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Influence of ethanol–diesel blended fuels on diesel exhaust emissions and mutagenic and genotoxic activities of particulate extracts

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Abstract

This study was aimed at evaluating the influence of ethanol addition on diesel exhaust emissions and the toxicity of particulate extracts. The experiments were conducted on a heavy-duty diesel engine and five fuels were used, namely: E0 (base diesel fuel), E5 (5%), E10 (10%), E15 (15%) and E20 (20%), respectively. The regulated emissions (THC, CO, NOx, PM) and polycyclic aromatic hydrocarbon (PAH) emissions were measured, and Ames test and Comet assay, respectively, were used to investigate the mutagenicity and genotoxicity of particulate extracts.

From the point of exhaust emissions, the introduction of ethanol to diesel fuel could result in higher brake specific THC (BSTHC) and CO (BSCO) emissions and lower smoke emissions, while the effects on the brake specific NOx (BSNOx) and particulate matters (BSPM) were not obvious. The PAH emissions showed an increasing trend with a growth of ethanol content in the ethanol–diesel blends.

As to the biotoxicity, E20 always had the highest brake specific revertants (BSR) in both TA98 and TA100 with or without metabolizing enzymes (S9), while the lowest BSR were found in E5 except that of TA98 − S9. DNA damage data showed a lower genotoxic potency of E10 and E15 as a whole.

Keywords: Ethanol–diesel blended fuels; Regulated emissions; Polycyclic aromatic hydrocarbons; Ames assay; Comet assay

1. Introduction

The global concern over vehicle emission pollutants and the increase of petroleum fuel prices have triggered awareness focused on the development of alternative fuel sources. Due to the advantages of biodegradability, low toxicity as well as high miscibility with diesel fuel relative to methanol, ethanol, as an oxygenous biomass fuel, has received considerable attentions. Particularly, the regenerative capability and cleaner burning characteristics make ethanol so attractive that it may be considered as a predominant alternative fuel for diesel engines. Researches indicated that the ethanol–diesel blended fuels were technically acceptable for existing diesel engines [1]. At present, there is a widespread interest in ethanol–diesel blended fuels for their potential to help reduce harmful exhaust emissions from current and future diesel engines.

The first studies on the use of ethanol in diesel engines were conducted in South Africa in the 1970s, and continued in Germany and the United States during the 1980s [2]. Numerous experimental results indicate that ethanol–diesel blends could significantly reduce particulate matter (PM) and smoke emissions. Spreen [3] and Kass et al. [4] concluded that the introduction of 10% and 15% ethanol could reduce PM emission by 20–27% and 30–40%, respectively. The blends containing 83–94% diesel fuel, 5–15% ethanol and 1–3% additive cetane improver could decrease 41% PM [5], and the 15% ethanol–diesel blends could produce a drop of 33.3% in smoke and 32.5% in the soot mass concentration [6]. However, the effects of ethanol addition on THC, CO, NOx emissions, which depend much on the test engines and test procedures, are less clear. Kass et al. [4] investigated the exhaust emissions from a 5.9 L, turbo-charged, direct injection (DI) diesel engine with

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AVL 8 mode test and the results showed that the addition of ethanol had no noticeable effect on the emission of NOx, but produced small increase in CO and HC. Li et al. [7] reported that ethanol–diesel blend fuels led to a decrease in CO and NOx emission and an increase in THC emission from a single-cylinder DI engine. According to Corkwell [8], who reviewed the existing published data from previous exhaust emissions testing on ethanol–diesel blends, the most frequent observations occurred around a 20% increase in the level of THC emission, a 20% reduction or no change in CO emission and almost no variation in NOx emission. Besides the regulated exhaust emissions, ethanol–diesel fuel blends could increase the emission of unburned hydrocarbons [9] and aldehyde emission [10].

Although many papers have shown the emission characteristics of ethanol–diesel engines, few research works have been carried out in the field of toxicity and environment-security of PM from diesel engine fueled with ethanol–diesel blends.

Diesel exhaust particulates (DEP) are mainly composed of carbon nuclei and absorbed organic compounds. Epidemiological studies have shown an association between exposure to diesel exhaust and an excess risk for lung cancer in humans [11]. The absorbed organic compounds consisting of some highly mutagenic chemicals, such as polycyclic aromatic hydrocarbons (PAHs) and nitro-PAHs [12,13] were shown to cause pulmonary tumors [14]. The nitro-PAHs induce mutations in bacterial and mammalian cells, sister chromatid exchanges and chromosomal aberrations in cultured mammalian cells [15]. In addition, there is evidence for carcinogenicity in rats, although it seems that the rat model is not appropriate to assess human risk [16,17].

The environment contains a wide variety of man-made genotoxic agents including mutagens and carcinogens. The development of short-term genetic bioassays in the mid-1970s rapidly led to the use of these assays in environmental monitoring [18]. The most widely used bacterial mutagenicity bioassay is the *Salmonella typhimurium* plate-incorporation assay described by Ames et al. [19] and validated as an initial bioassay to screen for potential carcinogens [19,20]. Ames' bacterial bioassay is now used as a short-term test to detect and quantify the mutagenicity associated with complex mixtures of harmful substances in air, water, industrial effluents and commercial products. The comet assay, i.e., single cell gel electrophoresis (SCGE), is a sensitive and rapid method for DNA damage detection in individual cells. In alkaline conditions, it involves the detection of cell DNA fragments which, on electrophoresis, migrate from the nuclear core and result in a "comet" formation. The SCGE assay is becoming a major tool in environmental pollutant biomonitoring, both in vivo and in vitro.

