

## PAH, NITRO-PAH, AND MUTAGENICITY ASSOCIATED WITH DIESEL EMISSIONS MODIFIED BY USE OF A CERAMIC PARTICLE TRAP.

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### INTRODUCTION

Whole diesel exhaust has recently been classified as a probable human carcinogen by the International Agency for Research on Cancer (1) and a potential occupational carcinogen by the National Institute for Occupational Safety and Health (2). Much of the research on the possible health effects of diesel emissions has focused on the particle phase and associated organics, which include many polynuclear aromatic hydrocarbons (PAH) with known biological activity (1,3). The particle-free vapor phase exhaust component has been less extensively studied, with mixed results as to its potential health effects (1). However, this phase has also been shown to contain many of the same types of PAH as found with the particles (1,4,5), some of which could adsorb onto atmospheric particles after leaving the diesel engine.

Various federal regulations have been established concerning allowable levels of oxides of nitrogen ( $\text{NO}_x$ ), hydrocarbons (HC), and particles or total particulate matter (TPM) for on-road heavy-duty diesel emissions for 1991 and 1994 (6). Engine manufacturers have generally been able to meet all of the 1991 standards and the 1994  $\text{NO}_x$  and HC standards by making engine modifications. However, it is likely that some type of exhaust aftertreatment device will be required on some engines to meet the 1994 TPM standards, even with the use of low sulfur fuels. One type of device that would likely be used with some types of engines is the uncatalyzed ceramic particle trap. Traps will also be used on buses and on underground mine vehicles. The exhaust must pass through the walls of the trap, which typically results in  $\geq 90\%$  removal of particles depending on the type of engine and how it is operated. However, studies with a 1979 heavy-duty diesel engine indicate that HC and vapor phase organics, including PAH, may be only minimally affected by trap usage (4).

This study was designed to assess the effects of an uncatalyzed ceramic particle trap on the regulated and unregulated emissions such as PAH from a late-model, heavy-duty diesel engine. In addition to monitoring effects on  $\text{NO}_x$ , HC, and TPM, detailed analyses were made of organic material from both the particle and vapor phases for levels of selected biologically active PAH and nitro-PAH as well as mutagenicity. These data were obtained as part of a larger study funded by the Health Effects Institute concerned with obtaining some of the quantitative data necessary for the evaluation of the toxicological impact of employing particle trap technology with modern heavy-duty diesel engines.

### EXPERIMENTAL

Engine Operation and Emissions Measurements - A 1988 Cummins Engine Co. LTA10-300 heavy-duty diesel engine was used throughout this study. This turbocharged and aftercooled, direct injection engine met the 1988 federal and California Air Resources Board emissions standards (6) without any exhaust aftertreatment and was considered to be representative of engines on the road in the 1990's. The uncatalyzed ceramic particle trap was a Corning type EX-54 four-piece "quad" (0.305 m long and 0.289 m diameter) with a cell density of  $15.5 \text{ cells/cm}^2$ , a wall thickness of 0.43 mm, and a mean pore size of  $26 \mu\text{m}$ . One batch of AMOCO Premier No. 2 diesel fuel was used, having a cetane index of 48.6, sulfur content of 0.316 wt %, and aromatics content of 22.0 vol %. The engine oil used was Cummins Premium Blue (SAE 15W-40).

Emissions with and without the trap were collected with the engine operating at two rated speed (1900 rpm) modes of the EPA 13-mode steady-state cycle, i.e., modes 9 (75% load)

and 11 (25% load). These modes were selected as being representative of on-highway operation, having similar emission characteristics to data for transient operation of this engine, and having exhaust temperatures below those at which trap self-regeneration should occur. (However, later testing indicated that some trap self-regeneration was occurring at mode 9 due to higher trap temperatures.)

Levels of HC, NO<sub>x</sub>, and nitric oxide (NO) were determined in the raw exhaust using a Beckman flame ionization detector HC analyzer and a chemiluminescence NO/NO<sub>x</sub> analyzer, respectively. A portion of the raw exhaust with or without (baseline) trap usage was split-off and injected into a dilution tunnel. The volume dilution ratio was calculated by the ratio of measured raw NO<sub>x</sub> to measured dilute NO<sub>x</sub>.

