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Particle-bound PAHs and Particle-extract-induced Cytotoxicity of Emission from a Diesel-generator Fuelled with Soy-biodiesel

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ABSTRACT

This study investigates the size distribution of nano/ultrafine particle-bound PAHs (polycyclic aromatic hydrocarbons) and the PAH-associated carcinogenic potency/cytotoxicity of the exhaust from a generator that is fuelled with D100 (pure petroleum diesel) and S20 (v/v = 20% soy-biodiesel/80% D100) and operated at stable energy output loads (0 and 3 kW). A micro-orifice uniform deposit impactor (MOUDI) and a Nano-MOUDI (with aerodynamic diameters of 0.01–18 μm) were used to collect PM samples. The cytotoxicity of the organic solvent extracts of PM samples to the human male monocytic cell strain (U937) was evaluated using the MTT (3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide) method. The results indicate that at both loads, using S20 in place of D100 effectively reduced the emissions of DEPs, PAHs in the DEPs, and PAHs-associated BaP_{eq}; furthermore, the unit mass cytotoxicity of ultrafine particles and nano-particles in the DEPs was also lowered (by an average of 52.6%). Therefore, soybean biodiesel (S20) can be used as an alternative fuel to petroleum diesel to reduce the hazards of emissions from diesel engines to human health.

Keywords: Polycyclic aromatic hydrocarbons; Biodiesel; Particle size distribution; Cytotoxicity.

INTRODUCTION

Diesel engines are important in industry, agriculture and transportation because they have a simple and strong mechanical structure, a low fuel cost and a high thermal efficiency. However, diesel engines are commonly responsible for the emission of large amounts of gaseous and particulate pollutants, which adversely affect health (Geiss *et al*., 2010; Wu *et al*., 2010; Avino *et al*., 2011). The gaseous pollutants from diesel engines mainly comprise CO (carbon monoxide), NO_x (nitrogen oxides), SO_x (sulfur oxides) and HC (hydrocarbons) (Colbeck *et al*., 2011; Ma *et al*., 2011). NO_x is usually generated in combustion at high temperature, and its concentration increases with the engine combustion efficiency (Yanowitz *et al*., 2000). Most SO_x from diesel engines is produced by the high-temperature oxidation of the sulfates in petroleum diesel. After they are emitted into the atmosphere, NO_x and SO_x become nitrates, sulfuric acid or sulfate aerosols that detrimentally affect the environment. Diesel exhaust particles (DEPs) mainly consist of carbon particles, ash, SOFs (soluble organic fractions) and sulfur-containing compounds. The carbon particles are chiefly formed by the combustion of an excess of fuel and are mostly in the form of solid carbon. Upon atomization, some of the fuel and the lubricating oil after vaporization will form volatile organic substances or SOFs. Accordingly, the PAHs on the DEPs comprise mostly carbon particles, ash, and SOFs (Ning and Sioutas, 2010). However, some PAHs are highly carcinogenic and mutation-causing compounds that are detrimental to both the natural environment and human health (IARC, 1987). The US EPA designated 16 non-substituent PAHs as major pollutants, and eight of these (BaA, BeP, CHR, BbF, BkF, IND, DBA and BghiP) are notable for their carcinogenic characteristics (Menzie *et al.*, 1992).

The particles in the exhaust from vehicle engines (diesel and gasoline engines) can be divided into coarse particles, fine particles, ultra-fine particles, and nanometer-scale particles. Kittelson *et al.* (2004) indicated that most of the particle mass is associated with the 0.05–1 μm accumulation mode. Although the nuclei mode, consisting of nanometer-scale particles with diameters of 5–50 nm, accounts for only 1–

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20% of total particle mass, it includes more than 90% of all particles. 70–90% of the particles that have a diameter that is smaller than 5 μm can be inhaled by humans (Katz *et al.*, 1980) and accumulate in the respiratory bronchi and pulmonary alveoli, causing respiratory diseases (Voutsa and Samara, 2002; USEPA, 2002; Health Effects Institute, 2002). As fine particles (D_p < 2.5 μm) have a higher surface area-to-volume ratio than coarse particles (2.5 μm < D_p < 10 μm), PAHs can be more easily adsorbed onto fine particles and nanometer particles; therefore, if the mass concentrations of the coarse and fine particles are equal, then the fine particles will have a higher PAH-toxicity than the coarse particles (Kahandawala *et al*., 2004; Westerdahl *et al*., 2005). Research has proven that both solid (organic matter) and gaseous (volatile organic compounds) matter in diesel particles can trigger the mutation of cells, resulting in teratogenesis and other hazards (Westerholm *et al.*, 1991). Exposure to diesel engine exhausts may increase the risk of lung cancer and neurological conditions in rats (Witten *et al.*, 2005). Lin *et al.* (2008a) observed that the cytotoxicity of traffic-related nano/ultrafine particle extracts (using 1:1 (v/v) n-hexane/dichloromethane) was significantly higher ($p < 0.05$) for nano (particularly 10–18 nm)/ultrafine particles than for coarser particles. Other epidemiological research has shown that exposure to a large amount of diesel particles causes an increase in morbidity and mortality from respiratory diseases, which has a negative impact on human health (Reynolds and Richards, 2001; Sultan *et al.*, 2007).

Diesel engines can be used for on-road or non-road (off-road) purposes. Despite the small number used, non-road diesel engines account for disproportionate proportions of particulate matter (PM) and NO_x emissions because their emissions are usually minimally controlled (Chung *et al.*, 2008). Recently, diesel-engine generators have been adopted as emergency electric power in large houses (Mayer, 2000; Singh, 2004; Budisan *et al.*, 2007). Additionally, diesel-engine generators are widely used, in which industrial development is rapid, and the expansion of the supply of electrical power has not kept up with the demand in some areas (Idjdarene *et al.*, 2008). Unfortunately, relevant emission control regulations or standards have not yet been instituted for non-road diesel engines. In general, the pollution from diesel engine emission can be reduced in the following three ways; 1) installing an exhaust processor at the engine exhaust pipe (such as a particulate trap or catalytic device), 2) redesigning the engine to increase combustion efficiency (as in high-pressure common rail injection systems and exhaust recirculation systems), and 3) improving the properties of fuel such as by using biodiesel and fuel additives. Methods 1 and 2 require the installation of additional devices that result in subsequent equipment-related costs, maintenance, and service problems. Method 3 causes the problem of an increased fuel price. Fast industrial growth and urbanization have increased the demand for energy. Limited oil reserves, unstable prices (subject to international events), and environmental concerns have increased the urgency of the demand for renewable resources, and the using of biodiesel is a feasible solution.

Biodiesel is a renewable resource with considerable potential for commercialization. In recent years, biodiesel has been extensively studied. Given its biodegradability, a high oxygen content (~10 wt%), and a relatively high cetane content, biodiesel can be directly used in unmodified diesel engines (Refaat, 2009; Wu *et al.*, 2009). Numerous studies have demonstrated that the use of biodiesel in appropriate mixing ratios can effectively reduce pollutants such as PMs, gaseous pollutants (SO_2, CO and CO_2), and PAHs (Lin *et al.*, 2006; Lin *et al.*, 2008b; Chien *et al.*, 2009; Lin *et al.*, 2010; Tsai *et al.*, 2010; Lee *et al.*, 2011; Tsai *et al.*, 2011). Most studies of biodiesel as an alternative fuel have focused on the reduction of solid and gaseous pollutants from the exhaust, as well as on increasing the energy efficiency of diesel engines (Table 1). However, little attention has been paid to the size distributions of nano/ultrafine particle-bound PAHs and the PAH-associated carcinogenic potency/cytotoxicity of the emissions from a diesel-engine generator when biodiesel is utilized as an alternative fuel.

In this study, to characterize DEPs, particle-bound PAHs, and their cytotoxicity properties in diesel engine exhaust, soybean biodiesel (S20 (20 vol% soy-biodiesel + 80 vol% diesel)) in the unload (0 kW) and load (3 kW) phases of a diesel engine power generator were tested. PMs, including $PM_{0.01–0.056}$ (nano particles), $PM_{0.01–0.1}$ (ultrafine particles), $PM_{0.01–1}$ (submicron particles), $PM_{0.01–2.5}$ (fine particles), $PM_{0.01–10}$ and $PM_{0.01–18}$ were sampled using MOUDI and Nano-MOUDI. The PMs, particle-bound PAHs, and their BaP_{eq} in the exhaust of the generator were analyzed. In addition, a human male monocytic cell strain (U937) was used in cytotoxicity assays on the organic solvent extracts of the PM (with various diameters) samples using the MTT (3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide) method.

