	8.73±1.91
116,124,132	200±40.9	2206±602	8.53±1.48
142,145,158,160	322±44.6	3664±227	6.47±1.31
168	242±34.0	4112±1191	5.43±1.39

TABLE 38. Mutagenic Activity of SOF of Diesel Exhaust Particles Collected During Cold-Start and Steady-State Portions of CRC Project VE-1 Expressed as Revertants per Hp-Hr.

Values are revertants per hp-hr x 10^{-3}, as in Table 36.

COLD-START:

#	FUEL	Revertants per Hp-Hr x 10^{-3} TA98+S9	TA98-S9	TA100+S9	TA100-S9
52	0.3/15/543	239±60.3	208±35.6	225±118	184±111
68	0.3/41/551	1240±161	2024±256	2654±630	1732±358
85	0.3/41/630	1575±252	2171±334	2793±827	685±349
95	0.3/16/635	337±119	349±48.6	253±150	298±159
76	0.3/31/600	578±73.0	868±85.0	984±520	604±323
113	0.05/31/600	545±69.6	984±145	682±167	811±329
129	0.17/31/600	509±161	775±232	677±343	455±304
142	0.04/10/598	101±31.6	239±34.3	264±102	236±71.2
160	0.06/41/602	555±132	806±103	697±158	619±175
180	0.3/31/600*	274±68.9	259±41.7	246±135	410±150
	STEADY-STATE				
55	0.3/15/543	198±52.0	338±95.8	179±130	120±247
71	0.3/41/551	908±241	1564±359	949±274	1027±323
88	0.3/41/630	1212±368	1249±224	957±499	340±192
98	0.3/16/635	521±150	780±305	1066±378	1064±708
79	0.3/31/600	423±94.6	641±145	496±318	391±345
116	0.05/31/600	214±102	531±91.7	242±203	473±118
132	0.17/31/600	198±92.0	238±66.7	194±194	259±203
145	0.04/10/598	104±40.7	174±37.9	127±113	108±127
158	0.06/41/602	314±48.0	667±150	720±145	802±244
168	0.3/31/600*	248±88.7	402±157	625±99.7	722±176

*Samples #168 and #180 are extracts of particles collected during Hot-Start tests with number 5 fuel, and are included for reference.

TABLE 39. Regression Coefficients (Slopes) of the Relationships Between Fuel Characteristics and Mutagenic Activity of Exhaust Particle Extracts from CRC Project VE-1.

Values are the regression coefficients ± the standard deviation of revertant values around the calculated regression line. "t" values are the t-statistic of the slope coefficient. "p" values are for 2-tailed test. n.s. = not significant.

Hot Start, First Test

% Aromatic Content: Revertants/hp-hr/% Aromatics x 10^{-3}

TA98+S9	TA98-S9	TA100+S9	TA100-S9
10.0 ± 5.28 t = 2.25 p<0.10; n.s.	19.4 ± 9.69 t = 2.38 p<0.05	25.8 ± 16.8 t = 1.83 p<0.20; n.s.	9.97 ± 10.8 t = 1.10 p<0.20; n.s.

90% Boiling Point: Revertants/hp-hr/90% B.P., ^{o}F x 10^{-3}

1.84 ± 0.313 t = 0.86 p<0.50; n.s.	2.05 ± 0.603 t = 0.50 p<0.70; n.s.	1.53 ± 0.963 t = 0.23 p<0.90; n.s.	-0.759 ± 0.558 t = 0.20 p<0.90; n.s.

Weight-Percent S: Revertants/hp-hr/wt % S x 10^{-3}

308 ± 901 t = 0.58 p<0.60; n.s.	-1216 ± 1562 t = 1.31 p<0.30; n.s.	1756 ± 2506 t = 1.18 p<0.30; n.s.	1015 ± 1450 t = 0.38 p<0.80; n.s.

Hot Start, Second Test

% Aromatics: Revertants/hp-hr/% Aromatics x 10^{-3}

7.07 ± 2.89 t = 2.91 p<0.10; n.s.	12.5 ± 3.34 t = 4.44 p<0.01	8.40 ± 3.99 t = 2.50 p<0.05	8.20 ± 5.01 t = 1.95 p<0.10; n.s.

90% Boiling Point: Revertants/hp-hr/90% B.P., ^{o}F x 10^{-3}

0.477 ± 0.199 t = 0.35 p<0.80; n.s.	0.709 ± 0.298 t = 0.35 p<0.80; n.s.	0.981 ± 0.252 t = 0.57 p<0.60; n.s.	2.11 ± 0.273 t = 1.13 p<0.30; n.s.

Weight-percent S: Revertants/hp-hr/wt% S x 10^{-3}

500 ± 478 t = 1.76 p<0.20; n.s.	-101 ± 8.42 t = 0.20 p<0.90; n.s.	652 ± 611 t = 1.79 p<0.20; n.s.	-94.4 ± 825 t = 0.19 p<0.90; n.s.

TABLE 39, Continued.

Cold-Start Transient Test

% Aromatic Content: Revertants/hp-hr/% Aromatics x 10^{-3}

TA98+S9	TA98-S9	TA100+S9	TA100-S9
31.3 ± 10.8 t = 3.47 p<0.02	48.8 ± 15.3 t = 3.82 p<0.01	60.8 ± 25.2 t = 2.89 p<0.05	27.2 ± 12.4 t = 2.62 p<0.05

Weight-percent S: Revertants/hp-hr/wt % S x 10^{-3}

1631 ± 2288 t = 1.22 p<0.30; n.s.	1867 ± 3598 t = 0.88 p<0.50; n.s.	3441 ± 4779 t = 1.23 p<0.30; n.s.	659 ± 2433 t = 0.46 p<0.70; n.s.

90% Boiling Point: Revertants/hp-hr/90% B.P. ^{o}F x 10^{-3}

1.51 ± 0.851 t = 0.26 p<0.90; n.s.	0.767 ± 1.29 t = 0.087 p<1.0; n.s.	-0.963 ± 1.79 t = 0.079 p<1.0; n.s.	-5.00 ± 0.792 t = 0.92 p<0.40; n.s.

Steady State Test

% Aromatic Content: Revertants/hp-hr/% Aromatics x 10^{-3}

18.1 ± 11.3 t = 1.91 p<0.10; n.s.	25.0 ± 13.1 t = 2.28 p<0.10; n.s.	14.4 ± 12.7 t = 1.36 p<0.30; n.s.	10.9 ± 12.8 t = 1.02 p<0.40; n.s.

Weight-percent S: Revertants/hp-hr/Wt% S x 10^{-3}

1843 ± 1580 t = 1.99 p<0.10; n.s.	1986 ± 2081 t = 1.62 p<0.20; n.s.	1607 ± 1728 t = 1.58 p<0.20; n.s.	639 ± 1899 t = 0.57 p<0.60; n.s.

90% Boiling Point: Revertants/hp-hr/90% B.P. ^{o}F x 10^{-3}

2.35 ± 0.658 t = 0.52 p<0.70; n.s.	-0.261 ± 0.829 t = 0.046 p<1.0; n.s.	4.34 ± 0.640 t = 0.99 p<0.40; n.s.	1.65 ± 0.653 t = 0.37 p<0.80; n.s.

the trap, as was the total mutagenic activity, calculated as revertants per mile of travel (Tables 16 and 17). From these studies it may be concluded that the Mercedes Benz trap is very effective in reducing the emission of particle-associated mutagenic activity. However, the presence of the trap shifts the proportion of hydrocarbons in the various phases of the exhaust stream. With the trap it may be expected that more of the hydrocarbons will be present in the vapor/gas phase of the exhaust (Dorie et al., 1987). In the present study these phases were not examined for mutagenic activity. In spite of this omission, the oxidizer trap can still be considered as an important tool for control of environmental pollution.

