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## Chemical and toxicological characterization of particulate emissions from diesel vehicles

--Manuscript Draft--

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<b>Corresponding Author:</b>	Kin Fai Ho The Chinese University of Hong Kong Hong Kong, HONG KONG
<b>First Author:</b>	Bei Wang
<b>Order of Authors:</b>	Bei Wang Yik-Sze Lau Yuhan Huang Bruce Organ Hsiao-Chi Chuang Steven Sai Hang Ho, PhD Linli Qu Shun-Cheng Lee Kin Fai Ho
<b>Abstract:</b>	<p>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.</p>



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23 particulate matter (PM) emitted from diesel vehicles running on a chassis dynamometer under  
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38 *Keywords: PM emission; OCEC; PAH; DCFH; Interleukin-6 (IL-6); Glutathione (GSH);*  
39 *Driving Cycle; Chassis dynamometer*

## 40        **1. Introduction**

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

48    A large quantity of PM contains carbonaceous aerosols (Hou et al., 2011), which are typically  
49    classified into two categories, namely elemental carbon (EC) and organic carbon (OC). Lee et al.  
50    (2006) reported that carbonaceous aerosols are the dominant species of fine particles in the  
51    roadside environment in Hong Kong and account for approximately 44% of the PM<sub>2.5</sub>  
52    concentration in Hong Kong. Vehicular emissions are the major source of carbonaceous aerosols  
53    in Hong Kong (Cheng et al., 2010, 2011; Ho et al., 2002; Lee et al., 2006). Zheng et al. (2006)  
54    reported that more than 60% of the OC measured at a roadside station in Hong Kong originated  
55    from vehicular emissions. The OC in vehicle-emission-derived PM comprises various toxic  
56    organic compounds, including polycyclic aromatic hydrocarbons (PAHs). Several studies have  
57    reported that vehicular emissions, particularly from diesel vehicles, are the largest sources of PAHs  
58    in urban areas (Cecinato et al., 2014; Chen et al., 2013; Shen et al., 2011). Therefore, various

59 studies have been conducted to characterize the emission factors (EFs) and chemical compositions  
60 of PAHs emitted from diesel vehicles under different conditions (Zielinska et al., 2004; Tsai et al.,  
61 2011; Hu et al., 2013; Cao et al., 2017; Lin et al., 2019, Hays et al., 2017). In Hong Kong, the  
62 PAHs in vehicle emissions have been mainly characterized through tunnel measurement and the  
63 collection of ambient air samples (Cheng et al., 2010; Ho et al., 2002, 2009; Ma et al., 2016).  
64 Limited studies have been conducted on direct tailpipe emissions (e.g., chassis dynamometer test  
65 or portable emission measurement system [PEMS] study) from diesel vehicles in Hong Kong.

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

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

## 77        **2. Methodology**

### 78        **2.1 Fleet overview and instrumentation setup**

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

86        Chassis dynamometer tests were conducted in the Jockey Club Heavy Vehicle Emissions Testing  
87        and Research Centre (JCEC), Hong Kong. **Fig. S1** of the Supporting Information depicts the  
88        schematic of the test setup. All the testing facilities in the JCEC comply with the European  
89        standards for type approval tests. Two chassis dynamometers were used to test the vehicles with  
90        different weights. PCs and LDVs were tested on a Mustang dynamometer with a 48” (121.92 cm)  
91        single roller, whereas MDVs and HDVs were tested on a Mustang dynamometer with a 17.2”  
92        (43.688 cm) triple roller.

### 93        **2.2 Driving cycles and testing conditions**

94        Four driving cycles, namely the cold start transient, hot start transient, idling, and steady-state  
95        cycles, were used to test each vehicle. For convenience, the cold and hot start transient cycles are

96 called cold start and hot start cycles in the following text. For each vehicle, the cold start cycle test  
97 was repeated two times and the hot start, idling, and steady-state cycle tests were repeated three  
98 times. Detailed descriptions of the four driving cycles and loading conditions are presented in the  
99 Supporting Information.

### 100 **2.3 PM sample collection**

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

### 112 **2.4 Chemical analysis**

113 The samples collected on the quartz filters were used for OC/EC and PAH analyses. Each filter  
114 was cut exactly in half with a specially designed chopper for the two analyses. The contents of OC



115 and EC were analyzed using a Desert Research Institute Model 2001 Thermal/Optical Carbon  
116 Analyzer with the IMPROVE-A protocol described by Chow et al. (2012). The PAH samples were  
117 analyzed using the thermal desorption-gas chromatography/mass spectrometry method (Ho et al.,  
118 2008). The chemical analyses procedures are described in the Supporting Information.

## 119 **2.5 Toxicological analysis**

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

## 124 **2.6 Calculation of the EF and statistical analysis**

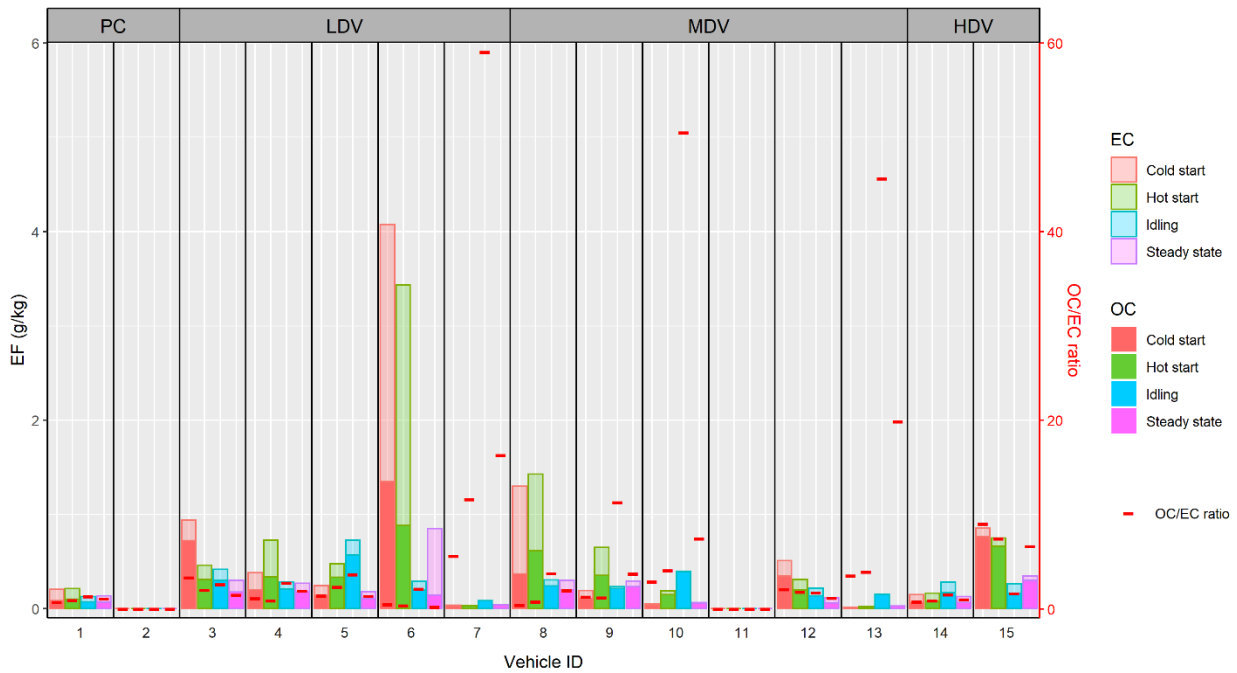
125 Details regarding the calculation of the EF and the statistical analysis performed in this study are  
126 presented in the Supporting Information.

# 127 **3. Results and discussion**

## 128 **3.1 OC and EC**

129 The EFs of OC and EC and the OC/EC ratios at different driving conditions are depicted in **Fig.**  
130 **1.** OC was the dominant fraction in all the collected samples except those from Vehicles 6 and 8,  
131 in which EC dominated. Several studies have indicated that EC is dominant in PM emissions from  
132 diesel vehicles (Chiang et al., 2012; Grieshop et al., 2006; Kleeman et al., 2000), whereas other

133 studies have reported contrasting results (Shah et al., 2004; Wu et al., 2016). Gali et al. (2017)  
 134 indicated that under cold idle, or low-engine-speed conditions, OC is the dominant fraction in PM,  
 135 which is consistent with our results.



136  
 137 **Fig. 1.** *EFs* of OC and EC and OC/EC ratios. Each bar represents the sum of OC and EC *EF*.

138 Light color bar represents EC *EF* and deep color bar represents OC *EF*.

139 The variation in the OC/EC ratio was caused by the difference in the emission standard, testing  
 140 weight, engine power, and capacity and maintenance conditions of the test vehicles. The EC  
 141 content in emissions from vehicles equipped with diesel particulate filters (DPFs) was less than  
 142 the detection limit (Vehicles 2 and 11) or extremely low ( $0.003 \pm 0.002$ ,  $0.017 \pm 0.014$ , and  $0.003$   
 143  $\pm 0.001$  g/kg for Vehicles 1, 10, and 13, respectively). This observation is consistent with the  
 144 findings of May et al. (2014a), who reported that DPFs can effectively decrease the EC emission  
 145 from diesel vehicles. The results also revealed that the EC removal by DPFs was satisfactory even

146 for vehicles with high odometer readings (e.g. Vehicles 10, 11, and 13). As depicted in **Fig. 1**, high  
147 OC/EC ratios were observed for Vehicles 7 (59.06), 10 (50.51), and 13 (45.63) under the idling  
148 condition. For these three vehicles, the EC concentration was very low and the OC concentration  
149 was high. The high OC content in the PM samples of Vehicles 7, 10, and 13 could be partially  
150 attributed to the property of quartz filters in collecting PM samples. As revealed by May et al.  
151 (2013), using quartz filters alone to collect PM sample results in the collection of small quantities  
152 of semivolatile and low-volatility organic compounds. Because these compounds are in the gas  
153 phase, they are not removed by the DPF. Therefore, the DPF-equipped vehicles (Vehicles 7, 10,  
154 and 13) emitted substantial quantities of OC, which was a possible reason for the higher OC/EC  
155 ratio obtained in this study than in other related studies.

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

161 The European emission standard assigned to the vehicle considerably affected OC and EC  
162 emissions. Typically, the EFs of OC and EC decreased with an increase in emission standards,  
163 except in the cases of Vehicles 6, 12, and 15. An extremely low level of EC and a measurable level  
164 of OC were recorded in vehicles with high emission standards (e.g., Vehicles 7 and 13). A possible

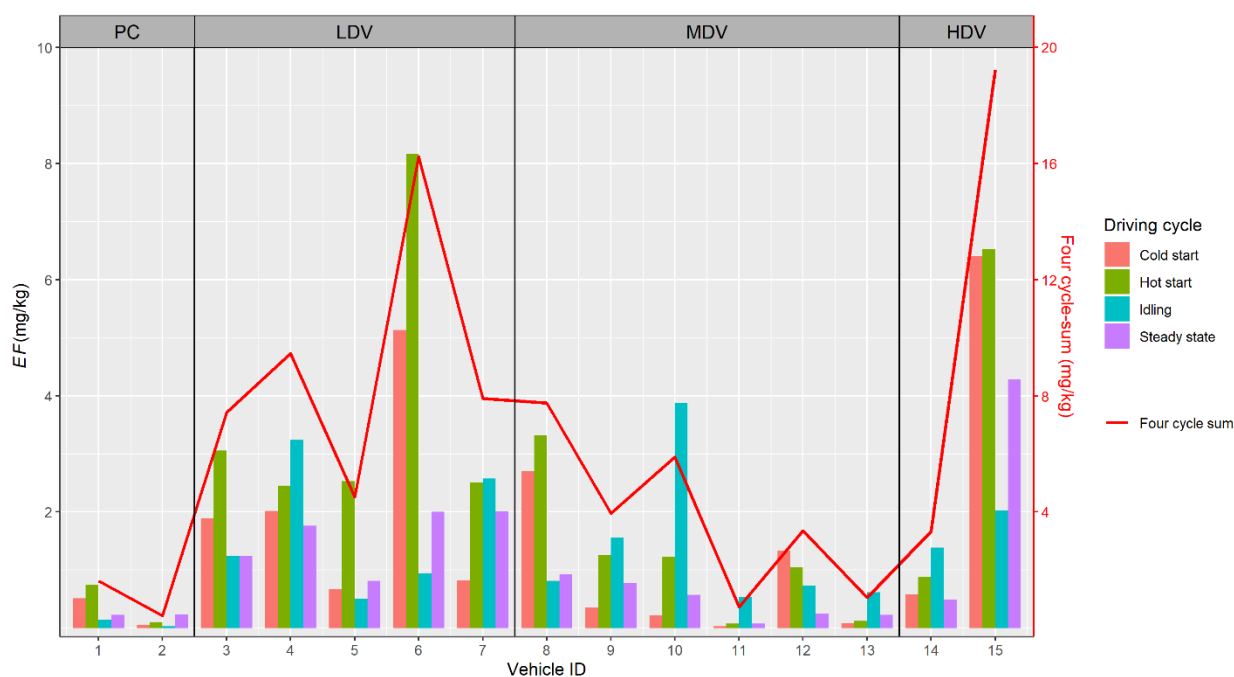
165 reason for this result is that the emission control technologies applied in new vehicles may not  
166 effectively remove OC from diesel vehicle exhausts. Because most of the toxic and mutagenic  
167 properties of diesel exhaust are associated with OCs (Claxton, 2015; Shah et al., 2004), our results  
168 suggest that the development of emission control technologies should focus on reducing the OC  
169 fraction of PM.

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

### 178 **3.2 PAHs**

179 A total of 31 PAHs (**Table S2**) were characterized, and their EFs were calculated. In all the tests,  
180 the EFs of acenaphthylene, acenaphthene, and fluoranthene were less than the detection limit  
181 because these three PAHs have low molecular weight and are mainly present in the gas phase.  
182 Therefore, the aforementioned three PAHs were excluded in the following analyses. **Fig. 2**  
183 presents an overview of the PAH EFs under different driving conditions. Each bar in **Fig. 2**

184 corresponds to the total PAH EF (sum of the EFs of all the PAHs tested) in a given driving cycle,  
 185 and the red solid line represents the sum of the total PAH EFs in the four driving cycles (denoted  
 186 as “four-cycle sum” in the following text). As displayed in **Fig. 2**, Vehicle 15 emitted the highest  
 187 four-cycle sum of 18.60 mg/kg, followed by Vehicle 6. Vehicle 2 emitted the lowest four-cycle  
 188 sum of 0.41 mg/kg, followed by Vehicles 11 and 13. A common feature of these three vehicles  
 189 with low four-cycle sums was that they were equipped with DPFs.



190  
 191 **Fig. 2.** Total PAH EFs in different driving cycles. The red solid line represents the sum of total  
 192 PAH EFs of the four driving cycles, denoted as “four cycle-sum”.

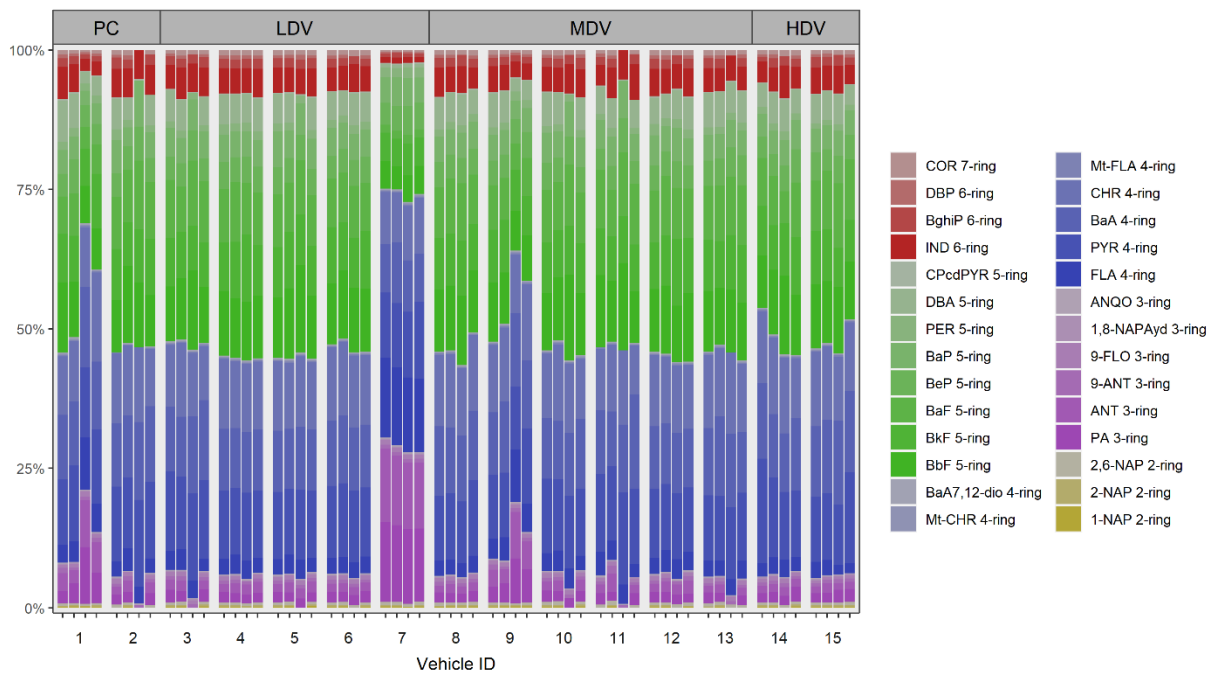
193 **Fig. 2** reveals that the MDVs had lower total PAH EFs than the LDVs did. Unexpectedly, vehicles  
 194 with greater testing weights emitted less PAHs. A possible explanation for this result is that two  
 195 DPF-equipped vehicles were present in the MDV class, whereas only one DPF-equipped vehicle  
 196 was present in the LDV class. Cao et al. (2017) observed the aforementioned pattern for their

197 vehicle fleet, which comprised 18 diesel trucks in China. The aforementioned observation was not  
198 an isolated event, which suggested that the effect of the vehicle size on the PAH emission should  
199 be further investigated.

