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► To cite this version:

J. Boudjema, B. Lima, C. Grare, Laurent Alleman, D. Rousset, et al.. Metal enriched quasi-ultrafine particles from stainless steel gas metal arc welding induced genetic and epigenetic alterations in BEAS-2B cells. *NanoImpact*, 2021, 23, pp.100346. 10.1016/j.impact.2021.100346 . hal-03321110

HAL Id: hal-03321110

<https://hal.science/hal-03321110v1>

Submitted on 22 Aug 2023

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1 **METAL ENRICHED QUASI-ULTRAFINE PARTICLES FROM STAINLESS STEEL**
2 **GAS METAL ARC WELDING INDUCED GENETIC AND EPIGENETIC**
3 **ALTERATIONS IN BEAS-2B CELLS**

4
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19 **ABBREVIATIONS**

20

21 4-HNE: 4-hydroxynonenal

22 8-OHdG: hydroxy-2'-deoxyguanosine

23 CCL5: C-C motif chemokine ligand 5

24 CIRC: international agency for research on cancer

25 CM-H₂DCFDA: chloromethyl derivative of 2',7'-dichlorodihydrofluorescein diacetate

26 DRG: differentially regulated gene

27 DRmiR: differentially regulated miRNA

28 FC: fold-change

29 GM-CSF: granulocyte macrophage colony stimulating factor

30 GMAW: gas metal arc welding

31 GRO- α : growth-regulated oncogene- α

32 HAT: histone acetyl transferase

33 HDAC: histone deacetylase

34 ICP-MS: inductively coupled plasma-mass spectrometry

35 IL-1 β : interleukin-1 beta

36 IL-6: interleukin-6

37 IL-8: interleukin-8

38 LOQ: Limit of quantitation

39 MCP-1: monocyte chemoattractant protein 1

40 miR: miRNA

41 MMS: methyl methanesulfonate

42 MS: mild-steel

43 NF- κ B: nuclear factor-kappa B

44 NRF2: nuclear factor erythroid 2-related factor 2

45 PM: particulate matter

46 PTM: post-translational modification

47 Q-UFP: quasi-ultrafine particles

48 RANTES: regulated upon activation, normal T cell expressed and presumably secreted

49 ROS: reactive oxygen species

50 SS: stainless-steel

51 t-BHP: tert-Butyl hydroperoxide

52 TNF- α : tumor necrosis factor

53 UFP: ultrafine particles

54 WF: welding fume

55

56 **ABSTRACT**

57
58 Recent evidence has supported welding fume (WF)-derived ultrafine particles (UFP) could be the driving force
59 of their adverse health effects. However, UFP have not yet been extensively studied and are currently not included in
60 present air quality standards/guidelines. Here, attention was focused on the underlying genetic and epigenetic
61 mechanisms by which the quasi-UFP (Q-UFP, i.e., $\leq 0.25 \mu\text{m}$) of the WF emitted by gas metal arc welding-stainless
62 steel (GMAW-SS) exert their toxicity in human bronchial epithelial BEAS-2B cells. The Q-UFP under study showed
63 a monomodal size distribution in number centered on $104.4 \pm 52.3 \text{ nm}$ and a zeta potential of $-13.8 \pm 0.3 \text{ mV}$. They
64 were enriched in $\text{Fe} > \text{Cr} > \text{Mn} > \text{Si}$, and displayed a relatively high intrinsic oxidative potential. Dose-dependent
65 activation of nuclear factor erythroid 2-related factor 2 and nuclear factor-kappa B signaling pathway, glutathione
66 alteration, and DNA, protein and lipid oxidative damage were reported in BEAS-2B cells acutely (1.5 and $9 \mu\text{g}/\text{cm}^2$,
67 24 h) or repeatedly (0.25 and $1.5 \mu\text{g}/\text{cm}^2$, $3 \times 24 \text{ h}$) exposed to Q-UFP ($p < 0.05$). Alterations of the Histone H3
68 acetylation were reported for any exposure ($p < 0.05$). Differentially regulated miRNA and mRNA indicated the
69 activation of some critical cell signaling pathways related to oxidative stress, inflammation, and cell cycle
70 deregulation towards apoptosis. Taken together, these results highlighted the urgent need to better evaluate the
71 respective toxicity of the different metals and to include the Q-UFP fraction of WF in current air quality
72 standards/guidelines relevant to the occupational settings.

73
74
75
76 **KEYWORDS:** Welding fume; Quasi-ultrafine particles; Metals; human bronchial epithelial BEAS-2B cells;
77 intrinsic oxidative potential; genetic and epigenetic alterations.

78

79 **INTRODUCTION**

80
81 Welding is commonly defined as an industrial process where two or more metal pieces are melted together by
82 means of heat to form a joint as the parts cool, leading to generation of complex and heterogenous aerosols of metal
83 fumes, gases, and solid particles. Worldwide, an estimated 11 million workers have a job title of welder, and around
84 110 million additional workers probably incur welding-related exposures. Welding generally involves the exposure
85 to high levels of fine- and ultrafine combustion-derived particulate matter (PM), UV radiation, and electromagnetic
86 fields, and, in some cases, welders are also co-exposed to asbestos and solvents (Guha et al. 2017). In March 2017, a
87 working group met at the International Agency for Research on Cancer (IARC) to evaluate the carcinogenicity of
88 welding. For a majority of the cohort and case-control studies across different countries, time periods, and
89 occupational settings they reported an elevated risk of lung cancer for workers employed as welders or
90 occupationally exposed to welding fume (WF). The risk for lung cancer was higher in welders that had a longer or
91 higher cumulative exposure (i.e., both mild-steel, MS and stainless-steel, SS welding). The working group concluded
92 that there is ‘sufficient evidence in humans’ that WF cause lung cancer and, therefore, classified welding as
93 Group1/carcinogenic to humans (IARC, 2017).

94 Recent works have identified combustion-derived PM emitted within WF as the driving force of the adverse
95 health effects that occur in humans (Gliga et al. 2017, McCarrick et al. 2019, Pega et al. 2020). While most of this
96 PM mass is found in the fine-sized particle range (i.e., PM_{2.5}, aerodynamic diameter $\leq 2.5\mu\text{m}$), the largest particle
97 number is in the ultrafine-sized particle range (i.e., PM_{0.1}, aerodynamic diameter $\leq 0.1\mu\text{m}$). Current health concerns
98 were initially focused on the coarse (i.e., PM₁₀, aerodynamic diameter $\leq 10\mu\text{m}$) and fine fractions, mainly because of
99 their ability to migrate deeply and be highly retained down towards the respiratory tract. Emphasis has since then
100 been clearly shifted from larger to ultrafine particles (UFP) (Stone et al. 2017). Epidemiological and toxicological
101 evidence has indeed incriminated their unique properties, compared to those of the larger size particle fractions,
102 including their higher lung deposition, particle number concentration, and surface area per mass. However, the UFP
103 fraction of the WF has not been extensively studied yet and is currently not included in current air quality standards
104 and/or guidelines relevant to the occupational settings, although it probably closely participates for the most part of
105 the human health effects induced by WF (Falcone et al. 2018).

106 Health effects of WF exposure are complex, as their composition is affected by the type of welding alloy used
107 (Chang et al. 2013; Erdem et al. 2020; Falcone et al. 2018; Leonard et al., 2010; Zheng et al., 2015). Welding involves
108 several processes (e.g., oxyfuel [gas], arc, and resistance welding) and materials (e.g., MS and SS). Arc welding,
109 including one type known as gas metal arc welding (GMAW), in which shielding gases are used to protect against
110 oxidation, is still the most common industrial welding process (Antonini et al. 1999, 2014). This strongest method of
111 joining metals creates a relatively high levels of WF, the composition of which largely depending on whether a MS
112 or a SS electrode is used: GMAW-MS fume contains primarily iron (Fe), zinc (Zn), and manganese (Mn), whereas
113 GMAW-SS fume contains largely Fe, chromium (Cr, also existing in both CrIII and CrVI oxidation states), nickel
114 (Ni), copper (Cu), and Mn (Antonini et al. 2014; Falcone et al. 2018; Ghanem et al. 2021; Stanisławska et al. 2011).

115 Welding exposures being somewhat complex owing to the diversity of welding processes, a majority of welders
116 generally using multiple welding processes and consumables throughout their lifetime, and some confounders or

117 additional exposures being possibly regarded in the workplace, associations are still difficult to established (Falcone
118 et al. 2017; Shoeb et al. 2017; Zeidler-Erdely et al. 2010, 2019). All the cellular and/or molecular underlying
119 mechanisms responsible for the toxicity and even the carcinogenicity of WF are still not fully understood. Therefore,
120 controlled experimental studies are crucial to better understand which WF type and which of their metal components
121 are the most toxic and have the greatest carcinogenic potential. Series of animal studies, by which the exposure to
122 WF was well-controlled, were undertaken to better elucidate the role of WF metal composition (e.g., SS versus MS)
123 on lung toxicity and cancer development. Some of them reported oxidative stress conditions, inflammatory
124 responses, and immune suppression (Falcone et al. 2018; Gliga et al. 2017; Grigg et al. 2017; Marongiu et al. 2016;
125 McCarrick et al. 2019; Pega et al. 2020; Stanisławska et al. 2020). In a two-stage initiation-promotion model of lung
126 carcinogenesis, GMAW-SS fume significantly increased lung tumor multiplicity after both an oropharyngeal
127 aspiration and inhalation exposure in A/J mice (Falcone et al. 2017; Zeidler-Erdely et al., 2010). An *in vivo* study
128 indicated that WF derived from SS welding acted as a tumor promoter and lead to lung cancer in mice initially
129 exposed to 3-methylcholanthrene (Zeidler-Erdely et al. 2013). Further demonstration of the greater toxic effect of
130 GMAW-SS fume than GMAW-MS fume was reported in other animal models. For example, Antonini et al. (2007,
131 2009) found that GMAW-MS fume caused no lung inflammation or lung injury in Sprague-Dawley rats 1, 4, or 11
132 days post-inhalation compared to GMAW-SS fume, which caused significant lung damage. Regardless, *in vivo*
133 studies investigating exposures to WF as occurring in GMAW-MS or -SS are still lacking to contribute to an entire
134 knowledge of the metal components closely responsible for their toxicity and/or their carcinogenicity. Two main
135 topics that need to be further evaluated in future experimental models are not only the contribution of their individual
136 chemicals and/or the specific roles of their UFP fraction in the development of lung toxicity and tumor formation,
137 but also the cellular and molecular underlying mechanisms by which they contribute to cause lung toxicity and even
138 lung tumorigenesis.

139 The better knowledge of the cellular and molecular underlying mechanisms involved in the pathogenicity of
140 lung toxicity and even lung cancers related to WF exposure is still essential and could notably rely on relevant lung
141 cell culture models (Leclercq et al., 2016). Indeed, clinical and basic science applications have focused on the
142 bronchial epithelium because of the chronic inflammatory diseases resulting from disruption of this region (Boublil
143 et al., 2013; Leclercq et al. 2017a). Up to now, only very few *in vitro* studies have been undertaken to better
144 elucidate the individual chemicals and/or UFP sized-fractions of WF and their critical roles in the harmful health
145 effects they induced.

146 As supported by the current literature, the underlying mechanism of post-inflammatory effects of WF is
147 certainly mostly launched by soluble intermediate metals through the excessive production of reactive oxygen
148 species (ROS) (Ghanem et al. 2021; Stanislawaka et al. 2020). One of the most well-described toxicological
149 mechanisms responsible for the lung adverse effects of combustion derived-PM is the pro-inflammatory response
150 driven by oxidative stress (Abbas et al. 2019; Badran et al. 2020a; Cazier et al. 2016; Garçon et al. 2001, 2006;
151 Leclercq et al. 2018; Saint-Georges et al. 2008; Sotty et al. 2020). Of course, ROS generation and pro-inflammatory
152 reaction have been reported among welders who are exposed to high levels of PM emitted within WF, but other
153 underlying mechanisms, not even fully elucidated, like critical epigenetic alterations, could probably interplay to

154 induce the adverse health effects. New toxicological research is therefore urgently requested to improve the current
155 knowledge about the specific role of the UFP fraction in the overall toxicity of WF.

156 Hence, in this work, we sought to better evaluate the toxicity of the quasi-UFP fraction (Q-UFP, i.e., $PM_{0.25}$) of
157 the WF emitted by GMAW-SS in human bronchial epithelial cells (BEAS-2B). Firstly, we aimed to physically and
158 chemically characterize this Q-UFP fraction. Secondly, attention was focused on the ability of this Q-UFP fraction to
159 induce cytotoxicity, pro-oxidative and/or pro-inflammatory responses, genetic and/or epigenetic alterations, and,
160 therefore, some related cell signaling pathway activation in BEAS-2B cells acutely or repeatedly exposed. These
161 relevant results will also contribute to new insights of the critical role played by the Q-UFP fraction in human
162 adverse effects induced by the exposure to GMAW-SS-derived WF.

163

164 **MATERIALS AND METHODS**

165

166 **Chemicals**

167 Merck-Millipore (St Quentin-en-Yvelines, France) provided BEAS-2B cells (ATCC® CRL-9609™),
168 cOmplete™, EDTA-free Protease Inhibitor Cocktail, Phosphatase Inhibitor Cocktail, RIPA buffer, CHAPS buffer,
169 MILLIPLEX® MAP human cytokine/chemokine magnetic bead panel-immunology multiplex assays, methyl
170 methanesulfonate (MMS), and all the other chemicals. ThermoFisher scientific (Villebon-sur-Yvette, France)
171 provided collagen type I from rat tail, chloromethyl derivative of 2',7'-dichlorodihydrofluorescein diacetate (CM-
172 H₂DCFDA), Pierce™ BCA protein assay kits, Single-channel dead cell apoptosis kits with Annexin V alexa fluor™
173 488, and SYTOX™ green dyes, and all the other reagents for chemistry, and cellular and molecular biology. Qiagen
174 (Courtaboeuf, France) provided RNeasy mini Kits. Promega (Charbonnière-les-Bains, France) provided CellTiter-
175 Glo® luminescent cell viability, GSH/GSSG-Glo™ assay, and Caspase Glo® 3/7, Caspase Glo® 8, and Caspase
176 Glo® 9 assays. Abcam (Cambridge, UK) provided 4-hydroxynonenal (4-HNE) modified bovine serum albumin,
177 rabbit polyclonal anti-4-HNE antibody, and Protein carbonyl content assay kit, and 8-hydroxy-2'-deoxyguanosine
178 (8OHdG) ELISA kit. Active Motif (La Hulpe, Belgium) provided Nuclear extract and TransAM® nuclear factor
179 erythroid 2-related factor 2 (NRF2) and nuclear factor-kappa B (NF-κB) kits, Global DNA Methylation LINE-1
180 Assay, Histone Purification Mini Kit, Histone total H3, H3K9ac, and H3K27ac ELISA kits, and histone acetyl
181 transferase (HAT) and histone deacetylase (HDAC) Assay kits. Bio-Techne (Lille, France) provided repair
182 endonuclease hOGG1.

183

184 **Collection of WF particles**

185 WF particles were produced according to GMAW-SS process using an automatic welding bench as described
186 elsewhere (Bonthoux et al. 2016). The bench is equipped with a Digiwave welding installation (SAF-FRO, Le Grand
187 Quevilly, France) using M21 as shielding gas (i.e., 82% Ar / 18% CO₂). Briefly, a stainless-steel plate (750 x 50 x 6
188 mm) moves under the torch feeded with a SS wire (Filinox 316LSi 1 mm in diameter, SAF-FRO) to produce a
189 regular weld bead. All the fumes are drawn into an extraction hood under a flow rate of 120 m³/h. A sampling probe
190 inside the duct was connected to a 4-way flow splitter (Palas GmbH, Karlsruhe, Germany) linked to the sampling
191 devices. The total sampling flow rate ranged between 36.8 and 37.8 L/min according to the sampling configuration.
192 For this study, several Sioutas cascade impactors (SKC Ltd, Blandford Forum, United-Kingdom) were used to
193 collect particles based on their aerodynamic diameter over 5 stages (< 0.25 μm, 0.25 - 0.50 μm, 0.50 - 1 μm, 1 - 2.5
194 μm, > 2.5 μm). A SG10-2 pump (GSA Germany GmbH, Frankfurt am Main, Germany) was used to maintain the
195 sampling flow rate of 9 L/min. Polycarbonate (PC) filters with 25-mm diameter and 0.8 μm pore size (Whatman,
196 Kent, United-Kingdom) were used as the impaction media. The finest particles (i.e. also called Q-UFP, aerodynamic
197 diameter ≤ 0.25 μm) were collected on a 37-mm diameter and 0.8 μm pore size PC filters (Whatman) placed onto a
198 37-mm cellulose pad to prevent any leakage. Generations of WF were repeated following the exact same procedure
199 until getting sufficient mass. For each generation, a way of the flow splitter was dedicated to real time monitoring of

200 number concentration and size distribution determined with an electrical low-pressure impactor ELPI⁺ (Dekati,
201 Kangasala, Finland).

