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

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## 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 (PM<sub>4</sub> and PM<sub>2.5</sub> 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<sup>DTT</sup>). The averaged mass concentration of respirable particles (PM<sub>4</sub>) at the Tunnel site was about twice the one at the Depot sites (173±103 µg/m<sup>3</sup> and 90±36 µg/m<sup>3</sup> respectively), whereas the OP<sup>DTT</sup> was practically identical for all the sites (10.6±7.2 pmol DTT.min<sup>-1</sup>.µg<sup>-1</sup> at the Tunnel site; 10.4±4.6 pmol DTT.min<sup>-1</sup>.µg<sup>-1</sup> at the Depot sites). The OP<sup>DTT</sup> of PM<sub>4</sub> was mostly present on the smallest PM<sub>2.5</sub> fraction (OP<sup>DTT</sup> PM<sub>2.5</sub>: 10.2±8.1 pmol DTT.min<sup>-1</sup>.µg<sup>-1</sup>; OP<sup>DTT</sup> PM<sub>4</sub>: 10.5±5.8

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

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31 Oxidative potential, DTT assay, occupational exposure, particulate matter, quinones  
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## 36 37 **1. Introduction**

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

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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  
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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.  
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## 10 11 **2. Materials and method**

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

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15 The particles OP methodology used was based on collecting particles on filters followed by  
16 assessing the OP<sup>DTT</sup> 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<sup>DTT</sup> (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.  
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## 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

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2  
3 determine their quinones, PAH and metal content, high volume PM<sub>4</sub> sampling was done by  
4 deploying a Digitel<sup>®</sup> DH77 (580 L/min, Digitel, Switzerland) and a GravikonPM4<sup>®</sup> (60 L/min,  
5 Strohlein, Germany) side by side (Table 1). The OP<sup>DTT</sup> for PM<sub>4</sub> was measured on punches  
6 from filters of the GravikonPM4<sup>®</sup>. Low volume samplers were also used at both locations for  
7 the determination of the PM<sub>4</sub> content of OC, EC and the OP<sup>DTT</sup> of PM<sub>2.5</sub> (Table 1).  
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10 Due to logistical constrains, the Digitel<sup>®</sup> 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).  
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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  
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3 a GC-MS system (Saturn 4000, Varian, Switzerland). Total iron and copper were determined  
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5 after ultrasonic digestion of the filter with HF and subsequently with HCl:HNO<sub>3</sub> 2:1. After  
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7 diluting the residue, the metal concentrations were measured with an Atomic Absorption  
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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*

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19 All statistical analyses were performed with STATA (STATA/IC 12.1, College Station USA).  
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21 Values smaller than the limit of detection (LOD, corresponding to three time the standard  
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23 deviation of a blank) were replaced by ½ LOD in the database for statistical treatment. Due  
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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  
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29 the PM physico-chemical characteristics and the OP<sup>DTT</sup> (expressed as air concentration) was  
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31 assessed separately for each workplace type (“Tunnel” or “Depot”) by using the Spearman  
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33 rank correlation with the associated probability. Association was considered significant with  
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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*

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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  
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50 four initial sampling days, when the wind came from the south through the tunnel. In contrast,  
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52 low concentrations were measured when the wind came from the north (“Tunnel” 5 and 6).  
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54 These two days were also particularly cold (average -2.7°C). Concentrations measured at  
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56 the three “Depot” sites were lower and less variable.

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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 ( $6.6\pm 1.3$   
5  $\mu\text{m}$ ) compared to the "Depot" sites ( $3.3\pm 0.6 \mu\text{m}$ ; Mann-Whitney test,  $p=0.004$ ). The particle  
6  
7 number concentration was rather constant at "Tunnel" ( $104'000\pm 17'000 \text{ cm}^{-3}$ ) and higher  
8  
9 compared to the different "Depot" workplaces ( $60'000\pm 30'000 \text{ cm}^{-3}$ ; Mann-Whitney test,  
10  
11  $p=0.025$ ). The geometric mean diameter (GMD) measured at the Tunnel site was quite  
12  
13 variable, with larger diameters on days with southerly winds and warmer temperatures  
14  
15 ( $52\pm 10 \text{ nm}$ ,  $n=4$ ) compared to situations with northerly wind ( $24\pm 3 \text{ nm}$ ,  $n=2$ ). At the "Depot"  
16  
17 sites, the GMD was quite similar for all sampling days (average  $47\pm 4 \text{ nm}$ ,  $n=6$ ). At both sites  
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19 the geometric standard deviation was about 2.3, which suggests that all sites presented  
20  
21 polydisperse particles. When the SMPS-derived surface concentration was considered, a  
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23 broad range of values were obtained (Table 2), with the smallest surface concentration for  
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25 "Tunnel" 5 and 6 (cold situation with north wind going into the tunnel), followed by all the  
26  
27 Depot sites and the largest values for the "Tunnel" 1 to 4. Typical averaged number- and  
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29 surface-based size distribution measured at the "Tunnel" and "Depot" sites are given in the  
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31 supplementary material (Figure S5).  
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### 34 35 3.1.2. Chemical characteristics

36  
37 Table 3 presents the different chemical compounds analysed. OC was the predominant  
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39 measured compound. At the "Tunnel" site on average  $48\pm 4\%$  of the measured  $\text{PM}_4$  mass  
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41 was explained by the sum of OC, EC, Fe and Cu, while at the "Depot" the same components  
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43 explained a larger part of the  $\text{PM}_4$  mass ( $70\pm 12\%$ ). For this calculation, we used an organic  
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45 molecular weight per carbon weight ratio of 1.6, as recommended by Turpin *et al.*, 2001 (see  
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47 supplementary material Figure S3 in online edition). The mass-based OC content of the  
48  
49 collected particles was significant lower in the "Tunnel" site (average  $33\pm 13\%$ ,  $n=12$ )  
50  
51 compared to the "Depot" sites (average  $45\pm 11\%$ ,  $n=12$ ; Mann-Whitney test,  $p=0.018$ ). The  
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53 ratio of elemental to total carbon (with total carbon TC corresponding to the sum of OC+EC),  
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55 differed significantly between the two sites (average "Tunnel":  $25\pm 9\%$ ,  $n=12$ ; average  
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57 "Depot":  $16\pm 9\%$ ,  $n=12$ ; Mann-Whitney test,  $p=0.021$ ) but also within the tunnel in function of  
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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.  
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10 The chemical compounds showed a different correlation pattern at the two workplaces (see  
11 supplementary material Table S1 and Fig S6-S9 in online edition). At the "Tunnel" site,  
12 consistent correlations were observed between OC and EC,  $\Sigma$  4 Quinones and metals (Fe,  
13 Cu); EC with metals (Fe, Cu) and  $\Sigma$  4 Quinones with Fe. At the "Depot" site, no association  
14 was observed between any measured chemical variables.  
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### 22 3.2. Oxidative potential

