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Biological Toxicities of Exhausts from a Diesel-Generator Fueled with Water-Containing Acetone/Butanol and Waste-Edible-Oil-Biodiesel Blends

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ABSTRACT

In this investigation, conventional diesel (D), 1–30 vol% waste-edible-oil-biodiesel (WEO-biodiesel, (W), 1–3 vol% pure/water-containing acetone (A/A" (5% water content)) or 1–50 vol% butanol (B/B' (2% water content)/B" (5% water content) were tested as fuels and their effects on the cytotoxicity of emissions from a generator at 3 kW load were studied. Human male single cells (U937) and the MTT (3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide) method were used to test the cell toxicity of gas- and particle-phase samples (which were obtained by organic-solvent extraction). The results revealed that adding 1–3% acetone/water-containing acetone to bio-dieselhols reduced the mortality of U937 that were exposed to exhaust emissions organic-solvent extraction of to U937 when the generator was loaded at 3 kW. Adding more acetone/water-containing acetone further reduced the mortality of cells that were exposed to the emission gas from organic solvent extraction Compared with water-free butanol, using water-containing butanol (2% or 5%) added WEO-biodiesel further reduced the cytotoxicity (to U937) of organic solvent extracts from the emissions. The mortality of U937 decreased as the added butanol percentage increased in the range of 10−30% but increased as the added butanol percentage was increased to 40% or 50%. Therefore, the water-containing and -free acetone/butanol blending could reduce the toxicity of diesel-engine exhausts.

Keywords: Biodiesel; Acetone; Butanol; Water-containing; Cytotoxicity.

INTRODUCTION

Mounting evidence has established that diesel engine exhaust (DEE) is hazardous to human health, so reducing diesel pollution has become a public priority (IARC, 2012; Chen *et al.*, 2013; Popovicheva *et al.*, 2014). Emissions from diesel engines mostly comprise gas-phase (carbon monoxide (CO), nitrogen oxides (NO_x), sulfur oxides (SO_x), hydrocarbons (HC), and volatile organic compounds (VOCs), particulate-phase pollutants (carbon particles, ash, soluble organic fractions (SOFs), and trace metals) (Durbin *et al.*, 2000; Lin *et al.*, 2008a), and toxic organic pollutants (polycyclic aromatic hydrocarbons (PAH), polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs), polychlorinated biphenyl (PCBs), polybrominated dibenzo-*p*-dioxins/dibenzofurans (PBDD/Fs), and polybrominated diphenyl ethers (PBDEs)) (Chuang *et al.*, 2010; Chang *et al.*, 2014). Therefore, the use of clean and renewable energy rather than fossil fuel in diesel engines is becoming increasingly favored to reduce their pollutant emissions without the need to modify the engines, because the fuel that is used in diesel engines is a critical factor which directly affects the particle and gas pollutants in DEE.

Biodiesel has similar combustion features to those of diesel but the former causes less air pollution and has a weaker greenhouse effect (US EPA, 2000). Many studies of biodiesel have shown that the use of fuels that contain appropriate percentages of biodiesel can reduce the amounts of pollutants, such as particulate matter (PM), particulate carbons, PAHs, CO, and CO_2, that are emitted from diesel engines (Lin *et al.*, 2010; Tsai *et al.*, 2010; Lu *et al.*, 2013; Shukla *et al.*, 2014). Biodiesel that is used in diesel engines has higher oxygen content and cetane number than fossil diesel, increasing engine combustion efficiency and reducing

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pollutant emissions. Bio-dieselhol (a blend of biodiesel, solvent, and fossil diesel) is another feasible oxygenated fuel that may improve combustion and reduce the emissions of CO, HC, and PM (Kwanchareon *et al.*, 2007; Lin *et al.*, 2010). Several studies have demonstrated that using oxygenated additives (i.e., acetone, methane, and ethane) could effectively reduce soot formation (Hong *et al.*, 2009; Burshaid and Hamdan, 2013) and even decrease the emissions of PM, NO_x, PAHs, and BaP_{eq} in diesel exhausts (Chang *et al.*, 2014; Tsai *et al.*, 2014). Several related studies are shown in Table 1. However, the high oxygen content of pure biodiesel or bio-dieselhol is associated with (approximately 10%) higher NO_x emissions (Lapuerta *et al.*, 2008; Tan *et al.*, 2015).

