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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.

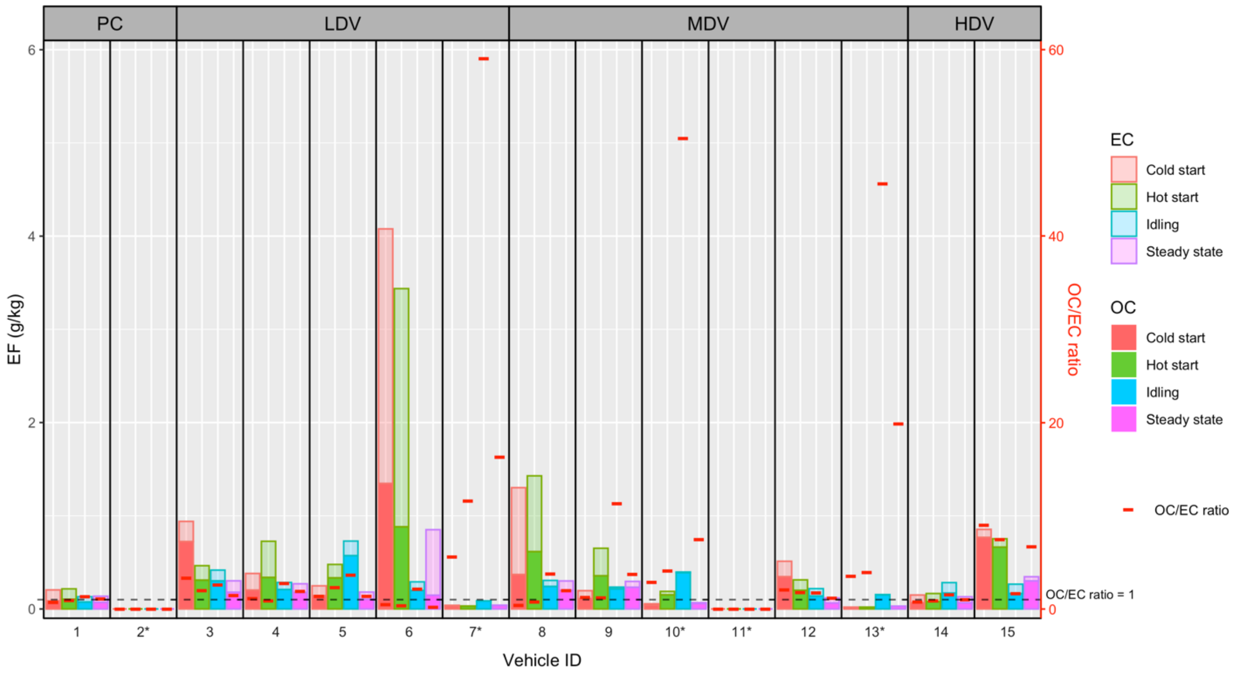

Fig. 1. *EFs* of OC and EC and OC/EC ratios. Each bar represents the sum of OC and EC *EF*. Light color bar represents EC *EF* and deep color bar represents OC *EF*. Vehicles with an asterisk next to their vehicle ID were equipped with DPF.

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 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.

