Respirable ambient particulate matter (PM) exposure has been associated with an increased risk of cardiovascular disease. Direct translocation of PM-associated metals from the lungs into systemic circulation may be partly responsible. We measured elemental content of lungs, plasma, heart, and liver of healthy male WKY rats (12–15 weeks old) 4 or 24 h following a single intratracheal (IT) instillation of saline or 8.33 mg/kg of oil combustion PM (HP-12) containing a variety of transition metals with differing water and acid solubility. Tissues were digested with a combination of quaternary acid, amine, and nitric acid and analyzed using inductively coupled plasma-atomic emission spectroscopy. Lung levels of metals were lower at 24 h than at 4 h. Metals with high water solubility and relatively high concentration in HP-12 were increased in extrapulmonary organs. Water-soluble nonessential metals, like vanadium and nickel, were increased in plasma, hearts, and livers of exposed animals at both time points. Exposure-related small increases in essential metals, like zinc and manganese, were also noted in extrapulmonary tissues at both time points. Lead, with low water solubility but high acid solubility, was detected in liver only at 24-h postinstillation. Elements with low water or acid solubility, like silicon and aluminum, were not detected in extrapulmonary tissues despite decreased levels in the lung suggesting mucociliary clearance. We have shown that HP-12–associated metals translocate to systemic circulation and extrapulmonary organs following IT exposure. This translocation is dependent upon their relative levels and water solubility. Thus, following inhalation, PM-associated metals deposited in the lung may be released into systemic circulation at different rates depending on their water/acid solubility, thereby providing a means by which metals may elicit direct extrapulmonary effects.

**Key Words:** particulate matter; cardiovascular injury; metals; translocation; bioavailability.

Numerous epidemiological and toxicological studies show that pulmonary exposure to ambient particulate matter (PM) results in increased cardiovascular and other extrapulmonary health effects (Campen et al., 2003; Dockery, 2001; Kodavanti et al., 2003). More recently, PM exposure has resulted in exacerbation of existing atherosclerotic plaques in atherosclerosis-prone ApoE−/− mice. Progression of atherosclerosis can lead to plaque rupture, leading to thrombus formation and possible myocardial infarction (Pope et al., 2006). The exact mechanism by which this occurs remains unclear. There are three primary hypotheses being investigated. Firstly, lung inflammation following PM exposure may induce a systemic inflammatory response via the release of inflammatory peptides and vasoactive substances, and by stimulating the blood coagulation cascade (Dubowsky et al., 2006). It has also been suggested that upon PM exposure, autonomic control of the heart may be altered, accounting for changes in heart rate variability and heart rate (Park et al., 2005). The third postulated mechanism involves translocation of PM components and mediation of extrapulmonary responses directly by interaction with effector cells and tissues. Metals have been hypothesized to be a primary injury causing component of PM (Costa and Dreher, 1997), and are likely to translocate to extrapulmonary organs, making them available to cause direct toxicity to the heart and other organ systems. The extrapulmonary toxicity probably depends upon the amount and type of water soluble, and therefore, bioavailable metals present in the PM (Gilmour et al., 2006; Kodavanti et al., 2003). Ambient particles contain a variety of metals and the metal composition of PM varies depending on the source and geographical location. Zinc is one of the common PM-associated element found in ambient air, especially near industrial sources (Dye et al., 2001) or roadways (Schauer et al., 2006). The water-soluble fraction of zinc has been postulated to be a causative agent in PM-induced injury (Adamson et al., 2000).
PM-associated vanadium and nickel have also been linked to adverse cardiopulmonary effects in cells and animals (Graff et al., 2004; Kodavanti et al., 1998). Recently, PM-associated nickel has been shown to contribute to progression of atherosclerosis in Apo-E−/− mice (Lippmann et al., 2006). Furthermore, the inflammatory effects of PM in vitro have been shown to be due to the soluble transition metals present (McNeilly et al., 2004).

