**State of California**
**California Environmental Protection Agency**

# STAFF REPORT

# Multimedia Evaluation of Biodiesel

**Prepared by the**
**Multimedia Working Group**

March 2015

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## Acknowledgements

Staff acknowledges the invaluable contributions from the University of California researchers who conducted the multimedia evaluation. This report was prepared by the Multimedia Working Group with the assistance and support from other individuals within the California Environmental Protection Agency and academia.



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*This report has been reviewed by the staff of the boards, departments, and offices of the California Environmental Protection Agency, as noted above, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the California Environmental Policy Council; any of the boards, departments, and offices noted above; or the California Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.*

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# GLOSSARY

| | |
|---|---|
| ADF | Alternative Diesel Fuel |
| ARB | Air Resources Board |
| BSFC | Brake Specific Fuel Consumption |
| Cal/EPA | California Environmental Protection Agency |
| CCR | California Code of Regulations |
| CEPC | California Environmental Policy Council |
| DTSC | Department of Toxic Substances Control |
| CARB | California Air Resources Board |
| CO | Carbon Monoxide |
| $CO_2$ | Carbon Dioxide |
| FAME | Fatty Acid Methyl Esters |
| FTP | Federal Test Procedure |
| GHG | Greenhouse Gas |
| HSC | Health and Safety Code |
| LCFS | Low Carbon Fuel Standard |
| MMWG | Multimedia Working Group |
| NOx | Oxides of Nitrogen |
| NTDE | New Technology Diesel Engine |
| OEHHA | Office of Environmental Health Hazard Assessment |
| PAH | Polycyclic Aromatic Hydrocarbon |
| PM | Particulate Matter |
| SCR | Selective Catalytic Reduction |
| SWRCB | State Water Resources Control Board |
| TAC | Toxic Air Contaminant |
| THC | Total Hydrocarbons |
| UDDS | Urban Dynamometer Driving Schedule |
| UL | Underwriters Laboratories |
| UST | Underground Storage Tanks |
| VOC | Volatile Organic Compounds |

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## I. Introduction

The staff of the Air Resources Board (ARB or Board) intends to establish new motor vehicle fuel specifications for biodiesel as part of the proposed regulation on the commercialization of new alternative diesel fuels (ADFs). The ADF regulation[1] is intended to provide a framework for low carbon diesel fuel substitutes to enter the commercial market in California, while mitigating any potential environmental or public health impacts. The proposed regulation order is provided in Appendix A.

Before new fuel specifications are established, Health and Safety Code (HSC) section 43830.8 requires a multimedia evaluation to be conducted and reviewed by the California Environmental Policy Council (CEPC). The CEPC must determine if the proposed regulation poses a significant adverse impact on public health or the environment.[2] Since the ARB intends to establish new fuel specifications for biodiesel, a comprehensive multimedia evaluation of the fuel was conducted in accordance to HSC section 43830.8.

The Multimedia Working Group (MMWG) was established to oversee the multimedia evaluation process and make recommendations to the CEPC regarding the acceptability of new fuel formulations proposed for use in the State. The purpose and scope of the multimedia evaluation is to inform the rulemaking process and provide the information needed for the development of fuel regulations.

For the proposed biodiesel specifications included as part of the ADF regulation, the MMWG prepared this staff report for submittal to the CEPC. The purpose of this report is to provide a summary of the multimedia evaluation and the MMWG's overall conclusions and recommendations to the CEPC based on the evaluation.

### A. Fuels Multimedia Evaluation

"Multimedia evaluation" is the identification and evaluation of any significant adverse impact on public health or the environment, including air, water, and soil, that may result from the production, use, or disposal of the motor vehicle fuel that may be used to meet the state board's motor vehicle fuel specifications.[3]

At a minimum, the evaluation must address impacts associated with the following:

- Emissions of air pollutants, including ozone forming compounds, particulate matter, toxic air contaminants, and greenhouse gases.
- Contamination of surface water, ground water, and soil.

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[1] Air Resources Board. *Proposed Regulation on the Commercialization of New Alternative Diesel Fuels Staff Report: Initial Statement of Reasons*. January 2, 2015. Appendix A: Proposed Regulation Order.
[2] California Air Pollution Control Laws. Health and Safety Code, Division 26, Part 5, Chapter 4, Section 43830.8(e).
[3] California Air Pollution Control Laws. Health and Safety Code, Division 26, Part 5, Chapter 4, Section 43830.8(b).

- Disposal or use of the byproducts and waste materials from the production of the fuel.

As specified in HSC 43830.8, a multimedia evaluation must be based on the best available scientific data, written comments, and any information collected by the Board in preparation for the proposed rulemaking. After the evaluation has been completed, the MMWG must prepare a written summary report, including the MMWG's overall conclusions and recommendations to the CEPC, and submit it for peer review pursuant to HSC section 57004. The staff report and results of the peer review will then be submitted to the CEPC for final review and approval.

1. Multimedia Working Group

The California Environmental Protection Agency (Cal/EPA) formed the inter-agency MMWG to oversee the multimedia evaluation process and make recommendations to the CEPC. The MMWG includes representatives from the ARB, State Water Resources Control Board (SWRCB), Office of Environmental Health Hazard Assessment (OEHHA), and Department of Toxic Substances Control (DTSC). The MMWG may also consult with other agencies and experts, as needed. The complete list of all members of the MMWG is provided in Appendix B.

The biodiesel multimedia evaluation includes an assessment of potential impacts on public health and the environment, including air, water, and soil, that may result from the production, use, and disposal of biodiesel. In this evaluation, ARB staff was responsible for the air quality impact assessment and overall coordination of the multimedia evaluation process. OEHHA staff was responsible for evaluating potential public health impacts, SWRCB staff was responsible for evaluating potential surface water and groundwater quality impacts, and DTSC staff was responsible for evaluating potential hazardous waste and soil impacts.

2. California Environmental Policy Council

Pursuant to Public Resources Code section 71017(b), the CEPC was established as a seven-member body comprised of the Secretary for Environmental Protection; the Chairpersons of ARB and SWRCB; and the Directors of OEHHA, DTSC, Department of Pesticide Regulation (DPR), and Department of Resources Recycling and Recovery (CalRecycle).

As previously stated, the CEPC must determine if the regulation poses a significant adverse impact on public health or the environment. In making its determination, the CEPC must consider the following:

- Emissions of air pollutants.
- Contamination of surface water, groundwater, and soil.
- Disposal of waste materials.

- MMWG recommendations contained in the staff report and peer review comments.

According to HSC section 43830.8(e), the CEPC shall complete its review of the evaluation within 90 calendar days following notice that the ARB intends to adopt a new regulation. If the CEPC determines that the regulation will cause a significant adverse impact on public health or the environment, or that alternatives exist that would be less adverse, the CEPC shall recommend alternatives or mitigating measures to reduce the adverse impact on public health or the environment.

## 3. Overview of the Multimedia Evaluation Process

A multimedia evaluation consists of three tiers. Tier I begins with a summary of what is known about the fuel and the information needed for the multimedia risk assessment. The Tier I Report, or Work Plan, identifies key knowledge gaps about the fuel, if any, and establishes the overall scope of the evaluation. Tier II is the development of the Tier II Report, or Risk Assessment Protocol, to fill in any knowledge gaps identified during Tier I. If key knowledge gaps are not identified in Tier I, no further Tier II testing or information are needed and the multimedia evaluation would then proceed directly to Tier III. Tier III is the implementation of the risk assessment, resulting in a final report of any significant adverse impacts on public health or the environment. The multimedia evaluation process is summarized in Table 1.[4]

**Table 1.** Summary of the Multimedia Evaluation Process

| | **Fuel Applicant** | **Multimedia Work Group Review** | **MMWG Consultation and Peer Review** |
|---|---|---|---|
| **Tier I** | Fuel Background Summary Report:<br>• Chemistry<br>• Release scenarios<br>• Environmental behavior | Screens applicant and establishes key assessment elements and issues | Technical consultation during development of Tier I Work Plan including identification of key risk assessment elements and issues |
| | Mutually-agreed upon Tier I Work Plan | | |
| **Tier II** | Risk Assessment Protocol Report | Comment on Risk Assessment Protocol | Technical consultation on Risk Assessment Design |
| **Tier III** | Execution of Risk Assessment and preparation of Multimedia Risk Assessment Report | Prepare recommendations to the Environmental Policy Council based on Multimedia Risk Assessment Report | Independent external peer review of the Multimedia Risk Assessment Report and Multimedia Working Group recommendations |

[4] U.C. Berkeley, U.C. Davis, and Lawrence Livermore National Laboratory. *Guidance Document and Recommendations on the Types of Scientific Information Submitted by Applicants for California Fuels Environmental Multimedia Evaluations*. June 2008, 9-10.

Each tier of the multimedia evaluation process is designed to provide input for the next stage of the decision-making process. After Tier III is complete, the MMWG prepares a summary of the multimedia evaluation and their conclusions and recommendations in a staff report to the CEPC.

4. External Scientific Peer Review

Under HSC section 43830.8(d), an external scientific peer review of the multimedia evaluation must be conducted pursuant to HSC section 57004. The purpose of the peer review is to determine whether the scientific portions of the MMWG staff report are based upon "sound scientific knowledge, methods, and practices."[5]

The peer review process is initiated by submittal of a request memorandum to the manager of the Cal/EPA Scientific Peer Review Program. The memorandum is prepared by ARB as the leading agency of the MMWG and includes a summary of the nature and scope of the requested review, descriptions of the scientific conclusions to be addressed, and list of recommended areas of expertise.

In November 2013, ARB requested external scientific peer review of the MMWG's assessment of the biodiesel multimedia evaluation. The review was completed in February 2014. After completion of the MMWG's review and assessment of new biodiesel studies published after the November 2013 release of the initial staff report, and additional references and publications from the initial peer review, the MMWG made updates and modifications to the staff report. Revisions include proposed modifications to the MMWG's assessment of the biodiesel multimedia evaluation, including updates to the air quality and public health evaluations from staff's assessment of new biodiesel studies and detailed peer reviews.

In January 2015, ARB staff requested supplemental external peer review of this revised staff report. The review will focus on the modifications to the MMWG's assessment of the biodiesel multimedia evaluation and the scientific basis for which the proposed modifications are based. Once all peer review comments are received, the MMWG will prepare written responses and make any revisions to the staff report, as needed. After all comments have been addressed, the MMWG will finalize the staff report for submittal to the CEPC. The Cal/EPA will then convene a public meeting of the CEPC to consider the results of the peer reviewers and the overall biodiesel multimedia evaluation. Based on the evaluation and peer review comments, the CEPC will determine if the proposed regulation will cause a significant adverse impact on public health or the environment.

