

Final Technical Report

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Center Name: Southern California Particle Center and Supersite (SCPCS)

Center Director: John R. Froines

Title: The Chemical Toxicology of Particulate Matter

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RFA: Airborne Particulate Matter (PM) Centers (1999)

Research Category: Particulate Matter

Topic A: Studies Emphasizing Investigation of the Biological Mechanisms of Particulate Matter (PM) Effects in Relation to PM Physical and Chemical Characteristics

Objective(s) of the Research Project: The research on chemical toxicology aimed at testing the hypothesis that PM contains reactive chemical species and they, either separately or as a mixture, are responsible for the toxicological phenomena associated with PM. The objective of this project was to characterize PM samples for the chemical properties that likely contribute to their adverse health effects. As the most general mechanism, the chemical basis for the induction of oxidative stress was the focus of the work. Reactive chemical species in PM can be organic and inorganic, and may act through several possible chemical reactions with biological substrates. We focused on two general mechanisms: redox and electrophilic reactions. Redox reactions involve the catalytic reduction of oxygen to reactive oxygen species by components of PM with electrons from biological sources. In the electrophilic reactions, a reactive function in PM reacts with nucleophilic functions in biological systems to form covalent bonds. These bonds are irreversible so that the affected biological molecule is destroyed. The thiol group is a likely target of the two reactions as this group participates in both redox and covalent bond forming reactions. Thiols serve key functions in proteins such as enzymes, transporters and receptors so their modification can result in substantial disruption of cell biochemistry. By characterizing and quantitatively determining the reactivity in a given PM sample with respect to these chemical properties, we intended to be able to predict its potential toxicity.

To test our hypotheses, we developed and continued to develop quantitative assay procedures that can be applied to PM samples to assess their chemical reactivity and potential toxicity as predicted by the considerations above. The development of the assays and their applications are described in the findings section below.

Summary of Findings:

Analytical Procedures

Gas Chromatography/Mass Spectrometry (GC/MS) Assay for Quinones (Cho, et al., 2004). The role of quinones in cellular toxicity has long been recognized and their contribution to the toxicity of air pollution was also recognized (Dellinger, et al., 2001; Squadrito, et al., 2001). In an effort to determine the quantities of quinones in PM, a GC/MS based procedure was developed which provided the first quantitative assessment of quinones in ambient air masses. The high sensitivity of the procedure allowed quantitative analysis of small sample sizes, and in the initial report, was applied to a National Institute of Standards and Technology (NIST) standard, diesel exhaust particles and ambient air samples collected in the Los Angeles Basin (LAB). The findings of that work are reported in more detail below. A recent report by another group has described a liquid chromatography/mass spectrometry procedure to analyze the same quinones. Their procedure requires larger samples (Valavanidis et al., 2006) but when applied to ambient samples, the results were consistent with ours.

Dithiothreitol Assay for Redox Activity (Cho, et al., 2005). The redox properties of airborne particulate matter have been recognized for some time (Sagai, et al., 1993; Kumagai, et al., 1997; Dellinger, et al., 2001; Squadrito, et al., 2001) and a role for redox activity in the cellular effects of PM has been proposed (Kumagai, et al., 1997; Li, et al., 2000; Squadrito, et al., 2001; Hirano, et al., 2003; Li, et al., 2004; Valavanidis, et al., 2005). Our dithiothreitol (DTT) assay was developed to assess the redox properties of a given PM sample in a quantitative manner and is based on the ability of the sample to catalyze the electron transfer between DTT and oxygen. The procedure determines the rate at which DTT is consumed, due to the oxidation of DTT as it transfers electrons to oxygen in a process catalyzed by the test sample. We have shown that redox active quinones such as the naphthoquinones and phenanthroquinone, diesel exhaust particles and the particulate and volatile fractions of ambient air exhibit this property. When applied to ambient air samples, the rate of DTT consumption is a function of the sample as a whole, so that an integrated metric of exposure to this redox activity is obtained. The specific compound(s) that catalyze the reaction are not determined by this assay. In its initial evaluation, ambient air samples were collected in the LAB with a VACES concentrator and a liquid impactor and the samples assayed with this procedure.

