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Reactive Oxygen Species Generation Linked to Sources of Atmospheric Particulate Matter and Cardiorespiratory Effects

Josephine T. Bates,[†] Rodney J. Weber,[‡] Joseph Abrams,[§] Vishal Verma,[∥] Ting Fang,[‡] Mitchel Klein,[§] Matthew J. Strickland,[§] Stefanie Ebelt Sarnat,[§] Howard H. Chang,[⊥] James A. Mulholland,[†] Paige E. Tolbert,[§] and Armistead G. Russell[*,†]

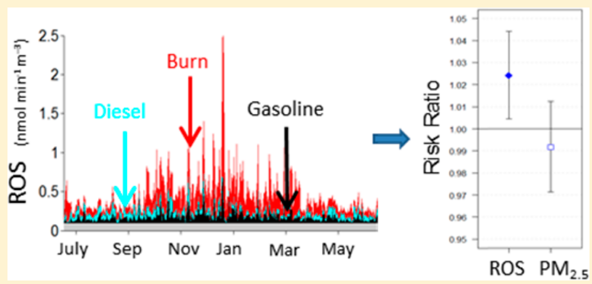
[†]School of Civil and Environmental Engineering, and [‡]School of Earth and Atmospheric Sciences, Georgia Institute of Technology, Atlanta, Georgia 30332, United States

[§]Department of Environmental Health, Rollins School of Public Health, and [⊥]Department of Biostatistics and Bioinformatics, Rollins School of Public Health, Emory University, Atlanta, Georgia 30322, United States

[∥]Department of Civil and Environmental Engineering, University of Illinois at Urbana−Champaign, Champaign, Illinois 61801, United States

Supporting Information

ABSTRACT: Exposure to atmospheric fine particulate matter ($PM_{2.5}$) is associated with cardiorespiratory morbidity and mortality, but the mechanisms are not well understood. We assess the hypothesis that $PM_{2.5}$ induces oxidative stress in the body via catalytic generation of reactive oxygen species (ROS). A dithiothreitol (DTT) assay was used to measure the ROS-generation potential of water-soluble $PM_{2.5}$. Source apportionment on ambient (Atlanta, GA) $PM_{2.5}$ was performed using the chemical mass balance method with ensemble-averaged source impact profiles. Linear regression analysis was used to relate $PM_{2.5}$ emission sources to ROS-generation potential and to estimate historical levels of DTT activity for use in an epidemiologic analysis for the period of 1998−2009. Light-duty gasoline vehicles (LDGV) exhibited the highest intrinsic DTT activity, followed by biomass burning (BURN) and heavy-duty diesel vehicles (HDDV) (0.11 ± 0.02, 0.069 ± 0.02, and 0.052 ± 0.01 nmol min^{-1} μg^{-1}_{source}, respectively). BURN contributed the largest fraction to total DTT activity over the study period, followed by LDGV and HDDV (45, 20, and 14%, respectively). DTT activity was more strongly associated with emergency department visits for asthma/wheezing and congestive heart failure than $PM_{2.5}$. This work provides further epidemiologic evidence of a biologically plausible mechanism, that of oxidative stress, for associations of adverse health outcomes with $PM_{2.5}$ mass and supports continued assessment of the utility of the DTT activity assay as a measure of ROS-generating potential of particles.



■ INTRODUCTION

Air pollution exposure is one of the world's leading environmental health risks, causing approximately 7 million deaths worldwide in 2010.[1] Fine particulate matter ($PM_{2.5}$) is a prevalent air pollutant, and epidemiologic studies show that exposure to $PM_{2.5}$ increases risk for cardiorespiratory morbidity and mortality.[2−4] However, the mechanisms of toxicity are not well understood. Reactive oxygen species (ROS) either transported on particles or catalytically generated by particles through redox reactions are suspected to cause injurious cellular responses. This work focuses on the catalytic generation of ROS by $PM_{2.5}$. It is hypothesized that $PM_{2.5}$ inhalation can induce oxidative stress, leading to a variety of health effects, by catalyzing the generation of ROS in excess of the antioxidant capacity of the body.[5,6] Small-scale health studies have linked particulate matter ROS-generating potential to inflammation and decreased lung capacity, although inconsistent results highlight the need for a more comprehensive population-level epidemiologic analysis.[7−9]

ROS-generating potential of $PM_{2.5}$ may vary by $PM_{2.5}$ composition. For example, quinones and transition metals have been shown to catalyze redox reactions in the body.[10] Biomass burning organic aerosols and highly oxidized organic aerosols have been identified as having high intrinsic toxicities with regard to ROS-generating abilities.[11] This study aims to identify emission sources of $PM_{2.5}$ that have the ability to catalytically generate ROS and investigate the relationship between these particles and human health. Identifying sources of redox-active $PM_{2.5}$ rather than species is important for policy

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development and provides an additional understanding of the compounds involved in ROS generation.

