Oxidant production from source-oriented particulate matter Part 1: Oxidative potential using the dithiothreitol (DTT) assay

Jessica G. Charrier¹, Nicole K. Richards-Henderson¹, Keith J. Bein^{2,3}, Alexander S. McFall¹, Anthony S. Wexler^{1,2,4}, Cort Anastasio^{1*}

¹ Department of Land, Air and Water Resources, University of California – Davis.

² Air Quality Research Center, University of California – Davis

³Center for Health and the Environment, University of California – Davis

⁴ Department of Mechanical and Aeronautical Engineering, University of California – Davis.

*Corresponding author.

Telephone: (530) 754-6095

Fax: (530) 752-1552

canastasio@ucdavis.edu

University of California, Davis

1 Shields Ave.

Davis, CA, USA 95616

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Abstract

Recent epidemiological evidence supports the hypothesis that health effects from inhalation of ambient particulate matter (PM) are governed by more than just the mass of PM inhaled. Both specific chemical components and sources have been identified as important contributors to mortality and hospital admissions, even when these endpoints are unrelated to PM mass. Sources may cause adverse health effects via their ability to produce reactive oxygen species in the body, possibly due to the transition metal content of the PM. Our goal is to quantify the oxidative potential of ambient particle sources collected during two seasons in Fresno, CA using the dithiothreitol (DTT) assay. We collected PM from different sources or source combinations into different ChemVol (CV) samplers in real time using a novel source-oriented sampling technique based on single particle mass spectrometry. We segregated the particles from each source-oriented mixture into two size fractions - ultrafine ($D_p \le 0.17 \mu m$) and submicron fine (0.17 μ m $\leq D_{p} \leq$ 1.0 μ m) - and measured metals and the rate of DTT loss in each PM extract. We find that the mass-normalized oxidative potential of different sources varies by up to a factor of 8 and that submicron fine PM typically has a larger mass-normalized oxidative potential than ultrafine PM from the same source. Vehicular Emissions, Regional Source Mix, Commute Hours, Daytime Mixed Layer and Nighttime Inversion sources exhibit the highest mass-normalized oxidative potential. When we apportion DTT activity for total PM sampled to specific chemical compounds, soluble copper accounts for roughly 50% of total air-volume-normalized oxidative potential, soluble manganese accounts for 20%, and other unknown species, likely including quinones and other organics, account for 30%. During nighttime, soluble copper and manganese largely explain the oxidative potential of PM, while daytime has a larger contribution from unknown (likely organic) species.

1 **1. Introduction**

2 Inhalation of particulate matter (PM) has been linked with adverse health effects and mortality 3 in humans (Dominici et al., 2006;Dockery et al., 1993;Pope et al., 2002;Sunyer and Basagana, 4 2001; Breysse et al., 2013). Current regulations to protect health are based on PM mass without regard 5 to source or chemical composition. While PM mass often exhibits good correlations with toxicity 6 (Brunekreef and Holgate, 2002), the toxicity of PM is likely more complicated than mass alone, and may 7 be defined by a combination of properties including number concentration, size, surface area and 8 chemical composition (Valavanidis et al., 2008;Adamson et al., 1999;Costa and Dreher, 1997;Stoeger et 9 al., 2006; Peters et al., 1997; Perrone et al., 2013). These properties determine where PM will deposit in 10 the lungs, how it will interact with the body, and how much oxidative stress it will produce (Churg and 11 Brauer, 1997; Aust et al., 2002; Oberdorster et al., 2005; Yang et al., 2009). Oxidative stress is 12 hypothesized as a primary mechanism for adverse health effects from PM (Ercan et al., 2006;Fearon and 13 Faux, 2009;Halliwell and Cross, 1994), and occurs when reactive oxygen species (ROS) or other oxidants 14 overwhelm the natural defenses of the body (Li et al., 2003a). The picture is further complicated by the 15 fact that PM is a complex mixture of particles from various primary and secondary sources with different 16 degrees of atmospheric processing. Aging changes both the size distribution and chemical composition 17 of PM (Ellison et al., 1999; Maring et al., 2003; Pirrone et al., 2000; Sullivan et al., 2007), making sources 18 harder to differentiate and potentially changing the toxicity of the aerosol over time. 19 Recent epidemiological studies have identified specific sources or chemical components of PM 20 that are associated with health outcomes even when PM mass is not, supporting the hypothesis that 21 specific chemicals or sources have higher toxicities. These results indicate that PM mass may 22 underestimate the extent of health effects from PM (Ito et al., 2011;Lall et al., 2011). Ostro et al. (2007) 23 identified that mortality from PM in California was strongly tied to particulate Cu and other compounds, 24 while Lall et al. (2011) identified steel and traffic PM in New York as sources associated with respiratory 25 and cardiovascular hospital emissions, respectively. Traffic and residual oil fly ash are two sources 26 commonly associated with adverse effects (Hoffmann et al., 2007;Peters et al., 2004;Dreher et al.,

27 1997), possibly due to the ability of transition metals in the PM to cause oxidative stress (Gasser et al.,

28 2009;Antonini et al., 2004;Aust et al., 2002;Dye et al., 1997;Costa and Dreher, 1997).

One of the primary tools used to identify sources of ambient PM is positive matrix factorization (PMF). PMF can be used in conjunction with *in vitro* (Zhang et al., 2008) or epidemiological (Lall et al., 2011) data to identify sources that are potentially important for human health. PMF was also recently applied to DTT data for particles collected in the Southeast U.S. (Verma et al., 2014). However, PMF

requires intensive sampling and chemical analysis to obtain enough data for accurate source identification, which limits its applicability. In this work we use a novel method (Bein et al., 2009) to identify, separate, and collect ambient particles from different sources in real time based on their chemical composition. The method uses single particle mass spectrometry coupled to a suite of particle samplers, which allows for source identification and collection on relatively short (minute) time scales. Each source-oriented mixture of particles is collected on individual substrates as ultrafine (UF, $D_p \le 0.17$ µm) and sub-micron fine (SMF, 0.17 µm $\le D_p \le 1.0$ µm) size fractions.

