MINING DIESEL mdec EMISSIONS COUNCIL

Biodiesel as a Control Strategy for Reducing Exposure of Underground Miners to Diesel Aerosols: Effects of Toxicological Properties of Emitted Aerosols

By
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The lung is constantly exposed to airborne particles.

- On a daily basis, a person can inhale 20 m^3 of air, resulting in deposition of airborne particles on the epithelial surface of the lung.
- Exposure to particulates increases risk for cardiopulmonary diseases and lung cancer, as well as exacerbation of asthma and development of allergy in the early years of life.
- Air pollutants are estimated to account for 800,000 premature deaths every year.
- In recent years, special awareness has been drawn to potential health effects of ultrafine (< 100 nm) and nanoparticles (<50 nm).
- Due to use of diesel-powered equipment in confined space, the concentrations of ultrafine and nanoparticle are relatively high in underground mining operations.

Respiratory Tract
(Particle Deposition)

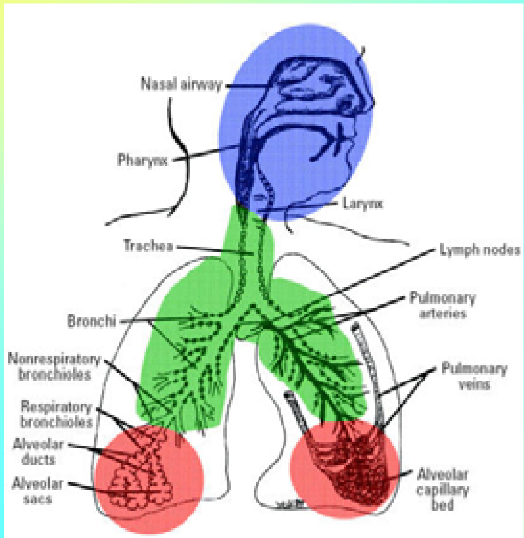
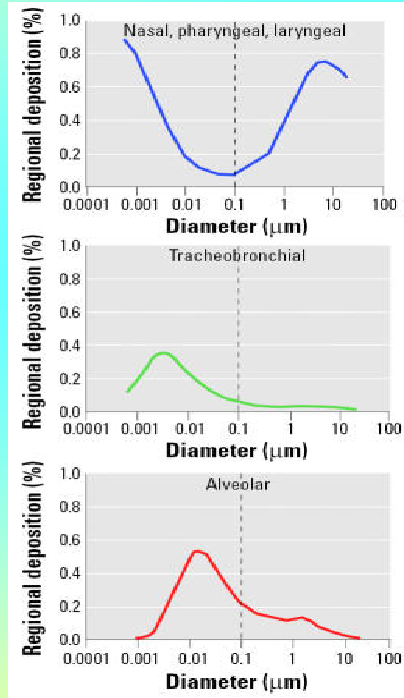
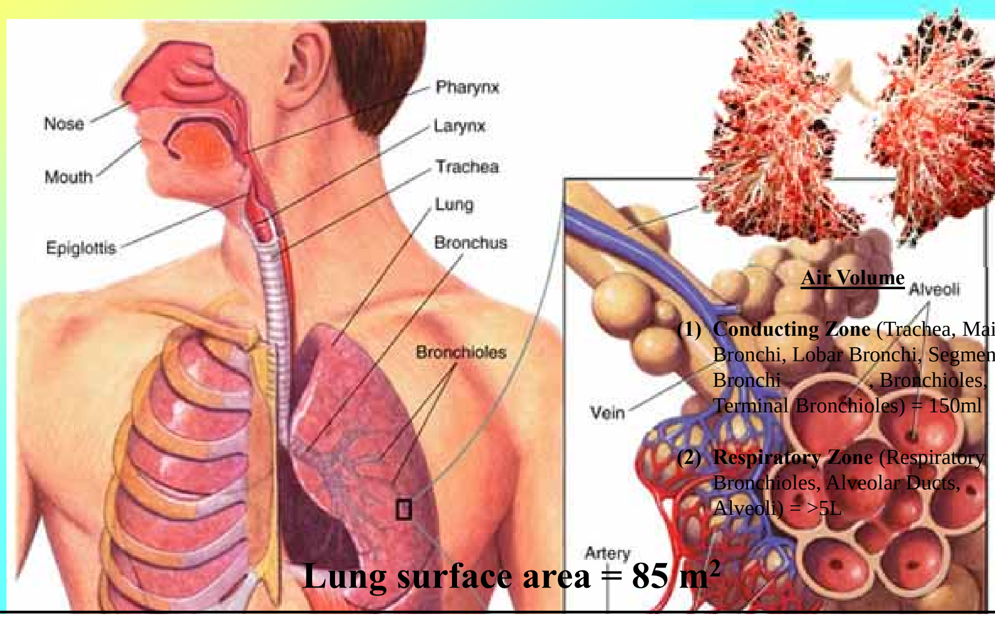


Figure: courtesy of Harkema; adapted from 1994



Branching of Human Airways



Air Volume

(1) **Conducting Zone** (Trachea, Main Bronchi, Lobar Bronchi, Segmental Bronchi , Bronchioles, Terminal Bronchioles) = 150ml

(2) **Respiratory Zone** (Respiratory Bronchioles, Alveolar Ducts, Alveoli) = >5L

Lung surface area = 85 m^2

Biological Effects of Biodiesel Aerosols vs. Diesel Aerosols:

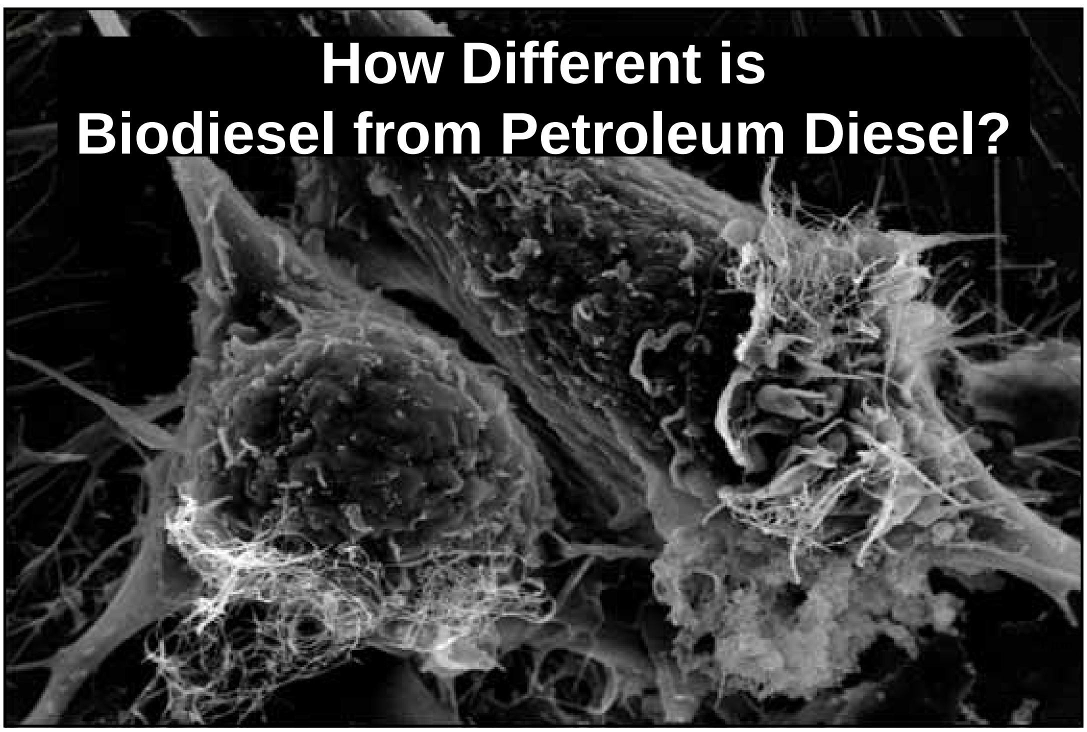
1) Does respiratory exposure to biodiesel and diesel aerosols cause pulmonary inflammation?

