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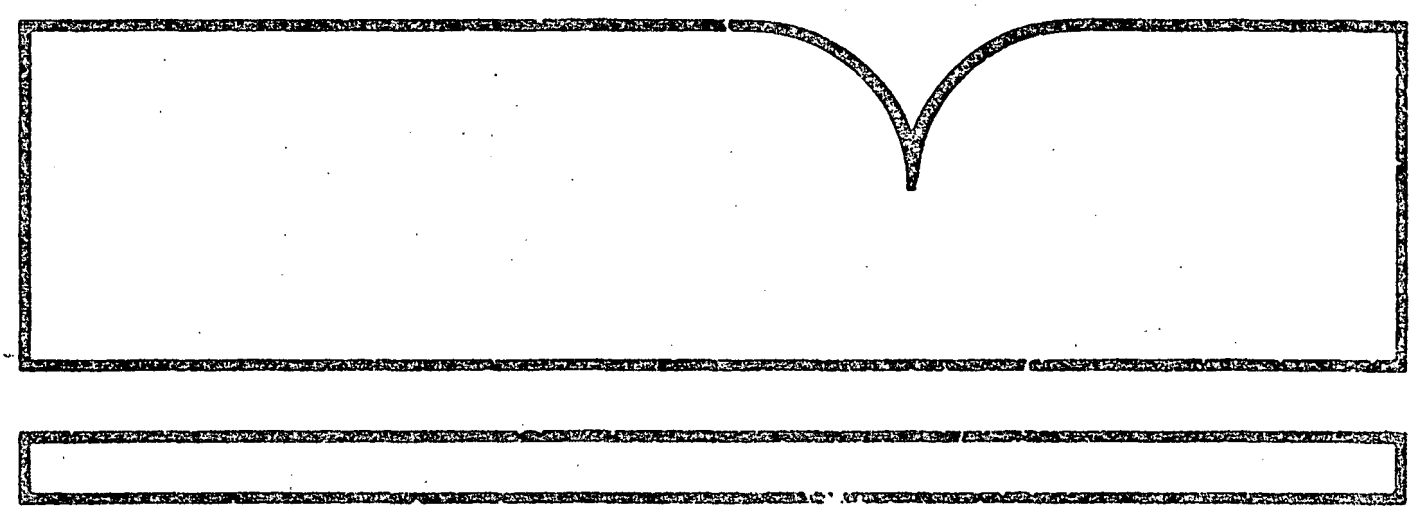

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16. ABSTRACT

Due to the growing numbers of diesel passenger autobiles in the United States, there has been an expanded effort to understand the health effects of airborne pollutants arising from increased automotive emissions. Bacterial mutagenicity testing has played an important role in the characterization of genotoxic effects and components arising from these combustion products. This review examines published material concerning the bacterial mutagenicity of automotive emissions. In addition, the paper explores factors that modify the mutagenicity of mobile-source emissions, the use of bacterial tests for the comparison of various mobile source emissions, and the use of bacterial tests to examine the phenomena of mammalian uptake and metabolism.

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Characterization of Automotive Emissions by Bacterial Mutagenesis Bioassay: A Review

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Due to the growing numbers of diesel passenger automobiles in the United States, there has been an expanded effort to understand the health effects of airborne pollutants arising from increased automotive emissions. Bacterial mutagenicity testing has played an important role in the characterization of genotoxic effects and components arising from these combustion products. This review examines published material concerning the bacterial mutagenicity of automotive emissions. In addition, the paper explores factors that modify the mutagenicity of mobile-source emissions, the use of bacterial tests for the comparison of various mobile source emissions, and the use of bacterial tests to examine the phenomena of mammalian uptake and metabolism.

Key words: diesel, gasoline, Ames test, Salmonella, fuel, combustion

INTRODUCTION

The United States has approximately 130 million passenger cars and light-duty trucks, nearly one light-duty vehicle registered for each adult. The sales and servicing of automobiles and trucks account for about 25% of the US retail market [Gray and von Hippel, 1981]. In 1980 this fleet of vehicles consumed approximately 2 billion barrels of oil. Since engineering tests have shown a 25% or greater improvement in fuel economy in light-duty vehicles equipped with diesel engines versus those equipped with gasoline engines, diesel vehicle sales are expected to increase from 4% (1980) to 15% (1985) of the new car market. This "dieselization" has sparked new interest in the health effects of mobile-source emissions. Although earlier work [Kotin et al. 195-, 1955] had demonstrated that diesel and gasoline emissions have potential carcinogenic activity, it was not until 1978 that industry and government expanded their efforts toward understanding whether or not mobile-source emissions could

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show any potential genotoxic health effects. This issue received priority after Huisingh et al [1978] and a cautionary notice for laboratory workers issued by the US Environmental Protection Agency (EPA) [Gage, 1977] reported that organic fractions from the exhaust particles of diesel vehicles were mutagenic in the Ames Salmonella typhimurium plate incorporation assay [Ames et al, 1975]. The purpose of this review is to recognize and document the role that bacterial mutation tests have played in characterizing mobile source emissions for genotoxic activity. A summary of this review was given at the EPA 1981 Diesel Emissions Symposium held October 5-7, 1981, in Raleigh, NC [Claxton, 1981b].

Although the original report by Huisingh et al [1978] was quite extensive—examining, for example, chemical and physical properties of fractions from exhaust organics, multiple vehicles, and multiple fuels—many questions remained unanswered. Researchers in government and industry, in the service of the public interest, have used bacterial mutagenicity tests to answer several critical questions concerning sample generation, collection, extraction, fractionation, bioassay, statistical significance, and relevance. For example, Wei et al [1980] postulated that ". . . controversies on the biological hazards of diesel emissions will remain unresolved until more is known about the chemical identities of the direct-acting mutagens." Fractionation directed by bacterial bioassay results enables investigators to follow the distribution of genotoxic activity among different chemical classes before compound identification is complete. In addition, microbial tests allow comparative measurements of genotoxic activity from roadside-exhaust, smog-chamber, and dilution-tunnel samples. The purpose of this paper, therefore, is to recognize and document the role that bacterial mutation tests have played in characterizing mobile-source emissions for genotoxic activity. The bacterial mutagenicity of mobile-source emissions is considered according to the following schema: (1) generalized observations; (2) generation of emissions; (3) collection of emissions samples; (4) extraction of particulate samples; (5) fractionation and identification of individual chemical components; (6) applicability and relevance of bacterial bioassays; and (7) data transformation and statistical analysis of research data.

GENERALIZED OBSERVATIONS

Huisingh et al [1978] and most other investigators have used the Salmonella typhimurium plate incorporation assay [Ames et al, 1975] as the primary test protocol. Although some investigators employ all five tester strains recommended by Ames for general screening, many investigators work exclusively with strains TA98 and TA100, primarily for two reasons: First, the sample amounts available have been relatively limited; and second, TA98 and TA100 have been the strains most responsive to the soluble organic fraction (SOF) extracted from mobile-source emission particles. Total extracts give negative results in tests with TA1535, which responds to base pair-substitution mutagens; however, since bioassay-directed chemical fractionation studies have not generally used this strain, any fractions containing mutagens that cause base pair substitution may be overlooked. Strain TA1538 provides even more interesting observations. TA1538 exhibits an increased response upon the addition of an Aroclor-induced liver homogenate, whereas TA98 exhibits the same or a decreased response under exogenous activation. The different responses of TA1538 and TA98 suggest either that (1) TA1538 (although not providing as many revertants per plate) distinguishes the presence of indirect-acting mutagens not readily detected by TA98;

or (2) TA98 detects frameshift promutagens that are S9-deactivated, which TA1538 is not capable of detecting. Researchers may thus be underestimating the importance of indirect-acting frameshift mutagens within mobile emissions.

Using the knowledge of mutagens available at that time, Wei et al [1980] surmised that nitro-substituted polycyclic aromatic hydrocarbons (PAH) were the probable mutagens associated with diesel exhaust emissions. Nitro derivatives of some PAHs have been identified in ambient air samples [Jäger, 1978; Wang et al, 1980; Talcott and Harger, 1981; Pitts et al, 1982b]. Rosenkranz et al [1980] and Mermelstein et al [1981] characterized nitro reductase-deficient bacterial strains that allowed for the initial recognition of nitroarenes in mobile emissions. Nitroarenes were identified in diesel exhaust through the use of these strains by Claxton [1981a], Claxton and Kohan [1981], and Löfroth [1981].

The 8-azaguanine mutation system has also shown positive results with various mobile source emissions. It can be used to give a more quantitative approach and identify a wider spectrum of compounds [Claxton and Kohan, 1981; Liber et al, 1980; Barfknecht et al, 1981b]. However, this system has not been generally employed.

The widely used liver homogenate systems generally reduce the mutagenic response of diesel organics (excepting TA1538). However, exogenous activation in gasoline exhaust organics produces an enhanced mutagenic response, a fact that has received little emphasis in the literature. Thus, diesel and gasoline vehicles are demonstrated to emit different mutagenic compounds.