The request memorandums for peer review, dated November 19, 2013 and January 21, 2015, are provided in Appendix H.

[5] California Air Pollution Control Laws. Health and Safety Code, Division 26, Part 5, Chapter 4, Section 57004(d)(2).

## B. Biodiesel Background Fuel Information

Biodiesel is defined as a fuel composed of mono-alkyl esters of long chain fatty acids derived from vegetable oils or animal fats and meeting ASTM International standard D6751. Pure biodiesel contains no petroleum but can be blended with petroleum diesel to create a biodiesel blend. In this report, CARB diesel blended with 10 volume percent (vol%), 20 vol% or 50 vol% biodiesel is denoted as B10, B20 or B50, respectively. Pure biodiesel is denoted as B100.

To produce biodiesel, a feedstock undergoes a transesterification reaction with methanol and a catalyst to produce methyl esters, which compose biodiesel, also known as Fatty Acid Methyl Esters or FAME. Primary biodiesel feedstocks expected to be used in California include soybean oil, palm oil, corn oil, yellow grease, animal tallow, trap (brown) grease, canola oil, and safflower oil.[6]

There are many steps involved in the transesterification production of biodiesel. Initially, the three components, alcohol, oil, and a catalyst, are mixed in a reactor. The next step involves separating the methyl esters and glycerin (a byproduct). The methyl esters are then neutralized with acid to remove any residual catalyst and to separate any soap that may have formed during the reaction. The mixture is washed with water and any alcohol is removed. The biodiesel may then be dried in a vacuum flash process that leaves a clear amber-yellow liquid with a viscosity similar to petroleum diesel. Some processes also distill the final product to remove undesirable impurities.[7]

Biodiesel feedstocks are classified by their fatty acid profile; the fatty acid composition greatly influences a fuel's characteristics, as esters of different fatty acids have different physical and chemical properties. Generally, the quality of the fuel is dependent on the quality and fatty-acid composition of the feedstock, the production process, and post-production handling. Biodiesel blends up to B5 must meet ASTM D975 standards. ASTM has also established ASTM D7467 for blends of B6 to B20, and ASTM D6751-12 for B100.[8]

## C. Multimedia Evaluation of Biodiesel

Pursuant to HSC section 43830.8, researchers from UC Davis and UC Berkeley conducted the multimedia evaluation of biodiesel. The evaluation is a relative comparison between biodiesel fuel and CARB diesel. CARB diesel is diesel fuel meeting ARB motor vehicle fuel specifications. The proposed ADF regulation defines "CARB diesel" as a light or middle distillate fuel that may be comingled with up to five (5) volume percent biodiesel and meets the definition and requirements for "diesel

[6] Ginn, T.R. et al. *California Biodiesel Multimedia Evaluation Final Tier III Report*. June 2014, I-1.
[7] Ginn, T.R. et al. *California Biodiesel Multimedia Evaluation Final Tier III Report*. June 2014, I-1.
[8] Ginn, T.R. et al. *California Biodiesel Multimedia Evaluation Final Tier III Report*. June 2014, I-1,I-2.

fuel" or "California nonvehicular diesel fuel" as specified in California Code of Regulations, title 13, section 2281 et seq.[9]

After each tier of the biodiesel multimedia evaluation, the UC researchers submitted a report and finalized it with the MMWG. The final reports from each tier are listed below:

- California Biodiesel Multimedia Evaluation Final Tier I Report (Final Tier I Report)[10]
- California Biodiesel Multimedia Evaluation Final Tier II Report (Final Tier II Report)[11]
- California Biodiesel Multimedia Evaluation Final Tier III Report (Final Tier III Report or Biodiesel Final Report)[12]

The Biodiesel Final Report is provided in Appendix G and includes both the Final Tier I Report and Final Tier II Report as attachments. In these reports by the UC researchers, "CARB diesel," "CARB Ultra Low Sulfur Diesel (ULSD)," and "conventional petroleum diesel," are used interchangeably.

As previously described, a multimedia evaluation may consist of a total of three tiers. During Tier I of the biodiesel evaluation, the UC researchers completed a detailed review of biodiesel, evaluated potential impacts, and determined key knowledge gaps. Upon completion of Tier I, the overall scope of the biodiesel evaluation was established. The knowledge gaps identified in Tier I necessitated further study, testing, and a more detailed impact assessment of biodiesel in Tier II. The biodiesel Tier II risk assessment design included various test plans and studies to fill in key knowledge gaps identified in Tier I. Tier III began with the implementation of the Tier II risk assessment protocols and concluded with the formal submittal of the Biodiesel Final Report.

Based on the biodiesel multimedia evaluation and the information provided in the Tier I, Tier II, and Tier III reports by UC Davis and UC Berkeley, the MMWG determined that the use of biodiesel, as specified in the multimedia evaluation and proposed regulation, does not pose a significant adverse impact on public health or the environment.

For the proposed biodiesel specifications included as part of the ADF regulation, the MMWG prepared this staff report for submittal to the CEPC. The purpose of this report is to provide a summary of the multimedia evaluation and the MMWG's overall conclusions and recommendations to the CEPC.

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[9] Air Resources Board. *Proposed Regulation on the Commercialization of New Alternative Diesel Fuels Staff Report: Initial Statement of Reasons*. January 2, 2015. Appendix A: Proposed Regulation Order. A-8.
[10] Ginn, T.R. et al. *California Biodiesel Multimedia Evaluation Final Tier I Report*, September 2009.
[11] Ginn, T.R. et al. *California Biodiesel Multimedia Evaluation Final Tier II Report*, January 2012.
[12] Ginn, T.R. et al. *California Biodiesel Multimedia Evaluation Final Tier III Report*, June 2014.

## II. Section Summaries

This section provides the multimedia evaluation summaries prepared by ARB, SWRCB, OEHHA, and DTSC. The evaluations are based on the relative differences between biodiesel and CARB diesel. The MMWG evaluated potential environmental and public health impacts from changes to air emissions, water quality, soil quality, and hazardous waste generation. The complete evaluations and supporting documentation are provided in the appendices of this report.

### A. Air Resources Board Evaluation

ARB staff completed an air quality assessment of biodiesel fuel. The evaluation includes a description of the biodiesel emissions test program and impact analysis on criteria pollutants, toxic air contaminants, and ozone precursors. The complete report is provided in Appendix C.

Staff's assessment is based on the data and information provided for the biodiesel multimedia evaluation, including the UC researchers' multimedia reports (Final Tier I, Tier II and Tier III reports); the "*CARB Assessment of the Emissions from the Use of Biodiesel as a Motor Vehicle Fuel in California*" (ARB Emissions Study)[13] by UC Riverside from emissions testing conducted at the College of Engineering – Center for Environmental Research and Technology (CE-CERT) and ARB emissions test facilities in Stockton and El Monte, California; and the "*CARB Comprehensive B5/B10 Blends Heavy-Duty Engine Dynamometer Testing*" (B5/B10 Study)[14] by ARB, UC Riverside, and UC Davis. The B5/B10 Study expands on the ARB Emissions Study to provide more comprehensive information on the emissions of lower biodiesel blends.

As part of the ARB Emissions Study and subsequent B5/B10 Study, emissions testing were conducted on biodiesel (B100) and various biodiesel blends (B5, B10, B20, B50) with CARB diesel as the baseline fuel. For the ARB Emissions Study, test fuels include five primary fuels that were subsequently blended at various levels to comprise the full test matrix. Two biodiesel feedstocks were used for testing, including one soy-based and one animal-based biodiesel fuel. These fuels were selected to provide a range of properties that are representative of typical feedstocks representing different characteristics of biodiesel in terms of cetane number and degree of saturation.[15] The results of the testing were straight averages of the difference between biodiesel and CARB diesel emissions.

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[13] Durbin, T.D. et al. *CARB Assessment of the Emissions from the Use of Biodiesel as a Motor Vehicle Fuel in California "Biodiesel Characterization and NOx Mitigation Study."* October 2011.
[14] Karavalakis, G. et al. *CARB Comprehensive B5/B10 Blends Heavy-Duty Engine Dynamometer Testing.* June 2014.
[15] Durbin, T.D. et al. *CARB Assessment of the Emissions from the Use of Biodiesel as a Motor Vehicle Fuel in California "Biodiesel Characterization and NOx Mitigation Study."* October 2011, xxv.

The ARB Emissions Study included both engine testing and chassis testing. Engine testing was performed on a 2006 Cummins ISM engine and a 2007 MBE4000 engine. Chassis testing was performed on the following test vehicles:

- 2006 International Truck equipped with a 2006 Cummins ISM engine
- 2008 Freightliner Truck equipped with a 2007 MBE4000 engine
- 2000 Freightliner Truck equipped with a 2000 Caterpillar C-15 engine
- Kenworth model T800 truck equipped with a 2010 Cummins ISX engine

Test engines included typical six cylinder, in-line, direct injection, turbocharged, heavy-duty diesel engines. The engines were selected from two model year categories; 2002-2006 and 2007-2009. The 2002-2006 engines were estimated to represent an important contribution to the emissions inventory from the present through 2017. The 2007-2009 model year engine represented the latest technology that was available at the time of testing.[16]

1. Air Emissions

The emissions measurements for the engine testing under the ARB Emissions Study focused primarily on regulated emissions, including particulate matter (PM), nitrogen oxides (NOx), total hydrocarbons (THC), carbon monoxide (CO), and carbon dioxide ($CO_2$). More extensive testing, including toxics analyses, was completed for the chassis testing.

Average PM emissions results showed consistent and significant reductions for all biodiesel blends, with the magnitude of reductions increasing with blend level. For the 2006 Cummins engine over the Federal Test Procedure (FTP) test cycle, PM reductions for soy-based biodiesel were approximately 6% for B5, 25% for B20, and 58% for B100. For animal-based biodiesel, PM reductions ranged from 19% for B20 to 64% for B100.

Average NOx emissions showed trends of increasing NOx emissions with increasing biodiesel blend level. Soy-based biodiesel blends showed a higher increase in NOx emissions for essentially all blend levels and test cycles compared to animal-based biodiesel blends. For soy-based biodiesel over the FTP cycle, results for the 2006 Cummins engine showed NOx increases of 2.2% for B5, 6.6% for B20, and 27% for B100. Animal-based biodiesel results showed NOx increases of 1.5% for B20 to 14% for B100. For the 2007 MBE4000 engine, NOx increases were greater than those of the 2006 engine for nearly all biodiesel blends and test cycles.