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

207 To identify the dominant PAHs emitted from each vehicle, the weighted percentage of each PAH  
208 emitted under different driving cycles was determined (**Fig. 3**). For most of the vehicles, the  
209 dominant PAHs were 4- and 5-ring PAHs, including pyrene (PYR), benz[*a*]anthracene (BaA),  
210 chrysene (CHR), benzo[*b*]fluoranthene (BbF), benzo[*k*]fluoranthene (BkF), and  
211 benzo[*a*]fluoranthene (BaF). This finding was consistent with those reported in previous studies  
212 (Cao et al., 2017; Hu et al., 2013). The difference in the PAH composition under different driving  
213 conditions is illustrated in **Fig. 3**. The distributions of individual PAHs did not exhibit considerable  
214 variations when a given vehicle was tested under different driving conditions, except when  
215 Vehicles 1 and 9 were tested under the idling and steady-state cycles. The variation in the PAH

216 composition among vehicles was not significant, except for Vehicle 7. Excluding the  
 217 aforementioned two exceptions, the PAHs collected in all the driving cycles were dominated by  
 218 4- and 5-ring PAHs for all the vehicles. This observation suggests that the driving conditions,  
 219 driving pattern (NEDC or FIGE), mileage, testing weight of the vehicle, and after-treatment  
 220 technologies do not considerably affect the composition of the emitted PAHs. Furthermore, in  
 221 general the collected PAH samples originated from the same source, probably fuel combustion,  
 222 because they all had similar compositions. Therefore, the PAH samples collected in the  
 223 exceptional cases (for Vehicle 7 and in the idling and steady-state cycles for Vehicles 1 and 9)  
 224 were probably affected by other sources. Further investigations are required to characterize the  
 225 sources of PAHs collected from the tailpipe emissions of diesel vehicles.



226

227 **Fig. 3.** Mass percentages of PAHs of each vehicle. The four bars under the same Vehicle ID

228

correspond to cold start, hot start, idling and steady state cycles from left to right.

### 229 3.3 Comparison with other studies

230 The PAH data obtained in this current study were compared with the results of similar studies.

231 Unlike the fuel-based EF used in the previous sections, distance-based EFs ( $EF_{sd}$ ) were used in

232 this section because most of the results in previous studies were presented in a distance-based

233 manner. The  $EF_{sd}$  value for each PAH was calculated as the ratio of the total quantity of PAHs

234 released in a driving cycle to the distance traveled in the driving cycle. The driving distances in

235 the NEDC, FIGE, and steady-state cycles were 11.0, 29.5, and 16.7 km, respectively. An averaged

236  $EF_{sd}$  for each vehicle class (i.e. PCs, LDVs, MDVs, and HDVs) was calculated. **Fig. 4** presents a

237 comparison of the averaged  $EF_{sd}$  of each PAH from each vehicle class in this study with the PAHs

238 in diesel vehicle emissions in a PEMS study in China (Cao et al., 2017), tunnel studies in China

239 (Chen et al., 2013) and Hong Kong (Ho et al., 2009), and a chassis dynamometer study in the US

240 (Hays et al., 2017). As depicted in **Fig. 4**, the results of the current study were in the range reported

241 in previous studies. Cao et al. (2017) reported the highest PAH  $EF_{sd}$  values among the compared

242 studies. Their vehicle fleet comprised 18 diesel trucks with China 3 and 4 emission standards

243 (equivalent to Euro 3 and 4, respectively). In general, the PAH  $EF_{sd}$  results of Cao et al. were an

244 order of magnitude higher than those obtained for the HDVs in this study (except for CHR). The

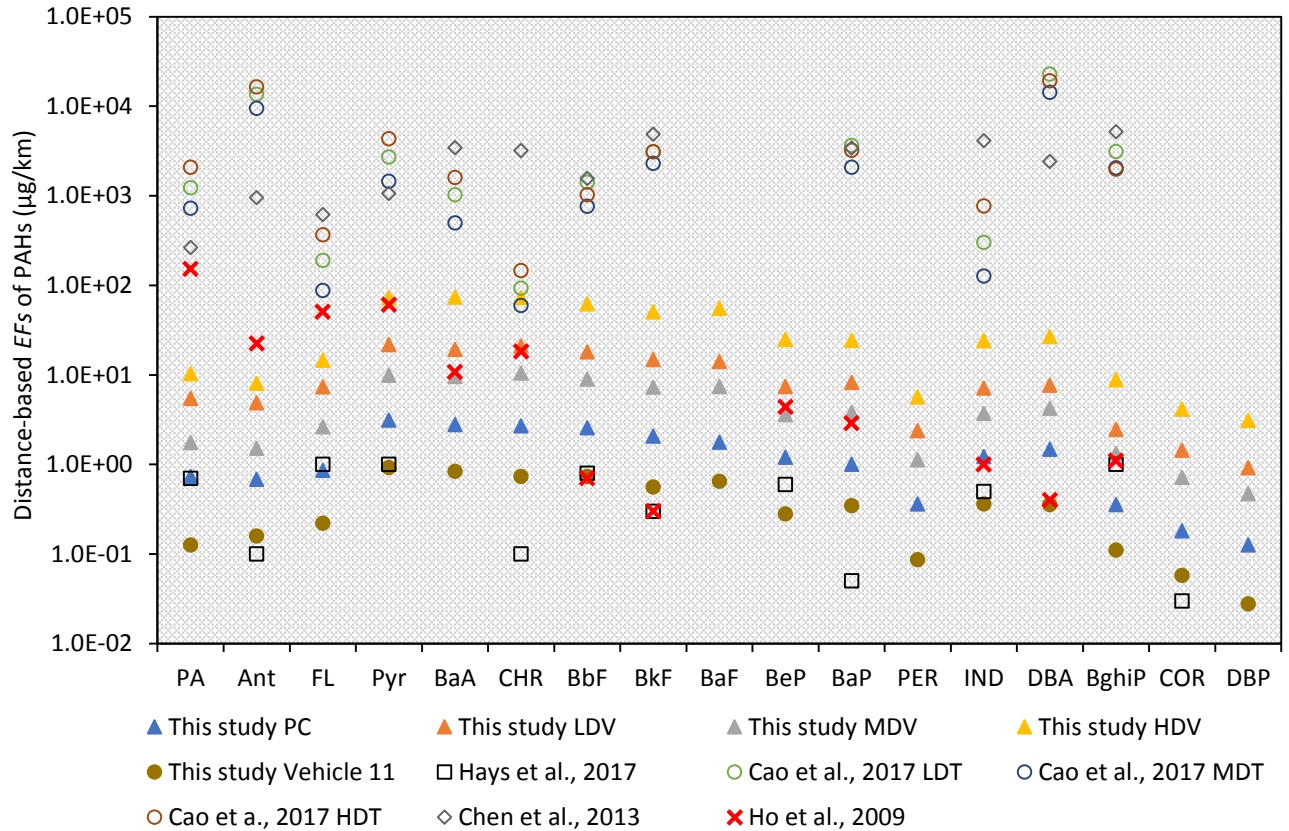
245  $EF_{sd}$  value of ANT in the study of Cao et al. (2017) was four orders of magnitude higher than that

246 in this study, which could be attributed to two main reasons. First, the emission standards of the

247 tested HDVs (Euro 4 and 5) in this study were higher than those of Cao's fleet. Second, Cao et al.



248 (2017) measured on-road emissions by using a PEMS. The emission EFs during on-road driving  
 249 are considerably higher than those in laboratory chassis dynamometer tests (Huang et al., 2018;  
 250 May et al., 2014b; Weiss et al., 2012) because the driving conditions in the real world are more  
 251 rigorous than the driving cycles tested in chassis dynamometer studies.



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

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

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

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

### 278 3.4 Toxicological analysis

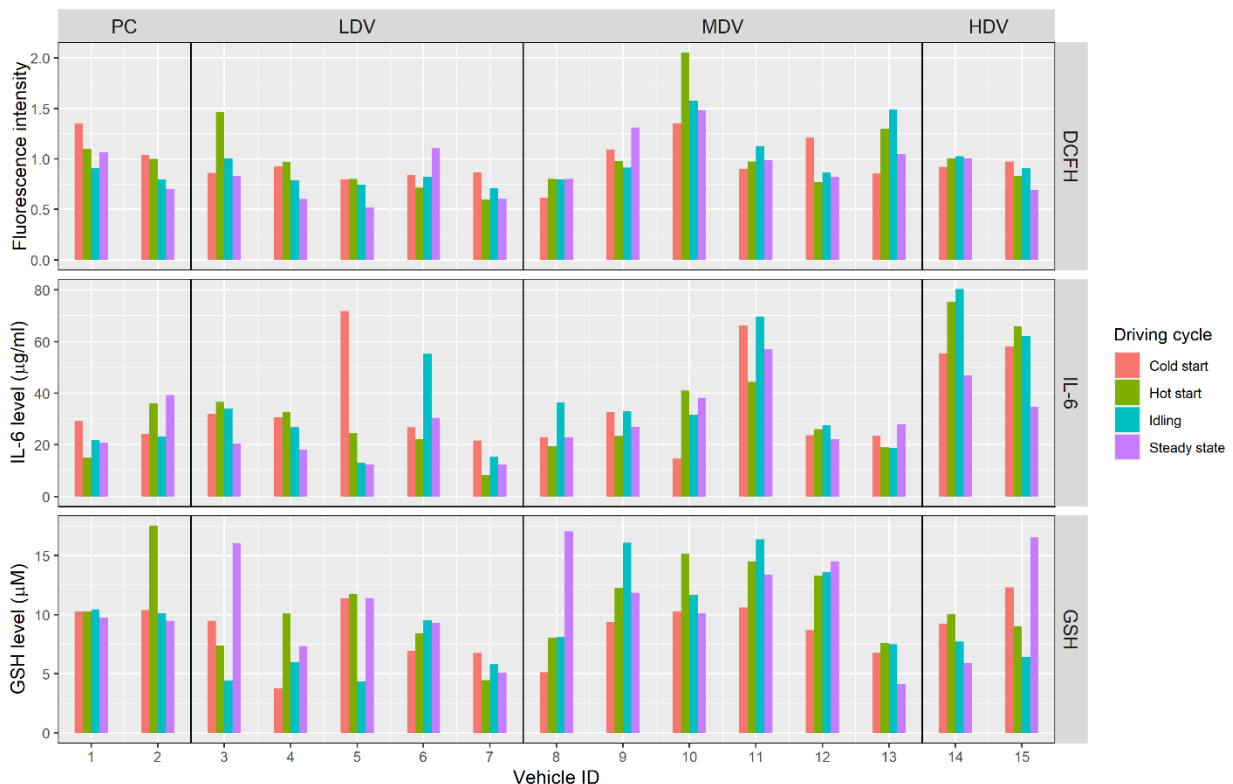
279 The levels of DCFH, IL-6, and GSH were analyzed to assess the cellular oxidative and  
280 inflammatory responses produced by the PM samples. The toxicological results of the current  
281 study cannot be compared with those of other studies because the cell culture conditions in this  
282 study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

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

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

297 DPF were  $9.89 \pm 3.97$  and  $9.85 \pm 3.37$   $\mu\text{M}$ , respectively. This result indicated that DPFs removed  
 298 PM mass without changing the GSH response of the PM; thus, DPFs probably did not significantly  
 299 change the morphology and composition of the PM samples.

300 IL-6 is a proinflammatory cytokine released in response to PM exposure. The IL-6 results are  
 301 illustrated in the middle panel of **Fig. 5**. The idling cycle for Vehicle 14 exhibited the highest IL-  
 302 6 level of 80.3, whereas the hot start cycle for Vehicle 7 exhibited the lowest IL-6 level of 8.30. In  
 303 general, no clear trend was observed for the effect of driving cycles on the IL-6 levels. HDVs had  
 304 the highest IL-6 concentrations among the vehicle classes, followed by MDVs. The IL-6 levels of  
 305 PCs, LDVs, and MDVs did not exhibit significant variations.



306

307 **Fig. 5.** Results of the production of ROS expressed as the fluorescent intensity of DCF, IL-6

308

level and GSH level of the 15 vehicles in different testing cycles.

309

310

311 *3.4.1 Effects of the vehicle type and driving cycle on the toxicological markers*

312 To determine the effects of the vehicle type on the three toxicological markers, the levels of each

313 toxicological marker for all vehicles were pooled and grouped according to their corresponding

314 vehicle type (i.e., PC, LDV, MDV, and HDV). The results are presented using boxplots in **Fig. 6**.

315 As depicted in **Fig. 6**, a certain degree of variation existed between vehicle types for the three

316 toxicological markers. The LDVs produced lower responses for DCFH and GSH than the other

317 three vehicle types did. The HDVs exhibited a higher IL-6 level than the other three vehicle types.

318 The results were verified by conducting a Kruskal–Wallis H test for each toxicological marker at

319 a significance level of  $p = 0.05$ . The Kruskal–Wallis H test results presented in the bottom left of

320 each boxplot indicated that statistically significant differences existed among the four vehicle types

321 for the three toxicological markers. The pairwise Wilcoxon rank-sum test was conducted as the

322 post-hoc test of the Kruskal–Wallis test to determine which vehicle type pair had significant

323 differences in their toxicological marker levels at a significance level of 0.05. The vehicle type

324 pairs with adjusted  $p$  values of  $<0.05$  are marked with an asterisk in **Fig. 6**. Significant differences

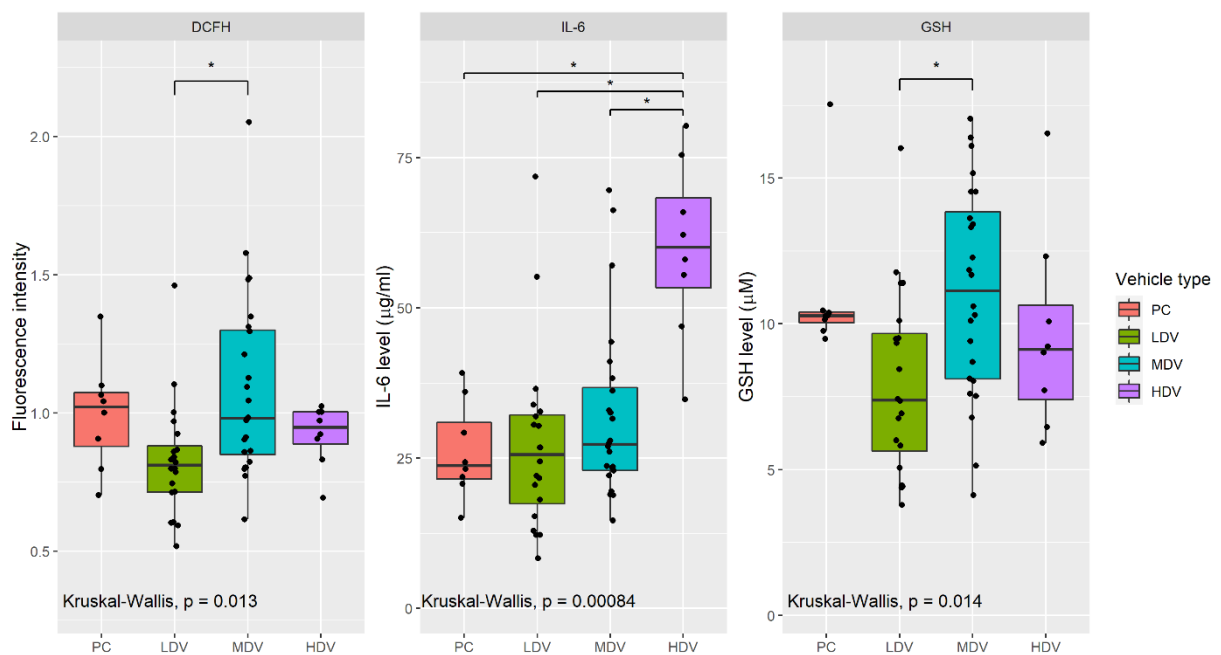
325 were identified in the DCFH and GSH levels of the LDVs and MDVs. Moreover, significant

326 differences were identified in the IL-6 levels of the HDVs and other types of vehicles. The

327 aforementioned results suggested that the vehicle type affected the response of the three

328 toxicological markers. Further investigations are required to determine the mechanisms or reasons  
 329 leading to this observation.

