FINAL REPORT

Evaluation of Factors That Affect Diesel Exhaust Toxicity

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EXECUTIVE SUMMARY

Diesel exhaust has been designated by the International Agency for Research on Cancer as a probable human carcinogen and is undergoing evaluation as a potential toxic air contaminant under the California Air Resources Board's (CARB's) air toxics program. Diesel exhaust comprises a complex mixture of hundreds of particle bound and vapor phase compounds. Extensive literature exists on the relationship between diesel fuel parameters and emissions of criteria pollutants; however, little is known about the impact of these fuel changes on the complex speciation of the gas-phase, semi-volatile, and particle-phase components and specifically on emissions of toxic components present in diesel exhaust. This study was designed to provide this information.

In 1991, CARB instituted regulatory efforts to reduce emissions of total hydrocarbons (THC), carbon monoxide (CO), oxides of nitrogen (NOx), and particulate matter (PM) from diesel fuel (CARB, 1988; 1991). The regulations took effect October 1, 1993. These regulations were based on a review of the available data regarding the impact of fuel properties on these emissions. The regulation limits the maximum sulfur content to 0.05%, the minimum cetane index to 40, and the maximum aromatic content to 10%. Because these changes could require fuel refiners to make major capital investments, CARB has allowed fuel producers the option of developing less costly alternatives with a higher aromatic content, if equivalent emissions can be demonstrated (CARB, 1988).

To evaluate the emissions impact of these regulations a team of researchers from UC Riverside College of Engineering Center for Environmental Research and Technology (CE-CERT) and Statewide Air Pollution Research Center (SAPRC), as well as the Department of Environmental Toxicology at UC Davis, conducted testing on three diesel fuels: a pre-1993 fuel, a low aromatic fuel, and alternative formulation blend, henceforth referred to as reformulated fuel. Emission testing was conducted at the Los Angeles County Metropolitan Transportation Authority (LACMTA) emission test facility. The test bed was a Cummins L10, 6-cylinder inline, turbocharged, 4-stroke direct injected diesel engine rated at 310 Bhp @ 1600 RPM. The engine was tested over the heavy-duty transient test cycle. Multiple samples were collected for each of the three fuels over a 4-week test interval. A total of 47 test cycles (7 cold and 40 hot) were run with the pre-1993 diesel fuel, 23 test cycles (4 cold and 19 hot) were run with the low aromatic fuel, and 39 test cycles (5 cold and 34 hot) were run with the reformulated diesel blend.

The results of the testing indicated the emission rates of THC and NOx for both cold and hot start cycles are greatest for pre-1993 fuel, followed by the reformulated and low aromatic fuels. The emission rates for CO, however, varied. The low aromatic fuel was slightly above the baseline, while the reformulated fuel was below the baseline. These differences were not significant at the 95% confidence level. Hot-start CO emissions for the reformulated fuel were 2.7% above the baseline, and those for the low aromatic fuel were 8.9% above the baseline. These figures were statistically significant at the 95% confidence level. Reductions in PM emission rates from the baseline pre-1993 fuel range from 17 to 25%. Size-resolved PM emission rates indicate that 99% of the total mass has an aerodynamic diameter of less than10 μm, and greater than 95% is smaller than 2.5μm [Summary Table 1].

Summary Table 1 Average Emission Test Results on Cummins L10 engine for Pre-1993, Low Aromatic, and Reformulated Fuel.

Pre-1993

	Cold-Start		**Hot-Start**		**Weighted Total (1)**	
	Mean gm/bhp-hr	**SDEV** gm/bhp-hr	**Mean** gm/bhp-hr	**SDEV** gm/bhp-hr	**Mean** gm/bhp-hr	**SDEV** gm/bhp-hr
NOx	4.98	0.13	4.74	0.12	4.77	0.1
THC	0.56	0.02	0.52	0.02	0.53	0.02
CO	2.38	0.11	2.24	0.19	2.26	0.16
CO2	547.3	8.1	518	3.9	522.1	3.5
PM	0.259	0.016	0.218	0.01	0.224	0.009
BSFC(lb/bhp-hr)	0.385	0.007	0.364	0.003	0.367	0.003
Work(bhp-hr)	22.254	0.087	22.327	0.046	22.317	0.041

Low Aromatic

	Cold-Start			**Hot-Start**			**Weighted Total (1)**		
	Mean gm/bhp-hr	**SDEV** gm/bhp-hr	**(2)** %	**Mean** gm/bhp-hr	**SDEV** gm/bhp-hr	**(2)** %	**Mean** gm/bhp-hr	**SDEV** gm/bhp-hr	**(2)** %
NOx	4.6*	0.06	-7.6	4.41	0.06	-7	4.44	0.05	-7.1
THC	0.41*	0.01	-27	0.48*	0.02	-7.7	0.47	0.02	-11
CO	2.47	0.01	3.8	2.44*	0.04	8.9	2.44	0.03	8.1
CO2	545.6	1.4	-0.3	511.7	6.2	-1.2	516.5	5.31	-1.1
PM	0.193*	0.003	-25	0.181*	0.008	-17	0.183	0.007	-18
BSFC(lb/bhp-hr)	0.387	0.001	0.5	0.364	0.004	0	0.367	0.003	0.1
Work(bhp-hr)	21.962	0.034	-1.3	22.035	0.222	-1.3	22.025	0.19	-1.3

Reformulated

	Cold-Start			**Hot-Start**			**Weighted Total (1)**		
	Mean gm/bhp-hr	**SDEV** gm/bhp-hr	**(2)** %	**Mean** gm/bhp-hr	**SDEV** gm/bhp-hr	**(2)** %	**Mean** gm/bhp-hr	**SDEV** gm/bhp-hr	**(2)** %
NOx	4.85*	0.05	-2.6	4.61	0.19	-2.7	4.64	0.16	-2.7
THC	0.47*	0.02	-16	0.5*	0.02	-3.8	0.5	0.02	-5.7
CO	2.3	0.06	-3.4	2.3*	0.05	2.7	2.3	0.04	1.8
CO2	549.2	2.2	0.3	511.6	4.4	-1.2	517	3.8	-1
PM	0.212*	0.019	-18	0.182*	0.012	-17	0.186	0.011	-17
BSFC(lb/bhp-hr)	0.385	0.002	0	0.359	0.003	-1.4	0.362	0.003	-1.2
Work(bhp-hr)	22.128	0.043	-0.6	22.198	0.047	-0.6	22.188	0.041	-0.6

(1) Wt. Tot .= (1/7 gm(cold) + 6/7 gm(hot))/(1/7 Bhp-hr(cold)+ 6/7 Bhp-hr(hot)); SDEV = √(1/6 SDEV(cold))^2 + (6/7SDEV(hot))^2

(2) Percent change in mean from the Pre-1993 fuel.

* Significant at 95% confidence limit using Fisher's Protected Least Significant Difference Test

There were no significant differences found in the PM_{10} and $PM_{2.5}$ size distribution as a function of fuel type. The composition of the PM was found to be dominated by elemental and organic carbon (97% of the mass identified). Organic carbon comprises 33 to 40% of the total carbon and remains relatively constant for all fuels tested. The remaining particulate mass recovered included increased nitrate emission rates for the low aromatic and reformulated fuels. This may result from the use of organonitrates as cetane improvers for the reformulated fuel, but is unexplained for the low aromatic fuel. Elemental sulfur and sulfate emission rates are consistent with sulfur content present in the fuel. The remaining elemental constituents — Mg, P, Ca, Zn, and Fe —are attributed to the engine oil and wear, with higher emission rates occurring during the cold-start tests. Silicon emissions rates are relatively constant for all fuels and cycles. Their source is unknown.

Carbonyl emission rates for cold and hot start tests are very similar for all fuels, with formaldehyde>acetaldehyde>acrolein>propionaldehyde. The low aromatic fuel has lower formaldehyde and acetaldehyde emission rates during cold start, but these differences are less than 20%. During hot start the pre-1993 fuel has approximately 10% lower acetaldehyde emissions than the low aromatic and reformulated fuels. The low aromatic fuel demonstrated an elevated emission rate of acrolein during cold start compared with the pre-1993 and reformulated fuels [Summary Table 2]. The emission rates for speciated gas phase hydrocarbons for all fuels demonstrate the same trends for all fuels. Benzene emission rates ranged from 5.65-8.15 mg/Bhp-hr. with 1,3-Butadiene, toluene, o-Xylene, m&p-Xylene, styrene, and naphthalene emission rates at or below 2.5 mg/Bhp-hr. The low aromatic fuel has higher hot-start 1,3-Butadiene, higher hot- and cold-start benzene, higher hot-start toluene, lower cold-start o-Xylene, and lower hot-start m&p-Xylene emissions than the other fuels. No statistically significant differences were observed between the pre-1993 and reformulated fuels [Summary Table 3].

Particle-bound polycyclic aromatic hydrocarbon (PAH) [Summary Table 4] and nitro-PAH [Summary Table 5] were collected with a high-volume sampling system using Teflon-impregnated glass fiber filters backed by two polyurethane foam plugs (PUF) in series, which allowed quantitative sampling of PAH with molecular weights greater than 178 Daltons. The PAH of molecular weights greater than 228 Daltons were found on the filters alone. The PAH of molecular weights between 178 and 202 Daltons were distributed between the filters and the front PUFs. Breakthrough of 2,3,5-trimethylnaphthalene onto the back PUF occurred, and the values measured for this alkyl-PAH must be viewed as lower limits. Separate samples for gas-phase PAH (including 2,3,5-trimethylnaphthalene) were also collected through the addition of an XAD resin adsorbent cartridge downstream of the PUF adsorbent [Summary Table 6].

The most pronounced differences in emission rates for particle-bound PAH occurred for the alkyl-PAH, 2,3,5-trimethylnaphthalene and the methylphenanthrenes. The trend in alkyl-PAH emissions followed the trend in PAH content of the fuels. That is, the highest PAH content and the highest alkyl-PAH emissions were for the pre-1993 fuel, followed by the reformulated fuel, and both PAH fuel content and alkyl-PAH emissions were substantially lower for the low aromatic fuel. These differences were statistically significant for all fuel paired comparisons [Summary Table 7]

Summary Table 2 Emission Rate for Carbonyls.

			Pre-1993			
	Cold-Start		Hot-Start		Weighted Total (1)	
	Mean	**SDEV**	**Mean**	**SDEV**	**Mean**	**SDEV**
	mg/Bhp-hr	mg/Bhp-hr	mg/Bhp-hr	mg/Bhp-hr	mg/Bhp-hr	mg/Bhp-hr
Formaldehyde	62.33	1.37	56.26	0.95	57.12	0.83
Acetaldehyde	20.23	0.31	17.81	0.2	18.15	0.18
Acrolein	2.18	1.12	2.13	0.74	2.14	0.65
Propionaldehyde	4.45	0.08	3.56	0.43	3.69	0.37

				Low Aromatic					
	Cold-Start			Hot-Start			Weighted Total (1)		
	Mean	**SDEV**	**(2)**	**Mean**	**SDEV**	**(2)**	**Mean**	**SDEV**	**(2)**
	mg/Bhp-hr	mg/Bhp-hr	%	mg/Bhp-hr	mg/Bhp-hr	%	mg/Bhp-hr	mg/Bhp-hr	%
Formaldehyde	54.75 *	2.1	-12	59.42	5.77	5.6	58.75	4.95	2.8
Acetaldehyde	17.66 *	0.45	-13	19.34 *	1.72	8.6	19.1	1.47	5.2
Acrolein	5.49 *	0.69	152	5.84 *	0.91	174	5.79	0.79	171
Propionaldehyde	3.9	0.22	-12	3.92	0.49	10	3.92	0.42	6.2

				Reformulated					
	Cold-Start			Hot-Start			Weighted Total (1)		
	Mean	**SDEV**	**(2)**	**Mean**	**SDEV**	**(2)**	**Mean**	**SDEV**	**(2)**
	gm/Bhp-hr	mg/Bhp-hr	%	mg/Bhp-hr	mg/Bhp-hr	%	mg/Bhp-hr	mg/Bhp-hr	%
Formaldehyde	59.69	2.71	-4.2	59.85	1	6.4	59.83	0.94	4.7
Acetaldehyde	20.1	1.1	-0.6	19.9 *	0.58	12	19.93	0.52	9.8
Acrolein	2.52	0.44	16	2.41	1.71	13	2.42	1.47	14
Propionaldehyde	4.28	0.84	-3.8	4.11	0.47	15	4.13	0.42	12

(1) Wt. Tot .= (1/7 mg(cold) + 6/7 mg(hot))/(1/7 Bhp-hr(cold)+ 6/7 Bhp-hr(hot)); SDEV = √(1/6 SDEV(cold))2 + (6/7SDEV(hot))2

(2) Percent change in mean from the Pre-1993 fuel.

* Significant at 95% confidence limit using Fisher's Protected Least Significant Difference Test

Summary Table 3 Emission Rates for Gas Phase Hydrocarbons.

			Pre-1993			
	Cold-Start		Hot-Start		Weighted Total (1)	
	Mean	SDEV	Mean	SDEV	Mean	SDEV
	mg/Bhp-hr	mg/Bhp-hr	mg/Bhp-hr	mg/Bhp-hr	mg/Bhp-hr	mg/Bhp-hr
1,3-Butadiene	2.08	0.52	1.75	0.11	1.8	0.12
Benzene	6.49	0.04	5.8	0.3	5.9	0.26
Toluene	2.17	0.1	1.89	0.27	1.93	0.23
Ethylbenzene	0.74	0.06	1.3	0.66	1.22	0.57
o-Xylene	0.85	0.09	0.77	0.09	0.78	0.08
m&p-Xylene	1.9	0.23	2.12	0.55	2.09	0.47
Styrene	0.97	0.34	1.32	0.31	1.27	0.27
Naphthalene	1.33	0.09	1.75	0.5	1.69	0.43

				Low Aromatic					
	Cold-Start			Hot-Start			Weighted Total (1)		
	Mean	SDEV	(2)	Mean	SDEV	(2)	Mean	SDEV	(2)
	mg/Bhp-hr	mg/Bhp-hr	%	mg/Bhp-hr	mg/Bhp-hr	%	mg/Bhp-hr	mg/Bhp-hr	%
1,3-Butadiene	2.19	0.53	5.3	2.5	0.13	42	2.46	0.13	37
Benzene	7.29 *	0.38	12	8.15 *	1.17	41	8.03	1	36
Toluene	1.96	0.23	-9.7	2.31 *	0.33	22	2.26	0.28	17
Ethylbenzene	0.54	0.11	-27	0.69	0.2	-47	0.67	0.17	-45
o-Xylene	0.58 *	0.05	-32	0.62	0.11	-19	0.61	0.09	-21
m&p-Xylene	1.24	0.2	-35	1.24 *	0.34	-42	1.24	0.29	-40
Styrene	2.1	1.6	116	1.49	0.21	13	1.58	0.29	24
Naphthalene	1.55	0.54	17	1.77	0.25	1.1	1.74	0.22	2.9

				Reformulated					
	Cold-Start			Hot-Start			Weighted Total (1)		
	Mean	SDEV	(2)	Mean	SDEV	(2)	Mean	SDEV	(2)
	gm/Bhp-hr	mg/Bhp-hr	%	mg/Bhp-hr	mg/Bhp-hr	%	mg/Bhp-hr	mg/Bhp-hr	%
1,3-Butadiene	1.72	0.04	-17	1.87	0.15	6.8	1.84	0.13	2.9
Benzene	6.78	0.19	4.5	5.65	0.8	-2.6	5.81	0.68	-1.5
Toluene	1.86	0.55	-14	1.86	0.25	-1.6	1.86	0.23	-3.6
Ethylbenzene	1.03	0.65	39	1.2	0.72	-7.7	1.18	0.62	-3.6
o-Xylene	0.99	0.11	16	0.86	0.24	12	0.88	0.21	12
m&p-Xylene	1.84	0.39	-3.1	2.19	0.36	3.3	2.14	0.31	2.5
Styrene	1.36	0.19	40	1.46	0.31	11	1.45	0.27	14
Naphthalene	1.18	0.07	-11	1.29	0.25	-26	1.27	0.21	-24

(1) Wt. Tot .= (1/7 mg(cold) + 6/7 mg(hot))/(1/7 Bhp-hr(cold)+ 6/7 Bhp-hr(hot)); SDEV = v(1/6 SDEV(cold))2 + (6/7SDEV(hot))2

(2) Percent change in mean from the Pre-1993 fuel.

* Significant at 95% Confidence limit using Fisher's Protected Least Significant Difference Test

Summary Table 4 Average Emission Rates for Polycyclic Aromatic Hydrocarbons from Three Hot Start Sample Collections per Fuel Type.

	Pre-1993 Diesel Fuel		Low Aromatic Diesel Fuel		Reformulated Diesel Blend		
PAH	Average µg/bhp-hr	SDEV µg/bhp-hr	Average µg/bhp-hr	SDEV µg/bhp-hr	Average µg/bhp-hr	SDEV µg/bhp-hr	P-value
2,3,5-trimethyl naphthalene [a,b]	283.68	5.27	14.77	2.42	56.21	2.82	<0.0001
phenanthrene [c]	336.71	9.08	160.92	15.54	220.73	52.68	0.002
anthracene [d]	38.89	1.43	18.54	2.13	26.16	6.86	0.003
Me-phenanthrenes/anthracenes [c,e]	331.32	16.07	25.17	1.41	111.98	28.74	<0.0001
fluoranthene [d]	128.45	7.60	132.36	18.30	123.07	26.21	0.839
pyrene [d]	193.03	16.51	211.19	37.35	206.82	39.04	0.784
benzo[c]phenanthrene	3.03	0.24	1.74	0.14	1.54	0.26	0.000
benzo[ghi]fluoranthene [f]	24.84	2.68	18.93	2.14	16.94	2.31	0.016
cyclopenta[cd]pyrene	21.44	4.11	26.15	3.12	21.25	3.46	0.245
benz[a]anthracene	16.42	1.67	10.57	1.15	10.96	2.42	0.013
chrysene + triphenylene [g]	17.36	1.66	10.38	0.54	12.20	2.72	0.009
benzo[b+j+k]fluoranthene [g]	31.05	4.17	23.17	1.98	29.18	7.93	0.242
benzo[e]pyrene	16.71	2.72	14.55	1.34	18.99	5.58	0.392
benzo[a]pyrene	20.46	3.27	16.48	1.56	20.59	5.75	0.403
perylene	4.32	0.88	3.71	0.74	4.18	1.16	0.718
indeno[1,2,3-cd]fluoranthene	0.34	0.07	0.21	0.02	0.17	0.00	0.005
benzo[c]chrysene	0.29	0.05	0.18	0.05	0.14	0.04	0.025
dibenz[a,j]anthracene	0.93	0.05	0.55	0.10	0.67	0.09	0.004
indeno[1,2,3-cd]pyrene	19.45	2.71	14.04	1.99	22.16	9.11	0.272
dibenz[a,h + a,c]anthracene [g]	1.54	0.15	0.87	0.12	1.48	0.67	0.154
benzo[b]chrysene	0.40	0.01	0.15	0.05	0.27	0.05	0.001
benzo[ghi]perylene	49.17	9.63	39.81	7.22	60.74	26.60	0.375
coronene	9.49	3.13	4.93	0.47	7.48	1.59	0.088
dibenzo[a,l]pyrene	2.84	0.45	1.25	0.15	2.31	0.48	0.007
dibenzo[a,e]pyrene	1.10	0.29	0.61	0.06	1.13	0.15	0.027
dibenzo[a,i]pyrene	0.91	0.21	0.27	0.09	0.71	0.15	0.007
dibenzo[a,h]pyrene	1.33	0.25	0.75	0.07	0.84	0.20	0.022

[a] Lower limit based on summing amounts on front PUF and back PUF (amount on filter negligible).

[b] The area of the molecular ion peak (m/z 170) and the response factor for 2,3,5-trimethylnaphthalene relative to deuterated phenanthrene were used to quantify 2,3,5-trimethylnaphthalene and a co-eluting isomer.

[c] Sum of amounts on filter, front PUF and back PUF.

[d] Sum of amounts on filter and front PUF; negligible amount found on back PUF.

[e] The areas of the molecular ion of the five isomers present were summed and the response factor for 1-methylphenanthrene relative to deuterated phenanthrene was used to quantify all isomers.

[f] Standard not available, response factor for cyclopenta[cd]pyrene relative to deuterated chrysene used for quantification.

[g] Co-eluting isomers.

Summary Table 5 Average Emission Rates for Nitro-Polycyclic Aromatic Hydrocarbons from Three Hot Start Sample Collections per Fuel Type.

	Pre-1993		Low Aromatic		Reformulated		
Nitro-PAH	**Average** μg/bhp-hr	**SDEV** μg/bhp-hr	**Average** μg/bhp-hr	**SDEV** μg/bhp-hr	**Average** μg/bhp-hr	**SDEV** μg/bhp-hr	**P-value**
1-nitronaphthalene	0.52	0.02	0.32	0.02	0.66	0.35	0.1988
2-nitronaphthalene	1.51	0.02	0.69	0.05	1.37	0.38	0.0078
methylnitronaphthalenes	<0.5		<0.5		<0.5		
2-nitrobiphenyl	<0.5		<0.5		<0.5		
4-nitrobiphenyl	<0.5		<0.5		<0.5		
5-nitroacenaphthene	<0.5		<0.5		<0.5		
2-nitrofluorene	<0.3		<0.3		<0.3		
9-nitroanthracene[a]	0.56	0.06	0.54	0.01	0.34	0.08	0.0083
1-nitropyrene	1.95	0.27	2.07	0.11	1.64	0.43	0.2670
3-nitrofluoranthene	<0.06		<0.06		<0.06		
4-nitropyrene	<0.06		<0.06		<0.06		
7-nitrobenz[a]anthracene	<0.1		<0.1		<0.1		
6-nitrochrysene	<0.1		<0.1		<0.1		
6-nitrobenzo[a]pyrene	0.31	0.08	0.42	0.04	0.39	0.08	0.1988

[a] For the Pre-1993 Fuel and the Reformulated Blend, interferences prevented quantification using the molecular ion at m/z 223 and the [M-NO$^+$] fragment ion was used.

Naphthalene emission rates were the highest of all vapor phase PAHs for all three fuels. All the targeted vapor phase PAHs except acenaphthene were detected in the exhaust of all three fuels. The emission rates of the vapor-phase alkyl-PAH, 1-and 2-methylnaphthalene, 2,6/2,7-dimethylnaphthalene, and 2,3,5-trimethylnaphthalene, consistent with the results obtained for the particle-bound alkyl-PAH, were greatest for the pre-1993 fuel with its high PAH fuel content. The vapor-phase alkyl-PAH, as occurred for the particle-bound PAH, were lowest in the emissions from the low aromatic fuel. Because alkyl-PAH are formed in lower abundance than their parent PAH in high temperature combustion processes, the high alkyl-PAH emissions from the high PAH content fuel suggests that a significant portion of the alkyl-PAH emissions are due to unburned fuel components, and the PAH formed during the combustion process are generally unsubstituted PAH. The lowered emissions of volatile alkyl-PAH with decreased fuel PAH content may be expected to lead to a decreased potential for the atmospheric formation of mutagenic nitro-PAH and nitro-PAH lactones.

Summary Table 6 Gas Phase PAH Emissions from Single Hot Start Samples.

Fuel Sample Identification	Pre-1993 H3 μg/Bhp-hr	Low Aromatic H4 μg/Bhp-hr	Reformulated H6 μg/Bhp-hr
naphthalene	2,921	1,905	2,078
2-methylnaphthalene	1,637	225	949
1-methylnaphthalene	1,118	158	547
biphenyl	481	110	379
2,6/2,7-dimethylnaphthalene	720	54	264
acenaphthylene	250	191	196
acenaphthene [a]	CoE	CoE	CoE
2,3,5-trimethylnaphthalene [b]	572	48	156

a. Another compound coelutes with acenaphthene and it cannot be quantitated.

b. 2,3,5-trimethylnaphthalene coelutes with another naphthalene, quantitation is based on trimethylnaphthalene.

Summary Table 7 Vapor-Phase and Particle-Associated Alkyl PAH Emissions

	Pre-1993 (ug/Bhp-hr.)	Low Aromatic (ug/Bhp-hr.)	Reformulated (ug/Bhp-hr.)
1-methylnaphthalene	1,118	158	547
2-methylnaphthalene	1,637	225	949
2,6/2,7-dimethylnaphthalenes	720	54	264
2,3,5-trimethylnaphthalene	572	48	156
methylphenanthrenes	331	25	112

The emission trends for unsubstituted PAH with four and more rings are similar for all fuels, with pyrene being the most abundant of those measured, followed by fluoranthene and benzo[ghi]perylene. Ten PAH showed no statistically significant difference in emission rate as a function of fuel type. These ten PAH were: fluoranthene, pyrene, cyclopenta[cd]pyrene, benzo[b+j+k]fluoranthenes, benzo[e]pyrene, benzo[a]pyrene, perylene, indeno[1,2,3-cd]pyrene, benzo[ghi]perylene, and dibenzo[a,h+a,c]-anthracene. Ten PAH showed statistically higher emission rates from the pre-1993 fuel than from either the reformulated or low aromatic fuel, but showed no difference between the latter two fuels. These ten PAH were: phenanthrene, anthracene, benzo[c]phenanthrene, benzo[ghi]fluoranthene, benz[a]anthracene, chrysene + triphenylene, indeno[1,2,3-cd]fluoranthene, benzo[c]chyrsene, dibenz[a,j]anthracene, and dibenzo[a,h]pyrene. Benzo[b]chrysene was the only PAH other than the alkyl-PAH which showed significant differences between all three fuel pairs and, as with the alkyl-PAH, the ranking of the emission rates was: pre-1993 fuel highest, reformulated intermediate and low aromatic fuel lowest. For dibenzo[a,l]pyrene, dibenzo[a,e]pyrene, and dibenzo[a,i]pyrene, the low aromatic fuel emission rates were significantly lower than both the pre-1993 and reformulated fuels, which were not different from one another. For coronene, the pre-1993 fuel had significantly higher emissions than the low aromatic fuel. In all cases where there was a statistically significant difference between the low aromatic fuel PAH emission rate and one or both of the other fuels, the low aromatic fuel PAH emission rate was lower.

The samples analyzed for nitro-PAH were those collected with the high-volume sampling system using filters backed by PUFs. 1-Nitropyrene, 6-nitrobenzo[a]pyrene, 9-nitroanthracene, and 1- and 2-nitronaphthalene were found in the emissions from all three test fuels. 1-Nitropyrene was the most abundant nitro-PAH measured. The nitronaphthalenes were obtained from PUF extracts, while the remaining nitro-PAH were particle-bound. There was no statistically significant difference in the emission rates as a function of fuel type for 1-nitronaphthalene, 1-nitropyrene and 6-nitrobenzo[a]pyrene . 9-Nitroanthracene was lower in the reformulated fuel emissions than either the low aromatic or pre-1993 fuels. The low aromatic fuel had lower emission rates of 2-nitronaphthalene than the pre-1993 and reformulated fuels.

Nitrosamines sampling was performed for the pre-1993 and reformulated fuels, as part of a scoping experiment to evaluate the validity of sampling method. The emission rate of N-nitrosodimethylamine was 6.41 mg/Bhp-hr for pre-1993 fuel while the reformulated fuel was slightly greater at 7.92 mg/Bhp-hr. N-nitrosodipropylamine was detected in the emissions of the pre-1993 and reformulated fuels. N-nitrosomorpholine was not detected in the emissions of the pre-1993 and reformulated fuels [Summary Table 8].

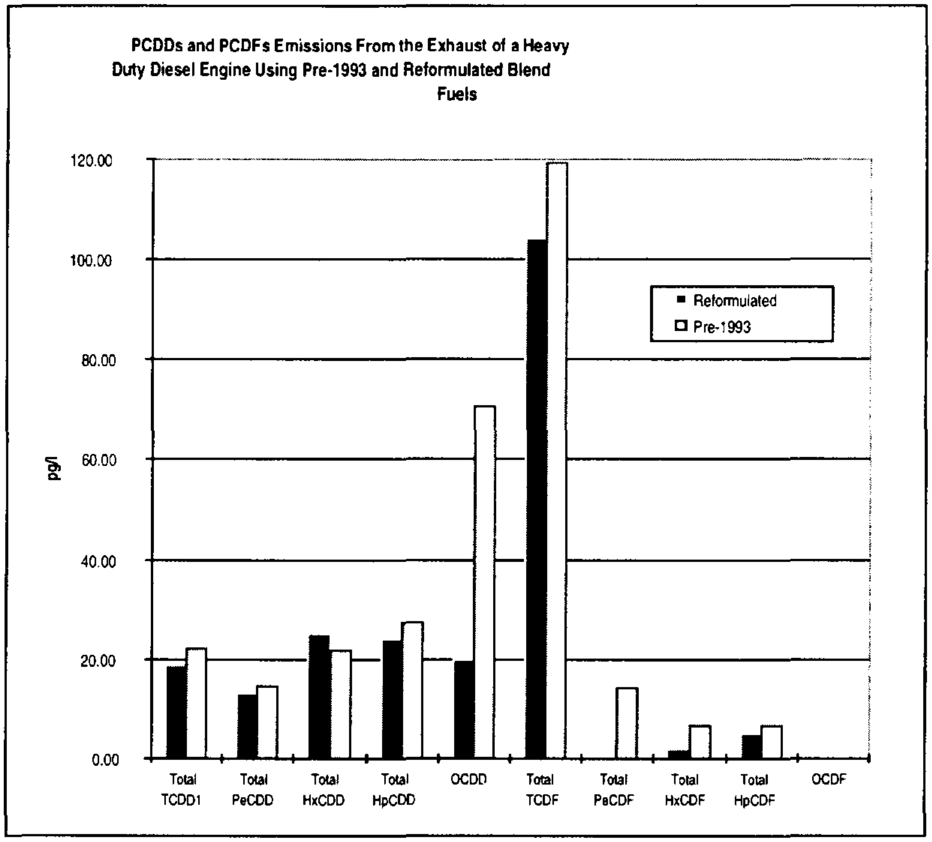
Dioxin sampling was conducted for the pre-1993 and reformulated fuels only, this is due to limintations in time available to collect additional samples for the low aromatic fuel. Although chlorinated dioxins and dibenzofurans, PCDDs and PCDFs, were detected, the most toxic isomers, 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD and 2,3,4,7,8-PeCDF were not detected in either the pre-1993 or reformulated fuel. TEQ profiles were incomplete due to the low level of PCDD and PCDF detected in the emission samples. This was the case even though detection limits were estimated to be 7-10 times lower than the EPA recommended 100 pg TEQ per liter of fuel target detection level. [Summary Figure 1].

Mutagenic activity was detected in the particle and vapor-phase emissions from all fuels tested. The pre-1993 fuel has higher specific mutagenic activity (activity per mg particulate matter or per vapor-phase extract) than the emissions from low aromatic and reformulated fuels. Higher mutagen emission values (mutagenic activity per brake-horsepower hr.) were observed in the particle and vapor-phase samples collected from pre-1993 fuel than in the low aromatic and reformulated fuels. Mutagenic activity of HPLC fractions provided mutagenic profiles or "mutagrams" of each fuel type. The most mutagenic fraction for the particulate matter is in a fraction that is different from the fractions where the PAHs and nitro-PAHs are present. For all fuels, one fraction accounts for approximately 60-70% of the total activity (+S9) and two fractions account for approximately 70-80% of activity without metabolic enzymes added (-S9) [Summary Table 9].

Summary Table 8 Nitrosamine Emission Rate.

	Pre-93		average	Reformulated		average
	76798	76806	(precision)	76803	76811	(precision)
Nitrosamine	µg/Bhp-hr.	µg/Bhp-hr.	µg/Bhp-hr.	µg/Bhp-hr.	µg/Bhp-hr.	µg/Bhp-hr.
N-nitrosodimethylamine (NDMA)	5.81	7.02	6.41 (9.41)	7.84	8	7.92 (1.01)
N-nitrosodiethylamine (NDEA)	<7.7	<7.9		<7.8	<7.9	
N-nitrosodipropylamine (NDPA)	8.28	<7.9		<7.8	7.2	
N-nitrosobutylamine (NDBA)	<12	<12		<12	<12	
N-nitrospiperidine (NPIP)	<7.7	<7.9		<7.8	<7.9	
N-nitrosopyrolidine (NPYR)	<7.7	<7.9		<7.8	<7.9	
N-nitrosomorpholine (NMOR)	<7.7	<7.9		<7.8	<7.9	

Summary Figure 1 Emission Profile of PCDDs and PCDFs Isomer Classes for Pre-93 and Reformulated Diesel.



Summary Table 9 Emission Rate of Mutagenic Compounds.

	Emission Rrate (+S9)	95% Confidence Interval (+S9)	Emission Rate (-S9)	95% Confidence Interval (-S9)
Fuel	(Rev/Bhp-hr. x 10^6)		(Rev/Bhp-hr. x 10^6)	
Pre-1993	5.65	0.61	7.4	1.41
Low Aromatic	2.33	0.29	3.75	0.48
Reformulated	3.42	0.65	4.66	0.88

Duplicate experiments

1.0 INTRODUCTION

1.1 Background

Diesel exhaust has been designated by the International Agency for Research on Cancer as a probable human carcinogen, and is undergoing evaluation as a potential toxic air contaminant under the California Air Resources Board's (CARB's) air toxics program. Diesel exhaust is a complex mixture consisting of hundreds of compounds. Effective October 1, 1993, the CARB mandated new highway diesel fuel regulations (CARB, 1991) which limited the maximum sulfur content to 0.05%, the minimum cetane index to 40, and the maximum aromatic content to 10%. These regulations were based upon a review of available data (CARB, 1988) on the impact of fuel properties on criteria pollutant emissions, total hydrocarbons (THC), carbon monoxide (CO), oxides of nitrogen (NOx), and particulate matter (PM). Since lowering the aromatic content from levels above 30% to those below 10% requires major capital investment and operating costs, CARB has allowed fuel producers the option of producing a less-costly alternative fuel with a higher aromatic content, if equivalent emissions can be demonstrated (CARB, 1988). There is an extensive literature on the effects of diesel fuel parameters on emissions of criteria pollutants, but little is know on the impact of these fuel changes on the complex speciation of the gas-, semi-volatile-, and particle-phase components and specifically on emissions of toxic components present in diesel exhaust. This study was designed to provide this information on three diesel fuels: a pre-1993 fuel, a low aromatic fuel, and an alternative formulation, henceforth refered to as reformulated. This work has been conducted by a team of researchers from the College of Engineering-Center for Environmental Research and Technology (CE-CERT), the Statewide Air Pollution Research Center (SAPRC) at the University of California (UC) Riverside, and the UC Davis Department of Environmental Toxicology. Emission testing was conducted at the Los Angeles County Metropolitan Transportation Authority (LACMTA) emission test facility.

1.2 Scope of Work

The scope of this project was to obtain a preliminary assessment of the potential impact of diesel fuel formulation on the speciation and toxic components of diesel exhaust. The test bed was a Cummins L10 engine operating over the heavy-duty transient test cycle using three diesel fuels: a pre-1993 diesel fuel, a low aromatic diesel fuel, and an alternative formulation diesel fuel. The sampling/analysis plan included:

- Determination of the criteria pollutant emission rates (THC, CO, NOx, and PM)
- Determination of PM_{10} and $PM_{2.5}$ emission rates
- Collection and analysis of particulate samples for elemental, inorganic ion and elemental/organic carbon analyses
- Collection of bag samples for VOC speciation analyses
- Collection of 2,4-dinitrophenylhydrazine (DNPH) cartridges for determination of oxygenates
- Collection of nitrosomorpholine with Thermosorb N cartridges
- Collection of semi-volatiles on PUF/XAD and particulate samples for PAH, nitro-PAH, and mutagenicity studies.
- Collection and analysis of dioxins for the pre-1993 and alternative formulation diesel fuels.

1.3 Intended Use of Data

The data collected in this study are to be used to assess the influence of diesel fuel formulation on the emission of toxic species and will also be used for designing the scope and direction of more comprehensive studies. This study provides information in a research area having limited existing data. The results from this study will be referenced in California's report "Proposed Identification of Diesel Exhaust as a Toxic Air Contaminant" to augment the discussion about the chemical composition of the exhausts of diesel powered engines using pre-1993 and post-1993 diesel fuels. An acknowledgement of the limited scope of the study will be included.

1.4 Project Objectives

The objective of this study was to collect toxic component related data from a Cummins L10 engine with pre-1993 diesel fuel, a low aromatic diesel fuel, and an alternative formulation diesel fuel, operating over the EPA heavy-duty diesel engine transient Federal Test Procedure (FTP). The data quality objectives are to determine the magnitudes of the differences in toxic emissions and their variability among the different fuel types. To meet these data quality objectives, multiple samples were collected during multi-day testing for each of the three fuels. For each fuel, the objectives were to:

- Measure all regulated emissions: THC, CO, CO_2, NOx, total particulates.
- Quantify the mass fraction of the particulate below 10 and 2.5 μm aerodynamic diameter.
- Collect, identify, and quantify at least 80% of the C_1-C_{12} VOC emissions.
- Collect, identify, and quantify at least 80% of carbonyl compounds.
- Collect, identify and quantify the elemental components and inorganic ion components in the particle phase.
- Quantify the elemental and inorganic carbon fractions of the particulate.
- Collect, identify and quantify the particle-bound PAH and nitro-PAH emissions.
- Collect, identify, and quantify the gas-phase PAH and nitro-PAH emissions.
- Determine mutagenicity.
- Collect, identify, and quantify the nitrosomorpholine emissions for the pre-1993 and reformulated fuel.
- Collect, identify, and quantify the dioxins for analytical method development for the pre-1993 and reformulated fuel.

2.0 PROCEDURES

2.1 Engine Set-Up

All testing was performed at the Los Angeles County Metropolitan Transportation Authority (LACMTA) engine dynamometer facility, which is equipped and calibrated to perform the EPA heavy-duty diesel engine transient FTP. Engine installation, set-up, and operation were performed by LACMTA staff. Specifications for the Cummins L10 test engine are listed below:

Engine Family	:	343E
Engine Model	:	L10-310
Engine S/N	:	34705128
Engine Type	:	In-line 6 cylinder, turbocharged direct injection
Displacement	:	10.0 liters (611 cu. in.)
Advertised HP (SAE)	:	310 @ 1600 RPM
Peak Torque	:	1150 ft-lb. @ 1200 RPM

This engine was provided by Cummins Engine Company, Inc. and had been certified to the EPA and California standards for the 1991 to 1993 model years (THC-1.3 g/Bhp-hr, CO-15.5 g/Bhp-hr, NOx-5.0 g/Bhp-hr, PM-0.25 g/Bhp-hr). Engine set-up, mapping procedures, and transient cycle generation was performed according to the procedures outlined in the Code of Federal Regulations (CFR), Title 40, Part 86, Subpart N and as outlined below:

1. Start engine and run at various speeds and loads until the pan and water temperatures have stabilized. Set up the engine at rated speed (1800 rpm)

Intake restriction (in. H_2O)	17.5 ±1.0
Exhaust restriction (in. Hg)	2.4±0.2
Max crossover restriction	4.0
Intake manifold temperature (°F)	115±5
Water supply temperature (°F)	77±4
Engine coolant temperature (°F)	165±10
Fuel in temperature (°F)	100±10

2. Record full load performance data at the following speeds (1800 & 1200 rpm)
3. Run reference torque map
4. Run practice cycles
5. Conduct natural soak until the engine oil sump is stabilized below 86 °F.
6. Run cold cycle, 20-minute soak, and hot cycle. Additional hot cycles run as required by the test matrix.
7. Repeat 5 and 6 to complete test matrix for each fuel.

2.2 Fuel and Fuel Change Procedures

The required specification ranges for the fuels are presented in Table 1. The pre-1993 diesel fuel was supplied by Paramount Petroleum, the low aromatic fuel by Chevron, and the alternative formulation, henceforth referred to as Reformulated fuel, consists of a splash blended mix of equal volumes of fuel from the three major retailers in the South Coast Air Basin (Chevron, ARCO, and Texaco). Fuel analyses provided by the supplier and determined by the ARB's EL Monte and Core Laboratories are presented in Table 2. All fuels met the required specification ranges.

Table 1. Diesel Fuel Specification Ranges

Fuel Type	Pre-1993	Low Aromatic	Reformulated
Aromatic HC-Vol. %	33	10 max.	20-25
Sulfur-ppm Wt.	<5000	500 max.	100-300
Cetane Number	>40	48 min.	50-55
PAH - Wt. %	8	1.4 max.	2-5
Nitrogen – ppm Wt.	300-600	10 max.	40-500

Fuel was stored in sealed 55-gallon drums prior to use. Prior to testing with each fuel, the engine oil was drained and filled with new oil, and the oil and fuel filters were changed. Oil analysis results are presented in Table 3. The engine mapping procedures and at least two practice cycles, as outlined in Section 2.1, were conducted at the start of testing with each new fuel. In addition to verifying that the statistical criteria for transient testing were satisfied, the practice cycles also served to precondition the CVS dilution tunnel for the changes in emissions associated with each fuel.

2.3 Test Sequence

To meet the sampling requirements for this project, each fuel was tested under a multi-cycle test sequence. For each fuel, the engine mapping procedures and the practice cycles were run and then the engine was allowed to stand overnight at soak temperatures of 68 to 86 °F in preparation for cold-start testing the following day. Due to the extensive engine preconditioning sequence, which included fuel, oil and filter changes followed by a series of engine mapping cycles, randomized fuel changeovers were minimized. Adopting this test procedure maximized the time allotted for collect test samples. During each day's testing, a cold-start transient test was followed at 20-minute intervals by a sequence of hot-start transient tests. At the end of the day's testing the engine was again allowed to soak overnight in preparation for the next day's testing. The inability to conduct more than one cold start transient test per day has limited the total number cold start samples. This limitation does not apply to the hot start transient tests, which can be replicated multiple times during each day of testing. To further maximize the time alloted for testing, the collection of tunnel blank samples was conducted in the morning prior to engine testing during the period normally used to warm-up and calibrate the emission test equipment. The fuel testing sequence was conducted in the following order: pre-1993, low aromatic, alternative formulation, retest with pre-1993. Testing at the beginning (initial) and end (final) of the sampling period with the pre-1993 fuel allowed an assessment of the effects of any drift in engine emissions during the testing period. Table 4 summarizes the test cycles conducted for each fuel. Test cycles, and samples collected during these cycles are designated by the Julian Date, the type of cycle (cold or hot), and for multiple hot cycles run on the same day, the cycle sequence (hot 1, hot 2, hot 3, etc.).

Table 2. Fuel Analyses.

	Pre-1993			Low Aromatic		Reformulated Blend			
Analytical Laboratory	Core Labs	Paramount	CARB	Core Labs	Chevron	Core Labs	CARB "A"	CARB "B"	CARB "C"
API Gravity	32.8	32.8		37.2	37.3	37.1			
Sulfur, ppm wt.	349.7	361		<0.1	<6	71.3	241	50.6	<10
Nitrogen, ppm wt.	352			<1	0.54 microgm/mL	205			
SFC, wt %									
Aromatics	31.0	30.5	28.8	4.3	4.0	22.3	22.5	23.3	23.3
Polynuclear Aromatics	5.9	7.4	6.17	0.7	0.7	4.1	4	4.33	2.4
Non-Aromatics	69.0			95.7		77.7			
Boiling Point Dist., °F									
IBP	389	382		345		386			
5% point	427			408		424			
10% point	448	453		443	440	440			
20% point	476	478		485		466			
30% point	494			509		489			
40% point	512			530		511			
50% point	529	530		546	548	530			
60% point	547			561		550			
70% point	567			575		568			
80% point	592			592		588			
90% point	627	628		616	616	614			
95% point	665			638		641			
EP	708	694		663		661			
% recovery	98.0	98.0		98.2		98.3			
% residue	0.9	1.0		1.0		1.0			
% loss	1.1	1.0		0.8		0.7			
Cetane Number D-613	43.5			54.2		54.4			
Viscosity @ 40°C, cSt	3.50	3.42		3.52		3.27			
Flash Point, °F	169	168		144		162			
Cloud Point, °F	12	20		10		14			
Pour Point, °F	10			10		10			
Ash Content, routine	<0.001			<0.001		<0.001			
Copper Strip Corrosion	1A	1A		1A		1A			
Elemental Analyses (mg/kg)									
Antimony (Sb)	ND			ND		ND			
Arsenic (As)	ND			ND		2.1			
Beryllium (Be)	ND			ND		ND			
Cadmium (Cd)	ND			ND		ND			
Chromium (Cr)	ND			ND		ND			
Cobalt (Co)	ND			ND		ND			
Copper (Cu)	ND			ND		ND			
Lead (Pb)	ND			ND		ND			
Manganese (Mn)	ND			ND		ND			
Mercury (Hg)	<0.20			<0.2		<0.2			
Nickel (Ni)	ND			ND		ND			
Selenium (Se)	<0.20			<0.1		<0.1			
Chloride	<10			20		56			

Table 3. Oil Analysis

OIL	
Analytical Laboratory	Core Labs
Elemental Analyses (mg/kg)	
Antimony (Sb)	ND
Arsenic (As)	ND
Beryllium (Be)	ND
Cadmium (Cd)	ND
Chromium (Cr)	ND
Cobalt (Co)	ND
Copper (Cu)	ND
Lead (Pb)	ND
Manganese (Mn)	ND
Mercury (Hg)	<0.2
Nickel (Ni)	ND
Selenium (Se)	0.3
Chloride	230

2.4 Los Angeles County Metropolitan Transit Authority (LACMTA) Dilution Tunnel Facility and Measurements of THC, CO, CO_2, NOx, and PM.

All sampling was performed at the LACMTA test facility using a Horiba CVS-48 HDD constant volume sampler (CVS). The Horiba CVS consists of an 18" primary dilution tunnel and three critical flow venturis providing for dilute bulkstream flow rates of 1500 scfm, 2500 scfm, 3000 scfm and 4000 scfm. 3000 scfm flow was utilized for this study. A Horiba FIA-43A-2 heated flame ionization detector (HFID) analyzer was used for the continuous measurement of HCs. A Horiba CLA-22A heated chemiluminesence (CL) analyzer was used for the continuous measurement of NOx. A Horiba AIA-23 nondispersive infrared (NDIR) analyzer was used for measurements of CO and CO_2. The Horiba Double Dilution Proportional Sampler was used to collect the total PM emitted during the transient emission test. Samples were extracted from the CVS primary dilution tunnel, diluted with filtered air in the secondary dilution tunnel, and collected on a pair (e.g., primary and secondary) of 70 mm filters. The secondary dilution tunnel is 5" in diameter and the 70-mm filters were Pallflex T60A20 glass fiber filters. Sampling and analysis procedures for THC, CO, CO_2, NOx, and particulate followed the procedures outlined in CFR 86.1310-1340.

Statistical Analysis Differences in means between the fuel types for the measured emissions were tested using the one-way Analysis of Variance (ANOVA). The one-way ANOVA is the best test for differences in means here because of the different sample sizes within treatments resulting from the vehicle test matrix. The one-way ANOVA also has the advantage of having the maximum number of error degrees of freedom for testing the mean differences between fuel types. For variables where the ANOVA indicated significant differences existed between fuel types, paired comparisons were conducted using the Fisher's Protected Least Significant Difference (PLSD) test for unequal sample sizes (Abacus Concepts, StatView). The Fisher PLSD test is a relatively liberal paired comparison test, which is "protected" by the requirement that the test of the main effect must be significant. The sample mean and variance were used to calculate the 95% confidence limits for each variable within each fuel type using the standard formula:

$$\text{Lower Limit} = \text{mean} - t_\alpha * \text{standard error}$$

$$\text{Upper Limit} = \text{mean} + t_\alpha * \text{standard error}$$

where t_α is the two-tailed critical value of the t distribution at level α.

Table 4. Test/Sampling Cycles Run for Each Fuel

Pre-1993 Diesel Fuel

Test Date	12/2/96	12/3/96	12/4/94	12/5/96	12/6/96	1/8/97	1/9/97
Test Cycles	337CS	338CS	339CS	340CS	341CS	8CS	9CS
	337H1	338H1	339H1	340H1	341H1	8H1	9H1
	337H2	338H2	339H2	340H2	341H2	8H2	9H2
	337H3	338H3	339H3	340H3	341H3	8H3	9H3
		338H4	339H4	340H4		8H4	9H4
		338H5	339H5			8H5	9H5
		338H6	339H6			8H6	9H6
		338H7	339H7			8H7	9H7
		338H8					
		338H9					

Low Aromatic Diesel Fuel

Test Date	12/9/96	12/10/96	12/11/96	12/12/19
Test Cycles	344H1	345CS	346CS	347CS
	344H2	345H1	346H1	347H1
	344H3	345H2	346H2	347H2
	344H4	345H3	346H3	347H3
		345H4	346H4	347H4
			346H5	347H5
			346H6	347H6

Reformulated Diesel Fuel

Test Date	12/16/96	12/17/96	12/18/96	12/19/96	12/20/96
Test Cycles	351CS	352CS	353CS	354CS	355CS
	351H1	352H1	353H1	354H1	355H1
	351H2	352H2	353H2	354H2	355H2
	351H3	352H3	353H3	354H3	355H3
	351H4	352H4	353H4	354H4	355H4
	351H5	352H5	353H5	354H5	355H5
	351H6	352H6	353H6	354H6	355H6
		352H6	353H7	354H7	355H7

Figure 1 presents a schematic of the LACMTA engine dynamometer dilution tunnel and the associated sampling systems utilized for collection of samples for detailed chemical and physical analysis. Specific procedures utilized are described below.

2.5 Speciated Hydrocarbons (C_1-C_{12})

Samples for the C_1-C_{12} HC speciation were collected in 8 L black Tedlar GC bags. At the end of a test run, a PTFE-coated diaphragm pump was utilized to transfer the samples from the CVS bag system to the GC bags. For each test run a background and emission sample were collected. Final reported results are background corrected. New sets of bags were utilized for each fuel to avoid cross contamination. Prior to use, bags were filled with vehicle emission zero (V.E.Z.) grade nitrogen and checked for impurities. Prior to reuse, bags were evacuated and filled with V. E. Z. grade nitrogen three times. Hydrocarbon speciation analyses utilized the Auto/Oil Phase II protocol (Siegl, et al., 1993). Light hydrocarbons (C_1 through C_4) were measured using a Hewlett-Packard (HP) 6880 Series GC with a flame ionization detector (FID) maintained to 250° C. A 15 m x 0.53 mm polyethylene glycol pre-column and a 50 m x 0.53 mm aluminum oxide "S" deactivation porous layer open tubular (PLOT) column were used. A 5-ml stainless steel sample loop was conditioned with the sample from the GC bag prior to analysis. A second HP 6880 Series GC with a FID maintained to 300° C was used to measure the C_4 to C_{12} hydrocarbons. A 2 m x 0.32 mm deactivated fused silica pre-column and a 60 m x 0.32 mm HP-1 column was used. A 5-ml stainless steel sample loop was conditioned with the sample from the GC bag prior to analysis. Analyses were completed within four hours of sample collection.

2.6 Carbonyls

Samples for analysis of carbonyls were collected utilizing Waters Sep-Pak Silica cartridges coated with acidified 2,4-dinitrophenylhydrazine (DNPH) from the CVS dilution tunnel through a heated sample line (110 °C) with the sample probe in the same location as the integrated (i.e., continuous) gaseous and particle sample probes, and from the test cell for a background ambient air measurement. All results presented are background corrected. Sample flows were maintained at approximately 1.0 L/min with needle valves and measured with calibrated dry gas meters located downstream of the DNPH cartridges. DNPH cartridges were sealed and stored at 0°C prior to use and after sampling until analyzed. DNPH cartridges were analyzed using the Auto/Oil Phase II protocol (Siegl, et al., 1993). Cartridges were eluted (via gravity feed) with 5 ml of acetonitrile and transferred to liquid chromatography (LC) vials and capped for analysis.

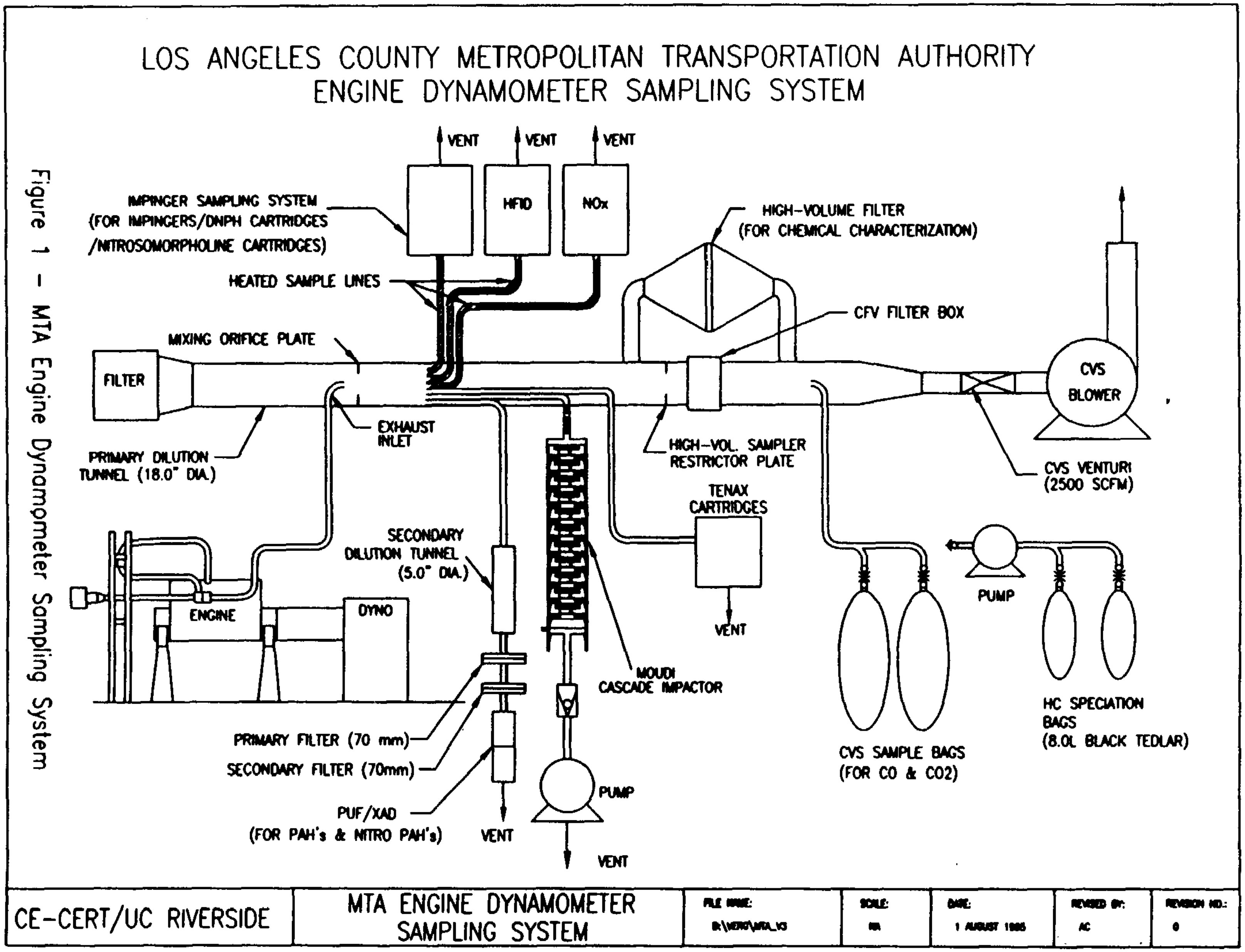


Figure 1. Schematic of LACMTA test facility

A Shimadzu SCL 10A high performance liquid chromatograph (HPLC) with a variable ultraviolet detector and a 5 µm Deltabond AK C18 column was used for analysis of carbonyls. Analyses were performed within 36 hours of sample collection.

