

Sauvain, J.J.; Deslarzes, S.; Storti, F.; Riediker, M. **Oxidative potential of particles in different occupational environments: a pilot study.** The Annals of Occupational Hygiene, 59(7):882-894, 2015.

Postprint version	Final draft post-refereeing
Journal website	http://annhyg.oxfordjournals.org/content/by/year
Pubmed link	http://www.ncbi.nlm.nih.gov/pubmed/25846363
DOI	doi:10.1093/annhyg/mev024

Oxidative potential of particles in different occupational environments: A Pilot study

Sauvain Jean-Jacques ¹§, Deslarzes Simon ¹, Storti Ferdinand ¹, Riediker Michael ^{1,2}

¹ Institute for Work and Health, University of Lausanne + Geneva, Route de la Corniche 2, 1066 Epalinges-Lausanne Switzerland

² SAFENANO, Institute of Occupational Medicine (IOM) Singapore, 30 Raffles Place, 048622 Singapore

§: corresponding author

Abstract

The oxidative potential (OP) of particulate matter has been proposed as a toxicologically relevant metric. This concept is already frequently used for hazard characterisation of ambient particles but it is still seldom applied in the occupational field. The objective of this study was to assess the OP in two different types of workplaces and to investigate the relationship between the OP and the physico-chemical characteristics of the collected particles. At a toll station at the entrance of a tunnel ("Tunnel" site) and at three different mechanical yards ("Depot" sites), we assessed particle mass (PM₄ and PM_{2.5} and size distribution), number and surface area, organic and elemental carbon, PAH and four quinones as well as iron and copper concentration. The OP was determined directly on filters without extraction by using the dithiothreitol assay (DTT assay-OP^{DTT}). The averaged mass concentration of respirable particles (PM₄) at the Tunnel site was about twice the one at the Depot sites (173±103 µg/m³ and 90±36 µg/m³ respectively), whereas the OP^{DTT} was practically identical for all the sites (10.6±7.2 pmol DTT.min⁻¹.µg⁻¹ at the Tunnel site; 10.4±4.6 pmol DTT.min⁻¹.µg⁻¹ at the Depot sites). The OP^{DTT} of PM₄ was mostly present on the smallest PM_{2.5} fraction (OP^{DTT} PM_{2.5}: 10.2±8.1 pmol DTT.min⁻¹.µg⁻¹; OP^{DTT} PM₄: 10.5±5.8

1
2
3 pmol DTT.min⁻¹.µg⁻¹ for all sites), suggesting the presence of redox inactive components in
4
5 the PM_{2.5-4} fraction. Although the reactivity was similar at the Tunnel and Depot sites
6
7 irrespective of the metric chosen (OP^{DTT}/µg or OP^{DTT}/m³), the chemicals associated with
8
9 OP^{DTT} were different between the two types of workplaces. The organic carbon, quinones
10
11 and/or metal content (Fe, Cu) were strongly associated with the DTT reactivity at the Tunnel
12
13 site whereas only Fe and PAH were associated (positively and negatively respectively) with
14
15 this reactivity at the Depot sites. These results demonstrate the feasibility of measuring of the
16
17 OP^{DTT} in occupational environments and suggest that the particulate OP^{DTT} is integrative of
18
19 different physico-chemical properties. This parameter could be a potentially useful exposure
20
21 proxy for investigating particle exposure related oxidative stress and its consequences.
22
23 Further research is needed mostly to demonstrate the association of OP^{DTT} with relevant
24
25 oxidative endpoints in humans exposed to particles.
26
27

28
29 Keywords:

30
31 Oxidative potential, DTT assay, occupational exposure, particulate matter, quinones
32
33
34
35

36 37 **1. Introduction**

38
39 Numerous epidemiological studies have associated particle mass exposure to adverse
40
41 health effects. However, particulate matter (PM) presents generally quite a complex, dynamic
42
43 and variable composition. It is known that at least for ambient PM, most of its mass is
44
45 biologically inactive (Borm *et al.*, 2007). Toxicologically important particle constituents like
46
47 polycyclic aromatic hydrocarbons (PAH) or metals represent only a very small fraction of the
48
49 particle mass. Studies about effects of PM suggest that oxidative stress plays an important
50
51 role in the biological effect induced by particles (Brook *et al.*, 2010). Oxidative stress is an
52
53 imbalance between production of reactive oxygen species (ROS) and antioxidants in cells
54
55 and tissues. This perturbation of the intra- and extra-cellular redox homeostasis could results
56
57 from i) the intrinsic presence on the particles surface of radicals entities (Hopke, 2008); ii) the
58
59 catalytic generation of radicals by redox active chemicals on the particle surface (Limbach *et*
60

1
2
3 *al.*, 2007) in presence of reducing compounds; iii) the activation of cellular responses by the
4 particles (Donaldson *et al.*, 2002); or iv) a combination of these different processes. Either of
5 these mechanism may lead to an overproduction of potentially toxic species like superoxide
6 ($O_2^{\bullet -}$) or hydroxide (OH^{\bullet}) radicals as well as hydrogen peroxide (H_2O_2), all resulting from the
7 reduction of oxygen and collectively called reactive oxygen species (ROS) (Halliwell and
8 Gutteridge, 2007). The cellular increase of ROS could explain at least partly the pro-
9 inflammatory effect of PM in the lungs (Donaldson *et al.*, 2005) and on the cardiovascular
10 system (Donaldson *et al.*, 2001). Therefore, the measurement of the ability of particles to
11 generate ROS, corresponding to their intrinsic oxidative potential (OP), could provide a better
12 health-based exposure metric than mass.

13
14
15
16
17
18
19
20
21
22
23 Several approaches were already used for quantifying the intrinsic OP of ambient micro- or
24 nanoparticles by purely acellular tests (reviewed by Ayres *et al.* (2008)). In order to mimic
25 what may happen when particles deposit in the reducing milieu of the lung (Cross *et al.*,
26 1994), one approach is to determine how fast particles are able to deplete reducing
27 compounds in solution. Whereas not a physiologically constituent of the lung lining fluid,
28 dithiothreitol (DTT) can be considered a surrogate of glutathione, as both compound oxidise
29 at a similar rate in phosphate buffer solutions (Held and Biaglow, 1994). In the DTT assay,
30 redox-active components of PM can be reduced by accepting the DTT electrons (Figure 1,
31 path 1). These PM components may then be regenerated (i.e reoxidised) by transferring the
32 gained electrons to dissolved oxygen, thus producing $O_2^{\bullet -}$ (Figure 1, path 2). This step
33 corresponds to the catalytical part of a redox-cycling process. The superoxide anion will
34 allow the production of H_2O_2 and OH^{\bullet} by dismutation (Figure 1, path 3) and Fenton-like
35 reactions respectively (Figure 1, path 4). The DTT assay was used before for
36 characterisation of ambient PM (Cho *et al.*, 2005), combustion engine emissions (Geller *et*
37 *al.*, 2006) and engineered nanoparticles (Sauvain *et al.*, 2008). No study so far described its
38 use in occupational situations. The objective of this study was to characterise the DTT-based
39 OP (OP^{DTT}) of particles in different occupational situations and to investigate the relationship
40 between this OP^{DTT} and other aerosol physico-chemical characteristics, including the particle
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 number and surface area concentration, components such as organic and elemental carbon,
4 metals, and PAH as well as four quinones. This would allow assessing the usefulness of this
5 method for measuring the OP of particles in occupational settings.
6
7

8 9 10 11 **2. Materials and method**

12 13 *2.1. Developments for the filter-based measurement of the redox activity of particles*

14
15 The particles OP methodology used was based on collecting particles on filters followed by
16 assessing the OP^{DTT} on this support. We sampled particles onto poly(1,1,2,2-
17 tetrafluoroethylene (PTFE) filters (Table 1). The DTT assay was done after a maximum of 48
18 hours storage at ambient temperature (20±3°C). These measurement conditions were
19 selected based on preliminary tests investigating the effect of the filter type (quartz or PTFE),
20 the storage time and the collected particle mass on the OP^{DTT} (see supplementary material in
21 online edition). The DTT consumption rate was evaluated without extracting the filter in
22 solvents, therefore the obtained blank-corrected reactivity corresponds to the total OP of the
23 sampled PM (Table 1). Briefly, a punch of the filter containing less than 1 mg particles was
24 immersed (loaded side facing the liquid) in a beaker containing 5 ml of 0.6 mg/L Tween 80[®]
25 (Fluka, Switzerland) and sonicated for 15 minutes at 180 W (Branson 5210, Milian,
26 Switzerland). Five ml of a DTT 100 µM solution (Sigma-Aldrich, Switzerland) was added. The
27 DTT loss over time at 37°C and under agitation was measured at different times (5, 10, 15,
28 20, 25, 30, 35 min). Each time, a 500 µl aliquot of the reacting solution was removed and
29 added to 0.5 ml of 10% trichloroacetic acid to stop the DTT oxidation. The oxidised amount
30 of DTT was determined by adding 2 ml of 0.4 M Tris buffer pH 8.4 than 50 µl of 10 mM 5,5'-
31 dithio-bis(2-nitrobenzoic acid) (DTNB, Fluka Switzerland). The resulting yellow 2-nitro-5-
32 thiobenzoic acid was quantified spectrophotometrically at 412 nm. The rate of DTT
33 consumption was obtained by calculating the slope of the linear regression of absorbance
34 against time, as described earlier (Sauvain et al., 2008). The average DTT consumption rate
35 of the loaded filters (done in duplicates) was corrected with the results obtained from a blank
36 filter treated in the same way.
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

2.2. Selected workplaces for measuring the particle oxidative potential

Two types of workplaces with PM presenting potentially different physico-chemical characteristics were assessed. Typical combustion-engine derived PM was collected in a toll station at the entrance of a tunnel between Switzerland and Italy. The air quality of this workplace (abbreviated "Tunnel") is strongly influenced by the wind direction. When southerly winds prevail, most of the vehicle's emissions inside the 5.8 km long tunnel are carried towards the toll station, strongly increasing the levels of pollutants at this site. In contrast, northerly winds will bring in clean air towards the measurement point and dilute the emissions from the passing traffic. During the sampling days, about 40 vehicles/hour circulated in this tunnel in each direction with 70% of the vehicles being light duty vehicles and the rest being heavy duty vehicles. Activities at this workplace include fees collection, tunnel infrastructures maintenance and custom controls. This site was sampled in January and February 2009 three times at two different locations near the same tunnel entrance during two consecutive days, resulting in a total of twelve 8 hour sampling events.

Several mechanical yards were studied as models for more complex and mixed aerosols. They originated from diesel bus emissions and were potentially enriched with metals and organics from repairing activities. Three mechanical yards (abbreviated "Depot") were sampled one time each at two different locations during two consecutive days, between March and Mai 2009. In "Depot 1", small repair activities like changing tires, washing and storing diesel buses and trams were observed during the sampling days. More important mechanical activities were performed in "Depot 2" including replacement of motor lubricant, use of solvents and metal grinding. The third site ("Depot 3") was used for mechanical repair and maintenance of diesel and electric busses as well as a bus parking.

2.3. Workplace sampling

Two different sampling locations were considered in each workplace to obtain a wide variability of PM characteristics. In order to collect a quantity of particle sufficient to

1
2
3 determine their quinones, PAH and metal content, high volume PM₄ sampling was done by
4 deploying a Digitel[®] DH77 (580 L/min, Digitel, Switzerland) and a GravikonPM4[®] (60 L/min,
5 Strohlein, Germany) side by side (Table 1). The OP^{DTT} for PM₄ was measured on punches
6 from filters of the GravikonPM4[®]. Low volume samplers were also used at both locations for
7 the determination of the PM₄ content of OC, EC and the OP^{DTT} of PM_{2.5} (Table 1).
8

9
10 Due to logistical constrains, the Digitel[®] DH77 and instruments for the characterization of the
11 particulate size distribution were used only at one location. The mass-size distribution was
12 determined with an Andersen Cascade Impactor in nine size fractions: 0-0.4 μm; 0.4-0.7 μm;
13 0.7-1.1 μm; 1.1-2.1 μm; 2.1-3.3 μm; 3.3-4.7 μm; 4.7-7 μm; 7-11 μm; and >11 μm. The
14 number-size distribution was assessed with a Scanning Mobility Particle Sizer (SMPS,
15 Grimm, Ainring, Germany, L-DMA classifier) using the manufacturer's recommended flow
16 settings, which allows sizing particles from 11 to 1083 nm. The particle surface area was
17 derived from these measurements using the Grimm software (5.477, vers 1.34).
18

19 Before gravimetric analysis, filters were conditioned for 24 hours at constant humidity (53 ±
20 4%) and ambient temperature (20±3°C). Laboratory field blanks were used for correcting the
21 measured loaded mass. After weighting, filters from the different high volume sampler were
22 sectioned to allow multiple chemical analyses (Table 1). The four toxic quinones 1,2-
23 naphthoquinone, 1,4-naphthoquinone, 9,10-anthraquinone and 9,10-phenanthraquinone were
24 determined as described by Cho et al (2004). Filters were ultrasonicated in toluene and the
25 organic extract was concentrated under nitrogen gas to 200 μl. The extracted quinones were
26 acetylated at 75°C in presence of zinc and acetic anhydride (Fluka, Switzerland). The
27 derivatization was stopped after 30 minutes by adding water in the reacting media. The
28 diacetylated quinones were extracted in pentane and quantified with a GC-MS system
29 (Saturn 4000, Varian, Switzerland). Seven different PAHs with molecular weight larger than
30 252 were determined using an earlier published method (Sauvain et al., 2003). Briefly, the
31 filters were Soxhlet extracted in toluene and the organic extract was subsequently purified
32 through solid phase extraction, fractionated with a semi-preparative HPLC column and the
33 PAH fraction finally partitioned by liquid-liquid extraction. The purified extract was injected on
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 a GC-MS system (Saturn 4000, Varian, Switzerland). Total iron and copper were determined
4
5 after ultrasonic digestion of the filter with HF and subsequently with HCl:HNO₃ 2:1. After
6
7 diluting the residue, the metal concentrations were measured with an Atomic Absorption
8
9 Spectrometer equipped with a graphite furnace (Perkin-Elmer HGA 700, Schwerzenbach,
10
11 Switzerland) following an accredited ISO/EN 17025 method. Finally, a coulometric-based
12
13 method (Perret *et al.*, 1999) was used for determining the organic carbon (OC) and
14
15 elemental carbon (EC) content of the particles.