A variety of methods are available for measuring the capacity of $PM_{2.5}$ to catalyze redox reactions. The dithiothreitol (DTT) assay is a commonly used acellular assay because its response correlates well with biological markers, such as hemeoxygenase (HO-1) expression in cells and exhaled nitric oxide fraction in human subjects.[9,10] Water-soluble and water-insoluble fractions of particulate matter exhibit different mechanisms of action in the body,[11] and while both components are important, this research focuses on the water-soluble fraction. A DTT assay was used in this study to quantify ROS-generation potential of water-soluble $PM_{2.5}$ (WS-$PM_{2.5}$). Source apportionment techniques were used with linear regression analyses to assess intrinsic DTT activity of $PM_{2.5}$ sources. The model was used to simulate historical trends in ambient DTT activity for use in epidemiologic studies. Developing better indicators of the potential of $PM_{2.5}$ to impact health facilitates optimization of air pollution control strategies.

MATERIALS AND METHODS

Sample Collection and Analysis. Atlanta is a major urban population center with relatively high emissions of $PM_{2.5}$ from a variety of sources (including mobile sources, biomass burning, etc.) and secondary aerosol formation, making it a suitable U.S. location to study particle toxicity. Additionally, it is the site of detailed work identifying source impacts on $PM_{2.5}$,[12] which have also been used in prior health studies.[13,14] Sampling was conducted at a Southeastern Aerosol Research and Characterization (SEARCH) site in Atlanta [Jefferson Street (JST)] from June 2012 to April 2013. Ambient $PM_{2.5}$ was collected using a high-volume sampler (HiVol, Thermo Anderson, non-denuded, nominal flow rate of 1.13 m^3/min, $PM_{2.5}$ impactor). Prebaked 8 × 10 in. quartz filters were used for particle collection. Samples were taken from 12 pm (noon) to 11 am, creating 23 h integrated samples. All samples were wrapped in prebaked aluminum foil and stored in freezers (−18 °C) until ROS-generation analysis. Filter extraction processes were similar to those described in detail by Verma et al.[15] In brief, 1 in. punches were extracted in 15 mL of deionized water via sonication in a water bath for 30 min. Each extract was filtered using a polytetrafluoroethylene (PTFE) 0.45 μm pore syringe filter to remove insoluble materials and fibers. These WS-$PM_{2.5}$ samples were used in the DTT analysis.

A semi-automated cell-free DTT assay instrument was developed and used to measure the rate at which ambient WS-$PM_{2.5}$ catalytically generates ROS,[16] creating a uniquely large data set of over 225 23 h integrated measurements. DTT acts as a surrogate of the biological reducing agents reduced nicotinamide adenine dinucleotide (NADH) and reduced nicotinamide adenine dinucleotide phosphate (NADPH), and the ROS-generating potential of ambient WS-$PM_{2.5}$ was quantified by the DTT consumption rate (i.e. DTT activity). The method of measuring DTT activity is described in detail by Cho et al.[17] Briefly, the rate of DTT consumption was measured from a mixture of 100 μM DTT, a WS-$PM_{2.5}$ filter sample (3.5 mL) collected using the HiVol sampler, and potassium phosphate buffer (0.1 mL, pH 7.4). This mixture was incubated in a ThermoMixer (Eppendorf North America, Inc., Hauppauge, NY) at 37 °C. DTT consumption was measured at five time steps (4, 13, 23, 30, and 41 min). For each, a 100 μL aliquot was mixed with 1 mL of 1% (w/v) trichloroacetic acid (TCA) to quench the reaction, and 0.5 mL of 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) and 2 mL of tris(hydroxymethyl)-aminomethane (Tris) buffer [0.08 M with 4 mM ethylenediaminetetraacetic acid (EDTA)] were added to form 2-nitro-5-mercaptobenzoic acid (TNB) by reacting with the residual DTT. A spectrometer was used to measure the light absorption of this product to quantify the remaining DTT concentrations. The measurements at the five time steps, along with the initial DTT concentration, were used to estimate a linear slope parameter representing the DTT consumption rate (nmol min^{-1}) associated with the aerosol sample collected from a known volume of ambient air. Volume-normalized DTT (DTTv) is the rate of moles of DTT consumed per minute per volume of air sampled (nmol min^{-1} m^{-3}). Mass-normalized DTT (DTTm), with units of nmol min^{-1} μg^{-1}, was determined by dividing DTTv for each day by the total 23 h $PM_{2.5}$ mass measured from a tapered elemental oscillating microbalance (TEOM). The recently developed analytical system allowed for automated measurements at a rate of one sample per hour and the ability to generate large DTT data sets at a reasonable cost.[16]

