MUTAGENICITY OF DIESEL EXHAUST PARTICLES FROM AN ENGINE WITH DIFFERING EXHAUST AFTER TREATMENTS

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This study was conducted to investigate the effects of engine operating conditions and exhaust aftertreatments on the mutagenicity of diesel particulate matter (DPM) collected directly in an underground mine environment. A number of after-treatment devices are currently used on diesel engines in mines, but it is critical to determine whether reductions in DPM concentrations result in a corresponding decrease in adverse health effects. An eddy-current dynamometer was used to operate naturally aspirated mechanically controlled engine at several steady-state conditions. The samples were collected when the engine was equipped with a standard muffler, a diesel oxidation catalytic converter, two types of uncatalyzed diesel particulate filter systems, and three types of disposable diesel particulate filter elements. Bacterial gene mutation activity of DPM was tested on acetone extracts using the Ames *Salmonella* assay. The results indicated strong correlation between engine operating conditions and mutagenic activity of DPM. When the engine was fitted with muffler, the mutagenic activity was observed for the samples collected from light-load, but not heavy-load operating conditions. When the engine was equipped with a diesel oxidation catalyst, the samples did not exhibit mutagenic activity for any of four engine operating conditions. Mutagenic activity was observed for the samples collected when the engine was retrofitted with three types of disposable filters and sintered metal diesel particulate filter and operated at light load conditions. However, those filtration systems substantially reduced the concentration-normalized mutagenic activity from the levels observed for the muffler.

It has been estimated that approximately 1.35 million workers are occupationally exposed to combustion products of diesel fuel in about 80,000 workplaces across the United States (NIOSH, 1988). Occupational exposure is common among transportation workers, among operators of diesel-powered equipment, and among miners operating diesel-powered equipment underground. Diesel exhaust consists of both gaseous and particulate fractions. Diesel particulate matter (DPM), composed mostly of carbon formed through the incomplete combustion of fuel, contains chemicals such as

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polycyclic aromatic hydrocarbons (PAH), nitro-PAH, oxygenated PAH, aliphatic hydrocarbons, ketones and aldehydes (McClellan, 1987; Lowenthal et al., 1994, Hammerle, et al., 1995, McDonald et al., 2002). According to the National Institute for Occupational Safety and Health (NIOSH, 1988), the International Agency for Research on Cancer (IARC, 1989), and the U.S. Environmental Protection Agency (U.S. EPA, 2002), DPM was declared a potential or probable human carcinogen. Therefore, DPM poses potential carcinogenic and other health hazards to workers where diesel engines are used.

In January 2001, the Mining Safety and Health Administration (MSHA) promulgated two rules (Mine Safety and Health Administration, 2001, 2006) limiting the exposure of metal and nonmetal underground miners and coal miners to DPM. As of May 20, 2008, the exposure of metal and nonmetal miners is limited to 160 $\mu g/m^3$ total carbon. The current regulations are established on feasibility of implementing diesel emissions control technologies and strategies rather than on firm understanding of the health risks associated with exposure to DPM. Extensive use of diesel-powered equipment makes reduction of underground miner exposure to particulate matter and gaseous emissions from diesel-powered equipment a major challenge for the mining industry in the United States and worldwide. Aforementioned MSHA regulations spurred implementation of advanced diesel engine and exhaust after-treatment technology and alternative fuels in underground mines. Diesel exhaust after-treatment technologies can dramatically change the physical and chemical properties of DPM (Maricq et al., 2002; Kittelson et al., 2005; Bugarski et al., 2006a) and potentially change their associated health effects (Bugarski et al., 2009). While use of a diesel particulate filter (DPF) systems was reported to reduce total mass concentration of emitted aerosols from 80 to 95% (Schnakenberg & Bugarski, 2002; Bugarski et al., 2006a, 2009), several investigators reported high concentrations of nucleation mode aerosols when DPF systems were used.

Organic solvents, such as acetone, have been used to extract PAH and other chemicals from DPM for toxicological studies (U.S. EPA, 2002). While methylene chloride was used in many studies, acetone was found to extract more mutagenic activity from airborne particulates than dichloromethane or other solvents (Krishna et al., 1983). Acetone has been used in recent motor vehicle exhaust mutagenicity studies (Seagrave et al. (2002), and in chemical characterization studies (McDonald et al., 2004), and has the advantage of low toxicity to tester cells and no mutagenic properties, unlike methylene chloride.

