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Redox and electrophilic properties of vapor- and particle-phase components of ambient aerosols ☆

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ABSTRACT

Particulate matter (PM) has been the primary focus of studies aiming to understand the relationship between the chemical properties of ambient aerosols and adverse health effects. Size and chemical composition of PM have been linked to their oxidative capacity which has been postulated to promote or exacerbate pulmonary and cardiovascular diseases. But in the last few years, new studies have suggested that volatile and semi-volatile components may also contribute to many adverse health effects. The objectives of this study were: (i) assess for the first time the redox and electrophilic potential of vapor-phase components of ambient aerosols and (ii) evaluate the relative contributions of particle- and vapor-fractions to the hazard of a given aerosol. To achieve these objectives vapor- and particle-phase samples collected in Riverside (CA) were subjected to three chemical assays to determine their redox and electrophilic capacities. The results indicate that redox active components are mainly associated with the particle-phase, while electrophilic compounds are found primarily in the vapor-phase. Vapor-phase organic extracts were also capable of inducing the stress responding protein, heme-oxygenase-1 (HO-1), in RAW264.7 murine macrophages. These results demonstrate the importance of volatile components in the overall oxidative and electrophilic capacity of aerosols, and point out the need for inclusion of vapors in future health and risk assessment studies.

1. Introduction

Although exposure to ambient air pollutants includes chemicals present in both the vapor- and particle-phases, most health assessment studies have focused on ambient particulate matter (PM) and diesel exhaust particles (DEP) (Brauer et al., 2002; Gottipolu et al., 2008; Hoek et al., 2002; Klemm et al., 2004; Mauderly and Chow, 2008; Naeher et al., 2007). Health effects observed after exposure to these pollutants include among others exacerbation of asthma and cardiovascular diseases (Brauner et al., 2007; Castorena-Torres et al., 2008; De Vizcaya-Ruiz et al., 2006; Pereira et al., 2007; Xia et al., 2004).

Recently, several studies have indicated that volatile (VOCs) and semi-volatile (SVOCs) organic compounds may also be involved in diverse health effects (Arif and Shah, 2007; Boeglin et al., 2006; Rumchev et al., 2004). For example, ambient levels of VOCs have been correlated with the frequency of hospital visits due to ischemic heart disease and myocardial infarctions (Klemm et al., 2004; Tolbert et al., 2001). In a study evaluating the cardiovascular effects of highway aerosols in rats, a decrease in heart rate was associated with the vapor-phase components and not the particles (Elder et al., 2007). A similar study conducted by Lund et al. (2007) showed that the effects of exposure to gasoline emissions on oxidative stress and pro-atherosclerotic tissue changes in aorta of mice were not reduced when PM was removed from the ambient air using high-efficiency particulate

Abbreviations: PM, particulate matter; DEP, diesel exhaust particles; VOCs, volatile organic compounds; SVOCs, semi-volatile organic compounds; ROS, reactive oxygen species; PAHs, polycyclic aromatic hydrocarbons; DCM, dichloromethane; DMSO, dimethyl sulfoxide; DTT, dithiothreitol; DTPA, diethylenetriaminepentaacetic acid; DHBA, dihydroxybenzoic acid; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HO-1, heme-oxygenase-1

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air (HEPA) filters. In addition, previous laboratory studies demonstrated that the toxic potency of freshly emitted SVOCs was higher than that of the emitted PM (Seagrave et al., 2001, 2003).

The ability of aerosols to induce a state of cellular oxidative stress has been suggested as a common pathway leading to these adverse health effects (Balakrishna et al., 2009; Blanchet et al., 2004; Delfino et al., 2005; Donaldson et al., 2003; MacNee and Donaldson, 2003). Oxidative stress is typically caused by reactive oxygen species (ROS) such as hydrogen peroxide and hydroxyl radical, and electrophiles such as α,β-unsaturated carbonyls. In this context, the chemical components of both vapors and particles are key players. Transition metals present in PM are capable of generating ROS through the Fenton reaction, and concentrations of iron and copper have been correlated with the ability of ambient PM and DEP to induce a state of oxidative stress (DiStefano et al., 2009; Gottipolu et al., 2008; Ohyama et al., 2007; Shinyashiki et al., 2009). Besides the contribution of transition metals, organic components of PM and ambient air have been shown to induce oxidative stress. The organic content of PM, in particular polycyclic aromatic hydrocarbons and quinones, has also been correlated with the ability of the particles to induce oxidative stress and inflammatory responses by the respiratory system (Chung et al., 2006; Hiyoshi et al., 2005; Inoue et al., 2007a, 2007b; Li et al., 2003). More recent studies have also found that ambient PM and DEP contain compounds that can inactivate thiol proteins through covalent bonding (Iwamoto et al., 2007; Rodriguez et al., 2005; Shinyashiki et al., 2008).

The main purpose of this study was to assess, for the first time, the redox and electrophilic potential of vapor-phase components of aerosols, and to evaluate the relative contributions of particle- and vapor-fractions to the overall redox potential and electrophilic content of ambient air. To achieve these goals three different chemical assays were used to: the dithiothreitol (DTT) assay, which measures the ability of the sample to generate ROS; the dihydroxybenzoic acid (DHBA) assay, which assesses the capacity of the sample to catalyze the Fenton reaction; and the glyceraldehyde-3-phosphate dehydrogenase (GADPH) assay, which measures electrophilic activity through thiol protein inactivation. We also conducted an in-vitro assay to measure the ability of the vapor-phase extract to induce the stress protein heme-oxygenase-1 (HO-1) in macrophages.