40 Using this technique we collected up to 10 sources over two sampling campaigns during 2008 41 and 2009 in Fresno, CA. In this paper we describe the soluble and total metal composition of the PM 42 sources and their oxidative potential as measured by the dithiothreitol (DTT) assay. It should be noted 43 that the DTT assay is insensitive to ROS production from iron (Fe) (Charrier and Anastasio, 2012;Lin and 44 Yu, 2011), though Fe effectively produces hydroxyl radical (OH) in particle extracts via the Fenton 45 reaction (Vidrio et al., 2009). Thus, use of the DTT assay may underestimate oxidative potential from 46 sources that are high in Fe. In our companion manuscript (Part 2), we quantitatively measure the rate of 47 production of OH and hydrogen peroxide (HOOH), from the same Fresno particles in a cell-free 48 surrogate lung fluid, and compare these results with our DTT outcomes (Richards-Henderson et al., 49 2015). In this current paper, we examine differences in soluble metal composition and oxidative 50 potential for different source-oriented particles collected in Fresno. We also quantify the contributions 51 of different chemical species and particle sources to the measured oxidative potential as a function of 52 season and particle size.

53

54 **2. Methods**

55 2.1 Source-oriented particulate matter

56 Source-oriented particles (i.e., particles primarily from one source or a mixture of a few sources) 57 were identified and collected using on-line single particle mass spectrometry (RSMS-II) as described in 58 Bein et al. (2009). Downstream of the RSMS-II was a bank of 10 ChemVol (CV) samplers – which are high 59 flow rate (900 Lpm) impactor-based samplers (Demokritou et al., 2002) – for PM collection, with each 60 CV assigned to one source or source combination. Table 1 lists the CVs for each collection season, with 61 corresponding source identification and sampling information. Prior to sample collection, Bein et al. 62 (2009) used a pre-study analysis in Fresno, CA to determine up to 8 major nighttime sources based on RSMS-II single particle mass spectra, diurnal trends, and back trajectory analysis. These pre-study data 63 64 were used to define the combination of spectra that identify a specific source. During later sample

65 collection, when the single particle mass spectrometer observed these spectra, sampling algorithms 66 automatically switched the flow of ambient aerosol to the appropriate CV. We continuously collected 67 PM over each sampling period, alternating between CVs as an individual source became dominant 68 during nighttime sampling. If the RSMS-II identified a mixture of sources or an unidentified source, the 69 flow was directed to the "Nighttime Inversion (CV 10)" sampler, which therefore represents a mixture of 70 many different particle origins and histories. Daytime PM were not separated into sources because the 71 lower PM mass and greater turbulent mixing in the boundary layer during the day did not allow for a 72 clear separation of sources (Bein et al., 2009). Daytime PM were segregated into one to three samples 73 based on time-of-day instead of chemical-composition. These samples include "Daytime Mixed Layer 74 (CV9)" for both seasons, along with "Morning Commute (CV8)" and "Evening Commute (CV7)" during 75 winter 2009 (specific sampling times listed in Table 1). These sampling adjustments were made in 76 Winter 2009 based on sampling experience gained during Summer 2008. "Evening Commute (CV 7)" 77 was only collected for the last five days of the winter 2009 study.

Particles were collected in Fresno, CA from September 11th 2008 to October 21st 2008 and from 78 March 1st 2009 to April 6th 2009. To maintain consistency with previous publications (Bein et al., 79 80 2009;Bein and Wexler, 2014;Bein et al., 2014;Carosino et al., 2014) these study periods are designated 81 "summer" and "winter", respectively, which reflect the timing of the corresponding pre-studies for the 82 single particle mass spectrometer, though actual sample collections were in fall and spring. For each CV 83 particles were collected separately as ultrafine (UF, $D_0 \le 0.17 \mu m$) and submicron fine (SMF, 0.17 < $D_0 \le$ 84 1.0 µm) size fractions. SMF samples were collected on polyurethane foam (PUF, Fisher Scientific) and UF were collected on Teflon-coated glass-fiber filters (Pall TX-40, Fisher Scientific). 85

86 **2.3 PM extraction and sample preparation**

87 The PM extraction is described in detail in Bein and Wexler (2014). Briefly, PM collection 88 substrates were extracted via sonication using a sequential series of solvents: Milli-Q (18.2 M Ω cm), 89 dichloromethane (HPLC grade, 99.9%, Acros) then hexane (Optima, HPLC grade, 95%, Fisher). The water 90 fraction was extracted in a separatory funnel with dichloromethane then hexane to remove organics 91 (especially semi-volatiles). The remaining water fraction was Lyophilized (freeze dried, Labconco 92 FreeZone[®]) to remove water ice leaving solid PM behind. The four dichloromethane and hexane 93 extracts were dried under nitrogen and the remaining solid from all extractions, including the water fraction, were combined. The solid was stored under vacuum in the freezer (-20 °C) in 10 ml serum 94 95 bottles with 20 mm rubber split stoppers (Labconco) until reconstitution. To obtain a total extracted 96 mass for each PM sample, the vacuum sealed serum bottles were gravimetrically weighed before and

97 after PM was added using an A&D model HR-202i semi-micro analytical balance (± 0.01 mg). This
98 method extracts over 90% of PM from the filters, and retains bulk chemical composition better than
99 traditional extraction techniques, especially in regards to organics (Bein and Wexler, 2014).

To divide each particle sample into smaller aliquots, we first reconstituted the material in
methanol (Fisher, HPLC grade, 99.9%), sonicated for ~10 min in a bath sonicator (Branson model 8510,
Bransonic[®]), and split the suspension into 8-ml amber glass (Thermo Scientific) vials for DTT analysis.
Methanol in each vial was evaporated and the resulting solids were stored in the dark at -20° C until the
day of each experiment.

