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RESEARCH ARTICLE



Smoldering and flaming biomass wood smoke inhibit respiratory responses in mice

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ABSTRACT

Background: Acute and chronic exposures to biomass wildfire smoke pose significant health risks to firefighters and impacted communities. Susceptible populations such as asthmatics may be particularly sensitive to wildfire effects. We examined pulmonary responses to biomass smoke generated from combustion of peat, oak, or eucalyptus in control and house dust mite (HDM)-allergic mice.

Methods: Mice were exposed 1 h/d for 2 consecutive days to emissions from each fuel type under smoldering or flaming conditions (~ 40 or ~ 3.3 mg PM/m³, respectively) while maintaining comparable CO levels (~ 60 – 120 ppm).

Results: Control and allergic mice reduced breathing frequency during exposure to all biomass emissions compared with pre-exposure to clean air. Smoldering eucalyptus and oak, but not peat, further reduced frequency compared to flaming conditions in control and allergic groups, while also reducing minute volume and peak inspiratory flow in control mice. Several biochemical and cellular markers of lung injury and inflammation were suppressed by all biomass emission types in both HDM-allergic and control mice. Control mice exposed to flaming eucalyptus at different PM concentrations (C) and times (T) with the same C $\times$ T product had a greater decrease in breathing frequency with high concentration acute exposure compared with lower concentration episodic exposure. This decrease was ameliorated by PM HEPA filtration, indicating that the respiratory changes were partially mediated by biomass smoke particles.

Conclusion: These data show that exposure to smoldering eucalyptus or oak smoke inhibits respiratory responses to a greater degree than peat smoke. Anti-inflammatory effects of CO may possibly contribute to smoke-induced suppression of allergic inflammatory responses.

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Introduction

Wildfires have increased in frequency and severity resulting in longer wildfire seasons over the last 30 years within the United States (Westerling et al. 2006). In 2017 alone, wildfires burned over 10 million acres (National Interagency Fire Center 2018). Wildfire smoke composition is influenced by several factors including fuel composition and moisture content, fire temperature, and wind conditions (Liu and Wimberly 2015). Wildfire smoke contains a number of air pollutants including particulate matter (PM), carbon monoxide (CO), carbon dioxide (CO₂), nitric oxide (NO), nitrogen dioxide (NO₂), ozone, volatile organic compounds (VOCs), and polycyclic aromatic compounds (PAHs) (Jerrett et al. 2005; Urbanski et al. 2009; Black et al. 2017).

Low temperature smoldering biomass burning releases high concentrations of PM, while high temperature flaming conditions produce higher CO₂ and nitrogen oxides at comparable emissions of CO (Kim et al. 2018). Air quality is affected by primary and secondary pollutants derived from wildfire smoke emissions. As wildfire smoke emissions continue to increase, susceptible populations such as children, older adults and individuals with preexisting cardiovascular and respiratory illnesses are at risk.

The all-cause mortality from biomass wildfire smoke globally has been estimated at 339,000 deaths annually (Johnston et al. 2012). Furthermore, wildfire smoke has significant impacts on respiratory and cardiovascular function. Although no association has been found between wildfire

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smoke exposure and respiratory mortality (Cascio 2018), epidemiological studies along with systemic reviews show wildfire smoke exposure is linked to respiratory morbidity (Youssef et al. 2014; Liu et al. 2015; Tinling et al. 2016) including asthma, bronchitis, and chronic obstructive pulmonary disorder (COPD) (Alman et al. 2016; Reid et al. 2016; Vicedo-Cabrera et al. 2016). For asthma in particular, wildfire smoke exposures are associated with increased numbers of physician and emergency room visits (Henderson et al. 2011; Johnston et al. 2014) and increased usage of emergency asthma medication (Reid et al. 2016).

Although several epidemiological and occupational health studies have assessed health effects of wildfire smoke, less is known about its toxicity relative to other forms of air pollution. Defining the fuel type and associated health effects for individual wildfire events presents challenges considering the wide variety of exposure types, durations, and concentrations. In this study, we generated wildfire smoke in a controlled quartz tube furnace system to test health effects of different fuel types and combustion conditions. Our objective was to assess pulmonary responses to smoke emissions from different biomass fuel types in an allergic mouse model with several features of human asthma. We hypothesized that exposure to biomass smoke in the allergic model would worsen pulmonary function and exacerbate lung inflammation.

Acute inhalation studies were conducted to assess the relative toxicity of emissions from three distinct biomass fuel types representative of woodland types (pocosin peat for the coastal plains of the Carolinas and Virginia, red oak for forests in the Eastern and Central United States forests, and eucalyptus for chaparral in Southern California) and provide a ranking of their effects on pulmonary and allergic asthmatic responses in mice. This assessment included evaluation of pulmonary function during biomass smoke exposures, and biochemical, pathological, and cellular changes in the lungs of control and house dust mite (HDM)-allergic mice following exposure. We also assessed the concentration–time relationship of biomass smoke toxicity by evaluating the effects of flaming eucalyptus exposures administered over different time periods with the same concentration $\times$ time product. The role of the smoke particulate fraction was assessed by repeating the high concentration exposure with a filter to remove the particulate fraction. Results from these studies inform the relative risks of exposure to different biomass emissions in healthy and at-risk populations related to respiratory and lung inflammatory responses.

Methods

Fuel types

Eucalyptus (*Eucalyptus globulus*) and Irish peat were purchased from commercial suppliers (Woodworkers Source, Phoenix, AZ and Glynn Brothers, Boston, MA, respectively), and northern red oak (*Quercus rubra*) was obtained from the Air and Energy Management Division of the U.S. Environmental Protection Agency (EPA)'s National Risk

Management Research Laboratory. Oak and eucalyptus were cut into ~ 2 cm-long chips, while peat was crumbled into similar sizes to promote consistent combustion conditions. All samples were stored in a temperature- and humidity-controlled room (23 °C; 39% RH).

Automated combustion system

A quartz-tube furnace system was used to control combustion phases and biomass smoke concentrations under constant conditions for 60 min, as previously described (Kim et al. 2019). Briefly, stable exposure conditions were maintained using an automated mass flow controller incorporating a proportional-integral-derivative (PID) feedback loop. Biomass smoke generated from the tube furnace system under smoldering (~ 500 °C) or flaming (~ 640 °C) conditions was diluted with air and delivered to a 64-port nose-only inhalation chamber (Laboratory Products Inc., Seaford, DE). Smoke PM concentrations were continuously monitored and adjusted in the chamber as changes were detected, maintaining the PM concentration within $\sim 10\%$ of the target set value. Exposure flow rates were maintained at ~ 1 L/min through each chamber port, while chamber temperature and RH were maintained at ~ 22 °C and $\sim 40\%$, respectively. Exposure atmosphere CO₂, CO, nitrogen oxides (NO and NO₂), VOCs, PM concentrations, particle size, temperature, relative humidity, static pressure, and flow rate were analyzed as previously described (George et al. 2014; Kim et al. 2014, 2019). Modified combustion efficiency (MCE), defined as $MCE (\%) = (\Delta CO_2 / (\Delta CO_2 + \Delta CO)) \times 100$, where ΔCO_2 and ΔCO are the excess concentrations of CO₂ and CO (Ward and Hardy 1991), is $>95\%$ for flaming and 65–85% for smoldering combustion conditions (Urbanski 2014), and MCE was calculated to confirm that the exposures met these criteria. Epidemiological studies suggest CO is associated with alterations in lung function (Canova et al. 2010) and inhibition of lower airway inflammation (Zhao et al. 2016), and CO inhibits pro-inflammatory cytokines in mice (Otterbein et al. 2000). Therefore we compared flaming and smoldering conditions based on equivalent CO concentrations. Accordingly, flaming (~ 4 mg/m³ PM) and smoldering (~ 40 mg/m³ PM) conditions were generated which produced equivalent CO levels of ~ 60 – 120 ppm for all 3 fuel types (Kim et al. 2019), enabling comparisons based on biomass source, PM levels, and other exposure characteristics.

Mouse allergy model

All experimental protocols using laboratory animals were approved by the local animal care and use committee of the U.S. EPA. Young adult (7–8 weeks old, 18–21 g) female BALB/cJ mice (Jackson Laboratories, Bar Harbor, ME) were housed for a minimum of 5 d before treatment in a barrier-isolated animal facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care. Food (Prolab RHM 3000; PMI Nutrition International, St. Louis, MO) and water were provided *ad libitum*. Mice were

weight-randomized into treatment groups. On days 0 and 7, mice were sensitized intranasally (i.n.) with 0.7 μ g HDM antigen (*D. pteronyssinus*, Greer Laboratories, Lenoir, NC) in 50 μ L sterile saline, while control (non-allergic) mice received saline only i.n. On day 21, all groups were challenged i.n. with 0.7 μ g HDM, and exposures to biomass smoke began the next day.

Effects of exposure to biomass smoke on real-time respiratory responses in allergic mice

Mice were acclimated to head-out plethysmograph (HOP) tubes (emka Technologies, Falls Church, VA) prior to exposure. Smoke-exposed mice were held in HOP tubes attached to ports of the inhalation chamber (Figure 1), while separate control groups were exposed to filtered air in a different chamber. Biomass smoke or clean air exposures were conducted for 1 h on days 22 and 23 of the allergy protocol (1 and 2 d after HDM challenge). HOP assessments were obtained on all mice in the biomass smoke-exposed groups, which were also assessed during exposure to clean air for 20 min immediately before smoke exposure to provide

baseline respiratory responses. Measurements of tidal volume (TV), frequency (F), peak inspiratory and expiratory flow rates (PIF, PEF), inspiratory and expiratory times (T_i , T_e), minute ventilation (MV), relaxation time (RT), enhanced pause (PenH), and mid-expiratory flow (EF_{50}) were determined using IOX 2.9.5 software (emka Technologies, Falls Church, VA, USA). Baseline values of F, MV, and PIF were averaged over the 20-min air exposure for each mouse and subtracted from each 5-min interval during the 1-h exposure, while 20-min baselines for other parameters were subtracted from values averaged over the entire exposure hour.

Effects of smoke concentration, timing, and particle fraction on respiratory responses

On an equal mass basis, PM isolated from flaming peat and eucalyptus atmospheres produced higher numbers of BALF neutrophils and other indices of toxicity after aspiration in control mice than flaming oak or smoldering biomass PM of any type (Kim et al. 2018). We chose flaming eucalyptus to assess respiratory and inflammatory endpoints following inhalation exposures at different PM concentrations (C) and