16 17 *2.4. Data treatment*

18
19 All statistical analyses were performed with STATA (STATA/IC 12.1, College Station USA).
20
21 Values smaller than the limit of detection (LOD, corresponding to three time the standard
22
23 deviation of a blank) were replaced by ½ LOD in the database for statistical treatment. Due
24
25 to the rather low sample size, the robust nonparametric Mann-Whitney test was used to
26
27 evaluate differences in aerosol characteristics at both study sites. The association between
28
29 the PM physico-chemical characteristics and the OP^{DTT} (expressed as air concentration) was
30
31 assessed separately for each workplace type (“Tunnel” or “Depot”) by using the Spearman
32
33 rank correlation with the associated probability. Association was considered significant with
34
35 the probability $p < 0.05$.

36 37 38 39 **3. Results**

40 41 42 *3.1 Physico-chemical characteristics of the particles at the different workplaces*

43 44 *3.1.1 Physical characteristics*

45
46 Table 2 presents the physical characteristics of the particles collected at the different
47
48 workplaces. High particle mass concentrations were measured at the “Tunnel” site during the
49
50 four initial sampling days, when the wind came from the south through the tunnel. In contrast,
51
52 low concentrations were measured when the wind came from the north (“Tunnel” 5 and 6).
53
54 These two days were also particularly cold (average -2.7°C). Concentrations measured at
55
56 the three “Depot” sites were lower and less variable.

57
58
59
60

1
2
3 All the mass-based size distributions were multimodal (see supplementary material, Figure
4 S4 in online edition), with a significant larger median diameter at the "Tunnel" site (6.6 ± 1.3
5 μm) compared to the "Depot" sites ($3.3\pm 0.6 \mu\text{m}$; Mann-Whitney test, $p=0.004$). The particle
6 number concentration was rather constant at "Tunnel" ($104'000\pm 17'000 \text{ cm}^{-3}$) and higher
7 compared to the different "Depot" workplaces ($60'000\pm 30'000 \text{ cm}^{-3}$; Mann-Whitney test,
8 $p=0.025$). The geometric mean diameter (GMD) measured at the Tunnel site was quite
9 variable, with larger diameters on days with southerly winds and warmer temperatures
10 ($52\pm 10 \text{ nm}$, $n=4$) compared to situations with northerly wind ($24\pm 3 \text{ nm}$, $n=2$). At the "Depot"
11 sites, the GMD was quite similar for all sampling days (average $47\pm 4 \text{ nm}$, $n=6$). At both sites
12 the geometric standard deviation was about 2.3, which suggests that all sites presented
13 polydisperse particles. When the SMPS-derived surface concentration was considered, a
14 broad range of values were obtained (Table 2), with the smallest surface concentration for
15 "Tunnel" 5 and 6 (cold situation with north wind going into the tunnel), followed by all the
16 Depot sites and the largest values for the "Tunnel" 1 to 4. Typical averaged number- and
17 surface-based size distribution measured at the "Tunnel" and "Depot" sites are given in the
18 supplementary material (Figure S5).
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33

34 3.1.2. Chemical characteristics

35
36 Table 3 presents the different chemical compounds analysed. OC was the predominant
37 measured compound. At the "Tunnel" site on average $48\pm 4\%$ of the measured PM_4 mass
38 was explained by the sum of OC, EC, Fe and Cu, while at the "Depot" the same components
39 explained a larger part of the PM_4 mass ($70\pm 12\%$). For this calculation, we used an organic
40 molecular weight per carbon weight ratio of 1.6, as recommended by Turpin *et al.*, 2001 (see
41 supplementary material Figure S3 in online edition). The mass-based OC content of the
42 collected particles was significant lower in the "Tunnel" site (average $33\pm 13\%$, $n=12$)
43 compared to the "Depot" sites (average $45\pm 11\%$, $n=12$; Mann-Whitney test, $p=0.018$). The
44 ratio of elemental to total carbon (with total carbon TC corresponding to the sum of OC+EC),
45 differed significantly between the two sites (average "Tunnel": $25\pm 9\%$, $n=12$; average
46 "Depot": $16\pm 9\%$, $n=12$; Mann-Whitney test, $p=0.021$) but also within the tunnel in function of
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 the wind direction. The sum of the four analysed quinones was always significant higher at
4 the "Tunnel" site (Mann-Whitney test, $p=0.01$). Among the four analysed quinones, 9,10-
5 anthraquinone was the most abundant; the two volatile naphthoquinones presented lower
6 concentrations. Iron was present in larger concentrations than Cu and both were strongly
7 influenced by the wind direction at the "Tunnel" site.
8

9
10
11 The chemical compounds showed a different correlation pattern at the two workplaces (see
12 supplementary material Table S1 and Fig S6-S9 in online edition). At the "Tunnel" site,
13 consistent correlations were observed between OC and EC, Σ 4 Quinones and metals (Fe,
14 Cu); EC with metals (Fe, Cu) and Σ 4 Quinones with Fe. At the "Depot" site, no association
15 was observed between any measured chemical variables.
16
17
18
19
20
21

22 3.2. Oxidative potential

23
24
25
26
27 The intrinsic mass-based OP^{DTT} ($OP^{DTT}/\mu\text{g}$) of the sampled particles is given in Figure 2 A.
28
29 The $OP^{DTT}/\mu\text{g}$ didn't differ significantly between both sites (PM_4 : 10.6 ± 7.2 pmol DTT/min/ μg
30 at "Tunnel", $n=11$; 10.4 ± 4.6 pmol DTT/min/ μg at "Depot", $n=12$; Mann Whitney test, $p=0.71$;
31
32 $PM_{2.5}$: 9.3 ± 5.4 pmol DTT/min/ μg at "Tunnel", $n=12$; 11.0 ± 10.0 pmol DTT/min/ μg at "Depot",
33
34 $n=12$; Mann Whitney test, $p=0.86$). The largest intrinsic reactivity was observed for
35 workplaces with very small particles ("Tunnel" 6) or with a high copper content ("Depot" 2 2).
36
37 A large fraction of the PM_4 reactivity was already present on the smaller $PM_{2.5}$ fraction
38 (Figure 3, average $PM_{2.5}/PM_4$ reactivity ratio for all sites: $97\pm 22\%$). The OP^{DTT} exposure level
39 to humans (Figure 2B, OP^{DTT}/m^3) was obtained by multiplying the $OP^{DTT}/\mu\text{g}$ with the
40 corresponding PM concentration. As for the $OP^{DTT}/\mu\text{g}$, no statistically significant difference
41 was observed between OP^{DTT}/m^3 at both sites (1.3 ± 0.7 nmol DTT/min/ m^3 at "Tunnel", $n=11$;
42
43 0.9 ± 0.4 nmol DTT/min/ m^3 at "Depot", $n=12$; Mann Whitney test, $p=0.20$ for PM_4 and 0.8 ± 0.4
44
45 nmol DTT/min/ m^3 at "Tunnel", $n=12$; 0.8 ± 0.7 nmol DTT/min/ m^3 at "Depot", $n=12$; Mann
46
47 Whitney test, $p=0.23$ for $PM_{2.5}$). For both PM_4 $OP^{DTT}/\mu\text{g}$ and OP^{DTT}/m^3 levels, large inter-day
48
49 variations were observed. A maximum of three-fold increase for the PM_4 $OP^{DTT}/\mu\text{g}$ was
50
51 observed between "Tunnel 2" and "Tunnel 6" (7.0 to 24.9 pmol DTT/min/ μg respectively)
52
53
54
55
56
57
58
59
60

1
2
3 whereas it decreased about the same order for these two sampling days when expressed as
4 air volume (1.27 nmol/min/m³ to 0.38 nmol/min/m³ respectively). Such variability could be
5 due to changes in the environmental conditions or workplaces activities. The identification of
6 the chemical compounds associated with the PM₄ OP^{DTT}/m³ was done by Spearman rank
7 correlation (Supplemental material, Table S2). At the “Tunnel” site, SMPS-based surface,
8 OC, Σ 4 quinones and metals (Fe, Cu) were clearly associated with the PM₄ OP^{DTT}/m³. At the
9 “Depot” site, significant associations were observed only between PM₄ OP^{DTT}/m³ and Fe
10 (positive) or Σ 7 PAH (negative association).
11
12
13
14
15
16
17
18
19
20
21

22 **4. Discussion**

23 *4.1. Physico-chemical characteristics of the aerosol.*

24
25 The mass concentrations measured at the “Tunnel” site are comparable to levels determined
26 in similar environments as busy road tunnel entrances (Larsson *et al.*, 2007) or tollbooths on
27 highways with high traffic density (Shih *et al.*, 2008). The “Depot” site presented slightly
28 higher values compared to the one previously reported for the same workplace (Sauvain *et*
29 *al.*, 2011) but were similar to levels found in Swedish bus depots (Lewne *et al.*, 2007). About
30 2/3 of the measured PM₄ mass at the “Depot” sites could be attributed to organic, elemental
31 carbon, Fe and Cu, while about half of the total PM₄ mass was not identified at the “Tunnel”
32 site (Supplemental material, Figure S3). It is possible that resuspended de-icing salts used
33 during winter time for the road maintenance or background ammonium, sulphate and nitrate
34 ions (Lanz *et al.*, 2010) contributed to a large extend to this mass. The measured number
35 concentration corresponded well to reported values for dieselised environments
36 (Ramachandran *et al.*, 2005; Park *et al.*, 2011). The surface concentration found in the
37 studied workplaces are similar to those reported for automotive engine manufacturing
38 (Heitbrink *et al.*, 2009) or milling processes in die cast facility (Park *et al.*, 2011), but about 5-
39 10 time larger compared to concentrations measured in garage mechanics (Ramachandran
40 *et al.*, 2005). This difference could be due to the larger size range (11-1083 nm) considered
41 in this study to calculate the surface area.
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 Whereas the physical properties of the particles mostly define their deposition site and their
4 interaction with pulmonary cells, the chemical composition has also to be considered for
5 understanding the particulate toxicity (Giechaskiel *et al.*, 2009). The aerosol present at the
6
7 “Tunnel” site corresponds to typical combustion-based processes. The predominantly
8
9 combustion origin of the PM sampled at the “Tunnel” site is suggested by their components.
10
11 The observed EC/TC ratio of 0.25 ± 0.08 is consistent with values reported for typical urban
12
13 traffic and fuel combustion sources (Vodicka *et al.*, 2013); the significant OC-EC correlation
14
15 (Supplemental material, Table S1) is also consistent with this. The relatively large
16
17 concentration of quinones also points to diesel particles which are much richer in quinones
18
19 than particles originating from light-duty gasoline vehicles (Jakober *et al.*, 2007). The strong
20
21 correlation between Fe and Cu at the “Tunnel” site is also in support of a vehicle source,
22
23 though more likely related to abraded particles from brakes and other vehicle parts (Riediker
24
25 *et al.*, 2003; Birmili *et al.*, 2006).
26
27

28
29 The lack of association between any chemical variables at the “Depot” sites is suggestive of
30
31 a more complex and mixed source of airborne compounds. The lack of OC and EC
32
33 correlation may be due to the presence of additional OC sources not related to combustion,
34
35 such as the observed use of solvents or lubricating oil during repair activities. The absence of
36
37 association between Fe and Cu at the “Depot” sites compared to the “Tunnel” site
38
39 (Supplemental material, Table S1) is also indicative of other independent sources of Fe or
40
41 Cu from activities like grinding or welding.
42
43

44 4.2. Oxidative potential of particles

45

46 All the collected samples were able to oxidise DTT in a more or less large extent and
47 suggest OP^{DTT} as a general property for such particles. The measured $OP^{DTT}/\mu\text{g}$ was about
48
49 2-3 times lower than reported values for urban $PM_{2.5}$ (Fang *et al.*, 2014; Delfino *et al.*, 2013;
50
51 Charrier and Anastasio, 2012; see supplemental material Table S3 for a detailed analysis).
52
53 This intrinsic smaller $OP^{DTT}/\mu\text{g}$ reactivity could be due to: i) different methodological
54
55 approaches as we put directly the filter into contact with the DTT solution and didn't extract
56
57 the particles in water or methanol, as done by the cited authors in Table S3 (supplemental
58
59
60

1
2
3 material). It is possible that the redox-active entities present on the filter are less available for
4 reacting with the DTT compared to when they are already extracted in a water or methanol
5 solution. This would be in line with the lower electron spin resonance-based reactivity
6 measured directly for PM loaded filters compared to the water-extracted filters (Hellack *et al.*,
7 2014); ii) the low contribution of photochemical processes at the studied workplaces, which
8 correspond to indoor situations. Such photochemical processes have been shown to be quite
9 important for modulating the outdoor PM reactivity, via the formation of secondary organic
10 aerosols (Verma *et al.*, 2009; Rattanavaraha *et al.*, 2011). Such processes are not likely to
11 take place in the confined studied workplaces, potentially explaining the lower reactivity
12 compared to ambient particles; iii) the presence of organic and inorganic redox-inactive
13 compounds adsorbed on the particles which may contribute to the mass but not to the PM
14 reactivity. Particularly at the “Depot” site, the important use of lubricating oil (containing
15 paraffinic redox-inactive chemicals) might explain the large variability of the OP^{DTT} at these
16 sites. Whereas the intrinsic $PM_{2.5}$ reactivity was low, the OP^{DTT}/m^3 for this size range was
17 quite comparable with other reported values (supplemental material Table S3). The fact that
18 larger PM mass were present in the studied workplaces compared to the levels reported for
19 urban situations compensate their lower intrinsic reactivity and could explain this similarity.
20
21 The OP^{DTT} of PM_4 was mostly present on the smallest $PM_{2.5}$ fraction (Figure 3). This is
22 consistent with published data for ambient PM indicating an increasing OP^{DTT} from the
23 coarse to the ultrafine fraction (Ntziachristos *et al.*, 2007).
24
25

