PAPER

In vitro genotoxicity of exhaust emissions of diesel and gasoline engine vehicles operated on a unified driving cycle

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Acetone extracts of engine exhaust particulate matter (PM) and of vapor-phase semi-volatile organic compounds (SVOCs) collected from a set of 1998–2000 model year normal emitter diesel engine automobile or light trucks and from a set of 1982–1996 normal emitter gasoline engine automobiles or light trucks operated on the California Unified Driving Cycle at 22 °C were assayed for *in vitro* genotoxic activities. Gasoline and diesel PM were comparably positive mutagens for *Salmonella typhimurium* strains YG1024 and YG1029 on a mass of PM extract basis with diesel higher on a mileage basis; gasoline SVOC was more active than diesel on an extracted-mass basis, with diesel SVOC more active on a mileage basis. For chromosomal damage indicated by micronucleus induction in Chinese hamster lung fibroblasts (V79 cells), diesel PM expressed about one-tenth that of gasoline PM on a mass of extract basis, but was comparably active on a mileage basis; diesel SVOC was inactive. For DNA damage in V79 cells indicated by the single cell gel electrophoresis (SCGE) assay, gasoline PM was positive while diesel PM was active at the higher doses; gasoline SVOC was active with toxicity preventing measurement at high doses, while diesel SVOC was inactive at all but the highest dose.

Introduction

Diesel engines are an important component of the industrial and transportation sectors, including numerous industrial, mining, agriculture and construction uses. There are concerns with diesel exhaust emissions in regards to potential adverse health effects from occupational and environmental exposures. Based on reviews of the literature, the National Institute for Occupational Safety and Health,[1] the International Agency for Research on Cancer[2] and the US Environmental Protection Agency,[3] have declared diesel exhaust a potential or probable human carcinogen.

Epidemiological studies of diesel emissions and lung cancer risk have been reviewed recently.[3,4] Studies of occupational exposures to diesel exhaust have included railroad workers[5,6] and truck drivers.[7–10] *In vivo* animal studies of diesel exhaust particulate material (DEP) have confirmed lung tumors in the rat from long-term inhalation exposures.[11,12] However, the genotoxicant role of DEP in inhalation tumorogenicity studies has been questioned after comparable tests of non-genotoxic carbon black resulted in tumorogenesis in the same animal models.[13,14] Numerous *in vitro* studies have shown mutations in bacterial strains, and chromosomal and DNA damage in mammalian cells from DEP solvent extracts.[2,15] While *in vitro* bioassays of organic solvent extracts of DEP appear not to model the biological availability in the lung of DEP-bound organic genotoxicants,[16] it has been demonstrated that DEP can express *in vitro* genotoxic activities as non-extracted particulate dispersions in the primary components of the surfactants which line the lung small airways and airspaces.[17–21]

Gasoline engine exhaust genotoxicities have been studied since the 1930s;[22] however, the literature is not as extensive as that for diesel engine emissions.[2] Bacterial mutagenicity for gasoline exhaust has been reviewed by Claxton,[23] and short-term bioassay results have been reviewed by Lewtas.[15] Most studies show positive results in bacterial mutation, as well as many higher-tier bioassays. While results were often quantitatively similar for gasoline or diesel engine exhaust particulate matter (PM) when normalized to mass of PM or mass of solvent extract of PM,[15] emissions per mile from diesel engine vehicles generally were much greater than from comparably-sized gasoline engine vehicles.[2]

In the current study, gene mutation in bacteria, chromosomal damage as micronucleus (MN) induction in mammalian cells and DNA damage by the single cell gel electrophoresis assay in mammalian cells were performed on solvent extracts of diesel or gasoline engine exhaust particulate matter (PM) or vapor-phase semi-volatile organic compounds (SVOCs), as part of a US Department of Energy multi-institutional study of the exhaust toxicities of modern gasoline, diesel and natural-gas mobile vehicle engines.[24–26] Diesel PM extract, diesel SVOC extract, gasoline engine PM extract and gasoline engine SVOC extract used for the study were obtained from automobile or light truck vehicles operated on the California Unified Driving Cycle[27] at 22 °C (72 °F). Comparison measurements were made also on the extract of a standard diesel

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exhaust particulate material: National Institute of Standards and Technology (NIST) Standard Reference Material SRM1650a.