FACTORS THAT MODIFY THE GENERATION OF MUTAGENS WITHIN MOBILE SOURCE EMISSIONS

In the generation of emissions from a combustion system, there are five fundamental components to consider: the fuel, the fuel's oxidant, the fuel's diluents, the type and degree of combustion, and the atmospheric and environmental conditions.

Fuels

Fuels could influence the mutagenicity of exhaust organics by either the direct contribution of mutagens or by supplying the precursors for mutagens created during the combustion process. The diesel fuel used by Huisingh et al [1978] was negative when tested directly in the Salmonella bioassay. Lebowitz et al [1979] also reported that diesel fuel was negative. The diesel fuel JP-4 and two types of gasoline were reported negative by Wang et al [1978a,b] when tested with TA98. Various crude oils and some of their distillates, however, were observed as positive in the Ames test [Brusick and Matheson, 1978a,b]. Positive results, for example, were reported for some natural, syncrude, and shale oil crudes (and some of their distillates) by Calkins et al [1980] and Calkins and Krahn [1979]. In each case, however, the naphtha distillate was negative. Epler et al [1978b] and Guerin et al [1980] demonstrated that coal-derived petroleum substitutes could provide a tenfold increase in bacterial mutagenicity over a similar natural product. They also reported that the petroleum crude activity was found primarily in the neutral fraction, while significant activity was found in both the neutral and basic fractions of derived fuels. For the neutral fraction, Guerin et al [1980] demonstrated that aromatic amines were the predominant mutagenic constituent.

In contrast to the above studies, when Henderson et al [1981] separated diesel fuel into an aromatic and an aliphatic fraction, they found both fractions mutagenic

using strain TA100. These varying reports may indicate vast differences between sources of diesel fuel and bioassay techniques employed. A further possible variable is the presence of minor components not detected unless fractionated components of the fuel are used for testing. In addition, Henderson et al [1982] noted that exposure to nitrogen dioxide (NO_2) dramatically increased the response of both fractions. This finding is supported by the work of Pitts [1979], who exposed an indirect-acting mutagen, benzo(a)pyrene, to NO_2 and generated a direct-acting derivative. Such a correlation was not unexpected, since some nitrous acid also could have been present during these exposure conditions. The precursor effect of the fuel appears to have been demonstrated by Huisingh et al [1978]. Upon testing the effects of seven different fuels in two different vehicles, they found a wide range of mutagenic activity in the emission organics. The results of McClellan [1980b] are similar, also suggesting that fuels high in aromatic content produce a more notable mutagenic response. Although results support the hypothesis that fuels mainly supply precursor material for the mutagens in exhaust emissions, more research would be needed to rule out any major concentrating effect.

The Fuel's Oxidant and Diluent

The oxidant for both spark-ignited (gasoline) engines and compression-ignited (diesel) engines is, of course, oxygen. Nitrogen, which composes approximately 78% of the atmosphere, is the most common diluent. Water vapor, other inert gases, and some inorganic ash are the other diluents present. At high temperature, some inert nitrogen enters into the combustion reaction, and nitrogen oxides are produced. In addition, excess oxygen, lubricating oils, and/or fuel behave as diluents. (For an introduction to combustion and emission chemistry, see the text by Edwards [1977].) As will be shown in a later section, nitrated and oxygenated components of incomplete combustion contribute to the mutagenicity of emission products. Crankcase oils have also been investigated for possible mutagenic activity. Wang et al [1978a], Hermann et al [1980], and Löfroth [1981] each reported that unused crankcase oils are nonmutagenic, but that used crankcase oils from gasoline engines give a positive response. In addition, Löfroth [1981] stated that (1) metabolic activation increased any mutagenic response seen; (2) the response increased with vehicle mileage; and (3) this positive response was not seen with used oil recovered from a diesel engine.

Type and Degree of Combustion

Within mobile sources, the type of combustion depends upon the type of power source that is used. In the United States, the most common power train for light-duty vehicles is the typical spark-ignited gasoline engine. Diesels, which are reciprocating-compression ignition engines, are most often the power source for heavy-duty trucks, buses, locomotives, and vessels. Other engines that have shown some utility or are undergoing further research are: gas turbine and Wankel internal-combustion, Rankins and Stirling cycle external-combustion, and electric.

The effect of type and degree of combustion on mutagenicity is examined by comparing the results from different power sources and/or vehicles. Although a few authors did not describe the engine or vehicle used in their research, most gave at least a limited description. Huisingh et al [1978] employed two heavy-duty engines and three light-duty engines. Although direct comparison of differing sources was not the primary purpose of that research, it provided a mobile source comparison

based on bacterial mutagenicity. Particle exhaust organics from heavy-duty engines were tested in TA98, TA1535, TA100, TA1537, and TA1538, both with and without exogenous activation. Both engines showed very similar qualitative results, with the four positive strains having decreasing activity in the order TA100 > TA98 > TA1538 > TA1537. Without activation, TA1535 was negative with samples from both engines; however, with activation, one engine (Caterpillar 3208, four-stroke V-8) produced a marginally positive response. Given the sample amounts available, exhaust organics from the three light-duty engines were tested using only strain TA1538. In this study, in which fuel comparison was a primary component, results for even a single vehicle (using different fuels) could vary greater than 100 times. Qualitatively, the results from other studies [Claxton, 1980, 1981a; Claxton and Kohan, 1981; Löfroth, 1981; Dukovitch et al, 1981; Dietzman et al, 1981] are in agreement with the report of Huisingh et al [1978].

The results of Claxton and Kohan [1981] demonstrating the effects of three different sample parameters are given in Table I. The three comparisons were made between (1) different runs with the same diesel engine; (2) gasoline vehicles of the same make, model, and configuration; and (3) different makes of diesel vehicle. The coefficients of variation for the revertants per mile for these three cases were 0.11, 0.49, and 0.59, respectively. Assuming normal distribution and that the coefficient of variation was, in this case, a good estimation of the true standard deviation, one can estimate confidence limits in all three cases. For the above three cases, a value could fall within 99% confidence limit values and vary by 33%, 147%, and 177%, respectively. If multiple testing facilities, fuels, and bioassay laboratories were used, the variation between results would be expected to increase. Because the Ames assay is a semiquantitative test for screening substances over a dynamic range of $\sim 10^6$ in a dose-response slope and because other parameters (such as percent of the particles extractable) show broad variation, the variation encountered here for a complex

TABLE I. Comparison of Summary Data Demonstrating the Effect of Differing Sampling Parameters

	Slope[a] rev/plate/μg	% Extractable	Rev × 10^5 g particle	PER[b] g/mi	Rev × 10^5/mi
Different runs within same automobile (diesel)					
Mean	3.68	11.8	4.35	0.524	2.27
Standard deviation	0.42	1.0	0.64	0.037	0.26
Coefficient of variation	0.11	0.09	0.15	0.07	0.11
Vehicles of same make, model, and configuration (gasoline)					
Mean	7.03	7.52	3.16	0.0102	0.032
Standard deviation	3.51	7.83	0.87	0.0048	0.016
Coefficient of variation	0.50	1.04	0.28	0.47	0.49
Different diesel vehicles					
Mean	1.98	36.6	6.96	0.687	4.38
Standard deviation	0.80	18.0	4.06	0.256	2.59
Coefficient of variation	0.41	0.49	0.58	0.37	0.59

[a]Slope of linear regression line.
[b]Particle emission rate.

testing situation should not be considered excessive. Together, these studies indicate the degree to which quantitative comparisons can be made within a single study and show that cautious qualitative comparisons can be made using results from multiple studies.

Ambient Environmental Conditions

Ambient conditions are known to affect the condensation of organic compounds onto particles, influence the interaction of organic compounds, alter the organic species emitted by a source, and provide the conditions for various other interactions [Pitts et al, 1982a]. Dilution-tunnel experiments examining the effect of crankcase oil temperatures upon test results were reported by Braddock [1981]. After the vehicles were maintained overnight at various ambient temperatures (ranging from 23°F to 82°F), the vehicles were tested at ambient temperatures. For the soluble organic fraction, a mild correlation between mutagenic activity and conditioning temperature was noted; however, this correlation did not exist for comparisons on a revertant per mile basis. Only a few investigators have explored ambient effects. Claxton and Barnes [1981] used the Calspan smog chamber to examine a variety of ambient factors. They found that the presence of ozone in the chamber tended to reduce the mutagenic response to the organic material collected. Those results also showed that ambient like irradiation without other mitigating factors such as ozone did not alter the mutagenic response.