2) Do biodiesel and diesel aerosols cause oxidative stress?

3) Do biodiesel and diesel aerosols cause reproductive toxicity?

4) Are biodiesel aerosols as harmful as diesel aerosols?

How Different is Biodiesel from Petroleum Diesel?

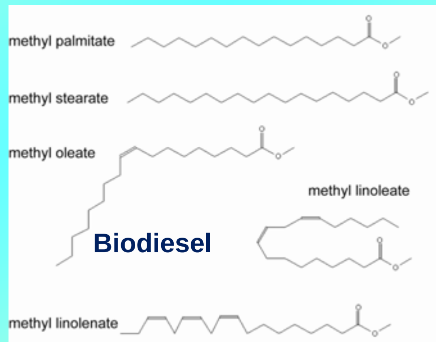


Chemical Composition of Biodiesel and Diesel

Components of Pre-combustion

Component	Biodiesel	Diesel
Fatty Acid Methyl Esters		
(a) 18:1	20-49%	0%
(b) 18:2	34-62%	0%
(c) 16:0	6-12%	0%
(d) 18:0	2-5%	0%
(e) 18:3	5-11%	0%
C8-C25 alkanes	0%	80-50%
Aromatic compounds	0%	20-35%
Sulfur	0%	<15 ppm

Diesel



Primarily composed of poly-unsaturated fatty acids, biodiesel is more susceptible to oxidation upon combustion, leading to formation of peroxide and other oxidation products.

Engine Specifications & Operating Conditions

(Diesel Lab at NIOSH OMSHR)

Isuzu C240 Engine	
Type	in-line 4
Cycles	4
Cooling	Water
Valves	Overhead
Injection	Indirect
Air intake system	naturally aspirated
Engine management	mechanical
Displacement [cm^3 (in^3)]	2369 (145)
Intermittent rating [kW (bhp)]	41.8 (56) @ 3000 rpm
Continuous rating [kW (bhp)]	36.5 (49) @ 3000 rpm
Peak torque [Nm (lb ft)]	146.4 (108) @ 2000
Moment of inertia [kg m^3 (lb ft^2)]	1499 (35.59)
Engine speed at idle [rpm]	700±50
Maximum engine speed at no load [rpm]	3260±50

Mode	Description	Engine Speed	Torque	Power
		rpm	Nm	kW
R50	Rated speed 50% load	2950	55.6	17.2
R100	Rated speed 100% load	2950	111.2	34.3
I50	Intermediate speed 50% load	2100	69.1	14.9
I100	Intermediate speed 100% load	2100	136.9	30.6

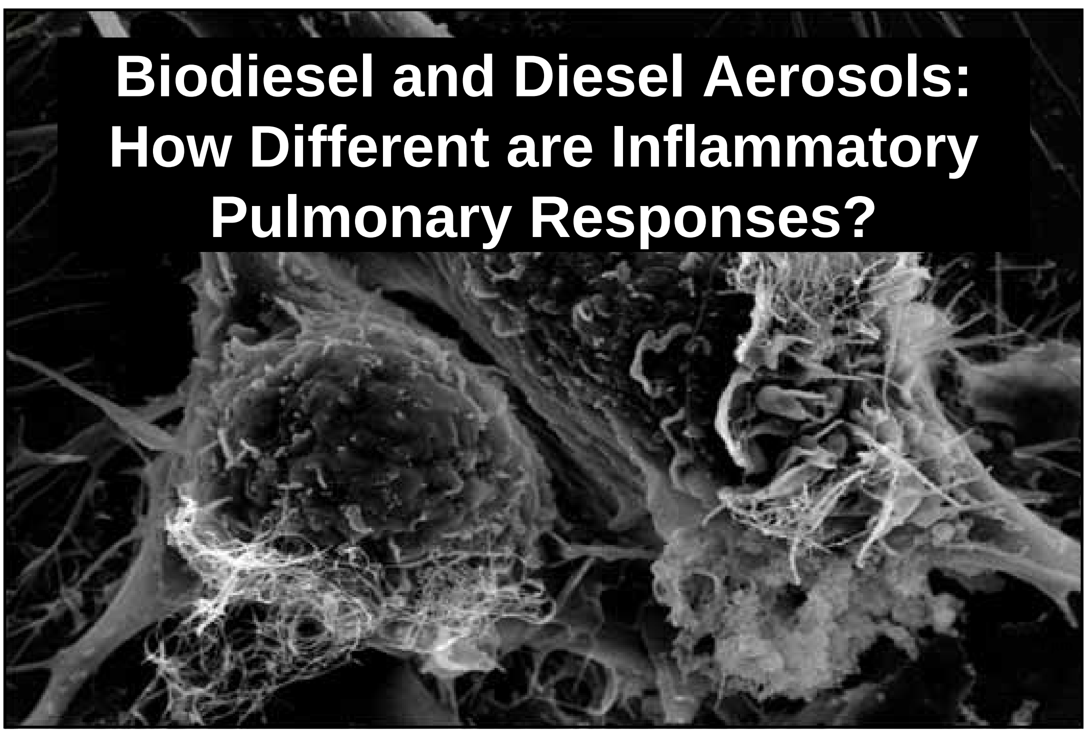
The diluted exhaust samples were collected in liquid medium using a custom designed sampling system, a slightly modified version of the versatile aerosol concentrator enrichment system (VACES) & BioSampler.