Studies with the Volkswagen Auto. The overall results and conclusions, with respect to reduction of particulate emission and particle-associated mutagenic activity are similar to those for the Mercedes Benz Auto. There was no significant change in the specific mutagenic activity of the material extracted from the particulate phase collected with the trap in place compared to that collected without the trap. The trap significantly reduced both total particulate as well as total extractable material. The question of the fate of the hydrocarbons not collected on the filters remains, as in the case of the Mercedes Benz. A further important question, unique to the Volkswagen, is the nature of the particulate phase collected with the trap in place. Since the particles are insoluble in DCM they may represent inorganic material which might act as a carrier for polycyclic hydrocarbons. The nature of these particles should be investigated if it is intended that the use of the Volkswagen trap is to become widespread.

Effect of Fuel Formulation on Mutagenic Activity in Diesel Exhaust. The results of the studies with the CRC VE-1 samples indicating increased mutagenic activity in exhaust particles when burning fuel with high aromatic content are in agreement with previous reports showing increased levels of polynuclear hydrocarbons in exhaust particles with similar fuels (e.g., Candeli et al., 1982). In the present study, differences of up to 10-fold or more in mutagenic activity were seen between fuels having either 10 or 41% aromatic content (Tables 35 and 36). These differences are considerably greater than have been reported by others who have made similar comparisons (e.g., Lewtas, 1982). The latter author reported 2- to 5-fold lower mutagenic activity in exhaust particles (Strain TA98 - S9) when burning 17% aromatic fuel vs 36% aromatic fuel in heavy duty diesel engines. These differences may be partly due to differences in the Ames assay methods, i.e., use of the

micromodification in the present work vs. the use of the standard assay by others. Nevertheless, the results of the studies reported here support the notion that fuel formulation may play an important role in control of environmental pollution by diesel engines. By using quantitative assays for mutagenic activity in diesel exhaust it may be possible to define an optimum fuel formula which will retain desired combustion characteristics while minimizing emission of pollutants by diesel-powered vehicles.

REFERENCES

Badger, G. M. Mode of formation of carcinogens in human environment. Natl. Cancer Inst. Monograph 9:1-16, 1962.

Bagley, S. T., Dorie, L. D., Leddy, D. G., and Johnson, J. H. An Investigation into the Effect of a Ceramic Particle Trap on the Chemical Mutagens in Diesel Exhaust. Health Effects Institute New Investigator Program Report No. 5., Health Effects Institute, Cambridge, MA, January, 1987.

Bernstein, L., Kaldor, J., McCann, J., and Pike, M.C. An empirical approach to the statistical analysis of mutagenesis data from the Salmonella test. Mutation Res. 97:267-281, 1982.

Candeli, A., Morazzi, G., Shapiro, M.A.: PAH content of exhaust gases with different aromatic fraction. In: Mobile Source Emissions Including Polycyclic Organic Species. D. Rondia, M. Cooke, R. Haroz, eds. NATO Advanced Science Inst. Series C, vol. 112. D. Reidel Publ. Co., Dordrecht, Holland, 1982, pp. 29-47.

Dorie, L. D., Bagley, S. T., Leddy, D. G., and Johnson, J. H. Characterization of mutagenic subfractions of diesel exhaust modified by ceramic particulate traps. Environ. Sci. Technol. 21:757-765, 1987.

Green, M.H.L., and Muriel, W.J. Mutagen testing using trp+ reversion in Escherichia coli. Mutation Res. 38:3-32, 1976.

Ham, R. G. Clonal growth of mammalian cells in a chemically defined, synthetic medium. Proc. Natl. Acad. Sci. U.S. 53:288-293, 1965.

Henderson, T.R., Royer, R.E., and Hanson, R.L. Concentration of mutagenic activity in diesel exhaust extracts for chemical analysis. Inhalation Toxicology Research Institute Annual Report (Lovelace Biomedical and Environmental Research Institute) pp. 202-205, December 1980.

Hsie, A.W., Casciano, D.A., Couch, D.B. et al. The use of Chinese hamster ovary cells to quantify specific locus mutation and to determine mutagenicity of chemicals. Mutation Res. 86:193-214, 1981.

Huisingh, J., Bradow, R. Jungers, R., Claxton, L., and Zweidinger, R. Application of bioassay to the characterization of diesel particle emissions. In: Application of Short-Term Bioassay in the Fractionation and Analysis of Complex Environmental Mixtures. M. D. Waters et al., eds. pp. 381-418. Plenum Press, New York, 1978.

Ishinishi, N., Koizumi, A., McClellan, R. O., and Stoeber, W., eds., Carcinogenicity and Mutagenicity of Diesel Engine Exhaust. Elsevier, Amsterdam, 1986.

Kado, N. Y., Langley, D., and Eisenstadt, E. A simple modification of the Salmonella liquid-incubation assay. Increased sensitivity for detecting mutagens in human urine. Mutation Res. 121:25-32, 1983.

Kado, N. Y., Guirguis, G. N., Flessel, C. P., Chan, R. C., Chang, K.-I., and Wesolowski, J. J. Mutagenicity of fine (<2.5 um) airborne particles: Diurnal variation in community air determined by a Salmonella micro preincubation (microsuspension) procedure. Environ. Mutagenesis 8:53-66, 1986.

Kaighn, M. E. Human Liver Cells. In: Tissue Culture: Methods and Applications. P. F. Kruse, Jr., and M. K. Patterson, Jr., eds. pp. 54-58. Academic Press, New York, 1973.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. Protein Measurement with Folin phenol reagent. J. Biol. Chem. 193:265-275, 1951.

Lewtas, J.: Evaluation of motor vehicle and other combustion emissions using short-term genetic bioassays. In: Mobile Source Emissions Including Polycyclic Organic Species. D. Rondia, M. Cooke, R. Haroz, Eds. NATO Advanced Science Inst. Series C, vol. 112. D. Reidel Publ. Co., Dordrecht, Holland, 1982, pp. 165-180.

Manabe, Y., Kinouchi, T., and Ohnishi, Y. Identification and quantification of highly mutagenic nitroacetoxypyrenes and nitrohydroxypyrenes in diesel exhaust particles. Mutation Res. 158:3-18, 1985.

Maron, D. M. and Ames, B. N. Revised methods for the Salmonella mutagenicity test. Mutation Res. 113:173-215, 1983.

Matsushima, T., Sawamura, M., Hara, K., and Sugimura, T. A safe substitute for polychlorinated biphenyls as an inducer of metabolic activation system. in: F.J. DeSerres, J.R. Fouts, J.R. Bend, and R.M. Philpot, (eds.), In Vitro Metabolic Activation in Mutagenesis Testing, pp. 85-88 Elsevier/North Holland, Amsterdam, 1976.

McClellan, R. O. Health Effects of Exposure to Diesel Exhaust Particles. Ann. Rev. Pharmacol. Toxicol. 27:279-300, 1987.

Pierson, W.R., Gorse, R.A., Jr., Szkariat, A.C., Brachaczek, W.W., Japar, S.M., and Lee, F.S.-C. Mutagenicity and chemical characteristics of carbonaceous particulate matter from vehicles on the road. Environ. Sci. Technol. 17:31-44, 1983.

Pitts, J. N., van Cauwenberge, K.A., Grosjean, D. et al. Atmospheric reactions of polycyclic aromatic hydrocarbons: Facile formation of aromatic nitro derivatives. Science 202:515-518, 1978.