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26 The intrinsic mass-based  $OP^{DTT}$  ( $OP^{DTT}/\mu g$ ) of the sampled particles is given in Figure 2 A.  
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28 The  $OP^{DTT}/\mu g$  didn't differ significantly between both sites ( $PM_4$ :  $10.6\pm 7.2$  pmol DTT/min/ $\mu g$   
29 at "Tunnel",  $n=11$ ;  $10.4\pm 4.6$  pmol DTT/min/ $\mu g$  at "Depot",  $n=12$ ; Mann Whitney test,  $p=0.71$ ;  
30  
31  $PM_{2.5}$ :  $9.3\pm 5.4$  pmol DTT/min/ $\mu g$  at "Tunnel",  $n=12$ ;  $11.0\pm 10.0$  pmol DTT/min/ $\mu g$  at "Depot",  
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33  $n=12$ ; Mann Whitney test,  $p=0.86$ ). The largest intrinsic reactivity was observed for  
34 workplaces with very small particles ("Tunnel" 6) or with a high copper content ("Depot" 2 2).  
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36 A large fraction of the  $PM_4$  reactivity was already present on the smaller  $PM_{2.5}$  fraction  
37 (Figure 3, average  $PM_{2.5}/PM_4$  reactivity ratio for all sites:  $97\pm 22\%$ ). The  $OP^{DTT}$  exposure level  
38 to humans (Figure 2B,  $OP^{DTT}/m^3$ ) was obtained by multiplying the  $OP^{DTT}/\mu g$  with the  
39 corresponding PM concentration. As for the  $OP^{DTT}/\mu g$ , no statistically significant difference  
40 was observed between  $OP^{DTT}/m^3$  at both sites ( $1.3\pm 0.7$  nmol DTT/min/ $m^3$  at "Tunnel",  $n=11$ ;  
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42  $0.9\pm 0.4$  nmol DTT/min/ $m^3$  at "Depot",  $n=12$ ; Mann Whitney test,  $p=0.20$  for  $PM_4$  and  $0.8\pm 0.4$   
43  
44 nmol DTT/min/ $m^3$  at "Tunnel",  $n=12$ ;  $0.8\pm 0.7$  nmol DTT/min/ $m^3$  at "Depot",  $n=12$ ; Mann  
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46 Whitney test,  $p=0.23$  for  $PM_{2.5}$ ). For both  $PM_4$   $OP^{DTT}/\mu g$  and  $OP^{DTT}/m^3$  levels, large inter-day  
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48 variations were observed. A maximum of three-fold increase for the  $PM_4$   $OP^{DTT}/\mu g$  was  
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50 observed between "Tunnel 2" and "Tunnel 6" ( $7.0$  to  $24.9$  pmol DTT/min/ $\mu g$  respectively)  
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3 whereas it decreased about the same order for these two sampling days when expressed as  
4 air volume (1.27 nmol/min/m<sup>3</sup> to 0.38 nmol/min/m<sup>3</sup> 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 PM<sub>4</sub> OP<sup>DTT</sup>/m<sup>3</sup> 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 PM<sub>4</sub> OP<sup>DTT</sup>/m<sup>3</sup>. At the  
9 “Depot” site, significant associations were observed only between PM<sub>4</sub> OP<sup>DTT</sup>/m<sup>3</sup> and Fe  
10 (positive) or Σ 7 PAH (negative association).  
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## 22 **4. Discussion**

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

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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 PM<sub>4</sub> mass at the “Depot” sites could be attributed to organic, elemental  
31 carbon, Fe and Cu, while about half of the total PM<sub>4</sub> 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.  
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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  
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7 “Tunnel” site corresponds to typical combustion-based processes. The predominantly  
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9 combustion origin of the PM sampled at the “Tunnel” site is suggested by their components.  
10  
11 The observed EC/TC ratio of  $0.25 \pm 0.08$  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 tough 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  
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#### 44 4.2. Oxidative potential of particles

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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  
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57 the particles in water or methanol, as done by the cited authors in Table S3 (supplemental  
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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 Although the two types of workplaces differed based on their physico-chemical  
26 characteristics, the  $OP^{DTT}$  of the particles was practically identical, irrespective of the metric  
27 used (mass or air volume). Whereas this observation might be different with other OP  
28 assays, this suggests that the potential generation of ROS in lungs of particle exposed  
29 workers would be similar at both workplaces even though their composition differs. The  $PM_4$   
30  $OP^{DTT}/m^3$  of the combustion type PM (“Tunnel” site) was associated with SMPS-based  
31 surface, organics (OC, quinones) and/or Fe, Cu (see supplementary material Table S2 in  
32 online edition), consistent with literature data relative to urban or combustion-based PM (Cho  
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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).  
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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  
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59 to respond to organics and also metals. These two compounds families are considered key  
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3 for the induction of PM-related oxidative stress and subsequent pro-inflammatory processes  
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5 (Donaldson *et al.*, 2005; Nawrot *et al.*, 2009). The measurement of redox activity of particles  
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7 as a new metric is appealing (Borm *et al.*, 2007). However, many questions have to be  
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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  
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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  
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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.  
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## 54 **5. Conclusion**

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56 The DTT assay allowed to quantitatively determine a redox activity for all the particles  
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58 collected in different occupational environments. Whereas the PM mass concentrations  
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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.  
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42 The authors declare no conflict of interest relating to the material presented in this article.  
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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 <sub>4</sub>	Quartz (Whatman QM-A, Milian, Switzerland, Ø 150 mm)	Gravimetry (PM <sub>4</sub> )	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 <sub>4</sub> , Strohle, Germany); 60 L/min; PM <sub>4</sub> cyclone	PTFE coated glass fiber (Pallflex, T60A20-HT, Pall Corporation, USA, Ø 70 mm)	Gravimetry (PM <sub>4</sub> )	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 <sup>®</sup> PM <sub>2.5</sub>	PTFE (SKC, Blanc-Labo, Switzerland; 2 µm, Ø 37 mm)	Gravimetry (PM <sub>2.5</sub> )	Whole filter	Oxidative potential	DTT assay
Low volume sampler (MSA, Pittsburgh, USA); 2 L/min; Cyclone Casella PM <sub>4</sub>	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)	-	-	-

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.

Site	Mass				Number			Surface		
	PM <sub>4</sub>	PM <sub>2.5</sub>	Median diameter <sup>a</sup>	σ <sub>g</sub> <sup>a</sup>	Number	Mean geometric diameter <sup>b</sup>	σ <sub>g</sub> <sup>b</sup>	Surface	Mean geometric diameter <sup>b</sup>	σ <sub>g</sub> <sup>b</sup>
	[μg/m <sup>3</sup> ]	[μg/m <sup>3</sup> ]	[μm]		[#/cm <sup>3</sup> ]	[nm]		[mm <sup>2</sup> /m <sup>3</sup> ]	[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	

<sup>a</sup>: Based on the Andersen measurement, n=1.

<sup>b</sup>: Based on SMPS measurement, using the same number of measurements (scans). σ<sub>g</sub>: 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 [ $\mu\text{g}/\text{m}^3$ ]	$\Sigma$ 4 quinones <sup>a, b</sup> [ $\text{ng}/\text{m}^3$ ]	$\Sigma$ 7 PAH <sup>b, c</sup> [ $\text{ng}/\text{m}^3$ ]	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 <sup>b</sup>	0.19 <sup>b</sup>
Tunnel 4	74±8 (4)	12±1 (4)	4.94	11.47	8.7 <sup>b</sup>	0.33 <sup>b</sup>
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. <sup>e</sup>
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 <sup>d</sup>	12	3	0.04	0.3	0.01	0.004

<sup>a</sup>: Sum of 1,2-Naphtoquinone + 1,4-Naphtoquinone + 9,10-Phenanthroquinone + 9,10-Anthraquinone.

<sup>b</sup>: n=1

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

<sup>d</sup>: LOD: Limit of detection, corresponding to three times the noise of a blank sample.

<sup>e</sup>: not detected (< LOD)

