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 characterization 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_4 and $PM_{2.5}$ and size distribution), number and surface area, organic and elemental carbon, polycyclic aromatic hydrocarbon (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 (PM_4) at the Tunnel site was about twice the one at the Depot sites (173 ± 103 and 90 ± 36 μg m^{-3}, 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 PM_4 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} PM_4: 10.5 ± 5.8 pmol DTT min^{-1} μg^{-1} for all sites), suggesting the presence of redox inactive components in the $PM_{2.5-4}$ fraction. Although the reactivity was similar at the Tunnel and Depot sites irrespective of the metric chosen (OP^{DTT} μg^{-1} or OP^{DTT} m^{-3}), the chemicals associated with OP^{DTT} were different between the two types of workplaces. The organic carbon, quinones, and/or metal content (Fe, Cu) were strongly associated with the DTT reactivity at the Tunnel site whereas only Fe and PAH were associated (positively and negatively, respectively) with this reactivity at the Depot sites. These results demonstrate the feasibility of measuring of the OP^{DTT} in occupational environments and suggest that the particulate OP^{DTT} is integrative of different physicochemical properties. This parameter could be a potentially useful exposure proxy for investigating particle exposure-related oxidative stress and its consequences. Further research is needed mostly to demonstrate the association of OP^{DTT} with relevant oxidative endpoints in humans exposed to particles.

KEYWORDS: DTT assay; occupational exposure; oxidative potential; particulate matter; quinones

INTRODUCTION

Numerous epidemiological studies have associated particle mass exposure to adverse health effects. However, particulate matter (PM) presents generally quite a complex, dynamic, and variable composition. It is known that at least for ambient PM, most of its mass is biologically inactive (Borm *et al.*, 2007). Toxicologically important particle constituents like polycyclic aromatic hydrocarbons (PAH) or metals represent only a very small fraction of the particle mass. Studies about effects of PM suggest that oxidative stress plays an important role in the biological effect induced by particles (Brook *et al.*, 2010). Oxidative stress is an imbalance between production of reactive oxygen species (ROS) and antioxidants in cells and tissues. This perturbation of the intra- and extracellular redox homeostasis could results from (i) the intrinsic presence on the particles surface of radicals entities (Hopke, 2008); (ii) the catalytic generation of radicals by redox active chemicals on the particle surface (Limbach *et al.*, 2007) in the presence of reducing compounds; (iii) the activation of cellular responses by the particles (Donaldson *et al.*, 2002); or (iv) a combination of these different processes. Either of these mechanism may lead to an overproduction of potentially toxic species like superoxide ($O_2^{\cdot-}$) or hydroxide ($OH^{\cdot}$) radicals as well as hydrogen peroxide (H_2O_2), all resulting from the reduction of oxygen and collectively called ROS (Halliwell and Gutteridge, 2007). The cellular increase of ROS could explain at least partly the proinflammatory effect of PM in the lungs (Donaldson *et al.*, 2005) and on the cardiovascular system (Donaldson *et al.*, 2001). Therefore, the measurement of the ability of particles to generate ROS, corresponding to their intrinsic oxidative potential (OP), could provide a better health-based exposure metric than mass.

Several approaches were already used for quantifying the intrinsic OP of ambient micro- or nanoparticles by purely acellular tests [reviewed by Ayres *et al.* (2008)]. In order to mimic what may happen when particles deposit in the reducing milieu of the lung (Cross *et al.*, 1994), one approach is to determine how fast particles are able to deplete reducing compounds in solution. Whereas not a physiologically constituent of the lung lining fluid, dithiothreitol (DTT) can be considered a surrogate of glutathione, as both compound oxidize at a similar rate in phosphate buffer solutions (Held and Biaglow, 1994). In the DTT assay,

