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Toxicity assessment of volatile organic compounds and polycyclic aromatic hydrocarbons in motorcycle exhaust

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Abstract

This study investigates the toxicity of various pollutant species from motorcycle exhaust via dose–response analysis and margin of safety using *Escherichia coli* DH5α. The toxicity evaluation of the major components of motorcycle exhaust volatile organic compounds (VOCs), collected with impinger, and polycyclic aromatic hydrocarbons (PAHs), collected with filter and XAD-2, is essential to determine emission standards for motorcycles. The toxicity of benzene (B), toluene (T), ethyl benzene (E) and xylene (X) was selected for comparison as standard VOCs emitted from motorcycles. In addition, three types of reformulated gasoline (high oxygenate and high benzene content (No. 1), low oxygen and high benzene (No. 2), and low oxygen and low benzene (No. 3) were prepared to reveal combined toxicity of individual compositions. Motorcycle exhaust is significantly more toxic than BTEX due to the highly toxic VOCs generated from incomplete combustion. Overall toxicity evaluation showed that the toxicity, indicated as EC_{50}, was approximately as follows: PAHs > two-stroke engines > four-stroke engines > BTEX.

Keywords: Dose–mortality curves; Volatile organic compounds; Polycyclic aromatic hydrocarbons; *Escherichia coli*

1. Introduction

Due to marked rises in congested traffic of metropolitan Taiwan, significant increases in waste gas have the inevitable risks to impact public health. In the highly populated Taiwan, people frequently use motorcycle as a common means of metropolitan transportation due to their higher convenience and mobility. According to the Taiwan Ministry of Transportation and Communications (http://www.motc.gov.tw/) [1], there were 13.2×10^6 motorcycles officially reported in Taiwan before December 2006. The motorcycle density in Taiwan (ca. 452 vehicles per square kilometer) almost ranked first in the world. In some cities, the exhaust gas from motorcycles is apparently the primary source of mobile pollutants. The exhaust gas is presumed to be harmful to human health as it is known to contain a range of volatile organic compounds (VOCs), polycyclic aromatic hydrocarbons (PAHs) particulate matter and other chemicals [2].

VOCs have been found to cause acute toxic effects (primarily neurological), cancer (such as leukemia), neurobehavioral effects and adverse effects on the kidney [3]. The major volatile components of motorcycle exhaust include benzene (B), toluene (T), ethyl benzene (E) and xylene (X) [4,5]. A portion of the absorbed benzene is excreted in exhaled air unmetabolized; however, a large portion of the absorbed compound is metabolized to benzoic acid. This compound is conjugated with glycine in the liver and eventually excreted in urine as hippuric acid [6,7]. The acute health effects associated with exposure to benzene include dizziness, muscle weakness, confusion and incoordination. These effects generally occur only a high concentrations and are not likely to be associated with motorcycle exhaust. The effects associated with exposure to benzene are similar to the physiological response following exposure to most organic solvents. With respect to chemicals, evidence for benzene leukemogenesis has been reported in both human populations [8,9] and in experimental animals [10].

In addition, ophthalmic effects of VOCs also have been noted; for instance, the injury of the optic nerves may have resulted from toluene as a metabolite [11]. Toluene has also been criminated as the cause of polyneuropathy in shoe factory workers

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[12]. Generally, toluene in vapor phase can be absorbed rapidly from the lungs, and liquid toluene is taken up readily from the gastrointestinal tract, but poorly through the skin [13,14]. Xylene is a powerful solvent, but is irritating to eyes, mucous membranes and skin [15]. It can pass through intact skin or can be absorbed from lungs and the gastrointestinal tract [16]. The euphoria that is a typical response to ethyl benzene toxicity is attractive to certain individuals who have made a practice of sniffing glue, paint thinner, or ethyl benzene itself, the latter instance resulting in a schizophreniform psychosis in some case(s) [17,18]. As BTEX are well-characterized pollutants, they are thus used as standard pollutants of petroleum hydrocarbons in comparison with other pollutants (e.g., motorcycle exhaust). Regarding toxic metabolites and/or suspected carcinogens in BTEX, their metabolic transformations are divided into four categories—oxidation, reduction, hydrolysis and conjugation. Among these, oxidation is the most common biotransformation reaction mediated by microsomal enzymes. For example, benzene converts to phenol via hydroxylation.