Salmeen, I.T., Pero, A.M., Zator, R., Schuetzle, D., and Riley, T.L. Ames assay chromatograms and the identification of mutagens in diesel particle extracts. Environ. Sci. Technol. 18:375-382, 1984.

Schuetzle, D. Sampling of vehicle emissions for chemical analysis and biological testing. Environ. Health Perspec. 47:65-80, 1983.

Schuetzle, D., Jensen, T.E., and Ball, J.C. Polar polynuclear aromatic hydrocarbon derivatives in extracts of particulates: Biological characterization and techniques for chemical analysis. Environment International 11:169-181, 1985.

Tokiwa, H., Nakagawa, R., Morita, K., and Ohnishi, Y. Mutagenicity of nitro derivatives induced by exposure of aromatic compounds to nitrogen dioxide. Mutation Res. 85:195-205, 1981.

Vogel, H.J., and Bonner, D.M. Acetylornithinase of Escherichia coli: partial purification and some properties. J. Biol. Chem. 218:97-106, 1956.

APPENDIX

TO

FINAL REPORT

CONTRACT NUMBER A5-130-33

GENOTOXICITY OF DIESEL EXHAUST PARTICLES AND VAPORS COLLECTED FROM ENGINES WITH AND WITHOUT PARTICULATE TRAP OXIDIZERS

REGENTS OF THE UNIVERSITY OF CALIFORNIA

UNIVERSITY OF CALIFORNIA, IRVINE

JULY 31, 1988

PREPARED FOR CALIFORNIA AIR RESOURCES BOARD

BY

RONALD E. RASMUSSEN, PH.D., PRINCIPAL INVESTIGATOR

TABLE OF CONTENTS

PAGE

List of Tables....78

Acknowledgements....79

Introduction and Summary....80

Experimental....82

Results....86

Discussion and Conclusions....99

References....101

LIST OF TABLES

TABLE PAGE

1. Collection and Extraction of Diesel Exhaust Particles from Heavy Duty Bus Engine....87

2. Comparison of Standard Ames Test with Micro Test Using Sample 34B....90

3. Summary of Microtest Results with Extracts of Particles from Bus Engine....91

4. Mutagenicity or Extracts of Bus Particles Expressed as Revertants per Pound of Fuel Burned....95

5. Mutagenic Action of Extracts of SRM-1650 Particles on ARL-14 Cells....97

6. Mutagenic Action of Extract of Sample 34B on ARL-14 Cells....97

7. Mutagenic Action of Extract of Sample 34B on CHO Cells....98

8. Mutagenic Action of DCM Extract of Sample 37D on CHO Cells....98

ACKNOWLEDGEMENTS

The staff of the Mobile Source Division (MSD) at El Monte were most helpful in collecting exhaust particles and vapor phase material during the dynamometer testing of a diesel bus. In particular, Drs. Amir Tacawy, Leo Zafonte, and William Howell provided equipment and expert consultation in this phase of the project.

INTRODUCTION AND SUMMARY

A diesel bus from the Los Angeles RTD system was equipped with a prototype particulate trap oxidizer and run on a dynamometer at the Mobile Source Division facility at El Monte, CA. The primary objective of the tests was to assess the durability and performance of the trap oxidizer under specified conditions. Testing was done at intervals during revenue service and was discontinued when the prototype trap failed. Because there is considerable question as to whether the trap was ever operating as intended, the results presented in this APPENDIX can only be considered as preliminary, and should not necessarily be used as a guide in estimating the usefulness of particulate trap oxidizers in general.

Exhaust particles were collected from an exhaust dilution tunnel on Pallflex teflon-coated glass fiber filters, and vapor phase material was collected on XAD-2 styrene resin beads. The presence of the trap on the bus affected the total weight of collected particles differently for the high and low sulfur fuels. With low sulfur fuel, the total weight of particles collected during the test run was reduced by about 68%, compared to the weight collected under the same conditions without the trap. On the other hand, when burning high sulfur fuel, the presence of the trap led to a 463% increase in the total weight of particles collected during the test run compared to the weight collected without the trap. The chemical nature of the particles was not examined in this study, however, high sulfur fuels are known to increase emission of sulfates. Further, the results with the bus engine demonstrated that the trap substantially reduced the amount of organic material that could be extracted from the particles with dichloromethane. With either high or low sulfur fuel, the weight of material extractable from particles collected under the same conditions was reduced by more than 90% (high sulfur fuel, 94% reduction; low sulfur fuel, 93% reduction). Thus, even though the total weight of particles collected with the trap in place and burning high sulfur fuel was more than 15 times that collected with low sulfur fuel, the total weight of extractable material was essentially the same (average 10.8 mg for high sulfur fuel vs 12.5 mg for low sulfur fuel, with the same amount of fuel burned in each case).

The specific mutagenic activity (mutants/microgram) of the material extracted from particles collected with the trap in place was as much as 100 times greater than that of material from particles collected without the trap. When mutagenic activity

was related to fuel consumption (mutants/pound of fuel burned), the results appeared to depend on the sulfur content of the fuel. With low sulfur fuel, the presence of the trap reduced the total emission of mutagenic substances by 2-5 fold. However, with high sulfur fuel, this was reversed, and more mutagenic material was emitted when the trap was in place. The explanation for these somewhat unexpected results is complex, and probably depends on several factors. A major problem may be that the test run conditions may have been incompatible with the design parameters of the trap. Consultation with the staff at the MSD and with Vince Pellegrin of the RTD Equipment Engineering Division revealed that trap temperatures during the tests may not have reached the regeneration level. Unburned hydrocarbons, i.e., soot accumulated inside the trap, and temperatures at the outlet reached a maximum of 430°C (800°F) during maximum load and were as low as 60°C (140°F) during idle. In addition, the characteristics of the Detroit Allison engine are such that lubricating oil may, from time to time, enter the exhaust stream.

Since it is well known that mutagenic polycyclic hydrocarbons are formed by pyrolytic synthesis in the range of 500°C to 800°C (900-1500°F) (Badger, 1962), and are destroyed only at the upper end of this range, these observations suggest that the trap may have actually been a source of the mutagenic substances which it was designed to eliminate. When the bus was run in service on a route which consisted of mostly high speed freeway driving, the trap appeared to operate as desired. However, under stop-and-go conditions typical of urban service, the trap appeared to be incapable of self-regeneration. Also, the specific mutagenic activity (mutants/pound of fuel consumed) varied as much as five-fold within a presumably identical set of samples, suggesting that the trap operation was somewhat erratic. Nevertheless, it can be concluded that the presence of the trap did, in some cases, significantly decrease the emission of particle-associated mutagens by the bus engine. Tests of the vapor-phase material collected on XAD-2 resin showed no evidence for mutagenic activity statistically above the background mutation rate of the Salmonella tester strains, using concentrations of the material up to the limit of its solubility. At this writing, further tests of trap efficiency with the bus engine have not been scheduled.

EXPERIMENTAL

The first phase of the project was concerned with collection of samples from a diesel bus undergoing dynamometer testing, periodically during in-service use, at the Mobile Source Division of CARB at El Monte. The original plan included collection of baseline samples without the trap, and then periodic collection of samples after installation of the trap and continuation in service in order to obtain information on the effects of aging on the trap efficiency. Baseline samples were collected without the trap, and after installation of the trap, while burning high or low sulfur diesel fuel. However, during these tests, the trap failed and no further samples were collected. At this time no further tests are scheduled. Those samples that were collected have been tested for mutagenic activity, and the results are presented below.

The test bus was from the Los Angeles RTD fleet and all dynamometer testing and exhaust sample collections were done at the Haagen-Smit Laboratory of the CARB at El Monte CA. Test conditions were as follows:

Bus Engine: Detroit Diesel Allison model 8V-71 TAC.

