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PREDICTING OXIDATIVE POTENTIAL OF VEHICULAR AIR POLLUTION IN METROPOLITAN ATLANTA USING MULTIVARIATE REGRESSION MODELING

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Abstract

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Background: Oxidative potential (OP) has been considered to be the new, more novel method of evaluating air pollution exposure, because it takes into account various aspects of air pollution, such as particle size, chemical components, and meteorology. The method for evaluating oxidative potential in this study is with DTT oxidation (OP^{DTT}), using different components of air quality as predictors, such as black carbon, organic carbon, and ozone.
Aims: 1) The first aim of this study is to create a predictive model for OP^{DTT} of air pollution in vehicular traffic of metropolitan Atlanta. The second aim is to use that model to estimate oxidative potential of particle pollution in another study. The third aim is to conduct preliminary analysis of the estimated oxidative potential data on predicting health response.
Methods: All Data was provided by the Atlanta Commuter Studies (ACE-1 and ACE-2). ACE-2 was used to create the predictive model, because it contained OP^{DTT} data. The model was used to create OP^{DTT} estimates for ACE-1. Those estimates were analyzed with changes and percent changes in exhaled nitric oxide (eNO) and forced expiratory volume in 1 second (FEV1) to evaluate the associations between OP^{DTT} and corresponding health response.
Results: The final predictive model for oxidative potential is:
$OP^{DTT} = 7.76 - 2.45WSOC + 0.19WSOC^2 + 0.019BC - 0.10NOISE + 0.033WSOC*NOISE - 0.0026WSOC^2*NOISE$.
The adjusted R^2 of 0.75 was one of the highest. The predictive model's predictor p-values were all statistically significant except for BC (p=0.34). The ACE-1 OP^{DTT} estimates (0.60±0.14 nmol/minute/m^3) created from the model did not appear to have a significant association with change in eNO and FEV1.
Conclusion: In conclusion, the predictive model has reliable fit statistics. However, further analyses involving longitudinal data should be done in regards to evaluating the relationship between OP^{DTT} and health response.

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INTRODUCTION

An estimated 45 million people in the United States reside within 300 feet of a major road, airport, or railroad (EPA, 2015). The transportation sector is a major contributor to outdoor air pollution, consisting of fine particulate matter ($PM_{2.5}$) and gaseous pollutants (e.g. nitrogen dioxide (NO_2), ozone (O_3), polycyclic aromatic hydrocarbons (PAHs)) (EPA, 2015). For these reasons, daily commuters are highly susceptible to various cardiorespiratory health consequences associated with oxidative stress, the steady state level of oxidative damage in cells, tissues, or organs caused by reactive oxygen species (ROS). To evaluate traffic-related, personal air pollution exposure, we analyzed data from the panel-based Atlanta Commuters Exposure (ACE) studies, which consists of two research protocols (ACE-1 and ACE-2). ACE-1 measured in-vehicle air quality and health response related to 2-hour highway commutes in metropolitan Atlanta in order to examine the associations between pollutant mixtures during automobile commuting and corresponding oxidative stress-mediated pathways of cardiorespiratory injury (Sarnat et al., 2014). In addition to those same pollutant and health measurements, ACE-2 also included measurements targeted to characterized oxidative potential (OP) of within sampled $PM_{2.5}$.

Numerous studies in the past have evaluated air pollution exposures using traditional, single-pollutant metrics, such as total $PM_{2.5}$ mass concentration (expressed in $\mu g/m^3$), and its corresponding associations with a variety of adverse health responses (Lin et al., 2002; Delfino et al., 2004). Recently, alternative metrics have emerged as potentially more sensitive and more biologically-relevant indicators of air pollution exposure. Particulate OP, specifically, has been proposed in recent years as means of assessing exposures to $PM_{2.5}$ components directly responsible for many observed health effects attributed to air pollutant emissions (Boogaard et al., 2012; Charrier et al., 2014; Daher et al., 2014; Delfino et al., 2013; Fang et al., 2015). In contrast to traditional indicators, such as elemental carbon (EC), black carbon (BC), and organic carbon (OC), OP may represent a means of accounting for potential health risk due to particle size, chemistry, and biology (Janssen et al., 2014; Szigeti et al., 2015; Steenhof et al., 2011).Various a-cellular assays have been used to evaluate OP, including dithiothreitol (DTT), electron spin resonance (ESR), and ascorbate depletion (AA) (Janssen et al., 2014; Fang et al., 2015; Liu et al., 2014; Jean-Jacques et al., 2015). Previous studies show that different air pollution components may elicit oxidative stress, such as carbon monoxide (CO) (Piantadosi et al., 2006), PAH, O_3, NO_2, $PM_{2.5}$, EC, and OC (Risom et al., 2005; Yang et al., 2009; Delfino et al., 2013). Specific attention has been given to oxidative stress caused by bio-available transition metals such as copper (Cu), vanadium (V), chromium (Cr), nickel (Ni), cobalt (Co), and iron (Fe) (Romieu et al., 2008), or non-transition metals lead (Pb), manganese (Mn), and zinc (Zn) (Ntziachristos et al., 2007).

To evaluate the OP of traffic-related air pollution exposure and corresponding health impact, we developed three aims for this analysis. The first aim consisted of examining the empirical relationship between traffic air pollution components and OP^{DTT} through multivariate linear regression modeling (using samples collected as part of ACE-2). We used a DTT assay, which can be considered as a chemical surrogate of cellular reactants, reducing oxygen (O_2) to superoxide anion (O_2^-) and inducing oxidative stress (Kumagai et al., 2002). The antioxidant loss rate can be interpreted as the ability of $PM_{2.5}$ to transfer electrons from DTT to O_2 (Fang et al., 2015). Previous studies have conducted simple linear regression models using single air pollution components to generate OP^{DTT} estimate, typically transition metal species to PAH

(Ntziachristos et al., 2007; Charrier et al., 2014). Results from these earlier efforts support using multivariate approaches to assess OP of air pollution.

As part of the second aim, we estimated OP^{DTT} in ACE-1, where the DTT assay was not conducted, using the model results on the ACE-2 samples. As a final aim, we examined whether OP^{DTT} was predictive of corresponding acute cardiorespiratory health response in ACE-1 participants. Accurate measurements of OP can help draw connections to corresponding health response, which can be measured with sub-clinical cardiorespiratory biomarkers, such as exhaled nitric oxide (eNO) and forced expiratory volume in 1 second (FEV1) that may be indicative of oxidative stress processes. eNO is orally exhaled nitric oxide that originates from respiratory epithelium (Alving et al., 2010). FEV1 measures how much air an individual can exhale during a forced breath to test lung function (Young et al., 2007).

METHODS

Sampling methods have been described elsewhere in detail (Sarnat et al., 2014). Briefly, traffic exposure data were obtained from measurements conducted in ACE-1 and ACE-2. Both studies consisted of in-vehicle pollutant exposures and corresponding biomarker measurements prior to and following the commutes. Of the two protocols, only ACE-2 measured OP in the $PM_{2.5}$ samples using the DTT assay method, which measures the rate at which DTT is oxidized when mixed with $PM_{2.5}$. A semi-automated system was used to quantitate OP^{DTT} values (Fang et al., 2014). Ambient air pollution data, consisting of O_3, NO_2, and CO, were taken from EPA monitoring stations in the counties of metropolitan Atlanta, consisting of Fulton, DeKalb, Gwinnett, Cobb, Clayton, Coweta, Douglas, Fayette, and Henry. O_3 values were assessed on a 24-hour lag when analyzing their predictive impact on OP, since O_3 forms, secondarily, from photochemical reactions of NO_2 and sunlight. O_3, NO_2, CO, and ACE-2 air pollutant components were used to construct a robust, predictive model for OP^{DTT}. The model was then used to create OP^{DTT} estimates in ACE-1. Using those values, initial semi-quantitative analyses were done to examine associations between estimated OP^{DTT} and several relevant health endpoints. For this step, eNO and FEV1 were selected as primary biomarkers, following previous literature has shown them having strong associations with air pollution exposure (Sarnat et al., 2014; Strak et al., 2012). All statistical analyses were performed via the Statistical Analysis Software (SAS version 9.4).