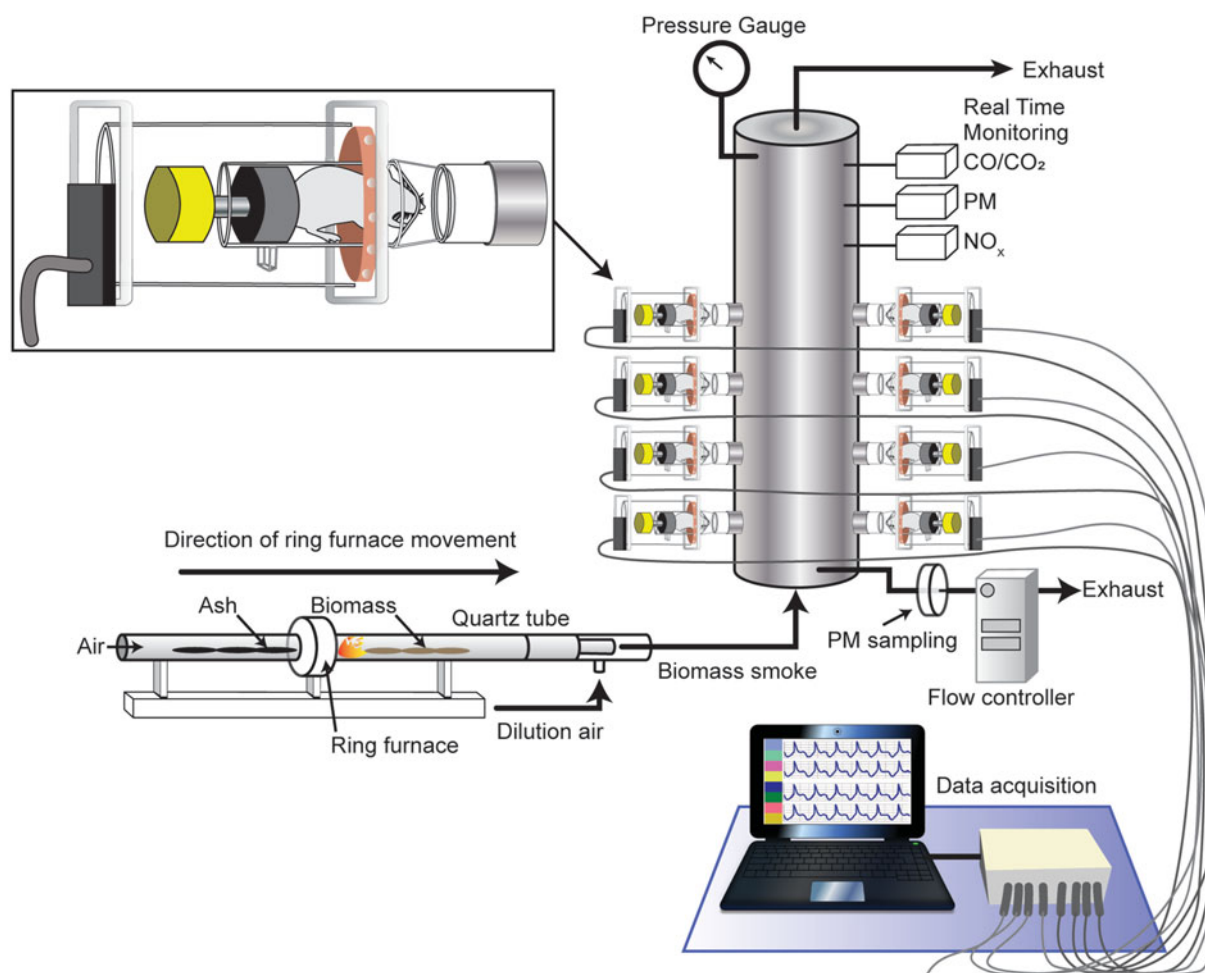


Figure 1. Assessment of respiratory responses in mice during exposure to biomass combustion emissions. Controlled combustion of peat, oak, or eucalyptus chips in a ring furnace under smoldering ($\sim 500^\circ\text{C}$) or flaming ($\sim 640^\circ\text{C}$) conditions generates biomass emissions delivered to a nose-only inhalation exposure tower. Gases (CO, CO₂, NO_x) and particles are continuously monitored, while time-integrated samples of PM, VOCs, and other chemical species are collected. Thorax pressure signals were recorded from mice in head-out plethysmographs attached to tower ports and processed to produce respiratory function parameters.

times (T) which generated the same PM C × T product, and examined the role of the particulate fraction in this response. Control mice (no i.n. dosing) were exposed to clean air or flaming eucalyptus at 3 mg PM/m³ for 1 h/d over 5 consecutive days (5-d), 3 mg PM/m³ for 1 h/d per week over 5 consecutive weeks (5-week), or 5 mg PM/m³ for three 1-h periods separated by 30-min rest periods between exposures in a single day (3-h). The latter exposure was also repeated with a high-efficiency particulate air (HEPA) filter to remove PM and assess the effects of gases alone (3F-h). HOP assessments were conducted on all flaming eucalyptus smoke-exposed groups with a 20-min clean air baseline before exposures, and data were analyzed as described above for the allergy study.

Bronchoalveolar lavage fluid (BALF) collection and analysis

All mice in the allergy study were euthanized 4 h after the second exposure day by i.p. injection of a phenytoin/pentobarbital mixture (>25 and 200 mg/kg, respectively; Virbac AH Inc., Fort Worth, TX, USA). Mice in the C × T flaming eucalyptus study were euthanized 24 h after the final exposure. Mice were exsanguinated via cardiac puncture, the left lung was clamped, and the right lobes were lavaged with 3 aliquots of Hanks' balanced salt solution (0.6 mL each). BALF cell numbers and differential cell types were quantified as previously described (McGee et al. 2015; Hargrove et al. 2018). BALF supernatant was assessed for lactate dehydrogenase (LDH), protein (reagents from Thermo Fisher diagnostics, Rockford, IL, USA), *N*-acetyl-β-D-glucosaminidase (NAG, Roche Diagnostics, Indianapolis, IN, USA), and γ-glutamyl transferase (GGT, Thermo Fisher Scientific, Pittsburgh, PA, USA). Assays were carried out using a Konelab 30 clinical chemistry analyzer (Thermo Clinical Lab Systems, Espoo, Finland).

Concentrations of murine interleukin (IL)-4, IL-5, IL-13 and eotaxin were quantified in BALF using Milliplex® MAP mouse cytokine fluorescent and magnetic bead panel kits with a Luminex 100 (Luminex Co., Austin, TX, USA) following the manufacturer's instructions (Millipore, Billerica, MA, USA). The lower limit of quantification for each cytokine (pg/mL) was as follows: IL-4: 0.4, IL-5: 1.0, IL-13: 7.8, and eotaxin: 1.8.

Lung histopathology

Left lung lobes were collected and fixed with 10% neutral buffered formalin at 20 cm water pressure. Lung tissues were embedded in paraffin, and 5 μm sections were stained with hematoxylin and eosin (H&E). One longitudinal section of the left lung lobe was evaluated for each animal. Stained sections were evaluated via light microscopy by a board-certified pathologist using established pathologic criteria for inflammatory, degenerative, metaplastic, and proliferative changes (Renne et al. 2009).

Statistical analyses

GraphPad Prism 6.0 (San Diego, CA) software was used to perform statistical analyses. Values were expressed as means ± standard error of the mean (SEM). Statistical analyses were conducted using analysis of variance (ANOVA) followed by Tukey or Sidak post-hoc test. A *p*-value <0.05 was considered statistically significant. Differences in histopathology lesion incidence between groups were evaluated using a two-sided Fisher's Exact Test.

Results

Biomass exposure characterization

Particulate and gaseous components of peat, oak, and eucalyptus combustion atmospheres under smoldering and flaming conditions are summarized in Table 1. Average PM levels were similar for each fuel type under smoldering (39.9 mg/m³) or flaming (3.3 mg/m³) phases, and particle sizes were consistently in the smaller fine PM range (0.13–0.18 μm MMAD) under all conditions except for a slightly larger size (0.25 μm) in smoldering eucalyptus smoke. Average CO levels were comparable (78 ppm for all exposures), though higher overall for peat and lower for oak exposures. Nitrogen oxides were 30- to 100-fold higher under flaming conditions compared with smoldering conditions, and 2- to 3-fold higher in flaming peat compared with flaming oak or eucalyptus atmospheres. The calculation of MCE confirmed that smoldering (84–86%) and flaming (97–99%) exposures met defined criteria for these exposures (Urbanski 2014). Further details of these smoke exposure atmospheres, including elemental and organic carbon, VOCs, ions, and elemental constituents have been reported elsewhere (Kim et al. 2019).

Table 1. Biomass smoke characteristics for peat, oak, and eucalyptus exposures.

Measurement	Peat		Oak		Eucalyptus	
	Smoldering	Flaming	Smoldering	Flaming	Smoldering	Flaming
PM (mg/m ³)	38.7 ± 0.4	3.4 ± 0.0	40.5 ± 0.8	3.5 ± 0.1	40.6 ± 1.0	3.1 ± 0.1
MMAD (μm)	0.15 [1.49]	0.13 [1.27]	0.18 [1.52]	0.16 [1.32]	0.25 [1.68]	0.15 [1.36]
CO (ppm)	115 ± 1	81 ± 1	56 ± 1	59 ± 2	73 ± 1	81 ± 2
CO ₂ (ppm)	721 ± 5	2,794 ± 40	297 ± 5	2,942 ± 65	404 ± 8	5,925 ± 74
NO (ppb)	217 ± 30	4,969 ± 592	39 ± 1	1,811 ± 161	31 ± 2	2,044 ± 74
NO ₂ (ppb)	0 ± 0	1,164 ± 165	0 ± 0	501 ± 98	0 ± 0	1,047 ± 88
NO _x (ppb)	217 ± 30	6,112 ± 729	39 ± 1	2,302 ± 256	31 ± 2	3,074 ± 97
MCE (%)	86.3 ± 0.1	97.2 ± 0.0	84.0 ± 0.3	98.0 ± 0.1	84.3 ± 0.3	98.7 ± 0.0

Values shown are means ± SEM. MCE: modified combustion efficiency (%) = $[\Delta\text{CO}_2/(\Delta\text{CO}_2 + \Delta\text{CO})] \times 100$. MMAD: mass median aerodynamic diameter of PM; bracketed values represent geometric standard deviation (σ_g).

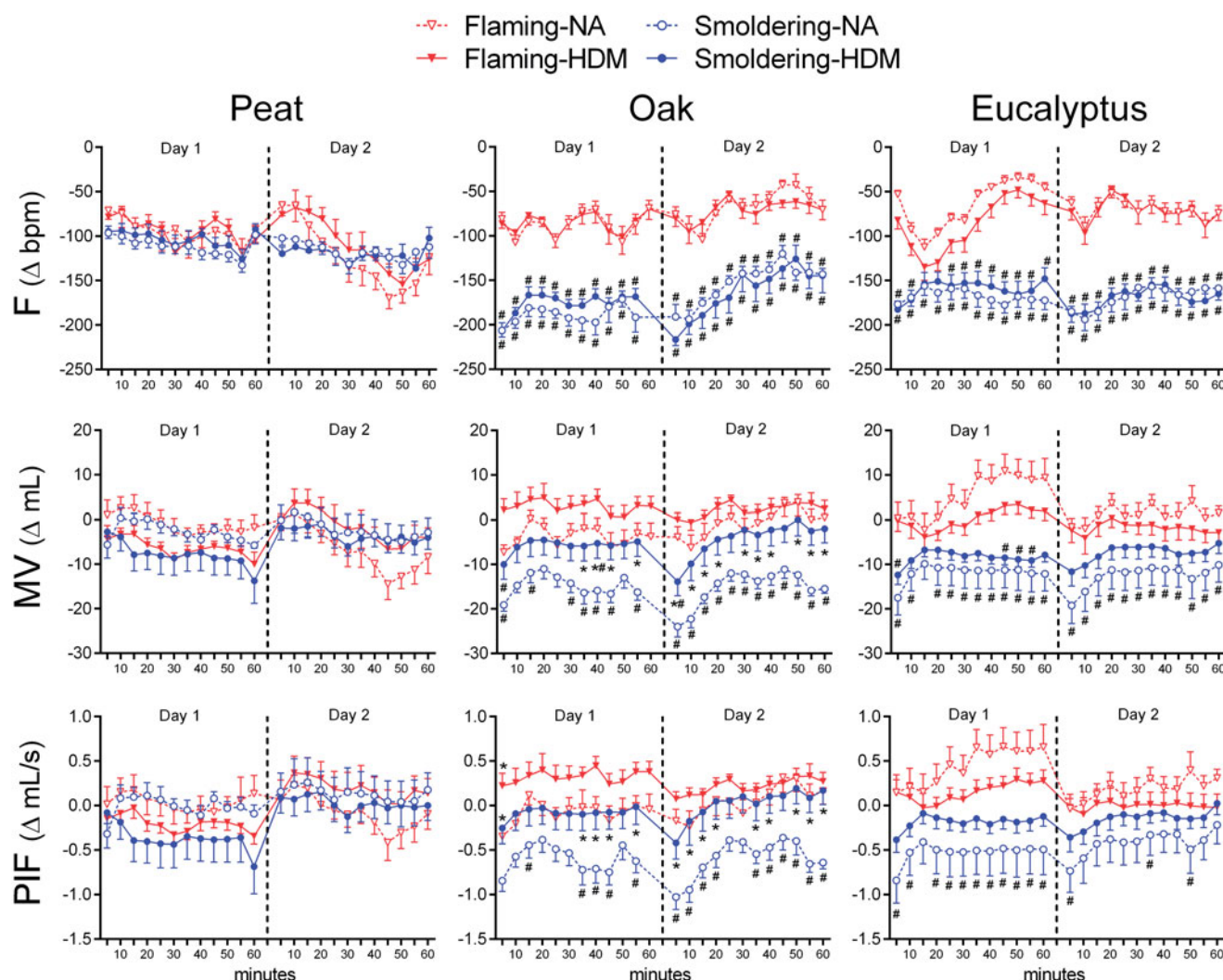


Figure 2. Changes in ventilatory parameters measured by head out plethysmography during 1-hour exposure on each of 2 d to peat, oak or eucalyptus smoke emissions under flaming or smoldering conditions in non-allergic (NA) and HDM-allergic (HDM) mice. Data show means and SEM ($n = 8$ mice/group) for differences in 5-min exposure average values compared with 20-min clean air averages recorded immediately before exposure, for breathing frequency (Δ breaths per minute, top panels), minute volume (MV, Δ mL, middle panels) and peak inspiratory flow (PIF, Δ mL/s, bottom panels). *Significant difference ($p < 0.05$) versus non-allergic group exposed to the same biomass smoke condition. # $p < 0.05$ versus flaming exposure condition in groups with the same fuel type and allergic status.