Average THC emissions for the 2006 Cummins showed consistent and significant reductions for all biodiesel blends, with the magnitude of reductions increasing with blend level. For soy-based biodiesel over the FTP cycle, THC emissions showed 6% reductions for B10, 11% for B20, and 63% for B100. For animal-based biodiesel, THC reductions ranged from 13% for B20 to 71% for B100.

[16] Durbin, T.D. et al. *CARB Assessment of the Emissions from the Use of Biodiesel as a Motor Vehicle Fuel in California "Biodiesel Characterization and NOx Mitigation Study."* October 2011, 5-6.

Average CO emissions also showed consistent and significant reductions for animal-based biodiesel, ranging from 7% for B5, 14% for B20, and 27% for B100. For soy-based biodiesel, CO trends were less consistent with some results not statistically significant.

Average tailpipe $CO_2$ emissions showed a slight increase for the higher biodiesel blends. For the 2006 Cummins engine, the increase ranged from about 1% to 4%, with increases being statistically significant for the B100 fuels for all tests, the B50 fuel for the cruise cycles, and other testing combinations. For the 2007 MBE 4000 engine, only the B100 showed consistent and statistically significant increases for the different cycles, ranging from 1% to 5%.

The biodiesel blends showed an increase in fuel consumption with increasing biodiesel blends. The fuel consumption differences were generally greater for the soy-based biodiesel in comparison with animal-based biodiesel for the 2006 Cummins engine, but not for the 2007 MBE 4000 engine. For the 2006 Cummins engine, changes in fuel consumption for soy-based biodiesel blends ranged from 1.4% to 1.8% for B20 and 6.8% to 9.8% for B100. Animal-based biodiesel blends ranged from no statistical difference to 2.6% for B20 and 4.4% to 6.7% for B100. For the 2007 MBE4000 engine, the differences in fuel consumption ranged from no change to 2.5% for B50 and lower blends, while the increases for B100 blends ranged from 5.6% to 8.3%.

Additionally, on a federal level, a U.S. EPA biodiesel exhaust emissions study found beneficial impacts associated with biodiesel use. Tailpipe emissions from heavy-duty engines were compiled and analyzed. Compared to federal diesel, data showed approximately 10% to 20% emissions reductions of PM, CO and HC from biodiesel blends of B20 and approximately 45% to 65% range reductions from B100.[17]

### 2. Toxic Air Contaminants

ARB identified diesel PM as a toxic air contaminant in 1998, and determined that diesel PM accounts for about 70% of the toxic risk from all identified toxic air contaminants. As previously stated, test results showed that the use of biodiesel reduces PM emissions with increasing blend levels.

Other toxic emissions tests were conducted for various carbonyls, volatile organic compounds (VOCs), and polycyclic aromatic hydrocarbons (PAHs). Overall, results show decreases in most PAHs and VOCs. Carbonyl emissions did not show consistent trends between different fuels. Genotoxicity assays were also performed and results showed either reduced toxicity compared to CARB diesel or no difference in toxicity.

[17] U.S. Environmental Protection Agency, *A Comprehensive Analysis of Biodiesel Impacts on Exhaust Emissions*, EPA420-P-02-001, October 2002.

### 3. Greenhouse Gas Emissions

Greenhouse gas (GHG) emissions are primarily $CO_2$, methane ($CH_4$), nitrous oxide ($N_2O$), and hydrofluorocarbons.[18] GHG emissions from the use of fuels are primarily $CO_2$.[19] The ARB Emissions Study included emissions measurements for $CO_2$. As previously stated, average tailpipe $CO_2$ emissions showed a slight increase for higher biodiesel blends. However, this measured increase in $CO_2$ emissions does not necessarily suggest that the fuels lead to an overall increase in carbon emissions. Most THC and CO convert to $CO_2$ in the atmosphere, so total $CO_2$ produced by the biodiesel combustion process is determined by direct $CO_2$ emissions, as well as THC and CO.

The fate of most fatty acids in plants or animals is metabolism by animals or microorganism to produce energy and $CO_2$. Production of biodiesel fuel reduces the amount of $CO_2$ produced by energy metabolism. Combustion of the fatty acid moiety of biodiesel produces an amount of $CO_2$ that is approximately equal this reduction in $CO_2$ production. The presence of methanol esterified to fatty acids may lead to a small increase in $CO_2$ releases from production and use of biodiesel fuel. However, the net increase in $CO_2$ releases from production and use of biodiesel is far less than net $CO_2$ releases from production and use of an equivalent amount of petroleum-based diesel.

Life cycle GHG emissions include emissions associated with the production, transportation, and use of a fuel in a motor vehicle. The life cycle analysis (LCA) of a fuel includes direct emissions from producing, transporting, and using the fuel, as well as other indirect effects, including land use change. Depending on the fuel, GHG emissions from each step of the life cycle can include $CO_2$, $CH_4$, $N_2O$, and other GHG contributors. The "carbon intensity" of a fuel represents the equivalent amount of $CO_2$ emitted from each stage of the fuel's life cycle and is expressed in terms of grams of $CO_2$ equivalent per megajoule ($gCO_2e/MJ$).[20]

In contrast, end-of-pipe or tailpipe emissions only include exhaust emissions associated with the use of a fuel in an internal combustion engine.[21] Tailpipe $CO_2$ emissions are only one component in determining a fuel's life cycle carbon emissions. Therefore, the measured increase in $CO_2$ emissions may not necessarily lead to an overall increase in carbon emissions. An increase in $CO_2$ reflects more complete combustion, and is an expected result of decreased THC and CO emissions.

Under the ARB Emissions Study, biodiesel blends also showed an increase in average brake specific fuel consumption (BSFC) with increasing levels of biodiesel. This is

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[18] Air Resources Board. *Staff Report: Initial Statement of Reasons for Proposed Rulemaking, Public Hearing to Consider Adoption of Regulations to Control Greenhouse Gas Emissions from Motor Vehicles*. August 6, 2004, i.

[19] Air Resources Board. *Proposed Re-Adoption of the Low Carbon Fuel Standard*. *Staff Report: Initial Statement of Reasons*. December 2014, ES-2.

[20] Air Resources Board. *Proposed Re-Adoption of the Low Carbon Fuel Standard. Staff Report: Initial Statement of Reasons.* December 2014.

[21] Air Resources Board. *Proposed Regulation to Implement the Low Carbon Fuel Standard. Staff Report: Initial Statement of Reasons.* 2009, IV-12.

consistent with expectations based on the lower energy density of biodiesel. The changes in fuel consumption for soy-based biodiesel blends for the 2006 Cummins engine range from 1.4% to 1.8% for B20 and 6.8% to 9.8% for B100. The changes in fuel consumption for animal-based biodiesel blends for the 2006 Cummins engine range from no statistical difference to 2.6% for B20 and 4.4% to 6.7% for B100.

For an alternative fuel, the determination of GHG emissions impact is the result of a full LCA of the fuel. For biodiesel, the outcome of the analysis is greatly dependent on the feedstock source. The LCA of biodiesel under the Low Carbon Fuel Standard showed reductions in GHG emissions of about 15% to 95% depending on feedstock source.[22]

4. Ozone Precursors

Ozone is produced by photochemical reactions. Its precursor components are primarily the result of road traffic. Unlike many of the other GHGs, ozone is a short-lived gas that is found in regionally varying concentrations. Nevertheless, it is the third most important anthropogenic GHG behind $CO_2$ and $CH_4$.[23]

Both THC and NOx emissions determine ozone concentrations. As previously stated, THC emissions showed consistent and significant reductions with the magnitude of the reductions increasing with blend level. However, NOx was found to increase at certain biodiesel blend levels.

The results of both the ARB Emissions Study and B5/B10 Study apply specifically to heavy-duty vehicles that do not use post-exhaust NOx emissions control. Therefore, the results of this study should not be extended to New Technology Diesel Engines (NTDEs) or light-duty and medium-duty vehicles.

Engines that meet the latest emission standards through the use of Selective Catalytic Reduction (SCR) systems have been shown to have no significant difference in NOx emissions based on the fuel used. A study conducted by the National Renewable Energy Laboratory looked at two Cummins ISL engines equipped with SCR systems.[24] Results showed that the use of SCR was effective at reducing NOx to near the detection limit on all duty cycles and fuels, including B100.[25]

Light-duty and medium-duty vehicles have similarly been found not to experience increases in NOx due to the use of biodiesel. For example, a study performed on three light-duty vehicles using different biodiesel blends found no significant and consistent

[22] Air Resources Board, *LCFS Carbon Intensity Lookup Table*, December 2012.
[23] Air Resources Board. Proposed Rulemaking, *Public Hearing to Consider Adoption of Regulations to Control Greenhouse Gas Emissions from Motor Vehicles*. August 6, 2004, i.
[24] Lammert et al., *Effect of B20 and Low Aromatic Diesel on transit Bus NOx emissions Over Driving Cycles with a Range of Kinetic Intensity*, SAE Int. J Fuels Lubr., 5(3):2012
[25] Air Resources Board. *Proposed Regulation on the Commercialization of New Alternative Diesel Fuels Staff Report: Initial Statement of Reasons*. January 2, 2015. Page 44.

pattern in NOx emissions based on blend levels across the different engines, blends, and cycles.[26]

### B. State Water Resources Control Board Evaluation

SWRCB staff completed an evaluation of potential surface water and groundwater impacts from biodiesel fuel. Staff based their assessment on the information provided in the UC Davis and UC Berkeley multimedia evaluation reports (Final Tier I, Tier II, and Tier III Reports). The multimedia evaluation and SWRCB's assessment of environmental impacts is specific to the difference between biodiesel and CARB diesel. Please refer to Appendix D for staff's complete evaluation.

#### 1. Water Impacts

Aquatic toxicity screening with unadditized and additized biodiesel and biodiesel blends showed an increase in toxicity for subsets of screening species compared to CARB diesel. Water allocation and agricultural impacts associated with the growing of feedstocks used in the production of biodiesel were not considered as part of the multimedia evaluation. A supplemental multimedia review may need to be performed in the future to evaluate any agricultural and water resource impacts if feedstocks are to be grown in California.

#### 2. Underground Storage Tank Material Compatibility and Leak Detection

Material compatibility testing has demonstrated that biodiesel and biodiesel blends are incompatible with various products commonly used in California's existing underground storage tank (UST) infrastructure. Incompatibility increases the risk of unauthorized releases. Therefore, material selection in UST equipment and leak detection technology is important to prevent releases.