Because PM-associated water-soluble metals can be leached off in the lung lining fluid, they are likely to be translocated to the pulmonary vasculature, heart, and other extrapulmonary organs before dilution in the systemic circulation and clearance by liver. Therefore, cardiac tissue is a potential target for PM-associated bioavailable metal-induced damage. Translocation of intratracheally instilled vanadium to systemic circulation has been documented (Sharma et al., 1987), and furthermore, an element’s solubility has been suggested to mediate translocation from pulmonary exposure (Rhoads and Sanders, 1985).

Retention of metals, such as lead and cadmium, in the lungs as well as translocation to other organs has been reported in exposed workers (Mehani, 1966; Satarug et al., 2001). Increases in lung levels of cadmium, copper, cobalt, nickel, and lead have also been documented in humans exposed to Mexico City air pollution (Fortoul et al., 1996). It is likely that these metals have direct extrapulmonary effects following pulmonary exposure. The amount and type of water-soluble metals present in PM has been suggested to modulate the amount of generation of free radicals, and therefore oxidative stress and toxicity (Ghio et al., 2002; Valavanidis et al., 2005). Since chronic diseases, including cardiovascular, are accompanied by an increased level of underlying oxidative stress (Abrescia and Golino, 2005), an additional increase resulting from exposure to PM metals could substantiate a level of oxidative stress that is physiologically significant and harmful.

The purpose of this study was to examine the pattern of translocation of PM-associated metals to systemic circulation and extrapulmonary organs, and investigate how water and acidic solubility of metals influence translocation. Our hypothesis was that PM-associated metals are able to translocate into systemic circulation, and that their ability to translocate would be related to their solubility in water or acid. For this study, we chose an oil combustion source PM (HP-12) with moderate levels of several water-leachable essential and nonessential metals, with the idea that the detection of changes in nonessential metals will be accurate in signifying translocation into extrapulmonary systems. Exposure to several Metals at once, as they exist in a given HP-12 sample, allows us to compare the relative abilities and rates of translocation to characteristics of a metal, such as solubility or absolute amount in the PM. Our study demonstrated that following pulmonary exposure to a metal-containing HP-12, a number of Metals are rapidly translocated to the circulation and accumulated in extrapulmonary organs such as heart and liver within 24 h, and that initial translocation is associated with the water solubility of metals.

**MATERIALS AND METHODS**

**Animals.** Healthy male Wistar Kyoto (WKY) rats, 12–15 weeks old were purchased from Charles River Laboratories Inc., Raleigh, NC. Animals were double housed in polycarbonate cages with beta chips bedding and acclimated for 1 week in an AAALAC-approved animal facility (21 ± 1°C, 50 ± 5% relative humidity, 12/12-h light/dark cycle) prior to and during the experimental period. All animals received standard Purina rat chow (Brentwood, MO) and water ad libitum.

**Selection of combustion source PM.** Elemental composition of oil combustion PM varies depending upon the source and grade of residual oil used and the conditions of oil combustion. We selected an oil combustion PM which contained moderate levels of a variety of transition metals, such that it can be comparable to ambient PM of highly polluted industrial areas. The PM used in this study (HP-12) was obtained from the precipitator unit of a Boston power plant burning residual oil through our collaboration with the Harvard School of Public Health. The process of collection and physicochemical characterization of bulk sample has been previously reported (Gilmour et al., 2004; Wichers et al., 2006a). HP-12 contained water-soluble, 1M HCl-leachable and non-leachable elements such as aluminum, iron, silicon, vanadium, nickel, lead, copper, manganese, and zinc; however, their concentrations by mass are much lower than their concentration in previously used highly soluble oil combustion PM (Costa and Dreher, 1997).

**Intratracheal instillations.** Rats were randomly assigned to different groups (n = 6) based on body weight. Bulk HP-12 sample was weighed and suspended in sterile saline at concentrations of 0.00 or 8.33 mg/ml to reflect earlier studies (Gilmour et al., 2004; Kodavanti et al., 1998). The reason we selected such a high concentration is that we have previously shown that this concentration does not result in severe lung toxicity and furthermore, if translocation was occurring, increases in metal levels would be detectable in extrapulmonary tissues. We assumed that the metals which leach off in saline suspension will also leach off in lung lining fluid upon inhalation; however, it is likely that the solubility may be greater in lung lining fluid, due to presence of polar and nonpolar surfactant components. The HP-12 samples were vortexed and mixed for at least 20 min prior to intratracheal (IT) instillations. Rats were anesthetized under light halothane and single IT instillations were given at a volume of 1 ml/kg (Costa et al., 1986); Control rats received 1 ml/kg of the sterile saline.