Respiratory responses during inhalation of biomass smoke

In comparison to baseline clean air exposure values (Table S1), breathing rate declined during exposures to flaming and smoldering peat, oak, and eucalyptus biomass smoke in HDM-allergic and control non-allergic mice on both days of exposure (Figure 2, top panels). Whereas flaming emissions from all three fuel types as well as smoldering peat produced consistent decrements of ~ 60 to 140 breaths per minute (bpm), the smoldering oak and eucalyptus exposures produced significantly greater decrements (~ 150 to 200 bpm) compared with the corresponding flaming conditions at nearly all times during exposure. Decreases in breathing frequency were reflected in corresponding increases in inspiratory, expiratory, and relaxation times, with significantly greater effects in smoldering vs. flaming oak and eucalyptus exposures (Table S2). The first day of smoldering oak exposure had significantly lower effects on T_i , T_e , and RT in HDM-allergic mice compared with non-allergic mice (closer

to air control baselines), but allergic status had no significant effects on other respiratory responses to biomass emissions.

Smoldering oak and eucalyptus exposures also significantly decreased minute volume (MV) and peak inspiratory flow (PIF) on both days in non-allergic mice compared with flaming exposures (Figure 2, middle and bottom panels). Smoldering oak, but not smoldering eucalyptus, also induced greater decrements in MV and PIF in non-allergic mice compared with HDM-allergic mice during most exposure time points. There were no significant effects of flaming or smoldering peat biomass exposures on MV or PIF in either non-allergic or HDM-allergic mice. Other parameters such as $PenH$ and EF_{50} (correlates of airway obstruction), tidal volume, and peak expiratory flow rate were virtually unaltered by any of the biomass exposures (Table S2). Overall, these data showed that HDM-allergic mice had comparable or slightly lesser respiratory responses to biomass exposures compared with non-allergic mice. Reduced responses to biomass smoke in allergic mice may be due to

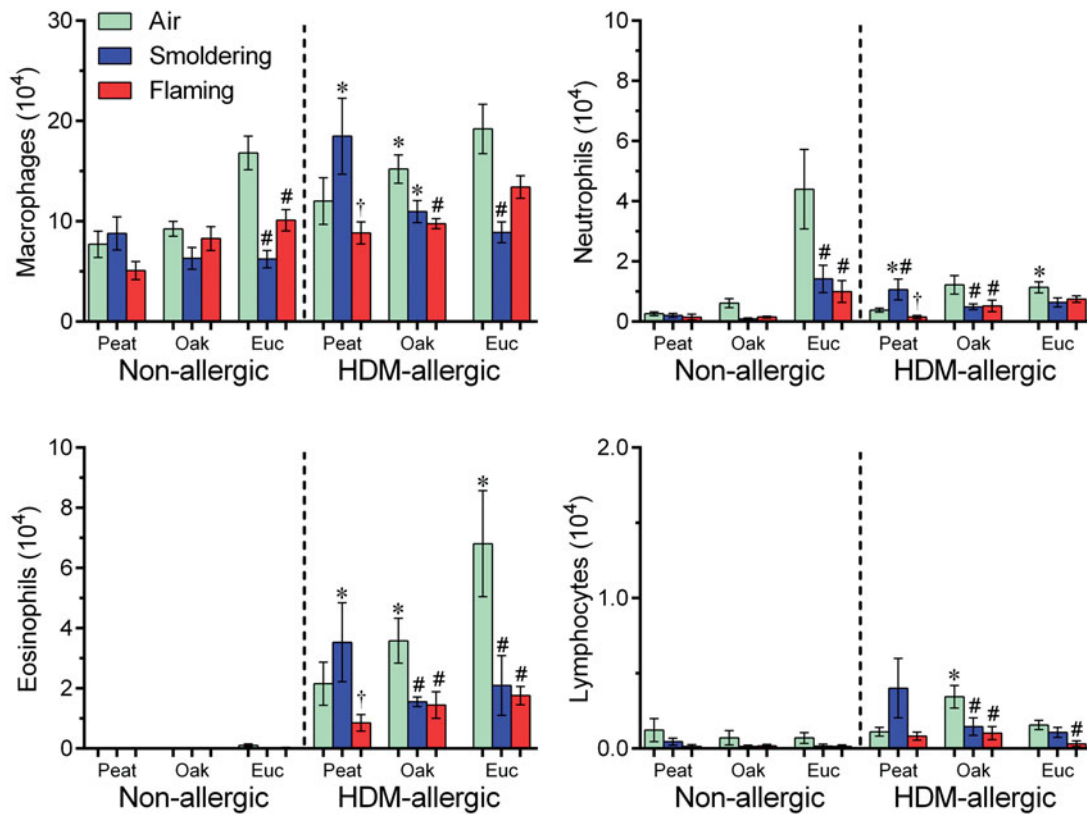


Figure 3. Numbers of BALF alveolar macrophages, neutrophils, and eosinophils in non-allergic and HDM-allergic mice recovered 4 h after final exposure to peat, oak, or eucalyptus smoke emissions under smoldering or flaming conditions. Values are means $\pm$ SEM of 6–8 mice/group. *Significantly different ($p < 0.05$) versus non-allergic group exposed to the same biomass smoke condition. # $p < 0.05$ versus air-exposed cohort with same allergic status. † $p < 0.05$ versus smoldering condition with the same fuel type and allergic status.

enhanced mucus production, which can provide a barrier to penetration of particulate and gaseous components of the exposure atmospheres.

BALF cells, cytokines, and biochemical indices

Exposure to flaming or smoldering peat or oak biomass smoke had no effects on BALF cell numbers in non-allergic groups (Figure 3). Macrophage and neutrophil numbers in non-allergic smoldering and flaming eucalyptus groups were comparable to corresponding peat or oak groups, but significantly reduced in comparison with an atypically elevated air control cohort. HDM sensitization and challenge produced mild allergic inflammation indicated by increases in most BALF cell types in air-exposed control groups, though responses were variable in the three air cohorts. Smoldering peat increased macrophages, neutrophils, and eosinophils at least 2-fold compared with flaming peat, and neutrophils were also greater compared with air-exposed allergic mice. In contrast, both smoldering and flaming oak and eucalyptus exposures reduced BALF eosinophils compared with air-exposed allergic cohorts, while smoldering and flaming oak also reduced other BALF cell types. Previous studies of allergic responses in mice exposed to hardwood smoke demonstrated minimal exacerbation of some indices of inflammation (Barrett et al. 2006).

All HDM-allergic air-exposed groups had significantly higher BALF levels of pro-allergic cytokines IL-4 and IL-5

compared with non-allergic air-exposed groups (Figure 4). No effects of biomass smoke exposures on BALF IL-4 or IL-5 were observed in non-allergic mice. However, smoldering and flaming exposures of all fuel types significantly reduced both cytokines in allergic mice, except for nearly significant reductions ($p = 0.06$ – 0.08) of IL-5 following smoldering oak or flaming eucalyptus exposures. Eotaxin and IL-13 levels were not significantly altered by any of the biomass smoke exposures (data not shown).

None of the biomass exposures significantly increased biochemical indicators of acute lung injury in BALF beyond levels found in air-exposed groups. In HDM-allergic mice, flaming and smoldering peat significantly reduced protein levels to about 60% of the air control group, while flaming eucalyptus suppressed GGT compared with air exposure (Figure 5). Smoldering peat reduced GGT activity in HDM-allergic mice compared with both air and flaming peat exposures. Smoldering oak slightly reduced LDH, a marker of cytotoxicity, in non-allergic mice. Both smoldering oak and eucalyptus produced slight decreases in NAG activity in non-allergic groups compared to their respective air controls, while smoldering oak also reduced NAG compared to the air control in HDM-allergic mice.

Histopathological changes in the lung

Independent of smoke exposure, HDM-allergic groups had increased mixed cell inflammation in perivascular,

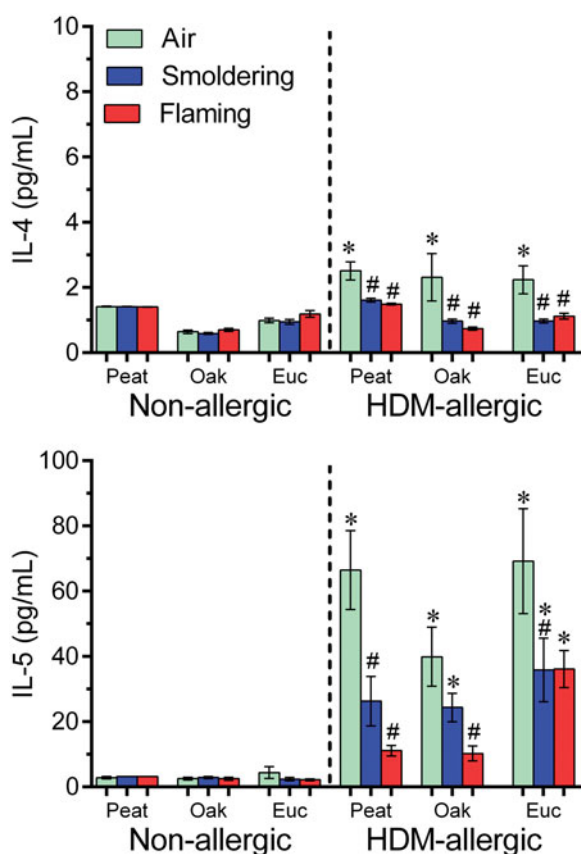


Figure 4. BALF supernatant IL-4 and IL-5 in non-allergic and HDM-allergic mice exposed to peat, oak, or eucalyptus smoke emissions under smoldering and flaming conditions. Values are means $\pm$ SEM of 6–8 mice/group. * $p < 0.05$ versus non-allergic group exposed to the same biomass smoke condition. # $p < 0.05$ versus air-exposed cohort with the same allergic status.

peribronchiolar, and alveolar regions (Figure 6). Inflammatory cells included eosinophils, neutrophils, lymphocytes, macrophages, and, in some cases, rare multinucleated giant cells. Other allergic changes included an increased incidence of mucus within bronchiolar airways, mucous cell metaplasia/hyperplasia of the bronchiolar epithelium, and peribronchiolar lymphoid hyperplasia (Table S3). Inflammation was also observed in some non-allergic groups; the cohort exposed to air concurrently with the eucalyptus exposures had a notably higher incidence of mixed cell inflammation compared with other non-allergic groups. Across all non-allergic and HDM-allergic groups, air control groups had a higher incidence of histiocytic and mixed cell inflammation, intrabronchiolar mucus, and lymphoid hyperplasia compared to flaming or smoldering biomass exposures (air > smoldering > flaming).

Effects of biomass concentration, frequency, and particulate fraction on pulmonary responses

Control mice were exposed to flaming eucalyptus over days, weeks, or hours (5-d, 5-week, or 3-h, respectively) with the same target PM $C \times T$ product (15 mg-h/m^3), and the latter exposure was repeated with a PM filter (3F-hour). Actual $C \times T$ products of the 3 exposure schedules ranged from $16.59\text{--}20.36 \text{ mg-h/m}^3$, and no PM was present in the 3F-

hour exposure (Table 2). Gaseous components of the 5-d and 5-week exposures were similar to those recorded for flaming eucalyptus in the HDM-allergy study (Table 1). Gaseous components of the 3-h and 3F-hour exposures were comparable and about twice as high as those in the 5-d and 5-week exposures, except for a much lower NO_2 concentration in the 3F-h exposure (Table 2).

Relative to clean air baseline values, mice in the 3-h exposure group had a significantly greater reduction in breathing frequency and corresponding increase in relaxation time over the course of each exposure hour compared with the 5-d and 5-week groups (Figure 7). The 3-h group responses were more pronounced near the midpoint of each of the exposure hours, which were separated by 30-min rest periods. Mice in the 3F-h group had significantly attenuated F and RT responses from 30 to 50 min during each hour of exposure, suggesting a significant role for biomass smoke PM in the respiratory response to flaming eucalyptus smoke. These flaming eucalyptus exposures elicited minimal changes in indices of inflammation in BALF and blood 24 h after the final exposure (Table S4), demonstrating that change in real-time pulmonary function was the most sensitive indicator of the adverse effects of these biomass smoke exposures.