The increasing occurrence of genotoxic pollutants in the environment has become a matter of interest as a complex public health problem. Therefore, when the effects of ethanol–diesel blended fuels on exhaust emissions are considered, special attentions should be also focused on the mutagenic and genotoxic activities of PM. In the current study, the regulated emissions (THC, CO, NOx, PM) and polycyclic aromatic hydrocarbon (PAH) emissions from a diesel engine using ethanol–diesel blended fuels have been measured. Meanwhile, the mutagenic activities of PM extracts were evaluated by means of Ames test using *S. typhimurium* strains TA98 and TA100 with and without S9mix, and the genotoxicity potency was measured with the comet assay on rat fibrocytes L-929 cells. The aims of our study were: (a) to evaluate the effects of different ethanol–diesel blended fuels on exhaust emissions, including regulated emissions and PAHs emissions; (b) to detect the genotoxicity of the assay samples on various genetic targets, assess the suitability of SCGE on rat fibrocytes for diesel exhaust pollution monitoring, together with standard short-term mutagenicity tests.

2. Experimental equipment and methods

2.1. Diesel engine and test procedure

A heavy-duty, non-catalyst, turbocharged inter-cooler commercial diesel engine, which was manufactured by Dongfeng Chaoyang Diesel Engine Ltd. of China, was used in this study. The engine featured a 17.5:1 compression ratio, six cylinders, direct injection, bore and stroke (102 mm × 118 mm), total displacement of 5785 mL. The maximum torque was 431 N m at 1700 rpm and the rated power was 107 kW at 2800 rpm. An AVL ALPHA350AF eddy current dynamometer was coupled to the engine and was controlled with PUMA control system.

The ECE R49-13 mode test procedure was chosen for emission test (Fig. 1). Before running the engine with a new blended fuel, the residual ethanol–diesel mixture left in the combustion chamber and fuel system was drained up. Then the engine was operated at a high idling condition with a new blended fuel to consume the remaining fuel. To collect the necessary quantity of PM for toxicological analysis, the ECE R49-13 mode was run for 15 cycles. Hereinto, three replicates cycles were used to measure the regulated emissions (THC, CO, NOx, PM).

2.2. Test fuels

The tests were conducted using five fuels. The base fuel (E0) was a light diesel fuel (0#) without any additive for the preparation of ethanol–diesel blended fuels and the others were blended fuels, containing 5%, 10%, 15% and 20% ethanol by volume,

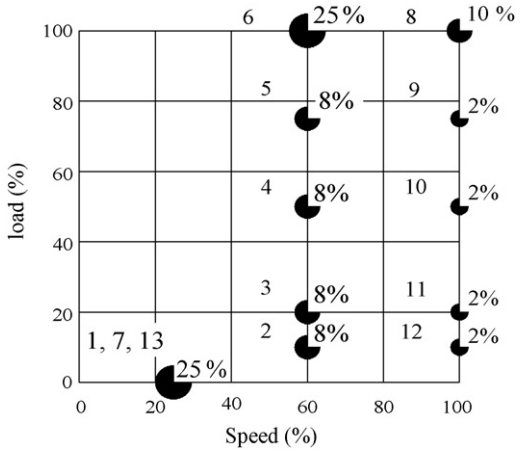

Fig. 1. The ECE R49-13 mode test procedure.

Table 1
Properties of ethanol and ethanol–diesel blended fuels

Parameter	Ethanol	E0	E5	E10	E15	E20
Density (g/cm^3 at 20 °C)	0.7893	0.8379	0.8349	0.8324	0.8301	0.8279
Cetane number	8	53.1	50.6	48.5	46.4	43.9
Gross heat content (MJ/kg)	26.778	42.845	42.013	41.219	40.416	39.628
Latent heat of vaporization (kJ/kg)	854	301	319	350	379	407
Oxygen content (wt%)	34.73	0.021	1.751	3.483	5.218	6.958
Liquid viscosity (cP at 20 °C)	1.2	5.18	–	–	–	–

marked E5, E10, E15 and E20, respectively. All the blends were prepared from the same batch of diesel fuel, and the ethanol was anhydrous to ensure the solubility in the base fuel. The blended fuels were confected in situ. Some physicochemical properties of the base fuel and ethanol–diesel blended fuels are shown in Table 1.

2.3. Sampling and chemical analysis

2.3.1. Regulated emission analysis

Gaseous emissions were drawn from the tailpipe and measured on line by an AVL CEB-II exhaust analyzer with a resolution of 0.1 ppm for CO, THC and NOx emissions. Specifically, CO was analyzed by a non-dispersive infrared (NDIR) analyzer, NOx by a chemiluminescent detector (CLD) and THC by a flame ionization detector (FID). The relative standard deviation is less than 3% for the CO emission, 2% for the THC and NOx emissions.

Exhaust smoke was analyzed by AVL415 and AVL439 smoke analyzer. The results were given in the form of the filter smoke number (FSN) and the smoke absorption coefficient (*K*), respectively. FSN indicates the relative reflectance of particulate collected on filter paper and *K* represents the relative quantity of light that passes through the exhaust. AVL 472 dilution sampling system was used to harvest PM on two 70-mm filters, and the temperature of the diluted mixture maintained below 52 °C. The PM mass on each filter was determined gravimetrically by the difference in mass before and after each test using an electronic analytical microbalance (Sartorius ME 5-F) with an accuracy of 0.001 mg.