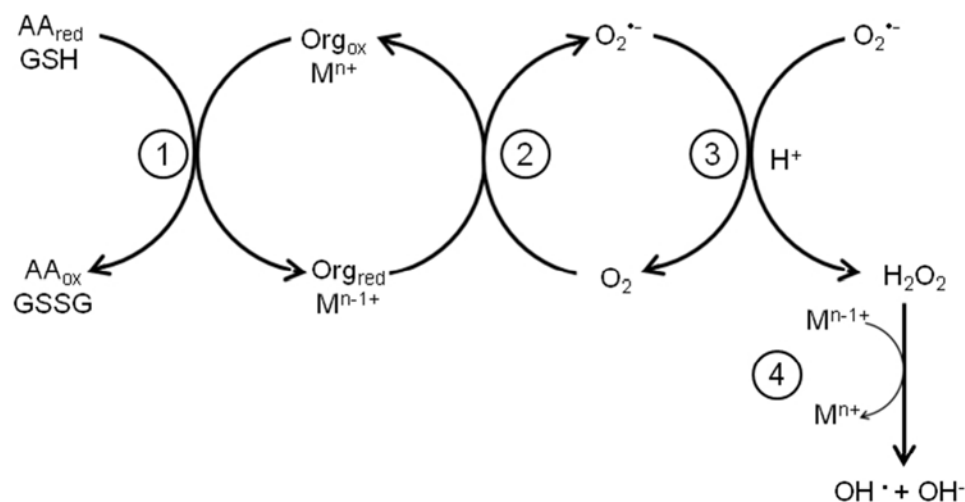


Figure 1: Cascade of possible reactions taking place in the reducing milieu of the lung lining fluid and generating reactive oxygen species ( $O_2^{\bullet-}$ ,  $H_2O_2$ ,  $OH^{\bullet}$ ) (adapted from Held and Biaglow, (1994)). AA<sub>red</sub> : ascorbic acid (reduced form); AA<sub>ox</sub> : ascorbic acid (oxidised form); RSH<sub>red</sub> : thiol-containing molecule like glutathione or DTT (reduced form); RSSR<sub>ox</sub> : thiol-containing molecule like glutathione or DTT (oxidised form); PM<sub>red</sub> : surface functions of particulate matter (reduced form); PM<sub>ox</sub> : surface functions of particulate matter (oxidised form); Org<sub>red</sub> : organic compound adsorbed on the particle (reduced form); Org<sub>ox</sub> : organic compound adsorbed on the particle (oxidised form); M<sup>(n-1)+</sup> : redox active metal (reduced form); M<sup>n+</sup> : redox active metal (oxidised form); O<sub>2</sub> : oxygen; O<sub>2</sub><sup>•-</sup> : superoxide ion; H<sub>2</sub>O<sub>2</sub> : hydrogen peroxide; OH<sup>•</sup> : peroxide radical.



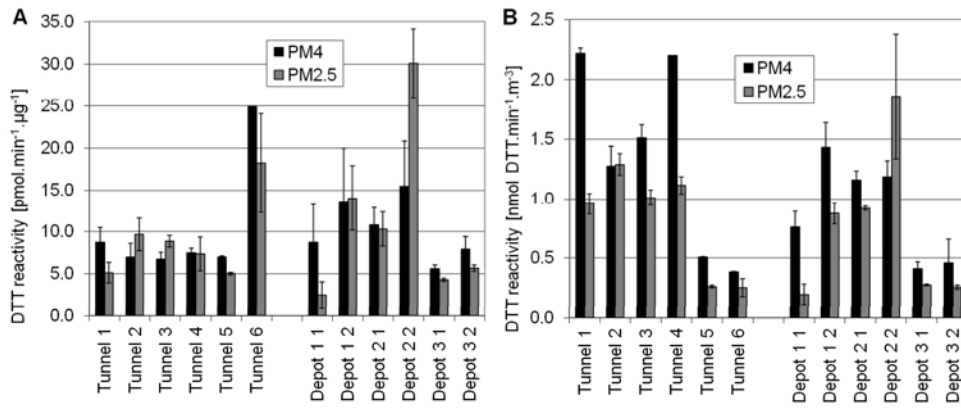


Figure 2. OPDTT per particle mass (A) and OPDTT expressed as an air concentration (B) for the particles collected in the different workplaces. Bars correspond to the standard deviation of duplicate measurements.

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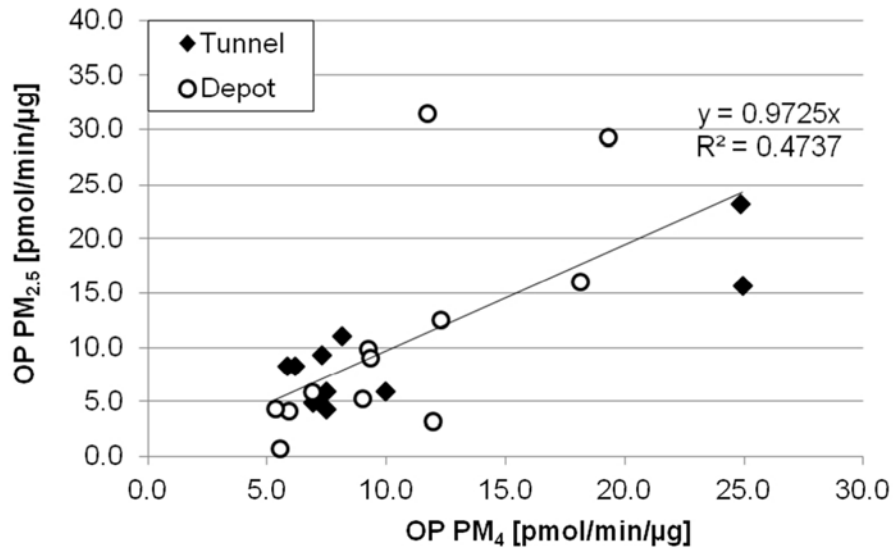


Figure 3: Relationship between the oxidative potential of PM<sub>2.5</sub> and PM<sub>4</sub> for the two types of workplaces. The equation for the regression line for all the sampling sites is also given, together with the corresponding regression coefficient (R<sup>2</sup>).

## Supplemental material

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

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#### *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 m<sup>3</sup>) 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<sup>®</sup>, SKC Inc, USA), allowing to collect PM<sub>2.5</sub> (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 PM<sub>4</sub> (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<sub>2.5</sub>) and on quartz filters (PM<sub>4</sub>) were 6.8±1.6 pmol DTT.min<sup>-1</sup>.µg<sup>-1</sup> (n=3) and 6.7±3.6 pmol DTT.min<sup>-1</sup>.µg<sup>-1</sup> (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).

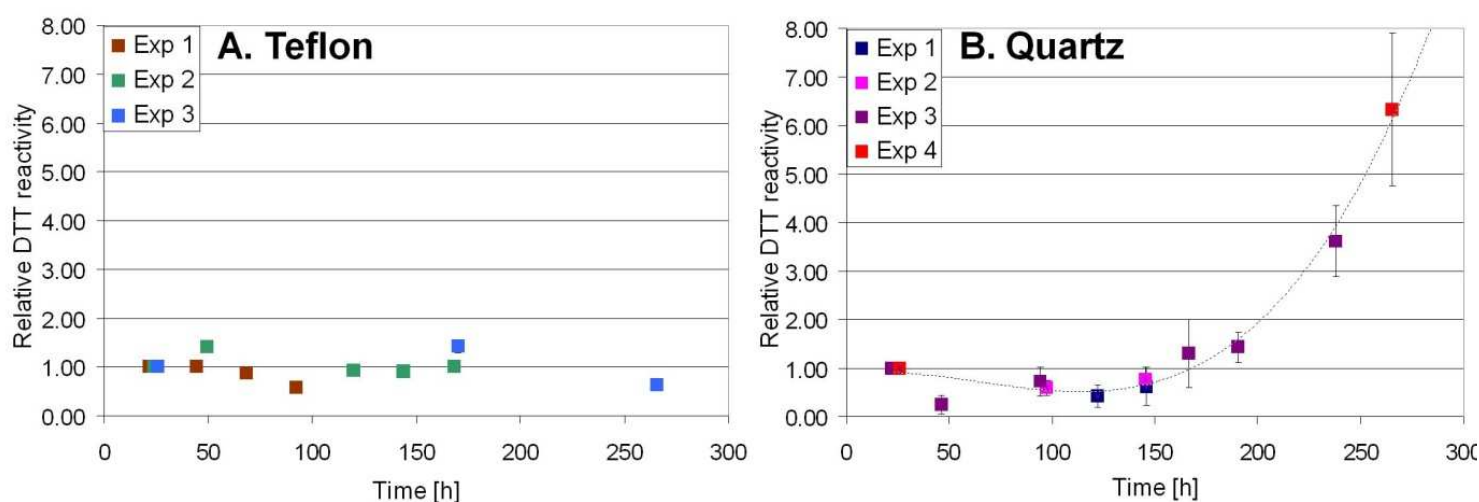


Figure S1: Effect of the storage duration on the DTT reactivity of particles collected from a diesel van. Reactivity of the loaded filter is expressed relative to the one determined after 24 hours stabilisation in a glovebox at 55% humidity. **A.** Teflon filter **B.** Quartz filter

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  $0.16 \text{ mg/cm}^2$ ).

