1 # Inflammatory Response and $PM_{2.5}$ Exposure of Urban
2 # Traffic Conductors
3

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Abstract

Human exposure to airborne $PM_{2.5}$ has been linked to increased risk of respiratory and cardiovascular diseases, possibly via activation of systemic inflammation. However, associations between airborne $PM_{2.5}$ and systemic inflammation in humans remained inconclusive. The traffic related air pollutants (TRAPs) are the major source of $PM_{2.5}$ in urban areas**; the adverse health effect of $PM_{2.5}$ from TRAPs is currently a critical issue of public concern.** The present cross-sectional study aimed to examine the relationship between $PM_{2.5}$ exposure and systemic inflammation for considering health impacts of **TRAP $PM_{2.5}$** on urban traffic conductors. All study participants, i.e., office policemen (the reference) and traffic conductors (the exposure), were requested to carry a personal sampler to determine individual $PM_{2.5}$ exposure. An adenovirus-based NF-κB luciferase reporter assay was used to determine proinflammatory activity in serum samples collected from **the** study participants**. The blood proinflammatory activity was presented as tumor necrosis factor-α (TNFα) equivalence (TNFα-EQ), which was extrapolated from the sigmoidal semi-logarithmic dose-response curve of NF-κB reporter assay by TNFα.** **Levels of** both personal $PM_{2.5}$ exposure and blood proinflammatory activity **(TNFα-EQ)** in the exposure group **(traffic conductors)** were significantly higher than that in the reference group **(office policemen) (p < 0.05)**. **The present study revealed** a positive **and significant** association between personal $PM_{2.5}$ exposure **levels** and blood **TNFα-EQ levels, in a linear regression model of y = 0.511x – 3.062 (y = log TNFα-EQ and x = log $PM_{2.5}$) (R = 0.231 and p = 0.047); the result** suggests that exposure to TRAP $PM_{2.5}$ significantly contributes to the increased systemic inflammation in humans. **The study provides clear evidence that long-term occupational exposure to TRAPs causes adverse health impacts, i.e., inflammation, on traffic conductors.**

***Keywords*:** Air Pollution; Health Effects/Risks; Human Exposure; Personal Exposure; Toxicology.

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52 # INTRODUCTION

53

54 For over decades, particulate matter (PM), particularly fine PM, has been
55 identified to have marked contribution to air pollution. Upon inhalation, PM tends to
56 accumulate in human respiratory tract and thus is classified as a severe health hazard
57 (Bilal *et al.*, 2017; Cai *et al.*, 2017; Morales Betancourt *et al.*, 2017). According to US
58 Environmental Protection Agency (EPA) (2016), $PM_{2.5}$ is the fine particle with a
59 diameter of 2.5 micrometers or less, such as the emissions from construction sites,
60 unpaved roads, smokestacks, fire, and vehicles. Both toxicological and clinical studies
61 revealed that acute exposure to high levels of PM led to immediate physiological
62 changes (Osornio-Vargas *et al.*, 2003; Chow *et al.*, 2015; McGrath *et al.*, 2017). The
63 peak concentration of air pollutants in the transportation environment could be up to
64 three times higher than that in the background (Morales Betancourt *et al.*, 2017).
65 Therefore, traffic related air pollutants (TRAPs) may cause significant health impacts
66 especially on those with routine exposure, e.g., drivers, commuters, and traffic
67 conductors.

68 Atmospheric $PM_{2.5}$ has been recognized as one of the major air pollutants in
69 urban areas because of its influence on public health, visibility deterioration, and
70 global climate change (Liang *et al.*, 2015; Chew *et al.*, 2016; Li *et al.*, 2016). $PM_{2.5}$
71 emission from internal combustion engines represents a major source of TRAPs in
72 urban areas with heavy traffic (Matawle, 2015; Lu *et al.*, 2016; Tseng, 2016; Fan *et*
73 *al.*, 2017; Fujitani *et al.*, 2017). It was found that the $PM_{2.5}$-bound pollutants included
74 polycyclic aromatic hydrocarbons (PAHs), carbonaceous species, heavy metals,
75 carbon black, and halogen persistent organic chemicals (e.g., polybrominated
76 diphenyl ethers (PBDEs)) in heavy-traffic areas (Chen *et al.*, 2016; Chao *et al.*, 2016;
77 Wang *et al.*, 2018). Importantly, there is evidence that airborne PM of TRAPs is

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78 linked to increased levels of inflammatory response (Kannan *et al.*, 2006; Ritz and
79 Wilhelm, 2008; Liu *et al.*, 2017).

80 Epidemiological studies have demonstrated an increased risk of pulmonary
81 disease, lung cancer, cardiovascular disease, or DNA damage in humans with
82 long-term exposure to airborne $PM_{2.5}$ (Pope *et al.*, 2002; Vinzents *et al.*, 2005; Miller
83 *et al.*, 2007; Cao *et al.*, 2012; Chu *et al.*, 2015; Wang *et al.*, 2015). During cold
84 weather, high levels of outdoor $PM_{2.5}$ were associated with increased emergency visits
85 for cardiovascular and respiratory diseases, particularly hypertension, heart failure,
86 and asthma (Rodopoulou *et al.*, 2015). Results from animal model studies revealed
87 the activation of lung inflammation in response to atmospheric PM (Mantecca *et al.*,
88 2010) or $PM_{2.5}$ collected in a residential area (Park *et al.*, 2011). Upon inhalation,
89 $PM_{2.5}$ is more capable than PM_{10} of reaching distal regions of the lung, where $PM_{2.5}$
90 may trigger the inflammatory response (Osornio-Vargas *et al.*, 2003; Ferguson *et al.*,
91 2013).