26
27 Although the two types of workplaces differed based on their physico-chemical
28 characteristics, the OP^{DTT} of the particles was practically identical, irrespective of the metric
29 used (mass or air volume). Whereas this observation might be different with other OP
30 assays, this suggests that the potential generation of ROS in lungs of particle exposed
31 workers would be similar at both workplaces even though their composition differs. The PM_4
32 OP^{DTT}/m^3 of the combustion type PM (“Tunnel” site) was associated with SMPS-based
33 surface, organics (OC, quinones) and/or Fe, Cu (see supplementary material Table S2 in
34 online edition), consistent with literature data relative to urban or combustion-based PM (Cho
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 *et al.*, 2005; Geller *et al.*, 2006; Ntziachristos *et al.*, 2007; Hu *et al.*, 2008; Kumagai *et al.*,
4
5 2002; Chung *et al.*, 2006; Verma *et al.*, 2009; Charrier and Anastasio, 2012). As we
6
7 observed an association between Cu and the OP^{DTT}/m^3 in the case of the “Tunnel” site and
8
9 as the DTT assay responds strongly to the presence of Cu ions (Charrier and Anastasio,
10
11 2012; Kachur *et al.*, 1997), we estimated its contribution to the OP^{DTT}/m^3 . By using a
12
13 bioavailable fraction of 25% for particulate Cu (Birmili *et al.*, 2006) and a published
14
15 relationship between the DTT consumption rate and the soluble Cu concentration in the DTT
16
17 assay (Charrier and Anastasio, 2012), we found that copper contributed importantly
18
19 ($78\pm 20\%$) to the $PM_4 OP^{DTT}/m^3$ at the “Tunnel” site. Redox active quinones may additionally
20
21 contribute to this reactivity (Charrier and Anastasio, 2012).
22

23
24 At the “Depot” sites, only Fe was significantly and positively associated with the PM_4
25
26 OP^{DTT}/m^3 . As Fe presents a low reactivity toward DTT (Charrier and Anastasio, 2012), this
27
28 association is probably due to other redox active components associated with Fe but not
29
30 measured in this study. Interestingly, we observed also a significantly negative association
31
32 between $\Sigma 7$ PAH and OP^{DTT}/m^3 . Such negative association has been reported only one time
33
34 to our knowledge, for urban quasi-ultrafine particles (Verma *et al.*, 2009). It has been
35
36 attributed to photo-oxidation of PAH, leading to the formation of oxy-PAH and quinones
37
38 which are reactive toward DTT. Such process is not likely to take place in the confined
39
40 studied workplaces. On the contrary, the presence of aerosolised used lubricating oil,
41
42 containing paraffinic non-redox active compounds and potentially enriched with PAH
43
44 (Brandenberger *et al.*, 2005) could mask reactive sites on particles and thus explain the
45
46 observed negative association between PAH and the OP^{DTT}/m^3 . Such quenching effect could
47
48 also explain the absence of relationship between OC and the OP^{DTT}/m^3 (see supplemental
49
50 material Table S2).
51

52
53 Due to the complexity of the PM present in the workplaces, the hazard assessment of such
54
55 mixtures requires an integrated assessment of the biologically important components. The
56
57 DTT assay used in this study may present suitable integrative characteristics, as it was found
58
59 to respond to organics and also metals. These two compounds families are considered key
60

1
2
3 for the induction of PM-related oxidative stress and subsequent pro-inflammatory processes
4
5 (Donaldson *et al.*, 2005; Nawrot *et al.*, 2009). The measurement of redox activity of particles
6
7 as a new metric is appealing (Borm *et al.*, 2007). However, many questions have to be
8
9 solved before it can be used in occupational exposure assessment. At the methodological
10
11 level, different acellular tests probing the oxidative potential of particles are available (for
12
13 example, electron spin resonance, ascorbic acid depletion, 2'7-dichlorodihydrofluorescein,
14
15 Ayres *et al.*, 2008) and may respond differently (Sauvain *et al.*, 2013; Zielinski *et al.*, 1999). It
16
17 may also be necessary to use more complex mixtures like synthetic respiratory tract lining
18
19 fluid (RTLFL) (Ayres *et al.*, 2008). All these assays are abiotic in nature and thus cannot take
20
21 into account other possible oxidative pathways resulting from particle-biological system
22
23 interactions such as, for example, biological activation of PAH to quinones (Xue and
24
25 Warshawsky, 2005), or upregulation of cellular/tissue enzymes producing antioxidants (Chan
26
27 *et al.*, 2013). Thus, before being proposed as an alternative metric to mass, the biologically
28
29 relevance of such assays has to be demonstrated. The relation between the DTT redox-
30
31 activity and some *in vitro* cellular endpoints has already been published (Hu *et al.*, 2008; Li *et*
32
33 *al.*, 2003; Steenhof *et al.*, 2011; Uzu *et al.*, 2011; Li *et al.*, 2009). For children exposed to
34
35 traffic related pollutants, an association between $PM_{2.5}$ OP^{DTT}/m^3 and exhaled NO,
36
37 considered as a marker of airway inflammation has been recently reported (Delfino *et al.*,
38
39 2013). This further supports the idea that the DTT assay could be a relevant indicator for the
40
41 potential of particles to induce oxidative stress in biological systems. Nevertheless, the
42
43 reported lack or low consistent associations between various OP assays and different
44
45 pulmonary and cardiovascular acute end-points for healthy volunteers exposed to different
46
47 particle type (Strak *et al.*, 2012; Steenhof *et al.*, 2013; Steenhof *et al.*, 2014; Janssen *et al.*,
48
49 2015) stress the importance of further studies in this field.
50
51
52
53

54 **5. Conclusion**

55
56 The DTT assay allowed to quantitatively determine a redox activity for all the particles
57
58 collected in different occupational environments. Whereas the PM mass concentrations
59
60

1
2
3 differed largely between the two studied sites, similar OP^{DTT} was determined in these
4 workplaces. This redox activity was associated to toxicologically relevant organics (OC,
5 quinones) and metals (Fe, Cu). Particulate copper and possibly quinones contributed largely
6 to this OP^{DTT}/m^3 at the "Tunnel" site. On the contrary, a combination of oxidative (attributable
7 to Fe or associated chemicals) and quenching effects (possibly lubricating oil) are postulated
8 to modulate the OP^{DTT}/m^3 at the "Depot" site. The DTT assay appears thus to be an
9 integrative and potentially relevant indicator of the oxidative hazard of particles to biological
10 systems. Nevertheless, the biological relevance of the OP^{DTT} approach for *in vivo* systems
11 has to be demonstrated. Considering the ongoing debate about which metrics are most
12 suitable to describe the hazards posed by particles to human health, the way forward in
13 occupational health research seems to be a comprehensive exposure assessment based on
14 multiple parameters such as mass, number, surface area, shape, a measurement of the
15 oxidative potential such as OP^{DTT} , and potentially other parameters, which can later be
16 distilled down to those factors that turn out to be most informative.
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33

34 **6. Acknowledgement**

35 We would like to thank each representative in both companies who allowed us to sample the
36 aerosol in their facilities. In addition, Dr Nancy Hopf, Dr Pascal Wild and Dr Michel Rossi are
37 acknowledged for their helpful comments on the manuscript as well as Christine Kohler and
38 Patricia Stephan for their help in the determination of the metal content in particles. We also
39 appreciated the comments of two anonymous reviewers who helped improve the manuscript.
40 The French Agence Nationale de Sécurité Sanitaire de l'Alimentation, de l'Environnement et
41 du Travail (ANSES) is acknowledged for its financial support through grant EST 2006/1/7.
42
43
44
45
46
47
48
49
50 The authors declare no conflict of interest relating to the material presented in this article.
51
52

53 **7. References**

54 Ayres JG, Borm P, Cassee FR, *et al.* (2008) Evaluating the toxicity of airborne particulate
55 matter and nanoparticles by measuring oxidative stress potential - A workshop report
56 and consensus statement. *Inhal Toxicol*; 20: 75-99.
57
58
59
60

- 1
2
3 Birmili W, Allen AG, Bary F, *et al.* (2006) Trace metal concentrations and water solubility in
4 size-fractionated atmospheric particles and influence of road traffic. *Environmental*
5 *Science & Technology*; 40: 1144-53.
6
7 Borm PJA, Kelly F, Kunzli N, *et al.* (2007) Oxidant generation by particulate matter: from
8 biologically effective dose to a promising, novel metric. *Occup Environ Med*; 64: 73-74.
9
10 Brandenberger S, Mohr M, Grob K, *et al.* (2005) Contribution of unburned lubricating oil and
11 diesel fuel to particulate emission from passenger cars. *Atmos Environ*; 39: 6985-94.
12
13 Brook RD, Rajagopalan S, Pope CA, *et al.* (2010) Particulate Matter Air Pollution and
14 Cardiovascular Disease An Update to the Scientific Statement From the American
15 Heart Association. *Circulation*; 121: 2331-78
16
17 Chan JKW, Charrier JG, Kodani SD, *et al.* (2013) Combustion-derived flame generated
18 ultrafine soot generates reactive oxygen species and activates Nrf2 antioxidants
19 differently in neonatal and adult rat lungs. *Part Fibre Toxicol*; 10: 34.
20
21 Charrier JG, Anastasio C. (2012) On dithiothreitol (DTT) as a measure of oxidative potential
22 for ambient particles: evidence for the importance of soluble transition metals.
23 *Atmospheric Chemistry and Physics*; 12: 9321-33.
24
25 Cho AK, Di Stefano E, You Y, *et al.* (2004) Determination of four quinones in diesel exhaust
26 particles, SRM 1649a, an atmospheric PM2.5. *Aerosol Sci Technol*; 38: 68-81.
27
28 Cho AK, Sioutas C, Miguel AH, *et al.* (2005) Redox activity of airborne particulate matter at
29 different sites in the Los Angeles Basin. *Environ Res*; 99: 40-47.
30
31 Chung MY, Lazaro RA, Lim D, *et al.* (2006) Aerosol-borne quinones and reactive oxygen
32 species generation by particulate matter extracts. *Environmental Science &*
33 *Technology*; 40: 4880-86.
34
35 Cross CE, Vandervliet A, Oneill CA, *et al.* (1994) Oxidants, Antioxidants, and Respiratory-
36 Tract Lining Fluids. *Environ Health Perspect*; 102: 185-91.
37
38 Delfino RJ, Staimer N, Tjoa T, *et al.* (2013) Airway inflammation and oxidative potential of air
39 pollutant particles in a pediatric asthma panel. *J Expo Sci Environ Epidemiol*; 23: 466-
40 73.
41
42 Donaldson K, Brown D, Clouter A, *et al.* (2002) The pulmonary toxicology of ultrafine
43 particles. *Journal of Aerosol Medicine-Deposition Clearance and Effects in the Lung*;
44 15: 213-20.
45
46 Donaldson K, Stone V, Seaton A, *et al.* (2001) Ambient particle inhalation and the
47 cardiovascular system: Potential mechanisms. *Environ Health Perspect*; 109: 523-27.
48
49 Donaldson K, Tran L, Jimenez LA, *et al.* (2005) Combustion-derived nanoparticles: a review
50 of their toxicology following inhalation exposure. *Part Fibre Toxicol*; 2: 10.
51
52 Fang T, Verma V, Guo H, *et al.* (2014) A semi-automated system for quantifying the
53 oxidative potential of ambient articles in aqueous extracts using the dithiothreitol (DTT)
54
55
56
57
58
59
60

1
2
3 assay: results from the Southeastern Center for Air Pollution and Epidemiology
4 (SCAPE). *Atmos Meas Tech Discuss*; 7: 7245 - 79.
5

6 Geller MD, Ntziachristos L, Mamakos A, *et al.* (2006) Physicochemical and redox
7 characteristics of particulate matter (PM) emitted from gasoline and diesel passenger
8 cars. *Atmos Environ*; 40: 6988-7004.
9

10 Giechaskiel B, Alfoeldy B, Drossinos Y. (2009) A metric for health effects studies of diesel
11 exhaust particles. *J Aerosol Sci*; 40: 639-51.
12

13 Halliwell B, Gutteridge J. (2007) Free radicals in biology and medicine. Oxford, UK: Oxford
14 University Press. ISBN 978-0-19-856868-1.
15

16
17 Heitbrink WA, Evans DE, Ku BK, *et al.* (2009) Relationships Among Particle Number,
18 Surface Area, and Respirable Mass Concentrations in Automotive Engine
19 Manufacturing. *J Occup Environ Hyg*; 6: 19-31.
20

21 Held KD, Biaglow JE. (1994) Mechanisms for the Oxygen Radical-Mediated Toxicity of
22 Various Thiol-Containing Compounds in Cultured-Mammalian-Cells. *Radiat Res*; 139:
23 15-23.
24

25 Hellack B, Yang A, Cassee FR, *et al.* (2014) Intrinsic hydroxyl radical generation
26 measurements directly from sampled filters as a metric for the oxidative potential of
27 ambient particulate matter. *J. Aerosol Sci*; 72: 47-55.
28

29
30 Hopke PK. (2008) New directions: Reactive particles as a source of human health effects.
31 *Atmos Environ*; 42: 3192-94.
32

33 Hu S, Polidori A, Arhami M, *et al.* (2008) Redox activity and chemical speciation of size
34 fractionated PM in the communities of the Los Angeles-Long Beach harbor. *Atmospheric
35 Chemistry and Physics*; 8: 6439-51.
36

37 Jakober CA, Riddle SG, Robert MA, *et al.* (2007) Quinone emissions from gasoline and
38 diesel motor vehicles. *Environmental Science & Technology*; 41: 4548-54.
39

40 Janssen NAH, Strak M, Yang A, *et al.* (2015) Associations between three specific a-cellular
41 measures of the oxidative potential of particulate matter and markers of acute airway
42 and nasal inflammation in healthy volunteers. *Occup Environ Med*; 72: 49-56.
43

44 Kachur AV, Held KD, Koch CJ, *et al.* (1997) Mechanism of production of hydroxyl radicals in
45 the copper-catalyzed oxidation of dithiothreitol. *Radiat Res*; 147: 409-15.
46

47
48 Kumagai Y, Koide S, Taguchi K, *et al.* (2002) Oxidation of proximal protein sulfhydryls by
49 phenanthraquinone, a component of diesel exhaust particles. *Chem Res Toxicol*; 15:
50 483-89.
51

52 Lanz VA, Prevot ASH, Alfarrar MR, *et al.* (2010) Characterization of aerosol chemical
53 composition with aerosol mass spectrometry in Central Europe: an overview.
54 *Atmospheric Chemistry and Physics*; 10: 10453-71.
55

