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Chemical and toxicological characterization of particulate emissions from diesel vehicles

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Chemical and toxicological characterization of particulate emissions from diesel vehicles

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ABSTRACT

This paper presents a detailed chemical and toxicological characterization of the diesel particulate matter (PM) emitted from diesel vehicles running on a chassis dynamometer under different driving conditions. Chemical analyses were performed to characterize the contents of organic carbon (OC), elemental carbon (EC), and 31 polycyclic aromatic hydrocarbons (PAHs) in the collected PM samples. The OC–EC analysis results revealed that PM emissions from diesel vehicles in this study were dominated by OC and that the emission of vehicles equipped with diesel particulate filters had high OC/EC ratios. The PAH analysis results revealed that 4- and 5-ring PAHs were the dominant PAHs in the OC fraction of the PM samples. Particle toxicity was evaluated through three toxicological markers in human A549 cells, namely (1) acellular 2,7-dichlorofluorescein (DCFH) for oxidative potential, (2) interleukin-6 (IL-6) for inflammation, and (3) glutathione (GSH) for antioxidation after exposure. Statistical analyses revealed that vehicle sizes have statistically significant effects on the concentrations of the markers. Correlation analysis between PAHs and toxicological markers revealed that significant correlations existed between specific compounds and markers. Our results can be used as a reference by policy makers to formulate emission control strategies and as a dataset for other modeling studies.

Keywords: PM emission; OCEC; PAH; DCFH; Interleukin-6 (IL-6); Glutathione (GSH); Driving Cycle; Chassis dynamometer

1. Introduction

Hong Kong is one of the most densely populated cities in the world, and the majority of the population is exposed to vehicular exhaust because many people work and live close to main roads and highways. By the end of 2017, more than 830,000 licensed motor vehicles were running on the roads, and the number of licensed motor vehicles increased by 35% from 2007 to 2017 (Hong Kong Transport Department, 2018). In particular, diesel vehicles are the main source of particulate matter (PM) pollution on the streets (Lee et al., 2006; Shen et al., 2014; Weiss et al., 2012; Yao et al., 2011).

A large quantity of PM contains carbonaceous aerosols (Hou et al., 2011), which are typically classified into two categories, namely elemental carbon (EC) and organic carbon (OC). Lee et al. (2006) reported that carbonaceous aerosols are the dominant species of fine particles in the roadside environment in Hong Kong and account for approximately 44% of the $PM_{2.5}$ concentration in Hong Kong. Vehicular emissions are the major source of carbonaceous aerosols in Hong Kong (Cheng et al., 2010, 2011; Ho et al., 2002; Lee et al., 2006). Zheng et al. (2006) reported that more than 60% of the OC measured at a roadside station in Hong Kong originated from vehicular emissions. The OC in vehicle-emission-derived PM comprises various toxic organic compounds, including polycyclic aromatic hydrocarbons (PAHs). Several studies have reported that vehicular emissions, particularly from diesel vehicles, are the largest sources of PAHs in urban areas (Cecinato et al., 2014; Chen et al., 2013; Shen et al., 2011). Therefore, various studies have been conducted to characterize the emission factors (EFs) and chemical compositions of PAHs emitted from diesel vehicles under different conditions (Zielinska et al., 2004; Tsai et al., 2011; Hu et al., 2013; Cao et al., 2017; Lin et al., 2019, Hays et al., 2017). In Hong Kong, the PAHs in vehicle emissions have been mainly characterized through tunnel measurement and the collection of ambient air samples (Cheng et al., 2010; Ho et al., 2002, 2009; Ma et al., 2016). Limited studies have been conducted on direct tailpipe emissions (e.g., chassis dynamometer test or portable emission measurement system [PEMS] study) from diesel vehicles in Hong Kong.

PM emissions from vehicles are toxic to human beings (Abdel-Shafy and Mansour, 2016; Chuang et al., 2012; Kim et al., 2013). Toxicological studies have revealed that reactive oxygen species (ROS) production is the main mechanism for the increased risk of adverse health effects due to PM exposure (Li et al., 2003; Nel, 2005). In a healthy biological system, glutathione (GSH) is increased to mitigate the PM-derived ROS in cells (Ghio et al., 2012). However, when in excess, ROS causes inflammatory responses such as interleukin 6 (IL-6) and interleukin 8 (IL-8) production, which lead to harmful health effects.

The objectives of the current study were to characterize the EFs of the OC, EC, and PAHs in diesel vehicle emissions, compare the differences in the chemical compositions and toxicological responses under different driving conditions, and investigate the correlation between the chemical composition and toxicological response of PM samples.

2. Methodology

2.1 Fleet overview and instrumentation setup

Fifteen vehicles of various classes and with different engine sizes, after-treatment technologies, and emission standards (**Table S1**) were studied. The vehicles were classified according to the United Nations Economic Commission for Europe (UNECE, 2011). Passenger cars (PCs) were defined as M-type vehicles. Light-duty vehicles (LDVs), medium-duty vehicles (MDVs), and heavy-duty vehicles (HDVs) were defined as N1-, N2-, and N3-type vehicles, respectively. Vehicle 15 was categorized as an HDV because it was a 10-ton tractor designed for carrying a trailer of up to 20 tons.

Chassis dynamometer tests were conducted in the Jockey Club Heavy Vehicle Emissions Testing and Research Centre (JCEC), Hong Kong. **Fig. S1** of the Supporting Information depicts the schematic of the test setup. All the testing facilities in the JCEC comply with the European standards for type approval tests. Two chassis dynamometers were used to test the vehicles with different weights. PCs and LDVs were tested on a Mustang dynamometer with a 48” (121.92 cm) single roller, whereas MDVs and HDVs were tested on a Mustang dynamometer with a 17.2” (43.688 cm) triple roller.

2.2 Driving cycles and testing conditions

Four driving cycles, namely the cold start transient, hot start transient, idling, and steady-state cycles, were used to test each vehicle. For convenience, the cold and hot start transient cycles are called cold start and hot start cycles in the following text. For each vehicle, the cold start cycle test was repeated two times and the hot start, idling, and steady-state cycle tests were repeated three times. Detailed descriptions of the four driving cycles and loading conditions are presented in the Supporting Information.

2.3 PM sample collection

PM samples were collected simultaneously on quartz (47 mm, Whatman, USA) and Teflon membrane filters (47 mm, Pall Corporation, USA) for different offline tests. The mass of samples collected on the filters were determined using a microbalance (MC5, Sartorius, Germany) with a readability of 0.001 mg. The filters were conditioned in a humidity-controlled chamber (i.e. relative humidity = 40%) for at least 24 h before weighing. Each filter was weighted at least twice before and after sample collection. The weighing result was accepted only if the difference between two consecutive weighings was less than 0.01% of the filter weight. Then, the filters were sealed in zip-zap bags and stored at −20 °C for chemical and toxicological analyses. Operational blanks and laboratory control blanks were processed simultaneously with the field samples during sample collection and analyses. All the filter data were corrected with the operation and laboratory blanks.

2.4 Chemical analysis

The samples collected on the quartz filters were used for OC/EC and PAH analyses. Each filter was cut exactly in half with a specially designed chopper for the two analyses. The contents of OC and EC were analyzed using a Desert Research Institute Model 2001 Thermal/Optical Carbon Analyzer with the IMPROVE-A protocol described by Chow et al. (2012). The PAH samples were analyzed using the thermal desorption-gas chromatography/mass spectrometry method (Ho et al., 2008). The chemical analyses procedures are described in the Supporting Information.

2.5 Toxicological analysis

The PM samples collected on Teflon filters were removed for toxicological analysis. The three toxicological markers included (1) acellular DCFH for oxidative potential, (2) IL-6 for inflammation, and (3) GSH for antioxidation after exposure. Details of the toxicological analysis procedures are presented in the Supporting Information.

2.6 Calculation of the EF and statistical analysis

Details regarding the calculation of the EF and the statistical analysis performed in this study are presented in the Supporting Information.

3. Results and discussion

3.1 OC and EC

The EFs of OC and EC and the OC/EC ratios at different driving conditions are depicted in **Fig. 1**. OC was the dominant fraction in all the collected samples except those from Vehicles 6 and 8, in which EC dominated. Several studies have indicated that EC is dominant in PM emissions from diesel vehicles (Chiang et al., 2012; Grieshop et al., 2006; Kleeman et al., 2000), whereas other studies have reported contrasting results (Shah et al., 2004; Wu et al., 2016). Gali et al. (2017) indicated that under cold idle, or low-engine-speed conditions, OC is the dominant fraction in PM, which is consistent with our results.

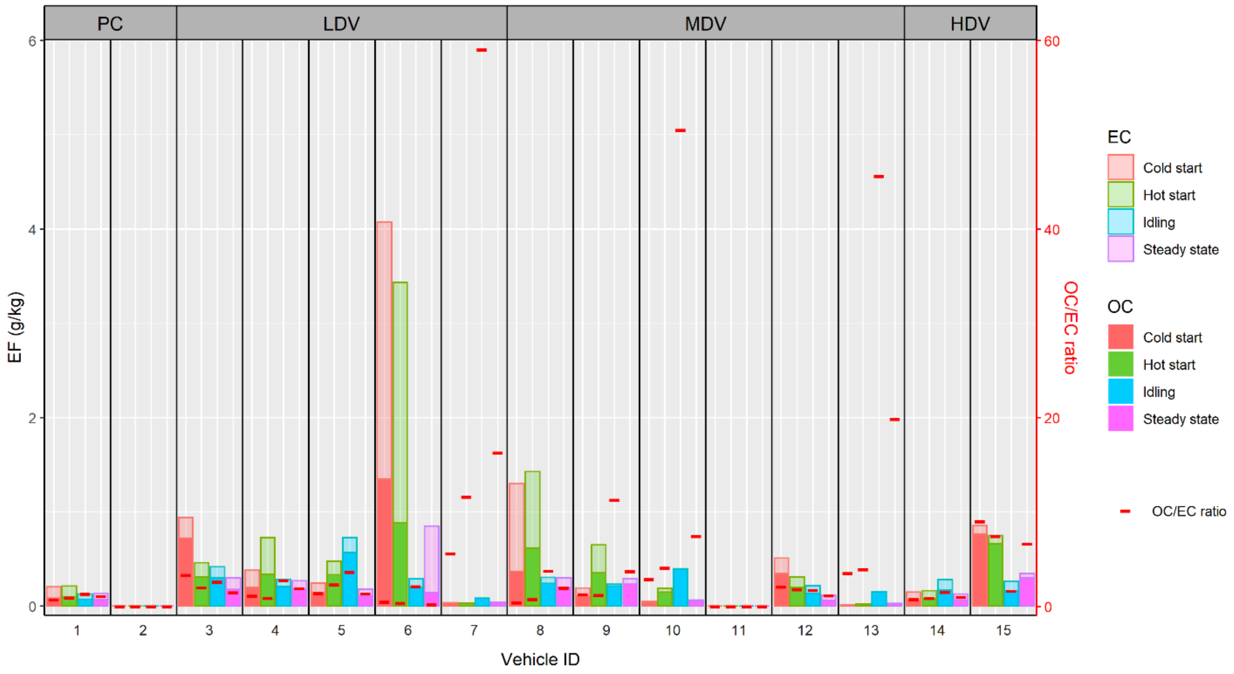

Fig. 1. *EFs* of OC and EC and OC/EC ratios. Each bar represents the sum of OC and EC *EF*. Light color bar represents EC *EF* and deep color bar represents OC *EF*.

The variation in the OC/EC ratio was caused by the difference in the emission standard, testing weight, engine power, and capacity and maintenance conditions of the test vehicles. The EC content in emissions from vehicles equipped with diesel particulate filters (DPFs) was less than the detection limit (Vehicles 2 and 11) or extremely low (0.003 ± 0.002, 0.017 ± 0.014, and 0.003 ± 0.001 g/kg for Vehicles 1, 10, and 13, respectively). This observation is consistent with the findings of May et al. (2014a), who reported that DPFs can effectively decrease the EC emission from diesel vehicles. The results also revealed that the EC removal by DPFs was satisfactory even for vehicles with high odometer readings (e.g. Vehicles 10, 11, and 13). As depicted in **Fig. 1**, high OC/EC ratios were observed for Vehicles 7 (59.06), 10 (50.51), and 13 (45.63) under the idling condition. For these three vehicles, the EC concentration was very low and the OC concentration was high. The high OC content in the PM samples of Vehicles 7, 10, and 13 could be partially attributed to the property of quartz filters in collecting PM samples. As revealed by May et al. (2013), using quartz filters alone to collect PM sample results in the collection of small quantities of semivolatile and low-volatility organic compounds. Because these compounds are in the gas phase, they are not removed by the DPF. Therefore, the DPF-equipped vehicles (Vehicles 7, 10, and 13) emitted substantial quantities of OC, which was a possible reason for the higher OC/EC ratio obtained in this study than in other related studies.

Among the four driving conditions tested in this study, idling generally produced the highest OC/EC ratio. EC mainly arises from fuel droplet pyrolysis, whereas OC mainly originates from unburned fuel and incomplete combustion (Shah et al., 2004). When the vehicles were in the idling condition, their engine temperature decreased, which resulted in “less complete” fuel combustion compared with that under other conditions.

The European emission standard assigned to the vehicle considerably affected OC and EC emissions. Typically, the EFs of OC and EC decreased with an increase in emission standards, except in the cases of Vehicles 6, 12, and 15. An extremely low level of EC and a measurable level of OC were recorded in vehicles with high emission standards (e.g., Vehicles 7 and 13). A possible reason for this result is that the emission control technologies applied in new vehicles may not effectively remove OC from diesel vehicle exhausts. Because most of the toxic and mutagenic properties of diesel exhaust are associated with OCs (Claxton, 2015; Shah et al., 2004), our results suggest that the development of emission control technologies should focus on reducing the OC fraction of PM.

In addition to the effects of emission standards, the results revealed that the EFs of OC and EC were considerably influenced by the conditions of the vehicles. For example, vehicles with advanced emission standards did not always exhibit OC or EC reduction. Compared with the HDVs depicted in **Fig. 1**, the OC EF of Vehicle 15 (Euro 5) was considerably higher than that of Vehicle 14 (Euro 4). During the chassis dynamometer testing of Vehicle 15, white smoke and pungent smell emanated from the exhaust, which indicated that Vehicle 15 was poorly maintained. The maintenance condition of Vehicle 15 had a larger influence than its emission standard on the emissions of OC and EC.

3.2 PAHs

A total of 31 PAHs (**Table S2**) were characterized, and their EFs were calculated. In all the tests, the EFs of acenaphthylene, acenaphthene, and fluoranthene were less than the detection limit because these three PAHs have low molecular weight and are mainly present in the gas phase. Therefore, the aforementioned three PAHs were excluded in the following analyses. **Fig. 2** presents an overview of the PAH EFs under different driving conditions. Each bar in **Fig. 2** corresponds to the total PAH EF (sum of the EFs of all the PAHs tested) in a given driving cycle, and the red solid line represents the sum of the total PAH EFs in the four driving cycles (denoted as "four-cycle sum" in the following text). As displayed in **Fig. 2**, Vehicle 15 emitted the highest four-cycle sum of 18.60 mg/kg, followed by Vehicle 6. Vehicle 2 emitted the lowest four-cycle sum of 0.41 mg/kg, followed by Vehicles 11 and 13. A common feature of these three vehicles with low four-cycle sums was that they were equipped with DPFs.

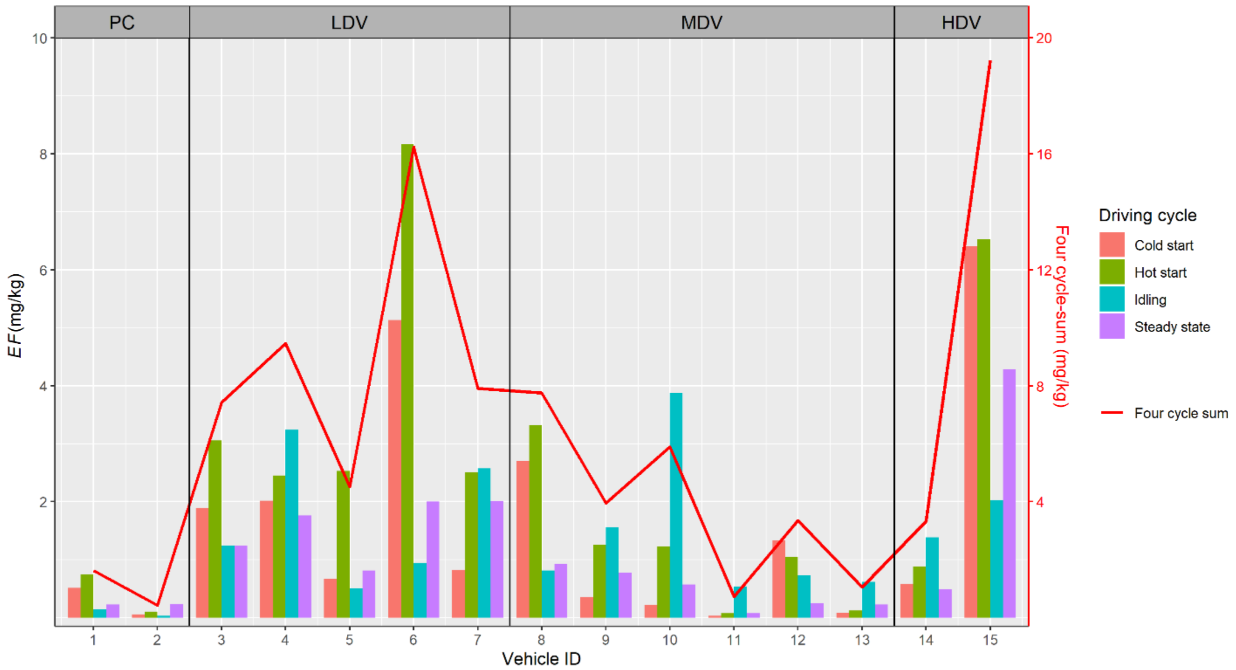

Fig. 2. Total PAH *EFs* in different driving cycles. The red solid line represents the sum of total PAH *EFs* of the four driving cycles, denoted as "four cycle-sum".

Fig. 2 reveals that the MDVs had lower total PAH EFs than the LDVs did. Unexpectedly, vehicles with greater testing weights emitted less PAHs. A possible explanation for this result is that two DPF-equipped vehicles were present in the MDV class, whereas only one DPF-equipped vehicle was present in the LDV class. Cao et al. (2017) observed the aforementioned pattern for their vehicle fleet, which comprised 18 diesel trucks in China. The aforementioned observation was not an isolated event, which suggested that the effect of the vehicle size on the PAH emission should be further investigated.

Fig. 2 also reveals that the steady-state cycle generally exhibited the least total PAH EFs, whereas the transient and idling cycles exhibited substantially higher PAH EFs. These results are consistent with those reported by Shah et al. (2005). Furthermore, the aforementioned figure indicates that the total PAH EFs of Vehicles 4, 7, 9, 10, 11, 13 and 14 under idling cycles were higher than those under transient cycles. This result suggested that the vehicle emission behavior under different driving conditions, especially under idling, should be studied because a substantial difference in EFs were observed between different driving cycles.

To identify the dominant PAHs emitted from each vehicle, the weighted percentage of each PAH emitted under different driving cycles was determined (**Fig. 3)**. For most of the vehicles, the dominant PAHs were 4- and 5-ring PAHs, including pyrene (PYR), benz[*a*]anthracene (BaA), chrysene (CHR), benzo[*b*]fluoranthene (BbF), benzo[*k*]fluoranthene (BkF), and benzo[*a*]fluoranthen (BaF). This finding was consistent with those reported in previous studies (Cao et al., 2017; Hu et al., 2013). The difference in the PAH composition under different driving conditions is illustrated in **Fig. 3**. The distributions of individual PAHs did not exhibit considerable variations when a given vehicle was tested under different driving conditions, except when Vehicles 1 and 9 were tested under the idling and steady-state cycles. The variation in the PAH composition among vehicles was not significant, except for Vehicle 7. Excluding the aforementioned two exceptions, the PAHs collected in all the driving cycles were dominated by 4- and 5-ring PAHs for all the vehicles. This observation suggests that the driving conditions, driving pattern (NEDC or FIGE), mileage, testing weight of the vehicle, and after-treatment technologies do not considerably affect the composition of the emitted PAHs. Furthermore, in general the collected PAH samples originated from the same source, probably fuel combustion, because they all had similar compositions. Therefore, the PAH samples collected in the exceptional cases (for Vehicle 7 and in the idling and steady-state cycles for Vehicles 1 and 9) were probably affected by other sources. Further investigations are required to characterize the sources of PAHs collected from the tailpipe emissions of diesel vehicles.

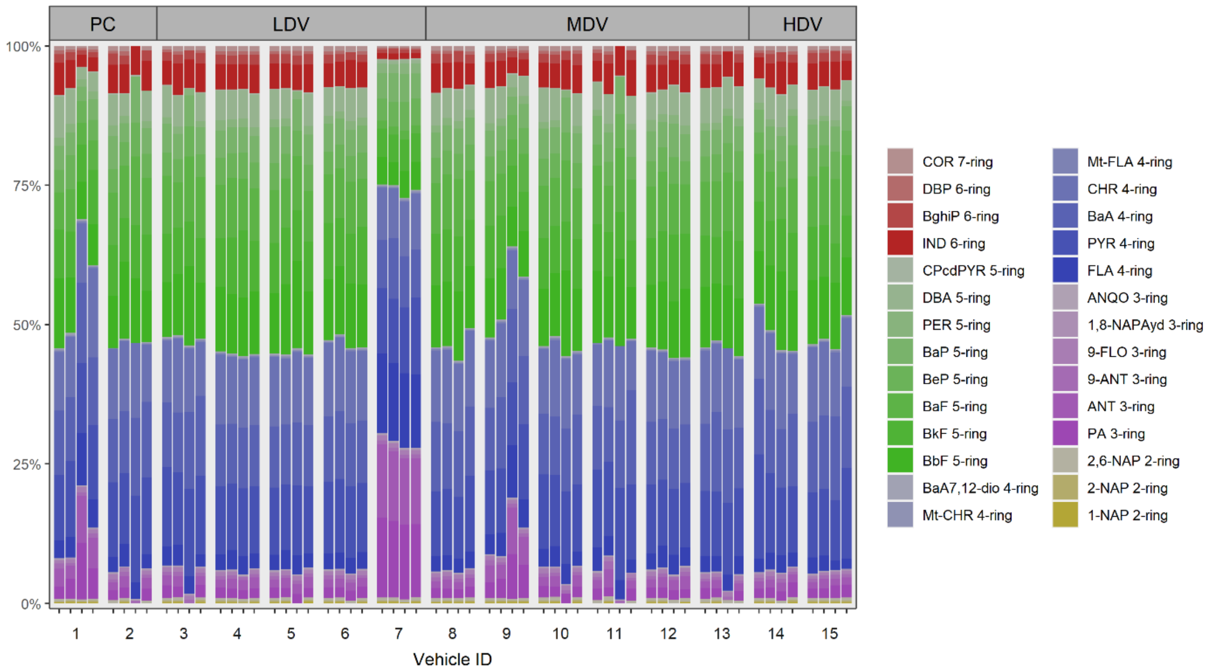

Fig. 3. Mass percentages of PAHs of each vehicle. The four bars under the same Vehicle ID correspond to cold start, hot start, idling and steady state cycles from left to right.

3.3 Comparison with other studies

The PAH data obtained in this current study were compared with the results of similar studies. Unlike the fuel-based EF used in the previous sections, distance-based EFs (EFs_d) were used in this section because most of the results in previous studies were presented in a distance-based manner. The EF_d value for each PAH was calculated as the ratio of the total quantity of PAHs released in a driving cycle to the distance traveled in the driving cycle. The driving distances in the NEDC, FIGE, and steady-state cycles were 11.0, 29.5, and 16.7 km, respectively. An averaged EF_d for each vehicle class (i.e. PCs, LDVs, MDVs, and HDVs) was calculated. **Fig. 4** presents a comparison of the averaged EFs_d of each PAH from each vehicle class in this study with the PAHs in diesel vehicle emissions in a PEMS study in China (Cao et al., 2017), tunnel studies in China (Chen et al., 2013) and Hong Kong (Ho et al., 2009), and a chassis dynamometer study in the US (Hays et al., 2017). As depicted in **Fig. 4**, the results of the current study were in the range reported in previous studies. Cao et al. (2017) reported the highest PAH EF_d values among the compared studies. Their vehicle fleet comprised 18 diesel trucks with China 3 and 4 emission standards (equivalent to Euro 3 and 4, respectively). In general, the PAH EF_d results of Cao et al. were an order of magnitude higher than those obtained for the HDVs in this study (except for CHR). The EF_d value of ANT in the study of Cao et al. (2017) was four orders of magnitude higher than that in this study, which could be attributed to two main reasons. First, the emission standards of the tested HDVs (Euro 4 and 5) in this study were higher than those of Cao's fleet. Second, Cao et al.

(2017) measured on-road emissions by using a PEMS. The emission EFs during on-road driving are considerably higher than those in laboratory chassis dynamometer tests (Huang et al., 2018; May et al., 2014b; Weiss et al., 2012) because the driving conditions in the real world are more rigorous than the driving cycles tested in chassis dynamometer studies.

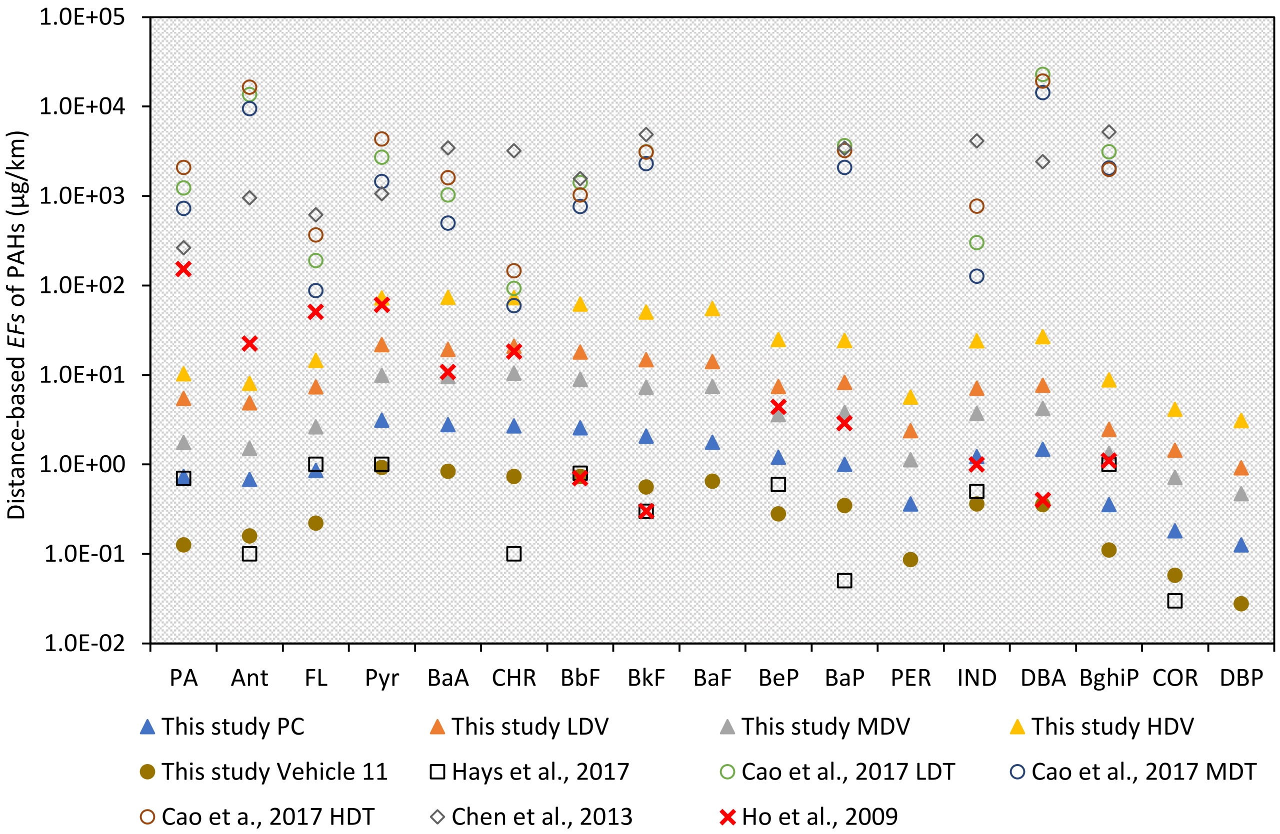

Fig. 4. Distance-based PAHs *EFs* of different vehicle classes.