Solvent extracts of DPM are known to be genotoxic to bacteria and mammalian cells (Wallace et al., 1987; Liu et al., 2005). Liu et al. (2005) found that exhaust emissions of diesel and gasoline engine vehicles operated on a unified driving cycle induce gene mutations in bacteria and induce micronucleus (MN) formation as well as DNA damage in cultured V79 cells (Chinese hamster lung fibroblasts). It was also shown that the mutagenic activity of DPM solvent extracts in bacteria varied with fuel type and engine speed (McMillian et al., 2002).

The objective of this study was to investigate the effects of engine operating conditions, a muffler, a diesel oxidation catalyst (DOC), two types of DPF systems, and three types of filtration systems with high-temperature disposable filter elements (DFE) on the mutagenicity of diesel aerosol solvent extracts in *Salmonella typhimurium*. The samples analyzed in this study were collected directly in an underground mining environment at the NIOSH Lake Lynn Experimental Mine, Fairchance, PA, using the in-mine NIOSH Diesel Laboratory.

METHODOLOGY

Experimental Setup

A schematic of the laboratory layout for the sample collection is shown in Figure 1. The D-drift is approximately 530 m long, 6 m wide, and 2 m high. The major components of the laboratory are an engine/dynamometer system, three sampling and measurement stations,

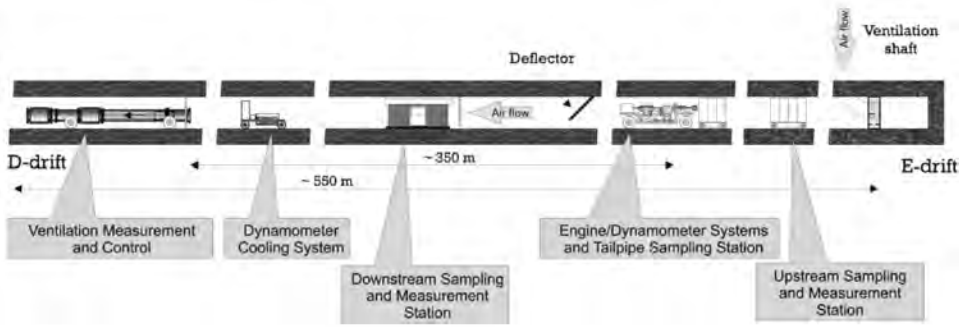

FIGURE 1. NIOSH Diesel Laboratory in D-drift of Lake Lynn Experimental Mine (not to scale).

and a ventilation measurement and control system.

The Isuzu C240, one of the most commonly used light-duty engines in U.S. underground coal mines (MSHA 2009), was operated at four steady-state engine operating modes (Table 1). The modes were selected to cover a wide range of engine operating parameters such as exhaust temperatures and emission rates. The R50 and I50 are considered to be representative of light load conditions, and R100 and I100 are considered to be representative of heavy load conditions. Fresh air was supplied to the underground facility via a ventilation shaft located in the E-drift (Figure 1). A series 1000 model 23017–3450 Axivane fan (Joy Technologies, New Philadelphia, OH) and a subsonic Venturi meter (Primary Flow Signal, Inc., Tulsa, OK) were used to maintain and measure constant flow of fresh air through the drift throughout the tests. The measurements showed an average volumetric flow rate of 5.69 ± 0.05 m^3/s. The low test-to-test variability in flow rate (0.86%) eliminated the need for normalization of the data with respect to it. The average exhaust dilution ratios for R50, R100, I50, and I100 engine operating modes were calculated to be 148, 149, 186, and 188, respectively. The average ambient temperatures upstream of the engine were between 10.5 and 18.7°C. The corresponding average ambient temperatures at the downstream measurement station ranged between 14.7 and 22.3°C.