Materials and methods

Sample collection

Filter-collected particulate material samples and sorbent resin-absorbed vapor phase semi-volatile organic compounds were collected from the exhausts of gasoline engine or diesel engine light trucks and automobiles, listed in Table 1, under a program sponsored by the US Department of Energy, Office of Heavy Vehicle Technologies (now, the Office of Freedom-CAR & Vehicle Technologies). The vehicles belonged to the staff of Southwest Research Institute (SwRI; San Antonio, TX).[24] Vehicles were operated on a chassis dynamometer using the California Unified Driving Cycle. Exhaust PM and SVOC were collected and pooled from five normal-emitter spark-ignition gasoline engine automobile or pickup truck vehicles, 1982 to 1996 models, operating at 72 °F ambient temperature. Samples were also similarly collected and pooled from three diesel engine automobile or pickup truck vehicles, 1998 to 2000 models operated at the same ambient temperature. Engine emission rates averaged for the pooled samples are listed in Table 2. Sample collection was performed by SwRI. Samples were acetone-extracted by Desert Research Institute (Reno, NV).[26] Collected exhaust PM was washed from filters with acetone and shipped to NIOSH as suspensions. SVOC were extracted from sorbent resins with acetone and shipped as solutions. Parallel samples were provided to the Lovelace Respiratory Research Institute (LRRI) for mutagenicity assay of combined PM and SVOC extract using *Salmonella typhimurium* TA98 and TA100, and for a set of *in vitro* and *in vivo* animal instillation toxicity assays.[25]

Treatment of samples

After receipt, PM samples in acetone were filtered through Whatman No. 1 filter paper and the extracts evaporated to dryness or a minimum volume under N_2. TWEEN 80 was added at twice the mass of a sample extract in a brown glass vial, the vial agitated for 30 min in a sonication bath, sterile de-ionized H_2O was added until the TWEEN 80 concentration was 4%, and sonication continued until homogenization of the sample extract was evident.[28] Before use each sample was diluted serially with sterile deionized water to desired concentrations.

SVOC fractions also were evaporated to dryness or a minimum volume under N_2 and the samples treated the same as the particulate samples with TWEEN 80 and sonication.

Table 1 Exhaust samples were collected from five gasoline engine vehicles and three diesel engine vehicles[a]

Vehicles	Year	Model	Odometer reading
Gasoline engine	1982	Nissan Maxima	190 203
	1993	Mercury Sable	70 786
	1994	GMC 1500 pickup truck	68 325
	1995	Ford Explorer	76 733
	1996	Mazda Millenia	35 162
Diesel engine	1998	Mercedes Benz E300	47 762
	1999	Dodge 2500 pickup truck	37 242
	2000	Volkswagen Beetle TDI	7455

[a] Four pooled samples were prepared for assay: gasoline exhaust PM or SVOC; diesel exhaust PM or SVOC.

Table 2 Emission rates and extraction fractions for motor vehicles

Gasoline PM	13.3 mg mile^{-1}	0.554 mg extract (mg PM)$^{-1}$
Gasoline SVOC	2.28 mg mile^{-1}	
Diesel PM	139 mg mile^{-1}	0.363 mg extract (mg PM)$^{-1}$
Diesel SVOC	19.0 mg mile^{-1}	

NIST SRM1650a, a standardized DEP positive control, was treated with acetone, filtered, and evaporated the same as the vehicle exhaust samples, and treated with TWEEN 80 and sonication as above.

Salmonella gene mutation assay

This study used the microsuspension preincubation method, with *Salmonella typhimurium* strains YG1024 and YG1029 obtained from Dr Takehike Nohmi at the National Institute of Hygiene Sciences, Tokyo, Japan. Positive controls were 2-aminoanthracene (2AA) (with activation) and 1-nitropyrene (1NP) (without activation), both from Sigma Aldrich, St. Louis, MO. The microsomal mixed function oxidase system (S9, from Arochlor 1254-induced male SD rats) was purchased from MOLTOX (Research Triangle Park, NC). Chemicals, culture media, and serum were from Sigma–Aldrich.