MATERIALS AND METHODS

Sampling Methods and Diesel-engine Generator

The diesel engine was a water-cooled, direct injection-single cylinder four-stroke diesel engine (Model: TF110E, made by YANMAR, Japan) (natural intake) with a cylinder bore of 88 mm and strokes of 96 mm, and a displacement of 583 cm^3. The power generator (Model: YSG-5SEN, YANMAR) had a 100/110 V (50/60 Hz) AC output (single-phase, two-wire type), with a maximum output of 4 kW. To prevent interruption in the experiment due to damage to the diesel engine power generator, 3 kW (or 75% of max output power) was used as the load.

The size distributions of particle-bound PAHs that were emitted from the generator using different fuels were measured using a micro-orifice uniform deposit impactor (MOUDI) and a Nano-MOUDI (with 0.01–18 μm aerodynamic diameters). The flow rates of the MOUDI and Nano-MOUDI were set to 30 and 10 L/min, respectively; these two samplers used 37 and 47 mm quartz filters (Pall Ltd., USA), respectively. The impactors in the MOUDIs and Nano-MOUDI separated the particulate matter into 13 size ranges (at 50% efficiency) with the following equivalent cutoff diameters 0.010–0.018, 0.018–0.032, 0.032–0.056, 0.056–0.1, 0.1–0.18, 0.18–0.32, 0.32–0.56, 0.56–1.0, 1.0–1.8, 1.8–3.2, 3.2–5.6, 5.6–10, and 10–18 μm. The particles

Table 1. Summary of tests of diesel-engines fuelled with biodiesel in literature.

Species of biodiesels	Types of tested diesel-engines	Measurements	References
Rapeseed-oil-based biodiesel	A heavy duty diesel engine (Model: EURO 2 IVECO 8360.46R) (Six cylinders; Turbocharged with indirect fuel injection; 130 mm bore× 112 mm stroke; Max output power: 158 kW)	Total PM; Soluble organic fraction (SOF); Gaseous pollutants (NOx and CO); PAH/nitro-PAH; Carbonyl compounds and light aromatics; Mutagenicity assays; Brake-specific fuel consumption (BSFC).	Turrio-Baldassarri *et al.*, 2004
Palm-biodiesel	Diesel-engine generator (No. QC495) (Four-stroke; Water cooled; 95 mm bore × 105 mm stroke; Max output power: 40 kW)	Total PM; PAHs; BaP_{eq}; SOF; Energy efficiency.	Lin *et al.*, 2006
Vegetable-oil-based biodiesel	A modern diesel-engine car (Model: Civilian U-BW40) (Model of diesel-engine: ED-35; Equipped with an oxidation catalyst; Displacement: 3465 cm^3)	Total PM; PAH/nitro-PAH; Gaseous pollutants (NOx and CO).	Kameda *et al.*, 2007
Emulsified Biosolution + Natural organic enzyme-7F (NOE-7F) + Soybean biodiesel + Fossil diesel	Diesel-engine generator (No. QC495) (Four-stroke; Water cooled; 95 mm bore × 105 mm stroke; Max output power: 40 kW)	Total PM; PAHs; BaP_{eq}; SOF; Energy efficiency.	Lin *et al.*, 2008b
Five typical biodiesels (cottonseed oil, soybean oil, rapeseed oil, palm oil and waste cooking oil)	A Euro-III diesel engine (Model: Cummins ISBe6) (Six cylinders; Turbocharged with indirect fuel injection; Rated output power: 136 kW/2500 rpm)	Total PM; Dry soot; Gaseous pollutants (NOx, CO, and HC).	Wu *et al.*, 2009
Waste-edible-oil biodiesel	A modern diesel engine (Mitsubishi, type 6M60-1AT2) (Six cylinders; Turbocharged with indirect fuel injection; 95 mm bore× 100 mm stroke; Max output power: 80.9 kW)	PM and PAH Emissions in nano, ultrafine, fine and coarse size ranges.	Chien *et al.*, 2009
Water-containing acetone + Neat soybean oil + Soybean biodiesel + Fossil diesel	Diesel-engine generator (No. TF110E, YANMAR) (Four-stroke; Single horizontal cylinder engine; 88 mm bore × 96 mm stroke; Max. output power: 5 kW)	Total PM; PAHs; BaP_{eq}; Gaseous pollutants (NOx, CO, and CO_2); Fuel stability; Energy savings.	Lin *et al.*, 2010
Soybean biodiesel	Diesel-engine generator (No. NM260L, Mitsubishi) (Four-stroke; Single horizontal cylinder engine; 113 mm bore × 115 mm stroke)	Total PM; Organic/elemental carbons; PAHs; BaP_{eq}; Energy efficiency; BSFC.	Tsai *et al.*, 2010
Soybean biodiesel	A Euro 2 compliant VW Golf 1.9 TDi (equipped with a turbocharged direct injection diesel engine and an oxidation catalyst)	Emissions of PAH, nitro-PAH, and oxy-PAH; Driving cycle influence; Cold-start influence	Karavalakis *et al.*, 2010
Water-containing ethanol + Soybean biodiesel	Diesel-engine generator (No. TF110E, YANMAR) (Four-stroke; Single horizontal cylinder engine; 88 mm bore × 96 mm stroke; Max. output power: 5 kW)	Total PM; PAHs; BaP_{eq}; Gaseous pollutants (NO_x and CO); Fuel stability; BSFC.	Lee *et al.*, 2011
Waste-edible-oil biodiesel	Diesel-engine generator (No. TF110E, YANMAR) (Four-stroke; Single horizontal cylinder engine; 88 mm bore × 96 mm stroke; Max. output power: 4 kW)	Total PM; Organic/elemental carbons; PAHs; BaP_{eq}; Energy efficiency; BSFC.	Tsai *et al.*, 2011

were divided into six size groups: $PM_{0.01–0.056}$ (nano particles: 0.01 μm < D_p < 0.056 μm), $PM_{0.01–0.1}$ (ultrafine particles: 0.01 μm < D_p < 0.1 μm), $PM_{0.01–1}$ (submicron particles: 0.01 μm < D_p < 1 μm), $PM_{0.01–2.5}$ (fine particles: 0.01 μm < D_p < 2.5 μm), $PM_{0.01–10}$ (0.01 μm < D_p < 10 μm), and $PM_{0.01–18}$ (0.01 μm < D_p < 18 μm). The pure petroleum diesel that was utilized in the experiments (D100) was purchased from CPC (Taiwan) and the soy-biodiesel, manufactured by the World Energy Corp. (U.S.A.), was produced from fresh soybean oil and methyl alcohol via a transesterification reaction. Table 2 lists the properties of fuel and Fig. 1 presents the sampling process.

Before they were used to sample the particles, the quartz fiber filters were heated for 2.5 h at 900°C to reduce their carbon blank. This process minimized the background concentration of carbon in the quartz fiber matrix, as it could influence the analysis. The treated paper filters were stored in a dry chamber at a temperature of 25 ± 3°C and a humidity of 45 ± 5% for one day both before and after sampling. A five-digit electronic balance (model HM-202; A&D Co. Ltd., Japan) that was accurate to within 0.01 mg was used to weigh the paper filters before and after sampling. The filter blanks were determined to correct all PM and particle-bound PAH concentrations.

PAH Analysis

The extraction of PAHs from the paper filters and the glass sleeves that were used in the sampling was performed using the *Soxhlet*-extraction method. The extraction solvent was a mixture of n-hexane and dichloromethane (1:1 volume ratio). The extraction reflux time was 24 h. After extraction, the extract was concentrated to 2 mL using highly pure nitrogen gas. The extract was then poured into a purification tube that contained pretreated silica gel (dried at 105°C for 8 h and then activated with distilled water for 24 h) and n-hexane to remove moisture and highly polar substances. The purified solution was further concentrated to 1 mL using a nitrogen gas stream and then stored in brown sample vials for the subsequent identification of 15 PAHs using a gas chromatograph/mass selective detector (GC/MSD; model: GC 6890N/HP 5973; Hewlett-Packard, Wilmington, DE, USA). The GC/MSD, equipped with a capillary column (HP Ultra 2, 50 m × 0.32 mm × 0.17 μm) and an automatic sampler (HP-7683), was operated under the following conditions; GC/MSD injection volume of 1 μL, splitless injection temperature of 310°C, and ion source temperature of 310°C. The oven temperature increased from 50°C to 100°C at a rate of 20 °C/min, and then from 100°C to 290°C at a rate of 3 °C/min; this temperature, 290°C, was maintained for 40 min. The GC/MSD was calibrated with a diluted standard solution of 16 PAH compounds (EPA610 PAH mixtures; Supelco, Bellefonte, PA, USA) and five additional individual PAHs (Pyr, CYC, BeP, BbC, and COR) obtained from Merck (Darmstadt, Germany). The

Table 2. Properties of the premium diesel and soybean biodiesel.

