

RESEARCH ARTICLE

# Cardiac and pulmonary oxidative stress in rats exposed to realistic emissions of source aerosols

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## Abstract

*In vivo* chemiluminescence (CL) is a measure of reactive oxygen species in tissues. CL was used to assess pulmonary and cardiac responses to inhaled aerosols derived from aged emissions of three coal-fired power plants in the USA. Sprague–Dawley rats were exposed to either filtered air or: (1) primary emissions (P); (2) ozone oxidized emissions (PO); (3) oxidized emissions + secondary organic aerosol (SOA) (POS); (4) neutralized oxidized emissions + SOA (PONS); and (5) control scenarios: oxidized emissions + SOA in the absence of primary particles (OS), oxidized emissions alone (O), and SOA alone (S). Immediately after 6 hours of exposure, CL in the lung and heart was measured. Tissues were also assayed for thiobarbituric acid reactive substances (TBARS). Exposure to P or PO aerosols led to no changes compared to filtered air in lung or heart CL at any individual plant or when all data were combined. POS caused significant increases in lung CL and TBARS at only one plant, and not in combined data from all plants; PONS resulted in increased lung CL only when data from all plants were combined. Heart CL was also significantly increased with exposure to POS only when data from all plants were combined. PONS increased heart CL significantly in one plant with TBARS accumulation, but not in combined data. Exposure to O, OS, and S had no CL effects. Univariate analyses of individual measured components of the exposure atmospheres did not identify any component associated with increased CL. These data suggest that coal-fired power plant emissions combined with other atmospheric constituents produce limited pulmonary and cardiac oxidative stress.

**Keywords:** Chemiluminescence, oxidative stress, heart, lung, ambient particles, source emissions

## Introduction

The involvement of reactive oxygen species (ROS) in the toxicity of particulate matter (PM) had been assumed for many years based on *in vitro* measurement of oxidant production (Baeza-Squiban et al., 1999; Donaldson et al., 1997; Frampton et al., 1999; Ghio et al., 1999; Goldsmith et al., 1998; Hitzfeld et al., 1997; Prahald et al., 1999; Shukla et al., 2000), and prevention of *in vitro* (Churg et al., 2005; Imrich et al., 2007; Jimenez et al., 2000; Kennedy et al., 1998; Shukla et al., 2000) and *in vivo* (Roberts et al., 2003) PM effects by antioxidants.

Organ chemiluminescence (CL) is a low intensity emission in the visible range mainly due to the decay of excited states of molecular oxygen (singlet oxygen) and excited carbonyls (Boveris et al., 1980; Cadenas & Sies

1984) formed during the termination steps of the chain reaction of lipid peroxidation (Halliwell & Gutteridge 1990). Organ CL has been successfully used previously to show increased oxidative stress in the lung of rats exposed to paraquat (Turrens et al., 1988) or hyperoxia (Evelson & González-Flecha 2000), in the perfused lung *ex vivo* (Barnard et al., 1993), in the liver and brain of rats exposed to hyperbaric hyperoxia (Boveris & Cadenas 1999), and in the heart of mice treated with coxorubicin (Lores Arnaiz & Llesuy 1993). In the present report as well as in previous studies, using a model of inhalation exposure to concentrated ambient particles (CAPs) we used this technique to assess pulmonary and cardiac oxidative stress induced by PM and in that way to study the role of ROS in the toxic effects of PMs

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CL is a sensitive and early marker of increases in ROS, and as such it can be used as a predictor of cellular damage caused by ROS (González-Flecha et al., 1991). In the model of inhalation exposure to CAPs aerosols in rats, increases in heart and lung CL preceded and were associated with mild tissue damage (Gurgueira et al., 2002), were prevented by removal of PM in rats exposed to filtered air, and were abrogated by antioxidants like *n*-acetyl cysteine (Rhoden et al., 2004) or MnTBAP, a membrane permeable SOD analog (Rhoden et al., 2008). Furthermore, PM-induced lung inflammation (Rhoden et al., 2004) and changes in cardiac rhythm (Rhoden et al., 2005) were abrogated by antioxidants, confirming that oxidative stress is directly involved in the mechanisms of damage by PM. In this study, we measured heart and lung CL as a marker of oxidative stress and a predictor of oxidative damage by PM derived from emissions of three different coal-fired power plants with or without photochemical modifications. The “aging” scenarios were chosen to mimic three specific atmospheric situations: namely, oxidation of SO<sub>2</sub> to H<sub>2</sub>SO<sub>4</sub>, neutralization of an oxidized plume by background ammonia, and mixing with a background biogenic volatile organic compound (VOC) to yield secondary organic aerosol (SOA).