TABLE 1. Engine Operating Modes

Mode	Description	Engine speed (rpm)	Torque (Nm)	Power (kW)
R50	Rated speed, light load (50% load)	2950	55.6	17.2
R100	Rated speed, heavy load (100% load)	2950	111.2	34.3
I50	Intermediate speed, light load (50% load)	2100	69.1	14.9
I100	Intermediate speed, heavy load (100% load)	2100	136.9	30.6

Exhaust Treatment Technologies and Fuel

The DPM samples were collected downstream of the engine operated with DPF and DFE systems and DOC representative of the devices currently available to the underground mining industry to curtail emissions from diesel-powered equipment. The scope of the project limited collection to the particulate fraction of the diesel emissions only; no vapor-phase fractions were collected

The DPF systems evaluated in this study were:

1. A DPF system manufactured by Catalytic Exhaust Products (CEP), Toronto, ON, model 912-SXT with uncatalyzed Corning EX-80 Cordierite element (31 cells/cm^2 and 0.3 mm wall thickness) (C DPF).
2. A DPF system manufactured by Mann+Hummel GMBH, Speyer, Germany, Model SMF-AR with an uncatalyzed sintered metal element (10 μm mean pore

size, 45% porosity, and 0.38 mm wall thickness) used with Satacen 3, an iron-based fuel additive (SM DPF).

Both DPF systems were installed at an identical location in the exhaust system for their respective tests.

Three types of high-temperature DFE with synthetic filtration media were tested using a filtration system consisting of custom filter housing and an air-to-air heat exchanger designed and built by Mac's Mining Repair Service (Huntington, UT). The heat exchanger was installed between the engine and the DFE housing to cool exhaust below 343°C. The following DFEs were evaluated:

1. A DFE manufactured by Donaldson Company, Minneapolis, MN, model P604516 (DFE-A).
2. A laundered DFE, identical to DFE-A, but laundered by Mac's Mining Repair Service, Huntington, UT, following their internal standard procedure (LDFE-A).
3. A DFE manufactured by FST Systems Corporation, Price, UT, model FST-115-26 (DFE-B).

The laundering process is used by some coal mine operators to extend the life cycle of the high-temperature DFE. The laundered version of DFE-A (LDFE-A) tested in this study was laundered only once. After installation, the two brand new elements (DFE-A and DFE-B) and the laundered element (LDFE-A) were conditioned prior to the 4-mode tests by running the engine for 13 h at the R50 mode.

A DOC used in this study was manufactured by Engine Control Systems Ltd., Newmarket, ON (model A16-0130) with a Cordierite substrate and a proprietary catalyst formulation. A generic muffler installed in place of the DPF system and the DOC was used as a baseline or control when the effects of aftertreatment devices were examined. Single-consignment ultra-low-sulfur diesel fuel (11 ppm S by weight) supplied by Guttman Oil (Belle Vernon, PA) was used for all tests in this study; synthetic 5W40 oil was used for engine lubrication.

Sample Collection

A custom sampling method was used to collect integrated DPM samples from the centrally located sampling point at the downstream sampling stations (Figure 1). Two sampling trains were used concurrently to collect the sample onto two pairs of series-connected 90-mm Teflon-coated glass fiber filters (TX40HI20-WW, Pall Corporation, Ann Arbor, MI). A URG cyclone was used at the inlet to each of the sampling streams to remove aerosols with aerodynamic diameter larger than 1 μm. A model SV25 rotary vane pump from Oerlikon Leybold Vacuum was used to draw sample at 90 L/min through each of the trains. The total mass flow rate through each of the trains was maintained using Sierra Instruments (Monterey, CA) mass flow controllers (model C100M).

The gravimetric analysis was performed using an ultra-microbalance (model UMX2, Mettler-Toledo, Columbus, OH), equipped with a special 110-mm-diameter weighing pan. The filters were conditioned and weighed in a climate-controlled room (20–22°C and 47–50% relative humidity [RH]). Thirteen samples were collected for the analysis. Table 2 shows the information on the exhaust configuration and engine operating mode pertinent to each of the samples.