56 Larsson BM, Sehlstedt M, Grunewald J, *et al.* (2007) Road tunnel air pollution induces
57 bronchoalveolar inflammation in healthy subjects. *Eur Respir J*; 29: 699-705.
58
59
60

- 1
2
3 Lewne M, Plato N, Gustavsson P. (2007) Exposure to particles, elemental carbon and
4 nitrogen dioxide in workers exposed to motor exhaust. *Ann Occup Hyg*; 51: 693-701.
5
6 Li N, Sioutas C, Cho A, *et al.* (2003) Ultrafine particulate pollutants induce oxidative stress
7 and mitochondrial damage. *Environ Health Perspect*; 111: 455-60.
8
9 Li N, Wang M, Bramble LA, *et al.* (2009) The Adjuvant Effect of Ambient Particulate Matter Is
10 Closely Reflected by the Particulate Oxidant Potential. *Environ Health Perspect*; 117:
11 1116-23.
12
13 Limbach LK, Wick P, Manser P, *et al.* (2007) Exposure of engineered nanoparticles to
14 human lung epithelial cells: Influence of chemical composition and catalytic activity on
15 oxidative stress. *Environmental Science & Technology*; 41: 4158-63.
16
17
18 Nawrot TS, Kuenzli N, Sunyer J, *et al.* (2009) Oxidative properties of ambient PM(2.5) and
19 elemental composition: Heterogeneous associations in 19 European cities. *Atmos*
20 *Environ*; 43: 4595-602.
21
22 Ntziachristos L, Froines JR, Cho AK, *et al.* (2007) Relationship between redox activity and
23 chemical speciation of size-fractionated particulate matter. *Part Fibre Toxicol*; 4: 5.
24
25 Park JY, Ramachandran G, Raynor PC, *et al.* (2011) Estimation of surface area
26 concentration of workplace incidental nanoparticles based on number and mass
27 concentrations. *J Nanopart Res*; 13: 4897-911.
28
29
30 Perret V, Huynh CK, Droz PO, *et al.* (1999) Assessment of occupational exposure to diesel
31 fumes - parameter optimization of the thermal coulometric measurement method for
32 carbon. *J Environ Monit*; 1: 367-72.
33
34 Ramachandran G, Paulsen D, Watts W, *et al.* (2005) Mass, surface area and number metrics
35 in diesel occupational exposure assessment. *J Environ Monit*; 7: 728-35.
36
37 Rattanavaraha W, Rosen E, Zhang H, *et al.* (2011) The reactive oxidant potential of different
38 types of aged atmospheric particles: An outdoor chamber study. *Atmos Environ*; 45:
39 3848-55.
40
41 Riediker M, Williams R, Devlin R, *et al.* (2003) Exposure to particulate matter, volatile organic
42 compounds, and other air pollutants inside patrol cars. *Environmental Science &*
43 *Technology*; 37: 2084-93.
44
45
46 Sauvain J-J, Rossi MJ, Riediker M. (2013) Comparison of Three Acellular Tests for
47 Assessing the Oxidation Potential of Nanomaterials. *Aerosol Sci Technol*; 47: 218-27.
48
49 Sauvain JJ, Deslarzes S, Riediker M. (2008) Nanoparticle reactivity toward dithiothreitol.
50 *Nanotoxicology*; 2: 121-29.
51
52 Sauvain JJ, Duc TV, Guillemin M. (2003) Exposure to carcinogenic polycyclic aromatic
53 compounds and health risk assessment for diesel-exhaust exposed workers. *Int Arch*
54 *Occup Environ Health*; 76: 443-55.
55
56
57
58
59
60

- 1
2
3 Sauvain JJ, Setyan A, Wild P, *et al.* (2011) Biomarkers of oxidative stress and its association
4 with the urinary reducing capacity in bus maintenance workers. *Journal of Occupational*
5 *Medicine and Toxicology*, 6: 1-13.
6
7 Shih T-S, Lai C-H, Hung H-F, *et al.* (2008) Elemental and organic carbon exposure in
8 highway tollbooths: A study of Taiwanese toll station workers. *Sci Total Environ*; 402:
9 163-70.
10
11 Steenhof M, Gosens I, Strak M, *et al.* (2011) In vitro toxicity of particulate matter (PM)
12 collected at different sites in the Netherlands is associated with PM composition, size
13 fraction and oxidative potential - the RAPTES project. *Part Fibre Toxicol*; 8: 26.
14
15 Steenhof M, Janssen NAH, Strak M, *et al.* (2014) Air pollution exposure affects circulating
16 white blood cell counts in healthy subjects: the role of particle composition, oxidative
17 potential and gaseous pollutants - the RAPTES project. *Inhal Toxicol*; 26: 141-65.
18
19 Steenhof M, Mudway IS, Gosens I, *et al.* (2013) Acute nasal pro-inflammatory response to
20 air pollution depends on characteristics other than particle mass concentration or
21 oxidative potential: the RAPTES project. *Occup Environ Med*; 70: 341-48.
22
23 Strak M, Janssen NAH, Godri KJ, *et al.* (2012) Respiratory Health Effects of Airborne
24 Particulate Matter: The Role of Particle Size, Composition, and Oxidative Potential-The
25 RAPTES Project. *Environ Health Perspect*, 120: 1183-89.
26
27 Uzu G, Sauvain J-J, Baeza-Squiban A, *et al.* (2011) In vitro Assessment of the Pulmonary
28 Toxicity and Gastric Availability of Lead-Rich Particles from a Lead Recycling Plant.
29 *Environmental Science & Technology*; 45: 7888-95.
30
31 Verma V, Ning Z, Cho AK, *et al.* (2009) Redox activity of urban quasi-ultrafine particles from
32 primary and secondary sources. *Atmos Environ*; 43: 6360-68.
33
34 Vodicka P, Schwarz J, Zdimal V. (2013) Analysis of one year's OC/EC data at a Prague
35 suburban site with 2-h time resolution. *Atmos Environ*; 77: 865-72.
36
37 Xue WL, Warshawsky D. (2005) Metabolic activation of polycyclic and heterocyclic aromatic
38 hydrocarbons and DNA damage: A review. *Toxicol Appl Pharmacol*; 206: 73-93.
39
40 Zielinski H, Mudway IS, Berube KA, *et al.* (1999) Modeling the interactions of particulates
41 with epithelial lining fluid antioxidants. *American Journal of Physiology-Lung Cellular*
42 *and Molecular Physiology*; 277: L719-L26.
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Table 1: Description of the different sampling instruments and the corresponding analyses.

Sampler	Filter type	Physical analysis	Chemical analysis		
			Support	Analyte	Methodology
High Volume sampler (Digitel DH 77, Hegnau, Switzerland); 580 L/min; Cascade impactor PM ₄	Quartz (Whatman QM-A, Milian, Switzerland, Ø 150 mm)	Gravimetry (PM ₄)	8 punches Ø 40 mm	4 quinones	Gas chromatography with mass detection
			Rest of the filter	7 PAH	Gas chromatography with mass detection
High Volume sampler (Gravikon PM ₄ , Strohle, Germany); 60 L/min; PM ₄ cyclone	PTFE coated glass fiber (Pallflex, T60A20-HT, Pall Corporation, USA, Ø 70 mm)	Gravimetry (PM ₄)	1-2 punches Ø 28-42 mm	Oxidative potential	DTT assay
			Rest of the filter	Metals (Fe, Cu)	Atomic absorption (graphite furnace)
Low volume sampler (Leland, SKC, Eighty Four, USA); 10 L/min; Impactor PEM [®] PM _{2.5}	PTFE (SKC, Blanc-Labo, Switzerland; 2 µm, Ø 37 mm)	Gravimetry (PM _{2.5})	Whole filter	Oxidative potential	DTT assay
Low volume sampler (MSA, Pittsburgh, USA); 2 L/min; Cyclone Casella PM ₄	Quartz (Whatman QM-A, WWR, Switzerland, Ø 37 mm)	-	Whole filter	Organic carbon (OC); elemental carbon (EC)	Coulometry
Cascade impactor Andersen; 28 L/min, 9 stages	Glass fibre (Whatman 934AH, WWR, Switzerland; Ø 81 mm)	Mass-based size distribution (<0.4 - > 11 µm)	-	-	-

1
2
3
4
5
6

Table 2: Average mass, number and surface concentration with standard deviation measured in the different workplaces (in bracket, number of measurements or scans – in the case of number and surface data). When only duplicate measurements were available, the median with minimum and maximum values in bracket are indicated.

8
9
10
11
12

14
15
16
17
19
20
21
22

24
25
26

27
28

30
31
32

33

35
36
37

39

40

41

42

43

44

45

46
47
48
49
49

Site	Mass				Number			Surface		
	PM ₄	PM _{2.5}	Median diameter ^a	σ _g ^a	Number	Mean geometric diameter ^b	σ _g ^b	Surface	Mean geometric diameter ^b	σ _g ^b
	[μg/m ³]	[μg/m ³]	[μm]		[#/cm ³]	[nm]		[mm ² /m ³]	[nm]	
Tunnel 1	260±57 (6)	190±27 (3)	5.0	2.6	94'200±26'000 (36)	61±10	2.2	3'700±1'200 (36)	197±17	2.1
Tunnel 2	184±18 (6)	135±18 (3)	6.2	2.5	108'400±57'000 (96)	57±14	2.2	3'500±1'200 (96)	186±27	2.1
Tunnel 3	225±10 (6)	114±2 (3)	5.9	2.6	119'500±50'000 (110)	37±9	2.2	1'920±960 (110)	146±28	2.3
Tunnel 4	278±23 (6)	156±33 (3)	6.3	2.8	118'900±54'000 (112)	54±16	2.3	4'140±1'670 (112)	199±28	2.1
Tunnel 5	73±2 (5)	53±2 (3)	7.2	2.0	75'800±45'000 (110)	26±5	2.0	740±590 (110)	149±26	2.7
Tunnel 6	15±2 (5)	13±2 (3)	8.9	1.1	104'200±57'000 (116)	22±4	2.0	670±410 (116)	137±45	3.0
All Tunnel sites	173±103	110±64	6.6±1.3		103'500±16'500	43±17		2'450±1500	169±28	
Depot 1 1	105±70 (7)	104±46 (3)	2.7	3.7	38'000±14'000 (102)	49±9	2.3	1'000±240 (102)	169±21	2.1
Depot 1 2	113±37 (7)	71±24 (3)	4.3	3.1	45'900±26'500 (160)	40±8	2.3	1'000±370 (160)	175±14	2.2
Depot 2 1	111±28 (6)	88±21 (3)	3.7	2.2	28'700±7'000 (95)	48±6	2.4	920±320 (95)	197±18	2.1
Depot 2 2	83±37 (6)	65±16 (3)	3.0	3.1	58'200±38'000 (146)	49±10	2.2	1'400±460 (146)	171±16	2.1
Depot 3 1	74±16 (7)	64 (65; 63)	2.6	4.1	84'100±83'000 (113)	51±12	2.3	2'120±610 (113)	181±19	2.1
Depot 3 2	57±14 (6)	45 (41; 50)	3.5	4.1	104'500±160'000 (169)	45±10	2.2	1'860±1'380 (169)	168±30	2.2
All Depot sites	90±36	73±26	3.3±0.6		60'000±29'000	47±4		1'390±500	177±11	

^a: Based on the Andersen measurement, n=1.

^b: Based on SMPS measurement, using the same number of measurements (scans). σ_g: Mean geometric standard deviation.

Table 3: Average concentrations with standard deviation for the different particle-bound chemicals determined in the studied workplaces (number of measurements in bracket). When only duplicate measurements were available, the median with minimum and maximum values in bracket are indicated.

Site	OC [$\mu\text{g}/\text{m}^3$]	EC [$\mu\text{g}/\text{m}^3$]	Σ 4 quinones ^{a, b} [ng/m^3]	Σ 7 PAH ^{b, c} [ng/m^3]	Fe [$\mu\text{g}/\text{m}^3$]	Cu [$\mu\text{g}/\text{m}^3$]
Tunnel 1	67±22 (3)	26±7 (3)	4.88	3.70	9.7 (9.6; 9.8)	0.42 (0.41; 0.43)
Tunnel 2	62±16 (4)	14±2 (4)	4.45	11.69	7.9 (7.7; 8.0)	0.39 (0.39; 0.40)
Tunnel 3	59±8 (4)	15±2 (4)	3.61	7.53	5.2 ^b	0.19 ^b
Tunnel 4	74±8 (4)	12±1 (4)	4.94	11.47	8.7 ^b	0.33 ^b
Tunnel 5	19±5 (4)	8±1 (4)	1.01	9.17	1.1 (0.9; 1.2)	0.02 (0.01; 0.03)
Tunnel 6	12±3 (4)	7±1 (4)	1.04	8.04	0.2 (0.2; 0.2)	n.d. ^e
All Tunnel sites	49±27	13±7	3.3±1.8	8.6±2.9	5.2±4.1	0.27±0.17
Depot 1 1	66±32 (4)	6±1 (4)	0.31	5.89	1.2 (0.9; 1.4)	0.06 (0.06; 0.06)
Depot 1 2	47±19 (4)	7±1 (4)	0.36	3.46	3.6 (3.4; 3.7)	0.05 (0.04; 0.05)
Depot 2 1	43±5 (3)	6±1 (3)	0.23	4.05	1.4 (1.0; 1.9)	0.06 (0.04; 0.08)
Depot 2 2	43±14 (3)	3±2 (3)	0.33	5.79	5.1 (1.8; 8.5)	0.77 (0.6; 1.0)
Depot 3 1	40±15 (4)	16±3 (4)	0.56	9.24	1.6 (1.6; 1.7)	0.11 (0.11; 0.11)
Depot 3 2	26±4 (4)	10±4 (4)	0.74	6.09	1.1 (1.1; 1.2)	0.07 (0.06; 0.07)
All Depot sites	44±17	8±5	0.42±0.19	5.8±2.0	2.3±2.1	0.18±0.29
LOD ^d	12	3	0.04	0.3	0.01	0.004

^a: Sum of 1,2-Naphtoquinone + 1,4-Naphtoquinone + 9,10-Phenanthroquinone + 9,10-Anthraquinone.

^b: n=1

^c: Sum of Benzo[a]Anthracen, Benzo[b+k+j]Fluoranthen, Benzo[a]Pyren, Indeno[c,d]Pyren, Dibenz[a,h]Anthracen.

