

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

INVOLVEMENT OF NITRO-COMPOUNDS IN THE MUTAGENICITY OF URBAN PM_{2.5} AND PM₁₀ IN TURIN

This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/90315> since 2016-11-30T16:14:10Z

Published version:

DOI:10.1016/j.mrgentox.2011.09.002

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

This Accepted Author Manuscript (AAM) is copyrighted and published by Elsevier. It is posted here by agreement between Elsevier and the University of Turin. Changes resulting from the publishing process - such as editing, corrections, structural formatting, and other quality control mechanisms - may not be reflected in this version of the text. The definitive version of the text was subsequently published in MUTATION RESEARCH, 726 (1), 2011, 10.1016/j.mrgentox.2011.09.002.

You may download, copy and otherwise use the AAM for non-commercial purposes provided that your license is limited by the following restrictions:

- (1) You may use this AAM for non-commercial purposes only under the terms of the CC-BY-NC-ND license.
- (2) The integrity of the work and identification of the author, copyright owner, and publisher must be preserved in any copy.
- (3) You must attribute this AAM in the following format: Creative Commons BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/deed.en>), 10.1016/j.mrgentox.2011.09.002

The publisher's version is available at:

<http://linkinghub.elsevier.com/retrieve/pii/S1383571811002671>

When citing, please refer to the published version.

Link to this full text:

<http://hdl.handle.net/2318/90315>

1 **TITLE PAGE:**

2

3 **NITRO-COMPOUND INVOLVEMENT IN THE MUTAGENICITY OF URBAN PM2.5 AND PM10**

4 **IN TURIN**

5

6 **AUTHORS:**

7 D Traversi (1), T Schilirò (1), R Degan (1), C Pignata(1), L. Alessandria (1), G Gilli (1)

8 Authors:

9 **AFFILIATIONS:**

10 (1) Department of Public Health and Microbiology, University of the Study of Turin,

11 via Santena 5 bis 10126, Turin, Italy

12 phone +390116705810

13 fax +390112605810

14 ***Corresponding author:** e-mail: deborah.traversi@unito.it

15

16 **KEY WORDS:** PM2.5, PM10, air pollution, mutagenicity, nitro-compounds

17 **FOUNDING:** This study was co-funded by the University of Turin and the Piedmont Region

18 (Italy) in the field of health projects.

19

20 **ABSTRACT:**

21 Fine particles can be active carriers of toxic compounds into the alveoli of lungs. Among
22 these compounds are numerous mutagens and carcinogens. The direct acting mutagenicity
23 per unit mass of fine particulate matter (PM) is significantly higher than those of coarse
24 particles, especially in urban areas. In this study, the mutagenic properties of urban PM_{2.5}
25 and PM₁₀ were evaluated, and the role of nitro-compounds was estimated. PM_{2.5} and
26 PM₁₀ samplings, NO_x and some PAHs determinations were performed daily in 2007 in Turin
27 following a consolidated in vitro test – the *Salmonella* assay – that was conducted with
28 PM_{2.5} and PM₁₀ organic extracts. The mutagenic properties were assessed for each month
29 of sampling with *S. typhimurium* TA98- and TA98-derived strains: a nitroreductase-less
30 mutant strain (TA98NR) and an added nitroreductase-producing plasmid strain (YG1021).
31 The annual measured mean levels of PM_{2.5} and PM₁₀ were 34±20 and 48±18 µg/m³. The
32 PM_{2.5}/PM₁₀ ratio ranged from 0.36 to 0.89. The *Salmonella* assay showed higher
33 mutagenicity in autumn/winter (20±15 TA98NR, 54±39 TA98, 173±161 YG1021 net
34 revertens/m³) with respect to spring/summer (2±2 TA98NR, 7±8 TA98, 24±27 YG1021 net
35 revertens/m³)(p<0.01). There are also statistically significant seasonal differences in the
36 gravimetric analysis data. The ratio between the TA98 net revertants per PM_{2.5} µg is 6.5
37 times greater than per PM₁₀ µg. Moreover, the bioassay results showed an amplified
38 response in the YG1021 strain and a reduced response in the TA98NR strain. The net
39 revertant ratio TA98NR/YG1021 is equal to 11±4 for PM_{2.5} and 13±6 for PM₁₀ organic
40 extracts (p<0.01). There is a significant correlation with NO_x and PAHs concentrations. These
41 findings can describe the relevant role of nitro compounds, and they underline the priority
42 in prevention improvements to reduce nitrated molecule air pollution.

43 **INTRODUCTION:**

44 Atmospheric pollution has significant effects on human health and, more generally, on a
45 population's quality of life [58]. Epidemiological studies have clearly associated negative
46 health effects, especially respiratory diseases, with exposure to air pollution [1]. Also, the
47 relationship between lung cancer and air pollution is widely debated [2], and various studies
48 have discussed the significance of this kind of correlation [3,4]. Borderline results are
49 justified by the complexity of the factors involved in this environmental problem. First of all,
50 the physiological effects of air pollution on lung function have only been studied since the
51 1990s by epidemiologic studies, and difficulties arise from the heterogeneity of air pollution
52 (gaseous phase and particle phase), the long-term appearance of the main effects, and the
53 heterogeneity of genetic and other environmental factors in the involved populations [5].
54 However, numerous experimental studies conducted in vitro and in vivo have shown
55 evidence of mutagenic and genotoxic effects [6]. These effects range from a simple
56 point mutation [7] to epigenetic effects [8]. In vitro toxicology evaluations have
57 reported significant negative effects attributable to the particulate fraction in relation to the
58 dose of exposure [9]. However, air pollution is the result of continuous interactions between
59 gaseous and particulate phases. The equilibrium depends on various factors, including
60 environmental temperature, primary compound emission sources, and dispersion ratio [10].
61 Among the more genotoxic and mutagenic compounds, we can distinguish nitro
62 compounds, such as nitro-polycyclic aromatic hydrocarbons (nitro-PAHs). These chemicals
63 can be both directly emitted into the atmosphere and produced by the interaction between
64 PAHs and NO_x. The presence of these two groups of chemicals is common in both outdoor
65 [11] and indoor environments [12], and their interactions have been observed in the

