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## **Toxicity of fine and quasi-ultrafine particles: Focus on the effects of organic extractable and non-extractable matter fractions**

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1     **Toxicity of fine and quasi-ultrafine particles: focus on the effects of organic extractable**  
2                                     **and non-extractable matter fractions**

3  
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23

## 24 Abstract

25

26 To date no study has been able to clearly attribute the observed toxicological effects of  
27 atmospheric particles (PM) to a specific class of components. The toxicity of both the organic  
28 extractable matter (OEM<sub>2.5-0.3</sub>) and non-extractable matter (NEM<sub>2.5-0.3</sub>) of fine particles  
29 (PM<sub>2.5-0.3</sub>) was compared to that of PM<sub>2.5-0.3</sub> in its entirety on normal human epithelial  
30 bronchial BEAS-2B cells in culture. The specific effect of the quasi-ultrafine fraction (PM<sub>0.3</sub>)  
31 was assessed, by comparing the responses of cells exposed to the PM<sub>2.5-0.3</sub> and PM<sub>0.3</sub> organic  
32 extractable matter, OEM<sub>2.5-0.3</sub> and OEM<sub>0.3</sub> respectively. Chemically, PAH, O-PAH, and  
33 N-PAH were respectively 43, 17, and 4 times more concentrated in PM<sub>0.3</sub> than in PM<sub>2.5-0.3</sub>,  
34 suggesting thereby a predominant influence of anthropogenic activities and combustion  
35 sources. BEAS-2B cells exposed to PM<sub>2.5-0.3</sub>, NEM<sub>2.5-0.3</sub>, EOM<sub>2.5-0.3</sub> and OEM<sub>0.3</sub> lead to  
36 different profiles of expression of selected genes and proteins involved in the metabolic  
37 activation of PAH, O-PAH, and N-PAH, and in the genotoxicity pathways. Specifically,  
38 OEM<sub>0.3</sub> was the most inducer for phase I and phase II enzymes implicated in the metabolic  
39 activation of PAH (*AHR*, *AHRR*, *ARNT*, *CYP1A1*, *CYP1B1*, *EPHX-1*, *GSTA-4*) thereby  
40 producing the highest DNA damage, felt by ATR and, thereafter, a cascade of protein  
41 phosphorylation (CHK1/CHK2/MDM2) closely related to the cell cycle arrest (P21 and P53  
42 induction). This study underlined the crucial role played by the organic chemicals present in  
43 PM<sub>0.3</sub>. These results should be considered in any future study looking for the main chemical  
44 determinants responsible for the toxicity of ambient fine PM.

45

46 **Keywords:** fine and quasi-ultrafine particles, organic extractable matter, physicochemical  
47 characteristics, toxicity, metabolic activation, genotoxicity.

48

### 49 List of abbreviations:

50 1-NPyr: 1-nitropyrene; 9-FluO: 9-Fluorenone; Ace: Acenaphthalene; Acy: Acenaphthylene;  
51 Ant: Anthracene; BaA: Benz[a]anthracene; BaP: Benzo[a]pyrene; BbF:  
52 Benzo[b]fluoranthene; BghiP: Benzo[g,h,i]perylene; BkF: Benzo[k]fluoranthene; Chr:  
53 Chrysene; DahA: Dibenz[a,h]anthracene; Fla: Fluoranthene; Flu: Fluorene; InPy:  
54 Indeno[1,2,3-c,d]pyrene; N-PAH: nitrated-PAH; Nap: Naphthalene; OEM: organic  
55 extractable matter; NEM: non-extractable matter; O-PAH: oxygenated-PAH; PAH: polycyclic  
56 aromatic hydrocarbons; Phe: Phenanthrene; PM<sub>2.5-0.3</sub>: particles with an equivalent  
57 aerodynamic diameter between 0.3 and 2.5  $\mu\text{m}$ ; PM<sub>0.3</sub>: particles with an equivalent

58 aerodynamic diameter below 0.3  $\mu\text{m}$  (quasi-ultrafine particles); Pyr: Pyrene; ROS: reactive  
59 oxygen species.

60

## 61 1. Introduction

62 Nowadays, air pollution represents one of the major risk factors for human health:  
63 91% of the world's population live in places where air quality exceeds world health  
64 organization (WHO) guideline limits and 7 million premature deaths are related to air  
65 pollution each year (WHO, 2018). An important part of this threat is due to the specific  
66 presence in the atmosphere of particles having an equivalent aerodynamic diameter (EAD)  
67 lower than 2.5  $\mu\text{m}$ , also called fine fraction ( $\text{PM}_{2.5}$ ). This latter is classified as carcinogenic  
68 group I for humans by the international agency for research on cancer (IARC) (Loomis et al.,  
69 2013).  $\text{PM}_{2.5}$  includes the ultrafine fraction ( $\text{PM}_{0.1}$ ) which has the ability, in addition to the  
70 high deposition potential in the lungs, to translocate to other parts of the body (Stone et al.,  
71 2017). Many studies have assessed the toxicity of ambient  $\text{PM}_{2.5}$ , but much less were  
72 interested specifically in ambient  $\text{PM}_{0.1}$ , mainly due to the technical limitations regarding its  
73 sampling and handling. Available studies have generally focused on the toxicity of ultrafine  
74 particles related to a well-defined source. Baldauf et al. (2016) have evaluated the toxicity of  
75 diesel exhaust particles, which include not only ultrafine particles, but also particles of other  
76 diameters, which puts in place an uncertainty to discriminate the respective roles played by  
77 the different size fractions. Loxham et al. (2015) were interested in studying the toxicity of  
78 size-fractionated underground railway PM. Bendtsen et al. (2018) evaluated the *in-vivo* effects  
79 of ultrafine particles related to airport emissions and some other authors such as Farina et al.  
80 (2019) reported the effects of biomass burning ultrafine particles. Few studies were dealing  
81 with airborne ultrafine PM originated from several source as it is encountered in the ambient  
82 air. Between them, we can cite the works done by Carter et al. (2018) considering ambient  
83 ultrafine particles retrieved from a filter using water, sonication and lyophilization, as well as  
84 the study performed by Gualtieri et al. (2018) who used an air liquid interface exposure  
85 system and a cut-off cyclone to expose cells to PM with a diameter estimated in the 8.9 nm to  
86 1.5  $\mu\text{m}$  size range.

87 Ambient  $\text{PM}_{2.5}$  is a mixture of particles emitted by natural and anthropogenic sources,  
88 and in most of the cases during combustion processes related to engine exhausts and biomass  
89 burning (Cheng et al., 2015). It forms a heterogeneous complex of inorganic components (*e.g.*  
90 metals, salts), organic ones (*e.g.* polycyclic aromatic hydrocarbons: PAH, polychlorinated  
91 dibenzo-*p*-dioxins (PCDD), polychlorinated dibenzofurans (PCDF), polychlorobiphenyls  
92 (PCB)), and biological materials (*e.g.* pollen, bacteria, fungi). The assumption that its toxicity  
93 depends mainly on its composition is a widely accepted hypothesis (Kelly and Fussell, 2012).  
94 However, both the chemical composition and the particle size are widely dependent of the

95 geographic location, meteorological conditions and emission sources, rendering thereby  
96 highly complex the identification and the prevision of their toxic effects (Loomis et al.  
97 2013). To date, a lot of researches have been devoted to the toxicological responses induced  
98 by airborne PM exposure. However, a lack of understanding remains about the specific role of  
99 PM chemical components and/or fractions involved in the PM toxicity. As well, there is a  
100 growing body of evidence that several parameters should be considered together, such as  
101 composition, origin or size of PM, to explain the response a cell models exposed to  
102 atmospheric PM (Abbas et al., 2018). It was also intended to gain information on the  
103 toxicological effects induced by PM<sub>2.5</sub> collected under rural, industrial or urban influences, in  
104 various seasons and having various physicochemical characteristics (Billet et al., 2018;  
105 Dergham et al., 2012, 2015; Melki et al., 2017).

106 Indeed, many studies have confirmed that heavy metals within PM were harmful to  
107 human health (de Kok et al., 2006). Among them, transition metals, such as Cr, As, Cd and Ni  
108 have been listed as carcinogenic to human (IARC, 2012). They can also induce cells to  
109 overproduce reactive oxygen species (ROS), which contribute to the lipid peroxidation of the  
110 cell membrane, DNA and protein damage, and even lead the cells to the death (Chen and  
111 Lippmann, 2009; Fortoul et al., 2015; Zhang et al., 2018). Among the organic constituents of  
112 PM, PAH are of great interest. They are mainly introduced in the atmosphere during  
113 combustion process and can be subject to atmospheric physicochemical reactions resulting in  
114 the formation of PAH derivatives such as nitrated PAH (N-PAH) and oxygenated PAH (O-  
115 PAH) (Abbas et al., 2018). Concentrations of the latter in airborne PM can be very low, but  
116 their toxic effects are often considered as higher than those of their parent compounds.  
117 Several works have shown that the PM organic extractable matter (OEM) contains toxic and  
118 carcinogenic compounds playing a role in the overall PM toxicity without being clearly able  
119 to identify the underlying mechanisms (Alessandria et al., 2014; Xiang et al., 2018).  
120 Accordingly, PM organic constituents could directly contribute to the induction of an  
121 oxidative stress and an inflammatory response in pulmonary cell models as well indirectly  
122 after their metabolization (Abbas et al., 2019; Longhin et al., 2013, 2016; Zhou et al., 2014).  
123 Even so, complementary works focusing on the critical role of the different PM constituent  
124 appears necessary to better understand the PM toxicity. Hence, in the present work, airborne  
125 PM with an EAD between 0.3 and 2.5  $\mu\text{m}$  (PM<sub>2.5-0.3</sub>) and quasi-ultrafine (PM with EAD < 0.3  
126  $\mu\text{m}$ , PM<sub>0.3</sub>) particles were considered. This study aimed at comparing the *in vitro* biological  
127 effects and, especially, the relative genotoxic effects of both OEM of PM<sub>2.5-0.3</sub>, (OEM<sub>2.5-0.3</sub>),  
128 non-extractable matter (NEM) of PM<sub>2.5-0.3</sub> (NEM<sub>2.5-0.3</sub>) and PM<sub>2.5-0.3</sub> considered in its entirety

129 using a relevant normal human epithelial bronchial BEAS-2B cell model. The specific effect  
130 of the quasi-ultrafine fraction was also assessed, by exposing cells to the  $PM_{2.5-0.3}$  and  $PM_{0.3}$   
131 organic extractable matter, also called  $OEM_{2.5-0.3}$  and  $OEM_{0.3}$ , respectively.