Hays et al. (2017) conducted chassis dynamometer testing on DPF-equipped diesel trucks in the US. Their findings agreed with the PAH EF_d values obtained for Vehicle 11 in this study, which are denoted by brown solid dots in **Fig. 4**. Vehicle 11 is a DPF-equipped MDV and is comparable in size to the vehicles tested by Hays et al. (2017). The agreement between the results of this study and Hays et al. (2017) confirmed that DPF can efficiently remove PAHs from vehicle exhausts.

Chen et al. (2013) conducted a tunnel study in Nanjing, and Ho et al. (2009) conducted a tunnel study in Hong Kong. As depicted in **Fig. 4**, the results of Chen et al. (2013) were in the range of those of Cao et al. (2017), whereas the results of Ho et al. (2009) were generally within the range of those of the current study. The EF_d values of the pollutants emitted from diesel vehicles were strongly related to regions, which can be attributed to the diesel fuel variation among regions. Studies have indicated that the fuel type (i.e., low-sulfur diesel vs. ultra-low-sulfur diesel) considerably affects the EFs of PAHs (Cheung et al., 2010, Lim et al., 2005).

The EFs_d of the PAHs in this study were expected be lower than those PAH EFs_d in the study of Ho et al. (2009) because according to the Environmental Protection Department of Hong Kong, the $PM_{2.5}$ emissions in Hong Kong have reduced by more than 50% (HKEPD, 2019). This observation can be explained by the driving condition for vehicles in tunnels, where vehicles travel at almost constant speed. This condition resembles the steady-state cycle in this study. **Fig. 2** indicates that the PAH EFs in the hot start cycle were higher than those in the steady-state cycle for all vehicles except Vehicles 2, 11, and 13. Also, the averaged total PAHs EF_d in steady-state cycle is 0.0834 ± 0.146 mg/km, which is lower than that in cold start (0.180 ± 0.303 mg/km) and hot start (0.239 ± 0.310 mg/km) cycles. This observation explains the higher-than-expected PAH EFs in this study compared with the study of Ho et al. (2009). Some high-emission vehicles (e.g., Vehicles 6 and 15) contributed considerably to the calculated average PAH values, which increased the PAH EFs in this study.

3.4 Toxicological analysis

The levels of DCFH, IL-6, and GSH were analyzed to assess the cellular oxidative and inflammatory responses produced by the PM samples. The toxicological results of the current study cannot be compared with those of other studies because the cell culture conditions in this study were not exactly the same as those in previous studies. Furthermore, the results were obtained using a fixed concentration of PM (i.e. 50 μg mL^{-1}), and the quantity of PM emitted by the vehicles was not considered.

The ROS production is expressed in terms of the fluorescent intensity of DCFH, as depicted in the upper panel of **Fig. 5**. In general, the fluorescent intensity did not vary significantly among the different driving cycles. Vehicle 10 exhibited the highest fluorescent intensity, with an average value of 1.62 ± 0.31. In addition to Vehicle 10, the cold start cycle for Vehicle 1, hot start cycle for Vehicle 3, and idling cycle for Vehicle 13 exhibited elevated levels of fluorescent intensity in response to the emitted PM. However, other than the aforementioned vehicles and cycles, the variation in the ROS among the remaining vehicles was not significant.

The lowest panel in **Fig. 5** illustrates the GSH levels produced after the A549 cells were exposed to the PM samples. Compared with the results of the DCFH level, a certain degree of variation was observed in the GSH levels for the tested vehicles. LDVs (Vehicles 3, 4, 5, 6, and 7) generally had lower GSH concentrations than the other classes of vehicles did. Other parameters did not significantly influence the GSH level. The average GSH levels for vehicles with and without a

DPF were 9.89 ± 3.97 and 9.85 ± 3.37 μM, respectively. This result indicated that DPFs removed PM mass without changing the GSH response of the PM; thus, DPFs probably did not significantly change the morphology and composition of the PM samples.

IL-6 is a proinflammatory cytokine released in response to PM exposure. The IL-6 results are illustrated in the middle panel of **Fig. 5**. The idling cycle for Vehicle 14 exhibited the highest IL-6 level of 80.3, whereas the hot start cycle for Vehicle 7 exhibited the lowest IL-6 level of 8.30. In general, no clear trend was observed for the effect of driving cycles on the IL-6 levels. HDVs had the highest IL-6 concentrations among the vehicle classes, followed by MDVs. The IL-6 levels of PCs, LDVs, and MDVs did not exhibit significant variations.

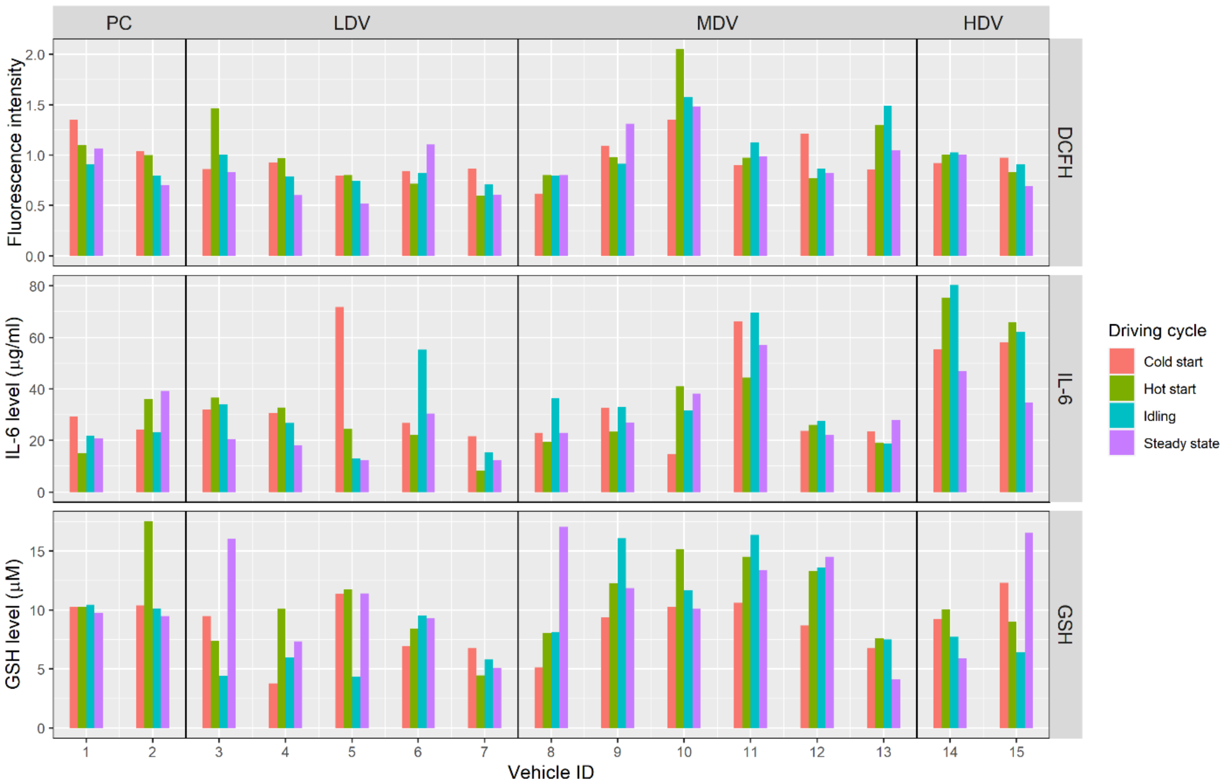

Fig. 5. Results of the production of ROS expressed as the fluorescent intensity of DCF, IL-6 level and GSH level of the 15 vehicles in different testing cycles.

3.4.1 Effects of the vehicle type and driving cycle on the toxicological markers

To determine the effects of the vehicle type on the three toxicological markers, the levels of each toxicological marker for all vehicles were pooled and grouped according to their corresponding vehicle type (i.e., PC, LDV, MDV, and HDV). The results are presented using boxplots in **Fig. 6**. As depicted in **Fig. 6**, a certain degree of variation existed between vehicle types for the three toxicological markers. The LDVs produced lower responses for DCFH and GSH than the other three vehicle types did. The HDVs exhibited a higher IL-6 level than the other three vehicle types. The results were verified by conducting a Kruskal–Wallis H test for each toxicological marker at a significance level of $p = 0.05$. The Kruskal–Wallis H test results presented in the bottom left of each boxplot indicated that statistically significant differences existed among the four vehicle types for the three toxicological markers. The pairwise Wilcoxon rank-sum test was conducted as the post-hoc test of the Kruskal–Wallis test to determine which vehicle type pair had significant differences in their toxicological marker levels at a significance level of 0.05. The vehicle type pairs with adjusted p values of <0.05 are marked with an asterisk in **Fig. 6**. Significant differences were identified in the DCFH and GSH levels of the LDVs and MDVs. Moreover, significant differences were identified in the IL-6 levels of the HDVs and other types of vehicles. The aforementioned results suggested that the vehicle type affected the response of the three toxicological markers. Further investigations are required to determine the mechanisms or reasons leading to this observation.

Unlike the vehicle type, the driving condition of the vehicle did not have a significant effect on the three toxicological markers. Statistical analyses (**Fig. S3**) confirmed that no statistically significant differences existed between the four driving cycles ($p > 0.05$) for the three toxicological markers. This result suggested that the driving condition had a minimal effect on the production of ROS and the proinflammatory cytokine IL-6. This finding is consistent with our PAH results, which revealed that the composition of PAHs did not vary significantly among the different driving cycles.

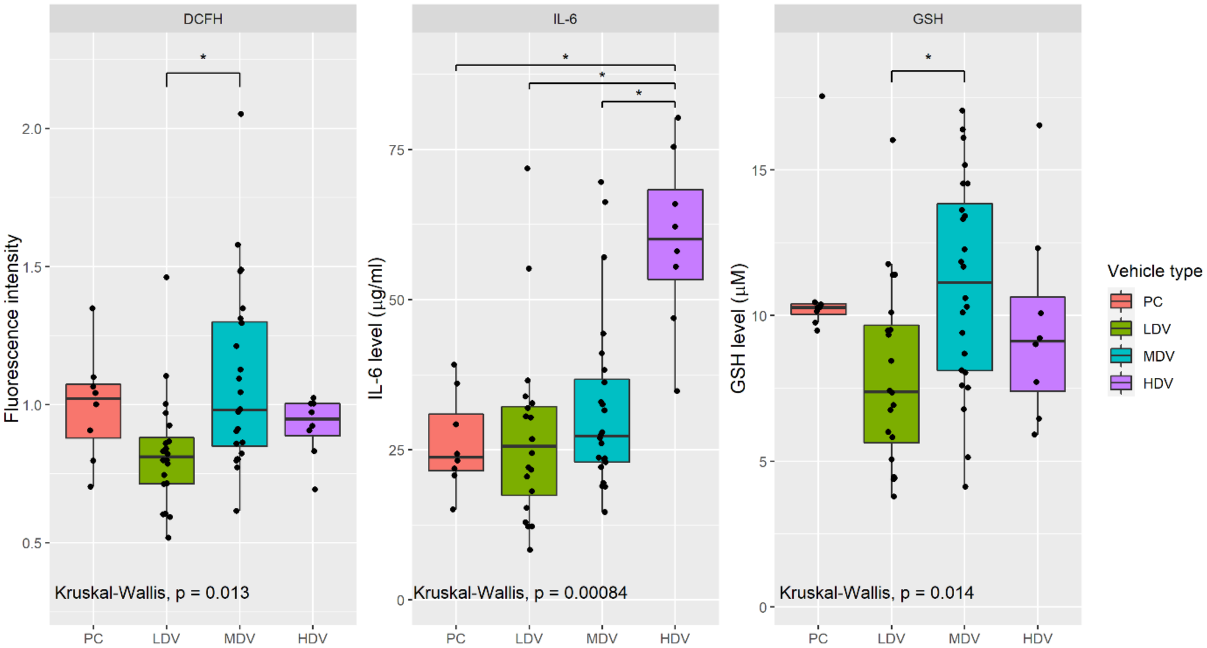

Fig. 6. Boxplots of the levels of DCFH, IL-6 and GSH grouped by vehicle type. Black dots represent the data points in the corresponding driving cycle. Asterisk represents the adjusted p-value in pairwise Wilcoxon rank sum test smaller than 0.05.

3.5 Correlation between the toxicity data and the PAH concentration

The chemical composition of PM samples has been reported to be related to the oxidative potential and the release of oxidative stress mediators (Chuang et al., 2012; Ho et al., 2016). In particular, certain PAHs have significant correlations with the vasoactive function and proinflammatory cytokines (Niu et al., 2017).

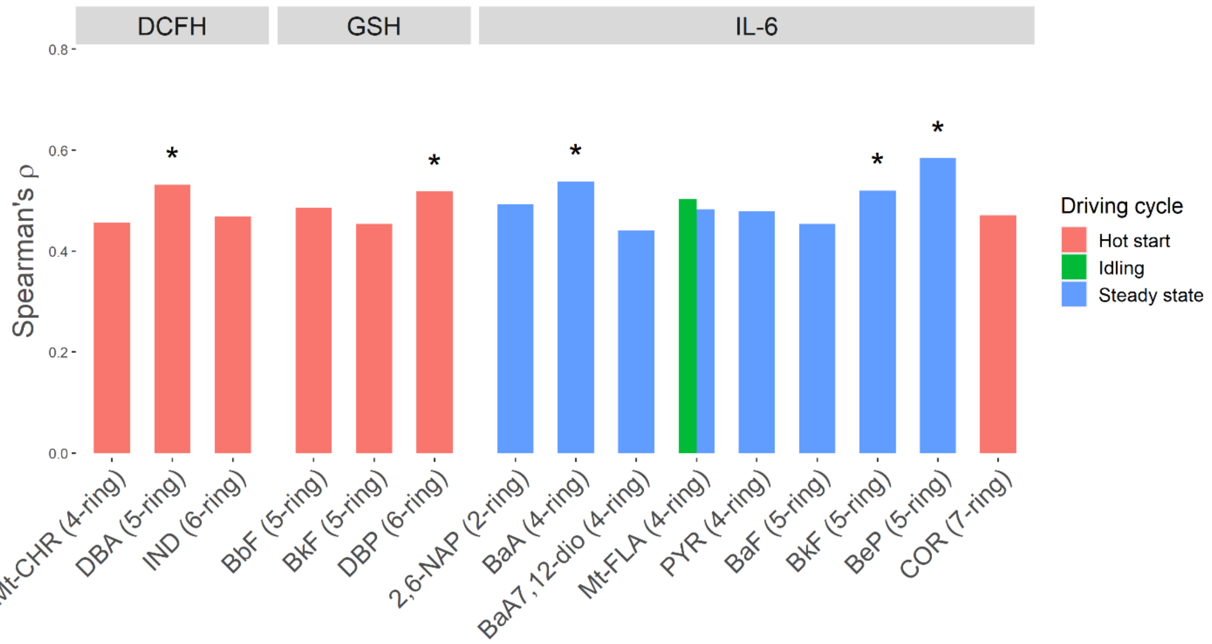

Fig. 7. Correlation between mass concentration of selected PAHs and toxicology test results. P-values of all the correlation coefficients in this figure are less than 0.1. Asterisk indicates that the p-value of the corresponding correlation coefficient is less than 0.05.

Correlation analysis was conducted between the concentrations of the PAHs and toxicological markers according to the method stated in the Methodology section. The correlation test results with a p value of <0.1 are presented in **Table S3** and **Fig. 7**. A total of 16 PAH–toxicological marker pairs had moderate or good correlations. In general, as depicted in **Fig. 7**, almost all the aforementioned pairs were associated with the hot start and steady-state cycles, with the exception of one pair that was associated with the idling cycle. No correlation was found between the PAH and toxicology data under the cold start condition. Thus, under hot engine conditions (hot start and steady state), some PAHs functioned as good indicators of ROS production or proinflammatory response. However, under low-engine-temperature conditions (i.e., cold start and idling), the PAH concentrations measured in this study were not the main driving forces for the observed toxicology responses.

As depicted in **Fig. 7**, the DCFH levels and PAH concentrations exhibited correlation only under the hot start condition. Moderate correlations were found between DCFH level and Mt-CHR (ρ = 0.46, $p < 0.1$) and between DCFH level and IND (ρ = 0.47, $p < 0.1$). Moreover, a good correlation was found between DCFH level and DBA (ρ = 0.53, $p < 0.05$). This result agreed with that of Wu et al. (2017), who found a strong correlation between ROS generation and DBA in petrol and diesel fuel combustion experiments. The GSH and PAH levels were correlated only in the hot start driving cycle. BbF (ρ = 0.48, $p < 0.1$) and BkF (ρ = 0.45, $p < 0.1$) exhibited moderate correlations with GSH, and DBP (ρ = 0.51, $p < 0.05$) exhibited a good correlation with GSH. Notably, both the DCFH and GSH levels were related to ROS generation. Moreover, all the reasonably good correlations of the PAH levels with DCFH and GSH levels were found in the hot start cycle.

Therefore, the relationship between ROS and PAHs in on-road driving conditions requires further investigation.

Fig. 7 displays the correlations between the proinflammatory mediator IL-6 and selected PAHs. Moderate correlation was found between IL-6 and COR ($\rho = 0.47$, $p < 0.1$) in the hot start cycle. The correlation between IL-6 and Mt-FLA ($\rho = 0.50$, $p < 0.1$) was the only significant correlation in the idling cycle. Except the aforementioned two correlations, all the observed correlations between IL-6 and PAHs were found in the steady-state cycle. The compound 2,6-NAP ($\rho = 0.49$, $p < 0.1$) was the only PAH with less than three rings that exhibited correlation with the toxicology results. IL-6 exhibited moderate correlation with Ba7,12-dio ($\rho = 0.44$, $p < 0.1$), Mt-FLA ($\rho = 0.48$, $p < 0.1$), PYR ($\rho = 0.48$, $p < 0.1$), and BaF ($\rho = 0.45$, $p < 0.1$). Moreover, IL-6 exhibited significant correlations with BaA ($\rho = 0.54$, $p < 0.05$), BkF ($\rho = 0.52$, $p < 0.05$), and BeP ($\rho = 0.58$, $p < 0.05$).

Several studies have assessed the correlations among IL-6 and particle-bounded PAHs from different sources; however, they have obtained different results. Niu et al. (2017) and Chowdhury et al. (2019) have investigated the correlation between PAHs in an atmospheric PM sample and the IL-6 responses in an A549 cell and a BEAS-2B cell (human bronchial epithelial cell), respectively. They have determined that certain PAHs are positively correlated with the IL-6 level. Lin et al. (2013) studied the effect of household particles on inflammation in human coronary artery endothelial cells (HCAECs) and revealed that PAHs were significantly correlated with the IL-6 level. Delfino et al. (2010) analyzed blood samples from 60 people and the air samples in their vicinity. Their results suggested a positive correlation between PAHs in air samples and the IL-6 level in human blood samples. Our findings are in agreement with those of the aforementioned studies, which support a positive correlation between PAHs and the IL-6 level. However, a study conducted by Skuland et al. (2017) could not establish a clear connection between the total or individual PAH levels in diesel exhaust particles and the IL-6 level in a BEAS-2B cell. Chuang et al. (2012) could not find a significant correlation between the PAHs in air samples and the IL-6 level in HCAECs. Moreover, Wang et al. (2016) found a significant negative correlation between the PAHs in atmospheric PM samples and the IL-6 level in BEAS-2B cells. The aforementioned studies suggest that in some cases, PAHs might not be the main inducer of proinflammatory response. Therefore, further investigations are essential to elucidate the reaction mechanism for the release of IL-6 and other proinflammatory mediators.

4. Conclusion

This paper presents a detailed chemical analysis of PM samples collected from diesel vehicles with various physical properties. This study is the first in Hong Kong to investigate the toxicity of PM samples through chassis dynamometer testing. The results indicated that PM emissions from the tested diesel vehicles were dominated by OC. DPF-equipped diesel vehicles had very high OC/EC ratios, which suggested that DPFs could effectively remove EC but not OC in PM. The EC removal efficiency of the DPFs was high even for vehicles with high odometer readings.

Among the identified PAHs, 4- and 5-ring PAHs were the most abundant species. The highest PAH EFs were exhibited by the HDVs, followed by the LDVs and MDVs. The driving cycle had a significant effect on the EFs of the PAHs. The steady-state cycle generally exhibited the lowest PAH EFs, and the transient and idling cycles exhibited substantially higher PAH EFs than the steady-state cycle did. Although different PAH EFs were observed under different driving conditions, the mass percentage of individual PAHs (i.e., the PAH composition of the PM samples) did not vary significantly with different driving conditions.

The cellular exposure experiments revealed that the PM emissions of diesel vehicles cause potential oxidative stresses, which emerge from ROS, for human lung cell activities. The statistical analysis results indicated that the MDVs produced significantly higher levels of DCFH and GSH than the LDVs did. Moreover, the HDVs produced significantly larger quantities of IL-6 than the other types of vehicles did. Correlation analysis between the PAHs and three toxicology markers revealed that statistically significant correlations existed between certain PAH–toxicological marker pairs, including DCFH and DBA ($\rho = 0.53$, $p < 0.05$), GSH and DBP ($\rho = 0.51$, $p < 0.05$), IL-6 and BaA ($\rho = 0.54$, $p < 0.05$), IL-6 and BkF ($\rho = 0.52$, $p < 0.05$), and IL-6 and BeP ($\rho = 0.58$, $p < 0.05$). Furthermore, the results suggested that new emission control technologies and policies should focus on OC and PAH reduction to reduce their adverse health effects on the human respiratory system.

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References

ABDEL-SHAFY, H. I. & MANSOUR, M. S. M. 2016. A review on polycyclic aromatic hydrocarbons: Source, environmental impact, effect on human health and remediation. *Egyptian Journal of Petroleum,* 25**,** 107-123.

CAO, X., HAO, X., SHEN, X., JIANG, X., WU, B. & YAO, Z. 2017. Emission characteristics of polycyclic aromatic hydrocarbons and nitro-polycyclic aromatic hydrocarbons from diesel trucks based on on-road measurements. *Atmospheric Environment,* 148**,** 190-196.

CECINATO, A., GUERRIERO, E., BALDUCCI, C. & MUTO, V. 2014. Use of the PAH fingerprints for identifying pollution sources. *Urban Climate,* 10**,** 630-643.

CHEN, F., HU, W. & ZHONG, Q. 2013. Emissions of particle-phase polycyclic aromatic hydrocarbons (PAHs) in the Fu Gui-shan Tunnel of Nanjing, China. *Atmospheric Research,* 124**,** 53-60.

CHENG, Y., LEE, S. C., HO, K. F., CHOW, J. C., WATSON, J. G., LOUIE, P. K. K., CAO, J. J. & HAI, X. 2010. Chemically-speciated on-road PM2.5 motor vehicle emission factors in Hong Kong. *Science of The Total Environment,* 408**,** 1621-1627.

CHENG, Y., ZOU, S. C., LEE, S. C., CHOW, J. C., HO, K. F., WATSON, J. G., HAN, Y. M., ZHANG, R. J., ZHANG, F., YAU, P. S., HUANG, Y., BAI, Y. & WU, W. J. 2011. Characteristics and source apportionment of PM1 emissions at a roadside station. *Journal of Hazardous Materials,* 195**,** 82-91.

CHEUNG, K. L., NTZIACHRISTOS, L., TZAMKIOZIS, T., SCHAUER, J. J., SAMARAS, Z., MOORE, K. F. & SIOUTAS, C. 2010. Emissions of Particulate Trace Elements, Metals and Organic Species from Gasoline, Diesel, and Biodiesel Passenger Vehicles and Their Relation to Oxidative Potential. *Aerosol Science and Technology,* 44**,** 500-513.

CHIANG, H.-L., LAI, Y.-M. & CHANG, S.-Y. 2012. Pollutant constituents of exhaust emitted from light-duty diesel vehicles. *Atmospheric Environment,* 47**,** 399-406.

CHOW, J. C., WATSON, J. G., CHEN, L. W. A., CHANG, M. C. O., ROBINSON, N. F., TRIMBLE, D. & KOHL, S. 2012. The IMPROVE_A temperature protocol for Thermal/Optical carbon analysis: maintaining consistency with a long-term database. *Journal of the Air & Waste Management Association,* 57**,** 1014-1023.

CHOWDHURY, P. H., HONDA, A., ITO, S., OKANO, H., ONISHI, T., HIGASHIHARA, M., OKUDA, T., TANAKA, T., HIRAI, S. & TAKANO, H. 2019. Effects of Ambient PM2.5 Collected Using Cyclonic Separator from Asian Cities on Human Airway Epithelial Cells. *Aerosol and Air Quality Research,* 19**,** 1808-1819.

CHUANG, H. C., FAN, C. W., CHEN, K. Y., CHANG-CHIEN, G. P. & CHAN, C. C. 2012. Vasoactive alteration and inflammation induced by polycyclic aromatic

hydrocarbons and trace metals of vehicle exhaust particles. *Toxicol Lett,* 214**,** 131-6.
CLAXTON, L. D. 2015. The history, genotoxicity, and carcinogenicity of carbon-based fuels and their emissions. Part 3: Diesel and gasoline. *Mutation Research/Reviews in Mutation Research,* 763**,** 30-85.
DELFINO, R. J., STAIMER, N., TJOA, T., ARHAMI, M., POLIDORI, A., GILLEN, D. L., KLEINMAN, M. T., SCHAUER, J. J. & SIOUTAS, C. 2010. Association of biomarkers of systemic inflammation with organic components and source tracers in quasi-ultrafine particles. *Environ Health Perspect,* 118**,** 756-62.
GALI, N. K., YANG, F., CHEUNG, C. S. & NING, Z. 2017. A comparative analysis of chemical components and cell toxicity properties of solid and semi-volatile PM from diesel and biodiesel blend. *Journal of Aerosol Science,* 111**,** 51-64.
GHIO, A. J., CARRAWAY, M. S. & MADDEN, M. C. 2012. Composition of air pollution particles and oxidative stress in cells, tissues, and living systems. *Journal of Toxicology and Environmental Health, Part B,* 15**,** 1-21.
GRIESHOP, A. P., LIPSKY, E. M., PEKNEY, N. J., TAKAHAMA, S. & ROBINSON, A. L. 2006. Fine particle emission factors from vehicles in a highway tunnel: Effects of fleet composition and season. *Atmospheric Environment,* 40**,** 287-298.
HAYS, M. D., PRESTON, W., GEORGE, B. J., GEORGE, I. J., SNOW, R., FAIRCLOTH, J., LONG, T., BALDAUF, R. W. & MCDONALD, J. 2017. Temperature and Driving Cycle Significantly Affect Carbonaceous Gas and Particle Matter Emissions from Diesel Trucks. *Energy Fuels,* 31.
HO, K.-F., HO, S. S. H., HUANG, R.-J., CHUANG, H.-C., CAO, J.-J., HAN, Y., LUI, K.-H., NING, Z., CHUANG, K.-J., CHENG, T.-J., LEE, S.-C., HU, D., WANG, B. & ZHANG, R. 2016. Chemical composition and bioreactivity of PM2.5 during 2013 haze events in China. *Atmospheric Environment,* 126**,** 162-170.
HO, K. F., HO, S. S. H., LEE, S. C., CHENG, Y., CHOW, J. C., WATSON, J. G., LOUIE, P. K. K. & TIAN, L. 2009. Emissions of gas- and particle-phase polycyclic aromatic hydrocarbons (PAHs) in the Shing Mun Tunnel, Hong Kong. *Atmospheric Environment,* 43**,** 6343-6351.
HO, K. F., LEE, S. C., YU, J. C., ZOU, S. C. & FUNG, K. 2002. Carbonaceous characteristics of atmospheric particulate matter in Hong Kong. *Science of The Total Environment,* 300**,** 59-67.
HO, S. S. H., YU, J. Z., CHOW, J. C., ZIELINSKA, B., WATSON, J. G., SIT, E. H. L. & SCHAUER, J. J. 2008. Evaluation of an in-injection port thermal desorption-gas chromatography/mass spectrometry method for analysis of non-polar organic compounds in ambient aerosol samples. *Journal of Chromatography A,* 1200**,** 217-227.

