Tier II Results for Multimedia Risk Assessment of Biodiesel: Relative Mobility, Biodegradation, and Aquatic Toxicity
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Multimedia Risk Assessment

Tier 1
Tier II
Tier III

Tier 1 Preliminary Review
- Define framework and approach
- Identify information needs and gaps
- Peer review

Tier 2 Multimedia Risk Assessment Design Review
- Experimental design developed and submitted
- Design peer reviewed, feedback provided for Tier 3

Tier 3 Final Multimedia Risk Review
- Final report is used as the basis for recommendations submitted to the Environmental Policy Council
- Final report is peer reviewed
Multimedia Risk Assessment¹
Tier I
Tier II²
Tier III

Tier 1
Preliminary
Review
- Define framework and approach
- Identify information needs and gaps
- Peer review

Tier 2 Multimedia
Risk Assessment
Design Review
- Experimental design
developed and submitted
- Design peer reviewed,
feedback provided for Tier 3
- Final report is used as the basis for
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Tier 3

¹ http://www.arb.ca.gov/fuels/multimedia/multimedia.htm
² http://www.arb.ca.gov/fuels/diesel/altdiesel/biodiesel.htm

Multimedia Risk Assessment¹
Tier II²
Experiments Performed

• Mobility
  - Side-by-side infiltration in 2D “ant farm” flow cells

• Biodegradation Tests
  - Microcosm respirometry in soil slurry, 29 day

• Aquatic Toxicity
  - Suite of freshwater/estuarine toxicity tests

¹ http://www.arb.ca.gov/fuels/multimedia/multimedia.htm
² http://www.arb.ca.gov/fuels/diesel/altdiesel/biodiesel.htm
Mobility

Fuel Blends

- Image analysis of biodiesel vertical infiltration in Sandbox
- 30x20x2cm, #20 (coarse) sand, water table
- Soy- and Animalfat-based 100% and 20% blends, 1 additive

- Sandbox preparation
  - Wet-pluviated sand
  - Drain to water table
  - simultaneous 50mL ULSD#2 and biodiesel side-by-side, both dyed

- Data collected
  - infiltration rate in vadose zone
  - redistribution
  - lens form & surface area, on water table
Mobility

Sample Results
Final Lenses

• Soy B20 least different

Soy B20a  CARB ULSD#2

Mobility

Sample Results
Final Lenses

• Animal fat B100
  strongest effect
  - similar traveltimes
  - Less lateral dispersion
  - thicker, deeper lens
  - more residual, less sfc area

AF B100a  CARB ULSD#2
Mobility

Summary

• Minor differences in travel times
• AF B100a only shows Moderate differences
  - thicker lens formation
  - more residual

Interfacial Tensions\(^1\) (mN/m):
  - ULSD: 7.4
  - Soy (B20/B100): 8.5/12.0
  - AF (B20/B100): 15.0/19.5

Viscosity\(^1\)

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Biodegradation Tests

- 29-day Respirometry using soil slurry inoculum
  - Soy- and Animalfat-based 100% and 20% blends, 2 additives
- Microcosm preparation
  - 250 mL flask that consists of 200 ml mineral medium
  - 2 g soil (Yolo silt loam) as bacterial inoculums
  - 5uL of test substrate
- For each fuel type:
  - triplicate batch
  - one sterilize control (1% sodium azide) - showed no CO2.

Biodegradation Tests

Fuel Blends

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Description</th>
<th>Fuel Type</th>
<th>Abbreviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>Diesel</td>
<td>Soy biodiesel 20% blend + bioextend</td>
<td>Soy B-20 A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Animal fat biodiesel 20% blend + bioextend</td>
<td>AF B-20 A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Soy biodiesel 20% blend -no additives</td>
<td>Soy B-20</td>
</tr>
<tr>
<td>#2</td>
<td>Diesel</td>
<td>Soy biodiesel 100% - no additives</td>
<td>Soy B-100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Animal fat biodiesel 20% blend - no additives</td>
<td>AF B-20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Animal fat biodiesel 100% - no additives</td>
<td>AF B-100</td>
</tr>
<tr>
<td>#3</td>
<td>Diesel</td>
<td>Soy biodiesel 20% blend + bioextend + biocide</td>
<td>Soy B-100 AA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Animal fat biodiesel 20% blend + bioextend + biocide</td>
<td>AF B-20 AA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Soy biodiesel 100% + bioextend + biocide</td>
<td>Soy B-100 AA</td>
</tr>
<tr>
<td>#4</td>
<td>Diesel</td>
<td>Soy biodiesel 100% + bioextend + biocide</td>
<td>Soy B-100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Animal fat biodiesel 100% + bioextend</td>
<td>AF B-100 A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Animal fat biodiesel 100% + bioextend</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Soy biodiesel 100% + bioextend</td>
<td>Soy B-100</td>
</tr>
</tbody>
</table>
Biodegradation Tests

Results

![Graph showing cumulative degradation percentages over 29 days for different fuel mixtures.](image)

- **ULSD**
- **Soy B-20**
- **AF B-20A**
- **Soy B-20 AA**
- **AF B-20 AA**

**29Day Cumulative degradation percentages**

- **Run #1**
  - ULSD: 80%
  - Soy B-20: 60%
  - AF B-20 A: 70%

- **Run #2**
  - Soy B-100 AA: 90%
  - AF B-20: 80%
  - AF B-20 AA: 95%

- **Run #3**
  - Soy B-100: 95%
  - AF B-20 AA: 90%

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Biodegradation Tests

Summary

• All fuel blends more readily degradable than ref. fuel
• Soy-based blends somewhat more degradable than Animalfat-based blends
• 20% biodiesel blends somewhat more degradable than 100% biodiesel
• Additives effect are minor

Aquatic Chronic Toxicity Tests

• 6 fuel blends
• 3 freshwater and 3 estuarine organisms
• 6 dilutions plus a control per species/fuel
• Using published USEPA chronic toxicity testing protocols
• “100% solutions” produced using the “slow stir” method, defining equilibrium solubility conditions
• All tests met protocol QA/QC requirements
Aquatic Chronic Toxicity Tests

Details

6 Blends in addition to reference fuel (ULSD)
- Animalfat biodiesel (100% 20%, 20% w/additive)
- Soy biodiesel (100% 20% 20% w/additive)

100% solubility solution by slow stir method
- solutions 100%, 50%, 25%, 10%, 5%, and 1%, w/stock
  # 2 samples/test archived frozen for later analysis
  # Replicates for particular combinations.