## Materials and methods

### Power plants

Three power plants utilizing different coal types, combustion conditions, and air pollution control devices were used for these studies. All three plants operated under similar boiler temperatures (~1500°C) and are described in more detail in Godleski et al (2011b), and Kang et al (2011). The first plant, located in the Upper Midwest (plant 1) was also described previously (Ruiz et al., 2007b). This plant burned a low sulfur (~0.2% S) coal from the Wyoming powder river basin and was equipped with electrostatic precipitators (ESP) to control particulate emissions. The second plant was located in the Southeast (plant 2), burned relatively low-to-medium sulfur (~1.0% S) bituminous coals from Kentucky, West Virginia, and South America. The plant had an ESP for particulate control and selective catalytic reduction (SCR) for NO<sub>x</sub> control. The third plant, in the Midwest (plant 3) burned a high sulfur (~3.0% S) bituminous coal from Indiana and had a forced oxidation wet flue gas desulfurization (FGD) scrubber to reduce SO<sub>2</sub> emissions, along with an ESP and SCR. The wet FGD scrubber used limestone as alkaline slurry to absorb SO<sub>2</sub> from the flue gas and produced calcium-sulfur compounds, with the primary product calcium sulfate.

### Animals

Adult male Sprague–Dawley rats (300 g body weight) were maintained and studied in accordance with the National Institutes of Health Guidelines for the care and use of animals in research. All protocols were approved by the Harvard Medical Area Standing Committee on Animals.

### Exposures to primary and aged aerosols

Detailed descriptions of the system have been reported by Ruiz et al (Ruiz et al., 2006; Ruiz et al., 2007a; Ruiz et al., 2007b) and are further described by Godleski et al (2011) and Kang et al (2011). Briefly, stack emissions composed primarily of SO<sub>2</sub>, NO<sub>x</sub>, and primary PM were diluted by addition of filtered (dry) air as needed. Sampling of fine PM was achieved by using a sampling tube with a size selective inlet to remove particles larger than 2.5 μm. The stack sampler was placed at a point where the emissions temperature was already starting to cool, and then the sampled emissions were diluted with dry filtered compressed air with dilution factors of 75–150. By the time the emissions reached the reaction chambers, they were at ambient temperature. To create the different exposure scenarios, the aerosols were aged in one or two photochemical chambers. In chamber 1 the hydroxyl radicals, generated by mixing stack emissions with ozone and water vapor in the presence of ultraviolet irradiation, oxidized SO<sub>2</sub> to sulfuric acid. In chamber 2, the oxidized plume was mixed with NH<sub>3</sub> to neutralize acid particles, and/or SOA was generated through the addition of α-pinene at a concentration of 1670 μg/m<sup>3</sup> plus 1000 ppb of ozone. Residence time in the dynamic chambers was set at 90 min. The gases in excess were removed by a counter current denuder (Ruiz et al., 2006) before the resulting aerosols were sent to the animal exposure chambers. Sham controls were exposed to room air filtered through a HEPA filter (using Millipore Opticap filters) at a flow of 1.5 l/min.

Particle mass was monitored both continuously and as an integrated, gravimetric measurement as described in Kang et al 2011. Particle count (serving as a proxy for ultrafine particles) was continuously monitored using a condensation particle counter (CPC, TSI Inc. Model 3022a), and particle size distribution was determined semi-continuously using a scanning mobility particle sizer (SMPS, TSI Inc. Model 3934). Sulfate, nitrate, and ammonium ion were measured by ion chromatography and particle strong acidity by pH analysis. Organic carbon was measured by the thermal optical reflectance method, and organic speciation of particle-phase pinene oxidation products was conducted by gas chromatography. Trace elements were quantified by X-ray fluorescence. Continuous measurement of gaseous pollutants was carried out: NO and NO<sub>2</sub> by CL; SO<sub>2</sub> by pulsed fluorescence; and O<sub>3</sub> by UV photometry.

Adult Sprague–Dawley rats obtained from Charles River Laboratories, Wilmington MA at ~300 g were exposed to either filtered air (sham) or each of the described aerosols (Table 1) simultaneously for 6 hours. For these studies with CL as an end point, two filtered air and two aerosol exposed animals per day were studied. This small number of animals has been found to be necessary so that the short-lived CL response can be optimally measured (Gurgueira et al., 2002). Thus, in order to obtain sufficient number of animals for each determination, at least four exposures of each scenario were

Table 1. Summary of aged particulate species derived from three coal-fired power plants for the TERESA study