Portions of the dilution tunnel flow were diverted simultaneously into one of two sampling systems, i.e., a 47-mm filter system used for determination of TPM and component levels [soluble organic fraction (SOF), sulfate, and solids (SOL)] and a 508 x 508-mm filter system used for collection of particle and vapor phase materials for chemical and biological characterization. Both systems contained the same type of Teflon-coated woven glass fiber filters (Pallflex TX40HI20-WW). A container with 40 g XAD-2 resin was located immediately behind the 508 x 508-mm filter to collect semi-volatile organics (XOC).

In order to minimize dilution tunnel sampling variability (7), the filter face temperature under all engine operating conditions was maintained at 45 ± 2°C by allowing the dilution ratio to vary as ambient temperatures (tunnel inlet temperatures) varied for different runs. In order to also minimize the potential for artifact formation, sampling times were held at ≤60 minutes and filter face nitrogen dioxide levels at <5 ppm.

Levels of TPM on the 47-mm filters were determined gravimetrically. Soluble organic fraction levels were determined gravimetrically after filter extraction for four hours with dichloromethane in a Soxhlet apparatus. Sulfate levels were then determined using ion chromatography. Any remaining mass on the filters was considered to be the SOL component. Levels of SOF and XOC associated with the 508 x 508-mm filters and XAD-2 resin, respectively, were determined after Soxhlet extraction for 24 hours with dichloromethane. The mass of each extract was determined gravimetrically by allowing the SOF or XOC to dry on a known mass of Florisil.

**Compound Quantitation** - The PAH and nitro-PAH selected for quantitation by high performance liquid chromatography (HPLC) were: benz[a]anthracene; benzo[b]fluoranthene; benzo[k]fluoranthene; benzo[a]pyrene; chrysene; fluoranthene; 7-nitrobenz[a]anthracene; 6-nitrochrysene; 3-nitrofluoranthene; 2-nitrofluorene; 1-nitropyrene; and pyrene. These compounds were selected for quantitation due to their known or suspected health effects (1,3), their known occurrence in diesel exhaust (1,3-5,7), the availability of suitable reference materials, and the ability to resolve them from apparent interferences in our quantitation procedure (7).

Separation of the SOF or XOC samples and compound quantitations were conducted using the scheme presented in Figure 1. Quantitation was accomplished by using internal standard method calculations using 2-chloroanthracene and comparison to the National Institute for Standards and Technology standard reference material 1647a as the reference (calibration) standard for PAH and standard reference material 1597 for the nitro-PAH calibration standard. Minimum detection levels (MDL) were determined using dilutions of the calibration standards, with MDL considered to be those quantities injected on the HPLC having a minimum of three times the background noise and areas which could be reproducibly measured. As 1-nitropyrene and 1,3-dinitropyrene coeluted by the method noted for nitro-PAH quantitation, the results are presented as "1-nitropyrene + 1,3-dinitropyrene" levels.

**Mutagenicity Analyses** - Mutagenicity associated with the SOF and XOC samples was determined using a small dish modification of the microsuspension version of the *Salmonella typhimurium*/microsome mutagenicity bioassay (8-10). Samples for the mutagenicity bioassays were prepared by dissolving a known mass of SOF or XOC in a known volume of dimethylsulfoxide. All samples were tested with tester strain TA98 ± S9 metabolic activation and retested with the nitroreductase-deficient tester strains TA98NR and TA98-1,8-DNP<sub>6</sub> if

responses were detected with TA98-S9. The appropriate solvent, positive control, genotype, sterility, and viability controls were conducted with each test (8).

Data Analysis - As data from two to six test dates per mode and condition were available for analysis, this study was considered to have an unbalanced design with test dates as replicates (11). There was an unequal number of samples per replicate date for each mode and condition; therefore, a mean of means was calculated for most emission parameters, except as noted below. A two-way analysis of variance (ANOVA) was used to compare mode (9 and 11) versus condition (baseline and trap) means at a significance level of 0.05. Where a significant interaction term occurred, a one-way ANOVA was used.