2.7 Particle Size Measurement

A Micro Orifice Uniform Deposit Impactor (MOUDI), manufactured by MSP Corporation (Minneapolis, MN), was used for particle size measurements. Due to limitations in the number of sampling ports, impactor samples were extracted from the primary dilution tunnel with an isokinetic sample probe in the same location as the integrated gaseous and particulate sample probes (see Figure 1). Measurement of the diluted exhaust gas temperature immediately in front of the MOUDI showed a maximum temperature of 38.5 °C, well below the 51.7 °C maximum temperature required by the CFR. The MOUDI was operated at a flow rate of 30 lpm with 4 stages having aerodynamic diameter cut points of 10, 5.6, 3.2, and 1.8 µm, together with a 47 mm 2.0 µm Gelman Teflon membrane backup filter for collection of particles less than 1.8 µm. Aluminum foil substrates were used for the impaction plates. Filter and substrate samples were stored in sealed and labeled plastic petri dishes in a cooler packed with blue ice for transport to and from the LACMTA test facility. Sample flow was controlled with a needle valve and calibrated orifice with total flow measured with a dry gas meter. Filter and aluminum substrate weights were determined with an ATI Orion Cahn ultra-microbalance to a precision of ±10 µg. Tare and final substrate weights were determined after at least 24 hours equilibration at 25±0.5 °C and a relative humidity of 40±5%.

2.8 Elemental, Inorganic Ion, and Elemental and Organic Carbon

Due to a limited number of available sampling ports, it was necessary to obtain some samples from the primary dilution tunnel using an isokinetic probe. The probe was located in the sample zone in a longitudinal and radial location equivalent to the integrated (i.e., continuous) and particulate sampling probes used in the secondary dilution system (see Figure 1). Measurement of the diluted exhaust gas temperature immediately in front of the filters showed a maximum temperature of 38.5 °C, well below the 51.7 °C maximum temperature required by the CFR. The sampling probe was fitted with a flow splitter mated to two 47-mm filter holders. One filter holder was fitted with Pallflex quartz-fiber 2500 QAP-UP filters prefired at 900 °C for three hours to remove background carbon for organic and elemental carbon analyses and determination of sulfate, nitrate, potassium and ammonium ions. A backup quartz fiber filter was collected to assess for artifact organic carbon collection. The other filter holder was fitted with 2.0 µm Gelman Teflon Membrane filters for elemental analysis. Sample flows were maintained at 30 lpm using needle valves in series with calibrated rotometers and a vacuum pump. All filters were stored in sealed and labeled plastic petri dishes in a cooler packed with blue ice for transport to and from the LACMTA test facility and stored in a refrigerator prior to shipment for analysis. Filter samples were shipped to the Desert Research Institute (DRI) in a cooler with blue ice packs.

Teflon membrane filters were analyzed for elements using x-ray fluorescence (XRF) at DRI. Quartz fiber filters were analyzed for elemental and organic carbon by DRI using the thermal/optical reflectance (TOR) method (Chow et al., 1993) from a 0.512 cm^2 punch from the filter. Sulfate, nitrate, and potassium analyses were performed on the remainder of the quartz fiber filter by ion chromatography, and ammonium analysis by automated colorimetry using the indophenol method.

2.9 Particle-Bound and Gas-Phase PAH and Nitro-PAH (SAPRC)

Sample Collection: The high volume sampling media for PAH and nitro-PAH analysis consisted of 8 in. x 10 in. Teflon-impregnated glass fiber (TIGF) filters backed up by polyurethane foam (PUF) adsorbent in a glass module. Two TIGF filters (front and back) were backed up by two PUF plugs (front and back). A schematic of the high volume sampling train is shown in Figure 2. The sampling assembly is similar to that shown in Figure 4 with the exception that the PUF sorbent module was not backed up by XAD sorbent. The position of the sampler in the dilution tunnel is as shown in Figure 5.

The TIGF filters (Pallflex T60A20) were pre-cleaned by a 20-hr Soxhlet extraction with dichloromethane (DCM, Fisher Optima) followed by drying (60°C, 3 hr) and a 20-hr Soxhlet extraction with methanol (MeOH, Fisher Optima). The filters were dried for four hours at 60 °C and placed onto a rack in a small weighing chamber for equilibration at 18-25°C and 40-50 percent relative humidity. After a 24-hr equilibration, the filters were weighed on a precision balance (Sartorius) and stored individually in pre-labeled glassine envelopes, which were placed in numbered manila envelopes.

The pre-cut PUF plugs were obtained from S&W Plastics (Riverside, CA). Each sampling train consisted of a front PUF plug (3 in. diameter. x 3.5 in. long) and a shorter back PUF plug (3 in. diameter. x 1 in. long). The purpose of the back PUF plug was to check for breakthrough from the main front plug. The PUF plugs were pre-cleaned by extraction with a large Soxhlet apparatus, first with DCM (20 hr) followed by drying (60°C, 3 hr) and Soxhlet extraction with MeOH (20 hr) and drying. The PUF plugs were wrapped in MeOH-rinsed foil and placed in Ziploc bags inside glass canning jars and stored in the dark at -20 °C. The jars containing the plugs were transported to the LACMTA Emissions Test Facility in a cooler and just prior to sampling jars were removed from the cooler and allowed to warm to room temperature before opening.

After sampling, the two PUF plugs were wrapped in foil and each was placed in a separate Ziploc bag before returning them to the glass jar. Each filter was folded in half with the particulate-laden faces together and returned to its respective glassine envelope. The glassine envelope containing the filters was then placed in a Ziploc bag, sealed, and returned to the manila envelope. PUF jars and filters were put promptly into a cooler with blue ice for transport to SAPRC. The samples were transported to SAPRC the same day they were taken and immediately placed in a freezer at -20 °C where they were stored until extracted. The samples taken were either from a single test cycle or from two consecutive test cycles as listed in Table 5.

High-Volume Sampler Particulate Weights: Samples were collected at the LACMTA facility in December 1996. Following collection of particulate and gas-phase emissions onto T60A20 high volume filters and PUF plugs, samples were stored in a freezer at SAPRC until analyzed. Filters were removed from the freezer and equilibrated in a small weighing chamber maintained at 18-25°C and relative humidity of 40-50 percent. Filters were weighed on a Sartorius precision balance after overnight equilibration.

Particulate and gas-phase samples collected on filters and PUFs with the high volume sampling apparatus at LACMTA, along with particulate weights and the hot-cycle samples designated for PAH and nitro-PAH analysis are listed in Table 5. Also noted are the samples that were extracted, fractionated by HPLC and shipped to UC Davis for mutagenicity testing.

Chemicals: HPLC grade acetonitrile, hexane, methanol and methylene chloride were purchased from Fisher Scientific (Optima grade). Standard Reference Material (SRM) 1491, aromatic hydrocarbons in hexane/toluene, was obtained from the National Institute of Standards and Technology (NIST). Other PAH standards included cyclopenta[cd]pyrene, indeno[1,2,3-cd]fluoranthene, dibenzo[a,h]pyrene, and dibenzo[a,i]pyrene (Dr. W. Schmidt, Sieker Landstrasse 19, 2070 Ahrensburg, Germany), benzo[c]phenanthrene, dibenzo[a,e]pyrene, dibenzo[a,l]pyrene, benzo[b]chrysene, benzo[c]chrysene, and benzo[ghi]fluoranthene (BCR), coronene, dibenz[a,c]anthracene, and dibenz[a,j]anthracene (Aldrich).

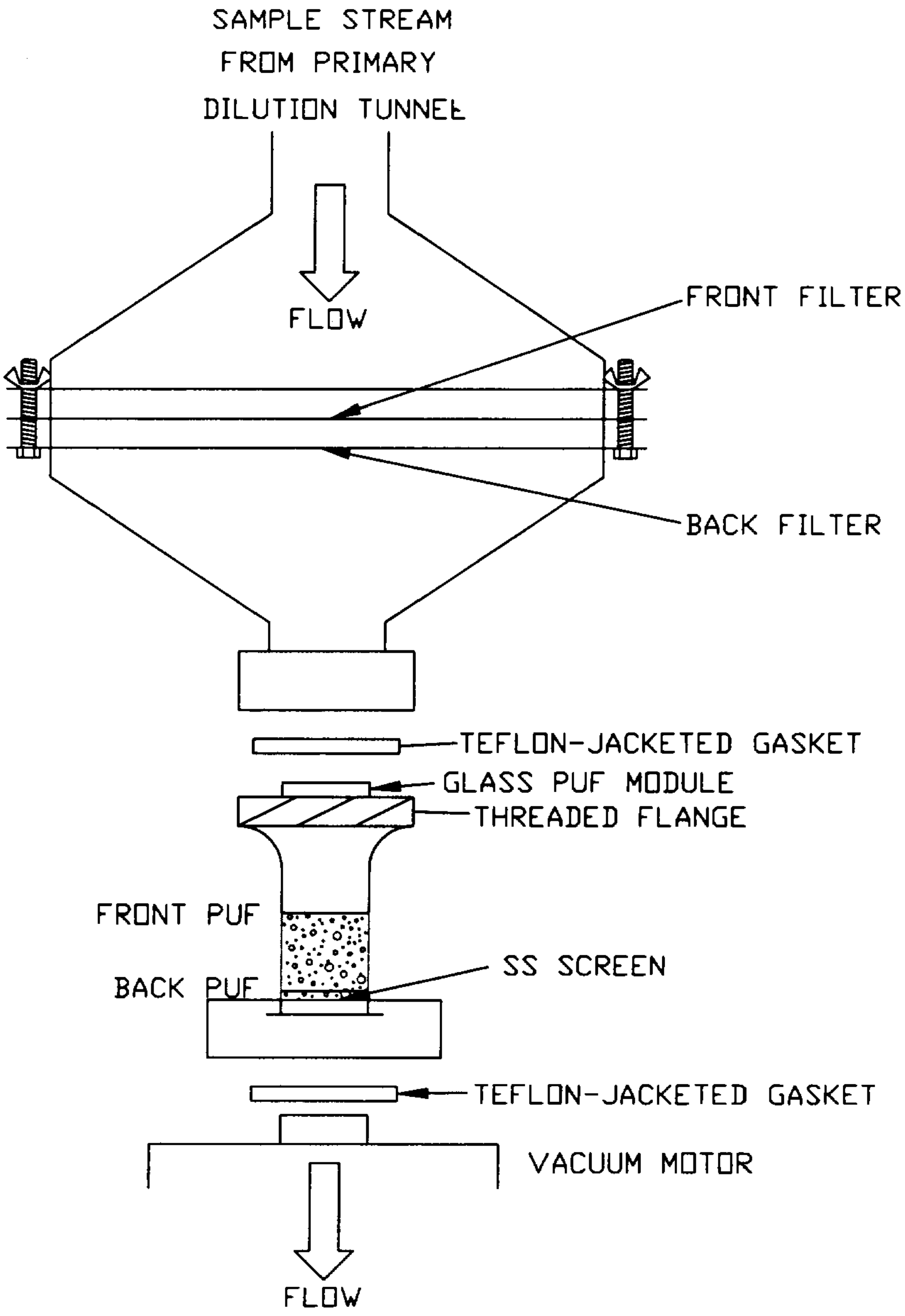


Figure 2. Schematic of high volume sampling system

Deuterated PAH standards included naphthalene-d_8 (Aldrich), acenaphthene-d_{10} and fluoranthene-d_{10} (MSD Isotopes), anthracene-d_{10}, phenanthrene-d_{10}, benzo[a]pyrene-d_{12}, perylene-d_{12}, and dibenz[a,h]anthracene-d_{14} (Cambridge Isotopes), chrysene-d_{12} and pyrene-d_{10} (ICN-KOR) and benzo[b]fluoranthene-d_{12} (C/D/N). Deuterated 1-nitronaphthalene-d_7 was purchased from Aldrich. 1-Nitropyrene-d_9 and 2-nitrofluorene-d_9 were previously synthesized in-house.

Sample Extraction and Fractionation of T60A20 Filters and PUF Samples: Three hot/hot cycle particulate samples were selected for chemical analysis for each fuel type (pre-1993 reference, low aromatic, and alternative formulation blend) as listed in Table 5. Prior to Soxhlet extracting these samples, a hot-cycle screening sample was chosen for each diesel fuel to determine the appropriate level of deuterated internal standards (IS) with the goal of adding IS to the 9 samples at levels comparable to the native PAH in the samples. For each sample, the front and back filters were combined, spiked with deuterated IS and Soxhlet extracted 16 hr in DCM. Front and back PUFs were treated as independent samples to allow quantitative collection of an individual PAH to be determined by the absence of break-through onto the back PUF. Each extract was concentrated by rotary evaporation and filtered through a Gelman Acrodisc CR-PTFE 0.2 mm. Table 6 shows the internal standard (IS) amounts of deuterated PAH and deuterated nitro-PAH added to the T60A20 filters prior to extraction, and the IS amounts added to the front and back PUFs are listed in Table 7.

For each filter analysis sample and each front PUF analysis sample, 25% of the Soxhlet extract was concentrated for PAH analysis by GC-MS, 50% was fractionated by HPLC for nitro-PAH analysis and 25% was reserved. For each back PUF sample, 25% was used for PAH analysis, 37.5% for fractionation and subsequent nitro-PAH analysis, and 37.5% was reserved..

Samples were fractionated prior to GC-MS nitro-PAH quantification and mutagenicity testing (UCD) using a Hewlett-Packard 1050 HPLC equipped with a Model 1040M Diode-array detector (DAD) interfaced to a Foxy 2200 fraction collector. The HPLC was fitted with a Spherisorb 5 µm Si column with a 7 micron Brownlee Si NewGuard precolumn. The solvent programming and sample collection times were as shown on Table 8.

Table 5 T60A20 High Volume Samples and PUF Samples Collected at LACMTA

Sampling Date	Fuel	Test Cycles	SAPRC Filter No.	Particulate Wt.[a](mg)	PUF No.[b]	Analysis
12/3/96	Pre-1993	338CS	96-12, 96-13		96-25	
	Pre-1993	338H2,H3	96-14, 96-15	99.6	96-26	HPLC, GC-MS
	Pre-1993	338H4,H5	96-16, 96-17	106.6	96-27	HPLC, GC-MS
	Pre-1993	338H6,H7	96-18, 96-19	88.6	96-28	HPLC, GC-MS
	Pre-1993	338H8,H9	96-20, 96-21	79.9	96-29	Mutagenicity [c]
12/4/96	Pre-1993	339CS	96-22, 96-23		96-30	
	Pre-1993	339H1	96-24, 96-25	34.1	96-31	GC-MS[d]
	Pre-1993	339H2,H3	96-26, 96-27	85.7	96-32	
	Pre-1993	339H4,H5	96-28, 96-29	79.7	96-33	
12/10/96	Low Aromatic	345CS	96-34, 96-35		96-36	
	Low Aromatic	345H1,H2	96-36, 96-37	71.5	96-37	
	Low Aromatic	345H3,H4	96-38, 96-39	55.9	96-38	
12/11/96	Low Aromatic	346CS	96-40, 96-41	38.9	96-39	
	Low Aromatic	346H1,H2	96-42, 96-43	97.3	96-40	
	Low Aromatic	346H3,H4	96-44, 96-45	65.5	96-41	Mutagenicity [c]
	Low Aromatic	346H5,H6	96-46, 96-47	62.2	96-42	HPLC, GC-MS
	Low Aromatic	346H7	96-48, 96-49	40.5	96-43	GC-MS [d]

Table 5. T60A20 High Volume Samples and PUF Samples Collected at LACMTA (cont.)

Sampling Date	Fuel	Test Cycles	SAPRC Filter No.	Particulate Wt.[a](mg)	PUF No.[b]	Analysis
12/12/96	Low Aromatic	347CS	96-50, 96-51		96-44	
	Low Aromatic	347H1,H2	96-52, 96-53	88.2	96-45	
	Low Aromatic	347H3,H4	96-54, 96-55	93.4	96-46	HPLC, GC-MS
	Low Aromatic	347H5,H6	96-56, 96-57	57.4	96-47	HPLC, GC-MS
12/16/96	Reformulated	351CS	96-60, 96-61		96-50	
	Reformulated	351H1,H2	96-62, 96-63	82.8	96-51	Mutagenicity[c]
	Reformulated	351H3,H4	96-64, 96-65	80.6	96-52	HPLC, GC-MS
	Reformulated	351H5,H6	96-66, 96-67	78.0	96-53	GC-MS[d]
12/17/96	Reformulated	352CS	96-68, 96-69		96-55	
	Reformulated	352H1,H2	96-70, 96-71	76.5	96-56	
	Reformulated	352H3,H4	96-72, 96-73	91.3	96-57	HPLC, GC-MS
	Reformulated	352H5,H6	96-74, 96-75	85.9	96-58	HPLC, GC-MS

[a] Total of front and back filter.

[b] Consists of front and back PUF.

[c] Extraction and HPLC separation performed at SAPRC. HPLC fractions and aliquot of whole extract sent to UC Davis for mutagenicity analysis.

[d] Internal standard screening sample used to determine levels for analysis samples.

Table 6. T60A20 High-Volume Filter Internal Standards (µg per sample).

	Pre-1993		Low Aromatic		Reformulated	
	Screening Sample[a]	Analytical Samples[b]	Screening Sample[a]	Analytical Samples[b]	Screening Sample[b]	Analytical Samples[b]
naphthalene-d_8	8.86	13.3	6.64	13.3	6.64	13.3
acenaphthene-d_{10}	10.5	15.75	7.88	15.75	7.88	15.75
phenanthrene-d_{10}	9.52	14.3	7.14	14.3	7.14	14.3
anthracene-d_{10}	10.19	15.28	7.64	15.28	7.64	15.28
fluoranthene-d_{10}	10.41	15.62	7.81	31.5	7.81	31.5
pyrene-d_{10}	9.73	14.6	7.3	33.7	7.3	33.7
chrysene-d_{12}	8.03	12	6.02	12	6.02	12
benzo[a]pyrene-d_{12}	9.66	14.5	7.24	14.5	7.24	14.5
perylene-d_{12}	10.55	15.82	7.91	15.82	7.91	15.82
dibenz[a,h]anthracene-d_{14}	10.14	15.21	7.6	15.21	7.6	15.21
1-nitronaphthalene-d_7	1.18	1.77	0.89	1.77	0.89	1.77
2-nitrofluorene-d_9	1.69	2.52	1.26	2.52	1.26	2.52
1-nitropyrene-d_9	9.69	14.5	7.27	14.5	7.27	14.5

[a] Single-cycle hot.
[b] Two-cycle hot.

Table 7. PUF Internal Standards (µg per PUF).

	Pre-1993 Reference			Low Aromatic			Reformulated		
	Screening Sample[a,b]	Analytical Samples[c]		Screening Sample[a,b]	Analytical Samples[c]		Screening Sample[b,c]	Analytical Samples[c]	
		Front	*Back*		*Front*	*Back*		*Front*	*Back*
naphthalene-d_8	5.43	10.8		5.43	10.8		10.8	10.8	
phenanthrene-d_{10}	5.14	109	10.9	5.14	109	10.9	10.2	109	10.9
anthracene-d_{10}	3.31	6.62	0.66	3.31	6.62	0.66	6.62	6.62	0.66
fluoranthene-d_{10}	5.94	11.9	1.19	5.94	11.9	1.19	11.9	11.9	1.19
pyrene-d_{10}	24	48.1	0.48	24	48.1	0.48	48.1	48.1	0.48
chrysene-d_{12}	1.92	3.84	0.38	1.92	3.84	0.38	3.84	3.84	0.38
1-nitronaphthalene-d_7	6.03	12.1	1.21	6.03	12.1	1.21	12.1	12.1	1.21
2-nitrofluorene-d_9	6.19	12.4	1.24	6.19	12.4	1.24	12.4	12.4	1.24

[a] Single-cycle hot.

[b] Front PUF only.

[c] Two-cycle hot.

Table 8 Hewlett Packard 1050 HPLC Solvent Program.

Time (min)	% Hexane	%CH_2Cl_2	% Acetonitrile
0	100	0	0
10	100	0	0
15	95	5	0
40	0	100	0
50	0	100	0
60	0	0	100
70	0	0	100
75	0	100	0
80	0	100	0
85	100	0	0
95	100	0	0

Foxy 2200 Fraction Program

Fraction	Time (min)	Compounds
waste	0 - 1	
1	1 - 10	
2	10 - 19	PAH
3	19 - 26	PAH
4	26 - 37	nitro-PAH
5	37 - 46	dinitro-PAH
6	46 - 55	
7	55 - 64	
8	64 - 73	
9	73 - 82	

GC-MS Analyses for PAH: The PAH were quantified using a Hewlett-Packard Model 5890 Series Gas Chromatograph equipped with a 7673A Automatic Sampler and interfaced to a Model 5970 quadruple mass selective detector operating in the selected ion monitoring (SIM) mode. As noted above, three replicate emission samples for each of the three fuel types were collected. The 27 samples (9 filter, 9 front PUF, 9 back PUF) were analyzed by triplicate injections onto the GC-MS. Compounds were identified based on their selected ion response and by matching their retention times with those of known standards using a 60m DB-5MS capillary column for separation. To correct for small retention time shifts between GC runs, the retention times were measured as the difference between the compound retention time and that of the nearest deuterated internal standard. Three sets of analyses with different GC-MS conditions and calibration standards were made to quantify the PAH (filter 128-278 Daltons; filter up to 302 Daltons; PUF PAH) and details of the analysis conditions are given in Table 9.

Calibration standards were run to determine response factors for each PAH quantified relative to the appropriate deuterated internal standard (IS). The PAH, ions monitored, and compounds used as IS for each PAH are given in Table 10. Response factors (RF) were calculated as (area/ng PAH) / (area/ng IS). The amount of a given PAH was calculated as [(area PAH) x ng IS]/[(area IS x RF)].

For each 2-cycle sample, the PAH concentrations were calculated on the basis of the weight of particulate matter (PM) collected (µg PAH/mg PM). For each PAH, the PAH emissions rate (µg PAH/Bhp-hr) was calculated by dividing the PAH concentration by the mean of the PM emission rate (g/Bhp-hr) over the two cycles.

GC-MS Analyses for Nitro-PAH: Two sets of analyses with different GC-MS conditions and standards were run to quantify the nitro-PAH in the filter and PUF samples (see Table 9). Nitro-PAH are more labile than the parent PAH and for this reason the nitro-PAH GC-MS analyses were made using cool on-column manual injections. Unlike the PAH, which generally exhibit only a single major ion peak, $[M]^+$, in their spectra, the nitro-PAH exhibit significant characteristic fragmentation in their mass spectra. Therefore, in addition to the molecular ion, $[M]^+$, characteristic fragment ions were also monitored. The ions monitored for the specific nitro-PAH and the deuterated internal standards are given in Table 11.

Table 9. GC-MS Conditions for Analysis of PAH and Nitro-PAH in Filter and PUF Extracts.

Column: 60 m DB-5MS, 0.25 mm i.d.; 0.25 µm film thickness.
Scan mode: SIM

Sample	Injector	Injector Mode	Inj. Temp.	Detector	Column Program
Filter PAH (128 to 278 Daltons)	Splitless	Autosampler	250 C	320 C	Initial 40 C, 8 C/min to 340 C; hold 10 min
Filter PAH (up to 302 Daltons)	Splitless	Autosampler	300 C	320 C	Initial 40 C, 15 C/min to 200 C; then 4 C/min to 340 C; hold 5 min
PUF PAH	Splitless	Autosampler	250 C	300 C	Initial 40 C, 8 C/min to 300 C; hold 15 min
Filter Nitro-PAH	On-column	Manual	Cool	300 C	Initial 40 C, 15 C/min to 200 C; 4 C/min to 320 C; hold 4.5 min
PUF Nitro-PAH	On-column	Manual	Cool	300 C	Initial 40 C, 8 C/min to 300 C

Table 10. Analytes and Internal Standards (IS) for GC-MS SIM Analyses.

Polycyclic Aromatic Hydrocarbon	SIM Ion	IS[a]	IS Ion
2,3,5-trimethylnaphthalene	170	PH-d10	188
phenanthrene	178	PH-d10	188
anthracene	178	AN-d10	188
Me-phenanthrenes/anthracenes	192	PH-d10	188
fluoranthene	202	FL-d10	212
pyrene	202	PY-d10	212
benzo[c]phenanthrene	228	CH-d12	240
benzo[ghi]fluoranthene	226	CH-d12	240
cyclopenta[cd]pyrene	226	CH-d12	240
benz[a]anthracene	228	CH-d12	240
chrysene + triphenylene	228	CH-d12	240
benzo[b+j+k]fluoranthene	252	BAP-d12	264
benzo[e]pyrene	252	BAP-d12	264
benzo[a]pyrene	252	BAP-d12	264
perylene	252	PRY-d12	264
indeno[1,2,3-cd]fluoranthene	276	DBA-d14	292
benzo[c]chrysene	278	DBA-d14	292
dibenz[a,j]anthracene	278	DBA-d14	292
indeno[1,2,3-cd]pyrene	276	DBA-d14	292
dibenz[a,h + a,c]anthracene	278	DBA-d14	292
benzo[b]chrysene	278	DBA-d14	292
benzo[ghi]perylene	276	DBA-d14	292
coronene	300	DBA-d14	292
dibenzo[a,l]pyrene	302	DBA-d14	292
dibenzo[a,e]pyrene	302	DBA-d14	292
dibenzo[a,i]pyrene	302	DBA-d14	292
dibenzo[a,h]pyrene	302	DBA-d14	292

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[a] Abbreviations: PH-d10, phenanthrene-d_{10}; AN-d10, anthracene-d_{10}; FL-d10, fluoranthene-d_{10}; PY-d10, pyrene-d_{10}; CH-d12, chrysene-d_{12}; BAP-d12, benzo[a]pyrene-d_{12}; PRY-d12, perylene-d_{12}; DBA-d14, dibenz[a,h]anthracene-d_{14}.

Table 11. Analytes and Deuterated Internal Standards for GC-MS SIM Analyses.

	SIM Ions				
Nitro-PAH	$[M]^+$	$[M\text{-}NO]^+$	$[M\text{-}NO_2]^+$	$[M\text{-}HNO_2]^+$	$[M\text{-}NO\text{-}CO]^+$
1-nitronaphthalene-d_7	180		134		
1-nitronaphthalene	173		127		115
2-nitronaphthalene	173		127		115
methylnitronaphthalenes	187				115[a]
2-nitrobiphenyl	199			152	
4-nitrobiphenyl	199			152	
5-nitroacenaphthene	199			152	
2-nitrofluorene-d_9	220		174		
2-nitrofluorene	211	194[b]	165		
9-nitroanthracene	223	193		176	165
1-nitropyrene-d_9	256	226	210		
1-nitropyrene	247	217	201	200	189
3-nitrofluoranthene	247	217	201	200	189
4-nitropyrene	247	217	201	200	189
7-nitrobenz[a]anthracene	273	243		226	
6-nitrochrysene	273	243		226	
6-nitrobenzo[a]pyrene	297	267		250	239

[a] Fragment ion is indenyl ion, characteristic of substituted naphthalenes; $[M\text{-}72]^+$ from methylnitronaphthalenes.
[b] Fragment ion is $[M\text{-}OH]^+$.

Quantification was made on the basis of the molecular ion, with the fragment ion(s) serving to confirm the identity of the nitro-PAH. As discussed above for the PAH, response factors relative to a deuterated IS were determined and used for quantification. 1-Nitronaphthalene-d_7 served as the IS for 1- and 2-nitronaphthalene. 1-Nitropyrene-d_9 was used as the internal standard to quantify 1-nitropyrene, 9-nitroanthracene and 6-nitrobenzo[a]pyrene. The emission rates of the nitro-PAH as µg/Bhp-hr were also calculated as described above for the PAH.

2.10 Vapor Phase PAH (U.C. Davis)

Chemicals: HPLC grade methanol, acetone, hexane, and water were obtained from Fisher Scientific. Dichloromethane (DCM, OmniSolve) was from EM Science. Acetonitrile (ChromPure) and hexane (UV, High Purity) were from Burdick and Jackson.

Deuterated standards included methylnaphthalene-d_{10}, fluorene-d_{10}, anthracene-d_{10}, fluoranthene-d_{10}, and pyrene-d_{10} were obtained from Cambridge Isotopes Laboratories (Andover, MA). Naphthalene-d_8, acenaphthene-d_{10}, phenanthrene-d_{10}, chrysene-d_{12} and perylene-d_{12} were from AccuStandard (New Haven, CT). Standard Reference Material SRM 2260, native PAHs in toluene (nominal 60 ng/µl), were obtained from NIST (Gaithersburg, Maryland).

Adsorbents Pre-cut PUF plugs were obtained from North Carolina Foam, Inc. (Mount Airy, NC). Two sizes of PUF plugs were used. The PUF plugs used for the low volume sampler were nominally 3" in length and 2" in diameter. The PUF plugs used for the high volume sampler were nominally 1" in length and 3" in diameter. All PUF plugs were pre-cleaned by sequential sonication in methanol (3x) and DCM (3x) and dried in a Baker BioChemgard hood, equipped with HEPA filters.

Purified XAD-4 resin was from Alltech (Philadelphia, PA). The XAD was further purified to remove potential background interferences by sequential 15 min sonications in methanol (3x) and dichloromethane (3x). The resin was dried for 5 days under vacuum at 50°C and then stored in solvent-cleaned amber glass jars.

Low Volume Sampling Apparatus PAH samples were collected from both a high volume sampler and a low volume sampler. The low volume sampling unit is illustrated in Figure 3 and consisted of two sampling trains that were connected by a stainless steel "Y" whereby two parallel samples were collected during a single dynamometer run for bioassay and PAH analysis. Each diesel exhaust sampling train consisted of two Teflon-coated glass fiber filters, a sorbent module containing three PUF plugs, one 40 ml bed of XAD, and a rotometer connected in series. Teflon sorbent modules containing PUF and XAD were from Savillex Corp. (Minnetonka, MN). Teflon tubing was used to connect both the filter holder assembly to the complete sampling train and to the vacuum pump.

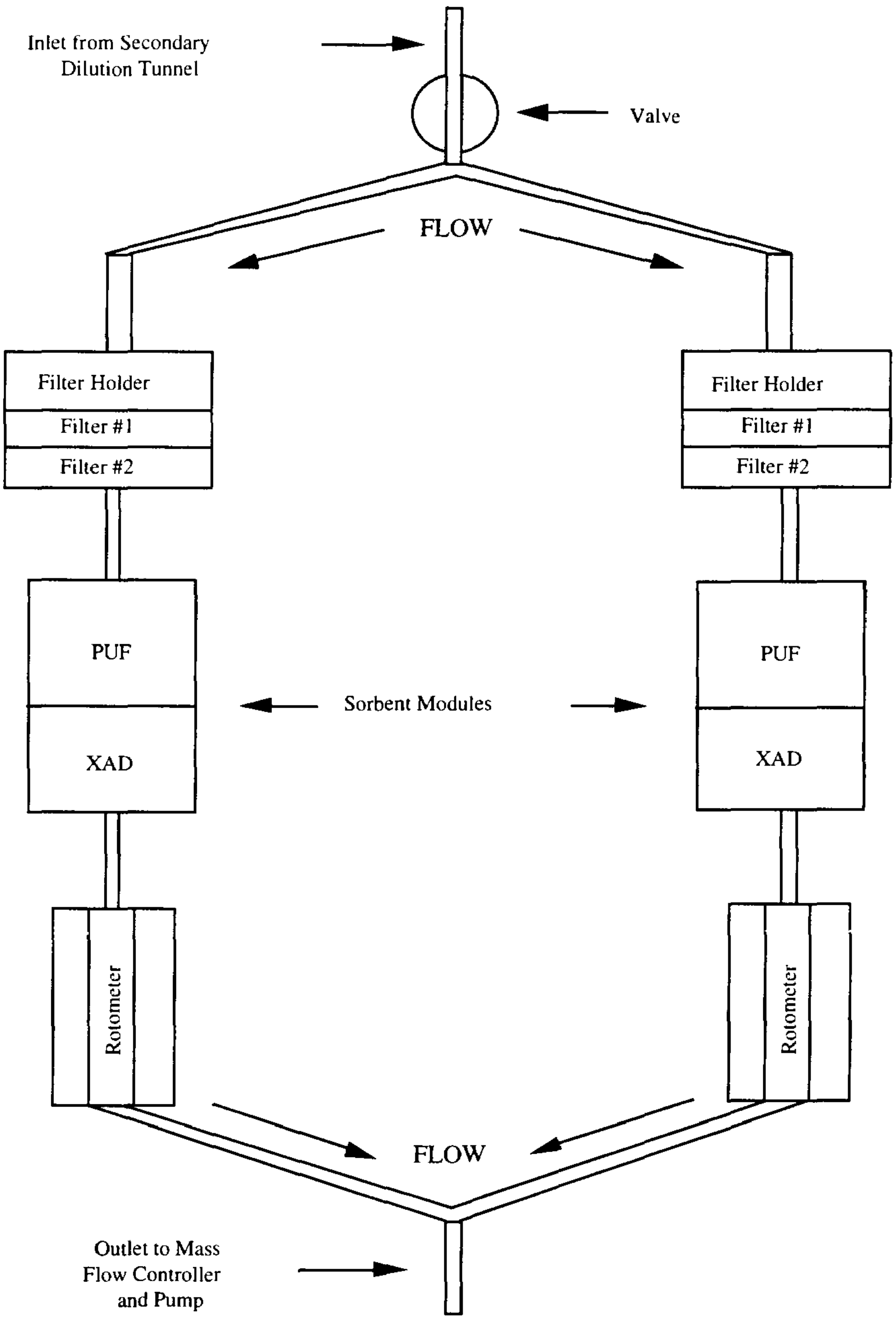

Figure 3. Particulate and Vapor-Phase Sampler.

High Volume Sampler: The high volume sampling train is illustrated in Figure 4 and consisted of a filter assembly that housed two Teflon-coated glass fiber filters, a sorbent module containing three 3" diameter x 1" thick PUF plugs and one 60 ml bed of XAD-4, a vacuum pump, and a laminar flow element connected in series. The sampling train was inserted into the six inch diameter by-pass on the primary tunnel. The filter assembly, sorbent holder, and vacuum pump were from Grasby GMW (Village of the Cleves, Ohio) and the Laminar Flow Element was from Meriam Instruments (Cleveland, Ohio). Glass sorbent modules (6 in. long x 76.2 mm ID) containing PUF and XAD were from R&D Glass (Berkeley, CA). A Variac was used to control the vacuum pump speed by varying the voltage, which in turn controlled the flow rate through the sampler. A laminar flow element was used to measure the flow rate. Two manometers were used to measure the delta P and pressure in the tunnel. The temperature probe from the digital thermometer was located near the inlet end of the laminar flow element. Pressure and temperature measurements were immediately put into a spreadsheet and the flow was calculated in standard cubic feet per minute. Using the updated flowrate, corrections to the voltage output from the Variac were made to ensure a consistent flowrate.

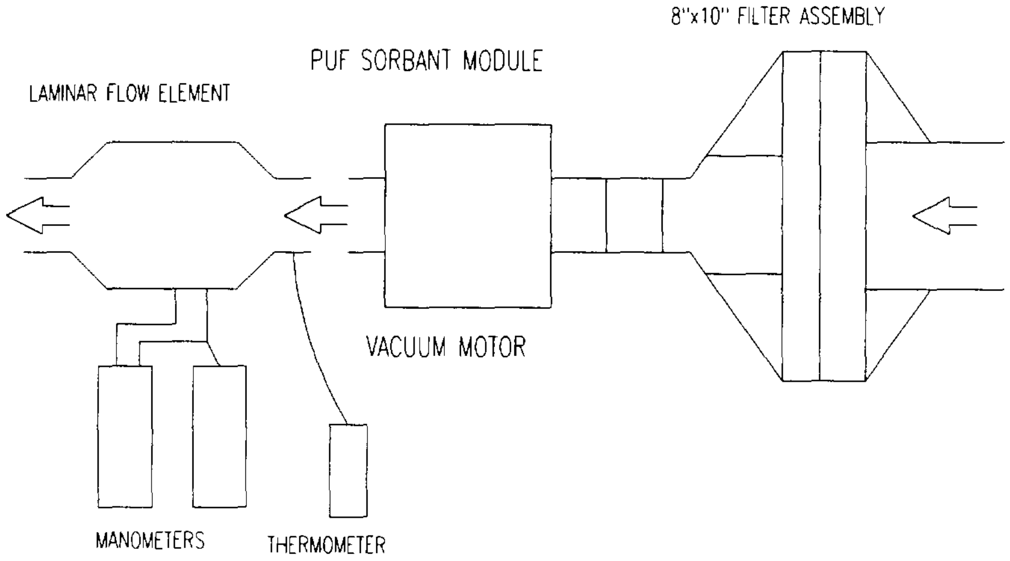

Figure 4. Schematic of High Volume Sampler used for PAH sampling.

Diesel Exhaust Sampling: In preparation for collecting diesel exhaust samples for the main study, a series of Pretest experiments were conducted at the Los Angeles County Metropolitan Transportation Authority (LACMTA) dynamometer facility. A diagram of the diesel exhaust sampling set-up at the LACMTA facility is illustrated in Figure 5.

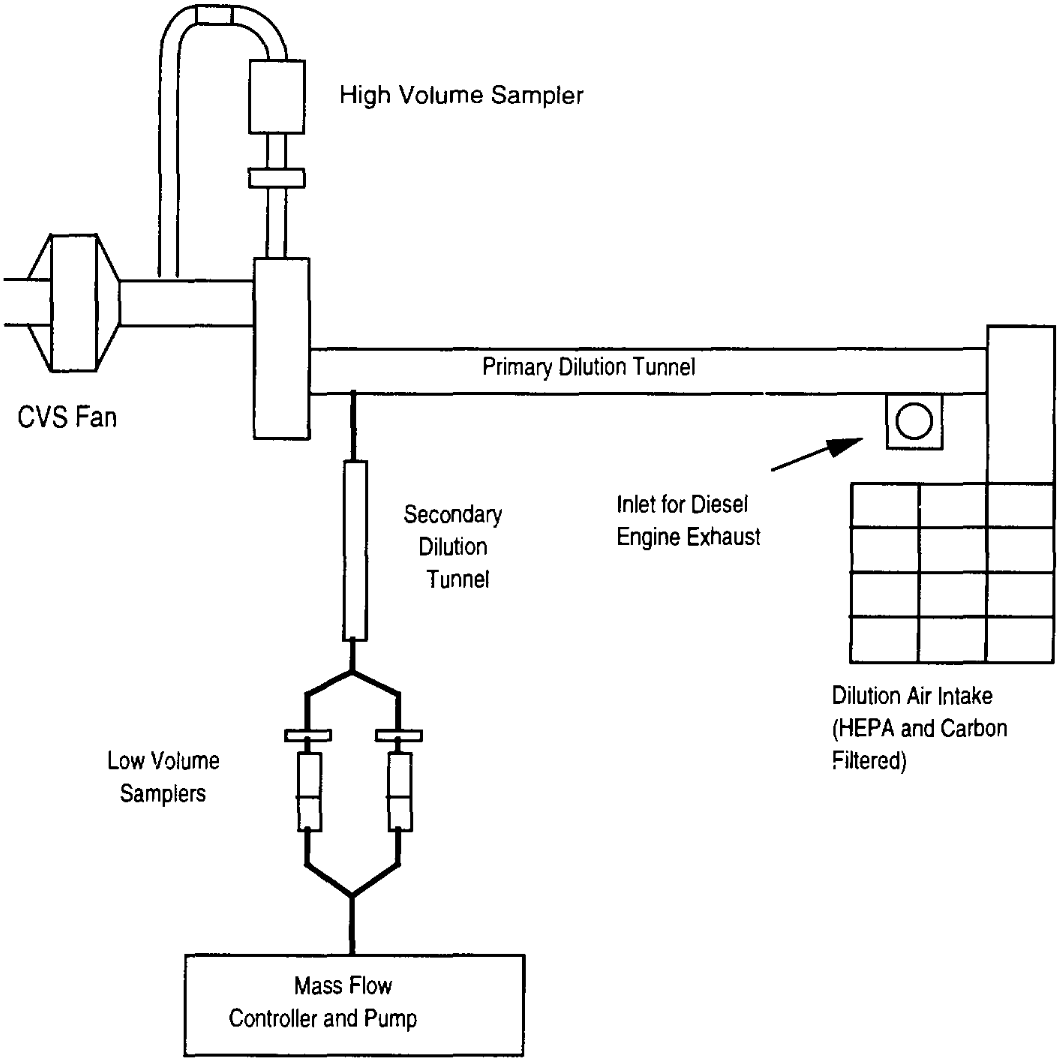

Figure 5 Dilution Tunnel for Heavy-Duty Diesel Exhaust and Sampler at the LACMTA Facility.

Initial pre-tests were conducted to test the feasibility of modifying the original Horiba high volume particulate sampler to a high volume sampler capable of collecting both particulates and semi-volatile compounds. This modification required that a sorbent module containing PUF and XAD be added to the sampling train. After numerous tests, it became apparent that the original sampler and flow meter were not compatible with the addition of the sorbent module. A substantial increase in the pressure drop, caused by the sorbent module, dropped the flow rate below the lower limit of the Venturi flow meter. Increasing the flow rate through the passive high volume sampler required that a flow restrictor be placed in the main tunnel. This increases the back pressure in the main tunnel thereby forcing more flow into the high volume sampler. Concerns were raised as to whether the restrictor caused the main tunnel venturi, which controls the flow rate in the main tunnel, to run sub-critical or out of calibration. Since this would be an unacceptable condition, the high volume sampler was redesigned as an active high volume sampler that did not require a flow restrictor.

In subsequent pre-tests, the flow rate through the system was increased by the addition of a high volume vacuum motor. Flow rate measurements were made by replacing the venturi (rated from 60-200 CFM) with a laminar flow element calibrated from 5 cfm to 60 cfm.

In the October 1996 pre-test, a blank and a sample were collected to estimate the concentration of PAHs found in the diesel exhaust. The Main study was also conducted at the LACMTA Emissions Test facility using 1) a Pre-October 1993 (Pre-Oct. '93) specification fuel, 2) a low aromatic fuel and 3) a blend of three fuels (Reformulated). Low volume Teflon sorbent modules and high volume glass sorbent modules containing the samples, spikes, and requisite blanks were wrapped in aluminum foil throughout the sampling period to minimize potential photo-oxidation. Immediately after each sampling period, the sample and blank modules were stored at 4°C, until they were transported back to the laboratory, where they continued to be stored at 4°C until further processing.

High Volume Sample Preparation: The high volume diesel exhaust vapor-phase samples consisted of two filters and two PUF plugs followed by 60 ml of XAD-4 resin. The PUF and XAD were extracted separately by SFE and the filters were extracted by sonication. High volume PUF plugs were cut in half, and one half of each PUF plug was used for PAH analysis.

Prior to extraction, one half of the PUF sample was spiked with 120 µl of a 20 ng/µl deuterated PAH internal standard mixture and 1 ml of methanol modifier was added. The PUF was extracted by SFE using supercritical carbon dioxide and the final volume of each extract was approximately 2 ml. Summary of the SFE conditions are given in Table 12.

Table 12. SFE Extraction Conditions for High Volume and Low Volume PUF and XAD-4 Samples.

Extraction Condition	PUF	XAD-4
Pressure	5000 psi	7000 psi
Temperature	75°C	100°C
Time	90 min	60 min
Modifier	10% methanol	10% methanol

A 660 μl aliquot of the SFE extract was loaded onto a Waters C18 SepPak cartridge to remove some of the hydrocarbons that interfere with GC/MS analysis. The C18 was extracted with 2 ml of methanol. The methanol extract was transferred to a Waters Silica SepPak cartridge. The methanol was allowed to evaporate on the cartridge before eluting 3 ml of hexane through the cartridge. The hexane fraction was saved for GC/MS analysis. DCM was added to a measured aliquot of SFE extract to adjust the final volume to 200 μL. The diluted extract was analyzed by GC/MS.

XAD-4 resin from the high volume sampler was well mixed and approximately 10 mls of XAD from each sample was prepared for GC/MS analysis. The XAD sample was spiked with 100 μl of a 20 ng/μl deuterated PAH internal standard mixture and 1 ml of methanol modifier was added. The XAD was extracted by SFE using supercritical carbon dioxide and the final volume of each extract was approximately 2.0 ml. Summary of the SFE conditions are given in Table 12. A 400 μl aliquot of the SFE extract was added to a Waters C-18 SepPak cartridge to remove some of the hydrocarbon interferences. The C-18 was extracted with 2 mls of methanol. The methanol extract was transferred to a Waters Silica SepPak cartridge.

The methanol was allowed to evaporate on the cartridge before eluting 3 mls of hexane through the cartridge. The hexane fraction was saved for GC/MS analysis. DCM was added to a measured aliquot of the methanol extract to adjust the final volume of 200 μl.

Prior to extraction the filters were divided in two with one half dedicated to bioassay analysis and the other half to PAH analysis. One half of the front and backup filter was cut into pieces and placed in an Erlenmeyer flask with a screw cap. 40 µl of 5ng/µl internal standards was added to the filters. The filters were extracted 4 times in 25 mls of DCM for 20 minutes. After each sonication the sample was filtered through a 2 A filter. The flask and filter were rinsed with DCM and the washes and extractions were combined. The combined extract was concentrated to 1 ml. One-half of the sample was then added to a Waters C-18 SepPak cartridge. The DCM from the extract was allowed to evaporate before the C-18 cartridge was eluted with 2mls methanol. The methanol extract was transferred to a Silica SepPak cartridge and the methanol was allowed to evaporate before eluting with 3 mls of hexane. The hexane extract was concentrated to 1 ml and prepared for GC/MS analysis.

Low Volume Sample Preparation: Six test cycles were collected on each low volume sample. Each low volume diesel exhaust vapor-phase sample consisted of 6 filters (the main and backup filters were changed after 2 test cycles), a three inch long PUF plug followed by 40 ml of XAD-4 resin. The PUF and XAD were extracted separately by SFE. The filters were extracted by sonication. The PUF samples were spiked with 100 µl of a 20 ng/µl deuterated PAH internal standard mixture and 1 ml of methanol modifier was added. The PUF was extracted by SFE and the final volume of each extract was approximately 2 mls. A summary of the SFE conditions is also given in Table 12. A 660 µl aliquot of the SFE extract was loaded onto a Waters C-18 SepPak cartridge to remove some of the hydrocarbons that interfere with GC/MS analysis. The C-18 was extracted with 2 mls of methanol. The methanol extract was transferred to a Waters Silica SepPak cartridge. The methanol was allowed to evaporate on the cartridge before eluting 3 mls of hexane through the cartridge. The hexane fraction was saved for GC/MS analysis. DCM was added to a measured aliquot of SFE extract to adjust the final volume to 200 µl. The diluted extract was injected into the GC/MS for analysis.

The low volume XAD-4 resin was well mixed and approximately 10 ml of XAD from each sample was prepared for SFE extraction and for GC/MS analysis. Prior to SFE, the XAD sample was spiked with 75 µl of a 20 ng/µl deuterated PAH internal standard mixture and 1 ml of methanol modifier was added. The XAD was extracted by SFE and the final volume of each extract was approximately 2 ml. A 660 µl aliquot of the SFE extract was loaded onto a Waters C-18 SepPak cartridge to remove some of the hydrocarbons that interfere with GC/MS analysis. The C-18 was extracted with 2 ml of methanol. The methanol extract was transferred to a Waters Silica SepPak cartridge. The methanol was allowed to evaporate on the cartridge before eluting 3 ml of hexane through the cartridge. The hexane fraction was saved for GC/MS analysis. DCM was added to a measured aliquot of the methanol extract to adjust the final volume to 200 µl and each diluted extract analyzed by GC/MS run in selective ion monitoring (SIM) mode.

The six filters from each sample were cut into pieces and placed in an Erlenmeyer flask with a screw cap. 100 µl of 5 ng/µl internal standards was added to the filters. The filters were extracted 4 times in 25 ml of DCM for 20 minutes. After each sonication the sample was filtered through a 2 A filter. The flask and filter were rinsed with DCM and the washes and extracts were combined. The combined extract was concentrated to 1 ml. One-half of the sample was then added to a Waters C-18 SepPak cartridge. The DCM from the extract was allowed to evaporate before the C-18 cartridge was eluted with 2ml methanol. The methanol extract was transferred to a Silica SepPak cartridge and the methanol was allowed to evaporate before eluting with 3 ml of hexane. The hexane extract was concentrated to 1 ml and prepared for GC/MS analysis.

Instrumental conditions and column selection: The SFE extracts were analyzed using a Hewlett-Packard Model 5890 Series II Gas Chromatograph (GC) equipped with a Model 8290 autosampler interfaced to a Hewlett-Packard Model 5970A quadrupole mass selective detector (MSD). The GC was equipped with a split/splitless injector and an electronic pressure controller. The injector was run in the splitless mode and the electronic pressure controller was programmed for vacuum compensation and constant flow mode. The GC was equipped with a 30 m x 0.25 mm i.d. DB-5MS fused silica capillary column (0.5 µm film thickness; Hewlett-Packard). The MSD was run in selective ion monitoring (SIM) mode. The final instrument conditions are summarized in Table 13.

The target and substituted PAHs that were quantitated are listed in Table 14. The PAHs were analyzed using an isotope dilution method to improve the quantitation. Note that the targeted compounds include the alkyl-substituted PAH, 1- and 2-methylnaphthalene, dimethylnaphthalenes and 1-methylphenanthrene.

Table 13. GC/MS Conditions for Analysis of PUF and XAD-4 Extracts

Carrier gas / velocity	He; 32 cm/sec
Temperature program	40°C hold for 10 min
	5°C/min to 270°C
	5 min hold @ 270°C
	10°C/min to 310°C
	10 min hold @ 310°C
Injector temperature	295°C
Detector temperature	285°C
Solvent delay	10 min
Run time	40 min
Scan mode	SIM

Table 14. List of Analytes and Internal Standards for PAH Analysis by GC/MS.

Compound No.	Compound	Quantitation ion
1	naphthalene-d$_8$*	136
2	naphthalene	128
3	methylnaphthalene-d$_{10}$*	152
5	2-methylnaphthalene	142
6	1-methylnaphthalene	142
8	2,6- & 2,7-dimethylnaphthalene	141
15	acenaphthene-d$_{10}$*	160
16	acenaphthylene	152
17	acenaphthene	154
18	fluorene-d$_{10}$*	176
19	fluorene	166
20	anthracene-d$_{10}$*	188
24	1-methylphenanthrene	192
28	phenanthrene-d$_{10}$*	188
29	phenanthrene	178
30	anthracene	178
31	fluoranthene-d$_{10}$*	212
32	fluoranthene	202
33	pyrene-d$_{10}$*	212
34	pyrene	202
35	chrysene-d$_{12}$*	240
36	benzo[a]anthracene	228
37	chrysene	228

* internal standard

Quality Assurance and Control: The collection and storage of samples followed specifically defined procedures. All testing conditions for the Main Study were recorded on Field Data Sheets. For each sampling run, the date, time, type of sample (bioassay or chemical analysis), flow rate, temperature, and pressure of the sampling system were recorded. The parameters for each cycle were also recorded. All samples were identified in a Master Log that was used throughout the main study. The Master Log contained sample ID, sample type, and date of collection. Finally, all sample transfers were tracked from collection to analysis using Chain of Custody forms. The Chain of Custody contained samples ID's, sample type, dates of transfer and acceptance, and the initials of the personnel who transferred and accepted the samples.

For the sampling and chemical analysis, adsorbent and solvent blanks were analyzed along with the actual samples in order to determine possible background interferences. Field samples were stored on blue ice during transport to the laboratory where they were stored at 4°C before sample work-up and extraction. All SFE extracts were chemically analyzed immediately following extraction or stored at -20°C until sample analysis and mutagenicity testing could be completed.

Prior to sample analysis, the MSD was manually tuned using perfluorotributylamine. The MSD was optimized for SIM analysis for PAHs by first injecting a reagent blank into the GC/MS to determine background contamination levels. If the background levels were acceptable, then a multi-point calibration curve was established by injecting 5 standards at 5 different concentrations. The actual samples were analyzed after the analysis of the calibration standards was completed. After every ten (10) samples, a calibration standard check sample was run to ensure that the instrument was still properly calibrated. If the target compounds were found at levels above the calibration curve, the samples were diluted and analyzed again. Duplicate analyses were performed for each sample. All PAH analysis incorporated adsorbent blanks. All PAH procedures were carried out in a room fitted with yellow fluorescent lights (G.E. F40Go) to minimize potential photo-oxidation of chemicals.

Samples: A summary of the PUF and XAD-4 samples collected from the emissions of a heavy-duty diesel engine using pre-1993, low aromatic, and a reformulated blend fuel and extracted by SFE is presented in Table 15. Samples were collected from the hot cycles for each fuel type. No cold start samples were collected.

Table 15. Summary of PUF and XAD-4 Samples Collected from Pre-1993, Low Aromatic and Reformulated Blend Fuels and Extracted by SFE.