132

133

## 134 2. Materials and Methods

135

### 136 2.1. PM Sampling

137 The sampling campaign was carried out in the southern suburb of Beirut, Lebanon  
138 (Figure S1). This region is characterized by huge road traffic, electricity generation by  
139 diesel-powered generators, and suffers from a problem of uncivilized incineration of  
140 household waste. The southern suburb of Beirut, belonging to the district of Baabda, has the  
141 largest population, reaching one million inhabitants as a result of successive migrations from  
142 villages towards the capital. The sampling device was placed on the roof of the Lebanese  
143 atomic energy commission (33°50'59" N; 35°29'57"E), at approximately 12 meters from the  
144 street level to prevent direct influence of soil particles resuspension. This site, located on the  
145 main road between the city center and Beirut airport, is a major axis of travel between Beirut  
146 and the southern part of the country. The PM sampling was done continuously from the 1<sup>st</sup> of  
147 January to the 3<sup>rd</sup> of March 2017 using a high volume five stages plus back-up cascade  
148 impactor (model 235, 68 m<sup>3</sup>/h, Tisch, USA), allowing the separation of collected particles,  
149 according to their equivalent aerodynamic diameter. PM with EAD > 2.5 μm was retained on  
150 a filter placed on the first stage and was not considered in this study. PM<sub>2.5-0.3</sub> was impacted  
151 on the different impaction plates (stage 2 to 5), while the quasi-ultrafine fraction (PM<sub>0.3</sub>) was  
152 retained on a pure quartz fiber filter (Pallflex® Tissuquartz 2500 QAT-UP, Pall, USA) placed  
153 after the impaction system. The system was changed every 5 days. After sampling, impaction  
154 plates were dried in a laminar flow hood during 48 h. PM<sub>2.5-0.3</sub> were recovered from stages 2  
155 to 5, weighted, and finally stored at -20 °C until further handling. Back-up filters were placed  
156 under laminar flow hood for 48 h as well and frozen at -20°C till further use. At the end of the  
157 sampling campaign, individual PM<sub>2.5-0.3</sub> samples were gathered and carefully homogenized to  
158 form an average PM<sub>2.5-0.3</sub> sample. In a similar manner, average PM<sub>0.3</sub> samples were  
159 constituted by considering a proportional mix of pieces of the individual back-up filters. The  
160 methodology for sampling, recovery, and homogenization of PM has been published  
161 elsewhere (Borgie et al., 2016). The meteorological conditions encountered during this  
162 sampling period were normal for the January and February months. As well, no dust storm  
163 was observed during the sampling period as it could suddenly occur in the region.

164

### 165 2.2. Physicochemical characterization of collected PM

166 Chemical characterization of PM was carried out using several methods. For both  
167 PM<sub>2.5-0.3</sub> and PM<sub>0.3</sub> samples, total carbon was determined using CHNS/O analyzer (FLASH



168 2000, Fisher Scientific, UK), water-soluble ions were quantified by ion chromatography (IC,  
169 model ICS 5000, ThermoElectron, UK) and inorganic composition (major and trace elements)  
170 was quantified using inductively coupled plasma- atomic emission spectrometry (ICP-AES,  
171 iCAP 6000 Series, ThermoElectron, UK) as published elsewhere (Borgie et al., 2015; Melki  
172 et al., 2017). Quantification of the 16 United-States Environmental Protection Agency  
173 (USEPA) priority PAH and n-alkane was carried out by gas chromatography-mass  
174 spectrometry (GC-MS, model 1200 TQ, Varian, USA), after ultrasonic extraction with  
175 dichloromethane (DCM) for 20 min at 20 °C. The obtained solution was concentrated under  
176 nitrogen flux until a volume of 1 mL for analysis. PCDD, PCDF, and PCB concentrations  
177 were analyzed by Micropolluants Technology SA (Saint Julien Les Metz, France), using the  
178 USEPA methods 1613 and 1668 using high resolution gas chromatography and high-  
179 resolution mass spectrometry (HRGC/HRMS). The concentration of 8 O-PAH and 15 N-PAH  
180 was investigated by gas chromatography-mass spectrometry with negative chemical  
181 ionization (GC-NCI-MS). This analysis was performed using a 7890B GC system coupled to  
182 a 7000D MS detector (Agilent technologies, USA) in Negative Chemical Ionization mode  
183 with a DB-5MS-UI capillary column (Agilent technologies). For PM<sub>2.5-0.3</sub> only, morphology  
184 and elemental composition at the particle scale was also studied by Scanning Electron  
185 Microscopy (438 VP; LEO Electron Microscopy Ltd, UK) coupled with energy dispersive X-  
186 ray analysis (IXRF, Oxford Instruments, UK), SEM-EDX. As well, Identification of PM  
187 crystalline phases was done by X-ray diffraction at room temperature (XRD AXS D8  
188 Advance, Brüker, Germany) as published elsewhere (Melki et al., 2017).

189

### 190 **2.3. Sample preparation for toxicological studies**

191 Toxicological approaches were performed on PM<sub>2.5-0.3</sub>, OEM<sub>2.5-0.3</sub> and NEM<sub>2.5-0.3</sub> as  
192 well as on the OEM<sub>0.3</sub>. PM<sub>0.3</sub> was collected on filters. As PM<sub>0.3</sub> was trapped within the quartz  
193 fibers, it was not possible to retrieve it to test the quasi-ultrafine particles (PM<sub>0.3</sub>) in its  
194 entirety (without filter). Consequently, only an organic extract (OEM<sub>0.3</sub>) was considered for  
195 studying the PM<sub>0.3</sub> effects. Briefly, to prepare OEM, known masses of PM<sub>2.5-0.3</sub>, and quasi-  
196 ultrafine particles (PM<sub>0.3</sub>) collected on filters were placed in a glass vial, with 10 mL  
197 dichloromethane (DCM), and subjected to a 20 min sonication assisted extraction step at  
198 20 °C. After the ultrasonic treatment, the obtained solution was centrifuged (1000 g) for  
199 20 min, 20 °C. Then, the supernatant solution containing extracted organics was carefully  
200 removed and transferred into a second vial. Remaining PM and filters were then rinsed two  
201 times using fresh DCM followed by centrifugation, and supernatant was retrieved again.

202 These extraction and rinse steps were repeated three times on the remaining PM and filters to  
203 ensure the recovery of all extractable matter. The remaining PM after the extraction procedure  
204 was considered as the non-extractable matter (NEM<sub>2.5-0.3</sub>) and is assumed as the PM skeleton  
205 on which the OEM was bound. Finally, the obtained extracts were gathered, and DCM was  
206 evaporated and replaced by dimethylsulfoxide (DMSO). Hence, two organic extracts from  
207 PM<sub>2.5-0.3</sub> (OEM<sub>2.5-0.3</sub>) and PM<sub>0.3</sub> (OEM<sub>0.3</sub>) in DMSO were obtained together with the  
208 non-extractable matter of PM<sub>2.5-0.3</sub> (NEM<sub>2.5-0.3</sub>). Concentrations of final OEM<sub>2.5-0.3</sub> and OEM<sub>0.3</sub>  
209 solution were respectively 98.1 and 96.4 mg PM-equivalent/mL (PM-eq/mL). The PM-eq/mL  
210 unit is in relation with the mass of PM extracted and the final volume of DMSO. Define  
211 volumes of these OEM stock solutions were diluted in the LHC-9 culture medium to expose  
212 BEAS-2B cells to the desired concentrations of OEM (expressed in  $\mu\text{g PM-eq/cm}^2$ ).

213

## 214 **2.4. Evaluation of the toxicity**

### 215 **2.4.1. Cell culture and exposure**

216 BEAS-2B cells (ATCC® CRL-9609™, ECACC, Wiltshire, UK) were used for the  
217 cytotoxicity experiments. BEAS-2B cell line originally derives from normal human bronchial  
218 epithelial cells (non-cancerous individuals). BEAS-2B cells used in all the presented tests  
219 derived from the same cell culture and were exposed before reaching the confluence (< 80%).  
220 Cell culture method has been detailed elsewhere (Leclercq *et al.* 2017a). Briefly, BEAS-2B  
221 cells were grown in LHC-9 medium at 37 °C in a humidified air (relative humidity 95%, 5%  
222 CO<sub>2</sub>) in 75 cm<sup>2</sup> CellBIND® surface plastic flasks (Corning, USA). Medium was refreshed  
223 every 2-3 days. BEAS cells were exposed to control (LHC-9 and DMSO 0.5% in LHC-9) or  
224 to one of the different samples (PM<sub>2.5-0.3</sub>, OEM<sub>2.5-0.3</sub>, NEM<sub>2.5-0.3</sub> or OEM<sub>0.3</sub>) suspended in  
225 LHC-9 at different concentrations ranging from 3 to 96  $\mu\text{g PM-eq/cm}^2$ . Non-exposed  
226 BEAS-2B cells were used as a negative control for all experiments. In addition,  
227 benzo[a]pyrene (BaP) (25  $\mu\text{M}$ ), 1-nitropyrene (1-NPyr) (25  $\mu\text{M}$ ), 9 fluorenone (9-FluO)  
228 (25  $\mu\text{M}$ ) were used as positive controls of PAH, N-PAH and O-PAH effects respectively.  
229 When exposing to OEM, the DMSO concentration in the culture medium was below 0.5%  
230 rendering its toxic effect insignificant (Billet *et al.*, 2018). After 6, 24, 48 and/or 72 h of  
231 exposure, aliquots of cell-free culture supernatants media were sampled and quickly frozen (-  
232 80 °C) till cytotoxicity assessment. Adherent BEAS-2B cells were washed twice with 1 mL  
233 cold sterile PBS, then quickly frozen (-80°C) for further analyses (gene expression and  
234 genotoxicity).