Ascorbate-Salicylate Based Assay for Metal Based Redox Activity. The apparent selectivity of the DTT assay for organic compound based redox activity and the need for assessment of metal based redox properties led us to modify an assay developed by Kelly measuring the consumption of ascorbic acid (Mudway, et al., 2004). By including salicylate in the reaction mixture, the transition metals in the test sample catalyze the Fenton reaction, in which hydrogen peroxide generated by the reduction of oxygen is converted to hydroxyl radical. This highly reactive oxygen species will rapidly hydroxylate salicylate to isomers of dihydroxybenzoic acid (DHBA) which is readily measured by high-performance liquid chromatography (HPLC) and an electrochemical detector. The use of this latter reaction to determine hydrogen peroxide and cellular oxidative stress is well established (e.g., Coudray and Favier, 2000; Liu, et al., 2003). We have applied it to ambient PM samples, collected with Teflon filters or with the biosampler and found measurable activities. This activity, expressed as nmoles of total DHBA formed per minute per microgram of sample, is completely blocked by the metal chelator, diethylene triamine pentaacetic acid (DTPA), consistent with the participation of metal ions in the reaction. The assay is currently being examined as a complementary analysis to the DTT assay that will

detect metal based redox activity, and has been applied in a comparative study of samples collected on the 110 freeway and on samples collected in the Caldecott Tunnel (see below).

GAPDH Based Assay for Electrophilic Properties in Samples. Electrophilic reactions represent an important chemical mechanism, in addition to generation of reactive oxygen species, by which PM and its reactive constituents could cause adverse cellular effects. However the electrophilic properties of airborne pollution have not yet been characterized. During our related work on the mechanisms of quinone toxicity in yeast, the thiol based enzyme, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was found to be a key cellular target (Rodriguez, et al., 2004). Our subsequent studies revealed two mechanisms of GAPDH inhibition that can be carried out by various quinones. The first mechanism is dependent on reactive oxygen species (ROS) generation. The second mechanism is oxygen independent and involves covalent interactions between the catalytic thiol in GAPDH and electrophilic quinones (Rodriguez, et al., 2005). Electrophilic inactivation is characterized by two parameters: a rate constant for inactivation ($k_{\text{inactivation}}$) and an affinity parameter, K_i , which reflects the concentration of inhibitor needed to decrease the overall rate by 50% under a standard set of conditions. This assay has been applied to two test quinones that are present in atmospheric samples, and a diesel exhaust extract (see below). Currently, a protocol for the assay that is applicable to samples from different ambient collection devices is being developed.

Application of Assays to Ambient PM Samples

As part of overall Southern California Particle Center and Supersite (SCPCS) activities, a variety of ambient PM samples were collected at various locations within the LAB. Selected samples were then transferred to our laboratory to be tested in our assays for quinones and chemical reactivity. The SCPCS conducted several sampling campaigns at sites heavily influenced by traffic, including freeway and tunnel sites. Seasonal differences in PM characteristics were also explored.

Application of DTT Assay to LAB Ambient Air Samples

Ambient air samples were collected with a VACES concentrator and a liquid impactor and DTT activity was assessed. Physical correlates of activity were evaluated to identify chemical species that might be involved and to compare the coarse (C), fine (F), and ultrafine (UF) fractions for their redox activity (Cho, et al., 2005). The study found:

1. The DTT-based redox activity did not correlate with any of the inorganic species measured, including metals (elemental and ions), sulfate and nitrate. The highest DTT activity was found in the UF fraction, which has the highest content of organic species.
2. The activities correlated with organic carbon ($r^2=0.53$), elemental carbon ($r^2 = 0.79$) and polycyclic aromatic hydrocarbon (PAH) levels in the PM sample. The highest correlation was with benzo[ghi]pyrene (BgP) ($r^2 = 0.82$). BgP is regarded as a marker for vehicular combustion emissions, especially from gasoline motor vehicles (Miguel, et al., 1998).