redox-active components of PM can be reduced by accepting the DTT electrons (Fig. 1, path 1). These PM components may then be regenerated (i.e. reoxidized) by transferring the gained electrons to dissolved oxygen, thus producing $O_2^{\cdot-}$ (Fig. 1, path 2). This step corresponds to the catalytical part of a redox-cycling process. The superoxide anion will allow the production of H_2O_2 and $OH^{\cdot}$ by dismutation (Fig. 1, path 3) and Fenton-like reactions, respectively, (Fig. 1, path 4). The DTT assay was used before for the characterization of ambient PM (Cho *et al.*, 2005), combustion engine emissions (Geller *et al.*, 2006), and engineered nanoparticles (Sauvain *et al.*, 2008). No study so far described its use in occupational situations. The objective of this study was to characterize the DTT-based OP (OP^{DTT}) of particles in different occupational situations and to investigate the relationship between this OP^{DTT} and other aerosol physicochemical characteristics, including the particle number and surface area concentration, components such as organic and elemental carbon (EC), metals, and PAH as well as four quinones. This would allow assessing the usefulness of this method for measuring the OP of particles in occupational settings.
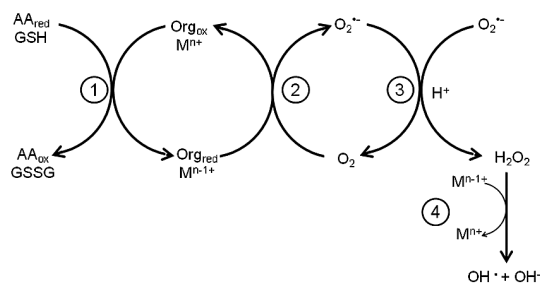

Figure 1 Cascade of possible reactions taking place in the reducing milieu of the lung lining fluid and generating reactive oxygen species ($O_2^{\cdot-}$, H_2O_2, $OH^{\cdot}$) [adapted from Held and Biaglow (1994)]. AAred: ascorbic acid (reduced form); AAox: ascorbic acid (oxidized form); RSHred: thiol-containing molecule like glutathione or DTT (reduced form); RSSRox: thiol-containing molecule like glutathione or DTT (oxidized form); PMred: surface functions of particulate matter (reduced form); PMox: surface functions of particulate matter (oxidized form); Orgred: organic compound adsorbed on the particle (reduced form); Orgox: organic compound adsorbed on the particle (oxidized form); $M^{(n-1)+}$: redox active metal (reduced form); M^{n+}: redox active metal (oxidized form); O_2: oxygen; $O_2^{\cdot-}$: superoxide ion; H_2O_2: hydrogen peroxide; $OH^{\cdot}$: peroxide radical.

MATERIALS AND METHOD

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

The particles OP methodology used was based on collecting particles on filters followed by assessing the OP^{DTT} on this support. We sampled particles onto poly(1,1,2,2-) tetrafluoroethylene (PTFE) filters (Table 1). The DTT assay was done after a maximum of 48 h storage at ambient temperature ($20 \pm 3°C$). These measurement conditions were selected based on preliminary tests investigating the effect of the filter type (quartz or PTFE), the storage time and the collected particle mass on the OP^{DTT} (see Supplementary Material is available at *Annals of Occupational Hygiene* online). The DTT consumption rate was evaluated

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⁻¹; Cascade impactor PM₄	Quartz (Whatman QM-A, Milian, Switzerland, Ø 150 mm)	Gravimetry (PM₄)	Eight punches Ø 40 mm	Four quinones	Gas chromatography with mass detection
			Rest of the filter	7 PAH	Gas chromatography with mass detection
High Volume sampler (Gravikon PM₄, Strohlein, Germany); 60 l min⁻¹; PM₄ cyclone	PTFE-coated glass fibre (Pallflex, T60A20-HT, Pall Corporation, USA, Ø 70 mm)	Gravimetry (PM₄)	1–2 punches Ø 28–42 mm	OP	DTT assay
			Rest of the filter	Metals (Fe, Cu)	Atomic absorption (graphite furnace)
Low volume sampler (Leland, SKC, Eighty Four, USA); 10 l min⁻¹; Impactor PEM˙ PM₂.₅	PTFE (SKC, Blanc-Labo, Switzerland; 2 μm, Ø 37 mm)	Gravimetry (PM₂.₅)	Whole filter	OP	DTT assay
Low volume sampler (MSA, Pitsburgh, USA); 2 l min⁻¹; Cyclone Casella PM₄	Quartz (Whatman QM-A, WWR, Switzerland, Ø 37 mm)	—	Whole filter	Organic carbon (OC); elemental carbon (EC)	Coulometry
Cascade impactor Andersen; 28 l min⁻¹, nine stages	Glass fibre (Whatman 934AH, WWR, Switzerland; Ø 81 mm)	Mass-based size distribution (<0.4–>11 μm)	—	—	—