^d: LOD: Limit of detection, corresponding to three times the noise of a blank sample.

^e: not detected (< LOD)

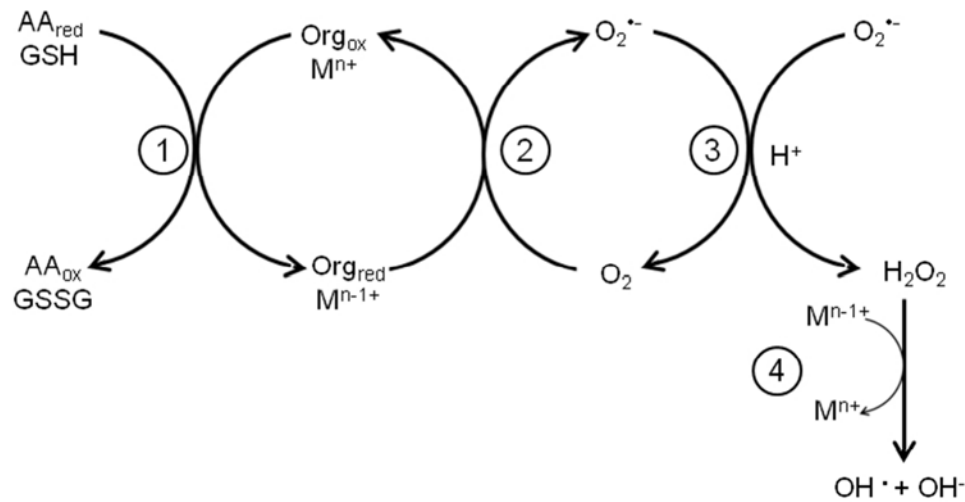


Figure 1: Cascade of possible reactions taking place in the reducing milieu of the lung lining fluid and generating reactive oxygen species ($O_2^{\bullet-}$, H_2O_2 , OH^{\bullet}) (adapted from Held and Biaglow, (1994)). AA_{red} : ascorbic acid (reduced form); AA_{ox} : ascorbic acid (oxidised form); RSH_{red} : thiol-containing molecule like glutathione or DTT (reduced form); $RSSR_{ox}$: thiol-containing molecule like glutathione or DTT (oxidised form); PM_{red} : surface functions of particulate matter (reduced form); PM_{ox} : surface functions of particulate matter (oxidised form); Org_{red} : organic compound adsorbed on the particle (reduced form); Org_{ox} : organic compound adsorbed on the particle (oxidised form); $M^{(n-1)+}$: redox active metal (reduced form); M^{n+} : redox active metal (oxidised form); O_2 : oxygen; $O_2^{\bullet-}$: superoxide ion; H_2O_2 : hydrogen peroxide; OH^{\bullet} : peroxide radical.

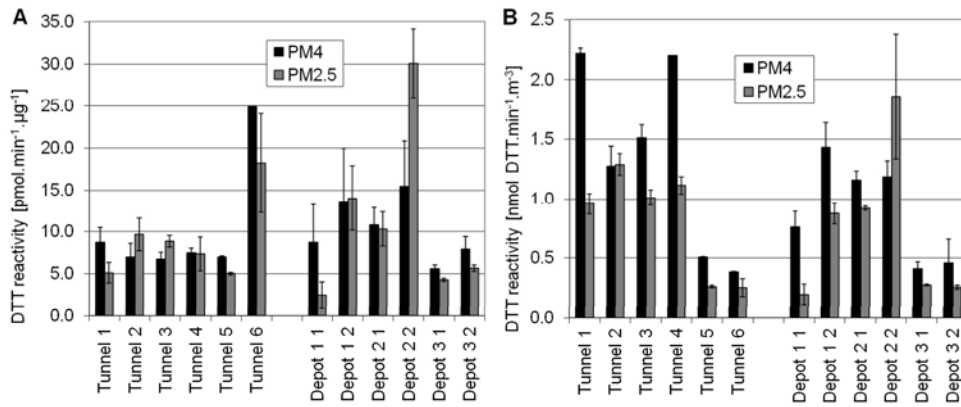


Figure 2. OPDTT per particle mass (A) and OPDTT expressed as an air concentration (B) for the particles collected in the different workplaces. Bars correspond to the standard deviation of duplicate measurements.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

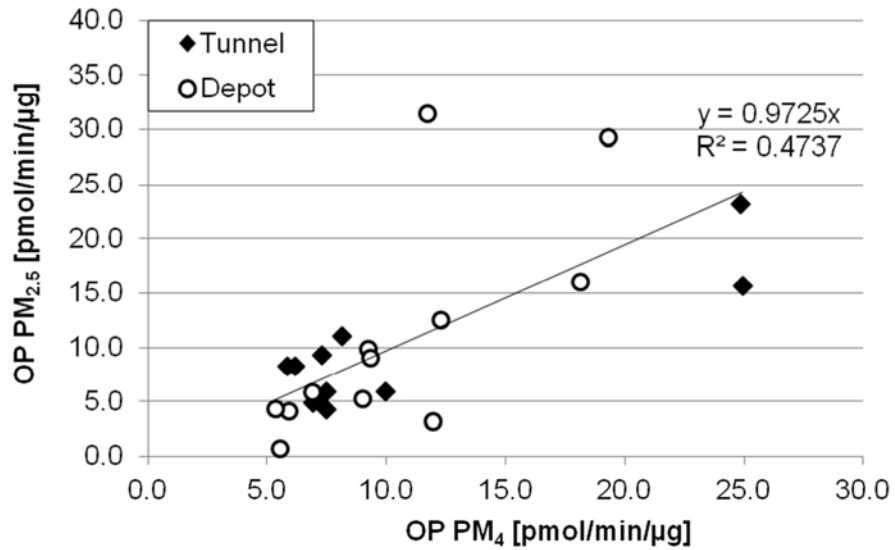


Figure 3: Relationship between the oxidative potential of PM_{2.5} and PM₄ for the two types of workplaces. The equation for the regression line for all the sampling sites is also given, together with the corresponding regression coefficient (R²).

Supplemental material

Oxidative potential of particles in different occupational environments: A Pilot study

Sauvain Jean-Jacques¹, Deslarzes Simon¹, Storti Ferdinand¹, Riediker Michael^{1,2}

1 Institute for Work and Health, University of Lausanne + Geneva, Route de la Corniche 2, 1066 Epalinges-Lausanne Switzerland

2 SAFENANO, Institute of Occupational Medicine (IOM) Singapore, 30 Raffles Place, 048622 Singapore

1. Developments for the filter-based measurement of the redox activity of particles

We studied the influence of the filter type (Teflon or quartz), the storage time and the particle load on the DTT reactivity. All these tests were done by collecting the freshly emitted particles from a 1986 diesel van (Mercedes Benz Model 309 D, operating at idling position) in a small box (about 0.125 m³) containing the sampling heads. Sampling on Teflon filters (37 mm, 2 µm, SKC Inc, Blanc-Labo Switzerland) were done during less than 10 minutes at 10 L/min (Leland Legacy pump, SKC Inc, USA) with an impaction head (PEM[®], SKC Inc, USA), allowing to collect PM_{2.5} (particle matter with 50% smaller than 2.5 µm). Sampling with quartz filters (37 mm, QM-A, Whatmann, Switzerland) were done at 2 L/min (Escort ELF pumps, MSA, USA) with a Casella cyclone head, allowing to collect PM₄ (particle matter with 50% smaller than 4 µm). By varying the sampling time, the mass loaded on the different filters could be modified. Once loaded, the filters were stored in a thermostated weighting zone (55 ±10 % relative humidity) for a maximum of 24 hours and weighted (Microbalance Sartorius MP; Switzerland; 1 µg sensibility).

After 24 hours stabilisation at ambient temperature and constant humidity (55%), the DTT reactivity of the diesel particles loaded on Teflon (PM_{2.5}) and on quartz filters (PM₄) were 6.8±1.6 pmol DTT.min⁻¹.µg⁻¹ (n=3) and 6.7±3.6 pmol DTT.min⁻¹.µg⁻¹ (n=4) respectively and didn't differ (p<0.05, Student test). Compared to this value, we observed that the reactivity of the particles sampled on Teflon filters didn't change after at least 170 hours storage at ambient temperature (Figure S1 A). On the contrary, the same particles sampled on quartz filters presented an increased reactivity toward DTT after about 150 hours storage at ambient temperature (Figure S1 B). Such an increase could be due to the presence of acidic hydroxyl surface functions (OH) on the quartz fibers which could favor the formation of oxidized compounds able to react with DTT. Such a phenomenon has been observed for PAH, which are easier oxidized when adsorbed on silica particles (Miet *et al.*, 2009).

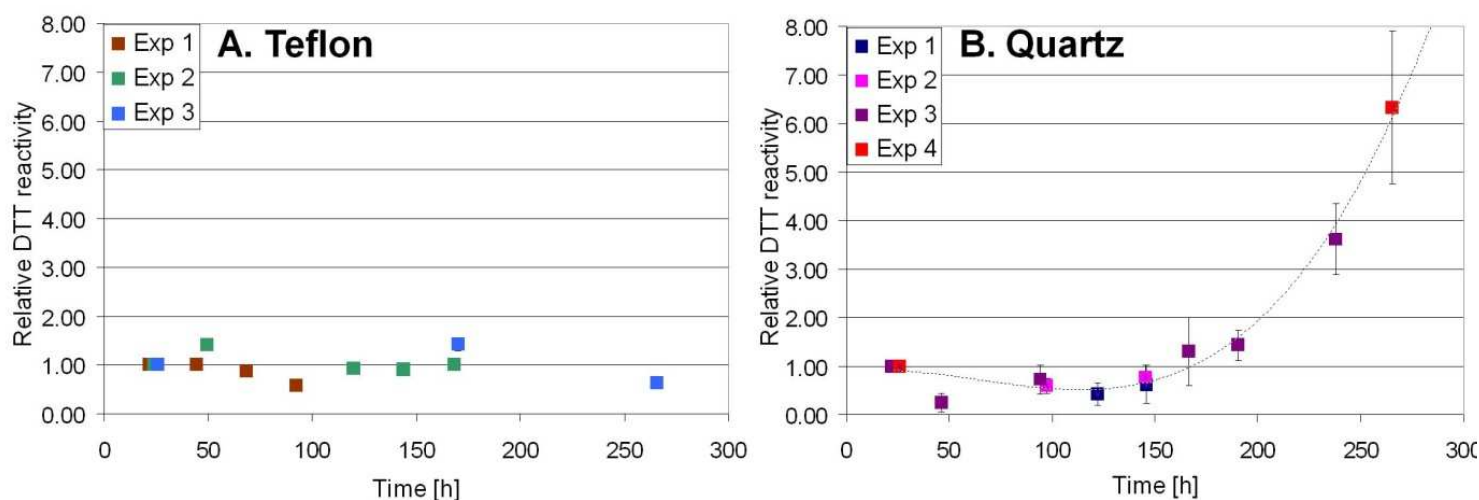


Figure S1: Effect of the storage duration on the DTT reactivity of particles collected from a diesel van. Reactivity of the loaded filter is expressed relative to the one determined after 24 hours stabilisation in a glovebox at 55% humidity. **A.** Teflon filter **B.** Quartz filter

In our experimental conditions, the DTT reactivity was linearly related to the particle mass on Teflon filters until about 1 mg (Figure S2). Based on these preliminary tests, we sampled particles in occupational environment with Teflon filters and the determination of the DTT

reactivity was done after a maximum of 48 hours storage at ambient temperature and on filters/punches loaded with less than 1 mg particles (corresponding to 0.16 mg/cm^2).

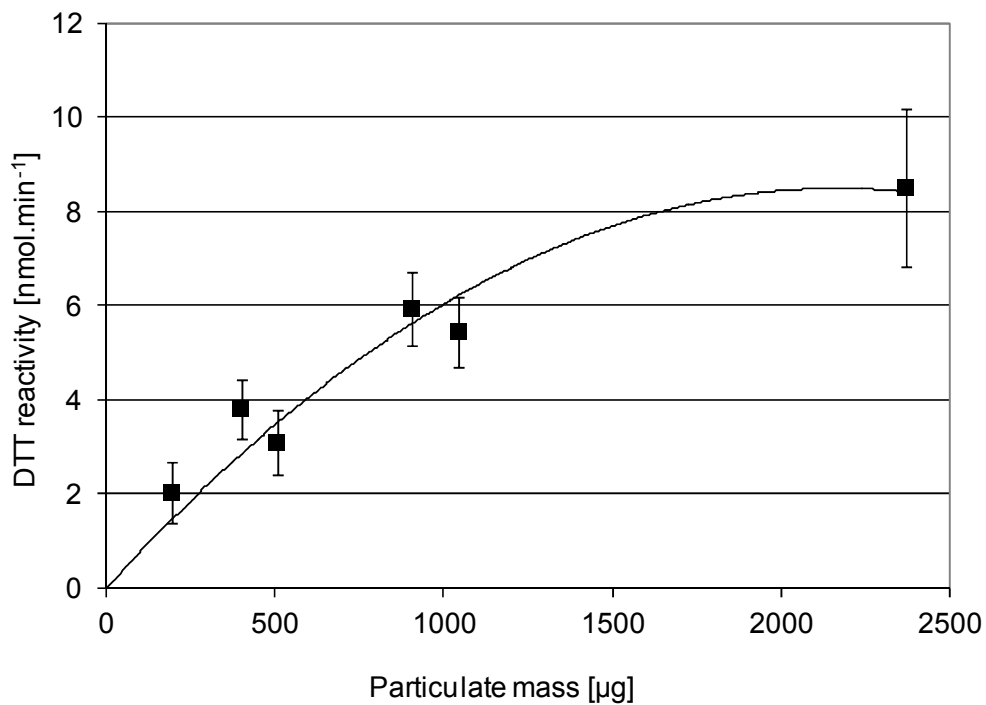


Figure S2: Relationship between the experimental DTT consumption and the mass of diesel particles loaded on a Teflon filter. Error bars corresponds to the uncertainty of the measurement.

2. Reconstructed mass

We reconstructed the PM₄ mass by considering only the OC, EC, Fe, Cu variables. In order to take into account for the presence of additional elements to carbon in the organic compounds, the OC concentrations were multiplied by a factor of 1.6 (Turpin and Lim, 2001). Figure S3 presents the reconstructed mass in function of the measured PM₄ mass.

