

Inflammatory response to assess toxicity of biodiesel emission samples

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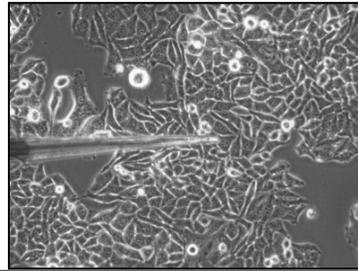
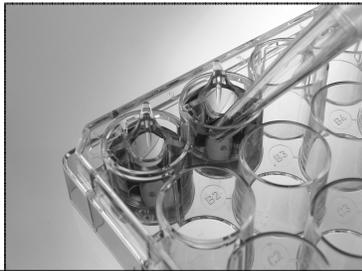
ARB December 8, 2010
Biodiesel and Renewable Diesel Multimedia
Evaluation Public Meeting

Inhalation of environmental and occupational pollutants in vivo

- Pulmonary inflammation, fibrosis, lung carcinomas
- Cardiovascular diseases like atherosclerosis
- Chronic inflammatory response as a main cause for adverse health effects

In vitro cell models

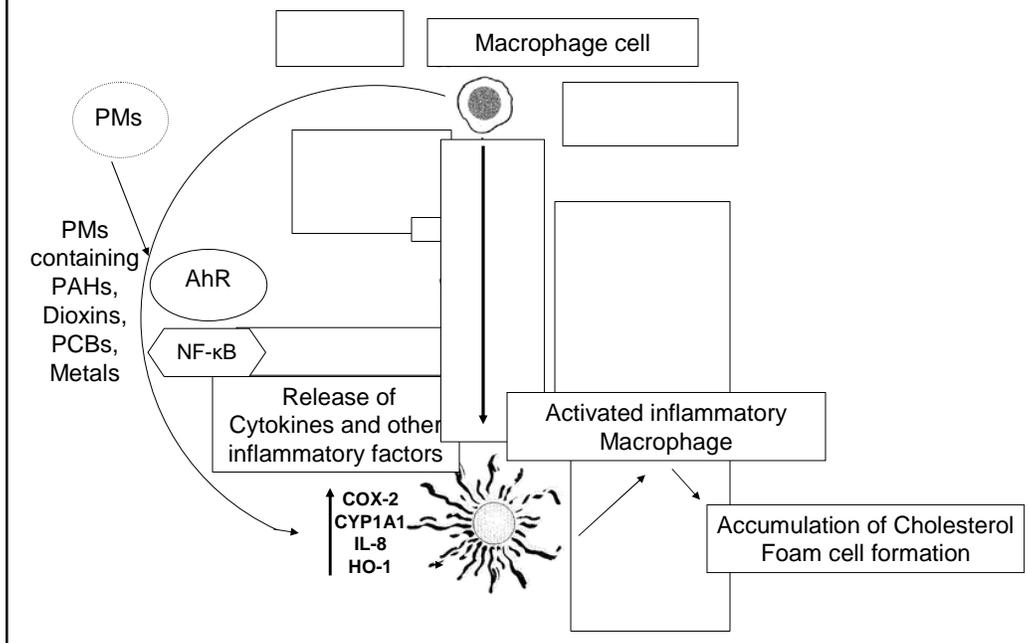
- Two main target cell types
 - a) Macrophages (U937), phagocyte, acts as first line of defense
 - b) Lung Clara cells from pulmonary epithelium (NCI H441)



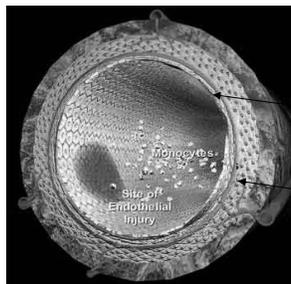
Biomarkers of PM exposure, inflammation and oxidative stress