without extracting the filter in solvents, therefore the obtained blank-corrected reactivity corresponds to the total OP of the sampled PM (Table 1). Briefly, a punch of the filter containing less than 1 mg particles was immersed (loaded side facing the liquid) in a beaker containing 5 ml of 0.6 mg l^{-1} Tween 80° (Fluka, Switzerland) and sonicated for 15 min at 180 W (Branson 5210, Milian, Switzerland). Five millilitres of a 100-μM solution DTT (Sigma-Aldrich, Switzerland) was added. The DTT loss over time at 37°C and under agitation was measured at different times (5, 10, 15, 20, 25, 30, and 35 min). Each time, a 500-μl aliquot of the reacting solution was removed and added to 0.5 ml of 10% trichloroacetic acid to stop the DTT oxidation. The oxidized amount 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′-dithio-bis(2-nitrobenzoic acid) (Fluka Switzerland). The resulting yellow 2-nitro-5-thiobenzoic acid was quantified spectrophotometrically at 412 nm. The rate of DTT consumption was obtained by calculating the slope of the linear regression of absorbance against time, as described earlier (Sauvain *et al.*, 2008). The average DTT consumption rate of the loaded filters (done in duplicates) was corrected with the results obtained from a blank filter treated in the same way.

Selected workplaces for measuring the particle OP

Two types of workplaces with PM presenting potentially different physicochemical 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 h^{-1} 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-h 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.

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 determine their quinones, PAH and metal content, high volume PM$_4$ sampling was done by deploying a Digitel° DH77 (580 l min^{-1}, Digitel, Switzerland) and a GravikonPM4° (60 l min^{-1}, Strohlein, Germany) side by side (Table 1). The OP^{DTT} for PM$_4$ was measured on punches from filters of the GravikonPM4°. Low volume samplers were also used at both locations for the determination of the PM$_4$ content of OC, EC, and the OP^{DTT} of PM$_{2.5}$ (Table 1).

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

Before gravimetric analysis, filters were conditioned for 24 h at constant humidity (53 ± 4%) and ambient temperature (20 ± 3°C). Laboratory field blanks

were used for correcting the measured loaded mass. After weighting, filters from the different high volume sampler were sectioned to allow multiple chemical analyses (Table 1). The four toxic quinones 1,2-naphtoquinone, 1,4-naphtoquinone, 9,10-anthraquinone and 9,10-phenanthraquinone were determined as described by Cho *et al.* (2004). Filters were ultrasonicated in toluene and the organic extract was concentrated under nitrogen gas to 200 µl. The extracted quinones were acetylated at 75°C in presence of zinc and acetic anhydride (Fluka, Switzerland). The derivatization was stopped after 30 min by adding water in the reacting media. The diacetylated quinones were extracted in pentane and quantified with a GC-MS system (Saturn 4000, Varian, Switzerland). Seven different PAHs with molecular weight larger than 252 were determined using an earlier published method (Sauvain *et al.*, 2003). Briefly, the filters were Soxhlet extracted in toluene and the organic extract was subsequently purified through solid phase extraction, fractionated with a semipreparative HPLC column and the PAH fraction finally partitioned by liquid–liquid extraction. The purified extract was injected on a GC-MS system (Saturn 4000, Varian, Switzerland). Total iron and copper were determined after ultrasonic digestion of the filter with HF and subsequently with $HCl:HNO_3$ 2:1. After diluting the residue, the metal concentrations were measured with an Atomic Absorption Spectrometer equipped with a graphite furnace (Perkin-Elmer HGA 700, Schwerzenbach, Switzerland) following an accredited ISO/EN 17025 method. Finally, a coulometric-based method (Perret *et al.*, 1999) was used for determining the organic carbon (OC) and EC content of the particles.

Data treatment

All statistical analyses were performed with STATA (STATA/IC 12.1, College Station, TX, USA). Values smaller than the limit of detection (LOD, corresponding to three time the standard deviation of a blank) were replaced by 1/2 LOD in the database for statistical treatment. Due to the rather low sample size, the robust nonparametric Mann–Whitney test was used to evaluate differences in aerosol characteristics at both study sites. The association between the PM physicochemical characteristics and the OP^{DTT} (expressed as air concentration) was assessed separately for each workplace type ('Tunnel' or 'Depot') by using the Spearmann rank correlation with the associated probability. Association was considered significant with the probability $P < 0.05$.

RESULTS

Physicochemical characteristics of the particles at the different workplaces

Physical characteristics

Table 2 presents the physical characteristics of the particles collected at the different workplaces. High particle mass concentrations were measured at the 'Tunnel' site during the four initial sampling days, when the wind came from the south through the tunnel. In contrast, low concentrations were measured when the wind came from the north ('Tunnel' 5 and 6). These two days were also particularly cold (average −2.7°C). Concentrations measured at the three 'Depot' sites were lower and less variable.