Other air quality data, including total species concentrations [organic carbon (OC), elemental carbon, ions, and metals], were used for source apportionment of total $PM_{2.5}$. Different measurements had to be used for source apportionment than the filters for DTT analysis because the source profiles used in the chemical mass balance (CMB) method are based on total species concentrations, whereas only water-soluble species were measured from the DTT filters. The measurements of total species concentrations were collected at JST over the same time period as the DTT filter collection (from June 2012 to April 2013) using methods detailed by Hansen et al.,[18] Edgerton et al.,[19] and Hansen et al.[20] In brief, total $PM_{2.5}$ was collected using a Rupprecht & Patashnick model 20205 sequential FRM monitor. The 24 h integrated samples (from midnight to midnight) were collected daily on Teflon filters (47 mm diameter and 2 μm pore size) and were collected, processed, and analyzed according to FRM protocols.[21] A particle composition monitor (PCM) built by Atmospheric Research & Analysis, Inc. was used to measure sulfate, nitrate, ammonium, OC, and black carbon (BC) every 3 days. The 24 h integrated samples of ions were collected on Teflon filters (47 mm diameter and 2-μm pore size) and analyzed using ion chromatography. The 24 h integrated samples of OC and BC were collected on quartz filters (37 mm diameter) and measured using thermal/optical reflectance. Denuders (annular for the ions and activated carbon honeycomb for OC and BC) were used for removal of select gases. Flow through the PCM was maintained at 16.7 L/min. Metals, including aluminum, calcium, copper, iron, potassium, manganese, lead, silicon, titanium, and zinc, were measured from the FRM filter samples using X-ray fluorescence. These measurements are available daily.

Source Apportionment. Using collocated measures of total $PM_{2.5}$ and $PM_{2.5}$ species (ions, carbon, and metals), $PM_{2.5}$ source impacts were constructed. Source apportionment was performed on the ambient $PM_{2.5}$ measurements using the CMB model, version 8.2, with ensemble-based source profiles for $PM_{2.5}$ that combined source impacts from three receptor-based models (CMB-LGO, CMB-MM, and positive matrix factorization) and a chemical transport model (community multi-scale air quality model).[12−14] Sources in this profile include light-duty gas vehicles (LDGV), heavy-duty diesel vehicles (HDDV), soil dust (SDUST), biomass burning (BURN), ammonium

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sulfate (AMSULF), ammonium bisulfate (AMBSULF), ammonium nitrate (AMNITR), and not otherwise apportioned organic carbon (OTHER_OC), which mostly contains secondary biogenic carbon. To correct for temporal misalignment between DTT measurements collected from noon to 11 am and source impact estimates from midnight to midnight, the sources identified were linearly interpolated to the time period over which the DTT filters were taken.

Linear Regression. The impact of specific sources of $PM_{2.5}$ on DTT activity was estimated using a multivariate linear regression (Figure 1). The regression identifies the relationship

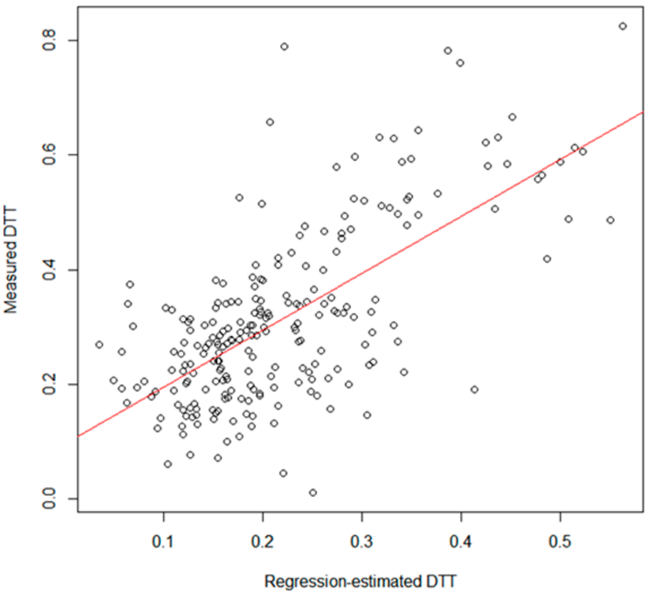

Figure 1. Measured DTT activity plotted against DTT activity estimated using the regression model for the 2012−2013 period. The R^2 between estimated and measured DTT is 0.45.

