Peer Review
Experimental Toxicology Division
NHEERL

PARTICULATE MATTER

Cardiac, Pulmonary, and Thermoregulatory Effects of Instilled Oil Combustion-derived, Fugitive Emission Particulate Matter (EPM) in Spontaneously Hypertensive (SH) Rats

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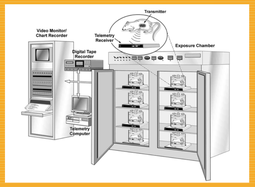
Introduction

- A host of epidemiological studies have presented convincing evidence of a small but consistent association between the levels of ambient particulate matter (PM) and the incidence of cardiopulmonary-related morbidity and mortality
- Persons with pre-existing cardiopulmonary disease appear to be more susceptible to the adverse effects of PM
- The frequency of acute episodes of increased ambient PM, when combined with the prevalence of cardiopulmonary disease in the general population, insures that substantial numbers of individuals are at risk due to exposure to PM
- Despite recognition of the potentially significant health concerns posed by such exposures, the specific constituents of PM and the underlying mechanism/s responsible for the reported adverse effects remain unclear

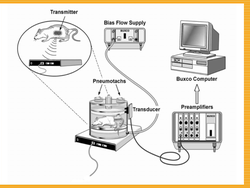
Methods

Experimental conditions:
- animals: male Spontaneously Hypertensive (SH) rats (a model of systemic hypertension)
- n=109; 4 dose groups (9-10 telemetry rats/group, 4-8 naïve rats/group per sacrifice time point)
- experimental treatment: EPM
- dose groups:
  1) Control (saline)
  2) Low dose (0.83 mg/kg)
  3) Mid dose (3.33 mg/kg)
  4) High dose (8.33 mg/kg)
- exposure: intratracheal instillation (IT)
- sacrifice: 24 hr, 96 hr, 192 hr post-IT

Procedure:
- implant radiotelemeters
- recovery period (10 d)
- control period (3 d)
- intratracheal instillation
- monitor (4-7 days post-IT)

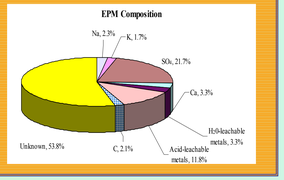


Endpoints assessed:
- heart rate (HR), blood pressure (BP), core temperature (T_{co}), QA interval
- activity
- body weight
- heart and lung weight
- ECG intervals/duration
- arrhythmias
- pulmonary function
- BALF constituents
- serum enzymes
- heart and lung histopathology

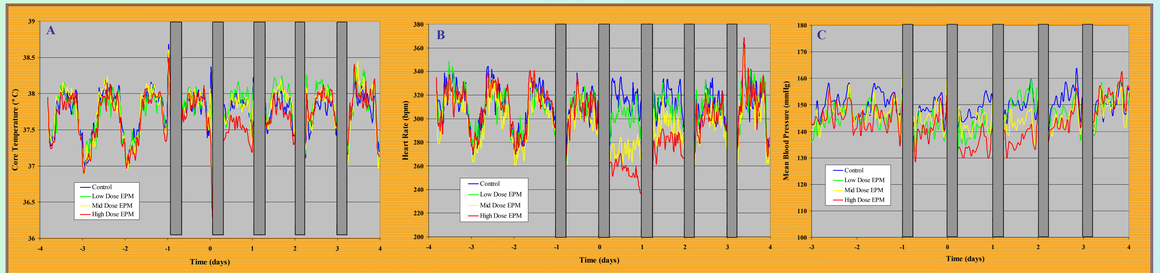


Concentrations of water-soluble transition metals:
- Zn 11.4 µg/mg
- Ni 6.9 µg/mg
- Fe 0.0* µg/mg
- V 1.3 µg/mg
- Cu 0.2 µg/mg
- Pb 0.0* µg/mg