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



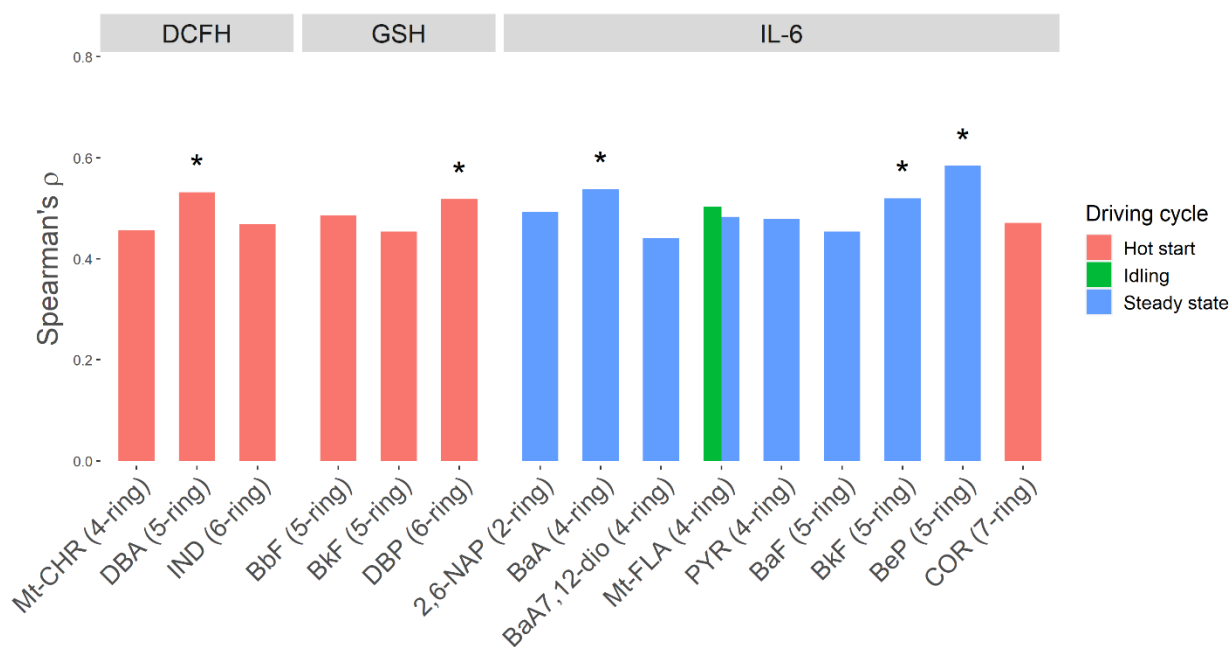
337

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

341

### 342 3.5 Correlation between the toxicity data and the PAH concentration

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



347

348 **Fig. 7.** Correlation between mass concentration of selected PAHs and toxicology test results. P-

349 values of all the correlation coefficients in this figure are less than 0.1. Asterisk indicates that the

350 p-value of the corresponding correlation coefficient is less than 0.05.

351 Correlation analysis was conducted between the concentrations of the PAHs and toxicological

352 markers according to the method stated in the Methodology section. The correlation test results

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

362 As depicted in **Fig. 7**, the DCFH levels and PAH concentrations exhibited correlation only under  
363 the hot start condition. Moderate correlations were found between DCFH level and Mt-CHR ( $\rho =$   
364  $0.46, p < 0.1$ ) and between DCFH level and IND ( $\rho = 0.47, p < 0.1$ ). Moreover, a good correlation  
365 was found between DCFH level and DBA ( $\rho = 0.53, p < 0.05$ ). This result agreed with that of Wu  
366 et al. (2017), who found a strong correlation between ROS generation and DBA in petrol and  
367 diesel fuel combustion experiments. The GSH and PAH levels were correlated only in the hot start  
368 driving cycle. BbF ( $\rho = 0.48, p < 0.1$ ) and BkF ( $\rho = 0.45, p < 0.1$ ) exhibited moderate correlations  
369 with GSH, and DBP ( $\rho = 0.51, p < 0.05$ ) exhibited a good correlation with GSH. Notably, both the  
370 DCFH and GSH levels were related to ROS generation. Moreover, all the reasonably good  
371 correlations of the PAH levels with DCFH and GSH levels were found in the hot start cycle.



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

374 **Fig. 7** displays the correlations between the proinflammatory mediator IL-6 and selected PAHs.  
375 Moderate correlation was found between IL-6 and COR ( $\rho = 0.47, p < 0.1$ ) in the hot start cycle.  
376 The correlation between IL-6 and Mt-FLA ( $\rho = 0.50, p < 0.1$ ) was the only significant correlation  
377 in the idling cycle. Except the aforementioned two correlations, all the observed correlations  
378 between IL-6 and PAHs were found in the steady-state cycle. The compound 2,6-NAP ( $\rho = 0.49,$   
379  $p < 0.1$ ) was the only PAH with less than three rings that exhibited correlation with the toxicology  
380 results. IL-6 exhibited moderate correlation with Ba7,12-dio ( $\rho = 0.44, p < 0.1$ ), Mt-FLA ( $\rho = 0.48,$   
381  $p < 0.1$ ), PYR ( $\rho = 0.48, p < 0.1$ ), and BaF ( $\rho = 0.45, p < 0.1$ ). Moreover, IL-6 exhibited significant  
382 correlations with BaA ( $\rho = 0.54, p < 0.05$ ), BkF ( $\rho = 0.52, p < 0.05$ ), and BeP ( $\rho = 0.58, p < 0.05$ ).  
383 Several studies have assessed the correlations among IL-6 and particle-bounded PAHs from  
384 different sources; however, they have obtained different results. Niu et al. (2017) and Chowdhury  
385 et al. (2019) have investigated the correlation between PAHs in an atmospheric PM sample and  
386 the IL-6 responses in an A549 cell and a BEAS-2B cell (human bronchial epithelial cell),  
387 respectively. They have determined that certain PAHs are positively correlated with the IL-6 level.  
388 Lin et al. (2013) studied the effect of household particles on inflammation in human coronary  
389 artery endothelial cells (HCAECs) and revealed that PAHs were significantly correlated with the  
390 IL-6 level. Delfino et al. (2010) analyzed blood samples from 60 people and the air samples in

391 their vicinity. Their results suggested a positive correlation between PAHs in air samples and the  
392 IL-6 level in human blood samples. Our findings are in agreement with those of the  
393 aforementioned studies, which support a positive correlation between PAHs and the IL-6 level.  
394 However, a study conducted by Skuland et al. (2017) could not establish a clear connection  
395 between the total or individual PAH levels in diesel exhaust particles and the IL-6 level in a BEAS-  
396 2B cell. Chuang et al. (2012) could not find a significant correlation between the PAHs in air  
397 samples and the IL-6 level in HCAECs. Moreover, Wang et al. (2016) found a significant negative  
398 correlation between the PAHs in atmospheric PM samples and the IL-6 level in BEAS-2B cells.  
399 The aforementioned studies suggest that in some cases, PAHs might not be the main inducer of  
400 proinflammatory response. Therefore, further investigations are essential to elucidate the reaction  
401 mechanism for the release of IL-6 and other proinflammatory mediators.

402

#### 403 **4. Conclusion**

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

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

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

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431

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- 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.



香港中文大學  
The Chinese University of Hong Kong

Dr. Kin Fai Ho

JC School of Public Health and Primary Care

The Chinese University of Hong Kong

Shatin, N.T., Hong Kong

Tel: 852-2252-8763, Fax: 852-2606-3500

Email: kfho@cuhk.edu.hk

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.



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## 25 **ABSTRACT**

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

## 45 INTRODUCTION

46 Hong Kong is one of the most densely populated cities in the world, and the vast majority of the  
47 population is exposed to vehicular exhaust, as many people work and live close to main roads and  
48 even highways. By the end of 2017, there were more than 830,000 licensed motor vehicles running  
49 on the roads, which was increased by 35% compared to 2007 (Hong Kong Transport Department,  
50 2018). Among all vehicles, diesel vehicles are the main sources of street-level particulate matter  
51 (PM) pollution (Lee et al., 2006, Shen et al., 2014, Weiss et al., 2012, Yao et al., 2011).

52 A large fraction of PM is comprised of carbonaceous aerosols (Hou et al., 2011). In general, the  
53 carbonaceous aerosols are classified into two categories: elemental carbon (EC) and organic  
54 carbon (OC). Lee et al. (2006) reported that the carbonaceous aerosols were the dominating species  
55 in fine particles in the roadside environment in Hong Kong, accounting for around 44% of PM<sub>2.5</sub>.  
56 Lee and his research team suggested that vehicular emissions were the major sources of  
57 carbonaceous aerosols (Cheng et al., 2010, Cheng et al., 2011, Ho et al., 2002, Lee et al., 2006).  
58 Zheng et al. (2006) reported that more than 60% of OC measured at a roadside station in Hong  
59 Kong came from vehicular emissions. The OC fraction in vehicle emission derived PM is made  
60 up of various toxic organic compounds, and polycyclic aromatic hydrocarbon (PAH) is one of  
61 them. PAHs are important toxic components of vehicle emitted organic species. They are  
62 identified as one of the major toxic air pollutants, mainly originated from anthropogenic processes,  
63 especially from incomplete combustion of organic matters. PAHs are semi-volatile organic

64 compounds that can be present in both particulate and gaseous phases. PAHs are present on the  
65 surface of airborne particles through condensation, adsorption or combustion processes (Yamasaki  
66 et al., 1982, Dimashki et al., 2001). Several studies have reported that vehicular emissions,  
67 particularly diesel vehicles, are the most important sources of PAHs in urban areas (Cecinato et  
68 al., 2014, Chen et al., 2013, Shen et al., 2011). Therefore, various studies have been done to  
69 characterize the *EFs* and chemical composition of PAHs emitted from diesel vehicles under  
70 different conditions (Zielinska et al., 2004, Tsai et al., 2011, Hu et al., 2013, Cao et al., 2017, Hays  
71 et al., 2017, Lin et al., 2019). In Hong Kong, the characterization of PAH derived from vehicle  
72 emission were mainly done by tunnel measurement and ambient air sample collection (Ho and  
73 Lee, 2002, Cheng et al., 2010, Ho et al., 2009, Ma et al., 2016). For example, Ho et al. (2009)  
74 conducted a tunnel study to investigate the PAHs emission from vehicles in Hong Kong. The  
75 authors concluded that two- and three-ring PAHs were the dominant fraction in gas phase, while  
76 four-ring PAHs were the most abundant in particle phase. Since studies of direct tailpipe emission  
77 (e.g. chassis dynamometer or PEMS study) from diesel vehicles in Hong Kong are scarce, carrying  
78 out such kind of research is needed to fill the knowledge gap.

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

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

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

102 secondary organic aerosol (SOA), which is, in many cases, more harmful than the precursor (Lin  
103 et al., 2019). Therefore, characterizing the *EF* of PAH is important to air quality control as well as  
104 the health of the general public.

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

## 109 **METHODOLOGY**

### 110 **Fleet overview and instrumentation set-up**

111 The vehicle fleet in this study was comprised of 15 vehicles covering a wide range of vehicle  
112 classes, engine sizes, after-treatment technologies, and emission standards (**Table S1**). The vehicle  
113 fleet was selected briefly in accordance to the emission standard distribution of diesel vehicles in  
114 Hong Kong, where most of them are with Euro 4 and 5, followed by Euro 3 and others. Vehicle  
115 categories were classified with reference to the United Nations Economic Commission for Europe  
116 (UNECE) categories (UNECE, 2011). Passenger cars (PCs) were defined as M type vehicles. Light  
117 Duty Vehicles (LDVs), Medium Duty Vehicles (MDVs) and Heavy Duty Vehicles (HDVs)  
118 corresponded to N1, N2 and N3 type vehicles, respectively. Vehicle 15 was exceptionally  
119 categorized as HDV since it was a 10-ton tractor designed for carrying a trailer up to 20 tons. All  
120 vehicles, except Vehicle 2 and 7, have taken and passed the annual vehicle examination required

121 by the Transport Department of the Hong Kong Government. The annual vehicle examination is  
122 mandatory for vehicles with first registration date over 6 years in order to make sure that vehicles  
123 running on road are in acceptable maintenance condition. The diesel fuel used by all vehicles in  
124 the current study is the same, which comply with the Euro 5 diesel fuel standard, as stated in **Table**  
125 **S3**.

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

### 133 **Driving cycles and testing conditions**

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

140 **PM sample collection**

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

151 **Chemical analysis**

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

## 159 **Toxicological analysis**

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

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

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

## 170 **RESULTS AND DISCUSSION**

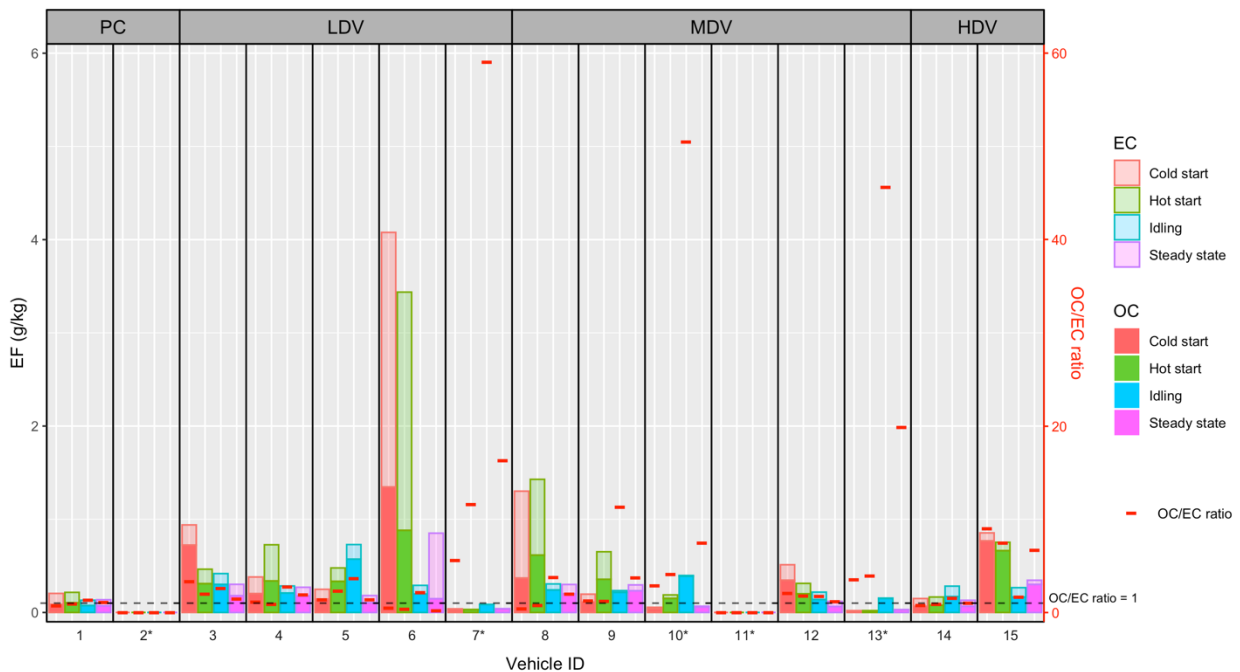
### 171 **[PM emission characteristics](#)**