235

#### 236 **2.4.2. Evaluation of cytotoxicity**

237 Cytotoxicity of PM<sub>2.5-0.3</sub>, NEM<sub>2.5-0.3</sub>, OEM<sub>2.5-0.3</sub>, and OEM<sub>0.3</sub> was evaluated, after  
238 exposure of cells to 3, 6, 12, 24, 48 and 96 µg PM-eq/cm<sup>2</sup> for 6, 24 and 72 h, by the  
239 quantification of intracellular ATP using the Cell-Titer-Glo Luminescent cell viability Assay  
240 (Promega).

241

#### 242 **2.4.3. Metabolic activation of PAH**

243 Evaluation of gene expression was carried out for several genes implicated in the PAH  
244 metabolic pathway: *cytochrome P4501A1: CYP1A1*, *cytochrome P4501B1: CYP1B1*, *Epoxide*  
245 *hydrolase 1: EPHX*, and *Glutathione S-Transferase Alpha 4: GSTA4*, in addition to *aryl*  
246 *hydrocarbon receptor: AHR*, *Aryl-Hydrocarbon Receptor Repressor: AHRR*, and *aryl*  
247 *hydrocarbon receptor nuclear translocator: ARNT*, using quantitative RT-PCR, Taqman™  
248 gene expression assays (*ARN18S*: Hs99999901, *AHR*: Hs00169233\_m1, *ARNT*:  
249 Hs01121918\_m1, *AhRR*: Hs01005075\_m1, *CYP1A1*: Hs01054797\_g1, *CYP1B1*:  
250 Hs00164383\_m1, *EPHX1*: Hs01116806\_m1, *GST-A4*: Hs01119249\_m1), as published  
251 elsewhere (Abbas et al. 2019). The fold changes (FC) in the mRNA expression levels of the  
252 target genes between exposed and control BEAS-2B cells, corrected by the level of RNA18S,  
253 were determined using the following equation: FC: 2<sup>-Δ(ΔCt)</sup>, where ΔCt = Ct(target gene) -  
254 Ct(RNA18S gene), and Δ(ΔCt) = ΔCt(exposed group) - ΔCt(control group). Only FC>1.5  
255 were considered as biologically significant.

256

#### 257 **2.4.4. Genotoxicity induced by atmospheric PM**

258 The genotoxicity was evaluated using the "Milliplex Map Kit- 7-plex DNA  
259 damage/Genotoxicity Magnetic Bead Kit 96-well Plate" based on the Luminex xMAP  
260 technology. This Milliplex Kit is used to detect changes in phosphorylated checkpoint kinase  
261 1 (CHK-1) (Ser345), phosphorylated checkpoint kinase 2 (CHK-2) (Thr68), phosphorylated  
262 H2A histone family member X (H2AX) (Ser139), and phosphorylated protein 53 (pP53)  
263 (Ser15) as well as total protein levels of ataxia telangiectasia and Rad3-related protein (ATR),  
264 mouse double minute 2 homolog (MDM2), and protein 21 (P21) in cell lysates, according to  
265 the manufacturer's recommendations.

266

#### 267 **2.4.5. Statistical analysis**

268 Results were normalized to control, and presented as mean values and standard  
269 deviations. For each incubation time, results from BEAS-2B cells exposed to PM<sub>2.5-0.3</sub>,

270 OEM<sub>2.5-0.3</sub>, NEM<sub>2.5-0.3</sub> and OEM<sub>2.5-0.3</sub> or to positive controls BaP, 1-NPyr, and 9-FluO were  
271 compared to those obtained in non-exposed cells using the Mann–Whitney U-test (SPSS  
272 software). Significant differences were reported with *p* values <0.05.

273

274

### 275 3. Results and discussion

#### 276 3.1. PM<sub>2.5</sub> atmospheric concentrations

277 Concentrations of atmospheric PM<sub>2.5-0.3</sub>, PM<sub>0.3</sub> and PM<sub>2.5</sub> were calculated taking into  
278 consideration the mass of collected PM during the two months sampling period, as well as the  
279 volume of air that passed through the impactor. In these conditions, the average daily PM<sub>2.5</sub>  
280 atmospheric concentrations, in the southern suburb of Beirut, was 54 µg/m<sup>3</sup>. This  
281 concentration largely exceeds the daily guideline value recommended by the WHO  
282 (25 µg/m<sup>3</sup>) for PM<sub>2.5</sub>. It was also higher than those reported by Abbas et al. (2019) at the same  
283 site in 2016 (48 µg/m<sup>3</sup>), by Borgie et al. (2015) in the Northern suburb of Beirut in 2011 (41  
284 µg/m<sup>3</sup>), and by Massoud et al. (2011) in three different locations in this city in 2010 (close to  
285 20 µg/m<sup>3</sup>).

286

#### 287 3.2. PM<sub>2.5-0.3</sub> and PM<sub>0.3</sub> physico-chemical characteristics

##### 288 3.2.1. Element and water soluble ion content

289 Table 1 represents the average concentration of major and trace elements, water  
290 soluble ions and total carbon in fine and quasi-ultrafine particles. Results showed that Ca  
291 (13.4% and 10% for PM<sub>2.5-0.3</sub> and PM<sub>0.3</sub> respectively), NO<sub>3</sub><sup>-</sup> (5.6% and 2.9%), Fe (2.6% and  
292 1.2%), Al (2.1% and 1.5%), Cl<sup>-</sup> (1.7% and 2.1%), Mg (1.2% and 1.8%) and Na (1.1% and  
293 4.1%), were the major constituents, in addition to SO<sub>4</sub><sup>2-</sup> (2.7%) in PM<sub>2.5-0.3</sub> and NH<sub>4</sub><sup>+</sup> (1.7%)  
294 in PM<sub>0.3</sub>. Then, concentrations of K (0.5% and 0.7%), Cu (0.2% and 0.3%), Ti (0.2% and  
295 0.1%) and P (0.1% and 0.1%) were between 0.1% and 1%, in addition to NH<sub>4</sub><sup>+</sup> (0.1%) and Ba  
296 (0.1%) in PM<sub>2.5-0.3</sub> and SO<sub>4</sub><sup>2-</sup> (0.7%) and Zn (0.1%) in PM<sub>0.3</sub>. Finally, the other species, Zn,  
297 Mn, Sr, Pb, Cr, V, Sn, Ni, Mo, Co, As, and Cd showed concentrations below 0.1% for both  
298 PM<sub>2.5-0.3</sub> and PM<sub>0.3</sub>, in addition to Ba in PM<sub>0.3</sub>. The presence of elements such as Al, Ca, Fe,  
299 Mg, Ca, and Ti within the atmospheric particles can be related to the resuspension of soil dust  
300 (Wedepohl, 1995). Particularly, the predominance of Ca element could be related to the soil  
301 type in Lebanon, which is typically calcareous (Verheye, 1973). It was also confirmed by  
302 XRD analysis that evidenced the presence of calcite (Figure S2). Calcium was also found in  
303 the form of gypsum, as confirmed by individual particle analysis done by SEM-EDX analysis,  
304 evidencing Ca and S containing particles (Figure S3). Using XRD, Al was also detected in the  
305 kaolinite form, which is one of the minerals commonly identified in Lebanese soils (Al-  
306 Zubaidi et al., 2011).

307 Minerals and metals are generally considered as the main components of PM collected  
308 from roadsides as a result of the re-suspension by traffic of road dust and particles generated

309 by the wear of mobile parts of vehicles, such as tires, brake or clutch disk (Putaud et al.,  
310 2004). Indeed, trace elements showing the influence of vehicular emissions were also detected  
311 (Ba, Ni, and Zn) (Gietl et al., 2010).

312  $\text{NO}_3^-$  and  $\text{SO}_4^{2-}$ , the major ions detected in PM, can be originated from gas to particle  
313 conversion of  $\text{NO}_x$  and  $\text{SO}_2$  precursor gases as well as from long range transport (Borgie et  
314 al., 2016; Luria et al., 1989). Indeed, in Lebanon, it was estimated that road transport  
315 contributes to 52% of the  $\text{NO}_x$  emissions. In addition,  $\text{SO}_2$  emissions are mainly related to  
316 industrial activities and electricity production from the combustion of heavy fuel oil, with  
317 sulfur content between 25,000 and 30,000 ppm. The latter are also used in domestic generator  
318 sets and diesel heavy vehicles (buses and trucks) (Waked et al., 2012).

319 The concentration of total carbon was about 7-fold higher in  $\text{PM}_{0.3}$  compared to  
320  $\text{PM}_{2.5-0.3}$  (126 vs 17.5 mg/g). This highest concentration in the quasi-ultrafine particles may be  
321 linked to the contribution of soot known to belong to this size fraction in relation with the  
322 combustion process largely influenced by the huge road traffic close to the sampling site.