2.3.2. Sample preparation

For this study, the collected samples of E0, E5, E10, E15 and E20 were extracted using the Soxhlet technique under yellow fluorescent lights. All solvents used in this study were Burdick and Jackson Distilled-In-Glass quality. The samples were extracted with the methylene chloride for 24 h. The resulting extracts were concentrated to 1 mL by rotary film and vortex evaporation and then were kept in the sealed bottles at −20 °C in the dark, respectively. The whole course of concentration was protected by nitrogen gas. When the methylene chloride volatilized completely, the dry filter papers were weighed. Then the mass of soluble organic fraction (SOF) was calculated according to the difference of the filter paper mass before and after the extraction. After having analyzed the PAHs, each SOF extract (E0, E5, E10, E15 and E20) was prepared for the Ames test and Comet assay.

2.3.3. Chemical analysis of PAHs

Analysis of PAHs was undertaken using a gas chromatograph (GC) (Hewlett-Packard 5890A) with a mass selective detector (MSD) (Hewlett-Packard 5971) and a computer workstation. The GC/MS was equipped with a Hewlett-Packard capillary column (HP-1, 12 m × 0.22 mm). Helium was employed as the carrier gas with head pressure 0.02 MPa, and the following temperature program from 100 °C (1 min) to 300 °C (4 min) at 15 °C/min was adopted. The masses of primary and secondary ions of PAHs were determined by means of the scan mode for pure PAH standards. The identification of target compounds was based on the detection of the molecular ion along with comparison of retention time relative to that of the PAH standards. Quantification of PAHs was performed by using the selected ion monitoring (SIM) mode.

For SOF samples of E0, E5, E10, E15 and E20, the 16 EPA-PAH compounds were quantified, namely: naphthalene (Nap), acenaphthylene (AcPy), acenaphthene (Acp), fluorene (Flu), anthracene (Ant), phenanthrene (PA), pyrene (Pyr), fluoranthene (FL), benzo[*a*]anthracene (BaA), chrysene (CHR), benzo[*a*]pyrene (BaP), benzo[*b*]fluoranthene (BbF), benzo[*k*]fluoranthene (BkF), benzo[*g*,*h*,*i*]perylene (B*ghi*P), dibenz[*a*,*h*]anthracene (DBA), and indeno[1,2,3-cd]pyrene (IND). The PAH recovery efficiencies ranged from 0.79 to 1.07 and averaged 0.85. Mean relative standard deviation (R.S.D.) was less than 13%. Analysis of blank filters showed no significant contamination from sampling throughout analysis (GC/MS integrated area < detection limit).

2.4. Biological analysis

2.4.1. Ames test

In Ames test, *S. typhimurium* strains TA98 and TA100 were selected, and plate-incorporation assays were abided by the method of Maron and Ames [20] and De Meo et al. [21]. The bacterial strains TA98 and TA100 detect frameshift mutagens and basepair substitutions, respectively. All the SOF, prepared as described above, were evaporated to dryness under nitrogen and then diluted with dimethyl sulfoxide (DMSO). Limited to the collected mass of particle, only three concentrations (0.025, 0.05, 0.1 mg/plate) of the SOF extracts were adopted to tested in TA98 and TA100 strains with or without the addition of rat liver S9 metabolic activation fraction. All determinations were made in triplicate in the independent experiments to obtain an average value of the experimental data. Induced and spontaneous revertants (SR) per plate were determined for each dose

with a bacterial colony counter. Dexon (0.5 mg/mL) and 2-aminofluorene (0.2 mg/mL) served as positive controls with and without S9mix, respectively.

2.4.2. Comet assay

In comet assays, the test procedure followed the original description of Singh et al. [22] with a few minor modifications [23,24]. Briefly, rat fibrocytes L-929 cells were plated onto multiwell systems at a density of 2×10^4 cells/mL culture medium. After 24 h of growth, the cells were exposed to the SOF of E0, E5, E10, E15 and E20, 20 μL, respectively, for another 24 h at 37 °C and 5% CO_2. After exposure, the viability of the cells was determined by the trypan blue method and only cultures with a cell viability of more than 80% were used for analysis. Then, a suspension of 10^3–10^4 cells was mixed with 75 μL of 0.8% LMA in PBS and transferred to normal melting agarose-coated (1.5%) slides. Three slides were prepared for each concentration of the sample tested. Then, the slides were covered with a coverslip and the agarose was allowed to solidify in a refrigerator at 4 °C. Thereafter, the coverslips were removed and the slides kept in a lysing solution (pH 10) containing 2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris, 1% Triton-X 100 and 10% DMSO over 1 h at 4 °C. After alkali unwinding (1 mM Na_2EDTA and 0.3 mM NaOH), pH 13.5, for 20 min, the slides were electrophoresed at 25 V and 300 mA for 30 min. Subsequently, the slides were rinsed two times with 400 mM Tris buffer (pH 7.5), stained with 40 μL ethidium bromide solution (13 μg/mL) and analyzed with a Leitz Diaplan fluorescence microscope (excitation filter, 515–650 nm, barrier filter, 590 nm).