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



176 **OC and EC**

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



185 **Fig. 1.** EFs of OC and EC and OC/EC ratios. Each bar represents the sum of OC and EC EF.  
186 Light color bar represents EC EF and deep color bar represents OC EF. Vehicles with an asterisk  
187 next to their vehicle ID were equipped with DPF.  
188

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

205 Among the four driving conditions tested in this study, idling generally produced the highest  
206 OC/EC ratio. As mentioned, EC mainly arises from fuel droplet pyrolysis, while OC is mainly  
207 originated from unburned fuel and incomplete combustion (Shah et al., 2004). When the vehicle

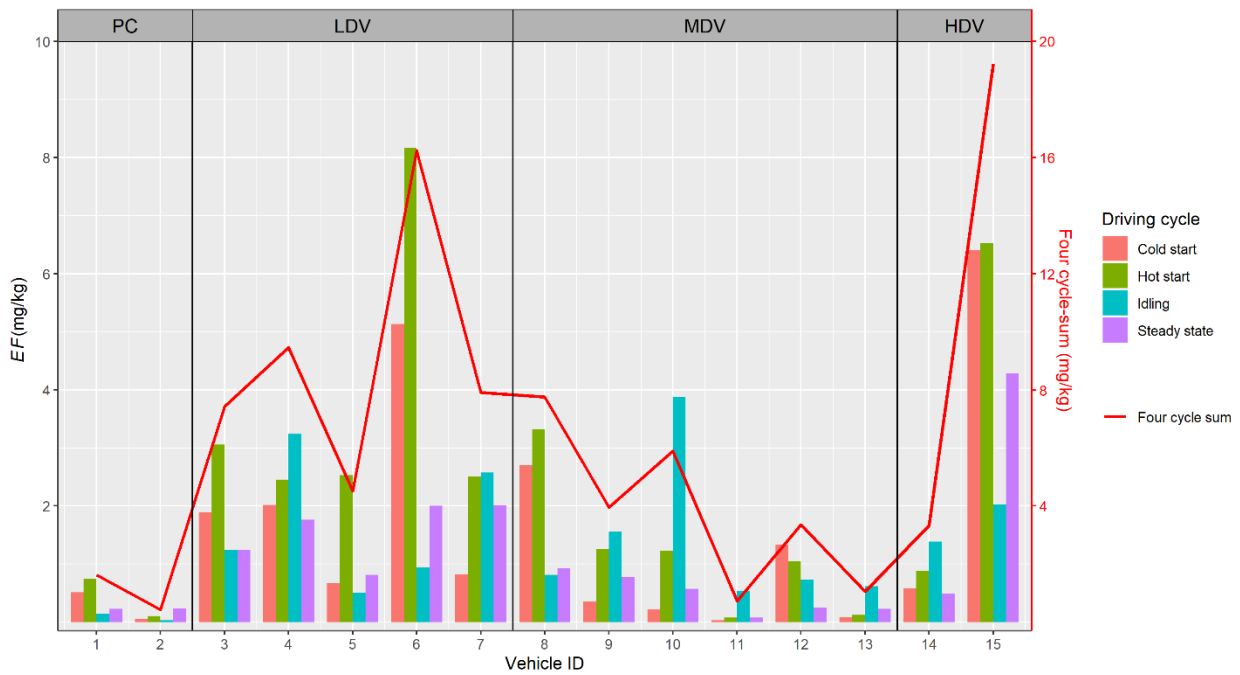
208 was in idling condition, its engine temperature was lower, resulting in “less complete” fuel  
209 combustion as compared to other conditions.

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

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

## 226 PAHs

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



238

239 **Fig. 2.** Total PAH *EFs* in different driving cycles. The red solid line represents the sum of total

240 PAH *EFs* of the four driving cycles, denoted as “four cycle-sum”.

241 **Fig. 2** also shows that MDVs had lower total PAHs *EFs* than LDVs did. Unexpectedly, vehicles

242 with greater testing weights emitted less PAHs. One possible explanation was that there were two

243 DPF-equipped vehicles in the MDV class, while there was only one DPF-equipped vehicle in the

244 LDV class. Cao et al. (2017) also observed the same pattern in their vehicle fleet, which was

245 comprised of 18 diesel trucks in China. The fact that this observation being not an isolated event

246 suggested that the effect of vehicle size on PAH emission should be further investigated.

247 Another feature observed in **Fig. 2** is that steady state cycle generally had the least total PAH *EFs*

248 while transient and idling cycles emitted substantially more PAHs, which was consistent with the

249 results reported by (Shah et al., 2005). **Fig. 2** shows that the total PAH *EFs* of Vehicles 4, 7, 9, 10,

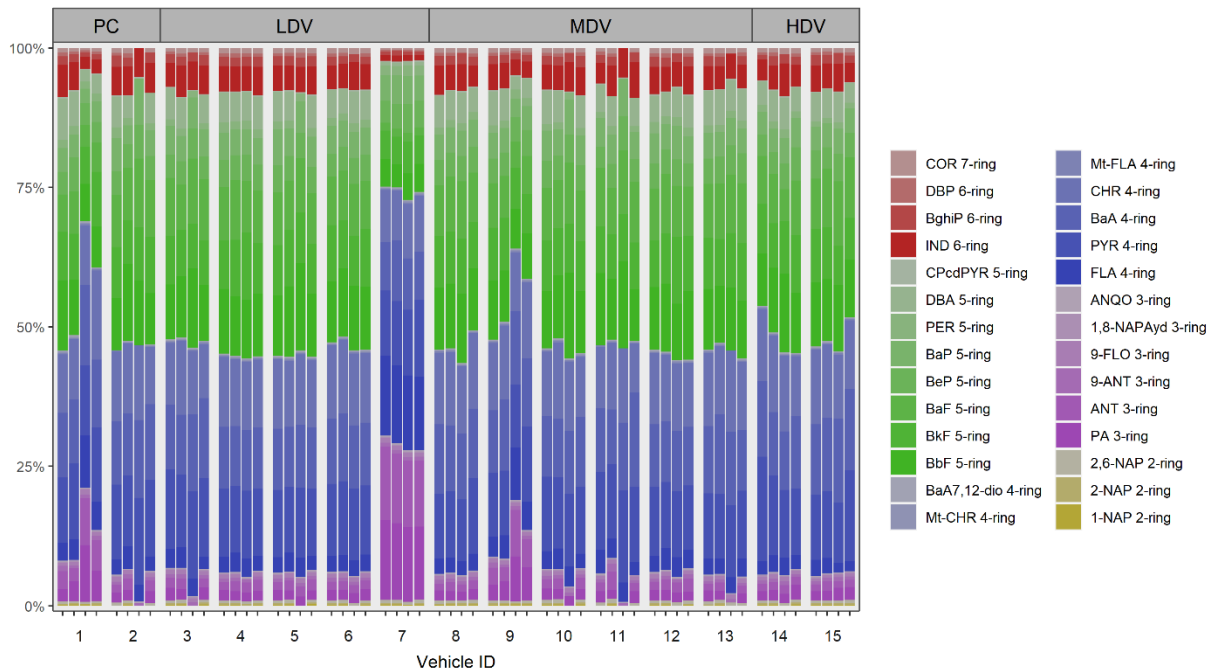
250 11, 13 and 14 under idling cycles were higher than those under transient cycles. This result

251 suggested that it is important to characterize the vehicle emission behaviour under different driving  
252 conditions, including idling, as studies of vehicle emission behaviour under idling condition are  
253 scarce compared to other conditions (e.g. transient and constant speed).

254 To identify the dominant PAHs emitted from each vehicle, the weighted percentage of each PAH  
255 emitted under different driving cycles is shown in **Fig. 3**. For most of the vehicles, the dominating  
256 PAHs were 4- and 5-ring PAHs including pyrene (PYR), benz[*a*]anthracene (BaA), chrysene  
257 (CHR), benzo[*b*]fluoranthene (BbF), benzo[*k*]fluoranthene (BkF) and benzo[*a*]fluoranthene (BaF).

258 This finding was consistent with those reported by previous studies (Cao et al., 2017, Hu et al.,  
259 2013). The difference in PAH composition under different driving conditions can also be  
260 compared in **Fig. 3**. From the figure, the distribution of individual PAH did not show a great  
261 variation among different driving conditions of a given vehicle, except for idling and steady state  
262 cycles of Vehicle 1 and 9. When comparison was made across vehicles, it can be observed that the  
263 variation in PAH composition among vehicles was not significant, except for Vehicle 7. Excluding  
264 the above two exceptions, PAHs collected in all driving cycles were dominated by the 4 and 5-  
265 ring PAHs for all vehicles. This observation suggests that driving conditions, driving pattern  
266 (NEDC or FIGE), mileage, testing weight of the vehicle, and after-treatment technologies do not  
267 have significant effects on the composition of PAHs. It also suggests that in general the PAH  
268 samples collected came from the same source, which was probably fuel combustion, since they all  
269 have similar composition. Therefore, the PAH samples collected in the exceptional cases (in

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



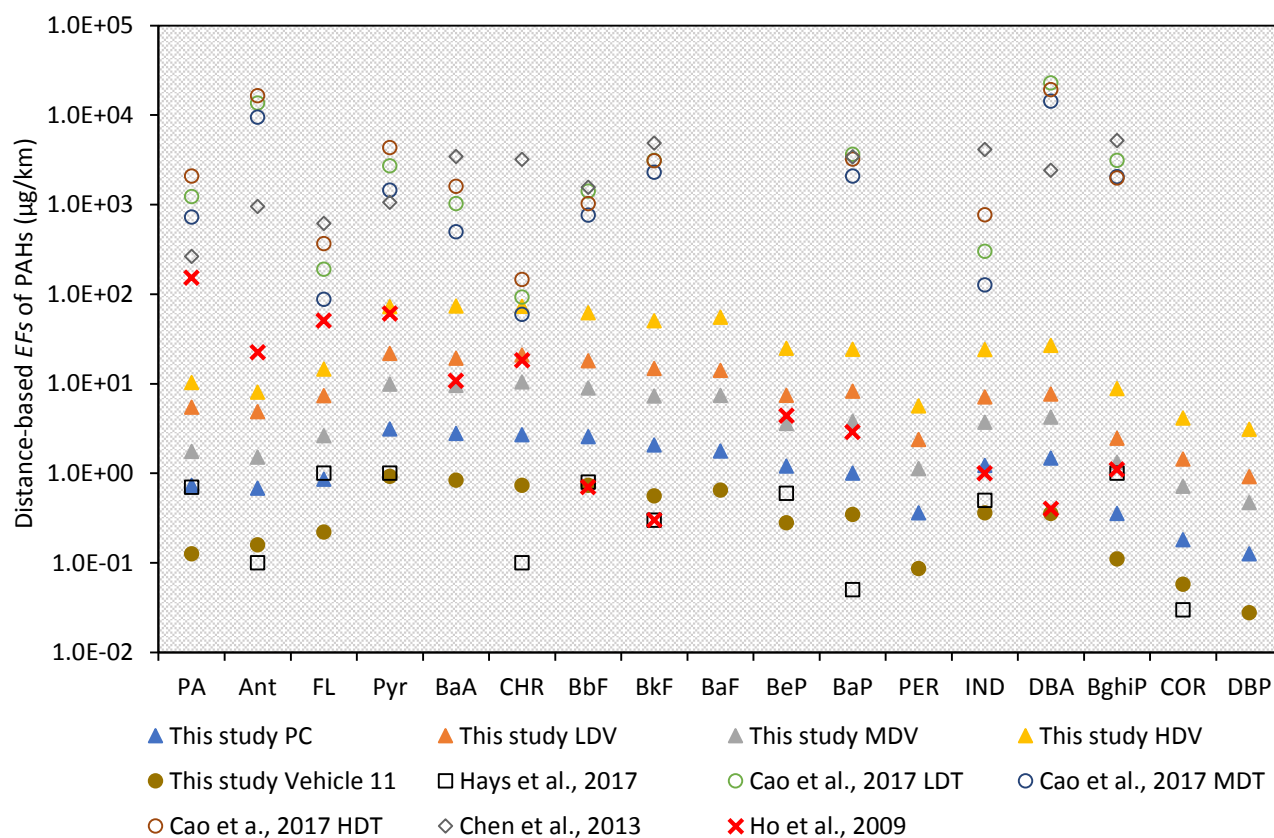
273  
 274 **Fig. 3.** Mass percentages of PAHs of each vehicle. The four bars under the same Vehicle ID  
 275 correspond to cold start, hot start, idling and steady state cycles from left to right.

276 **Comparison with other studies**

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

283 16.7 km, respectively. An averaged  $EF_d$  for each vehicle class (i.e. PC, LDV, MDV and HDV)  
284 was calculated. **Fig. 4** summarizes the averaged  $EF_d$  of each PAH from each vehicle class, in  
285 comparison to diesel vehicles PAHs reported by previous studies. These studies included a PEMS  
286 study in China (Cao et al., 2017), tunnel studies in China (Chen et al., 2013) and Hong Kong (Ho  
287 et al., 2009), and a chassis dynamometer study in the US (Hays et al., 2017). As shown in **Fig. 4**,  
288 the results of the current study fell in ranges of the previous studies. The highest PAH  $EF_d$  was  
289 from Cao et al. (2017), in which the vehicle fleet comprised of 18 diesel trucks with emission  
290 standards of China 3 and 4 (equivalent to Euro 3 and 4). In general, their results were an order of  
291 magnitude higher than the results of HDVs in the current study (except for CHR). As for ANT,  
292 the  $EF_d$  in Cao et al. (2017) was 4 orders of magnitude higher than that in this study. There were  
293 two main reasons. Firstly, the emission standard of the tested HDVs (Euro 4 and 5) in this study  
294 was higher than that of Cao's fleet. Secondly, Cao et al. (2017) measured real driving emissions  
295 using PEMS. It has well documented that the real-driving emissions (RDE)  $EF_s$  were much higher  
296 than laboratory chassis dynamometer results (Huang et al., 2018, May et al., 2014b, Weiss et al.,  
297 2012), due to more vigorous driving conditions in real world compared to the driving cycles tested  
298 in chassis dynamometer studies.





299

300

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

301

Hays et al. (2017) conducted chassis dynamometer testing on DPF-equipped diesel trucks in the

302

US. Their findings agreed well with the PAHs  $EF_d$  of Vehicle 11 in this study, as outlined by

303

brown solid dots in **Fig. 4**. Vehicle 11 was a DPF-equipped MDV with comparable size as the

304

vehicles tested by Hays et al. (2017). The agreement between studies confirmed that DPF was very

305

efficient to remove PAHs in vehicle exhausts.

306

Chen et al. (2013) conducted a tunnel study in Nanjing, while Ho et al. (2009) conducted their

307

tunnel study in Hong Kong. As shown in **Fig. 4**, the results of Chen et al. (2013) fell in the range

308

of the results of Cao et al. (2017), while the results of Ho et al. (2009) generally fell within the

309

range of the current study. It showed that  $EF_d$  of pollutants from diesel vehicles were strongly

310 regional related. The possible reason was that diesel fuels varied among regions since previous  
311 studies pointed out that fuel type (i.e. low-sulfur vs ultra-low-sulfur diesel) had significant effect  
312 on the emission factors of PAHs (Cheung et al., 2010, Lim et al., 2005).