92 Epidemiological studies have also revealed the association between TRAPs and
93 systemic inflammation. Occupational exposure of taxi drivers with air pollutants
94 resulted in elevated levels of proinflammatory cytokines, e.g., TNFα, in blood
95 (Brucker *et al.*, 2013). Effects of $PM_{2.5}$/$PM_{2.5}$-bound chemicals on activation of
96 systemic inflammation were observed in urban residents (Liu *et al.*, 2017; Wang *et al.*,
97 2018). However, it was also noted that some epidiological results did not support the
98 idea that TRAPs could cause inflammation. A study in healthy adults in commuting
99 indicated that TRAPs exposure was not consistently associated with acute changes in
100 serum inflammation markers, i.e., IL6, IL8, TNFα, and C-reactive protein (CRP)
101 (Zuurbier *et al.*, 2011). Another study in highway maintenance workers suggested that
102 $PM_{2.5}$ exposure was positivly associated with CRP, but was negatively associated with

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103 TNFα (Meier *et al.*, 2014). Moreover, short-term diesel exhaust exposure caused no
104 significat effects on proinflammatory cytokines (i.e., IL6 and TNFα) in healthy adults
105 (Cliff *et al.*, 2016). Indeed, exposure scenarios of TRAPs, such as dosage, exposure
106 duration, pollutant types, etc., may partly explain the difference of these studies.
107 Importantly, plasma samples collected from human volunteers with diesel exhaust
108 exposure were demonstrated to enhance inflammatory gene expreesion *in vitro*,
109 suggesting the elevated proinflammatory factors in circulating (Channell *et al.*, 2012).
110 Therefore, improving measurement of the *total* proinflammatory activity, instead of a
111 selected proinflammtory marker or a panel of inflammatory cytokines, in blood
112 samples is a promising alternative to further justify the finding.

113 In the present cross-sectional study in traffic conductors (the exposure) and
114 office policemen (the reference), personal airborne $PM_{2.5}$ sampling and an
115 adenovirus-based NF-κB luciferase reporter assay were used to determine the
116 individual $PM_{2.5}$ exposure and the *total* proinflammatory activity in blood,
117 respectively. Results of the study clearly demonstrated the association between
118 personal $PM_{2.5}$ exposure and systemic proinflammatory activity in humans with
119 occupational exposure to TRAPs.

120

121 # METHODS

122

123 ***Study participants***

124 The cross-sectional study was designed in this research. Study participants were
125 invited to have a health examination survey of the Taipei polices (HESTP) from April
126 2009 to June 2011. The HESTP cohort information was previously described in detail
127 (Huang *et al.*, 2012; Huang *et al.*, 2013). Briefly, there was a total of 144 participants
128 in the HESTP cohort, including 91 traffic conductors as the exposure group (case) and

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129 53 indoor office policemen as the reference group (control), at ages between 20 and
130 63 years old. The HESTP cohort were healthy and had been in their current job for
131 more than 3 months. With their agreement, the HESTP cohort were required to
132 complete self-administered questionnaires (including demographic parameters,
133 lifestyle, smoking/drinking habit, and disease history), to undergo health examination,
134 and to collect urine and serum samples.

135 **The present study participants were selected using convenience sampling. As**
136 **summarized in Table 1, Population 1 (N = 115), i.e., 69 traffic conductors (the**
137 **exposure group) and 46 office policemen (the reference group), was sampled**
138 **from the HESTP cohort. Population 2 (N = 75), i.e., 35 traffic conductors (the**
139 **exposure group) and 40 office policemen (the reference group), was sampled**
140 **from the Population 1.** The study protocol was reviewed and approved by the
141 Institutional Review Board of the Human Ethical Committees in National Health
142 Research Institutes, Taiwan in 2009. Ethical standards formulated in Declarations of
143 Helsinki in 1964 and revised in 2008 (sixth revision) were followed. The informed
144 consent was written by the participants after receiving detailed explanation of the
145 study and potential consequences prior to enrollment (Huang *et al.*, 2012; Huang *et*
146 *al.*, 2013).

147 ***Airborne $PM_{2.5}$ sampling and personal $PM_{2.5}$ exposure determination***

148 Following the standard method by United States Environmental Protection
149 Agency (US EPA) (EPA Method IP-10A), personal airborne $PM_{2.5}$ sampling was
150 conducted to determine individual $PM_{2.5}$ exposure in the daily work shift as
151 previously described (Huang *et al.*, 2012). Briefly, the Personal Environmental
152 Monitor (PEM) (761-203) (SKC Inc., PA, USA), with a 2.5-μm single-stage impactor
153 for $PM_{2.5}$ air sampling, was connected to a Gilian GilAir 5 pump (Sensidyne Inc.,

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154 Clearwater, FL, USA); before each test, the pump was calibrated at a flow rate of 2
155 liter min^{-1}. For airborne $PM_{2.5}$ sampling, the study participants were equipped with a
156 PEM for 9-10 working hours per day. Airborne fine particulate ($PM_{2.5}$) was collected
157 on a 2.5-μm 50%-cutting-size Teflon filter (37 mm diameter) (Biotech Line, Lynge,
158 Denmark). To reduce sampling bias, the Teflon filters were conditioned in a
159 temperature/humidity-controlled space (before and after each sampling) before
160 weighing on a Micro Balance MT5 (Mettler-Toledo, Glostrup, Denmark). Personal
161 $PM_{2.5}$ exposure (μg m^{-3}) was defined as the collected $PM_{2.5}$ mass on the filter divided
162 by the sampled air volume.