Sample ID	Fuel Type	Cycle/Start Type [a]	Sampler type	Chem. Analysis	
				XAD-4	PUF
H3	Pre-1993	2 FTP/Hot[b]	High Vol	X	X
H4	Low Aromatic	2 FTP/Hot	High Vol	X	X
H6	Reformulated	2 FTP/Hot	High Vol	X	X
UV2	No Diesel	Tunnel Blank[c]	High Vol	X	X
UV3	No Diesel	Tunnel Blank	High Vol	X	X
UV4	No Diesel	Tunnel Blank	High Vol	X	X
TR1	No Diesel	Trip Blank	High Vol	X	X
H110	Pre-1993	6 FTP/Hot[d]	Low Vol	X	X
H127	Low Aromatic	6 FTP/Hot	Low Vol	X	X
H145	Reformulated	6 FTP/Hot	Low Vol	X	X
H150	Reformulated	6 FTP/Hot	Low Vol	X	X
H170	Pre-1993	6 FTP/Hot	Low Vol	X	X
UL100	No Diesel	Tunnel blank	Low Vol	X	X
UL120	No Diesel	Tunnel blank	Low Vol	X	X
UL134	No Diesel	Tunnel blank	Low Vol	X	X
TRL1	No Diesel	Trip Blank	Low Vol	X	X

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[a] All data are from federal transient engine test cycle runs.

[b] All data are from 2 federal transient engine test cycle runs in the hot start mode.

[c] Tunnel blank = sample dilution tunnel without engine running.

[d] All data are from 6 federal transient engine test cycles run in the hot start mode.

X Sample has been extracted for chemical analysis.

2.11 Nitrosamines

Materials: Thermosorb-N cartridges were purchased from Thermedics (Woburn, Mass). Pretest-Calibration calibrated against Gilmont Flow meters.

Sampling: The sampler consisted of a filter module, sorbent module, rotometer, and sample pump. The filter module consisted of the stainless steel filter holder used at the LACMTA facility. The holder housed two 70 mm Teflon membrane filters separated by a stainless steel screen. A 1/8" diameter Teflon tube connected the Filter holder to two Thermosorb-N cartridges connected in series. The second cartridge was connected to a rotometer. The purpose of the second cartridge was to trap any NAs that were not trapped by the front cartridge. A bellows pump was attached to the exit of the rotometer. Nitrosamine samples were taken from a side port on the secondary dilution tunnel. See Figure 6.

The sampling flow was set at a nominal 2 liters per minute. Samples were collected during FTP cycles. To obtain a sufficient sample, emissions from six FTP cycles were collected on each set of cartridges. Each cycle lasted approximately 20 minutes with a total sample time of 120 minutes. This resulted in a total sample volume of 240 liters corresponding to a quantitation limit of 2.5 $\mu g/m^3$ in the undiluted diesel exhaust for all NA samples. Filters were changed after every cycle. A leak check was performed prior to each series of sampling runs and after each time the cartridges were disconnected from the sampling train.

The particles collected on the filters were archived. The volatile emissions were collected on the Thermosorb-N cartridges. After sampling the cartridges were immediately capped and sealed in aluminum foil packages until shipped to Lancaster laboratory for analysis. All the front cartridges were analyzed. One backup cartridge was analyzed to determine whether breakthrough had occurred.

Analytical Procedure: A mixture of 25% methanol and 75% dichloromethane was eluted through a Thermosorb-N cartridge. The first 1.5-1.8 mls of eluant was collected for GC/TEA analysis. 4 µls of sample was injected for analysis. The GC is equipped with a 10' long x 1/8" O.D. stainless steel column packed with 10% Carbowax 20M and 2% KOH on Chromsorb W-AH and a Thermal Energy Analyzer Detector. Lancaster Laboratories analyzed the samples for N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA), N-nitrosodipropylamine (NDPA), N-nitrosodibutylamine (NDBA), N-nitrosopiperidine (NPIP), N-nitrosopyrolidine (NPYR), and N-nitrosomorpholine (NMOR).

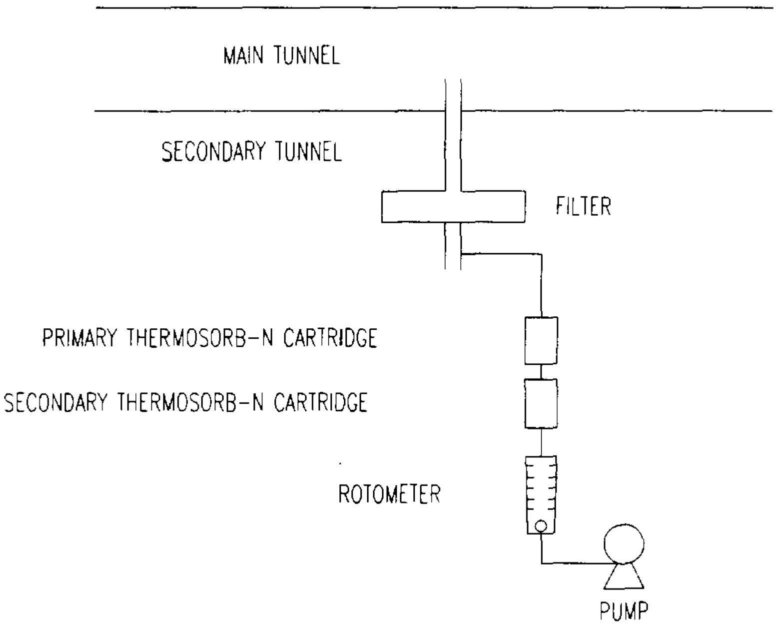


Figure 6. Nitrosamine Sampler at the LACMTA Facility

2.12 Dioxins

Chemicals: HPLC grade hexane and methanol were obtained from Fisher Scientific. PUF sheets were obtained from Graseby GMW (Village of Cleves, Ohio). The sheets were cut into 3" diameter x 1" thick PUF plugs. 8"x10" Teflon coated glass fiber filters were obtained from Pallflex (Putnam, CT). 76.2 mm x 6" diameter Glass PUF holders were obtained from R&D glass (Berkeley, CA). The filters, PUF, and glass holders were pre-cleaned by Alta Laboratories (El Dorado Hills, CA). All utensils and aluminum foil used in the test were pre-cleaned in methanol. The filter holder, sorbent holder and vacuum motor were obtained from Graseby GMW (Village of Cleves, OH). The laminar flow element was obtained from Meriam Instruments (Cleveland, OH).

Sampling-EPA Proposed Test Procedure: No standardized testing procedures were available for PCDD and PCDF testing. PCDD and PCDF methodologies developed for this study were adapted from a test plan outlined in a USEPA Memo, "Method Validation and Confirmatory Testing for the Identification and Quantification of Dioxin and Dioxin-Like Compounds from Diesel Emissions" (Jan 31, 1996). The test plan was based on recommendations made by a USEPA Dioxin work group. Modifications to the Test plan were made based on comments by the ARB/CE-CERT Technical Advisory Committee which was formed for this study.

Test Plan: The Test plan was based on the criteria for Dioxin sampling in diesel exhaust as outlined in a USEPA Memo, "Method Validation and Confirmatory Testing for the Identification and Quantification of Dioxin and Dioxin-Like Compounds from Diesel Emissions" (Jan 31, 1996). The memo was issued by the National Vehicle and Fuel Emissions Laboratory of the USEPA. Much of this test plan was based on Phase 1 of that memo. Most of the criteria listed in the memo were implemented into this plan. Listed below are the criteria adopted from the USEPA test plan:

1. The test engine was connected to a engine dynamometer and run using a FTP cycle.

2. Dioxin diesel exhaust samples were collected from a dilution tunnel.

3. A high volume sampler was used to collect the diesel exhaust samples.

4. Chlorine, a Dioxin precursor, and copper, a catalyst, were measured in the emissions, fuel and oil.

5. A qualified commercial laboratory using standardized Dioxin EPA method was used to analyze the samples.

6. EPA criteria and assumptions for estimating sample size were adapted. The sampler was designed to collect enough sample to obtain a toxic equivalent factor detection limit of 100 pg/l.

Additional validation not included in the EPA plan but incorporated into the current study included:

1. Analysis of Dioxins in the fuel and oil.

2. Validation of the sampler in a pretest trial.

3. Test for breakthrough of the PUF.

4. Incorporation of additional QA procedures such as tunnel blanks.

Other criteria could not be included because they required major modifications to the main test plan. The major differences are summarized below.

1. *Engine Selection.* The EPA memo recommends a Detroit Diesel Series 60 or a Caterpillar 3176 engine. For this test a Cummins L10 engine was used. The selection of the engine for this test was determined by the ARB/CE-CERT Technical Advisory Committee.

2. *Ambient Chlorine.* EPA recommends measuring atmospheric chlorine at the air intake of the engine. Atmospheric chlorine could not be measured for this test. Modifications to the system were beyond the resources and scope of this project. No practical way could be found to measure atmospheric chlorine without affecting the operation of the engine. This did not affect this study since the purpose of the test was not to characterize the mechanism of Dioxin formation that would require a significantly larger matrix.

3. *Tunnel Conditioning Time.* Due to time constraints, conditioning time in the tunnel was changed from 30 hours to 2 hours.

4. *Selection of Fuels.* A pre-1993 California fuel and a reformulated blend of post-October 1993 fuels was used in this study instead of a low chlorine and a high chlorine diesel certification fuel. This change was made since the objective of this study was to develop methods to characterize differences in reformulated and pre-1993 fuels rather than to determine the effects of chlorine in the fuel on Dioxin emissions.

To acquire sufficient sample, a high volume sampler equipped with a filter and sorbent module was interfaced to the dilution tunnel. Multiple cycles were collected on one sample train. The pooled samples were analyzed for PCDDs and PCDFs.

Characterization of dioxins not only requires analysis of diesel emissions but also of dioxins in the fuel and oil. Also needed is the characterization of chlorine and copper in the emissions, fuel, and oil. Chlorine is a dioxin precursor that is found in diesel fuel and oil. Copper is a catalyst that has been shown to increase the rates of formation of dioxins in diesel exhaust (1).

Estimating Sample Size: Initial estimates of sample size were based on USEPA estimates that 0.5% of the total diesel emissions need to be collected in a single sampling run and that sufficient samples would be collected in two FTP cycles. The following assumptions were used in this estimation. 1) The diesel emission factor is 100 pg toxic equivalence per liter of diesel fuel (TEQ/l). This emission factor is based on a study by Marklund et al., 1987. 2) The congener profiles for diesel exhaust resembles the congener profile for municipal waste incinerators. 3) The amount of fuel used per test is 4 liters. 4) Assumes four congeners, TCDD, PeCDD, HxCDD, and PeCDF make up over 80% of the body burden TEQ for the general population. 5) Five tenths of a percent of the total diesel emissions can be collected in a single FTP cycle. 6) The overall extraction efficiency of the method is 80%. 7) The GC/MS can quantify 2-4 pg of 2,3,7,8 TCDD and 20-40 pg for other congeners.

For the sampling setup at the LACMTA facility, an estimated 1.5% of the total diesel emissions can be collected in a single FTP cycle. This requires a high volume sampler with a sampling rate of 39 cubic feet per minute and a 32:1 dilution of the diesel exhaust. Sufficient sample should be collected in pooling 1-2 cycles on a single sampling train to achieve a 100 pg TEQ/l. Lower detection limits can be achieved by pooling more cycles on a single sample. The maximum number of cycles is limited by the number of filter changes required. Filter changes occur after every 2 or 3 cycles because of the increased pressure drop due to particle loading. The number of filters is limited to three because of the amount of space in the Soxhlet extraction apparatus used for extracting the sample. The maximum number of cycles per pooled sample is eight.

Sampling System: The Horiba High Volume Sampler installed on the dilution tunnel was not suitable for dioxin emission sampling. It was removed for this diesel test and was replaced by a modified Grasby GMW high volume sampler. The sampler consisted of a filter module, sorbent module, vacuum motor and laminar flow element as shown in Figure 7. The unit was connected to the main tunnel by a bypass. Part of the diesel emissions were diverted through the bypass into the sampler and then channeled back into the main tunnel.

The sampler collected particulates on a 8"x10" Pallflex T60A20 rectangular filter. Gaseous emissions were trapped by two 1" thick x 3" diameter PUF plugs. Sample was pulled from the main tunnel by a motor and flow was measured by a laminar flow element. Pressure was measured by a U- tube oil manometer and a water manometer. Flow was regulated by using a Variac to control the voltage on the motor. Flow rates were measured and adjusted every two minutes. Temperature in the laminar flow element was measured by electronic digital thermometer.

Sample Preparation: The PUF sheets were cut to size using a three inch diameter dye. 3" id. x 6" long Glass sorbent modules were used to house the PUF. PUF, sorbent modules, and filters were cleaned by Alta Laboratories. Alta loaded the PUF in the glass modules. Prior to sampling, the upstream PUF was spiked with a cocktail of labeled dioxins and furans, and the list of surrogates are presented in Table 16.

Sample Tracking and Chain of Custody: The spiked sorbents were wrapped in aluminum foil and labeled. Filters were cleaned, wrapped in pre-cleaned foil, and labeled prior to sampling. The samples were packed on blue ice and shipped via over-night delivery to LACMTA. After delivery to LACMTA, the sorbent modules were stored in a freezer at 4°C until use.

Leak checks were performed before a sample was collected and after each filter change. Emissions from seven hot and one cold start FTC cycles were collected on a single set of PUFs. The filters were changed after the first 2 cycles and every 3 cycles thereafter. After emission sampling, the filters were folded and put into the glass sorbent module. The module was then wrapped in aluminum foil, labeled, and sealed in plastic bags. The samples were stored in a freezer at 4°C. The samples were packed with blue ice and shipped by overnight delivery to Alta Laboratories for analysis. Samples were analyzed within 1 month of sampling.

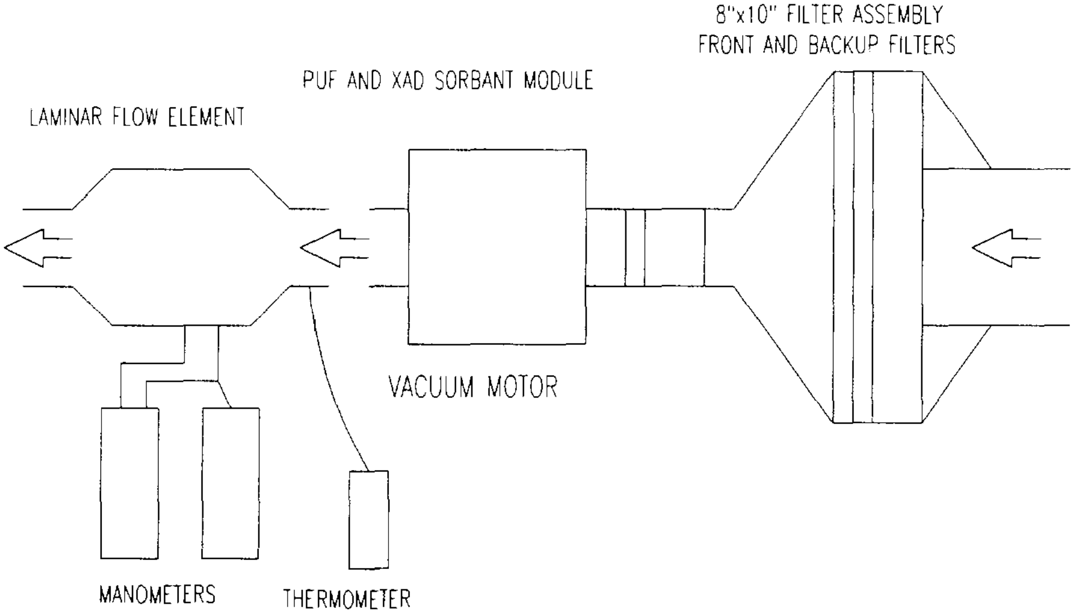


Figure 7. Diagram of PCDD and PCDF high volume sampler.

Table 16. Surrogate Standards

^{37}Cl-2,3,7,8-TCDD

^{13}C-1,2,3,4,7,8, -HxCDD

^{13}C-2,3,4,7,8-PeCDF

^{13}C-1,2,3,4,7,8-HxCDF

^{13}C-1,2,3,4,7,8,9 -HpCDF

Fuel, oil, and particle samples were collected in pre-cleaned Teflon lined screw cap bottles. Fuel and particle samples were shipped from MTA to UCD, where they were processed and prepared for shipping to Alta for analysis.

All samples were labeled, all pertinent information was put in a master log, and chain of custody procedures were used to track all samples.

Analysis of Diesel Exhaust Samples: The analytical procedures used for the analysis of PCDDs and PCDFs emission samples followed the analytical procedures given in Air Resources Board Stationary Source Test Method 428: Determination of Polychlorinated Dibenzo-p-Dioxin (PCDD), Polychlorinated Dibenzofuran (PCDF), and Polychlorinated Biphenyl Emissions from Stationary Sources, (ARB Stationary Source Test Methods Vol. 3).

The PUF, PUF module, and filters from a pooled sample were put into a Soxhlet extraction apparatus. The samples were spiked with PCDDs and PCDFs internal standards and then extracted in toluene for 24 hours. The sample extract was concentrated by roto-evaporation. Half the extract was further concentrated by nitrogen evaporation and the other half was archived. The concentrated extract was cleaned up by using silica gel, alumina, and carbopak/celite. Recovery standards were added to the sample and the analysis was performed by HRGC/HRMS. The GC was equipped with a 60 meter x .25 mm ID DB-5MS column. The mass spectrometer was capable of accurately resolving masses to four decimal places and a resolving power of >10,000. The PCDDs and PCDFs analyzed are listed in Table 17.

Table 17. List of Target Compounds

PCDDs	PCDFs
2,3,7,8-TCDD	2,3,7,8-TCDF
total TCDD	total TCDF
1,2,3,7,8-PeCDD	1,2,3,7,8-PeCDF
total PeCDD	2,3,4,7,8-PeCDF
1,2,3,4,7,8-HxCDD	total PeCDF
1,2,3,6,7,8-HxCDD	1,2,3,4,7,8-HxCDF
1,2,3,7,8,9-HxCDD	1,2,3,6,7,8-HxCDF
total HxCDD	1,2,3,7,8,9-HxCDF
1,2,3,4,6,7,8-HpCDD	total HxCDF
total HpCDD	1,2,3,4,6,7,8-HpCDF
total OCDD	1,2,3,4,7,8,9-HpCDF
	total HpCDF
	total OCDF

Analysis of Diesel Fuel, Oil, and Tunnel Particulate Matter: Fuel and oil samples were prepared and analyzed by EPA method 8270. 10 to 100 mls of fuel was spiked with internal standards and diluted with dichloromethane. Clean-up by column chromatography was performed to remove hydrocarbon interferences. One-half of each sample was concentrated by roto-evaporation. Tetradecane, a keeper solvent, was added and each sample was further concentrated by nitrogen evaporation. Recovery standards were added and each sample was analyzed by HRGC/HRMS.

Tunnel particulates and manifold particulate samples were spiked with internal standards and Soxhlet extracted in toluene. Further clean-up was done by column chromatography. One-half of each sample was concentrated by roto-evaporation. Tetradecane, a keeper solvent, was added and each sample was further concentrated by nitrogen evaporation. Recovery standards were added and each sample was analyzed by HRGC/HRMS.

2.13 Bioassay

Chemicals: All organic solvents were HPLC grade or at least this equivalent grade in purity. Methanol, acetone, hexane, and water (HPLC grade) were obtained from Fisher Scientific. For mutagenicity experiments, benzo(a)pyrene and dimethylsulfoxide (DMSO; spectrophotometric grade) were from Aldrich Chemical Co. and were used without further purification. Dichloromethane (DCM, OmniSolve) was from EM Science. For solid phase extractions (SPE), acetonitrile (ChromPure) and hexane (UV, High Purity) were from Burdick and Jackson.

Filter Samples: Particulate samples extracted for bioassay analyses were collected from a high-volume sampler described in the methods section on PAH. In a typical sampling procedure, particles were collected on pre-cleaned 8" x 10" Teflon-coated glass fiber filters (T60A20 - Pallflex, Putnam, Conn.). Particulate matter from each sample were collected on primary and secondary (backup) filter. Filters were extracted either by SAPRC using DCM and Soxhlet extraction (48 hr), or by UC Davis using DCM and sonication. The DCM extract was further concentrated using roto- and nitrogen-evaporation. The mass of particulate matter which was collected and extracted is summarized in Table 18. The entire scope of samples processed and tested in bioassay are also presented in this table.

All particle masses represent the particulate matter collected during 2 consecutive FTP transient cycles. The total particulate mass extracted, the particulate mass used for HPLC fractionation, and the particulate mass of unfractionated extract are reported in Table 19. HPLC fraction blanks (no sample injected onto the HPLC) were also tested. The HPLC was used to fractionate the whole extract into nine fractions, with the least polar compounds eluting in fraction 1, and the most polar fractions eluting in fraction 9. Details of the procedure are presented by SAPRC for the analyses of nitro-PAHs in the Methods section. Filter blanks were also incorporated. These blanks included: 1) trip blanks - filters handled and extracted as experimental samples, and 2) field or tunnel blanks - filters used to collect tunnel air without the engine running.

Table 18. Samples extracted and tested in bioassay.

Fuel Type	Test Cycle ID	Ave Particle Emissions (g/Bhp-hr)	Laboratory Sample ID	Particle Wt Per Hi Vol Filter (2 cycles each) (mg)	Samples Tested
Pre-1993	338H8,H9	0.201	96-20,96-21[a]	79.9	PM, HPLC
Pre-1993	337H1,H2	0.212	H1FV[b]	76.6	PM, VP
Pre-1993	339H6,H7	0.223	H3FV[b]	76.1	PM, VP
Low Aromatic	346H3, H4	0.177	96-44,96-45[a]	65.5	PM, HPLC
Low Aromatic	344H1, H2	0.1979	H4FV[b]	55.9	PM, VP
Low Aromatic	344H3, H4	0.187	H5FV[b]	60.2	PM, VP
Reformulated	351H1, H2	0.172 [c]	96-62, 96-63[a]	82.8	PM, HPLC
Reformulated	348H1,H2	0.172[c]	H6FV[b]	46.7 [d]	PM, VP
Reformulated	351H7,352H7	0.171 [c]	H7FV[b]	60.6	VP

a SAPRC UC Riverside ID. Sample extracted and fractionated by SAPRC.
b UC Davis ID. Sample extracted and fractionated by UC Davis.
PM=particulate matter; VP=Vapor-phase, HPLC = high performance liquid chromatography.
c Values from secondary dilution tunnel per CFR.
d Sampler leaked see Table 41 for PAHs for details

Table 19. Mass of Particulate Matter from the Hi Volume Sampler Collected and Extracted for the HPLC Bioassay Analyses.

Fuel	Total [a] Collected Particulate mass (mg)	Particulate mass Fractionated (HPLC) (mg)	Particulate mass Unfractionated (mg)
Pre-1993	79.9	26.6	13.3
Low Aromatic	65.5	21.8	10.9
Reformulated	82.8	27.6	13.8

a Particulate matter collected for two consecutive federal transient engine test cycles. All of the particulate matter was extracted and portions indicated were divided for fractionation, unfractionated, and archived samples. HPLC fractionation was conducted at UC Riverside.

The unfractionated extract (neat complex mixture extract) was used to determine relative potencies of each extract. A portion of the extract was transferred to a pre-cleaned 0.5 dram vial and taken to dryness under a gentle flow of nitrogen. The extract was then redissolved in DMSO for the bioassay. Doses were developed by serially diluting the redissolved solution in DMSO. To prepare the HPLC fractions for bioassay, a known aliquot of each fraction was transferred to a pre-cleaned 1/2 dram vial, dried under a gentle stream of nitrogen, and redissolved in DMSO. Dose-response relationships were developed for each fraction.

Collection of Vapor-Phase Sample: Samples were collected using the Hi-Vol system detailed in the Methods section for the collection of PAHs. The sampling train consisted of two 8" x 10" Teflon-coated glass fiber filters (T60A20; Pallflex, Putnam, Conn.), followed in series by two PUF plugs, and 60 ml of XAD-4 resin. The filters were extracted by solvent as described above, while the PUF and XAD were extracted using supercritical carbon dioxide (supercritical extraction).

Extraction of Vapor-phase Samples - Supercritical Fluid Extraction: Supercritical fluid extraction (SFE) of the organic compounds from the PUF and XAD samples was accomplished using liquid carbon dioxide (CO_2) as the extraction solvent at pressures and temperatures above its critical point of 72.9 atm and 31°C. Since CO_2 is a gas at atmospheric pressure, this technique produces a concentrated extract without residual solvent. The concentrated extract can be directly evaluated by using GC/MS analyses as well as bioassay analyses.

All supercritical fluid extractions were carried out using an ISCO Model 260D syringe pump (Lincoln, NE), an ISCO SFX2-10 extractor, and SFC/SFE grade CO_2 (Air Products, Allentown, PA) under a helium headspace of 2000 psi. Prior to sample extractions, the PUF and XAD adsorbents were placed into a 10 ml stainless steel extraction cell. For chemical recovery studies, the adsorbents were spiked with either target or substituted PAHs (for example alkyl-PAHs) in DCM. After spiking, the DCM was allowed to evaporate for a period of 10 minutes., after which approximately 1 mL of methanol was added as a modifier to the top of the adsorbent. For actual samples, approximately 1 mL of methanol was added as a modifier to the top of the adsorbent. The cell was then closed and placed into the extractor for a 5 minute temperature equilibration period. A static extraction (SFE without CO_2 flow into the collection vial) was conducted for 10 minutes prior to conducting the dynamic extraction (SFE with CO_2 flow into the collection vial). The syringe pump was operated at 5000 psi, 75°C, 90 min for the PUF samples and at 7000 psi, 100°C, 60 min for XAD samples. A length of stainless steel capillary tubing was used as a depressurizing flow restrictor to maintain supercritical conditions within the extraction cell. As the supercritical CO_2 (s-CO_2) exits the restrictor, it expands to a gas upon reaching ambient pressure. The effluent from the outlet of the capillary was directed into a graduated collection vial with a screw top fitted with a Teflon septum. The collection vial contained approximately 2 mL of methanol and was placed in a dry ice-ethanol bath to trap and concentrate the semi-volatile chemicals for direct mutagenicity and chemical analyses.

Fractionation of Samples: Samples were fractionated using solid phase extraction (SPE) to develop mutagenic profiles of the extracts. The extracts are processed through columns packed with C18 and silica sorbents. The solvent series and volumes used are summarized in Table 20. Each fraction was stored at -20° C in amber glass vials fitted with a Teflon-lined screw cap.

Table 20. Fractionation Sorbents and Solvents for Vapor-Phase Samples.

Sorbent	Solvent	Vol (mL)
C18 - Fraction 1	Methanol	2
C18 - Fraction 2	Methanol:Acetonitrile (1:1)	1
C18 - Fraction 3	Acetonitrile	1
C18 - Fraction 4	Dichloromethane	1
C18 - Fraction 5	Hexane	1
Silica - Fraction 1	Hexane	4
Silica - Fraction 2	Dichloromethane	1
Silica - Fraction3	Dichloromethane:Methanol (1:1)	1
Silica - Fraction 4	Methanol	1

Bioassay: Bioassay experiments were conducted to determine the specific mutagenic activity of the particulate and vapor-phase extracts. For these samples, the specific mutagenic activity is reported as the number of revertants per microgram equivalent of sample extract (rev/μg equiv.) or per μl equivalent solvent volume (rev/μl equiv.), respectively. The term "equivalent" refers to the addition of an extract of particulate matter that represents the original mass of particulate matter collected, or in the case of a vapor-phase sample, the addition of a volume of extract that represents a specified volume of the original extract. The specific mutagenic activity is determined using the slope obtained from the linear portion of the dose-response curve. All particulate extracts were then tested for mutagenicity using the microsuspension bioassay procedure (Kado et al, 1983) with tester strain TA98. This tester strain is the primary strain used for detecting the mutagens present in diesel exhaust. Particulate extracts were prepared for bioassay by first taking a portion of the extract which is in DCM, evaporating the DCM to dryness under a gentle stream of nitrogen, and redissolving the extract in DMSO. Doses of the extract were prepared by serially diluting the redissolved extract in DMSO. Vapor-phase samples were tested in tester strains TA98 and TA100.

For the microsuspension procedure, bacteria were grown overnight in Oxoid Nutrient Broth No. 2 (Oxoid Ltd., Hants, England) to approximately 1 - 2 x 10^9 cells/ml and harvested by centrifugation (5,000 x gravity, 4°C, 10 min). Cells were resuspended in ice-cold phosphate-buffered saline (0.15M phosphate-buffered saline, pH 7.4) to a concentration of approximately 1 x 10^{10} cells/ml. The S9 (metabolic enzymes) and S9 mix (enzyme co-factors) were prepared and 300 μg S9/ml final concentration was used. The S9 from Aroclor 1254 pretreated male Sprague-Dawley rats was obtained from Molecular Toxicology, Inc. (Annapolis, MD.) and contained 39.2 mg protein/ml. For the microsuspension assay, the following ingredients were added, in order, to 12 x 75 mm or 12 x 65 mm (screw cap) sterile glass culture tubes kept on ice: 0.1 mL S9 mix, 0.005 mL sample in DMSO, and 0.1 mL concentrated bacteria in PBS (1 x 10^{10} / mL phosphate-buffered saline). The mixture was incubated in the dark at 37°C with rapid shaking. After 90 min, the tubes were placed in an ice bath and taken out one at a time immediately before adding 2 ml molten top agar containing 90 nanomoles of histidine and biotin. The combined solutions were vortex-mixed and poured onto minimal glucose plates. Plates were incubated at 37°C in the dark for 48 hrs and counted using an automatic plate counter. Benzo(a)pyrene, 2-nitrofluorene, and nitroquinoline-N-oxide were used as a positive controls. Tester strain markers were routinely determined for each experiment.

3.0 Results and Discussion

3.1 Emission Rates for NOx, THC, CO, and PM

Attachment A contains NOx, THC, CO, and PM emission results for individual test cycles, run with pre-1993 fuel in Table A1, low aromatic fuel in Table A2, and the reformulated blend in Table A3. All cold- and hot-start transient emissions data reported were verified to meet the FTP validation criteria for transient engine operation. Table 21 presents the means and standard deviations for each fuel during the cold- and hot-start transient cycles. Also presented in Table 21 are the weighted total emission rates calculated from the means of the cold- and hot-starts. Table 21 also presents the percent change in emission rates for the low aromatic and reformulated fuels compared to the pre-1993 fuel. Plots of the means and 95% confidence intervals for cold– and hot–start results are presented in Figures 8 and 9, respectively. The means and standard deviations for the pre-1993 fuel were calculated from all test results obtained with this fuel, including the initial and final (repeat) tests run at the end of the main sampling study as described in Section 3.2. Table 22 summarizes statistical results from an analysis of variance (ANOVA) and an analysis of pairwise differences between fuels, using the Fisher's protected least square difference method. Values of $p \leq 0.05$ indicate significant differences among the fuel types at the 95% confidence level. Statistically significant differences at the 95% confidence level for pairwise comparisons are indicated by an S in the table. Statistical analysis of the weighted total emission rates was not performed. There were considerably fewer cold-start cycles run compared to hot-start cycles. This makes a meaningful statistical analysis of the weighted total difficult.

Table 21. Average Emission Test Results on Cummins L10 engine for Pre-1993, Low Aromatic, and Reformulated Fuel.

Pre-1993

	Cold-Start		Hot-Start		Weighted Total (1)	
	Mean gm/bhp-hr	SDEV gm/bhp-hr	Mean gm/bhp-hr	SDEV gm/bhp-hr	Mean gm/bhp-hr	SDEV gm/bhp-hr
NOx	4.98	0.13	4.74	0.12	4.77	0.1
THC	0.56	0.02	0.52	0.02	0.53	0.02
CO	2.38	0.11	2.24	0.19	2.26	0.16
CO2	547.3	8.1	518	3.9	522.1	3.5
PM	0.259	0.016	0.218	0.01	0.224	0.009
BSFC(lb/bhp-hr)	0.385	0.007	0.364	0.003	0.367	0.003
Work(bhp-hr)	22.254	0.087	22.327	0.046	22.317	0.041

Low Aromatic

	Cold-Start			Hot-Start			Weighted Total (1)		
	Mean gm/bhp-hr	SDEV gm/bhp-hr	(2) %	Mean gm/bhp-hr	SDEV gm/bhp-hr	(2) %	Mean gm/bhp-hr	SDEV gm/bhp-hr	(2) %
NOx	4.6*	0.06	-7.6	4.41	0.06	-7	4.44	0.05	-7.1
THC	0.41*	0.01	-27	0.48*	0.02	-7.7	0.47	0.02	-11
CO	2.47	0.01	3.8	2.44*	0.04	8.9	2.44	0.03	8.1
CO2	545.6	1.4	-0.3	511.7	6.2	-1.2	516.5	5.31	-1.1
PM	0.193*	0.003	-25	0.181*	0.008	-17	0.183	0.007	-18
BSFC(lb/bhp-hr)	0.387	0.001	0.5	0.364	0.004	0	0.367	0.003	0.1
Work(bhp-hr)	21.962	0.034	-1.3	22.035	0.222	-1.3	22.025	0.19	-1.3

Reformulated

	Cold-Start			Hot-Start			Weighted Total (1)		
	Mean gm/bhp-hr	SDEV gm/bhp-hr	(2) %	Mean gm/bhp-hr	SDEV gm/bhp-hr	(2) %	Mean gm/bhp-hr	SDEV gm/bhp-hr	(2) %
NOx	4.85*	0.05	-2.6	4.61	0.19	-2.7	4.64	0.16	-2.7
THC	0.47*	0.02	-16	0.5*	0.02	-3.8	0.5	0.02	-5.7
CO	2.3	0.06	-3.4	2.3*	0.05	2.7	2.3	0.04	1.8
CO2	549.2	2.2	0.3	511.6	4.4	-1.2	517	3.8	-1
PM	0.212*	0.019	-18	0.182*	0.012	-17	0.186	0.011	-17
BSFC(lb/bhp-hr)	0.385	0.002	0	0.359	0.003	-1.4	0.362	0.003	-1.2
Work(bhp-hr)	22.128	0.043	-0.6	22.198	0.047	-0.6	22.188	0.041	-0.6

1) Wt. Tot .= (1/7 gm(cold) + 6/7 gm(hot))/(1/7 Bhp-hr(cold)+ 6/7 Bhp-hr(hot)); SDEV = $\surd(1/6\ \mathrm{SDEV(cold)})^2 + (6/7\mathrm{SDEV(hot)})^2$

2) Percent change in mean from the Pre-1993 fuel.

* Significant at 95% confidence limit using Fisher's Protected Least Significant Difference Test

Figure 8. Cold-Start Emission Rates and 95% Confidence Intervals

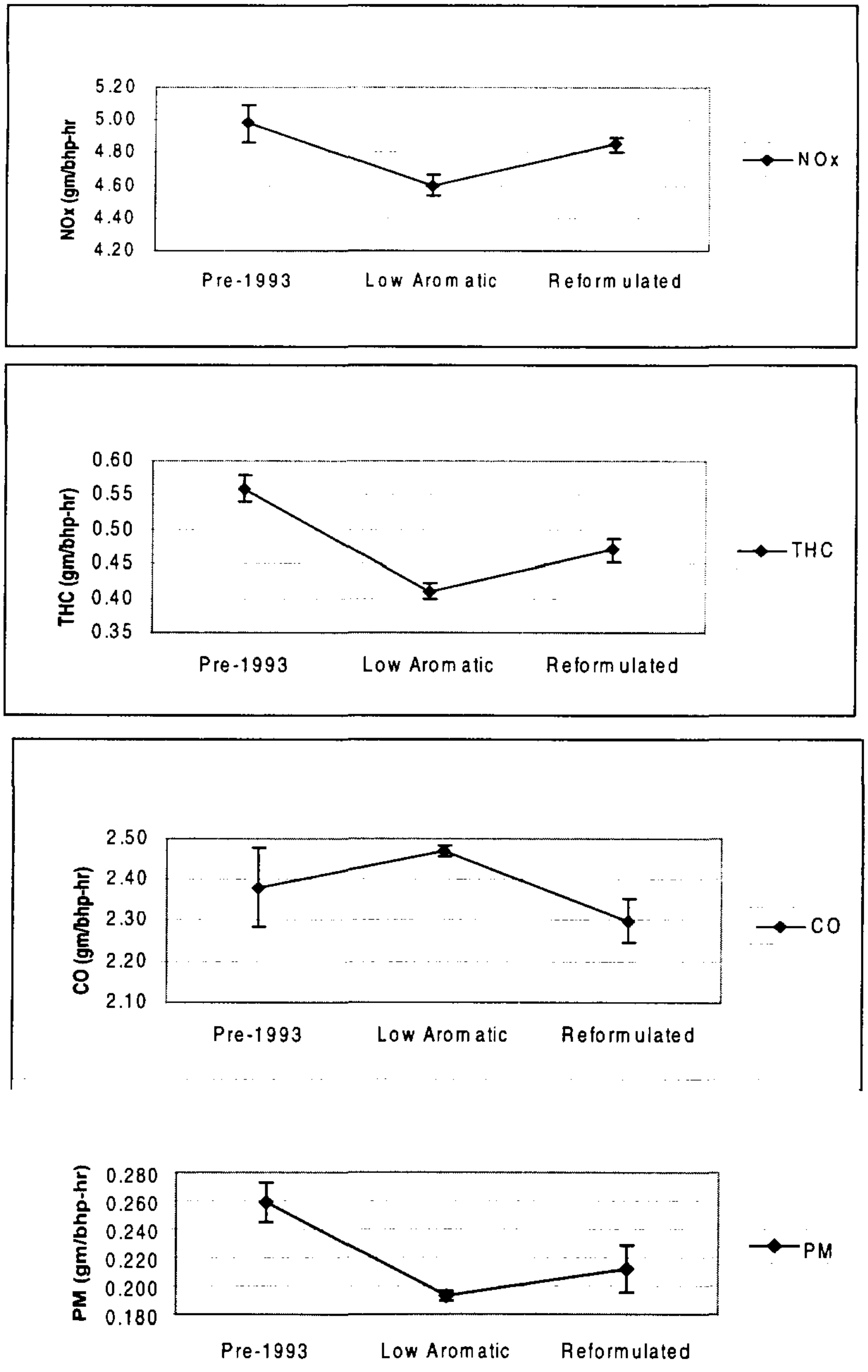


Figure 9. Hot-Start Emission Rates and 95% Confidence Intervals

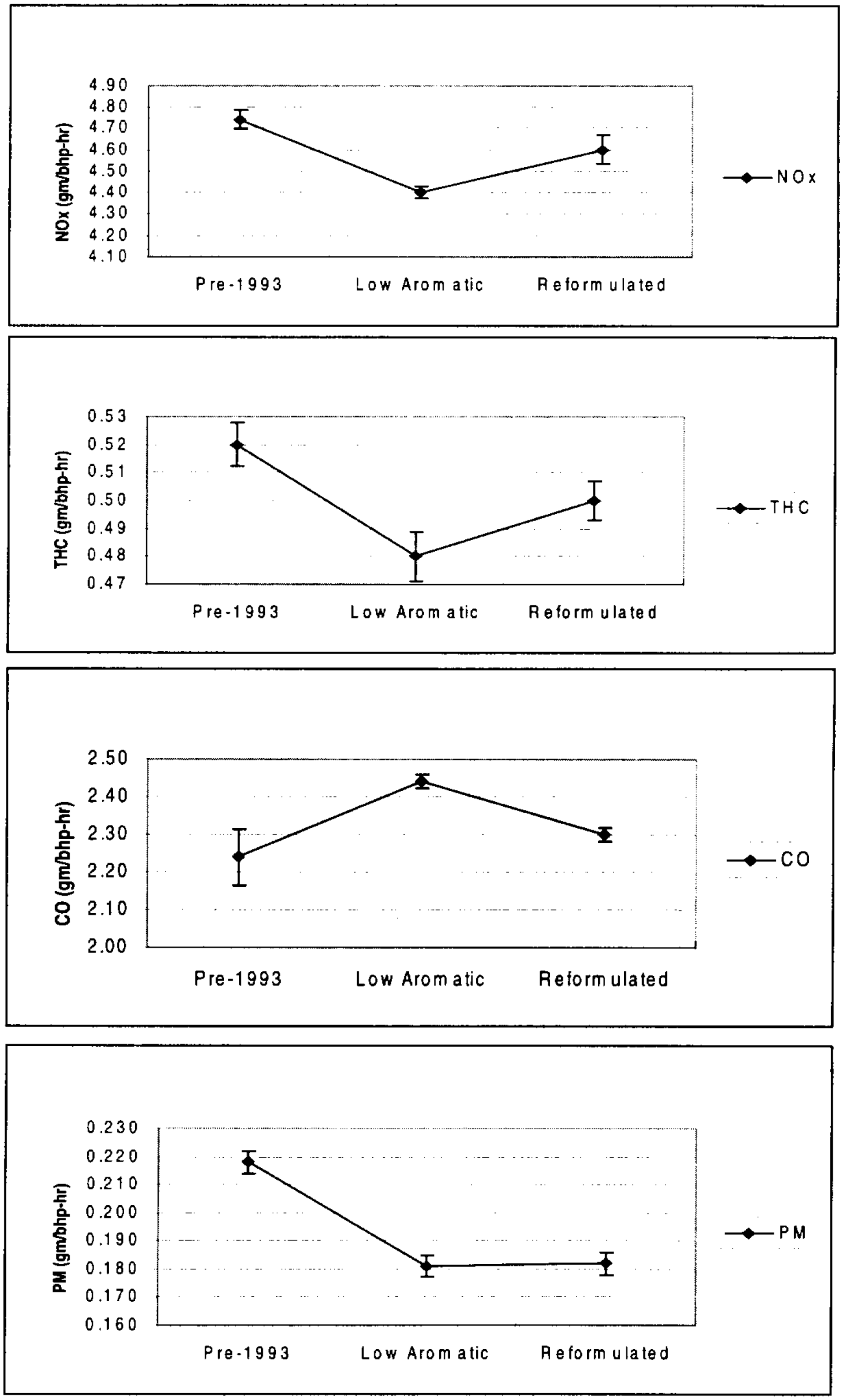


Table 22. **Analysis of variance (ANOVA) and pairwise Fisher's protected least square difference results for NOx, THC, CO, and PM**

	NOx		THC		CO		PM	
Cycle	**Cold**	**Hot**	**Cold**	**Hot**	**Cold**	**Hot**	**Cold**	**Hot**
P-value	.005	<.0001	<.0001	<.0001	.0579	<.0001	<.0001	<.0001
Low Ar. vs. Pre-93	S	S	S	S		S	S	S
Low Ar. vs. Reform.	S	S	S	S		S		
Pre-93 vs. Reform.	S	S	S	S		S	S	S

NOx, THC, and PM show reductions in emission rates for both the cold- and hot-start cycles with the low aromatic and reformulated fuels in comparison to the pre-1993 fuel. For NOx and THC, all of the pairwise differences between fuel types are statistically significant at the 95% confidence level. For PM, the low aromatic and reformulated fuels show a statistically significant difference compared to the pre-1993 fuel, but are not different from each other. These same trends in NOx, THC, and PM emission rates are observed for the weighted total emission rates.

The low aromatic fuel indicates an increase and the reformulated fuel a decrease in cold-start CO emissions compared to the pre-1993 fuel, but none of the differences are significant at the 95% confidence level. Both the low aromatic and reformulated fuels show increases in hot-start CO emission rates in comparison to the pre-1993 fuel. All of the differences in hot-start CO emission rates are statistically significant at the 95% confidence level. The weighted total CO emission rates for the low aromatic and reformulated fuels are higher than the pre-1993 fuel. For the reformulated fuel this results from the higher weighting of the hot-start emissions.

The reductions in NOx emission rates with the low aromatic and reformulated fuels range from 2.6 to 7.6%. Cold-start THC emissions were reduced by 27% with the low aromatic fuel and 16% with the reformulated fuel, while hot-start THC emissions were reduced by 7.7 and 3.8% with the low aromatic and reformulated fuels, respectively. The low aromatic and reformulated fuels have the largest impact on PM emission rates, with reductions ranging from 17 to 25% compared to the pre-1993 fuel.

The present study was designed to examine differences between specific fuels rather than the effects of fuel parameters on emissions. As a consequence, there is a high correlation between fuel parameters for the three fuels studied which makes it difficult to conduct a detailed analysis of the impact of specific fuel parameters on emissions. The emission results are consistent with the general trends reported for the effects of changes in diesel fuel parameters on emissions. CARB's original decision to limit the sulfur and aromatic content of diesel fuel was based upon available data at that time which indicated these parameters had a significant impact upon emissions. This included data generated in a Coordinating Research Council (CRC) study to assess the impact of aromatic content, 90% boiling point, and sulfur content (Ullman, 1989a, 1989b). These results indicated that reduction of the aromatic content reduced HC, CO, NOx, and PM emissions, and that reduced sulfur levels resulted in lower PM emissions. During this phase of the CRC project, the aromatic content and fuel cetane number were highly correlated. Work done in the next phase of the CRC project (Ullman et. al., 1990a, 1990b) separated the effects of aromatic content and cetane number and concluded that increased cetane number was the key to reducing HC and CO emissions, and that both increased cetane number and lowered aromatic content decreased NOx and PM emissions. From the reformulated fuel analysis results presented in Table 2, it is apparent that refiners have utilized both reduced aromatic content and increased cetane number to certify alternative formulations (Nikanjam, 1993). It should be noted that the emissions results for the reformulated blend should not necessarily be expected to be equivalent to or less than the low aromatic fuel studied in this project. First, the equivalency testing for CARB certification is specified to be performed on a Detroit Diesel Corporation Series 60 engine and it has been shown that the effects of fuel changes are dependent upon engine type (Ullman, 1989a, 1989b). Second, the low aromatic fuel has substantially lower aromatic content than the maximum allowed for CARB reference fuel (4.3% vs. 10%) and higher cetane number than the minimum allowed for the reference fuel (54.2 vs. 48).

3.2 Assessment of Engine Emissions Drift during Testing

The main sampling phase of this project was conducted over a three week period in December, 1996 with the following sequence: pre-1993 fuel tested the week of December 1, low aromatic fuel tested the week of December 8, and reformulated fuel tested the week of December 16. To assess whether any drift occurred with the engine emissions over this test period that would affect interpretation of the results, emission tests were also conducted with the pre-1993 fuel the week of January 5, 1997.

This also provided an additional opportunity to collect additional samples for analysis Complete results are presented in Table A1 in Appendix A. Means and 95% confidence intervals for NOx, THC, CO, and PM emission rates obtained during the initial (week of 12/1/96) and final (week of 1/5/97) tests with the pre-1993 fuel are presented in Figure 10. An analysis of variance (ANOVA) shows that the increase in hot-start NOx ($p<0.001$) and decrease in cold-start CO ($p=0.0133$) are statistically significant at the 95% confidence level. None of the other emission differences were found to be statistically significant at the 95% confidence level. The statistically significant emission trends associated with fuel changes are independent to the time sequence trends. The emission rate drift is attributed to variations in the extensive preconditioning sequence performed during fuel changeover. In the absence of a statistically significant relationship between time sequence and emission rate, it is concluded the effect of engine drift on emission rate is negligible.

3.3 Particle Size Distributions

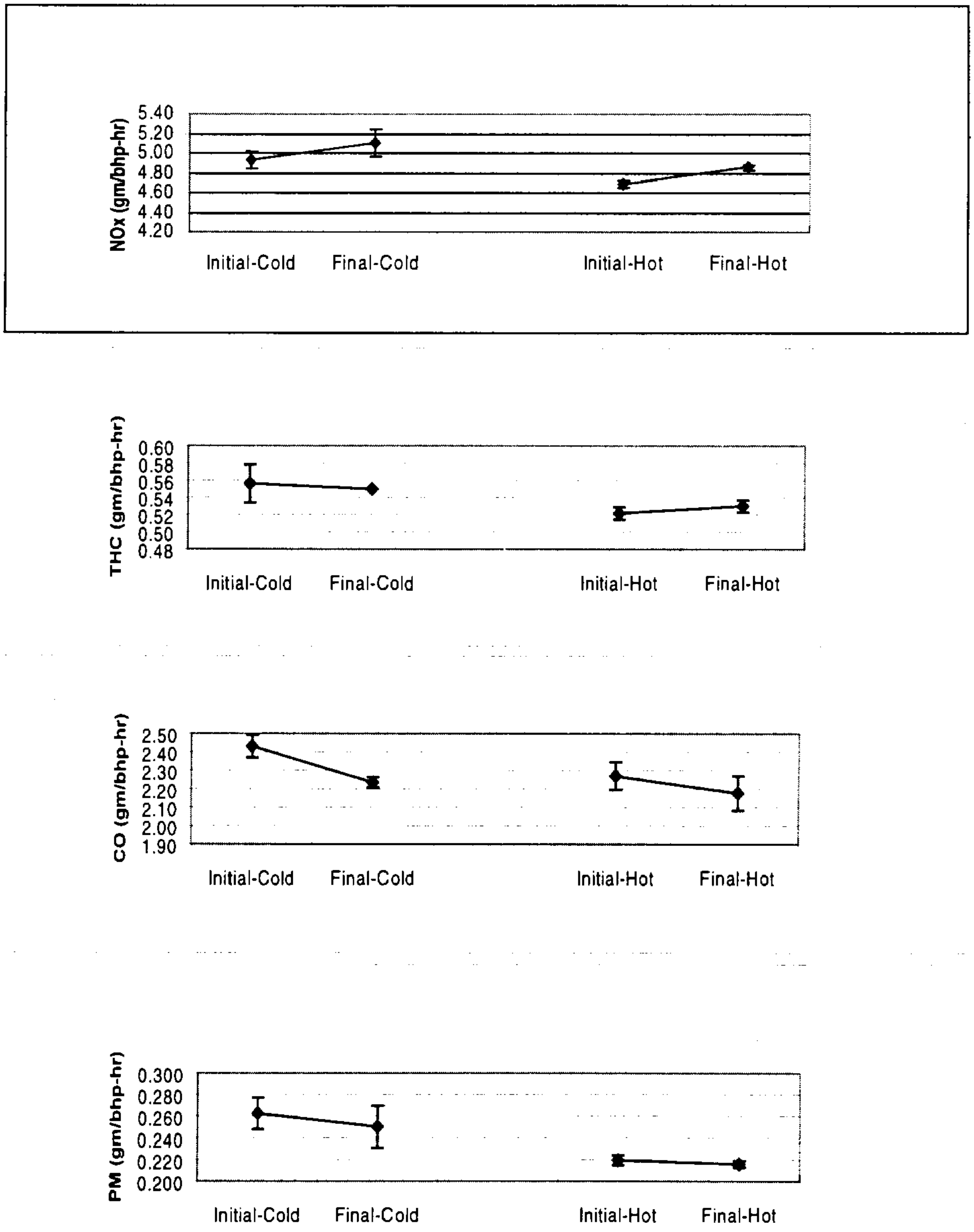
Particle size distribution results form the MOUDI cascade impactor presented as per cent of total particulate mass below 10 and 2.5 μm for each fuel are presented in Table 23.

Table 23. Particle Size Distributions

Fuel	Percent <10μm	SDEV	Percent <2.5 μm	SDEV
		Cold-Start		
Pre-1993	**99.6**	**0.1**	**98.0**	**0.4**
Low Aromatic	**99.1**	**0.5**	**95.0**	**0.8**
Reformulated	**99.2**	**0.7**	**97.3**	**0.8**
		Hot-Start		
Pre-1993	**99.8**	**0.6**	**98.7**	**0.7**
Low Aromatic	**99.3**	**0.2**	**96.9**	**0.4**
Reformulated	**99.3**	**0.6**	**97.9**	**1.4**

No significant differences were noted in the particle size distributions between the three fuels and as expected the majority of the particles (>95%) were found to be less than 2.5 µm in aerodynamic diameter.

Figure 10. Initial and Final Emissions with Pre-1993 Diesel Fuel



3.4 Elemental and Organic Carbon, Ion, and Elemental Analyses of Particulates

Complete ion and organic and elemental carbon analysis results are summarized in Table A4 of Appendix A. Elemental analysis results are summarized in Table A5 of Appendix A. Table 24 presents results for components that were detected at levels at least twice the analytical uncertainty. All results have been background corrected. Elemental and organic carbon dominate the composition of the particulate matter for all fuels and represent more than 97% of the total identified mass, consistent with previous studies of diesel particulate (Watson, et al., 1994, Hildemann, et al., 1991). The major changes in emission rates associated with the fuel are found in nitrate, sulfur and sulfate, and elemental and organic carbon. Although the low aromatic and reformulated fuels show lower elemental and organic carbon emission rates associated with the lower total PM emission rates for these fuels, the organic carbon as a percent of total carbon is relatively constant for all three fuels and ranges from 33 to 40%. Selected backup quartz fiber filters were analyzed by the TOR method to assess the contribution of organic carbon artifact formation. These results show that the backup filters contain organic carbon equivalent to 14-20% of the primary filter organic carbon contents which can be ascribed to sampling artifacts resulting from adsorption of gas phase organics on the filter media.

Nitrate emission rates are higher for the low aromatic and reformulated fuels. This may be a result of the use of organonitrates as cetane improvers in the reformulated fuel, but is unexplained for the low aromatic fuel. Sulfur and sulfate emissions follow the trend pre-1993 > reformulated > low aromatic. This is the same order as the fuel sulfur levels. Mg, P, Ca, and Zn emission rates are relatively constant. This is expected as these are known engine oil components. The emission rates of these oil derived components and Fe (due to engine wear) are consistently higher during the cold-start cycles than the hot-start cycles. Si emissions are relatively constant for all fuels and all cycles with the source of these emissions being unknown. Cl and Cu are minor components and are observed at levels close to or slightly above twice the analytical uncertainties.

The sum of the identified species represents 83 to 95% of the total PM mass for these results. Similar results have been reported in previous studies (Lowenthal, et al., 1994) and have been ascribed to non-uniform filter deposits and to the fact that these analyses do not measure species such as hydrogen and oxygen which contribute to the total PM mass.