66 combustion rooms of diesel motors [13]. Environmentally higher levels of PAHs and NO_x
67 with a low dispersion rate entail relevant formations of nitro-derived compounds [10,14,15].
68 The **particulate phase or gas phase** form of specific nitro-PAHs depends on molecular
69 weight, temperature and pollution entity [14,16]. Fine particles present a higher number of
70 particles per volume unit, and they have major superficial extension. They may be active
71 carriers of toxic compounds into the lung alveoli [17]. These toxic compounds may also
72 cause DNA damage and complex genotoxic effects. Particulate matter shows a higher
73 toxicity indirectly proportional to the aerodynamic diameter of the particles. Thus, fine
74 particles seem to be more genotoxic than coarse particles [18]. Although the effects of
75 particle sizes below 10 µm (PM₁₀) were investigated in the initial studies, even smaller
76 particles have recently been regarded as questionable, and research has been conducted on
77 the health effects of minute particulate matter below 2.5 µm (PM_{2.5}). Ultrafine particles
78 have been sporadically measured [19,20].

79 Air pollution levels are tightly linked to climate and topography [21]. Air pollution episodes
80 can be particularly troublesome if the affected city is located in a valley surrounded by
81 mountains, as in the Meuse Valley in Belgium [22] and the Valley of Mexico [23], but they
82 are also a concern in the Padana Plain, where Torino is located [24]. In this study, we
83 analyse the NO_x levels and the particulate pollution in Torino's metropolitan area over the
84 course of a full year before the new European Regulation (2008/50/CE) and its Italian
85 introduction (D. Lgs. 155, 30/07/2010) were promulgated. The mutagenic properties of
86 urban PM_{2.5} and PM₁₀ were evaluated while focusing on the role of nitro-compounds by a
87 biological approach and related NO_x **and PAHs** levels that are able to influence the presence
88 of nitro-compounds in the air [25].

89

90 **MATERIALS & METHODS:**

91

92 ***PM10 and PM2.5 sampling***

93 Sampling was performed from January 2007 to December 2007 at two meteorological–
94 chemical stations of the Environmental Protection Regional Agency (Piedmont A.R.P.A.)
95 located at Torino. Torino is placed in the northwest of the Padana Plain (Italy). The first
96 sampling site (station 1), Lingotto, is located outdoors in a small green area within an
97 enclosure zone classified as urban background. The second sampling site was located in the
98 centre of the city (station 2) in a traffic-regulated street, and it is classified as a traffic station
99 [26]. Turin has a population density of 7,000 inhabitants per km², so the pressure correlated
100 to human activity on the territory is very high [56]. Moreover, the climate and topography
101 characteristics of the area contribute to critical air pollution [27]. PM10 (PM passing through
102 a size-selective inlet with a 50% efficiency cut-off at a 10 µm aerodynamic diameter) was
103 sampled on glass microfibre filters (Type A/E, 8 in×10 in, Gelman Sciences, Michigan, USA)
104 with a Sierra Andersen High Volume Sampler 1200/VFC (Andersen Samplers, Atlanta,
105 Georgia, USA) using a flow of 1,160 L/min. The sample duration was controlled by a timer
106 accurate to ±15 min over a 24-h sample period. The PM2.5 filters were glass micro-fibre
107 filters (Type A/E 47 mm \square , Gelman Sciences, Michigan, USA), and the PM2.5 sampler was a
108 PM2.5 MicroVol 1100 Low Volume Air Sampler using a flow of approximately 32 L/min. This
109 sampler is certified in compliance with EN-14907 norm requirements. The exact flow was
110 calculated daily and was corrected for variations in atmospheric pressure and actual
111 differential pressure across the filter. The filters were conditioned for 48 hours and were
112 weighted with an analytical balance ($\pm 10 \mu\text{g}$) before and after sampling to calculate the PM

113 mass concentration. The procedures were conducted according to the European Committee
114 for Standardization [55], as previously described [28].

115

116 ***Extractions and biological assays***

117 Daily filters were pooled to obtain one monthly sample in each of the three cities.
118 Extractions of each pooled sample were carried out with a Soxhlet apparatus for at least 80
119 cycles with acetone. Subsequent evaporation was induced by a Rotavapor instrument, and
120 the re-suspension of the sample was performed with dimethyl-3-sulfoxide (DMSO) to obtain
121 an equivalent concentration of $0.1 \text{ m}^3/\mu\text{l}$. The mutagenicity assay was executed according to
122 [29]. Definite concentrations of PM_{2.5} organic extract were tested to generate a dose-
123 response curve (20, 50, 100 μl of the DMSO $0.1 \text{ m}^3/\mu\text{l}$ suspension). The slope of the dose-
124 response curve (revertants/ m^3) was calculated by the least squares linear regression from
125 the first linear portion of the dose-response curve [30]. All experiments were done in
126 triplicate with at least three doses. The results were expressed as total revertants minus
127 spontaneous revertants to obtain net revertants per cubic metre (rev/m^3), and they were
128 calculated by the dose-response curve [31-33]. The mutagenic activity of airborne
129 particulate extracts was studied using *S. typhimurium* strains TA98, TA98NR and YG1021.
130 YG1021 is a 'classical' nitroreductase-overproducing strain that is obtained by cloning the
131 nitroreductase gene of *S. typhimurium* TA1538 into pBR 322 and introducing the
132 recombinant plasmid into TA98 [34]. YG1021 has a nitrofurazone reductase activity more
133 than 50 times higher than the original TA98 strain, permitting an efficient detection of
134 mutagenic nitroarenes. TA98NR lacks of 'classical' nitroreductase, and thus, the bioassays
135 describe a reduced mutagenicity proportionally to the nitroarene amounts [35,36]. The
136 spontaneous revertants obtained during 12 bioassay sessions, one for each sampling month,