323

### 324 **3.2.2. Organic composition**

325 The determination of the 16 USEPA priority PAH showed that  $\text{PM}_{2.5-0.3}$  and  $\text{PM}_{0.3}$  had  
326 substantially similar profiles with an important representation of the higher molecular weight  
327 PAH (i.e., 4 aromatic rings or more) (Table 2). The concentration of PAH found in  $\text{PM}_{0.3}$  was  
328 about 43 times higher than in  $\text{PM}_{2.5-0.3}$ , proving thereby a major influence of anthropogenic  
329 activities and combustion sources (industries, road traffic and electric generators) on the  
330 emission of quasi-ultrafine particles. Among them, dibenz[a,h]anthracene,  
331 benzo[b]fluoranthene, indeno[1,2,3-cd]pyrene, benzo[a]pyrene, and chrysene were  
332 predominant. They are usually emitted by combustion sources including gasoline motor  
333 vehicles, biomass burning, and industrial process (INERIS, 2005). To investigate the PAH  
334 emission sources, PAH diagnostic ratios were calculated and compared with those available in  
335 the current literature (Table 3). The Fla/(Fla+Pyr) ratio value was ranging from 0.4 to 0.5  
336 indicating fossil fuel combustion as a source of PAH. The (Fla/Pyr) ratio higher than 0.6 (0.92  
337 and 0.79 respectively for  $\text{PM}_{2.5-0.3}$  and  $\text{PM}_{0.3}$  as well as the BaP/(BaP+Chry) higher than 0.35  
338 (0.43 and 0.59) suggested the influence of traffic emissions. Finally, the Flu/(Flu+Pyr) ratio  
339 values, 0.44 and 0.48 respectively, were slightly lower than 0.5 indicating a major influence  
340 of gasoline emissions versus diesel ones. The use of BaP-toxic equivalent factor (BaP-TEQ)  
341 to PAH concentrations can provide an indicator of the carcinogenic risk related to PAH  
342 environmental exposure. PAH concentrations were converted in BaP-TEQ concentration

343 using the factors published by (Kumar et al., 2018) and (Nisbet and LaGoy, 1992). This  
344 calculation showed that the considered PM<sub>2.5</sub> (PM<sub>2.5-0.3</sub> + PM<sub>0.3</sub>) had a huge carcinogenic  
345 potential with 8.2 ng/m<sup>3</sup> BaP-TEQ. This value is clearly higher than the BaP WHO guideline  
346 (0.12 ng/m<sup>3</sup>; cancer risk 1/100 000). The highest BaP-TEQ values were for  
347 dibenz[a]anthracene (3.9 ng/m<sup>3</sup> BaP-TEQ) followed by BaP (2.5 ng/m<sup>3</sup> BaP-TEQ) and  
348 indeno[1,2,3-cd]pyrene (0.7 ng/m<sup>3</sup> BaP-TEQ).

349 Moreover, six O-PAH and nine N-PAH were detected in PM (Table 2). Total  
350 concentration of O-PAH was higher than those of N-PAH as already reported in other studies  
351 (Abbas et al., 2018; Albinet et al., 2007). In addition, concentrations of O-PAH and N-PAH  
352 were respectively 17 and 4-fold higher in PM<sub>0.3</sub> compared to PM<sub>2.5-0.3</sub>. Among O-PAH  
353 congeners, 1,8-naphthamic anhydride, 7H-benzo(de)anthracen-7-one, and 9,10-anthraquinone  
354 were the most abundant in PM<sub>2.5</sub> (45, 28 and 21 µg/g, respectively). The 2-nitroanthracene  
355 and 7-nitrobenz(a)anthracene were the most abundant nitro-PAH (4.9 µg/g and 3.7 µg/g  
356 respectively). The emission sources of these compounds are still not well explored in the  
357 literature; O-PAH and N-PAH also originate as direct or indirect products of incomplete  
358 combustion (Abbas et al., 2018). Indeed, 1 nitropyrene is the most abundant pyrene in diesel  
359 exhaust emissions (Bamford et al., 2003). Some N-PAH were used in industries such as the  
360 5-nitroacenaphthene, which is an intermediate in the synthesis of naphthalimide dyes that  
361 are used in biomedical applications or as fluorescent whitening agents and photochemical  
362 agents (WHO, 2003; Zhang et al., 2016). Concentrations and profiles of n-alkanes are  
363 presented in Figure S4. As observed for PAH and PAH derivatives, quasi-ultrafine particles  
364 were as well more concentrated in n-alkanes (10-fold higher) than fine ones. Two different  
365 distribution profiles were evidenced with a main difference for C<sub>29</sub> and C<sub>31</sub>. Odd n-alkanes  
366 are known to mainly derive from natural origins whereas even ones can be related to  
367 anthropogenic origins (Waples, 1985). The carbon maximum number (C<sub>max</sub>) has been used to  
368 evaluate the sources of n-alkanes (Chen et al., 2014). Higher value of C<sub>max</sub> indicated that n-  
369 alkanes primary originated from terrestrial plants, while lower C<sub>max</sub> was associated with  
370 anthropogenic sources. For fine particles, C<sub>max</sub> was 31, while for quasi-ultrafine particles, it  
371 was 24, showing the influence of anthropogenic emissions in the latter's composition. The  
372 carbon preference index (CPI), can be used to determine the sources of n-alkanes and was  
373 calculated as published in Melki et al. (2017). Values of overall CPI<sub>21-31</sub> and high CPI were  
374 respectively 1.35 and 1.65 for the fine particles and 1.06 and 1.41 for the quasi-ultrafine  
375 particles. These ratio value suggested that alkanes were originated from both natural and  
376 anthropogenic sources (1.5 < high CPI < 3 and overall CPI > 1) in fine particles, while they

377 mainly derived from anthropogenic sources (high CPI < 1.5 and overall CPI close to 1) in  
378 quasi-ultrafine ones (Figure S4).

379 The total concentrations of PCDD, PCDF and PCB were respectively 30.7 ng/g (1100  
380 fg/m<sup>3</sup>), 40.4 ng/g (1170 fg/m<sup>3</sup>), and 27.9 ng/g (770 fg/m<sup>3</sup>) in fine particles. It should be  
381 noticed that concentrations of these compounds were higher in the quasi-ultrafine fraction  
382 than in the fine one (PCB: 15.7 vs 12.2 ng/g, PCDD: 25.2 vs 5.5 ng/g, and PCDF: 26.4 vs  
383 14 ng/g respectively) (Table 2). By comparing the values found in this study with previous  
384 published ones, the concentrations of PCDD and PCDF were almost twice higher than those  
385 determined at the same site in 2015 by Abbas et al (2019): 1100 vs 449 fg/m<sup>3</sup> and 1170 vs 560  
386 fg/m<sup>3</sup> respectively. Furthermore, the concentration of dioxins and furans were respectively  
387 about 4.4 (770 vs 214 fg/m<sup>3</sup>) and 13-fold (1170 vs 87 fg/m<sup>3</sup>) higher than that found in 2015 in  
388 the northern suburb of Beirut by Borgie et al. (2015). These higher concentrations could be  
389 related to the worst solid waste management crisis in Lebanon, since 2015, when open air  
390 waste burning has become a common practice in Beirut (Baalbaki et al., 2018).

391

### 392 **3.3. Evaluation of PM toxicity**

393 The comparison of the effects of OEM<sub>2.5-0.3</sub> and NEM<sub>2.5-0.3</sub> vs PM<sub>2.5-0.3</sub> on the one hand,  
394 and OEM<sub>0.3</sub> vs OEM<sub>2.5-0.3</sub> on the other hand, were thereafter carried out to better investigate  
395 the critical role of chemicals related to a specific size fractions and/or to the extractable/non-  
396 extractable fractions involved in the cellular and molecular damages induced by PM.  
397 Cytotoxicity, pathways of metabolic activation of the PAH and other related compounds, as  
398 well as pathways of genotoxicity-DNA damage were also the critical toxicological endpoints  
399 under study.

400

#### 401 **3.3.1. Cytotoxicity**

402 Evaluation of cytotoxicity was carried out using the quantification of intracellular ATP  
403 (Figure S5). The exposure of BEAS-2B cells to PM<sub>2.5-0.3</sub> and NEM<sub>2.5-0.3</sub> showed a significant  
404 decrease in ATP after 6 and 24 h of exposure to 6, 12, 24, 48 and 96 µg/cm<sup>2</sup> (dose-  
405 dependent). For the time corresponding to 72 hours of exposure, ATP concentrations slightly  
406 increased when BEAS-2B cells were exposed to low and moderate concentrations (3, 6, 12,  
407 24 and 48 µg PM-eq/cm<sup>2</sup>) and decreased significantly when exposed to the highest  
408 concentration of PM<sub>2.5-0.3</sub> (96 µg/cm<sup>2</sup>) and NEM<sub>2.5-0.3</sub> (48 and 96 µg/cm<sup>2</sup>). The increase of  
409 ATP concentrations in exposed cells for longest time (> 24 hours which is the minimum time  
410 required for cell division) is related to the proliferation of cells which is naturally



411 accompanied by an increase of ATP concentration. Decrease of ATP concentration indicated  
412 a mitochondrial dysfunction and proved the ability of PM<sub>2.5-0.3</sub> to induce toxicity, even at the  
413 lowest doses and the shortest times. A more pronounced effect was observed for the highest  
414 doses. The toxicity of the OEM<sub>0.3</sub>, after 72 h of exposure to the two highest concentrations (48  
415 and 96 µg PM-eq/cm<sup>2</sup>) was the most remarkable. The ATP concentration decrease was  
416 significantly higher for cells exposed to OEM<sub>0.3</sub> in comparison with cells exposed to  
417 OEM<sub>2.5-0.3</sub> (p<0.020 and p<0.034 for the two doses respectively). This could be explained by  
418 the highest concentration of PAH and organic compounds in PM<sub>0.3</sub> compared to PM<sub>2.5-0.3</sub>.

419 Based on the above-detailed results, two concentrations of exposure ensuring  
420 sufficient doses to observe some harmful effects without a massive cell death were selected  
421 for the study of the other toxicological endpoints. Indeed, the two considered doses, 3 and  
422 12 µg PM-eq/cm<sup>2</sup> were also among the lowest reported in the literature to give harmful effects  
423 whilst limiting a massive cell death (Boublil et al. 2013, Leclercq et al. 2016, 2017b, Gualtieri  
424 et al., 2010, Longhin et al. 2013).

425

### 426 3.3.2. *Metabolic activation*

427 PAH are known to enter into the cell through cytoplasmic membrane before binding to  
428 the aryl hydrocarbon receptor (AHR). Following ligand binding, the PAH-AHR complex  
429 translocates to the nucleus and forms a heterodimer with its nuclear translocator (aryl receptor  
430 nuclear translocator, ARNT). This latter then binds to a specific region of the DNA called  
431 xenobiotic response element (XRE), recruits the RNA polymerase II and induces the  
432 transcription of several genes, included in the AhR gene battery. It is also implicated in the  
433 metabolic activation of PAH into more easily eliminable metabolites (Puga et al., 2009).  
434 Among them, phase I enzymes (CYP1A1 and CYP1B1) and phase II enzymes (EPHX-1 and  
435 GSTA-4) are closely involved (Dietrich, 2016; Rogers et al., 2017). The main function of the  
436 AHR signaling pathway is to activate parent PAH and thereafter to conjugate their highly  
437 electrophilic intermediate metabolites with highly hydrophilic groups in order to easily  
438 eliminate them from cells and organism thereby avoiding severe toxic effects.