202

203 **Determination of the mass of the collected WF particles**

204 The mass concentration of particles collected on the PC filters was measured gravimetrically using a XP2U
205 Ultra Micro balance (Mettler-Toledo, Viroflay, France) placed in a particle-controlled room. After storage in the
206 room atmosphere for at least 12 h, filters were weighed before and after aerosol sampling. Static charges were
207 discharged using an anti-static ionizing bar (Elcowa SA, Mulhouse, France). The mass of the collected particles was
208 calculated by difference and corrected with field blank filters to adjust for humidity and temperature-related
209 variations. Limits of quantitation (LOQ) were below 30 µg and 75 µg for 25 mm and 37 mm PC filters, respectively.

210

211 **Extraction of WF particles**

212 The samples of WF collected on weighed PC filters were water-extracted in a clean room with an ultrasound
213 system (Vibracell 75455, 500W, 20 KHz) equipped with cold circulating water. Three successive MilliQ water baths
214 were used and combined before being distributed among the different teams. The Q-UFP fraction accumulated from
215 WF emissions (100.9 mg in 50 mL of MilliQ water) was stored at -20°C until its further use for the physical and
216 chemical characterization and the toxicological study.

217

218 **Physical and chemical characterization of WF-derived Q-UFP**

219 The size distribution and the zeta potential of the Q-UFP fraction of the WF emitted by GMAW-SS process
220 were studied using a Zetasizer Nano ZSP™ (Malvern Instruments SARL, Orsay, France) after a sonication (45W, 40
221 kHz, 2 min) with a Branson Sonifier SFX 150 (ThermoFisher Scientific).

222 The metal composition of the Q-UFP was studied by inductively coupled plasma-mass spectrometry (ICP-MS,
223 Perkin Elmer NeXion 300X, Perkin Elmer, Villebon-sur-Yvette, France). The Q-UFP suspension was weighed and
224 quantitatively mineralized in an acidic medium (HNO₃/H₂O₂/HF) at 220°C in a microwave oven (Ultrawave,
225 ThermoFisher scientific). According to Mbengue et al. (2014) and Leclercq et al. (2017b), the analyses were carried
226 out in triplicates for three sub-samples of 100 µg of Q-UFP and for two different dilutions (i.e., 1/2 and 1/10).
227 Repeated measurements of blanks and quality control (QC) standards attached to the NIST were performed during
228 each analytical run. A mixed internal standard (⁶⁹Ga, ¹⁰³Rh) was added (1µg/L) to each analyzed solution to correct
229 for the drift of the ICP-MS signal. Moreover, in order to validate the extraction procedure, several samples (~ 1 mg)
230 of NIST SRM 1648a (i.e., Urban particulate matter, NIST, Gaithersburg, MA, USA) were systematically tested as
231 standard reference material. Thirty trace elements and 7 major elements were determined: Ag, As, Ba, Be, Bi, Cd,
232 Ce, Co, Cr, Cs, Cu, La, Li, Mn, Mo, Ni, Pb, Pd, Pt, Rb, Sb, Se, Sn, Sr, Th, Ti, Tl, U, V, Zn, Al, Ca, Fe, K, Mg, Na,
233 and Si.

234 During 7 experiments, WF were collected for hexavalent chromium (CrVI) determination in a Millipore®
235 cassette (opening diameter 4 mm) fitted with a 37 mm quartz fiber filter (QMA, Whatman) impregnated with
236 magnesium sulfate and sodium carbonate. The CrVI was determined by ion chromatography (Dionex, ThermoFisher

237 scientific) after extraction in a $\text{Na}_2\text{CO}_3/\text{NaOH}$ solution with the one extraction protocol Métropol M-43 (INERIS,
238 2020).

239

240 **Intrinsic oxidative potential of WF-derived Q-UFP**

241 The intrinsic oxidative potential (OP) of WF-derived Q-UFP was determined through CM- H_2DCFDA acellular
242 assay and glutathione oxidation (i.e., ratio between oxidized form and reduced form, GSSG/GSH), according to
243 Crobeddu et al. (2020). Briefly, for CM- H_2DCFDA acellular assay, ROS-produced by Q-UFP, ranging from 10 to
244 100 $\mu\text{g/mL}$, convert the non-fluorescent CM- H_2DCFDA probe (20 μM) into the fluorescent 2',7'-dichlorofluorescein
245 (DCF). Fluorescence generated by probe oxidation was kinetically monitored for 30 min at $\lambda_{\text{ex}} = 485$ nm and $\lambda_{\text{em}} =$
246 520 nm, at 37 °C, with Spark® 10M multimode plate reader (TECAN France SASU, Lyon, France). For glutathione
247 status, Q-UFP from 10 to 100 $\mu\text{g/mL}$ were incubated for 4 h at 37 °C in a solution containing physiological
248 concentrations (200 μM) of GSH in PBS at pH 7.4 with constant mixing. After incubation, both the GSH and GSSG
249 concentrations, and also the glutathione status, were evaluated thanks to the GSH/GSSG-Glo™ assay (Promega).
250 Hydrogen peroxide (H_2O_2) at 100 μM was used as positive control.

251

252 **Cell culture and exposure to WF-derived Q-UFP**

253 Normal human bronchial epithelial BEAS-2B cells (ATCC® CRL-9609™), which derived from normal human
254 bronchial epithelial cells obtained from autopsy of non-cancerous individual, were cultured as published elsewhere
255 (Badran et al. 2020b, Leclercq et al. 2017, Sotty et al. 2020). Briefly, depending on the toxicological endpoints under
256 study, BEAS-2B cells were cultured in 95 % humidified air with 5 % CO_2 at 37°C in 75 cm^2 CellBIND® surface
257 plastic flasks (Corning; Sigma-Aldrich), in LHC-9 serum-free medium supplemented with 1% (v/v) penicillin
258 (10,000 U/mL)-streptomycin (10,000 $\mu\text{g/mL}$) solution (ThermoFisher Scientific) once cells had reached 80 % of
259 confluence. Thereafter, BEAS-2B cells were seeded for 24 h at 25,000 cells/well on 96-well CellBIND® surface
260 plastic microplates coated with collagen type I from rat tail (0.03 mg/mL) or at 200,000 cells/well on 6-well
261 CellBIND® surface plastic microplates coated with collagen type I from rat tail (0.03 mg/mL), in LHC-9 serum-free
262 medium supplemented with 1% (v/v) penicillin (10,000 U/mL)-streptomycin (10,000 $\mu\text{g/mL}$) solution, and 2% (v/v)
263 amphotericin B solution (20 $\mu\text{g/mL}$) (ThermoFisher Scientific). Immediately prior the exposure, WF-derived Q-UFP
264 solution (i.e., 1.91 $\mu\text{g}/\mu\text{l}$) was diluted in sterile HBSS supplemented with amphotericin B solution (20 $\mu\text{g/mL}$) at 2 %
265 (v/v) in order to obtained final concentrations ranging from 0.33 $\text{ng}/\mu\text{L}$ (i.e., 0.1 $\mu\text{g}/\text{cm}^2$) to 165 $\text{ng}/\mu\text{L}$ (i.e., 50
266 $\mu\text{g}/\text{cm}^2$), and sonicated for 2 x 1 min. Thereafter, for cytotoxicity, BEAS-2B cells were exposed one or three times,
267 for 24 h, as negative controls (i.e., sterile HBSS supplemented with 2 % v/v amphotericin B) or Q-UFP-exposed cells
268 (i.e., concentrations ranging from 0.1 to 50 μg Q-UFP/ cm^2 , suspended in sterile HBSS supplemented with 2 % v/v
269 amphotericin B). For the Comet assay, cells were seeded at a density of 150,000 to 400,000 cells/well in LHC-9 in 6-
270 well CellBIND® surface plastic microplates coated with collagen type I from rat tail (0.03 mg/mL) in LHC-9 serum-
271 free medium supplemented with 1 % (v/v) penicillin (10,000 U/mL)-streptomycin (10,000 $\mu\text{g/mL}$) solution and 2%
272 (v/v) amphotericin B solution (20 $\mu\text{g/mL}$), prior to BEAS-2B cell exposure, acutely (i.e., 1 time for 4 h or 24 h) or
273 repeatedly (i.e., 3 times for 24 h) to WF-derived Q-UFP. For all the other toxicological endpoints, BEAS-2B cells

274 were exposed one or three times, for 24 h, as negative controls (i.e., sterile HBSS supplemented with 2 % v/v
275 amphotericin B solution) or Q-UFP-exposed cells (i.e., at two concentrations: calculated lethal dose at 10%, LD₁₀,
276 and 6 x DL₁₀, suspended in sterile HBSS supplemented with 2 % v/v amphotericin B solution). Hydrogen peroxide
277 (H₂O₂, 100µM), tert-Butyl hydroperoxide (t-BHP, 10 µM), lipopolysaccharide (LPS) from E. coli (50 µg/mL),
278 methyl methanesulfonate (MMS, 10 or 15 µg/mL), or staurosporin (1 µM) served as positive controls. Twenty-four
279 hours later, 1 mL-aliquots of cell-free culture media were sampled and quickly frozen at -80°C. Adherent cells were
280 washed once with 1mL-aliquots of cold sterile PBS, and either quickly fixed or quickly frozen at -80°C. For mRNA
281 and miRNA analyses, adherent cells were washed once with 1mL-aliquots of cold sterile PBS and thereafter lysed
282 with 700 µL of QIAzol lysis buffer and quickly frozen at -80°C.

283

284 **WF-derived Q-UFP-induced cytotoxicity**

285 Intracellular ATP concentrations of BEAS-2B cells were determined using the CellTiter-Glo® luminescent cell
286 viability (Promega).

287

288 **WF-derived Q-UFP-induced other toxicological endpoints**

289 The further study of the toxicological endpoints needed the preparation of different cell lysates: (i) nuclear
290 factor erythroid 2-related factor 2 (NRF2) DNA binding activity binding activity, nuclear factor-kappa B (NFκB)
291 DNA binding activity, histone H3 post-translational modifications (PTM), and caspase activities were studied after
292 cell lysis with RIPA buffer supplemented with cOmplete™EDTA-free Protease Inhibitor Cocktail and Phosphatase
293 Inhibitor Cocktail (Merck-Millipore), (ii) 8-OHdG and global DNA methylation were studied after DNA extraction
294 with the QIAamp DNA Mini Kit (Qiagen), (iii) histone acetyl transferase (HAT) and histone deacetylase (HDAC)
295 activities were studied after cell lysis with CHAPS buffer (Merck-Millipore), and (iv) mRNA and miRNA
296 expression profiles were studied after total RNA extraction with the miRNeasy Mini Kit (Qiagen).

297 ROS production and oxidative damage: Firstly, the fluorescence of CM-H₂DCFDA (1 µM) was kinetically
298 monitored at 37°C within BEAS-2B cells (Sotty et al. 2020). Secondly, the NRF2 DNA binding activity was studied
299 using TransAM® NRF2 from Active Motif. Associated gene expressions of some members of the NRF2 signaling
300 pathway (i.e., *heme oxygenase 1*, *HMOX*, and *NAD(P)H quinone dehydrogenase 1*, *NQO1*) were evaluated by RT-
301 qPCR using specific Taqman™ gene expression assays (*HMOX*: Hs01110250_m1, *NQO1*: Hs01045993_g1, and
302 *RNAI8S*: Hs99999901_s1), a StepOnePlus™ Real-Time PCR System, and the Expression Suite Software
303 (ThermoFisher scientific). Thirdly, the concentrations of 4-hydroxynonenal (4-HNE), carbonylated protein (CO-
304 protein), 8-hydroxy-2'-deoxyguanosine (8-OHdG), and glutathione were studied respectively thanks to a home-made
305 competitive ELISA, based on MSD technologies (MesoScale Discovery, Rockville, MD, USA), using 4-HNE-
306 modified BSA and rabbit polyclonal anti-4-HNE antibody (Abcam), Protein carbonyl content assay kits (Abcam), 8-
307 hydroxy-2'-deoxyguanosine ELISA kit (Cell Biolabs, and GSH/GSSG-Glo™ assay (Promega), as published
308 elsewhere (Anthérieu et al. 2017; Garçon et al. 2000; Leclercq et al. 2016). Hydrogen peroxide (i.e., H₂O₂, 100 µM,
309 4 h)-treated BEAS-2B cells served as positive controls.

310 Cytokine and chemokine secretion: The NF- κ B DNA binding activity was studied using TransAM® NF- κ B
311 from Active Motif. The secretion of C-C motif chemokine ligand 5 (CCL5) or regulated upon activation, normal T
312 cell expressed and presumably secreted (RANTES), granulocyte macrophage colony stimulating factor (GM-CSF),
313 growth-regulated oncogene- α (GRO- α), interleukin-1 beta (IL-1 β), interleukin-6 (IL-6), interleukin-8 (IL-8),
314 monocyte chemoattractant protein 1 (MCP-1), and tumor necrosis factor (TNF- α), in the cell-free culture
315 supernatants of BEAS-2B cells was detected by MILLIPLEX® MAP Human Cytokine/Chemokine Magnetic Bead
316 Panel-Immunology Multiplex Assay (Merck-Millipore) (Devos et al. 2019). Lipopolysaccharide (LPS from *E. coli*,
317 50 μ g/mL, 24 h)-treated BEAS-2B cells served as a positive control.

318 Genetic alterations: The comet assay was performed under alkaline conditions (pH > 13) in compliance with a
319 previously described protocol (Singh et al., 1988; Tice et al., 2000; Witte et al., 2007). Slight modifications using the
320 repair endonuclease hOGG1 (R&D systems, Minneapolis, USA) were carried out to detect specifically oxidative
321 DNA damage, based on Collins' and Smith's procedures (Collins et al. 1993; Smith et al., 2006). For each group, 4
322 duplicate slides per culture were prepared (i.e., 2 slides for the standard comet assay and 2 slides for the hOGG1-
323 modified comet assay). When scoring for DNA migration, slides were independently coded and analyzed after the
324 addition of 25 μ L of propidium iodide at 20 μ g/mL and a coverslip. Slides were then examined at 250 X
325 magnification using a fluorescence microscope (Leica Microsystems SAS - DM 2000, Heerbrugg, Switzerland)
326 equipped with an excitation filter of 515-560 nm and a 590 nm barrier filter, connected through a gated monochrome
327 CCD IEEE1394 FireWire video camera (Allied Vision Technologies) to the Comet Assay IV Image Analysis
328 System, version 4.11 (Perceptive Instruments Ltd, Suffolk, United Kingdom). One hundred randomly selected cells
329 per slide were scored. DNA damage was expressed as percentage of DNA in the tail (% tail intensity) (Burlinson et
330 al., 2007; Lovell and Omori, 2008). Positive control was MMS at 10 or 15 μ g/mL (MMS, Merck-Millipore) and
331 negative control was culture medium. For the comet assay, cytotoxicity was assessed at harvest using the Trypan
332 Blue dye exclusion assay. To exclude cytotoxicity as a confounding factor, concentrations which led to more than
333 70% of cell viability were then submitted to a genotoxicity assessment (Tice et al. 2000).

334 Cell signaling pathways: Total RNA has been isolated using the miRNeasy Mini Kit (Qiagen). After the reverse
335 transcription of 200 ng of total RNA in single-stranded cDNA using the Superscript™ IV VILO™ Mastermix Kit,
336 the expression profile of 672 target mRNA was carried out using TaqMan® OpenArray® Human Signal
337 Transduction Panel and TaqMan® OpenArray® Real-time MasterMix with a QuantStudio™ 12K Flex Real-Time
338 PCR System equipped with the AccuFill™ robot, and the Relative Quantification application from the ThermoFisher
339 Scientific's cloud (i.e., Fold-Change: FC > 1.5 or < 0.66, p value < 0.05) according to the manufacturer's
340 recommendations (ThermoFisher Scientific). Functional analysis of the differentially regulated gene (DRG) was
341 carried out in silico using Cytoscape v 3.8.2. software (<https://cytoscape.org/>).