Discussion

In this study, we investigated comparative effects of emissions from three biomass fuel types (Irish peat, red oak, and eucalyptus) on respiratory and inflammatory responses in a mouse model of allergic airway disease representing several features of human asthma. Our results show the following: (1) all flaming exposures as well as smoldering peat caused decrements in respiration, while smoldering oak and eucalyptus reduced respiration to a significantly greater degree; (2) while respiratory effects of biomass smoke were observed in both non-allergic and HDM-allergic groups, allergic groups had attenuated responses to smoldering oak; (3) biomass smoke exposures reduced several lung inflammatory cell types, allergic cytokines, and markers of lung injury in both non-allergic and HDM-allergic mice compared with their respective air-exposed cohorts; and (4) biomass smoke exposure concentration, timing, and PM and gaseous components significantly influence respiratory responses. Overall, the data showed that exposures to smoldering oak and eucalyptus smoke led to greater reductions in respiration and subsequent inflammation than exposure to smoldering peat smoke.

Wildfire smoke contains several respiratory irritants including particulate matter, acrolein, and other aldehydes known to reduce lung function and exacerbate asthma symptoms. Particulate matter is one of the primary pollutants of concern during acute exposures to biomass smoke (Liu et al. 2017). Previous studies have evaluated effects of biomass emission PM samples via oropharyngeal aspiration (Kim et al. 2014, 2018). Our recent study of inhaled biomass smoke in mice also included assessment of respiratory parameters using whole body plethysmography before and

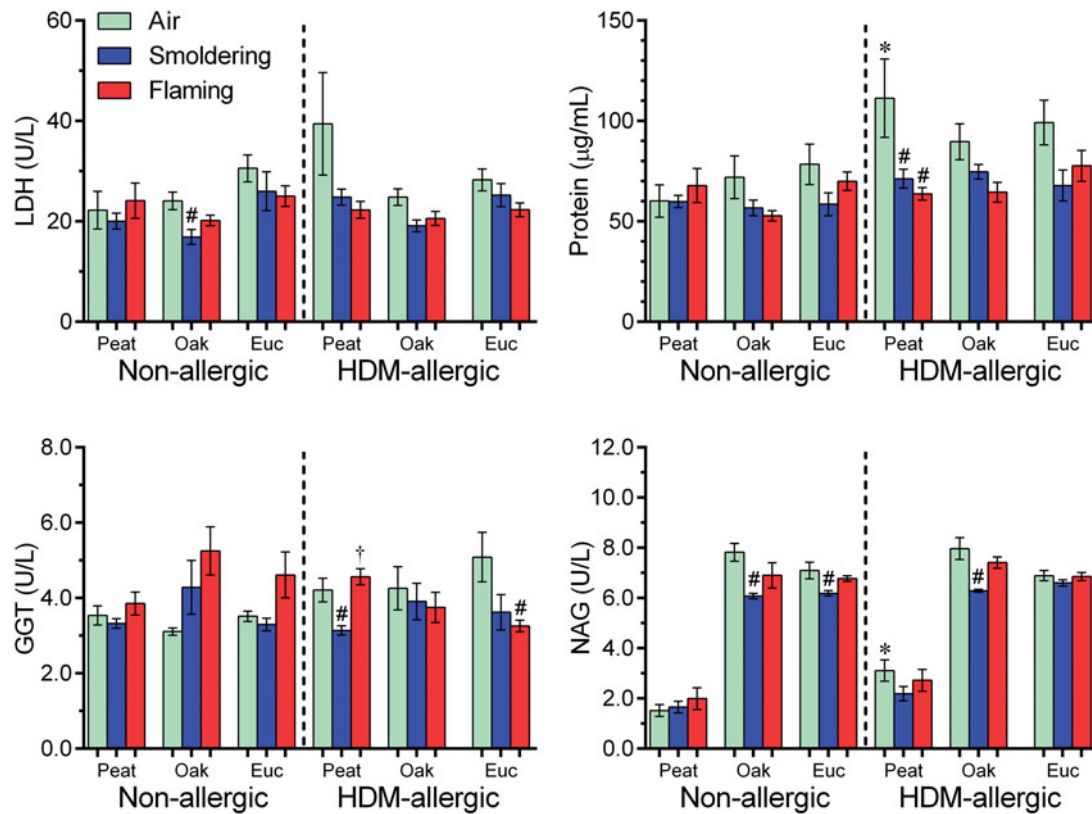


Figure 5. BALF supernatant biochemical indicators of acute injury (LDH: lactate dehydrogenase; protein; GGT: γ -glutamyl transferase; NAG: *N*-acetyl- β -D-glucosaminidase) in non-allergic and HDM-allergic mice exposed to peat, oak, or eucalyptus smoke emissions under smoldering or flaming conditions. Values are means $\pm$ SEM of 6–8 mice/group. * $p < 0.05$ versus non-allergic group exposed to the same biomass smoke condition. # $p < 0.05$ versus air-exposed cohort with same allergic status. † $p < 0.05$ versus smoldering condition with same fuel type and allergic status.

after biomass smoke exposures (Kim et al. 2019). Here we report for the first time respiratory responses obtained during acute biomass exposures in both healthy and allergic mice. Both flaming and smoldering combustion conditions altered breathing frequency, minute volume, peak inspiratory flow, and other respiratory parameters, and responses were significantly greater after exposure to smoldering oak and eucalyptus. Although PM concentrations were 10-fold higher in smoldering vs. flaming conditions, smoldering peat did not cause further declines in respiratory function beyond flaming conditions, indicating that biomass smoke PM composition is an important factor in toxicity. Recently reported detailed analyses of the particulate and gaseous chemical constituents of these biomass smoke atmospheres do not suggest chemical species which might clearly explain the differences in respiratory responses (Kim et al. 2019).

Relatively few studies have evaluated respiratory and inflammatory effects of biomass smoke exposures on exacerbation of asthma (Zelikoff et al. 2002). Oak wood smoke had minimal effects on allergic airway inflammation in mice using two ovalbumin allergy and exposure protocols (Barrett et al. 2006). We recently assessed respiratory parameters in control mice using whole body plethysmography one day before, immediately after biomass exposures (1 h/d for 2 d), and one day post-exposure (Kim et al. 2019). Increases in PenH (a correlate of airflow obstruction) were observed after each day of flaming or smoldering eucalyptus smoke

exposures, while flaming peat or oak exposure produced only transient increases. In contrast, we did not observe significant changes in PenH using head-out plethysmography during real-time exposure to any biomass smoke, suggesting that airflow obstruction may develop some time after exposure.

Exposure to smoldering and flaming biomass emissions decreased several types of injury markers, inflammatory cells, and pro-allergic cytokines compared with air controls. Reductions in lavage macrophage cell numbers were also observed in mice instilled with coarse or fine wildfire PM, suggesting cytotoxic effects of the PM (Wegesser et al. 2009). Alternatively, CO is known to exert strong anti-inflammatory effects in several experimental systems (Ryter and Choi 2016). Inhaled CO (250 ppm) reduced macrophages and neutrophils in a rat model of ventilator-induced lung injury (Dolinay et al. 2004), and also reduced BALF eosinophils and IL-5 in allergic mice (Chapman et al. 2001). The same concentration of CO also increased serum anti-inflammatory cytokines and reduced pro-inflammatory cytokines in a mouse model of endotoxemia (Otterbein et al. 2000). Since the wildfire smoke atmospheres in this study had relatively high concentrations of CO (56–115 ppm), it is possible that CO may have reduced inflammation in the biomass smoke-exposed control and HDM-allergic mice. Inhaled NO (20 ppm) has been shown to improve gas exchange and moderate respiratory failure in a pig model

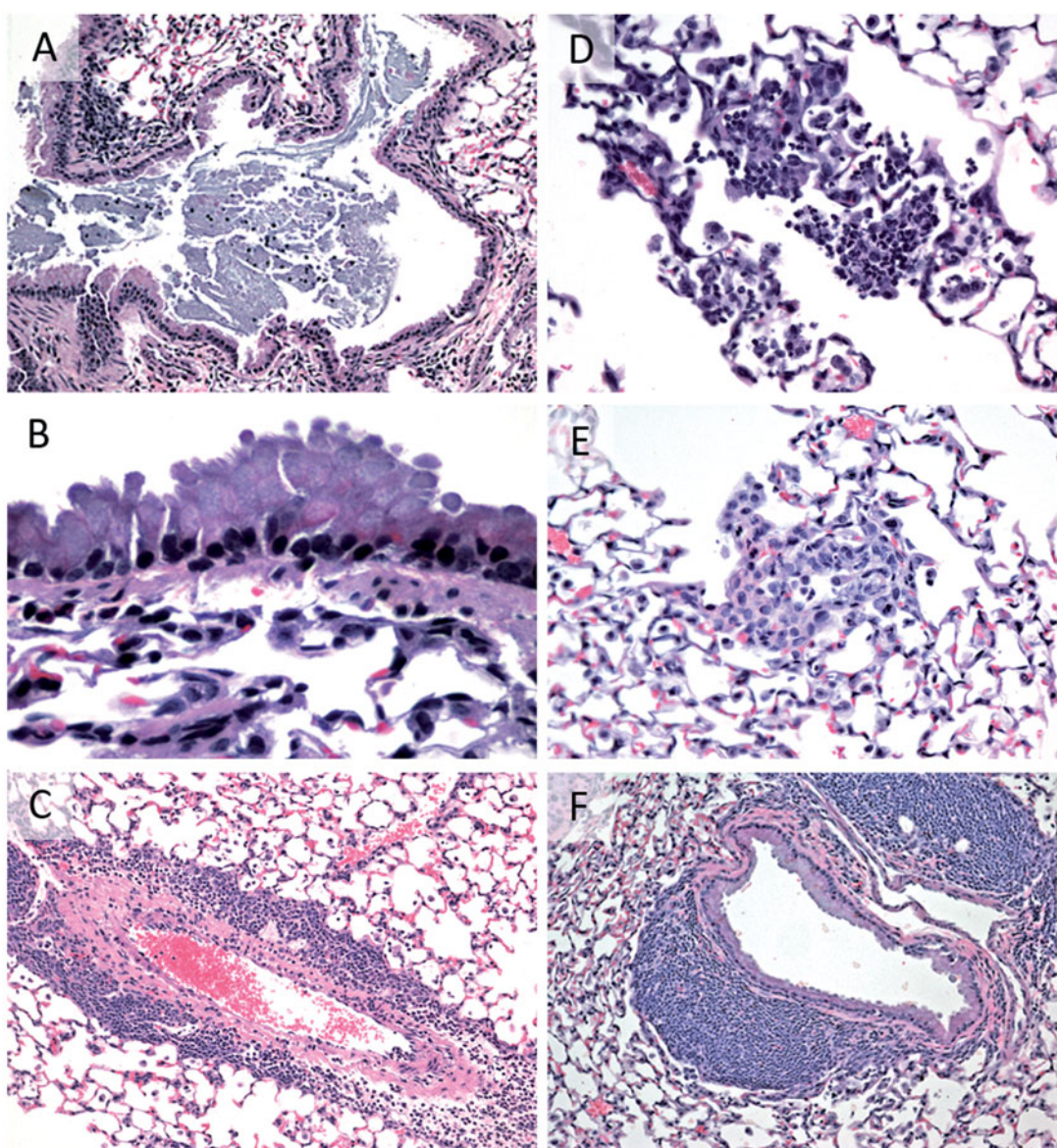


Figure 6. Representative lung findings following exposure of non-allergic and HDM-allergic mice to biomass emissions. (A) Intrabronchiolar mucus (HDM-allergic: flaming peat); (B) mucous cell metaplasia (HDM-allergic: flaming oak); (C) perivascular mixed cell inflammation (HDM-allergic: air); (D) alveolar mixed cell inflammation composed of neutrophils, alveolar macrophages, and lymphocytes (non-allergic: air); (E) alveolar histiocytic inflammation (non-allergic: smoldering oak); and (F) peribronchiolar lymphoid hyperplasia (HDM-allergic: air). Objective magnification = $10\times$ (A, C, F), $20\times$ (D, E), and $40\times$ (B). All images are from H&E-stained slides.

Table 2. Biomass smoke characteristics of flaming eucalyptus exposures designed to assess effects of concentration, time, and particle fraction on respiratory responses.