Motorcycle exhaust also contains PAHs which are known to be toxic and genotoxic. In addition, as Cerna [19] indicated the mutation response of gaseous PAHs are more significant than those of solid PAHs, and their toxicity are strongly dependent upon concentration [20]. The reason is that the gaseous PAHs are easy to be absorbed into blood than that of the solid PAHs. Boffetta et al. [21] also demonstrated that cancer of the skin, lungs and bladder can result from exposure to PAHs. PAHs have been associated with elevated levels of DNA adducts (e.g., AH–DNA adducts) and p53 mutations in persons who smoke and/or are exposed to PAH in the either workplace or ambient air [22,23]. PAH–DNA adducts formed by the carcinogen B[a]P diol epoxide (B[a]PDE) have been strongly linked to an increased risk of lung cancer.

Although the concentration of highly toxic VOCs is low in motorcycle exhausted waste gas, it might affect human health seriously due to long-term exposure and/or bioaccumulation. Therefore, from the perspective of air pollution control, the determination of toxicity of VOCs and PAHs is extremely crucial for how to take actions of toxicity attenuation for motorcycle exhaust. As known, there are some standard methods (e.g., Microtox® [24], Mutatox and Ames test) that can be quantitatively evaluated toxicity of pollutants. However, these methods are not cost-effective to be a first screening protocol of assessment upon suspected toxicants for public uses. Thus, we intentionally selected a genetically defective and environmentally sensitive strain *Escherichia coli* DH5α as a probing microorganism [25] to reveal dose–mortality relationships of VOCs and PAHs in motorcycle exhaust using well-known BTEX as standards for comparison. With this plausible method with economic feasibility, industrial sectors can adopt a more open policy toward diverse waste sources to consider possible alternatives for reduction, reuse or recycling. Results were also confirmed with literature data, revealing the practicability with cost effectiveness as first screening assessment of toxicity for general use. Serial dilution–agar plating procedures were carried out in duplicate for quality assurance and control (QA/QC).

2. Materials and methods

2.1. Tested pollutants, engine and gasoline

2.1.1. Challenge pollutants

Major compositions of motorcycle exhaust are BTEX (benzene (B), toluene (T), ethyl benzene (E) and xylene (X)). First, to obtain bases for toxicity comparison, individual BTEX solvents were serially diluted with deionized water to obtain solutions in different concentrations. The VOCs analysis method was related to use of gas chromatography (GC)-flame ionisation detector (FID) to analyze concentration of species. The HNU Portable Gas Chromatography, model-311, with GC column SP-l200, 1.75% Bentonite 34, 6 in. × 1/8 in. was used to analyze VOCs composition. The sampling procedures composed of preparing and preheating the sampling system, connecting the auto-analysis system and combining the data-log system. The sampling volume was about 1 L for each run. However, the waste gas injected to GC was only 300 mL for analysis. Before assessing the toxicity of VOCs, the concentrations of main toxic VOCs in the motorcycle were measured and shown in Table 1. The test results shows that the main toxic VOCs are benzene, toluene, ethyl benzene and xylene.

PAHs were also extracted from the motorcycle exhaust. The sampled PAHs can be classified into two phases, gaseous PAHs and solid PAHs.

Samples of PAHs were taken from the exhaust gas of motorcycle engine. Impactor sampling combined with quartz filter and XAD-2 adsorbent was used to collect solid phase and gaseous phase PAHs and carried out in triplicate. The air was drawn at 10 L/min first through a 2.5 μm cutoff cyclone, then through two 47-mm quartz filters (Whatman) that collected particles and finally through two XAD-2 resins (Supleco Co.) for gas sampling [26,27]. According to literatures [28,29], quartz filters were treated after the humidity was removed in a desiccator. The XAD-2 was cleaned by Soxhlet extraction first with dichrolomethane (24 h) and then with methanol (24 h). The extraction procedure was repeated twice. The XAD-2 was then heated at 90 °C to remove moisture. The cleaned XAD-2 (ca. 7.5 g) was packed in a glass resin (6 cm × 1.5 cm I.D.) and kept in a clean dessicator until sampling. The filters and XAD-2 were

Table 1
The concentration of main toxic VOCs components in the exhaust waste gas with two-stroke engine

Compounds	No. 1 gasoline (ppm-v)	No. 2 gasoline (ppm-v)	No. 3 gasoline (ppm-v)
Methyl *tert*-butyl ether	14.4	14.2	13.6
Benzene	101.6	103.2	65.3
n-Heptane	14.1	15.1	13.4
Toluene	77.1	86.5	66.8
Ethylbenzene	69.0	68.3	58.6
p-Xylene	53.3	56.4	58.4
m-Xylene	13.2	14.3	13.6
O-Xylene	12.9	12.0	12.5
Isopropylbenzene	1.2	1.1	1.5
1,2,4-Trimethylbenzene	1.6	1.8	1.3

analyzed with GC–MS, Hp GCD. The instrumentation as well as the analytical procedure was established and conducted as described elsewhere [30,31]. A standard PAH mixture (Z-014G-R) containing 17 compounds in CH_2Cl_2/benzene (1:1 v/v) was purchased from Supleco company for quantitative analysis.