342 Epigenetic alterations: The Global DNA Methylation LINE-1 Assay provided a measure of global DNA
343 methylation shifts in BEAS-2B cells (Active Motif). Active motif's Histone Purification Mini Kit was used to purify
344 core histones from BEAS-2B cells and ELISA kits were used to determine specific H3 post-translational
345 modifications (i.e., H3K9ac and H3K27ac versus total H3). HAT and HDAC activities of BEAS-2B cells were
346 determined using HAT and HDAC assays from Active motif, according to the manufacturer's recommendations

347 (Leclercq et al. 2017, Sotty et al. 2019). Moreover, miRNA expression profiles were studied in BEAS-2B cells.
348 Briefly, total RNAs were isolated using the miRNeasy Mini Kit (Qiagen). After the reverse transcription of 100 ng of
349 total RNA in single-stranded cDNA using the TaqMan® MicroRNA Reverse Transcription Kit and the pre-
350 amplification of these cDNA using the TaqMan® PreAmp MasterMix), the expression profile of 754 target miRNA
351 was carried out using Taqman® OpenArray® Human miRNA Panel and TaqMan® OpenArray® Real-time
352 MasterMix with a QuantStudio™ 12K Flex Real-Time PCR System equipped with the AccuFill™ robot, and the
353 Relative Quantification application from the ThermoFisher Scientific's cloud (i.e., Fold-Change: FC > 1.5 or < 0.66,
354 p value < 0.05), according to the manufacturer's recommendations (ThermoFisher Scientific). Functional analysis of
355 the differentially regulated miRNA (DRmiR) was carried out in silico using TargetScanHuman v7.2
356 (http://www.targetscan.org/vert_72/), miRNA target Prediction Database (miRDB; <http://mirdb.org>), and et DIANA
357 TOOLS Tarbase V8 (http://carolina.imis.athena-innovation.gr/diana_tools/web/index.php?r=tarbasev8%2Findex).

358 Apoptotic events: Activities of both some initiator caspases (caspase 8 and caspase 9) and executioner caspases
359 (i.e., caspase 3 and caspase 7) were determined with Caspase Glo® 3/7, Caspase Glo® 8, and Caspase Glo® 9
360 Assays, as recommended by the manufacturer (Promega). Single-channel dead cell apoptosis kit with Annexin V
361 Pacific Blue™ 488 and SYTOX™ AAdvanced dyes for flow cytometry applications were used as recommended by
362 the manufacturer (ThermoFisher scientific), before BEAS-2B cell analysis using an Attune™ NxT Acoustic
363 Focusing Cytometer (ThermoFisher scientific) to study apoptotic events. Staurosporine (2.5 µM, 24 h)-treated
364 BEAS-2B cells served as a positive control.

365

366 **Statistical analysis**

367 Results were expressed as mean values and standard deviations. Comparisons were performed between data
368 from exposed BEAS-2B cells (i.e., WF-derived Q-UFP) and those from negative controls. After having checked for
369 the Normal distribution of the variables under study, the Student's t-test ($p < 0.05$) was used to perform statistical
370 analyses (Software: SPSS v27 for windows). For the in vitro comet assay, it has previously been shown that the tail
371 intensity does not follow a Gaussian distribution. Thus, the non-parametric Mann-Whitney U-test was used to
372 evaluate the statistical difference between groups (i.e. between each concentration vs. the respective negative
373 control). All statistical analyses were performed with StatView Software (version 5.0, SAS Institute Inc., SAS
374 Campus Drive, Cary, North Carolina 27513, USA). Differences with a $p < 0.05$ were considered statistically
375 significant.

376

377 **RESULTS**

378

379 **Physical and chemical characteristics of WF-derived Q-UFP**

380 The campaign to produce WF according to GMAW-SS process using an automatic welding bench was realized
381 between the 10th^h and the 13th of September 2018, with the aim of collecting the Q-UFP (PM_{0.25}). The average mass
382 concentration of the total particles was 40.1 ± 3.9 mg/m³. Fifty-seven welding cords were used to collect 133 mg of
383 Q-UFP. Concentrations in numbers ranged from 6.6 to 11.7×10^6 /cm³ on the entire collection. The Q-UFP represented
384 about 87 % of the total amount of the particles from each generation. The size distribution in number of the WF-
385 derived Q-UFP showed a monomodal distribution centered on a aerodynamic diameter of 104.4 ± 52.3 nm as
386 measured by ELPI+. Analysis of the zeta potential of Q-UFP suspended in MilliQ water (i.e., 1.91 µg/µL) yielded to
387 an average result of -13.8 ± 0.3 mV. As shown in Table 1, total element concentration and total oxide concentration
388 of Q-UFP were 646.9 µg/mg and 944 µg/mg, respectively. Moreover, Q-UFP had high enrichment degrees of certain
389 elements (i.e., Fe > Cr > Mn > Si > Ni) and accordingly certain oxides (i.e., Fe₂O₃ > CrO₃ > MnO > SiO₂ > NiO).
390 Regarding CrVI, the concentration within WF was 160 ± 10 µg/m³.

391

392 **Intrinsic oxidative potential of WF-derived Q-PUF**

393 The intrinsic OP of Q-UFP was firstly determined through the CM-H₂DCFDA acellular assay. After the
394 incubation of the CM-H₂DCFDA probe in the continuous presence of Q-UFP at 10, 50 and 100 µg/mL, the relative
395 fluorescence intensities were respectively 7.57, 21.14, and 57.97-fold increased, versus negative control ($p < 0.001$).
396 The relative fluorescence intensity of positive control (i.e., H₂O₂, 100µM) was 27.74-fold increased, versus negative
397 control ($p < 0.001$). Secondly, the intrinsic OP of Q-UFP was determined through glutathione oxidation. There were
398 respectively 14.86, 58.36, and 122.43-fold increases of the GSSG/GSH ratio in presence of Q-UFP at 10, 50 and 100
399 µg/mL, versus negative control. The positive control lead to a 50.70-fold increase of the ratio.

400

401 **WF-derived Q-UFP-induced cytotoxicity**

402 The Figures 1-A and 1-B respectively show the intracellular ATP concentrations of BEAS-2B cells after acute
403 or repeated exposures to Q-UFP at concentrations ranging from 0.1 to 50 µg/cm². There were statistically significant
404 dose-dependent decreases of the ATP contents within BEAS-2B cells acutely exposed to Q-UFP at concentrations \geq
405 1 µg/cm², or repeatedly exposed to Q-UFP at concentrations ≥ 0.25 µg/cm², versus negative control ($p < 0.05$).
406 Taken together, these results suggested to use the lowly- (i.e., lethal concentration at 10 %, LC₁₀; red dotted lines)
407 and highly (i.e., lethal concentration at 40 %, LC₄₀; black dotted lines)-cytotoxic doses of 1.5 and 9 µg/cm² for the
408 acute scenario of exposure, and the use of the lowly- (i.e., LC₁₀; red dotted lines) and highly (i.e., LC₄₀; black dotted
409 lines)-cytotoxic doses of 0.25 and 1.5 µg/cm² for the repeated scenario of exposure, in order to further investigate the
410 other toxicological endpoints under study.

411

412 **WF-derived Q-UFP-induced oxidative stress**

413 The Figure 2-A indicates that there was a significant dose-dependent-production of ROS by BEAS-2B cells
414 acutely exposed to Q-UFP at 1.5 or 9 µg/cm², and, to a lesser extent, repeatedly exposed to Q-UFP at 0.25 and 1.5

415 $\mu\text{g}/\text{cm}^2$, versus negative control ($p < 0.001$). Moreover, as shown in the Figures 2-B, 2-C, and 2-D, NRF2 signaling
416 pathway was significantly activated in a dose-dependent manner in Q-UFP-exposed BEAS-2B cells, versus negative
417 control. There were also statistically significant increases of NRF2 binding activity in BEAS-2B cells acutely
418 exposed to Q-UFP, only at the highest dose, or repeatedly exposed to Q-UFP, at the two doses ($p < 0.01$). Moreover,
419 the expression of the NRF2-downstream target genes, such as *heme oxygenase-1 (HMOX)* and *NADPH quinone*
420 *oxydoreductase 1 (NQO-1)*, was further increased in BEAS-2B cells acutely exposed to Q-UFP at 1.5 or 9 $\mu\text{g}/\text{cm}^2$,
421 and, to a lesser degree, repeatedly exposed to Q-UFP at 0.25 and 1.5 $\mu\text{g}/\text{cm}^2$ ($p < 0.05$). Remarkably, for any
422 exposure, despite the early activation of NRF2 signaling pathway, oxidative alterations of glutathione status (i.e.,
423 oxidation of GSH into GSSG), DNA (i.e., 8-OHdG), proteins (i.e., carbonylated proteins), and lipids (i.e., 4-HNE)
424 significantly occurred in a dose-dependent manner, versus negative control ($p < 0.05$; Figures 3-A, 3-B, 3-C, and 3-
425 D). However, it is noteworthy that Q-UFP-induced oxidative damage to all these critical macromolecules, except for
426 lipids (i.e., 4-HNE) was generally highest after the acute exposure scenario than after the repeated exposure scenario.

427

428 **WF-derived Q-UFP-induced cytokine secretion**

429 As shown in Figure 4 A, there were statistically significant increases of the NF- κ B binding activity in BEAS-
430 2B cells only after their acute or repeated exposures to the highest dose of Q-UFP, versus negative control ($p < 0.01$).
431 Figures 4-B, 4-D, and 4-F showed the only very slight induction of the gene expression of *TNF- α* in BEAS-2B cells
432 acutely exposed to the highest dose of Q-UFP, and *IL-1 β* in BEAS-2B acutely exposed to the two doses of Q-UFP
433 and repeatedly exposed to the highest one ($p < 0.05$). For any exposure, no significant difference of the secretion by
434 BEAS-2B cells of all the inflammatory mediators under study (i.e., RANTES, GM-CSF, GRO- α , IL-1 β , IL-6, IL-8,
435 MCP-1, and TNF- α) was reported versus negative control. Figures 4-C, 4-E, and 4-G give some examples for TNF-
436 α , IL-1 β , and IL-6.

437

438 **WF-derived Q-UFP-induced genetic alterations**

439 The *in vitro* comet assay under alkaline conditions was applied to assess primary DNA damage. The cell
440 viability should be more than 70 % to exclude cytotoxicity as a confounding factor. Results of the mean of medians
441 of % DNA in the comet tail and the corresponding relative cell viability obtained in BEAS-2B cells are shown in
442 Figure 5-A, 5-B, and 5-C. For both the standard (i.e., dark grey histogram bars) and the hOGG1-modified (i.e., slight
443 grey histogram bars) comet assays, no increase in DNA damage was observed in acutely exposed BEAS-2B cells
444 (i.e., 4 h or 24 h) or repeatedly exposed BEAS-2B cells (i.e., 3 x 24h) to Q-PUF at doses ranging from 0.25 until 9
445 $\mu\text{g}/\text{cm}^2$, versus negative control.

446

447 **WF-derived-Q-UFP-induced cell signaling pathways**

448 To better decipher the underlying mechanisms triggered by Q-UFP in BEAS-2B cells, DRG known as closely
449 implicated to some cell signaling pathways were investigated. Figures 6-A and 6-B show the down and up- regulated
450 genes, respectively. Indeed, 6 DRG (i.e., 6 down-regulated) were reported in BEAS-2B cells acutely exposed to the
451 lowest dose of Q-UFP, 24 DRG (i.e., 19 down- and 5 up-regulated) in BEAS-2B cells acutely exposed to the highest

452 dose of Q-UFP, and 39 DRG (i.e., 30 down- and 9 up-regulated) in BEAS-2B cells repeatedly exposed to the highest
453 dose of Q-UFP, versus negative controls. As shown in Figures 6-A and 6-B, only 2 DRG, also down-regulated, (i.e.,
454 *Cyclin-dependent kinase 4: CDK4*, and *Serine/threonine-protein kinase MTOR: MTOR*) were reported as common to
455 all the exposure conditions of BEAS-2B cells to Q-UFP. Moreover, *Heat Shock Protein Family D (Hsp60) Member
456 1(HSPD1)* and *Ras Homolog, MTORC1 Binding (REHB)* were the two down-regulated genes common to BEAS-2B
457 cells acutely exposed to the lowest or the highest doses of Q-UFP. Only *REHB* was down-regulated at the same time
458 in BEAS-2B cells acutely exposed to the lowest dose of Q-UFP and in BEAS-2B cells repeatedly exposed to the
459 highest dose of Q-UFP. At least, 6 DRG, 5 down-regulated (i.e., *Janus Kinase 2: JAK2*, *Hydroxymethylbilane
460 Synthase: HMBS*, *Lymphoid Enhancer Binding Factor: 1LEF1*, *BCL2 Apoptosis Regulator: BCL2*, *Proenkephalin:
461 PENK*) and 1 up-regulated (*Pyruvate Dehydrogenase Kinase 1: PDK1*) were common to BEAS-2B cells acutely and
462 repeatedly exposed to the highest dose of Q-UFP.
463

464 **WF-derived Q-UFP-induced epigenetic alterations**

465 Firstly, the Figures 7-A, 7-B, and 7-C show both the HDAC and HAT activities, and their ratio, in BEAS-2B
466 cells, acutely and repeatedly exposed to Q-UFP. In BEAS-2B cells acutely exposed to the highest dose of Q-UFP or
467 repeatedly exposed to the two doses of Q-UFP, there were statistically significant increases of the HDAC activity,
468 versus negative control ($p < 0.01$). Only the repeated scenario of exposure to Q-UFP lead to significant increases of
469 the HAT activity in BEAS-2B cells ($p < 0.001$). Overall, the figure 7-C revealed significant increases of the HDAC
470 activity/HAT activity ratio in BEAS-2B cells repeatedly exposed to the lowest dose of Q-UFP, and acutely or
471 repeatedly exposed to the highest dose of Q-UFP ($p < 0.05$). Moreover, Figures 7-D and 7-E indicated significant
472 decreases of the acetylation of the H3 histone at Lys 9 and Lys 27 in BEAS-2 B cells acutely exposed to the two
473 doses of Q-UFP, but only at Lys 27 in BEAS-2 B cells repeatedly exposed to the two doses of Q-UFP, versus
474 negative control ($p < 0.05$). In addition, no significant modification of the global DNA methylation was observed in
475 BEAS-2B cells for any exposure.

476 Secondly, to go further in deciphering the underlying mechanisms induced by Q-UFP in BEAS-2B cells,
477 DRmiR were investigated (Figures 8-A and 8-B). Accordingly, 4 DRmiR (i.e., 2 down- and 2-up-regulated) were
478 reported in BEAS-2B cells acutely exposed to the lowest dose of Q-UFP, 7 DRmiR (i.e., 1 down-regulated and 6 up-
479 regulated) in BEAS-2B cells acutely exposed to the highest dose of Q-UFP, 4 DRmiR (i.e., 1 down- and 3 up-
480 regulated) in BEAS-2B cells repeatedly exposed to the lowest dose of Q-UFP, and 5 DRmiR (i.e., 3 down- and 2 up-
481 regulated) in BEAS-2B cells repeatedly exposed to the highest dose of Q-UFP, versus negative controls. As shown in
482 Figures 8-A and 8-B, only 2 DRmiR, also 1 down- and 1-up-regulated (i.e., hsa-miR-551b# and hsa-miR-331-5p,
483 respectively) were reported as common to BEAS-2B cells acutely exposed to the lowest or the highest doses of Q-
484 UFP. Only hsa-miR-210-3p was up-regulated at the same time in BEAS-2B cells acutely exposed to the highest dose
485 of Q-UFP and in BEAS-2B cells repeatedly exposed to the lowest dose of Q-UFP.

