EPA/AA/CTAB/PA/81-19

Summary of EPA and Other Programs on the Potential Carcinogenicity of Diesel Exhaust

by

Penny M. Carey

August 1981

NOTICE

Technical reports do not necessarily represent final EPA decisions or positions. They are intended to present technical analyses of issues using data which are currently available. The purpose in the release of such reports is to facilitate the exchange of technical information and to inform the public of technical developments which may form the basis for a final EPA decision, position or regulatory action.

Control Technology Assessment and Characterization Branch
Emission Control Technology Division
Office of Mobile Source Air Pollution Control
U.S. Environmental Protection Agency
2565 Plymouth Road
Ann Arbor, Michigan 48105

Table of Contents

		Page
I.	Summary and Conclusions	4
II.	Introduction	8
III.	Summary of Major Diesel Health Effects Publications	10
	A. EPA	10
	B. National Academy of Sciences (NAS)	10
	C. Health Effects Institute (HEI)	11
IV.	Description of EPA's Diesel Emissions Research Program	12
V.	Mutagenicity	14
	A. *In vitro* studies	14
	B. *In vivo* studies	19
VI.	Carcinogenicity	22
	A. *In vitro* studies	22
	B. *In vivo* studies	24
	C. Bioavailability	30
VII.	Non-genetic Effects	31
VIII.	Characterization	33
	A. Gas-phase	34
	B. Particulate	37

Page

IX. Epidemiology 39

X. Risk Assessment 42

References 47

Tables 54

I. Summary and Conclusions

Much research has been performed to evaluate the health effects associated with exposure to Diesel emissions. The research performed falls into five general areas: mutagenicity, carcinogenicity, non-genetic effects, characterization and epidemiology. EPA is conducting a massive research program that includes studies in each area. Since epidemiological data for Diesel emissions are limited, a major portion of EPA's research effort involves determining the relative mutagenic and carcinogenic potency of Diesel emissions compared to potencies of comparative emissions for which epidemiological data are available. EPA will use the results of these studies with epidemiological data for the comparative sources to assess the human health risk associated with exposure to Diesel emissions.

This report summarizes most of the studies EPA plans to use to formulate a Diesel risk assessment including significant studies by researchers outside EPA. The National Academy of Sciences (NAS) has completed an evaluation of the existing health effects data base on Diesel exhaust products; the general conclusions reached in this study are also included.

Based on the studies summarized, the following generalizations can be made:

1. Mutagenic compounds, both direct and indirect acting in the Ames and other bioassay tests, are associated with Diesel particulate emissions. Most of these particulate emissions represent particulates which are small enough to be inhaled and deposited deep within the human lung.

2. Diesel exhaust particulate extracts and whole particulates contain some known carcinogenic materials such as benzo(a)pyrene. The extracts have been shown to be mutagenic and carcinogenic in a number of in vitro and in vivo bioassay tests. Whether whole (i.e. unextracted) engine exhaust particulates are carcinogenic to any significant extent is not known yet; however, work is currently in

progress on two studies: the intratracheal instillation of Diesel particulate and extract with Syrian Golden hamsters and the intraperitoneal injection of Diesel particulate and extract with Strain A mice. The results of these studies could provide important information about the bioavailability of the organics on the particulate, in addition to determining the carcinogenicity of whole Diesel exhaust particulates.

3. To date, whole Diesel exhaust (particulate and gas phase) has not been found to be carcinogenic when inhaled by laboratory animals. Inhalation experiments with dilute Diesel exhaust possibly do not permit a sufficient dose of the active portion to enter the lung. In addition, inhalation experiments designed to detect in vivo mutagenicity have produced generally negative results with the exception of one study designed to detect sister chromatid exchange.

4. The mutagenic and carcinogenic potencies of the Diesel particulate samples are generally but not always less than the potencies of the comparative samples (coke oven emissions, roofing tar emissions, and cigarette smoke condensate) based on a variety of tests including skin tumorigenesis initiation; however, they all fall within the same order of magnitude (per unit weight of material tested). The four Diesel samples tested (Caterpillar 3304, Datsun Nissan 220C, Oldsmobile 350 and Volkswagen Rabbit) exhibited a wide range of potencies (i.e. sometimes an order of magnitude difference). The potencies of the Diesel and comparative samples appear to be two to three orders of magnitude less than that of pure benzo(a)pyrene, a carcinogenic and mutagenic polynuclear aromatic hydrocarbon (PAH).

5. While extensive Ames and other bioassay testing for mutagenicity is being performed on the organics extracted from the particulate, the relative mutagenicity of the gas phase organics remains unknown primarily because an analytical method for collection had not been developed. EPA is currently working to develop artifact-free methods to collect gas phase hydrocarbons in exhaust for future bioassay testing. Some work has been done by EPA-OMSAPC in Ann Arbor, with the bulk of the work being performed at EPA-ORD-RTP.

6. The chemical composition of the organics adsorbed on Diesel exhaust particulate is complex. Polynuclear aromatic hydrocarbons (PAH) and numerous PAH derivatives have been identified thus far. It appears that nitro-PAHs may account for a significant portion of the direct-acting mutagenicity, as measured by the Ames test.

7. Few conclusions can be made regarding non-carcinogenic pulmonary and systemic effects of Diesel particulates. In one study, mice exposed to diluted Diesel exhaust exhibited enhanced susceptibility to infection when subsequently exposed to a bacterial pathogen. The significance of this finding is not yet clear and requires further research.

8. Epidemiological data for Diesel emissions are limited. The London Transit Worker study, an epidemiology study of Diesel bus workers in London, has been cited as a strong indication that Diesel emissions result in no excess cancer risk. There are many inconsistencies in this study including the lack of considering the "healthy worker" effect, as well as some doubt as to whether the study population was exposed to a greater amount of Diesel emissions than the general population to which it was compared. EPA's statistical analysis of this study indicates it would still be possible to have lung cancer deaths numbering in the thousands each year in the U.S. due to Diesel engine emissions and not be inconsistent with the results obtained in the London Transit Worker study.

9. A risk assessment performed by Lovelace Research Institute for the Department of Energy estimates 30 lung cancer deaths per year may be attributable to exposure to light-duty Diesel exhaust. Their calculations were based on the year 1995 and beyond, assuming 20% of the light-duty vehicle fleet will be Diesel-powered and controlled to 0.16 gm/mile particulate. An earlier preliminary risk assessment performed by EPA's Carcinogen Assessment Group (CAG) estimated 346 lung cancer deaths attributable to exposure to light-duty Diesel

exhaust and 668 lung cancer deaths due to heavy-duty Diesel exhaust. EPA's calculations were based on 1990, assuming 10% of the light-duty vehicle fleet will be Diesel-powered (a best estimate) and uncontrolled (1.08 gm/mile particulate). Both the EPA and DOE-Lovelace assessments hypothesized that Diesel emissions were as potent as coke oven emissions. Allowing for the different population exposure estimates, the two assessments agree reasonably well. It should be mentioned that both the EPA and Lovelace Research Institute risk assessments are tentative.

EPA intends to release a revised risk assessment in the future, based on the results of the relative potency study. The data available to date indicate that coke oven emissions are, in fact, more potent than Diesel particulate extract. Based on the potencies obtained from the skin tumorigenesis assay only, a reevaluation of the preliminary EPA risk assessment was made by CAG. It was estimated that 19 cancer deaths per year in the U.S. may be attributable to Diesel exhaust, assuming 15% of the automotive fleet is Diesel-powered and uncontrolled (1.0 gm/mile particulate). It should be noted that the revised risk assessment that EPA plans to release in the future is expected to incorporate the results of a variety of mutagenesis and carcinogenesis assays for estimation of relative potencies.

10. The most apparent research gaps in the Diesel health program are in the following areas:

- determination of the *in vitro* and *in vivo* bioavailability of the organics adsorbed on inhaled or ingested Diesel exhaust particulate,

- determination of the mutagenic and carcinogenic activity of the gas phase components; identification of the major components or classes of compounds in the gas phase of Diesel exhaust,

- effect of inhaled or ingested Diesel exhaust on susceptible subpopulations (e.g. pulmonary and cardiovascularly impaired),

- synergistic and potentiative effects of Diesel exhaust with other environmental pollutants,

- short- and long-term deposition and clearance of inhaled or ingested Diesel exhaust particulates,

- further characterization of the particulate soluble organic fractions responsible for the mutagenic and carcinogenic activity, and

- a more definitive estimate of the potential carcinogenic risk associated with Diesel particulate emissions.

II. Introduction

The projected increase in the production of light-duty Diesel-equipped vehicles has raised concern over possible adverse health effects associated with this increase. Attention has been primarily focused on particulate emissions from Diesel-equipped vehicles. The particles in Diesel exhaust differ both in quantity and composition from particles in gasoline engine exhaust. Currently, Diesel-equipped vehicles emit from 30 to 100 times more particulate mass (grams per mile) than gasoline-powered, catalyst-equipped vehicles. These Diesel exhaust particulates are small enough to be inhaled and deposited deep within the lungs. Gasoline particulate emissions from catalyst-equipped vehicles using unleaded fuel are primarily sulfates, while Diesel exhaust particulates are composed of carbonaceous soot with high molecular weight organic compounds adsorbed on the surface. These "particle bound organics", or PBO, can account for 10-50% of the particulate weight.

In 1977, EPA tested organic extracts of Diesel exhaust particulate and found that the extracts contained materials that were mutagenic to strains of

Salmonella typhimurium in the Ames bioassay. Since the Ames assay has been shown to be indicative in detecting substances that are carcinogenic (or non-carcinogenic) in whole animal studies, EPA felt that the positive test result warranted the issuance of an informal Precautionary Notice in 1977 (1)*. This notice mentioned EPA's preliminary findings and suggested that persons working with Diesel emissions in a laboratory setting exercise caution to avoid exposure to the emissions.

EPA has since launched a massive research program to evaluate the health effects associated with exposure to Diesel emissions. Since epidemiological data for Diesel emissions are very limited, a major portion of this research effort involves determining the relative mutagenic and carcinogenic potency of Diesel emissions (specifically, the particle-bound organics) compared to potencies of particle-bound organics from other emissions for which epidemiological data are available. The comparative sources selected were coke oven emissions, roofing tar emissions and cigarette smoke condensate (CSC). Benzo(a)pyrene, a known carcinogen, was used as a standard. The mobile source samples evaluated included a heavy-duty Diesel engine (Caterpillar 3304), three light-duty Diesel passenger cars (Datsun Nissan 220C, Oldsmobile 350 and Volkswagen turbocharged Rabbit) and a gasoline catalyst-equipped car (Ford Mustang II). The results from this study and others will be used to assess the human health risk associated with increased use of the Diesel engine.

An extensive amount of research has been performed by EPA and others. A comprehensive summary of the existing health effects data base on Diesel exhaust products entitled, "Health Effects of Exposure to Diesel Exhaust" (prepublication) was completed by the National Academy of Sciences (NAS) in 1980 (2).

---

*Numbers in parentheses designate references listed at end of paper.