OP^{DTT} Model Creation

A subset of ACE-2 data was used to create the OP^{DTT} model. Three extremely low OP^{DTT} values, sampled during highway commutes, were excluded from model creation. These values were considerably lower than other data collected on highways, side-streets, and indoors, and highly implausible, resulting from potential, unspecified sampling errors that may have occurred during the data collection process. Several major components of vehicular emissions were assessed (Table 1), initially in a Spearman's rank correlation analysis, for their correlation with OP^{DTT}. These components included $PM_{2.5}$, BC, EC, OC, water-soluble organic carbon (WSOC), particle number count (PNC), and PAH. Ambient gaseous pollutants considered were O_3, NO_2, and CO. In-vehicle noise was also considered as a physical pollutant indicator that may also be associated with oxidative stress response (Demirel et al., 2009; Samson et al., 2007). Metals taken from previous literature were considered initially; those taken from ACE-2 consisted of Cu, V, Cr, Ni, Co, Fe, Pb, Mn, and Zn. Statistically significant associations with OP^{DTT} were determined at $p\text{-value}<0.05$. All of the metals had very weak correlations with corresponding OP^{DTT} levels, indicated by the high p-values and low Spearman's rank correlation coefficients; none were subsequently considered for inclusion in the predictive model. Before running any models, we excluded CO from model buildering, since it too was weakly correlated with OP^{DTT} ($\rho = -0.079$, p-value = 0.54).

With the remaining variables, we initially conducted a stepwise regression, which yielded WSOC as the sole significant predictor of OP^{DTT}. Using stepwise backwards elimination methods as a complimentary approach, results showed that O_3, OC, EC, PAH, and WSOC all remained in the model as significant predictors.

Given these contrasting results, we assessed the variables in multivariate regression analyses without the use of SAS model building functions. We decided that total OC and WSOC should not be included simultaneously, since total OC includes WSOC. We chose WSOC over total OC due to its water solubility, and the likelihood that this OC fraction is both more biologically-available. NO_2 was ultimately excluded given the collinearity with O_3, resulting from its role within photochemical smog production processes. Between NO_2 and O_3, O_3 was shown to be a much stronger independent predictor of OP^{DTT} and has been shown more closely associated with ROS generation in previous studies (Mustafa et al., 1978; Rietjens et al., 1986). $PM_{2.5}$ was removed because BC, EC, and WSOC are more specific chemical components included with total $PM_{2.5}$, and was each correlated with those components. EC and BC are equivalent measures of elemental carbon in literature (Ho et al., 2006); of the two, EC was removed because its correlation with OP^{DTT} was much weaker than that of BC's with OP^{DTT}. With PNC, WSOC, BC, PAH, O_3, and noise as the remaining predictors, each were entered in individual plots with OP^{DTT}. Out of all the potential predictors, WSOC appeared to have the stronger, albeit non-linear relationship with OP^{DTT} (Figure 1). Although the relationship between WSOC and OP^{DTT} appears quadratic, a linear spline can be applied at WSOC = 10 μg/m^3 to evaluate the variables with linear functions at different intervals. A linear spline and quadratic trend were applied to WSOC in separate analyses, both yielding improved model fit diagnostics; WSOCspline and $WSOC^2$ variables were created to evaluate WSOC with a linear spline (Figure 7) and a quadratic trend (Figure 8), respectively. Two-way interaction terms were created between each of the variables. We evaluated the models using the adjusted R^2 values, as well as the statistical significance of the individual predictors in the model, as a collective measure of the model fit. The final model with the best combination of high adjusted R^2 value and number of significant predictors included WSOC, $WSOC^2$, noise, BC, an interaction term for WSOC and noise, and an interaction term for $WSOC^2$ and noise.

Analysis of Model OP^{DTT} Estimates on Health Response

Using the final predictive model, ACE1 predictor values were inputted to create OP^{DTT} estimates for the ACE1 dataset. Descriptive analysis of the predicted OP^{DTT} values showed a low extreme outlier (0.0088 nmol/minute/m^3) and high extreme outlier (6.78 nmol/minute/m^3). These extreme values were highly unlikely to be accurate and were, thus, excluded from further analysis. Table 4 shows the descriptive statistics of the remaining OP^{DTT} estimates. These estimates were categorized into three groups, based on their 33.33% (at 0.51 nmol/minute/m^3) and 66.67% (at 0.64 nmol/minute/m^3) cut points. Box-and-whisker plots were created to graphically display differences in absolute eNO levels, percent change in eNO levels, absolute change in FEV1 change, and percent change in FEV1, by the three OP^{DTT} categories. Category one consists of OP^{DTT} estimates less than 0.51 nmol/minute/m^3. Category two consists of OP^{DTT} estimates between 0.51 and 0.64 nmol/minute/m^3. Category three consists of OP^{DTT} estimates greater than 0.64 nmol/minute/m^3. Regarding changes in the eNO and FEV1, the values represent the difference between measurements taken immediately before a given commute compared to measurements immediately following the commute.

RESULTS

Using ACE-2 data, we created various regression models and compared their adjusted R^2 values and individual predictor p-values. Weighing these factors, we selected a model with the best combination of fit statistics and statistical significance of predictors. After selecting a predictive model, we created OP^{DTT} estimates by inputting values of the corresponding ACE-1 pollutant variables into the model. After categorizing ACE-1 OP^{DTT} values into tertiles, we compared the changes and percent changes in eNO and FEV1 before and right after the commutes to evaluate the predictive ability of OP^{DTT} on health response.

OP^{DTT} Model Justification

The final predictive model in table 2, also showing its adjusted R^2 value and individual predictor p-values, was:

$OP^{DTT} = 7.76 - 2.45WSOC + 0.19WSOC^2 + 0.019BC - 0.10NOISE + 0.033WSOC*NOISE - 0.0026WSOC^2*NOISE$.

Although the model is difficult to interpret given the quadratic and interaction terms, there are several significant relationships. The results show that there was a significant interaction between WSOC and NOISE in predicting corresponding variability in OP^{DTT} ($p=0.0015$ for WSOC*NOISE and $p=0.0003$ for $WSOC^2$*NOISE). The adjusted R^2 of 0.75 was one of the highest. Table 2 shows that all of its predictors, with the exception of BC ($p=0.34$), were statistically significant. Omitting BC however, led to a decrease in the overall model fit. The adjusted R^2 without BC, for example, dropped to 0.62. The sample size of 38 for the final model is fairly low, but it is not considerably lower than other models tested.

When adding other variables such as BC and noise in a model with $WSOC^2$, a higher number of predictors was statistically significant compared to adding those same variables in a model with WSOCspline; the adjusted R^2 values comparing models with $WSOC^2$ and those with

WSOCspline, however, were fairly close. Other predictors were also analyzed for nonlinear relationships OP^{DTT}, shown in Figures 2 to 6, although there did not appear to be any clear nonlinear relationships beyond those observed for WSOC and OP^{DTT}. Importantly, the plots clearly exhibit non-linear associations between WSOC and OP^{DTT}, justifying our use of quadratic terms in the final model (Figures 7 and 8).

PAH, O_3, and PNC were not included in the final model because including them in any form, whether as individual predictors or interaction terms with each other or with BC, WSOC, and/or noise, did not improve the adjusted R^2 and yielded mostly predictors that were not statistically significant. Any combination with those three variables in the model did not appear beneficial to the model from a statistical standpoint.

Preliminary Analysis of Predicted OP^{DTT} Estimates on Health Response

The OP^{DTT} estimates created for ACE-1 using the final predictive model ranged from 0.35 to 1.06 nmol/minute/m^3. The average OP^{DTT} was 0.60±0.14 nmol/minute/m^3. Comparing eNO change, eNO percent change, FEV1 change, and FEV1 percent change over the three OP^{DTT} tertile strata (strata 1 average=0.47±0.048; strata 2 average=0.58±0.036; strata 3 average=0.75±0.11), ANOVA tests indicated no statistical difference between the tertile groups. Separate analyses were done comparing eNO and FEV1 over dichotomized OP^{DTT} groups, also yielded statistically insignificant differences, based on two-tailed t-tests.