Ohnishi et al [1980] examined road side particles collected in a highway tunnel. They found a 60- to 88-revertants/m^3 response for particles collected during daytime hours and tested with TA100 in the presence of an activating system. In the same study, particles collected at night with a high density of diesel traffic exhibited 121 to 238 revertants/m^3. Alfheim and Moller [1981] found that the contribution of traffic to the mutagenicity of air samplers is significant by comparing samples from a roadside site, on a roof, and at a park. In an Allegheny tunnel study conducted by Pierson et al [1982], the diesel aerosol organics were similar in activity to organics recovered in dilution tunnel studies. Furthermore, it was shown that the mutagenicity of diesel engine exhaust is several times that of gasoline engine exhaust when expressed as revertants per mile. Studies such as these demonstrate that the production, chemical alteration, distribution, and concentration of mutagenic mobile source particles are dependent upon traffic patterns, amounts of reactive gases and vapors, level of ozone present, meteorological conditions, and the presence or absence of other ambient air particles.

Effect of Sample Collection Upon the Mutagenicity of Mobile Source Emissions

The influence of particle collection methods upon the chemical composition and biological activity of diesel-particle extracts was investigated by Chan et al [1981]. They noted that filter sampling allowed potential chemical conversion of organic compounds by the nitrogen oxides in the exhaust gases, whereas electrostatic precipitation (ESP) collection methods provided for ozone generation and interaction at the time of collection. In their experimental results, they found 11% extractable organics for the ESP sample and 6.2% for the filter collected sample. The chemical profiles for the two collection methods were similar except that the ESP sample contained greater amounts of an acid salt fraction. Although the overall biological activity of the ESP and filter samples was comparable, subtle but consistent differences sug-

gested that different direct-acting mutagens could be found in the two sample types. As seen from the studies of Chan et al [1981], the most serious obstacle in collection methodology is the generation of artifacts, ie, the generation of substances that do not exist in the natural situation or the elimination of substances that would normally exist. A number of investigators [Claxton, 1980; Claxton and Kohan, 1981; Löfroth, 1981; Gibson et al, 1980; Pederson and Siak, 1980, 1981a,b] have demonstrated that mutagenic nitroarenes are contained in organic extracts of filter-collected particles. However, since diesel and gasoline also emit varying levels of nitrogen oxides that pass across the filters and collected particles, these nitroarenes may be artifacts. They may be produced under three possible circumstances: (1) the combustion process; (2) the exhaust process, as organics interact and condense upon the particles; or (3) the collection process as an artifact. The passage of nitrogen oxides across a PAH compound upon a filter can generate a nitroarene [nitrogen dioxide (NO_2)-PAH] that is direct-acting in the Ames bacterial assay [Pitts, 1979].

Henderson et al [1981] generated direct-acting mutagens for strain TA100 by exposing 1-g samples of fuel aromatics and fuel aliphatics to excess NO_2 at 25°C. The aromatic NO_2 fraction was the most active and nitro-PAH compounds were identified in this fraction. In some preliminary experiments, Bradow [1980] and Claxton [1980] reported passing artificial gas streams containing high levels of NO_2 across filters with diesel particles and observing increased mutagenic activity of the extracted organics. Gibson et al [1980] reexposed filter-collected diesel particles to the gas-phase portion of similar diesel emissions and found increased levels of 1-nitropyrene, nitrobenzo(a)pyrene, and mutagenic activity. Although the issue of the extent and relevance of artifacts has not been fully resolved, bacterial testing has paved the way in identifying and providing methods for examining the problems.

Since sample collection must occur during some type of test cycle (running test modes of the vehicle or engine), the test cycle may affect the generation, transformation, condensation, and collection of emitted particles and organics. Only a few researchers have published any direct comparison of test cycles. When reporting data as revertants per microgram of organic material, Gabele et al [1981] found no great differences between six different test cycles. Gibbs et al [1980] examined five different cycles with six different automobiles. When expressing the data as revertants per gram of particulates, they found "widely divergent" results; however, when expressing the data as revertants per mile, "cycle-to-cycle" trends were more pronounced and reproducible. For cycles ranked by revertants per mile, activity decreased in the order Federal Testing Procedure (FTP) > Congested Freeway Driving Schedule (CFDS) > Highway Fuel Economy Test (HFET) > 50-Mile-an-Hour Cruise Procedure (50C), and a general reduction in revertants per mile was found as the mileage of the vehicle increased. Upon close examination of the data of Gibbs et al [1980], it was noted that very low-mileage cars (< 4,000 miles) demonstrated a greatly enhanced mutagenic response for all cycles except idle. McClellan [1980b] examined four test cycles using a single automobile and reported that the cycles with lower speeds and more stops and starts resulted in higher mutagenic activity.

INTEGRATION OF PHYSIOCHEMICAL INFORMATION AND PROCEDURES WITH BACTERIAL BIOASSAY PROCEDURES

A review of the literature prior to 1979 provided a list of 184 chemicals identified as being in diesel exhaust [Claxton, 1982]. Of these 184 compounds, 44

were listed in published mutagenicity reports and 21 were acceptable positives in one or more mutagenicity assays. Seven of the 184 were reported as carcinogens. Since 1979, more research activity has been devoted to bioassay-directed fractionation than to the pairing of chemical and biological literature reports. For showing the mutagenic response of different chemical fractions from the organic emissions of a diesel engine, an organic extract from emission particles of two heavy-duty engines was initially used [Huisingh et al, 1978]. The two most active fractions, the transitional and the oxygenate, were eluted from a silica gel column after dichloromethane (DCM) extraction from the exhaust particles. Choudhury and Doudney [1981] fractionated organic emission into three primary fractions—acid, basic, and neutral—and subsequently fractionated the neutral fraction into seven subfractions. All three major fractions and five of the seven subfractions showed some type of mutagenic activity. The paraffinic subfraction was negative.

Upon examining emissions from both a diesel and a gasoline vehicle, Löfroth [1981] noted that the aromatic and an oxygenate fraction were the most mutagenic. McClellan's work [1980a], using a Fiat under varying conditions, showed that upon Sephadex fractionation three of five fractions were mutagenic to bacteria. The classes of compounds reported as contributing to the mutagenicity of these fractions were alkyl-substituted PAH compounds and oxygenated PAH. Ohnishi et al [1980] investigated the fractionated emissions of two heavy-duty vehicles and one small diesel and found the fractions to be positive. Rappaport et al [1980] studied 16 liquid chromatography fractions of organic emissions from a Cummins turbodiesel engine and postulated that pyrene-3,4-dicarboxylic acid anhydride and similar compounds accounted for a sizeable portion of the mutagenic activity.

In summarizing their fractionation study with emission organics from a GM 5.7-liter diesel engine, Siak et al [1979] stated that "more than 90% of the biological activity was accounted for in the neutral-nonpolar II, neutral polar, weak and strong acid fractions." Using nitroreductase-deficient strains of the Salmonella tester strains, Claxton [1980a] and Löfroth [1981] demonstrated the presence of nitroarenes in diesel exhaust organics. Pederson and Siak [1981a,b], using normal-phase and reverse-phase thin layer chromatography, showed that monosubstituted nitro-PAH compounds were present in diesel exhaust extracts. A number of investigators have reported the isolation and identification of these potentially mutagenic nitro-PAH compounds. Xu et al [1982b] reported the tentative identification of more than 50 nitro-PAHs in an extract of diesel exhaust particles. Schuetzle et al [1982] reported the analytical methods and identification of specific nitro-PAHs associated with diesel particles. The report of Pitts et al [1982b] provided the quantitative level and bacterial mutagenicity of 3 nitro-PAHs [nitropyrene, 9-nitroanthracene, and 6-nitrobenzo(a)pyrene] and 5H-phenanthro (4,5-a,b,d)pyran-5-one for an exhaust particle extract from a six-cylinder, light-duty diesel engine. Yergey et al [1982] employed a unique and highly controlled model system in attempting to identify mutagens arising from diesel combustion. To simplify the combustion process, they used a single cylinder diesel engine, a defined fuel (1:1 volume ratio of n-tetradecane and 2,2,4 trimethylpentane), a synthetic ashless lubricating oil (polyalkylene glycol), and, for some experiments, an argon/oxygen oxidant system. Their results indicate two useful observations: (1) Nitropyrene was isolated and identified in each of the air oxidant samples and one of the argon/oxygen oxidant samples; and (2) the compounds observed are similar to those identified by investigators using typical diesel fuels. (Note that the nitropyrene

associated with the argon/oxygen sample was observed when the experiment was conducted with a cracked piston ring allowing entry of nitrogen-containing air to the chamber.) These results suggest that the formation of nitroarenes is not dependent upon fuel-bound or lubricant-bound nitrogen and that some of the PAH compounds are products of the combustion process.

At EPA's 1981 Diesel Emissions Symposium, Raleigh, NC, several investigators provided lists of compounds recently identified in diesel exhaust. Since a listing of these results may not be readily available, a compilation of the compounds is given in Table II. Table II also provides a summary of the bacterial mutagenicity associated with the compounds tested and reported. It is interesting to note that in bioassay-directed fractionation, very few investigators used the indicator strains TA1535 and TA1538; therefore, some mutagens that cause base pair substitution and that need activation to be frameshift mutagens could be overlooked. In any event, bioassay-directed fractionation has aided in the identification of several mutagens that previously have not been recognized in mobile source emissions.

