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$PM_{2.5}$ and Associated Polycyclic Aromatic Hydrocarbon and Mutagenicity Emissions from Motorcycles

Hsi-Hsien Yang · Shu-An Lee · Dennis P. H. Hsieh · Mu-Rong Chao · Che-Yu Tung

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Abstract In this study, $PM_{2.5}$ in diluted exhausts of motorcycles are collected and emission characteristics of $PM_{2.5}$-associated polycyclic aromatic hydrocarbons (PAHs) and mutagenicities are investigated. The measured mutagenicity emission factors with metabolic activation for new fuel injection, used fuel injection, new carburetor and used carburetor motorcycles are 7.77×10^4, 1.18×10^5, 1.32×10^5 and 1.15×10^5 rev/km, respectively. The mutagenicity emission factors with metabolic activation are higher than the corresponding values without metabolic activation. The average PAH emission factors are 12.3, 16.3, 25.5 and 26.5 μg/km for new and used fuel-injection motorcycles, and new and used carburetor-operated motorcycles, respectively. The correlation coefficients between PAHs and mutagenicity emission factors are higher with metabolic activation (0.59) than that without metabolic activation (0.31).

H.-H. Yang (✉) · C.-Y. Tung
Department of Environmental Engineering and Management, Chaoyang University of Technology, Wufong, Taichung 41349, Taiwan, ROC
e-mail: hhyang@cyut.edu.tw

S.-A. Lee
Department of Environmental Engineering and Science, Feng Chia University, Taichung 100, Taiwan, ROC

D. P. H. Hsieh
Division of Environmental Health and Occupational Medicine, National Health Research Institutes, 35 Keyan Road, Miaoli 35053, Taiwan, ROC

M.-R. Chao
Department of Occupational Safety and Health, Chung Shan Medical University, Taichung 402, Taiwan, ROC

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There is an increased concern about human exposure to air particulate matter (PM) originating from vehicle traffic. Combustion processes in vehicle engines produce a wide variety of PM in urban areas, and are a significant source of particles with diameters smaller than 2.5 μm ($PM_{2.5}$) (Lightly et al. 2000). Evidence of a high PM concentration in two-wheel vehicle emissions have been reported (Rijkeboer et al. 2005). Previous research on particulate size distribution has also shown that the particulate emitted from motorcycles is primarily in size ranges smaller than 2.5 μm (Yang et al. 2005a). Because of its deeper penetration into the gas exchange region of the lung, $PM_{2.5}$ has been associated with increasing the risk of lung cancer and other respiratory-related problems (Pope et al. 2002; Greaves 2006).

Motor vehicles are also a significant emission source of polycyclic aromatic hydrocarbons (PAHs) (Larsen and Baker 2003). Some PAHs are potential mutagens and carcinogens, and are probably a significant cause of cancer (IARC 1987). Most carcinogenic PAHs have been found to associate with particulates, predominately fine particulates (Westerholm et al. 1991).

PAHs and PM are co-pollutants emitted during combustion processes in engines (Spencer et al. 2006) and convincing evidence exists that PAHs are significant toxic components of $PM_{2.5}$ leading to adverse human health risks (Deng et al. 2006).

Numerous studies have been conducted on PM and PAH emissions for diesel vehicles (e.g., Maricq 2007 and references in this review article). However, only limited studies have focused on motorcycles (Rijkeboer et al. 2005; Yang et al. 2005a, b). Since diesel PM and motorcycle PM

(Rijkebeor et al. 2005) differ significantly, it is reasonable to infer that motorcycle PM would exhibit different biological effects than diesel PM. Motorcycles are important transportation means for many Asian and European countries. To control and mitigate $PM_{2.5}$ and associated PAHs with a view of reducing health and environmental risks, a good understanding of their contribution from motorcycle emissions is necessary. In addition to PM and PAHs, to the best of our knowledge, the characteristics of mutagenicity for $PM_{2.5}$ in motorcycle exhaust have never been investigated. In this study, $PM_{2.5}$ in diluted exhaust from motorcycles was collected and associated mutagenicity and PAHs were analyzed.