Exposure name	Flaming eucalyptus exposures			
	5-d	5-week	3-h	3F-h
Exposure schedule (total exposure hours)	1 h/d $\times$ 5 d (5)	1 h/d (1 d/week) $\times$ 5 weeks (5)	3 $\times$ 1 h; single day (3)	Filtered 3-h exposure (3)
Target PM (mg/m^3)	3	3	5	0
Recorded PM (mg/m^3)	3.32 ± 0.04	3.14 ± 0.04	6.87 ± 0.07	0
Target PM $\text{C} \times \text{T}$ ($\text{mg}\cdot\text{h}/\text{m}^3$)	15	15	15	0
Recorded $\text{C} \times \text{T}$ ($\text{mg}\cdot\text{h}/\text{m}^3$)	16.59	15.70	20.36	0
CO (ppm)	92 ± 2	83 ± 2	182 ± 6	174 ± 5
CO ₂ (ppm)	3749 ± 46	4013 ± 32	6546 ± 74	7430 ± 72
NO (ppb)	1272 ± 161	1379 ± 129	2429 ± 179	3295 ± 371
NO ₂ (ppb)	778 ± 87	898 ± 57	972 ± 130	74 ± 10
NO _x (ppb)	2041 ± 216	2267 ± 173	3383 ± 292	3350 ± 359
MCE (%)	97.5 ± 0.1	98.0 ± 0.0	97.3 ± 0.1	97.7 ± 0.1

Exposure PM $\text{C} \times \text{T}$ targets were $15 \text{ mg}\cdot\text{h}/\text{m}^3$ (except for filtered 3-h exposure). Values shown are means $\pm$ SEM. MCE: modified combustion efficiency, defined in Table 1.

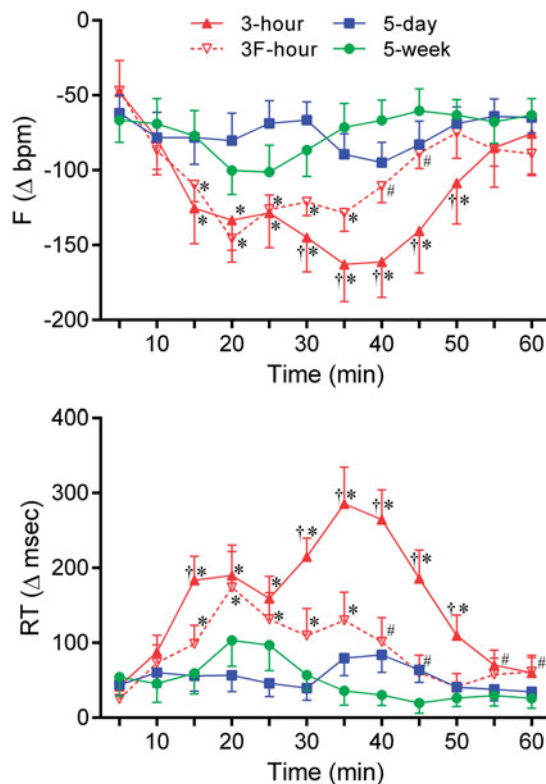


Figure 7. Effects of concentration, time, and particulate fraction on changes in breathing frequency (A) and relaxation time (B). Control mice were exposed to flaming eucalyptus biomass at 3 mg PM/m³ 1 h/d for 5 d (5-d), 3 mg PM/m³ 1 h/d per week over 5 weeks (5-week), 5 mg PM/m³ for three consecutive 1-h periods in a single day (3-h), or the latter exposure filtered to remove PM (3F-h). Data show means and SEM of changes from 20-min clean air averages recorded daily immediately before exposures, and averaged for all hours of exposure ($n=8$ mice/group). * $p < 0.05$ versus both 5-d and 5-week groups. # $p < 0.05$ versus 5-week group. † $p < 0.05$ versus 3F-h group.

(Sison et al. 2000). The same concentration of NO also had anti-inflammatory properties in rats (McElroy et al. 1997; Howlett et al. 1999). NO concentrations in flaming smoke exposures here were in the range of 2–5 ppm, with much lower concentrations in the smoldering phase, suggesting that NO is less likely to exert anti-inflammatory effects compared with CO.

Elevated levels of T-helper 2 (TH₂) cytokines including IL-4, IL-5, IL-13, and eotaxin are found in asthmatic airways compared with healthy airways (Robinson et al. 1993; Ponath et al. 1996; Garlisi et al. 1997). The pro-allergic cytokines IL-4 and IL-5 were suppressed following exposure to all fuel types under both combustion conditions within HDM-allergic mice. Wood smoke exposure has been associated with inflammation including local and systemic pro-inflammatory cytokine production (Van Eeden et al. 2001; Swiston et al. 2008). High CO levels attenuate the production of the pro-inflammatory cytokines IL-6, tumor necrosis factor- α , and macrophage inhibitory protein-2, suppressing inflammation (Ryter and Choi 2016). We observed minimal changes in these cytokines following exposure of control mice to these smoldering and flaming smoke atmospheres (Kim et al. 2019). Relatively high CO levels within biomass smoke are likely to inhibit inflammatory responses, but may not prevent injury induced by chronic or repeated smoke exposures.

Wildfire biomass smoke exposure concentration, frequency, and duration are important determinants of health effects (Liu et al. 2015; Alman et al. 2016; Tinling et al. 2016). Repeated low-level exposures may lead to adaptation, while single high concentration exposures could overwhelm pulmonary defense mechanisms leading to toxicity. Here we found that flaming eucalyptus exposures at ~ 3.3 mg PM/m³ had relatively consistent effects on breathing frequency and relaxation time in control mice whether given over 2 or 5 consecutive days (Figures 3 and 7 and Table S2). However, flaming eucalyptus given over 3 exposure hours in a single day produced greater effects on F and RT than either the 5-d or 5-week protocols despite a similar $C \times T$ product. The effect may be more likely due to the higher PM concentration alone (6.9 mg PM/m³) rather than $C \times T$ product since the respiratory effects were observed even during the first of the 3 exposure hours. Removal of the particulate fraction with a filter significantly reduced but did not eliminate respiratory responses in the 3-h exposures, indicating that both particulate and gaseous components are toxicologically significant.

A limitation of the present study is that only 1-h exposures repeated 2–5 times were evaluated; further investigations are warranted to include subchronic and chronic exposures, along with other animal models representative of susceptible populations to fully assess health impacts. In addition, animals were assessed 4 h following biomass smoke exposures in the allergy study and 24 h after exposures in the $C \times T$ study, typical of previous studies to allow for induction of inflammation. However, fibrotic or remodeling responses may occur at later time points, especially with repeated exposures. Future studies are needed to examine other fuel types based on regional vegetation types and their potential health impacts. The application of our findings to real-world exposures in wildland firefighters is uncertain. Assessment in wildland firefighters have been conducted in several occupational health studies, but do not provide adequate data for pre- and post-shifts adjusting for factors such as gender, race, burn days, smoker status, and job type. Further, different ventilatory parameters are measured during *in vivo* experiments when compared to clinical studies. Decrements in lung function including forced vital capacity and forced expiratory volume in 1 s along with increased respiratory symptoms in wildland firefighters have been demonstrated (Swiston et al. 2008; Adetona et al. 2011; Gaughan et al. 2014).

The products of wildfire smoke are numerous and depend on the nature of the fuel and combustion conditions. Burning each of the three fuels (peat, oak, or eucalyptus) under either flaming or smoldering conditions produced modifications in pulmonary and inflammatory responses. However, the smoldering phase (involving incomplete combustion) emits a higher ratio of pollutants to the fuel consumed and higher PM content, yielding overall greater respiratory and inflammatory effects. Smoldering combustion of oak and eucalyptus produced greater effects on respiratory responses (breathing frequency, minute volume, and peak inspiratory flow) indicating the importance

of PM composition in toxicity of biomass combustion. Changes in real-time pulmonary function were more sensitive than other indices of toxicity produced by biomass smoke exposures. Biomass smoke suppressed inflammatory cell numbers and pro-allergic cytokines under both combustion conditions from all fuel types. Collectively, these findings suggest smoldering oak or eucalyptus smoke exposures lead to greater reductions in respiration and inflammatory responses than smoldering peat.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Data availability

Data supporting the results and analyses from the published paper will be available on the Environmental Dataset Gateway of the U.S. EPA: <https://edg.epa.gov/metadata/catalog/main/home.page>. The DOI 10.23719/1503946 has been reserved for the data and will be active when the publication process is complete and a public page for the dataset has been generated.

Disclaimer

The research described in this article has been reviewed by the National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency and approved for publication. Approval does not signify that the contents necessarily reflect the views or the policies of the Agency nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

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Supplementary File

Smoldering Eucalyptus and Oak Smoke Inhibit Respiration in an Allergic Mouse Model

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Fuel	Burn Type	Model-Day	F (bpm)	MV (mL)	PIF (mL/s)
Peat	SM	NA-1	314 ± 6	32 ± 2	1.93 ± 0.17
		NA-2	299 ± 6	31 ± 5	1.86 ± 0.29
		HDM-1	304 ± 8	30 ± 4	1.86 ± 0.27
		HDM-2	299 ± 7	23 ± 2	1.38 ± 0.11
	FL	NA-1	304 ± 7	32 ± 4	1.90 ± 0.22
		NA-2	293 ± 6	33 ± 4	1.87 ± 0.19
		HDM-1	288 ± 5	28 ± 2	1.61 ± 0.12
		HDM-2	272 ± 5	21 ± 2	1.30 ± 0.11
Oak	SM	NA-1	315 ± 7	31 ± 1	1.91 ± 0.09
		NA-2	296 ± 6	35 ± 2	2.04 ± 0.11
		HDM-1	317 ± 5	21 ± 3	1.27 ± 0.16
		HDM-2	308 ± 13	22 ± 4	1.38 ± 0.20
	FL	NA-1	310 ± 9	36 ± 1	2.19 ± 0.08
		NA-2	304 ± 6	31 ± 2	1.88 ± 0.14
		HDM-1	299 ± 8	21 ± 3*	1.31 ± 0.18*
		HDM-2	287 ± 8	21 ± 3	1.30 ± 0.15
Euc	SM	NA-1	315 ± 4	34 ± 5	2.12 ± 0.34
		NA-2	303 ± 9	32 ± 5	1.94 ± 0.31
		HDM-1	294 ± 13	24 ± 2	1.43 ± 0.11
		HDM-2	295 ± 9	22 ± 3	1.33 ± 0.20
	FL	NA-1	293 ± 7	31 ± 5	1.82 ± 0.27
		NA-2	293 ± 10	32 ± 3	1.94 ± 0.18
		HDM-1	300 ± 13	23 ± 2	1.32 ± 0.11
		HDM-2	281 ± 12	22 ± 4	1.30 ± 0.21

Table S1. Baseline ventilatory parameters during 20-minute exposures to clean air measured immediately prior to each of 2 days exposure to biomass smoke emissions under smoldering (SM) or flaming (FL) conditions in non-allergic (NA) and HDM-allergic (HDM) mice. Data show means ± SEM (n = 8 mice/group) for breathing frequency (F, breaths per minute), minute volume (MV), and peak inspiratory flow (PIF). Prior to the first day of exposure, breathing frequency of all non-allergic mice together was equivalent to that of all HDM-allergic mice (309 ± 3 vs. 300 ± 4, respectively, $P = 0.09$, n = 48 each). At the same time, both MV and PIF were ~25% lower in HDM-allergic mice compared with non-allergic mice (MV: 32.7 ± 1.4 (NA) vs. 24.6 ± 1.2 (HDM); PIF: 1.98 ± 0.09 (NA) vs. 1.47 ± 0.07 (HDM), $P < 0.0001$ for each). As the individual groups had smaller sizes (n = 8), these differences were only significant for the single indicated matching pair. *Significant difference ($P < 0.05$) vs. non-allergic group in the same day-exposure cohort. Euc = Eucalyptus.