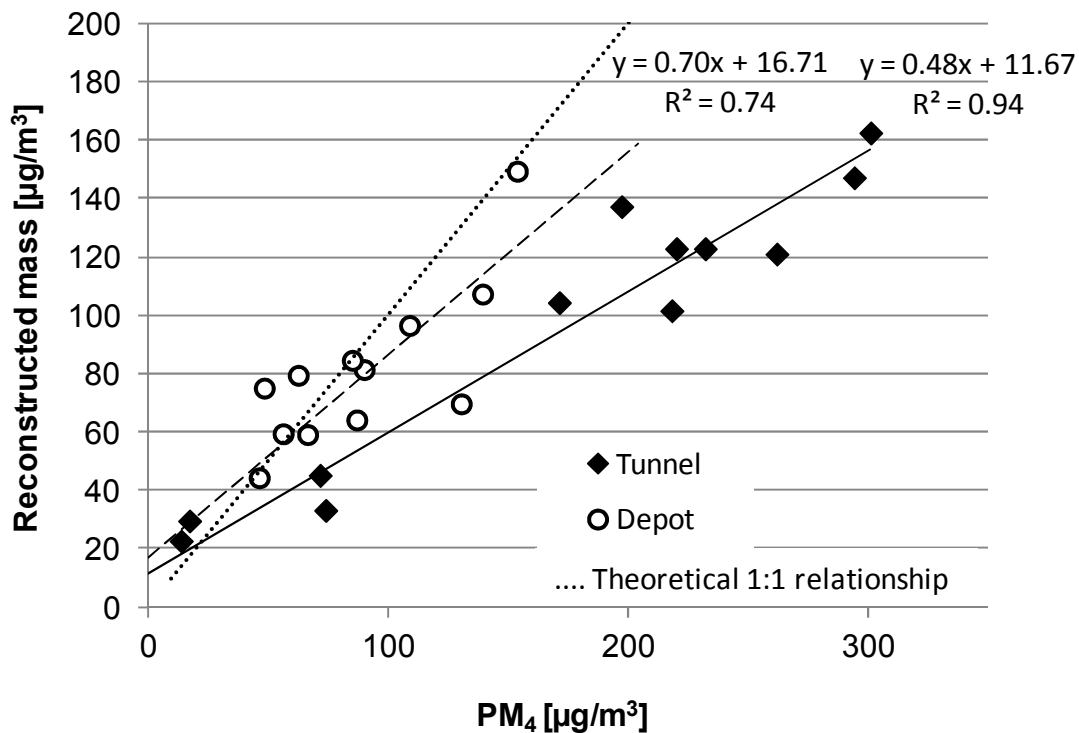


Figure S3: Comparison between the reconstructed PM₄ mass (based on OC, EC, Fe and Cu) and the experimental one for each sampling site. The dotted line corresponds to the theoretical 1:1 line. The equation for the regression line for each sampling site is also given, together with the corresponding regression coefficient (R^2).

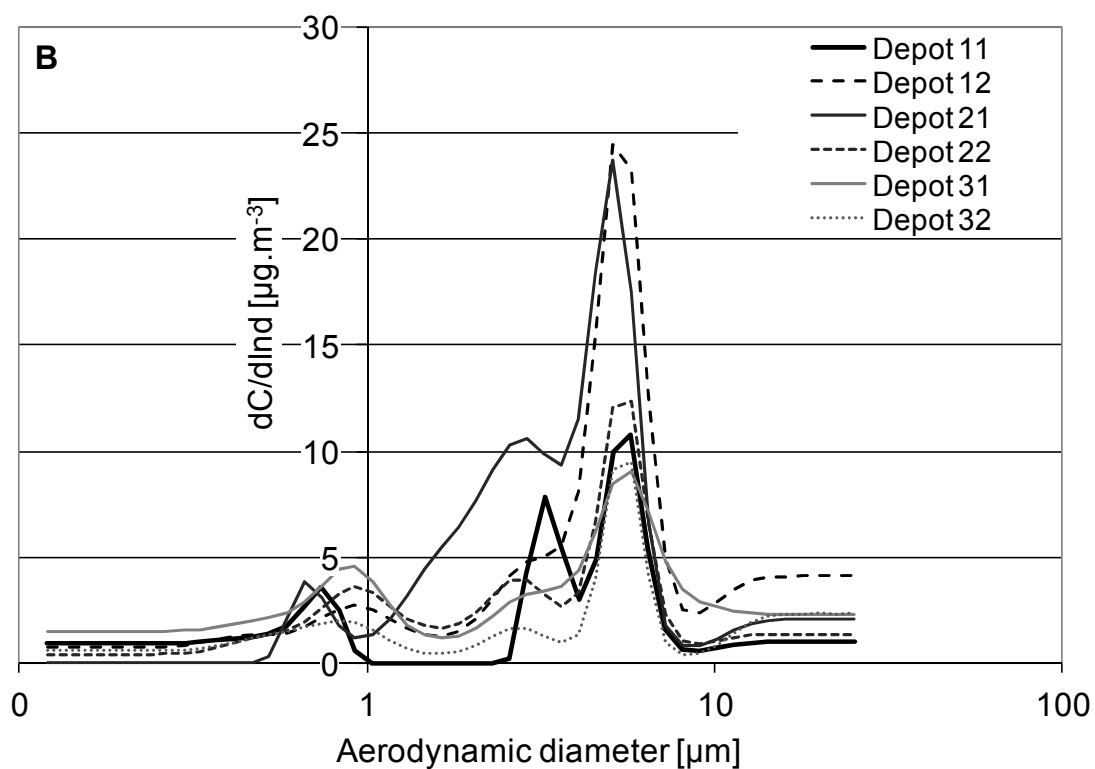
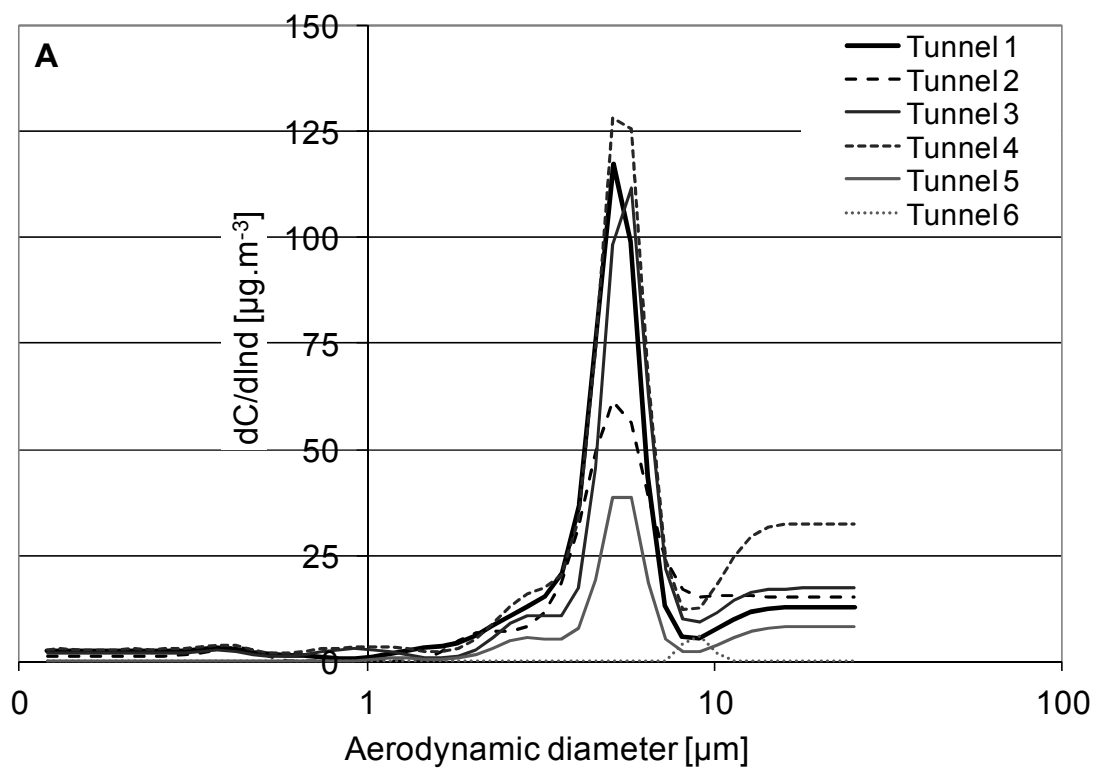


Figure S4 : Mass-based size distribution (Andersen impactor) of the particles sampled at **A**. Tunnel site; **B**. Depot site.

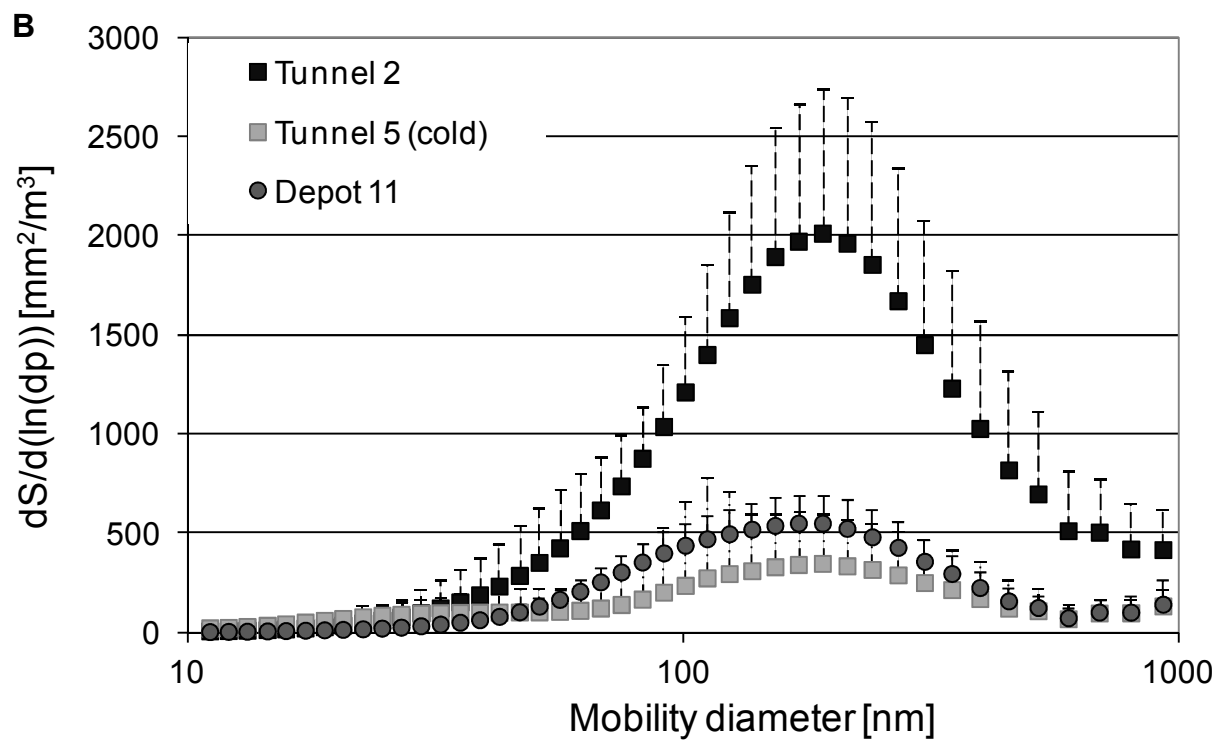
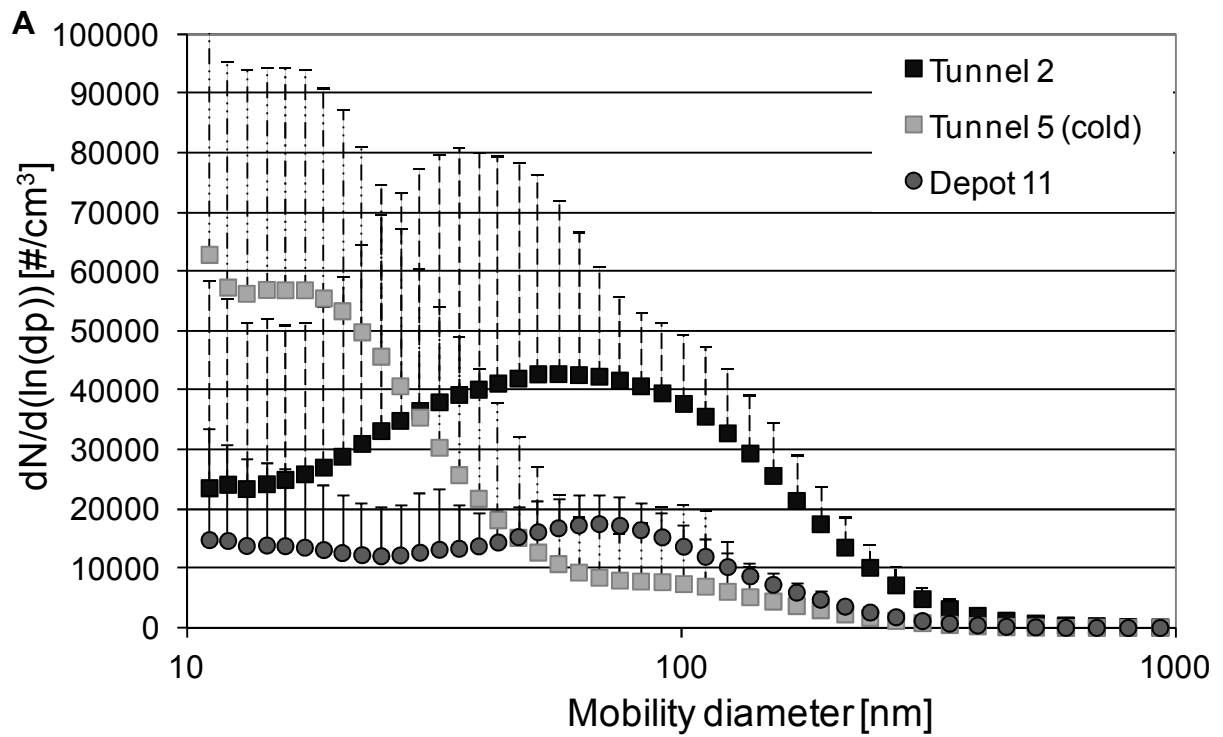


Figure S5: Typical examples of the averaged number- (A) and surface (B) size distribution measured at the “Tunnel” site (south wind conditions for Tunnel 2 and north wind conditions for Tunnel 5) and at the “Depot 11” site. Error bars correspond to the standard deviation of all the measured size distribution during a 7 hour sampling duration ($n \geq 115$ scans).