Item	D100 (pure fossil diesel)	S20 (20 vol% soy-biodiesel)	S100 (pure soy-biodiesel)	Test method
Cetane index	56	55	51	ASTM D976
Heating value (Cal/g)	11036	10727	9492	
Density at 15°C (g/cm^3)	0.83	0.84	0.87	CNS 12017
Viscosity, at 40°C (cSt)	2.66	2.71	3.60	ASTM D445
C (wt%)	86.13	83.72	77.21	
H (wt%)	13.93	13.11	12.34	
O (wt%)	~ 0*	2.27	10.16	
S (ppmw)	36	29	N.D.	ASTM D4294

*: obtained by O (wt%) = 100% – C (wt%) + H (wt%) assuming that the content of other components is negligible.

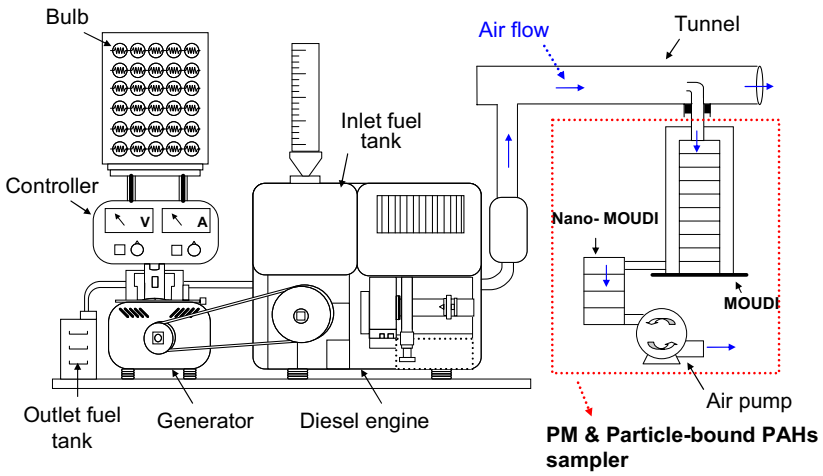

Fig. 1. Generator/engine and sampling system.

method detection limits (MDLs) for the 21 PAH compounds were 0.023–0.106 ng, and the R^2 values of calibration curves applied for GC/MSD analysis were 0.991–0.999. The recovery efficiencies were 83.9–92.6% (average = 86.4%).

The 15 identified PAH species were four 4-ring (fluoranthene (FL), pyrene (Pyr), benzo(a)anthracene (BaA), chrysene (CHR)), six 5-ring (cyclopenta(c,d)pyrene (CYC), benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF), benzo(e)pyrene (BeP), benzo(a)pyrene (BaP), perylene (PER)), four 6-ring (indeno(1,2,3,cd)pyrene (IND), dibenzo(a,h)anthrance (DBA), benzo(b)chrycene (BbC), benzo(ghi)perylene (BghiP)), and one 7-ring (Coronene (COR)) PAH compound. The 4-ring and 5-/6-/7-ring PAHs are referred to as having a middle molecular weight (MMW) and a high molecular weight (HMW) PAHs, respectively. Tables 3 and 4 list the calculated equivalent toxicity (BaP_{eq}) of each PAH species by multiplying the PAH concentration by its corresponding Toxicology Equivalent Factor (TEF) that were recommended by Malcom and Dobson (1994).

Preparation of Particle Extracts

The DEPs of D100 and S20 were extracted by 1:1 (v/v) n-hexane/dichloromethane. Subsequently, the organic solvent that was used to extract the particles was evaporated in a

Table 3. Concentrations of tested 4–7 ring PAHs in DEPs emitted from diesel-engine generator fuelled with D100 and S20 under 0 (unload) kW.

@ unload / 15 PAHs	Mean Concentrations (μg/m^3) (n = 3)												TEF*
	$PM_{0.01–18}$		$PM_{0.01–10}$		$PM_{0.01–2.5}$		$PM_{0.01–1}$		$PM_{0.01–0.1}$		$PM_{0.01–0.056}$		
	D100	S20	D100	S20	D100	S20	D100	S20	D100	S20	D100	S20	
FL	31.7 (± 8.23)	5.88 (± 1.32)	30.7 (± 7.74)	5.54 (± 1.35)	27.2 (± 6.14)	4.78 (± 1.76)	22.8 (± 3.18)	4.19 (± 1.86)	5.63 (± 1.25)	2.40 (± 1.50)	4.77 (± 1.35)	2.15 (± 1.46)	0.001
Pyr	35.6 (± 15.7)	5.76 (± 2.03)	34.4 (± 14.7)	5.50 (± 2.06)	29.5 (± 10.6)	4.75 (± 2.35)	24.0 (± 7.70)	4.20 (± 2.36)	5.71 (± 3.78)	2.44 (± 1.87)	4.84 (± 3.49)	2.22 (± 1.84)	0.001
CYC	7.46 (± 2.09)	2.36 (± 2.53)	7.25 (± 2.27)	2.34 (± 2.53)	6.86 (± 2.52)	2.18 (± 2.60)	6.12 (± 2.50)	2.02 (± 2.57)	0.70 (± 0.21)	0.19 (± 0.05)	0.53 (± 0.22)	0.14 (± 0.04)	0.1
BaA	3.19 (± 1.53)	0.44 (± 0.19)	3.12 (± 1.55)	0.43 (± 0.20)	3.02 (± 1.57)	0.38 (± 0.24)	2.69 (± 1.33)	0.33 (± 0.25)	0.19 (± 0.06)	0.18 (± 0.23)	0.17 (± 0.06)	0.17 (± 0.23)	0.1
CHR	9.92 (± 4.41)	3.14 (± 1.38)	9.71 (± 4.54)	2.97 (± 1.50)	9.04 (± 4.63)	2.67 (± 1.73)	7.98 (± 3.72)	2.33 (± 1.77)	0.78 (± 0.15)	0.34 (± 1.50)	0.60 (± 0.28)	0.21 (± 1.46)	0.01
BbF	5.22 (± 2.03)	1.68 (± 1.60)	5.13 (± 2.04)	1.65 (± 1.61)	4.94 (± 2.05)	1.35 (± 1.34)	4.31 (± 1.39)	1.28 (± 1.34)	1.41 (± 0.39)	0.93 (± 1.27)	1.24 (± 0.33)	0.90 (± 1.26)	0.1
BkF	5.81 (± 2.60)	1.73 (± 0.54)	5.77 (± 2.62)	1.64 (± 0.56)	5.31 (± 2.78)	1.39 (± 0.57)	4.30 (± 2.28)	1.20 (± 0.58)	0.61 (± 0.23)	0.54 (± 0.28)	0.47 (± 0.16)	0.47 (± 0.31)	0.1
BeP	3.63 (± 1.48)	0.82 (± 0.36)	3.62 (± 1.48)	0.80 (± 0.36)	3.35 (± 1.35)	0.69 (± 0.41)	2.91 (± 1.13)	0.58 (± 0.41)	0.26 (± 0.06)	0.17 (± 0.10)	0.22 (± 0.00)	0.16 (± 0.10)	0.01
BaP	9.50 (± 2.43)	2.45 (± 0.81)	9.43 (± 2.42)	2.38 (± 0.85)	8.92 (± 2.40)	2.11 (± 1.11)	7.82 (± 1.15)	1.81 (± 1.08)	0.49 (± 0.34)	0.48 (± 0.38)	0.39 (± 0.39)	0.42 (± 0.35)	1
PER	4.48 (± 2.41)	2.30 (± 2.26)	4.32 (± 2.39)	2.27 (± 2.29)	3.57 (± 2.41)	2.14 (± 2.33)	2.93 (± 1.69)	1.99 (± 2.26)	0.91 (± 0.71)	1.62 (± 2.22)	0.71 (± 0.49)	1.59 (± 2.24)	0.001
IND	2.00 (± 1.33)	0.87 (± 0.99)	1.97 (± 1.34)	0.82 (± 0.95)	1.90 (± 1.36)	0.67 (± 0.82)	1.78 (± 1.31)	0.53 (± 0.72)	0.58 (± 0.27)	0.41 (± 0.59)	0.35 (± 0.18)	0.39 (± 0.59)	0.1
DBA	4.54 (± 1.19)	0.76 (± 0.90)	4.49 (± 1.22)	0.72 (± 0.91)	4.22 (± 1.41)	0.55 (± 0.65)	3.56 (± 1.08)	0.50 (± 0.68)	1.11 (± 0.18)	0.44 (± 0.64)	1.00 (± 0.21)	0.43 (± 0.64)	1
BbC	6.23 (± 2.44)	4.94 (± 7.92)	5.87 (± 2.28)	4.92 (± 7.93)	5.30 (± 2.04)	4.01 (± 6.45)	3.41 (± 1.25)	3.94 (± 6.38)	2.05 (± 0.42)	3.68 (± 6.03)	1.79 (± 0.34)	3.65 (± 6.01)	–
BghiP	6.21 (± 2.33)	0.79 (± 0.58)	6.19 (± 2.32)	0.79 (± 0.59)	5.74 (± 2.06)	0.70 (± 0.55)	4.52 (± 1.17)	0.66 (± 0.54)	1.16 (± 0.34)	0.26 (± 0.27)	1.02 (± 0.33)	0.25 (± 0.27)	0.01
COR	3.60 (± 0.54)	1.39 (± 0.11)	3.42 (± 0.50)	1.30 (± 0.07)	2.96 (± 0.37)	1.06 (± 0.03)	2.65 (± 0.27)	0.94 (± 0.03)	1.70 (± 0.36)	0.62 (± 0.07)	1.53 (± 0.35)	0.54 (± 0.07)	0.001
MMW-PAHs	80.4 (± 24.2)	15.2 (± 4.92)	77.9 (± 22.7)	14.4 (± 5.10)	68.7 (± 17.4)	12.6 (± 6.08)	57.5 (± 10.4)	11.0 (± 6.24)	12.3 (± 4.90)	6.36 (± 5.10)	10.4 (± 4.78)	5.76 (± 4.99)	
HMW-PAHs	58.7 (± 17.3)	20.1 (± 17.2)	57.5 (± 17.2)	19.6 (± 17.2)	53.1 (± 17.7)	16.8 (± 15.4)	44.3 (± 11.3)	15.5 (± 15.3)	11.0 (± 1.70)	9.33 (± 11.1)	9.24 (± 1.13)	8.94 (± 11.1)	
Total-PAHs	139 (± 37.7)	35.3 (± 16.7)	135 (± 36.1)	34.1 (± 17.0)	122 (± 32.1)	29.4 (± 16.3)	102 (± 18.2)	26.5 (± 16.4)	23.3 (± 5.69)	15.7 (± 9.98)	19.6 (± 5.74)	14.7 (± 9.78)	
BbF+BaP+DBA	19.2 (± 5.63)	4.89 (± 2.29)	19.0 (± 5.65)	4.75 (± 2.34)	18.1 (± 5.80)	4.01 (± 2.22)	15.7 (± 3.49)	3.60 (± 2.32)	3.01 (± 0.56)	1.85 (± 1.68)	2.62 (± 0.58)	1.74 (± 1.68)	
Total-BaP_{eq}	16.7 (± 4.57)	3.99 (± 1.35)	16.5 (± 4.61)	3.85 (± 1.39)	15.6 (± 4.84)	3.31 (± 1.56)	13.5 (± 3.07)	2.90 (± 1.64)	1.99 (± 0.24)	1.18 (± 0.67)	1.69 (± 0.41)	1.08 (± 0.67)	