Exceptions had to be made to the statistical analysis procedures (as described above) for many of the PAH and nitro-PAH measurements and the mutagenic activity data. The latter could not be analyzed statistically because the revertants/ $\mu\text{g}$  value was calculated using a power function model that was dependent upon the interaction of both the slope and the y-intercept (12). The frequent occurrence of "less than MDL" values in the compound quantitation data sets meant that mean daily values typically could not be calculated. Therefore, mean of median values were determined for each mode/condition for use in the ANOVA. If the majority of values for any test date was above the MDL, then that median value was used for that test date. If 50% or less of the values for each test date were above the MDL, then a value was assigned to each "less than MDL" value for median determinations using several different techniques. The technique that appeared to have the least scattering of residuals was the replacement of the "less than MDL" value with "MDL/2". All ANOVA results reported in this paper involving mean of median analyses were conducted using this technique.

## RESULTS AND DISCUSSION

This paper summarizes the results of one of the few large heavy-duty diesel emission sample sets comprising multiple test dates and engine operating modes and conditions. It also represents the first detailed quantitative study specifically of biologically active PAH and nitro-PAH compounds associated with both the particle and vapor phase exhaust from a late-model diesel engine.

Gaseous and Particle-Associated Emissions - Baseline emissions from this 1988 Cummins heavy-duty diesel engine were notably different from older engines tested at Michigan Technological University (MTU) (4, 7, 12). Not only were TPM levels much lower with the 1988 engine, but the proportion of the HC recovered on XAD-2 resin (as XOC) compared to the filters (SOF) was greatly increased.

As indicated in Table 1, use of the trap with the Cummins engine had no significant effects on  $\text{NO}_x$  (or NO) concentrations at either mode but exerted its effects on the HC (and XOC), TPM, and TPM-associated exhaust components. The greatest effects were found with reductions in SOL concentrations of up to 95%, as this type of control device acts primarily to physically remove this material via engine mode-independent mechanisms. These results are in agreement with previous studies with other types of diesel engines (4, 12-14). The reductions in SOF can be related both to some significant effects on HC levels (at mode 9) and to the decrease in SOL surface sites for adsorption of organic material found at both modes. Sulfates were also decreased with trap use, although a significant change was found only at mode 9. These combined reductions resulted in 92% and 80% TPM-removal efficiencies at modes 9 and 11, respectively. There were consistent mode-dependent differences in trap effects on HC and XOC concentrations, with the latter representing a portion of the vapor phase HC. The significant changes in HC and XOC concentrations (reductions of 43 and 67%, respectively) at mode 9 but virtually no change at mode 11 were most likely related to differences in exhaust temperature between the two modes. The higher trap temperature at mode 9 (450°C) versus mode 11 (325°C) probably resulted in greater oxidation of HC within the trap and, thus, lower HC and XOC levels after the trap. A high proportion of the recoverable HC were still vapor-phase associated (as XOC) even with trap use at mode 9.

Mutagenicity Effects - A summary of the trap and mode effects on TPM and XOC-associated mutagenic activities is presented in Table 2. All detected mutagenicity was direct-acting in nature, regardless of mode, trap usage or particle or vapor phase association, as has

typically been found for diesel exhaust-associated organics. However, in contrast to previous studies with older model engines at MTU (4,12) and elsewhere (13,14) with this same type of control device, trap use with this 1988 engine did not produce increases in TPM-associated mutagenic activities (revertants/ $\mu\text{g}$ ). The 93% and 86% decreases in exhaust mutagenic concentrations (revertants/ $\text{m}^3$ ) at modes 9 and 11, respectively, were similar to the reductions found in TPM and SOF concentrations (Table 1). Similar to our earlier study with a 1979-model heavy-duty diesel engine (4), about 50% of the recovered mutagenicity (TPM plus XOC) was associated with the XOC. Unlike the earlier study, no mutagenicity was detected at either mode with trap usage with this 1988 engine. Differences in engine configurations and operation, emissions levels (such as for  $\text{NO}_x$  and NO), and sampling procedures may account for these differences in trends between the present and previous studies.