105 2.4 Dithiothreitol assay

106 Dithiothreitol (DTT) is commonly used to measure the oxidative potential of PM (Cho et al., 107 2005; Charrier and Anastasio, 2012). This method measures the rate of loss of 100 μM DTT at 37 °C upon 108 incubation with PM. The magnitude of the rate of DTT loss is proportional to the oxidative potential of 109 the PM. We used DTT analysis methods identical to Charrier and Anastasio (2012). We warm the PM 110 sample of known mass (Table S2) for 2 minutes in a 7.0 mL amber glass vial. We start the reaction by 111 adding 100 μ M DTT in 0.10 M phosphate buffer (77.8 mM Na₂H₂PO₄ and 22.2 mM KH₂PO₄, pH 7.4; 112 Chelex-treated) and measure the concentration of DTT over time to calculate the linear rate of DTT loss. 113 Each experiment includes a solution blank and positive control ($0.50 \mu M Cu(II)SO_4$) to ensure 114 reproducibility from day to day: our average ($\pm 1 \sigma$, n=25) blank is 0.19 \pm 0.05 μ M DTT / min and our 115 average blank-corrected positive control is $0.76 \pm 0.05 \mu M DTT / min$. The average solution-blank-116 corrected filter blank ($\pm 1 \sigma$, n=3 each) was 0.23 ± 0.018 and 0.22 $\pm 0.003 \mu$ M DTT / min for the SMF and 117 UF samples. Each PM sample and filter blank is analyzed in triplicate. All sample rates are corrected by 118 their daily solution blank and average filter blank (which was already solution-blank corrected). The 119 final DTT rate for a given size fraction of source-oriented mixture (i.e., CV) is reported as a PM-mass-120 normalized rate (pmols DTT / minute / μ g PM) according to: 121 Mass-normalized DTT rate = Blank corrected rate of loss (pmols DTT / min) (Eqn 1) 122 PM mass added to the vial (µg) 123 124 We also calculate the air-volume-normalized rate for a given season and size fraction (pmols DTT / 125 minute $/ m^3$ of air sampled) according to: 126 Air-volume-normalized DTT rate = $\sum ([Mass-normalized DTT rate CV_i] * [total mass CV_i (µg)]) (Eqn 2)$ 127 Total air volume for that season (m^3) 128

where the numerator term is summed over each source-oriented mixture *i* and the total air volume collected is 41,568 m³ for summer and 40,029 m³ for winter. The error of each sample includes the standard deviation of replicates (n = 2 – 3), the error of mass in the sample (supplemental Table S2), and propagated error of the filter blank (n = 3 – 5).

133 As we discuss in supplemental Section S1, the DTT response from PM is non-linearly related to 134 PM mass when Cu and Mn are significant contributors to the oxidative potential, a consequence of the 135 non-linear concentration-response curves for these metals (Charrier and Anastasio, 2012). Thus the 136 mass-normalized oxidative potential (Eqn 1) will sometimes vary with PM mass added to the vial, which 137 complicates comparison between samples, and confounds the interpretation of DTT data. We are in the 138 process of developing and validating a method to normalize DTT data for the PM mass extracted (data 139 not yet published). Application of this preliminary method to the Fresno samples in this current work 140 had little effect on the DTT results (Figures S2 and S3), so we did not adjustment the DTT responses for 141 these samples (Section S1).

142 **2.5 Transition metal analysis**

143 Soluble metals were measured by extracting the PM solid in acid-washed PTFE containers 144 containing 5.0 ml of a surrogate lung fluid (SLF). The SLF is composed of PBS (114 mM NaCl (A.C.S., 145 Fisher), 2.2 mM KH₂PO₄ (A.C.S., 99%, Fisher), and 7.8 mM Na₂HPO₄ (A.C.S., Fisher) at pH 7.4 that is 146 Chelex-treated to remove transition metals (Charrier and Anastasio, 2012)) and four anti-oxidants (200 147 μM ascorbic acid (99%, Fluka), 300 μM citric acid (99.7%, Sigma Aldrich), 100 μM glutathione (98%, 148 Sigma Aldrich) and 100 µM uric acid (A.C.S., Sigma Aldrich). After 1.5 or 24 hours of incubation on a 149 wrist-action shake table, 400 µl of the PM extract was filtered (0.22 µm, PTFE syringe filter, Tisch 150 Scientific) into a 15-ml acid-washed polypropylene centrifuge vial (Fisher Scientific) containing 3.6 ml of 151 3% nitric acid (Optima, Fisher Scientific) and then refrigerated until analysis. Samples were analyzed for 152 Ba, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, V, and Zn by ICP-MS (Agilent 7500 CE); further details of ICP-MS 153 analysis along with total metals analysis are given in the supplemental Sect. S3. Soluble metal 154 concentrations were corrected for the metal concentration in the solution blank and the corresponding 155 solution-blank-corrected field blank. Errors were calculated from the variance between replicates and the propagated error of the solution and field blanks. 156

157 Conditions of the DTT assay differ from the SLF conditions under which the PM was extracted 158 for metals analysis, i.e., temperature and the presence of antioxidants. Since these factors may affect 159 soluble metal concentrations in the extract, we compared metals content in separate SLF and DTT 160 extracts for 12 samples. We found very good agreement between the two techniques, except that Fe

- 161 was sometimes higher in the SLF than DTT assay (Supplemental section S2), possibly because SLF
- 162 contains citrate, which solubilizes particulate Fe (Aust et al., 2002). Although our soluble Fe
- 163 concentrations in SLF are likely upper-bound estimates of the amounts in the DTT assay, Fe is a relatively
- 164 weak oxidant for DTT (Charrier and Anastasio, 2012) and is a negligible contributor to DTT loss in our
- 165 Fresno source-oriented samples, as we describe later.

166 **3. Results and Discussion**

167 **3.1 Sample characteristics**

168 Table 1 summarizes the sampling characteristics and source identifications for each CV. 169 Detailed source identification information can be found in Bein et al. (2009, 2014). Briefly, sources were 170 identified based on chemical composition measured by the single-particle mass spectrometer, the 171 temporal pattern of the source, and wind roses indicating the direction of the source. In addition to the 172 timed daytime CVs, seven other sources were identified in summer and six in winter. Two distinct 173 cooking sources were identified: "Cooking – NE (CV1)" is from the northeast, characterized by a high K 174 content, while "Cooking – W (CV6 or CV7)" comes from the west and is characterized by K and mixed 175 Na/K particles. In summer the "Unknown – Metals (CV 6)" source is of unknown origin and was 176 identified based on high concentrations of metals, especially Zn and Pb (Bein et al., 2014).

