United States
Environmental Protection
Agency

Research and Development

EPA

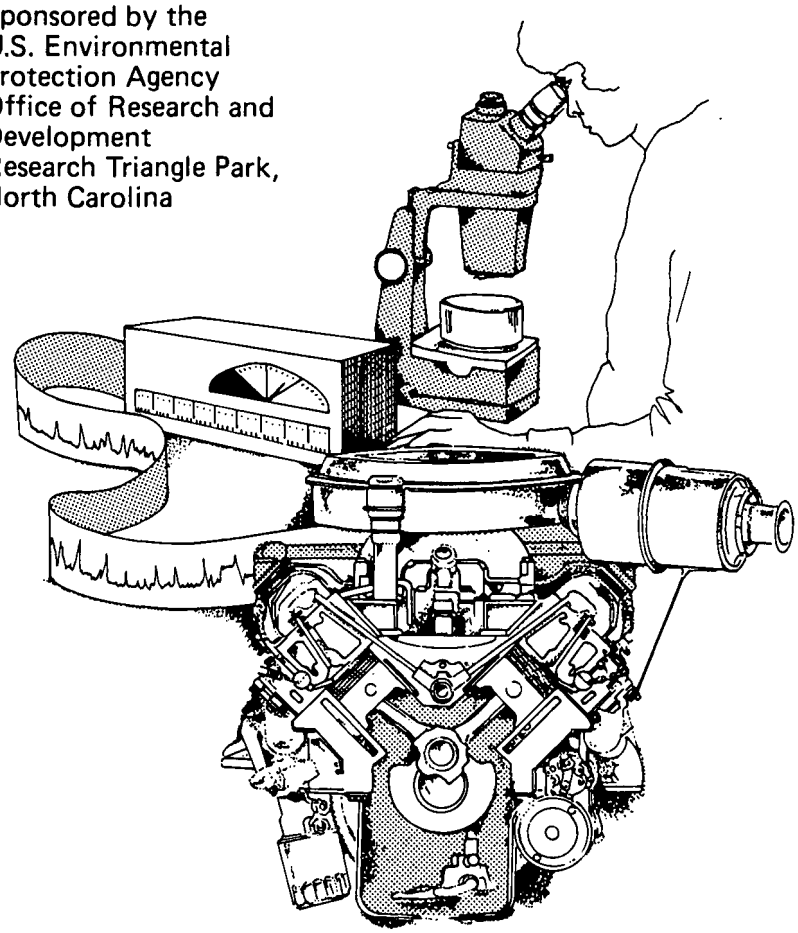
1981 DIESEL EMISSIONS SYMPOSIUM

Registration and Abstract Book

October 5-7, 1981

The Royal Villa Hotel
Raleigh, North Carolina

Sponsored by the
U.S. Environmental
Protection Agency
Office of Research and
Development
Research Triangle Park,
North Carolina



Foreword

The high fuel efficiency of diesel engines is expected to result in a significant increase in the production of diesel-powered passenger cars. Major research programs were initiated in the late 1970s by governments, industry, and the academic community in order to understand the physical and chemical characteristics of emissions from the diesel engine, and the potential biological effects of these emissions.

In December 1979 the U.S. Environmental Protection Agency Health Effects Laboratory at Cincinnati, Ohio, sponsored the first symposium on the Health Effects of Diesel Engine Emissions. The purpose of this 1981 symposium, sponsored by the Office of Research and Development, is to foster exchange of the more recent scientific and technical information derived from the various research programs.

This registration volume contains a compilation of extended abstracts of each presentation to be made at the 1981 Diesel Emissions Symposium on October 5-7 at Raleigh, North Carolina. The symposium is organized into the following sessions:

- Diesel Emissions Characterization and Control Technology
- Chemical and Bioassay Characterization
- Pulmonary Function
- Pulmonary Toxicology and Biochemistry
- Mutagenesis and Carcinogenesis
- Exposure and Risk Assessment

The 96 short papers included here represent the oral and poster presentations. The invited overview presentations and selected papers will be published after the meeting to provide an in-depth review of each of these topics.

F. Gordon Hueter
Director
Health Effects Research Laboratory
U.S. Environmental Protection Agency
Research Triangle Park, NC

Acknowledgements

The assistance of the many individuals who contributed to the planning of this symposium is gratefully acknowledged. Special appreciation is due to Ms. Olga Wierbicki and Ms. Barbara Elkins of Northrop Services who serve as symposium coordinators.

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PROGRAM

October 5: Session 1: Diesel Emissions Characterization and Control Technology
Session 2: Chemical and Bioassay Characterization

Poster Session 1

October 6: Session 3: Pulmonary Function
Session 4: Pulmonary Toxicology and Biochemistry

Poster Session 2

October 7: Session 5: Mutagenesis and Carcinogenesis
Session 6: Exposure and Risk Assessment

ABSTRACTS

The abstracts of the oral and poster presentations appear in the order that they are listed in the program.

General Information

GENERAL CHAIRMAN	James Smith, Acting Director, RASSO, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711, (919) 541-2909.
ORGANIZING CHAIRMAN	Joellen Lewtas, Health Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711, (919) 541-3849.
ORGANIZING COMMITTEE	Stephen Nesnow, Larry Claxton, and Ronald Bradow, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711.
SYMPOSIUM COORDINATORS	Olga Wierbicki and Barbara Elkins, Northrop Services, Inc., P.O. Box 12313, Research Triangle Park, NC 27709, (919) 549-0411. The Symposium Coordinators and their staff will be happy to help you in case you have any questions or problems.
LOCATION	The symposium is being hosted at the Royal Villa Hotel and Convention Center, 5339 Glenwood Avenue, Raleigh, NC 27662, (919) 782-4433.
REGISTRATION	All participants are asked to register either Sunday, October 4, 7-9 pm, in the Main Hall or Monday, October 5, starting at 7:30 am, also in the Main Hall. Participants arriving after commencement of the symposium should register on arrival.
LUNCHEONS	A luncheon has been catered for all three days. Participants are encouraged to sign up for these luncheons due to the limits of time and available restaurants. The meal fee is $37.00. This fee also includes your coffee/tea. Those participating for one day only may pay $13.00 for lunch and coffee. The meals will be in Royal King's Hall III.
COFFEE/TEA	Coffee, tea, and sanka will be provided for the participants. Soda will also be available for the afternoon break. Anyone who has not paid a meal fee but wants coffee, etc., is asked to pay a fee of $10.00 for the three days. Those here for only one day are asked to contribute $5.00 for coffee.

SYMPOSIUM PUBLICATIONS	An abstract book will be distributed to each attendee at the time of the symposium. A proceedings will be published in book form consisting of the overview presentations and selected papers. This proceedings is available at a discount for $35.00 if it is ordered at the time of the symposium. Participants may order a copy at the registration table.
CHECKS	Please make checks for the meal or coffee fees and/or for the proceedings out to Diesel Emissions Symposium.
MEETING ROOM	Sessions 1-6 will be held in Royal King's Hall I and II.
POSTER SESSIONS	The poster sessions are an integral part of the program. They will be held October 5 and 6 from 5:30-7:30 pm in Royal King's Hall III. Poster presenters are asked to pin up their poster during the afternoon break of the day.
SPECIAL EVENTS	Cocktails will be available on a cash basis during the poster sessions.
TOURS	A tour of selected research facilities in the Research Triangle Park is planned for October 8. Since space in the bus is limited, please sign up early at the registration table. More detailed information will be announced during the symposium.
MESSAGES	Callers should dial (919) 782-4433, the Royal Villa Hotel and Convention Center in Raleigh, NC. All incoming messages will be posted near the registration table.

Program

OCTOBER 5

SESSION 1 DIESEL EMISSIONS CHARACTERIZATION AND CONTROL TECHNOLOGY
Chairman: Ronald Bradow, Environmental Sciences Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park

8:30 am	Opening Remarks
8:45 am	Diesel Emissions, a Worldwide Concern K. Springer, Southwest Research Institute
9:15 am	Diesel Particle and Organic Emissions; Engine Simulation, Sampling, and Artifacts R. Bradow, U.S. Environmental Protection Agency, Research Triangle Park
9:45 am	Diesel Particulate Emissions: Composition, Concentration, and Control R. Williams, General Motor Research Laboratories
10:15 am	Morning coffee break
10:45 am	Particulate Emissions from Spark-Ignition Engines T. Naman, U.S. Department of Energy
11:00 am	Particulate Emission Characterization Studies of In-Use Diesel Automobiles R. Gibbs, NY State Dept. of Environmental Conservation
11:15 am	Diesel Exhaust Treatment Devices: Effects on Gaseous and Particulate Emission and on Mutagenic Activity R. Gorse, Jr., Ford Motor Company
11:30 am	Characterization and Oxidation of Diesel Particulate D. Trayser, Battelle-Columbus Laboratories
11:45 am	Heavy-Duty Diesel Engine Emissions: Some Effects of Control Technology J. Perez, Caterpillar Tractor Company
12:00 pm	Lunch

OCTOBER 5

SESSION 2 CHEMICAL AND BIOASSAY CHARACTERIZATION

Chairman: Joellen Lewtas, Health Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park

1:30 pm Methodology of Fractionation and Partition of Diesel Exhaust Particulate Samples
B. Petersen, Battelle-Columbus Laboratories

2:00 pm The Utility of Bacterial Mutagenesis Testing in the Characterization of Mobile Source Emissions: A Review
L. Claxton, U.S. Environmental Protection Agency, Research Triangle Park

2:30 pm Emission Factors from Diesel- and Gasoline-Powered Vehicles; Correlation with the Ames Test
R. Zweidinger, U.S. Environmental Protection Agency, Research Triangle Park

3:00 pm Afternoon coffee break

3:30 pm Analyses of Volatile Polycyclic Aromatic Hydrocarbons in Heavy-Duty Diesel Exhaust Emission
W. Eisenberg, IIT Research Institute

3:45 pm The Chemical Characterization of Diesel Particulate Matter
J. Yergey, Johns Hopkins University

4:00 pm The Analysis of Nitrated Polynuclear Aromatic Hydrocarbons in Diesel Exhaust Particulates by MS/MS Techniques
T. Riley, Ford Motor Company

4:15 pm Contribution of 1-Nitropyrene to Direct-Acting Ames Assay Mutagenicities of Diesel Particulate Extracts
I. Salmeen, Ford Motor Company

4:30 pm Dinitropyrenes: Their Probable Presence in Diesel Particle Extracts and Consequent Effect on Mutagen Activations by NADPH-Dependent S9 Enzymes
T. Pederson, General Motors Research Laboratories

OCTOBER 5

5:30-7:30 pm POSTER SESSION 1
Convener: Larry Claxton, Health Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park

Mutagenicity of Particle-Bound Organic Chemical Fractions from Diesel and Comparative Emissions
A. Austin, U.S. Environmental Protection Agency, Research Triangle Park

Emission of Diesel Particles and Particulate Mutagens at Low Ambient Temperature
J. Braddock, U.S. Environmental Protection Agency, Research Triangle Park

Chemical Characterization of Mutagenic Fractions of Diesel Particulate Extracts
D. Choudhury, NY State Dept. of Health

Influence of Driving Cycle and Car Type on the Mutagenicity of Diesel Exhaust Particle Extracts
C. Clark, Lovelace Inhalation Toxicology Research Institute

The Rapid Analysis of Diesel Emissions Using the Taga™ 6000 Triple Quadrupole Mass Spectrometer
J. Fulford, Sciex, Canada

Compounds in City Air Compete with ^{3}H-2, 3,7,8-Tetrachloro-dibenzo-p-Dioxin for Binding to the Receptor Protein
J-Å Gustafsson, Karolinska Institute, Sweden

GC/MS and MS/MS Studies of Direct-Acting Mutagens in Diesel Emissions
T. Henderson, Lovelace Inhalation Toxicology Research Institute

Evaluation of the Release of Mutagens and 1-Nitropyrene from Diesel Particles in the Presence of Lung Macrophage Cells in Culture
L. King, U.S. Environmental Protection Agency, Research Triangle Park

Bacterial Mutagenicity of a Diesel Exhaust Extract and Two Associated Nitroarene Compounds After Metabolism and Protein Binding
M. Kohan, U.S. Environmental Protection Agency, Research Triangle Park

OCTOBER 5

5:30-7:30 pm POSTER SESSION 1 (continued)

Characterization of Particulate Emissions from In-Use Gasoline-Fueled Motor Vehicles
J. Lang, Northrop Services, Inc., Research Triangle Park

Surface Reactivity of Diesel Particle Aerosols
M. Lenner, University of Gothenburg, Sweden

Effects of Ozone and Nitrogen Dioxide Present During Sampling of Genuine Particulate Matter as Detected by Two Biological Test Systems and Analysis of Polycyclic Aromatic Hydrocarbons
G. Löfroth, University of Stockholm, Sweden

Alumina-Coated Wool as a Particulate Filter for Diesel-Powered Vehicles
M. McMahon, Texaco, Inc.

Isolation and Identification of Mutagenic Nitroarenes in Diesel Exhaust Particulates
J. Nachtman, University of California at Berkeley

Comparison of Nitro-PNA Content and Mutagenicity of Diesel Emissions
M. Nishioka, Battelle-Columbus Laboratories

Capillary Column GC/MS Characterization of Diesel Exhaust Particulate Extracts
T. Prater, Ford Motor Company

Physico-Chemical Properties of Diesel Particulate Matter
M. Ross, Johns Hopkins University

Some Factors Affecting the Quantitation of Ames Assays
I. Salmeen, Ford Motor Company

Chemical and Mutagenic Characteristics of Diesel Exhaust Particles from Different Diesel Fuels
D. Sklarew, Battelle Northwest Laboratories

Fractionation and Characterization of the Organics from Diesel and Comparative Emissions
C. Sparacino, Research Triangle Institute, Research Triangle Park

Trapping Gaseous Hydrocarbons
F. Stump, U.S. Environmental Protection Agency, Research Triangle Park

OCTOBER 5

5:30-7:30 pm POSTER SESSION 1 (continued)

Analytical Methods for Nitroaromatic Compounds
S. Tejada, U.S. Environmental Protection Agency, Research Triangle Park

Total Luminescence Spectroscopy of Diesel Exhaust Particulate
R. Whitby, NYS Department of Environmental Conservation

Evaluation of the Metabolic Requirements of Diesel and Comparative Source Samples in the Salmonella Typhimurium Plate Incorporation Assay
K. Williams, U.S. Environmental Protection Agency, Research Triangle Park

MS/MS Characterization of Diesel Particulates
K. Wood, Purdue University

OCTOBER 6

SESSION 3 PULMONARY FUNCTION
Chairman: Donald Gardner, Health Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park

8:30 am Inhalation Toxicology of Diesel Exhaust Particles
R. McClellan, Lovelace Inhalation Toxicology Research Institute

9:00 am EPA's Inhalation Toxicology Study
W. Pepelko, U.S. Environmental Protection Agency, Cincinnati

9:30 am Pulmonary Function Testing of Rats Chronically Exposed to Diluted Diesel Exhaust for 612 Days
K. Gross, General Motors Research Laboratories

9:45 am Pulmonary Functional Response in Cats Following Two Years of Diesel Exhaust Exposure
W. Moorman, National Institute for Occupational Health and Safety

10:00 am Deposition and Retention of Surrogate and Actual Diesel Particles
R. Wolff, Lovelace Inhalation Toxicology Research Institute

10:15 am Lung Clearance of Radioactively Labelled Inhaled Diesel Exhaust Particles
P. Lee, General Motors Research Laboratories

10:30 am Morning coffee break

11:00 am Compartmental Analysis of Diesel Particle Kinetics in the Respiratory System of Exposed Animals
S. Soderholm, General Motors Research Laboratories

11:15 am A Subchronic Study of the Effects of Exposure of Three Species of Rodents to Diesel Exhaust
H. Kaplan, Southwest Research Institute

11:30 am Response of Pulmonary Cellular Defenses to the Inhalation of High Concentration of Diesel Exhaust
K. Strom, General Motors Research Laboratories

OCTOBER 6

SESSION 3 PULMONARY FUNCTION (continued)

11:45 am The Effect of Diesel Exhaust on Cells of the Immune System
D. Dziedzic, General Motors Research Laboratories

12:00 pm The Participation of the Pulmonary Type II Cell Response to Inhalation of Diesel Exhaust Emission: Late Sequelae
H. White, General Motors Research Laboratories

12:15 pm Lunch

OCTOBER 6

SESSION 4 PULMONARY TOXICOLOGY AND BIOCHEMISTRY

Chairman: Judy Graham, Health Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park

1:45 pm Pulmonary Deposition, Retention, Inactivation, and Clearance of Inhaled Diesel Particles: The Role of the Pulmonary Defense System
J. Vostal, General Motors Research Laboratories

2:15 pm Investigations of Toxic and Carcinogenic Effects of Diesel Exhaust in Long-Term Inhalation Exposures of Rodents
W. Stöber, Fraunhofer Institute fuer Toxikologie und Aerosolforschung, West Germany

2:45 pm Biochemical Alterations in Bronchopulmonary Lavage Fluids After Intratracheal Administration of Diesel Particulates to Rats
C. Eskelson, University of Arizona Health Science Center

3:00 pm Lipid Changes in Lungs of Rats After Intratracheal Administration of Diesel Particulates
C. Eskelson, University of Arizona Health Science Center

3:15 pm Afternoon coffee break

3:45 pm Bioavailability of Diesel Particle Bound [G-^{3}H]-Benzo(a)pyrene After Intratracheal Instillation
S. Dutta, Wayne State University

4:00 pm The Potential for Aromatic Hydroxylase Induction in the Lung by Inhaled Diesel Particles
K. Chen, General Motors Research Laboratories

4:15 pm Xenobiotic Metabolizing Enzyme Levels in Mice Exposed to Diesel Exhaust or Diesel Exhaust Extract
W. Peirano, U.S. Environmental Protection Agency, Cincinnati

4:30 pm Morphometric Ultrastructural Analysis of Alveolar Lungs of Guinea Pigs Chronically Exposed by Inhalation to Diesel Exhaust
M. Barnhart, Wayne State University School of Medicine

OCTOBER 6

5:30-7:30 pm POSTER SESSION 2
Convener: Joellen Lewtas, Health Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park

Scanning Electron Microscopy of Terminal Airways of Guinea Pigs Chronically Inhaling Diesel Exhaust
M. Barnhart, Wayne State University School of Medicine

The Design of the Long-Term Inhalation Program within the CCMC's Health Effects Research Program
J. Brightwell, Committee of Common Market Automobile Constructors, Battelle Geneva Research Center, Switzerland

Chronic Inhalation Oncogenicity Study of Diesel Exhaust in Sencar Mice
K. Campbell, U.S. Environmental Protection Agency Cincinnati

Species Differences in Deposition and Clearance of Inhaled Diesel Exhaust Particles
T. Chan, General Motors Research Laboratories

Species Comparisons of Bronchoalveolar Lavages from Guinea Pigs and Rats Exposed In Vivo to Diesel Exhaust
S-t. Chen, Wayne State University School of Medicine

Preliminary Report of Systemic Carcinogenic Studies on Diesel and Gasoline Particulate Emission Extracts Applied to Mouse Skin
N. Clapp, Oak Ridge National Laboratory

CCMC's Health Effects Research Program
Committee of Common Market Automobile Constructors Emissions Research Committee, Belgium

Effects of Chronic Diesel Exposure on Pulmonary Protein Synthesis in Rats
S. Dutta, Wayne State University School of Medicine

Fractionation and Identification of Organic Components in Diesel Exhaust Particulate
M. Erickson, Research Triangle Institute

Preparation of Diesel Exhaust Particles and Extracts as Suspensions for Bioassays
J. Graf, IIT Research Institute

OCTOBER 6

5:30-7:30 pm POSTER SESSION 2 (continued)

Research Plans for Diesel Health Effects Study
H. Kachi, Japan Automobile Research Institute, Japan

Neurodepressant Effects of Uncombusted Diesel Fuel
R. Kainz, Tulane University

The Effect of Exposure to Diesel Exhaust on Pulmonary Protein Synthesis
R. McCauley, Wayne State University School of Medicine

Respiratory Health Effects of Exposure to Diesel Exhaust Emissions
R. Peger, National Institute of Occupational Safety and Health

SWRI-SFRE Diesel Health Effects Exposure Facility
K. Springer, Southwest Research Institute

Post-Exposure Diesel Particle Residence in the Lungs of Rats Following Inhalation of Dilute Diesel Exhaust for Six Months
K. Strom, General Motors Research Laboratories

OCTOBER 7

SESSION 5 MUTAGENESIS AND CARCINOGENESIS
Chairman: Stephen Nesnow, Health Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park

8:00 am Mutagenic Activity of Diesel Emissions
J. Lewtas, U.S. Environmental Protection Agency

8:30 am Mutagenicity of Diesel and Spark Ignition Engine Exhaust Particulate Extract Components to Salmonella Typhimurium and Human Lymphoblasts
T. Barfknecht, Massachusetts Institute of Technology

8:45 am Cytotoxicity, Mutagenicity, and Co-Mutagenicity of Diesel Exhaust Particle Extracts on Chinese Hamster Ovary Cells In Vitro
A. Li, Lovelace Inhalation Toxicology Research Institute

9:00 am Induction of In Vivo Sister Chromatid Exchange by Diesel Particulate and Diesel Extract
M. Pereira, U.S. Environmental Protection Agency, Cincinnati

9:15 am Mutagenic Activity of Diesel Particles in Alveolar Macrophages from Rats Exposed to Diesel Engine Exhaust
J-S. Siak, General Motors Research Laboratories

9:30 am Morning coffee break

10:00 am Skin Carcinogenesis Studies of Emission Extracts
S. Nesnow, U.S. Environmental Protection Agency, Research Triangle Park

10:30 am Dermal Carcinogenesis Bioassays of Diesel Particulates and Dichloromethane Extract of DP
L. DePass, Bushy Run Research Center

11:00 am Pespiratory Carcinogenicity of Diesel Fuel Emissions: Interim Results
A. Shefner, IIT Research Institute

11:30 am Carcinogenicity of Extracts of Diesel and Related Environmental Emissions Upon Lung Tumor Induction in Strain 'A' Mice
R. Laurie, U.S. Environmental Protection Agency, Cincinnati

OCTOBER 7

SESSION 5 MUTAGENESIS AND CARCINOGENESIS (continued)

11:45 am The Influence of Inhaled Diesel Engine Emissions Upon Lung Tumor Induction in Strain 'A' Mice
W. Pepelko, U.S. Environmental Protection Agency, Cincinnati

12:00 pm Objectives and Experimental Conditions of VW/Audi Diesel Exhaust Inhalation Study
W. Stöber, Fraunhofer Institute fuer Toxikologie und Aerosolforschung, West Germany

12:15 pm Lunch

OCTOBER 7

SESSION 6 EXPOSURE AND RISK ASSESSMENT
Chairman: Robert Jungers, Environmental Monitoring Systems Laboratory, U.S. Environmental Protection Agency, Research Triangle Park

1:30 pm Projected Human Health Risks from Increased Use of Diesel Light-Duty Vehicles in the United States
R. Cuddihy, Lovelace Biomedical and Environmental Research Institute

2:00 pm Health Effects of Exposure to Diesel Fumes and Dust in Two Trona Mines
M. Attfield, National Institute of Occupational Safety and Health

2:15 pm Mutagenicity and Chemical Characterization of Carbonaceous Particulate Matter from Vehicles on the Road
W. Pierson, Ford Motor Company

2:45 pm Afternoon coffee break

3:15 pm Emissions of Gases and Particulates from Diesel Trucks on the Road
R. Kiyoura, Research Institute of Environmental Science, Japan

3:30 pm Diesel Bus Terminal Study: Effects of Diesel Emission on Air Pollutant Levels
R. Burton, U.S. Environmental Protection Agency, Research Triangle Park

3:40 pm Diesel Bus Terminal Study: Characterization of Volatile and Particle-Bound Organics
R. Jungers, U.S. Environmental Protection Agency, Research Triangle Park

3:50 pm Diesel Bus Terminal Study: Mutagenicity of the Particle-Bound Organics and Organic Fractions
J. Lewtas, U.S. Environmental Protection Agency, Research Triangle Park

4:00 pm Nitro Derivatives of Polynuclear Aromatic Hydrocarbons in Airborne and Source Particulate
T. Gibson, General Motors Research Laboratories

OCTOBER 7

SESSION 6 EXPOSURE AND RISK ASSESSMENT (continued)

4:15 pm Risk Assessment of Diesel Emissions
R. Albert, U.S. Environmental Protection Agency, Washington, DC

4:30 pm Perspectives on Diesel Emission Health Research
N. Nelson, New York University Medical Center

5:00 pm Close

Abstracts

DIESEL EMISSIONS, A WORLDWIDE CONCERN

by
Karl J. Springer
Department of Emissions Research
Southwest Research Institute
6220 Culebra Road
San Antonio, Texas

Recent visits to Japan and Europe plus scores of visitors from other countries have convinced me that there is a worldwide concern over the possible health effects of diesel exhaust. Not all of these visitors come to San Antonio to visit the Alamo or stroll by the river.

Laboratory tests with bacteria, animal cells and tissues have shown some components of diesel exhaust to be toxic, mutagenic or carcinogenic. In addition to gas phase compounds that have both direct and secondary effects in the atmosphere, diesel exhaust contains particulates of both solid (soot) and liquid (aerosol) type. The soot particles are less than one millionth of a meter in size and provide a surface for the aerosols to condense or absorb. For example, benzo(a)pyrene, a well known carcinogen, is but one of the materials that are in diesel particulate.

Studies in 1977 by Southwest Research Institute's Emissions Research Department proved that diesel passenger cars produce particulate 50 to 80 times their gasoline-fueled counterparts. A 1981 report gave 0.31 g/km (0.5 g/mile) as an emission rate from cars with 15 percent being soluble organics (i.e., extractable with dichloromethane solvent). It is the soluble fraction of the particulate that has sounded the alarm since this contains the materials that have been found to be direct acting mutagens by the Ames bioassay test.

It is this fraction, first evaluated in the Ames test in 1977, that resulted in the precautionary notice published by EPA that same year. It is this fraction which has caused this symposium to be held and the previous CRC Dearborn meeting in March 1981, the EPA Cincinnati Symposium in December 1979, and the EPA Ann Arbor Symposium in May 1978. It is this fraction which has caused the legislators, Federal officials in the DOE, DOT, EPA, Bureau of Mines and others so much frustration and confusion. It is this fraction which has given executives of diesel engine manufacturers and car makers chronic nightmares. It is this fraction which has given the voices for environmental protection another item for argument. It is also this

fraction which has given many of us a scientific challenge.

I loosely gather the governmental policymakers, the corporate executives, and all those for whom you work as "THEY Who Must Be Obeyed." THEY have supported us with money, facilities and the opportunity to investigate diesel particulate. Until recently, THEY have been, on the surface at least, patient, realizing that such research is tedious and long term in nature. Underneath that thin veneer of patience I sense the growing imperative to decide, to rule, and to move on. It is that basic impatience of the U.S. or us.

Can we decide at this conference if there is or is not a problem with that organic fraction? If there is, do we have enough data to prove it? If we don't, what is needed in time and money? Can we convince ourselves and others that the studies should continue? In the meantime, what about dieselization of the passenger cars and trucks in the U.S.? Is it business as usual? Do we suggest diesels be limited in urban and congested areas? Do we give diesels the green light?

THEY, who must be obeyed, are faced with these and related questions. What to do? Do not think that because your research is incomplete or that you need another five years to complete a health survey of a specific population that THEY will necessarily wait. Diesels in cars and trucks are a quick way to reduce fuel consumption, energy costs and foreign dependence. It is clear from recent changes in environmental thinking that a new policy is emerging. We have to prove to THEY, who must be obeyed, that there is a clear and present danger from diesel particulate. I wonder if we are able to do this in the next few days.

THEY, who must be obeyed, have a standard approach which goes essentially like this. A broad program has been in progress for, say, three to four years with no proof that diesel exhaust is hazardous beyond the positive Ames test (which we knew of in 1977). What are the chances of proving a hazard exists, given another three to four years?

It is the same question we ask of ourselves on many topics every day. I submit that the delegates to this conference have a job of convincing THEY, who must be obeyed, of the need to continue. Otherwise, THEY, who must be obeyed, quite likely will become impatient and make those decisions for us using whatever facts are available at the time. You may not agree, but the decision point is very near.

The remainder of this keynote address deals with the macroscopic or big picture particulate contributions of light- and heavy-duty vehicles. For example, given no control and a 25 percent sales penetration (by 1985 and thereafter) of cars powered by diesel engines, it will be 1998 before diesel cars equal diesel truck and bus particulates. With moderate particulate control of both classes of vehicles, particulate parity is not reached until after the year 2000. Similar projections are given based on lower and higher penetration of diesel cars.

To add further perspective to the diesel issue, specifically the

substitution of many diesel cars for gasoline ones, some recent results from microscale modeling are summarized. Self-contamination can occur in parking garages, street canyons, tunnels, expressways, or almost anywhere crowded conditions occur with limited dilution. The current maximum 24 hour limit for total suspended particulate (TSP) is 260 $\mu g/m^3$. For example, in a typical above ground multilevel parking garage, the TSP can be exceeded by having as few as 12 percent of the cars diesel-powered. These localized concentrations may be important, if not from health, from public welfare, such as odor and eye irritation and reduced visibility, increased soiling, etc.

The challenge to this meeting is quite simply to assimilate and ruminate and then communicate. We must absorb, digest, compare and then think about it and get the meaning into simple terms so that THEY, who must be obeyed, will understand. Only then, will we have done our job well.

Diesel Particle and Organic Emissions: Engine Simulation, Sampling and Artifacts

by

Ronald L. Bradow

The major reason for this paper is to summarize for the health scientists among us some of the technical factors and reasoning underlying the samples so many of us in automotive research have been generating. In virtually all cases where material has been collected for biological testing, procedures used in generating samples have been well-accepted engineering practices simulating the road performance of vehicles,the emission of exhaust into ambient air, and recovery of particles or gaseous organics on collecting media as inert as possible to chemical changes.

In large part, the conditions were selected before any artifact problems were known because of the time constraints in the early part of the EPA work on diesel particles. As more becomes known about the chemical makeup of diesel exhaust particles and organics, the potential artifacts in collection become simpler to hypothesize. Still experiments conducted so far suggest that these are not a major problem for samples collected in the past.

There are a number of vehicle categories which have significantly different uses in transporting people and things. Because passenger cars, trucks and buses are operated under different conditions of speed and load and pollutant emission characteristics for each type of vehicle vary often dramatically, with operating conditions, a variety of testing procedures have been developed. These attempt to simulate, while a vehicle is stationary, those forces which would have been experienced had the vehicle been moving down a roadway.

With passenger cars, the standard way of achieving this simulation uses a twin roll chassis dynamometer. Mechanisms are available to simulate the aerodynamic drag, tire rolling resistance and inertial forces on a vehicle, all as a function of vehicle speed. The nature of these simulations with the commonly used dynamometer types are shown.

A variety of driving schedules are currently available for use in passenger car testing. Since the dynamometer set up takes care of the resistive force simulation, the driving schedules are merely speed-time listing or charts which can be readily driven repeatably. The most commonly used of these schedules is the Federal Test Procedure urban driving schedule. This route

simulates a home-to-work commuter route in Los Angeles and in fact, the route actually exists in that city. Other simulations for very slow central business district driving, crowded urban expressways, and two-lane country highways are also available for use. Literature values for particle and organic emission rates are available for a variety of gasoline and passenger car types and these are presented in the usual form, mass per unit distance driven. This form is commonly used because the mass emission rate of the air pollutant is compared in this ratio to an index of social value, the distance driven.

There are alternative means of expressing emission results, though, and of these, fuel-specific emission rates are the most revealing because vehicle size tends to be normalized in this approach. Some emission rates of diesel trucks, buses and cars are fairly close when fuel consumption is normalized. Literature values of diesel and gasoline truck emissions of particles and of organic matter are compared on this basis.

The compliance testing of trucks and buses is somewhat controversial right now and, apparently, there is not wide-spread agreement on the propriety of testing measures. Usually, just the engines and not whole vehicles are tested and the simulation with respect to road performance is somewhat more difficult to achieve. Further, the index of social value used to compare emissions is different from that used for passenger cars. In this case, emissions are normalized by the useful work done and emissions are expressed in mass/unit work (grams/kw-hr. or grams/hp-hr). Emissions expressed on this basis are somewhat difficult to compare with conventional passenger car emission rates and usually one resorts to fuel-specific comparisions.

However, it is possible, for research purposes, to test trucks on a chassis dynamometer in road simulations for central business district, arterial roadway, and expressway driving routes, just as passenger cars are tested. Here, all the same problems of load simulation and representational character of the driving schedule are encountered. Since this procedure is fairly new, there are still some uncertainties associated with its use. One experimental program has reported some results with such a procedure, however, and these results are summarized in comparison with engine dynamometer and passenger car data.

The emissions experienced on these schedules are conventionally measured using constant volume sampling procedures. These sampling methods seek to accommodate two important test requirements. One is to provide an integrated portional sample of auto exhaust and the other is to achieve some simulation of the air-dilution process which occurs when the exhaust enters ambient air. This process is important because the organic substances are partitioned by this process; some enter the condensed or particle phase on cooling while others remain gaseous.

The physical chemistry of this process has recently been an active topic of research and current results of static and dynamic experiments are presented. The absorption of organic substances by fine carbon particles is an important key to understanding these processes and results from the work of Kittleson,

Ross, Black and Pierson are discussed along with a model presented by McDonald. It appears that both temperature and dilution ratio can have some effect on the amount of organic material in the condensed phase. However, no serious objection to the commonly used dilution tube procedure seems to be in evidence. The contributions of diesel vehicles both to airborne carbon and particulate organics appear to be important.

It is possible to separate the gas and particle phase material by several methods and some comparison data for several methods is reported. To insure that only a physical separation is made and not some recovery of gaseous material has been no small task. While many of the currently used procedures involve some art and guess-work, they do appear generally adequate for the task of sampling. Equipment currently in use is described.

Recovery of heavier gaseous organics for biological sampling has been a more serious problem. Condensation procedures used by Grimmer and by Lofroth in Europe and by Gross in the United States are quite different in philosophy from the air dilution procedures used in vehicle sampling. Results from a variety of these procedures are compared and equipment for recovery of large amounts of these organics is described.

These collection methods have some potential for artifactual generation of biologically active material, particularly with diesel exhaust. Some preliminary results from experiments with NO_2 addition to diluted exhaust and results from correlations of bioactivity with NO_2 level from the work of Leddy and Johnson are compared. It appears that NO_2 levels below about 5ppm to not constitute a serious threat to diesel particle organic integrity but higher levels, particularly those above about 20 ppm, cause serious artifacts. For this reason, a reasonably high dilution ratio is needed to avoid artifactual generation of nitroaromatics. It appears that none of the diesel samples generated thus far has been seriously jeopardized by this effect, but caution might be warranted in interpreting condensation experiment data from diesel vehicles.

Gasoline-fueled cars seem to have very low levels of NO_2 and no major problems within nitroaromatic formation are known.

Thus, a reasonably useful set of engineering practices and equipment exists to capture samples of both particle and gaseous organic materials. While there could still be some improvements, it appears that estimates of biological activity and chemical composition using existing samples are reasonably correct.

DIESEL PARTICULATE EMISSIONS: COMPOSITION, CONCENTRATION, AND CONTROL

by

Ronald L. Williams
Environmental Science Department
General Motors Research Laboratories
Warren, Michigan

The application of new techniques and approaches to study diesel emissions has increased our understanding of the formation, atmospheric impact, and health significance of diesel particulate emissions. This paper will review recent work on the composition of diesel particulate and compare it with particulate from other combustion sources. Then, estimates of the current and projected concentration of diesel particulate in urban areas will be discussed. Finally, the limited information available on the effects of experimental particulate-control systems will be presented.

COMPOSITION

The size distribution of diesel particles has been studied by a variety of instruments, including, electron microscopes, electrical aerosol analyzers, and inertial impactors. While the mass median diameter of diesel particulate is a few tenths of a micrometer, the number median diameter is considerably smaller. Therefore, on a frequency basis, the particles deposited in animal lungs will be dominated by the smaller particles.

Despite the application of the best analytical methods, no feature of diesel particulate has been identified which clearly distinguishes it from particulate emitted by gasoline-burning engines or from particulate emitted by other combustion sources. Figure 1 shows the relative amounts of organic and elemental carbon in combustion particulate from several different sources (1,2). The ratio for each source type appears to depend on the air-to-fuel ratio during combustion. Measurements of the polynuclear aromatic compounds in the organic material likewise fail to show source-specific components of the particulate.

CONCENTRATION

Since diesel vehicles emit larger amounts of particulate carbon on a per mile basis than gasoline vehicles, it is important to project the increase in the ambient concentration of particulate carbon from expanded use of diesel-powered cars and trucks. Estimates of the current input of diesel particulate to the nation's atmosphere range from 80 to 120 x 10^9 grams per year. Ambient measurements show that diesel particulate currently accounts for about 25% of the elemental carbon in urban air (3), or 1 to 2 micrograms per cubic meter in most major United States cities.

Estimates of diesel particulate concentrations for the future depend on the total amount of diesel particulate emitted and on the distribution of diesel vehicles geographically. Particulate emissions from light-duty diesels result in proportionately larger increases in urban particulate concentrations than do particulate emissions from heavy-duty diesels which accumulate a smaller percentage of their mileage in urban areas. We estimate that 6 to 9 million light-duty diesels with an average emission rate of 0.6 g/mile would contribute an additional 1 to 2 micrograms per cubic meter of diesel particulate to the air of most major United States cities.

CONTROL

The prospect for increased use of diesel engines has stimulated efforts to develop new technologies for reducing diesel particulate emissions. Two different control approaches have been examined to determine the effects on the composition of the particulate as well as on the total quantity. Because the health effects studies being conducted predate the availability of particulate-control systems, it is important to make a preliminary assessment of the composition changes, even though these systems are still experimental.

One of the particulate-control systems lowered the FTP total particulate to 160 milligrams per mile (0.16 g/mile). With this system organic carbon was 3 to 8 milligrams per mile and benzo(a)pyrene emissions were more than 90% lower than normally emitted by diesels. However, enhanced sulfate emissions occurred in higher-speed driving cycles, apparently due to the oxidation of sulfur dioxide by the control system. During regeneration of the particle trap of this system, a visible white cloud of sulfate was emitted.

A second particulate-control system also reduced the FTP total particulate substantially. More than 99% of the elemental carbon was removed from the exhaust. Organic carbon emissions were 10 to 30 milligrams per mile and benzo(a)pyrene emissions were more than 90% lower than normally emitted by diesels. Higher-speed driving cycles and regeneration of the particulate trap in this case gave similarly reduced emission rates of organic carbon and benzo(a)pyrene.

Diesel particulate is currently the subject of intense chemical and biological study. While typical diesel particulate is not distinguishable from particulate from other combustion sources, the limited evidence available indicates that particulate-control systems may markedly change the composition of diesel particulate in the future.

REFERENCES

1. Muhlbaier, J. L. and R. L. Williams. 1981. Fireplaces, furnaces, and vehicles as emission sources of particulate carbon. In: Particulate Carbon: Atmospheric Life Cycle. G. T. Wolff and R. L. Klimisch, eds. Plenum Press; New York.

2. Cadle, S. H. and P. J. Groblicki. 1981. An evaluation of methods for the determination of organic and elemental carbon in particulate samples. Ibid.

3. Wolff, G. T., P. J. Groblicki, S. H. Cadle, and R. J. Countess. 1981. Particulate carbon at various locations in the United States. Ibid.

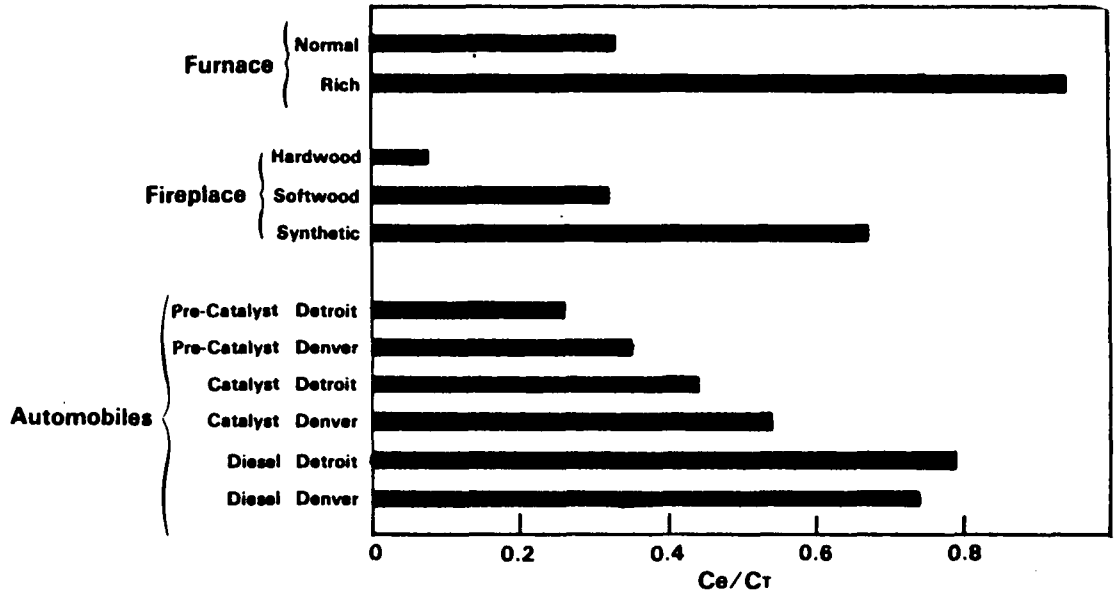

Figure 1. The ratio of elemental carbon to total carbon from selected sources.

PARTICULATE EMISSIONS FROM SPARK-IGNITION ENGINES

by

Ted M. Naman
D. E. Seizinger
U.S. Department of Energy
Bartlesville Energy Technology Center
Bartlesville, Oklahoma

Charles R. Clark
Inhalation and Toxicology Research Institute
Albuquerque, New Mexico

ABSTRACT

Experiments were conducted at the U.S. Department of Energy's Bartlesville (Okla.) Energy Technology Center to quantify particulate and gaseous emissions from current-production vehicles equipped with spark-ignition engines, to determine the influence of fuel and ambient temperature on particulate emissions, and to characterize particulates in terms of their carbon content, soluble organic fractions, and biological activity.

Four 1980-81 model-year vehicles equipped with oxidation and three-way catalysts and spark-ignition engines ranging from 1.6 liter, 4-cylinder to 4.3 liter, V8 (see table 1) were tested on a climate-controlled chassis dynamometer using the driving cycles of the 1975 Federal Test Procedure. The vehicles were operated at 20°, 50°, 75°, and 100° F (-7°, 10°, 24°, and 38° C, respectively) ambients on gasoline and at 75° F ambient on four fuel blends: 90 percent gasoline/10 percent ethanol, 90 percent gasoline/10 percent methanol, 93 percent gasoline/7 percent methyl tertiary butyl ether, and a commercial gasohol.

Particulate matter was collected on 40- by 40-inch filters using the total volume of the exhaust, and on conventional 47 mm filters using a sampling probe and a portion of the exhaust. Bioassays of dichloromethane extracts of the samples were carried out using the Salmonella mutagenicity (Ames) test at the Lovelace Inhalation Toxicology Research Institute.

The results from the Federal Test Procedure (figure 1) showed a significant reduction in particulate emissions with the alcohol/gasoline fuel blends when compared to gasoline alone. The methyl tertiary butyl ether/gasoline fuel blend showed a slight reduction in particulate emissions. The carbon monoxide emissions were slightly reduced with the alcohol/gasoline fuel blends. Hydrocarbon emissions remained relatively unchanged, oxides of nitrogen emissions were slightly increased, and fuel economy was 2 to 3 percent lower (data not shown). Ambient temperature seemed to have a slight effect on particulate emissions. Overall, particulates emitted from the vehicles with spark-ignition engines were 90 to 100 times lower than particulates emitted from current-production diesel vehicles.

Samples of particulate extracts from vehicles fueled with gasoline and gasohol were separated using a silica column. The results obtained from the separation showed a higher percentage of polar compounds in the gasohol than in the gasoline samples (figure 2).

Benzo(a)pyrene and nitropyrene levels in the gasohol particulate extracts were at least 50 percent lower than the measured levels in particulate extracts from gasoline-fueled vehicles.

Dichloromethane extracts of the particulate exhaust from vehicles operating on gasoline were evaluated in Salmonella strain TA-100, with and without the addition of a liver enzyme preparation (S-9) as a source of metabolic enzymes. Extracts of particulate exhaust from the four vehicles operated on gasoline and/or on alcohol/gasoline fuel blends produced direct, dose-related increases in mutagenicity (see table 2). The addition of S-9 either decreased or did not alter the observed direct mutagenicity (data not shown).

The addition of 10 percent ethanol to gasoline either decreased or did not significantly change the mutagenicity of the resultant exhaust particulate extracts (table 2). Operation of the Mercury Monarch on the methanol blend increased the mutagenicity of the exhaust particulate extracts but decreased mutagenicity in the Chevrolet Citation. Commercially available gasohol resulted in particulate extracts that were less mutagenic in the Ford Escort but not significantly different in the other cars.

The mutagenic potencies of the extracts do not reflect differences among cars in the mass of mutagenic material associated with the particulate emission rates. Therefore, the mutagenicity data were normalized so that comparisons of the amount of mutagenicity could be made among cars. This was done by dividing the mass of dichloromethane extractable material from each filter by the number of miles of vehicle operation (26), to yield mg/mile of particulate associated organic material. This number was multiplied by revertants per microgram (μg) to estimate the amount of mutagenicity emitted from each car (revertants per mile).

When compared to gasoline, the addition of 10 percent ethanol or methanol to gasoline, or operating the vehicles on the commercial gasohol, reduced the mass emission rate (mg/mi) of organic materials associated with the particulates. This resulted in significant reductions in revertants per mile for all of the alcohol fuel blends tested (table 2).

Table 1. Test vehicles

	Ford Escort	Oldsmobile Cutlass	Chevrolet Citation	Mercury Monarch
Engine displacement, CID (liters)	98 (1.6)	263 (4.3)	151 (2.5)	250 (4.1)
Carburetion	2 bbl	2 bbl	2 bbl	1 bbl
Compression ratio	8.8	7.5	8.2	8.6
Transmission	Manual 4-spd	Auto	Auto	Auto
Emission Control System:				
EGR	Yes	Yes	Yes	Yes
Air pump	Yes	Yes	No	Yes
Air injection	No	No	Yes	No
Oxidation catalyst	No	No	Yes	Yes
Three-way catalyst	Yes	Yes	No	No
Charcoal canister	Yes	Yes	Yes	Yes
Axle ratio	3.59	2.29	2.84	2.79
Inertia weight, lb	2375	3750	2875	3625
Actual dyno load, hp	6.4	11.5	6.6	11.1

Table 2. Influence of alcohol fuel blends on mutagenicity of spark-ignition engine exhaust particulate extracts

Vehicle and Fuel	Revertants/μg Extract TA-100[a]	Emission of Particulate Associated Organic Material (mg/mi)	Revertants per Mile
Ford Escort			
Gasoline	10	1.5	15,000
Ethanol blend	9	1.1	9,900
Commercial gasohol	4	1.2	4,800
Oldsmobile Cutlass			
Gasoline	10	1.7	17,000
Ethanol blend	5	0.6	3,000
Commercial gasohol	13	0.6	7,800
Chevrolet Citation			
Gasoline	17	1.9	32,300
Ethanol blend	14	0.9	12,600
Methanol blend	11	0.8	8,800
Commercial gasohol	10	1.0	10,000
Mercury Monarch			
Gasoline	16	7.1	114,000
Ethanol blend	12	2.8	34,000
Methanol blend	26	3.3	86,000
Commercial gasohol	20	2.2	44,000

[a]Slope of linear portion of dose-response curve, without S-9.

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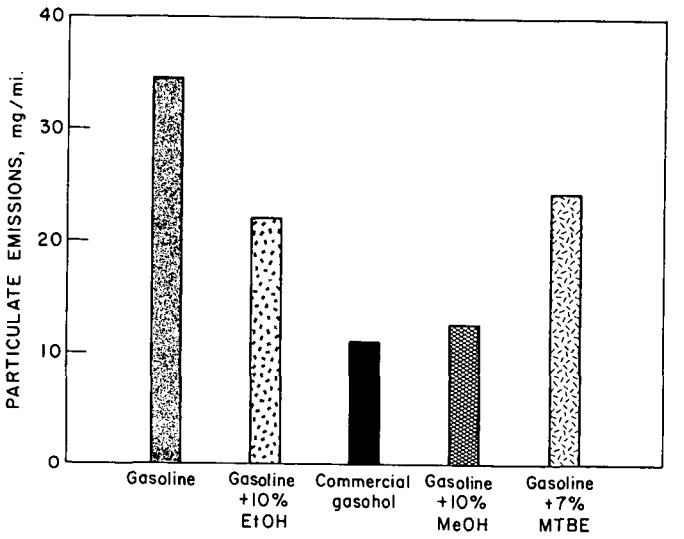


Figure 1. Influence of fuel extenders on particulate emissions.

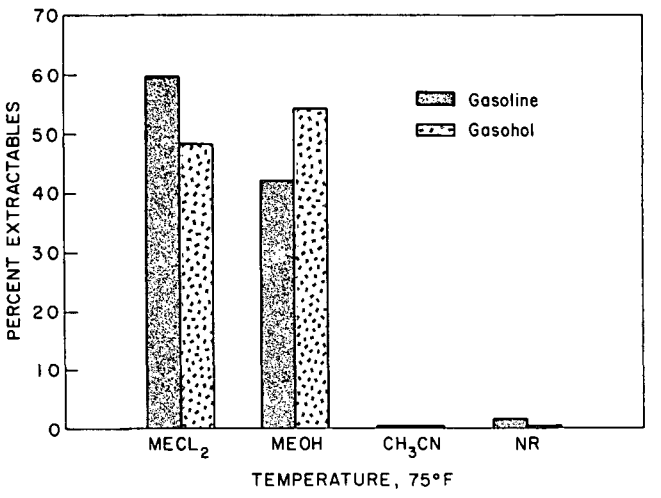


Figure 2. Particulate extracts from vehicles operating on gasoline and gasohol.

PARTICULATE EMISSION CHARACTERIZATION STUDIES OF IN-USE DIESEL AUTOMOBILES

by

Richard Gibbs, James Hyde, and Robert Whitby
Division of Air
New York State Department of Environmental Conservation
Albany, New York

A sample of 20 in-use diesel automobiles has been repeatedly tested over a two-year period to accumulate emissions characterization data with major emphasis on particulate. Each vehicle test included replicate sample collection for "as received" and "control fuel, control oil" vehicle conditions. Driving cycles tested at each vehicle condition included: FTP, CFDS (CUE), HFET, 50 mph cruise, NYCC, and Idle. Measurements in each test cycle included gaseous emissions, fuel economy, and particulate emissions; also, individual particulate samples were collected for each individual test cycle in sufficient quantity for subsequent chemical and bioassay analyses. These particulate samples were quantified for soluble organic fraction (SOF) and Ames test direct-acting mutagenic response by TA98 (-). These parameters are examined for 60 vehicle tests to indicate particulate character effects for vehicle types and test cycles. Results will be presented for three vehicles in the sample group which have been tested over sufficient mileage accumulation intervals to provide limited insight into vehicle aging effects.

Table I gives average values of the mutagenic activity of the SOF directly. In general, FTP samples exhibit a higher specific activity than samples from the same vehicle for other driving cycles. Expressed as a percent of total particulate, however, the SOF (%) is generally less for the FTP than other cycles as seen in Table II.

When SOF mutagenic response and SOF (%) data are combined to express mutagenic response on a particulate mass basis, these differences are mostly indiscernable as shown in Table III.

From Table III, with some exceptions, it can be seen that the bio-activity on a total particulate basis is quite uniform within a vehicle type. This is in spite of rather large variations in activity among the various vehicles tested to generate the averages. Thus, we conclude that, as a first

approximation, for a given vehicle test a "gram of particulate is a gram of particulate" regardless of driving cycle. When variations in vehicle particulate emission rate per distance travelled are incorporated to the data of Table III, the per-mile emission of bio-active material can be calculated as given in Table IV.

Since all test cycles except the FTP begin with a warm vehicle, the foregoing conclusions may not include parameters related to vehicle start-up. A battery of tests were performed on a VW diesel in the Winter of 1981 to preliminarily investigate cold-start effects on particulate, SOF, and mutagenic activity. Overnight vehicle soak at laboratory and outdoor ambient conditions were followed by: FTP, Bag I, 10 min. pause, FTP, Bag III to give separate particulate samples for each bag at each condition. The cold-ambient tests were repeated, and average values from two runs are reported. Continuous temperature recordings of ambient temperature, crankcase lubricating oil, and fuel temperature (between pump and injectors) were obtained. When results for the normal FTP Bag III were used as a basis for comparison, the other three test conditions yield ratios as given in Table V.

Comparison of the 0°C Bag I results to the 20°C base condition showed a 74% increase in particulate emission, 11% increase in SOF emission corresponding to a 36% decrease in SOF expressed as a percentage of particulate. SOF mutagenic response was increased by a factor of 3.6 and 4.0 when expressed as revertants/mile. These results are in general agreement with those presented above in comparison of FTP and other driving cycles for the in-use sample group.

Table I. Mutagenic Activity Per Microgram SOF
(Revertants/μg SOF)

	FTP	HFET	50mph Cruise	CFDS	NYCC	IDLE	Sample n
GM	3.8	2.4	2.1	2.7	1.4	2.4	57
VW	11.3	10.9	8.1	11.0	16.8	3.4	36
MB	4.8	4.4	3.5	5.9	1.8	2.7	18

Table II. SOF (%)

	FTP	HFET	50mph Cruise	CFDS	NYCC	IDLE	Sample n
GM	25.1	34.1	39.3	31.2	31.7	24.6	57
VW	20.0	21.5	20.7	22.7	33.4	55.0	36
MB	14.0	13.6	15.2	14.6	14.8	14.6	18

Table III. Mutagenic Activity Per Microgram Particulate
(Revertants/μg Particulate)

	FTP	HFET	50mph Cruise	CFDS	NYCC	IDLE	Sample n
GM	0.77	0.75	0.72	0.74	0.36	0.43	57
VW	1.95	2.06	1.53	2.26	5.82	1.94	36
MB	0.53	0.45	0.40	0.68	0.27	0.39	18

Table IV. Mutagenic Activity Per Vehicle Mile
(10^5 Revertants/Mile)

	FTP	HFET	50mph Cruise	CFDS	NYCC	IDLE*	Sample n
GM	6.5	2.7	2.2	3.8	8.6	0.74	57
VW	6.9	6.1	4.4	7.4	27.0	0.47	36
MB	2.8	1.7	1.3	3.0	6.2	0.17	18

*10^5 Revertants/Minute

Table V. VW Cold Start Particulate Comparisons

Vehicle Test Condition	Mean Temperatures °C			Particulate	SOF	SOF	TA98 (-) Revertants		
	Overnight Soak	Injector Fuel Line	Crankcase Lube	(g/m)	(%)	(g/m)	R/μg SOF	R/μg Part.	10^5 R/mi
Base Condition = (FTP Bag III)	20	25	90	0.34	24.8	0.084	5.8	1.44	4.9
Note: Results below shaded areas are ratios to base condition									
Bag III after ambient Cold Soak Bag I	0	18	98	1.0	0.87	0.87	1.5	1.3	1.3
Normal FTP Bag I	20	21	48	1.18	0.79	0.93	1.8	1.4	1.7
Cold Ambient Soak FTP Bag I	0	5	38	1.74	0.64	1.11	3.6	2.3	4.0

CHARACTERIZATION AND OXIDATION OF DIESEL PARTICULATE

by

David A. Trayser and Louis J. Hillenbrand
Battelle-Columbus Laboratories
505 King Avenue
Columbus, Ohio 43201

INTRODUCTION

A study was recently completed by Battelle-Columbus Laboratories for the U.S. Environmental Protection Agency to evaluate emissions control on light-duty diesel vehicles by postcylinder oxidation. The primary objective of this program was to determine the feasibility of thermal or catalytic oxidation as a means of diesel particulate emissions control.

Two major efforts in this program involved characterization of the particulate for chemical and physical parameters relating to ignition and oxidation, and development of a catalytic ignition concept for reducing the ignition temperature of the particulate in the exhaust system.

CHARACTERIZATION OF THE PARTICULATE

Particulate characteristics were measured using samples collected directly from the surface of the exhaust pipe and samples collected by filter from a dilution system.

Physical Properties

The particulate physical properties included surface area, size distribution, and mass concentration. These were measured using dilution system samples, hence, representing the particulate after entering the atmosphere. The surface area was approximately 100 m^2/g, the mass median particle diameter was in the range of 0.1 to 0.3 μm and was observed to increase in size as engine speed and load increased, and the mass concentration in the engine tail pipe was observed also to vary with engine speed and load. Mass concentrations of particulate in the exhaust gas varied from 20 mg/m^3 at the lowest speed and lightest load to 500 mg/m^3 at maximum speed and load. These values translate to 0.1 to 3.8 grams per mile, respectively, a fairly "dirty" exhaust for an automotive diesel (and not representative of current diesel engines).

Chemical Properties

The chemical properties were measured using the exhaust-pipe particulate samples and included soluble organics (using toluene), carbon, hydrogen, oxygen, ash, and trace minerals. The soluble organics ranged from 2 to 10 percent, with no evident correlation with engine speed/load conditions. By comparison, the soluble organic content measured in particulate collected in the dilution system, for the same range of engine speed and load conditions, varied from 3 to 25 percent, increasing as speed and load decreased.

Carbon content ranged between 73 and 93 percent, hydrogen content varied from 0.5 to 1.8 percent, and ash content ranged from 0.1 to 2.2 percent. Again, no correlation with engine conditions was noted for any of these parameters. About 7 percent oxygen was found in two particulate samples, and 23 percent in a third sample. Part of the oxygen is present in the particulate as SO_4 and part may represent partial oxidation (to CO_2, perhaps) but with the products still bound in the particulate.

The trace mineral analyses revealed considerable calcium, iron, phosphorus, and magnesium in the particulate. Similar analyses of samples of the fuel and lube oil indicated that the lube oil was most likely the source of these elements as well as of chromium, copper, manganese, and lead, found in lesser quantities in the particulate. These results imply that a substantial portion of the particulate may derive from the lube oil.

Ignition Properties

A Differential Thermal Analyzer technique was used to measure ignition temperatures and maximum oxidation temperatures for the particulate samples obtained in the engine exhaust pipe. The mean ignition temperature for the samples evaluated was 594 C, with a variation range of 583 to 604 C. The only significant correlations with engine operating conditions and with physical and chemical properties appeared to be with the presence of hydrocarbons in the exhaust and with aluminum and lead in the particulate. Lower ignition temperatures occurred with higher exhaust gas hydrocarbons and higher particulate aluminum and lead.

More significant changes in ignition temperature were observed when the bulk density and the amount of the particulate sample in the DTA were altered. Increasing the sample size and the bulk density by factors of 10 resulted in a decrease in ignition temperature of about 150 C. This result suggests that if a method could be devised for compacting the particulate in a trap, it would be more easily ignited under normal exhaust-gas conditions.

CATALYTIC IGNITION

In this study the catalytic ignition of diesel particulate was initially developed in bench-scale experiments. Final experiments were conducted in the exhaust system of an engine to verify the applicability of the concept to the actual engine environment.

Bench-Scale Experiments

The bench-scale experiments were carried out using a hot-tube reactor in which small samples of the diesel particulate were subjected to a gradually increasing temperature until ignition and oxidation occurred. Experiments were conducted to identify potentially catalytic materials, to explore methods of catalyst application, and to determine the magnitude of the catalytic effect in relation to the amount of catalytic material used.

Metal salts such as copper chloride, manganese chloride, and cobalt chloride were found to be capable of reducing the ignition temperature of the particulate by as much as 200 C. The addition of sodium or ammonium salts to the metal salt reduced the ignition temperature another 50 C, for a total reduction of 250 C (resulting in an ignition temperature of about 350 C for the catalyzed particulate). For most of this work, copper chloride equivalent to 7.5 mg Cu/g soot was used to achieve the catalyzed ignition.

Exhaust System Experiments

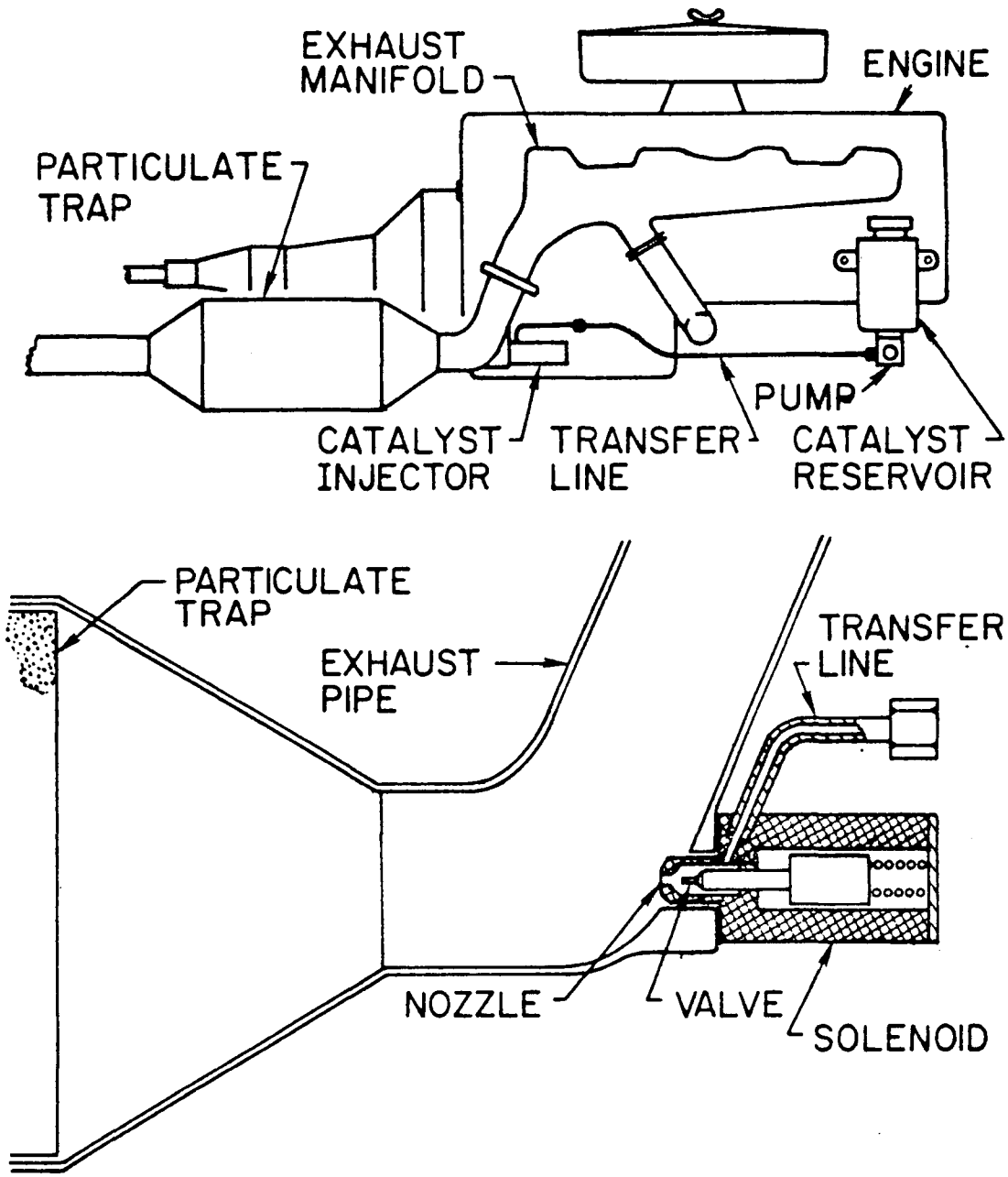
Final experiments were conducted in the exhaust pipe of a production diesel engine, where the results of the bench-scale experiments were confirmed. Various particulate trap materials and configurations were tried, including quartz wool, stainless steel wool, foamed alumina, and porous ceramic honeycomb. The porous ceramic honeycomb traps proved to be the most suitable, providing high surface area in a small volume, good collection efficiency at low pressure loss, and tolerance to the high temperatures reached during oxidation of the particulate.

Successful regeneration of the trap in the engine exhaust was achieved by intermittent injection of a water solution of the copper chloride/sodium chloride catalyst mixture into the exhaust stream immediately upstream of the trap, after a period of particulate collection on the trap. The actual ignition temperature in these tests ranged from 350 to 400 C. The amount of soot burned in each of these final trials is unknown but the copper concentration is believed to have been somewhat greater than used in most of the bench-scale tests.

CONCLUSIONS

The concept of using a catalyst material introduced into the exhaust of a diesel engine in a manner which allows the catalyst to associate with particulate collected on a trap has been successfully demonstrated. The catalyst acts to reduce the ignition temperature of the trapped particulate by about 250 C, which results in particulate burnoff and trap regeneration at significantly lower exhaust gas temperatures than would be required otherwise. This concept could provide more highly controlled burnoff of the trapped particulate, leading to lower peak oxidation temperatures in the trap (promoting longer trap life and improved trap reliability) and to minimization of the danger of unscheduled trap burnoff as a fire hazard.

Practical application of this concept requires development of suitable hardware and identification of the optimum operation cycle for the trap-oxidizer/catalyst injection system. The enclosed figure illustrates, in simplified form, a particulate control system that might be developed based on the metal-salt-catalyst concept briefly described in this paper.



DIESEL ENGINE PARTICULATE CONTROL SYSTEM BASED ON BATTELLE METAL-SALT-CATALYST CONCEPT

HEAVY-DUTY DIESEL ENGINE EMISSIONS -- SOME EFFECTS OF CONTROL TECHNOLOGY

by

J.M. Perez and R.V. Bower
Research Department
Caterpillar Tractor Co.
Peoria, Illinois

Use of various control technology methods to reduce specific emissions such as NO_x or particulates usually result in changes to other constituents in the exhaust of diesel engines. The effects of control technology on EPA Advisory Circular No. 76 emissions are reported. Unregulated emission trade-offs as a result of timing, EGR, catalysts, and engine modifications are discussed. Fuel consumption increased with most changes.

Although the emission levels are changed as a result of the control technology, the emissions pose no obvious health risk based on estimated exposure levels and available health effects data.

Table 1. Engine Change Tradeoffs

↑ = Increase ↓ = Decrease ⟷ = No Change

Change	Particulates Total	Particulates SEF	HC	NO_x	ALD	BAP	Fuel Cons	Power
PC→DI	↑	↑↓	↑↓	↑	↑↓	↑	↓	⟷
EGR	↑	↓	↓	↓	↓	↓	↑	↓
Timing								
Advance	↑↓	↑	⟷	↑	↑↓	↑	↑	⟷
Retard	↑↓	↑	↑↓	↓	↑	↑	↑	↓
Aftercooling	↑↓	↑↓	↑↓	↑↓	↓	↓	↑↓	↑
Injector SAC Volume Increase	↑	↑	↑	↑	↑	↑	⟷	⟷
Catalyst*	↑↓	↑↓	↓	↓	↓	↓	↑	⟷
Fuel (BaP Increase)	—	—	—	—	—	↑	—	—

* SO_4 ↑↓, NH_3 ↑

METHODOLOGY OF FRACTIONATION AND PARTITION OF DIESEL EXHAUST PARTICULATE SAMPLES

by

Bruce A. Petersen
Battelle-Columbus Laboratories
Columbus, Ohio

Diesel exhaust is a complex mixture of carbonaceous matter and gaseous compounds. As the exhaust is cooled, the gases condense or adsorb onto the solid particles. Because of the complex nature of diesel exhaust particulate, fractionation of the diesel exhaust extract into different compound classes is required before identification of individual components.

A fractionation method was developed by Battelle in 1978 to separate the chemical classes in diesel particulate extract samples. Several refinements in the original procedure have been made, and considerable experience in its use has been obtained on a wide variety of particulate sample types. There are two primary advantages associated with this procedure which are pertinent to the analysis of the organic material extracted from particulate samples:

- It is sensitive enough to fractionate small quantities of extracted mass. Particulate extracts containing 1 to 4 mg of total extracted mass have been sufficient for compound class separation and chemical analysis

- It can be conveniently scaled up for work with much larger samples. Particulate extracts containing several grams of extracted mass have been successfully fractionated without significant loss of material.

The procedure has already been used to separate about 800 diesel particulate filter extracts into six specific compound classes, without significant loss of material. The range of total organic mass was 1 to 143 mg. Recovery of extracted mass through this procedure ranged from 75 to 104 percent.

This procedure has also been scaled up in order to separate gram quantities of extracts from carbon black, urban air particulate, and wood stove emissions. Typical recovery of total mass using the scaled-up procedure has ranged from 90 to 101 percent.

THE FRACTIONATION PROCEDURE

Using the Battelle fractionation method, the particulate extract is separated into acidic, basic, and neutral components. Acids and bases are first separated by liquid-liquid partitioning. The neutrals are further partitioned by means of silica gel column chromatography. A schematic representation of the entire fractionation procedure is shown in Figure 1, and the generated fractions are listed in Table 1. For purposes of discussion, the fractions are referred to numerically as #1, #2, etc. In this discussion, the method describes the procedure to fractionate approximately 50 mg of extract.

The solvent extract of the particulate samples is first concentrated to 50 ml and an aliquot of 10-100 μl is removed to measure the organic mass in the extract before fractionation into the compound classes. In the fractionating scheme, the bases are first separated from the extract by liquid-liquid partition with a 5 percent sulfuric acid solution. Then, the acid and phenolic compounds are extracted from the organic solution with a 5 percent sodium hydroxide solution. Both the basic and acidic fractions are back extracted with methylene chloride. The remaining organic solution containing the neutral compounds is further partitioned into four fractions by open column chromatography on 5 percent H_2O-deactivated silica gel.

The silica gel columns are packed with 20 g of the silica gel in a hexane slurry. An additional column is also prepared to check the accuracy of the silica gel deactivation before partitioning the neutral organic solution. This is done by measuring the volume of hexane required to elute 500 ng of anthracene. The migration of the anthracene is monitored by a 366-nm UV lamp, and the volume of hexane is measured during the migration. When the silica gel is deactivated 5 percent, anthracene starts to elute from the column after the addition of 140 $\pm$ 10 ml of hexane. For 3 percent and 7 percent deactivation, the volume of hexane required is 270 $\pm$ 12 ml and 115 $\pm$ 8 ml, respectively.

Upon assurance that the silica gel is 5 percent H_2O deactivated, the neutral organic compounds are further fractionated. Four elution solvents are used. They are applied to the column and the eluent is collected in the following sequence: 60 ml hexane, 100 ml hexane/benzene (1:1), 100 ml methylene chloride, and finally, 200 ml methanol. The collected fractions correspond to fraction numbers 3, 4, 5 and 6.

Alkanes and alkenes are present in the aliphatic fraction (#3). The PAH compounds, nitrogen heterocycles and mono-nitrated PAH are presented in the aromatic fraction (#4). Polynitrated PAH, sulfur heterocycles, and oxygenated 2 and 3 rings PAH are found in the moderately polar fraction (#5). The highly polar fraction (#6) typically contains PAH with more than one functional group, oxygenated with more than 3 rings.

In this presentation, methods for fractionation of diesel exhaust particulate samples are reviewed and Battelle-developed methods are discussed. Experimental results using Battelle methods from two light-duty diesel engines and

one heavy duty diesel engine are presented. Data will be given to illustrate the quantity of mass in each fraction as well as the material balance for each particulate sample extract. Identification of selected compounds such as the nitro-PAH within the various fractions will also be presented.