All the mass-based size distributions were multimodal (see Supplementary Fig. 4 is available at *Annals of Occupational Hygiene* online), with a significant larger median diameter at the 'Tunnel' site (6.6 ± 1.3 µm) compared with the 'Depot' sites (3.3 ± 0.6 µm; Mann–Whitney test, $P = 0.004$). The particle number concentration was rather constant at 'Tunnel' ($104\ 000 \pm 17\ 000\ cm^{-3}$) and higher compared to the different 'Depot' workplaces ($60\ 000 \pm 30\ 000\ cm^{-3}$; Mann–Whitney test, $P = 0.025$). The geometric mean diameter measured at the Tunnel site was quite variable, with larger diameters on days with southerly winds and warmer temperatures (52 ± 10 nm, $n = 4$) compared to situations with northerly wind (24 ± 3 nm, $n = 2$). At the 'Depot' sites, the geometric mean diameter was quite similar for all sampling days (average 47 ± 4 nm, $n = 6$). At both sites, the geometric standard deviation was about 2.3, which suggests that all sites presented polydisperse particles. When the SMPS-derived surface concentration was considered, a broad range of values were obtained (Table 2), with the smallest surface concentration for 'Tunnel' 5 and 6 (cold situation with north wind going into the tunnel), followed by all the Depot sites and the largest values for the 'Tunnel' 1–4. Typical averaged number- and surface-based size distribution measured at the 'Tunnel' and 'Depot' sites are given in the Supplementary

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_4 ($\mu g\,m^{-3}$)	$PM_{2.5}$ ($\mu g\,m^{-3}$)	Median diameter[a] (μm)	$\sigma_g{}^a$	Number (# cm^{-3})	Mean geometric diameter[b] (nm)	$\sigma_g{}^b$	Surface ($mm^2\,m^{-3}$)	Mean geometric diameter[b] (nm)	$\sigma_g{}^b$
Tunnel 1	260±57 (6)	190±27 (3)	5.0	2.6	94 200±26 000 (36)	61±10	2.2	3700±1200 (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	3500±1200 (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	1920±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	4140±1670 (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		2450±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	1000±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	1000±370 (160)	175±14	2.2
Depot 2 1	111±28 (6)	88±21 (3)	3.7	2.2	28 700±7000 (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	1400±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	2120±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	1860±1380 (169)	168±30	2.2
All Depot sites	90±36	73±26	3.3±0.6		60 000±29 000	47±4		1390±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.

Fig. 5 is available at *Annals of Occupational Hygiene* online).

Chemical characteristics

Table 3 presents the different chemical compounds analysed. OC was the predominant measured compound. At the 'Tunnel' site on average $48 \pm 4\%$ of the measured PM_4 mass was explained by the sum of OC, EC, Fe, and Cu, while at the 'Depot' the same components explained a larger part of the PM_4 mass ($70 \pm 12\%$). For this calculation, we used an organic molecular weight per carbon weight ratio of 1.6, as recommended by Turpin and Lim (2001) (see Supplementary Fig. 3 available at *Annals of Occupational Hygiene* online). The mass-based OC

content of the collected particles was significant lower in the 'Tunnel' site (average $33 \pm 13\%$, $n = 12$) compared to the 'Depot' sites (average $45 \pm 11\%$, $n = 12$; Mann–Whitney test, $P = 0.018$). The ratio of elemental to total carbon (with total carbon TC corresponding to the sum of OC + EC), differed significantly between the two sites (average 'Tunnel': $25 \pm 9\%$, $n = 12$; average 'Depot': $16 \pm 9\%$, $n = 12$; Mann–Whitney test, $P = 0.021$) but also within the tunnel in function of the wind direction. The sum of the four analysed quinones was always significant higher at the 'Tunnel' site (Mann–Whitney test, $P = 0.01$). Among the four analysed quinones, 9,10-anthraquinone was the most abundant; the two volatile naphtoquinones presented lower

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 $(\mu g\,m^{-3})$	EC $(\mu g\,m^{-3})$	Σ 4 quinones[a,b] $(ng\,m^{-3})$	Σ 7 PAH[b,c] $(ng\,m^{-3})$	Fe $(\mu g\,m^{-3})$	Cu $(\mu g\,m^{-3})$
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.[d]
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[e]	12	3	0.04	0.3	0.01	0.004

[a]Sum of 1,2-Naphtoquinone + 1,4-Naphtoquionone + 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]Not detected (<LOD).
[e]LOD: Limit of detection, corresponding to three times the noise of a blank sample.

concentrations. Iron was present in larger concentrations than Cu and both were strongly influenced by the wind direction at the 'Tunnel' site.