The purpose of this report is to summarize the significant health effects studies of Diesel exhaust with an emphasis on 1) research EPA will use to formulate a risk assessment, 2) research results available after the printing of the NAS report, and 3) significant studies by researchers outside EPA. Important ongoing research will also be discussed. Topics covered in this report include mutagenicity, carcinogenicity, non-genetic effects, characterization, epidemiology and risk assessment.

III. Summary of Major Diesel Health Effects Publications

A. EPA

EPA has published much information on the health effects of Diesel engine emissions. A description of some parts of the program is contained in a pamphlet entitled, "The Diesel Emissions Research Program" (3). An EPA review of the health effects data entitled, "Health Effects Associated with Diesel Exhaust Emissions" was published in November 1978 (4). EPA's Health Effects Research Laboratory (HERL) sponsored the first International Symposium on the Health Effects of Diesel Emissions in December 1979. The proceedings of this international symposium are contained in a two volume publication (5, 6) and are referenced throughout this report.

B. National Academy of Sciences (NAS)

NAS recently published a report entitled, "Health Effects of Exposure to Diesel Exhaust" (2). This report was prepared by the Health Effects Panel of the Diesel Impacts Study Committee under contract to the Environmental Protection Agency, the Department of Energy and the Department of Transportation. The report is a comprehensive summary of the health effects of exposure to Diesel exhaust in four areas - mutagenesis, carcinogenesis, pulmonary and systemic effects and epidemiology. The principal conclusions drawn by the Panel are summarized below.

- The available epidemiologic information does not reveal an excess risk of human cancer of the lung or any other site in the populations studied. This information is based entirely on occupational studies that have numerous deficiencies in the research design.

- There is no convincing evidence that inhaled whole Diesel exhaust is mutagenic or carcinogenic in laboratory animals. However, in animal cell and whole animal skin application tests, organic extracts of Diesel exhaust particulates have been found to contain substances that have mutagenic and carcinogenic potencies similar to extracts of gasoline engine exhaust, roofing tar, and coke oven effluent. It is possible that Diesel exhaust is carcinogenic or mutagenic in animals or humans exposed by inhalation but at a level too low to be detected in studies conducted to date.

- From available epidemiologic, clinical, and laboratory animal studies, no firm conclusions can be drawn about possible pulmonary and systemic effects of Diesel exhaust exposure. However, evidence based on laboratory animal studies suggests that inhaled Diesel exhaust affects the lung clearance mechanisms, produces nonspecific histopathologic changes in the lung that may or may not be reversible, and adversely affects the pulmonary defense mechanisms.

The panel emphasized that much of the information and data evaluated in the report were incomplete or unpublished; therefore, the conclusions should be regarded as tentative.

C. Health Effects Institute (HEI)

In late 1980, HEI was formed to conduct health research on mobile source emissions. HEI is jointly funded by industry and EPA but is set up as an independent entity, i.e. HEI Panel members will not be affiliated with either industry or EPA. HEI represents a unique opportunity for EPA and industry to function together in a cooperative mode to complete needed research.

At this time, HEI is formulating plans for the type of research they would implement. EPA and industry will be suggesting the type of work that is important from their perspectives. While HEI has not initiated any studies to date, it was included here because it is expected to be a major source of Diesel health effects data in the future.

IV. Description of EPA's Diesel Emissions Research Program

For the past several years, EPA has conducted a large research program to assess the potential carcinogenicity of Diesel exhaust. A major segment of EPA's health effects research on Diesel exhaust involves the comparison of the mutagenic and carcinogenic activity of various Diesel samples and coke oven, roofing tar and cigarette smoke samples. If a consistent pattern of data can be obtained on relative potencies among these samples, then the epidemiological data from coke oven, roofing tar, and cigarette smoke probably can be used to estimate human effects from exposure to Diesel exhaust. This approach is used because EPA has found no usable Diesel epidemiologic data on which to base a risk assessment.

The mobile source samples tested include three light-duty Diesel-powered vehicles (Oldsmobile 350, VW turbocharged Rabbit, and Nissan 220C), one heavy-duty Diesel-powered engine (Caterpillar 3304) and one gasoline-powered catalyst-equipped vehicle (Ford Mustang II). Coke oven emissions, roofing tar emissions, cigarette smoke condensate and benzo(a)pyrene (B(a)P) were selected as the comparative samples. The organics extracted from the particulates emitted from these sources were used to determine relative potencies.

The light-duty vehicles were operated on a chassis dynamometer, using the highway fuel economy test cycle (HWFET). The HWFET cycle has an average speed of 48 miles per hour over a length of 10.24 miles. The heavy-duty Caterpillar engine was mounted on an engine dynamometer and operated at steady-state using mode 2 of the 13-mode heavy-duty test procedure. Operation at this lightly loaded mode (2% load) apparently resulted in low

activity for the Caterpillar sample in the mutagenesis and carcinogenesis bioassays. These results are consistent with those from other samples from heavy-duty Diesels at lightly loaded modes. Additional information on the collection of the mobile source samples can be found in reference 6.

The gasoline-powered vehicle was operated at a richer-than-normal stoichiometry in order to generate enough sample; however, the hydrocarbon and extractable organic emission rates are not considered high when compared to in-use catalyst-equipped vehicles. The Nissan Diesel-powered vehicle had an injector design defect resulting in considerable "after injection" of Diesel fuel. Newer Nissan vehicles have redesigned injectors to eliminate this problem. When the injector was corrected, the biological activity as measured by the Ames bioassay was about 2 revertants/ug extract versus 10-15 revertants/ug extract with the earlier injector system. These factors should be taken into consideration when evaluating the results.

The coke oven samples were collected on top of a coke oven at Republic Steel in Gadsden, Alabama. The samples were collected in a location that was upwind of the coke ovens a large fraction (as much as 98%) of the time; however, large sample masses were obtained for the 2% of the time that the wind was blowing in the right direction. It is also thought that some of the sample was from road traffic; there was a paved road about 750 to 1000 feet from the sampler. Thus, an unknown portion of the sample may have been from the urban environment rather than the coke oven.

There was not enough ambient coke oven sample for every type of *in vivo* carcinogenesis testing required so additional samples were obtained in the coke oven main. Workers have not been and are not exposed to the material in the main. The main contains the volatilized organics emitted from the coke oven as coal is coked. Biological testing of the coke oven main reveals that the coke oven main sample is slightly more active than the coke oven ambient sample.

The cigarette smoke condensate was generated by Oak Ridge National Laboratory. Kentucky Reference 2R1 cigarettes were used (non-filter, approximately 30 mg tar per cigarette).

The roofing tar emissions were obtained by collecting a particulate sample above a hot pot of roofing tar. Pitch-base tar was used which is the type of tar in use at the time the epidemiological data were generated.

The dichloromethane extracted organics were subjected to a battery of mutagenesis and carcinogenesis bioassays. The results of these bioassays are reported in this paper. This work involves studies on particulate extract rather than total vehicle exhaust containing both particulate and gas phase emissions. EPA has also done some animal inhalation experiments with whole exhaust (discussed later) but these inhalation experiments were generally negative.

EPA is developing a method to collect the gas phase hydrocarbons present in Diesel exhaust for mutagenicity testing so that an assessment of the relative mutagenicity of the particulates versus the gas phase hydrocarbons in Diesel exhaust can be made. EPA has also done some work to identify some of the compounds present in the extractable organics. A summary of the progress in these areas can be found in section VIII.

V. Mutagenicity

A. In vitro studies

Chemical mutagens are toxic substances that cause changes in the primary structure of the DNA. A high correlation exists between an agent's ability to cause mutations in bacteria and cancer in animals. Two bacterial assays designed to detect gene mutations are the *Salmonella typhimurium* (Ames) and *Saccharomyces cerevisiae* assays. They are discussed below.

One of the most frequently used bacterial assays is the Ames assay, developed by Ames et al. (7). The Ames test involves specially constructed strains of the bacterium *Salmonella typhimurium*. Each strain contains unique types of DNA damage (base pair substitutions, frame shift mutations). The tester strains all require an exogeneous supply of the amino acid histidine for growth. Different doses of the material to be

tested are combined directly on a Petri dish along with a bacterial tester strain. A trace of histidine, which is not enough to permit colonies to form but which will allow sufficient growth for expression of mutations is added. Homogenates of rat liver (S-9 mix) can also be added directly to the Petri plates to detect carcinogens that require metabolic activation (metabolic conversion to an active mutagenic form). The bacteria will grow only if the material is mutagenic, since a mutagen will cause one or more of the tester strains to revert so that they no longer require exogeneous histidine for growth. The number of revertant bacteria are measured by counting the revertant colonies on the plate after two or three days incubation. The potency of compounds can be compared by determining the number of revertants per microgram of sample generated in the linear portion of the dose-response curve.

EPA has measured the mutagenic activity of particle bound organics from Diesel and related environmental emissions using the Ames assay (8). Dichloromethane was used to extract the organics from the particulate. Four strains (TA98, TA100, TA1535 and TA98-NRD) were used in the study. Strain TA98 is used to detect frameshift mutagens and was chosen because of its high overall sensitivity to mutagens. Strains TA100 and TA1535 are designed to detect mutations due to base-pair substitutions and tend to respond selectively to mutagens such as alkylating agents. Strain TA98-NRD is nitroreductase deficient, i.e. not sensitive to nitro compounds.

The results can be found in Tables 1 and 2 (following the text). Table 1 gives the specific activities for each sample. The specific activity is defined as the expected response at 100 ug of organic material and is used as a convenient method of comparison. The activity of each sample was compared to the activity of the Nissan sample by arbitrarily assigning the Nissan sample a relative value of 100. These comparisons are referred to as "relative potency" and are found in Table 2. Results for strains TA98 and TA100 are included in the tables. All samples were negative with strain TA1535. Only two samples, the Diesel Nissan and cigarette smoke condensate samples, provided sufficient material for testing with the nitroreductase strain, TA98-NRD.

As seen in Table 1, cigarette smoke condensate, roofing tar and benzo(a)pyrene required metabolic activation to achieve a positive response. These samples are said to contain indirect acting mutagens. The other samples, including the Diesel samples contain direct acting mutagens; they do not require activating agents. The majority of the activity associated with the Diesel samples appears to be direct acting. This indicates that the mutagenic activity does not reside primarily in the polynuclear aromatic hydrocarbon (PAH) fraction because the PAH's require addition of activating agents to produce responses. The negative response in strain TA1535 suggests that most of the activity is due to polynuclear frameshift mutagens rather than alkylating agents. When the Diesel Nissan and cigarette smoke condensate samples were tested with the nitroreductase strain, TA98-NRD the responses differed. The responses of TA98 and TA98-NRD were quite different with the Diesel Nissan sample but similar with the cigarette smoke condensate sample. This suggests that the particle bound organics from the Nissan Diesel contain nitroarene compounds. In general, results from the Ames bioassay indicate that mutagenic compounds, both direct and indirect acting adhere to carbonaceous Diesel exhaust particulates.