2.1.2. Challenge gasolines

The toxicity of PAHs in different kinds of gasolines was also assessed in the exhaust waste gas since the characteristics of reformulated PAHs was dependent on the content of oxygen and benzene [24]. Three types of reformulated gasolines, No. 1 gasoline (high oxygen and high benzene), No. 2 gasoline (low oxygen and high benzene), and No. 3 gasoline (low oxygen and low benzene) (Table 2) were intentionally used for study. Note that according to US CARB [26], the content of oxygen and benzene above 1.8 and 0.8, respectively are defined as high content.

2.1.3. Challenge engine

To have toxicity figures for exhaust waste gas of motorcycles in heavy traffic for city dwellers, two kinds of motorcycle engine, two-stroke (2T) and four-stroke (4T), were used as model engines for assessment. The ages of the two-stroke are the same and are 3 years old. The powers of two-stroke and four-stroke engines are 1.5 and 5.0 horse power, respectively.

2.2. Dose–mortality assessment

E. coli DH5α (generously provided by Professor Jo-Shu Chang, National Cheng-Kung University, Taiwan) was used as an indicator strain for biotoxicity assessment. A loopful of the indicator strain seed taken from an isolated colony in LB-streak plate was precultured in 50 mL Luria-Bertani medium (LB broth, Miller, Difco) for 12 h overnight at 37 °C, 200 rpm. To ensure the synchronous growth activity and maximum metabolic functioning in the same growth phase for bioassay, 5% (v/v) cultured broth was then inoculated to fresh sterile LB medium and a cell culture was harvested at approximately mid-exponential growth phase (ca. 4 h) for further toxicity assessment. The 1.0 mL cell culture was then serially diluted and well mixed with 9.0 mL sterile saline solution (SSS; NaCl 10.0 g L^{-1}) and only the diluent with appropriate cell concentrations (ca. 1500–15,000 cells/mL) was chosen as the test seed (TS) for later uses.

2.3. Biotoxicity assessment

Biotoxicity assessment was specifically designated through a modification of dose–response analysis [32–39] as follows: To exclude the presence of unwanted bacterial contaminants, all of the solutions for sampled chemicals (e.g., BTEX, PAHs) were first sterilized via moist-heat method (121 °C at 15 psi for 20 min) and then tested materials (e.g., BTEX, PAHs) sterilized through 0.2 μm sterile filter were added. Thus, the sample concentration defined herein was the concentration of tested chemicals and their serial diluents well mixed with SSS. It was postulated that the sodium chloride solution used for serial dilutions of various solution was the toxicity-free control. To

Table 2
The composition of gasoline

	Volume %
(a) The detailed chemical analysis of gasoline	
n-Paraffins	
n-Butane	4.82
n-Pentane	3.62
n-Hexane	1.75
n-Heptane	0.51
n-Octane	0.30
Total	11.00
Branched paraffins	
Isobutane	1.14
Isopentane	10.26
2-Methylpentane	4.39
3-Methylpentane	2.59
2,3-Dimethylbutane	1.83
2-Methylhexane	1.39
3-Methylhexane	1.39
2,3-Dimethylpentane	0.84
2,4-Dimethylpentane	0.93
2,2,4-Trimethylpentane	5.51
2,3,4-Trimethylpentane	3.04
2,3,3-Trimethylpentane	2.87
2,2,3-Trimethylpentane	0.33
2,4-Dimethylhexane	1.00
2,5-Dimethylhexane	0.84
2,3-Dimethylhexane	0.98
3-Methylheptane	0.90
2-Methylheptane	0.71
2-Methyloctane	0.30
3-Methyloctane	0.30
2,2,5-Trimethylhexane	0.72
Total	42.26
Cycloparaffins	
Methylcyclopentane	0.92
cis-1,3-Dimethylcyclopentane	0.30
Methylcyclohexane	0.30
Total	1.52
Olefins	
t-Butene-2	0.49
t-Pentene-2	0.64
c-Pentene-2	0.33
2-Methylpentene-l	0.48
2-Methylpentene-2	0.78
Total	2.72
Aromatics	
Benzene	1.69
Toluene	3.99
Ethylbenzene	1.69
o-Xylene	1.89
m-Xylene	4.79
p-Xylene	1.46
1-Methyl,3-ethylbenzene	1.30
1-Methyl,4-ethylbenzene	1.31
1,2,4-Trimethylbenzene	2.72
Total	20.84