Oxoid #2 nutrient media (50 ml) containing 0.2 ml ampicillin solution (6.25 mg ml^{-1}), 0.2 ml tetracycline solution (1.56 mg ml^{-1} and 0.1 ml of thawed tester strains (YG1024 or YG1029) were added into each of two 125 ml culture flasks. The cultures were placed in a 37 °C gyrating incubator, and shaken at approximately 120 rpm for 16 h. These overnight cultures were transferred to 50 ml centrifuge tubes and centrifuged at 3000 rpm for 20 min at 4 °C, the supernatant liquid discarded, and the cultures were resuspended in physiological saline to a concentration of approximately 4×10^9 cells ml^{-1}. Test samples (10 µl), along with 65 µl of physiological saline or S9 mix, and 25 µl of the overnight cultures were pre-incubated at 37 °C for 30 min on a rotary shaker. Following pre-incubation, 2.5 ml of molten top agar (at 45 °C) were added to each sample tube, the contents mixed, and immediately poured onto a Vogel–Bonner minimal glucose plate, and incubated at 37 °C for 48 h (YG1029) or 66 h (YG1024). Three readings of revertant colonies on each plate were scored by automatic colony counter (Accucount 1000, Biologics Inc., Gainsville, VA), and the average of the readings was recorded. The average number of revertant colonies and standard deviation per plate for at least two plates were calculated. Each sample was tested twice in separate experiments. The background bacterial lawn was checked regularly by light microscopy. The criterion for a positive test result was that the number of revertant colonies on the plates containing test sample was at least twice the number for the solvent control and that a clear dose–response relationship was evident.

Single cell gel electrophoresis (SCGE) assay

Exponentially growing V79 cells were seeded into 25 cm^2 flasks (1.5×10^6 per flask) with 5 ml of complete Dulbecco's minimal essential medium (DMEM; Gibco, Grand Island, NY) containing 10% of heat-denatured fetal bovine serum (FBS; Sigma–Aldrich), 2 mM of L-glutamine (Sigma–Aldrich), 100 U ml^{-1} of Penicillin (Gibco) and 100 µg ml^{-1} of Streptomycin (Gibco). Following overnight incubation, cells were challenged with samples suspended in complete DMEM for 20 h, then rinsed with phosphate-buffered saline (PBS; Gibco) and harvested by trypsinization. Cytotoxicity of V79 cells challenged by the exhaust samples was detected by cell density measurement and viability was determined using the trypan blue exclusion assay. The cell suspensions were centrifuged for 10 min at $300 \times g$ and the cell pellets were resuspended in 0.5 ml of complete

DMEM medium (approx. 1.5–2 × 10^6 ml^{-1}). Tubes containing the resuspended cells were kept on ice for the experiments described below. The SCGE assay was performed according to the procedure of Tice *et al.*[29] with minor modifications. Each slide well for each treatment in the assay used 1.5–2 × 10^4 cells. The electrophoresis was conducted at exactly 300 mA, at approximately 25 volts (0.7 V cm^{-1}), for 30 min at room temperature. Tail length measurements were done using fluorescence microscopy with a calibrated reticule, and were done for 25 cells per slide, with 4 replicate slides per treatment.

Micronucleus assay

Cell suspensions used to make slides in SCG assay were diluted 1 : 4 in physiological saline (approx. 4–5.0 × 10^5 cells ml^{-1}). A Cytospin 3 (Shandon, Pittsburgh, PA) was used to make the slides. An aliquot of cell suspension (75 µl) was loaded into the Cytospin chamber and spun at 500 rpm for 5 min. The slides were dried on a slide warmer at 37 °C for 10 min, fixed and stained with Diff-Quick solution (Dade–Behring, Deerfield, IL), using steps of 10 s fixation, 20 s in Diff-Quick solution I, 20 s in Diff-Quick solution II and one rinse in distilled water. Four slides were made from each treatment, two from each of the duplicate treatment flasks. One thousand cells were scored from each slide; 4000 cells were scored for each treatment. The criteria for MN scoring were similar to those reported by Countryman and Heddle[30] and Roberts *et al.*[31] Briefly, the diameter of the MN must be no larger than one-third the main nuclei. The MN must be non-refractile, thus excluding small stain particles. The color of MN must be the same as or brighter than the main nuclei, and MN must be located within the cytoplasm but not in contact with the main nuclei.

Data analyses

Analyses of the *Salmonella* assay results used a lack-of-fit test repeatedly applied to each sample; and the data for higher doses were deleted until there was an adequate linear trend for a subset of the data with at least three doses.[32] This serves to eliminate the effect of cellular toxicity from the mutagenicity–dose relationship. Three to six dose levels were retained for each of the samples. The remaining data produced a statistically significant linear positive slope estimate for each of the groups and factor combinations. Weighted least-squares were used in the slope estimation, because the variances were not homogeneous over the dose ranges. Table 4 contains the slope estimates, in terms of revertants per µg for each factor combination.