3. The DTT activity in ambient samples was found to correlate with the ability of a given PM sample to induce the stress protein, hemeoxygenase-1 in tissue culture cells (Li, et al., 2003), so that the UF fraction was associated with chemical and biological activity. These findings support the hypotheses of the project that toxic properties of PM may be attributable, in part, to the organic carbon compounds with which the assay findings were most highly correlated.
4. The observed redox activity was normalized to mass to enable comparison of the potency of samples from each site, and normalized to volume of air to enable comparison of exposure to reactive chemical species. When normalized to mass, Claremont, an area east and downwind from central Los Angeles, exhibited the highest activity. Claremont is considered a “receptor” site because it receives an air mass that has been subjected to photochemical reactions during its movement across the basin. Oxidation by photochemical reactions could account for its higher activity, since redox active compounds such as quinones are photo-oxidation products.
5. When the activity is normalized to volume of air, the area adjacent to the University of Southern California (USC) campus had the highest exposure levels, due to the high particle count.

Redox Properties of PM From the 110 Freeway

The 110 Freeway was used predominantly by small vehicle traffic in the downtown area of Los Angeles. Two collection campaigns were conducted, one during the summer of 2004 and a second in the winter of 2005. The redox activity of both sets of samples was determined by the DTT-based assay, and the winter samples were additionally assayed by both the DTT and ascorbate-dihydroxybenzoate assay procedures, in a pilot application of this assay to ambient PM samples. The results are summarized in Table 1, with activity expressed per mass in the first two columns and DTT activity expressed per volume of air in the last column.

Table 1. Redox Activity of PM (Summer 2004 and Winter 2005)

	DTT nmol*min ⁻¹ *mg ⁻¹	DHBA nmol*min ⁻¹ *mg ⁻¹	PM Mass mg*m ⁻³	DTT Activity nmol*min ⁻¹ *m ⁻³
110 Summer C	0.008	ND	123.300	0.9864
110 Summer F	0.031	ND	33.900	1.0509
110 Summer UF	0.056	ND	9.600	0.5376
110 Winter C	0.017	0.024	10.600	0.1802
110 Winter F	0.025	0.018	13.000	0.325
110 Winter UF	0.042	0.045	14.700	0.6174

ND: Not Determined

C: Coarse; F: Fine; UF: Ultrafine

The DTT activity per mass is the highest in the UF fraction in both the summer and winter freeway samples. This is consistent with samples from non-roadway source and receptor sites in

the LAB (above). When the 110 Freeway samples were compared for exposure to redox activity per volume, the C and F contributed the greatest exposure concern in the summer whereas the UF contributed the highest exposure levels in the winter. This reflects a shift in the size distribution of PM with season.

DHBA formation was also the highest in the UF fraction, but activity was also clearly present in C samples, consistent with higher metal concentrations in C. It is possible that DHBA formation by UFs may be reflective of hydrogen peroxide levels rather than metal content.

Caldecott Tunnel Study

The assays for chemical reactivity were applied in a study of the air mass in the Caldecott Tunnel, located in Northern California. This tunnel provides access to an enclosed environment of vehicular traffic. The Caldecott has two bores, one of which is used by gasoline and diesel vehicles (CB1, mixed) and a second which is used exclusively by gasoline vehicles (CB2, gasoline). Liquid impinger samples were collected in the summer and winter seasons and assayed for redox activities. The concentrations of PM in the tunnels were very high, roughly an order of magnitude greater than at the 110 Freeway. Tunnel concentrations of C were higher in the summer, while F and UF levels in the tunnel remained more consistent between seasons.