137 ranged from 13 to 20 (18±2) for TA98, from 12 to 20 (16±3) for TA98NR, and from 19 to 29
138 (25±2) for YG1021. The genotype of each tester strain was routinely confirmed, and in each
139 assay session, positive and negative controls were included. 2-nitrofluorene (1 µg/plate) was
140 tested in each assay as a known mutagen positive control.

141

142 **NO_x and PAHs data**

143 The NO_x and PAHs data were extracted from a specialised database by the Regional System
144 for the real time monitoring of Air Quality, AriaWeb (Regione Piemonte, 2007). The data are
145 referred to the same day of PM samplings and to the same sampling station, and for NO_x
146 they represent a monthly mean of hourly data collected with the standard monitoring
147 method EN 14211:2005 (2008/50/EC, annex VI, section B), while for PAHs they represent a
148 monthly mean of a daily data collected on the PM_{2.5} for the station 1 and on the PM₁₀ for
149 the station 2. The determined PAHs are benzo(a)pyrene (b(a)p), benz(a)anthracene (b(a)a),
150 benzo(b)fluoranthene, benzo(j)fluoranthene and benzo(k)fluoranthene (benzo(bjk)f). The
151 adopted method consisted of extraction in toluene under sonication in a water bath
152 followed by HRGC/LRMS analysis using the internal method UT2.M128 R01 2002 (Pereira et
153 al., 2001). The benzo(b)fluoranthene, benzo(j)fluoranthene and benzo(k)fluoranthene as
154 expressed as the sum of the three compounds by the synthetic term b(b,j,k)f.

155

156 **Statistics**

157 The seasons were designated as winter/autumn (January–March and October–December),
158 and spring/summer (April–June and July–September). Statistical analyses were performed
159 using the SPSS Package, version 17.0. In particular, 1) a log transformation of non-normally
160 distributed data was carried out, 2) the Spearman rank order correlation coefficient was

161 used to assess relationships between variables, 3) a Wilcoxon test was used to compare
162 means, and 4) ANOVA was used for multivariate analysis in which we assumed an equal
163 variance while using Tukey as post-hoc multiple comparisons. The mean differences and
164 correlations were considered significant at $p < 0.05$.

165

166 **RESULTS and DISCUSSION:**

167 A description of the statistics of the measured variables for each sampling station is detailed
168 in Table 1. The particulate matter levels exceeded the yearly WHO Air Quality Guideline
169 values [37] for 7 and 6 months for PM_{2.5} and PM₁₀, respectively. The annual measured
170 mean levels of PM_{2.5} and PM₁₀ were 34 ± 20 and 48 ± 18 $\mu\text{g}/\text{m}^3$. The PM₁₀ annual mean was
171 2.4 times higher than the levels proposed as safe for human health. This ratio for PM_{2.5} was
172 3.4, which is more troubling. Station 1 showed higher pollution as expected because of its
173 zone characteristics [26]. The PM_{2.5}/PM₁₀ ratio ranged from 0.36 to 0.89, and it was higher
174 in winter/autumn than in spring/summer. This ratio is affected by the different sampling
175 sites in terms of traffic pressure, but it is comparable to those reported in the literature [38].
176 Also, the nitrogen dioxide yearly mean is higher than the reference level, but the ratio is
177 much less (1.2). The guideline of hourly value was never observed (Table 1). B[a]P is taken as
178 a marker of the PAH mixture [60]. Only for the benzo(a)pyrene (classified as IARC group 1
179 compound) there is an air guideline evaluation, the corresponding concentrations for
180 lifetime exposure producing excess lifetime cancer risks of 1/10000, 1/100000 and
181 1/1000000 are approximately 1.2, 0.12 and 0.012 ng/m^3 , respectively [62]. The figure 1
182 showed data lower than the highest risk corresponding evaluation. Moreover other PAHs
183 are classified by the IARC and the benzo(a)anthracene, benzo(b)fluoranthene,

184 benzo(k)fluoranthene and benzo(j)fluoranthene are classified as 2B group. At the moment it
185 isn't defined a real exhaustive risk evaluation on the PAHs air pollution [61].

186 The mutagenicity is much higher for the PM2.5 organic extracts and with the YG1021 strain,
187 especially in the first months of the year. The biological effects observed in the samples with
188 the strain TA98NR were limited (Table 1, Figures 1 and 3). The trend of mutagenicity and
189 NOx are shown in Figure 1/A for the first sampling station and Figure 1/B for the second
190 sampling station. The measured PAHs showed a similar level distribution during the year.