Materials and Methods

Two kinds of motorcycles were tested in this study: a 4-stroke carburetor motorcycle and a 4-stroke fuel injection motorcycle, both with displacements of 125 cc. To investigate the influences of driving mileage on emission characteristics, new and used motorcycles were tested for both motorcycle types (i.e., overall four motorcycles were tested in this study). The used motorcycles were chosen mainly on the basis of accumulated mileage of more than 15,000 km. All motorcycles were produced and supplied by the same manufacturer (Motive Power Industry). The test fuel used was a commercial fuel produced by the Chinese Petroleum Company with an octane number of 95, the most widely used unleaded gasoline in Taiwan.

The test motorcycles were driven on a Schenck GS-530 GS 30 chassis dynamometer. The dynamometer system was comprised of a fan, a dynamometer, a dilution tunnel, a constant-volume sampler (CVS) unit, a gas analyzer and a personal computer (Fig. 1). The European driving cycle (ECE) is the legistive cycle used for automotive emission certification in Taiwan. One complete test cycle (780 s) includes idle time (240 s), acceleration (168 s), cruising (228 s) and deceleration (144 s). Four different cruising speeds (15, 32, 35 and 50 km/h) are applied in these tests. Diluted exhaust gas was extracted by the Apex Instruments source sampling system (Model MC-500). Apex Instruments' cyclone was attached to the probe to determine particulate emissions at 2.5 μm. $PM_{2.5}$ in diluted motorcycle exhaust was separated by the cyclone and was collected by pre-weighed PTFE filters (Advantec) at temperatures of approximately 25–30°C. The filter was stored in a desiccator for at least 8 h for moisture equilibrium before weighing. After the experiment, the filter was brought back to the laboratory and put in a desiccator for 8 h to remove moisture, then weighed again to determine the net mass of particulates collected.

Each PAH-containing filter was Soxhlet-extracted with a mixed solvent (*n*-hexane and dichloromethane, 200 mL/L each) for 24 h. The extract was then concentrated by purging with ultra-pure nitrogen to 2 mL for the cleanup procedure. The cleanup procedure was to remove pollutants which would coelute with PAHs from the GC column. The cleanup procedure was described in detail in our previous

