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Pulmonary and gastric lead burden assessment for lead-recycling plant workers

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Key words: bioaccessibility, DTT, lead, PM, oxidative stress, toxicity.

Abstract
As a function of PM size ($PM_{10-2.5}$, $PM_{2.5-1}$ and PM_1) and origin (furnace, refining and channeled emissions), lead toxicity and bioaccessibility assessment were performed in a lead recycling plant using complementary biologic and chemical acellular tests. According to their origin, process PM displays differences in metal contents, granulometry and percentage of inhalable fraction. Lead gastric bioaccessibility was relatively low (maximum 25%) in comparison with previous available studies, though due to their high total lead concentrations, significant metal quantities could reach the circulatory system. Whatever their origin, finest PM_1 particles induced the greatest pro-inflammatory response from human bronchial epithelial cells. Moreover, this biological response was correlated with the acellular DiThioThreitol (DTT) assay, suggesting some biological predictive value for such acellular test.

Introduction
Among numerous epidemiological studies showing that atmospheric particulate matter (PM) increases respiratory and cardiovascular pathologies in urban areas, many deal with PM from transport. But, the fate and effects of PM from industry enriched with metals and emitted in the atmosphere, focused in the present work, was poorly studied (Uzu et al., 2011). PM of different size ($PM_{10-2.5}$, $PM_{2.5-1}$ and PM_1) and origin (furnace, refining and channeled emissions), lead toxicity and bioaccessibility assessment was performed in a lead recycling plant using complementary biological and chemical acellular tests.

Materials and Methods
Various size fractions of PM were separated and characterized according to Uzu et al. (2009, 2010 & 2011). Size distribution (laser granulometry), total lead concentration (ICP-OES after acid digestion) and reactivity were studied. Bioacessibility was measured according to Unified Barge Method (UBM) (Cave et al., 2006). DTT Assay was performed according to Sauvain et al. (2008). In vitro assays of cytotoxicity and inflammation were performed according to Val et al. (2009).

Results and Discussion
As determined by their origin, process PM presented differences in metal contents, granulometry and percentage of inhalable fraction. Percentages of soluble lead in gastric fluids (pH~2) ranged from 10 to 16% for PM_{tot} and 15 to 23% for $PM_{2.5-1.}$ Positive dose-dependent relations were observed for GM-CSF cytokine release by 16HBE14o- cells after 24h exposure to PM (pro-inflammatory response). At the lowest non-cytotoxic dose (5 µgPM.cm^{-2}), PM_1 let appear the most important GM-CSF induction potential, suggesting that this fraction strongly contribute to pro-inflammatory effects. Considering PM_1, the most prone to interact with bronchial cells, *Emissions* showed a higher pro-inflammatory response than *Refining* and *Furnace* at non cytotoxic concentrations. Irrespective of PM origin, GM-CSF secretion increased in parallel to total lead content and was inversely related to lead solubility. Moreover, this biological response was correlated with the acellular DiThioThreitol (DTT) assay, suggesting some biological predictive value for such acellular tests.

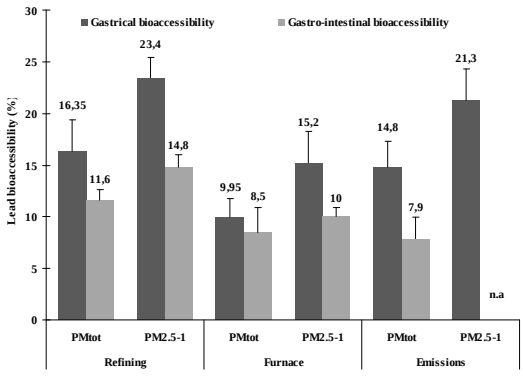

Figure 1. Gastric and gastro-intestinal bioaccessibility values for PM_{tot} and $PM_{2.5-1}$. Results are expressed as the percentage of the soluble lead and the total lead content.
*different from the respective PM_{tot} for gastrical values.

+different from the respective PM_{tot} for gastro-intestinal values ($p<0.05$ ANOVA one factor, LSD Fisher test).

Knowing PMtot concentrations at Furnace (average 350 µg.m-3) and Refining (average 125 µg.m-3), together with size distributions and lead contents in each fraction, we evaluated the lung and gastric burden due to inhalation of such lead-rich particles. Results clearly indicate that ingestion is the most important source of bioavailable lead. Total lead deposited in lungs during 8 hour shift is 6 time lower than the inhaled fraction (compare 92 µg total Pb deposited in lungs to 539 µg total Pb ingested for Furnace). When looking for the bioaccessible Pb^{2+} fraction, this ratio increases to 11-16 (compare 5 µg Pb^{2+} in lung to 54 µg Pb^{2+} ingested for Furnace). The Pb^{2+} fraction in lungs is a minimum, knowing that internalized PM is found in phagolysosomes where low pH values can favor their dissolution.

Conclusions

Taken together, our data suggest that biological behavior for such lead-rich PM could be driven by a combination of both lead solubility (related to speciation) and particulate effect. Finally, according to pro-inflammatory effect, DTT test and PM size distribution, Emissions and Furnace PM were found to be the most of concern for health. These findings are promising in a view to combine different acellular and cellular assays as recommended by REACH legislation.

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