177 The volume and mass of the collected PM for each sample varies widely depending on how 178 often a source was dominant. During most nights, no single dominant source was identified, and 179 sampling was directed to "Nighttime Inversion (CV10)" which accounts for 40 – 60% of PM mass in each 180 season (Table 1). The difference in the percent of total mass attributed to "Nighttime Inversion (CV 10)" 181 between seasons is partially driven by different sampling times: nighttime sampling was 18 hours per 182 day during summer and 10 hours per day during winter (Table 1). The total air volume and total UF 183 mass concentration are similar between seasons, approximately 40,000 m³ and \sim 80 – 100 mg, 184 respectively. In contrast, the total SMF mass collected is three times larger in summer than in winter. In 185 summer, UF mass concentrations for the CVs are relatively similar (2.0 – 3.6 μ g/m³), while there is more variability in mass concentrations for PM sources in the other seasons and sizes: $5.1 - 11.9 \,\mu\text{g/m}^3$ for 186

187 SMF summer, $1.9 - 8.2 \mu g/m^3$ for UF in winter, and $2.3 - 11.5 \mu g/m^3$ for SMF in winter (Table 1).

188 **3.2 Metals composition by CV**

Figure 1 shows the soluble metals content for each source, season, and size fraction in units of μg metal per gram of PM; total metals are in Supplemental Fig. S5. Ambient concentrations (ng/m³) of total and soluble metals are summarized by season and size fraction in Table S1. The median soluble metals concentrations are at or below median levels from other regions of the U.S. (Charrier and 193 Anastasio, 2012). The general trend in relative abundance follows Zn >> Ba ~ Fe > Cu ~ Mn > Ni > Pb ~ V 194 ~ Cr > Cd ~ Co. On average, Zn, Ba, Fe, Cu and Mn account for 66, 11, 10, 5.6, and 3.0% by mass of the 195 all measured soluble metals, respectively, while other metals each account for less than 3%. In the total 196 metals data Fe is slightly more important, accounting for 20% of the total on average, while Zn still 197 dominates with an average of 57% of the total. Total and soluble metals are generally well correlated 198 $(R^2 = 0.4 - 0.8)$ likely due to high solubility (50 - 100%) in our system (Supplemental Figures S3 and S4). Fe is the exception, showing poor correlation between total and soluble measurements ($R^2 = 0.15$) and 199 200 much lower solubility (median solubility of 27%). A large percent of each particulate metal is soluble, 201 with an average of approximately 100% for all species besides Fe, which has an average solubility of 202 29%. Metal solubility in ambient PM depends on a number of factors including pH, ligands, particle size, 203 morphology, and extraction technique. A large range of metal solubilities in ambient PM have been 204 reported (Connell et al., 2006). However, the percent of particulate Fe that is soluble is generally low in 205 ambient PM, with reports ranging from <1% to 6% solubility (Costa and Dreher, 1997;Upadhyay et al., 206 2011;Connell et al., 2006). The high metal solubilities in the source-oriented particles might be due to 207 the extraction procedure used to remove PM from their substrates required a number of analytical 208 steps which may have changed some properties of the PM, e.g. sonication of particles may have 209 preferentially removed soluble metals, and left some fraction of insoluble metals on the filter.

210 In general, the soluble metals content in μg metal / g PM is similar for the two size fractions of a 211 given source. Notable exceptions are "Diesel Enriched (CV 3)", "Vehicular Emissions (CV 5)", and 212 "Unknown – Metals (CV 6)" in summer and "Regional Source Mix (CV 5)" in winter, all of which have a 213 higher concentration of metals in the SMF size fraction compared to the UF, especially for Fe, Cu and 214 Mn. The metals content of PM (Figure 1) and the ambient concentration of metals (Table S1) are often 215 higher in Summer sampling. Because the sampling periods were actually Spring and Fall, we do not 216 expected the mixing height to affect concentrations differently between these two seasons. It is 217 possible that periodic rain during the Winter sampling explains some of the reduction in ambient 218 concentrations of both PM mass and metals. NOAA precipitation data indicates no rain during summer 219 sampling and 5 days of rain during winter sampling (NOAA, 2014).

220 **3.3 Mass-normalized oxidative potential**

We start by examining the rate of DTT loss for each source normalized by PM mass (Eqn. 1), which is a measure of the intrinsic oxidative potential of each source. Figure 2 shows that, for a given source, the submicron fine particles (SMF) are generally more reactive than the accompanying ultrafine (UF) particles: for approximately half of the PM sources the SMF reactivity is a factor of 2 larger than

225 that for the UF fraction. This higher reactivity of the submicron fine particles is in contrast to past 226 results, where ultrafine particles (typically $D_p < 0.1 \,\mu$ m) are generally reported to be more reactive than 227 PM_{2.5} (Li et al., 2003b;Cho et al., 2005;Ntziachristos et al., 2007;Hu et al., 2008). All of these past 228 measurements of size-segregated DTT responses are for California's South Coast Air Basin, while our 229 results are the first for the Central Valley of California. Thus this difference in the mass-normalized 230 oxidative potential between SMF and UF particles might reflect differences in sampling site. 231 Across both seasons, we observe a range of DTT rates for both UF and SMF PM, with values of 11 -232 60 and 24 – 92 pmols DTT / min / μ g PM, respectively; i.e., the mass-normalized oxidative potential of 233 different sources varies by up to a factor of 5.5 for UF and 3.8 for SMF particles. Our mass-normalized 234 rates of DTT loss are similar to those reported in the literature (restricted to those studies using the

same DTT method), which range from 5.0 to 170 pmol DTT / min / μg PM (Verma et al.,

236 2009;Ntziachristos et al., 2007;De Vizcaya-Ruiz et al., 2006;Verma et al., 2012).