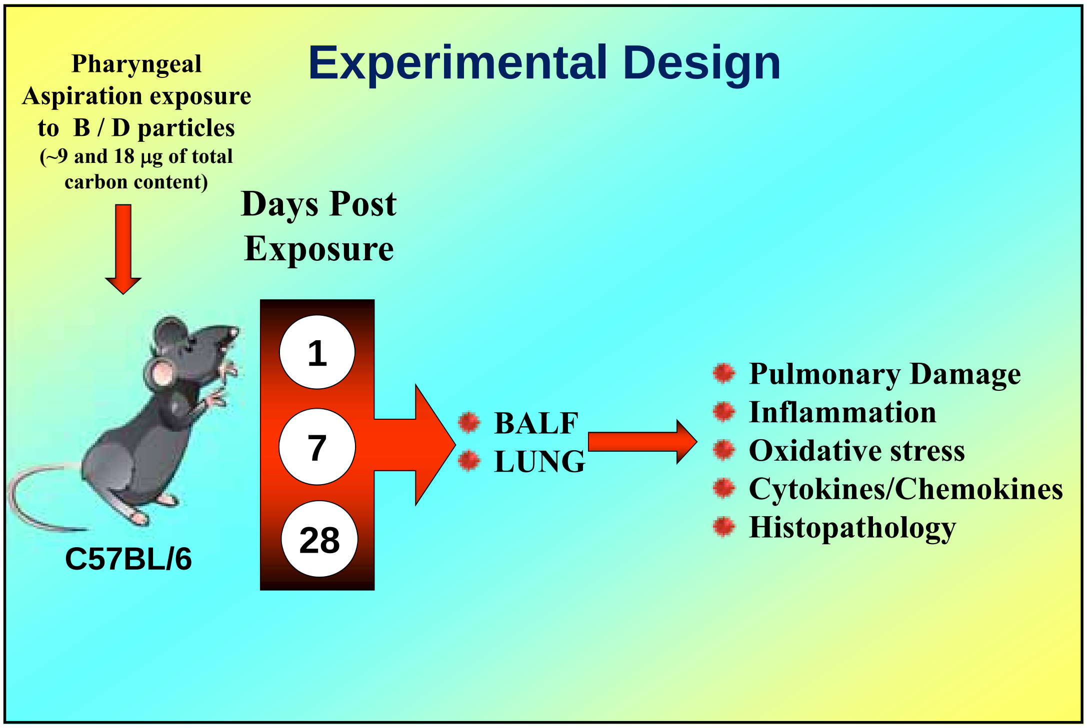
Modified VACES System

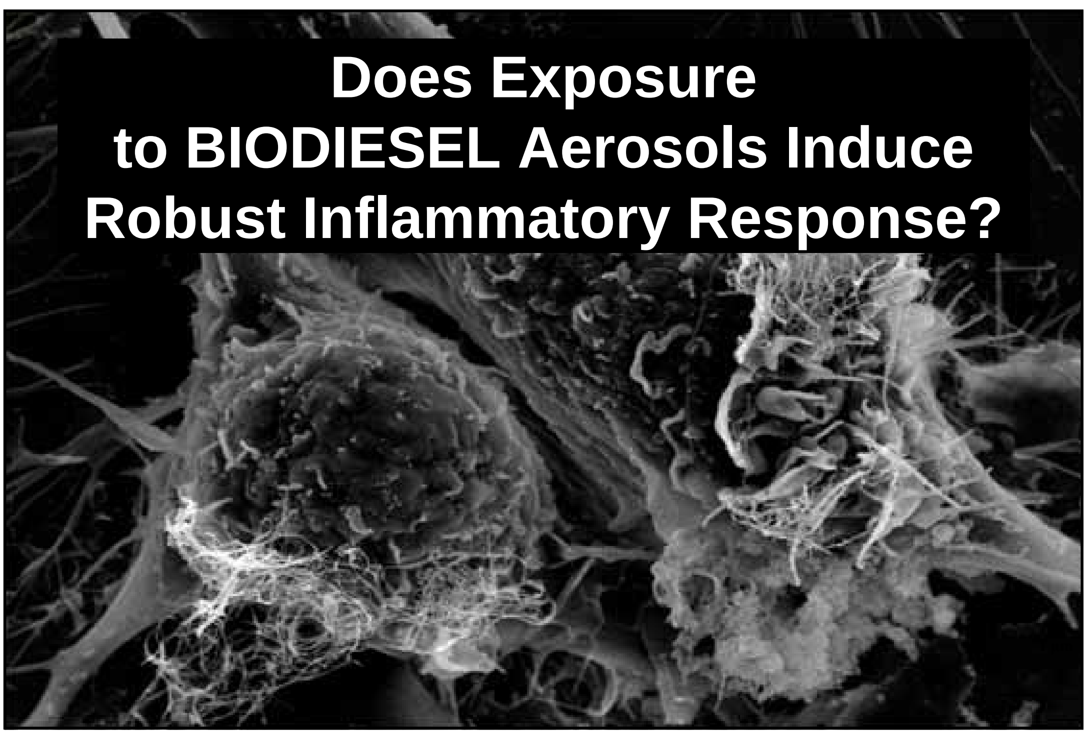
BioSampler with DPM sample

Collection of nano and ultrafine DPM aerosols in liquid media, preserves their physical and chemical characteristics. Collecting and assaying DPM in water minimizes non-physiologic agglomeration, dissolution, and surface conditioning of DPM and destruction of the particulate properties that can occur in filter collection, and solvent extraction.

Biodiesel and Diesel Aerosols: How Different are Inflammatory Pulmonary Responses?

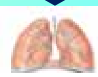



Experimental Design
Pharyngeal
Aspiration exposure
to B / D particles
(~9 and 18 μg of total
carbon content)
Days Post
Exposure
1
7
28
C57BL/6
BALF
LUNG
Pulmonary Damage
Inflammation
Oxidative stress
Cytokines/Chemokines
Histopathology


Does Exposure
to BIODIESEL Aerosols Induce
Robust Inflammatory Response?

Typical Features of (Pulmonary) Inflammatory Response

Exposure to Biodiesel (B) or Diesel (D) Aerosols



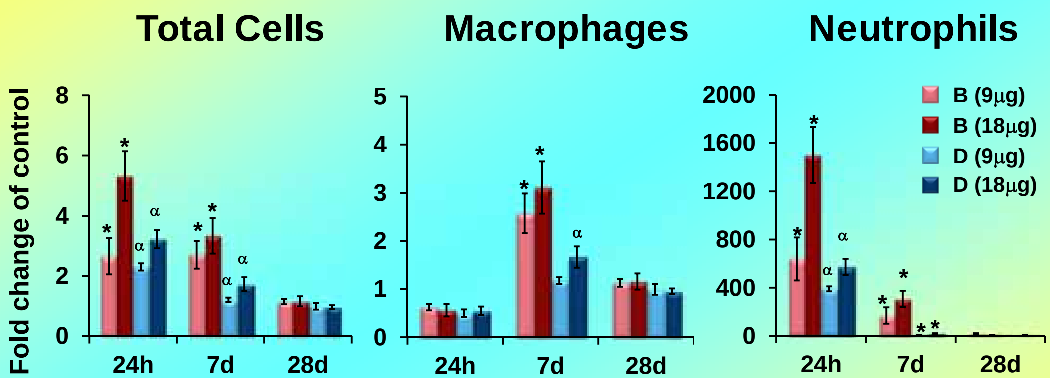
LUNG

Bronchoalveolar lavage (BAL)
Lung homogenate

Inflammatory response

- Recruitment and activation of inflammatory cells (neutrophils, macrophages, polymorphonuclear leukocytes)
- Cytokine production and release (pro-/anti-)
- Cell death (total protein and lactate dehydrogenases (LDH) release)
- Oxidative stress (antioxidant depletion, oxidation of proteins, DNA, and lipids)