Some investigations have found that the addition of appropriate amounts of water to fuels eliminates combustion temperature and reduces the formation of NO_x (Monyem and Gerpen, 2001; Lin *et al.*, 2012; Chang *et al.*, 2013). Chen and Lee (2008) suggested that using wastewater/oil emulsified fuel that contains 20% wastewater and 0.1% surfactant in boilers reduces the emissions of PM, NO_x, SO_2, and CO, below those from conventional heavy oil fuel. Accordingly, in this study, the ability of water-containing bio-dieselhol blends to reduce the toxicity of diesel-engine exhausts was tested.

Although the use of biodiesel/bio-dieselhol may significantly reduce PM emission from diesel engines (Lin *et al.*, 2008b; Marina *et al.*, 2012), the toxicity of diesel exhausts on the environment and human health must be evaluated. In this study, the cytotoxicity of the emissions from a diesel-engine generator that is fuelled with various percentages of water-free acetone/butanol or water-containing acetone/ butanol, WEO-biodiesels, and petro-diesel blends was tested at a 3 kW load. The human male monocyte cell line (U937) and MTT (3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay were used in cell toxicity tests on the organic-solvent-extracted contents of gas-/particle-phase samples in the generator exhaust.

MATERIALS AND METHODS

Sampling Methods and Fuel Compositions

The diesel-engine generator that was used herein was a four-stroke, water-cooled, single fuel-injection cylinder (bore: 88.0 mm, stroke: 96.0 mm), manufactured by YANMAR Ltd., Japan (Model: TF110E&YSG-5SEN). The generator has one phase/two wires, an output frequency of 50/60 Hz and a maximum output power of 4 kW. An auto-detector flow sampling system, equipped with a quartz fiber filter (Model: 2500 QAT-UP, 47 mm; Pall Corporation), was installed downstream of the diesel generator exhaust to measure the emitted concentrations of particulate-phase pollutants. Gas-phase emissions were collected using two connected cartridges that were filled with XAD-16 resin. Further details of the sampling programs can be found elsewhere (Tsai *et al.*, 2010, 2011a).

The premium diesel fuel was obtained from the Chinese Petroleum Corporation, Taiwan; the WEO-biodiesel was manufactured by the Taiwan NJC Corp.; the tested acetone,

Table 1. Summary of researches concerning the air pollutant emission when using water-containing diesel blends.

Fuel blends	Comparison with regular diesel					References
	PM	PAHs	NO_x	CO	HC	
20% acetone-butanol-ethanol (ABE) solution + 0.5% water	↓5.82–61.6%	↓0.699–31.1%	↓3.69–16.4%	↑34.1–117%	–	Chang *et al.*, 2013
Water content emulsified diesel fuels (WD, including WD-0, WD-5, WD-10, and WD-15)	–	–	↓18.3–45.4%	–	–	Syu *et al.*, 2014
25% water-containing ABE solution + 25–75% biodiesel	↓10.9–63.1%	↓26.7–67.6%	↓4.30–30.7%	–	–	Chang *et al.*, 2014
2% Microalgae biodiesel + 20% butanol + 0.5% water	↓59.5%	↓22.8%	↓28.2%	↑1.72%	↑69.8%	Mwangi *et al.*, 2015
20% waste-edible-oil-biodiesel + 1–40% butanol containing 2% of water	↓26.1–47.9%	↓35.9–59.2%	–	–	–	Tsai *et al.*, 2015
20% waste-edible-oil-biodiesel + 1–40% butanol containing 5% of water	↓44.9–56.4%	↓48.3–58.2%	–	–	–	Tsai *et al.*, 2015

↓: Decrease; ↑: Increase.

butanol, and isopropyl alcohol (IPA) solvents were obtained from Taiwan Merck Ltd. Deionized (DI) water was added to pure acetone and butanol to form 5 vol% water-containing acetone (A") and 2 and 5 vol% water-containing butanols (B' and B"), and 2% isopropyl alcohol (IPA) was used as a co-solvent to stabilize the water content in the B" fuel blends. Further details of the compositions of the fuel blends are provided elsewhere (Tsai *et al.*, 2014, 2015).