Material compatibility and leak detection functionality with a stored substance is a requirement of the UST laws and regulations, and verified by the local permitting agency with the UST owner or operator. Recently revised UST regulations allow the storage of substances not certified as compatible by an independent testing organization, typically Underwriters Laboratories (UL), if the manufacturer of the components provides affirmative statements of compatibility. This option however is limited to double-walled USTs. UL's current certification status of biodiesel blends only include blends up to B5. Therefore, biodiesel blends up to B5 can be stored in both single or double-walled petroleum approved USTs. Blends above B5 may be stored in double-walled petroleum USTs when the manufacturer provides affirmative statements of compatibility.

[26] Air Resources Board. *Proposed Regulation on the Commercialization of New Alternative Diesel Fuels Staff Report: Initial Statement of Reasons*. January 2, 2015. Page 45.

### 3. Biodegradability and Fate and Transport

The biodiesel multimedia evaluation identifies that unadditized biodiesel and biodiesel blends consistently show increased biodegradation as compared to CARB diesel, and that additized biodiesel and biodiesel blends can result in decreased biodegradation. These biodegradability scenarios are influenced by the additives used and biodiesel blend concentration.

### 4. Waste Discharge from Manufacturing

Chemicals used in biodiesel production and byproducts are required to comply with hazardous waste laws and regulations. No significant areas of concern have been identified by staff when comparing the waste streams of biodiesel to CARB diesel.

## C. Office of Environmental Health Hazard Assessment Evaluation

OEHHA staff evaluated potential public health impacts from the use of biodiesel. Staff based their evaluation on a number of relevant biodiesel toxicity studies. Please refer to Appendix E for the complete evaluation.

A number of studies found that biodiesel combustion emissions are more potent than petroleum diesel combustion emissions in eliciting responses associated with inflammation and oxidative stress in cell-based assays and *in vivo*. OEHHA notes that in most studies, the toxicity per mass unit of PM is evaluated, and the smaller mass of PM generally associated with biodiesel is not included in the comparison. At this time, it is not clear whether biodiesel combustion emissions are more potent than petroleum diesel combustion emissions when compared using doses based on PM emissions per mile (or per horsepower hour). Nonetheless, the results of these reports are important to consider and raise concern regarding the relative reactivity in biological systems of the many components associated with PM from biodiesel and conventional diesel.

The data from recent *in vitro* and *in vivo* animal studies indicate that biodiesel combustion emissions exposure can induce enhanced inflammatory responses relative to petroleum diesel particulate when measured on a mass basis. This may be offset by biodiesel's lower mass emissions of PM and other constituents. However, the recent research increases concern regarding health impacts associated with an inflammatory response. Further research is warranted to determine whether the increased toxicity of biodiesel combustion emissions, at least related to oxidative stress and the inflammatory response, might outweigh the beneficial reduction of particulate mass (and associated toxicity) that would result from the use of biodiesel. Of note, volatile constituents of combustion emissions vary by fuel type and are likely involved in the oxidative stress and inflammatory responses measured in these studies.

In conclusion, OEHHA cannot determine with certainty whether replacing petroleum diesel by biodiesel or biodiesel-petroleum diesel blends for on-road motor vehicle use will reduce adverse human health impacts attributable to oxidative stress and

inflammation from toxic chemicals in diesel-engine emissions. The emissions of toxic substances that result in oxidative stress and inflammation (and other cellular damage leading to a variety of health outcomes) from both biodiesel combustion and petroleum diesel combustion depend on many factors including the type of engine used in the test, the workload protocol, the source of the biodiesel used in the test, and the type of petroleum diesel (e.g., CARB diesel, low sulfur diesel, high sulfur diesel, etc.). For example, several studies showed increases in carbonyls in the emissions with certain biodiesel fuels while a few showed decreases. Application of oxidative catalysts also affected the emissions of organic compounds including aldehydes. Most studies indicated decreases in PAH emissions with biodiesel versus petroleum diesel combustion. The types of published studies evaluating potential toxicity of biodiesel versus petroleum diesel emissions include both *in vitro* protocols (exposing cells) and *in vivo* animal exposures. Extrapolation, particularly from *in vitro* studies to human health effects, has limitations because physiological conditions of cells exposed *in vitro* can be very different from physiological conditions of cells exposed *in vivo*. Finally, the mix of toxic chemicals in exhaust, which varies as noted above by fuel, engine, and operating conditions, affects the overall toxicity of engine exhaust in ways that cannot be fully characterized. The most useful comparisons of the toxicity of combustion emissions for ARB's purposes would be to compare emissions from CARB diesel-fueled engines to biodiesel-fueled engines under conditions (workload patterns) typical of heavy duty diesel use in California. Except for the ARB Emissions Study, the toxicity studies mostly do not look at CARB diesel, use a variety of engines, use different biodiesel fuels, and employed different workload patterns than may be typical of heavy-duty engines in California.

Some, but not all, of the more recent studies published in 2013 and 2014 raise concerns that use of biodiesel will result in increased emissions of substances that induce oxidative stress and/or indicators of inflammation. While some studies indicate combustion emissions from biodiesel are more potent than combustion emissions from petroleum diesel in producing these particular effects when compared on a per µg of PM basis, the reduced mass of PM produced per unit of engine work from biodiesel may offset the increase in potency per µg of PM. Thus, a comparison of oxidative stress, or other toxicity, induced in a biological system per µg of PM emission needs to be placed in context of decreased overall emissions. Another complication is the role of non-PM constituents in induction of oxidative stress. These volatile components of emissions have not necessarily been measured in the *in vitro* and *in viv*o toxicology studies; rather, PM is usually used as the exposure metric. Thus, the responses cannot be evaluated in terms of exposure to particular volatile or semi-volatile components. Further, it must be noted that oxidative stress may be just one of the mechanisms involved in the toxicity of diesel exhaust emissions, which include respiratory and cardiovascular health effects, immunotoxicity, and carcinogenicity.

### D. Department of Toxics Substances Control Evaluation

DTSC staff assessed potential impacts to human health and the environment from the production, use, transport, storage, and disposal of biodiesel compared to CARB diesel.

Specifically, staff's evaluation focused on: (1) hazardous waste generation during the production, use, storage, and disposal of biodiesel and biodiesel blends, and (2) impacts on the fate and transport of biodiesel and biodiesel blends in subsurface soil from unauthorized spills or releases. Please refer to Appendix F for DTSC's complete evaluation.

Biodiesel's chemical composition depends on the feedstock's fatty acid content that has a significant bearing on final biodiesel characteristics. Generally, biodiesel is more acidic and reactive to certain plastics, some rubbers, and metals than CARB diesel.

Based on the feedstock, hazardous waste can be generated during biodiesel production, storage, distribution, and use. Potential hazardous wastes from production include, but are not limited to: hexane, ethanol, methanol, potassium/sodium hydroxides, sulfuric acid, and phosphoric acid. Furthermore biodiesel and production chemical releases into ground surface and subsurface environments are expected because of ruptures or leaks to above and below ground storage tanks; production equipment; piping and fittings; and/or transport, delivery, and spills during use. Large feedstock extraction will probably occur out of state, but could include the possibility of in-state manufacture. Such release scenarios should be evaluated on a case-by-case basis. Mitigation strategies should be established to avoid such spills, limit the migration of biodiesel and biodiesel blends, and comply with the appropriate hazardous waste management standards.

Tests were conducted using pure biodiesel, CARB diesel, and biodiesel blends. Additionally, tests were done with two additives (Kathon FP-1.5 as a biocide, and Bioextend-30 as an antioxidant). The results of the experiments were reported in the Tier II report. When comparing the data of CARB diesel to that of biodiesel, the biodiesel has the following characteristics:

(1) Biodiesel aerobically biodegrades more readily.

(2) Biodiesel with Bioextend-30 (an antioxidant) preliminarily has a higher aquatic toxicity for a small subset of tested species.

(3) Biodiesel, in general, has no significant difference in vadose zone infiltration rate. Biodiesel's infiltration rate from animal fat appeared to be similar to CARB diesel; however, biodiesel left a noticeable increase in the residual's vertical dimension and spread less extensive horizontally.

Based on the tests performed, biodiesel appears to react differently in the environment than CARB diesel. The assumption made was that additives used in the tests would be the baseline for bringing biodiesel to market. As biodiesel or biodiesel blends are brought into the market, distribution chain monitoring would occur, and that information presented to the MMWG. Follow-up from unforeseen releases or impacts would be addressed by the affected agencies. If new or different additives from those tested are proposed for use, appropriate evaluation through the MMWG process should occur.

## III. Conclusions

This section provides the conclusions of each of the evaluations conducted by ARB, SWRCB, OEHHA, and DTSC. The conclusions on the impacts of biodiesel on public health and the environment are summarized below:

### A. Conclusions on Air Emissions Impact

Based on a relative comparison between biodiesel and CARB diesel, staff concludes that with in-use requirements biodiesel, as specified in the multimedia evaluation and proposed regulation, does not pose a significant adverse impact on public health or the environment from potential air quality impacts.

Staff also makes the following general conclusions:

- Biodiesel reduces PM emissions in diesel exhaust.
- Biodiesel reduces emissions and health risk from PM in diesel exhaust, a toxic air contaminant identified by ARB.
- Biodiesel reduces CO emissions in diesel exhaust.
- Biodiesel reduces THC emissions in diesel exhaust.
- Biodiesel at certain blend levels increases NOx emissions in diesel exhaust.
- In consideration of NOx, in-use requirements incorporated in the proposed ADF regulation will not result in any significant adverse impacts from biodiesel use.

In general, studies have found environmental benefits associated with biodiesel use as compared to use of CARB diesel fuel. Biodiesel is considered a low carbon fuel and supports GHG emission reductions. Biodiesel emits less CO, PM, THC, and air toxics than CARB diesel.

### B. Conclusions on Water Impacts

SWRCB staff concludes that given the information provided by the UC researchers, there are minimal additional risks to beneficial uses of California waters posed by biodiesel than that posed by CARB diesel. SWRCB staff supports the multimedia evaluation of biodiesel which meets the ASTM fuel specifications and the finding of no significant adverse impacts on public health or the environment.

### C. Conclusions on Public Health Impact

Based upon the information presented in the biodiesel multimedia evaluation, the substitution of biodiesel for CARB diesel appears to reduce the rate of addition of carbon dioxide to the atmosphere and the amount of PM, benzene, ethyl benzene, and PAHs released into the atmosphere.

However biodiesel use may increase NOx emissions for certain blends. Further, biodiesel combustion may produce higher levels of some toxic constituents, such as

1,2-napthoquinone and acrolein, as well as a larger proportion of total particles as ultrafine particles relative to petroleum diesel.

