

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.