The *Saccharomyces cerevisiae* (yeast) D3 assay was used to evaluate the *in vitro* mutagenic effects of extracted particle-bound organics of Diesel and related environmental emissions. The work was performed for EPA by Stanford Research Institute (SRI) International (9). The samples were from similar engines used in the *Salmonella typhimurium* assay with the exception of the heavy-duty Caterpillar sample.

Homozygous mutants of the yeast *S. cerevisiae* D3 can be generated from the heterozygotes by mitotic recombination. The homozygous mutants are easily distinguished because they produce a red pigment. The frequency of mitotic recombinations may be increased by incubating the organisms with various carcinogenic or recombinogenic agents. The recombinogenic activity of a test sample is determined by recording the number of red-pigmented colonies appearing on the test plates. All testing was performed with and without metabolic activation.

The results for the seven emission samples indicate a slight increase in the number of recombinants at one or two concentrations; however, the results were neither reproducible nor dose-related. It was concluded that this assay was not sufficiently sensitive for this evaluation.

The mammalian cell in vitro assays used by EPA to compare the potencies of Diesel and related environmental emissions are the following: L5178Y mouse lymphoma assay, Balb/c 3T3 fibroblast assay and the Chinese hamster ovary (CHO) cell assay. They are discussed below.

The L5178Y mouse lymphoma assay was performed for EPA by SRI International (9). EPA supplied the samples for testing. The L5178Y mouse lymphoma assay measures the effects of chemicals on the forward mutation frequency of the cells at the thymidine kinase (TK) locus. Unlike the heterozygous cells, mutated homozygous cells can not utilize exogenous thymidine. The mutated homozygous cells can not utilize thymidine analogs as well, such as trifluorothymidine (TFT), but are able to survive and grow in their presence; the heterozygous cells, on the other hand, can not survive in the presence of thymidine analogs. Hence, the mutagenic activity of a chemical in this assay is determined by the number of colonies found growing in the presence of the thymidine analog, TFT.

All of the emission sample extracts gave positive mutagenic responses with and without metabolic activation. When B(a)P was tested, mutagenicity was detected only in the presence of activation. Good dose-dependent dose response curves were found. The comparative potency rankings for the samples using the L5178Y mouse lymphoma assay can be found in Table 3. Table 3 also includes comparative potency rankings for other mutagenesis and carcinogenesis assays and will be referred to frequently throughout this document.

As seen in Table 3, the comparative potencies have been evaluated for the mutagenesis assays using the data obtained with metabolic activation. This is because better dose response curves have been obtained when metabolic activation was used. Both the Ames and lymphoma assays show the same relative potency within the Diesel samples with Nissan>Olds>Rabbit>Cat.

The BALB/c 3T3 assay, like the mouse lymphoma assay, detects gene mutations. This assay was performed by Microbiological Associates under contract to EPA using the Diesel and comparative samples provided by EPA (10). The comparative potency rankings for this assay can be found in Table 3. The BALB/c 3T3 assay responded in a dose-dependent fashion when the B(a)P was used as the positive control. However, when the complex emissions extracts were assayed none yielded good dose-response curves. It was determined that this particular assay does not seem to work for complex mixtures and should not be used for comparative potency.

The sister chromatid exchange (SCE) assay was performed by SRI International under contract to EPA (9). The SCE assay uses Chinese hamster ovary cells to detect DNA damage. The induction of DNA lesions by chemical mutagens leads to the formation of sister chromatid exchanges. In this study, particulate extracts from Diesel and related environmental samples were tested to determine whether they increase SCE frequencies. The samples were tested both with and without metabolic activation.

DNA damage was induced by most of the emission samples both with and without metabolic activation. Table 3 presents the potency rankings when metabolic activation was used. The cigarette smoke condensate and two Diesel samples, the Caterpillar and Oldsmobile, gave a negative response in the SCE assay, unlike the Ames and mouse lymphoma assays. According to the researchers, the negative response may be related to the relatively short exposure time used for SCE testing with activation. The SCE assay did not seem very sensitive or responsive, in comparison with the mouse lymphoma and Ames assays.

From the above data, it can be concluded that, in general, the Diesel samples gave positive responses in the *in vitro* mutagenesis assays. Further work is needed to determine which portion of the Diesel exhaust causes the mutational events to occur. Another major area of concern is how the above mutagenesis data can be used, if at all, to determine human carcinogenic risk to current and future levels of Diesel exhaust emissions.

B. In vivo studies

A number of in vivo studies have been conducted to determine the mutagenic effects of Diesel exhaust. Biological endpoints include gene mutation, sister chromatid exchange and clastogenicity. A selection of in vivo studies are discussed below.

Two in vivo studies designed to detect gene mutations were conducted with male fruit flies of the species Drosophila melanogaster. In one study, approximately 200 flies were exposed eight hours to whole Diesel exhaust (i.e. gases and particulate) diluted five-fold with filtered ambient air (11). The flies were mated and successive generations were investigated for the occurrence of recessive lethal events. The Diesel engine used in this study was a 6-cylinder Nissan engine. Particulate levels in the Drosophila chamber were 2.2 mg/m^3. Results indicate that, under the conditions tested, the Diesel exhaust did not increase the mutation frequency of the exposed flies when compared to the control flies.

In another study, Drosophila were fed 1 mg/ml in a sugar solution of the most polar neutral (oxygenated) fraction of Diesel particulate extract generated from a Caterpillar 3208 heavy-duty engine (12). This was done for three days. Difficulties were encountered in administering the organic fraction and the results were negative.

Male Chinese hamsters were exposed by inhalation to whole diluted Diesel exhaust daily (8 hrs) for 6 months to determine if the exposure would result in sister chromatid exchange (SCE) in the bone marrow cells of these animals (13). The Diesel exhaust was generated from a Nissan 6-cylinder Diesel engine. Six animals were exposed to Diesel exhaust, six animals to clean air and four animals to benzo(a)pyrene (B(a)P) as a positive control. The Diesel exhaust was diluted to achieve a particulate mass concentration as near 6 mg/m^3 as possible. The B(a)P positive control group showed an increase in the number of SCE/cell compared to the clean air control group. Diesel exhaust, on the other hand, did not cause an increase in the frequency of SCE in Chinese hamster bone marrow cells.

Another in vivo assay with sister chromatid exchange as a biological endpoint uses Syrian Hamster lung cells (14). Like the previous assay discussed, a Nissan 6-cylinder Diesel engine was used to generate the Diesel particulate. One set of animals was intratracheally instilled with Diesel particulate, administered in a range from 0 to 20 mg per hamster over a 24-hour exposure period. Lung tissues from these animals were later analyzed for chromatid exchange. SCE increases were induced with intratracheal instillation of Diesel exhaust particulate, producing a linear SCE dose response.

A separate group of animals were chronically exposed to Diesel exhaust for 8 hrs/day, 7 days/week for a period of about 3 months. The particulate concentration in the exhaust emission chambers during the exposure was approximately 6 mg/m^3. The results indicated that a 3-month exposure to 6 mg/m^3 of Diesel exhaust particulates was insufficient to produce measurable mutagenic changes in lung cells. Results for the exposed and control groups were similar.

A subsequent study involved sister chromatid exchange analysis of Syrian hamster lung cells from a total of 33 treated and control animals (15). The treated animals were exposed via inhalation for 3 months to 12 mg/m^3 of Diesel exhaust particulate (DEP) in contrast to 6 mg/m^3 DEP in the previous study. Preliminary results indicate a positive response for the animals exposed to 12 mg/m^3 DEP. The SCE level for the treated hamsters was about double that for the control hamsters.

An attempt was made recently to measure the effect of Diesel particulate extract upon SCE (16). Instillation of extract in dimethyl sulfoxide (DMSO) resulted in a very short residence time in the lung due to its extreme solubility, and resulted in only a marginal increase in SCE. By attaching the extract to an inert carbon carrier particle, however, they were able to once again achieve a dose response curve. This test could be regarded as a useful in vivo test for determining comparative potencies of Diesel particulate extract and extracts from other comparative sources. Future

work could thus involve using other samples, including more Diesel and possibly coke oven, cigarette smoke, and roofing tar samples. A complete analysis of the SCE work should be available later this year.

The Chinese hamster bone marrow bioassay to detect SCE was discussed earlier. This bioassay was a portion of a larger study using Chinese hamster bone marrow (13). Various endpoints were examined. Clastogenicity endpoint bioassays included the chromosome aberrations bioassay and the micronucleus bioassay. Male Chinese hamsters were exposed to diluted Diesel exhaust daily (8 hrs/day, 7 days/wk) for 6 months. There was some increase in the frequency of micronuclei in the animals exposed to the Diesel exhaust. No increase in chromosomal abnormalities was observed in bone marrow cells of the hamsters exposed to Diesel exhaust.

In another study to assess the potential risk from heritable effects in human populations, mice were exposed by inhalation to diluted Diesel exhaust (particulate and gas phase) and a number of genetic endpoints were studied (17). The genetic endpoints included point mutations in males, chromosome damage in males and chromosome damage in females. In addition, various parameters were used to assess reproductive performance in females and histological analyses of germ-cell survival were done in males. The results of this study have recently been released.

A six-cylinder Nissan engine was used to generate the exhaust; the exhaust was diluted with air at the ratio of 1:18. The Diesel particulate concentration in the chambers averaged 6 mg/m^3 during the exposure period of 8 hours per day and 7 days per week. Exposure times in different groups varied from 5 to 10 weeks. The authors calculate that, during the 10-week exposure period, the total intake of exhaust per mouse was about 85 times what a person in an average U.S. environment (urban-rural) would intake in 30 years.

The results of all genetic assays in both sexes were negative. Slight effects on the reproductive performance of females of one strain were

observed, consisting of a decrease in the number of ovulations and an increase in the interval between mating opportunity and copulation. There was no detectable effect of Diesel exposure on the number and distribution of cell types in the testis.

The results indicate that transmitted genetic effects are not a major hazard from exposure to Diesel exhaust; however, the authors stress that the findings should not be used to draw any conclusions about possible risks to the exposed individual himself.

In contrast to the in vitro results, results for the in vivo mutagenesis assays were generally negative with the exception of one study designed to detect sister chromatid exchange.

VI. Carcinogenicity

A number of in vitro and in vivo tests for carcinogenicity have been performed; selected studies will be described in this section. Also included in this section are the results of studies examining the bioavailability of the organics bound to Diesel particulate.

A. In vitro studies

Michigan State University researchers have performed an in vitro study on the effects of Diesel particulate on human normal and xeroderma pigmentosum cells (18). Their investigation found that normal human fibroblasts and excision repair deficient xeroderma pigmentosum fibroblasts are sensitive to the cytotoxic (toxic to cells) action of a direct-acting agent(s) of Diesel particulate. The xeroderma fibroblasts were significantly more sensitive than normal fibroblasts to both the organic solvent extracts of the Diesel particulate and the whole particulate itself. The organic extracts from Diesel exhaust particulate and the non-extracted organics on the particulate interfere with the human cell DNA. The xeroderma pigmentosum cells are

subsequently unable to repair the DNA damage caused by the Diesel organics. In the case of whole particulate, the cells absorb the particulate as demonstrated by electron microscopy and the blackish appearance of the Diesel particulate-treated cells. The surface of the cells is almost clear of particulate. Since the differentially cytotoxic material in Diesel particulate is only slightly soluble in tissue culture medium containing serum, the data suggest that the Diesel particulates are taken up by the cells and then the differentially cytotoxic material elutes from the cell surface and attacks the cell's DNA.