SCGE assay data for DNA damage were transformed from a continuous variable (tail length) to a categorical variable. If a cell had a positive tail in the comet assay it was assigned a value of 1. If it had no tail it was assigned a value of 0. This eliminates any problem of scale and variability from one SCGE assay to the next. Analysis of variance techniques were used for analysis of the transformed data.

Micronucleus induction was plotted as the number of micronucleated cells per 1000 cells, *versus* dose in µg ml^{-1}, and also normalized to dosage expressed in vehicle miles traveled. A positive dose–response relationship was found for all samples except the diesel SVOC, but a linear relationship was evident only for the diesel PM samples.

Results

Results of the *Salmonella* gene mutation assay are shown in Table 3, based on two replicate plates per experiment for each treatment, and two duplicate experiments. Results are presented for diesel exhaust particulate extract (DP), for spark-ignition gasoline engine exhaust particulate extract (GP), for diesel exhaust SVOC extract (DSVOC), for gasoline engine SVOC extract (GSVOC), and for NIST SRM 1650a PM extracts. Results for all positive controls, both with and without microsomal activation were acceptable. All samples induced mutations in both YG1024 and YG1029. The increase in mutation frequencies was highly significant and increased in a dose-related manner for all of the samples tested. In most cases, the increase was higher with S9 than without S9 activation. The PM extract is more mutagenic than the SVOC extract.

Table 4 contains the slope estimates for the rate of change in the revertants with dose for each factor combination and group. The estimates for GP, DP and SRM 1650a in the first factor combination, 59.5, 56.8 and 72.2 are joined by the letter 'A' to show that they are not statistically different from one another ($p = 0.76$, 0.51 and 0.42, respectively). The estimates for GP and DP in the next factor combination are significantly different with p-values of 0.03 and 0.02, respectively, but neither was significantly different from the NIST sample (codes B and C in Table 4; $p = 0.66$ and 0.41, respectively). All other estimates within the third and fourth factor combinations are statistically different with p-values <0.001, except that the SRM 1650a samples are not different from the GP sample for the YG1029−S9 assay ($p = 0.24$), and the SRM 1650a sample was not different from the DP sample in the YG1029+S9 assay ($p = 0.08$); these are codes D and E in Table 4. Results were converted to revertants per vehicle mile from revertants per µg of extract by multiplying by the µg of extract per mg of particulate material, and then multiplying by the mg of total PM per mile;[26] the values used in this study are included in Table 2. SVOC mass amounts per vehicle mile were taken from the same report.

Results from the SCGE assay of DNA damage in V79 cells were scored and recorded as tail lengths in µm; but due to the high frequency of zero readings in some samples, the data were transformed into a categorical variable that had two states, "damaged" and "undamaged"; any cell with a nonzero tail was classified as damaged, and zero length tails were classified as undamaged. Data from replicate experiments were pooled and the results are displayed in Fig. 1a and 1b; these show the percent of cells expressing DNA damage in the single-cell gel electrophoresis assay *versus* dose as extract mass or vehicle miles. Analysis of variance established a positive dose-response of DNA damage with the gasoline and diesel particulate extract samples, as did the gasoline SVOC sample, but the diesel SVOC fraction did not exhibit a significant dose–response relationship under the conditions of this study.

All of the samples had a dose-related response in the micronucleus assay, except the diesel SVOC sample. The PM and SVOC fractions of gasoline exhaust and the NIST PM were stronger inducers of micronuclei in V79 cells in comparison with the diesel PM sample, as shown in Fig. 2a and 2b. However, the activities for gasoline PM and diesel PM extracts were more comparable when converted to micronucleated cells per mile. The diesel SVOC sample did not induce significant micronuclei, even at very high concentrations. Due to the nonlinear nature of the dose–response patterns (excepting the diesel PM), numerical comparisons of the strengths of dose–response relationships were not possible.