486 Thirdly, functional analysis of the up-regulated DRmiR founded in BEAS-2B acutely or repeatedly exposed to
487 Q-PUF was carried out in silico using three complementary miRNA target Prediction Database (i.e.,
488 TargetScanHuman v7.2, miRDB, and DIANA TOOLS Tarbase V8), and thereafter compared with the DRG
489 previously identified. Figures 9-A to 9-J showed the results as Venn Diagrams of hsa-miR-331-5p, hsa-miR-210-3p,

490 hsa-miR-424-5p, hsa-miR-597-5p, hsa-miR-212-3p, hsa-miR-1260a, hsa-miR-1248, hsa-miR-625-5p, hsa-miR-744-
491 3p, and hsa-miR-766-3p, respectively. Indeed, 19 target genes, only predicted by TargetScanHuman v7.2, were
492 founded within the DRG reported in BEAS-2B cells acutely and/or repeatedly exposed to Q-UFP: *Apoptosis*
493 *Regulator: BCL2, Janus kinase 2: JAK2, SMAD Family Member 4: SMAD4, Cyclin-dependent kinase 4: CDK4,*
494 *Inhibitor Of Nuclear Factor Kappa B Kinase Regulatory Subunit Gamma: IKBKG, RAS P21 Protein Activator 1:*
495 *RASAI, caspase 1: CASP1, MYD88 Innate Immune Signal Transduction Adaptor:MYD88, Mitogen-Activated*
496 *Protein Kinase Kinase Kinase 7: MAP3K7, Transcription Factor 7: TCF7, Cyclin B1: CCNB1, Signal Transducer*
497 *And Activator Of Transcription 5A: STAT5A, Myocyte Enhancer Factor 2C: MEF2C, Lysophosphatidic Acid*
498 *Receptor 2: LPAR2, Toll Like Receptor 4T: LR4, Toll Like Receptor 6T LR6, NKD Inhibitor Of WNT Signaling*
499 *Pathway 1: NKD1, Solute Carrier Family 44 Member 2: SLC44A2, and Calcitonin Receptor Like Receptor:*
500 *CALCRL. Galanin Receptor 2 (GALR2) was the target gene only predicted by miRDB, also reported within the DRG*
501 *reported in BEAS-2B cells acutely and/or repeatedly exposed to Q-UFP. Moreover, 10 target genes, only predicted*
502 *by DIANA TOOLS Tarbase V8, were among the DRG of BEAS-2B cells acutely and/or repeatedly exposed to Q-*
503 *UFP: SMAD Family Member 4: SMAD4, Peroxisome Proliferator Activated Receptor Gamma: PPARG, NKD*
504 *Inhibitor Of WNT Signaling Pathway 1: NKD1, Apoptosis Regulator: BCL2, Cyclin-dependent kinase 4: CDK4, Toll*
505 *Like Receptor 6T LR6, Lymphoid Enhancer Binding Factor 1: LEF1, Lysophosphatidic Acid Receptor 2: LPAR2,*
506 *Adenosine A1 Receptor: ADORA1, and Calcitonin Receptor Like Receptor: CALCRL. Some target genes also*
507 *founded among the DRG of BEAS-2B cells acutely and/or repeatedly exposed to Q-UFP were predicted by two of*
508 *the three miRNA target Prediction Databases: Transcription Factor 7: TCF7, Proenkephalin: PENK, Apoptosis*
509 *Regulator: BCL2, Lymphoid Enhancer Binding Factor 1: LEF1, Wnt Family Member 5B: WNT5B by*
510 *TargetScanHuman v7.2 and miRDB, on the one hand, and Inhibitor Of Nuclear Factor Kappa B Kinase Regulatory*
511 *Subunit Gamma: IKBKG and Kinase Suppressor Of Ras: KSR1 by TargetScanHuman v7.2 and DIANA TOOLS*
512 *Tarbase V8, on the other hand. At least, only three target genes also founded within the DRG of BEAS-2B cells*
513 *acutely and/or repeatedly exposed to Q-UFP were predicted by all the three miRNA target Prediction Databases:*
514 *Neural Cell Adhesion Molecule 1: NCAM1, RAS P21 Protein Activator 1: RASAI, Adenosine A1 Receptor:*
515 *ADORA1.*

516

517 **WF-derived Q-UFP-induced apoptotic events**

518 Figure 10-A revealed significant increases of the Annexin V labelling for any exposure scenario of BEAS-2B
519 cells to Q-PUF ($p \leq 0.05$). Moreover, acute and repeated exposure of BEAS-2B cells to the highest doses of Q-UFP
520 significantly activate initiator caspase-8, initiator caspase-9, and effector caspase-3/7, versus negative controls ($p \leq$
521 0.01 , Figures 10-B, 10-C, and 10-D).

522

523 **DISCUSSION**

524
525 Although the current literature supported that the combustion-derived PM emitted within WF will represent the
526 driving force of this occupational hazard, researchers are still far from having a fully detailed mechanistic
527 explanation for its respiratory toxicity. Using a human bronchial epithelial cell model, we also try to better decipher
528 the cellular and molecular underlying mechanisms of toxicity triggered by the WF-derived Q-UFP fraction.

529 Firstly, WF were produced according to GMAW-SS process with an automatic welding bench and Q-UFP were
530 collected based on their aerodynamic diameter. The nanometric monomodal size distribution by number of these
531 WF-derived Q-UFP was centered on 104.4 ± 52.3 nm, in keeping with the size characteristics published by
532 Kirichenko et al. (2018). Antonini et al. (2017), studying the size distribution of all the particles within WF, indicated
533 a multi-modal distribution because of the agglomeration, chain-like structures, of the majority of the nanometer-size
534 primary particles collected in the submicron size range, and only a very small amount ($< 5\%$) of non-agglomerated
535 UFP. The metal composition of the WF-derived Q-UFP under study revealed high concentrations of certain elements
536 (i.e., $Fe > Cr > Mn > Si > Ni$) and, accordingly, oxides (i.e., $Fe_2O_3 > Cr_2O_3 > MnO > SiO_2 > NiO$), as published by
537 Antonini et al. (2017), Badding et al. (2014), Falcone et al. (2018a,2018b), Leonard et al. (2010), and McCarrick et
538 al. (2019). According to McCarrick et al. (2019), because of the use of SS, high concentrations of CrVI (i.e., $160 \pm$
539 $10 \mu\text{g}/\text{m}^3$) were found, representing 3% of the total mass of Cr. Taken together, the physical and chemical
540 characteristics of the WF-derived Q-UFP emitted by GMAW-SS were close to those generally encountered in
541 occupational settings, proving once again that welding operations are procedures with high levels of Q-UFP hazard
542 for human health.

543 Thereafter, in order to contribute to the better knowledge of the cellular and molecular underlying mechanisms
544 by which WF-derived Q-UFP exerts their toxicity, BEAS-2B cells were acutely or repeatedly exposed to such Q-
545 UFP at relatively low doses (i.e., 1.5 and 9 $\mu\text{g}/\text{cm}^2$, and 0.25 and 1.5 $\mu\text{g}/\text{cm}^2$, respectively). The doses we applied
546 were among the lowest reported to give harmful effects whilst limiting a massive cell death (Boublil et al. 2013;
547 Gualtieri et al. 2018; Leclercq et al. 2016, 2017, 2018; Longhin et al. 2019; Platel et al; 2019; Sotty et al. 2019,
548 2020). Indeed, ATP concentrations within BEAS-2B cells acutely or repeatedly exposed to Q-UFP supported both
549 the use of a low (i.e., DL_{10}) and a moderate (i.e., DL_{40}) doses, thereby allowing to keep sufficient effective doses to
550 study these underlying mechanisms while contributing to the effort to be as closer as possible to the human exposure
551 levels at the workplaces.

552 Oxidative stress is one of the underlying mechanisms generally involved in air pollutant-induced adverse health
553 effects (Abbas et al. 2010, 2013, 2016, 2019; Badran et al. 2020; Bocchi et al. 2016; Garçon et al. 2006; Gualtieri et
554 al. 2010, 2011; Leclercq et al. 2016, 2017a, 2018; Longhin et al. 2013, 2016, Sotty et al. 2019, 2020). Firstly, the
555 intrinsic OP of Q-UFP was reported through the dose-dependent oxidation of both CM-H₂DCFDA and glutathione in
556 acellular assays. Of course, this result must be related to the metal (e.g., $Fe > Cr > Mn > Si > Ni$) and oxide (e.g.,
557 $Fe_2O_3 > Cr_2O_3 > MnO > SiO_2 > NiO$) composition of the Q-UFP under study. Badding et al. (2014) indicated that Fe
558 makes up the majority of GMAW-SS metals, and that transition metals such as Fe and Cr, in addition to Mn, Ni, and
559 Cu (i.e., 259.8, 129.4, 127.6, 43.2, and 4 $\text{ng}/\mu\text{g}$, respectively, in the Q-UFP we studied) participate in Fenton
560 reduction/oxidation cycling and are known to mediate ROS production. Ghanem et al. (2021) recently reported that

561 transition metals in the both the SS-derived WF and MS-derived WF, mainly composed of Fe, Mn, Zn, Cu, Cr, and
562 Ni in various proportions, have shown different solubility and also different contributions to their intrinsic OP, Mn
563 (II), Cu (II), and Ni (II) being the most active. Garçon et al. (2001) showed the important role of iron oxides (i.e.,
564 Fe₂O₃ and Fe₃O₄) in the over-production of ROS in several cell and animal models. Leonard et al. (2010)
565 demonstrated that WF were able to induce ROS over-production over a range of particle sizes, the smaller particle
566 size (i.e., 0.032-0.180 µm) showing the most reactivity and the greater ROS potential, and that WF-derived PM
567 emitted by GMAW-SS showed a higher reactivity and ROS capacity. Taken together, all these results supported the
568 crucial role played by the chemical composition of SS on the ROS generation capacity thanks to the presence of Cr
569 and Ni, and other transition metals, such as Fe, Mn, and Cu. Secondly, after having shown the intrinsic OP of Q-
570 UFP, we sought to evaluate their ability to effectively produce massive oxidative damage in a relevant cell model.
571 Accordingly, we demonstrated that Q-UFP highly oxidized all the cellular macromolecules in a dose-dependent
572 manner in BEAS-2B cells acutely or repeatedly exposed. As a consequence, there was a dose-dependent activation
573 of the specific antioxidant NRF2 signaling pathway, as assessed by the NRF2 binding activity and the expression of
574 *HMOX* and *NQO-1*, two of the NRF2-downstream target genes, in BEAS-2B cells acutely and, to a lesser extent,
575 repeatedly exposed to Q-UFP. Because of its key role as master regulator of the cell redox homeostasis, NRF-2 is
576 also well-equipped to counteract ROS production and is critical for maintaining the redox balance in the cell
577 (Wardyn et al. 2015). Leclercq et al. (2018) and Sotty et al. (2020), studying the NRF2 signaling pathway activation
578 within BEAS-2B cells repeatedly exposed to fine or ultrafine particles supported the key role played by this
579 transcriptional factor to counteract ROS over-production. However, despite the early activation of NRF2 signaling
580 pathway, there were massive oxidative alterations of the cellular constituents, such as DNA, proteins, and/or lipids,
581 in BEAS-2B cells after any exposure to WF-derived Q-PUF. Hence, in this work, because of the inability of the
582 NRF2 signaling pathway to totally ward off ROS over-production, the antioxidant defenses-induced by NRF2 were
583 exceeded in BEAS-2B cells acutely and/or repeatedly exposed. Oxidative damage to DNA, proteins and/or lipids can
584 also disrupt the cell homeostasis, thereby contributing to gene expression deregulation, DNA mutation, protein
585 alteration with loss of function, and lipid peroxidation with loss of membrane integrity and/or fluidity (Abbas et al.
586 2019; Badran et al. 2020; Garçon et al., 2006; Gualtieri et al. 2010, 2011; Leclercq et al. 2016, 2017a, 2018; Longhin
587 et al. 2013, 2016; Sotty et al. 2020). Accordingly, Grazyk et al. (2016), indicated that, 3 h after the short-term
588 exposure to GMAW-SS WF, even in controlled and well-ventilated settings, there were acute oxidative stress
589 reactions in healthy, non-smoking individuals not chronically exposed to WF, as shown by significant increases in
590 plasma and urinary-H₂O₂ concentrations, and plasma and urinary-8-OHdG concentrations.

591 In order to maintain essential coordinated cellular responses needed to resolve the unbalanced status of the cells
592 and/or tissues, NRF2 and NF-κB cell signaling pathways, that respectively regulate cellular responses to oxidative
593 stress and inflammation, must closely interplay through multiple molecular interactions, which can often depend on
594 the cell types and tissue contexts (Wardyn et al. 2015). However, in this work, despite the significant increase of the
595 anti-inflammatory NRF2 binding to ARE, there was a significant activation of the pro-inflammatory NF-κB cell
596 signaling pathway in BEAS-2B cells repeatedly exposed to Q-UFP at their highest dose. Nevertheless, only a very
597 slight induction of the gene expression of *TNF-α* and *IL-1β* in BEAS-2B cells acutely and repeatedly exposed to the

598 highest dose of Q-UFP was reported, without any significant secretion of their proteins, or those of other pro-
599 inflammatory mediators (i.e., RANTES, GM-CSF, GRO- α , IL-6, IL-8, and MCP-1). Despite our efforts to quantify
600 the protein expression of these inflammatory mediators in the cell-free supernatants of BEAS-2B cells using different
601 methods (i.e., Luminex and MSD assays), we failed to obtain consistent results in the BEAS-2B cells exposed to
602 WF-derived Q-UFP. Interestingly, other authors, having already encountered the same difficulties, have incriminated
603 some artefactual interactions of UFP with these inflammatory mediators, thereby creating non-biological artifacts
604 when measuring their concentrations in biological matrices (Brown et al. 2010; Saleh et al. 2019; Veranth et al.
605 2007). Accordingly, Badding et al. (2014), testing the hypothesis that some pro-inflammatory mediators may be
606 produced by unprimed RAW 264.7 cells 24 h after their exposure to GMAW-SS-derived WF, reported some non-
607 significant increases (i.e., *TNF- α* and IL-6), thereby supporting the general statement that WF are not able to
608 robustly produce pro-inflammatory mediators from various cell models, including unprimed macrophages. In
609 contrast, other authors evaluating the pro-inflammatory potent of WF-derived particles through animal experiments,
610 observed transient but significant elevated markers of lung inflammation, as shown by total cells and/or neutrophils
611 in bronchioalveolar lavage fluid and cytokine and/or chemokine secretion (Antonini et al. 2017; Ederly et al. 2011;
612 Falcone et al. 2018a). Moreover, Krishnaraj et al. (2017a, 2017b), investigating the effect of WF in rats, reported
613 increased levels ROS in lung tissues associated with accumulation of 8-OHdG and, at the same time, activation of
614 both NRF2 and NF κ B signaling pathways, thereby leading to the significant secretion of pro-inflammatory
615 mediators. In addition, Jarvela et al. (2012) investigating occupational exposure to WF generated from grinding MS
616 plates or pieces in current workplaces, reported a slight acute inflammation in metal workers by means of alterations
617 of the total blood leukocytes and neutrophils, as well as IL-1 β and E-selectin, but no changes of the pulmonary
618 function, after the work shift.

619 Because oxidative DNA damage, such as 8-OHdG, occurs not only in cell models but also in humans exposed
620 to WF, we next sought to elucidate whether WF-derived Q-UFP contribute to other genetic alterations. However,
621 under our experimental conditions, there was no significant primary DNA damage, as evaluated using the *in vitro*
622 standard comet assay under alkaline conditions and the hOGG1-modified version. Other authors, reported the ability
623 of GMAW-MS and, to a higher extent, GMAW-SS-derived WF to produce DNA double-strand breaks, and
624 incriminated the high levels of CrVI, Ni and/or Mn (Badding et al. 2014; Leonard et al. 2010; McCarrick et al.
625 2019). Accordingly, Antonini et al. (2005) showed that CrVI within WF emitted by MMA-SS induced DNA
626 damage, that was similar to what they observed when using a soluble Cr salt, likely as a result from hydroxyl radical
627 formation. However, here, the doses of Q-UFP we applied on the BEAS-2B cell model seemed to be too low to
628 produce any significant primary DNA damage.

629 Thereafter, to go further in better deciphering the molecular mechanisms underlying the toxicity of WF-derived
630 Q-UFP, some critical DRG involved in the activation of some key cell signaling pathways were identified in BEAS-
631 2B cells acutely and repeatedly exposed. Accordingly, 6 DRG (i.e., 6 down-regulated) were reported in BEAS-2B
632 cells acutely exposed to the lowest dose of Q-UFP, and 24 DRG (i.e., 19 down- and 5 up-regulated) in BEAS-2B
633 cells acutely exposed to the highest dose of Q-UFP, whereas 39 DRG (i.e., 30 down- and 9 up-regulated) were found
634 in BEAS-2B cells repeatedly exposed to the highest dose of Q-UFP, thereby supporting the highest effects of this

635 later. Interestingly, 2 DRG (i.e., *Cyclin-dependent kinase 4: CDK4*, and *Serine/threonine-protein kinase MTOR:*
636 *MTOR*), generally described as closely involved in cell cycle regulation, were reported as down-deregulated for any
637 exposure (Guo et al. 2021). Moreover, the 6 DRG, 5 down-regulated (i.e., *Janus Kinase 2: JAK2*,
638 *Hydroxymethylbilane Synthase: HMBS*, *Lymphoid Enhancer Binding Factor: ILEF1*, *BCL2 Apoptosis Regulator:*
639 *BCL2*, *Proenkephalin: PENK*) and 1 up-regulated (*Pyruvate Dehydrogenase Kinase 1: PDK1*), common to BEAS-
640 2B cells acutely and repeatedly exposed to the highest dose of Q-PUF, supported the alteration of some key
641 regulators of cell cycle, generally members of anti-apoptotic signaling pathways (Tandawy et al. 2020, Yildirim et al.
642 2021, Tungskruthai et al. 2021). Zeilder-Ederly et al. (2010), in their comprehensive transcriptional profiling, also
643 revealed differences in the DRG and gene networks triggered by WF according to mouse strains: gene expression
644 was more deregulated in the susceptible A/J strain, and exposure to WF-emitted by GMAW-SS was associated with
645 overexpression of immunomodulatory genes. Erdely et al. (2011) reported some DRG closely involved in
646 inflammation (e.g., *TNF- α* , *IL1- β*), stress (e.g., *HMOX*, *NQO1*), coagulation, adhesion, and remodeling/growth
647 factors in mice exposed to WF, but with some difference according to their emission by MMA-SS, GMAW-SS, and
648 GMAW-MS. Moreover, Falcone et al. (2018a), exposing mice to WF emitted by GMAW-SS supported the above-
649 mentioned results and the differential inflammatory and/or stress responses elicited depending on the type of WF. Oh
650 et al. (2011), exposing rats to WF emitted by MMA-SS, also reported DRG profiles related to inflammation and
651 repair processes, rapidly, dynamically, and stringently regulated in many biological diseases and pathological
652 processes. At least, exposure to WF emitted by GMAW-SS induced 37 DRG coding for DNA damage sensors, cell
653 cycle arrest in G1/S phase, DNA repair enzymes, and caspase-mediated apoptosis within rat lungs (Krishnaraj et al.
654 2017a, 2017b).

