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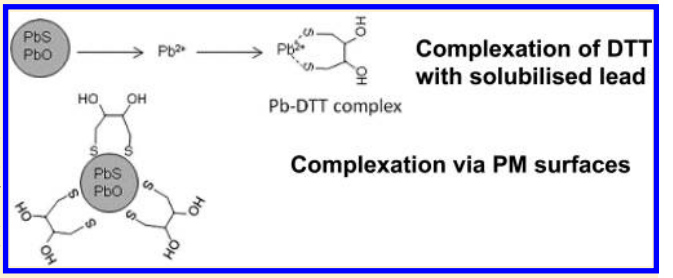
ABSTRACT: Epidemiological studies in urban areas have linked increasing respiratory and cardiovascular pathologies with atmospheric particulate matter (PM) from anthropic activities. However, the biological fate of metal-rich PM industrial emissions in urban areas of developed countries remains understudied. Lead toxicity and bioaccessibility assessments were therefore performed on emissions from a lead recycling plant, using complementary chemical acellular tests and toxicological assays, as a function of PM size ($PM_{10-2.5}$, $PM_{2.5-1}$ and PM_1) and origin (furnace, refining and channeled emissions). Process PM displayed differences in metal content, granulometry, and percentage of inhalable fraction as a function of their origin. Lead gastric bioaccessibility was relatively low (maximum 25%) versus previous studies; although, because of high total lead concentrations, significant metal quantities were solubilized in simulated gastrointestinal fluids. Regardless of origin, the finest PM_1 particles induced the most significant pro-inflammatory response in human bronchial epithelial cells. Moreover, this biological response correlated with pro-oxidant potential assay results, suggesting some biological predictive value for acellular tests. Pulmonary effects from lead-rich PM could be driven by thiol complexation with either lead ions or directly on the particulate surface. Finally, health concern of PM was discussed on the basis of pro-inflammatory effects, accellular test results, and PM size distribution.



INTRODUCTION

Particulate matter (PM) can induce adverse effects on human health,[1−3] depending on its characteristics.[4] Lead is frequently present in anthropogenic atmospheric PM, and in particular from metal treatment plants.[5] The environmental impact of particles emitted by a lead recycling plant has been reported, revealing foliar lead uptake by vegetables in the surroundings.[6] In contrast with urban PM, largely derived from traffic emissions, which is mainly associated with short-term effects resulting in airway inflammation and respiratory diseases, lead-rich PM is fundamentally different in terms of composition and poses both short and long-term health risks because of its toxicity and persistence in ionic and particulate form. Therefore, lead-rich PM emissions from metal treatment plants are an important health issue for workers and local residents.

Lead PM enters the human body mainly through inhalation and ingestion; with inhalation being the major exposure route at the workplace.[7] Following lead-rich PM inhalation, deposition rates in lungs are estimated to be 30−50%,[7] with an alveolar absorption rate of >90% of the deposited amount. One of the main toxic effects of lead is caused by its ability to bind to biomembrane structures, altering their function.[8,9] Lead(II) can then quickly combine with thiol groups, leading to depletion of glutathione (GSH) (a cellular antioxidant) and disruption of the prooxidant/antioxidant cellular balance.[10] Thus, oxidative stress is a secondary mechanism of lead toxicity.[11] In fact, both acellular assays (Dithiothreitol (DTT) test)[12] and in vitro biological assays of common PM target cells (e.g., macrophages or respiratory epithelial cells)[13] have been used to measure PM-induced oxidative stress.

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Although lead PM ingestion is a secondary exposure route in adults; it is the main pathway by which children are exposed to lead: via ingestion of contaminated dust and soil.[14] Moreover, inhaled PM can be secondarily ingested during removal of particles from the respiratory tract by the mucociliary escalator.[15] Thus, the lead fraction that reaches the circulatory system (i.e., that which is bioavailable) following exposure to Pb-rich PM is controlled by the amount of Pb that is solubilized into gastric and intestinal fluids.[16] Lead bioaccessibility is defined as the maximal in vitro amount of lead solubilized by sequential extraction with synthetic digestive fluids.[17]

Because the mechanisms involved in PM-mediated health effects are complex, numerous complementary tests must be used. In addition, most studies have dealt with urban PM,[18,19] while knowledge of industrial lead-rich process PM is limited. Moreover, available studies have focused on a single exposure route or biological end point, such as oxidative stress,[20] inflammation,[2] or bioaccessibility.[21] Therefore, the aim of the present study was to characterize the fate and the impact of lead-rich PM following inhalation or ingestion, combining acellular assays of bioaccessibility and the oxidative potential of lead-rich process PM with an in vitro cellular assay of pro-inflammatory potential using bronchial epithelial cells. Moreover, hypotheses for mechanisms involved were proposed and correlation between biological and chemical in vitro tests was studied.

MATERIALS AND METHODS

Particle Sampling and Characterization. A secondary lead smelter for battery recycling in urban area was studied. Process PM associated with this plant has been previously described in Uzu et al.[22] Three sources of harmful particles directly impacting workers and the environment were identified: (i) ambient air from rotary furnaces (hereafter specified as *Furnace PM*); (ii) ambient air from refinery where lead is purified from unwanted metals or enriched (*Refining PM*); and (iii) channeled emissions which evacuate fumes and gases from the furnace to outside of the factory (*Emissions PM*). PM_{tot} in the ambient air in the Furnace and Refinery working units was 100−700 $\mu g \cdot m^{-3}$ and 70−200 $\mu g \cdot m^{-3}$, respectively. Three months after complete cleaning of the two work units, 1 kg of settled down (2 m high sheet) PM from ambient air from furnaces and refinery working units was collected in polyethylene bags by surface wiping. According to Uzu et al.[22] that PM collection of fallouts on beams (not only larger particles) is representative of three months emission period. Emission PM was also collected from electrofilters used in the facility. PM samples were passed through a certified 2-mm stainless sieve before storage at 4 °C in sealed opaque containers with Merck desiccant.