USE AND EFFECT OF VARIOUS BIOLOGICAL AND ASSAY PROCEDURES

Since initial testing involved organic chemicals extracted from particles with strong organic solvents, researchers questioned whether chemicals bound to carbonaceous particles would be released into physiological fluids in vivo. McGrath et al [1978], using the Ames bioassay, tested whole particles suspended in dimethylsulfoxide (DMSO) and obtained results ranging from negative to moderately positive. However, DMSO is a moderately effective solvent. Siak et al [1981] reported extracting particles with four simulated biological fluids: fetal calf serum, 0.5% bovine serum albumin, lung surfactant, and saline. The assay of each biological fluid in the Ames test was negative except for a positive response with the fetal calf serum. The fetal calf serum extract provided only about 6% of the response found with extraction by DCM. Brooks et al [1980] found similar results with dog serum, lung lavage fluid, saline, dipalmitoyl lecithin, and albumin. However, they state that "the minimal mutagenic activity . . . may be due to a lack of removal of mutagens from the particles or an inactivation of removed mutagens by binding or some other process." Clark and Vigil [1980] tested a DCM diesel extract under the following conditions: Aroclor 1254-induced rat liver S9, an uninduced S9, an S9 without nicotinamide adenine dinucleotide (NAD), bovine serum albumin, and fetal calf serum. They found a decreased mutagenic response in each case. That result suggests that protein binding of mutagenic components was at least partially responsible for the lack of activity seen with incubated particles. By following the mutagenic activity of the DCM extracts in serum, lung cytosol, protease-treated serum, protease-treated lung cytosol, and extracted particles, King et al [1981] demonstrated the release of mutagens from diesel particles and postulated that the lack of mutagenic response is due to either protein binding or metabolism. Siak and Strom [1981] exposed rats to diesel particles, recovered the lung macrophages, and extracted the macrophages with DCM. They showed that although the particles continued to contain mutagens, "seven days after exposure, DCM extracts of alveolar macrophages had no detectable mutagenic activity, even though more diesel particles were recovered." These effects may be due to either protein binding or metabolism. Wang and Wei [1981] and Wang et al [1981] gave evidence that the antimutagenic effect of S9 is not enzymatic by

TABLE II. Salmonella Mutagenicity Results for Compounds Identified in Diesel Exhaust Emissions and Presented at the EPA's 1981 Diesel Emissions Symposium, Raleigh, NC

No.	Compound	CAS No.	Reference for diesel identification[a]	Bioassay reference[b]	Bioassay result[c]
1.	Acenaphthalene	34493-60-2	Yergey et al [1981] Riley et al [1982]	Kaden et al [1979]	+ (8-Az)
2.	Acenaphthalene, nitro	—	Riley et al [1982] Xu et al [1982a]	—	—
3.	Acenaphthalene, nitromethyl	—	Riley et al [1982]	—	—
4.	Anthracene	102-12-7	Prater and Schuetzle [1982] Yergey et al [1981]	Anderson and Styles [1978]	Neg
				Epler et al [1978a, 1979]	Neg
				Florin et al [1980]	Neg
				Gibson et al [1978]	Neg
				Lavoie et al [1979]	Neg
				Probst and Hill [1980]	Neg
				Salamone et al [1979]	Neg
5.	Anthracene, methyl	—	Prater and Schuetzle [1982]	—	—
	2-methyl	613-12-7	—	Gibson et al [1978]	Neg
	9-methyl	779-02-2	—	Kaden et al [1979]	+ (8-Az)
			—	Epler et al [1978a]	?
			—	Gibson et al [1978]	Neg
			—	Kaden et al [1979]	+ (8-Az)
6.	Anthracene, dimethyl	29063-00-1	Prater and Schuetzle [1982]	Hubbard et al [1981]	+
7.	Anthracene, trimethyl	27358-28-7	Prater and Scheutzle [1982]	—	—
8.	Anthracene, tetramethyl	—	Prater and Schuetzle [1982]	—	—
9.	Anthracene, nitro	—	Riley et al [1982] Xu et al [1982a]	—	—
	9-nitro	602-60-8	—	Ho et al [1981]	+
				Matsushita [1980]	+
				Pederson and Siak [1980, 1981a]	+
				Tokiwa et al [1981]	+
	2-nitro	—	—	Claxton and Kohan [1981]	+
10	Anthracene, nitromethyl	—	Riley et al [1982]	—	—

11.	Anthracene, nitrodimethyl	—	Riley et al [1982]	—	—
12.	Anthracene, carboxyaldehyde	—	Prater and Schuetzle [1982]	—	—
13.	Anthracene, carboxyaldehyde, nitromethyl	—	Riley et al [1982]	—	—
14.	Anthracene, benz(a)	56-55-3	Prater and Schuetzle [1982]	de Flora [1981]	+
				Glatt et al [1981]	+
				Probst et al [1981]	+
15.	Anthracene, dione, benz	—	Prater and Schuetzle [1982]	—	—
16.	9,10-anthraquinone	84-65-1	Erickson et al [1982]	Anderson and Styles [1978]	Neg
				Brown et al [1977]	Neg
				Gibson et al [1978]	Neg
				Kaden et al [1979]	Neg
				Salamone et al [1979]	Neg
17.	Anthraquinone, nitro	—	Xu et al [1982a]	—	—
	1-nitro	82-34-8	—	Matsushita [1980]	+
18.	Anthrone	90-44-8	Prater and Schuetzle [1982]	Anderson and Styles [1978]	Neg
			Erickson et al [1982]	Brown et al [1977]	Neg
				Gibson et al [1978]	Neg
				Kaden et al [1979]	Neg
19.	Anthrone, nitro	—	Riley et al [1982]	—	—
20.	Anthrone, methyl	—	Prater and Schuetzle [1982]	—	—
21.	Anthrone, dimethyl	—	Prater and Schuetzle [1982]	—	—
22.	Anthrone, trimethyl	—	Prater and Schuetzle [1982]	—	—
23.	Benzo(a)pyrene	50-32-8	Prater and Schuetzle [1982]	Epler et al [1978a]	+
				Florin et al [1980]	+
				Pederson and Siak [1981a]	+
				Lavoie et al [1979]	+
				Pitts et al [1978]	+
				Pitts [1979]	+
				Salamone et al [1979]	+

Continued

TABLE II. Salmonella Mutagenicity Results for Compounds Identified in Diesel Exhaust Emissions and Presented at the EPA's 1981 Diesel Emissions Symposium, Raleigh, NC (Continued)

No.	Compound	CAS No.	Reference for diesel identification[a]	Bioassay reference[b]	Bioassay result[c]
24.	Benzo(a)pyrene, nitro	—	Riley et al [1982]	—	—
	1-nitro	—	—	Pitts [1979]	+
	3-nitro	—	—	Pitts [1979]	+
	6-nitro	63041-90-7	—	Pitts et al [1978]	+
				Tokiwa et al [1981]	+
				Wei et al [1978]	
25.	Benzo(e)pyrene, nitro	—	Riley et al [1982]	—	—
26.	Biphenyl	92-52-4	Yergey et al [1981]	Anderson and Styles [1978]	Neg
				Bronzetti et al [1981]	Neg
				Epler et al [1978a]	Neg
				Kawachi et al [1980]	Neg
				Probst and Hill [1980]	Neg
				Probst et al [1981]	Neg
27.	Biphenyl, nitro	—	Riley et al [1982]	—	—
			Xu et al [1982a]		
	2-nitro	86-00-0	—	Anderson and Styles [1978]	+
				El-Bayoumy et al [1981]	+
				Matsushita [1980]	Neg
				McMahon et al [1979]	Neg
				Probst and Hill [1980]	Neg
				Tokiwa et al [1981]	+
	3-nitro	2113-58-8	—	El-Bayoumy et al [1981]	+
				Matsushita [1980]	Neg
				McMahon et al [1979]	Neg
	4-nitro	92-93-3	—	Anderson and Styles [1978]	+
				El-Bayoumy et al [1981]	+
				Matsushita [1980]	+
				McMahon et al [1979]	+
				Probst and Hill [1980]	+
				Probst et al [1981]	+