The responses of the two nitroreductase-deficient tester strains (Table 2) indicated more of the observed TPM-associated activity was due to nitro-substituted compounds under baseline than under trap conditions. The same general relationship was found between baseline TPM and XOC-associated mutagenicity, with the exception of the TA98-1,8-DNP<sub>6</sub> responses at mode 11. This likely indicates differences in the proportion of nitro-substituted compounds in the particle versus vapor phases under baseline conditions and, perhaps, lower levels of these mutagenic compounds with the trap.

**PAH and Nitro-PAH Constituents** - The ranges of PAH and nitro-PAH concentrations found for each operating condition with and without the trap are presented in Table 3. The baseline particle-associated concentrations for many of these compounds are lower than those in our earlier study with an older engine using similar quantitation techniques (7), in part due to the lower TPM levels with the 1988 engine. However, comparisons to literature values for other studies are difficult due to differences in engines, operating and sampling conditions, and analytical procedures. As noted in the "Experimental - Data Analysis" section, presentation and analysis of most of these data sets were complicated by the presence of "less than MDL" values. With the exception of the fluoranthene TPM and XOC, pyrene TPM and XOC, and chrysene TPM data sets, all of the PAH and nitro-PAH data were analyzed using procedures to estimate medians. Even so, several of these ANOVA comparisons did result in significant differences between either trap use or mode.

Trap usage had significant effects on fluoranthene, benzo[b]fluoranthene, and 7-nitrobenzo[a]anthracene TPM-associated concentrations at mode 9 and pyrene and 7-nitrobenzo[a]anthracene at mode 11. With the exception of pyrene, the concentrations of these compounds decreased with trap use. The general trend, which was more pronounced at mode 9 than 11, was for decreases in concentrations with use of the trap. Pyrene was the only compound with significant differences in concentrations between modes, although the general trend was for higher concentrations at mode 11 than at mode 9. This trend is in agreement with the higher exhaust temperatures and greater reductions in HC and SOF found at mode 9.

In contrast, the trap was found to have no significant effects on vapor phase compound concentrations. There were significant differences in fluoranthene, 6-nitrochrysene, and 1-nitropyrene + 1,3-dinitropyrene concentrations between modes, with the lower concentrations again found at mode 9.

Several PAH, such as the benzofluoranthenes and benzo[a]pyrene, were found almost exclusively with particles and some such as fluoranthene and pyrene were proportionally much higher in the vapor phase; these associations were generally in accordance with the known vapor pressures for these compounds (5,15). However, all of the other PAH and nitro-PAH were detected in the vapor phase emissions of this engine with or without the particle trap; a general trend was for an increase in the vapor phase concentration of many of these compounds with trap usage.

## CONCLUSIONS

This uncatalyzed ceramic particle trap had consistently significant effects (up to 95% reductions) on TPM and component concentrations regardless of engine operating condition. The PAH and nitro-PAH particle-associated concentrations generally were reduced with trap use, but this was not necessarily true with the vapor phase-associated concentrations. Some

compounds appeared to shift from the particle to vapor phase with the trap. Mutagenicity associated with the particles was generally unaffected with the traps, but mutagenic concentrations decreased due to decreases in particle concentrations. All detectable vapor phase mutagenicity was eliminated with trap use. Therefore, this type of emissions control device appeared to reduce particle concentrations from this late-model heavy-duty diesel engine without increasing particle-associated levels of biologically active PAH or nitro-PAH or mutagenicity. However, the impact of increases in certain types of compounds in the vapor phase should be investigated further.

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Table 1. Effect of a Ceramic Particle Trap on Emissions.