Exhaust Trap: Johnson-Matthey Model _____, consisting of a wire mesh catalyst system.

Fuels: Baseline fuel was #2 diesel with the following specifications:

Gravity, ^{o}API: 33-37
90% B.P.: 550-610^{o}F
Total Sulfur: 0.02-0.05 wt.%
Aromatics, vol. %: 27% min.
Flashpoint: 130^{o}F min.
Viscosity, cSt @ 100^{o}F; 2.0-3.2.
Cetane number: 42-50.

The High Sulfur Fuel had the following analysis:

Gravity, °API: 35.2
90% B.P.: 588°F
Total Sulfur: 0.244 wt.%
Aromatics, vol.%: 46.3
Flashpoint, °F: 150°
Viscosity, cSt @ 100°F: 2.7
Cetane Number: 47.1

Test Cycles: An "8-mode" test procedure was used during collection of samples for the mutagenesis tests. Each mode lasted 3 min, and were performed in the sequence given. Particles from each mode were not collected separately, but were collected on a single filter over the period of the test procedure (approx. 24 min total time). The modes were as follows:

Mode 1. Engine idle, in neutral.

Mode 2. 75% of full load at 2100 rpm (maximum brake horsepower).

Mode 3. 50% of full load at 2100 rpm.

Mode 4. Engine idle, in gear.

Mode 5. 100% load at maximum torque (approx. 1200 rpm).

Mode 6. 75% load at maximum torque.

Mode 7. 50% load at maximum torque.

Mode 8. 30% load at maximum torque.

Number of tests performed in which particles were collected for Ames testing.

High sulfur fuel with trap: 3
High sulfur fuel without trap: 3

Low sulfur fuel with trap: 3
Low sulfur fuel without trap: 2

Collection of Exhaust Particles. The methods were essentially as described in the main body of the Final Report. In brief, the raw exhaust was diluted approximately 1:10 in a stainless steel dilution tunnel. A constant volume pump was used to drawn a known volume of the diluted exhaust through a 508 x 508 mm teflon-coated glass fiber filter (Pallflex T60A20) held in a specially-constructed holder. Particle weight were determined by weighing the filters before and after collection of the particles. After collection of the particles, the filters were carefully folded and sealed in a nitrogen atmosphere in tedlar bags. The filters were held at refrigerator temperature until transfer to UC Irvine, where they were stored at -40°C until extraction. The extraction methods are described in the main body of the report, and consisted of Soxhlet extraction with dichloromethane, followed by reduction in volume under nitrogen. The solvent was exchanged with dimethylsulfoxide (DMSO) for testing of mutagenic activity.

Collection of Vapor Phase. Styrene resin beads (XAD-2; Rohm and Haas) were used to collect vapor-phase material from the bus engine exhaust. This resin will collect hydrocarbons with 7 or more carbon atoms with reasonably good efficiency, but smaller molecules and highly volatile compounds will not be trapped. A special stainless steel holder which held a cylindrical resin bed approximately 6 inches in diameter and 2 inches deep was constructed at the MSD. The holder had conical end sections, and the central portion containing the resin beads was defined by stainless steel mesh and sealed with neoprene rubber gaskets. Prior to use, the resin was washed 3 times with 5 volumes of glass-distilled water, 3 times with methanol, and 3 times with reagent-grade dichloromethane (DCM). Adequacy of washing was checked by HPLC of a sample of the final DCM wash for the presence of materials having the characteristics of PAH, and also for the presence of mutagenic activity using the Ames test, as decribed below. The washed resin was stored under absolute methanol until use. Immediately before use, the resin holder was filled with the

methanol-resin slurry, allowed to drain thoroughly, and dried with a stream of clean air. Because of mechanical and equipment constraints, the resin holder was not in series with the Pallflex filter used to collect particles from the bus exhaust, but was instead in series with an existing absolute particle filter and a constant-volume sampler. This arrangement allowed sampling of a known volume of exhaust vapor phase, consistent with the flow capabilities of the resin bed. Following the test runs, the resin was recovered and stored dry and in the dark in a refrigerator the original sealed glass containers. The resin samples were transferred to UC Irvine within one week, and there were stored at -40°C until extraction.The XAD-2 resin samples containing vapor phase material were extracted in a manner similar to that for the filters. A weighed portion of the resin was placed in the extraction thimble and extracted with DCM, as above. A nylon mesh held in place with a teflon ring was used to contain the resin in the extraction thimble. The resin was then allowed to drain, but not dry, and extracted with absolute methanol. The extracts were reduced in volume and tested as for the filter extracts. It should be noted that the resin will not retain compounds having appreciable volatility at room temperature. Also, the extraction and mutagenesis testing procedures do not prevent loss of such compounds.

Mammalian Cell Mutagenicity Tests were done on some samples using established procedures (Hsie et al., 1981). Two cell strains were used: the Chinese hamster ovary (CHO) line and a line of rat lung cells, designated ARL-14, established in this laboratory. The cells were maintained in Ham's medium F12 as modified by Kaighn (1973) supplemented with 5% dialysed, heat inactivated fetal bovine serum, and gentamicin (50 micrograms/ml) to minimize microbial contamination. The medium was formulated without thymidine or hypoxanthine, which would interfere with the mutagenesis assays. The procedure followed was the same for both cell lines, with the exception that the mutation expression period for the ARL-14 cells was 14-16 days and that for the CHO cells was 8-10 days. This difference is due to the differing generation times for the cells. The cells were treated as subconfluent monolayers by adding DMSO solutions of the test materials to serum-free medium or buffer, and incubation of the cells for specififed periods. The DMSO concentration never exceeded 1%, and control cultures received medium containing the DMSO vehicle at the same concentration as the treated cultures. The density of the cultures was such that at least 10^6 cells survived the treatment. Following the treatment, a sample of the culture was taken to determine cytotoxicity of the test material, and

the remaining cells maintained in culture for the expression period, with subculture upon reaching confluence. At each subculture, at least 10^6 cells were used to initiate the new culture. To test for mutation, the cells were seeded in 10 replicate dishes at a density of 2.5×10^5 cells per 100 mm dish in complete medium containing 6-thioguanine (6TG) at 10^{-5}M. Plating efficiency was determined by seeding 5 or 10 replicate 60 mm dishes with 200 cells per dish. After 6-8 days (CHO cells) or 8-10 days (ARL-14 cells) the cells were fixed with absolute methanol, stained with crystal violet, and the colonies counted (Hsie et al., 1981). Mutation rate was expressed as the number of 6TG-resistant clones per 10^6 colony-forming cells.

Mutagenesis Testing with the Ames Salmonella System. The methods used for testing and data analysis followed established procedures (Maron and Ames, 1983; Kado et al., 1983, 1986; Bernstein et al., 1982) and are described in detail in the main body of the report. Briefly, dose-response experiments were done using a range of concentrations of the particle extracts, as indicated in the individual tables. The specific mutagenic activity was derived from the slope of the linear portion of the dose-response curves, and is presented in the tables as revertants/microgram of extract or revertants/pound of fuel burned.

RESULTS

Mutagenesis Tests of Extracts of Bus Exhaust Particles.

The schedule of particle collection, extraction, and mutagenesis testing for the bus exhaust particles is given in Table 1. Part 1A gives the dates of particle collection, together with the total weight of particles collected and the particle density on the filters as indicated by comparison with a Kodak photographic gray scale. Part 1B gives the dates of particle extraction and amounts of material recovered. In the case of the low sulfur fuel, the Kodak gray scale estimates and the amounts of extractable material were clearly related to the presence or absence of the oxidizer trap. However, with the high sulfur fuel, the weight of particles collected did not agree with the Kodak gray scale or the amount of extractable material. Less than 1% of the particulate material collected with high sulfur fuel and with the trap in place could be extracted with DCM, while about 42% of the particulate collected without the trap was souble in DCM. The nature of the

TABLE 1. Collection and Extraction of Diesel Exhaust Particles from Heavy Duty Bus Engine.