Preparation of Exhaust Samples Extracts

The gas-/particle-phase samples that were collected from diesel-generator exhausts were extracted using 1:1 (v/v) n-hexane/dichloromethane. Then, the organic solvent that was used to extract the particles was evaporated in a stream of nitrogen. The residues thus obtained were then resuspended in dimethyl sulfoxide (DMSO) and stored in a freezer at –80°C until the cytotoxicity assay was performed using porcine human male monocytic cell strain (U937) as part of an *in vitro* screening system. Further details of the preparation programs can be found in our earlier works (Tsai *et al.*, 2011b, 2012).

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 in each well maintained at 4×10^4. Then, 1 µL of the organic-solvent-extracts that had been pre-treated by DMSO was added to each well. Then, the fluid was transferred to a 5% CO_2 incubator at 37°C 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 crystals at the bottom of each well. 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 utilized to measure the Formazan crystallization OD (optical density) at a wavelength of 550 nm. The cell viability rate of the samples was the obtained ratio of the OD of the sample group to that of the negative control group (with 1 µL pure DMSO added). Bleomycin (BLM) may cause pulmonary cell fibrosis (Schein and Winokur, 1975) and pulmonary macrophage apoptosis (Zhao *et al.*, 2004), although it is a glycopeptide antibiotic that is commonly used as an anticancer drug. In this study, 75 mU μL^{-1} BLM was added in the positive control group to evaluate the cytotoxicity of gas-/particle-phase samples organic-solvent extracted materials. Six tests were performed on each group of samples.

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

$$\text{Cell viability rate (\%)} = \frac{OD_{sample}}{OD_{control}} \times 100\% \qquad (1)$$

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

RESULTS AND DISCUSSION

Using Biodieselhol Additive in Acetone/Water-Containing-Acetone

To measure toxicity, various ratios of acetone or water-containing acetone, WEO-biodiesels, and petro-diesel blends (1–30 vol% waste-edible-oil-biodiesel + 1–3 vol% pure acetone or water-containing acetone + 1 vol% IPA + 66–99 vol% diesel) were tested as the fuel in the generator under a 3 kW load. Fig. 1 presents the data on U937 mortality obtained using the organic solvent extracts of gas-/particle-phase samples that were collected from the generator exhaust. The U937 cell cytotoxicity of organic solvent extracts of gas-/particle-phase samples was highest for D100, for which it was slightly less than that of Bleomycin (BLM, 44.7 ± 3.6% mortality). BLM is a common antitumor medicine that causes pulmonary fibrosis and pulmonary macrophage death. BLM was used for comparison in evaluating the U937 cell cytotoxicities of organic-solvent extracts of generator emissions. The used BLM concentration (75 mU μL^{-1} per well) was that found by Lin *et al.* (2008a) to be cytotoxic to the macrophage of pulmonary alveoli.

As presented in Fig. 1, the generator under 3 kW load with W1, W3, W5, W10, W20 and W30 as fuel yielded organic solvent extracts of gas-/particle-phase emission samples whose U937 cell mortality were (about 10.4–36.4%, and on average 22.2%) lower than obtained using D100. Among these extracts, the one obtained using W10 exhibited the least cytotoxicity to U937, followed by that from W20. When percentage of added WEO-biodiesel did not exceed 10%, the U937 cell cytotoxicity of organic solvent extracts of gas-/particle-phase samples from the generator emissions declined as the percentage increased. As the percentage of WEO-biodiesel added increased from 20% to 30%, the U937 cell mortality of the organic solvent extracts increased, although it was always less than that of D100. Adding 1–3% pure acetone to W1, W3, W5, W10, and W20 reduced the U937 cell mortality of the organic solvent extracts of the gas-/particle-phase samples from the generator emission (on average by 12.9%), and the mortality decreased as the amount of acetone increased. Adding 1–3% water-containing acetone to W1, W3 or W5 further reduced the U937 cell mortality of organic solvent extracts from generator emissions, and