Table 24. Ion, Elemental, and Organic and Elemental Carbon

Pre-1993

	Cold Start		Hot Start	
	Emission Rate mg/Bhp-hr	SDEV mg/Bhp-hr	Emission Rate mg/Bhp-hr	SDEV mg/Bhp-hr
Nitrate	*0.07*	0.08	*0.08*	0.01
Sulfate	1.10	0.97	1.11	0.09
Ammonium	0.59	0.19	0.58	0.12
Organic Carbon	89.00	9.02	66.51	8.57
Elemental Carbon	133.98	4.24	125.87	7.96
Mg	0.081	0.044	0.066	0.034
Si	0.619	0.109	0.748	0.092
P	0.079	0.017	0.044	0.011
S	1.725	0.187	1.349	0.060
Cl	*0.022*	0.001	*0.026*	0.025
Ca	0.084	0.046	0.079	0.045
Fe	0.451	0.339	0.213	0.135
Cu	0.012	0.006	0.008	0.013
Zn	0.156	0.032	0.096	0.019
Sum	227.90		196.70	
Total PM	259		218	

Low Aromatic

	Cold Start		Hot Start	
	Emission Rate mg/Bhp-hr	SDEV mg/Bhp-hr	Emission Rate mg/Bhp-hr	SDEV mg/Bhp-hr
Nitrate	0.37	0.04	0.39	0.05
Sulfate	0.39	0.10	*0.10*	0.02
Ammonium	0.37	0.04	0.30	0.05
Organic Carbon	56.28	1.73	51.29	3.33
Elemental Carbon	109.86	7.98	118.87	6.54
Mg	0.080	0.049	*0.039*	0.012
Si	0.674	0.081	0.637	0.081
P	0.089	0.018	0.060	0.013
S	0.530	0.068	0.264	0.034
Cl	0.048	0.012	0.039	0.004
Ca	0.064	0.013	0.030	0.008
Fe	0.441	0.041	0.067	0.024
Cu	0.006	0.001	*0.000*	0.001
Zn	0.174	0.042	0.107	0.013
Sum	169.38		172.19	
Total PM	193		181	

Reformulated

	Cold Start		Hot Start	
	Emission Rate mg/Bhp-hr	SDEV mg/Bhp-hr	Emission Rate mg/Bhp-hr	SDEV mg/Bhp-hr
Nitrate	0.19	0.07	0.17	0.04
Sulfate	1.05	0.81	0.43	0.04
Ammonium	0.43	0.20	0.34	0.05
Organic Carbon	58.27	5.83	51.24	7.06
Elemental Carbon	113.87	18.75	103.70	14.82
Mg	*0.077*	0.046	*0.051*	0.041
Si	0.628	0.185	0.512	0.088
P	0.110	0.056	0.072	0.018
S	0.925	0.327	0.570	0.033
Cl	*0.028*	0.013	0.033	0.010
Ca	0.095	0.066	0.045	0.011
Fe	0.331	0.311	0.089	0.026
Cu	0.009	0.005	*0.005*	0.004
Zn	0.267	0.136	0.153	0.019
Sum	176.28		157.41	
Total PM	212		182	

Values in Italics are < twice the analytical uncertainty

3.5 Carbonyls and Speciated Hydrocarbons

The comprehensive carbonyl compound (aldehydes and ketones) emission results are presented in Table A6 of Attachment A. Emission rates for formaldehyde, acetaldehyde, acrolein, and propionaldehyde are presented in Table 25. Means and standard deviations for each fuel during the cold- and hot-start transient cycles are presented together with the weighted total emission rates calculated from the means of the cold- and hot-starts. Table 25 also presents the percent change in emission rates for the low aromatic and reformulated fuels compared to the pre-1993 fuel. Means and 95% confidence limits are plotted in Figure 11. Table 26 summarizes statistical results from an analysis of variance (ANOVA) and an analysis of pairwise differences between fuels using the Fisher's protected least square difference method. Statistical analysis of the weighted total emission rates was not performed. There were fewer cold-start cycles run compared to hot-start cycles. This makes a meaningful statistical analysis of the weighted total difficult.

Results presented in Table 25 show that the same patterns and similar emission rates are observed for each fuel with the highest emissions observed for formaldehyde followed by acetaldehyde. The low aromatic fuel has lower formaldehyde and acetaldehyde emissions than the pre-1993 and reformulated fuels during the cold-start cycle which are statistically significant at the 95% confidence level. This trend is not observed for the hot-start. The pre-1993 fuel has lower hot-start acetaldehyde emissions than the low aromatic and reformulated fuels. The other statistically significant difference is higher cold- and hot-start acrolein emissions for the low aromatic fuel in comparison to the pre-1993 and reformulated fuels.

Complete gas-phase hydrocarbon speciation results are presented in Table A7 in Appendix A. Emission rates for components of interest to the ARB (1,3-butadiene, benzene, toluene, ethylbenzene, o-xylene, m&p-xylene, styrene, and naphthalene) are presented in Table 27. Means and standard deviations for each fuel during the cold- and hot-start transient cycles are presented together with the weighted total emission rates calculated from the means of the cold- and hot-starts. Table 27 also presents the percent change in emission rates for the low aromatic and reformulated fuels compared to the pre-1993 fuel. Means and 95% confidence limits are plotted in Figure 12. Table 28 summarizes statistical results from an analysis of variance (ANOVA) and an analysis of pairwise differences between fuels using the Fisher's protected least square difference method. Statistical analysis of the weighted total emission rates was not performed. There were fewer cold-start cycles run compared to hot-start cycles. This makes a meaningful statistical analysis of the weighted total difficult.

As with the carbonyl results, the hydrocarbon emissions show the same emission trends and similar emission rates for all fuels. The low aromatic fuel has higher hot-start 1,3-butadiene, higher cold- and hot-start benzene, higher hot-start toluene, lower cold-start o-xylene, and lower hot-start m&p-xylene emissions than the other fuels. All of these trends are statistically significant at the 95% confidence level. These same trends are observed with the weighted total emissions. No statistically significant differences are observed between the pre-1993 and reformulated fuels.

Very little data exists on the possible effects of diesel fuel composition on carbonyl and speciated hydrocarbon emissions. Spreen, et al., 1995, have reported limited results from heavy-duty engine dynamometer studies with the conclusion that lowering the aromatic content did not appear to have a significant impact on emission rates for individual carbonyl or hydrocarbon species. Hublin, et al., 1996, have reported results on the effects of diesel fuel parameters on emissions of benzene, 1,3-butadiene, formaldehyde, and acetaldehyde from studies of light-duty diesel vehicles on a chassis dynamometer. The overall observation was that emissions of these species varied more with engine technology than with fuel composition. The analytical results for the carbonyls and hydrocarbons do not differ sufficiently to allow for a meaningful analysis, especially when combined with limited differences in analytical results.

Table 25. Emission Rates for Carbonyls.

Pre-1993

	Cold-Start		Hot-Start		Weighted Total (1)	
	Mean	**SDEV**	**Mean**	**SDEV**	**Mean**	**SDEV**
	mg/Bhp-hr	mg/Bhp-hr	mg/Bhp-hr	mg/Bhp-hr	mg/Bhp-hr	mg/Bhp-hr
Formaldehyde	62.33	1.37	56.26	0.95	57.12	0.83
Acetaldehyde	20.23	0.31	17.81	0.2	18.15	0.18
Acrolein	2.18	1.12	2.13	0.74	2.14	0.65
Propionaldehyde	4.45	0.08	3.56	0.43	3.69	0.37

Low Aromatic

	Cold-Start			Hot-Start			Weighted Total (1)		
	Mean	**SDEV**	**(2)**	**Mean**	**SDEV**	**(2)**	**Mean**	**SDEV**	**(2)**
	mg/Bhp-hr	mg/Bhp-hr	%	mg/Bhp-hr	mg/Bhp-hr	%	mg/Bhp-hr	mg/Bhp-hr	%
Formaldehyde	54.75 *	2.1	-12	59.42	5.77	5.6	58.75	4.95	2.8
Acetaldehyde	17.66 *	0.45	-13	19.34 *	1.72	8.6	19.1	1.47	5.2
Acrolein	5.49 *	0.69	152	5.84 *	0.91	174	5.79	0.79	171
Propionaldehyde	3.9	0.22	-12	3.92	0.49	10	3.92	0.42	6.2

Reformulated

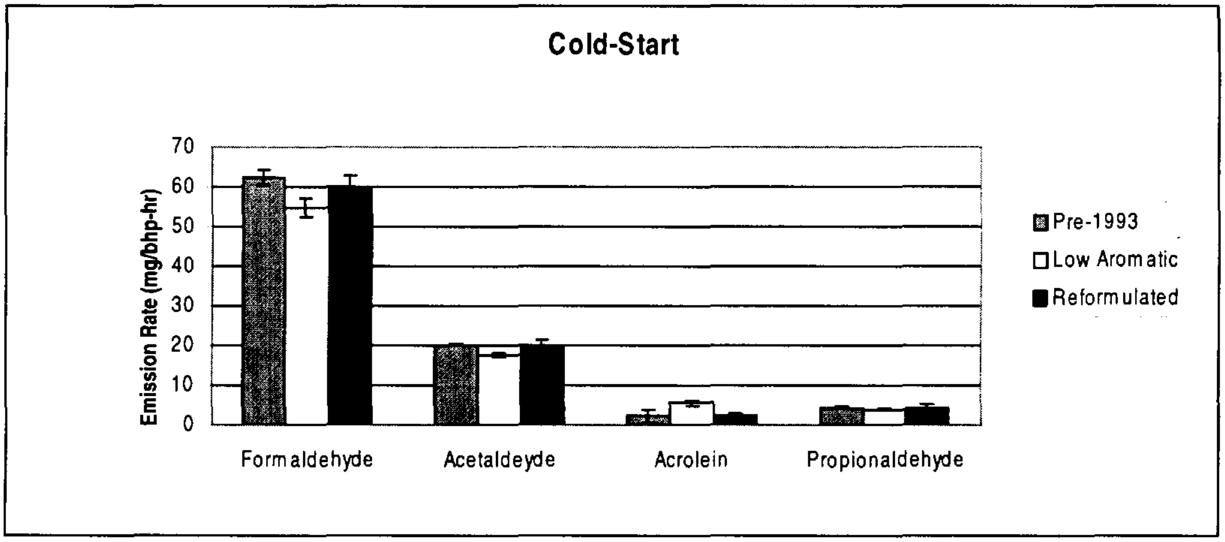
	Cold-Start			Hot-Start			Weighted Total (1)		
	Mean	**SDEV**	**(2)**	**Mean**	**SDEV**	**(2)**	**Mean**	**SDEV**	**(2)**
	gm/Bhp-hr	mg/Bhp-hr	%	mg/Bhp-hr	mg/Bhp-hr	%	mg/Bhp-hr	mg/Bhp-hr	%
Formaldehyde	59.69	2.71	-4.2	59.85	1	6.4	59.83	0.94	4.7
Acetaldehyde	20.1	1.1	-0.6	19.9 *	0.58	12	19.93	0.52	9.8
Acrolein	2.52	0.44	16	2.41	1.71	13	2.42	1.47	14
Propionaldehyde	4.28	0.84	-3.8	4.11	0.47	15	4.13	0.42	12

(1) Wt. Tot .= (1/7 mg(cold) + 6/7 mg(hot))/(1/7 Bhp-hr(cold)+ 6/7 Bhp-hr(hot)); SDEV = v(1/6 SDEV(cold))2 + (6/7SDEV(hot))2

(2) Percent change in mean from the Pre-1993 fuel.

* Significant at 95% confidence limit using Fisher's Protected Least Significant Difference Test

Figure 11. Means and 95% Confidence Intervals for Carbonyls.



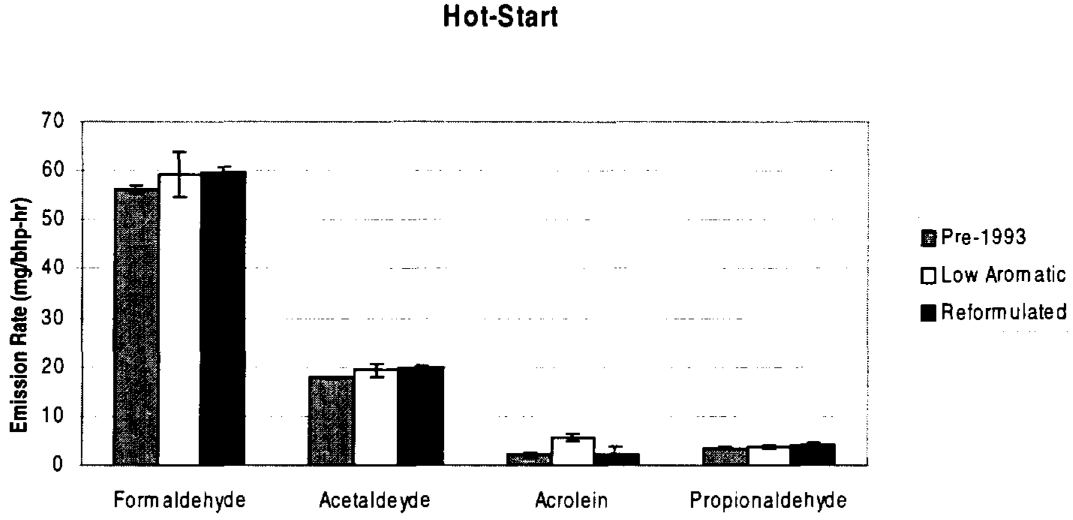


Table 26. Analysis of variance (ANOVA) and pairwise Fisher's protected least square difference results for carbonyls.

	Formaldehyde		Acetaldehyde		Acrolein		Propionaldehyde	
	Cold	**Hot**	**Cold**	**Hot**	**Cold**	**Hot**	**Cold**	**Hot**
P-value	.0316	.2581	.0178	.0245	.0055	<.0001	.5384	.1746
Low Ar. vs. Pre-93	S		S	S	S	S		
Low Ar. vs. Reform.	S		S		S	S		
Pre-93 vs. Reform.				S				

Table 27. Emission Rates for Gas Phase Hydrocarbons

	Pre-1993					
	Cold-Start		Hot-Start		Weighted Total (1)	
	Mean mg/Bhp-hr	**SDEV** mg/Bhp-hr	**Mean** mg/Bhp-hr	**SDEV** mg/Bhp-hr	**Mean** mg/Bhp-hr	**SDEV** mg/Bhp-hr
1,3-Butadiene	2.08	0.52	1.75	0.11	1.8	0.12
Benzene	6.49	0.04	5.8	0.3	5.9	0.26
Toluene	2.17	0.1	1.89	0.27	1.93	0.23
Ethylbenzene	0.74	0.06	1.3	0.66	1.22	0.57
o-Xylene	0.85	0.09	0.77	0.09	0.78	0.08
m&p-Xylene	1.9	0.23	2.12	0.55	2.09	0.47
Styrene	0.97	0.34	1.32	0.31	1.27	0.27
Naphthalene	1.33	0.09	1.75	0.5	1.69	0.43

	Low Aromatic								
	Cold-Start			Hot-Start			Weighted Total (1)		
	Mean mg/Bhp-hr	**SDEV** mg/Bhp-hr	**(2)** %	**Mean** mg/Bhp-hr	**SDEV** mg/Bhp-hr	**(2)** %	**Mean** mg/Bhp-hr	**SDEV** mg/Bhp-hr	**(2)** %
1,3-Butadiene	2.19	0.53	5.3	2.5	0.13	42	2.46	0.13	37
Benzene	7.29 *	0.38	12	8.15 *	1.17	41	8.03	1	36
Toluene	1.96	0.23	-9.7	2.31 *	0.33	22	2.26	0.28	17
Ethylbenzene	0.54	0.11	-27	0.69	0.2	-47	0.67	0.17	-45
o-Xylene	0.58 *	0.05	-32	0.62	0.11	-19	0.61	0.09	-21
m&p-Xylene	1.24	0.2	-35	1.24 *	0.34	-42	1.24	0.29	-40
Styrene	2.1	1.6	116	1.49	0.21	13	1.58	0.29	24
Naphthalene	1.55	0.54	17	1.77	0.25	1.1	1.74	0.22	2.9

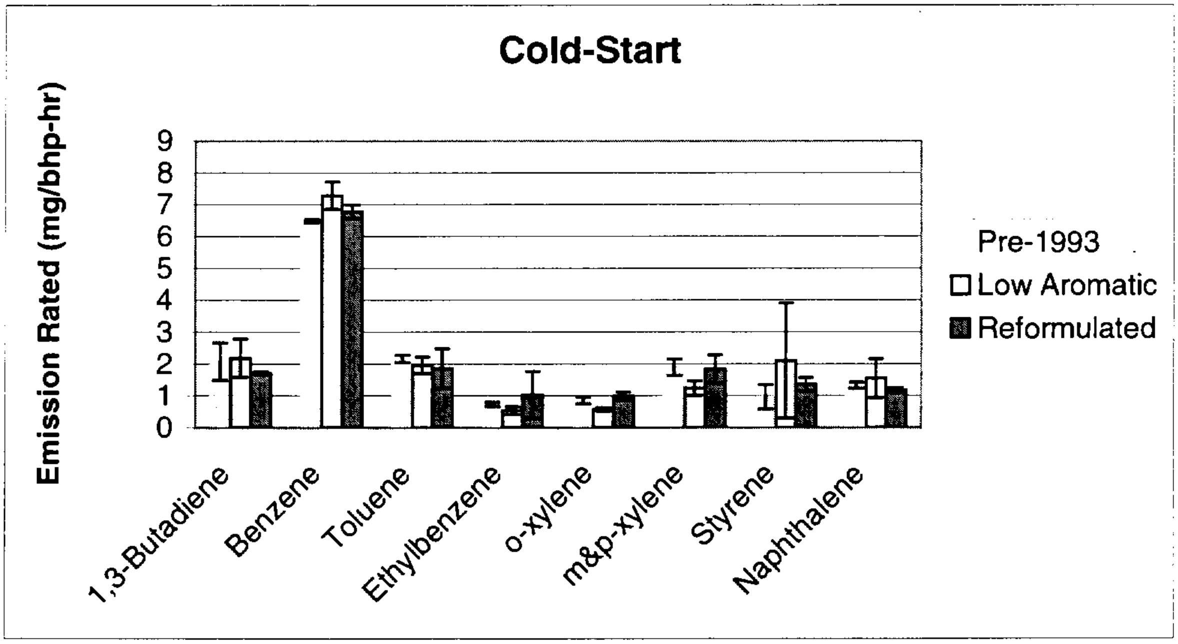
	Reformulated								
	Cold-Start			Hot-Start			Weighted Total (1)		
	Mean gm/Bhp-hr	**SDEV** mg/Bhp-hr	**(2)** %	**Mean** mg/Bhp-hr	**SDEV** mg/Bhp-hr	**(2)** %	**Mean** mg/Bhp-hr	**SDEV** mg/Bhp-hr	**(2)** %
1,3-Butadiene	1.72	0.04	-17	1.87	0.15	6.8	1.84	0.13	2.9
Benzene	6.78	0.19	4.5	5.65	0.8	-2.6	5.81	0.68	-1.5
Toluene	1.86	0.55	-14	1.86	0.25	-1.6	1.86	0.23	-3.6
Ethylbenzene	1.03	0.65	39	1.2	0.72	-7.7	1.18	0.62	-3.6
o-Xylene	0.99	0.11	16	0.86	0.24	12	0.88	0.21	12
m&p-Xylene	1.84	0.39	-3.1	2.19	0.36	3.3	2.14	0.31	2.5
Styrene	1.36	0.19	40	1.46	0.31	11	1.45	0.27	14
Naphthalene	1.18	0.07	-11	1.29	0.25	-26	1.27	0.21	-24

(1) Wt. Tot .= (1/7 mg(cold) + 6/7 mg(hot))/(1/7 Bhp-hr(cold)+ 6/7 Bhp-hr(hot)); SDEV = v(1/6 SDEV(cold))2 + (6/7SDEV(hot))2

(2) Percent change in mean from the Pre-1993 fuel.

* Significant at 95% confidence limit using Fisher's Protected Least Significant Difference Test

Figure 12. Means and 95% Confidence Intervals for Hydrocarbons



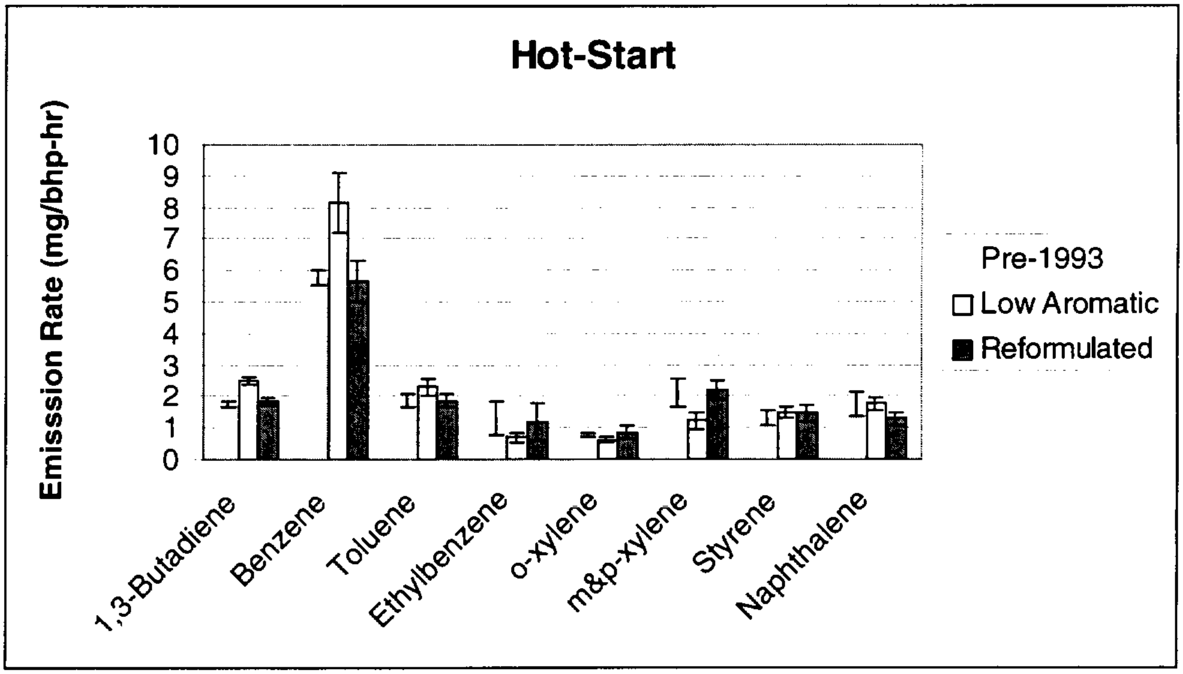


Table 28. Analysis of variance (ANOVA) and pairwise Fisher's protected least square difference results for hydrocarbons.

	1,3-but.	ben.	tol.	e-ben.	o-xyl.	m&p-xyl.	Sty.	Naph.
	Cold-Start							
P-value	.4293	.0195	.5877	.3527	.0034	.0553	.3898	.4123
Low Ar. vs. Pre-93		S			S			
Low Ar. vs. Reform.		S			S			
Pre-93 vs. Reform.								
	Hot-Start							
P-value	<.0001	<.0001	.0247	.1769	.0627	.0024	.5426	.0526
Low Ar. vs. Pre-93	S	S	S			S		
Low Ar. vs. Reform.	S	S	S			S		
Pre-93 vs. Reform.								

3.6 PAH and Nitro-PAH (SAPRC)

Gas-Particle Distribution of PAH under High Volume Sampling Conditions: In order to collect the total PAH emissions from the diesel exhaust, high volume and low volume emission samples were collected for analysis at SAPRC (see Section 2.9 for experimental details) and UCD (see Section 2.10 for experimental details). Experience with ambient sampling using high volume collection suggested that for the 4-ring and smaller PAH, some fraction of these PAH will be gas-phase and not particle-associated (Arey et al., 1987; 1989; Coutant et al., 1988; Wilson et al., 1995). The high volume sampling system used to collect the emission samples analyzed at SAPRC and reported below was designed to be complementary to the low and high volume sampling system whose samples were analyzed and reported on by UCD. The high volume sampling system, configured for SAPRC, consisted of two T60A20 Teflon-coated glass fiber filters backed up by two polyurethane foam plugs (PUFs), with the smaller back PUF allowing compound breakthrough from the first PUF to be determined. In the UCD low and high volume systems (see Section 3.7 for results) the addition of XAD adsorbent after a PUF was designed for quantitative collection of even the most volatile PAH naphthalene (Westerholm et al., 1991).

The distributions of the PAH between the filters and PUFs are shown in Table 29 and Figure 13. The 4-ring PAH, benz[a]anthracene and chyrsene (m.w. 228 Daltons) were quantitatively collected on the filters with no significant amount (<5% of total) found in the front PUF extracts. The 4-ring PAH, fluoranthene and pyrene (m.w. 202 Daltons) were distributed between the filter and the front PUF, with no detectable fluoranthene or pyrene on the back PUF. Interestingly, a consistently higher fraction of the PAH was found on the particles from the low aromatic fuel samples (see Figure 13).

Because of the lack of available standards, the areas of the five methylphenanthrene/ methylanthracene peaks (m.w. 192 Daltons) were summed and the response factor for 1-methylphenanthrene relative to deuterated phenanthrene was used to quantify all isomers. Prior reports have suggested that methylphenanthrenes may be used as a marker for diesel exhaust (Benner et al., 1989), and methylphenanthrene isomers have previously been quantified in heavy-duty diesel emissions (Westerholm and Li, 1994). Summing the areas of the five peaks may

Table 29. Distribution of PAH between Filter and PUFs during High Volume Sampling.

Pre- 1993 Fuel

PAH	Average % on Filter	SDEV	Average % on Front PUF	SDEV	Average % on Back PUF	SDEV
2,3,5-Trimethylnaphthalene	0.0	0.0	88.2	1.5	11.8	1.5
Phenanthrene	8.6	1.3	91.2	1.3	0.2	0.2
Anthracene	13.7	1.9	86.3	1.9	0.0	0.0
Methylphenanthrenes	18.9	3.3	81.0	3.3	0.1	0.0
Fluoranthene	43.3	7.2	56.7	7.2	0.0	0.0
Pyrene	52.7	7.6	47.3	7.6	0.0	0.0

Low Aromatic Fuel

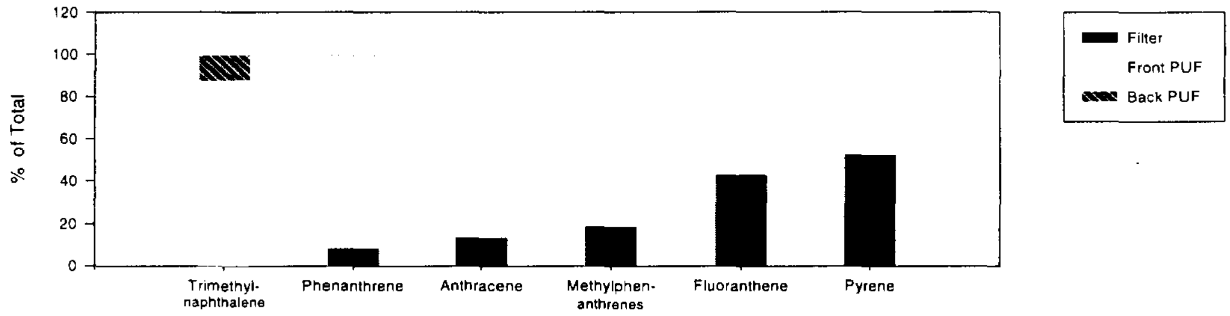
PAH	Average % on Filter	SDEV	Average % on Front PUF	SDEV	Average % on Back PUF	SDEV
2,3,5-Trimethylnaphthalene	0.0	0.0	84.2	2.4	15.8	2.4
Phenanthrene	16.0	1.5	83.7	1.3	0.3	0.2
Anthracene	28.1	2.8	71.9	2.8	0.0	0.0
Methylphenanthrenes	27.6	3.3	71.6	3.9	0.8	1.1
Fluoranthene	71.7	7.9	28.3	7.9	0.0	0.0
Pyrene	78.4	6.4	21.6	6.4	0.0	0.0

Reformulated Diesel

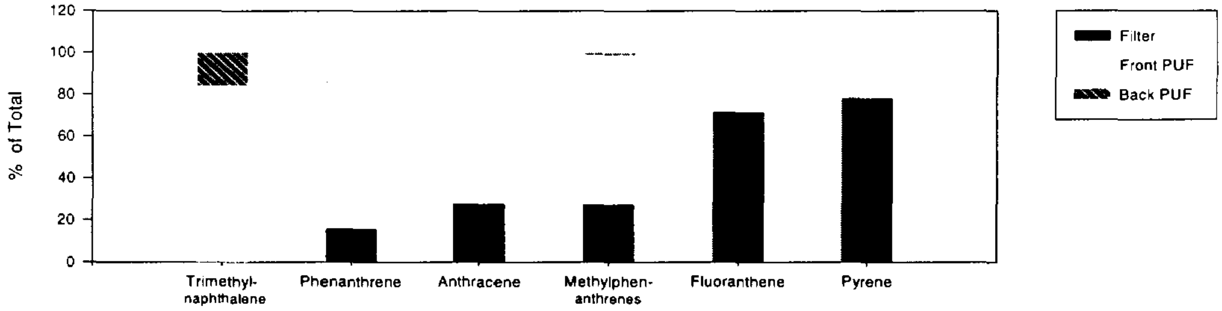
PAH	Average % on Filter	SDEV	Average % on Front PUF	SDEV	Average % on Back PUF	SDEV
2,3,5-Trimethylnaphthalene	0.0	0.0	81.4	3.3	18.6	3.3
Phenanthrene	7.6	0.6	91.8	0.6	0.6	0.6
Anthracene	13.0	1.4	87.0	1.4	0.0	0.0
Methylphenanthrenes	12.4	1.8	87.4	1.8	0.2	0.1
Fluoranthene	41.7	3.3	58.3	3.3	0.0	0.0
Pyrene	51.3	3.6	48.7	3.6	0.0	0.0

Figure 13. Distribution of PAH between Filter and PUFs during High Volume Sampling

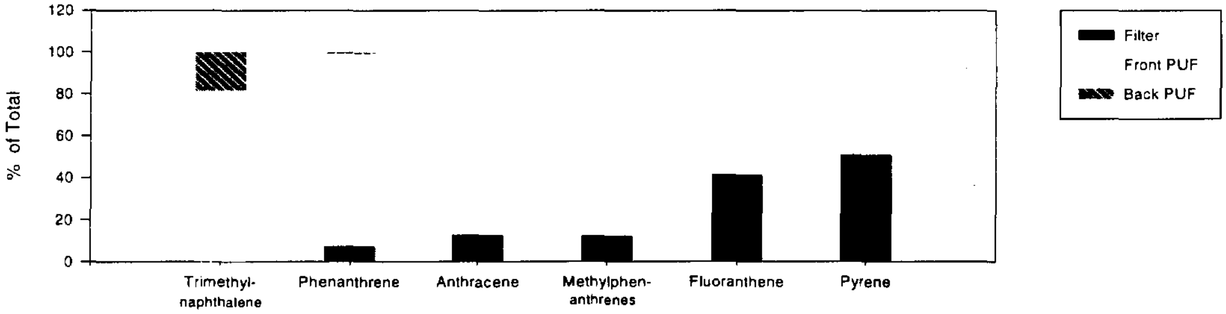
Pre-1993 Fuel



Low Aromatic Fuel



Reformulated Blend Fuel



cause some systematic error in the quantification, but since the pattern of isomers present was consistent, the relative amounts from each of the fuel types can be reliably determined. Methylphenanthrene/methylanthracenes were found mainly on the front PUFs, but with a significant fraction (up to 30%) on the filters and minimal breakthrough (<0.3%, except for a single sample of 2%) on the back PUF (see Figure 13).

The 3-ring PAH phenanthrene and anthracene (m.w. 178 Daltons) were again mainly collected on the front PUFs with up to 17% for phenanthrene and 31% for anthracene present on the low aromatic fuel filters and minimal (≤0.5%, except for a single sample of 1.3%) present on the back PUF.

Breakthrough onto the back PUF became significant for the alkylnaphthalenes. Figure 13 shows the distribution of 2,3,5-trimethylnaphthalene (m.w. 170 Daltons) between the front and back PUF. Therefore, the quantification of C_3-naphthalenes using the SAPRC configured high volume sampling system strictly gives only lower limits to the true emissions.

In summary, the fraction of the PAH present on the PUFs decreased with the volatility of the PAH in the order: C_3-naphthalene > phenanthrene > anthracene > C_1-phenanthrene/anthracene > fluoranthene > pyrene. The PAH more volatile than the C_3-naphthalenes were not quantitatively collected using the filter/PUF sampling system. Only analysis of the filter samples was required to quantify the PAH of molecular weight 228 Daltons and above.

PAH in the Emissions are Fuel-derived and Formed *in-situ*: Table A8 in Attachment A lists the results for micrograms of individual PAH emissions per brake horse power hour (sum of high volume filter plus PUF) for the three replicate hot-start samples from the three fuel types: pre-1993 fuel, low aromatic fuel, and blend of reformulated fuels. Table 30 lists the average PAH emissions with their standard deviations (n=3) for the three fuels. Figure 14 through Figure 19 show the means and 95% confidence intervals for the PAH in the emissions from the three fuel types. Table 30a through Table 30f summarize statistical results for each PAH from an analysis of variance (ANOVA) and analysis of pairwise differences between fuels, using the Fisher's protected least square difference method. Values of $p \leq 0.05$ indicate significant differences among the fuel types at the 95% confidence level. Statistically significant differences at the 95% confidence level for pairwise comparisons are indicated by an S in the tables.

Table 30. Average Emission Rates for Polycyclic Aromatic Hydrocarbons from Three Hot Start Sample Collections per Fuel Type (see Table 5 for specific test cycles, Table A8 for individual emissions values, and Tables 30a through 30f for ANOVA results).

	Pre-1993 Diesel Fuel		Low Aromatic Diesel Fuel		Reformulated Diesel Blend		
PAH	**Average** µg/bhp-hr	**SDEV** µg/bhp-hr	**Average** µg/bhp-hr	**SDEV** µg/bhp-hr	**Average** µg/bhp-hr	**SDEV** µg/bhp-hr	**P-value**
2,3,5-trimethylnaphthalene[a,b]	283.68	5.27	14.77	2.42	56.21	2.82	<0.0001
phenanthrene[c]	336.71	9.08	160.92	15.54	220.73	52.68	0.002
anthracene[d]	38.89	1.43	18.54	2.13	26.16	6.86	0.003
Me-phenanthrenes/anthracenes[c,e]	331.32	16.07	25.17	1.41	111.98	28.74	<0.0001
fluoranthene[d]	128.45	7.60	132.36	18.30	123.07	26.21	0.839
pyrene[d]	193.03	16.51	211.19	37.35	206.82	39.04	0.784
benzo[c]phenanthrene	3.03	0.24	1.74	0.14	1.54	0.26	0.000
benzo[ghi]fluoranthene[f]	24.84	2.68	18.93	2.14	16.94	2.31	0.016
cyclopenta[cd]pyrene	21.44	4.11	26.15	3.12	21.25	3.46	0.245
benz[a]anthracene	16.42	1.67	10.57	1.15	10.96	2.42	0.013
chrysene + triphenylene[g]	17.36	1.66	10.38	0.54	12.20	2.72	0.009
benzo[b+j+k]fluoranthene[g]	31.05	4.17	23.17	1.98	29.18	7.93	0.242
benzo[e]pyrene	16.71	2.72	14.55	1.34	18.99	5.58	0.392
benzo[a]pyrene	20.46	3.27	16.48	1.56	20.59	5.75	0.403
perylene	4.32	0.88	3.71	0.74	4.18	1.16	0.718
indeno[1,2,3-cd]fluoranthene	0.34	0.07	0.21	0.02	0.17	0.00	0.005
benzo[c]chrysene	0.29	0.05	0.18	0.05	0.14	0.04	0.025
dibenz[a,j]anthracene	0.93	0.05	0.55	0.10	0.67	0.09	0.004
indeno[1,2,3-cd]pyrene	19.45	2.71	14.04	1.99	22.16	9.11	0.272
dibenz[a,h + a,c]anthracene[g]	1.54	0.15	0.87	0.12	1.48	0.67	0.154
benzo[b]chrysene	0.40	0.01	0.15	0.05	0.27	0.05	0.001
benzo[ghi]perylene	49.17	9.63	39.81	7.22	60.74	26.60	0.375
coronene	9.49	3.13	4.93	0.47	7.48	1.59	0.088
dibenzo[a,l]pyrene	2.84	0.45	1.25	0.15	2.31	0.48	0.007
dibenzo[a,e]pyrene	1.10	0.29	0.61	0.06	1.13	0.15	0.027
dibenzo[a,i]pyrene	0.91	0.21	0.27	0.09	0.71	0.15	0.007
dibenzo[a,h]pyrene	1.33	0.25	0.75	0.07	0.84	0.20	0.022

[a] Lower limit based on summing amounts on front PUF and back PUF (amount on filter negligible).

[b] The area of the molecular ion peak (m/z 170) and the response factor for 2,3,5-trimethylnaphthalene relative to deuterated phenanthrene were used to quantify 2,3,5-trimethylnaphthalene and a co-eluting isomer.

[c] Sum of amounts on filter, front PUF and back PUF.

[d] Sum of amounts on filter and front PUF; negligible amount found on back PUF.

[e] The areas of the molecular ion of the five isomers present were summed and the response factor for 1-methylphenanthrene relative to deuterated phenanthrene was used to quantify all isomers.

[f] Standard not available, response factor for cyclopenta[cd]pyrene relative to deuterated chrysene used for quantification.

[g] Co-eluting isomers.

Table 30a. Analysis of variance (ANOVA) and pairwise Fisher's protected least square difference results for 2-4-ring PAH and Alkyl-PAH

	2,3,5-Tri-methyl-naphthalene	Phenan-threne	Anthra-cene	Methyl-phenan-threnes	Fluoran-thene	Pyrene
Cycle	Hot	Hot	Hot	Hot	Hot	Hot
P-value	<.0001	.0015	0.003	<.0001	0.8385	0.7842
Low Ar. vs. Pre-93	S	S	S	S		
Low Ar. vs. Reform.	S			S		
Pre-93 vs. Reform.	S	S	S	S		

Table 30b. Analysis of variance (ANOVA) and pairwise Fisher's protected least square difference results for 226-228 Dalton 4-ring PAH

	Benzo[c]-phenan-threne	Benzo[ghi]-fluoran-thene	Cyclo-penta[cd]-pyrene	Benz[a]-anthracene	Chrysene and Triphenylene
Cycle	Hot	Hot	Hot	Hot	Hot
P-value	0.0003	.0159	0.2453	0.0133	0.0093
Low Ar. vs. Pre-93	S	S		S	S
Low Ar. vs. Reform.					
Pre-93 vs. Reform.	S	S		S	S

Table 30c. Analysis of variance (ANOVA) and pairwise Fisher's protected least square difference results for 5-ring PAH of 252 Daltons

	Benzo[b+j+k]-fluoranthenes	Benzo[e]-pyrene	Benzo[a]-pyrene	Perylene
Cycle	Hot	Hot	Hot	Hot
P-value	0.2418	.3919	0.403	0.7179
Low Ar. vs. Pre-93				
Low Ar. vs. Reform.				
Pre-93 vs. Reform.				

Table 30d. Analysis of variance (ANOVA) and pairwise Fisher's protected least square difference results for most abundant PAH of 276 and 300 Daltons

	Indeno[1,2,3-cd]-pyrene	Benzo[ghi]-perylene	Coronene
Cycle	Hot	Hot	Hot
P-value	0.2719	.3748	0.0881
Low Ar. vs. Pre-93			S
Low Ar. vs. Reform.			
Pre-93 vs. Reform.			

Table 30e. Analysis of variance (ANOVA) and pairwise Fisher's protected least square difference results for PAH of 276 and 278 Daltons

	Indeno-[1,2,3-cd]-fluoranthene	Benzo[c]-chrysene	Dibenz[a,j]-anthracene	Dibenz-[a,h + a,c]-anthracene	Benzo[b]-chrysene
Cycle	Hot	Hot	Hot	Hot	Hot
P-value	0.0054	.0250	0.0038	0.154	0.0011
Low Ar. vs. Pre-93	S	S	S		S
Low Ar. vs. Reform.					S
Pre-93 vs. Reform.	S	S	S		S

Table 30f. Analysis of variance (ANOVA) and pairwise Fisher's protected least square difference results for isomeric dibenzopyrenes

	Dibenzo[a,l]-pyrene	Dibenzo[a,e]-pyrene	Dibenzo[a,i]-pyrene	Dibenzo[a,h]-pyrene
Cycle	Hot	Hot	Hot	Hot
P-value	0.0068	.0266	0.0068	0.0215
Low Ar. vs. Pre-93	S	S	S	S
Low Ar. vs. Reform.	S	S	S	
Pre-93 vs. Reform.				S

Table 30a summarizes statistical results for the 2-4 ring PAH and alkyl-PAH whose mean emission rates and confidence levels are given in Figure 14. As may be seen from Table 30a and from Figure 14, the alkyl-PAH, 2,3,5-trimethylnaphthalene and the methylphenanthrenes, have statistically different emission rates among fuel types with a confidence level of >99.99%. The pre-1993 fuel had the highest emissions followed by the reformulated blend, and the low aromatic fuel had the lowest emission rates for these alkyl-PAH. Alkyl-PAH reportedly are formed in lower abundance than their parent PAH in high temperature combustion processes (Benner *et al.*, 1989) such as that presumably occurring in a properly running diesel engine. Fuel analysis of the pre-1993 fuel showed the presence of alkylnaphthalenes, phenanthrene and methylphenanthrenes derivatives. The concentrations of the alkyl-PAH present in the exhaust from the pre-1993 fuel are consistent with the majority of their emissions resulting from the alkyl-PAH initially present in the fuel. The ranking of the alkyl-PAH emissions and the emissions of phenanthrene and anthracene from the three fuels follows the ranking of fuel PAH content (pre-1993 fuel, 6.9 mass% PAH; reformulated fuel, 4.1 mass% PAH; and low aromatic fuel, 0.7 mass% PAH). However, while the phenanthrene and anthracene emissions in the pre-1993 fuel are statistically significantly higher than both the reformulated blend and the low aromatic fuel, the reformulated and low aromatic fuels are not significantly different in these emissions. This is consistent with phenanthrene and anthracene being present at highest levels in the pre-1993 fuel, but also being formed *in situ* in the combustion process. The similarity in emission rates of fluoranthene and pyrene for the three fuel types (no significant difference for any fuel pair, Table 30a) suggests that these PAH are mainly being formed *in situ* during the combustion process.

The PAH showing no statistically significant difference in emission rate with fuel type are: fluoranthene, pyrene, cyclopenta[cd]pyrene, benzo[b+j+k]fluoranthenes, benzo[e]pyrene, benzo[a]pyrene, perylene, indeno[1,2,3-cd]pyrene, benzo[ghi]perylene, and dibenzo[a,h+a,c]-anthracene. PAH showing statistically higher emission rates from the pre-1993 fuel than from either the reformulated or low aromatic fuel, but showing no difference between the latter two fuels are: phenanthrene, anthracene, benzo[c]phenanthrene, benzo[ghi]fluoranthene, benz[a]anthracene, chrysene + triphenylene, indeno[1,2,3-cd]fluoranthene, benzo[c]chrysene, dibenz[a,j]anthracene, and dibenzo[a,h]pyrene.

Benzo[b]chrysene was the only PAH other than the alkyl-PAH which showed significant differences between all three fuel pairs and, as with the alkyl-PAH, the ranking of the emission rates was: pre-1993 fuel highest, reformulated blend intermediate and low aromatic fuel lowest. For dibenzo[a,l]pyrene, dibenzo[a,e]pyrene, and dibenzo[a,i]pyrene, the low aromatic fuel emission rates were significantly lower than both the pre-1993 and reformulated blend fuels, which were not different from one another. For coronene, the pre-1993 fuel had significantly higher emissions than the low aromatic fuel.

In summary, the most dramatic differences in emission rates with fuel type occurred for the alkyl-PAH and the emission rate rankings followed the PAH content of the fuels. Ten of the 27 PAH quantified, ranging in molecular weight from 202 to 278 Daltons, showed no statistically significant difference in emission rate with fuel type. For another 10 PAH, ranging in molecular weight from 178 to 302 Daltons, the emission rates from the pre-1993 fuel were highest and there was no statistically significant difference between the reformulated blend and low aromatic fuel emission rates. Where there was a statistically significant difference between the low aromatic fuel emission rates and one or both of the other fuels, the low aromatic fuel emission rates were always lower.

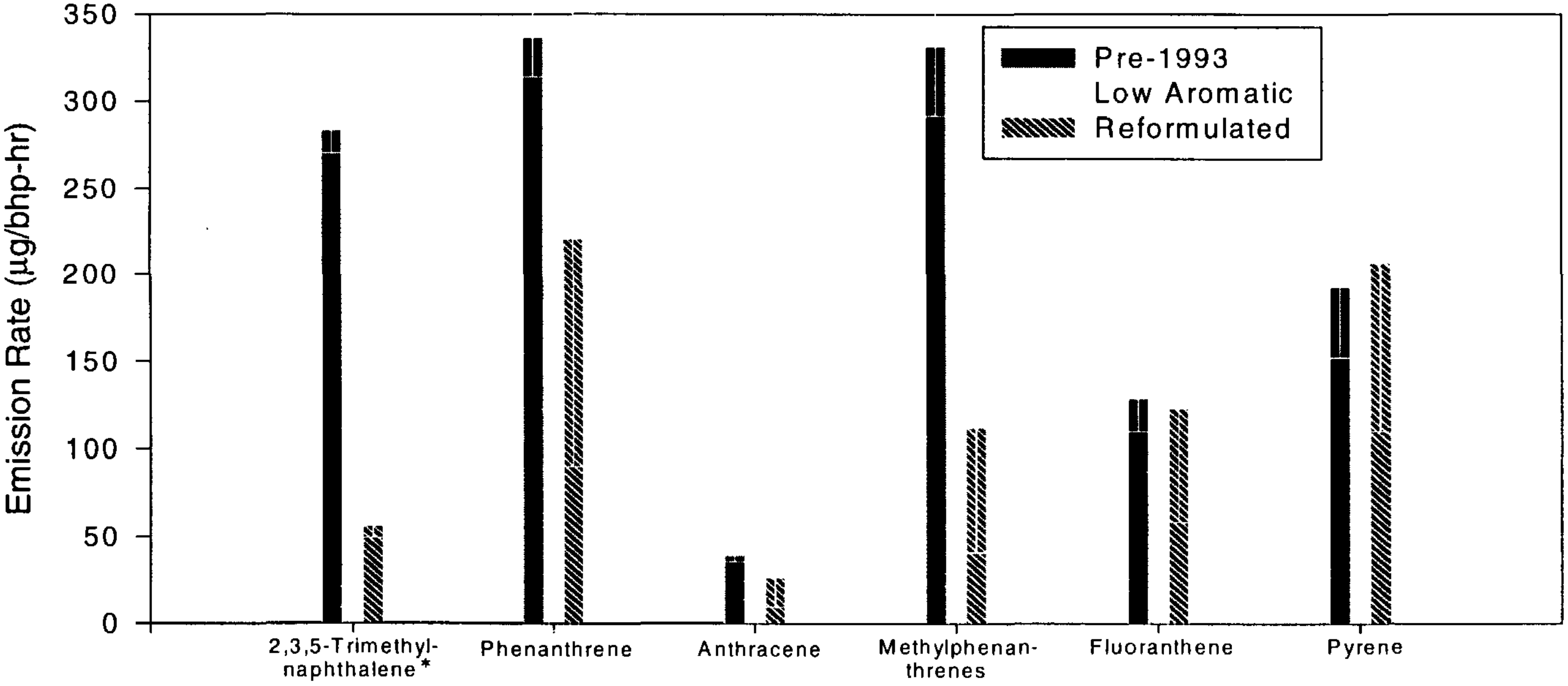
The observed PAH emission profiles show higher emission rates for the 2-, 3- and 4-ring PAH than for the larger PAH which generally have higher toxicities. If one assumes that the PAH emissions from the low aromatic fuel are mainly formed *in situ* in the engine rather than originating in the fuel, phenanthrene, fluoranthene and pyrene emissions are of comparable rates and exceed the emissions of the other PAH measured by at least an order of magnitude. The sums of the total PAH emissions in Table 30 for the three fuel types are: pre-1993 fuel, 1555 µg/Bhp-hr; reformulated blend fuel, 979 µg/Bhp-hr; and low aromatic fuel, 752 µg/Bhp-hr. This may be interpreted to suggest that, for those PAH listed in Table 30, approximately half of the total PAH emissions from the pre-1993 fuel are fuel-derived rather than formed in the engine. The pre-1993 fuel is abundant in alkylnaphthalenes as well as methylphenanthrenes and for these C_1 through C_4-naphthalenes, differences in emissions with fuel type may be even more dramatic than for the PAH listed in Table 30 (see the discussion of vapor-phase PAH in section 3.7).

While the toxicities of the more volatile PAH are generally less than, for example, benzo[a]pyrene, the ultimate health consequences of their emissions must also consider their fate in the atmosphere. These volatile 2-4 ring PAH and 2-3 ring alkyl-PAH will be present predominantly in the gas phase under typical ambient conditions (Arey et al., 1987; 1989; Coutant et al., 1988; Wilson et al., 1995). The atmospheric lifetimes for the majority of these gas-phase PAH will be <1 day (Atkinson and Arey, 1994; Arey, 1998) and nitro-PAH and nitro-PAH lactones have been found to be products of the gas-phase atmospheric reactions of PAH (Atkinson and Arey, 1994; Arey, 1998). PAH found to produce nitro-PAH products in the atmosphere include naphthalene, 1- and 2-methylnaphthalene, fluoranthene and pyrene (Atkinson and Arey, 1994; Arey, 1998), while phenanthrene and pyrene have been shown to yield nitro-lactones (Helmig et al., 1992a,b; Sasaki et al., 1995).

The significance of atmospheric nitro-PAH formation may be judged by comparing ambient 1-nitropyrene (as discussed below, the most abundant nitro-PAH observed in the emissions from all three fuels) concentrations with that of 2-nitrofluoranthene, an isomeric nitro-PAH whose presence in the atmosphere is attributed to radical-initiated atmospheric reactions of gas-phase fluoranthene (Atkinson and Arey, 1994; Arey, 1998). World-wide ambient 2-nitrofluoranthene concentrations have been observed to exceed 1-nitropyrene concentrations (Atkinson et al., 1988; Zielinska et al., 1989; Ciccioli et al., 1995; 1996), indicating that atmospheric nitro-PAH formation generally dominates over direct emission of nitro-PAH from sources such as diesel exhaust.

Nitro-PAH and nitro-PAH lactones are highly mutagenic in bacterial assays and their health effects remain largely unknown (see Arey, 1998 and references therein). Thus, the observed dramatic decreases in emissions of alkylnaphthalenes, methylphenanthrenes and, to a lesser extent, phenanthrene with decreased PAH content of the fuel may have a significant beneficial effect by decreasing the potential for atmospheric formation of nitro-PAH and nitro-PAH lactones through the removal of the precursor volatile PAH and alkyl-PAH.

Figure 14. Means and 95% Confidence Intervals for 2-4 Ring PAH and Alkyl-PAH



* Because significant trimethylnaphthalene was found on the back PUF, these values must be considered as lower limits to the true emissions.

Figure 15. Means and 95% Confidence Intervals for 4-Ring PAH of Molecular Weight Range 226-228 Daltons

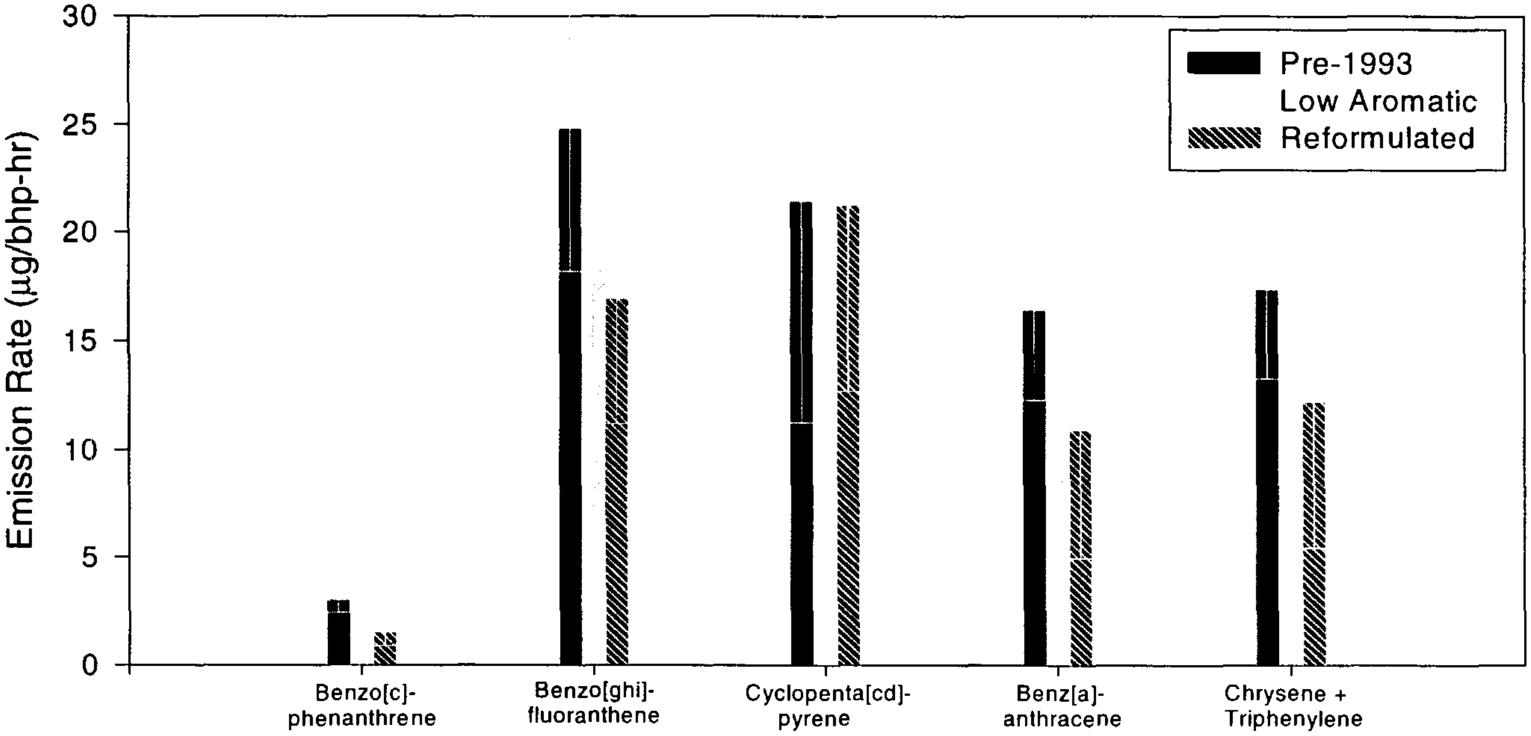


Figure 16. Means and 95% Confidence Intervals for 5-Ring PAH of Molecular Weight 252 Daltons.

