OXIDATIVE STRESS, INFLAMMATORY BIOMARKERS AND TOXICITY IN MOUSE LUNG AND LIVER AFTER INHALATION EXPOSURE TO 100% BIODIESEL OR PETROLEUM DIESEL EMISSIONS.

Supplemental Methods.

Combustion emissions (CE) generation system and conditions. Resistance heating elements provided a constant 3 kW load. From the engine, approximately 85 L/min of the CE was directed to a cone diluter and mixed with 595 L/min (7:1 dilution) of high efficiency particulate air (HEPA) filtered room air. The diluted CE then traveled approximately 12m through 7.6cm diameter stainless steel tubing to three Hazelton 1000 (984 L, Lab Products, Inc., H-1000, Seaford, DE) exposure chambers housed in an isolated animal exposure room. Target CE concentrations in the three chambers were 50 μg (low), 150 μg and 500 μg (high) of PM/m^3. Control animals were placed in a fourth chamber supplied with the same HEPA filtered room air. All four chambers were operated at the same flow rate (283 L/min), which resulted in approximately 17 air exchanges per hour. Integrated 4 h samples from Teflon filters/Tedlar bag were collected daily from each chamber and analyzed gravimetrically to determine particle concentrations (Table S2). Analysis of PAHs was conducted from filters of emissions collected in the chambers not during exposures of animals. Gas and particulate samples were collected via probes directed to the center top of the inhalation chamber. Continuous emission monitors were used to measure chamber concentrations of PM by a tapered element oscillating microbalance, (TEOM, Rupprecht and Patashnick Co., series 1400, Albany, NY), oxygen (O_2, Beckman Corp., model 755, La Habra, CA), carbon monoxide (CO, Thermo Electron Corp, model 48, Franklin, MA), nitrogen oxides (NO and NO_2, Teledyne Technology Co., model 200A4, San Diego, CA),

and sulfur dioxide (SO_2, Thermo Electron Corp, model 43c, Franklin, MA). Samples were extracted through fixed stainless steel probes in the exposure chambers. Gas samples were passed through a particulate filter prior to the individual gas analyzers. Dilution air was adjusted periodically to maintain target PM concentrations as measured by the TEOM. Particle size distributions were characterized during each exposure using an engine exhaust particle sizer (EEPS, TSI Inc., model 3091, St. Paul, MN). **A summary of all the parameters evaluated in the animal chambers is listed in supplementary Table S2.**

Supplemental Material, Table S1: Pre-combustion components of B100 and D100 fuels. [1]GC-MS analysis of Hexane/Isopropanol/Benzene extract. [2]Generic composition of number 2 diesel fuel.

Component	B100 (Soy Biodiesel [1])	D100 (Petroleum Diesel [2])
Methyl linoleate	46%	0%
Methyl oleate	14%	0%
Methyl linolenate	8%	0%
Stearic acid methyl ester	3%	0%
Hexadecanoic acid	9%	0%
Oleic acid	4%	0%
Octadecanoic acid	3%	0%
Unidentified	13%	0%
C8-C25 alkanes	0%	50-80%
Aromatic compounds	0%	20-50%
Sulfur	0%	<15 ppm

Supplemental Material, Table S2: Summary of inhalation chamber concentrations and particle size distributions of the D100 and B100 combustion emission particles and selected gases within the animal inhalation chambers at all target concentrations.