Power plant	Exposure scenario	Aged particle mass ( $\mu\text{g}/\text{m}^3$ )	Particle number ( $\#/\text{cm}^3$ )	Total sulfate ( $\mu\text{g}/\text{m}^3$ )	Acidic sulfate <sup>a</sup> sulfate <sup>b</sup> ( $\mu\text{g}/\text{m}^3$ )	Neutralized sulfate <sup>b</sup> sulfate <sup>c</sup> ( $\mu\text{g}/\text{m}^3$ )	Organic carbon ( $\mu\text{g}/\text{m}^3$ )
Plant 1	P ( $n=4$ ) <sup>c</sup>	1.0 $\pm$ 0.9	1726 $\pm$ 1277	0.2 $\pm$ 0.3	2.3 $\pm$ 0.4	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
	PO ( $n=3$ )	46.0 $\pm$ 12.6	6723 $\pm$ 3550	36.1 $\pm$ 7.7	27.6 $\pm$ 9.5	8.4 $\pm$ 2.6	2.6 $\pm$ 4.5
	POS ( $n=4$ )	123.3 $\pm$ 28.4	16,924 $\pm$ 4495	55.8 $\pm$ 22.8	50.2 $\pm$ 21.6	5.6 $\pm$ 3.4	51.6 $\pm$ 8.6
	PONS ( $n=12$ )	154.9 $\pm$ 41.7	52,109 $\pm$ 11,951	68.2 $\pm$ 28.8	14.7 $\pm$ 13.6	53.6 $\pm$ 16.8	30.2 $\pm$ 16.4
Plant 2	P ( $n=4$ )	1.7 $\pm$ 1.8	910 $\pm$ 964	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
	PO ( $n=4$ )	115.5 $\pm$ 18.5	4281 $\pm$ 1911	100.3 $\pm$ 16.3	71.6 $\pm$ 17.0	28.8 $\pm$ 1.3	0.0 $\pm$ 0.0
	POS ( $n=8$ )	212.1 $\pm$ 39.7	11,473 $\pm$ 3774	146.0 $\pm$ 36.7	107.9 $\pm$ 31.7	38.1 $\pm$ 12.0	59.0 $\pm$ 20.2
	PONS ( $n=4$ )	257.1 $\pm$ 10.0	40,811 $\pm$ 2179	154.8 $\pm$ 12.4	15.7 $\pm$ 3.8	139.1 $\pm$ 15.5	35.1 $\pm$ 10.1
Plant 3	P ( $n=4$ )	43.2 $\pm$ 14.6	55,947 $\pm$ 11,769	34.0 $\pm$ 13.3	12.8 $\pm$ 7.1	21.2 $\pm$ 9.2	1.9 $\pm$ 3.8
	PO ( $n=4$ )	82.3 $\pm$ 15.6	69,372 $\pm$ 8523	77.9 $\pm$ 14.5	66.6 $\pm$ 16.8	10.3 $\pm$ 2.8	0.0 $\pm$ 0.0
	POS ( $n=8$ )	144.4 $\pm$ 31.6	40,446 $\pm$ 6657	83.3 $\pm$ 21.3	68.9 $\pm$ 18.2	14.5 $\pm$ 7.1	54.7 $\pm$ 27.5
	PONS ( $n=4$ )	173.5 $\pm$ 20.9	38,483 $\pm$ 3651	85.0 $\pm$ 12.9	2.5 $\pm$ 2.0	82.5 $\pm$ 13.5	52.0 $\pm$ 23.0
	OS ( $n=4$ )	137.8 $\pm$ 9.3	35,959 $\pm$ 6290	47.2 $\pm$ 14.6	30.3 $\pm$ 11.6	16.9 $\pm$ 11.6	83.6 $\pm$ 9.6
	O ( $n=4$ )	43.8 $\pm$ 3.5	29,294 $\pm$ 2392	40.6 $\pm$ 3.8	31.7 $\pm$ 5.8	8.9 $\pm$ 2.3	0.0 $\pm$ 0.0
	S ( $n=4$ )	61.4 $\pm$ 6.6	7574 $\pm$ 1598	1.3 $\pm$ 0.4	1.0 $\pm$ 1.3	0.7 $\pm$ 0.5	59.7 $\pm$ 6.1

All data presented in this table are the same as data presented in Kang et al 2011, Diaz et al 2011, and Godleski et al 2011, all values are average  $\pm$  standard deviation.

<sup>a</sup>Acidic sulfate was calculated from strong acidity (pH) measurements as the equivalent concentration of H<sub>2</sub>SO<sub>4</sub> aerosol, <sup>b</sup>Neutralized sulfate = total sulfate – acidic sulfate, <sup>c</sup>Number of exposure days

needed. Animals were exposed inside individual polycarbonate cylindrical chambers (10 cm diameter  $\times$  18 cm long), specially designed for this experiment, chambers are clear to allow light to come in and let the investigator monitor the animal during the exposure. The size of the chambers was big enough for the animal to be able to turn around and stretch at will, reducing the amount of stress and the sensation of confinement. Flow through each chamber was maintained at 1.5 LPM. Immediately after exposure, CL of the lung and heart were measured. Tissues were then excised, frozen and later assayed for thiobarbituric acid reactive substances (TBARS).