HONG KONG TRANSPORT DEPARTMENT 2018. THE ANNUAL TRAFFIC CENSUS 2017.

HOU, B., ZHUANG, G., ZHANG, R., LIU, T., GUO, Z. & CHEN, Y. 2011. The implication of carbonaceous aerosol to the formation of haze: revealed from the characteristics and sources of OC/EC over a mega-city in China. *J Hazard Mater,* 190**,** 529-36.

HU, S., D HERNER, J., ROBERTSON, W., KOBAYASHI, R., CHANG, O., HUANG, S.-M., ZIELINSKA, B., KADO, N., F COLLINS, J., RIEGER, P., HUAI, T. & AYALA, A. 2013. Emissions of polycyclic aromatic hydrocarbons (PAHs) and nitro-PAHs from heavy-duty diesel vehicles with DPF and SCR. *Journal of the Air & Waste Management Association (1995),* 63**,** 984-96.

HUANG, Y., ORGAN, B., ZHOU, J., SURAWSKI, N., HONG, G., CHAN, E. & YAM, Y. S. 2018. Emission measurement of diesel vehicles in Hong Kong through on-road remote sensing: Performance review and identification of high-emitters. *Environmental Pollution,* 237**,** 133-142.

KIM, K. H., JAHAN, S. A., KABIR, E. & BROWN, R. J. 2013. A review of airborne polycyclic aromatic hydrocarbons (PAHs) and their human health effects. *Environ Int,* 60**,** 71-80.

KLEEMAN, M. J., SCHAUER, J. J. & CASS, G. R. 2000. Size and composition distribution of fine particulate matter emitted from motor vehicles. *Environmental Science & Technology,* 34**,** 1132-1142.

LEE, S., CHENG, Y., HO, K. F., CAO, J., LOUIE, P. K. K., CHOW, J. & WATSON, J. 2006. PM 1.0 and PM 2.5 Characteristics in the Roadside Environment of Hong Kong. *Aerosol Science and Technology,* 40**,** 157-165.

LI, N., SIOUTAS, C., CHO, A., SCHMITZ, D., MISRA, C., SEMPF, J., WANG, M., OBERLEY, T., FROINES, J. & NEL, A. 2003. Ultrafine Particulate Pollutants Induce Oxidative Stress and Mitochondrial Damage. *Environmental Health Perspectives,* 111**,** 455-460.

LIM, M. C. H., AYOKO, G. A., MORAWSKA, L., RISTOVSKI, Z. D. & ROHAN JAYARATNE, E. 2005. Effect of fuel composition and engine operating conditions on polycyclic aromatic hydrocarbon emissions from a fleet of heavy-duty diesel buses. *Atmospheric Environment,* 39**,** 7836-7848.

LIN, L.-Y., LIU, I. J., CHUANG, H.-C., LIN, H.-Y. & CHUANG, K.-J. 2013. Size and composition effects of household particles on inflammation and endothelial dysfunction of human coronary artery endothelial cells. *Atmospheric Environment,* 77**,** 490-495.

LIN, Y. C., LI, Y. C., AMESHO, K. T. T., CHOU, F. C. & CHENG, P. C. 2019. Characterization and quantification of PM2.5 emissions and PAHs concentration in PM2.5 from the exhausts of diesel vehicles with various accumulated mileages. *Sci Total Environ,* 660, 188-198.

MA, Y., CHENG, Y., QIU, X., LIN, Y., CAO, J. & HU, D. 2016. A quantitative assessment of source contributions to fine particulate matter (PM2.5)-bound polycyclic aromatic hydrocarbons (PAHs) and their nitrated and hydroxylated derivatives in Hong Kong. *Environ Pollut,* 219, 742-749.

MAY, A. A., NGUYEN, N. T., PRESTO, A. A., GORDON, T. D., LIPSKY, E. M., KARVE, M., GUTIERREZ, A., ROBERTSON, W. H., ZHANG, M., BRANDOW, C., CHANG, O., CHEN, S., CICERO-FERNANDEZ, P., DINKINS, L., FUENTES, M., HUANG, S.-M., LING, R., LONG, J., MADDOX, C., MASSETTI, J., MCCAULEY, E., MIGUEL, A., NA, K., ONG, R., PANG, Y., RIEGER, P., SAX, T., TRUONG, T., VO, T., CHATTOPADHYAY, S., MALDONADO, H., MARICQ, M. M. & ROBINSON, A. L. 2014a. Gas- and particle-phase primary emissions from in-use, on-road gasoline and diesel vehicles. *Atmospheric Environment,* 88, 247-260.

MAY, A. A., PRESTO, A. A., HENNIGAN, C. J., NGUYEN, N. T., GORDON, T. D. & ROBINSON, A. L. 2013. Gas-particle partitioning of primary organic aerosol emissions: (1) Gasoline vehicle exhaust. *Atmospheric Environment,* 77, 128-139.

MAY, J., BOSTEELS, D. & FAVRE, C. 2014b. An Assessment of Emissions from Light-Duty Vehicles using PEMS and Chassis Dynamometer Testing. SAE International.

NEL, A. 2005. Air pollution-related illness: effects of particles. *Science,* 308, 804-806.

NIU, X., HO, S. S. H., HO, K. F., HUANG, Y., SUN, J., WANG, Q., ZHOU, Y., ZHAO, Z. & CAO, J. 2017. Atmospheric levels and cytotoxicity of polycyclic aromatic hydrocarbons and oxygenated-PAHs in PM2.5 in the Beijing-Tianjin-Hebei region. *Environ Pollut,* 231, 1075-1084.

SHAH, S. D., COCKER, D. R., MILLER, J. W. & NORBECK, J. M. 2004. Emission Rates of Particulate Matter and Elemental and Organic Carbon from In-Use Diesel Engines. *Environmental Science & Technology,* 38, 2544-2550.

SHAH, S. D., OGUNYOKU, T. A., MILLER, J. W. & COCKER, D. R. 2005. On-Road Emission Rates of PAH and n -Alkane Compounds from Heavy-Duty Diesel Vehicles. *Environmental science & technology,* 39, 5276-84.

SHEN, H., TAO, S., WANG, R., WANG, B., SHEN, G., LI, W., SU, S., HUANG, Y., WANG, X., LIU, W., LI, B. & SUN, K. 2011. Global time trends in PAH emissions from motor vehicles. *Atmospheric Environment,* 45, 2067-2073.

SHEN, X., YAO, Z., HUO, H., HE, K., ZHANG, Y., LIU, H. & YE, Y. 2014. PM2.5 emissions from light-duty gasoline vehicles in Beijing, China. *Science of The Total Environment,* 487, 521-527.

SKULAND, T. S., REFSNES, M., MAGNUSSON, P., OCZKOWSKI, M., GROMADZKA-OSTROWSKA, J., KRUSZEWSKI, M., MRUK, R., MYHRE, O., LANKOFF, A. & OVREVIK, J. 2017. Proinflammatory effects of diesel exhaust particles from moderate blend concentrations of 1st and 2nd generation biodiesel in BEAS-2B bronchial epithelial cells-The FuelHealth project. *Environ Toxicol Pharmacol,* 52**,** 138-142.

TSAI, Y. I., YANG, H.-H., WANG, L.-C., HUAN, J.-L., YOUNG, L.-H., CHENG, M.-T. & CHIANG, P.-C. 2011. The Influences of Diesel Particulate Filter Installation on Air Pollutant Emissions for Used Vehicles. *Aerosol and Air Quality Research,* 11**,** 578-583.

UNECE 2011. Consolidated Resolution on the Construction of Vehicle (R.E.3), revision. United Nations Economic Commission for Europe, Geneva, Switzerland.

WANG, B., LI, N., DENG, F., BUGLAK, N., PARK, G., SU, S., REN, A., SHEN, G., TAO, S. & GUO, X. 2016. Human bronchial epithelial cell injuries induced by fine particulate matter from sandstorm and non-sandstorm periods: Association with particle constituents. *J Environ Sci (China),* 47**,** 201-210.

WEISS, M., BONNEL, P., KÜHLWEIN, J., PROVENZA, A., LAMBRECHT, U., ALESSANDRINI, S., CARRIERO, M., COLOMBO, R., FORNI, F., LANAPPE, G., LE LIJOUR, P., MANFREDI, U., MONTIGNY, F. & SCULATI, M. 2012. Will Euro 6 reduce the NOx emissions of new diesel cars? – Insights from on-road tests with Portable Emissions Measurement Systems (PEMS). *Atmospheric Environment,* 62**,** 657-665.

WU, B., SHEN, X., CAO, X., YAO, Z. & WU, Y. 2016. Characterization of the chemical composition of PM2.5 emitted from on-road China III and China IV diesel trucks in Beijing, China. *Science of The Total Environment,* 551-552**,** 579-589.

WU, D., ZHANG, F., LOU, W., LI, D. & CHEN, J. 2017. Chemical characterization and toxicity assessment of fine particulate matters emitted from the combustion of petrol and diesel fuels. *Sci Total Environ,* 605-606**,** 172-179.

YAO, Z., HUO, H., ZHANG, Q., STREETS, D. G. & HE, K. 2011. Gaseous and particulate emissions from rural vehicles in China. *Atmospheric Environment,* 45**,** 3055-3061.

ZHENG, M., HAGLER, G. S. W., KE, L., BERGIN, M. H., WANG, F., LOUIE, P. K. K., SALMON, L., SIN, D. W. M., YU, J. Z. & SCHAUER, J. J. 2006. Composition and sources of carbonaceous aerosols at three contrasting sites in Hong Kong. 111.

ZIELINSKA, B., SAGEBIEL, J., MCDONALD, J. D., WHITNEY, K. & LAWSON, D. R. 2004. Emission rates and comparative chemical composition from selected in-use diesel and gasoline-fueled vehicles. *J Air Waste Manag Assoc,* 54**,** 1138-50.

Highlights

- PM samples from the exhaust were dominated by OC and 4,5-ring PAHs.
- Diesel particulate filter equipped vehicles have high OC/EC ratios.
- Steady-state cycle exhibited the lowest PAH EF.
- Vehicle size has significant effect on the toxicity of the PM sample.
- Significant correlations were found between certain PAHs and toxicological markers.

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Dear Editor,

I am writing to submit the original research manuscript entitled "***Chemical and toxicological characterization of particulate emissions from diesel vehicles***".

Hong Kong is one of the most densely populated cities in the world, and the majority of the population is exposed vehicular exhaust. It is therefore important to **closely monitor the change in vehicle emission behavior, and the in chemical and toxicological properties of the vehicular exhaust.**

The main objectives of this work are to characterize the chemical and toxicological properties of the PM samples collected in diesel vehicle exhaust, and to examine the correlation between the chemical composition and toxicological response of the PM samples. The results showed that PM emissions from diesel vehicles were dominated by organic carbon (OC) and 4- and 5-ring polycyclic aromatic hydrocarbons (PAHs). Statistical analyses revealed that significant correlations existed between specific PAHs and toxicological markers. **This study suggested that new emission control technologies and policies should focus on OC and PAH reduction to reduce their adverse health effects on the human respiratory system.**

This is an original research article that has not been previously published, in whole or in part, and it is not under consideration by any other journals. We believe this manuscript fits perfectly with the scope of your journal.

We look forward to the comments from reviewers. Should you have any questions to this work, please do not hesitate to contact me. Thank you for your consideration.

Yours faithfully,

Kin Fai Ho, Ph.D.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Statement of novelty

This novel study targeted the chemical and toxicological properties of particulate matters (PM) emitted from diesel vehicles in Hong Kong, which was rarely reported. The results of this study are important for the design and implementation of new emission control policies and technologies.

The current study characterized the toxicological effects of diesel vehicle PM in cell-based assays. Statistical analyses of the results showed that significant correlation exist between specific chemical compounds in the PM samples and their toxicological responses. Therefore the content of the current study matches with the scope of the Journal of Hazardous Materials.

Revised Manuscript

Chemical and Toxicological Characterization of Particulate Emissions from Diesel Vehicles

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ABSTRACT

This study presents a detailed chemical and toxicological characterization of diesel particulate matter (PM) emitted from diesel vehicles running on a chassis dynamometer under different driving conditions. Chemical analyses were carried out to characterize the organic carbon (OC), elemental carbon (EC) and 31 polycyclic aromatic hydrocarbons (PAHs) contents of the PM samples collected. OCEC analysis results showed that PM emissions from diesel vehicles in the current study were dominated by OC. DPF-equipped vehicles were found with high OC/ EC ratios. PAH analysis results showed that 4- and 5-ring PAHs were the dominating PAHs in the OC fraction of the PM sample. Emission factor (EF) of PAHs were lower in steady state cycle compared to transient and idling cycles. Particle toxicity was examined by three toxicological markers in human A549 cells, including (1) acellular DCFH for oxidative potential, (2) interleukin-6 (IL-6) for inflammation, and (3) glutathione (GSH) for anti-oxidation after exposure. Statistical analyses showed that driving conditions have minimal effects on the three toxicological markers, while vehicle size have statistically significant effects on the concentrations of the markers. Correlation analysis between PAHs and toxicological markers revealed that significant correlation existed between specific compounds and markers, including DCFH and dibenzo[a,h]anthracene, GSH and phenanthrene, and anthracene, as well as IL-6 and benzo[a]anthracene, benzo[k]fluoranthene, and benzo[e]pyrene. Our results served as scientific evident for policy makers to setup new emission control strategies and data sets for other modelling studies.

INTRODUCTION

Hong Kong is one of the most densely populated cities in the world, and the vast majority of the population is exposed to vehicular exhaust, as many people work and live close to main roads and even highways. By the end of 2017, there were more than 830,000 licensed motor vehicles running on the roads, which was increased by 35% compared to 2007 (Hong Kong Transport Department, 2018). Among all vehicles, diesel vehicles are the main sources of street-level particulate matter (PM) pollution (Lee et al., 2006, Shen et al., 2014, Weiss et al., 2012, Yao et al., 2011).

A large fraction of PM is comprised of carbonaceous aerosols (Hou et al., 2011). In general, the carbonaceous aerosols are classified into two categories: elemental carbon (EC) and organic carbon (OC). Lee et al. (2006) reported that the carbonaceous aerosols were the dominating species in fine particles in the roadside environment in Hong Kong, accounting for around 44% of $PM_{2.5}$. Lee and his research team suggested that vehicular emissions were the major sources of carbonaceous aerosols (Cheng et al., 2010, Cheng et al., 2011, Ho et al., 2002, Lee et al., 2006). Zheng et al. (2006) reported that more than 60% of OC measured at a roadside station in Hong Kong came from vehicular emissions. The OC fraction in vehicle emission derived PM is made up of various toxic organic compounds, and polycyclic aromatic hydrocarbon (PAH) is one of them. PAHs are important toxic components of vehicle emitted organic species. They are identified as one of the major toxic air pollutants, mainly originated from anthropogenic processes, especially from incomplete combustion of organic matters. PAHs are semi-volatile organic compounds that can be present in both particulate and gaseous phases. PAHs are present on the surface of airborne particles through condensation, adsorption or combustion processes (Yamasaki et al., 1982, Dimashki et al., 2001). Several studies have reported that vehicular emissions, particularly diesel vehicles, are the most important sources of PAHs in urban areas (Cecinato et al., 2014, Chen et al., 2013, Shen et al., 2011). Therefore, various studies have been done to characterize the *EFs* and chemical composition of PAHs emitted from diesel vehicles under different conditions (Zielinska et al., 2004, Tsai et al., 2011, Hu et al., 2013, Cao et al., 2017, Hays et al., 2017, Lin et al., 2019). In Hong Kong, the characterization of PAH derived from vehicle emission were mainly done by tunnel measurement and ambient air sample collection (Ho and Lee, 2002, Cheng et al., 2010, Ho et al., 2009, Ma et al., 2016). For example, Ho et al. (2009) conducted a tunnel study to investigate the PAHs emission from vehicles in Hong Kong. The authors concluded that two- and three-ring PAHs were the dominant fraction in gas phase, while four-ring PAHs were the most abundant in particle phase. Since studies of direct tailpipe emission (e.g. chassis dynamometer or PEMS study) from diesel vehicles in Hong Kong are scarce, carrying out such kind of research is needed to fill the knowledge gap.

Another important concern of PM is their adverse effects to human health (Abdel-Shafy and Mansour, 2016, Chuang et al., 2012, Kim et al., 2013). In previous toxicological studies, it is believed that reactive oxygen species (ROS) production by PM exposure is the main mechanisms for increased risk of adverse health effects (Li et al., 2003, Nel, 2005). For instance, studies by

Geller et al. (2006) and Ntziachristos et al. (2007) revealed strong correlations between the redox activities of their PM sample and several PM species, including OC, EC and PAHs. In a healthy biological system, glutathione (GSH) is produced to mitigate the PM-derived ROS in cells (Ghio et al., 2012). However, the overloaded ROS is able to incur inflammatory responses such as interleukin 6 (IL-6) and interleukins 8 (IL-8) production, consequently leading to harmful health effects. Various studies have been done to investigate the inflammatory response triggered by the exposure of vehicle-derived PM (Mazzarella et al., 2007, Gerlofs-Nijland et al., 2013, Bengalli et al., 2017). Results from these studies showed that vehicle-derived PM emission is closely related to the release of pro-inflammatory cytokines, like IL-6, while the use of advanced emission control technologies, such as DPF, will significantly change the inflammatory responses in the studied cell.

Many PAHs are known or suspected carcinogens in human (IARC 2010; Song et al., 2012), while some PAHs are believed to trigger pro-inflammatory response (Lin et al., 2013; Niu et al., 2017). The relationship between PAHs and different toxicology markers have also been investigated by various studies. Cheung et al (2010) and Wu et al (2017) have investigated the relationship between ROS level and diesel fuel burning particles, while Vattanasit et al (2014) and Totlandsdal et al (2014) studied the ability of PAHs in diesel exhaust particles to induce pro-inflammatory response. All these studies showed correlation between PAHs and adverse health effects. Moreover, gas phase PAHs can undergo oxidation reactions in the atmosphere, producing secondary organic aerosol (SOA), which is, in many cases, more harmful than the precursor (Lin et al., 2019). Therefore, characterizing the *EF* of PAH is important to air quality control as well as the health of the general public.

The objectives of the current study are to characterize the *EFs* of OC, EC and PAHs of the recruited diesel vehicles, to compare the differences in chemical compositions and toxicological responses under different driving conditions, and to investigate the correlation between the chemical composition and toxicological response of the PM samples.

METHODOLOGY

Fleet overview and instrumentation set-up

The vehicle fleet in this study was comprised of 15 vehicles covering a wide range of vehicle classes, engine sizes, after-treatment technologies, and emission standards (**Table S1**). The vehicle fleet was selected briefly in accordance to the emission standard distribution of diesel vehicles in Hong Kong, where most of them are with Euro 4 and 5, followed by Euro 3 and others. Vehicle categories were classified with reference to the United Nations Economic Commission for Europe (UNECE) categories (UNECE, 2011). Passenger cars (PCs) were defined as M type vehicles. Light Duty Vehicles (LDVs), Medium Duty Vehicles (MDVs) and Heavy Duty Vehicles (HDVs) corresponded to N1, N2 and N3 type vehicles, respectively. Vehicle 15 was exceptionally categorized as HDV since it was a 10-ton tractor designed for carrying a trailer up to 20 tons. All vehicles, except Vehicle 2 and 7, have taken and passed the annual vehicle examination required by the Transport Department of the Hong Kong Government. The annual vehicle examination is mandatory for vehicles with first registration date over 6 years in order to make sure that vehicles running on road are in acceptable maintenance condition. The diesel fuel used by all vehicles in the current study is the same, which comply with the Euro 5 diesel fuel standard, as stated in **Table S3.**

Chassis dynamometer tests were carried out in the Jockey Club Heavy Vehicle Emissions Testing and Research Centre (JCEC) in Hong Kong. **Fig. S1** of Supporting Information shows the schematic diagram of the instrumental set-up. All testing facilities in JCEC complied with the European standards for type approval tests. Two chassis dynamometers were used to test the vehicles with different weights. PCs and LDVs were tested on a Mustang Dynamometer with 48" single roller, while MDVs and HDVs were tested on a Mustang Dynamometer with 17.2" triple roller.

Driving cycles and testing conditions

Four driving cycles were used to test each vehicle, namely cold start transient cycle, hot start transient cycle, idling cycle, and steady state cycle. For the sake of convenience, cold start and hot start transient cycles will be called cold start and hot start cycle in the following parts. For each vehicle, cold start cycle was repeated two time while hot start, idling, and steady state cycle tests were repeated three times. Detailed descriptions of the four driving cycles and loading conditions can be found in Supporting Information.

PM sample collection

PM samples were collected simultaneously on a quartz (Whatman, 47 mm) and a Teflon membrane filters (Pall, 47 mm) for different offline tests. The mass of sample collected on the filters were determined by a microbalance (Sartorius, MC5) with readability of 0.001 mg. The filters were conditioned in a humidity-controlled chamber (i.e. relative humidity = 40%) for at least 24 hours before weighing. Each filter was weighted at least twice before and after sample collection. The weighing result was accepted only if the difference between two consecutive weighing was less than 0.01% of the filter weight. After obtaining the weights of PM samples, the filters were sealed in zip-zap bags and stored at -20°C for further chemical and toxicological analyses. Operational blanks and laboratory control blanks were processed simultaneously with the field samples during sample collection and analyses. All filter data were corrected with operation and laboratory blanks.

Chemical analysis

Samples collected on the quartz filters were used for OC/EC and PAH analyses. Each filter was cut exactly in half with a specially designed chopper for the two analyses. The contents of OC and EC were analysed by a Desert Research institute (DRI) Model 2001 Thermal/Optical Carbon Analyzer with the IMPROVE-A protocol as described by Chow et al. (2012). PAH samples were analyzed by the thermal desorption-gas chromatography/mass spectrometer (TD-GC/MS) method (Ho et al., 2008). Description of the chemical analyse procedures can be found in Supporting Information.

Toxicological analysis

PM samples collected on Teflon filters were removed for toxicological analysis. The three toxicological markers included (1) acellular DCFH for oxidative potential, (2) interleukin-6 (IL-6) for inflammation, and (3) glutathione (GSH) for anti-oxidation after exposure. Description of the toxicological analysis procedures can be found in Supporting Information.

Calculation of emission factor (*EF*) and statistical analysis

In the current study, the fuel-based EF of OC, EC, and individual PAHs were calculated to compare the emission characteristics between different vehicles. The relationship between toxicological markers and chemical species were examined by correlation studies. Detailed description of the calculation of *EF* and statistical analysis used in the current study were given in Supporting Information.

RESULTS AND DISCUSSION

PM emission characteristics

The result of PM *EF* was published elsewhere (Wang et al., 2019). The *EF* of PM was calculated from the mass of PM sample collected on the Teflon filter. In general, the emission characteristic of PM mass is close to that of total carbon content (OC+EC), which will be discussed in the following section.

OC and EC

The EFs of OC and EC and the OC/EC ratios at different driving conditions are shown in **Fig. 1**. OC was the dominant fraction in most of the collected samples, except for Vehicles 6 and 8 in which EC dominates. Previous studies pointed out that EC was the dominating fraction in PM emission from the diesel vehicles (Chiang et al., 2012, Grieshop et al., 2006, Kleeman et al., 2000), while some studies reported a wide range of OC and EC contents emitted from their fleets (Shah et al., 2004, Wu et al., 2016). In a more recent study, Gali et al. (2017) showed that under cold idle or lower engine speed conditions, OC is the dominating fraction in PM sample, which is quite similar to our results.

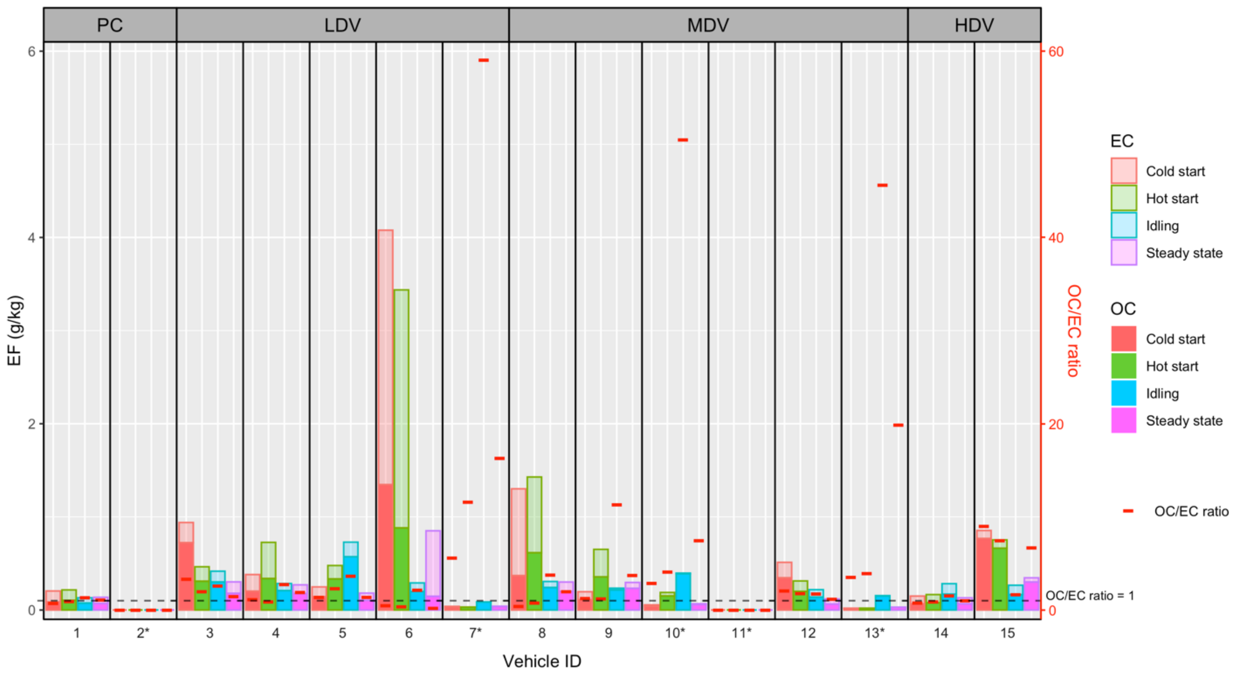

Fig. 1. *EFs* of OC and EC and OC/EC ratios. Each bar represents the sum of OC and EC *EF*. Light color bar represents EC *EF* and deep color bar represents OC *EF*. Vehicles with an asterisk next to their vehicle ID were equipped with DPF.