**Necropsy and tissue collection.** Four or 24-h post-HP-12 instillation, rats were weighed and anesthetized with an overdose of sodium pentobarbital (50–100 mg/kg, ip). Blood was collected through abdominal aortic puncture directly into a vacutainer containing sodium heparin. These blood samples were spun at 3000 × g for 10 min. An aliquot of plasma was stored at −80°C. Following exsanguination, the heart was quickly excised, blotted with gauze, and a piece of the left ventricle (0.1–0.3 g in weight) was quick frozen in liquid nitrogen and saved for metal analysis. The accessory lobe of the lung and a piece of the liver were excised, blotted with gauze, weighed, quick frozen in liquid nitrogen, and saved for metal analysis.

**Tissue elemental analysis.** We digested all plasma and tissue samples using tetramethyl ammonium hydroxide/nitric acid and analyzed for elemental content using inductively coupled plasma-atomic emission spectroscopy (ICP-AES) (Model 4300DV, PerkinElmer Instruments, Shelton, CT) following EPA Method 200.11 (Martin et al., 1991). Method 200.11 was originally developed for analysis of fish tissue, but the digestion is robust and used to solubilize all types of biological fluids and tissues prior to elemental analysis. For this study we extended the analyte list for Method 200.11 to monitor the following elements found in combustion PM: boron (B), barium (Ba), cobalt (Co), manganese (Mn), molybdenum (Mo), silicon (Si, expressed as SiO2), strontium (Sr), titanium (Ti), and vanadium (V); in addition to elements originally covered by the method: aluminum (Al), calcium (Ca), chromium (Cr), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), sodium (Na), nickel (Ni), phosphorus (P), and zinc (Zn). Method detection limits were improved.
RESULTS

**HP-12 Elemental Composition**

The elemental composition of the bulk sample of HP-12 has been previously reported (Gilmour et al., 2004; Wichers et al., 2006a). HP-12 contained both water and HCl-leachable fractions of several transition metals such as zinc, vanadium, nickel, and aluminum. Table 1 lists the elemental composition of HP-12 by order of the absolute amount that is water soluble. For example, HP-12 contained the most amount of water-soluble zinc (11.55 μg/mg particle), vanadium (7.18 μg/mg particle), and nickel (6.16 μg/mg particle) and the least amount of water-soluble chromium (0.05 μg/mg particle). It also lists the absolute amount of metals that leached off in a 1M HCl solution, as well as metals that did not leach off in the water or acid fraction, such that one can view the concentration of each specific metal and its water or acid solubility. Based on overall relative levels and differing water solubility, HP-12 contained high levels of water-soluble zinc, vanadium and nickel. In HP-12, lead was not water soluble but existed in the 1M HCl-leachable fraction. On the other hand, silicon and aluminum were abundant in absolute amounts in HP-12, but are primarily in insoluble form.

Table 2 lists the amount of each element instilled based on an HP-12 suspension at 8.33 mg/ml and 1 ml/kg body weight instillation dose for a 375 g rat at the time of instillation. Rat weights averaged 374 ± 32 g. The determination of metal instilled in the whole lung at time zero allows us to identify its relationship with the levels translocated at 4 versus 24 h. Further, since metal content was measured in the accessory (cardiac) lung lobe, the whole lung levels were estimated based on 1 g lung weight for a 375 g rat, and subsequent correction for the degree of edema in exposed rats (Fig. 1). The elements are listed in order by absolute amount instilled, without regards to water or acid solubility. Lung levels of all HP-12–associated metals, including essential and nonessential, are high at 4- and 24-h postinstillation, reflecting the fact that the majority of the low-water-soluble fraction of metals remain associated with particles within the lung. Note that the level of metals in the