A part of sampled PM was size-segregated by air resuspension in Teflon bags (compressed air) and impaction in a $PM_{10/2.5/1}$ cascade using a Dekati inertial impactor, leading to nine PM sets (3 sources × 3 sizes): coarse ($PM_{10-2.5}$ particles with an aerodynamic diameter between 2.5 and 10 μm), fine ($PM_{2.5-1}$), and PM_1 (diameter <1 μm).[23] The impactor consists of successive stages, with aerodynamic cutoff diameters of 10, 2.5, and 1 μm when operated with 10 L min^{-1} airflow. The other part of PM was not-size segregated (kept as raw PM) and was noted as PM_{tot}. Supplementary details on particle sampling and size segregation are also available in Uzu et al. 2009, 2010, and 2011[6,22,24]. Finally, size-segregated PM sets ($PM_{10-2.5/2.5-1/<1}$) and PM_{tot} were kept dry in black containers with desiccant at 4 °C before use for acellular bioaccessibility and oxidative potential assays, as well as for cellular in vitro toxicity tests.

PM_{tot} was used for size distribution measure by laser granulometric analysis after dispersion in ethanol and a 10 min sonication using a Malvern Mastersizer S (in the range 0.05−900 μm).

The elemental lead content of $PM_{tot/10-2.5/2.5-1/<1}$ samples was determined by inductively coupled plasma-mass spectrometry ICP-MS, Agilent Technologies, after heated digestion with standard acid (0.4 g of PM in a 3 mL mixture of 37% HCl:70% HNO_3 (3:1) and 20 μL of HF) in a PTFE vessel. In previous studies, the main species identified included PbS, $PbSO_4$, $PbO \cdot PbSO_4$, PbO, $PbCO_3$, Pb^0, Na_2SO_4, and $ZnSO_4$.[22,24]

Gastric/Gastrointestinal Bioaccessibility Assay. Lead bioaccessibility was assessed using the Unified Barge Method (UBM)[25] validated with an in vivo model (young swine) for Pb, Cd, and As.[26] UBM test performed at 37 °C first simulates chemical processes occurring in the mouth and stomach (gastric phase, pH 1.0), followed by the intestines (gastrointestinal phase, pH 6.5), using synthetic digestive solutions.[27] UBM bioaccessibility tests were conducted in triplicate only on PM_{tot} and $PM_{2.5-1}$ fractions (insufficient quantities were available for the other size fractions). Briefly, 0.1 g of PM was added to 9 mL of artificial saliva (pH 6.5), shaken for 5 min, then 13.5 mL of gastric solution (pH 1.0) was added and the pH of the solution was adjusted to 1.2 using HCl suprapur 37%. The suspension was mixed using an end-over-end rotation at 37 °C for 1 h and the pH was adjusted with HCl 37% to 1.2−1.7. The gastric phase was then extracted by centrifugation at 3000*g* for 5 min and Pb concentration in the solution was then measured by inductively coupled plasma-optical emission spectrometry (ICP-OES; Jobin Yvon). In parallel, a gastrointestinal extraction was carried out on a second sample of the same particles. Following the stomach step as described above, bile and intestinal solution[27] were added and pH was subsequently increased to reach a pH in the range 5.8−6.8. Solutions were mixed end-over-end for a further 4 h and centrifuged at 3000*g*. Pb concentration was measured in the supernatant (gastro-intestinal phase). The accuracy of the UBM extraction was checked using the NIST reference materials SMR 2710 and 2711 (Pb contents 5510 mg $\cdot kg^{-1}$ and 1096 mg $\cdot kg^{-1}$). According to the in vitro bioaccessibility test used in this work, Pb recovery in the two reference materials was 100% for SMR 2710 and 98% for 2711. In a manner similar \to the test soil samples, the bioaccessibility values were significantly higher for the saliva-gastric phase than for the intestinal phase (α = 5%). For each phase, bioaccessibility was significantly different among soil samples (α = 5%). For the saliva-gastric phase, the bioaccessibility value reached 87.9% of the total Pb content for NIST 2710 and 85% for NIST 2711. In the intestinal phase, the bioaccessibility value reached 25% of the total Pb content for NIST 2710 and 13% for NIST 2711.

Assay of the Intrinsic Oxidative Potential of the PM. *Particle Suspensions.* A stock suspension of 50 mg PM $\cdot L^{-1}$ was prepared in surfactant media (Tween 80 0.6 mg $\cdot L^{-1}$, Fluka BioChemika) and sonicated for 15 min at 180 W in a 30 °C water bath (Ultrason Bransonic 5210) just prior the assay.

DTT Assay. The DTT chemical assay provides a measure of the intrinsic capacity of particles to catalyze electron transfer between reducing DTT (DTT E_0 = −0.33 V) and oxygen. The procedure used is described in Sauvain et al.,[28] and further detailed in the Supporting Information.

Table 1. Total Lead Content in Process Particles According to Their Size and Their Solubility in Tween 0.6 mg·L^{-1}

	emissions				refining				furnace			
size (μm)	PM_{tot}	$PM_{10-2.5}$	$PM_{2.5-1}$	PM_1	PM_{tot}	$PM_{10-2.5}$	$PM_{2.5-1}$	PM_1	PM_{tot}	$PM_{10-2.5}$	$PM_{2.5-1}$	PM_1
Pb contents (mass%) ± 0.5%	61.2[a]	57[a]	59.4	59.0+	41.3[a]	45.0[a]	41.8[a]	24.7+	45.0[a]	45.2[a]	39.9[a]	31.4+
solubility in Tween 0.6 mg·L^{-1} (% of total lead) ± 0.5%	n.a	3.2	3.5	3.3	n.a	4.8	3.4	8.5	n.a	n.a	4.5	6.6

[a] * Different from respective PM_1; + different from other PM_1 ($p < 0.01$, $n = 3$) as measured by a LSD fisher test (ANOVA one factor). n.a: not analyzed