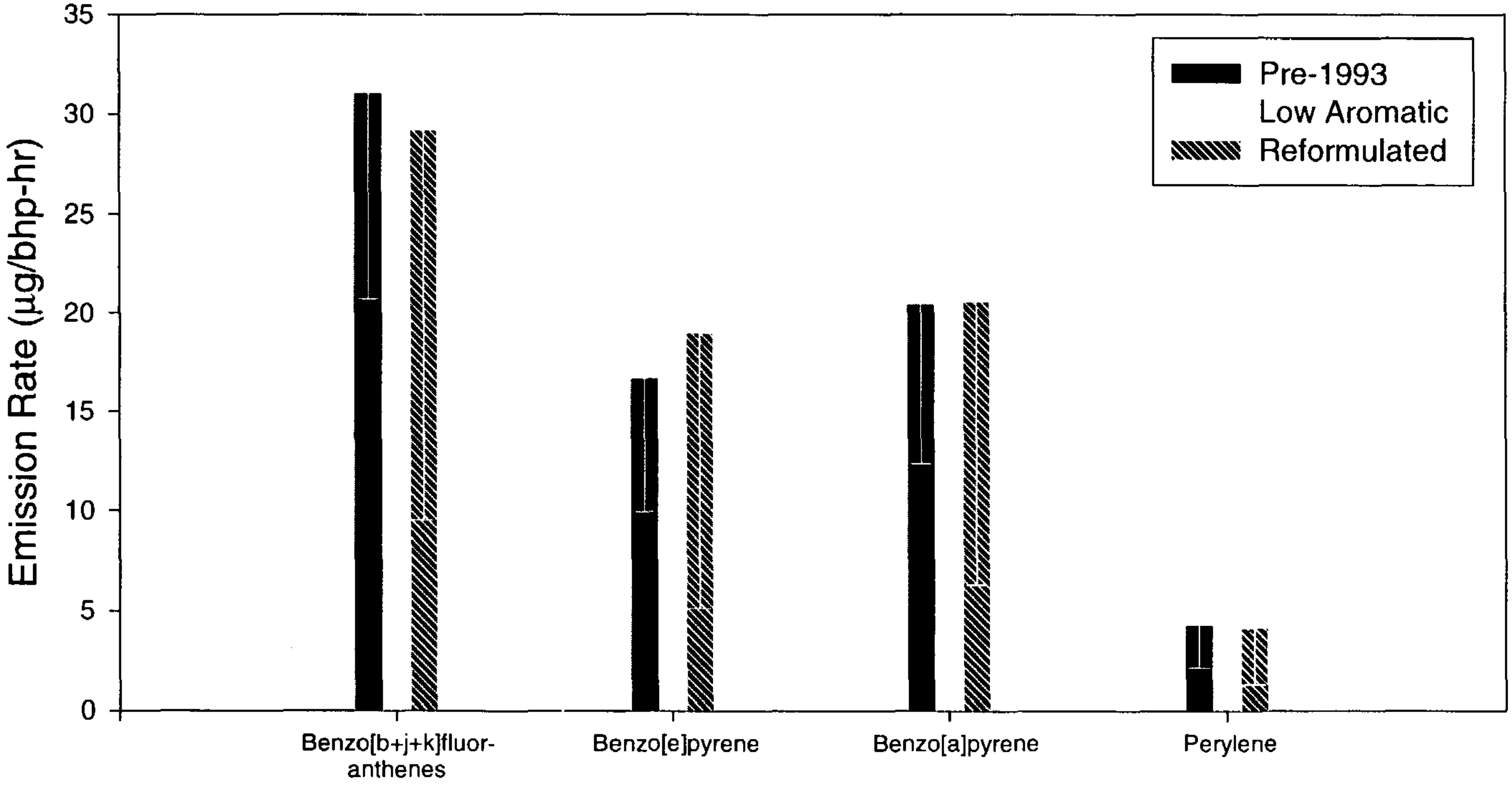


Figure 17. Means and 95% Confidence Intervals for Most Abundant PAH of Molecular Weights 276 and 300 Daltons.

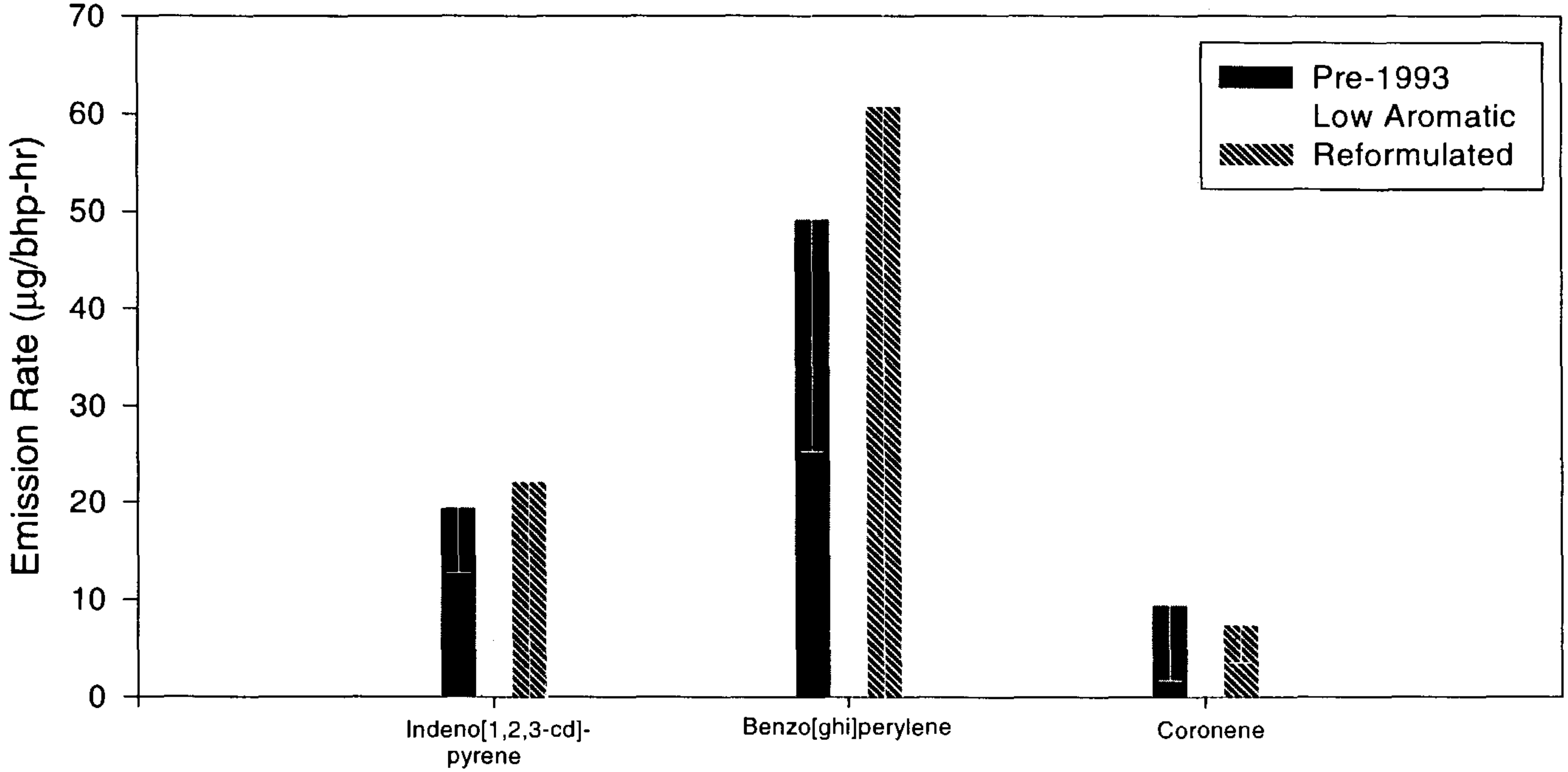


Figure 18. Means and 95% Confidence Intervals for PAH of Molecular Weights 276 and 278 Daltons.

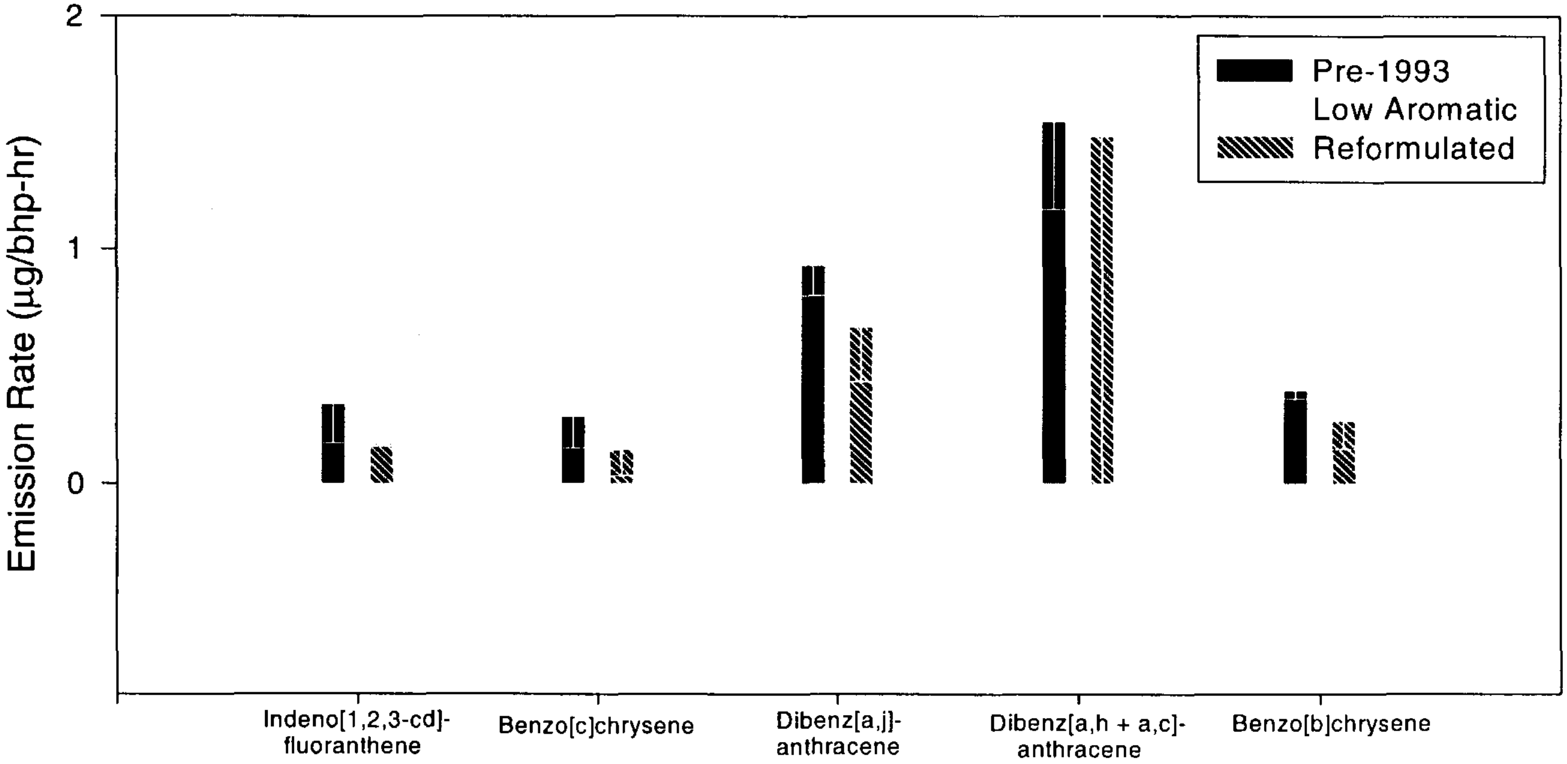
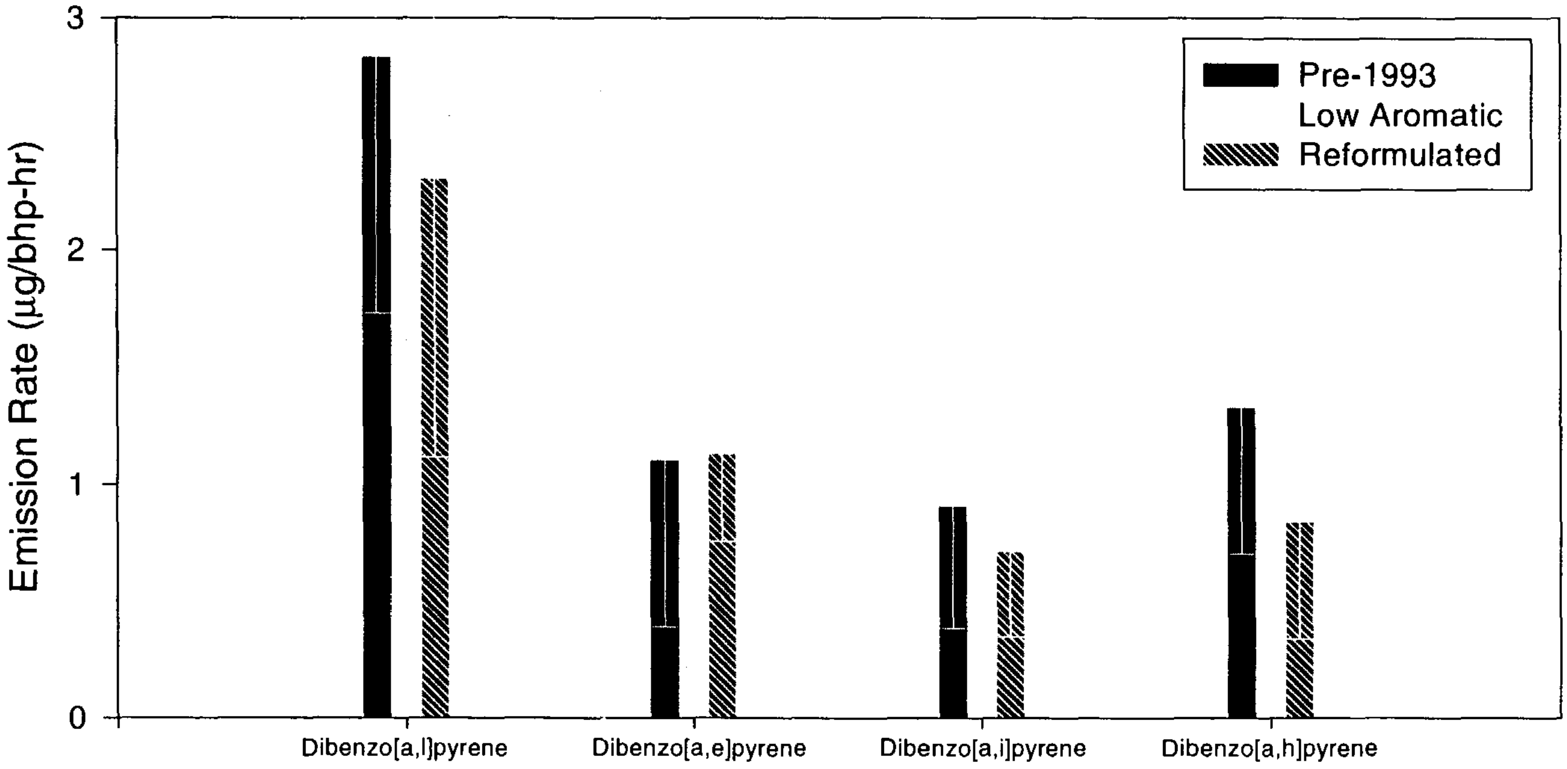


Figure 19. Means and 95% Confidence Intervals for Isomeric Dibenzopyrenes (302 Daltons)



Nitro-PAH: Table A9 in Attachment A lists the results for micrograms of individual nitro-PAH emissions per brake horsepower hour for the three replicate samples from the three fuel types. Because the distribution of the PAH between the filter and PUF plugs closely resembled the distribution previously observed under ambient sampling conditions (Arey *et al.*, 1987), any nitronaphthalenes, methylnitronaphthalenes, nitrobiphenyls and nitroacenaphthene were expected to be on the PUFs. The three-ring nitro-PAH were expected to be particle-associated. 2-Nitrofluorene was looked for in both the filter and PUF extracts.

The average nitro-PAH emissions are given in Table 31. Only five nitro-PAH were detected. 1-Nitronaphthalene and 2-nitronaphthalene were quantified in the PUF plug extracts and analysis of the back PUF showed that no breakthrough during sampling had occurred. The most abundant nitro-PAH was 1-nitropyrene. 6-Nitrobenzo[a]pyrene and 9-nitroanthracene were also quantified in each of the filter extracts. The means and 95% confidence intervals for the nitro-PAH are shown in Figure 20 and Table 31a summarizes the statistical results for each nitro-PAH from an analysis of variance (ANOVA) and analysis of pairwise differences between fuels, using the Fisher's protected least square difference method. The pairwise tests of the fuels showed no statistically significant differences in the emission rates of 1-nitronaphthalene,1-nitropyrene and 6-nitrobenzo[a]pyrene. 9-Nitroanthracene was lower in the reformulated fuel emissions than either the low aromatic or pre-1993 fuels. The low aromatic fuel had lower emission rates of 2-nitronaphthalene than the pre-1993 and reformulated fuels. The fact that there were no significant differences in the emissions of pyrene and benzo[a]pyrene among the fuel types is consistent with the lack of difference among the fuels in the nitro-derivatives of these PAH. The apparent differences in the emissions of the 2- and 3-ring nitro-PAH are not readily explained.

Upper limits are given in Table 31 for the particle-associated nitro-PAH that were not detected. These limits are based on the response from injections of a standard solution made from SRM 1587 and containing sub-ng levels of the nitro-PAH analytes, or in the case of 4-nitropyrene and 3-nitrofluoranthene, estimating what fraction of the observed isomeric 1-nitropyrene would clearly be detected. Figure 21 shows GC-MS ion traces of the molecular ion, m/z 247, for the nitropyrenes (NP) and nitrofluoranthenes (NF) for a solution containing all eight isomers (upper trace) and an injection of the filter extract from the reformulated fuel sample (351H3,H4) showing the 1-nitropyrene present and arrows at the retention times for 3-NF and 4-NP (lower trace). Assuming that a peak with 3% of the area of the 1-nitropyrene peak would be readily detected, an upper limit for 3-NF and 4-NP of 0.06 µg/Bhp-hr is given. Solutions containing the 14 methylnitronaphthalenes and the isomeric 199 Daltons species (nitrobiphenyls and nitroacenaphthene) were used to determine the retention times for these analytes and their characteristic fragmentation patterns. Careful comparisons with the ion chromatograms from the PUF sample extracts showed no evidence for the presence of these volatile nitro-PAH. A conservative upper limit of 0.5 µg/Bhp-hr is reported in Table 31 for these volatile nitro-PAH.

The nitro-PAH emitted from combustion sources such as diesel exhaust are the electrophilic nitration products of the PAH (Schuetzle, 1983; HEI, 1995 and references therein; see Final Report to CARB Contract No. 93-307 for a discussion of the sources of nitro-PAH in ambient air, including atmospheric formation). The specific nitro-PAH observed in this study are indeed the isomers produced by electrophilic nitration of pyrene, benzo[a]pyrene, anthracene and naphthalene, although 1-nitronaphthalene is more abundant than 2-nitronaphthalene in the electrophilic nitration of naphthalene. The most abundant nitro-PAH generally reported in diesel exhaust are 1-nitropyrene and 2-nitrofluorene (Schuetzle and Perez, 1983; Beije and Möller, 1988 and references therein). Emission rates of these and other nitro-PAH vary with engine type, operating conditions and fuels (Schuetzle and Frazier, 1986; IARC, 1989). Nitro-PAH have been shown to contribute 20-55% of the direct-acting mutagenicity of diesel exhaust particle extracts, with 1-nitropyrene and 1,3-; 1,6-; and 1,8-dinitropyrenes (DNP) being the major contributors (Schuetzle, 1983; IARC, 1989 and references therein).

2-Nitrofluorene was not found (<0.3 µg/Bhp-hr) in any of the filter or PUF samples analyzed. Generally, the amount of the parent PAH and its reactivity toward electrophilic nitration (Nielsen, 1984) would be expected to predict the amount of nitro-PAH found in the emission samples. Reports of high 2-nitrofluorene in diesel emissions may reflect fuel/engine combinations in which fluorene is a dominant emission.

The other noteworthy difference between this study and previous work is found in the mutagen profiles discussed below. In this study, the dominant mutagenicity was found in HPLC fractions containing compounds more polar than the nitro- or dinitro-PAH (see Section 3.10).

Table 31. Average Emission Rates for Nitro-Polycyclic Aromatic Hydrocarbons from Three Hot Start Sample Collections per Fuel Type (see Table 5 for specific test cycles, Table A9 for individual emissions values and Table 31a for ANOVA results).

	Pre-1993 Diesel Fuel		Low Aromatic Diesel Fuel		Reformulated Diesel Blend		
Nitro-PAH	**Average** µg/bhp-hr	**SDEV** µg/bhp-hr	**Average** µg/bhp-hr	**SDEV** µg/bhp-hr	**Average** µg/bhp-hr	**SDEV** µg/bhp-hr	**P-value**
1-nitronaphthalene	0.52	0.02	0.32	0.02	0.66	0.35	0.1988
2-nitronaphthalene	1.51	0.02	0.69	0.05	1.37	0.38	0.0078
methylnitronaphthalenes	<0.5		<0.5		<0.5		
2-nitrobiphenyl	<0.5		<0.5		<0.5		
4-nitrobiphenyl	<0.5		<0.5		<0.5		
5-nitroacenaphthene	<0.5		<0.5		<0.5		
2-nitrofluorene	<0.3		<0.3		<0.3		
9-nitroanthracene[a]	0.56	0.06	0.54	0.01	0.34	0.08	0.0083
1-nitropyrene	1.95	0.27	2.07	0.11	1.64	0.43	0.2670
3-nitrofluoranthene	<0.06		<0.06		<0.06		
4-nitropyrene	<0.06		<0.06		<0.06		
7-nitrobenz[a]anthracene	<0.1		<0.1		<0.1		
6-nitrochrysene	<0.1		<0.1		<0.1		
6-nitrobenzo[a]pyrene	0.31	0.08	0.42	0.04	0.39	0.08	0.1988

[a] For the Pre-1993 Fuel and the Reformulated Blend, interferences prevented quantification using the molecular ion at m/z 223 and the $[M-NO]^+$ fragment ion was used.

Table 31a. Analysis of variance (ANOVA) and pairwise Fisher's protected least square difference results for Nitro-PAH

	1-Nitro-naphthalene	2-Nitro-naphthalene	9-Nitro-anthracene	1-Nitro-pyrene	6-Nitro-benzo[a]-pyrene
Cycle	**Hot**	**Hot**	**Hot**	**Hot**	**Hot**
P-value	0.1988	.0078	0.0083	0.267	0.1988
Low Ar. vs. Pre-93		S			
Low Ar. vs. Reform.		S	S		
Pre-93 vs. Reform.			S		

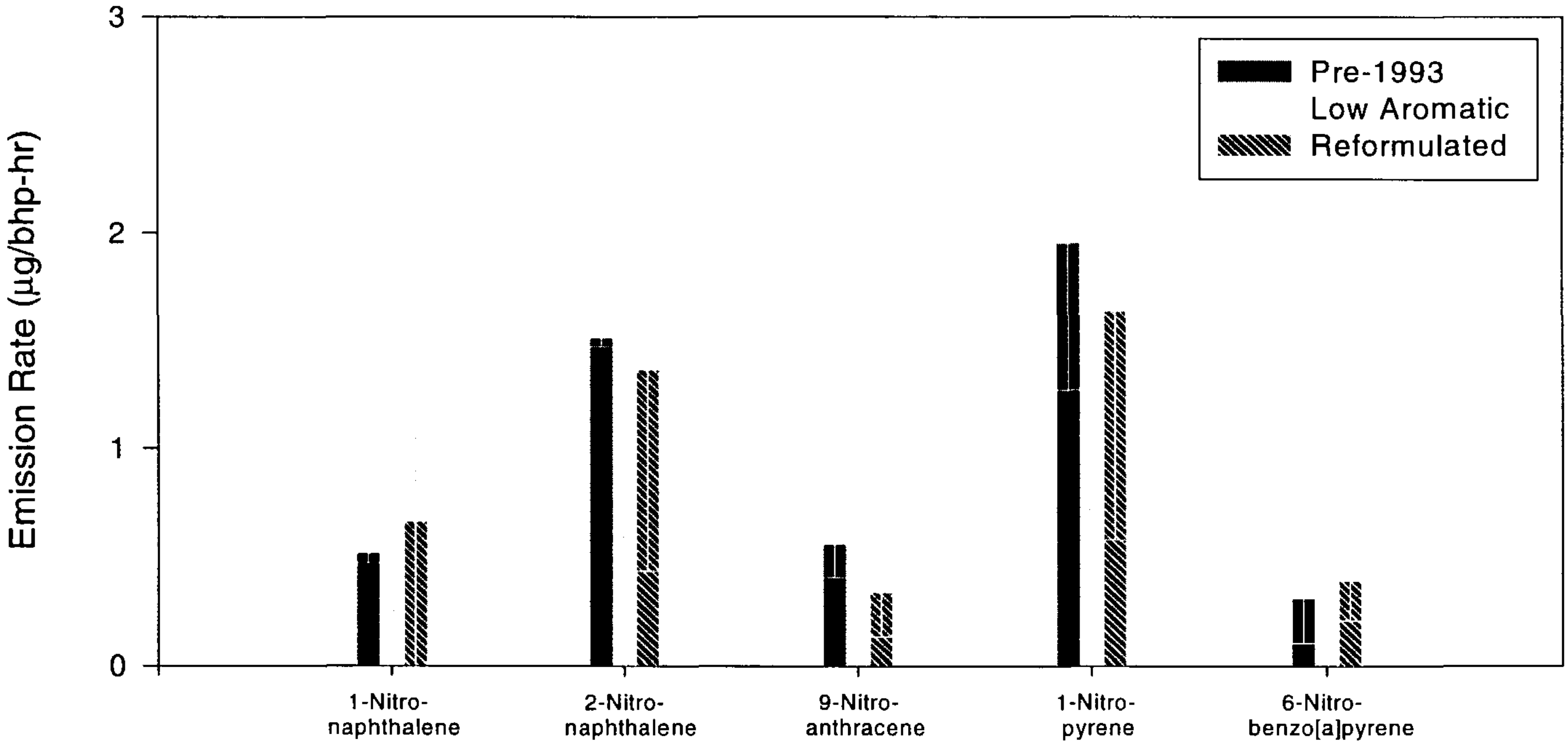


Figure 20. Means and 95% Confidence Intervals for the Nitro-PAH

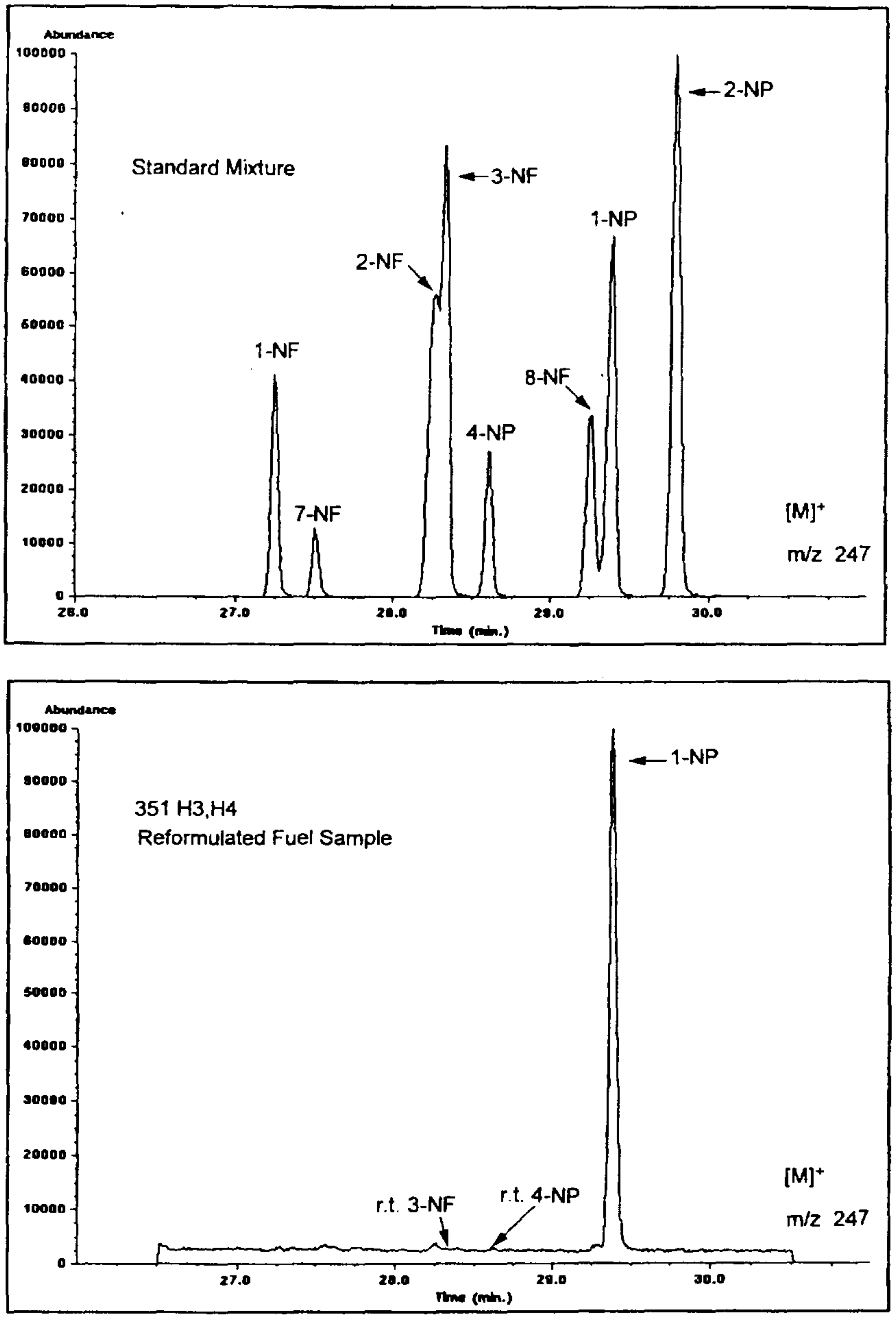


Figure 21 GC-MS SIM Traces of the Molecular Ion for the Nitrofluoranthenes (NF) and Nitropyrenes (NP).

3.7 Vapor Phase PAH (UC Davis)

High volume and low volume emission samples were collected from the exhaust of the diesel engine using three fuels. In addition requisite trip blanks, tunnel blanks, and trip spikes were collected. Included in this section are the vapor-phase PAH results from the low volume and high volume samplers configured with filters, PUFs, and XAD adsorbent (see Section 2.10 for experimental details).. For the purposes of this report, PAHs distributed between the filter, XAD, and PUF are considered vapor phase PAHs. In all samples the filter, PUF and XAD were analyzed separately. To estimate the total emissions of individual vapor phase PAHs the chemical results from the filter, PUF and XAD were combined.

2,6-Dimethylnaphthalene co-elutes with 2,7 dimethylnaphthalene and is reported as the sum of the two dimethylnaphthalenes. Also 2,3,5-trimethylnaphthalene co-elutes with another trimethylnaphthalene and they are reported as the sum of the two trimethylnaphthalenes. A compound that co-elutes with acenaphthene prevented its quantitation. In some of the samples there were interferences with one of the qualifier ions of fluorene and the identification was based on two of the three ions.

<u>PAHs measured in the PUF portion of the low volume sample:</u> Presented in Table 32 are the PAH emission rates of the PAHs collected on the PUF. Naphthalene, 1-methylnaphthalene, 2-methylnaphthalene, biphenyl, dimethylnaphthalene, trimethylnaphthalene, acenaphthylene, fluorene, phenanthrene, anthracene, fluoranthene, and pyrene were detected in all the samples. Fluoranthene and pyrene were detected in all the samples with none reported in the PUF between the PUF trip blank. In general the tunnel blanks PUF contained fluoranthene and pyrene that were greater than 20% of the sample levels. The dilution tunnel blanks are higher than the trip blank, which indicates that the fluoranthene and pyrene are being stripped off the tunnel during sampling of the tunnel blanks. This happens even after the tunnel has been conditioned by running ambient air through the tunnel for 2 hours prior to collecting the tunnel blanks. It is uncertain how to correct for tunnel blanks since they are not conducted under identical conditions as the samples. For example, tunnel blanks are run at cooler temperatures and when the engine is not running.

The PUF extract contained many other alkyl PAHs that were not quantified. To quantify the target compounds the PUF extracts were cleaned up using C-18 and Silica cartridges. The PAH fractions revealed a whole series of alkylated PAHs. Among them a series of C1, C2, C3, - naphthalenes were detected in the analysis of the target compounds.

Table 32. PAH emissions from the PUF in the low volume sampler.

	pre-1993[a]	pre-1993	reformulated[b]	reform	low aromatic[c]
	H110[d]	H170	H145	H150	H127
	ug/Bhp-hr[e]	ug/Bhp-hr	ug/Bhp-hr	ug/Bhp-hr	ug/Bhp-hr
Naphthalene	241	282	168	240	140
2-methyl naphthalene	573	610	287	356	71
1-methyl naphthalene	424	434	181	217	54
biphenyl	345	327	266	268	61
2,6/2,7-dimethyl naphthalene	848	727	277	294	58
acenaphthylene	355	399	358	294	263
acenaphthene	CoE[f]	CoE	CoE	CoE	CoE
2,3,5-trimethyl naphthalene[g]	347	301	106	102	22
fluorene	197	184	129	71	40
phenanthrene	285	217	184	140	89
anthracene	34	29	12[h]	17	9[h]
fluoranthene[h]	18	12	13	7	4
pyrene[h]	22	12	11	9	6

a. pre-1993 fuel
b. reformulated blend
c. low aromatic fuel
d. Sample Identification
e. brake horse power hour
f. Another compound coelutes with acenaphthene and it cannot be quantitated.
g. 2,3,5-trimethylnaphthalene coelutes with another naphthalene, quantitation is based on 2,3,5-trimethylnaphthalene
h. Tunnel blank levels in PUF higher than 20% of the sample value in PUF.

PAHs measured in XAD portion of the low volume sampler: The XAD background corrected PAH emission rates for the XAD resin are presented in Table 33. PAH emission rates for the XAD resin are presented in Table 33. The main PAHs in the XAD samples were naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene, although many other unquantified alkylnaphthalenes were present in the samples. Other quantitated PAHs included biphenyl, 2,4-dimethylnaphthalene, and acenaphthylene.

One low volume trip blank was taken and one tunnel blank was taken prior to the sample collection of each fuel. Analysis of the trip blank and tunnel blanks showed high levels of naphthalene contamination. Naphthalene in the trip blank was at 25%, 28.5% and 53% of naphthalene emission levels for the pre-1993, low aromatic, and reformulated blend respectively. In the low aromatic fuel emission XAD tunnel blank, levels of naphthalene contributed to over half the total naphthalene measured. Naphthalene XAD blank levels for the pre-1993 and reformulated blend emission XAD samples were lower, but still significant at 23% and 42% respectively of the total naphthalene measured. In Table 33. Naphthalene levels are XAD background corrected. Levels of acenaphthene, 2,3,5-trimethylnaphthalene, fluorene, phenanthrene, anthracene, fluoranthene, and pyrene were comparable in the trip blank, tunnel blanks and samples, suggesting measured levels of these PAHs are due to contamination in the XAD. The fact that these are artifacts and not from the diesel exhaust is further supported by the fact that in the sampler the PUF is upstream of the XAD which should prevent phenanthrene, anthracene, pyrene, and fluoranthene from breaking through to the XAD and are not reported in Table 34. PAHs are contaminants inherent in XAD and cannot be completely removed by cleaning.

In order to obtain the total emission rates of PAHs found in the vapor phase, the diesel particulate filter samples needed to be analyzed. 2,3,5-Trimethylnaphthalene, fluorene, phenanthrene, anthracene, fluoranthene, and pyrene can partition between the filter and PUF. Presented in Table 34 are the PAH emission results from the filter analysis.

Table 33. PAH emissions from the XAD in the low volume sampler

	pre-1993[a]	pre-1993	reformulated[b]	reform	low aromatic[c]
	H110[d] ug/Bhp-hr.[e]	H170 ug/Bhp-hr.	H145 ug/Bhp-hr.	H150 ug/Bhp-hr.	H127 ug/Bhp-hr.
Naphthalene[f]	1611	1196	1108	1260	529
2-methyl naphthalene	1043	703	555	542	74
1-methyl naphthalene	631	396	282	263	46
biphenyl	58	17	61	25	9
2,6/2,7-dimethyl naphthalene	118	45	56	33	11
acenaphthylene	0	0	0	0	0
acenaphthene	CoE[f]	CoE	CoE	CoE	CoE
2,3,5-trimethyl naphthalene[h.]	Bkg[i]	Bkg	Bkg	Bkg	Bkg
fluorene	Bkg	Bkg	Bkg	Bkg	Bkg
phenanthrene	Bkg	Bkg	Bkg	Bkg	Bkg
anthracene	Bkg	Bkg	Bkg	Bkg	Bkg
fluoranthene	Bkg	Bkg	Bkg	Bkg	Bkg
pyrene	Bkg	Bkg	Bkg	Bkg	Bkg

a. pre-1993 fuel
b. reformulated blend
c. low aromatic fuel
d. Sample identification
e. brake horse power hour
f. naphthalene levels corrected for naphthalene in the XAD trip spike.
g. Another compound coelutes with Acenaphthene and it cannot be quantitated.
h. 2,3,5-trimethyl naphthalene coelutes with another naphthalene, quantitation is based on 2,3,5-trimethyl naphthalene
i. Bkg = Background. Levels measured are due to artifacts in the XAD

Table 34. PAH emissions from the Filter in the low volume sampler

	pre-1993[a]	pre-1993	reformulated[b]	reformulated	low aromatic[c]
	H110[d]	H170	H145	H150	H127
	ug/Bhp-hr.[e]	ug/Bhp-hr.	ug/Bhp-hr.	ug/Bhp-hr.	ug/Bhp-hr.
2,3,5-trimethyl naphthalene[f]	5	4	1	1	2
Fluorene	2	2	1	1	1
phenanthrene	50	44	29	27	35
anthracene	7	7	4	5	5
fluoranthene	54	60	57	47	63
pyrene	85	87	93	89	98

a. pre-1993 fuel
b. reformulated blend
c. low aromatic fuel
d. Sample Identification
e. brake horse power hour
f. 2,3,5-trimethyl naphthalene coelutes with another naphthalene, quantitation is based on 2,3,5-trimethyl naphthalene

Total PAH Emission Rates of the Combined Filter/XAD/PUF from the Low Volume Samples: PAH emission rates determined from the low volume samples for the heavy-duty diesel engine using the three fuels are given in Table 35. Emission rates were calculated by summing the PAHs measured in the Filter, PUF and XAD. Fluorene, 2,3,5-trimethylnaphthalene, phenanthrene, anthracene, fluoranthene, and pyrene emission rates were XAD background corrected. The Pre-1993 fuel had the highest emissions rates for all the alkyl-PAHs, including 1-and 2-methylnaphthalene, 2,4-dimethylnaphthalene, and trimethylnaphthalene, while the low aromatic fuel had the lowest emission rates for these alkyl PAHs. For the reformulated fuel, the emission rates of these alkyl PAHs were intermediate between the low aromatic and pre-1993 fuel.

Under the low volume sampling conditions employed here, naphthalene, 2-methylnaphthalene, 1-methylnaphthalene, biphenyl, 2,4-dimethylnaphthalene, and acenaphthylene are distributed between the PUF and XAD, the 2,3,5 trimethylnaphthalene, phenanthrene, and anthracene are mainly in the PUF, and the fluoranthene and pyrene are mainly in the particulate matter.

Table 35. Sum of PAH emissions from the Filter, PUF and XAD in the low volume sampler

	pre-1993[a]	pre-1993	reformulated[b]	reformulated	low aromatic[c]
	H110[d]	H170	H145	H150	H127
	ug/Bhp-hr.[e]	ug/Bhp-hr.	ug/Bhp-hr.	ug/Bhp-hr.	ug/Bhp-hr.
naphthalene[h]	1852	1478	1270	1500	529
2-methyl naphthalene	1617	1313	842	899	145
1-methyl naphthalene	1055	830	463	480	101
biphenyl	403	345	326	293	69
2,6/2,7-dimethyl naphthalene	966	771	333	327	68
acenaphthylene	355	399	358	294	263
acenaphthene	CoE[f]	CoE	CoE	CoE	CoE
2,3,5-trimethyl naphthalene[g,h]	351	305	107	103	24
fluorene[h]	199	186	130	72	41
phenanthrene[h]	335	261	213	167	124
anthracene[h]	41	36	17	22	15
fluoranthene[h]	72	72	70	54	67
pyrene[h]	107	100	104	98	104

a. pre-1993 fuel
b. reformulated blend
c. low aromatic fuel
d. Sample Identification
e. brake horse power hour
f. Another compound coelutes with Acenaphthene and it cannot be quantitated.
g. 2,3,5-trimethyl naphthalene coelutes with another naphthalene, quantitation is based on 2,3,5-trimethylnaphthalene
h. Values XAD background corrected.

High Volume PAH: Table 36 shows the total PAHs emitted in the diesel exhaust of the three fuels. The high volume PAH emission profile is similar to the low volume emission profile. The results from the reformulated blend must be considered suspect since the sample leaked during collection. The magnitude of the leak cannot be determined and it is not known if it affected the integrity of the sample. One trip blank and one tunnel blank for each fuel was taken for the high volume samples. All levels measured in the blanks were significantly less than 20% of the sample.

The volatile PAH emissions rates for the PUF are presented in Table 37 and volatile PAHs emission rates for the XAD are presented in Table 38. Table 39 presents emission rates of PAHs found in the high volume filter. In general, the PUF samples contained naphthalene, acenaphthylene, fluorene, phenanthrene, anthracene, fluoranthene and pyrene. Alkyl naphthalenes quantitated were 1- and 2-methylnaphthalene, 2,4-dimethylnaphthalene, and 2,3,5-trimethylnaphthalenes. Other C2 and C3 naphthalenes were detected at similar levels. PAHs with a molecular weight greater than 202 were not detected in the PUF samples.

Table 36. High volume Total PAH emissions.

	pre-1993[a]	reformulated[b]	low aromatic[c]
	H3[d] µg/Bhp-hr.[e]	H6 µg/Bhp-hr.	H4 µg/Bhp-hr.
Naphthalene	2921	2078	1905
2-methylnaphthalene	1637	949	225
1-methylnaphthalene	1118	547	158
biphenyl	481	379	110
2,6/2,7-dimethylnaphthalene	720	264	54
acenaphthylene	250	196	191
acenaphthene[f]	CoE	CoE	CoE
2,3,5-trimethylnaphthalene[g]	572	156	48
fluorene	159	74	43
phenanthrene	248	169	166
anthracene	32	25	18
fluoranthene	155	76	93
pyrene	242	145	189

a. pre-1993 fuel
b. reformulated blend
c. low aromatic fuel
d. Sample Identification
e. brake horse power hour
f. Another compound coelutes with acenaphthene and it cannot be quantitated.
g. 2,3,5-trimethylnaphthalene coelutes with another naphthalene, quantitation is based on 2,3,5-trimethylnaphthalene

Table 37. PUF high volume PAHs Emission rates of a diesel engine using three fuels.

	pre-1993[a]	reformulated[b]	low aromatic[c]
	H3[d]	H6	H4
	µg/Bhp-hr.[e]	µg/Bhp-hr.	µg/Bhp-hr.
naphthalene	12	32	22
2-methylnaphthalene	14	48	5
1-methylnaphthalene	11	30	4
biphenyl	17	49	7
2,6/2,7-dimethylnaphthalene	25	39	4
acenaphthylene	25	130	119
acenaphthene[f]	CoE	CoE	CoE
2,3,5-trimethylnaphthalene[g]	68	82	12
fluorene	55	55	20
phenanthrene	187	123	92
anthracene	23	17	8
fluoranthene	104	22	12
pyrene	134	26	13

a. pre-1993 fuel
b. reformulated blend
c. low aromatic fuel
d. Sample ID
e. brake horse power hour
f. Another compound coelutes with acenaphthene and it cannot be quantitated.
g. 2,3,5-trimethylnaphthalene coelutes with another naphthalene, quantitation is based on 2,3,5-trimethylnaphthalene

Table 38. Emission rates of PAHs found in the high volume XAD.

	pre-1993[a]	reformulated[b]	low aromatic[c]
	H3[d]	H6	H4
	µg/Bhp-hr.[e]	µg/Bhp-hr.	µg/Bhp-hr.
naphthalene	2910	2046	1893
2-methylnaphthalene	1626	901	219
1-methylnaphthalene	1106	517	154
biphenyl	465	330	104
2,6/2,7-dimethylnaphthalene	695	225	50
acenaphthylene	224	66	73
acenaphthene[f]	CoE	CoE	CoE
2,3,5-trimethylnaphthalene[g]	495	73	35
fluorene	104	19	23
phenanthrene	24	23	41
anthracene	4	3	5
fluoranthene	0	2	0
pyrene	6	15	9

a. pre-1993 fuel
b. reformulated blend
c. low aromatic fuel
d. Sample Identification
e. brake horse power hour
f. Another compound coelutes with acenaphthene and it cannot be quantitated.
g. 2,3,5-trimethylnaphthalene coelutes with another naphthalene, quantitation is based on 2,3,5-trimethylnaphthalene

Table 39. Emission rates of PAHs found in the High Volume Filters.

	pre-1993[a]	reformulated[b]	low aromatic[c]
	H3[d]	H6	H4
	µg/Bhp-hr.[e]	µg/Bhp-hr.	µg/Bhp-hr.
2,3,5-trimethylnaphthalene[f]	8	2	2
phenanthrene	36	23	34
anthracene	6	5	5
fluoranthene	50	52	81
pyrene	102	113	162

a. pre-1993 fuel
b. reformulated blend
c. low aromatic fuel
d. Sample Identification
e. brake horse power hour
f. 2,3,5-trimethylnaphthalene coelutes with another naphthalene, quantitation is based on 2,3,5-trimethylnaphthalene

Note: sample not identical sample conditions on Table 41

Compared to the PUF samples, the XADs sample contained higher concentrations of naphthalene, 1- and 2-methylnaphthalene. The XAD had lower levels of acenaphthylene, fluorene, phenanthrene, and anthracene than the PUF. The reformulated fuel emission sample contained fluoranthene, and all three emission samples contained pyrene which may indicate that some of these PAHs broke through the PUF.

<u>Comparison of PAH results from the High Volume and Low Volume Sampler</u>: During a series of pretests the high volume sampler was compared with the low volume sampler. Particulate emission rates from both samplers were compared. The pretest results showed particle emission rates in the high volume samplers were about 15% lower than in the low volume sampler. Results were sufficiently close to allow for sample collection utilizing both samplers.

During the main test, UCD collected vapor phase PAH samples from both the high and low volume sampler. Since most of UCD's previous work was done on the low volume sampler, low volume samples were collected to serve as a baseline for comparison between the low volume and high volume samplers. UCD collected a single high volume sample for each fuel type. The following caveats apply to the comparison between the high volume and low volume samples, since the sampling times and conditions between the two sets of samples were not identical. To collect sufficient sample for the low volume sampler, six test cycles were collected for a single sample. The high volume sampling consisted of two test cycles. High volume samples were collected off the main dilution tunnel, while the low volume samples were collected off the second dilution tunnel, which resulted in a higher dilution volume and a lower sampling temperature for the low volume sampler. Also, the low volume sampler was about 10 feet upstream of the high volume sampler. Due to scheduling limitations, high and low volume samples were not collected in parallel. Two low volume samples were collected for the pre-1993 and reformulated fuel. A single high volume sample was collected per fuel and a single low volume low sample was collected for the low aromatic fuel. For the low volume sampler results, the acenaphthylene, 3,5-trimethylnaphthalene, fluorene, phenanthrene, anthracene, fluoranthene, and pyrene are XAD blank corrected, that is, the amount listed in the table is the amount in the sampler minus the amount found in the XAD blank. No corrections for tunnel blanks were made.

Presented in Table 40 is the comparison of the high volume sample results with the XAD corrected low volume sample results. Levels for 2,3,5-trimethylnaphthalene, fluorene, phenanthrene, anthracene, fluoranthene, and pyrene are slightly lower with the XAD correction.

Table 40. Comparison of the high volume sample results with the XAD corrected low volume sample results.

Fuel:	Pre 1993					Low Aromatic				Reformulated				
Sample ID:	H3	H110	H170			H4	H127			H6	H145	H150		
Sampler Type:	Hi Vol	Low Vol	Low Vol	Average	Sdev	Hi Vol	Low Vol	Average	Sdev	Hi Vol	Low Vol	Low Vol	Average	Sdev
			(µg/Bhp-hr.)				(µg/Bhp-hr.)					(µg/Bhp-hr.)		
naphthalene	2,921	2,340	1,956	2,406	272	1,915	980	1,448	661	2,078	1,784	1,980	1,947	150
2-methyl naphthalene	1,637	1,617	1,313	1,522	215	225	145	185	56	949	842	899	896	53
1-methyl naphthalene	1,118	1,055	830	1,001	159	158	101	129	40	547	463	480	497	44
biphenyl	481	403	345	410	41	110	69	90	29	379	326	293	333	44
2,6/2,7-dimethyl naphthalene	720	966	771	819	138	54	68	61	10	264	333	327	308	38
acenaphthylene	250	355	399	335	32	191	263	227	51	196	358	294	282	82
2,3,5-trimethyl naphthalene	572	351	305	409	33	48	24	36	17	156	107	103	122	30
fluorene	159	199	186	181	9	43	41	42	2	74	130	72	92	33
phenanthrene	248	335	261	281	52	166	124	145	30	169	213	167	183	26
anthracene	32	41	36	36	4	18	15	16	2	25	17	22	21	4
fluoranthene	155	72	72	100	0	93	67	80	18	76	70	54	67	11
pyrene	242	107	100	150	5	189	104	146	60	145	104	97	116	25

Low volume and high volume samples were taken at different times and conditions

ug/Bhp-hr. = micrograms per brake horsepower hour.

For the main test, naphthalene, 1-methylnaphthalene, 2-methylnaphthalene, dimethylnaphthalene, biphenyl, acenaphthylene, fluorene, trimethylnaphthalene, and phenanthrene, anthracene, fluoranthene, and pyrene were detected in all the high and low volume samples. Generally high volume volatile PAH emission rates were similar to the low volume volatile PAH emission rates.

The trends for emission rates of the three fuels in the low and high volume samples were similar. For both the high and low volumes samples, individual alkyl PAH emission rates were highest in the pre-1993 fuel and lowest in the low aromatic fuel. High volume naphthalene emission rates were higher than the low volume naphthalene emission rates in spite of the high blank contribution to the low volume naphthalene.

Blanks: Tunnel blank levels are not directly comparable to the sample levels. The procedure for taking tunnel blanks into account requires that the tunnel is first conditioned for two hours by running ambient air through the tunnel. The tunnel blank is taken only when the CVS is running and at ambient temperature, a temperature lower than during an FTP run. Alkyl PAH exhaust levels from the low aromatic fuel were considerably lower than in the exhausts of the other two fuels and closer to the background levels found in the tunnel blank. Some PAHs appeared to be desorbing off the tunnel. Continued PAH desorption off the tunnel continued after the tunnel blank was taken could account for the tunnel blank PUFs levels being higher than the trip blank PUFs.

Unlike the low volume sampler blank levels, the high volume blank levels were an insignificant part of the measured PAH emissions. Two factors explain the differences. The first is that sufficient sample was collected in the high volume sampler (approximately 9 times more sample than the low volume sampler) to obtain PAH values significantly higher than the blank levels. Second, because the high volume sampler was operated at a higher flow rate and temperature, more of the heavy PAHs broke through the filter and were trapped on the PUF. This resulted in a greater fraction of the heavy PAHs trapped on the PUF and proportionally increased sample levels above the blank levels. Both these factors contributed to the increase in levels of heavier PAHs in the PUF compared to levels in the tunnel blank.

Discussion: Bagley, et al. (1987) chemically characterized compounds in diesel particulate matter and in the vapor-phase as trapped on XAD-2 resin using an ultra-high volume sampler. The lowest boiling point compounds reported included parent and substituted naphthalenes, fluorenones, anthracenes, phenanthrenes, and fluorenes. Westerholm et al. (1991) reported a number of semi-volatile PAHs collected using PUF and XAD-2 including phenanthrene or substituted phenanthrenes, fluoranthene, pyrene, and anthracene. The authors used volumetric sampling flow rates of 240 and 340 LPM for XAD-2 and PUF sorbents, respectively.