As part of EPA's comparative potency study, particulate extracts from Diesel, gasoline and comparative sources were tested for morphological transformation in BALB/c 3T3 cells (10). The extracts were also tested simultaneously for mutagenic activity in BALB/c 3T3 cells; this was discussed earlier in the mutagenesis section. This assay did not yield a dose dependent increase in either mutation frequency or transformation frequency. As mentioned previously, this particular assay, as performed, does not work for complex mixtures and should not be used for comparative potency.

Another *in vitro* carcinogenicity test, performed as part of EPA's comparative potency study, is designed to detect enhancement of viral transformation in Syrian hamster embryo cells (19). This assay produced a positive dose-response with all samples; however, the particulate extracts prepared from Caterpillar emissions failed to induce a significant increase in the transformation frequency. The comparative potencies of the test samples for this assay can be found in Table 3. The Diesel and gasoline particulate extracts, with the exception of the Caterpillar appear to be within the same range, with the Nissan the highest. The comparative sources were clearly more active, particularly the roofing tar. A substance that repeatedly scores positive in one or more transformation assays is highly suspect of being carcinogenic *in vivo* (2).

B. In vivo studies

Many of the in vivo tests for carcinogenicity are currently being performed. This section summarizes the available results from a selection of in vivo studies, with an emphasis on those studies EPA will use to perform a risk assessment.

The objective of a study performed at the EPA-Cincinnati facility is to determine the relative carcinogenicity of Diesel exhaust particulates using the pulmonary adenoma assay on Strain A mice. In the initial study, Strain A mice were exposed to Diesel particulate by intraperitoneal injection (20). The animals were injected 3 times weekly for 8 weeks to the Diesel particulate. At the time of this study, Strain A mice were also being exposed to diluted Diesel exhaust by inhalation. The particulate concentration in the inhalation exposure chambers was approximately 6 mg/m^3. In an attempt to correlate the inhalation and intraperitoneal injection studies, the highest particulate dose level for the intraperitoneally injected mice was chosen to correspond to the inhalation dose assuming 50% retention. Thus, the highest dose group received 235 ug/injection, 705 ug/week. The animals were sacrificed after approximately 9 months. Results showed no significant difference between the incidence of tumors in the injected and control mice. (The inhalation study will be discussed in more detail later in this section.)

The intraperitoneal injection study with Strain A mice has since been expanded. More animals and test materials have been employed. The test materials include: control injected (control chemicals are urethane and benzo(a)pyrene), cigarette smoke condensate, roofing tar, coke oven, Oldsmobile Diesel particulate extract, Nissan Diesel particulate extract and Nissan particulate. Except for the control chemicals, the test materials are injected three times per week for eight weeks (1 mg of test material per injection). Fifty-five (55) mice per test material are being injected. The animals are to be sacrificed at 9 months of age to determine the number of pulmonary adenomas. Currently, sacrifice on all but one group of animals is complete. Results should be available later this year.

An intratracheal instillation lifetime study with Syrian golden hamsters is currently underway to compare the potential carcinogenicity of Diesel particulates, Diesel particulate extract, coke oven main extract, roofing tar extract, cigarette smoke condensate, and benzo(a)pyrene. The study is being performed by the Illinois Institute of Technology Research Institute (IITRI) and sponsored by EPA. Hamsters were used for intratracheal instillation because they are susceptible to lung tumors, yet have a low spontaneous rate of lung tumors. The advantages of intratracheal instillation as a route of exposure are that it allows high doses of samples when compared to inhalation studies and is a natural route of exposure (21).

The lifetime study includes positive, solvent, and untreated controls. The treatment schedule is once per week for 15 weeks, beginning at 12 to 13 weeks of age. Each material is being tested at three dose levels, 1.25, 2.5 and 5 mg/week. These doses were chosen after performing a preliminary dose-range study with Diesel particulates (22). The Diesel particulate extract, coke oven main extract, cigarette smoke condensate and roofing tar extract are being administered with a ferric oxide carrier. The ferric oxide carrier offers the advantage of keeping the sample in contact with the lung tissue longer than an emulsion would because the particles take longer to be cleared out of the lung. Also, if the same amounts of extract in emulsion and extract plus carrier are injected, the sample plus carrier represents a lower dose of extract due to the slow release of the extract from the carrier (21). The Diesel particulate and solvent are administered with and without the ferric oxide carrier. The study is being conducted in two replicates of half the animals in each test or control group.

As of May 30, 1981, the first replicate is in the forty-ninth week of the study (23). The hamsters in the first replicate are 14 months of age. When the hamsters were 12 months of age, a scheduled sacrifice of 245 animals was conducted. All treatment groups and control groups were represented. Organ weight determinations failed to indicate a toxic effect that could be attributed directly to the test articles used in this study. IITRI is working on the histopathological evaluation.

The second replicate is in the thirtieth week. The hamsters are now 10 months of age. All dose levels have continued to gain weight; however, the Diesel particle group (5 mg), the Diesel extract plus ferric oxide group (5 mg and 1.25 mg) and the benzo(a)pyrene plus ferric oxide group (2 mg) are below the colony controls with respect to the percent increase in mean body weight.

During the period July 20-30, 1981, 255 randomly selected, twelve-month old hamsters from the second replicate were sacrificed for histopathological evaluation. Selective organ weight determinations will also be made at this time. The remainder will be allowed to live out their normal lifespan. Final results for this study are not expected until 1983.

Both the intratracheal instillation study with Syrian golden hamsters and the intraperitoneal injection study with Strain A mice include the testing of Diesel particulate and Diesel particulate extract. The results of these studies could provide important information about the bioavailability of the organics on the particulate, in addition to providing comparative potency data.

An important in vivo assay in progress that will be used to help formulate a risk assessment is the mouse skin carcinogenesis assay. This work is being sponsored by EPA and conducted at Oak Ridge National Laboratory. The objective of this study is to evaluate the ability of Diesel particulate extracts to act as tumor initiators, tumor promoters, cocarcinogens and complete carcinogens and to compare the potency of these extracts to other emission extracts and pure carcinogens.

Tumor initiation is the first step of the carcinogenic process. A tumor initiator converts a normal cell to a pre-malignant cell. A tumor promoter is one which, when applied repeatedly after a single dose of a tumor initiator, will result in tumors. Tumor promoters can be either weak carcinogens or noncarcinogens. Pre-malignant (initiated) cells can become malignant, even after 1 year from the time the initiator is applied to the time the promotor is applied. To determine if an agent is a cocarcinogen,

the agent is given concurrently with a tumor initiator. A complete carcinogen is both an initiator and a promoter. A good qualitative and quantitative correlation exists between complete carcinogenesis and tumor initiation in mouse skin (24).

Two strains of mice are being used, one sensitive strain (SENCAR) and one resistant strain (C57 black). The mobile source samples consisted of particulate extracts from three light-duty Diesel-fueled vehicles (Datsun-Nissan 220-C, Volkswagen Rabbit and Oldsmobile 350), one gasoline-fueled vehicle (Mustang II) and one heavy-duty Diesel engine (Caterpillar 3304). The comparative sources employed were cigarette smoke condensate, coke oven ambient samples, roofing tar emissions and residential home furnace samples. The pure carcinogen tested was benzo(a)pyrene. Forty male and forty female mice per dose were used for each strain. The extracts were applied to the mouse skin at five dose levels per agent.

Recent results with the C-57 black mice were negative (i.e. no carcinogenic response) with the exception of one dose of the roofing tar samples (25). Work with the more sensitive Sencar mouse does show a positive response. At the present time, the tumor initiation papilloma data are available(24,26). A statistical analysis was performed with these data. Slopes of the dose response curves were determined for each complex mixture sample (in terms of papillomas/mouse/mg dose). A square root analysis was then performed. The data did not follow a Poisson distribution (which assumes independent events) because it was discovered that the chances are greater that a mouse with an existing tumor will get another versus a mouse with no tumor getting an initial tumor. Hence, the Probit model was used to rank the complex mixtures.

The samples have been scored 26 weeks after treatment according to potency (papillomas/mouse/mg) and then ranked. The results are included in Table 3. Both the cigarette smoke condensate and the heavy-duty Caterpillar sample gave a negative response. The cigarette smoke condensate was not concentrated to the same extent as the other samples. The detectability limit of this particular assay is above the doses and concentrations tested for the cigarette smoke condensate (24).

The ranking indicates that the potency of pure benzo(a)pyrene was greater than the coke oven sample, which was, in turn, greater than the roofing tar and Nissan samples. The Diesel samples exhibited a wide range of potencies.

Samples from other sources are currently being tested. These sources include a Mercedes Diesel, 1970 Ford van (non-catalyst), roofing tar condensate, coke oven main, and cigarette smoke condensate. The cigarette smoke condensate sample is being retested at 10 times the original dose. The coke oven main sample has been discussed in section IV. The initial roofing tar sample consisted of an extract of roofing tar emissions trapped in the collecting device. This sample was subsequently replaced with an extract of roofing tar emissions which condensed on the funnel just prior to the collecting device. This new sample has been called roofing tar condensate.

Improved statistical models have since been developed to analyze the data. Tumor initiation data for the coke oven main and roofing tar condensate samples should be available later this year. Carcinoma data for the original samples, which will supplement the papilloma initiation data, will be presented at a Diesel emissions symposium in October 1981. Pathology results will also be presented.

General Motors is sponsoring a skin carcinogenesis study on Diesel particulates and Diesel particulate extract. The study is being conducted by the Bushy Run Research Center, Carnegie-Mellon University. Male C3H mice are being used in three types of studies, initiation, promotion and complete carcinogenesis. Studies have been in progress for close to 2 years and results should be available in about a year.

Other <u>in vivo</u> carcinogenesis studies have been performed using inhalation as the route of exposure. In inhalation studies, the test animal is exposed to the whole exhaust (particulate and gas phase) rather than just one component of the exhaust. A selection of studies are described in detail below.

A number of inhalation studies have been conducted at the EPA-Cincinnati facility. Strain A mice were exposed to either diluted Diesel exhaust or clean control air 8 hours per day, 7 days per week for up to 30 weeks. The particulate concentration in the Diesel exhaust inhalation chambers was approximately 6 mg/m^3. There was no increase in incidence of lung tumors in the animals exposed to 6 mg/m^3 Diesel particulate compared to the control animals (20).

In a subsequent study, Strain A mice were divided into the following four exposure groups: control (no treatment), control plus urethane (5 mg), Diesel exhaust (particulate concentration of 12 mg/m^3) and Diesel exhaust plus urethane (5 mg). The urethane was administered by the intraperitoneal route. The animals were exposed 30 weeks. Results indicate that the urethane treatment had more effect than the Diesel exhaust treatment. The number of tumors per mouse for the Diesel exhaust group were similar to the control group. The groups given urethane had more tumors than the control group. The group given urethane alone had the greatest number of tumors per mouse.