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Elemental Composition of HP-12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Element</td>
<td>Total μg/mg</td>
</tr>
<tr>
<td>Zn</td>
<td>29.86</td>
</tr>
<tr>
<td>V</td>
<td>62.95</td>
</tr>
<tr>
<td>Ni</td>
<td>43.10</td>
</tr>
<tr>
<td>Al</td>
<td>47.40</td>
</tr>
<tr>
<td>SiO₂</td>
<td>138.80</td>
</tr>
<tr>
<td>Ba</td>
<td>2.52</td>
</tr>
<tr>
<td>Fe</td>
<td>47.05</td>
</tr>
<tr>
<td>Cu</td>
<td>2.49</td>
</tr>
<tr>
<td>Sr</td>
<td>0.45</td>
</tr>
<tr>
<td>Co</td>
<td>1.59</td>
</tr>
<tr>
<td>Mn</td>
<td>0.56</td>
</tr>
<tr>
<td>Pb</td>
<td>2.75</td>
</tr>
<tr>
<td>Cr</td>
<td>0.49</td>
</tr>
</tbody>
</table>

*Note. Values given are in μg/mg HP-12, while values in parentheses represent percentage of total metal content of the particle that is water or HCl soluble, or insoluble. Zn = zinc, V = vanadium, Ni = nickel, Al = aluminum, SiO₂ = silicon, Ba = barium, Fe = iron, Cu = copper, Sr = strontium, Co = cobalt, Mn = manganese, Pb = lead, Cr = chromium. These values were extracted from previously published papers from our labs at EPA (Gilmour et al., 2004; Wichers et al., 2006b).*

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>Theoretical Total Amount of Metals Instilled as They Exist in HP-12, and Lung Burden at 4 and 24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Element</td>
<td>Theoretical amount instilled /µg/rat</td>
</tr>
<tr>
<td>SiO₂</td>
<td>433.57</td>
</tr>
<tr>
<td>V</td>
<td>196.63</td>
</tr>
<tr>
<td>Al</td>
<td>148.08</td>
</tr>
<tr>
<td>Fe</td>
<td>146.96</td>
</tr>
<tr>
<td>Ni</td>
<td>134.62</td>
</tr>
<tr>
<td>Zn</td>
<td>93.62</td>
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<tr>
<td>Pb</td>
<td>8.60</td>
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<tr>
<td>Ba</td>
<td>7.86</td>
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<td>Cu</td>
<td>7.79</td>
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<td>Co</td>
<td>4.98</td>
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<tr>
<td>Mn</td>
<td>1.75</td>
</tr>
<tr>
<td>Cr</td>
<td>1.54</td>
</tr>
<tr>
<td>Sr</td>
<td>1.42</td>
</tr>
</tbody>
</table>

*Note. Values were calculated with the assumption that instilled HP-12 was uniformly distributed in the lung. Instillation values were calculated from total metal content in 8.33 mg HP-12/kg body weight for a 375 g rat. For lung calculations, total lung weight was assumed to be 1 g, and values were divided by a ratio of accessory lung lobe weight between control and exposed animals, at 4- or 24-h postinstillation (see Fig. 1). Measured amounts in lung represent differences in measured amounts between saline and HP-12-exposed animals. Zn = zinc, V = vanadium, Ni = nickel, Al = aluminum, Fe = iron, SiO₂ = silicon, Ba = barium, Cu = copper, Sr = strontium, Co = cobalt, Mn = manganese, Pb = lead, Cr = chromium.*
lung decreased significantly at 4 from IT instilled absolute amount, and further from 4 to 24 h suggesting clearance via pulmonary capillaries, lymphatics, or the mucociliary escalator. Four- and 24-h time points were selected to focus primarily on translocation of metals, since clearance, especially of watersoluble elements, may be readily apparent within this short time. The percent of metal retained in the lung at 24 h was calculated by dividing the amount measured at 24 h by the theoretical amount instilled. This correlates with the percent of metal in HP-12 which is insoluble (Fig. 2).