163 ***Reagents and cell culture***

164 RPMI medium 1640, fetal bovine serum (FBS) (10091-148), and
165 penicillin/streptomycin (15140-122) were purchased from Gibco/Invitrogen
166 (Carlsbad, CA, USA). Sodium bicarbonate (S5761) was from Sigma Aldrich and
167 tumor necrosis factor-α (TNFα) (1371843) was from Roche. Human promonocytic
168 leukemia HL-CZ cells (BCRC-60043) was purchased from Bioresource Collection
169 and Research Center (BCRC) (Hsinchu, Taiwan). HL-CZ cells were cultured in RPMI
170 supplemented with 10% FBS, 1% penicillin/streptomycin, and 1.5 mg ml^{-1} sodium
171 bicarbonate.

172 ***Adenovirus-based NF-κB luciferase reporter assay***

173 Blood samples were collected from the study participants within half an hour
174 after the end of a work-shift on two consecutive days. Following centrifugation,
175 serum was collected and kept at -20°C until use. NF-κB luciferase reporter assay was
176 performed by using the recombinant adenovirus AdV-NFκB-Luc as previously
177 described in detail (Tsou *et al.*, 2011). Briefly, HL-CZ cells (1 x 10^4 cells per well in
178 96-well plates) were infected with AdV-NFκB-Luc at multiplicity of infection (MOI)

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179 of 0.2 pfu cell^{-1} for 16 hours. Then, the infected cells were treated with serum samples
180 or different levels of TNFα for 6 hours. Luciferase activity was determined with the
181 Luciferase Assay System (Promega, Madison, WI, USA) according to the
182 manufacturer's instructions.

183 ***Data and statistical analysis***

184 Experimental data were presented as means ± standard error (SE). Both personal
185 $PM_{2.5}$ exposure and blood proinflammatory activity levels were compared between
186 reference and exposure groups by the non-parametric Mann-Whitney *U* test due to the
187 non-normal distribution of data. *P*-values less than 0.05 were considered statistically
188 significant. After logarithmic transformation, levels of personal $PM_{2.5}$ exposure and
189 blood proinflammatory activity were fitted into a normal (Gaussian) distribution for
190 correlation analysis. All statistical analyses were carried out with the IBM SPSS
191 Statistics 21.0 (IBM Corp., Armonk, NY, USA).

192

193 ## RESULTS AND DISCUSSION

194

195 ***Descriptive analysis of demographic characteristics of study participants***

196 Descriptive analysis of demographic characteristics of study participants was
197 summarized in Table 1. In Population 1 (N = 115), the mean ages of the exposed and
198 reference groups were 48.9 and 42.8 years old, respectively; in Population 2 (N = 75),
199 the mean ages of the exposed and reference groups were 47.5 and 42.6 years old,
200 respectively. In Population 1, body mass index (BMI) of the exposure group (25.1 kg
201 m^{-2}) was significantly lower than that of the reference group (29.1 kg m^{-2}); in
202 Population 2, BMI of the exposure group (25.0 kg m^{-2}) was significantly lower than
203 that of the reference group (29.1 kg m^{-2}). Gender was significantly different between
204 the exposed and reference groups, with 74.3% to 75.4% of male in the exposure group

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205 and 77.5% to 78.3% of female in the reference group. In both Population 1 and
206 Population 2, the reference group had higher education levels than the exposure group
207 (p<0.001); no significant difference was observed in smoking habit, cooking habit,
208 drinking alcohol, and vitamin supplement consumption between the two groups.
209 Regarding the variables listed in Table 1, no significant difference was detected
210 between Population 1 and Population 2 and both Populations showed similar
211 characteristics with the previous HESTP cohort studies (Huang *et al.*, 2012; Huang *et*
212 *al.*, 2013).

213 ***Establishment of a dose-response curve of NF-κB luciferase activation by TNFα***

214 **NF-κB, a pivotal transcription factor of inflammatory responses, regulates**
215 **multiple aspects of innate and adaptive immune functions. Recent in-vitro**
216 **evidence revealed the cause-effect relationship between $PM_{2.5}$ and inflammatory**
217 **responses, where $PM_{2.5}$ treatments induce gene expression of NF-κB family and**
218 **activate NF-κB signaling (Dou et al., 2018; Marano et al., 2002; Zhang et al.,**
219 **2018). In this study, an adenovirus-based NF-κB luciferase reporter assay (Tsou**
220 ***et al.*, 2011) was adopted to determine the *total* proinflammatory activity in**
221 **serum samples collected from the study participants. Because NF-κB**
222 **transcription factors responds to most proinflammatory stimuli, the NF-κB**
223 **luciferase reporter assay provides a superior alternative to the conventional**
224 **ELISA, which allows detection of only one or a panel of selected cytokines.**

225 **First of all, the responsiveness of the NF-κB luciferase reporter assay to**
226 **proinflammatory stimuli was validated by using TNFα, a multifunctional**
227 **proinflammatory cytokine, i.e., a *positive* mediator of inflammation.** The
228 AdV-NFκB-Luc-infected HL-CZ cells were treated with TNFα (0, 0.003, 0.01, 0.03,
229 0.1, 0.3, 1.0, and 3.0 pg ml^{-1}) for 6 hours and then luciferase activity was determined.