between the measured time series of WS-$PM_{2.5}$ DTT activity and the estimated time series of source impacts on total $PM_{2.5}$. The use of total $PM_{2.5}$ for sources in the regression was required for applying the regression to previous years for which source impacts are only available for total $PM_{2.5}$ and not WS-$PM_{2.5}$. $PM_{2.5}$ sources rather than species were investigated in this work for the following reasons: First, the ROS-generation potential of emission sources circumvents the problem that some of the bulk species measurements actually represent many species that are expected to have different health impacts. For example, what is measured as OC encompasses many different specific chemical species, including those expected to significantly generate ROS (e.g., quinones and other humic-like substances) versus those less so.[17,22,23] Additionally, the chemical composition of OC varies seasonally; therefore, the ROS-generation potential per unit of OC is not stable over time.[11] This leads to the second reason for using sources: stability of the model over time. The composition of sources, such as vehicle emissions and biomass burning, is more constant over time, and the source profiles used in this work are consistent from 1998 to 2013; therefore, a model trained on 2012−2013 data can be used to backcast estimates to earlier years. Finally, using source impacts rather than species significantly reduces the number of independent variables in the model, many of which are highly correlated when using just species.

DTT activity was treated as the dependent variable, with sources as the independent variables. All programming was conducted using the statistical software package R 3.0.2. The assumption of a linear relationship between DTT activity and sources was supported by graphs of joint probability density functions (jpdf) between measured DTT activity of WS-$PM_{2.5}$ and concentrations of each individual source impact that illustrated linear relationships. An F test was used to assess the significance of each source impact in the model on estimating DTT activity. Coefficients of sources with p values of the F statistic >0.05 were not considered statistically significant. Using a backward elimination approach, the least significant source was removed from the model and least squares was performed again with one fewer independent variable. This process was repeated until only significant coefficients remained (Table S1). Ammonium sulfate was not included in the model because additional work performed during this study and by others show that sulfate alone is not DTT-active.[21] This was supported by direct laboratory experiments of a sulfate-containing solution in which DTT activities of blank filters and filters with only sulfate were compared and found to be similar (Figure S1). We also performed regression analyses in which source impacts had been normalized to a standard deviation of 1 to investigate the sensitivity of DTT activity to each source and provide further evidence of the differing contributions to DTT activity of each source impact.

Historical DTT Estimation. The DTT activity model was used to backcast estimated water-soluble DTT activity of daily $PM_{2.5}$ for the greater metropolitan Atlanta area for the period from August 1998 to December 2009 (a period during which DTT activity measurements were not available but daily speciated air quality measurements were available, allowing for source apportionment of $PM_{2.5}$). It was assumed that the chemical composition of $PM_{2.5}$ source impacts has not changed significantly over time, so that source profiles could be applied to all years from 1998 to 2013. Vegetation in Georgia has not changed significantly over the past decade, making biomass burning source profiles relatively stable. Additionally, the EC/OC ratio (the main driver of vehicle source profiles) of average vehicle fleets in the 20 county non-attainment Atlanta area calculated using MOVES 2010 is only ∼20% different between previous years (averaged over 1998−2009) and 2012.[24] These reasons, along with the reasons for using source impacts in the linear regression, support using a model trained on 2012−2013 data to backcast DTT estimates for earlier years. Additionally, it was assumed that the DTT activity estimated at one location (JST) using the regression model applies to the 5 county metro Atlanta area used in the epidemiologic study because DTT activity per volume of air has been shown to be spatially uniform.[15] To obtain estimates of DTT activity from 1998 to 2009, daily concentrations of $PM_{2.5}$ from each source were multiplied by their respective coefficients in the model and then summed to estimate daily total DTT activity. This resulting DTT time series was then used in an epidemiologic analysis to investigate the link between estimated DTT activity and emergency department (ED) visits for asthma/wheezing and congestive heart failure.

Epidemiologic Modeling. We conducted time series analyses using modeling methods and control for confounding variables employed in our previously reported epidemiologic analyses of these data[13,25,26] to characterize epidemiologic associations between health events and $PM_{2.5}$, estimated DTT activity, and measures of $PM_{2.5}$ species at JST. The outcome

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Table 1. Pearson Correlation Coefficients between DTT Activity and Sources for the 2012−2013 Period[a]

	total $PM_{2.5}$	LDGV	HDDV	SDUST	BURN	AMSULF	AMBSLF	AMNITR	OTHER_OC
DTTv (nmol min^{-1} m^{-3})	0.51	0.54	0.27	0.11	0.61	0.30	0.003	0.30	0.11
DTTm (nmol min^{-1} μg^{-1})	−0.25	0.34	−0.29	0.25	0.25	−0.16	−0.29	0.22	−0.47