Discussion

Exhaust products of gasoline and diesel engine vehicles have been examined for their potential for induction of adverse genetic effects for almost 50 years. In this time, engine designs and emissions controls have undergone several evolutions, and many *in vitro*, animal model, and epidemiological studies have been performed. This study was designed to address in part the question of the *in vitro* genotoxic activities of exhaust emission materials from some typical current-use spark-ignition gasoline engine and diesel engine light-duty vehicles. Multiple genotoxic

Table 3 Induction of mutation in *Salmonella* by extracts of gasoline engine exhaust particulate GP, diesel particulate DP, gasoline exhaust semi-volatile organic compounds GSVOC, diesel exhaust semi-volatile organic compounds DSVOC, and NIST SRM 1650a standard diesel particulate material[a]

		YG1024		YG1029	
Sample	Concentration/μg plate^{-1}	−S9 Colony number[b]	+S9 Colony number	−S9 Colony number	+S9 Colony number
TWEEN 80	0	38 ± 5	56 ± 13	115 ± 18	135 ± 28
GP	1.48	197 ± 50	90 ± 21	159 ± 4	148 ± 14
GP	4.44	355 ± 115	211 ± 42	224 ± 22	204 ± 14
GP	13.3	667 ± 190	590 ± 59	375 ± 34	461 ± 32
GP	40.0	1011 ± 189	1171 ± 188	583 ± 27	853 ± 48
GP	120.0	1298 ± 49	1373 ± 95	882 ± 101	1030 ± 96
DP	1.48	141 ± 35	113 ± 15	155 ± 7	185 ± 34
DP	4.44	273 ± 58	209 ± 23	191 ± 7	323 ± 17
DP	13.3	459 ± 107	548 ± 75	299 ± 33	882 ± 53
DP	40.0	797 ± 85	1238 ± 163	552 ± 21	1485 ± 119
DP	120.0	719 ± 88[c]	1625 ± 41	725 ± 363[c]	1256 ± 222[c]
TWEEN 80	0	44 ± 3	50 ± 7	101 ± 14	105 ± 16
GSVOC	4.44	76 ± 23	67 ± 10	130 ± 17	134 ± 16
GSVOC	13.3	122 ± 19	109 ± 15	179 ± 6	170 ± 8
GSVOC	120.0	165 ± 14	390 ± 32	264 ± 44[c]	509 ± 4
GSVOC	360.0	262 ± 63[d]	257 ± 19[c]	409 ± 64[d]	525 ± 24[c]
DSVOC	13.3	73 ± 10	65 ± 8	110 ± 13	127 ± 6
DSVOC	40.0	118 ± 5	102 ± 6	145 ± 11	184 ± 28
DSVOC	120.0	188 ± 16	245 ± 21	177 ± 9	303 ± 38
DSVOC	360.0	362 ± 15	459 ± 27	331 ± 23	447 ± 22
DSVOC	1080.0	635 ± 64	755 ± 52	533 ± 12	562 ± 24
TWEEN 80	0	62 ± 8	65 ± 5	99 ± 3	97 ± 12
NIST	1.48	186 ± 6	138 ± 27	120 ± 6	141 ± 12
NIST	4.4	353 ± 52	250 ± 69	159 ± 12	256 ± 12
NIST	13.3	776 ± 103	706 ± 175	395 ± 13	782 ± 58
NIST	40.	1360 ± 303	1523 ± 623	929 ± 100	1705 ± 117
NIST	120	2470 ± 586	2324 ± 623	2220 ± 215	2270 ± 37

[a] Values are given ± one standard deviation with $N = 4$. [b] Average number of revertant colonies per plate. [c] Toxic. [d] Highly toxic.

endpoints were examined for pooled exhaust materials from a set of currently-utilized diesel or gasoline engine automobiles or light-duty trucks. It should be noted that mobile vehicle engines are in a state of rapid evolution and improvement in emissions reduction technology so the vehicles sampled in this study do not necessarily represent the best emission control capabilities now available.

The diesel engine vehicles sampled consisted of 2 low-mileage late model autos and 1 light truck, model years 1998–2000, each with mileage <50 000 miles; the spark-ignition engine vehicles consisted of 2 autos and 3 light trucks or vans ranging from a 1982 model with >190 000 miles to 1996 models with <36 000 miles. None of the vehicles were "white smokers" or "black smokers". While ideally the gasoline and diesel vehicles would be more closely matched in number of vehicles and age and mileages, this selection of vehicles from those available for the study is reasonably typical of personal vehicles in use *ca*. 2000. In particular, all the gasoline engine vehicles used catalytic converter exhaust aftertreatment, while one diesel vehicle used an oxidation catalyst aftertreatment, and the other two diesel vehicles used no exhaust aftertreatment. Diesel PM emission rates observed in this study are much higher than those which will be permitted under USEPA regulations being phased-in through 2009 for new diesel vehicles. Comparisons were made also with similarly extracted standard diesel exhaust particulate material SRM 1650a from NIST, typical of heavy-duty diesel engine particulate emissions of the early 1980s.