The variation in OC/EC ratio can be caused by different factors, including emission standard, testing weight, engine power and capacity and maintenance condition of the test vehicles. EC from vehicles equipped with diesel particulate filters (DPF) were either below the detection limit (Vehicles 2 and 11) or extremely low (0.003 ± 0.002 g/kg in Vehicle 7, 0.017 ± 0.014 g/kg in Vehicle 10, and 0.003 ± 0.001 g/kg in Vehicle 13). This observation was consistent with the finding of May et al. (2014a), which reported that DPF could effectively lower the emission of EC from diesel vehicles. Our results also showed that even for vehicles with high odometer readings (e.g. Vehicle 10, 11 and 13), the ability of the DPF to remove EC was still satisfactory. As shown in **Fig. 1**, high OC/EC ratios were found in Vehicle 7 (59.06), Vehicle 10 (50.51) and Vehicle 13 (45.63) under idling condition. For these three cases, the amounts of EC were very low, while considerable amounts of OC were measured. This observation is in agreement with the gaseous total hydrocarbon (THC) result of the current study as presented in Wang et al. (2019). For idling cycles of Vehicle 7, Vehicle 10 and Vehicle 13, substantial amount of THC was measured. Since THC reflects the gas phase OC content, and it is possible for some high molecular weight hydrocarbons to partition to the particle phase, OC present in the aforementioned cycles were probably originated from the gas phase, which was not removed by the DPF.

Among the four driving conditions tested in this study, idling generally produced the highest OC/EC ratio. As mentioned, EC mainly arises from fuel droplet pyrolysis, while OC is mainly originated from unburned fuel and incomplete combustion (Shah et al., 2004). When the vehicle was in idling condition, its engine temperature was lower, resulting in “less complete” fuel combustion as compared to other conditions.

The European emission standard assigned to the vehicle had significant effects on the OC and EC emissions. In general, the *EFs* of OC and EC decreased with increasing emission standards, except for Vehicles 6, 12 and 15. Extremely low level of EC but measurable OC were recorded in vehicles with high emission standard (e.g. Vehicle 7 and 13). A possible reason is that the emission control technologies applied on the new vehicles might not effectively remove OC from the diesel vehicle exhaust. Since most of the toxic and mutagenic properties of diesel exhaust were associated with the OCs (Claxton, 2015, Shah et al., 2004), our results suggest that the development of emission control technologies should focus on reducing the OC fraction of PM.

Apart from the effects of emission standard, the results also showed that the *EFs* of OC and EC could be greatly influenced by the maintenance conditions of the vehicles. For example, vehicles with advanced emission standard did not guarantee reduction in either OC or EC. Comparisons with the HDVs as shown in **Fig. 1**, the OC *EF*s of Vehicle 15 (Euro 5) was much higher than that of Vehicle 14 (Euro 4). During the chassis dynamometer testing of Vehicle 15, it was observed that white smoke and pungent smell came out from the exhaust, indicating that Vehicle 15 was not well maintained. In this case, the maintenance condition of the vehicle was more important than its emission standard in controlling emissions of OC and EC.

PAHs

A total of 31 PAHs (**Table S2**) were characterized and their *EFs* were calculated. For all tests, *EFs* of Acenaphthylene (AcPy), Acenaphthene (AcP), and Fluoranthene (FL) were below the detection limit, since these three PAHs have low molecular weight and mainly present in gas phase. Therefore, these three PAHs will be excluded in the following analysis and discussion. **Fig. 2** provides an overview of the PAH EFs under different driving conditions. Each bar in **Fig. 2** corresponds to the total PAH *EF* (sum of *EFs* of all PAHs tested) in the given driving cycle, and the red solid line represents the sum of total PAH *EFs* of the four driving cycles (denoted as "four cycle-sum" in the following parts). As noted in **Fig. 2**, Vehicle 15 had the highest four cycle-sum of 18.60 mg/kg, followed by Vehicle 6. Vehicle 2 had the lowest four cycle-sum of 0.41 mg/kg, followed by Vehicle 11 and 13. One common feature of these three vehicles with lower four cycle-sum was that they were all equipped with DPF.

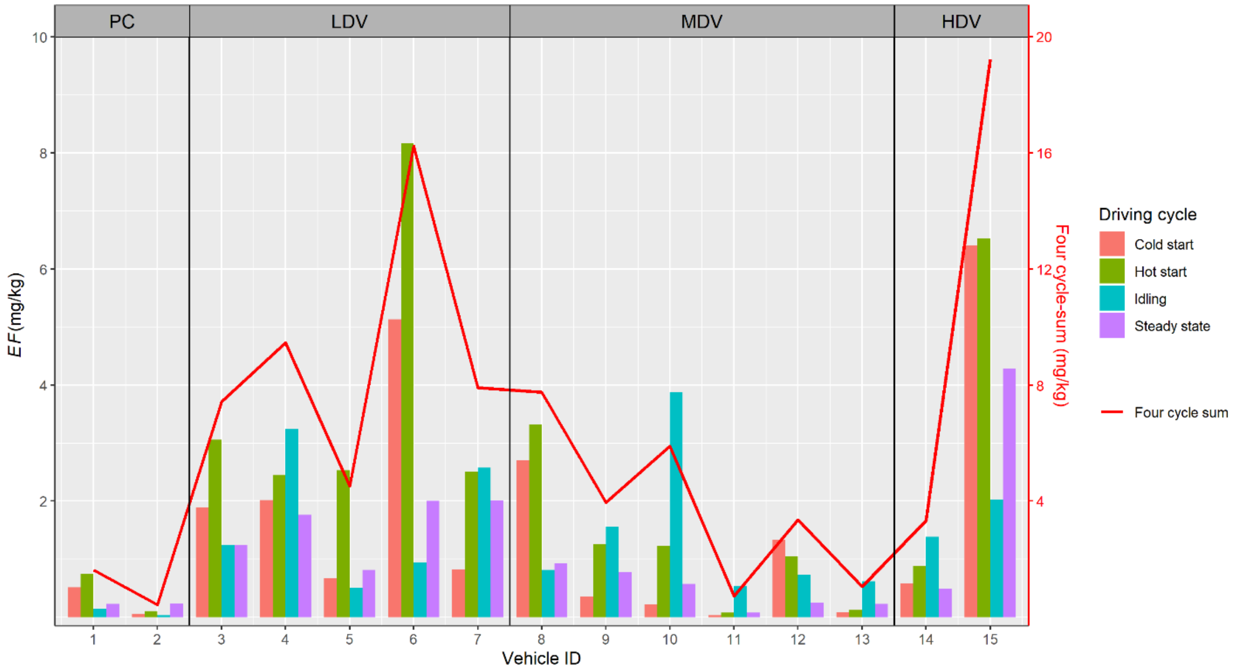

Fig. 2. Total PAH *EFs* in different driving cycles. The red solid line represents the sum of total PAH *EFs* of the four driving cycles, denoted as "four cycle-sum".

Fig. 2 also shows that MDVs had lower total PAHs *EF*s than LDVs did. Unexpectedly, vehicles with greater testing weights emitted less PAHs. One possible explanation was that there were two DPF-equipped vehicles in the MDV class, while there was only one DPF-equipped vehicle in the LDV class. Cao et al. (2017) also observed the same pattern in their vehicle fleet, which was comprised of 18 diesel trucks in China. The fact that this observation being not an isolated event suggested that the effect of vehicle size on PAH emission should be further investigated.

Another feature observed in **Fig. 2** is that steady state cycle generally had the least total PAH *EF*s while transient and idling cycles emitted substantially more PAHs, which was consistent with the results reported by (Shah et al., 2005). **Fig. 2** shows that the total PAH *EFs* of Vehicles 4, 7, 9, 10, 11, 13 and 14 under idling cycles were higher than those under transient cycles. This result suggested that it is important to characterize the vehicle emission behaviour under different driving conditions, including idling, as studies of vehicle emission behaviour under idling condition are scarce compared to other conditions (e.g. transient and constant speed).

To identify the dominant PAHs emitted from each vehicle, the weighted percentage of each PAH emitted under different driving cycles is shown in **Fig. 3**. For most of the vehicles, the dominating PAHs were 4- and 5-ring PAHs including pyrene (PYR), benz[*a*]anthracene (BaA), chrysene (CHR), benzo[*b*]fluoranthene (BbF), benzo[*k*]fluoranthene (BkF) and benzo[*a*]fluoranthen (BaF). This finding was consistent with those reported by previous studies (Cao et al., 2017, Hu et al., 2013). The difference in PAH composition under different driving conditions can also be compared in **Fig. 3**. From the figure, the distribution of individual PAH did not show a great variation among different driving conditions of a given vehicle, except for idling and steady state cycles of Vehicle 1 and 9. When comparison was made across vehicles, it can be observed that the variation in PAH composition among vehicles was not significant, expect for Vehicle 7. Excluding the above two exceptions, PAHs collected in all driving cycles were dominated by the 4 and 5-ring PAHs for all vehicles. This observation suggests that driving conditions, driving pattern (NEDC or FIGE), mileage, testing weight of the vehicle, and after-treatment technologies do not have significant effects on the composition of PAHs. It also suggests that in general the PAH samples collected came from the same source, which was probably fuel combustion, since they all have similar composition. Therefore, the PAH samples collected in the exceptional cases (in

Vehicle 7 and in the idling and steady state cycle of Vehicle 1 and 9) were probably affected by other sources. Further investigations are needed to characterize the sources of PAHs collected from the tailpipe emission of diesel vehicles.

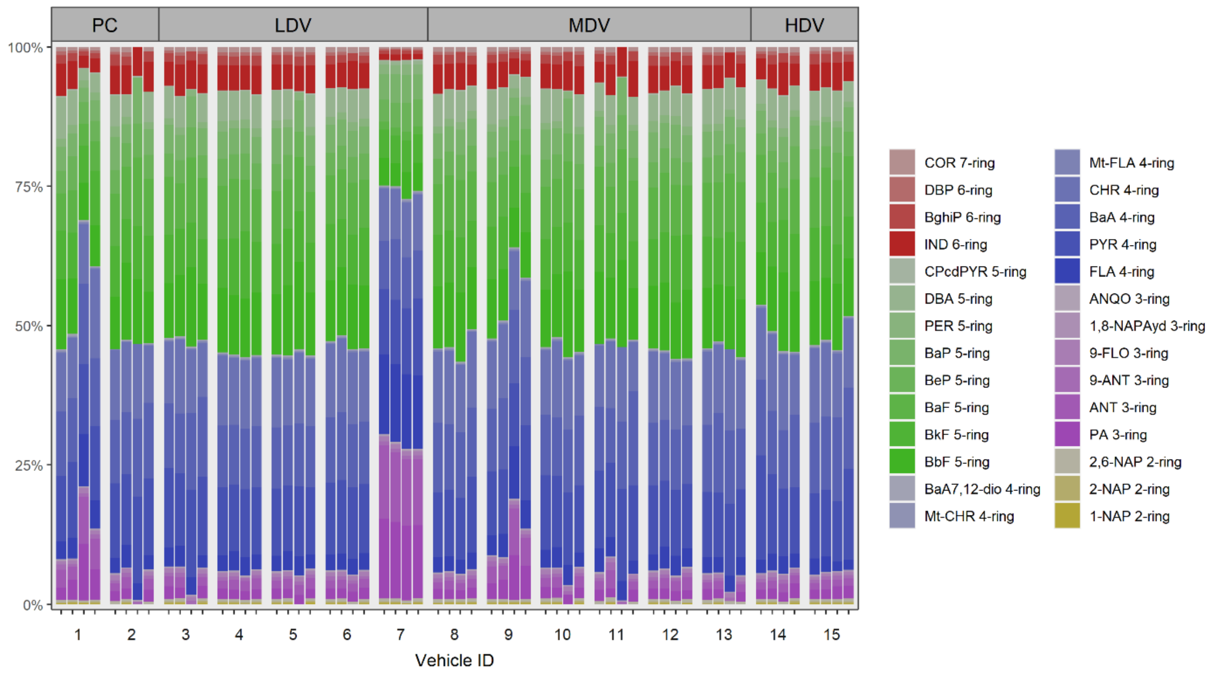

Fig. 3. Mass percentages of PAHs of each vehicle. The four bars under the same Vehicle ID correspond to cold start, hot start, idling and steady state cycles from left to right.

Comparison with other studies

In this section, PAH data obtained in the current study were used to compared with the results of similar studies conducted by other research groups. Rather than the fuel-based *EF* used in the previous parts, distance-based EFs (EF_d) were used in this session since most of the results in the previous studies were presented in a distance-based manner. EF_d for each PAH was calculated by dividing the total amount of PAHs released in a driving cycle by the distance travelled in the driving cycle. The driving distances of NEDC, FIGE and steady state cycles were 11.0, 29.5 and

16.7 km, respectively. An averaged EF_d for each vehicle class (i.e. PC, LDV, MDV and HDV) was calculated. **Fig. 4** summarizes the averaged EF_d of each PAH from each vehicle class, in comparison to diesel vehicles PAHs reported by previous studies. These studies included a PEMS study in China (Cao et al., 2017), tunnel studies in China (Chen et al., 2013) and Hong Kong (Ho et al., 2009), and a chassis dynamometer study in the US (Hays et al., 2017). As shown in **Fig. 4**, the results of the current study fell in ranges of the previous studies. The highest PAH EF_d was from Cao et al. (2017), in which the vehicle fleet comprised of 18 diesel trucks with emission standards of China 3 and 4 (equivalent to Euro 3 and 4). In general, their results were an order of magnitude higher than the results of HDVs in the current study (except for CHR). As for ANT, the EF_d in Cao et al. (2017) was 4 orders of magnitude higher than that in this study. There were two main reasons. Firstly, the emission standard of the tested HDVs (Euro 4 and 5) in this study was higher than that of Cao's fleet. Secondly, Cao et al. (2017) measured real driving emissions using PEMS. It has well documented that the real-driving emissions (RDE) *EFs* were much higher than laboratory chassis dynamometer results (Huang et al., 2018, May et al., 2014b, Weiss et al., 2012), due to more vigorous driving conditions in real world compared to the driving cycles tested in chassis dynamometer studies.

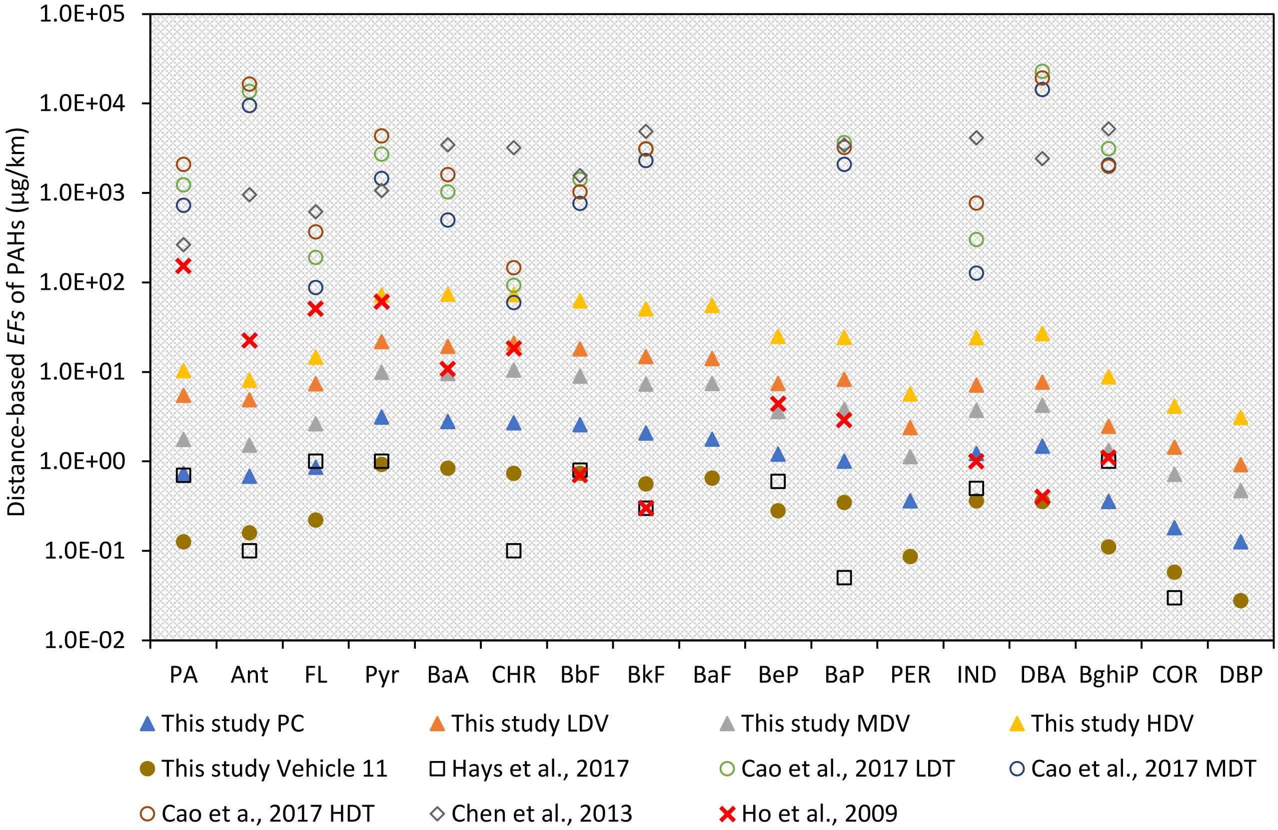

Fig. 4. Distance-based PAHs *EFs* of different vehicle classes.

Hays et al. (2017) conducted chassis dynamometer testing on DPF-equipped diesel trucks in the US. Their findings agreed well with the PAHs EF_d of Vehicle 11 in this study, as outlined by brown solid dots in **Fig. 4**. Vehicle 11 was a DPF-equipped MDV with comparable size as the vehicles tested by Hays et al. (2017). The agreement between studies confirmed that DPF was very efficient to remove PAHs in vehicle exhausts.

Chen et al. (2013) conducted a tunnel study in Nanjing, while Ho et al. (2009) conducted their tunnel study in Hong Kong. As shown in **Fig. 4**, the results of Chen et al. (2013) fell in the range of the results of Cao et al. (2017), while the results of Ho et al. (2009) generally fell within the range of the current study. It showed that EF_d of pollutants from diesel vehicles were strongly regional related. The possible reason was that diesel fuels varied among regions since previous studies pointed out that fuel type (i.e. low-sulfur vs ultra-low-sulfur diesel) had significant effect on the emission factors of PAHs (Cheung et al., 2010, Lim et al., 2005).

On the other hand, it is anticipated that the EF of PAHs in this study would be lower than that in Ho et al. (2009), because according to the Environmental Protection Department of Hong Kong, the emission of $PM_{2.5}$ nowadays reduced more than 50% compared to early 2000s (HKEPD 2019). This observation can be explained by the driving condition of vehicles in tunnel, where most of them travel in almost constant speed. This condition resembles the steady state cycle in this study. It has been shown in the previous discussion that transient cycles produce higher EFs of PAHs than steady state cycle. Referring to **Fig. 2**, the PAH *EFs* in hot start cycle are higher than EFs in steady state cycle for all vehicles, except vehicle 2, 11 and 13. This observation helps to explain the higher-than-expected PAH *EFs* in our results compared to Ho et al. (2009). Also, some high emitting vehicles (e.g. Vehicle 6 and 15) contributed a lot to the average PAH values calculated, and this will drag up the PAH *EFs* in this study.

Toxicological analysis

The levels of DCFH, IL-6 and GSH were analyzed to assess the cellular oxidative and inflammatory responses produced by the PM samples. It should be noticed that results of the current study cannot be used to compare with other studies, since the cell culture conditions will not be exactly the same. Also, as all the results were obtained by a fixed concentration of PM (i.e.

50 μg ml^{-1}), comparison between vehicles only accounted for the compositional difference of PM. Although the concentration specific toxicology results for DCFH and IL-6 can be normalized to the amount of PM emitted under different conditions to study the PM toxicity under these conditions, preliminary analysis showed that the normalized toxicology result strongly depend on the EF of PM. **Fig. S5** in Supporting Information illustrated that DCFH and IL-6 results normalized by the fuel-based PM *EF* showed the same pattern as PM *EF*. Therefore, the aforementioned result was not presented in the main context. Moreover, for Vehicle 2 under hot start condition, the amount of PM collected was under detection limit by gravimetric method. Therefore, there is no toxicological test results for Vehicle 2 under hot start condition.

The production of ROS is expressed by the fluorescent intensity of DCFH**,** as shown in the upper panel of **Fig. 5**. In general, the fluorescent intensity did not show significant variation among different driving cycles. Vehicle 10 was detected with the highest value of fluorescent intensity, with an average value of 1.62 ± 0.31. Apart from Vehicle 10, the cold start cycle of Vehicle 1, the hot start cycle of Vehicle 3, and the idling cycle of Vehicle 13 were also found with elevated levels of fluorescent intensity in response to the emitted PM. However, other than the above-mentioned specific vehicles and cycles, the variation of ROS among the remaining vehicles was not significant.

The lowest panel in **Fig. 5** shows the GSH levels after the A549 cells exposed to the PM samples. GSH is an antioxidant of which the concentration will decrease in response to oxidative stress. In general, GSH levels were depleted compared to the blank filter sample outlined in Fig.S3 for all PM samples. Compared with the results of DCFH level, a certain degree of variation among the tested vehicles was observed. LDVs (Vehicle 3,4,5,6 and 7) generally had lower GSH concentrations (stronger oxidative stresses) as compared to other classes of vehicles. Other parameters did not have great influence on the GSH level. The average GSH levels for vehicles with DPF and without DPF were 9.89 ± 3.97 μM and 9.85 ± 3.37 μM, respectively. This result showed that DPF removes PM mass without changing the GSH response of the PM, and this probably shows that DPF does not significantly change the morphology and composition of PM sample.

IL-6 is a pro-inflammatory cytokine released in response to PM exposure. The IL-6 results were illustrated in the middle panel of **Fig. 5**. It can be seen that the idling cycle of Vehicle 14 had the highest IL-6 level of 80.3 while hot start cycle of Vehicle 7 had the lowest IL-6 level of 8.30. In general, no clear trend can be observed for the effect of driving cycles on IL-6 levels. HDVs had higher IL-6 concentrations compared with other vehicle classes, followed by MDVs. On the other hand, the IL-6 levels of PC, LDV and MDV did not show great variation with each other.

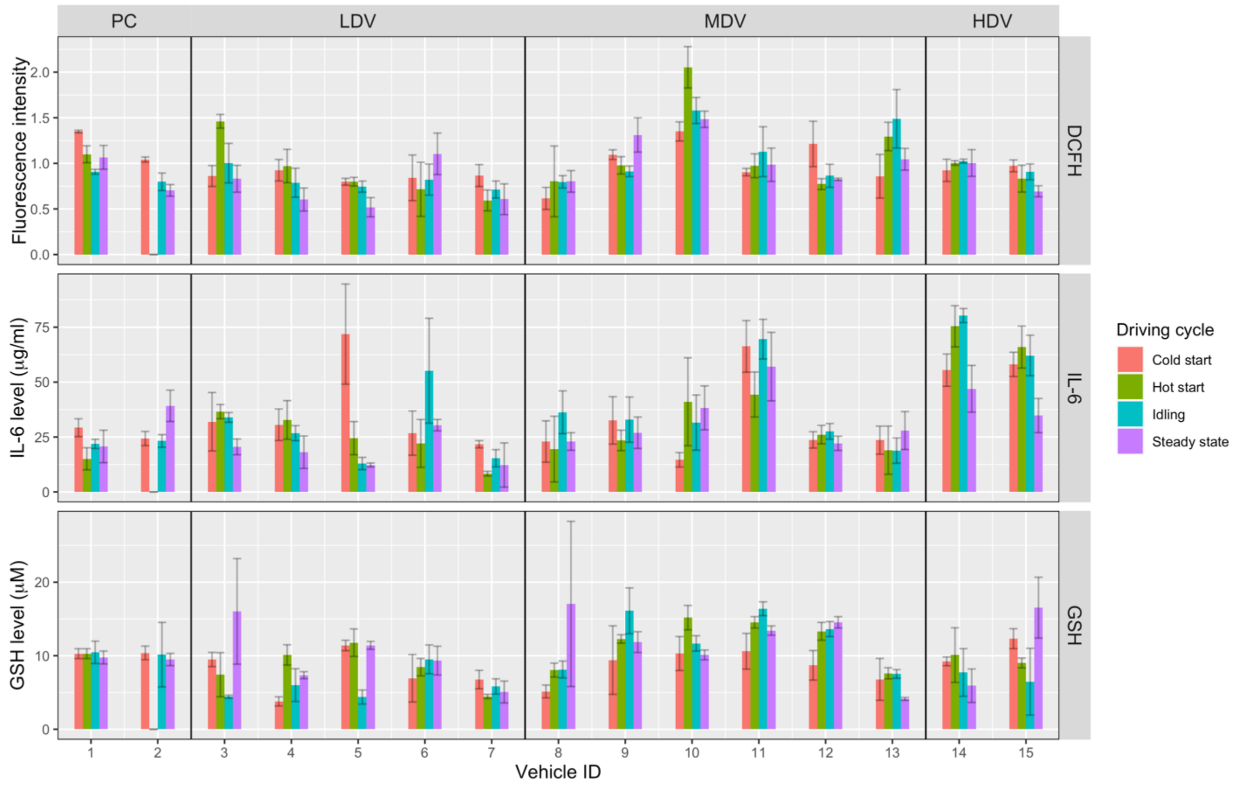

Fig. 5. Results of the production of ROS expressed as the fluorescent intensity of DCF, IL-6 level and GSH level of the 15 vehicles in different testing cycles.

Effect of vehicle type and driving cycle

To better illustrate the effect of vehicle type on the three toxicological markers, levels of each toxicological marker for all vehicles were pooled together and grouped according to their corresponding vehicle type (i.e. PC, LDV, MDV, HDV), and the results were presented by the boxplots in **Fig. 6**. As shown in **Fig. 6**, a certain degree of variation existed between vehicle types for all three toxicological markers. For DCFH and GSH, LDV produced lower responses compared to the other three types of vehicle. In the case of IL-6, HDV produced the highest IL-6 level among the four vehicles types. The results were further verified by conducting a Kruskal-Wallis H test for each toxicological marker at a significance level of $p = 0.05$. The Kruskal-Wallis H test results presented in the bottom left of each boxplot indicated that statistically significant difference exist among the four vehicle types for all three toxicological markers. Pairwise Wilcoxon rank sum test was carried out as the post-hoc test of Kruskal-Wallis test to find out which vehicle type pair of has significant difference at a significance level of 0.05. The vehicle type pairs with adjusted p-values < 0.05 were marked with an asterisk in **Fig. 6**. In the case of DCFH and GSH, significant difference was found between LDV and MDV while for IL-6, significant differences were identified between HDV and the other three types of vehicles. These results suggested that the response of the three toxicological markers would be affected by vehicle type. Moreover, it should be noticed that a decrease in GSH level indicates an increase of oxidative stress, which means that GSH level should show an opposite trend when compared to DCFH level. However, result of the aforementioned statistical analysis showed that increase of vehicle weight (from LDV to MDV) increased both GSH and DCFH levels. To find out the mechanisms or reasons behind this observation, further investigations are needed.