DNA damage results in increasing DNA migration away from individual cells and produces a characteristic comet shape. The scoring was done by randomly scanning and measuring 100 comets per slide. The comets selected for scoring were of uniform nuclear size. The scoring was done by visual inspection under the microscope, and measurements of the head and tail lengths of comets were made with an eyepiece micrometer and accorded a numeric value with regard to the following damage classed: undamaged—no tail visible (class 1); low damage—tail length not more than 30 μm and with low fluorescence and head still round and brightly fluorescent (class 2); medium damage—tail length between 30 and 50 μm and head and tail about equally brightly fluorescent (class 3); high damage—tail length between 50 and 70 μm and bright and head small and weakly fluorescent (class 4); and extreme damage—tail length more than 70 μm and head not a round unit anymore (class 5). Comets where the head had disintegrated fully with only the tail visible were deemed to be apoptotic and were not counted [25]. For each treatment 100 comets per slide of three slides per treatment were scored. Results were analyzed by SPSS statistical software followed by Student's *t*-tests for the comparisons. $P < 0.01$ was considered statistical significance.

3. Results and discussion

3.1. Regulated emissions

3.1.1. Regulated gaseous emissions

To investigate the exhaust emission level of the whole test cycle, the brake specific regulated gaseous emissions (BSRG) with the ECE R49-13 test mode are presented in Table 2. It can be seen that the BSTHC and BSCO emissions are increased with increasing the ethanol volume percent. The maximal increment of BSTHC and BSCO could reach 53.1% and 70.5% relative to E0, respectively. However, the addition of ethanol to base diesel has little effect on NOx emission and the increment only ranges from −7.5% to 6.8%.

These are attributed to the chemistry and properties of the blended fuels. The latent heats of vaporization of ethanol and diesel are 854 and 301 kJ/kg, respectively, and the gross heat contents of ethanol and diesel are 26.778 and 42.845 MJ/kg, respectively. Due to the lower gross heat contents and higher vaporization cooling effect, ethanol blends generally have lower flame temperatures and lower burning velocities than those of the base diesel fuel, which suppress NOx formation. On the other hand, the addition of ethanol not only lowers the cetane number of fuels and prolongs ignition delay, but also supplies oxygenated fraction in fuels, which contribute to NOx formation. As a result of the interaction, the variation of ethanol content has no significant effect on NOx emission. Higher BSTHC and BSCO emissions result from low combustion temperature throughout the cylinder and thick quenching layer caused by high ethanol vaporization cooling effects.

3.1.2. PM emission

Diesel particulate matters principally consist of dry soot (DS) and SOF, and SOF mainly results from incomplete combustion of fuel hydrocarbon. Fig. 2 shows the characteristics of the brake specific SOF (BSSOF) and the brake specific DS (BSDS) when different ethanol–diesel fuels are used. It is noted that BSDS decreases gradually with the increasing ethanol in blends, whereas the trend of BSSOF is quite opposite. Especially, BSSOF emission for E20 is up to 0.108 g/(kW h) which is significantly higher than that for the other blend fuels. That is because the introduction of 20% ethanol is so much that the com-

Table 2
Effects of different ethanol–diesel blended fuels on BSRG emissions

Fuel	THC (g/kW h)	CO (g/kW h)	NOx (g/kW h)	Change in THC (%)	Change in CO (%)	Change in NOx (%)
E0	1.112	1.583	7.934	–	–	–
E5	1.356	1.817	8.471	21.9	14.8	6.8
E10	1.375	2.265	8.172	23.7	43.1	3.0
E15	1.415	2.280	8.133	27.2	44.0	2.5
E20	1.702	2.699	7.350	53.1	70.5	−7.5

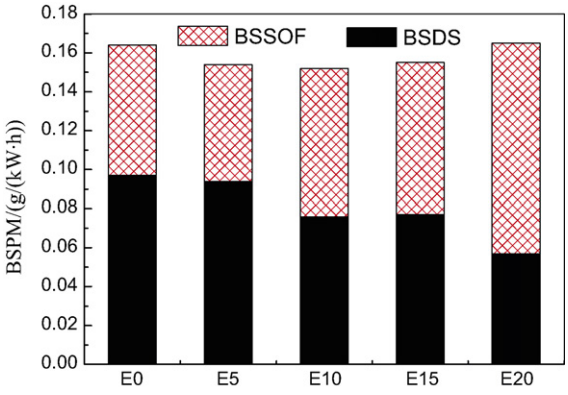

Fig. 2. BSSOF and BSDS emissions for different ethanol–diesel blended fuels.

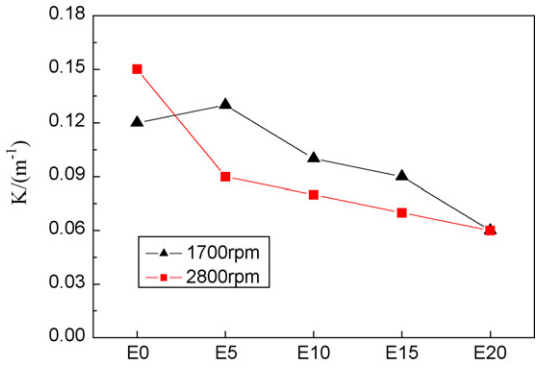

Fig. 3. Effects of different ethanol–diesel blended fuels on smoke absorption coefficient.

bustion process deteriorates, and incomplete combustion leads to produce more unburned hydrocarbon, thereby, resulting in high SOF emission. It also can be obtained from Fig. 2 that the brake specific PM (BSPM) is little affected by the addition of ethanol. This is due to a reduction in DS emission and a growth in SOF. The lowest value of BSPM is 0.152 g/(kW h) with E10 and the highest value is 0.165 g/(kW h) with E20.