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230 Results in Fig. 1 summarized the dose-dependent activation of NF-κB luciferase
231 reporter gene by TNFα; the sigmoidal semi-logarithmic dose-response curve
232 (R^2>0.95, p<0.001) was fitted by using a non-linear equation, $y = a_0 + (c_0 - a_0)/(1 +$
233 $10^{[(logEC50 - x)*\theta]})$ (see figure legend for details). The relative standard deviations (RSD)
234 from triplicate measurements in each test was below 20%. The limit of detection
235 (LOD) for TNFα was 0.00087 pg ml^{-1}, as defined by 3 times SD above the average
236 RLU value of the zero standard. **It was noted that the LOD of our NF-κB reporter**
237 **assay was lower than those of the enzyme-linked immunosorbance assay**
238 **(ELISA) (Dieme *et al.*, 2012; Brucker *et al.*, 2013; Hüls *et al.*, 2017; Zhou *et al.*,**
239 **2017). Taken together, the results suggest that the NF-κB luciferase reporter**
240 **assay is very sensitive to proinflammatory stimuli. Moreover, with TNFα as a**
241 **reference proinflammatory cytokine, the reporter assay was used thereafter to**
242 **determine the total proinflammatory activity in serum samples collected from**
243 **the study participants.**
244 ***Personal $PM_{2.5}$ exposure and blood proinflammatory activity***
245 Taxi drivers are constantly exposed to TRAPs, a heterogeneous mixture of
246 hazardous chemicals, and have been demonstrated to have significantly higher levels
247 of proinflammatory biomarkers, such as TNFα, in blood (Brucker *et al.*, 2013). For
248 the present HESTP cohort, we used personal airborne $PM_{2.5}$ sampling to determine
249 individual $PM_{2.5}$ exposure and NF-κB luciferase reporter assay to determine the blood
250 proinflammatory activity. It was of importance that the personal sampling and the
251 reporter assay in the present study provided high-quality data of the $PM_{2.5}$ exposure
252 and the *total* proinflammatory activity, respectively, of each study participant for
253 further analysis.
254 Results in Fig. 2 (A) indicated that the personal $PM_{2.5}$ exposure of the exposure

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255 group (150 ± 15.4 μg m^{-3}) (mean ± SE) was significantly higher than that of the
256 reference group (82.0 ± 4.53 μg m^{-3}) ($p < 0.001$). A previous study in the same
257 HESTP cohort revealed that the median values of $PM_{2.5}$ (82.9 μg m^{-3}) and
258 $PM_{2.5}$-bound PAHs (13.1 ng m^{-3}) of the exposure group were significantly higher than
259 that of the reference group ($PM_{2.5}$ = 70.8 μg m^{-3} and PAHs = 8.24 ng m^{-3}); in the
260 exposure group, a statistically significant positive correlation between the personal
261 $PM_{2.5}$ exposure and the $PM_{2.5}$-bound PAHs was observed (R = 0.42, $p < 0.001$)
262 (Huang *et al.*, 2012). **The NF-κB luciferase reporter assay was used here to**
263 **determine *total* proinflammatory activity in blood samples. The resulted**
264 **induction of NF-κB activity (RLU) by blood samples was converted into TNFα**
265 **equivalence (TNFα-EQ) (pg ml^{-1}) with the equation shown in Fig. 1.** The blood
266 proinflammatory activity in the exposure group (0.0302 ± 0.0048 pg ml^{-1} TNFα-EQ)
267 (mean ± SE) was significantly higher than that of the reference group (0.00440 ±
268 0.000800 pg ml^{-1} TNFα-EQ) ($p < 0.001$) (Fig. 2 (B)). **For example, blood samples**
269 **with proinflammatory activity of 0.1 pg ml^{-1} TNFα-EQ could induce the same**
270 **levels of NF-κB luciferase activity as 0.1 pg ml^{-1} TNFα.** Results in Fig. 2 together
271 showed that the exposure group exhibited higher levels of $PM_{2.5}$ exposure and blood
272 proinflammatory activity than the reference group, suggesting the potential positive
273 association between TRAPs (i.e., $PM_{2.5}$) and systemic inflammation (i.e., NF-κB
274 luciferase activity or TNFα-EQ).

275 ***Associations between blood proinflammatory activity and personal $PM_{2.5}$ exposure***

276 However, it was noted that both personal $PM_{2.5}$ exposure and blood
277 proinflammatory activity levels exhibited a non-normal distribution (Figs. 3(A) and
278 3(B)). Following logarithmic transformation, both results were shown in Figs. 3(C)
279 and 3(D). The normality of data in Fig. 3 was assessed with skewness and kurtosis as

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280 previously described (Kim, 2013), where data were considered as a normal
281 distribution when both $Z_{skewness}$ and $Z_{kurtosis}$ scores were between -3.29 and 3.29. The
282 skewness, kurtosis, and Z scores of both $PM_{2.5}$ and blood proinflammatory activity
283 (TNFα-EQ) before and after logarithmic transformation were summarized in Table 2.
284 Clearly, the log-transformed data of both $PM_{2.5}$ exposure ($Z_{skewness}$ = 2.29 and $Z_{kurtosis}$
285 = 1.61) and blood TNFα-EQ ($Z_{skewness}$ = -0.390 and $Z_{kurtosis}$ = -1.903) were considered
286 as a normal distribution.

287 Results in Fig. 4 revealed a statistically significant positive association between
288 blood proinflammatory activity and personal $PM_{2.5}$ exposure after logarithmic
289 transformation. The relationship between log TNFα-EQ and log $PM_{2.5}$ was fitted into
290 the linear equation y = 0.511x - 3.062 (y = log TNFα-EQ and x = log $PM_{2.5}$), with R =
291 0.231 and p = 0.047. As the scatterplot summarized in Fig. 4, most data in the
292 exposure group were above the regression line, whereas most data in the reference
293 group were below the line. The present finding supports the idea that TRAPs is able to
294 cause systemic inflammation in urban traffic conductors.