Unlike vehicle type, driving condition of the vehicle does not has significant effect on the three toxicological markers. Statistical analyses (**Fig. S3**) showed that there was no statistically significant difference between the four driving cycles ($p > 0.05$) for all toxicological markers. This suggested that the effect of driving condition on the production of ROS and the pro-inflammatory cytokine IL-6 was minimal. This observation was consistent with our PAH results, that the composition of PAHs did not show great variation among different driving cycles.

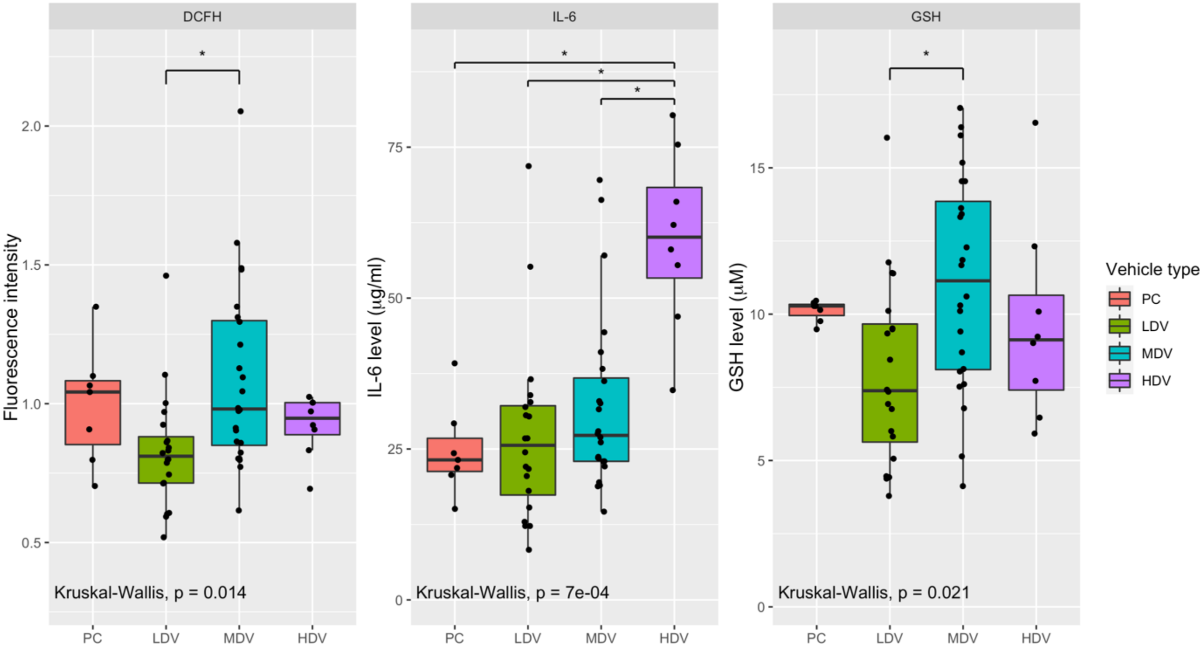

Fig. 6. Boxplots of the levels of DCFH, IL-6 and GSH grouped by vehicle type. Black dots represent the data points in the corresponding driving cycle. Asterisk represents the adjusted p-value in pairwise Wilcoxon rank sum test smaller than 0.05.

Correlation between toxicity data and PAHs concentration

It was reported that the chemical composition of the PM sample was related to the oxidative potential and the release of oxidative stress mediators (Chuang et al., 2012, Ho et al., 2016). In particular, certain PAHs were found to have significant correlations with the vasoactive function and pro-inflammatory cytokines (Niu et al., 2017).

Table 1. Results of spearman's correlation between PAHs and toxicological markers with p-value smaller than 0.1. Asterisk indicates p-value <0.05.

		DCFH *Spearman's ρ (p-value)*	**IL-6** *Spearman's ρ (p-value)*	**GSH** *Spearman's ρ (p-value)*
Hot start	IND	0.468 (<0.1)	-	-
	DBA	0.532 (<0.05) *	-	-
	Mt-CHR	0.457 (<0.1)	-	-
	COR	-	0.471 (<0.1)	-
Idling	Mt-FLA	-	0.503 (<0.1)	-
	2-NAP	-	-	-0.900 (0.037) *
Steady state	PA	-	-	-0.539 (<0.05) *
	ANT	-	-	-0.514 (<0.05) *
	FLA	-	-	-0.479 (<0.1) *
	BaA	-	0.538 (<0.05) *	-
	PYR	-	0.479 (<0.1)	-
	BkF	-	0.52 (<0.05) *	-
	BaF	-	0.454 (<0.1)	-
	BeP	-	0.584 (<0.05) *	-
	2,6-NAP	-	0.493 (<0.1)	-
	Mt-FLA	-	0.483 (<0.1)	-
	BaA7,12-dio	-	0.441 (<0.1)	-

Correlation analysis between PAHs and toxicological markers was carried out according to the method stated in the methodology part. The correlation test results with p-value <0.1 were summarized in **Table 1**. A total of 17 PAH-toxicological marker pairs were found to have moderate or good correlations. In general, as shown in **Table 1**, almost all these pairs were found in hot-start and steady state tests, except two in idling cycle. There is no correlation found between PAH and toxicology data under cold-start condition. In other words, under hot engine conditions (hot-start and steady state), some PAHs serve as good indicators of ROS production or pro-inflammatory response. However, in conditions with lower engine temperature compared to hot-start and steady state (i.e. cold-start and idling), the concentration of PAH measured in the current study seems not to be the main driving force for the observed toxicology responses.

As shown in **Table 1**, correlation between DCFH levels and PAHs were only observed under hot-start condition. Moderate correlations were found between DCFH and Mt-CHR ($\rho = 0.46$, $p < 0.1$), as well as IND ($\rho = 0.47$, $p < 0.1$). A relatively good correlation was found between DCFH and DBA ($\rho = 0.53$, $p < 0.05$). This result agreed with Wu et al. (2017), who found a strong correlation between ROS generation and DBA in petrol and diesel fuel combustion experiments. Correlation coefficients between GSH and PAHs were expected to be negative since the decrease in GSH shows the increase of oxidative stress posed by the corresponding PAH. Correlations of GSH with PAHs were found in idling and steady state cycle. PA ($\rho = -0.539$, $p < 0.05$) and ANT ($\rho = -0.514$, $p < 0.05$) showed good correlation with GSH and FLA ($\rho = -0.479$, $p < 0.1$) showed moderate correlation with GSH in steady state cycle. In idling cycle, GSH showed good correlation with 2-NAP ($\rho = -0.900$, $p < 0.05$).

Table 1 also showed the correlation between the pro-inflammatory mediator IL-6 and selected PAHs. Moderated correlation between IL-6 and COR ($\rho = 0.47$, $p < 0.1$) was found in hot-start cycle. Correlation between IL-6 and Mt-FLA ($\rho = 0.50$, $p < 0.1$) is the only data set that gives considerable correlation in idling cycle. Other than these two combinations, all observed correlation between IL-6 and PAHs come from steady state cycle. 2,6-NAP ($\rho = 0.49$, $p < 0.1$) is the only PAH with less than 3 rings that showed correlation with toxicology results in our study. Moderate correlations were found between IL-6 and Ba7,12-dio ($\rho = 0.44$, $p < 0.1$), Mt-FLA $\rho = 0.48$, $p < 0.1$), PYR ($\rho = 0.48$, $p < 0.1$), and BaF ($\rho = 0.45$, $p < 0.1$), while significant correlations were found between IL-6 and BaA ($\rho = 0.54$, $p < 0.05$), BkF ($\rho = 0.52$, $p < 0.05$) and BeP ($\rho = 0.58$, $p < 0.05$).

Several studies have assessed the correlation between IL-6 and particle bounded PAHs from different sources, but they did not come to the same conclusion. Niu et al. (2017) and Chowdhury et al. (2019) investigated the correlation between PAHs in atmospheric PM sample and IL-6 response in A549 cell and BEAS-2B cell (human bronchial epithelial cell) respectively. They found that certain PAHs were positively correlation with IL-6 level. Lin et al. (2013) studied the effect of household particles on inflammation in human coronary artery endothelial cells (HCAECs) and found that PAHs have significant correlation with IL-6 level. Delfino et al. (2010) analysed blood samples from 60 people and the air sample in vicinity to them. Their results also suggested positive correlation between PAHs in air samples and IL-6 in human blood samples. Our findings agree with the above studies, which support a positive correlation between PAHs and IL-6 level. However, a study conducted by Skuland et al. (2017) suggested that there is no clear connection between total or individual PAH levels in diesel exhaust particles with IL-6 in BEAS-2B cell, and Chuang et al. (2012) also cannot find any significant correlation between PAHs in air samples with IL-6 level in HCAECs. Moreover, Wang et al. (2016) found a significant negative correlation between PAHs in atmospheric PM samples and IL-6 level in BEAS-2B cells. These studies suggested that in some cases PAHs might not be the main inducer of pro-inflammatory response and further investigations are needed to elucidate the reaction mechanism of the release of IL-6 and other pro-inflammatory mediators.

Conclusion

The current study presents a detailed chemical analysis of PM samples collected from diesel vehicles with various physical properties. It is also the first study on the toxicity of PM samples collected by chassis dynamometer testing in Hong Kong. The results showed that PM emission from the tested diesel vehicles were dominated by OC. DPF-equipped diesel vehicles were found with very high OC/EC ratios, suggesting that DPF could effectively remove the EC, but not OC, fraction of PM. The EC removing efficiency of DPF is high even for vehicles with high odometer readings.

Among the identified PAHs, 4- and 5-ring PAHs were the most abundant species. Highest PAH *EFs* were found in HDVs, followed by LDV and MDV. Driving cycle has significant effects on the *EFs* of PAHs. Steady state cycle generally has the lowest PAH *EFs* while transient and idling cycle produce substantially higher PAH *EFs*. Although difference in PAH *EFs* were observed under different driving conditions, percentage by mass of individual PAH (i.e. PAH composition of the PM sample) did not show significant variation among different driving conditions.

The cellular exposure experiments revealed that the diesel vehicle PM emissions exerted potential oxidative stresses emerged from the ROS to human lung cell activities. Statistical analysis results showed that MDV produced significantly higher levels of DCFH and GSH than LDV, while HDV produced significantly larger amount of IL-6 than other types of vehicles. Correlation analysis between PAHs and the three toxicology markers were also carried out. Results showed that statistically significant correlation exist between certain PAH-toxicological marker pairs, including DCFH and DBA ($\rho = 0.53$, $p < 0.05$), GSH and PA ($\rho = -0.539$, $p < 0.05$), and ANT ($\rho = -0.514$, $p < 0.05$), as well as IL-6 and BaA ($\rho = 0.54$, $p < 0.05$), BkF ($\rho = 0.52$, $p < 0.05$), and BeP ($\rho = 0.58$, $p < 0.05$). Our results suggested that new emission control technologies and policies should focus on OC and PAHs reduction in order to reduce the adverse health effects on human respiratory system.

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References

ABDEL-SHAFY, H. I. & MANSOUR, M. S. M. 2016. A review on polycyclic aromatic hydrocarbons: Source, environmental impact, effect on human health and remediation. *Egyptian Journal of Petroleum,* 25, 107-123.

BENGALLI, R., LONGHIN, E., MARCHETTI, S., PROVERBIO, M. C., BATTAGLIA, C. & CAMATINI, M. 2017. The role of IL-6 released from pulmonary epithelial cells in diesel UFP-induced endothelial activation. *Environmental Pollution,* 231, 1314-1321.

CAO, X., HAO, X., SHEN, X., JIANG, X., WU, B. & YAO, Z. 2017. Emission characteristics of polycyclic aromatic hydrocarbons and nitro-polycyclic aromatic hydrocarbons from diesel trucks based on on-road measurements. *Atmospheric Environment,* 148, 190-196.

CECINATO, A., GUERRIERO, E., BALDUCCI, C. & MUTO, V. 2014. Use of the PAH fingerprints for identifying pollution sources. *Urban Climate,* 10, 630-643.

CHEN, F., HU, W. & ZHONG, Q. 2013. Emissions of particle-phase polycyclic aromatic hydrocarbons (PAHs) in the Fu Gui-shan Tunnel of Nanjing, China. *Atmospheric Research,* 124, 53-60.

CHENG, Y., LEE, S. C., HO, K. F., CHOW, J. C., WATSON, J. G., LOUIE, P. K. K., CAO, J. J. & HAI, X. 2010. Chemically-speciated on-road PM2.5 motor vehicle emission factors in Hong Kong. *Science of The Total Environment,* 408, 1621-1627.

CHENG, Y., ZOU, S. C., LEE, S. C., CHOW, J. C., HO, K. F., WATSON, J. G., HAN, Y. M., ZHANG, R. J., ZHANG, F., YAU, P. S., HUANG, Y., BAI, Y. & WU, W. J. 2011. Characteristics and source apportionment of PM1 emissions at a roadside station. *Journal of Hazardous Materials*, 195, 82-91.

CHEUNG, K. L., NTZIACHRISTOS, L., TZAMKIOZIS, T., SCHAUER, J. J., SAMARAS, Z., MOORE, K. F. & SIOUTAS, C. 2010. Emissions of Particulate Trace Elements, Metals and Organic Species from Gasoline, Diesel, and Biodiesel Passenger Vehicles and Their Relation to Oxidative Potential. *Aerosol Science and Technology,* 44, 500-513.

CHIANG, H.-L., LAI, Y.-M. & CHANG, S.-Y. 2012. Pollutant constituents of exhaust emitted from light-duty diesel vehicles. *Atmospheric Environment,* 47, 399-406.

CHOW, J. C., WATSON, J. G., CHEN, L. W. A., CHANG, M. C. O., ROBINSON, N. F., TRIMBLE, D. & KOHL, S. 2012. The IMPROVE_A temperature protocol for Thermal/Optical carbon analysis: maintaining consistency with a long-term database. *Journal of the Air & Waste Management Association,* 57, 1014-1023.

CHOWDHURY, P. H., HONDA, A., ITO, S., OKANO, H., ONISHI, T., HIGASHIHARA, M., OKUDA, T., TANAKA, T., HIRAI, S. & TAKANO, H. 2019. Effects of Ambient PM2.5 Collected Using Cyclonic Separator from Asian

Cities on Human Airway Epithelial Cells. *Aerosol and Air Quality Research,* 19**,** 1808-1819.

CHUANG, H. C., FAN, C. W., CHEN, K. Y., CHANG-CHIEN, G. P. & CHAN, C. C. 2012. Vasoactive alteration and inflammation induced by polycyclic aromatic hydrocarbons and trace metals of vehicle exhaust particles. *Toxicol Lett,* 214**,** 131-6.

CLAXTON, L. D. 2015. The history, genotoxicity, and carcinogenicity of carbon-based fuels and their emissions. Part 3: Diesel and gasoline. *Mutation Research/Reviews in Mutation Research,* 763**,** 30-85.

DELFINO, R. J., STAIMER, N., TJOA, T., ARHAMI, M., POLIDORI, A., GILLEN, D. L., KLEINMAN, M. T., SCHAUER, J. J. & SIOUTAS, C. 2010. Association of biomarkers of systemic inflammation with organic components and source tracers in quasi-ultrafine particles. *Environ Health Perspect,* 118**,** 756-62.

DIMASHKI, M., LIM, L. H., HARRISON, R. M. & HARRAD, S. 2001. Temporal trends, temperature dependence, and relative reactivity of atmospheric polycyclic aromatic hydrocarbons. *Environmental Science & Technology,* 35**,** 2264-2267.

GALI, N. K., YANG, F., CHEUNG, C. S. & NING, Z. 2017. A comparative analysis of chemical components and cell toxicity properties of solid and semi-volatile PM from diesel and biodiesel blend. *Journal of Aerosol Science,* 111**,** 51-64.

GELLER, M. D., NTZIACHRISTOS, L., MAMAKOS, A., SAMARAS, Z., SCHMITZ, D. A., FROINES, J. R. & SIOUTAS, C. 2006. Physicochemical and redox characteristics of particulate matter (PM) emitted from gasoline and diesel passenger cars. *Atmospheric Environment,* 40**,** 6988-7004.

GERLOFS-NIJLAND, M. E., TOTLANDSDAL, A. I., TZAMKIOZIS, T., LESEMAN, D. L. A. C., SAMARAS, Z., LÅG, M., SCHWARZE, P., NTZIACHRISTOS, L. & CASSEE, F. R. 2013. Cell Toxicity and Oxidative Potential of Engine Exhaust Particles: Impact of Using Particulate Filter or Biodiesel Fuel Blend. *Environmental Science & Technology,* 47**,** 5931-5938.

GHIO, A. J., CARRAWAY, M. S. & MADDEN, M. C. 2012. Composition of air pollution particles and oxidative stress in cells, tissues, and living systems. *Journal of Toxicology and Environmental Health, Part B,* 15**,** 1-21.

GRIESHOP, A. P., LIPSKY, E. M., PEKNEY, N. J., TAKAHAMA, S. & ROBINSON, A. L. 2006. Fine particle emission factors from vehicles in a highway tunnel: Effects of fleet composition and season. *Atmospheric Environment,* 40**,** 287-298.

HAYS, M. D., PRESTON, W., GEORGE, B. J., GEORGE, I. J., SNOW, R., FAIRCLOTH, J., LONG, T., BALDAUF, R. W. & MCDONALD, J. 2017. Temperature and Driving Cycle Significantly Affect Carbonaceous Gas and Particle Matter Emissions from Diesel Trucks. *Energy Fuels,* 31.

HO, K.-F., HO, S. S. H., HUANG, R.-J., CHUANG, H.-C., CAO, J.-J., HAN, Y., LUI, K.-H., NING, Z., CHUANG, K.-J., CHENG, T.-J., LEE, S.-C., HU, D., WANG, B. & ZHANG, R. 2016. Chemical composition and bioreactivity of PM2.5 during 2013 haze events in China. *Atmospheric Environment,* 126**,** 162-170.

HO, K. F., HO, S. S. H., LEE, S. C., CHENG, Y., CHOW, J. C., WATSON, J. G., LOUIE, P. K. K. & TIAN, L. 2009. Emissions of gas- and particle-phase polycyclic aromatic hydrocarbons (PAHs) in the Shing Mun Tunnel, Hong Kong. *Atmospheric Environment,* 43**,** 6343-6351.

HO, K. F. & LEE, S. C. 2002. Identification of atmospheric volatile organic compounds (VOCs), polycyclic aromatic hydrocarbons (PAHs) and carbonyl compounds in Hong Kong. *Science of The Total Environment,* 289**,** 145-158.

HO, K. F., LEE, S. C., YU, J. C., ZOU, S. C. & FUNG, K. 2002. Carbonaceous characteristics of atmospheric particulate matter in Hong Kong. *Science of The Total Environment,* 300**,** 59-67.

HO, S. S. H., YU, J. Z., CHOW, J. C., ZIELINSKA, B., WATSON, J. G., SIT, E. H. L. & SCHAUER, J. J. 2008. Evaluation of an in-injection port thermal desorption-gas chromatography/mass spectrometry method for analysis of non-polar organic compounds in ambient aerosol samples. *Journal of Chromatography A,* 1200**,** 217-227.

HONG KONG TRANSPORT DEPARTMENT 2018. THE ANNUAL TRAFFIC CENSUS 2017.

HOU, B., ZHUANG, G., ZHANG, R., LIU, T., GUO, Z. & CHEN, Y. 2011. The implication of carbonaceous aerosol to the formation of haze: revealed from the characteristics and sources of OC/EC over a mega-city in China. *J Hazard Mater,* 190**,** 529-36.

HU, S., D HERNER, J., ROBERTSON, W., KOBAYASHI, R., CHANG, O., HUANG, S.-M., ZIELINSKA, B., KADO, N., F COLLINS, J., RIEGER, P., HUAI, T. & AYALA, A. 2013. Emissions of polycyclic aromatic hydrocarbons (PAHs) and nitro-PAHs from heavy-duty diesel vehicles with DPF and SCR. *Journal of the Air & Waste Management Association (1995),* 63**,** 984-96.

HUANG, Y., ORGAN, B., ZHOU, J., SURAWSKI, N., HONG, G., CHAN, E. & YAM, Y. S. 2018. Emission measurement of diesel vehicles in Hong Kong through on-road remote sensing: Performance review and identification of high-emitters. *Environmental Pollution,* 237**,** 133-142.

KIM, K. H., JAHAN, S. A., KABIR, E. & BROWN, R. J. 2013. A review of airborne polycyclic aromatic hydrocarbons (PAHs) and their human health effects. *Environ Int,* 60**,** 71-80.

KLEEMAN, M. J., SCHAUER, J. J. & CASS, G. R. 2000. Size and composition distribution of fine particulate matter emitted from motor vehicles. *Environmental Science & Technology,* 34**,** 1132-1142.

LEE, S., CHENG, Y., HO, K. F., CAO, J., LOUIE, P. K. K., CHOW, J. & WATSON, J. 2006. PM 1.0 and PM 2.5 Characteristics in the Roadside Environment of Hong Kong. *Aerosol Science and Technology,* 40**,** 157-165.

LI, N., SIOUTAS, C., CHO, A., SCHMITZ, D., MISRA, C., SEMPF, J., WANG, M., OBERLEY, T., FROINES, J. & NEL, A. 2003. Ultrafine Particulate Pollutants Induce Oxidative Stress and Mitochondrial Damage. *Environmental Health Perspectives,* 111**,** 455-460.

LIM, M. C. H., AYOKO, G. A., MORAWSKA, L., RISTOVSKI, Z. D. & ROHAN JAYARATNE, E. 2005. Effect of fuel composition and engine operating conditions on polycyclic aromatic hydrocarbon emissions from a fleet of heavy-duty diesel buses. *Atmospheric Environment,* 39**,** 7836-7848.

LIN, L.-Y., LIU, I. J., CHUANG, H.-C., LIN, H.-Y. & CHUANG, K.-J. 2013. Size and composition effects of household particles on inflammation and endothelial dysfunction of human coronary artery endothelial cells. *Atmospheric Environment,* 77**,** 490-495.

LIN, Y. C., LI, Y. C., AMESHO, K. T. T., CHOU, F. C. & CHENG, P. C. 2019. Characterization and quantification of PM2.5 emissions and PAHs concentration in PM2.5 from the exhausts of diesel vehicles with various accumulated mileages. *Sci Total Environ,* 660**,** 188-198.

MA, Y., CHENG, Y., QIU, X., LIN, Y., CAO, J. & HU, D. 2016. A quantitative assessment of source contributions to fine particulate matter (PM2.5)-bound polycyclic aromatic hydrocarbons (PAHs) and their nitrated and hydroxylated derivatives in Hong Kong. *Environ Pollut,* 219**,** 742-749.

MAY, A. A., NGUYEN, N. T., PRESTO, A. A., GORDON, T. D., LIPSKY, E. M., KARVE, M., GUTIERREZ, A., ROBERTSON, W. H., ZHANG, M., BRANDOW, C., CHANG, O., CHEN, S., CICERO-FERNANDEZ, P., DINKINS, L., FUENTES, M., HUANG, S.-M., LING, R., LONG, J., MADDOX, C., MASSETTI, J., MCCAULEY, E., MIGUEL, A., NA, K., ONG, R., PANG, Y., RIEGER, P., SAX, T., TRUONG, T., VO, T., CHATTOPADHYAY, S., MALDONADO, H., MARICQ, M. M. & ROBINSON, A. L. 2014a. Gas- and particle-phase primary emissions from in-use, on-road gasoline and diesel vehicles. *Atmospheric Environment,* 88**,** 247-260.

MAY, J., BOSTEELS, D. & FAVRE, C. 2014b. An Assessment of Emissions from Light-Duty Vehicles using PEMS and Chassis Dynamometer Testing. SAE International.

MAZZARELLA, G., FERRARACCIO, F., PRATI, M. V., ANNUNZIATA, S., BIANCO, A., MEZZOGIORNO, A., LIGUORI, G., ANGELILLO, I. F. & CAZZOLA, M. 2007. Effects of diesel exhaust particles on human lung epithelial cells: An in vitro study. *Respiratory Medicine,* 101**,** 1155-1162.

NEL, A. 2005. Air pollution-related illness: effects of particles. *Science,* 308**,** 804-806.

NIU, X., HO, S. S. H., HO, K. F., HUANG, Y., SUN, J., WANG, Q., ZHOU, Y., ZHAO, Z. & CAO, J. 2017. Atmospheric levels and cytotoxicity of polycyclic aromatic hydrocarbons and oxygenated-PAHs in PM2.5 in the Beijing-Tianjin-Hebei region. *Environ Pollut,* 231**,** 1075-1084.

NTZIACHRISTOS, L., FROINES, J. R., CHO, A. K. & SIOUTAS, C. 2007. Relationship between redox activity and chemical speciation of size-fractionated particulate matter. *Particle and Fibre Toxicology,* 4**,** 5.

SHAH, S. D., COCKER, D. R., MILLER, J. W. & NORBECK, J. M. 2004. Emission Rates of Particulate Matter and Elemental and Organic Carbon from In-Use Diesel Engines. *Environmental Science & Technology,* 38**,** 2544-2550.

SHAH, S. D., OGUNYOKU, T. A., MILLER, J. W. & COCKER, D. R. 2005. On-Road Emission Rates of PAH and n -Alkane Compounds from Heavy-Duty Diesel Vehicles. *Environmental science & technology,* 39**,** 5276-84.

SHEN, H., TAO, S., WANG, R., WANG, B., SHEN, G., LI, W., SU, S., HUANG, Y., WANG, X., LIU, W., LI, B. & SUN, K. 2011. Global time trends in PAH emissions from motor vehicles. *Atmospheric Environment,* 45**,** 2067-2073.

SHEN, X., YAO, Z., HUO, H., HE, K., ZHANG, Y., LIU, H. & YE, Y. 2014. PM2.5 emissions from light-duty gasoline vehicles in Beijing, China. *Science of The Total Environment,* 487**,** 521-527.

SKULAND, T. S., REFSNES, M., MAGNUSSON, P., OCZKOWSKI, M., GROMADZKA-OSTROWSKA, J., KRUSZEWSKI, M., MRUK, R., MYHRE, O., LANKOFF, A. & OVREVIK, J. 2017. Proinflammatory effects of diesel exhaust particles from moderate blend concentrations of 1st and 2nd generation biodiesel in BEAS-2B bronchial epithelial cells-The FuelHealth project. *Environ Toxicol Pharmacol,* 52**,** 138-142.

TSAI, Y. I., YANG, H.-H., WANG, L.-C., HUAN, J.-L., YOUNG, L.-H., CHENG, M.-T. & CHIANG, P.-C. 2011. The Influences of Diesel Particulate Filter Installation on Air Pollutant Emissions for Used Vehicles. *Aerosol and Air Quality Research,* 11**,** 578-583.

UNECE 2011. Consolidated Resolution on the Construction of Vehicle (R.E.3), revision. United Nations Economic Commission for Europe, Geneva, Switzerland.

WANG, B., LAU, Y.-S., HUANG, Y., ORGAN, B., LEE, S.-C. & HO, K.-F. 2019. Investigation of factors affecting the gaseous and particulate matter emissions from diesel vehicles. *Air Quality, Atmosphere & Health,* 12**,** 1113-1126.

WANG, B., LI, N., DENG, F., BUGLAK, N., PARK, G., SU, S., REN, A., SHEN, G., TAO, S. & GUO, X. 2016. Human bronchial epithelial cell injuries induced by fine particulate matter from sandstorm and non-sandstorm periods: Association with particle constituents. *J Environ Sci (China),* 47**,** 201-210.