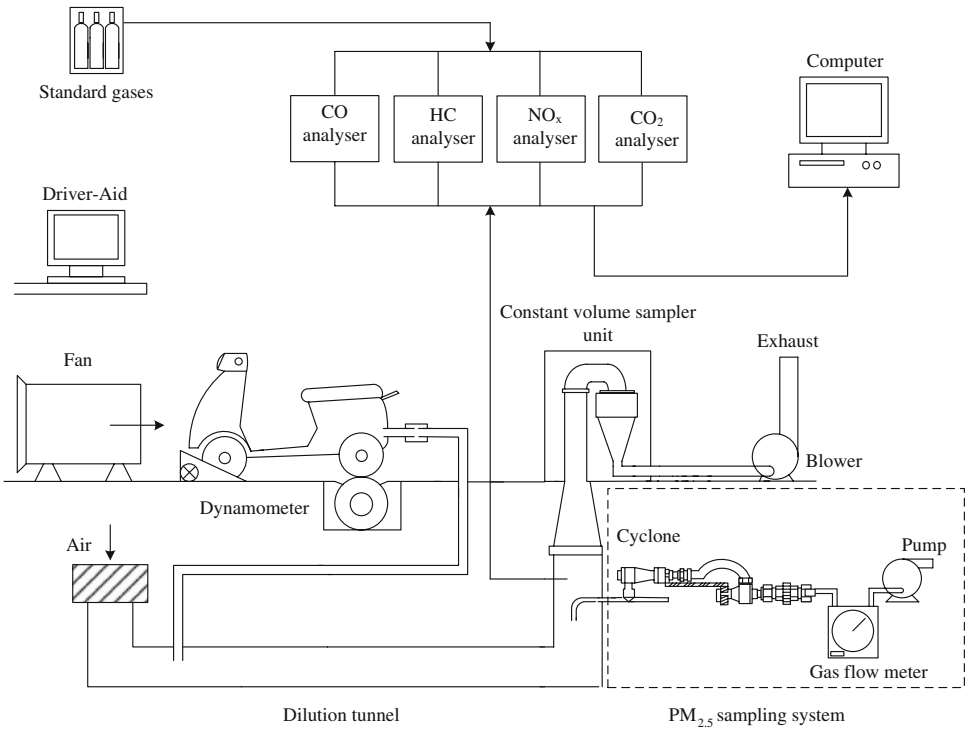

Fig. 1 Schematic diagram of the test equipment and $PM_{2.5}$ sampling system

study (Yang et al. 2005b). The collected eluent from the cleanup procedure was reconcentrated to 1 mL with ultrapure nitrogen. One-half (0.5 mL) of the eluent was analyzed for PAHs and the other-half was used for mutagenicity testing. The concentrations of the following 21 PAHs were determined: naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, cyclopenta[*c,d*]pyrene, benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*e*]pyrene, benzo[*a*]pyrene, perylene, indeno[*1,2,3-cd*]pyrene, dibenz[*a,h*]anthracene, benzo[*b*]chrysene, benzo[*ghi*]perylene, and coronene. A gas chromatography (GC) (Agilent 6890) with a mass selective detector (MSD) (Agilent 5973N) and a computer workstation were used for PAH analysis.

In this study, two internal standards (phenanthrene-d_{10} and perylene-d_{12}) were used to check the response factors and the recovery efficiencies for PAH analysis. The recovery efficiencies of 21 individual PAHs and these two internal standards were determined by processing a solution containing known PAH concentrations through the same experimental procedure used for the samples. This study showed that the recovery efficiency of PAHs varied between 78% and 113%, and averaged 86%. The recovery efficiencies of the two internal standards were between 83.0% and 88.0%. Blank tests for PAHs were accomplished by performing the same procedure as the recovery-efficiency tests without adding the standard solution before extraction. Analysis of the field blank filter found no significant contamination.

The other-half of the extracted eluent was evaporated to complete dryness by high-purity nitrogen. The residue was subsequently re-dissolved in 1 mL of dimethylsulfoxide (DMSO) for mutagenicity testing. Mutagenicity testing was performed following the procedure described by Maron and Ames (1983). In this study, 10 μL of DMSO sample solution, 10 μL of S9 mix (only when metabolic activation was desired), 200 μL of histidine–biotin solution and 100 μL of bacterial strain *Salmonella typhimurium* TA100 solution were preincubated for 60 min at 37°C, then added into 2 mL of top agar and the content of the tube poured onto minimal agar plates. Plates were counted after a 72 h incubation at 37°C. In this study, mutagenicity was determined both in the presence and in the absence of activating enzymes from rat liver microsomal fractions (S9), so as to determine the relative contribution of direct-acting and indirect-acting mutagens. Three dilutions of the DMSO solution were applied to the plates: 1.0, 0.5 and 0.25. Five replicates were performed, except in the case of spot tests, where plates were prepared in triplicate.

Benzo[*a*]pyrene and 2-nitrofluorene were used as a positive control under conditions with and without S9. The DMSO blanks were used as negative controls for spontaneous revertants in the Ames assay. According to the procedure, the sample was considered mutagenic when its mutagenicity ratio was higher than 2 and when it showed a linear dose–response (Maron and Ames 1983). The mutagenicity ratio is the ratio of the number of revertants induced by the sample and the number of spontaneous revertants.

Results and Discussion

Emission factors of $PM_{2.5}$ were calculated from the mass of $PM_{2.5}$ emitted, divided by the mileage traveled during the whole driving test. The average $PM_{2.5}$ emission factors measured in this study were 1.38, 3.18, 7.02 and 8.48 mg/km for the new and used fuel-injection motorcycles, and new and used carburetor motorcycles, respectively. $PM_{2.5}$ emission factors of used motorcycles were higher than those of new ones for all test motorcycles. The results indicate that deterioration of the motorcycle engines would lead to the higher $PM_{2.5}$ emissions.

The same as $PM_{2.5}$, the emission factors of PAHs were expressed as the mass of PAHs emitted per kilometer traveled. The average PAH emission factors are 12.3, 16.3, 25.5 and 26.5 μg/km for the new and used fuel-injection motorcycles, and the new and used carburetor motorcycles, respectively. The trends between the test motorcycles are the same as those of $PM_{2.5}$ (i.e., carburetor and used motorcycles have higher emissions than fuel injection and new motorcycles, respectively). These results are expected since the PAHs were adsorbed on $PM_{2.5}$. Higher $PM_{2.5}$ emission offers more surface area for PAHs to be adsorbed onto PM particles. Since $PM_{2.5}$ can penetrate deeply into the lungs through inhalation, the $PM_{2.5}$-associated PAHs could contact with lungs and potentially negatively effect human health. Stricter emission regulations are gradually being implemented all over the world. In Taiwan, for example, the Phase 5 emission regulations for CO, HC and NO_x have been reduced to 2, 0.8 and 0.15 g/km, respectively. Only fuel-injection motorcycles can meet the strict criteria. Carburetor motorcycles will not be produced and sold in Taiwan after 2009. According to the results in this study, not only the regulated air pollutants (CO, HC and NO_x), but $PM_{2.5}$, as well as the associated PAH emissions, could also be reduced while carburetor motorcycles are replaced by the fuel-injection motorcycles.