The data from recent *in vitro* and *in vivo* animal studies indicates that biodiesel emission particulate exposure can induce enhanced oxidative stress and inflammatory responses relative to petroleum diesel particulate when measured on a mass basis. This may be offset by lower mass emissions of PM and other constituents. The study results are complicated by different types of biodiesel and petroleum diesel, as well as engine and workload protocols used to generate the exhaust for the exposures. The studies were not all comparing CARB diesel with specific biodiesel meeting CARB specifications. However, this research increases uncertainty regarding health impacts associated with oxidative stress and inflammation. Further research is warranted to determine whether the increased toxicity of biodiesel emission particulate observed in some studies might outweigh the beneficial reduction of particulate mass and other constituents such as PAHs (and associated toxicity) that would result from the use of biodiesel.

Switching from petroleum diesel to biodiesel is likely to reduce cancer risks since biodiesel emissions contains significantly less PM, PAHs and benzene. These are well characterized carcinogens, and the risk reduction is real. However, this benefit must be measured against a less certain increase of adverse health outcomes due to biodiesel emission particulate-induced oxidative stress and inflammation (e.g., possible exacerbation of asthma conditions, increased cardio-vascular episodes, and premature mortality). Increased NOx emissions from the use of biodiesel may also produce increased adverse respiratory and cardiovascular impacts, if not controlled.

In summary, the information currently available to OEHHA indicates a reduction in cancer risk from use of biodiesel, and a reduction in greenhouse gas emissions, which in itself is associated with myriad environmental and public health impacts. It is difficult to state with certainty that the use of biodiesel will decrease cardiovascular or respiratory health risks because of the uncertainty introduced by recent studies that provide evidence for increased oxidative stress and inflammatory response to biodiesel emissions relative to petroleum diesel particles on a mass basis. As noted above, the reduction in PM and other emissions may offset this potential increased inflammatory response. CEPC may want to emphasize in its determination the continued importance of emissions controls for biodiesel fueled engines, as has been the emphasis for petroleum diesel fueled engines.

### D. Conclusions on Soil and Hazardous Waste Impact

Based on biodiesel and CARB diesel data, DTSC staff concludes that biodiesel aerobically biodegrades more readily than CARB diesel. Also, some additized biodiesel preliminarily has a higher aquatic toxicity for a small subset of tested species. In general, biodiesel has no significant difference in vadose zone infiltration rate. Biodiesel's infiltration rate from animal fat appeared to be similar to CARB diesel. However, biodiesel left a noticeable increase in the residual's vertical dimension and spread less extensive horizontally.

## IV. Recommendations

The Multimedia Working Group recommends that the CEPC:

1. Find that the use of biodiesel fuel in California, as specified in this multimedia evaluation and the proposed regulation, does not pose a significant adverse impact on public health or the environment compared to CARB diesel fuel.

2. Condition the finding on the following:

    a. Biodiesel that does not meet the specifications in the ADF regulation must undergo an emissions equivalence comparison certification program.

    b. Any hazardous substances and hazardous waste used in production, storage, and transportation of biodiesel will be handled in compliance with applicable California laws and regulations.

    c. Specific fuel formulations and additives that were not included within the scope of this multimedia evaluation must be reviewed by the MMWG for consideration of appropriate action.

    d. In the event that relevant available information indicate the potential for significant risks to public health or the environment, the specific use of biodiesel will be reviewed by the MMWG for appropriate action.

## V. References

Note: References are listed according to the corresponding footnote in the staff report. For references available online, electronic links have been provided. References used more than once are indicated as a duplicate (e.g., "Same as Footnote 2"), excluding specific page numbers, and are listed to maintain the order and numbering of the footnotes in the report.


1. Air Resources Board. *Proposed Regulation on the Commercialization of New Alternative Diesel Fuels Staff Report: Initial Statement of Reasons*. Appendix A: Proposed Regulation Order. January 2, 2015. http://www.arb.ca.gov/regact/2015/adf2015/adf15isor.pdf.

2. California Air Pollution Control Laws. Health and Safety Code, Division 26, Part 5, Chapter 4, Section 43830.8. http://www.arb.ca.gov/bluebook/bb11/hea/hea-43830_8.htm (accessed February 23, 2015).

3. Same as Footnote 2.

4. U.C. Berkeley, U.C. Davis, and Lawrence Livermore National Laboratory. *Guidance Document and Recommendations on the Types of Scientific Information Submitted by Applicants for California Fuels Environmental Multimedia Evaluations*, June 2008. http://www.arb.ca.gov/fuels/multimedia/guidancedoc.pdf (accessed February 23, 2015).

5. California Air Pollution Control Laws. Health and Safety Code, Division 26, Part 5, Chapter 4, Section 57004(d)(2). http://www.arb.ca.gov/bluebook/bb11/hea/hea-57004.htm (accessed February 23, 2015).

6. Ginn, T.R. et al. *California Biodiesel Multimedia Evaluation Final Tier III Report*, June 2014. http://www.arb.ca.gov/fuels/multimedia/meetings/Biodiesel_FinalReport_Jun2014.pdf (accessed February 24, 2015).

7. Same as Footnote 6.

8. Same as Footnote 6.

9. Same as Footnote 1.

10. Ginn, T.R. et al. *California Biodiesel Multimedia Evaluation Final Tier I Report*, September 2009. http://www.arb.ca.gov/fuels/multimedia/meetings/Biodiesel_FinalTierIReport_Sep2009_110413.pdf (accessed November 4, 2013).

11. Ginn, T.R. et al. *California Biodiesel Multimedia Evaluation Final Tier II Report*, January 2012. http://www.arb.ca.gov/fuels/multimedia/meetings/Biodiesel_FinalTierII_Jan2012_110413.pdf (accessed November 4, 2013).

12. Same as Footnote 6.

13. Durbin, T.D. et al. *CARB Assessment of the Emissions from the Use of Biodiesel as a Motor Vehicle Fuel in California "Biodiesel Characterization and NOx Mitigation Study."* October 2011. http://www.arb.ca.gov/fuels/diesel/altdiesel/20111013_CARB%20Final%20Biodiesel%20Report.pdf (accessed February 23, 2015).

14. Karavalakis, G. et al. *CARB Comprehensive B5/B10 Blends Heavy-Duty Engine Dynamometer Testing.* June 2014. http://www.arb.ca.gov/fuels/diesel/altdiesel/20140630CARBStudyB5_B10.pdf (accessed February 23, 2015).

15. Same as Footnote 13.

16. Same as Footnote 13.

17. U.S. Environmental Protection Agency, *A Comprehensive Analysis of Biodiesel Impacts on Exhaust Emissions*, Draft Technical Report. EPA420-P-02-001, October 2002. http://www.epa.gov/otaq/models/analysis/biodsl/p02001.pdf (accessed February 23, 2015).

18. Air Resources Board. *Staff Report: Initial Statement of Reasons for Proposed Rulemaking, Public Hearing to Consider Adoption of Regulations to Control Greenhouse Gas Emissions from Motor Vehicles*. August 6, 2004. http://www.arb.ca.gov/regact/grnhsgas/isor.pdf (accessed February 23, 2015).

19. Air Resources Board. *Proposed Re-Adoption of the Low Carbon Fuel Standard. Staff Report: Initial Statement of Reasons*. December 2014. http://www.arb.ca.gov/regact/2015/lcfs2015/lcfs15isor.pdf (accessed February 23, 2015).

20. Same as Footnote 19.

21. Air Resources Board. *Proposed Regulation to Implement the Low Carbon Fuel Standard. Staff Report: Initial Statement of Reasons. Volume I.* March 2009. http://www.arb.ca.gov/regact/2009/lcfs09/lcfsisor1.pdf (accessed February 23, 2015).

22. Air Resources Board, LCFS Carbon Intensity Lookup Table, December 2012. http://www.arb.ca.gov/fuels/lcfs/lu_tables_11282012.pdf (accessed February 24, 2015).

23. Same as Footnote 18.

24. Lammert et al. National Renewable Energy Laboratory. *Effect of B20 and Low Aromatic Diesel on transit Bus NOx emissions Over Driving Cycles with a Range of Kinetic Intensity*, SAE Int. 2012-01-1984. Published September 2012. http://www.nrel.gov/docs/fy13osti/55672.pdf (accessed February 23, 2015).

25. Same as Footnote 1.

26. Same as Footnote 1.

# APPENDIX A

## Proposed Regulation on the Commercialization of Alternative Diesel Fuels

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# APPENDIX A. PROPOSED REGULATION

## REGULATION ON COMMERCIALIZATION OF ALTERNATIVE DIESEL FUELS

Amend sections 2290, 2291, and 2293; renumber sections 2293 and 2293.5; adopt new sections 2293, 2293.1, 2293.2, 2293.3, 2293.4, 2293.5, 2293.6, 2293.7, 2293.8, 2293.9, and Appendix 1; and create new subarticles 1, 2, and 3, in title 13, chapter 5, article 3, California Code of Regulations, to read as follows:

[Note: The entire text of sections 2293, 2293.1, 2293.2, 2293.3, 2293.4, 2293.5, 2293.6, 2293.7, 2293.8, 2293.9, and Appendix 1 is new language. Existing sections 2290, 2291, 2292.1, 2292.2, 2292.3, 2292.4, 2292.5, 2292.6, and 2292.7 would be grouped as indicated under new subarticle 1 (Specifications for Current Alternative Motor Vehicle Fuels) and sections 2290 and 2291 would be revised as indicated. Existing sections 2293 and 2293.5 would be revised as indicated, renumbered to 2294 and 2295, and grouped as indicated under new subarticle 3 (Ancillary Provisions). The proposed amendments to existing text are shown in underline to indicate addition and ~~strikeout~~ to show deletions. All other portions of the article remain unchanged and are indicated by the symbol ******.]

## Chapter 5. Standards for Motor Vehicle Fuels

## Article 3. Specifications for Alternative Motor Vehicle Fuels

## Subarticle 1. Specifications for Alternative Motor Vehicle Fuels

**§2290. Definitions.**

(a) For the purposes of this ~~article~~subarticle, the following definitions apply:

(1) "Alternative fuel" means any fuel which is commonly or commercially known or sold as one of the following: M-100 fuel methanol, M-85 fuel methanol, E-100 fuel ethanol, E-85 fuel ethanol, compressed natural gas, liquefied petroleum gas, or hydrogen.
(2) "ASTM" means the American Society for Testing Materials.
(3) "Motor vehicle" has the same meaning as defined in section 415 of the Vehicle Code.
(4) "Supply" means to provide or transfer a product to a physically separate facility, vehicle, or transportation system.