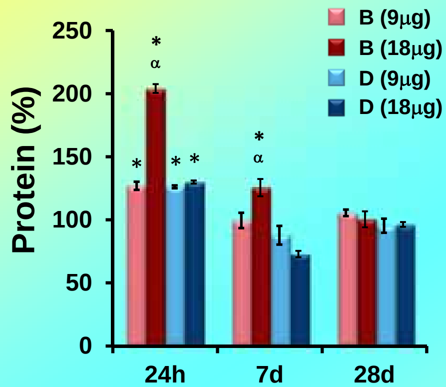
Profile of Immune Cells in the BAL Fluid

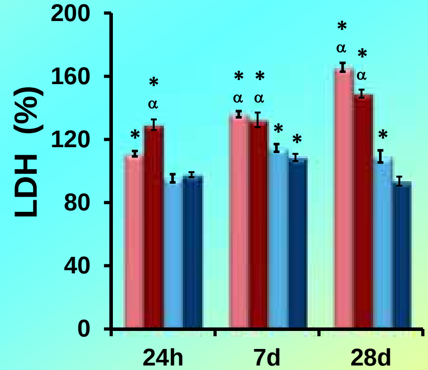


Enhanced recruitment of phagocytic immune cells was found at 24h, 7 and 28 days. The recruitment was more pronounced after exposure to biodiesel than diesel aerosols.

Yanamala et. al. *TAAP.* 2013.

Tissue Damage and Permeability of Lung

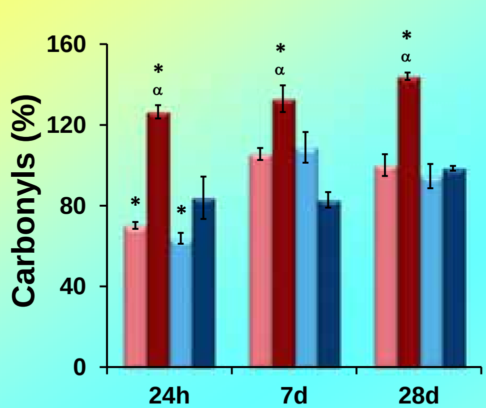


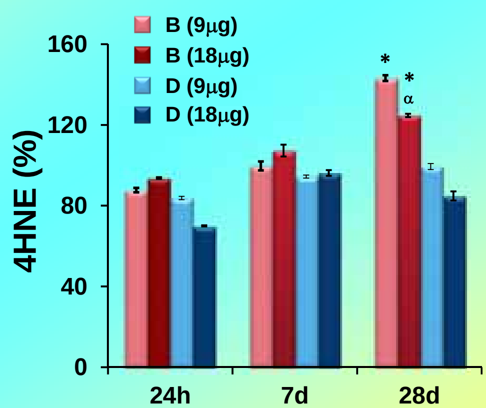


Biodiesel (B) aerosols were more potent in inducing acute pulmonary cell damage and increased lung permeability than diesel (D) aerosols.

Yanamala et. al. *TAAP.* 2013.

Oxidative Stress Responses in the Lungs Measured by Carbonyls and 4-Hydroxy-2-Nonenal (4HNE)



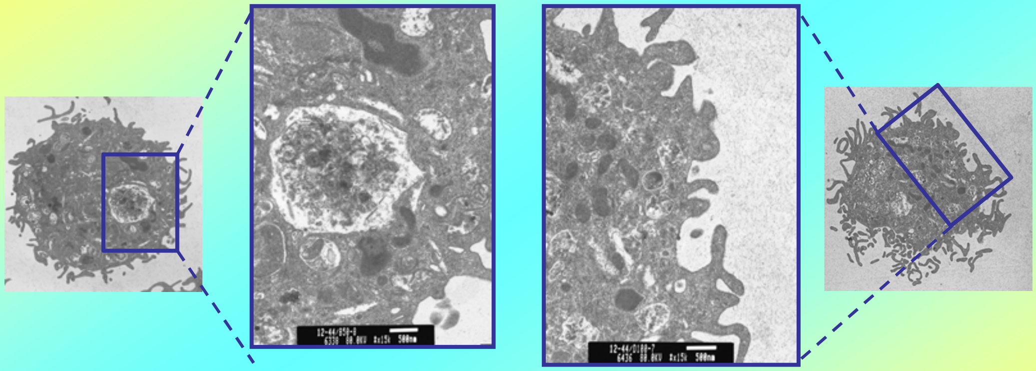


The magnitude of oxidative damage in the lungs is more pronounced in mice exposed to biodiesel (B) aerosols.

Yanamala et. al. *TAAP.* 2013.

TEM of BALF MΦs after 7 Days Post Exposure

Biodiesel **Diesel**

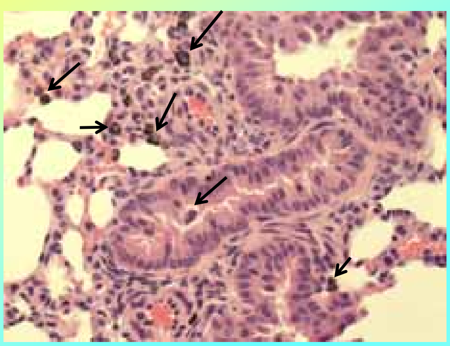


Electron micrographs indicate localization of biodiesel particulates to spherical organelles (ranging 1-2μm in diameter) in MΦs, mimicking lipid droplets.

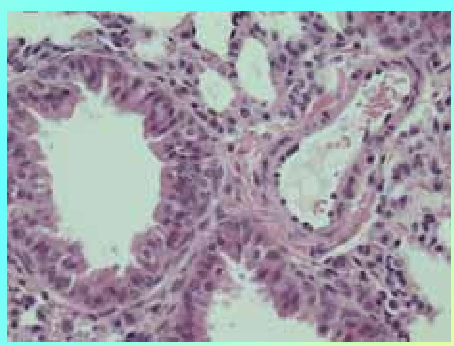
Yanamala et. al. *TAAP.* 2013.