[a]DTTv is the volume-normalized DTT activity, and DTTm is the mass-normalized DTT activity (the number of samples was 227 for DTTv and 132 for DTTm). The sources are light-duty gasoline vehicles (LDGV), heavy-duty diesel vehicles (HDDV), soil dust (SDUST), biomass burning (BURN), ammonium sulfate (AMSULF), ammonium bisulfate (AMBSLF), ammonium nitrate (AMNITR), and all remaining organic carbon (OTHER_OC).

was the daily number of ED visits with a primary International Classification of Disease, 9th Revision, (ICD-9) code of asthma or wheeze (493 and 786.07) or congestive heart failure (428) for a patient with a home ZIP code within the Atlanta 5 county metro area (Fulton, DeKalb, Gwinnett, Cobb, and Clayton) recorded in an Atlanta hospital from Aug 1, 1998 to Dec 31, 2009 (other outcome groups that are not presented here include chronic obstructive pulmonary disease, pneumonia, and ischemic heart failure; in preliminary examinations, none of these showed significant associations with estimated DTT activity or with $PM_{2.5}$ mass). Over the study period, 263 665 and 70 587 ED visits were recorded for asthma/wheezing and congestive heart failure, respectively. The primary air quality variables of interest were 24 h averaged estimated DTT activity and 24 h averaged $PM_{2.5}$ from JST (models were run for each of these variables separately as well as models with both variables included). Because health effects of pollution may be observed over several subsequent days, the exposures were modeled as lag 0−2 (average pollutant level for that day, the previous day, and the day before). This analysis was modeled as a Poisson generalized linear regression allowing for over-dispersion using SAS 9.4 statistical software (SAS Institute, Cary, NC).

The covariates in this model were chosen on the basis of prior knowledge of variables that could potentially act as temporal confounders between ED visits and daily pollution levels. Models controlled for temporal trends using transformed cubic splines with monthly knots. Linear, quadratic, and cubic terms were included for mean daily dew point (lag 0−2), maximum daily temperature, and minimum daily temperature (lag 1−2). Other variables included indicators of hospital contribution time periods (not all hospitals provided data for the whole time period from August 1998 to December 2009; the model controlled for the subset of days that the data for each hospital was available), season of year, day of week, and holiday indicators. Interaction terms were included between season and maximum temperature (linear, quadratic, and cubic) and between season and day of week because the effects of the latter variables can change according to season. The use of the maximum temperature from that day as well as the lagged minimum temperature was based on previous analyses showing that this temperature control was highly predictive of ED visits while minimizing covariate collinearity. Sensitivity analyses were conducted varying age category (pediatric ED visits versus all ED visits), geographic extent (20 county Atlanta area versus 5 county Atlanta area), and ICD code priority (any asthma/wheeze or congestive heart failure code versus primary AS_WHZ or CHF code). Results for these analyses remained similar to those presented.

Risk ratios and their 95% confidence intervals were calculated for estimated DTT activity in a single pollutant model, total $PM_{2.5}$ in a single pollutant model, and estimated DTT activity and total $PM_{2.5}$ in a two-pollutant model for lag 0−2. The risk ratios represent the relative risk of ED visits per unit increase in DTT activity or $PM_{2.5}$ mass. The interquartile ranges (IQR, a measure of variability that reflects the difference between the 75th percentile and the 25th percentile of a distribution) of DTT activity and $PM_{2.5}$ were used as exposure units. For example, if the risk ratio was 1.08, an increase in pollution by 1 IQR unit would increase the risk of ED visits for the illness in question by 8%. The two-pollutant model allows for the assessment of health outcome associations of DTT activity controlling for total $PM_{2.5}$ mass, providing additional evidence that any measured association with health outcomes represents the effects of ROS-generating potential.

■ RESULTS AND DISCUSSION

Source Contribution to DTT Activity. This paper investigates the differing ROS-generation capabilities of various source impacts on $PM_{2.5}$ and their relationship to acute health effects. First-level analysis on the relationship between DTT activity and $PM_{2.5}$ looked at the correlation coefficients between DTT activity, species, and $PM_{2.5}$ source impacts (Table 1 and Table S1). Volume-normalized DTT activity (DTTv, nmol min^{-1} m^{-3}) was positively correlated with $PM_{2.5}$ (R = 0.51), indicating that one or more components of this pollutant contribute to its ROS-generating capability. The negative correlation between DTT activity normalized by $PM_{2.5}$ mass (DTTm, nmol min^{-1} μg^{-1}) and total $PM_{2.5}$ (R = −0.25) suggests that certain species, such as sulfate, that contribute to total $PM_{2.5}$ mass do not on their own contribute much to the ROS-generating capability. These correlations support the regression results.