Detection of Vanadium in Lung, plasma, Heart and Liver

Because vanadium exists in water soluble, acid leachable and also insoluble forms, a significantly higher amount of vanadium was detected in each tissue at both time points in HP-12 exposed animals compared to saline controls (Fig. 3). In the plasma and heart of HP-12–exposed animals, the amount of vanadium detected at 4-h postinstillation was significantly higher than the amount detected at 24 h, suggesting rapid uptake of water-soluble vanadium. Vanadium was detected at the same level in the liver at both 4- and 24-h postexposure, most likely due to the rapid accumulation of the water-soluble form in the liver by 4 h and then its retention at the later time point.

Detection of Nickel in Lung, Plasma, Heart and Liver

Similar to the kinetics of vanadium, significantly higher amounts of nickel were measured in lung, plasma, heart, and liver of HP-12–exposed animals compared to saline controls at 4- and 24-h postinstillation (Fig. 4). In the plasma and heart, the amount of nickel detected at 24 h was less than the amount detected at 4 h again suggesting that soluble nickel rapidly translocated to these organs. At 24-h postinstillation, plasma nickel concentrations approached baseline values, reflecting its clearance and then eventual accumulation in the liver, as the amount of nickel found in livers of HP-12–exposed animals remained significantly elevated 4- and 24-h postinstillation.

Concentrations of Zinc in Lung, Plasma, Heart and Liver

As expected, relatively high levels of baseline zinc were detected in all four tissues of control animals. Because of these high baseline values of zinc and manganese over controls, % increase was calculated to obtain the relative changes in these two metals (Table 3). At 4-h postinstillation a significantly higher amount of zinc was found in the lung, and although not statistically significant, plasma levels of zinc in HP-12–exposed rats were 14.6% higher than in controls (Table 3). Note that the value for lung levels of zinc and all other metals (Table 2) represent levels subtracted from background tissue levels. By 24-h postexposure, the increase in plasma zinc was attenuated, and the amount of zinc found in the lung was significantly lower than at the 4-h time point. At 4-h postexposure there was no difference between saline and HP-12–exposed animals in the amount of liver zinc; however, at 24-h postexposure, levels were significantly higher than the amount detected in saline-exposed animals (Table 3). No significant differences between exposure groups were observed in the amount of zinc detected in heart tissue at either time point.

Concentrations of Manganese in Lung, Plasma, Heart and Liver

Similar to zinc measurements, levels of manganese found in control animals were high. Because of this, it is harder to detect HP-12–induced changes; however, some interesting trends were noted. Significantly higher amounts of manganese were found in the lungs of PM-exposed animals than controls 4- and 24-h postinstillation, and the amount of manganese found in
the lung 24-h postexposure was significantly lower than the amount found at 4-h postexposure (Table 3). Plasma levels of manganese were over 30% higher than control levels at 4 h, but by 24-h postexposure, the levels dropped to control. A similar pattern was observed in the heart, with a significant increase in the amount of manganese 4-h postexposure, but no significant increase 24-h postexposure. This pattern was also observed in the liver, although changes in this tissue were not statistically significant (Table 3). This pattern of manganese distribution is different from other metals such as vanadium, nickel, and zinc, which accumulated in the liver 24-h postinstillation. This is probably reflective of a different mechanism of its storage and distribution.

**Measurements of Other Metals in Lung, Plasma, Heart and Liver**

Several other essential metals were detected in extrapulmonary tissues, including iron, copper, selenium, and magnesium;
however, most changes could not be attributed to HP-12 exposure. As expected, baseline levels of essential elements such as copper, iron, and selenium were high in saline-exposed rats (data not shown). Although not significant, a higher amount of lead was noted in the liver of HP-12–exposed animals compared to control 24-h postexposure (Fig. 5). Because the absolute amount of water-soluble lead was low in HP-12 (Table 1), and a larger fraction was 1M HCl leachable, it seems likely that acid-leachable fraction translocates to extrapulmonary organs slowly. Small and nondetectable changes may have occurred in plasma lead and subsequently, at 24 h its accumulation in the liver reached a detectable value. Lead also binds to red blood cells (Church et al., 1993) and thus the blood uptake differences may account for lack of increased delectability in plasma.