Parameter <sup>a</sup>	Mode 9		Mode 11	
	Baseline (mg/m <sup>3</sup> )	Trap Effect (% change)	Baseline (mg/m <sup>3</sup> )	Trap Effect (% change)
NO <sub>x</sub>	1,390	+1.4	430	+8.8
HC	41.8	-43*	87.2	-6.0
TPM	27.5	-92*	34.0	-80*
SOF	4.40	-89*	7.95	-75*
SOL	17.8	-95*	23.3	-86*
SO <sub>4</sub>	5.08	-80*	2.77	-40
XOC	28.0	-67*	42.8	-4.0

<sup>a</sup>NO<sub>x</sub> = oxides of nitrogen; HC = hydrocarbons; TPM = total particulate matter; TPM components - SOF = soluble organic fraction, SOL = solids, and SO<sub>4</sub> = sulfates; and XOC = XAD-2 resin organic component.

\*Mean significantly different from comparable baseline mean (p ≥ 0.05).

Table 2. Effect of a Ceramic Particle Trap on Mutagenic Activity.

Mode/ Condition	Component <sup>a</sup>	Mutagenic Activity, TA98-S9 <sup>b</sup>		% of TA98-S9 Response		
		Revertants/μg	kRevertants/m <sup>3</sup>	TA98+S9	TA98NR-S9	TA98DNP-S9 <sup>c</sup>
9 Baseline	TPM	0.60	20.6	55	15	15
	XOC	0.67	18.6	105	55	64
9 Trap	TPM	0.42	1.39	92	60	46
	XOC	NR <sup>d</sup>	NR	NR	NT	NT
11 Baseline	TPM	0.68	27.3	72	24	24
	XOC	0.56	24.7	75	40	13
11 Trap	TPM	0.39	3.96	78	60	61
	XOC	NR	NR	NR	NT	NT

<sup>a</sup>TPM = total particulate matter; XOC = XAD-2 resin organic component.

<sup>b</sup>Revertants/μg based on power function model analysis of dose-response data.

<sup>c</sup>TA98DNP = TA98-1,8-DNP<sub>8</sub>.

<sup>d</sup>NR = no response above spontaneous revertant levels; NT = not tested as no activity with TA98-S9.

Table 3. Range of PAH and Nitro-PAH Concentrations.

Compound	Concentration Range (ng/m <sup>3</sup> ) <sup>a</sup>							
	9 Baseline		9 Trap		11 Baseline		11 Trap	
	TPM	XOC	TPM	XOC	TPM	XOC	TPM	XOC
Benz[a]anthracene	24-310	82-190	<1.1-76	<0.37-48	120-310	160-280	88-220	8.1-400
Benzo[b]fluoranthene	6.1-190	≤85 <sup>e</sup>	<0.97-52 <sup>*</sup>	<0.16-11	16-68	<4.6 <sup>d</sup>	<1.2-95	<0.96-180
Benzo[k]fluoranthene	<1.7-24	<2.8 <sup>d</sup>	<2.4 <sup>d</sup>	<3.0 <sup>d</sup>	<1.9-6.7	<4.6 <sup>d</sup>	<1.9 <sup>d</sup>	≤34 <sup>e</sup>
Benzo[a]pyrene	<1.4-22	<2.0 <sup>d</sup>	<0.14-1.3	<3.1 <sup>d</sup>	<1.3-5.2	<4.6 <sup>d</sup>	<1.4-13	<2.9 <sup>d</sup>
Chrysene <sup>b</sup>	24-340	1.4-49	4.6-110	<0.35-45	23-360	<0.31-40	12-230	<8.7-450
Fluoranthene <sup>c</sup>	520-23,000	1,300-2,200	57-2,100 <sup>*</sup>	88-3,000	410-780	2,100-3,500	190-2,900	730-6,500
Pyrene <sup>c</sup>	8.1-84	120-590	13-380	99-580	69-130	390-650	260-790 <sup>*</sup>	690-1,600
7-Nitrobenz[a]-anthracene	0.60-3.9	2.5-9.5	0.14-2.1 <sup>*</sup>	≤0.34-60	<4.0-7.2	14-63	≤1.3-18 <sup>*</sup>	<1.1-14
6-Nitrochrysene	2.1-13	1.7-2.5	<0.88-4.3 <sup>e</sup>	0.69-8.0	≤0.27-0.76	≤0.65-12	1.6-11	<0.95-7.9
3-Nitrofluoranthene	≤1.4 <sup>e</sup>	<2.8 <sup>d</sup>	<2.1 <sup>d</sup>	0.016-13	0.17-2.0	3.4-12	≤0.56-4.2	<1.1-7.5
2-Nitrofluorene	5.3-23	2.1-9.9	<2.0-21	<0.12-69	5.2-5.6	1.8-28	7.4-18	≤2.1-26
1-Nitropyrene + 1,3-Dinitropyrene	<2.1-21	<2.7 <sup>d</sup>	0.86-25	0.13-45	<3.8-38	12-190	<3.8-47	<1.1-65