	Oxygenate content (%)	Benzene content (%)
(b) The oxygenate and benzene content of gasoline		
No. 1 gasoline	3.0	1.0
No. 2 gasoline	1.0	1.0
No. 3 gasoline	1.0	0.5

exclude confounding interferences, phosphate buffered saline (PBS) solution, which is regularly used for biological assay, was not used here, since metallic phosphate precipitates might be formed during serial dilution. The initial concentration C_0 for toxicity tests of all chemicals was appropriately chosen under criteria of maximal solubility. Serial-half dilution of initial concentration C_0 (i.e., 1/2 C_0, 1/4 C_0, 1/8 C_0, 1/16 C_0, 1/32 C_0, ..., $1/2^n$ C_0) was carried out by using 50 mL solution to be tested or its derived diluents mixed with 50 mL SSS. The 9.0 mL resulted serial diluents (RSD) were all placed in sterile test tubes for use in quantification of viable cells afterwards. The test seed (TS) was then well mixed with RSD to form serial plate count diluents (SPCD) via serial dilution technique. Meanwhile, 1.0 mL fresh TS mixed with 9.0 mL pure SSS was used as the toxicity-free control. The numbers of viable DH5α in SPCD or the control were estimated by the standard plate count method [34,35]. Standard plate count in LB medium was carried out as follows: SPCD were serially diluted with SSS immediately after sampling, and then appropriate volumes (ca. 0.20 mL) of SPCD were spread onto agar Petri plates. Note that all cells in SPCD would be assumed metabolically viable and culturable on LB-medium plates due to fresh preparation of fast-growing *E. coli* cells in all steps. The LB-medium plates were then incubated at 37 °C for ca. 16–24 h to form observable colonies for enumeration. Only plates with between 30 and 300 colonies were statistically appropriate for counting. Serial dilution–agar plating procedures were carried out in duplicate for quality assurance and control (QA/QC). The microbial population in the original RSD can then be calculated using the following formula (CC: cell count):

$$\text{Cells per liter of broth (CC)} = \frac{\text{number of colonies}}{\text{amount plated} \times \text{dilution factor}} \quad (1)$$

To have quantitative toxicity for comparison, CC_0 was denoted as the CC at zero-toxicity control. The ratio CC/CC_0 of 0 and 1 directly indicated complete inhibition and no inhibitory toxicity to bacterial cell, respectively. The unity of this ratio simply suggests that the present toxicity of this diluent at this concentration is nearly equal to the toxicity of SSS (i.e., zero toxicity). The toxicity of SSS can be used to indicate the main toxicity of air pollutants that many species were mixed together. The concentration range for the ratio jumped from 1.0 to 0.0 in dose–response curves [37] is defined here as the "threshold toxicity" (TT) range. The comparison on TT range could provide an obvious diagram of toxicity ranking for various tested chemicals. For example, if the TT range for chemical A is much less than that for chemical B, chemical A is inevitable much more toxic than chemical B and this indicated that much higher dilution factor must be carried out for chemical A in order to have "zero" toxicity as same as control (SSS) (Fig. 1).

2.4. Dose–response analysis

The conversion formulae for dose–response analysis were

$$Y = A + B \log Z, \quad (2)$$

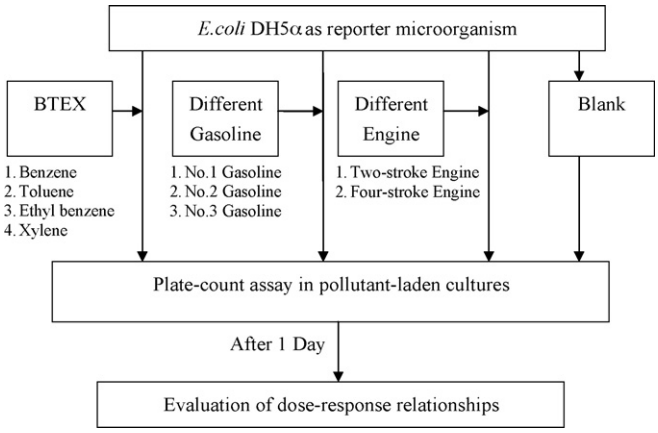

Fig. 1. Schematic of toxicity assessment (dose–mortality relationships) using plate count method.