439 In this study, the expression of some selected genes involved in the AhR signaling  
440 pathway was evaluated after exposure of BEAS-2B cells to PM, NEM and OEM at 3 and 12  
441 µg PM-eq/cm<sup>2</sup> for 6 and 24 h.

442 Exposure of BEAS-2B cells to PM<sub>2.5-0.3</sub>, NEM<sub>2.5-0.3</sub>, EOM<sub>2.5-0.3</sub> and EOM<sub>0.3</sub> revealed  
443 different gene expression profiles of some key proteins involved in the metabolic activation of  
444 PAH (Table 4). PM<sub>2.5-0.3</sub> in its entirety was able to induce significant gene expressions of

445 *AHR, AHRR, ARNT, CYP1A1, CYP1B1* and *GSTA-4*, 6 and 24 h after cell exposure to the two  
446 doses. Results obtained for positive controls (BaP, 1-Npyr and 9-FluO) confirmed the  
447 activation of the AHR signaling pathway by exogenous chemicals in BEAS-2B cells. In  
448 general, metabolic activation helps to decrease the toxicity of pollutants. However, this  
449 metabolism can form metabolites with higher toxicity than initial molecules (Saint-  
450 Georges et al., 2008, 2009). Gualtieri et al. (2011) also reported that the AHR signaling  
451 pathway was activated in BEAS-2B cells exposed to PM<sub>2.5</sub> and PM<sub>10</sub> collected in urban  
452 background area in relation with their high PAH contents. In contrast, no significant induction  
453 of these genes was observed in BEAS-2B cells exposed to NEM<sub>2.5</sub>, whatever the tested dose  
454 and time. In comparison, Billet et al. (2006, 2008) have used PM having undergone a thermal  
455 desorption under vacuum (dPM) before studying the metabolic activation. The technique used  
456 preserve the PM inorganic structure while organic chemicals were desorbed. Somewhat  
457 surprising result was the effective transcription of *CYP1A1* gene cells derived from a  
458 type II-like human alveolar epithelial carcinoma (A549 cell line) exposed to dPM, thereby  
459 supporting that the 400°C thermal desorption under vacuum was not enough sufficient for  
460 removing totally the organic fraction. In contrast, as NEM<sub>2.5-0.3</sub> did not significantly induce  
461 metabolic activation, the method used here seemed to be more efficient to recover both OEM  
462 and organics almost free NEM.

463 For OEM<sub>2.5-0.3</sub>, a significant increase of the gene expression of *CYP1A1* and *CYP1B1*  
464 was observed only 6 h after the exposure and only for the highest dose. Accordingly, Oh *et al.*  
465 (2011) indicated that OEM which contain aliphatic chlorinated hydrocarbons, PAH/their alkyl  
466 derivatives and nitro-AH/ketones/quinones, induced CYP1A1-dependent EROD activity,  
467 through AHR signaling pathway activation. After 24 h of exposure to OEM<sub>2.5-0.3</sub>, no  
468 significant gene expression was observed. In comparison, PM<sub>2.5-0.3</sub> was able to induce  
469 *CYP1A1* and *CYP1B1* gene expression after 6 h of exposure at the lowest dose and after 24h  
470 of exposure as well. One explanation could be related to both the kinetics of metabolic  
471 activation, the difference regarding the bio-accessibility of PAHs in PM<sub>2.5-0.3</sub> and OEM<sub>2.5-0.3</sub>  
472 (these points will be further discussed below) and the contribution of some metallic species  
473 only present in PM<sub>2.5-0.3</sub>. For this latter, Garçon et al. (2004a, 2004b) reported that the  
474 concurrent exposure to Fe<sub>2</sub>O<sub>3</sub> particles and BaP favors dramatically the metabolic activation  
475 of PAH by phase I enzymes (i.e., CYP1A1) to the detriment of the inactivation of PAH  
476 metabolites by phase II enzymes (i.e., GST-YA). They finally concluded that the kinetics of  
477 the adverse effects might be different depending on the presence or absence of BaP on Fe<sub>2</sub>O<sub>3</sub>  
478 particles. Korashy et al. (2005) also reported that *CYP1A1* gene expression could be induced

479 by heavy metals (Korashy and El-Kadi, 2004). Nevertheless, the NEM<sub>2.5-0.3</sub> fraction was not  
480 able to induce metabolic activation allowing to reject this hypothesis.

481 Regarding the quasi-ultrafine fraction, OEM<sub>0.3</sub> was able to induce a significant  
482 expression of all tested genes involved in the AHR signaling pathway 6 and 24 h after  
483 exposure, mainly to the highest tested dose (Table 4). Compared with OEM<sub>2.5-0.3</sub>, OEM<sub>0.3</sub> led  
484 to a higher expression of the proteins involved in the AHR signaling pathway. This could be  
485 closely related to the much higher contents of PAH and other related compounds in OEM<sub>0.3</sub>  
486 compared with OEM<sub>2.5-0.3</sub> (till 43 times higher for PAH). This result is in agreement with a  
487 recent study showing the significant induction of the two AHR-related CYP, *CYP1A1* and  
488 *CYP1B1*, in BEAS-2B cells 6 and 24h after their exposure to increasing concentrations of  
489 PM<sub>2.5</sub> OEM, ranging from 3 to 30 µg PM-eq/cm<sup>2</sup> (Abbas et al., 2019). Using the same  
490 concentrations as those used in this study, Borgie et al. (2015) reported also the effective up-  
491 regulation of the gene transcription of *CYP1A1* and *CYP1B1*, 24, 48, and 72 h after the  
492 exposure of BEAS-2B cells to OEM<sub>0.3</sub>.

493 Taken together, these results supported not only the significant activation of the AHR  
494 signaling pathway by the PAH but also put into question both the bioavailability of PM-  
495 linked-PAH vs PAH in an organic extract, the kinetics of the metabolic activation and the  
496 dose parameter. PM<sub>2.5-0.3</sub> and OEM<sub>2.5-0.3</sub> have the same PAH composition. Nevertheless,  
497 *CYP1A1* and *CYP1B1* expression were not the same for both samples. Especially, no  
498 expression was observed for 6 h of exposure at the lowest dose for OEM<sub>2.5-0.3</sub>, as well at 24 h,  
499 for all the doses. In OEM<sub>2.5-0.3</sub>, PAH are highly bio-accessible compared to PAH linked to  
500 PM<sub>2.5-0.3</sub>. This higher bio-accessibility could be responsible for a quicker metabolic activation  
501 of PAH. It can be suggested that this one took less than 6 hours to be completed especially  
502 when doses (and PAH concentrations) are low. This hypothesis is reinforced by the fact that  
503 no metabolic activation was observed at 24 hours as well. The early metabolic activation of  
504 PAH was also observed by Totlandsdal et al. (2010) who have studied the *CYP1A1* expression  
505 of BEAS-2B cells exposed to Diesel Exhaust Particles (DEP) for 2, 4 and 8 h. They found that  
506 the *CYP1A1* gene expression occurred at early time with a pronounced increase after 2 hours  
507 of exposure and a levelling off at 4 and 8 h. Then, after 24 h, the level of this gene was lower  
508 than the one observed at 8 h. Also testing DEP but considering HBEC3 cells, Rynning et al.  
509 (2018) measured a *CYP1A1* gene expression after 24 hours. It was the shortest time  
510 considered in their study. However, Brinchmann et al. (2018) have considered DEP exposure  
511 using a 3D tri-culture (A549/THP-1/EA.hy926) model for 2 h and 20 h. They have also  
512 studied the DEP-OEM exposure using HMEC-1 cells for 2 and 24 h. They observed a

513 *CYP1B1* significant expression in the epithelial side of the 3D model after only 2 h of  
514 exposure at DEP. This gene expression was also much higher after 20 h of exposure  
515 compared to 2 h. DEP-OEM exposed HMEC-1 cells also show a significant *CYP1A1* and  
516 *CYP1B1* gene expression after 2 h, staying quite constant after 24 h of exposure. Taken  
517 together these results are in agreement with an early metabolic activation of PAH that can be  
518 spread out over time especially when PAH are linked to PM (reduced accessibility and slow  
519 release). The fact that the metabolization of PAH in PM<sub>2.5-0.3</sub> appears to be time-shifted and  
520 spread-out compared to PAH in OEM<sub>2.5-0.3</sub> is also consistent with this statement. For higher  
521 PAH content such as that in OEM<sub>0.3</sub>, the metabolization of PAH seems to be extended  
522 compared to OEM<sub>2.5-0.3</sub>.

523 Finally and in agreement with previous works done by Garçon et al. (2004a, 2004b),  
524 these results involve also two hypotheses based on the bioavailability and bioaccessibility of  
525 organic compounds, whether those linked to PM, or found in the organic extract. The first one  
526 support the idea that particles represent a vector which facilitates penetration of organic  
527 compounds into the cell by endocytosis (Chen et al., 2016). The second hypothesis is based  
528 on the lipophilic properties of organic compounds. They can easily and even quickly penetrate  
529 the cell through the cytoplasmic membrane to be freely metabolized within the cell.  
530 Accordingly, Brinchmann et al (2018) reported that the fast induction of AhR-regulated genes  
531 in the basolateral endothelial cells of their alveolar 3D tri-culture model exposed to relevant  
532 concentrations of DEP (0.12 µg/cm<sup>2</sup>) seemed mainly to be due to the soluble lipophilic  
533 constituents rather than particle translocation. Indeed, DEP-EOM, next fractionated with  
534 increasing polarity, chemically characterized, and examined for direct effects on  
535 AhR-regulated genes in human microvascular endothelial cells and primary human  
536 endothelial cells, supported that the most lipophilic EOM, that contained the majority of AhR  
537 agonists such as PAH, had the clearest low-concentration effects. The different kinetics  
538 observed for PM and OEM could also be related to the fact that PAH linked to PM could be  
539 only progressively released in cell cytoplasm after particle internalization, and subsequently  
540 metabolized, while free PAH in the organic extract can be metabolized directly once in the  
541 cell.