Our current study showed greater differences in some volatile PAH emission rates from a heavy duty diesel engine using three different fuels. Comparisons between fuels can be made from data obtained from the high volume samples, low volume samples and a combination of the high volume and low volume samples. A comparison of the total PAH emissions rates based on the high volume sampler is given in Figure 22. The high volume sampler data was chosen because the sampling system is more similar to the SAPRC sampling system used to characterize PUF and particulate associated PAHs

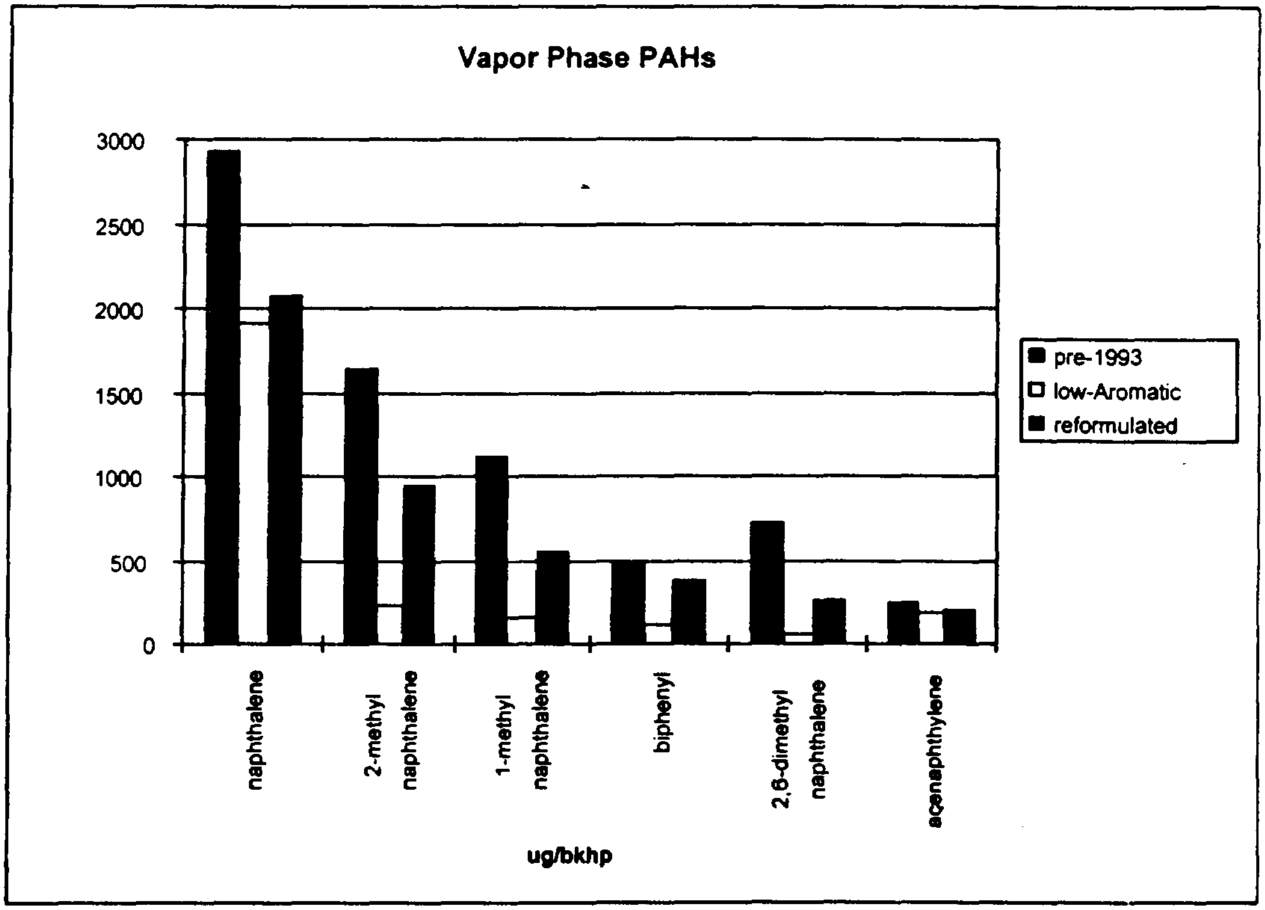


Figure 22 – Vapor phase PAHs emissions from the diesel engine using pre-1993, low-aromatic and reformulated fuel. Data was obtained from the high volume sampler.

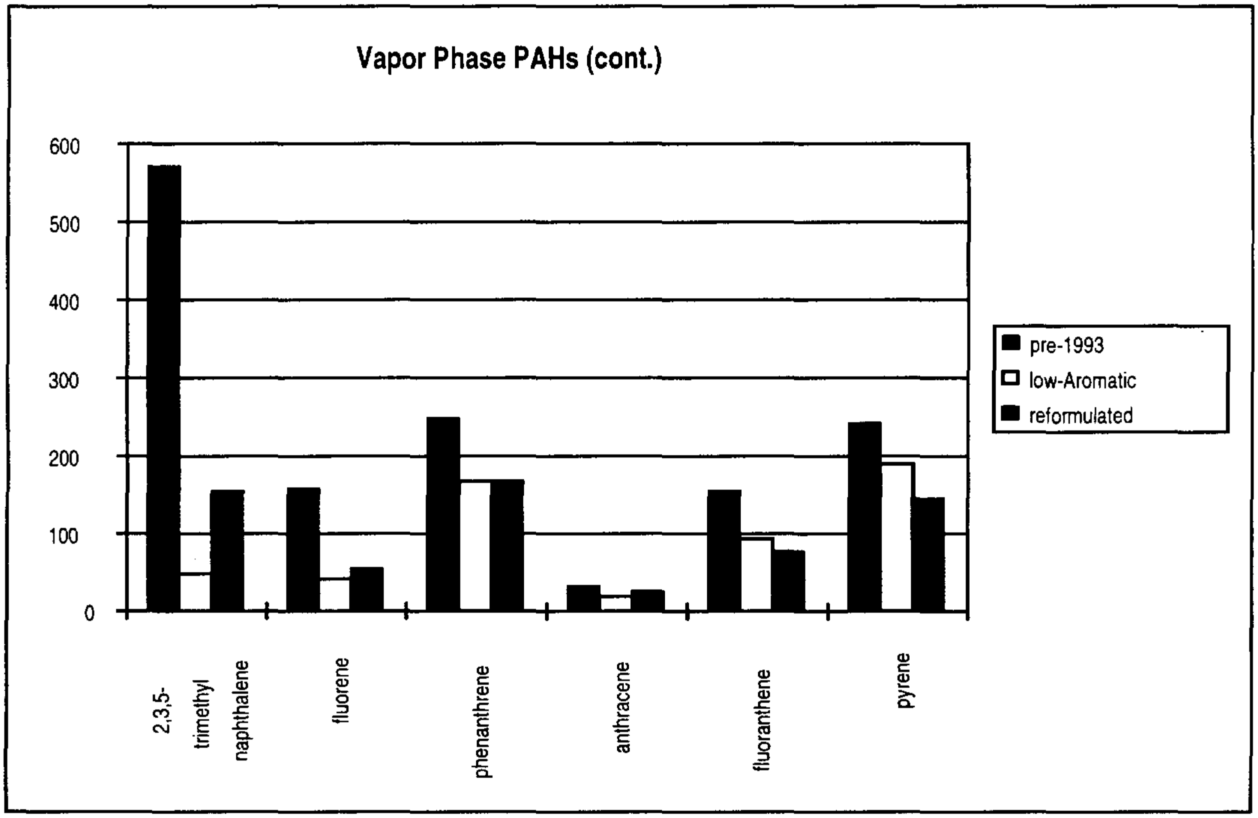


Figure 22 cont. – Vapor phase PAH emissions from the diesel engine using pre-1993, low aromatic and reformulated fuel. Data was obtained from the high volume sampler.

The pre-1993 fuel had the highest emissions rates for the all the alkyl PAHs including 1 and 2 methylnaphthalene, 2,4 dimethyl naphthalene, and trimethylnaphthalene while the low aromatic fuel had the lowest emission rates for these alkyl naphthalenes. For the reformulated fuel the emission rates of these alkyl PAHs were intermediate between the low aromatic and pre-1993 fuel. The profiles of the two and three ring unsubstituted PAH such as naphthalene, acenaphthylene, phenanthrene and anthracene do not show such a marked fuel dependence suggesting greater fraction of these PAHs are formed during the combustion process. For fluoranthene and pyrene, the two-four ring PAHs that were measured, there appears to be little if no fuel dependence. The exact magnitude of PAH fuel dependence cannot be measured since insufficient data is available for statistical analysis.

Conclusions: Our goal was two fold: 1) to investigate the vapor-phase emissions from a heavy duty engine using three different fuel formulations, a pre-1993, low aromatic and a reformulated blend and 2) to design and run a high volume sampler capable of collecting sufficient sample to detect and quantitate low level PAHs, dioxins and nitro-PAHs. Both goals were achieved by comparing volatile PAH emission rates of a heavy-duty diesel engine using three fuels and using data collected from both high and low volume samplers. A high volume sampler designed to collect larger sample volumes was successfully designed and tested at the LACMTA facility.

The sampler was used to collect samples for PAHs, nitro-PAHs, mutagenicity, and PCDDs and PCDFs. A comparison of the emission profiles of the heavy-duty diesel engine using three different fuels shows a similar distribution of volatile alkyl PAHs but at significantly different emission rates. Two- and three-ring PAHs show some fuel dependence, although the majority of these PAHs may be formed during combustion. Four-ring PAHs such as fluoranthene and pyrene do not show any fuel dependence and appear to be formed mainly from combustion.

A high volume sampler was designed and used to collect PAH samples as well as mutagenicity, dioxin, and nitro-PAH samples. The purpose of the high volume sampler was to collect a larger sample mass critical for the detection and quantitation of low level nitro-PAHs, dioxins, and some low level PAHs. The main advantage of the high volume sampler over the low volume sampler was the detection and quantification of low level PAHs that could not be done by low volume sampling. PAH results between the low and high volume samplers are comparable.

3.7 a. Comparison of SAPRC and UCD Results.

Semi-volatile PAH Comparison: The SAPRC high-volume sampling system consisted of two Teflon-coated glass fiber filters backed up by two polyurethane foam plugs. The UCD high-volume system was similar except for the addition of an XAD resin adsorbent downstream of the PUF adsorbent. The XAD adsorbent assured quantitative collection of all gas-phase PAH, including the most volatile, naphthalene. A number of PAHs were quantified independently by both laboratories and a comparison of the results is given in Table 41.

Table 41. A Comparison of Selected 2-4 Ring PAH Emission Rates as Determined by SAPRC (average values for 3 replicates, taken from Table 30) and UCD (high-volume sampler results from Table 36).

	Pre-1993 Diesel Fuel		Low Aromatic Diesel Fuel		Reformulated Diesel Blend	
PAH	SAPRC µg/Bhp-hr	UCD µg/Bhp-hr	SAPRC µg/Bhp-hr	UCD µg/Bhp-hr	SAPRC µg/Bhp-hr	UCD µg/Bhp-hr
2,3,5-trimethylnaphthalene	284[a]	572	15[a]	48	56[a]	156[b]
Phenanthrene	337	248	161	166	221	169[b]
Anthracene	39	32	19	18	26	25[b]
Fluoranthene	128	155	132	93	123	76[b]
Pyrene	193	242	211	189	207	145[b]

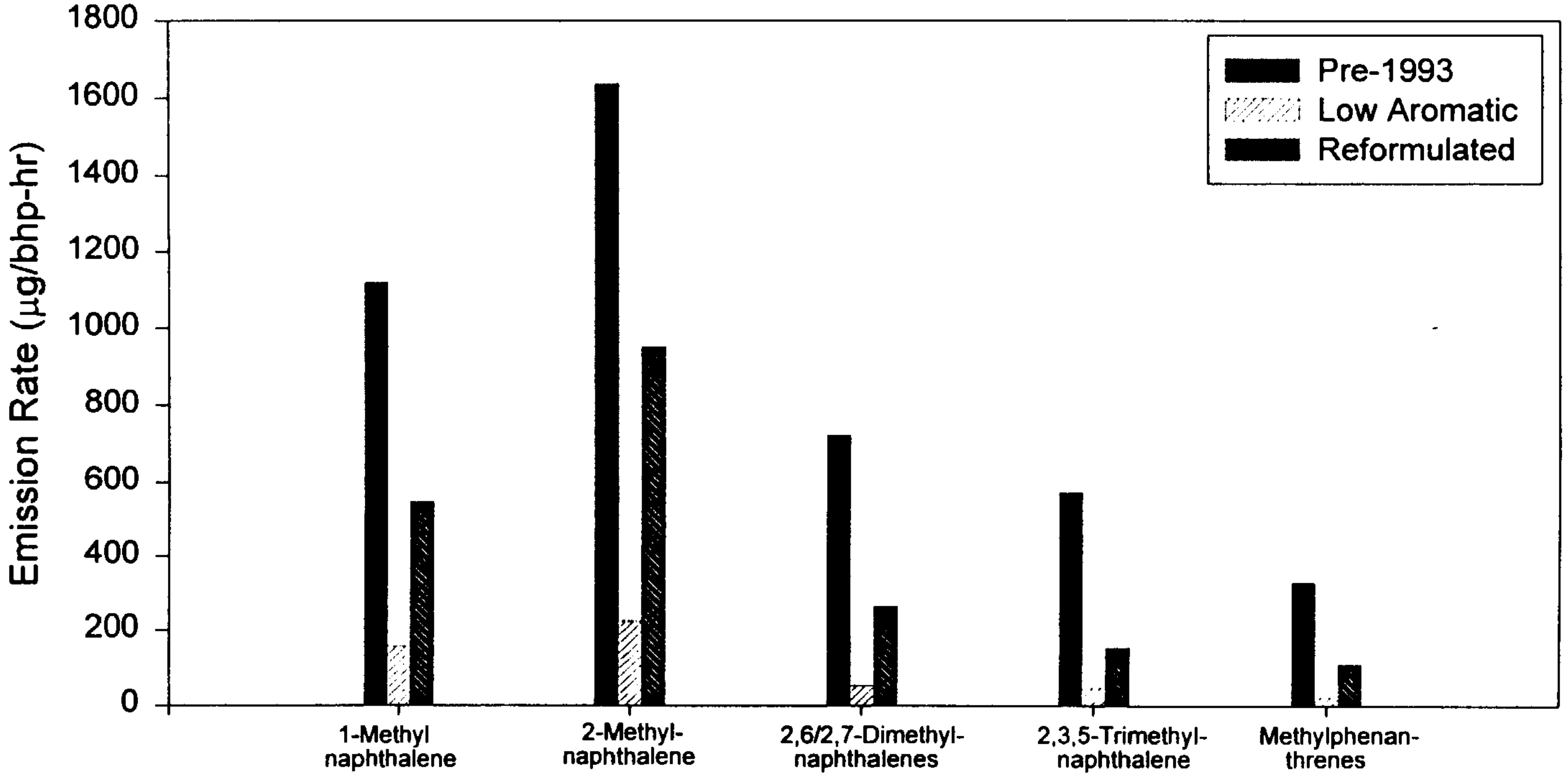
[a] Value is a lower limit, breakthrough onto the back PUF occurred (see Figure 13).
[b] Sample leaked during collection.

There is reasonable agreement between the two laboratories with the exception of the 2,3,5-trimethylnaphthalene data, where the SAPRC numbers are consistently lower. As noted on Table 41, the SAPRC 2,3,5-trimethylnaphthalene values are lower limits because breakthrough of this alkyl-naphthalene onto the back PUF occurred (see Table 13).

Alkyl-PAH:

It has been noted that the most pronounced differences in PAH emissions among the three fuel types occurred for the alkyl-PAH. The data suggests that emission levels of alkyl PAHs are highly fuel dependent. Figure 22a shows the combined particle- associated (methylphenanthrenes) and vapor-phase alkyl-PAH data. As noted previously, unsubstituted PAH are expected to be formed preferentially in high temperature combustion processes and for many of the particle-associated PAH quantified little fuel-dependent differences in emissions were found. The high alkyl-PAH emissions from the high PAH content fuel (pre-1993 fuel), therefore, suggests that significant fractions of these alkyl-PAH emissions are unburned fuel components present in the exhaust. As noted in Section 3.6, decreased fuel PAH content which lowers the emissions of volatile alkyl-PAH may be expected to lead to decreased potential for atmospheric formation of mutagenic nitro-PAH and nitro-PAH lactones.

Figure 22a. Vapor-Phase and Particle-Associated Alkyl-PAH



3.8 Nitrosamines

Nitrosamines sampling was performed for the pre-1993 and reformulated fuels only.as part of a scoping experiment to evaluate the validity of sampling method. The sampling for nitrosamines from the reformulated blend and pre-1993 fuels were conducted in conjunction with engine testing along with a tunnel blank and a trip blank. Samples, date sampled, type of fuel, and sample identification are summarized in Table 42.

Table 42. Summary of Nitrosamine samples obtained from the emissions of a heavy duty diesel engine using pre-1993 and reformulated blend fuels.

Sample ID	Fuel Type	Date Sampled	Cycle/Start Type
76798	Pre-1993	12/05/96	FTP/Hot
76806	Pre-1993	01/08/97	FTP/Hot
76815	Reformulated	12/16/97	FTP/Hot
76803	Reformulated	12/16/96	FTP/Hot
76813	none	12/13/97	Tunnel Blank
76800	none	02/04/97	Trip Blank

a. 6 hot start federal transient engine cycles.

Hydrocarbon, nitrogen oxides, carbon monoxide, and carbon dioxide pollutant levels taken during NA sampling are presented in Table 43. All samples were sent to Lancaster Laboratories and analysis was completed by 2/27/97. In addition to the analysis of the front cartridges, one backup cartridge was analyzed. No NA were detected in the backup cartridge so none of the other backup cartridges were analyzed. The results of NA are given in µg per brake horsepower hour are reported in Table 44.

Table 43. Summary of the average pollutant levels for the pooled NA samples.

Sample	Fuel	HC[a] g/Bhp-hr.	SDEV[b] g/Bhp-hr.	NOx[c] g/Bhp-hr.	SDEV g/Bhp-hr.
	Pre-1993	.53	.012	4.63	.052
	Pre-1993	.53	.010	4.88	.034
	Reformulated	.50	.010	4.56	.026
	Reformulated	.50	.019	4.60	.054
	Reformulated	.50	.019	4.60	.054

	Fuel	CO[d] g/Bhp-hr.	SDEV g/Bhp-hr.	CO_2[e] g/Bhp-hr.	SDEV g/Bhp-hr.
	Pre-1993	2.39	.063	520.9	1.01
	Pre-1993	2.25	.040	494.8	2.57
	Reformulated	2.30	2.17	510.7	6.58
	Reformulated	2.26	.051	512.9	1.39

a. Total Hydrocarbon Emissions
b. Standard Deviation
c. Nitrogen Oxide Emissions
d. Carbon Monoxide Emissions
e. Carbon Dioxide Emissions

Table 44. Nitrosamine emission rate

Nitrosamine	Pre-1993 76798 µg/Bhp-hr.	Pre-1993 76806 µg/Bhp-hr.	average (precision) µg/Bhp-hr.	Reformulated 76803 µg/Bhp-hr.	Reformulated 76811 µg/Bhp-hr.	average (precision) µg/Bhp-hr.
N-nitrosodimethylamine (NDMA)	5.81	7.02	6.41 (9.41)	7.84	8.00	7.92 (1.01)
N-nitrosodiethylamine (NDEA)	<7.7	<7.9		<7.8	<7.9	
N-nitrosodipropylamine (NDPA)	8.28	<7.9		<7.8	7.2	
N-nitrosobutylamine (NDBA)	<12	<12		<12	<12	
N-nitrospiperidine (NPIP)	<7.7	<7.9		<7.8	<7.9	
N-nitrosopyrolidine (NPYR)	<7.7	<7.9		<7.8	<7.9	
N-nitrosomorpholine (NMOR)	<7.7	<7.9		<7.8	<7.9	

The average NDMA emission per cycle was 143 µg (6.41 µg/hp-hr) in the pre-1993 fuel and 176 µg (7.92 µg/hp-hr) in the reformulated blend fuel. One pre-1993 fuel emission sample and one reformulated blend fuel emission sample contained measurable levels of NDPA at 185 µg (8.28 µg/hp-hr) and 160 µg (7.2 µg/hp-hr) respectively. No other nitrosamines, including NMOR, were detected in any of the samples.

To test for breakthrough one backup cartridge was tested. No NAs were detected in the backup cartridge. No NAs were detected in either the tunnel blank or in the trip blank.

Discussion: As reported in the literature, NA have been detected in the emissions from diesel and gasoline powered vehicles. Most of the studies have centered on emissions from catalyst equipped vehicles. NDMA has been detected when in malfunctioning catalyst equipped vehicles.

No other study has reported the emission of NDPA which in this study was detected in two samples and emitted at similar levels to NDMA. Only one sample from the emission of each fuel showed measurable levels of NDPA.

Conclusion: The objective of the nitrosamine component of this study was to determine whether NA were present in diesel exhaust. NDMA and NDPA were detected in the exhaust near the detection limit of the method. If other NAs are present at lower levels then larger sample volumes need to be collected to detect them. Additional QA procedures to test for NA artifacts and GC/MS confirmation are recommended but were not included in this study. GC/MS confirmation would have required a larger sample volume than was collected at this test and would have required designing and validating a sampler with a significantly higher flow rate. Artifacts are attenuated by the nitrogen dioxide suppressing layer in the Thermosorb-N sampler but additional information could be obtained by running a parallel sample train to sample for dialkyl amines. Dialkyl amines can react with nitrogen dioxide to form NA. Detection of dialkyl amines would help in calculating the upper limit of artifact NA formation.

3.9 Dioxins

Dioxin sampling was conducted for two of the three fuels due to the limited availability of the dynamometer facilities. There are 210 polychlorinated dibenzo-p-dioxin (PCDD) and dibenzofuran (PCDF) isomers. Each isomer has a different cancer potency. To assess cancer risk of PCDD and PCDF exposure, a system based on toxic equivalence factors (TEQ) has been developed. The isomer 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) which is considered the most potent isomer is given a TEQ of 1. PCDD and PCDF isomers with chlorines in the 2,3,7,8 position are considered carcinogenic and are also given TEQ factors. Isomers of PCDD and PCDF not chlorinated in the 2,3,7,8 position are not given TEQ factors. A number of TEQ schemes have been proposed. Currently the US EPA, Department of Toxic Substance Control (DTSC) and other nations use the international system (I-TEQ), and Cal/EPA and OEHHA use a different a different system, although Cal/EPA and OEHHA are (as of January 1998) considering adopting the I-TEQ system. The two TEQ systems are shown in Table 45.

Table 45. CA EPA/OEHHA and International Toxic Equivalence Factors for PCDDs and PCDFs.

Toxic Equivalence Factors (TEQ)

	CAL EPA/OEHHA Factors	US EPA/Int. Factors
Dioxins		
2,3,7,8-TCDD	1	1
Total TCDD	0	0
1,2,3,7,8-PeCDD	1	0.5
Total PeCDD	0	0
1,2,3,4,7,8-HxCDD	0.03	0.1
1,2,3,6,7,8-HxCDD	0.03	0.1
1,2,3,7,8,9-HxCDD	0.03	0.1
Total HxCDD	0	0
1,2,3,4,6,7,8-HpCDD	0.03	0.01
Total HpCDD	0	0
OCDD	0	0.001
Furans		
2,3,7,8-TCDF	1	0.1
Total TCDF	0	0
1,2,3,7,8-PeCDF	1	0.05
2,3,4,7,8-PeCDF	1	0.5
Total PeCDF	0	0
1,2,3,4,7,8-HxCDF	0.03	0.1
1,2,3,6,7,8-HxCDF	0.03	0.1
2,3,4,6,7,8-HxCDF	0.03	0.1
1,2,3,7,8,9-HxCDF	0.03	0.1
Total HxCDF	0	0
1,2,3,4,6,7,8-HpCDF	0.03	0.01
1,2,3,4,7,8,9-HpCDF	0.03	0.01
Total HpCDF	0	0
OCDF	0	0.0001

Pretest High Volume Sampler and Diesel Exhaust Studies: A series of pretests were conducted at the MTA Engine Emissions Test Facility located in Los Angeles in August and September of 1996. The purpose of the first set of pre-tests was to modify the existing Horiba high volume particulate sampler which is part of the dilution tunnel. The sampler was modified to include a sorbant module to collect semi-volatile compounds including PCDDs and PCDFs in the vapor phase. In October of 1996 a main pre-test was performed to 1) Evaluate the performance of the high volume sampler, 2) Collect a diesel emission sample to determine whether dioxins could be detected, and 3) Test for breakthrough of the PUF. A tunnel blank was collected during the main pre-test. A tunnel blank is when the blower in the tunnel is run but the engine is not.

PreTest: A tunnel blank was taken to determine background levels of dioxins in the main dilution tunnel. Prior to collecting the tunnel blank, the tunnel was conditioned for approximately two hours by running the CVS fan without running the engine. Only HEPA and charcoal filtered ambient air at ambient temperature was used to purge the tunnel.

Diesel exhaust from six hot start FTP cycles was collected on a single high volume sample. The high volume sample consisted of filters and a PUF module. The flow rate was approximately 60 CFM. A large volume of sample needed to be collected to achieve the required detection limit. This raised concerns for breakthrough of dioxins. Breakthrough was tested by spiking the front half of the PUF with a cocktail of PCDDs and PCDFs. The front half and the back half of the PUF were analyzed separately to test for breakthrough. Also native PCDDs and PCDFs were analyzed in the backup PUF to determine the amount of PCDDs and PCDFs that broke through the front PUF.

Presented in Table 46 are the PCDDs and PCDFs detected in the front half PUF, in the back PUF, and in the tunnel blank. Virtually all the PCDDs and PCDFs were detected in the front half PUF. The back half PUF PCDD and PCDF levels were virtually the same as in the tunnel blank.

Table 46. Pretest PCDDs and PCDFs Analytical Results for the Front and Back Half of Sample 96101 and the Blank.

	PUF Front Half Sample ID: 96101		PUF Back Half Sample ID: 96101		Tunnel Blank Sample ID: 96101-1.2	
Compound	Conc.[a] pg/sample	DL[b]	Conc. pg/sample	DL	Conc. pg/sample	DL
2,3,7,8-TCDD	ND[c]	4.2	ND	2.8	ND	2.4
Total TCDD	60		ND	2.8	16	
1,2,3,7,8-PeCDD	ND	2.1	ND	4.1	ND	2.2
Total PeCDD	19		ND	4.1	ND	2.2
1,2,3,4,7,8-HxCDD	ND	1.7	ND	2.5	ND	5.1
1,2,3,6,7,8-HxCDD	ND	1.7	ND	3.2	ND	4.6
1,2,3,7,8,9-HxCDD	ND	1.5	ND	2.9	ND	5
Total HxCDD	15		ND	3.2	ND	5.1
1,2,3,4,6,7,8-HpCDD	7.7		ND	2.6	ND	2.5
Total HpCDD	14		ND	2.6	ND	2.5
OCDD	37		6.9		12	
2,3,7,8-TCDF	4.7		ND	2.6	ND	2
Total TCDF	200		ND	2.6	26	
1,2,3,7,8-PeCDF	2.2		ND	2.3	ND	2.8
2,3,4,7,8-PeCDF	2.2		ND	2.2	ND	2.7
Total PeCDF	28		ND	2.3	ND	2.8
1,2,3,4,7,8-HxCDF	2.1		ND	1.6	ND	1.4
1,2,3,6,7,8-HxCDF	1.2		ND	1.4	ND	1.4
2,3,4,6,7,8-HxCDF	1		ND	1.7	ND	1.5
1,2,3,7,8,9-HxCDF	ND	0.61	ND	1.9	ND	1.7
Total HxCDF	10		ND	1.9	ND	1.7
1,2,3,4,6,7,8-HpCDF	4.1		ND	1.3	ND	1.5
1,2,3,4,7,8,9-HpCDF	ND	1.3	ND	1.7	ND	1.9
Total HpCDF	4.1		ND	1.7	ND	1.9
OCDF	4.5		ND	5.7	ND	3.7

a. Conc. = concentration
b. D.L. = detection limit
c. ND = not detected

A cocktail of 5 isotopically labeled PCDDs and PCDFs were spiked on the front half of PUF sample 96101. After the sample was collected, the PUF was analyzed to determined the amount of recovery standards that were lost from the front cartridge. Recoveries were from 89% to 115%, suggesting that there was no loss of recovery standards due to breakthrough. (Table 47).

Table 47. Recoveries of pre-spike standards added to the front half of the PUF.

Pre-spike Recovery Standard	%R
^{37}Cl-2,3,7,8-TCDD	89
^{13}C-2,3,4,7,8-PeCDF	104
^{13}C-1,2,3,4,7,8-HxCDD	111
^{13}C-1,2,3,4,7,8-HxCDF	115
^{13}C-1,2,3,4,7,8,9-HpCDF	114

*%R = Recovery of standards

After the main pretest it was determined that the original Horiba high volume sampler was not suitable for dioxin sampling. Modifications to the high volume sampling system (see PAH section) were successfully completed in November for subsequent testing.

Summary of Main Test Sampling: The main test was conducted at the MTA Emissions Test Facility. Testing started on 12/2/97, was suspended for the holidays on 12/19/97, resumed on 1/8/97 and was completed on 1/9/97. Fuel and oil samples were collected throughout the test. Tunnel and manifold particulate samples were collected on 1/9/97 and again on 5/1/97. A summary of the PCDD and PCDF samples are given in Table 48.

Criteria Pollutant Results: A total of six PCDDs and PCDFs diesel exhaust samples were collected. Diesel exhaust samples D1, D5, and D6 were collected when the engine was running on pre-1993 fuel. Diesel exhaust samples D2, D3, and D4 were collected when the diesel engine was running on the reformulated blend. Each sample consisted of 7 hot and 1 cold FTP cycles. For each pollutant the sample's average emission rate of regulated pollutants for all 8 cycles is given in Table 49.

Table 48. Summary information of PCDDs and PCDF samples collected at the LACMTA.

Sample ID	Lab ID	Date sampled	Type	Matrix	sample type	fuel
	Pretest					
96101-1,2	96101-1,2	10/2/97	filter/PUF	emission	6HFTP[a]	pre-1993
96101-1,2	96101-1,2		Back PUF	emission	6HFTP	pre-1993
96101	96101	10/2/97	filter/PUF	emission	blank[b]	pre-1993
	Main test					
ULFV0	ULFV1	12/2/96	filter/PUF	emission	blank	pre-1993
D1FV0	D1FV1	12/5/96	filter/PUF	emission	1C/7HFTP[c]	pre-1993
D5	Sample 1	1/8/97	filter/PUF	emission	1C/7HFTP	pre-1993
D6	Sample 2	1/9/97	filter/PUF	emission	1C/7HFTP	pre-1993
DVL2	DVL	12/13/96	filter/PUF	emission	blank	reformulated
D2	D2	12/18/96	filter/PUF	emission	1C/7HFTP	reformulated
D3	D3	12/19/96	filter/PUF	emission	1C/7HFTP	reformulated
D4	D4	12/20/96	filter/put	emission	1C/7HFTP	reformulated
	Fuel/Oil					
DPF-0	DPF-1	12/6/96	fuel	fuel		pre-1993
DMF-1	DMF-2	12/19/96	fuel	fuel		reformulated
DO-1	DO-1	12/6/97	oil	new oil		
DPO-2	DPO-2	12/6/96	oil	used oil		pre-1993
DMO-3	DMO-3	12/19/96	oil	used oil		reformulated
	Tunnel and Manifold					
DEP-1	DEP-1	1/9/97	particulates	exhaust line	exhaust	near manifold[d]
97051-2	97051-2	5/1/97	particulates	exhaust line	exhaust	near tunnel[e]
DTP-1	DTP-1	1/9/97	particulates	tunnel	exhaust	by 2nd dilution tunnel
97051-1	97051-1	5/1/97	particulates	tunnel	exhaust	by 2nd dilution tunnel
97051-3	97051-3	5/1/97	particulates	tunnel	exhaust	by bag filter

a.. 6 hot federal transient engine test cycles
b. Tunnel blank
c. 1 cold and 7 hot federal transient engine test cycles
d. Sample taken near engine manifold
e. Sample taken just before main tunnel

Table 49. Summary of Regulated Pollutant Emission Taken Concurrently During the Collection of Dioxin Samples.

Sample	Date	HC[a] emission rate	standard deviation	NO_X[b] emission rate	standard deviation
		g/Bhp-hr.[c]	g/Bhp-hr.	g/Bhp-hr.	g/Bhp-hr.
D1 (pre)[d]	12/5/97	0.53	0.015	4.64	0.097
D5 (pre)	1/8/97	0.54	0.7	4.87	0.074
D6 (pre)	1/9/97	0.52	0.017	4.84	0.13
D2 (MB)[e]	12/18/97	0.52	0.31	4.61	0.11
D3 (MB)	12/19/97	0.5	0.025	4.62	0.09
D4 (MB)	12/20/97	0.49	0.013	4.6	0.111

Sample	Date	CO[f] Emission Rate	Standard Deviation	CO_2[g] Emission Rate	Standard Deviation
		g/Bhp-hr.	g/Bhp-hr.	g/Bhp-hr.	g/Bhp-hr.
D1 (pre)	12/5/97	2.36	0.086	525	10.917
D5 (pre)	1/8/97	2.23	0.022	496	13.17
D6 (pre)	1/9/97	2.12	0.229	502.3	10.461
D2 (MB)	12/18/97	2.27	0.037	510.4	14.9
D3 (MB)	12/19/97	2.28	0.048	512.3	13.12
D4 (MB)	12/20/97	2.3	0.036	511.6	14.76

a. Total hydrocarbon emissions
b. Total Nitrogen Oxide emissions
c. grams per brake-horsepower hour
d. pre-Oct. '93 fuel
e. Reformulated
f. Total Carbon Monoxide Emissions
g. Total Carbon Dioxide Emissions

Dioxin Exhaust Sample Volume: Due to the low level of PCDDs and PCDFs found in the pre-test, a large sample volume was needed to detect their levels in the diluted diesel exhaust. Sample flow rates and the fraction of a single FTP cycle is given in Table 51. Approximately 10 % of the total exhaust was collected in each pooled sample of 8 FTP cycles. The flow rate averaged between 36.15 and 39.63 CFM. The flow rate decreased as the particulates loading on the 8"x10" filter increased. Also, it was observed that as the particulate loading on the main tunnel bag filter increased, the high volume sampler flow rate concurrently decreased.

Fuel Consumption during the FTP Cycle: Fuel consumption during a cycle was determined by two methods. In the first method, hydrocarbons (HC) from the diesel exhaust were used to calculate the mass of fuel consumed during a test. The second method was to directly measure the weight loss of the fuel drum by using a scale. The weight before and after a test was recorded to obtain the fuel consumption during each test. Initial weight measurements drifted when the scale was exposed to direct sunlight. Shade was provided for the scale which resulted in more reproducible data. The fuel weight determined directly was in good agreement with the HC calculated weight. Since there was a complete data set for the calculated HC weight the calculated fuel consumption data was used. Average calculated fuel consumption per sample is also reported in Table 50.

Table 50. Average Flow Rate and Fuel Consumption for PCDDs and PCDFs Samples.

	D1	D2	D3	D4	D5	D6
Avg. (SCFM)[a]	39.63	33.61	36.59	36.15	35.85	37.32
SDEV.[b]	3.81	4.05	4.68	4.90	5.07	5.38
Total Volume (CF)[c]	6,341	5,377	5,855	5,784	5,736	5,970
Total CVS Volume (CF)[d]	61,078	60,985	60,765	60,576	60,776	60,954
Fraction Sampled[e]	0.10	0.09	0.10	0.10	0.09	0.10
Fuel Consumed (L)[f]	7.27	6.27	6.27	6.27	7.24	7.27

a. Average sample flow rate in standard cubic feet per minute of 8 federal transient engine test cycles.
b. Standard Deviation of flow rate.
c. Total volume collected per sample.
d. Average total exhaust per federal transient engine test cycles.
e. Fraction of total volume collected per sample.
f. Average fuel consumed per cycle.

PCDDs and PCDFs in Diesel Emission Results: For each fuel tested, 3 diesel exhaust samples (8 cycle equivalents) and one tunnel blank were collected. For the tunnel blank, the CVS tunnel was run with dilution air only and the sample volume collected was comparable to the emissions samples. The emission sample consisted of 8 FTP cycles. The filter was changed after the completion of the first set of a cold and a hot cycle. Subsequent filters were changed after every third cycle.

Sample D1 had lower than expected concentrations of dioxins. A re-extraction and analysis of the sample D1 was performed to recheck the extraction procedure. Analysis of the re-extraction (sample D1RA) detected no additional PCDDs or PCDFs. This data is shown in Table 51 along with emission PCDDs and PCDFs data from samples ULFV1, D5, and D6. A comparison of the blank with the emission samples reveal that the total TCDDs, 1,2,3,4,6,7,8-HpCDD, 1,2,3,4,6,7,8-HpCDD, total HpCDD, OCDD, 1,2,3,4,6,7 HpCDF, and total HpCDF in the blank are between 25 and 50 percent of the emission samples. Levels in the tunnel blank may be a significant part of the total dioxin emissions measured in the emission samples. Concentration of PCDDs and PCDFs in the blank for the tunnel are also reported.

Samples D2, D3, and D4 and the tunnel blank DVL were collected from the CVS tunnel with the diesel engine running on the reformulated fuel. The reformulated fuel was a blend of three commercially available fuels. Levels of some PCDDs and PCDFs detected in the diesel exhaust were also found in the blank. A comparison of the blank with the emission samples (see Table 52) show that the total TCDDs, 1,2,3,4,6,7,8-HpCDD, 1,2,3,4,6,7,8-HpCDD, total HpCDD, and OCDD are between 25 and 50 percent of the emission samples. Again the tunnel blank levels may be a significant part of the emission sample.

The diesel exhaust from the reformulated blend contained lower levels of all PCDDs and PCDFs isomer classes than in the pre-1993 fuel. In the pre-1993 fuel, TCDFs were found in the highest concentration of all the PCDD and PCDF classes. OCDDs were found in the highest levels of all the PCDD classes. In the reformulated blend, TCDFs were also found in the highest concentration for all PCDD and PCDF classes. For PCDD classes, THxCDDs were detected at the highest concentration. This is unusual in that OCDDs are usually found in the highest level. A comparison of PCDD and PCDF isomer classes between the pre-1993 and reformulated blend are given in Figure 23.

Table 51. PCDDs and PCDFs Concentrations in the Exhaust of Heavy Duty Diesel Engine Using Pre-1993 Fuel.

	Sample ID: UL1FV1		Sample ID: D1		Sample ID: D1RA		Sample ID: D5		Sample ID: D6	
Compound	**Con**	**D.L.**[a]	**Con.**	**D.L.**	**Con.**	**D.L.**	**Con.**	**D.L.**	**Con.**	**D.L.**
2,3,7,8-TCDD	ND	2.3	ND	4.4	ND	2.5	ND	2.2	ND	3.2
Total TCDD	5.1		24		ND	2.5	13		11	
1,2,3,7,8-PeCDD	ND	1	ND	1.9	ND	0.52	ND	2.6	ND	1.7
Total PeCDD	2.8		11		ND	0.52	11		9.4	
1,2,3,4,7,8-HxCDD	ND	1.3	ND	1.5	ND	1.1	ND	3.1	ND	1.8
1,2,3,6,7,8-HxCDD	ND	1.3	2.4		ND	1.1	ND	3.0	ND	3.1
1,2,3,7,8,9-HxCDD	ND	1.2	ND	1.5	ND	1	ND	2.7	ND	2.2
Total HxCDD	ND	3	19		ND	1.1	14.0		14.0	
1,2,3,4,6,7,8-HpCDD	5.5		9		ND	0.8	12		8.4	
Total HpCDD	11		18		ND	0.8	23		18	
OCDD	35		42		7.8		69		40	
2,3,7,8-TCDF	ND	2.1	ND	3.7	ND	1.7	3.6		ND	2.6
Total TCDF	ND	2.1	91		ND	1.7	90		75	
1,2,3,7,8-PeCDF	ND	0.94	ND	1.6	ND	0.69	ND	1.7	ND	1.7
2,3,4,7,8-PeCDF	ND	0.91	ND	1.6	ND	0.67	ND	1.6	ND	2.1
Total PeCDF	ND	1.2	13		ND	0.69	12		5.7	
1,2,3,4,7,8-HxCDF	ND	0.65	1.9		ND	0.25	ND	2.8	ND	1.7
1,2,3,6,7,8-HxCDF	ND	0.56	ND	0.88	ND	0.22	ND	2.5	ND	1.1
2,3,4,6,7,8-HxCDF	ND	0.7	ND	1.1	ND	0.3	ND	1.5	ND	1.2
1,2,3,7,8,9-HxCDF	ND	0.77	ND	0.68	ND	0.3	ND	1.6	ND	1.2
Total HxCDF	ND	1.2	8.8		ND	0.3	3.2		3	
1,2,3,4,6,7,8-HpCDF	1.9	1.2	3.5		ND	0.35	6.3		4.6	
1,2,3,4,7,8,9-HpCDF	ND		ND	1.1	ND	0.45	ND	1.4	ND	2.4
Total HpCDF	1.9	3.2	3.5		ND	0.45	6.3		4.6	
OCDF	ND		ND	2.9	ND	2.5	ND	9.4	ND	6

a. Detection limit in picograms per sample

Table 52. PCDDs and PCDFs in exhaust samples of a diesel engine using reformulated blend.

	Sample ID: DVL		Sample ID: D2		Sample ID: D3		Sample ID: D4	
Compound	Conc.[a]	D.L.[b]	Conc.	D.L.	Conc.	D.L.	Conc.	D.L.
2,3,7,8-TCDD	ND[c]	2.2	ND	3.0	ND	4.7	ND	3.5
Total TCDD	6		10		12		11	
1,2,3,7,8-PeCDD	ND	2.8	ND	2.8	ND	3.8	ND	1.5
Total PeCDD	ND	2.8	7.2		7.8		8.4	
1,2,3,4,7,8-HxCDD	ND	2.6	ND	2.0	ND	2.1	ND	2.9
1,2,3,6,7,8-HxCDD	ND	2.5	ND	2.0	ND	2.1	ND	2.9
1,2,3,7,8,9-HxCDD	ND	2.3	ND	1.8	ND	1.9	ND	2.6
Total HxCDD	ND	2.6	14		17.0		13	
1,2,3,4,6,7,8-HpCDD	2.5		9.0		5.4		6.8	
Total HpCDD	4.8		17		11		14	
OCDD	13		33		22		37	
2,3,7,8-TCDF	ND	2.0	3.3		ND	3.5	ND	3.2
Total TCDF	6.8		66		55		61	
1,2,3,7,8-PeCDF	ND	1.5	ND	3.8	ND	4.0	ND	4.0
2,3,4,7,8-PeCDF	ND	1.5	ND	3.7	ND	3.8	ND	3.9
Total PeCDF	ND	1.5	ND	3.8	ND	4.0	ND	4.2
1,2,3,4,7,8-HxCDF	ND	0.96	ND	2.0	ND	2.3	ND	1.7
1,2,3,6,7,8-HxCDF	ND	0.84	ND	1.7	ND	2.0	ND	1.5
2,3,4,6,7,8-HxCDF	ND	1.0	ND	2.0	ND	2.3	ND	1.8
1,2,3,7,8,9-HxCDF	ND	1.1	ND	2.3	ND	2.7	ND	2
Total HxCDF	ND	1.1	ND	2.3	ND	2.7	3.1	
1,2,3,4,6,7,8-HpCDF	ND	1.2	2.8		3.0		3.0	
1,2,3,4,7,8,9-HpCDF	ND	1.6	ND	1.4	ND	1.5	ND	2.1
Total HpCDF	ND	1.6	2.8		3.0		3.0	
OCDF	ND	6.5	ND	6.2	ND	4.2	ND	6.1

a. Concentration in picograms per sample
b. Detection limit in picograms per sample
c. Not detected

One of the goals of this study was to collect sufficient sample to obtain a detection limit of 100 pg TEQ/L. This goal was exceeded with the lowest TEQ measured for the pre-1993 fuel emission samples being 10 pg TEQ/l and for the Market Basket blend being 15 pg TEQ/l. These values were calculated by adding the TEQ of compounds detected with the TEQ of the compounds not detected. The upper limit TEQ of undetected compounds was calculated by multiplying its TEQ factor with the detection limit of that compound. Even with the lower detection limit obtained in this study many of the PCDDs and PCDFs with TEQ factors were not detected. The average TEQ profiles (three samples each) of the pre-1993 and reformulated blend emission samples are illustrated in Figure 24. The TEQ profile of the market basket blend emission samples were lower than the pre-1993 emission samples.

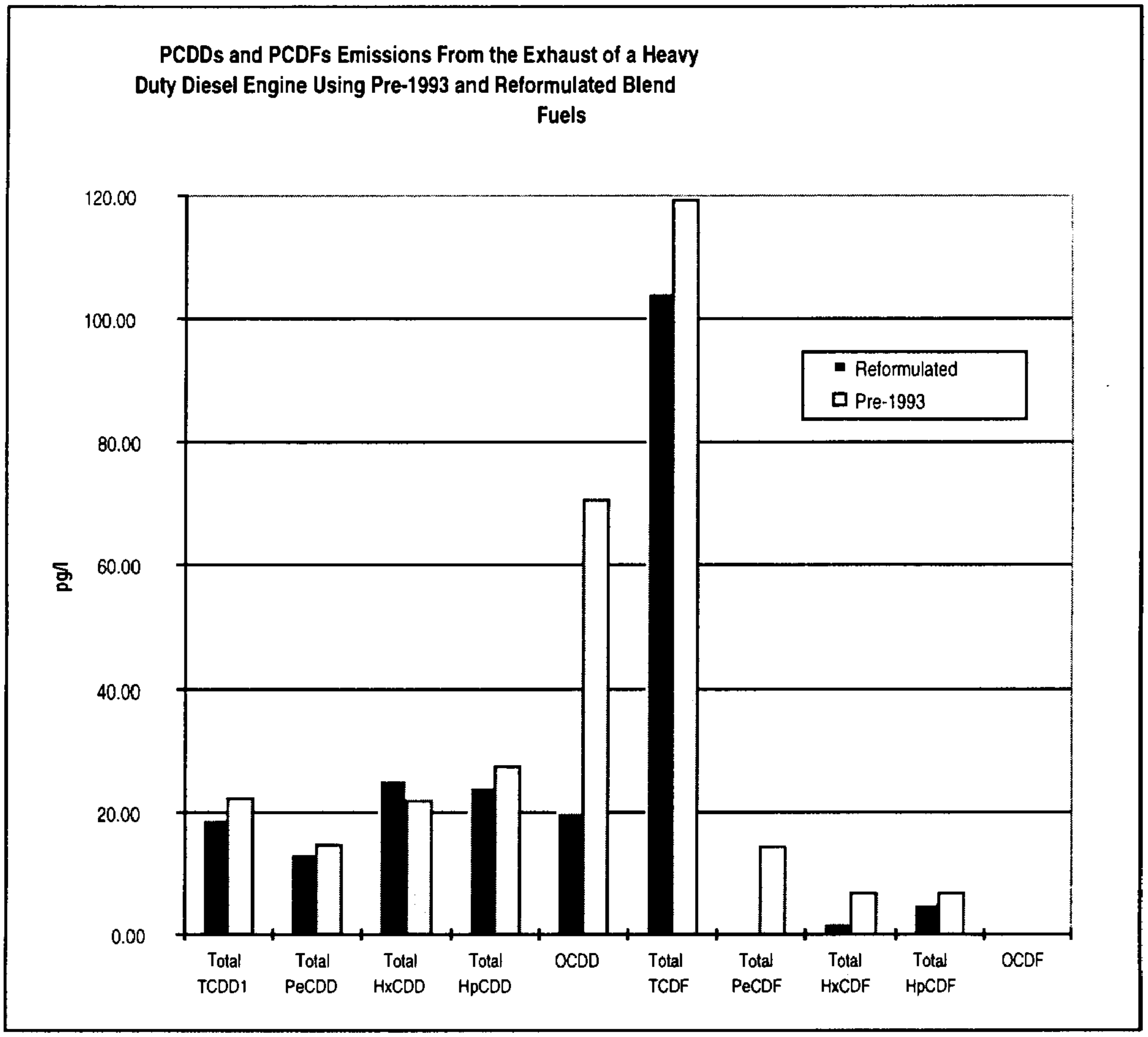


Figure 23. Profile of PCDDs and PCDFs isomer classes from the emissions from the exhaust of a heavy duty engine using Pre-1993 and Reformulated blend.

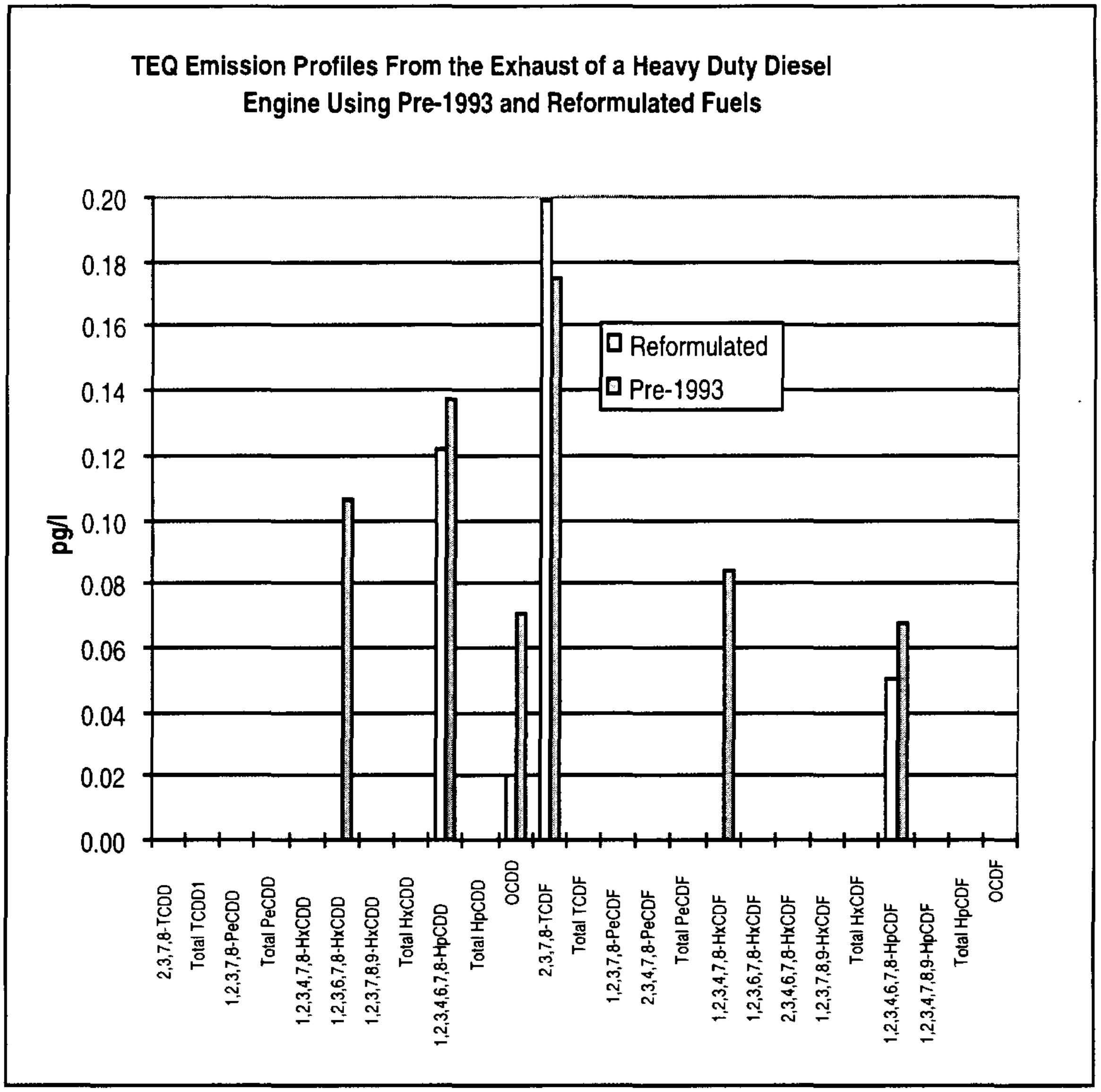


Figure 24. TEQ profiles for the pre-1993 and reformulated blend diesel emission samples taken at the LACMTA Test Emissions Facility.

Fuel and Oil Dioxin Results: PCDDs and PCDFs present in the fuel may be detected in the exhaust because some of the fuel is not burned during combustion. Both the pre-1993 and the reformulated blend fuels were analyzed for PCDDs and PCDFs. The results are presented in Table 53. OCDD levels in the pre-1993 fuel and the reformulated blend were 1400 pg/l and 720 pg/l, respectively. This corresponds to a TEQ of 1.4 pg/l for the pre-1993 fuel and .72 pg/l for the reformulated blend. The measured TEQ in the fuels can be misleading because not all the PCDDs and PCDFs with TEQ factors were above the detection limit. This is magnified because the detection limit in the pre-1993 fuel is much higher than in the reformulated blend. Levels below the detection limit of the pre-1993 fuel and above that of the reformulated blend could raise the TEQ for the pre-1993 fuel.

Table 53. PCDD and PCDF levels in the pre-1993 and Reformulated Blend Fuels.

	Sample ID: DPF-1 Pre-1993		Sample ID: DMF-1 Reformulated blend	
Compound	Conc.[a] (pg/l)[c]	D.L.[b] (pg/l)	Conc. (pg/l)	D.L. (pg/l)
2,3,7,8-TCDD	ND [d]	100	ND	26
Total TCDD	ND	100	ND	26
1,2,3,7,8-PeCDD	ND	89	ND	21
Total PeCDD	ND	89	ND	21
1,2,3,4,7,8-HxCDD	ND	110	ND	21
1,2,3,6,7,8-HxCDD	ND	120	ND	23
1,2,3,7,8,9-HxCDD	ND	110	ND	20
Total HxCDD	ND	120	ND	23
1,2,3,4,6,7,8-HpCDD	ND	230	ND	47
Total HpCDD	ND	230	ND	76
OCDD	1400		720	
2,3,7,8-TCDF	ND	21	ND	11
Total TCDF	ND	21	ND	11
1,2,3,7,8-PeCDF	ND	110	ND	8.1
2,3,4,7,8-PeCDF	ND	100	ND	11
Total PeCDF	ND	110	ND	11
1,2,3,4,7,8-HxCDF	ND	36	ND	6.4
1,2,3,6,7,8-HxCDF	ND	34	ND	6.1
2,3,4,6,7,8-HxCDF	ND	66	ND	17
1,2,3,7,8,9-HxCDF	ND	36	ND	7.5
Total HxCDF	ND	66	ND	17
1,2,3,4,6,7,8-HpCDF	ND	92	ND	48
1,2,3,4,7,8,9-HpCDF	ND	98	ND	50
Total HpCDF	ND	98	ND	50
OCDF	ND	260	ND	41

a. Concentration in picograms per liter
b. Detection limit in picograms per liter
c. Picograms per liter
d. Not detected

PCDDs and PCDFs Level in Oil: Samples of the new oil and used oil were collected and analyzed for PCDDs and PCDFs. After each fuel change on the diesel engine the oil was also changed. Used oil samples from the pre-1993 fuels test and reformulated blend were collected and analyzed for PCDDs and PCDFs. Levels in the new oil were the lowest, while levels in the used market basket oil were the highest. The TEQ of the used market basket oil was 13 pg/l of oil. This sample contained 1,2,3,4,6,7,8-HpCDD at 810 pg/l and OCDD at 5200 pg/l. Table 55 summarizes the oil results. From our current study, it is uncertain how dioxin levels in the oil affect levels in diesel exhaust.

Table 54. Summary of PCDDs and PCDFs measured in new and used oil.