191 Both figures highlight a marked seasonal trend for every variable. As shown in Table 2, all
192 the pollutants, the nitrogen oxides, PAHs and particulate matter, are highly correlated with
193 mutagenicity with the exception of the correlation between benzo(a)anthracene and
194 YG1021 mutagenicity that is significant at the $p < 0.05$ level. The lower value of the Spearman
195 rho, observed between YG1021 mutagenicity and PAHs on the PM2.5 organic extract, seems
196 to indicate other responsibility than maternal PAHs. Recently data nitro-PAHs were
197 definitely encounter as environmental genotoxic/mutagenic hazards confirming that
198 environmental aromatic nitration reactions lead to a relevant increase in genotoxicity and
199 mutagenicity properties [39]. The interactions of nitro-aromatic compounds with DNA and
200 the resulting mutagenicity have been characterized extensively and reviewed for a wide
201 variety of monocyclic, polycyclic, and heterocyclic nitroaromatic compounds [40]. These
202 compounds produce transitions, transversions, and frameshift mutations in gene coding
203 sequences moreover oxidation and reduction products of nitroaromatic compounds can
204 damage DNA. Structural and spectroscopic studies have found that the position of the nitro
205 group on the aromatic ring and the presence of other functional groups can influence the
206 mutagenicity and carcinogenicity potency of these chemicals [41]. The NO levels are
207 characterized by a more emphatic seasonal trend as with the mutagenicity (Figure 1). The

208 *Salmonella* assay showed higher mutagenicity in autumn/winter (20±15 TA98NR, 54±39
209 TA98, 173±161 YG1021 net revertants/m³) with respect to spring/summer (2±2 TA98NR,
210 7±8 TA98, 24±27 YG1021 net revertants/m³) (T-test; p<0.01). The mean levels analysis is
211 shown in the Figure 2, and they are subdivided by particulate fraction and season. There are
212 also statistically significant seasonal differences in the gravimetric analysis both for PM2.5
213 and PM10 levels (p<0.01). The ratio between the TA98 net revertants per PM2.5 µg and per
214 PM10 µg is meanly equal to 6.5. Moreover, the bioassay results show an amplified response
215 in the YG1021 strain and a reduced response in the TA98NR strain. The determined PAH
216 levels were included on the figure 3A. The PAH levels in the PM2.5 mixtures are as equal as
217 in the PM10. Furthermore is widely supported in literature that carcinogenic compounds
218 were for the most part present in the fine particles [42]. In particular the b(a)p is often
219 higher in the PM2.5 than in the PM10, this happened for example on February (1.03 vs 0.93
220 ng/m³), March (0.28 vs 0.27 ng/m³) and December (2.25 vs 2.22 ng/m³). This supports the
221 evidence of the higher mutagenicity properties of the PM2.5 organic extract. Firstly we have
222 with a 2.5 µm cut-off during the sampling the collection of an higher number of fine
223 particles, than the total particle surface is more extensive and suitable for the adhesion of
224 the organic pollutants [59]. Successively these characteristic influence the removability
225 during the organic extraction protocol both in terms of solvent-extractable organic fraction
226 [43,44] than semi-volatile mass [45], at the end also little differences in the amount of
227 potent mutagens such as 1-nitropyrene, 1.3-dinitropyrene, 1.8-dinitropyrene are able to
228 produce a great increase of the more sensible *Salmonella* strain revertants [40]. This
229 increase is not proportional to the increase of the single pollutant, there are probably
230 synergic action that produces a biological response amplification [46]. The mutagenicity
231 data are shown by season and particulate fraction in Figure 3. Although the results show an

232 appraisable mutagenic activity for the PM organic extract, the higher mutagenicity observed
233 per PM_{2.5} µg in winter for the YG1021 strain is 6 times lower than the mutagenicity
234 observed with the same strain in urban areas of South America [47]. The ratio between
235 TA98NR/YG1021 net revertants is equal to 11±4 for PM_{2.5} and 13±6 for PM₁₀ organic
236 extracts (p<0.01), while the ratio between the mutagenicity recorded with the PM_{2.5} and
237 PM₁₀ organic extract in winter with the YG1021 strain is 5.75. These results highlight the
238 critical role of urban fine and ultra-fine PM pollution, and they show the predominant role
239 of bacterial nitroreductase on induced mutagenicity. The high ratio between YG1021 and
240 TA98NR mutagenicity recorded with the PM_{2.5} organic extract in winter/autumn of 8.9
241 (Figure 3B) suggests a reduction in photodecay of nitro compounds during the
242 autumn/winter that promoted the accumulation of these kinds of pollutants.

243

244 **CONCLUSION:**

245 The north of Italy is an area of widespread air pollution [48]. The weak dispersion rate
246 observed during winter due to the conformation of the territory represents a relevant factor
247 [49]. Various air pollution indicators are above the WHO guidelines, especially for fine
248 particulate matter, or PM_{2.5}. Also, the exposure concentration obligation value for PM_{2.5}
249 of 20 µg/m³ defined by the European Directive 2008/50/EC, and that is to be met by 2015,
250 was clearly exceeded, and it is difficult to identify improvement safety margins. The
251 pollutant emission reductions are obtainable by action, focusing on the winter/autumn
252 seasons, which involve, for example, heating systems and traffic regulation. Various
253 improvements are in progress on these crucial points. First, centralised district heating is
254 being introduced that covers almost all the urban area, reducing the difficulty of
255 controllable emission points. Second, the limited green traffic zone has been expanded [57].

256 Today, the mutagenicity recorded with the *Salmonella* assay associated with pollution is
257 relevant especially during the winter or in industrial areas, as previously described [47,50]
258 and it is mainly due to the fine fraction of the particulate pollution [51,52]. The reduction of
259 NO₂ functional groups and the generation of more reactive intermediates is fundamental to
260 the production of DNA mutations.