TEF: toxic equivalent factor; *: Malcom and Dobson, 1994; –: No TEF has been suggested.

Table 4. Concentrations of tested 4–7 ring PAHs in DEPs emitted from diesel-engine generator fuelled with D100 and S20 under 3 kW load.

@ 3 kW / 15 PAHs	Mean Concentrations (μg/m^3) (n = 3)												TEF*
	$PM_{0.01–18}$		$PM_{0.01–10}$		$PM_{0.01–2.5}$		$PM_{0.01–1}$		$PM_{0.01–0.1}$		$PM_{0.01–0.056}$		
	D100	S20	D100	S20	D100	S20	D100	S20	D100	S20	D100	S20	
FL	60.4 (± 9.67)	35.6 (± 9.62)	57.9 (± 9.64)	33.8 (± 9.63)	50.8 (± 10.5)	28.7 (± 8.66)	43.5 (± 9.67)	25.1 (± 7.73)	16.9 (± 4.04)	12.9 (± 2.44)	14.3 (± 3.69)	10.6 (± 1.82)	0.001
Pyr	51.0 (± 11.4)	30.2 (± 8.10)	49.2 (± 11.5)	28.8 (± 8.25)	43.9 (± 12.1)	24.8 (± 7.54)	37.6 (± 11.1)	21.9 (± 6.78)	12.5 (± 2.84)	9.86 (± 2.44)	10.5 (± 2.61)	8.37 (± 1.28)	0.001
CYC	14.8 (± 17.2)	12.7 (± 14.8)	13.7 (± 15.9)	12.5 (± 14.8)	9.61 (± 9.79)	12.1 (± 14.9)	6.33 (± 5.01)	11.8 (± 15.0)	3.26 (± 2.07)	1.16 (± 0.40)	2.69 (± 1.66)	1.00 (± 0.35)	0.1
BaA	2.57 (± 1.72)	1.19 (± 0.61)	2.46 (± 1.65)	1.14 (± 0.61)	2.13 (± 1.37)	1.03 (± 0.58)	1.83 (± 1.12)	0.94 (± 0.56)	0.71 (± 0.32)	0.39 (± 0.17)	0.60 (± 0.26)	0.33 (± 0.16)	0.1
CHR	16.8 (± 2.52)	15.1 (± 2.88)	16.2 (± 2.05)	14.5 (± 2.90)	13.7 (± 1.78)	12.6 (± 3.06)	11.0 (± 1.55)	11.2 (± 2.54)	2.95 (± 1.01)	1.67 (± 0.52)	2.18 (± 1.22)	1.48 (± 0.61)	0.01
BbF	11.4 (± 10.1)	5.47 (± 2.50)	11.2 (± 10.1)	5.28 (± 2.53)	10.6 (± 10.0)	4.72 (± 2.25)	10.1 (± 10.1)	4.40 (± 2.25)	7.75 (± 11.1)	1.45 (± 0.55)	6.46 (± 9.15)	1.24 (± 0.50)	0.1
BkF	16.7 (± 0.27)	9.30 (± 2.95)	16.1 (± 0.28)	8.68 (± 3.04)	14.9 (± 0.23)	7.17 (± 2.65)	12.7 (± 0.91)	6.39 (± 2.41)	5.13 (± 3.69)	3.34 (± 1.48)	4.31 (± 3.12)	2.60 (± 1.47)	0.1
BeP	3.02 (± 1.26)	2.49 (± 0.91)	2.96 (± 1.30)	2.38 (± 0.93)	2.68 (± 1.20)	2.11 (± 0.87)	2.35 (± 1.23)	1.91 (± 0.80)	1.39 (± 1.24)	0.87 (± 0.23)	1.20 (± 1.00)	0.71 (± 0.17)	0.01
BaP	9.91 (± 4.55)	6.72 (± 3.37)	9.80 (± 4.48)	6.26 (± 3.57)	9.24 (± 4.30)	5.45 (± 3.32)	7.95 (± 3.52)	4.90 (± 3.01)	2.86 (± 2.06)	1.98 (± 0.65)	2.43 (± 1.75)	1.66 (± 0.47)	1
PER	34.2 (± 25.1)	5.64 (± 3.39)	33.2 (± 24.6)	5.53 (± 3.36)	25.4 (± 18.8)	5.22 (± 3.29)	18.2 (± 19.4)	4.77 (± 2.77)	14.8 (± 21.2)	2.09 (± 1.59)	11.5 (± 16.4)	1.95 (± 1.51)	0.001
IND	12.6 (± 10.1)	4.28 (± 3.02)	12.3 (± 10.0)	4.11 (± 2.98)	10.3 (± 8.09)	3.82 (± 2.91)	9.35 (± 7.10)	3.72 (± 2.89)	8.56 (± 7.26)	1.74 (± 1.86)	7.69 (± 6.82)	1.69 (± 1.84)	0.1
DBA	22.5 (± 18.8)	2.79 (± 2.09)	22.4 (± 18.8)	2.64 (± 2.11)	20.7 (± 17.2)	2.38 (± 1.94)	19.2 (± 15.7)	2.23 (± 1.90)	18.5 (± 15.5)	0.57 (± 0.32)	15.5 (± 13.1)	0.51 (± 0.30)	1
BbC	44.1 (± 50.6)	8.63 (± 4.90)	43.5 (± 50.8)	8.09 (± 4.52)	42.2 (± 51.1)	6.93 (± 4.04)	41.2 (± 51.7)	6.49 (± 3.94)	38.7 (± 52.1)	2.46 (± 2.42)	32.1 (± 43.1)	2.10 (± 2.33)	–
BghiP	8.84 (± 6.76)	2.06 (± 0.71)	8.74 (± 6.68)	1.96 (± 0.69)	8.09 (± 6.54)	1.51 (± 0.50)	7.48 (± 6.56)	1.27 (± 0.45)	6.67 (± 6.50)	0.64 (± 0.24)	6.04 (± 6.10)	0.53 (± 0.21)	0.01
COR	12.7 (± 2.27)	8.13 (± 1.23)	11.8 (± 2.15)	7.61 (± 1.15)	10.0 (± 1.74)	6.29 (± 0.89)	8.81 (± 1.50)	5.52 (± 0.75)	5.57 (± 0.55)	3.41 (± 0.40)	4.72 (± 0.35)	2.91 (± 0.33)	0.001
MMW-PAHs	131 (± 24.7)	82.2 (± 20.8)	126 (± 24.4)	78.2 (± 20.9)	111 (± 25.2)	67.1 (± 19.4)	93.9 (± 23.0)	59.1 (± 17.2)	33.0 (± 7.65)	28.8 (± 5.00)	27.6 (± 7.28)	23.8 (± 2.96)	
HMW-PAHs	191 (± 108)	68.2 (± 7.23)	186 (± 108)	65.0 (± 7.21)	164 (± 101)	57.7 (± 4.48)	144 (± 102)	53.4 (± 4.08)	113 (± 106)	19.7 (± 6.86)	94.7 (± 86.3)	16.9 (± 6.25)	
Total-PAHs	321 (± 109)	150 (± 27.6)	312 (± 109)	143 (± 27.7)	274 (± 97.5)	125 (± 23.4)	238 (± 94.8)	112 (± 20.1)	146 (± 102)	48.5 (± 11.6)	122 (± 82.4)	40.7 (± 9.01)	
BbF+BaP+DBA	43.8 (± 27.0)	15.0 (± 7.94)	43.4 (± 26.9)	14.2 (± 8.21)	40.6 (± 25.6)	12.6 (± 7.49)	37.2 (± 24.6)	11.5 (± 7.14)	29.1 (± 22.6)	4.00 (± 1.21)	24.4 (± 18.5)	3.41 (± 1.04)	
Total-BaP_{eq}	38.7 (± 26.1)	13.1 (± 5.25)	38.2 (± 25.9)	12.3 (± 5.44)	35.1 (± 23.3)	10.9 (± 4.96)	31.5 (± 20.7)	10.1 (± 4.60)	24.1 (± 16.8)	3.45 (± 1.09)	20.3 (± 14.0)	2.94 (± 0.91)	