#### Organ chemiluminescence

Spontaneous CL of the surface of the lung and heart was measured as previously described (Gurgueira et al., 2002). A Thorn EMI CT1 single-photon counting apparatus with an EMI 9816B photomultiplier cooled at  $-20^\circ\text{C}$  was used. Rats were anesthetized with sodium pentobarbital (50 mg/kg i.p.) and connected to an animal ventilator (5 ml/breath, 60 breaths/min, Harvard Apparatus, Cambridge, MA). The chest was opened, the animals were placed in the measurement compartment, and 10 measurements were made at the surface of the lung and heart and averaged. Body temperature was kept at  $37^\circ\text{C}$  using isothermal pads (Braintree Scientific, Braintree, MA). Emission data were expressed as counts per second per unit of tissue surface (cps/cm<sup>2</sup>).

#### Tissue preparation

At the end of the exposure, animals were removed from the exposure chambers. The hearts and lungs were removed, frozen in a dry-ice bath, and sent to the main laboratory for analysis of TBARS. Samples were stored at  $-80^\circ\text{C}$  until processing. Samples for the determination of

TBARS were homogenized in 120 mM KCl, 30 mM phosphate buffer (pH 7.2) added with protease inhibitors (1  $\mu\text{g}/\text{ml}$  leupeptin, 1  $\mu\text{g}/\text{ml}$  aprotinin, 10  $\mu\text{g}/\text{ml}$  soybean trypsin inhibitor, 1  $\mu\text{g}/\text{ml}$  pepstatin, and 0.5 mM PMSF) at  $0-4^\circ\text{C}$ . The suspensions were centrifuged at  $600 \times g$  for 10 min at  $0-4^\circ\text{C}$  to remove nuclei and cell debris. The pellets were discarded and the supernatants were used as homogenates.

#### Determination of TBARS

For measurements of TBARS, homogenates were precipitated with 10% TCA, centrifuged, and incubated with thiobarbituric acid (Sigma, Chem. Co.) for 1 hour at  $100^\circ\text{C}$ . TBARS were extracted using butanol (1:1). After centrifugation, the fluorescence of the butanol layer was measured at 515 nm excitation and 555 nm emission using a PTI spectrofluorometer (Photon Technology International, Lawrenceville, NJ, USA). The amount of TBARS formed was expressed in picomoles per milligram of protein. Malondialdehyde standards were prepared from 1,1,3,3-tetramethoxypropane (Esterbauer and Cheeseman 1990). Protein concentration in homogenates was measured by Lowry method (Lowry et al., 1951) using bovine serum albumin as standard. Measurements were carried out in a Perkin Elmer Lambda 40 spectrophotometer.

#### Statistics

The numbers in tables and the bars in figures indicate the mean value  $\pm$  standard error of the mean (SEM) of 4–12 independent experiments. Statistical approaches used are described in more detail in Coull et al (2011). Exploratory analysis showed that the CL data were log-normally distributed. Therefore, before analyses, each response was log-transformed to satisfy the normality assumptions in regression analyses outlined below. As

a result, regression parameters represent log multiplicative-fold increases due to exposure, and we report the percent change in this outcome associated with exposure. In addition, exploratory analyses also revealed significant day-to-day variability among sham animals. Therefore, in models for these outcomes, response levels in exposed animals were compared to those in the filtered air controls from the same day.

A multi-layered approach to statistical analysis was used, whereby multiple analyses exploited exposure metrics of increasing sensitivity. ANOVA techniques that treat exposure as a categorical variable were used first. Overall differences between exposed and filtered air responses were assessed (i.e. a binary exposure covariate), and then whether these differences varied by exposure scenarios were determined. The ANOVA models assessed the effects of exposure for each scenario, and used a Bonferroni correction for multiple comparisons. Thus, we consider  $p$ -values  $<0.007$  as strong evidence of an exposure effect for a given scenario, and a  $p$ -value greater than 0.007 but less than 0.05 as marginal evidence of an effect (Coull et al., 2011). Second, univariate associations between mass levels or exposure composition and CL were analyzed. Single-component analyses were conducted in which a separate regression model was fitted using ratios between exposed and filtered air (log) responses as the outcome and either mass, particle number, or a single concentration as the exposure metric. The resulting  $p$ -values from these models were used to rank the strength of associations between each component and health.

## Results

At each of the three power plants adult healthy Sprague-Dawley rats were exposed to four different scenarios:

primary emissions (P), oxidized unneutralized emissions (PO), oxidized emissions + SOA (POS), and neutralized oxidized emissions + SOA (PONS) (Table 1). At plant 3, a subset of animals was exposed to three additional control scenarios: oxidized emissions + SOA in the absence of primary particles (OS), oxidized emissions alone (O), and SOA alone (S) (Table 1). A complete description of the scenarios can be found in Godleski et al. (2011) and Kang et al. (2011). The basic characterization of the aerosols is shown in Table 1. Further characterization of exposure concentrations can be found in Kang et al. (2011).