WEISS, M., BONNEL, P., KÜHLWEIN, J., PROVENZA, A., LAMBRECHT, U., ALESSANDRINI, S., CARRIERO, M., COLOMBO, R., FORNI, F., LANAPPE, G., LE LIJOUR, P., MANFREDI, U., MONTIGNY, F. & SCULATI, M. 2012. Will Euro 6 reduce the NOx emissions of new diesel cars? – Insights from on-road tests with Portable Emissions Measurement Systems (PEMS). *Atmospheric Environment,* 62**,** 657-665.

WU, B., SHEN, X., CAO, X., YAO, Z. & WU, Y. 2016. Characterization of the chemical composition of PM2.5 emitted from on-road China III and China IV diesel trucks in Beijing, China. *Science of The Total Environment,* 551-552**,** 579-589.

WU, D., ZHANG, F., LOU, W., LI, D. & CHEN, J. 2017. Chemical characterization and toxicity assessment of fine particulate matters emitted from the combustion of petrol and diesel fuels. *Sci Total Environ,* 605-606**,** 172-179.

YAMASAKI, H., KUWATA, K. & MIYAMOTO, H. 1982. Effects of ambient temperature on aspects of airborne polycyclic aromatic hydrocarbons. *Environmental Science & Technology,* 16**,** 189-194.

YAO, Z., HUO, H., ZHANG, Q., STREETS, D. G. & HE, K. 2011. Gaseous and particulate emissions from rural vehicles in China. *Atmospheric Environment,* 45**,** 3055-3061.

ZHENG, M., HAGLER, G. S. W., KE, L., BERGIN, M. H., WANG, F., LOUIE, P. K. K., SALMON, L., SIN, D. W. M., YU, J. Z. & SCHAUER, J. J. 2006. Composition and sources of carbonaceous aerosols at three contrasting sites in Hong Kong. 111.

ZIELINSKA, B., SAGEBIEL, J., MCDONALD, J. D., WHITNEY, K. & LAWSON, D. R. 2004. Emission rates and comparative chemical composition from selected in-use diesel and gasoline-fueled vehicles. *J Air Waste Manag Assoc,* 54**,** 1138-50.

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Chemical and Toxicological Characterization of Particulate Emissions from Diesel Vehicles

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ABSTRACT

This paper presents a detailed chemical and toxicological characterization of the diesel particulate matter (PM) emitted from diesel vehicles running on a chassis dynamometer under different driving conditions. Chemical analyses were performed to characterize the contents of organic carbon (OC), elemental carbon (EC), and 31 polycyclic aromatic hydrocarbons (PAHs) in the collected PM samples. The OC–EC analysis results revealed that PM emissions from diesel vehicles in this study were dominated by OC and that the emission of vehicles equipped with diesel particulate filters had high OC/EC ratios. The PAH analysis results revealed that 4- and 5-ring PAHs were the dominant PAHs in the OC fraction of the PM samples. Particle toxicity was evaluated through three toxicological markers in human A549 cells, namely (1) acellular 2,7-dichlorofluorescein (DCFH) for oxidative potential, (2) interleukin-6 (IL-6) for inflammation, and (3) glutathione (GSH) for antioxidation after exposure. Statistical analyses revealed that vehicle sizes have statistically significant effects on the concentrations of the markers. Correlation analysis between PAHs and toxicological markers revealed that significant correlations existed between specific compounds and markers. Our results can be used as a reference by policy makers to formulate emission control strategies and as a dataset for other modeling studies.

Keywords: PM emission; OCEC; PAH; DCFH; Interleukin-6 (IL-6); Glutathione (GSH); Driving Cycle; Chassis dynamometer

INTRODUCTION

Hong Kong is one of the most densely populated cities in the world, and the majority of the population is exposed to vehicular exhaust because many people work and live close to main roads and highways. By the end of 2017, more than 830,000 licensed motor vehicles were running on the roads, and the number of licensed motor vehicles increased by 35% from 2007 to 2017 (Hong Kong Transport Department, 2018). In particular, diesel vehicles are the main source of particulate matter (PM) pollution on the streets (Lee et al., 2006, Shen et al., 2014, Weiss et al., 2012, Yao et al., 2011).

A large quantity of PM contains carbonaceous aerosols (Hou et al., 2011), which are typically classified into two categories, namely elemental carbon (EC) and organic carbon (OC). Lee et al. (2006) reported that carbonaceous aerosols are the dominant species of fine particles in the roadside environment in Hong Kong and account for approximately 44% of the $PM_{2.5}$ concentration in Hong Kong. Vehicular emissions are the major source of carbonaceous aerosols in Hong Kong (Cheng et al., 2010, Cheng et al., 2011, Ho et al., 2002, Lee et al., 2006). Zheng et al. (2006) reported that more than 60% of the OC measured at a roadside station in Hong Kong originated from vehicular emissions. The OC in vehicle-emission-derived PM comprises various toxic organic compounds, including polycyclic aromatic hydrocarbons (PAHs). PAHs are important toxic components of vehicle emitted organic species. They are identified as one of the major toxic air pollutants, mainly originated from anthropogenic processes, especially from incomplete combustion of organic matters. PAHs are semi-volatile organic compounds that can be present in both particulate and gaseous phases. PAHs are present on the surface of airborne particles through condensation, adsorption or combustion processes (Yamasaki et al., 1982, Dimashki et al., 2001). Several studies have reported that vehicular emissions, particularly diesel vehicles, are the most important sources of PAHs in urban areas (Cecinato et al., 2014, Chen et al., 2013, Shen et al., 2011). Therefore, various studies have been done to characterize the *EFs* and chemical composition of PAHs emitted from diesel vehicles under different conditions (Zielinska et al., 2004, Tsai et al., 2011, Hu et al., 2013, Cao et al., 2017, Hays et al., 2017, Lin et al., 2019). In Hong Kong, the characterization of PAH derived from vehicle emission were mainly done by tunnel measurement and ambient air sample collection (Ho and Lee, 2002, Cheng et al., 2010, Ho et al., 2009, Ma et al., 2016). For example, Ho et al. (2009) conducted a tunnel study to investigate the PAHs emission from vehicles in Hong Kong. The authors concluded that two- and three-ring PAHs were the dominant fraction in gas phase, while four-ring PAHs were the most abundant in particle phase. Since studies of direct tailpipe emission (e.g. chassis dynamometer or PEMS study) from diesel vehicles in Hong Kong are scarce, carrying out such kind of research is needed to fill the knowledge gap.

Another important concern of PM is their adverse effects to human health (Abdel-Shafy and Mansour, 2016, Chuang et al., 2012, Kim et al., 2013). In previous toxicological studies, it is believed that reactive oxygen species (ROS) production by PM exposure is the main mechanisms for increased risk of adverse health effects (Li et al., 2003, Nel, 2005). For instance, studies by Geller et al. (2006) and Ntziachristos et al. (2007) revealed strong correlations between the redox activities of their PM sample and several PM species, including OC, EC and PAHs. In a healthy biological system, glutathione (GSH) is produced to mitigate the PM-derived ROS in cells (Ghio et al., 2012). However, the overloaded ROS is able to incur inflammatory responses such as interleukin 6 (IL-6) and interleukins 8 (IL-8) production, consequently leading to harmful health effects. Various studies have been done to investigate the inflammatory response triggered by the exposure of vehicle-derived PM (Mazzarella et al., 2007, Gerlofs-Nijland et al., 2013, Bengalli et al., 2017). Results from these studies showed that vehicle-derived PM emission is closely related to the release of pro-inflammatory cytokines, like IL-6, while the use of advanced emission control technologies, such as DPF, will significantly change the inflammatory responses in the studied cell.

Many PAHs are known or suspected carcinogens in human (IARC 2010; Song et al., 2012), while some PAHs are believed to trigger pro-inflammatory response (Lin et al., 2013; Niu et al., 2017). The relationship between PAHs and different toxicology markers have also been investigated by various studies. Cheung et al (2010) and Wu et al (2017) have investigated the relationship between ROS level and diesel fuel burning particles, while Vattanasit et al (2014) and Totlandsdal et al (2014) studied the ability of PAHs in diesel exhaust particles to induce pro-inflammatory response. All these studies showed correlation between PAHs and adverse health effects.

Moreover, gas phase PAHs can undergo oxidation reactions in the atmosphere, producing secondary organic aerosol (SOA), which is, in many cases, more harmful than the precursor (Lin et al., 2019). Therefore, characterizing the *EF* of PAH is important to air quality control as well as the health of the general public.

The objectives of the current study were to characterize the EFs of the OC, EC, and PAHs in diesel vehicle emissions, compare the differences in the chemical compositions and toxicological responses under different driving conditions, and investigate the correlation between the chemical composition and toxicological response of PM samples.

METHODOLOGY

Fleet overview and instrumentation set-up

Fifteen vehicles of various classes and with different engine sizes, after-treatment technologies, and emission standards (**Table S1**) were studied. The vehicle fleet was selected briefly in accordance to the emission standard distribution of diesel vehicles in Hong Kong, where most of them are with Euro 4 and 5, followed by Euro 3 and others. The vehicles were classified according to the United Nations Economic Commission for Europe (UNECE, 2011). Passenger cars (PCs) were defined as M-type vehicles. Light-duty vehicles (LDVs), medium-duty vehicles (MDVs), and heavy-duty vehicles (HDVs) were defined as N1-, N2-, and N3-type vehicles, respectively. Vehicle 15 was categorized as an HDV because it was a 10-ton tractor designed for carrying a trailer of up to 20 tons. All vehicles, except Vehicle 2 and 7, have taken and passed the annual vehicle examination required by the Transport Department of the Hong Kong Government. The annual vehicle examination is mandatory for all commercial vehicles, as well as passenger cars and light duty vehicles (vehicle weight under 1.9 tons) with first registration date over 6 years. The annual examination policy is to make sure that vehicles running on road are in acceptable maintenance condition. The diesel fuel used by all vehicles in the current study is the same, which comply with the Euro 5 diesel fuel standard, as stated in **Table S3.**

Chassis dynamometer tests were conducted in the Jockey Club Heavy Vehicle Emissions Testing and Research Centre (JCEC), Hong Kong. **Fig. S1** of the Supporting Information depicts the schematic of the test setup. All the testing facilities in the JCEC comply with the European standards for type approval tests. Two chassis dynamometers were used to test the vehicles with different weights. PCs and LDVs were tested on a Mustang dynamometer with a 48" (121.92 cm) single roller, whereas MDVs and HDVs were tested on a Mustang dynamometer with a 17.2" (43.688 cm) triple roller.

Driving cycles and testing conditions

Four driving cycles, namely the cold start transient, hot start transient, idling, and steady-state cycles, were used to test each vehicle. For convenience, the cold and hot start transient cycles are called cold start and hot start cycles in the following text. For each vehicle, the cold start cycle test was repeated two times and the hot start, idling, and steady-state cycle tests were repeated three times. Detailed descriptions of the four driving cycles and loading conditions are presented in the Supporting Information.

PM sample collection

PM samples were collected simultaneously on quartz (47 mm, Whatman, USA) and Teflon membrane filters (47 mm, Pall Corporation, USA) for different offline tests. The mass of samples collected on the filters were determined using a microbalance (MC5, Sartorius, Germany) with a readability of 0.001 mg. The filters were conditioned in a humidity-controlled chamber (i.e. relative humidity = 40%) for at least 24 h before weighing. Each filter was weighted at least twice before and after sample collection. The weighing result was accepted only if the difference between two consecutive weighings was less than 0.01% of the filter weight. Then, the filters were sealed in zip-zap bags and stored at −20 °C for chemical and toxicological analyses. Operational blanks and laboratory control blanks were processed simultaneously with the field samples during sample collection and analyses. All the filter data were corrected with the operation and laboratory blanks.

Chemical analysis

The samples collected on the quartz filters were used for OC/EC and PAH analyses. Each filter was cut exactly in half with a specially designed chopper for the two analyses. The contents of OC and EC were analyzed using a Desert Research Institute Model 2001 Thermal/Optical Carbon Analyzer with the IMPROVE-A protocol described by Chow et al. (2012). The PAH samples were analyzed using the thermal desorption-gas chromatography/mass spectrometry method (Ho et al., 2008). The chemical analyses procedures are described in the Supporting Information.

Toxicological analysis

PM samples collected on Teflon filters were removed for toxicological analysis. The three toxicological markers included (1) acellular DCFH for oxidative potential, (2) interleukin-6 (IL-6) for inflammation, and (3) glutathione (GSH) for anti-oxidation after exposure. Description of the toxicological analysis procedures can be found in Supporting Information.

Calculation of emission factor (*EF*) and statistical analysis

In the current study, the fuel-based EF of OC, EC, and individual PAHs were calculated to compare the emission characteristics between different vehicles. The relationship between toxicological markers and chemical species were examined by correlation studies. Details regarding the calculation of the EF and the statistical analysis performed in this study are presented in the Supporting Information.

RESULTS AND DISCUSSION

PM emission characteristics

The result of PM *EF* was published elsewhere (Wang et al., 2019). The *EF* of PM was calculated from the mass of PM sample collected on the Teflon filter. In general, the emission characteristic of PM mass is close to that of total carbon content (OC+EC), which will be discussed in the following section.

OC and EC

The EFs of OC and EC and the OC/EC ratios at different driving conditions are depicted in **Fig. 1**. OC was the dominant fraction in all the collected samples except those from Vehicles 6 and 8, in which EC dominated. Several studies have indicated that EC is dominant in PM emissions from diesel vehicles (Chiang et al., 2012, Grieshop et al., 2006, Kleeman et al., 2000), whereas other studies have reported contrasting results (Shah et al., 2004, Wu et al., 2016). Gali et al. (2017) indicated that under cold idle, or low-engine-speed conditions, OC is the dominant fraction in PM, which is consistent with our results.

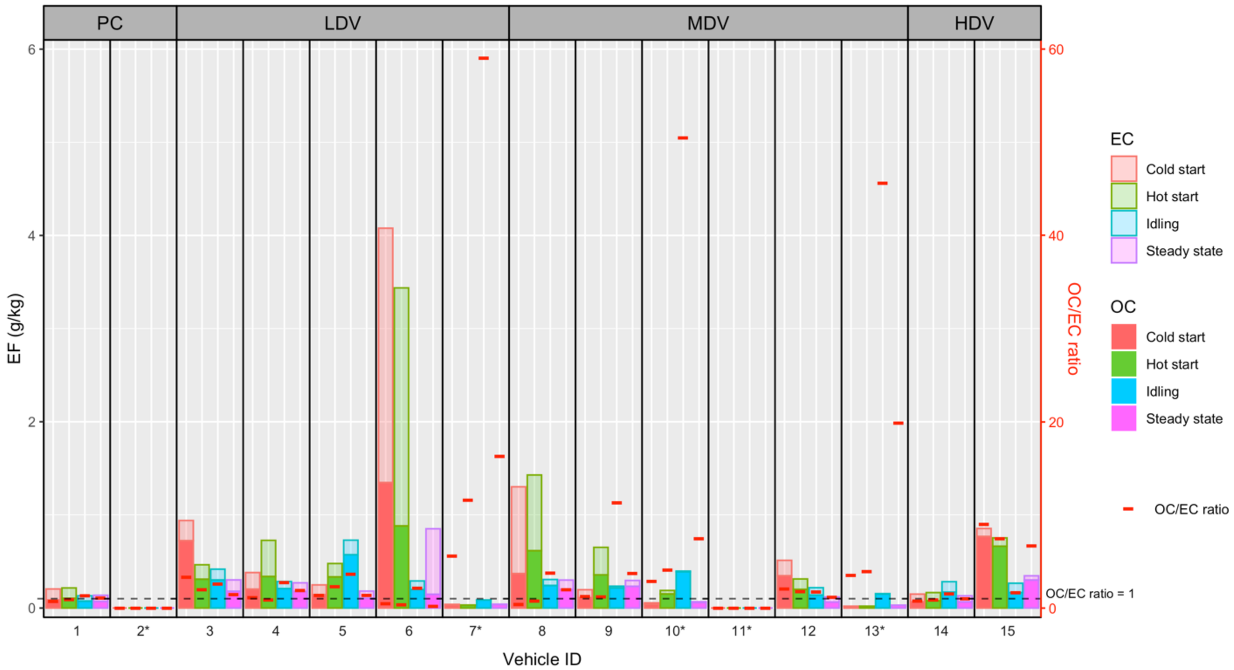

Fig. 1. *EFs* of OC and EC and OC/EC ratios. Each bar represents the sum of OC and EC *EF*. Light color bar represents EC *EF* and deep color bar represents OC *EF*. Vehicles with an asterisk next to their vehicle ID were equipped with DPF.

The variation in OC/EC ratio can be caused by different factors, including emission standard, testing weight, engine power and capacity and maintenance condition of the test vehicles. The EC content in emissions from vehicles equipped with diesel particulate filters (DPFs) was less than the detection limit (Vehicles 2 and 11) or extremely low (0.003 ± 0.002, 0.017 ± 0.014, and 0.003 ± 0.001 g/kg for Vehicles 1, 10, and 13, respectively). This observation is consistent with the findings of May et al. (2014a), who reported that DPFs can effectively decrease the EC emission from diesel vehicles. The results also revealed that the EC removal by DPFs was satisfactory even for vehicles with high odometer readings (e.g. Vehicles 10, 11, and 13). As depicted in **Fig. 1**, high OC/EC ratios were observed for Vehicles 7 (59.06), 10 (50.51), and 13 (45.63) under the idling condition. For these three vehicles, the EC concentration was very low while considerable amount of OC was measured. This observation is in agreement with the gaseous total hydrocarbon (THC) result of the current study as presented in Wang et al. (2019). For idling cycles of Vehicle 7, Vehicle 10 and Vehicle 13, substantial amount of THC was measured. Since THC reflects the gas phase OC content, and it is possible for some high molecular weight hydrocarbons to partition to the particle phase, OC present in the aforementioned cycles were probably originated from the gas phase, which was not removed by the DPF.

Among the four driving conditions tested in this study, idling generally produced the highest OC/EC ratio. EC mainly arises from fuel droplet pyrolysis, whereas OC mainly originates from unburned fuel and incomplete combustion (Shah et al., 2004). When the vehicles were in the idling condition, their engine temperature decreased, which resulted in “less complete” fuel combustion compared with that under other conditions

The European emission standard assigned to the vehicle considerably affected OC and EC emissions. Typically, the EFs of OC and EC decreased with an increase in emission standards, except in the cases of Vehicles 6, 12, and 15. An extremely low level of EC and a measurable level of OC were recorded in vehicles with high emission standards (e.g., Vehicles 7 and 13). A possible reason for this result is that the emission control technologies applied in new vehicles may not effectively remove OC from diesel vehicle exhausts. Because most of the toxic and mutagenic properties of diesel exhaust are associated with OCs (Claxton, 2015, Shah et al., 2004), our results suggest that the development of emission control technologies should focus on reducing the OC fraction of PM.

In addition to the effects of emission standards, the results revealed that the EFs of OC and EC were considerably influenced by the conditions of the vehicles. For example, vehicles with advanced emission standards did not always exhibit OC or EC reduction. Compared with the HDVs depicted in **Fig. 1**, the OC EF of Vehicle 15 (Euro 5) was considerably higher than that of Vehicle 14 (Euro 4). During the chassis dynamometer testing of Vehicle 15, white smoke and pungent smell emanated from the exhaust, which indicated that Vehicle 15 was poorly maintained. The maintenance condition of Vehicle 15 had a larger influence than its emission standard on the emissions of OC and EC.

PAHs

A total of 31 PAHs (**Table S2**) were characterized, and their EFs were calculated. In all the tests, the EFs of acenaphthylene, acenaphthene, and fluoranthene were less than the detection limit because these three PAHs have low molecular weight and are mainly present in the gas phase. Therefore, the aforementioned three PAHs were excluded in the following analyses. **Fig. 2** presents an overview of the PAH EFs under different driving conditions. Each bar in **Fig. 2** corresponds to the total PAH EF (sum of the EFs of all the PAHs tested) in a given driving cycle, and the red solid line represents the sum of the total PAH EFs in the four driving cycles (denoted as “four-cycle sum” in the following text). As displayed in **Fig. 2**, Vehicle 15 emitted the highest four-cycle sum of 18.60 mg/kg, followed by Vehicle 6. Vehicle 2 emitted the lowest four-cycle sum of 0.41 mg/kg, followed by Vehicles 11 and 13. A common feature of these three vehicles with low four-cycle sums was that they were equipped with DPFs.

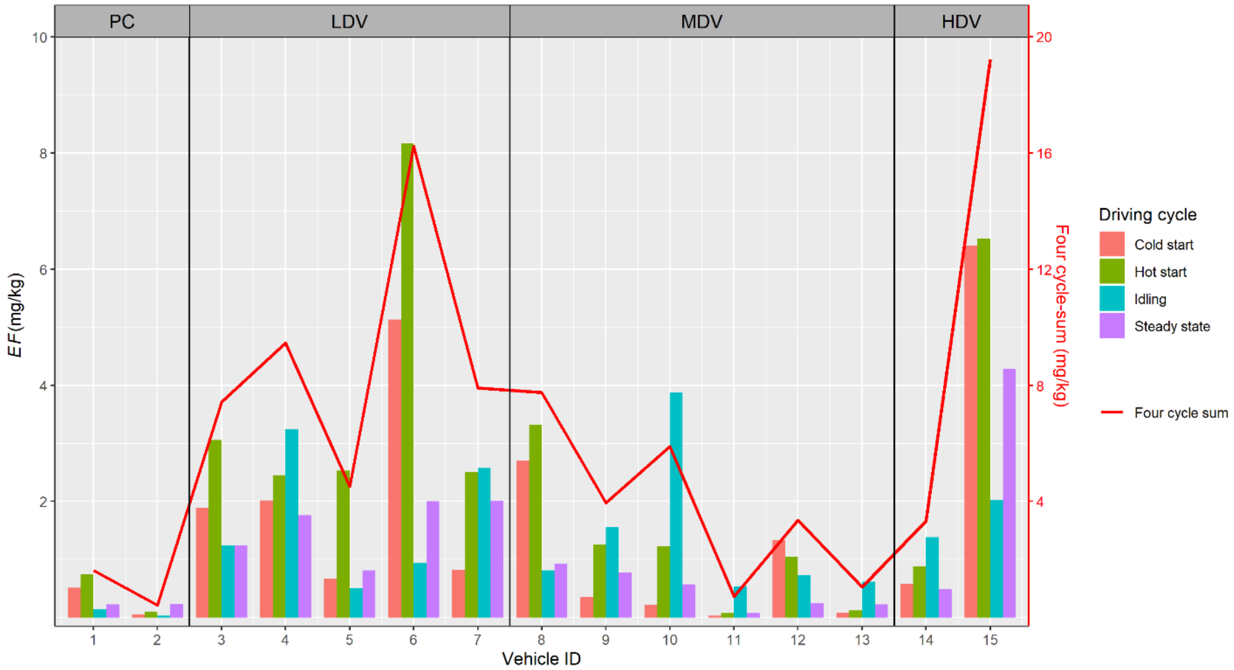

Fig. 2. Total PAH *EFs* in different driving cycles. The red solid line represents the sum of total PAH *EFs* of the four driving cycles, denoted as "four cycle-sum".

Fig. 2 reveals that the MDVs had lower total PAH EFs than the LDVs did. Unexpectedly, vehicles with greater testing weights emitted less PAHs. A possible explanation for this result is that two DPF-equipped vehicles were present in the MDV class, whereas only one DPF-equipped vehicle was present in the LDV class. Cao et al. (2017) observed the aforementioned pattern for their vehicle fleet, which comprised 18 diesel trucks in China. The aforementioned observation was not an isolated event, which suggested that the effect of the vehicle size on the PAH emission should be further investigated.

Fig. 2 also reveals that the steady-state cycle generally exhibited the least total PAH EFs, whereas the transient and idling cycles exhibited substantially higher PAH EFs. These results are consistent with those reported by (Shah et al., 2005). Furthermore, the aforementioned figure indicates that the total PAH EFs of Vehicles 4, 7, 9, 10, 11, 13 and 14 under idling cycles were higher than those under transient cycles. This result suggested that the vehicle emission behavior under different driving conditions, especially under idling, should be studied because a substantial difference in EFs were observed between different driving cycles.

To identify the dominant PAHs emitted from each vehicle, the weighted percentage of each PAH emitted under different driving cycles was determined (**Fig. 3)**. For most of the vehicles, the dominant PAHs were 4- and 5-ring PAHs, including pyrene (PYR), benz[*a*]anthracene (BaA), chrysene (CHR), benzo[*b*]fluoranthene (BbF), benzo[*k*]fluoranthene (BkF), and benzo[*a*]fluoranthen (BaF). This finding was consistent with those reported in previous studies (Cao et al., 2017, Hu et al., 2013). The difference in the PAH composition under different driving conditions is illustrated in **Fig. 3**. The distributions of individual PAHs did not exhibit considerable variations when a given vehicle was tested under different driving conditions, except when Vehicles 1 and 9 were tested under the idling and steady-state cycles. The variation in the PAH composition among vehicles was not significant, except for Vehicle 7. Excluding the aforementioned two exceptions, the PAHs collected in all the driving cycles were dominated by 4- and 5-ring PAHs for all the vehicles. This observation suggests that the driving conditions, driving pattern (NEDC or FIGE), mileage, testing weight of the vehicle, and after-treatment technologies do not considerably affect the composition of the emitted PAHs. Furthermore, in general the collected PAH samples originated from the same source, probably fuel combustion, because they all had similar compositions. Therefore, the PAH samples collected in the exceptional cases (for Vehicle 7 and in the idling and steady-state cycles for Vehicles 1 and 9) were probably affected by other sources. Further investigations are required to characterize the sources of PAHs collected from the tailpipe emissions of diesel vehicles.

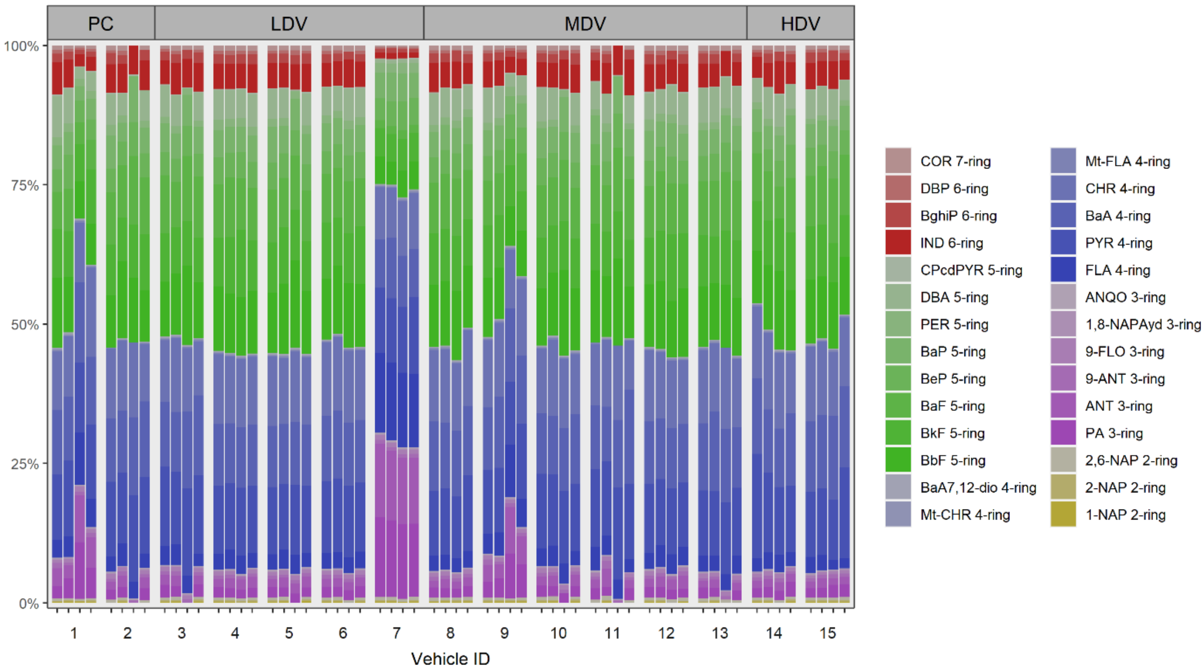

Fig. 3. Mass percentages of PAHs of each vehicle. The four bars under the same Vehicle ID correspond to cold start, hot start, idling and steady state cycles from left to right.