The chemical compounds showed a different correlation pattern at the two workplaces (see Supplementary Table 1 and Supplementary Figs 6–9 are available at *Annals of Occupational Hygiene* online). At the 'Tunnel' site, consistent correlations were observed between OC and EC, Σ4 Quinones and metals (Fe, Cu); EC with metals (Fe, Cu) and Σ4 Quinones with Fe. At the 'Depot' site, no association was observed between any measured chemical variables.

Oxidative potential

The intrinsic mass-based OP^{DTT} (OP^{DTT} μg^{-1}) of the sampled particles is given in Fig. 2A. The OP^{DTT} μg^{-1} did not differ significantly between both sites (PM_4: 10.6 ± 7.2 pmol DTT min^{-1} μg^{-1} at 'Tunnel', $n = 11$; 10.4 ± 4.6 pmol DTT min^{-1} μg^{-1} at 'Depot', $n = 12$; Mann–Whitney test, $P = 0.71$; $PM_{2.5}$: 9.3 ± 5.4 pmol DTT min^{-1} μg^{-1} at 'Tunnel', $n = 12$; 11.0 ± 10.0 pmol DTT min^{-1} μg^{-1} at 'Depot', $n = 12$; Mann–Whitney test, $P = 0.86$). The largest intrinsic reactivity was observed for workplaces with very small particles ('Tunnel' 6) or with a high copper content ('Depot' 2 2). A large fraction of the PM_4 reactivity was already present on the smaller $PM_{2.5}$ fraction (Fig. 3, average $PM_{2.5}/PM_4$ reactivity ratio for all sites: $97 \pm 22\%$). The OP^{DTT} exposure level to humans (Fig. 2B, OP^{DTT} m^{-3})

was obtained by multiplying the OP^{DTT} μg^{-1} with the corresponding PM concentration. As for the OP^{DTT} μg^{-1}, no statistically significant difference was observed between OP^{DTT} m^{-3} at both sites (1.3 ± 0.7 nmol DTT min^{-1} m^{-3} at 'Tunnel', $n = 11$; 0.9 ± 0.4 nmol DTT min^{-1} m^{-3} at 'Depot', $n = 12$; Mann–Whitney test, $P = 0.20$ for PM_4 and 0.8 ± 0.4 nmol DTT min^{-1} m^{-3} at 'Tunnel', $n = 12$; 0.8 ± 0.7 nmol DTT min^{-1} m^{-3} at 'Depot', $n = 12$; Mann–Whitney test, $P = 0.23$ for $PM_{2.5}$). For both PM_4 OP^{DTT} μg^{-1} and OP^{DTT} m^{-3} levels, large interday variations were observed. A maximum of 3-fold increase for the PM_4 OP^{DTT} μg^{-1} was observed between 'Tunnel 2' and 'Tunnel 6' (7.0–24.9 pmol DTT min^{-1} μg^{-1}, respectively) whereas it decreased
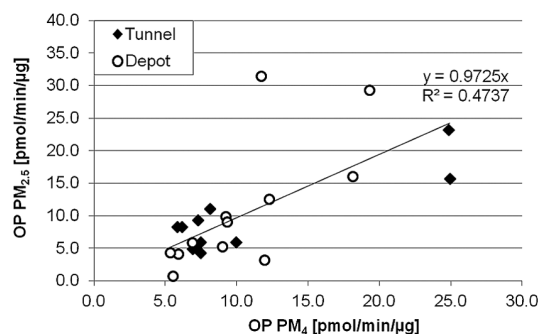