DISCUSSION

The final predictive model for OP^{DTT} was selected based on adjusted R^2 values and individual predictor p-values. Throughout the model selection process, there were few combinations of variables that produced higher adjusted R^2 values than those selected for inclusion in the final, but most of the parameter estimate p-values were also very high, indicating very little association between the parameters and corresponding OP^{DTT}. Although the final model included the best combination of independent predictors, it is difficult to interpret the parameter estimates, given the quadratic terms in the hierarchical model. Using a linear spline instead would have afforded greater interpretability because there would not be any downward trends to interpret at different predictor intervals; the linear spline model would show varying, consistent linear trends at the different spline cut points. However, using a spline WSOC predictor yielded a greater number of pollutant predictor estimates that are not statistically significant. Additionally, we did not observe any additional improvement in the model fit diagnostics, in particular the adjusted R^2 value, when using the splines. Regarding the final predictive model used to create estimated OP^{DTT} values for ACE-1, not every possible modeling method was used. A linear function could have been applied from WSOC = 3 to 10 $\mu g/m^3$, followed by a quadratic spline from WSOC = 10 to 16 $\mu g/m^3$.

Regarding interpretation of the parameter estimates of the final predictive model, it would have been preferable if the results yielded only positive values for all the variables. However, the current data did not include WSOC values within a 3 to 4 $\mu g/m^3$ range, leading to the creation of parabolic association. If ACE-2 had WSOC values within a 3 to 4 $\mu g/m^3$ range, we expect corresponding OP^{DTT} estimates to yield a flatter initial trend for the quadratic function, as opposed to a parabola. However, without data points for those values, regression analysis fit a trend that led to the initial downward trend. The parameter estimate of NOISE was also negative to compensate for the negative curve at WSOC within the 3 to 4 $\mu g/m^3$ range; the curve created for WSOC influenced the NOISE parameter estimate, making it also negative at certain intervals. With a greater sample size of measurements, presumably covering a wider range of WSOC values, the quadratic relationship may yield all positive parameter estimates, as we expected *a priori*. A greater number of observations may be also able to influence the final variables that are kept in the model, along with their parameter estimates. It would have allowed us to evaluate more predictors and interaction terms without concern over model oversaturation. Other air pollutants measured such as PAH might have been in the model if a larger sample size was available for analysis (Ntziachristos et al., 2007).

Previous studies have shown that O_3 and CO are strong predictors of oxidative stress (Kelly 2003), which suggests that they should correspondingly be associated with OP^{DTT}. These variables, however, were not directly measured in the ACE studies. Ambient O_3 and CO data were retrieved from the EPA air monitoring sites, which may not be representative of the personal exposure levels within the vehicles. Future personal air pollution exposure studies conducted in vehicle traffic should target the measurements of gaseous pollutants in addition to particles in order to evaluate the degree to which O_3, NO_2, and CO contribute to OP formation and exposures. Our results, which show a lack of association between OP and any of the measured transition metals, are not consistent with reported findings showing Mn, Fe, Cu, and Zn to be strongly associated with OP (Ntziachristos et al., 2007; Fang et al., 2015; Hellack et al., 2015). In these studies, transition metals were only evaluated in a simple linear regression setting. Future research should look to include the metals within multivariate linear regression models with OP to assess potential interactions between different metals.

This study was conducted as preliminary analysis of in-vehicle OP^{DTT} as a component of vehicular air pollution. Further analysis should be done for assessing the relationship between OP^{DTT} and health response. The box plots in Figures 3 to 6 showed no discernable or statistically significant differences among the OP^{DTT} categories, which only displayed changes in the biomarkers measured immediately before and following the 2-hour commute protocol. Data are currently available for eNO and FEV1 1, 2, and 3 hours post commute as well, which may include more biologically-relevant exposure windows to accurately reflect true exposure-response relationships.

CONCLUSION

In conclusion, the final predictive model for OP^{DTT} using ACE-2 data demonstrated reliable model fit statistics expressed as a strong adjusted R^2 value. The p-values for the individual predictors, with the exception of BC, indicate significant associations with OP^{DTT}. The preliminary testing of the model done with using the predicted OP^{DTT} estimates for ACE-1 to evaluate eNO and FEV1 change revealed no significant relationship. However, further research should be conducted to account for the biomarker concentrations at various time points after the commutes. Finally, the air quality data used was all from vehicular traffic. Future studies that involve creating a predictive model for oxidative potential can be done for other settings, such as occupational ones. The goal should be to make a generalizable model for the general population while using directly measured personal air pollution exposure as opposed to modeling personal exposure using ambient air quality data from more distant ambient site monitors.

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Table 1. Correlation analysis between OP^{DTT} and all the potential predictors.

	OP^{DTT}	
In-Vehicle Pollutant	**Spearman's Rank Correlation Coefficient (ρ)**	**P-Value**
OC (µg/m^3)	0.34	0.001
Particle Number Count (n/cm^3)	0.47	0.0001
EC (µg/cm^3)	0.29	0.13
WSOC (µg/m^3)	0.59	<.0001
PM (µg/m^3)	0.47	0.0001
BC (µg/m^3)	0.49	<.0001
NOISE (dB)	0.31	0.02
PAH (ng/m^3)	0.41	0.006
Gases from EPA Monitoring Sites		
O_3 (ppm)	0.23	0.07
NO_2 (ppb)	0.18	0.16
CO (ppm)	-0.01	0.94
Metals (ng/m^3)		
Chromium	-0.08	0.61
Copper	0.09	0.48
Iron	-0.002	0.99
Manganese	-0.02	0.88
Nickel	-0.03	0.86
Lead	-0.03	0.85
Vanadium	0.11	0.38
Zinc	0.09	0.55
Cobalt	-0.20	0.19

Table 2. Statistics of the Final Predictive Model.

Final Model	Variable	Parameter Estimate	P-Value	Confidence Interval
Adjusted R^2 = 0.75 n=38	Intercept	7.76	0.0017	(3.17, 12.35)
	WSOC	-2.45	0.0005	(-3.74, -1.16)
	$WSOC^2$	0.19	0.0001	(0.10, 0.27)
	NOISE	-0.10	0.0074	(-0.17, -0.029)
	BC	0.019	0.34	(-0.021, 0.058)
	WSOC*NOISE	0.033	0.0015	(0.014, 0.054)
	$WSOC^2$*NOISE	-0.0026	0.0003	(-0.0039, -0.0013)

Table 3. Descriptive statistics for the ACE-1 predicted OP^{DTT} estimates attained from the ACE-2 final predictive model.

Predicted OP^{DTT} Estimates (n=59)	
Mean ± Standard Deviation	0.60 ± 0.14
Minimum	0.35
Maximum	1.06