TEF: toxic equivalent factor; *: Malcom and Dobson, 1994; –: No TEF has been suggested.

stream of nitrogen. The residues that were obtained after the mixed solvent was evaporated were then resuspended in dimethyl sulfoxide (DMSO) and stored in a freezer at –80°C until the cytotoxicity assay was conducted using porcine human male monocytic cell strain (U937) as an *in vitro* screening system.

MTT Assay

The MTT assay is an MTT-based colorimetric assay, which is simple, fast, economic, and free of radioactive elements. The MTT assay is commonly used to analyze indicators of cell survival, toxicity, proliferation, and activation (Mosmann, 1983; Cory *et al.*, 1991). The MTT reagent (thiazolyl blue tetrazolium bromide) is a water-soluble, yellow tetrazolium salt. If it reacts with the dehydrogenase of the mitochondria in living cells, then the tetrazolium ring is eliminated from the reagent, forming a purple insoluble formazan crystalline precipitate.

To perform the MTT assay, first, the cultivated U937 cell culture fluid was moved to a well plate, with the number of cells controlled at 4×10^4 cells in each well. Subsequently, 1 μL of the particle extracts pre-treated by DMSO was added to each well. Then, the fluid was transferred to a 37°C, 5% CO_2 incubator for 24 hours. Afterwards, 10 μL of the MTT

reagents was added to each well before the fluid was placed in the incubator for one hour. The mitochondria of the living cells restored the MTT reagent to form purple-blue insoluble Formazan crystallizations at the bottom of the well. Next, after the reaction, the well plate was placed in a centrifugal machine (model CS-6R, Beckman Coulter Inc., Fullerton, California, USA), which was operated at 1200 rpm. A straw was used to remove the remaining fluid from the wells, leaving the Formazan crystals. Then, 100 μL of DMSO was added to each well to dissolve the crystals. Finally, an ELISA Reader (Multiskan Spectrum model, Thermo Electron Co., Vantaa, Finland) was used to measure the Formazan crystallization OD (optical density) at a wavelength of 550 nm. The obtained ratio of the OD of the sample group to that of the control group was the cell viability rate of the samples. The control group was pure DMSO (1 μL addition). Six tests were carried out on each group of samples.

A larger OD value corresponds to a higher cell viability rate, because only the mitochondria of living cells have active dehydrogenase. Hence, the Formazan crystallization output is proportional to the number of living cells. Eqs. (1) and (2) yield the cell viability and death rates), respectively.

$$\text{Cell viability rate (\%)} = \frac{\text{OD}_{sample}}{\text{OD}_{control}} \times 100\% \quad (1)$$

$$\text{Cell death rate (\%)} = 100\% - \text{Cell viability rate (\%)} \quad (2)$$

RESULTS AND DISCUSSION

Effect of DEPs from the Biodiesel-engine Generator

Fig. 2 shows the mass concentrations of the PM ($PM_{0.01–0.056}$, $PM_{0.01–0.1}$, $PM_{0.01–1.0}$, $PM_{0.01–2.5}$, $PM_{0.01–10}$ and $PM_{0.01–18}$) in the exhaust of the diesel engine power generator in which fuels D100 and S20 were used at 0 (unload) and 3 kW loads. In the unloaded state, S20 yields lower concentrations of $PM_{0.01–0.056}$, $PM_{0.01–0.1}$, $PM_{0.01–1.0}$, $PM_{0.01–2.5}$, $PM_{0.01–10}$, and $PM_{0.01–18}$ than does D100 by 75.8, 77.0, 55.7, 53.4, 50.9, and 50.4%, respectively; the corresponding reductions under

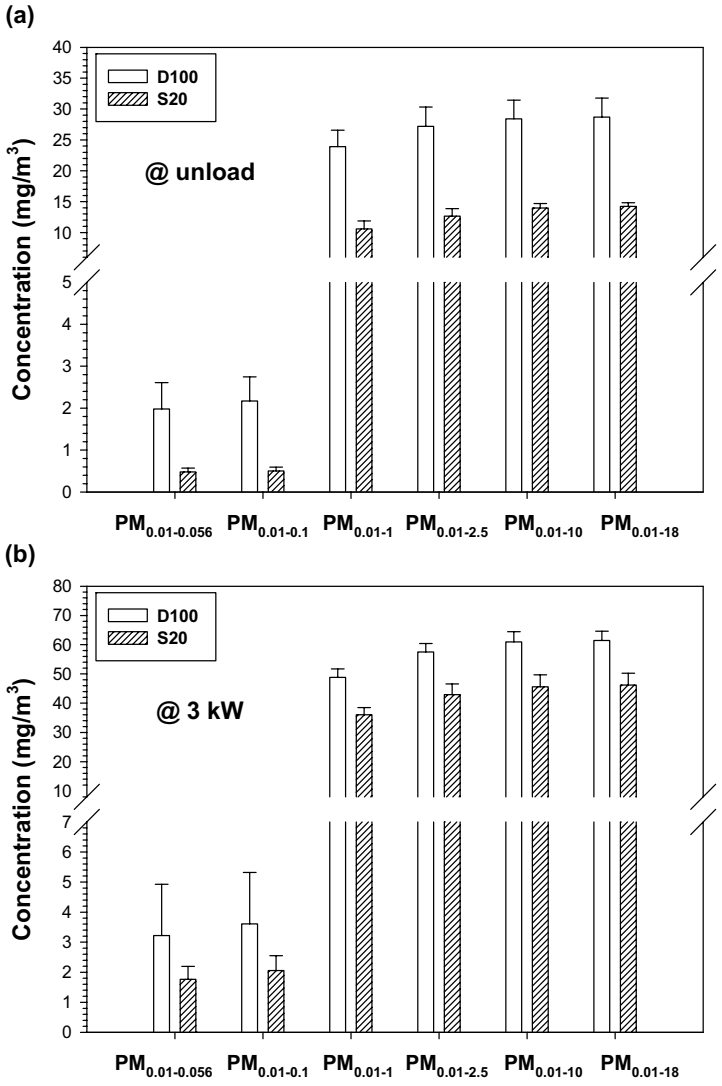

Fig. 2. Mass concentrations of $PM_{0.01–0.056}$, $PM_{0.01–0.1}$, $PM_{0.01–1.0}$, $PM_{0.01–2.5}$, $PM_{0.01–10}$ and $PM_{0.01–18}$ emitted from diesel-engine generator fuelled with D100 and S20 under (a) 0 (unloaded) and (b) 3 kW loads.

the 3kW load were 45.4, 43.0, 26.3, 25.4, 25.1, and 24.8%, respectively. The results indicate that the use of S20 biodiesel effectively reduced the masses of nano-particles ($PM_{0.01–0.056}$) and the ultrafine particles ($PM_{0.01–0.10}$) that were emitted from the diesel-engine generator (by an average of 60.3%), independently of generator load. This finding is probably associated with the fact that the sulfur content of D100 exceeded that of S20, because the numbers of nuclei and Aitken mode particles were lower when S20 was used. That the oxidation of SO_2 to SO_3 increases the number of nucleation mode particles has been confirmed elsewhere (Maricq *et al.*, 2002; Vaaraslahti *et al.*, 2004; Maricq, 2007; Zhang *et al.*, 2009). Therefore, under both unloaded and 3 kW load conditions, the use of S20 biodiesel markedly reduced the emission of DEPs from the diesel-engine generator.