- CYP1A1: Cytochrome P450 monooxygenase, xenobiotic metabolizing enzyme, Arylhydrocarbon-Receptor regulated
- COX-2: Cyclooxygenase, key enzyme for production of prostaglandins involved in inflammation
- IL-8: Interleukin 8, chemoattractant peptide for neutrophils, major mediator of inflammatory response
- HO-1: Hemeoxygenase 1, essential enzyme in heme catabolism, protect cells against oxidative injury. Induced by exposure to various forms of oxidative stress

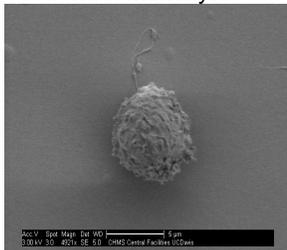
Macrophage Model to measure Inflammation caused by Diesel PM



Formation of foam cells

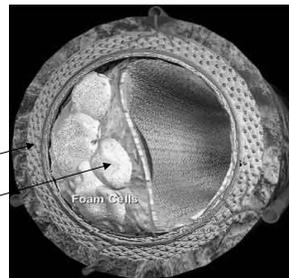


Control monocyte

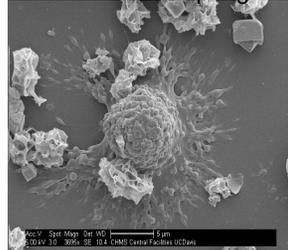


Swelling of the intima in the wall of the artery which pushes the endothelium into the lumen of the artery

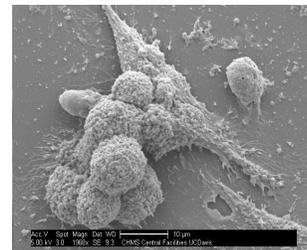
Arterial wall
Foam cells



Dioxin Foam Cells



Control Macrophage

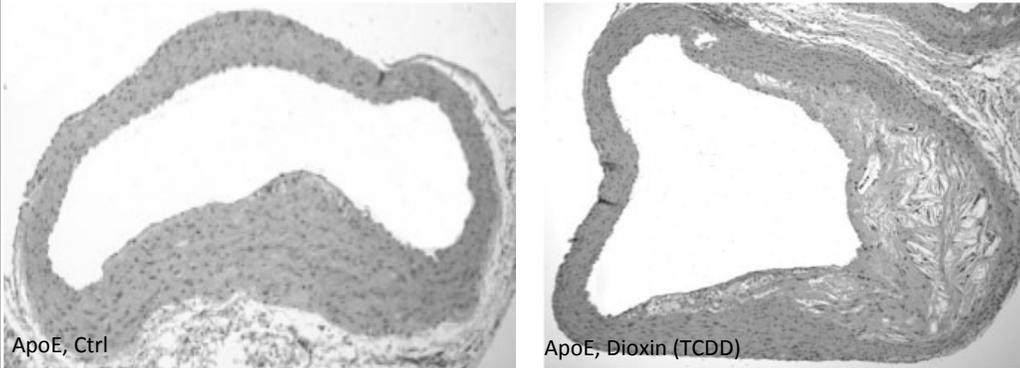


Acc.V Spot Magn Det WD 5.0µm 3.0kV X30 40216 EB 6.0 CHMS Central Facilities UCDavis

Acc.V Spot Magn Det WD 5.0µm 3.0kV X35 30995 SE 10.4 CHMS Central Facilities UCDavis

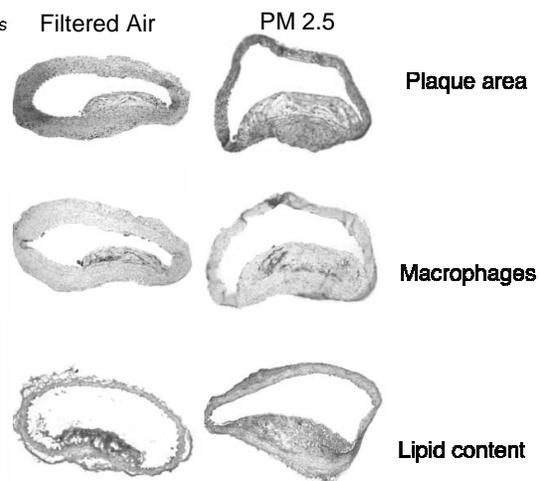
Acc.V Spot Magn Det WD 10µm 5.00kV X30 19069 SE 8.3 CHMS Central Facilities UCDavis