Sample Preparation

Each of the concurrently collected filter pairs was treated as a single sample. The gravimetric analysis showed that the mass of the DPM collected on all of the secondary filters was negligible. Therefore, the secondary filters were not used in the further analysis. The DPM was extracted simultaneously from both primary filters using acetone and sonication for 2 h. The extract was reduced to less than 40 ml by evaporation under N_2, transferred to a 40-ml sterile glass centrifuge tube, and centrifuged at 6000 × g for 40 min. The supernatant was removed and filtered through

TABLE 2. DPM Samples Collected With Pertinent Information on Engine Operating Conditions Configurations and Engine Operating Conditions

Sample number	Exhaust after-treatment	Engine operating conditions
1	Muffler	R50
2	Muffler	R100
3	Muffler	I50
4	Muffler	I100
5	Diesel oxidation catalyst (DOC)	R50
6	Diesel oxidation catalyst (DOC)	R100
7	Diesel oxidation catalyst (DOC)	I50
8	Diesel oxidation catalyst (DOC)	I100
9	Disposable filter element (DFE-A)	R50
10	Laundered disposable filter element (LDFE-A)	R50
11	Disposable filter element (DFE-B)	R50
12	Sintered metal diesel particulate filter (SM DPF)	R50
13	Cordierite diesel particulate filter (C DPF)	I100

25-mm sterile PTFE filters. Removal of particles is essential since the particles often fluoresce and interfere with electronic colony counting. The filtrate was transferred to a tared 40-ml sterile brown bottle and evaporated to dryness under N_2. Twelve milligrams DPM extract per milliliter dimethyl sulfoxide (DMSO) solutions was made as stock solution. Samples were diluted further with DMSO to obtain the required concentrations.

Ames *Salmonella* Assay

The mutagenicity of the extracted DPM samples was determined using the preincubation variant of the Ames *Salmonella* assay system (Maron & Ames, 1983; Kado et al., 1983). Overall results from previous studies indicate that TA98 and YG1024 gave better mutagenic responses than TA100 and YG1029 to DPM tested, and that S9 was not necessary for mutagenic activity of DPM (Liu et al., 2005; McMillian, 2002; Shi et al., 2009). Since only limited amounts of sample were available, only *Salmonella typhimurium* TA 98 without S9 was used in this study.

The cell suspensions of *Salmonella typhimurium* TA98 were prepared by overnight incubation of a small piece of frozen culture in 50 ml nutrient broth. Subsequently, 25 μl of that TA 98 overnight culture broth along with 10 μl of DPM-extracted sample solution and 65 μl physiological saline were added and preincubated at 37°C for 30 min on a rotary shaker. Two tubes were prepared for four different concentrations of each sample tested. The concentrations previously used in DPM mutagenicity studies (Shi et al., 2009) of 13.3, 40, 120, or 180 μg per treatment were adopted for this study. The control was established using DMSO in place of sample solution. After incubation, 2.5 ml of molten top agar was added to each tube, and the contents were mixed and poured onto a plate. All plates were incubated at 37°C for 48 h prior to counting. The conditions of the bacterial background lawn were examined during every experiment in order to know whether cytotoxicity appeared in any of the samples. Three readings of the revertant colonies per plate were scored by an automatic colony counter (Accucount 1000, Biologics, Inc., Gainsville, VA). The average number of revertant colonies and corresponding standard deviation from both plates were calculated for each concentration. A repeat experiment was performed to confirm the results obtained from the original experiment.

Statistical Treatment

Samples were considered to be mutagenic if a positive concentration-response relationship was observed and for at least one concentration the sample number of revertants was twice that of the respective solvent control (Ames et al., 1975). A concentration was considered to be cytotoxic if it produced a thin lawn and reduced the number of revertants per plate.

RESULTS

The results of the *Salmonella* gene mutation tests are shown in Table 3, with notations of concentrations that were positive for mutagenicity and any samples that were cytotoxic to the tester strain.