Figure 1. Graph of OP^{DTT} and WSOC

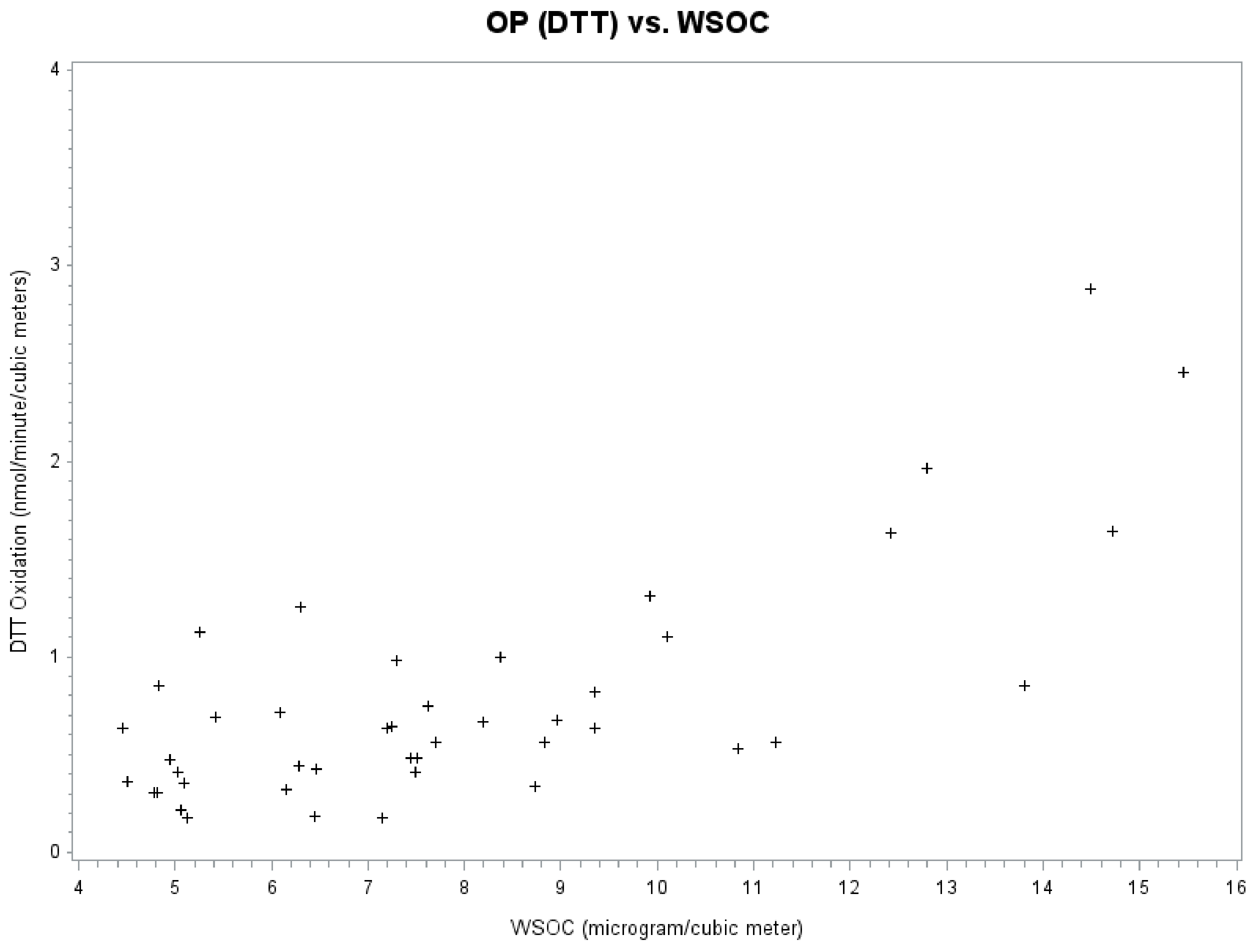

Figure 2. Graph of OP^{DTT} and BC

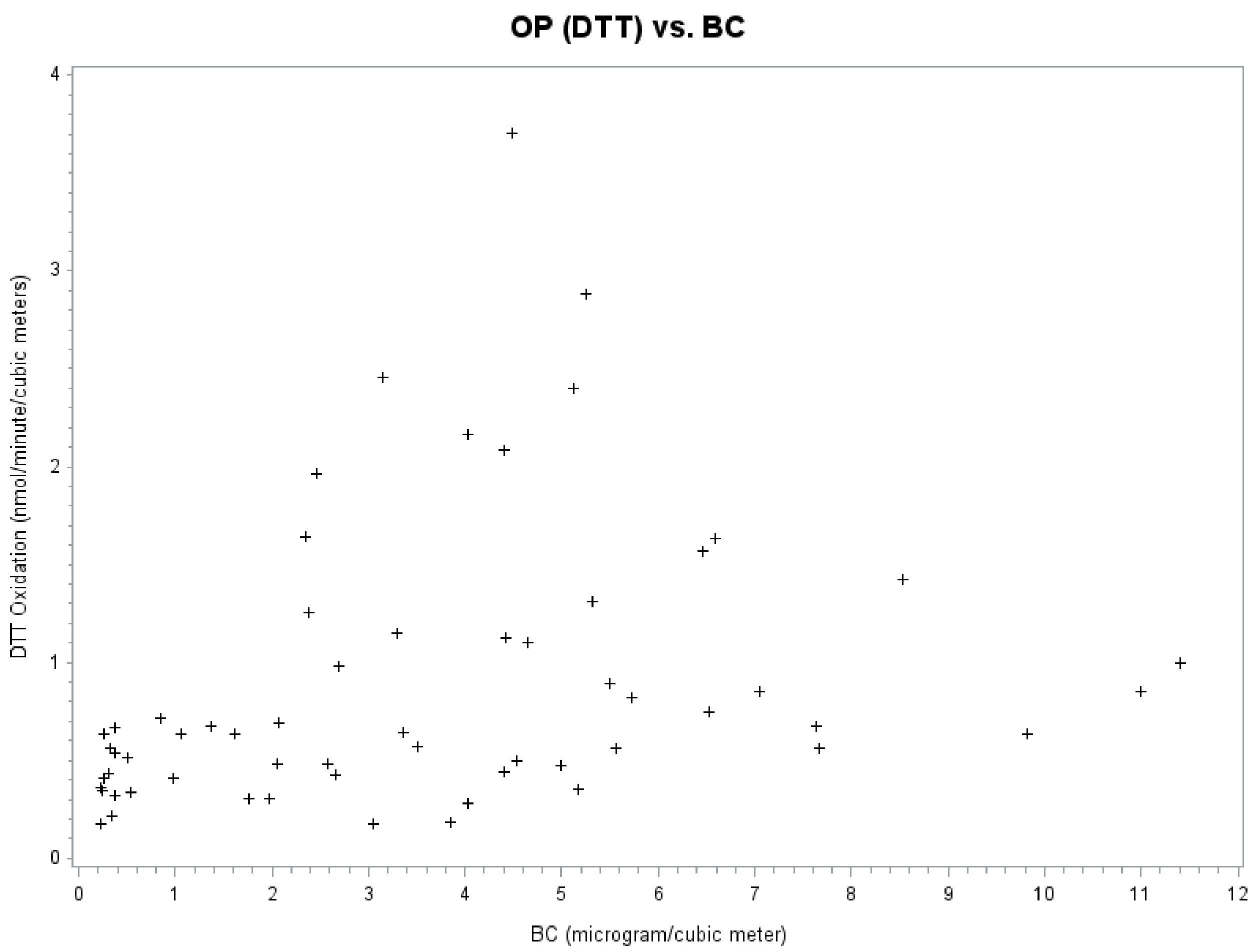

Figure 3. Graph of OP^{DTT} and Noise

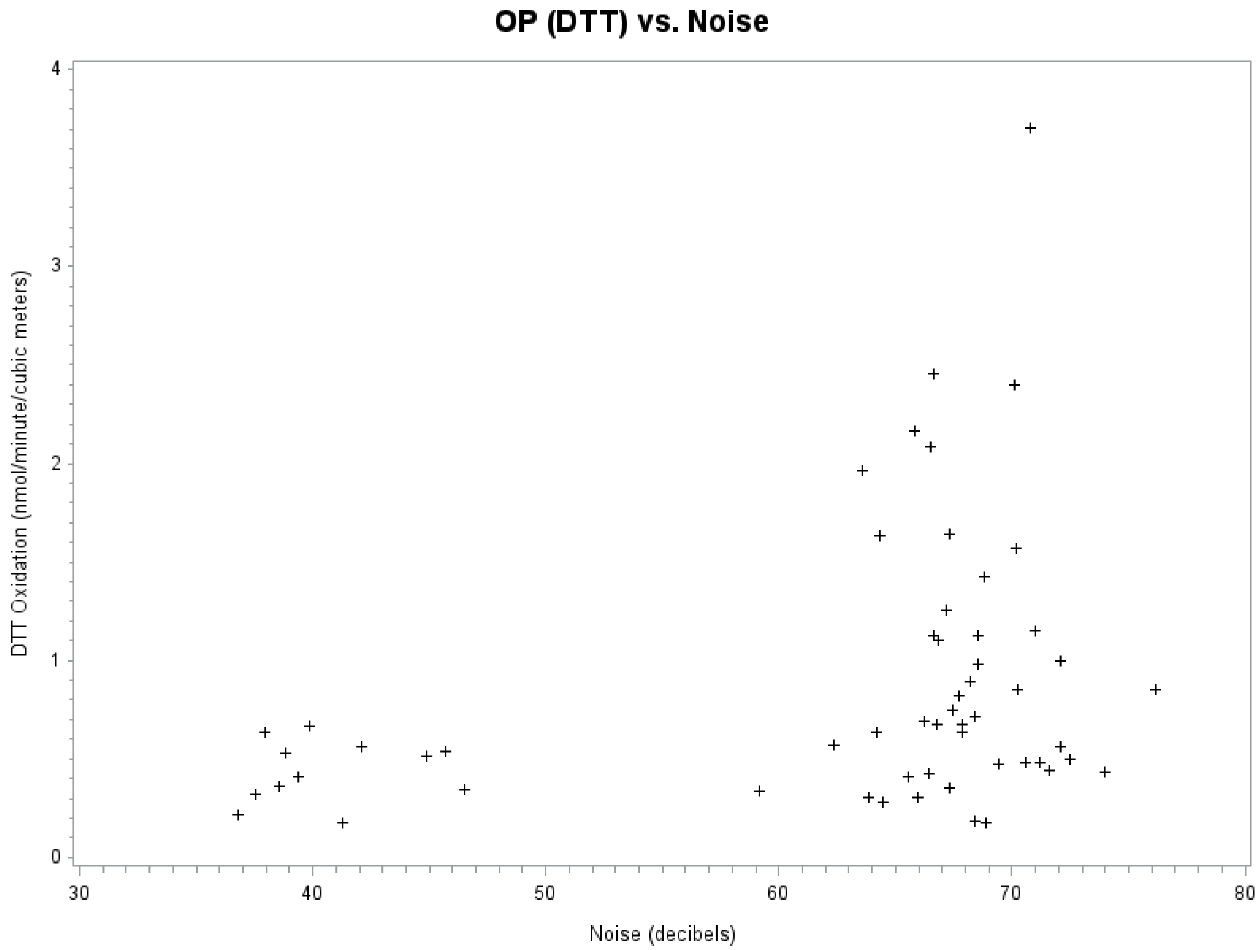