Additional Strain A mice were exposed to either clean air, clean air plus urethane, Diesel exhaust (particulate concentration of 12 mg/m^3), or Diesel exhaust plus urethane. This study differed from the previous study in that the mice were exposed during darkness. This was expected to increase the exposure since the animals were awake and active during exposure. The animals have been sacrificed and gross adenomas have been counted. The final analysis has not yet been performed; however, the preliminary results are similar to those of the previous study. There appear to be significantly fewer tumors in the Diesel exhaust group and the Diesel exhaust plus urethane group compared to the group given urethane alone. The reasons for this are not clear yet.

Sencar mice are being tested to determine the effect of lifetime inhalation of diluted Diesel exhaust (particulate concentration of 12 mg/m^3) on tumor induction. The mice were exposed 15 months to the exhaust. One test group was also given an initiator (urethane) and another was also given a promotor

(butylated hydroxytoluene). The animals have been sacrificed. Tumors and histology examinations are currently being done. Gross lung examination did not show any obvious difference between the exposed and control animals with respect to lung surface tumors.

General Motors Research Laboratories is sponsoring a large-scale inhalation study with Strain A/Jackson mice, Fischer 344 rats and Syrian Golden hamsters. The work is being conducted by the Southwest Foundation for Research and Education and the Southwest Research Institute, San Antonio, Texas. In a preliminary short-term study, the three species are exposed either to diluted Diesel exhaust (1500 ug/m^3 particulates) or to filtered air for 20 hours per day, 7 days per week for 3 months. The animals will be monitored for 6 months following exposure to determine recovery rates. Subsequently, a large-scale, 15 month study has been initiated with mice, rats and hamsters. The animals will be exposed to filtered air or diluted Diesel exhaust containing 250, 750 or 1500 ug/m^3 of particulate. This study is in progress. Histopathologic examination of the rats exposed for up to one year of exposure revealed no lung tumors.

When the NAS Health Effects Panel reviewed this study, they suggested that the investigators consider increasing the concentration of Diesel exhaust particulates because studies have shown that Strain A mice will tolerate up to 6400 ug/m^3 particulates when exposed 20 hours per day, 5 days per week (20). Syrian golden hamsters have been shown to accept approximately 7000 to 8000 ug/m^3 when exposed 7-8 hours per day, 5 days per week (27).

C. Bioavailability

An important issue is the bioavailability of the organics bound to Diesel particulate. As mentioned previously, both the intratracheal instillation study and the intraperitoneal injection study include in their design the testing of Diesel particulate and the Diesel particulate extract. These *in vivo* studies should provide some insight into the question of bioavailability.

A number of in vitro studies related to bioavailability have been performed. McCormick et al. (18) showed that the organic extract from the Diesel exhaust particulate and the non-extracted organics on the particulate interfere with the human cell DNA when xeroderma pigmentosum cells were used.

Siak, et al. (28) used the Ames mutagenicity assay to examine the effects of various extraction solvents and biological fluids on the mutagenic activity of Diesel particulate extracts. Of the various solvent extracts examined, the dichloromethane extract exhibited the highest activity in the Ames test, although methanol yielded the largest extractable mass. (EPA uses dichloromethane to extract the organics from the particulate.) The results of this study indicate that the mutagenic activity in Diesel particles is not readily removable by simulated biological fluids. Fetal calf serum (FCS) was the only simulated biological fluid which eluted mutagenic activity from the particles; however, FCS only extracted 3.6 to 12.6% of the activity found in the dichloromethane extracts.

The objective of a study by King et al. (29) was to evaluate the release of mutagens bound to Diesel particles in the presence of organic solvents, lung fluids and human serum. The mutagenic activity of the organics was evaluated using the Ames assay. Organic solvents were found to be the most efficient at removing mutagens from Diesel particles, with dichloromethane extracted organics having the greatest mutagenic activity of the solvent systems examined. The mutagenic activity of Diesel particle organics pre-extracted with dichloromethane is greatly reduced upon the addition of serum and lung cytosol to organics. Subsequent incubation of serum and lung cytosol bound Diesel organics with protease (an enzyme that digests proteins) increases the mutagenic activity. This research suggests that substantial mutagenic activity is released from Diesel particles upon incubation with serum and lung cytosol.

VII. Non-genetic effects

Non-genetic effects include pulmonary and systemic effects. Inhalation studies are generally used to determine the pulmonary and systemic health effects of Diesel exhaust.

One study was designed to determine the effect of inhalation of Diesel exhaust on sperm-shape abnormalities in mice (30). Strain A mice were exposed to either clean air or diluted Diesel exhaust (particulate concentration of 6 mg/m^3) for 31 or 39 weeks. The results show that inhalation exposure to diluted Diesel exhaust did not increase spermhead abnormalities in the mice.

Twenty-five male Fischer 344 laboratory rats were exposed to diluted Diesel exhaust (particulate concentration of 1500 ug/m^3) for 20 hours/day, 5 1/2 days/week for 267 days to determine the effect of chronic inhalation of Diesel exhaust on pulmonary function (31). Twenty-five control animals were exposed to clean, filtered air. There was no significant difference in pulmonary function between the control and experimental animals.

A series of experiments was conducted to determine if mice exposed to dilute Diesel exhaust exhibit enhanced susceptibility to infection (32). Female albino mice were first exposed for various durations (involving acute, subacute and chronic exposure periods) to diluted Diesel exhaust with a particulate concentration of 6 mg/m^3. The animals were then briefly exposed to a bacterial pathogen (Streptococcus). Typically, post-infection mortality was significantly greater in groups exposed to Diesel exhaust than in their corresponding control groups exposed to purified air only.

Limited data on acute tests of NO_2 and acrolein vapor alone suggest that the infectivity-enhancing effect of Diesel exhaust could be accounted for in large part by these components. Comparison of these data with past experiments involving Diesel-powered and gasoline catalyst-equipped vehicles indicate a somewhat greater excess mortality from bacterial infection in mice exposed to Diesel exhaust than in those exposed to catalytic gasoline exhaust. Exposures to Diesel exhaust, NO_2, or acrolein did not enhance the mortality response to a viral pathogen (A/PR8-34).

Chronic inhalation studies are currently being conducted with cats (33). The cats have been exposed to diluted Diesel exhaust emissions for approximately 27 months. Toxicological effects to be examined include

pulmonary function, pathology, blood enzyme levels and sperm abnormalities. No important changes in pulmonary function were detected after one year of exposure; however, since that time significant pulmonary function decrements have occurred. Half the cats were sacrificed following completion of the 27 month exposure. The other half will be sacrificed after six months recovery in clean air. The complete pathological evaluation is scheduled to be completed December 1981.

The deposition and clearance of inhaled Diesel particles in the respiratory tract was studied by Chan et al. (34). Twenty-four male Fischer 344 rats were exposed in a "nose-only" inhalation chamber for 40-45 minutes to diluted Diesel exhaust generated from Diesel engines burning fuel containing either ^{131}Ba or ^{14}C radioactive tracers. Immediately after exposure, the deposition efficiency of inhaled Diesel particles in the respiratory tract was determined to be $15 \pm 6\%$ by counting of ^{131}Ba and $17 \pm 2\%$ by counting of ^{14}C in the lung tissue samples. The ^{14}C tracer was used for the long-term clearance study. Two distinct phases of clearance were evident in the experimental data collected up to 105 days. Clearance half-times of 1 day and 62 days were found for mucociliary and alveolar clearance, respectively. Approximately 6% of the initial deposition was found in the mediastinal lymph nodes after 28 days, demonstrating that the lymphatic system was also involved in the removal of Diesel particles from the pulmonary airways. After the monitoring period of 105 days, 27% of the initial dose still remained in the lung.

VIII. Characterization

Diesel exhaust consists of gaseous compounds and particulate matter. Much research has been performed in an attempt to characterize these emissions. Of particular interest is the identification of the mutagenic organics bound to the exhaust particulate. This section gives a brief overview of this work, summarizing some of the significant results obtained to date.

A. Gas-phase

The principal combustion gases (hydrocarbons, nitrogen oxides and carbon monoxide) emitted from Diesel-powered vehicles are similar to those emitted from gasoline-powered vehicles. Table 4 gives the emission rates of these regulated compounds along with some unregulated compounds from Diesel-powered light-duty vehicles and heavy-duty engines studied in EPA programs. Gasoline counterparts are also included for comparison. The data in this table were taken from a study performed by Southwest Research Institute under contract to EPA (35).

The Diesel-powered light-duty vehicles appear to emit more hydrocarbons than their gasoline counterparts but, in turn, emit less carbon monoxide and oxides of nitrogen. Particulate emissions are much higher from Diesel-powered vehicles (This will be discussed in more detail in the following section). The unregulated emissions of sulfates and total aldehydes were higher from the light-duty Diesel-powered vehicles. As expected, the Diesel-powered vehicles are more fuel efficient than their gasoline counterparts.

The heavy-duty engines can be examined in the same fashion. The Diesel-powered Caterpillar 3208 engine and the gasoline-powered Chevrolet 366 engine are used in many identical truck applications. The Diesel-powered engine emits more oxides of nitrogen, particulate and sulfates than the gasoline-powered engine. The gasoline-powered engine, in turn, emits more hydrocarbons, carbon monoxide and total aldehydes. The brake specific fuel consumption (BSFC) was lower for the heavy-duty Diesel-powered engines.

EPA's Office of Research and Development (ORD) has attempted to identify the individual hydrocarbon compounds in the gas phase of Diesel- and gasoline-powered vehicle exhaust. This work has been described by ORD in several publications and was recently summarized in a report prepared by EPA's Office of Mobile Source Air Pollution Control (OMSAPC) (36).

In this report, gas phase hydrocarbon emissions from light-duty gasoline- and Diesel-powered vehicles were examined and compared. The hydrocarbon composition of gasoline exhaust consists to a large extent of components of carbon numbers 1 through about 10. In contrast, the gas phase organics from Diesel-powered vehicles range from C_1 to about C_{40}, the majority being below C_{25}. The C_1-C_{10} hydrocarbons result almost entirely from the combustion process. It is postulated that the C_{10}-C_{25} hydrocarbons result, to a large extent, from uncombusted fuel, and the C_{15}-C_{40} hydrocarbons from lubricants (37).

It is possible to identify the individual hydrocarbons with carbon numbers 1 through 10 with a gas chromatograph; therefore, the gas phase hydrocarbons emitted from gasoline-powered vehicles can be readily identified. The gas chromatograph used to measure the hydrocarbon compounds with carbon numbers greater than 10 does not have adequate resolution to permit identification of each individual compound in this range. It is, however, possible to determine the molecular weight distribution of the compounds of interest. Since it is not currently possible to identify individual components of Diesel-powered vehicle exhaust above C_{10}, Diesel hydrocarbon analyses must be done in terms of carbon number. Results indicate that the gas phase emissions from Diesel-powered vehicles contain small quantities of high molecular weight organics. These organics have not, as yet, been identified.