PART 1A. Dates of Particle Collection and Particle Weights.

Particle weights were determined at the Mobile Source Division, El Monte, by weighing filters before and after collection of particles. Relative particle density on filters is indicated by comparison with a Kodak photographic gray scale chart, over a scale of 0 (white) to 19 (Black).

Test #	Fuel/Trap	Date Coll.	Wt. Coll	Kodak Scale
40D	Hi S/yes	5-9-86	0.89 g	6-7
41D	Hi S/yes	5-12-86	3.6 g	9
42D	Hi S/yes	5-19-86	1.89 g	8
34B	Hi S/no	4-4-86	0.55 g	10
35B	Hi S/no	4-7-86	0.44 g	10
36B	Hi S/no	4-7-86	0.39 g	8
37D	Lo S/yes	5-8-86	0.11 g	8
38D	Lo S/yes	5-8-86	0.02 g	6
39D	Lo S/yes	5-9-86	0.28 g	7-8
45D	Lo S/no	7-31-86	0.57 g	18
46D	Lo S/no	7-31-86	0.30 g	15

PART 1B. Effect of Trap on Weight of Particles Collected.

Values are the mean ± S.D. of the weights of particles.

Fuel/Trap	Particle Weight	With Trap as % of Without
High Sulfur Fuel		
Hi S/yes	2.13 ± 1.37 g	463%
Hi S/no	0.46 ± 0.082 g	--
Low Sulfur Fuel		
Lo S/yes	0.137 ± 0.132	31.5%
Lo S/no	0.435 ± 0.191	--

PART 1C. Extraction of Collected Particles

The weights of nonvolatile extracted material (Wt. Extr.) were determined by gravimetric methods as described in the Experimental section. The amounts of fuel burned (Fuel), in pounds, were calculated from fuel consumption and exhaust dilution data provided by the MSD, and are the amounts producing the fraction of total exhaust which passed through the filtration apparatus, giving rise to the particles which were extracted. The mg of extract per pound of fuel burned (Mg Extr./Fuel) were calculated by dividing the total of nonvolatile material extracted from the particles by the amount of fuel.

Test#/Fuel/Trap	Date Extr.	Wt. Extr.	Fuel	Mg Extr./Fuel
40D/Hi S/yes	3-3-87	12 mg	0.031	391
41D/Hi S/yes	3-4-87	12 mg	0.032	381
42D/Hi S/yes	3-5-87	8.4 mg	0.032	266
34B/Hi S/no	12-4-86	203 mg	0.032*	6375
35B/Hi S/no	2-9-87	210 mg	0.032*	6562
36B/Hi S/no	1-20-87	160 mg	0.032*	5000
37D/Lo S/yes	2-17-87	22 mg	0.033	667
38D/Lo S/yes	2-23-87	7.6 mg	0.032	240
39D/Lo S/yes	3-2-87	8.0 mg	0.035	229
45D/Lo S/no	3-9-87	247 mg	0.024	10,335
46D/Lo S/no	3-10-87	125 mg	0.023	5342

*Estimated

PART 1D. Effect of Trap on Amount of Material Extracted from Collected Particles.

Values are the mean ± S.D. of the weights of extracted material as indicated in Part 1C.

Fuel/Trap	Extr. Material	With Trap as % of Without
Hi S/yes	10.8 ± 2.08	5.65%
Hi S/no	191 ± 27.1	---
Lo S/yes	12.5 ± 8.20	6.72%
Lo S/no	186 ± 86.3	---

particles collected with the trap in place while burning high sulfur fuel was not further investigated. In the case of the low sulfur fuel, there was also a difference in extractable material between particles collected with and without the trap. About 9% of the particle weight collected with the trap was extracted with DCM, while without the trap, about 43% was extractable.

The delay between collection of the particles and extraction may be of concern, however, the National Bureau of Standards guarantees the stability of their SRM-1650 for at least three years, suggesting that change in the chemical makeup proceeds at a slow rate. Also, it has been reported that particle-associated PAH are relatively stable in the dry state and in the dark (Scheutzle, 1983). Once the particles were extracted, mutagenesis testing was done as soon as possible.

A comparison of the standard Ames test and the micro test was made with the extract of sample 34B (high sulfur fuel, no trap), and the results are shown in Table 2. It was clear that the micro test was much more sensitive, and this modification was therefore used in all further tests.

Tables 3 and 4 summarize the data from the tests of bus exhaust particle extracts. In Table 3, Part 3A through 3D are presented the values for specific mutagenic activity, calculated as revertants per microgram of extracted material, together with the correlation coefficients and number of data points used in the calculation, each data point representing a single test plate. It is clear that the specific mutagenic activity of the extracts of particles collected with the trap in place is much greater than that of those collected without the trap. In most cases the correlation coefficients indicate a strong dose-response relationship for the data points included in the calculation. Table 4, Part 4A, shows the data in terms of the amount of fuel consumed during the collection of the particles. When the data are expressed as revertants per pound of fuel burned, the interpretation becomes less straightforward. With the high sulfur fuel, there is an apparent increase in the total mutagenic activity toward strain TA98 when the trap is in place. With strain TA100, there is little difference between mutagenic activity with or without the trap. With low sulfur fuel, it seems clear that the trap reduced the mutagenic activity toward both strains TA98 and TA100, however the differences were statistically marginal. Statistical comparison of the data in Part 6B showed only a few significant differences. The most active material was that from particles

TABLE 2. Comparison of Standard Ames Test with Micro Test Using Sample 34B (Hi S, No Trap).

Values are revertants per microgram of extract, calculated on the basis of dose-response curves obtained as desribed in the Experimental section. The numbers in parentheses associated with each value are the concentrations, in micrograms per plate, of the material used in the test which gave revertant values which were used to define the linear protion of the dose-response curve, as described in the Experimental section. Three test plates were prepared at each concentration. 2AF = 2-aminofluorene; 2NF = 2-nirtrofluorene; MMS = methyl methanesulfonate.

Standard Ames Test

Tester Strain	Revertants per Microgram
TA98 + S9	0.433 ± 0.233 (0-10-25-50)
TA98 - S9	0.500 ± 0.228 (")
TA100 + S9	0.943 ± 0.380 (")
TA100 - S9	1.35 ± 0.942 (0-10-25)

Positive Controls for Standard Test.

TA98 + S9 + 2AF: 272 ± 67.5 (0-0.1-1-5)
TA98 - S9 + 2NF: 160 ± 16.7 (0-0.1-1-5-10)

TA100 - S9 + MMS: 11.2 ± 0.882 (0-1-5-10-20)

Negative Controls for Standard Test.

TA98 + DMSO + S9: 34.3 ± 2.52 TA100 + DMSO + S9: 103 ± 9.85
TA98 + DMSO - S9: 21.3 ± 7.57 TA100 + DMSO - S9: 104 ± 8.62

Micro Ames Test

Tester Strain	Revertants per Microgram
TA98 + S9:	3.76 ± 1.87 (0-1-2.5-5)
TA98 - S9:	3.61 ± 3.14 (")
TA100 + S9	13.7 ± 9.81 (0-1-2.5)
TA100 - S9	12.0 ± 9.41 (")

Positive Controls for Microtest

TA98 + S9 + 2AF: 814 ± 223 (0-0.005-0.05-0.25)
TA98 - S9 + 2NF: 13610 ± 1454 (0-0.005-0.05)

TA100 - S9 + MMS: 11.8 ± 6.00 (0-1-5)

Negative Controls for Microtest

TA98 + S9: 25.0 ± 2.65 TA100 + S9: 102 ± 9.71
TA98 - S9: 22.7 ± 1.53 TA100 - S9: 110 ± 11.2

TABLE 3. Summary of Microtest Results with Extracts of Particles Collected from Bus Engine.