Lung histology 28 days post exposure

Biodiesel



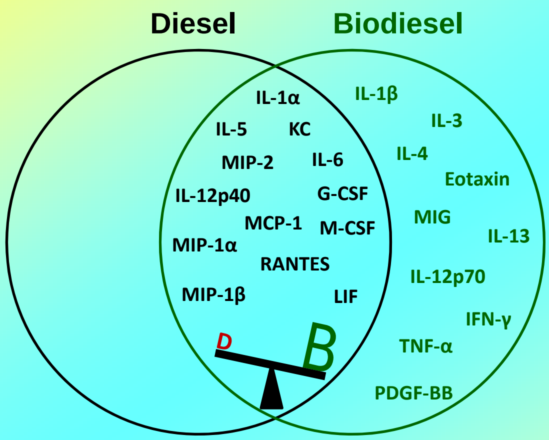
Diesel



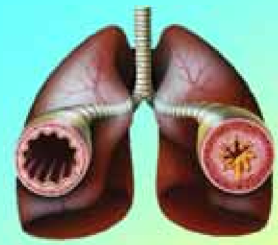
Lung sections indicated presence of lymphocytic infiltrate and impaired clearance with prolonged retention of biodiesel particulates in pigment laden macrophages (as indicated by arrows). No significant inflammation was observed after 28 days post exposure to diesel particulates.

Yanamala et. al. *TAAP.* 2013.

Differential Responses in Inflammatory Mediators



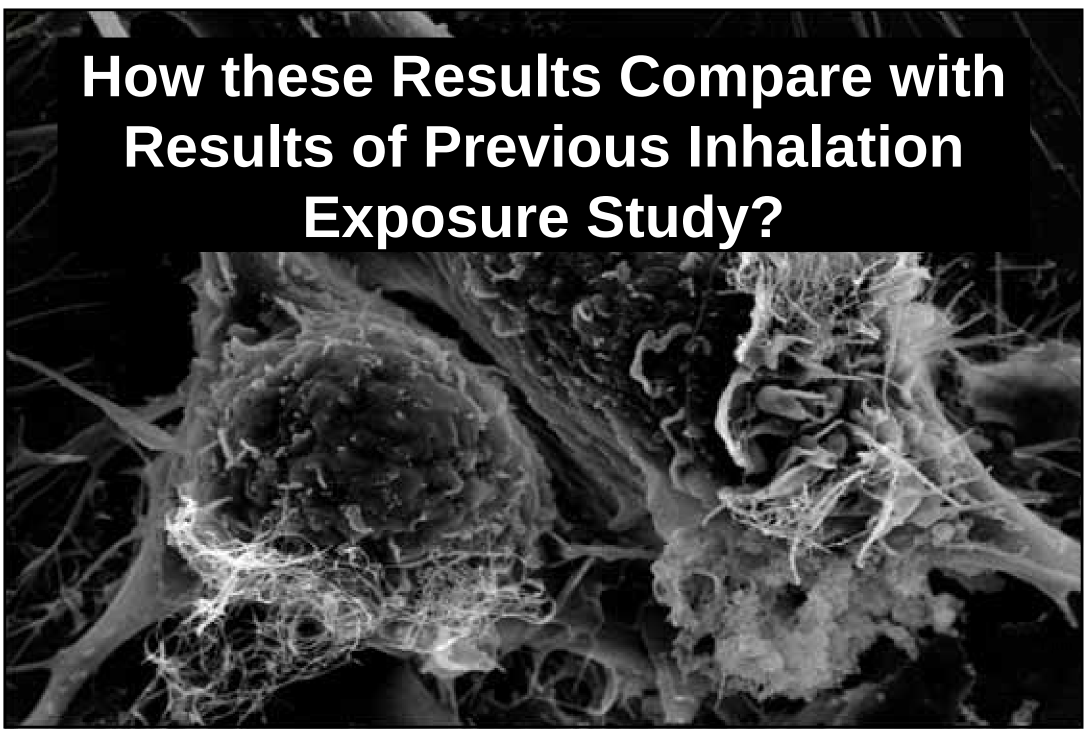
Asthma



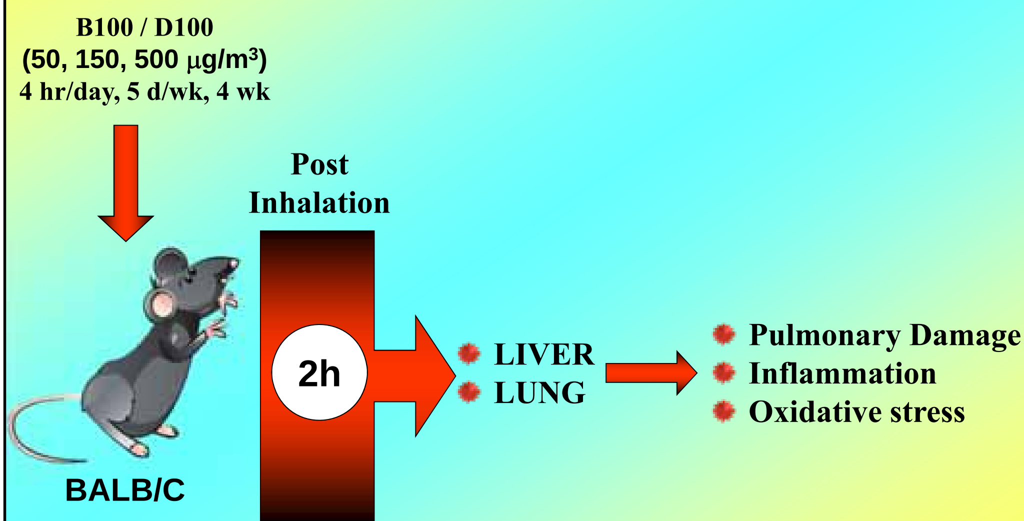
Accumulation of cytokines - IL-1β, IL-4, IL-13, IL-12p70, IFN-γ, TNF-α - seen only upon exposure to biodiesel aerosols could be associated with allergic inflammation and induction of type 1 (Th1) and 2 T helper cell (Th2) responses.

Yanamala et. al. *TAAP.* 2013.

How these Results Compare with Results of Previous Inhalation Exposure Study?