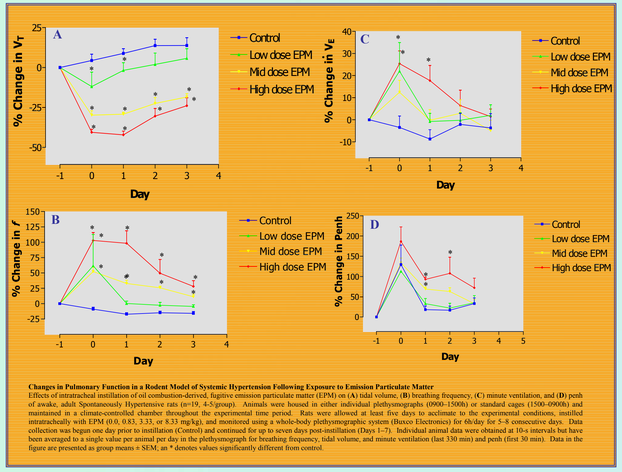
*Below detection limit



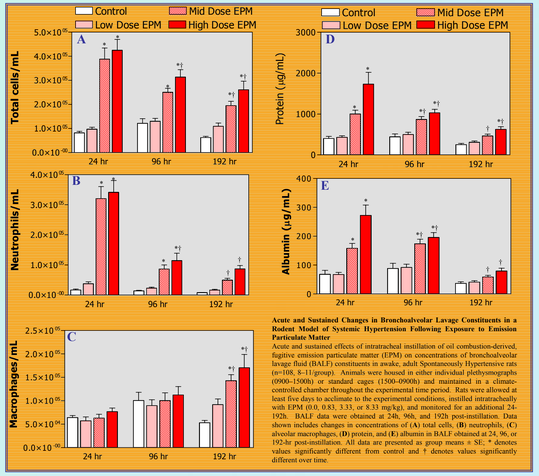
Results



Changes in Core Temperature, Heart Rate, and Systemic Blood Pressure in a Rodent Model of Systemic Hypertension Following Exposure to Emission Particulate Matter
Effects of intratracheal instillation of oil combustion-derived, fugitive emission particulate matter (EPM) on (**A**) core temperature (**B**) heart rate, and (**C**) mean systemic blood pressure of awake, adult Spontaneously Hypertensive rats (**A**,**B**: n=37, 9-10/group; **C**: n=20, 5/group). Animals were housed in either individual plethysmographs (0900–1500h) or standard cages (1500–0900h) and maintained in a climate-controlled chamber throughout the experimental time period. Core temperature, heart rate, and systemic blood pressure were continually monitored using previously implanted radiotelemetry transmitters. Rats were allowed five days to acclimate to the experimental conditions, instilled intratracheally with EPM (0.0, 0.83, 3.33, or 8.33 mg/kg), and monitored for 96–192h postinstillation. Data were obtained at 5-min intervals but for clarity of presentation have been averaged across 30-min (**A**,**B**) or 60-min periods (**C**). Data are presented as group means. Time periods during which the animals were maintained in the plethysmograph are indicated by the shaded vertical bands along the abscissa.
NOTE: Instillations were conducted at 0900h (shown as Time=0).



Changes in Pulmonary Function in a Rodent Model of Systemic Hypertension Following Exposure to Emission Particulate Matter
Effects of intratracheal instillation of oil combustion-derived, fugitive emission particulate matter (EPM) on (**A**) tidal volume, (**B**) breathing frequency, (**C**) minute ventilation, and (**D**) penh of awake, adult Spontaneously Hypertensive rats (n=19, 4-5/group). Animals were housed in either individual plethysmographs (0900–1500h) or standard cages (1500–0900h) and maintained in a climate-controlled chamber throughout the experimental time period. Rats were allowed at least five days to acclimate to the experimental conditions, instilled intratracheally with EPM (0.0, 0.83, 3.33, or 8.33 mg/kg), and monitored using a whole-body plethysmographic system (Buxco Electronics) for 6h/day for 5–8 consecutive days. Data collection was begun one day prior to instillation (Control) and continued for up to seven days post-instillation (Days 1–7). Individual animal data were obtained at 10-s intervals but have been averaged to a single value per animal per day in the plethysmograph for breathing frequency, tidal volume, and minute ventilation (last 330 min) and penh (first 30 min). Data in the figure are presented as group means + SEM; an * denotes values significantly different from control.



Acute and Sustained Changes in Bronchoalveolar Lavage Constituents in a Rodent Model of Systemic Hypertension Following Exposure to Emission Particulate Matter
Acute and sustained effects of intratracheal instillation of oil combustion-derived, fugitive emission particulate matter (EPM) on concentrations of bronchoalveolar lavage fluid (BALF) constituents in awake, adult Spontaneously Hypertensive rats (n=108; 8–11/group). Animals were housed in either individual plethysmographs (0900–1500h) or standard cages (1500–0900h) and maintained in a climate-controlled chamber throughout the experimental time period. Rats were allowed at least five days to acclimate to the experimental conditions, instilled intratracheally with EPM (0.0, 0.83, 3.33, or 8.33 mg/kg), and monitored for an additional 24-192h. BALF data were obtained at 24h, 96h, and 192h post-instillation. Data shown includes changes in concentrations of (**A**) total cells, (**B**) neutrophils, (**C**) alveolar macrophages, (**D**) protein, and (**E**) albumin in BALF obtained at 24, 96, or 192-hr post-instillation. All data are presented as group means + SE; * denotes values significantly different from control and † denotes values significantly different over time.

Conclusions and Impact

- Substantial deficits in cardiopulmonary function were observed in SH rats after IT exposure to EPM
- Significant dose-related increases in BAL indices of pulmonary injury were also observed
- Toxicity appears to be greater than that reported previously in healthy rats and comparable to that observed in other compromised rodent models
- EPM used in this study was more representative of ambient PM than the ROFA particles used previously
- The predominance of zinc among the transition metals in the EPM suggests an important role in PM–induced toxicity
- While the mechanism/s responsible for the adverse actions of PM in man are far from resolved, this study supports biologically-plausible modes of action
- These data provide important insights into the possible actions and effects of PM in humans

Future Directions

The next phase of this project will be an inhalation study using the same EPM. Exposures will be conducted in a whole-body plethysmograph system, thus enabling the collection of real-time ventilatory parameters during exposures. Rats will also be implanted with radiotelemeters to provide continuous monitoring and acquisition of physiological parameters in awake, unrestrained animals.

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