Comparison with other studies

The PAH data obtained in this current study were compared with the results of similar studies. Unlike the fuel-based EF used in the previous sections, distance-based EFs (EFs_d) were used in this section because most of the results in previous studies were presented in a distance-based manner. The EF_d value for each PAH was calculated as the ratio of the total quantity of PAHs released in a driving cycle to the distance traveled in the driving cycle. The driving distances in the NEDC, FIGE, and steady-state cycles were 11.0, 29.5, and 16.7 km, respectively. An averaged EF_d for each vehicle class (i.e. PCs, LDVs, MDVs, and HDVs) was calculated. **Fig. 4** presents a comparison of the averaged EFs_d of each PAH from each vehicle class in this study with the PAHs in diesel vehicle emissions in a PEMS study in China (Cao et al., 2017), tunnel studies in China (Chen et al., 2013) and Hong Kong (Ho et al., 2009), and a chassis dynamometer study in the US (Hays et al., 2017). As depicted in **Fig. 4**, the results of the current study were in the range reported in previous studies. Cao et al. (2017) reported the highest PAH EF_d values among the compared studies. Their vehicle fleet comprised 18 diesel trucks with China 3 and 4 emission standards (equivalent to Euro 3 and 4, respectively). In general, the PAH EF_d results of Cao et al. were an order of magnitude higher than those obtained for the HDVs in this study (except for CHR). The EF_d value of ANT in the study of Cao et al. (2017) was four orders of magnitude higher than that in this study, which could be attributed to two main reasons. First, the emission standards of the tested HDVs (Euro 4 and 5) in this study were higher than those of Cao's fleet. Second, Cao et al. (2017) measured on-road emissions by using a PEMS. The emission EFs during on-road driving are considerably higher than those in laboratory chassis dynamometer tests (Huang et al., 2018, May et al., 2014b, Weiss et al., 2012) because the driving conditions in the real world are more rigorous than the driving cycles tested in chassis dynamometer studies.

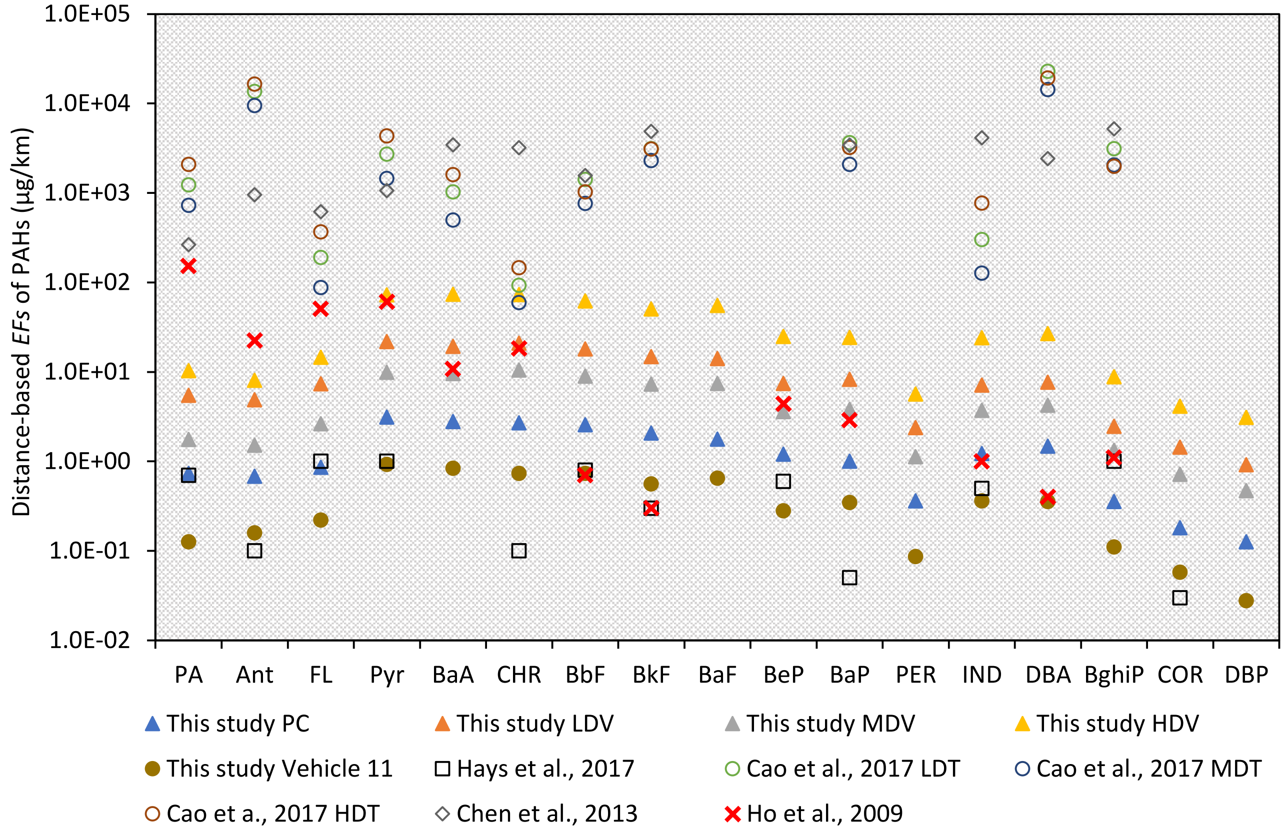

Fig. 4. Distance-based PAHs *EFs* of different vehicle classes.

Hays et al. (2017) conducted chassis dynamometer testing on DPF-equipped diesel trucks in the US. Their findings agreed with the PAH EF_d values obtained for Vehicle 11 in this study, which are denoted by brown solid dots in **Fig. 4**. Vehicle 11 is a DPF-equipped MDV and is comparable in size to the vehicles tested by Hays et al. (2017). The agreement between the results of this study and Hays et al. (2017) confirmed that DPF can efficiently remove PAHs from vehicle exhausts.

Chen et al. (2013) conducted a tunnel study in Nanjing, and Ho et al. (2009) conducted a tunnel study in Hong Kong. As depicted in **Fig. 4**, the results of Chen et al. (2013) were in the range of those of Cao et al. (2017), whereas the results of Ho et al. (2009) were generally within the range of those of the current study. The EF_d values of the pollutants emitted from diesel vehicles were strongly related to regions, which can be attributed to the diesel fuel variation among regions. Studies have indicated that the fuel type (i.e., low-sulfur diesel vs. ultra-low-sulfur diesel) considerably affects the EFs of PAHs (Cheung et al., 2010, Lim et al., 2005).

The EFs_d of the PAHs in this study were expected be lower than those PAH EFs_d in the study of Ho et al. (2009) because according to the Environmental Protection Department of Hong Kong, the $PM_{2.5}$ emissions in Hong Kong have reduced by more than 50% (HKEPD, 2019). This observation can be explained by the driving condition for vehicles in tunnels, where vehicles travel at almost constant speed. This condition resembles the steady-state cycle in this study. **Fig. 2** indicates that the PAH EFs in the hot start cycle were higher than those in the steady-state cycle for all vehicles except Vehicles 2, 11, and 13. Also, the averaged total PAHs EF_d in steady-state cycle is 0.0834 ± 0.146 mg/km, which is lower than that in cold start (0.180 ± 0.303 mg/km) and hot start (0.239 ± 0.310 mg/km) cycles. This observation explains the higher-than-expected PAH EFs in this study compared with the study of Ho et al. (2009). Some high-emission vehicles (e.g., Vehicles 6 and 15) contributed considerably to the calculated average PAH values, which increased the PAH EFs in this study.

Toxicological analysis

The levels of DCFH, IL-6, and GSH were analyzed to assess the cellular oxidative and inflammatory responses produced by the PM samples. The toxicological results of the current study cannot be compared with those of other studies because the cell culture conditions in this study were not exactly the same as those in previous studies. Also, as all the results were obtained by a fixed concentration of PM (i.e. 50 μg ml^{-1}), comparison between vehicles only accounted for the compositional difference of PM. Although the concentration specific toxicology results for DCFH and IL-6 can be normalized to the amount of PM emitted under different conditions to study the PM toxicity under these conditions, preliminary analysis showed that the normalized toxicology result strongly depend on the EF of PM. **Fig. S5** in Supporting Information illustrated that DCFH and IL-6 results normalized by the fuel-based PM *EF* showed the same pattern as PM *EF*. Therefore, the aforementioned result was not presented in the main context. Moreover, for Vehicle 2 under hot start condition, the amount of PM collected was under detection limit by gravimetric method. Therefore, there is no toxicological test results for Vehicle 2 under hot start condition.

The ROS production is expressed in terms of the fluorescent intensity of DCFH, as depicted in the upper panel of **Fig. 5**. In general, the fluorescent intensity did not vary significantly among the different driving cycles. Vehicle 10 exhibited the highest fluorescent intensity, with an average value of 1.62 ± 0.31. In addition to Vehicle 10, the cold start cycle for Vehicle 1, hot start cycle for Vehicle 3, and idling cycle for Vehicle 13 exhibited elevated levels of fluorescent intensity in response to the emitted PM. However, other than the aforementioned vehicles and cycles, the variation in the ROS among the remaining vehicles was not significant.

The lowest panel in **Fig. 5** shows the GSH levels after the A549 cells were exposed to the PM samples. GSH is an antioxidant of which the concentration will decrease in response to oxidative stress. In general, GSH levels were depleted compared to the blank filter sample outlined in Fig.S3 for all PM samples. Compared with the results of the DCFH level, a certain degree of variation was observed in the GSH levels for the tested vehicles. LDVs (Vehicles 3, 4, 5, 6, and 7) generally had lower GSH concentrations (stronger oxidative stresses) than the other classes of vehicles did. Other parameters did not significantly influence the GSH level. The average GSH levels for vehicles with DPF and without DPF were $9.89 \pm 3.97\ \mu M$ and $9.85 \pm 3.37\ \mu M$, respectively. This result indicated that DPFs removed PM mass without changing the GSH response of the PM; thus, DPFs probably did not significantly change the morphology and composition of the PM samples.

IL-6 is a proinflammatory cytokine released in response to PM exposure. The IL-6 results are illustrated in the middle panel of **Fig. 5**. The idling cycle for Vehicle 14 exhibited the highest IL-6 level of 80.3, whereas the hot start cycle for Vehicle 7 exhibited the lowest IL-6 level of 8.30. In general, no clear trend was observed for the effect of driving cycles on the IL-6 levels. HDVs had the highest IL-6 concentrations among the vehicle classes, followed by MDVs. The IL-6 levels of PCs, LDVs, and MDVs did not exhibit significant variations.

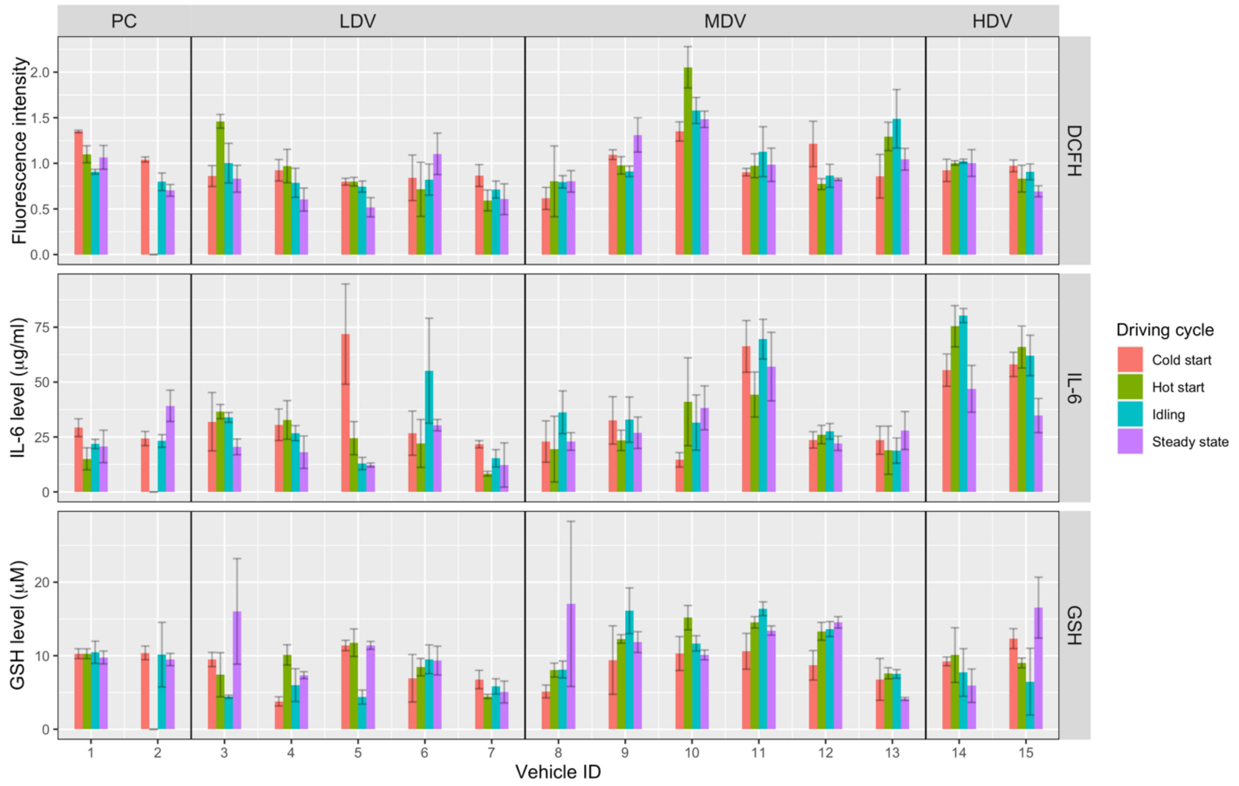

Fig. 5. Results of the production of ROS expressed as the fluorescent intensity of DCF, IL-6 level and GSH level of the 15 vehicles in different testing cycles.

Effect of vehicle type and driving cycle

To determine the effects of the vehicle type on the three toxicological markers, the levels of each toxicological marker for all vehicles were pooled and grouped according to their corresponding vehicle type (i.e., PC, LDV, MDV, and HDV). The results are presented using boxplots in **Fig. 6**. As depicted in **Fig. 6**, a certain degree of variation existed between vehicle types for the three toxicological markers. The LDVs produced lower responses for DCFH and GSH than the other three vehicle types did. The HDVs exhibited a higher IL-6 level than the other three vehicle types.

The results were verified by conducting a Kruskal–Wallis H test for each toxicological marker at a significance level of $p = 0.05$. The Kruskal–Wallis H test results presented in the bottom left of each boxplot indicated that statistically significant differences existed among the four vehicle types for the three toxicological markers. The pairwise Wilcoxon rank-sum test was conducted as the post-hoc test of the Kruskal–Wallis test to determine which vehicle type pair had significant differences in their toxicological marker levels at a significance level of 0.05. The vehicle type pairs with adjusted p values of <0.05 are marked with an asterisk in **Fig. 6**. Significant differences were identified in the DCFH and GSH levels of the LDVs and MDVs. Also, significant differences were identified in the IL-6 levels of the HDVs and other types of vehicles. The aforementioned results suggested that the vehicle type affected the response of the three toxicological markers. Moreover, it should be noticed that a decrease in GSH level indicates an increase of oxidative stress, which means that GSH level should show an opposite trend when compared to DCFH level. However, result of the aforementioned statistical analysis showed that increase of vehicle weight (from LDV to MDV) increased both GSH and DCFH levels. Further investigations are required to determine the mechanisms or reasons leading to this observation.

Unlike the vehicle type, the driving condition of the vehicle did not have a significant effect on the three toxicological markers. Statistical analyses (**Fig. S3**) confirmed that no statistically significant differences existed between the four driving cycles ($p > 0.05$) for the three toxicological markers. This result suggested that the driving condition had a minimal effect on the production of ROS and the proinflammatory cytokine IL-6. This finding is consistent with our PAH results, which revealed that the composition of PAHs did not vary significantly among the different driving cycles.

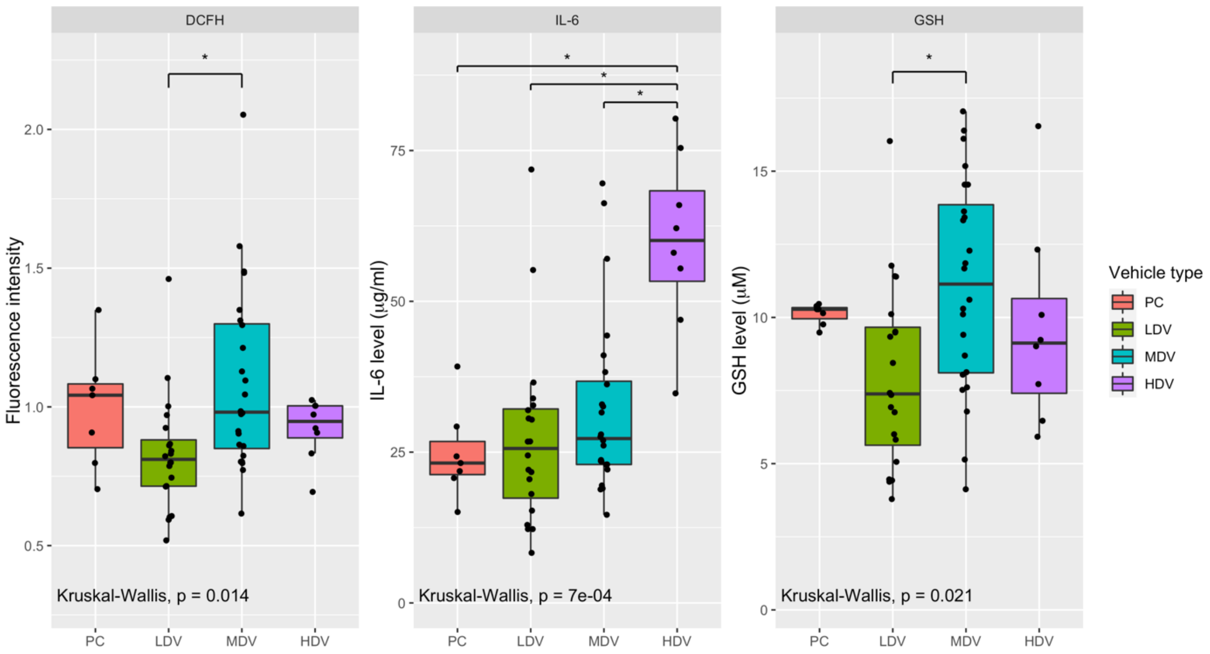

Fig. 6. Boxplots of the levels of DCFH, IL-6 and GSH grouped by vehicle type. Black dots represent the data points in the corresponding driving cycle. Asterisk represents the adjusted p-value in pairwise Wilcoxon rank sum test smaller than 0.05.

Correlation between toxicity data and PAHs concentration

The chemical composition of PM samples has been reported to be related to the oxidative potential and the release of oxidative stress mediators (Chuang et al., 2012, Ho et al., 2016). In particular, certain PAHs have significant correlations with the vasoactive function and proinflammatory cytokines (Niu et al., 2017).

Table 1. Results of spearman's correlation between PAHs and toxicological markers with p-value smaller than 0.1. Asterisk indicates p-value <0.05.

		DCFH *Spearman's ρ (p-value)*	**IL-6** *Spearman's ρ (p-value)*	**GSH** *Spearman's ρ (p-value)*
Hot start	IND	0.468 (<0.1)	-	-
	DBA	0.532 (<0.05) *	-	-
	Mt-CHR	0.457 (<0.1)	-	-
	COR	-	0.471 (<0.1)	-
Idling	Mt-FLA	-	0.503 (<0.1)	-
	2-NAP	-	-	-0.900 (0.037) *
Steady state	PA	-	-	-0.539 (<0.05) *
	ANT	-	-	-0.514 (<0.05) *
	FLA	-	-	-0.479 (<0.1) *
	BaA	-	0.538 (<0.05) *	-
	PYR	-	0.479 (<0.1)	-
	BkF	-	0.52 (<0.05) *	-
	BaF	-	0.454 (<0.1)	-
	BeP	-	0.584 (<0.05) *	-
	2,6-NAP	-	0.493 (<0.1)	-
	Mt-FLA	-	0.483 (<0.1)	-
	BaA7,12-dio	-	0.441 (<0.1)	-

Correlation analysis was conducted between the concentrations of the PAHs and toxicological markers according to the method stated in the Methodology section. The correlation test results with a *p* value of <0.1 are presented in **Table 1**. A total of 17 PAH-toxicological marker pairs had moderate or good correlations. In general, as depicted in **Table 1**, almost all the aforementioned pairs were associated with the hot start and steady-state cycles, with the exception of two pair that was associated with the idling cycle. There is no correlation found between PAH and toxicology data under cold-start condition. No correlation was found between the PAH and toxicology data under the cold start condition. Thus, under hot engine conditions (hot start and steady state), some PAHs functioned as good indicators of ROS production or proinflammatory response. However, under low-engine-temperature conditions (i.e., cold start and idling), the PAH concentrations measured in this study were not the main driving forces for the observed toxicology responses.

As depicted in **Table 1**, the DCFH levels and PAH concentrations exhibited correlation only under the hot start condition. Moderate correlations were found between DCFH level and Mt-CHR ($\rho = 0.46$, $p < 0.1$) and between DCFH level and IND ($\rho = 0.47$, $p < 0.1$). Moreover, a good correlation was found between DCFH level and DBA ($\rho = 0.53$, $p < 0.05$). This result agreed with that of Wu et al. (2017), who found a strong correlation between ROS generation and DBA in petrol and diesel fuel combustion experiments. Correlation coefficients between GSH and PAHs were expected to be negative since the decrease in GSH shows the increase of oxidative stress posed by the corresponding PAH. Correlations of GSH with PAHs were found in idling and steady state cycle. PA (ρ = -0.539, p < 0.05) and ANT (ρ = -0.514, p < 0.05) showed good correlation with GSH and FLA (ρ = -0.479, p < 0.1) showed moderate correlation with GSH in steady state cycle. In idling cycle, GSH showed good correlation with 2-NAP (ρ = -0.900, p < 0.05).

Table 1 also showed the correlation between the pro-inflammatory mediator IL-6 and selected PAHs. Moderate correlation was found between IL-6 and COR ($\rho = 0.47$, $p < 0.1$) in the hot start cycle. The correlation between IL-6 and Mt-FLA (ρ = 0.50, $p < 0.1$) was the only significant correlation in the idling cycle. Except the aforementioned two correlations, all the observed correlations between IL-6 and PAHs were found in the steady-state cycle. The compound 2,6-NAP (ρ = 0.49, $p < 0.1$) was the only PAH with less than three rings that exhibited correlation with the toxicology results. IL-6 exhibited moderate correlation with Ba7,12-dio (ρ = 0.44, $p < 0.1$), Mt-FLA (ρ = 0.48, $p < 0.1$), PYR (ρ = 0.48, $p < 0.1$), and BaF (ρ = 0.45, $p < 0.1$). Moreover, IL-6 exhibited significant correlations with BaA (ρ = 0.54, $p < 0.05$), BkF (ρ = 0.52, $p < 0.05$), and BeP (ρ = 0.58, $p < 0.05$).

Several studies have assessed the correlations among IL-6 and particle-bounded PAHs from different sources; however, they have obtained different results. Niu et al. (2017) and Chowdhury et al. (2019) have investigated the correlation between PAHs in an atmospheric PM sample and the IL-6 responses in an A549 cell and a BEAS-2B cell (human bronchial epithelial cell), respectively. They have determined that certain PAHs are positively correlated with the IL-6 level. Lin et al. (2013) studied the effect of household particles on inflammation in human coronary artery endothelial cells (HCAECs) and revealed that PAHs were significantly correlated with the IL-6 level. Delfino et al. (2010) analyzed blood samples from 60 people and the air samples in their vicinity. Their results suggested a positive correlation between PAHs in air samples and the IL-6 level in human blood samples. Our findings are in agreement with those of the aforementioned studies, which support a positive correlation between PAHs and the IL-6 level.

However, a study conducted by Skuland et al. (2017) could not establish a clear connection between the total or individual PAH levels in diesel exhaust particles and the IL-6 level in a BEAS-2B cell. Chuang et al. (2012) could not find a significant correlation between the PAHs in air samples and the IL-6 level in HCAECs. Moreover, Wang et al. (2016) found a significant negative correlation between the PAHs in atmospheric PM samples and the IL-6 level in BEAS-2B cells. The aforementioned studies suggest that in some cases, PAHs might not be the main inducer of proinflammatory response. Therefore, further investigations are essential to elucidate the reaction mechanism for the release of IL-6 and other proinflammatory mediators.

Conclusion

This paper presents a detailed chemical analysis of PM samples collected from diesel vehicles with various physical properties. This study is the first in Hong Kong to investigate the toxicity of PM samples through chassis dynamometer testing. The results indicated that PM emissions from the tested diesel vehicles were dominated by OC. DPF-equipped diesel vehicles had very high OC/EC ratios, which suggested that DPFs could effectively remove EC but not OC in PM. The EC removal efficiency of the DPFs was high even for vehicles with high odometer readings.

Among the identified PAHs, 4- and 5-ring PAHs were the most abundant species. The highest PAH EFs were exhibited by the HDVs, followed by the LDVs and MDVs. The driving cycle had a significant effect on the EFs of the PAHs. The steady-state cycle generally exhibited the lowest

PAH EFs, and the transient and idling cycles exhibited substantially higher PAH EFs than the steady-state cycle did. Although different PAH EFs were observed under different driving conditions, the mass percentage of individual PAHs (i.e., the PAH composition of the PM samples) did not vary significantly with different driving conditions.

The cellular exposure experiments revealed that the PM emissions of diesel vehicles cause potential oxidative stresses, which emerge from ROS, for human lung cell activities. The statistical analysis results indicated that the MDVs produced significantly higher levels of DCFH and GSH than the LDVs did. Moreover, the HDVs produced significantly larger quantities of IL-6 than the other types of vehicles did. Correlation analysis between the PAHs and three toxicology markers revealed that statistically significant correlations existed between certain PAH–toxicological marker pairs, including DCFH and DBA ($\rho = 0.53$, $p < 0.05$), GSH and PA ($\rho = -0.539$, $p < 0.05$), and ANT ($\rho = -0.514$, $p < 0.05$), as well as IL-6 and BaA ($\rho = 0.54$, $p < 0.05$), BkF ($\rho = 0.52$, $p < 0.05$), and BeP ($\rho = 0.58$, $p < 0.05$). Furthermore, the results suggested that new emission control technologies and policies should focus on OC and PAH reduction to reduce their adverse health effects on the human respiratory system. Nevertheless, the currently study focused on the chemical and toxicological analysis of pollutants in particle phase only. Further studies on volatile and semi-volatile organic pollutants from diesel vehicle exhaust are warranted. Another aspect to be considered in future studies is the effect of particle size distribution and particle morphologies on the chemical and toxicological properties of the diesel PM samples. An integration of the outcomes of the aforementioned studies and the data in this paper can provide a more comprehensive picture of the chemical composition and toxicological properties of diesel emission in the future.