Fuel	Burn Type	Model-Day	Ti (Δ msec)	Te (Δ msec)	RT (Δ msec)	TV (Δ ml)	PEF (Δ ml/s)	EF ₅₀ (Δ ml/s)	PenH (Δ units)
Peat	SM	NA-1	25 $\pm$ 3	-21 $\pm$ 9	60 $\pm$ 4	0.05 $\pm$ 0.01	0.05 $\pm$ 0.07	0.00 $\pm$ 0.07	-0.14 $\pm$ 0.04
		NA-2	32 $\pm$ 8	106 $\pm$ 13	92 $\pm$ 9	0.06 $\pm$ 0.01	0.13 $\pm$ 0.17	0.11 $\pm$ 0.16	-0.18 $\pm$ 0.06
		HDM-1	25 $\pm$ 3	-28 $\pm$ 7	56 $\pm$ 6	0.02 $\pm$ 0.01	-0.26 $\pm$ 0.14	-0.24 $\pm$ 0.11	-0.11 $\pm$ 0.03
		HDM-2	32 $\pm$ 4	82 $\pm$ 23	64 $\pm$ 22	0.04 $\pm$ 0.01	-0.02 $\pm$ 0.12	-0.10 $\pm$ 0.10	-0.15 $\pm$ 0.04
	FL	NA-1	23 $\pm$ 7	-2 $\pm$ 12	45 $\pm$ 10	0.05 $\pm$ 0.01	0.07 $\pm$ 0.08	0.09 $\pm$ 0.09	-0.03 $\pm$ 0.03
		NA-2	47 $\pm$ 8	150 $\pm$ 23	120 $\pm$ 23	0.05 $\pm$ 0.01	0.08 $\pm$ 0.09	0.02 $\pm$ 0.09	-0.08 $\pm$ 0.03
		HDM-1	27 $\pm$ 4	-6 $\pm$ 11	63 $\pm$ 9	0.02 $\pm$ 0.01	-0.12 $\pm$ 0.06	-0.15 $\pm$ 0.06	-0.06 $\pm$ 0.04
		HDM-2	41 $\pm$ 8	143 $\pm$ 27	113 $\pm$ 27	0.05 $\pm$ 0.01	0.15 $\pm$ 0.09	0.00 $\pm$ 0.06	-0.17 $\pm$ 0.03
Oak	SM	NA-1	90 $\pm$ 18#	321 $\pm$ 68#	257 $\pm$ 48#	0.04 $\pm$ 0.01	-0.14 $\pm$ 0.11	-0.21 $\pm$ 0.11	-0.07 $\pm$ 0.10
		NA-2	61 $\pm$ 7#	229 $\pm$ 21#	206 $\pm$ 19#	0.03 $\pm$ 0.01	-0.29 $\pm$ 0.09	-0.29 $\pm$ 0.09	-0.09 $\pm$ 0.03
		HDM-1	60 $\pm$ 6*#	119 $\pm$ 87*	95 $\pm$ 82*	0.05 $\pm$ 0.01	0.07 $\pm$ 0.12	0.03 $\pm$ 0.12	-0.16 $\pm$ 0.08
		HDM-2	52 $\pm$ 5#	191 $\pm$ 44#	179 $\pm$ 54#	0.06 $\pm$ 0.01	0.00 $\pm$ 0.13	-0.05 $\pm$ 0.13	-0.28 $\pm$ 0.13
	FL	NA-1	21 $\pm$ 2	61 $\pm$ 6	52 $\pm$ 5	0.04 $\pm$ 0.01	-0.03 $\pm$ 0.07	-0.01 $\pm$ 0.06	-0.05 $\pm$ 0.03
		NA-2	18 $\pm$ 4	37 $\pm$ 14	25 $\pm$ 13	0.03 $\pm$ 0.01	0.06 $\pm$ 0.10	0.05 $\pm$ 0.09	0.04 $\pm$ 0.07
		HDM-1	21 $\pm$ 4	43 $\pm$ 28	30 $\pm$ 31	0.05 $\pm$ 0.01	0.23 $\pm$ 0.10	0.13 $\pm$ 0.11	-0.07 $\pm$ 0.03
		HDM-2	18 $\pm$ 4	55 $\pm$ 12	45 $\pm$ 9	0.03 $\pm$ 0.00	0.13 $\pm$ 0.06	0.09 $\pm$ 0.06	-0.08 $\pm$ 0.02
Euc	SM	NA-1	64 $\pm$ 7#	185 $\pm$ 26#	158 $\pm$ 24#	0.05 $\pm$ 0.01	-0.15 $\pm$ 0.17#	-0.13 $\pm$ 0.10#	-0.08 $\pm$ 0.03
		NA-2	69 $\pm$ 14#	229 $\pm$ 35#	197 $\pm$ 28#	0.05 $\pm$ 0.01	-0.22 $\pm$ 0.13	-0.14 $\pm$ 0.16	-0.10 $\pm$ 0.02
		HDM-1	54 $\pm$ 6#	226 $\pm$ 21#	195 $\pm$ 19#	0.04 $\pm$ 0.01	-0.08 $\pm$ 0.09	-0.16 $\pm$ 0.08	-0.16 $\pm$ 0.03
		HDM-2	64 $\pm$ 7#	217 $\pm$ 37#	183 $\pm$ 28#	0.04 $\pm$ 0.01	-0.11 $\pm$ 0.18	-0.09 $\pm$ 0.09	-0.13 $\pm$ 0.09
	FL	NA-1	15 $\pm$ 1	41 $\pm$ 6	34 $\pm$ 7	0.06 $\pm$ 0.01	0.31 $\pm$ 0.14	0.30 $\pm$ 0.12	-0.07 $\pm$ 0.02
		NA-2	18 $\pm$ 3	50 $\pm$ 10	43 $\pm$ 10	0.04 $\pm$ 0.01	0.20 $\pm$ 0.10	0.22 $\pm$ 0.10	-0.03 $\pm$ 0.01
		HDM-1	22 $\pm$ 3	78 $\pm$ 9	62 $\pm$ 9	0.04 $\pm$ 0.01	0.02 $\pm$ 0.09	-0.03 $\pm$ 0.08	-0.09 $\pm$ 0.02
		HDM-2	16 $\pm$ 2	55 $\pm$ 17	47 $\pm$ 17	0.02 $\pm$ 0.01	-0.04 $\pm$ 0.10	-0.08 $\pm$ 0.09	-0.10 $\pm$ 0.03

Table S2. Changes in ventilatory parameters measured for 1 hour during each of 2 days exposure to biomass smoke emissions under smoldering (SM) or flaming (FL) conditions in non-allergic (NA) and HDM-allergic (HDM) mice. Data show means $\pm$ SEM (n = 8 mice/group) for differences in 1-hour exposure average values compared with 20-minute clean air averages recorded immediately before exposure, for inspiratory time (Ti), expiratory time (Te), relaxation time (RT), tidal volume (TV), peak expiratory flow (PEF), mid-expiratory flow (EF₅₀), and enhanced pause (PenH). *Significant difference ($P < 0.05$) vs. non-allergic group exposed to the same fuel type condition. # $P < 0.05$ vs. flaming exposure condition in groups with the same fuel type and allergic status. Euc = Eucalyptus.

Fuel-Burn	Model	Particulate Matter	Histiocytic Inflammation ¹	Mixed Cell Inflammation	Intra-bronchiolar Mucus	Mucous Cell Metaplasia/Hyperplasia	Multi-nucleated Giant Cells	Intrapulmonary Foreign Material ²	Lymphoid Hyperplasia
Air (Peat)	NA	2/8	7/8 (0.9)	3/8 (0.4)	1/8 (0.1)	1/8 (0.1)	0/8	2/8	1/8 (0.1)
	HDM	1/8	8/8 (1.3)	8/8* (2.0)	8/8* (1.4)	8/8* (2.1)	1/8	0/8	8/8* (1.8)
Peat-SM	NA	1/8	2/8 (0.3)	0/8 (0.0)	0/8 (0.0)	0/8 (0.0)	0/8	0/8	0/8 (0.0)
	HDM	0/8	3/8 (0.4)	8/8* (1.5)	7/8* (1.5)	7/8* (1.9)	0/8	3/8	6/8* (0.9)
Peat-FL	NA	0/8	0/8 (0.0)	0/8 (0.0)	0/8 (0.0)	0/8 (0.0)	0/8	0/8	0/8 (0.0)
	HDM	0/8	2/8 (0.3)	7/8* (1.4)	7/8* (1.4)	7/8* (1.6)	0/8	0/8	4/8 (0.5)
Air (Oak)	NA	4/8	7/8 (1.0)	0/8 (0.0)	2/8 (0.3)	0/8 (0.0)	0/8	1/8	2/8 (0.3)
	HDM	1/8	7/8 (1.5)	8/8* (2.1)	8/8* (1.5)	8/8* (2.4)	4/8	0/8	8/8* (2.0)
Oak-SM	NA	0/8	2/8 (0.3)	0/8 (0.0)	1/8 (0.1)	0/8 (0.0)	0/8	0/8	0/8 (0.0)
	HDM	1/8	7/8* (0.9)	8/8* (1.9)	8/8* (1.3)	8/8* (1.9)	2/8	1/8	5/8* (0.8)
Oak-FL	NA	2/8	3/8 (0.4)	0/8 (0.0)	0/8 (0.0)	1/8 (0.1)	0/8	1/8	0/8 (0.0)
	HDM	1/8	3/8 (0.4)	7/8* (1.3)	5/8* (1.0)	6/8* (1.5)	2/8	2/8	4/8 (0.8)
Air (Euc)	NA	3/8	7/8 (1.0)	8/8 (1.5)	3/8 (0.5)	2/8 (0.3)	2/8	1/8	1/8 (0.1)
	HDM	1/8	7/8 (0.9)	8/8 (1.8)	7/8 (1.1)	7/8* (1.8)	1/8	0/8	6/8* (1.3)
Euc-SM	NA	2/8	5/8 (0.6)	0/8 (0.0)	0/8 (0.0)	0/8 (0.0)	0/8	1/8	0/8 (0.0)
	HDM	2/8	3/8 (0.4)	8/8* (1.8)	7/8* (1.1)	8/8* (1.8)	1/8	1/8	6/8* (0.9)
Euc-FL	NA	0/8	2/8 (0.3)	0/8 (0.0)	0/8 (0.0)	0/8 (0.0)	0/8	1/8	0/8 (0.0)
	HDM	0/8	2/8 (0.3)	8/8* (1.1)	7/8* (1.0)	7/8* (1.3)	1/8	2/8	6/8* (0.8)

Table S3. Incidence summary table of histopathological changes in the lung after exposure to smoldering (SM) or flaming (FL) biomass emissions in non-allergic (NA) and HDM-allergic (HDM) mice (n=8 mice/group). Values represent incidence with average severity score across all individuals in the group in parentheses; severity scores were based on a qualitative 0-4 scale.

¹Histiocytic inflammation included macrophage aggregates with or without lesser numbers of neutrophils and lymphocytes. ²Foreign material was consistent with aspiration of bedding or feed material. * $P < 0.05$ vs. respective non-allergic control group (same treatment) by 2-tailed Fisher's Exact Test. Euc = Eucalyptus.