TABLE 3. Revertants Induced by DPM Samples

Sample number	Exhaust after-treatment	Engine mode	Revertants/plate[a] (μg/plate)				
			Control	13.3	40	120	180
1	Muffler	R50	13 ± 1.1	26 ± 1.1	37 ± 5.5[b]	61 ± 1.6[b]	46 ± 6.0[b,c]
2	Muffler	R100	13 ± 1.1	13 ± 0.6	22 ± 1.6	22 ± 1.6	16 ± 0.6[c]
3	Muffler	I50	24 ± 3.8	29 ± 2.2	41 ± 2.7	61 ± 5.0	70 ± 1.6[b]
4	Muffler	I100	13 ± 1.1	13 ± 2.2	18 ± 3.3	13 ± 0.6	16 ± 1.1[c]
5	DOC	R50	14 ± 1	18 ± 1.6	20 ± 3.3	31 ± 5.9	15 ± 1.1
6	DOC	R100	14 ± 1	17 ± 1	18 ± 0.5	24 ± 2.3	24 ± 1.2
7	DOC	I50	14 ± 1	14 ± 1.6	17 ± 1.5	22 ± 4	19 ± 0.4
8	DOC	I100	10 ± 1.1	18 ± 6.9	18 ± 6.8	23 ± 1.2	10 ± 2.4[c]
9	DFE-A	R50	13 ± 1.1	15 ± 0.6	36 ± 3.8[c]	44 ± 2.2[d]	41 ± 8.8[b,c]
10	LDFE-A	R50	13 ± 1.2	29 ± 3.3	51 ± 2.7[b]	108 ± 16.6[b]	56 ± 4.4[b,c]
11	DFE-B	R50	13 ± 1.1	21 ± 0.8	47 ± 1.1[b]	93 ± 5.5[b]	99 ± 14.2[b,c]
12	SM DPF	R50	13 ± 1.2	20 ± 0.8	41 ± 1.8[b]	90 ± 12.3[b]	70 ± 10.6[b,c]
13	C DPF	I100	15 ± 2.1	14 ± 1.4	20 ± 7.9	31 ± 3.9	23 ± 3.8

[a]Mean value of two plates ± standard deviation.
[b]Positive response (the observation average exceeds twice the respective negative control).
[c]Cytotoxicity.

The effects of the four engine operating conditions were examined using the results of the analysis performed on the samples collected for the engine equipped with the muffler; the mutagenic activity was found to be positive for the samples collected for the R50 and I50 conditions, but the samples collected at the I100 and R100 conditions were not mutagenic (Figure 2).

The effects of the DOC were compared with those of the muffler for all four test conditions. Figure 3 shows that for the R50 and I50 conditions the muffler samples showed a positive mutagenicity while the DOC samples exhibited a negative response. For the R100 and I100 conditions, the mutagenic activity was negative and did not measurably differ between the DOC and muffler samples.

The effects of the muffler, three types of DFE, and SM DPF were compared using the results obtained for the cases when the engine was equipped with those devices and operated at R50 conditions (Figure 4). The results showed positive mutagenic activity for DFE-A, LDFE-A, DFE-B, and SM DPF samples.

A comparison of normalized mutagenic activities based on the number of revertants/m^3 of air for Muffler, DFE-A, LDFE-A, DFE-B, and SM DPF for mode R50 are shown in Table 4. DFE-A, LDFE-A, DFE-B, and SM DPF samples showed a 71, 34, 30 and 62% reduction respectively in the number of mass-normalized revertants from the muffler samples.

DISCUSSION

This study demonstrates that DPM emitted by a naturally aspirated, indirectly injected engine in a realistically simulated mining workplace environment was mutagenic for some steady-state operating conditions. The results indicate that the engine operating condition is one of the dominant parameters affecting the mutagenic activity of DPM. For the engine equipped with the muffler, the DPM samples collected at light-load engine operating modes (R50 and I50) were mutagenic. No mutagenic activity was found for samples collected at heavy-load engine operating modes (R100 and I100). While the study used only four speed and load conditions, the dependence of mutagenicity on operating conditions is similar to a systematic study of an engine evaluated over a full range of operating variables (McMillian et al., 2002). In the limiting case of high speed and high loading, the mutagenicity of the DPM in that study was not eliminated, as in this study, but did become minimal. The maximum in the