Development of atherosclerotic lesions in ApoE mice



Long-term Air Pollution Exposure and Acceleration of Atherosclerosis and Vascular Inflammation in an Animal Model

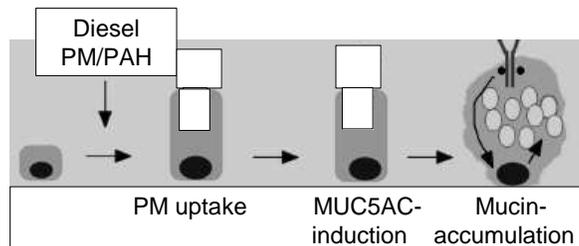
6 hrs/day, 5 days/wk x 6 months
Mean levels only 15.2 $\mu\text{g}/\text{m}^3$



Air pollution exposed mice developed more ATHEROSCLEROSIS

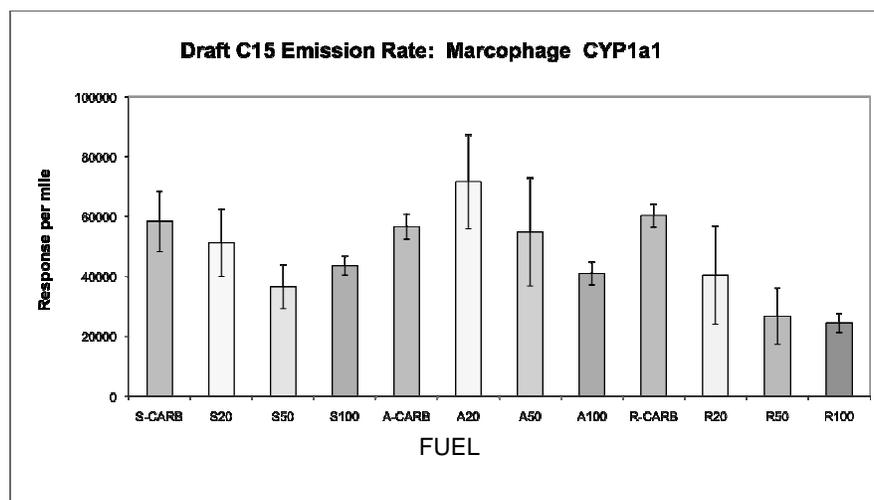
JAMA. 2005;294:3003-3010

Lung Clara cell model (NCIH441)