Note: Authority cited: Sections 39600, 39601, 43013, 43018, and 43101, Health and Safety Code; and *Western Oil and Gas Ass'n. v. Orange County Air Pollution Control District*, 14 Cal. 3d 411, 121 Cal.Rptr. 249 (1975). Reference: Sections 39000, 39001, 39002, 39003, 39010, 39500, 40000, 43000, 43016, 43018 and 43101, Health and Safety Code: and *Western Oil and Gas Ass'n. v. Orange County Air Pollution Control District*, 14 Cal. 3d 411, 121 Cal.Rptr. 249 (1975).

## §2291. Basic Prohibitions.

(a) Starting January 1, 1993, no person shall sell, offer for sale or supply an alternative fuel intended for use in motor vehicles in California unless it conforms with the applicable specifications set forth in this ~~article 3~~subarticle.

(b) An alternative fuel shall be deemed to be intended for use in motor vehicles in California if it is:

(1) stored at a facility which is equipped and used to dispense that type of alternative fuel to motor vehicles, or

(2) delivered or intended for delivery to a facility which is equipped and used to dispense that type of alternative fuel to motor vehicles, or

(3) sold, offered for sale or supplied to a person engaged in the distribution of motor vehicle fuels to motor vehicle fueling facilities, unless the person selling, offering or supplying the fuel demonstrates that he or she has taken reasonably prudent precautions to assure that the fuel will not be used as a motor vehicle fuel in California.

(c) For the purposes of this section, each retail sale of alternative fuel for use in a motor vehicle, and each supply of alternative fuel into a motor vehicle fuel tank, shall also be deemed a sale or supply by any person who previously sold or supplied such alternative fuel in violation of this section.

Note: Authority cited: Sections 39600, 39601, 43013, 43018, and 43101, Health and Safety Code; and *Western Oil and Gas Ass'n. v. Orange County Air Pollution Control District*, 14 Cal. 3d 411, 121 Cal.Rptr. 249 (1975). Reference: Sections 39000, 39001, 39002, 39003, 39010, 39500, 40000, 43000, 43016, 43018 and 43101, Health and Safety Code: and *Western Oil and Gas Ass'n. v. Orange County Air Pollution Control District*, 14 Cal. 3d 411, 121 Cal.Rptr. 249 (1975).

## §2292.1 Fuels Specifications for M100 Fuel Methanol.

* * * * *

Note: Authority cited: Sections 39600, 39601, 43013, 43018, and 43101, Health and Safety Code; and *Western Oil and Gas Ass'n. v. Orange County Air Pollution Control District*, 14 Cal. 3d 411, 121 Cal.Rptr. 249 (1975). Reference: Sections 39000, 39001, 39002, 39003, 39010, 39500, 40000, 43000, 43016, 43018 and 43101, Health and Safety Code: and *Western Oil and Gas Ass'n. v. Orange County Air Pollution Control District*, 14 Cal. 3d 411, 121 Cal.Rptr. 249 (1975).

## §2292.2 Specifications for M-85 Fuel Methanol.

* * * * *

Note: Authority cited: Sections 39600, 39601, 43013, 43018, and 43101, Health and Safety Code; and *Western Oil and Gas Ass'n. v. Orange County Air Pollution Control District*, 14 Cal. 3d 411, 121 Cal.Rptr. 249 (1975). Reference: Sections 39000, 39001, 39002, 39003, 39010, 39500, 40000, 43000, 43016, 43018 and 43101, Health and Safety Code: and *Western Oil and Gas Ass'n. v. Orange County Air Pollution Control District*, 14 Cal. 3d 411, 121 Cal.Rptr. 249 (1975).

## §2292.3 Specifications for E-100 Fuel Ethanol.

* * * * *

Note: Authority cited: Sections 39600, 39601, 43013, 43018, and 43101, Health and Safety Code; and *Western Oil and Gas Ass'n. v. Orange County Air Pollution Control District*, 14 Cal. 3d 411, 121 Cal.Rptr. 249 (1975). Reference: Sections 39000, 39001, 39002, 39003, 39010, 39500, 40000, 43000, 43016, 43018 and 43101, Health and Safety Code: and *Western Oil and Gas Ass'n. v. Orange County Air Pollution Control District*, 14 Cal. 3d 411, 121 Cal.Rptr. 249 (1975).

## §2292.4 Specifications for E-85 Fuel Ethanol.

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Note: Authority cited: Sections 39600, 39601, 43013, 43018, and 43101, Health and Safety Code; and *Western Oil and Gas Ass'n. v. Orange County Air Pollution Control District*, 14 Cal. 3d 411, 121 Cal.Rptr. 249 (1975). Reference: Sections 39000, 39001, 39002, 39003, 39010, 39500, 40000, 43000, 43016, 43018 and 43101, Health and Safety Code: and *Western Oil and Gas Ass'n. v. Orange County Air Pollution Control District*, 14 Cal. 3d 411, 121 Cal.Rptr. 249 (1975).

## §2292.5 Specifications for Compressed Natural Gas.

* * * * *

Note: Authority cited: Sections 39600, 39601, 43013, 43018, and 43101, Health and Safety Code; and *Western Oil and Gas Ass'n. v. Orange County Air Pollution Control District*, 14 Cal. 3d 411, 121 Cal.Rptr. 249 (1975). Reference: Sections 39000, 39001, 39002, 39003, 39010, 39500, 40000, 43000, 43016, 43018 and 43101, Health and Safety Code: and *Western Oil and Gas Ass'n. v. Orange County Air Pollution Control District*, 14 Cal. 3d 411, 121 Cal.Rptr. 249 (1975).

## §2292.6 Specifications for Liquefied Petroleum Gas.

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Note: Authority cited: Sections 39600, 39601, 43013, 43018, and 43101, Health and Safety Code; and *Western Oil and Gas Ass'n. v. Orange County Air Pollution Control District*, 14 Cal. 3d 411, 121 Cal.Rptr. 249 (1975). Reference: Sections 39000, 39001, 39002, 39003, 39010, 39500, 40000, 43000, 43016, 43018 and 43101, Health and Safety Code: and *Western Oil and Gas Ass'n. v. Orange County Air Pollution Control District*, 14 Cal. 3d 411, 121 Cal.Rptr. 249 (1975).

## §2292.7 Specifications for Hydrogen.

* * * * *

Note: Authority cited: Sections 39600, 39601, 43013, 43018, and 43101, Health and Safety Code; and *Western Oil and Gas Ass'n. v. Orange County Air Pollution Control District*, 14 Cal. 3d 411, 121 Cal.Rptr. 249 (1975). Reference: Sections 39000, 39001, 39002, 39003, 39010, 39500, 40000, 43000, 43016, 43018 and 43101, Health and Safety Code: and *Western Oil and Gas Ass'n. v. Orange County Air Pollution Control District*, 14 Cal. 3d 411, 121 Cal.Rptr. 249 (1975).

# Subarticle 2. Commercialization of Alternative Diesel Fuels

## §2293. Purpose.

The purpose of this regulation is to establish a comprehensive, multi-stage process governing the commercialization of alternative diesel fuels (ADF) in California, ranging from the initial limited sales of an ADF while it undergoes a screening evaluation; through expanded sales governed by enhanced monitoring, testing, and multimedia evaluations; and ending with full-scale commercial sales as warranted. This regulation is intended to foster the introduction and use of innovative ADFs in California while preserving or enhancing public health, the environment and the emissions benefits of the existing motor vehicle diesel fuel regulations.

Note: Authority cited: Sections 39600, 39601, 39667, 43013, 43018, and 43101, Health and Safety Code; and *Western Oil and Gas Ass'n. v. Orange County Air Pollution Control District*, 14 Cal. 3d 411, 121 Cal.Rptr. 249 (1975). Reference: Sections 39000, 39001, 39002, 39003, 39010, 39500, 40000, 43000, 43016, 43018 and 43101, 43865, Health and Safety Code: and *Western Oil and Gas Ass'n. v. Orange County Air Pollution Control District*, 14 Cal. 3d 411, 121 Cal.Rptr. 249 (1975).

## §2293.1. Basic Prohibitions.

(a) Starting January 1, 2016, no person shall sell, offer for sale or supply an ADF for use in California unless that person is in compliance with this subarticle and with the terms of any approved and current Executive Order issued under section 2293.5 that is applicable to the person or the ADF.

(b) For the purposes of this subarticle, each retail sale of ADF for use in a motor vehicle and each supply of ADF into a motor vehicle fuel tank constitutes a separate sale or supply by each and every person who previously sold or supplied such ADF in violation of this subarticle.

Note: Authority cited: Sections 39600, 39601, 39667, 43013, 43018, and 43101, Health and Safety Code; and *Western Oil and Gas Ass'n. v. Orange County Air Pollution Control District*, 14 Cal. 3d 411, 121 Cal.Rptr. 249 (1975). Reference: Sections 39000, 39001, 39002, 39003, 39010, 39500, 39515, 40000, 43000, 43016, 43018 and 43101, 43865, Health and Safety Code: and *Western Oil and Gas Ass'n. v. Orange County Air Pollution Control District*, 14 Cal. 3d 411, 121 Cal.Rptr. 249 (1975).

## § 2293.2. Definitions.

(a) For the purposes of this subarticle, the definitions in Health and Safety Code sections 39010 through 39060 shall apply, except as otherwise specified in this subarticle. The following definitions shall also apply to this subarticle:

(1) "Alternative diesel fuel" or "ADF" means any fuel used in a compression ignition engine that is not petroleum-based, does not consist solely of hydrocarbons, and is not subject to a specification under subarticle 1 of this article.

(2) "Biodiesel" means a fuel comprised of mono-alkyl esters of long chain fatty acids derived from vegetable oils or animal fats that is 99-100 percent biodiesel by volume (B100 or B99) and meets the specifications set forth by ASTM International in the latest version of *Standard Specification for Biodiesel Fuel Blend Stock (B100) for Middle Distillate Fuels* D6751 contained in the ASTM publication entitled: Annual Book of ASTM Standards, Section 5, as defined in California Code of Regulations, title 4, section 4140(a), which is hereby incorporated by reference.

(3) "Biodiesel Blend" means biodiesel blended with petroleum-based CARB diesel fuel or non-ester renewable diesel.

(4) "Blend Level" means the ratio of an ADF to the CARB diesel it is blended with, expressed as a percent by volume. The blend level may also be expressed as "AXX," where "A" represents the particular ADF and "XX" represents the percent by volume that ADF is present in the blend with CARB diesel (e.g., a 20 percent by volume biodiesel/CARB diesel blend is denoted as "B20").

