Chemical & Bioassay Analyses of Emissions from Biodiesel Fuel Combustion

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Research Objectives

- Study biodiesel and renewable diesel emissions:
  - Chemical characterization of toxics
  - Toxicity studies of emissions
Unregulated Toxic Emissions

- PAHs
- Alkyl PAHs
- Nitro-PAHs
- Selective reactive aldehydes
Toxicity Studies of Emissions

- Tests for markers of inflammation in human cells
- Tests for genotoxicity
  - Mutagenicity
  - Chromosomal Damage
## Test Vehicles

<table>
<thead>
<tr>
<th>Vehicle/Engine</th>
<th>Engine Displacement (L)</th>
<th>Control Devices</th>
<th>Test Cycle</th>
<th>Fuels Tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000 Freightliner C15 Caterpillar</td>
<td>15</td>
<td>-</td>
<td>UDDS</td>
<td>CARB Diesel, Soy, Animal, and Renewable @ 20%, 50% and 100%</td>
</tr>
<tr>
<td>2008 Freightliner Mercedes Benz MBE 4000</td>
<td>12.8</td>
<td>DOC, DPF, EGR</td>
<td>UDDS</td>
<td>CARB Diesel, Soy, Animal, and Renewable @ 20%, 50% and 100%</td>
</tr>
</tbody>
</table>
Methods

[Diagram showing sampling system with labels for High Volume Sampler and Medium Volume Sampler]
PM Filter Preparation for Analyses

Bioassay

Hi Vol Filter 8” x 10”

Pressurized Fluid Extraction

Bioassay Analyses

Chemistry

Med Vol Filter 90 mm

Internal std

Pressurized Fluid Extraction

Cleanup

Chemical Analyses
PUF XAD Preparation for Analyses

Bioassay

PUF for Bioassay

Pressurized Fluid Extraction

Bioassay Analyses

Chemistry

PUF for Chem

Pressurized Fluid Extraction

Internal std

Chemical Analyses

Liquid Extraction with Shaking

Internal std

Cleanup

Cleanup

Chemical Analyses

Chemical Analyses
Methods

Chemical Analyses

PAHs, Alkyl PAHs  Nitro-PAHs

↓  ↓

GC/MS - EI  GC/MS - NCl
Methods

Bioassay Analyses

Genotoxicity

Human Cell Markers of Inflammation
Chemical Analyses

- PAHs
- Alkyl PAHs
- Nitro-PAHs
### Compounds Analyzed

<table>
<thead>
<tr>
<th>PAHs</th>
<th>Alkyl PAHs</th>
<th>Nitro-PAHs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>2-Methylnaphthalene</td>
<td>1N-naphthalene</td>
</tr>
<tr>
<td>Acenaphthylene</td>
<td>1-Methylnaphthalene</td>
<td>2N-naphthalene</td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>2.6-Dimethylnaphthalene coelute</td>
<td>5N-acenaphthene</td>
</tr>
<tr>
<td>Fluorene</td>
<td>1,6-Dimethylnaphthalene</td>
<td>2N-fluorene</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>2,3,5-Trimethylnaphthalene coelute</td>
<td>9N-anthracene</td>
</tr>
<tr>
<td>Anthracene</td>
<td>3-Methylphenanthrene</td>
<td>3N-phenanthrene</td>
</tr>
<tr>
<td>Fluorantheine</td>
<td>2-Methylphenanthrene</td>
<td>2N-phenanthrene</td>
</tr>
<tr>
<td>Pyrene</td>
<td>9-Methylphenanthrene</td>
<td>3N-fluorantheine</td>
</tr>
<tr>
<td>Benz(a)anthracene</td>
<td>1-Methylphenanthrene</td>
<td>1N-pyrene</td>
</tr>
<tr>
<td>Chrysene+triphenylene</td>
<td>2-Methylanthracene</td>
<td>7N-BaA</td>
</tr>
<tr>
<td>Benzo(b+j+k)fluoranthenes coelute</td>
<td>2-Methylfluoranthenes</td>
<td>6N-chrysene</td>
</tr>
<tr>
<td>Benzo(e)pyrene</td>
<td>1-Methyl &amp; 3-Methylfluoranthenes</td>
<td>6N-BaP+1N-BeP</td>
</tr>
<tr>
<td>Benzo(a)pyrene</td>
<td>4-Methylpyrene</td>
<td></td>
</tr>
<tr>
<td>Perylene</td>
<td>1-Methylpyrene</td>
<td></td>
</tr>
<tr>
<td>Indeno(1,2,3-cd)pyrene</td>
<td>7,12-Dimethylbenz(a)anthracene</td>
<td></td>
</tr>
<tr>
<td>Dibenz(a,h)anthracene</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzo(g,h,i)perylen e</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dibenzo(a,l)pyrene</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
PAH Emissions
PM Associated PAHs
C15 Vehicle