655 Moreover; recent evidence supported that critical alterations of the epigenome can occur after exposure to air
656 pollution-derived PM and thereafter lead to severe dysregulation of gene expression within the lung (Leclercq et al.
657 2017a; Sotty et al. 2019). Therefore, a better knowledge of these epigenetic changes may provide an additional tool
658 for analyzing the association between WF-derived Q-UFP exposure and the development and/or exacerbation of
659 chronic inflammatory lung diseases, including cancers. However, here, no significant change of the global DNA
660 methylation was observed in BEAS-2B cells after any exposure to Q-PUF. Shoeb et al. (2017), trying to identify
661 potential biomarkers of epigenetic changes in isolated peripheral blood mononuclear cells of animal models after
662 their exposure to WF, including GMAW-MS and MMA-SS, reporting similar results. With regards to histone H3
663 PTM, the significant deacetylation of the H3 histone at Lys 9 and Lys 27 in BEAS-2 B cells acutely exposed to the
664 two doses of Q-PUF, but only at Lys 27 in BEAS-2 B cells repeatedly exposed to the two doses of Q-PUF, were
665 consistent with the related enzyme. However, to our current knowledge, only very few data are available about
666 histone H3 PTM by WF. Histone acetylation/deacetylation could be closely related to the development or
667 exacerbation of chronic inflammatory lung diseases, and even cancers (Li et al. 2015).

668 While miRNA are now well-known regulators in almost all cellular signaling pathways and can target up to
669 several hundred mRNA, studying the aberrant miRNA expression and their predicted interactions with the ahead-
670 identified DRG. appeared to be highly relevant (Acunzo et al. 2015). For any exposure, the number of DRmiR
671 reported in BEAS-2B remained relatively weak, ranging from 4 to 7. Among them, only 2 DRmiR, also 1 down- and

672 1 up-regulated (i.e., has-miR-551b# and has-miR-331-5p, respectively) were reported as common to BEAS-2B cells
673 acutely exposed to the lowest or the highest doses of Q-UFP, and only one (i.e., has-miR-210-3p) was up-regulated
674 at the same time in BEAS-2B cells acutely exposed to the highest dose of Q-UFP and repeatedly exposed to the
675 lowest dose of Q-UFP. Xu et al. (2014) founds that miR-551b# up-regulation in apoptotic-resistant cells inhibited the
676 expression of antioxidant enzymes, and potentiated ROS accumulation and mucin expression. While no information
677 is currently available about the role of has-miR-331-5p in lung cells, Ren et al. (2019), studying and comparing the
678 expression of miR-210-3p in different non-small cell lung cancer cell (NSCLC) lines and BEAS-2B cell line,
679 indicated that it can regulate the proliferation and apoptosis by targeting *Transcription Regulator Family Member A*.
680 However, as highlighted by Jardim et al. (2011) and Vrijens et al. (2105), although miRNA changes may be sensitive
681 indicators of the effects of acute and chronic environmental exposure such as WF-derived Q-UFP, further studies
682 should be conducted to elucidate the role of the mediation effect of miRNA between the exposure and the effect to
683 provide a more accurate evaluation of the consequences of these miRNA changes.

684 The functional analysis of the up-regulated DRmiR founded in BEAS-2B acutely or repeatedly exposed to Q-
685 PUF, carried out in silico using three complementary miRNA target Prediction Database (i.e., TargetScanHuman
686 v7.2, miRDB, and DIANA TOOLS Tarbase V8), and compared to the above-mentioned DRG, supported the
687 deregulation of some relevant cellular signaling pathways closely involved in cell inflammation (e.g., *IKBKKG*,
688 *NCAM1*, *MYD88*, *TCF7*, *TLR4*, *TLR6*, *LPAR2*, and *CALCRL*), and, to a higher extent, cell cycle deregulation (e.g.,
689 *BCL2*, *JAK2*, *SMAD4*, *RASA1*, *CASP1*, *CCND1*, *ADORA1*, *STAT5*, *KSRI*, *MEF2C*, *NKD1*, and *WNT5B*). Some of
690 the identified DRmiR and target DRG being closely implicated in the regulation of cell cycle and, in particular, in the
691 fate of the cell to enter into apoptosis, some key events of this regulated cell death were thereafter investigated.
692 Annexin V labelling together with initiator caspase-8, initiator caspase-9, and effector caspase 3/7 activation within
693 BEAS-2B cells whatever their exposure to Q-PUF revealed some apoptotic cells. Accordingly, after 12 weeks of
694 exposure to WF emitted by GMAW-SS, Krishnaraj et al. (2017a, 2017b) reported that the earlier triggered DNA
695 domain repair (DDR) was also compromised as reflected by resumption of the cell cycle, repair inhibition, and
696 failure of apoptosis. Their data clearly supported that exposure to WF influences two crucial layers of inclusive DDR
697 regulation, phosphorylation of key proteins in nonhomologous end-joining (NHEJ), homologous recombination
698 (HR), as well as the ncRNAs that epigenetically modulate DDR, thereby highlighting the concomitant occurrence of
699 severe DNA damage coupled with non-productive DNA repair and apoptosis avoidance, which could contribute to
700 neoplastic transformation. Moreover, Antonini et al. (2005) using an animal model, observed a time-dependent
701 increase in the number of apoptotic cells in the lung airspaces and parenchyma of animals intratracheally instilled
702 with the MMA-SS welding sample.

703 Overall, the present original results clearly demonstrated for the first time, in a relevant human bronchial
704 epithelial cell model, the crucial role played by the specific metal enriched Q-UFP fraction of the WF emitted by
705 GMAW-SS in ROS over-production, thereby triggering massive oxidative damage, genetic and/or epigenetic
706 alteration, and, therefore, some critical cellular signaling pathways related to oxidative stress, inflammation, and cell
707 cycle deregulation in favor of apoptosis cell death. Future works are still needed to go further in the better knowledge
708 of the respective toxicity potentials of the different metals (e.g., Fe, Cr, Mn, Ni, Cu) within WF-derived Q-UFP.
709 Nonetheless, all these new findings underlined the urgent need to include the Q-UFP fraction of WF in current air

710 quality standards and/or guidelines relevant to the occupational settings, because Q-UFP were certainly shown as
711 participating for a very large part of the human health effects induced by WF.
712

713 **FUNDING SOURCES**

714
715 This work benefited from grants or contributions from “Association de Santé au Travail 59-62”, “Institut National de
716 Recherche et de Sécurité”, “Institut Mines Telecom Lille Douai”, University of Lille, and the CLIMIBIO and CaPPA
717 projects. The CaPPA project (Chemical and Physical Properties of the Atmosphere) is funded by the French National
718 Research Agency (ANR) through the PIA (Programme d'Investissement d'Avenir) under contract “ANR-11-LABX-
719 0005-01” and by the Regional Council “Hauts-de-France”, and the “European Funds for Regional Economic
720 Development” (FEDER).
721

722 **CONFLICT OF INTEREST**

723

724 All authors of this manuscript do not have any financial and personal relationships with other people or

725 organizations that could inappropriately influence their work.

726

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977

978 **TABLES**
979

980

Table 1: Elements and oxides within welding fumes-derived quasi-ultrafine particles

ELEMENT #	MW (g/mol)	Concentration (ng/μg)	OXIDE¹	MW (g/mol)	Concentration (ng/μg)	Proportion (%)
Al	27.0	1.5	Al ₂ O ₃	102.0	2.8	0.3%
Ca	40.1	7.6	CaO	56.1	10.7	1.1%
Cr	52.0	129.4	Cr ₂ O ₃	152.0	189.1	20.0%
Cu	63.5	4.0	CuO	79.5	5.0	0.5%
Fe	55.8	259.8	Fe ₂ O ₃	159.7	371.4	39.4%
K	39.1	1.2	K ₂ O	94.2	1.4	0.1%
Mg	24.3	0.9	MgO	40.3	1.5	0.2%
Mn	54.9	127.6	MnO	70.9	164.7	17.5%
Mo	95.9	13.1	MoO ₃	143.9	19.7	2.1%
Na	23.0	1.5	Na ₂ O	62.0	2.1	0.2%
Ni	58.7	43.2	NiO	74.7	54.9	5.8%
Si	28.1	55.6	SiO ₂	60.1	118.9	12.6%
Zn	65.4	1.4	ZnO	81.4	1.8	0.2%
Total		646.9			944.0	100%

981
982[#] refers to the most likely main metal oxide in welding fumes (> 1 ng/μg).

983 **FIGURE LEGEND**

984

985 **Figure 1:** ATP concentrations in normal human bronchial epithelial BEAS-2B cells 24 h after one (Figure 1-A) or
986 three (Figure 1-B) 24 h-exposures to increasing concentrations (i.e., ranging from 0.1 to 50 $\mu\text{g}/\text{cm}^2$) of the quasi-
987 ultrafine particle (Q-UFP) fraction of welding fumes emitted by gas metal arc welding-stainless steel. As indicated
988 by the red black dotted lines on Figure 1-A and 1-B, respectively, the lowly- (i.e., lethal concentration at 10 %, LC_{10})
989 and highly (i.e., lethal concentration at 40 %, LC_{40})-cytotoxic doses of 1.5 and 9 $\mu\text{g}/\text{cm}^2$ were used for the acute
990 scenario of exposure, and the lowly- (i.e., LC_{10}) and highly (i.e., LC_{40})-cytotoxic doses of 0.25 and 1.5 $\mu\text{g}/\text{cm}^2$ were
991 used for the repeated scenario of exposure, in order to further investigate the other toxicological endpoints under
992 study. Values are depicted as means and standard deviations ($n = 5$). (Student t-test versus negative controls: *: $p <$
993 0.05; ***: $p < 0.001$).

994

995 **Figure 2:** Chloromethyl derivative of 2',7'-dichlorodihydrofluorescein diacetate (CM- H_2DCFDA) fluorescence
996 (Figure 2-A), binding activity of nuclear factor erythroid 2 p45-related factor 2 (NRF2) to antioxidant response
997 elements (ARE) (Figure 2-B), and NRF2 target gene expression (i.e., heme oxygenase: HMOX and -NADPH
998 quinone oxido-reductase-1: NQO-1; Figures 2-C and 2-D) in normal human bronchial epithelial BEAS-2B cells 24 h
999 after one (white histogram bars) or three (grey histogram bars) 24 h-exposures to the quasi-ultrafine particle (Q-UFP)
1000 fraction of welding fumes emitted by gas metal arc welding-stainless steel. Values are depicted as means and
1001 standard deviations ($n = 5$). (Student t-test versus negative controls: *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$).

1002

1003 **Figure 3:** Glutathione status (i.e. ratio between the oxidized and the reduced forms; Figure 3-A), 8-hydroxy-2'-
1004 deoxyguanosine (8-OHdG; Figure 3-B), carbonylated protein (CO-PROT; Figure 3-C), and 4-hydroxynonenal (4-
1005 HNE; Figure 3-D) in normal human bronchial epithelial BEAS-2B cells 24 h after one (white histogram bars) or
1006 three (grey histogram bars) 24 h-exposures to the quasi-ultrafine particle (Q-UFP) fraction of welding fumes emitted
1007 by gas metal arc welding-stainless steel. Values are depicted as means and standard deviations ($n = 5$). (Student t-test
1008 versus negative controls: *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$).

1009

1010 **Figure 4:** Binding activity of nuclear factor-kappa B (NF- κB) to DNA κB motifs (Figure 2-A), and tumor necrosis
1011 factor-alpha (TNF- α), Interleukin-1 beta (IL-1 β) and interleukin-6 (IL-6) gene expression (Figure 4-B, 4-D, and 4-F,
1012 respectively) and protein secretion (Figure 4-C, 4-E, and 4-G, respectively) in normal human bronchial epithelial
1013 BEAS-2B cells 24 h after one (white histogram bars) or three (grey histogram bars) 24 h-exposures to the quasi-
1014 ultrafine particle (Q-UFP) fraction of welding fumes emitted by gas metal arc welding-stainless steel. Values are
1015 depicted as means and standard deviations ($n = 5$). (Student t-test versus negative controls: **: $p < 0.01$; ***: $p <$
1016 0.001).

1017

1018 **Figure 5:** Primary DNA damage assessed with the alkaline comet assay in normal human bronchial epithelial
1019 BEAS-2B cells after 4 h, 24 h or 3 x 24 h (Figures 6-A, 6-B and 6-C, respectively) of exposure to the quasi-ultrafine

1020 particle (Q-UFP) fraction of welding fumes emitted by gas metal arc welding-stainless steel. Both the standard (i.e.,
1021 dark grey histogram bars) and the hOGG1-modified (i.e., slight grey histogram bars) comet assays were applied.
1022 Values are depicted as means of medians of % of tail intensity and standard deviations (n = 4). (Non-parametric
1023 Mann-Whitney U-test *versus* negative controls: *: $p < 0.05$). Cytotoxicity was assessed at harvest using the Trypan
1024 Blue dye exclusion assay. Results were expressed as percent of relative cell viability (i.e., the percent ratio of viable
1025 unstained cells to non-viable stained cells in exposed *versus* control groups).
1026

1027 **Figure 6:** Venn diagrams representing the differentially regulated genes down- and up-regulated (Figures 6-A and 6-
1028 B, respectively) in normal human bronchial epithelial BEAS-2B cells 24 h after one or three 24 h-exposures to the
1029 quasi-ultrafine particle (Q-UFP) fraction of welding fumes emitted by gas metal arc welding-stainless steel. (i.e.,
1030 Fold-Change: $FC > 1.5$ or < 0.66 , $p < 0.05$).
1031

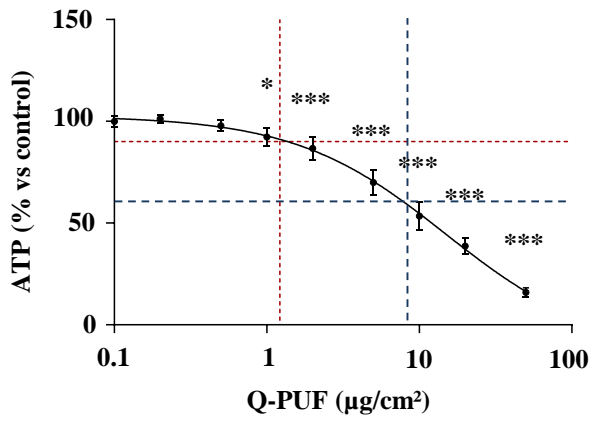
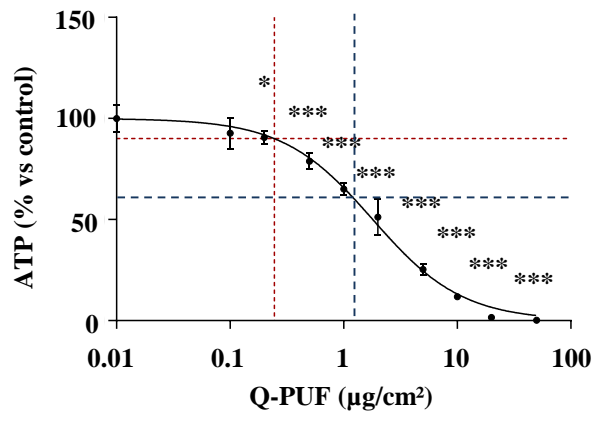
1032 **Figure 7:** Histone deacetylase (HDAC) activity (Figure 7-A), histone acetyl transferase (HAT) activity (Figure 7-B),
1033 ratio between HDAC activity and HAT activity (HDAC/HAT; Figure 7-C), histone H3Lys27ac concentration
1034 (Figure 7-D), histone H3Lys9ac concentration (Figure 7-E), and DNA global methylation (Figure 7-F) in normal
1035 human bronchial epithelial BEAS-2B cells 24 h after one (white histogram bars) or three (grey histogram bars) 24 h-
1036 exposures to the quasi-ultrafine particle (Q-UFP) fraction of welding fumes emitted by gas metal arc welding-
1037 stainless steel. Values are depicted as means and standard deviations (n = 5). (Student t-test *versus* negative controls:
1038 *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$).
1039