261 **In this study** the relationship among the mutagenic results for YG1021:TA98:TA98NR **was**
262 **quantified** as approximately 9:2.7:1 for PM_{2.5} and 7.7:2.8:1 for PM₁₀ (p<0.001). **Other**
263 **previous published PM_{2.5} data showed comparable ratios [53].** These findings further
264 highlight the important role nitro compounds play, and they underline the necessity of
265 primary prevention improvement to reduce nitrated molecule air pollution. In our work
266 without nitroreductase activity, the genotoxicity was limited. Today, the known sources of
267 these kinds of compounds are various combustion processes, especially in diesel motors,
268 where certain nitro-PAHs are formed in DPFs; however, other compounds are reduced, and
269 nitration is not a general trend [13]. **A mutagenic/carcinogenic activities is linked mainly to**
270 **o-nitroanisole, 1-nitropyrene, 4-nitropyrene, 1,6-dinitropyrene, 1,8-dinitropyrene, 6-**
271 **nitrochrysene, and nitrofen [60].** Moreover, concentrations of nitro-PAHs and other nitro
272 compounds produced from gas-phase reactions are generally correlated to NO_x pollution
273 [25], **and our work with the obtained mutagenicity data highlight this finding.** Despite their
274 critical role in the genotoxic properties of fine particulate pollution, there is currently no
275 monitoring procedure for these kinds of pollutants. **On the other hands known mutagens**
276 **accounted for only 20% of the total mutagenicity of the fine particulate extracts [52,54].**
277 **New control and monitoring strategies are auspicious in the new directives in order to**
278 **evaluate nitro-compounds but also with a biologic approach mutagenic/genotoxic**
279 **properties of the airborne mixtures.**

280 ACKNOWLEDGMENTS: This research was co-funded by the University of Turin as a local
281 project and by the Piedmont Region. The authors thank the Environmental Protection
282 Agency of Piedmont, especially dott. F. Lollobrigida and dott.ssa M. Maringo, for their
283 collaboration in the sample collection and Dr. T. Nohmi of the National Institute of Hygienic
284 Sciences of Tokyo for the *S. typhimurium* YG1021 and TA98NR supplies.

285 **TABLES:**

286 **Table 1** – Descriptive analysis of the collected data at the two sampling stations.

287

288 **Table 2** – Correlation between the collected pollution indicators ($\mu\text{g}/\text{m}^3$ for NO_x and ng/m^3
289 for PAHs) and mutagenicity (net revertants/ m^3). All Spearman's rho correlations are highly
290 significant ($p < 0.01$) with the exception of data labelled with *, in this case the correlation is
291 significant at $p < 0.05$ level.

292

293 **FIGURE LEGENDS**

294 **Figure 1** - Trend of the mutagenicity evaluated with the three *Salmonella* strains and NO_x
295 values with their standard deviations. **A:** data collected at station 1, the particulate indicator
296 is PM_{2.5}; **B:** data collected at station 1, the particulate indicator is PM₁₀.

297

298 **Figure 2** - Seasonal gravimetric analysis for PM_{2.5} (station 1) and PM₁₀ (station 2). Data are
299 divided by particles cut-off and by season. Cold period (autumn-winter) is set against hot
300 period (spring-summer). The squares indicate the mean value while the up and down lines
301 the standard deviations.

302

303 **Figure 3** - Seasonal analysis of the data for each strain for PM_{2.5} (station 1) and PM₁₀
304 (station 2). Cold period (autumn-winter) is set against hot period (spring-summer). **(A)** PAHs
305 data are divided by particles cut-off, by season and by each determined compounds The
306 circles indicate the mean value while the up and down lines the standard deviations. **(B)**
307 Mutagenicity data are divided by particles cut-off, by season and by each *Salmonella* strain.
308 The rhombus indicate the mean value while the up and down lines the standard deviations.

309

310 **REFERENCES:**

311

312 [1] C. Raheison and P.O. Girodet Epidemiology of COPD, Eur Respir Rev 18 (2009) 213-
313 221.

314 [2] P. Vineis and K. Husgafvel-Pursiainen Air pollution and cancer: biomarker studies in
315 human populations, Carcinogenesis 26 (2005) 1846-1855.

316 [3] B. Brunekreef, R. Beelen, G. Hoek, L. Schouten, S. Bausch-Goldbohm, P. Fischer, B.
317 Armstrong, E. Hughes, M. Jerrett and P. van den Brandt Effects of long-term
318 exposure to traffic-related air pollution on respiratory and cardiovascular mortality
319 in the Netherlands: the NLCS-AIR study, Res Rep Health Eff Inst (2009) 5-71;
320 discussion 73-89.

321 [4] K. Katanoda, T. Sobue, H. Satoh, K. Tajima, T. Suzuki, H. Nakatsuka, T. Takezaki, T.
322 Nakayama, H. Nitta, K. Tanabe and S. Tominaga An association between long-term
323 exposure to ambient air pollution and mortality from lung cancer and respiratory
324 diseases in Japan, J Epidemiol 21 (2011) 132-143.

325 [5] A. Landera and A.M. Mebel Mechanisms of formation of nitrogen-containing
326 polycyclic aromatic compounds in low-temperature environments of planetary
327 atmospheres: A theoretical study, Faraday Discussions 147 (2010) 479-494.

328 [6] M. Villarini, C. Fatigoni, L. Dominici, S. Maestri, L. Ederli, S. Pasqualini, S. Monarca
329 and M. Moretti Assessing the genotoxicity of urban air pollutants using two in situ
330 plant bioassays, Environ Pollut 157 (2009) 3354-3356.