Increases in all elements, both essential and nonessential, in the lungs of exposed animals verify that delivery of HP-12 to the lung did occur. Some of the metals, such as aluminum, although water-soluble to a small degree, were not detected in extrapulmonary organs. This may be due to the sensitivity of the ICP-AES for that metal, or differences in binding kinetics within the lung, which may not allow translocation. Most of the elements, which we were unable to detect outside of the lung were found in relatively small amounts in water-soluble form in HP-12. These elements include aluminum, as well as silicon. Interestingly, although barium existed in the HP-12 suspension largely in water-soluble form, we were unable to detect it in extrapulmonary tissues, despite a nearly 50% decrease in barium in the lungs from 4- to 24-h postinstillation. This suggests that the mechanisms by which soluble barium is handled and cleared by the body may be different than other metals, such as nickel and vanadium.

### DISCUSSION

Pulmonary exposure to metals has led to systemic changes in metal homeostasis and development of extrapulmonary pathologies (Fortoul et al., 1996; Mehani, 1966; Satarug et al., 2001). More recently, exposure to PM has also been associated with cardiac, neuronal, and reproductive dysfunction (Hahn et al., 1996; Sjogren et al., 2002; Warfvinge et al., 1992). Although metals have been postulated to be causative in PM-induced pulmonary and extrapulmonary health effects, the systemic translocation of PM-associated metals, metal bioavailability, and their potential direct effect on extrapulmonary organs has not been well characterized. In this study we demonstrate that water-leachable metals from oil combustion PM (HP-12) are rapidly translocated to systemic circulation and extrapulmonary organs following pulmonary exposure. This is evident by the detection of nonessential metals such as vanadium and nickel in the plasma, heart, and liver, and also increases in the concentration of essential metals such as zinc and manganese. Based on the detection of lead, which is largely acid leachable in HP-12, in the liver at later time point of 24 h, it is likely that a small fraction of acid-leachable metals may also translocate, but at a slower rate. Correlation analysis of lung metal retention and the percent insoluble metal content demonstrated that less water-soluble metals are retained in the lung longer.

The aim of this study was to monitor the translocation of HP-12–associated metals from the lungs, without the contribution of nasal uptake (Oberdorster et al., 2004), so we chose IT instillation as a method of pulmonary exposure. Furthermore, IT instillation allowed us to deliver a precisely controlled lung dose at one time point. Although the dose and the exposure methods used are environmentally irrelevant, and the clearance kinetics and HP-12 effects are likely different than those induced by continuous inhalation of PM at ambient level, this study design allowed us to demonstrate detectible changes in metals in multiple organs and provide evidence that particle

### TABLE 3

<table>
<thead>
<tr>
<th>Metal (increase over control)</th>
<th>Lungs</th>
<th>Plasma</th>
<th>Heart</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn (4 h) %</td>
<td>200.0*</td>
<td>14.6</td>
<td>3.6</td>
<td>2.7</td>
</tr>
<tr>
<td>Zn (24 h) %</td>
<td>84.1*</td>
<td>7.3</td>
<td>0.8</td>
<td>10.5*</td>
</tr>
<tr>
<td>Mn (4 h) %</td>
<td>416.4*</td>
<td>32.8</td>
<td>14.0</td>
<td>5.1</td>
</tr>
<tr>
<td>Mn (24 h) %</td>
<td>216.9*</td>
<td>0.8</td>
<td>1.6</td>
<td>0</td>
</tr>
</tbody>
</table>

Note. Values given are percent increases over control, calculated by dividing the absolute amount of increase in exposed rats over control by the control values, times 100. Zn = zinc, Mn = manganese.
*Indicates significantly different from time-matched saline control (p ≤ 0.05).
†Indicates significantly different from 4-h time point (p ≤ 0.05).