Advanced analysis involved the development of a regression relating DTT activity of WS-$PM_{2.5}$ to total $PM_{2.5}$ source impacts. The model was developed using DTT activity measurements in Atlanta, GA from June 2012 to April 2013 and source impacts estimated from the CMB method applied to collocated total $PM_{2.5}$ and species measurements, leading to a linear relationship between the ROS activity of WS-$PM_{2.5}$ (DTTa) and source impacts on total $PM_{2.5}$ (Figure 1).

$$\mathrm{DTTa} = 0.095 + 0.11\mathrm{LDGV} + 0.052\mathrm{HDDV} + 0.069\mathrm{BURN}$$

DTTa acts as a multi-pollutant, multi-source indicator, a measure integrating across species and sources with respect to their oxidative potential. This equation has an R^2 of 0.45 and mean-squared error of DTT activity of 0.013 nmol min^{-1} m^{-3} (4.2% of mean of measurements). Cross-validation was performed to evaluate this model. A total of 10% of the DTT measurements was removed; the regression coefficients were reevaluated; and the mean-squared error was calculated. This process was repeated 50 times, and the average mean-squared error over 50 iterations is 0.079 nmol min^{-1} m^{-3} (25.5% of the mean of measurements).

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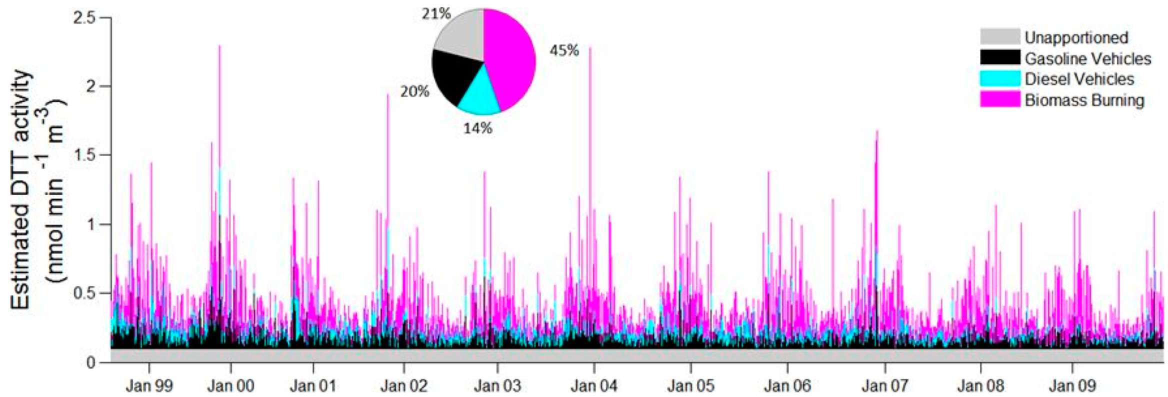

Figure 2. Daily estimated DTT activity of WS-$PM_{2.5}$ from Aug 1998 to Dec 2009 calculated using the regression. The pie chart shows the average contribution of each source.

Table 2. Regression Coefficients and Standard Error for Each Source in the DTT Regressions with Normalized Data[a]

	LDGV	HDDV	BURN	AMSULF	AMBSLF	OTHER_OC
DTTv (nmol min^{-1} m^{-3})	0.31 (0.058)	0.094 (0.051)	0.39 (0.058)			
DTTm (nmol min^{-1} μg^{-1})	0.22 (0.068)		0.21 (0.078)	−0.25 (0.077)	−0.28 (0.077)	−0.32 (0.092)

[a]Standard errors are in parentheses. The coefficients for SDUST and AMNITR were not statistically significant and were not included in the regressions.