Figure 4. Graph of OP^{DTT} and PAH

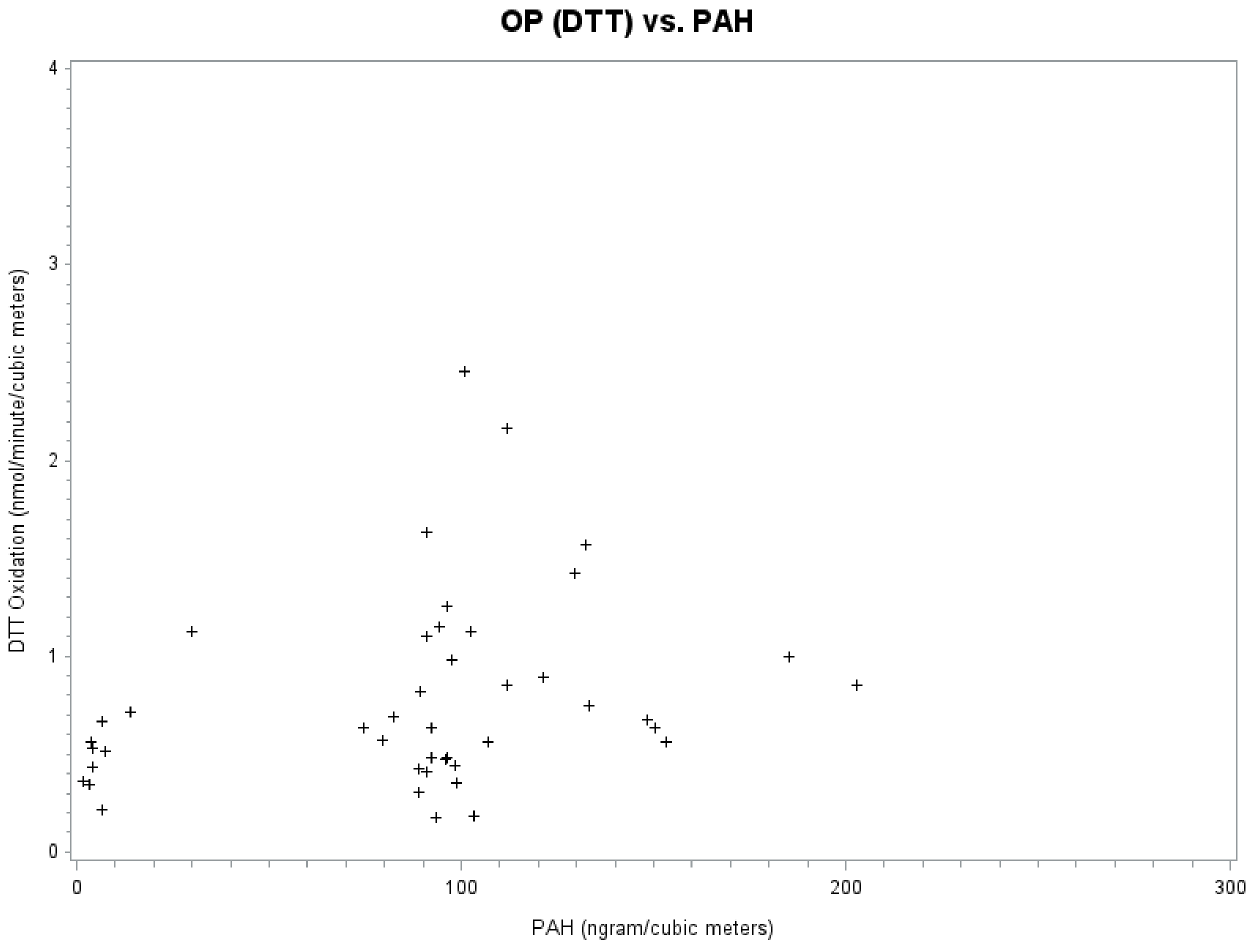

Figure 5. Graph of OP^{DTT} and PNC

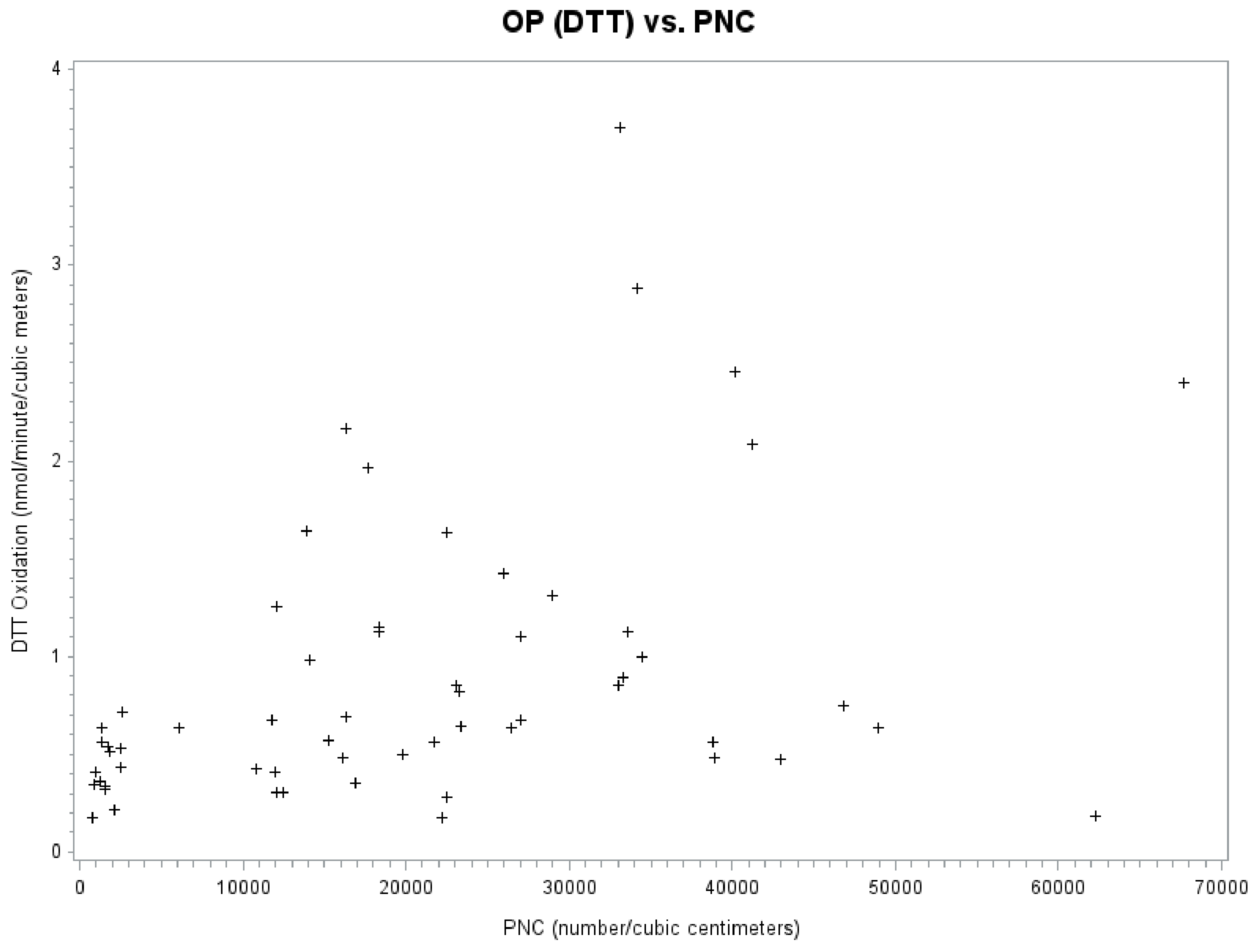

Figure 6. Graph of OP^{DTT} and O_3