The values for mutagenic activity are revertants/microgram of extract ± one standard deviation, determined from dose-response curves as described in the Experimental section. The concentrations used for determination of the dose-response were 0, 10, 25, 50, and 75 micrograms/plate, with 3 plates at each concentration. The values for "n" represent the number of plates used in calculation of the dose-response curves, and also indicate the concentration range involved. Thus, an "n" of 6 indicates 0-10 micrograms; "n" of 9 indicates 0-25 micrograms; "n" of 12 indicates 0-50 micrograms; and "n" of 15 indicates 0-75 micrograms. The "r" value is the correlation coefficient for the calculated slope. "Blank" values are revertants per plate ± 1 S. D., based on triplicate plates which received the DMSO vehicle only.

PART 3A. Results with Strain TA98 with S9.

Sample	Fuel/Trap	Revs./ug ± S.D.	r	n	Blank ± S.D.
40D	Hi S/yes	117 ± 11.7	0.997	6	28.3 ± 1.53
41D	Hi S/yes	90.4 ± 12.1	0.994	6	35.0 ± 5.29
42D	Hi S/yes	61.1 ± 17.1	0.975	6	35.0 ± 5.29
34B	Hi S/no	0.531 ± 0.218	0.913	15	38.0 ± 6.24
35B	Hi S/no	1.18 ± 0.277	0.967	15	44.7 ± 2.52
36B	Hi S/no	1.15 ± 0.336	0.954	15	39.3 ± 2.52
37D	Lo S/yes	75.5 ± 8.17	0.996	6	37.7 ± 6.51
38D	Lo S/yes	30.6 ± 4.29	0.994	6	40.3 ± 7.09
39D	Lo S/yes	55.5 ± 3.58	0.998	6	28.3 ± 1.53
45D	Lo S/no	9.35 ± 2.67	0.961	9	23.7 ± 5.03
46D	Lo S/no	10.8 ± 4.41	0.950	6	23.7 ± 5.03

Positive Controls. All values are revertants per microgram ± S.D. obtained with strain TA98 with S9 and 2AF. "Associated Samples" are the samples assayed at the same time as the positive controls. Other notations are as in Table 2.

Associated Sample(s)	Revertants per Microgram
34B (Hi S/no)	593 ± 126 (0-0.25)
35B (Hi S/no)	639 ± 94.7 (0-0.005-0.05-0.25)
36B (Hi S/no)	469 ± 138 (0-0.005-0.05-0.25)
37D (Lo S/yes)	581 ± 154 (0-0.005-0.05-0.25)
38D (Lo S/yes)	484 ± 134 (0-0.25)
39D (Lo S/yes) and 40D (Hi S/yes)	428 ± 66.4 (0-0.25)
41D and 42D (Hi S/yes)	533 ± 78.5 (0-0.25)
45D and 46D (Lo S/no)	1055 ± 143 (0-0.25)

PART 3B. Results with Strain TA98 Without S9.

Sample	Fuel/Trap	Revs./ug ± S.D.	r	n	Blank ± S.D.
40D	Hi S/yes	82.1 ± 7.57	0.997	6	23.7 ± 5.51
41D	Hi S/yes	67.8 ± 2.14	0.999	6	30.3 ± 3.21
42D	Hi S/yes	55.3 ± 1.35	0.999	6	30.3 ± 3.21
34B	Hi S/no	0.664 ± 0.258	0.921	15	30.0 ± 1.00
35B	Hi S/no	1.03 ± 0.215	0.977	15	46.0 ± 5.57
36B	Hi S/no	1.33 ± 0.238	0.982	15	39.0 ± 5.20
37D	Lo S/yes	72.3 ± 3.64	0.999	6	27.7 ± 5.51
38D	Lo S/yes	34.3 ± 7.19	0.986	6	31.3 ± 4.16
39D	Lo S/yes	53.5 ± 8.10	0.993	6	23.7 ± 5.51
45D	Lo S/no	12.6 ± 4.11	0.961	6	21.7 ± 3.21
46D	Lo S/no	8.50 ± 3.24	0.955	6	21.7 ± 3.21

Positive Controls. All values are revertants per microgram ± S.D. obtained with strain TA98 without S9 with 2NF. "Associated Samples" are as in Part 5A.

Associated Sample(s)	Revertants per Microgram
34B (Hi S/no)	1455 ± 129 (0-0.25)
35B (Hi S/no)	8548 ± 809 (0-0.01-0.1-0.5)
36B (Hi S/no)	11710 ± 927 (0-0.005-0.05)
37D (Lo S/yes)	13376 ± 1438 (0-0.005-0.05-0.25)
38D (Lo S/yes)	12227 ± 1839 (0-0.05)
39D (Lo S/yes) and 40D (Hi S/yes)	9267 ± 720 (0-0.05)
41D and 42D (Hi S/yes)	9553 ± 1957 (0-0.05)
45D and 46D (Lo S/no)	10040 ± 982 (0-0.05)

PART 3C. Results with Strain TA100 With S9

Sample	Fuel/Trap	Revs./ug ± S.D.	r	n	Blank ± S.D.
40D	Hi S/yes	128 ± 5.96	0.999	6	101 ± 3.51
41D	Hi S/yes	78.6 ± 20.2	0.978	6	103 ± 1.53
42D	Hi S/yes	51.3 ± 30.0	0.902	6	103 ± 1.53
34B	Hi S/no	0.834 ± 0.772	0.704	15	127 ± 5.13
35B	Hi S/no	1.05 ± 0.803	0.786	12	112 ± 3.06
36B	Hi S/no	2.71 ± 2.27	0.766	9	142 ± 31.2
37D	Lo S/yes	47.7 ± 7.29	0.993	6	74.7 ± 10.0
38D	Lo S/yes	39.5 ± 5.63	0.994	6	70.7 ± 26.1
39D	Lo S/yes	32.6 ± 15.1	0.936	6	101 ± 3.51
45D	Lo S/no	5.80 ± 5.40	0.796	6	90.7 ± 4.93
46D	Lo S/no	3.04 ± 2.32	0.794	9	90.7 ± 4.93

Positive Controls

No positive controls for TA100 + S9 were done.

PART 3D. Results with Strain TA100 Without S9.

Sample	Fuel/Trap	Revs./ug ± S.D.	r	n	Blank ± S.D.
40D	Hi S/yes	16.1 ± 8.86	0.911	6	96.0 ± 12.1
41D	Hi S/yes	16.3 ± 4.72	0.961	9	115 ± 7.09
42D	Hi S/yes	31.3 ± 31.4	0.774	6	115 ± 7.09
34B	Hi S/no	1.29 ± 1.00	0.788	9	125 ± 12.5
35B	Hi S/no	1.25 ± 0.915	0.806	9	133 ± 10.0
36B	Hi S/no	2.71 ± 2.27	0.630	6	147 ± 11.1
37D	Lo S/yes	20.3 ± 3.67	0.984	9	128 ± 6.03
38D	Lo S/yes	23.5 ± 3.43	0.993	6	66.3 ± 14.6
39D	Lo S/yes	16.8 ± 6.95	0.947	6	96.0 ± 12.1
45D	Lo S/no	2.00 ± 0.673	0.945	12	114 ± 9.54
46D	Lo S/no	3.21 ± 1.33	0.905	9	114 ± 9.54

Positive Controls. Values are revertants per microgram ± S.D. obtained with strain TA100 without S9 with MMS. "Associated Samples" are as in Part 5A.