Figs. 3 and 4 plot the size distributions and accumulations of DEPs that were emitted from the diesel engine power generator using D100 and S20 (under 0 and 3 kW loads), respectively. In Fig. 3, for all fuels and loads, the size distributions of DEPs were unimodal, peaking in the sub-micron range (aerodynamic diameter < 1 μm). Under both loaded and unloaded conditions, the main peaks were in the range 0.18–0.32 μm, for both fuels (D100 and S20). As shown in Fig 4, regardless of the fuel and load, more than 74% of DEPs (with 0.01–18 μm aerodynamic diameters) were sub-micron particles, and more than 89% of DEPs were fine particles (aerodynamic diameter < 2.5 μm) . These results are consistent with previous observations made elsewhere that diesel engines may emit large amounts of fine and ultra-fine particles (Harris and Maricq, 2001; Jacobson and Seinfeld, 2004; Rose *et al.*, 2006), including those smaller than 50 nm (Maricq *et al.*, 1999; Sakurai *et al.*, 2003).

Effect of Particle-bound PAHs in DEPs from the Biodiesel-engine Generator

Tables 3 and 4 present the concentrations of tested 4- to 7-ring PAHs in DEPs that are emitted from the power generator using D100 and S20, at 0 and 3 kW loads. In the unloaded state, S20 yielded total-PAHs concentrations of $PM_{0.01–0.056}$, $PM_{0.01–0.1}$, $PM_{0.01–1.0}$, $PM_{0.01–2.5}$, $PM_{0.01–10}$, and

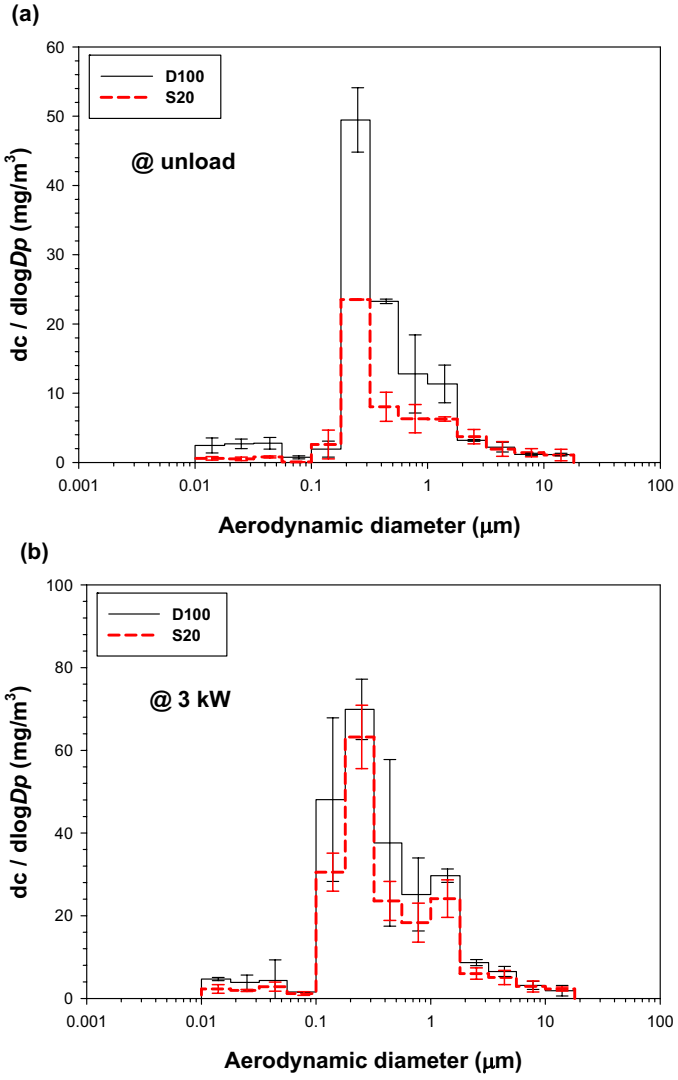

Fig. 3. Size distribution of DEPs emitted from diesel-engine generator fuelled with D100 and S20 under (a) 0 (unloaded) and (b) 3 kW loads.

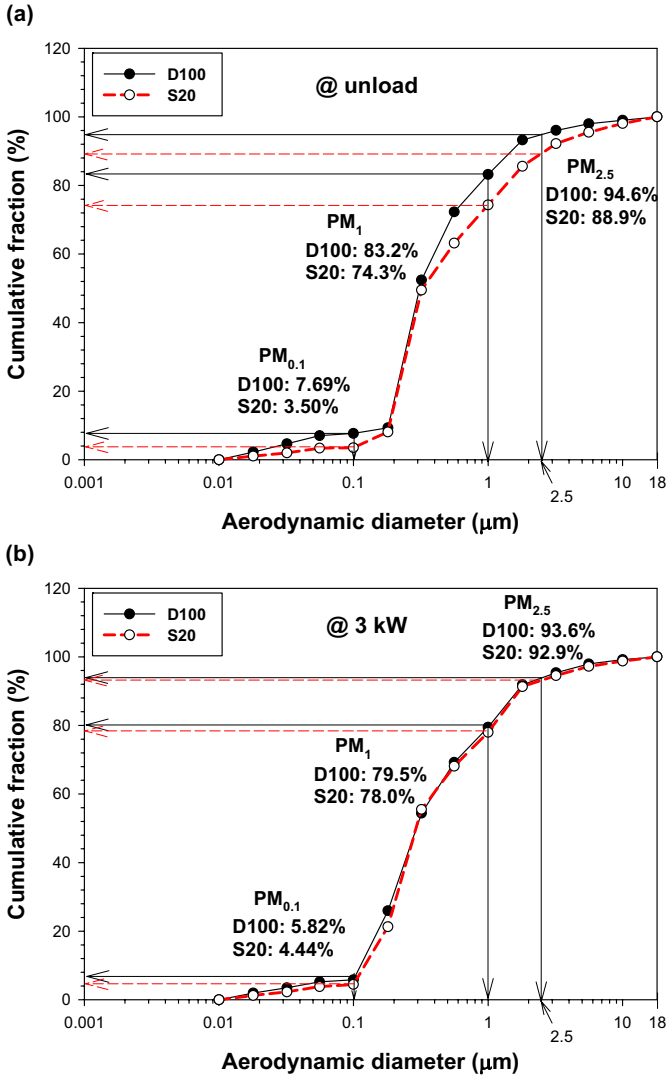

Fig. 4. Accumulation rate of DEPs emitted from diesel-engine generator fuelled with D100 and S20 under (a) 0 (unloaded) and (b) 3 kW loads.

$PM_{0.01–18}$ that were 25.0, 32.6, 74.0, 75.9, 74.7, and 74.6% lower, respectively, than those obtained using D100, and the concentrations of BbF + BaP + DBA (PAHs with relatively higher TEFs) were 33.6, 38.5, 77.1, 77.8, 75.0, and 74.5% lower, respectively; the concentrations of total BaP_{eq} (namely, the total-BaP_{eq} concentration) of the 15 PAHs were 36.1, 40.7, 78.5, 78.8, 76.7, and 76.1% lower, respectively. Under the 3 kW load, replacing D100 with S20 reduced the concentrations of total-PAHs in DEPs (PM with the six size ranges) by 66.6, 66.8, 52.9, 54.4, 54.2, and 53.3%, respectively; those of BbF + BaP + DBA by 86.0, 86.3, 69.1, 69.0, 67.3, and 65.8%, respectively, and those of total-BaP_{eq} by 85.5, 85.7, 67.9, 68.9, 67.8, and 66.1%, respectively.