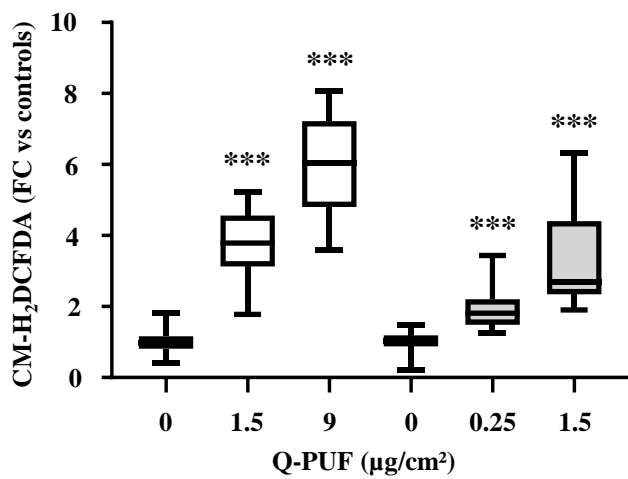
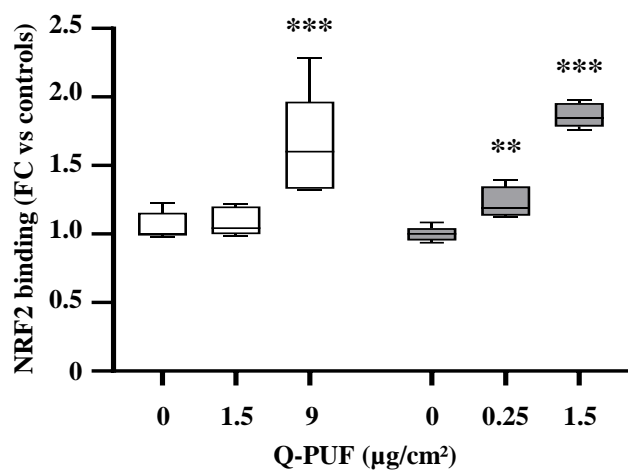
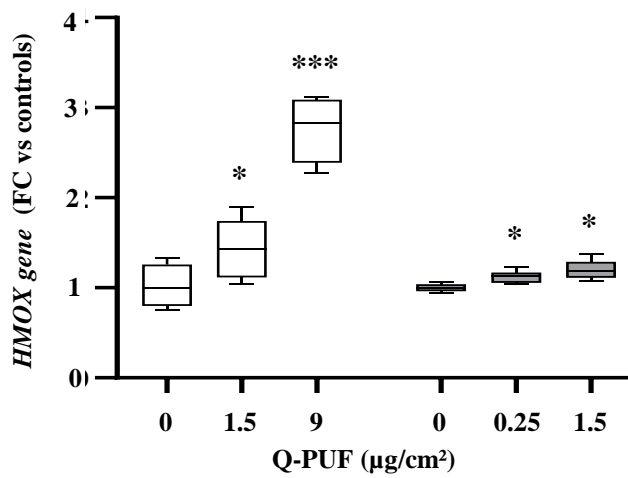
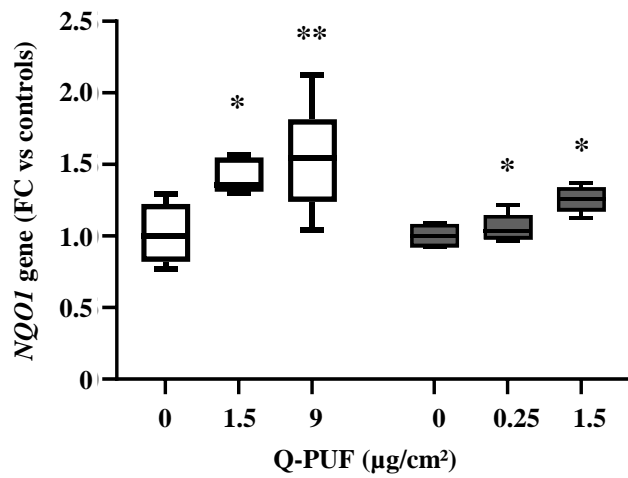
1040 **Figure 8:** Venn diagrams representing the differentially regulated microRNA down- and up-regulated (Figures 8-A
1041 and 8-B, respectively) in normal human bronchial epithelial BEAS-2B cells 24 h after one or three 24 h-exposures to
1042 the quasi-ultrafine particle (Q-UFP) fraction of welding fumes emitted by gas metal arc welding-stainless steel. (i.e.,
1043 Fold-Change: $FC > 1.5$ or < 0.66 , $p < 0.05$).
1044

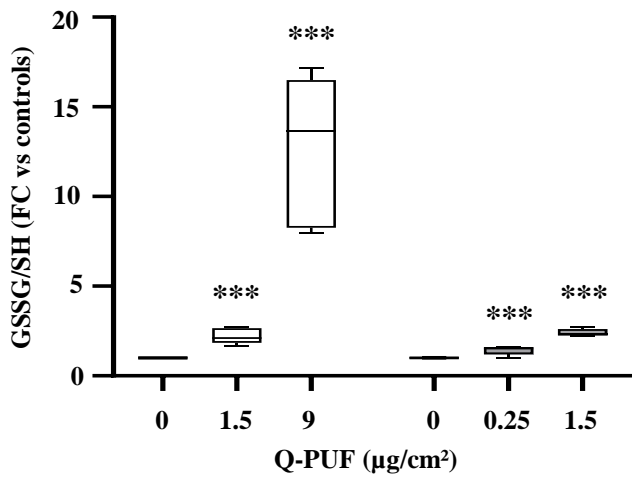
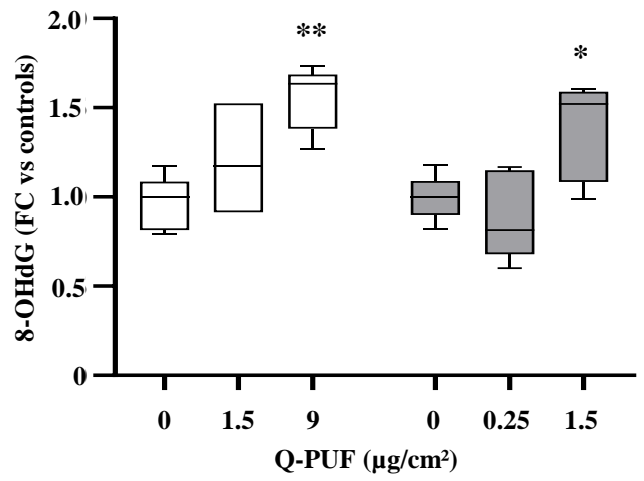
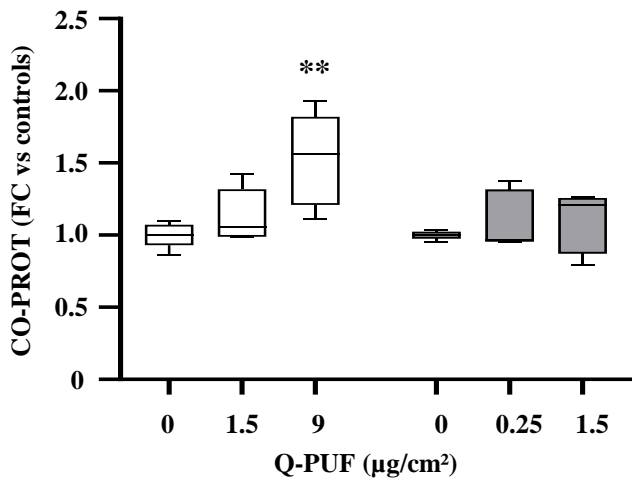
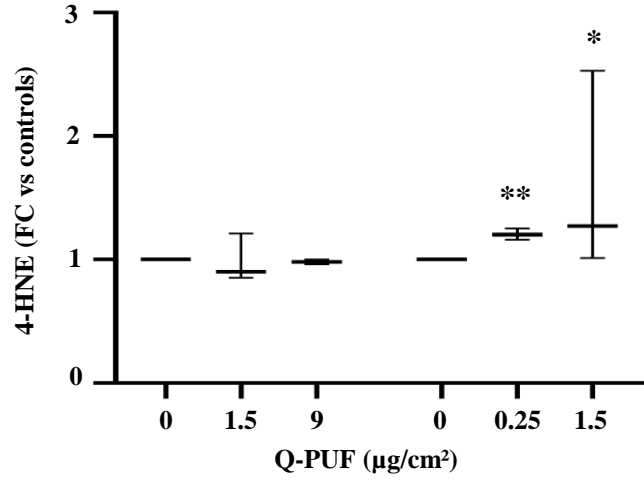
1045 **Figure 9:** Venn diagrams representing the target genes of differentially regulated microRNA (i.e., has-miR-331-5p,
1046 Figure 9-A; has-miR-210-3p, Figure 9-B; has-miR-424-5p, Figure 9-C; has-miR-597-5p, Figure 9-D; has-miR-212-
1047 3p, Figure 9-E; has-miR-1260a, Figure 9-F; has-miR-1248, Figure 9-G; has-miR-625-5p, Figure 9-H; has-miR-744-
1048 3p, Figure 9-I; and has-miR-755-3p, Figure 9-J), as assessed using TargetScanHuman v7.2
1049 (http://www.targetscan.org/vert_72/), miRNA target Prediction Database (miRDB; <http://mirdb.org>), and DIANA
1050 TOOLS Tarbase V8 (http://carolina.imis.athena-innovation.gr/diana_tools/web/index.php?r=tarbasev8%2Findex),
1051 and crossed with differentially deregulated genes by quasi-ultrafine particle (Q-UFP) fraction of welding fumes
1052 emitted by gas metal arc welding-stainless steel.
1053

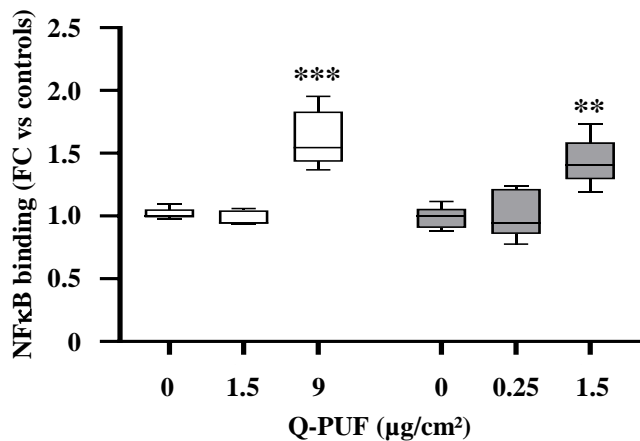
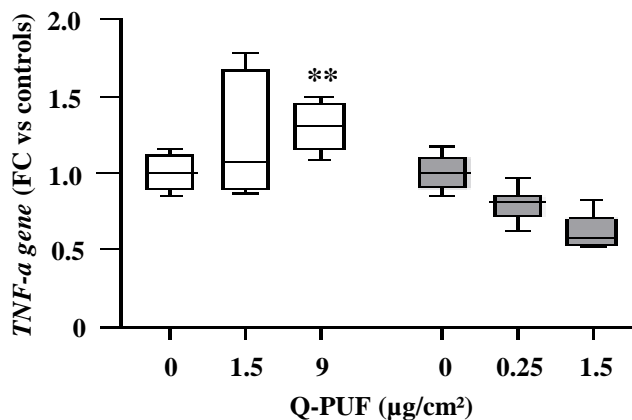
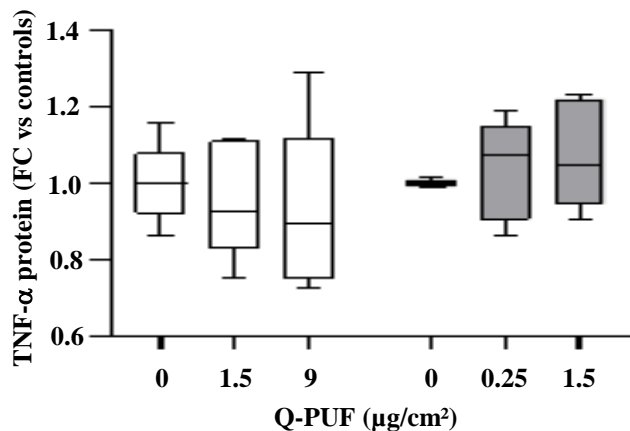
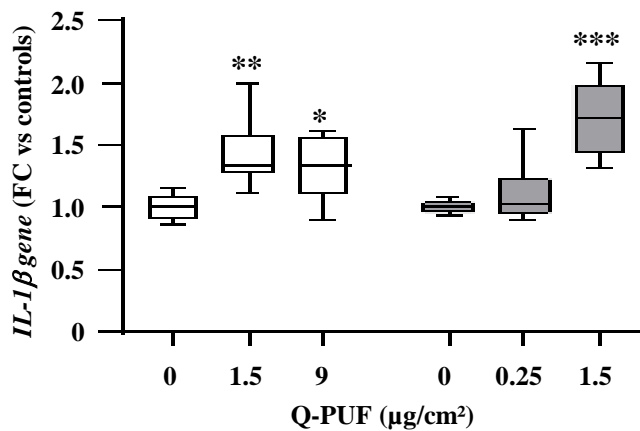
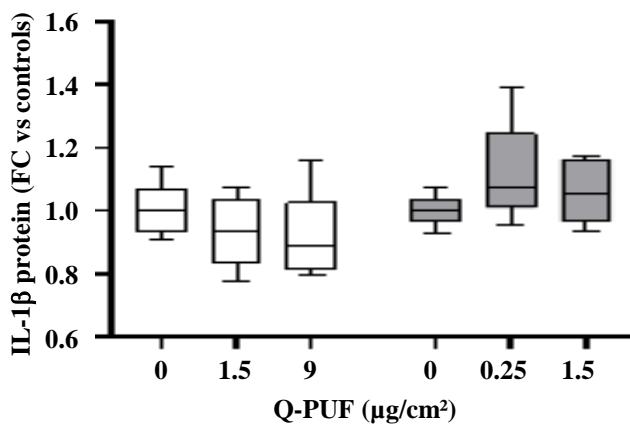
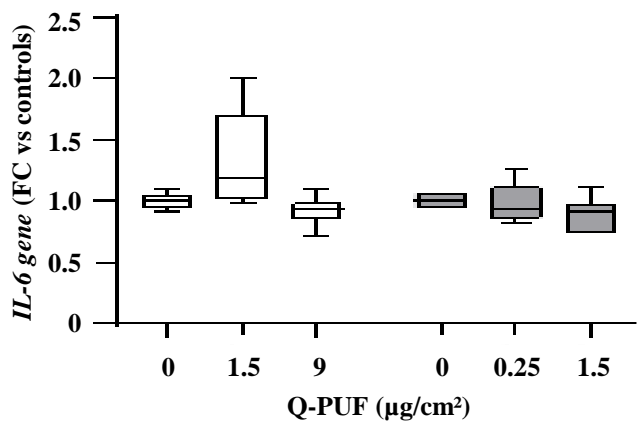
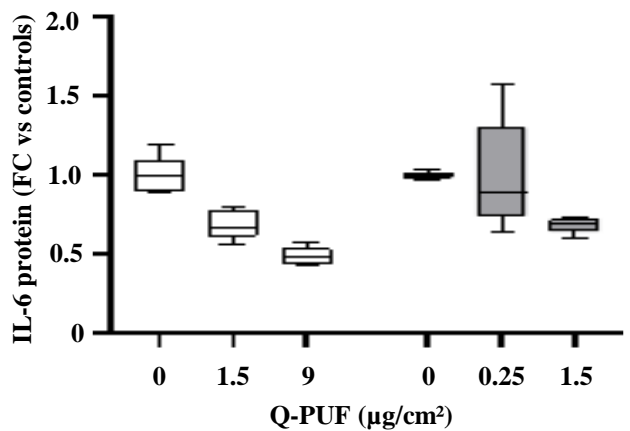
1054 **Figure 10:** Annexin V labelling (Figure 10-A) and activities of caspases 3/7, 8, and 9 (Figure 10-B, 10-C, and 10-D,
1055 respectively), in normal human bronchial epithelial BEAS-2B cells 24 h after one (white histogram bars) or three
1056 (grey histogram bars) 24 h-exposures to the quasi-ultrafine particle (Q-UFP) fraction of welding fumes emitted by