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

#### 324 **Toxicological analysis**

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

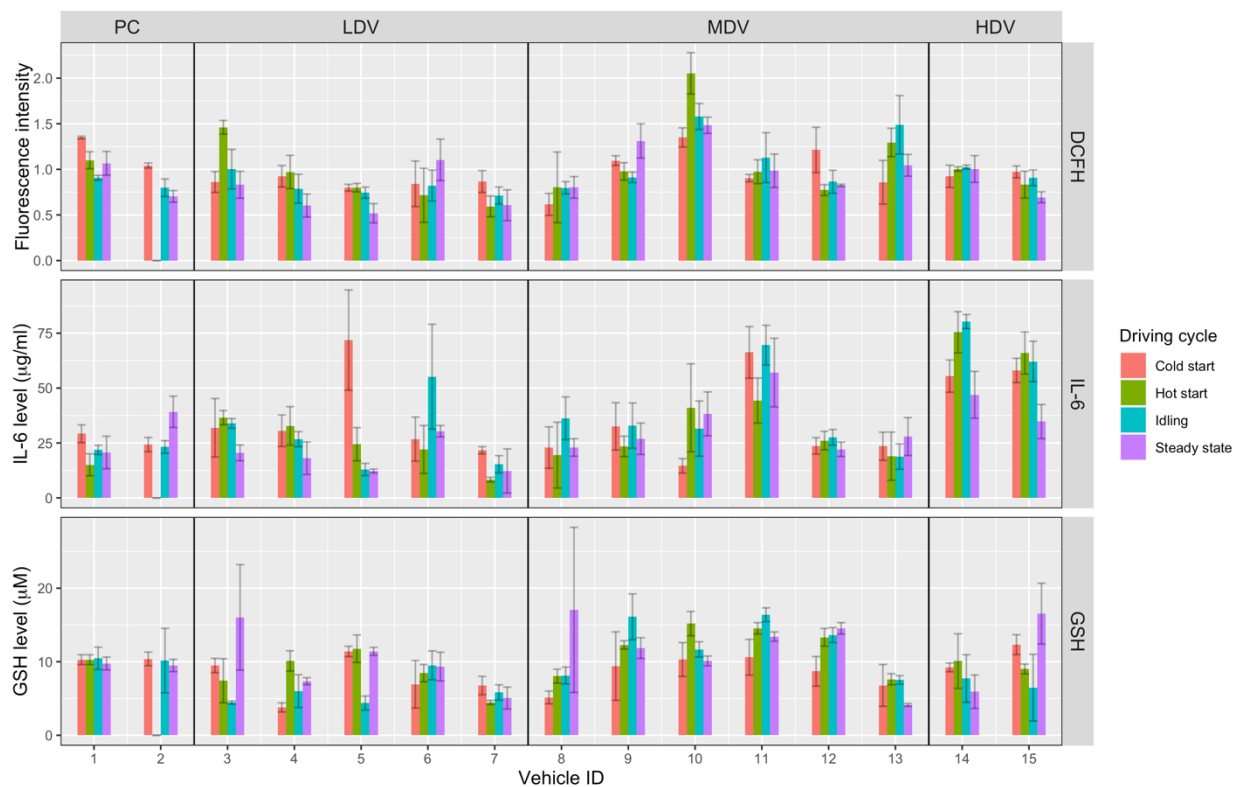
329 50  $\mu\text{g ml}^{-1}$ ), comparison between vehicles only accounted for the compositional difference of PM.  
330 Although the concentration specific toxicology results for DCFH and IL-6 can be normalized to  
331 the amount of PM emitted under different conditions to study the PM toxicity under these  
332 conditions, preliminary analysis showed that the normalized toxicology result strongly depend on  
333 the EF of PM. **Fig. S5** in Supporting Information illustrated that DCFH and IL-6 results normalized  
334 by the fuel-based PM *EF* showed the same pattern as PM *EF*. Therefore, the aforementioned result  
335 was not presented in the main context. Moreover, for Vehicle 2 under hot start condition, the  
336 amount of PM collected was under detection limit by gravimetric method. Therefore, there is no  
337 toxicological test results for Vehicle 2 under hot start condition.

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

346 The lowest panel in **Fig. 5** shows the GSH levels after the A549 cells exposed to the PM samples.  
347 GSH is an antioxidant of which the concentration will decrease in response to oxidative stress. In

348 general, GSH levels were depleted compared to the blank filter sample outlined in Fig.S3 for all  
349 PM samples. Compared with the results of DCFH level, a certain degree of variation among the  
350 tested vehicles was observed. LDVs (Vehicle 3,4,5,6 and 7) generally had lower GSH  
351 concentrations (stronger oxidative stresses) as compared to other classes of vehicles. Other  
352 parameters did not have great influence on the GSH level. The average GSH levels for vehicles  
353 with DPF and without DPF were  $9.89 \pm 3.97 \mu\text{M}$  and  $9.85 \pm 3.37 \mu\text{M}$ , respectively. This result  
354 showed that DPF removes PM mass without changing the GSH response of the PM, and this  
355 probably shows that DPF does not significantly change the morphology and composition of PM  
356 sample.

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



363

364 **Fig. 5.** Results of the production of ROS expressed as the fluorescent intensity of DCF, IL-6

365 level and GSH level of the 15 vehicles in different testing cycles.

366

367 *Effect of vehicle type and driving cycle*

368 To better illustrate the effect of vehicle type on the three toxicological markers, levels of each

369 toxicological marker for all vehicles were pooled together and grouped according to their

370 corresponding vehicle type (i.e. PC, LDV, MDV, HDV), and the results were presented by the

371 boxplots in **Fig. 6.** As shown in **Fig. 6,** a certain degree of variation existed between vehicle types

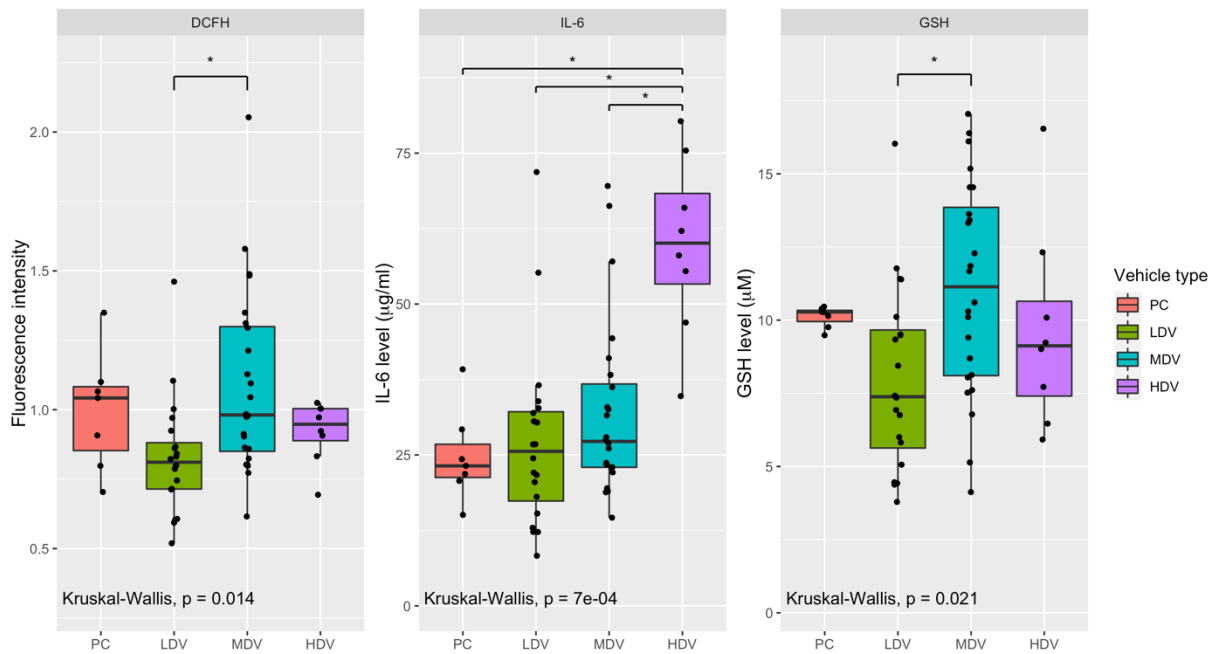
372 for all three toxicological markers. For DCFH and GSH, LDV produced lower responses compared

373 to the other three types of vehicle. In the case of IL-6, HDV produced the highest IL-6 level among

374 the four vehicles types. The results were further verified by conducting a Kruskal-Wallis H test  
375 for each toxicological marker at a significance level of  $p = 0.05$ . The Kruskal-Wallis H test results  
376 presented in the bottom left of each boxplot indicated that statistically significant difference exist  
377 among the four vehicle types for all three toxicological markers. Pairwise Wilcoxon rank sum test  
378 was carried out as the post-hoc test of Kruskal-Wallis test to find out which vehicle type pair of  
379 has significant difference at a significance level of 0.05. The vehicle type pairs with adjusted p-  
380 values  $< 0.05$  were marked with an asterisk in **Fig. 6**. In the case of DCFH and GSH, significant  
381 difference was found between LDV and MDV while for IL-6, significant differences were  
382 identified between HDV and the other three types of vehicles. These results suggested that the  
383 response of the three toxicological markers would be affected by vehicle type. **Moreover, it should**  
384 **be noticed that a decrease in GSH level indicates an increase of oxidative stress, which means that**  
385 **GSH level should show an opposite trend when compared to DCFH level. However, result of the**  
386 **mentioned statistical analysis showed that increase of vehicle weight (from LDV to MDV)**  
387 **increased both GSH and DCFH levels.** To find out the mechanisms or reasons behind this  
388 observation, further investigations are needed.

389 Unlike vehicle type, driving condition of the vehicle does not has significant effect on the three  
390 toxicological markers. Statistical analyses (**Fig. S3**) showed that there was no statistically  
391 significant difference between the four driving cycles ( $p > 0.05$ ) for all toxicological markers. This  
392 suggested that the effect of driving condition on the production of ROS and the pro-inflammatory

393 cytokine IL-6 was minimal. This observation was consistent with our PAH results, that the  
394 composition of PAHs did not show great variation among different driving cycles.



395  
396 **Fig. 6.** Boxplots of the levels of DCFH, IL-6 and GSH grouped by vehicle type. Black dots  
397 represent the data points in the corresponding driving cycle. Asterisk represents the adjusted p-  
398 value in pairwise Wilcoxon rank sum test smaller than 0.05.

399  
400 **Correlation between toxicity data and PAHs concentration**  
401 It was reported that the chemical composition of the PM sample was related to the oxidative  
402 potential and the release of oxidative stress mediators (Chuang et al., 2012, Ho et al., 2016). In  
403 particular, certain PAHs were found to have significant correlations with the vasoactive function  
404 and pro-inflammatory cytokines (Niu et al., 2017).

405

406 **Table 1.** Results of spearman's correlation between PAHs and toxicological markers

407 with p-value smaller than 0.1. Asterisk indicates p-value &lt;0.05.

		<b>DCFH</b> <i>Spearman's <math>\rho</math></i> <i>(p-value)</i>	<b>IL-6</b> <i>Spearman's <math>\rho</math></i> <i>(p-value)</i>	<b>GSH</b> <i>Spearman's <math>\rho</math></i> <i>(p-value)</i>
<b>Hot start</b>	IND	0.468 (<0.1)	-	-
	DBA	0.532 (<0.05) *	-	-
	Mt-CHR	0.457 (<0.1)	-	-
	COR	-	0.471 (<0.1)	-
<b>Idling</b>	Mt-FLA	-	0.503 (<0.1)	-
	2-NAP	-	-	-0.900 (0.037) *
<b>Steady state</b>	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)	-

408

409 Correlation analysis between PAHs and toxicological markers was carried out according to the

410 method stated in the methodology part. The correlation test results with p-value &lt;0.1 were

411 summarized in **Table 1**. A total of 17 PAH-toxicological marker pairs were found to have412 moderate or good correlations. In general, as shown in **Table 1**, almost all these pairs were found413 in hot-start and steady state tests, except **two** in idling cycle. There is no correlation found between

414 PAH and toxicology data under cold-start condition. In other words, under hot engine conditions



415 (hot-start and steady state), some PAHs serve as good indicators of ROS production or pro-  
416 inflammatory response. However, in conditions with lower engine temperature compared to hot-  
417 start and steady state (i.e. cold-start and idling), the concentration of PAH measured in the current  
418 study seems not to be the main driving force for the observed toxicology responses.

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

430 **Table 1** also showed the correlation between the pro-inflammatory mediator IL-6 and selected  
431 PAHs. Moderated correlation between IL-6 and COR ( $\rho = 0.47$ ,  $p < 0.1$ ) was found in hot-start  
432 cycle. Correlation between IL-6 and Mt-FLA ( $\rho = 0.50$ ,  $p < 0.1$ ) is the only data set that gives  
433 considerable correlation in idling cycle. Other than these two combinations, all observed

434 correlation between IL-6 and PAHs come from steady state cycle. 2,6-NAP ( $\rho = 0.49$ ,  $p < 0.1$ ) is  
435 the only PAH with less than 3 rings that showed correlation with toxicology results in our study.  
436 Moderate correlations were found between IL-6 and Ba7,12-dio ( $\rho = 0.44$ ,  $p < 0.1$ ), Mt-FLA  $\rho =$   
437  $0.48$ ,  $p < 0.1$ ), PYR ( $\rho = 0.48$ ,  $p < 0.1$ ), and BaF ( $\rho = 0.45$ ,  $p < 0.1$ ), while significant correlations  
438 were found between IL-6 and BaA ( $\rho = 0.54$ ,  $p < 0.05$ ), BkF ( $\rho = 0.52$ ,  $p < 0.05$ ) and BeP ( $\rho =$   
439  $0.58$ ,  $p < 0.05$ ).

440 Several studies have assessed the correlation between IL-6 and particle bounded PAHs from  
441 different sources, but they did not come to the same conclusion. Niu et al. (2017) and Chowdhury  
442 et al. (2019) investigated the correlation between PAHs in atmospheric PM sample and IL-6  
443 response in A549 cell and BEAS-2B cell (human bronchial epithelial cell) respectively. They  
444 found that certain PAHs were positively correlation with IL-6 level. Lin et al. (2013) studied the  
445 effect of household particles on inflammation in human coronary artery endothelial cells  
446 (HCAECs) and found that PAHs have significant correlation with IL-6 level. Delfino et al. (2010)  
447 analysed blood samples from 60 people and the air sample in vicinity to them. Their results also  
448 suggested positive correlation between PAHs in air samples and IL-6 in human blood samples.  
449 Our findings agree with the above studies, which support a positive correlation between PAHs and  
450 IL-6 level. However, a study conducted by Skuland et al. (2017) suggested that there is no clear  
451 connection between total or individual PAH levels in diesel exhaust particles with IL-6 in BEAS-  
452 2B cell, and Chuang et al. (2012) also cannot find any significant correlation between PAHs in air

453 samples with IL-6 level in HCAECs. Moreover, Wang et al. (2016) found a significant negative  
454 correlation between PAHs in atmospheric PM samples and IL-6 level in BEAS-2B cells. These  
455 studies suggested that in some cases PAHs might not be the main inducer of pro-inflammatory  
456 response and further investigations are needed to elucidate the reaction mechanism of the release  
457 of IL-6 and other pro-inflammatory mediators.

458

## 459 **Conclusion**

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

467 Among the identified PAHs, 4- and 5-ring PAHs were the most abundant species. Highest PAH  
468 *EFs* were found in HDVs, followed by LDV and MDV. Driving cycle has significant effects on  
469 the *EFs* of PAHs. Steady state cycle generally has the lowest PAH *EFs* while transient and idling  
470 cycle produce substantially higher PAH *EFs*. Although difference in PAH *EFs* were observed

471 under different driving conditions, percentage by mass of individual PAH (i.e. PAH composition  
472 of the PM sample) did not show significant variation among different driving conditions.

473 The cellular exposure experiments revealed that the diesel vehicle PM emissions exerted potential  
474 oxidative stresses emerged from the ROS to human lung cell activities. Statistical analysis results  
475 showed that MDV produced significantly higher levels of DCFH and GSH than LDV, while HDV  
476 produced significantly larger amount of IL-6 than other types of vehicles. Correlation analysis  
477 between PAHs and the three toxicology markers were also carried out. Results showed that  
478 statistically significant correlation exist between certain PAH-toxicological marker pairs,  
479 including DCFH and DBA ( $\rho = 0.53, p < 0.05$ ), [GSH and PA \( \$\rho = -0.539, p < 0.05\$ \)](#), and [ANT \( \$\rho\$   
480  \$= -0.514, p < 0.05\$ \)](#), as well as IL-6 and BaA ( $\rho = 0.54, p < 0.05$ ), BkF ( $\rho = 0.52, p < 0.05$ ), and  
481 BeP ( $\rho = 0.58, p < 0.05$ ). Our results suggested that new emission control technologies and policies  
482 should focus on OC and PAHs reduction in order to reduce the adverse health effects on human  
483 respiratory system.