Table 1. Compound Classes Generated by Fractionating Scheme

Fraction	Fraction #
Bases	1
Acids and Phenols	2
Aliphatic Hydrocarbons	3
Aromatic Hydrocarbons	4
Moderately Polar Neutrals	5
Highly Polar Neutrals	6

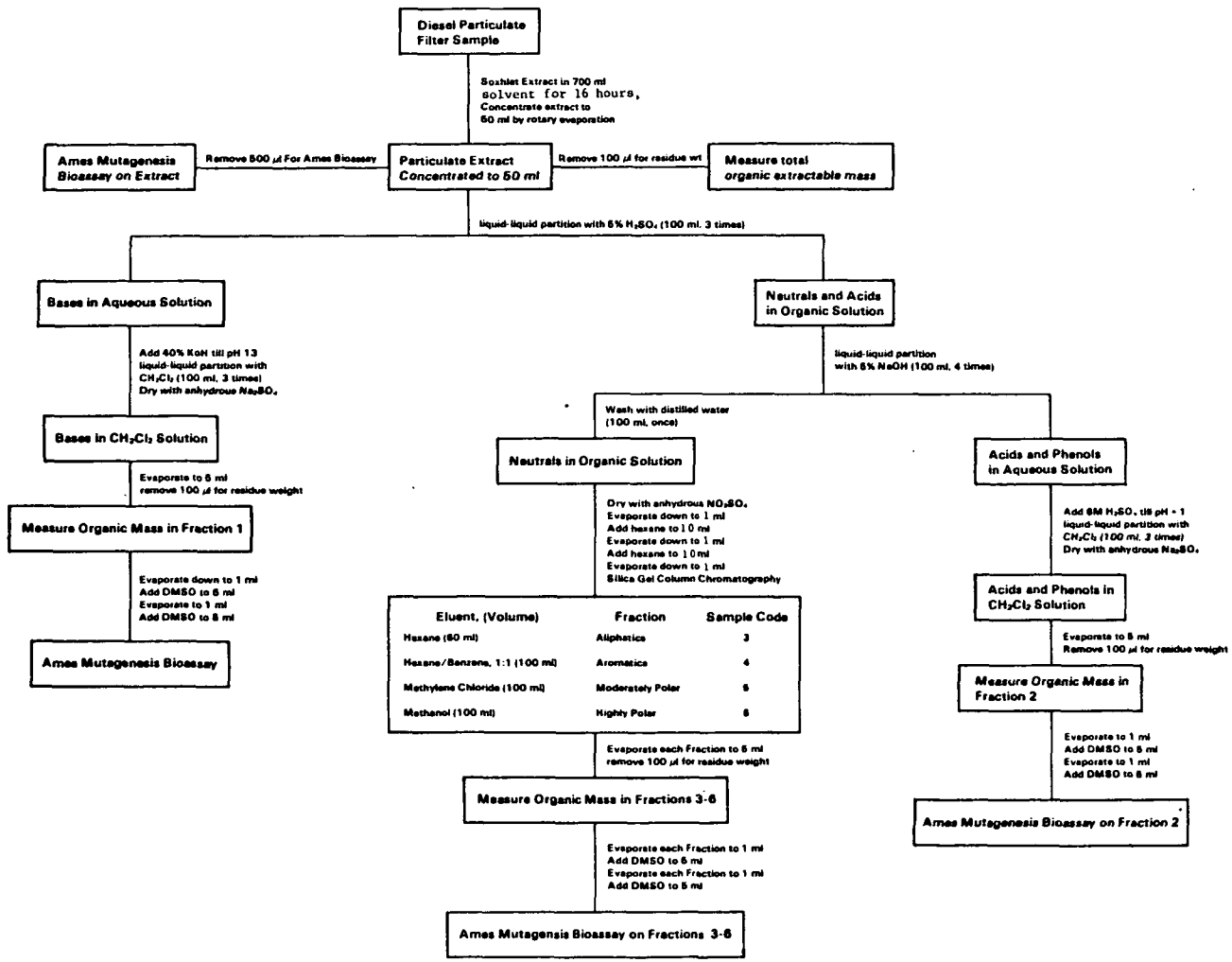


FIGURE 1. EXTRACTION AND FRACTIONATION PROCEDURE

THE UTILITY OF BACTERIAL MUTAGENESIS TESTING IN THE CHARACTERIZATION OF MOBILE SOURCE EMISSIONS: A REVIEW

by

Larry D. Claxton
Genetic Toxicology Division
Health Effects Research Laboratory
U.S. Environmental Protection Agency
Research Triangle Park, North Carolina

Since humans are and may be increasingly exposed to whole diesel exhaust, it is important to identify, characterize, and evaluate the biological activity of exhaust materials associated with mobile source emissions. It is also important to understand the effects of factors such as fuel, driving cycles, engine modifications, etc. that modify the emission products. The purpose of this paper is to review the use of bacterial mutagenicity testing in gathering information about the organics associated with mobile source emissions. The mutagenic activity of organic extracts from both diesel and gasoline exhaust particles was first established with the Salmonella typhimurium plate incorporation assay. Efforts to date demonstrate that mutagenic compounds condense upon and adhere to the central carbonaceous core of mobile source (diesel and gasoline engine) particles. In addition, bacterial mutagenesis work demonstrated that a S9 activating system containing the mixed function oxidase enzyme system was not needed in order to provide a mutagenic response. The response of the various tester strains provide evidence that the mutagens are of the frameshift type. By integrating microbial tests with chemical fractionation procedures, the more biologically active chemical fractions (e.g., polar neutrals) have been identified. This, in turn, has led to the identification of specific mutagens within diesel exhaust organics — such as 1-nitropyrene. Bacterial mutagenicity studies have aided in demonstrating that the mutagens are removed from the carbonaceous particles and become protein-bound when incubated with physiological fluids. By exposing diesel exhaust to various ambient-like conditions within a smog chamber and comparing the resulting mutagenic activity, one study demonstrated an alteration of mutagenic activity by ambient levels of ozone. Although bacterial mutagenesis studies provide exceptional insight into the nature of the genotoxic activity of mobile source organics, caution must also be observed in interpreting the data since differences between bacterial and mammalian cells exist with respect to metabolic activation, cell permeability, and particulate processing.

Emission Factors from Diesel and Gasoline Powered Vehicles; Correlation with the Ames Test

by

Roy B. Zweidinger
Mobile Source Emissions Research Branch
U.S. Environmental Protection Agency
Research Triangle Park, N.C. 27711

In 1978, initial findings on the mutagenic nature of diesel extracts were reported (1). Since that time, the Ames salmonella typhimurium bioassay has been used extensively in the investigation of mobile source emissions. Both government and industry have carried out numerous studies on the many variables effecting the mutagenicity of mobile source samples. These studies for the most part fall into six general categories: 1) Sampling-exhaust dilution ratios, gas phase vs particulate phase collection and examination of filter types; 2) Bioassay sample preparation-extraction solvents, extraction procedures, solvent exchange studies and sample storage; 3) Vehicle types-light duty diesels, heavy duty trucks and gasoline powered cars; 4) Operational characteristics-driving cycles, fuel types, temperature, mileage and engine malfunction conditions; 5) Artifacts; 6) Characterization studies-qualitative and quantitative studies on the nature of mobile source mutagens and their precursors.

The findings and results of some of these studies are given below. While problems exist in the quantitative comparison of Ames test results from different laboratories or even the same laboratory over a given period of time (2), camparisons should be valid at least in the qualitative sense.

Table 1 lists some average emission factors and Ames data for several vehicle classes. The heavy duty trucks were operated over the proposed 1983 transient driving cycle while the light duty vehicles were operated over the Federal Test Procedure driving cycle. Data for the light duty gasoline cars (3) and light duty diesels (4) are from in-use vehicle studies. A number of the gasoline cars had some form of emission system malfunction as evidenced by the high regulated emissions observed in some cases (eg. 1977 Dodge Aspen, NO_x emissions were 6.1 g/mile). The heavy duty trucks (5) were all 1979 models with the exception of the

2 cycle which was a 1977 Detroit Diesel city bus. The heavy duty truck data is limited and one must realize that "average" values may be strongly affected by outliers (eg. the TA 98, +S9 data for the heavy duty gasoline trucks is an average of two vehicles having values of 604 and 253 revertants/mile ($x10^{-3}$). One does not know which is the more representative value).

Correlations carried out on the gasoline cars indicated that benzo(a)-pyrene (BAP) emissions of the catalyst (unleaded) vehicles correlated with TA 98, +S9 (r^2 = 0.81). This is also in accord with the fact that, in general, gasoline cars showed higher activity with activation. A positive correlation was also found for the soluble organic fraction (SOF) vs TA 98, +S9 (r^2 = 0.84). The THC, CO, and NOX emissions showed no correlation with activity.

The levels of 1-nitropyrene in the light duty gasoline vehichles were only about 3% of that observed for diesels and show no correlation with TA 98, without activation. Emission levels of 1-nitropyrene have not been determined for a large number of diesels. It has been found, however, to correlate fairly well with TA 98, -S9 in cycle studies conducted on the same vehicle (r^2 =0.96) and in some artifact experiments (r^2 = 0.97).

Recently, some low temperature experiments were conducted on light duty diesels (6). Results indicated indicated increased particulate and SOF emissions wih decreasing FTP test temperatures. The major portion of the increased SOF emissions appeared to be unburned fuel and no correlation of temperature and TA 98 activity was observed.

While the majority of work has been conducted on diesel and gasoline particulate extracts, attempts have also been made to examin the gas phase organics for mutagenic activity (7). Using the porous polymer resin XAD2 for trapping followed by elution of the organics wiht dichloro-methane, activity in TA 98 was found to be very low, at least an order of magnitude less than the SOF. In addition, control experiments indicated that much of the observed activity may have been due to artifacts.

References

1. Huisingh, J., R. Bradow, R. Jungers, L. Claxton, R. Zweidinger, S. Tejada, J. Bumgarner, R. Duffield, M. Waters, V. Simmon, C. Hare, C. Rodriguez, and L. Snow, "Application of Bioassay to the Characterization of Diesel Particulate Emissions: Parts I, II." in Application of Short-term Bioassays in the Fractionation and Analysis of Complex Environmental Mixtures, EPA 600/9-78-027, November 1978.

2. Salmeen, I. and A. M. Durisin, "Some Effects of Bacteria Population on Quantitation of the Ames Salmonella Histidine Reversion Mutagenisis Assays." Mutation Research 85, 109-118, 1981.

3. Lang, J. L. Snow, R. Carlson, F. Black, R. Zweidinger and S. Tejada, "Characterization of Particulate Emissions from In-use Gasoline Fueled Motor Vehicles", Paper 81186 to be presented at SAE Fuels and Lub. Meeting, Tulsa, October 1981.

4. Gibbs, R.E., J.D. Hyde and S. M. Byer, "Characterization of Particulate Emissions from In-Use Vehicles", Paper 801372 presented at SAE Fuels and Lub. Meeting, Baltimore, October 1980.

5. Dietzman, H.E. and M.A. Parness, "Study of Emissions From Trucks Over Transient Driving Cycles", Final report to EPA under contract No. 68-02-2993, September 1981.

6. Braddock, "Emissions of Diesel Particles and Particulate Mutagens at Low Ambient Temperature", EPA Diesel Emissions Symposium, Raleigh, N.C. October 5-7, 1981.

7. Stump, F., "Trapping Gaseous Hydrocarbons", Ibid.

Table 1. Emission and Particulate Characteristics by Vehicle Class

	Heavy Duty Trucks[a]			Light Duty Vehicles[b]		
Emission Factors	Gasoline (2)[c]	4-cycle Diesel(3)	2-cycle Diesel	Diesel(6)	Leaded Gas(4)	Unleaded Gas(15)
THC, g/mi.	13.5	1.88	2.13	0.38	2.74	1.05
CO, g/mi.	116.3	7.53	75.1	1.27	28.5	12.2
NOX, g/mi.	13.4	25.5	35.5	1.27	3.52	2.31
Total Particulate, g/mi.	0.74	1.49	3.33	0.61	0.10	0.03
SOF, mg/mi.	27.6	335	537	124	21.1	14.4
BAP, ug/mi.	39.5	2.61	1.33	4.5[d]	14.5	3.3
1-Nitropyrene, ug/mi.	----	8.26[e]	-----	7.4[d]	0.20	0.24
TA 98, -S9, rev./mi.(10^{-3})	111	392	13	509	152	42.1
TA 98, +S9, rev./mi.(10^{-3})	428	356	48.3	-----	258	79.3

a. 1983 Transient Cycle
b. FTP cycle
c. ( ) denotes number of vehicles
d. Values derived from other vehicle data
e. One vehicle only, 1979 Cummins Formula 290

ANALYSIS OF VOLATILE POLYCYCLIC AROMATIC HYDROCARBONS IN HEAVY-DUTY DIESEL EXHAUST EMISSIONS

by

Walter C. Eisenberg and Sydney M. Gordon
Analytical Research Section
IIT Research Institute
Chicago, Illinois 60616

Joseph M. Perez
Research Department
Caterpillar Tractor Company
Peoria, Illinois 61629

The breakthrough and/or loss of polycyclic aromatic hydrocarbons (PAH) from particulate filters was investigated during the collection of heavy-duty diesel exhaust emissions. The concentration of PAH associated with heavy-duty diesel particulate has been monitored for several years. During these studies it was observed that appreciable quantities of organics including PAH pass through the particulate filter during the sample collection (1). The emissions from a heavy-duty diesel engine were sampled using 70-mm Pallflex TX40HI20WW filters. A portion of the gas passing through the particulate filter was sampled using 4.0-mm x 4.0-cm sorbent cartridges. Chromosorb 102 and Tenax GC were used to collect gas phase organics. The particulate filter was extracted in a micro-Soxhlet apparatus for 6 hours with methylene chloride. The sorbent cartridges were extracted by passing hexane/benzene (90/10, v/v) through the cartridge at a flow rate of 0.5 ml/min. Following concentration the PAH fraction was isolated using open column silica gel chromatography. The PAH in particulate and gas phase samples were analyzed using gas chromatography/mass spectrometry (GC/MS) and high performance liquid chromatography (HPLC).

In the GC/MS analysis the samples were eluted on a 15.0-m x 0.31-mm i.d. SE-54 fused silica capillary column directly into the ion source of a Varian MAT 311A mass spectrometer operating in the repetitive scanning mode. The data were enhanced using a computer program by Dromey et al. (2) to locate peaks in the raw data and provide a set of clean mass spectra of the sample components free of contributions from background and overlapping peaks. Compounds were identified with a library matching search algorithm, and in the case of complex spectra the data was manually interpreted.

Twelve parent PAH were measured in the gas phase and particulate samples using reverse phase HPLC. The analysis was performed using two coupled 4.6-mm x 25.0-cm Zorbax ODS columns and an acetonitrile water gradient. The eluant were monitored using an ultraviolet absorbance detector at λ = 254 and 280 nm and a fluorescence detector, λ_{ex} = 280 nm and λ_{em} = 389 nm.

Over 40 compounds were tentatively identified in the gas phase after a particulate filter during the collection of heavy-duty diesel exhaust emissions. Thirty-five of these compounds were volatile PAH and included parent- and aldyl-substituted compounds ranging in size from two to five fused rings. A distribution quotient was defined as the ratio of the concentration of the PAH in the gas phase to their concentration in the particulate phase. It ranged from ~ 56 for fluorene to ~ 1 for benz[a]anthracene. Experiments to date show that at some operating conditions the concentrations of volatile PAH were significantly lower when measured on the particulate only. Since the role of these compounds in the formation of artifacts and mutagens is unresolved, the methods for measuring PAH in exhaust streams need to be modified to account for gas phase compounds.

REFERENCES

1. W.E. Pepelko, R.M. Danner, and N.A. Clarke, eds. 1980. Health Effects of Diesel Engine Emissions. EPA-600/9-8-057a. Health Effects Research Laboratory, U.S. Environmental Protection Agency: Cincinnati, OH 45268. pp. 138-174.

2. R.G. Dromey, M.J. Stefik, T.C. Rindfleisch, and A.M. Duffield. 1976. Anal. Chem. 48:1368.

THE CHEMICAL CHARACTERIZATION OF DIESEL PARTICULATE MATTER

by

James Alan Yergey and Terence H. Risby
School of Hygiene and Public Health
Johns Hopkins University
Baltimore, Maryland

Samuel S. Lestz
Department of Mechanical Engineering
Pennsylvania State University
University Park, Pennsylvania

INTRODUCTION

A great deal of research has been directed toward elucidating the potential health hazards of Diesel particulate matter. The potential health risks of the emmitted particles are due to a number of important factors. The mass median diameter of the agglomerated particles found in Diesel exhaust is less than 1 um (1-4), and their surface areas greater than 50 m^2/g (3,4). The large surface areas facilitate the adsorption of gas phase combustion products, while the small diameters allow appreciable residence times in the atmosphere. In addition, it is generally accepted that particles less than 1 um in diameter can be respired by humans, with a significant portion depositing in the pulmonary regions of the lung (5-7). An assessment of the potential health effects of Diesel particles must also consider the fact that many of the surface adsorbed species which have been isolated from the soluble organic fraction (SOF) of Diesel particulate matter are potentially carcinogenic (8-10).

A number of references have appeared in the literature in recent years regarding the identification of the individual constituents of the SOF (11-15). The commercial Diesel fuels and lubricating oils which were used in each of these studies contain such a wide variety of compounds that a study of the combustion mechanisms which lead to the particle-bound products becomes extremely difficult. An understanding of the mechanisms governing the formation of the adsorbed species is a requisite for complete comprehension of the potential health effects of Diesel particulate matter. The primary objective of this research was to simplify the combustion chemistry in order to better understand the overall mechanisms governing the formation of the particle-adsorbed species.

EXPERIMENTAL

Diesel particulate matter was generated from a single-cylinder engine, operated on a 1:1 by volume blend of n-tetradecane and 2,2,4-trimethylpentane. This prototype fuel was used in conjuntion with a synthetic lubricating oil, in order to simplify the exhaust chemistry. Air and argon/oxygen oxidant systems were employed. The Ar/O_2 was used in order to investigate the products of nitrogen-free combustion. Particle samples were collected on 142 mm Pallflex filters in an isokinetically drawn sample line, and gas phase emissions were monitored. Filters were Soxhlet extracted with dichloromethane, and the resulting extract blown to dryness under nitrogen and weighed. Samples were analyzed by the Ames Salmonella and Comptest (16) bacterial assays in order to assess their mutagenic and potential carcinogenic capaity.

The simplifications introduced in this study allow the separation and analysis of the entire SOF in a single pass. Particle extracts were analyzed by capillary gas chromatography, using flame ionization and thermionic specific detectors, and high performance liquid chromatography, using UV detection. Both positive and negative chemical ionization mass spectrometry were employed for direct analysis of the gas chromatographic effluents, and for analysis of collected HPLC fractions, using a Pt-filament, direct-insertion probe. Capillary gas chromatography/electron impact mass spectrometry was utilized for substantiating identifications. In addition, Diesel particles were heated under vacuum, and the evolved gases analyzed by chemical ionization mass spectrometry.

RESULTS

Figure 1 portrays a typical GC/PCIMS total ion profile for an air oxidant sample. Table 1 illustrates the compounds which have been identified in the air oxidant samples. Identifications were based upon gas chromatographic retention indices (17), molecular weights derived from CIMS, and EIMS library searchs. In general, the Ar/O_2 samples exhibited the same major components, with lower concentrations. GC/TSD results indicated a lower number of nitrogen containing compounds in the Ar/O_2 samples, however, specific identifications of the nitrogenous species were not possible. HPLC separations coupled with the mass spectra generated using the Pt-filament probe were especially useful for identifying higher molecular weight PAH, while GC/NCIMS data were particularly sensitive to the oxygenated species found in the SOF

Results indicate that unsubstituted, non-linear polynuclear aromatic compounds are the primary particle-bound combustion products from aliphatic hydrocarbon fuel components. The identified compounds are demonstrated to be fuel-independent products of the diffusion controlled combustion process which exists in the Diesel engine. This fact should be considered in any future health effects studies of Diesel particulate matter. The potent carcinogen, nitropyrene, was tentatively identified in the soluble organic fraction of the particles, and could possibly account for a large portion of the observed mutagenic properties of the extractable organics.

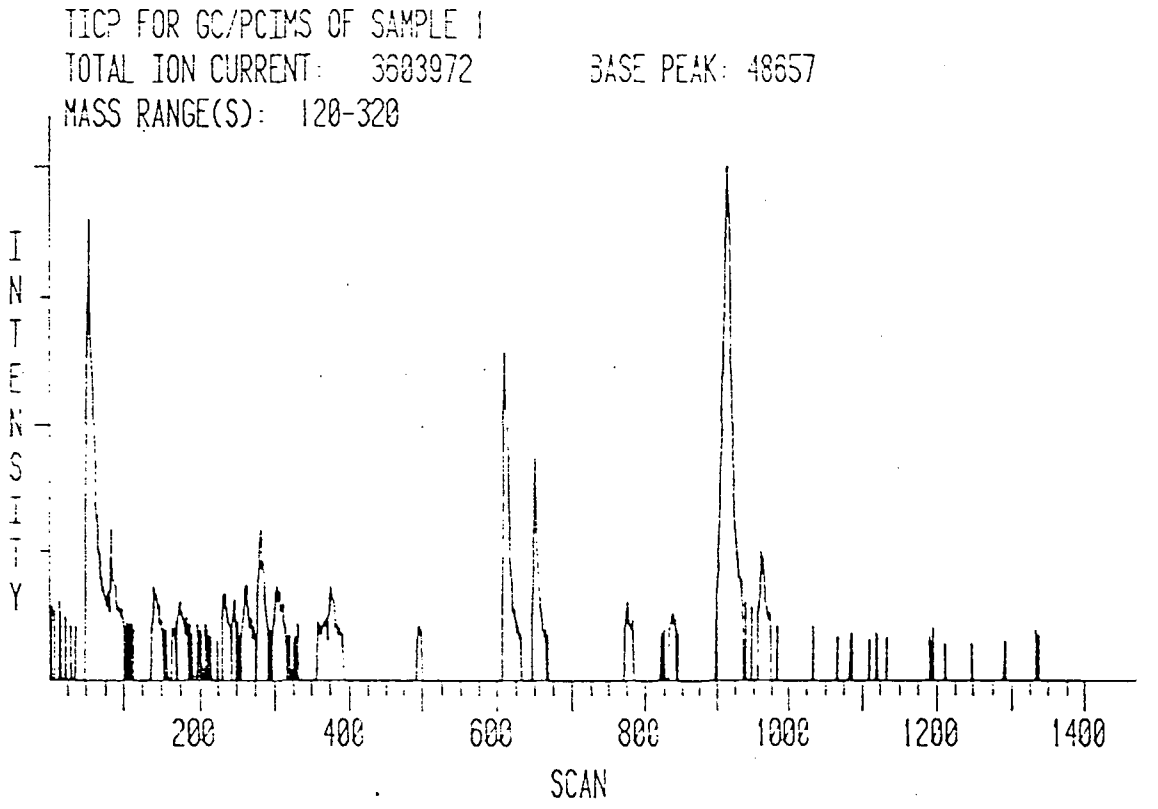


FIGURE 1. Total Ion Current Profile for GC/PCIMS of Air Oxidant Sample

Naphthalene	Phenanthrene
Benzofuran,7-methyl-	Methyl-9-Fluorenones
Inden-1-one,2,3-dihydro-	Benzo[c]cinnoline
Methylnaphthalenes	Fluorene Quinone
Phthalate-anhydride	Phenanthrene Quinone
Biphenyl	Cyclopenta-phenanthrene-5-one
n-Tetradecane	Naphtho[1,8-cd]pyran-1,3-dione-
1-Benzopyran-2-one	Fluoranthrene
Biphenylene, or Acenaphthylene	Pyrene
Acenaphthene	Methylpyrenes
Dibenzofuran	Benzo[ghi]fluoranthene
Fluorene	Cyclopenta[cd]pyrene
9-Fluorene	Chrysene or Triphenylene
Anthracene	Benzofluoranthrathenes or Benzopyrenes

TABLE 1. Compounds Identified in Air Oxidant SOF

REFERENCES

1. W. H. Lipkea, J. H. Johnson and C. T. Vuk. 1978. The Physical and Chemical Character of Diesel Particulate Emissions- Measurement Tecnniques and Fundamental Considerations. S.A.E. Paper No. SP-430.

2. J. A. Verrant and D. A. Kittelson. 1977. Sampling and Physical Characterization of Diesel Exhaust Aerosols. S.A.E. Paper No. 770720.

3. J. W. Frey and M. Corn. 1967. Nature. 216:615-617.

4. M. M. Ross. 1981. Physicochemical Characterization of Diesel Particulate Matter. Ph. D. Thesis. The Pennsylvania State University.

5. T. F. Hatch and P. Gross. 1964. Pulmonary Deposition and Retention of Inhaled Aerosols. Academic Press: New York.

6. Task Group on Lung Dynamics. 1966. Health Physics. 12:173-208.

7. P. Kotin and H. L. Falk. 1959. Cancer. 12:147-163.

8. D. J. Barth and S. M. Blacker. 1978. Journal of the Air Pollution Control Association. 28:769-771.

9. J. McCann, B. N. Ames, E. Choi and E. Yanasaki. 1975. Proccedings of the National Academy of Sciences U.S.A. 72:5135-5139.

10. M. Dukovich, R. E. Yasbin, S. S. Lestz, T. H. Risby and R. E. Zweidinger. 1981. Environmental Mutagenisis. In press.

11. E. F. Funkenbusch, D. G. Leddy and J. H. Johnson. 1979. The Characterization of the Soluble Organic Fraction of Diesel Particulate Matter. S.A.E. Paper No. 79418.

12. D. Schuetzle, F. S. Lee, T. J. Prater and S. B. Tejeda. 1981. International Journal of Environmental Analytical Chemistry. 9:93-144.

13. F. W. Karasek, R. J. Smythe and R. J. Laub. 1974. Journal of Chromatography. 101:125-136.

14. M. D. Erikson, D. L. Newton, E. D. Pellizzari, K. B. Tomer and D. Dropkin. 1979. Journal of Chromatographic Science. 17:449-454.

15. F. Black and L. High, Methodology for Determining Particulate and Gaseous Diesel Hydrocarbon Emissions. 1979. S.A.E. Paper No. 79042.

16. M. Dukovich. The Mutagenic and "SOS" Inducing Activity of Diesel Particulate. M. S. Thesis. (1981). The Pennsyvania State University.

17. M. L. Lee, D. L. Vassilaros, C. M. White and M. Novotny. 1979. Analytical Chemistry. 51:768-773.

THE ANALYSIS OF NITRATED POLYNUCLEAR AROMATIC HYDROCARBONS IN DIESEL EXHAUST PARTICULATES BY MASS SPECTROMETRY/MASS SPECTROMETRY TECHNIQUES[1]

by

T. Riley, T. Prater and D. Schuetzle,
Ford Motor Co, Scientific Research Lab., Dearborn, MI 48121;

T. M. Harvey and D. Hunt
Dept. of Chem., Univ. of Virginia, Charlottesville, VA 22901

INTRODUCTION

Recent investigations have indicated that organic solvent extracts of light-duty diesel exhaust particulates exhibit direct-acting mutagenicity when tested using the Ames assay. Preliminary estimates indicate that a significant portion of this direct-acting mutagenicity may be due to the presence of nitrated polynuclear aromatic hydrocarbons (nitro-PAH).[2] Mass analyzed ion kinetic energy spectrometry (MIKES) and triple stage quadrupole (TSQ) analytical techniques used to characterize these compounds in diesel exhaust are described.

EXPERIMENTAL

Light duty diesel exhaust particulate samples were collected on T60A20 Pallaflex filters using a dilution tube and a chassis dynamometer test facility. Filter samples were extracted with dichloromethane.

MIKES analyses were performed on a Vacuum Generators ZAB-2F mass spectrometer using both electron impact (EI) and negative ion methane chemical ionization (NICI) procedures. All experiments were conducted using a magnetic sector resolution of approximately 2000 and helium as a collision gas (1×10^{-7} torr).

A Finnigan TSQ mass spectrometer was used to perform collisionally activated dissociation (TSQ-CAD) and constant neutral loss studies. All experiments were conducted using positive ion methane chemical ionization (PICI) procedures. In the TSQ-CAD studies the first quadrupole was set to transmit a parent ion of interest into the second quadrupole which functioned as a collision cell (N_2, 5×10^{-3} torr). The third quadrupole was scanned repetitively to collect daughter ion spectra. In the constant neutral loss studies, the first and third quadrupoles were scanned in parallel with a 17 amu mass deficit. Under these conditions, only ions which experience the loss of a 17 amu neutral fragment when collisionally dissociated in the second quadruple are detected. This ion reaction was found to be characteristic of nitro-PAH compounds.

RESULTS AND DISCUSSION

The TSQ constant neutral loss analysis was found to be a very useful screening procedure for nitro-PAH compounds. Table I lists 20 different nitro-PAH derivatives which were tentatively identified in diesel particulate extract using this procedure. It must be emphasized that this technique only monitors a reaction characteristic of nitro-PAH compounds and does not confirm their presence. The specificity of the constant neutral loss analysis was assessed by examining the response of several compounds representative of the classes of PAH derivatives observed in diesel exhaust. The results of this study, as shown in Table II, indicate that the technique exhibits good selectivity for the nitro-PAH derivatives.

Table III illustrates the concentration of 1-NP in the exhaust particulate extract from four different diesel engines as determined by MIKES and TSQ-CAD analysis. These quantitation studies indicated that both MS/MS techniques lacked sufficient resolution on the first mass filter to eliminate positive interferences in the daughter ion spectra completely. The M-16 daughter ion fragment (M+1 - OH) was found to be specific for 1-NP and was used for quantitation by TSQ-CAD, but the electrostatic sector of the MIKES instrument did not resolve this daughter ion adequately. It was necessary to prefractionate the OP-1 and PG-1 samples by preparative scale high performance liquid chromatography and to use NICI techniques to accomplish an interference-free MIKES analysis of 1-NP.

Neither MS/MS technique distinguished between nitro-PAH isomers. This information was obtained by capillary GC-MS.

1 Riley, T., T. Prater, D. Schuetzle, T. Harvey and D. Hunt. 1981. The analysis of nitrated polynuclear aromatic hydrocarbons in diesel exhaust particulates by mass spectrometry/mass spectrometry techniques. Presented at the 29th Annual Conference on Mass Spectrometry and Allied Topics, Minneapolis, MN.

2 Schuetzle, D., T. Prater, T. Riley, A. Durisin and I. Salmeen. 1980. Analysis of nitrated derivatives of PAH and determination of their contribution to Ames assay mutagenicity for diesel particulate extracts. Presented at the Fifth International Symposium on Polynuclear Aromatic Hydrocarbons, Columbus, OH.

Table I. Nitro-PAH Derivatives Tentatively Identified in Diesel Particulate Extracts by TSQ Constant Neutral Loss Analysis

Nitroacenaphthylenes
Nitro(acenaphthlenes, biphenyls)
Nitronaphthaquinones
Nitrodihydroxynaphthalenes
Nitrofluorenes
Nitro(methylacenaphthalenes, methylbiphenyls)
Nitro(trimethylnaphthalenes)
Nitro(naphthalic acid)
Nitro(anthracenes and phenanthrenes)
Nitro(fluorenones and methylfluorenes)
Nitro(methylanthracenes and methylphenanthrenes)
Nitro(anthrones and phenanthrones)
Nitro(pyrenes and fluoranthenes)
Nitro(dimethylanthracenes and dimethylphenanthrenes)
Nitro(methylpyrenes and methylfluoranthenes)
Nitro(pyrones and fluoranthones)
Nitro(pyrene and fluoranthene)quinones
Nitro(dimethylphenanthrene and dimethylanthracene) carboxaldehydes
Nitro(methylbenzo(a)anthracenes, methylchrysenes and methyltriphenylenes)
Nitro(benzo(a)pyrenes, benzo(e)pyrenes and perylenes)

Table II. Selectivity of the TSQ Constant Neutral Loss Analysis for Nitro-PAH

Compound Class	Selectivity Ratio[a] (Nitro-PAH/Compound Class)
Amines	50/1
Aldehydes	200/1
Quinones	
Carboxylic Acids	
Acids	

[a] interference level <5%

Table III. Quantitation of 1-NP in Diesel Exhaust Particulate Extract using MS/MS Techniques

Engine Sample	Instrument	Ionization	Concentration (ppm)
N1-1	TSQ	PICI	2285±230
	MIKES	EI	2080±220
OL-1	TSQ	PICI	204±30
OP-1	TSQ	PICI	77±15
	MIKES	EI	≤105
	MIKES	NICI	55±11
PG-1	MIKES	NICI	150±30

CONTRIBUTION OF 1-NITROPYRENE TO DIRECT ACTING AMES ASSAY MUTAGENICITIES OF DIESEL PARTICULATE EXTRACTS

by

Irving Salmeen, Anna Marie Durisin,
Thomas J. Prater, Timothy Riley, and Dennis Schuetzle
Engineering and Research Staff
Research
Ford Motor Company
Dearborn, Michigan

We have determined the percentage contribution, P, of 1-nitropyrene (1-NP) to the direct acting Ames assay mutagenicities of dichloromethane extracts of exhaust particles collected from three different diesel powered passenger vehicles. For strains TA98, 100, and 1538, respectively, P was (16, 1, and 7%) for vehicle 1; (24, 9, and 7%) for vehicle 2; and (13, 4, and 13%) for vehicle 3. We assumed:

$$P = C \times (M1/M2) \times 100\%$$

M1 and M2 are the slopes of the linear portion of the Ames assay dose-response functions for 1-NP and for diesel particulate extract respectively. C, the mass of 1-NP in the particulate extracts/mass of extract, was determined by collisionally activated dissociation mass analyzed ion kinetic energy spectrometry (CAD-MIKES). (CAD-MIKES is a double mass spectrometer technique with which compounds often can be analyzed in complex mixtures directly without chromatographic separation.) C for vehicles 1, 2, and 3, respectively, was 55 $\pm$ 11, 2030 $\pm$ 220, and 150 $\pm$ 30 ppm. We used fused-silica capillary column GC-MS to show that the mass 247 (1-NP) CAD-MIKES spectrum was due to 1-NP and not to some other mass 247 isomer. Several isomers of nitrofluoranthene and nitropyrene were synthesized to assure adequate GC-MS separation of these isomers from 1-NP.

The slopes, M1 and M2, are approximately proportional to μN, where μ is the mutation rate per concentration of mutagen and N is the total number of histidine auxotrophs in the background lawn (1). N is proportional to the initial inoculum and the average number of auxotrophs per individual background colony. N cannot be measured in the Ames assay and it is not necessarily the same for M1 and M2. Consequently, whenever we carried out an Ames assay of diesel particulate extract we concurrently carried out an assay of 1-NP using equal amounts of the same broth culture, thereby ensuring that the initial inocula were equal. We then obtained photomicrographs of the

background lawn (100X) on each plate at the time of counting revertants and from these photomicrographs counted the background colonies and estimated their average size. If the background colony counts and their average sizes were the same for both 1-NP and diesel particulate extracts, we took this as evidence that N was the same and then used the corresponding slopes to calculate P.

Finally, we did experiments in which various known amounts of 1-NP were added to a constant amount of extract and obtained the Ames assay dose-response function of the 1-NP in the presence of the diesel extract. The added amounts of 1-NP were chosen to be within the same order of magnitude as that of the 1-NP already in the particulate extracts as measured by mass spectrometry. We found that the slope of the 1-NP dose-response function in the presence of particulate extract was the same as that for 1-NP alone in solution. This result supports an assumption, implicit in the above equation, that the mutagenicity of 1-NP is not altered by the other components in the mixtures.

These data show that 1-NP is an important contributor to the mutagenicity of these diesel particulate extracts. Even a 1% contribution is important, considering the thousands of compounds present in the particulate extracts. This observation is very encouraging to experimenters seeking to identify mutagens in complex mixtures because it suggests the possibility that a small number of compounds may account for the majority of the mutagenicity of these complex mixtures.

REFERENCES

1. Salmeen, I. and Durisin, A., Mutat. Res., 85, 101-118, 1981.

DINITROPYRENES: THEIR PROBABLE PRESENCE IN DIESEL PARTICLE EXTRACTS AND CONSEQUENT EFFECT ON MUTAGENIC ACTIVATIONS BY NADPH-DEPENDENT S9 ENZYMES

T. C. Pederson and J-S. Siak
Biomedical Science Department
General Motors Research Laboratories
Warren, MI 48090

The direct-acting mutagenic activity of diesel exhaust particle extracts in the *Salmonella* mutation assay may be mostly due to nitroaromatic compounds activated by bacterial nitroreductase enzymes. Chromatographic separations have demonstrated that much of the extract's mutagenic activity is associated with components that are more polar than 1-nitropyrene or other monosubstituted nitro-PAH (Pederson and Siak, 1981, J. Appl. Tox., 1(2):54-60). The present studies, employing TLC and HPLC separation techniques and the recently developed dinitropyrene-resistant *Salmonella* strains (Rosenkranz et al, 1981, Mutation Res., 91:103-105), investigate the probable presence of dinitro-, trinitro-, or tetranitropyrenes in the polar mutagenic fractions of diesel particle extracts.

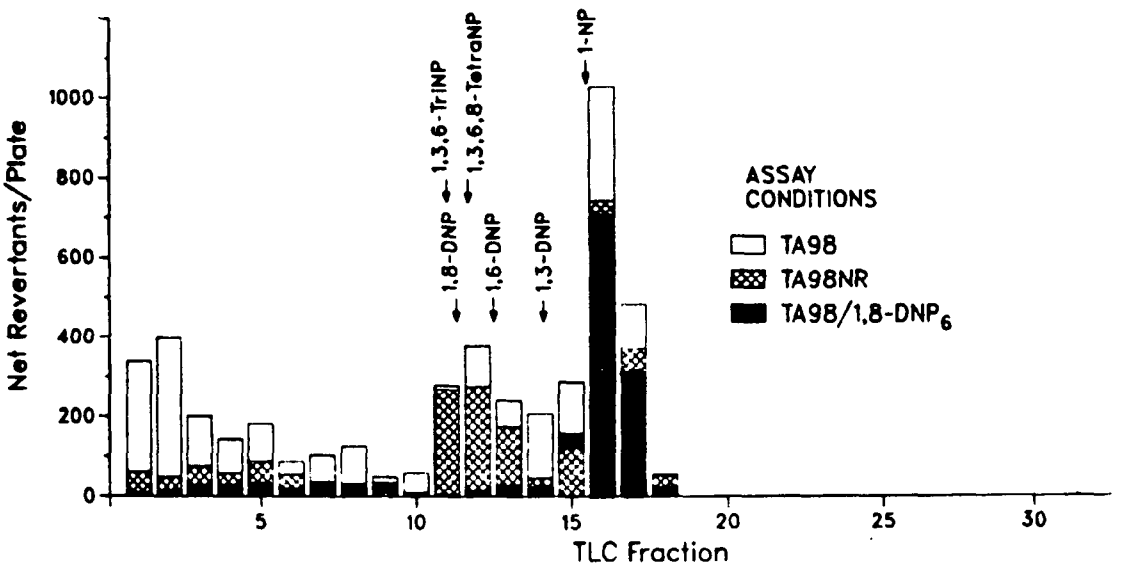
The direct-acting mutagenic activity of a diesel particle extract, reduced by 40% in the niridazole-resistant strain TA98NR, was decreased by 70% in the dinitropyrene-resistant strain TA98/1,8-DNP_6. After fractionation of the extract by silica gel TLC, all mutagenic fractions exhibited reduced activity in TA98/1,8-DNP_6, as shown in the figure. The most marked reduction occurred with the fractions which co-chromatographed with reference samples of the multisubstituted nitropyrenes. The mutagenicity of these fractions and multinitropyrenes was markedly reduced in TA98/1,8-DNP_6, but not in TA98NR. HPLC separations on a cyano phase-bonded silica column indicate that 1,8-dinitro-and 1,6-dinitropyrene are the predominant mutagenic components in the material recovered from TLC fractions 11 through 15. Some mutagenic activity was also attributed to 1,3-dinitro- and 1,3,6-trinitropyrene. The dinitropyrenes may account for 15-20% of the mutagenic activity in the particle extract, but they are very potent bacterial mutagens and would be present at concentrations of less than 1 ppm in the exhaust particulate.

The nitroreductase-deficient bacteria have also been used in the *Salmonella*/S9 mutation assay to examine the effect of mammalian enzyme activities on the mutagenicity of diesel particle extracts and the nitropyrenes. Under appropriate conditions, the activation of mutagens in diesel particle extract by rat liver S9 enzymes was evident as a difference between assays with and without NADPH (Pederson and Siak, 1981, J. Appl. Tox., 1(2):61-66). 1-Nitropyrene was similarly activated by S9 enzyme activity. The activation of 1-nitropyrene is located in the microsomal fraction of the S9 preparation, but activation of diesel particle extract was more evident with the cytosol fraction.

The mutagenicity of each dinitropyrene was greatly reduced by the NADPH-dependent activity of S9 enzymes. As shown in the table below, the NADPH-dependent inactivation is catalyzed by microsomal enzymes, but with the cytosol fraction, the mutagenic activity of the dinitropyrenes is increased. The NADPH-dependent increase in mutagenicity of the dinitropyrenes includes both a cytosol-independent reaction, which is probably a direct reduction of the compounds by reduced pyridine nucleotide, and an enzyme-catalyzed reaction. NADH is just as effective as NADPH in both the cytosol-dependent and independent activation reactions.

The NADPH-dependent increase in mutagenicity of diesel particle extract in the *Salmonella*/S9 assay involves both multiple extract components and multiple S9 enzymes. The dinitropyrenes presumably contribute to the cytosol-catalyzed activation. The much smaller effect of microsomal enzymes on the mutagenicity of the particle extract must reflect competing activation and inactivation reactions as evidenced by the difference between 1-nitropyrene and the dinitropyrenes.

		Net TA98NR Revertants/Plate	
		plus S9 microsomes	plus S9 cytosol
1,6-Dinitropyrene	-NADPH	282 +17	347 ± 16
10 ng/plate	+NADPH	19 ± 5	878 ± 98
	Change	-95%	+150%
1,8-Dinitropyrene	-NADPH	196 ±13	392 ± 21
2 ng/plate	+NADPH	7 ± 6	1129 ± 51
	Change	-95%	+190%
Diesel Particle	-NADPH	326 ± 6	344 ± 17
30 µg/plate	+NADPH	415 ± 8	605 ± 20
	Change	+25%	+75%



MUTAGENICITY OF PARTICLE-BOUND ORGANIC CHEMICAL FRACTIONS FROM DIESEL AND COMPARATIVE EMISSIONS

by

Ann Austin, Larry Claxton, and Joellen Lewtas
Genetic Toxicology Division
Health Effects Research Laboratory
U.S. Environmental Protection Agency
Research Triangle Park, North Carolina

A variety of mobile and stationary sources emit particle-bound organics that have demonstrated mutagenicity. The objective of this study was to measure the mutagenicity of the chemical class fractions derived from the total extracted organics for diesel and several comparative emission sources.

The four sources of combustion organics were diesel engine exhaust particles, a cigarette smoke condensate, a coke oven main sample, and roofing tar emissions. The diesel exhaust particles were collected from a 1978 Oldsmobile 350 diesel vehicle operated on the highway fuel economy test cycle (HWFET) with No. 2 diesel (Union 76) fuel. The particles were collected on Pallflex T60A20 filters and the organics were removed by Soxhlet extraction with methylene chloride as previously described (1). The 2RI Kentucky reference cigarette smoke condensate was generated according to the method of Patel (1977) at Oak Ridge National Laboratory (1). The coke oven main sample was collected from a separator located between the gas collector main and the primary coolers within a coke oven battery at Republic Steel in Gadsden, AL, about 60 miles northeast of Birmingham. The roofing tar sample was generated and collected using a conventional tar pot containing pitch-based tar, enclosed within a chamber and heated to 360° to 380°F, a normal temperature for commercial use. The evaporative emissions were collected using a small bag house fitted with Teflon filter bags (1). The solvents used to extract or condense the organics from each of these samples were removed by evaporation under nitrogen. The total extracted organics were class-fractionated into organic acids, organic bases, cyclohexane insolubles, polar neutrals, non-polar neutrals, and polynuclear aromatics (PNA). The PNA fraction was further fractionated chromatographically using gradient elution on high pressure liquid chromatography (HPLC) such that a purified fraction containing PNA hydrocarbons was obtained (PNA1) by elution with 2% dichloromethane (DCM) in hexane. Elution with more polar solvents resulted in three additional fractions (PNA2-4) that contained compounds of intermediate to higher polarity. Each class of organics was chemically characterized using GC/MS (2).

The chemical fractions for each emission source were bioassayed using the Salmonella typhimurium/microsomal plate incorporation test as developed by Ames et al. (3). Due to the large number of samples to be bioassayed at one time in this study and the limited amounts of some of the samples, only the mutant strain (TA98) of Salmonella typhimurium was used. The protocol described by Ames et al. (3) was followed with minor modifications (4). The data was analyzed using a non-linear model (5) to determine the slope of the dose-response curve. Weighted mutagenicities were determined for each fraction based on the mutagenicity model slope (rev/μg) and the percent of the total mass recovered from each fraction represented. The weighted mutagenicities were then used to determine the percent of mutagenicity attributed to each chemical fraction. Based on this data as summarized in Table 1 the following summary can be made:

1. Olds Diesel. Although the non-polar neutral fraction (NPN) represented the greatest percent of the total mass recovered upon fractionation, it accounted for very little (< 2%) of the mutagenic activity. From 45 to 50% of the mutagenic activity was found in the polar neutral fraction (PN). Polar neutral compounds having limited solubility in cyclohexane would appear in the cyclohexane insoluble fraction (CI), which contained from 15 to 31% of the mutagenic activity. Both of these fractions (PN and CI) contained direct-acting mutagens.

2. Cigarette Smoke. The cyclohexane insoluble fraction (CI) represented the greatest percent of the total mass recovered upon fractionation. The purified polynuclear aromatic fraction (PNA1) was the most active fraction; however, when the model slopes were weighted according to the percentage each fraction represented of the total organic sample, the basic fraction (BASE) accounted for the majority of the mutagenic activity in the presence of metabolic activation (57%), and a polar neutral PNA contaminant (PNA4) accounted for the majority of the mutagenic activity in the absence of metabolic activation (87%).

3. Coke Oven Mains. The greatest percent of the total mass recovered after fractionation was represented by the cyclohexane insoluble fraction (CI). The basic fraction (BASE) and the cyclohexane insoluble fraction (CI) contained the largest percentage of the mutagenic activity in the presence of metabolic activation (41% and 34%, respectively). A polar neutral PNA contaminant (PNA4) accounted for the majority of the mutagenic activity in the absence of metabolic activation (76%).

4. Roofing Tar. The chemical fractions representing the greatest percent mass were the non-polar neutrals (NPN) and a purified PNA fraction (PNA1). Although mutagenic activity was associated with several of the fractions, the cyclohexane insoluble fraction (CI) accounted for the majority (> 50%) of the mutagenic activity both in the presence and absence of metabolic activation.

This study demonstrated significant biological differences among the four emission sources. Within each source, the relative mutagenicity of each fraction was significantly different in the presence and absence of metabolic activation. The two sources which showed some similarities were the cigarette smoke and the coke oven mains. These sources had similar profiles in the percent of mutagenic activity attributed to each fraction both with and without metabolic activation; however, chemical characterization showed significant differences in the compounds identified in these two sources (2). Further chemical characterization of the constituents of each fraction is required to determine which specific chemicals are biologically active within a single source.

REFERENCES

1. Huisingh, J.L., R.L. Bradow, R.H. Jungers, B.D. Harris, R.B. Zweidinger, K.M. Cushing, B.E. Gill, and R.E. Albert. 1980. Mutagenic and Carcinogenic Potency of Extracts of Diesel and Related Environmental Emissions: Study Design, Sample Generation, Collection, and Preparation. EPA Report EPA-600/9-80-057b. U.S. Environmental Protection Agency, Research Triangle Park, NC. pp. 788-800.

2. Sparacino, C.M., R. Williams, and K. Brady. 1981. Fractionation and characterization of the organics from diesel and comparative emissions. Presented as a poster abstract at the U.S. Environmental Protection Agency Diesel Emissions Symposium, Raleigh, NC.

3. Ames, B.N., J. McCann, and E. Yamasaki. 1975. Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. Mutat. Res. 31:347-364.

4. Claxton, L.D. 1980. Mutagenic and Carcinogenic Potency of Diesel and Related Environmental Emissions: Salmonella Bioassay. EPA Report EPA-600/9-80-057b. U.S. Environmental Protection Agency, Research Triangle Park, NC. pp. 801-809.

5. Stead, A.G., V. Hasselblad, J.P. Creason, and L. Claxton. 1981. Modeling the Ames test. Mutat. Res. 85:13-27.

Table 1. Percent of Mutagenic Activity Attributed to Each Chemical Fraction from Comparative Sources (Reported as Percent of Weighted Slope)[a]

	TA98 Without Activation				TA98 With Activation			
Fractions	Olds Diesel	Cigarette Smoke	Coke Oven	Roofing Tar	Olds Diesel	Cigarette Smoke	Coke Oven	Roofing Tar
Acid	9.56	2.22	0.00	0.00	3.88	0.46	0.15	1.09
Base	4.63	0.00	0.00	0.00	3.66	57.39	40.89	5.91
PN	44.90	0.00	0.00	4.67	49.94	0.00	8.99	17.22
NPN	0.00	0.00	0.00	0.00	1.26	1.99	7.04	4.21
PNA1	0.00	0.00	0.00	0.00	0.19	0.20	4.31	4.79
PNA2	9.44	0.00	0.00	6.85	15.87	0.00	0.00	7.84
PNA3	0.74	10.84	24.14	0.03	9.67	0.18	2.90	0.14
PNA4	0.21	86.94	75.86	0.07	0.30	8.37	1.84	0.96
CI	30.53	0.00	0.00	88.38	15.23	31.41	33.87	57.83

[a]Percent of mutagenic activity (% M) determined by: weighted mutagenicity of each fraction (model slope [rev/μg] x % mass of fraction) x 100 ÷ total weighted mutagenicities of all the fractions.

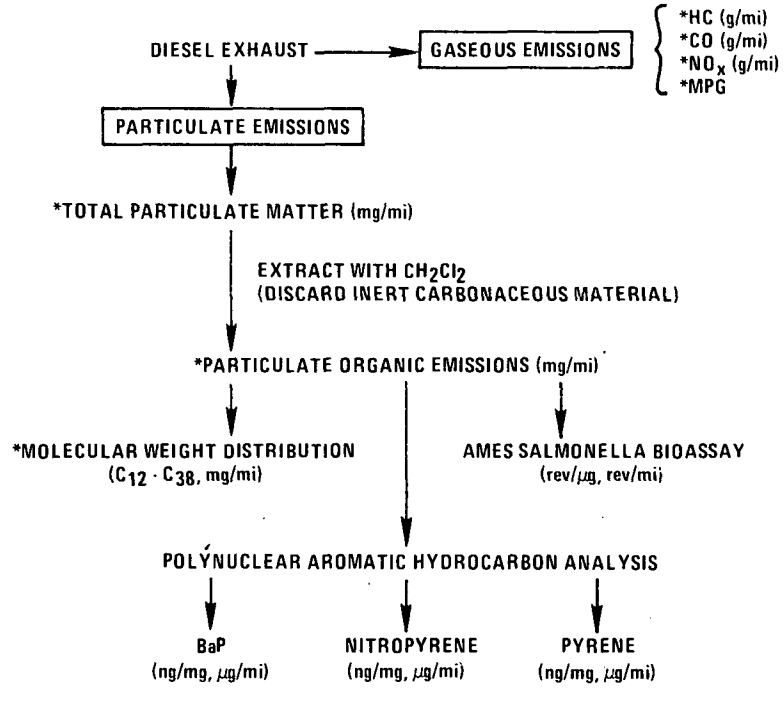
EMISSION OF DIESEL PARTICLES AND PARTICULATE MUTAGENS AT LOW AMBIENT TEMPERATURE

by

James N. Braddock
Environmental Sciences Research Laboratory
U.S. Environmental Protection Agency
Research Triangle Park, North Carolina

Gaseous and particulate exhaust emissions from two 1980 model diesel-powered passenger cars, an Oldsmobile 98 and a Volkswagen Rabbit, were measured over the urban dynamometer driving schedule of the Federal Test Procedure (FTP) as a function of ambient temperature (23°F-84°F). Gaseous emissions analysis included total hydrocarbons (HC), carbon monoxide (CO), nitrogen oxides (NO_x), and fuel economy (MPG). All were measured as a function of the 3 individual test phases (i.e., cold transient-phase 1, stabilized-phase 2, and hot transient-phase 3) of the FTP. Particulate emissions analysis included total particulate matter, particulate organic emissions, and molecular weight distribution measurements (also measured as a function of FTP individual test phase), polynuclear aromatic hydrocarbon measurements including benzo-a-pyrene (BaP), nitropyrene, and pyrene, and Ames Salmonella bioassay of the particulate organics in strain TA-98. See figure detailing diesel emissions analysis scheme below:

DIESEL EMISSIONS ANALYSIS SCHEME



*MEASURED AS A FUNCTION OF FTP PHASE 1, 2, 3 AND COMPOSITE FTP.

The data trends may be conveniently divided into two general categories, gaseous emissions and particulate emissions. Gaseous emission trends: HC, CO, and NO_x emissions increased slightly ($0.10 < r^2 < 0.75$) with decreasing test temperature. Oldsmobile HC emissions ranged from 0.20 to 0.29 g/mi, CO emissions from 1.1 to 1.4 g/mi, and NO_x emissions from 1.2 to 1.4 g/mi; Volkswagen HC emissions ranged from 0.26 to 0.32 g/mi, CO emissions from 1.0 to 1.2 g/mi, and NO_x emissions from 1.0 to 1.2 g/mi. Fuel economy was more temperature dependent ($r^2 > 0.80$) with the Oldsmobile's decreasing from 21.6 to 18.0 mpg and the Volkswagen's decreasing from 45.7 to 41.0 mpg.

Particulate emission trends: total particulate matter and particulate organic emissions (i.e., the CH_2Cl_2 extractable particulate matter) increased with decreasing test temperature. Comparison of overall FTP data at 82°F (median high temperature) versus 45°F (median low temperature) indicates that Oldsmobile total particulate emissions increased from ~567 to 739 mg/mi (+30%) and particulate organics increased from ~94 to ~153 mg/mi (+63%). For the Volkswagen, total particulate emissions increased from ~361 to ~423 mg/mi (+17%) and particulate organics increased from ~72 to ~101 mg/mi (+40%). Molecular weight distributions of the particulate organics in the C_{12}-C_{38} carbon number range, determined by gas chromatography, indicated that much of the organic matter associated with the particulate appears to be uncombusted diesel fuel. This is evident when comparing a lower temperature FTP to a higher temperature FTP. Using the Oldsmobile for example, at 32°F, 61% of the overall FTP particulate organic emission rate of 127 mg/mi is attributable to C_{13}-C_{22} while at 82°F, only 40% of the particulate emission rate of 83 mg/mi is attributable to C_{13}-C_{22}. This uncombusted diesel fuel effect is even more pronounced in the FTP test phase 1 molecular weight distributions: at 32°F, 68% of the Oldsmobile organic emission rate of 137 mg/mi is attributable to C_{13}-C_{22} while at 82°F, only 35% of the particulate emission rate of 105 mg/mi is attributable to C_{13}-C_{22}.

Polynuclear aromatic hydrocarbon (PAH) analysis of selected FTPs indicated decreasing (r^2 ~0.60) BaP, nitropyrene, and pyrene emissions (in ng/mg extract) with decreasing FTP test temperature. Overall Oldsmobile BaP emissions ranged from 0.5 to 1.1 μg/mi (average 0.7 ± 0.2); nitropyrene emissions ranged from 6.6 to 13.6 μg/mi (average 9.8 ± 2.2); pyrene emissions ranged from 82.1 to 133.7 μg/mi (average 106.6 ± 7.4). Overall Volkswagen BaP emissions ranged from 15.5 to 20.2 μg/mi (average 18.2 ± 1.5); nitropyrene emissions ranged from 4.2 to 12.6 μg/mi (average 8.3 ± 2.8); pyrene emissions ranged from 260.4 to 354.5 μg/mi (average 293.9 ± 33.7).

Ames activity levels, in terms of revertants per microgram of particulate organic emissions, correlated moderately ($r^2 = 0.73$) with FTP test temperature indicating decreasing mutagenic activity with decreasing test temperature. Activity levels also correlated moderately (r^2 ~ 0.73) with PAH emissions indicating decreasing mutagenicity (rev/μg) with decreasing PAH emissions (ng/mg extract). Mutagenic activity was greater without metabolic activation (-S9). For the Oldsmobile, activity with metabolic activation ranged from 1.1 to 1.6 rev/μg (average of 1.3 ± 0.1) and without metabolic activation from 2.4 to 3.8 rev/μg (average of 3.4 ± 0.9). Corresponding

Oldsmobile rev/mi x 10^3 ranges and rates were 105 to 185 (average of 152 ± 29) with S9 and 297 to 475 (average of 383 ± 66) without S9. The Volkswagen displayed slightly greater mutagenic activity than the Oldsmobile. Activity with metabolic activation ranged from 1.8 to 2.9 rev/μg (average of 2.2 ± 0.3)and without metabolic activation from 3.2 to 5.7 rev/μg (average of 4.5 ± 0.80. Corresponding Volkswagen rev/mi x 10^3 ranges and rates were 163 to 226 (average of 192 ± 23) with S9 and 341 to 426 (average of 385 ± 28) without S9.

CONCLUSIONS

1. The regulated gaseous emissions (HC, CO, and NO_x) of diesel-powered passenger cars were slightly temperature dependent with decreasing FTP test temperature slightly increasing HC, CO, and NO_x emissions. Fuel economy was moderately temperature dependent with decreasing FTP test temperature decreasing fuel economy.

2. Total particulate matter and particulate organic emissions were moderately temperature dependent. Decreasing FTP test temperature increased total particulate and particulate organic emissions.

3. Increases in particulate organic emission rates at low FTP test temperatures may be primarily attributed to uncombusted diesel fuel.

4. There appeared to be moderate correlation between polynuclear aromatic hydrocarbon emissions and FTP test temperature with PAH emissions decreasing with decreasing test temperature.

5. There appeared to be moderate correlation between Ames test mutagenic activity (without metabolic activation) and FTP test temperature with mutagenic activity decreasing with decreasing test temperature. There also appeared to be moderate correlation between mutagenic activity and PAH emissions with mutagenicity decreasing with decreasing PAH emissions.

CHEMICAL CHARACTERIZATION OF MUTAGENIC FRACTIONS OF DIESEL PARTICULATE EXTRACTS

by

Dilip R. Choudhury
Toxicology Institute
Division of Laboratories and Research
New York State Department of Health
Albany, New York

Projected increased use of diesel-powered automobiles has stimulated considerable interest in research on health effects of the particulates and identification of deleterious compounds adsorbed to the particulates. Diesel particulates are highly respirable and may present significant inhalation health hazard. It is now well recognized that organic extracts of diesel emission particulates exhibit significant mutagenicity as detected by Ames Salmonella bioassay and several other short-term mutagenicity assays. A great deal of effort has been directed to identification of known as well as hitherto unrecognized mutagens in the particulate extracts.

We have applied Ames Salmonella reversion assay to determine mutagenic potencies of diesel particulate extracts and to aid in isolation of mutagenic fractions for in-depth chemical characterization. In this presentation I will discuss the chemical characterization of mutagenic fractions employing a combination of complementary analytical techniques. Extracts of particulates collected from three vehicles run on a chassis dynamometer-dilution tube have been examined. On-line high performance liquid chromatography-mass spectrometry (HPLC-MS), HPLC-ultraviolet spectroscopy, and gas chromatography-MS provided definitive characterization of a number of compounds in mutagenic fractions. Several polar derivatives of polycyclic aromatic hydrocarbons (PAHs) including some nitrated PAHs have been identified. Some NO_2-PAHs are presently known to be bacterial mutagens. However, it is likely that several polycyclic carbonyl compounds detected in the extracts may also be mutagenic.

INFLUENCE OF DRIVING CYCLE AND CAR TYPE ON THE MUTAGENICITY OF DIESEL EXHAUST PARTICLE EXTRACTS

by

C. R. Clark, A. L. Brooks, R. O. McClellan
Lovelace Inhalation Toxicology Research Institute
P. O. Box 5890, Albuquerque, NM 87185

and

T. M. Naman and D. E. Seizinger
U. S. Department of Energy, Bartlesville Energy Technology Center
P. O. Box 1398, Bartlesville, OK 74003

Solvent extracts of particles collected from the exhaust of diesel trucks and automobiles are known to be mutagenic in bacterial test systems. To reasonably predict the potential health hazard of diesel exhaust emissions, differences in toxicity likely to be produced by different cars, or under various driving conditions, were studied. Extracts of exhaust particles collected from Oldsmobile, Peugeot, Fiat, Mercedes, Audi and Volkswagen diesel automobiles were evaluated for mutagenicity in standard and dinitropyrene-resistant Salmonella tester strains.

EXPERIMENTAL

Diesel exhaust particles were collected from the exhaust of cars operated on a climate-controlled chassis dynomometer at the Bartlesville (Oklahoma) Energy Technology Center. Cars were acquired new from the dealer or on loan from the manufacturer and allowed a 4000 mile break-in period before testing. All test vehicles were operated according to the EPA Federal Test Procedure (FTP). To study the influence of driving cycle, exhaust samples were also collected while driving the Oldsmobile on the Highway Fuel Economy Test (HFET) and the New York City Cycle (NYCC). The exhaust was diluted in a tunnel sized to cool the air/exhaust mixture to below 125°F, and the particulate portion collected on 40 x 40 inch Pallflex T60A20 filters. All tests were conducted with the same standard #2 diesel fuel.

The organic material associated with the exhaust particles was extracted by ultrasonication in dichloromethane and the extract evaporated to dryness under nitrogen. A portion of the extract was fractionated by high pressure liquid chromatography (HPLC) on a Biosil A column and eluted in a gradient from 94% isooctane to 100% dichloromethane. Only the gamma-1 fraction (1)

was evaluated in these studies. The extracts were evaluated for mutagenicity in Salmonella strains TA 100, TA 98, or TA 98-1,8-DNPR, without the addition of a liver enzyme homogenate. Five concentrations of each sample were tested in triplicate, and the results reported as the slope of the dose-response curve (revertants/μg) calculated by linear regression analysis. Since results of mutagenicity testing reflect only the genetic toxicity of the material extended from the filters, the results were normalized for differences in the amount of extractable material associated with the particles (extractable fraction) and particle emission rates. This provided an estimate of the amount of mutagenic activity emitted from the exhaust per mile of vehicle operation (revertants per mile).

RESULTS

Influence of Car Type - Extracts of exhaust particles collected from all six cars demonstrated direct, dose-related increases in mutagenicity in TA 100 (Table 1). The amount of dichloromethane extractable material associated with the exhaust particles (extractable fraction) produced by the cars varied markedly, and was inversely related to mutagenic potency of the extracts. The particle emission rates varied by about 3-fold in the six cars. Normalizing the mutagenic potency for extractable fraction and particle emission rates yielded "revertants per mile" values of a different ranking than that shown by the revertants per μg value.

Influence of Driving Cycle - Operating the Oldsmobile on highway, urban and congested urban driving cycles did not markedly influence the mutagenicity of exhaust particle extracts but dramatically changed the particle emission rate and extractable fraction (Table 2). Increasing the extent of stop and start driving increased the particle emission rate but decreased the amount of extractable material associated with the particles. Thus estimates of mutagenicity emitted from the exhaust (revertants per mile) were similar for the three driving cycles.

Mutagenicity in a Nitroreductase-Deficient Strain of Salmonella - Because of the occurrence of nitro-substituted polycyclic aromatic hydrocarbons (PAH) in diesel exhaust extracts (1) and the extreme potency of nitro-PAH in Salmonella, presumably due to their high nitroreductase activity (2), the extracts were evaluated in a strain shown to be resistant to the mutagenicity of some nitro-PAH. Mutagenicity of the gamma-1 HPLC fractions of the extracts, previously shown to contain nitro-PAH (1), were markedly lower (20-60%) in TA 98-1,8 DNPR than the standard TA 98 tester strain.

CONCLUSIONS

The similar mutagenic potencies of extracts of particles collected from six different diesel cars operated on the same fuel suggest that engine design has very little influence on mutagenicity of the particle associated organic materials. Driving cycle also did not significantly alter mutagenicity of the particle extracts. The large variability in extractable fraction and particle emission rates between different cars, and in the same car operated on different driving cycles, emphasizes the need to include these variables

when estimating the quantities of potentially hazardous materials emitted from the exhaust. The lower response of nitro-PAH containing fractions of the extracts in a nitroreductase deficient Salmonella strain is difficult to interpret since it is not known if the enzymes which activate nitro-PAH to mutagenic metabolites in Salmonella are unique to bacteria. (Research performed in part under DOE Contract Number DE-AC04-76EV01013.)

REFERENCES

1. Scheutzle, D., F. S. C. Lee, T. J. Prater, and S. B. Tejada. 1981. The identification of polynuclear aromatic hydrocarbon (PAH) derivatives in mutagenic fractions of diesel particulate extracts. Int. J. Environ. Anal. Chem. 9:93-145.

2. Mermelstein, R., K. K. Demosthenes, M. Butler, E. C. McCoy and H. S. Rosenkranz. 1981. The extraordinary mutagenicity of nitropyrenes in bacteria. Mutat. Res. 89:187-196.

Table 1. Influence of Car Type on Mutagenicity of Diesel Exhaust Particle Extracts

Test Vehicle	Rev/μg Extract (TA-100)	Extractable Fraction (%)	Particle Emissions (g/mi)	Revertants per mile (x 10^3)
Fiat 131	6	71	.34	1500
Peugeot 504	13	29	.21	800
Audi 5000	13	43	.51	2900
Oldsmobile D-88	17	20	.38	1300
VW Rabbit	16	26	.17	700
Mercedes 300	15	13	.26	500

Federal Test Procedure, hot start used for all tests

Table 2. Influence of Driving Cycle on Mutagenicity of Diesel Exhaust Particle Extracts

Driving Cycle[a]	Average Speed (mph)	Rev/μg Extract (TA-100)	Extractable Fraction (%)	Particle Emissions (g/mi)	Revertants per mile (x 10^3)
HFET	50	15	35	.22	1100
FTP	20	16	21	.33	1100
NYCC	7	13	12	1.23	1900

[a]Oldsmobile Detla-88 used in all tests

THE RAPID ANALYSIS OF DIESEL EMISSIONS USING THE TAGA 6000 TRIPLE QUADRUPOLE MASS SPECTROMETER

by

J.E. Fulford, T. Sakuma, and D.A. Lane
SCIEX, Inc.
Thornhill, Ontario, Canada

Because of the increasing consumption of diesel fuels by cars, trucks, heavy equipment, and industry, the environmental scientist is concerned with the atmospheric loading of toxic combustion products and their detrimental biological effects.

The conventional analysis of diesel fuel combustion products is very time-consuming and difficult, since the quantity of toxic pollutants such as polycyclic aromatic hydrocarbons (PAH) and their nitro derivatives is low, and since they are often associated with other contaminants (for example, unburned diesel fuel). The analysis entails sample trapping, extraction, substantial clean-up, and determination by capillary column gas chromatography combined with mass spectrometry.

Using MS/MS, the particulate extract can be directly analyzed for nitropolycylic aromatic hydrocarbons without sample clean-up. The sample is deposited unto a temperature program direct insertion probe operated at atmospheric pressure, and is desorbed over a period of twenty minutes. In the negative mode, parent ions which yield a daughter ion of m/z 46 [NO_2^-] can be scanned, or target compounds can be quantitated by integrating the desorption curve or the m/z 46 daughter ion in the multiple ion monitoring mode. Calibration plots for nitropyrene in spiked samples of diesel particulate extract are linear ($r = 0.99$), and the extrapolated detection limit for nitropyrene in the diesel extract is in the ppb range.

Since the vapor phase emission of diesel engines are also of interest, the exhaust has been sampled directly by the TAGA 6000 MS/MS. In this study, emissions of a diesel-powered vehicle were transported through a heated (150°C) Teflon pipe at 2 L/sec, and a small portion of this flow was admitted directly into the atmospheric pressure ion source. The mass spectrometric analysis was based on:

(i) the identification of particular compounds in the exhaust gas;
(ii) the rapid screening of the exhaust gas for nitro compounds;
(iii) the analysis of the exhaust gas for specific nitro-PAH.

COMPOUNDS IN CITY AIR COMPETE WITH ^{3}H-2,3,7,8-TETRACHLORO-DIBENZO-p-DIOXIN FOR BINDING TO THE RECEPTOR

J.-Å. Gustafsson, R. Toftgård, J. Carlstedt-Duke, G. Löfroth
Dept. of Medical Nutrition and Pharmacology,
Karolinska institute, S-104 01 Stockholm, and Dept. of
Radiobiology, University of Stockholm, S-106 91 Stockholm

It is well known that filter collected urban particulate matter contain compounds which are mutagenic in the Ames' Salmonella assay in the absence of rat liver metabolic activation, showing that these compounds are different from conventional polycyclic aromatic hydrocarbons (PAH). In the present study, we have shown that such particulate matter also contains compounds with an affinity for the rat liver receptor protein which specifically binds 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). The content of conventional PAH cannot account for the degree of binding.

Urban particulate matter used in the investigation was collected on glass fiber filters by high volume sampling at roof top levels in central Stockholm and a suburban site. Collection, Soxhlet extraction with acetone, extract preparation in dimethyl sulfoxide (DMSO) and mutagenicity testing of the extracts in the Salmonella assay have been described elsewhere. The affinity of the particulate fraction of the air samples to the rat liver receptor protein was measured by competition for the binding of ^{3}H-TCDD, as described below.

Liver cytosol was prepared and diluted to 3.5 $A_{280-310}$/ml 2-2.5 mg protein/ml). Each experiment consisted of a series of ten incubations of one ml of cytosol with 1.5 nM ^{3}H-TCDD. Two experiments were carried out in the presence of 20 µl of DMSO (total binding). Two incubations contained 150 nM radioinert 2,3,7,8-tetrachlorodibenzofuran (TCDBF) (non-specific binding). The remaining six incubations contained varying amounts of the extract of the particulate fraction of an air sample. 2.5-20 µl of the extracts, dissolved in DMSO, was added to the incubation mixture and the volume of DMSO was then adjusted to 20 µl/incubation. After incubation at 0°C for 60 min, the incubations were treated with dextran-coated charcoal and the amount of ^{3}H-TCDD bound to the receptor in each of the ten incubations was measured by isoelectric focusing carried out in polyacrylamide gel as described previously.

The non-specific binding of ^{3}H-TCDD was obtained from the two incubations in the presence of 150 nM radioinert TCDBF and was subtracted from the total binding in each of the other incubations (= specific binding). The specific binding of ^{3}H-TCDD in the presence of extracts of the air samples was expressed as a percentage of the specific binding in the control incubation (^{3}H-TCDD + DMSO). The relative binding affinity for the individual air samples was calculated from log-logit plots of the competition for TCDD-binding, where logit $b = \ln(b/1-b)$. The log-logit plots were calculated using linear regression analysis. Each extract was analysed 3-4 times at a suitable dilution.

An unused air filter (blank filter) was extracted in the same manner as

the other samples and tested for competition for receptor binding. The results were expressed as an equivalent volume of air. Benzo(a)pyrene and TCDBF were dissolved in dioxane and the competition for binding to the receptor measured.

The results are shown in Table 1, in which are given the mutagenic effects in Salmonella TA 98 and TA 100 in the absence of mammalian metabolic activation and the relative binding affinities expressed as the concentration of air sample extract that competes for 50% of the TCDD-binding to the receptor (ED_{50}; m^3 air/ml cytosol). Log-logit plots for the different air samples indicated that they competed for the same binding site. There seemed to be a gross correlation between the binding affinity and the pollution level as measured by mutagenic effects. Samples collected in the summer had higher ED_{50}'s than samples collected in the winter and spring.

Several compounds are known to bind to the receptor protein including many PAH present in urban particulate matter. Some of the particulate samples were analysed for PAH. Assuming that all PAH present at concentrations above 0.1 ng/m^3 have the same binding affinity for the receptor protein as benzo(a)pyrene (B(a)P), it can be calculated that the ED_{50} value should be 1.7, 15.3 and 0.07 m^3 of air for sample 148, 173 and S-258, respectively. The observed values were 0.035, 0.14 and 0.015 m^3, i.e. known PAH may account for about 2.4, 0.8 and 22.4% of the binding, indicating that other types of compounds are of major importance.

TCDD and TCDBF have the highest binding affinities for the receptor among investigated compounds. Two samples have been analysed for TCDD and TCDBF, and the concentrations were below the detection levels of 2 pg/m^3. Consequently, these compounds cannot account for the binding.

The affinity of a compound for the TCDD receptor is well correlated to the magnitude of aryl hydrocarbon hydroxylase (AHH) induction caused by that compound. Strains of mice with high AHH inducibility are more susceptible to pulmonary cancers caused by 3-methylcholanthrene than strains with low inducibility, indicating a link between AHH activity and appearance of pulmonary tumors. The rat lung has a high content of receptor protein, and the capability of the human lung to metabolise B(a)P indicates the presence of the receptor protein also in this tissue. Although TCDD has been shown to be a potent carcinogen in chronic feeding studies, it is apparent that TCDD shows no or very little mutagenic activity in *in vitro* bacterial test systems such as the Ames' test and a very low covalent binding to DNA *in vivo*. In a recent study, however, it was shown that TCDD is a potent promoting agent for hepatocarcinogenesis initiated by diethylnitrosamine. TCDD and possibly also other compounds with an affinity for the same receptor may thus better be described as cocarcinogens and tumor promoters rather than carcinogens. The presence of compounds with affinity for the receptor in urban particulate matter may be of importance with regard to the health implications of urban air pollution. This type of compounds may or may not be similar to the components that are mutagenic in the absence of mammalian metabolic activation in the Ames' Salmonella assay.

Conclusions

Acetone extracts of filter-collected urban atmospheric particulate matter contain compounds which can displace ^3H-2,3,7,8-tetrachlorodibenzo-p-dioxin from the rat liver receptor protein. The concentration of conventional polycyclic aromatic hydrocarbons or chlorinated dioxins and dibenzofurans cannot account for more than 0.8-22% of the displacement.

Table 1. Competition for TCDD receptor-binding and mutagenicity in Salmonella TA 98 and TA 100 in the absence of mammalian metabolic activation by extracts of filter-collected urban particulate matter. Samples were collected at roof top levels for 24 h starting about 6 a.m. Samples 148 and 174 were collected at a suburban site 22 km NNW Stockholm and the others in central Stockholm. The ED_{50} is the concentration of competitor that competes for 50% of the specific binding of ^{3}H-TCDD. The ED_{50}'s (in nM) for 2,3,7,8-tetrachlorodibenzofuran (TCDBF), benzo(a)pyrene (B(a)P), β-naphthoflavone (BNF), 3-methylcholanthrene (3-MC) and benz(a)anthracene (BA) are given for comparison. The mutagenic response is highest in the absence of mammalian metabolic activation and decreases by addition of S9 from rat liver; the decrease is dependent on the amount of S9 added. Samples 258, 264 and 268 were tested for mutagenicity both prior to (Feb. 1980) and after (Sept. 1980) the completion of the TCDD receptor analyses; there were no detectable changes in the mutagenic response.

Sample	Receptor affinity[a] ED_{50} (m^3 air/ml cytosol)	Mutagenic response (revertants/m^3) TA 98	TA 100	Sampling date and site
149	0.049 ± 0.014	19	11	79 04 10 inner city
148	0.035 ± 0.017	14	8	79 04 10 suburban
173	0.137 ± 0.073	3	n.d.	79 07 05 inner city
174	0.302 ± 0.147	1	n.d.	79 07 05 suburban
S-258	0.015 ± 0.007	59	79	80 02 04 inner city
T-262	n.d.	92	102	80 02 05 inner city
S-264	0.039 ± 0.010	30	20	80 02 06 inner city
T-268	0.049 ± 0.026	9	7	80 02 07 inner city
Blank filter[b]	2.18 ± 0.640	<0.2	<0.4	
TCDBF	2.69 ± 1.91 nM			
B(a)P	18.21 ± 9.88 nM			
BNF	7.0 nM			
3-MC	2.9 nM			
BA	3.8 nM			

[a]Values represent the means ± standard deviation (three to four determinations).