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**FIG. 5.** Analysis of lung (A) and liver (B) levels of lead following IT instillation of either saline or 8.33 mg/kg HP-12 in WKY rats. Data represent mean lead concentrations (ng Pb/g tissue or plasma) ± SEM for six rats per group. *Indicates significantly different from time-matched saline control (p ≤ 0.05). †Indicates significantly different from 4-h time point (p ≤ 0.05).
associated metals clear from lung and translocate to extra-pulmonary organs based on their solubility.

The exposure dose chosen for this study (8.33 mg/kg body weight) was high, and reflects earlier IT doses from our lab (Gilmour et al., 2004) and others (Wichers et al., 2004). However, because the levels of water-soluble metals were lower than previously used oil combustion PM (Costa and Dreher, 1997), the extent of lung injury was lower. A small degree of lung edema was induced, indicating increased vascular permeability, which may affect metal translocation to some extent. However, metal translocation to liver and kidney was also observed without apparent lung edema in rats exposed to a metal-containing fly ash for 28 days (Mani et al., 2006), indicating that although the rate of translocation may change with increased pulmonary leakage, translocation still occurs. For this study, our aim was to not only be able to detect changes in metal levels outside of the lung following pulmonary PM exposure, but also to relate the ability of a metal to translocate to its solubility. Therefore, we chose this high dose to get a greater sensitivity for detection of more metals in extra-pulmonary organs.

Ambient PM samples may differ in their elemental composition from HP-12 dependent upon the emission source contribution, geographical location, and atmospheric factors. We chose an oil combustion PM (HP-12) containing a variety of metals, generally detected in ambient PM, with differing water and acid solubility to gain broader perspective on the translocation of more metals as they exist in a complex PM sample. Although the levels of acid-leachable and nonleachable metals were high in HP-12, concentrations of some of the water-soluble metals were similar to previously used baghouse ambient PM (Adamson et al., 1999). The composition of this bulk PM has been well characterized in our labs at the Environmental Protection Agency (EPA) (Gilmour et al., 2004, Wichers et al., 2006a), allowing us to use that information to relate to translocation efficiency.

The amounts of each metal retained in the lung from instillation to 4-h postexposure are more variable than the amount retained from 4- to 24-h postexposure, especially in case of more water-soluble metals, suggesting a mechanism of rapid removal via either pulmonary capillaries or lung associated lymph nodes into the blood stream along with mucociliary clearance. Radioactivity can be measured outside of the lung within 1-min postexposure to radiolabeled ultrafine carbon particles (Nemmar et al., 2002). Inhaled elemental silver was detected outside of the lung within 30-min to 2-h postexposure (Takenaka et al., 2001). Drown et al. (1986) showed that levels of the soluble form of manganese in extra-pulmonary organs peak at 4-h post-IT instillation, while levels of the insoluble form do not peak until 3 days postexposure. Our observation that lead which existed primarily in acid-soluble form was found to be increased only at 24 h in the liver is consistent with the fact that less soluble metals may translocate more slowly than readily water-soluble metals. This relationship between solubility and the rate of translocation after instillation has been shown using two forms of cadmium (Aihara et al., 1985). We have previously shown that a single IT instillation of water-soluble zinc sulfate not only increased circulating levels of zinc, but also induced gene expression changes in the heart within 1 h (Gilmour et al., 2006). These studies along with our measurement of nonessential metals in the plasma and heart at 4 h (Figs. 3 and 4) provide evidence that water-soluble HP-12–associated metals rapidly translocate to systemic circulation and extrapulmonary organs.

The decrease in insoluble metals between 4 and 24 h in the absence of their appearance in the circulation and other storage sites, such as liver, suggests clearance via the mucociliary escalator. Earlier studies demonstrating mucociliary clearance of particles from rat lungs substantiate this suggestion (Hofmann and Asgharian, 2003; Snipes et al., 1983). Conversely, Wichers et al. (2006b) reported cumulative metal accumulation in the lung, but no clearance, following 4 consecutive days (6 h/day) of whole body inhalation of the same PM sample in rats. In the absence of measurement of metals in extrapulmonary organs, and just based on pulmonary metal burden following inhalation exposure, which does not allow actual deposition quantification, it is difficult to determine if translocation did occur in that particular study. This lack of clearance may also be explained in part by the differences in method of exposure and exposure dose encountered by pulmonary tissue at a given time.