Figure 3　Relationship between the oxidative potential of $PM_{2.5}$ and PM_4 for the two types of workplaces. The equation for the regression line for all the sampling sites is also given, together with the corresponding regression coefficient (R^2).
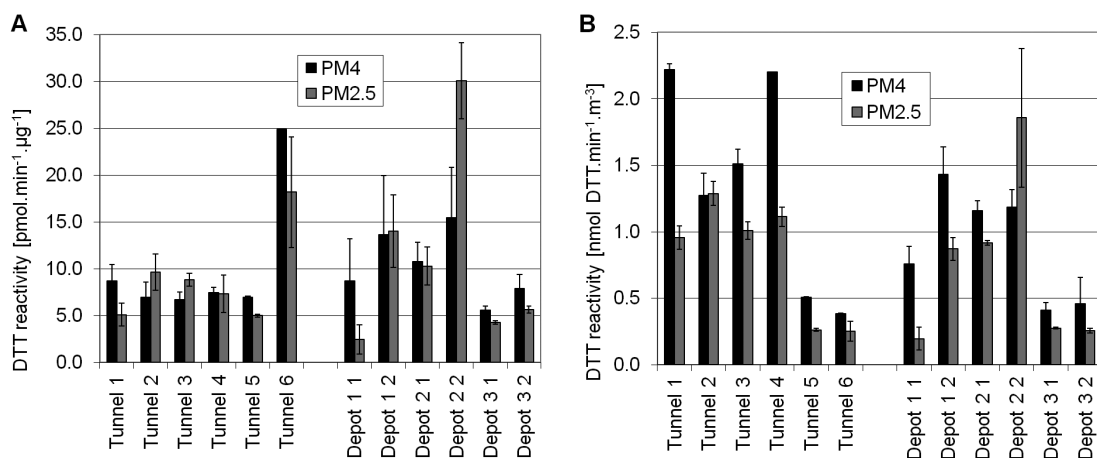

Figure 2　OP^{DTT} per particle mass (A) and OP^{DTT} expressed as an air concentration (B) for the particles collected in the different workplaces. Bars correspond to the standard deviation of duplicate measurements.

about the same order for these two sampling days when expressed as air volume (1.27–0.38 nmol min^{-1} m^{-3}, respectively). Such variability could be due to changes in the environmental conditions or workplaces activities. The identification of the chemical compounds associated with the PM$_4$ OP^{DTT} m^{-3} was done by Spearman rank correlation (Supplementary Table 2 is available at *Annals of Occupational Hygiene* online). At the 'Tunnel' site, SMPS-based surface, OC, Σ 4 quinones and metals (Fe, Cu) were clearly associated with the PM$_4$ OP^{DTT} m^{-3}. At the 'Depot' site, significant associations were observed only between PM$_4$ OP^{DTT} m^{-3} and Fe (positive) or Σ 7 PAH (negative association).

DISCUSSION

Physicochemical characteristics of the aerosol

The mass concentrations measured at the 'Tunnel'site are comparable to levels determined in similar environments as busy road tunnel entrances (Larsson *et al.*, 2007) or tollbooths on highways with high traffic density (Shih *et al.*, 2008). The 'Depot' site presented slightly higher values compared to the one previously reported for the same workplace (Sauvain *et al.*, 2011) but were similar to levels found in Swedish bus depots (Lewné *et al.*, 2007). About 2/3 of the measured PM$_4$ mass at the 'Depot' sites could be attributed to organic, EC, Fe, and Cu, while about half of the total PM$_4$ mass was not identified at the 'Tunnel' site (Supplementary Figure 3 are available at *Annals of Occupational Hygiene* online). It is possible that resuspended de-icing salts used during winter time for the road maintenance or background ammonium, sulphate and nitrate ions (Lanz *et al.*, 2010) contributed to a large extend to this mass. The measured number concentration corresponded well to reported values for dieselized environments (Ramachandran *et al.*, 2005; Park *et al.*, 2011). The surface concentration found in the studied workplaces are similar to those reported for automotive engine manufacturing (Heitbrink *et al.*, 2009) or milling processes in die cast facility (Park *et al.*, 2011), but about 5–10 times larger compared with concentrations measured in garage mechanics (Ramachandran *et al.*, 2005). This difference could be due to the larger size range (11–1083 nm) considered in this study to calculate the surface area.

Whereas the physical properties of the particles mostly define their deposition site and their interaction with pulmonary cells, the chemical composition has also to be considered for understanding the particulate toxicity (Giechaskiel *et al.*, 2009). The aerosol present at the 'Tunnel' site corresponds to typical combustion-based processes. The predominantly combustion origin of the PM sampled at the 'Tunnel' site is suggested by their components. The observed EC/TC ratio of 0.25 ± 0.08 is consistent with values reported for typical urban traffic and fuel combustion sources (Vodicka *et al.*, 2013); the significant OC–EC correlation (Supplementary Table 1 is available at *Annals of Occupational Hygiene* online) is also consistent with this. The relatively large concentration of quinones also points to diesel particles which are much richer in quinones than particles originating from light-duty gasoline vehicles (Jakober *et al.*, 2007). The strong correlation between Fe and Cu at the 'Tunnel' site is also in support of a vehicle source, tough more likely related to abraded particles from brakes and other vehicle parts (Riediker *et al.*, 2003; Birmili *et al.*, 2006).