- 331 [7] P. Moller, J.K. Folkmann, L. Forchhammer, E.V. Brauner, P.H. Danielsen, L. Risom and
332 S. Loft Air pollution, oxidative damage to DNA, and carcinogenesis, *Cancer Letters*
333 266 (2008) 84-97.
- 334 [8] I. Romieu, H. Moreno-Macias and S.J. London Gene by environment interaction and
335 ambient air pollution, *Proc Am Thorac Soc* 7 (2010) 116-122.
- 336 [9] L.D. Claxton and G.M. Woodall, Jr. A review of the mutagenicity and rodent
337 carcinogenicity of ambient air, *Mutat Res* 636 (2007) 36-94.
- 338 [10] D. Yaffe, Y. Cohen, J. Arey and A.J. Grosovsky Multimedia analysis of PAHs and nitro-
339 PAH daughter products in the Los Angeles Basin, *Risk Anal* 21 (2001) 275-294.
- 340 [11] K. Srogi Monitoring of environmental exposure to polycyclic aromatic hydrocarbons:
341 a review, *Environmental Chemistry Letters* 5 (2007) 169-195.
- 342 [12] M. Ciganek, J. Raszyk, J. Kohoutek, A. Ansorgova, J. Salava and J. Palac Polycyclic
343 aromatic hydrocarbons (PAHs, nitro-PAHs, oxy-PAHs), polychlorinated biphenyls
344 (PCBs) and organic chlorinated pesticides (OCPs) in the indoor and outdoor air of pig
345 and cattle houses, *Veterinari Medicina* 45 (2000) 217-226.
- 346 [13] N.V. Heeb, P. Schmid, M. Kohler, E. Gujer, M. Zennegg, D. Wenger, A. Wichser, A.
347 Ulrich, U. Gfeller, P. Honegger, K. Zeyer, L. Emmenegger, J.L. Petermann, J.
348 Czerwinski, T. Mosimann, M. Kasper and A. Mayer Secondary effects of catalytic
349 diesel particulate filters: Conversion of PAHs versus formation of Nitro-PAHs,
350 *Environmental Science & Technology* 42 (2008) 3773-3779.
- 351 [14] A. Albinet, E. Leoz-Garziandia, H. Budzinski, E. Villenave and J.L. Jaffrezo Nitrated and
352 oxygenated derivatives of polycyclic aromatic hydrocarbons in the ambient air of two
353 French alpine valleys - Part 1: Concentrations, sources and gas/particle partitioning,
354 *Atmospheric Environment* 42 (2008 a) 43-54.

- 355 [15] A. Albinet, E. Leoz-Garziandia, H. Budzinski, E. Villenave and J.L. Jaffrezo Nitrated and
356 oxygenated derivatives of polycyclic aromatic hydrocarbons in the ambient air of two
357 French alpine valleys - Part 2: Particle size distribution, *Atmospheric Environment* 42
358 (2008 b) 55-64.
- 359 [16] A. Cecinato, C. Balducci, G. Nervegna, S. Pareti, G. Tagliacozzo and A. Brachetti YEAR
360 TIME MODULATION OF n-ALKANES, PAH, NITRO-PAH AND ORGANIC ACIDS AT
361 MONTELIBRETTI ROME, ITALY, *Polycyclic Aromatic Compounds* 28 (2008) 500-517.
- 362 [17] C. Terzano, F. Di Stefano, V. Conti, E. Graziani and A. Petroianni Air pollution ultrafine
363 particles: toxicity beyond the lung, *European Review for Medical and*
364 *Pharmacological Sciences* 14 (2010) 809-821.
- 365 [18] U. Franck, O. Herbarth, S. Roder, U. Schlink, M. Borte, U. Diez, U. Kramer and I.
366 Lehmann Respiratory effects of indoor particles in young children are size
367 dependent, *Sci Total Environ* 409 (2011) 1621-1631.
- 368 [19] A. Can, M. Rademaker, T. Van Renterghem, V. Mishra, M. Van Poppel, A. Touhafi, J.
369 Theunis, B. De Baets and D. Botteldooren Correlation analysis of noise and ultrafine
370 particle counts in a street canyon, *Sci Total Environ* 409 (2011) 564-572.
- 371 [20] Y. Wang, P.K. Hopke, D.C. Chalupa and M.J. Utell Long-term characterization of
372 indoor and outdoor ultrafine particles at a commercial building, *Environ Sci Technol*
373 44 (2010) 5775-5780.
- 374 [21] J. Wallace, D. Corr and P. Kanaroglou Topographic and spatial impacts of
375 temperature inversions on air quality using mobile air pollution surveys, *Sci Total*
376 *Environ* 408 (2010) 5086-5096.
- 377 [22] B. Nemery, P.H.M. Hoet and A. Nemmar The Meuse Valley fog of 1930: an air
378 pollution disaster, *Lancet* 357 (2001) 704-708.