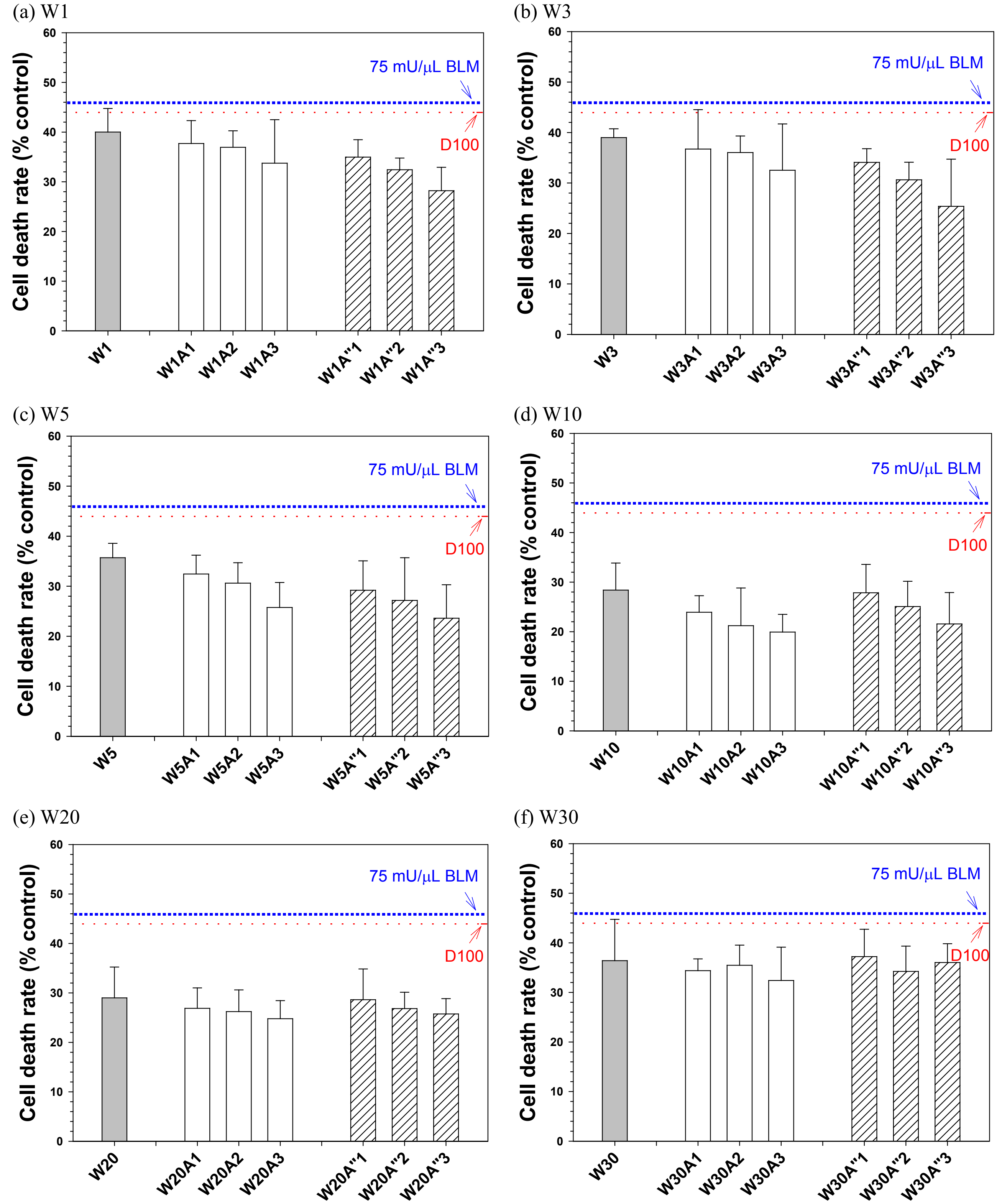

Fig. 1. U937 mortalities of organic solvent extracts of gas-/particle-phase samples from generator emissions from engine under 3 kW load and fueled with WEO-biodiesels to with added pure or water-containing acetone.

the mortality further decreased as the percentage of added water-containing acetone increased (Figs. 1(a)–(c)). The addition of less than 20% of WEO-biodiesel (W1, W3, W5, W10 and W20) reduced the U937 cell mortality of organic solvent extracts from generator emissions of the generator under a 3 kW load with water-containing acetone biodiesel as fuel to an extent that decreased as the percentage of added water-containing acetone increased.