237 We also calculated the overall oxidative potential for each size fraction in each of the two 238 seasons. This metric sums the mass-normalized oxidative potential of each particle type, weighted by 239 the mass of that PM collected:

240

Mass Weighted Oxidative Potential = $\sum (F_{i,k} \times DTT_{i,k})$ (Eqn. 3)

241 where *i* refers to season (summer or winter), *k* refers to size fraction (UF or SMF), *F* is the fraction of 242 total mass collected (Table 1) and DTT is the mass-normalized oxidative potential (Fig. 2, bars). This 243 metric is similar to Eqn.2, which calculated the DTT response of the total PM relative to air volume, but 244 instead calculates the DTT response of the total PM relative to PM mass. If we consider each season 245 across both size fractions (i.e., summing over k in Eqn. 3), the overall oxidative potentials for summer 246 and winter PM_{1.0} are 71 ± 9 and 45 ± 2 pmols DTT / min / μ g PM, respectively. Thus, the average mass-247 normalized oxidative potential of summer PM is 60% higher than that of winter for our study periods. 248 Broken down by season and size fraction, the weighted averages are 79 ± 12 and 47 ± 4 pmols DTT / min 249 / μ g PM for summer SMF and UF size fractions, respectively, and 49 ± 3 and 39 ± 3 pmols DTT / min / μ g 250 PM for winter SMF and UF, respectively; these values are plotted as lines in Fig. 2. Thus the overall 251 oxidative potential of SMF PM is higher than UF in both seasons, by 68% in summer and 26% in winter. 252 Comparing the two seasons, the oxidative potential of summer SMF is 61% larger than winter SMF, 253 while UF PM exhibited similar oxidative potential between the seasons. Based on this data, summer 254 SMF PM stands out as having a substantially higher mass-normalized oxidative potential relative to the 255 other PM.

When comparing each source-oriented mixture to the weighted average for season and size (bars versus lines in Fig. 2), most summer sources have an oxidative potential that is at or below the weighted average. This is because "Nighttime Inversion (CV 10)" accounts for most of the PM mass and has a high oxidative potential in summer for both UF and SMF PM, so it dominates the weighted average. In winter, there is less variability between the oxidative potentials of the different sources, but most sources are still at or below the weighted average defined by the "Daytime Mixed Layer (CV 9)" and "Nighttime Inversion (CV 10)", which make up most of the PM mass.

263 **3.4 Contribution of transition metals to DTT activity**

264 DTT responses in Fig. 2 often mirror the soluble metals concentrations (Fig. 1), e.g., summer SMF "Vehicular Emissions (CV 5)" and "Unknown – Metals (CV 6)" have some of the highest DTT 265 266 responses, and also have substantially higher soluble metals concentrations. Other sources, such as summer SMF "Daytime Mixed Layer (CV 9)" and "Nighttime Inversion (CV 10)" also have a high DTT 267 268 response, but lower soluble metals per PM mass, indicating metals may not explain the DTT response in 269 these cases. To quantify the contribution of each metal to our measured DTT response, we use our 270 measured metal concentrations in each PM extract in conjunction with concentration-response curves 271 for individual transition metals and quinones (Charrier and Anastasio, 2012). In this prior work we 272 determined that the DTT responses from these individual species are additive: mixtures of metals and 273 quinones give the same DTT response as the sum of the DTT responses from the individual species. Therefore, to calculate the total DTT response from soluble metals, we sum the calculated DTT response 274 275 from each individual species.

276 Figure 3 shows the measured DTT rate (in grey) in each PM extract compared to the calculated 277 DTT responses from Cu (purple), Mn (green), and Fe, V and Pb (orange). If metals are primarily 278 responsible for the DTT response, then the measured and calculated bars will be equal. If the measured 279 DTT rate is larger than the calculated rate, it indicates that other compounds, such as guinones, 280 contribute to DTT loss. In 36 out of the 38 samples the measured and calculated rates of DTT loss are 281 not statistically different (p < 0.05), highlighting the importance of copper and manganese. This 282 agreement between measured and calculated DTT loss is sometimes due to large errors, which primarily 283 from uncertainties in the soluble metals measurement and in PM masses added to the vial 284 (Supplemental Table S2). However, visual inspection of most pairs shows good agreement. Although 285 generally not statistically different, there are a number of cases where the measured DTT rate is larger 286 than the DTT rate calculated from soluble metals. In these cases there is likely some contribution from 287 other species, probably organic compounds, but the difference is encompassed by large error bars.

288 Even in these samples metals generally account for half or more of the DTT although there is a small but 289 significant contribution from other species, as would be expected from typical ambient concentrations 290 of guinones (as discussed below). There are also a few cases where the calculated DTT response is 291 larger than the measured DTT response, but large error bars in the soluble metals data indicate these 292 differences are generally due to experimental error. One exception is winter UF "Nighttime Inversion 293 (CV10)", which has a much larger calculated DTT response than we measured from PM. We don't 294 currently have an explanation for this result, but it could be an outlier in either the DTT or soluble metals 295 data.

296 On average ($\pm \sigma$), soluble Cu and Mn account for 83 \pm 40 % and 26 \pm 14 % of the measured DTT 297 response, respectively, in our Fresno particles. Fe, V, and Pb each contribute less than 1% to DTT 298 response; these contributions are too small to be seen for most samples in Figure 3. These results 299 represent the DTT response for the specific chemical composition of PM collected at this site. The DTT 300 response from PM measured at other sites will depend on the composition of the particles and may not 301 be dominated by transition metals. On the other hand, our interpretation of recent results from Atlanta 302 and other sites in the southeast U.S. also indicate Cu and Mn may play important roles in the DTT 303 response (Verma et al., 2014; see reviewer's discussion at http://www.atmos-chem-phys-304 discuss.net/14/19625/2014/acpd-14-19625-2014-discussion.html). The DTT results for any site 305 represent the specific chemical composition of the collected PM and the contributions to the resulting 306 DTT response depend on the relative amounts of Cu, Mn, quinones, and other redox-active species. 307 Sources of Cu and Mn in Fresno are incompletely understood, as indicated by recent modeling 308 that showed poor correlation between predicted and measured concentrations of Cu and Mn in PM2.5 309 and $PM_{0.1}$ at Fresno (Hu et al., 2014). The model underpredicts Cu and overpredicts Mn. In the US, 310 industrial Cu processing is thought to be the largest source of Cu, followed by brake lining dust, 311 agricultural soil, sintering furnaces, and process gas combustion, while Mn is primarily emitted by 312 unpaved road dust, agricultural soil, electric arc furnaces, and cast iron cupola furnaces in foundries 313 (Reff et al., 2009). Bukowiecki et al. (2009) observed Cu emissions from brake wear in the submicron 314 range, especially from heavy duty vehicles. The relative importance of industrial sources will likely be 315 smaller in California, while break lining dust and agricultural sources will likely be larger due to high 316 vehicle miles traveled and intense agricultural activity. For example, some dairies use copper baths to 317 treat cows for fungus, and pour the remaining solution into manure lagoons which may then be spread 318 to soils (Jokela et al., 2010).