[b]Blank filter extracted with the same volume of acetone. The competition is expressed as an equivalent volume of air.

n.d. Not determined.

GC/MS AND MS/MS STUDIES OF DIRECT-ACTING MUTAGENS IN DIESEL EMISSIONS

by

T. R. Henderson, J. D. Sun, R. E. Royer and C. R. Clark
Lovelace Inhalation Toxicology Research Institute
Albuquerque, New Mexico

T. M. Harvey and D. F. Hunt
Department of Chemistry
University of Virginia
Charlottesville, Virginia

J. E. Fulford, A. M. Lovett, and W. R. Davidson
Sciex, Inc.
Toronto, Canada

While the direct-acting mutagens in diesel emissions have not been unequivocally identified, evidence has accumulated showing that nitro-PAHs (polycyclic aromatic hydrocarbons) may be a major source of mutagenic activity (1,2). We have found that diesel fuel-PAHs are devoid of mutagenic activity in Salmonella test strains. After reaction with NO_2, the nitro-fuel-PAH mixtures are direct-acting, frameshift mutagens (200-1000 revertants/μg) in TA98, but were not active in TA1535, a base-pair substitution indicator strain (2). The cytotoxicity to CHO cells also increases after reaction with NO_2(2). These biological responses are very similar to those observed with diesel soot extract fractions although the activity of diesel extracts is much lower.

The fuel-nitro-PAH mixtures have been found useful as a positive control and as a reference mixture for MS/MS (triple stage quadrapole mass spectrometry) analyses. Although the fuel-nitro-PAHs are not resolvable by capillary GC/MS, the fuel-PAHs before reaction with NO_2 are readily separated and identified by GC/MS. Since the fuel-PAHs extracted with DMSO (Me_2SO) are readily identified by GC/MS, correlations can be made with the nitro-PAH masses observed in MS/MS spectra of nitro-PAH mixtures. These are used for MS/MS interpretation since MS/MS does not differentiate between isomers of the same molecular weight in most cases. When possible, these interpretations should be confirmed by independent methods (1).

MS/MS was carried out to compare the relative intensities of ions in samples of widely different mutagenic potencies. In this way, it may be possible to estimate which of the nitro-PAHs detected in diesel soot extracts (1) may make major contributions to the total mutagenic activity.

The MS/MS analyses involved: $R\text{-}NO_2 \rightarrow R\text{-}NO_2\text{-}H^+$ (ionization under isobutane chemical ionization conditions) $\rightarrow R\text{-}N^+O$ (collisionally induced dissociation by collision with N_2 in quadrupole #2). The MS/MS instrument was a modified Finnigan 3200, which has been described previously(3). The first quadrupole was scanned from 80 to 350 amu with a scan time of 1.3 sec. The second quadrupole was operated (with RF voltage only) as a collision chamber. The third quadrupole was scanned the same as #1, but 17 amu behind it. In this way, only ions which lost 17 amu in passing through the collision chamber were detected. The instrument was tuned with 1-nitropyrene (parent-H^+ ion m/z 248) and the N_2 pressure adjusted for maximum m/z 231 daughter ion (m - 17). Extract samples (40 μg) were volatilized into the source using a thermal desorber (temperature programmed from 50 to 350° C in 10 minutes).

MS/MS analyses were done on selected samples with an APCI/MS/MS (triple quadrupole MS/MS with an atmospheric pressure chemical ionization source) at the laboratories of Sciex, Inc., Toronto, Canada. The reaction monitored was:

$$R\text{-}NO_2 + O_2^- \dashrightarrow R\text{-}NO_2^- \xrightarrow[Ar]{CID} R + NO_2^-$$

The first quadrupole was scanned over a 100 to 300 amu range or single ion monitoring was done at m/z 247^-. The third quadrupole was set for constant daughter scans at m/z 46^-. The corona discharge current was 6 μamp constant current at 4 to 6 kV and an ion energy of 65 eV. Thus in this reaction, only negative ions of 100 to 300 molecular weight were detected which yielded NO_2 on collision with argon gas.

Diesel soot extracts were fractionated with DMSO to yield aliphatic, aromatic and polar fractions for MS/MS analyses. This fractionation method has been described previously (4-6). The aromatic fraction from a single-cylinder Swan diesel engine was used for comparison with an Oldsmobile exhaust aromatic fraction because of differences in mutagenic activity. The aromatic fraction recovered by DMSO fractionation of diesel soot extracts typically contains 50-80% of the direct mutagenic activity of different extracts and is concentrated 5- to 10-fold in specific activity. The aromatic fraction of diesel exhaust is very similar to the γ fraction isolated by Schuetzle _et al_. (1) in containing mononitro-PAHs and dinitro PAHs of 2 ring PAHs.

The mutagenic activities of the extracts in TA98 (no S-9) under standard Ames bioassay conditions (7) were: nitro-fuel-PAHs--435 rev/μg; Swan diesel soot aromatic fraction--57 rev/μg; Olds diesel soot aromatic fraction--21 revertants/μg. These samples, differing by about 20-fold in mutagenic specific activity, were compared by MS/MS. The unfractionated extracts were low in the intensity of certain (M - 27) ions, the polar fractions contained only a minor part of the total mutagenicity (< 20%) and the aliphatic fractions contained very low intensities of nitro-PAH ions.

Figure 1 shows that the even-mass ions (dinitro-PAHs) appeared to correlate with increased mutagenic activity of the three extracts, while odd-mass ions (mononitro-PAHs) negatively correlated with mutagenic activity

with the exception of m/z 171. The even mass ions of particular significance and their tentative identification were: m/z 252, dinitrophenanthrenes; m/z 256, dinitromethylbiphenyls; m/z 230, 244 and 258, dinitronaphthalenes containing 2, 3 and 4-methyl groups. These compounds might be particularly important in the total mutagenicity of diesel soot extracts. Nitropyrenes (m/z 231) did not significantly correlate with differences in the mutagenicity of these samples, although these compounds may account for 5-10% of the total mutagenicity(4).

Figure 1 also shows that the same nitro-PAH (M - 17) masses were present in all three types of samples. The fuel-nitro-PAHs, having been treated with excess NO_2, appeared to be lower in mono-nitro-PAH type masses and higher in the even masses (dinitro-PAHs) discussed above. Since most of the nitro-PAH ions present in diesel exhaust are also present in fuel aromatic fractions treated with NO_2 and these masses are relatable to the PAHs present in the fuel burned by the two engine types, this suggests that the fuel PAHs contribute to the exhaust nitro-PAHS. One possible mechanism is reaction of unburned fuel PAHs with NO_2.

Studies involving the addition of pyrene to the fuel for the single cylinder diesel engine was performed to further test the hypothesis that fuel PAHs may contribute to the formation of nitro-PAHs in exhaust soot extracts. It was found that addition of 0.01 to 1.0% w/v pyrene to diesel fuel (less than 0.01% pyrene by GC/MS) resulted in increased pyrene/phenanthrene ratios in soot extracts. With no addition of pyrene, this ratio was less than 2, but increased to 12 with 1% pyrene addition to the fuel. The mutagenicity in TA100 increased 2 to 3 fold by 0.01 to 0.1% pyrene addition, but decreased somewhat with 1% pyrene addition. MS/MS analyses of these extracts by APCI/MS/MS showed increased ion intensities of mono- and dinitropyrenes in the soot extract for pyrene additions up to 0.1% in the fuel, but the intensities of dinitropyrenes decreased at 1% pyrene addition to the fuel. (Supported in part by U.S. Department of Energy under DOE Contract No. DE-AC04-76EV01013.)

REFERENCES

1. Schuetzle, D., T. Riley, T. J. Prater, T. M. Harvey and D. F. Hunt. 1981. The identification of nitrated derivatives of PAH in diesel particulates. Anal. Chem., in press.

2. Henderson, T. R., A. P. Li, R. E. Royer and C. R. Clark. 1981. Increased cytotoxicity and mutagenicity of diesel fuel after reaction with NO_2. Environ. Mutag. 3: 211-220.

3. Hunt, D. F., J. Shabanowitz and A. B. Giordani. 1980. Collision Activited Decompositions of negative ions in mixture analysis with a triple quadrupole mass spectrometer. Anal. Chem. 52: 386-390.

4. Henderson, T. R., R. E. Royer and C. R. Clark. 1981. MS/MS Characterization of Diesel Emissions. Proceedings of 29th Annual Conference of Mass Spectrometery and Allied Topics (in press-extended abstract).

5. Henderson, T. R., C. R. Clark, R. L. Hanson and R. E. Royer. 1980. Fractionation of environmental organic extracts with dimethylsulfoxide. Applications to diesel exhaust particulates. Proceedings of 28th Annual Conference on Mass Spectrometry and Allied Topics. p. 243-244. (extended abstract).

6. Henderson, T. R., C. R. Clark, T. C. Marshall, R. L. Hanson and C. H. Hobbs. 1981. Heat degradation studies of Solar Heat Transfer Fluids. Solar Energy (in press).

7. Ames, B. N. 1979. Identifying environmental chemicals causing mutations and cancer. Science 204: 587-593.

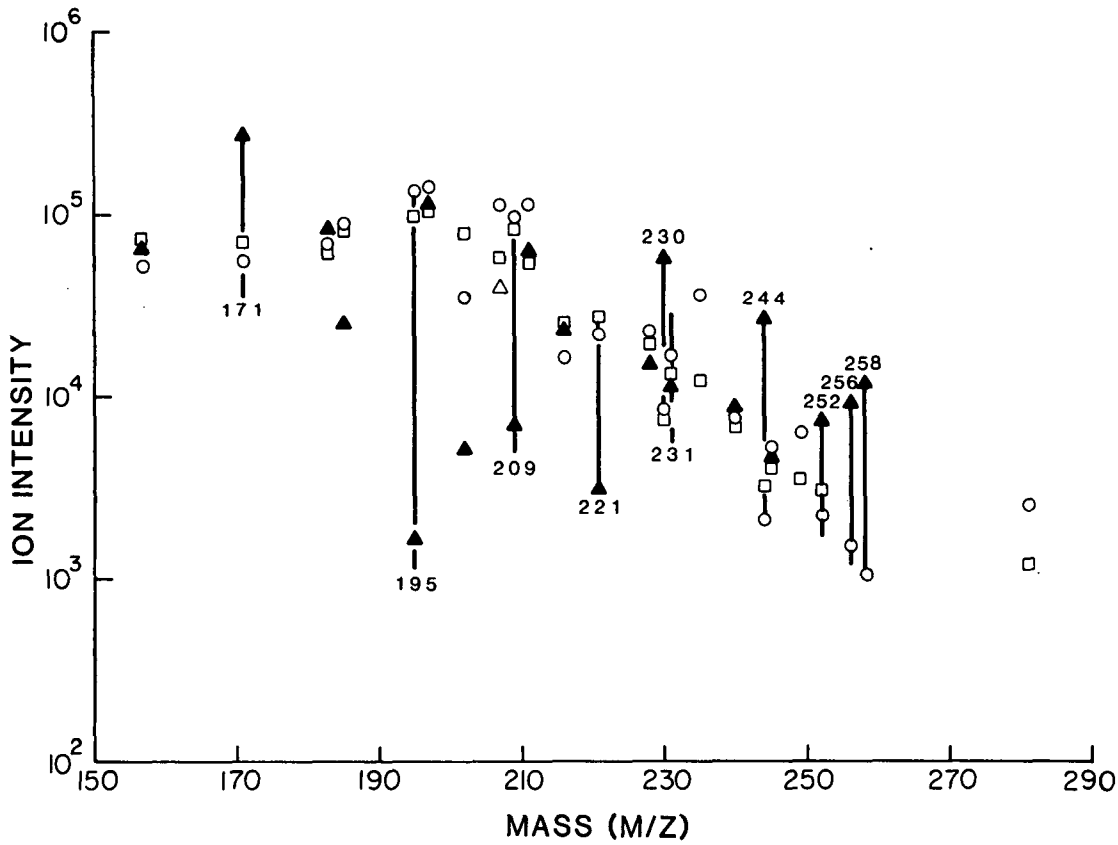


FIGURE 1

Figure 1. MS/MS Comparisons of Nitro-PAHs from Diesel Fuel and Exhaust Filter Extracts. --Δ-- Nitro-fuel-PAHs; --□-- Aromatic Fraction from Swan Engine Exhaust Soot Extracts; --O-- Aromatic Fraction, Olds Exhaust Soot Extracts. The masses represent the (M - 17) ions, the parent nitro-PAH being 17 amu more. The ion intensity is the total ion counts summed over the entire run, usually 300 scans.

EVALUATION OF THE RELEASE OF MUTAGENS AND 1-NITROPYRENE FROM DIESEL PARTICLES IN THE PRESENCE OF LUNG MACROPHAGE CELLS IN CULTURE

by

Leon C. King, Silvestre B. Tejada, and Joellen Lewtas
U.S. Environmental Protection Agency
Research Triangle Park, North Carolina

Diesel particles have been shown to contain organic components which are mutagenic in short-term bioassays (1). Nitroaromatics including 1-nitropyrene now appear to account for a portion of the mutagenic activity observed in these organics (2). It is important to determine whether these potentially carcinogenic components are released from particles under physiological conditions. We have shown in a previous study (3) using the Ames Salmonella typhimurium plate incorporation assay that serum and lung cytosol were very effective in removing mutagenic activity from diesel particles (3). This was determined by measuring the activity extractable from the particles before and after treatment of the particles with serum or cytosol. Direct addition of serum or lung cytosol to these organics reduced the mutagenic activity detectable by 80 to 90%. The objective of this study was to evaluate the removal and release of mutagenic activity and 1-nitropyrene from diesel particles in the presence of lung cells in culture. The lung cells used in these studies were alveolar macrophages obtained from rabbits by lung lavage. The diesel particles used in this study were obtained from a Datsun Nissan 220C, 4-cylinder passenger car previously described (3). The particles were sonicated in tissue culture medium (M199 with Hank's Salts) at 37°C for 30 min to deagglomerate and disperse the particles. Particle size analysis showed the majority of the particles to be 2.0 to 2.5 μm after this treatment.

Rabbit alveolar macrophage (RAM) cells were lavaged and cultured with the diesel particles according to previously published procedures (4) except that the final serum concentration was reduced to 10% and the culture time was increased to 40 h. In brief, RAM cells were added to individual wells of cluster dishes containing suspensions of the diesel particles from 15 to 1,500 μg/ml. Under these conditions, at concentrations of diesel particles above 75 μg/ml, over 95% of the particles were phagocytized after 20 h of culture. After 40 h of culture, cells were harvested by trypsinization. The final exposure conditions for the mutagenesis and 1-nitropyrene analyses were selected to maximize cellular exposure to the diesel particles while minimizing cellular toxicity. The final concentration of diesel particles selected was 375 μg of particles/ml. At this concentration, RAM cells engulf over 95% of the particles and after 40 h of exposure, less than 7% cell lysis was observed and cell viability was 63% of the control cultures.

In order to evaluate the effect of the lung macrophage cells on the removal of mutagens and 1-nitropyrene from diesel particles, particles were exposed to the culture medium at 375 μg/ml with and without lung macrophages and cultured for 40 h. After incubation the medium control treatment dishes were combined and the medium was separated by centrifugation from the particles which were washed once with water. The harvested macrophages were separated from the culture medium and sonicated to release engulfed particles. Dichloromethane and methanol (DCM:MeOH, 1:1) were used to extract the medium, free particles, engulfed particles, cell sonicate, and water washes. Each of these fractions was analyzed for 1-nitropyrene by LC/fluorescence (5) and compared to untreated particles extracted with DCM:MeOH.

Each of the fractions was also assayed for mutagenicity in the Ames *Salmonella typhimurium* plate incorporation assay in TA98 as previously described (3). The media, cells, and washes were assayed without extraction and due to a high background of activity in both the medium and cells, no detectable activity was observed as a function of the treatment group. Mutagenicity was detected in the DCM:MeOH extract of the macrophage-engulfed particles; however, it was only 2% of the mutagenicity originally present on the particles. Comparison of this activity with that of the media control particles, in which 6% of the mutagenicity was recovered, showed that the presence of macrophages decreased the mutagenicity 62%.

Nitropyrene analysis of the medium control group showed 96% recovery of the 1-nitropyrene with 31% of the nitropyrene found in the medium and washes and 66% remaining on the particles. Significantly less 1-nitropyrene was recovered in the macrophage treatment group (76%). Since over 95% of the particles were phagocytized and only 27% of the original 1-nitropyrene was recovered from the engulfed particles, it appears that the macrophages may have metabolized the 1-nitropyrene to a nondetectable form.

Comparison of the recoveries shows a greater loss of mutagenicity than 1-nitropyrene suggesting that other compounds detected in these particles may contribute more to the mutagenicity than the 1-nitropyrene alone.

REFERENCES

1. Huisingh, J., R. Bradow, R. Jungers, L. Claxton, R. Zweidinger, S. Tejada, J. Bumgarner, F. Duffield, M. Waters, U. Simmon, C. Hare, C. Rodriguez, and L. Snow. 1978. Application of short-term bioassay to the characterization of diesel particle emissions. In: Application of Short-Term Bioassays in the Fractionation and Analysis of Complex Environmental Mixtures. M.D. Waters, S. Nesnow, J.L. Huisingh, S.S. Sandhu, and L. Claxton, eds. Plenum Press: New York. pp. 383-418.

2. Nishioka, M., B. Peterson, and J. Lewtas. 1981. Comparison of nitro-PNA content and mutagenicity of diesel emissions. Presented at the U.S. Environmental Protection Agency Diesel Emissions Symposium, Raleigh, North Carolina.

3. King, L.C., M.J. Kohan, A.C. Austin, L.D. Claxton, and J.L. Huisingh. 1981. Evaluation of the release of mutagens from diesel particles in the presence of physiological fluids. Environ. Mutagen. 3:109-121.

4. Garrett, N.E., J.A. Campbell, H.F. Stack, M.D. Waters, and Joellen Lewtas. 1981. The utilization of the rabbit alveolar macrophage and Chinese hamster ovary cell for evaluation of the toxicity of particulate material. Environ. Res. 24:345-365.

5. Tejada, S.B., R.B. Zweidinger, J.E. Sigsby, Jr., and R.L. Bradow. 1981. Identification and measurement of nitro derivatives of PAH in diesel exhaust particulate extract. Presented at the Chemical Characterization of Diesel Exhaust Emissions Workshop, Dearborn, MI.

BACTERIAL MUTAGENICITY OF A DIESEL EXHAUST EXTRACT AND TWO ASSOCIATED NITROARENE COMPOUNDS AFTER METABOLISM AND PROTEIN BINDING

by

Mike Kohan and Larry Claxton
Health Effects Research Laboratory
U.S. Environmental Protection Agency
Research Triangle Park, North Carolina

Previous work has demonstrated that nitroarenes are associated with organic extracts from diesel exhaust (1,2,3). This study was designed to characterize two nitroarenes and to determine whether these compounds demonstrate the same type of mutagenic response as diesel exhaust organics. An extract of the particulate from exhaust of a VW diesel automobile, 2,7-dinitrofluorenone, and 1-nitropyrene were tested in the Salmonella mutagenicity assay using strain TA98 and two nitroreductase-deficient strains (TA98FR1 and TA98/1,8DNP$_6$) to establish a proper dose for a multivariant experiment. Each of the three samples was tested with and without a 9000g liver homogenate (S9) prepared from Aroclor 1254-induced rats. In the multivariant experiment, the following treatments (both with and without the NADPH-generating system) were used:

(a) no activating system (-S9),

(b) microsomes derived from the original S9 by centrifuging for 90 min at 100,000g,

(c) the cytosol fraction of the original S9, and

(d) boiled S9.

In addition, the samples were tested in the presence of both boiled and unboiled bovine serum albumin (BSA). Salmonella tester strains TA98 and TA98FR1 were used in the multivariant study. These treatments were used to determine the similarities of the three samples in the presence and absence of treatments with differing enzymatic and protein binding capabilities. This work is an extension of similar efforts by Wang et al. (4) and Pederson et al. (5).

The results using the diesel exhaust sample are seen in Table 1. When compared to the untreated (no activating system) situation, all of the treatments with one exception gave reduced mutagenic activity. Only the cytosol fraction gave an increase in activity. In comparison to the non-activated situation the cytosol activation gave a relative increase to 111%

for strain TA98 and to 164% for strain TA98FR1. In addition, it was noted that TA98 was the strain most responsive to the diesel exhaust extract, followed by TA98FR1, and then TA98/1,8DNP$_6$. 1-Nitropyrene activity was decreased by half in TA98/1,8DNP$_6$ but almost abolished in TA98FR1.

As in the diesel exhaust particulate sample, the mutagenic activity of 2,7-dinitrofluorenone was reduced in the majority of the different treatments. An increase to 170% of the untreated situation was seen when using the cytosol fraction with strain TA98FR1.

A reduction in the mutagenic activity of 1-nitropyrene was observed in all of the treatments without the generating system. However, in contrast to 2,7-dinitrofluorenone and the diesel exhaust particulate sample, a twenty-fold increase in the mutagenic activity of 1-nitropyrene was seen with the microsome treatment when using the generating system and strain TA98FR1. Smaller increases in activity were also observed with the S9 and cytosol fractions when using this same strain and generating system. The mutagenic activity of 1-nitropyrene also was increased by the microsome fraction with the generating system in strain TA98. This microsomal activation of 1-nitropyrene, in addition to the response pattern obtained with the nitroreductase-deficient tester strains, indicates that 1-nitropyrene may not be the major mutagenic component in this sample of extractable organics from VW Rabbit Diesel particle emissions.

REFERENCES

1. Pederson, T.C., and J.S. Siak. 1981. The role of nitroaromatic compounds in the direct-acting mutagenicity of diesel particle extracts. J. Appl. Toxicol. 1:54.

2. Claxton, L.D., and J.L. Huisingh. 1980. Comparative mutagenic activity of organics from combustion sources. In: Pulmonary Toxicology of Respirable Particles. Proceedings of the Nineteenth Annual Hanford Life Sciences Symposium at Richland, WA. P.L. Sanders, F.T. Cross, G.E. Dable, and J.A. Mahaffey, eds. pp. 453-465.

3. Lofroth, G. 1980. Salmonella/microsome-mutagenicity assays of extract from diesel and gasoline-powered motor vehicles. In: Health Effects of Diesel Engine Emissions: Proceedings of an International Symposium. EPA-600/9-80-057a, Vol. 1. U.S. Environmental Protection Agency: Cincinnati, OH. pp. 327-342.

4. Wang, Y.Y., R.E. Talcott, D.A. Seid, and E.T. Wei. 1980. Antimutagenic properties of liver homogenates, proteins, and glutathione on diesel exhaust particulates. Cancer Lett. 11:265-275.

5. Pederson, T.C., and J.-S. Siak. 1981. The activation of mutagens in diesel particle extract with rat liver S9 enzymes. J. Appl. Toxicol. 1:61-66.

Table 1. Mutagenic Activity of Diesel Exhaust Particle Extracts Using Different Treatment Conditions

	Without Generating System		With Generating System[a]	
Treatment	TA98	TA98FR1	TA98	TA98FR1
Untreated	1039 (100)[b]	788 (100)[c]	1001 (100)	680 (100)
S9	467 (45)	242 (31)	696 (70)	649 (95)
Microsomes	781 (75)	488 (62)	826 (82)	557 (82)
Cytosol	687 (66)	332 (42)	1110 (111)	1118 (164)
Boiled S9	595 (57)	369 (47)	577 (58)	305 (45)
BSA	354 (33)	264 (34)		
Boiled BSA	343 (33)	232 (29)		

[a]NADPH-generating system: NADPH, G-6-P, $MgCL_2$, and KCL.
[b]Average net revertants per plate at 100 μg organic extract.
[c]Relative response to untreated sample expressed as percent.

CHARACTERIZATION OF PARTICULATE EMISSIONS FROM IN-USE GASOLINE FUELED MOTOR VEHICLES

by

John M. Lang, Roy A. Carlson, Linda Snow
Northrop Services, Inc.
Research Triangle Park, North Carolina

Frank M. Black, Roy Zweidinger, Silvester Tejada
U.S. Environmental Protection Agency
Research Triangle Park, North Carolina

One of the primary tasks of those concerned with the study of environmental quality is estimating population exposure to air pollutants and determining the risk associated with exposure. Mobile sources generally contribute significantly to the population's exposure to hydrocarbons (HC), carbon monoxide (CO), oxides of nitrogen (NO_x), and fine particulate matter. Much of the mobile source data available in the literature has been obtained from well-maintained engineering test vehicles. However, emissions from consumer-operated vehicles can vary considerably from well-maintained vehicles.

Because of growing interest in diesel power for light-duty motor vehicles, it has become necessary to study particulate emissions comprehensively to determine the potential impact of dieselization on the public health. Assessment of exposure and risk requires knowledge of the emission rates and composition of diesel particulate matter and of the gasoline particulate matter being replaced by diesels.

Our study examined particulate emissions from twenty consumer-operated, light-duty gasoline fueled cars and trucks. The emissions characteristics observed were compared with those previously reported by Gibbs, et al., for nineteen consumer-operated, light-duty diesels (1).

A test fleet of twenty light-duty gasoline passenger cars and trucks was obtained from local residents and rental agencies. Four of the vehicles were fueled by leaded gasoline; the remaining sixteen fueled by unleaded

gasoline. Vehicles from model years 1970 to 1981 were represented and ranged in mass from 907 kg (2,000 lbs) to 2,268 kg (5,000 lbs) and accumulated mileage from 565 km (353 mi.) to 130,490 km (81,559 mi.). A variety of emission control configurations were represented, including oxidation catalysts, oxidation catalysts with air pumps, and three-way catalysts. All vehicles were tested as received.

Three gasoline fuels were purchased locally for the program; two leaded and one unleaded. Road load simulation was achieved with a Clayton CTE-50-0 direct-drive, water brake dynamometer. The vehicles were tested with a daily routine involving a cold-start Federal Test Procedure (FTP) cycle followed by repetitive Highway Fuel Economy Test cycles (HWFET). The FTP simulates city driving after the car has not been started for at least twelve (12) hours. The HWFET simulates highway driving after the car has been warmed up.

A positive-displacement pump Constant Volume Sampling (CVS) system collected the exhaust gases and allowed dilution air to flow through the sample train in order to maintain the dilution exhaust temperature at or below 52° C (125° F). Before entering the CVS system, the dilution exhaust flow passed through three 20 x 20 inch filters for collection of particulate matter (2). Pallflex T60A20 PTFE glass fiber filters were used for collecting the particulate.

The 20 x 20 filters were soxhlet extracted for eight hours with dichloromethane (DCM) to remove the soluble organic fraction (SOF). The extracted organics were examined for nitro-pyrene (NO_2-P), benzo(a)pyrene (BaP), and pyrene (Py) content by high pressure liquid chromotography (HPLC), and bioassayed with Ames Salmonella strain TA-98 for mutagenic potency. The activity (revertants/ microgram SOF) was determined as the slope in the linear portion of the dose-response curve. Five organic doses, 0 (solvent blank) to 100 ug, were used to define the dose-response curve.

A review of the regulated emission rates indicate that the test fleet included properly functioning vehicles and vehicles with a variety of emission control malfunction conditions. HC emission rates ranged from 0.07 to 24.5 g/mi., CO had a range of 0.08 to 60.95 g/mi., and NO_x was from 0.37 to 6.43 g/mi.. Comparison with the diesels indicate that the gasoline fleet average emission rates exceed the diesel rates and a broader range was seen in the gasoline data.

The total particulate emission rates from leaded gasoline vehicles were 2.7 times greater during the HWFET than the FTP. On the other hand, HWFET total particulate emission rates from unleaded gasoline vehicles were 81% of the FTP value. Overall, the leaded vehicles emitted more particulate than the unleaded vehicles during both cycles, 3.2 times more during the FTP and 10.8 times more during the HWFET.

The light-duty diesel total particulate emission rates reported by Gibbs, et al., compared to the gasoline vehicle rates as follows:

(FTP) diesel $\sim$ 5.9 x leaded rate; 19.1 x unleaded rate.
(HWFET) diesel $\sim$ 1.3 x leaded rate; 13.5 x unleaded rate.

Fleet average DCM soluble particulate organic emission rates during the FTP and HWFET compared with the light-duty diesels as follows:

(FTP) diesel ~ 5.9 x leaded rate; 8.6 x unleaded rate.
(HWFET) diesel ~ 3.4 x leaded rate; 6.9 x unleaded rate.

Nitro-pyrene emission rates were similar for leaded and unleaded gasoline vehicles. Diesels emit about 20 to 30 times as much NO_2-P as gasoline vehicles.

Gasoline vehicle BaP emissions were greater during cold-start FTP driving than during either hot-start FTP or HWFET driving. Functioning catalyst systems appeared to effectively reduce polynuclear aromatic hydrocarbon (PAH) emissions. During the cold-start FTP, BaP emission rates from leaded vehicles exceeded BaP rates from diesels; BaP emission rates from unleaded vehicles were similar to the diesel results. During the HWFET, BaP emission rates from both categories of gasoline vehicles were less than the rates from diesels.

Generally, the Ames TA-98 mutagenic activity of the gasoline particulate organics was higher with metabolic activation than without metabolic activation. Ames TA-98 revertant per mile levels were substantially higher for the leaded gasoline vehicles than for the unleaded gasoline vehicles under both FTP and HWFET conditions.

The higher total particulate and particulate organic emission rates of diesels are compensated somewhat by lower Ames TA-98 mutagenic activities when compared to gasoline vehicles. Relative activities (without metabolic activation) were as follows:

(FTP) diesel ~ 0.6 x leaded activity; 0.5 x unleaded activity.
(HWFET) diesel ~ 0.4 x leaded activity; 0.4 x unleaded activity.

Fleet average Ames TA-98 revertant per mile levels (without metabolic activation) for FTP and HWFET conditions compared with light-duty diesels as follows:

(FTP) diesel ~ 3.4 x leaded rate; 12.1 x unleaded rate.
(HWFET) diesel ~ 1.5 x leaded rate; 9.1 x leaded rate.

In conclusion, replacing the gasoline passenger cars represented by the test fleet of this program with diesel passenger cars would decrease HC, CO, and NO_x population exposures, and increase total particulate and mutagenic particulate organic exposures (as indicated by Ames Salmonella strain TA-98).

Table 1. Characterization of Particulate Emissions,
Diesel versus Gasoline

	Diesel *		Leaded		Unleaded	
	FTP	HWFET	FTP	HWFET	FTP	HWFET
Total particulate, mg/mi.	607(1)	345(1)	103	276	31.7	25.6
Dichloromethane soluble organics, mg/mi.	124(1)	79.7(1)	21.1	23.5	14.4	11.5
Benzo-a-pyrene, ug/mi.	4.5(3-7)	2.7(3-7)	14.5	0.89	3.3	0.61
Nitro-pyrene, ug/mi.	7.4(8)	6.8(8)	0.20	0.39	0.24	0.16
TA-98,-S9, rev/mg	4.1(1)	3.0	7.31	8.55	7.57	7.39
TA-98,+S9, rev/ug	**	**	12.5	10.6	13.4	7.43
TA-98,-S9, rev/mi.(x 10^{-3})	509(1)	239	152	163	42.1	26.4
TA-98,+S9, rev/mi.(x 10^{-3})	**	**	258	232	79.3	25.2

* Number in parenthesis indicates reference.
** Information unavailable at this time.

REFERENCES

1. Gibbs, R.E., J.D. Hyde, and S.M. Byer. Characterization of particulate emissions from in-use diesel vehicles. SAE paper number 810081, February, 1981.

2. Killough, P., and J. Watson. Filter-type, high volume particulate samples for automotive diesel emission studies. ES-TN-79-13. Northrop Services, Inc., December 1979.

3. Huisingh, J.L., and R.L. Bradow, R.H. Jungers, B.D. Harris, R.B. Zweidinger, K.M. Cushing, B.E.Gill, and R.E. Albert. Mutagenic and carcinogenic potency of extracts of diesel and related environmental emissions: study design, sample generation, collection and preparation. Health Effects of Diesel Engine Emissions: Proceedings of an International Symposium, EPA-600/9-80-056b. November 1980.

4. Kraft, J., and K.H. Lies. Polycyclic aromatic hydrocarbons in the exhaust of gasoline and diesel vehicles. SAE paper number 810082. February 1981.

5. Williams, R.L.,and D.P. Chock. Characterization of diesel particulate exposure. Health Effects of Diesel Engine Emissions: Proceedings of an International Symposium. EPA-600/9-80-057a. November 1980.

6. Hare, C.T., and T.M. Baines. Characterization of particulate and gaseous emissions from two diesel automobiles as a function of fuel and driving cycle. SAE paper number 790424. February 1979.

7. Williams, R.L., and S.J. Swarin. Benzo(a)pyrene emissions from gasoline and diesel automobiles. SAE paper number 790419. February 1979.

8. Tejada, S.. Particulate NO_2-pyrene emissions from a 1980 Oldsmobile and a 1980 VW diesel, FTP and HWFET cycles. Unpublished data. June 1981.

SURFACE REACTIVITY OF DIESEL PARTICLE AEROSOLS

by

Magnus Lenner, Oliver Lindqvist
and Evert Ljungström
Department of Inorganic Chemistry
University of Gothenburg and
Chalmers University of Technology

Inger Lundgren and Åke Rosén
Volvo Car Corporation

Gothenburg, Sweden

INTRODUCTION

In 1979 a research project, concerning exhaust emissions from passenger cars, was initiated by Volvo Car Corporation in collaboration with the University of Gothenburg and Chalmers University of Technology. The first report (1), which appeared in September 1979, comprised of investigations of exhaust particulates both from gasoline powered and from diesel powered passenger cars, as well as chemical analyses by several methods, of gaseous and particulate matter in exhaust samples from Volvo cars.

The present work deals with the influence of diesel particles on the oxidation of nitric oxide to nitrogen dioxide at different conditions of load, temperature and dilution. Secondly, spectroscopic investigations of how the chemical composition of diesel particle surfaces may be modified in the atmosphere have been performed. A full report (2) will be published in October 1981.

EXPERIMENTAL

Determination of Conversion Rates

To determine the effect of diesel particles on the conversion $NO \rightarrow NO_2$, two series, each comprising of eight rate constant determinations were made in bag samples of exhausts from a Volvo passenger diesel. The samples were

analysed for nitrogen oxides concentrations at intervals during ~24 h after sample collection. A Monitor Labs. 8440 Nitrogen Oxides Analyser was used.

For the first series of samples the engine of the car was run at approximately 1500 rpm idle, while for the second series the car was driven at 40 km/h with a road load of 12.5 horse powers in a chassis dynamometer. The parameters temperature (0° or 23° C), dilution rate (~1/60 or ~1/120) and presence/absence of diesel particles were varied. The samples were collected in Tedlar bags contained in an aluminum barrel, which could be evacuated to make the bag extract the appropriate volume of exhaust gases. Dry air from a gas cylinder was used for dilution. Diesel particles were removed by a filter for the particle-free samples.

Spectroscopic Methods

For infrared spectroscopy, samples were collected with an Electrical Cororna Sampler (3) on gold covered metal plates and analysed by a reflection-IR method, with a Nicolet MX-1 FTIR instrument.

ESCA samples were collected on Millipore filters and the electron spectra were recorded with a Hewlett-Packard 5960 A electron spectrometer.

RESULTS AND DISCUSSION

Conversion Rates

The formation of NO_2 from NO obeys the relationship:- $\frac{d[NO]}{dt} = k[NO]^2[O_2]$. The rate constant k is commonly given in either of the dimensions $1/[M]^2$ per second or 1/ppm per minute.

The values for k ($[M]^{-2} \times sec.^{-1}$), calculated for the respective 16 experiments, are summarized in Table 1. The rate constant has a negative temperature dependence, especially at higher dilution rates. The reaction is enhanced by the presence of diesel particles. The latter effect, though, is not as strong as the catalytic effect of street and wall surfaces, reported by Lindqvist et al. (4).

Spectroscopic Results

ESCA measurements were made on three kinds of samples of diesel particles, namely unexposed samples, samples which had been exposed to 2 ppm NO_2 for 48 h and finally samples which had been exposed simultaneously to NO_2 and UV-light for 6 h. Nitrogen (1s) responses were obtained only from the latter kind of sample. Signals at 400 eV and at 402 eV were assigned to emanate respectively from N_x and NH_4 by comparison with the results of Chang & Novakov (5).

The infrared spectra obtained by reflection of IR-light through diesel particles precipitated on a gold film showed absorption peaks at 1290/cm and at 860/cm, corresponding respectively to C - N stretch in primary

aromatic amines and to N - O stretch in aromatic nitro compounds. Unexposed samples gave the same kind of spectra as samples which had been exposed to NO_2 and UV-light.

REFERENCES

1. Lundgren, I., Rosén, Å. and Lindqvist, O. 1979. Unregulated pollutants. Measurements and analysis of exhaust gas and particulates from Volvo light-duty vehicles. Volvo Car Corporation: Gothenburg. 80 pp.

2. Lenner, M., Ljungström, E., Lindqvist, O., Lundgren, I., and Rosén, Å. 1981. Reactivity and catalytic activity of diesel particles. Studies of NO_x and particle emissions from a Volvo passenger diesel. Volvo Car Corporation: Gothenburg. 63 pp. In press.

3. Van de Vate, J. F., Plomp, A., de Jong, C. and Vrins, E. L. M. 1977. A battery-operated portable unit for electrostatic and impaction sampling of ambient aerosols for electron microscopy. Presented at the 5th Conference of the Gesellschaft für Aerosolforschung, Karlsruhe, W. Germany.

4. Lindqvist, O., Ljungström, E. and Svensson, R. 1981. Low temperature thermal oxidation of nitric oxide in polluted air. Atm. Environment. In press.

5. Chang, S. G. and Novakov, T. 1975. Formation of pollution particulate nitrogen compounds by NO-soot and NH_3-soot gas-particle surface reactions. Atm. Environment 9:495-504.

Table 1. Calculated Rate Constants. k is the slope of the function $1/[NO]_t - 1/[NO]_0 = kt$, calculated from measurements of [NO] at intervals after the start of an experiment at t = 0. The values have been multiplied by 10^{-4}.

1500 rpm idle							
Particles				No particles			
No.	Temp. (°C)	Dil. rate	k	No.	Temp. (°C)	Dil. rate	k
1	0	1/60	2.11	5	0	1/60	1.74
2	0	1/120	2.39	6	0	1/120	2.04
3	23	1/60	1.81	7	23	1/60	1.56
4	23	1/120	1.82	8	23	1/120	1.57
40 km/h. Road load: 12.5 hp							
Particles				No particles			
No.	Temp. (°C)	Dil. rate	k	No.	Temp. (°C)	Dil. rate	k
9	0	1/60	2.18	13	0	1/60	1.75
10	0	1/120	2.43	14	0	1/120	1.99
11	23	1/60	1.97	15	23	1/60	1.59
12	23	1/120	1.92	16	23	1/120	1.53

EFFECTS OF OZONE AND NITROGEN DIOXIDE PRESENT DURING SAMPLING OF GENUINE PARTICULATE MATTER AS DETECTED BY TWO BIOLOGICAL TEST SYSTEMS AND ANALYSIS OF POLYCYCLIC AROMATIC HYDROCARBONS

by

G. Löfroth
Department of Radiobiology
University of Stockholm
S-106 91 Stockholm

R. Toftgård, J. Carlstedt-Duke and J-Å. Gustafsson
Departments of Medical Nutrition and Pharmacology
Karolinska Institute
S-104 01 Stockholm

E. Brorström, P. Grennfelt and A. Lindskog
Swedish Water and Air Pollution Research Laboratory
S-402 24 Gothenburg

Urban particulate matter was collected in wintertime at -5 - 0 ^{o}C during 24 h periods on glass fiber filter with two simultaneously operating high volume samplers. One of the samplers was equipped with an ozone or nitrogen dioxide dosage system enhancing the concentration with about 200 ppb ozone or 960 ppb nitrogen dioxide. Filters were Soxhlet-extracted with acetone and the extracts analyzed with respect to eight polycyclic aromatic hydrocarbons (PAH), mutagenicity in the Salmonella/microsome assay and ability to displace 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) from the rat liver receptor protein.

Polycyclic Aromatic Hydrocarbons

A part of or the whole acetone extract of each filter was subjected to a clean up procedure with respect to PAH and eight components were quantified by gas chromatography on a Carlo Erba equipment with a SE-54 glass capillary column. The range of concentrations detected is given in Table 1.

Exposure to ozone had very little effect on the concentrations of the PAH. Significant degradation occurred only in one of the experiments. At this occasion the concentrations of nitric oxide and nitrogen dioxide in ambient air were rather high and it seems likely that the degradation may have been caused by nitrogen oxides formed by ozone oxidation of nitric oxide.

Statistical analysis between the nitrogen dioxide exposed samples and the simultaneously collected non-exposed samples showed a significant degradation for pyrene, benz(a)anthracene and benzo(a)pyrene being on the average about 20, 40 and 60 %, respectively.

Salmonella Mutagenicity

Portions of the acetone extracts were reduced in volume, but not to dryness, at < 40 °C and then diluted with dimethyl sulfoxide (DMSO). Each of the DMSO samples was assayed at at least three different occasions for mutagenicity by the Salmonella plate incorporation method with bacterial cultures fully grown overnight. Tests were performed with the strains TA 98 and TA 100 in the absence and presence of the microsome containing liver supernatant from Aroclor 1254-induced male rats (S9) and with the nitroreductase deficient strains TA 98 NR, TA 98/1,8 DNP_6 and TA 100 NR in the absence of S9. The S9 was used at a level of 20 µl per plate and was added together with necessary co-factors.

The response expressed as revertants per cu. m was calculated from the linear or approximately linear dose response curves. The results for assays in the absence of S9 are given in Table 2. The addition of S9 either decreased or did not change the mutagenic response.

Exposure to ozone did not generally alter the mutagenic response except for an increase in the experiment which also resulted in degradation of PAH.

Exposure to nitrogen dioxide increased the mutagenic response in nitroreductase proficient as well as nitroreductase deficient strains. The average enhancement found in the three investigated experiments was a 3 - 4-fold increase.

Affinity to the Rat Liver TCDD-Receptor Protein

Assays for the ability to displace TCDD from the rat liver receptor protein were performed by adding different amounts of the DMSO samples to one ml of rat liver cytosol containing tritium-labeled TCDD and determining the amount of TCDD which remained bound to the receptor. After correction for non-specific binding, the relative specific binding was calculated from log-logit plots and was expressed as the amount that competes for 50 % of the specific TCDD-binding, EC_{50}, cu. m per ml cytosol.

Two experiments from the nitrogen dioxide and one from the ozone exposure were analyzed and the results are given in Table 2. Neither nitrogen dioxide nor ozone altered the affinity.

Conclusions

High volume sampling of ambient particulate matter on glass fiber filter in the presence of a high level of ozone does not cause a significant degradation of PAH, alter the mutagenic response which is detected by the Salmonella assay or change the affinity to the TCDD-receptor protein.

Sampling in the presence of a high level of nitrogen dioxide causes a significant degradation of reactive PAH and increases the mutagenic response which is detected by the Salmonella assay, but does not change the affinity to the TCDD-receptor protein.

Simultaneous sampling of genuine particulate matter without and with enhanced concentrations of reactive gases may be the best method for studying artifact reactions during sampling. Further studies are in progress with nitrogen dioxide, nitrous acid and nitric acid.

Table 1. Concentration ranges of analyzed PAH components; ng / m^3.

PAH component	8 samples non-exposed	3 samples O_3-exposure 200 ppb	5 samples NO_2-exposure 960 ppb
Phenanthrene	0.42 - 2.2	0.66 - 2.0	0.39 - 1.9
Fluoranthene	0.64 - 22	1.7 - 3.6	0.81 - 4.5
Pyrene	1.1 - 8.0	1.5 - 3.6	0.81 - 5.8
Benz(a)anthracene	1.1 - 5.2	0.93 - 2.0	0.82 - 3.8
Chrysene / Triphenylene	0.8 - 4.0	1.1 - 2.1	1.1 - 4.0
Benzo(b&k)fluoranthenes	1.6 - 7.8	2.0 - 3.5	2.2 - 7.6
Benzo(e)pyrene	0.39 - 3.6	0.48 - 1.0	0.36 - 2.2
Benzo(a)pyrene	0.17 - 2.4	0.16 - 0.66	0.07 - 1.0

Table 2. Mutagenic response in the absence of mammalian metabolic activation and affinity to the TCDD-receptor protein of extracts of particulate matter collected without and with enhanced concentrations of nitrogen dioxide or ozone; n.d. not determined.

Sample		Revertants per m^3					Receptor affinity EC_{50} ± s.d. (n = 4) m^3 / ml
		TA 98	TA 98 NR	TA 98/ 1,8 DNP_6	TA 100	TA 100 NR	
800225	AMB	62	37	28	76	36	n.d.
	NO_2	87	48	32	142	62	n.d.
800226	AMB	14	8	3.2	14	5	0.17 ± 0.04
	NO_2	37	24	6.5	41	20	0.09 ± 0.04
800228	AMB	11	6	5	20	5	0.07 ± 0.02
	NO_2	76	35	20	110	41	0.10 ± 0.03
800326	AMB	4.3	n.d.	n.d.	2.9	n.d.	n.d.
	O_3	4.6	n.d.	n.d.	3.3	n.d.	n.d.
800327	AMB	28	11	4.5	21	5	0.08 ± 0.02
	O_3	31	12	4.4	25	7	0.06 ± 0.04
800331	AMB	10	n.d.	n.d.	8.4	n.d.	n.d.
	O_3	16	n.d.	n.d.	14	n.d.	n.d.

ALUMINA COATED METAL WOOL AS A PARTICULATE FILTER FOR DIESEL POWERED VEHICLES

by

M. A. McMahon, W. T. Tierney,
K. S. Virk, and C. H. Faist

Pending federal regulations will probably require 1985 and later diesel powered vehicles to be equipped with aftertreatment devices to control particulate emissions. Filtering devices which employ alumina coated steel wool as the filter matrix show promise of being a practical means for removing submicron size particles from diesel exhaust. Our diesel exhaust filters (DEF's) consist of two alumina coated stainless-steel-wool filled cartridges inserted into an outer housing about the same size as a conventional muffler (Figure 1). The alumina coating, which is applied to the steel wool by a proprietary process, characteristically retains soot particles which come in contact with it.

In our design, metal wool packing density, alumina coating thickness and the physical dimensions are the dominant performance determinants for the collection efficiency and exhaust back pressure. The surface area of the alumina in the DEF, which is a function of all of these, correlates well with collection efficiency.

Our tests have demonstrated that particulate removal in the 70% range in FTP testing can be achieved at acceptable backpressures. Measurements also show that trapping efficiency is constant over a gas flow range of 0.7 to 20 ft/sec. The trapping efficiency of a given size filter can be increased above 80% but only at an increased backpressure penalty. Satisfactory filters have been made for diesel engines varying in size from 2 to 14 litres.

Generally, filters capable of trapping soot with efficiencies in excess of 70% have a pressure drop of 3 to 4 inches of water per inch of filter at gas velocities of 15 ft/sec. For example, a filter mounted on a vehicle equipped with a 1980 Oldsmobile engine, having a trapping efficiency in this range demonstrated a pressure drop of 31 inches of water at 40 mph level road conditions.

In addition to removing soot from diesel exhaust, alumina coated steel wool filters remove significant amounts of hydrocarbons and sulfates. Importantly, a significant percentage (50%) of the polynuclear aromatics, as indicated by benzo (a) pyrene measurements, is removed. Noise is attenuated to levels equivalent to those observed using conventional mufflers.

Since the volume of soot generated by current diesel engines is quite large, soot collected on DEF's must be periodically removed for the filter to trap effectively for extended mileage. Burning at temperatures above 1000°F is the most feasible method for doing this. Since diesel exhaust temperatures at moderate loads are generally below 1000°F, consideration is being given to mounting torches in the exhaust system to increase exhaust gas temperature when regeneration is required. In that conventional torches present difficult operating problems when employed in exhaust systems, a catalytic torch is being developed in our laboratories. With this kind of torch, hydrocarbons injected into the exhaust are catalytically oxidized to increase the exhaust gas to regeneration temperature. The catalytic converter used was made by applying a noble metal catalyst to alumina coated steel wool. Although the torch performed satisfactorily using either propane or diesel fuel, propane was used in most of the development work because of experimental convenience.

To date, about 3,000 miles have been accumulated on a 1980 Oldsmobile vehicle equipped with a diesel exhaust filter. During this test, the filter was regenerated at 150-200 mile intervals with our catalytic torch and a trapping efficiency of greater than 65% was maintained throughout the entire test. Since the amount of heat released from burning soot in the limited DEF volume can be large, the regeneration interval is determined by this factor rather than by the potential for high backpressure due to filter loading.

In addition to the durability experience achieved with diesel engines, it is worth noting that alumina coated mesh filters have undergone millions of miles of durability testing as a lead particulate filter in the exhaust of a variety of gasoline engines.

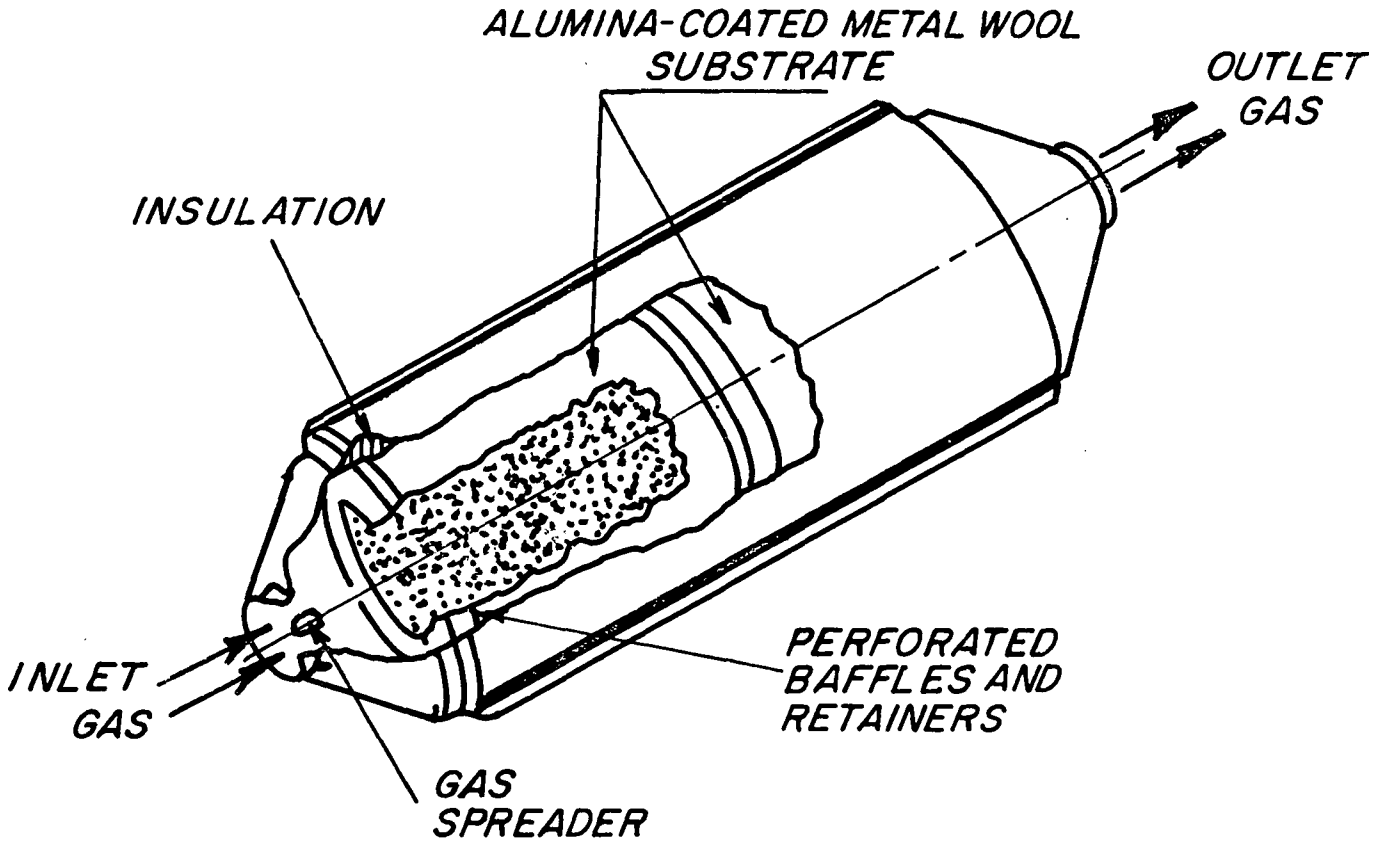


FIGURE 1 - Typical Texaco Diesel Exhaust Filter Design

ISOLATION AND IDENTIFICATION OF MUTAGENIC NITROARENES IN DIESEL-EXHAUST PARTICULATES

by

X.B. Xu*, Joseph P. Nachtman, Z.L. Jin*, E.T. Wei,
and Stephen Rappaport
Department of Biomedical and Environmental Health Sciences
School of Public Health
University of California, Berkeley, CA

and

A.L. Burlingame
Space Sciences Laboratory
University of California, Berkeley, CA, and
Department of Pharmaceutical Chemistry
School of Pharmacy
University of California, San Francisco, CA

Particulate matter emitted from diesel engines contains chemicals which are active in the Ames *Salmonella typhimurium* assay. The major portion of this mutagenic activity is liver enzyme independent and thus indicates that diesel exhaust contains a different class of mutagens than unsubstituted polynuclear aromatic hydrocarbons, which are liver enzyme dependent.

Diesel exhaust particulates were collected on glass fiber filters from heavy-duty engine test apparatus. A total of 14 filters were extracted for 24 hours with 6 L dichloromethane in a Soxhlet apparatus, the extract filtered and concentrated by rotary evaporator. The combined extracts contained approximately 225 g of organic matter which yielded 0.46 net TA98 revertants/μg (1.0×10^8 net TA98 revertants).

The CH_2Cl_2 extract was further fractionated on a preparative silica column with successively increasing solvent strength: hexane, chloroform, and methanol. The intermediate polarity fraction had the highest specific mutagenic activity and was further separated on gel permeation, high performance normal and reverse phase chromatography. Mutagenic activity was detected in virtually all fractions, so that fractions containing the highest specific activity were selected for further analysis.

*Members of the Institute of Environmental Chemistry, Chinese Academy of Sciences, Beijing, People's Republic of China.

High resolution mass spectrometry (HRMS) was performed on selected subfractions from the reverse phase separation. Each sample was evaporated under N_2 in the probe which was inserted into the electron impact source. Identification of nitroarenes was based upon accurate mass determinations (± 15 ppm at a resolution of 9000) of the molecular ion and of fragment ions corresponding to losses of the neutral fragments NO and/or NO_2. Some nitroarenes tentatively identified are listed in Table 1. Those with an asterisk (*) were found to have the same HRMS spectrum and high pressure liquid chromatography (HPLC) retention volume as their corresponding standard.

In this study, about 50 nitroarenes have been tentatively identified by HRMS. The variety of nitroarenes in diesel exhausts is extensive and, because only a few of the mutagenic fractions were examined, it is likely that more nitroarenes will be characterized. This complexity is not surprising if one considers the numerous PAH substrates available for aromatic ring nitration: a chemical process which will readily occur in the presence of even low concentrations of nitrogen oxides. Positive identification of each nitroarene is made difficult by the small quantity of nitroarenes relative to co-eluting oxygenated and some sulfur-containing PAH and also by the absence of synthetic standards. Comparison of mass spectra and retention volumes with available standards, however, support the identifications which have been suggested.

A number of nitroarenes are potent mutagens in the Ames Salmonella assay, because nitroreductases in the tester strains facilitate reduction of nitroarenes of electrophilic intermediates which, in turn, react with nucleic acids. This raises the question of whether nitroarenes in diesel exhaust or any other environmental source pose a significant human health hazard. From a qualitative viewpoint, nitroarenes are hazardous because compounds such as 2-nitronaphtalene and 2-nitrofluorene are carcinogenic to animals. However, from a quantitative viewpoint, issues of the human dose and the biologic potency of nitroarenes have not yet been established. It is also not known if nitroarene emissions are independent of or bear a reciprocal relationship to the amount of unsubstituted PAH that is emitted. The development of convenient techniques to separate and quantify nitroarenes and metabolites may expedite the acquisition of data to answer these questions.

TABLE 1

Mass	Possible Compound
161.048	Nitroindene
197.048	Nitroacenaphthylene
199.063	Nitrobiphenyl*
211.063	Nitrofluorene*
213.079	Nitro-methylbiphenyl
223.063	Nitroanthracene*
225.079	Nitro-methylfluorene
247.063	Nitropyrene*
261.079	Nitro-methylpyrene
287.095	Nitro-methylchrysene
225.043	Nitro-fluorenone*
241.074	Nitro-hydroxymethylfluorene
253.038	Nitro-anthraquinone
256.048	Dinitrofluorene*
340.143	Dinitro-(C_6)alkylfluorene
348.111	Dinitro-(C_4)alkylpyrene
371.112	Trinitro-(C_5)alkylfluorene

COMPARISON OF NITRO-PNA CONTENT AND MUTAGENICITY OF DIESEL EMISSIONS

by

Marcia G. Nishioka and Bruce A. Petersen
Battelle Columbus Laboratories
Columbus, Ohio

and

Joellen Lewtas
Health Effects Research Laboratory
U.S. Environmental Protection Agency
Research Triangle Park, North Carolina

INTRODUCTION

The increasing number of automobiles and light-duty trucks powered by diesel engines has generated concern over the emissions associated with these engines. The diesel engines have a higher particulate emission rate than gasoline catalyst engines. The extractable organics from both diesel and gasoline particle emissions have been found to be mutagenic and carcinogenic (1). Recently, nitro-substituted polynuclear aromatic hydrocarbons (nitro-PNA) have been identified in diesel particle extracts (2). Several of these nitro-PNAs are very potent bacterial mutagens (3).

A study was carried out to identify and quantitate nitroaromatic compounds in the extract of particulate material from three different diesel engines (Datsun Nissan diesel 220C, Oldsmobile diesel 350, and VW Rabbit diesel) and one gasoline engine (Ford Mustang II). The operating and sampling conditions have been described elsewhere (4). Mutagenic assay data was also collected on these extracts using the Salmonella typhimurium TA98 bioassay. The results of these two studies were compared to determine whether the amount of nitroaromatics detected can fully account for the direct-acting mutagenic activity indicated by the bioassay data.

METHODS AND RESULTS

Chemical

Two separate methods were developed to analyze the emission extracts: 1) combined high performance liquid chromatography/mass spectrometry (HPLC/MS) with positive chemical ionization (PCI); and 2) on-column injection high resolution gas chromatography/mass spectrometry (HRGC/MS) with negative

chemical ionization (NCI). The total dichloromethane extract was screened first by the PCI HPLC/MS method, which consisted of a Supelco normal phase HPLC column (Supelcosil LC-Si, 5 μm) interfaced to a Finnigan 4000 mass spectrometer via a microbore capillary and a polyimide moving belt. Nitropyrene and nitrophenanthrene/anthracene were detected by this method in all of the diesel engine extracts, but not in the gasoline engine extract.

The extracts were fractionated on silica gel by open-bed liquid chromatography into four compound class fractions: 1) hexane - aliphatic hydrocarbons, 2) hexane:benzene - polycyclic aromatic hydrocarbons and mononitroaromatics, 3) methylene chloride - moderately polar neutrals, including di- and trinitroaromatics, and 4) methanol-highly polar neutrals, primarily oxygenated compounds. Over 94% of the total extract was recovered for all four engines.

The three fractions expected to contain nitro-PNA (2,3,and 4) were analyzed by the NCI HRGC/MS system, which consisted of a JαW DB-5 bonded fused silica capillary column interfaced to a Finnigan 4000 mass spectrometer. The quantitation of nitroaromatics was based on response factors for eight standard nitroaromatics relative to the internal standard d_7-nitronaphthalene calculated over a concentration range of 100. More than twenty nitroaromatics were detected in the diesel engine extracts but only 1-nitropyrene was detected in the gasoline engine extract. In all cases the 1-nitropyrene was the nitroaromatic detected in greatest quantity and its concentration in the extracts is shown in Table 1. Quantitative data on the other nitroaromatics will be presented.

At masses higher than nitropyrene, the mono nitro derivatives of the molecular weight 228 (benz[a]anthracene, chrysene) and molecular weight 252 (B[a]P, B[e]P, perylene, benz[o]fluoranthenes) PNAs were also detected. Two nitropyrenone isomers were tentatively identified in the Nissan and Oldsmobile samples and three dinitropyrene isomers were identified in the VW sample.

Most of the compounds detected in the methanol fraction of the Nissan and VW samples were quinones. The methanol fraction represented 30% and 17% of the total organic mass for the Nissan and VW extracts, respectively.

Biological

The total dichloromethane extractable organics from each of the emission samples were bioassayed in the *Salmonella typhimurium* plate incorporation assay with minor modifications as reported elsewhere (5). The slope of the dose-response curve (rev/μg) for each of these samples with and without S9 activation was determined using a non-linear model (6) and is shown in Table 1. The emissions from the gasoline catalyst (Mustang II) differ from the diesel emissions in that these organics are significantly more mutagenic in the presence of the S9 activation system.

DISCUSSION

Both the PCI HPLC/MS and NCI HRGC/MS methods are capable of detecting nitroaromatic compounds. However, the greater chromatographic resolution and lower detection limit of the NCI HRGC/MS method favor the use of this method over the PCI HPLC/MS method. The limit of detection by NCI HRGC/MS is approximately 0.05 ng for the mononitroaromatics, but only about 100 ng for nitropyrene by the PCI HPLC/MS method.

The concentration of 1-nitropyrene detected in each of the samples in not highly correlated with the direct-acting mutagenic activity. The total nitroaromatic content does account for a substantive portion of the direct-acting mutagenicity of the Olds diesel, VW Rabbit diesel, and Mustang II gasoline vehicles. However, the nitroaromatics detected cannot account for the significantly higher mutagenic activity associated with the Nissan diesel extract.

It is possible that the quinones detected in the Nissan and VW extracts are the oxidation productions of nitroaromatics in the stored extracts, as similar extracts were shown to increase in toxicity with time (6). A greater concentration of quinones in the Nissan sample may indicate that a greater concentration of nitroaromatics may have been originally present in the extract. This is consistent with the fact that the Nissan extract originally had higher mutagenic activity than the VW extract.

REFERENCES

1. Nesnow, S., and J.L. Huisingh. 1980. Mutagenic and carcinogenic potency of extracts of diesel and related environmental emissions: Summary and discussion of the results. In: Health Effects of Diesel Engine Emissions, Vol. II. W.E. Pepelko, R.M. Danner, and N.A. Clarke, eds. EPA-600/9-80-057b. U.S. Environmental Protection Agency: Cincinnati, OH.

2. Petersen, B.A., C. Chuang, W. Margard, and D. Trayser. 1981. Identification of mutagenic compounds in extracts of diesel exhaust particulates. Proceedings of the 74 annual APCA Meetings, Philadelphia, PA.

3. Rosenkranz, H.S., E.C. McCoy, D.R. Sanders, M. Butler, D.K. Kiriazides, and R. Mermelstein. 1980. Nitropyrenes: Isolation, identification and reduction of mutagenic impurities in carbon black and toners. Science 209:1039-1043.

4. Huisingh, J.L., R.L. Bradow, R.H. Jungers, B.D. Harris, R.B. Zweidinger, K.M. Cushing, B.E. Gill, and R.E. Albert. 1980. Mutagenic and carcinogenic potency of extracts of diesel and related environmental emissions: Study design, sample generation, collection, and preparation. In: Health Effects of Diesel Engine Emissions, Vol. II. W.E. Pepelko, R.M. Danner, and N.A. Clarke, eds. EPA-600/9-80-057b. U.S. Environmental Protection Agency: Cincinnati, OH. pp. 788-800.

5. Claxton, Larry D. 1980. Mutagenic and carcinogenic potency of diesel and related environmental emissions: Salmonella bioassay. In: Health Effects of Diesel Engine Emissions, Vol. II. W.E. Pepelko, R.M. Danner, and N.A. Clarke, eds. EPA-600/9-80-057b. U.S. Environmental Protection Agency: Cincinnati, OH. pp. 801-807.

6. Huisingh, J., R. Bradow, R. Jungers, L. Claxton, R. Zweidinger, S. Tejada, J. Bumgarner, F. Duffield, V.F. Simmon, C. Hare, C. Rodriguez, L. Snow, and M. Waters. 1979. Application of bioassay to the characterization of diesel particle emnissions. Part II. Application of a mutagenicity bioassay to monitoring light-duty diesel particle emissions. In: Application of Short-term Bioassays in the Fractionation and Analysis of Complex Environmental Mixtures, Environmental Science Research, Vol. 15. M.D. Waters, S. Nesnow, J.L. Huisingh, S.S. Sandhu, and L. Claxton, eds. Plenum Press: New York. pp. 400-418.

Table 1. Quantification of 1-Nitropyrene in Engine Exhaust Extracts and Mutagenic Activity of the Extracts

Sample	Conc. 1-Nitropyrene wt ppm	Mutagenic Activity, rev/μg	
		TA98/-S9	TA98/+S9
Nissan diesel	407	20.8	15.1
Olds diesel	107	2.1	1.4
VW Rabbit diesel	589	5.2	6.1
Mustang II gasoline	2.5	2.1	8.6

CAPILLARY COLUMN GC/MS CHARACTERIZATION OF DIESEL EXHAUST PARTICULATE EXTRACTS[1]

by

T. J. Prater, T. Riley and D. Schuetzle
Analytical Sciences Department
Scientific Research Laboratory
Dearborn, Michigan 48121

INTRODUCTION

Previous studies have shown that most of the direct-acting Salmonella typhimurium mutagenic activity (>50%) in diesel exhaust particulate extracts is concentrated in chemical fractions which contain compounds of moderate polarity (2). Analytical-scale normal phase high performance liquid chromatography(NP-HPLC) (3), packed-column GC/MS and high resolution MS analysis were used in that work to determine that these moderately-polar fractions consisted primarily of polynuclear aromatic hydrocarbon (PAH) derivatives. The objective of this study was to extend the previous work by developing preparative scale NP-HPLC fractionation followed by fused silica capillary GC/MS analysis in order to improve component resolution.

EXPERIMENTAL

Light duty diesel exhaust particulate samples were collected on T60A20 Pallaflex filters using a dilution tube and a chassis dynomometer test facility. Filter samples were soxhlet extracted with dichloromethane (DCM).

HPLC analysis was performed on a Varian Model 5600 LC equipped with a 7.8 mm i.d. x 30 cm long Microporasil 10 μ column. The solvent program consisted of 100% hexane for 5 min, then 1% DCM/min for 5 min, followed by a linear gradient to 100% DCM in the next 25 min, 100% DCM for 10 min, then 10% acetonitrile/min for 10 min, and a final 10 min flush with 100% acetonitrile. The chromatographic separation was monitored by UV at 254 nm and by fluorescence at 254/320 nm. Further details of this technique are presented elsewhere (3).

GC/MS analyses were performed on a VGMM ZAB-2F mass spectrometer equipped with a 30 m long x 0.25 mm i.d. SE54 fused silica capillary column interfaced directly to the mass spectrometer source. Samples were injected directly on-column and temperature programming was 80° to 270° at 4°/min. Electron impact ionization techniques were used.

RESULTS AND DISCUSSION

This investigation emphasized the analysis of HPLC fractions containing nonpolar and moderately polar PAH derivatives. Nonpolar aliphatic and highly polar HPLC fractions were not characterized by GC/MS. The nonpolar and moderately polar PAH derivatives which were identified are listed respectively in Tables I and II. Many of the compounds identified have a large number of isomers as indicated by the parentheses in the tables. This is illustrated by the mass chromatograms in Fig. 1 which show the increasing complexity of the isomer series as methyl substituents are added to anthracene and phenanthrene. There are probably more isomers for the methylated anthracene and phenanthrenes than we were able to resolve even with the high-resolution fused-silica capillary column. It would be difficult and of limited utility to identify every specific isomer present in these fractions. For this reason, synthesis of standards and identification of isomers are only being undertaken on those groups of PAH and PAH-derivatives which yield a relatively high level of direct- or indirect-acting Ames activity. This has been found to be the case for the nitrated-PAH derivatives which tend to show high levels of direct-acting mutagenicity compared to other PAH and PAH derivatives in these samples.

The combination of normal phase HPLC fractionation followed by capillary GC/MS analysis proved to be a very useful approach to the qualitative characterization of diesel exhaust particulate extracts. The quantitative analysis of these diesel extracts by capillary GC/MS is complicated by the labile nature of some of the PAH derivatives. The quantitation technique found to be most accurate and presently being used in our laboratory employs the deuterated analog of the PAH derivative of interest which is added to filter samples prior to extraction. The deuterated standard exhibits the same chemical characteristics as the native compound, but it can be distinguished mass spectrometrically. Therefore, losses which occur during sample workup and analysis can be accounted for.

1. Prater, T. J., T. Riley, and D. Schuetzle. 1981. Capillary column GC/MS characterization of diesel exhaust particulate extracts. Presented at the 29th Annual Conference on Mass Spectrometry and Allied Topics, Minneapolis, MN.

2. Schuetzle, D., F.S.-C. Lee, T. J. Prater, and S. B. Tejada. 1981. The identification of polynuclear aromatic hydrocarbon (PAH) derivatives in mutagenic fractions of diesel particulate extracts. Intern. J. Environ. Anal. Chem. 9:93-144.

3. Schuetzle, D., and J. M. Perez. 1981. A CRC cooperative comparison of extraction and HPLC techniques for diesel particulate emissions. Presented at the 74th Annual Meeting of the Air Pollution Control Association, Paper #81-564, Philadelphia, PA.

4. Levine, S. P., and L. Skewes. 1981. High performance semi-preparative liquid chromatography of diesel engine emission particulate extracts. J. Chromatogr. In preparation.

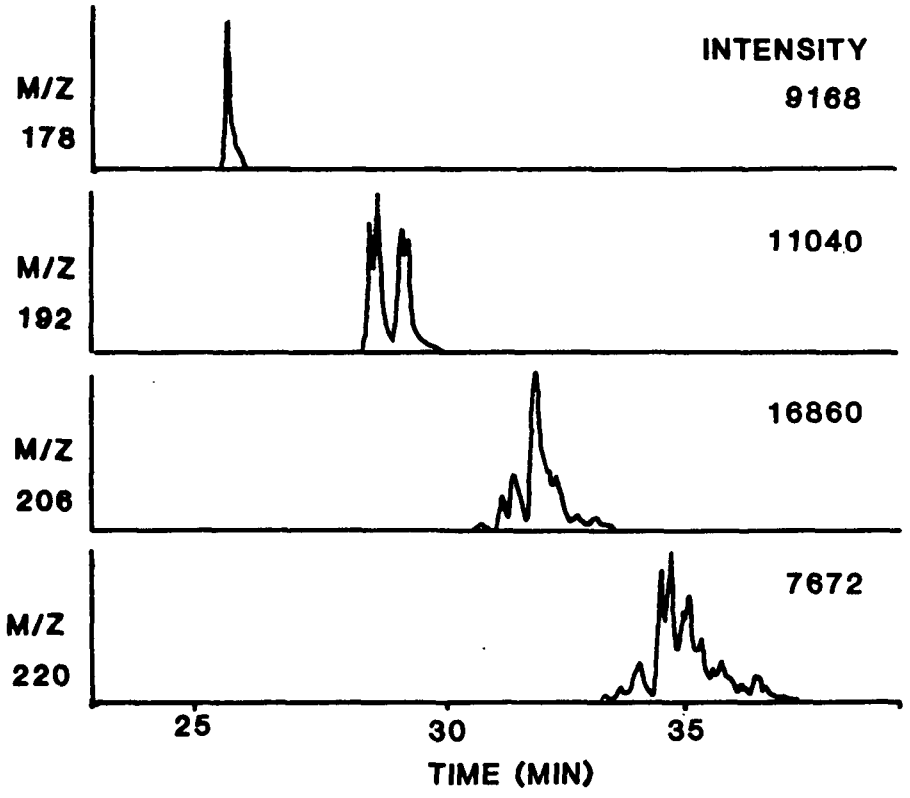
Table I. Nonpolar PAH Identified in Diesel Exhaust Particulate Extract

dibenzothiophene
anthracene and phenanthrene
methyl dibenzothiophene isomers(3)
methyl (phenanthrene and anthracene) isomers(4)
dimethyl dibenzothiophene isomers(7)
dimethyl (phenanthrene and anthracene) isomers(13)
fluoranthene and pyrene
trimethyl dibenzothiophene isomers(9)
BaP, BeP, perylene,and isomers(3)
trimethyl (phenanthrene and anthracene) isomers(15)
tetramethyl dibenzothiophene isomers(12)
tetramethyl (phenanthrene and anthracene) isomers(16)
benzo(g,h,i) fluoranthene
benz(a)anthracene, chrysene, benzo(c)phenanthrene, triphenylene isomers(2)
methyl benz(a)anthracene isomers(4)
pentamethyl dibenzothiophene isomers(4)
dimethyl benz(a)anthracene isomers(2)
methyl (fluoranthene and pyrene)isomers(7)

Table II. Moderately Polar PAH Derivatives Identified in Diesel Exhaust Particulate Extract

benz(a)anthracenedione
methyl (anthrone and phenanthrone) isomers
thiozanthone isomer
dimethyl (anthrone and phenanthrone) isomers
pyrenone
trimethyl (anthrone and phenanthrone) isomers
methyl thioxanthone
dimethyl thioxanthone isomers(2)
benz(d,e)anthrone and isomers(3)
1-nitropyrene
1,1' biphenyl-ol
9-fluorenone
(pyrene or fluoranthene) carboxaldehyde
dibenzofuran carboxaldehyde
phenanthrone
anthrone isomer
9-xanthone
xanthene carboxaldehyde
(anthracene or phenanthrene)dione
dibenzothiophene carboxaldehyde
methyl (anthracene or phenanthrene)dione
phenanthrene carboxaldehyde
anthracene carboxaldehyde
methyl (anthracene and phenanthrene) carboxaldehyde isomers(9)
dimethyl (anthracene and phenanthrene) carboxaldehyde isomers(8)

Figure 1. Mass chromatograms of phenanthrene(P) and anthracene(A) (178), methyl-A and -P (192), dimethyl-A and -P (206), and trimethyl-A and -P (220)



PHYSICO-CHEMICAL PROPERTIES OF DIESEL PARTICULATE MATTER

by

Mark M. Ross and Terence H. Risby
Division of Environmental Chemistry
Department of Environmental Health Sciences
The Johns Hopkins University School of Hygiene and Public Health
Baltimore, Maryland 21205

Samuel S. Lestz
Department of Mechanical Engineering
Pennsylvania State University
University Park, Pennsylvania 16802

Ronald E. Yasbin
Department of Microbiology
Pennsylvania State University
University Park, Pennsylvania 16802

Numerous studies have dealt with the identification and quantification of the compounds sorbed onto Diesel particulate matter. The ultimate environmental significance of these sorbed species depends upon the relative bioavailabilities which, in turn, depend upon the nature and strength of interactions prevailing at the gas-solid interface. This research focused on the fundamental adsorptive properties and surface characteristics of Diesel particulate matter. In addition, the free radical nature of the particles and the associated reactivity with selected stimuli was investigated.