<sup>a</sup>TPM = total particulate matter; XOC - XAD-2 resin organic component.

<sup>b</sup>Values above minimum detection limits (MDL) for TPM samples.

<sup>c</sup>Values above MDL for all TPM and XOC samples.

<sup>d</sup>No values > MDL.

<sup>e</sup>Above MDL value for one sample only.

<sup>\*</sup>Mean significantly different from comparable baseline mean (p = 0.05).

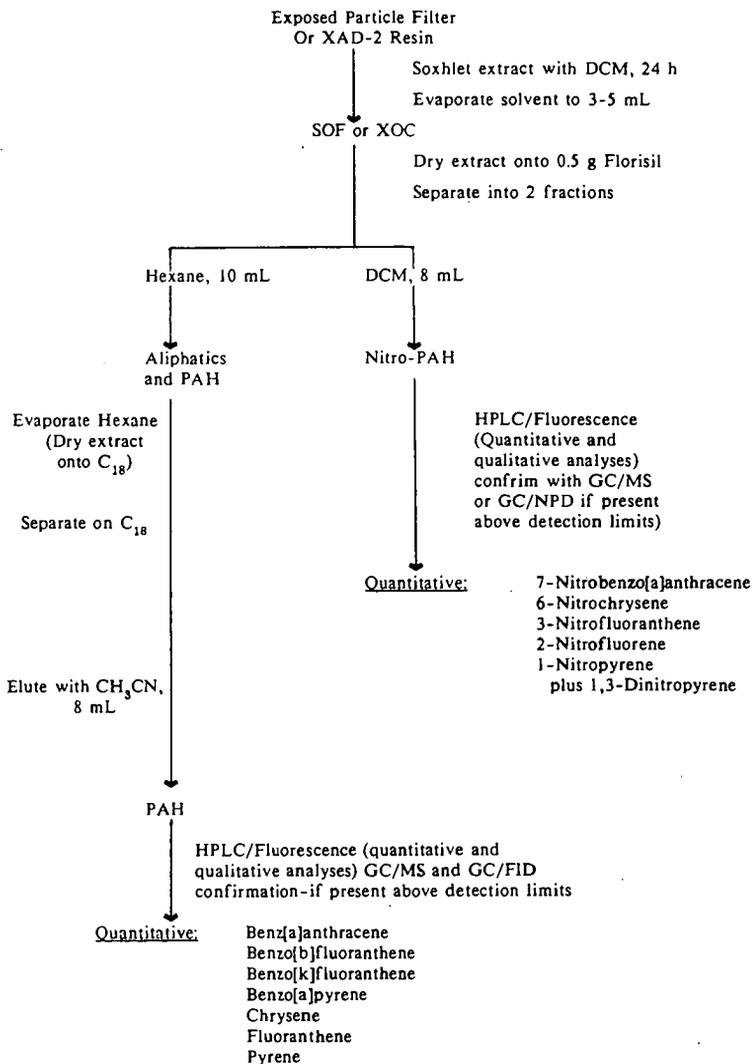


Figure 1. Flow chart for the extraction of SOF and XOC and compound quantitation. DCM = dichloromethane; SOF = soluble organic fraction; XOC = XAD-2 resin organic component; PAH = polynuclear aromatic hydrocarbons; CH<sub>3</sub>CN = acetonitrile.