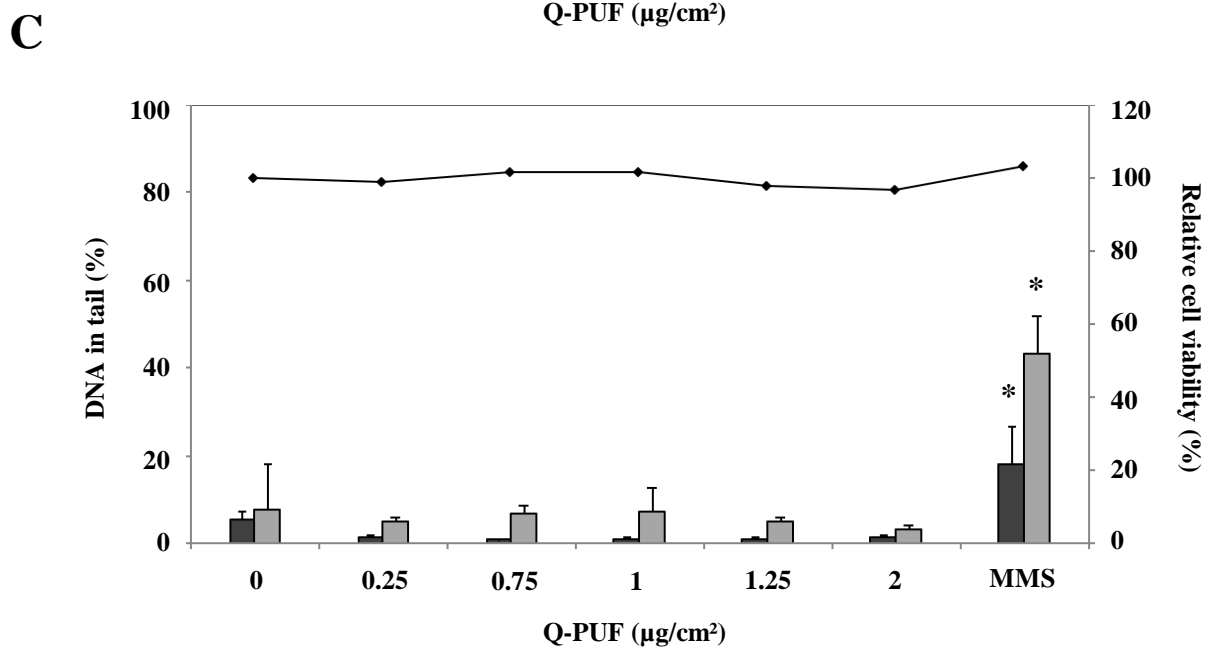
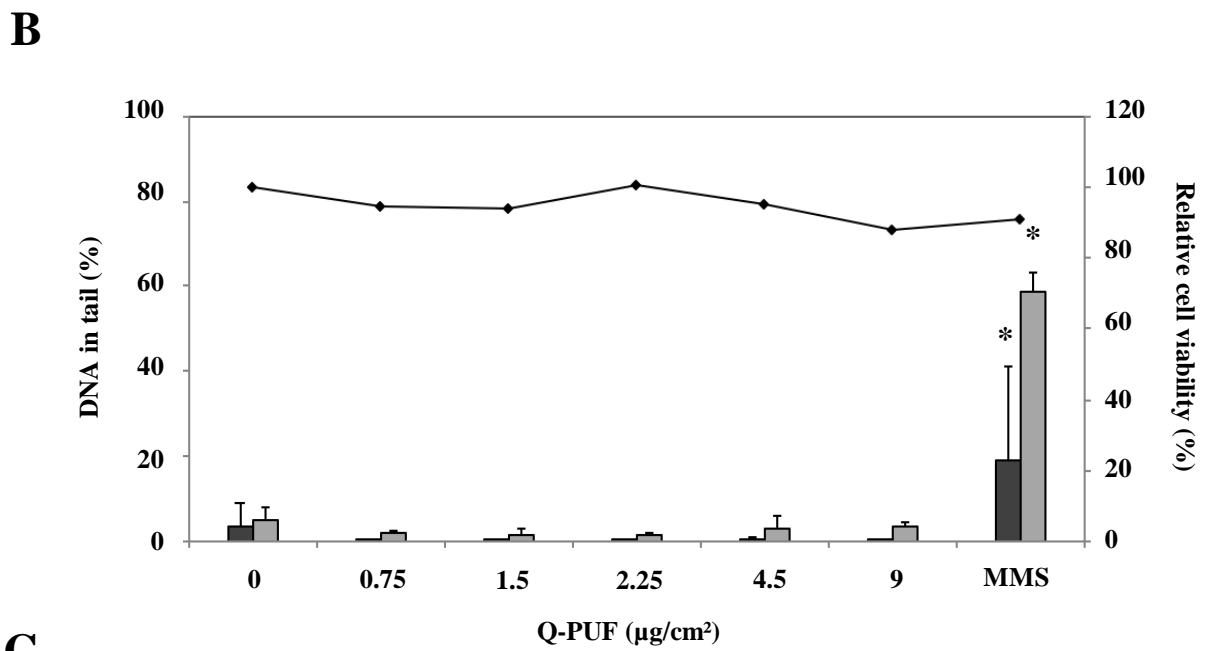
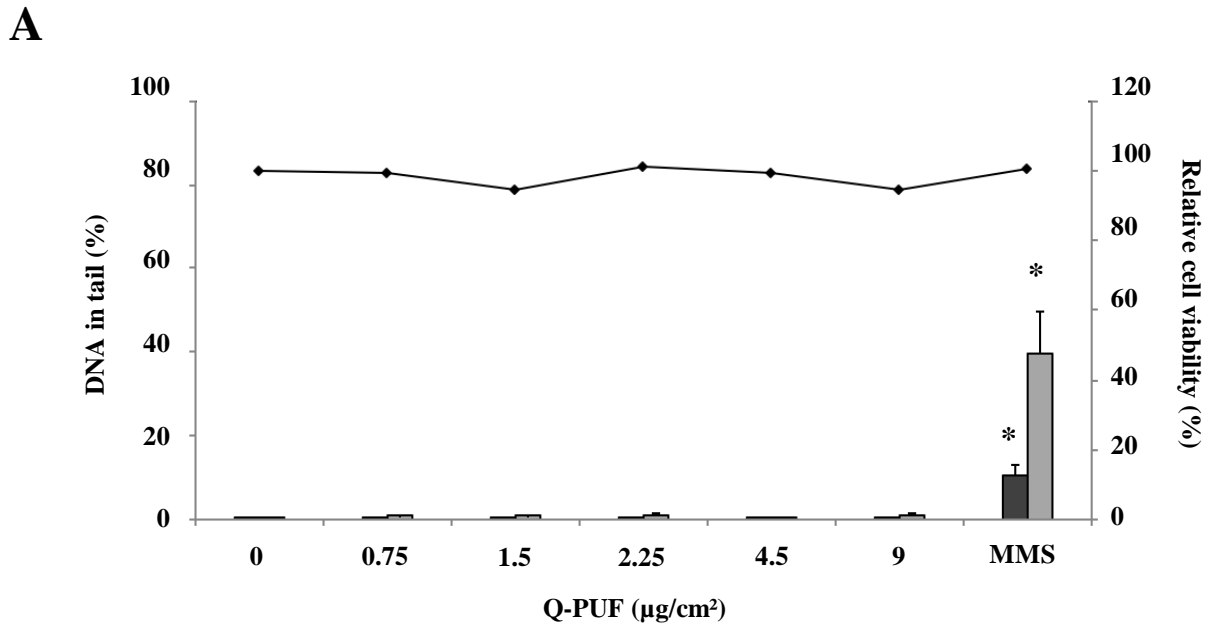
1057 gas metal arc welding-stainless steel. Values are depicted as means and standard deviations (n = 5). (Student t-test
1058 versus negative controls: *: p<0.05; **: p<0.01; ***: p<0.001).
1059

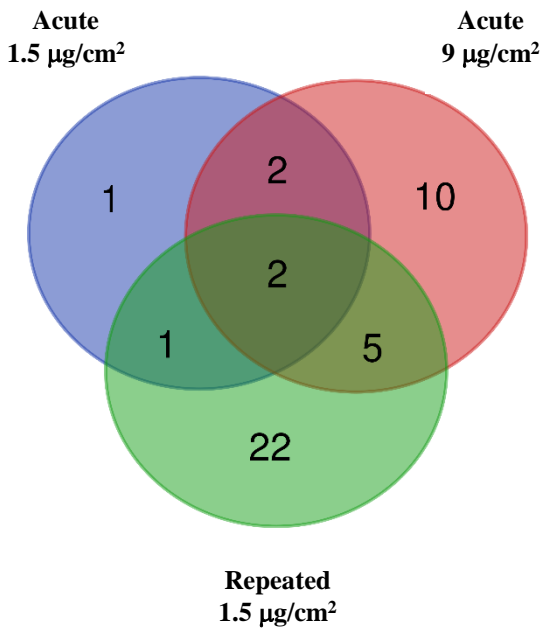
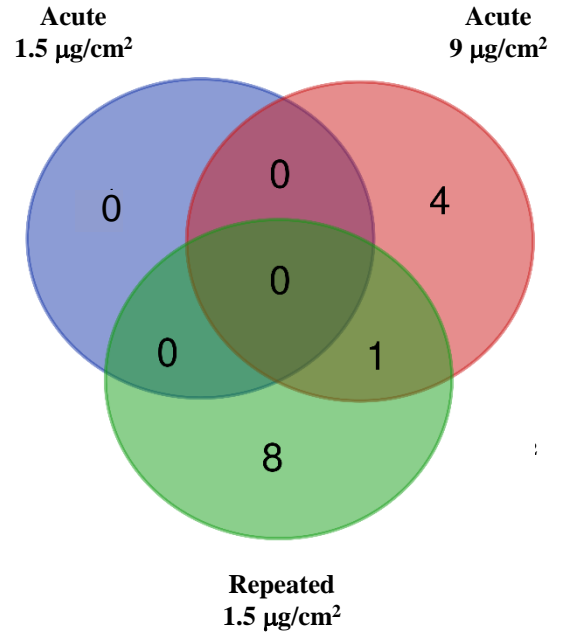
A**B**

A**B****C****D**

A**B****C****D**

A**B****C****D****E****F****G**



A**B**

Exposure/Dose (µg/cm ²)	Down-regulated genes
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Acute 1.5 µg/cm ² Acute 9 µg/cm ² Repeated 1.5 µg/cm ²	<i>CDK4, MTOR</i>
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Acute 1.5 µg/cm ² Acute 9 µg/cm ²	<i>HSPD1, RHEB</i>
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Acute 1.5 µg/cm ² Repeated 1.5 µg/cm ²	<i>RHEB</i>
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Acute 9 µg/cm ² Repeated 1.5 µg/cm ²	<i>JAK2, HMBS, LEF1, BCL2, PENK</i>
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Acute 1.5 µg/cm ²	<i>SMAD4</i>
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Acute 9 µg/cm ²	<i>PP3CA, IKBKG, AREG, MYD88, PPARG, MAP3K7, CASP1, RASA1, TCF7, NCAM1</i>
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Repeated 1.5 µg/cm ²	<i>LPAR2, CCNB1, TLR4, TLR6, MAP4K1, CDKN2A, GALR2, HSPA5, NKD1, CALCRL, IL12A, MAPKAPK3, SCL44A2, KSRI, GATA3, ODC1, WISP3, MEF2C, SOXS2, WNT5B, ADORA1, INHA</i>
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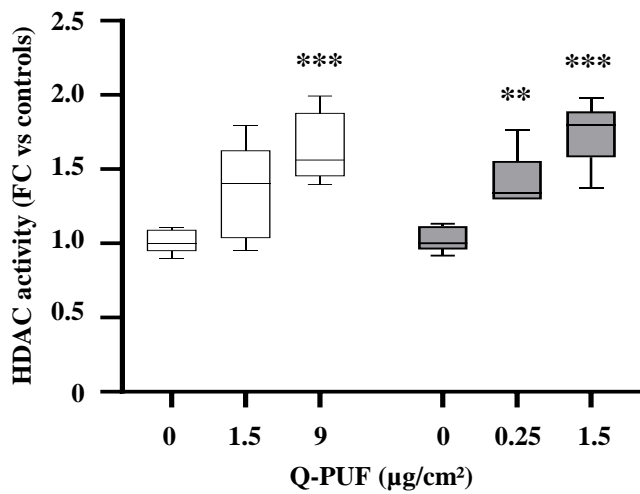
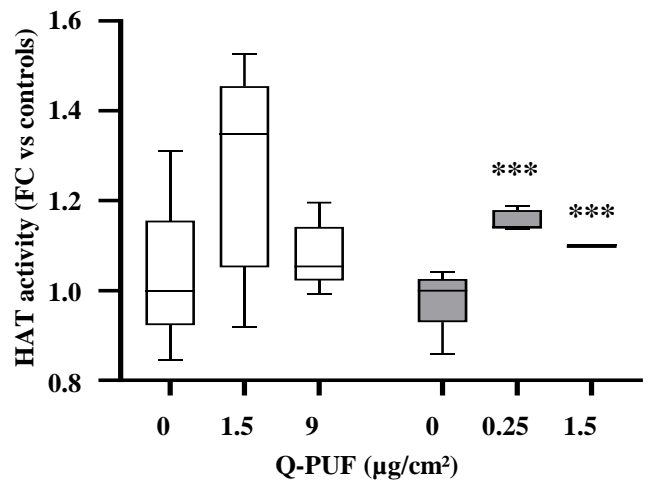
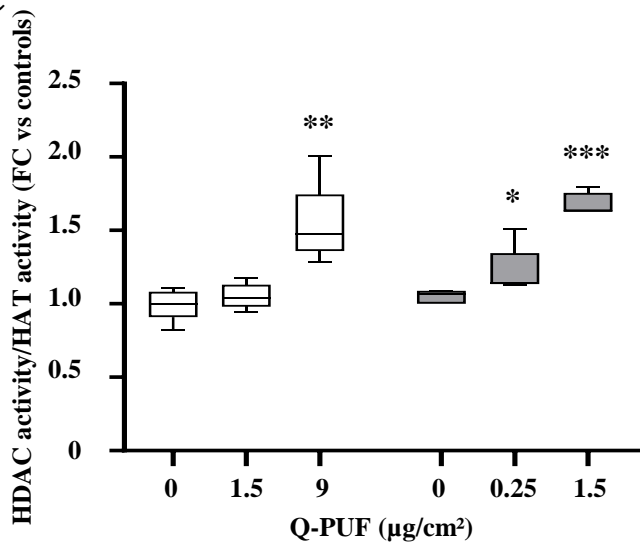
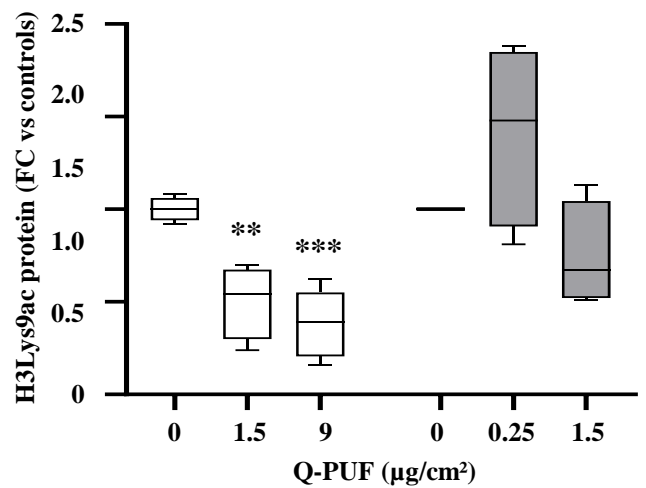
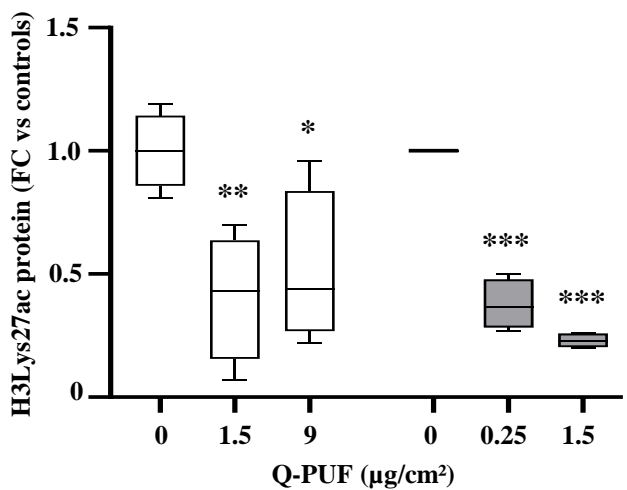
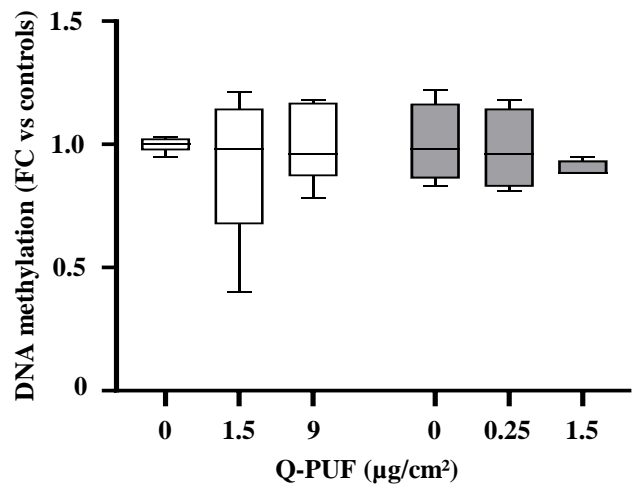
Exposure/Dose (µg/cm ²)	Up-regulated genes
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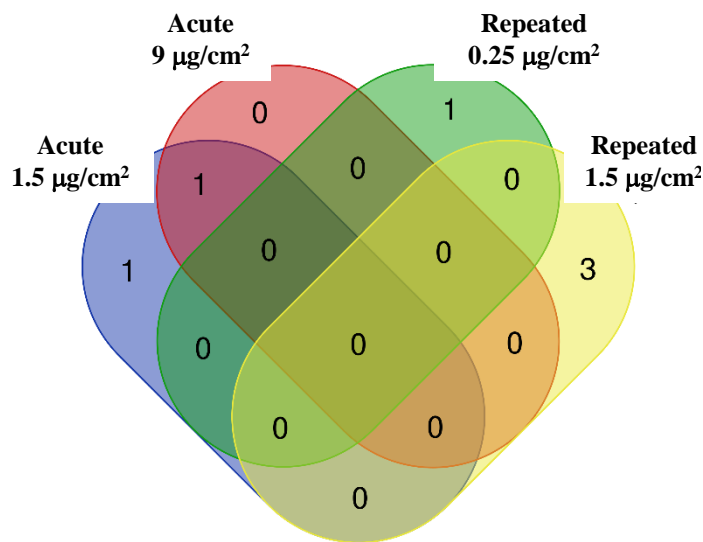
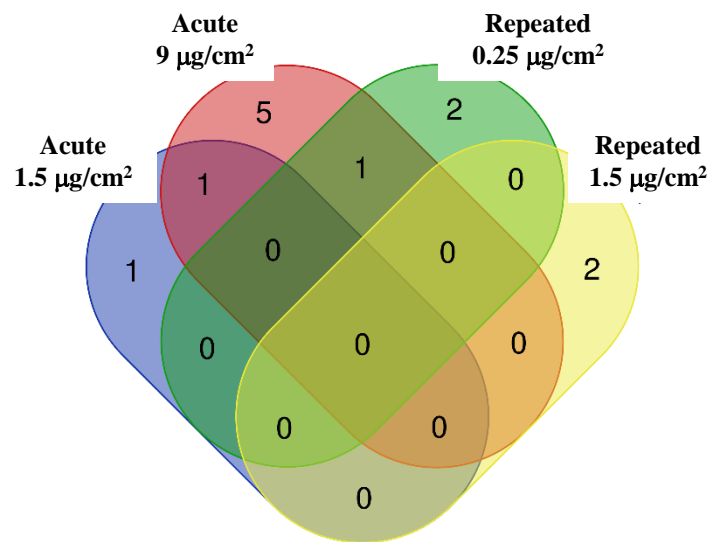
Acute 9 µg/cm ² Repeated 1.5 µg/cm ²	<i>PDK1</i>
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Acute 1.5 µg/cm ²	-
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Acute 9 µg/cm ²	<i>HMOX1, FOSB, MAP4K1, MYC</i>
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Repeated 1.5 µg/cm ²	<i>FZD8, CCL20, HK2, ENO2, GAPDH, IL8, CSF2, GYS1</i>
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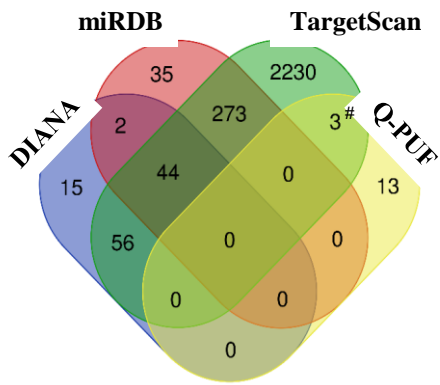
A**B****C****D****E****F**