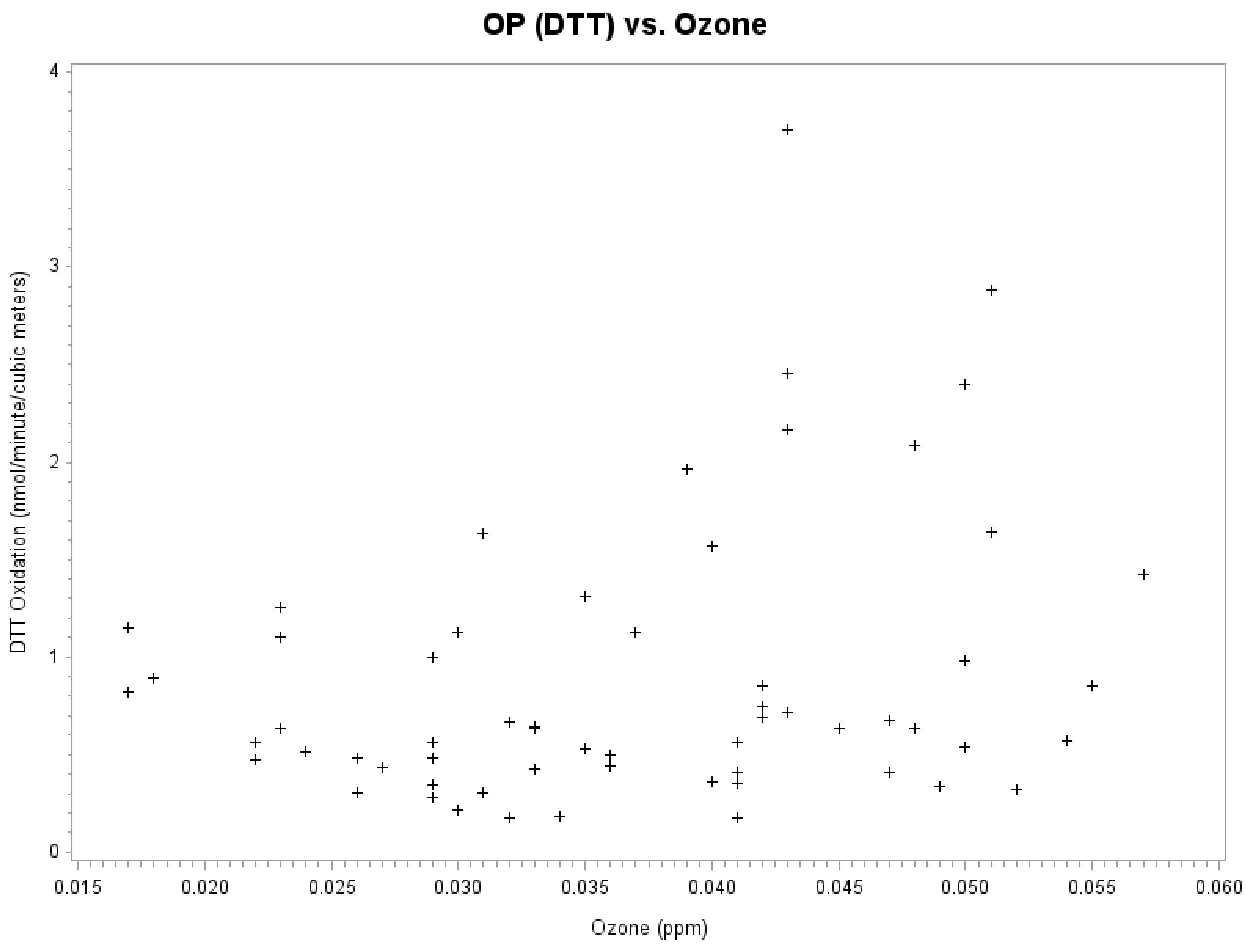

Figure 7. Graph of OP^{DTT} and WSOC Fit with Linear Spline

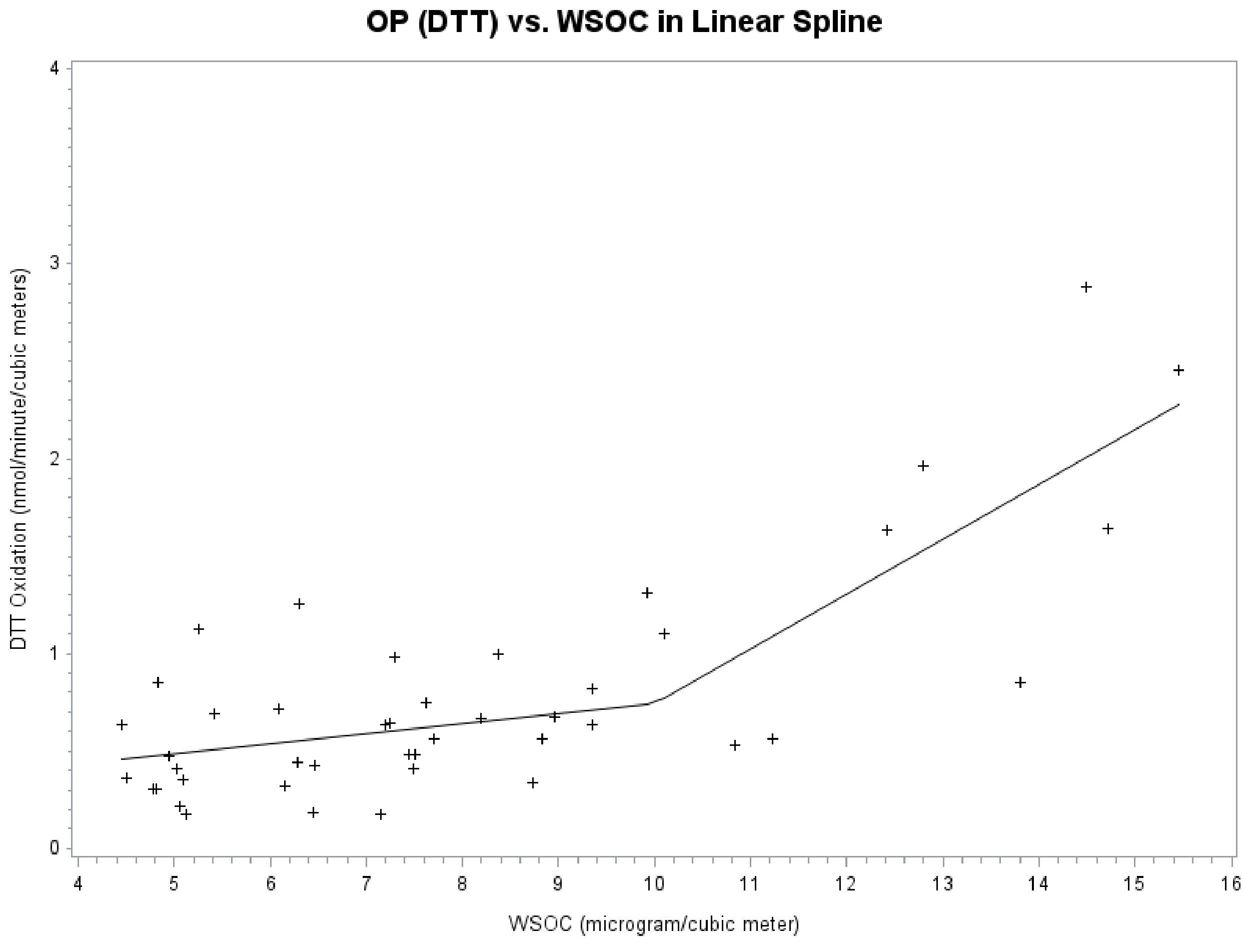

Figure 8. Graph of OP^{DTT} and WSOC Fit with Quadratic Trend

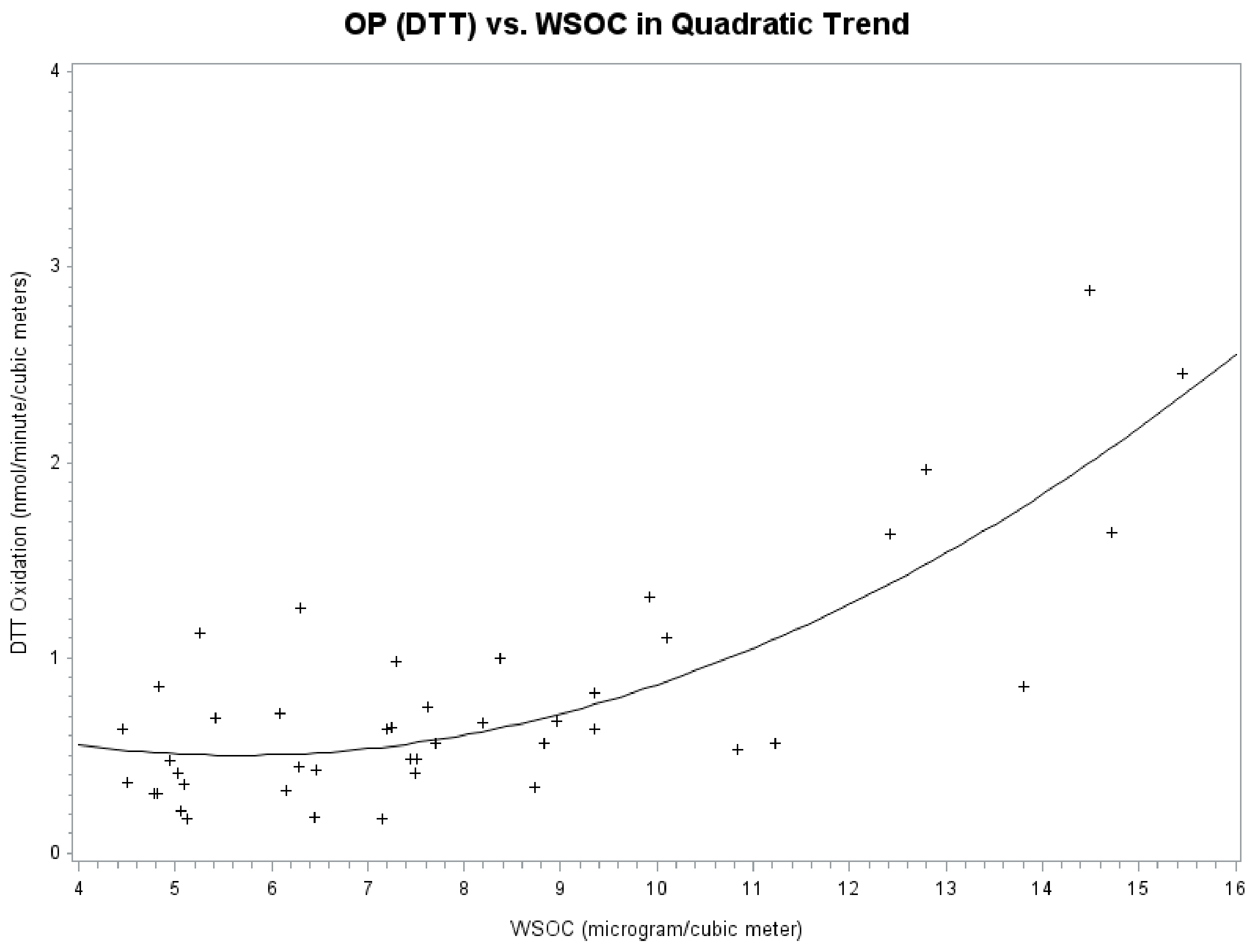