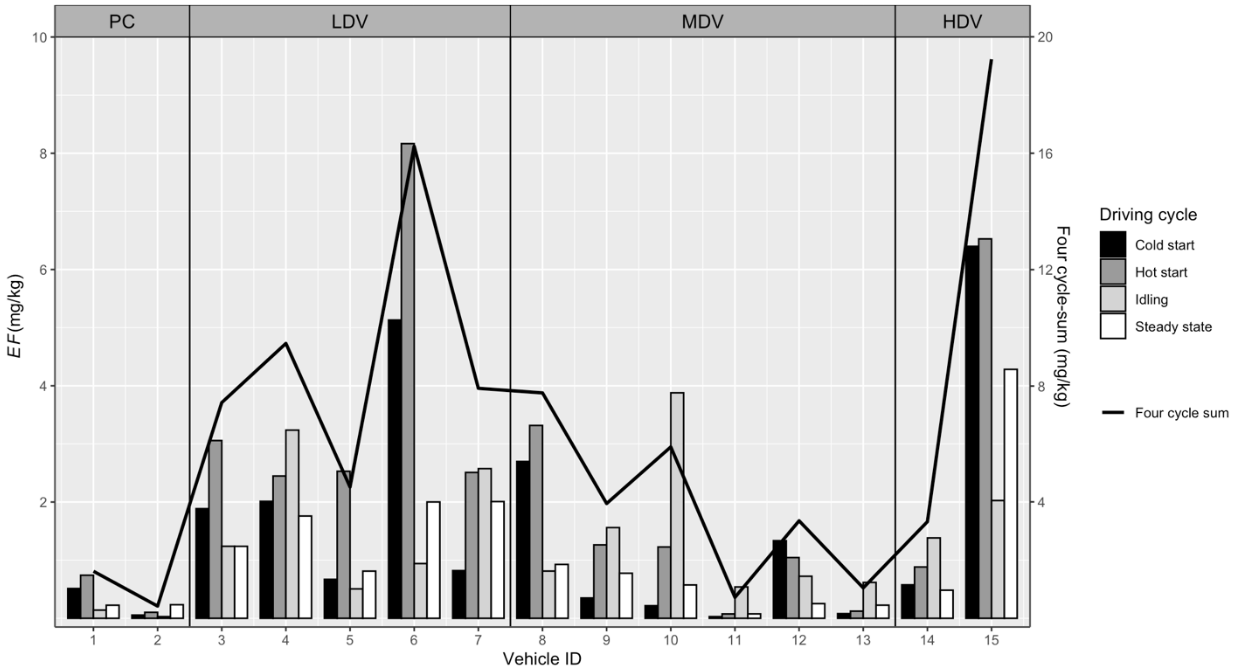

Fig. 2. Total PAH *EFs* in different driving cycles. The solid line represents the sum of total PAH *EFs* of the four driving cycles, denoted as "four cycle-sum".

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.

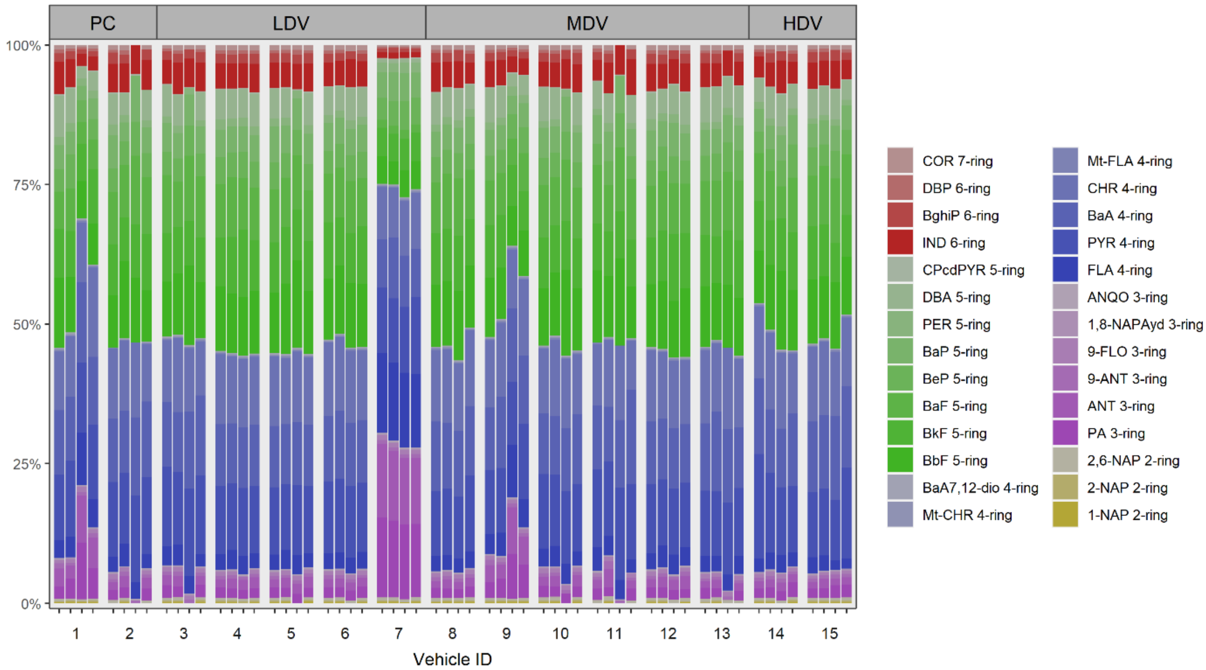

Fig. 3. Mass percentages of PAHs of each vehicle. The four bars under the same Vehicle ID correspond to cold start, hot start, idling and steady state cycles from left to right.

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.