Bacterial gene mutation and mammalian cell chromosomal and DNA damage were assayed on the acetone extract of a pooled particulate sample and on a pooled semi-volatile organics sample for the diesel and for the spark-ignition gasoline engine sets of vehicles operated on the California Unified Driving Cycle, and on a similarly prepared extract of NIST SRM 1650a standard diesel exhaust particulate material. Results indicate many similarities as well as differences between the *in vitro* genotoxic activities of the two sets of vehicles and between the particulate and SVOC materials. All fractions of all materials, PM and SVOC, were highly mutagenic in the *Salmonella* reversion assays in both tester strains and conditions of the assay, with and without microsomal activation. Mutagenic activities expressed were similar on a mass basis for the gasoline and diesel engines particulate extracts; mutagenic activities of the NIST SRM particulate material extract, which represents older, 1980s diesel technology, were somewhat stronger. When normalized to doses based on vehicle miles, however, the mutagenicity *versus* dose slope estimates become much larger for the diesel exhaust, based on the greater engine exhaust emission production rate, often almost an order of magnitude greater for the diesels *versus* the spark-ignition engine emissions. The SVOC extracts in general were far less potent mutagens than were the PM extracts. The effect of the S9 microsomal fraction was complex, with the YG1024 strain generally showing very similar or smaller dose–response mutagenicity slopes with the S9 addition, while the YG1029 strain

Table 4 Mutagenic activity per mass of extract and per vehicle mile for GP, DP, GSVOC, DSVOC and NIST SRM 1650a

Factor combination	Group	Slope estimate/ revertants (μg extract)$^{-1}$	Slope estimate/ revertants × 10^3 mile^{-1}
YG1024−S9	GP (gasoline)	59.5^{A}	440
	DP (diesel)	56.8^{A}	2866
	G-SVOC	6.1	13.9
	D-SVOC	1.9	36
	NIST (SRM 1650a)	72.2^{A}	—
YG1024+S9	GP	39.0^{B}	289
	DP	32.5^{C}	1640
	G-SVOC	4.9	11.2
	D-SVOC	1.4	26.6
	NIST	46.3^{BC}	—
YG1029−S9	GP	19.4^{D}	144
	DP	13.1	661
	G-SVOC	5.7	13
	D-SVOC	1.0	19
	NIST	21.8^{D}	—
YG1029+S9	GP	17.7	131
	DP	43.3^{E}	2185
	G-SVOC	5.2	11.9
	D-SVOC	1.8	34.2
	NIST	35.3^{E}	—

Table entries having the same superscript labels (A through E) are not significantly different ($p < 0.05$).

did show significantly increased responses for the diesel particulate and SVOC extracts with microsomal fraction addition.

Parallel exhaust materials were tested for *Salmonella* gene mutation using tester strains TA98 and TA100, by other labs in the US Department of Energy study of these exhaust materials.[25] That study design also differed from the current study by testing the PM extract recombined with the SVOC extract in the case of both gasoline and diesel exhaust. Other variations were that the solid residue of acetone-rinsed PM was left in the test preparations, and test materials were transferred into dimethylsulfoxide (DMSO) as the test medium while the current study used TWEEN 80. In the current study, YG bacterial strains were selected for their enhanced sensitivity, and for their enhanced *o*-acetyltransferase activity, in the order of 100-fold of that in the parent TA98 and TA100 strains, so S9 activation may be less necessary.[33] In general, all materials were active for bacterial gene mutation in both studies, with gasoline exhaust activity about equal to diesel in TA98 or YG1024 assays, and diesel exhaust generally stronger than the gasoline in the TA100 or YG1029 assays. In the TA98 or YG1024 system, gasoline exhaust activity was approximately equal to the diesel in the absence of S9 activation, and was slightly increased above diesel with S9 activation; however, the overall effect of S9 activation was to increase activity in the TA98 system and to decrease activity in the YG1024 system. In the TA100 system, diesel exhaust activity was about 3-fold greater than gasoline, and S9 activation did not significantly alter the activities; while in the YG1029 system the diesel exhaust activity was slightly lower than the gasoline without S9; but the diesel was increased 3-fold with S9 activation while the gasoline exhaust activity was not increased. This suggests that the gasoline and diesel exhaust extracts induced both frameshift and base-pair substitution mutations, with the diesel more active for the latter type. These results generally indicate that *in vitro* bacterial genotoxic activities of engine exhaust materials can be comparable on a mass of extract basis for spark-ignition gasoline engines and diesel engines, but are much stronger for diesel on a mileage basis because of the much higher emission rates per mile for the diesel vehicles in this study.