542

### 543 **3.3.3. Evaluation of genotoxicity biomarkers**

544 Given the complexity of atmospheric particles, the identification of the chemical  
545 compounds responsible for the genotoxic effect is still not clear. Several studies suggested the  
546 role of PAH, and therefore of the organic fraction, while other studies suggested the role of

547 water soluble (Pralhad et al., 2001) and non-water soluble metals (Ghio, 1999; Knaapen et  
548 al., 2002) in the initiation of oxidative stress and DNA damage. The different DNA lesions  
549 can be caused by a wide variety of agents. If they are still present during replication, tolerance  
550 mechanisms can be put in place. For each type of lesion, specific repair mechanisms exist.  
551 Thus, to maintain the integrity of the genome, eukaryotic cells have established signaling  
552 pathways that are activated in response to DNA damage. In case of DNA damage, the ATR  
553 and ATM sensors phosphorylate a number of downstream proteins, such as CHK1, CHK2 and  
554 P53, in addition to the activation of MDM2 and P21, allowing the cell cycle to be stopped, in  
555 order to provide the cell to repair its DNA with time, but also to induce apoptosis in the case  
556 of cells with serious lesions (Asimuddin and Jamil, 2012).

557 Proteins involved in the genotoxicity pathway including phosphorylated ones,  
558 p-CHK1, p-CHK2, p-H2AX, p-P53, and total proteins, ATR, MDM2, and P21, have been  
559 tested after exposure of BEAS-2B cells to the different fraction of PM<sub>2.5-0.3</sub> and OEM<sub>0.3</sub> for 24  
560 and 72 h. A very weak induction of the proteins involved in this pathway following the  
561 exposure of BEAS-2B cells to the different fractions of PM<sub>2.5-0.3</sub>, including NEM<sub>2.5-0.3</sub> (Table  
562 5) was observed. This indicates that most of the biologically active metabolites resulting from  
563 the metabolic activation of PM, their organic extract and the non-extractable fraction, are all  
564 conjugated during the phase II of this process and removed from the cell. However, different  
565 results were reported with OEM<sub>0.3</sub>. The highest dose of OEM<sub>0.3</sub> was able to induce a  
566 significant increase of ATR, p-CHK1 and p-CHK2, MDM2, P21, and p-P53, almost only  
567 after 72 h (Table 5). EOM<sub>0.3</sub> contains relatively high proportion of organic compounds  
568 compared to OEM<sub>2.5-0.3</sub>. It is clearly observed that EOM<sub>0.3</sub> induced DNA damage felt by ATR  
569 and followed by a cascade of protein phosphorylation, thereby contributing to the cell cycle  
570 arrest (i.e., P21 and P53 induction). Accordingly, higher levels of phosphorylated P53, CHK1  
571 and CHK2 were generally related to the cell cycle arrest (Fragkos et al., 2009).

572 In addition, this study showed a significant decrease in phosphorylated H2AX in  
573 BEAS-2B exposed for 72 h to the two doses of OEM<sub>2.5-0.3</sub>. In addition, the same decrease was  
574 observed after 72 hours of exposure to NEM<sub>2.5-0.3</sub> at 3 and 12 µg PM-eq/cm<sup>2</sup>, and to  
575 3 µg PM-eq/cm<sup>2</sup> of OEM<sub>0.3</sub>. These results are in agreement with those published by Abbas et  
576 al. (2019) and Bouquet et al. (2006) showing that, at low doses of exposure, the DNA repair  
577 mechanism was highly active leading to the degradation of phosphorylated H2AX. They also  
578 observed that, at high doses of exposure, the DNA repair mechanism seemed to be less active  
579 leading to phosphorylated H2AX accumulation, which is also the case in this study as shown  
580 for OEM<sub>0.3</sub>, 72 h and 12 µg PM-eq/cm<sup>2</sup>.

581 With regards to the different chemical compounds tested as positive controls, BaP was  
582 able to activate the DNA damage pathway by inducing the expression of several studied  
583 proteins including p-CHK2, p-H2AX, and p-P53, after 24 h, and p-CHK1 and p-CHK2 after  
584 72 h. Moreover, 1-NPyr significantly induced expression of p-CHK2 and p-P53, after 24 h,  
585 and of p-P53 after 72 h. In contrast, cells exposed to the 9-FluO didn't show any significant  
586 expression of the studied proteins.

587 Taken together, these results showed that the exposure to OEM<sub>0.3</sub> was able to induce  
588 the expression of most of the studied proteins related to cell cycle arrest and DNA damages.  
589 In addition, these results showed that BaP and 1-NPyr were able to induce DNA damages,  
590 while 9-FluO, the positive control used for O-PAH toxicity evaluation was not.

591

#### 592 4. Conclusion

593 In the present work, PM was collected at the southern suburbs of Beirut. Chemical  
594 analysis clearly showed the influence of soil resuspension and also traffic emissions. Organic  
595 fraction analysis showed that the contents of PAH and n-alkanes were far higher in PM<sub>0.3</sub> than  
596 PM<sub>2.5-0.3</sub>, which supports a strongly relation between the combustion process emission and the  
597 ultrafine particle composition. Toxicological studies suggested that the metabolic activation of  
598 PAH contained in PM<sub>2.5-0.3</sub> in its entirety was delayed in comparison with PAH in OEM. The  
599 NEM was not able to significantly express genes involved in the metabolic activation pathway  
600 of organic compounds. All the tested PM fractions, including the NEM were able to increase  
601 the expression of the studied proteins involved in the genotoxicity pathway. In addition, the  
602 PM-induced genotoxicity appeared to be strongly related to the organic chemical contents.  
603 The quasi-ultrafine particles were the most genotoxic due the predominance of organic  
604 compounds such as PAH in this size fraction. The obtained results confirmed that considering  
605 only OEM or NEM might lead to results regarding mechanisms and kinetics of toxicity that  
606 would be different of those obtained from cells exposed to PM considered in its entirety. This  
607 work evidenced that the co-presence of organic compounds and other inorganic elements  
608 (such as metals and trace elements) may determine the biological effects of fine PM as a  
609 whole. It also shows that organic compounds associated to the quasi-ultrafine fraction are the  
610 main drivers of the fine PM genotoxicity and phase I and phase II enzymes implicated in the  
611 metabolic activation of PAH. Finally, this study underlined the importance of evaluating the  
612 biological impact of ultrafine and quasi-ultrafine particles for a better understanding of the  
613 health effects related to exposure to the fine PM.

614

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## Table captions

**Table 1:** Elemental, water-soluble ions and total carbon concentrations in PM<sub>2.5-0.3</sub> and PM<sub>0.3</sub> collected in the suburb of Beirut (Lebanon).

**Table 2:** Concentrations of PAH, N-PAH, O-PAH (in µg/g) and PCB, PCDD and PCDF (in ng/g) detected in PM<sub>2.5-0.3</sub> and PM<sub>0.3</sub> collected in the southern suburbs of Beirut. The relative percentage of each compound in the corresponding class of organic compounds is also indicated.

**Table 3:** PAH diagnostic ratios calculated for PM<sub>2.5-0.3</sub> and PM<sub>0.3</sub> collected at the southern suburb of Beirut and compared to the bibliography, in order to determine the possible emission sources of PAH.

**Table 4:** Metabolic activation: gene expression of aryl hydrocarbon receptor (AHR), Aryl-Hydrocarbon Receptor Repressor (AHRR) and aryl hydrocarbon receptor nuclear translocator (ARNT), cytochrome P4501A1 (CYP1A1), cytochrome P4501B1(CYP1B1), epoxide hydrolase 1 (EPHX), glutathione S-transferase alpha 4 (GSTA4) enzymes in BEAS-2B cells exposed 6 and 24 h to PM<sub>2.5-0.3</sub>, organic extract matter (OEM<sub>2.5-0.3</sub>), non-extractable matter fraction (NEM<sub>2.5-0.3</sub>) and organic extractable matter of the quasi-ultrafine fraction (OEM<sub>0.3</sub>) at 3 and 12 µg PM-eq/cm<sup>2</sup> and positive controls (BaP, 1-NPyr and 9-FluO). These values are depicted as mean values and standard deviations of 3 replicates for controls and 3 replicates for exposed cells (Mann–Whitney U-test; vs. Controls, \*: P<0.05).

**Table 5:** Genotoxicity: Protein expression of ataxia telangiectasia and Rad3-related protein (ATR), phosphorylated checkpoint kinase 1 (p-CHK-1), phosphorylated checkpoint kinase 2 (p-CHK-2), phosphorylated H2A histone family member X (p-H2AX), total proteins of mouse double minute 2 homolog (MDM2), total protein 21 (P21), phosphorylated protein 53 (p-P53), in BEAS-2B cells exposed 6 and 24 h to PM<sub>2.5-0.3</sub>, organic extractable matter (OEM<sub>2.5-0.3</sub>), non-extractable matter fraction (NEM<sub>2.5-0.3</sub>) and organic extractable matter of the quasi-ultrafine fraction (OEM<sub>0.3</sub>) at 3 and 12 µg PM-eq/cm<sup>2</sup> and positive controls (BaP, 1-NPyr and 9-FluO). These values are depicted as mean values and standard deviations of 3 replicates for controls and 3 replicates for exposed cells (Mann–Whitney U-test; vs. Controls, \*: P<0.05).