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487

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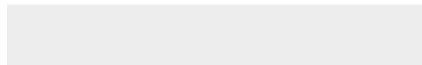
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**Supplementary Material**

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## Credit author statement

**Bei Wang:** Conceptualization, Funding acquisition, Writing – Original Draft, Supervision

**Yik-Sze Lau:** Investigation, Writing – Original Draft, Review and Editing, Visualization, Formal analysis

**Yuhan Huang:** Writing – Review and Editing

**Bruce Organ:** Investigation

**Hsiao-Chi Chuang:** Investigation, Writing – Review and Editing

**Steven Sai Hang Ho:** Investigation

**Linli Qu:** Investigation

**Shun-Cheng Lee:** Supervision

**Kin-Fai Ho:** Conceptualization, Methodology, Formal analysis, Writing – Review and Editing, Supervision



25 **ABSTRACT**

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

42 *Keywords: PM emission; OCEC; PAH; DCFH; Interleukin-6 (IL-6); Glutathione (GSH);*

43 *Driving Cycle; Chassis dynamometer*

## 45 INTRODUCTION

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

53 A large quantity of PM contains carbonaceous aerosols (Hou et al., 2011), which are typically  
54 classified into two categories, namely elemental carbon (EC) and organic carbon (OC). Lee et al.  
55 (2006) reported that carbonaceous aerosols are the dominant species of fine particles in the  
56 roadside environment in Hong Kong and account for approximately 44% of the PM<sub>2.5</sub>  
57 concentration in Hong Kong. Vehicular emissions are the major source of carbonaceous aerosols  
58 in Hong Kong (Cheng et al., 2010, Cheng et al., 2011, Ho et al., 2002, Lee et al., 2006). Zheng et  
59 al. (2006) reported that more than 60% of the OC measured at a roadside station in Hong Kong  
60 originated from vehicular emissions. The OC in vehicle-emission-derived PM comprises various  
61 toxic organic compounds, including polycyclic aromatic hydrocarbons (PAHs). PAHs are  
62 important toxic components of vehicle emitted organic species. They are identified as one of the  
63 major toxic air pollutants, mainly originated from anthropogenic processes, especially from

64 incomplete combustion of organic matters. PAHs are semi-volatile organic compounds that can  
65 be present in both particulate and gaseous phases. PAHs are present on the surface of airborne  
66 particles through condensation, adsorption or combustion processes (Yamasaki et al., 1982,  
67 Dimashki et al., 2001). Several studies have reported that vehicular emissions, particularly diesel  
68 vehicles, are the most important sources of PAHs in urban areas (Cecinato et al., 2014, Chen et al.,  
69 2013, Shen et al., 2011). Therefore, various studies have been done to characterize the *EFs* and  
70 chemical composition of PAHs emitted from diesel vehicles under different conditions (Zielinska  
71 et al., 2004, Tsai et al., 2011, Hu et al., 2013, Cao et al., 2017, Hays et al., 2017, Lin et al., 2019).  
72 In Hong Kong, the characterization of PAH derived from vehicle emission were mainly done by  
73 tunnel measurement and ambient air sample collection (Ho and Lee, 2002, Cheng et al., 2010, Ho  
74 et al., 2009, Ma et al., 2016). For example, Ho et al. (2009) conducted a tunnel study to investigate  
75 the PAHs emission from vehicles in Hong Kong. The authors concluded that two- and three-ring  
76 PAHs were the dominant fraction in gas phase, while four-ring PAHs were the most abundant in  
77 particle phase. Since studies of direct tailpipe emission (e.g. chassis dynamometer or PEMS study)  
78 from diesel vehicles in Hong Kong are scarce, carrying out such kind of research is needed to fill  
79 the knowledge gap.

80 Another important concern of PM is their adverse effects to human health (Abdel-Shafy and  
81 Mansour, 2016, Chuang et al., 2012, Kim et al., 2013). In previous toxicological studies, it is  
82 believed that reactive oxygen species (ROS) production by PM exposure is the main mechanisms



83 for increased risk of adverse health effects (Li et al., 2003, Nel, 2005). For instance, studies by  
84 Geller et al. (2006) and Ntziachristos et al. (2007) revealed strong correlations between the redox  
85 activities of their PM sample and several PM species, including OC, EC and PAHs. In a healthy  
86 biological system, glutathione (GSH) is produced to mitigate the PM-derived ROS in cells (Ghio  
87 et al., 2012). However, the overloaded ROS is able to incur inflammatory responses such as  
88 interleukin 6 (IL-6) and interleukins 8 (IL-8) production, consequently leading to harmful health  
89 effects. Various studies have been done to investigate the inflammatory response triggered by the  
90 exposure of vehicle-derived PM (Mazzarella et al., 2007, Gerlofs-Nijland et al., 2013, Bengalli et  
91 al., 2017). Results from these studies showed that vehicle-derived PM emission is closely related  
92 to the release of pro-inflammatory cytokines, like IL-6, while the use of advanced emission control  
93 technologies, such as DPF, will significantly change the inflammatory responses in the studied  
94 cell.

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

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

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

## 110 **METHODOLOGY**

### 111 **Fleet overview and instrumentation set-up**

112 Fifteen vehicles of various classes and with different engine sizes, after-treatment technologies,  
113 and emission standards (**Table S1**) were studied. The vehicle fleet was selected briefly in  
114 accordance to the emission standard distribution of diesel vehicles in Hong Kong, where most of  
115 them are with Euro 4 and 5, followed by Euro 3 and others. The vehicles were classified according  
116 to the United Nations Economic Commission for Europe (UNECE, 2011). Passenger cars (PCs)  
117 were defined as M-type vehicles. Light-duty vehicles (LDVs), medium-duty vehicles (MDVs),  
118 and heavy-duty vehicles (HDVs) were defined as N1-, N2-, and N3-type vehicles, respectively.  
119 Vehicle 15 was categorized as an HDV because it was a 10-ton tractor designed for carrying a  
120 trailer of up to 20 tons. All vehicles, except Vehicle 2 and 7, have taken and passed the annual

121 vehicle examination required by the Transport Department of the Hong Kong Government. The  
122 annual vehicle examination is mandatory for all commercial vehicles, as well as passenger cars  
123 and light duty vehicles (vehicle weight under 1.9 tons) with first registration date over 6 years. The  
124 annual examination policy is to make sure that vehicles running on road are in acceptable  
125 maintenance condition. The diesel fuel used by all vehicles in the current study is the same, which  
126 comply with the Euro 5 diesel fuel standard, as stated in **Table S3**.

127 Chassis dynamometer tests were conducted in the Jockey Club Heavy Vehicle Emissions Testing  
128 and Research Centre (JCEC), Hong Kong. **Fig. S1** of the Supporting Information depicts the  
129 schematic of the test setup. All the testing facilities in the JCEC comply with the European  
130 standards for type approval tests. Two chassis dynamometers were used to test the vehicles with  
131 different weights. PCs and LDVs were tested on a Mustang dynamometer with a 48” (121.92 cm)  
132 single roller, whereas MDVs and HDVs were tested on a Mustang dynamometer with a 17.2”  
133 (43.688 cm) triple roller.

#### 134 **Driving cycles and testing conditions**

135 Four driving cycles, namely the cold start transient, hot start transient, idling, and steady-state  
136 cycles, were used to test each vehicle. For convenience, the cold and hot start transient cycles are  
137 called cold start and hot start cycles in the following text. For each vehicle, the cold start cycle test  
138 was repeated two times and the hot start, idling, and steady-state cycle tests were repeated three

139 times. Detailed descriptions of the four driving cycles and loading conditions are presented in the  
140 Supporting Information.

#### 141 **PM sample collection**

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

#### 153 **Chemical analysis**

154 The samples collected on the quartz filters were used for OC/EC and PAH analyses. Each filter  
155 was cut exactly in half with a specially designed chopper for the two analyses. The contents of OC  
156 and EC were analyzed using a Desert Research Institute Model 2001 Thermal/Optical Carbon  
157 Analyzer with the IMPROVE-A protocol described by Chow et al. (2012). The PAH samples were

158 analyzed using the thermal desorption-gas chromatography/mass spectrometry method (Ho et al.,  
159 2008). The chemical analyses procedures are described in the Supporting Information.

### 160 **Toxicological analysis**

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

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

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

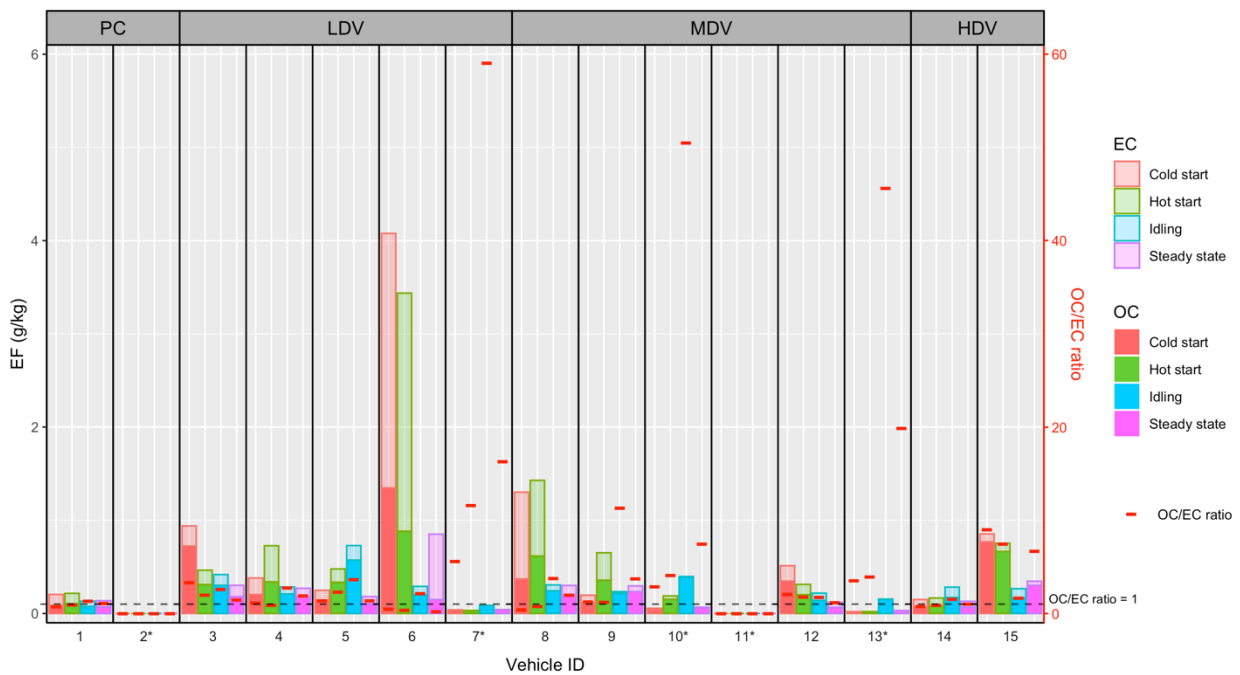
## 171 **RESULTS AND DISCUSSION**

### 172 **PM emission characteristics**

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

177 **OC and EC**

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



185 **Fig. 1.** EFs of OC and EC and OC/EC ratios. Each bar represents the sum of OC and EC EF.  
 186 Light color bar represents EC EF and deep color bar represents OC EF. Vehicles with an asterisk  
 187 next to their vehicle ID were equipped with DPF.  
 188

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

205 Among the four driving conditions tested in this study, idling generally produced the highest  
206 OC/EC ratio. EC mainly arises from fuel droplet pyrolysis, whereas OC mainly originates from  
207 unburned fuel and incomplete combustion (Shah et al., 2004). When the vehicles were in the idling

208 condition, their engine temperature decreased, which resulted in “less complete” fuel combustion  
209 compared with that under other conditions

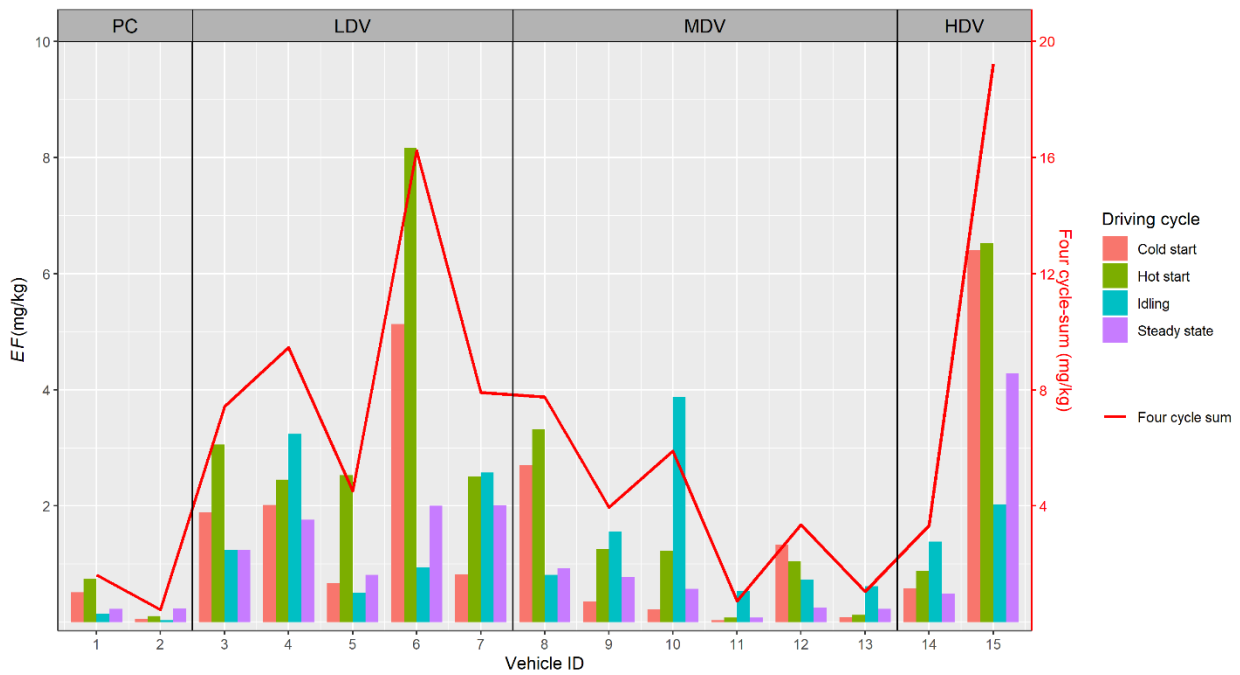
210 The European emission standard assigned to the vehicle considerably affected OC and EC  
211 emissions. Typically, the EFs of OC and EC decreased with an increase in emission standards,  
212 except in the cases of Vehicles 6, 12, and 15. An extremely low level of EC and a measurable level  
213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

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



## 227 PAHs

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



239  
 240 **Fig. 2.** Total PAH *EFs* in different driving cycles. The red solid line represents the sum of total  
 241 PAH *EFs* of the four driving cycles, denoted as “four cycle-sum”.

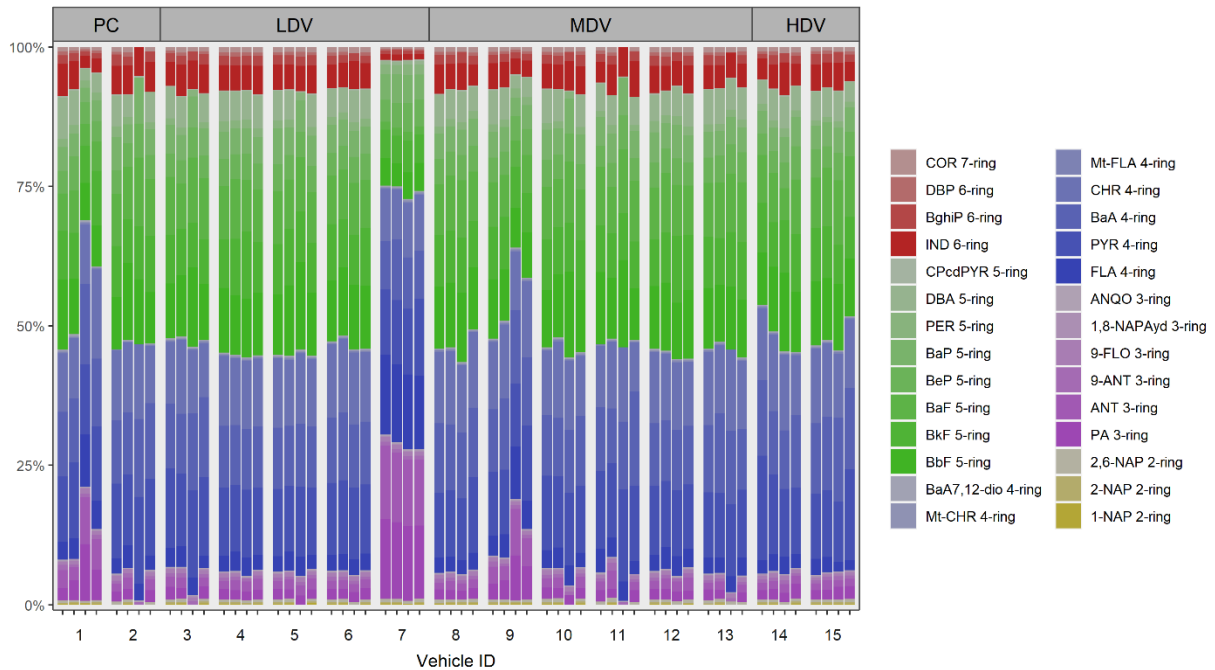
242 **Fig. 2** reveals that the MDVs had lower total PAH *EFs* than the LDVs did. Unexpectedly, vehicles  
 243 with greater testing weights emitted less PAHs. A possible explanation for this result is that two  
 244 DPF-equipped vehicles were present in the MDV class, whereas only one DPF-equipped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

249 **Fig. 2** also reveals that the steady-state cycle generally exhibited the least total PAH *EFs*, whereas  
 250 the transient and idling cycles exhibited substantially higher PAH *EFs*. These results are consistent  
 251 with those reported by (Shah et al., 2005). Furthermore, the aforementioned figure indicates that

252 the total PAH EFs of Vehicles 4, 7, 9, 10, 11, 13 and 14 under idling cycles were higher than those  
253 under transient cycles. This result suggested that the vehicle emission behavior under different  
254 driving conditions, especially under idling, should be studied because a substantial difference in  
255 EFs were observed between different driving cycles.