Mutagenicity emission factors are shown in Fig. 2. The results show that all $PM_{2.5}$ extracts in this study are mutagenic, which allows one to conclude that the samples contain mutagens causing base-pair mutations. The emission factors of mutagenicities without metabolic activation (−S9) are 2.03×10^4, 1.14×10^5, 8.05×10^4 and 1.04×10^5 rev/km for the new fuel injection, used fuel injection, new carburetor and used carburetor motorcycles, respectively. With

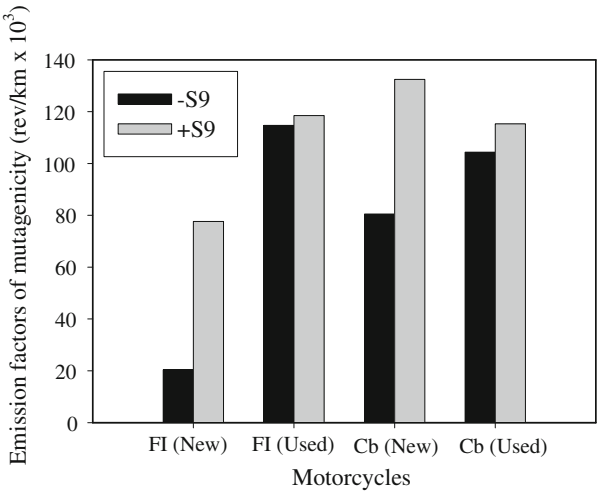

Fig. 2 Emission factors of mutagenicity. FI: fuel injection; Cb: carburetor

metabolic activation (+S9), the mutagenicity emission factors are 7.77×10^4, 1.18×10^5, 1.32×10^5 and 1.15×10^5 rev/km, respectively. The mutagenicity emission factors are higher for the used fuel-injection motorcycle than the new one. For carburetor motorcycles, the mutagenicity emission factors are similar for new and used motorcycles, which agree with PAH emission factors. With metabolic activation, the mutagenicity emission factors are higher than the corresponding values without metabolic activation. Higher values in the assay with S9 are expected due to the contribution of both direct- (−S9) and indirect- (+S9) acting mutagenicity; whereas, in assays without S9 only the direct-acting component contributes. In the presence of NO_x, direct-acting mutagens, such as nitro-PAHs, can be formed by transformation of PAHs during the passage of the emissions through the exhaust pipe and dilution tunnel and lead to higher contribution of direct-acting mutagenicity (DeMarini et al. 2004). The formation of direct-acting nitro-PAHs might be the reason for the higher mutagenicity without metabolic activation found in this study.

The correlation coefficients are 0.31 and 0.59 between PAHs and mutagenicity emission factors for the tests with and without metabolic activation. The higher correlation between PAHs and indirect-acting mutagenicity can also be used to explain the higher mutagenicity with metabolic activation than that without activation, due to the presence of indirect-acting compound PAHs. Only moderate correlations are found between PAHs and indirect-acting mutagenicity. It is suggested that, in addition to PAHs, other mutagenic compounds are contained in $PM_{2.5}$. A complete chemical characterization of $PM_{2.5}$ is practically impossible. In addition, there are also possible synergistic and antagonistic effects of the chemical compounds. In this study, only 21 PAHs were analyzed (i.e., just a small fraction of mutagenic compounds in $PM_{2.5}$). It is therefore not expected to reveal the true relationship between the chemical compounds in $PM_{2.5}$ and its mutagenicity; even though the moderate correlation between PAHs and indirect acting mutagenicity in this study indicate that PAHs play an important role in causing mutagenicity in $PM_{2.5}$ from motorcycle exhaust.

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