Figs. 3 and 4 present the effects of different ethanol–diesel fuels on the *K* and the FSN, respectively. It can be observed that under the operating conditions of 1700 and 2800 rpm, both the *K* and the FSN reduce as the increase in ethanol content of the fuels, except the *K* at the speed of 1700 rpm with E5. The general

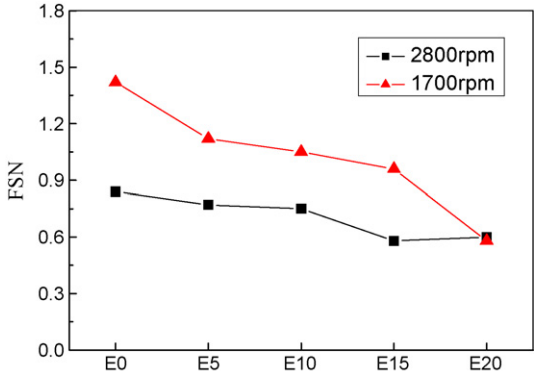

Fig. 4. Effects of different ethanol–diesel blended fuels on filter smoke number.

trends can be explained primarily by the longer ignition delay with the addition of ethanol. A longer ignition delay means an increased proportion of premixed combustion, which benefits accumulating more fuel/air mixture, and hence reducing the DS emission. Furthermore, high oxygen content of blends combined with low C/H ration also help to reduce DS formation [26].

3.2. PAH emissions

According to the analytical results, the brake specific emissions of the 16 kinds of PAHs (BSPAH) in each assay sample were obtained. As illustrated in Table 3, for ethanol–diesel blends, BSPAH emissions gradually grow with increasing the ethanol content. Among the BSPAH emissions, E5 is the lowest down to 62.432 μg/(kW h) and E20 is the highest up to 107.148 μg/(kW h). As compared with E0, the BSPAH emissions from E5 decrease by 19.1% and those from E20 increase by 38.8%. Meanwhile, the effect of using E10 is similar to E0 according to the BSPAH emissions. Previous investigations have shown that the source of PAHs in diesel exhaust emissions originates from unburned fuel, lubricating oil and the formation from pyrosynthetic and pyrolysis reactions [27,28]. Under the condition of the same diesel engine, the PAHs come from lubricating oil are almost the same, while those from fuels have a primarily impact on the content of PAHs in the exhaust. Theoretically, as to the same base fuel, the more ethanol addition, the less PAHs contents in the fuels of the same volume, and the smaller PAHs fraction in the diesel exhaust. But, in this study, the BSPAH emissions for the ethanol–diesel blends show gradually increasing trend as the more amount of ethanol added. Two different factors may be responsible for this. The first one is that high latent heat of vaporization of ethanol contributes to low combustion temperature and thick quenching layer, promoting SOF formation, and hence probably increasing the PAH emissions. The second one is that the power output of diesel engine decreases due to the lower energy content reduction by approximately 2% for each 5% of the ethanol addition [1].

3.3. Mutagenicity and genotoxicity of PM extracts

3.3.1. Mutagenicity of PM extracts by Ames test

The mutagenicities of the PM extracts with the five different fuels were examined by the *S. typhimurium* strains TA98 and TA100 both with and without S9mix. TA98 + S9 and TA100 + S9 are used to test the indirect mutagens. Meanwhile, the direct acting mutagens are detected by TA98 − S9 and TA100 − S9. At 0.025 mg/plate, the Ames tests for all extracts are negative and it is invalid for discussing mutagenic characteristic at this concentration. For the mutagenic activity of PM extracts at 0.05 and 0.1 mg/plate, it can be observed from Figs. 5 and 6. At the concentration of 0.05 mg/plate, each sample presents a visible mutagenicity (more than twofold spontaneous revertants) in TA98 + S9, but in TA98 − S9 and TA100 ± S9 still show negative results. At 0.1 mg/plate, positive results can be found for all the five fuels using both strains with and without S9mix. Therefore, the revertant numbers at 0.1 mg/plate were chosen to evaluate mutagenic activities in this study. In test strain TA98,

Table 3
Sixteen kinds of BSPAH emissions for each blended fuel

Number	PAHs	Assay Sample (μg/(kW h))				
		E0	E5	E10	E15	E20
1	Naphthalene	8.350	7.197	9.045	10.575	10.366
2	Acenaphthylene	4.641	3.771	5.224	7.177	6.412
3	Acenaphthene	3.956	3.25	3.599	5.214	5.960
4	Fluorene	2.605	2.273	2.266	2.550	4.786
5	Anthracene	6.952	4.892	6.513	8.036	11.972
6	Phenanthrene	1.685	1.418	1.464	2.010	2.844
7	Pyrene	7.575	4.931	4.556	7.381	9.471
8	Fluoranthene	6.031	4.665	5.389	7.166	8.688
9	Benzo(*a*)anthracene	2.315	1.752	1.697	2.496	2.215
10	Chrysene	8.902	6.344	6.765	9.568	7.303
11	Benzo(*a*)pyrene	1.678	1.032	1.017	1.529	1.159
12	Benzo(*b*)fluoranthene	7.658	6.691	8.986	8.904	11.379
13	Benzo(*k*)fluoranthene	3.361	3.107	4.151	4.164	5.170
14	Benzo(*g*,*h*,*i*)perylene	4.026	3.997	6.151	6.573	6.798
15	Dibenz(*a*,*h*)anthracene	4.635	4.274	6.514	6.935	8.010
16	Indeno(1,2,3-cd)pyrene	2.817	2.837	3.721	3.770	4.615
Sum		77.187	62.432	77.058	94.048	107.148