Table 1

Species	PM <sub>2.5-0.3</sub> (mg/g)	PM <sub>0.3</sub> (mg/g)
Ca	134	100
NO <sub>3</sub> <sup>-</sup>	56	28.8
SO <sub>4</sub> <sup>2-</sup>	27.1	7.11
Fe	25.8	12.2
Al	21.4	14.9
Cl <sup>-</sup>	16.5	21.2
Mg	12.3	17.6
Na	10.7	40.5
K	5.27	6.67
Cu	2.38	2.73
Ti	2.01	1.21
Ba	1.02	0.40
NH <sub>4</sub> <sup>+</sup>	1.01	17.4
P	0.58	1.39
Zn	0.40	1.12
Mn	0.30	0.19
Sr	0.23	0.13
Pb	0.15	0.32
Cr	0.14	0.08
V	0.11	0.32
Sn	0.11	0.06
Ni	0.05	0.16
Mo	0.04	0.34
Co	0.14	0.01
As	<DL <sup>a</sup>	0.01
Cd	<DL <sup>a</sup>	0.01
Total carbon	17.5	126

<sup>a</sup> DL : Detection limit



Table 2

	Abbreviation	PM <sub>2.5-0.3</sub>	Relative %	PM <sub>0.3</sub>	Relative %
<b>PAH (µg/g)</b>					
Naphthalene	Nap	0.3	0.9	1.1	0.1
Acenaphthylene	Acy	0.1	0.2	0.7	0.1
Acenaphthene	Ace	0.01	0.0	0.2	0.0
Fluorene	Flu	0.1	0.2	0.5	0.1
Phenanthrene	Phe	1.2	3.3	8.8	0.9
Anthracene	Ant	1.7	4.6	1.4	0.1
Fluoranthene	Fla	2.5	6.7	19	1.8
Pyrene	Pyr	2.7	7.3	23	2.3
Benzo[a]Anthracene	BaA	3.4	9.2	64	6.6
Chrysene	Chr	3.6	9.7	51	5.2
Benzo[b]Fluoranthene	BbF	5.6	15.2	173	17.6
Benzo[k]Fluoranthene	BkF	1.5	4.0	38	3.8
Benzo[a]Pyrene	BaP	2.7	7.4	73	7.5
Indeno[1.2.3-c.d]Pyrene	InPy	4.7	12.8	206	21.0
Dibenzo[a.h]Anthracene	DahA	0.4	1.1	22.9	2.3
Benzo[g,h,i]Perylene	BghiP	6.4	17.4	299	30.5
<b>Σ PAH</b>		<b>37</b>	<b>100</b>	<b>980</b>	<b>100</b>
<b>O-PAH<sup>a</sup> (µg/g)</b>					
1.8-naphthalic anhydride		2.3	32.9	43	40.6
7H-benz(de)anthracen-7-one	19-BAntO	0.8	11.4	27	25.5
9.10-anthraquinone	9.10-AntQ	2.5	35.7	19	17.9
9-fluorenone	9-FluO	0.7	10.0	3.3	3.1
Benzo(a)fluorenone	BaFluO	0.8	11.4	6.8	6.4
Benz(a)anthracen-7.12-dione	7.12-BaAQ	-	-	6.7	6.3
<b>Σ O-PAH</b>		<b>7.0</b>	<b>100</b>	<b>106</b>	<b>100</b>
<b>N-PAH<sup>b</sup> (µg/g)</b>					
2-nitroanthracene	2-Nant	0.2	4.9	4.8	31.2
7-nitrobenz(a)anthracene	7-NBaA	1.1	26.8	2.7	17.5
9-nitroanthracene	9-NAnt	0.05	1.2	1.5	9.7
6-nitrochrysene	6-NChry	0.3	7.3	1.1	7.1
1-nitropyrene	1-NPyr	0.6	14.6	1.0	6.5
1.3-dinitronaphthalene	1.3-DNNap	0.6	14.6	1.2	7.8
5-nitroacenaphthene	5-NAce	0.14	3.4	1.1	7.1
3-nitrophenanthrene	3-NPhe	0.6	14.6	1.1	7.1
2-nitrofluorene	2-NFlu	0.4	9.8	0.9	5.8
<b>Σ N-PAH</b>		<b>4.1</b>	<b>100</b>	<b>15.4</b>	<b>100</b>
<b>Σ PCDD (ng/g)</b>		<b>5.5</b>		<b>25.2</b>	
<b>Σ PCDF (ng/g)</b>		<b>14.0</b>		<b>26.4</b>	
<b>Σ PCB (ng/g)</b>		<b>12.2</b>		<b>15.7</b>	

<sup>a</sup>1-naphthaldehyde and phenanthrene-9-carboxyaldehyde were not detected.

<sup>b</sup>1-nitronaphthalene; 2-nitrobiphenyl; 1.5-dinitronaphthalene and 1.8-dinitropyrene were not detected.

Table 3:

PAH diagnostic ratios	PM <sub>2.5-0.3</sub>	PM <sub>0.3</sub>	Characteristic ratio values and sources		
InPy / (InPy+ BghiP) <sup>a</sup>	0.42	0.41	0.18 Cars	0.62 Wood burning	0.35-0.70 Diesel burning
Flu/(Flu+Pyr) <sup>a</sup>	0.48	0.44	<0.5 Gasoline		>0.5 Diesel
BaP/(BaP+Chry) <sup>b</sup>	0.43	0.59	> 0.35 Vehicular emission		0.2-0.35 Coal combustion
BaP/ BghiP <sup>a</sup>	0.42	0.25	0.5-0.6 Traffic emission		>1.25 Brown coal or lignite
Anth/(Anth+Phe) <sup>b</sup>	0.59	0.14	>0.1 pyrogenic	<0.1 petrogenic	
InPy / BghiP <sup>a</sup>	0.74	0.69	<0.4 Gasoline	~1 Diesel	
Fla/(Fla+Pyr) <sup>b</sup>	0.48	0.44	<0.4 petrogenic	0.4-0.5 Fossil fuel combustion	>0.5 Grass , wood , coal combustion
Fla/Pyr <sup>b</sup>	0.92	0.79	< 0.6 Non traffic emission	> 0.6 traffic emission	
CPAH/TPAH <sup>a</sup>	0.98	0.98		~1 combustion	

<sup>a</sup> Ravindra et al., 2008<sup>b</sup> Tobiszewski and Namiesnik, 2012

Table 4:

		PM <sub>2.5-0.3</sub>			NEM <sub>2.5-0.3</sub>		OEM <sub>2.5-0.3</sub>		OEM <sub>0.3</sub>		BaP	1-NPyr	9-FluO
		Control	3 µg PM- éq/cm <sup>2</sup>	12 µg PM- éq/cm <sup>2</sup>	3 µg PM- éq/cm <sup>2</sup>	12 µg PM- éq/cm <sup>2</sup>	3 µg PM- éq/cm <sup>2</sup>	12 µg PM- éq/cm <sup>2</sup>	3 µg PM- éq/cm <sup>2</sup>	12 µg PM- éq/cm <sup>2</sup>	25 µM	25 µM	25 µM
6 h	<i>AHR</i>	1 ± 0.02	<b>1.96*</b> ± 0.2	<b>2.93*</b> ± 0.31	0.99 ± 0.06	1.06 ± 0.15	1 ± 0.03	1 ± 0.08	1.18 ± 0.25	<b>2.79*</b> ± 0.38	0.9 ± 0.13	<b>4.18*</b> ± 0.41	<b>277.7*</b> ± 63.9
	<i>AhRR</i>	1 ± 0.08	<b>2.10*</b> ± 0.14	<b>2.86*</b> ± 0.88	0.99 ± 0.11	1.43 ± 0.33	1.2 ± 0.30	1.44 ± 0.46	1.19 ± 0.50	<b>2.21*</b> ± 0.60	0.94 ± 0.22	<b>3.15*</b> ± 1.05	<b>61.3*</b> ± 12.9
	<i>ARNT</i>	1 ± 0.02	1.41 ± 0.33	<b>1.91*</b> ± 0.53	1.13 ± 0.15	1.21 ± 0.26	0.9 ± 0.06	0.9 ± 0.13	1.45 ± 0.44	<b>1.66*</b> ± 0.14	1.05 ± 0.2	<b>2.36*</b> ± 0.54	<b>134.8*</b> ± 39.0
	<i>CYP1A1</i>	1 ± 0.07	<b>1.78*</b> ± 0.46	<b>3.65*</b> ± 0.82	1 ± 0.10	1 ± 0.12	0.97 ± 0.01	<b>1.55*</b> ± 0.10	<b>2.18*</b> ± 0.75	1.48 ± 0.33	1.03 ± 0.2	<b>2.28*</b> ± 0.35	<b>37.3*</b> ± 3.71
	<i>CYP1B1</i>	1 ± 0.04	<b>2.26*</b> ± 0.49	<b>4.51*</b> ± 0.83	1.14 ± 0.38	1.5 ± 0.50	0.98 ± 0.04	<b>2.37*</b> ± 0.91	<b>1.59*</b> ± 0.31	<b>2.06*</b> ± 0.15	0.69 ± 0.26	<b>2.55*</b> ± 0.67	<b>46.4*</b> ± 16.1
	<i>EPHX-1</i>	1 ± 0.12	0.99 ± 0.26	1.68 ± 0.58	1 ± 0.28	1.01 ± 0.26	0.87 ± 0.05	0.87 ± 0.06	<b>1.80*</b> ± 0.13	1.75 ± 0.38	0.82 ± 0.06	<b>2.44*</b> ± 0.45	<b>114.5*</b> ± 19.5
	<i>GSTA-4</i>	1 ± 0.09	1.30 ± 0.18	<b>2.59*</b> ± 0.75	0.84 ± 0.09	1.32 ± 0.30	1.21 ± 0.24	1.18 ± 0.10	2.30 ± 0.87	<b>1.71*</b> ± 0.15	0.81 ± 0.20	<b>3.04*</b> ± 1	<b>202.7*</b> ± 34.5
24 h	<i>AhR</i>	1 ± 0.01	1.31 ± 0.34	<b>4.04*</b> ± 0.8	0.95 ± 0.06	1.03 ± 0.16	0.94 ± 0.03	0.92 ± 0.10	0.43 ± 0.08	<b>1.67*</b> ± 0.22	<b>1.50*</b> ± 0.16	<b>2.48*</b> ± 0.72	<b>152.1*</b> ± 25.4
	<i>AhRR</i>	1 ± 0.04	1.45 ± 0.12	<b>4.13*</b> ± 0.79	0.93 ± 0.14	1 ± 0.20	0.86 ± 0.20	1 ± 0.02	1 ± 0.07	<b>1.64*</b> ± 0.36	<b>2.50*</b> ± 0.93	<b>1.64*</b> ± 0.11	<b>30.3*</b> ± 5.23
	<i>ARNT</i>	1 ± 0.05	1.30 ± 0.39	<b>3.56*</b> ± 1.16	0.92 ± 0.14	1.2 ± 0.36	1.17 ± 0.51	1.11 ± 0.34	0.89 ± 0.14	<b>1.98*</b> ± 0.22	<b>1.63*</b> ± 0.10	1.34 ± 0.60	<b>117.0*</b> ± 27.7
	<i>CYP1A1</i>	1 ± 0.04	<b>2.59*</b> ± 0.87	<b>7.46*</b> ± 2.70	0.9 ± 0.10	1.49 ± 0.42	1.01 ± 0.12	1.40 ± 0.28	<b>2.45*</b> ± 0.26	<b>4.3*</b> ± 0.97	<b>7.70*</b> ± 1.64	<b>1.86*</b> ± 0.46	<b>18.1*</b> ± 3.14
	<i>CYP1B1</i>	1 ± 0.13	<b>1.97*</b> ± 0.29	<b>4.07*</b> ± 0.68	1.19 ± 0.50	1.26 ± 0.41	1.26 ± 0.33	1.23 ± 0.41	1.09 ± 0.16	<b>3.21*</b> ± 1	<b>6.02*</b> ± 1.57	<b>1.67*</b> ± 0.17	<b>17.1*</b> ± 3.16
	<i>EPHX-1</i>	1 ± 0.02	1.38 ± 0.32	<b>4.04*</b> ± 1.02	1.05 ± 0.45	1.38 ± 0.38	1.000 ± 0.02	1.24 ± 0.45	1.15 ± 0.43	<b>1.69*</b> ± 0.14	<b>1.70*</b> ± 0.50	<b>1.75*</b> ± 0.26	<b>58.1*</b> ± 9.65
	<i>GSTA-4</i>	1 ± 0.04	1.03 ± 0.02	3.62 ± 1.06	0.84 ± 0.18	1.17 ± 0.34	1 ± 0.03	1 ± 0.048	1.16 ± 0.27	<b>1.65*</b> ± 0.19	1.46 ± 0.26	1.29 ± 0.28	<b>94.9*</b> ± 18.6

