Effects of Intratracheal Instillation of Tire and Road Wear Particles (TRWP) and Tread Particles (TP) on Inflammation and Cytotoxicity in Rat Lung: A Comparative Toxicity Study

a - ChemRisk LLC, Pittsburgh, PA; b - Lovelace Respiratory Research Institute, Albuquerque, NM; c - ChemRisk LLC, New York, NY

Marisa L. Kreider[a], Julie M. Panko[a], Jacob D. McDonald[b], Britt L. McAtee[a], Brent L. Finley[c], and JeanClare Seagrave[b]

ABSTRACT

Recent literature has implicated a role for tire and road wear particles (TRWP) in cardiopulmonary morbidities and mortalities associated with ambient particulate matter (PM) exposures. In this study, we compared the response to TRWP and tire tread particles (TP) to other particle types, including respirable titanium dioxide (TiO_2), a relatively non-toxic particle; respirable silica, a relatively inflammogenic particle; and diesel exhaust particles, known to induce inflammation and oxidative stress and found in ambient PM. Rats were intratracheally instilled with 1 or 2 mg of size-fractionated TP or TRWP or 1 mg of one of the comparative particle types suspended in 0.1% Tween-80 in phosphate buffered saline (PBS) and sacrificed at 24 hours or 7 days post-instillation. Bronchoalveolar lavage fluid (BALF) was analyzed for changes in markers of inflammation (cell differential profile and cytokine expression), and cytotoxicity (total protein, lactate dehydrogenase, alkaline phosphatase); lung tissue homogenates were analyzed for markers of oxidative stress (hemeoxygenase-1 and thiobarbituric acid reactive substances [TBARS]). For nearly every marker of inflammation and cytotoxicity, the response to TP or TRWP was not statistically different than the relevant controls, whereas equivalent doses of both diesel exhaust particles and crystalline silica produced significant increases in markers of both inflammation (i.e. influx of inflammatory cells and increase of inflammatory cytokines in BALF) and cytotoxicity (i.e. increases in LDH activity and total protein levels in BALF). These results indicate that tire wear particles are less potent inducers of adverse effects associated with exposure to ambient PM, compared to other constituents of ambient PM.

INTRODUCTION

- Fluctuations in ambient particulate matter (PM) levels have been associated with increases in cardiopulmonary morbidities and mortalities in humans (Dockery, et al. 1993; Pope, et al. 1995).
- While the mode of action of these adverse events following PM exposure are still under investigation, researchers have indicated there are roles for inflammation and cytotoxicity in the pulmonary system for inducing cardiopulmonary events following exposure. The generation of reactive oxygen species has been identified as a potential contributor to the inflammatory and toxic effects of particulate matter exposure.
- Ambient PM includes contributions from vehicle emissions, both exhaust and non-exhaust in origin. While exhaust emissions have been extensively studied in relation to adverse health effects, researchers are beginning to investigate non-exhaust vehicle emissions such as brake wear and tire wear.
- Recent studies (Mantecca, et al. 2009, 2010; Gottipolu, et al. 2008) have focused on potential adverse effects associated with inhalation of ground tread particles as a surrogate for understanding tire wear.
- As a tire interacts with the pavement, the particulate generated includes a mixture of tread particles embedded with pavement and free pavement particles; these particles, identified as tire and road wear particles (TRWP) are the focus of this study.
- The purpose of this study is to understand the relative potency of TRWP in inducing cardiopulmonary toxicity including inflammation, cytotoxicity, and oxidative stress when compared to particles of known toxicity, including constituents of ambient PM, known inflammatory particles, and particles considered to be relatively inert.

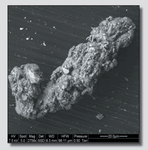
METHODS

Particle collection and separation

- TRWP were collected at the German Federal Highway Research Institute (Bundesanstalt für Straßenwesen [BASt]) using a predetermined and variable driving course that included acceleration, deceleration and cornering. The pavement used to generate the TRWP was standardized asphalt concrete with 6.1% proportion of bitumen (B50/70) according to ISO 10844. TRWP were collected from three tires and mixed into a composite at the ratios denoted below:
  - Summer carbon black tire (2 parts)
  - Winter silica tire (1 part)
  - Summer silica tire (1 part)
- Tread particles (TP) were also collected from each of the above tires and composited using the same ratios. TP are devoid of any contributions from the pavement sources.
- TRWP and TP were separated into the PM10 fraction through sieving; particle size was verified using an aerodynamic particle sizer (APS)