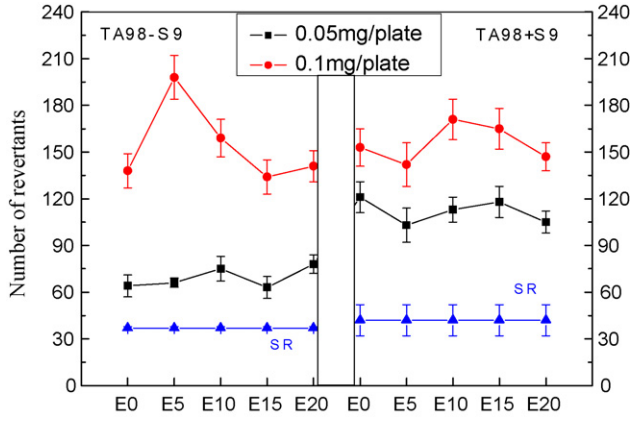

Fig. 5. Mutagenic activities of PM extracts in strain TA98 with and without S9.

the PM extracts with E0, E5, E10, E15, and E20 can induce higher mutational response (three- to fivefold spontaneous revertants). The extracts in strain TA100 result in two- to threefold spontaneous revertants. These indicate that the five fuel samples contain more considerable amounts of basepair substituting mutagens than frameshift mutagens. Under the condition without S9mix in both strains, it is found that for the four blended fuels, the more ethanol blended, the less mutagenicity except the slight increase of E20 for TA98 − S9, which may be attributed to the addition of ethanol suppressing the direct-acting mutagenicity. As compared to the other extracts, E5 can induce both higher basepair substituting and frameshift mutagens. Under the condition with S9mix in both strains, the trend of the mutagenicity is not obvious with increasing the proportion of ethanol to diesel. Furthermore, it can be evidently seen that mutagenicity for TA100 + S9 is lower than that for TA100 − S9 in all extracts.

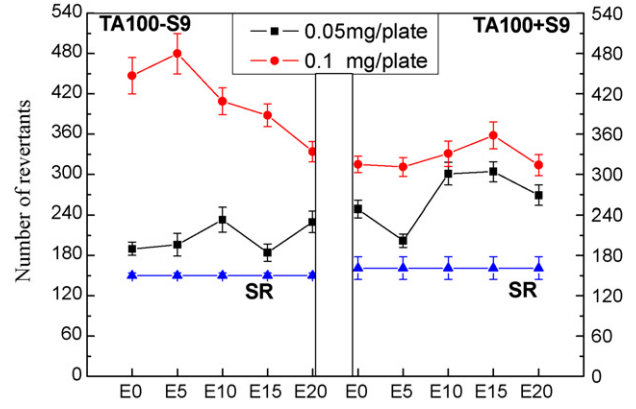

Fig. 6. Mutagenic activities of PM extracts in strain TA100 with and without S9.

The addition of the metabolizing system, S9, leads to a strong reduction in the number of revertants for TA100. The nature of this effect, often observed in similar experiments, is not fully clarified. A likely explanation is that the mutagenicity of nitroPAH is diminished by S9. Nevertheless, the occurrence of, so far unknown, directly reactive mutagens that are eliminated in reactions with S9 components cannot be ruled out [29]. In addition, there are marked differences among the mutagenic activities of the assay samples in two strains with or without S9. In these cases, toxicity cannot be used to explain the different behaviors of the two strains. Some compounds might react differently in the two strains, or there may be different types of compounds responsible for the mutagenic activity. However, this requires further study and confirmation. The trend and regularity of the mutagenicity is not obvious for increasing the proportion of ethanol to diesel. Moreover, some unknown compounds, or some interaction among mixture compounds, or different amount of each compound in each sample could have different mutagenic potency on the two strains [30]. And those differential sensitivities of the tester strains may indicate differences in sample composition, test and sampling conditions. Further tests are required to chemically characterize the compound or the class of compounds responsible for this activity [31].

Considering the effect of engine operating mode on mutagenicity of PM extracts, the brake specific revertants (BSR) was introduced. The BSR were calculated according to the following formula:

$$\text{BSR}\left(\frac{\text{rev}\times 10^3}{\text{kW h}}\right) = A \times B$$

where A denotes the mutagenic activity of the PM extracts, revertants/mg; B denotes the brake specific emission of SOF, g/(kW h) [32].