The above results suggest that regardless of load, the use of S20 biodiesel effectively reduced not only the total-PAHs in DEPs (with various aerodynamic diameters) that were emitted from the diesel engine power generator (by 25.0–75.9%) but also the total-BaPeq (by 36.1–85.7%). When S20 was used, the decreases in BbF + BaP + DBA, total-PAHs, and total-BaP_{eq} of the nano-particles and ultrafine particles ($PM_{0.01–0.056}$ and $PM_{0.01–0.1}$, respectively) that were emitted at 3 kW were more (by an average factor of 2.0–2.7) than those in the unload state; nevertheless, the reductions of BbF + BaP + DBA, total-PAHs, and total-BaP_{eq} in larger particles ($PM_{0.01–1.0}$, $PM_{0.01–2.5}$, $PM_{0.01–10}$ and $PM_{0.01–18}$) were smaller (average = 0.7–0.9 times). This phenomenon is possibly related to the fact that the PAH content of soybean oil biodiesel was close to or below the detection limit, and the oxygen content of the biodiesel (~10 wts%) was higher than that in the fossil diesel.

Lin *et al.* (2008c), who used palm-biodiesel obtained similar experimental results. They found that the mean total particle-phase PAH and BaP_{eq} concentrations from the exhaust of a heavy-duty diesel generator in a steady state (at 75% of maximum power) obtained using B5 (5 vol% palm-biodiesel), B10, B15, B20, B25, and B30 were (10.1%, 12.2%), (16.7%, 20.9%), (20.9%, 23.7%), (28.0%, 30.1%), (37.6%, 39.0%) and (41.2%, 43.1%) lower, respectively, than those obtained using B0. Kameda *et al.* (2007) studied the characteristics of particle-bound PAHs/nitro-PAHs that were

emitted from a diesel engine that was fuelled by biodiesel blends, and found that the amount of particulate PAHs/nitro-PAHs in the exhaust was reduced by 44–89% from that obtained using only fossil diesel, as the proportion of added biodiesel increased. One of our earlier papers also demonstrated that the emission factors of total-PAHs decreased (by an average of 72%) as the ratio of soy-biodiesel to premium diesel increased, and that the use of biodiesel with soybean oil methyl ester could considerably reduce the BaP_{eq} of PAHs that were emitted from a generator (Tsai *et al.*, 2010).

Cytotoxicty of DEP Extract

In this study, human male monocyte cell strains (U937) were exposed to various extract samples for 24 hours after various organic solvents had been replaced by DMSO (dimethyl sulfoxide). The MTT assay was adopted to analyze the cell viability rates of various PM particle extracts. The cell viability rate of U937 that was exposed to DMSO for 24 hours as a control was used to determine the cytotoxicities (cell death rates) of various organic solvent extracts of the DEPs (with various aerodynamic diameters) (Fig. 5). Under a 3 kW load, except for $PM_{1.8–3.2}$ and $PM_{10–18}$, the cytotoxicity of the organic solvent extracts of DEPs to U937 when S20 biodiesel was used, was generally lower (by 18–96%, average = 67%) than that when D100 was used. Bunger *et al.* (1998) also found that the mutagenic potency of diesel fuel was higher than that using rapeseed oil methyl esters (RME, biodiesel) in a modern passenger car, probably because the exhaust of an engine that is fuelled with RME contains a smaller amount of polycyclic aromatic compounds (PACs). Turrio-Balassarri *et al.* (2004) used petroleum diesel and B20 biodiesel to investigate the PAHs in diesel engine exhaust and their toxicities. These research findings suggest that the emission of PAHs and the bio-toxicity of DEPs were lower when biodiesel was used than when petroleum diesel was used. Other investigations have also found that the use of biodiesel can reduce the PAH content of the particles in the diesel engine exhaust as well as the bio-toxicity of the particles that are emitted from diesel engines (Carraro *et al.*, 1997; Bagley *et al.*, 1998; Bunger *et al.*, 2006).

As presented in Fig. 5, regardless of fuel or load, the cytotoxicity of different DEP organic solvent extracts varied little with the aerodynamic diameter of the DEPs. The cytotoxicty of DEP extracts obtained when D100 and S20 were used was positively correlated with the concentrations of total-PAHs and total-BaP_{eq} in DEPs of various particle aerodynamic diameters (r^2 = 0.659–0.749, Fig. 6). This phenomenon is associated with the fact that the DEP organic solvent extract contained no DEP particles, making the particle aerodynamic diameter irrelevant to the bio-toxicity (Zhao *et al.*, 2006). However, when the cytotoxicity was converted into unit mass cytotoxicity (cell death rate per unit DEP mass), as displayed in Fig. 7, the DEPs with aerodynamic diameters of less than 0.1 μm (ultrafine particles and nanometer-particle) were found to have noticeably higher unit mass cytotoxicity than the DEPs with particles of other sizes (for D100, 4.92–5.60 times; for S20, 2.70–6.56 times). Additionally, under no load, the distribution pattern of unit mass cytotoxicity for particles of various DEPs obtained using S20 was similar to that obtained using D100. At 3 kW load, the unit mass cytotoxicity values of the extracts of nano/ultrafine DEPs (0.010–0.018, 0.018–0.032, 0.032–0.056, and 0.056–0.1 μm) were clearly lower when S20 was used than when D100 was used (by 47.7%, 46.5%, 63.3% and 52.8%, respectively), while those of larger DEPs (0.1–18 μm) were similar for. The above results suggest that replacing D100 (petroleum diesel) with S20 (a mixture of 80% petroleum diesel with 20% soybean biodiesel) helps to reduce the bio-toxicity of DEPs that are emitted from the power generator.

In addition to PAHs, a variety of complex organics (e.g., VOCs, SOFs, and even dioxins) (Schmitz *et al.*, 2000; Chuang *et al.*, 2010a; Chuang *et al.*, 2010b; Kim *et al.*, 2011; Hsieh *et al.*, 2011) and inorganics (such as trace metals) (Lin *et al.*, 2005; Lin *et al.*, 2008a; Hu *et al.*, 2009) in DEPs may also be responsible for the cytotoxicity of DEP extracts because of additivity, synergy, and antagonism. The SOF of diesel is toxic and causes generate inflammation and oxidative stress (Liu *et al.*, 2008; Swanson *et al.*, 2007). Shima *et al.* (2006) indicated that the organic compounds that are adsorbed on DEPs had high oxidative capacity; furthermore, many functional groups of the organic compounds were involved in oxygenation and contributed greatly to oxidative stress, cytotoxicity, and inflammatory response. These effects are strongly related to the organic fraction of the diesel exhaust particles and the association of these particles with oxidative stress and the magnitude of the response of cytokines (Bonvallot *et al.*, 2001).

The higher bio-toxicity of DEP extracts to U937 is also possibly due to the aldehyde/ketone components formed during the injection of diesel fuel into the high-temperature combustion chamber followed by thermal cleavage. Yang *et al.* (2007) confirmed that the average fraction of particulate *trans,trans*-2,4-decadienal (*tt*-DDE) (a toxic aldehyde) in cooking oil fumes was 83% for 16 investigated restaurants. Toxicological studies have evaluated that *tt*-DDE may induce human bronchial epithelial cells proliferation, and then lead to lung carcinogenesis (Chang and Lin, 2008; Young *et al.*, 2010). The biodiesel used in the world is mainly transformed from vegetable oils which are comprised of unsaturated methyl esters such as linoleic and linolenic (C18:3) (Karavalakis *et al.*, 2010), so the aldehyde/ketone components should be formed during the biodiesel burning process. This inference has been proved by Ballesteros *et al.* (2011). Moreover, Brito *et al.* (2010) concluded that higher addition of biodiesel might be more toxic than diesel fuel. They observed that 50 vol% and 100 vol% soy-biodiesel in diesel promoted cardiovascular alterations as well as pulmonary and systemic inflammation. The difference between the observations made in the cited studies and the present investigation may be related to the use of different proportions of biodiesel.

CONCLUSIONS

This investigation characterized particle-bound PAHs and particle-extract-induced cytotoxicity of the emissions from a diesel-generator that was fuelled with petroleum diesel

and soy-biodiesel. Under two loads, the use of S20 biodiesel instead of D100 effectively reduced the concentration of PM (by 16.2–77.0% (for different sizes), particle-bound PAHs (by 52.9–82.9%) and BaP_{eq} (by 56.6–92.9%). Independently of load and fuel, about three quarters of the PM mass of $PM_{0.01–18}$ in the power generator exhaust was that of nano-particles that had diameters of less than 1 μm. Whether loaded or unloaded, the use of S20 biodiesel was less cytotoxic to U937 than was D100 (by 18–96%, with an average of 67%), based on the organic solvent extracts of PM with various diameters. Therefore, the soybean biodiesel (S20) can serve as an alternative to fossil-diesel, to reduce PM emissions from diesel power generators and PM-associated cytotoxicity.