### Cardiac and pulmonary oxidative stress in rats exposed to aged PM

#### Plant 1 (Upper Midwest)

No significant changes were observed in either CL or TBARS measurements in any of the scenarios carried out at this plant (Table 2).

#### Plant 2 (Southeast)

Unneutralized oxidized emissions + SOA (POS) showed marginally significant increases in lung CL (sham  $10 \pm 1$  cps/cm<sup>2</sup>; exposed  $15 \pm 2$  cps/cm<sup>2</sup>;  $p < 0.05$ ) and TBARS (sham  $17 \pm 1$  pmol/mg protein; exposed  $28 \pm 4$  pmol/mg protein;  $p < 0.05$ ) (Figure 1A). Heart CL was marginally significantly increased in rats exposed to neutralized oxidized emissions with SOA (PONS) (sham  $12 \pm 1$  cps/cm<sup>2</sup>; exposed  $25 \pm 6$  cps/cm<sup>2</sup>;  $p < 0.05$ ) and these changes were also reflected in TBARS accumulation (sham animals  $142 \pm 10$  pmol/mg protein; exposed  $180 \pm 6$  pmol/mg protein;  $p < 0.05$ ) (Figure 1B). Exposure to P or PO led to no changes in lung or heart CL (Table 2).

#### Plant 3 (Midwest)

Although there were trends toward an increase in the levels of cardiac oxidants in the POS and O scenarios,

Table 2. Lung and heart CL for different exposure scenarios in three coal-fired power plants.

Power plant	Exposure scenario	Heart CL (cps/cm <sup>2</sup> )		Lung CL (cps/cm <sup>2</sup> )	
		Filtered air	Aerosol	Filtered air	Aerosol
Plant 1	P (8) <sup>a</sup>	ND	ND	ND	ND
	PO (6)	ND	ND	ND	ND
	POS (8)	14 ± 2	9 ± 1	10 ± 1	6 ± 1
	PONS (24)	11 ± 1	11 ± 1	8 ± 1	11 ± 2
Plant 2	P (8)	14 ± 3	15 ± 2	12 ± 2	12 ± 2
	PO (8)	12 ± 1	15 ± 1	11 ± 1	12 ± 1
	POS (16)	16 ± 3	21 ± 4	10 ± 1	18 ± 3*
	PONS (8)	12 ± 1	23 ± 2*	11 ± 1	14 ± 2
Plant 3	P (8)	12 ± 2	12 ± 2	9 ± 1	10 ± 1
	PO (8)	ND	ND	ND	ND
	POS (16)	14 ± 1	17 ± 1	10 ± 1	12 ± 2
	PONS (8)	12 ± 2	14 ± 2	11 ± 1	16 ± 2
	OS (8)	14 ± 2	14 ± 1	12 ± 2	11 ± 1
	O (8)	9 ± 1	11 ± 2	7 ± 1	6 ± 1
	S (8)	13 ± 1	13 ± 1	10 ± 1	9 ± 1