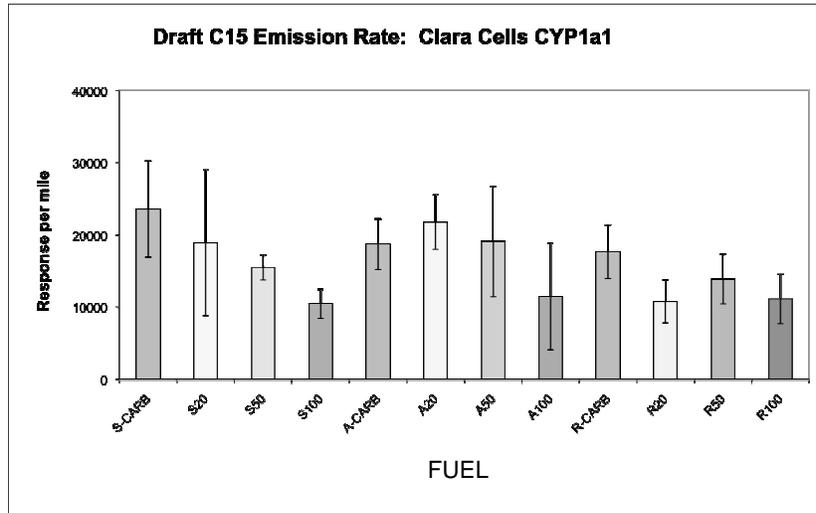


- Chronic obstructive pulmonary disease (COPD)
- Emphysema
- Asthma

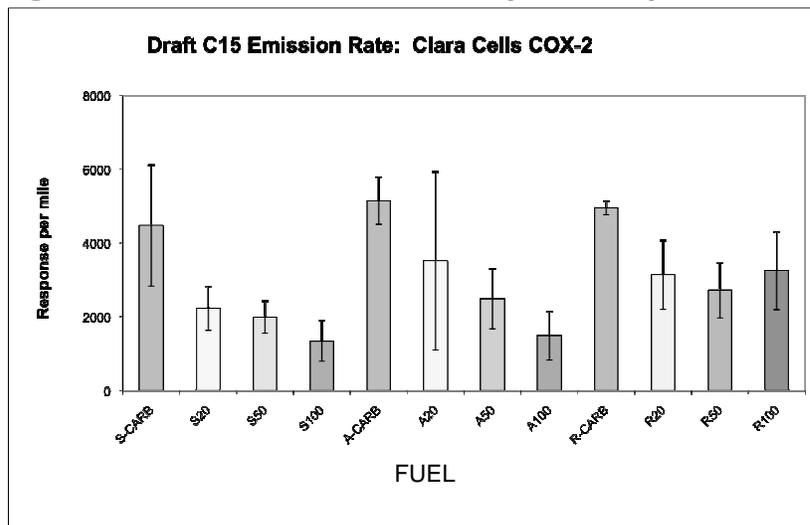
Draft C15 Emission Rate: Macrophage CYP1a1 response per mile



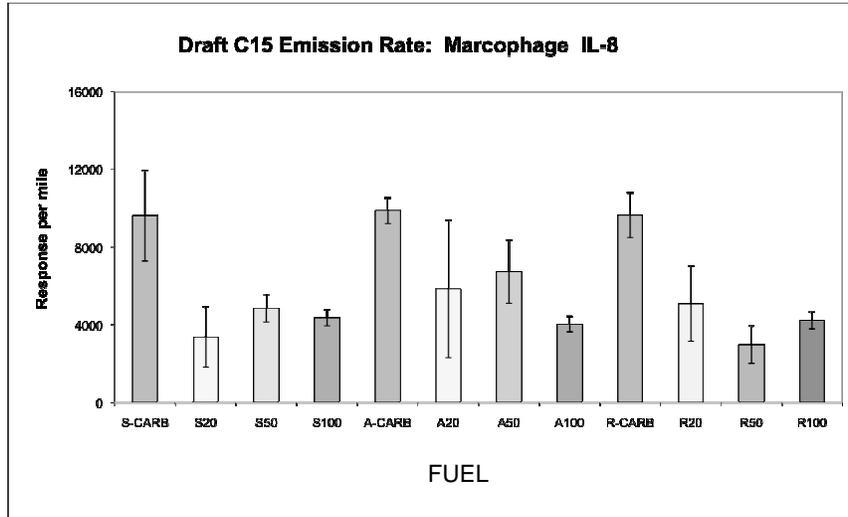
Draft C15 Emission Rate: Lung Clara cells CYP1a1 response per mile