*AHR* : aryl hydrocarbon receptor, *AhRR*: Aryl-Hydrocarbon Receptor Repressor, *ARNT*: aryl hydrocarbon receptor nuclear translocator, *CYP1A1*: cytochrome P4501A1, *CYP1B1* : cytochrome P4501B1, *EPHX-1* : Epoxide hydrolase 1, *GSTA-4* : Glutathione S-Transferase Alpha 4, PM<sub>2.5-0.3</sub>: fine particulate matter, NEM<sub>2.5-0.3</sub>: Non-extractable matter fraction, OEM<sub>2.5-0.3</sub>: fine organic extractable fraction, OEM<sub>0.3</sub>: quasi-ultrafine organic extractable matter, BaP : Benzo[a]pyrene, 1-NPyr: 1-nitropyrene, 9-FluO : 9-Fluorenone.

Table 5:

		PM <sub>2.5-0.3</sub>			NEM <sub>2.5-0.3</sub>		OEM <sub>2.5-0.3</sub>		OEM <sub>0.3</sub>		BaP	1-NPyr	9-FluO
		Control	3 µg PM- éq/cm <sup>2</sup>	12 µg PM- éq/cm <sup>2</sup>	3 µg PM- éq/cm <sup>2</sup>	12 µg PM- éq/cm <sup>2</sup>	3 µg PM- éq/cm <sup>2</sup>	12 µg PM- éq/cm <sup>2</sup>	3 µg PM- éq/cm <sup>2</sup>	12 µg PM- éq/cm <sup>2</sup>	25 µM	25 µM	25 µM
24 h	ATR	1 ± 0.07	1.07 ± 0.06	1.08 ± 0.18	1.02 ± 0.13	1.05 ± 0.1	0.92 ± 0.07	1.22 ± 0.29	1.014 ± 0.137	1.03 ± 0.13	1 ± 0.224	1.06 ± 0.01	1 ± 0.209
	p-CHK1	1 ± 0.09	0.97 ± 0.16	0.94 ± 0.13	0.94 ± 0.14	0.92 ± 0.07	0.93 ± 0.06	1.369 ± 0.517	1.038 ± 0.179	<b>1.24*</b> <b>± 0.12</b>	1.23 ± 0.22	1.16 ± 0.08	1.03 ± 0.21
	p-CHK2	1 ± 0.09	1 ± 0.14	1 ± 0.18	0.93 ± 0.13	0.94 ± 0.09	0.95 ± 0.09	1.464 ± 0.622	0.984 ± 0.152	1.08 ± 0.13	<b>1.34*</b> <b>± 0.19</b>	<b>3.63*</b> <b>± 0.29</b>	1.04 ± 0.22
	p-H2AX	1 ± 0.13	0.88 ± 0.25	0.756 ± 0.164	0.92 ± 0.12	0.83 ± 0.1	0.95 ± 0.26	1.110 ± 0.383	0.806 ± 0.137	0.85 ± 0.18	<b>1.42*</b> <b>± 0.08</b>	1.19 ± 0.24	0.83 ± 0.27
	MDM2	1 ± 0.12	<b>1.27*</b> <b>± 0.11</b>	<b>1.34*</b> <b>± 0.24</b>	1.02 ± 0.13	<b>1.21*</b> <b>± 0.1</b>	0.92 ± 0.107	1.251 ± 0.408	0.918 ± 0.131	<b>1.22*</b>	1.24 ± 0.20	1.15 ± 0.03	1.06 ± 0.25
	P21	1 ± 0.10	0.89 ± 0.2	0.55 ± 0.1	0.8 ± 0.07	0.61 ± 0.08	0.88 ± 0.05	1.065 ± 0.215	0.881 ± 0.128	<b>1.60*</b> <b>± 0.10</b>	0.88 ± 0.15	0.95 ± 0.07	1.17 ± 0.26
	p-P53	1 ± 0.18	1.17 ± 0.16	1.11 ± 0.3	1.11 ± 0.26	1.06 ± 0.12	1.16 ± 0.15	<b>2.53*</b> <b>± 1.639</b>	<b>1.574*</b> <b>± 0.384</b>	<b>1.76*</b> <b>± 0.24</b>	<b>4.29*</b> <b>± 0.47</b>	<b>1.71*</b> <b>± 0.09</b>	1.12 ± 0.29
72 h	ATR	1 ± 0.14	0.98 ± 0.19	0.916 ± 0.182	1.14 ± 0.05	0.73 ± 0.13	0.97 ± 0.08	0.74 ± 0.14	0.886 ± 0.077	<b>1.29*</b> <b>± 0.75</b>	1.1 ± 0.15	0.93 ± 0.33	0.88 ± 0.14
	p-CHK1	1 ± 0.15	1.07 ± 0.19	0.916 ± 0.195	1.26 ± 0.13	1 ± 0.27	1.21 ± 0.11	0.98 ± 0.09	<b>1.37*</b> <b>± 0.18</b>	<b>5.21*</b> <b>± 2.85</b>	<b>1.56*</b> <b>± 0.16</b>	1.24 ± 0.21	1.37 ± 0.32
	p-CHK2	1 ± 0.13	1.05 ± 0.2	0.889 ± 0.161	1.24 ± 0.18	1.01 ± 0.32	1.25 ± 0.10	1.08 ± 0.08	<b>1.83*</b> <b>± 0.32</b>	<b>4.21*</b> <b>± 2.31</b>	<b>1.65*</b> <b>± 0.17</b>	1.31 ± 0.27	1.46 ± 0.37
	p-H2AX	1 ± 0.13	1.36 ± 0.38	1.005 ± 0.224	<b>0.8*</b> <b>± 0.09</b>	<b>0.49*</b> <b>± 0.06</b>	<b>0.42*</b> <b>± 0.03</b>	<b>0.43*</b> <b>± 0.04</b>	<b>0.74*</b> <b>± 0.11</b>	<b>1.36*</b> <b>± 0.73</b>	0.94 ± 0.38	0.65 ± 0.15	0.46 ± 0.11
	MDM2	1 ± 0.12	1.16 ± 0.15	1.054 ± 0.259	<b>1.37*</b> <b>± 0.20</b>	1.01 ± 0.22	1 ± 0.07	0.79 ± 0.07	0.94 ± 0.16	<b>3.13*</b> <b>± 1.98</b>	1.03 ± 0.11	0.80 ± 0.193	0.79 ± 0.15
	P21	1 ± 0.24	<b>1.11*</b> <b>± 0.25</b>	0.912 ± 0.199	1.42 ± 0.29	0.79 ± 0.06	0.86 ± 0.07	0.7 ± 0.15	1.03 ± 0.23	<b>18.49*</b> <b>± 7.89</b>	1.40 ± 0.20	0.88 ± 0.26	0.94 ± 0.25
	p-P53	1 ± 0.20	0.96 ± 0.39	0.860 ± 0.132	<b>1.67*</b> <b>± 0.41</b>	0.94 ± 0.17	0.77 ± 0.08	0.89 ± 0.18	<b>2.24*</b> <b>± 0.33</b>	<b>14.91*</b> <b>± 9.31</b>	<b>3.51*</b> <b>± 0.56</b>	<b>1.42*</b> <b>± 0.17</b>	1.51 ± 0.63

CHK-1: checkpoint kinase 1, CHK-2: checkpoint kinase 2, H2AX: H2A histone family member X, P53: protein 53, ATR: ataxia telangiectasia and Rad3-related protein, MDM2: mouse double minute 2 homolog P21: protein P21, PM<sub>2.5-0.3</sub>: fine particulate matter, NEM<sub>2.5-0.3</sub>: Non-extractable matter fraction, OEM<sub>2.5-0.3</sub>: fine organic extractable fraction, OEM<sub>0.3</sub>: quasi-ultrafine organic extractable matter, BaP: Benzo[a]pyrene, 1-NPyr: 1-nitropyrene, 9-FluO: 9-Fluorenone.