	5-day		5-week		3-hour		
	Air	FE	Air	FE	Air	FE	Filtered FE
BALF Data							
Macrophages (10 ⁴)	11.40 ± 1.60	8.20 ± 0.83	11.48 ± 0.91	10.11 ± 1.45	9.90 ± 0.88	10.21 ± 0.62	14.33 ± 1.04*
Neutrophils (10 ⁴)	0.12 ± 0.04	0.03 ± 0.01	0.03 ± 0.02	0.09 ± 0.05	0.10 ± 0.03	0.09 ± 0.03	0.10 ± 0.04
Lymphocytes (10 ⁴)	0.01 ± 0.01	0 ± 0	0 ± 0	0 ± 0	0.02 ± 0.01	0.04 ± 0.02	0.02 ± 0.01
Protein (mg/mL)	70.07 ± 6.34	70.42 ± 3.07	68.82 ± 3.18	76.85 ± 7.93	59.09 ± 7.16	55.78 ± 3.25	58.07 ± 2.90
Albumin (mg/mL)	21.85 ± 1.80	17.78 ± 0.83	21.21 ± 1.58	21.64 ± 1.66	19.20 ± 2.54	15.65 ± 0.83	15.21 ± 0.54#
GGT (U/L)	3.80 ± 0.18	3.72 ± 0.12	3.66 ± 0.10	3.36 ± 0.17	3.75 ± 0.07	3.84 ± 0.35	3.41 ± 0.35
LDH (U/L)	13.73 ± 1.53	12.11 ± 1.19	12.49 ± 0.81	13.32 ± 1.22	15.35 ± 1.93	12.63 ± 0.56	13.61 ± 0.68
NAG (U/L)	7.16 ± 0.06	7.15 ± 0.05	6.44 ± 0.21	6.58 ± 0.05	7.16 ± 0.41	6.43 ± 0.03§	6.96 ± 0.09
MIP-2 (pg/mL)	48.86 ± 5.35	47.03 ± 1.89	40.12 ± 5.22	48.55 ± 1.18	61.69 ± 5.79	58.79 ± 7.86	53.46 ± 4.22
TNF-α (pg/mL)	2.98 ± 0.48	2.61 ± 0.56	1.62 ± 0.50	1.62 ± 0.51	6.70 ± 0.54	5.87 ± 0.73	3.70 ± 0.62
IFN-γ (pg/mL)	1.64 ± 0.35	1.57 ± 0.42	0.64 ± 0	0.64 ± 0	2.38 ± 0.87	1.78 ± 0.44	1.39 ± 0.42
IL-6 (pg/mL)	1.28 ± 0	1.28 ± 0	1.28 ± 0	1.28 ± 0	1.74 ± 0.46	1.28 ± 0	1.28 ± 0
Blood Data							
RBC (10 ⁶ /μL)	7.39 ± 0.48	7.11 ± 0.73	7.34 ± 0.17	7.45 ± 0.17	7.11 ± 0.26	8.24 ± 0.20	6.93 ± 0.08
MCV (fL)	49.66 ± 1.16	48.41 ± 0.31	48.20 ± 0.15	47.91 ± 0.20	48.03 ± 0.19	48.42 ± 0.26	48.35 ± 0.19
Hct (%)	36.80 ± 2.83	34.53 ± 3.64	35.40 ± 0.84	35.71 ± 0.82	34.16 ± 1.32	39.91 ± 1.01	33.49 ± 0.49
Hgb (g/dL)	12.6 ± 1.0	12.2 ± 1.3	12.5 ± 0.4	12.6 ± 0.4	11.9 ± 0.5	13.2 ± 0.3	11.5 ± 0.2
MCH (pg)	17.0 ± 0.2	17.1 ± 0.1	17.0 ± 0.2	16.8 ± 0.2	16.7 ± 0.3	16.0 ± 0.1*#	16.6 ± 0.1
MCHC (g/dL)	35.0 ± 0.2	35.2 ± 0.2	35.2 ± 0.4	35.1 ± 0.4	34.7 ± 0.7	33.1 ± 0.3*#	34.4 ± 0.2
WBC (10 ³ /μL)	3.6 ± 0.5	1.9 ± 0.2§	3.4 ± 0.5	2.5 ± 0.5	2.9 ± 0.3	2.2 ± 0.2	2.2 ± 0.2
Lym (10 ³ /μL)	2.7 ± 0.3	1.3 ± 0.2§	2.8 ± 0.4	2.0 ± 0.4	2.3 ± 0.2	1.7 ± 0.2	1.6 ± 0.2
Platelets (10 ³ /μL)	406 ± 25	385 ± 60	436 ± 14	432 ± 17	454 ± 19	419 ± 56	453 ± 32
CRP (mg/L)	0.03 ± 0.02	0.03 ± 0.02	0.02 ± 0.01	0.03 ± 0.01	0.04 ± 0.02	0.12 ± 0.03*#	0.10 ± 0.03

Table S4. Inflammatory markers and cellular endpoints in BALF and blood 24 hours after varying exposures to flaming eucalyptus (FE) with the same PM C×T target (see Table 2 in main paper for exposure characterization). Additional BALF assays for albumin, macrophage inflammatory protein (MIP)-2, tumor necrosis factor (TNF)-α, interferon (IFN)-γ, and IL-6 were carried out as described in Methods for other markers, with reagents from the same manufacturers. Approximately 0.5-0.8 ml of blood was drawn by cardiac puncture into a syringe primed with sodium citrate and deposited into a microcentrifuge tube containing 17 μl sodium citrate. Hematological data including total red blood cells (RBC), mean corpuscular volume (MCV), hematocrit (Hct), hemoglobin (Hgb), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cells (WBC), lymphocytes (Lym), and platelets were measured using a Coulter AcT 10 Hematology Analyzer (Beckman Coulter Inc., Miami, FL). Blood plasma was analyzed for C-reactive protein (CRP) using a kit from DiaSorin (Stillwater, MN). Data show means ± SEM (n = 8 mice/group). *Significant difference ($P < 0.05$) vs. 5-day FE exposure. # $P < 0.05$ vs. 5-week FE exposure. § $P < 0.05$ vs. air control cohort from the same exposure.

Supplementary File

Smoldering Eucalyptus and Oak Smoke Inhibit Respiration in an Allergic Mouse Model

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Fuel	Burn Type	Model-Day	F (bpm)	MV (mL)	PIF (mL/s)
Peat	SM	NA-1	314 ± 6	32 ± 2	1.93 ± 0.17
		NA-2	299 ± 6	31 ± 5	1.86 ± 0.29
		HDM-1	304 ± 8	30 ± 4	1.86 ± 0.27
		HDM-2	299 ± 7	23 ± 2	1.38 ± 0.11
	FL	NA-1	304 ± 7	32 ± 4	1.90 ± 0.22
		NA-2	293 ± 6	33 ± 4	1.87 ± 0.19
		HDM-1	288 ± 5	28 ± 2	1.61 ± 0.12
		HDM-2	272 ± 5	21 ± 2	1.30 ± 0.11
Oak	SM	NA-1	315 ± 7	31 ± 1	1.91 ± 0.09
		NA-2	296 ± 6	35 ± 2	2.04 ± 0.11
		HDM-1	317 ± 5	21 ± 3	1.27 ± 0.16
		HDM-2	308 ± 13	22 ± 4	1.38 ± 0.20
	FL	NA-1	310 ± 9	36 ± 1	2.19 ± 0.08
		NA-2	304 ± 6	31 ± 2	1.88 ± 0.14
		HDM-1	299 ± 8	21 ± 3*	1.31 ± 0.18*
		HDM-2	287 ± 8	21 ± 3	1.30 ± 0.15
Euc	SM	NA-1	315 ± 4	34 ± 5	2.12 ± 0.34
		NA-2	303 ± 9	32 ± 5	1.94 ± 0.31
		HDM-1	294 ± 13	24 ± 2	1.43 ± 0.11
		HDM-2	295 ± 9	22 ± 3	1.33 ± 0.20
	FL	NA-1	293 ± 7	31 ± 5	1.82 ± 0.27
		NA-2	293 ± 10	32 ± 3	1.94 ± 0.18
		HDM-1	300 ± 13	23 ± 2	1.32 ± 0.11
		HDM-2	281 ± 12	22 ± 4	1.30 ± 0.21

Table S1. Baseline ventilatory parameters during 20-minute exposures to clean air measured immediately prior to each of 2 days exposure to biomass smoke emissions under smoldering (SM) or flaming (FL) conditions in non-allergic (NA) and HDM-allergic (HDM) mice. Data show means ± SEM (n = 8 mice/group) for breathing frequency (F, breaths per minute), minute volume (MV), and peak inspiratory flow (PIF). Prior to the first day of exposure, breathing frequency of all non-allergic mice together was equivalent to that of all HDM-allergic mice (309 ± 3 vs. 300 ± 4, respectively, $P = 0.09$, n = 48 each). At the same time, both MV and PIF were ~25% lower in HDM-allergic mice compared with non-allergic mice (MV: 32.7 ± 1.4 (NA) vs. 24.6 ± 1.2 (HDM); PIF: 1.98 ± 0.09 (NA) vs. 1.47 ± 0.07 (HDM), $P < 0.0001$ for each). As the individual groups had smaller sizes (n = 8), these differences were only significant for the single indicated matching pair. *Significant difference ($P < 0.05$) vs. non-allergic group in the same day-exposure cohort. Euc = Eucalyptus.

Fuel	Burn Type	Model-Day	Ti (Δ msec)	Te (Δ msec)	RT (Δ msec)	TV (Δ ml)	PEF (Δ ml/s)	EF ₅₀ (Δ ml/s)	PenH (Δ units)
Peat	SM	NA-1	25 $\pm$ 3	-21 $\pm$ 9	60 $\pm$ 4	0.05 $\pm$ 0.01	0.05 $\pm$ 0.07	0.00 $\pm$ 0.07	-0.14 $\pm$ 0.04
		NA-2	32 $\pm$ 8	106 $\pm$ 13	92 $\pm$ 9	0.06 $\pm$ 0.01	0.13 $\pm$ 0.17	0.11 $\pm$ 0.16	-0.18 $\pm$ 0.06
		HDM-1	25 $\pm$ 3	-28 $\pm$ 7	56 $\pm$ 6	0.02 $\pm$ 0.01	-0.26 $\pm$ 0.14	-0.24 $\pm$ 0.11	-0.11 $\pm$ 0.03
		HDM-2	32 $\pm$ 4	82 $\pm$ 23	64 $\pm$ 22	0.04 $\pm$ 0.01	-0.02 $\pm$ 0.12	-0.10 $\pm$ 0.10	-0.15 $\pm$ 0.04
	FL	NA-1	23 $\pm$ 7	-2 $\pm$ 12	45 $\pm$ 10	0.05 $\pm$ 0.01	0.07 $\pm$ 0.08	0.09 $\pm$ 0.09	-0.03 $\pm$ 0.03
		NA-2	47 $\pm$ 8	150 $\pm$ 23	120 $\pm$ 23	0.05 $\pm$ 0.01	0.08 $\pm$ 0.09	0.02 $\pm$ 0.09	-0.08 $\pm$ 0.03
		HDM-1	27 $\pm$ 4	-6 $\pm$ 11	63 $\pm$ 9	0.02 $\pm$ 0.01	-0.12 $\pm$ 0.06	-0.15 $\pm$ 0.06	-0.06 $\pm$ 0.04
		HDM-2	41 $\pm$ 8	143 $\pm$ 27	113 $\pm$ 27	0.05 $\pm$ 0.01	0.15 $\pm$ 0.09	0.00 $\pm$ 0.06	-0.17 $\pm$ 0.03
Oak	SM	NA-1	90 $\pm$ 18#	321 $\pm$ 68#	257 $\pm$ 48#	0.04 $\pm$ 0.01	-0.14 $\pm$ 0.11	-0.21 $\pm$ 0.11	-0.07 $\pm$ 0.10
		NA-2	61 $\pm$ 7#	229 $\pm$ 21#	206 $\pm$ 19#	0.03 $\pm$ 0.01	-0.29 $\pm$ 0.09	-0.29 $\pm$ 0.09	-0.09 $\pm$ 0.03
		HDM-1	60 $\pm$ 6*#	119 $\pm$ 87*	95 $\pm$ 82*	0.05 $\pm$ 0.01	0.07 $\pm$ 0.12	0.03 $\pm$ 0.12	-0.16 $\pm$ 0.08
		HDM-2	52 $\pm$ 5#	191 $\pm$ 44#	179 $\pm$ 54#	0.06 $\pm$ 0.01	0.00 $\pm$ 0.13	-0.05 $\pm$ 0.13	-0.28 $\pm$ 0.13
	FL	NA-1	21 $\pm$ 2	61 $\pm$ 6	52 $\pm$ 5	0.04 $\pm$ 0.01	-0.03 $\pm$ 0.07	-0.01 $\pm$ 0.06	-0.05 $\pm$ 0.03
		NA-2	18 $\pm$ 4	37 $\pm$ 14	25 $\pm$ 13	0.03 $\pm$ 0.01	0.06 $\pm$ 0.10	0.05 $\pm$ 0.09	0.04 $\pm$ 0.07
		HDM-1	21 $\pm$ 4	43 $\pm$ 28	30 $\pm$ 31	0.05 $\pm$ 0.01	0.23 $\pm$ 0.10	0.13 $\pm$ 0.11	-0.07 $\pm$ 0.03
		HDM-2	18 $\pm$ 4	55 $\pm$ 12	45 $\pm$ 9	0.03 $\pm$ 0.00	0.13 $\pm$ 0.06	0.09 $\pm$ 0.06	-0.08 $\pm$ 0.02
Euc	SM	NA-1	64 $\pm$ 7#	185 $\pm$ 26#	158 $\pm$ 24#	0.05 $\pm$ 0.01	-0.15 $\pm$ 0.17#	-0.13 $\pm$ 0.10#	-0.08 $\pm$ 0.03
		NA-2	69 $\pm$ 14#	229 $\pm$ 35#	197 $\pm$ 28#	0.05 $\pm$ 0.01	-0.22 $\pm$ 0.13	-0.14 $\pm$ 0.16	-0.10 $\pm$ 0.02
		HDM-1	54 $\pm$ 6#	226 $\pm$ 21#	195 $\pm$ 19#	0.04 $\pm$ 0.01	-0.08 $\pm$ 0.09	-0.16 $\pm$ 0.08	-0.16 $\pm$ 0.03
		HDM-2	64 $\pm$ 7#	217 $\pm$ 37#	183 $\pm$ 28#	0.04 $\pm$ 0.01	-0.11 $\pm$ 0.18	-0.09 $\pm$ 0.09	-0.13 $\pm$ 0.09
	FL	NA-1	15 $\pm$ 1	41 $\pm$ 6	34 $\pm$ 7	0.06 $\pm$ 0.01	0.31 $\pm$ 0.14	0.30 $\pm$ 0.12	-0.07 $\pm$ 0.02
		NA-2	18 $\pm$ 3	50 $\pm$ 10	43 $\pm$ 10	0.04 $\pm$ 0.01	0.20 $\pm$ 0.10	0.22 $\pm$ 0.10	-0.03 $\pm$ 0.01
		HDM-1	22 $\pm$ 3	78 $\pm$ 9	62 $\pm$ 9	0.04 $\pm$ 0.01	0.02 $\pm$ 0.09	-0.03 $\pm$ 0.08	-0.09 $\pm$ 0.02
		HDM-2	16 $\pm$ 2	55 $\pm$ 17	47 $\pm$ 17	0.02 $\pm$ 0.01	-0.04 $\pm$ 0.10	-0.08 $\pm$ 0.09	-0.10 $\pm$ 0.03