(5) "Blendstock" means a component that is either used alone or is blended with another component(s) to produce a finished fuel used in a motor vehicle. A blendstock that is used directly as a transportation fuel in a vehicle is considered a finished fuel.

(6) "B5" means a biodiesel blend containing no more than five percent biodiesel by volume.

(7) "B20" means a biodiesel blend containing more than five and no more than 20 percent biodiesel by volume.

(8) "Candidate ADF" means a fuel that is in the Stage 1 or Stage 2 evaluation process in this subarticle.

(9) "CARB diesel" means a light or middle distillate fuel that may be comingled with up to five (5) volume percent biodiesel and meets the definition and requirements for "diesel fuel" or "California nonvehicular diesel fuel" as specified in California Code of Regulations, title 13, section 2281 et seq. "CARB diesel" may include: non-ester renewable diesel; gas-to-liquid fuels; Fischer-Tropsch diesel; diesel fuel produced from renewable crude; CARB diesel blended with additives specifically formulated to reduce emissions of one or more criteria or toxic air contaminants relative to reference CARB diesel; and CARB diesel specifically formulated to reduce emissions of one or more criteria or toxic air contaminants relative to reference CARB diesel.

(10) "Criteria Pollutant" means any air pollutant for which a California ambient air quality standard (CAAQS) or a national ambient air quality standard (NAAQS) has been established.

(11) "Diesel Substitute" means any liquid fuel that is intended for use as a neat fuel, with CARB diesel or CARB diesel blends in a compression ignition engine. "Diesel substitute" includes, but is not limited to, non-ester renewable diesel; gas-to-liquid fuels; Fischer-Tropsch fuels; CARB diesel blended with additives specifically formulated to reduce emissions of one or more criteria or toxic air contaminants relative to reference CARB diesel; and CARB diesel specifically formulated to reduce emissions of one or more criteria or toxic air contaminants relative to reference CARB diesel.

(12) "Executive Officer" means the Executive Officer of the Air Resources Board, or his or her designee.

(13) "Executive Order" or "EO" means a document signed by the Executive Officer or his or her designee under this subarticle that: a) provides an exemption from in-use requirements, b) approves a formulation under the certification procedures as an equivalent CARB diesel formulation, or c) specifies the stage at which a regulated party(ies) for an ADF or candidate ADF is or will be operating under. An Executive Order includes any enforceable terms, conditions, and requirements that the regulated party(ies) must meet in order to sell, offer for sale, or supply that ADF or candidate ADF for use in California.

(14) "Finished Fuel" means a fuel that is used directly in a vehicle for transportation purposes without requiring additional chemical or physical processing.

(15) "Hydrocarbon" means any chemical or mixture that is composed solely of hydrogen and carbon.

(16) "Importer" has the same meaning as defined in the Low Carbon Fuel Standard regulation at California Code of Regulations, title 17, section 95481(a).

(17) "Multimedia Evaluation" has the same meaning as defined in Health and Safety Code section 43830.8(b).

(18) "Multimedia Evaluation Guidance Document" means the procedure described in chapter 5, 6 and 7, governing the Executive Officer's multimedia evaluation conducted prior to establishing a motor vehicle fuel specification. The multimedia evaluation guidance document chapters 5, 6, and 7 ("Guidance Document and Recommendations on the Types of Scientific Information Submitted by Applicants for California Fuels Environmental Multimedia Evaluations") are available at www.arb.ca.gov/fuels/multimedia/guidancedoc.pdf, June 2008, and are incorporated herein by reference.

(19) "New Technology Diesel Engine" or "NTDE" means a diesel engine that meets at least one of the following criteria:

(A) Meets 2010 ARB emission standards for on-road heavy duty diesel engines under section 1956.8.

(B) Meets Tier 4 emission standards for non-road compression ignition engines under sections 2421, 2423, 2424, 2425, 2425.1, 2426, and 2427.

(C) Is equipped with or employs a Diesel Emissions Control Strategy (DECS), verified by ARB pursuant to section 2700 et seq., which uses selective catalytic reduction to control Oxides of Nitrogen (NOx).

(20) "Non-ester renewable diesel" means a diesel fuel that is produced from nonpetroleum renewable resources but is not a mono-alkyl ester and which is registered as a motor vehicle fuel or fuel additive under 40 Code of Federal Regulations part 79.

(21) "Offsetting factors" means any factors in the commercial market that serve to offset the emissions of a pollutant from the use of an ADF. Offsetting factors may include, but are not limited to, the use of:

(A) Specific vehicle technologies such as NTDEs that have been proven to reduce emissions of the pollutant;

(B) Diesel substitutes that reduce emissions of the pollutant; and

(C) Feedstocks that have been shown to reduce or eliminate increases in the pollutant.

(22) "Person" has the same meaning as defined in Health and Safety Code section 39047 and includes, but is not limited to, ADF producers, importers, marketers and blenders. "Person" includes the plural when two or more persons are subject to an Executive Order executed or an interim or final fuel specification issued pursuant to the requirements of this subarticle.

(23) "Pollutant Control Level" means a blend level of an ADF above which per gallon in-use requirements have been established by regulation to ensure there will be no increases in one or more criteria pollutants when compared to emissions from Reference CARB Diesel.

(24) "Potential Adverse Emissions Impacts" means for any given ADF or ADF blend, any criteria pollutant for which testing during a multimedia evaluation results in statistically significant increases of that criteria pollutant above an appropriate baseline for that ADF.

(25) "Producer" has the same meaning as defined in the Low Carbon Fuel Standard regulation at California Code of Regulations, title 17, section 94581(a).

(26) "Reference CARB Diesel" has the same meaning as "reference fuel" as that term is defined in section 2282(g)(3).

(27) "Toxic Air Contaminant" means any substance identified or designated by the Air Resources Board as a toxic air contaminant pursuant to Health and Safety Code section 39657, or is designated as a hazardous air pollutant under section 112 of the federal Clean Air Act (42 U.S.C. § 7412).

(28) "Trade Secret" has the same meaning as defined in Government Code section 6254.7.

(b) List of Acronyms and Abbreviations

| | |
|---|---|
| AAQS | Ambient Air Quality Standards |
| ADF | Alternative Diesel Fuel or Fuels |
| API | American Petroleum Institute |
| ARB or Board | California Air Resources Board |
| ASTM | ASTM International formerly known as American Society for Testing and Materials |
| CCR | California Code of Regulations |

| | |
|---|---|
| CI | Carbon Intensity |
| EO | Executive Order |
| EmFAC | ARB's Emission (Em) Factors (FAC) Model |
| FAME | Fatty Acid Methyl Esters |
| GVWR | Gross Vehicle Weight Rating |
| H&SC | California Health and Safety Code |
| LRT | Low Carbon Fuel Standard Reporting Tool |
| MMWG | Multimedia Working Group |
| NOx | Oxides of Nitrogen |
| NTDE | New technology diesel engines |
| PM | Particulate Matter |
| ppm | Parts per Million |
| U.S. EPA | U.S. Environmental Protection Agency |

Note: Authority cited: Sections 39600, 39601, 39667, 43013, 43018, and 43101, Health and Safety Code; and *Western Oil and Gas Ass'n. v. Orange County Air Pollution Control District*, 14 Cal. 3d 411, 121 Cal.Rptr. 249 (1975). Reference: Sections 39000, 39001, 39002, 39003, 39010, 39500, 39515, 40000, 43000, 43016, 43018 and 43101, 43830.8, 43865, Health and Safety Code: and *Western Oil and Gas Ass'n. v. Orange County Air Pollution Control District*, 14 Cal. 3d 411, 121 Cal.Rptr. 249 (1975).

## §2293.3. Exemptions.

This subarticle does not apply to any of the following, as specified:

(a) Fuels that have a specification under subarticle 1 of this article (commencing with section 2292);

(b) CARB diesel blends comprised solely of CARB diesel and one or more diesel additives comprising in the aggregate no more than 1.0 percent by volume of the CARB diesel blend. This exemption does not apply to additives used pursuant to the in use requirements specified in Appendix 1;

Note: Authority cited: Sections 39600, 39601, 39667, 43013, 43018, and 43101, Health and Safety Code; and *Western Oil and Gas Ass'n. v. Orange County Air Pollution Control District*, 14 Cal. 3d 411, 121 Cal.Rptr. 249 (1975). Reference: Sections 39000, 39001, 39002, 39003, 39010, 39500, 40000, 43000, 43016, 43018 and 43101, 43865, Health and Safety Code: and *Western Oil and Gas Ass'n. v. Orange County Air Pollution Control District*, 14 Cal. 3d 411, 121 Cal.Rptr. 249 (1975).

## §2293.4. General Requirements Applicable to All ADFs.

Starting January 1, 2016, any person who sells, offers for sale or supplies an ADF for use in motor vehicles in California must first meet the requirements in this subarticle and must also:

(a) Have the ADF registered with U.S. EPA under 40 Code of Federal Regulations part 79.

(b) Meet all applicable regulatory requirements of the California Department of Food and Agriculture (including, but not limited to, those in Cal. Code Regs., tit. 4, §§ 4140—4148, 4200, and 4202—4205).

(c) Meet all other applicable local, State, and federal requirements.

Note: Authority cited: Sections 39600, 39601, 39667, 43013, 43018, and 43101, Health and Safety Code; and *Western Oil and Gas Ass'n. v. Orange County Air Pollution Control District*, 14 Cal. 3d 411, 121 Cal.Rptr. 249 (1975). Reference: Sections 39000, 39001, 39002, 39003, 39010, 39500, 40000, 43000, 43016, 43018 and 43101, 43865, Health and Safety Code: and *Western Oil and Gas Ass'n. v. Orange County Air Pollution Control District*, 14 Cal. 3d 411, 121 Cal.Rptr. 249 (1975).

## §2293.5. Phase-In Requirements.

[Note: The goal of this comprehensive process is to foster the introduction of new, lower polluting ADF fuels by allowing the limited sales of innovative ADFs in stages while emissions, performance, and environmental impacts testing is conducted. This testing is intended to develop the necessary real-world information to quantify the environmental and human health benefits from using new ADFs, determine whether these fuels have adverse environmental impacts relative to conventional CARB diesel, and identify any vehicle/engine performance issues such fuels may have.]

An ADF that has not been approved for commercialized sales under subsection (c) for Stage 3A fuels or subsection (d) for Stage 3B fuels may only be sold, offered for sale, or supplied for use in motor vehicles in California pursuant to an approved Executive Order (EO) for candidate ADF issued under subsection (a) for a Stage 1 pilot program or under subsection (b) for a Stage 2 ADF.