Whole Body Inhalation Exposure with 100% Soy Biodiesel (B100) and Diesel (D100) Exhaust



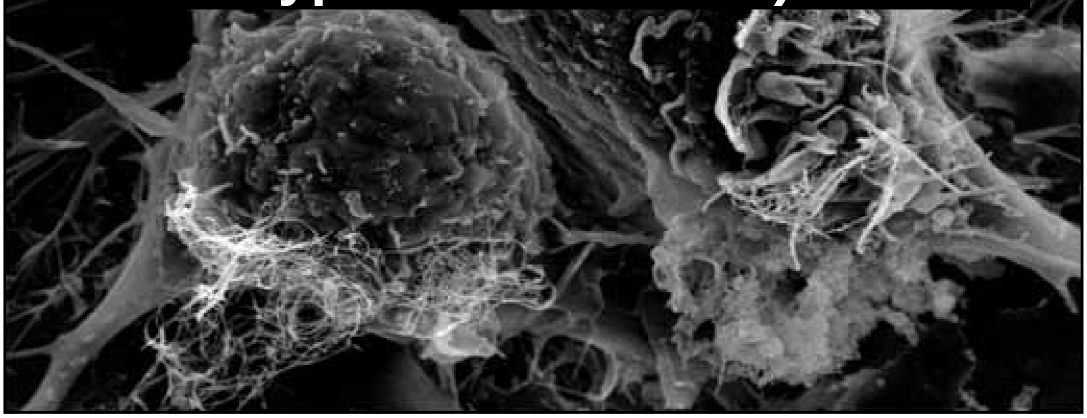
Collaboratively with ***EPA***

Results of Inhalation Exposure Studies

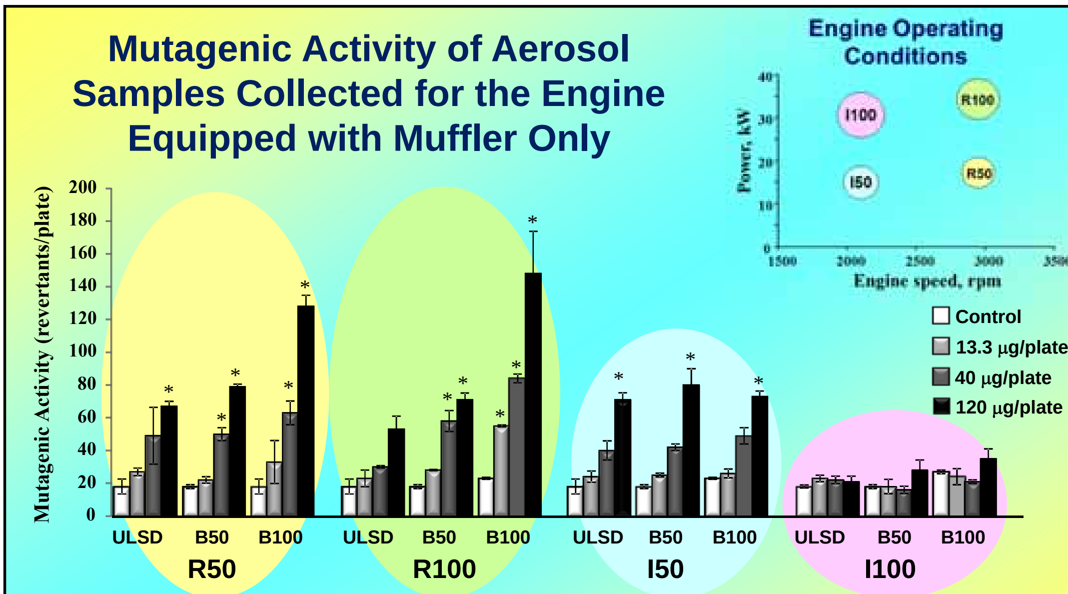
- Biomarkers of tissue damage (LDH) and inflammation (myeloperoxidase, MPO) were significantly elevated in the lungs and liver of mice exposed to B100 exhaust as compared to D100.
- In comparison to D100 inhalation exposure, a significant accumulation of oxidatively modified proteins (carbonyls), an increase in 4-hydroxy-2-nonenal (4-HNE), a reduction of protein thiols, and depletion of antioxidant - gluthatione (GSH) were observed in mice exposed to B100.
- Cytokines, IL-6, IL-10 and IL-12p70 were significantly increased in both lung and the liver upon B100 exposure, and a significant increase in interferon ϒ (IFNϒ) levels was only seen upon D100 exposure.
- Overall, our data suggest that B100, despite its decreased levels of PM, HC and PAH emissions, might cause more adverse effects compared to diesel exhaust particles.

Shvedova et. al. *J Toxicol Environ Health A.* 2013;76(15):1-15.

Do Biodiesel Aerosols Induce Mutagenicity? (Ames Test – Salmonella typhimurium TA98)?

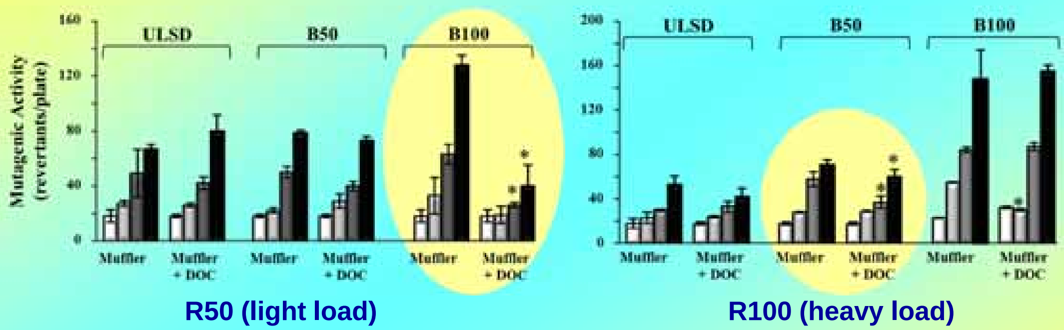


Mutagenic Activity of Aerosol Samples Collected for the Engine Equipped with Muffler Only



Mutagenic activity of diesel aerosols was strongly affected by fuels and engine operating conditions. While the mutagenic activity was found in B50 and B100 samples collected from both light- and heavy-load operating conditions, the ULSD samples were mutagenic only at light-load conditions. **Mutagenicity was increased with the fraction of biodiesel in the fuel.**

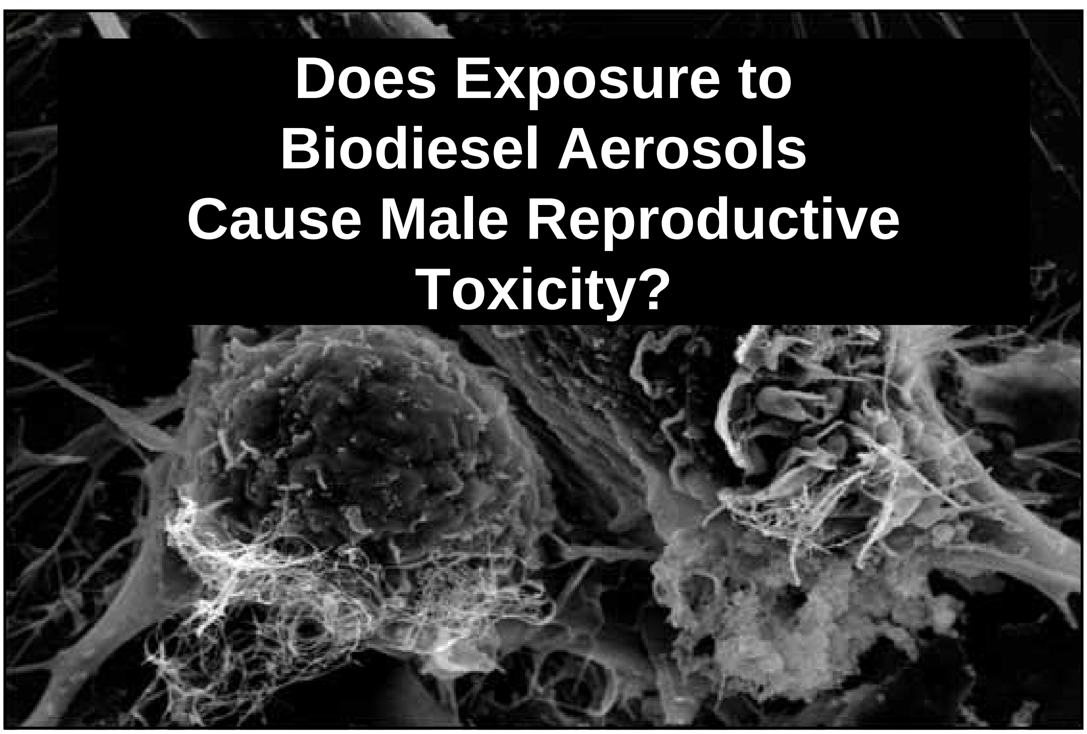
Effect of DOC on Mutagenic Activity of DPM Samples