This study was carried out using a graphitized carbon black, Spheron 6, as a "reference" solid. Two Diesel particulate samples were used. The first, DPM-PSU, was collected from a single-cylinder engine operated with a prototype fuel and a lubricant free of trace inorganic compounds. The second, DPM-EPA, was collected from an Oldsmobile 350 engine operated with a commercial fuel and lubricant at the US E.P.A.

Electron micrographs and elemental compositions revealed the common spherical carbon particle structure of all the samples. Yet, the Diesel samples had lower bulk densities and higher external surface areas, as calculated from mean particle diameters. Nitrogen B.E.T. surface areas

were measured in order to determine internal plus external surface areas. The surface area of DPM-EPA was found to be dependent on outgassing pretreatment. The surface area accessible to nitrogen increased from 41 M^2/g at 50°C degassing to 112 M^2/g at 400°C degassing. DPM-PSU was measured to have a surface area of approximately 104 M^2/g and showed little change upon increasing activation temperature. Nitrogen porosity experiments revealed the existence of pores of 100-200Å in diameter in both Diesel samples.

Isosteric heats of adsorption of a variety of organic compounds on the particulate samples were measured with gas-solid chromatography. The heats on DPM-PSU were consistently greater than those on graphitized carbon blacks (gcb). The variations of adsorption energies with adsorbate surface coverage were determined by measurement of adsorption isotherms at different temperatures. DPM-PSU exhibited adsorption characteristics similar to those of gcb and the few differences are attributed to a more polar and energetically heterogeneous DPM-PSU surface. DPM-EPA was determined to have markedly different properties due to the increased quantity of presorbed material. The DPM-EPA surface was found to be relatively non-polar and homogenous. Removal of the presorbed species caused the surface to become more active and similar to that of DPM-PSU. The significance of these results is that compounds close to the carbon surface will be more difficult to remove than those adhering to presorbed layers. Sorbed compound bioavailability and surface properties of Diesel particulate matter are dependent upon the nature and amount of presorbed material.

A related study of the reactivity of Diesel particulate matter with respect to atmospheric stimuli was performed using electron paramagnetic resonance (EPR) spectrometry. The EPR signals of the three samples were monitored after selected heat and evacuation treatments, gas (O_2, NO, NO_2) exposures, and ultraviolet/visible irradiation. The sample signals differed with respect to line widths but all signals narrowed upon sample evacuation and heat treatment and broadened upon exposure to oxygen and nitric oxide. The Diesel particulate sample signals were extremely sensitive to nitrogen dioxide and irradiation. Exposure of NO_2 caused an increase in the free radical concentration in the Diesel samples. Irradiation effects were varied depending on sample conditions but the greatest signal increase occurred with evacuated Diesel particles. With EPR, the existence of free radicals in Diesel particulate matter and the reactivity of these species with respect to selected treatments were demonstrated. The results provide evidence of potential photochemical reactivity of airborne particulate matter.

SOME FACTORS AFFECTING THE QUANTITATION OF AMES ASSAYS

by

Irving Salmeen and Anna Marie Durisin
Engineering and Research Staff
Research
Ford Motor Company
Dearborn, Michigan

The simple theory of bacterial mutation experiments of Luria and Delbrück (1) starts with the assumption

$$\frac{dm}{dt} = \mu n(t) \qquad (1)$$

where m is the number of mutants, n is the number of wild type, and μ is the mutation rate coefficient. We have shown (2) that, in the absence of cell killing, this equation predicts for the Ames assay

$$M = \alpha CN \qquad (2)$$

where M is the number of revertants per plate, α is the mutation rate per concentration, C, of mutagen and N is the total number of histidine auxotrophs in the background lawn. Thus the dose-response function is linear, but the slope is proportional to N. In the Ames assay, $N \approx n_0 P$, where n_0 is the initial inoculum and P is the average number of bacteria per background colony of histidine auxotrophs.

In a series of experiments we determined dose-response curves as a function of n_0; estimated n_0 by counting background colonies in photomicrographs (100X) of the background lawn; and estimated the volume of individual background colonies which is proportional to P. We found that P depends nonlinearily on n_0; P is much larger at low n_0 than it is at high n_0 presumably because at lower n_0 there is less competition among the colonies for the trace histidine. We observe that N decreases by about 1/3 when n_0 is decreased from 10^8 to 10^7 bacteria/plate. N is roughly independent of n_0 when n_0 is less than about 5×10^6 bacteria/plate. For merely detecting mutagens, neglect of the dependence of slopes on N, while conceptually incorrect, may have no serious effect. For quantitative experiments, such as determining the contribution of a compound to the mutagenicity of a mixture, failure to take into account the dependence of the slopes on N may

cause errors by factors of 2 to 3.

When the test compound causes killing, then equation (1) becomes:

$$\frac{dm}{dt} = \mu n(t) - k_b m \tag{3}$$

where k_b is the killing rate coefficient for mutants and, now, n(t) must include a specific killing term for the histidine auxotrophs. If we assume that

$$n(t) = n_o \exp[(\gamma - k_a)t] \tag{4}$$

where γ is the growth rate coefficient and k_a is the killing coefficient for histidine auxotrophs, and if we also assume that killing and mutations are independent events and that the killing coefficients of auxotrophs and revertants are equal and proportional to the concentration of mutagen, i.e., $k_a=k_b=kC$, then the number of revertants per plate is of the general functional form

$$M \sim \alpha CN' \exp(-kC) \tag{5}$$

where N' is the total number of histidine auxotrophs in the background population when $kC<<1$. The concentration which yields the maximum in the dose-response function is $C_m=1/k$.

We have obtained dose-response functions using diesel particulate extract which have a maximum followed by a monotonic decrease to zero. We determined the killing coefficient k in three ways: (1) from the value of the concentration corresponding to the maximum of the dose-response function; (2) from a classical dilution-plating killing curve; and (3) from a killing curve developed from counting colonies in photomicrographs of the background lawn. The killing rates determined from these 3 methods agree to within about 15%, suggesting that this simple model is a good approximation to the mutation-killing kinetics, at least for these samples.

This model is free of adjustable parameters in the sense that the two parameters can be determined directly from the data. Functional forms similar to equation (5) have sometimes been used ad hoc in statistical curve fitting routines to describe Ames assay data; the above derivation provides theoretical support for use of this function. We will show data to illustrate that the apparent slope of the initial approximately linear portion of non-linear dose-response functions obtained with several diesel-particulate extracts can over estimate, by as much as a factor of 2, the actual mutation frequency in the Ames assay.

REFERENCES

1. Luria, S. and Delbrück, M., Genetics 28, 491 1943.

2. Salmeen, I. and Durisin, A., Mutat. Res. 85, 109 1981.

CHEMICAL AND MUTAGENIC CHARACTERISTICS OF DIESEL EXHAUST PARTICLES FROM DIFFERENT DIESEL FUELS

by

D. S. Sklarew, R. A. Pelroy and S. P. Downey
Pacific Northwest Laboratory operated by Battelle
P. O. Box 999
Richland, Washington 99352

R. H. Jungers and J. Lewtas
U. S. Environmental Protection Agency
Research Triangle Park, North Carolina 27711

A potential for increased use of a wide variety of diesel fuels has increased the importance of studies to determine whether the nitrogen content of different fuels affects the chemical and mutagenic characteristics of the particles produced during combustion. In this study, the exhaust particles from five diesel fuels with various nitrogen and aromatic hydrocarbon contents were examined. The fuels included minimum quality #2 (Min. Qual.), jet fuel (JP-7), shale diesel fuel-marine (DFM), a base fuel plus heavy aromatics and hexylnitrate (BF+HAN+HN), and the base fuel plus isoquinoline (BF+IQ). Table 1 indicates the aromatic and nitrogen contents of these fuels. Particles used in the study were generated by Southwest Research Institute for EPA using a Mercedes 240D vehicle driven through five consecutive Highway Fuel Economy Tests.

Experimental

The particle samples were soxhlet extracted with methylene chloride and these extracts and the fuel samples were fractionated by acid-base and silica gel column chromatography methods. Six fractions resulted from each material: base, acid, aliphatic hydrocarbon, aromatic HC, moderately polar neutral, and highly polar neutral. Several fractions were analyzed by selective detector gas chromatography and by gas chromatography-mass spectrometry. A 30m fused silica Carbowax column was used for the moderately polar and highly polar fractions, and a 30m SE-52 column was used for the aromatic hydrocarbon fraction.

All fractions were assayed for mutagenicity in the Ames histidine reversion test with *Salmonella typhimurium* TA98 as the target cell. All samples were

tested with and without metabolic activation with Aroclor induced rat liver (S9) homogenates.

Because of the current interest in nitroaromatics in diesel particles (1,2,3), an experiment was done to estimate the recovery of smaller ring nitroaromatics from filters. 2-Nitrofluorene was added to one of the filter samples (BF+HAN+HN) and this "spiked" filter as well as a control filter of the same fuel were extracted, fractionated, and assayed in the Ames test with TA98 minus S9.

Results and Discussion

The amount of material extracted with DCM from the diesel particles ranged from 9% to 16%. Recovery of material in the fractionation procedure ranged from 82% to 105%. The weight distribution in the six chemical class fractions did not appear to correlate with either the aromatic or nitrogen content of the parent (uncombusted) fuel. Nitroaromatic standards distributed between the aromatic hydrocarbon and moderately polar neutral fractions.

Gas chromatography with a nitrogen selective detector was used to compare the nitrogen-containing components from the five particle samples. The moderately polar fractions showed similar patterns in all five samples, as did the highly polar fractions (Fig. 1). However, particles from the diesel fuel marine and minimum quality fuels appeared to contain more nitrogen compounds than did the other fuels. There did not appear to be a correlation between the major nitrogen-containing peaks in the fractions and the nitrogen content of the fuel.

The aromatic hydrocarbon fraction of the shale diesel fuel-marine particles was analyzed by GC-MS. It contained numerous polycyclic aromatic hydrocarbons including phenanthrene, anthracene, fluoranthene, pyrene, benz(a)anthracene, chrysene, benzofluoranthenes, and benzpyrenes. The moderately polar fraction of the particles from minimum quality #2 contained a number of carboxylic acids, carboxylic acid methyl esters, phenanthrenequinone, alkylated phenols, fluorenone, alkylated fluorenones, and possible alkanones. Compounds tentatively identified in the highly polar fraction of particles from the base fuel plus isoquinoline include a number of phenols, carboxylic acids, benzoic acid, quinoline, xanthenone, and benzofuranone. Another peak with a probable molecular weight of 179 is tentatively identified as an anhydride, possibly, a nitrogen-containing phthalic anhydride.

The fractions from the parent fuels showed no mutagenic activity in the Ames assay. The mutagenic activity of the fractions from the diesel particles is shown in Figure 2. After fractionation mutagens were recovered in four fractions: acid, moderately polar neutrals, strongly polar neutrals, and aromatic hydrocarbons. Recoveries of mutagenic activity in the diesel fractions were low; however, standard reference mutagens, both direct and indirect, did not appear to be destroyed by the fractionation procedure. In particular, in the experiment with the 2-nitrofluorene spike, much of the mutagenic activity was recovered in the aromatic hydrocarbon and moderately polar neutral fractions. It is of interest to note that ∿50% of the recovered mutagenic

activity from the samples is not in the fractions in which the 2-nitrofluorene was concentrated but instead is in the highly polar neutral fraction.

The three most mutagenically active crude DCM extracts were derived from the diesel fuels that contained relatively high concentrations of aromatic compounds. No correlation was observed between increased mutagenicity and nitrogen content of the parent diesel fuels.

References

1. Wang, Y. Y., S. M. Rappaport, R. F. Sawyer, R. E. Talcott, E. T. Wei. 1978. Direct-acting mutagens in automobile exhaust. Cancer Letters 5: 39-47.

2. Yu, M. L., and R. A. Hites. 1981. Identification of organic compounds on diesel engine soot. Anal. Chem. 53:951-954.

3. Schuetzle, D., T. Riley, T. J. Prater, T. M. Harvey, D. F. Hunt. 1981. The identification of nitrated derivatives of PAH in diesel particulates. Anal. Chem., in press.

Table 1. Aromatic and Nitrogen Content of Diesel Fuels

	Aromatics (vol %)	Nitrogen (ppm)
DFM	29.9	5
Min. Qual.	34.6	240
BF+HAN+HN	30.8	718
BF+IQ	6.6	930
JP-7	2.7	<1

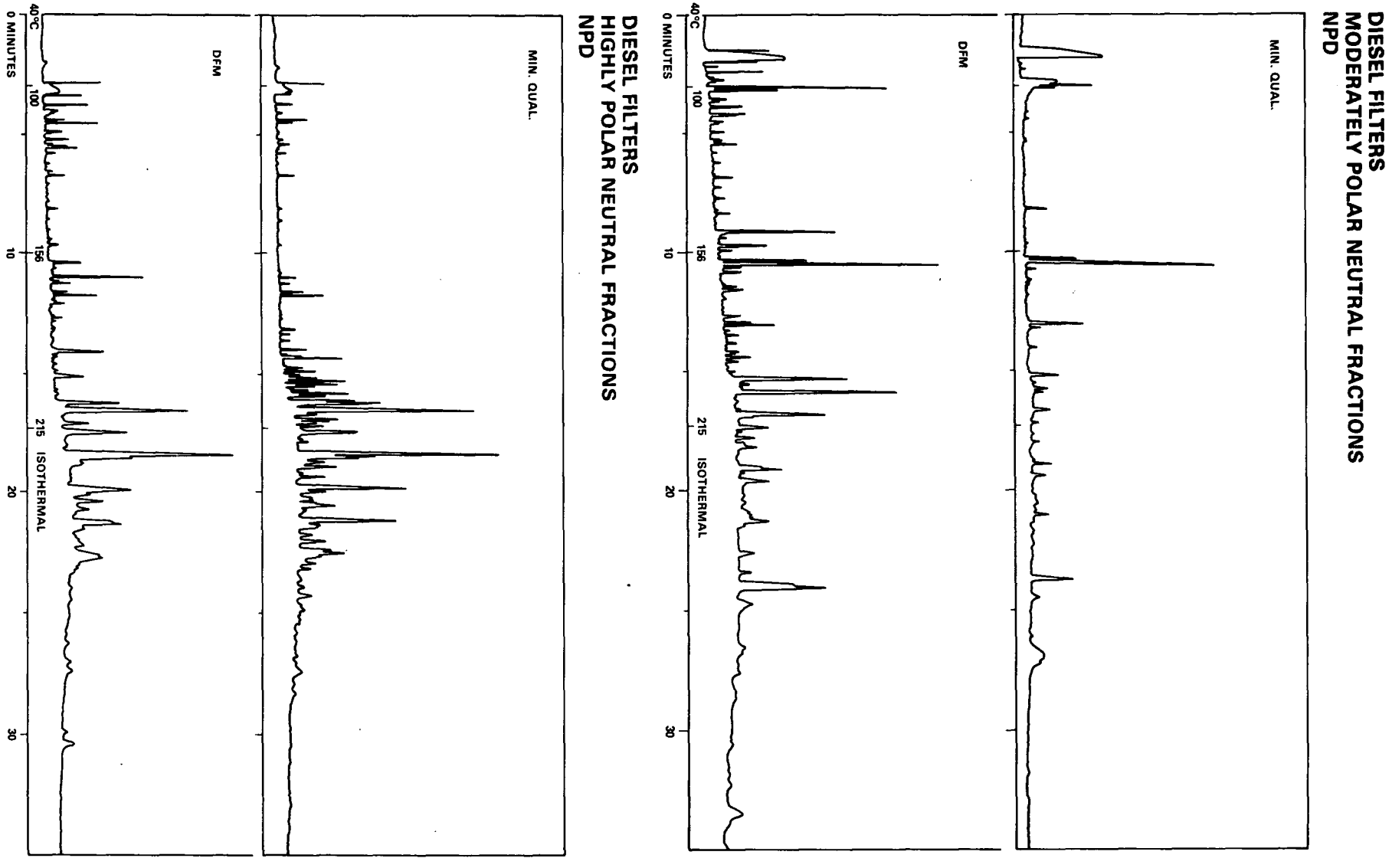


Fig. 1. Gas chromatogram with nitrogen selective detector of the moderately polar and highly polar fractions from two diesel particulate samples.

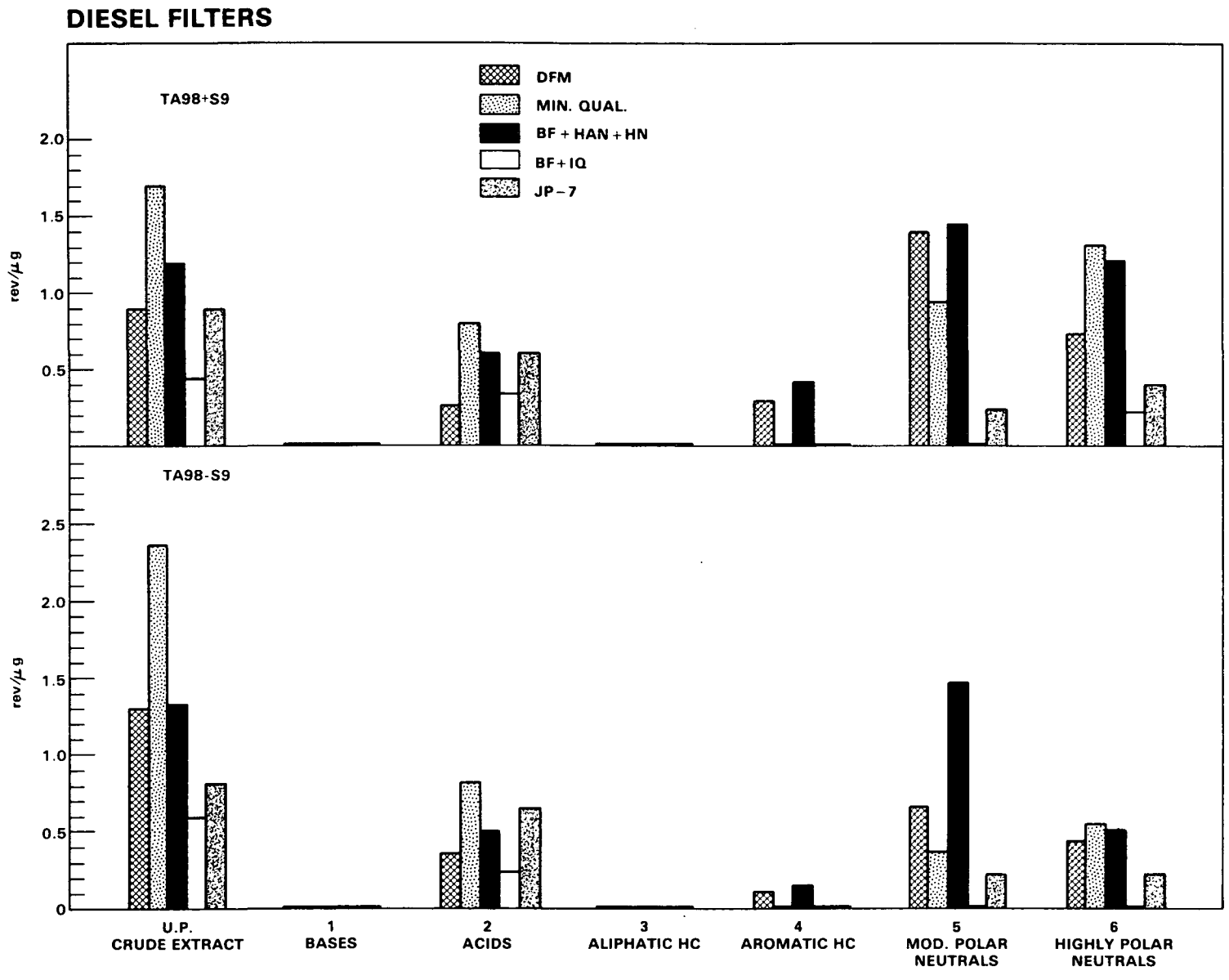


Fig. 2. Ames mutagenicity data of fractions from five diesel filters.

FRACTIONATION AND CHARACTERIZATION OF THE ORGANICS FROM DIESEL AND COMPARATIVE EMISSIONS

by

C. Sparacino, R. Williams, K. Brady
Research Triangle Institute
Research Triangle Park, North Carolina

R. Jungers
Environmental Protection Agency
Research Triangle Park, North Carolina

Semi-volatile materials were analyzed from various media including diesel soot, tar from coke oven residues, roofing tar and cigarette smoke condensate (CSC).

The diesel exhaust particles were collected from a 1978 Oldsmobile 350 diesel vehicle operated on the Highway Fuel Economy Test Cycle (HWFET) with No. 2 diesel (Union 76) fuel. The particles were collected on Pallflex T60A20 filters and the organics were removed by Soxhlet extraction with methylene chloride as previously described (1).

The 2RI Kentucky Reference cigarette smoke condensate was generated according to the method of Patel (1977) at Oak Ridge National Laboratory (1).

The coke oven main sample was collected from a separator located between the gas collector main and the primary coolers within a coke oven battery at Republic Steel in Gadsden, Alabama, about 60 miles northeast of Birmingham.

The roofing-tar sample was generated and collected using a conventional tar pot containing pitch-based tar, enclosed within a chamber and heated to 360°-380°F, a normal temperature for commercial use. The evaporative emissions were collected using a small bag house fitted with Teflon filter bags (1).

The solvents used to extract or condense the organics from each of these samples was removed by evaporation under nitrogen.

The nature and number of organic compounds associated with samples of the type addressed in this program render them among the most complex of environmental samples. At the present time no direct determinant approach, regardless of the resolving power, is available for routine characerization. The analytical problems can be minimized by pretreatment of the sample in order to distribute the sample compounds into fractions of similar chemical or physical properties. This class division provides useful information regarding the sample's overall make-up, yields convenient and meaningful subfractions for biotesting purposes, and lessens the analytical burden for ultimate characterization. This procedure is

based on work by Novotny et al., (2) which, after significant alteration, was used for this study. The entire scheme is depicted in Figure 1.

Each sample was subjected to this fractionation procedure to yield six fractions of various chemical properties and polarities. The acid fraction contains both weak (e.g., phenols) and strong (e.g., carboxylic acids) acids. The base fraction contains organic, Bronsted bases (e.g., amines). The neutral fraction is subdivided into 3 main fractions based on compound polarity. The non-polar neutral (NPN) fraction is comprised of compounds less polar than ∿ naphthalene. Paraffinic materials are characteristic of this fraction. The PNA fraction contains compounds of intermediate polarity, and is selective for condensed ring aromatics. All neutral materials with polarities greater than PNA hydrocarbons are found in the polar neutral (PN) fraction. Prior to the subfractionation of the neutral fraction, the latter must be dissolved in cyclohexane. All components are not soluble in this solvent. The insoluble material is collected as a separate fraction (CI), and is comprised of intermediate and highly polar compounds.

Spillover of various compounds into all fractions is a natural feature of solvent partitioning processes. Polar neutral material was removed from the PNA fraction by silica gel chromatography. The PNAs were chromatographed using gradient elution such that a fraction containing only PNA hydrocarbons was obtained (PNA-1). Other fractions (PNA 2-4) were collected that contained compounds of intermediate to high polarity.

Most fractions were directly analyzed by capillary GC/MS. The fractions enriched in polynuclear aromatic hydrocarbons (PNAs) were further purified by column chromatography, and the collected subfractions were analyzed by GC/MS. A large portion of each sample, after fractionation, was submitted to the EPA for biotesting. The remainder of each sample was used for all analytical work.

Approximately 1 g of each sample was partitioned. Recovery of material after application of the fractionation scheme was generally ca. 80%. Overall mass balances are shown in Table 1. The recovery for CSC was uncharacteristically low (47.6%). Extensive emulsions were not formed during partition; the formation of insoluble material upon dissolution in methylene chloride prior to fractionation may account for the low figure.

The mass distribution for each sample is shown in Table 2. These results represent approximate quantities since any solvent partition process is a rough separation method. The cyclohexane insoluble (CI) fraction contains significant proportions of material in some samples. This fraction is a measure of the amount of sample, after acid/base removal, that is not soluble in cyclohexane, and is therefore presumably polar neutral material. The CSC and coke oven samples both contain major quantities of such material.

The CSC sample contained a significant amount of organic bases, while the acid fraction was more important for the diesel soot extract. The large amount of non-polar neutral material associated with the diesel soot is not unexpected; aliphatic hydrocarbons are known to constitute a major proportion of such mixtures (3). The roofing tar sample is notable for the size of the PNA fraction.

The partition scheme proved effective in providing some fractions that were amenable to direct GC/MS analysis. Acid fractions require derivatization before comprehensive GC/MS analysis can be carried out. The bases, NPN, PNA and CI fractions can be successfully approached via GC/MS, although clean-up is required for some fractions. The PN fraction represents a very difficult analytical problem that probably requires LC/MS, derivatization, further fractionation, etc. The actual extent to which <u>any</u> fraction can be comprehensively analyzed by any GC technique is unknown. More work is clearly required in this area.

The partition scheme fractions showed, after GC/MS analysis, more or less the expected compositions. The acid fractions were shown to contain phenolic materials; carboxylic acids such as fatty acids were detected infrequently (derivatization required). The non-polar neutral fraction was, for all sample types, highly enriched in saturated and unsaturated aliphatic hydrocarbons. The compounds covered a molecular weight range corresponding to <u>ca</u>. C_{10}-C_{24} paraffins. The CSC fractions contained several plant natural products. The PNA fraction from CSC was too small to permit both adequate bioassay and complete analysis. The PNA fraction from all other samples showed the presence of PNAs and methylated PNAs containing 2-5 rings. Other compounds found were dibenzothiophene, dibenzofuran and oxygenated fluorenes.

The preparative chromatography of the PNA fraction provided separation of PNA hydrocarbons from the more polar contaminants of that fraction. The amount of material recovered in the chromatographic fractions (PNA 2-4) was usually quite small; only phthalate esters and unknown species were indicated. Based on similar chromatographic schemes (4), nitroarenes, oxygenated PNAs and non-basic nitrogen containing PNAs (e.g., carbazoles) would be expected constituents of these fractions.

The polar neutral fractions showed, for all samples, significant spillover of PNAs. Some oxygenated PNAs (e.g., benzanthrone, anthraquinones, hydroxyaromatics) were also identified in this fraction.

References

1. Huisingh, J. L., R. L. Bradow, R. J. Jungers, B. D. Harris, R. B. Zweidinger, K. M. Cushing, B. E. Gill, R. E. Albert. 1980 Mutagenic and Carcinogenic Potency of Extracts of Diesel and Related Environmental Emissions: Study Design, Sample Generation, Collection, and Preparation. EPA Reports (EPA Report EPA-600/9-80-057b, pp. 788-800.

2. Novotny, M., P.L. Lee, K.D. Bartle. 1974. J. Chrom. Sci. 12:606.

3. Rodriguez, C.F., J.B. Fisher, and D.E. Johnson. 1980. Health Effect of Diesel Engine Emissions: Proceedings of an International Symposium, Vol. 1. EPA-600/9-80-057a. U.S. Environmental Protection Agency: Cincinnati, OH.

4. Erickson, M.D., D.L. Newton, K.B. Tomer. 1980. Analytical Charactierization of Diesel Exhaust Particulate Extracts. Third Annual Report. EPA Contract No. 68-02-2767.

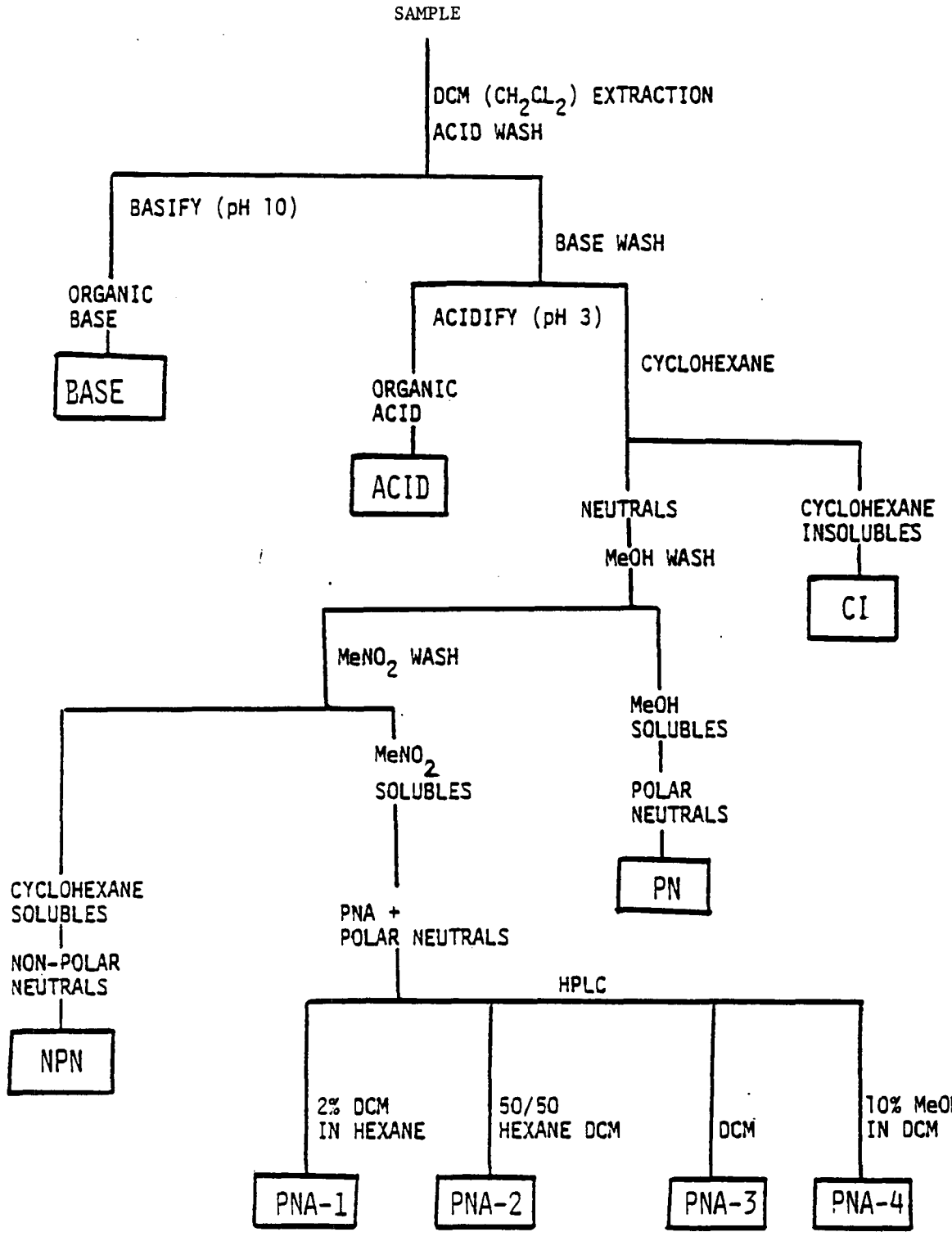
Table 1. Mass Balance Results from Fractionation of Comparative Samples

	Diesel Vehicle	Cigarette Smoke	Coke Oven	Roofing Tar
Amount fractionated (mg)	803.0	913.8	936.8	1071.2
Fractional totals (mg)	696.9	435.0	783.1	895.9
Mass balance (%)	86.8	47.6	83.6	83.6

Table 2. Percent of Total Mass Recovered Upon Fractionation of Comparative Samples

Fractions	Diesel Vehicle	Cigarette Smoke	Coke Oven	Roofing Tar
Acid	3.6	1.0	0.4	2.3
Base	1.0	12.1	4.3	2.9
PN	7.6	11.5	7.1	13.1
NPN	74.2	0.6	15.2	40.4
PNA1	1.4	0.02	4.9	31.8
PNA2	1.7	0.0	0.0	1.2
PNA3	0.9	0.1	4.0	0.02
PNA4	1.6	0.9	0.7	0.3
CI	7.8	73.7	63.3	8.0

Figure 1. SOLVENT PARTITIONING FRACTIONATION SCHEME



TRAPPING GASEOUS HYDROCARBONS

by

Fred Stump
Environmental Sciences Reserach Laboratory
U.S. Environmental Protection Agency
Research Triangle Park, North Carolina

The gas phase mutagen research was initiated at the request of the Office of Mobile Source Air Pollution Control for the development of a procedure to trap gas phase hydrocarbons (HC) and then to use the method in a series of studies with the objective of comparing the Ames Bioassay activity of the gas phase with the particle-bound vehicle emissions.

Several of the hydrocarbon absorbents in use by other researchers were procured for evaluation as to applicability for HC collection in the diesel fuel range. A thorough examination of the known physical characteristis of the mediums resulted in the selection of coconut charcoal and XAD-2 resin (divinylbenzene polystyrene polymer) as the best possible candidates.

Preliminary evaluations consisted of making injections of diesel fuel into a small dilution tunnel and trapping the hydrocarbons with subsequent recovery determinations proving both to be excellent HC absorbers but the XAD-2 was the better release medium. Although, several different solvents and solvent combinations were used as extraction agents to improve recovery efficiency from the charcoal, a recovery greater than 60% could not be achieved and work with charcoal was discontinued. Further recovery work showed the XAD-2 to be quantitative by both chromatographic and gravimetric analysis for diesel fuel range hydrocarbons. The recovery and qualification experiments were performed using A.D. Little (ADL) developed odor traps, 3/8" O.D. x 2" length, filled with the absorbents.

With completion of the qualification tests several samples were taken from both a 1978 Oldsmobile and a 1978 Datsun 220C diesel powered passenger car with the resin filled ADL traps. An extended sample time in the order of 5-10 hours was necessary, due to the low trap flow rate and limited resin capacity, to obtain sufficient materials for Ames Bioassay. This long sample time not only tied up equipment and personnel excessively but also posed some

question as to the possibility of artifact formation on the resin. In order to help clarify the artifact question, an experiment was conducted by making injections of diesel fuel and NO_x on resin filled traps (ADL) and then submitting the extract for Ames testing using the TA 98 strain. The test results indicated that considerable artifact, sample level in some situations, could be generated under these long exposure conditions and further hydrocarbon collecting with these tubes was discontinued.

To eliminate the low flow and the long sample time problems, a larger 2" x 2" trap with about 100 times the resin capacity of the ADL tubes was fabricated and carried through the same qualification experiments as the smaller traps with comparable recovery efficiencies (greater than 95%).

These large traps were then used to sample a series of different cycles (FTP's, NYCC, HWFET's) from a VW Rabbit diesel passenger car. These samples were submitted for Ames testing with the results showing the gas phase materials to be active but this activity was only 9-16% (depending on test cycle) of the particle-bound activity. Although, artifact formation is probably still present the trap activity data indicates that it had been substantially reduced.

These tests completed the diesel studies. Since the gasoline system had little NO_2 present, with possible minimum artifact formation, an XAD-2 trapping system, 20" x 20" x 2" bed, having 100 times (due to low gasoline emissions) the resin capacity of the 2" x 2" traps was fabricated to collect the hydrocarbons. A series of three gasoline powered vehicles were tested: (1) a 1972 Chevrolet Impala using unleaded fuel; (2) a 1981 Dodge van with a light duty catalyst system also using unleaded fuel; and (3) a 1970 Ford van using commercially available leaded fuel.

A sample from each of the vehicles was then submitted for Ames Bioassay testing with the XAD-2 trapping results being quite different from that observed in the diesel vehicle. The activity of the gaseous materials trapped by the XAD-2 was at background level for all three vehicles. The Dodge van particle-bound HC, without S9 activation, had about twice the activity in reverents/microgram as the Impala, and four times the activity of the Ford, with the higher Dodge activity density probably due to the oxidative properties of the emissions control system since the catalyst was the major parameter difference between the Dodge and Impala. The Ford had the lowest activity of the three vehicles and this could possibly be attributed to the lower fuel aromatic content (44.4% unleaded and 27.8% leaded).

A comparison of the diesel and gasoline vehicles activity (reverents/microgram) indicates that for the HWFET cycle,(the only cycle tested common to all vehicles) without activation the diesel and 1972 Chevrolet are about the same activity, the Dodge has twice the diesel activity, and the Ford about one half the diesel activity. Although the gasoline emissions have activities comparable on a revertent/microgram basis, when the emissions are observed on a reverent/mile basis, without activation, the worst of the gasoline vehicle emitters (Ford) has an activity that is only 11.8% and, with activation, only 4.5% of the diesel particle-bound activity.

These trapping studies have clearly indicated the low level of activity associated with the diesel gas phase hydrocarbons and the extremely low or background levels present in gasoline gas phase emissions.

The ability of XAD-2 to effectively collect diesel fuel range HC has been well demonstrated and studies are currently in progress to characterize the XAD-2 collectability of gasoline fuel range hydrocarbons.

ANALYTICAL METHODS FOR NITROAROMATIC COMPOUNDS

by

Silvestre B. Tejada
Mobile Source Emissions Research Branch
U.S. Environmental Protection Agency
Research Triangle Park, N.C. 27711

A number of methods have been used to detect and/or measure nitroaromatic compounds in environmental samples (1-5). The analysis usually involves a combination of fractionation schemes - solvent-solvent extraction, thin layer chromatography (TLC), open column chromatography, high performance liquid chromatography (HPLC) - and followed by analytical finish using TLC, HPLC with UV and fluorescence detection, gas chromatography (GC), and a variety of mass spectrometric (MS) techniques. Most of these methods are labor intensive and some are plagued by poor sensitivity and interference problems.

We have developed a reverse phase HPLC-fluorescence method using water-methanol solvent for the detection, identification and measurement of selected nitroaromatic compounds with sensitivity at low and sub-nanogram levels. The detection technique is based on on-column catalytic conversion of the non-fluorescent nitroaromatic compounds to the highly fluorescent amine analogs. Compound selectivity is achieved by appropriate choice of wavelengths for fluorescence measurements. Stop-flow techniques and spectral scanning of the trapped peaks were used to establish chemical identify by comparison with spectra of standard samples.

The heart of our analytical system is a platinum-rhodium catalyst column (maintained at 60-80 degrees Celsius) between two reverse phase ODS columns. Initial separation is achieved in the first ODS column, reduction to amine analog is immediately accomplished in the catalyst column and the final analytical separation of the aminoaromatic compounds from interferring components is achieved in the second ODS column. By allowing only selected amino-aromatic peaks through the second ODS column, we have managed to conveniently eliminate the tedious sample clean-up prior to analysis. Figures 1 and 2 illustrate the use of this technique in the analysis of nitro-pyrene. Precision of ± 3% at 1 nanogram level is routinely obtained for nitro-pyrene analysis. Minimum detectable quantity of nitropyrene under our present analytical configuration is about 20 picograms.

The catalyst has been observed to reduce nitro compounds to the corresponding amine reproducibly under fixed conditions of flow rate, temperature and solvent composition. The following nitroaromatic compounds are converted with better than 99% conversion efficiency: nitro-naphthalenes, nitro-anthrance, nitro-fluorene, nitro-chrysene, nitro-BaP, dinitro-pyrenes and nitro-fluorenones. We have obtained fluorescence spectra of the amine analogs of most of the nitroaromatic compounds available to us. The amines, especially the diamines, were observed to be unstable under UV light. Adjustment of the solvent pH to about 8 with NaOH helped to stabilized the diamines adeqately to make reproducible spectral scans of the trapped peaks.

The present analytical system has been used to measure nitro pyrene in complex matrices such as diesel exhaust particulate extracts, leaded and non-leaded automotive exhaust particulate extracts, gas trap extracts, fly ash extracts as well as biological extracts. Samples dissolved in DMSO intended for Ames tests are likewise amenable to analysis without additional sample clean-up. Other nitroaromatic compounds can be detected and measured by appropriate choice of chromatographic elution windows coupled with the optimum wavelengths for fluorescence measurements.

REFERENCES

1. Jager, J., "Detection and characterization of nitro derivatives of some polycyclic aromatic hydrocarbons by fluorescence quenching after thin layer chromatography: Application to air pollution analysis", J. Chrom. 152, 575-578 (1978).

2. Schuetzle, D., Lee, F.S. -C., Prater, T.J., Tejada, S.B., "The Identification of polynuclear aromatic hydrocarbon derivatives in mutagenic fractions of diesel particulate extracts", Intern. J. Environ. Anal. Chem. 9, 1-53, (1981).

3. Gibson, T.L., Ricci, A. I., Williams, R.L., "Measurement of Polynuclear Aromatic Hydrocarbons, Their Derivatives and Their Reactivity in Diesel Automobile Exhaust" in "Chemical Analysis and Biological Fate: Polynuclear Aromatic Hydrocarbons", Cooke, M. and Dennis, A.J., eds., Battle Press, 1981, p.707.

4. Schuetzle, D., Riley, T., Prater, T.J., Harvey, T.M. and Hunt, D., " The Identification of Nitrated Derivatives of PAH in Diesel Particulates", in press.

5. Rozenkranz, H.J., McCoy, E.C., Sanders, D.R., Butler, M., Kiriazides, D.K., Mermelstein, R.,"Niropyrenes: Isolation, identificatin, and reduction of mutagenic impurities in carbon black and toners", Science 202, 515-519 (1978).

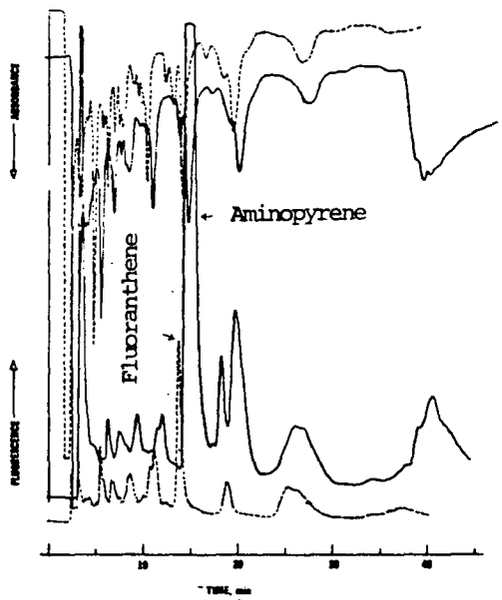


Figure 1

Neat diesel extract (25 ug) through ODS column only (——); through ODS and catalyst columns (——). Note peak enhancements due to formation of aminocompounds. Detection wavelengths: Excitation (360 nm), Emission (430 nm), UV (254 nm).

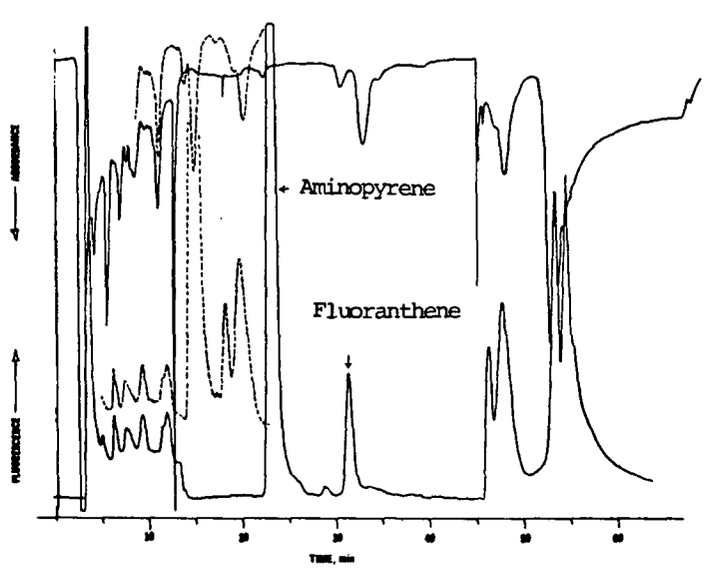


Figure 2

Neat diesel extract (25 ug) through ODS-Catalyst-ODS columns. Only the main aminopyrene peak was injected into the second ODS column. (——) Uv and fluorescence profiles of the sample through ODS-Catalyst columns. Note removal of interferrent peaks after passage through second ODS column.

TOTAL LUMINESCENCE SPECTROSCOPY OF DIESEL EXHAUST PARTICULATE

by

Gregory Wotzak, Ph.D.
Cleveland State University
Cleveland, Ohio

Robert Whitby, P.E.
New York State Department of Environmental Conservation
Division of Air
Albany, New York

Total Luminescence Spectroscopy (TLS) analysis of organic extract material from diesel exhaust particulate matter has been previously described by Wotzak et al[1]. TLS is the determination of the luminescence intensity as a function of all accessible excitation and emission wavelengths. TLS data are typically obtained from between 50 and 200 emission spectra, each taken at a specific excitation wavelength. Contour mapping of the points of equal luminescence intensity on an excitation vs. emission wavelength grid has been chosen as a convenient means of representation for TLS data. TLS analysis thus encompasses the computerized data acquisition, manipulation, display, and interpretation of such luminescence data.

The New York State Department of Environmental Conservation, Automotive Emission Evaluation (AEE) unit has been studying the utilization of TLS in the characterization of diesel particulate organic extract using a Baird Corporation SFR-100 Ratio Recording Spectrofluorometer. Data acquisition and scan control for this instrument is locally provided by a Baird MP-100 microprocessor controller which are linked to a host Data General Nova 3 computer system with 128K words of core and a 10 megabyte disk. Software was provided by Baird Corporation and modified by AEE computer personnel to include data smoothing algorithms. Contour plots are produced on a Houston Instruments Complot™ X-Y recorder.

More recent advancement in raw data reduction has been achieved by wavelength correction and digital smoothing using Fast Fourier Transform methods. Extract samples, fractionated by column chromatography, have been analyzed, generating TLS contour maps for each fraction. These fraction contours may be added by computer software routines, using appropriate weighting by cut recovery factors, and compared with spectra for the original bulk sample.

This reconstitution work was performed as an internal consistency test for both the acidic, basic and neutral cuts of the raw extract, as well as the sub-cuts of the neutral fraction.

A variety of tasks have been performed in order to obtain a general indication of the utility of this relatively new analytical procedure. Sequential dilutions of extract sub-fractions were performed in order to determine the extent of internal absorption of fluorescent radiation. TLS spectra of the diesel fuel and lubricant were subtracted from appropriate spectra in order to facilitate further analysis, and determine the partition of unburned fuel and lubricant among extract sub-fractions. The neutral fraction was analyzed qualitatively and quantitatively for several known compounds.

(1) Wotzak, G., R. Gibbs, and J. Hyde, 1980, A Particulate Characterization Study of In-Use Diesel Vehicles. In: Health Effects of Diesel Engine Emissions: Proceedings of an International Symposium, Volume 1. W.E. Pepelko, R.M. Danner, and N.A. Clarke, editors. U.S. Environmental Protection Agency, EPA-600/9-80-057a. pp. 113-137.

EVALUATION OF THE METABOLIC REQUIREMENTS OF DIESEL AND COMPARATIVE SOURCE SAMPLES IN THE *SALMONELLA TYPHIMURIUM* PLATE INCORPORATION ASSAY

by

Katherine Williams and Joellen Lewtas
Genetic Toxicology Division
Health Effects Research Laboratory
U.S. Environmental Protection Agency
Research Triangle Park, North Carolina

The mutagenic activity of a mobile source sample (Nissan diesel exhaust extract) and three comparative source samples (coke oven mains, cigarette smoke condensate, and roofing tar extract) were examined in the Ames plate incorporation assay using strain TA98. The mutagenic and carcinogenic activity of these complex mixtures had previously been determined in a bioassay test matrix (1). The comparative sources were less mutagenic in the Ames assay compared to the mobile source sample. However, they were more active in mammalian cell mutation and mouse tumor initiation bioassays. The objective of this study was to determine whether modifications in the S9 activation system would alter the mutagenic activity in the Ames bioassay. The modifications examined included altering both the species from which the liver S9 was prepared and the concentration of S9 on the plate.

Both Aroclor 1254-induced and -uninduced S9 were prepared from CD rats and Syrian golden hamsters. Livers were pooled from groups of at least 6 male animals for each preparation. The protein concentration of each S9 was determined by the method of Lowry et al. (2). Four doses of S9 were run: 0.31, 0.63, 1.25, and 2.5 mg/plate, chosen to encompass the usual concentration in the Ames test (ca 1.5 mg/plate). Aliquots were adjusted to contain the required dose in a 0.5-ml volume as used in the Ames test.

Three doses of each source sample (a low, medium, and high range) were tested at all four S9 concentrations for each S9 preparation. The dose varied between samples and were selected to be below the toxic level for each, yet high enough to have mutagenic activity. For Diesel Nissan and cigarette smoke, the doses were 30, 100, and 300 μg/plate. For coke oven mains and roofing tar, they were 5, 50, and 100 μg/plate.

Experiments were run in duplicate, on different days, using triplicate plates for each point. In any experiment all 3 doses of 2 samples were tested with all 4 concentrations of both uninduced and induced S9 from one species. For each experiment fresh dilutions were prepared from aliquots of the S9

preparations, which were held at -80°C. The time between duplicate experiments ranged from 3 to 9 days. The data is presented as the mean ± SE for duplicate experiments.

The optimum S9 dose for all samples, with the exception of the Diesel Nissan which did not require metabolic activation, was either 1.25 or 2.5 mg/plate for both induced and uninduced rat and hamster S9, as shown in Table 1 for the induced rat liver S9.

Recently, several studies have noted that induced hamster S9 is more effective than induced rat S9 in activating such compounds as aromatic amines (3) phenacetin (4) and diethylnitrosamine (5), while Aroclor-induced rat S9 is more effective than hamster S9 with polycyclic aromatic hydrocarbons (3). This study showed no difference in effectiveness between hamster S9 and rat S9 in activating the Diesel or the comparative source samples.

The mutagenic activity of the comparative source samples was higher when Aroclor-induced S9 was the metabolic activator as compared to uninduced S9, whether rats or hamsters were the source of the S9. The Diesel Nissan sample was again the exception; the mutagenic activity was higher with uninduced S9 than with induced.

In conclusion, for the three comparative source samples, all of which require metabolic activation for maximum mutagenic activity in the Ames test: the optimum S9 dose is 1.25 to 2.5 mg/plate; Aroclor-induced S9 is more effective as an activator than uninduced S9 regardless of species; and rat and Syrian golden hamster S9 are equally effective in activating these complex mixtures. These results would suggest the lower mutagenic activity of the comparative source samples in the Ames test as compared with other mutagenicity or carcinogenicity bioassays was not due to the exogenous metabolic activation system.

REFERENCES

1. Nesnow, S., and J.L. Huisingh. 1980. Mutagenic and carcinogenic potency of extracts of diesel and related emissions: Summary and discussion of the results. In: Health Effects of Diesel Engine Emissions. Proceedings of an International Symposium, Vol. 2. W.E. Pepelko, R.M. Danner, and N.A. Clarke, eds. EPA-600/9-80-057b. U.S. Environmental Protection Agency: Cincinnati, OH. pp. 898-912.

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

3. Raineri, R., J.A. Poiley, R.J. Pienta, and A.W. Andrews. 1981. Metabolic activation of carcinogens in the Salmonella mutagenicity assay by hamster and rat liver S9 preparations. Environ. Mutagen. 3:71-84.

4. Nagao, M., T. Sugimura, and T. Matsushima. 1978. Environmental mutagens and carcinogens. Ann. Rev. Genet. 12:117-159.

5. Prival, M.J., V.D. King, and A.T. Sheldon. 1979. The mutagenicity of diabyl nitrosamines in the Salmonella plate assay. Environ. Mutagen. 1:95-104.

Table 1. Effect of Metabolic Activation Dose in Mutagenicity of Diesel and Comparative Source Samples[a]

Sample	Revertants/plate[b]			
	0.31[c]	0.63[c]	1.25[c]	2.5[c]
Diesel Nissan	816 ± 26	625 ± 39	491 ± 50	334 ± 31
Coke oven mains	492 ± 57	727 ± 8	861 ± 21	874 ± 27
Cigarette smoke	68 ± 0	83 ± 4	74 ± 6	64 ± 3
Roofing tar	59 ± 1	86 ± 2	94 ± 3	113 ± 9

[a]Salmonella typhimurium TA98. Samples at 100 μg/plate.
[b]Mean ± SE of two experiments with triplicate plates.
[c]Rat Aroclor 1254-induced S9, mg/plate.

MS/MS CHARACTERIZATION OF DIESEL PARTICULATES

by

Karl V. Wood
Fuels Analysis Laboratory-Chemistry Building
Purdue University
West Lafayette, Indiana 47907

James D. Ciupek and R. Graham Cooks
Department of Chemistry
Purdue University
West Lafayette, Indiana 47907

Colin F. Ferguson
School of Mechanical Engineering
Purdue University
West Lafayette, Indiana 47907

INTRODUCTION

Analysis of the organic constituents adsorbed on diesel exhaust particulates has become increasingly important with the increase in the number of diesel engine automobiles. Extraction followed by GC/MS has been the usual analytical method of choice for these unknown organic constituents. Mass spectrometry/mass spectrometry (MS/MS) offers a possible means of direct analysis with either minimal or no separation. This technique enables a fast characterization of the organic constituents of the whole diesel particulates. Selectivity and specificity of particular compounds or classes of compounds of interest using MS/MS can be improved by the appropriate choice of the chemical ionization reagent gas as well as the choice of positive or negative ion detection.

EXPERIMENTAL

The MS/MS experiments described in this study were run using a Finnigan triple stage quadrupole mass spectrometer.[1] The diesel particulate sample is introduced into the source with the direct insertion probe which is heated in steps to obtain temperature profiles of the organics adsorbed on the particulates. The sample is ionized using the chemical ionization technique. The ion of interest is mass selected by quadrupole 1, undergoes collisionally induced dissociations in quadrupole 2 with the resulting

fragment ions being mass analyzed with quadrupole 3. Typcial ion axial energies into quadrupole 2 are 20 eV relative to the source. The collision gas used for these studies was argon at a pressure ca. 2.2 mTorr.

The diesel engine employed in this study was an AVL model[2] 520.005 naturally aspirated single cylinder direct injection engine. The diesel exhaust particulates are sampled using a mini dilution tunnel system.[3] As this system was designed both the dilution ratio and the temperature of the particulate filter can be varied.

RESULTS

The initial emphasis of this study was to identify constituents of diesel exhaust particulates by direct analysis using MS/MS. This identification is done by comparing the MS/MS spectrum of a particular ion in the diesel exhaust particulate sample with the MS/MS spectrum of the corresponding ion of a standard reference compound. For example, the MS/MS spectrum of the m/z 143 ion from a diesel exhaust particulate sample has two major fragment ions, m/z 128 (100%) m/z 115 (15%) besides the main beam ion m/z 143 (20%). These ion ratios are nearly identical to that found in the MS/MS spectrum of 2-methylnaphthalene, suggesting its presence in the diesel exhaust particulate sample. It is not possible to say which methylnaphthalene is present, if only one is, or the relative concentration of the two in a probable mixture of both. The case of the m/z 143 ion suggesting the presence of predominately only one constituent and its positional isomers is not unique. However, as would be expected the MS/MS spectra of many ions are suggestive of the presence of more than one type of structure. An example of this is the ion at m/z 139 in the diesel exhaust particulate sample which is strongly indicative of the presence of hydroxybenzoic acid isomers through comparison with standard reference compounds. However, there are other relatively intense fragment ions in the MS/MS spectrum which cannot be resulting from hydroxybenzoic acid. One other compound that may account for the remaining fragment ions is decalin.

Along these lines is the investigation of the selectivity and specificity of the MS/MS technique as it relates to the direct analysis of diesel exhaust particulates. For example the comparison of positive and negative ion isobutane chemical ionization of a diesel exhaust particulate sample can yield information about specific classes of compounds. An example of this is the identification of carboxylic acids in the diesel exhaust particulate samples. In positive ion chemical ionization a carboxylic acid will be protonated as will an aromatic hydrocarbon at the same nominal mass. However, in negative ion chemical ionization the carboxylic acid will lose a proton to give a $(M-H)^-$ ion whereas the aromatic hydrocarbon will be ionized by electron transfer to give a M^- ion. This technique was used to confirm the identification of the components in the m/z 139 ion MS/MS spectrum discussed previously. Thus the use of negative ion chemical ionization allows the separation of different classes of compounds for MS/MS identification in the direct analysis of diesel exhaust particulates.

Another example of selective ionization to allow a more accurate MS/MS identification to be made is the use of differing reagent gases. While

isobutane chemical ionization is a general protonating agent, ammonia can be used to protonate relatively basic compounds, like amines or aza compounds. While this technique has been used successfully in the analysis of coal-derived liquids[4] it has not been as useful with diesel exhaust particulates.

Besides these studies, two variables associated with sampling the diesel exhaust particulates have also been investigated. These variables, dilution ratio and particulate filter tmperature were utilized to gain a better insight into the complex problem of particulate sampling.

CONCLUSION

MS/MS provides a means for the rapid direct analysis of diesel exhaust particulates. The use of selective ionization techniques further enhances the positive identification when different types of compounds are present at the same nominal mass.

REFERENCES

1. Slayback, J.R.B. and M.S. Story. 1981. Chemical Analysis Problems Yield to Quadrupole MS/MS. Industrial Research FEB; 129-134.

2. Pischinger, R. and W. Cartellieri. 1972. Combustion System Parameters and Their Effect Upon Diesel Engine Exhaust Emissions. SAE Paper 720756.

3. MacDonald, J.S., S.L. Plee, J.B. O'Arcy, and R.M. Schreck. 1980. Experimental Measurements of the Independent Effects of Dilution Ratio and Filter Temperature on Diesel Exhaust Particulate Samples. SAE Paper 730834.

4. Zakett, D., V.M. Shaddock and R.G. Cooks. 1979. Analysis of Coal Liquids by Mass Analyzed Ion Kinetic Energy Spectrometry. Anal. Chem. 51:1849-1852.

INHALATION TOXICOLOGY OF DIESEL EXHAUST PARTICLES

by

R. O. McClellan, A. L. Brooks, R. G. Cuddihy, R. K. Jones,
J. L. Mauderly and R. K. Wolff
Lovelace Inhalation Toxicology Research Institute
P. O. Box 5890
Albuquerque, NM 87185

Studies of the inhalation toxicology of diesel exhaust particles (DEP) have been directed toward answering several inter-related questions. Does exposure of laboratory animals or people to high levels of DEP place them at increased risk for developing health effects? If health effects are observed, what are the mechanisms by which DEP produces health effects? If health effects are observed, does consideration of the specific kinds of health effects and the mechanisms by which they are produced provide a basis for extrapolating these health effects to exposure levels of DEP likely to be encountered in occupational or environmental settings?

With these questions in mind, an idealized experimental approach to assessing the toxicity of inhaled DEP will be presented. Using this approach as a reference point, the current state of our knowledge on the toxicity of DEP will be reviewed. This will include consideration of the deposition and retention of DEP; dissociation, detoxification and activation of organic species associated with DEP; the effective dose of the organic species to various tissues of the respiratory tract and other organs; and carcinogenic and other effects produced by exposure to DEP. Each of these areas will be reviewed to determine the adequacy of our current knowledge and to identify information needs that have not yet been adequately addressed relative to answering the questions that have been posed.

ACKNOWLEDGEMENTS

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U.S. ENVIRONMENTAL PROTECTION AGENCY'S INHALATION TOXICOLOGY STUDY

by

William E. Pepelko
Toxicology Division
Health Effects Research Laboratory
U.S. Environmental Protection Agency
Cincinnati, Ohio

Due to increasing use of diesel engines in passenger cars and light trucks, the U.S. Environmental Protection Agency (EPA) over the past several years has undertaken an investigation of the health effects of diesel engine emissions. This program included studies in the areas of epidemiology, bioassays of collected diesel particulate matter, and animal inhalation studies. The inhalation studies described are those carried out by the EPA Health Effects Research Laboratory at Cincinnati, Ohio. The types of studies include an assessment of the effects of inhaled diesel engine emissions upon a variety of toxicological, carcinogenic, and heritable mutagenic endpoints. Nonheritable mutation will be described elsewhere.

Due to limitations on chamber space resulting from the wide variety of planned experiments, a single exposure level was selected. Because of the Agency's interest in carcinogenic and mutagenic risk assessment and because of its plans to use linear non-threshold models to make risk assessments, an exposure level near the maximum chronically tolerated concentration was selected. After a two-month preliminary study to aid in estimating tolerance levels, an exhaust concentration was selected such that the total suspended particulate matter equaled 6 mg/m^3. This was achieved by a daily adjustment of the dilution ratio which was usually near 18:1. After about one year of exposure, it became apparent that this concentration was not producing overt signs of toxicity, such as decreased food consumption and body weight gain. As a result, a decision was made to decrease the exhaust dilution ratio to about 9:1, and to increase the particulate concentration to 12 mg/m^3. The particulate concentration was maintained at this level until completion of exposures, approximately 14 months later.

The animals were housed in wire cages and exposed in 24 100 cubic-foot stainless steel chambers. Exhaust was produced by a 6-cylinder 198 cubic-inch displacement Nissan diesel engine developing 90 horsepower. City driving conditions were simulated by operating the engine under the Federal Short Cycle mode. The engine was operated 8 h/day, 7 days/week. Carbon dioxide, carbon monoxide, nitrogen oxides, nitrogen dioxide, sulfur dioxide, total hydrocarbons, total suspended particulate matter, temperature, and relative

humidity were monitored regularly. Aliphatic aldehydes, ammonia, and sulfates were measured periodically.

A wide range of experiments were carried out during the 124 weeks the engines were operated. Carcinogenesis studies included lung tumor induction in Strain "A" mice and Syrian hamsters, tumor induction in SENCAR mice treated with promoters and initiators, and liver island assays. No conclusions could be reached from the hamster study due to the loss of a large number of animals from Tyzzer's disease during the exposure.

The other three studies have been completed and will be reported on at this conference. The effects of exhaust exposure on heritable mutations was investigated using mice and fruit flies. These studies were reported at the 1979 Diesel Health Effects Symposium held at Cincinnati. Other toxicological endpoints studied included reproductive effects in mice; teratological effects in rats and rabbits; pulmonary function changes in mice, Chinese hamsters, and cats; lung pathology in mice, rats, hamsters, and cats; behavioral and neurophysiological effects in rats; biochemical alterations in the lungs of mice, rats, and cats; resistance to infection in mice; deposition and clearance in rats; and enzyme induction in mice. The majority of these experiments have been completed and many were reported on at the previous symposium. Two that will be presented here include pulmonary function changes in cats exposed to diesel exhaust for two years and the enzyme induction studies. Two other experiments, a multigeneration reproduction study in mice and detailed morphometric analysis of chronically exposed cats' lungs, are not yet completed.

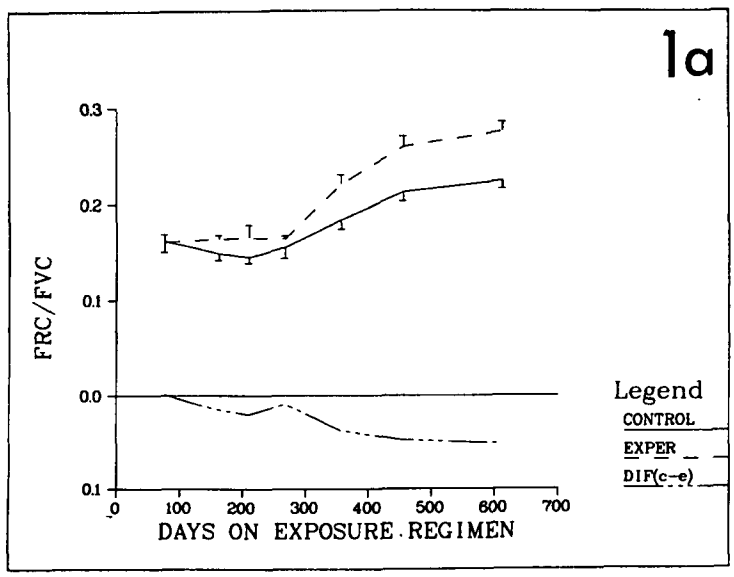
Following completion of these studies, no further inhalation exposures are planned at EPA's Cincinnati facility.

PULMONARY FUNCTION TESTING OF RATS CHRONICALLY EXPOSED TO DILUTED DIESEL EXHAUST FOR 612 DAYS

K. B. Gross
Biomedical Science Department
General Motors Research Laboratories
Warren, MI 48090

Diesel engine emissions contain particulate matter composed of a multitude of organic compounds, and of a size that may be readily inhaled and retained by the lung. It thus has the potential for interacting with the deep lung, and possibly altering the structure and function of this organ. The study was designed to address the question of whether the chronic inhalation of diluted diesel exhaust may affect pulmonary function. Twenty-five Fischer-344 rats were exposed to diesel exhaust, diluted with clean air at a ratio of 1:15 (particulate concentration = 1500 $\mu g/m^3$) for 20 hrs/day, 5-1/2 days/week, for 612 days. Twenty-five control animals were treated in a similar manner, but exposed to clean filtered air. Noninvasive pulmonary function testing, which produced no apparent harmful effects, were performed on the animals at mean times of 11, 23, 30, 38, 51, 65, and 87 weeks on the exposure regimen. Animals were anesthetized (Halothane, 5%), transorally intubated, and placed in a plethysmograph. Measurements of lung volumes and flows were made while the animals were spontaneously breathing and during forced expiratory maneuvers. Functional residual capacities were computed using the Boyles law principle. All data was normalized by each animal's own forced vital capacity in order to compensate for animal growth and interindividual variability. Through the first year of testing, no significant differences between the two groups were found for any of the measured parameters. During the second year of exposure, seven of the eighteen measured indices displayed a significant difference as indicated by analysis of variance. The normalized functional residual capacity (FRC) and its component volumes - expiratory reserve (ER) and residual volume (RV) - maximum expiratory flows at 40% (MEF_{40}) and 20% (MEF_{20}) of the lung volume remaining, and the forced expiratory volume in 0.1 sec ($FEV_{.1}$) were all greater in the diesel-exposed animals. The normalized inspiratory capacity (IC) was significantly larger in the control group, but the test point of greatest difference for this parameter does not exceed 4%, and at all other test points is 2% or less. The statistical significance of the IC is interpreted as being the result of the very low variability of this parameter, rather than a result of a clinically important change in the pulmonary function. The significantly larger values for relative normalized FRC, ER, and RV in the diesel-exposed animals could be indicative of chronic obstructive lung disease and are similar to the changes seen in other studies in which an emphysema-like condition was induced in rats by intratracheal instillation of elastase, as well as in reported clinical data on chronic obstructive lung disease. However, this interpretation of the changes in the three lung volumes is contradicted by the MEF_{40}, MEF_{20}, and $FEV_{.1}$ values. If chronic lung disease was occurring, these parameters would be expected to decrease in the

diesel-exposed animals compared to the controls. The fact that they increased in the diesel group, suggesting an improvement in airway caliber, is not consistent with what would be expected based on studies reported in the literature for human pulmonary disease. In conclusion, the majority of the measured parameters did not differ significantly between the control and diesel-exposed groups, and while one cannot exclude the possibility that the differences that were observed in this experiment between the diesel-exposed and clean air controls may be attributable to the chronic inhalation of the diesel exhaust, the results are not consistent with documented clinical findings on chronic lung disease.



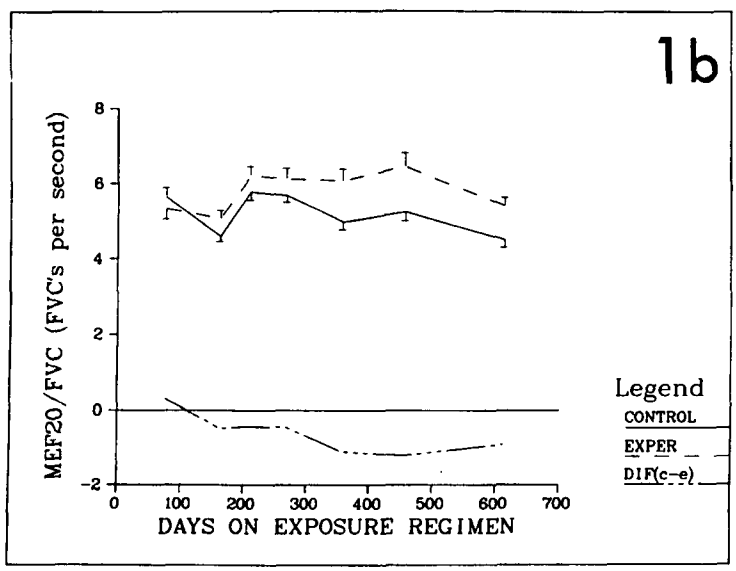


Figure 1a Functional residual capacity (FRC) of diesel exposed (-----) and control (———) animals as a fraction of each animal's forced vital capacity (FVC). The bottom curve (— - - - —) is the difference between the means of the two groups (control - experimental).

Figure 1b Normalized maximum expiratory flow rate at 20% of vital capacity (MEF_{20}). Legend same as Figure 1a.

PULMONARY FUNCTIONAL RESPONSE IN CATS FOLLOWING TWO YEARS OF DIESEL EXHAUST EXPOSURE

William J. Moorman and John C. Clark
National Institute for Occupational Safety and Health
Division of Biomedical and Behavioral Science
4676 Columbia Parkway
Cincinnati, OH 45226

and

William E. Pepelko and Joan Mattox
U.S. Environmental Protection Agency
Health Effects Research Laboratory
Cincinnati, OH 45268

INTRODUCTION:

Both NIOSH and the EPA have responsibilities to assess potential health effects from exposure to diesel engine exhaust. These responsibilities are mandated under both the OSH and Clean Air Acts, respectively. While EPA's responsibility relates to the general population, NIOSH's responsibility is to the worker, especially those at high exposure risk in the mining and transportation industries.

The advantages of diesel power are manyfold; however, the prime motivation for the use of diesels relates to the reduced cost of operation due to longer engine life, decreased maintenance, cheaper fuel, increased specific energy (2.4 X that of gasoline), and increased safety. The increase in safety is related to diesel fuel's lower volatility, flash point with a concomitantly reduced explosive hazard when compared to gasoline. As a result of our proximal locations in Cincinnati, the NIOSH, DBBS Cardiopulmonary Laboratory participated with EPA-HERL Laboratory to apply comprehensive pulmonary function testing to the cats exposed to the controlled diesel atmospheres.

BACKGROUND:

Evaluation of pulmonary function responses to experimentally controlled diesel exhaust is primarily confined to investigation of the past few years. Previous studies are largely epidemiologic and while they are important guides, they lack qualitative and quantitative characterization, essential for analytical, dose-response toxicology.

At the last symposium on Health Effects of Diesel Engine Emissions, Gross reported pulmonary function findings in Fischer 344 rats following 38 weeks of exposure at 1500 $\mu g/m^3$ for 5-1/2 days/week, 20 hrs/day(1). He found no differences in mechanical properties, lung volumes, or dynamic ventilatory performance. In a later report presented at the Society of Toxicology meeting (1980), he found higher function residual capacity and maximum expiratory

flow rates at 20% of vital capacity in the diesel exposed group. These results are not interpretable or consistent with dysfunction. His conclusion was that no clinically important alterations were observed after 65 weeks of exposure(2).

In another report, O'Neil et al., reported on functional and morphological consequences of diesel exhaust exposure in mice following 3 months of exposure to a 1:18 dilution (diesel and air) of diesel exhaust with particulate levels of 6.4 mg/m^3. He found no statistically significant findings in lung volume or diffusing capacity(3).

In a third report on functional responses in rodents, Vinegar et al., described significant decrements in vital capacity, residual volume, and diffusing capacity in Chinese hamsters exposed to a dilution of diesel exhaust with a particulate level of 6.4 mg/m^3(4).

In the only larger animal exposure, we reported findings from one year of exposure in cats(5). The exposure was conducted at the Cincinnati EPA, HERL laboratory. A 1:18 dilution diesel exhaust with 6.4 mg/m^3 was used. Our results indicated no response in mechanical properties, lung volumes, distribution, diffusing capacity or ventilatory performance following one year of exposure.

METHODS:

In an effort to enhance functional response characteristics, the diesel exposure was increased by decreasing the dilution ratio from 1:18 to 1:9 after the first year. A summary of exposure is provided in Table I. During this second year, 19 of the original 21 male cats were exposed to 11.7 mg/m^3 particulate, 4.37 ppm nitrogen dioxide, 5.03 ppm sulfur dioxide, and 33.30 ppm carbon monoxide with total hydrocarbons of 7.72 ppm. Twenty male cats served as controls. All cats were young adult males obtained from Liberty Laboratories and were born and maintained in a disease-free environment and inbred for several generations. They were of uniform size (3.63 ± 0.46 kg) and within two weeks of the same age.

Prior to pulmonary function testing, the cats were fasted for one day. The testing followed 18-20 hours of no diesel exposure. On a random schedule, the cats to be tested were anesthesized with ketamine and acepromizine at a dose of 42 mg/kg. Following induction of the anesthesia, an esophageal balloon was placed in the lower third of the esophagus and an 18-22 F endotracheal tube (largest possible) was inserted into the trachea with the aid of a laryngoscope. All testing, except compliance (CL) and resistance (RL) was performed with the cats placed in the prone position in a variable pressure plethysmograph. CL and RL tests were performed with the cats recumbent to facilitate measurement of transpulmonary pressure. Figure I shows the general pulmonary testing situation diagrammatically.