As mentioned above, the data of revertants at 0.1 mg/plate was still used to determine the BSR because of a visible mutagenicity in either strain for each sample at this concentration. The BSR of PM extracts as tested in TA98 and TA100 with or without S9mix are summarized for all samples in Fig. 7 and Fig. 8. In general, the BSR in TA98 is lower than in TA100 for all test samples. For TA98, the highest BSR is observed in E20, followed by E10, E5, E15 and E0 without S9 while E10, E15, E0 and E5 with S9. For TA100, the highest BSR is also observed in E20 in agreement with TA98, followed by E10, E15, E0 and E5 without S9 while E15, E10, E0 and E5 with S9. From the both strains with S9, E5 has lower BSR than the other samples, which is the same as the result in TA100 − S9. The E5 averages approximately 83.8%, 89.1% and 97.0% of BSR for E0 with TA98 + S9, TA100 + S9 and TA100 − S9, respectively, whereas the lowest BSR in TA98 − S9 is achieved by E0.

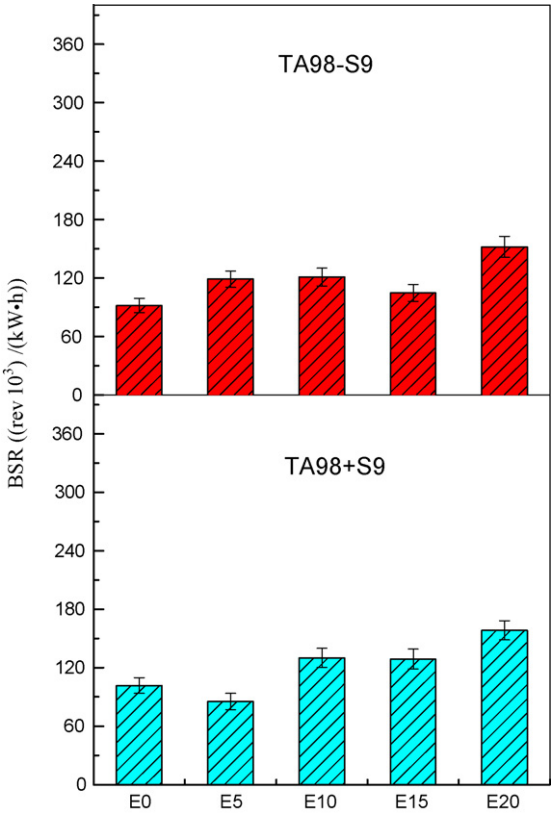

Fig. 7. Effects of different ethanol–diesel blended fuels on BSR with TA98 ± S9.

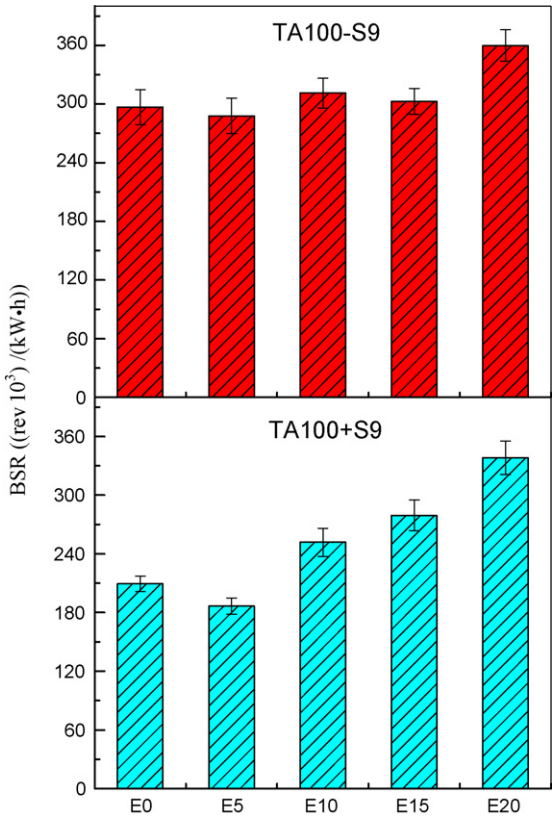

Fig. 8. Effects of different ethanol–diesel blended fuels on BSR with TA100 ± S9.

3.3.2. Genotoxicity of PM extracts by Comet assay

The comet tail lengths, as the measurement results of DNA damage, using the in vitro exposure, are given in Table 4. Significant differences ($P < 0.01$) between the assay samples and the negative control clearly indicated the effectiveness and integrity of the method. The responses of each samples to the four exposure concentrations using damage classes is in accordance with the results using the Student's t-test. The effect of the lowest exposure concentration (0.125 mg/mL) is not significantly different from that of the negative control. For the other three concentrations, the data show a higher genotoxic potency of the five samples as compared to 0.125 mg/mL ($P < 0.01$). The responses to 0.25 mg/mL, 0.5 mg/mL and 1.0 mg/mL differed statistically from negative control but not from each other. No dose-related response is evident for the three exposure concentrations and it might therefore be necessary to do a further research for the damage classes. According to the damage classes described previously, the data show lower genotoxic potencies of E10 and E15 than those of E0, E5 and E20 at 0.25 mg/mL. At 0.5 mg/mL, E0 and E20 belong to the high damage, while the other three samples all belong to the medium damage. Meanwhile, there is a similar significant increase of comet tail lengths for five assay samples at 1.0 mg/mL, which belong to the extreme damage. The data of the DNA damage show a lower genotoxic potency of E10 and E15 which have similar effects to E5 except 0.25 mg/mL, and E0 and E20 have a similar effect for all the concentrations and are more genotoxic than the other fuel blends. Concentration-dependent trends in DNA damage are obvious, i.e. increasing sample con-