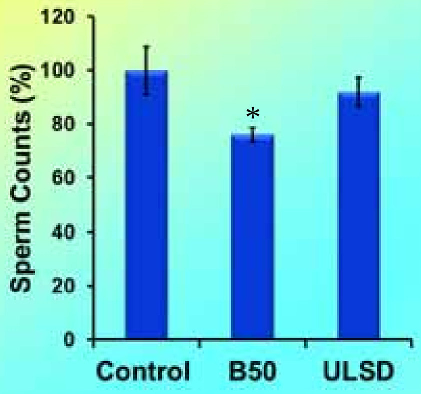
The presence of DOC resulted in decreased mutagenicity (by 69%) when engine was fueled with B100 and operated at light-load conditions (R50). Heavy-load operating condition (R100) lead to a reduction of mutagenicity when engine was fueled with B50 or B100 (low concentration). **The decrease in mutagenicity can be explained by the fact that DOC effectively removed hydrocarbons and the organic carbon fraction of DPM.**

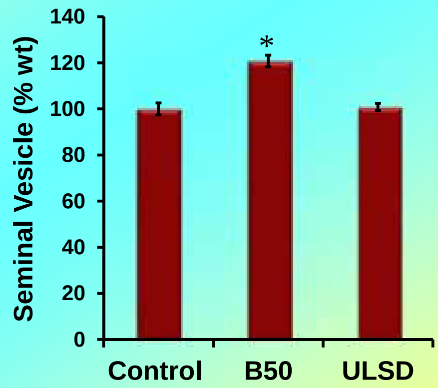
Kisin et. al. *J. of Env. Eng. Eco. Sci.* 2013, 2:3.
http://www.hoajonline.com/jeees/2050-1323/2/3

Does Exposure to Biodiesel Aerosols Cause Male Reproductive Toxicity?



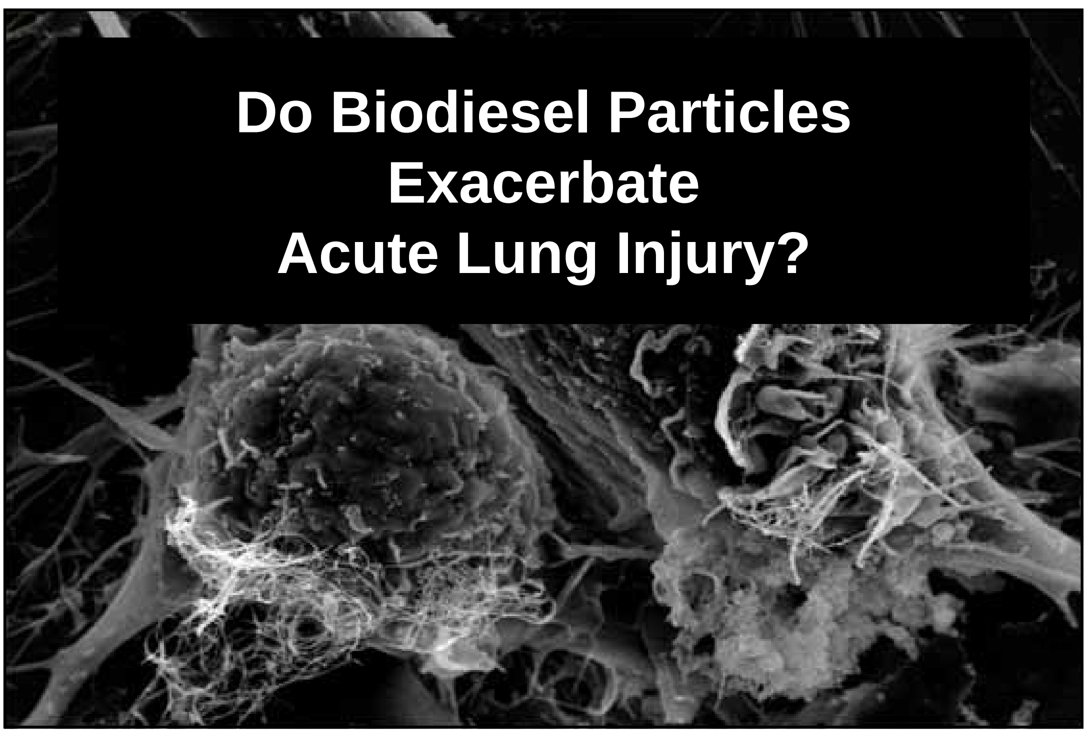
Reproductive Toxicity
(Studies in Male Mice after 24h Post Exposure)



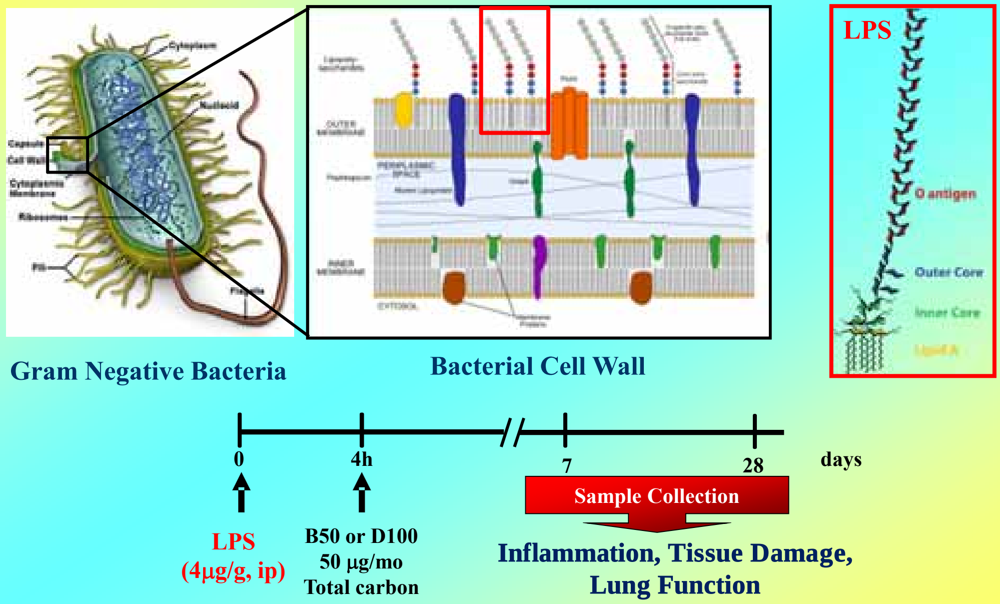


A significant decrease in sperm counts and an increase in seminal vesicle weight was observed upon exposure to biodiesel (B50, 50µg of total carbon) aerosols.