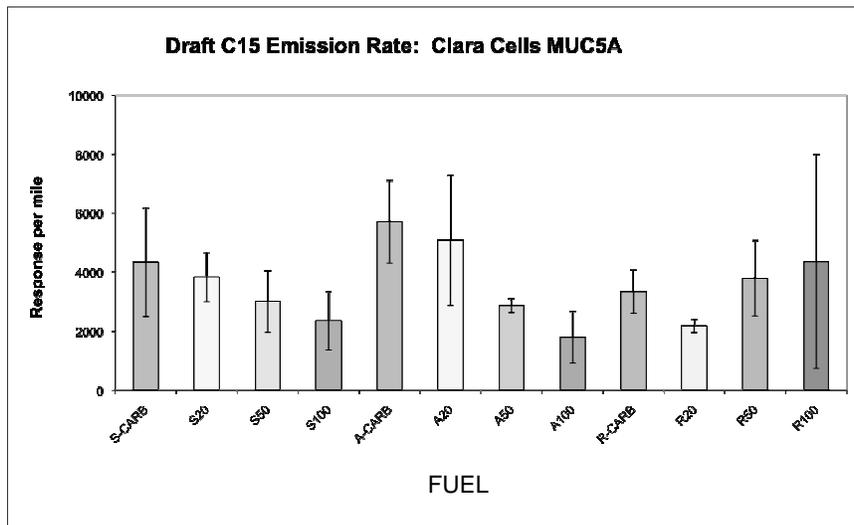
Draft C15 Emission Rate: Lung Clara cells COX-2 response per mile



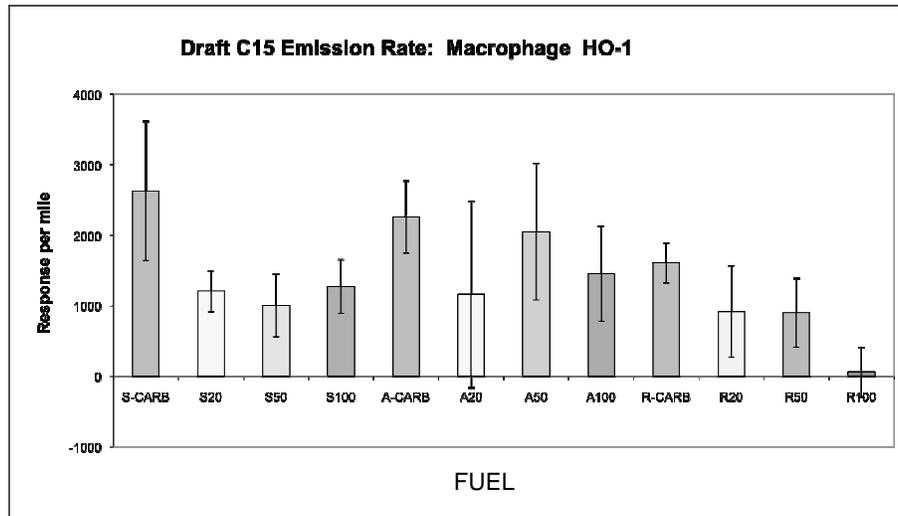
Draft C15 Emission Rate: Macrophage IL-8 response per mile