Table S1: Spearman correlation coefficient (rho) with number of data used (n) and the associated level of significance p (in bracket: n; p) between the different assessed chemical components PM₄ (normalised based on volume), in the two different sites. Significant correlations are indicated in bold.

“Tunnel”	OC	EC	∑ 4 quinones	∑ 7 PAH	Fe	Cu
OC		0.59 (12; 0.045)	0.89 (6; 0.019)	-0.09 (6; 0.872)	0.84 (10; 0.002)	0.75 (10; 0.012)
EC			0.54 (6; 0.266)	-0.43 (6; 0.396)	0.88 (10; 0.001)	0.96 (10; 0.001)
∑ 4 quinones				0.08 (6; 0.872)	0.88 (6; 0.019)	0.77 (6; 0.072)
∑ 7 PAH					-0.08 (6; 0.872)	-0.03 (6; 0.957)
Fe						0.95 (10; 0.001)
Cu						

“Depot”	OC	EC	∑ 4 quinones	∑ 7 PAH	Fe	Cu
OC		-0.23 (12; 0.469)	-0.48 (6; 0.329)	0.14 (6; 0.787)	0.32 (12; 0.313)	-0.01 (12; 0.983)
EC			0.54 (6; 0.266)	-0.43 (6; 0.396)	-0.14 (12; 0.656)	-0.15 (12; 0.636)
∑ 4 quinones				0.54 (6; 0.266)	0.43 (6; 0.396)	0.41 (6; 0.425)
∑ 7 PAH					-0.31 (6; 0.544)	0.64 (6; 0.173)
Fe						0.30 (12; 0.335)
Cu						

Figure S6: Relationship between OC and the other PM₄ chemical variables, expressed as air concentrations. Labels correspond to the “Tunnel” site or the “Depot” sites. **A:** OC=f(EC); **B:** OC=f(Σ 4 Quinones); **C:** OC=f(Σ 7 PAH); **D:** OC =f(Fe); **E:** OC =f(Cu).

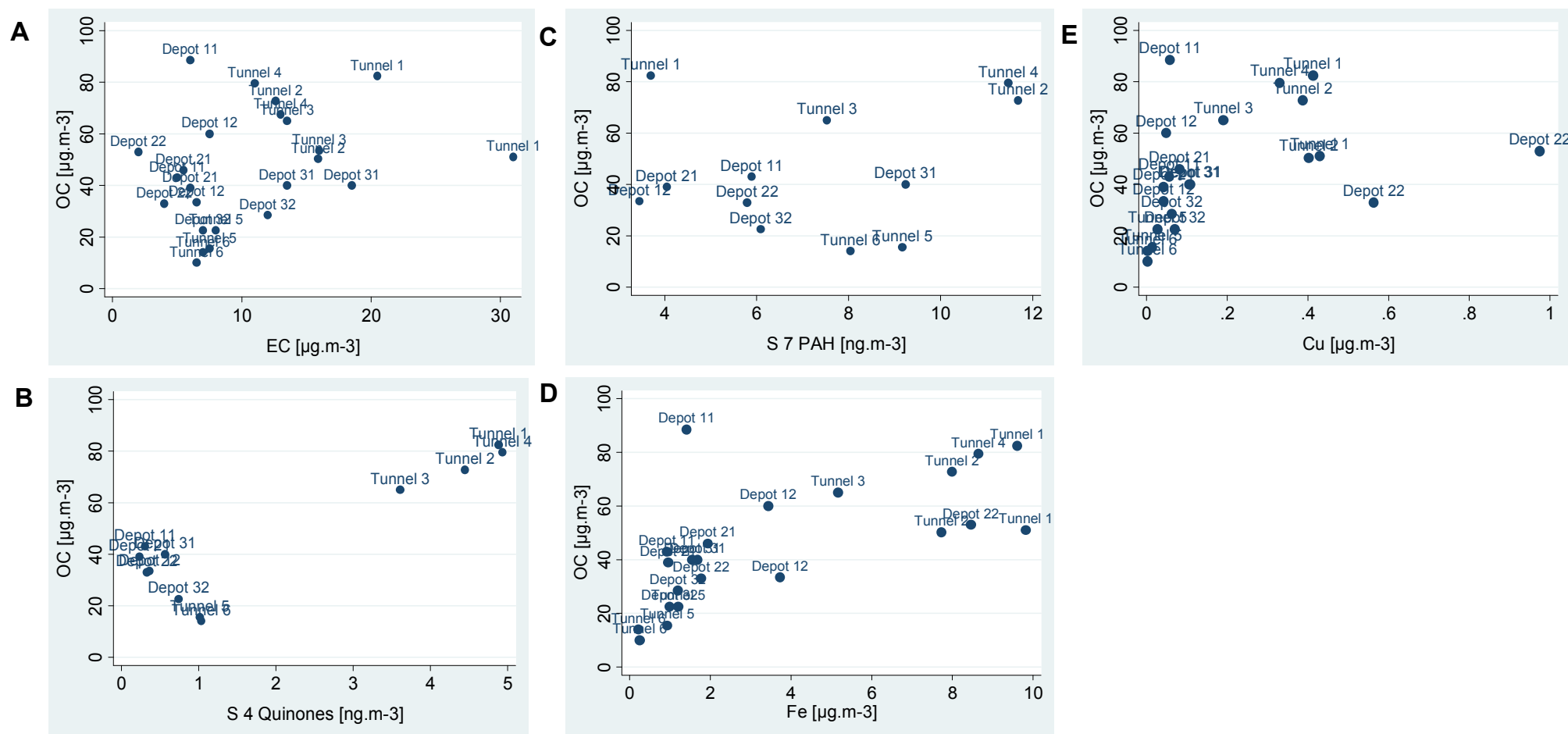


Figure S7: Relationship between Fe and the other PM₄ chemical variables, expressed as air concentrations. Labels correspond to the “Tunnel” site or the “Depot” sites. **A:** Fe=f(EC); **B:** Fe=f(Σ 4 Quinones); **C:** Fe=f(Σ 7 PAH); **D:** Fe =f(Cu).

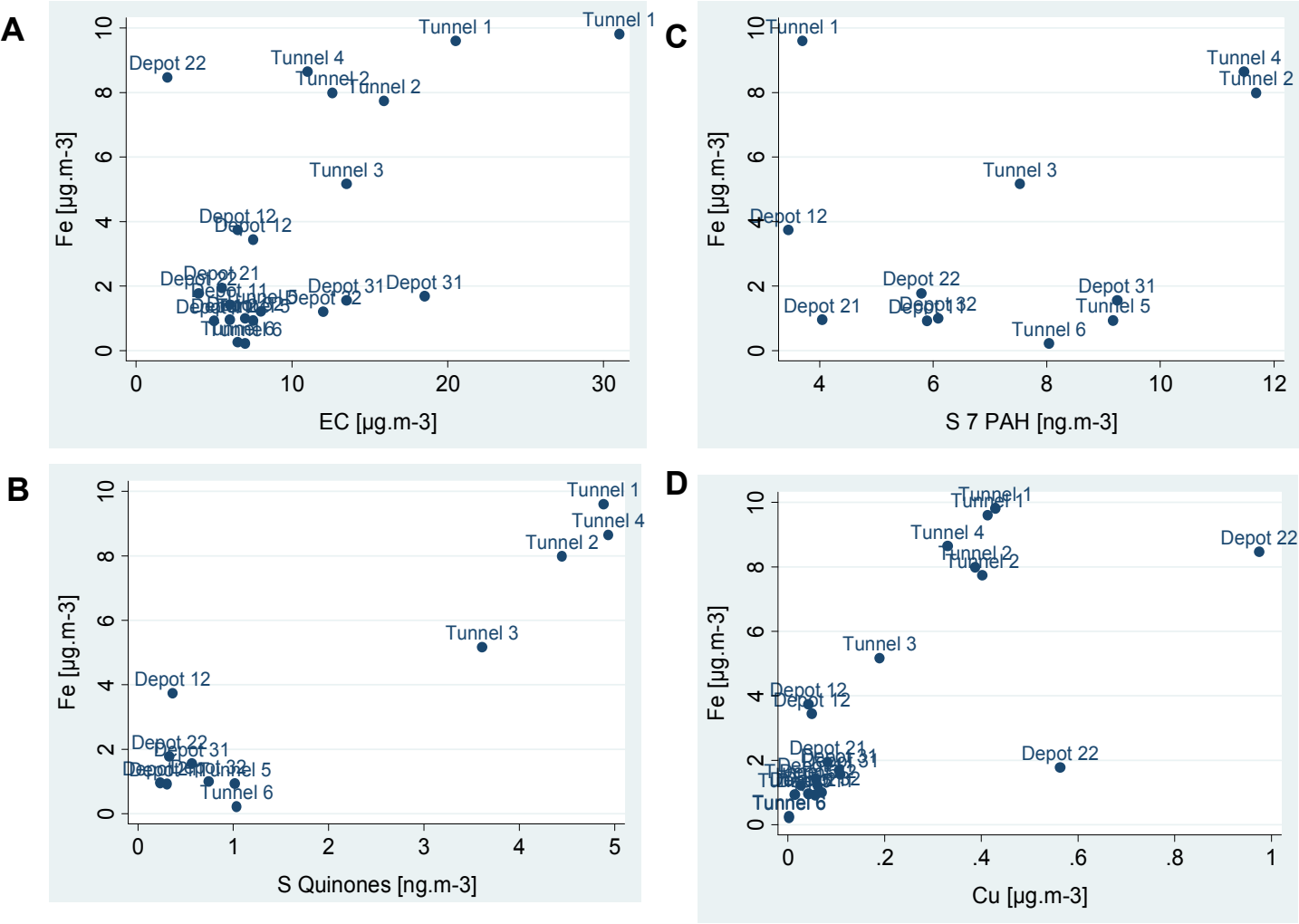


Figure S8: Relationship between EC and the other PM₄ chemical variables, expressed as air concentrations. Labels correspond to the “Tunnel” site or the “Depot” sites. **A:** EC=f(Σ 4 Quinones); **B:** EC=f(Σ 7 PAH); **C:** EC =f(Cu).

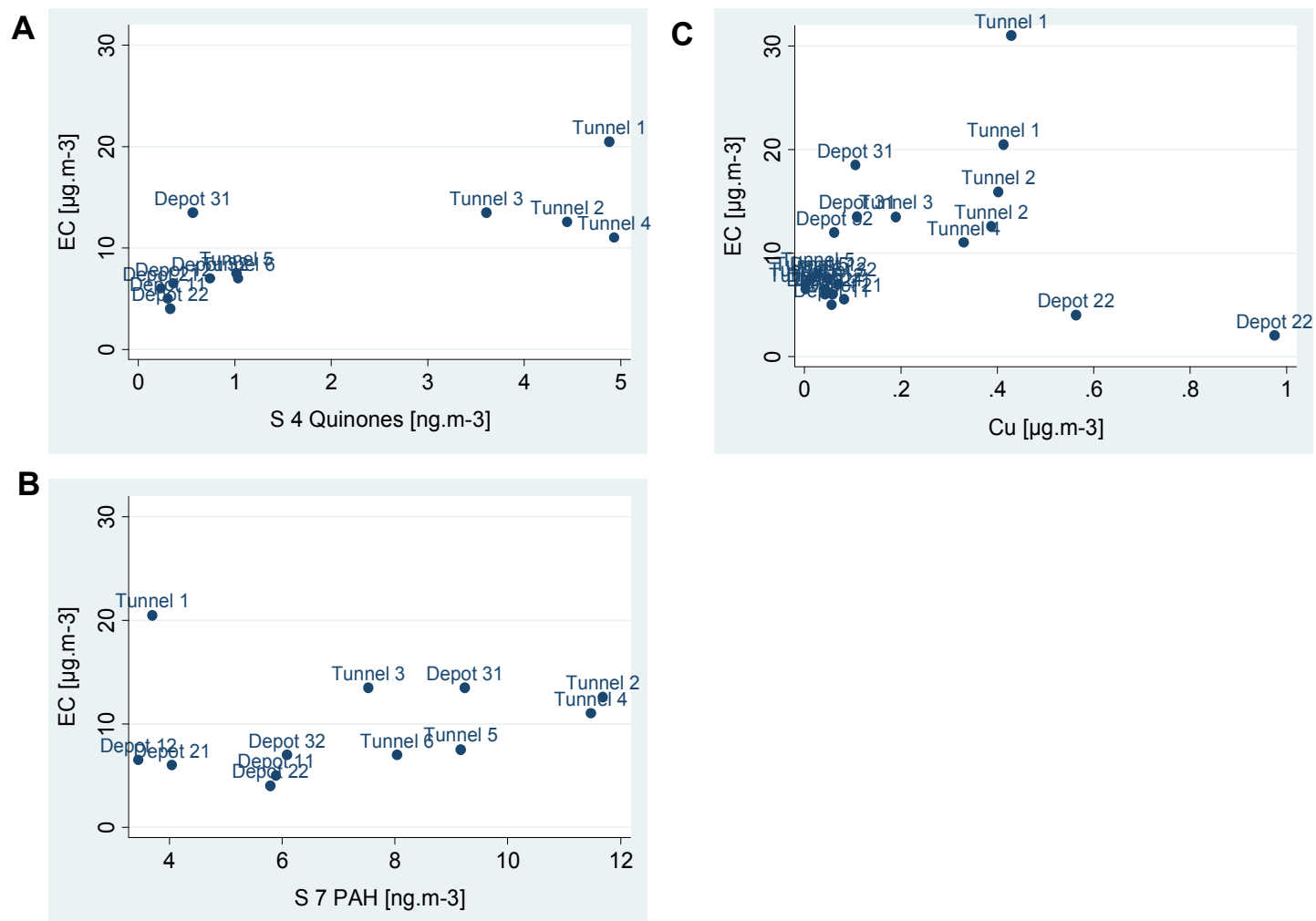


Figure S9: Relationship between Cu or Σ 4 Quinones and the other PM₄ chemical variables, expressed as air concentrations. Labels correspond to the “Tunnel” site or the “Depot” sites. **A:** Cu=f(Σ 4 Quinones); **B:** Cu=f(Σ 7 PAH); **C:** Σ 4 Quinones = f(Σ 7 PAH).