Draft Benzo(a)pyrene Emission
- C15 Vehicle -

(nɡ/miℓe)
PAH Emissions
PM Associated PAHs

Draft Indeno(1,2,3-cd)pyrene Emission
- C15 Vehicle -

(ng/mile)
PAH Emissions
Vapor-Phase PAHs

Draft Naphthalene Emission
- C15 Vehicle -

(nm/mile)
PAH Emissions
Vapor-Phase PAHs

Draft Fluoranthene Emission
- C15 Vehicle -

(ng/mile)
PAH Emissions
Nitro-PAHs
Vapor-Phase

Draft 1-Nitronaphthalene Emission
- C15 Vehicle -

<table>
<thead>
<tr>
<th>CARB-S</th>
<th>S20</th>
<th>S50</th>
<th>S100</th>
<th>CARB-A</th>
<th>A20</th>
<th>A50</th>
<th>A100</th>
<th>CARB-R</th>
<th>R20</th>
<th>R50</th>
<th>R100</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tr>
</tbody>
</table>

(nm/mile)
Summary PAHs
C15 Vehicle

- PM and Semi-Volatile PAHs and Nitro-PAHs decreased with increasing blend level of biodiesels.
PAH Emissions
PM Associated PAHs
MBE 4000

Draft Pyrene Emission
- MBE4000 Vehicle -

- CARB
- S20
- S50
- S100

(ng/mile)
PAH Emissions
Vapor-Phase PAHs
MBE 4000

Draft Naphthalene Emission
- MBE4000 Vehicle -

(ng/mile)
PAH Emissions
Vapor-Phase PAHs
MBE 4000

Draft 2-Methylnaphthalene Emission
- MBE4000 Vehicle -

<table>
<thead>
<tr>
<th>CARB</th>
<th>S20</th>
<th>S50</th>
<th>S100</th>
</tr>
</thead>
<tbody>
<tr>
<td>4000</td>
<td>3000</td>
<td>2000</td>
<td>1000</td>
</tr>
</tbody>
</table>
Draft PAH Emissions
Nitro-PAHs
MBE 4000

Draft 1-Nitronaphthalene Emission
- MBE4000 Vehicle -

(nc/mile)
Summary PAHs
MBE 4000 Vehicle

- Low Levels of PM associated PAHs and Nitro-PAHs in CARB and Biodiesel Fuel Emissions
- Lower levels of Vapor-phase PAHs and Nitro-PAHs emissions
Reactive Carbonyls
Reactive Carbonyl Sampling

- Sampling from Dilution Tunnel to Mist Chamber
- Samples in parallel to Filtered/Charcoal dilution air
- Samples for single UDDS test cycle
Reactive Carbonyl Analyses

- Stable carbonyls formed through reaction with bisulfite
- Carbonyls liberated from bisulfite
- Free carbonyls derivatized by o-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine (PFBHA*)
- Derivatives detected & quantitated by GC/MS - NCI
Carbonyl Emissions

C15 Vehicle

Draft Acrolein Emissions
C15 Vehicle

Emissions (µg/mile)

Fuel

CARB-S  S50  S100  CARB-A  A50  A100  CARB-R  R50  R100
Carbonyl Emissions

C15 Vehicle

Draft o,m-Tolualdehyde Emissions
C15 Vehicle

Emissions (µg/mile)

Fuel

CARB-S, S50, S100, CARB-A, A50, A100, CARB-R, R50, R100
### Carbonyl Emissions

**MBE 4000 Vehicle**

<table>
<thead>
<tr>
<th>Fuel</th>
<th>Emission (µg/mile)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CARB</td>
<td>60</td>
</tr>
<tr>
<td>S50</td>
<td>40</td>
</tr>
<tr>
<td>S100</td>
<td>140</td>
</tr>
</tbody>
</table>

**Draft Acrolein Emissions**

**MBE 4000**

![Graph showing emissions for different fuels]
Summary
Reactive Carbonyls

- C15 Vehicle S50, S100 and A50, A100 were higher in certain carbonyls such as acrolein
- C15 Vehicle Renewable diesel no change over Carb
- MBE4000 Vehicle – carbonyls lower
Genotoxicity Tests

- Microbial
  eg. Ames Salmonella test
- Mammalian cell
  eg. Chinese hamster ovary (CHO)
- *In vivo*
  eg. Big Blue transgenic rodent
Genotoxicity Tests
Two Questions

• How consistent is it to hypothesized mechanisms of action for carcinogens?