256 To identify the dominant PAHs emitted from each vehicle, the weighted percentage of each PAH  
257 emitted under different driving cycles was determined (**Fig. 3**). For most of the vehicles, the  
258 dominant PAHs were 4- and 5-ring PAHs, including pyrene (PYR), benz[*a*]anthracene (BaA),  
259 chrysene (CHR), benzo[*b*]fluoranthene (BbF), benzo[*k*]fluoranthene (BkF), and  
260 benzo[*a*]fluoranthene (BaF). This finding was consistent with those reported in previous studies  
261 (Cao et al., 2017, Hu et al., 2013). The difference in the PAH composition under different driving  
262 conditions is illustrated in **Fig. 3**. The distributions of individual PAHs did not exhibit considerable  
263 variations when a given vehicle was tested under different driving conditions, except when  
264 Vehicles 1 and 9 were tested under the idling and steady-state cycles. The variation in the PAH  
265 composition among vehicles was not significant, except for Vehicle 7. Excluding the  
266 aforementioned two exceptions, the PAHs collected in all the driving cycles were dominated by  
267 4- and 5-ring PAHs for all the vehicles. This observation suggests that the driving conditions,  
268 driving pattern (NEDC or FIGE), mileage, testing weight of the vehicle, and after-treatment  
269 technologies do not considerably affect the composition of the emitted PAHs. Furthermore, in  
270 general the collected PAH samples originated from the same source, probably fuel combustion,

271 because they all had similar compositions. Therefore, the PAH samples collected in the  
 272 exceptional cases (for Vehicle 7 and in the idling and steady-state cycles for Vehicles 1 and 9)  
 273 were probably affected by other sources. Further investigations are required to characterize the  
 274 sources of PAHs collected from the tailpipe emissions of diesel vehicles.

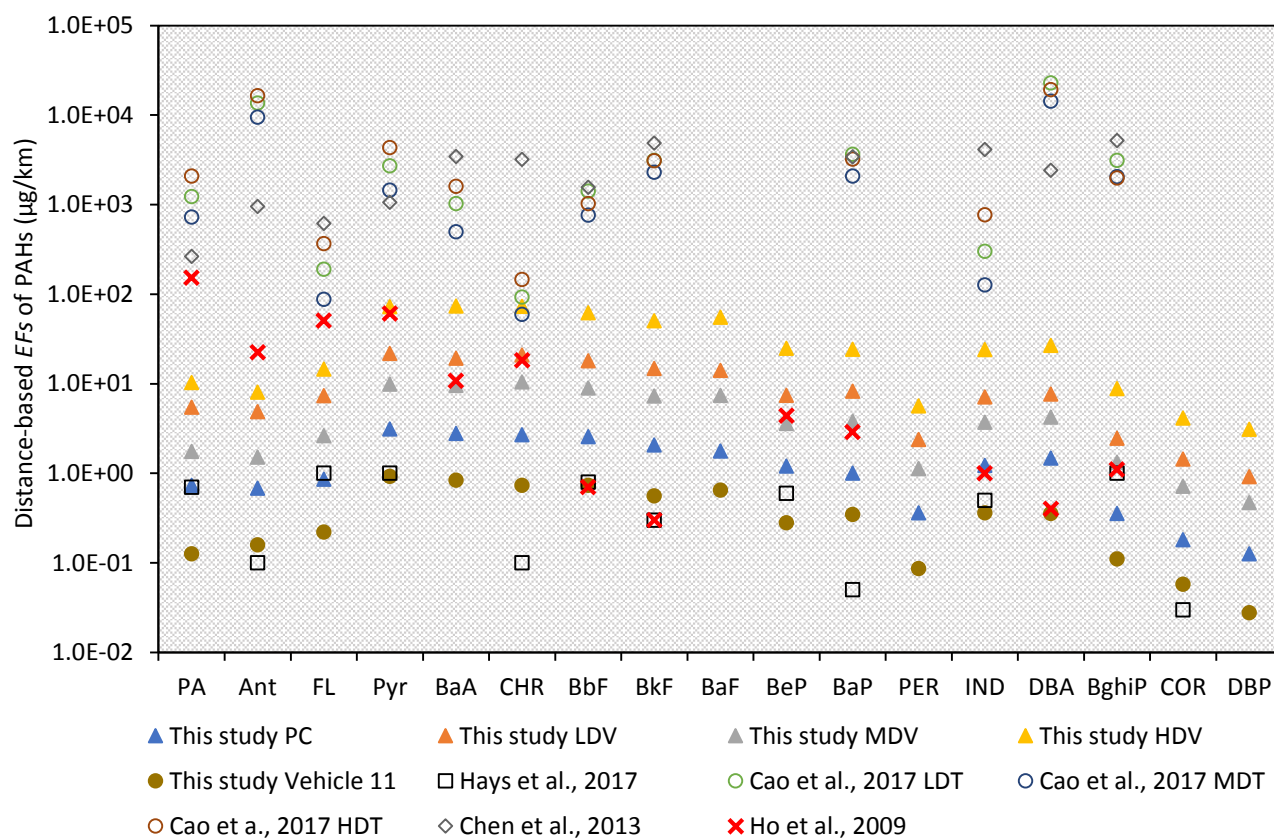


275  
 276 **Fig. 3.** Mass percentages of PAHs of each vehicle. The four bars under the same Vehicle ID  
 277 correspond to cold start, hot start, idling and steady state cycles from left to right.

278 **Comparison with other studies**

279 The PAH data obtained in this current study were compared with the results of similar studies.  
 280 Unlike the fuel-based EF used in the previous sections, distance-based EFs ( $EF_d$ ) were used in  
 281 this section because most of the results in previous studies were presented in a distance-based  
 282 manner. The  $EF_d$  value for each PAH was calculated as the ratio of the total quantity of PAHs  
 283 released in a driving cycle to the distance traveled in the driving cycle. The driving distances in

284 the NEDC, FIGE, and steady-state cycles were 11.0, 29.5, and 16.7 km, respectively. An averaged  
285  $EF_d$  for each vehicle class (i.e. PCs, LDVs, MDVs, and HDVs) was calculated. **Fig. 4** presents a  
286 comparison of the averaged  $EF_{s_d}$  of each PAH from each vehicle class in this study with the PAHs  
287 in diesel vehicle emissions in a PEMS study in China (Cao et al., 2017), tunnel studies in China  
288 (Chen et al., 2013) and Hong Kong (Ho et al., 2009), and a chassis dynamometer study in the US  
289 (Hays et al., 2017). As depicted in **Fig. 4**, the results of the current study were in the range reported  
290 in previous studies. Cao et al. (2017) reported the highest PAH  $EF_d$  values among the compared  
291 studies. Their vehicle fleet comprised 18 diesel trucks with China 3 and 4 emission standards  
292 (equivalent to Euro 3 and 4, respectively). In general, the PAH  $EF_d$  results of Cao et al. were an  
293 order of magnitude higher than those obtained for the HDVs in this study (except for CHR). The  
294  $EF_d$  value of ANT in the study of Cao et al. (2017) was four orders of magnitude higher than that  
295 in this study, which could be attributed to two main reasons. First, the emission standards of the  
296 tested HDVs (Euro 4 and 5) in this study were higher than those of Cao's fleet. Second, Cao et al.  
297 (2017) measured on-road emissions by using a PEMS. The emission EFs during on-road driving  
298 are considerably higher than those in laboratory chassis dynamometer tests (Huang et al., 2018,  
299 May et al., 2014b, Weiss et al., 2012) because the driving conditions in the real world are more  
300 rigorous than the driving cycles tested in chassis dynamometer studies.



301

302

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

303

Hays et al. (2017) conducted chassis dynamometer testing on DPF-equipped diesel trucks in the

304

US. Their findings agreed with the PAH  $EF_d$  values obtained for Vehicle 11 in this study, which

305

are denoted by brown solid dots in **Fig. 4**. Vehicle 11 is a DPF-equipped MDV and is comparable

306

in size to the vehicles tested by Hays et al. (2017). The agreement between the results of this study

307

and Hays et al. (2017) confirmed that DPF can efficiently remove PAHs from vehicle exhausts.

308

Chen et al. (2013) conducted a tunnel study in Nanjing, and Ho et al. (2009) conducted a tunnel

309

study in Hong Kong. As depicted in **Fig. 4**, the results of Chen et al. (2013) were in the range of

310

those of Cao et al. (2017), whereas the results of Ho et al. (2009) were generally within the range

311

of those of the current study. The  $EF_d$  values of the pollutants emitted from diesel vehicles were

312 strongly related to regions, which can be attributed to the diesel fuel variation among regions.  
313 Studies have indicated that the fuel type (i.e., low-sulfur diesel vs. ultra-low-sulfur diesel)  
314 considerably affects the EFs of PAHs (Cheung et al., 2010, Lim et al., 2005).  
315 The EF<sub>s,d</sub> of the PAHs in this study were expected be lower than those PAH EF<sub>s,d</sub> in the study of  
316 Ho et al. (2009) because according to the Environmental Protection Department of Hong Kong,  
317 the PM<sub>2.5</sub> emissions in Hong Kong have reduced by more than 50% (HKEPD, 2019). This  
318 observation can be explained by the driving condition for vehicles in tunnels, where vehicles travel  
319 at almost constant speed. This condition resembles the steady-state cycle in this study. **Fig. 2**  
320 indicates that the PAH EFs in the hot start cycle were higher than those in the steady-state cycle  
321 for all vehicles except Vehicles 2, 11, and 13. Also, the averaged total PAHs EF<sub>d</sub> in steady-state  
322 cycle is  $0.0834 \pm 0.146$  mg/km, which is lower than that in cold start ( $0.180 \pm 0.303$  mg/km) and  
323 hot start ( $0.239 \pm 0.310$  mg/km) cycles. This observation explains the higher-than-expected PAH  
324 EFs in this study compared with the study of Ho et al. (2009). Some high-emission vehicles (e.g.,  
325 Vehicles 6 and 15) contributed considerably to the calculated average PAH values, which  
326 increased the PAH EFs in this study.

### 327 **Toxicological analysis**

328 The levels of DCFH, IL-6, and GSH were analyzed to assess the cellular oxidative and  
329 inflammatory responses produced by the PM samples. The toxicological results of the current  
330 study cannot be compared with those of other studies because the cell culture conditions in this

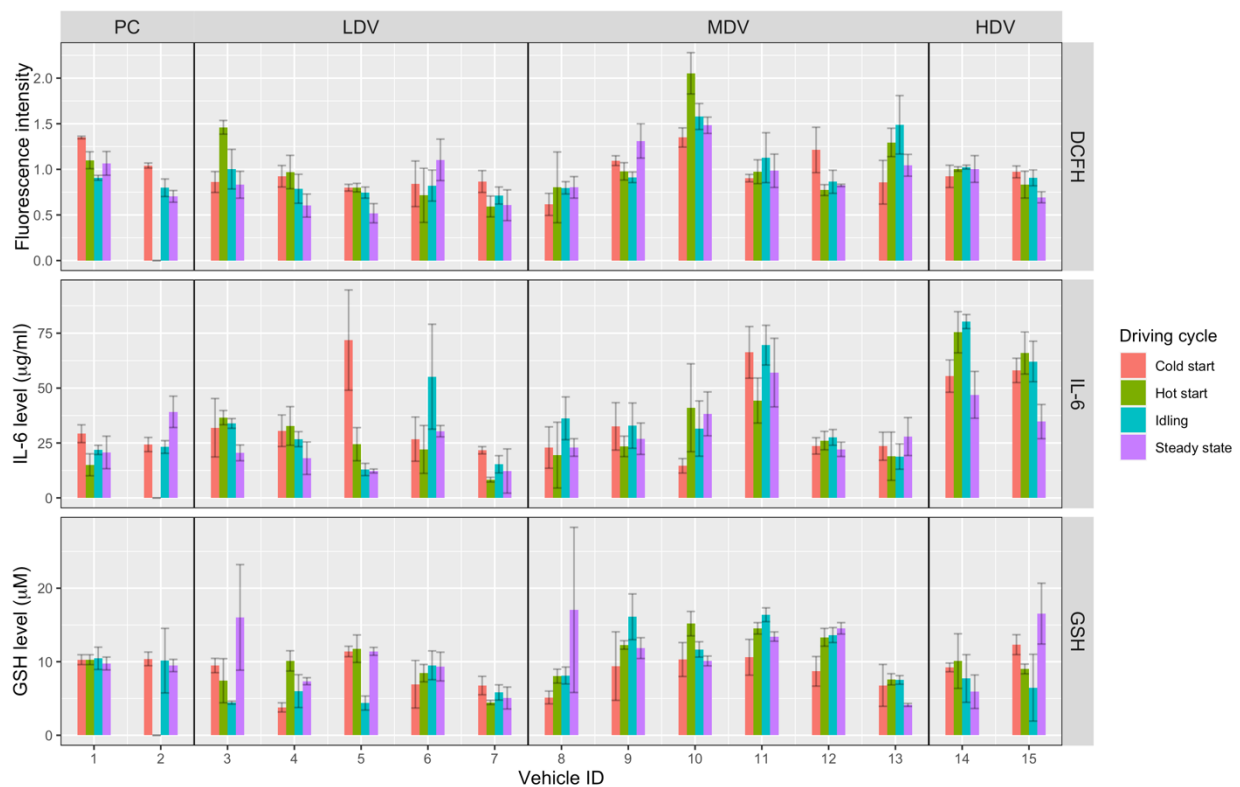
331 study were not exactly the same as those in previous studies. Also, as all the results were obtained  
332 by a fixed concentration of PM (i.e.  $50 \mu\text{g ml}^{-1}$ ), comparison between vehicles only accounted for  
333 the compositional difference of PM. Although the concentration specific toxicology results for  
334 DCFH and IL-6 can be normalized to the amount of PM emitted under different conditions to  
335 study the PM toxicity under these conditions, preliminary analysis showed that the normalized  
336 toxicology result strongly depend on the EF of PM. **Fig. S5** in Supporting Information illustrated  
337 that DCFH and IL-6 results normalized by the fuel-based PM *EF* showed the same pattern as PM  
338 *EF*. Therefore, the aforementioned result was not presented in the main context. Moreover, for  
339 Vehicle 2 under hot start condition, the amount of PM collected was under detection limit by  
340 gravimetric method. Therefore, there is no toxicological test results for Vehicle 2 under hot start  
341 condition.

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



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

359 IL-6 is a proinflammatory cytokine released in response to PM exposure. The IL-6 results are  
360 illustrated in the middle panel of **Fig. 5**. The idling cycle for Vehicle 14 exhibited the highest IL-  
361 6 level of 80.3, whereas the hot start cycle for Vehicle 7 exhibited the lowest IL-6 level of 8.30. In  
362 general, no clear trend was observed for the effect of driving cycles on the IL-6 levels. HDVs had  
363 the highest IL-6 concentrations among the vehicle classes, followed by MDVs. The IL-6 levels of  
364 PCs, LDVs, and MDVs did not exhibit significant variations.