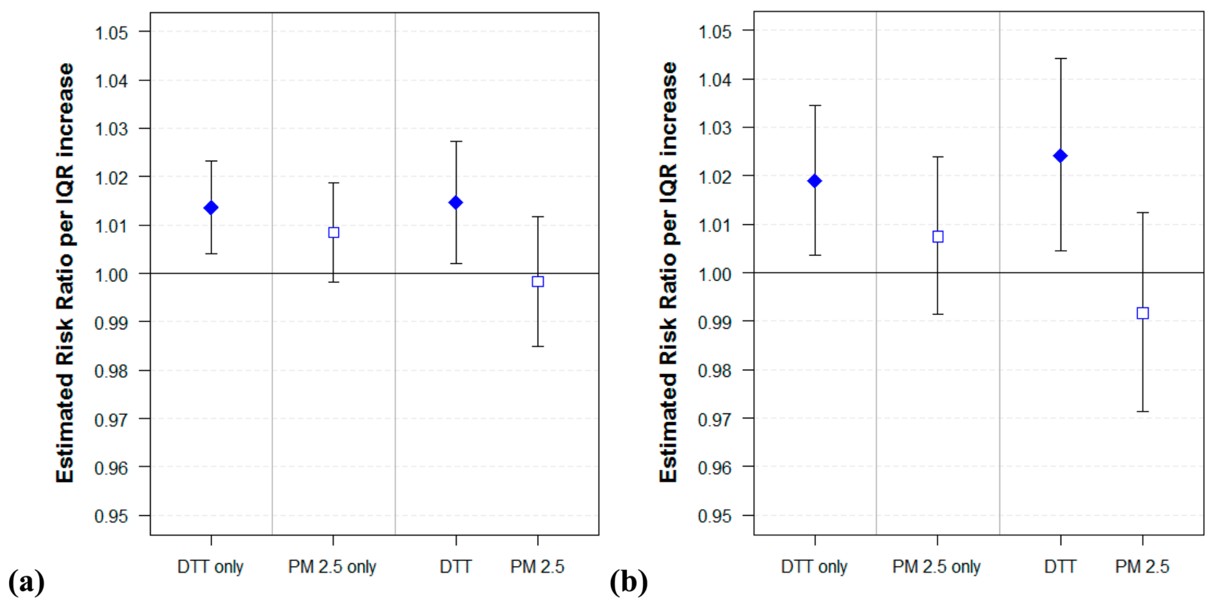

Figure 3. Effect of ROS (by estimated DTT activity) on ED visits for (a) asthma/wheeze and (b) congestive heart failure in the Atlanta 5 county area, from Aug 1, 1998 to Dec 31, 2009, with 0−2 day lag. The model labeled "DTT only" or "$PM_{2.5}$ only" illustrates the results from the model run only with DTT activity or total $PM_{2.5}$ mass as the exposure variable, respectively. The model labeled "DTT and $PM_{2.5}$" illustrates the results from the two-pollutant model, in which both DTT activity and total $PM_{2.5}$ mass were used as exposure variables.

From this analyses, we find that gasoline vehicle emissions exhibited the highest intrinsic DTT activity, as shown by their coefficient in the regression (nmol min^{-1} $\mu g^{-1}{}_{source}$), which is consistent with studies showing links between DTT activity and oxygenated OC and metals.[11,15,17] Diesel particles have been shown to be DTT-active; however, most of the DTT activity occurs in the water-insoluble fraction of diesel particles[23] and, thus, was not measured in this work and is not captured by our approach, consistent with its relatively low intrinsic activity (i.e., lowest coefficient in the model). The high intrinsic DTT activity of biomass burning is most likely driven by its high oxygenated OC content.[11,15]

While gasoline vehicles exhibited the highest intrinsic toxicity in this analysis, biomass burning accounted for the highest fraction of total estimated DTT activity on a per volume of air basis (Figure 2). Biomass burning, light-duty gasoline vehicles, and heavy-duty diesel vehicles contributed 45, 20, and 14% (standard deviations of 15, 10, and 9%), respectively. Results from measurements and estimated historical DTT activity suggest a strong seasonal trend, with higher values in the winter than in the summer, driven mostly by biomass burning emissions. The largest source of biomass burning $PM_{2.5}$ in Atlanta is prescribed burns, occurring primarily from winter to early spring.[27] This, along with the lower wintertime mixing heights, results in a strong seasonal trend in DTT activity.

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Mobile source emissions are more evenly spread throughout the year.

Normalized regressions were used to further investigate sensitivity of DTT activity to each source, and results suggest that gasoline vehicles and biomass burning drive the variation in DTT activity of WS-$PM_{2.5}$ (Table 2). The negative coefficients in the normalized DTTm regression provide further evidence that ammonium sulfate is not significantly DTT-active. Although OC is usually correlated with DTT activity, OTHER_OC (linked to secondary OC formation)[12] identified during source apportionment was not found to be a significant contributor to DTT activity, likely because it consists mostly of biogenic components derived from isoprene and terpene oxidation, which are not as DTT-active as biomass burning and mobile source WS-$PM_{2.5}$.[11]

Uncertainties in the DTTa regression arise from measurement error, interpolation of data, source apportionment, and possible incomplete source information in the model. A non-zero intercept in the DTTa regression is an indicator of model misspecification, which could arise from a missing source. The intercept is partly due to artifacts collected on the undenuded filters used for the DTT analysis and smaller contributions from other sources. Regressions between OC from the undenuded filters used for DTT analysis and OC from the denuded SEARCH data set used for source apportionment were created to investigate the presence of positive artifacts that may affect DTT activity, leading to the following relationship:

$$OC_{\text{DTT-Filter}} = 1.48 OC_{\text{SEARCH-Filter}} \qquad (R^2 = 0.72)$$

This indicates that the filters used for DTT analysis had on average ~1.5 times the concentration of OC as the denuded filters used for source apportionment, consistent with the presence of a positive OC artifact on the DTT filters.