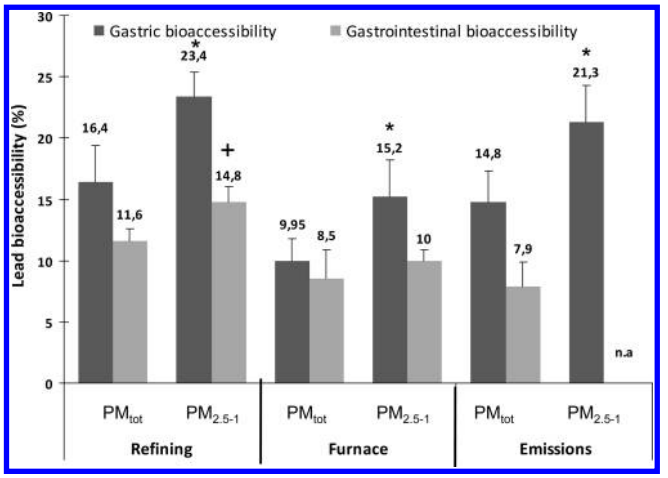

Figure 1. Gastric and gastrointestinal bioaccessibility values for PM_{tot} and $PM_{2.5-1}$. Results are expressed as the percent of total lead content that was soluble. n.a: not-analyzed because of lack of material. Error bars correspond to the standard deviation of 3 replicates.* statistically different from respective PM_{tot} for gastric values, + statistically different from respective PM_{tot} for gastrointestinal values ($p < 0.05$, one-way ANOVA, LSD Fisher test).

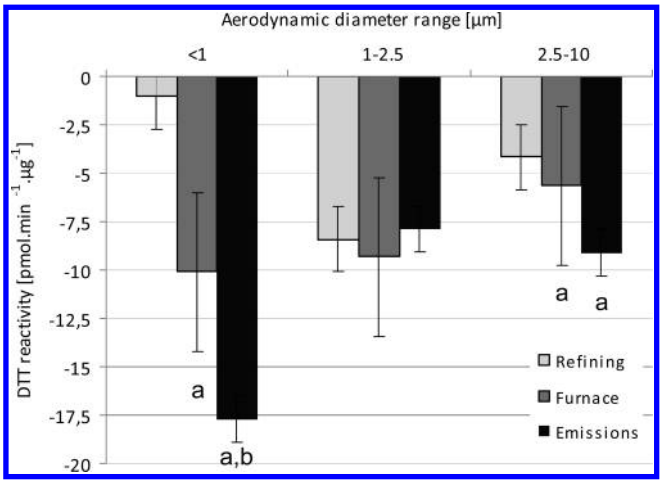

Figure 2. DTT consumption (pmol DTT·min^{-1}·μg^{-1}) for different particle sizes suspended in Tween 80 0.6 mg·L^{-1}. Error bars correspond to standard deviations for a minimum of 3 repetitions. (a) Statistically significant difference versus the corresponding Refining size fraction; (b) statistically significant difference versus the corresponding Furnace size fraction (ANOVA with Bonferroni adjustment, $p < 0.05$).

Lead Solubility from PM Samples during the DTT Test. The solubility of lead from PM samples in surfactant media (Tween 80 0.6 mg·L^{-1}) during the DTT test was assessed to better understand the mechanisms involved in the assay (details in the Supporting Information).

In vitro Cytotoxicity and Inflammation Assays. *Particles.* Stock PM suspensions were made at a concentration of 2 mg·mL^{-1} in DMEM/F12 cell culture media (Dulbecco Modified Eagle Medium Nutrient Mix F-12, Invitrogen) and stored at −20 °C before use. Suspensions were sonicated three times for 20 s at 60 kW (to favor desegregation) and diluted in cell culture medium without Ultroser G.

Cell Culture Conditions, Cytotoxic Assay, and Cytokine Assay. Cells were grown to subconfluence and exposed to PM suspensions. A cell proliferation reagent, the water-soluble tetrazolium salt (WST-1) was incubated with PM to determine (a) whether Refining, Furnace, and Emissions PM exhibit cytotoxicity against 16HBE14o- human bronchial epithelial cells exposed for 24 h to PM, from 0 to 100 μg·cm^{-2}; and (b) to determine noncytotoxic concentrations for which the pro-inflammatory response could be considered to be an adaptive response to particle exposure. This pro-inflammatory response was evaluated by the release of pro-inflammatory cytokine granulocyte monocyte colony-stimulating factor (GM-CSF), known to be triggered by oxidative stress induced by diesel particles or urban PM.[29,30] All experiments were performed according to Val et al.,[31] with methodological details provided in Supporting Information.

Statistical Analysis. Data represented as mean ± SD were evaluated by analysis of variance (ANOVA) followed by Dunnet's t test to examine differences between different treated groups with respect to control, whereas the Newmann−Keuls test was used to evaluate difference among treated groups, with $p < 0.05$ considered significant.

RESULTS

Particle Characterization. *Size Distribution.* A broad process PM size distribution (0.1−300 μm) was observed, regardless of origin (Figure S1, Supporting Information). Channelled emissions differed from Furnace and Refining PM, which were characterized by a major peak at sizes >10 μm. Refining PM displayed a Gaussian distribution, with a mean geometric diameter of 21.4 ± 0.5 μm and a maximum at 19.3 μm, whereas the distribution of Furnace PM was trimodal, with a mean geometric diameter of 24.6 ± 0.6 μm, and local maxima at 0.67, 3.59, and 26.20 μm. In contrast with the coarser Refining and Furnace PM, Emissions PM consisted mainly of fine particles (mean geometric diameter 3.9 ± 0.8 μm), characterized by a bimodal distribution, with local maxima at 0.57 and 4.19 μm: $PM_{10-2.5}$ accounted for 50%; $PM_{2.5-1}$ 20%; and PM_1 21%. The respirable PM fraction <10 μm represented 21%, 27%, and 90% of the total mass of Refining, Furnace, and Emissions PM, respectively.