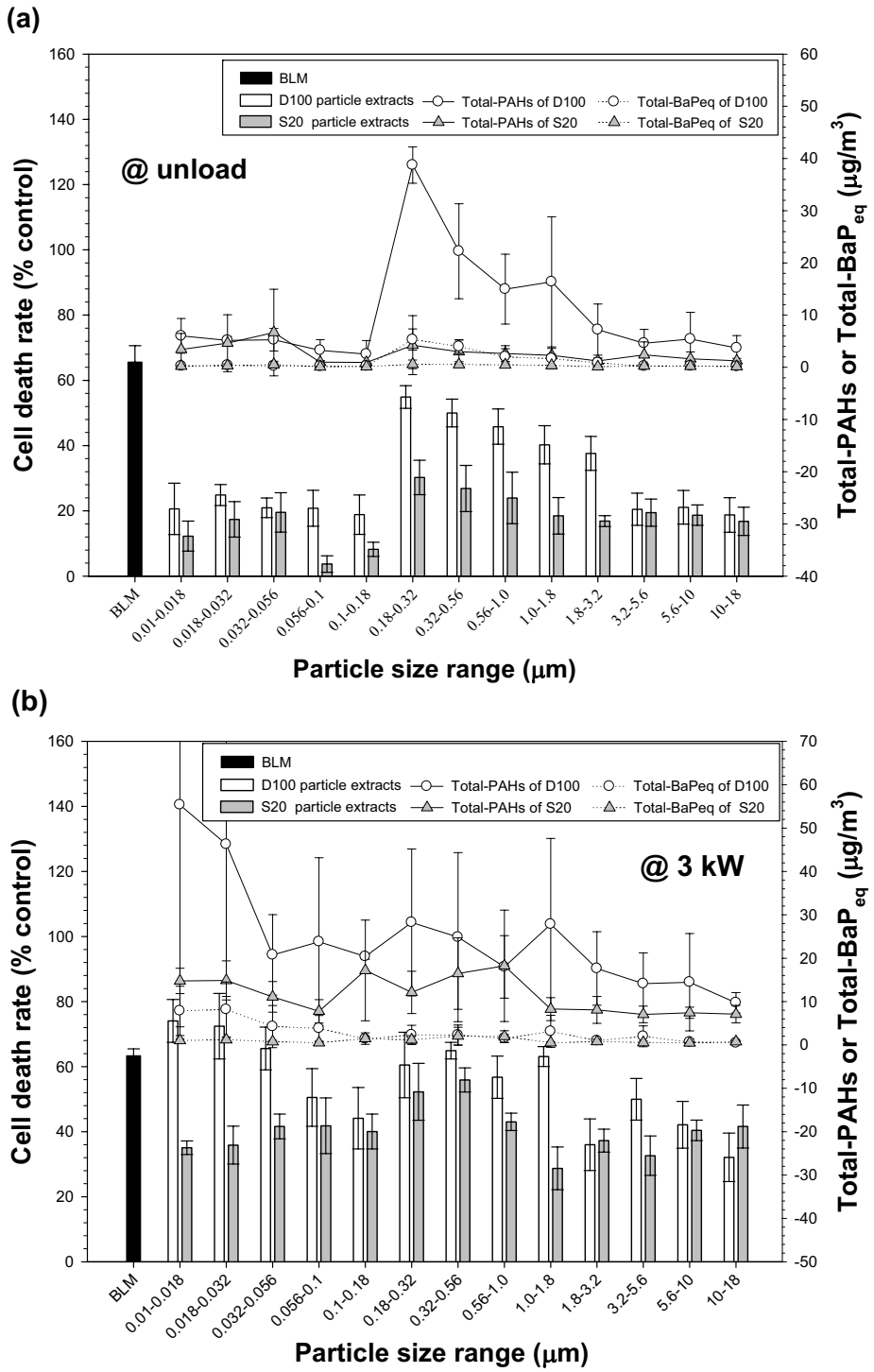

Fig. 5. PAH-associated BaP_{eq} and extract cytotoxicties (cell death rates) of DEPs emitted from diesel-engine generator fuelled with D100 and S20 under (a) 0 (unloaded) and (b) 3 kW loads. Cells (4×10^4/100 μL/well) were treated with 1 μL of bleomycin (BLM, 75 U/mL) or particle extracts, and incubated in CO_2 incubator at 37°C for 24 h.

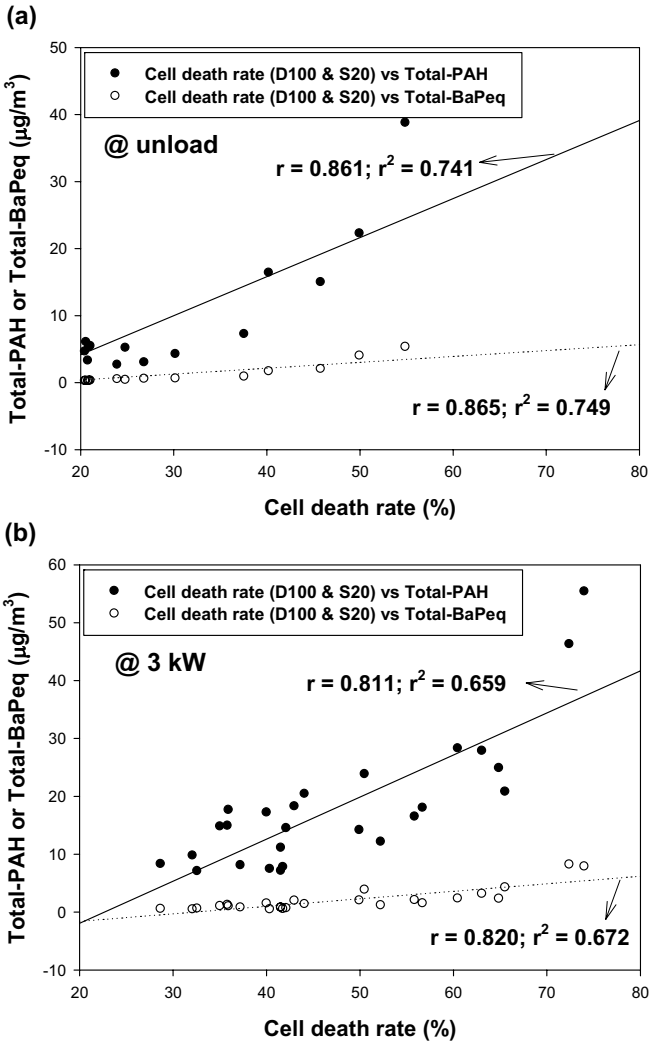

Fig. 6. DEPs extract cytotoxicty (cell death rate) against total-PAH and total-BaP_{eq} for variously sized DEPs emitted from diesel-engine generator fuelled with D100 and S20 under (a) 0 (unloaded) and (b) 3 kW loads.

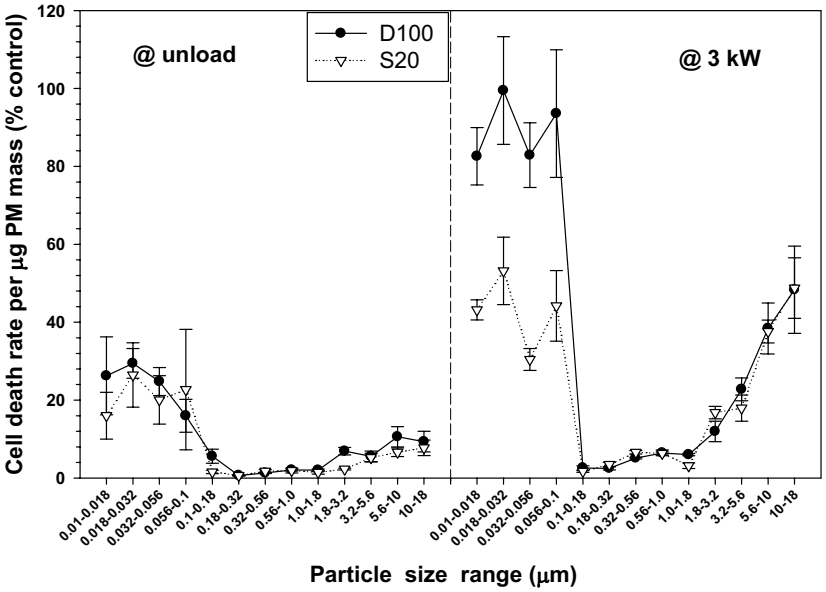

Fig. 7. Unit mass cytotoxicity (cell death rate per unit mass) of DEPs emitted from diesel-engine generator fuelled with D100 and S20 under (a) 0 (unloaded) and (b) 3 kW loads.

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