Inflammatory response to assess toxicity of biodiesel emission samples

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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

PMs containing PAHs, Dioxins, PCBs, Metals

PMs

AhR

NF-κB

Release of Cytokines and other inflammatory factors

Activated inflammatory Macrophage

Accumulation of Cholesterol
Foam cell formation

Macrophage cell

COX-2
CYP1A1
IL-8
HO-1
Formation of foam cells

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

Arterial wall

Foam cells

Control monocyte

Control Macrophage

Dioxin Foam Cells
Development of atherosclerotic lesions in ApoE mice

ApoE, Ctrl

ApoE, Dioxin (TCDD)
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 μg/m³

Filtered Air  PM 2.5

Plaque area

Macrophages

Lipid content

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

![Chart showing response per mile for different fuels: S-CARB, S20, S50, S100, A-CARB, A20, A50, A100, R-CARB, R20, R50, R100. The y-axis represents response per mile, and the x-axis represents different fuel types.](chart.png)
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: Clara Cells MUC5A

![Graph showing response per mile for different fuels](image-url)
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 Assay Overview

DNA damage (Chemical, UV or γ-irradiation)

Cells with damaged (relaxed) DNA having single-strand / double-strand breaks

Single cells are embedded on agarose-coated slide and lysed

After electrophoresis and fluorescent staining, the damaged DNA is separated from the intact DNA (the "head") and generates a comet "tail".

Living cells from culture media, blood, or tissue
Comet Standard Cells 3
Undamaged DNA retains a highly organized association with matrix proteins in the nucleus
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
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

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