28.	Biphenyl, nitromethyl	—	Riley et al [1982]	—	—
			Xu et al [1982a]	—	—
	2-methyl-4-nitro	33350-73-1	—	El-Bayoumy et al [1981]	+
	3-methyl-4-nitro	69314-47-2	—	El-Bayoumy et al [1981]	+
29.	Biphenyl, dinitromethyl	—	Henderson et al [1982]	—	—
30.	Biphenylene	259-79-0	Yergey et al [1981]	—	—
31.	Chrysene	218-01-9	Yergey et al [1981]	Barfknecht et al [1981b]	
				Epler et al [1978a,1979]	+
			Prater and Schuetzle [1982]	Florin et al [1980]	+
				Gibson et al [1978]	Neg
				Lavoie et al [1979]	+
				McCoy and Rosenkranz [1980]	Neg
				Salamone et al [1979]	+
32.	Chrysene, nitromethyl	—	Yergey et al [1981]	—	—
33.	Cinnoline, benzo(c)	230-17-1	Yergey et al [1981]	—	—
34.	Dibenzo(b,d)thiophene	132-65-0	Prater and Schuetzle [1982]	Kaden et al [1979]	Neg (8-Az)
				Nakamura and Kashimoto [1979]	Neg
35.	Dibenzothiophene, methyl	30995-64-3	Prater and Schuetzle [1982]	—	—
36.	Dibenzothiophene, dimethyl	70021-47-5	Prater and Schuetzle [1982]	—	—
37.	Dibenzothiophene, tetramethyl	—	Prater and Schuetzle [1982]	—	—
38.	Dibenzothiophene, carboxyaldehyde	—	Prater and Schuetzle [1982]	—	—
39.	Fluoranthene	206-44-0	Riley et al [1982]	Barfknecht et al [1981a]	+ (8-Az)
			Yergey et al [1981]	Epler et al [1979]	?
				Florin et al [1980]	Neg
				Gatehouse [1980]	Neg
				Kaden et al [1979]	+ (8-Az)
				Lavoie et al [1979]	+
				Rao et al [1976]	+
				Salamone et al [1979]	Neg

Continued

TABLE II. Salmonella Mutagenicity Results for Compounds Identified in Diesel Exhaust Emissions and Presented at the EPA's 1981 Diesel Emissions Symposium, Raleigh, NC (Continued)

No.	Compound	CAS No.	Reference for diesel identification[a]	Bioassay reference[b]	Bioassay result[c]
40.	Fluoranthene, methyl	—	Prater and Schuetzle [1982]	—	—
	1-methyl	25889-60-5	—	Lavoie et al [1979]	+
	2-methyl	33543-31-6	—	Lavoie et al [1979]	+
	3-methyl	1706-01-0	—	Lavoie et al [1979]	+
	7-methyl	23339-05-1	—	Lavoie et al [1979]	+
	8-methyl	20485-57-8	—	Lavoie et al [1979]	+
41.	Fluoranthene, nitromethyl	—	Riley et al [1982]	—	—
42.	Fluoranthene, quinone, nitro	—	Yergey et al [1981]	—	—
43.	Fluoranthene, benzo (ghi)	203-12-3	Yergey et al [1981]	Lavoie et al [1979]	+
44.	Fluorene	86-73-7	Yergey et al [1981]	Epler et al [1978a]	Neg
				Gibson et al [1978]	Neg
				Kawachi et al [1980]	Neg
				Lavoie et al [1981a]	Neg
				Probst et al [1981]	Neg
				Probst and Hill [1980]	Neg
45.	Fluorene, nitro	—	Xu et al [1982a]	—	—
	2-nitro	609-57-8	—	Anderson and Styles [1978]	+
				McCoy et al [1981]	+
				Pederson and Siak [1981a]	+
				Probst and Hill [1980]	+
				Tokiwa et al [1981]	+
46.	Fluorene, dinitro	—	Xu et al [1982a]	—	—
	2,5-dinitro	15110-74-4	—	Matsushita [1980]	+
	2,7-dinitro	5405-53-8	—	Levin et al [1979]	+
				Matsushita [1980]	+
				McCoy et al [1981]	+
				Pederson and Siak [1981a]	+
				Probst and Hill [1980]	+
				Tokiwa et al [1981]	+

47.	Fluorene, nitromethyl	—	Xu et al [1982a]	—	—
48.	Fluorene, quinone	—	Yergey et al [1981]	—	—
49.	Fluorenone	486-25-9	Erickson et al [1982] Prater and Schuetzle [1982]	Florin et al [1980]	Neg
50.	Fluorenone, benzo	76723-60-9	Riley et al [1982]	—	—
51.	Fluorenone, nitro(s)	—	Riley et al [1982] Xu et al [1982a]	—	—
	3-nitro	42135-22-8	—	Pederson and Siak [1981a]	+
	2,7-dinitro	31511-45-8	—	Levin et al [1979]	+
				Probst and Hill [1980]	+
52.	Fluorenone, nitromethyl	—	Riley et al [1982]	—	—
53.	Furan, dibenzo	132-64-9	Yergey et al [1981]	—	—
54.	Furan, 7-methyl benzo	17059-52-8	Yergey et al [1981]	—	—
55.	Indene, nitro	—	Xu et al [1982a]	—	—
56.	Indene-1-one, dihydro	—	Yergey et al [1981]	—	—
57.	Naphthalene	91-20-3	Yergey et al [1981]	Anderson and Styles [1978]	Neg
				Florin et al [1980]	Neg
				Ho et al [1981]	Neg
				Kaden et al [1979]	Neg
				Epler et al [1979]	Neg
58.	Naphthalene, dinitromethyl	—	Henderson et al [1982]	—	—
59.	Napthalene, nitromethyl	—	Henderson et al [1982] Xu et al [1982a]	—	—
				El-Bayoumy et al [1981]	+
	1-nitro-2-methyl	881-03-8	—	Matsushita [1980]	+
	1-methyl-2-nitro	6,3017-87-8	—	El-Bayoumy et al [1981]	+
	3-methyl-2-nitro	—	—	El-Bayoumy et al [1981]	+
60.	Naphthalene, nitrodihydroxy	—	Riley et al [1982]	—	—
61.	Naphthalene, nitrotrimethyl	—	Riley et al [1982]	—	—
62.	Naphthalene dicarboxylic acid, nitro	—	Riley et al [1982]	—	—
63.	Naphthaquinone, nitro	—	Riley et al [1982]	—	—

Continued

TABLE II. Salmonella Mutagenicity Results for Compounds Identified in Diesel Exhaust Emissions and Presented at the EPA's 1981 Diesel Emissions Symposium, Raleigh, NC (Continued)

No.	Compound	CAS No.	Reference for diesel identification[a]	Bioassay reference[b]	Bioassay result[c]
64.	Phenanthrene	85-01-8	Prater and Schuetzle [1982]	Barfknecht et al [1981b]	
			Yergey et al [1981]	Epler et al [1978a,1979]	?
				Florin et al [1980]	Neg
				Probst and Hill [1980]	Neg
65.	Phenanthrene, nitro	—	Henderson et al [1982]	—	—
			Riley et al [1982]		
66.	Phenanthrene, methyl	—	Henderson et al [1982]	—	—
			Prater and Schuetzle [1982]		
			Riley et al [1982]		
	1-methyl	832-69-9	—	Gibson et al [1978]	Neg
				Lavoie et al [1981b]	+
	2-methyl	2581-84-2	—	Gibson et al [1978]	Neg
				Lavoie et al [1981b]	Neg
	3-methyl	832-71-3	—	Lavoie et al [1981b]	Neg
	4-methyl	832-64-4	—	Lavoie et al [1981b]	Neg
	9-methyl	883-20-5	—	Gibson et al [1978]	Neg
				Lavoie et al [1981b]	+
67.	Phenanthrene-5-one, cyclopenta	—	Yergey et al [1981]	—	—
68.	Phenanthrene, quinone	—	Yergey et al [1981]	—	—
69.	Phenanthrone	—	Erickson et al [1982]	—	—
			Prater and Schuetzle [1982]		
70.	Phenanthrene, methyl	—	Prater and Schuetzle [1982]	—	—
71.	Phenanthrone, nitro	—	Riley et al [1982]	—	—
72.	Perylene	198-55-0	Prater and Schuetzle [1982]	Anderson and Styles [1978]	+
				Florin et al [1980]	+
				Ho et al [1980]	+
				Lavoie et al [1979]	+
				Salamone et al [1979]	Neg

73.	Perylene, nitro	—	Riley et al [1982]	—	—
	3-nitro	20589-63-3	—	Ho et al [1981]	+
				Pitts et al [1978]	+
				Pitts [1979]	+
74.	Pyrene	129-00-0	Prater and Schuetzle [1982]	Epler et al [1978a, 1979]	+
			Yergey et al [1981]	Florin et al [1980]	Neg
				Gibson et al [1978]	Neg
				Ho et al [1981]	Neg
				Kawachi et al [1980]	+
				Lavoie et al [1979]	Neg
				Probst ad Hill [1980]	Neg
75.	Pyrene, cyclopenta (c,d)	27208-37-3	Yergey et al [1981]	Gold and Eisenstadt [1980]	+
76.	Pyrene, cyclopenteno (c,d)	—	Barfknecht et al [1981b]	—	—
77.	Pyrene, methyl	—	Prater and Schuetzle [1982]	—	—
			Yergey et al [1981]		
	1-methyl	—	—	Kaden et al [1979]	+ (8-Az)
78.	Pyrene, nitromethyl	—	Riley et al [1982]	—	—
			Xu et al [1978a]		
79.	Pyrenequinones, nitro	—	Riley et al [1982]	—	—
80.	Thioxanthones	—	Prater and Schuetzle [1982]	—	—
81.	Triphenylenes	—	Riley et al [1982]	Epler et al [1979]	+
			Yergey et al [1981]	Gibson et al [1978]	Neg
82.	Xanthones	—	Erickson et al [1982]	—	—
			Prater and Schuetzle [1982]		

[a]All references for diesel identification are from Smith [1982].