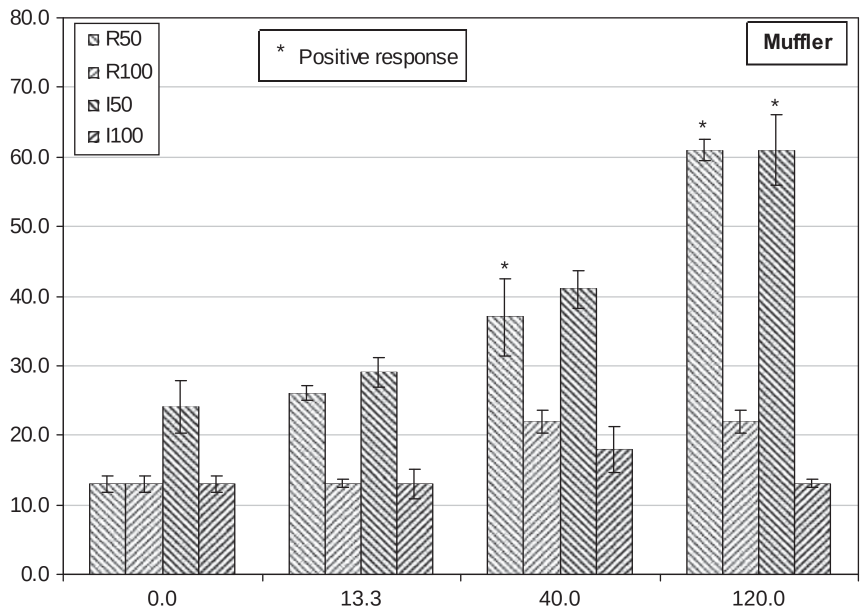

FIGURE 2. Mutagenic activity of DPM samples collected for the engine equipped with the muffler and at four engine operating conditions. Positive response was when the observation exceeded twice the respective negative control.

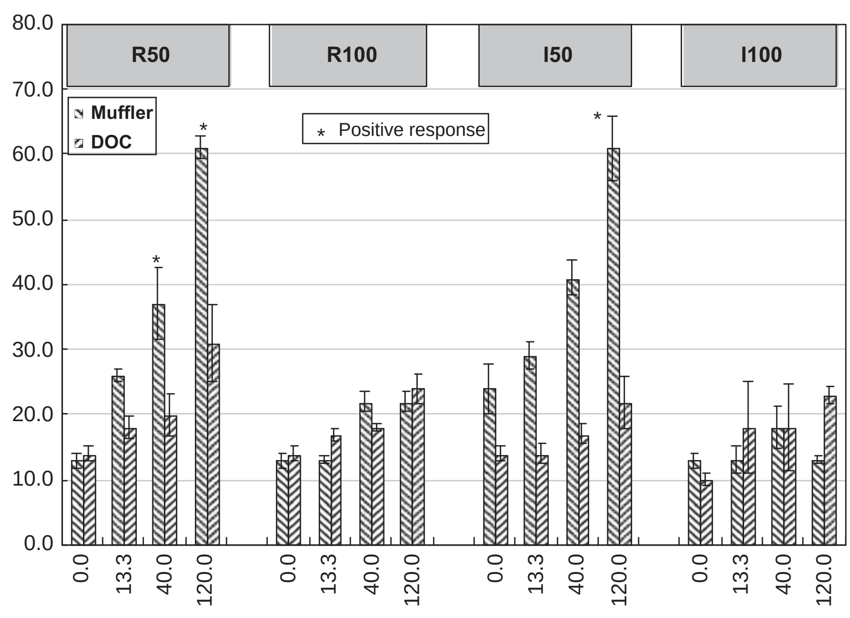

FIGURE 3. Comparison of the mutagenic activity between DOC and muffler samples for all four engine operating conditions. Positive response was when the observation exceeded twice the respective negative control.

graph of mutagenicity versus load also peaked near 50% load in that study. The potential interpretation is that the combustion at partial loads is less complete than that at higher loads, resulting in the higher emissions of organic compounds that exhibit higher mutagenicity. A detailed explanation of this effect is outside the scope of this study and should be addressed in combustion chemistry studies.

The DFE systems and SM DPF system reduced the concentration of DPM in the sampled aerosol, ranging from 51 to 71%. The