Animal treatment and endpoint evaluation

- Rats (male, F344, n=8/treatment group) were treated via intratracheal instillation with the following particles suspended in a solution of 0.01% Tween-80 in PBS:
  - TRWP at 1 or 2 mg/rat;
  - TP at 1 or 2 mg/rat;
  - Diesel exhaust particles (DEP; NIST SRM 2975) at 1 mg/rat;
  - Titanium dioxide (<5 µm in size) at 1 mg/rat, or;
  - Crystalline silica (MinUSil5, 97% < 5 µm in size) at 1 mg/rat
- Animals were sacrificed at either 24 hours or 7 days post-instillation
- Bronchoalveolar lavage fluid was collected from each rat for analysis for markers of cytotoxicity (total protein, lactate dehydrogenase [LDH], and alkaline phosphatase [ALP]) and markers of inflammation (cell differential profile, cytokine markers including interleukin-6 [IL-6], keratinocyte chemoattractant (GRO-KC), and tumor necrosis factor alpha [TNFα]).
- Lung tissue homogenates from each rat were analyzed for markers of oxidative stress, including hemeoxygenase (HO-1) and thiobarbituric acid reactive substances (TBARS).
- Continuous variables were plotted and analyzed by ANOVA with Dunnett's multiple comparison post-test, comparing all test articles with, first: the air control (1 day and 1 wk results), and second: the vehicle-instilled control (1 day results only) or the TiO_2-treated animals (1 wk results only).



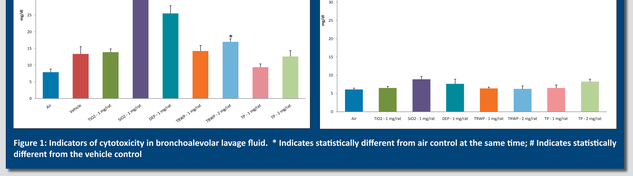


Figure 1: Indicators of cytotoxicity in bronchoalveolar lavage fluid. * Indicates statistically different from air control at the same time; # Indicates statistically different from the vehicle control

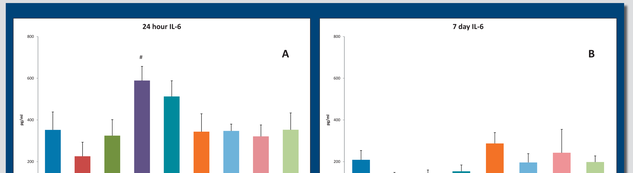


Figure 3: Cytokines in bronchoalveolar lavage fluid. * Indicates statistically different from air control at the same time; # Indicates statistically different from the vehicle control; + Indicates statistically different from titanium dioxide

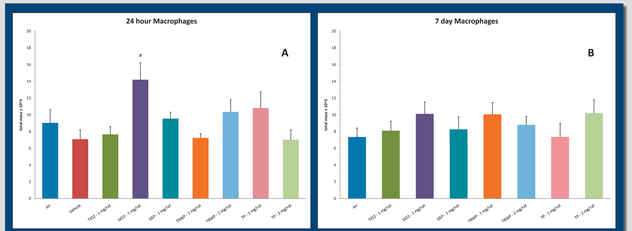


Figure 2: Cell differential profile in bronchoalveolar lavage fluid. * Indicates statistically different from air control at the same time; # Indicates statistically different from the vehicle control; + Indicates statistically different from titanium dioxide

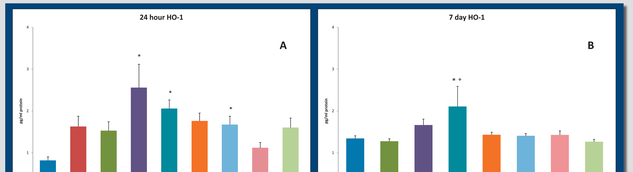


Figure 4: Oxidative stress markers in lung tissue homogenate. * Indicates statistically different from air control at the same time; # Indicates statistically different from the vehicle control; + Indicates statistically different from titanium dioxide

RESULTS

Particle size

- Average particle size by number (number median aerodynamic diameter [NMAD]) and mass (mass median aerodynamic diameter [MMAD]) of particles for TRWP and TP are reported in Table 1. TP were typically larger than TRWP.