Health Analysis. The model used daily $PM_{2.5}$ source impacts from 1998 to 2009 to produce daily DTT activity estimates for an epidemiologic analysis on acute health effects. The average estimated DTT activity over the time period was 0.44 nmol min^{-1} m^{-3}, with a standard deviation of 0.24 nmol min^{-1} m^{-3}. The IQR for estimated DTT activity and $PM_{2.5}$ were 0.213 nmol min^{-1} m^{-3} and 8.3 μg m^{-3}, respectively. Time series analyses using ED visit data showed that estimated DTT activity was positively associated with ED visits for both asthma/wheeze and congestive heart failure (Figure 3). The risk ratio for DTT activity of WS-$PM_{2.5}$ in a two-pollutant model (with DTT activity and total $PM_{2.5}$) for asthma/wheeze was 1.015 [95% confidence interval (CI) = 1.002−1.027] per IQR increase. The risk ratio for WS-$PM_{2.5}$ DTT activity with total $PM_{2.5}$ in a two-pollutant model for congestive heart failure was 1.024 (95% CI = 1.004−1.044) per IQR increase. Each risk ratio was significant at a 95% level. Estimated DTT activity was the only single pollutant measured out of several tested pollutant measures ($PM_{2.5}$, O_3, elemental carbon, and OC) that exhibited a significant link to congestive heart failure. Further, in two-pollutant models with DTT activity and $PM_{2.5}$, included in the model simultaneously, estimated DTT activity was significantly associated with asthma/wheeze and congestive heart failure, while $PM_{2.5}$ was not associated with these outcomes (Figure 3). These results are interesting in that DTT activity is likely not estimated as well as $PM_{2.5}$ is measured, and overall, they provide support that DTT activity may be a driver of health effects from $PM_{2.5}$.

The observed associations between estimated DTT activity and cardiorespiratory ED visits are consistent with the hypothesis that oxidative stress is a mechanism of particle toxicity. Furthermore, the association between estimated DTT activity and asthma/wheeze and congestive heart failure and a lack of association between $PM_{2.5}$ and these health effects in the two-pollutant models supports the interpretation that endogenous ROS-generation potential may be a property of particulate matter responsible, in part, for detrimental health effects. Thus, DTT activity may be a helpful multi-pollutant, multi-source indicator of the potential health consequences of $PM_{2.5}$ exposure, complementary to $PM_{2.5}$ mass.

DTT-active components of ambient WS-$PM_{2.5}$ potentially pose a larger risk to human health than the components that do not significantly generate ROS, which is supported by the observed association between estimated DTT activity and asthma/wheeze and congestive heart failure ED visits. Given the potential implications of these results on assessing the effectiveness of control strategies and regulations, verification of the study results and advancements in this research area are important. Much of the mass in $PM_{2.5}$ is sulfate and, in many locations, biogenically derived secondary OC,[28] which have water-soluble fractions that are not as DTT-active as other species. The links between ROS, biomass burning and vehicles as sources, and both respiratory and cardiovascular health outcomes can focus controls on those sources.

This study is the first to estimate population-level health effects of a measure of ROS activity and to use an epidemiologic approach to linking $PM_{2.5}$ ROS to health end points. Our results suggest that reducing pollutant emissions associated with WS-$PM_{2.5}$ DTT activity may measurably decrease ED visits associated with asthma and wheezing attacks and congestive heart failure. Additional studies exploring DTT/health associations in other populations or using spatially distributed measurements could also be useful for elucidating the relationship between DTT activity and health outcomes. By presenting further evidence of oxidative stress as a potential mechanism for particle toxicity, this work provides epidemiologic evidence of a biologically plausible mechanism for the observed associations of $PM_{2.5}$ concentration with cardiorespiratory effects.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.5b02967.

Results from an experiment comparing the DTT activity of a blank filter (filter 1) to filters with water-soluble sulfate (filters 2−7) showing that there is no significant difference between the blank filters and water-soluble sulfate filters (Figure S1), Pearson correlation coefficients between volume-normalized DTT (DTTv, nmol min^{-1} m^{-3}) and species concentration and mass-normalized DTT (DTTm, nmol min^{-1} μg^{-1}) and species concentration measured from June 2012 to April 2013 (Table S1), and coefficients, standard errors (in parentheses), and p values for the DTT regression with all sources (row 1) and for the reduced regression with only sources that have statistically significant coefficients used to create the historical DTT estimates for the epidemiologic study (row 3) (Table S2) (PDF)

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■ AUTHOR INFORMATION

Corresponding Author

*Telephone: 404-894-3079. E-mail: ted.russell@gatech.edu.

Notes

The authors declare no competing financial interest.

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