319 The daytime samples summer SMF CV9 and winter SMF and UF CV8, and CV9, stand out as 320 having a relatively high contribution from unknown species (i.e., the measured rate of DTT loss is larger 321 than the rate calculated from metals). The measured DTT response in both summer and winter SMF 322 "Daytime Mixed Layer (CV 9)" is statistically larger than calculated from transition metals (Fig. 3 b and d, 323 marked with stars). In both cases, unknown species account for 49% of the measured rates of DTT loss. 324 This indicates that compounds other than the soluble metals we measured, likely guinones or other 325 organics, sometimes make significant contributions to the PM oxidative potential. It is possible that 326 insoluble metals also contribute to DTT loss by "unknown species", but this is less likely since the 327 insoluble fraction of diesel PM (which has a low metal content) oxidizes DTT quickly, while insoluble dust 328 (which has high metals) oxidizes DTT very slowly (Akhtar et al., 2010). Furthermore, based on our total 329 and soluble metals data, most DTT active metals (except Fe) have a high solubility (supplemental Fig. 330 S7), while Fe is less soluble but has a very low DTT activity (Charrier and Anastasio, 2012). Hu et al. 331 (2008) found a strong correlation between DTT response and organic carbon (OC) and observed that 332 concentrations of OC are more than 4 times larger during the day than at night. This could explain our 333 observed increase in DTT activity from compounds other than metals during the day. OC is made up of a 334 wide variety of organic compounds, not all of which are DTT active. Except for quinones (Kumagai et al., 335 2002; Charrier and Anastasio, 2012; Chung et al., 2006), it is largely unknown which organic compounds 336 are active in the DTT assay. Recent research has identified humic-like substances (HULIS) as DTT-active 337 (Verma et al., 2012;Lin and Yu, 2011), so these types of compounds may contribute to the oxidative 338 potential of OC.

339 We can estimate the likely contribution of quinones to our Fresno PM samples by using 340 previously measured particulate concentrations of phenanthrenequinone (PQN), the most DTT-active quinone tested (Kumagai et al., 2002; Charrier and Anastasio, 2012; Chung et al., 2006). PQN has a 341 median particulate-phase concentration of 0.32 ng/m³ based on (limited) measurements in Southern 342 343 California (Cho et al., 2004). If we assume this ambient concentration for all of our Fresno source-344 oriented mixtures (SMF + UF), and that all PQN is soluble, the resulting PQN concentration in solution is between 1 - 12 nM. This would correspond to DTT responses in the range of $0.01 - 0.16 \mu$ M DTT / min, 345 346 which represents 2 - 36 % of the measured DTT rates (with an average $\pm 1\sigma$ contribution of 13 ± 9 %). 347 This result agrees well with the observations in Figure 3, which show a consistent but relatively small 348 contribution of species besides Cu and Mn to measured DTT response. This is also consistent with our 349 previous calculations for PM_{2.5} based on typical literature concentrations of DTT-active species (Charrier 350 and Anastasio, 2012), where Cu, Mn, and quinones accounted for 47%, 28% and 18% of the calculated

DTT response of a hypothetical "typical" fine particle sample. The specific organic species that contribute to DTT response are still incompletely understood, and may include species other than the PQN. These hypothetical results are a lower estimate for organic contribution to DTT response if other DTT-active organic species are present. It is also possible that PM samples underestimate the quinone contribution because semi-volatile organics might be lost during sampling or particle extraction, but we do not have any evidence that this occurred for the present study.

357 In contrast to our mechanistic approach to identify the important DTT-active species in PM, 358 previous studies generally use correlation analysis. However, correlations between our DTT results and 359 measured soluble metal concentrations (Supplemental Fig. S8.a-c) reveal some of the difficulties with 360 the correlation approach. Cu, Mn, and Fe are modestly correlated with DTT loss (R^2 values of 0.40, 0.56 361 and 0.43, respectively) and the correlation with Cu is the weakest, even though our mechanistic 362 approach identifies Cu as the dominant contributor to DTT loss. The correlation understates the role of 363 copper because this metal has a non-linear concentration-response curve (see the green line in 364 Supplemental Fig. S8.a). The positive correlation between DTT loss and Mn agrees with our mechanistic 365 results, but the correlation with Fe is spurious (since Fe is nearly inactive in the DTT assay), and is 366 possibly due to the covariance between Fe and Mn (Supplemental Fig. S8.f). These plots illustrate that 367 correlation analysis is unlikely to identify the metals most important for DTT loss, which might explain 368 the inconsistent correlations between DTT response and Cu in the literature (Ntziachristos et al., 369 2007;Hu et al., 2008).

370 **3.5 Air-volume-normalized oxidative potential**

371 While the mass-normalized oxidative potential in Figures 2 and 3 is useful for identifying sources 372 that have the highest potential for harm, the oxidative burden associated with an air parcel also 373 depends on the particle mass concentration of each source. A source aerosol with a very high mass-374 normalized oxidative potential may not be important if its atmospheric mass concentration is low. As a 375 first step to address this, we use Eqn. 2 to calculate the air-volume-weighted DTT response for each size 376 fraction and season. This metric weights both the intrinsic oxidative potential of a source and the 377 relative abundance of that source. The volume-normalized oxidative potentials from both size fractions 378 (i.e., all PM with $D_0 \le 1.0 \,\mu\text{m}$) for summer and winter are 746 ± 90 and 217 ± 10 pmols DTT / min / m³-379 air, respectively. The DTT activity in the summer particles is over 3 times higher than in the winter 380 because both the PM mass concentration and mass-normalized oxidative potential of that PM are 381 higher in summer (Table 1 and Fig. 2), by factors of 2.2 and 1.6, respectively. The winter SMF and UF volume-normalized oxidative potentials are 135 and 83 pmols DTT / min / m³-air, respectively, while the 382

corresponding summer SMF and UF values are 623 and 123 pmols DTT / min / m³-air, respectively. SMF
 PM has a higher volume-normalized oxidative potential in both seasons, with summer SMF exhibiting
 very high oxidative potential due to high mass concentrations and high intrinsic oxidative potentials.