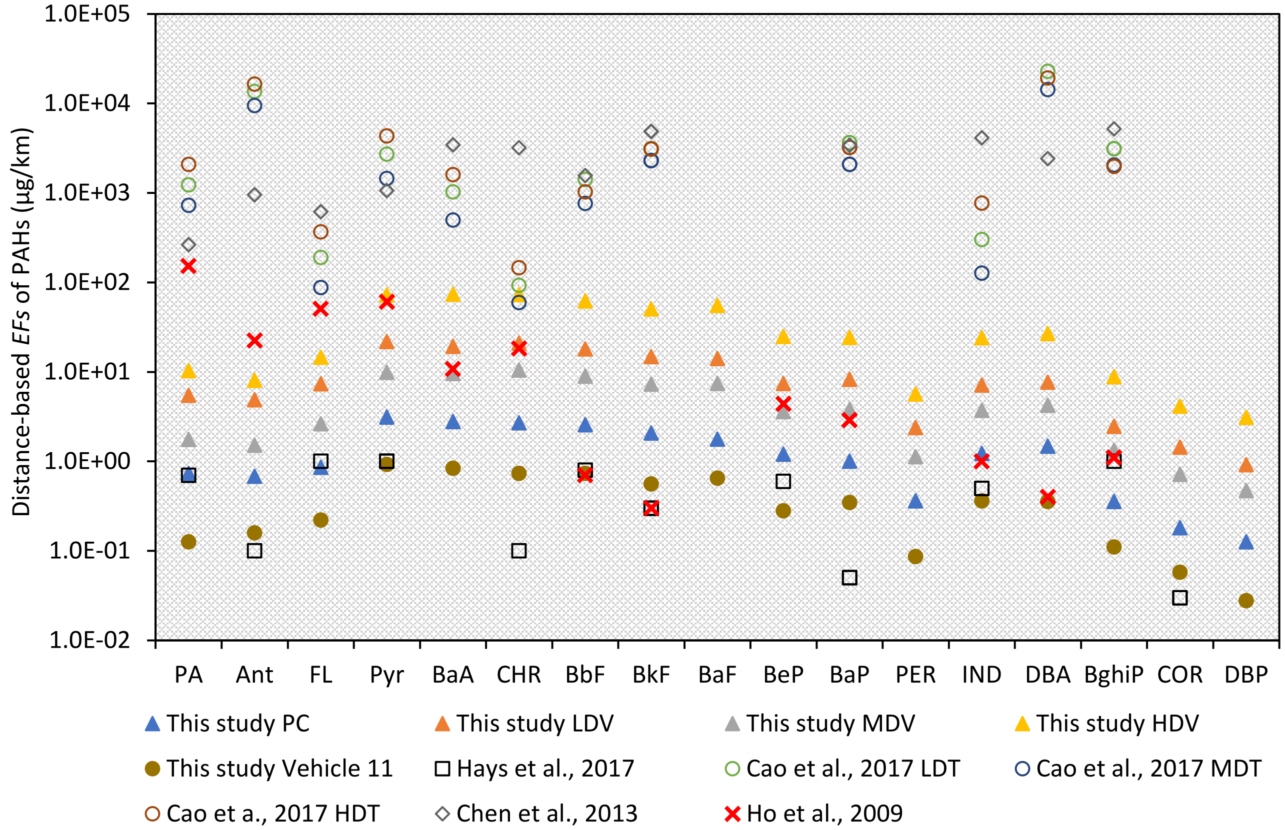

Fig. 4. Distance-based PAHs *EFs* of different vehicle classes.

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.