The lack of association between any chemical variables at the 'Depot' sites is suggestive of a more complex and mixed source of airborne compounds. The lack of OC and EC correlation may be due to the presence of additional OC sources not related to combustion, such as the observed use of solvents or lubricating oil during repair activities. The absence of association between Fe and Cu at the 'Depot' sites compared with the 'Tunnel' site (Supplementary Table 1 is available at *Annals of Occupational Hygiene* online) is also indicative of other independent sources of Fe or Cu from activities like grinding or welding.

OP of particles

All the collected samples were able to oxidize DTT in a more or less large extent and suggest OP^{DTT} as a general property for such particles. The measured OP^{DTT} µg^{-1} was about 2–3 times lower than reported values for urban PM$_{2.5}$ (Charrier and Anastasio, 2012; Delfino *et al.*, 2013; Fang *et al.*, 2014; see Supplementary Table 3 is available at *Annals of Occupational Hygiene* online). This intrinsic smaller OP^{DTT} µg^{-1} reactivity could be due to: (i) different methodological approaches as we put directly the filter into contact with the DTT solution and did not extract the particles

in water or methanol, as done by the cited authors in Supplementary Table 3 (available at *Annals of Occupational Hygiene* online). It is possible that the redox-active entities present on the filter are less available for reacting with the DTT compared to when they are already extracted in a water or methanol solution. This would be in line with the lower electron spin resonance-based reactivity measured directly for PM loaded filters compared to the water-extracted filters (Hellack *et al.*, 2014); (ii) the low contribution of photochemical processes at the studied workplaces, which correspond to indoor situations. Such photochemical processes have been shown to be quite important for modulating the outdoor PM reactivity, via the formation of secondary organic aerosols (Verma *et al.*, 2009; Rattanavaraha *et al.*, 2011). Such processes are not likely to take place in the confined studied workplaces, potentially explaining the lower reactivity compared to ambient particles; (iii) the presence of organic and inorganic redox-inactive compounds adsorbed on the particles which may contribute to the mass but not to the PM reactivity. Particularly at the 'Depot' site, the important use of lubricating oil (containing paraffinic redox-inactive chemicals) might explain the large variability of the OP^{DTT} at these sites. Whereas the intrinsic $PM_{2.5}$ reactivity was low, the OP^{DTT} m^{-3} for this size range was quite comparable with other reported values (Supplementary Table 3 is available at *Annals of Occupational Hygiene* online). The fact that larger PM mass were present in the studied workplaces compared to the levels reported for urban situations compensate their lower intrinsic reactivity and could explain this similarity. The OP^{DTT} of PM_4 was mostly present on the smallest $PM_{2.5}$ fraction (Fig. 3). This is consistent with published data for ambient PM indicating an increasing OP^{DTT} from the coarse to the ultrafine fraction (Ntziachristos *et al.*, 2007).

Although the two types of workplaces differed based on their physicochemical characteristics, the OP^{DTT} of the particles was practically identical, irrespective of the metric used (mass or air volume). Whereas this observation might be different with other OP assays, this suggests that the potential generation of ROS in lungs of particle exposed workers would be similar at both workplaces even though their composition differs. The PM_4 OP^{DTT} m^{-3} of the combustion type PM ('Tunnel' site) was associated with SMPS-based surface, organics (OC, quinones) and/or Fe,

Cu (see Supplementary Table 2 is available at *Annals of Occupational Hygiene* online), consistent with literature data relative to urban or combustion-based PM (Kumagai *et al.*, 2002; Cho *et al.*, 2005; Chung *et al.*, 2006; Geller *et al.*, 2006; Ntziachristos *et al.*, 2007; Hu *et al.*, 2008; Verma *et al.*, 2009; Charrier and Anastasio, 2012). As we observed an association between Cu and the OP^{DTT} m^{-3} in the case of the 'Tunnel' site and as the DTT assay responds strongly to the presence of Cu ions (Kachur *et al.*, 1997; Charrier and Anastasio, 2012), we estimated its contribution to the OP^{DTT} m^{-3}. By using a bioavailable fraction of 25% for particulate Cu (Birmili *et al.*, 2006) and a published relationship between the DTT consumption rate and the soluble Cu concentration in the DTT assay (Charrier and Anastasio, 2012), we found that copper contributed importantly ($78 \pm 20\%$) to the PM_4 OP^{DTT} m^{-3} at the 'Tunnel' site. Redox active quinones may additionally contribute to this reactivity (Charrier and Anastasio, 2012).