Mammalian cell assays for DNA or chromosomal damage exhibited some qualitative differences between the diesel and gasoline extract samples distinct from the bacterial cell findings. DNA damage as measured by the SCGE "comet" assay was positive for gasoline engine exhaust PM, but diesel PM was comparatively weak on a mass basis, while comparable on a mileage basis, as shown in Fig. 1. Micronucleus induction in V79 cells did not parallel the SCGE results for all samples: the gasoline PM and gasoline SVOC extract activities for micronucleus induction were similar on a mass basis; this was the only case where SVOC activity approached that of the corresponding PM extract activity. Gasoline PM was much stronger than diesel PM extract for micronucleus induction on a mass basis, but was comparable on a mileage basis. However, diesel SVOC was inactive for micronucleus induction. The NIST sample was positive in this micronucleus assay, but with complex dose–response behavior, with activity initiating at the middle dose level. The results indicate that these gasoline engine exhaust materials were much stronger than the diesel exhaust materials on a mass basis for some mammalian cell genotoxic activities, with the gasoline exhaust activities comparable to or sometimes greater than the diesel exhaust activities on a mileage basis. This is not inconsistent with some past studies of earlier generation vehicles,[34] which saw significant mammalian cell genotoxic activities for gasoline engine exhaust extracts. One significant difference in the older studies was their use of leaded gasoline fuel, standard at the time. The current study suggests that leaded fuel use is not necessary for the production of gasoline exhaust extract genotoxic activity.

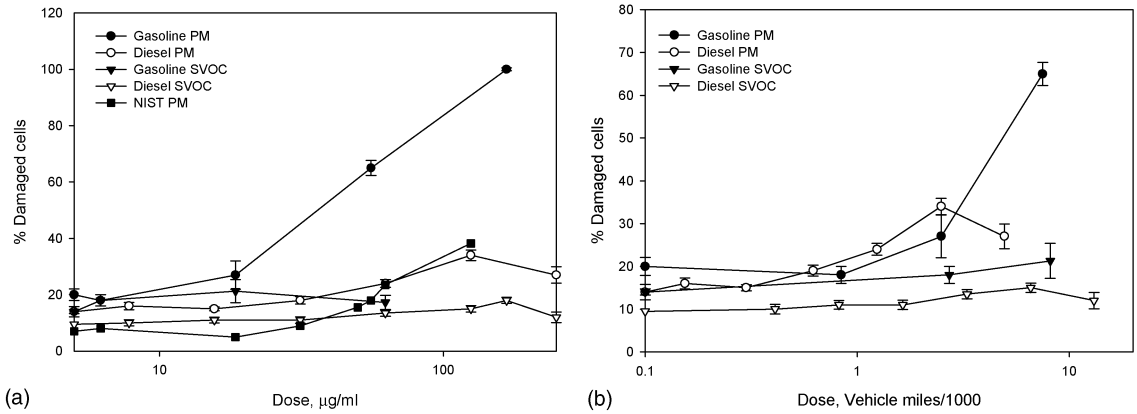

Fig. 1 Fraction of damaged cells from the SCGE assay *vs.* dose: (a) dose as μg extract per ml of culture medium; (b) dose as vehicle miles per ml of culture medium. Error bars are standard error of the means: $N = 8$ for all gas and diesel PM and SVOC; $N = 12$ for NIST. The abscissa scale is logarithmic. Values for the negative controls (zero dose) are shown on the ordinate.