$$P = 0.5\left\{1 + \text{erf}\left(\frac{(Y-5)}{\sqrt{2}}\right)\right\}, \quad (3)$$

$$\text{erf}(x) \equiv \left(\frac{2}{\sqrt{\pi}}\right)\int_0^x e^{-\xi^2}\, d\xi, \quad (4)$$

where B and A denoted the Hill slope (i.e., steepness or slope factor) and intercept of dose–response relation, Z and Y were metal concentration (mg/L) and probit unit, respectively; P was the response (%) corresponding to administered metal, erf(x) was an error function. It should be noted that the response variable was normalized to be located between 0 and 1. The conversion relation between the probit unit and provoked response $1 - VCC/VCC_0$ was calculated. The VCC and VCC_0 denote the lethal concentration and initial lethal concentration, respectively. To consider the non-biased experimental design for dose–response analysis in log-normal distribution, pollutant concentrations were approximately equally spaced on a log scale (e.g., 1.0, 3.16, 10, 31.6, 100, 316 mg/L).

3. Results and discussion

3.1. BTEX

The toxicity of a compound can be expressed by the effective concentration (EC_x) administered to the bacterial population to provoke x% response (e.g., $x = 50$ as EC_{50}). The EC_0 and EC_{100} can be defined as the maximum concentration to have a detectable response (i.e., 0^+%) and minimum concentration to have 100% response, respectively. The discrepancy among ranks of effective concentrations at different levels (e.g., EC_0, EC_{20} and EC_{50}) are response effect of each level. The higher EC_0 can be illustrated with lower toxicity of maximum concentration to have a detectable response. Whereas, the EC_{50} are the 50% detectable response of the bacterial population. It is more strictly to use EC_{50} than to use EC_{20} as an indicator in regulation. The target compounds over and under their EC_{100} obviously result in extinction and survival of viable cells, respectively. EC_x quantitatively provides a more clear indication for toxicity comparison among chemicals; the smaller the EC_x, the more toxic

Table 3
Critical effective concentrations and related parameters of toxicity predicted from the probit model for BTEX

	EC_0 (mg/L)	EC_{20} (mg/L)	EC_{50} (mg/L)	$Y = A + B \times \log C$
Benzene	8.18×10^{-2}	9.65×10^{-1}	3.64	$Y = 4.183 + 1.456 \times \log C$
Toluene	9.52×10^{-3}	5.63×10^{-1}	5.07	$Y = 4.380 + 0.880 \times \log C$
Ethyl benzene	5.74×10^{-2}	7.89	111.78	$Y = 3.506 + 0.730 \times \log C$
Xylene	1.66×10^{-1}	1.42	4.50	$Y = 3.907 + 1.675 \times \log C$

the compound. As lower EC_x clearly revealed higher toxic characteristics of pollutants in comparison, the toxicity ranking, in increasing order, based upon EC_{20} and EC_{50} are listed as follows (Table 3; Fig. 2):

EC_0: toluene > ethyl benzene > benzene > xylene,
EC_{20}: toluene > benzene > xylene > ethyl benzene,
EC_{50}: benzene > xylene > toluene > ethyl benzene.

Note that the difference in the ranking of EC_x values was due to diverse tolerance to the suspected toxic material to be tested. Moreover, the slope factor B of probit model for dose–mortality curves (see Eqs. (2)–(4)) is also another indicator to the toxicity of a target compound. Note that a standard dose–response curve has a slope factor B of unity. Since all slope factors B of the curves were greater than unity, steeper curves thus indicate the toxic characteristics of all cases. The toxicity ranking, in increasing order, based upon slope B is listed as follows (Table 3; Fig. 2):

Slope B: xylene > benzene > (1.0) > toluene > ethyl benzene.

Note that if the slope B (e.g., less than unity) of the dose–response curve is shallow, there is considerable variation in susceptibility to that particular chemical within probing microorganism. The largest value (1.675) of slope B for xylene suggests the smallest tolerance range from EC_0 to EC_{100} for toxicity tolerance (Fig. 2). In contrast, the smallest value (0.73) of slope B for ethyl benzene simply implies the widest range of tolerance to ethyl benzene toxicity from the threshold dose (EC_0) to a maximum effect dose (EC_{100}). As many chemicals do not become airbone easily, oral LD_{50} in acute dose–mortality curve was used to estimate the quantity of chemical that would be potentially lethal for a human [36]. According to Taiwan EPA (e.g., http://flora2.epa.gov.tw/toxicweb/ToxicUC4/database/1330207.doc for xylene), oral LD_{50} (in g/kg) in rats were almost in the same order of magnitude (OM) (e.g., benzene (4.70), ethylbenzene (5.46), toluene (5.00), xylene (5.10 ± 3.5)). According to US EPA (e.g., http://www.epa.gov/ttn/atw/hlthef/xylenes.html for xylene), air LD_{50} (in g/m^3) in mice almost followed identical OM trends (e.g., benzene (32.0), toluene (33.2), xylene (27.6), ethylbenzene (17.4)). Thus, this suggested that EC_{50} might be relatively viable for parallel OM trends (except ethylbenzene) and could be used to define the margin of safety for suspected pollutants (see afterwards).