[b]References resulting from a Environmental Mutagen Information Center (EMIC) search in September 1982. References are found within the paper's reference list.

[c]Bioassay results as reported by the authors and summarized as follows: +, positive; Neg, negative; ?, questionable or ± result; (8-Az), 8-azaquanine forward mutation system used.

examining S9, heat-deactivated S9, S9 minus cofactors, and albumin effects. Somewhat in contrast, Pederson and Siak [1981b] used a nitroreductase-deficient bacterial strain to show that some mutagens in diesel particle extracts are activated by S9 and that 1-nitropyrene is also activated by NAD phosphate-dependent S9 enzymes.

These studies demonstrate that substances are released from diesel exhaust particles into certain physiological fluids and cells. Physiological fluids and S9 apparently decrease the mutagenic activity of diesel extracts and particles primarily because of protein binding; however, some mutagenic components (eg, 1-nitropyrene) are activated by the microsomal fraction of S9, while other components are activated by the cytosol fraction.

In addition to the above concerns of scientists regarding biological parameters, investigators have questioned whether the Salmonella bioassay correlates well enough with other bioassays to use as a routine screen. This knowledge would be useful in the development of new combustion and control technologies. Lewtas showed in her review [1981] that when no exogenous activation system was used, the Salmonella bioassay data had a greater than 90% degree of correlation with the following assay data: mouse lymphoma, sister chromatid exchange in Chinese hamster ovary cells, viral enhancement, and skin tumorigenesis. When exogenous activation was used, however, the correlation with viral enhancement and skin tumorigenicity data was 79% and 72% (respectively). It will be interesting to see whether these high correlations are maintained, as the body of data and information grows.

SUMMARY

In summary, the work presented demonstrates that rapid, in vitro indicators of genotoxicity continue to play a valuable role in our understanding of the toxicity of mobile source emissions. Bacterial assays have had tremendous importance in the characterization of mobile source emissions. Specifically they have had four major uses: (1) comparative screening, (2) analyzing factors that alter the genotoxicants found in emission products, (3) directing the chemical fractionation of emission organics for the identification of specific genotoxicants, and (4) analyzing the interaction of complex emission products with various mammalian systems.

REFERENCES

Alfheim S, Moller N (1981): Mutagenicity of airborne particulate matter in relation to traffic and meteorological conditions. In Waters MD, Sandhu SS, Huisingh JL, Claxton LD, Nesnow S (eds): "Short-Term Bioassays in the Analysis of Complex Environmental Mixtures II." New York: Plenum Press, pp 85–99.

Ames BN, McCann J, Yamasaki E (1975): Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. Mutat Res 31:437–364.

Anderson D, Styles JA (1978): An evaluation of 6 short-term tests for detecting organic chemical carcinogens. Appendix 2. The bacterial mutation test. Br J Cancer 37:924–930.

Barfknecht TR, Andon BM, Bishop WW, Thilly WG (1981a): Radiosterilization of rat liver microsome containing postmitochondrial supernatant for mutation assays. Environ Mutagen 3:565–573.

Barfknecht TR, Andon BM, Thilly WG, Hites RA (1981b): Soot and mutation in bacteria and human cells. In Cooke M, Dennis AJ (eds): "Chemical Analysis and Biological Fate: Polynuclear Aromatic Hydrocarbons." Columbus, Ohio: Batelle Press, pp 231–242.

Braddock JN (1981): Emissions of diesel particles and particulate mutagens at low ambient temperatures. Paper presented at EPA Symposium on the Application of Short-Term Bioassays in the Analysis of Complex Environmental Mixtures, Chapel Hill, North Carolina, January 25–27, 1981.

Bradow RL (1980): Diesel particle emissions. Bull NY Acad Med 56:797-811.

Bronzetti G, Esposito A, Panago G, Quinto I (1981): Comparative study of biphenyl and diphenyl ether in sea urchin, S. typhimurium and S. cerevisiae. Mutat Res 85:233.

Brooks AL, Wolff RK, Royer RE, Clark CR, Sanchez A, McClellan RO (1980): Biological availability of mutagenic chemicals associated with diesel exhaust particles. In Pepelko WE, Danner RM, Clarke NA (eds): "Health Effects of Diesel Engine Emissions: Proceedings of an International Symposium," EPA-600/9-80-057a, Cincinnati, Ohio: U.S. Environmental Protection Agency, pp 345-358.

Brown JP, Dietrich PS, Brown RJ (1977): Frameshift mutagenicity of certain naturally occurring phenolic compounds in the Salmonella/microsome test. Biochem Soc Trans 5:1489-1492.

Brusick D, Matheson DW (1978a): Mutagen and oncogen study on JP-8. AMRL-TR-78-20. Wright-Patterson Air Force Base, Ohio: Aerospace Medical Research Laboratory.

Brusick D, Matheson DW (1978b): Mutagen and oncogen study on JP-8. AMRL-TR-78-24. Wright-Patterson Air Force Base, Ohio: Aerospace Medical Research Laboratory.

Calkins WH, Krahn DF (1979): Synthetic crude oils carcinogenicity screening tests: Progress report. DOE/COO-4758-2. Washington, DC: U.S. Dept of Energy.

Calkins W, Deye C, King C (1980): Synthetic crude oils carcinogenicity screening test. E.I. DuPont Report 10127-3:1-7.

Chan TL, Lee PS, Siak JS (1981): Diesel-particulate collection for biological testing. Comparison of electrostatic precipitation and filtration. Environ Technol 15(1):89-93.

Choudhury DR, Doudney CO (1981): Mutagenic activity of diesel emission particulate extracts and isolation of mutagenic fractions. Environ Int 5(4-5):389-392.

Clark CR, Vigil CL (1980): Influence of rat lung and liver homogenates on the mutagenicity of diesel exhaust particulate extracts. Toxicol Appl Pharmacol 56:110-115.

Claxton LD (1980): Detection and comparison of mutagens associated with complex environmental substances. Dissertation. Raleigh, North Carolina: North Carolina State University.

Claxton LD (1981a): Mutagenic and carcinogenic potency of diesel and related environmental emissions: Salmonella bioassay. In Pepelko WE, Danner RM, Clarke NA (eds): "Health Effects of Diesel Engine Emissions: Proceedings of an International Symposium."EPA-600/9-80-057b, Cincinnati, Ohio: U.S. Environmental Protection Agency, pp 801-809.

Claxton LD (1981b): The utility of bacterial mutagenesis testing in the characterization of mobile source emissions. In Lewtas JL (ed): "Toxicological Effects of Emissions From Diesel Engines." New York: Elsevier, pp 69-82.

Claxton LD (1982): The integration of bioassay and physiochemical information for complex mixtures. Paper presented at the EPA Symposium on the Application of Short-Term Bioassays in the Analysis of Complex Environmental Mixtures. Chapel Hill, North Carolina, January 25-27, 1982.

Claxton LD, Barnes HM (1981): The mutagenicity of diesel-exhaust particle extracts collected under steady chamber conditions using the Salmonella typhimurium test system. Mutat Res 88:255-272.

Claxton LD, Kohan M (1981): Bacterial mutagenesis and the evaluation of mobile source emissions. In Waters MD, Sandhu SS, Huisingh JL, Claxton LD, Nesnow S (eds): "Short-Term Bioassays in the Analysis of Complex Environmental Mixtures II." New York: Plenum Press, pp 299-317.

de Flora S (1981): A "spiral test" applied to bacterial mutagenesis assays. Mutat Res 82:213-227.

Dietzman HE, Parness MA, Bradow RL (1981): Emissions from gasoline and diesel delivery trucks by chassis transient cycle. ASME Publ No. 81-DGP-6. New York: American Society of Mechanical Engineers.

Dukovich M, Yasbin RE, Lestz SS, Risby JH, Zweidinger RB (1981): The mutagenic and SOS-inducing potential of the soluble organic fraction collected from diesel particulate emissions. Environ Mutagen 3:253-264.

Edwards JB (1977): "Combustion: Formation and Emission of Trace Species." Ann Arbor: Ann Arbor Science Publications, Inc.

El-Bayoumy K, Lavoie EJ, Hecht SS, Fow EA, Hoffman D (1981): The influence of methyl substitution on the mutagenicity of nitronapthalenes and nitrobiphenyls. Mutat Res 81:143-153.

Epler JL, Larimer FW, Rao TK, Nix CE, Ho T (1978a): Energy-related pollutants in the environment: Use of short-term tests for mutagenicity in the isolation and identification of biohazards. Environ Health Perspect 27:11-20.

Epler JL, Rao TK, Guerin MR (1979): Evaluation of feasibility of mutagenic testing of shale oil products and effluents. Environ Health Perspect 30:179-184

Epler JL, Young JA, Hardigree AA, Ra... JK, Guerin MR, Rubin JB, Ho CH, Clark BR (1978b): Analytical and biological analyses of test materials from the synthetic fuel technologies: I. Mutagenicity of crude oils determined by the Salmonella typhimurium microsomal activation system. Mutat Res 57:265–276.