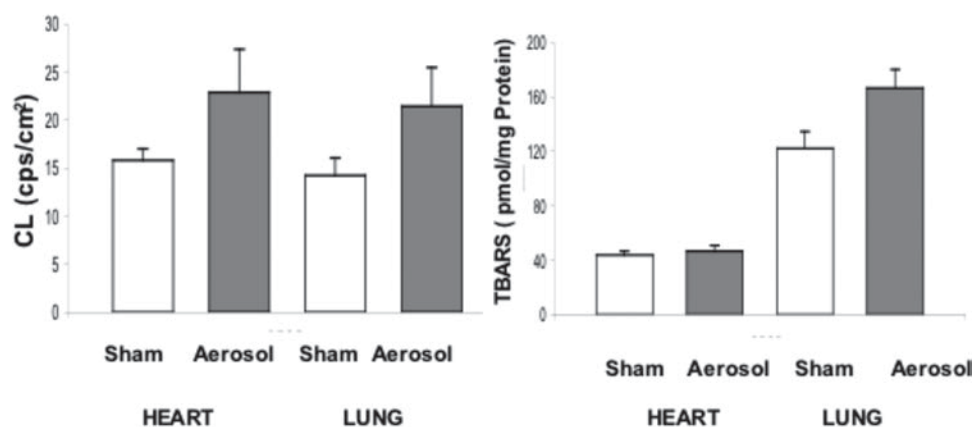
ND, not determined

<sup>a</sup>number of rats

\*  $p < 0.05$



### A. Scenario: POS



### B. Scenario: PONS

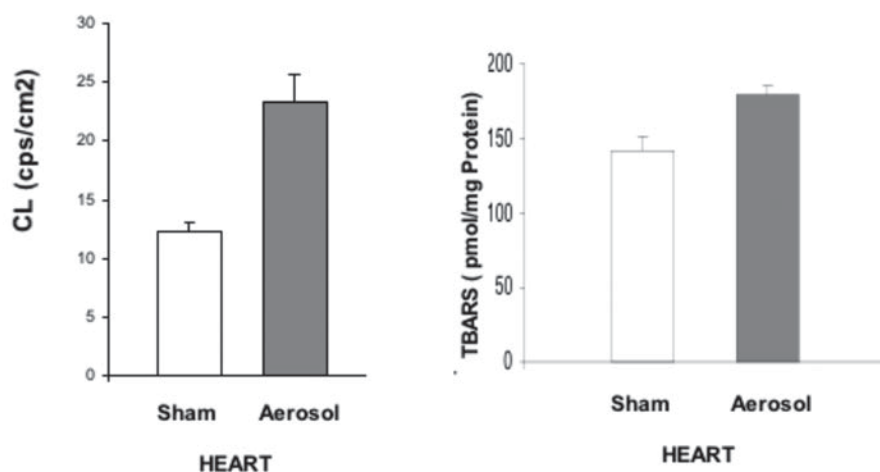


Figure 1. Cardiac and pulmonary oxidative stress in rats exposed to selected scenarios at plant 2. Adult Sprague–Dawley rats were exposed to aged PM (aerosol) or filtered air (sham) and assessed for heart and lung CL or accumulation of TBARS as described. (A) POS scenario, (B) PONS scenario. Values represent the mean of 4–6 independent determinations  $\pm$  SEM.

and in pulmonary oxidants in the PONS scenario, these changes did not reach statistical significance. No significant differences were observed between any of the measured parameters in any of the scenarios evaluated at this power plant (Table 2).

#### All power plants combined

Comparisons of the exposed/sham ratios for heart and lung CL for each scenario including all three power plants showed a significant increase in heart CL in rats exposed to POS ( $p < 0.0006$ ). Lung CL for the PONS scenario ( $p < 0.04$ ) was marginally significant. A trend of increase was also seen for lung CL in the POS scenario ( $p < 0.06$ ). The same analysis performed on normalized (log) ratios yielded  $p$ -values of 0.046 for exposure to PONS in the lung and 0.009 for exposure to POS in the heart. No other significant differences were observed. The  $p$ -values for the interaction between mean values by scenario were 0.6 for the lung and 0.02 for the heart for

the raw data and 0.7 for the lung and 0.1 for the heart for the normalized data.

#### Associations of oxidant effects with aerosol components

Univariate regression analyses show significant associations of increasing lung CL with decreasing Al and Mg concentrations (Table 3). No significant associations were seen for heart CL. Given these findings, multivariate analyses as described in Coull et al (2011) were not done with the data from this study.

#### Discussion

This study was aimed at determining whether exposure to primary or aged PM from power plants, plus common atmospheric constituents, leads to cardiac and/or pulmonary oxidant stress. Our results show that: (i) emissions from different power plants subjected to the

same photochemical aging triggered different oxidant responses in the heart and lung of rats, (ii) in the power plant where responses were observed, different scenarios produced different effects on either the heart or the

Table 3. Univariate regression of lung CL and concentrations of specific components.

Component	Coefficient/SEM	
	Heart	Lung
O <sub>3</sub>	-0.0035	0.0662
NO	0.1002	0.0298
NO <sub>2</sub>	-0.0911	0.0118
SO <sub>2</sub>	-0.0159	-0.0697
Formaldehyde	0.0191	0.0349
Acetaldehyde	0.0380	0.0640
Acetone	0.0658	0.0405
Total Aldehydes	0.0504	0.0443
Pinene	0.0619	0.0267
Mass	0.0728	0.0293
NC	-0.0862	-0.0028
SO <sub>4</sub>	0.0936	0.0298
NO <sub>3</sub>	-0.0443	0.0546
NH <sub>4</sub>	0.0307	0.0299
Acidic SO <sub>4</sub>	0.0538	-0.0075
Neutralized SO <sub>4</sub>	0.0436	0.0360
OC	0.0334	-0.0204
EC	-0.0001	0.0416
TC	0.0312	-0.0130
Al	-0.0672	-0.0973 <sup>a</sup>
Si	0.0408	0.0497
Mg	-0.3737	-0.3980 <sup>a</sup>
Na	-0.0103	-0.0162
S	0.0936	0.0298
Fe	-0.0366	-0.0688
Ni	0.0008	-0.0634
Zn	-0.0580	-0.0602
Pb	0.0234	-0.0046

lung, (iii) neither P alone nor PO produced any changes in heart or lung CL whereas POS and PONS showed tissue-specific responses in one power plant, and (iv) the pulmonary responses showed specific, but inverse, associations with Al and Mg concentrations.