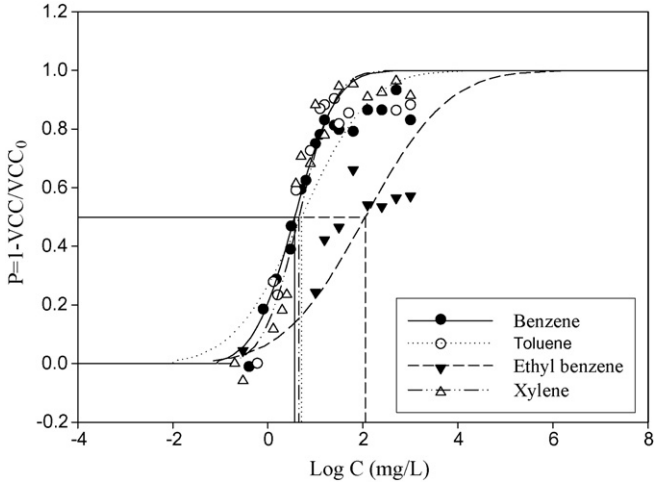

Fig. 2. Dose–mortality curve of BTEX toxicity predicted from the probit model (curves) (lines indicated EC_{50} values).

3.2. Motorcycle exhaust using different reformulated gasolines

3.2.1. Different reformulated oxygen and benzene gasoline

Literature [15,37–39] indicated that increases in the oxygen and aromatic hydrocarbon (HC) contents lead to marked rises in the concentrations of PAHs in motorcycle exhaust. The concentration of PAHs composition profiles were listed in Fig. 3. The highest mass concentration of the main composition, naphthalene, is approximately ranged from 45 to 80 μg/Nm^3. Furthermore, the highest toxic concentration of the main composition, benzo(a)pyrene, is roughly ranged from 20 to 30 μg/Nm^3. This also suggested that increases in these contents might also augment the toxicity of motorcycle exhaust to be generated. As shown in Fig. 4 and Table 4, toxicity rankings of these reformulated gasolines (in increasing order) were shown as follows:

EC_0: No. 1 $PAHs_{(S)}$ > No. 2 $PAHs_{(S)}$ > No. 3 $PAHs_{(G)}$ > No. 3 $PAHs_{(S)}$,
EC_{20}: No. 1 $PAHs_{(S)}$ > No. 3 $PAHs_{(G)}$ > No. 2 $PAHs_{(S)}$ > No. 3 $PAHs_{(S)}$,
EC_{50}: No. 1 $PAHs_{(S)}$ > No. 3 $PAHs_{(G)}$ > No. 2 $PAHs_{(S)}$ > No. 3 $PAHs_{(S)}$,
Slope B: No. 3 $PAHs_{(G)}$ > No. 1 $PAHs_{(S)}$ > No. 2 $PAHs_{(S)}$ > 1.0 > No. 3 $PAHs_{(S)}$.

These rankings in toxicity seemed to be in parallel with an increasing order in oxygen and benzene in reformulated gasolines. The rankings can be also verified with the data in Fig. 3 since the most toxic compound, benzo(a)pyrene, concentration of No. 1 is larger than that of the other gasolines. In addition, literature [36] indicated that PAHs may react with strong oxidative