Acknowledgements

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References

ABDEL-SHAFY, H. I. & MANSOUR, M. S. M. 2016. A review on polycyclic aromatic hydrocarbons: Source, environmental impact, effect on human health and remediation. *Egyptian Journal of Petroleum,* 25**,** 107-123.

BENGALLI, R., LONGHIN, E., MARCHETTI, S., PROVERBIO, M. C., BATTAGLIA, C. & CAMATINI, M. 2017. The role of IL-6 released from pulmonary epithelial cells in diesel UFP-induced endothelial activation. *Environmental Pollution,* 231**,** 1314-1321.

CAO, X., HAO, X., SHEN, X., JIANG, X., WU, B. & YAO, Z. 2017. Emission characteristics of polycyclic aromatic hydrocarbons and nitro-polycyclic aromatic hydrocarbons from diesel trucks based on on-road measurements. *Atmospheric Environment,* 148**,** 190-196.

CECINATO, A., GUERRIERO, E., BALDUCCI, C. & MUTO, V. 2014. Use of the PAH fingerprints for identifying pollution sources. *Urban Climate,* 10**,** 630-643.

CHEN, F., HU, W. & ZHONG, Q. 2013. Emissions of particle-phase polycyclic aromatic hydrocarbons (PAHs) in the Fu Gui-shan Tunnel of Nanjing, China. *Atmospheric Research,* 124**,** 53-60.

CHENG, Y., LEE, S. C., HO, K. F., CHOW, J. C., WATSON, J. G., LOUIE, P. K. K., CAO, J. J. & HAI, X. 2010. Chemically-speciated on-road PM2.5 motor vehicle emission factors in Hong Kong. *Science of The Total Environment,* 408**,** 1621-1627.

CHENG, Y., ZOU, S. C., LEE, S. C., CHOW, J. C., HO, K. F., WATSON, J. G., HAN, Y. M., ZHANG, R. J., ZHANG, F., YAU, P. S., HUANG, Y., BAI, Y. & WU, W. J. 2011. Characteristics and source apportionment of PM1 emissions at a roadside station. *Journal of Hazardous Materials,* 195**,** 82-91.

CHEUNG, K. L., NTZIACHRISTOS, L., TZAMKIOZIS, T., SCHAUER, J. J., SAMARAS, Z., MOORE, K. F. & SIOUTAS, C. 2010. Emissions of Particulate Trace Elements, Metals and Organic Species from Gasoline, Diesel, and Biodiesel Passenger Vehicles and Their Relation to Oxidative Potential. *Aerosol Science and Technology,* 44**,** 500-513.

CHIANG, H.-L., LAI, Y.-M. & CHANG, S.-Y. 2012. Pollutant constituents of exhaust emitted from light-duty diesel vehicles. *Atmospheric Environment,* 47**,** 399-406.

CHOW, J. C., WATSON, J. G., CHEN, L. W. A., CHANG, M. C. O., ROBINSON, N. F., TRIMBLE, D. & KOHL, S. 2012. The IMPROVE_A temperature protocol for Thermal/Optical carbon analysis: maintaining consistency with a long-term database. *Journal of the Air & Waste Management Association,* 57**,** 1014-1023.

CHOWDHURY, P. H., HONDA, A., ITO, S., OKANO, H., ONISHI, T., HIGASHIHARA, M., OKUDA, T., TANAKA, T., HIRAI, S. & TAKANO, H. 2019. Effects of Ambient PM2.5 Collected Using Cyclonic Separator from Asian

Cities on Human Airway Epithelial Cells. *Aerosol and Air Quality Research,* 19, 1808-1819.

CHUANG, H. C., FAN, C. W., CHEN, K. Y., CHANG-CHIEN, G. P. & CHAN, C. C. 2012. Vasoactive alteration and inflammation induced by polycyclic aromatic hydrocarbons and trace metals of vehicle exhaust particles. *Toxicol Lett,* 214, 131-6.

CLAXTON, L. D. 2015. The history, genotoxicity, and carcinogenicity of carbon-based fuels and their emissions. Part 3: Diesel and gasoline. *Mutation Research/Reviews in Mutation Research,* 763, 30-85.

DELFINO, R. J., STAIMER, N., TJOA, T., ARHAMI, M., POLIDORI, A., GILLEN, D. L., KLEINMAN, M. T., SCHAUER, J. J. & SIOUTAS, C. 2010. Association of biomarkers of systemic inflammation with organic components and source tracers in quasi-ultrafine particles. *Environ Health Perspect,* 118, 756-62.

DIMASHKI, M., LIM, L. H., HARRISON, R. M. & HARRAD, S. 2001. Temporal trends, temperature dependence, and relative reactivity of atmospheric polycyclic aromatic hydrocarbons. *Environmental Science & Technology,* 35, 2264-2267.

GALI, N. K., YANG, F., CHEUNG, C. S. & NING, Z. 2017. A comparative analysis of chemical components and cell toxicity properties of solid and semi-volatile PM from diesel and biodiesel blend. *Journal of Aerosol Science,* 111, 51-64.

GELLER, M. D., NTZIACHRISTOS, L., MAMAKOS, A., SAMARAS, Z., SCHMITZ, D. A., FROINES, J. R. & SIOUTAS, C. 2006. Physicochemical and redox characteristics of particulate matter (PM) emitted from gasoline and diesel passenger cars. *Atmospheric Environment,* 40, 6988-7004.

GERLOFS-NIJLAND, M. E., TOTLANDSDAL, A. I., TZAMKIOZIS, T., LESEMAN, D. L. A. C., SAMARAS, Z., LÅG, M., SCHWARZE, P., NTZIACHRISTOS, L. & CASSEE, F. R. 2013. Cell Toxicity and Oxidative Potential of Engine Exhaust Particles: Impact of Using Particulate Filter or Biodiesel Fuel Blend. *Environmental Science & Technology,* 47, 5931-5938.

GHIO, A. J., CARRAWAY, M. S. & MADDEN, M. C. 2012. Composition of air pollution particles and oxidative stress in cells, tissues, and living systems. *Journal of Toxicology and Environmental Health, Part B,* 15, 1-21.

GRIESHOP, A. P., LIPSKY, E. M., PEKNEY, N. J., TAKAHAMA, S. & ROBINSON, A. L. 2006. Fine particle emission factors from vehicles in a highway tunnel: Effects of fleet composition and season. *Atmospheric Environment,* 40, 287-298.

HAYS, M. D., PRESTON, W., GEORGE, B. J., GEORGE, I. J., SNOW, R., FAIRCLOTH, J., LONG, T., BALDAUF, R. W. & MCDONALD, J. 2017. Temperature and Driving Cycle Significantly Affect Carbonaceous Gas and Particle Matter Emissions from Diesel Trucks. *Energy Fuels,* 31.

HO, K.-F., HO, S. S. H., HUANG, R.-J., CHUANG, H.-C., CAO, J.-J., HAN, Y., LUI, K.-H., NING, Z., CHUANG, K.-J., CHENG, T.-J., LEE, S.-C., HU, D., WANG, B. & ZHANG, R. 2016. Chemical composition and bioreactivity of PM2.5 during 2013 haze events in China. *Atmospheric Environment,* 126**,** 162-170.

HO, K. F., HO, S. S. H., LEE, S. C., CHENG, Y., CHOW, J. C., WATSON, J. G., LOUIE, P. K. K. & TIAN, L. 2009. Emissions of gas- and particle-phase polycyclic aromatic hydrocarbons (PAHs) in the Shing Mun Tunnel, Hong Kong. *Atmospheric Environment,* 43**,** 6343-6351.

HO, K. F. & LEE, S. C. 2002. Identification of atmospheric volatile organic compounds (VOCs), polycyclic aromatic hydrocarbons (PAHs) and carbonyl compounds in Hong Kong. *Science of The Total Environment,* 289**,** 145-158.

HO, K. F., LEE, S. C., YU, J. C., ZOU, S. C. & FUNG, K. 2002. Carbonaceous characteristics of atmospheric particulate matter in Hong Kong. *Science of The Total Environment,* 300**,** 59-67.

HO, S. S. H., YU, J. Z., CHOW, J. C., ZIELINSKA, B., WATSON, J. G., SIT, E. H. L. & SCHAUER, J. J. 2008. Evaluation of an in-injection port thermal desorption-gas chromatography/mass spectrometry method for analysis of non-polar organic compounds in ambient aerosol samples. *Journal of Chromatography A,* 1200**,** 217-227.

HONG KONG TRANSPORT DEPARTMENT 2018. THE ANNUAL TRAFFIC CENSUS 2017.

HOU, B., ZHUANG, G., ZHANG, R., LIU, T., GUO, Z. & CHEN, Y. 2011. The implication of carbonaceous aerosol to the formation of haze: revealed from the characteristics and sources of OC/EC over a mega-city in China. *J Hazard Mater,* 190**,** 529-36.

HU, S., D HERNER, J., ROBERTSON, W., KOBAYASHI, R., CHANG, O., HUANG, S.-M., ZIELINSKA, B., KADO, N., F COLLINS, J., RIEGER, P., HUAI, T. & AYALA, A. 2013. Emissions of polycyclic aromatic hydrocarbons (PAHs) and nitro-PAHs from heavy-duty diesel vehicles with DPF and SCR. *Journal of the Air & Waste Management Association (1995),* 63**,** 984-96.

HUANG, Y., ORGAN, B., ZHOU, J., SURAWSKI, N., HONG, G., CHAN, E. & YAM, Y. S. 2018. Emission measurement of diesel vehicles in Hong Kong through on-road remote sensing: Performance review and identification of high-emitters. *Environmental Pollution,* 237**,** 133-142.

KIM, K. H., JAHAN, S. A., KABIR, E. & BROWN, R. J. 2013. A review of airborne polycyclic aromatic hydrocarbons (PAHs) and their human health effects. *Environ Int,* 60**,** 71-80.

KLEEMAN, M. J., SCHAUER, J. J. & CASS, G. R. 2000. Size and composition distribution of fine particulate matter emitted from motor vehicles. *Environmental Science & Technology,* 34**,** 1132-1142.

LEE, S., CHENG, Y., HO, K. F., CAO, J., LOUIE, P. K. K., CHOW, J. & WATSON, J. 2006. PM 1.0 and PM 2.5 Characteristics in the Roadside Environment of Hong Kong. *Aerosol Science and Technology,* 40**,** 157-165.

LI, N., SIOUTAS, C., CHO, A., SCHMITZ, D., MISRA, C., SEMPF, J., WANG, M., OBERLEY, T., FROINES, J. & NEL, A. 2003. Ultrafine Particulate Pollutants Induce Oxidative Stress and Mitochondrial Damage. *Environmental Health Perspectives,* 111**,** 455-460.

LIM, M. C. H., AYOKO, G. A., MORAWSKA, L., RISTOVSKI, Z. D. & ROHAN JAYARATNE, E. 2005. Effect of fuel composition and engine operating conditions on polycyclic aromatic hydrocarbon emissions from a fleet of heavy-duty diesel buses. *Atmospheric Environment,* 39**,** 7836-7848.

LIN, L.-Y., LIU, I. J., CHUANG, H.-C., LIN, H.-Y. & CHUANG, K.-J. 2013. Size and composition effects of household particles on inflammation and endothelial dysfunction of human coronary artery endothelial cells. *Atmospheric Environment,* 77**,** 490-495.

LIN, Y. C., LI, Y. C., AMESHO, K. T. T., CHOU, F. C. & CHENG, P. C. 2019. Characterization and quantification of PM2.5 emissions and PAHs concentration in PM2.5 from the exhausts of diesel vehicles with various accumulated mileages. *Sci Total Environ,* 660**,** 188-198.

MA, Y., CHENG, Y., QIU, X., LIN, Y., CAO, J. & HU, D. 2016. A quantitative assessment of source contributions to fine particulate matter (PM2.5)-bound polycyclic aromatic hydrocarbons (PAHs) and their nitrated and hydroxylated derivatives in Hong Kong. *Environ Pollut,* 219**,** 742-749.

MAY, A. A., NGUYEN, N. T., PRESTO, A. A., GORDON, T. D., LIPSKY, E. M., KARVE, M., GUTIERREZ, A., ROBERTSON, W. H., ZHANG, M., BRANDOW, C., CHANG, O., CHEN, S., CICERO-FERNANDEZ, P., DINKINS, L., FUENTES, M., HUANG, S.-M., LING, R., LONG, J., MADDOX, C., MASSETTI, J., MCCAULEY, E., MIGUEL, A., NA, K., ONG, R., PANG, Y., RIEGER, P., SAX, T., TRUONG, T., VO, T., CHATTOPADHYAY, S., MALDONADO, H., MARICQ, M. M. & ROBINSON, A. L. 2014a. Gas- and particle-phase primary emissions from in-use, on-road gasoline and diesel vehicles. *Atmospheric Environment,* 88**,** 247-260.

MAY, J., BOSTEELS, D. & FAVRE, C. 2014b. An Assessment of Emissions from Light-Duty Vehicles using PEMS and Chassis Dynamometer Testing. SAE International.

MAZZARELLA, G., FERRARACCIO, F., PRATI, M. V., ANNUNZIATA, S., BIANCO, A., MEZZOGIORNO, A., LIGUORI, G., ANGELILLO, I. F. & CAZZOLA, M. 2007. Effects of diesel exhaust particles on human lung epithelial cells: An in vitro study. *Respiratory Medicine,* 101, 1155-1162.

NEL, A. 2005. Air pollution-related illness: effects of particles. *Science,* 308, 804-806.

NIU, X., HO, S. S. H., HO, K. F., HUANG, Y., SUN, J., WANG, Q., ZHOU, Y., ZHAO, Z. & CAO, J. 2017. Atmospheric levels and cytotoxicity of polycyclic aromatic hydrocarbons and oxygenated-PAHs in PM2.5 in the Beijing-Tianjin-Hebei region. *Environ Pollut,* 231, 1075-1084.

NTZIACHRISTOS, L., FROINES, J. R., CHO, A. K. & SIOUTAS, C. 2007. Relationship between redox activity and chemical speciation of size-fractionated particulate matter. *Particle and Fibre Toxicology,* 4, 5.

SHAH, S. D., COCKER, D. R., MILLER, J. W. & NORBECK, J. M. 2004. Emission Rates of Particulate Matter and Elemental and Organic Carbon from In-Use Diesel Engines. *Environmental Science & Technology,* 38, 2544-2550.

SHAH, S. D., OGUNYOKU, T. A., MILLER, J. W. & COCKER, D. R. 2005. On-Road Emission Rates of PAH and n -Alkane Compounds from Heavy-Duty Diesel Vehicles. *Environmental science & technology,* 39, 5276-84.

SHEN, H., TAO, S., WANG, R., WANG, B., SHEN, G., LI, W., SU, S., HUANG, Y., WANG, X., LIU, W., LI, B. & SUN, K. 2011. Global time trends in PAH emissions from motor vehicles. *Atmospheric Environment,* 45, 2067-2073.

SHEN, X., YAO, Z., HUO, H., HE, K., ZHANG, Y., LIU, H. & YE, Y. 2014. PM2.5 emissions from light-duty gasoline vehicles in Beijing, China. *Science of The Total Environment,* 487, 521-527.

SKULAND, T. S., REFSNES, M., MAGNUSSON, P., OCZKOWSKI, M., GROMADZKA-OSTROWSKA, J., KRUSZEWSKI, M., MRUK, R., MYHRE, O., LANKOFF, A. & OVREVIK, J. 2017. Proinflammatory effects of diesel exhaust particles from moderate blend concentrations of 1st and 2nd generation biodiesel in BEAS-2B bronchial epithelial cells-The FuelHealth project. *Environ Toxicol Pharmacol,* 52, 138-142.

TSAI, Y. I., YANG, H.-H., WANG, L.-C., HUAN, J.-L., YOUNG, L.-H., CHENG, M.-T. & CHIANG, P.-C. 2011. The Influences of Diesel Particulate Filter Installation on Air Pollutant Emissions for Used Vehicles. *Aerosol and Air Quality Research,* 11, 578-583.

UNECE 2011. Consolidated Resolution on the Construction of Vehicle (R.E.3), revision. United Nations Economic Commission for Europe, Geneva, Switzerland.

WANG, B., LAU, Y.-S., HUANG, Y., ORGAN, B., LEE, S.-C. & HO, K.-F. 2019. Investigation of factors affecting the gaseous and particulate matter emissions from diesel vehicles. *Air Quality, Atmosphere & Health,* 12, 1113-1126.

WANG, B., LI, N., DENG, F., BUGLAK, N., PARK, G., SU, S., REN, A., SHEN, G., TAO, S. & GUO, X. 2016. Human bronchial epithelial cell injuries induced by fine particulate matter from sandstorm and non-sandstorm periods: Association with particle constituents. *J Environ Sci (China)*, 47**,** 201-210.

WEISS, M., BONNEL, P., KÜHLWEIN, J., PROVENZA, A., LAMBRECHT, U., ALESSANDRINI, S., CARRIERO, M., COLOMBO, R., FORNI, F., LANAPPE, G., LE LIJOUR, P., MANFREDI, U., MONTIGNY, F. & SCULATI, M. 2012. Will Euro 6 reduce the NOx emissions of new diesel cars? – Insights from on-road tests with Portable Emissions Measurement Systems (PEMS). *Atmospheric Environment*, 62**,** 657-665.

WU, B., SHEN, X., CAO, X., YAO, Z. & WU, Y. 2016. Characterization of the chemical composition of PM2.5 emitted from on-road China III and China IV diesel trucks in Beijing, China. *Science of The Total Environment*, 551-552**,** 579-589.

WU, D., ZHANG, F., LOU, W., LI, D. & CHEN, J. 2017. Chemical characterization and toxicity assessment of fine particulate matters emitted from the combustion of petrol and diesel fuels. *Sci Total Environ*, 605-606**,** 172-179.

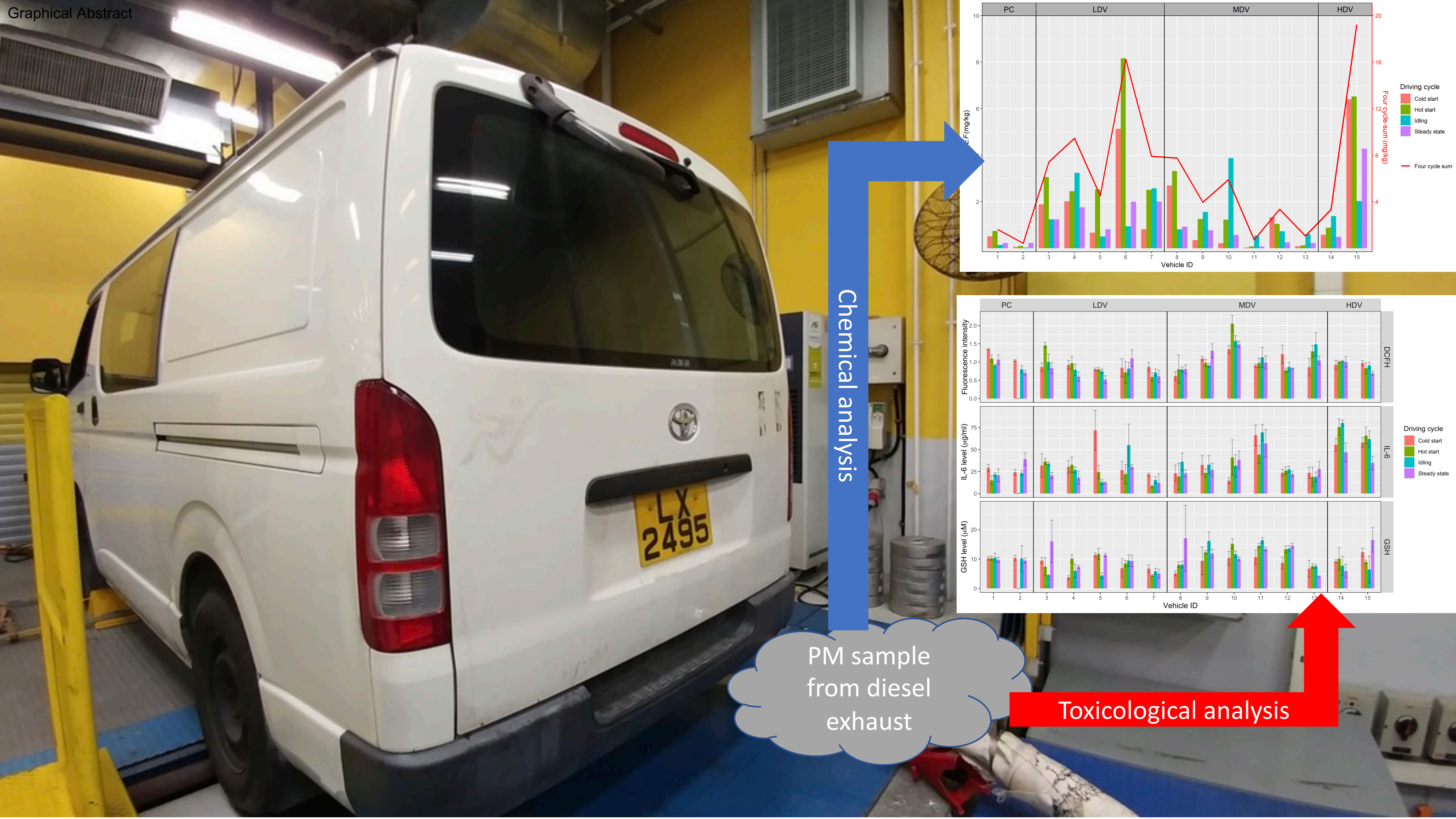
YAMASAKI, H., KUWATA, K. & MIYAMOTO, H. 1982. Effects of ambient temperature on aspects of airborne polycyclic aromatic hydrocarbons. *Environmental Science & Technology*, 16**,** 189-194.

YAO, Z., HUO, H., ZHANG, Q., STREETS, D. G. & HE, K. 2011. Gaseous and particulate emissions from rural vehicles in China. *Atmospheric Environment*, 45**,** 3055-3061.

ZHENG, M., HAGLER, G. S. W., KE, L., BERGIN, M. H., WANG, F., LOUIE, P. K. K., SALMON, L., SIN, D. W. M., YU, J. Z. & SCHAUER, J. J. 2006. Composition and sources of carbonaceous aerosols at three contrasting sites in Hong Kong. 111.

ZIELINSKA, B., SAGEBIEL, J., MCDONALD, J. D., WHITNEY, K. & LAWSON, D. R. 2004. Emission rates and comparative chemical composition from selected in-use diesel and gasoline-fueled vehicles. *J Air Waste Manag Assoc*, 54**,** 1138-50.

Graphical Abstract



ABSTRACT

This paper presents a detailed chemical and toxicological characterization of the diesel particulate matter (PM) emitted from diesel vehicles running on a chassis dynamometer under different driving conditions. Chemical analyses were performed to characterize the contents of organic carbon (OC), elemental carbon (EC), and 31 polycyclic aromatic hydrocarbons (PAHs) in the collected PM samples. The OC–EC analysis results revealed that PM emissions from diesel vehicles in this study were dominated by OC and that the emission of vehicles equipped with diesel particulate filters had high OC/EC ratios. The PAH analysis results revealed that 4- and 5-ring PAHs were the dominant PAHs in the OC fraction of the PM samples. Particle toxicity was evaluated through three toxicological markers in human A549 cells, namely (1) acellular 2,7-dichlorofluorescein (DCFH) for oxidative potential, (2) interleukin-6 (IL-6) for inflammation, and (3) glutathione (GSH) for antioxidation after exposure. Statistical analyses revealed that vehicle sizes have statistically significant effects on the concentrations of the markers. Correlation analysis between PAHs and toxicological markers revealed that significant correlations existed between specific compounds and markers. Our results can be used as a reference by policy makers to formulate emission control strategies and as a dataset for other modeling studies.

Response to reviews:

Reviewer #1: Most comments have been replied and only a few issues should be clarified or revised.

1. The authors have explained the test fuel used in this study. Since fuel plays a crucial role on air pollutant emissions. I suggest to add the descriptions in maintext but only in supplementary materials.
   R: Thanks for your suggestion. Description of the diesel fuel used in the current study was added in the main text, from line 125 to 126:
   - "The diesel fuel used by all vehicles in the current study is the same, which comply with the Euro 5 diesel fuel standard, as stated in **Table S3."**

2. The authors explained that calculation of emission factors does not require exhaust dilution ratio. Since dilution ratio is an important parameter and can be obtained easily after dyno test, I suggest to add these parameters in the manuscript.
   R: Thanks for your suggestion. Description of the dilution factor was added to the Supporting Information part, from line 45 to 50:
   - *"Dilution factor (DF)*
     The dilution factor (DF) of exhaust is defined as the volume ratio of diluted exhaust in the dilution tunnel and the raw exhaust gas from the vehicle at a certain moment of the driving cycle. In the current study, the averaged DFs in transient and steady state cycles for PC, LDV, MDV and HDV are around 20, 20, 10 and 5 respectively. The DFs of exhaust for PC, LDV, and MDV in idling cycles are around 50, while the DFs for HDV in idling cycle are around 20."

Reviewer #3: The literature review was improved significantly.

Most of the comments were addressed satisfactorily.

1. Relevant data were added, or the corresponding paper is named. Only the particle size distribution and number concentration are still missing. Maybe this data can be considered in further work.
   R: We agree that data of particle size distribution, number concentration and particle morphology would help to provide a more comprehensive picture of how the PM emission from diesel vehicle would affect human health. We have included this point in the suggestion for further study in the conclusion part.

2. line 481-483: As mentioned in review nr. 1 (general comment) the relevance of gaseous compounds (organic and inorganic) for health effects of diesel emissions is high. A significant proportion of vehicle emissions will enter the human body directly without significant changes due to atmospheric processes (proximity of pedestrians and vehicles). Therefore, more effects and data have to be considered when making suggestions to policy makers. Maybe the conclusion could be address uncertainties and further work that has to be done to evaluate the correlation between emissions and health effects.

R: Thanks for your suggestion. We agree that assessment of gaseous and semi-volatile compound toxicity has great importance and should be the next research topic. We have added this point in the conclusion part accordingly.
The corresponding changes for question 1 and 2 were added to the manuscript, from line 487 to 493:

- "Nevertheless, the currently study focused on the chemical and toxicological analysis of pollutants in particle phase only. Further studies on volatile and semi-volatile organic pollutants from diesel vehicle exhaust are warranted. Another aspect to be considered in future studies is the effect of particle size distribution and particle morphologies on the chemical and toxicological properties of the diesel PM samples. An integration of the outcomes of the aforementioned studies and the data in this paper can provide a more comprehensive picture of the chemical composition and toxicological properties of diesel emission in the future."

Other changes made in the manuscript:

A more precise description of the annual vehicle examination policy adopted by the HK government was given from line 121 to 125:

- "The annual vehicle examination is mandatory for all commercial vehicles, as well as passenger cars and light duty vehicles (vehicle weight under 1.9 tons) with first registration date over 6 years. The annual examination policy is to make sure that vehicles running on road are in acceptable maintenance condition."

13 November 2020

Dear Editor,

Attached please find our revised manuscript entitled "*Chemical and Toxicological Characterization of Particulate Emissions from Diesel Vehicles*", revised supplementary material (Revised Supporting Information_2nd revision), and a thorough, point-by-point response to each question raised by reviewers (Response to Reviewers). Thanks for your consideration of accepting our manuscript. We believe that our manuscript is suitable for publication in your journal.

Please don't hesitate to contact me at kfho@cuhk.edu.hk if you need additional information.

Sincerely,

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