386 To identify which species and sources drive the air-volume-normalized responses, we next 387 apportion the volume-normalized oxidative potential in two ways - by chemical composition and by 388 source (Fig. 4). Fig. 4a shows the contributions of soluble metals and unknown species for each size 389 fraction and season as an absolute value, while Fig. 4b shows the data as a percent of the total DTT loss. 390 Summer SMF particles have the greatest oxidative potential, approximately 6 times larger than the 391 other conditions, and this response is due to Cu (47%), Mn (21%), and unknown species (31%). Even 392 though the other conditions have much lower air-volume-normalized rates of DTT loss, the percent 393 contributions from Cu, Mn, and unknowns are similar (Fig. 4b), although winter UF does not have a 394 contribution from unknown species. Based on these results, unknown species, likely quinones or other 395 organics, are important to the oxidative potential of PM in Fresno, but copper and manganese are the 396 dominant redox-active species. The unknown contribution to PM is primarily from daytime sources (Fig. 397 3), consistent with secondary formation of quinones from PAHs (Eiguren-Fernandez et al., 2008; Wang et 398 al., 2007). DTT response from most segregated nighttime sources, on the other hand, is completely 399 explained by metals, with little unknown contribution (Fig. 3).

400 Apportionment of volume-normalized oxidative potential by source is more difficult because 401 most (>79%) of the mass collected was during times when sources were not segregated (daytime), or 402 were collected as part of the catch-all "Nighttime Inversion (CV 10)" source. To more accurately 403 quantify the prevalence of each source, we use modeling results for Fresno based on the California Air 404 Resources Board's primary $PM_{2.5}$ and $PM_{0.1}$ emissions inventory (Hu et al., 2014). We match sources 405 that we measured with emissions inventory sources, then use the modeling results that quantify the 406 relative abundance of each emissions inventory source. There is additional uncertainty not explicitly 407 accounted for due to the assumptions of matching our data to that in Hu et al. (2014), which are 408 discussed in more detail in supplemental section S6. The inventory in Hu et al. (2014) is the best 409 available information of Fresno PM sources because it has been validated using ambient measurements, 410 contains temporal information that allows matching to our specific measurement season, and is the only 411 source of ultrafine PM source contributions available. A detailed summary of the source matching 412 method is in the Supplemental Material (Section S6 and Supplemental Tables S3 and S4). 413 While there is significant uncertainty in our results, and major sources are unidentified or

unknown, Figures 4c and 4d show that cooking and mobile sources make important individual

contributions, accounting for 18 – 29 % and 16 – 28 %, respectively, of the volume-normalized oxidative 415 416 potential. Biomass/Wood Smoke was only identified during winter in our sampling, and contributes 6 % 417 to the volume-normalized oxidative potential for both the SMF and UF size fractions. Though our 418 sample is designated as "winter", since we only caught the tail end of the wood burning season (Hu et 419 al., 2014), these results contain relatively low ambient concentrations of wood burning and do not 420 represent true winter conditions, when much more burning occurs. Heating was only identified during 421 winter in the SMF size fraction, and contributes 5 % to the volume-normalized oxidative potential. 422 Unknown sources are significant, accounting for 43 – 45 % of winter and 58 – 65 % of summer volume-423 normalized oxidative potential. It is important to consider that this "Unknown" result contains a 424 combination of modeling and atmospheric PM mass that was either not identified as a specific source, 425 did not match between the model and ambient sampling, or constituted a mixture of all identified 426 sources. Additionally, modeling results used to identify the relative abundance of each source type use 427 only primary PM mass, without an accounting for the secondary mass present in our ambient samples. 428 Thus, secondary organic aerosol (including reactions that produce quinones) are automatically included 429 in the "Unknown" category. In fact, most of the measured ambient Cu and Mn are apportioned to this 430 category since most measured PM mass resides in this category. In Summer, 76% of Cu and 83% of Mn 431 are apportioned to this category, while in Winter 77% of Cu and 90% of Mn are in the unknown 432 categories. This is why it was necessary to use modeling results to properly apportion source 433 contributions. These results also point out that the sources of Cu and Mn are poorly constrained in the 434 current emissions inventory, especially in the "ultrafine" particles.

435 **3.6 Vehicular emissions**

436 Vehicular (mobile) emissions sources exhibited some of the highest mass-normalized oxidative 437 potentials (Fig. 2), high soluble metals concentrations (Fig. 1), and a large contribution to total volume-438 normalized oxidative potential of PM (Fig. 4c and 4d). This agrees with literature results that have 439 consistently linked vehicular emissions to both high oxidative potential (Cheung et al., 2010;Delfino et 440 al., 2013; Gasser et al., 2009) and adverse health effects and mortality in humans (Lall et al., 2011; Hoek 441 et al., 2002;Hoffmann et al., 2007). Vehicular emissions contain multiple toxic chemical classes across 442 size ranges, including many metal and organic species. Metals can be emitted from brake wear, 443 lubricating oil and catalytic converters (Lough et al., 2005; Cheung et al., 2010; Zhao et al., 2006). 444 Particulate organics from vehicles are both primary and secondary and are present across multiple size 445 classes (Kam et al., 2012). Paved road dust is a large contribution to PM emissions in urban areas and

consists of many sources of PM including soil, biogenic material, and tire and brake wear (Rogge et al., 446 447 1993). Though not directly emitted by vehicles, this source is correlated with vehicle traffic volume. 448 In our summer data, "Vehicular Emissions (CV5)" showed high oxidative potential per mass of 449 PM compared to other sources (Fig. 2a), and also high soluble metals concentrations (Fig. 1a). This 450 source also showed significantly higher oxidative potential and soluble metals in the SMF size fraction 451 versus UF, which is not true for a majority of other sources. Summer SMF "Vehicular Emissions (CV 5)" 452 was enriched in soluble Zn, Fe and Cu, which were 46 %, 79 % and 188 % higher than the average 453 summer SMF concentrations for the other sources, while concentrations of soluble V, and Mn were 454 similar to the average for the same size and season. Zn and Cu are known to be enriched in brake wear 455 and road dust (Cheung et al., 2010), which have the largest concentrations in coarse sizes but also 456 contribute to submicron PM (Kam et al., 2012). Thus the enhancement in soluble metals in the larger 457 size fraction of summer Vehicular Emissions suggest brake wear or paved road dust can be important 458 contributors to the oxidative potential of SMF vehicular emissions. Winter "Vehicular Emissions (CV3)" 459 did not exhibit the same pronounced enhancement in either soluble metals (Fig. 1b) or oxidative 460 potential (Fig. 2b), or show a difference between the two size fractions. We do not currently have a 461 definitive explanation for this result; however, there was periodic rain during winter sampling which 462 may have suppressed paved road dust.