The results reveal that independently of load, the generator with pure /water-containing acetone biodiesels yielded emissions whose organic solvent extracts had a lower U937 cell mortality than that with D100. Adding 1–3% pure /water-containing acetone biodiesels further reduced the U937 cell mortality of organic-solvent extracts from emissions from the 3 kW-loaded generator that was fueled W1, W3, W5, W10 and W20, to an extent that increased with the percentage added. As the addition percentages of pure acetone and water-containing acetone increased to 3%, the U937 cell mortalities of organic solvent extracts from generator emissions were further reduced by 24.46–55.37% and 19.32–51.71%, respectively. The reductions of mortality followed the order WnA"3 > WnA"2 > WnA"1.

Using Bio-Dieselhols and Butanol/Water-Containing Butanol Additive

Fig. 2 shows the U937 mortalities for the organic solvent extracts of gas-/particle-phase emission samples from the generator that was operated under a 3 kW load with bio-dieselhols that comprised 10–50 vol% pure (or dehydrated) butanol, 10–40 vol% water-containing butanol (2% and 5% water content), 20 vol% WEO-biodiesel, and 30–80 vol% petro-diesel. The U937 cell mortalities of the organic solvent extracts of gas-/particle-phase samples from generator emissions using D100 and 75 mU μL^{-1} BLM (per well) under a 3 kW load were 47.3±5.5% and 52.7 ± 3.2%, respectively, which exceeded those using fuels with various additives. Using W20 reduced U937 cell mortality by 13.5% below that of the organic solvent extracts of generator emission. The U937 mortality obtained using W20 was 40.7 ± 5.5%. B10, B20, B30, B40 and B50 bio-dieselhols (with added water-free butanol) yielded generator emissions whose organic solvent extracts had U937 cell mortalities that were 15.6, 21.6, 25.2, 16.5 and 14.2% lower, respectively. Water-containing B'10, B'20, B'30, B'40, B"10, B"20, B"30 and B"40 bio-dieselhols yielded generator emissions whose organic solvent extracts had U937 cell mortalities that were 23.9, 46.3, 54.8, 44.6, 24.7, 47.6, 49.3 and 35.3% lower, respectively.

The results reveal that under 3 kW load, B10, B20, B30, B40, B50, B'10, B'20, B'30, B'40, B"10, B"20, B"30 and B"40 bio-dieselhols yielded generator emissions whose the organic solvent extracts had a lower U937 cell mortality than obtained using D100. When the percentage of added butanol (either pure or water-containing) did not exceed 30%, the U937 cell cytotoxicity of the organic solvent extract of the generator emissions decreased as the percentage increased. This finding follows from the fact that adding an oxygenate (such as an alcohol or ester) to the petro-diesel increased the fuel combustion efficiency of their engine and thereby reduced the emission of hazardous pollutants (Basha *et al.*, 2009; Shukla *et al.*, 2014). Yang *et al.* (2007) stated that using biodiesels reduced the emission of high-ring PAHs. Other investigations have stated that biodiesels reduce emission of pollutants (such PAHs and PCDD/Fs) from diesel engines (Chang *et al.*, 2014) and the toxicology of the emitted particles (Bünger *et al.*, 2006; Tsai *et al.*, 2012). Zhang and Balasubramanian (2014) found that adding 5–15% butanol to 20% palm-oil biodiesel reduced the cell cytotoxicity of the emitted particles below that of those emitted from an engine that runs on pure diesel.

Using water-containing butanol bio-dieselhols (B'10, B'20, B'30, B'40, B"10, B"20, B"30 and B"40) reduced the U937 cell mortalities of the organic solvent extracts of generator emissions by 9.77, 31.5, 39.6, 33.7, 10.8, 33.2, 32.2 and 22.5%, respectively, relative to those obtained from water-free butanol (B10, B20, B30 and B40). These results reveal that using water-containing butanol (2% or 5%) bio-dieselhols reduced the U937 cell mortalities of the organic solvent extracts from generator emissions by 9.8–39.5% below those obtained using water-free butanol bio-dieselhols. This result is related to the presence of a small amount of water in butanol, which favors the micro-explosion (secondary atomization) of blended fuel (oil droplets) (Chen and Lee, 2008; Tsai *et al.*, 2015), the more