Associated Sample(s)	Revertants per Microgram
34B (Hi S/no)	69.7 ± 23.0 (0-1)
35B (Hi S/no)	19.1 ± 11.2 (0-0.75-1.5)
36B (Hi S/no)	28.0 ± 20.8 (0-0.1-0.5-1.0)
37D (Lo S/yes)	19.8 ± 38.2 (0-0.015-0.15-0.75-1.5)
38D (Lo S/yes)	39.3 ± 5.41 (0-5)
39D (Lo S/yes) and 40D (Hi S/yes)	27.4 ± 5.08 (0-5)
41D and 42D (Hi S/yes)	64.3 ± 10.8 (0-5)
45D and 46D (Lo S/no)	12.2 ± 4.71 (0-5)

TABLE 4. Mutagenic Activity in Extracts of Particles Collected from Bus Exhaust Expressed as Revertants/Pound of Fuel Consumed.

The values were calculated using the values for mg of material per mile (Table 1) and the revertants per microgram given in Table 3.

Part 4A. Values for Revertants/Pound of Fuel Burned by Bus Engine.

Revertants per Pound ± S.D. x 10^{-6}

Sample/Group	TA98 + S9	TA98 - S9	TA100 + S9	TA100 - S9
40D/Hi S/yes	45.7 ± 4.57	32.1 ± 2.96	50.0 ± 2.33	6.30 ± 3.46
41D/Hi S/yes	34.4 ± 4.61	25.8 ± 0.815	29.9 ± 7.70	6.21 ± 1.80
42D/Hi S/yes	16.2 ± 4.55	14.7 ± 0.359	13.6 ± 7.98	8.33 ± 8.35
34B/Hi S/no	3.38 ± 1.39	4.23 ± 1.64	5.31 ± 4.92	8.22 ± 6.37
35B/Hi S/no	7.75 ± 1.82	6.76 ± 1.41	6.89 ± 5.27	8.19 ± 6.00
36B/Hi S/no	5.75 ± 1.68	6.65 ± 1.19	13.6 ± 11.4	11.9 ± 17.8
37D/Lo S/yes	50.4 ± 5.45	48.2 ± 2.43	31.8 ± 4.86	13.5 ± 2.45
38D/Lo S/yes	7.34 ± 1.03	8.23 ± 1.73	9.48 ± 1.35	5.64 ± 0.823
39D/Lo S/yes	12.7 ± 0.820	12.2 ± 1.85	7.46 ± 3.46	3.85 ± 1.59
45D/Lo S/no	96.6 ± 27.6	130 ± 42.5	59.9 ± 55.8	20.7 ± 6.96
46D/Lo S/no	57.7 ± 23.6	45.4 ± 17.3	16.2 ± 12.4	17.1 ± 7.10

PART 4B. Means ± S.D. of values for revertants/pound of fuel from Part 4A.

Sample Group	TA98 ± S9	TA98 - S9	TA100 ± S9	TA100 - S9
40D.41D,42D Hi S, + Trap	32.1 ± 14.9[a]	24.2 ± 8.81[a]	31.2 ± 18.2[a]	6.95 ± 5.32
34B,35B,36B Hi S, - Trap	5.63 ± 2.19	5.88 ± 1.43	8.60 ± 7.79	9.44 ± 11.4
37D,38D,39D Lo S, + Trap	23.5 ± 23.5	22.9 ± 22.0	16.2 ± 13.5	7.66 ± 5.13
45D,46D Lo S, - Trap	77.2 ± 27.5[b]	87.7 ± 59.8[c]	38.1 ± 40.4	18.9 ± 7.03

Statistical comparisons are for data obtained with the individual tester strains, and not between tester strains.

[a]Greater than Hi S, + trap; $p<0.05$.

[b]Greater than Lo S + trap, Hi S + trap, and Hi S - trap; $p<0.05$.

[c]Greater than Hi S - trap; $p<0.05$.

Other differences seen within each tester strain are not significant.

collected with low sulfur fuel, without the trap. The material extracted from particles collected with high sulfur fuel, with the trap was significantly more active that that from particles collected without the trap while burning this fuel. In summary, it is very clear that the oxidizer trap reduced both the total particulate emissions as well as the amount of material extractable from the particles. On the other hand, the specific mutagenic activity of the material extracted from the particles collected with the trap in place was much greater than that from particles collected without the trap. On balance, the total emission of mutagenic material seems to be reduced by the trap, but, given the variability of the mutagenesis test results, further studies may be necessary to firmly establish this conclusion.

Studies with Mammalian Cells.

The DCM and methanol extracts of the SRM-1650 particles were tested for mutagenic activity using the ARL-14 cell line. Cells were exposed to the extracts for 6 hr, followed by subculture and testing for 6TG resistance as described. The results are shown in Table 5. A modest level of mutagenic activity was present in the DCM extracts, but no significant activity was detected in the methanol extracts. The DCM extract was fractionated into aromatic and aliphatic subfractions, and these tested in the ARL-14 line. The aromatic fraction showed no mutagenic activity at a concentration of 1 microgram/ml, and was lethal to the cells at 5 micrograms/ml. The aliphatic fraction showed no mutagenic or cytotoxic action up to the limit of its solubility in the culture medium, approximately 10 micrograms/ml.

The DCM extract of sample 34B (high sulfur, no trap) was tested for toxicity and mutagenic activity with cell line ARL-14, and the results are given in Table 6. To provide metabolic activation for the extract, S9 mix was used as the treatment medium. The liver S9 protein concentration was 0.5 mg/ml. The extract was of low toxicity, and showed a low, but measureable level of mutagenic activity. As a positive control, benzo(a)pyrene (BaP) was included in the test.

The DCM extract of sample 34B (high sulfur, no trap) was also tested in cell line CHO with S9 mix to provide metabolic activation. The results, shown in Table 7, indicated a low level of mutagenic activity at a concentration of 25 micrograms/ml, comparable to the activity of BaP at 1 microgram/ml. At higher concentrations of

TABLE 5. Mutagenic Action of Extracts of SRM 1650 Particles on ARL-14 Cells.

Cell cultures were exposed for 6 hr to the indicated concentrations of extracts and mutation to 6-thioguanine (6TG) resistance assayed as described in the Experimental section. The results shown are from a single experiment. The values are the calculated mutant clones per 10^6 viable cells at the time of mutant assay and are the mean ± S.D. of 10 replicate dishes.

Treatment	Mutant Clones/10^6 Viable Cells
DMSO, 0.005 ml/ml	29 ± 4.7
DCM Extract, 5 ug/ml	43 ± 6.6
DCM Extract, 10 ug/ml	75 ± 22
Methanol Ext., 5 ug/ml	6.5 ± 0.95
Methanol Ext., 10 ug/ml	31 ± 2.9

TABLE 6. Mutagenic Action of DCM Extract of Sample 34B on ARL-14 Cells.

Cell cultures were exposed for 2 hr to the indicated material in the presence of S9 mix (0.5 mg protein/ml) and assayed for survival and mutation as described in the Experimental section. The values are the mean ± S.D. of 5 replicate dishes for the survival assay and 10 replicate dishes for the mutation assay. Values for survival are for immediately after treatment. Mutation rate is expressed as mutants/ 10^6 viable cells at the time of mutant assay.