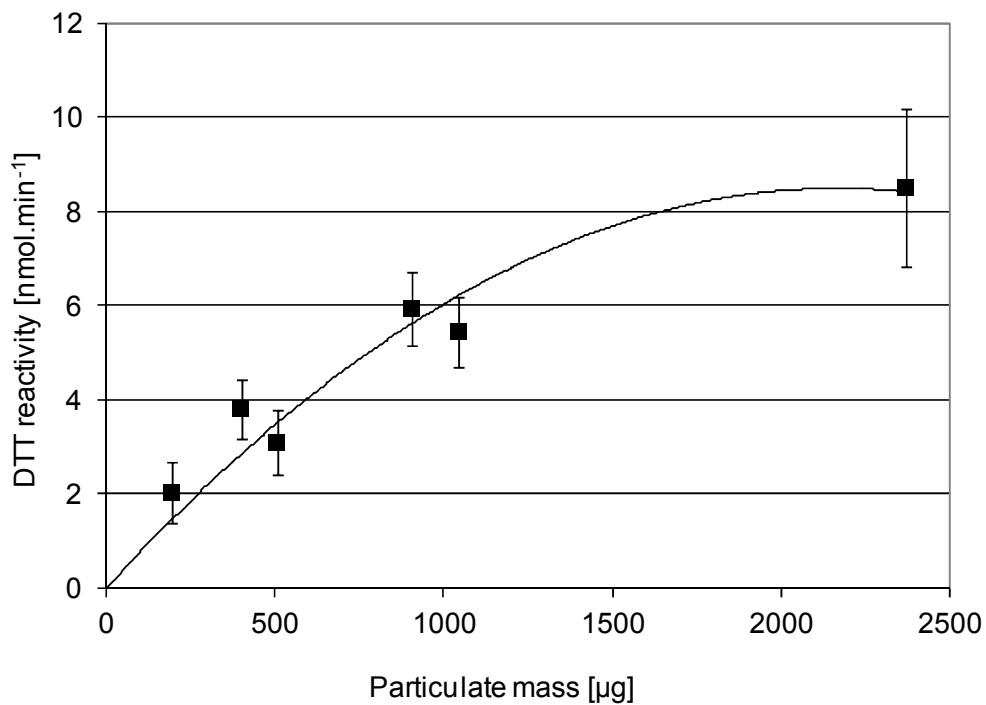


Figure S2: Relationship between the experimental DTT consumption and the mass of diesel particles loaded on a Teflon filter. Error bars corresponds to the uncertainty of the measurement.

## 2. Reconstructed mass

We reconstructed the PM<sub>4</sub> 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 PM<sub>4</sub> mass.

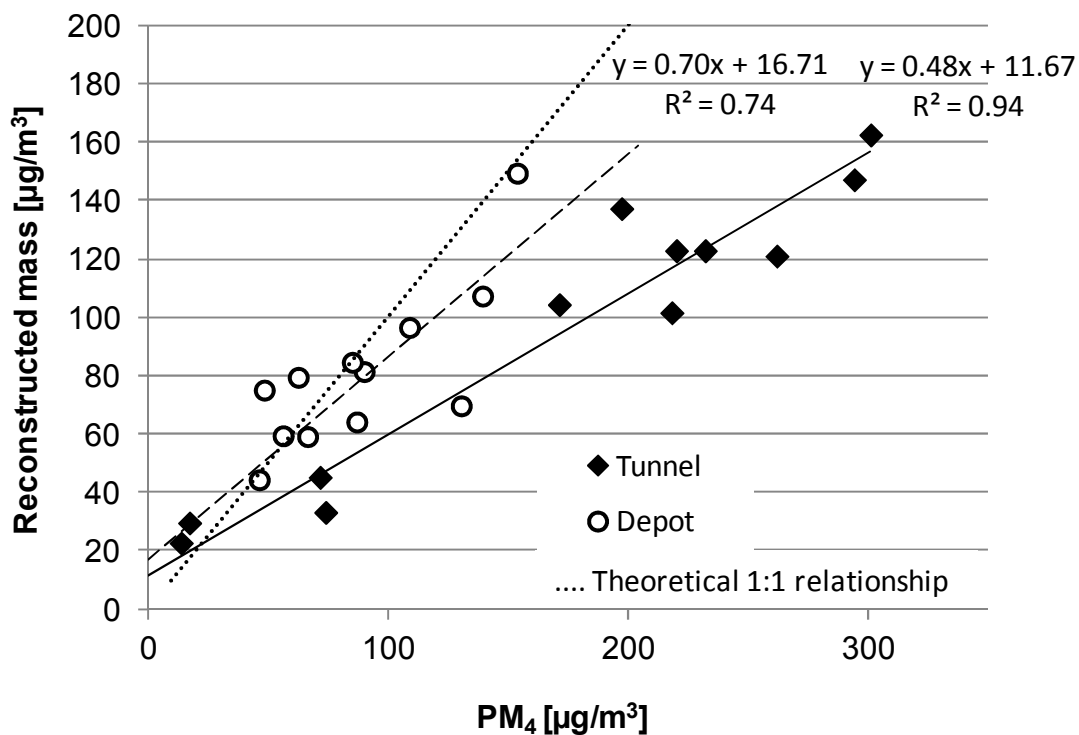


Figure S3: Comparison between the reconstructed PM<sub>4</sub> mass (based on OC, EC, Fe and Cu) and the experimental one for each sampling site. The dotted line corresponds to the theoretical 1:1 line. The equation for the regression line for each sampling site is also given, together with the corresponding regression coefficient ( $R^2$ ).