Subsequent to the collections, the glass containers used for the winter samples were found to produce a redox artifact of ascorbate consumption because of variable but low levels of trace metals that contribute to the blank. Assay findings are presented in Table 2 for summer 2004 samples.

Table 2. Redox Properties of PM From the Caldecott Tunnel (Summer 2004)

	DTT nmol*min ⁻¹ *mg ⁻¹	Ascorbate nmol*min ⁻¹ *mg ⁻¹	DHBA Total nmol*min ⁻¹ *mg ⁻¹	PM Mass mg*m ⁻³	DTT Activity nmol*min ⁻¹ *m ⁻³
Bore 1 Coarse	0.019	0.051	0.0050	988.000	18.772
Bore 1 Fine	0.068	0.041	0.0039	644.000	43.792
Bore 1 UF	0.111	0.117	0.0002	230.000	25.530
Bore 2 Coarse	0.032	0.061	0.0052	581.000	18.592
Bore 2 Fine	0.075	0.081	0.0070	477.000	35.775
Bore 2 UF	0.172	0.115	0.0041	130.000	22.360

UF: Ultrafine

The UF possessed higher DTT activity relative to the F and C, consistent with the findings at the 110 Freeway and non-roadway sites. Interestingly, we found somewhat higher DTT activity in the UFs collected in bore 2, which is limited to light duty traffic (primarily gasoline), relative to samples from bore 1. We compared DTT results to chemical composition analyses of the samples; neither elemental nor organic carbon levels correlated well with DTT activity in this set of samples.

When expressed per mass, the activities for the 110 and Caldecott samples were comparable, but the large mass found in the tunnels resulted in much greater total redox activity in the air mass.

Tunnel commuters may have high overall exposure to potentially toxic materials during tunnel transit.

Activity in the ascorbate assay was essentially in agreement with DTT results in the summer tunnel samples. We consider that these assays may provide some redundancy in measuring the levels of redox activity. However, it is important to note that the results of the two assays have not been congruent in all sample sets tested. The DHBA data suggest an inverse association with particle size in the tunnel bore bearing mixed heavy and light duty traffic, consistent with the greater level of metals expected in C vs. UF. We found somewhat higher overall DHBA production in the samples from the gasoline only tunnel, although the association with greater particle size was not as pronounced.

Quinone Concentrations in Particle and Volatile Fractions

Caldecott Tunnel. Filter and volatile air samples were collected on Teflon filters (PM_{2.5}) with XAD resin beds below the filters, during the 2004 Caldecott campaign. The filters and XAD beds were extracted with dichloromethane and the concentrations of four quinones determined by GC/MS (Cho, et al., 2004) (Figure 1). The results from this analysis show that all four of the quinones were present with high levels of 9,10-AQ in the PM_{2.5} fraction and high levels of 1,4-NQ in the volatile, XAD, fraction. No clear seasonal pattern was apparent. The substantial day to day variability is shown by the standard deviations observed for 9,10-AQ in particular. This variability limits the ability to evaluate differences in levels for their significance.