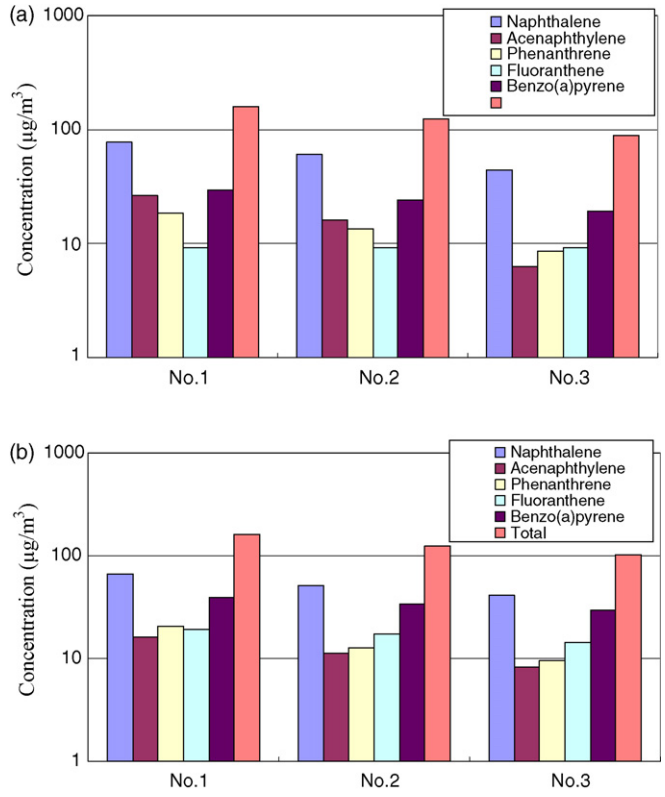

Fig. 3. The main composition of PAHs in the motorcycle exhaust with two-stroke engine.

compounds (e.g., O_3, NO_x) in air to form highly toxic pollutant species. Moreover, due to less mass transfer resistance, chemical species present in gas phase would have a significantly higher frequency to collide with species for reaction, leading to a higher toxicity in gas phase for No. 3 PAHs [40,41].

3.2.2. *Motorcycle exhaust using different stroke engines*

To Consider evaluation upon practical cases, motorcycles in two-stroke engine (2T engine) and four-stroke engine (4T engine) were used (Table 5 and Fig. 5). The toxicity rankings (i.e., EC_0, EC_{20}, EC_{50}, slope B) of these two engines all showed that 2T engine > 4T engine. The reasons of this result are straightforward. Compared to 4T engine, 2T engine is relatively

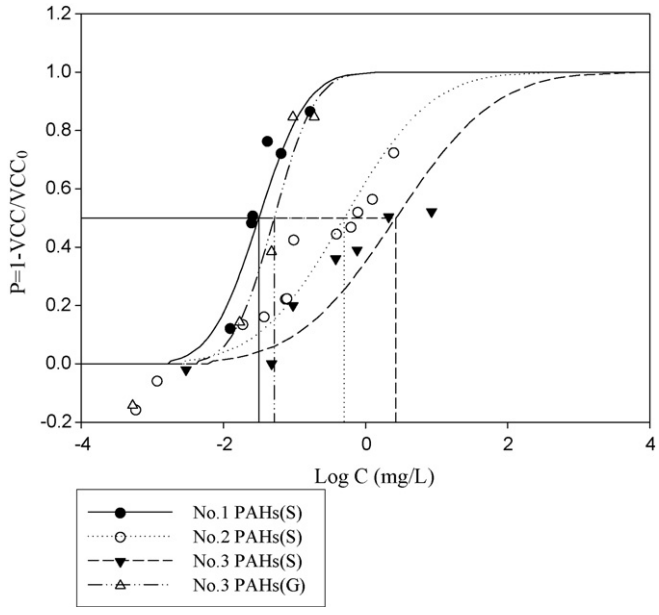

Fig. 4. Dose–mortality curve of toxicity of motorcycle exhaust using No. 1, No. 2 and No. 3 gasolines.

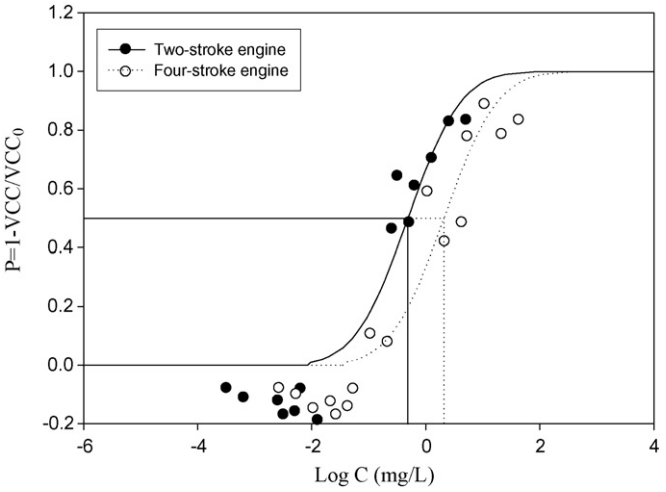

Fig. 5. Dose–mortality curve of toxicity of motorcycle exhaust using two-stroke and four-stroke engines.