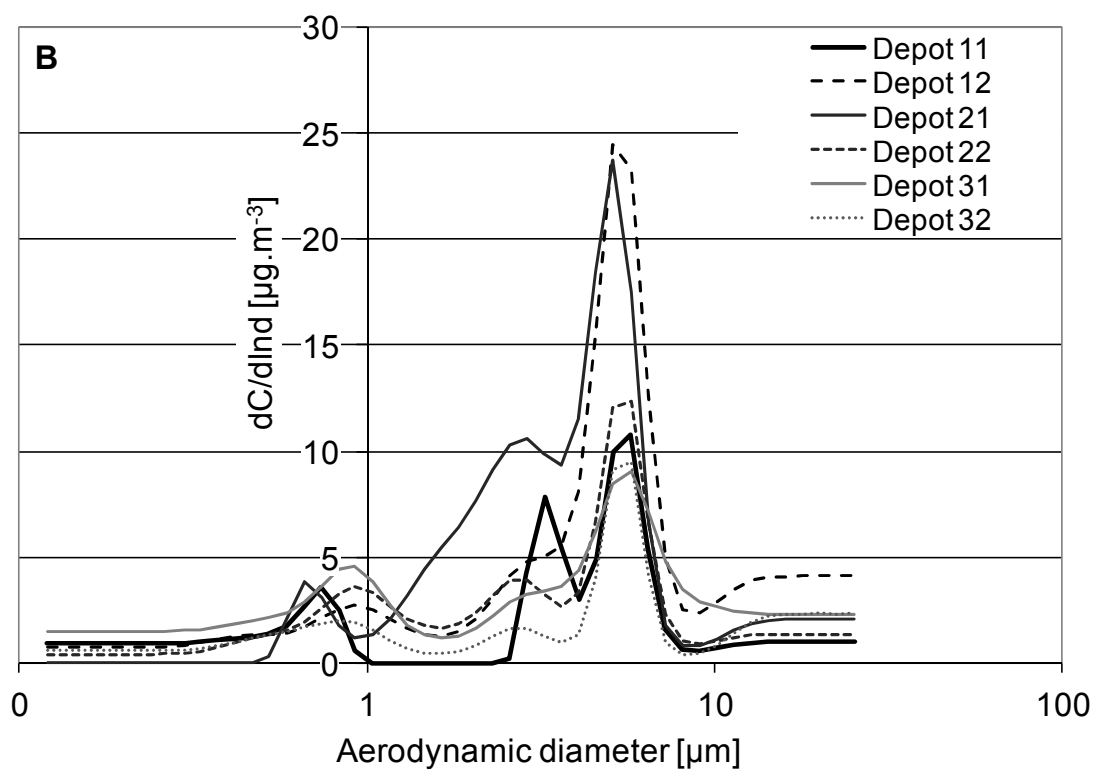
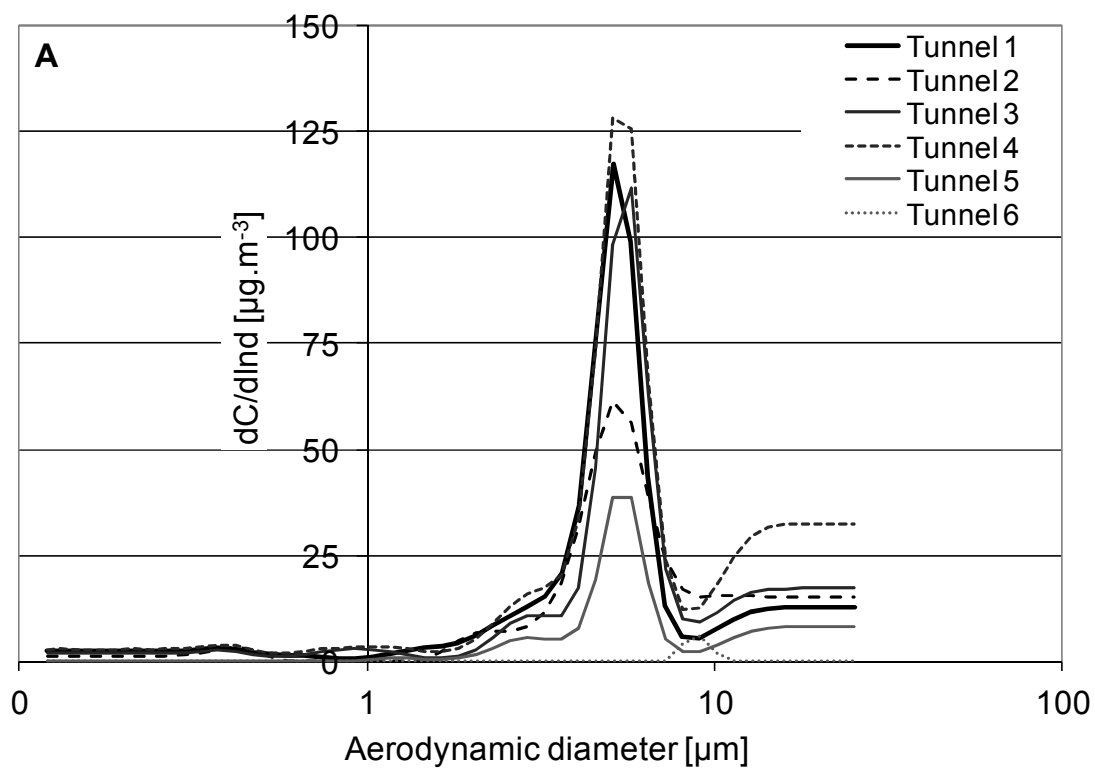


Figure S4 : Mass-based size distribution (Andersen impactor) of the particles sampled at **A**. Tunnel site; **B**. Depot site.

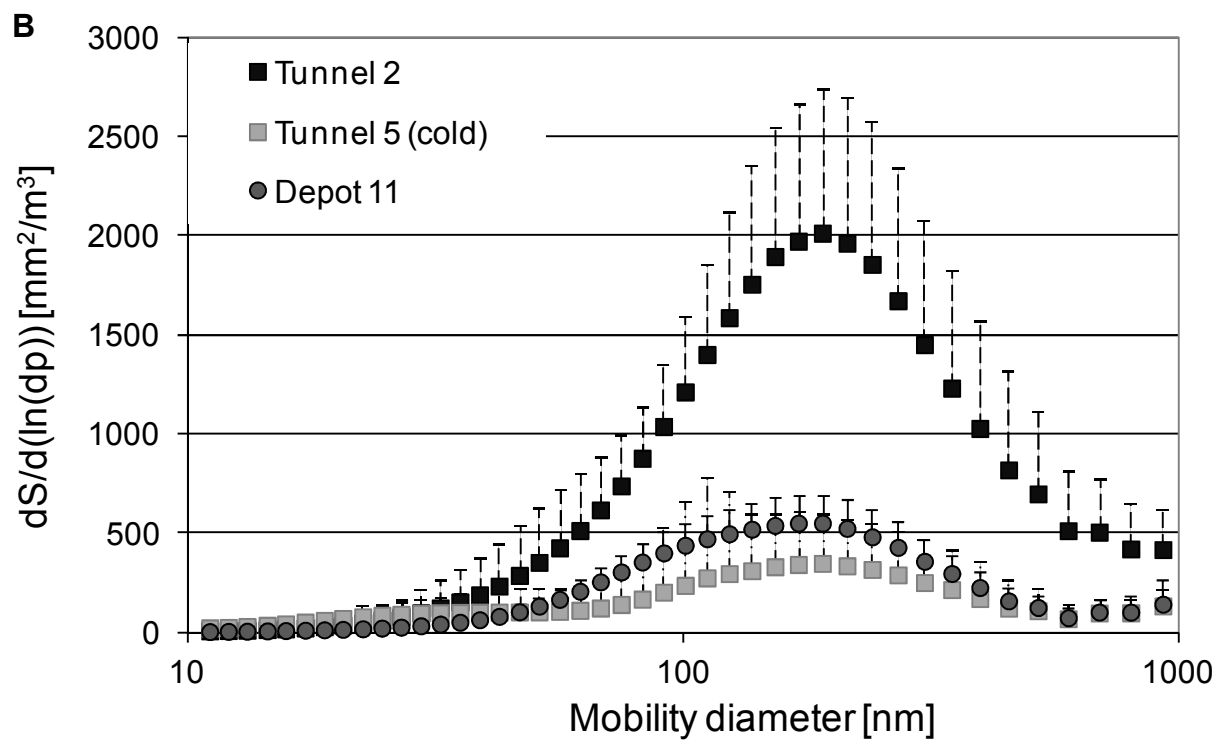
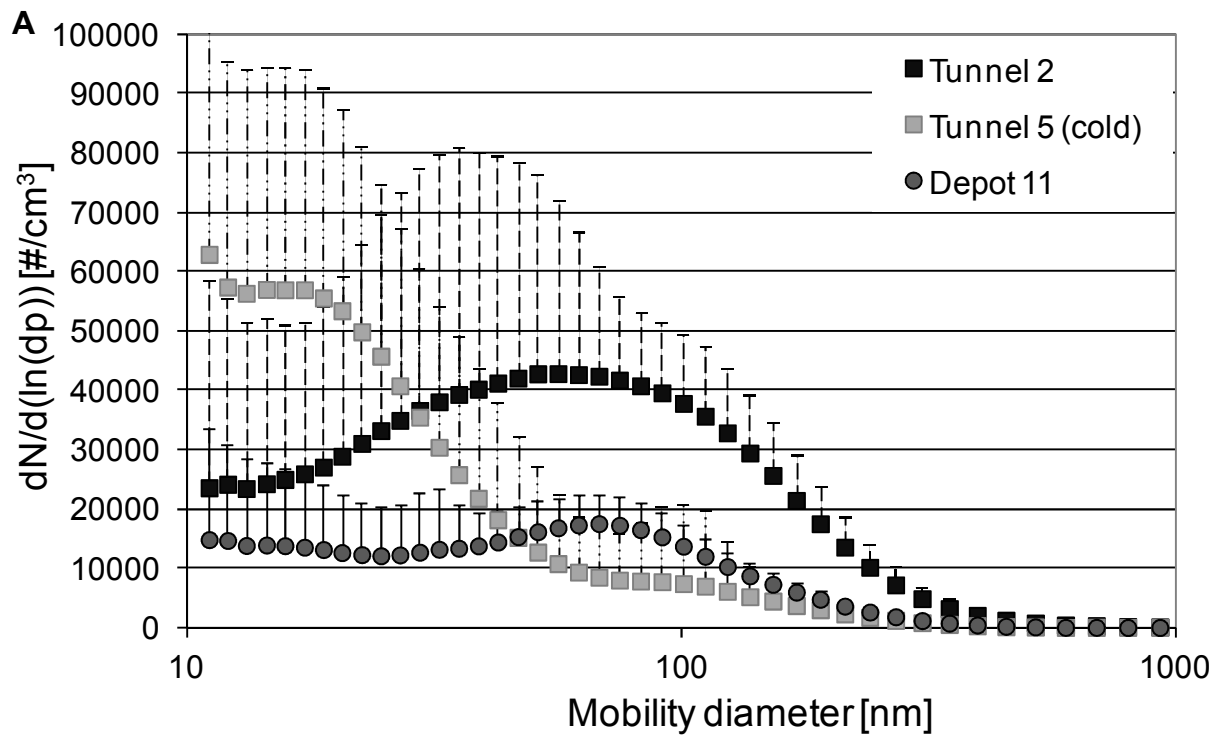