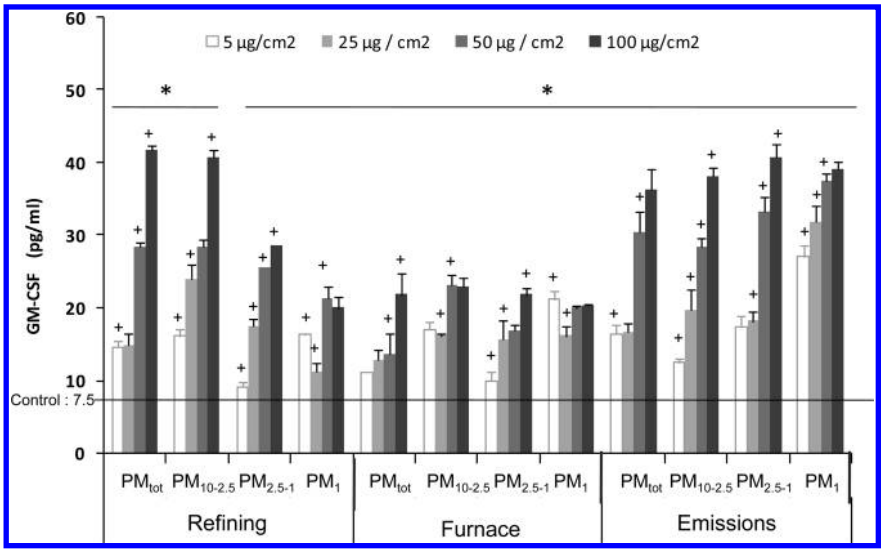

Figure 3. Concentration of GM-CSF, a pro-inflammatory cytokine, in 16HBE14o- cell culture media following exposure to various concentrations of PM. Error bars correspond to the standard deviation of 5 replicates. Data are averages for 3 wells ± SD (* statistically different from control, $p < 0\,0.05$; + statistically different from the treated groups for one size, one origin at various doses, $p < 0.05$).

Total Lead Concentrations. All process PM displayed high Pb levels (25−60% dry weight), regardless of their origin or size as displayed in Table 1. Although no general trend appeared as a function of size; the concentration of lead in PM_1 was significantly lower than in other corresponding fractions ($p < 0.05$, one-way ANOVA).

Bioaccessibility. The bioaccessibility of gastric lead by UBM method in Figure 1 was greater than that of gastrointestinal lead, regardless of particle size or origin; the highest lead bioaccessibilities were observed for Refining and Emissions $PM_{2.5-1}$. The percentage of soluble lead in gastric fluids (pH ∼2) ranged from 10 to 16% for PM_{tot} and 15 to 23% for $PM_{2.5-1}$. In addition, with the exception of furnace PM_{tot}, gastric bioaccessibility was always greater ($p < 0.05$, one-way ANOVA) for $PM_{2.5-1}$ versus PM_{tot}.

Intrinsic Oxidative Potential of the PM Samples. In Figure 2 intrinsic DTT reactivity was obtained by subtracting the control consumption rate from DTT consumption rates in the presence of particles, then dividing by the particle mass in the reaction tube. The observed negative values indicate that all lead-rich PM samples slowed down DTT oxidation versus the control. For PM_1 and $PM_{2.5-10}$, mass-based DTT reactivity strongly depended on their origin: the most negative values were observed for Emission PM, followed by Furnace and Refining PM samples. Assessments of PM dissolution were performed in the presence of DTT because similar reducing conditions are found in the lung lining fluid.[32] In fact, significant dissolution (3−8% Table 1) took place under such conditions, which was larger than that reported in $CaCl_2$.[24] Pb^{2+} can be complexed by DTT, and based on the Pb−DTT complexation stability constant ($\log \beta = 13.9$[33]), the calculated percentage of complexed lead was between 81 and 88%. Refining and Furnace PM_1 fractions displayed statistically higher solubility (8.5 ± 0.2 and 6.6 ± 0.02%, respectively) than other fractions (average 3.8 ± 0.7%; Kruskal−Wallis test, $p = 0.002$). An inverse linear relationship was observed between the total lead content in the PM samples and lead solubility in Tween media (Figure S2). Increased DTT stabilization (more negative values) was observed with increasing total Pb content in PM (Figure S3A), whereas an inverse relationship between DTT reactivity and Pb solubility was observed (Figure S3B): suggesting that the less soluble the PM, the greater the stabilization of DTT.

Cytotoxicity and Pro-Inflammatory Response to PM. The cytotoxic response induced in 16HBE14o- cells by 24 h exposure to the different PM samples was found to be a function of the work unit: (i) Refining PM did not induce cytotoxicity, regardless of particle size and tested concentrations; (ii) Furnace PM only induced cytotoxicity for fine ($PM_{2.5-1}$) and PM_1 fractions at high concentrations (50 and 100 $\mu g \cdot cm^{-2}$); and (iii) Emissions PM induced cytotoxicity at the highest concentration tested (100 $\mu g \cdot cm^{-2}$) for all size fractions. Based on the above, we conclude that exposure of 16HBE14o- cells to particle concentrations up to 25 $\mu g \cdot cm^{-2}$ does not induce cytotoxicity.

Positive dose-dependent relationships were observed for GM-CSF cytokine release by 16HBE14o- cells (Figure 3) after 24 h exposure to PM (pro-inflammatory response). At the lowest noncytotoxic dose (5 μg PM $\cdot cm^{-2}$), PM_1 displayed the most significant GM-CSF induction potential, suggesting that this fraction strongly contributes to pro-inflammatory processes. Considering fraction PM_1, which is the most prone to interact with bronchial epithelial cells, Emissions PM induced a higher pro-inflammatory response than Refining and Furnace PM at noncytotoxic concentrations. Regardless of PM origin, GM-CSF secretion increased in parallel with total lead content (Figure S4A), and was inversely related to lead solubility (Figure S4B).