Pulmonary mechanics were obtained from simultaneous volume, flow and transpulmonary pressure. Dynamic compliance (CL_{dym}) was measured from volume and transpulmonary pressure at points of no flow. Average flow resistance ($RL_{ave.flow}$) was measured from change in transpulmonary pressure at equal

volumes, divided by the sum inspiratory and expiratory flow. All mechanics were obtained while the cats were spontaneously breathing (15-25 breaths/min). The pulmonary function tests requiring breathing maneuvers [lung volumes, forced expiratory flows (FEF% Vol), diffusing capacity ($DL_C{}^{18}{}_O$), nitrogen washout (ΔN_2), and closing volume (CV)] were performed using a variable pressure plethysmographic chamber previously described(6). The methods of Brashear et al.(7) and Mitchell et al.(8) were combined to obtain values for $DL_C{}^{18}{}_O$ and total lung capacity (TLC). The calculations for $DL_C{}^{18}{}_O$ were performed according to the methods described by Wagner et al., for $C^{18}O$(9). All gas analyses were done using a respiratory mass spectrometer (Perkin-Elmer MGA1100). Distribution was studied using the single-breath nitrogen washout and closing volume adapted from the human methods described by Buist and Ross(10). All data was tested statistically by nonparametric, Kruskal-Wallis one-way rank analysis of variance.

RESULTS:

Following the first year of exposure, no significant differences were found in mechanical properties, diffusing capacity, uniformity of distribution or ventilatory performance. In contrast to the negative findings following the first year, we now have clearly defined responses at the end of two years. Table II presents all parameters studied for control and exposed cats contrasting the values for one and two years.

The reduction in inspiratory capacity, vital capacity, and total lung capacity with normal values for ventilatory function (mechanics of breathing) indicates that a lesion is present which restricts breathing but does not cause airway obstruction or loss of elasticity. This restrictive disease found in this study is compatible with a diagnosis of pulmonary fibrosis of the interstitial or intraalveolar type. Concurrent status may include chronic inflammation, interstitial edema, or vascular engorgement. Additional support for the diagnosis of interstitial disease is the finding of impaired diffusing capacity. Distribution of this disease appears nonuniform as indicated by the significantly elevated nitrogen washout values for the exposed group.

DISCUSSION:

Pathological description of pulmonary responses to diesel exhaust at similar concentrations has been previously characterized(11,12). The observations include: (1) marked accumulation of black pigment laden macrophage in the interstitum localizing around blood vessels and respiratory bronchioles; (2) hyperplasia of the alveolar lining cells with focal thickening of the interstitium; (3) interstitial pneumonitis; (4) traces of, or no emphysema or peribronchiolitis. While the pathological examination of the cats' lung not complete, the above description is consistent with our physiologic finding of restrictive lung disease.

REFERENCES:

1. Gross, K.B. 1980. Pulmonary Function Testing of Animals Chronically Exposed to Diluted Diesel Exhaust. Presented at the Environmental Protection Agency International Symposium on Health Effects of Diesel Engine Emissions. Cincinnati, Ohio.

2. Gross, K.B. 1981. Noninvasive Pulmonary Function Testing of Fischer 344 Rats Chronically Exposed to Diluted Diesel Exhaust for Fifteen Months. The Toxicologist. Vol. 1, No. 1.

3. O'Neil, J.J. et al., 1980. Functional and Morphological Consequences of Diesel Exhaust Inhalation in Mice. Presented at the Environmental Protection Agency International Symposium on Health Effects of Diesel Engine Emissions. Cincinnati, Ohio.

4. Vinegar, A., et al. 1980. Pulmonary Function Changes in Chinese Hamsters Exposed Six Months to Diesel Exhaust. Presented at the Environmental Protection Agency International Symposium on Health Effects of Diesel Engine Emissions. Cincinnati, Ohio.

5. Pepelko, W.E., et al. 1980. Pulmonary Function Evaluation of Cats After One Year of Exposure to Diesel Exhaust. Presented at the Environmental Protection Agency International Symposium on Health Effects of Diesel Engine Emissions. Cincinnati, Ohio.

6. Moorman, W.J., et al. 1975. Maximum Expiratory Flow Volume Studies on Monkeys Exposed to Bituminous Coal Dust. J. Appl. Physiol. 39:444-448.

7. Brashear, R.E., et al. 1966. Pulmonary Diffusion and Capillary Blood Volume in Dogs at Rest and With Exercise. J. Appl. Physiol. 21:520-526.

8. Mitchell, N.M., et al. 1968. Application of the Single-Breath Method of Total Lung Capacity Measurement to the Calculation of Carbon Monoxide Diffusing Capacity. Am. Rev. Resp. Dis. 97:581-584.

9. Wagner, P.D., et al. 1971. Diffusing Capacity and Anatomic Dead Space for Carbon Monoxide [$C^{18}O$]. J. Appl. Physiol. 31:817-852.

10. Buist, A.S., et al. 1973. Quantitative Analysis of Alveolar Plateau in the Diagnosis of Early Airway Obstruction. Am. Rev. Resp. Dis. 108:1078-1087.

11. Wiester, M.J., et al. 1980. Altered Function and Histology in Guinea Pigs After Inhalation of Diesel Exhaust. Environ. Res. 22:285-297.

12. Karagiones, M.T., et al. 1981. Effects of Inhaled Diesel Emissions and Coal Dust in Rats. Am. Ind. Hyg. Assoc. J. 42:382-391.

Table I. EXPOSURE CHAMBER COMPONENT CONCENTRATIONS, STUDY AVERAGES

		Weeks #1-61	Weeks 62-124
Dilution Factor (air:diesel)	DF	18.16 ± 1.72:1	9.37 ± 1.13:1
Particulate Mass, mg/m^3	M	6.34 ± 0.81	11.70 ± 0.99
Nitrogen Oxides	NO_x		
Nitric Oxide, ppm	NO	11.64 ± 2.34	19.49 ± 3.80
Nitrogen Dioxide, ppm	NO_2	2.68 ± 0.80	4.37 ± 1.19
Sulfur Dioxide, ppm	SO_2	2.12 ± 0.58	5.03 ± 1.03
Total Hydrocarbons, ppn	THCcorr.	4.15 ± 0.97	7.22 ± 0.85
Carbon Monoxide, ppm	CO	20.17 ± 3.01	33.30 ± 2.94
Carbon Dioxide, %	CO_2	0.30 ± 0.04	0.52 ± 0.04

Table II. PULMONARY FUNCTION PARAMETERS COMPARING THE CONTROL GROUP TO THE DIESEL EXPOSED GROUP AFTER 1 YEAR AND 2 YEARS

Mechanical Properties	One Year		Two Years	
	Exposed	Control	Exposed	Control
$CL_{dyn.}$	23.5 ± 7.2	23.7 ± 9.3	27.5 ± 4.9	26.2 ± 7.1
$RL_{ave.flow}$	10.7 ± 4.6	10.3 ± 4.4	5.6 ± 3.2	5.7 ± 2.3
Lung Volumes				
TLC	415 ± 56.0	449 ± 74.5	428 ± 56.3↓*	484 ± 68.3
FVC	348 ± 43.5	368.9 ± 42.1	369 ± 42.3↓*	410 ± 57.6
FRC	158 ± 35.6	165 ± 42.2	145 ± 26.2↓*	163 ± 36.9
ERV	69 ± 24.6	67 ± 19.0	79 ± 24.0	83 ± 34.5
RV	86 ± 36.9	104 ± 37.7	67 ± 14.3	80 ± 28.2
RV/TLC%	20.3 ± 6.9	22.7 ± 5.9	15.6 ± 1.9	16.4 ± 4.5
IC	279 ± 44.8	301 ± 49.6	291 ± 44.1↓*	328 ± 58.6
Ventilatory Performance				
$FEV_{.5\%}$	84.3 ± 8.4	81.6 ± 6.4	86.9 ± 6.1	86.9 ± 5.9
PEFR	1016 ± 185	1042 ± 174	887 ± 98 ↓*	952 ± 110.7
FEF_{50}	728 ± 196	761 ± 160	802 ± 125	864 ± 121
FEF_{25}	490 ± 186.8	481 ± 199.5	518 ± 154	574 ± 153
FEF_{10}	196 ± 107.4	222 ± 156.8	223 ± 109	234 ± 102
$FEF_{40\%}TLC$	486 ± 252.6	557 ± 248.0	586 ± 173	625 ± 213
Diffusion				
DLCO	1.18 ± .43	1.22 ± .40	0.89 ± .27 ↓*	1.01 ± .14
Distribution and Closing Volume				
$\%N_2/25\%/VC$	0.32 ± .20	0.29 ± .30	0.39 ± .27 ↑*	0.21 ± .181
CV	25.6 ± 13.4↓*	36.0 ± 16.1	27 ± 17.6	25 ± 19.3

*Statistically significant $P < 0.05$

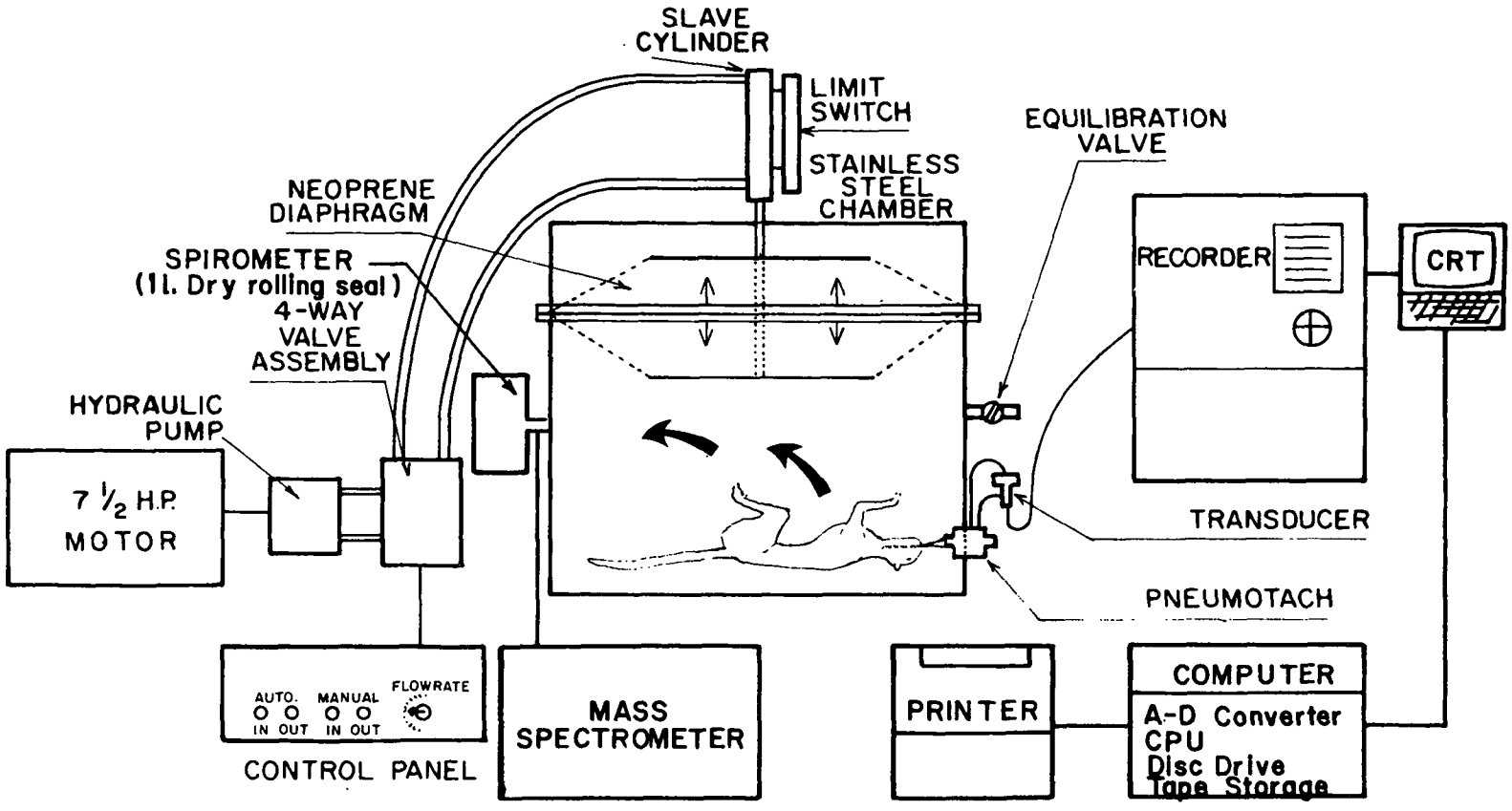


Diagram of Pulmonary Function Lab.

DEPOSITION AND RETENTION OF SURROGATE AND ACTUAL DIESEL PARTICLES

by
R. K. Wolff, L. C. Griffis, G. M. Kanapilly and R. O. McClellan
Lovelace Inhalation Toxicology Research Institute
P.O. Box 5890
Albuquerque, New Mexico 87185

INTRODUCTION

Data on deposition and retention of diesel particles are an important need in assessing their toxicological impact. Deposition and retention experiments were carried out with ^{67}Ga aggregate aerosols (surrogate diesel particles) in Beagle dogs to provide good estimates for human deposition. Experiments were also carried out with these particles in Fischer-344 rats to provide comparative information in a small laboratory animal and also to estimate lung burdens in chronic exposures to diesel exhaust. A method was developed to quantitate actual diesel soot burdens in rats exposed to diesel exhaust. These values were found to be comparable to these predicted from the surrogate particle deposition experiments.

METHODS

Aggregated particles of $^{67}Ga_2O_3$, 0.02 and 0.1 μm volume median diameter (VMD), were produced using heat treatment of ^{67}Ga tetramethylheptanedione using methods described previously (1). Ten Beagle dogs from the Institute's colony were exposed for 1/2 hr in a nose-only exposure unit equipped with a plethysmograph for pulmonary function monitoring. Whole body counting and gamma camera analysis were used to measure total amounts of activity deposited and their regional distribution. A total of 144 Fischer-344 rats were exposed 5 hrs/day for either 1 or 3 days in the same style multi-tiered exposure chamber used in the chronic diesel exhaust exposure study. Whole body counting and also sacrifice and tissue counting methods were used to measure deposited radioactivity.

The diesel exhaust exposure system consisted of an Oldsmobile 5.7l diesel engine connected to a dynamometer with the engine load and speed determined by an analog control system. The engine was operated on a 7 mode urban cycle and the fuel was U.S. Department of Energy Reference fuel 8007 (Phillips Chemical Co.). The entire engine exhaust was diluted in a large stainless steel tunnel and then further diluted in 3 stages with filtered air which produced average particulate concentrations of 4150, 990 and 200 $\mu g/m^3$. The diluted exhaust was drawn through the 2.2 m^3 exposure chambers (Hazleton Systems, Inc., Aberdeen, MD) at 560 l/min. The exposure schedule was 7 hrs/day, 5 days/week.

Laboratory reared Fischer-344 rats were 12-13 weeks old at the initiation of the study. They were exposed for a cumulative period of 541 hours over 18 weeks. At the end of this period 8 rats from each exposure group were sacrificed. Lungs were removed, homogenized, and centrifuged to produce a cell pellet. This tissue pellet was dissolved in 1 ml H_2O and 2 ml tetramethylammonium hydroxide. The remaining "soot" particles were suspended in 5 ml H_2O. Light absorbance at 690 nm was measured and compared against standards prepared from known weights of diesel particles collected on filters from the dilution tunnel of the inhalation exposure system. For these collections the same engine cycle was used as for the animal exposures.

RESULTS AND DISCUSSION

Table 1 shows the mean total and regional deposition values measured in the Beagle dogs for the 0.02 and 0.01 μm $^{67}Ga_2O_3$ particles. Deposition was higher in all compartments for the 0.02 μm particles. Despite the overall high variability in deposition 9 of the 10 dogs had higher deposition at 0.02 μm than 0.1 μm and the difference was statistically significant ($P < .05$). Although most of the material was deposited in the pulmonary region, deposition in the nasopharyngeal, and tracheobronchial regions was becoming increasingly significant as particle size decreased. Figure 1 shows pulmonary deposition of the 0.02 and 0.1 μm particles is in good agreement with the trends in deposition observed previously in humans (2,3) and Beagle dogs (4) at larger particle sizes. The deposition values are lower than predicted by the ICRP Task Group on Lung Dynamics (5) but are in good agreement with predictions by Yeh and Schum (6) and also Yu (7).

Deposition of the 0.1 μm $^{67}Ga_2O_3$ particles was somewhat lower in rats than had been found in dogs. Lung deposition (bronchial and pulmonary) was estimated to be 15 ± 3%. This estimate was based on measured lung burdens and assuming minute volumes as measured in free standing Fischer-344 rats at this Institute. Absolute lung deposition was found to be 2.4 μg/hr for a particle mass concentration of 1000 μg/m^3. Using these initial deposition values and a measured lung half-life of 75 days for $^{67}Ga_2O_3$, lung burdens could be calculated for various exposure periods. Table 2 shows the predictions for the exposure period and concentrations experienced by the animals in the chronic diesel exhaust exposures.

Measured diesel soot burdens following the 18 week exposure are also shown in Table 2. The observed values were in good agreement at the highest exposure level but overestimated deposition at the two lower levels. Either deposition was higher or clearance was slower at the high exposure level compared to the lower levels.

The degree of agreement between predicted and observed burdens shows that deposition and retention behavior of the surrogate particles is similar to actual diesel particles. The observed long-term retention of $^{67}Ga_2O_3$ in rats was very similar to the 62-day half-time reported by Chan, et al., (8) following acute exposures to ^{14}C-labeled diesel particles. These observations give confidence to extrapolations made from observations with

0.1 μm $^{67}Ga_2O_3$ aggregate particles. These data do show that pulmonary deposition is relatively high for diesel particles, and retention times are relatively long.

ACKNOWLEDGEMENTS

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REFERENCES

1. Wolff, R.K., G.M. Kanapilly, P.B. DeNee, and R.O. McClellan. 1981. Deposition of 0.1 μm chain aggregate aerosols in Beagle dogs. J. Aerosol Sci. 12:119-129.

2. Lippmann, M. 1977. Regional Deposition of Particles in the Human Respiratory Tract. Handbook of Physiology, Section A: Reactions to Environmental Agents, The American Physiological Society, Chaper 14, pp. 213-232.

3. Chan, T.L., and M. Lippmann. 1980. Experimental measurements and emperical modelling of the regional deposition of inhaled particles in humans. Am. Ind. Hyg. Assoc. J. 41:399-409.

4. Cuddihy, R.G., D.G. Brownstein, O.G. Raabe, and G.M. Kanapilly. 1973. Respiratory Tract Deposition of Inhaled Polydisperse Aerosols in Beagle Dogs. J. of Aerosol Sci., 5, 35-43.

5. Task Group on Lung Dynamics. 1966. Deposition and Retention Models for Internal Dosimetry of the Human Respiratory Tract. Health Physics, 12, p. 173-207.

6. Yeh, H.C., and G.M. Schum. 1980. Models of Human Lung Airways and Their Application to Inhaled Particle Deposition. Bulletin of Mathematical Biology, 42, pp. 461-480.

7. Yu, C.P. 1978. A Two-Component Theory of Aerosol Deposition in Lung Airways. Bulletin of Mathematical Biology, 40, p. 693-706.

8. Chan, T.L., P.S. Lee, and W.E. Hering. 1981. Deposition and clearance of inhaled diesel exhaust particles in the respiratory tract of Fischer rats. J. Appl. Toxicol. 1:77-82.

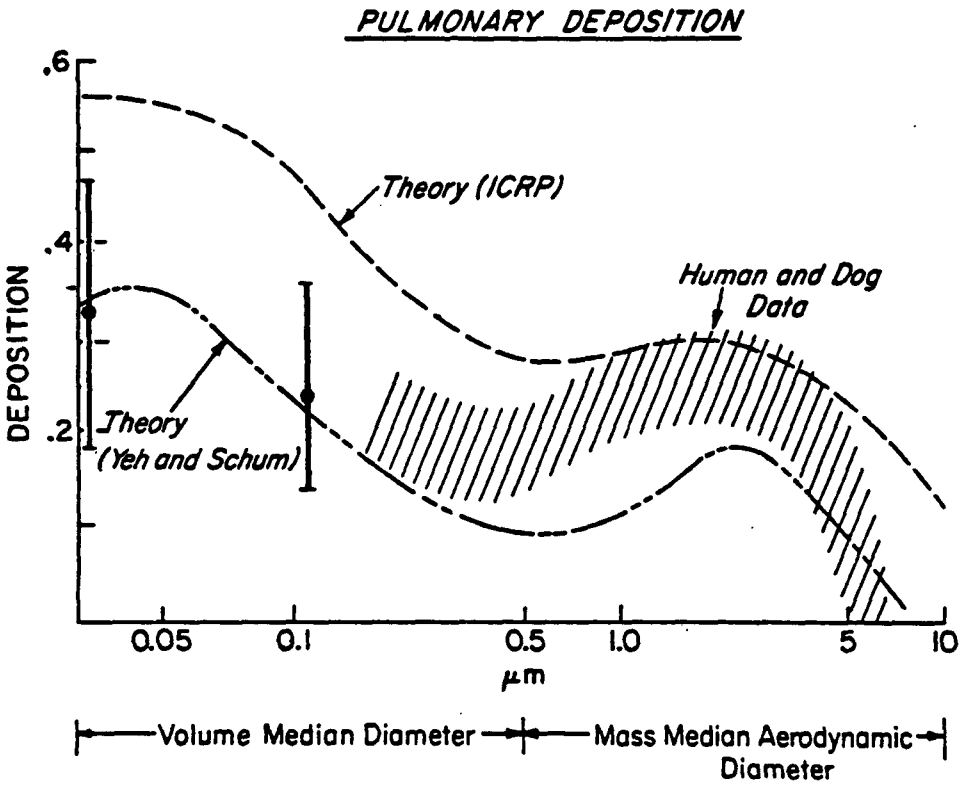


Figure 1. Comparison of mean pulmonary deposition (± S.D.) of 0.1 μm irregularly shaped polydisperse aerosols (●) with that of spherical monodisperse aerosols. The mid range of deposition data (////) taken from human experiments (Lippmann 1977 (2); Chan and Lippmann, (3)) and dog experiments (Cuddihy et al. 1973, (4)) is shown. Also shown are theoretical predictions for depositions in humans by ICRP Task Group on Lung Dynamics (5) and also by Yeh and Schum (6).

Table 1. Comparison of Total and Regional Deposition of $^{67}Ga_2O_3$ Particles in Beagle Dogs

Compartment	Particle Size	
	0.1 μm	0.2 μm
Nasopharyngeal	7%	9%
Tracheobronchial	7%	12%
Pulmonary	25%	32%
TOTAL	39%	53%

Table 2. Lung Burdens of Diesel Soot in Rats One Day After 18 Weeks Exposure to Diluted Exhaust

Average Aerosol Concentration[a] (μg/m^3)	Lung Burden (μg)	
	Predicted	Observed
200 ± 70	100	36 ± 8
990 ± 390	500	224 ± 39
4150 ± 1460	2100	1926 ± 335

[a] ± S.D. of average daily values.

LUNG CLEARANCE OF RADIOACTIVELY LABELLED INHALED DIESEL EXHAUST PARTICLES

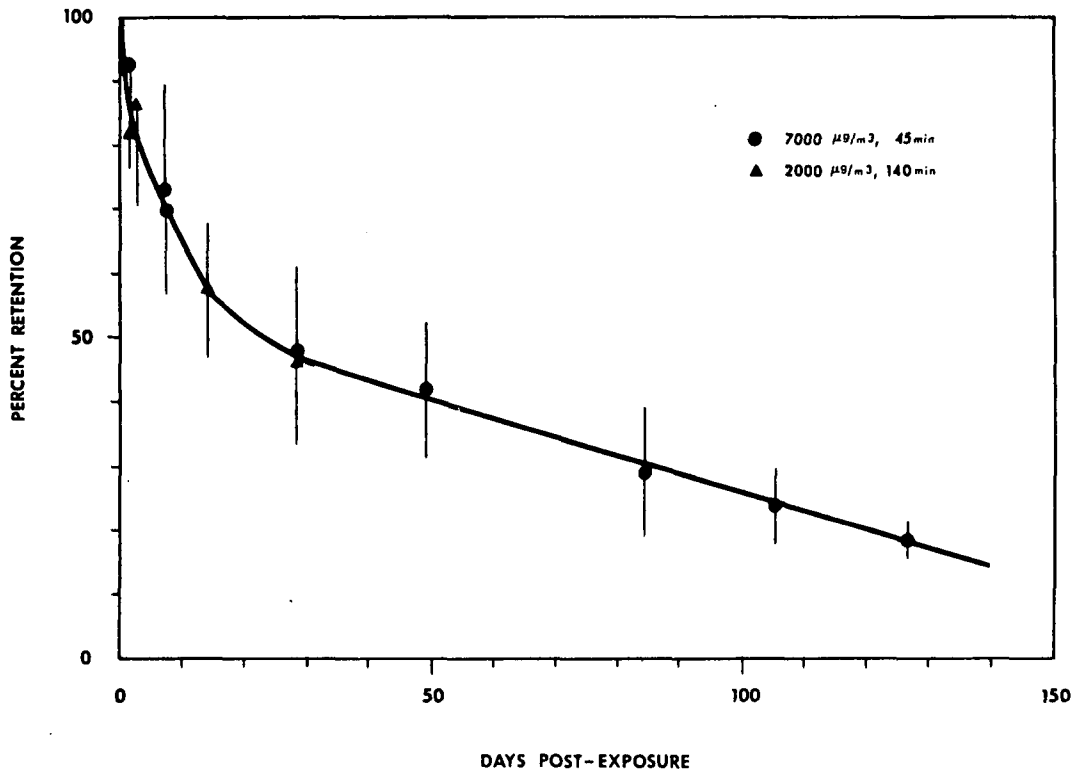
P. S. Lee, T. L. Chan, and W. E. Hering
Biomedical Science Department
General Motors Research Laboratories
Warren, MI 48090

The fate of inhaled diesel exhaust particles was studied in male Fischer 344 rats using radioactive diesel particles generated from a single cylinder diesel engine and tagged in the insoluble particulate core with radioactive ^{14}C. The particle size, extractability and ^{14}C distribution in the diesel exhaust were characterized at various engine load conditions. At full load, only 1% of the radioactivity of diesel particles was extractable by dichloromethane and the mass median aerodynamic diameter of the particles was 0.12 μm [J. Appl. Tox., 1(2):77-82, 1981]. Radioactive carbon dioxide was removed from the exhaust by a diffusion scrubber prior to exposures via a "nose-only" inhalation chamber. Rapid elimination of the inhaled $^{14}CO_2$ from the blood in the expired air, and urine of test animals indicated that the correction for increased radioactivity due to the inhaled carbon dioxide was necessary only for the initial deposition measurement. The amount of blood and its contribution of $^{14}CO_2$ activity was accounted for in the excised organs.

Test animals were exposed to diluted diesel exhaust at two particulate concentrations with similar total inhaled dose (7000 μg/m^3 for 45 minutes, and 2000 μg/m^3 for 140 minutes) and had comparable deposition efficiencies. After the exposure, the animals were housed in a clean air environment and the clearance of the radioactively tagged particles was determined over an extended period of time. Up to the period of 28 days after exposure, no significant difference in particle clearance has been observed. This indicates that thus far, the differences in the concentration of inhaled particles did not cause any significant alteration in the alveolar clearance process after a single, short-term inhalation exposure, at least within the studied concentration range.

The retention of inhaled particles in animals exposed to radioactive diesel exhaust at 7000 μg/m^3 particulate concentration has been investigated, thus far, to 126 days after the exposure. The particle retention data (Figure 1), analyzed by a curve stripping procedure, indicated three components with approximate half-times of 1 day, 8 days, and 80 days, respectively. The biological meaning of these components can be understood in terms of clearance mechanisms. The first mechanism deals preferentially with particles deposited in the tracheobronchial tree and represents their rapid transport by the mucociliary escalator. The particles are finally cleared through the gastrointestinal tract, and their elimination is clearly documented by the presence of ^{14}C activity in the feces. The second mechanism is interpreted as the transport of material deposited in the most proximal respiratory bronchioles, where only a short

distance is required for transferring the particulates to the mucociliary escalator. The third mechanism removes the particulate matter from the alveolar region, and the clearance mechanism may involve endocytosis, passive and active absorption, and dissolution or metabolism. The extended data base in continuing studies is expected to provide further information on the presence of additional clearance phase(s) of inhaled diesel particles and to assess their clinical significance.



Clearance of inhaled diesel exhaust particles in Fischer 344 rats. The vertical lines represent standard deviations.

COMPARTMENTAL ANALYSIS OF DIESEL PARTICLE KINETICS IN THE RESPIRATORY SYSTEM OF EXPOSED ANIMALS

S. C. Soderholm
Biomedical Science Department
General Motors Research Laboratories
Warren, MI 48090

One key element in assessing the potential health effects of diesel engine emissions is determining to what extent inhaled particles deposit in the respiratory system and how long they remain before being cleared. The deposition and clearance of diesel particulate is also of interest as an example of a seldom studied interaction between the lung and submicron particles which are assumed to be insoluble. Several investigators have collected data relevant to the deposition and clearance of diesel particulate in animal models. The development of a model of the kinetics of insoluble submicron particles in the respiratory system should provide a basis for organizing, explaining, and comparing these experimental data.

A review of available information on the deposition, transport, and clearance of insoluble submicron particles suggests a model with compartmental divisions illustrated in Figure 1. The model consists of a set of differential equations specifying the rate of transport of particulate mass among compartments. The kinetics are assumed to be first order, since it is the simplest assumption which can be made and since it leads to exponential clearance curves, the type found experimentally.

The major conceptual difference between this model of particulate kinetics in the lung and some previous ones [1,2] is that the model describes a wide range of experimental inhalation conditions, and provides compartments and parameters that apply even to exposures using nearly maximum tolerated doses. Although the ICRP clearance model includes a nasopharyngeal compartment and emphasizes particle dissolution, these features were not considered in the present model because they are irrelevant to the character of insoluble diesel particulates. Inclusion of the "free particulate" compartment does not significantly influence the fit to experimental data, but serves as a reminder that particulate resides on the lung surface for a short time.

General solutions of the model for two types of experiments are presented. In a Type I ("post-exposure") experiment, the animals are exposed for only a short period, and their changing particulate lung burdens are measured starting at the end of the exposure. In a "long exposure" experiment, the animals are exposed continuously or nearly continuously over a long period, and particulate lung burdens are periodically measured.

The major contribution of the particulate kinetics model to the analysis of the experimental data is to point out that the long-term clearance phase may be

interpreted in two ways: it may be clearance of particulate out of the deep lung with a half-time of 62 days as originally interpreted, or it may be a combination of deep lung clearance and sequestering. The amount of particulate in the lungs of chronically exposed animals suggested a linear build-up over time and indicated that after excessive exposure some portion of the deposited particulates may be retained in the lung for longer times. Consequently, the new model emphasizes that the slope of the buildup may be related to the clearance half-time from the "macrophage" to the "sequestered particulate" compartment, to the overall clearance rate from the "macrophage" compartment, and to the deep lung particulate deposition rate.

The application of the model has so far shown that no single set of model parameters fit all the data available from all experimental approaches. The differences may be related to deficiencies in the model or to actual differences in the respiratory system's response to particulate in the different exposure situations.

REFERENCES

1. Task Group on Lung Dynamics (Paul E. Morrow, Chairman), Deposition and retention models for internal dosimetry of the human respiratory tract. Health Physics, 12:173, 1966.

2. R. G. Thomas, An interspecies model for retention of inhaled particles. Assessment of Airborne Particles, W. Stober et al, eds., Springfield, IL. Charles C. Thomas, p. 405, 1972.

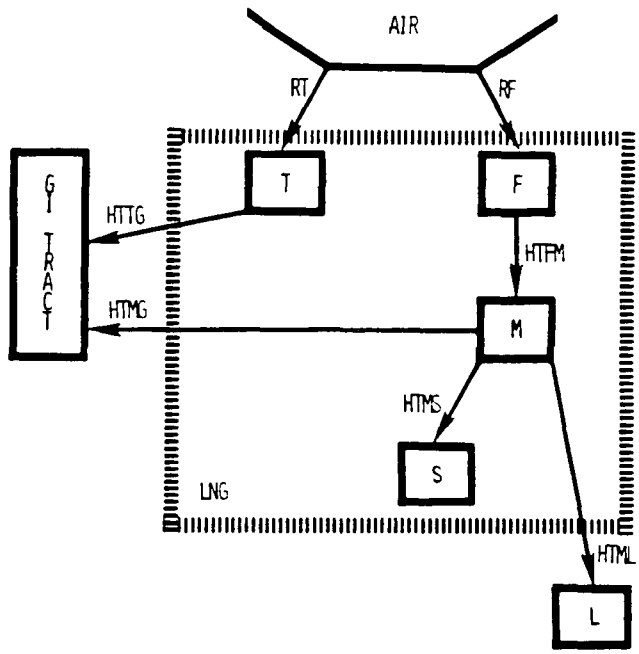


Figure Compartments and parameters in the model.

Compartments:

T = "tracheo-bronchial"
F = "free particulate" on deep lung surfaces
M = "macrophages" and other scavenger cells
S = "sequestered particulate"
L = "lymph nodes" draining lung
G = "GI tract"
LNG = "total lung"

Parameters:

RT = deposition rate into T
RT = SDT * C
SDT = specific deposition rate into T
SDT = DET * VM
DET = deposition efficiency of compartment T
C = concentration of airborne particulate
RF = deposition rate into F
RF = SDF * C
SDF = specific deposition rate into F
SDF = DEF * C
DEF = deposition efficiency of compartment F
VM = minute volume
HTxy = clearance half-time from compartment "x" to compartment "y"

A SUBCHRONIC STUDY OF THE EFFECTS OF EXPOSURE OF THREE SPECIES OF RODENTS TO DIESEL EXHAUST

by

Harold L. Kaplan, Ph.D.
Department of Fire Technology
Southwest Research Institute
San Antonio, Texas

William F. MacKenzie, D.V.M.
Department of Comparative Medicine
University of Texas Medical School
Houston, Texas

Karl J. Springer
Department of Emissions Research
Southwest Research Institute
San Antonio, Texas

Richard M. Schreck, Ph.D.
and
Jaroslav J. Vostal, M.D., Ph.D.
Biomedical Science Department
General Motors Research Laboratories
Warren, Michigan

A subchronic study of toxicologic and carcinogenic effects of inhalation exposure of rodents to diesel exhaust was conducted in preparation for a 15-month chronic investigation of three dose levels of the exhaust. More than five hundred rodents, consisting of male Fischer 344 rats, Syrian hamsters and Strain A/J mice, were exposed to diluted diesel exhaust containing 1500 $\mu g/m^3$ of particulate 20 hours per day, seven days per week. A 5.7-liter Oldsmobile engine was operated continuously at 40 mph to generate the diesel exhaust. Hydrocarbons, CO, CO_2, NO_x and particulates were monitored on a periodic basis and dilution was adjusted, as necessary, to maintain the 1500 $\mu g/m^3$ particulate level. Control groups of animals were exposed to the same filtered air used to dilute the raw exhaust. At the end of three months of exposure, a portion of each species of animals was randomly selected for measurement of organ weights (lungs, heart, kidneys, liver and spleen) and for histopathological examination. The remaining animals were held for a six-month post-exposure recovery period, with the exception of the majority of mice which were held for four and one-half

months following termination of exposure. Growth rate of all animals was monitored by biweekly measurements of body weights during the exposure and recovery periods. At the end of four and one-half months of recovery, most of the Strain A/J mice were sacrificed at nine months of age for evaluation of pulmonary adenoma response. After six months of recovery, animals of each species were sacrificed for histopathology and organ weight measurements.

Exposure to diesel exhaust did not affect animal health, as indicated by mortality, growth rate and organ weights, in any of the three species. In the Strain A/J mice, neither the average number of adenomas per animal nor the incidence of these tumors was significantly different in control and exposed animals. In contrast, intraperitoneal injection of urethane at a dose of 1 mg/g of body weight produced a large increase in both tumor parameters. The results of light microscopic examination of tissues were consistent in the three species. After three months of exposure, particulate was distributed diffusely and in foci in the alveolar spaces of the lungs, both in free form and in macrophages, and in the regional lymph nodes. After six months of recovery, partial removal of particulate from the lungs and redistribution into focal accumulations had occurred. Most of the particulate was found inside macrophages or within protean matrices. Clearance of the particulate via lymphatic channels was evident in both the exposed and recovered animals. Species differences in clearance of particulate were apparent, with hamsters exhibiting the largest clearance of particles and mice the least. In both exposed and recovered animals of each species, alveolar fibrosis associated with large accumulations of particulate was minimal and not considered of clinical significance. Lesions in organs other than the respiratory system were also minimal, of similar incidence in control, exposed and recovered animals and not considered exposure-related.

RESPONSE OF PULMONARY CELLULAR DEFENSES TO THE INHALATION OF HIGH CONCENTRATIONS OF DIESEL EXHAUST

Kenneth A. Strom
Biomedical Science Department
General Motors Research Laboratories
Warren, MI 48090

Bronchopulmonary lavage was used to explore the responses of the pulmonary phagocytic defense in rats exposed to diesel exhaust at concentratons of 250, 750, or 1500 $\mu g/m^3$ diesel particles for 26 to 48 weeks. Figure 1 shows the quantities of alveolar macrophages, polymorphonuclear leukocytes and lymphocytes obtained by lavage of the respiratory airways (millions of cells in 40 mL). There is no difference in the number or kinds of cells obtained from control and from 250 $\mu g/m^3$ exposed animals after either 6 or 11 months of exposure. However, after exposure to 750 or 1500 $\mu g/m^3$, the number of alveolar macrophages increases by 25-33% and 100-150%, respectively. When the inhaled particulate concentration exceeds 250 $\mu g/m^3$, the quantity of the lavaged alveolar macrophages shows dependence on the inhaled concentration of the diesel particulate after both 6 and 11 months of exposure. In contrast, polymorphonuclear leukocytes, which are not observed in lavage fluid from control rat lungs, are obtained in high numbers from rats exposed to 750 or 1500 $\mu g/m^3$ for 11 months or 1500 $\mu g/m^3$ for 6 months. The quantities of lavaged cells are, therefore, dependent primarily on the concentration of exposure and secondarily on the length of exposure.

Since equal amounts of diesel particulate are delivered to the lungs during exposure to 1500 μg DP/m^3 for 6 months or to 750 μg DP/m^3 for 12 months, comparison of the quantities and kinds of cells obtained by lavage points out differences in the response of the phagocytic defense to the inhaled concentrations. When identical amounts of particulates are administered over twice the length of time, the number of alveolar macrophages is increased by 25% above control values, compared to 150% after shorter exposure to a higher concentration. The counts of polymorphonuclear leukocytes are $5.1 \pm 1.5 \times 10^6$ compared to $19.1 \pm 4.4 \times 10^6$ at the shorter time of higher level exposure. This comparison demonstrates that the pulmonary cellular systems respond primarily to the rate of submicron particles entering the lungs, rather than to the total amount of particulate delivered to the lung. Lymphocytes which are not obtained from control rat lungs are lavaged from animals exposed for 11 months to both 750 and 1500 μg DP/m^3, but not to 250 μg DP/m^3. The presence of the nonphagocytic lymphocytes in the lavage fluid may represent a slow immune response to the presence of large numbers of polymorphonuclear leukocytes and particle-filled alveolar macrophages in the lung. The enzyme contents of the acid phosphatase and β-glucuronidase in alveolar macrophages lavaged from control and 250 $\mu g/m^3$ exposed animals were identical. At higher inhaled concentrations, the cellular enzyme content per mL of lavage fluid increased, but due to the variability of the cell counts and types with the continuing diesel exposure, the contribution of each cell type to the total enzyme activity was not determined.

Under the exposure regime used in these experiments (20 hrs/day, 5-1/2 days/week), a threshold rate for response of the phagocytic defense occurs between inhaled concentrations of 250 and 750 $\mu g/m^3$ diesel particles. The response consists of compensative immigration into the lungs, at first, of alveolar macrophages and later on, also, of polymorphonuclear leukocytes. After excessive exposures (11 months of exposure to 750 or 1500 $\mu g/m^3$ diesel particles), mononuclear leukocytes (lymphocytes) are also lavaged in high numbers. The probable cause of the observed effects is the continuous phagocytosis of the diesel particles by the macrophage, which results in the cellular accumulation of excessive amounts of "indigestible" carbonaceous particle nuclei. When the macrophage renewal rate in the lungs is too slow to cope with the influx of particles, the macrophages eventually become overloaded. Release of humoral factors from active macrophages may stimulate the recruitment of more alveolar macrophages. Polymorphonuclear and mononuclear leukocytes most probably respond to other not yet known humoral agents from degenerating particle-laden macrophages.

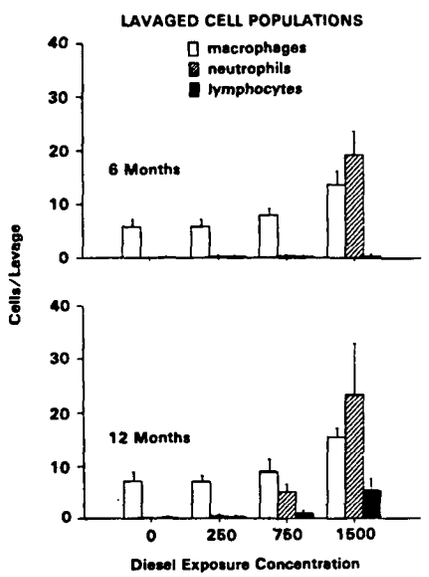


Figure Lavaged cell populations. The proportion of each cell type in lavaged cells was multiplied times the total number of cells obtained from each rat. The numbers were averaged for each exposure group (six rats) and charted as millions of cells in the total lavage volume vs. the diesel exposure concentration. The line shows the standard deviation range of the data.

THE EFFECT OF DIESEL EXHAUST ON CELLS OF THE IMMUNE SYSTEM

D. Dziedzic
Biomedical Science Department
General Motors Research Laboratories
Warren, MI 48090

Inhalation of diesel engine exhaust results in the deposition of submicron carbonaceous particles in the respiratory airways. The particles are phagocytized by the pulmonary alveolar macrophages, and are cleared from the respiratory tract via the mucociliary escalator or through lymphatic channels. Lung clearance via lymphatics results in an accumulation of particles in the regional lymph nodes, and literature data suggest that the presence of hydrocarbons or carbonaceous particles in high doses might affect immune functions [1,2].

Two approaches have been used in the present work to determine whether inhalation of diesel exhaust could be immunotoxic. In one series of experiments, dichloromethane extract of diesel particles was injected in massive doses (10-50 mg/kg) intraperitoneally over a 7 day period into C_{57} Bl mice in two separate protocols. First, the splenic lymphocytes were isolated and studied for ability to respond to polyclonal stimulation of B or T cells by E. coli lipopolysaccharide or concanavalin A, respectively. Secondly, dinitrofluorobenzene-induced contact hypersensitivity reaction was measured as a reflection of T cell function by quantifying changes in ear thickness after an irritative challenge. In both experiments, a small deterioration in the immuno-defensive ability of lymphocytes from extract-treated animals was observed. In mitogen response assays, lipopolysaccharide response (LPS) was reduced by about 20% compared to vehicle control groups. Similarly, a 20-50% reduction was seen in concanavalin A (CON A) stimulated cultures. In T cell mediated contact hypersensitivity reaction, all of the treated animals showed decreased ear thickness response. In none of the experiments, however, was a direct dose-response relationship observed. Furthermore, fluctuations in liver weights from experimental animals indicated the possibility that hepatic changes induced by the excessive doses of injected hydrocarbons may be involved in the observed effects.

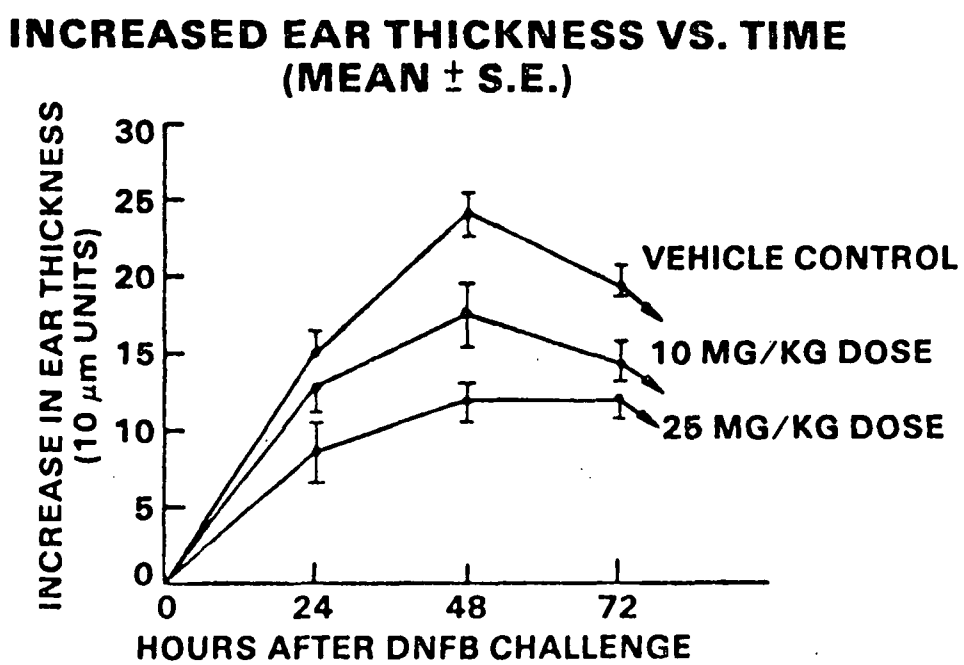
The approach used in this series of experiments is clearly limited, since 1) by use of high doses of diesel particle extract, the question of bioavailability of hydrocarbon from particles is ignored; 2) the large doses of extract may overwhelm normal defense mechanisms; 3) the route of exposure allows for system distribution of material which may not occur when inhalation occurs; and 4) in the case of mitogen responsiveness, splenic rather than lymph node lymphocytes were studied. Nonetheless, the possibility that the diesel exhaust particle extract administered in high doses may potentially affect the immune system is at least tentatively raised, and should be further verified in inhalation studies.

The second approach was used to detect immunotoxicity of diesel particles by studying lymph nodes, blood and spleen from guinea pigs exposed to diluted diesel exhaust at a particulate concentration of 1500 $\mu g/m^3$. In this experiment, immune system organs were studied for shifts in lymphocyte subpopulations counts. Alterations in this parameter have been observed in several forms of human diseases, including active forms of lupus nephritis or chronic glomerulonephritis, inflammatory bowel disease, and other disorders. In addition, exposures to environmental toxins such as lead, polybrominated biphenyls, and cigarette smoke may also be associated with changes in subpopulation proportions. The data from the present experiments, however, show that no shift in subpopulation occurred in the mediastinal lymph node, the site of primary diesel particle deposition. In spleen and blood, small fluctuations of no more than ± 5% were observed, which is well within the limits of variability described in the literature, and no other significant biological effects were identified.

In summary, small functional differences were seen in lymphocyte responsiveness of $C_{57}Bl$ mice after treatment with diesel particle extracts. However, limitations of the experimental protocol preclude direct extrapolation to possible findings during inhalation exposure to diluted diesel exhaust. In a test system where the more realistic inhalation mode of exposure was employed, no major effects were seen, and more studies are needed to focus on lung immune system reactivity before the immunotoxic potential of diesel particulates deposited in the mediastinal lymph nodes can be definitely assessed.

REFERENCES

1. D. E. Bice, et al, Drug Chem. Toxic., 2, 1979.
2. A. Zarkower, Arch. Environ. Health, 26, 1972.



THE PARTICIPATION OF THE PULMONARY TYPE II CELL RESPONSE TO INHALATION OF DIESEL EXHAUST EMISSION: LATE SEQUELAE

H. J. White and B. D. Garg
Biomedical Science Department
General Motors Research Laboratories
Warren, MI 48090

Among the earliest cellular responses of the lung to inhalation of diluted diesel exhaust is the focal proliferation of the alveolar Type II cell, the cell responsible for the synthesis of surfactant. This reaction takes place relatively within the same time frame as that for phagocytosis by the alveolar macrophage, and can be seen in rats as early as twenty-four hours post-exposure to a diesel particle concentration of 6000 $\mu g/m^3$ [1]. The proliferation is focally within alveoli, sometimes showing several cells in a line. The variation in the staining of their nuclei supports the idea of a fairly rapid proliferation, although mitoses have not been seen. There is no evidence in our hands that this proliferative response is of a reparative nature secondary to damage of the Type I cell. The Type II cells release considerably increased amounts of surfactant, which accounts for a morphologic change in the phagocytic alveolar macrophage which now takes on a more foamy appearance. The transition between the early macrophage (one-day exposure to diesel at 6000 $\mu g/m^3$ to that of 6 weeks' exposure) reveals the gradual accumulation of surfactant material. Some of the phagosomes can be seen to contain recognizable myelin figures of Type II provenance changing to a more filamentous form, suggesting an unraveling of the more compact tightly-wound phospholipid. The mechanism of accumulation of cholesterol is certainly not clear, although plate-like crystalline structures can be easily identified within the phagosomes, suggesting cholesterol ester formations. The excess cholesterol could well have its origin from the phagocytosed surfactant material which then at some later stage is de-esterified to form the familiar elongated acicular structures of cholesterol. This foamy cell is apparently more sluggish, and tends to accumulate near the terminal bronchioles where further Type II cell activity is elicited.

With prolonged exposures to 9 weeks at 6000 $\mu g/m^3$ and 1500 $\mu g/m^3$ for two years, cholesterol also begins to accumulate within the phagosome. Later, the crystals grow and tend to become extracellular. This can be demonstrated by both light and electron microscopy. Build-up of the cholesterol deposits in these late stages has been found to be associated with increased collagenosis of the septal wall in which mast cells are also present. The reaction of mast cells to the release of extracellular cholesterol is also obscure. One could speculate that the heparin of the mast cell is involved in activation of lipoprotein lipase in an attempt to clear the excess of lipid release. In some way, the amine component of the mast cell is also released at this time to provoke the laying down of collagen. In addition, there is an apparent intimate association between laying-

down of extracellular cholesterol, lipids, septal mast cells and collagenosis; the collagenosis is, however, focal and the integrity of the septal architecture is preserved. Occasionally, focal cholesterol granulomas have been observed. These are quite similar in appearance to those seen after prolonged inhalation exposure to Sb_2O_3 [2] and marihuana smoke [3]. The phenomenon of "benign" focal alveolar collagenosis seems to be a consequence of high particulate burden that stimulates an increased production of phospholipids, and not directly of the effect of the diesel particle per se. The observation that diesel particulates, sequestered in the thoracic lymph nodes for up to two years, do not provoke a fibrotic reaction and supports this contention.

REFERENCES

1. White, H.J. and Garg, B.D. (1981), Early pulmonary response of the rat lung to inhalation of high concentrations of diesel particles. J. Appl. Tox. 1:104-110.

2. Gross, P., Brown, J.H., and Hatch, T.F. (1952), Experimental endogenous pneumonia. Am. J. Path., 28:211-221.

3. Fleischman, R.W., Baker, J.R., and Rosenkrantz, H. (1979), Pulmonary pathologic changes in rats exposed to marihuana smoke for one year. Tox. and Appl. Pharmacol., 47:557-566.

PULMONARY DEPOSITION, RETENTION, INACTIVATION AND CLEARANCE OF INHALED DIESEL PARTICLES: THE ROLE OF THE PULMONARY DEFENSE SYSTEM

J. J. Vostal
Biomedical Science Department
General Motors Research Laboratories
Warren, MI 48090

The submicron size of diesel particles permits an easy penetration of the particles into the deepest parts of the respiratory system, and allows the possibility of their prolonged contact with the sensitive cells at the lowest levels of the bronchopulmonary regions. Since a knowledge of the level of the interaction between the particle surface or of the adsorbed hydrocarbons with the intimately adjacent cells of the respiratory epithelium is essential for the assessment of their potential toxicity and adverse respiratory effects, the exact determination of the inhaled dose and residence time of particles retained in the respiratory airways is important in predicting the potential health impact of the wider use of diesel-powered vehicles on our roads.

As a direct continuation to our previous program studying the health effects of long-term, high-level exposures in extensive laboratory experiments, the deposition, retention, inactivation, and clearance of inhaled particles have been intensively investigated in animal models during the past year. Specifically, investigations were concentrated on the detailed analysis of the conditions responsible for the handling of the particles by the pulmonary defense system.

In various animal models, investigations with freshly-generated, radioactively-labelled particulates indicated that even under conditions of extremely high exposures, not more than approximately 15-17% of the inhaled dose is retained in the respiratory tract of most laboratory animals used in the studies. After a single short-term exposure, approximately one-fifth and one-third of the retained dose is cleared from the respiratory tract of Fischer 344 rats within the first 48 hours, and during the first two weeks, respectively; the remaining portion of the lung burden is removed more slowly with a clearance half-time of approximately 60-80 days [Lee et al, 1981]. The fact that other species of laboratory animals may clear the particulates from the lung by different mechanisms and at different clearance rates [Chan et al, 1981a] makes a direct comparison among animal species and the extrapolation of the laboratory data to man particularly difficult. Consequently, our conclusions are restricted to a relative assessment of specific clearance or deposition mechanisms in individual animal models with the final intention of correlating the administered dose with the observed effects.

Unfortunately, most of the accumulated data were obtained after local administration of high doses or inhalation of highly elevated concentrations of diesel particulates.

Although the first comparisons of single short-term exposures between either extremely high or moderately high concentrations did not reveal a significant effect of inhaled concentration on the rate of clearance of diesel particles [Lee et al, 1981], other data obtained in animals after prolonged exposures to high doses of particulates (particulate lung burden > 10 mg) seems to indicate that the removal of the particles may be significantly slowed in excessive exposures and contribute to an artifically exaggerated accumulation of particles in the respiratory system - a phenomenon which is not expected after breathing expected ambient levels of diesel particulates [Chan et al, 1981b].

A similar threshold effect of the inhaled concentrations has also been observed in the response of pulmonary cellular defense mechanisms, in which the first measurable quantitative increase of the macrophage cell counts occurred in the bronchopulmonary lavage only when the inhaled concentrations were higher than 250-750 μg of particulates per cubic meter [Strom, 1981]. Similarly, qualitative changes in the composition of the lavageable mobile cells (immigration of polymorphonuclear leukocytes, and later on, of lymphocytes) in the lavage fluid were observed only after excessively high concentrations were inhaled.

Furthermore, a high rate of particulate influx to the deep alveolar region can potentially overload the physiological clearance mechanisms, and significantly influence the rate of accumulation and storage of the inhaled particulates. This appears to happen via the creation of a new sequestering compartment in the form of focally aggregated macrophages with particulates in the subpleural and terminal bronchiolus regions of the lung [White et al, 1981a]; again a phenomenon which probably does not exist after inhalation of low concentrations.

However, even if we assume that the macrophage sequesteration and aggregation are not typical physiological mechanisms, it is important to note that the sequestered particles are well enclosed in the aggregated macrophages, and form a stationary deposit which may persevere in the lung tissue without any negative reaction for a long period of time [Strom et al, 1981; Soderholm, 1981]. Although it can significantly contribute to a measurable burden of retained particles, the macrophage aggregate effectively prevents the contact of particles with the sensitive respiratory epithelium. The tissue reaction to the presence of the aggregates remains negative for a long period of time. Only after long and excessive exposures can we observe an intracellular build-up of phospholipids and cholesterol deposits, which may later be accompanied by a locally increased number of collagen fibers in the septal walls, as well as by focal mast cell activation. Even then, the tissue reaction remains focal and without significant clinical impact [White et al, 1981b]. Therefore, this tissue reaction must be classified as a non-specific reaction to the high level of particulate burden, rather than a direct effect of the presence of the diesel particle or its adsorbed hydrocarbons.

The pulmonary alveolar macrophage is obviously not only capable of scavenging and sequestering particles which penetrate deeply into the respiratory system, but can also contribute to the inactivation of their biological activity, as indicated by the observed loss of mutagenic activity of diesel particulate extracts from particles which had been incorporated into the macrophage phagosomes [Siak et al, 1981]. Although we cannot clearly determine at this time if the inactivation occurs either by dissolution, metabolism, or simple relocation of mutagenic hydrocarbons from the particulate surface, it is also important to note that this process prevents the immediate contact of sensitive respiratory cells with the concentrated hydrocarbons on the particle surface.

Inactivation of the biological effects of diesel particulates deposited deeply in the lung is further corroborated by the absence of hydrocarbon metabolizing enzyme induction in the lung, even after long-term inhalation exposures to high concentrations of diesel particulates [Chen et al, 1981]. Since published studies of the structural and functional responses of the respiratory system do not reveal changes considered to be clinically significant, despite conditions of excessive exposures, the effective role of the pulmonary defense mechanisms, which provide significant protection against the effects of inhaled diesel particles, has been clearly demonstrated.

INVESTIGATIONS OF TOXIC AND CARCINOGENIC EFFECTS OF DIESEL EXHAUST IN LONG-TERM INHALATION EXPOSURES OF RODENTS

by

U. Heinrich, L. Peters, W. Funcke, F. Pott, U. Mohr and W. Stöber
Fraunhofer-Institut für Toxikologie und Aerosolforschung
Münster, Federal Republic of Germany

Extended Abstract

INTRODUCTION

The purpose of this study was to provide experimental evidence of the theoretically expected carcinogenicity of diesel exhaust. It is known that the emissions from internal combustion engines contain minute amounts of polycyclic aromatic hydrocarbons (PAH), some of which are proven to be carcinogens in animals. These carcinogenic PAH show rather low vapor pressures and tend to condense on airborne particles. Since diesel engines produce much larger amounts of finely dispersed particles during fuel combustion than gasoline engines, the particulate matter in diesel exhaust with its surface condensate of various organics is suspected to constitute a special carcinogenic potential which deserves a careful investigation into the chronic effects of continuous inhalation exposure.

EXPERIMENTAL

A total number of 864 female Syrian golden hamsters [Hoe: SYHK(SPF Ars), breeding farm of Frankfurt-Hoechst, West Germany] was subdivided into three groups of 288 animals each. One group was to inhale clean air, the second group was exposed to the diluted original emissions of a diesel engine, the third one breathed the same diluted emissions but void of any particulate matter. The animals were exposed to the exhaust in wire cages placed into a chamber ventilated with the diluted exhaust atmosphere. The exposures lasted for 7 to 8 hours per day, 5 days per week for about two years.

In addition to the golden hamsters, two groups of 20 rats were exposed for 18 to 24 months for subsequent testing of some lung functions in comparison to a control group of the same size.

The exhaust was generated under constant operating conditions with a Daimler-Benz DB 200 D diesel engine on a test bench. The emissions were cooled and, without passing the dew point, diluted with refrigerated clean air (1 part of exhaust into 7 parts of air) before drawing them through the inhalation exposure chambers. Two locations were selected for sampling the exposure atmosphere : one port was inserted into the ducts about 5 meters upstream of the flow entrance of the inhalation chamber, the other probe drew directly from within the chamber. Table 1 gives the results for all measured components as average values of all data obtained over the whole study period. Apparently, there were no significant differences between the gaseous components of the original exhaust and the exhaust void of particles. In addition to these results, the concentrations of 14 PAH on the particles in the inhalation chambers were recorded and found to be low (e.g. 7.0 μg of benzo(a)-pyrene per gram of particles). The particulate matter in the exposure chambers was highly dispersed, the mass median aerodynamic diameter being below 0.1 μm.

In designing the experiments, it was anticipated that the probability of directly proving an existence of the theoretically expected carcinogenicity of diluted diesel exhaust with a limited number of animals was rather low. Thus, an attempt was made with some of the test animals to produce a base line tumor induction rate by applying a known carcinogen at the beginning of the inhalation exposure and to investigate the influence of the exhaust inhalation on this base line rate. Each of the three exposure groups of 288 hamsters was subdivided into 6 sub-groups (48 hamsters each) and pre-treated in the following manner :

(1) no additional treatment (controls)
(2) 2 mg pyrene in 20 weekly intratracheal instillations of 0.1 mg each (second controls)
(3) 2 mg dibenzo(a,h)anthracene, intratracheally instilled as under (2)
(4) 6 mg dibenzo(a,h)anthracene, intratracheally instilled as under (2), but 0.3 mg each
(5) 1.5 mg diethyl nitrosamine per kg body weight, subcutaneously injected
(6) 4.5 mg diethyl nitrosamine per kg body weight, subcutaneously injected

During the course of the long-term exposure, 11 biochemical and 7 hematological parameters were determined repeatedly. In addition, the impact of the diesel exhaust inhalation on the lung clearance of a radioactively labelled iron oxide aerosol and on some respiratory parameters was measured for the rats.

RESULTS

A detailed report on the results will be given in the final paper. Two of the findings of the long-term study were particularly conspicuous :

- the tumor induction rate in the respiratory tract produced by the subcutaneous injection of diethyl nitrosamine was enhanced for the hamster groups exposed to the exhaust atmospheres (Fig. 1),

- the lung clearance of the chronically exposed rats was decreased (Fig. 2).

It appears to be premature to draw firm conclusions from these preliminary results obtained with rather small animal groups. The findings certainly need a confirmation by results of additional experiments.

	MEASUREMENT			
	IN THE CHAMBERS		BEFORE THE CHAMBERS	
	A	B	A	B
CO [ppm]	16,9 (±3,0)	17,9 (±3,3)	16,3 (±3,2)	18,0 (±3,4)
CO_2 [Vol%]	0,63 (±0,14)	0,67 (±0,14)	0,52 (±0,14)	0,57 (±0,16)
SO_2 [ppm]	4,7 (±1,6)	4,9 (±0,9)	7,9 (±3,8)	7,0 (±1,4)
C_NH_M [ppm]	8,5 (±3,4)	8,9 (±2,8)	5,3 (±3,8)	4,2 (±1,7)
CH_4 [ppm]	2,1 (±0,8)	2,5 (±0,9)	1,0 (±0,33)	1,0 (±0,32)
C_NH_M - CH_4 [ppm]	6,3 (±3,0)	6,3 (±2,6)	4,3 (±3,7)	3,3 (±1,4)
NO [ppm]	15,8 (±7,1)	15,6 (±6,9)	17,6 (±8,5)	16,0 (±8,8)
NO_X [ppm]	16,3 (±7,2)	15,9 (±6,5)	17,7 (±10,2)	16,4 (±10,3)
NO_2 [ppm]	0,45 (±0,42)	0,40 (±0,28)	0,10 (±0,09)	0,21 (±0,27)
O_2 [vol%]	20,0 (±0,7)	19,5 (±0,6)	20,0 (±0,7)	19,1 (±0,5)
PARTICLES [mg/m³]	–	4,2 (±0,3)	–	–

A: EXHAUST WITHOUT PARTICLES B: TOTAL EXHAUST

Table 1 : Analytic average data on emission concentrations during exposure

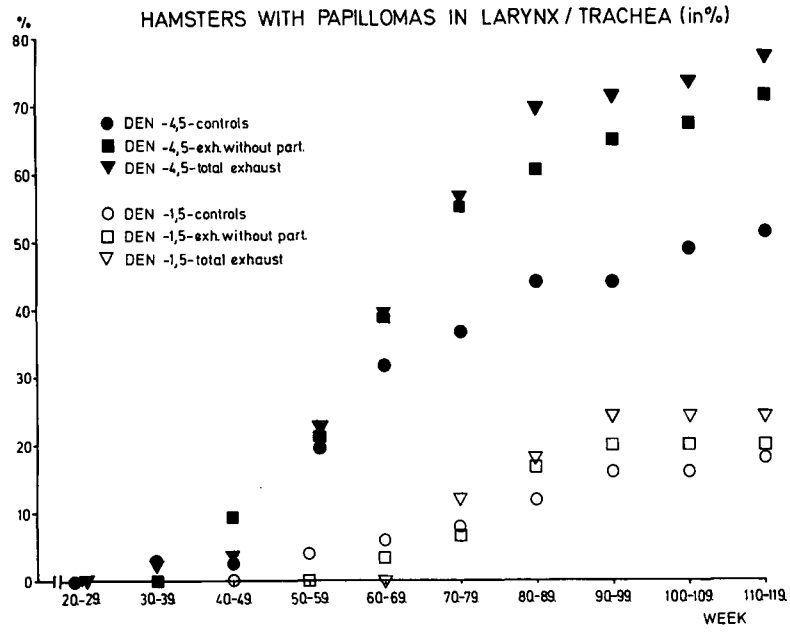


Figure 1 : Papillomas in pre-treated Syrian golden hamsters

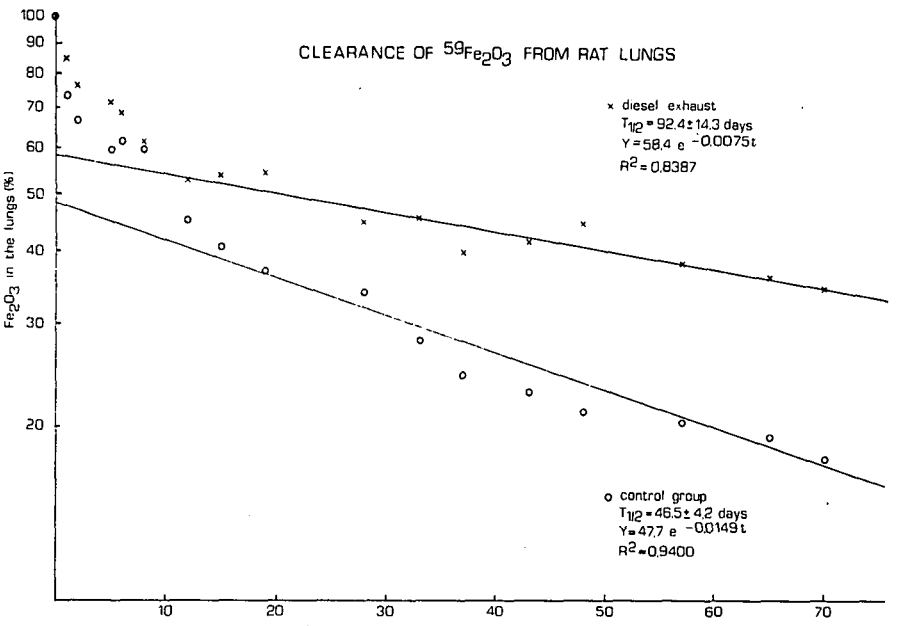


Figure 2 : Lung clearance of $^{59}Fe_2O_3$ aerosols in rats

BIOCHEMICAL ALTERATIONS IN BRONCHOPULMONARY LAVAGE FLUID AFTER INTRATRACHEAL ADMINISTRATION OF DIESEL PARTICULATES TO RATS

by

C.D. Eskelson, M. Chvapil, E. Barker, J.A. Owen
Department of Surgery, Division of Surgical Biology
University of Arizona Health Sciences Center
Tucson, Arizona 85724

J.J. Vostal
Biomedical Science Department
General Motors Research Laboratories
Warren, Michigan 48090

Male Sprague Dawley rats weighing 180-200 g were given intratracheally 5 mg of diesel particulates (DP) in 0.75 ml saline. Control rats were given saline. Five days after administering the DP and three hours before the rats were sacrificed they were injected with a pulse of 20 μC of ^{14}C-acetate.

The lungs were intubated before they were removed and lavaged 3 times with 5 ml of saline. The combined lavage fluids were lyophylized. The lipids were extracted from the lyophylized lavage fluid with chloroform: methanol (2:1) and its cholesterol (C) and phospholipid (PL) content determined. The radioactivity incorporated into the lipids was determined by separating the lipids by a TLC method and scraping the spots of interest into counting vials.

Pulmonary lavage fluid from the rats given DP contained 4 times more PL, C, and protein than in control rats (Table 1). Total radioactivity incorporated into lecithin was twice that of controls and was 3.5 times greater than the radioactivity found in the other PL studied. The lavaged lungs from the control and experimental rats were lyophylized and homogenized in a chloroform:methanol (2:1) solution. The lipid analysis showed no difference in the PL and C levels between the control and experimental lungs (Table 2).

The fatty acid (FA) profile of the lavage fluid determined by a GLC method indicated a three-fold increase in palmitic acid and arachidonic acid. Stearic, oleic and linoleic acids were not significantly altered (Table 3).

These studies imply that the lipid loading observed in lungs exposed to 5 mg of DP for 5 days are a result of increased deposition of pulmonary surfactant (extracellular lipids) and are not a result of intracellular lipids.

Table 1. Analysis of Pulmonary Lavage Fluid from Rats Intratracheally Exposed to 5 mg of Diesel Particulate

	Phospholipids		Cholesterol		Protein	
	mg	SD	mg	SD	mg	SD
Experimental	1.90	0.48	.539	.040	8.47	0.33
Control	0.49	0.18	.151	.081	2.09	1.04
Student's t	7.82	P < .001	6.37	P < .001	8.25	P < .001

Results expressed as mg of lipids in the total lavage fluid

Table 2. Analysis of Lavaged Lungs from Rats Intratracheally Exposed to 5 mg of Diesel Particulate

	Phospholipids		Cholesterol	
	mg	SD	mg	SD
Experimental	34.9	1.34	15.9	1.02
Control	31.30	10.6	13.1	3.23
Student's t	.453	NS	1.18	NS

Results expressed as mg of lipid per lung

Table 3. Fatty Acid Profile from Lung Lavage Fluid of Rats Exposed to 5 mg Diesel Particulate

	C_{16}	C_{18}	$C_{18:1}$	$C_{18:2}$	$C_{20:4}$
Experimental	1.77	0.115	0.152	0.152	0.104
SD	.07	.033	.016	.030	.015
Control	0.541	.097	0.152	0.225	.030
SD	0.195	.103	.015	.322	.021
Student's t	8.50	NS	NS	NS	4.61
	$P < .001$				$P < .01$

Results expressed as mg of the fatty acid methyl ester per total lavage fluid

LIPID CHANGES IN LUNG OF RATS AFTER INTRATRACHEAL ADMINISTRATION OF DIESEL PARTICULATES

by

C.D. Eskelson, E. Barker, M. Chvapil, J.A. Owen
Department of Surgery, Division of Surgical Biology
University of Arizona Health Sciences Center
Tucson, Arizona 85724

J.J. Vostal
Biomedical Science Department
General Motors Research Laboratories
Warren, Michigan 48090

Lung, liver and serum from 180-200 g male Sprague Dawley rats were analyzed for various lipids 5 days after the rats were given intertracheally in a saline solution 1 mg of diesel particulates (DP). The lipogenic activity occurring in these rats was studied by giving i.p. 20 μC of ^{14}C-acetate one hour before they were sacrificed.

Phospholipids and cholesterol content of the lungs were significantly increased while that of triacylglycerols were not changes significantly (see Table 1).

Contrariwise to pulmonary lipids, hepatic phospholipids and cholesterol levels were decreased in rats exposed to diesel dust while hepatic triacylglycerol (TG) levels were not significantly altered. Accompanying the loss of hepatic lipids are an increased phospholipid, cholesterol and TG specific activity indicating increased hepatic lipogenesis. To determine if the loss of hepatic lipids were due to their being mobilized to the serum from the liver serum lipids were determined and were found not to be significantly altered. However, the specific activity of serum phospholipids, cholesterol and triacylglycerols were all significantly increased in rats intratracheally given the diesel particulates. A corresponding doubling of radioactivity in pulmonary phospholipids and cholesterol was also detected in these animals.

The results obtained here are similar to those reported earlier for rats intratracheally given silica dust (1-3) and suggest that a particulate insult to the lungs results in the lung producing lipotrophic factors which stimulate the liver to increase lipogenesis and lipid export to the blood. The lung in turn picks up the lipids from the serum and remodels them to meet pulmonary lipid need. To further study this hypothesis, rat hepatocytes were isolated and incubated in a pH 7.0 phosphate buffer containing 2 μC

^{14}C-acetate and several cofactors. To this hepatocyte suspension was added lung slices and the system thence incubated for 2 hours at 37°C.

Phosphatidyl choline (PC) was isolated from each sample by TLC and the PC spots from each TLC scraped into counting vials. The amount of radioactivity incorporated into the PC spots of the 5 samples for each experimental manipulation was averaged and is presented in Figure 1.

This study clearly demonstrated increased lipogenesis above that of the sum of lung slice PCgenesis and hepatocyte PCgenesis. The results from the *in vitro* studies strongly support the concept that a pulmonary lipotropic factor exists which stimulates lipgenesis in the liver and that these *de novo* synthesized lipids are utilized in part to maintain lipid homeostasis in the lung.