- 379 [23] K.E. Kelly, I.C. Jaramillo, M. Quintero-Nunez, D.A. Wagner, K. Collins, H.L. Meuzelaar
380 and J.S. Lighty Low-wind/high particulate matter episodes in the Calexico/Mexicali
381 region, *J Air Waste Manag Assoc* 60 (2010) 1476-1486.
- 382 [24] D. Traversi, R. Degan, R. De Marco, G. Gilli, C. Pignata, M. Ponzio, M. Rava, F.
383 Sessarego, S. Villani and R. Bono Mutagenic properties of PM_{2.5} air pollution in the
384 Padana Plain (Italy) before and in the course of XX Winter Olympic Games of "Torino
385 2006", *Environ Int* 34 (2008) 966-970.
- 386 [25] H.A. Bamford, D.Z. Bezabeh, M.M. Schantz, S.A. Wise and J.E. Baker Determination
387 and comparison of nitrated-polycyclic aromatic hydrocarbons measured in air and
388 diesel particulate reference materials, *Chemosphere* 50 (2003) 575-587.
- 389 [26] J.P. Putaud, R. Van Dingenen, A. Alastuey, H. Bauer, W. Birmili, J. Cyrys, H. Flentje, S.
390 Fuzzi, R. Gehrig, H.C. Hansson, R.M. Harrison, H. Herrmann, R. Hitzenberger, C.
391 Hüglin, A.M. Jones, A. Kasper-Giebl, G. Kiss, A. Kousa, T.A.J. Kuhlbusch, G. Loschau,
392 W. Maenhaut, A. Molnar, T. Moreno, J. Pekkanen, C. Perrino, M. Pitz, H. Puxbaum, X.
393 Querol, S. Rodriguez, I. Salma, J. Schwarz, J. Smolik, J. Schneider, G. Spindler, H. ten
394 Brink, J. Tursic, M. Viana, A. Wiedensohler and F. Raes A European aerosol
395 phenomenology-3: Physical and chemical characteristics of particulate matter from
396 60 rural, urban, and kerbside sites across Europe, *Atmospheric Environment* 44
397 (2010) 1308-1320.
- 398 [27] M.E. Hazenkamp-Von Arx, T. Gotschi, U. Ackermann-Liebrich, R. Bono, P. Burney, J.
399 Cyrys, D. Jarvis, L. Lillienberg, C. Luczynska, J.A. Maldonado, A. Jaen, R. de Marco,
400 Y.H. Mi, L. Modig, L. Bayer-Oglesby, F. Payo, A. Soon, J. Sunyer, S. Villani, J. Weyler
401 and N. Kunzli PM_{2.5} and NO₂ assessment in 21 European study centres of ECRHS II:

- 402 annual means and seasonal differences, *Atmospheric Environment* 38 (2004) 1943-
403 1953.
- 404 [28] G. Gilli, D. Traversi, R. Rovere, C. Pignata and T. Schiliro Chemical characteristics and
405 mutagenic activity of PM10 in Torino, a northern Italian City, *Science of the Total*
406 *Environment* 385 (2007 b) 97-107.
- 407 [29] D.M. Maron and B.N. Ames Revised methods for the Salmonella mutagenicity test,
408 *Mutat Res* 113 (1983) 173-215.
- 409 [30] G. Gilli, C. Pignata, T. Schiliro, R. Bono, A. La Rosa and D. Traversi The mutagenic
410 hazards of environmental PM2.5 in Turin, *Environmental Research* 103 (2007 a) 168-
411 175.
- 412 [31] A. Buschini, F. Cassoni, E. Anceschi, L. Pasini, P. Poli and C. Rossi Urban airborne
413 particulate: genotoxicity evaluation of different size fractions by mutagenesis tests
414 on microorganisms and comet assay, *Chemosphere* 44 (2001) 1723-1736.
- 415 [32] F. Cassoni, C. Bocchi, A. Martino, G. Pinto, F. Fontana and A. Buschini The Salmonella
416 mutagenicity of urban airborne particulate matter (PM2.5) from eight sites of the
417 Emilia-Romagna regional monitoring network (Italy), *Science of the Total*
418 *Environment* 324 (2004) 79-90.
- 419 [33] L.D. Claxton, P.P. Matthews and S.H. Warren The genotoxicity of ambient outdoor
420 air, a review: Salmonella mutagenicity, *Mutat Res* 567 (2004) 347-399.
- 421 [34] P.D. Josephy, P. Gruz and T. Nohmi Recent advances in the construction of bacterial
422 genotoxicity assays, *Mutat Res* 386 (1997) 1-23.
- 423 [35] P. Einisto, M. Watanabe, M. Ishidate, Jr. and T. Nohmi Mutagenicity of 30 chemicals
424 in *Salmonella typhimurium* strains possessing different nitroreductase or O-
425 acetyltransferase activities, *Mutat Res* 259 (1991) 95-102.

- 426 [36] P. Hrelia, C. Fimognari, F. Maffei, D. Spinelli, L. Lamartina, M.C. Sarva and G. Cantelli
427 Forti Influence of nitroreductase and O-acetyltransferase on the mutagenicity of
428 substituted nitrobenzothiophenamines in *Salmonella typhimurium*, *Chem Biol*
429 *Interact* 118 (1999) 99-111.
- 430 [37] M. Krzyzanowski WHO Air Quality Guidelines for Europe, *J Toxicol Environ Health A*
431 71 (2008) 47-50.
- 432 [38] X. Querol, A. Alastuey, C.R. Ruiz, B. Artinano, H.C. Hansson, R.M. Harrison, E. Buringh,
433 H.M. ten Brink, M. Lutz, P. Bruckmann, P. Straehl and J. Schneider Speciation and
434 origin of PM10 and PM2.5 in selected European cities, *Atmospheric Environment* 38
435 (2004) 6547-6555.
- 436 [39] A. Bonnefoy, S. Chiron and A. Botta Environmental nitration processes enhance the
437 mutagenic potency of aromatic compounds, *Environ Toxicol* (2010).
- 438 [40] V. Purohit and A.K. Basu Mutagenicity of nitroaromatic compounds, *Chem Res*
439 *Toxicol* 13 (2000) 673-692.
- 440 [41] K.S. Ju and R.E. Parales Nitroaromatic compounds, from synthesis to biodegradation,
441 *Microbiol Mol Biol Rev* 74 (2010) 250-272.
- 442 [42] K. Slezakova, D. Castro, M.C. Pereira, S. Moralis, C. Delerue-Matos and M.C. Alvim-
443 Ferraz Influence of traffic emissions on the carcinogenic polycyclic aromatic
444 hydrocarbons in outdoor breathable particles, *J Air Waste Manag Assoc* 60 (2010)
445 393-401.
- 446 [43] B.L. Valle-Hernandez, V. Mugica-Alvarez, E. Salinas-Talavera, O. Amador-Munoz,
447 M.A. Murillo-Tovar, R. Villalobos-Pietrini and A. De Vizcaya-Ruiz Temporal variation
448 of nitro-polycyclic aromatic hydrocarbons in PM10 and PM2.5 collected in Northern
449 Mexico City, *Sci Total Environ* 408 (2010) 5429-5438.