Treatment	Relative Survival	Mutation Rate
No Treatment	1.0	4.9 ± 5.7
S9 + 5 ul DMSO/ml	0.46 ± 0.12	<1
5 ug BaP/ml	0.23 ± 0.07	9.4 ± 9.4
10 ug BaP/ml	0.24 ± 0.03	16 ± 14
25 ug 34B extract/ml	0.34 ± 0.08	3.4 ± 5.9
50 ug 34B extract/ml	0.49 ± 0.11	13 ± 7.3

TABLE 7. Mutagenic Action of Extract of Sample 34B on CHO Cells.

CHO cell cultures were treated for 2 hr with the indicated materials in the presence of S9 mix (0.5 mg protein/ml) and assayed for survival and mutation as described in the Experimental section. Relative survival values are for immediately after treatment. Mutation rate is expressed as 6TG-resistant clones per 10^6 viable cells at the time of assay. Values are the mean ± S.D., and are based on 10 replicate dishes for each treatment.

Treatment	Relative Survival	Mutation Rate
S9 + 5 ul DMSO/ml	1.0	8.1 ± 5.6
0.5 ug BaP/ml	1.2 ± 0.094	8.4 ± 5.8
1.0 ug BaP/ml	1.0 ± 0.12	21 ± 5.4
25 ug extract/ml	1.0 ± 0.076	24 ± 11
50 ug extract/ml	0.96 ± 0.066	7.2 ± 5.7
100 ug extract/ml	1.2 ± 0.10	2.2 ± 3.8

TABLE 8. Mutagenic Action of DCM Extract of Sample 37D on CHO Cells.

CHO cell cultures were treated either for 3 hr without S9 or for 2 hr with S9 mix together with the indicated materials. Values are as in Table 9.

Treatment Without S9	Relative Survival	Mutation Rate
Untreated	1.0	17 ± 8.6
5 ul DMSO/ml	1.1 ± 0.13	14 ± 8.7
1 ul Ethyl Methanesulfonate/ml	0.72 ± 0.07	393 ± 46*
25 ug Extract/ml	0.97 ± 0.04	15 ± 9.4
50 ug Extract/ml	0.94 ± 0.08	17 ± 9.1
100 ug Extract/ml	1.1 ± 0.08	20 ± 13

*Greater than DMSO control; $p<0.005$. Other values are not significantly different from control.

Treatment With S9		
5 ul DMSO/ml	1.0	9.2 ± 5.1
5 ul Dimethylnitrosamine/ml	0.54 ± 0.08	57 ± 28*
25 ug Extract/ml	0.80 ± 0.11	23 ± 13
50 ug Extract/ml	0.68 ± 0.07	29 ± 8.6
100 ug Extract/ml	0.97 ± 0.12	31 ± 12

the extract, a lower mutagenic activity was observed, which is unexplained. There was no immediate toxic effect of the extract.

The DCM extract of sample 37D (low sulfur, with trap) was tested for mutagenic activity in cell line CHO with and without S9 mix for metabolic activation, and the results are given in Table 8. Without S9 mix, no mutagenic or toxic effects were seen, up to a concentration of 100 micrograms of extract/ml, which was greater than the solubility of the extract in the test medium. In the presence of S9 mix, a low level of mutagenic activity was seen. Dimethylnitrosamine (DMNA), with S9, and ethyl methanesulfonate (EMS) without S9 were included as positive controls. DMNA and EMS are usually considered to be relatively weak mutagens.

Because of the low activity shown by the extracts in the mammalian cells, and the number of samples requiring assay, a decision was made to concentrate on the bacterial mutagenesis assays. Consequently, no further tests were done with particle extracts on mammalian cells. No tests were done using vapor phase material collected on XAD-2 resin.

DISCUSSION AND CONCLUSIONS

The results of the above studies suggest that the presence of an oxidizer trap in the exhaust system may be capable of reducing the total particulate emission as well as the amount of DCM-extractable material by 90% or more, when measured in relation to pounds of fuel burned. However, because of the failure of the trap during the test runs, no firm conclusions can be drawn. Nevertheless, there are some points of potential interest. A possibly significant finding in this work was that the specific mutagenic activity of the material extracted from the particles collected with the trap in place, calculated as Salmonella revertants/mg of extract, was actually increased compared to the extracts of particles collected without the trap. For example, in the case of the high sulfur fuel, the specific mutagenic activity, measured with strain TA98 + S9, of the material extracted from particles collected with the trap in place was more than 40 times that of extracts of particles collected without the trap (see Table 3). This increased specific mutagenic activity of material extracted from particles collected with the trap in place was seen in all tests, although not to the same degree. The explanation for this observation appears to be related to the test conditions, the characteristics of the

bus engine, and the trap design. Consultation with the staff at the MSD and with Vince Pellegrin of the LARTD Equipment Engineering Division indicated that the internal trap temperature during the tests may not have reached the regeneration level. Measurements at the exhaust outlet reached a maximum of 430°C (800°F) under maximum test load, and were as low as 60°C (140°F) at idle, suggesting that temperatures within the trap may have been in the range known to favor pyrolytic synthesis of polynuclear aromatic hydrocarbons (500-800°C) (Badger, 1962). The observation that soot accumulated inside the trap during the tests supports this conjecture. Also, the design of Detroit Allison diesel engine may have permitted significant amounts of lubricating oil to enter the exhaust stream, providing a source of precursors for pyrolytic synthesis. Together, these observations suggest that the trap may actually have been a source for mutagenic materials during the tests. Under sustained high load conditions, the trap may operate as intended. When the bus was put in service on a route which consisted mostly of high speed freeway driving, the trap appeared to operate as intended. Thus, the results of the dynamometer tests, with respect to emission of mutagenic materials, must be considered inconclusive. The reduced emission of particulate material and increased specific mutagenic activity tended to cancel each other out, leaving a modest overall reduction in the total mutagenic activity emitted with the trap in place. However, compared to the automobile exhaust traps, the performance of the bus trap was not impressive.

00002881
ASSET

REFERENCES

Badger, G. M. Mode of formation of carcinogens in human environment. Natl. Cancer Inst. Monograph 9:1-16, 1962.

Bernstein, L., Kaldor, J., McCann, J., and Pike, M.C. An empirical approach to the statistical analysis of mutagenesis data from the Salmonella test. Mutation Res. 97:267-281, 1982.

Ham, R. G. Clonal growth of mammalian cells in a chemically defined, synthetic medium. Proc. Natl. Acad. Sci. U.S. 53:288-293, 1965.

Hsie, A.W., Casciano, D.A., Couch, D.B. et al. The use of Chinese hamster ovary cells to quantify specific locus mutation and to determine mutagenicity of chemicals. Mutation Res. 86:193-214, 1981.

Kado, N. Y., Langley, D., and Eisenstadt, E. A simple modification of the Salmonella liquid-incubation assay. Increased sensitivity for detecting mutagens in human urine. Mutation Res. 121:25-32, 1983.

Kado, N. Y., Guirguis, G. N., Flessel, C. P., Chan, R. C., Chang, K.-I., and Wesolowski, J. J. Mutagenicity of fine (<2.5 um) airborne particles: Diurnal variation in community air determined by a Salmonella micro preincubation (microsuspension) procedure. Environ. Mutagenesis 8:53-66, 1986.

Kaighn, M. E. Human Liver Cells. In: Tissue Culture: Methods and Applications. P. F. Kruse, Jr., and M. K. Patterson, Jr., eds. pp. 54-58. Academic Press, New York, 1973.

Maron, D. M. and Ames, B. N. Revised methods for the Salmonella mutagenicity test. Mutation Res. 113:173-215, 1983.

Schuetzle, D. Sampling of vehicle emissions for chemical analysis and biological testing. Environ. Health Perspec. 47:65-80, 1983.

CARB LIBRARY
07163