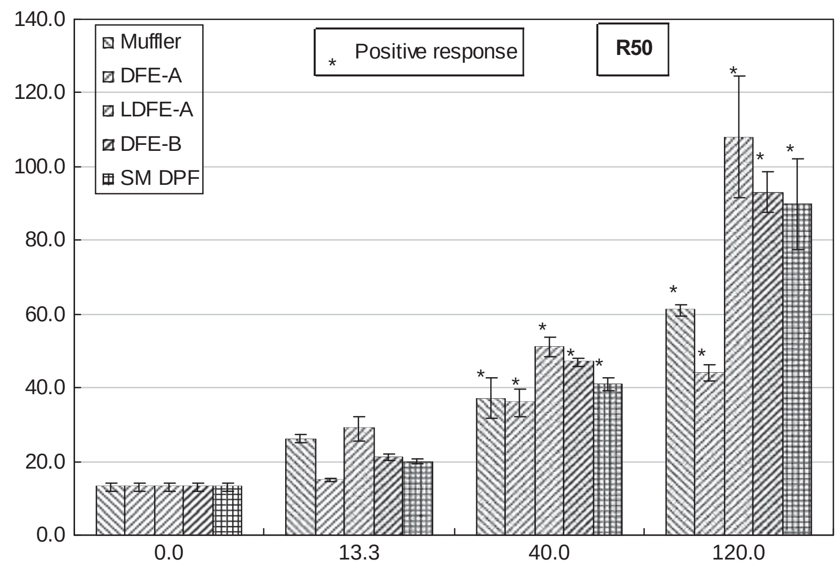

FIGURE 4. Effects of muffler, DFE-A, LDFE-A, DFE-B, and SM DPF on the mutagenicity of samples collected for R50 engine operating condition. Positive response was when the observation exceeded twice the respective negative control.

TABLE 4. Reductions in Mass-Normalized Ames *Salmonella*/Microsomal Assay Mutagenic Activity for R50 Test Conditions

Exhaust system	Average concentrations (μg/m^3)	Mutagenic activity (Revertants/μg)	Mutagenic activity (Revertants/m^3)	Reduction in concentration (%)	Reduction in mutagenic activity (%)
Muffler	239	0.5	117.1	N/A	N/A
DFE-A	107.9	0.3	34.5	54.9	71.
LDFE-A	89.5	0.9	76.9	62.6	34.3
DFE-B	117.9	0.7	82.5	50.7	29.5
SM DPF	69.4	0.7	45.1	71.0	61.5

reductions in mutagenicity did not always parallel the reductions in DPM concentrations in the exhausts. In one case, the laundered DFE lowered the concentration of DPM more than the unused DFE, but the mutagenicity increased. When the DOC was used, the samples showed no marked mutagenic activity, although the reduction in DPM concentration was comparable to the other devices. Those results can be explained by catalytic oxidation of the organic fraction of particulate matter by DOC (Pataky et al., 1994). The results of this study suggest that despite only moderate effects on mass concentrations (Bugarski et al., 2009), the DOC might play important role in reducing potential adverse health effects associated with exposure to diesel aerosols.

When compared with DFE systems and DPF systems, the DOC exerted minor effects on aerosol mass and number concentrations (Bugarski et al., 2009). However, the DOC were found to be effective in reducing soluble organic fraction (SOF)/organic carbon (OC) fractions of diesel particulate matter (DPM) and vapor-phase organics (XOC)/gaseous-phase hydrocarbons emitted by heavy-duty (Bagley et al., 1998; Shah et al., 2007) and light-duty diesel engines (Klein et al., 1998). The DOC lowered concentrations of PAH associated with SOF fraction and OC (Pataky et al., 1994; Khair & McKinnon, 1999). The effectiveness of the DOC control was dependent on the fraction of OC present in the engine exhaust, with total PM reduction efficiency increasing with increasing OC content (Shah et al., 2007). Previous studies (Pataky et al., 1994; Bagley et al., 1998) showed that use of DOC reduced both particle- and vapor-phase-associated mutagenic activity by 50% or more. The DOC was found to generally increase the mutagenic activity per mass of the SOF and XOC; however, the rise was offset by the decrease in the SOF and XOC levels (Pataky et al., 1994). The 50% or more of the detected mutagenic activity was attributed to XOC (Pataky et al., 1994).

The DFE and DPF systems were found to be effective in reducing mass emissions of DPM (Shah et al., 2007) and consequently can be effectively used to lower concentrations of DPM in mine air (Bugarski et al. 2009). This control technology was effective in reducing both the elemental carbon (EC; 97%) and organic carbon (OC; 92%) fraction (Shah et al., 2007). The mutagenic activity of the DPM samples collected for DFE and the uncatalyzed SM DPF was primarily associated with hydrocarbon-rich aerosols that passed through DFE and DPF in a vapor phase and nucleate after leaving tailpipe. Those aerosols are semivolatile in nature (Bugarski et al., 2009). Further research is needed to investigate the effects of DOC and DPF systems and to extend these findings to other engine types and potential alternative diesel fuels.

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