Table 1: Average particle size of TRWP and TP (based on three samples)

Particle Type	NMAD (GSD) µm	MMAD (GSD) µm
TRWP	1.21 (1.67)	3.92 (1.79)
TP	2.35 (1.95)	6.66 (1.63)

Markers of cytotoxicity

- Neither TRWP nor TP produced statistically significant increases in markers of cytotoxicity at 24 hours or 7 days post-instillation at either 1 mg or 2 mg per rat when compared to vehicle control (Figure 1).
- Both diesel exhaust and crystalline silica induced LDH activity and increased total protein in the lavage fluid 24 hours post-instillation when compared to vehicle control (Figure 1A and 1C).
- No treatment had any effect on levels of alkaline phosphatase in the bronchoalevolar lavage fluid (data not shown).

Markers of inflammation

Cell differential

- For most cell types, TRWP and TP did not cause a statistically significant influx in inflammatory cells into the bronchoalevolar lavage fluid at 1 mg or 2 mg per rat when compared to appropriate controls at 24 hours or 7 days post-instillation (Figure 2). There was a statistically significant increase in lymphocytes when compared to air control in response to 1 mg/rat TRWP exposure at 7 days post-instillation, but this response was not dose dependent (i.e. no effect was noted at 2 mg/rat) (Figure 2F).
- Crystalline silica caused an influx in polymorphonuclear cells (PMNs) into the bronchoalevolar lavage fluid at both 24 hours and 7 days post-instillation when compared to appropriate controls (Figures 2C and 2D). Crystalline silica also increased the influx of macrophages at 24 hours post instillation when compared to vehicle control and lymphocytes at 7 days post-instillation when compared to both air control and titanium dioxide (Figure 2A).
- Diesel exhaust particles induced an increase in PMNs when compared to air and titanium dioxide controls at 7 days post-instillation (Figure 2D).

Cytokines

- Neither TRWP nor TP caused a statistically significant increase in inflammatory cytokines in the bronchoalevolar lavage fluid at 24 hours or 7 days post-instillation when compared to controls at either 1 mg/ rat or 2 mg/rat (Figure 3).
- Crystalline silica increased IL-6 when compared to vehicle control at 24 hours post-instillation (Figure 3A); crystalline silica also increased GRO-KC at 7 days post-instillation when compared to both air control and titanium dioxide (Figure 3D).
- Diesel exhaust particles increased GRO-KC when compared to vehicle control at 24 hours post-instillation (Figure 3C).
- No treatment resulted in TNFα production.

Oxidative stress

- At 1 mg/rat, neither TRWP nor TP increased hemeoxygenase-1 (HO-1) in lung tissue homogenates at 24 hours or 7 days post-instillation when compared to vehicle control where equivalent doses of crystalline silica and diesel exhaust particles did increase HO-1 (Figure 4). The effect of diesel exhaust particles on HO-1 persisted to 7 days post-instillation (Figure 4B). At 2 mg/rat, HO-1 was increased 24 hours post-instillation in TRWP-exposed rats; this effect did not persist to 7 days post-instillation.
- There were no treatment-related effects on TBARS in any animals.

DISCUSSION

- Neither TP nor TRWP produced evidence of cytotoxicity, inflammation, or oxidative stress following intratracheal instillation in rats.
- At equivalent doses to those administered in the TP and TRWP-exposed animals, both diesel exhaust particles and crystalline silica, historically known to produce inflammatory responses in animal models, caused increases in markers of cytotoxicity, inflammation, and oxidative stress.
- The doses of TRWP and TP administered in this study are far larger than are anticipated to be inhaled from exposure to PM in ambient air. This study suggests that wear particles from tires likely contribute minimally if at all to the adverse effects associated with ambient PM.

ACKNOWLEDGEMENTS

This work was funded by the Tire Industry Project, an industry-wide initiative organized under the World Business Council for Sustainable Development that aims to understand the human health and environmental risks associated with the tire throughout its lifecycle.

REFERENCES

Available upon request.