DISCUSSION

Influence of Lead Solubility and Chemical Speciation on PM Reactivity. With the exception of Emissions PM, lead size-segregation was observed for process PM, with decreased Pb amounts in PM_1. Spear et al.[34] previously reported high lead association (99%) with the coarse fraction (5.8−10 μm) in lead smelting factories. While lead is poorly soluble in Tween 0.6 $mg \cdot L^{-1}$ (<8%, see Table 1) used for DTT test, we found that the finest PM readily dissolved, in agreement with Spear et al.[34] The decrease in solubility observed with increasing lead content

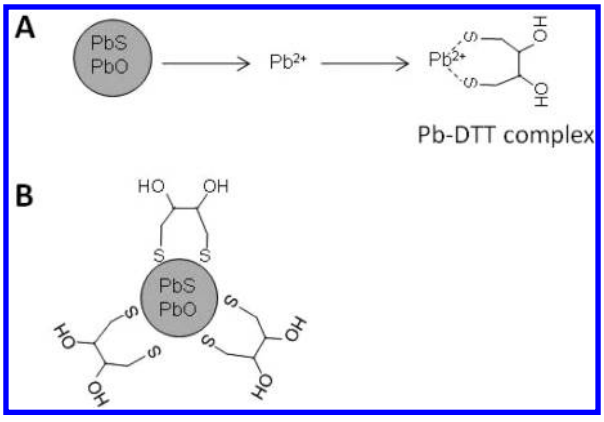

Figure 4. Potential mechanisms of DTT stabilization through complexation with solubilized lead (A), or via PM surfaces (B).

suggests that (with the exception of Emissions PM), lead speciation is PM size dependent. Yokel et al.[35] concluded that lead solubility strongly influences Pb bioavailability. As, PbS, $PbSO_4$, $PbO \cdot PbSO_4$, PbO, $PbCO_3$, and Pb^0 are the major lead compounds of PM process,[22] the highly water-soluble sulfates and carbonates lead compounds[36] could therefore reach higher percentages in finest PM_1 fractions.

Higher bioaccessibilities of gastric lead (25%, pH ~1.9) versus gastrointestinal lead (15%, pH = 7) are in agreement with UBM validations, and are possibly due to pH differences which may induce lead precipitation in the intestines.[37] Caboche[26] observed a greater correlation between in vivo lead absorption by young pigs and UBM gastric bioaccessibility versus gastrointestinal bioaccessibility. Refining and Emissions PM fraction displaying the highest gastric bioaccessibility could therefore represent a greater sanitary risk by ingestion. Measured lead bioavailabilities for process PM were relatively low compared to previous studies on various hazardous materials: 52.4−77.2% bioavailability in gastric fluid and 4.9−32.1% bioavailability in intestinal fluid for <75 μm house dust;[38] >65% bioavailability in gastric fluid and <20% bioavailability in duodenal fluid for soluble lead from polluted soils.[39] However, by no means are they the lowest in literature, considering 3% of gastric bioaccessibility of mine wastes (PBET test) in Bruce et al.[40] and could result from solubility limit. But, considering the high total particle lead content, Pb quantities which may reach systemic circulation should not be neglected.

Potential Effects Induced by Lead-Rich PM during Inhalation. When 16HBE14o- human bronchial epithelial cells were exposed to noncytotoxic PM concentrations (<25 $\mu g \cdot cm^{-2}$), a significant increase in released GM-CSF was observed for PM_1, regardless of origin. Airway inflammation is the most often observed short-term effect of human exposure to PM, and most studies agree that the intrinsic inflammation potential is increased from coarse to fine particles when compared on a similar mass basis.[30,41] Interestingly, a positive relationship between cytokine release and total lead content (Figure S4A), and a negative relationship between Pb^{2+} and cytokine release (Figure S4B), was observed for the studied PM samples with relatively low Pb solubility. The inflammatory response could therefore be mainly driven by PM in correlation with the total lead content.

Negative DTT reactivities were observed with process PM enriched with lead (Figure 2), contradicting the positive reactivities reported for organic PM.[12,28,42] Lead-rich PM may stabilize DTT (negative reactivity) and inhibit its oxidation via DTT−Pb complexation, which can take place either with Pb^{2+} [33] or directly on the particle surface as described in Figure 4A and B.

In addition, DTT−Pb complexation could hinder the formation of disulfur bonds in DTT. In the experimental reaction media (which contained a large excess of DTT), more than 80% of the dissolved Pb^{2+} was calculated to be in complex with DTT. The same stabilization effect was observed with lead acetate solution (−189 ± 14 pmol/min·μg Pb; these results are not comparable with the data shown in Figure 2, which are expressed as the total particle mass). Thus, if only Pb^{2+} ions were responsible for DTT stabilization (via complexation), DTT reactivity should decrease as a function of lead solubility, however, the opposite was observed (Figure S3B). This suggests that DTT sulphydryl groups−PM surfaces interactions are mainly involved.

The biological effects of PM lead following lung deposition may thus be due to reaction of sulfur-containing antioxidants or proteins in lung lining fluids with Pb, via a mechanism similar to that of DTT. Indeed, oxidative stress was proposed as a mechanism taking place in the pathophysiology of lead poisoning[11] or cardiovascular disease.[43] Lead toxicity is currently attributed to its ability to bind biomembranes, altering their functions.[9] Indeed, complex formation between lead and reduced glutathione or glutathione reductase inhibits their activity, suggesting a plausible mechanism explaining the decreased antioxidant activity of such vital peptides/enzymes.[8] Such inhibitory effects would shift the oxidant−antioxidant balance in the direction of oxidative stress, thus rendering cells more vulnerable to oxidative attack.[44]

Comparison between Acellular DTT and in Vitro Pro-Inflammatory Cell Culture Tests. Because GM-CSF release is triggered by oxidative stress,[45,46] the linear relationship between DTT reactivity and GM-CSF ratios (Figure S5) suggests that the DTT assay correlates with biological responses. With the exception of Refining $PM_{2.5-10}$, increased negative DTT reactivities correlate with an increased release of GM-CSF. This is particularly evident for PM_1. Li et al.[19] observed a correlation between DTT activity in ambient PM and cellular oxidant stress response. Our results strongly suggest that lead-rich process PM induces inflammation in the lungs. Intriguingly, such cytokine induction (by PM addition) is not due to the release of reactive oxygen species (as negative DTT values were observed), but rather could be due to an imbalance between pro and antioxidants, via depletion of sulfur-containing antioxidants by lead complexation as previously discussed. Together, these results suggest that the DTT assay not only responds to oxidants, but also provides some clues about the complexation abilities of lead-rich PM with other thiol containing compounds.