Draft C15 Emission Rate: Lung Clara cells MUC5AC response per mile

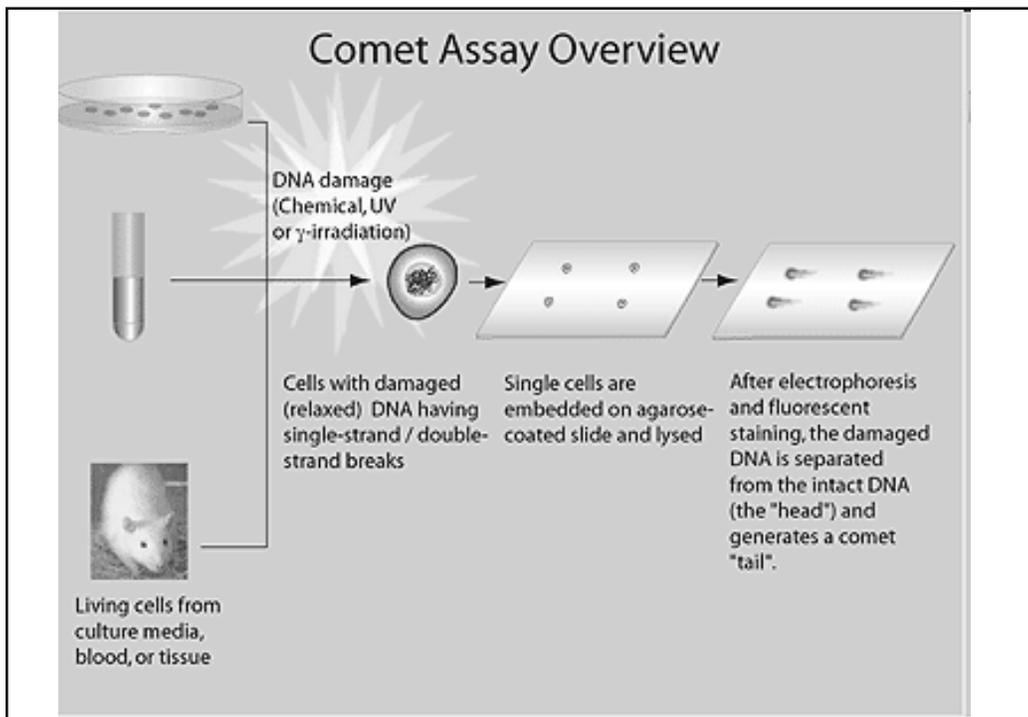


Draft C15 Emission Rate: Macrophage HO-1

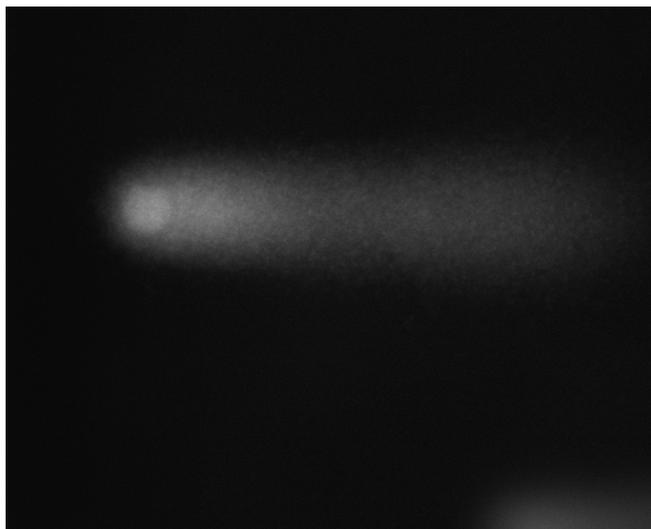


Comet Assay or Single-Cell-Gel- Electrophoresis assay

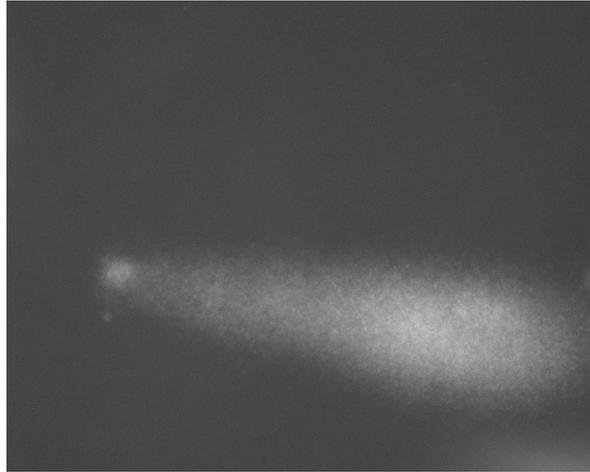
- sensitive technique for the detection of DNA damage at the level of the individual eukaryotic cell
- standard technique for evaluation of DNA damage, biomonitoring genotoxicity