295 TNFα has been used as a biomarker to evaluate proinflammatory response by
296 $PM_{2.5}$ exposure both *in vitro* and *in vivo*. $PM_{2.5}$ exposure markedly induced the
297 expression and release of TNFα in culture cells (Dieme *et al.*, 2012; Liu *et al.*, 2014;
298 Pardo *et al.*, 2015; Pope *et al.*, 2016;Yan *et al.*, 2016; Niu *et al.*, 2017). In rodents
299 with $PM_{2.5}$ exposure, increased TNFα levels were detected in blood circulation,
300 hippocampus, and prefrontal cortex (Li *et al.*, 2015; Hu *et al.*, 2017; Li *et al.*, 2018).
301 Therefore, the NF-κB luciferase activity in this study was converted to TNFα-EQ for
302 evaluation of proinflammatory activation in response to TRAPs. In the process, the
303 $PM_{2.5}$-bound metals (Dieme *et al.*, 2012; Liu *et al.*, 2014; Pardo *et al.*, 2015; Yan *et*
304 *al.*, 2016) and PAHs (Dieme *et al.*, 2012; Niu *et al.*, 2017) could be the active

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components for TNFα induction.

TRAP $PM_{2.5}$-bound chemicals contributed to the formation of reactive oxygen species (ROS)-related biomarkers (i.e., 8-oxo-7,8-dihydroguanine (8-oxodG) and 1-hydroxypyrene glucuronide (1-OHPG)) and activation of inflammatory mediators (i.e. interleukin 6 and TNFα) (Zuurbier *et al.*, 2011; Brucker *et al.*, 2013; Meier *et al.*, 2014; Cliff *et al.*, 2016; Dai *et al.*, 2018; Hüls *et al.*, 2017). Our previous studies in HESTP cohort revealed that $PM_{2.5}$-bound PAHs were significantly correlated with both 1-OHPG (a PAH metabolite) and 8-oxodG (an oxidative DNA damage biomarker) in urine (Huang *et al.*, 2012), and urinary 8-oxodG was significantly associated with urinary levels of cadmium and 1-OHPG (Huang *et al.*, 2013). The present study in healthy adults with occupational TRAPs exposure indicated that the personal $PM_{2.5}$ exposure was significantly associated with the blood proinflammatory activity (TNFα-EQ). Moreover, it is noted that NF-κB is a redox-sensitive transcription factor involved in regulating metabolism gene expression in response to heavy metal exposure (Korashy and El-Kadi, 2008). **These studies together suggest that cellular metabolism of $PM_{2.5}$-bound pollutants, e.g., heavy metals and PAHs, from TRAPs may contribute to the induction of proinflammatory cytokine genes via ROS production and/or NF-κB activation. Results of previous (Huang et al., 2012; Huang et al., 2013) and present studies in the HESTP cohort highlight the finding that long-term occupational exposure to TRAPs is able to cause adverse health impacts, e.g., inflammation and oxidative stress, on traffic conductors.**

The limitations of this study are mentioned here for further consideration of research. Firstly, the $PM_{2.5}$ levels collected by personal samplers of both exposure and reference groups in the present study were pretty high. When the airborne $PM_{2.5}$ levels meet the indoor air quality standards (IAQs), the present NF-κB

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330 **luciferase reporter system may not be sensitive enough to differentiate the**
331 **difference of blood proinflammatory activity between exposure and reference**
332 **groups. Secondly, PM-bound chemicals of different characteristics may activate**
333 **different biological responses; therefore, chemicals of $PM_{2.5}$ emitted from the**
334 **other sources may not be able to activate NF-κB as well as those of TRAP $PM_{2.5}$.**
335 **Thirdly, convenience sampling, instead of random sampling, was used for**
336 **recruiting the study participants in the present study.**

337

338 ## CONCLUSIONS

339

340 The study in traffic conductors (the exposure) and office policemen (the
341 reference) revealed that the exposure group exhibited elevated levels of both $PM_{2.5}$
342 exposure and proinflammatory activity than the reference group. A significant positive
343 association between personal $PM_{2.5}$ exposure and blood proinflammatory activity was
344 observed. These results suggest the potential involvement of TRAPs in activation of
345 systemic inflammation in urban traffic conductors.

346

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348

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352

353 ## DISCLAIMER

354

355 The authors declare no conflicts of interest.

356

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560 **Figure Captions**

561 **Fig. 1.** Sigmoidal semi-logarithmic dose-response curve of NF-κB luciferase
562 induction by TNFα. NF-κB luciferase activity is expressed as relative light units
563 (RLU). Data from three independent experiments (n = 3) are presented in a bar chart
564 with means ± SE. The dose-response curve ($R^2 > 0.95$, $p < 0.001$) was fitted by using
565 a non-linear equation: $y = a_0 + (c_0 - a_0)/(1 + 10^{[(\log EC50 - x)*\theta]})$, in which y is the NF-κB
566 luciferase activity, c_0 is the maximal NF-κB luciferase activity, a_0 is the basal NF-κB
567 luciferase activity, EC50 is the half-maximal effective TNFα concentration, x is the
568 TNFα concentration, and θ is the hillslope.