Figure 9. Box Plots of Change in eNO Stratified by OP^{DTT} Tertile Categories

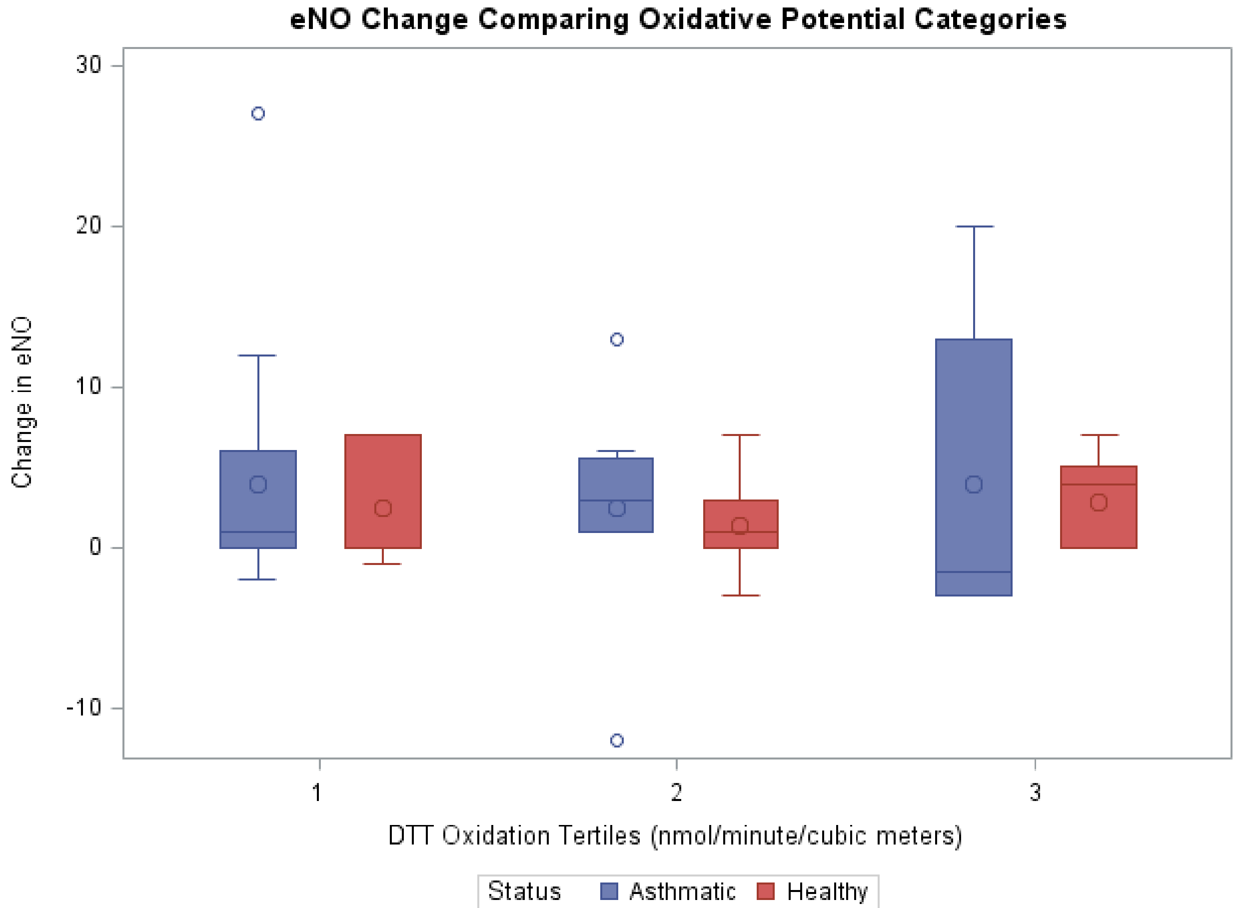

Figure 10. Box Plots of Percent Change in eNO Stratified by OP^{DTT} Tertile Categories