Table 1. Pulmonary Lipids from Rats Intratracheally Exposed to 1 mg of Diesel Particulate

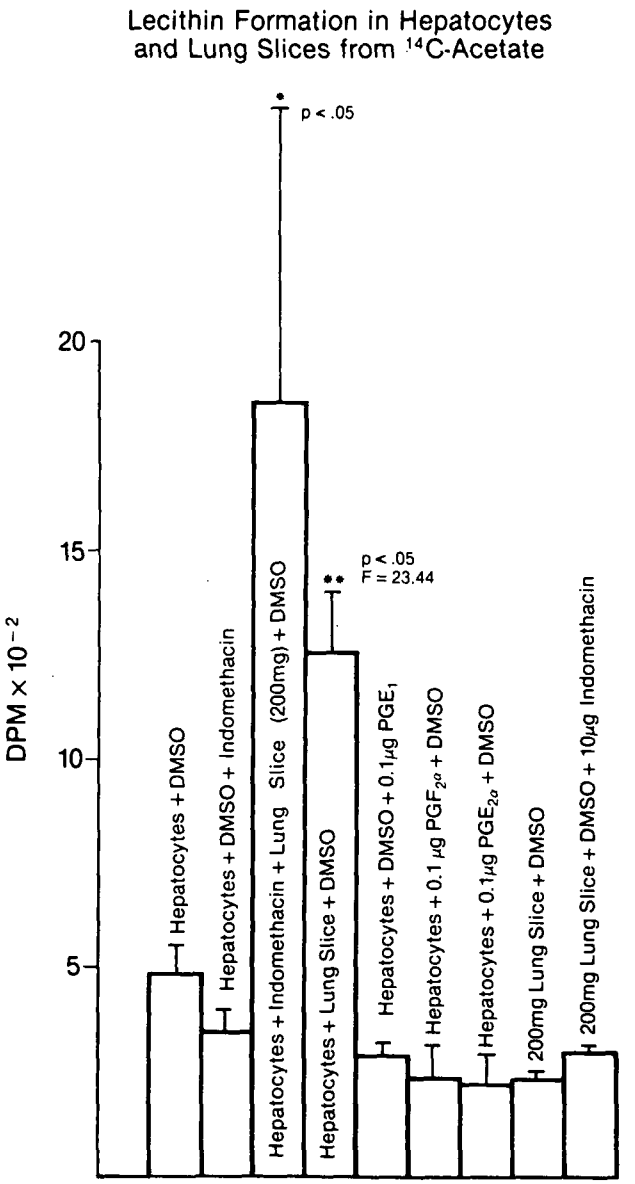
	Phospholipids		Cholesterol		Triacylglycerols	
	mg	SD	mg	SD	mg	SD
Experimental	51.7	4.64	11.95	1.11	20.00	3.15
Control	30.8	4.66	8.27	0.63	24.52	1.24
Student's t	6.15	P < .001	5.15	P < .005	2.32	NS

Results expressed as mg of lipids per lung

Table 2. Hepatic Lipids from Rats Intractracheally Exposed to 1 mg of Diesel Particulate (mg/g of liver)

	Phospholipids		Cholesterol		Triacylglycerols	
	mg	SD	mg	SD	mg	SD
Experimental	27.9	1.9	3.36	0.10	8.57	1.43
Control	31.3	1.7	4.02	0.11	9.07	0.88
Student's t	2.52	P < .05	8.60	P < .001	.53	NS

Figure 1.



References

1. Eskelson, C.D., Stiffel, Virginia, Owen, J.A. and Chvapil, M. The importance of the liver in normal and silicotic lung-lipid homeostasis. 2. Cholesterol. Environ Res 19:432-441(1979).

2. Eskelson, C.D., Stiffel, Virginia, Owen, J.A. and Chvapil, Milos. The importance of liver in normal and silicotic lung-lipid homeostasis: 3. Triacylglcerols. Physiol Chem Physics 11:135-141(1979).

3. Eskelson, C.D., Stiffel, Virginia, Owen, J.A., and Chvapil, Milos. The importance of liver in normal and silicotic lung-lipid homeostasis. 1. Phospholipids. Accepted for publication in J Environ Path Tox.

BIOAVAILABILITY OF DIESEL PARTICLE BOUND [G-^{3}H-] BENZO(a)PYRENE (^{3}H-BP) AFTER INTRATRACHEAL INSTILLATION

by

P.K. Medda, Sukla Dutta and Saradindu Dutta
Wayne State University School of Medicine
Detroit, Michigan 48201

Recently, we have investigated whether pulmonary prostaglandin dehydrogenase (PGDH) activity is affected by long-term exposure to low doses of diesel exhaust (1) and whether high doses (6.0 mg/m^3) for a short period (2-8 weeks) can affect such biochemical functions as (a) blood methemoglobin level, (b) reduced glutathione levels, (c) angiotensin coverting enzyme activity, and (d) mixed function oxidase activity (2, 3). In general these studies have shown that as far as the above set of biochemical parameters is concerned, there exists no particular adverse effect of the diesel exhaust in the exposed animal. Because of these findings and the observation made by Siak et al., (4) that simulated biological fluids elute no significant mutagenic activity from the diesel particles, we have contended that the many polycyclic hydrocarbons, such as benzo(a)pyrene (BP) which are known to be present in the diesel particles (DP), probably remain unavailable to the pulmonary tissue. In order to provide support for this contention attempts have been made to determine the bioavailability of ^{3}H-BP following intratracheal administration of this agent bound to diesel particles in an albumin-saline suspension.

In order to determine the bioavailability of benzo(a)pyrene as bound to diesel particles, ^{3}H-BP (120 μCi/1.79μg/1.0 ml ethanol) was allowed to bind diesel exhaust particles by adsorption. After removal of free ^{3}H-BP by repeated suspension and centrifugation, nearly 90-95 μCi of ^{3}H-BP remained bound to 1 mg of DP. Tightness of the binding was tested by continuously washing 125 μg of ^{3}H-BP bound DP with 6.0 ml either Krebs-Henseleit (K-H) solution or K-H with 3.2% albumin solution or DMSO or dichloromethane for one hour. Results showed the least dissociation in K-H solution (2.1%) and the highest in the presence of dicholoromethane (72%). For the bioavailability experiments, female guinea pigs (Hartley) were lightly anesthetized with ether in preparation for instillation of 1 mg labelled DP. When animals were appropriately anesthetized, diesel particles as suspended in 0.2 ml K-H solution containing 3.2% albumin was introduced slowly into the trachea over a period of 10-15 minutes by inserting a PE10 polyethylene tubing through the tracheal ring and pushing deep inside the broncheal tree. After intratracheal instillation of labelled particles guinea pigs were divided into two groups

Five guinea pigs of one group were immediately sacrificed within 20-25

minutes, while six guinea pigs belonging to the second group were moved to Nalgene(R) metabolic cages, held there individually for collection of urine and feces for 48 hours and then sacrificed for excision of selected organs such as liver, kidney, intestine and collection of blood for measurement of radioactivity.

Results of these studies showed that intratracheal instillation of 1 mg labelled DP did not produce any obvious symptoms in these animals. Furthermore, except for individual difference, no abnormality was noted in feeding and excretory profiles of these guinea pigs. At autopsy, lobar localization of DP was clearly visible as distinct patches of black marks of 1-2 cm diameters. Percentages of radioactivity retained by guinea pig lungs immediately after intratracheal instillation of DP bound to ^{3}H-BP, when calculated on the basis of the administered dose, showed wide variability in that 77-95% of the theoritical radioactivity (90-95 μCi ^{3}H-B(a)P/mg DP) was actually measured in the lungs of these guinea pigs that were sacrificed within 20-25 minutes of instillation. As observed by Henry and Kaufmann (5), in the present study also the discrepancy between the amount of dose actually intended for delivery and the amount actually measured in the lungs after intratracheal instillation could not be accounted for any loss due to regurgitation of the suspension.

Percentages of radioactivity retained by six guinea pig lungs at 48 hours following intratracheal instillation of DP bound ^{3}H-BP, when calculated on the basis of the administered dose, showed a mean disapperance of 42% ± 6 of radioactivity during the 48 hour time period. However, the observation that actual delivered dose was somewhat less than the dose intended for instillation meant that the lungs might have lost much more radioactivity during the 48 hours time interval. It was also noted that at 48 hours radioactivity had distributed widely in that all the organs studied such as liver, kidney and intestine showed about 1-2% as much ^{3}H-BP content per gram in comparison to the radioactivity retained per gram of lung tissue. Furthermore, during 48 hours 25% ± 3 of the radioactivity was excreted in urine and feces in these animals.

In conclusion, these studies show that ^{3}H-BP dissociates from the labelled diesel particles upon instillation in the lungs and appears in urine and feces. This rapid dissociation of ^{3}H-BP from the diesel particles implies that by the existing method of labelling of DP by adsorption with ^{3}H-BP we may not have simulated the forces by which benzo(a)pyrene binds to diesel particles under engine condition.

REFERENCES

1. Chaudhari, A., R.G. Farrer and S. Dutta. 1981. Effect of exposure of diesel exhaust of pulmonary prostaglandin dehydrogenase (PGDH) activity. J. Appl. Toxicol. 1: 132-134.

2. Chaudhari, A. and S. Dutta. 1982. Alteration in tissue glutathione and angiotensin converting enzyme due to inhalation of diesel exhaust. J. Toxicol. Envir. Heal. (In Press).

3. Navarro, C., J. Charboneau and R. McCauley. 1981. The effect of in vivo exposure to diesel exhaust of rat hepatic and pulmonary microsomal activities. J. Appl. Toxicol. 1: 124-127.

4. Siak, J., T.L. Chani and P. Lee. 1979. Diesel particulate extracts in bacterial test system. Presented at the U.S. Environmental Protection Agency Symposium on Health Effects of Diesel Engine Emissions. Cincinnati, Ohio.

5. Henry, M.C. and D.G. Kaufman. 1973. Clearance of benzo(a)pyrene from hamster lungs after administration of coated particles. J. Nat. Canc. Inst. 51: 1961-1964.

THE POTENTIAL FOR AROMATIC HYDROXYLASE INDUCTION IN THE LUNG BY INHALED DIESEL PARTICLES

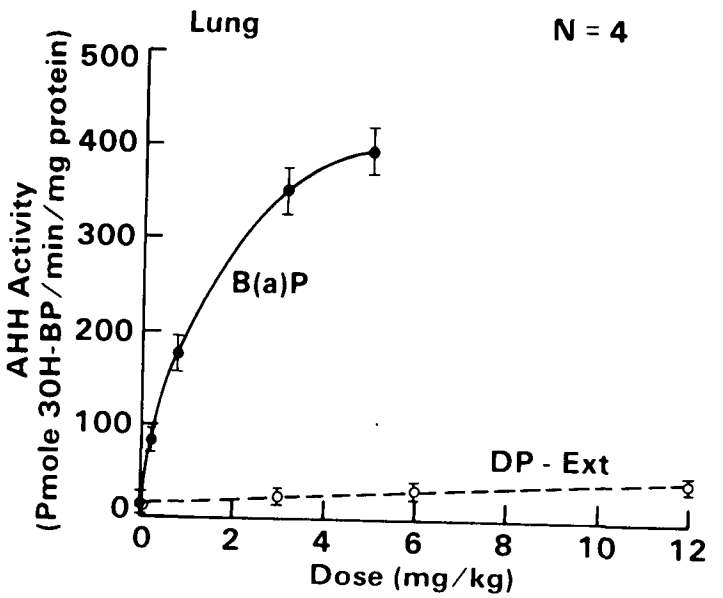
K. C. Chen, and J. J. Vostal
Biomedical Science Department
General Motors Research Laboratories
Warren, MI 48090

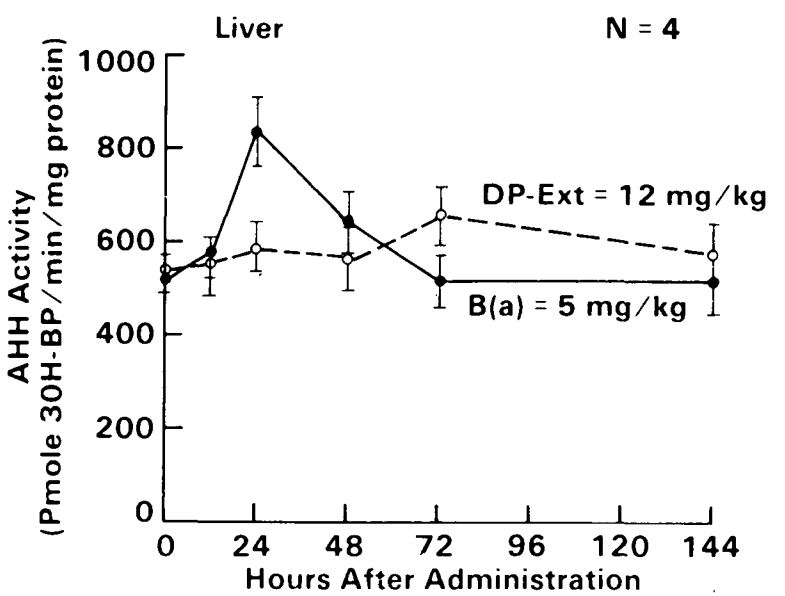
Diesel exhaust particles contain trace amounts of a wide spectrum of polyaromatic hydrocarbons (PAH) adsorbed on the surface and, when extracted by an organic solvent, produce mutagenic effects in short-term microbial laboratory tests. Since the mutagenic or carcinogenic effects of hydrocarbons are frequently initiated by a metabolite rather than by the parent molecule, and since the activity of the metabolizing enzymes can be easily increased by preceding administration of powerful inducers, the enzyme induction could theoretically predetermine the potential for the adverse health effects of inhaled diesel exhaust emissions.

The effects of long-term inhalation of diluted diesel exhaust on aryl hydrocarbon activity (AHH) and cytochrome P450 content in lung and liver microsomes were investigated in male Fischer-344 rats and compared with repeated parenteral administration of organic solvent extracts of hydrocarbon adsorbed on the diesel particulate surface during the combustion process. No significant effects of long-term inhalation exposure were observed in liver microsomal AHH activity. The animals were exposed to concentrations of 750 $\mu g\ m^{-3}$ or 1500 $\mu g\ m^{-3}$ of diesel particulates from a 5.7 GM diesel engine at 20 hours per day, 5-1/2 days per week for up to 9 months, or treated by repeated IP injections of diesel particulate extract dissolved in corn oil, from the same engine at the several dose levels for 4 days. A decrease in lung microsomal AHH activity was found in rats following 9 months of exposure to diesel exhaust at the particulate concentration of 1500 $\mu g\ m^{-3}$. In contrast, 1.4- to 9-fold increases in AHH activity were observed in liver and lung microsomes of rats pretreated by intraperitoneal doses 10-15 times larger (25-125 mg/kg BW) than the most conservative estimate of the deposited lung burden [J. Appl. Tox., 1(2):27, 1981].

Since the intraperitoneal injection of diesel particle extract may not fully represent the activity of PAH deposited on the inhaled diesel particles in the respiratory airways, direct intratracheal instillation (ITI) of various doses of extract was used, and microsomal enzyme induction was investigated in the lung as well as in the liver in order to detect the local and systemic response to hydrocarbons deposited in the respiratory system. Diesel particulate extract or pure benzo[a] pyrene, dissolved in a gelatin-saline solution and used as a reference compound, were administered by ITI at several dose levels. The results show that direct intratracheal administration of the diesel particle extract required doses as high as 6 mg/kg BW before the activity of the induced enzyme in the lung was barely doubled (Figure 1). The induction was slow and

occurred selectively in lung only (Figure 2), indicating that diesel particulate extract probably does not absorb easily into the lung circulation, and is not distributed to other organs. The data suggest that the absence of AHH activity induction in rat lung exposed to diesel exhaust is due to the inavailability of hydrocarbons for distribution in the body and insufficient quantities for enzyme induction. All data seem to indicate that the inhaled diesel particles would not be capable of inducing aromatic hydroxylase in the lung unless the total deposited dose in the lung reaches approximately 6-8 mg of the particle extract per kilogram of body weight. Since the extractable portion represents only 10-15% of the total particulate mass, the required pulmonary deposits of diesel particles in a 70 kg man would be excessive to become a significant step in promotion of a potential neoplastic process.





XENOBIOTIC METABOLIZING ENZYME LEVELS IN MICE EXPOSED TO DIESEL EXHAUST OR DIESEL EXHAUST EXTRACT

by

William Bruce Peirano
Health Effects Research Laboratory
U.S. Environmental Protection Agency
Cincinnati, Ohio

Diesel exhaust (DE) contains organics, specifically polycyclic aromatic hydrocarbons (PAH), which are mutagenic[1] and also potentially carcinogenic. These PAH, for the most part, must be metabolized via the xenobiotic metabolizing enzymes to become active mutagens and/or carcinogens. The primary functions of these enzymes are to detoxify and/or to make these PAH more readily excretable. Unfortunately, a percentage of the metabolites formed are reactive electrophiles, which can bind to proteins, RNA and/or DNA to cause mutations and/or cancer. Moreover, the body can respond to an environmental PAH assault by increasing the levels of the metabolizing enzymes; thus, potentially increasing the formation of active metabolites and the potential risk of cancer. Therefore, the effects of DE on the xenobiotic metabolizing enzymes levels were determined as one approach to assessing the potential carcinogenic risk from DE exposure.

The inhalation study involved the chronic exposure of Strain A/J male mice 8 hours/day, 7 days/week for periods of 6 and 8 months to clean air or DE diluted to 6 mg/m^3 of particulates at the U.S. Environmental Protection Agency, Center Hill facility in Cincinnati, Ohio[2]. In this study, lung and liver microsomal aryl hydrocarbon hydroxylase (AHH) activities and liver microsomal cytochrome $P_{448/450}$ levels were determined using the slightly modified methods of Van Cantfort (1977) et al., and Omura and Sato (1964), respectively. The results (Table 1) indicated that there were no statistical differences in the liver microsomal cytochrome $P_{448/450}$ levels and liver microsomal AHH activites between clean air and DE exposed mice both at 6 and 8 months. Small differences were noted in the lung microsomal AHH activities, but these are believed to be artifactual differences, due to increases in non-microsomal lung protein present in the microsomal preparations. The only significant differences found were in the weight of the animals' lungs. The DE exposed mice were found to have significantly increased wet lung weights, which could be attributed, in

part, to the deposition of diesel particulates in the lungs and possibly to lung physiological and biochemical changes caused by the DE insult on the lungs.

A follow-up study was conducted to assess the ability of extracted diesel particulate organics to cause changes in the levels of liver cytochrome $P_{448/450}$. This was done to see if changes in these enzymes levels could be produced from intraperitoneal (i.p.) injections of diesel exhaust extract (DEE) given at a maximum tolerated dose to mice. The extract of the diesel particulates was used because it should be fully bioavailable to the body's systems, whereas there are still doubts as to the degree of bioavailability of the DE organics when adsorbed onto diesel carbon particulates and then deposited in the body. The experimental approach involved the i.p. injection of male and female Strain A/J mice with DEE, the positive enzyme inducing compounds phenobarbital (PB, a cytochrome P_{450} inducer) or 3-methylcholanthrene (MC, a cytochrome P_{448} inducer), or the appropriate vehicle controls for two days and sacrificing the animals the third day. Liver microsomal preparations were immediately prepared after sacrificing and the liver microsomal cytochrome $P_{448/450}$ levels were determined. The total doses given per kg body weight were DEE - 500 mg, PB - 160 mg, MC - 40 mg, and vehicle controls (DMSO and saline) - 300 ul. The DEE was derived from the 24-hour soxhlet extraction, without cellulose thimble, of DE particulates collected on teflon coated pallflex TbOA20 type filters, using methylene chloride as an elutant. The extract was then made up to the desired injectable concentration in DMSO via solvent exchange using a nitrogen atmosphere for methylene chloride removal. The results (Table 2) showed that PB and MC did result in the induction of the respective P_{448} and P_{450} enzymes in both sexes as expected. The injected DEE caused significant increases in the liver cytochrome $P_{448/450}$ levels in male but not female mice. This increase, however, was smaller than those seen in the PB and MC induced animals, and the male DEE induced enzymes were found spectrally between where the cytochrome P_{448} and P_{450} enzymes were found for PB and MC, respectively.

It is therefore concluded, from the chronic inhalation and the i.p. injection studies, that enzyme inducing chemicals are present in DE and that the absence of enzyme changes found in the mice exposed to DE via inhalation may be due to 1) the enzyme inducing organics associated with DE were not bioavailable to the body system and/or 2) the inhalation dose was not sufficient to elicit a detectable change in the enzyme levels.

REFERENCES

1. Pitts, Jr., J.N., K. Van Cauweberghe, A.M. Winer, and W.L. Belser. 1979. Chemical Analysis and Bioassay of Diesel Emission Particulates. U.S. Environmental Protection Agency Report of Contract No. R806042.

2. Hinners, R.G., J.K. Burkart, M. Malanchuk, and W.D. Wagner. 1980. Animal Exposure Facility for Diesel Exhaust Studies. In: Generation of Aerosols. K. Willeke, ed. Ann Arbor Science Publishes: Ann Arbor, Mich., pp. 525-540.

3. Van Cantfort, J., J. DeGraeve, and J.E. Gielen. 1977. Radioactive Assay for Aryl Hydrocarbon Hydroxylase. Improved Method and Biological Importance. Biochem. Biophys. Res. Comm. 79: 505-512.

4. Omura, T., and R. Sato. 1964. The Carbon Monoxide - Binding Pigment of Liver Microsomes. J. Biol. Chem. 239: 2370-2378.

Table 1. Liver Cytochrome $P_{448/450}$ Levels and Liver Aryl Hydrocarbon Hydroxylase Activity in Mice Exposed to Clean Air or Diesel Exhaust

Months Exposed	Cytochrome $P_{448/450}$ Level Values = $\bar{X} \pm$ SEM in (nMoles/mg microsomal protein) *n = sample size		Aryl Hydrocarbon Hydroxylase Activity Values = $\bar{X} \pm$ SEM (pMoles/min./mg microsomal protein) *n = sample size	
	Control	Diesel Exposed	Control	Diesel Exposed
6	1.52 ± 0.073 n = 10	1.54 ± 0.066 n = 11	48.41 ± 2.40 n = 10	44.08 ± 1.26 n = 11
8	1.61 ± 0.067 n = 9	1.62 ± 0.081 n = 8	50.84 ± 2.57 n = 9	49.04 ± 1.76 n = 8

*Each sample consisted of pooled microsomes from two mice.

Table 2. LIVER CYTOCHROME $P_{448/450}$ LEVELS

Values = $\overline{X} \pm$ SEM in (nMoles/mg microsomal protein)

n = Sample Size

	Saline (control) 100 μl/30gm BW	Phenobarbital 160 mg/Kg BW in 100 μl saline	DMSO (control) 300 μl/30gm BW	Diesel Exhaust Extract 500 mg/KG BW in 300 μl DMSO	3-Methylcholanthrene 40 mg/KG BW in 300 μl DMSO
Males	1.386 ± 0.049	2.426 ± 0.020	1.096 ± 0.059	1.346 ± 0.080	1.524 ± 0.077
	n = 4	n = 4	n = 8	n = 6	n = 6
Females	1.302 ± 0.067	3.013 ± 0.127	1.106 ± 0.056	1.186 ± 0.066	1.512 ± 0.056
	n = 4	n = 4	n = 7	n = 5	n = 6

MORPHOMETRIC ULTRASTRUCTURAL ANALYSIS OF ALVEOLAR LUNGS OF GUINEA PIGS CHRONICALLY EXPOSED BY INHALATION TO DIESEL EXHAUST (DE)

Marion I. Barnhart, Steven O. Salley*,
Shan-te Chen and Henry Puro**
Departments of Physiology, Anesthesiology* & Pathology**
Wayne State University School of Medicine
Detroit, MI 48201

Sixty-four Hartley guinea pigs were sacrificed at intervals up to two years during chronic inhalation exposure to 0, 250, 750, 1500 or 6000 μg DE/m^3. Preliminary findings are published on short term experiments with chronic inhalation of DE (1,2). The cellular uptake of DE particulates (DEP) was strikingly accomplished by alveolar and interstitial macrophages as well as by epithelial type I cells (Epi I) and recruited eosinophils (Fig. 1). Cellular DEP (bulls-eye profiles of 0.073 ± 0.01 μm diameter) was confined to phagolysosomes and there was no evidence of cytotoxicity. Arithmetic and harmonic mean tissue thicknesses of the air-blood barrier were occasionally but significantly increased ($p < 0.05$) during DE exposures greater than 250 μg; the 6 mon 1500 μg set was 2 fold greater than controls. However, morphometric diffusion capacity was relatively unaffected. Epithelial type II cell (Epi II) prominence and increased interstitial thickness were evident as early as 2 weeks with 750 μg DE. Increased interstitial cellularity (up to 4 fold control values) suggests the presence of local chemotactic substances. However, adaption to the DE challenge and burden may be occurring since comparison of the duration effects reveal a reduced but still abnormal interstitial cellularity; eg. 183 x 10^6 interstitial cells occurred by 6 mon contrasting with only 64 x 10^6 cells by 18 mon 1500 μg DE. Non-cellular interstitium of 250 and 750 μg sets was not significantly different in absolute volume from controls through 6 mon but 1500 μg DE promoted more interstitial fibers. Epithelial type II cells increased in both numbers (3.2 times control of 18 x 10^6 cells/cm^3) and absolute mass (1.7 x controls). In contrast, Epi I by 3 mon at 750 μg DE had decreased numbers (30-60% of controls) but cell volumes were increased. Endothelial cell numbers were generally increased up to 3 fold the control values and volumes were decreased. Alveolar macrophages increased 2-3 times control numbers with little significant changes in volume per cell. Particularily notable are comparative data from animals experiencing possibly equivalent dose-duration DE exposures. One comparative set contrasts 6 mon 1500 μg DE with 12 mon 750 μg DE. There was a 2-4 fold increase in cellularity in the short duration high dose partner or the longer duration equivalent partner set was less responsive. Although certain responses to chronic DE were dose dependent the absence of linearity suggests adaptive responses to DE challenge. (Aided in part by General Motors Research Laboratory, Warren, MI).

REFERENCES

1. Barnhart, M.I., Chen, S. and H. Puro. 1980. Impact of diesel engine exhaust (DEE) particles on the structural physiology of the lung. Health Effects of Diesel Engine Emissions: Proc. Internat. Symp., Vol. 2, pp. 649-672. Center for Environ. Research Information EPA, Cincinnati, OH.
2. Barnhart, M.I., Chen, S., Salley, S.O. and H. Puro. 1981. Ultrastructure and morphometry of the alveolar lung of guinea pigs chronically exposed to diesel engine exhaust: Six month's experience. J. App. Tox. 1: 88-103.

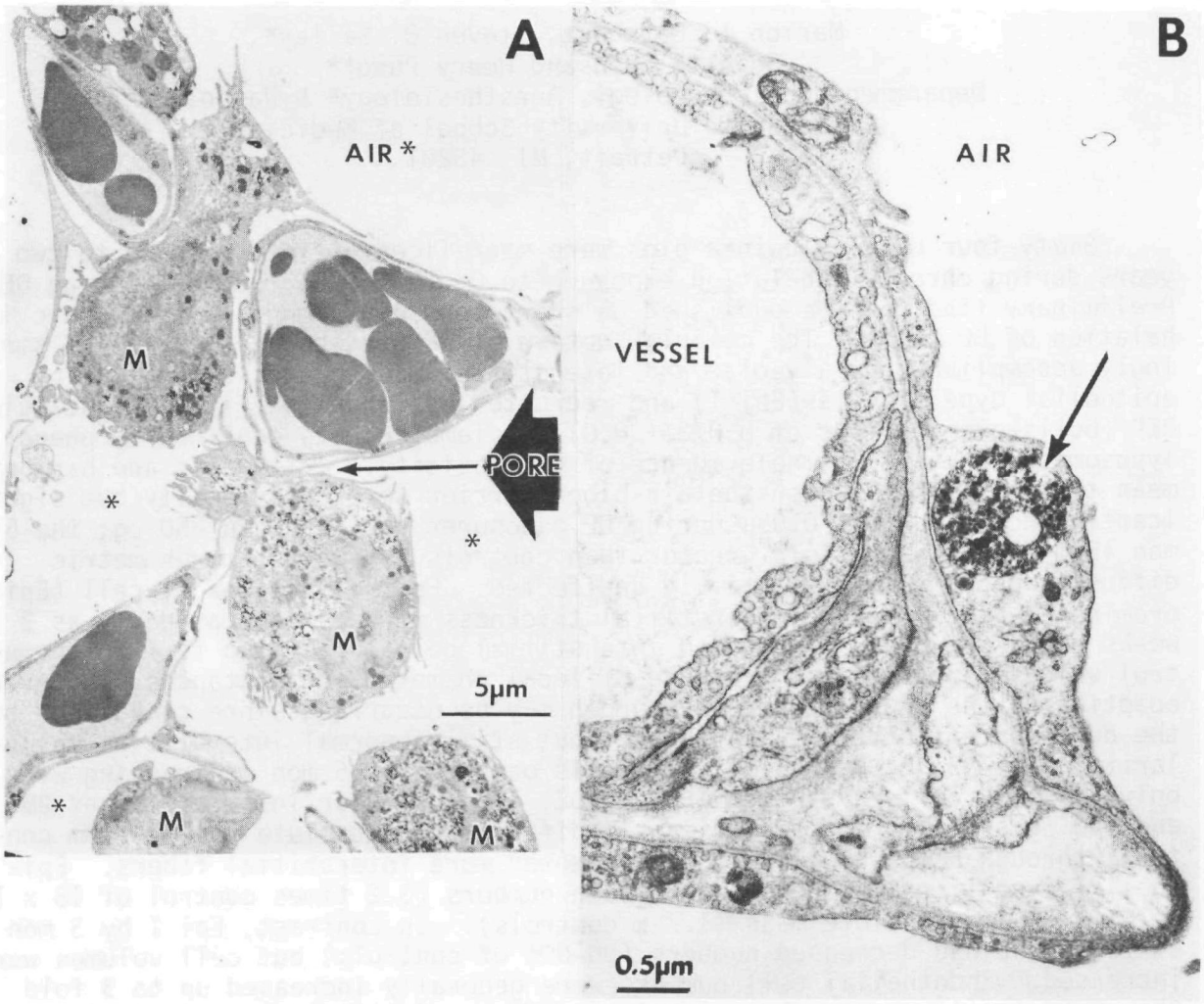


Fig. 1. Uptake of DEP by lung parenchymal cells.

1A - DE-laden alveolar macrophages can pass through pores of Kohn in alveolar lung.

1B - DEP in epithelial type I cell of lung from 6 mon 250 μg DE exposure.

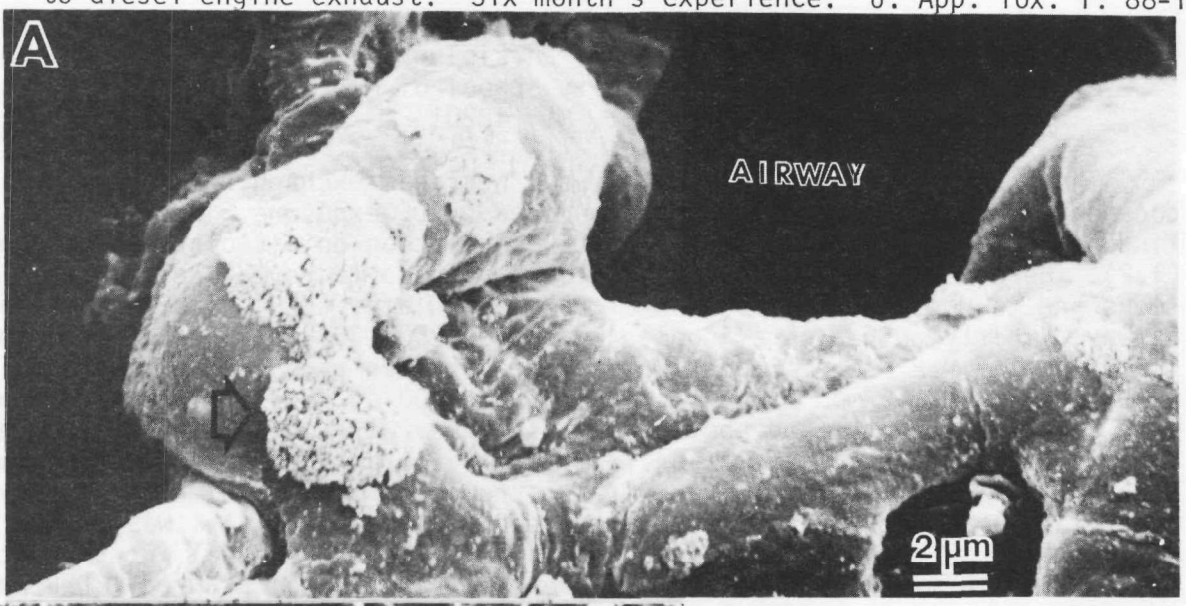
SCANNING ELECTRON MICROSCOPY OF TERMINAL AIRWAYS OF GUINEA PIGS CHRONICALLY INHALING DIESEL EXHAUST (DE)

Marion I. Barnhart, Fatma Mohamed and Ahmet Kucukcelebi
Department of Physiology
Wayne State University School of Medicine
Detroit, MI 48201

The structural physiology of airways near gas exchanging alveoli was documented to establish any changes induced by DE exposure. Preliminary findings are published on effects of DEP inhalation on alveolar macrophages (1,2). Here scanning electron microscopy was used to reveal cell interrelations and to resolve distribution of DE particulates (DEP) along the terminal airway. Thirty guinea pigs inhaled either 0, 250, 750 or 1500 μg DE/m^3 for 110 hr/week for 2 weeks, 3 and 12 mon while fifteen rats were exposed for 10 weeks 6000 μg DE, 6 mon 750 μg DE and 12 mon 1500 μg DE. Peripheral airways were selected for study and photography when they were of sufficient length to provide structural information from terminal bronchiolus to alveolar outpockets. Airways were evaluated without knowledge of the animal's history. The relative amount and distribution of deposited particulate, was graded on a scale of 1 to 5+. Decoding was done later followed by final interpretations. At least 10 terminal airways/animal were extensively photographed. DEP was identified as free individual particles, 0.1 + 0.03 μm, (Fig. 1). DEP was adherent to epithelium and irregular patches of particles were prominent at airway bifurcations (Fig. 1A). Proximal airway, characterized by an epithelium of secretory and ciliated cells, had even larger agglomerates of particulates especially in 12 mon 1500 and 10 week 6000 μg DE sets. These agglomerates consisted of various sized particles, only some of which had sizes appropriate for DEP. Quite likely some of the admixture was secretory granules and congealed proteins; which tend to be larger and more irregular than particles suspected of being DEP (Fig. 1B). Terminal bronchioles often were crowded with macrophages and granulocytes, exiting the lung. Surface domes were prominent on Clara cells which may be increased in number in DE sets. More pneumocyte II cell clusters occurred at bronchiolar-alveolar junctions in heavily exposed than in age-matched control animals. Broad expanses of terminal airway in DE exposed animals appeared relatively clean, but still contained more particulate than companion controls. Alveoli opening off terminal bronchioles had more particulates than other alveoli. The morphology and 0.1 μm size of the spherical particles and relative sparcity of such in controls suggests that this is a visualization of the DEP burden but is insufficient for absolute identification. However the highest DE exposure conditions were associated with the dustier terminal airways. (This study was aided in part by General Motors Research Laboratory, Warren, Michigan).

REFERENCES

1. Barnhart, M.I., Chen, S. and H. Puro. 1980. Impact of diesel engine exhaust (DEE) particles on the structural physiology of the lung. Health Effects of Diesel Engine Emissions: Proc. Internat. Symp., Vol. 2, pp 649-672. Center for Environ Research Information EPA, Cincinnati, OH.
2. Barnhart, M.I., Chen, S., Salley, S.O. and H. Puro. 1981. Ultrastructural and morphometry of the alveolar lung of guinea pigs chronically exposed to diesel engine exhaust: Six month's experience. J. App. Tox. 1: 88-103.



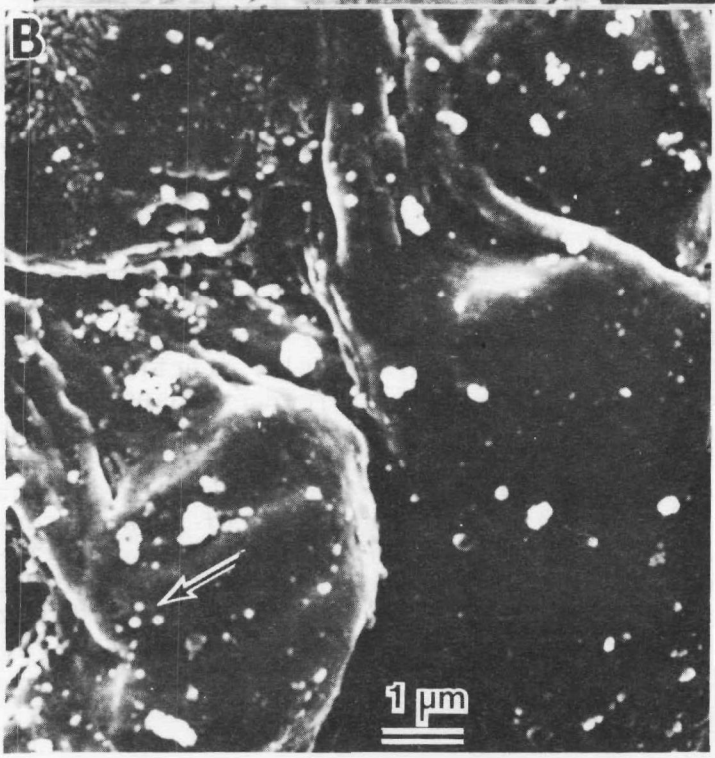


Fig. 1A. Terminal airway of guinea pig exposed to 1500 µg DE for 12 mon. Note patches of particles whose individual size is 0.1 µm and could be DE deposits.

Fig. 1B. Terminal bronchiole adjacent to alveolus in rat exposed to 6000 µg DE for 2 mon. Small dust particles are 0.1 µm in diameter and probably DEP.

THE DESIGN OF THE CCMC'S LONG-TERM INHALATION PROGRAM TO INVESTIGATE THE POSSIBLE TOXICOLOGICAL EFFECTS OF DIESEL AND GASOLINE ENGINE EXHAUST EMISSIONS

by

J. Brightwell, R.D. Cowling, X. Fouillet, R.K. Haroz,
H. Pfeifer, and J.C. Shorrock
Center for Toxicology and Biosciences
BATTELLE
Geneva Research Centres
Switzerland

The health effects program of the Committee of Common Market Automobile Constructors (CCMC) on diesel and gasoline engine emissions is presented in another poster. Part of this program, the long-term inhalation study, is being carried out by Battelle-Geneva, and in this poster we present the design of the equipment and the experimental protocol.

Four different types of emissions--diesel (D), filtered diesel (DF), gasoline (G), and gasoline with convertor (GC)--are generated by three engines (VW Rabbit 1.5 litre diesel and two Renault R18 1.6 litre gasoline) running on the FTP (US-72 hot start cycle). Two species of animals (Syrian hamsters and Fischer-344 rats) will be exposed for up to 24 months, 16 hours per day, 5 days per week.

These emissions can be diluted to three dose levels: high (H), medium (M), and low (L). From this 4 x 3 matrix of exhaust types and dose levels, three have been omitted as being of little potential interest. An indicative matrix of dose levels selected for exposure are shown in Table 1.

Table 1. Matrix of Dose Levels

Dose Levels	D	DF	G	GC
H	8.3	8.3	3.6	3.6
M	2.8	2.8	1.2	1.2
L	0.92	-	-	-

The figures given in the above table are the calculated mean exhaust gas concentrations (% volume) when the exhaust gases from one cycle are diluted by the same volume of air for all engines. In this example the dilution air volume per cycle for the high dose levels is 300 Nm^3 (equivalent to 40 Nm^3/mile). The M and L dose levels have been set at 1/3 and 1/9 of the H levels.

Such dose levels are considered to be directly comparable across the table since they are based on equal mileage. This comparison takes into account the different internal dilution taking place in the engines and also the different fuel efficiences of the automobiles being compared.

In Table 2, the high dose levels, HD and HG, are expected to correspond to the following concentrations of the regulated components (running the gasoline engine at lambda = 1).

Table 2. High Dose Level and Concentration Correspondence

Components	Units	Diesel (HD)	Gasoline (HG)
Exhaust gas in air	%	8.3	3.5
Particulate matter	mg/m^3	5.5	-
CO	ppm	20	203
No_x (NO_2 equivalent)	ppm	15	49
THC	mg/m^3	9.2	37

The figures given in these tables are all calculated from the data supplied by the automobile manufacturers. They are currently being evaluated in our system and, if necessary, the flow rate of air to the dilution tunnels will be modified with a view to keeping concentrations of the biologically critical components in HD and HG at as high a level as is considered compatible with a chronic study.

Although slight differences exist in the distribution systems for each engine, the basic principle remains the same and is described below for one engine.

The exhaust gases are injected from the tail pipe directly into a dilution tunnel where they are mixed with a constant flow of conditioned air (filtered and dried to a water content of 7 g/kg air). The air is dried to compensate, at least partially, for the high water vapour content of the gasoline engine emissions so that condensation does not take place in the dilution tunnel, and to ensure that the relative humidity in the inhalation chambers is not too high.

A fraction of the diluted exhaust gas is drawn off from the mixing tunnel into a buffer tank where it is held for about one minute. The need for this tank is being investigated, and it will be removed from the system if not required. Its role is to attenuate the high peak values of NO_2 and CO that are produced by the engines over short periods during the US-72 cycle to a level at which they do not appreciably affect the breathing pattern of the animals.

The dilution tunnels are run at the high dose level. The medium and low dose levels are achieved by further diluting the high dose level with air. The dose levels are computed directly from flow rate measurements using rotameters.

The high dose level streams will be continuously monitored for CO and NO_x for safety purposes. The concentrations of the regulated components and certain non-regulated components will be checked in the inhalation chambers at regular intervals.

Battelle-Northwest designed Hazleton-1000 inhalation exposure chambers will be used, each chamber housing one treatment group of 72 male and 72 female rats or 156 male and 156 female hamsters. The control groups (fresh air) will contain 288 rats and 624 hamsters.

Initial and interim sacrifices will be made on 8 animals of each sex from each group after 0, 6, 12, and 18 months of exposure. These animals will be used for respiratory physiology, haematology, urinalysis, and blood chemistry investigations.

Complete autopsies will be carried out on all animals in the study and the animals in the highest dose levels and the control groups will be subjected to a histopathological examination of the respiratory tract. Any anomalies detected during autopsies will also be subjected to a histopathological diagnosis. Other organs will be stored in formalin and be available for further examination if required.

System testing of the experimental facility and some short-term exposures at the high dose levels were carried out up to August 1981. The plan to start up the long-term exposure using staggered intakes of animals during the months of September to December 1981 has unfortunately been delayed by several months due to an accident. Rebuilding and testing of part of the facility is now necessary before the long-term exposure can be started.

CHRONIC INHALATION ONCOGENICITY STUDY OF DIESEL EXHAUST IN SENCAR MICE

by

K. I. Campbell, E. L. George, I. S. Washington, Jr.,
P. K. Roberson, and R. D. Laurie
Health Effects Research Laboratory
U. S. Environmental Protection Agency
Cincinnati, Ohio

ABSTRACT

A large number of Sencar mice were used in an investigation to assess the long term potential inhalation oncogenicity of automotive diesel emissions. After exposure of parental mice from before mating on through gestation, the offspring continued in exposure 8 hours daily for 15 months to an atmosphere of diluted automotive diesel engine exhaust bearing 6 mg particulate/m^3. Subgroups for testing initiation (A), promotion (B), and whole carcinogen (C) potentials of diesel exposure were administered, respectively, weekly i.p. injections of promoter (butylated hydroxytoluene) for about 1 year, an initial single i.p. injection of initiator (urethan), and neither promoter or initiator. Matching controls were exposed to purified air. Each subgroup initially numbered 260, equally divided by sex. Additional negative, positive, and vehicle control groups were used.

Over all groups, survival was 13 percent less in diesel-exposed than in control mice (75 vs 65 percent), the initiation test group males being the most affected. Survival was least in initiation-test mice, due apparently in large part to consequences of frequent i.p. BHT-in-oil injections. In all subgroups except A females survival was greater in control than in diesel-exposed mice. In general, males were more susceptible than females to reduced-survival effects of diesel exposure, and survival effects were least severe in mice receiving diesel exhaust only (i.e., not receiving promoter or initiator).

In periodically weighed sample groups, body weight gain was depressed in diesel-exposed mice of both sexes in all subgroups compared to corresponding clean air controls, the effect ranging from 11 to 24 percent of control mean weight gain. Similarly, mean terminal body weights taken for all survivors showed lower values (ranging 7 to 17%) for both sexes in all subgroups of diesel-exposed compared to control mice.

Brief inspection of histopathology results indicate no remarkably consistent differences in lung or other tumor incidences associated with diesel exhaust exposure. However, for several types of respiratory lesions there were consistently and greatly increased incidences in diesel-exposed compared to control mice. These lesions included: alveolar macrophages, black alveolar pigment material, perivascular and peribronchial mononuclear cells, focal fibrosis, alveolar interstitial thickening, rhinitis (females), and black pigment in mediastinal lymph nodes. Predictably, serositis was a common lesion in mice of the BHT-in-oil injected groups of both control and diesel atmospheres.

SPECIES DIFFERENCES IN DEPOSITION AND CLEARANCE OF INHALED DIESEL EXHAUST PARTICLES

T. L. Chan and P. S. Lee
Biomedical Science Department
General Motors Research Laboratories
Warren, Michigan 48090

Deposition of inhaled diesel particles and their subsequent clearance from the gas exchange regions of the respiratory tract may play important roles in the question of potential health impact of diesel emissions. The initial particulate deposition in the lungs depends on the physical characteristics of the particles and the airways morphometry may also affect the regional deposition within the lungs. For example, the narrow nasal passages can shift the particle deposition in the nasal region proximally for particles larger than a micron in small experimental animals, and the final deposition patterns in the lungs will be significantly different from those of larger species or man. Although diesel particles are not large enough to deposit by impaction in the upper respiratory tract, species differences can still exist in alveolar clearance mechanisms, clearance pathways and kinetics. Table 1 compares the estimated initial particulate dose to the lungs in different species exposed to 0.1 μm particles at 250 $\mu g/m^3$ for an hour. Although the absolute particulate burden by weight is highest in man, the immediate local dose to the lung tissues is expected to be five times higher in the dog and guinea pig. The relative dose is even higher in rats and hamsters by at least a factor of ten.

Male Hartley guinea pigs and Fischer 344 rats were exposed in a nose-only inhalation chamber to radioactive diesel exhaust particles. The particles, tagged in the insoluble carbonaceous core with ^{14}C, were generated by combustion of (1-^{14}C)-n-hexadecane in a single cylinder diesel engine operated at full load [1]. The ^{14}C activity in the lungs and lymph nodes were determined for groups of exposed animals sacrificed immediately after the 45-minute exposure and others at scheduled intervals for an extended period of time. Although the initial lung deposition efficiencies and mucociliary clearance half-times were comparable in both species, the amount of inhaled diesel particles cleared from the upper respiratory airways in the guinea pig accounted for only 17% of the initial lung burden, compared to 34% in the rat. Furthermore, the alveolar clearance of diesel particles in the guinea pig was extremely slow, with more than 80% of the initial dose retained after 105 days (Figure 1). The pulmonary clearance half-time for inhaled diesel particles in the guinea pig is estimated to exceed 300 days which strongly contrasts with 60-80 days in rats (determined by fitting experimental data collected so far to two- or three-phase clearance models.) The differences observed in this study demonstrate a greater long-term retention of inhaled diesel particles in the guinea pig possibly caused by slower clearance processes in the deep lung of this species. The actual biological dose to the respiratory epithelium would also be

different in both species. This clearly indicates the difficulty in comparing studies on potential health effects of inhaled diesel particles among different species and in extrapolating experimental animal data to man.

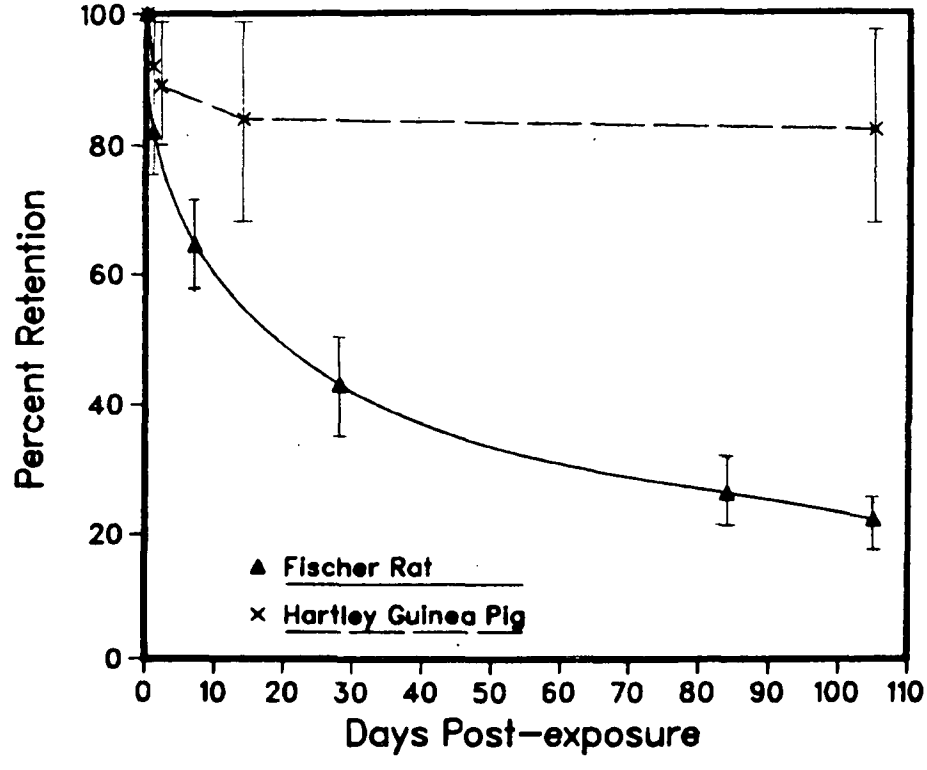
TABLE 1. ESTIMATED INITIAL LUNG DEPOSITION DOSE IN DIFFERENT SPECIES AFTER INHALATION OF 0.1 μm PARTICLES AT 250 $\mu g/m^3$ FOR 1 HOUR

Species	Body Weight (g)	Minute Volume (mL)	Deposition Efficiency (%)	Lung Wt (g)	Particulate Burden (μg)	Particles/g Lung Tissue (μg/g)
Man	70K	7000	25	1000	26	0.025
Dog	12K	3100	27	80	12	0.15
G. Pig	400	125	20	3.0	0.4	0.15
Rat	250	150	17	1.5	0.4	0.25
Hamster	92	61	20	0.4	0.2	0.50

REFERENCES

1. T. L. Chan, P. S. Lee, and W. H. Hering, Deposition and clearance of inhaled diesel exhaust particles in the respiratory tract of Fischer rats. J. Appl. Tox., 1:77-82, 1981.

Lung Retention of Inhaled Diesel Particles



SPECIES COMPARISONS OF BRONCHOALVEOLAR LAVAGES FROM GUINEA PIGS AND RATS EXPOSED IN VIVO TO DIESEL EXHAUST (DE)

Shan-te Chen, Mary Ann Weller and
Marion I. Barnhart
Department of Physiology
Wayne State University School of Medicine
Detroit, MI 48201

This ultrastructural, biochemical and functional study involved 93 Hartley guinea pigs and 63 Fischer rats divided into control and DE exposed sets of 3 animals. Preliminary reports on certain aspects of this study have been published (1-3). Clean air or DE exposures of 250, 750, 1500 and 6000 μg DE particulates/m^3 were inhaled for a few days or chronically up to 18 mon. Broncholavage with Dulbecco's phosphate buffered saline provided free cells, which in controls, were 89-100% macrophages. Viability according to dye exclusion was 90-99% in all specimens. The absolute number of alveolar macrophages increased 1.4 to 2 times control values (7.63 ± 1.11 million cells) by 6 and 12 mon exposure to 750 and 1500 μg DE in guinea pigs. In a short term study in rats after 2 mon 6000 μg DE macrophage number was 5 times controls. Macrophage size also increased, excepting 250 μg DE sets. Maximum diameters and surface areas were measured on scanning electron micrographs of cytocentrifuged lavage specimens. Calculated macrophage volume increased up to 4 times the controls. After the *in vivo* DE exposure there were ultrastructural signs of macrophage activation. DE particulates were phagocytized without cytotoxic effects since phagosomal membranes remained intact and macrophage lactic dehydrogenase activity (a signal of cell lysis) was not in lavage fluids. Cytofluorometry revealed partial blockade of macrophage phagocytosis of ex vivo fed latex. Macrophages from 12 mon 750 and 1500 μg DE/m^3 exposures had reduced staining for acid phosphatase while the cell free lavage fluids showed 2-5 fold increases in that enzyme. Comparison of equivalently dosed animals revealed similar magnitudes of change in macrophage number and granulocyte recruitment. Upon DE exposure granulocytes became a significant percent of the free cell population by 1 mon at 6000 μg DE, 2 mon at 750 and 1500 μg DE and 12 mon at 250 μg DE. In rat lavages neutrophilic granulocytes appeared contrasting with eosinophilic granulocyte mobilization in guinea pigs. Lymphocytes appeared in lavages of both species after 2 mon. While rats appear to be less responsive to the DE challenge, both species show elevations of albumin, IgG and total protein in the longer duration and higher exposure sets (Table 1). Both granulocyte emigration and elevations in proteins at the high DE doses are features of a classic inflammatory response, but the 250 μg DE exposures even to 18 mon elicited few and generally insignificant changes over controls. Defense capabilities of these healthy rodent species appear adequate to cope with chronic DE challenges at the tested concentrations.

REFERENCES

1. Chen, S., Weller, M.A. and M.I. Barnhart. 1980. Effects of diesel engine exhaust on pulmonary alveolar macrophages. Scanning Electron Microsc. 3:327-338.
2. Weller, M.A., Chen, S. and M.I. Barnhart. 1981. Acid phosphatase in alveolar macrophages exposed *in vivo* to diesel engine exhaust. Micron 12: 89-90.
3. Barnhart, M.I., Chen, S. and H. Puro. 1980. Impact of diesel engine exhaust (DEE) particles on the structural physiology of the lung. Health Effects of Diesel Engine Emissions: Proc. Internat. Symp., Vol. 2, pp. 649-672. Center for Environ. Research Information EPA, Cincinnati, OH.

Table 1. Comparison of Dose-Duration Effects of Diesel Exhaust Exposure on Protein & Enzyme Content of Acellular Broncholavage Fluids

ANIMALS (#) & CONDITIONS	TOTAL PROTEIN mg/ml	ALBUMIN mg/ml	IgG mg/ml	ACID PHOSPHATASE n M/hr/mg prot
GUINEA PIGS (58)				
CONTROLS (16)				
2 WK-18 MON	4.78 ± 1,35	1.55 ± 0.66	0.14 ± 0.10	7.97 ± 4.58
250 μg DE (15)				
2 WK & 2,4,6 MON	4.82 ± 0.93	1.27 ± 0.65	0.12 ± 0.10	10.32 ± 5.07
12 & 14.5 MON	4.74 ± 1.01	1.41 ± 0.66	0.26 ± 0.17	12.60 ± 6.74
18 MON	10.57 ± 4.19	3.95 ± 2.59	0.72 ± 0.19	11.54 ± 5.85
750 μg DE (14)				
2,6 & 8 MON	7.89 ± 2.64	2.19 ± 0.94	0.39 ± 0.27	16.41 ± 3.25
12 MON	11.50 ± 3.77	5.00 ± 2.61	0.54 ± 0.21	29.18 ± 7.94
1500 μg DE (13)				
2 WK, 2 & 6 MON	8.55 ± 0.80	2.17 ± 0.17	0.25 ± 0.07	13.72 ± 3.07
12 MON	17.88 ± 5.64	4.75 ± 0.90	0.92 ± 0.43	61.76 ±23.48
RATS (38)				
CONTROLS (12)				
2-18 MON	1.94 ± 0.21	0.34 ± 0.19	0.05 ± 0.01	19.10 ±12.34
250 μg DE (11)				
2 MON	1.99 ± 0.45	0.28 ± 0.15	0.04 ± 0.02	29.24 ±15.58
12 & 14.5 MON	1.84 ± 0.18	0.28 ± 0.16	0.06 ± 0.01	28.30 ±10.88
18 MON	2.75 ± 0.08	0.33 ± 0.08	0.11 ± 0.03	23.40 ± 3.56
750 μg DE (9)				
2, 5 & 8 MON	2.38 ± 0.15	0.41 ± 0.18	0.07 ± 0.01	27.26 ± 6.95
12 MON	3.55 ± 0.41	0.89 ± 0.26	0.08 ± 0.0	42.14 ± 6.7
1500 μg DE (6)				
2 MON	2.06 ± 0.23	0.59 ± 0.41	0.04 ± 0.03	43.07 ± 2.98
12 MON	7.26 ± 1.09	4.18 ± 2.67	0.27 ± 0.02	70.93 ±10.82

ACKNOWLEDGEMENTS: This work was partially supported by General Motors Research Laboratories.

PRELIMINARY REPORT OF SYSTEMIC CARCINOGENIC STUDIES ON DIESEL AND GASOLINE PARTICULATE EMISSION EXTRACTS APPLIED TO MOUSE SKIN

by

N.K. Clapp, M.A. Henke, T.L. Shock, T. Triplett, and T.J. Slaga
Biology Division
Oak Ridge National Laboratory
Oak Ridge, Tennessee

and

S. Nesnow
Carcinogenesis and Metabolism Branch
U.S. Environmental Protection Agency
Research Triangle Park, North Carolina

Emission particulates may constitute a potential health hazard to persons constantly exposed. We are determining if emission components given by skin application might cause carcinogenesis in other organs. To the skin of SENCAR mice, we applied dichloromethane extracts from particulates collected by filtration of cooled diluted emissions from Oldsmobile (OLDS) (1 mg/mouse), Nissan (10 mg/mouse), and Volkswagen (VW) (10 mg/mouse) diesel, and Mustang V-8 (3 mg/mouse) gasoline fueled engines. Appropriate controls including benzo(a)pyrene (BP) (0.054 mg/mouse), 12-O-tetradecanoylphorbol-13-acetate (TPA), and aging untreated mice (CONTROLS) were maintained. In each treatment group, 40 male and 40 female 6-week-old mice were treated for 52 weeks following one of two protocols: 1) A single initiation dose of the compound was followed twice weekly by applications of TPA (2 μg); or 2) the test compound was applied twice weekly (OLDS only, 4 mg/mouse/week). Surviving mice were killed 52 weeks after initiation and examined grossly; tissues from 20 different organs were routinely taken for histologic examination. The doses chosen were those that gave maximal tumor-initiating activity (1,2).

Survival and tumorigenesis for the experimental groups are shown in Table 1. The numbers of surviving mice were significantly reduced by TPA alone and all experimental groups given TPA reflected this treatment with decreased survival as compared with controls. The only group that was further reduced in survival by the test compound was Nissan + TPA, which had the lowest survival. Lung tumor incidences varied with treatment groups but were not different from controls and TPA lone. Tumors of other organs were observed randomly in the treated groups but showed no consistent increased incidences associated with treatments; tumors were found in the mammary gland, uterus, pituitary gland, cervix, and liver. No evidence of leukemogenesis was seen in killed animals, although 35% (6/17) of dead males had leukemias in BP + TPA group. In mice

that died prior to the kill date significant numbers exhibited squamous cell carcinomas of the skin with 10 to 25% metastases to regional lymph nodes and lungs in Nissan mice. We have also observed high incidences (40 to 60%) of amyloidosis, primarily in the spleen and liver, and pyelonephritis and papillary necrosis in the kidneys of mice given TPA with or without test compounds as initiators. The relationships and pathogenesis of these diseases are now under investigation. The sex effect, which shows a difference in survival as well as tumorigenesis, is not consistent between treatment groups, and its relationship to the tumor process is unclear at this time. When OLDS was given repeatedly over the 52-week treatment period (protocol 2), tumor incidences were not different from untreated controls; it was not effective as a complete carcinogen with this dose and protocol.

Further analysis of remaining treatment groups and complete of observations on mice through 24 months of age will provide information about temporal advancement and tumor incidence modifications by various treatments.

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REFERENCES

1. Slaga, T.J., L.L. Triplett, and S. Nesnow. 1980. Mutagenic and carcinogenic potency of extracts of diesel and related environmental emission: Two-stage carcinoegenesis in skin tumor sensitive mice (SENCAR). In: Health Effects of Diesel Engine Emissions. Proceedings of an International Symposium, Vol. 2. W.E. Pepelko, R.M. Danner, and N.S. Clarke, eds. EPA-600/9-80-057b. U.S. Environmental Protection Agency: Cincinnati, OH. pp. 874-987.

2. Nesnow, S., L.L. Triplett, and T.J. Slaga. (in press). Tumorigenesis of diesel exhaust and related emission extracts on SENCAR mouse skin. In: Short-Term Bioassays in the Analysis of Complex Environmental Mixtures, 1980. Michael D. Waters, Shahbeg S. Sandhu, Joellen Lewtas Huisingh, Larry Claxton, and Stephen Nesnow, eds. Plenum Press: New York.

Table 1. Survival and Tumor Induction in SENCAR Mice Surviving 1 Year Given Diesel and Gasoline Particulate Emission Extracts to the Skin

			Tumors					
			Skin					
Treatment	Sex[a]	No. surviving mice (%)	T-positive (paps) (%)	No. paps/ No. surviving	T-positive carcinomas	Lung (%)	Misc. (%)	Leukemias (%)
Untreated controls	F	100	0	--	0	3	0	3
	M	97	0	--	0	0	0	0
TPA	F	67	19	0.23	0	4	0	0
	M	71	4	0.04	4	4	4	0
BP + TPA	F	81	74	3.10	0	6	13	0
	M	56	100	4.70	14	4	0	0
OLDS + TPA	F	74	27	0.38	3	14	3	0
	M	95	42	0.67	3	19	0	6
Mustang + TPA	F	80	31	0.46	9	3	0	0
	M	71	12	0.12	0	8	0	0
Nissan + TPA	F	47	94	4.50	11	0	5	0
	M	46	89	3.40	5	5	0	0
VW + TPA	F	68	54	1.00	8	0	4	0
	M	75	50	1.00	3	3	0	0
OLDS (no TPA)	F	97	0	--	0	0	3	0
	M	97	0	--	0	3	3	0

F = female; M = male.

CCMC'S HEALTH EFFECTS RESEARCH PROGRAM

by

the Members of the Emissions Research Committee of the
CCMC (Committee of Common Market Automobile Constructors)
Brussels - Belgium (*)

1. GENERAL

In the past decade much progress has been achieved in cleaning the air by a concerted effort of the governments and the automotive industry. With more health effect data becoming available, further legislative measures involving more stringent car emission standards become more and more questionable. Before stricter standards for emissions of motor vehicles are legislated, resources for research programs on health effects must be deployed on an increased scale.

Already in 1974 in the U.S. the National Academy of Sciences (NAS) established that there were virtually no results available relating to the effects of automobile emissions. Since then the situation has not altered greatly. It is only with the concern about cancer with regard to diesel-powered cars that larger research programs have now been started in the U.S.

Although a limited number of research investigations have shown no carcinogenic effects of human exposure to diesel-engined vehicles' exhaust gases up to now, the European car manufacturers have also initiated a large scale research project on "an investigation into possible toxicological and carcinogenic effects of diesel and gasoline engine exhaust emissions". The project is sponsored by the Committee of Common Market Automobile Constructors (CCMC).

(*) Mrs. Chevrier (Renault), A. Henriet (Peugeot S.A.),
H. Klingenberg (VW), H. Metz (BMW), O. Montabone (Fiat),
N. Pelz (Daimler-Benz), A. Piccone (Alfa Romeo),
S. Wallman (Volvo), J.H. Weaving (BL)

2. OBJECTIVES

This program is considered to be a first step in what will probably be a continuing investigation by the European industry on the potential hazards associated with the emissions from automobiles.

The objectives of this first CCMC program are

- to compare potential toxic and carcinogenic effects of diesel and gasoline engine emissions,
- to check out the presumed beneficial effect of the catalytic converter of gasoline engines,
- to check out the often assumed relative harmlessness of the gaseous fraction of the diesel emissions,
- to investigate the mutagenic properties of diesel and gasoline engine particulates and condensates.

3. PROGRAMS

The project is divided into two parts:

(1) a "long term" inhalation exposure of rats and hamsters to gasoline and diesel engine exhausts;

(2) a "short term" program of in vivo and in vitro tests on the effects of particulate extracts and condensates from gasoline and diesel engine exhausts.

3.1 Long Term Program

The "long term" inhalation program has been contracted out by the CCMC to the Geneva Division of the Battelle Memorial Institute.

With about 6000 hamsters and rats the combined effects of different concentrations of total exhaust from gasoline engines with and without catalyst, and a diesel engine with and without particulate matter, removed by filtration, operating according to the U.S. FTP cycle, are being investigated to determine the dose/response relationship, and to make a carefully controlled comparison of diesel and gasoline engines, with respect to toxicity and carcinogenicity. The running time of the project is 3 years and costs will amount to 4 million U.S. dollars.

The program is described in detail in another paper in this Symposium.

3.2 Short Term Program

The "short term" program, including three main approaches, is being conducted in separate laboratories in England, France and Italy.

In different short term in vivo and in vitro tests with bacteria, mammalian cells, rats and mice, the effects of particulate extracts and condensates from gasoline and diesel engine exhaust, collected at Fiat, Italy, are being investigated in an endeavour to identify the mutagenic components.

The running time of this project is three years and the costs will amount to 0.73 million U.S. dollars.

Three main approaches were taken:

3.2.1 "in vitro" assays - detection and identification of mutagens (Microtest, University of York, United Kingdom)

The four basic preparations (particulate and condensate, both diesel and gasoline) in total and additionally fractionated in accordance with the EPA procedure, are subjected to a modified AMES procedure (bacterial mutation assay) using five strains of salmonella typhimurium (TA 1535, TA 1537, TA 1538, TA 98 and TA 100). The preparations are also tested for the ability to elicit unscheduled DNA synthesis (UDS) in cultured human fibro-blast, (DNA repair assay using Hela cells in culture) and in vitro transformation in rodent cells (mammalian cell mutation assay using mouse lymphoma L 5178 Y cells). All these assays have been combined with a liver monoxygenase enzyme fraction.

The tests are also carried out with extracts of the particulate fractions.

3.2.2 skin painting tests - detection and identification of promoters (Institute of Scientific Research on Cancer (CNRS), Paris, France)

In the short term work sebaceous gland and hyperplasia tests are performed with the different exhaust preparations. Investigation is being undertaken to separate potential cancer initiators and cancer promoters.

Female 45-day old Swiss mice housed individually and randomised between the different groups receive, on well delineated skin areas previously clipped (3 days before), 0.05 ml of acetone solution of the studied substance. The treatment is repeated on alternate days up to a total of 3 skin applications (more applications may be necessary). Eight days after the first treatment, the mice are killed and the areas of treated skin fixed, sectioned and stained for histological examination. The thickness of the epidermis and the number of sebaceous glands present are determined in 2 to 4 microscopic fields of each of 6 sections that are cut from each skin specimen. The microscopic examination is carried out on code-numbered slides which carry no details of treatment given.

For each term or treatment, 25 to 30 mice are being used. To control the model and to evaluate the relative activities of the test compounds, adequate positive (benzo-a-pyrene and forbol ester-tpa) and negative (solvent) control groups will be provided for each assay.

3.2.3 in vivo tests - possible extraction of mutagens from particulate matter in the whole animal (bioavailability) (University of Naples, Department of Biochemistry, Italy)

An important aspect of the CCMC short term project is the bioavailability of any mutagen or possible carcinogen and although inhalation is the preferred test method, due to the long term nature of this process, additional short term tests are being conducted with animals.

These tests consist of assimilating the sample (diesel particulates) into an appropriate medium, such as corn oil, bovine serum albumen and human blood serum and injecting this into the peritoneum and subsequently subjecting the urine to appropriate AMES assays to test for mutagenicity. These tests are being carried out using male and female Sprague-Dawley rats. Three animals for each dose are being used.

FRACTIONATION AND IDENTIFICATION OF ORGANIC COMPONENTS IN DIESEL EXHAUST PARTICULATE

Mitchell D. Erickson, David L. Newton, Michael C. Saylor, Kenneth B. Tomer, and E. D. Pellizzari
Research Triangle Institute
P. O. Box 12194
Research Triangle Park, NC 27709

Roy B. Zweidinger and Sylvestre Tejada
Mobile Source Emissions Research Branch
Environmental Protection Agency
Research Triangle Park, NC

Diesel exhaust particulate, generated using production model passenger car engines on a chassis dynamometer, was extracted from Teflon-coated glass fiber filters with methylene chloride and fractionated using either a solvent partition scheme partition scheme or low pressure liquid chromatography (LPLC). The solvent partition scheme(1) generated two acid, two base, a cyclohexane insoluble, polar neutral, non-polar neutral, and PNA fractions. The LPLC scheme generated fractions which elute from a silica gel (Lobar) column with 10% CH_2Cl_2/90% hexane (Fraction F1 and part of F2), 50% CH_2Cl_2/ 50% hexane (part of F2 and F2A), 100% CH_2Cl_2 (F3 and F4), 10% CH_3OH/90% CH_2Cl_2 (F5 and F6), 50% CH_3OH/50% CH_2Cl_2 (F7 and F8) and 100% CH_3OH (F9 and F10). A hexane-insoluble (HI) fraction was analyzed using the same preparative LC technique. The fractions generated by both schemes were analyzed by normal phase HPLC, glass capillary GC/MS/DS (electron impact [EI], chemical ionization, and negative ion chemical ionization [NICI]), direct probe NICIMS, direct probe EI high resolution MS, FTIR and $(GC)^2$/FTIR.

Including isomers, 52 polycylic aromatic hydrocarbons and alkyl derivatives, 35 PNA-ketone and di-ketone derivatives, 20 aromatic aldehydes and cyclic anhydrides, 26 nitrogen-containing PNAs (including nitro PNAs) and 30 other compounds (including alkanes and some possible background contaminants) were identified.

The compounds of particular interest are the various PNA ketones (e.g., fluorenone) and the nitro PNAs(e.g., nitropyrene) and their alkyl-substituted homologs. These compounds are listed in Tables 1 and 2. The nitro PNAs are of interest in light of recent findings that some of them are highly mutagenic.(2-4) The nitro PNAs were found in the more mutagenic fractions. Thus, it may be speculated that these compounds are contributing much or possibly most of the mutagenic activity to these fractions and therefore to the diesel exhaust particulate itself.

REFERENCES

1. Hughes, T.J., L.W. Little, E.D. Pellizzari, C.M. Sparacino, G. McCue, L. Claxton, and M. Waters. Mutation Res., 76, 51-83 (1980).
2. Schuetzle, D., J.S.-C. Lee, T.J. Prater, and S.B. Tejada, Int. J. Environ. Anal. Chem., 9, 93-144 (1981).
3. Löfroth, G., E. Hefner, I. Alfheim, M. Møller, Science, 209, 1037-1039 (1980).
4. Rosenkranz, H.S., E.C. McCoy, D.R. Sanders, M. Butler, D.K. Kiriazides, R. Mermelsteim, Science, 209, 1039-1043 (1980).

Table 1. POLYCYCLIC KETONES AND DIONES IDENTIFIED IN NISSAN DIESEL EXHAUST PARTICULATE

Identified Compound	Number of Isomers Identified	Analysis					Fraction(s) Containing Compound Identified
		GC/EIMS	GC/NICIMS	HRMS	GC/FTIR	Other	
naphthoquinone	1	x					F4
9-fluorenone or $C_{13}H_8O$[2] isomer	1	x	x				F4;F3;F2
methylfluorenone isomer or $C_{14}H_{10}O$ isomer	3	x	x				F4;F3;F2
anthrone or phenanthrone isomer	1	x					F2
C_2-alkyl fluorenone isomers or $C_{15}H_{12}O$ isomers	4	x					F3;F2
C_3-alkyl-fluorenone isomers or $C_{16}H_{14}O$ isomers (tent)	4	x	x				F3;F2
C_4-alkyl fluorenone isomers or $C_{17}H_{16}O$ isomers (tent)	2	x	x				HI;F2
xanthone (tent)	1	x					F3
anthraquinone	1	x			?[3]		F3;F2
4H-cyclopenta(def)phenanthrene-4-one (tent)	1	x					F3;F2
benzanthrone isomers	3	x					F3;F2
methyl-4H-cyclopenta(def)-phenanthrene-4-one isomer (tent)	2	x					F2
benzofluorenone isomers (tent)	2	x					F2
$C_{18}H_{12}O$ ketone isomers[3] (tent)	2	x	x				F2
$C_{18}H_{10}O_2$ dione isomer[4] (tent)	1	x	x				F2
6H-benzo(cd)pyrenone isomers or $C_{19}H_{10}O$ isomer (tent)	3	x	x				F2
C_2-alkyl-4H-cyclopenta (def)phenanthren-4-one isomer (tent)	2		x				F2
C_5-alkyl fluorenone isomer or $C_{18}H_{18}O$ isomer (tent)	1		x				F2

[1] See text for fraction identifications.
[2] Other possible isomers include perinaphthenone and benzoindenone isomers.
[3] Tentative identification.
[4] Possible isomers include naphthacenone, triphenylenone, chrysenone, and methylbenzanthrone isomers.
[5] Possible isomers include di-ketones of naphthacene, chrysene, and triphenylene.

Table 2. NITROGEN CONTAINING AROMATICS IDENTIFIED IN NISSAN DIESEL EXHAUST PARTICULATE

Identified Compound	Number of Isomers Identified	Analysis					Fraction(s) Containing[5] Compound Identified
		GC/EIMS	GC/NICIMS	HRMS	GC/FTIR	Other	
N-phenylnaphthylamine isomer	1	x			x		F4;F2;F5;F6;F3;F8;F1G
C_2-alkyl-N-phenylnaphthylamine isomer (tent)	1	x					F5
benzo(c)cinnoline[2]	1	x					F5;HI
methylbenzo(c)cinnoline[2] isomers	3	x					F5
$C_{13}H_9O$ isomer[3] (tent)	1	x					F6
nitroanthracene isomer or nitrophenanthrene isomer	1	x	x		x		F1G;F2
methylnitroanthracene or methylnitrophenanthrene isomers (tent)	4	x	x				F1G;F2
C_2-alkyl nitroanthracene or C_2-alkyl nitrophenanthrene isomers (tent)	7	x	x				F1G
C_3-alkyl nitroanthracene or C_3-alkyl nitrophenanthrene isomers (tent)	4	x					F1G
nitropyrene isomer or $C_{16}H_9NO_2$ isomer	1	x	x		x		F2
methylnitropyrene isomer or nitrobenzofluorene isomer (tent)	1	x	x				F1G;F2
$C_{18}H_{11}NO_2$ isomer[4]	1	x	x				F2

[1] See text for fraction identifications.
[2] It is possible that these are polycyclic ketones of the formulas $C_{13}H_8O$ and $C_{14}H_{10}O$. However, their mass spectra more closely resembled those for benzo(c)cinnoline in standard spectra. These compounds were also later eluting than 9-fluorenone and its alkyl homologs. Further elucidation of these compounds is currently underway for fraction F5 of the refractionated HI sample by means of GC/FTIR and HRMS to determine whether these are indeed benzo(c)cinnolines.
[3] This eluant gave a mass spectrum similar to that of acridine or benzoquinoline, but only a trace quantity of the compound was present.
[4] Possible isomers include nitrochrysene, nitronaphthacene, and nitrotriphenylene isomers.
[5] Fraction F1 was further fractionated to yield subfractions F1A through F1G.