• How does it compare to animal or human carcinogenicity tests?
Salmonella Tester Strains

- TA 98
  - Frameshift mutation in the HisD gene coding for histidinol dehydrogenase
  - Target site: series of 8 GCGCGCGC’s
Salmonella Tester Strains

- TA 100

- Base-pair mutation in the His G gene coding for His biosynthesis

- Target site: $\text{GGG}$ (proline) His dependent
Salmonella/microsome Test

- A feature of the Test:
  - Metabolic enzymes can be added to detect activation
Salmonella/microsome Test

- Metabolic enzymes needed for activation of certain compounds – eg. PAHs
- Enzymes from various tissues can be used – e.g. Lung, liver
Urine Mutagenicity

Fig. 1. The kinetics of mutagen excretion in the urine of an occasional smoker (smokes about 1 cigarette per week). A single filter-tipped cigarette (17 mg tar per cigarette) was smoked at the times indicated. TA98 revertant equivalents/h = TA98 revertants per ml urine multiplied by the total volume of urine donated and divided by the number of hours elapsed since providing the last specimen. TA98 revertants per ml of urine was determined from the slope of the linear portion of the dose–response curve for each urine specimen. Water extracted in an identical manner as urine was used as the control.

Mut Res 157:227, 1985
Mutagen Emissions TA98 (+S9)
C15 Vehicle PM
Mutagen Emissions TA98 (–S9)

C15 Vehicle PM

Draft C15 PM Mutagen Emissions TA98 -S9

- Mutagen Emissions (Rev/mi x 10^3)
- Fuel:
  - S-CARB
  - S20
  - S50
  - S100
  - A-CARB
  - A20
  - A50
  - A100
  - R-CARB
  - R20
  - R80
  - R100
Mutagen Emissions TA100 (+S9) C15 Vehicle VP

Draft C15 Vapor Phase Mutagen Emissions TA100+S9

Mutagen Emissions (Rev/mile x 10^3)

Fuel

- CARB
- S20
- S50
- S100
- CARB
- A20
- A50
- A100
- CARB
- R20
- R50
- R100
Mutagen Emissions

MBE 4000 Vehicle PM

![Chart showing mutagen emissions for different fuel types (CARB, S20, S50, S100)]
Mutagen Emissions

MBE 4000 Vehicle VP

Draft Mutagen Emissions TA 98+S9
MBE 4000 Vapor Phase

<table>
<thead>
<tr>
<th>Fuel</th>
<th>Mutagen Emissions (Revs/mile x 10^-3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CARB</td>
<td>6.0</td>
</tr>
<tr>
<td>S20</td>
<td>6.0</td>
</tr>
<tr>
<td>S50</td>
<td>6.0</td>
</tr>
<tr>
<td>S100</td>
<td>4.0</td>
</tr>
</tbody>
</table>
Bus Emissions
Compressed Natural Gas

### Mutagen Emissions TA98

**Vehicle Configuration**

- PM
- VP

<table>
<thead>
<tr>
<th>Vehicle Configuration</th>
<th>+S9</th>
<th>-S9</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNG.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ECD.OC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ECD.CR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CNG.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Mutagen Emission (Rev/mile) x 10^5**

- 0.0
- 4.0
- 8.0
- 12.0
- 16.0

ES&T 39:7638, 2005
Summary

- For C15 Vehicle: Generally decrease in Mutagen emissions with blend level
- For C15 PM Samples TA98 (+ or – S9) more sensitive than TA100 for all fuels
- Vapor Phase samples lower mutagen emissions than PM TA100 slightly more sensitive
- MBE Mutagen Emissions considerably lower than C15
Summary

- Chemical and Biological tests were overall very consistent with each other regarding emission results for the fuels tested
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UCD – Etox
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