569

570 **Fig. 2.** Comparisons of personal $PM_{2.5}$ exposure and blood proinflammatory activity
571 between reference and exposure groups. (A) Personal $PM_{2.5}$ exposure of Population 1
572 (N = 115) and (B) blood proinflammatory activity in Population 2 (N = 75) were
573 shown in scatterplots with means and SE error bars. Number in parentheses means
574 sample size. ****p* < 0.0001, with the non-parametric Mann-Whitney U test.

575

576 **Fig. 3.** Distribution of $PM_{2.5}$ exposure and proinflammatory activity of the study
577 participants. Histograms of (A) $PM_{2.5}$ exposure of Population 1 (N = 115) and (B)
578 TNFα-EQ levels in Population 2 (N = 75) were summarized. Logarithmic (C) $PM_{2.5}$
579 exposure and (D) TNFα-EQ levels were respectively converted from the original data
580 in (A) and (B).

581

582 **Fig. 4.** Association between blood proinflammatory activity (in TNFα-EQ) and
583 personal $PM_{2.5}$ exposure. Both TNFα-EQ and $PM_{2.5}$ data of the 75 participants in
584 Population 2 (40 in reference group and 35 in exposure group) were summarized in

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585 the scatterplot of log TNFα-EQ versus log $PM_{2.5}$. The relationship between log

586 TNFα-EQ and log $PM_{2.5}$ was fitted into the linear equation $y = 0.511x - 3.062$ (y = log

587 TNFα-EQ and x = log $PM_{2.5}$), with $R = 0.231$, $F(1,73) = 4.097$, $^{*}p = 0.047$, and the 95%

588 confidence interval for mean of slope between 0.008 and 1.015.

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1 **Table 1**. Descriptive analysis of demographic characteristics of study participants

Variables	Population 1 (N=115) [a] Exposure group (N=69) Mean (SD) or N (%) [b]	Population 1 (N=115) [a] Reference group (N=46) Mean (SD) or N (%) [b]	*p* values	Population 2 (N=75) [a] Exposure group (N=35) Mean (SD) or N (%) [b]	Population 2 (N=75) [a] Reference group (N=40) Mean (SD) or N (%) [b]	*p* values
Age in years [c]	48.9 (9.08)	42.8 (8.84)	<0.001	47.5 (9.33)	42.6 (7.09)	0.036
BMI in kg m^{-2} [c]	25.1 (3.54)	29.1 (6.49)	<0.001	25.0 (3.59)	29.1 (6.49)	<0.001
Gender [d]			<0.001			<0.001
Male	52 (75.4)	10 (21.7)		26 (74.3)	9 (22.5)	
Female	17 (24.6)	36 (78.3)		9 (25.7)	31 (77.5)	
Education levels [d]			<0.001			<0.001
College	26 (37.7)	38 (82.6)		13 (37.1)	33 (82.5)	
High school	43 (62.3)	8 (17.4)		22 (62.9)	7 (17.5)	
Smoking habit [d]			0.196			0.223
Smokers	16 (23.2)	6 (13.0)		8 (22.9)	6 (15.0)	
Nonsmokers	53 (76.8)	40 (87.0)		27 (77.1)	34 (85.0)	
Cooking habit [d]			0.107			0.134
Yes	29 (42.0)	24 (52.2)		15 (42.9)	21 (52.5)	
No	40 (58.0)	22 (47.8)		20 (57.1)	19 (47.5)	
Drinking alcohol [d]			0.997			0.997
Yes	12 (17.4)	8 (17.4)		6 (17.1)	7 (17.5)	
No	57 (82.6)	38 (82.6)		29 (82.9)	33 (82.5)	
Vitamin supplement [d]			0.151			0.284
Yes	39 (56.5)	19 (41.3)		20 (57.1)	17 (42.5)	
No	30 (43.5)	27 (58.7)		15 (42.9)	23 (57.5)	

2 [a] Population 1 (N = 115), i.e., 69 traffic conductors (the exposure group) and 46 office policemen (the reference group), was sampled from the HESTP
3 cohort. Population 2 (N = 75), i.e., 35 traffic conductors (the exposure group) and 40 office policemen (the reference group), was sampled from the
4 Population 1.
5 [b] Mean (SD) or N (%): mean (standard deviation) or number (percentage)
6 [c] Mean (standard deviation)
7 [d] Number (percentage)
8

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1 **Table 2.** Skewness, kurtosis, and Z scores for personal $PM_{2.5}$ exposure (in Population 1) and
2 blood proinflammatory activity (TNFα-EQ) (in Population 2) before and after logarithmic
3 transformation

	N	Skewness	$SE_{Skewness}$	$Z_{Skewness}$ [a]	Kurtosis	$SE_{Kurtosis}$	$Z_{Kurtosis}$ [a]
Population 1	115						
$PM_{2.5}$		3.22	0.226	14.3	14.1	0.447	31.4
Log $PM_{2.5}$		0.517	0.226	2.29	0.766	0.447	1.61
Population 2	75						
TNFα-EQ		4.62	0.277	16.7	28.5	0.548	52.0
Log TNFα-EQ		-0.108	0.277	-0.390	-1.043	0.548	-1.903

4 [a] $Z_{Skewness}$ = Skewness/$SE_{Skewness}$; $Z_{Kurtosis}$ = Kurtosis/$SE_{Kurtosis}$