EFFECT OF CHRONIC DIESEL EXPOSURE OF PULMONARY PROTEIN SYNTHESIS IN RATS

by

R. G. Farrer, Sukla Dutta and S. Dutta
Wayne State University School of Medicine
Department of Pharmacology
Detroit, Michigan 48201

There is evidence that when rats are subjected to acute exposure to cigarette smoke, hepatic protein synthesis is inhibited and the extent of inhibition is positively correlated with the dosage of smoke. The present study has been undertaken to determine the effect of diesel smoke on pulmonary protein synthesis. For this study, male Fischer rats have been exposed to diesel exhaust (6 mg/m^3) for 2, 4 and 8 weeks. At the end of the desired exposure periods, the lungs have been removed respectively from the exposed and time-matched control rats and placed in an isolated lung apparatus.

The apparatus was devised by modifying the design of Fisher *et al.*, (2) for the perfusion of lung excised from rats. Briefly, for perfusion of each lung, the animal was anesthetized with pentobarbital intraperitoneally and trachea was cannulated and connected to a Harvard respirator. At this point, by means of a pressure transducer placed in the in-flow route of the respirator, we recorded *in situ* ventilation pressure for 3-4 minutes in an eight channel recorder. This allowed us to compare *in vivo* tracheo-bronchiolar air resistance under inspiratory pressure of 10 cm H_2O with that of the resistance when the lungs would be under *ex vivo* condition. Following this procedure, thoracotomy was conducted and the lungs were excised by carefully separating pulmonary artery from the aorta. Once the pulmonary artery was clearly dissected out from the other mediastinal structures, heparin (0.5 units/g) was injected through this. After a few minutes of circulation of heparin, the pulmonary artery was separated from the right ventricle and the open end was cannulated for delivery of perfusate at 15 ml/min by means of a Harvard peristaltic pump from the reservior. While perfusate was going through the lungs, a small incision was made in the left artrium so that the perfusate might flow freely and wash blood out of the pulmonary vascular bed. When the lungs were cleared of blood, they were transferred to a water-jacketed perfusion chamber maintained at 35°C. During the transfer, we kept respiring the isolated lung while interrupting the perfusate only for a few seconds. The peristaltic pump was then switched on and upon perfusion of the isolated lungs the perfusate freely drained into the perfusion chamber and from there by means of Tygon tubing back to the reservoir. Thus, a fixed volume of

perfusate continuously recirculated through the lungs for the entire duration of the experiment. Moment to moment performance of the preparation was monitored by recording continuously pH and pO_2 of the perfusate before it entered the lungs and the pressures required for tidal ventilation and for delivery of perfusate at 15 ml/min, through the pulmonary artery.

One exposed and one control Fischer rat was tested in one day, and two such runs were conducted each week. It required approximately two hours to complete one perfusion experiment which consisted of 30 minutes of equilibration followed by one hour of ^3H leucine incorporation. During the equilibration period, the lungs were perfused by recirculation with 50 ml Leibovitz solution and watched for any change in pH, pO_2, ventilation and perfusion pressures. If all these parameters looked normal, which was the case for all the 24 experiments needed to complete the series, the perfusion medium was changed to fresh 50 ml Liebovitz solution containing approximately 1 μCi/μ mol/ml of ^3H-leucine. Thus, the perfusion technique made it possible to provide a constant supply of substrate, ^3H leucine, with a desired specific activity all throughout the incorporation period.

At the end of ^3H leucine incorporation period, samples were taken for autoradiography and, then, the rest of the lungs were subjected to homogenization in 0.02 KH PO_4 and TCA precipitation of protein which was washed with polar and non-polar solvents. The washed protein residue was dissolved in 4.0 ml of 2.0 N NaOH at 50°C. The protein was determined by the method of Sedmak and Grossberg (3). Total pulmonary DNA was determined by using the modified diphenylamine technique of Burton (4) following precipitation of DNA. Radioactivity obtained from incorporated ^3H-leucine was measured in 1.0 ml samples of the dissolved protein using a Beckman LS-100 counter. Because there were differences in quenching between the diesel exposed (very dark due to presence of diesel particles) and control samples, internal standard (^3H tolune) was used to correct the observed CPM before expressing the results in DPM which was converted to nmole ^3H-leucine by using the known specific activity. Results were normalized on the bases of mg protein as well as mg DNA as obtained per gram of lung tissue. The results of ^3H leucine incorporation as shown in table 1 revealed that 8 weeks of exposure of male Fisher rats to 6.0 mg particulates/m^3 of diesel engine exhaust had no significant effect on the lungs to incorporate ^3H-leucine into the TCA insoluble protein. Similar results were obtained after 2 and 4 weeks of exposure to diesel exhaust in comparison to respective time matched controls. Also, electron microscopic autoradiographic grain counts as obtained from the diesel exposed vs. control rats showed no particular difference among various groups.

Table 1. Incorporation of ^3H-leucine by the perfused lungs as obtained from rats after 8 weeks of exposure and their time matched controls.

Experiment	nmoles leucine per mg protein	nmoles leucine per mg DNA	Protein/DNA
8 weeks of air exposure	3.53 ± 0.23*	41.3 ± 3*	11.8 ± 0.4*
8 weeks of diesel	3.30 ± 0.48	46.3 ± 6	13.9 ± 1.1

* Means ± S.E.

REFERENCES

1. Garrett, R.J.B., and M.A. Jackson 1979. Effect of acute smoke exposure on hepatic protein synthesis. J. Pharm. Expt. Therap. 209: 215-218.

2. Fischer, A.B., C. Dodia and J. Linadk 1980. Perfusate composition and edema formation in isolated rat lungs. Expt. Lung Res. 1: 13-21.

3. Sedmark, J.J. and S.E. Grossberg 1977. A rapid, sensitive and versatile assay of protein using Coomassie Brilliant Blue G250. Anal. Biochem. 79: 544-522.

4. Burton, K. 1955. The relation between the synthesis of deoxyribonucleic acid and the synthesis of protein in the multiplication of bacteriophage T_2. Biochem. J. 61: 473-483.

THE EFFECT OF EXPOSURE TO DIESEL EXHAUST ON PULMONARY PROTEIN SYNTHESIS

by

C. Filipowitz, C. Navarro, and R. McCauley
Department of Pharmacology
Wayne State University School of Medicine
Detroit, Michigan

Previous work performed in collaboration with the Biomedical Research Division of General Motors Corporation had indicated that exposure of rats to diluted diesel exhaust for periods of up to one year did not induce the activity of microsomal benzo[a]pyrene-oxidizing enzymes in lung tissue. Several explanations for this observation, including the possibility that exposed animals are unable to respond to inducing agents, have been suggested. In this report, we will discuss the ability of animals which have been exposed to exhaust in the concentration of 6 mg/m^3 of diesel particles to synthesize pulmonary proteins as judged by *in vivo* ^3H-leucine incorporation and to respond to 3-methylcholanthrene by the induction of pulmonary oxidative metabolism of benzo[a]pyrene.

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PREPARATION OF DIESEL EXHAUST PARTICLES AND EXTRACTS AS SUSPENSIONS FOR BIOASSAY

by

Jean L. Graf
IIT Research Institute
Fine Particles Research Section
10 West 35th Street
Chicago, Illinois 60616

A bioassay program is being conducted at IITRI to evaluate the acute toxicities and carcinogenic potentials of diesel engine exhaust components, cigarette smoke condensates and organic solvent extracts of roofing tar volatiles and coke oven emissions. The test materials were administered to hamsters by the intratracheal route. Administrations in both the acute toxicity and carcinogenic potential bioassay experiments have been completed.

The test materials were supplied through the U.S. EPA Biomedical Research Branch. The diesel engine exhaust components supplied were a whole particle exhaust consisting of carbonaceous soot with adsorbed liquid and gaseous species, and a dichloromethane extract of the whole particle exhaust. The cigarette smoke condensate was supplied as a concentrated solution in acetone. Both the roofing tar and coke oven emission extracts were supplied as dichloromethane solutions.

The intratracheal administration route required preparation of stable suspensions and emulsions of the test materials in fluids compatible with the hamster respiratory tract fluids. Saline was the obvious suspending fluid to be used but additional ingredients were required to enable suspension of the particles and the solvent-free extracts. Examination of the as-received whole particle exhaust revealed that very large (up to 150 μm) diesel particle aggregates were present. These large particles hindered suspension preparation and were not suitable for intratracheal suspension. Therefore, a research program was conducted to develop methods for preparing saline suspensions of the whole particle exhaust in particle size ranges amenable to intratracheal instillation and saline emulsions (liquid-liquid suspensions) of the various extracts.

For the short-term acute toxicity studies, a simple wet ball milling

method was developed which both reduced the size of the diesel engine exhaust particles and suspended them in saline. The hydrophilic nature of the diesel particles required that they first be wetted with propylene glycol in the glass milling jar before the saline suspending fluid and the glass milling beads were added. Gelatin was also added to the saline to serve as a protective colloid.

Simple hand emulsifying techniques were used to prepare the diesel engine exhaust extracts and the other extracts condensate as stable emulsions in the gelatin-saline fluid for the short-term acute toxicity studies. Standard glass tissue grinders proved to adequately emulsify the four types of organic extracts, once the solvent had been removed and they were wetted with propylene glycol and a surface active agent. To maintain the emulsion stabilities for more than 30 minutes, gum arabic was also added to the saline to provide a stronger protective colloid.

The greater materials requirements for the larger scale carcinogenic potential bioassay experiments required development of a semi-mechanized method to prepare the emulsions of the test materials. Trial emulsion preparations with a Polytron® tissue homogenizer proved successful and a unit was purchased. The Polytron® is a high speed mixing device which employs both mechanical shear action and ultrasonics to accomplish homogenization of liquid samples. Various types of homogenizing probes are available and provide a wide range of shear and ultrasonic energies. One probe design provides sufficient shear energy to reduce particle sizes of soft solid materials such as the diesel engine exhaust particles.

Thus, for the long-term carcinogenicity bioassay experiments, development of protocols to prepare the suspensions and emulsions with the Polytron® were conducted. The whole particle diesel engine exhaust suspensions were easily prepared as stable suspensions in gelatin-saline, once the particles had been wetted with propylene glycol. Particle concentrations as high as 25 mg/ml were attainable. The primary advantages of the Polytron® milling over ball milling to prepare the particle suspensions were shorter preparation times (one hour versus 10 days elapsed time), elimination of glass milling contaminants, and reduction of the reagglomeration tendency after milling was completed. The diesel engine exhaust extract was also easily prepared as an emulsion in gelatin-saline once the extract had been wetted with propylene glycol (after solvent removal). The use of the high energy Polytron® to emulsify the diesel extract as well as the other organic extracts in saline eliminated the necessity of adding a surface active agent. However, gum arabic was still required as an additional protective colloid to maintain emulsion stability.

Assay and characterization methods for the prepared suspensions were also developed. While the emulsions could be characterized microscopically, no practical assay methods could be developed.

RESEARCH PLANS FOR DIESEL HEALTH EFFECTS STUDY

by

Hironari Kachi and Tadao Suzuki
First Research Department
Japan Automobile Research Institute, Inc.
Yatabe-cho, Tsukuba-Gun, Ibaraki-ken
305 Japan

1) PAST RESEARCHES ON AUTOMOBILE EXHAUST IN JAPAN AUTOMOBILE RESEARCH INSTITUTE, INC. (JARI)

JARI was granted research contracts from the Japan Automobile Manufacturers Association, Inc. (JAMA) for studying the reduction of automobile exhaust and their health effects around 1970 when automobile exhaust were a wide public concern in Japan on the grounds that they may cause atmospheric pollutions typically exemplified by the phenomenon of photochemical smogs.

JARI started research on these contracts in 1971 including field surveys using a mobile smog chamber, tracing of photochemical reactions using a stationary smog chamber and chemical analysis of emission components. A behavioral assessments on animals were started in 1975, and basic researches on health effects of NO_x and O_3 were started in 1976. Preliminary researches on diesel emissions were started around this time. Small-scale animal inhalation system with modified Rochester type inhalation chambers were designed and tested. Studies on the health effects of diesel emissions to rats were done preliminary for one month and then three months. Investigations on respiratory system revealed that morphological changes in early stage of exposure are attributed mostly depend to the gaseous components, and it seems that particulate matters amplify the changes depending on the particulate concentrations.

The facility for Ames test was completed in 1980. A preliminary Ames test was conducted on extracts from diesel particulates using Salmonella Typhimurium TA100 and TA98. It was found that PAHs contained as neutral fraction components showed a relatively high mutagenicity. Examinations of sampling methods which would allow to obtain artifact-free diesel particulates are currently conducted comparing results of Ames tests and those of chemical analysis.

2) HEALTH EFFECTS RESEARCH PROGRAM (HERP)

In recent years, the health effects of diesel emissions has become an important subject in Japan. However, the research works in this field is still in an embryonic stage. Under these circumstance, JAMA referred this subject to some researchers of automobile engineering, medical and chemical fields. As a result, HERP has been drafted in 1981.

An outline of the HERP is presented below.

Period : From 1981 to 1985

Organization (Committee of HERP)

Chairman : Dr. Atsushi WATARI
President of the JARI
Prof. Emeritus of Univ. of Tokyo

Chairman of Steering Committee : Dr. Noburu ISHINISHI
Prof. of Hygiene & Public Health
Kyushu University

Subcommittees :
1. Diesel Exhaust Generation and Sampling
2. Analysis and Custoday
3. Inhalation Studies
4. Small Animal Experiment
5. Culture Cell Experiment (I)
6. Culture Cell Experiment (II)
7. Mutagenicity Test using Microorganisms
8. Miscellaneous including environmental assessment

Secretariat : JARI Officier

Research items :

Facilities for particulate generation, sampling and analysis and a full-scale facility for the inhalation experiment will be built at JARI. Preparations are underway to complete these facilities in 1982.

It is planned that JARI will participate principally sampling, analysis, storage and delivery of diesel tars and the inhalation experiment. The various *in vitro* and *in vivo* tests on diesel emission samples will be conducted in some research institutes and laboratories of the national and private universities.

Two types (large and small) of diesel engines will be used in the project considering that properties of emission materials might depend on diesel engines.

Chronic toxicity tests and carcinogenicity tests will be conducted by the inhalation for over two years. Intratracheal instillations, skin painting and other tests of the extracts from diesel particulates will be conducted

on small animals. Mutagenic test of the extracts from diesel particulates will be conducted on culture cells and microorganisms (such as the *Salmonella Typhimurium*).

NEURODEPRESSANT EFFECTS OF UNCOMBUSTED DIESEL FUEL

BY

Robert J. Kainz, Sc.D.
Environmental Industrial Safety Consultants
Fredrick, Maryland

LuAnn E. White, Ph.D.
Tulane University School of Public Health and Tropical Medicine
Department of Environmental Health Sciences
New Orleans, Louisiana

INTRODUCTION

Studies were conducted to characterize the short term neurotoxic effects of the inhalation of uncombusted diesel fuel vapors. Since diesel fuel contains indentified harmful hydrocarbon constituents which may exert neurological effects, the studies in this research effort were designed to screen for neurotoxic effects of the uncombusted diesel vapors. Mice were exposed at concentrations of 0.204 mg/1, 0.135 mg/1 and 0.065 mg/1 of uncombusted diesel vapor for 8 hours/day, 5 consecutive days.

METHODS

Three groups of mice were maintained throughout the experiment: an exposure chamber group, a control chamber group, and a vivarium control group. Ten mice each were in the exposure chamber and the control chamber groups; five mice were in the vivarium control group. Conditions of the exposure and control chamber groups were identical except for the presence of the uncombusted diesel vapor.

Five tests were selected to identify changes in performance which are related to interference of the nervous system. These tests were: the square box activity test, used to evaluate activity of the mice by suggesting either depression or stimulation of activity; the rota rod test which indicates alterations of the integrity of neuromuscular junctions and coordination; the inclined plane test, which serves to evaluate neuromuscular junction integrity of neuromuscular strength or paralysis; the corneal reflex test, used to screen for spinal cord depressant activity; and the hot plate test, a test of analgesic response. General observations were made during testing and for 30 minutes after mice were returned to their cages. The tests were administered to exposure and control chamber groups 24 hours prior to the first day of exposure, after completion of each day of exposure, and 24 hours after the last day of exposure. Results of the tests were compared between the exposure chamber and control chamber groups and expressed as percent of control within standard error. Exposure was conducted using an inhalation chamber which exposed mice primarily via the respiratory system with minimal ingestion and cutaneous exposures. Vapor generation was directly from complex liquid state to the vapor state and varied $\pm$ 10% for the duration of the study.

RESULTS

Square Box Activity Test: Mice exposed to 0.204 mg/1 showed 50 to 90% less activity than the control chamber mice. Those mice exposed to 0.135 mg/1 demonstrated activity comparable to that of the control chamber mice, while mice exposed to 0.065 mg/1 had increased activity as high as 150 percent as that of the control chamber mice. Comparison 24 hours after removal from exposure to the diesel vapor resulted in similar values between exposure and control chamber groups.

Rota Rod Test: When compared to the control chamber group, mice exposed to 0.204 mg/1 initially demonstrated a slight increase in performance which drastically deteriorated as exposure continued. Mice exposed to 0.135 mg/1 had a slight increase in performance followed by a slight decrease in performance when compared to control chamber mice. Those mice exposed to 0.065 mg/1 showed no relative difference in performance between exposure and control chamber mice. Comparison after 24 hours of recovery from last exposure indicated little change between exposure and control chamber mice.

Hot Plate Test: Results of the exposure to 0.204 mg/1 indicated an initial increase in heat sensitivity followed by tolerance to heat. At 0.135 mg/1 a slight increase in heat sensitivity was observed for the entire exposure. Exposure to 0.065 mg/1 identified a substantial increase in heat sensitivity for the exposure mice as compared to the control mice in the chambers. No relative difference could be observed between exposure and control chamber groups 24 hours after termination of exposure.

Corneal Reflex Test: No difference in response was noted between groups for any concentrations.

Incline Plane Test: Both the exposure and control chamber groups had negative results at each concentration.

All test result variations are depicted in Figure 1.

General Observations: All mice exposed to 0.204 mg/1 displayed severe discoloration of the tail indicating vasodilation after three days of exposure. Severe dehydration was observed in all mice. Grooming habits deteriorated after day two of the exposure. While walking, five (50%) of the mice displayed tremors through day three. Half of the mice with tremors died at day three or sooner; the rest continued to have tremors but recovered. A weight loss of 30% was observed in the exposure group. The mice in the exposure chamber group were generally less active than the control chamber group when returned to their cages.

At an exposure of 0.135 mg/1, five (50%) of the mice displayed tail discoloration after 3-4 days of exposure; slight dehydration was also apparent. Tremors were evident in three (30%) of the mice while in motion; however, no deaths occurred. Grooming was poor but less so as compared to the 0.204 mg/1 exposure group.

In mice exposed to the 0.065 mg/1 concentration no appreciable difference were displayed between the exposure and control chamber groups.

CONCLUSIONS

The exposure of the mice identified general trends in the effect of uncombusted diesel vapor on the nervous system. Comparison of exposure to control chamber groups suggests a positive central nervous system involvement. Exposure appears to be concentration and duration dependent. At the concentration 0.204 mg/1 depression of the inhibitory neuron occurs followed by extensive depression of the stimulatory neuron. Concentrations of 0.135 mg/1 appear to have little effect when compared to controls and suggest depression of inhibitory neuron and slight depression of the stimulatory neurons while the concentration of 0.065 mg/1 causes depression in the inhibitory neuron.

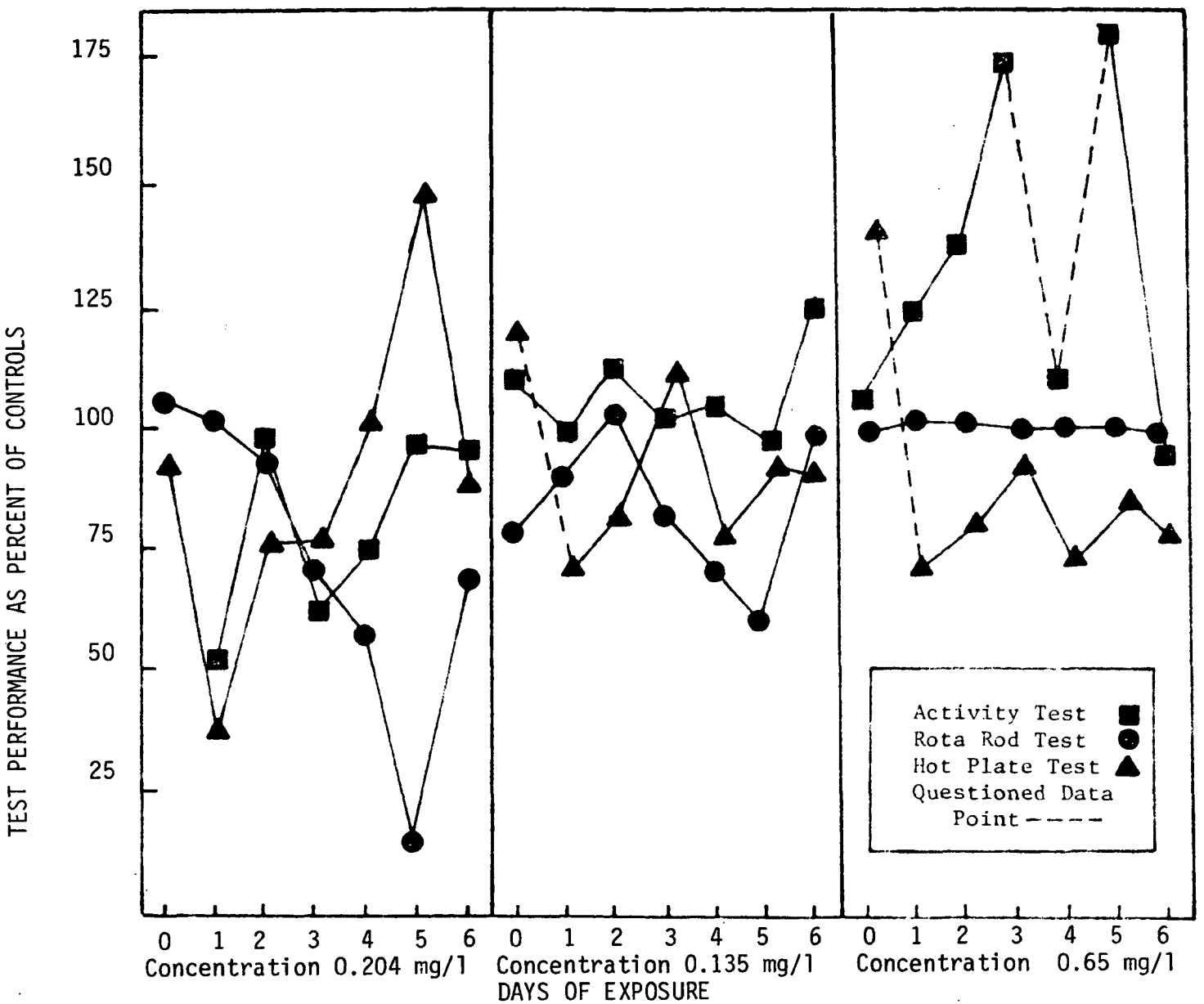

Figure 1. Results of Screening Tests as Percent Control for All Three Concentrations of Uncombusted Deisel Vapor

RESPIRATORY HEALTH EFFECTS OF EXPOSURE TO DIESEL EXHAUST EMISSIONS
(Bus Garage Mechanics; Salt, Potash, Metal, and Coal Miners)

by

R.B. Reger
Epidemiological Investigations Branch
National Institute for Occupational Safety and Health
Appalachian Laboratories
Morgantown, West Virginia

A comprehensive research program has been mounted relating to chronic and acute respiratory health effects of diesel emissions exposure. Special attention has focused on occupational groups exposed in enclosed spaces. This study involves over 5,000 workers engaged in various types of mining occupations as well as bus garage mechanics. These subjects were given chest radiographs, asked questions on respiratory symptoms, smoking and occupational histories, and given spirometric tests. These data have been coupled with industrial hygiene information to evaluate relationships between selected health parameters and component measures of diesel exhaust emissions. This paper reports the results for each group of workers separately.

SwRI-SFRE DIESEL HEALTH EFFECTS EXPOSURE FACILITY

by
Karl J. Springer
Department of Emissions Research
Southwest Research Institute
6220 Culebra Road
San Antonio, Texas

The future of the fuel efficient automotive diesel passenger car engine has been clouded by the possibility that its exhaust particulates have carcinogenic properties. To determine whether this is a possibility, auto manufacturers and the federal government are seeking to determine the possible long term health effects of diesel exhaust particulates. What the National Research Council called the most comprehensive effort in this area is being conducted by Southwest Research Institute and its sister organization, Southwest Foundation for Research and Education. The project is sponsored by General Motors Corporation.

The facility is the largest and most advanced of its kind. The building consists of three rooms. The engine room houses the diesel engine and mechanical equipment for conditioning the dilution air. The exposure levels are monitored and the operation of the experiment is handled from the control room. The four large exposure chambers are located in the chamber room and are in close proximity to the diesel engine exhaust, yet isolated by a soundproof wall.

Each chamber is 8 ft wide by 8 ft long by 8 ft high. They are large enough to handle about 1250 rats, mice, and hamsters for long term exposure to diesel exhaust for a total of about 5000 animals. We wished to simulate levels of exposure not unlike those that might be experienced on the street. From previous experience we know that the maximum dose one might experience behind a city bus is about 1 part of exhaust in 120 parts of air. So, one chamber is operating at that level. Another chamber is operating at twice that dose, or 1 part of exhaust to 60 parts of air. A third chamber is operating at about one-third that level, 1 part of exhaust to 360 parts of air. A fourth chamber receives no diesel exhaust at all, just purified air and therefore is the control group for comparison to the other three.

Two Oldsmobile 5.7 liter diesel engines are mounted on individual stationary dynamometers. One is operated and the other is a back-up when

necessary. The engine operates at an equivalent 40 mph cruise condition (1,350 rpm and 71 ft lbs torque) for 20 hours each day. Individual samples of exhaust are directed to the top entrance of each of the three pyrimidal shaped chambers for subsequent dilution and mixing. The engine operates on a type 2D emissions test fuel. The engine room also houses the air conditioning and air filtration equipment used to condition and purify the dilution air.

The control room contains the automatic controls to maintain engine speed and power output, the environmental controls for maintaining the chamber temperature at 74°F and relative humidity at 50 percent with a slight negative pressure of 0.5 in H_2O. Monitoring of the gaseous emissions is on a semi-continuous basis. Each chamber is automatically monitored for 10 minutes each hour for hydrocarbons by heated FID, CO and CO_2 by non-dispersive infrared analyzers, and for NO/NO_x by chemiluminescence analyzer. These and pertinent engine and chamber environment data are recorded each 10 minutes.

The most important measurement is that of total particulate in each chamber. This is performed by collecting a sample of the chamber atmosphere on a 47 mm diameter plastic coated fiberglass filter media. The weight gain is used with the sample volume to compute the particulate concentration within each chamber. These measurements are made at least once each day. The amount of exhaust sample admitted into the mixing area prior to the chamber is adjusted as required to maintain the concentration of particulates as close to specification as possible.

The entire facility was designed for long term, continuous, trouble-free operation. Redundancies are provided in terms of backup engine dynamometer and controls, backup power for emergency air conditioning, backup pumps and air conditioning units and backup controllers etc., that may malfunction and result in an emergency condition. The totally integrated design located equipment items to simulate exhaust exposures as close to that in the field as possible. The system typically operates 20 hours each day (4 hours for animal and cage hygiene) and on a 7 day per week basis.

POST-EXPOSURE DIESEL PARTICLE RESIDENCE IN THE LUNGS OF RATS FOLLOWING INHALATION OF DILUTE DIESEL EXHAUST FOR 6 MONTHS

K. A. Strom and B. D. Garg
Biomedical Science Department
General Motors Research Laboratories
Warren, MI 48090

Due to its submicron size, 15-17% of inhaled diesel particulate deposits in the airways of the lung. In the alveoli of the lung, the alveolar macrophages scavenge the diesel particles, phagocytize them and diesel particulate-laden macrophages were found in lung lavage fluid even 90 days following an exposure of 16 days to diesel emissions at 6 mg/m^3 [1,2]. This indicated that the diesel particles may have a long residence time in the macrophages within the lung, rather than being rapidly eliminated via the ciliated airways or lymphatics. The studies describe the results of biochemical, morphological and physical measurements on the alveolar macrophages, as well as the histology of the lung after exposure of rats to 250 μg DP/m^3 for 6 months, and serial sacrifice up to 16 months post-exposure.

Male Fischer 344 rats (COBS CDF F-344/CrlBR) were exposed to diesel exhaust particulate concentration of 250 $\mu g/m^3$ for 20 hrs/day, 5-1/2 days/week for 6 months. Control animals were exposed to clean air. Lungs of exposed rats were lavaged *in situ* with Hank's Balanced Salt Solution (without calcium or magnesium). Differential cell counts and assays of the enzymatic activities of acid phosphatase and beta-glucuronidase were performed on the lavaged cells.

Light microscopic investigations of the lung revealed that immediately after the exposure, diesel-laden macrophages were diffusely distributed throughout the pulmonary alveoli and also focally aggregated in some alveoli. Some macrophages containing diesel particles were also observed in the bronchus-associated lymphoid tissue (BALT) and in the lymphatics. Subpleural pigmentation consisted primarily of aggregations of alveolar macrophages containing diesel particulate. Initially, ninety-five percent of the lavaged macrophages were completely pigmented with phagocytized diesel particles, such that only the nucleus of the cell was visible and the cytoplasm was opaque. The percentage of these macrophages declined with time, showing an exponential decay with a 6 week half-time. The percentage of lavaged macrophages which were free of diesel particulates rose linearly at a rate of 2.5 percent per week. The rest of the lavaged macrophages contained some diesel particulate-filled phagosomes within the cytoplasm. The macrophages were obtained in the same numbers as those from control animals, and had comparable cell size and activities of the lysosomal enzymes, beta-glucuronidase and acid phosphatase.

With increasing time post-exposure, diesel-laden macrophages were no longer observed diffusely distributed throughout the lung, but remained even after 16 months (life span) in focal accumulations in the alveoli (some of which had thickened alveolar walls), particularly noted in the subpleural regions, also in the BALT and in the lymphatics. Initially, the lung tissue contained 0.663 ± 0.075 mg (n=2) diesel particulate. After 16 months of clearance, diesel particulate in the lung had declined to 0.250 ± 0.090 mg (n=5) with only 0.014 mg (n=10) in the regional lymph nodes.

The rapid decline of the diesel-laden macrophages which was much faster than the overall diesel particulate removal from the lung, suggests that under conditions of prolonged exposures to high concentrations disappearance of particulate-laden macrophages from the lavageable pool of cells seems to be due to the formation of aggregates of alveolar macrophages rather than transport out of the lung. The overall clearance of diesel particulate after extensive exposures seems to be slow and proceeds by as yet unknown mechanisms. After 16 months post-exposure, alveolar macrophages containing small amounts of diesel particulate can still be identified in the lavage fluid. In addition, polymorphonuclear leukocytes are present among the aggregated macrophages suggesting that the incoming alveolar macrophages and polymorphonuclear leukocytes may be involved in or contribute to the breakdown of the macrophages aggregates.

REFERENCES

1. S. D. Lee, K. I. Campbell, D. Laurie, R. G. Hinners, M. Malanchuk, W. Moore, R. J. Bhatnagar and I. Lee, Toxicological assessment of diesel emissions. Abstract of presentation to Air Pollution Control Assoc., 71st Annual Meeting, Houston, TX, 25-30 June 1978.

2. W. Moore, J. Orthoefer, J. Burkart, and M. Malanchuk, Preliminary findings on the deposition and retention of automotive diesel particulate in rat lungs. Abstract of presentation to Air Pollution Control Association, 71st Annual Meeting, Houston, TX. 25-30 June 1978.

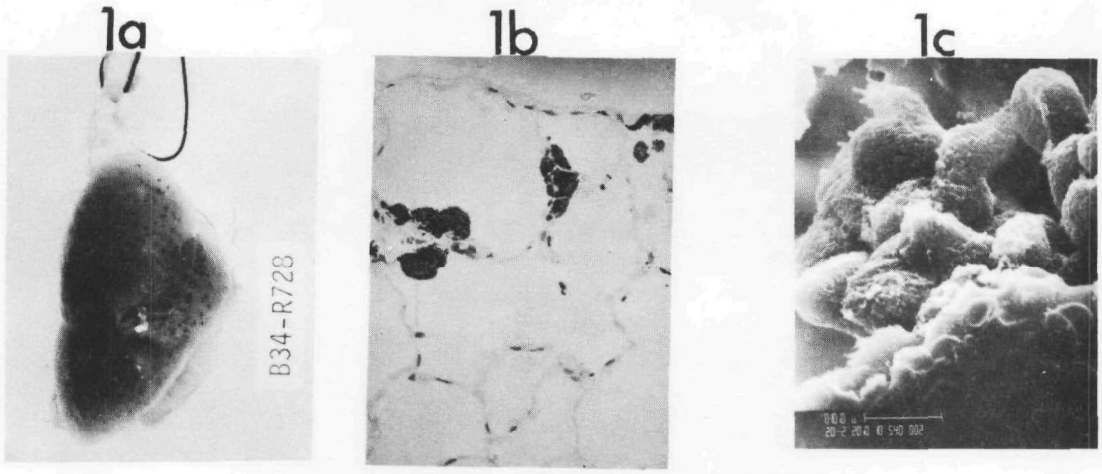

FIGURE LEGENDS

Fig. 1a 250 $\mu g/m^3$ for twenty-five weeks and 8 weeks post-exposure: speckled appearance of the exposed lung.

Fig. 1b 250 $\mu g/m^3$ for twenty-five weeks and sixty-nine weeks post-exposure. Diesel-laden macrophages are still present in association with the pleural surface region.

Fig. 1c 250 $\mu g/m^3$ for twenty-six weeks and forty-five weeks post-exposure. Scanning electron micrograph of macrophage aggregation in a pleural region from an area shown in Figure 1b.

MUTAGENIC ACTIVITY OF DIESEL EMISSIONS

by

Joellen Lewtas
Genetic Toxicology Division
Health Effects Research Laboratory
U.S. Environmental Protection Agency
Research Triangle Park, North Carolina

Incomplete combustion of fuel in diesel vehicles results in the emission of very small carbonaceous particles which, after cooling and dilution, contain varying quantities (e.g., 5 to 50%) of extractable organic constituents. These organics generally have been found to be mutagenic in bacteria.

Bioassay-directed fractionation and chemical characterization studies suggest that polar neutral compounds in general and nitrated polynuclear aromatic (NO_2-PNA) compounds in particular may account for a significant portion of the bacterial mutagenicity.

Confirmatory bioassays in mammalian cells provide the capability of detecting chromosomal and DNA damage in addition to gene mutations. Those assays performed in cell lines, however, usually require the addition of microsomal enzymes to metabolize polynuclear aromatic hydrocarbons (PAH) and may lack the nitroreductases responsible for metabolizing the NO_2-PNAs present in Salmonella strains. Mammalian cells capable of directly engulfing whole particles also provide a means of measuring cellular availability of the organics. In order to evaluate the mutagenicity of these organics in mammalian cells, extractable organics from particle emissions from a series of diesel and gasoline emissions have been compared in a battery of microbial mammalian cells and in vivo bioassays shown in Table 1. The mammalian cell mutagenicity bioassays were selected to detect gene mutations, DNA damage, and chromosomal effects. Carcinogenesis bioassays conducted included short-term assays for oncogenic transformation and skin tumorigenesis.

Diesel and gasoline particle emissions collected on Pallflex T68-20 Teflon-coated filters after dilution and cooling in a standard dilution tunnel were Soxhlet-extracted with dichloromethane and prepared for bioassay as previously described (1). The samples examined were obtained from heavy- and light-duty diesel and gasoline-powered vehicles and engines.

The bioassays examined in Table 1 were generally conducted in a similar manner. Assays were conducted at 5 to 7 doses, after a preliminary toxicity range-finding test to select the proper doses. Data analysis was performed to

determine the slope of the dose-response curve. In some assays increasing responses were not obtained by increasing the exposure concentration.

The objective of this presentation is to review these results, comparing different cell systems (e.g., CHO vs L5178Y) and different biological endpoints (e.g., gene mutation vs DNA damage). Mammalian cell data available on chemical fractions and individual compounds (e.g., benzo[a]pyrene and 1-nitropyrene) will be compared to the mutagenicity of the total extractable organics, the whole particles, and gaseous emissions.

REFERENCES

1. Lewtas Huisingh, J., R.L. Bradown, R.H. Jungers, D.R. Harris, R.B. Zweidinger, K.M. Cushing, B.E. Gill, and R.E. Albert. 1980. Mutagenic and carcinogenic potency of extracts of diesel and related environmental emissions: Study design, sample general, collection, and preparation. In: Health Effects of Diesel Engine Emissions. EPA 600/9-80-057b. U.S. Environmental Protection Agency: Cincinnati, OH. pp. 788-800.

Table 1. Mutagenesis and Carcinogenesis Bioassays Used in the Evaluation of Diesel Emissions

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A. Mutagenesis Bioassays

1. Gene Mutation Assays
   a. Bacterial assay
      1. *Salmonella typhimurium*, reverse mutation at the histidine locus

   b. Mammalian cell assays
      1. Mouse lymphoma L5178Y cells, forward mutation at the thymidine kinase locus using tri-flourothymidine resistance
      2. Mouse embryo fibroblasts, Balb/C 3T3 cells, forward mutation using ouabain resistance
      3. Chinese hamster ovary, CHO cells, forward mutation using 6-thioguanine resistance

2. DNA Damage Assays
   a. Yeast assay
      1. *Saccharomyces cerevisiae* D3 recombinogenic assay

   b. Mammalian cell assays
      1. Syrian hamster primary cells, DNA strand breaks by sedimentation in alkaline sucrose gradients
      2. Rat liver primary cells, DNA repair assay using autoradiographic unscheduled DNA synthesis
      3. Chinese hamster ovary, sister chromatid exchange assay

3. Chromosomal Aberrations
   a. Mammalian cell assays
      1. Chinese hamster ovary, chromosomal aberrations after *in vitro* exposure
      2. Human lymphocytes, chromosomal aberrations after *in vitro* exposure

B. Carcinogenesis Bioassays

1. Oncogenic Transformation Assays
   a. Mouse embryo fibroblasts, Balb/C 3T3 cells using morphological transformation
   b. Syrian hamster primary embryo cells using viral (SA7) enhancement of morphological transformation

2. Skin Carcinogenesis Assays

MUTAGENICITY OF DIESEL AND SPARK IGNITION ENGINE EXHAUST PARTICULATE EXTRACT COMPONENTS TO SALMONELLA TYPHIMURIUM AND HUMAN LYMPHOBLASTS

by

Thomas R. Barfknecht, Barbara M. Andon and William G. Thilly
Toxicology Group
Department of Nutrition and Food Science
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139

Ronald A. Hites
Department of Chemistry
School of Public and Environmental Affairs
Indiana University
Bloomington, Indiana 47405

Ercole L. Cavalieri
Eppley Institute for Research in Cancer
University of Nebraska Medical Center
Omaha, Nebraska 68105

MUTAGENICITY OF DIESEL EXHAUST COMPONENT PAH

In a previous report it was shown that a methylene chloride extract of diesel exhaust particulate collected from a 350 CID Oldsmobile diesel engine was mutagenic in a Salmonella typhimurium 8-azaguanine resistance forward mutation assay in the presence or absence of Aroclor-induced rat liver postmitochondrial supernatant (PMS) (1). In addition, the same diesel exhaust particulate extract was mutagenic to human lymphoblasts only in the presence of PMS.

In order to determine what components of the diesel exhaust extract were responsible for its mutagenicity, a fractionation was carried out based on polarity. An equal parts hexane/toluene polycyclic aromatic hydrocarbon (PAH) containing fraction was found to be most mutagenic to S. typhimurium in the presence of PMS (1,2). Analysis of this fraction by gas chromatography/mass spectrometry has identified 27 major PAH and derivatives (2).

Initial testing of PAH in human lymphoblasts has shown that fluoranthene, 1-methylphenanthrene and 9-methylphenanthrene induced significant trifluorothymidine resistant mutant fractions at concentrations of 2 μM, 5 μM and 4 μM, respectively (Table 1). These three PAH represent approximately 0.4% by weight of the total methylene chloride extractable organic matter of our diesel exhaust sample; however, these same PAH can account for up to 40% of the total mutability of this sample to human lymphoblasts. Fluoranthene alone may be responsible for up to 30% of the total mutagenicity of our extract.

Because fluoranthene plays a major role in determining the mutagenicity of our diesel exhaust extract, we have initiated studies to determine what metabolites of fluoranthene are responsible for its mutagencity. One of the two possible trans-2,3-dihydrodiols of fluoranthene was found to be significantly mutagenic to human lymphoblasts in the presence of PMS at a concentration of 2 μM, however, this same dihydrodiol was much less active in the bacterial mutation assay system requiring a concentration of 33 μM to induce a significant mutant fraction (Table 1). A trans-2,3-dihydrodiol-1,10B-epoxide of fluoranthene proved to be an ultimate mutagen to S. typhimurium inducing a significant mutant fraction at a concentration of 0.5 μM without metabolic activation. However, this diol-epoxide derivative of fluoranthene was not mutagenic to the human lymphoblasts up to a concentration of 1.0 μM (Table 1).

MUTAGENICITY OF CYCLOPENTENO(c,d)PYRENE AND DERIVATIVES

Cyclopenteno(c,d)pyrene is a major PAH component of spark ignition engine exhaust condensate (3,4). We have found that cyclopenteno(c,d)pyrene (CPEP) was mutagenic to S. typhimurium and human lymphoblasts at the concentrations of 6-7 μM with PMS activation. CPEP was significantly mutagenic to bacteria at a concentration of 1 μM when activation was by rat liver microsomes indicating that microsomes are a more efficient source of metabolic activation for CPEP than PMS. Cyclopentano(c,d)pyrene (CPAP) which lacks the 3,4 double bond of CPEP was significantly less mutagenic to both bacteria and human cells (Table 1). Incubation with microsome did not increase the mutagenicity of CPAP to bacteria.

A 3,4-arene-oxide of CPEP was significantly mutagenic to both S. typhimurium and human lymphoblast without activation at the concentrations of 0.7 μM and 0.4 μM respectively. These data indicate that CPAP-3,4-oxide is an ultimate mutagen of CPEP.

CPAP-3,4-trans-diol was weakly active in the bacterial system inducing a significant mutant fraction at a concentration of 90 μM. However, it was not mutagenic to human lymphoblast up to a concentration of 80 μM. In contrast, CPAP-3,4-cis-diol was as mutagenic as CPEP inducing a significant mutant fraction at a concentration of 6 μM in both bacterial and human cell mutation assay systems. When activated by microsomes, CPAP-3,4-cis-diol was as mutagenically potent as the direct-acting CPAP-3,4-oxide in the S. typhimurium mutation assay inducing a significant mutant fraction at a concentration of 0.5 μM.

CPAP-3-OH, CPAP-4-OH, CPAP-3-one and CPAP-4-one were significantly mutagenic to S. typhimurium at the μM concentrations of 14, 20, 14 and 20 respectively. When microsomes were utilized for activation, CPAP-3-OH and CPAP-4-OH were mutagenically active at the concentrations 90 μM and 10 μM respectively. CPAP-3-one and CPAP-4-one were inactive up to a concentration of 120 μM. Only CPAP-4-OH was significantly mutagenic to human lymphoblasts (Table 1).

These data suggest that there are four distinct metabolic pathways for CPEP and its derivatives: 1) the formation of CPAP-3,4-oxide which is the predominant pathway of activation for CPEP, 2) a pathway specific to CPAP-3,4-cis-diol, 3) a pathway of activation for CPAP and CPAP-4-OH, and 4) metabolic activation of CPAP-3-OH, CPAP-3-one and CPAP-4-one requiring the cytosolic fraction of PMS. This final pathway apparently does not produce metabolites that are mutagenic to human cells.

REFERENCES

1. Liber, H. L., B. M. Andon, R. A. Hites and W. G. Thilly. 1980. Diesel soot: Mutation measurements in bacteria and human cells. U.S. Environmental Protection Agency. EPA-600/9-80-0576a. Cincinnati, OH 404 pp.

2. Yu, M.-L., and R. A. Hites. 1981. Identification of organic compounds on diesel engine soot. Anal. Chem. 53:951-954.

3. Grimmer, G. 1977. Analysis of automobile exhaust condensates. I.A.R.C. Sci. Pub. 16:29-39.

4. Grimmer, G., K.-W. Naujack, and D. Schneider. 1980. Changes in PAH - profiles in different areas of a city during the year. In: Polynuclear Aromatic Hydrocarbons. A. Bjorset and A. J. Dennis, eds. Battelle Press: Columbus, OH pp. 107-125.

TABLE 1. MUTAGENICITY OF POLYCYCLIC AROMATIC HYDROCARBONS AND DERIVATIVES TO *SALMONELLA TYPHIMURIUM* AND DIPLOID HUMAN LYMPHOBLASTS[a]

Compound	*S. typhimurium* μM Concentration	Human lymphoblasts μM Concentration
Phenanthrene	- 300[b]	+ 100
1-methylphenanthrene	+ 80	+ 5
2-methylphenanthrene	+ 40	- 200
3-methylphenanthrene	-1000	NT[c]
9-methylphenanthrene	+ 80	+ 4
Pyrene	+ 90	- 300
1-methylpyrene	+ 180	- 100
Cyclopenteno(c,d)pyrene	+ 6	+ 7
CPAP[d]	+ 12	+ 40
CPAP-3,4-oxide (-PMS)[a]	+ 0.7	+ 0.4
CPAP-3,4-*cis*-diol	+ 6	+ 6
CPAP-3,4-*trans*-diol	+ 90	- 80
CPAP-3-OH	+ 14	- 80
CPAP-4-OH	+ 20	+ 40
CPAP-3-one	+ 14	- 80
CPAP-4-one	+ 20	- 80
Benz(a)anthracene	+ 65	+ 9
Chrysene	+ 45	+ 6
Triphenylene	+ 44	+ 20
Fluoranthene	+ 5	+ 2
Fluoranthene-2,3-*trans*-diol	+ 33	+ 2
Fluoranthene-2,3-*trans*-diol-1, 10B epoxide (-PMS)[a]	+ 0.5	- 1

(continued)

TABLE 1. (continued)

Compound	*S. typhimurium* μM Concentration	Human lymphoblasts μM Concentration
Benzo(a)pyrene	+ 4	+ 1

[a]Experiments were performed in the presence of 5% v/v Aroclor-induced rat liver postmitochondrial supernatant (PMS) except where indicated.

[b](+) the tested compound induced a significant mutant fraction at the indicated μM concentration, or in the case of a (-) negative response, the highest concentration tested is indicated.

[c]NT, not tested.

[d]CPAP, cyclopentano(c,d)pyrene.

CYTOTOXICITY, MUTAGENICITY AND COMUTAGENICITY IN DIESEL EXHAUST PARTICLE EXTRACTS ON CHINESE HAMSTER OVARY CELLS IN VITRO

by

A. P. Li, R. E. Royer, A. L. Brooks, and R. O. McClellan
Lovelace Inhalation Toxicology Research Institute
P. O. Box 5890, Albuquerque, NM 87185

Diesel exhaust particle extracts were found cytotoxic to Chinese hamster ovary (CHO) cells. Extracts from cars of different manufacturers had different cytotoxicity. The emission rates of cytotoxic chemicals were calculated for the different cars using the cytotoxicity of the extracts, the percentage of extractable chemicals on the exhaust particles, and particulate emission rates. The ranking of emission rates of cytoxic chemicals for the different cars were found to be the reverse of the ranking of the cytotoxicity of the extracts (1). Our data indicate the need to include emission data other than the activities of the extracts, when the emission of noxious agents from different vehicles are compared.

The cytotoxicity of diesel exhaust particle extracts is antagonized by serum, lung and liver cytosols, and sulfhydryl agents in vitro (2) [Figures 1, 2]. The detoxifying effects of the cytosols is enhanced further by the addition of cofactors (NADP and glucose-t-phosphate); therefore, suggesting enzymatic detoxification in addition to protein binding. Our data suggest that similar detoxification of the toxic chemicals associated diesel exhaust particles may occur in vivo.

All diesel exhaust particle extracts had low mutagenicity towards CHO cells. This low activity was observed using different endpoints including sister-chromatid-exchange and mutation at the hypoxanthine-guanine phosphoribosyl (HGPRT) gene locus (3). The mutagenicity was slightly enhanced by the addition of exogenous aroclor 1254-induced liver S9. Although the extracts had only low mutagenicity, they were found to have definite co-mutagenic activities (4). Treatment of CHO cells with a combination of a mutagen (N-methyl, N'-nitro, N-nitrosoguanidine or benzo(a)pyrene) and diesel exhaust extract yielded a 2-3 fold higher mutant frequency than that calculated by the mutagenicity of the mutagen and the diesel exhaust extract alone [Table 1]. This co-mutagenicity was observed for all extracts tested, using three different endpoints: mutation at the HGPRT gene locus, mutation at the Na^+-Ka^+-ATPase gene locus, and sister-chromatid-exchange. We have shown that diesel exhaust particles are associated with chemicals with cytotoxic, mutagenic, and co-mutagenic properties. Engineering variables, biological detoxifying molecules, and other environment mutagens/carcinogens, all could possibly modify the health-effect of the diesel exhaust emission.

REFERENCES

1. Li, A. P., R. E. Royer, A. L. Brooks, R. O. McClellan, W. F. Marshall, and T. M. Naman. Cytotoxicity of diesel exhaust particle extract--a comparison among five diesel passenger cars of different manufacturers. Manuscript in preparation.

2. Li, A. P. 1981. Antagonistic effects of animal sera, lung and liver cytosols, and sulfhydryl compounds on the cytotoxicity of diesel exhaust particle extracts. Toxicol. Appl. Pharmacol. 57:55-62.

3. Li, A. P., and A. L. Brooks. 1981. Use of Chinese hamster ovary cells in the evaluation of potential hazards from energy effluents--application to diesel exhaust emission. Lewis, M. (ed.). "Proceedings, the International Symposium of Health Impact of Different Sources of Energy", jointly sponsored by WHO/UNEP/IAEA, Nashville, TN, June 22-26, 1981. In press.

4. Li, A. P., and R. E. Royer. 1981. Diesel exhaust particle extract enhancement of chemical-induced mutagenesis in cultured Chinese hamster ovary cells: Possible interaction of diesel exhaust with environmental carcinogens. Mutat. Res. In press.

Table 1. Co-mutagenicity of Diesel Exhaust Particle Extracts in the Presence of Exogenous Activation System[a] (Li and Royer, 1981)

Treatment	Mutant Frequency -B(a)P	Mutant Frequency +B(a)P (o.5 μg/ml) Expected[b]	Mutant Frequency +B(a)P (o.5 μg/ml) Observed	Enhancement[c]
Solvent (DMSO) Control	7 (A)	-	56 (B)	-
Exhaust Extracts (60 μg/ml)				
Car A	28	77	218	2.8
Car B	6	56	170	3.0
Car C	11	60	194	3.2
Car D	9	58	184	3.2
Car E	25	74	229	3.1

[a]An Aroclor 1254 - induced rat liver cytosol/cofactors mixture was used for exogenous activation.

[b]Expected mutant frequency = mutant frequency (B(a)P alone) (B) + mutant frequency (diesel exhaust particle extract alone) - mutant frequency (DMSO alone) (A).

[c]Enhancement = observed mutant frequency ÷ expected mutation frequency.

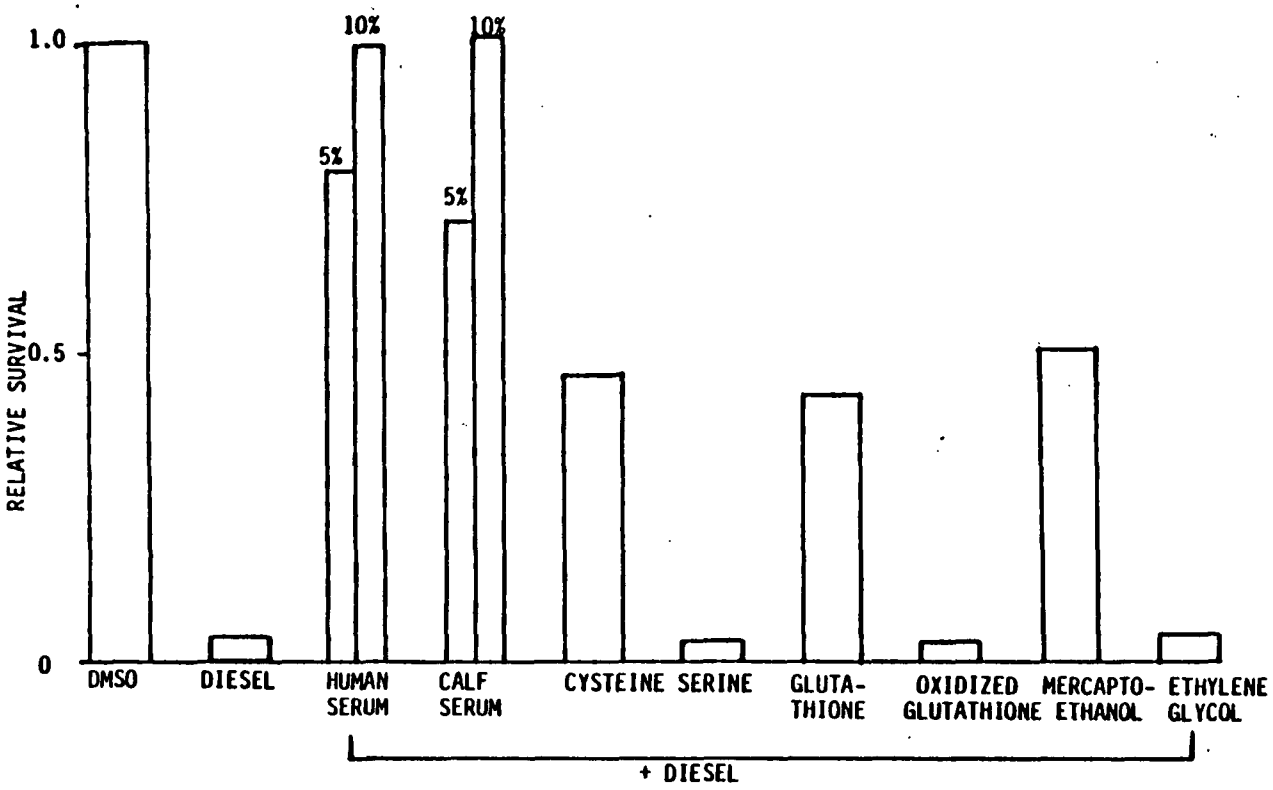


Figure 1 (Li, 1981). Effects of animal sera, sulfhydryl compounds and their non-sulfhydryl analogs, on the cytotoxicity of diesel exhaust extract.

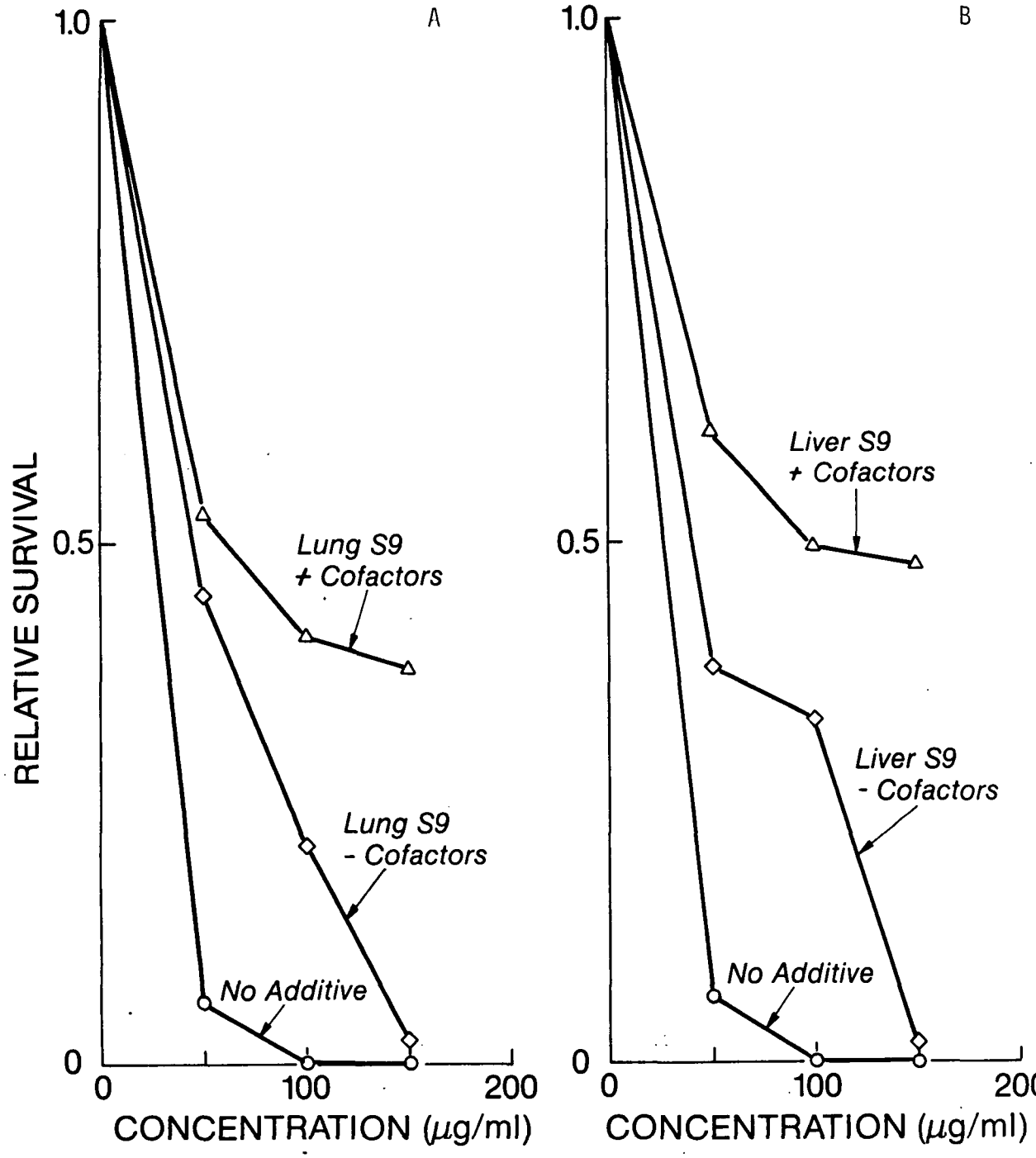


Figure 2 (Li, 1981). Effects of lung (A) and liver (B) cytosols on the cytotoxicity of diesel exhaust extract.

INDUCTION OF IN VIVO SISTER CHROMATID EXCHANGE BY DIESEL PARTICULATE AND DIESEL EXTRACT

by

Michael A. Pereira and Lofton McMillan
Health Effects Research Laboratory
U.S. Environmental Protection Agency
Cincinnati, Ohio 45268

D.K. Gulati, C.L. Stephens and P. Kaur
Environmental Health Research and Testing Inc.
Cincinnati, Ohio 45220

The use of diesel fuel is no longer limited to heavy duty engines because our energy consious society is gradually shifting to diesel powered automobiles. The safety of this change has been questioned because diesel engines generate 30 to 50 times more particulate matter in comparison to gasoline engines (J. Air Poll. Control Assoc., 28: 760, 1980). Investigations were carried out to study the effect of diesel particulate and its methylene chloride extract on the induction of sister chromatid exchanges (SCE) in the bone marrow cells of mice. Diesel exhaust particles were generated by a Nissan CN-6 diesel six cylinder engine and a Chrysler torgue-flite automatic transmission Model A-727 along with an Eaton-Dynamomter Model 758-DG, operated by Federal Short Cycle-type driving modes. The diesel exhaust particles were collected on teflon coated pallflex T60A20 type filters. An extract of the diesel particulate was prepared in a soxhlet extraction apparatus using methylene chloride as the elutant. The extract was transfered to dimethyl sulfoxide by solvent exchange using a stream of nitrogen to remove the methylene chloride.

Three month old male mice were injected intraperitoneally with diesel particulate matter at 300 mg/Kg body weight or diesel extract at 800 mg/Kg body weight. Each animal received a single intrapentoneal injection. After 1, 2, 5, and 14 days of treatment, mice were sacrificed by cervical dislocation and intact femurs removed. Six animals were sacrificed at each time point. The bone marrow cells were flushed from the canal with 0.075 M KCl. The cell suspension was centrifuged at 1,000 g for 10 minutes and the cells fixed. After two additional fixations, the cell suspension was dropped onto cold wet slides. A combination of Hoechst dye and Giemsa was used to stain the cells. Twenty metaphases were evaluated for the number of SCE. The mitotic index was estimated as total number of dividing cells per 1,000 cells.

Diesel particulate matter when administered ip, caused a substantial enhancement in the number of SCE per metaphase and per chromosome in the bone marrow cells. In mice treated with diesel particulate matter and sacrificed 1, 2, 5, and 14 days post-treatment, average frequency of SCE per metaphase in the bone marrow cells was 5.4, 11.3, 6.7, and 5.1, respectively. The average number of SCE in the bone marrow cells of solvent treated animals was approximately 6 per metaphase. It is evident from these observations that the genotoxic effect of diesel particulate matter appears on the second day of treatment. By the fifth day post-treatment, the genotoxic substances on the diesel particles were no longer capable of inducing SCE. The methylene chloride extract of diesel particulate matter also substantially enhanced the frequency of SCE/metaphase in the bone marrow cells. Experimental animals sacrificed 1 and 2 days after the treatment, revealed approximately 60% higher frequency of SCE than the corresponding controls. When the treatment was extended to 5 or 14 days, the number of SCE was equivalent to the spontaneous rate. These findings suggest that diesel particulates and its methylene chloride extract contain genotoxic substances.

MUTAGENIC ACTIVITY OF DIESEL PARTICLES IN ALVEOLAR MACROPHAGES FROM RATS EXPOSED TO DIESEL ENGINE EXHAUST

J-S. Siak and K. A. Strom
Biomedical Science Department
General Motors Research Laboratories
Warren, MI 48090

Diesel engine exhaust contains submicron size carbonaceous particles. Dichloromethane extracts of these particles collected by filtration or electrostatic precipitation elicited mutagenic responses in bacterial mutagenicity assay. Currently, there are no data on the mutagenic properties of inhaled diesel particles that are deposited directly in the lung. The purpose of this experiment was to determine the mutagenicity of the inhaled diesel particles and the interaction between the particles and alveolar macrophages.

Adult male Fischer 344 rats were exposed to 6 mg/m^3 of diesel particles for 3 days (20 hrs/day). Alveolar macrophages were obtained by bronchopulmonary lavage immediately after exposure and at 1, 4, and 7 days thereafter. Macrophages from forty animals were pooled for each data point, sized and counted. The mass of diesel particles phagocytized in alveolar macrophages was determined by a spectrophotometric method (Rudd and Strom, J. Appl. Tox., 1(2):83-87, 1981). The alveolar macrophages were concentrated by filtration on pre-washed fiberglass filters and dried at room temperature to constant weight. The filters were extracted with dichloromethane in a Soxhlet apparatus for 4 hours (20-25 solvent cycles). The resulting extracts were oily, indicating cellular lipids and surfactant were extracted from the macrophages. The *Salmonella typhimurium* strain TA98 was used for mutagenicity assay. For thin layer chromatography, Whatman LK6 plates were used and the developing solvent was toluene:hexane (5:1).

Table 1 shows the diesel particle mass recovered in alveolar macrophages from exposed rats. The mass of diesel particle recovered from the lavage accounted for 45-50% of the particles deposited in rat lungs. The extracts of diesel particles (DPE) collected from the exposure chamber by filtration were used as reference for the thin layer chromatographic and mutagenic analysis of the macrophage extracts. The TLC fluorescence banding pattern of the samples from macrophages obtained immediately or one day post-exposure were similar to that of chamber DPE. However, the extracts of macrophages recovered on the fourth and seventh day post-exposure lost their fluorescence patterns. Figure 1 shows the results of the mutagenicity assay. The data indicate that the cellular lipids extracted from macrophages mitigated the mutagenic response of the airborne DPE, but a positive result was still detectable in the extracts of the macrophages obtained immediately, and one day after the exposure. In contrast, the mutagenic activity of extracts from macrophages obtained on the fourth and seventh

day after the exposure was undetectable. The data indicate that: 1) inhaled diesel particles contain extractable mutagenic compounds - whether they are the same as those found in the particles collected by other means has yet to be resolved; 2) alveolar macrophages have the ability to release or transform the fluorescent and mutagenic extractable hydrocarbons from phagocytized diesel particles over a period of several days and thus may significantly influence their biological activity in the respiratory system.

Table 1

DIESEL PARTICLE MASS RECOVERY
IN ALVEOLAR MACROPHAGES FROM EXPOSED RATS

GROUPS	DP ug/mL Lavage Fluid	DP ug/10^6 Macrophages	Total Recovery (mg)
IMMEDIATELY	5.0	41.3	8.4
1 DAY-POST	5.9	34.3	10.3
4 DAY-POST	6.3	25.5	10.6
7 DAY-POST	6.6	25.8	11.1

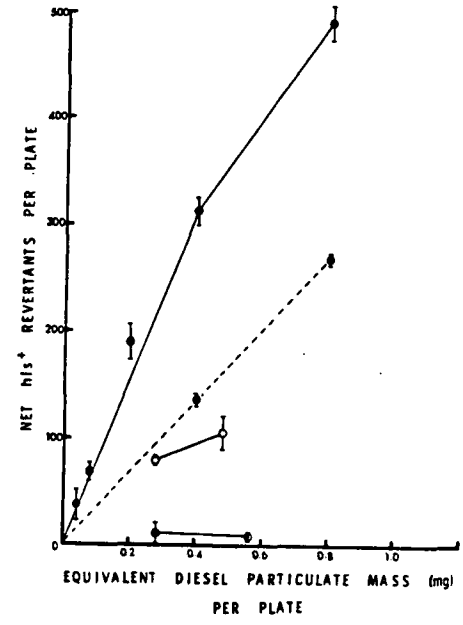
Mutagenic activity of airborne diesel particle extract and macrophage extracts.

—●— Airborne diesel particle extract.

- - -●- - - Airborne diesel particle extract + 800 μg control macrophage extract.

—○— Macrophage extract from exposed rats immediately after exposure.

—⊙— Macrophage extract from exposed rats 7 days after exposure.



DERMAL CARCINOGENESIS BIOASSAYS OF DIESEL PARTICULATES (DP) AND DICHLOROMETHANE (DCM) EXTRACT OF DP.

by

Linval R. DePass, Lynn G. Peterson and Carrol S. Weil,
Bushy Run Research Center, Export, Pennsylvania

K.C. Chen
Biomedical Science Department, General Motors
Research Laboratories, Warren, Michigan

INTRODUCTION

The present studies were designed to assess the potential of DP and DCM extract of DP as complete carcinogens and as initiators or promoters of carcinogenesis using a mouse skin model.

MATERIALS AND METHODS

The test agents were applied as suspensions in acetone to the dorsal skin of 40 male C3H/HeJ mice (Jackson Laboratory, Bar Harbor, Maine) per group at the highest concentrations that were sufficiently flowable for dosing. In some studies lower concentrations were also used to obtain information on dose-response relationships. The samples were shipped on dry ice from General Motors to Bushy Run Research Center on a regular basis throughout the study. Suspensions for dosing were stored at -12°C in amber-colored bottles except during application to the animals. Dosing was performed 3 times weekly in the initiation and complete carcinogenesis studies and 5 times weekly in the promotion studies. Positive control groups received repeated applications, 38 ug each, of benzo[a]pyrene (BaP, Eastman Kodak) for complete carcinogenesis or a single application of BaP,230 ug, followed by repeated applications, 1.5 ug each, of phorbol myristate acetate (PMA, PL Biochemicals) for the promotion studies. The test agents were applied in place of BaP in the complete carcinogenesis and initiation studies and in place of PMA in the promotion studies.

Mortality and tumor incidence data were analyzed by methods based on the Kaplan-Meier distribution (1). Comparisons were made using the Mantel-Cox (1) and Breslow (1) tests with a probability of 0.05 (2-tailed) required for rejection of the null hypothesis.

RESULTS

Complete Carcinogenesis Studies

One tumor-bearing animal has been observed at the highest dosage of DCM extract (12 mg/day) in the complete carcinogenesis study. The tumor was in the treatment area and was diagnosed as a squamous cell carcinoma. One mouse which received 1 mg/day DCM extract died with a subcutaneous sarcoma of the left lateral surface. Thirty eight tumor-bearing animals were observed in the positive control (BaP) group. No tumors were observed in the negative controls or in any other dosage group.

Initiation Studies

Six and five tumor-bearing mice were observed in the mice initiated with DP (2.0 mg) and DCM extract (12.0 mg) respectively. Four tumor-bearing animals have been observed in a negative control group initiated with acetone, and one tumor-bearing animal was seen in a second negative control group initiated with PMA.

Promotion Studies

In the promotion studies, one and two tumor-bearing animals have been observed in the groups which received 12.0 and 5.1 mg/day of DCM extract, respectively. No tumors have been observed in the groups treated with DP (2.0 mg/day), acetone, or a group which was untreated after the initiating dose of BaP.

In a positive control group which received repeated applications of PMA after an initiating dose of BaP, 19 tumor-bearing animals were observed.

DISCUSSION

The results to date suggest that DP and DCM extract of DP have little, if any, tumor-initiating or tumor-promoting activity under the conditions of this assay. This conclusion is based on the absence of a statistically significant increase in tumor incidence (or reduction in time to tumor) in any dosage group in the initiation and promotion studies.

However, the observation of a tumor-bearing mouse in the high dosage DCM extract group of the complete carcinogenesis study must be considered in assessing the oncogenic potential of diesel emissions. Although the presence of a single tumor is clearly not statistically significant, its importance must be considered in the light of extensive historical control data. The C3H/HeJ strain has been found to have an extremely low spontaneous skin tumor incidence in this laboratory. Of 474 acetone-treated controls, only a single mouse with a squamous cell carcinoma of the eyelid has been observed. No tumors have been seen in the treatment area. Thus, the tumor in the treat-

ment area of a DCM extract-treated mouse may have toxicological significance.

Since some of these studies are still in progress, it would be inappropriate to draw any final conclusions at this time. Preliminary conclusions based on gross observations will be possible after the death of all the animals.

REFERENCE

1. Gart, J.J., K.C. Chu and R.E. Tarone 1979. Statistical issues in interpretation of chronic bioassay tests for carcinogenicity. J. Natl. Cancer Inst. 62:957-974.

RESPIRATORY CARCINOGENICITY OF DIESEL FUEL EMISSIONS - INTERIM RESULTS

by

Alan M. Shefner, Bobby R. Collins, Arsen Fiks,
Lawrence Dooley and Maurline M. Preache
IIT Research Institute
Life Sciences Research Division
10 West 35th Street
Chicago, Illinois 60616

An experiment is in progress in which diesel engine emission particles (DP), organic solvent extracts of diesel particles (DE), extracts of roofing tar volatiles (RT) and of coke oven emissions (CO), and cigarette smoke condensate (CS) are being evaluated for their carcinogenic potential when administered by intratracheal instillation to hamsters. Appropriate control animal groups including untreated colony controls (CC), solvent (SV), solvent plus ferric oxide (SF), benzo(a)pyrene (BP) as a positive control, and gel-saline plus ferric oxide (GS) were included in the study. Because of the number of hamsters being treated, the experiment was conducted in two replicates of identical design with the exception of the inclusion of a gel-saline control in the second replicate.

All five test materials were prepared as mixtures with equal weights of ferric oxide and delivered as suspensions or emulsions in appropriate solvents. Diesel emission particles were also prepared without admixed ferric oxide and constituted the sixth test material. It was prepared as a suspension in SV. Test materials were delivered at doses of 5, 2.5, and 1.25 mg/treatment/week with additional equal weights of ferric oxide for five of the test materials. Intratracheal administration was carried out once weekly for fifteen weeks for each test and control treated group. Hamsters were twelve-to-thirteen weeks of age at initiation of treatment. Only male hamsters were used in the study and animals were obtained from Charles River Breeding Laboratories. Group sizes in each replicate consisted of 60 hamsters per dose level for each test group, 60 SV controls, 150 SF controls, 60 BP animals and 120 colony controls. An additional 60 GS controls were included in the second replicate. Thus an initial group of 1470 hamsters was included in the first replicate and 1530 hamsters in the second. When the hamsters reached 12 months of age a sacrifice of randomly selected animals from each group was carried out in each replicate. This consisted of 10 hamsters per dose level for each test group, 10 SV controls, 25 SF controls, 10 BP controls, 20 CC animals and

10 GS controls. Sacrificed animals were subjected to a complete gross pathology examination, major organs were weighed and an extensive group of tissues were submitted for histologic processing. Histopathologic examination of tissue slides from the scheduled sacrifice animals from the first replicate has been carried out and similar studies on second replicate animals are in progress.

High dose hamsters in most treatment groups gained weight more slowly than their respective controls during the treatment period. Upon cessation of treatment high dose animals generally gained weight at a faster rate and body weight differentials gradually disappeared. At 61 weeks on test in the first replicate and 44 weeks on test in the second there are no consistent treatment-related trends in body weights in surviving hamsters.