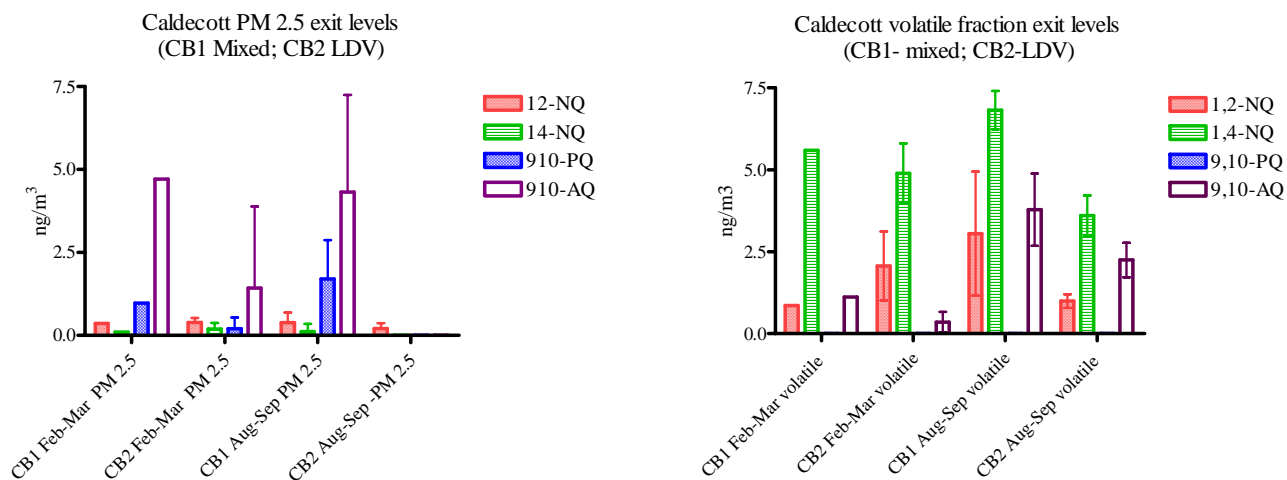


Figure 1. Quinone Concentrations in the Caldecott Tunnel

1. The wide variability in the quinone content for a given day was such that seasonal differences were not significant. Differences in the distribution of the quinones between particle and vapor phases were noted, with the three ring quinone, 9,10-phenanthroquinone (9,10-PQ) found mostly in the particle fraction while the naphthoquinones, 1,2- and 1,4-naphthoquinone (1,2- and 1,4-NQ) were found in the vapor phase at levels substantially greater than 9,10-PQ

2. The consistently high concentration of the naphthoquinones in the volatile fraction reflects the high concentration of the parent aromatic hydrocarbon in exhaust and in ambient air (Fraser, et al., 1998). Their presence in the tunnel would suggest that these quinones are formed from combustion. Their levels did not appear to differ between the two bores
3. There appears to be a trend toward higher levels of the three ring quinones, 9,10-PQ and 9,10-AQ in the particle phase of bore 1, with mixed traffic than bore 2. This could reflect the presence of the heavy-duty vehicles (HDVs) in bore 1

Other Studies

The Redox Properties of Diesel Exhaust (Pan, et al., 2004). Diesel exhaust particles, obtained from the laboratories of Professor Sagai in Tsukuba, Japan, have been used in many of our assay procedures (Li et al., 2003; Cho et al., 2004; Cho et al., 2005) as a standard. This sample is available in large quantity and diesel exhaust particles (DEP) are directly relevant to the urban aerosols of major interest to the SCPCS. This material was examined for its redox properties using DTT and ascorbate as electron sources. In the presence of either of these reducing agents, whole particle suspensions were able to reduce oxygen to superoxide or peroxide in a catalytic process that was dependent on the particle concentration. To characterize the nature of the contributing species, the particles were subsequently extracted with an organic solvent (dichloromethane) and dilute acid (1 M HCl) to remove organic and metal species, respectively, that contribute to the overall activity. The results of this study showed that while the dichloromethane extract contained species that exhibit redox activity, a considerable proportion of the original activity remained in the particle residue after extraction. Furthermore, when the extract and the particles were combined to reconstitute the original preparation, the overall activity was greater than that found before extraction. The extraction with dilute acid also reduced the activity of the original particles, but a reconstitution experiment was not performed. These observations indicate that while a fraction of the redox activity associated with diesel exhaust particles is extractable by both organic solvent and acid, most of the redox activity is associated with the particles themselves. Furthermore, extraction of DEP with organic solvents appears to alter the chemical properties of the particles such that their reactivity increases, indicating a complex interaction between particle structure and extractable components.