While extensive Ames and other bioassay testing for mutagenicity is being performed on the organics extracted from the particulate, the relative mutagenicity of the gas phase organics remains uncertain. Methods are currently being developed to collect artifact-free samples of gas phase hydrocarbons in motor vehicle exhaust for bioassay testing. These methods are discussed below.

EPA-ORD has done some preliminary testing with both a condensate and a filter cartridge method. The condensate method involves filtering the exhaust particulates and then condensing the components in the gas stream. The condensate appears to have low Ames test activity. If the filter upstream of the condenser is removed, the condensate contains some Diesel particulate and has somewhat higher Ames test activity.

The filter cartridge method involves passing a gas stream sample after a conventional particulate filter through a cartridge or bed of treated XAD-2 resin. After the gas stream is passed through the XAD-2, the hydrocarbons absorbed onto the XAD-2 are removed from the resin by methylene chloride extraction. The lower molecular weight hydrocarbons (e.g. below C-10) are sufficiently volatile that they are probably lost during the extraction. However, hydrocarbons above C-10 are retained and can then be subjected to the Ames test. Since the conventional particulate filter will generally retain hydrocarbon compounds above C-15, the XAD-2 traps could provide a good method to collect hydrocarbons in the C-10 to C-15 range. A preliminary result of Ames testing on the gas phase hydrocarbon collected by this method for a VW Diesel Rabbit shows that the activity may be low compared to that of the particulate.

EPA-OMSAPC has also been developing a technique to collect Diesel gaseous hydrocarbons. The procedure involves collecting particulate on 20" by 20" filters using dilute Diesel exhaust from a vehicle run on the EPA Highway Fuel Economy Test (HFET). A portion of the loaded filters were then baked in an oxygen-free oven to drive off extractable organics. Two of the baked filters were then installed behind a double primary filter and an FTP was run. The particulate is caught by the double primary filter, enabling the gas phase hydrocarbons to pass through to the pre-baked back-up filters. The procedure was repeated for the HFET cycle. As a control, background air was passed through the filters with no car installed. Filter weights were measured during each step of the process. In addition, filters from each step of the process were extracted and submitted for Ames testing.

Preliminary Ames results with strain TA98 indicate that, <u>without</u> metabolic activation, the mutagenic activity of the extractables from the loaded (unbaked) filters is roughly equal to the extractables from the baked back-up filters (used to collect the gas phase hydrocarbons). When metabolic activation was used, the mutagenic activity of the loaded filter extractables was much greater that that of the baked back-up filter extractables. The same trends were apparent with strain TA100. With strain TA1538, the mutagenic activity of the loaded filter extractables was much

greater than that of the baked back-up filter extractables, regardless of whether or not metabolic activation was used. It must be emphasized that these results are very preliminary; however, they appear to indicate the presence of direct-acting mutagens in the gas phase of Diesel exhaust.

B. Particulate

The chemical composition of particulate matter from Diesel exhaust is complex. Diesel particulate consists of a carbonaceous core with organic compounds adsorbed on the surface. Particulate emission rates for some Diesel-powered vehicles can be found in Table 5. The sources in Table 5 are those being tested as part of EPA's health effects program. A gasoline-powered vehicle is also included for comparison. It can be seen that the particulate emission rates of the Diesel-powered vehicles differ from one another but, in all cases, exceed the particulate emission rate of the gasoline-powered vehicle by more than an order of magnitude. Typically, more than 85% of the Diesel particulate emitted is under 1 micron (10^{-6} meter) in size; as a result, the particulate is small enough to be inhaled and deposited deep within the lungs.

Because Diesel particulate is easily respirable, considerable effort has been spent in an attempt to identify the organic compounds adsorbed on the particulate surface, in particular those responsible for the mutagenic activity observed in the Ames test. The organic compounds are extracted from the particulate using a solvent such as dichloromethane. Table 5 includes the percentage of particulate composed of extractable (soluble) organics for each sample. Like the particulate emission rate, the percentage of extractable matter varies from vehicle to vehicle.

The soluble organic fraction (SOF) of particulate from Diesel exhaust has been separated by high performance liquid chromatography into three major fractions: acid, base and neutral. The neutral fraction, in turn, has been further separated into nonpolar, moderately polar and highly polar fractions designated as the polynuclear aromatic hydrocarbon (PAH), transition and oxygenate fractions, respectively. The Ames test has been used to determine

the mutagenicity of these fractions. It was found that the transition and oxygenate fractions account for most of the Ames test activity. The transition fraction alone accounts for more than 65% of the direct acting mutagenicity for the total extract. The mutagenicity of the total extract was found to be equivalent to the summation of its fractions.

The nonpolar (PAH) fraction has been well characterized and consists of PAH and aliphatic hydrocarbons. Benzo(a)pyrene (B(a)P) is a PAH that has been identified. Table 5 reports the results of B(a)P analyses performed on those samples.

The transition and polar fractions are more difficult to characterize. The polar fraction consists primarily of carboxylic acid PAHs.

Approximately 60% by weight of the material in the transition fraction consists of oxygenated PAH derivatives (including hydroxy, ketone, carboxaldehyde, quinone, dihydroxy, acid anhydride and nitro derivatives). The carboxaldehyde PAH derivatives were among the most abundant PAH derivatives found in the transition fractions. A total of about eighty PAH derivatives have been identified in this fraction, including a nitro-PAH, 1-nitropyrene. The 1-nitropyrene was found to account for roughly 45% of the direct-acting mutagenicity for the transition fraction and 30% of the direct-acting mutagenicity for the total extract. Two other nitro-PAH were tentatively identified in the transition fraction but their mutagenicity is not known. The investigators conclude that the nitro-PAH may account for a significant portion of the direct-acting mutagenicity for the transition fraction (38).

General Motors conducted a study to determine whether nitro-PAH are formed in the combustion process or by chemical reactions during exhaust sampling by filtration (39). They found that the levels of nitro-PAH were higher in particulate samples collected over longer sampling times or reexposed to additional Diesel exhaust gases. The samples reexposed to exhaust gases also showed higher direct-acting mutagenic activity in the Ames test (using strain TA98). The authors conclude that much of the nitro-PAH could be formed as artifacts of filter sampling.

EPA has conducted experiments recently in which additional NO_2 was introduced into the dilution tunnel to determine if high levels of NO_2 in the dilution tunnel would result in a reaction of NO_2 with some of the hydrocarbons present in the gas stream or on the particulate filter. If so, the reaction products (artifacts), including various nitro-PAH compounds would cause an artifically high Ames test response. Results indicate that artifact (e.g. excess 1-nitropyrene) is formed when the NO_2 levels are above 5 ppm in the dilute exhaust. The NO_2 levels in the dilute exhaust are normally no higher than 3 ppm (40).

EPA has also conducted experiments with a single cylinder Diesel engine with artificial combustion air containing no nitrogen and a nitrogen free fuel. Subsequently, no NOx would be formed in the exhaust gas. By comparing the results of these tests to tests with conventional air (79% N_2, 21% O_2) and regular Diesel fuel (which contains traces of nitrogen), one can determine if there were artifact formation due to NO_2. The results of these tests have not yet been published.

IX. Epidemiology

The following section summarizes selected epidemiology studies performed to date.

Of the several epidemiological studies evaluating the effects of Diesel exhaust the London Transit Worker study has received much attention in the last few years. Lung cancer incidence among male employees of the London Transport Authority, aged 45 to 64, was reported during 1950 to 1974 (41). (The results for 1950 to 1954 were originally reported by Raffle in 1957 and updated by Waller in 1979 to cover the period from 1950 to 1974.) In a presumed highly exposed group, i.e., the "engineers" servicing buses in garages who were exposed to Diesel exhaust in an enclosed area, a total of 177 cases of lung cancer were observed in 86,054 man-years at risk where 197.1 were expected based upon greater London death rates in the 1950 to 1974 time frame.

Both studies, the original and the follow-up, suffer many weaknesses in design. When the NAS Diesel Health Effects Panel reviewed this study, they cited the following weaknesses:

- no measure of individual worker exposure, merely a gross estimate of pollutant concentrations in the garage on a few separate days over the 25 years,
- no smoking habits/histories were known,
- not following employees who left the London Transport Authority for other jobs,
- not considering the impact on lung cancer of social and ethnic differences between the workers and the general population (the comparison group), and
- not investigating the "healthy worker effect".

In addition, the cause of death of employees who retired from the London Transport Authority was not investigated. If a retired employee learned that he had lung cancer the day after his retirement, he would not be included in the data base.

This epidemiology study has been cited by many individuals as a strong indication that Diesel emissions result in no excess cancer risk. Todd Thorslund, of EPA's Carcinogen Assessment Group evaluated the London Transit Worker Study (LTWS) (42). His analysis has been reviewed both inside and outside EPA by various experts. Thorslund analyzed the study parameters statistically to obtain an upper bound potency estimate which could then be translated into an upper bound measure for the total potential cancer effect in the U.S. population. He concluded that "...it would still be possible to have lung cancer deaths numbering in the thousands each year in the U.S. due to Diesel engine emissions and not be inconsistent with the results obtained in the LTWS.".

Dr. Jeff Harris of the Analytical Panel of the NAS Diesel Impacts Study Committee also performed an analysis of the LTWS (43). Like Thorslund, Harris obtained a statistical upper bound potency estimate. He concludes, with 95 percent confidence, that the undetected incidence of lung cancer among Diesel bus garage workers was no greater than 160 percent of the incidence of lung cancer among other unexposed employees. Harris then asked the question: with 95% confidence that the risk will not be any higher, what is the possible lung cancer risk in the general population, based on my treatment of the uncertainies present in the London Transport data base? Harris found that the upper 95% confidence limit represents a 0.05 percent increase in lung cancer incidence per unit of exposure, where one unit of exposure is equivalent to inhaling a concentration of 1 microgram of particulates per cubic meter for one year.

Based on the analyses by Thorslund and Harris, it is possible that significant excess cancer deaths could result in the general population even though the LTWS showed no excess cancer deaths in the exposed group.

Two major epidemiology studies are currently planned. One is a study of heavy equipment operators in the Operating Engineers Union (44). This study is sponsored by the Coordinating Research Council (CRC). Eligible subjects are those who have worked for one year or more between January 1958 and December 1978. It is estimated that there will be from 25,000 to 40,000 subjects with up to 500,000 man-years of work experience. Mortality and cancer incidence will be recorded.

The second study, sponsored by EPA under a Research Grant with the Harvard School of Public Health, will examine railroad workers exposed to Diesel exhaust (45). Railroad Retirement Board records will be used to identify about 80,000 subjects exposed to Diesel exhaust. These subjects have worked 10 to 19 years as railroad workers in 1964 and will be followed through 1978.

Both proposed studies have attempted to eliminate the shortcomings present in the London Transit Worker Study.