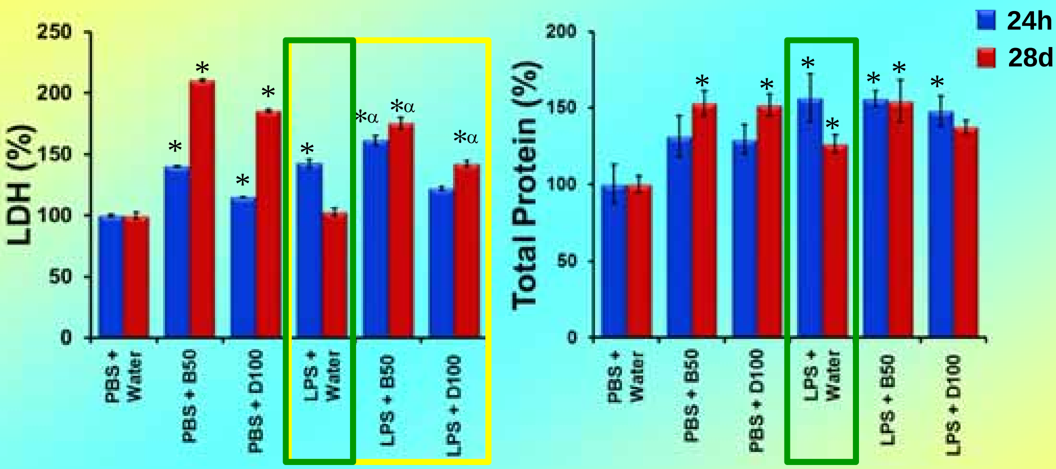
Exposure to biodiesel aerosols could affect male fertility.



Experimental Design for Lung Injury

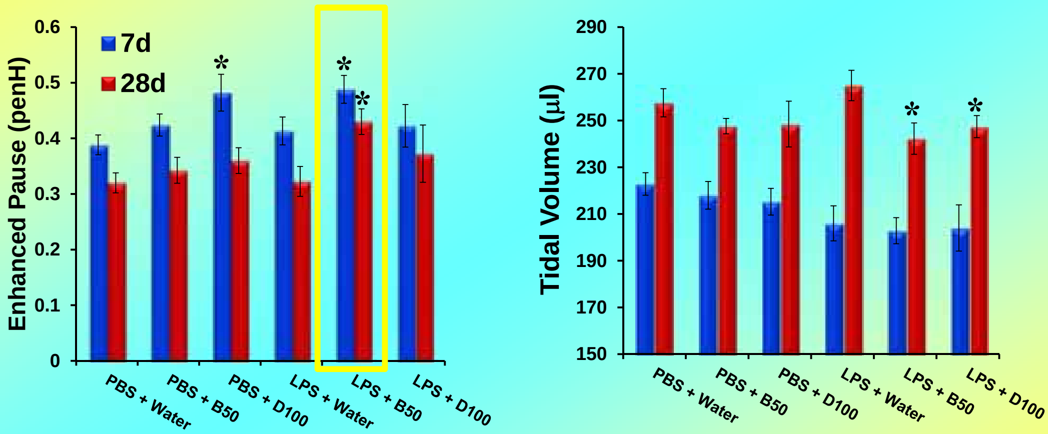


Tissue Damage and Permeability of Lung



Compared to diesel (D100) aerosols, biodiesel (B50) aerosols significantly enhanced LPS-induced lung injury in mice.

Pulmonary Function Test



Compared to diesel (D100) aerosols, biodiesel (B50) aerosols increased airway resistance in mice injected with LPS.

Estimation of Human Equivalent Exposure

Given that :

- MSHA DPM standard **(A)** = 160 µg/m^3 of total carbon
- Inhaled volume **(B)** = 20 L/min = 20000 ml/min
- Deposition efficiency for particles of size ~250nm **(C)** = 15%
- Total alveolar surface area of human lung **(D)** = 102 m^2
- Total alveolar surface area of mouse lung **(E)** = 0.06 m^2

Dosage used in mice = 18 µg of TC/mouse

Total Lung Burden = 18 µg/0.06 (total of mouse lung in m^2) = 300 µg/m^2

Equivalent Exposure in humans : 300 µg/m^2 in mice = **(A x B x C x duration)/D**

Exposure duration in humans (duration) = (300 x D)/(A x B x C)

= (300 x 102)/(160 µg/m^3 x (20000 ml/min * 10^{-6} m^3/ml) x 0.15)

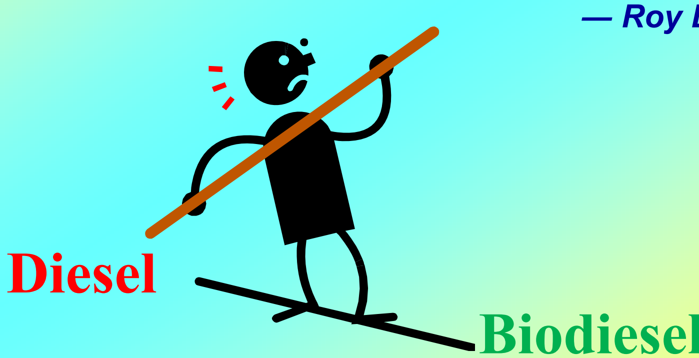
= (300 x 102) / 0.48 min

= 63750 min = (1062.5 hours)/8 hours per day = ~133 working days

At workplace concentrations of 160 µg/m^3 of total carbon, human equivalent exposure to a deposited dose of 18µg of total carbon can be achieved in ~133 working days.

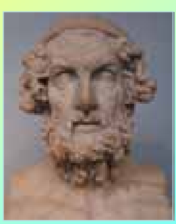
“It's not hard to make decisions when you know what your values are.”

— Roy Disney



“Once Harm Has Been Done, Even a Fool Understands It.”

Homer



The Iliad Ἰλιάς, 9-8th century BC

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