Figure S5: Typical examples of the averaged number- (**A**) and surface (**B**) size distribution measured at the “Tunnel” site (south wind conditions for Tunnel 2 and north wind conditions for Tunnel 5) and at the “Depot 11” site. Error bars correspond to the standard deviation of all the measured size 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 PM<sub>4</sub> (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		<b>0.59 (12; 0.045)</b>	<b>0.89 (6; 0.019)</b>	-0.09 (6; 0.872)	<b>0.84 (10; 0.002)</b>	<b>0.75 (10; 0.012)</b>
EC			0.54 (6; 0.266)	-0.43 (6; 0.396)	<b>0.88 (10; 0.001)</b>	<b>0.96 (10; 0.001)</b>
∑ 4 quinones				0.08 (6; 0.872)	<b>0.88 (6; 0.019)</b>	0.77 (6; 0.072)
∑ 7 PAH					-0.08 (6; 0.872)	-0.03 (6; 0.957)
Fe						<b>0.95 (10; 0.001)</b>
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 PM<sub>4</sub> chemical variables, expressed as air concentrations. Labels correspond to the “Tunnel” site or the “Depot” sites. **A:** OC=f(EC); **B:** OC=f( $\Sigma$  4 Quinones); **C:** OC=f( $\Sigma$  7 PAH); **D:** OC =f(Fe); **E:** OC =f(Cu).

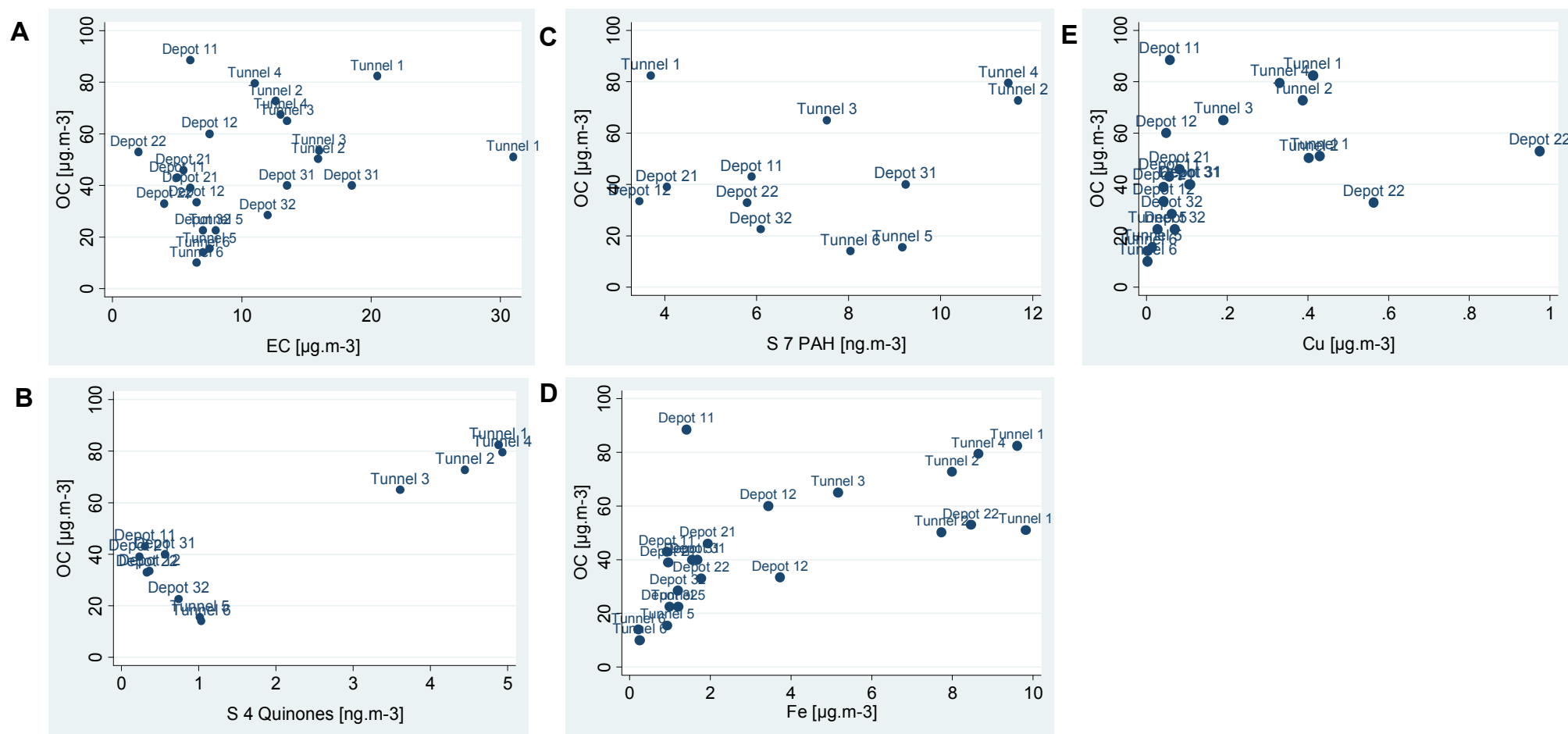


Figure S7: Relationship between Fe and the other PM<sub>4</sub> chemical variables, expressed as air concentrations. Labels correspond to the “Tunnel” site or the “Depot” sites. **A:** Fe=f(EC); **B:** Fe=f( $\Sigma$  4 Quinones); **C:** Fe=f( $\Sigma$  7 PAH); **D:** Fe =f(Cu).