Erickson MD, Newton DL, Saylor MC, Tomer KB, Pellizzari ED, Zweidinger RB, Tejada S (1982): Fractionation and identification of organic components in diesel exhaust particulate. In Smith JR (ed): "Diesel Emissions Symposium Proceedings." EPA-600/9-82-014, Research Triangle Park, North Carolina: U.S. Environmental Protection Agency, pp 509–512.

Florin I, Rutberg L, Curvall M, Enzell CR (1980): Screening of tobacco smoke constituents for mutagenicity using the Ames test. Toxicology 15:219–232.

Gabele PA, Black FM, King FG, Zweidinger RB, Brittain RA (1981): Exhaust emission patterns from two light-duty diesel automobiles. SAE Technical Report 810081, Warrendale, Pennsylvania: Society of Automotive Engineers.

Gage SJ (1977): Precautionary notice on laboratory handling of exhaust products from diesel engines. Washington, DC: Office of Research and Development, U.S. Environmental Protection Agency.

Gatehouse D (1980): Mutagenicity of 1,2 ring-fused acenaphthenes against S. typhimurium TA1537 and TA1538: Structure-activity relationships. Mutat Res 78:121–135.

Gibbs RE, Hyde JD, Byer SM (1980): Characterization of particulate emissions from in-use vehicles. SAE Technical Report 801372, New York: Society of Automotive Engineers.

Gibson TL, Smart VB, Smith LL (1978): Non-enzymatic activation of polycyclic hydrocarbons as mutagens. Mutat Res 49:153–161.

Gibson T, Ricci A, Williams RL (1980): Measurement of polynuclear aromatic hydrocarbons, their derivatives and their reactivity in diesel automobile exhaust. Research Publ No. GMR3478, Warren, Michigan: General Motors, Inc.

Glatt H, Vogel K, Bentley P, Sims P, Oesch F (1981): Large differences in metabolic activation and inactivation of chemically closely related compounds: Effects of pure enzymes and enzyme induction on the mutagenicity of the twelve monomethylated benz(a)anthracenes, 7,12-dimethylbenz(a)anthracene, and benz(a)anthracene in the Ames test. Carcinogenesis (London) 2:813–821.

Gold A, Eisenstadt E (1980): Metabolic activation of cyclopentacdipyrene to 3,4-epoxy cyclopentacdipyrene by rat liver microsomes. Cancer Res 40:3940–3944.

Gray C, von Hippel F (1981): The fuel economy of light vehicles. Sci Am 244:48–59.

Guerin MR, Ho CH, Rao JK, Clark BR, Epler JL (1980): Polycyclic aromatic primary amines as determinant chemical mutagens in petroleum substitutes. Environ Res 23:42–53.

Henderson TR, Li AP, Royer RE, Clark CR (1981): Increased cytotoxicity and mutagenicity of diesel fuel after reaction with NO_2. Environ Mutagen 3:211–220.

Henderson TR, Sun JD, Royer RE, Clark CR, Harvey TM, Hunt DF, Fulford JE, Lovett AM, Davidson WR (1982): GC/MS and MS MS studies of direct-acting mutagens in diesel emissions. In Smith JR (ed): "Diesel Emissions Symposium Proceedings." EPA-600/9-82-014, Research Triangle Park, North Carolina: U.S. Environmental Protection Agency, pp 523–527.

Hermann M, Chaude O, Weill N, Bedouelle H, Hofnung M (1980): Adaptation of the Salmonella/mammalian-microsome test to the determination of the mutagenic properties of mineral oils. Mutat Res 77:327–339.

Ho C-H, Clark BR, Guerin MR, Barkenbus BD, Rao TK, Epler JL (1981): Analytical and biological analyses of test materials from the synthetic fuel technologies. Mutat Res 85:335–345.

Hubbard SA, Bridges JW, Green MHL (1981): Freshly isolated hepatocytes for metabolic activation in a bacterial mutation assay. Mutat Res 85:264.

Huisingh JL, Bradow R, Jungers R, Claxton LD, Zweidinger R, Tejada S, Bumgarner J, Duffield F, Waters M, Simmon VF, Hare C, Rodriguez C, Snow L (1978): Application of bioassay to the characterization of diesel particle emissions. In Waters MD, Nesnow S, Huisingh JL, Sandhu SS, Claxton LD (eds): "Application of Short-Term Bioassays in the Fractionation and Analysis of Complex Environmental Mixtures II." EPA-600/9-78-027, Research Triangle Park, North Carolina: U.S. Environmental Protection Agency, pp 1–32.

Jäger J (1978): Detection and characterization of nitroderivatives of some polycyclic aromatic hydrocarbons by fluorescence quenching after thin-layer chromatography. Application to air pollution analysis. Chromatography 152:575–578.

Kaden DA, Hites RA, Thilly WG (1979): Mutagenicity of soot and associated polycyclic aromatic hydrocarbons to Salmonella typhimurium. Cancer Res 39:4152–4159.

Kawachi T, Yahugi T, Kada T, Tazima Y, Ishidate M, Sasaki M, Sugiyama T (1980): Cooperative program on short-term assays for carcinogenicity in Japan. IARC Sci Publ 27:323-330.

King LC, Kohan MJ, Austin AC, Claxton LD, Huisingh JL (1981): Evaluation of the release of mutagens from diesel particles in the presence of physiological fluids. Environ Mutagen 3:109-121.

Kotin P, Falk HL, Thomas M (1954): Aromatic hydrocarbons. II. Presence in the particulate phase of gasoline-engine exhausts and the carcinogenicity of exhaust extracts. AMA-Arch Ind Hyg Occup Med 9:164-177.

Kotin P, Falk HL, Thomas M (1955): Aromatic hydrocarbons. III. Presence in the particulate phase of diesel-engine exhausts and the carcinogenicity of exhaust extracts. AMA Arch Ind Health 11:113-120.

Lavoie E, Bedenko V, Hirota N, Hecht SS, Hoffman D (1979): A comparison of the mutagenicity, tumor-initiating activity and complete carcinogenicity of polynuclear aromatic hydrocarbons. In Jones PW, Leber P (eds): "Polynuclear Aromatic Hydrocarbons." Michigan: Ann Arbor Science Publishers, Inc., pp 705-721.

Lavoie E, Tulley L, Bedenko V, Hoffman D (1981a): Mutagenicity of methylated fluorenes and benzofluorenes. Mutat Res 91:167-176.

Lavoie E, Tulley-Freiler L, Bedenko V, Hoffman D (1981b): Mutagenicity, tumor-initiating activity, and metabolism of methyl phenanthrenes. Cancer Res 41:3441-3447.

Lebowitz H, Brusick D, Matheson D, Jagannath DR, Reed M, Goode S, Roy G (1979): Commonly used fuels and solvents evaluated in a battery of short-term bioassays. Environ Mutagen 1:172-173.

Levin DE, Barnes WS, Klekowski E (1979): Mutagenicity of fluorene derivatives: A proposed mechanism. Mutat Res 63:1-10.

Lewtas JL (1981): Mutagenic activity of diesel emissions. In Lewtas JL (ed): "Toxicological Effects of Emissions from Diesel Engines." New York: Elsevier, pp 243-264.

Liber HL, Andon BM, Hites RA, Thilly WG (1980): Diesel soot: Mutation measurements in bacterial and human cells. In Pepelko WE, Danner RM, Clarke NA (eds): "Health Effects of Diesel Engine Emissions: Proceedings of an International Symposium." EPA-600/9-80-057a, Cincinnati, Ohio: U.S. Environmental Protection Agency, pp 404-412.

Löfroth G (1981): Salmonella/microsome mutagenicity assays of exhaust from diesel and gasoline powered motor vehicles. Environ Int 5(4-5):255-262.

Matsushita H (1980): Mutagenicity of atmospheric nitrogen oxides (NO, NO_2) and their reaction products in air. Hen'igen to Dokusei (Mutagens and Toxicology) 11:39-46.

McClellan RO (ed) (1980a): "Diesel Exhaust Emissions." Toxicology program status report—January 1980. DOE/EY-76-C-04-1013, Washington, DC: U.S. Dept of Energy.

McClelland RO (ed) (1980b): "Diesel Exhaust Emissions." Toxicology program status report—July 1980. DOE/DE-AC04-76VO-1013, Washington, DC: U.S. Dept of Energy.

McCoy EC, Rosenkranz HS (1980): Activation of polycyclic aromatic hydrocarbons to mutagens by singlet oxygen: An enhancing effect of atmospheric pollutants? Cancer Lett 9:35-42.

McCoy EC, Rosenkranz EJ, Rosenkranz HS, Mermelstein R (1981): Nitrated fluorene derivatives are potent frameshift mutagens. Mutat Res 90:11-20.

McGrath JJ, Schreck RM, Siak JS (1978): Mutagenic screening of diesel particulate material. Paper presented at the 71st Annual Meeting of the Air Pollution Control Association, Reprint No. 78-33-6, Houston, Texas, June 25-30, 1978.