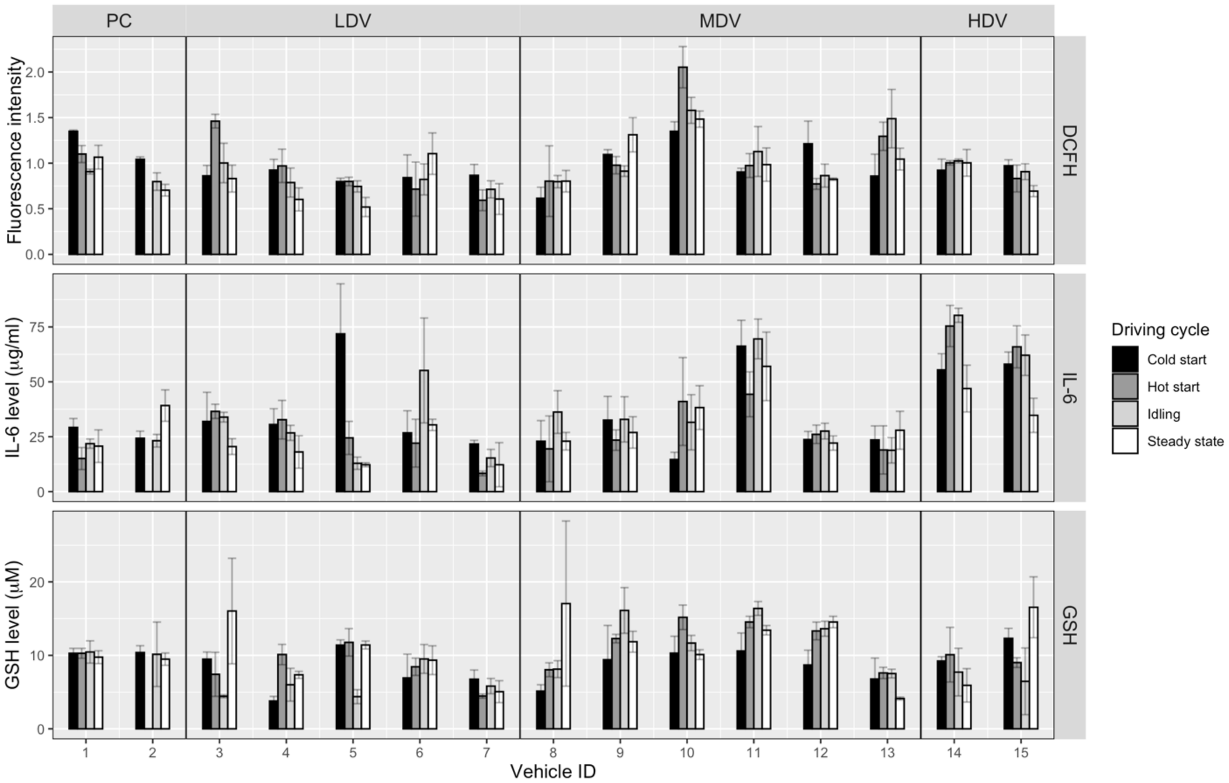

Fig. 5. Results of the production of ROS expressed as the fluorescent intensity of DCF, IL-6 level and GSH level of the 15 vehicles in different testing cycles.

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.

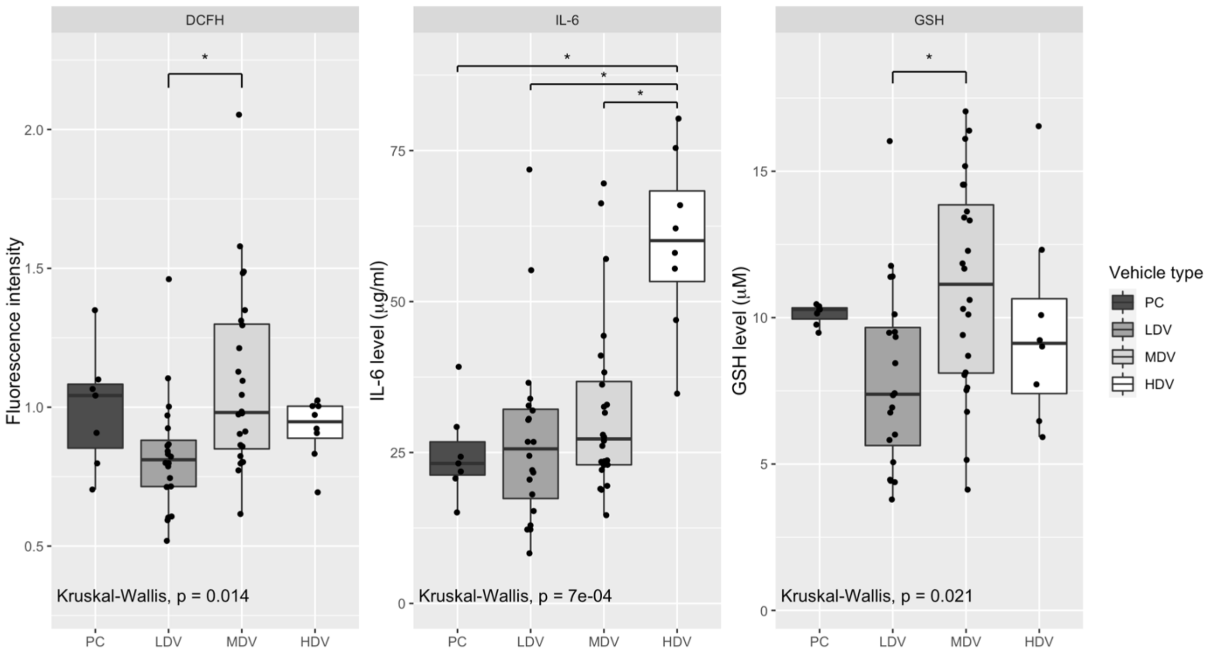

Fig. 6. Boxplots of the levels of DCFH, IL-6 and GSH grouped by vehicle type. Black dots represent the data points in the corresponding driving cycle. Asterisk represents the adjusted p-value in pairwise Wilcoxon rank sum test smaller than 0.05.

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	**IL-6**	**GSH**
		Spearman's ρ (p-value)	*Spearman's ρ (p-value)*	*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 (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. 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 (ρ = 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.

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