Finally, the potential toxicity of the studied PM_1 fraction can be classified as follows: Emissions > Furnace > Refining.

Sanitary Risk Assessment of Lead-Rich PM: Calculation and Comparison of the Lead Burden from Inhalation and Ingestion. Using the PM_{tot} concentrations at the Furnace (average 350 $\mu g \cdot m^{-3}$) and Refining (average 125 $\mu g \cdot m^{-3}$) work units, coupled with the size distributions (Figure S1) and lead content in each fraction (Table 1); we evaluated the lung and gastric burden due to inhalation of lead-rich particles in Table 2. For calculation of the deposited dose, we hypothesized that (i) workers inhale 10 m^3 during a typical work shift;[47] (ii) only 10.5% of the inhaled PM deposit in the alveolar region, and all PM deposited in the head and trachea−bronchial regions of the pulmonary tree will finally be ingested; (iii) lead solubility is

Table 2. Evaluation of the Inhaled and Ingested Dose of Total and Soluble Lead

	furnace	refining	remarks
PM_{tot} [$\mu g \cdot m^{-3}$]	350	125	typical concentrations measured on site
inhalable PM [$\mu g \cdot m^{-3}$]	235	85.5	using the inhalable convention and size distribution (Figure S1)[49]
inhaled volume for a shift (8 h) [m^3]	10	10	
calculated inhaled PM mass per shift [μg]	2348	855	
calculated deposition fraction			calculated with the MPPD software (CIIT, RIVM, http://www.thehamner.org/mppd/helpfiles/index.htm
head [%]	54.6	57.4	
conducting airways [%]	7.0	7.2	
alveolar [%]	10.5	11.0	
particle mass deposited in the head region per shift [μg]	1282	491	
particle mass deposited in the conducting airways per shift [μg]	164	62	
particle mass deposited in the alveolar region per shift[μg]	247	94	
total pulmonary particle burden per shift [μg]	247	94	
total ingested particle burden per shift [μg]	1446	552	Σ deposited mass in head + conducting airways region
average lead content in particle [%]	38	36	
average Pb solubility in the lung lining fluid [%]	5	5	estimated from the solubilities in Tween (see Table 1)
average Pb solubility in gastric juice [%]	10	16	see Figure 1
total lead burden in lungs per shift [μg]	96	35	
total gastric lead burden per shift [μg]	562	205	
total bioaccessible lead burden in lungs per shift [μg]	5	2	
total bioaccessible lead burden in digestive track per shift [μg]	56	33	
ratio lead ingested to inhaled	6	6	
ratio dissolved lead in digestive track to lung	11	16	

approximately 5% in the lung lining fluid (assuming the value in the surfactant media Tween, 0.6 $mg \cdot L^{-1}$, see Table 1) and 10−16% in gastric juice (Figure 1). According to these calculations, ingestion is the most important source of lead bioavailability. Total lead deposited in the lungs during a typical 8-h work shift is 6 times lower than the ingested fraction (e.g., compare 96 μg total Pb deposited in lungs vs 562 μg total Pb ingested for Furnace, see Table 2); while this ratio for bioaccessible Pb^{2+} increases to 11−16 times (e.g., compare 5 μg Pb^{2+} in lung to 56 μg Pb^{2+} ingested for Furnace, Table 2). The Pb^{2+} fraction in the lungs is minimal, given that internalized PM is typically found in phagolysosomes, where low pH values favor their dissolution.[48]

In addition to an increased lead content, Emission particles contain the largest proportion of fine PM. Regardless of origin, the finest PM_1 fractions are the most efficient at inducing pro-inflammation responses. Moreover, among the studied fractions (PM_{tot} and $PM_{2.5-1}$), the highest gastric bioaccessibility was observed for $PM_{2.5-1}$. The increased reactivity of Emission PM with SH groups (as suggested by the DTT test), coupled with their pro-inflammatory potential toward epithelial cells, suggests that Emission PM should be classified as the most hazardous PM from the studied plant. According to Turner and Radford and Ridgway[21,50] the present study illustrates the interest of a combination of complementary acellular and cell-based assays for evaluating the biological effects of complex hazardous particles.

■ ASSOCIATED CONTENT

Supporting Information. Additional information on the methodology, particle characterization, and relationships between particle properties and their reactivity. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ REFERENCES

(1) Brook, R. D.; Franklin, B.; Cascio, W.; Hong, Y.; Howard, G.; Lipsett, M.; Luepker, R.; Mittleman, M.; Samet, J.; Smith, S. C.; Tager, I. Air pollution and cardiovascular disease: A statement for healthcare professionals from the expert panel on population and prevention science of the American heart association. *Circulation* **2004**, *109* (21), 2655–2671.

(2) Perrone, M. G.; Gualtieri, M.; Ferrero, L.; Lo Porto, C.; Udisti, R.; Bolzacchini, E.; Camatini, M. Seasonal variations in chemical composition and in vitro biological effects of fine PM from Milan. *Chemosphere* **2010**, *78* (11), 1368–1377.

(3) Polichetti, G.; Cocco, S.; Spinali, A.; Trimarco, V.; Nunziata, A. Effects of particulate matter (PM10, PM2.5 and PM1) on the cardiovascular system. *Toxicology* **2009**, *261* (1−2), 1–8.

(4) Aust, A. E.; Ball, J. C.; Hu, A. A.; Lighty, J. S.; Smith, K. R.; Straccia, A. M.; Veranth, J. M.; Young, W. C. Particle characteristics responsible for effects on human lung epithelial cells. *Res. Rep. (Health Effects Institute)* **2002**, (110), 1−65; discussion 67−76.