Measurement	Units	D100 (petroleum diesel) Target Concentration ($\mu g/m^3$)				B100 (100% soy) Target Concentration ($\mu g/m^3$)			
		0	50	150	500	0	50	150	500
Particle Mass Concentration (TEOM)	$\mu g/m^3$	1.2 ± 0.3	49.3 ± 1.2	147 ± 4	490 ± 6	1.5 ± 0.1	54.6 ± 2.4	155 ± 2	521 ± 10
Particle Number (by TSI SMPS)	#/cc	ND	ND	ND	3.5 ± 0.7 x e5	ND	ND	ND	5.0 ± 0.6 x e5
Nitrogen oxide (NO)	ppm	<1	3.6 ± 0.3	6.7 ± 0.2	17.2 ± 0.5	<1	3.8 ± 0.3	9.3 ± 0.6	19.9 ± 0.8
Nitrogen dioxide (NO_2)	ppm	<1	<1	<1	2.2 ± 0.1	<1	<1	<1	<1
Carbon monoxide (CO)	ppm	<1	4.9 ± 0.4	8.9 ± 0.4	27.7 ± 1.1	<1	1.6 ± 0.2	5.2 ± 0.6	12.3 ± 0.4
Sulfur dioxide (SO_2)	ppm	<1	<1	<1	<1	<1	<1	<1	<1
Oxygen (O_2)	%	20.0 ± 0.9	20.8 ± 0.0	20.7 ± 0.0	20.3 ± 0.0	21.0 ± 0.0	20.9 ± 0.3	20.7 ± 0.0	20.4 ± 0.0
Nitrogen oxides (NO_x)	ppm	<1	4.1 ± 0.3	7.7 ± 0.2	19.5 ± 0.6	<1	4.0 ± 0.4	9.9 ± 0.7	21.1± 0.8
Temperature	°F	74.8 ± 0.1	76.8 ± 0.3	75.0 ± 0.3	73.6 ± 0.2	75.9 ± 0.7	76.7 ± 0.4	75.6 ± 0.3	74.7 ± 0.1
Humidity	%	43.1 ± 0.5	40.0 ± 0.7	44.0 ± 0.5	53.5 ± 0.5	34.5 ± 1.0	32.7 ± 1.3	42.4 ± 1.6	42.0 ± 1.3
Number median$^{\Psi}$ *	nm	ND	ND	ND	74 ± 2	ND	ND	ND	61 ± 3
Volume median$^{\Psi}$ *	nm	ND	ND	ND	166 ± 9	ND	ND	ND	109 ± 4
Surface median$^{\Psi}$ *	nm	ND	ND	ND	118 ± 5	ND	ND	ND	87 ± 3
Pyrene @	µg/g	125.3 ± 2.6				27.9 ± 0.6			
Total PAH @	µg/g	251.5 ± 5.3				94.6 ± 3.6			

All particle mass and gas concentrations for B100 and D100 are reported means± standard errors based on 20 exposure days. ND: Not determined.

$^{\Psi}$: indicates particle number, surface area, and volume median diameters for particle size distributions ± geometric standard deviation. The volume median was calculated from number median based on mobility diameters assuming spherical particles and a density of 1.2 g/ml. The sample size for B100 and D100 were 12 and 23 samples, respectively.

***:** particle size distributions were determined with a Scanning Mobility Particle Sizer (SMPS) by TSI. This instrument was available for the B100 portion of the study but was unavailable for the D100 portion. D100 data was retrieved from a previous study using the same instrument, fuel and operating conditions over a 33-day period. The previous study was of similar duration. Dates were March 3 to April 11 2008.

@: Data for pyrene and total PAH is based on analysis of samples taken during higher concentration exposures and indicate mean + SD.

Table S3: Total protein in the lungs and liver of BALB/cJ mice in response to inhalation exposure of neat biodiesel (B100) and diesel (D100) combustion exhaust particles for a total of 4 weeks at a rate of 4hr/day and 5days/week. Data are presented as mean ± SE.

Organ type	Fuel Type	control	50 μg/m^3	150 μg/m^3	500 μg/m^3
Liver	B100	93.04 ± 6.76	86.73 ± 9.16	87.84 ± 9.49	92.82 ± 3
	D100	96.04 ± 4.01	103.44 ± 3.29	112.19 ± 6.09	101.03 ± 5.94
Lung	B100	13.87 ± 1.61	13.73 ± 1.45	11.27 ± 0.64	13.43 ± 0.69
	D100	18.36 ± 1.01	18.03 ± 2.08	21.8 ± 1.21	20.66 ± 1.73