X. Risk Assessment

This section summarizes the assessments that have been performed in an attempt to determine the risk associated with exposure to Diesel emissions. EPA's revised risk assessment will also be discussed.

Dr. Harris has estimated the potential risk of lung cancer from Diesel emissions (43). His results, based on information from the London Transit Worker Study, were given in the previous section. Estimates of the risk of human lung cancer from exposure to Diesel emissions were also made using relative carcinogenic potencies for Diesel emissions and two comparative source emissions, coke oven and roofing tar. Data from three short-term bioassays (using the soluble organic fraction) were used to estimate the relative carcinogenic potencies. The bioassays included tumor initiation in SENCAR mice by skin painting, enhancement of viral transformation in Syrian hamster embryo cells, and mutagenesis with and without metabolic activation in L5178Y mouse lymphoma cells. (The bioassays and sources were tested as part of EPA's Diesel emissions research program. More information on this work can be found in previous sections.) The 95% confidence limit of potential risk was found to be a 0.03 percent proportional increase in lung cancer incidence per unit of exposure. This is comparable to the 0.05 percent increase in lung cancer incidence per unit of exposure derived from the EPA analysis of the LTWS.

Lovelace Inhalation Toxicology Research Institute prepared a report for the Department of Energy summarizing the potential environmental effects and human health risks for projected increased use of Diesel-powered light-duty vehicles in the United States (46). The authors estimated the lung cancer deaths associated with exposure to light-duty Diesel exhaust. Their calculations were based on 1995 and beyond. It was assumed that all Diesel-powered automobiles would be controlled to 0.16 gm/mile (0.1 gm/km) particulate. The projected increase in Diesel-powered light-duty vehicles used was 20% of the light-duty vehicle fleet, an upper level based on restrictions on oil refinery processes. Estimates were made of the

concentration of Diesel exhaust particulate to which various numbers of people would be exposed. The routes of exposure estimated were inhalation and ingestion. Only inhalation and resulting lung cancer deaths were quantified.

To estimate lung cancers from light-duty Diesel exhaust, coke oven and smoking epidemiological data were used. These data were standardized to annual lung cancer risk per 100,000 people per ug/m^3 of benzo(a)pyrene (B(a)P), and per mg/m^3 of ambient air particulate matter. Assuming that Diesel exhaust was not significantly more potent than the worst estimates obtained from the coke oven and smoking data, estimates of lung cancers associated with breathing Diesel exhaust were made. These values are:

using B(a)P

10 excess lung cancers from 20% Diesel-powered automobiles controlled to 0.16 gm/mile particulate, in 189 million people exposed

using particulate

30 excess lung cancers from 20% Diesel-powered automobiles controlled to 0.16 gm/mile particulate, in 189 million people exposed.

This report was issued in late December 1980 and is the first of a series of annual reports on this topic. Future reports will include evaluations of additional health risks and examination of the health risks for people with existing respiratory diseases.

An initial risk assessment was performed by EPA's Cancer Assessment Group (CAG) in June 1979 (47). The CAG assessment was based on the following major assumptions:

1) that Diesel exhaust products measured by organic extractables, and coke oven emissions measured by organic extractables have the same carcinogenic potency on a unit mass basis,

2) that the entire U.S. population, estimated to be 220 million in number, is exposed and that the exposures were log-normally distributed, and

3) that the shape of the dose-response curve for lung cancer due to high level coke oven industrial exposures are extrapolatable in a linear fashion to low-level environmental exposures.

The following estimates of excess cancer deaths per year were obtained assuming two Diesel market penetration scenarios (10% and 25% of the light-duty vehicles and 68% and 99% of the heavy-duty vehicles being Diesel-powered by 1990, which represent the best estimate and maximum growth estimate, respectively):

Excess Cancer Deaths Per Year

	Best Estimate	Maximum Growth Estimate
Light-duty Diesels	346	625
Heavy-duty Diesels	668	1185

The light-duty Diesel particulate emission factor used was 1.08 gm/mile; this assumes no particulate standard and allows for a penalty in particulate emissions to compensate for further control of NOx emissions. The particulate emission factors used for the heavy-duty vehicles equipped with 2 stroke and 4 stroke cycle engines, and buses were 4.08, 2.44 and 4.87 gm/mile, respectively. The CAG intended this document to provide a crude estimate of the magnitude of the potential Diesel exhaust problem and would rely on the results from ongoing health effects research before an improved analysis could be made.

Both the EPA-CAG and DOE-Lovelace assessments assumed that Diesel emissions were at least as potent but not significantly more potent compared to coke oven emissions. Allowing for the different population exposure estimates, the two assessments agree reasonably well.

EPA has been conducting a massive Diesel health effects research program to determine the carcinogenic potency of the particle-bound organics from Diesel emissions as well as the potency of particle-bound organics from other environmental emissions (including coke oven emissions) for which human epidemiological data are available. The mobile source samples selected for this program were collected from a heavy-duty Diesel engine, a series of light-duty Diesel passenger cars and a gasoline catalyst-equipped automobile. The comparative source samples include cigarette smoke condensate, coke oven emissions, roofing tar emissions and benzo(a)pyrene. Further information on the mobile source and comparative source samples can be found in section IV. The latest comparative potency rankings are presented in Table 3. This information, together with the available epidemiological data for the comparative sources, will be used to assess the human health risk associated with increased use of the Diesel engine. The major assumption to be used in formulating the revised risk assessment is that the relative carcinogenic potencies of Diesel engine emissions and the related environmental emissions are preserved across human and non-human biological systems so that the available data can be used for human exposures.

Todd Thorslund of EPA's Carcinogen Assessment Group has used some of the potency data to formulate highly tentative first cut estimates of the population risk parameters (48). The SENCAR mouse skin tumorigenesis data were used to estimate relative potencies of the Diesel samples and coke oven emissions. These data indicate that Diesel particulate extract has a potency roughly 10% of that of coke oven emissions; the 1979 EPA preliminary risk assessment assumed the two substances to have equal potency. The unit risk estimate developed for coke oven emissions, together with the relative potency data were used to estimate a unit risk for Diesel emissions. The unit risk estimate for individuals living in cities of 1 million or more is estimated to be 2.5×10^{-5}. Applying this unit risk estimate with rough population exposure estimates of Diesel particulate and particle-bound organics (supplied by S. Blacker, EPA, OMSAPC) Thorslund estimates about 19 respiratory cancer deaths/year in the U.S. population will be attributable to Diesel exhaust. This is based on uncontrolled Diesel automobiles (i.e. 1.0 gm/mile particulate, 15% organics on the

particulate by weight) comprising approximately 15% of the automobile fleet. This estimate is probably far below the ability of an epidemiological study to detect.

It should be noted that this latest risk estimate of Todd Thorslund's is highly tentative and only uses the results of one test, skin tumorigenesis initiation as measured by papillomas, to estimate relative potencies. A point to consider is that skin tumorigenesis may respond more strongly to the types of mutagens present in coke oven emissions than the types of mutagens present in Diesel particulate extract. The revised risk assessment is expected to incorporate the results of a variety of mutagenesis and carcinogenesis tests for estimation of relative potencies.

References

1. "Precautionary Notice on Laboratory Handling of Exhaust Products from Diesel Engines", 4 November 1977.

2. "Health Effects of Exposure to Diesel Exhaust", the report of the Health Effects Panel of the Diesel Impacts Study Committee, National Research Council, National Academy of Sciences, 1981.

3. "The Diesel Emissions Research Program", EPA-625/9-79-004, December, 1979.

4. "Health Effects Associated with Diesel Exhaust Emissions - Literature Review and Evaluation", EPA-600/1-78-063, November, 1978.

5. "Health Effects of Diesel Engine Emissions: Proceedings of an International Symposium - Volume 1", EPA-600/9-80-057a, November, 1980.

6. "Health Effects of Diesel Engine Emissions: Proceedings of an International Symposium - Volume 2", EPA-600/9-80-057b, November, 1980.

7. B.N. Ames, J. McCann, and E. Yamasaki, "Methods for Detecting Carcinogens and Mutagens with the Salmonella/Mammalian-Microsome Mutagenicity Test", Mutation Research, 31:347-364, 1975.

8. Larry D. Claxton, "Mutagenic and Carcinogenic Potency of Diesel and Related Environmental Emissions: Salmonella Bioassay", In: "Health Effects of Diesel Engine Emissions: Proceedings of an International Symposium - Volume 2", 1980.

9. Ann D. Mitchell, et al., "Mutagenic and Carcinogenic Potency of Extracts of Diesel and Related Environmental Emissions: In Vitro Mutagenesis and DNA Damage", In: "Health Effects of Diesel Engine

Emissions: Proceedings of an International Symposium - Volume 2", 1980.

10. R.D. Curren, et al., "Mutagenic and Carcinogenic Potency of Extracts from Diesel Related Environmental Emissions: Simultaneous Morphological Transformation and Mutagenesis in Balb/c 3T3 Cells", In: "Health Effects of Diesel Engine Emissions: Proceedings of an International Symposium - Volume 2", 1980.

11. Ronald L. Schuler and Richard W. Niemeier, "A Study of Diesel Emissions on Drosophila", In: "Health Effects of Diesel Engine Emissions: Proceedings of an International Symposium - Volume 2", 1980.

12. J. Lewtas Huisingh, "Short-term Carcinogenesis and Mutagenesis Bioassays of Unregulated Automotive Emissions", Bulletin of the New York Academy of Medicine, In press, 1981.

13. M.A. Pereira, et al., "In Vivo Detection of Mutagenic Effects of Diesel Exhaust by Short-Term Mammalian Bioassays", In: "Health Effects of Diesel Engine Emissions: Proceedings of an International Symposium - Volume 2", 1980.

14. R.R. Guerrero, et al., "Sister Chromatid Exchange Analysis of Syrian Hamster Lung Cells Treated In Vivo with Diesel Exhaust Particulates", In: "Health Effects of Diesel Engine Emissions: Proceedings of an International Symposium - Volume 2", 1980.

15. EPA, Health Effects Research Laboratory - Cincinnati Quarterly Report, July-September, 1980.

16. William E. Pepelko, EPA, Letter to Gerald Rausa on Sister Chromatid Exchange, 10 March, 1981.

17. L.B. Russell, et al., "Evaluation of Mutagenic Effects of Diesel Emissions I. Tests for Heritable and Germ-Cell Effects in the Mouse", 24 December, 1980.

18. J. Justin McCormick, et al., "Studies on the Effects of Diesel Particulate on Normal and Xeroderma Pigmentosum Cells", presented at EPA's Second Symposium on Application of Short-Term Bioassays in the Analysis of Complex Environmental Mixtures, Williamsburg, Virginia, 4-7 March, 1980.

19. Bruce C. Casto, et al., "Mutagenic and Carcinogenic Potency of Extracts of Diesel and Related Environmental Emissions: In Vitro Mutagenesis and Oncogenic Transformation", In: "Health Effects of Diesel Engine Emissions: Proceedings of an International Symposium - Volume 2", 1980.