McMahon RE, Cline JC, Thompson CZ (1979): Assay of 855 test chemicals in ten tester strains using a new modification of the Ames test for bacterial mutagens. Cancer Res 39:682-693.

Mermelstein R, Rosenkranz HS, McCoy EC (1981): The microbial mutagenicity of nitroarenes. In Tice RR, Costa DL, Schaich KM (eds): "Genotoxic Effects of Airborne Agents." New York: Plenum Press, pp 369-396.

Nakamura A, Kashimoto T (1979): Toxicological assessment of heavy oil and sulfur-containing components by various conventional biological tests. 2. Studies on heavy oil components in food. Shokuhin Eiseigaku Zasshi (J Food Hyg Soc Jpn) 20:161-165.

Ohnishi Y, Kachi K, Sato K, Tahara S, Takeyoshi H, Tokiwa H (1980): Detection of mutagenic activity in automobile exhaust. Mutat Res 77:229-240.

Pederson JC, Siak JS (1980): Characterization of direct-acting mutagens in diesel exhaust particulates by thin layer chromatography. Research Publ No GMR-3265, Warren, Michigan: General Motors, Inc.

Pederson JC, Siak JS (1981a): Role of nitroaromatic compounds in the direct-acting mutagenicity of diesel particle extracts. J Appl Toxicol 1:54–60.

Pederson JC, Siak JS (1981b): The activation of mutagens in diesel particles with rat liver S9 enzymes. J Appl Toxicol 1:61–66.

Pierson WR, Gorse RA, Szkarlat AC, Brachaczek WW, Japar SM, Lee SC, Zweidinger RB, Claxton LD (1982): Mutagenicity of extracts of particulate matter from vehicles on the road. In Smith JR (ed): "Diesel Emissions Symposium Proceedings." EPA-600/9-82-014. Research Triangle Park, North Carolina: U.S. Environmental Protection Agency, pp 453–456.

Pitts JN Jr (1979): Photochemical and biological implications of the atmospheric reactions of amines and benzo(a)pyrene. Philos Trans R Soc Lon [a]290:551–556.

Pitts JN Jr, van Cauwenberghe KA, Grosjean D, Schmid JP, Fitz DR, Belser WL, Knudson G, Hynds P (1978): Atmospheric reactions of polycyclic hydrocarbons: Facile formation of mutagenic nitro derivatives. Science 202:515–519.

Pitts JN Jr, Harger W, Lokensgard DM, Fitz DR, Scorziell GM, Mejia V (1982a): Diurnal variations in the mutagenicity of airborne particulate organic matter in California's south coast air basin. Mutat Res 104:35–41.

Pitts JR Jr, Lokensgard DM, Harger W, Fisher TS, Mejia V, Schuler JJ, Scorziell GM, Katzenstein YA (1982b): Mutagens in diesel exhaust particulate. Identification and direct activities of 6-nitrobenzo(a)pyrene, 9-nitroanthracene, 1-nitropyrene, and 5H-phenanthro[4,5-bcd]pyran-5-one. Mutat Res 103:241–249.

Prater TJ, Schuetzle D (1982): Capillary column GC/MS characterization of diesel exhaust particulate extracts. In Smith JR (ed): "Diesel Emissions Symposium Proceedings." EPA-60/9-82-014. Research Triangle Park, North Carolina: U.S. Environmental Protection Agency, pp 584–587.

Probst GS, Hill LE (1980): Chemically-induced DNA repair synthesis in primary rat hepatocytes: Correlation with bacterial mutagenicity. Ann NY Acad Sci 349:405–406.

Probst GS, McMahon RE, Hill LE, Thompson CZ, Epp JK, Neal SB (1981): Chemically-induced unscheduled DNA synthesis in primary rat hepatocyte cultures: A comparison with bacterial mutagenicity using 218 compounds. Environ Mutagen 3:11–32.

Rao TK, Rubin IB, Guerin MR, Epler JL (1976): Environmental mutagenesis of energy-related effluents. Genetics 83(5):60.

Rappaport SM, Wang YY, Wei EJ, Sawyer R, Watkings BE, Rappaport H (1980): Isolation and identification of a direct-acting mutagen in diesel exhaust particulates. Environ Sci Technol 14(12):1505–1509.

Riley T, Prater T, Schuetzle D, Harvey TM, Hunt DF (1982): The analysis of nitrated polynuclear aromatic hydrocarbons in diesel exhaust particulates by MS/MS technology. In Smith JR (ed): "Diesel Emissions Symposium Proceedings." EPA-60/9-82-014. Research Triangle Park, North Carolina: U.S. Environmental Protection Agency, pp 115–119.

Rosenkranz HS, McCoy EC, Sanders DR, Butler M, Kinazides DK, Mermestein R (1980): Nitropyrenes: isolation, identification and reduction of mutagenic impurities in carbon black and tones. Science 209:1039–1043.

Salamone MF, Heddle JA, Katz M (1979): The mutagenic activity of thirty polycyclic aromatic hydrocarbons and oxides in urban airborne particulates. Environ Int 2:37–43.

Schuetzle D, Riley TL, Prater TJ (1982): Analysis of nitrated polycyclic aromatic hydrocarbons in diesel particulates. Anal Chem 54:265–271.

Siak JS, Chan TL, Lee PS (1979): Diesel particulate extracts in bacterial test systems. Research Publ No. GMR-3171. Warren, Michigan: General Motors, Inc.

Siak JS, Chan JL, Lee PS (1981): Diesel particulate extracts in bacterial test systems. Environ Int 5(4–5):243–248.

Siak JS, Strom KA (1981): Biological fate of inhaled diesel particles. Paper presented at the Annual Meeting of the Society of Toxicology, San Diego, California, Feb 1981.

Smith JR (ed) (1982): Diesel Emissions Symposium Proceeedings, EPA-600/9-82-014. Research Triangle Park, North Carolina: U.S. Environmental Protection Agency.

Talcott RE, Harger W (1981): Chemical characterization of direct-acting airborne mutagens: The Functional Group. Mutat Res 91:433–436.

Tokiwa H, Nakagawa R, Ohnishi Y (1981): Mutagenic assay of aromatic nitro compounds with Salmonella typhimurium. Mutat Res 91:321–325.

Wang CY, Lee MS, King CM, Warner PO (1980): Evidence for nitroaromatics as direct-acting mutagens of airborne particulates. Chemosphere 9:83–87.

Wang YY, Wei ET (1981): Ability of liver homogenates and proteins to reduce the mutagenic effect of diesel exhaust particulates. In Waters MD, Sandhu SS, Huisingh JL, Claxton LD, Nesnow S (eds): "Short-Term Bioassay in the Analysis of Complex Mixtures II." New York: Plenum Press, pp 359–368

Wang YY, Rappaport SM, Sawyer RF, Talcott RD, Wei EJ (1978a): Direct-acting mutagens in automobile exhaust. Cancer Lett 5:39–47.

Wang YY, Sawyer RF, Wei ET (1978b): Mutagens in automobile exhaust. In Waters MD, Nesnow S, Huisingh JL, Sandhu SS, Claxton LD (eds): "Application of Short-Term Bioassays in the Fractionation and Analysis of Complex Environmental Mixtures." EPA-600/9-78-027, Research Triangle Park, North Carolina: U.S. Environmental Protection Agency, pp 587.

Wang YY, Talcott RE, Seid DA, Wei EJ (1981): Antimutagenic properties of liver homogenate protein and glutathione on diesel exhaust particulates. Cancer Lett 11:265–275.

Wei ET, Wang YY, Rappaport SM (1980): Diesel emissions and the Ames test: A commentary. J Air Pollut Control Assoc 30(3):267–271.

Wei ET, Wang YY, Talcott RE, Sawyer RF, Rappaport SM (1978): Mutagens in automobile exhaust. Fed Proc Fed Am Soc Exp Biol 37:247.

Xu XB, Nachtman JP, Jin ZL, Wei ET, Burlingame AL (1982a): Isolation and identification of mutagenic nitroarenes in diesel exhaust particulates. In Smith JR (ed): "Diesel Emissions Symposium Proceedings." EPA-600/9-82-014, Research Triangle Park, North Carolina: U.S. Environmental Protection Agency, pp 556–558.

Xu XB, Nachtman JP, Jin ZL, Wei ET, Rappaport SM (1982b): Isolation and identfication of mutagenic nitro-PAH in diesel-exhaust particulates. Anal Chim Acta 136:163–174.

Yergey JA, Risby TH, Lestz SS (1981): The chemical characterization of diesel particulate matter. In Smith JR (ed): "Diesel Emissions Symposium Proceedings." EPA-600/9-82-014, Research Triangle Park, North Carolina: U.S. Environmental Protection Agency, pp 111–114.

Yergey JA, Risby TH, Lestz SS (1982): Chemical characterization of organic adsorbates on diesel particulate matter. Anal Chem 54(3):354–357.