2. Materials and methods

2.1. Sample collection

Samples were collected at the Riverside Agricultural Center (CA) using $PM_{2.5}$ medium-vol samplers (Tisch Environmental Model 1202, Cleves, OH) during April–May and October–December 2007, and June–August 2008 (Table 1). $PM_{2.5}$ was collected on Teflon-coated glass fiber filters (Pall Corp., East Hills, NY) and vapors in XAD-4 resin (Acros, Thermo Fisher Scientific). Two parallel instruments were run at 113 l/min for 6 h/day over 6-day period. Samples were collected from 7:00 am to 1:00 pm parallel to animal exposure studies. Sampling details and matrix cleaning procedures have been previously published (Eiguren-Fernandez et al., 2004). During the last week of October 2007, Southern California had a significant fire, which emissions impacted the Riverside area where the samples had been collected.

Table 1
Sampling information and total sample volume (m^3)

Sample ID	Sampling dates	Total volume (m^3)
RIV041607	April 16–24, 2007	399
RIV042507	April 25–May 3, 2007	423
RIV050707	May 7–16, 2007	415
RIV102507	October 25–November 8, 2007	578
RIV111307	November 11–21, 2007	339
RIV112607	November 26–December 18, 2007	808
RIV062608	June 26–July 3, 2008	339
RIV070708	July 7–15, 2008	401
RIV072108	July 21–29, 2008	400
RIV811108	August 11–19, 2008	402

As samples were collected during several days, and although they were kept in the freezer between sample collection periods, it is important to acknowledge that sampling artifacts may have occurred. As Teflon-coated filters where used for the sample collection no positive artifacts were expected. To minimize losses due to negative artifacts (volatilization of organic compounds associated with the collected PM) a double filter system was used. However, the volatilized organic carbon is not always recaptured by the backup filter; if this was the case volatiles would have been captured by the downstream XAD bed.

2.2. Sample extraction

Vapor-phase samples collected in parallel were pooled and extracted by sonication (30 min) with dichloromethane (DCM) (Fisher Scientific, PA). The suspension was filtered through a 0.45 μm nylon filter (Millipore, Billerica, Massachusetts), volume reduced, and solvent exchanged to dimethyl sulfoxide (DMSO) (Fisher Scientific, PA). Final concentration of the organic extract was ~250 m^3/mL. Aliquots of the DMSO solution were used for posterior chemical and toxicological analyses. Blank XAD was prepared as described previously and used as control.

$PM_{2.5}$ samples were extracted by two different methods depending on the study: (i) by sonicating filter punches in water and obtaining water suspensions of particles and (ii) extracting filter punches with DCM followed by filtration through 0.45 m nylon filter to obtain an organic extract, which was solvent exchanged to DMSO prior to the assay. The chemical assays were conducted using aliquots of the suspension and organic extract. Highly polar substances associated with the vapor-phase would not be included in the analysis as the XAD resin bed as some of these species would not be extracted with DCM.

2.3. DTT assay

This method assesses the redox activity of the sample based on its ability to transfer electrons from dithiothreitol (DTT) to oxygen (Cho et al., 2005; Kumagai et al., 2002). In this assay, aliquots of vapor-phase and $PM_{2.5}$ organic extracts and $PM_{2.5}$ water suspensions were incubated with DTT (Sigma Chemical Co., MO) for times varying from 10 to 30 min. The reaction was quenched at specific times and, after addition of 5,5′-dithiobisbis-(2-dinitro)benzoic acid (DTNB) (Sigma Chemical Co., MO) to complex with the remaining DTT, the absorption at 412 nm measured. Rates are calculated averaging duplicate runs, and are blank corrected.

Since DTT can be oxidized by high concentrations of metal ions (Kachur et al., 1997; Netto and Stadtman, 1996), the contribution of metals to the DTT-based redox activity was also determined adding the metal chelator diethylenetriaminepentaacetic acid (DTPA) (10 M) to one set of the samples

2.4. DHBA assay

This analytical procedure was developed to quantify metal-based redox activity by measuring the ability of transition metals associated with the ambient particles to catalyze the Fenton reaction in which hydrogen peroxide is converted to hydroxyl radical (DiStefano et al., 2009). Briefly, aliquots of $PM_{2.5}$ water suspensions were incubated with ascorbic acid and salicylate (Sigma Chemical Co., MO) for times varying from 15 to 45 min. At each time point, the reaction was quenched by the addition of metaphosphoric acid (Sigma Chemical Co., MO). A second set of tubes containing the metal chelator, DTPA was included in each condition to evaluate the participation by metal ions such as iron and copper in the reaction. Concentrations of 2, 3- and 2, 5-DHBA were then determined by high-performance liquid chromatography–electrochemical detector. All samples are run in duplicate and blank corrected.

The DHBA assay was performed only on the aqueous suspensions of filters as the DCM extracts would be devoid of metals.

2.5. GAPDH assay

This assay measures the content of electrophiles in the sample, based on their ability to inhibit or inactivate the thiolate enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH), through covalent bonding. Inhibition of GAPDH by vapor and $PM_{2.5}$ samples was determined under anaerobic conditions according to the method described previously (Shinyashiki et al., 2008). In brief, chicken GAPDH (Sigma Chemical Co., MO) or 1 unit of rabbit GAPDH was incubated with aliquots of the organic extracts of vapors and particles or water suspension under argon gas at 25 °C for 120 min. At this time point the reaction was quenched by adding an equal volume of cold DTT solution, and GAPDH activity, measured as rate of