Table 4
Genotoxic activities of assay samples on rat fibrocytes using comet tail length (μm)

Sample	Dose (mg/mL)			
	0.125	0.25	0.5	1.0
E0 PM extracts	11.5 ± 2.7[a]	33.3 ± 3.2[b,*]	55.4 ± 4.8[c,*]	93.3 ± 6.5[d,*]
E5 PM extracts	9.5 ± 2.3[a]	30.2 ± 2.9[b,*]	49.4 ± 4.1[b,*]	88.6 ± 6.1[d,*]
E10 PM extracts	9.1 ± 2.1[a]	26.6 ± 2.7[a,*]	45.4 ± 3.8[b,*]	73.3 ± 5.3[d,*]
E15 PM extracts	9.6 ± 2.1[a]	25.6 ± 2.8[a,*]	46.4 ± 3.7[b,*]	74.3 ± 5.2[d,*]
E20 PM extracts	12.5 ± 2.8[a]	32.2 ± 3.3[b,*]	53.4 ± 4.9[c,*]	90.6 ± 6.1[d,*]
Negative control		6.6 ± 1.4[a]		
Positive control		113.3 ± 7.9[d]		

[a]low damage; [b]medium damage; [c]high damage; [d]extreme damage. Effects are considered significantly positive with respect to untreated group (*t*-test). *$P < 0.01$. Negative control: DMSO; positive control: potassium biochromate.

centrations caused a corresponding increase in DNA damage, but the genotoxicity is variable with the increasing ethanol fraction of the diesel fuel.

Using the Comet assay, all the assay samples give rise to genotoxicity at relatively higher concentration; however, the DNA damage to 0.25 mg/mL, 0.5 mg/mL and 1.0 mg/mL differs statistically from negative control but not from each other. E0 and E20 may cause more significant DNA damage than the others, whereas E10 and E15 show lower genotoxic potency. Meanwhile, E5 has a similar effect to E10 and E15 except 0.25 mg/mL using damage class. In fact, the assay samples are able to cause significant DNA damage, although each to a different extent. The slight difference in effects on DNA damage among the assay samples may be related to the difference of the chemical compositions of SOF. In our experiments, the assay samples could cause DNA damage to the different extents, indicating that these samples contain a genotoxic fraction. Considering the possible concentrations of assay samples in the air, it is conceivable that the DNA damage may occur at the lowest concentrations, namely real-life concentration, the meaning of which could be the subject of future investigation. The comet assay provides an advantage over other strand break assays because measurements are made on individual cells. Scoring these cells on slides provides an independent measure of the toxicity of a test compound. Dead cells can be identified by their distinct morphology compared to cells exhibiting DNA damage. The comet assay could represent a useful test to evaluate the biological consequences of environmental contamination, being sensitive to cellular damage [23,33].

The wide variability of bioassay response indicates that compound genotoxicity is detected with varying sensitivity in each assay, which is probably due to innate differences in the cells, different mechanisms expressing the effects, and differences in test conditions. The data demonstrate the limitations in predicting genotoxic potential of diesel fuel and blends based on only one biological system. The two biological systems used appear to be sensitive and be able to monitor the pollution arising from the fuel blends. Because of wide variability between test results in the different assays, it is not possible to evaluate whether one bioassay is more specific than other test systems. This paper does not intend to provide a quantitative risk assessment. Rather, it is a comparative characterization of the exhaust pollutants from the diesel engine burning ethanol–diesel blends.

4. Conclusions

Engine experiments were carried out to compare the effects of different ethanol–diesel blend fuels on regulated emissions (THC, CO, NOx, PM) and PAH emissions. The experimental results indicated that under the ECE R49-13 test mode, the BSTHC and BSCO emissions tended to increase with the addition of ethanol, and the maximum increment could be up to 53.1% and 70.5% relative to E0, respectively. The BSNOx and BSPM emissions were observed little variation. But, the ethanol–diesel blends showed significant benefit in terms of smoke reduction. The more ethanol was added, the less smoke emitted. For PAHs emissions, it presented an increasing trend with a growth of ethanol content in the ethanol–diesel blends. Comparing with E0, only E5 showed the advantage of reducing BSPAH emissions by 19.1%.

For the sake of evaluating the mutagenicity and genotoxicity of particulate extracts, Ames test and Comet assay were used. The results of the Ames mutagenicity test showed that the PM extracts contained both direct-acting and indirect-acting compounds, and all the five samples showed more mutagenicity in TA100 than in TA98 with or without S9, which indicated that the five fuel samples contained more considerable amounts of basepair substituting mutagens than frameshift mutagens. For the both strains with or without S9, the highest BSR were all observed in E20. Meanwhile, the lowest BSR was found in E5 except that of TA98 − S9. DNA damage on rat fibrocytes, analysed by the comet assay, showed a wide sensitivity. The data suggested a lower genotoxic potency of E10 and E15 as a whole, which is consistent with E5 except 0.25 mg/mL using damage classes, and E0 and E20 had a similar effect for all the concentrations and were more genotoxic than the other three assay samples. Overall, the conclusion, established on the integration of the results of *Salmonella*/microsome test and Comet assay, is that the lower toxicity can be achieved by E5, which is in agreement with the PAHs emission analysis.

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