Table S2. Changes in ventilatory parameters measured for 1 hour during each of 2 days exposure to biomass smoke emissions under smoldering (SM) or flaming (FL) conditions in non-allergic (NA) and HDM-allergic (HDM) mice. Data show means $\pm$ SEM (n = 8 mice/group) for differences in 1-hour exposure average values compared with 20-minute clean air averages recorded immediately before exposure, for inspiratory time (Ti), expiratory time (Te), relaxation time (RT), tidal volume (TV), peak expiratory flow (PEF), mid-expiratory flow (EF₅₀), and enhanced pause (PenH). *Significant difference ($P < 0.05$) vs. non-allergic group exposed to the same fuel type condition. # $P < 0.05$ vs. flaming exposure condition in groups with the same fuel type and allergic status. Euc = Eucalyptus.

Fuel-Burn	Model	Particulate Matter	Histiocytic Inflammation ¹	Mixed Cell Inflammation	Intra-bronchiolar Mucus	Mucous Cell Metaplasia/Hyperplasia	Multi-nucleated Giant Cells	Intrapulmonary Foreign Material ²	Lymphoid Hyperplasia
Air (Peat)	NA	2/8	7/8 (0.9)	3/8 (0.4)	1/8 (0.1)	1/8 (0.1)	0/8	2/8	1/8 (0.1)
	HDM	1/8	8/8 (1.3)	8/8* (2.0)	8/8* (1.4)	8/8* (2.1)	1/8	0/8	8/8* (1.8)
Peat-SM	NA	1/8	2/8 (0.3)	0/8 (0.0)	0/8 (0.0)	0/8 (0.0)	0/8	0/8	0/8 (0.0)
	HDM	0/8	3/8 (0.4)	8/8* (1.5)	7/8* (1.5)	7/8* (1.9)	0/8	3/8	6/8* (0.9)
Peat-FL	NA	0/8	0/8 (0.0)	0/8 (0.0)	0/8 (0.0)	0/8 (0.0)	0/8	0/8	0/8 (0.0)
	HDM	0/8	2/8 (0.3)	7/8* (1.4)	7/8* (1.4)	7/8* (1.6)	0/8	0/8	4/8 (0.5)
Air (Oak)	NA	4/8	7/8 (1.0)	0/8 (0.0)	2/8 (0.3)	0/8 (0.0)	0/8	1/8	2/8 (0.3)
	HDM	1/8	7/8 (1.5)	8/8* (2.1)	8/8* (1.5)	8/8* (2.4)	4/8	0/8	8/8* (2.0)
Oak-SM	NA	0/8	2/8 (0.3)	0/8 (0.0)	1/8 (0.1)	0/8 (0.0)	0/8	0/8	0/8 (0.0)
	HDM	1/8	7/8* (0.9)	8/8* (1.9)	8/8* (1.3)	8/8* (1.9)	2/8	1/8	5/8* (0.8)
Oak-FL	NA	2/8	3/8 (0.4)	0/8 (0.0)	0/8 (0.0)	1/8 (0.1)	0/8	1/8	0/8 (0.0)
	HDM	1/8	3/8 (0.4)	7/8* (1.3)	5/8* (1.0)	6/8* (1.5)	2/8	2/8	4/8 (0.8)
Air (Euc)	NA	3/8	7/8 (1.0)	8/8 (1.5)	3/8 (0.5)	2/8 (0.3)	2/8	1/8	1/8 (0.1)
	HDM	1/8	7/8 (0.9)	8/8 (1.8)	7/8 (1.1)	7/8* (1.8)	1/8	0/8	6/8* (1.3)
Euc-SM	NA	2/8	5/8 (0.6)	0/8 (0.0)	0/8 (0.0)	0/8 (0.0)	0/8	1/8	0/8 (0.0)
	HDM	2/8	3/8 (0.4)	8/8* (1.8)	7/8* (1.1)	8/8* (1.8)	1/8	1/8	6/8* (0.9)
Euc-FL	NA	0/8	2/8 (0.3)	0/8 (0.0)	0/8 (0.0)	0/8 (0.0)	0/8	1/8	0/8 (0.0)
	HDM	0/8	2/8 (0.3)	8/8* (1.1)	7/8* (1.0)	7/8* (1.3)	1/8	2/8	6/8* (0.8)

Table S3. Incidence summary table of histopathological changes in the lung after exposure to smoldering (SM) or flaming (FL) biomass emissions in non-allergic (NA) and HDM-allergic (HDM) mice (n=8 mice/group). Values represent incidence with average severity score across all individuals in the group in parentheses; severity scores were based on a qualitative 0-4 scale.

¹Histiocytic inflammation included macrophage aggregates with or without lesser numbers of neutrophils and lymphocytes. ²Foreign material was consistent with aspiration of bedding or feed material. * $P < 0.05$ vs. respective non-allergic control group (same treatment) by 2-tailed Fisher's Exact Test. Euc = Eucalyptus.

	5-day		5-week		3-hour		
	Air	FE	Air	FE	Air	FE	Filtered FE
BALF Data							
Macrophages (10 ⁴)	11.40 ± 1.60	8.20 ± 0.83	11.48 ± 0.91	10.11 ± 1.45	9.90 ± 0.88	10.21 ± 0.62	14.33 ± 1.04*
Neutrophils (10 ⁴)	0.12 ± 0.04	0.03 ± 0.01	0.03 ± 0.02	0.09 ± 0.05	0.10 ± 0.03	0.09 ± 0.03	0.10 ± 0.04
Lymphocytes (10 ⁴)	0.01 ± 0.01	0 ± 0	0 ± 0	0 ± 0	0.02 ± 0.01	0.04 ± 0.02	0.02 ± 0.01
Protein (mg/mL)	70.07 ± 6.34	70.42 ± 3.07	68.82 ± 3.18	76.85 ± 7.93	59.09 ± 7.16	55.78 ± 3.25	58.07 ± 2.90
Albumin (mg/mL)	21.85 ± 1.80	17.78 ± 0.83	21.21 ± 1.58	21.64 ± 1.66	19.20 ± 2.54	15.65 ± 0.83	15.21 ± 0.54#
GGT (U/L)	3.80 ± 0.18	3.72 ± 0.12	3.66 ± 0.10	3.36 ± 0.17	3.75 ± 0.07	3.84 ± 0.35	3.41 ± 0.35
LDH (U/L)	13.73 ± 1.53	12.11 ± 1.19	12.49 ± 0.81	13.32 ± 1.22	15.35 ± 1.93	12.63 ± 0.56	13.61 ± 0.68
NAG (U/L)	7.16 ± 0.06	7.15 ± 0.05	6.44 ± 0.21	6.58 ± 0.05	7.16 ± 0.41	6.43 ± 0.03§	6.96 ± 0.09
MIP-2 (pg/mL)	48.86 ± 5.35	47.03 ± 1.89	40.12 ± 5.22	48.55 ± 1.18	61.69 ± 5.79	58.79 ± 7.86	53.46 ± 4.22
TNF-α (pg/mL)	2.98 ± 0.48	2.61 ± 0.56	1.62 ± 0.50	1.62 ± 0.51	6.70 ± 0.54	5.87 ± 0.73	3.70 ± 0.62
IFN-γ (pg/mL)	1.64 ± 0.35	1.57 ± 0.42	0.64 ± 0	0.64 ± 0	2.38 ± 0.87	1.78 ± 0.44	1.39 ± 0.42
IL-6 (pg/mL)	1.28 ± 0	1.28 ± 0	1.28 ± 0	1.28 ± 0	1.74 ± 0.46	1.28 ± 0	1.28 ± 0
Blood Data							
RBC (10 ⁶ /μL)	7.39 ± 0.48	7.11 ± 0.73	7.34 ± 0.17	7.45 ± 0.17	7.11 ± 0.26	8.24 ± 0.20	6.93 ± 0.08
MCV (fL)	49.66 ± 1.16	48.41 ± 0.31	48.20 ± 0.15	47.91 ± 0.20	48.03 ± 0.19	48.42 ± 0.26	48.35 ± 0.19
Hct (%)	36.80 ± 2.83	34.53 ± 3.64	35.40 ± 0.84	35.71 ± 0.82	34.16 ± 1.32	39.91 ± 1.01	33.49 ± 0.49
Hgb (g/dL)	12.6 ± 1.0	12.2 ± 1.3	12.5 ± 0.4	12.6 ± 0.4	11.9 ± 0.5	13.2 ± 0.3	11.5 ± 0.2
MCH (pg)	17.0 ± 0.2	17.1 ± 0.1	17.0 ± 0.2	16.8 ± 0.2	16.7 ± 0.3	16.0 ± 0.1*#	16.6 ± 0.1
MCHC (g/dL)	35.0 ± 0.2	35.2 ± 0.2	35.2 ± 0.4	35.1 ± 0.4	34.7 ± 0.7	33.1 ± 0.3*#	34.4 ± 0.2
WBC (10 ³ /μL)	3.6 ± 0.5	1.9 ± 0.2§	3.4 ± 0.5	2.5 ± 0.5	2.9 ± 0.3	2.2 ± 0.2	2.2 ± 0.2
Lym (10 ³ /μL)	2.7 ± 0.3	1.3 ± 0.2§	2.8 ± 0.4	2.0 ± 0.4	2.3 ± 0.2	1.7 ± 0.2	1.6 ± 0.2
Platelets (10 ³ /μL)	406 ± 25	385 ± 60	436 ± 14	432 ± 17	454 ± 19	419 ± 56	453 ± 32
CRP (mg/L)	0.03 ± 0.02	0.03 ± 0.02	0.02 ± 0.01	0.03 ± 0.01	0.04 ± 0.02	0.12 ± 0.03*#	0.10 ± 0.03

Table S4. Inflammatory markers and cellular endpoints in BALF and blood 24 hours after varying exposures to flaming eucalyptus (FE) with the same PM C×T target (see Table 2 in main paper for exposure characterization). Additional BALF assays for albumin, macrophage inflammatory protein (MIP)-2, tumor necrosis factor (TNF)-α, interferon (IFN)-γ, and IL-6 were carried out as described in Methods for other markers, with reagents from the same manufacturers. Approximately 0.5-0.8 ml of blood was drawn by cardiac puncture into a syringe primed with sodium citrate and deposited into a microcentrifuge tube containing 17 μl sodium citrate. Hematological data including total red blood cells (RBC), mean corpuscular volume (MCV), hematocrit (Hct), hemoglobin (Hgb), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cells (WBC), lymphocytes (Lym), and platelets were measured using a Coulter AcT 10 Hematology Analyzer (Beckman Coulter Inc., Miami, FL). Blood plasma was analyzed for C-reactive protein (CRP) using a kit from DiaSorin (Stillwater, MN). Data show means ± SEM (n = 8 mice/group). *Significant difference ($P < 0.05$) vs. 5-day FE exposure. # $P < 0.05$ vs. 5-week FE exposure. § $P < 0.05$ vs. air control cohort from the same exposure.