5

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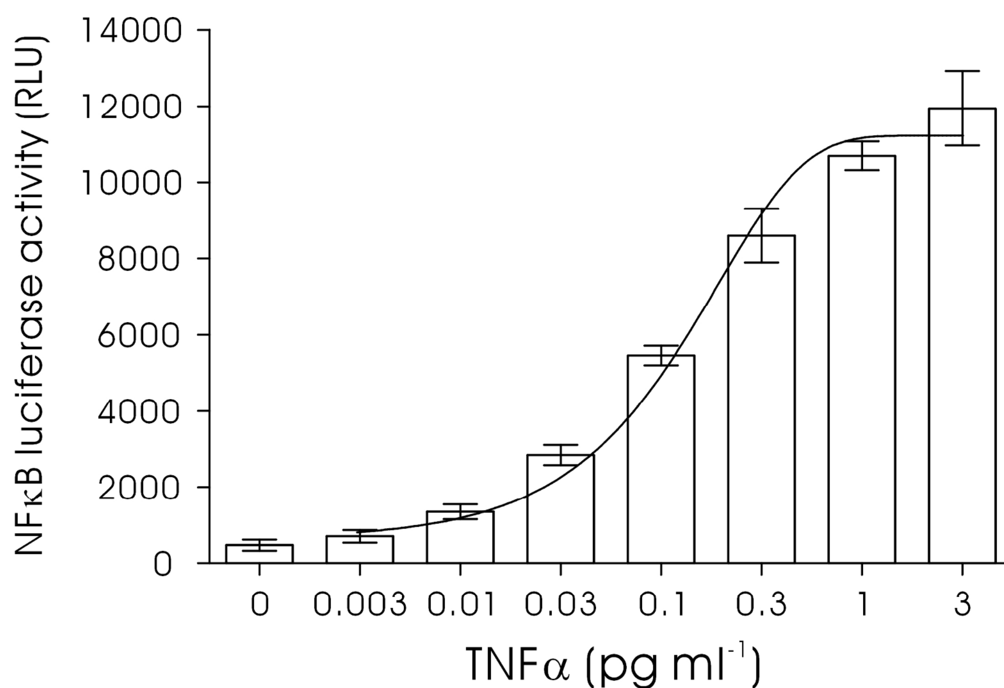

Fig. 1. Sigmoidal semi-logarithmic dose-response curve of NF-κB luciferase induction by TNFα. NF-κB luciferase activity is expressed as relative light units (RLU). Data from three independent experiments (n = 3) are presented in a bar chart with means ± SE. The dose-response curve ($R^2 > 0.95$, $p < 0.001$) was fitted by using a non-linear equation: $y = a0 + (c0 - a0)/(1 + 10^{[(\log EC50 - x)*\theta]})$, in which y is the NF-κB luciferase activity, c0 is the maximal NF-κB luciferase activity, a0 is the basal NF-κB luciferase activity, EC50 is the half-maximal effective TNFα concentration, x is the TNFα concentration, and θ is the hillslope.

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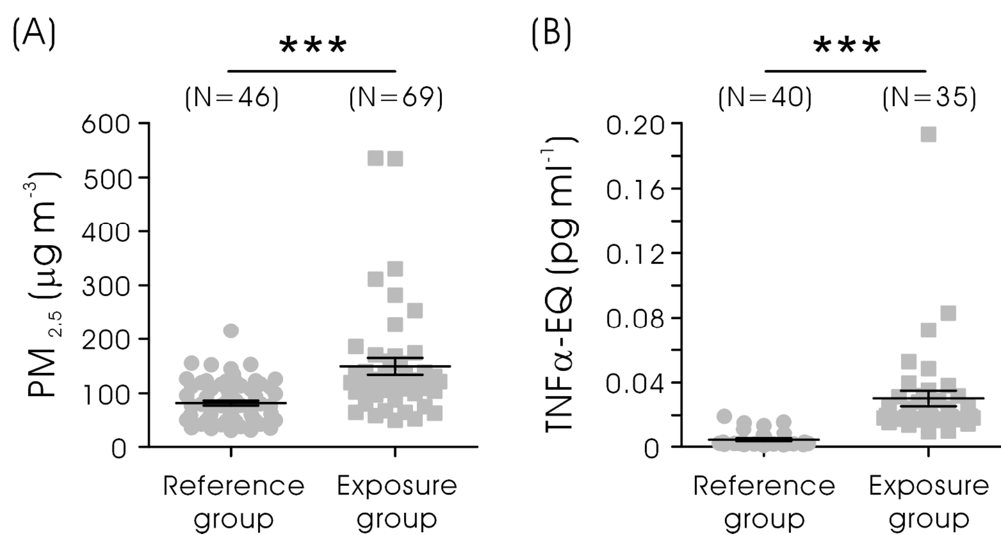

Fig. 2. Comparisons of personal PM2.5 exposure and blood proinflammatory activity between reference and exposure groups. (A) Personal PM2.5 exposure of Population 1 (N = 115) and (B) blood proinflammatory activity in Population 2 (N = 75) were shown in scatterplots with means and SE error bars. Number in parentheses means sample size. ***p < 0.0001, with the non-parametric Mann-Whitney U test.