The toxicity of PM-associated metals may be attributable to the total mass of bioavailable metals present, as well as their chemical and physical properties (Gutierrez-Castillo et al., 2005). The physical form of the metal in HP-12 could play a role in its solubility, and therefore its bioavailability. Adamson et al. (1999) reported that the soluble fraction of PM containing metals may mediate pulmonary injury. Metal sulfates are more water-soluble than metal oxides, and therefore are likely to translocate more easily into systemic circulation following pulmonary exposure (Kyotani and Iwatsuki, 1998). We observed that the less water soluble an element is in HP-12, the longer it was retained in the lung, with the exception of barium. Although most barium existed in a water-soluble form, no barium was detected in the plasma or liver despite its near 50% depletion in the lung between 4 and 24 h, suggesting a unique kinetic property. It is not clear what factors may have played a role in the case of HP-12–associated barium. One study reports barium translocation to the plasma and urine following long-term occupational exposure (Zschiesche et al., 1992), suggesting that longer exposures may be needed to detect a significant extrapulmonary translocation. Lymphatic clearance of barium to the spleen is likely (Donaldson et al., 2005). Although the water solubility of a metal may be a good predictor of its bioavailability, many other factors, such as concentration, interactions with other PM components, interactions with endogenous proteins, cell uptake kinetics, and composition of lung lining fluid may affect the bioavailability.
Trafficking of essential, and perhaps nonessential metals in cells may occur via special transport mechanisms involving metal binding proteins, transporters, and storage proteins, such as metallothioneins (Napier et al., 2005; Nath et al., 2000). Induction of metallothionein protein synthesis in the liver and other organs has been well characterized (Jacob et al., 1999). This protective mechanism is essential for avoiding the potentially harmful effects of free metals. We have previously reported that pulmonary exposure of rats to high levels of zinc induces expression of metallothionein proteins in not only the lung, but also in the liver and heart (Gilmour et al., 2006). Thus, it is likely that PM-associated essential and nonessential metals within the lung or other tissues may disturb systemic metal balance by induction of metallothioneins and sequestration of metals from their catalytically active protein sites from different tissues, resulting in systemic toxicity.

Specific metal–metal interactions may be causing biological changes within the organs and cells. In the blood, lead replaces zinc in heme enzymes (Goyer, 1997). Copper and zinc may similarly replace each other in metallothionein proteins, which normally regulate the homeostasis of both of these metals (Cai et al., 2005). Interactions between nickel, vanadium, and zinc have been suggested (Campen et al., 2001; Costa and Dreher, 1997; Kodavanti et al., 1998). A synergistic relationship between inhaled vanadium and nickel has been proposed (Campen et al., 2001); however, in this study iron seemed to attenuate the toxic response to inhaled vanadium or vanadium and nickel (Campen et al., 2002). Thus, the kinetics of translocation and biological actions of metals found in HP-12 and other ambient PM may be influenced by the presence of several metals as a mixture and their interaction with biological molecules.

We have demonstrated that a single IT instillation of HP-12, a combustion source PM containing moderate amounts of water-soluble, acid-leachable and nonsoluble metals, induces rapid changes in lung as well as extrapulmonary tissue metal levels. The presence of water-soluble nonessential metals outside the lung indicates metal translocation and subsequent sequestration in the liver. We also observed that a small quantity of acid-leachable lead (low water solubility) was translocated to the liver only at 24-h postexposure, while no increase in circulating or extrapulmonary levels was noted among insoluble elements such as silicon and aluminum. Thus, the ability of metals to translocate from the lungs into systemic circulation appears to be related to their solubility in water. In describing the extrapulmonary translocation of PM-associated metals following pulmonary exposure, we hope to better define the exact mechanism of PM-induced cardiovascular injury.

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