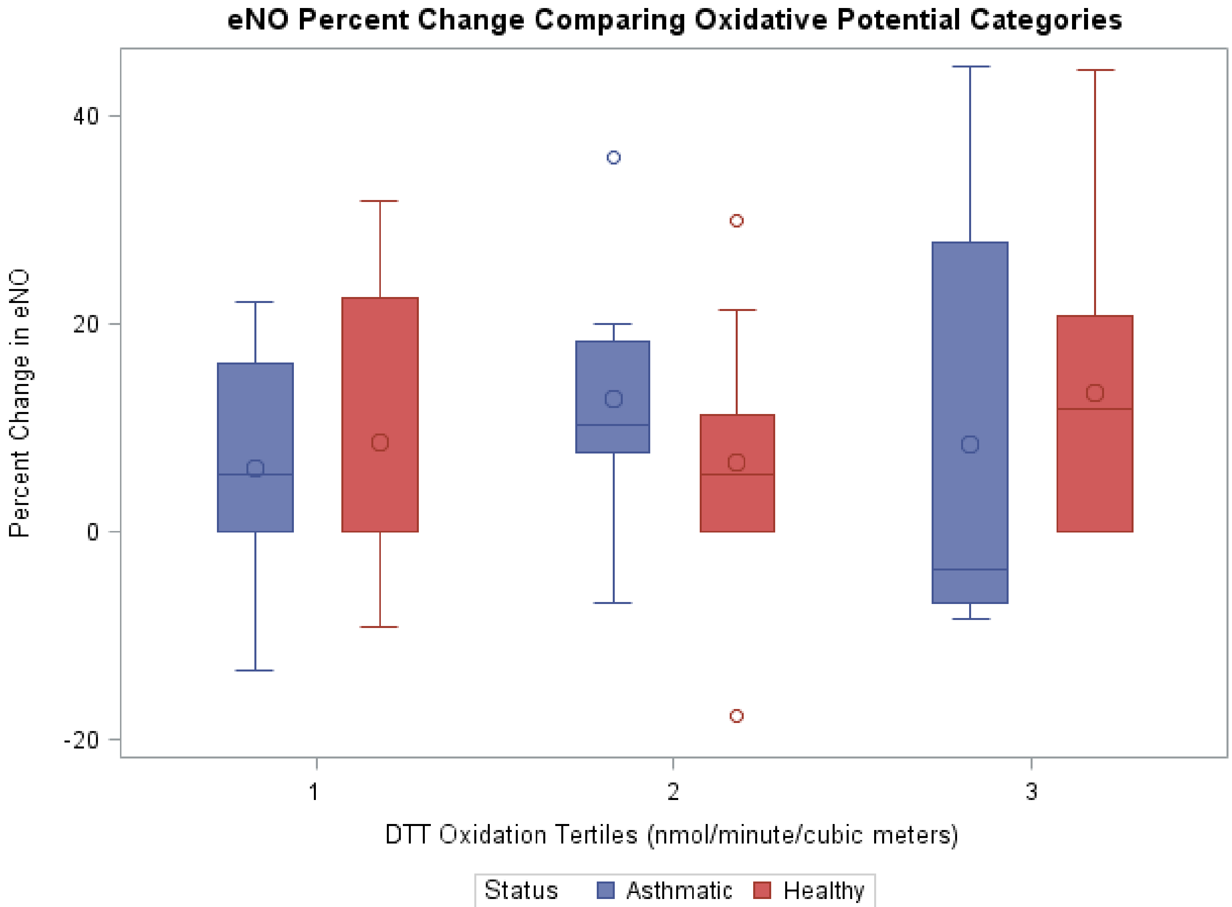

Figure 11. Box Plots of Change in FEV1 Stratified by OP^{DTT} Tertile Categories

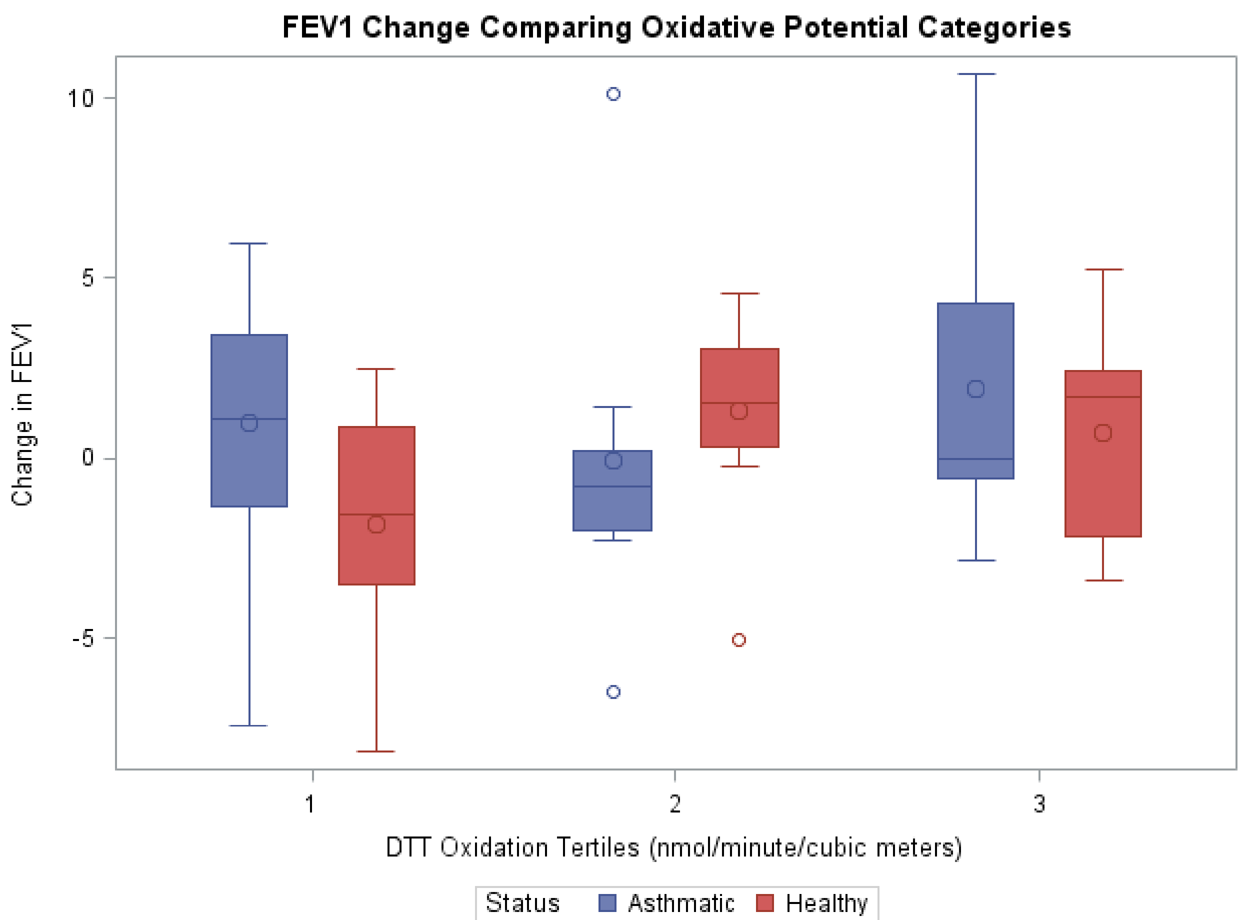

Figure 12. Box Plots of Percent Change in FEV1 Stratified by OP^{DTT} Tertile Categories