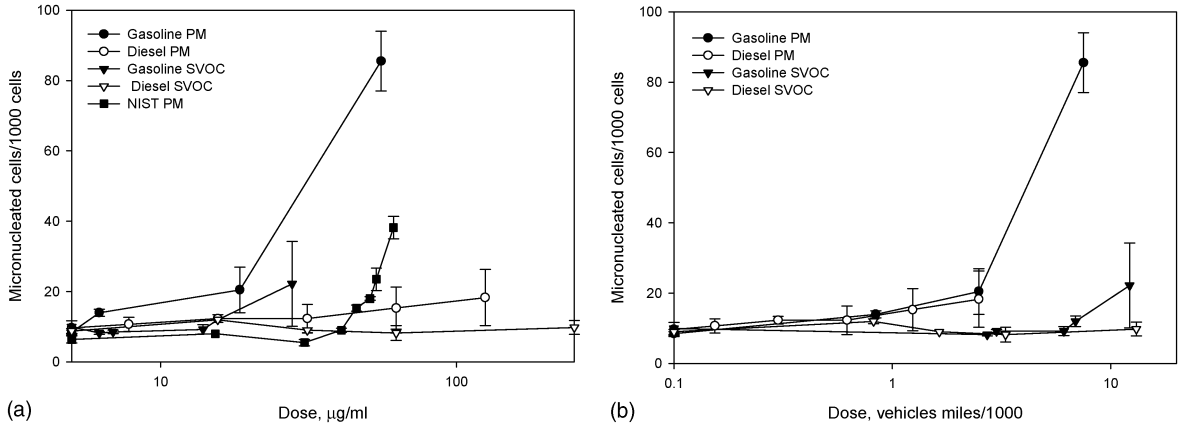

Fig. 2 Micronucleus induction expressed as micronucleated cells per 1000 V79 cells *vs.* dose expressed as (a) μg extract per ml of culture medium or as (b) vehicle miles per ml of culture medium. Error bars are standard error of the means: *N* ranging from 2 to 5. The abscissa scale is logarithmic. Values for the negative controls (zero dose) are shown on the ordinate.

Chemical characterization was done for this same set of vehicles in a separate study,[26] which included both particulate and gas-phase sampling. One finding was that the diesel exhaust particulate fraction contained much higher levels of nitro-polyaromatic hydrocarbon compounds (nitro-PAHs), which have been demonstrated to be a major contributor to the mutagenicity of diesel exhaust in the *Salmonella* mutagenicity assay.[35–37] The detailed chemical analysis of the gasoline engine exhaust showed generally lower concentrations of most analytes,[26] relative to diesel exhaust, with the exception of PAHs, especially high molecular weight compounds such as indeno[*cd*]perylene, benzo[*ghi*]perylene and coronene, which has also been observed in earlier studies.[38,39] This may at least partially explain why the micronucleus and SCGE assay results were consistently stronger for the gasoline exhaust PM, while the diesel PM was comparable on a mass basis in the *Salmonella* mutagenicity assay. Additional fractionation and preparation would be required before genotoxicity studies could test this hypothesis.

The use of a unified driving cycle in these tests provides a reasonable representation of average emission materials under one considered model of typical vehicle use. The California Unified Driving Cycle used in this study is not the Federal Test Procedure (US Code of Federal Regulations, Title 40, Part 86), which is used by manufacturers for USEPA emissions certification. Genotoxic activities of exhaust products from these or any vehicles can be expected to change with the specific mode of operation. Under steady state operating conditions, diesel exhaust genotoxic activity can be strongly affected by conditions of operation, *e.g.*, engine torque, rpm and fuel injection timing.[40] Detailing the effects on exhaust genotoxicant composition of these engine operational parameters, or of changing these parameters during vehicle operation as in acceleration, deceleration, or conditions of changing load, requires a different experimental design. Other factors that may affect the emissions include the fuel used and the effects of engine and exhaust aftertreatment system design; and vehicle age and maintenance can greatly affect emissions, *e.g.*, "white smokers" and "black smokers".[25] Diesel engine exhaust control technology is rapidly evolving; compared to the PM emission rates seen in this study, significant reductions are mandated by the USEPA for 2007 to 2009 model years. Determination of the effects of engine design and operational parameters and new emission control technologies on exhaust PM genotoxic activity, measured so as to retain particulate phase properties and effects on genotoxicant bioavailability, should provide information readily enough to help design, test, and evaluate control options for genotoxicant emissions from mobile vehicle diesel and spark-ignition gasoline engines. The results of this study, along with findings from related chemistry,[26] and toxicology[25] studies, will be followed by additional studies that will encompass low temperature-operated diesel and gasoline exhausts, white and black smoker gasoline exhausts, high-emitter diesel exhausts and ultra-low emitter engine exhausts, and compare and contrast the findings to pinpoint critical issues for control strategies.

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