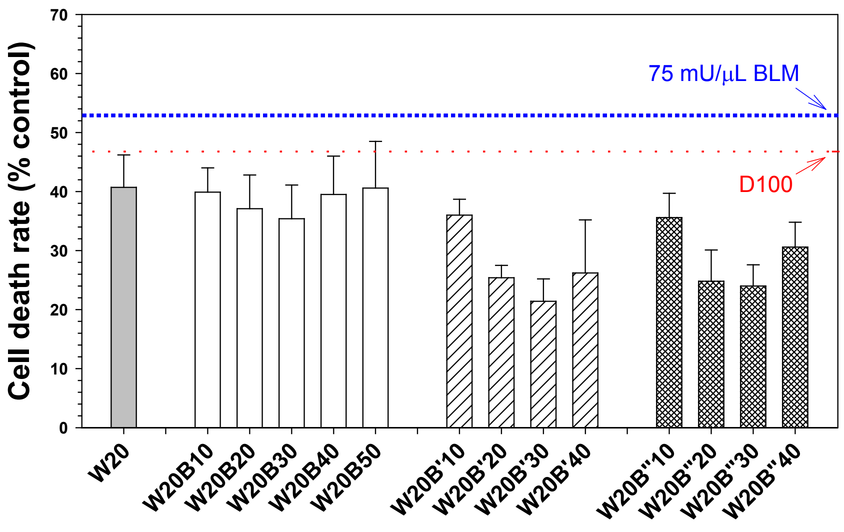

Fig. 2. U937 mortalities of organic solvent extracts of gas-/particle-phase samples from generator emissions from engine under 3 kW load and fueled with WEO-biodiesels with added pure butanol/water-containing butanol.

complete combustion of fuel, and the reduction of pollution emissions (Ribeiro *et al.*, 2007; Chang *et al.*, 2013). The difference in the U937 cell mortality between the organic-solvent extracts from generator emissions obtained using fuels with 2% and 5% added water-containing butanol was insignificant. Some researchers have reported that the abundant oxygen content in diesel blends could further reduce harmful emissions (aldehydes, ketones, PAHs, PBDE, PCDD, etc.) by promoting more complete oxidation of aromatic rings and their precursors (CH_2 radical) (Hoon *et al.*, 2003; Ballesteros *et al.*, 2011; Chang *et al.*, 2014). In addition, some secondarily atomized water-containing fuel droplets in high-temperature combustion may be pyrolyzed to form ion-stated carbon particles, leading to the water-gas-shift reaction ($C_{(s)} + H_2O_{(g)} \rightarrow CO_{(g)} + H_{2(g)}$; $CO_{(g)} + H_2O_{(g)} \rightarrow CO_{2(g)} + H_{2(g)}$). The formed CO and H_2, which are flammable gases, also help to reduce the formation of toxic pollutants (Tsolakis and Megaritis, 2004; Leung *et al.*, 2009).

CONCLUSIONS

This investigation examined the toxicity of the exhaust of a 3 kW-loaded generator that was fueled with WEO-biodieselhols (with added pure/water-containing acetone or pure/water-containing butanol) using the MTT method. The following conclusions are drawn.

1. Under a 3 kW load, using pure /water-containing acetone bio-dieselhols effectively reduced the U937 cell mortality of the organic solvent extracts from the generator emissions below that obtained using D100.
2. Under a 3 kW load, adding 1–3% pure /water-containing acetone biodiesels to W1, W3, W5, W10 and W20, reduced the U937 cell mortalities of the organic solvent extracts from the generator emissions to a degree that increased with the percentage of pure /water-containing acetone added. When the addition percentage of pure /water-containing acetone reached 3%, the U937 cell mortalities of the organic solvent extracts from the generator emissions were reduced by 24.5–55.4% /19.3–51.7%, respectively. The reductions of mortality followed the order WnA"3> WnA"2> WnA"1.
3. Each bio-dieselhol with added butanol reduced the U937 cell mortality of the organic solvent extract from the generator emission below that obtained using D100. When the percentage of added butanol (either pure or water-containing) did not exceed 30%, the U937 cell cytotoxicities of the organic-solvent extracts from the generator emissions decreased as the percentage increased.
4. Using water-containing butanol (2% or 5%) bio-dieselhols reduced the U937 cell mortalities of the organic solvent-extracts from the generator emissions below that obtained using water-free butanol bio-dieselhols.

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