(5) Donisa, C.; Mocanu, R.; Steinnes, E.; Vasu, A. Heavy metal pollution by atmospheric transport in natural soils from the northern part of eastern carpathians. *Water, Air Soil Poll.* **2000**, *120* (3), 347–358.

(6) Uzu, G. l.; Sobanska, S.; Sarret, G. r.; Muñoz, M.; Dumat, C. Foliar lead uptake by lettuce exposed to atmospheric fallouts. *Environ. Sci. Technol.* **2010**, *44* (3), 1036–1042.
(7) Lauwerys, R. R.; Haufroid, V.; Hoet, P.; Lison, D. *Toxicologie Industrielle Et Intoxications Professionnelles*; Elsevier: Masson; **2007**.
(8) Gurer, H.; Ercal, N. Can antioxidants be beneficial in the treatment of lead poisoning? *Free Radical Biol. Med.* **2000**, *29* (10), 927–945.
(9) Halliwell, B. Biochemistry of oxidative stress. *Biochem. Soc. Trans.* **2007**, *35* (5), 1147–1147.
(10) Gurer-Orhan, H.; Sabir, H. U.; Ozgünes, H. Correlation between clinical indicators of lead poisoning and oxidative stress parameters in controls and lead-exposed workers. *Toxicology* **2004**, *195* (2−3), 147–154.
(11) Garçon, G.; Leleu, B.; Zerimech, F.; Marez, T.; Haguenoer, J.-M.; Furon, D.; Shirali, P. Biologic markers of oxidative stress and nephrotoxicity as studied in biomonitoring of adverse effects of occupational exposure to lead and cadmium. *J. Occup. Environ. Med.* **2004**, *46* (11), 1180–1186.
(12) Cho, A. K.; Sioutas, C.; Miguel, A. H.; Kumagai, Y.; Schmitz, D. A.; Singh, M.; Eiguren-Fernandez, A.; Froines, J. R. Redox activity of airborne particulate matter at different sites in the Los Angeles basin. *Environ. Res.* **2005**, *99* (1), 40–47.
(13) Baulig, A.; Poirault, J.-J.; Ausset, P.; Schins, R.; Shi, T.; Baralle, D.; Dorlhene, P.; Meyer, M.; Lefevre, R.; Baeza-Squiban, A.; Marano, F. Physicochemical characteristics and biological activities of seasonal atmospheric particulate matter sampling in two locations of Paris. *Environ. Sci. Technol.* **2004**, *38* (22), 5985–5992.
(14) Landrigan, P. J.; Todd, A. C. Lead poisoning. *Western J. Med.* **1994**, *161*, 153–159.
(15) Roth, J. A. Homeostatic and toxic mechanisms regulating manganese uptake, retention, and elimination. *Biol. Res.* **2006**, *39* (1), 45–57.
(16) Kelley, M. E.; Brauning, S. E.; Schoof, R. A.; Ruby, M. V. *Assessing Oral Bioavailability of Metals in Soil*; Battelle Press: Columbus, OH, 2002.
(17) Oomen, A. G.; Hack, A.; Minekus, M.; Zeijdner, E.; Cornelis, C.; Schoeters, G.; Verstraete, W.; Van de Wiele, T.; Wragg, J.; Rompelberg, C. J. M.; Sips, A. J. A. M.; Van Wijnen, J. H. Comparison of five in vitro digestion models to study the bioaccessibility of soil contaminants. *Environ. Sci. Technol.* **2002**, *36* (15), 3326–3334.
(18) Annesi-Maesano, I.; Dab, W. Pollution atmosphérique et poumon: Approche épidémiologique. *Med Sci (Paris)* **2006**, *22* (6), 589–594.
(19) Li, N.; Sioutas, C.; Cho, A.; Schmitz, D.; Misra, C.; Sempf, J.; Wang, M.; Oberley, T.; Froines, J.; Nel, A. Ultrafine particulate pollutants induce oxidative stress and mitochondrial damage. *Environ. Health Perspect.* **2003**, *111* (4), 455−460–455−460.
(20) Ntziachristos, L.; Froines, J. R.; Cho, A. K.; Sioutas, C., Relationship between redox activity and chemical speciation of size-fractionated particulate matter. *Part. Fibre Toxicol.* **2007**, *4* (5).
(21) Turner, A.; Radford, A. Bioaccessibility of trace metals in boat paint particles. *Ecotoxicol. Environ. Saf.* **2010**, *73* (5), 817–24.
(22) Uzu, G.; Sobanska, S.; Aliouane, Y.; Pradere, P.; Dumat, C. Study of lead phytoavailability for atmospheric industrial micronic and sub-micronic particles in relation with lead speciation. *Environ. Pollut.* **2009**, *157* (4), 1178–1185.
(23) Batonneau, Y.; Bremard, C.; Gengembre, L.; Laureyns, J.; Le Maguer, A.; Le Maguer, D.; Perdrix, E.; Sobanska, S. Speciation of PM10 sources of airborne nonferrous metals within the 3-km zone of lead/zinc smelters. *Environ. Sci. Technol.* **2004**, *38* (20), 5281–5289.
(24) Uzu, G.; Sobanska, S.; Sarret, G.; Sauvain, J. J.; Pradere, P.; Dumat, C. Characterization of lead-recycling facility emissions at various workplaces: Major insights for sanitary risks assessment. *J. Hazard. Mater.* **2011**, *186* (2−3), 1018–1027.
(25) Cave, M.; Wragg, J.; Klinck, B.; Grön, C.; Oomen, T.; Van de Wiele, T.; Ollson, I.; Koch, K.; Reimer, N.; Basta, N.; Tack, K.; Casteel, S. Preliminary assessment of a unified bioaccessibility method for arsenic in soils. In *International Conference in Epidemiology and Environmental Exposure*, Paris, 2006.
(26) Caboche, J. Validation d'un test de mesure de bioaccessibilité. Application a quatre elements traces metalliques dans les sols: As, Cd, Pb et sb. Ph.D. Dissertation, Institut National Polytechnique de Lorraine, Nancy, France, 2009.