Table 4
Critical effective concentrations and related parameters of toxicity predicted from the probit model for different motorcycle exhaust using reformulated gasoline

	EC_0 (mg/L)	EC_{20} (mg/L)	EC_{50} (mg/L)	$Y = A + B \times \log C$
No. 1 $PAHs_{(S)}$	1.66×10^{-3}	1.12×10^{-2}	3.13×10^{-2}	$Y = 7.846 + 1.879 \times \log C$
No. 2 $PAHs_{(S)}$	2.48×10^{-3}	7.80×10^{-2}	4.99×10^{-1}	$Y = 5.315 + 1.042 \times \log C$
No. 3 $PAHs_{(S)}$	5.95×10^{-3}	3.14×10^{-1}	2.66	$Y = 4.615 + 0.906 \times \log C$
No. 3 $PAHs_{(G)}$	4.16×10^{-3}	2.14×10^{-2}	5.16×10^{-2}	$Y = 7.824 + 2.194 \times \log C$

Table 5
Critical effective concentrations and related parameters of toxicity predicted from the probit model for motorcycle exhaust using 2T, 4T engines

	EC_0 (mg/L)	EC_{20} (mg/L)	EC_{50} (mg/L)	$Y = A + B \times \log C$
Two-stroke engine	8.46×10^{-3}	1.18×10^{-1}	4.87×10^{-1}	$Y = 5.427 + 1.364 \times \log C$
Four-stroke engine	3.47×10^{-2}	4.98×10^{-1}	2.09	$Y = 4.568 + 1.349 \times \log C$

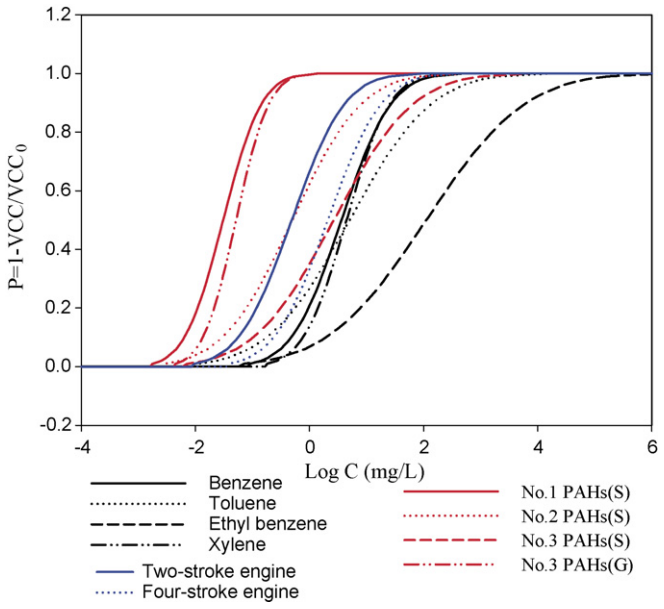

Fig. 6. Comparison of toxicity potency for all tested pollutants (e.g., BTEX and PAHs).

inexpensive to generate larger power output than 4T engine in the same size. However, the 2T engine has disadvantages of incomplete combustion and less efficiency due to mixed combustion of engine oil and gasoline. According to EC_{50}, compared to 2T engine evidently using 4T engine at least reduced fourfold toxicity even toxicity tolerance (i.e., slope *B*) seems to be identical. This could explain why Taiwan EPA tended to strongly encourage 4T engine in place of 2T engine for new environmental regulations [42].

3.3. Overall toxicity comparison

The ranking of toxicity tolerance (slope *B*) for various pollutants are No. 3 $PAHs_{(G)}$ (2.194) > No. 1 $PAHs_{(S)}$ (1.879) > xylene (1.675) > benzene (1.456) > 2T engine (1.364) > 4T engine (1.349) > No. 2 $PAHs_{(S)}$ (1.042) > 1.0 > No. 3 $PAHs_{(S)}$ (0.906) > toluene (0.880) > ethyl benzene (0.730). In addition, the toxicity series based on EC_{50} (in mg/L) are No. 1 $PAHs_{(S)}$ (0.0313) > No. 3 $PAHs_{(G)}$(0.0516) > 2T engine (0.487) > No. 2 $PAHs_{(S)}$ (0.499) > 4T engine (2.09) > No. 3 $PAHs_{(S)}$(2.60) > benzene (3.64) > xylene (4.50) > toluene (5.07) ≫ ethylbenzene (111.78). Obviously, motorcycle exhaust is significantly more toxic than typical VOCs (e.g., BTEX) likely due to highly toxic VOCs generated from incomplete combustion. EC_{50}s also indicated that all the pollutants were apparently more toxic than BTEX. Regarding margin of safety, overall toxicity evaluation upon all pollutants showed that the toxicity series (EC_{50} in mg/L) was approximately as follows: PAHs (ca. 0.05–0.5) >2T, 4T engines (0.5–2) >BTEX (3.6–5.0) (No. 3 PAHs(s) and ethylbenzene excluded) (Tables, Figs. 2–6).

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