A limitation of this study is that the P scenario at plants 1 and 2 had mass concentrations that were several orders of magnitude below the concentrations in the other scenarios. The PO exposures were less than the POS and PONS exposure doses, but in the same range and definitely within a dose range in which we have seen changes with CAPs (Gurgueira et al., 2002). The P concentrations were considerably lower, and, indeed, in some cases much lower than average US ambient levels. Although the actual primary particle aerosol concentrations from power plant emissions provide a realistic view of these emissions and a view of the aerosol to which people may be exposed, it lacks the direct dose comparison one would prefer to have in a toxicological study.

Organ CL has been successfully used to detect increases in the steady-state concentrations of ROS in several experimental models in animals (Barnard et al., 1993; Boveris & Cadenas 1999; Evelson & González-Flecha 2000; Ghelfi et al., 2008; Gurgueira et al., 2002; Lores Arnaiz & Llesuy 1993; Rhoden et al., 2005; Turrens et al., 1988) and humans (Ferreira et al., 1989; Ferreira et al., 1988; González-Flecha et al., 1991) (Table 4). Increases in CL are associated with increased oxidative damage in the tissue under study and a ratio treated/control of 1.4 is considered the threshold between oxidative stress and damage (González-Flecha et al., 1991). Consistently, the treated/control ratio was reported to be 2.1 and 1.8 in the lung of animals exposed to paraquat and 85% O<sub>2</sub>, respectively, and 10 and 1.7 for the heart of rats exposed to adryamicin and 85% O<sub>2</sub> (Table 4). In a model of inhalation exposure to Boston CAPs, the treated/control ratio for both lung and heart CL

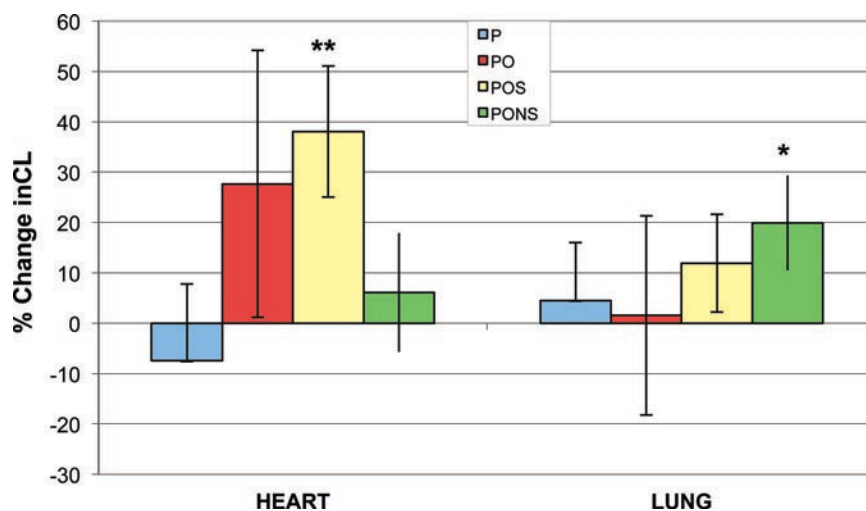


Figure 2. Changes in heart and lung CL for different exposure scenarios, all power plants combined. The bars represent average percentages of change in heart and lung CL for each scenario at all three power plants  $\pm$  SEM. Statistical differences among mean values were calculated as described in the Methods section. P: primary particles. PO: oxidized PM, POS: oxidized PM + organics, PONS: Oxidized Neutralized PM + organics. \*  $p < 0.05$ , \*\*  $p < 0.01$ . Should include  $n$ .

Table 4. Treated/control ratio for lung and heart CL in different experimental models.

Species/Tissue	Model	Treated/Control CL	Reference
Rat lung	Paraquat (30 mg/Kg)	2.1	Turrens <i>et al.</i> 1988
	85% Oxygen (1 day)	1.8	Evelson and González-Flecha 2000
	Boston CAPs		Gurgueira <i>et al.</i> 2002
	1 hour	1	
	3 hours	1.2	
	5 hours	1.7	
		Plant 2 SE	Average all plants
	Power Plant		Present study
	P	1.0	1.0
	PO	1.1	1.0
	POS	1.8	1.1
	PONS	1.3	1.2
Mice heart	Doxorubicin (15 mg/Kg)	10.0	Lores Arnaiz and Llesuy 1993
Rat heart	85% Oxygen (1 day)	1.7	Evelson and González-Flecha 2000
	Boston CAPs		Gurgueira <i>et al.</i> 2002
	1 hour	0.9	
	3 hours	1.5	
	5 hours	1.9	
	Boston CAPs		Rhoden <i>et al.</i> 2005
	5 hours	2.0–3.8	
	+ NAC	1.0	
	Boston CAPs (5 hours)	1.9–2.8	Ghelfi <i>et al.</i> 2008
	Power plant emissions		Present study
	P	1.1	0.9
	PO	1.2	1.3
	POS	1.3	1.4
	PONS	1.9	1.1

was found to be dependent on the length of inhalation exposure and on the composition of the CAPs aerosol (Gurgueira *et al.*, 2002). Exposures of 3 hours or longer showed significant increases in lung and heart CL, with values for the exposed/control ratio ranging 1.9–3.8 for the heart and ~1.7 for the lung. As expected, increases in the treated/control ratio in heart CL were prevented by pretreatment with antioxidants such as mannitol or NAC (Rhoden *et al.*, 2004) (Table 4).