At the 'Depot' sites, only Fe was significantly and positively associated with the PM_4 OP^{DTT} m^{-3}. As Fe presents a low reactivity toward DTT (Charrier and Anastasio, 2012), this association is probably due to other redox active components associated with Fe but not measured in this study. Interestingly, we observed also a significantly negative association between Σ 7 PAH and OP^{DTT} m^{-3}. Such negative association has been reported only one time to our knowledge, for urban quasi-ultrafine particles (Verma *et al.*, 2009). It has been attributed to photo-oxidation of PAH, leading to the formation of oxy-PAH and quinones which are reactive toward DTT. Such process is not likely to take place in the confined studied workplaces. On the contrary, the presence of aerosolized used lubricating oil, containing paraffinic non-redox active compounds and potentially enriched with PAH (Brandenberger *et al.*, 2005) could mask reactive sites on particles and thus explain the observed negative association between PAH and the OP^{DTT} m^{-3}. Such quenching effect could also explain the absence of relationship between OC and the OP^{DTT} m^{-3} (see Supplementary Table 2 is available at *Annals of Occupational Hygiene* online).

Due to the complexity of the PM present in the workplaces, the hazard assessment of such mixtures requires an integrated assessment of the biologically important components. The DTT assay used in this study may present suitable integrative characteristics,

as it was found to respond to organics and also metals. These two compounds families are considered key for the induction of PM-related oxidative stress and subsequent proinflammatory processes (Donaldson *et al.,* 2005; Nawrot *et al.,* 2009). The measurement of redox activity of particles as a new metric is appealing (Borm *et al.,* 2007). However, many questions have to be solved before it can be used in occupational exposure assessment. At the methodological level, different acellular tests probing the OP of particles are available (e.g. electron spin resonance, ascorbic acid depletion, 2′7-dichlorodihydrofluorescein, Ayres *et al.,* 2008) and may respond differently (Zielinski *et al.,* 1999; Sauvain *et al.,* 2013). It may also be necessary to use more complex mixtures like synthetic respiratory tract lining fluid (RTLF) (Ayres *et al.,* 2008). All these assays are abiotic in nature and thus cannot take into account other possible oxidative pathways resulting from particle-biological system interactions such as, e.g. biological activation of PAH to quinones (Xue and Warshawsky, 2005), or upregulation of cellular/tissue enzymes producing antioxidants (Chan *et al.,* 2013). Thus, before being proposed as an alternative metric to mass, the biologically relevance of such assays has to be demonstrated. The relation between the DTT redox-activity and some *in vitro* cellular endpoints has already been published (Li *et al.,* 2003; Li *et al.,* 2009; Steenhof *et al.,* 2011; Uzu *et al.,* 2011). For children exposed to traffic-related pollutants, an association between $PM_{2.5}$ OP^{DTT} m^{-3} and exhaled NO, considered as a marker of airway inflammation has been recently reported (Delfino *et al.,* 2013). This further supports the idea that the DTT assay could be a relevant indicator for the potential of particles to induce oxidative stress in biological systems. Nevertheless, the reported lack or low consistent associations between various OP assays and different pulmonary and cardiovascular acute end-points for healthy volunteers exposed to different particle type (Strak *et al.,* 2012; Steenhof *et al.,* 2013; Steenhof *et al.,* 2014; Janssen *et al.,* 2015) stress the importance of further studies in this field.

CONCLUSION

The DTT assay allowed to quantitatively determine a redox activity for all the particles collected in different occupational environments. Whereas the PM mass concentrations differed largely between the two studied sites, similar OP^{DTT} was determined in these workplaces. This redox activity was associated to toxicologically relevant organics (OC, quinones) and metals (Fe, Cu). Particulate copper and possibly quinones contributed largely to this OP^{DTT} m^{-3} at the 'Tunnel' site. On the contrary, a combination of oxidative (attributable to Fe or associated chemicals) and quenching effects (possibly lubricating oil) are postulated to modulate the OP^{DTT} m^{-3} at the 'Depot' site. The DTT assay appears thus to be an integrative and potentially relevant indicator of the oxidative hazard of particles to biological systems. Nevertheless, the biological relevance of the OP^{DTT} approach for *in vivo* systems has to be demonstrated. Considering the ongoing debate about which metrics are most suitable to describe the hazards posed by particles to human health, the way forward in occupational health research seems to be a comprehensive exposure assessment based on multiple parameters such as mass, number, surface area, shape, a measurement of the OP such as OP^{DTT}, and potentially other parameters, which can later be distilled down to those factors that turn out to be most informative.

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