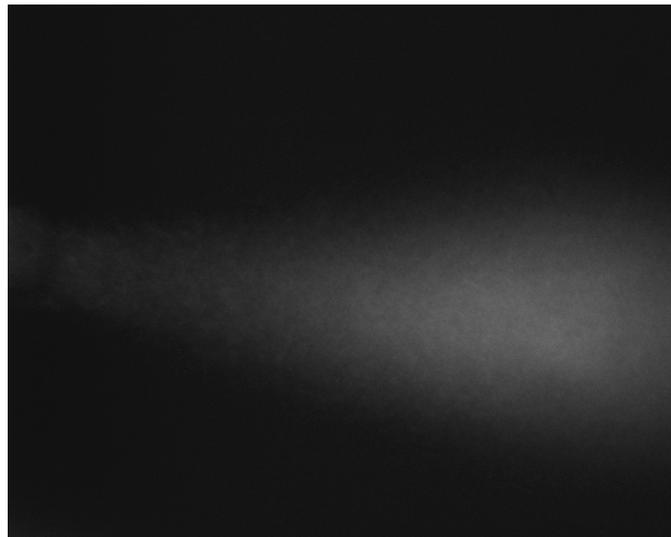
Comet Standard Cells 1



Comet Standard Cells 2



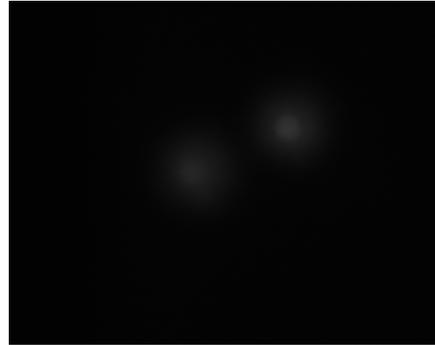
Comet Standard Cells 3



Control

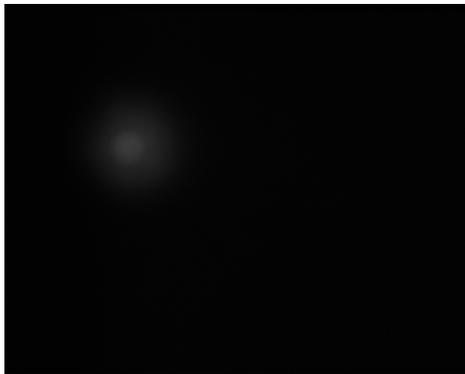


Carb



Undamaged DNA retains a highly organized association with matrix proteins in the nucleus

Soy 100



Animal 100



Renewable 100

NIST SRM1650

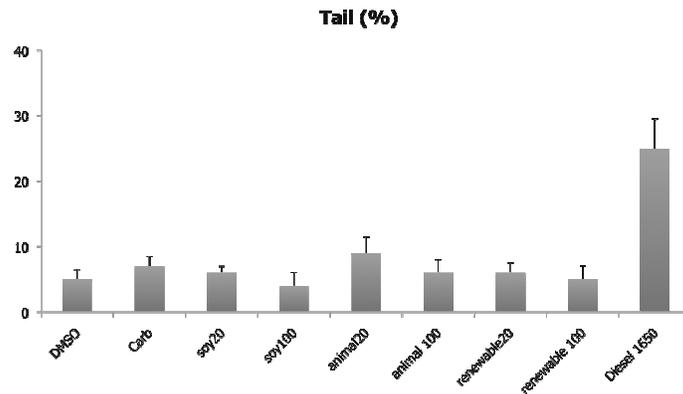


Circular "head" corresponding to the undamaged DNA and a "tail" of damaged DNA, the brighter and longer the tail, the higher the level of damage

NIST SRM1650



DNA damage measured by the comet assay



Percent Tail DNA was measured after 3-h treatment of U937 cells under serum-free conditions with 200 µg/ml extracts of PMs.

Summary

- Carb and Biodiesel blends induce CYP1A1 through PAHs which bind to and activate the Ah-Receptor
- Carb and Biodiesel blends induce inflammatory markers like COX-2 and IL-8 in macrophages and MUC5AC in lung Clara cell type (NCI H441)
- Effect of Biodiesel blends on inflammatory markers like COX-2 and IL-8 tend to be lower than Carb diesel
- No genotoxic effects of biodiesel blends in Comet assay

Thank you

Bob Okamoto
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Helen Woldai
Pat Wong
Fumio Matsumura