463 **4. Conclusions**

464 We measured the oxidative potential of 38 source-oriented particle samples – separated into 465 submicron fine (SMF) and ultrafine (UF) size fractions - from Fresno, CA in summer 2008 and winter 466 2009 using the DTT assay. We also quantified total and soluble metals in each sample. Fe and Zn are 467 the most abundant metals, followed by Cu and Mn. V and Pb concentrations are extremely low, and Co, 468 Cr, Cd and Ni are below detection. PM collected in summer 2008 has a higher metal content on average 469 and a three times higher ambient SMF mass concentration. As a result, the oxidative potential of 470 summer PM is 60% higher than winter PM per µg of particle mass. SMF samples generally show higher 471 oxidative potentials on a mass-normalized basis compared to UF particles. Summer SMF sources with high oxidative potential include "Vehicular Emissions (CV 5)", "Unknown – Metals (CV 6)", "Daytime 472 Mixed Layer (CV 9)", and "Nighttime Inversion (CV 10)". UF sources with the highest oxidative potential 473 474 per mass of PM include summer "Cooking – NE (CV1)", summer "Nighttime Inversion (CV 10)", winter "Morning Commute (CV 8)" and winter "Daytime Mixed Layer (CV 9)". On an air-volume-normalized 475 476 basis useful for considerations of human exposure, summer PM produces 240% more oxidative potential 477 than winter PM per cubic meter of air. 83% of summer oxidative potential is from the SMF size fraction.

478 Our results indicate that Cu, and to a lesser extent Mn, account for essentially all of the DTT 479 response for most of our individual nighttime sources. DTT rates in the daytime sources have a large (up 480 to 50%) contribution from unknown compounds, likely quinones and other organics with the remaining 481 activity due to metals. When calculating the overall volume-normalized oxidative potential for each 482 season (Fig. 4), Cu accounts for approximately 50%, unknown species account for approximately 30 %, 483 and Mn accounts for 20%. These results are supported by recent epidemiological studies that found a 484 statistical relationship between the Cu content of PM and mortality in California (Ostro et al., 2007). 485 When we apportion our volume-normalized oxidative potential to sources, unknown/unconstrained 486 sources make the largest contribution (43 - 65 %), while Cooking accounts for 18 - 29 %, Mobile sources 487 account for 14 – 28 %, and heating and biomass combustion make minor contributions (though they will 488 be larger in the winter months).

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500

501 Figure Captions

502

Figure 1. Soluble metals concentrations for a) summer and b) winter. For each CV, the first bar is the
result for the UF size fraction and the second bar is for the SMF size fraction. Zn concentrations are
divided by 10.

506

507 Figure 2. PM-mass-normalized rate of DTT loss for a) summer and b) winter. Lines represent the mass-

508 weighted, average DTT loss over all CVs for UF (dashed line) and SMF (solid line) in a given season. Error

bars are one standard deviation of replicates (n = 2-3) with the propagated error of the filter blank.

510

511 Figure 3. Measured rate of DTT loss (grey bars) compared to the calculated rate from soluble transition

512 metals (colored stacked bars). Asterisks identify cases where the measured and calculated rates are

513 statistically different ($p \le 0.05$). Errors for the calculated bars are ± 1 standard deviation, propagated

- 514 from the uncertainty in each of the five metals.
- 515
- 516 Figure 4. Panels (a) and (b) show the contributions of transition metals to the volume-normalized DTT
- 517 loss for each season and size fraction, in terms of rates (panel a) and percent contribution (panel b).
- 518 Panels (c) and (d) show the contributions of known (and unknown) particle emission sources to DTT loss,
- both in terms of rate (panel c) and percent contribution (panel d). "Unknown" in (a) and (b) indicates
- 520 chemical species that contribute to DTT response besides measured metals. "Unknown" in (c) and (d)
- 521 indicate unknown or other sources.

Sample Time	CV	Source	% of total volume	% of total UF mass	% of total SMF mass	UF mass concentration	SMF mass concentration $(ug (m^3))$
			Summo	~ 2000		(µg / m)	(µg / m)
Summer 2000							
Night - source segregated samples 3 pm - 11 am	1	Cooking - NE	11	13	11	3.0	7.8
	2	Secondary	8	11	13	3.5	11.9
	3	Diesel enriched	0.3	0.4	0.4	3.0	9.3
	4	Regional source mix	0.3	0.4	0.3	3.4	9.0
	5	Vehicular emissions	0.07	0.1	0.1	3.6	7.6
	6	Unknown - metals	0.8	1.0	0.9	3.5	9.2
	7	Cooking - W	0.2	0.1	0.1	2.0	5.1
	10	Nighttime Inversion	61	58	60	2.5	7.8
Day 11 am - 3 pm	9	Daytime mixed layer	18	16	14	2.3	6.2
		Summer Total:	41,568 m ³	108 mg	327 mg	2.6	7.9
Winter 2009							
Night - source segregated samples 8 pm - 6 am	1	Residential heating	0.9	3.1	2.9	6.9	8.6
	2	Secondary	0.8	1.5	2.8	4.0	9.2
	3	Vehicular emissions	0.6	1.2	1.8	4.2	8.4
	4	Processed biomass	0.2	0.9	0.7	8.2	8.9
	5	Regional source mix	0.6	1.6	1.5	5.2	6.5
	6	Cooking - W	0.3	1.0	1.1	8.2	11.5
	10	Nighttime inversion	48	44	43	1.9	2.4
Day 6 am - 9 am	8	Morning commute	12	13	14	2.3	3.1
Day 9 am - 5 pm	9	Daytime mixed layer	35	32	30	2.0	2.3
Day 5 pm - 8 pm	7	Evening commute	1.2	1.3	1.7	2.3	4.0
		Winter Total:	40,029 m ³	84.5 mg	109 mg	2.1	2.7

Table 1. CV sample characteristics^a

a) Data from (Bein et al., 2009)

b) Totals are the sum of all CVs. Total mass concentrations were calculated from the total PM mass divided by the total volume collected for all sources.









Figure 3



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