20. John G. Orthoefer, et al., "Carcinogenicity of Diesel Exhaust as Tested in Strain 'A' Mice", In: "Health Effects of Diesel Engine Emissions: Proceedings of an International Symposium - Volume 2", 1980.

21. Robert W. Dickinson, EPA, "Trip to Illinois Institute of Technology (IIT) Research Institute in Chicago", OMSAPC memo, 11 December, 1980.

22. Alan M. Shefner, et al., "Carcinogenicity of Diesel Exhaust Particles by Intratracheal Instillation - Dose Range Study", In: "Health Effects of Diesel Engine Emissions: Proceedings of an International Symposium - Volume 2", 1980.

23. Bobby R. Collins, "Respiratory Carcinogenicity of Diesel Fuel Emissions", IITRI Quarterly Report - March 1, 1981 through May 30, 1981 (dated June, 1981).

24. Stephen Nesnow, et al., "Tumorigenesis of Diesel Exhaust, Gasoline Exhaust, and Related Emission Extracts on Sencar Mouse Skin", EPA-HERL Report, 1981.

25. Stephen Nesnow, "Report on Skin Tumorigenesis Studies of Diesel Emissions and Related Samples on C57 Black Mice", EPA-HERL Report, December, 1980.

26. Stephen Nesnow, "Report on Skin Tumorigenesis Studies of Volkswagen Turbo Rabbit and Home Heater Samples", EPA-HERL Report, December, 1980.

27. U. Heinrich, et al., "Long Term Diesel Exhaust Inhalation Studies with Hamsters", In: "Health Effects of Diesel Engine Emissions: Proceedings of an International Symposium - Volume 2", 1980.

28. J.S. Siak, et al., "Diesel Particulate Extracts in Bacterial Test Systems", In: "Health Effects of Diesel Engine Emissions: Proceedings of an International Symposium - Volume 1", 1980.

29. Leon C. King, et al., "Evaluation of the Release of Mutagens from Diesel Particles in the Presence of Physiological Fluids", Environmental Mutagenesis, in press, 1981.

30. M.A. Pereira, et al., "The Effect of Diesel Exhaust on Spermshape Abnormalities in Mice", In: "Health Effects of Diesel Engine Emissions: Proceedings of an International Symposium - Volume 2", 1980.

31. Kenneth B. Gross, "Pulmonary Function Testing of Animals Chronically Exposed to Diluted Diesel Exhaust", In: "Health Effects of Diesel Engine Emissions: Proceedings of an International Symposium - Volume 2", 1980.

32. K.I. Campbell, et al., "Enhanced Susceptibility to Infection in Mice After Exposure to Dilute Exhaust from Light Duty Diesel Engines", In: "Health Effects of Diesel Engine Emissions: Proceedings of an International Symposium - Volume 2", 1980.

33. William E. Pepelko, et al., "Pulmonary Function Evaluation of Cats After One Year of Exposure to Diesel Exhaust", In: "Health Effects of Diesel Engine Emissions: Proceedings of an International Symposium - Volume 2", 1980.

34. T.L. Chan, et al., "Deposition and Clearance of Inhaled Diesel Particles in the Respiratory Tract", presented at the Society of Toxicology Poster Session, 3 March, 1981.

35. Karl J. Springer, "Characterization of Sulfates, Odor, Smoke, POM and Particulates from Light and Heavy Duty Engines - Part IX", EPA-460/3-79-007, June, 1979.

36. Penny Carey and Janet Cohen, "Comparison of Gas Phase Hydrocarbon Emissions From Light-Duty Gasoline Vehicles and Light-Duty Vehicles Equipped with Diesel Engines", EPA-OMSAPC Report, EPA/AA/CTAB/PA/80-5, September, 1980.

37. Frank Black and Larry High, "Methodology for Determining Particulate and Gaseous Diesel Hydrocarbon Emissions", Society of Automotive Engineers Paper 790422, 1979.

38. Dennis Schuetzle, et al., "The Identification of Polynuclear Aromatic Hydrocarbon (PAH) Derivatives in Mutagenic Fractions of Diesel Particulate Extracts", Intern. J. Environ. Anal. Chem., 9:93-144, 1981.

39. T.L. Gibson, et al., "Determination of Nitro Derivatives of PNA in Diesel Automobile Exhaust", presented at the CRC Chemical

Characterization of Diesel Exhaust Emissions Workshop, Dearborn, Michigan, 2-4 March, 1981.

40. Discussion by Dr. Ron Bradow, EPA during the Workshop on the Evaluation of Research in Support of the Carcinogenic Risk Assessment for Diesel Engine Exhaust, Research Triangle Park, NC, 24-25 February, 1981.

41. R.E. Waller, "Trends in Lung Cancer in London in Relation to Exposure to Diesel Fumes", In: "Health Effects of Diesel Engine Emissions: Proceedings of an International Symposium - Volume 2", 1980.

42. Todd Thorslund, "Answer to the posed question: '"Are the results obtained in the London Transit Worker Study sufficient to dismiss any concern regarding the potential cancer hazard for the U.S. population in the future, due to Diesel engine exhaust?'", EPA-ORD memo, 29 January, 1981.

43. Jeffrey E. Harris, "Potential Risk of Lung Cancer from Diesel Engine Emissions", National Academy Press, Washington, D.C., 1981.

44. "Health Effects of Exposure to Diesel Exhaust", National Academy of Sciences, 1980, p. 155.

45. Marc B. Schenker and Frank E. Speizer, "A Retrospective Cohort Study of Diesel Exhaust Exposure in Railroad Workers: Study Design and Methodologic Issues", In: "Health Effects of Diesel Engine Emissions: Proceedings of an International Symposium - Volume 2", 1980.

46. R.G. Cuddihy, et al., "Potential Health and Environmental Effects of Diesel Light Duty Vehicles", Lovelace Inhalation Toxicology Research Institute, October, 1980.

47. Roy E. Albert, "Carcinogen Assessment Group's Initial Review on Potential Carcinogenic Impact of Diesel Engine Exhaust", EPA Report, 11 June, 1979.

48. Todd W. Thorslund, "A Suggested Approach for the Calculation of the Respiratory Cancer Risk Due to Diesel Engine Exhaust", EPA-ORD Report, February, 1981.

TABLE 1. AMES TEST SPECIFIC ACTIVITIES AT 100 ug OF ORGANIC MATERIAL

Sample	TA98 +S9	TA98 -S9	TA100 +S9	TA100 -S9
		Diesel		
Caterpillar	59.3	65.9	115.2	167.8
Nissan	1367.1	1225.2	881.7	1270.1
Oldsmobile	318.7	614.8	169.9	247.5
VW	297.5	399.2	426.0	641.6
		Gasoline		
Mustang	341.9	137.8	228.0	196.5
		Comparative Samples		
Cigarette	98.2	Neg	?	Neg
Coke Oven	251.6	164.1	265.6	259.4
Roofing Tar	98.7	Neg	420.0	Neg
		Control Compound		
B(a)P	15202.3*	NT	26438.0*	NT

*Extrapolation
NT = Not tested
Neg = Negative.

TABLE 2. RELATIVE POTENCY OF ORGANIC MATERIAL BASED ON AMES TEST RESULTS

Sample	TA98 +S9	TA98 -S9	TA100 +S9	TA100 -S9
		Diesel		
Caterpillar	4.3	5.4	13.0	13.2
Nissan	100.0	100.0	100.0	100.0
Oldsmobile	22.3	50.2	19.3	19.5
VW	21.8	32.6	48.3	50.6
		Gasoline		
Mustang	25.0	11.3	25.9	15.5
		Comparative Samples		
Cigarette	7.2	Neg	?	Neg
Coke Oven	18.4	13.4	30.2	20.4
Roofing Tar	7.2	Neg	47.6	Neg
		Control Compound		
B(a)P	1112.1	NT	2997.5*	NT

*Extrapolation.
NT = Not tested
Neg = Negative

Table 3

COMPARATIVE POTENCY RANKINGS

	MUTAGENESIS				CARCINOGENESIS		
	AMES[a]	SCE[a]	L-5178Y[a]	BALB[a]	VIRAL ENHANCEMENT	BALB[a]	TUMOR INITATION[b]
DIESEL: CAT	4.3	0	1[c]	0	0	0	0
NISSAN	100	100	100	100	100	100	100
OLDS	23	0	64	750	25	0	28
VW RAB	22	50	50	NT[d]	50	NT	6
GASOLINE:							
MUSTANG	25	1	36	750	50	200	16
COMPARATIVE SOURCES							
CIGARETTE	7	0	21	300	200	200	0
COKE	18	44	339	15	800	500	355
ROOF TAR	7	291	850	750	2016	500	120
HOME HEATER							7
STANDARDS:							
B(a)P	1112	1750	189	25000	52000	16700	16500

[a]In the presence of an Aroclor-1254 induced rat hepatic S-9

[b]Mouse skin tumor initiation in male and female sencar mice after 26 weeks of treatment

[c]Testing incomplete at this time.

[d]Not tested.

Table 4

Exhaust Emissions From Diesel- and Gasoline-Powered Light-Duty Vehicles and Heavy-Duty Engines

	Emission Rate, g/km				Emission Rate, mg/km		Fuel Econ.
	HC	CO	NOx	Part.	Sulfates	Total Aldehydes	mpg
Light-duty Vehicles[a]							
Oldsmobile Cutlass							
350 Diesel	0.47	1.24	0.70	0.573	9.962	82.5	21.7
260 Gasoline	0.24	1.34	0.85	0.006	1.373	14.0	15.6
Volkswagen Rabbit							
Diesel	0.23	0.49	0.54	0.182	3.662	39.6	42.7
Gasoline	0.14	2.30	0.63	0.004	0.041	37.8	24.6

	Emission Rate, g/hp-hr				Emission Rate, mg/hp-hr		BSFC
	HC	CO	NOx	Part.	Sulfates	Total Aldehydes	lbs/bhp-hr
Heavy-duty Engines							
Diesel[b]							
Mack ETAY(B)673A	0.476	1.588	6.613	0.612	33.467	64.29	0.399
Caterpillar 3208/EGR	1.163	6.200	3.747	2.208	16.725	161.34	0.472
Gasoline[c]							
Chevrolet 366	2.49	55.00	3.39	0.207	1.033	1190.12	0.761

(a) 1975 FTP cycle used for all light-duty vehicles
(b) 13-mode FTP cycle used for the Diesel-powered heavy-duty engines
(c) 23-mode FTP cycle used for the gasoline-powered heavy-duty engine

Table 5

Particulate Emission Rates

Sample	Particulate Emission Rate (g/km)	Extractable Matter (%)	B(a)P (ng/mg extract)	B(a)P (ng/mg part)
Diesel:				
Caterpillar	0.72*	27	2	0.5
Nissan	0.205	8	1173	96.2
Olds	0.32	17	2	0.4
VW Rabbit	0.11	18	26	4.6
Gasoline:				
Mustang	0.003	43	103	44.1
Comparative sources:				
Cigarette		--	<1	--
Coke		5-10	478	31.5
Roofing tar		>99	889	889

* g/hp-hr. The Caterpillar is a heavy-duty engine.