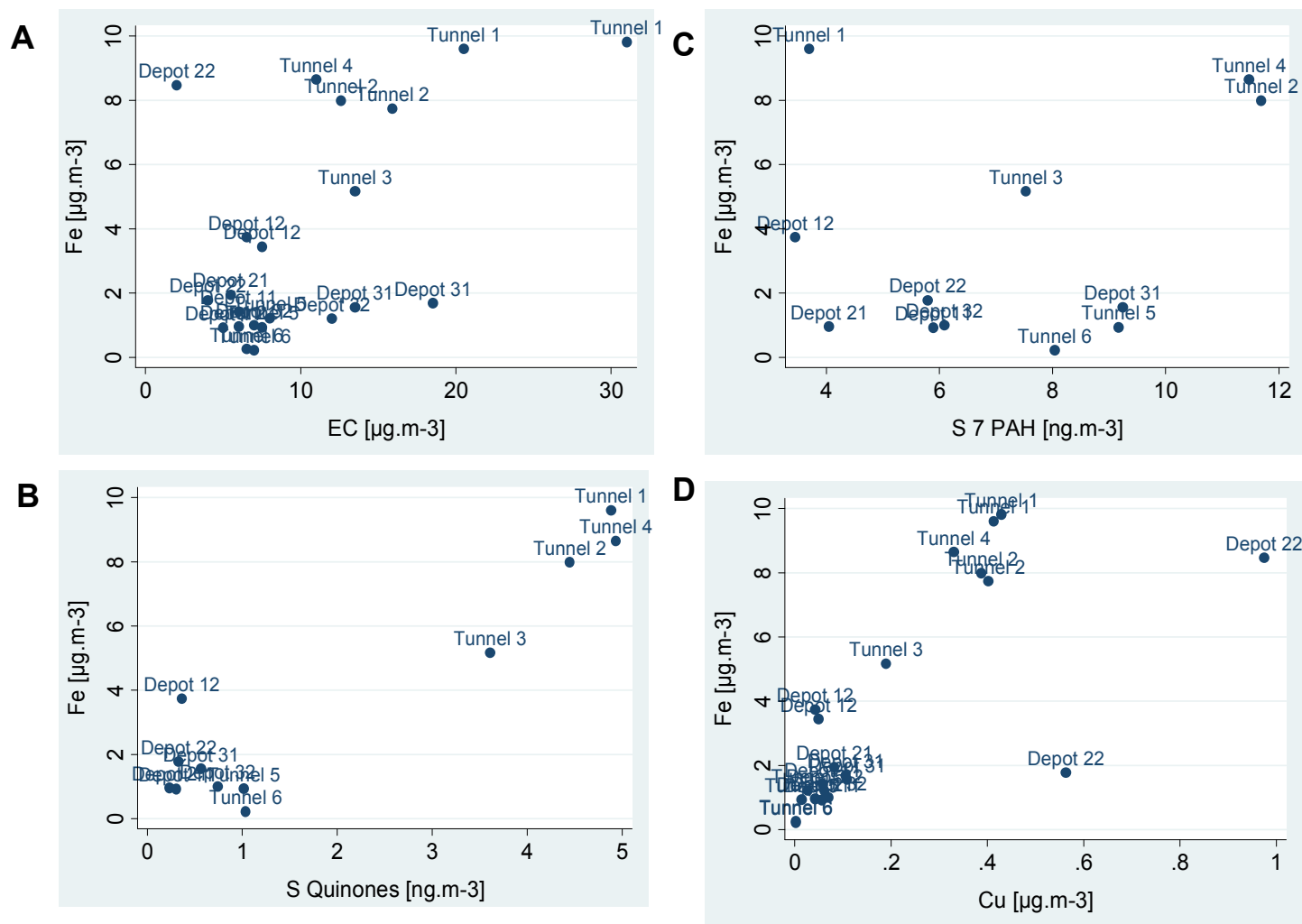


Figure S8: Relationship between EC and the other PM<sub>4</sub> chemical variables, expressed as air concentrations. Labels correspond to the “Tunnel” site or the “Depot” sites. **A:** EC=f( $\Sigma$  4 Quinones); **B:** EC=f( $\Sigma$  7 PAH); **C:** EC =f(Cu).

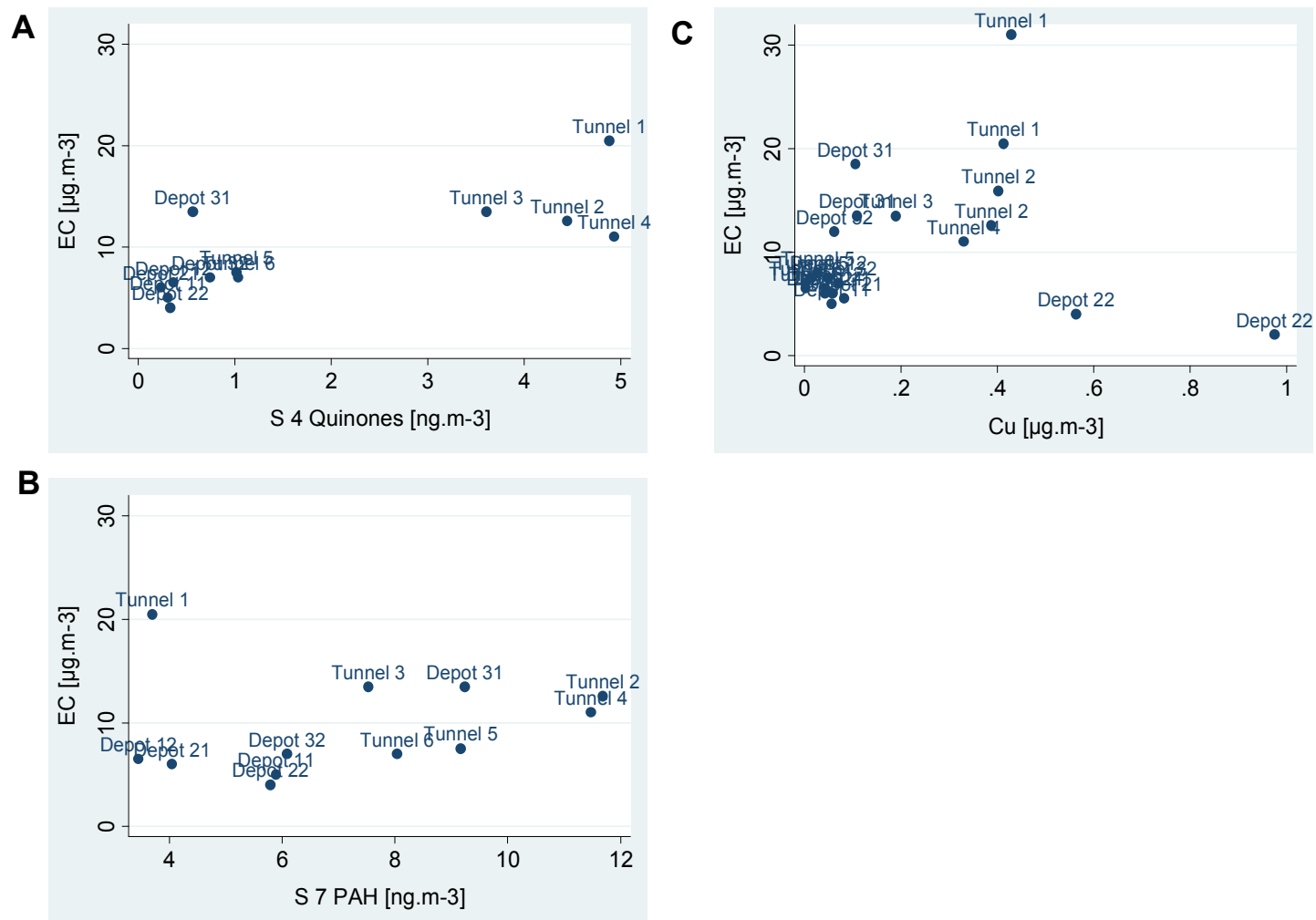


Figure S9: Relationship between Cu or  $\Sigma$  4 Quinones and the other PM<sub>4</sub> chemical variables, expressed as air concentrations. Labels correspond to the “Tunnel” site or the “Depot” sites. **A:** Cu=f( $\Sigma$  4 Quinones); **B:** Cu=f( $\Sigma$  7 PAH); **C:**  $\Sigma$  4 Quinones =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