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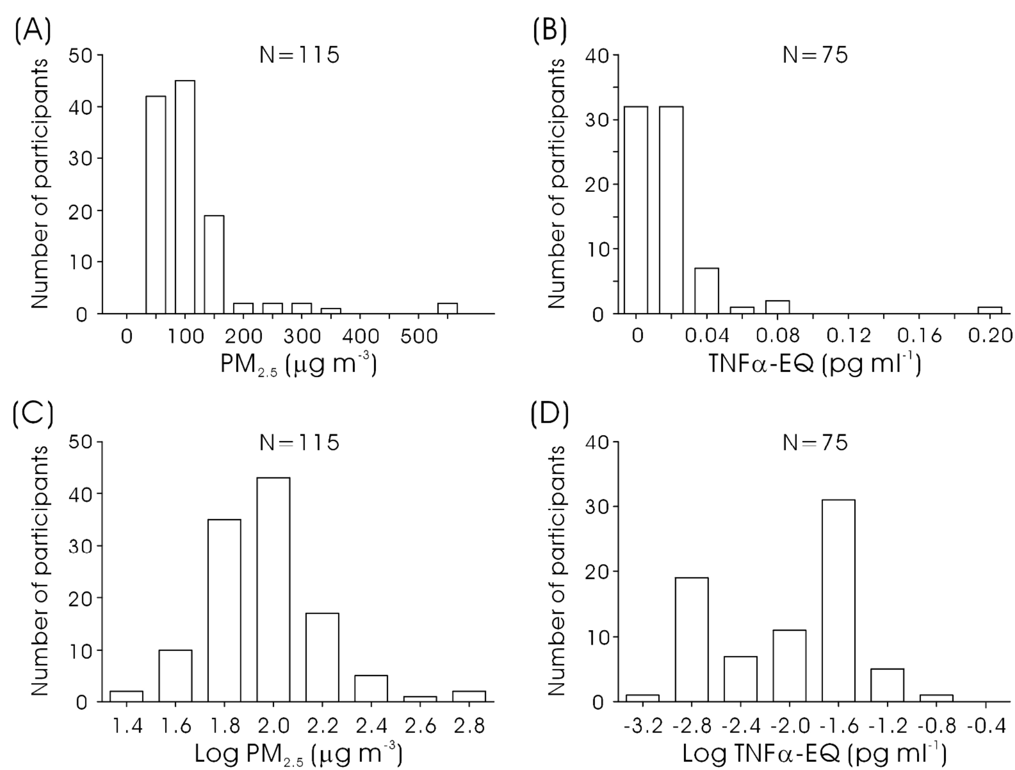

Fig. 3. Distribution of PM2.5 exposure and proinflammatory activity of the study participants. Histograms of (A) PM2.5 exposure of Population 1 (N = 115) and (B) TNFα-EQ levels in Population 2 (N = 75) were summarized. Logarithmic (C) PM2.5 exposure and (D) TNFα-EQ levels were respectively converted from the original data in (A) and (B).

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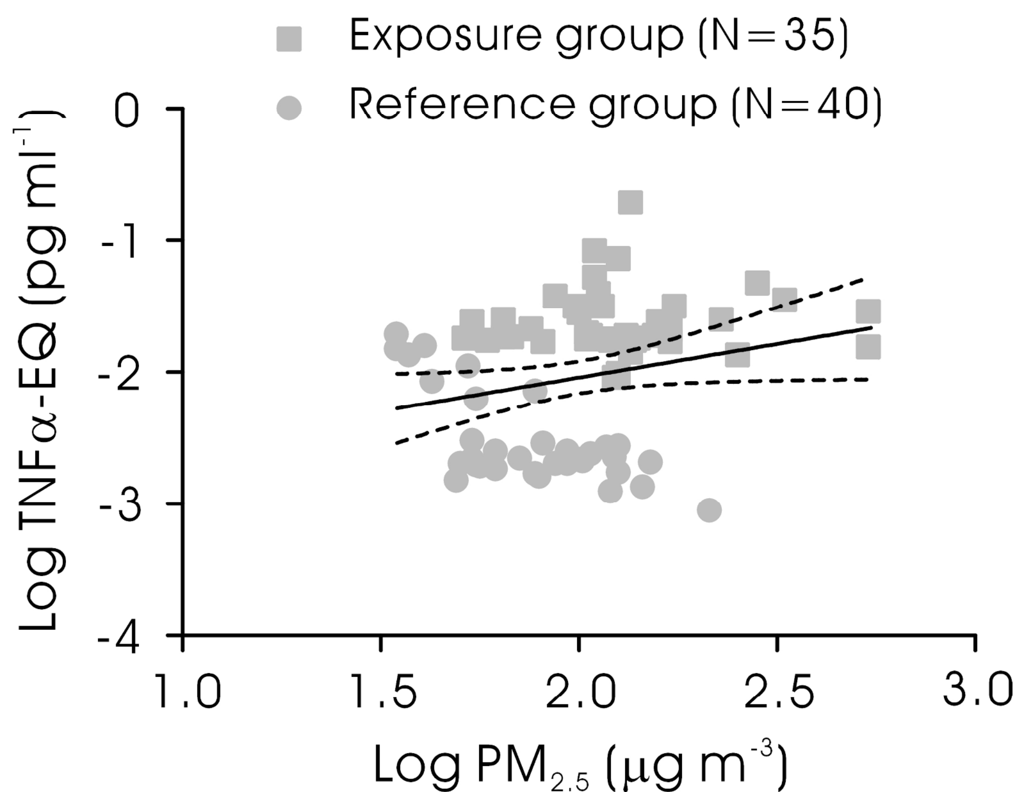

Fig. 4. Association between blood proinflammatory activity (in TNFα-EQ) and personal PM2.5 exposure. Both TNFα-EQ and PM2.5 data of the 75 participants in Population 2 (40 in reference group and 35 in exposure group) were summarized in the scatterplot of log TNFα-EQ versus log PM2.5. The relationship between log TNFα-EQ and log PM2.5 was fitted into the linear equation $y = 0.511x - 3.062$ (y = log TNFα-EQ and x = log PM2.5), with $R = 0.231$, $F(1,73) = 4.097$, $*p = 0.047$, and the 95% confidence interval for mean of slope between 0.008 and 1.015.

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