The exposed/sham ratios found for the different exposure scenarios tested here range from 1.0–1.8 for the lung and 0.9–1.9 for the heart, indicating that some of the aged aerosols used in this study have an oxidant effect above 1.4. Interestingly, the effects of POS and PONS aerosols in the heart and lung are comparable to those observed for some Boston CAPs. For example, the 1.9 exposed/sham ratio found in the heart of rats exposed to PONS aerosols at plant 2 are analogous to those reported to produce significant electrophysiological alterations when using the CAPs inhalation model (Ghelfi *et al.*, 2008). Similarly, increases in lung CL of 1.8 were associated with significant lung inflammation in a model of instillation exposure to PM from Washington DC (Rhoden *et al.*, 2008). The PONS scenario, where lung CL was increased non-significantly in all plants, had significant decreases in expiratory air flow (Diaz *et al.*, 2011) in the same animals at plants 1

and 2, but not plant 3. When data from all plants were combined, change in lung CL increased only with the PONS scenario, and it was the PONS scenario that was most strongly associated with decreases in expiratory air flow when all data were combined (Diaz *et al.*, 2011). The PONS scenario had the highest mass concentrations among all the scenarios studied, and the lack of any positive univariate associations raises the possibility that the CL effect with the PONS scenario in the lung combining all data could represent a response to inhalation of particulate mass.

Indeed, the lack of positive univariate associations for measured components with the effects observed is most surprising in our study, because the number of exposure days in our study and in the study of respiratory assessments (Diaz *et al.*, 2011) were the same, and positive univariate associations were found in that study. At the same time, positive univariate associations were found with cellular responses and components of the PONS scenario (Godleski *et al.*, 2011a), and in that study, the number of exposure days for broncho-alveolar lavage outcomes was approximately half. The numbers of animals assessed in the respiratory studies was about two times more than in this study, but the numbers of animals assessed for the cellular response by broncho-alveolar lavage was slightly less than in our study. Therefore, neither the differences in exposure days nor differences in numbers of animals

can account for finding a lack of positive univariate associations. The finding of significant negative univariate associations with measurements of elemental Aluminum and Magnesium in the exposure aerosol are explained by the fact that plant 3 in all scenarios had at least 5-fold higher levels of these elements compared to plants 1 and 2 (Kang et al., 2011), and plant 3 had no significant increases in lung CL in any scenario (Table 2).

The POS scenario had strongest effects on lung CL in plant 2, yet the POS scenario had no respiratory or inflammatory cellular effects at plant 2 (Diaz et al., 2011, Godleski et al., 2011a). When all data for lung CL were combined, the POS scenario had no significant effect. Heart CL was non-significantly increased in plants 2 and 3 with the POS scenario. When all data across the plants were combined, Heart CL significantly increased with the POS scenario. Again there were no significant univariate associations with measured components and heart CL. An empiric choice to use the POS scenario with the MI model studies was made, and at plant 2, there was increased frequency of ventricular arrhythmias and decreased heart rate found (Wellenius et al., 2011).

In the CAPs studies individual sets of exposures within a given study led to different exposed/sham ratios for CL (Table 4). Also, the particle components associated with oxidant effects on the lung and the heart were different, with lung CL being associated with the CAPs concentrations of redox-active metals (Fe, Cu, Mn and Zn) and heart CL showing strong associations with metals considered as tracers of crustal components of PM (Al, Si, Fe and Ti) (Gurgueira et al., 2002). Recent studies (Ghelfi et al., 2008) have shown that the cardiac CL signal can be blocked by blocking pulmonary vanilloid receptors using inhalation or systemic injection of receptor blockers. Rhoden et al (2005) showed that the cardiac signal can be generated by agonists of the autonomic nervous system. These findings suggest that the cardiac CL effects are not direct and may be related to neural signals arising from the lung.

In the present study, different scenarios gave rise to different responses in the lung and heart, and the same scenario produced different responses across power plants. The lack of association with specific PM components seen for these outcomes may be another instance where the effect on cardiac and pulmonary oxidants is more related to the scenarios rather than a specific component of the exposure as in Diaz et al (2011).

## Declaration of interest

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