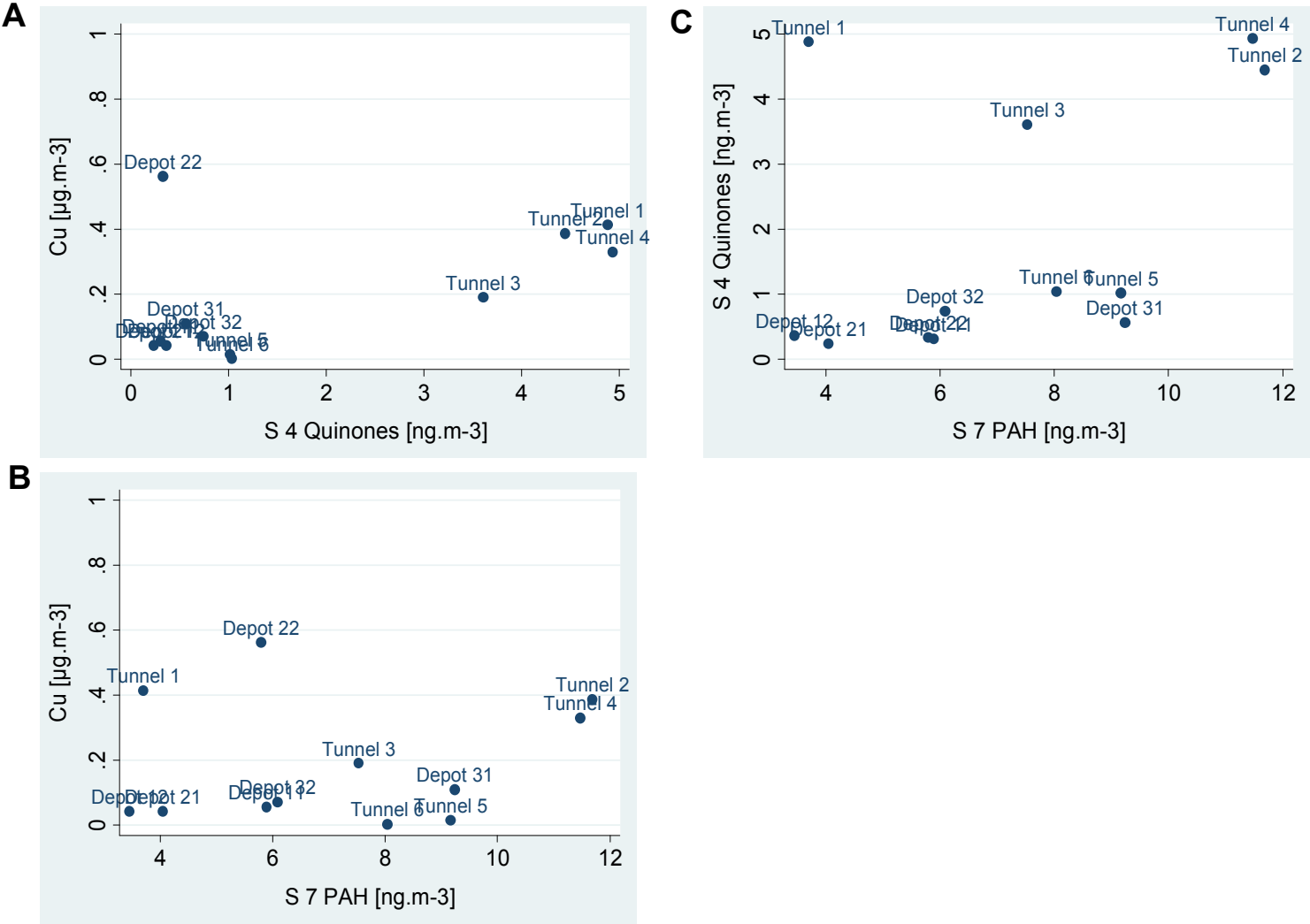


Figure S10: Relationship between the OP^{DTT} for PM_4 and the corresponding composition variables, expressed as air concentrations. Labels correspond to the “Tunnel” site or the “Depot” sites. **A:** $OP^{DTT}=f(OC)$; **B:** $OP^{DTT}=f(EC)$; **C:** $OP^{DTT}=f(\sum 4 \text{ Quinones})$; **D:** $OP^{DTT}=f(\sum 7 \text{ PAH})$; **E:** $OP^{DTT}=f(Fe)$; **F:** $OP^{DTT}=f(Cu)$.

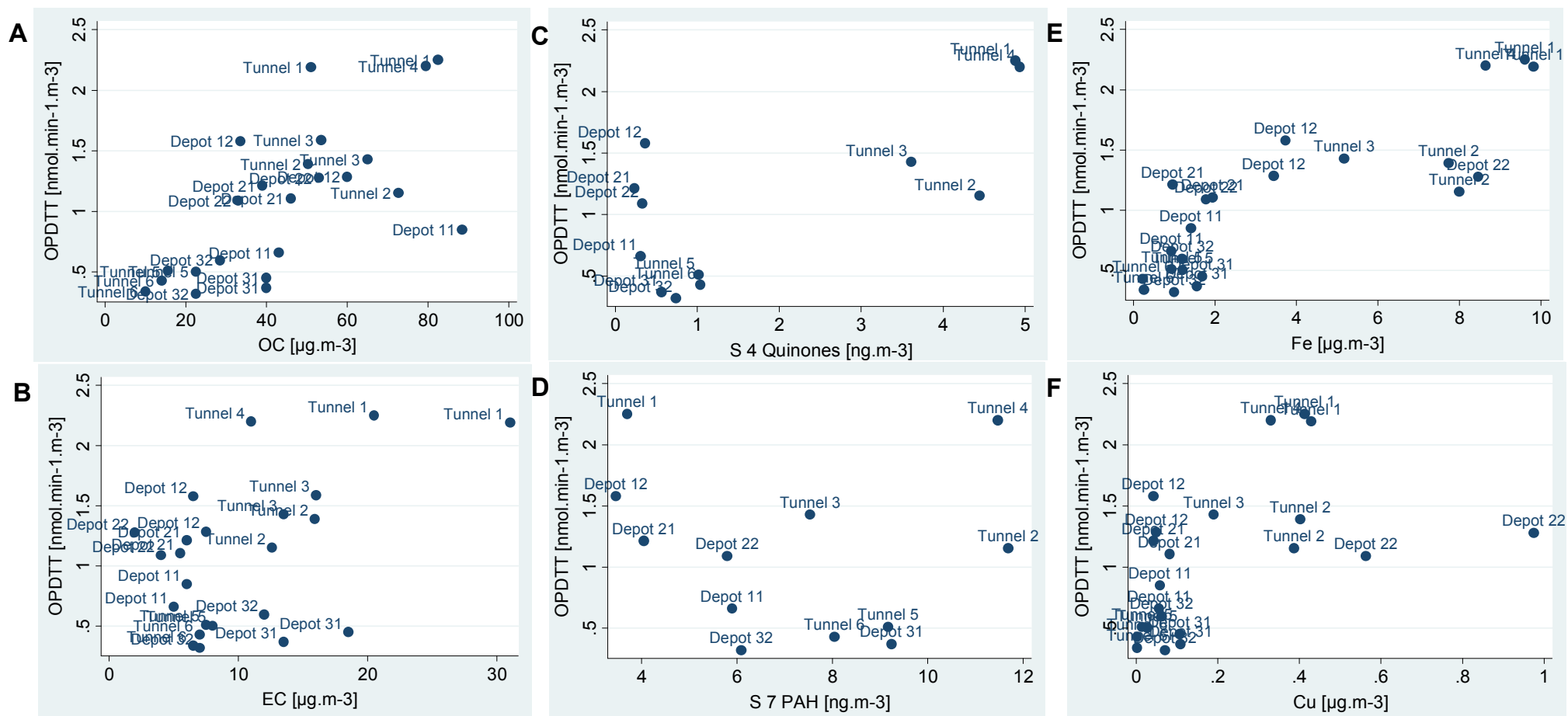


Table S2: Spearman correlation coefficient (ρ) with the number of data used (n) and the associated level of significance (p) between the different chemical variables and the PM₄ DTT redox reactivity (normalised based on air concentration) in the two different workplace type. Significant correlation is indicated in bold.

"Tunnel"	PM ₄ redox reactivity		
	Spearman rho	n	p
Number	0.257	6	0.623
Surface	0.886	6	0.019
OC	0.864	11	0.001
EC	0.827	11	0.002
∑ 4 quinones	0.829	6	0.042
∑ 7 PAH	-0.314	6	0.544
Fe	0.891	10	0.001
Cu	0.827	10	0.003

"Depot"	PM ₄ redox reactivity		
	Spearman rho	n	p
Number	-0.714	6	0.111
Surface	-0.754	6	0.084
OC	0.375	12	0.230
EC	-0.452	12	0.140
∑ 4 quinones	-0.600	6	0.208
∑ 7 PAH	-0.943	6	0.005
Fe	0.615	12	0.033
Cu	-0.375	12	0.230

Table S3: Reported OP^{DTT} values for ambient PM_{2.5} expressed either as mass (OP^{DTT}/μg) or volume (OP^{DTT}/m³). Either the observed range (with median in bracket) or the averaged values (with standard deviation) are presented for water-extracted filters from different locations.

OP _M ^{DTT} /μg [pmol.min ⁻¹ .μg ⁻¹]	OP ^{DTT} /m ³ [nmol.min ⁻¹ .m ⁻³]	Location type	Reference
14 – 24 (19)	0.04 – 1.3 (0.32)	Road side	(Delfino <i>et al.</i> , 2013)
18 – 55 (32)	0.10 - 0.16 (0.14)	Urban and Port area	(Hu <i>et al.</i> , 2008)
	0.20 – 0.43 (0.29)	Rural and urban	(Fang <i>et al.</i> , 2014)
	1.4 ^a	Urban background	(Janssen <i>et al.</i> , 2014)
	1.7 ^a	Stop&Go	(Janssen <i>et al.</i> , 2014)
	3.3 ^a	Continuous traffic	(Janssen <i>et al.</i> , 2014)
	0.58 – 1.67 (0.96) ^b	Riverside agriculture center	(Eiguren-Fernandez <i>et al.</i> , 2010)
5 – 24 (14)	0.08 – 0.80 (0.49)	Urban/Wildfire	(Verma <i>et al.</i> , 2009)
22±6			(Verma <i>et al.</i> , 2011)
21 – 75 (27) ^b			(Ntziachristos <i>et al.</i> , 2007)
15 - 39 (24) ^b		Urban	(De Vizcaya-Ruiz <i>et al.</i> , 2006)
27 – 61 (39)		Urban	(Charrier and Anastasio, 2012)
20 – 25 (23)		Rural	(Charrier and Anastasio, 2012)
10 – 48 (26) ^b		Traffic/receptor site	(Cho <i>et al.</i> , 2005)
4.3 – 23.2 (8.3)	0.17 – 1.35 (0.99)	Tunnel	This study
0.9 – 31.5 (7.5)	0.12 – 2.40 (0.57)	Depots	This study

^a: Geometric mean

^b: Water suspension

References

- Charrier JG, Anastasio C. (2012) On dithiothreitol (DTT) as a measure of oxidative potential for ambient particles: evidence for the importance of soluble transition metals. *Atmospheric Chemistry and Physics*; 12: 9321-33.
- Cho AK, Sioutas C, Miguel AH, *et al.* (2005) Redox activity of airborne particulate matter at different sites in the Los Angeles Basin. *Environ Res*; 99: 40-47.
- De Vizcaya-Ruiz A, Gutierrez-Castillo ME, Uribe-Ramirez M, *et al.* (2006) Characterization and in vitro biological effects of concentrated particulate matter from Mexico City. *Atmos Environ*; 40: S583-S92.
- Delfino RJ, Staimer N, Tjoa T, *et al.* (2013) Airway inflammation and oxidative potential of air pollutant particles in a pediatric asthma panel. *J Expo Sci Environ Epidemiol*; 23: 466-73.
- Eiguren-Fernandez A, Shinyashiki M, Schmitz DA, *et al.* (2010) Redox and electrophilic properties of vapor- and particle-phase components of ambient aerosols. *Environ Res*; 110: 207-12.

- Fang T, Verma V, Guo H, *et al.* (2014) A semi-automated system for quantifying the oxidative potential of ambient articles in aqueous extracts using the dithiothreitol (DTT) assay: results from the Southeastern Center for Air Pollution and Epidemiology (SCAPE). *Atmos Meas Tech Discuss*; 7: 7245 - 79.
- Hu S, Polidori A, Arhami M, *et al.* (2008) Redox activity and chemical speciation of size fractionated PM in the communities of the Los Angeles-Long Beach harbor. *Atmospheric Chemistry and Physics*; 8: 6439-51.
- Janssen NAH, Yang A, Strak M, *et al.* (2014) Oxidative potential of particulate matter collected at sites with different source characteristics. *Sci Total Environ*; 472: 572-81.
- Miet K, Le Menach K, Flaud PM, *et al.* (2009) Heterogeneous reactions of ozone with pyrene, 1-hydroxypyrene and 1-nitropyrene adsorbed on particles. *Atmos Environ*; 43: 3699-707.
- Ntziachristos L, Froines JR, Cho AK, *et al.* (2007) Relationship between redox activity and chemical speciation of size-fractionated particulate matter. *Part Fibre Toxicol*; 4: 5-5.
- Turpin BJ, Lim HJ. (2001) Species contributions to PM_{2.5} mass concentrations: Revisiting common assumptions for estimating organic mass. *Aerosol Sci Technol*; 35: 602-10.
- Verma V, Pakbin P, Cheung KL, *et al.* (2011) Physicochemical and oxidative characteristics of semi-volatile components of quasi-ultrafine particles in an urban atmosphere. *Atmos Environ*; 45: 1025-33.
- Verma V, Polidori A, Schauer JJ, *et al.* (2009) Physicochemical and Toxicological Profiles of Particulate Matter in Los Angeles during the October 2007 Southern California Wildfires. *Environmental Science & Technology*; 43: 954-60.