First replicate hamsters were housed three to a cage in suspended polycarbonate cages with filter sheets over the shelves of the racks. A considerable amount of fighting occurred between cage mates resulting in a 94-100 percent incidence of lumbosacral skin lesions secondary to fighting. Early deaths in these hamsters were not treatment or dose related and were attributed largely to wound-related causes. Cage dividers of stainless steel were designed and fabricated and the hamsters were rehoused two per cage separated by a solid partition. Skin lesions cleared up over time after the hamsters were physically isolated from cage mates. The hamsters in the second replicate were individually housed in these partitioned cages from the fourth week of test onward. Overall survival rates in the second replicate have been consistently higher than that observed in the first replicate. This absence of fighting-associated deaths in the second replicate should make possible the determination of test material effects on survival time should such exist.

Group mean organ weights of test hamsters from the first replicate showed no significant effects of test materials as compared with their solvent controls. Histopathologic findings on these animals were generally less severe than those observed in earlier dose response studies where hamsters were sacrificed five weeks after the end of treatment. This observation tends to reinforce the apparent recovery from immediate treatment effects implied by the increased body weight gains observed following cessation of treatment.

Various lesions of the lung were noted and those which appeared to be treatment related generally reflected the quantity of particulate material administered as well as showing differences in reactivity to specific test substances. In general inflammatory reactions and granuloma were more prevalent in diesel particle, diesel extract and coke oven extract animals than in cigarette smoke or roofing tar treated groups. Adenomatous hyperplasia and papillary hyperplasia were either found in a low incidence or not observed in solvent control and colony control hamsters and to a trace to mild degree in solvent plus ferric oxide animals. The incidence and severity of these lesions was greatest in both diesel particulate groups, intermediate in the coke oven extract treated animals, and lowest in the diesel extract, cigarette smoke, and roofing tar groups.

Bronchiolization was greatest in high dose diesel particle groups, found to a lesser degree in coke oven and roofing tar animals, least frequent in diesel extract and cigarette smoke animals and absent in control hamsters. Squamous metaplasia was observed primarily in coke oven treated animals and the few adenomas that were found were present in diesel particle and diesel extract treated animals. In general the frequency and severity of hyperplastic and metaplastic changes in the lung were greatest in the two diesel emission particle test groups, somewhat lower in the coke oven extract and in the diesel extract groups, and least in the cigarette smoke and roofing tar extract hamsters.

Particles were found in high density in the thoracic lymph nodes of particle treated animals and reactions to the presence of these particles were common in all treatment groups but appeared more commonly in diesel particle treated hamsters.

Whether any of these lesions will progress over the lifespan of the remaining hamsters now on test, and whether such progression will be related to test material and dose remains to be seen.

(This study was supported by EPA Grant No. R806929-01).

CARCINOGENICITY OF EXTRACTS OF DIESEL AND RELATED ENVIRONMENTAL EMISSIONS UPON LUNG TUMOR INDUCTION IN STRAIN 'A' MICE

by

R. D. Laurie, W. B. Peirano, W. Crocker,
F. Truman, J. K. Mattox and W. E. Pepelko
Health Effects Research Laboratory
U.S. Environmental Protection Agency
Cincinnati, Ohio

INTRODUCTION

The predicted increase in the use of diesel engines has resulted in a regulatory need for data assessing the relative carcinogenicity of diesel exhaust. Since cigarette smoke, roofing tar and coke oven emissions have been shown to be carcinogenic, a matrix of experiments was designed to compare the relative potency of these pollutants with diesel exhaust particulate. The present study is one of several, including skin painting of Sencar mice, intratracheal instillation in hamsters and *in vitro* testing, designed to provide such a comparison.

METHODS

Compounds

Nissan diesel particulate matter was collected with a high volume sampler using Pallflex T60A20 (teflon coated) filters. The samples were collected from a large mixing chamber containing exhaust diluted with about 9 parts clean air to produce a particulate matter concentration of 12 mg/m^3. Exhaust was produced with a 6 cylinder, 90 cubic inch displacement Nissan diesel engine run on the Federal Short Cycle. For details see Hinners et al (1979). The Oldsmobile sample differed from the Nissan sample in that it was generated at a steady state (40 mph). Both samples were Soxhlet extracted with dichloromethane. Cigarette smoke condensate (CSC) was supplied by the Chemical Repository and Tobacco Smoke Chemistry Division of the Tobacco and Health Research Institute, University of

Kentucky. The CSC was produced from Kentucky Reference 2RI Cigarettes. For details of collection and generation see Patel (1977). The coke oven emissions were collected using massive volume samplers from the top of a coke oven battery at Republic Steel in Godsden, Alabama. The roofing tar emissions were collected using a baghouse filter fitted with special nonreacting filter bags. Details of collection procedures for both coke oven and roofing tar have been described in detail by Huisingh et al (1979). Most samples were dissolved in DMSO: for the Nissan particulate matter 5% of the DMSO solution was EL620.

Animals Used and Experimental Design

Strain A/Jax mice approximately 8 weeks of age, were randomly assigned to 9 groups: 1) uninjected controls, 2) vehicle controls, 3) positive controls, 4) Nissan generated particulate matter, 5) Nissan particulate extract, 6) Oldsmobile particulate extract, 7) cigarette smoke condensate, 8) coke oven emissions, and 9) roofing tar. The doses for each group are listed in Table 1. Because of limited availability of Oldsmobile extract only males were injected. Due to high mortality rates among mice injected with Nissan particulate the dose level was halved in the second experiment. The experiment was carried out in 2 parts because of the limited availability of animals and manpower. The mice were injected 3 times weekly for 8 weeks with the test substances. The injection volume was 50 microliters, delivered by Hamilton syringes fitted with 26 G needles. The urethane positive controls received only one injection as the start of the experiment.

Collection and Analysis of Data

The mice were sacrificed at 9 months of age with an overdose of nembutal. The lungs were removed and placed in buffered formalin. After 2 weeks the lobes were detached from the bronchi and the number of adenomas visible on the surface counted. Questionable areas were examined microscopically. Analysis of variance compared the number of tumors per mouse among groups; Chi Square analysis compared the frequency of mice with tumors.

RESULTS

From the data presented in Table 1 it is clear that a significant increase in number of lung adenomas per mouse and percent of mice with tumors occurred in the positive controls (urethane injected). In experiment 1 a significant increase in number of tumors per mouse was noted in males injected with Nissan diesel extract compared with controls or those injected with Oldsmobile extract. In experiment 2, a significant increase in lung tumor rates was detected in females injected with coke oven emissions. After combining the data from both experiments, no statistically significant differences were noted, except for the positive controls.

DISCUSSION

There were no consistent, statistically significant differences between the exposed and control groups with respect to either number of adenomas per mouse or number of mice with adenomas. The response to urethane was equivalent to the numbers predicted from numerous previous studies indicating that the animals were sensitive to tumor induction (Shimkin and Stoner, 1975). It was concluded that either the carcinogens present in the test substances were very weak or that an insufficient concentration reached the lungs to produce a positive result.

REFERENCES

Hinners, R.G., J.K. Burkart, M. Malanchuk and W.D. Wagner. Animal exposure facility for diesel exhaust studies. Health Effects of Diesel Engine Emissions: Proceedings of an International Symposium, Vol. 2: 681-697, 1979.

Huisingh, J.L., R.L. Bradow, R.H. Jungers, B.D. Harris, R.B. Zweidinger, K.M. Cushing, B.E. Gill and R.E. Albert. Mutagenic and carcinogenic potency of extracts of diesel and related environmental emissions: Study design, sample generation, collection and preparation. Health Effects of Diesel Engine Emissions: Proceedings of an International Symposium. Vol. 2: 788-800, 1979.

Patel, A.R. Preparation and monitoring of cigarette smoke condensate samples. In Report No. 3, Toward less hazardous cigarettes. The third set of experimental cigarettes, G.B. Gori, Ed., DHEW Publication No. (NIH) 77-1280.

Shimkin, N.B. and G.D. Stoner. Lung tumors in mice: Application to carcinogenesis bioassay. Adv. Cancer Res. 21:1-58, 1975.

Table 1 - Strain A Mouse Data for Induction of Adenomas by Environmental Mixtures

Group	Sex	Percent of Mice with Tumors	Av. Number of Tumors per Mouse	Percent Surviving	Dose
Uninjected Controls	M	40	0.6 ± 0.2	75	-
	F	47	0.6 ± 0.2	85	-
DMSO + 5% EL620	M	40	0.9 ± 0.5	50	0.05 ml/injection
	F	47	0.7 ± 0.2	75	
Urethane	M	100[A]	22.5 ± 1.9[A]	75	20 mg/mouse
	F	100[A]	21.8 ± 1.5[A]	85	
Nissan Diesel Particulate	M	40	0.4 ± 0.1	33	4 mg/injection
	F	20	0.5 ± 0.1	33	
Nissan Diesel Extract	M	73	1.4 ± 0.3[B]	50	1 mg/injection
	F	55	1.0 ± 0.3	67	
Olds. Diesel Extract	M	37	0.4 ± 0.1	63	1 mg/injection
	F	-	-	-	
Cigarette Smoke Condensate	M	59	0.8 ± 0.3	73	0.20 mg/injection
	F	54	1.1 ± 0.2	80	
Coke Oven	M	54	1.2 ± 0.3	87	0.02 mg/injection
	F	31	0.5 ± 0.2	87	
Roofing Tar	M	41	0.7 ± 0.2	73	0.02 mg/injection
	F	44	0.7 ± 0.3	47	

Experiment 1

Injected Controls	M	13	0.2 ± 0.1	100	-
	F	26	0.4 ± 0.2	100	-
DMSO + 5% EL620	M	44	0.5 ± 0.2	80	0.05 ml/injection
	F	26	0.3 ± 0.1	95	
Urethane	M	95[A]	7.3 ± 0.7[A]	100	10 mg/mouse
	F	100[A]	11.3 ± 0.9[A]	96	
Nissan Diesel Particulate	M	27	0.3 ± 0.1	68	2 mg/injection
	F	32	0.3 ± 0.1	42	
Nissan Diesel Extract	M	33	0.4 ± 0.1	60	1 mg/injection
	F	50	0.7 ± 0.1	71	
Olds. Diesel Extract	M	33	0.4 ± 0.1	69	1 mg/injection
	F	-	-	-	
Cigarette Smoke Condensate	M	30	0.4 ± 0.1	77	0.20 mg/injection
	F	21	0.2 ± 0.1	80	
Coke Oven	M	31	0.4 ± 0.1	83	0.02 mg/injection
	F	65	0.9 ± 0.2[A]	89	
Roofing Tar	M	44	0.7 ± 0.2	91	0.02 mg/injection
	F	22	0.3 ± 0.1	91	

Experiment 2

[A] Significantly different from uninjected and injected controls ($p < 0.05$).

[B] Significantly different from uninjected controls and Olds. diesel extract ($p < 0.05$).

THE INFLUENCE OF INHALED DIESEL ENGINE EMISSIONS UPON LUNG TUMOR INDUCTION IN STRAIN'A' MICE

by

William E. Pepelko, John G. Orthoefer, W. Bruce Peirano,
Walden Crocker, and Freda Truman
Health Effects Research Laboratory
U. S. Environmental Protection Agency
Cincinnati, Ohio

The Strain 'A' mouse was one of several animal models chosen by the Environmental Protection Agency to assess the carcinogenic risk of exposure to diesel engine emissions. The Strain 'A' mouse was selected because: it has been one of the most extensively used models for assessment of lung tumor induction; the test is well validated; exposure times are fairly short for a cancer assay; the test is relatively straight forward to perform and evaluate and finally; it is one of the most sensitive lung tumor bioassays available (1). The results presented are from experiments in which mice were exposed to exhaust with a particulate concentration of 12 mg/m^3. These studies are a continuation of earlier work in which exposure levels were about one half those used in the present experiments (2).

Details of the exposure conditions and experimental procedures have been published previously (2, 3). Briefly, Strain 'A' mice obtained from Jackson or Strong Laboratories were exposed 8 hrs/day, 7 days/week from 6 weeks to either 9 or 12 months of age. The mice were housed in wire cages and exposed in 100 cubic feet stainless steel chambers. Exhaust was produced by a 6 cylinder, 90 cu inch Nissan diesel engine. In order to simulate city driving conditions, the engine load and speed were varied cyclically using the Federal Short cycle. After completion of exposure, the animals were sacrificed and the lungs fixed and observed for the presence of pulmonary adenomas.

Three experiments were carried out. In the first, 360 animals, 180 of each sex, were exposed to clean air or diesel exhaust. One half of each group received a single intraperitoneal injection of 5 mg urethane prior to the start of exhaust exposure. In the second study, 115 males and 143 females were exposed to diesel exhaust, while 108 males and 142 females were

exposed to clean air. Conditions differed from that of the first study in that the exposure occurred during the dark portion of the daily cycle when the animals were presumably awake, active and respiring at a greater level. In the final study in which only males were used, the mice were sacrificed at 12 months instead of 9 months of age. The number of mice with tumors were compared using Chi Square testing. Results are shown in Table 1. Exposure of mice to diesel exhaust until 9 months of age resulted in a significant decrease in the mean tumor incidence in females, and in males and females combined ($P < .05$). Decreases were also noted in males, but differences were not significant. In comparing groups treated with an initiating dose of 5 mg urethane prior to exposure, a decreased tumor incidence was again noted in the exhaust exposed mice, ($P < .10$) for males, ($P < .001$) for females, and ($P < .001$) for males and females combined. Exposure to diesel exhaust during the dark portion of the daily cycle also resulted in a decreased tumor incidence compared with clean air controls ($P < .01$) for males, ($P < .001$) for females, and ($P < .001$) for males and females combined. Finally, exposure of males to diesel exhaust until 12 months of age again resulted in a decrease in lung tumor incidence ($P < .01$).

Overall, there was no indication that exposure of Strain 'A' mice to diesel engine emissions resulted in an increase in lung tumor incidence. On the contrary, the studies consistently showed that tumor rates were decreased in exhaust exposed mice. Such a decrease following exposure to a potentially carcinogenic pollutant is rare, but is not unknown. Nettesheim et al., (4) reported that inhalation of a combination of ozonized gasoline and ferric oxide particles inhibited the tumorigenic effects of injected diethylnitrosamine on the respiratory tract. Kotin and Folk (5) also showed that exposure of C57BL mice for a lifetime to an atmosphere of ozonized gasoline resulted in a significantly lower incidence of malignant lymphomas and hepatomas compared to mice breathing clean air. Finally, Pereira (6) found fewer gamma glutamyl transpeptidase positive liver islands in rats following exposure to diesel exhaust than in clean air controls.

An explanation of the present results must await further study. Possibly, diesel exhaust inhalation inhibits the induction of enzymes responsible for converting procarcinogens to their active forms. The immunocompetence of the animals could also have been altered as a result of the inflammatory reaction to deposited exhaust particulate. The results cannot be explained by increased mortality of mice susceptible to tumor induction since survival rates were not significantly altered by the exposure positions.

REFERENCES

1. Shimkin, M.B., and C.D. Stoner. 1975. Lung Tumors In mice: Application to Carcinogenesis Assay. Adv. Cancer. Res. 21:1-58.

2. Orthoefer, J.G., W.Moore, D. Kraemer, F. Truman, W. Crocker, and Y.Y. Yang. 1980. Carcinogenicity of Diesel Exhaust as Tested in Strain 'A' Mice. Presented at the U.S. Environmental Protection Agency International Symposium on Health Effects of Diesel Engine Emission. Cincinnati, Ohio.

3. Hinners, R.G., J.K. Burkart, M. Malanchuk, and W.D. Wagner. 1980. Facilities for Diesel Exhaust Studies. Presented at the U. S. Environmental Protection Agency International Symposium on Health Effects of Diesel Engine Emissions. Cincinnati, Ohio.

4. Nettesheim, P., D.A. Creasia, and T.J. Mitchell. 1975. Carcinogenic and Co-Carcinogenic Effects of Inhaled Synthetic Smog and Ferric Oxide particles. J. NCI. 55: 159-169.

5. Kotin, P. and H.L. Falk. 1956. The Experimental Induction of Pulmonary Tumors and Changes in the Respiratory Epithelium in C57BL Mice Following Their Exposure to an Atmosphere of Ozonized Gasoline. Cancer. 11: 473-481.

6. Pereira, M.A., H. Shinozuka, and B. Lombardi. 1980. Test of Diesel Exhaust Emissions In the Rat Liver Foci Assay. Presented at the U. S. Environmental Protection Agency International Symposium on Health Effects of Diesel Engine Emission. Cincinnati, Ohio.

Table 1. Effects of Inhaled Diesel Engine Emissions Upon Lung Tumor Incidence in Strain 'A' Mice

Treatment	Age at Sacrifice (Months)	Illumination During Exposure	Sex	Number of Mice with Tumors / Number of Surviving Mice	P Values
Clean Air	9	Light	M	10/44	
			F	11/43	
			M+F	21/87	
Diesel Exhaust	9	Light	M	5/37	NS
			F	4/43	< .05
			M+F	9/80	< .05
Clean Air + 5 mg Urethane	9	Light	M	32/38	
			F	34/37	
			M+F	66/75	
Diesel Exhaust + 5 mg Urethane	9	Light	M	26/39	< .10
			F	16/36	< .001
			M+F	42/75	< .001
Clean Air	12	Light	M	22/38	
Diesel Exhaust	12	Light	M	11/44	< .01
Clean Air	9	Dark	M	28/97	
			F	31/140	
			M+F	59/237	
Diesel Exhaust	9	Dark	M	13/111	< .01
			F	9/139	< .001
			M+F	22/250	< .001

OBJECTIVES AND EXPERIMENTAL CONDITIONS OF A VW/AUDI DIESEL EXHAUST INHALATION STUDY

by

U. Heinrich, F. Pott and W. Stöber
Fraunhofer-Institut für Toxikologie und Aerosolforschung
Hannover, Federal Republic of Germany

H. Klingenberg
Volkswagenwerk AG, Forschungsabteilung Meßtechnik
Wolfsburg, Federal Republic of Germany

Extended Abstract

OBJECTIVES

The rationale of this project is to make a most sophisticated effort into contesting the contention that, even under aggravated conditions, exposure to automobile exhaust, particularly to emissions of a VW diesel engine, does not produce irreversible long-term health effects and does not warrant emission regulations resulting in very expensive motor vehicle technologies. Therefore, this investigation is designed to reveal the existence of potential toxic effects of the exhaust of a VW diesel engine in long-term inhalation studies with rats, Syrian golden hamsters and mice. In particular, attention will be focussed on the impact on lung functions and the incidence of lung tumors. The study takes advantage of the results of an earlier investigation on the long-term diesel exhaust inhalation exposure of Syrian golden hamsters and is related to a program on the inhalation exposure to gasoline engine exhaust presently in progress.

EXHAUST GENERATION AND ANALYTICS

The exhaust is taken from a VW diesel engine on a test bench. The engine is operated by a computer simulating continuously the US-72 (hot start) Federal Test Procedure (FTP) cycle. The exposure chamber air is sampled for the analysis of a number of gaseous emission components as well as a variety of polycyclic aromatic hydrocarbons (PAH) and the physical parameters and the chemical composition of the airborne diesel soot particles.

EXPERIMENTAL

Nine stainless steel exposure chambers of special design, sized 3 x 2 x 2 meters each (depth x width x height), were built into a new inhalation toxicology facility. The design provides a horizontal ventilation of the chambers by the diluted exhaust emissions. The total number of animals utilized in the project is about 850 rats, 1150 Syrian golden hamsters and 1700 mice. The rats and mice are held under barrier conditions, while the hamsters are kept conventionally.

The exhaust inhalation exposure lasts 16 to 18 hours daily on 5 days per week and is to be continued for about 2 years. In a range-finding test experiment of 3 months, 4 different exhaust dilutions are applied and the maximum exhaust concentration that may not reduce the natural life expectancy of the 3 animal species will be determined by means of some clinical-chemical measurements and histological evaluations. If the species will respond differently, correspondingly different exhaust concentrations will be employed.

During the long-term experiments, periodical tests are performed to provide data on the clinical chemistry of blood and liquids of lung lavages. Furthermore, lung function tests are conducted on hamsters and rats.

The focus of the experimental design is on the question of a potential carcinogenicity of the exhaust of this diesel engine. Since the chances of inducing tumors in a straightforward exposure of healthy animals are rather slim, some of the test animals will be pre-treated with either a nitrosamine or a carcinogenic PAH similar to an earlier experimental design already described elsewhere. This pre-treatment should produce a base line tumor induction rate of about 20 percent so that a possibly existing disproportionate additional effect on the tumor induction by the exhaust emissions stands a better chance of statistical significance.

PROJECTED HUMAN HEALTH RISKS FROM INCREASED USE OF DIESEL LIGHT DUTY VEHICLES IN THE UNITED STATES

by

R. G. Cuddihy, R. O. McClellan, W. C. Griffith,
F. A. Seiler and B. R. Scott
Lovelace Inhalation Toxicology Research Institute
P. O. Box 5890
Albuquerque, NM 87185

Diesel light duty vehicles are expected to comprise 20% of the total light duty vehicle fleet in the United States within 15 years. Their use should grow because of a perceived advantage over gasoline engine vehicles in fuel costs, but it will be limited by the capacity to produce sufficient diesel fuel economically. To estimate the potential health risks from exposures of people to diesel vehicle emissions in the environment, it is necessary to identify any toxic components in their exhaust, to project future levels of these pollutants in congested urban environments and to estimate the potential magnitude of the related human health risks.

Chemical analyses of diesel emissions have identified potentially toxic gases including nitrogen oxides and carbon monoxide along with particles that contain known carcinogenic compounds including polyaromatic hydrocarbons and nitroaromatics. However, other industrial and transportation sources of these pollutants contribute substantially more to the environment than can be projected from the increased use of diesel light duty vehicles. Therefore, in well-mixed urban atmospheres little of the total health risk attributable to inhaled pollutants could be related to the future use of light duty diesel vehicles.

In congested urban areas with limited air circulation such as street canyons and parking garages, vehicle exhausts may reach sufficiently high levels to produce respiratory symptoms. Both gasoline engine vehicles and diesel vehicles contribute to these problem areas, however, diesel exhaust contains significantly higher concentrations of nitrogen oxides and particles. Empirical relationships between measured levels of carbon monoxide in vehicle exhaust and its concentrations in urban street canyons have been developed based upon studies near streets in New York, St. Louis and San Jose. These relationships indicate that if diesels were to comprise 20% of the total light duty vehicles, they may add 10 $\mu g/m^3$ to the particle concentrations of air and more than 50 $\mu g/m^3$ to the nitrogen oxide concentrations. Other models indicate that in parking garages, diesel particle concentrations could reach 200 $\mu g/m^3$ and nitrogen oxide concentrations 1000 $\mu g/m^3$ during

periods of high vehicle activity. The average concentrations of diesel particles over large city areas are expected to be between 1 and 2 $\mu g/m^3$. Diesel emissions of nitrogen oxides are not expected to produce measurable changes in these ambient air concentrations.

The potential health effects of exposures to nitrogen oxides have been summarized in two recent reports by the National Academy of Sciences and the Electric Power Research Institute. In general, prolonged exposures to nitrogen dioxide above 100 $\mu g/m^3$ can lead to chronic bronchitis in children and adults, and above 150 $\mu g/m^3$ respiratory function changes may occur. Exposures to nitrogen dioxide above 1000 $\mu g/m^3$ for several hours can lead to acute metabolic and respiratory function changes. Therefore, people who work in congested urban environments with restricted air circulation are likely to be exposed to irritant levels of nitrogen oxides. This is especially true on days with little sunlight when the chemical balance favors formation of nitrogen dioxide over formation of nitric oxide. However, it should also be noted that the contribution of gasoline engine vehicles to nitrogen oxides in these atmospheres is also significant.

The potential health effects of diesel particle emissions are more difficult to evaluate due to very limited observations of their effects on people or laboratory animals. Because diesel vehicles are expected to add less than 2 $\mu g/m^3$ to urban environments, their emissions will not add significantly to health risks that are solely related to the general levels of particulate pollution.

Concern has also been expressed for health risks due to carcinogenic organic compounds associated with diesel particles. Diesel particle extracts have been shown to be mutagenic to cells in culture, to cause cell transformations and to induce tumors in the skin of rats. Further studies have also shown that diesel particle extracts are not markedly different from extracts or condensates of cigarette smoke, coke oven emissions, or urban soot in their ability to cause these biological effects per unit mass. Therefore, we summarized information on the exposures of these human populations to airborne particles and on their lung cancer incidences (Table 1).

Table 1. Summary of Population Exposures to Airborne Particles and Annual Cancer Risks

Study Population	Average Air Concentration mg Particles/m^3	Annual Lung Cancer Risk Per 100,000 People	Annual Cancer Risk Per 100,000 People Per mg/m^3
Rural Nonsmokers	0.03	3	100
Urban Nonsmokers	0.1	7	70
Smokers (cigarettes/day)			
1 - 9	2-16	26	3
10 - 19	18-35	47	2
20 - 39	36-71	80	2
40 +	73+	107	1
Coke Oven Workers	3	400	130

By assuming that all of the lung cancers in these populations were caused by their exposures to the particles, we obtained an upper estimate of lung cancer risk that would be expected in people exposed to diesel exhaust particles. The risk estimator was taken to be 0.0015 cancers per year per mg/m^3 lifetime exposure to diesel particles. Combining this risk factor with projected air concentrations of diesel particles in urban environments in future years, we estimated that less than 30 lung cancers per year could be related to the use of diesel light duty vehicles in the United States.

Two additional analyses of the carcinogenic risks from exposures to diesel emissions became available during 1981. The first analysis was contained in a report to the Diesel Impacts Study Committee of the National Research Council (1). This analysis was mainly based upon studies of London bus garage workers who were exposed to high levels of diesel engine exhausts between 1930 and 1974. Their lung cancer incidences were compared to other transit workers including engineers, bus drivers, conductors and subway motormen. Although the studies failed to show a definite increased lung cancer risk in the garage workers, based upon statistical considerations, Harris calculated an upper limit for their lung cancer risks. The upper 95% confidence limit on the increased lung cancer risk was 1% per $\mu g/m^3$ particulate exposures.

A second study was completed by DuMouchel and Harris (2) that estimated lung cancer risks from diesel emissions based upon laboratory studies of mutagenesis and viral cell transformations produced by diesel particle extracts. The relative potency of the diesel particle extracts in these test cell systems was estimated and compared to roofing tar vapors and coke oven emissions. The results of epidemiology studies were also used to estimate the absolute lung cancer risks per unit of exposure to roofing tars and coke oven emissions. By this technique DuMouchel and Harris (2) estimated an upper 95% confidence limit for exposures of people to diesel particles at 1.8% increase in lifetime lung cancer risks per μg of particles/m^3 of exposure. As shown in Table 2, all of these estimates of lung cancer risk to people exposed to diesel emissions are reasonably similar.

Table 2. Estimates of the Proportional Increased Risk of Lung Cancer Using Three Approaches

Data Sets Used	Proportional Increased Risk of Lung Cancer Per $\mu g/m^3$ Particulate	Reference
Cigarette Smokers and Coke Oven Workers	0.5%*	Cuddihy *et al.* (3)
London Garage Workers	1.0%**	Harris (2)
London Garage Workers, Viral Cell Transformations and *Salmonella* Tester Strains	1.8%**	DuMouchel and Harris (2)

*Largest estimate
**Upper 95% confidence limit

ACKNOWLEDGEMENTS

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REFERENCES

1. Harris, J. E. 1981. Potential risk of lung cancer from diesel engine emissions. Report to the Diesel Impacts Study Committee, Assembly of Engineering, National Research Council, National Academy Press, Washington, DC.

2. DuMouchel, W. H. and J. E. Harris. 1981. Bayes and empirical bayes methods for combining cancer experiments in man and other speices. Technical Report No. 24, Department of Mathematics, Massachusetts Institute of Technology.

3. Cuddihy, R. G., F. A. Seiler, W. C. Griffith, B. R. Scott and R. O. McClellan. 1980. Potential health and environmental effects of diesel light duty vehicles. Lovelace Inhalation Toxicology Research Institute Report LMF-82, UC-48.

HEALTH EFFECTS OF EXPOSURE TO DIESEL FUMES AND DUST IN TWO TRONA MINES

by

M.D. Attfield
Appalachian Laboratories
National Institute of Occupational Safety and Health
Morgantown, West Virginia

The industrial environment is often a useful situation in which to study the effect of health hazards, as workers usually receive higher exposures than does the general population. This is particularly so for miners exposed to diesel fumes underground, since the restriction on ventilation acts to concentrate the fumes. This study involves 700 workers engaged in trona ($Na_2CO_3 \cdot NaHCO_3 \cdot 2H_2O$) mining. These miners were given chest radiographs, asked questions on chest symptoms, smoking and work history, and given spirometric tests. In addition, comprehensive industrial hygiene surveys were undertaken at the two mines which were studied. The data available from these surveys is being explored for dose-response relationships between health indices and measures of diesel-engine-related pollutants. This paper will report the results.

MUTAGENICITY AND CHEMICAL CHARACTERISTICS OF CARBONACEOUS PARTICULATE MATTER FROM VEHICLES ON THE ROAD

by

William R. Pierson, Robert A. Gorse, Jr., Ann Cuneo Szkarlat,
Wanda W. Brachaczek, Steven M. Japar, and Frank S.-C. Lee*
Engineering & Research Staff - Research
Ford Motor Company
P.O. Box 2053
Dearborn, Michigan 48121

Roy B. Zweidinger and Larry D. Claxton
U.S. Environmental Protection Agency
Research Triangle Park, North Carolina 27711

Two experiments were conducted in the eastbound tunnel of the Allegheny Mountain Tunnel of the Pennsylvania Turnpike in 1979 to evaluate the bacterial mutagenicity of the organic solvent extracts of particulate emissions from heavy-duty Diesels and from (predominantly light-duty) gasoline-powered vehicles in highway operation. Filter samples (PTFE-Teflon-impregnated glass fiber and PTFE membrane) collected during periods dominated by Diesel traffic as well as periods dominated by gasoline-powered vehicles were Soxhlet-extracted with dichloromethane (CH_2Cl_2) followed by acetonitrile (CH_3CN). Concurrently collected ambient-air samples (in the ventilation intake fan rooms and at a tower on the mountaintop) were treated the same way in order to distinguish between vehicle and ambient contributions to the mutagenic activity of the tunnel samples, and also to compare mutagenic activity of vehicle and ambient particulate-matter extracts. Total tunnel air flow, traffic volume, and traffic composition were monitored to permit calculation of emission rates per unit distance driven, for Diesels and for gasoline-powered vehicles (e.g., Fig. 1).

Mutagenicity was determined by the *Salmonella typhimurium* plate incorporation assay [Ames test (1)] using several tester strains, with and without microsomal activation by S9 rat-liver homogenate. The number of revertant colonies per km travelled was calculated for each sampling run and plotted against traffic composition (e.g., Fig. 2) to obtain revertants/km averages for gasoline- and Diesel-powered vehicles separately. High performance liquid chromatography (HPLC) profiles were obtained on the CH_2Cl_2 and CH_3CN

*Present address: Amoco Research Center, Standard Oil Company of Indiana, P.O. Box 400, Naperville, Illinois 60566

extracts. Gas-chromatographic (GC) molecular-weight distributions (retention-time distributions) were obtained on the CH_2Cl_2 extracts and resolved as above according to vehicle type.

The main findings are as follows:

(1) The Diesel-produced aerosol in the Allegheny Tunnel is similar to that encountered in dilution tubes, with respect to all criteria, viz., percentage extractable into CH_2Cl_2 (24 $\pm$ 3%), Ames mutagenicity in revertants per km travelled or revertants per μg of CH_2Cl_2-extracted material, HPLC fluorescence profile, and molecular-weight distribution.
(2) Expressed as revertants per μg of CH_2Cl_2-extracted material, the mutagenic activities of the Diesel-produced aerosol in the Allegheny Tunnel are of the same order of magnitude as the mutagenic activities of the ambient aerosol in the vicinity at Allegheny.
(3) Expressed as revertants per km travelled, the mutagenicity of the CH_2Cl_2 extract of the particulate emissions from Diesels is several times that from gasoline-powered vehicles.

Some of the mutagenicity results are summarized in Tables 1 and 2.

REFERENCES

1. Ames, B. N., J. McCann, and E. Yamasaki, 1975. Methods for determining carcinogens as mutagens with the Salmonella/mammalian microsome mutagenicity test. Mutation Research 31:347-364.

Table 1. Mutagenicities, Thousands of TA98 Revertants per Kilometer Travelled; CH_2Cl_2 Extracts, Allegheny Mountain Tunnel 1979.

		Gasoline-powered Vehicles	Diesel Trucks (a)
Without S9	May/June	39±24[b]	211±113
	Aug/Sept	19±10	80±20
With S9	May/June	26±14	181±40
	Aug/Sept	12±4	51±7

[a]Average gross weight approximately 35 tons.

[b]Error quoted is the standard deviation.

Table 2. Mutagenicities, TA98 Revertants per Microgram of CH_2Cl_2-extracted Material, Allegheny Mountain Tunnel 1979.

		Vehicles			Ambient Air	
		Gasoline-powered	Diesel Trucks	Over-all	Fan Rooms	Tower
Without S9	May/June	3±2	1.1±0.6	1.3	0.9	-
	Aug/Sept	4±3	0.4±0.1	0.6	0.6	0.2
With S9	May/June	2±1	0.9±0.2	1.0	0.4	-
	Aug/Sept	2.4±1.6	0.27±0.04	0.4	0.4	0.08

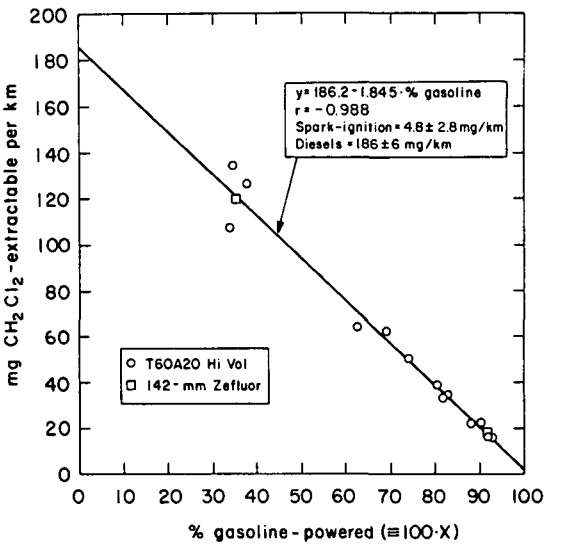

Figure 1. Plot of mg/km emission rate of CH_2Cl_2-extractable particulate matter vs. traffic composition, Allegheny Mountain Tunnel August/September 1979. Intercept at 0% gasoline-powered vehicles is the emission rate (186± mg/km) from Diesels.

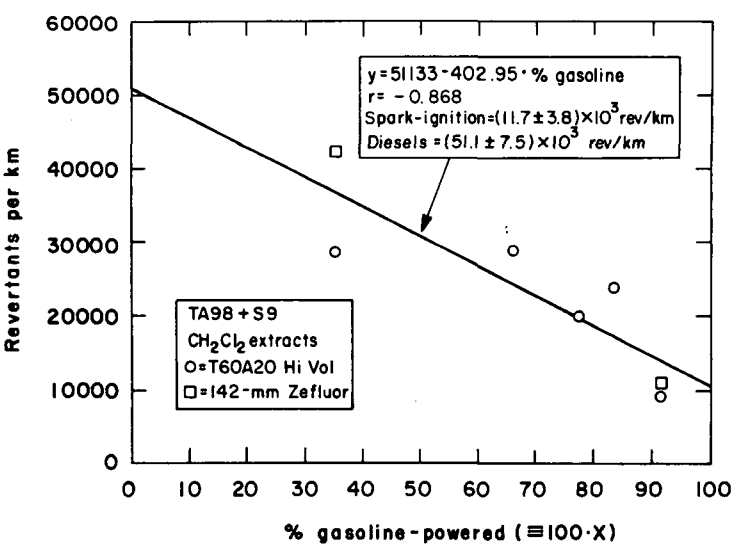

Figure 2. Plot of revertants/km vs. traffic composition, CH_2Cl_2 extracts, tester strain TA98 with microsomal activation (+S9), Allegheny Mountain Tunnel August/September 1979.

EMISSIONS OF GASES AND PARTICULATES FROM DIESEL TRUCKS ON THE ROAD (2)

by

Raisaku Kiyoura
Research Institute of Environmental Science
4, 5-Chome, Kojimachi, Chiyoda-ku
Tokyo, JAPAN

(A) Experiments have been conducted to estimate the diesel trucks emission rates at the Nihonzaka Tunnel. The Tunnel is a 4-lane dual tunnel (2 eastbound lanes through one tube, 2 westbound lanes through the other), 2 km long, on a slight grade upward (2.5%, 1.2 km), then downward (-1.84%, 0.8 km toward the east), located 170 km west of Tokyo. The vehicle traffic in the tunnel is very high with an average 1,107 - 1,148 cars/h; the percent of diesel trucks is 30 - 85%. Magnetic counts of eastbound traffic are by car length of 6 ± 0.5 m. (Over 6 m is almost diesel-truck.) The 90% of the diesel trucks is 6.5 - 22 t car weight. The 53% is 10 - 22 t. Intake fans above each of the tunnels force ventilation air into the tunnel through overhead louvers at 303 m^3/s. Air is drawn in also through the vehicle entrance portal by the ramming action of the traffic. All of the air leaves via the vehicle exit portal, at volumes averaging 380 m^3/s. Truck speed was 80 km/h. Sulphur content of fuel oil was 0.4%. Measurement procedures are almost similar to the Allegheny tunnel study by William R. Pierson and Wanda W. Brachaczek (1). The preliminary study was done in 1972; the present study was started in 1979, and will continue to 1981.

(B) Average emissions rates of diesel truck were found as in Table 1 and Table 2. Particulates are ~0.03 μm by electronmicroscope.

(C) The overall sulphur dioxide conversion to sulphate in the Tunnel was 2% (1980), 3% (1979). The measurements of the ambient are on the way. Sulfuric acid particulates of 2 - 10 micron spheres were observed on the thymol blue dye coated films exposed in the ambient 40 meters distant from the Tunnel portal, when relative humidity was +90%.

REFERENCES

1. Pierson, W.R., and W.W. Brachaczek. Particulate matter associated with vehicles on the road. Automotive Engineering Congress and Exposition, Detroit, MI, Feb. 23-27, 1976. Paper No. 760039.

Table 1. Diesel Gaseous Emission Rates on the Road (g/km)[a]

Year	1980-Oct.	1979-Oct.
NO	6.42 ± 9.7%	5.02 ± 7.4%
NO_2	0.62 ± 15 %	0.77 ± 6.5%
NO_x	7.03 ± 8.8%	5.79 ± 7.3%
CO	5.04 ± 17 %	--
SO_2	1.27 ± 20 %	1.14 + 27 %
T-HC	1.73 ± 9.7%	--
CH_4	1.08 ± 9.2%	--
NM-HC	0.63 ± 17 %	--

[a]In 1980, numbers of measurements: n=36.
In case of SO_2 measurement, n=12. Pearson's correlation coefficient, $p < 0.05$.
In 1979, n=24. In case of SO_2 measurement, n=8, $p < 0.05$.

Table 2. Diesel Particulates Emission Rates on the Road (g/km)

Year	1980-Oct.	1979-Oct.
Total particulates[a]	1.03 ± 4.9%	0.92 ± 5.4%
Sulphate particulates	0.041 ± 25 %	0.051 ± 19 %
Nitrate particulates	0.003 ± 37 %	0.003 ± 15 %
Ammoniate particulates	0.005 ± 44 %	0.004 ± 33 %

[a]Particulates of under -10 μm were measured by high-volume air sampler.
In 1980, numbers of measurement: n=36. Pearson's correlation coefficient, $p < 0.05$.
In 1979, numbers of measurement: n=24, $p < 0.05$.

DIESEL BUS TERMINAL STUDY
EFFECTS OF DIESEL EMISSIONS ON AIR POLLUTANT LEVELS

BY

Robert M. Burton, Robert Jungers, Jack Suggs
Environmental Monitoring Systems Laboratory
U.S. Environmental Protection Agency
Research Triangle Park, North Carolina

INTRODUCTION

The New York City Bus Terminal Diesel Study was designed to measure, collect, chemically characterize, and bioassay diesel exhaust as it exists after becoming resident in the ambient atmosphere. Concentration levels of size fractionated particle mass, organic vapors and inorganic vapors were determined. Gram amounts of size fractionated particle samples were collected for detailed chemical analysis and bioassay (1) screening.

STUDY DESIGN

A semi-enclosed area of the NYC bus terminal in which approximately 1400 buses operate daily was used for the diesel exhaust collection site (indoor, Site #1); a second site (outdoor, Site #2) on 9th Avenue upwind of the terminal, was used as a particle and organic vapor background sampling and collection site. The background site was used for ambient control and for comparative chemical and physical characterization of the terminal diesel enriched pollutants and the unaffected outdoor pollutants. Background inorganic gaseous data were obtained from nearby ambient monitoring stations which were also unaffected by the terminal pollutants.

The terminal site was selected so that the majority of the ambient pollutant loading would be specifically concentrated diesel exhaust originating from diesel vehicles operating in a specified area. Street canyons have the disadvantage of being traffic pattern and meteorology dependent and are confounded by stationary sources and gasoline powered vehicles.

The traffic count of buses passing by the indoor site remained consistent throughout the study with the weekday daily count averaging 1,415 (1,336 minimum; 1,440 maximum) and the weekend daily count averaging 615 (579 minimum; 643 maximum). Monitoring and sample collection began July 16, 1979 and ran daily on a 24-hour schedule through July 30, 1979.

TSP hi vol (2), Dichotomous (2), and massive air volume (3) particle samples were collected; volatile organic compounds were collected on Tenax cartridges; and primary pollutant inorganic vapors were measured by continuous sensors.

The Tradescantia plant system (4) developed by Brookhaven National Laboratories for on-site detection of toxic air pollutants, was also operated at the indoor site. The Tradescantia system allows ambient air to be screened for the presence of mutagenic chemical vapors.

The majority of buses using the terminal were equipped with Detroit two-stroke heavy diesel engines.

RESULTS AND DISCUSSION

Results of aerometric measurements reveal that some pollutants were elevated considerably at the indoor site, while others there remained at background level. Particle measurements revealed very high levels of fine particulate matter being generated in the terminal building.

The average Total Suspended Particulate (TSP) 24-hour average level in the terminal was 325 $\mu g/m^3$ while the same TSP outdoors was 120 $\mu g/m^3$. Dichotomous measurements gave a 240 $\mu g/m3$ 24-hour average for indoor particles less than 2.5 microns in diameter compared to 60 $\mu g/m^3$ at outdoor Site #2. The 24-hour average coarse fraction of the dichtomous sample (2.5-15 microns) averaged 46 $\mu g/m^3$ indoors and 20 $\mu g/m^3$ outdoors. Weekend particle mass levels dropped considerably more indoors than outdoors. Results of 24-hour TSP sulfate, nitrate, and lead levels indoors were at the same approximate concentration as at background Site #2. The outdoor levels were influencing the indoor air for the three pollutants. Ratios of sulfate, nitrate, and lead to TSP were all lower indoors than at the outdoor background site.

The massive air volume size fractionating particle collectors were operated at both sites throughout the study. The amount of material collected is shown in Table 1.

Like the size distribution of the particle mass collected by the dichotomous samplers, the massive volume samplers show the majority of the elevated particulate mass at the indoor site to be aerosol in the fine size range.

Comparison of Daily Maximum Hourly Averages for the Gaseous Primary Pollutants

Real time continuous measurements of the gaseous primary pollutants revealed peaks to occur at 8 a.m. and 5 p.m. at the indoor site each day when bus traffic was at a maximum. More variation in maximum hourly averages occured at the indoor site than outdoors for all gases measured. A summary of gaseous pollutant measurements follows:

Sulfur dioxide (SO_2). Based on 15-day averages, there was no difference (statistically) between indoor and outdoor maximum hourly averages. Both the Mable Dean Bacon School (outdoor) site and the Central Park (outdoor) site SO_2 levels are equivalent to those found inside the terminal, thus indicating very low SO_2 emissions from the buses inside the terminal. There is no significant difference between weekday and weekend maximum hourly values inside the terminal.

Nitrogen dioxide (NO_2). For NO_2 levels, the data indicate more variation in maximum hourly averages for the indoor site than outdoors. The maximum hourly average indoor values (mean of 1.36 ppm) are on the average significantly higher than those for outdoors (mean of .09 ppm) based on 15-day average of maximum hourly values. Weekend maximum hourly values indoors are significantly lower compared to weekdays. The NO_2 mean of 1.36 ppm is 10 times higher than the maximum 24-hour level of the National Ambient Air Quality Standard.

Nitric oxide (NO). NO maximum hourly values indoors throughout the study were significantly much higher than those outdoors. This is consistent with other diesel exhaust products measurements where NO has been shown to be emitted at high concentration levels.

Ozone (O_3). There were no detectable O_3 levels indoors at the bus terminal during the study. Outdoors, the maximum hourly O_3 values ranged from 0.0 to .12 ppm with an average of .04 ppm over the sample period. With the NO levels exceeding 7 ppm and NO_2 levels exceeding 1.0 ppm, it is safe to assume all of the O_3 at indoor Site #1 was reacting with NO to form NO_2.

Carbon monoxide (CO). There was significantly more day-to-day variation in the maximum hourly CO values for the indoor site as compared to the outdoor site. This was apparently due to lower weekend values for indoor Site #1 maximum hourly averages. Averaged over the sampling period, the maximum hourly average for indoors (17.79 ppm) was significantly higher than the outdoor 10-day average of 2.6 ppm.

Total hydrocarbons (THC). No ambient THC data were available for comparison with indoor Site #1 levels. Peak hourly maximum averages for indoor Site #1 was at the 10 ppm level.

Comparison of Indoor Diurnal Patterns for the Gaseous Primary Pollutants

The indoor generated gaseous pollutants were also analyzed for diurnal variation of concentration. Weekday (Monday-Friday) hourly averages and standard deviations for the study period were computed; weekend (Saturday-Sunday) hourly averages with standard deviations were also computed. As described below for each gaseous pollutant, the diurnal concentration levels for weekdays (1400 buses) were always considerably higher than for weekends (600 buses) for all gaseous pollutants except SO_2.

Sulfur dioxide (SO_2). Indoor SO_2 diurnal patterns are similar for both weekends and weekdays. Both were influenced by peak hour traffic

activity occurring at 8 a.m. and 5 p.m. each day. The indoor average 5 p.m. value was significantly higher during the weekday as compared to weekend levels. The ambient background SO_2 levels did not experience 8 a.m. and 5 p.m. peaks. As mentioned earlier, there were no significant differences between indoor and outdoor peak daily hourly averages for the duration of the study.

Nitrogen dioxide (NO_2). Diurnal patterns indicate significantly higher average levels for weekdays compared to weekends for the 8 a.m. and 5 p.m. indoor hourly averages. For weekdays the hourly averages range from .06 ppm during early morning hours (3-4 a.m.) to 1.55 ppm during 5 p.m. rush hour. For weekends the hourly averages range from .08 ppm during morning hours (3-5 a.m.) to 0.45 ppm during 5 p.m. averaging time. Had more ozone been present in the terminal, much higher NO_2 levels may have been expected.

Nitric oxide (NO). Trends indicate a higher indoor NO average level during peak hours 8 a.m. and 5 p.m. for weekdays compared to weekends. The values for weekday peaks were beyond the range of the instrument but are estimated to be approximately 10 ppm.

Carbon monoxide (CO). Indoor weekday 8 a.m. and 5 p.m. hourly averages were significantly higher than weekend averages during the same averaging times for weekends. During weekdays, hourly averages were slightly elevated compared to weekend averages for hours before 8 a.m. to after 5 p.m. This cannot be concluded about other gaseous pollutants examined in the study. The differences for CO were not significant on an hour-by-hour basis during this time period, even though the hourly averages at 8 a.m. and 6 p.m. were elevated above the rest of the hours in the day.

Total hydrocarbons (THC). Diurnal patterns indicate elevations in indoor hourly averages during rush hour activity (8 a.m. to 5 p.m.) for weekday measurements. The 5 p.m. measurement averaged 10.3 ppm during weekdays and is significantly higher than the weekend average of 3.6 ppm.

Effect of Buses on Particle Levels

A statistical analysis for describing the relationship between the bus activity and particulate levels both indoors and outdoors was performed. A paired t-test was used to statistically examine the difference between the indoor and outdoor sites for TSP, dichotomous (fine, coarse, and total), sulfate, nitrate, and lead. The inside measurements were on the average significantly higher ($\alpha = .05$) than outside for TSP, (0-2.5μ) and (0-15μ) particles. These were the only significant differences. There was no significant difference between indoor and outdoor (2.5-15μ) coarse fraction particles.

Correlation coefficients between daily bus activity and pollutant levels were calculated. Several correlations were determined to be significantly different from zero. These were specifically TSP indoors .84; (0-

2.5) indoors, -.74; (0-15μ) indoors, .87; sulfate indoors, -.65; sulfate outdoors, -.65.

An important observation is that the bus activity does not correlate very highly with indoor sulfate, nitrate, and lead. Indoor levels of these pollutants are essentially the same as outdoors. Obviously the bus emissions contribution to sulfate, nitrate, and lead are lower than levels of these pollutants already resident in the atmosphere.

CONCLUSIONS

Sulfate, nitrate, and lead emissions from the buses were at a low level. Sulfur dioxide from the bus emissions were also at a low level, since no significant difference between the indoor and outdoor SO_2 levels was found. Small particles below 2.5μ aerodynamic diameter, and the gaseous pollutants of NO, NO_2, THC, and CO were all emitted at high levels from the buses. The indoor site was somewhat shielded from ultraviolet radiation, and its absence could have an effect on the organic exhaust products found in the atmosphere (5). Ozone was below detectable limits due to its use in the production of NO_2.

TABLE 1. Amount of Size-Separated Ambient Particles Collected by the Massive Air Volume Sampler

	STAGE I (20-3.5μ)	Stage II (3.5-1.7μ)	Stage III (1.7-0μ)
Site #1 Indoors	7.67 gm	1.72 gm	61.89 gm
Site #2 Outdoors	6.06 gm	1.18 gm	14.68 gm

REFERENCES

1. Huisingh, J. et. al. "Application of Bioassay to the Characterization of Diesel Particle Emissions." In: Application of Short-Term Bioassays in the Fractionation and Analysis of Complex Environmental Mixtures, M. Waters, et. al. eds. Plenum Press, New York, 1979.

2. Rodes, C. "Inhalable Particulate Network Operations and Quality Assurance Manual," U.S. EPA Office of Research and Development, Environmental Monitoring Systems Laboratory, Research Triangle Park, N.C. 27711, May 1980.

3. Mitchell, R.I., et. al. "Massive Volume Sampler for Gram Quantities of Respirable Aerosols." APCA Proceedings, June 22-24, 1977, Toronto, Canada.

4. Schairer, L.A., et. al. "Measurement of Biological Activity of Ambient Air Mixtures Using a Mobile Laboratory for *In Situ* Exposures: Preliminary Results from the *Tradescantia* Plant Test System." pp. 419-440 in Application.

5. Claxton, L. and H.M. Barnes. "The Mutagenicity of Diesel Exhaust Exposed to Smog Chamber Conditions as Shown by Salmonella Typhimurium," submitted to Mutation Research for publication.

DIESEL BUS TERMINAL STUDY: CHARACTERIZATION OF VOLATILE AND PARTICLE BOUND ORGANICS

by

Robert H. Jungers and Joseph E. Bumgarner
U. S. Environmental Protection Agency
Research Triangle Park, North Carolina 27711

Charles M. Sparacino and Edo D. Pellizzari
Research Triangle Institute
Research Triangle Park, North Carolina 27709

The New York City Bus Terminal was selected as a unique source of heavy duty diesel bus engines. To evaluate emissions from these bus engines in comparison to ambient air a semi-enclosed site was selected inside the terminal where approximately 1400 diesel buses operate daily and a second site was selected outside and upwind of the terminal. Volatile organic compounds were collected on Tenax cartridges, recovered by thermal desorption and introduced into a high resolution gas chromatographic column for separation. Characterization and quantification of these compounds were accomplished by mass spectrometry measuring total ion current and mass fragmentography. Volatile chemicals selected for quantitation were benzene; toluene; xylenes; ethylbenzene; 1,1,1-trichloroethane; trichloroethylene; tetrachloroethylene; benzaldehyde; n-octane; and n-hepane. These were selected to represent chemicals associated with diesel engines, i.e., octane, heptane, benzaldehyde, chemicals not associated with diesels, i.e., chlorinated hydrocarbons and general aromatic chemicals which could be found in the atmosphere.

The quantitation of the volatile organic compounds collected over a two-week period showed that the chemicals which had consistently higher concentration inside were n-octane, n-heptane, 1,1,1-trichloroethane, xylenes, and toluene. The chemical which was higher outside was tetrachloroethylene while benzaldehyde, trichloroethylene, benzene, and ethylbenzene were about the same concentration. Week day (1400 buses/day) concentrations were higher for all ten chemicals than on weekends (600 buses/day).

Air particles were collected inside and outside (at the same site as the volatile samplers). Total suspended particulate (TSP) measurement was done by the standard Hi-Volume sampler method (1) and size fractionated particles were collected using a massive air volume sampler (MAVS) which separates the particles into three size ranges. Table 1 presents data on samplers, mass, organic extractable and benzo-α-pyrene analysis.

The smallest size range (1.7 μm and below) of all samples collected inside the terminal were combined into a single sample. This procedure was also followed for the outside samples. This was done to insure sufficient quantity of sample for chemical and biological analysis.

The air particle concentration, organic concentration and the percent organic extractables were, generally, considerably higher inside than outside the terminal while the benzo-α-pyrene concentration was higher outside than inside the terminal.

The air particle samples were subjected to a fractionation procedure to yield six fractions of various chemical properties and polarities. The acid fraction contains both weak (e.g., phenols) and strong (e.g., carboxylic acids) acids. The base fraction contains organic, Bronsted bases (e.g., amines). The neutral fraction is subdivided into three main fractions based on compound polarity. The non-polar neutral (NPN) fraction is comprised of compounds less polar than ∿ naphthalene. Paraffinic materials are characteristic of this fraction. The PNA fraction contains compounds of intermediate polarity, and is selective for condensed ring aromatics. All neutral materials with polarities greater than PNA hydrocarbons are found in the polar neutral (PN) fraction. Prior to the subfractionation of the neutral fraction, the latter must be dissolved in cyclohexane. All components are not soluble in this solvent. The insoluble material is collected as a separate fraction (CI), and is comprised of intermediate and highly polar compounds.

Spillover of various compounds into all fractions is a natural feature of solvent partitioning processes. Polar neutral material was removed from the PNA fraction by silica gel chromatography. The PNAs were chromatographed utilizing HPLC such that a fraction containing only PNA hydrocarbons was obtained (PNA-1). Other fractions (PNA 2-4) were collected that contained compounds of intermediate to high polarity.

Most fractions were directly analyzed by capillary GC/MS. The fractions enriched in polynuclear aromatic hydrocarbons (PNAs) were further purified by column chromatography, and the collected subfractions were analyzed by GC/MS. A portion of each sample, after fractionation, was prepared for bioassay by removal of the fractionating solvent and addition of dimethylsulfoxide (DMSO).

Comparison of the mass distribution of each chemical fraction inside and outside the bus terminal showed several significant differences. The organics from the outside air contained a higher percent mass of one of the PNA subfractions (PNA-3), the polar neutral fraction as well as the acids and bases. The non polar neutral fraction was present at a higher percent mass inside the bus terminal. This appears to be due to higher concentrations of alkanes from unburned fuel. Bioassay analysis of the non polar neutral fraction (2) suggests that this fraction may contain substantial amounts of polynuclear aromatic hydrocarbons.

REFERENCES

1. Code of Federal Regulations. 1980. Title 40, part 50, appendix B. Reference Method for Determination of Suspended Particulates in the Atmosphere (High Volume Method). General Service Administration: Washington, DC. pp. 531-535.

2. Huisingh, J., R. Bradow, R. Jungers, L. Claxton, R. Zweidinger, S. Tejada, J. Bumgarner, F. Duffield, V.F. Simmon, C. Hare, C. Rodriguez, L. Snow, and M. Waters. 1978. Application of bioassay to the characterization of diesel particle emissions. Part I. Characterization of Heavy Duty Diesel Particle Emissions. Part II. Application of a mutagenicity bioassay to monitoring light duty diesel particle emissions. Application of Short-term Bioassays in the Fractionation and Analysis of Complex Environmental Mixtures. M.D. Waters, S. Nesnow, J.L. Huisingh, S.S. Sandhu, and L. Claxton, eds. Plenum Press: New York. pp. 381-418.

Table 1

	Particle Concentration µg Particle/M^3		Organic Concentration µg Organics/M^3		% Organic Extractable µg Organics/ 100 µg Particle		BaP µg BaP/g Particle	
SAMPLER particle size range	I	O	I	O	I	O	I	O
Hi-Vol (0-50 µm)	325.0	120.0	14.95	4.08	4.6	3.4	15.4	32.0
MAVS I (3.5-20 µm)	58.5	24.0	7.37	0.98	12.6	4.1	5.9	25.2
MAVS II (1.7-3.5 µm)	6.5	6.0	0.84	0.31	13.0	5.2	11.9	34.3
MAVS III (0-1.7 µm)	214.5	66.0	60.49	9.57	28.2	14.5	0.8	5.9

I = Inside
O = Outside

DIESEL BUS TERMINAL STUDY: MUTAGENICITY OF THE PARTICLE-BOUND ORGANICS AND ORGANIC FRACTIONS

by

Joellen Lewtas, Ann Austin, and Larry Claxton
Health Effects Research Laboratory
U.S. Environmental Protection Agency
Research Triangle Park, North Carolina

Emission testing of both heavy-duty diesel engines and light-duty diesel cars using tunnel dilution and filtration to collect diesel particles has shown the organics associated with these collected particles to be mutagenic in the Ames Salmonella typhimurium assay (1). The mutagenicity of these organics has been shown to be dependent on fuel quality (2) as well as engine type (3). The organics associated with particle emissions from heavy-duty diesel engines have generally been less mutagenic than the organics from light-duty cars. Fractionation and bioassay studies suggest this is due to a greater concentration of nonmutagenic aliphatic compounds emitted from unburned fuel. Over 90% of the mutagenic activity has been observed in the polar neutral fractions not requiring metabolic activation (1). Mutagenicity studies of the organics associated with urban ambient air particles have also reported mutagenic activity in the organics extracted from particles (4,5).

In order to evaluate the impact of emissions from heavy-duty buses on the mutagenicity of ambient air, this study was designed to compare the mutagenic activity of the total extractable organics from size-fractionated air particles and chemical class fractions both inside and outside a diesel bus terminal.

The mutagenicity of the ambient air inside and outside the New York Port Authority Bus Terminal was compared using a microbial mutagenesis bioassay. Approximately 1400 diesel buses operate daily in the semi-enclosed bus terminal. Air particles were collected simultaneously using both the Massive Air Volume Sampler (MAVS) (6) and the standard Hi-Volume air sampler (Hi-Vol). The dichloromethane-extractable organics from these air particles were bioassayed in the Salmonella typhimurium plate incorporation assay (7) in TA98 with and without metabolic activation with minor modifications (3). The slope of the dose-response curve (rev/μg) was determined using a nonlinear model (8). The air particle concentration inside the bus terminal was nearly 3 times the outside concentration based on the Hi-Vol TSP. Comparison of the Hi-Vol and MAVS data showed the increased concentration of particles inside was due primarily to increased concentrations of particles less than 1.7 micron in size. These small (less than 1.7 micron) particles inside the terminal had a higher concentration of extractable organics than the small particles outside the terminal. Although both the small particle and organic concentrations

were lower outside the terminal, the mutagenicity of the organics from outside was significantly greater (nearly 10 times) than inside the terminal. Both direct-acting and indirect-acting mutagens were detected in these samples. The mutagenic activity of the air in revertants per cubic meter provides a direct comparison of the mutagenicity of the inside and outside air. Using data from either the Hi-Vol samples or the smallest particles from the MAVS, the outside air was approximately twice as mutagenic as the air inside the bus terminal.

Fractionation and mutagenesis bioassay of the organics from the less-than-1.7-micron particles were conducted to compare the chemical composition inside and outside. The mutagenicity (rev/μg) of each fraction and the mass percentage of each fraction were used to calculate weighted mutagenicities. The percent of the total mutagenicity attributable to each chemical fraction was determined and compared inside and outside the terminal. The diesel emissions inside the bus terminal increased the concentration of aliphatic hydrocarbons found in the non-polar neutral fraction. The higher mutagenicity in the outside ambient air appears to be due to higher concentrations of organic acids and direct-acting moderately polar neutral compounds. The highly polar neutral fraction showed more direct-acting mutagenic activity inside the terminal.

REFERENCES

1. Huisingh, J., R. Bradow, R. Jungers, L. Claxton, R. Zweidinger, S. Tejada, J. Bumgarner, F. Duffield, V.F. Simmon, C. Hare, C. Rodriguez, L. Snow, and M. Waters. 1979. Application of bioassay to the characterization of diesel particle emissions. Part I. Characterization of heavy duty diesel particle emissions. In: Application of Short-term Bioassays in the Fractionation and Analysis of Complex Environmental Mixtures, Environmental Science Research, Vol. 15. M.D. Waters, S. Nesnow, J.L. Huisingh, S.S. Sandhu, and L. Claxton, eds. Plenum Press: New York. pp. 382-400.

2. Huisingh, J., R. Bradow, R. Jungers, L. Claxton, R. Zweidinger, S. Tejada, J. Bumgarner, F. Duffield, V.F. Simmon, C. Hare, C. Rodriguez, L. Snow, and M. Waters. 1979. Application of bioassay to the characterization of diesel particle emissions. Part II. Application of a mutagenicity bioassay to monitoring light duty diesel particle emissions. In: Application of Short-term Bioassays in the Fractionation and Analysis of Complex Environmental Mixtures, Environmental Science Research, Vol. 15. M.D. Waters, S. Nesnow, J.L. Huisingh, S.S. Sandhu, and L. Claxton, eds. Plenum Press: New York. pp. 400-418.

3. Claxton, L.D. 1980. Mutagenic and Carcinogenic Potency of Diesel and Related Environmental Emissions: Salmonella Bioassay. EPA Report EPA-600/9-80-057b, U.S. Environmental Protection Agency: Research Triangle Park, NC. pp. 801-809.

4. Tokiwa, H., H. Takiyoshi, K. Morita, K. Takahashi, N. Soruta, and Y. Ohnishi. 1976. Detection of mutagenic activity in urban air pollutants. Mutat. Res. 38:351-359.

5. Lewtas Huisingh, J. (in press). Bioassay of particulate organic matter from ambient air. In: Short-term Bioassays in the Analysis of Complex Environmental Mixtures. 1980. Michael D. Waters, Shahbeg S. Sandhu, Joellen Lewtas Huisingh, Larry Claxton, and Stephen Nesnow, eds. Plenum Press: New York.

6. Jungers, R., R. Burton, L. Claxton, and J. Lewtas Huisingh. (in press). Evaluation of collection and extraction methods for mutagenesis studies on ambient air particulate. In: Short-term Bioassays in the Analysis of Complex Environmental Mixtures, 1980. Michael D. Waters, Shahbeg S. Sandhu, Joellen Lewtas Huisingh, Larry Claxton, and Stephen Nesnow, eds. Plenum Press: New York.

7. Ames, B.N., J. McCann, and E. Yamasaki. 1975. Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. Mutat. Res. 31:347-364.

8. Stead, A.G., V. Hasselblad, J.P. Creason, and L. Claxton. 1981. Modeling the Ames test. Mutat. Res. 85:13-27.

NITRO DERIVATIVES OF POLYNUCLEAR AROMATIC HYDROCARBONS IN AIRBORNE AND SOURCE PARTICULATE

by

Thomas L. Gibson
Environmental Science Department
General Motors Research Laboratories
Warren, Michigan

INTRODUCTION

Direct mutagenic activity is observed in the Ames Salmonella bioassay of organic extracts from ambient airborne particulate and source emissions. Nitro derivatives of polynuclear aromatic hydrocarbons (nitro-PNA), some of which are strong direct-acting mutagens, are considered to be probable contributors to this activity.

SAMPLING AND ANALYSIS

Particulate samples were collected on dexiglas filters and extracted with benzene-ethanol (80:20 v/v) with a Soxhlet apparatus. Automobile exhaust samples were obtained using a chassis dynamometer and dilution tube. An analytical method for nitro-PNA was recently developed which involves reduction of these compounds to the corresponding amino-PNA and their determination by HPLC with fluorescence detection.[1] With HPLC methods, the concentrations of 1-nitropyrene, 6-nitro-BaP, pyrene, and BaP were measured in samples of the various particulates. In all of these samples, part of the nitro-PNA may have resulted from the reaction of oxides of nitrogen found in emissions and in ambient air with PNA bound to the particles on the filter. For example, an increase in nitro-PNA was measured when diesel exhaust particulate was exposed to filtered diesel exhaust gases, suggesting a strong likelihood that nitro artifacts are formed during filter sampling.[1] The direct mutagenic activity was determined by the Ames Salmonella bioassay (Litton Bionetics, Kensington, MD) using tester strain TA-98 without metabolic activation. Each particulate extract was dissolved in DMSO and tested at five doses using triplicate plates with equal numbers of bacteria from the same starting culture. The slope of the initial linear part of the dose response curve was considered as the specific activity.

AMBIENT AIRBORNE PARTICULATE

Ambient particulate collected at an urban and a suburban site during Spring and Summer contained 0.2-0.6 ppm (i.e., ng/mg particulate) of 1-nitropyrene corresponding to airborne concentrations of 0.016 to 0.030 ng/m^3, and also contained 0.9-2.5 ppm 6-nitro-BaP corresponding to 0.04-0.28 ng/m^3. As shown in Figure 1, the fluorescence spectra of the nitro-PNA were scanned to determine their identity by comparison with authentic standards. The direct mutagenic activity of the suburban samples in the Ames bioassay was 0.15-0.56 revertants/microgram of particulate corresponding to 7-20 revertants/m^3 airborne mutagenicity. Based on its reported specific activity, the 1-nitropyrene in the particulate could account for less than 0.3% of the total activity.

AUTOMOBILE EXHAUST PARTICULATES

Samples were collected from a few of the numerous sources of particulate emissions including a 1981 2.5-L 4-cylinder catalyst car, a 1980 4.3-L 8-cylinder car with no catalyst using unleaded gasoline, a 1974 5.7-L 8-cylinder car with leaded gasoline, and 5.7-L 1980 8-cylinder diesel cars made by General Motors divisions. The catalyst car gave particulate with a lower concentration of 1-nitropyrene (0.63 ppm) than the noncatalyst cars (3.9-4.3 ppm) and production diesel car (8.0 ppm). The catalyst car particulate had 0.21 ppm 6-nitro-BaP, the noncatalyst cars 17-33 ppm, and the diesel car less than 0.4 ppm. On a per mile basis also, the catalyst car emitted less of the PNA, nitro-PNA, and direct mutagenic activity than the other vehicles. The level of direct acting mutagenicity seemed to be directly related to the total nitro-PNA concentration of the particulate and not to PNA concentrations.

An experimental (noncommercial) low emission diesel car was also tested. Compared to the production model of the 1980 diesel, the low emission diesel gave much lower particulate, PNA, nitro-PNA, and mutagenic emissions.

STATIONARY SOURCES

Particulate samples from a wood-burning fireplace did not contain levels of the nitro-PNA above the minimum detection limits (less than 0.1 ppm). The concentrations of pyrene and BaP found in these samples were low compared to automobile particulates and depended on how the samples were collected: averages of 3-4 ppm in particles collected from the raw flue gases with an EPA Method 5 sampling train (heated filter and impingers) compared to 30-60 ppm when collected from emissions diluted 25-fold with air. The increased levels of PNA in particulate from cool, diluted fireplace emissions suggests that much of the organics remain in the vapor phase in emissions sampled by the EPA method. Particulate emissions measured by this method should not be compared to vehicle emissions determined by the dilution tube method and may lead to

erroneous estimates of emissions from stationary sources (sampled from raw flue gases). Similarly, a particulate sample from a coal-fired boiler, collected from hot, undiluted flue gases, showed low or undetectable levels of PNA (less than 0.5 ppm) and nitro-PNA (less than 0.02 ppm).

CONCLUSIONS

Nitro-PNA are found in ambient airborne particles and various source emissions. Because of the very limited data from only a few of the possible sources and the complicating effects of differences in sampling methods, filter artifact formation, and atmospheric reactions, source allocation for PNA derivatives in ambient particulate is not feasible at the present time.

1. T. L. Gibson, A. I. Ricci, and R. L. Williams, "Measurement of Polynuclear Aromatic Hydrocarbons, Their Derivatives and Their Reactivity in Diesel Automobile Exhaust," in Polynuclear Aromatic Hydrocarbons: Chemistry and Biological Effects, A. Bjorseth and A. J. Dennis, Eds., Battelle Press (in press), presented at the 5th International symposium on Polynuclear Aromatic Hydrocarbons, Columbus, OH, Oct. 28, 1980.

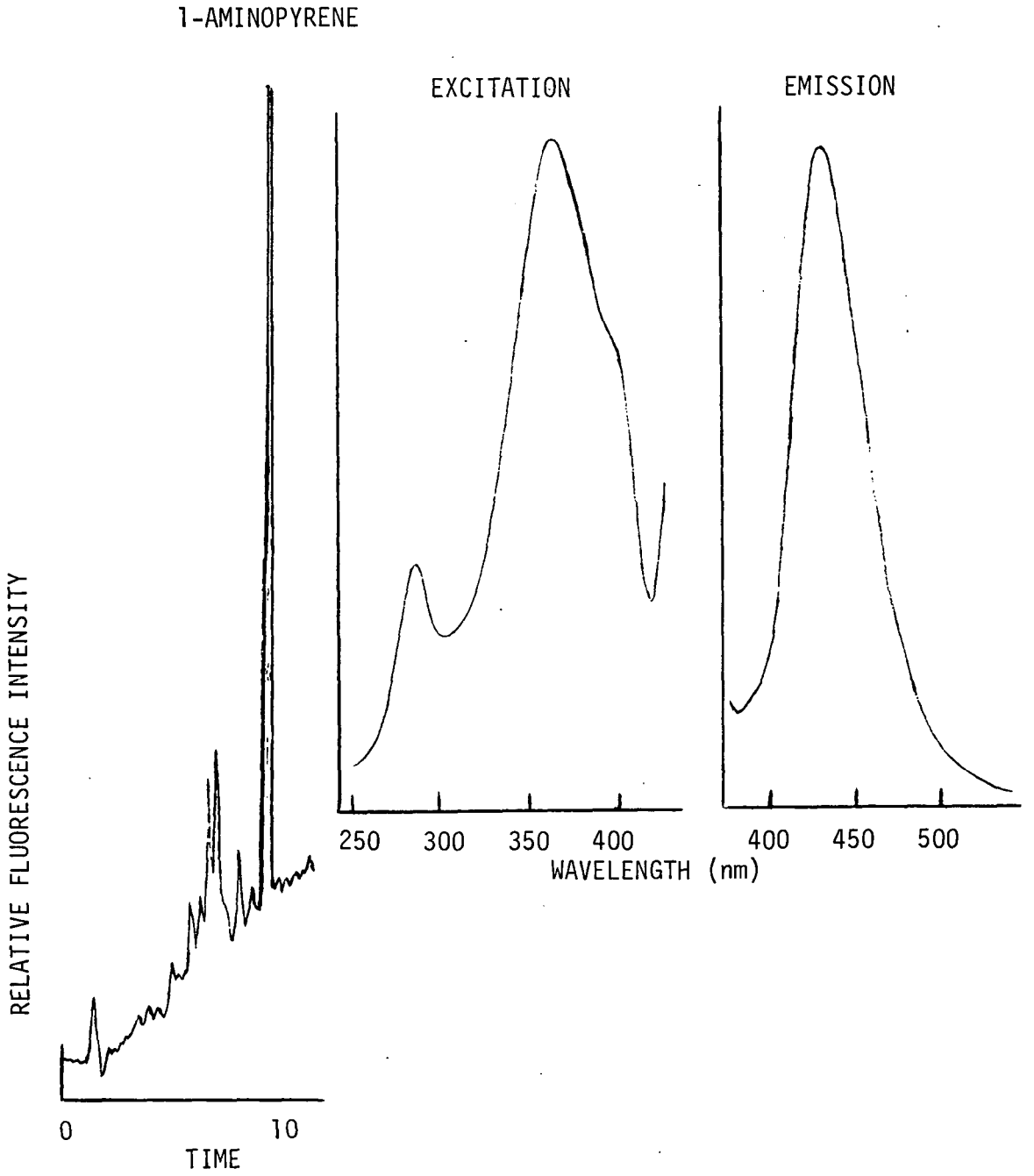

Figure 1. HPLC chromatogram of ambient particulate extract after treatment with a reducing agent (Conditions, see reference[1]) Fluorescence Detector -- excitation 365 nm, emission 430 nm. Stop-flow scanning gave the emission and excitation spectra shown, which match those of authentic 1-aminopyrene.

RISK ASSESSMENT OF DIESEL EMISSIONS

by

R. Albert
Institute of Environmental Medicine
New York University Medical Center
New York, New York

and

T. Thorslund
U.S. Environmental Protection Agency
Washington, District of Columbia

The observations made a number of years ago, which have since been verified, to the effect that diesel exhaust particulates are mutagenic and contain agents that are recognized carcinogens, established the position that diesel particulates are likely to be carcinogenic in humans. However, the unanswered question is how potent are these particulates and what is the magnitude of the cancer hazard to the general population. In view of the absence of any direct animal experiments or epidemiologic data, an approach to risk assessment several years ago which seemed reasonable was to use the available epidemiologic data that involved exposure to combustion products having similarities to diesel particulates and to compare the relative potency of these materials with diesel exhaust particulates. The epidemiologic studies that were chosen involved cigarette smoking, coke oven emissions, and roofing tar. An extensive series of studies including mutagenesis, cell transformation, skin painting, inhalation, and intratracheal intubation have been undertaken to compare these materials with diesel particulates. The present status of the carcinogen risk assessment in terms of the epidemiologic and laboratory studies will be presented.

Notes