A**B**

Exposure/Dose ($\mu\text{g}/\text{cm}^2$)	Down-regulated miR
Acute 1.5 $\mu\text{g}/\text{cm}^2$ Acute 9 $\mu\text{g}/\text{cm}^2$	hsa-miR-551b#
Acute 1.5 $\mu\text{g}/\text{cm}^2$	hsa-miR-92a-3p
Repeated 0.25 $\mu\text{g}/\text{cm}^2$	hsa-miR-548-3p
Repeated 1.5 $\mu\text{g}/\text{cm}^2$	hsa-miR-1233, hsa-miR-339-3p, hsa-miR-378a-5p

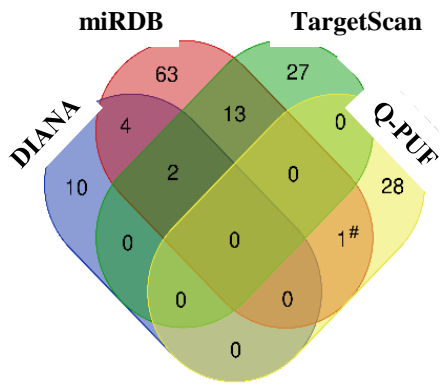
Exposure/Dose ($\mu\text{g}/\text{cm}^2$)	Up-regulated miR
Acute 1.5 $\mu\text{g}/\text{cm}^2$ Acute 9 $\mu\text{g}/\text{cm}^2$	hsa-miR-331-5p
Acute 9 $\mu\text{g}/\text{cm}^2$ Repeated 0.25 $\mu\text{g}/\text{cm}^2$	hsa-miR-210-3p
Acute 1.5 $\mu\text{g}/\text{cm}^2$	hsa-miR-424-5p
Acute 9 $\mu\text{g}/\text{cm}^2$	hsa-miR-597-5p, hsa-miR-212-3p, hsa-miR-1260a, hsa-miR-1248, hsa-miR-625-5p
Repeated 0.25 $\mu\text{g}/\text{cm}^2$	hsa-miR-494-3p, hsa-miR-1274b
Repeated 1.5 $\mu\text{g}/\text{cm}^2$	hsa-miR-744-3p, hsa-miR-766-3p

A – hsa-miR-331-5p



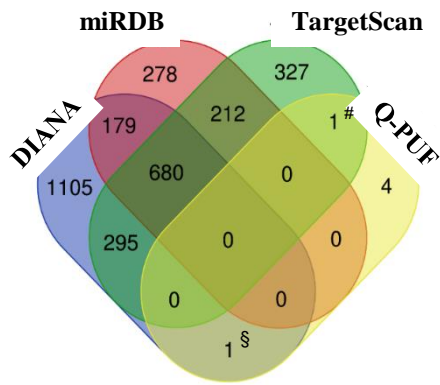
#*BCL2, JAK2, SMAD4*

B – hsa-miR-210-3p



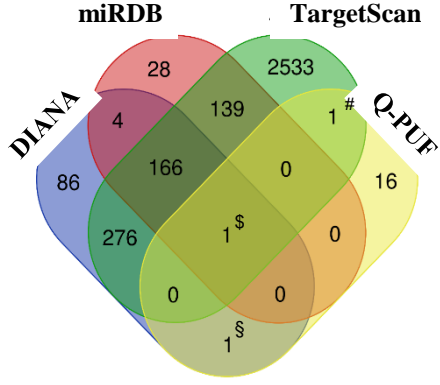
#*GALR2*

C – hsa-miR-424-5p



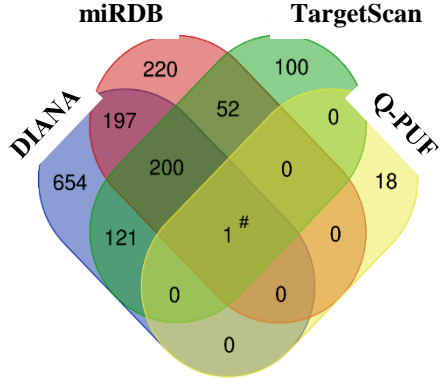
#*CDK4* §*SMAD4*

D – hsa-miR-597-5p



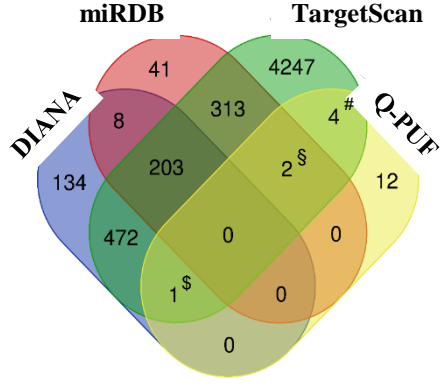
#*IKBK* §*PPARG* §*NCAM1*

E – hsa-miR-212-3p



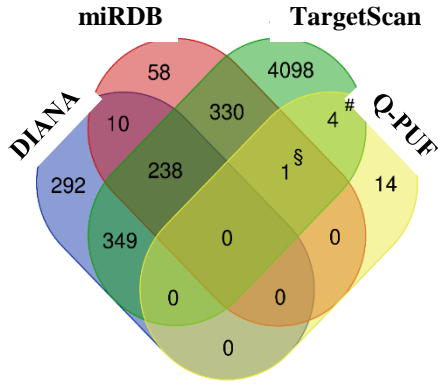
#*RASA1*

F – hsa-miR-1260a



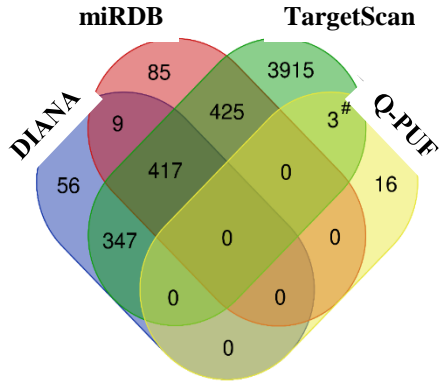
#*BCL2, RASA1, CASP1, MYD88*
 §*TCF7, PENK* §*IKBK*

G – hsa-miR-1248



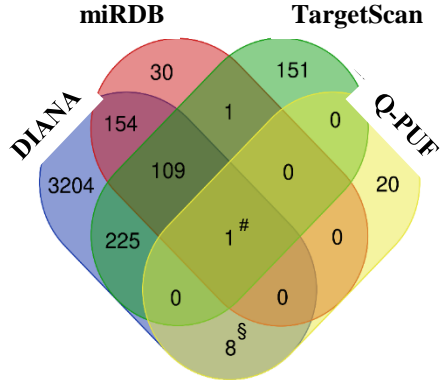
#*IMAP3K7, CDK4, IKBK, MYD88*
 §*BCL2*

H – hsa-miR-625-5p



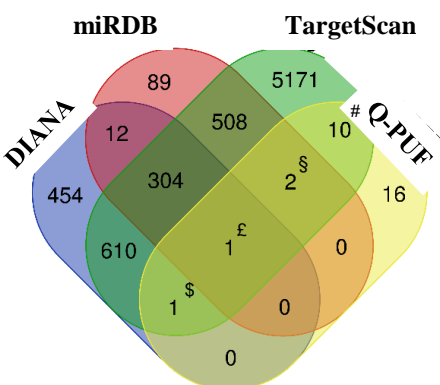
#*TCF7, IKBK, CDK4*

I – hsa-miR-744-3p

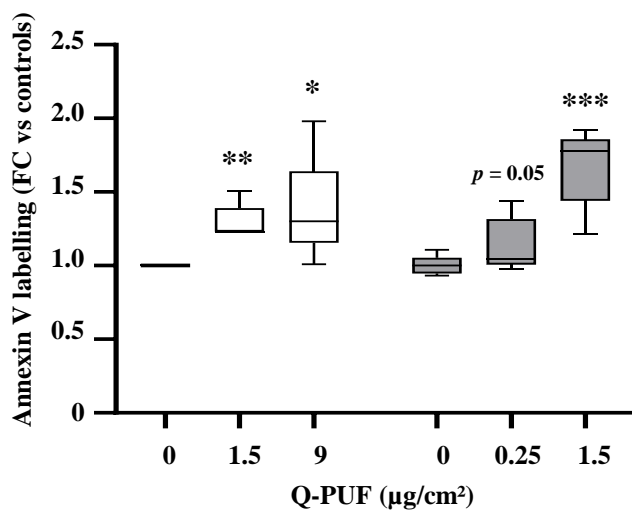
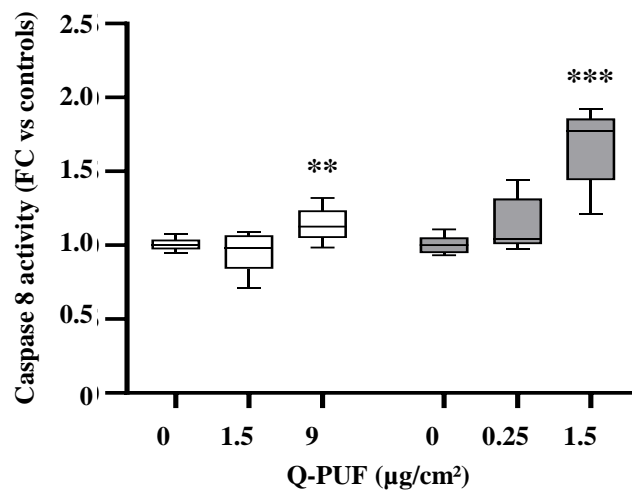
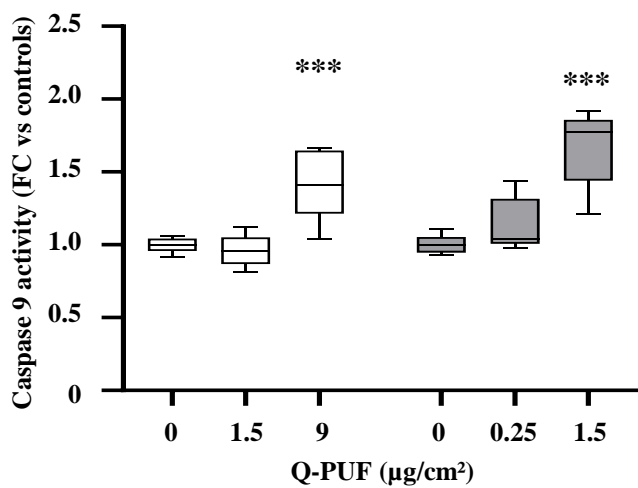
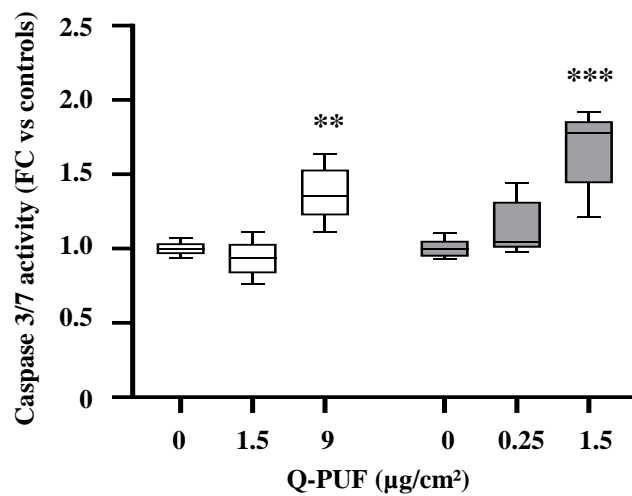


#*MEF2C* §*NKD1, BCL2, CDK4, TLR6, LEF1, LPAR2, ADORA1, CALCRL*

J – hsa-miR-766-3p

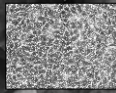


#*CCNB1, BCL2, STAT5A, MEF2C, LPAR2, TLR4, NKD1, SLC44A2, TLR6, CALCRL*
 §*LEF1, WNT5B* §*KSRI* £*ADORA1*

A**B****C****D**

GMAW-SS WF-derived Q-UFP

Normal human epithelial
bronchial BEAS-2B cells



ROS overproduction
Cell signaling pathways
Epigenetic alterations