	Sample DPO-1 new oil		Sample DPO-2 used after pre-1993 test		Sample DMO-3 used after reformulated fuel test	
Compound	Conc.[a] (pg/l)[c]	D.L.[b] (pg/l)	Conc. (pg/l)	D.L. (pg/l)	Conc. (pg/l)	D.L. (pg/l)
2,3,7,8-TCDD	ND[d]	96	ND	46	ND	55
Total TCDD	ND	96	ND	46	ND	55
1,2,3,7,8-PeCDD	ND	300	ND	210	ND	210
Total PeCDD	ND	300	ND	210	ND	210
1,2,3,4,7,8-HxCDD	ND	300	ND	130	ND	150
1,2,3,6,7,8-HxCDD	ND	340	ND	150	ND	180
1,2,3,7,8,9-HxCDD	ND	290	ND	130	ND	160
Total HxCDD	ND	340	ND	150	ND	180
1,2,3,4,6,7,8-HpCDD	ND	370	ND	220	810	0
Total HpCDD	ND	370	ND	220	810	0
OCDD	2000	0	1100	0	5200	0
2,3,7,8-TCDF	ND	77	ND	64	ND	76
Total TCDF	370	0	320	0	330	0
1,2,3,7,8-PeCDF	ND	160	ND	180	ND	150
2,3,4,7,8-PeCDF	ND	170	ND	150	ND	150
Total PeCDF	ND	170	ND	180	ND	150
1,2,3,4,7,8-HxCDF	ND	110	ND	64	ND	62
1,2,3,6,7,8-HxCDF	ND	120	ND	78	ND	74
2,3,4,6,7,8-HxCDF	ND	120	ND	80	ND	71
1,2,3,7,8,9-HxCDF	ND	160	ND	110	ND	100
Total HxCDF	ND	160	ND	110	ND	100
1,2,3,4,6,7,8-HpCDF	ND	480	ND	140	ND	160
1,2,3,4,7,8,9-HpCDF	ND	470	ND	140	ND	210
Total HpCDF	ND	480	ND	140	ND	210
OCDF	ND	700	ND	470	ND	450

a. Concentration in picograms per liter
b. Detection limit in picograms per liter
c. Picograms per liter
d. Not detected

Emission Analysis Results of Dioxin Precursors and Catalyst: Emissions of chloride, chlorine (Cl), iron (Fe), and copper (Cu) are presented in Table 55. Emission samples were taken by CE-CERT personnel. The data listed in this table are pertinent to the PCDD and PCDF study. Listed in this table are the total chloride, chlorine, copper and iron levels. Chlorine is considered a dioxin precursor and copper and iron are considered catalysts for dioxin formation.

Table 55. Summary of Dioxin precursors and catalyst that were measured in the diesel exhaust.

Species	Pre-1993 Cold Start Cycle Emission Rate mg/Bhp-hr.	SDEV mg/Bhp-hr.	Pre-1993 Hot Start Cycle Emission Rate mg/Bhp-hr.	SDEV mg/Bhp-hr.
Chloride	-	-	0.01	0.01
Cl	0.022	0.001	0.026	0.025
Fe	0.451	0.339	0.213	0.135
Cu	0.012	0.006	0.008	0.013

Species	Reformulated Blend Cold Start Cycle Emission Rate mg/Bhp-hr	SDEV mg/Bhp-hr	Reformulated Blend Hot Start Cycle Emission Rate mg/Bhp-hr	SDEV mg/Bhp-hr
Chloride	0.03	0.03	0.07	0.11
Cl	0.028	0.013	0.033	0.010
Fe	0.331	0.311	0.089	0.026
Cu	0.009	0.005	0.005	0.004

Isotope Recoveries: Part of the Quality Assurance procedures is to measure the recoveries of the internal standards spiked on the samples. Percent recoveries are calculated by dividing the amount measured in the sample by the amount added, multiplied by 100. Recoveries between 60-140% are considered adequate and levels outside this range are flagged. Because isotope dilution compensates for recoveries, internal standard levels outside this range are considered acceptable as long as there is sufficient signal to accurately measure the internal standard. Internal standard recoveries in diesel emission samples are presented in Table 56. ^{13}C-1,2,3,7,8-PeCDD in samples D2 and D4, ^{13}C-

1,2,3,6,7,8-HxCDD in sample D2 and ^{13}C-1,2,3,6,7,8-HxCDF in sample D2, D3, and D4 had recoveries greater than 140%. Recoveries of the alternate standard for samples D1, D5 and D6 were less than 60%.

Table 56. Recoveries of Internal Standards in Diesel Emission Samples.

	D1	D2	D3	D4	D5	D6	UL1FV1	UL2FV1
Internal Standard:	%R[a]	%R	%R	%R	%R	%R	%R	%R
^{13}C-2,3,7,8-TCDD	109	116	115	118	86	80	99	92
^{13}C-1,2,3,7,8-PeCDD	138	143[b]	139	142[b]	104	96	116	103
^{13}C-1,2,3,6,7,8-HxCDD	96	143[b]	132	137	117	105	98	99
^{13}C-1,2,3,4,6,7,8-HpCDD	89	133	136	133	89	94	110	96
^{13}C-OCDD	69	88	93	88	50	81	104	90
^{13}C-2,3,7,8-TCDF	106	92	92	93	89	79	97	91
^{13}C-1,2,3,7,8-PeCDF	112	98	100	99	91	84	104	92
^{13}C-1,2,3,6,7,8-HxCDF	91	148[b]	149[b]	146[b]	111	102	94	96
^{13}C-1,2,3,4,6,7,8-HpCDF	87	128	123	127	71	77	97	96
Pre-spike Recovery Standard:								
^{37}Cl-2,3,7,8-TCDD	100	106	108	108	105	106	99	107
^{13}C-2,3,4,7,8-PeCDF	98	106	104	108	98	98	99	106
^{13}C-1,2,3,4,7,8-HxCDD	95	110	124	123	90	96	98	91
^{13}C-1,2,3,4,7,8-HxCDF	101	106	114	116	101	105	100	99
^{13}C-1,2,3,4,7,8,9-HpCDF	108	111	121	114	118	120	114	112
Alternate Recovery Standard:								
^{13}C-1,2,3,7,8,9-HxCDF	11[b]	70	73	73	49[b]	47[b]	106	106

a. Recovery of internal standard
b. Recovery of internal standard flagged because outside specified range of 60-140%

Recoveries of internal standards spiked in the fuel and oil samples are presented in Table 57. ^{13}C-2,3,7,8-TCDD in sample DPF-1 and ^{13}C-2,3,4,6,7,8-HxCDF and ^{13}C-OCDD in sample DMO-3 are flagged.

Table 57. Recoveries of internal standards in fuel and oil samples.

	DPF-1	DMF-2	DO-1	DPO-2	DMO-3
Internal Standard:	%R[a]	%R	%R	%R	%R
^{13}C-2,3,7,8-TCDD	53[b]	74	97	100	100
^{13}C-1,2,3,7,8-PeCDD	69	69	71	74	63
^{13}C-1,2,3,4,7,8-HxCDD	99	131	127	129	130
^{13}C-1,2,3,6,7,8-HxCDD	84	105	104	96	106
^{13}C-1,2,3,4,6,7,8-HpCDD	95	91	96	92	90
^{13}C-OCDD	73	64	75	83	49[b]
^{13}C-2,3,7,8-TCDF	65	111	101	103	100
^{13}C-1,2,3,7,8-PeCDF	77	134	87	81	77
^{13}C-2,3,4,7,8-PeCDF	82	129	74	77	68
^{13}C-1,2,3,4,7,8-HxCDF	108	144[b]	148[b]	139	52
^{13}C-1,2,3,6,7,8-HxCDF	95	129	117	112	130
^{13}C-2,3,4,6,7,8-HxCDF	114	137	129	130	148[b]
^{13}C-1,2,3,7,8,9-HxCDF	121	138	118	116	136
^{13}C-1,2,3,4,6,7,8-HpCDF	88	96	102	89	104
^{13}C-1,2,3,4,7,8,9-HpCDF	116	101	118	112	116
Cleanup Recovery Standard:					
37Cl-2,3,7,8-TCDD	121	98	119	128	112

a. Recovery of internal standard

b. Recovery of internal standard flagged because outside specified range of 60-140%

Internal standard recoveries for tunnel and manifold particulate matter are presented in Table 58.. ^{13}C-1,2,3,7,8-PeCDD recovery for sample DTP-2 was 148%.

Comparisons to Other Studies: Very few studies of dioxin incorporating sampling from a dynamometer have been reported. Marklund et al; (1987) conducted a test of a heavy-duty diesel vehicle on a chassis dynamometer. Due to difficulties in the analytical procedures the detection limit (I-TEQs) was less than 100 pg/km. Essers, Hutziner and Hagenmaier (1992) (Hagenmaier) conducted two runs of a heavy-duty diesel engine on an engine dynamometer and reported I-TEQs of 70 and 81 pg/l. In a transportation study, Gertler et al; (1996) had reported I-TEQs of 170 pg/km emitted from a fleet of vehicles consisting of 20% heavy-duty diesel vehicles. The study was conducted in the Fort McHenry tunnel. Oehme's transportation study conducted in Norway reported I-TEQs at 5100 pg/km where there were leaded gasoline vehicles and only 3% heavy-duty diesel vehicles.

Table 58. Recoveries of internal standards in tunnel and manifold particulate matter.

	DEP-1	DTP-2	97051-1	97051-2	97051-3
Internal Standard:	%R[a]	%R	%R	%R	%R
^{13}C-2,3,7,8-TCDD	100	120	106	108	105
^{13}C-1,2,3,7,8-PeCDD	117	146[b]	114	109	109
^{13}C-1,2,3,4,7,8-HxCDD	100	137	117	116	118
^{13}C-1,2,3,6,7,8-HxCDD	105	137	90	91	88
^{13}C-1,2,3,4,6,7,8-HpCDD	97	124	95	96	96
^{13}C-OCDD	74	108	80	82	86
^{13}C-2,3,7,8-TCDF	99	128	110	106	108
^{13}C-1,2,3,7,8-PeCDF	113	113	132	150	136
^{13}C-2,3,4,7,8-PeCDF	102	128	123	126	125
^{13}C-1,2,3,4,7,8-HxCDF	87	122	99	90	99
^{13}C-1,2,3,6,7,8-HxCDF	86	112	81	72	79
^{13}C-2,3,4,6,7,8-HxCDF	84	120	84	80	84
^{13}C-1,2,3,7,8,9-HxCDF	96	62	98	102	97
^{13}C-1,2,3,4,6,7,8-HpCDF	80	106	79	82	81
^{13}C-1,2,3,4,7,8,9-HpCDF	83	109	90	106	95
Cleanup Recovery Standard:					
^{37}Cl-2,3,7,8-TCDD	99	122	103	104	106

a. Recovery of internal standard
b. Recovery of internal standard flagged because outside specified range of 60-140%

The concentration of individual PCDDs and PCDFs in diesel exhaust in this study were at lower levels than in other studies. Total PCDDs and PCDFs from the diesel exhaust for the market basket blend and the pre-1993 fuels were at 250 pg/l and 320 pg/l, respectively. This is an order of magnitude less than Hagenmaier et al reported where there was 4340 pg/l and 2778 pg/l of PCDDs and PCDFs emitted from two diesel exhaust runs.

In this study, levels of some PCDDs and PCDFs congeners could not be detected and therefore the corresponding TEQs could not be determined. This problem was compounded because the congeners with the highest potency factors, 2,3,7,8 TCDD, 1,2,3,7,8 PeCDD, and 2,3,4,7,8 PeCDF, were not detected. Using the detection limit as an estimate of the upper limit for the concentration of these compounds, the maximum I-TEQ in the diesel exhaust would be 9.3 pg/l for the pre-1993 fuel and 15.7 pg/l for the reformulated blend. A summary of each TEQ measured and a calculated maximum TEQ of undetected PCDDs and PCDFs are summarized in Table 59.

The calculated maximum TEQ levels are still considerably lower than that which was reported in the Hagenmaier et al (1992) study.

A comparison of total PCDDs and PCDFs in diesel emissions from the two fuels of this study and the two heavy-duty diesel emissions runs from the Hagenmaier et al (1992) study are illustrated in Figure 25. Because PCDDs and PCDFs levels in the Hagenmaier study are much higher than in this study, the profile of PCDDs and PCDFs are given as a fraction to the total PCDD and PCDF emissions. The Hagenmaier et al (1992) study profile shows PCDFs as a larger fraction of the total mass than in this study, except for total TCDFs which are a smaller fraction in the Hagenmaier study. In this study, TCDFs were the largest fraction whereas in the Hagenmaier study OCDDs were the largest fraction. Absolute levels of total PCDDs and PCDFs diesel emissions from the two fuels used for this study and the two heavy duty diesel runs from the Hagenmaier study are illustrated in Figure 26. The figure shows the higher PCDD and PCDF emission rates from Hagemaier than from this study.

Table 59. Measured and potential TEQ for diesel emissions from pre-1993 and Reformulated fuel.

	Pre-1993		Reformulated	
Compound	I-TEQ Measured pg/l	I-TEQ D.L. pg/l	I-TEQ Measured pg/l	I-TEQ D.L. pg/l
2,3,7,8-TCDD	0.00	4.33	0.00	6.38
Total TCDD	0.00	0.00	0.00	0.00
1,2,3,7,8-PeCDD	0.00	1.37	0.00	2.31
Total PeCDD	0.00	0.00	0.00	0.00
1,2,3,4,7,8-HxCDD	0.00	0.28	0.00	0.40
1,2,3,6,7,8-HxCDD	0.11	0.40	0.00	0.40
1,2,3,7,8,9-HxCDD	0.00	0.28	0.00	0.36
Total HxCDD	0.00	0.00	0.00	0.00
1,2,3,4,6,7,8-HpCDD	0.13	0.00	0.12	0.00
Total HpCDD	0.00	0.00	0.00	0.00
OCDD	0.07	0.00	0.05	0.00
2,3,7,8-TCDF	0.16	0.00	0.19	0.38
Total TCDF	0.00	0.00	0.00	0.00
1,2,3,7,8-PeCDF	0.00	0.11	0.00	0.34
2,3,4,7,8-PeCDF	0.00	1.17	0.00	3.25
Total PeCDF	0.00	0.00	0.00	0.00
1,2,3,4,7,8-HxCDF	0.08	0.30	0.00	0.34
1,2,3,6,7,8-HxCDF	0.00	0.20	0.00	0.30
2,3,4,6,7,8-HxCDF	0.00	0.17	0.00	0.35
1,2,3,7,8,9-HxCDF	0.00	0.15	0.00	0.40
Total HxCDF	0.00	0.00	0.00	0.00
1,2,3,4,6,7,8-HpCDF	0.06	0.00	0.05	0.00
1,2,3,4,7,8,9-HpCDF	0.00	0.02	0.00	0.03
Total HpCDF	0.00	0.00	0.00	0.00
OCDF	0.00	0.01	0.00	0.01
Total	0.61	8.79	0.41	15.23

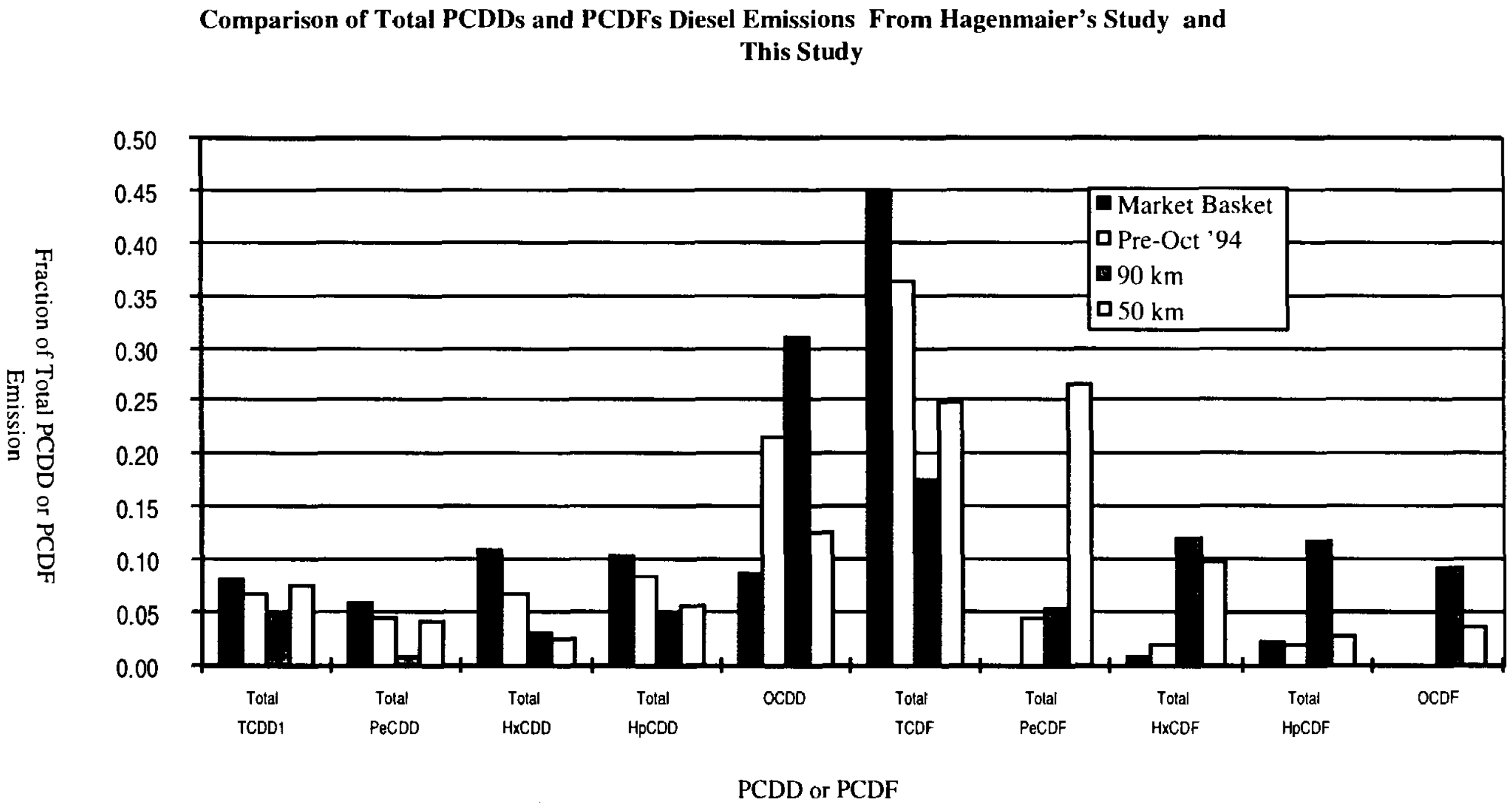

Comparison of Total PCDDs and PCDFs Diesel Emissions From Hagenmaier's Study and This Study

Figure 25. Results are normalized to total PCDD and PCDF emissions in order to determine relative differences in PCDD and PCDF class distributions. Market Basket and pre-1993 refer to reformulated and pre-1993 diesel fuel used for this study. 90 km refers to the Hagenmaier study where emissions were collected from a heavy duty diesel engine run at 90 km/hr and 50 km refers to the same diesel engine run at 50 km/hr.

In the pre-test, PCDDs and PCDFs were not detected in the tunnel blank, except for low levels of OCDDs, which is a common background contaminant that is often found even in the method blanks. The tunnel blanks taken during the main test had elevated background levels, with some as high as 50% of the emission samples. It is unknown why there was an increase in tunnel blank level since the same protocols were used. Corrections for the tunnel blanks is not a simple subtraction from sample levels since they were not taken under identical conditions as the emission samples. No blank corrections were applied to the emission data, and tunnel background levels are reported.

Other Factor Affecting Low Emission Levels: To address the significantly lower results of this study than have been reported in other dynamometer studies, a comprehensive review of the test including a review of all sampling methodologies and procedures was undertaken. Flow rate measurements were validated against a low volume sampler and the results were similar. Breakthrough of dioxins through the PUF was investigated in the pre-test by analyzing the front and backup PUF separately. No breakthrough was detected in the backup PUF. In every sample a pre-spike of dioxin and furan surrogates was added to the front PUF. All recoveries of spiked surrogate standards were excellent, which also indicates that breakthrough was not a problem.

In this study and in the studies by Marklund et al (1987) and Hagenmaier et al (1992), the emissions samples were collected differently. Both Marklund and Hagenmaier collected the exhaust using a Grimmer's full-stream collection method where no dilution tunnel was used. The hot diesel exhaust was collected directly into the sampler by cryogenically cooling the exhaust. In this study, samples from a dilution tunnel were collected by a high volume sampler equipped with a filter and PUF. In a dilution tunnel the exhaust is cooled with filtered ambient air. To determine whether tunnel losses were a problem, particles which had adhered to the side of the tunnel were collected and analyzed.

The concentrations of PCDDs and PCDFs in tunnel scrapings taken at two locations in the tunnel and at two locations in the exhaust line that connected the engine to the main tunnel are presented in Table 60.

Two manifold samples were taken. DM-1 was taken in an area after the exhaust manifold and DM-2 was taken just before the juncture to the main tunnel. Samples DTP-1A and DTP-1B were taken in the main tunnel near the secondary dilution probe. DTP-2 was taken at a site located after the high volume sampler and just before the bag filters. The tunnel particulate matter showed high concentration of PCDDs and PCDFs. In the main tunnel I-TEQ concentrations were 125 pg/g, a concentration significantly higher than in the high volume samples. The second tunnel particulate matter was collected downstream of the first sample and it had a concentration of 42 pg TEQ/g. Higher concentrations of PCDDs and PCDFs were found in particulate matter taken from the walls of the tunnel than on particulate matter collected from the samples. Further research is needed to characterize how the particulates coated on the inside wall of the dilution tunnel affect the sampling results. Also, a dioxin concentration gradient through the tunnel is needed. To estimate the tunnel losses per run the number of FTP cycles run, types of fuels, and types of engine tested are needed.

Table 60. PCDDs and PCDFs Concentrations in Tunnel, Engine Manifold, and Engine Exhaust Line Particulate Matter.

	Sample ID: DTP-1A Conc. pg[a]/g	Sample ID: DTP-1B Conc. pg/g	Sample ID: DTP-2 Conc. pg/g	Sample ID: DM-1 Conc. pg/g	Sample ID: DM-2 Conc. pg/g
Compound					
2,3,7,8-TCDD	ND	ND	ND	ND	ND
Total TCDD	460	600	190	ND	ND
1,2,3,7,8-PeCDD	33	46	ND	ND	ND
Total PeCDD	1200	1200	250	ND	ND
1,2,3,4,7,8-HxCDD	56	50	12	ND	ND
1,2,3,6,7,8-HxCDD	110	100	24	ND	ND
1,2,3,7,8,9-HxCDD	42	54	19	ND	ND
Total HxCDD	1800	1500	330	ND	ND
1,2,3,4,6,7,8-HpCDD	400	550	130	4	ND
Total HpCDD	750	1100	260	4	ND
OCDD	940	2500	670	61	120
2,3,7,8-TCDF	11	24	20	ND	ND
Total TCDF	450	520	320	ND	ND
1,2,3,7,8-PeCDF	51	55	22	ND	ND
2,3,4,7,8-PeCDF	80	83	34	ND	ND
Total PeCDF	880	730	280	ND	ND
1,2,3,4,7,8-HxCDF	94	96	30	ND	ND
1,2,3,6,7,8-HxCDF	110	110	29	ND	ND
2,3,4,6,7,8-HxCDF	78	96	74	14	29
1,2,3,7,8,9-HxCDF	17	20	ND	ND	ND
Total HxCDF	1200	680	260	14	29
1,2,3,4,6,7,8-HpCDF	210	310	98	ND	ND
1,2,3,4,7,8,9-HpCDF	10	ND	ND	ND	ND
Total HpCDF	280	330	98	ND	ND
OCDF	60	180	61	ND	ND

a. picogram

Total dioxin and furans in this study are about an order of magnitude lower than in Hagenmaier's study but TEQ levels were two orders of magnitude lower. Part of the reason is that none of the 2,3,7,8 TCDD, 1,2,3,7,8 PeCDD, and 2,3,4,7,8 PCDF were detected in any of the samples. If these compounds were present at levels below the detection limits, the TEQs would certainly increase. Even in the tunnel scraping, 2,3,7,8 TCDD was not detected although 1,2,3,7,8 PeCDD and 2,3,4,7,8 PCDF were detected. This certainly adds to the possibility that the latter two compounds were present in the exhaust at levels below the detectable limit of the method.

Another factor that may contribute to lower emissions was that the engine was brand new, and the system was in peak operating condition. The oil was changed after each week of testing. Chlorine levels in the pre-1993 fuel were less than 10 mg/kg and in the reformulated blend were 56 mg/kg. The lower chlorine pre-1993 fuel had higher levels of PCDDs and PCDFs in the diesel engine exhaust. Oil used for the test had chlorine concentrations of 230 mg/kg which is relatively high for lubricating oil and would contribute to increased PCDDs and PCDFs emission levels.

Conclusions: Achieved detection limits for the samples collected were between 6 and 10 times lower than the target detection limits of 100 pg I-TEQ/l. Evaluation of the emission data suggest PCDDs and PCDFs levels in the diesel exhaust were at very low levels. TEQ profiles were incomplete due to the low level detected. An estimated 10 times more sample would be needed to obtain a complete TEQ profile.

Analysis of the particulate matter removed from the interior surface of the dilution tunnel indicates there may be losses to the particulates adhering to the wall. Using a crude mass balance that accounts for these losses, levels in the exhaust are lower than in other studies. Analysis of particulate matter coating the inner wall of the dilution tunnel needs to be further investigated to determine the effects of dioxin emission sampling.

3.10 Bioassay Analyses

Diesel exhaust is composed of a complex mixture of compounds present in the particle as well as the vapor phase. These compounds include known and unknown genotoxic compounds. Important classes of compounds such as the polycyclic aromatic hydrocarbons (PAHs) can be quantitated in these complex mixtures as detailed in this report. There are however, as yet unidentified compounds in these mixtures that are important in human exposure to toxic compounds. Therefore, a supplemental approach or approaches to help identify and help screen for toxic compounds present in the complex mixture of diesel exhaust would be an important assessment tool.

One approach for examining compound classes and to identify potentially toxic compounds, is to use bioassay in conjunction with chemical analyses. The approach, termed "bioassay-directed chemical analyses" has been used in the chemical analyses of diesel particulate as well as airborne particulate matter (Schuetzle, et al., 1986; Arey et al, 1992). In a typical experiment, particulate matter is extracted using organic solvents, concentrated by evaporation, and the concentrate is fractionated by using HPLC. The HPLC fractions are then individually tested in a bioassay. The bioassay generally used is the Salmonella/microsome test of Ames et al. (1975) or more recently, its modifications that requires less material (Kado et al., 1983, 1986). Mutagenic activity of each fraction is then obtained resulting in a profile of relative mutagenic activities, or "mutagrams". These mutagrams are profiles of different compound classes present in the complex mixture of the particulate extract. The mutagrams can be used to compare mutagenic activities and compound profiles from different sources, locations, or collection variables. In the current study, the mutagrams were developed to examine the mutagen profiles from different diesel fuel particulate phase and the vapor-phase.

Prior to developing the profiles, the mutagenic potency of the sample, referred to as specific mutagenic activity, was determined. The specific mutagenic activity is reported as the mutagenic activity (revertants) per µg of particulate matter or per µl of extract. Although the potency value is important to consider in assessing toxicity, an index of exposure requires emission values. Emissions for genotoxic compounds will be reported as the level of mutagenic activity emitted per unit of work of the engine, or per brake horsepower-hr.

Particulate Matter Genotoxic or mutagenic activity is reported as "revertants per plate". Revertants are the number of mutated bacterial cells present per incubation plate, and is representative of the level of DNA damage to the target cells. Dose-response relationships are established by measuring the number of cells that mutate (number of revertants) over a series of doses. The dose-response relationships of the particle extracts collected from the combustion of each fuel are presented in Figures 26 and 27 for the all fuels and blanks when using tester strain TA98 with (+S9) and without (-S9) metabolic enzymes added, respectively. These enzymes are routinely used in this type of bioassay and are known to metabolically activate toxic compounds to their reactive forms where they can damage target molecules such as DNA.

The hi-volume particulate samples were extracted using two methods: 1) Soxhlet extraction (samples designated by the prefix 96) and 2) sonication (samples designated H1, H3, numbers). The blanks were collected for equivalent times as the samples from the tunnel, except that the engine was not operating. The samples were extracted by the two methods because one set of extracts (the Soxhlet extracted samples) was used for HPLC fractionation and one set was used exclusively for mutagenicity testing. Both methods are considered comparable for purposes of bioassay analyses.

The most potent extracts (revertants per mg of particle extracted - or particle equivalents) were the Pre-1993 fuel particulate matter, when tested with and without metabolic enzymes added. The potencies of the reformulated blend as well as the low aromatic fuel were similar in potency with or without the addition of metabolic enzymes, and were lower than the Pre-1993 fuel particulate matter. The overall potency of the particulate emission extracts was higher without metabolic enzymes added, especially for Soxhlet-extracted samples. The blanks had very little or no mutagenic activity. Relative genotoxic potencies of the extracts are summarized in Table 61. The specific mutagenic activities and the r^2 values for the slopes are provided. The slopes for the Pre-1993 fuel particulate matter extracts were the highest relative to the other two fuels. The comparisons of potencies between the fuels should be made with samples extracted in an identical manner (for example, Soxhlet-extracted samples for all three fuels). For the Soxhlet extracted samples, the Pre-1993 fuel had the highest specific mutagenic activity (+ or -S9) relative to the Low Aromatic and Reformulated fuels. For the samples extracted using sonication, the Pre-1993 fuel had the greatest activity, followed by the Low Aromatic fuel and by the Reformulated fuel particle sample. The specific activities are also presented in Table 61 as a percent of the activity of the Pre-1993 fuel within each extraction procedure used.

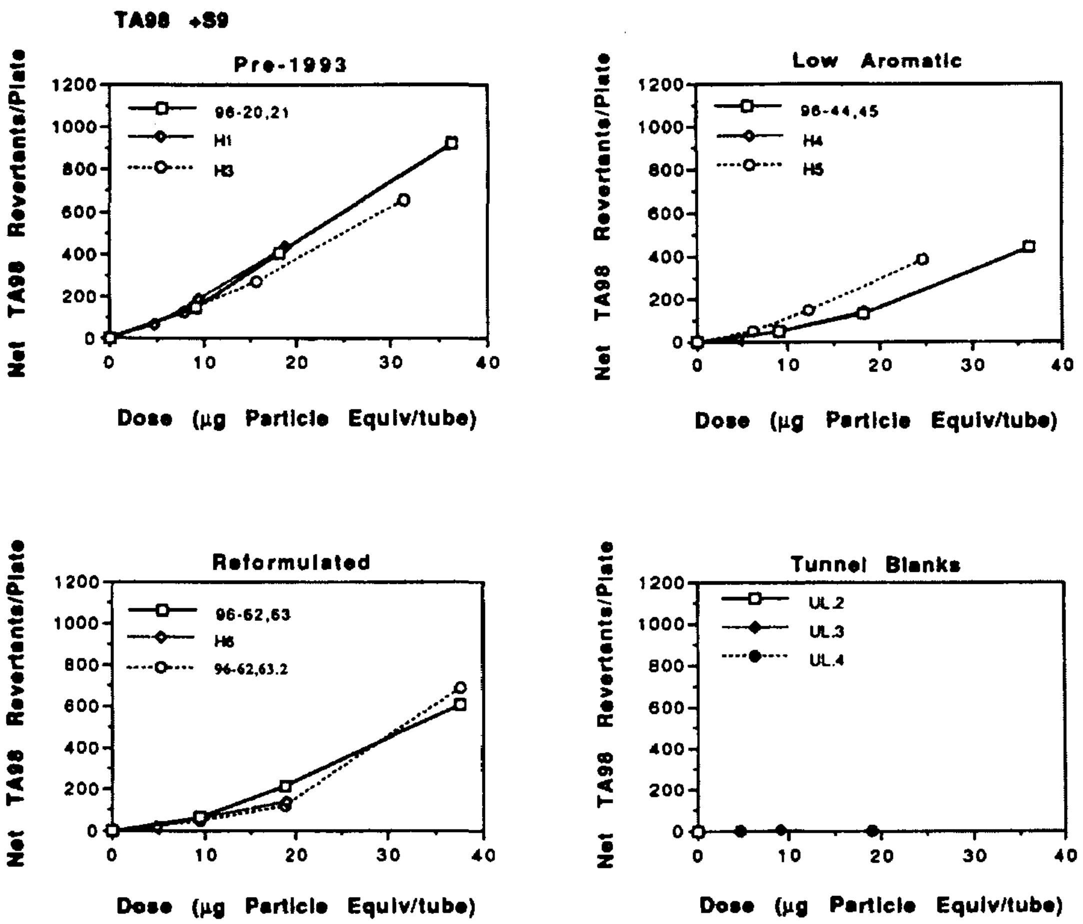


Figure 26. Dose-response curves for pre-1993, low aromatic, and reformulated fuel particulate matter. Samples were tested using tester strain TA98 with metabolic enzymes added (+S9)

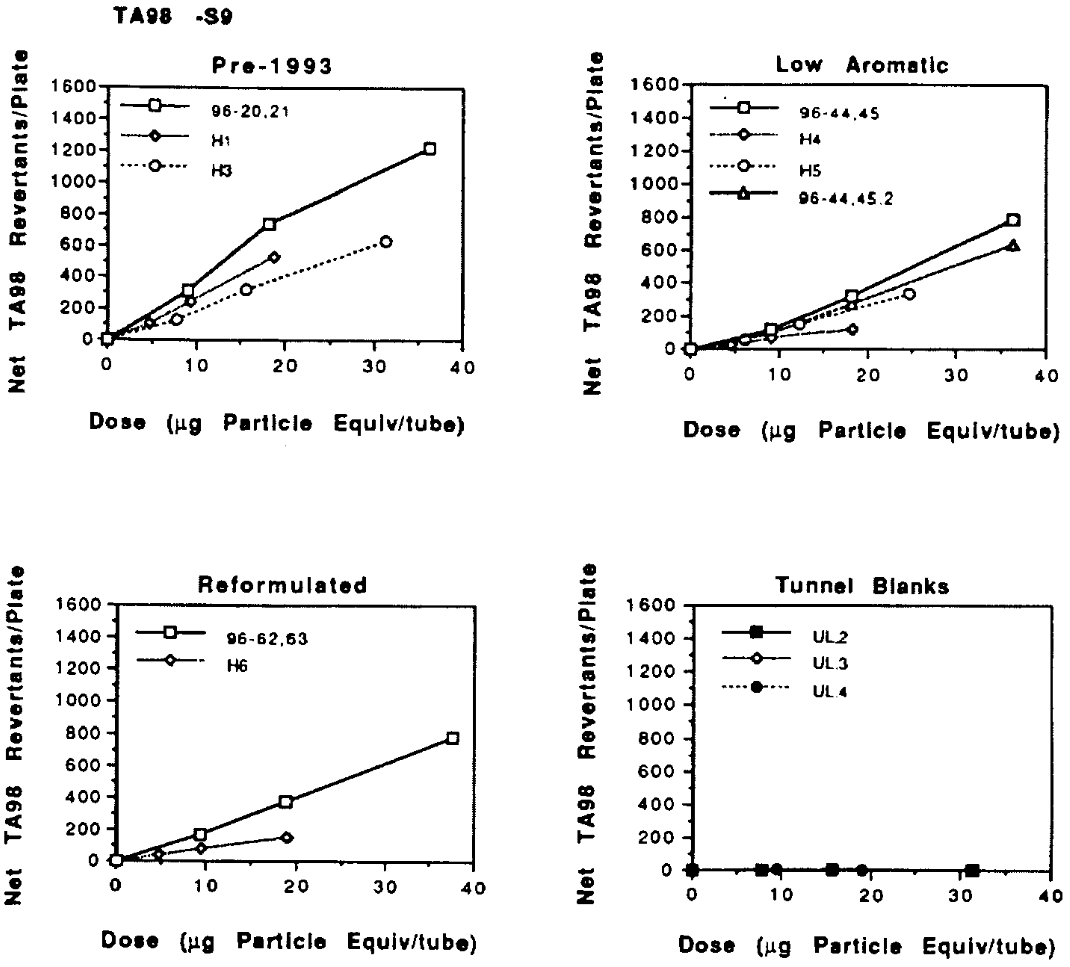


Figure 27. Dose-response curves for pre-1993, low aromatic, and reformulated fuel particulate matter. Samples were tested using tester strain TA98 without metabolic enzymes added (-S9).

Table 61. Specific Mutagenic Activities and Emissions of the Particulate Matter Collected from the High Volume Sampler.

Fuel Type	Test Cycle ID	Lab Sample ID	Sp. Act Rev/µg (+)	Sp. Act r 2	Sp. Act Percent of Pre-1993	Emission TA98 (+ S9) **Rev/hp-hr (x10 ^{6})**	Sp. Act Rev/µg (-)	Sp. Act r2	Sp. Act Percent of Pre-1993	Emission TA98 (- S9) **Rev/hp-hr (x10 ^{6})**
Pre-1993	338H8,H9	96-20,96-21	28.332	0.959	100	**5.69**	32.729	0.964	100	**6.58**
Pre-1993	337H1,H2	H1FV	24.498	0.968	100	**5.19**	29.006	0.988	100	**6.15**
Pre-1993	339H6,H7	H3FV	21.738	0.989		**4.85**	20.797	0.99		**4.64**
Low Aro	346H3, H4	96-44,96-45	12.886	0.927	45[d]	**2.28**	20.731	0.96	63[d]	**3.67**
Low Aro	344H1, H2	H4FV	8.445	0.969	54 emission	**1.67**	6.538	0.985	41[e]	**1.29**
Low Aro	344H3, H4	H5FV	16.659	0.965		**3.11**	14.079	0.984		**2.63**
Reform	351H1, H2	96-62, 96-63	18.81	0.918	66[d]	**3.23**	25.6	0.911	78[d]	**4.4**
Reform	348H1,H2	H6FV	7.74	0.974	33[e]	**1.33**	7.955	0.992	32[e]	**1.37**

[a] Particulate mass collected per filter from 2 consecutive hot start cycles.

[b] Specific mutagenic activity is the number of revertants per microgram of particulate matter. Sample added for the assay is actually an extract of the particulate matter. Therefore, the mass is an equivalent mass.

[c] + or - S9 indicates that the test included or did not include adding metabolic enzymes.

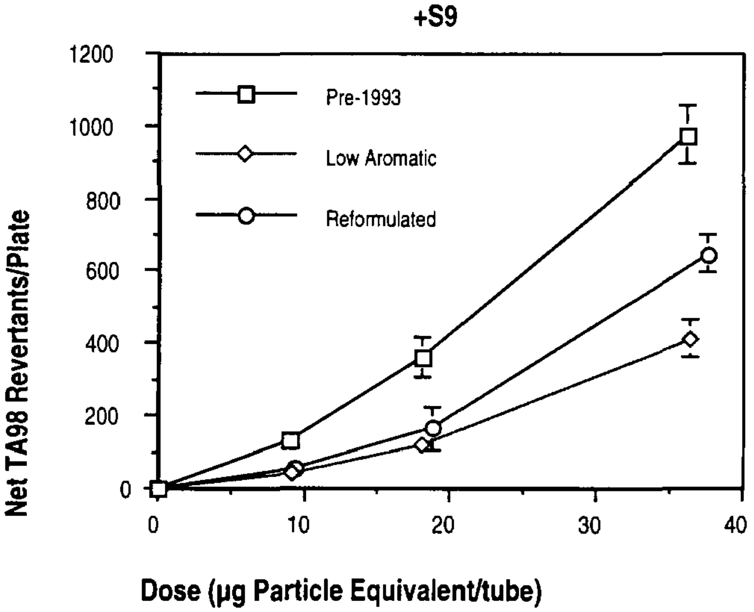
d Compared to the Pre-1993 sample extracted by Soxhlet.

e Compared to the Pre-1993 sample extracted by sonication. Average when there are two values.

Although genotoxic potency is an important component in the evaluation of potential toxicity, an index for the levels of genotoxic compounds being emitted is needed to help evaluate potential exposure to diesel emissions. As mentioned, the emissions are a complex mixture of compounds, and only a few toxic genotoxic compounds can realistically be measured. Mutagenic activity could therefore serve as an index for some of these complex emissions. An emission value that incorporates both the potency and yield of these compounds would serve to meet this need. The emissions based on the bioassay results can be presented as mutagenic activity emitted per mile or per brake horsepower hour (Bhp-hr.), or more specifically as revertants / Bhp-hr. These emission values were calculated by using the specific mutagenic activity and emissions of particulate matter for each fuel.

The emissions for genotoxic activity for each fuel type are summarized in Table 61. The pre-1993 fuel particulate matter had the highest emissions both with and without the addition of metabolic enzymes. The low aromatic and reformulated fuel particulate matter had similar emissions based on the average values from all extracts within a fuel type.

To test the reproducibility of the bioassay for these complex samples, a specific series of samples from each fuel were tested. The samples used for this analyses were extracted by Soxhlet extraction using DCM (Sample ID prefixes "96" for all fuel types) and were tested for dose-response relationships. The results are presented in Figure 28. The experiments were conducted on separate days. The dose-response curves for these samples using the microsuspension bioassay were reproducible. Also, within these samples, the differences between the pre-1993 fuel and the low aromatic and reformulated fuels can be seen. To further examine the variability between assays, the specific mutagenic activity derived from these specific samples (rev/μg particulate matter) and the particle emissions (g/Bhp-hr.) were used calculate the average mutagen emissions and 95 percent confidence interval for each fuel (Figure 29). The emissions for the pre-1993 fuel for these samples was higher than emissions from the other two fuels, with and without S9. Although the Reformulated fuel emissions appear higher than the low aromatic fuel emissions, the 95% confidence intervals overlap, especially without S9 added. To investigate the chemical characteristics of the mutagenic compounds, hi-volume samples were fractionated using HPLC.



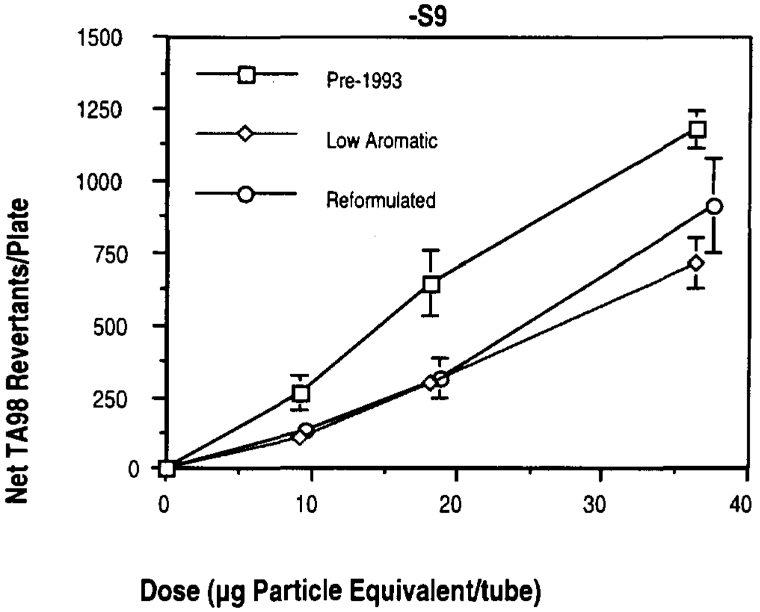


Figure 28. Dose-response curves to check for bioassay reproducibility of testing particulate matter from the combustion of Pre-1993, low aromatic, and reformulated fuel formulations. Samples were extracted using Soxhlet and the extracts tested using tester strain TA98 with metabolic enzymes added (+S9) and no metabolic enzymes added (-S9). Results represent data from two separate experiments with average (± SD) responses.

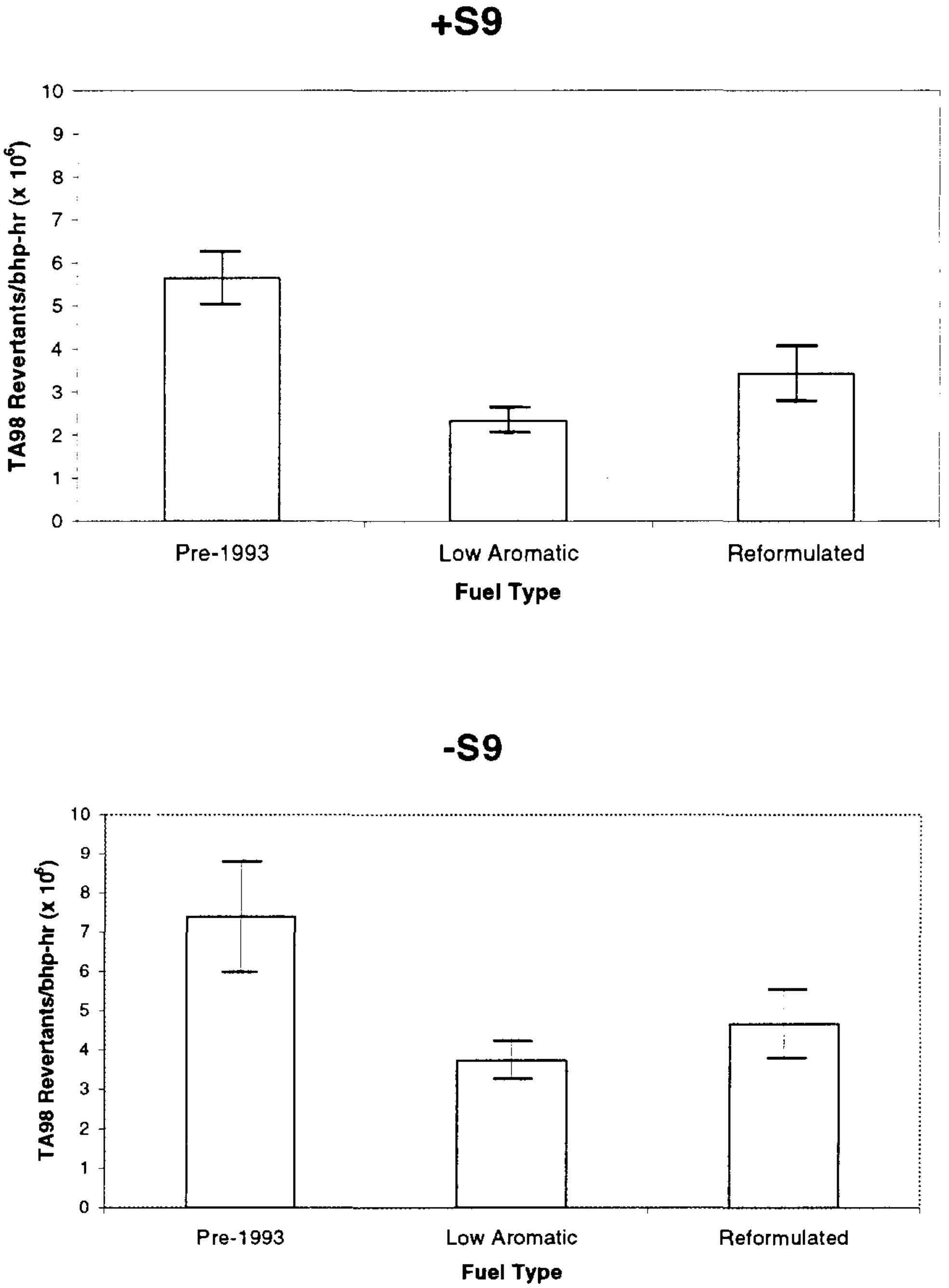


Figure 29. Emissions of mutagenic compounds for the three fuel types from Hi volume samples represented in Figure 28. The rectangles represent the average emissions (and 95 % confidence intervals) determined from two separate experiments from samples extracted with Soxhlet (Sample No. with "96" prefix). Each sample represents two consecutive cycles collected. Samples were tested using tester strain TA98 with (+S9) and without (-S9) the addition of metabolic enzymes.

HPLC Fractions and Mutagrams The HPLC fractions from each particulate sample were tested for mutagenic activity using three doses, in duplicate. The objective for testing the fractions is to help to further focus the chemical analyses for the most mutagenic fraction(s) and to develop patterns or mutagrams for each fuel emission. Dose-response curves were developed for each HPLC fraction and the specific genotoxic activities were determined. The relative genotoxic activity of each fraction is presented in a bar graph, or "mutagram", where the relative percent of activity is illustrated. The mutagrams for all the fuel particulate matter are presented in Figures 30 and 31 with (+S9) and without (-S9) metabolic enzymes added, respectively. For the pre-1993 fuel, HPLC Fraction 7 typically had the highest mutagenic activity, accounting for approximately 64% of the total mutagenic activity with S9 added and approximately 50% of the total mutagenic activity without S9 added. Relative to Fraction 7, the next most mutagenic fraction was Fraction 6, which accounted for approximately 12 % and 28% with and without S9, respectively. The rest of the activity is spread over the other fractions, especially Fractions 3,4 and 5. The relative activity in Fraction 6 without S9 is approximately 2 times the activity when S9 is added.

The mutagram for the low aromatic fuel particulate matter also has Fraction 7 with the greatest mutagenic activity. The relative activity with metabolic activation (+S9) was approximately 66% and was approximately 41% without activation (-S9). Unlike the Pre-1993 fuel, the low aromatic fuel has a higher relative mutagenic activity in Fraction 3, especially when S9 was added. Fraction 3 represented approximately 23 % of the total mutagenic activity measured in the fractions. There was virtually no activity in Fraction 3 without S9. Fraction 6 (-S9) contained approximately 38% of the activity. The remainder of the activity was spread out over Fractions 4 and 5 (+S9) and 8 and 9 (-S9).

Fraction 7 is also the most mutagenic fraction from the reformulated blend fuel particulate matter accounting for approximately 73% and 57% of the activity, with and without S9, respectively. Fraction 4 contained approximately 12 % of the total activity with metabolic activation. Without metabolic enzymes added, Fraction 6 is the second highest peak with approximately 21% of the total activity. The mutagrams for the particulate matter extracts appear to be unique for each fuel, but there are similarities such as for the Pre-1993 and Reformulated fuel mutagrams.

The mutagrams representing actual plate counts for near identical high dose of extract tested are presented in Figures 32 and 33, with and without S9 added, respectively. For the mutagenic activity with S9 added, and for all fuel types, there appear to be distinct differences in Fractions 3 through 7. In Fraction 3, the low aromatic fuel appears to have higher activity compared to the other fuels, while in Fraction 4 (the nitro-PAH fraction), the Low Aromatic appears to have the lowest activity and the

Pre-1993 fuel has the highest. In Fraction 6 and 7, the Pre-1993 fuel has the highest activity. Fraction 7 is the dominant mutagenic fraction. For the mutagenic activity without S9, the Reformulated fuel has decreased numbers of revertants in Fraction 6 compared to the other two fuels. However, all three fuels appear similar in Fraction 4, 5, and 7. Fraction 7 is the dominant mutagenic fraction, followed by Fraction 6.

The mutagrams representing actual plate counts for near identical high dose of extract tested is presented in Figures 32 and 33, with and without S9 added, respectively. For the mutagenic activity with S9 added, and for all fuel types, there appear to be distinct differences in fractions 3 through 7. In fraction 3, the low aromatic fuel appears to have higher activity compared to the other fuels, while in fraction 4 (the nitro-PAH fraction), the low aromatic appears to have the lowest activity and the Pre-1993 fuel has the highest. In fraction 6 and 7, the Pre-1993 fuel has the highest activity. Fraction 7 is the dominant mutagenic fraction. For the mutagenic activity without S9, the reformulated fuel has decreased numbers of revertants in Fraction 6 compared to the other two fuels. However, all three fuels appear similar in fraction 4, 5, and 7. Fraction 7 is the dominant mutagenic fraction, followed by fraction 6.

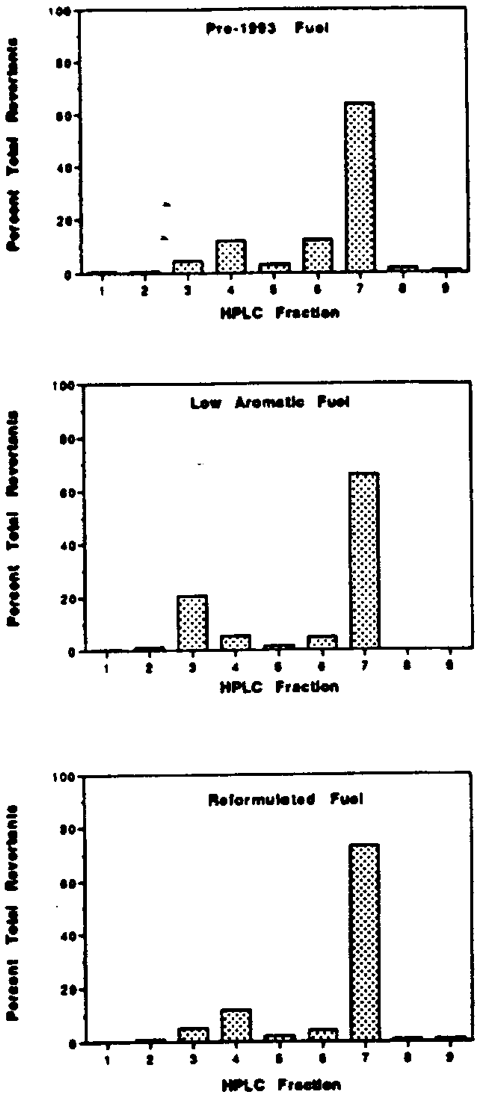


Figure 30. Percent total mutagenic activity of HPLC fractions ("mutagram") of particle extract collected from combustion emission of Pre-1993, low aromatic, and reformulated fuel. Tester strain TA98 used with metabolic enzymes added (+S9). HPLC fractions are presented in order of increasing polarity. Fraction 1 represents the most non-polar fraction and Fraction 9 represents the most polar fraction. The PAHs are typically present in fractions 2 and 3, the nitro-PAHs in fraction 4, and the dinitro-PAHs in fraction 5 (Arey, and colleagues, 1994).