- 450 [44] R. Ladji, N. Yassaa, C. Balducci, A. Cecinato and B.Y. Meklati Distribution of the
451 solvent-extractable organic compounds in fine (PM1) and coarse (PM1-10) particles
452 in urban, industrial and forest atmospheres of Northern Algeria, *Sci Total Environ*
453 408 (2009) 415-424.
- 454 [45] J.C. Hansen, W.R. Woolwine, 3rd, B.L. Bates, J.M. Clark, R.Y. Kuprov, P. Mukherjee,
455 J.A. Murray, M.A. Simmons, M.F. Waite, N.L. Eatough, D.J. Eatough, R. Long and B.D.
456 Grover Semicontinuous PM2.5 and PM10 mass and composition measurements in
457 Lindon, Utah, during winter 2007, *J Air Waste Manag Assoc* 60 (2010) 346-355.
- 458 [46] S.J. de Andrade, S.D. Varella, G.T. Pereira, G.J. Zocolo, M.R. de Marchi and E.A.
459 Varanda Mutagenic activity of airborne particulate matter (PM10) in a sugarcane
460 farming area (Araraquara city, southeast Brazil), *Environ Res* 111 (2011) 545-550.
- 461 [47] T.S. Pereira, G.N. Gotor, L.S. Beltrami, C.G. Nolla, J.A.V. Rocha, F.P. Broto, L.R.
462 Comellas and V.M.F. Vargas Salmonella mutagenicity assessment of airborne
463 particulate matter collected from urban areas of Rio Grande do Sul State, Brazil,
464 differing in anthropogenic influences and polycyclic aromatic hydrocarbon levels,
465 *Mutation Research-Genetic Toxicology and Environmental Mutagenesis* 702 (2010)
466 78-85.
- 467 [48] E. Cadum, G. Berti, A. Biggeri, L. Bisanti, A. Faustini and F. Forastiere [The results of
468 EpiAir and the national and international literature], *Epidemiol Prev* 33 (2009) 113-
469 119; 123-143.
- 470 [49] S. Poncino, S. Bande and M. Muraro [Meteo-diffusive analysis: a case-study of Turin],
471 *Epidemiol Prev* 33 (2009) 27-33.

- 472 [50] M.V. Coronas, R.C. Horn, A. Ducatti, J.V. Rocha and V.M.F. Vargas Mutagenic activity
473 of airborne particulate matter in a petrochemical industrial area, *Mutation Research-
474 Genetic Toxicology and Environmental Mutagenesis* 650 (2008) 196-201.
- 475 [51] G. Gilli, D. Traversi, R. Rovere, C. Pignata and T. Schiliro Airborne particulate matter:
476 Ionic species role in different Italian sites, *Environ Res* 103 (2007 c) 1-8.
- 477 [52] D.U. Pedersen, J.L. Durant, B.W. Penman, C.L. Crespi, H.F. Hemond, A.L. Lafleur and
478 G.R. Cass Human-cell mutagens in respirable airborne particles in the northeastern
479 United States. 1. Mutagenicity of fractionated samples, *Environ Sci Technol* 38 (2004)
480 682-689.
- 481 [53] D. Traversi, R. Degan, R. De Marco, G. Gilli, C. Pignata, S. Villani and R. Bono
482 Mutagenic properties of PM2.5 urban pollution in the Northern Italy: The nitro-
483 compounds contribution, *Environment International* 35 (2009) 905-910.
- 484 [54] D.U. Pedersen, J.L. Durant, K. Taghizadeh, H.F. Hemond, A.L. Lafleur and G.R. Cass
485 Human cell mutagens in respirable airborne particles from the northeastern United
486 States. 2. Quantification of mutagens and other organic compounds, *Environ Sci
487 Technol* 39 (2005) 9547-9560.
- 488 [55] CEN. Air quality - determination of the PM10 fraction of suspended particulate
489 matter – reference method and field test procedure to demonstrate reference
490 equivalence of measurement methods. Brussels: European Committee for
491 Standardization; (European Standard EN 12341) 1998.
- 492 [56] Istituto Nazionale di Statistica, 2008. *Annuario statistico 2008*.
- 493 [57] Comune di Torino, 2008. *Piano Urbano della Mobilità Sostenibile*.
- 494 [58] World Health Organization Europe. 2006. *Air Quality Guidelines — Global Update
495 2005*.

- 496 [59] Buser, M.D., Parnell, C.B., Lacey R.E., Shaw, B.W., Auvermann, B.W. 2001. Inherent
497 biases of PM₁₀ and PM_{2.5} samplers based on the interaction of particle size and
498 sampler performance characteristics. An ASAE Meeting Presentation.
- 499 [60] U.S. Department of Health and Human Services Secretary Kathleen Sebelius. 2011.
500 12th Report on Carcinogens (RoC).
- 501 [61] World Health Organization Europe. 2010. WHO guidelines for indoor air quality:
502 selected pollutants.
- 503 [62] World Health Organization Europe. 2000. Air quality guidelines for Europe 2nd
504 edition.
- 505