365

366 **Fig. 5.** Results of the production of ROS expressed as the fluorescent intensity of DCF, IL-6

367 level and GSH level of the 15 vehicles in different testing cycles.

368

369 *Effect of vehicle type and driving cycle*

370 To determine the effects of the vehicle type on the three toxicological markers, the levels of each

371 toxicological marker for all vehicles were pooled and grouped according to their corresponding

372 vehicle type (i.e., PC, LDV, MDV, and HDV). The results are presented using boxplots in **Fig. 6.**

373 As depicted in **Fig. 6,** a certain degree of variation existed between vehicle types for the three

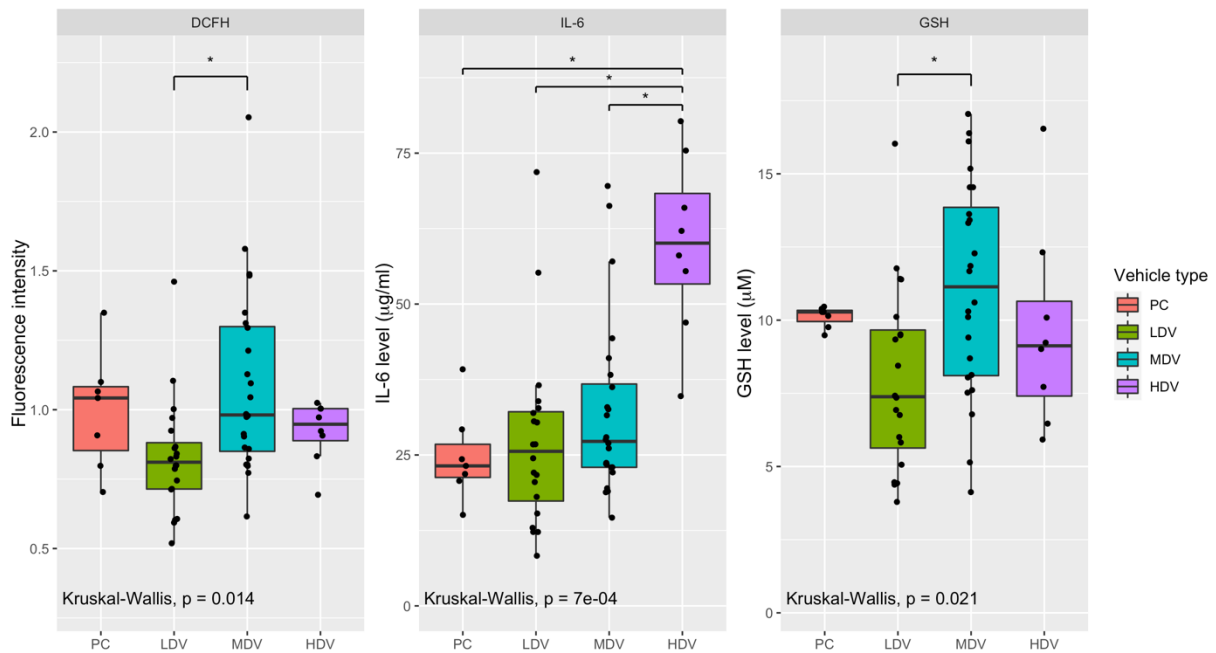
374 toxicological markers. The LDVs produced lower responses for DCFH and GSH than the other

375 three vehicle types did. The HDVs exhibited a higher IL-6 level than the other three vehicle types.

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

391 Unlike the vehicle type, the driving condition of the vehicle did not have a significant effect on  
392 the three toxicological markers. Statistical analyses (**Fig. S3**) confirmed that no statistically  
393 significant differences existed between the four driving cycles ( $p > 0.05$ ) for the three toxicological  
394 markers. This result suggested that the driving condition had a minimal effect on the production

395 of ROS and the proinflammatory cytokine IL-6. This finding is consistent with our PAH results,  
396 which revealed that the composition of PAHs did not vary significantly among the different driving  
397 cycles.



398  
399 **Fig. 6.** Boxplots of the levels of DCFH, IL-6 and GSH grouped by vehicle type. Black dots  
400 represent the data points in the corresponding driving cycle. Asterisk represents the adjusted p-  
401 value in pairwise Wilcoxon rank sum test smaller than 0.05.

402  
403 **Correlation between toxicity data and PAHs concentration**  
404 The chemical composition of PM samples has been reported to be related to the oxidative potential  
405 and the release of oxidative stress mediators (Chuang et al., 2012, Ho et al., 2016). In particular,

406 certain PAHs have significant correlations with the vasoactive function and proinflammatory  
 407 cytokines (Niu et al., 2017).

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

		<b>DCFH</b>	<b>IL-6</b>	<b>GSH</b>
		<i>Spearman's <math>\rho</math></i>	<i>Spearman's <math>\rho</math></i>	<i>Spearman's <math>\rho</math></i>
		<i>(p-value)</i>	<i>(p-value)</i>	<i>(p-value)</i>
<b>Hot start</b>	IND	0.468 (<0.1)	-	-
	DBA	0.532 (<0.05) *	-	-
	Mt-CHR	0.457 (<0.1)	-	-
	COR	-	0.471 (<0.1)	-
<b>Idling</b>	Mt-FLA	-	0.503 (<0.1)	-
	2-NAP	-	-	-0.900 (0.037) *
<b>Steady state</b>	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)	-

410

411 Correlation analysis was conducted between the concentrations of the PAHs and toxicological  
 412 markers according to the method stated in the Methodology section. The correlation test results  
 413 with a *p* value of <0.1 are presented in **Table 1**. A total of 17 PAH-toxicological marker pairs had  
 414 moderate or good correlations. In general, as depicted in **Table 1**, almost all the aforementioned  
 415 pairs were associated with the hot start and steady-state cycles, with the exception of two pair that

416 was associated with the idling cycle. There is no correlation found between PAH and toxicology  
417 data under cold-start condition. No correlation was found between the PAH and toxicology data  
418 under the cold start condition. Thus, under hot engine conditions (hot start and steady state), some  
419 PAHs functioned as good indicators of ROS production or proinflammatory response. However,  
420 under low-engine-temperature conditions (i.e., cold start and idling), the PAH concentrations  
421 measured in this study were not the main driving forces for the observed toxicology responses.

422 As depicted in **Table 1**, the DCFH levels and PAH concentrations exhibited correlation only under  
423 the hot start condition. Moderate correlations were found between DCFH level and Mt-CHR ( $\rho =$   
424  $0.46, p < 0.1$ ) and between DCFH level and IND ( $\rho = 0.47, p < 0.1$ ). Moreover, a good correlation  
425 was found between DCFH level and DBA ( $\rho = 0.53, p < 0.05$ ). This result agreed with that of Wu  
426 et al. (2017), who found a strong correlation between ROS generation and DBA in petrol and  
427 diesel fuel combustion experiments. Correlation coefficients between GSH and PAHs were  
428 expected to be negative since the decrease in GSH shows the increase of oxidative stress posed by  
429 the corresponding PAH. Correlations of GSH with PAHs were found in idling and steady state  
430 cycle. PA ( $\rho = -0.539, p < 0.05$ ) and ANT ( $\rho = -0.514, p < 0.05$ ) showed good correlation with  
431 GSH and FLA ( $\rho = -0.479, p < 0.1$ ) showed moderate correlation with GSH in steady state cycle.  
432 In idling cycle, GSH showed good correlation with 2-NAP ( $\rho = -0.900, p < 0.05$ ).

433 **Table 1** also showed the correlation between the pro-inflammatory mediator IL-6 and selected  
434 PAHs. Moderate correlation was found between IL-6 and COR ( $\rho = 0.47, p < 0.1$ ) in the hot start

435 cycle. The correlation between IL-6 and Mt-FLA ( $\rho = 0.50, p < 0.1$ ) was the only significant  
436 correlation in the idling cycle. Except the aforementioned two correlations, all the observed  
437 correlations between IL-6 and PAHs were found in the steady-state cycle. The compound 2,6-NAP  
438 ( $\rho = 0.49, p < 0.1$ ) was the only PAH with less than three rings that exhibited correlation with the  
439 toxicology results. IL-6 exhibited moderate correlation with Ba7,12-dio ( $\rho = 0.44, p < 0.1$ ), Mt-  
440 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  
441 exhibited significant correlations with BaA ( $\rho = 0.54, p < 0.05$ ), BkF ( $\rho = 0.52, p < 0.05$ ), and BeP  
442 ( $\rho = 0.58, p < 0.05$ ).

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

454 However, a study conducted by Skuland et al. (2017) could not establish a clear connection  
455 between the total or individual PAH levels in diesel exhaust particles and the IL-6 level in a BEAS-  
456 2B cell. Chuang et al. (2012) could not find a significant correlation between the PAHs in air  
457 samples and the IL-6 level in HCAECs. Moreover, Wang et al. (2016) found a significant negative  
458 correlation between the PAHs in atmospheric PM samples and the IL-6 level in BEAS-2B cells.  
459 The aforementioned studies suggest that in some cases, PAHs might not be the main inducer of  
460 proinflammatory response. Therefore, further investigations are essential to elucidate the reaction  
461 mechanism for the release of IL-6 and other proinflammatory mediators.

462

## 463 **Conclusion**

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

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



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

477 The cellular exposure experiments revealed that the PM emissions of diesel vehicles cause  
478 potential oxidative stresses, which emerge from ROS, for human lung cell activities. The statistical  
479 analysis results indicated that the MDVs produced significantly higher levels of DCFH and GSH  
480 than the LDVs did. Moreover, the HDVs produced significantly larger quantities of IL-6 than the  
481 other types of vehicles did. Correlation analysis between the PAHs and three toxicology markers  
482 revealed that statistically significant correlations existed between certain PAH–toxicological  
483 marker pairs, including DCFH and DBA ( $\rho = 0.53, p < 0.05$ ), GSH and PA ( $\rho = -0.539, p < 0.05$ ),  
484 and ANT ( $\rho = -0.514, p < 0.05$ ), as well as IL-6 and BaA ( $\rho = 0.54, p < 0.05$ ), BkF ( $\rho = 0.52, p <$   
485  $0.05$ ), and BeP ( $\rho = 0.58, p < 0.05$ ). Furthermore, the results suggested that new emission control  
486 technologies and policies should focus on OC and PAH reduction to reduce their adverse health  
487 effects on the human respiratory system. Nevertheless, the currently study focused on the chemical  
488 and toxicological analysis of pollutants in particle phase only. Further studies on volatile and semi-  
489 volatile organic pollutants from diesel vehicle exhaust are warranted. Another aspect to be  
490 considered in future studies is the effect of particle size distribution and particle morphologies on  
491 the chemical and toxicological properties of the diesel PM samples. An integration of the outcomes

492 of the aforementioned studies and the data in this paper can provide a more comprehensive picture  
493 of the chemical composition and toxicological properties of diesel emission in the future.

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497

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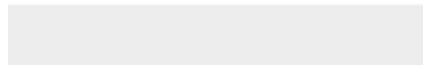


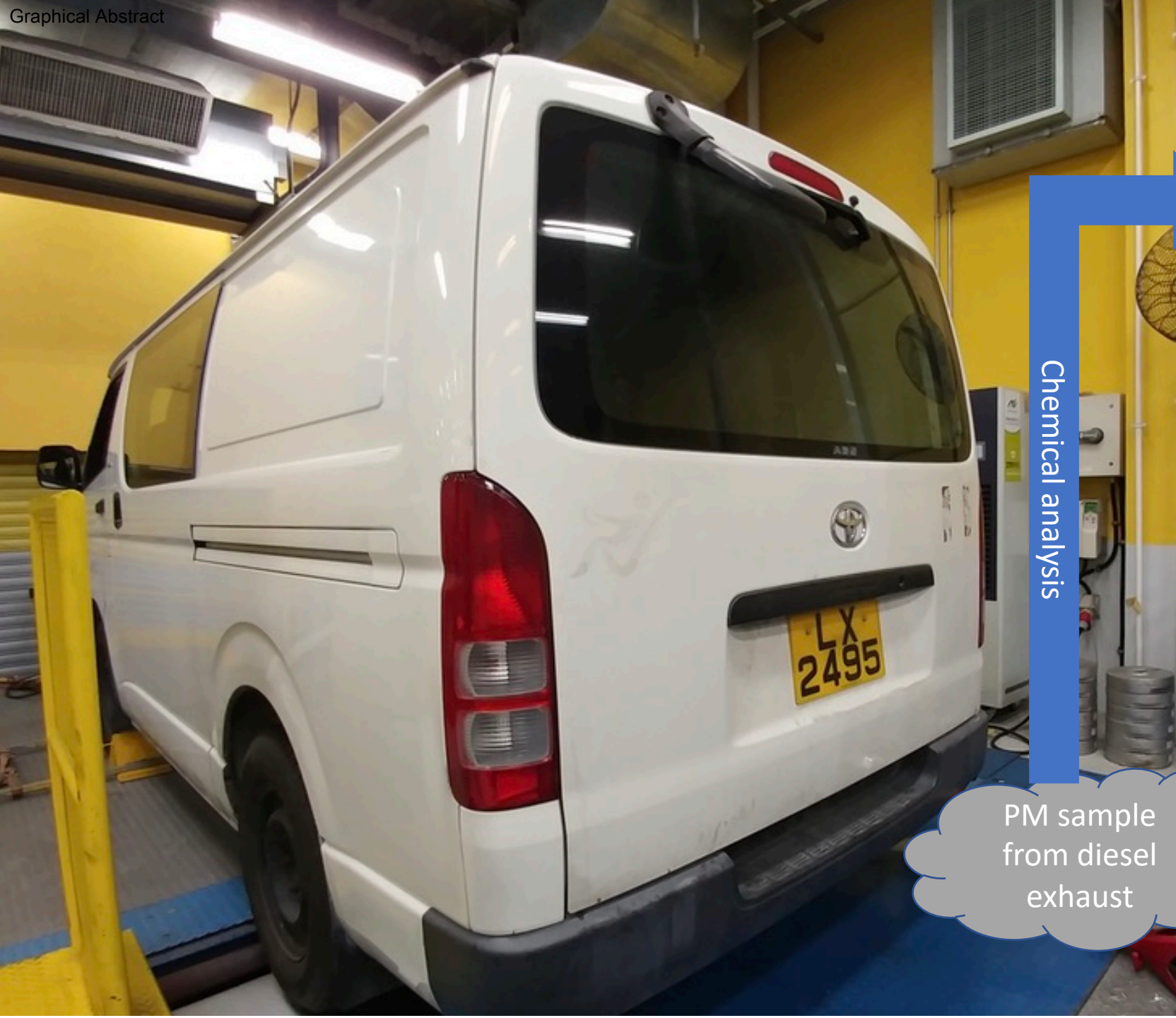


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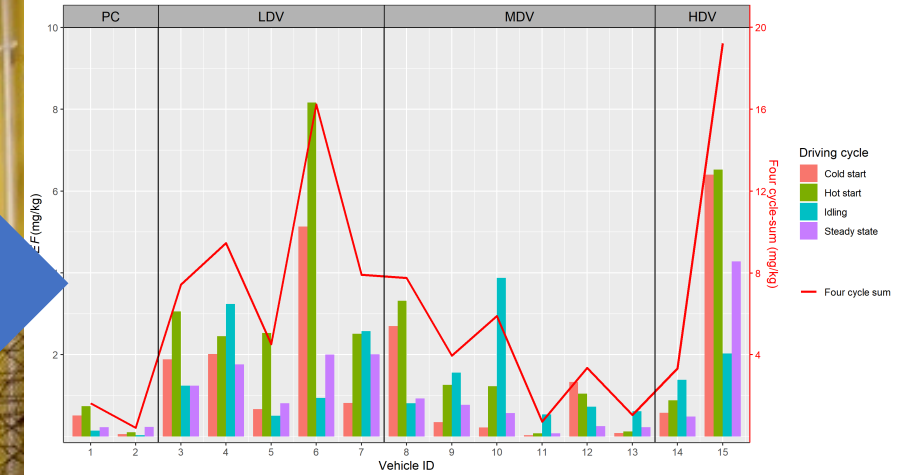
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Chemical analysis

PM sample from diesel exhaust



Toxicological analysis

**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](mailto:kfho@cuhk.edu.hk) if you need additional information.

Sincerely,

Kin Fai Ho

The Jockey Club School of Public Health and Primary Care,

The Chinese University of Hong Kong,

Hong Kong, China

+852 22528763

+852 26063500

[kfho@cuhk.edu.hk](mailto:kfho@cuhk.edu.hk)