(27) Denys, S.; Tack, K.; Caboche, J.; Delalain, P. Bioaccessibility, solid phase distribution, and speciation of Sb in soils and in digestive fluids. *Chemosphere* **2009**, *74* (5), 711–716.
(28) Sauvain, J.-J.; Deslarzes, S.; Riediker, M. Nanoparticle reactivity toward dithiothreitol. *Nanotoxicology* **2008**, *2* (3), 121–129.
(29) Baulig, A.; Sourdeval, M.; Meyer, M.; Marano, F.; Baeza-Squiban, A. Biological effects of atmospheric particles on human bronchial epithelial cells. Comparison with diesel exhaust particles. *Toxicol. in Vitro* **2008**, *17* (5−6), 567–573.
(30) Ramgolam, K.; Chevaillier, S.; Marano, F.; Baeza-Squiban, A.; Martinon, L. Proinflammatory effect of fine and ultrafine particulate matter using size-resolved urban aerosols from Paris. *Chemosphere* **2008**, *72* (9), 1340–1346.
(31) Val, S.; Hussain, S.; Boland, S.; Hamel, R.; Baeza-Squiban, A.; Marano, F. Carbon black and titanium dioxide nanoparticles induce pro-inflammatory responses in bronchial epithelial cells: Need for multi-parametric evaluation due to adsorption artifacts. *Inhalation Toxicol.* **2009**, *21* (s1), 115–115.
(32) Borm, P. J. A.; Kelly, F.; Kunzli, N.; Schins, R. P. F.; Donaldson, K. Oxidant generation by particulate matter: From biologically effective dose to a promising, novel metric. *Occup. Environ. Med.* **2007**, *64* (2), 73–74.
(33) Krężel, A.; Maret, W. Different redox states of metallothionein/thionein in biological tissue. *Biochem. J.* **2007**, *402* (3), 551–558.
(34) Spear, T. M.; Svee, W.; Vincent, J. H.; Stanisich, N. Chemical speciation of lead dust associated with primary lead smelting. *Environ. Health Perspect.* **1998**, *106* (9), 565–571.
(35) Yokel, R. A.; Lasley, S. M.; Dorman, D. C. The speciation of metals in mammals influences their toxicokinetics and toxicodynamics and therefore human health risk assessment. *J. Toxicol. Environ. Health, Part B* **2006**, *9* (1), 63–85.
(36) Sammut, M. L.; Noack, Y.; Rose, J.; Hazemann, J. L.; Proux, O.; Depoux, M.; Ziebel, A.; Fiani, E. Speciation of Cd and Pb in dust emitted from sinter plant. *Chemosphere* **2010**, *78* (4), 445–450.
(37) Denys, S.; Caboche, J.; Tack, K.; Delalain, P. Bioaccessibility of lead in high carbonate soils. *J. Environ. Sci. Health, Part A* **2007**, *42* (9), 1331–1339.
(38) Chang, Ho, Y.; Lih-Ming, Y.; Paul, J. L. The bioaccessibility of lead (Pb) from vacuumed house dust on carpets in urban residences. *Risk Anal.* **2006**, *26* (1), 125–134.
(39) Bosso, S.; Enzweiler, J.; Angélica, R. Lead bioaccessibility in soil and mine wastes after immobilization with phosphate. *Water, Air Soil Pollut.* **2008**, *195* (1), 257–273.
(40) Bruce, S. N.; Barry; Matanitobua, V.; Ng, J. In vitro physiologically based extraction test (pbet) and bioaccessibility of arsenic and lead from various mine waste materials. *J. Toxicol. Environ. Health, Part A* **2007**, *70*, 1700–1711.
(41) Takano, H.; Ichinose, T.; Miyabara, Y.; Shibuya, T.; Lim, H.-B.; Yoshikawa, T.; Sagai, M. Inhalation of diesel exhaust enhances allergen-related eosinophil recruitment and airway hyperresponsiveness in mice. *Toxicol. Appl. Pharmacol.* **1998**, *150* (2), 328–337.
(42) Li, N.; Hao, M.; Phalen, R. F.; Hinds, W. C.; Nel, A. E. Particulate air pollutants and asthma: A paradigm for the role of oxidative stress in pm-induced adverse health effects. *Clin. Immunol.* **2003**, *109* (3), 250–265.
(43) Navas-Acien, A.; Guallar, E.; Silbergeld, E. K.; Rothenberg, S. J. Lead exposure and cardiovascular disease - a systematic review. *Environ. Health Perspect.* **2006**.
(44) Flora, S. J. S.; Mittal, M.; Mehta, A. Heavy metal induced oxidative stress & its possible reversal by chelation therapy. *Indian J. Med. Res.* **2008**, *128* (4), 501–523.
(45) Bonvallot, V.; Baeza-Squiban, A.; Baulig, A.; Brulant, S.; Boland, S.; Muzeau, F.; Barouki, R.; Marano, F. Organic compounds from diesel

exhaust particles elicit a proinflammatory response in human airway epithelial cells and induce cytochrome p450 1a1 expression. *Am. J. Respir. Cell Mol. Biol.* **2001**, *25* (4), 515–521.

(46) Mastruzzo, C.; Crimi, N.; Vancheri, C. Role of oxidative stress in pulmonary fibrosis. *Monaldi Arch. Chest. Dis.* **2002**, *57* (3−4), 173–6.

(47) IRCP: International Commission on Radiological Protection. *Report of the task group on reference man*; Pergamon Press: Oxford, UK, 1992; 23, 343−347.

(48) Lundborg, M.; Falk, R.; Johansson, A.; Kreyling, W.; Camner, P. Phagolysosomal pH and dissolution of cobalt oxide particles by alveolar macrophages. *Environ. Health Perspect.* **1992**, *97*, 153–157.

(49) CEN. Workplace atmospheres − size fraction definitions for measurement of airborne particles. In Vol. European Standard EN 481, Bruxelles, 1993.

(50) Ridgway, P. The promotion of alternatives under reach: A political and regulatory perspective. *Rep. Toxicol.* **2009**, *28* (2), 141–141.