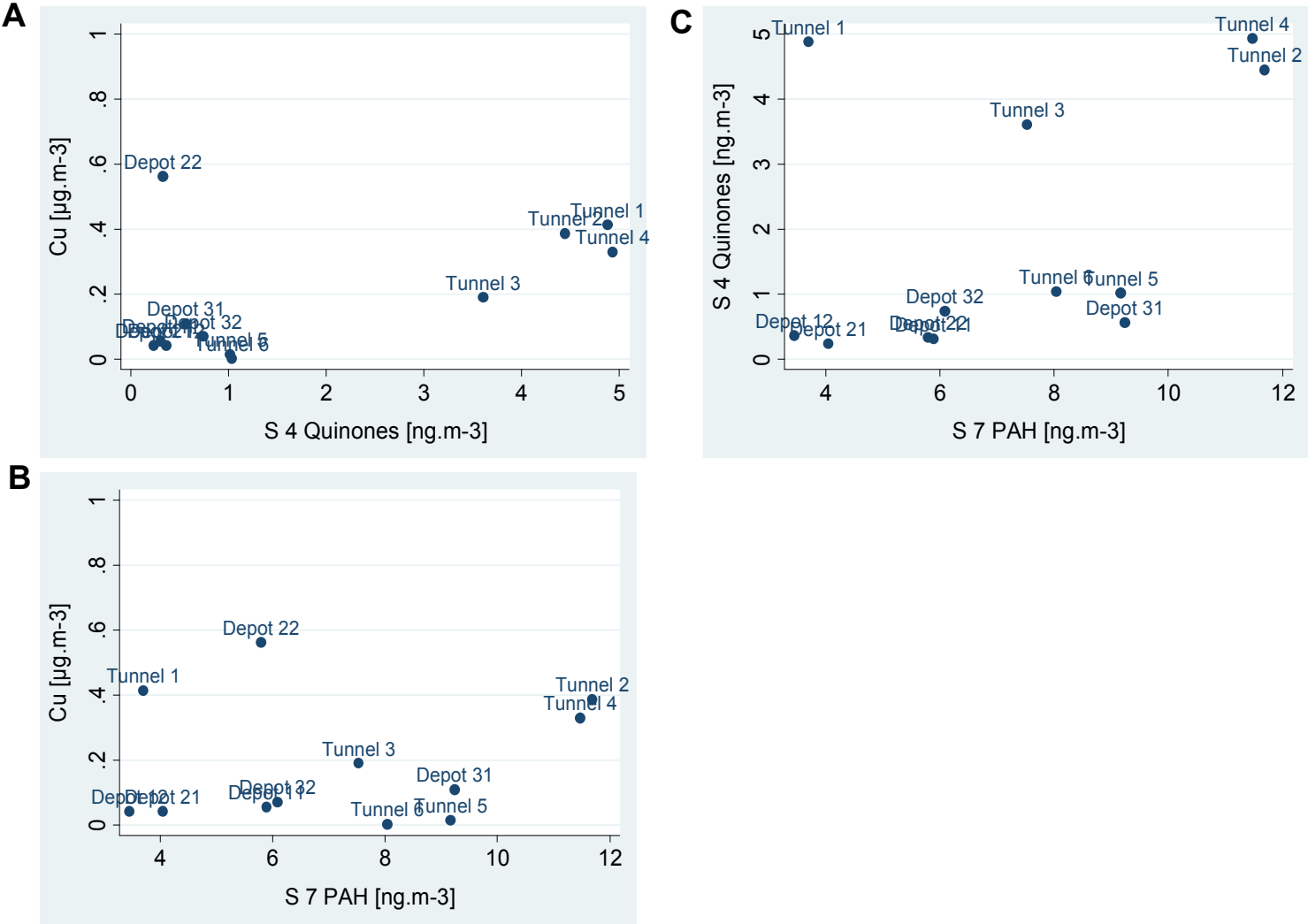


Figure S10: Relationship between the  $OP^{DTT}$  for  $PM_4$  and the corresponding composition variables, expressed as air concentrations. Labels correspond to the “Tunnel” site or the “Depot” sites. **A:**  $OP^{DTT}=f(OC)$ ; **B:**  $OP^{DTT}=f(EC)$ ; **C:**  $OP^{DTT}=f(\sum 4 \text{ Quinones})$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

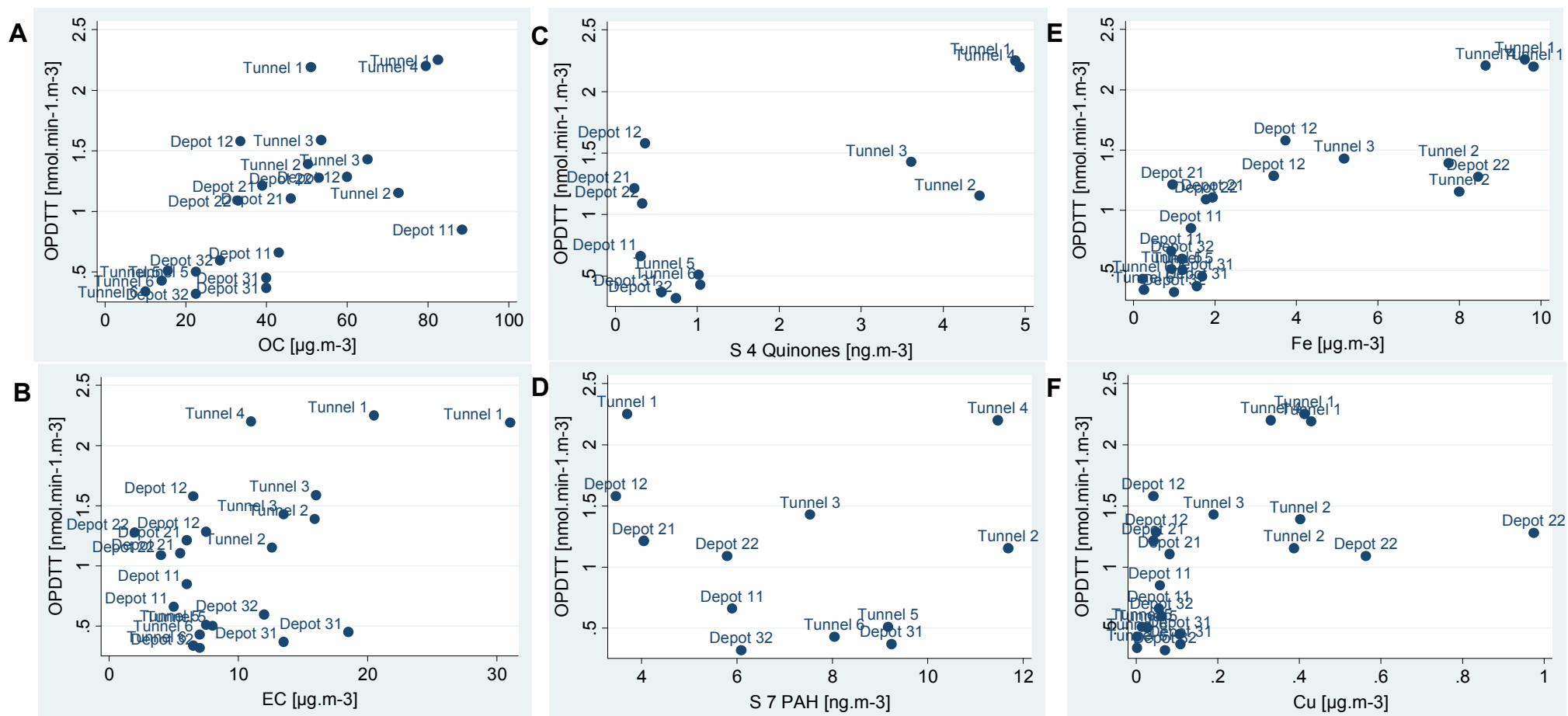


Table S2: Spearman correlation coefficient (rho) with the number of data used (n) and the associated level of significance (p) between the different chemical variables and the PM<sub>4</sub> DTT redox reactivity (normalised based on air concentration) in the two different workplace type. Significant correlation is indicated in bold.

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

"Depot"	PM <sub>4</sub> 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	<b>-0.943</b>	<b>6</b>	<b>0.005</b>
Fe	<b>0.615</b>	<b>12</b>	<b>0.033</b>
Cu	-0.375	12	0.230

Table S3: Reported OP<sup>DTT</sup> values for ambient PM<sub>2.5</sub> expressed either as mass (OP<sup>DTT</sup>/μg) or volume (OP<sup>DTT</sup>/m<sup>3</sup>). 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 <sub>M</sub> <sup>DTT</sup> /μg [pmol.min <sup>-1</sup> .μg <sup>-1</sup> ]	OP <sup>DTT</sup> /m <sup>3</sup> [nmol.min <sup>-1</sup> .m <sup>-3</sup> ]	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 <sup>a</sup>	Urban background	(Janssen <i>et al.</i> , 2014)
	1.7 <sup>a</sup>	Stop&Go	(Janssen <i>et al.</i> , 2014)
	3.3 <sup>a</sup>	Continuous traffic	(Janssen <i>et al.</i> , 2014)
	0.58 – 1.67 (0.96) <sup>b</sup>	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) <sup>b</sup>			(Ntziachristos <i>et al.</i> , 2007)
15 - 39 (24) <sup>b</sup>		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) <sup>b</sup>		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

<sup>a</sup>: Geometric mean

<sup>b</sup>: Water suspension

## References

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