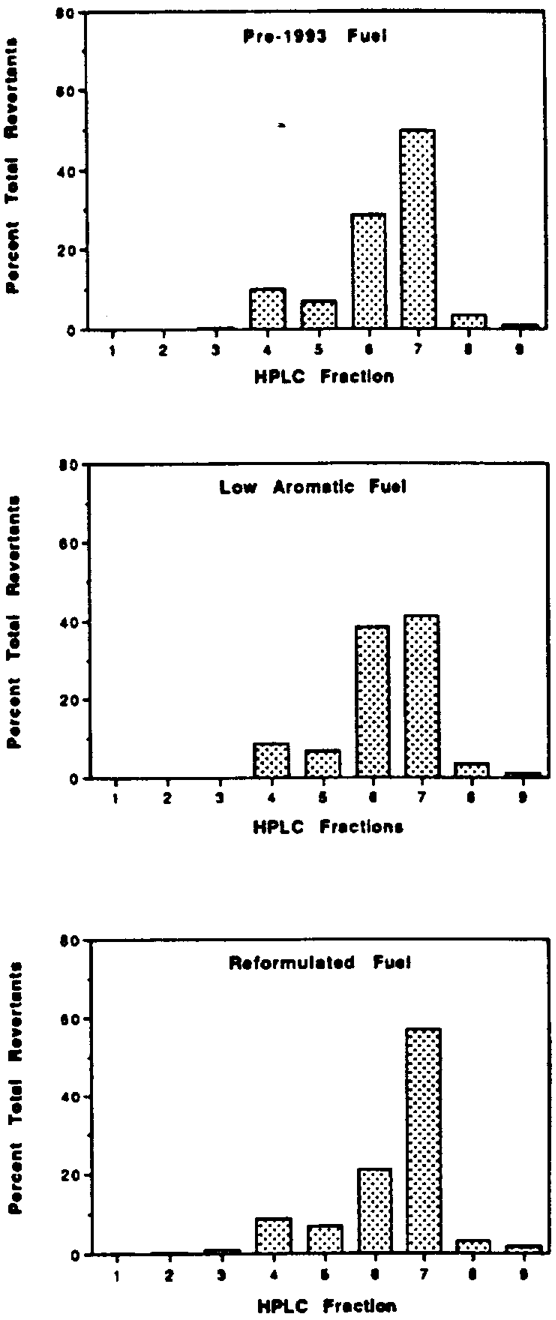


Figure 31. Percent Total Mutagenic activity of HPLC fractions ("mutagram") of particle extract collected from combustion emission of Pre-1993, low aromatic, and reformulated fuel. Tester Strain TA98 used without the addition metabolic enzymes (-S9). HPLC fractions are presented in order of increasing polarity. Fraction 1 represents the most non-polar fraction and Fraction 9 represents the most polar fraction.

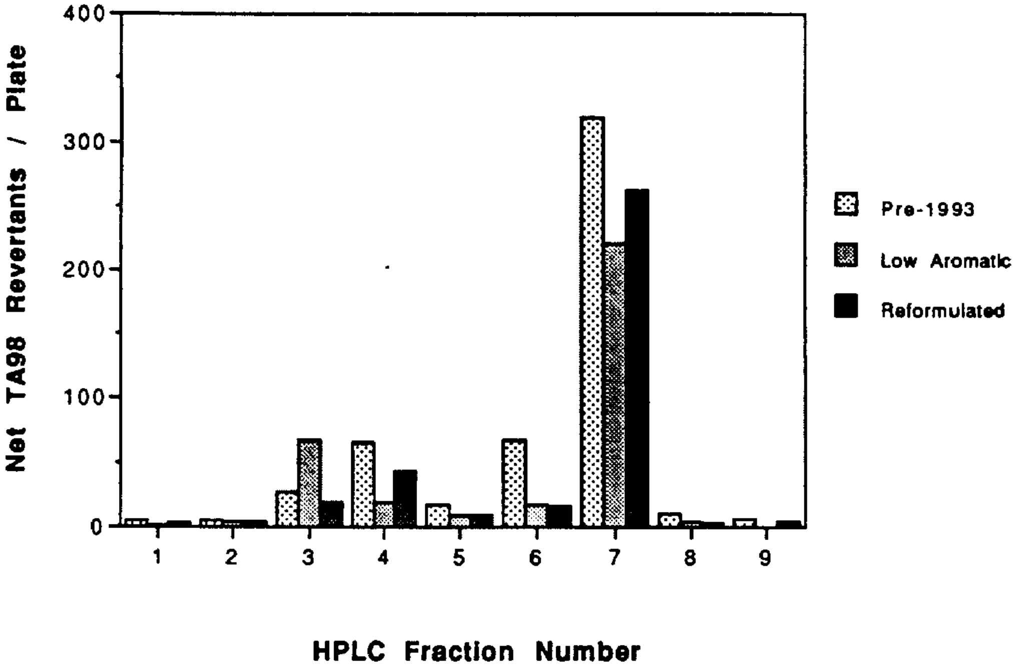


Figure 32. Mutagenic profile (mutagram) of particulate matter from all fuels. The bars represent the mutagenic activity of the fraction (net revertants) at the highest dose of the particle equivalent tested (nominally 36 µg equivalent of particulate matter per tube). TA98 was used with the addition of metabolic enzymes (+S9).

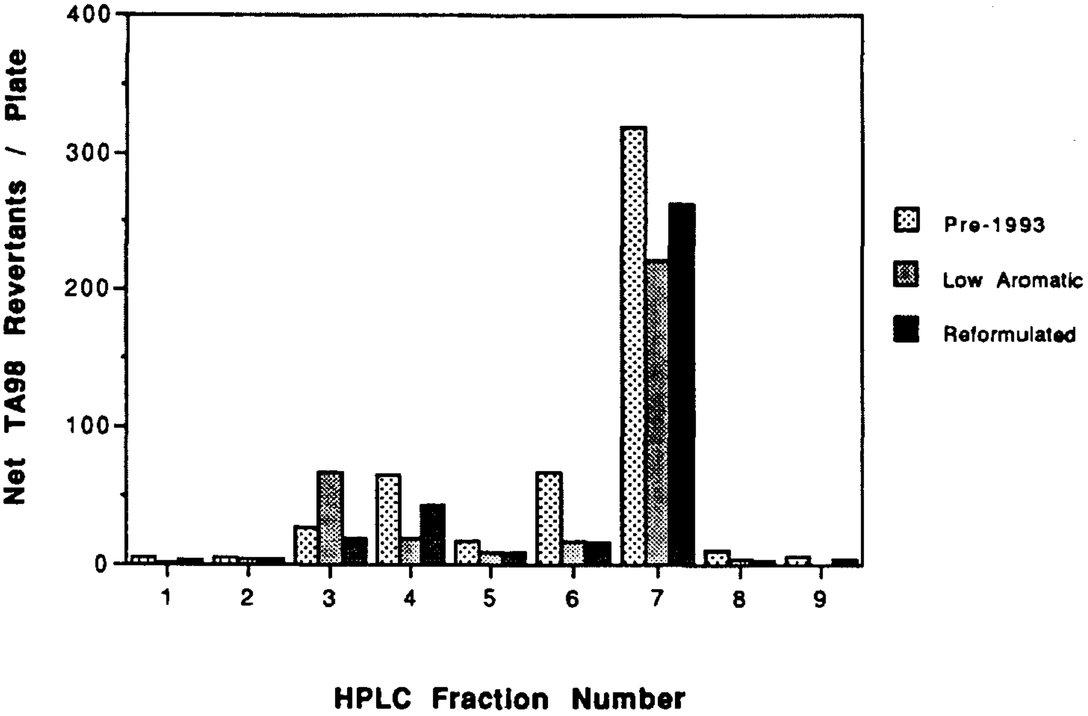


Figure 33. Mutagenic profile (mutagram) of particulate matter from all fuels. The bars represent the mutagenic activity of the fraction (net revertants) at the highest dose of the particle equivalent tested (nominally 36 µg equivalent of particulate matter per tube). TA98 was used without the addition of metabolic enzymes (-S9).

Vapor-Phase Samples: Vapor-phase samples were collected onto PUF and XAD adsorbents, extracted by supercritical fluid extraction, and tested in the bioassay. Dose response curves were developed for each fuel type in duplicate or triplicate using tester strains TA98 and TA100. Each PUF sample represents two consecutive hot cycles and the dose-response curves for each fuel type are presented in Figures 32 and 33. The dose response relationships for the trip blanks and tunnel blanks are also presented. For the Pre-1993 fuel, the extracts were mutagenic in both TA98 and TA100. In tester strain TA98 however, there is a decrease in the response at the highest dose tested (10 µl). This might indicate that there is some toxicity to the tester strain used. This plateau was not observed for the samples tested with strain TA100. The samples, H1 to H6 are from two consecutive hot start cycles and were extracted and tested independently. The dose response curves for each fuel have slopes that are very reproducible.

The slope of the linear portion dose-response curve was used to determine the specific mutagenic activity of each sample and are reported as revertants per µl equivalent. These values reflect the potency of the samples. The specific mutagenic activities are presented in Table 62. An increase in the slope of the dose-response curve results in an increase in an increase the potency of the extract. The highest specific mutagenic activity in both tester strains were observed in emissions from the Pre-1993 fuel. For all fuels, tester strain TA100 is more sensitive than TA98 for the mutagens present in these extracts. For example, specific activities for the Pre-1993 PUF samples increased approximately 3-fold using TA98 as compared to using TA100.

Total emission of mutagenic activity for the vapor-phase samples were determined and are reported as revertant per brake-horsepower hour (rev /Bhp-hr.) in Table 62. The highest relative mutagenic emissions for TA98 were found in the Pre-1993 fuel, followed by the Reformulated fuel and the Low Aromatic fuel which like the particulate matter. Tester strain TA100 had higher emission values compared to TA98, and the relative order of emissions was similar to that observed for TA98 for the PUF samples. However, for TA100, there is very little difference in the emissions for the XAD samples across all fuels. For the Low Aromatic and Reformulated fuels, the XAD sample is higher relative to the matching PUF sample. The XAD samples appeared to have greater activity in TA100 compared to TA98. The emission value from a PUF sample and its matching XAD sample are also presented as the sum of the two values. The XAD was placed in series behind the PUF adsorbent. The combined values represent the mutagenic vapor-phase compounds adsorbed and extracted from the adsorbents. Based on the sum of emission values determined using TA100, the Pre-1993 fuel has the highest emissions, and the Low Aromatic and Reformulated fuels are similar in emissions.

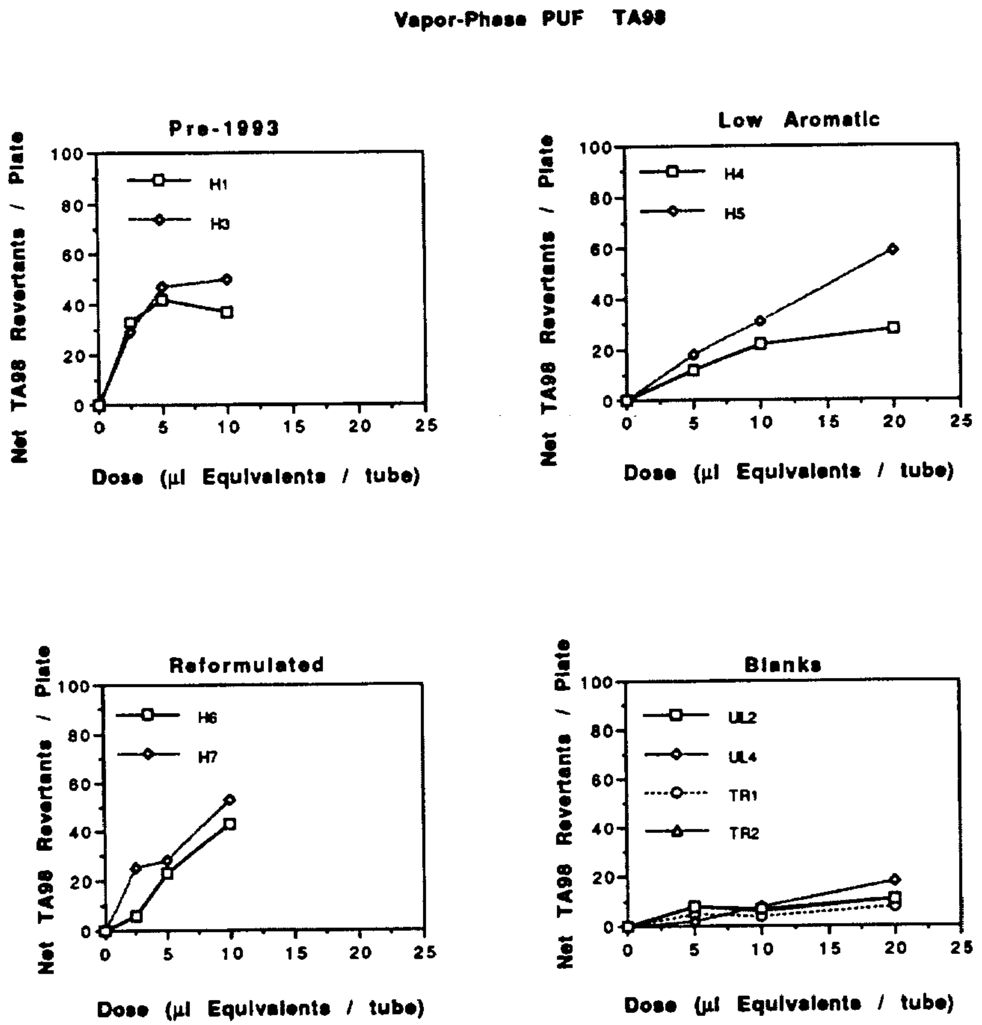


Figure 34. Dose-response curves for fuel types and blanks in the vapor-phase PUF samples using tester strain TA98 with the addition of metabolic enzymes. Each extract that was tested represents two transient engine cycles run consecutively and each data point represents the values obtained from duplicate determinations. UL= tunnel blanks; TR = trip blanks (blanks taken on the trip to the dynamometer facility and not used for sampling.

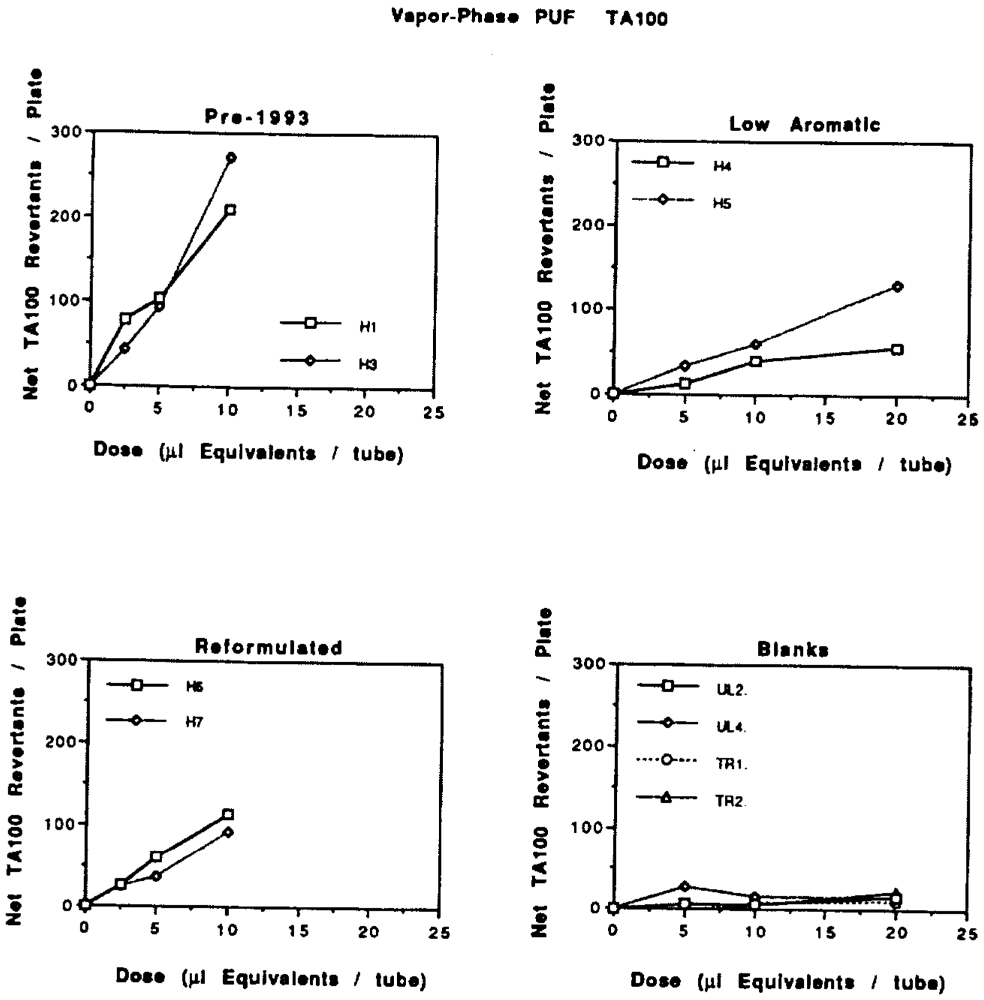


Figure 35. Dose-response curves for fuel types and blanks in the vapor-phase PUF samples using tester strain TA98 without the addition of metabolic enzymes. Each extract that was tested represents two transient engine cycles run consecutively and each data point represents the values obtained from duplicate determinations.

Table 62. Specific Mutagenic Activity and Emissions of Mutagenicity in PUF and XAD samples.

Fuel Type	Test Cycle ID	Lab Sample ID	Sample Type a	Sp. Act TA98 Rev/µl	Sp.Act r 2	Emissions (+ S9) TA98 Rev/hp-hr (x10^{5})	Sp. Act TA100 Rev/µl	Sp.Act r2	Emissions (+ S9) TA100 Rev/hp-hr (x10^{5})
Pre-1993	337H1,H2	H1PV	PUF	8.50	0.831	0.96	20.14	0.964	2.30
Pre-1993	339H6,H7	H3PV	PUF	9.50	0.925	1.16	27.57	0.969	3.42
Pre-1993	337H1,H2	H1XV	XAD	0.70	0.564	0.26	4.79	0.755	2.80
		Total b	Vapor	Phase	H1	1.22			5.10
Low Aromatic	344H1, H2	H4PV	PUF	1.22	0.757	0.19	2.88	0.792	0.44
Low Aromatic	344H3, H4	H5PV	PUF	2.92	0.985	0.37	6.45	0.996	0.81
Low Aromatic	344H1, H2	H4XV	XAD	0.45	0.893	0.19	6.21	0.869	2.70
		Total b	Vapor	Phase	H4	0.38			3.14
Reformulated	348H1,H2	H6PV	PUF	4.47	0.963	0.73	11.58	0.967	1.89
Reformulated	351H7, 352H7	H7PV	PUF	4.91	0.891	0.68	9.19	0.907	1.27
Reformulated	351H7, 352H7	H7XV	XAD	2.76	0.971	0.70	8.55	0.975	2.24
		Total b	Vapor	Phase	H7	1.38			3.51

a All samples represent 2 consecutive hot start cycles

.b Total of PUF and XAD sample (sample no. H1, H4, or H7).

Mutagenic profiles - Mutagrams of Vapor Phase Samples. The extracts from PUF and XAD samples were fractionated using SPE as described in the Methods section. The five fractions from C18 were individually tested in the bioassay. Fraction 1 was subfractionated using a silica SPE cartridge with a series of solvents that included hexane, DCM, DCM:methanol, and finally methanol. These subfractions were also tested in bioassay. The results for the C18 fractions and silica subfractions tested in TA98 for the PUF samples are presented in Figures 36 and 37. The results for the C18 fractions and silica subfractions tested in TA100 are presented in Figures 38 and 39. Fraction 1 (from the C18 SPE) was selected for subfractionation since this fraction had higher relative mutagenic activity for the Pre-1993 and Reformulated fuel vapor phase emissions compared with the other C18 fractions. The Low Aromatic fuel emissions had higher relative activities in C18 Fraction 5 (non-polar fraction). The C18 Fraction 1 (methanol) was analyzed for PAHs and the results are presented in the PAH analyses section. Most of the PAHs were present in this fraction. When comparing the mutagrams between the fuel types, each fuel type had distinct distributions of the percent total mutagenic activity. For the Pre-1993 fuel tested in both TA98 and TA100 tester strains, C18 Fraction 1 and silica subfraction 3 from were the dominant peaks of mutagenic activity. Subfraction 3 is a polar fraction. For the Low Aromatic fuel, C18 Fraction 5 silica subfraction 2 were the most active fractions. For the reformulated fuel, C18 Fractions 1 and 5 were the most active, with Fraction 5 having more activity with metabolic enzymes added (+S9). For the Reformulated fuel, silica subfraction 1 was the most active (hexane fraction). These differences in mutagrams are indicative of different classes of compounds representing a range of polarities, or are indicative of different concentrations of compounds present in the various fractions.

Mutagrams were also developed for the XAD samples and are presented in Figures 40 through 43. In Figure 37, the primary mutagenic activity was observed in Fraction 5 for the Pre-1993 and the Reformulated fuels. The mutagram is similar to the trip blank (blank XAD taken on the trip and returned, stored, extracted and tested in an identical manner as the samples). The low aromatic fuel SPE fractions were toxic. The silica SPE subfractions were tested using TA98 with metabolic enzymes added (Figure 40). The emission samples did not follow the same pattern as the mutagram for the blank, where most of the activity was in silica subfraction 3. All the fuels had their own specific mutagram. In the more sensitive of the two tester strains used (TA100), the C18 mutagram followed the TA98 mutagram including the observation that the Low Aromatic fuel was toxic when fractionated. The reformulated fuel fractions in C18 were also toxic to the tester strains. The mutagram for the silica subfractions are illustrated in Figure 42. No two fuel mutagrams were alike for the silica subfractions.

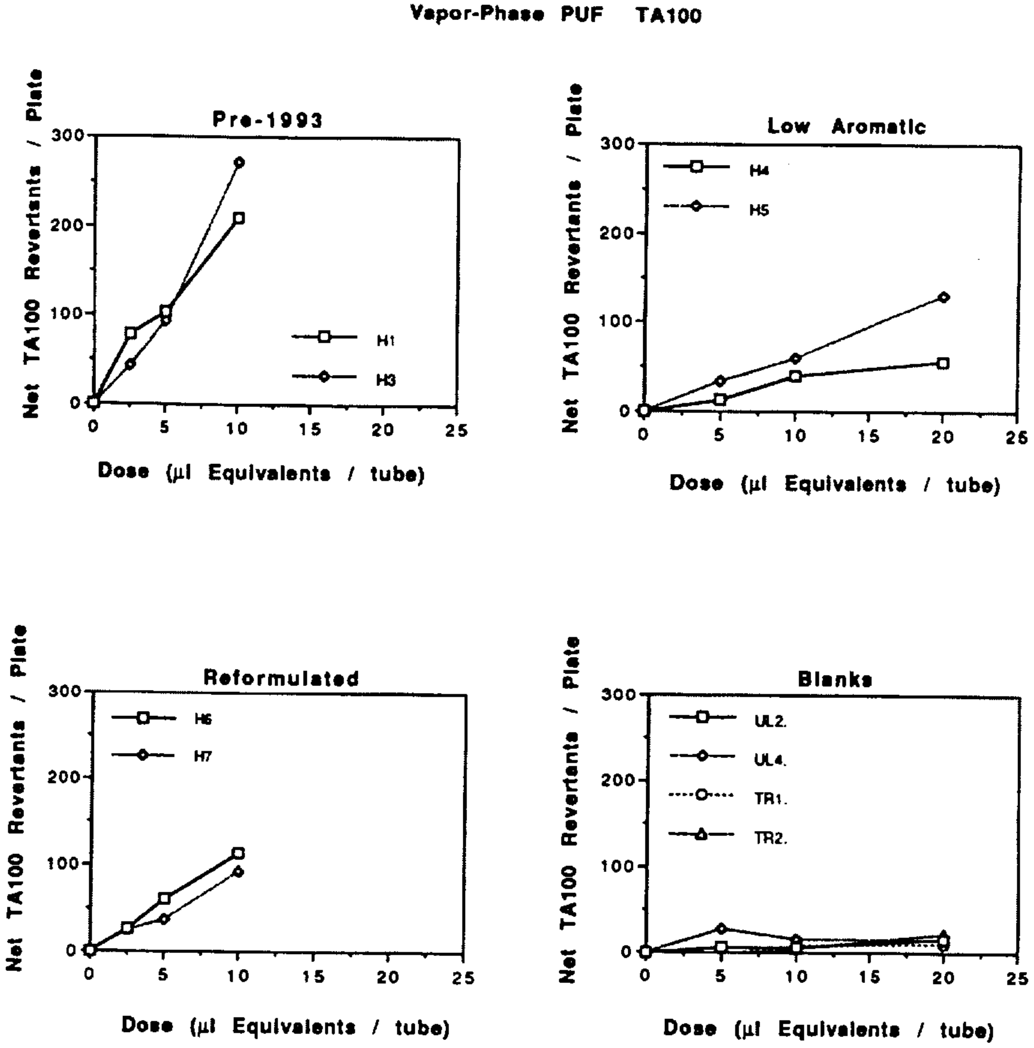


Figure 36. Mutagrams of Pre-1993, low aromatic, and reformulated fuel vapor-phase emissions collected on PUF and fractionated using C18 solid phase extraction (SPE). Fractions tested in tester strain TA98 with metabolic enzymes added.

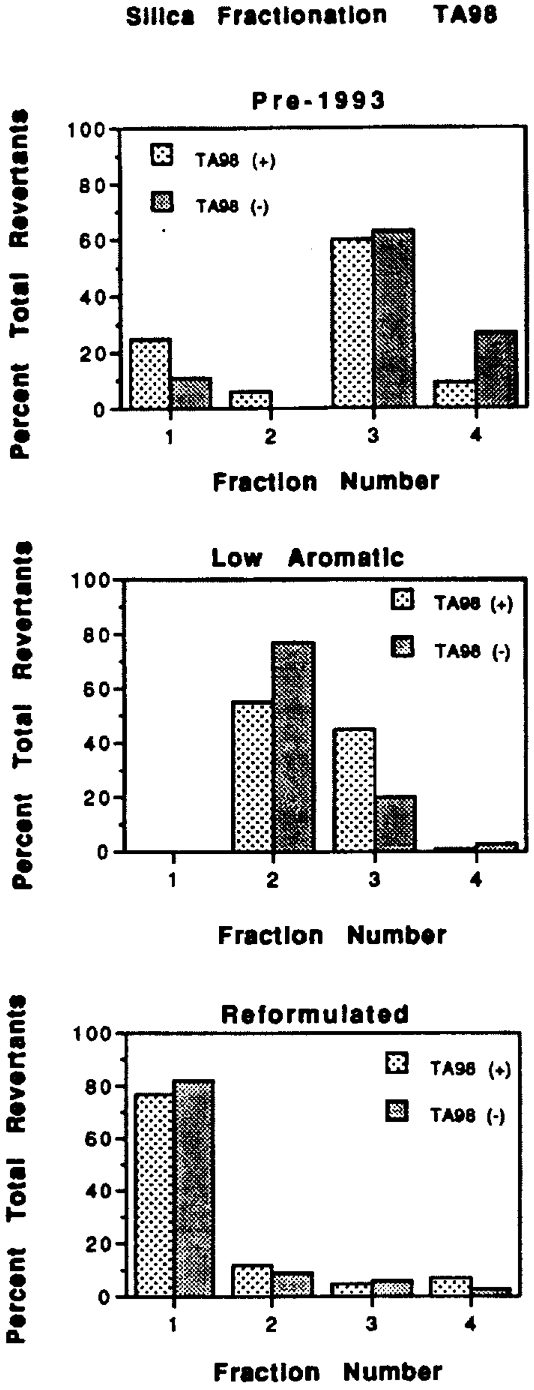


Figure 37. Mutagrams of Pre-1993, low aromatic, and reformulated fuel vapor-phase emissions collected on PUF and fractionated using silica solid phase extraction (SPE). Fractions tested in tester strain TA98 with metabolic enzymes added.

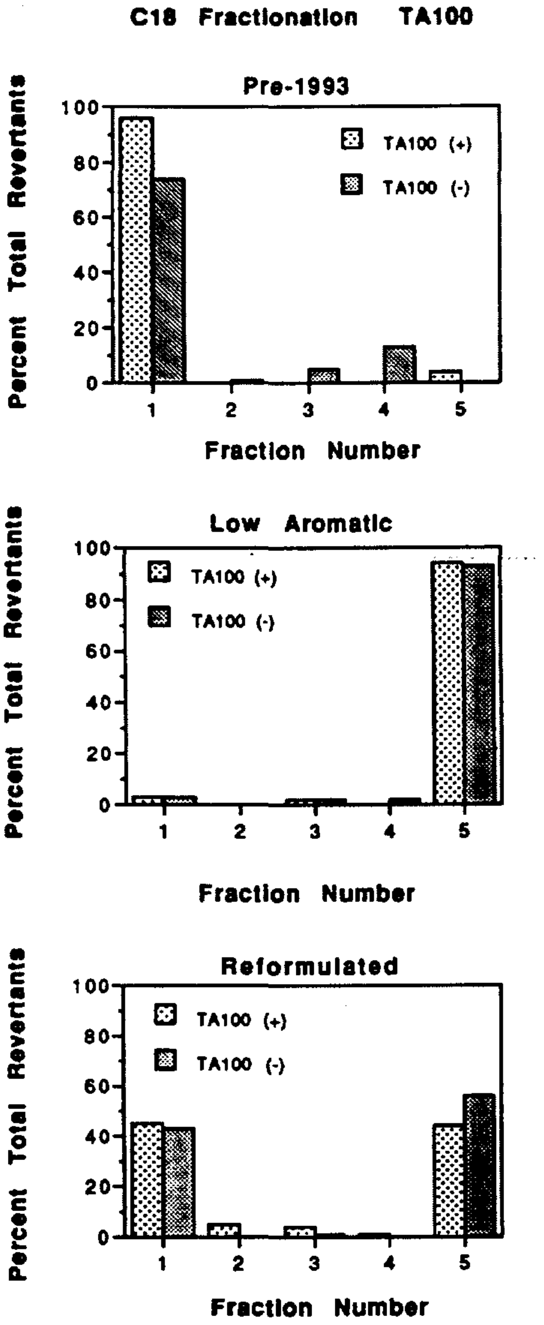


Figure 38. Mutagrams of Pre-1993, low aromatic, and reformulated fuel vapor-phase emissions collected on PUF and fractionated using C18 solid phase extraction (SPE). Fractions tested in tester strain TA100 with metabolic enzymes added.

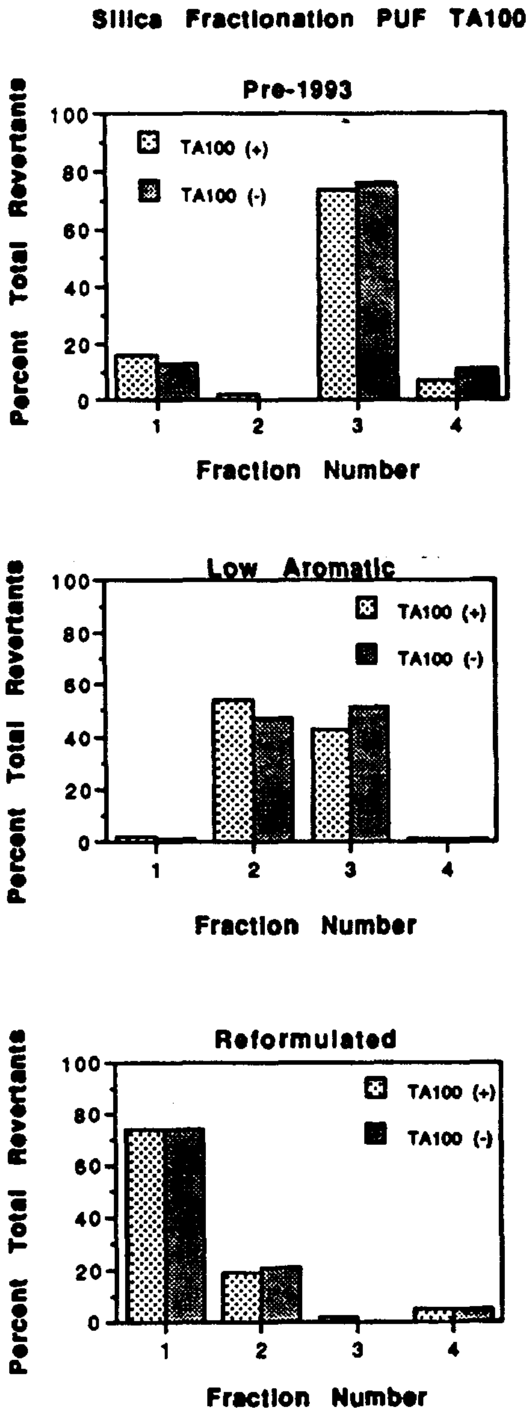


Figure 39. Mutagrams of Pre-1993, low aromatic, and reformulated fuel vapor-phase emissions collected on PUF and fractionated using silica solid phase extraction (SPE). Fractions tested in tester strain TA100 with metabolic enzymes added.

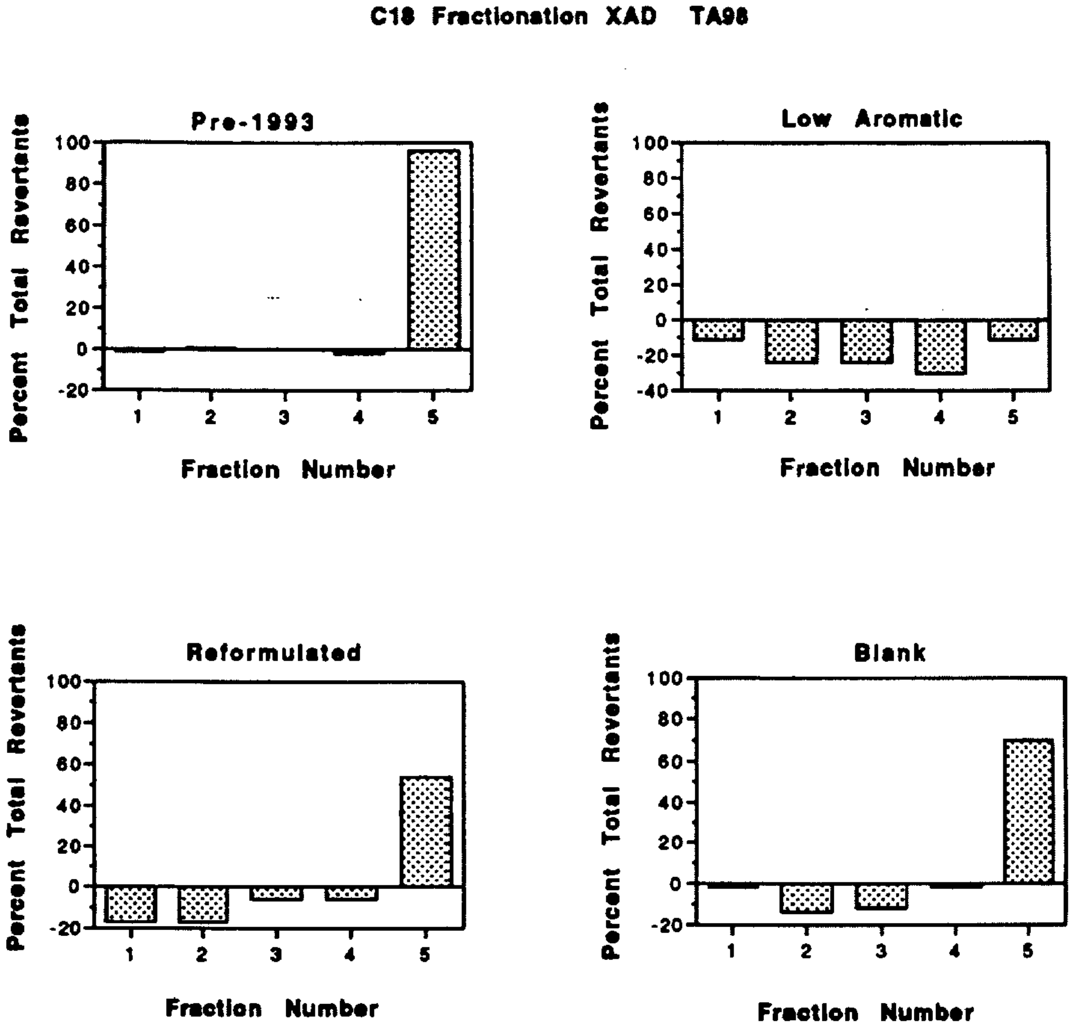


Figure 40. Mutagrams of Pre-1993, low aromatic, and reformulated fuel vapor-phase emissions collected on XAD and fractionated using C18 solid phase extraction (SPE). Fractions tested in tester strain TA98 with metabolic enzymes added.

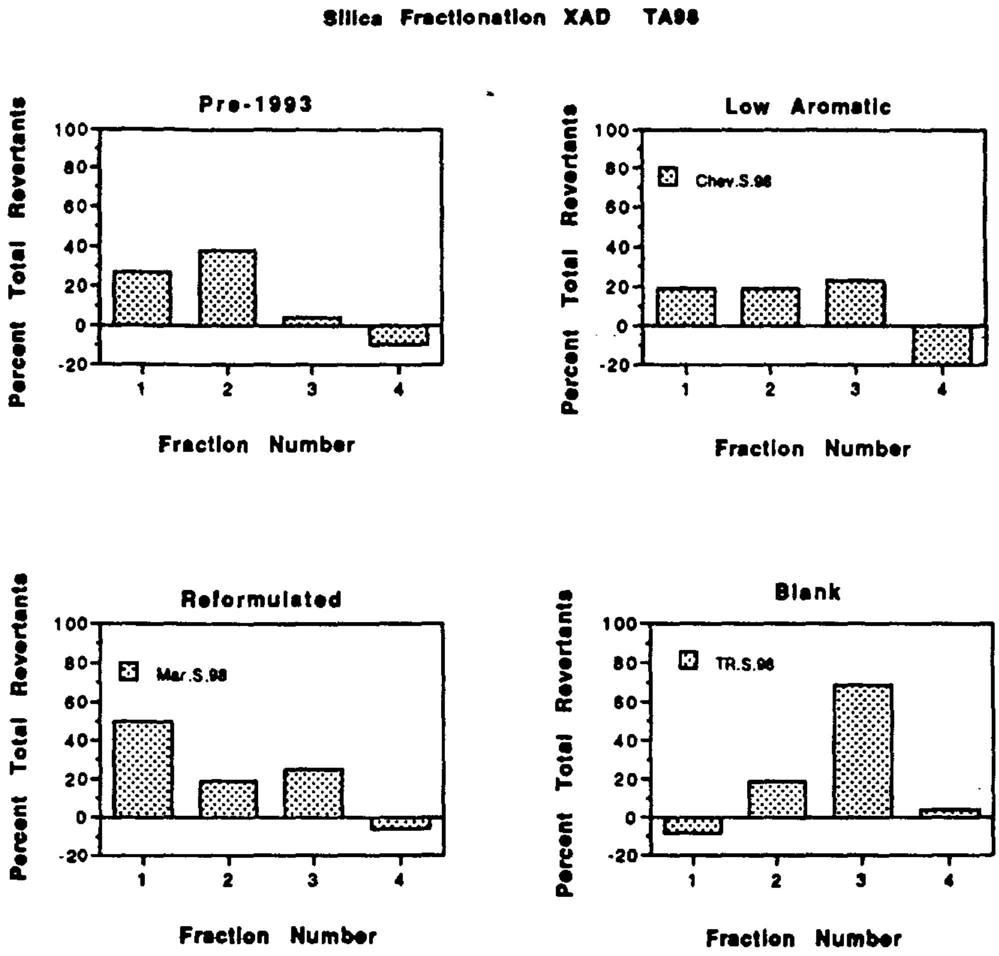


Figure 41. Mutagrams of Pre-1993, low aromatic, and reformulated fuel vapor-phase emissions collected on XAD and fractionated using silica solid phase extraction (SPE). Fractions tested in tester strain TA98 with metabolic enzymes added.

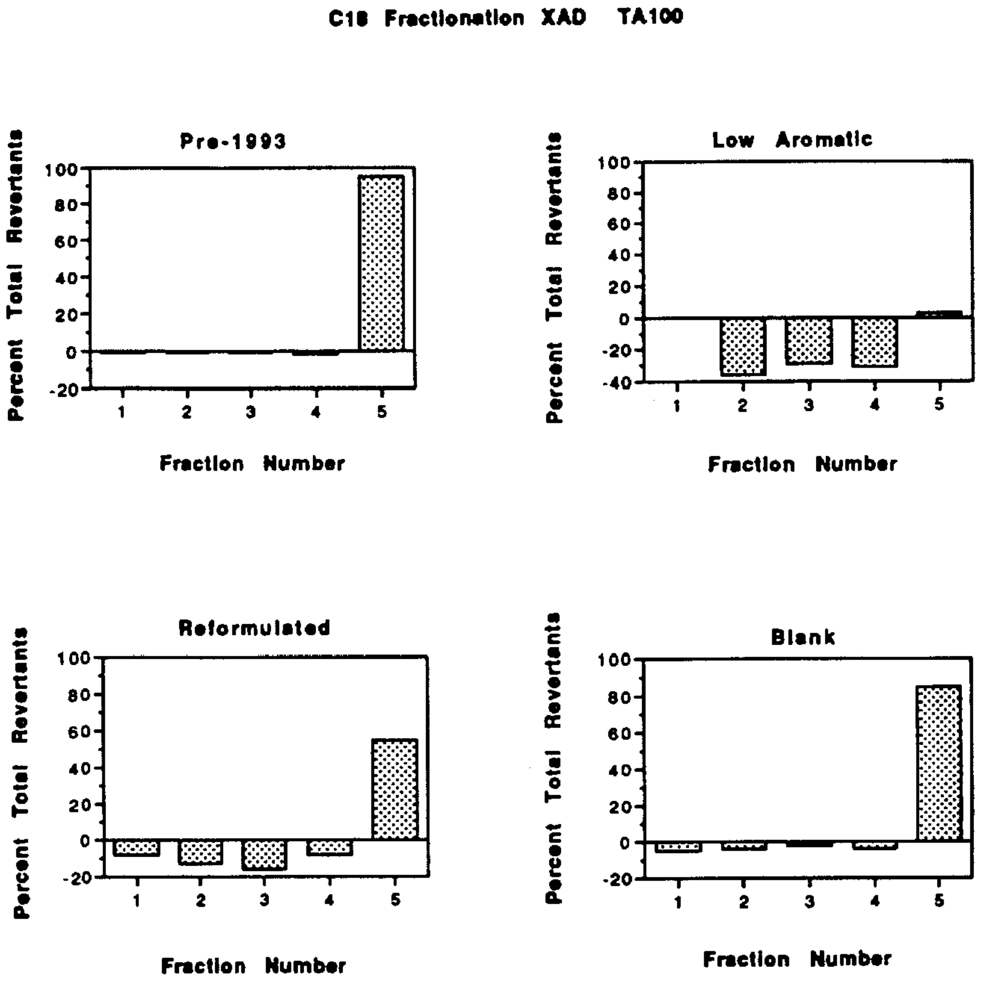


Figure 42. Mutagrams of Pre-1993, low aromatic, and reformulated fuel vapor-phase emissions collected on XAD and fractionated using C18 solid phase extraction (SPE). Fractions tested in tester strain TA100 with metabolic enzymes added.

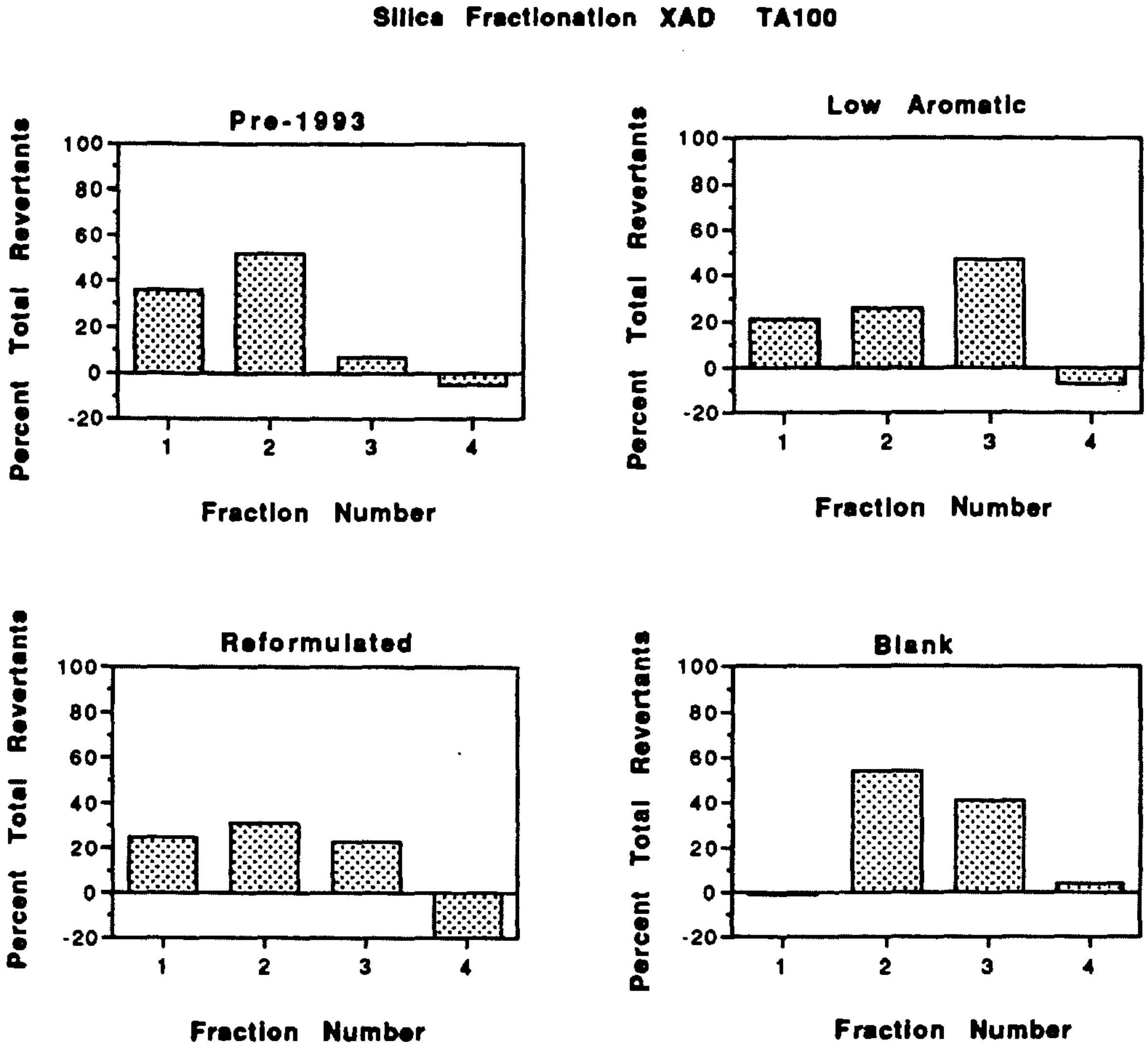


Figure 43. Mutagrams of Pre-1993, low aromatic, and reformulated fuel vapor-phase emissions collected on XAD and fractionated using silica solid phase extraction (SPE). Fractions tested in tester strain TA100 with metabolic enzymes added.

Discussion: The dose response curves for the particulate matter and vapor-phase samples allowed us to determine a potency value, which was used to calculate emissions for all fuels tested. For the particulate matter, the specific mutagenic activity (revertants per μg of particulate matter extracted) for the Pre-1993 fuel was higher both with and without S9 compared to the other fuels. The activity for the Reformulated blend fuel particulate matter was similar to the activity for the Low Aromatic fuel when tested with S9 or without S9. The difference in specific activities between fuels, with and without S9, support the hypothesis that different compounds may be present in the extracts, or that there are different concentrations of similar compounds. For example, nitro-PAHs are potent mutagens often detected without S9. Compound classes can further be analyzed using HPLC fractionation with subsequent bioassay analyses of the fractions. These mutagrams can be informative with respect to compound class identification.

HPLC fractionation using a normal phase (silica) column produces fractions that range from a typically non-polar fraction (Fraction 1) and progressively increases in polarity to Fraction 9 which contains the most polar compounds. HPLC Fraction 7 was the most mutagenic fraction for the particulate matter from all 3 fuels, both with and without S9. Each fuel did have distinctive mutagrams, however. For example, for the Pre-1993 fuel, HPLC Fractions 4 and 6 each had levels of mutagenic activity that were similar to each other and were the next highest peak in the mutagram relative to Fraction 7 with S9 added. For the Reformulated blend fuel particulate matter, Fraction 4 had the next highest mutagenic activity peak and Fraction 6 was considerably lower than Fraction 4. For the low aromatic fuel, Fraction 3 had the next highest peak in the mutagram. Fractions 2 and 3 typically contain the PAHs.

Differences in the mutagrams are observed when comparing activity with and without metabolic enzymes added. For example, for all three fuels, Fraction 6 has relatively low mutagenic activity compared to Fraction 7 when metabolic enzymes are added. Fraction 6 dramatically increases relative to Fraction 7 when no metabolic enzymes are added. This is indicative of what is referred to as "direct-acting" mutagenic compounds, or compounds that do not typically require the addition of enzymes for their detection. Arey and colleagues (1992) have reported that the nitro-PAHs are typically present in HPLC Fraction 4, and the di-nitro PAHs are in Fraction 5.

Historic work by Arey and colleagues (1994) with ambient samples and controlled chamber experiments, have isolated and identified highly mutagenic nitro-phenanthrene-lactone compounds that are present in HPLC Fraction 6. The most mutagenic compounds present in HPLC Fraction 7 remain unknown, and are considerably more polar than classical PAHs or nitro-PAHs. One possible candidate class of compounds that may be present in this fraction are substituted PAHs (such as oxygenated PAH) that are more polar than the classical nitro-PAHs.

Mutagenicity of fractions were reported by Westerholm et al. (1991) on a diesel truck equipped with a 14.2 L engine and run on a chassis dynamometer. There were five fractions collected from the extract from particulate matter and these fractions were tested in the standard Salmonella/microsome test of Ames et al. (1975). The fractions with the highest mutagenic activity were in fractions 3 and 4 containing the nitro- and dinitro-PAHs. Our results indicated that the most mutagenic fractions contain compounds that are more polar than fractions containing the nitro-PAHs. However, this may be due to differences in the fractionation procedures used and separation of the compounds in each fractionation procedure. The emission rates for mutagenicity were reported by Westerholm in revertants per km and were therefore not directly comparable to our emission rates which are reported as revertants per Bhp-hr. Crebeli et al. (1991) examined the mutagenicity spectrum of diesel and gasoline engine particulate matter. Organic extracts were fractionated into acidic, neutral and basic fractions. For all particulate matter, the neutral compounds accounted for approximately 80% of the mutagenic activity.

Bagley et al. (1996) investigated the emissions including mutagenic activity from a Cummins L10 engine (1988 model year) and samples were collected using steady state modes. The investigators studied the effects of a ceramic particle trap on emissions as well as the examining the influence of sulfur content in the fuel. The investigators reported that the trap lowered the levels of mutagenic activity associated with particles, and there were the same or lower levels of mutagenic activity with lower sulfur fuel. These mutagenicity results could not be compared with the current reported study since the units of emissions were not directly or conveniently comparable.

The levels of activity and the relative activities of emissions using different fuels was evaluated from other studies. Rasmussen et al. (1990) tested particulate matter collected from diesel exhaust and used the Salmonella microsuspension assay. The test engine was a Cummins NTCC 400 with in-line 6 cylinder and 14 L displacement. The test was conducted using the 1988 Federal Test Procedures for transient testing of heavy-duty diesel engines. A number of fuels of varying aromaticity were tested. They reported that the levels of activity were generally related to the level of aromaticity.

In our work, we observed that this relationship of fuel aromaticity to mutagenicity was not necessarily related quantitatively based on either potency and emission values. The Pre-1993, Low Aromatic, and Reformulated fuels contained approximately 30, 4, and 23 percent aromatics, respectively. The specific mutagenic activity and emission values for the Pre-1993 fuel were higher than the two other fuels. However, the specific mutagenic activity for the Low Aromatic and Reformulated fuels were very similar both in the particle- and vapor- phases, without S9. The Low Aromatic fuel itself also had the lowest total polyaromatics as determined by fuel analyses (0.7%) compared to the Pre-1993 (approximately 6-7%) and the Reformulated fuel (approximately 4%). The studies by Rasmussen et al. were not originally designed to test for fuel aromaticity differences and mutagenic activity. However, since the microsuspension assay was used and the testing was conducted on an engine dynamometer with a transient cycle, we could compare some of the emission results for mutagenic activity from the current study. For the nine fuels tested, the range for emissions of mutagenic activity for particulate matter were approximately 5 to 8 x 10 5 TA98 revertants/Bhp-hr. (with or without S9). These emission values are approximately 5 to 10 times lower than the emission rates we found for the fuels and engine tested in the current study.

With respect the vapor-phase compounds collected and tested in the bioassay, the testing of the complex mixture of the vapor-phase was challenging in many regards. First, the extraction, handling, and bioassay testing of the extracts or their fractions had to be carefully completed since the compounds are semi-volatile. Second, there appeared to be toxicity or concurrent masking of mutagenic activity in some of the extracts. For example, the supercritical fluid extracts from the Pre-1993 PUF sample were toxic to TA98 at very low concentrations of the extract and SPE fractions of the XAD sample were toxic. Finally, collection of the sample is highly labor intensive starting from the preparation and pre-testing of the media (PUF and XAD), to collecting a high volume sample from the dynamometer, to extraction of the sample, to chemical and bioassay testing of the sample. Alternative approaches for this entire process could be investigated. Vapor-phase samples were previously tested for emissions collected from a medium heavy duty diesel truck (Kado et al, 1996). Tester strain TA100 was the most sensitive strain for these and current vapor-phase samples. Tester strain TA98 is the more sensitive strain when testing the diesel particulate matter. These results are consistent with the results of Westerholm et al. (1991).

With respect to the mutagrams of the vapor-phase compounds, a number of factors should be noted. For example, the mutagrams for the C18 fractions are different between the fuels. As indicated, these differences could be due to differences in the chemical composition of the vapor-phase mutagenic compounds, and varying relative concentration of these compounds. The mutagrams between strains for a given fuel type are very similar, but the relative mutagenic activities are higher in tester strain TA100.

The emission values from the vapor-phase are difficult to compare directly with the emissions of the particle phase due to differences in tester strain sensitivity to each phase. The tester strains appear to be sensitive to different compounds in the extracts, and therefore the vapor-phase activity detected is an indication that there are different mutagenic compounds or changes in compound concentrations. For the Pre-1993 fuel, for example, when comparing the most sensitive strain for particle or vapor-phase samples, the vapor-phase mutagen emissions are approximately 10 times lower (10^5 vs 10^6 Rev/Bhp-hr.) than the particle phase. To estimate the total number of revertant equivalents emitted per cycle, the emission rate can be multiplied by the number of Bhp-hr. in the cycle (nominally 22).

The emission levels of both particle and vapor-phase associated mutagenic compounds are important to evaluate as an index of potential exposure to the complex mixture of compounds present. The mutagenic activity is also used in conjunction with bioassay to chemically characterize the most genotoxic compounds or groups of compounds present in the particle and vapor phase. These integrated approaches using chemical analyses and bioassay could prove to be valuable in evaluating human and ecological health risks.

Future studies for comparing different fuels and engines (and vehicles) would provide valuable information regarding the factors important in affecting mutagen emissions. The factors which affect these emissions could also be evaluated.