Interpolate among dilutions to determine EC_{25}
- "Toxicity" as TU = 100/EC_{25}
  # TU<1 no effects
  # TU = 1 effects seen only at 100% solution
  # TU = 100 effects seen at 1% solution

Aquatic Chronic Toxicity Tests

Fuel Blends

<table>
<thead>
<tr>
<th>Fuel Type</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>100% Ultra-Low Sulfur Diesel</td>
<td>ULSD</td>
</tr>
<tr>
<td>100% Soy Biodiesel</td>
<td>Soy B-100</td>
</tr>
<tr>
<td>20% Soy Biodiesel + 80% ULSD (w/w)</td>
<td>Soy B-20</td>
</tr>
<tr>
<td>20% Soy + 80% ULSD (w/w) amended with additive</td>
<td>Soy B-20A</td>
</tr>
<tr>
<td>100% Animal Fat Biodiesel</td>
<td>AF B-100</td>
</tr>
<tr>
<td>20% Animal Fat Biodiesel + 80% ULSD (w/w)</td>
<td>AF B-20</td>
</tr>
<tr>
<td>20% Animal Fat + 80% ULSD (w/w) amended with additive</td>
<td>AF B-20A</td>
</tr>
</tbody>
</table>
Aquatic Chronic Toxicity Tests

Test Species

<table>
<thead>
<tr>
<th>Category</th>
<th>Test Species</th>
<th>Test Type</th>
<th>Test Endpoints</th>
<th>Replicates</th>
<th>Temp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freshwater</td>
<td>Green algae (S. capricornutum)</td>
<td>96-hour static</td>
<td>Cell growth</td>
<td>10,000 cells/rep 4 rep/conc</td>
<td>25 ± 1 °C</td>
</tr>
<tr>
<td></td>
<td>Water flea (C. dubia)</td>
<td>7-day daily renewal</td>
<td>Survival Reproduction</td>
<td>1 flea/rep 10 reps/conc</td>
<td>25 ± 1 °C</td>
</tr>
<tr>
<td></td>
<td>Fathead minnow (P. promelas)</td>
<td>7-day daily renewal</td>
<td>Survival Growth</td>
<td>10 fish/rep 4 reps/conc</td>
<td>25 ± 1 °C</td>
</tr>
<tr>
<td>Estuarine/Marine</td>
<td>Red abalone (H. rufescens)</td>
<td>48-hour static</td>
<td>Normal shell development</td>
<td>5 reps/conc 2000 embryos/rep</td>
<td>15 ± 1 °C</td>
</tr>
<tr>
<td></td>
<td>Mycid shrimp (M. balthica)</td>
<td>7-day daily renewal</td>
<td>Survival Growth Fecundity</td>
<td>5 fish/rep 8 reps/conc</td>
<td>25 ± 1 °C</td>
</tr>
<tr>
<td></td>
<td>Topsmelt (A. offinis)</td>
<td>7-day daily renewal</td>
<td>Survival Growth</td>
<td>5 fish/rep 5 reps/conc</td>
<td>20 ± 1 °C</td>
</tr>
</tbody>
</table>

Aquatic Chronic Toxicity Tests

Results

- ULSD - low but detectable toxicity on mysid growth (1.0 TU) and Ceriodaphnia reproduction (1.8 TUC) only.

- No unadditized Animalfat or Soy Biodiesel blends produced detectable toxicity to the mysid, topsmelt or fathead minnow.

- Animal Fat and Soy B-100 and B-20 mixtures caused toxicity to algae cell growth, abalone shell development, and Ceriodaphnia survival and/or growth.

- Except for algae, the additized Biodiesel B-20 test materials were substantially more toxic than the corresponding unadditized material.
Aquatic Chronic Toxicity Tests

Examples

Red Abalone (*Haliotis Rufecens*) shell development

Water flea (*Ceriodaphnia dubia*) survival and reproduction
Aquatic Chronic Toxicity Tests

Summary Toxicity with additive

Toxicity apparent in all 6 species per growth endpoint

Aquatic Chronic Toxicity Tests

Summary

- Biodiesel blends are significantly more toxic than CARB ULSD#2
  - algae cell growth
  - abalone shell development
  - *Ceriodaphnia* survival and growth

- Biodiesel 20% blends with antioxidant additive were substantially more toxic than the corresponding unadditized blend
  - abalone shell development
  - *Ceriodaphnia* survival and growth
Tier II for Biodiesel Blends Tested

Summary

• Mobility
  - AFB100a shows thicker lens, more residual
  - due to higher viscosity, IFT

• Biodegradation
  - All biodiesel blends more readily degradable than ULSD
  - Soy-based blends, or 20%gs, somewhat more degradable
  - Additives effect are minor

• Aquatic Toxicity
  - Biodiesel blends are more toxic than ULSD#2
  - Biodiesel 20% blends with antioxidant additive are more toxic than the corresponding unadditized blend