Using electron paramagnetic resonance spectrometry, DEP were shown to have inherent paramagnetic properties. This suggests unpaired electrons that may be important to the ability to catalyze the formation of reactive oxygen species and at least partially responsible for their toxicity. The paramagnetic species identified was resistant to extraction, as described above.

Electrophilic Properties of PM and Related Quinones. Our assay for inhibition of GAPDH was performed on two quinones that have been detected in atmospheric samples, and a diesel exhaust extract to compare the mechanisms by which these compounds inhibit the enzyme (Rodriguez, et al., 2005). The redox active 9,10-PQ inactivates the enzyme by both oxygen dependent and independent mechanisms whereas 1,4-BQ, a pure electrophile in biological systems, inactivates the enzyme by an electrophilic, oxygen independent mechanism. The rate constants for affinity (K_i) and inactivation ($k_{\text{inactivation}}$) for the two quinones, determined under anaerobic conditions,

are shown in Table 3, together with the $k_{\text{inactivation}}$ determined for a diesel exhaust extract that we have used in all of our experiments as a “standard.” Experiments with DEP extracts showed an analogous time dependent inactivation that could be prevented by coincubation with high concentrations of alternate thiols, consistent with covalent attachment to the enzyme thiol. These results are the first to demonstrate the electrophilic nature of DEP constituents and indicate that with this source of PM, the induction of cellular stress by pathways other than reactive oxygen generation is possible.

Table 3. GAPDH Inactivation

Compound	K_i (μM)	$k_{\text{inactivation}}$
9,10-PQ	22.54	1.47 ($\mu\text{M}^{-1} \cdot \text{min}^{-1}$)
1,4-BQ	.67	0.57 ($\mu\text{M}^{-1} \cdot \text{min}^{-1}$)
DEP extract	ND	.0028 ($\mu\text{g}/\text{mL})^{-1} \cdot \text{min}^{-1}$

Summary

The objective of this project was to characterize PM samples for the chemical properties that likely contribute to their adverse health effects. The project developed assays designed to assess these chemical properties on a quantitative basis. The assays were based on known redox and electrophilic properties of quinones whose presence in PM had been reported in qualitative terms (Kumagai, et al., 1995; Koeber, et al., 1999). Our assays used quinones as standards in the development process, and the assays were developed for samples of particulate material on filters, in aqueous suspensions or as extracts of XAD resins containing volatile substances. Key findings from this project include:

1. We determined the concentration of four quinones found in both particulate and volatile fractions of ambient air samples and demonstrated that the assay can be used to determine changes in these quinones as an air mass moves across the LAB. The results are consistent with the notion that at least two of the quinones, 1,4-NQ and 9,10-PQ are formed by photochemical processes in the LAB.
2. We have determined the redox activity of air samples collected at different sites in the LAB and found that while the activity per mass does not vary over a wide range, the particle concentration variability is such that exposure can be vastly different at different sites and in different seasons. UF in general have a higher activity per mass, while the greater mass concentration of F often renders this fraction responsible for the greatest exposure to material that is active in the DTT assay.
3. We have found that samples of PM from roadways possess redox activity. In a study of the redox properties of diesel exhaust particles, we have observed that the organic and acid extractable materials present in the particles possess redox activity but that the particles retain significant activity after extraction.

4. We have obtained preliminary evidence to support the notion that particles and their constituents exhibit electrophilic properties, as evidenced by the inhibition of the nucleophilic enzyme, GAPDH.

Our studies demonstrate that UFs have greater potency in redox cycling assays, and that this potency may be attributable to organic chemicals associated with combustion processes. Chemical properties that derive from the particle structure, not only the constituent compounds, are relevant and our DTT assay reflects the behavior of whole particles not limited to any specific constituent. Other research in the SCPCS found that cell biological effects of PM that are related to oxidative stress and mitochondrial damage are greater with UF, indicating that activity in our chemical assays may predict biological toxicity of PM samples.

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