(a) Stage 1: Pilot Program.

[Note: The purpose of this stage is to allow limited, small fleet use of innovative fuels while requiring screening tests and assessments to quickly determine whether there will be unreasonable potential impacts on air quality, the environment and vehicular performance. Such data will help inform more extensive testing and analysis to be conducted in Stage 2. This Stage 1 is modeled after the existing ARB regulation that provides limited, fuel test program exemptions under 13 CCR 2259.]

(1) Stage 1 Application.

A person seeking a Stage 1 Executive Order (EO) for an ADF must submit an application to the Executive Officer that includes all the following information:

(A) Expected program duration, not to exceed one year except as provided in section 2293.5(a)(4)(B) below;

(B) An estimate of the maximum number of vehicles or engines involved in the program;

(C) The mileage duration per vehicle involved in this stage;

(D) The quantity of fuel expected to be used in the pilot program, not to exceed the energy equivalent of one million gallons of diesel fuel per year, per ADF total;

(E) The site(s) in which the testing during this stage will be conducted (including the street address, city, county, and zip code);

(F) The manner in which the distribution pumps will be labeled to ensure proper use of the test fuel;

(G) The name, address, telephone number, title of the person(s) and the name of the company or organization requesting entry into a Stage 1 pilot program; and

(H) If different from the information in (G) above, the name, address, telephone number and title of the person(s) and the name of the company or organization responsible for recording and making the information specified above available to the Executive Officer and the location in which such information will be maintained.

(I) Chemical and physical properties of the candidate ADF: complete chemical speciation, Chemical Abstract Services (CAS) numbers (if available), density, energy content, vapor pressure, oxidative potential, distillation curve, log $K_{ow}$ (water-octanol partition coefficient), and Henry's law coefficient.

(J) Environmental information about the ADF: Material Safety Data Sheet(s) (MSDS) for all components of the candidate ADF, production process diagram, identification of potential human health effects, lifecycle flow diagram (including all stages of the process-raw material extraction, manufacturing, distribution, use and disposal including all intervening transportation steps), and potential release scenarios during production (including by-products), transportation and use.

(K) Identify whether the fuel is intended to be blended with diesel, whether it can be used as a neat fuel, or whether it can be used either way.

(L) Plan for commercialization under this regulation.

(M) Emissions testing completed on criteria pollutants.

(N) Attestation that the vehicles to be used in the pilot program are owned by the applicant or the applicant has received written consent from their owners.

(O) The vehicle identification number (VIN) of each vehicle participating in the pilot program.

(P) Affirmative statement that the owner(s) of all vehicles to be used in the applicant's pilot program are aware of any possible warranty issues that may arise from the use of the candidate ADF or candidate ADF/CARB diesel blend in their engines.

(Q) A declaration by the applicant that, either:

1. there is an existing fuel standard for the ADF as required by Business and Professions Code Chapter 14, sections 13400 to 13460; or if no such standard exist,

2. a copy of the developmental fuel variance the applicant has submitted to the California Department of Food and Agriculture pursuant to Business and Professions Code section 13405 and proof of its approval; and,

a. the requirements of Business and Profession Code Section 12001– 13800 other than fuel quality have been met; and,

b. the California Department of Food and Agriculture received a copy of the application required to be submitted under 13 CCR §2293.5.

(R) Proof that the candidate complies with the U.S. Environmental Protection Agency under 40 CFR 79.

It is the responsibility of the applicant to identify any specific portion of the information submitted above as trade secret. Any such trade secret information identified by the applicant shall be treated pursuant to 17 CCR 91000—91022 and the California Public Records Act (Government Code sec. 6250 et seq.).

(2) Stage 1 Application Completeness Determination.

(A) After receiving a pilot program application, the Executive Officer shall advise the applicant in writing within 30 business days either that the application is provisionally complete or that specified additional information is required to make it provisionally complete.

(B) After receiving the additional information required under (A), the Executive Officer shall advise the applicant in writing within 15 business days either that the application is now provisionally complete or that specified additional information is still required to make it complete.

(C) If additional information is required and not received within 60 days the application will be deemed incomplete.

(3) Public Comment and Final Action on a Stage 1 Application.

(A) After deeming an application provisionally complete, the Executive Officer shall post the application on ARB's internet web site for 15 business days for public comments. Only comments related to potential factual or methodological errors may be considered by the Executive Officer. Within 30 calendar days, the applicant shall either make revisions to its application and submit those revisions to the Executive Officer, or submit a detailed written response to the Executive Officer explaining why no revisions are necessary.

(B) Within 30 business days of receiving the applicant's response to the public comments under (A), the Executive Officer shall either approve or disapprove the pilot program. The Executive Officer shall notify the applicant of his/her decision in writing and provide, if the application is denied, the reasons for the denial.

(C) The Executive Officer shall disapprove a proposed pilot program if he/she determines the use of the candidate ADF, under the terms and conditions of the pilot program as proposed, poses an unacceptable risk to the community in which the pilot program is proposed to be conducted, or its risks substantially outweigh the putative benefits of using the candidate ADF.

(D) No approval of a pilot program shall be effective without an approved Executive Order (EO) executed between the Executive Officer and the applicant(s). The EO shall include terms and conditions that the applicant must meet in order to provide the candidate ADF fuel in California during the term of the EO. The terms and conditions shall be based on the information specified in (1)(A)--(R) above, as well as require the following:

1. any additional information the Executive Officer determines is necessary to fill in data gaps that may have been identified during the application process;

2. additional toxicity and other testing the Executive Officer determines is necessary and appropriate to better characterize any substance in the candidate ADF; and

3. evidence of substantial progress in working in good faith with the original equipment/engine manufacturers of the engines involved in the EO, consensus standards organizations (e.g., ASTM), regulatory agencies, and other interested parties toward developing a consensus set of fuel specifications for the candidate ADF.

4. The use of adequate controls to ensure appropriate fuel quality and performance in consideration of vehicle performance, impact on the environment and fuel production. Appropriate controls include but are not limited to the use of interim fuel specifications and consensus standards.

(4) Operation under a Stage 1 EO.

(A) For the duration of the EO, the applicant must meet all the terms and conditions specified therein;

(B) The Executive Officer may terminate or modify a EO, with 30 days written notice to the applicant(s), for failure of the applicant(s) to comply with any of the terms and conditions of the EO, failure to comply with any other applicable provision in this subarticle, or for good cause. Good cause includes, but is not limited to, a determination by the Executive Officer that the information submitted in the application was inaccurate or incomplete and that the use of the ADF, under the terms and conditions of the approved pilot program, may pose an unacceptable risk to the community in which the pilot program is being conducted, or its risks substantially outweigh the putative benefits of using the candidate ADF;

(C) The Executive Officer shall not revoke or modify an approved Stage 1 EO without first affording the applicant an opportunity for a hearing in accordance with 17 CCR 60040 et seq., but the Executive Officer may temporarily suspend an EO without a hearing and prior to revocation or modification if the Executive Officer determines that continued operations under the EO may adversely affect human health;

(D) In the event an applicant cannot complete an approved pilot program within the allotted time, the applicant(s) may request a six month extension, renewable up to three times; and

(E) Upon successful completion of the pilot program, the applicant(s) may submit an application for a Stage 2 EO, as specified in section 2293.5(b) below.

(b) Stage 2: Development of Fuel Specification.

[Note: The purpose of this stage is to allow limited but expanded fleet use of an ADF that has successfully undergone the Stage 1 pilot program. Stage 2 candidate ADFs undergo additional emissions and performance testing to better characterize potential impacts on air quality, the environment and vehicular performance. This testing and assessment will be conducted pursuant to a formal multimedia evaluation leading to the development of a fuel specification, as appropriate. Further, the multimedia evaluation will be the basis for determining whether the candidate ADF has potential adverse emissions impacts. The determination of potential adverse emissions impacts determines whether the candidate ADF can proceed to Stage 3A or Stage 3B.]

A person who has successfully completed a Phase 1 pilot program for a candidate ADF under subsection (a) may apply for a Stage 2 EO for that candidate ADF.

(1) Stage 2 Application.

An applicant for Stage 2 must submit an application to the Executive Officer that includes all the following information:

(A) Planned duration for this stage, not to exceed one year, renewable up to four times or as otherwise provided in section 2293.5(b)(4);

(B) An estimate of the maximum number of vehicles or engines involved in this stage along with a description of the emissions control technology;

(C) The mileage duration per vehicle involved in this stage;

(D) The quantity of the candidate ADF fuel expected to be used in this stage, not to exceed the energy equivalent of 30 million gallons of diesel fuel per year;

(E) The site(s) in which the testing during this stage will be conducted (including the street address, city, county, and zip code);

(F) Any changes or updates to the information submitted under 2293.5(a)(1)(F)—(S) to reflect the expanded scope of vehicles, locations, fuel volume, timeframe, and other aspects of operation under Stage 2. For each of these items, the applicant must specify whether there has been no change or update, or if there has been a change or update, what that change or update is; and

(G) Identification of the test lab and principal investigator, including his/her curriculum vitae, who will be conducting the multimedia evaluation for the candidate ADF.

It is the responsibility of the applicant to identify any specific portion of the information submitted above as trade secret. Any such trade secret information identified by the applicant shall be treated pursuant to 17 CCR 91000—91022 and the California Public Records Act (Government Code sec. 6250 et seq.).

(2) Stage 2 Application Completeness Determination

(A) After receiving a Stage 2 application, the Executive Officer shall advise the applicant in writing within 30 business days either that the application is provisionally complete or that specified additional information is required to make it provisionally complete;

(B) After receiving the additional information required under (A), the Executive Officer shall advise the applicant in writing within 15 business days either that the application is now provisionally complete or that specified additional information is still required to make it provisionally complete.

(3) Public Comment and Final Action on a Stage 2 Application

(A) After deeming an application provisionally complete, the Executive Officer shall post the application on ARB's internet web site for 30 calendar days for public comments. Only comments related to potential factual or methodological errors or information regarding vehicle performance may be considered by the Executive Officer. Within 30 days, the applicant shall either make revisions to its application and submit those revisions to the Executive Officer, or submit a detailed written response to the Executive Officer explaining why no revisions are necessary;

(B) Within 30 business days of receiving the applicant's response to the public comments under (A), the Executive Officer shall either approve or disapprove the Stage 2 application. The Executive Officer shall notify the applicant of his/her decision in writing and provide, if the application is denied, the reasons for the denial;

(C) The Executive Officer shall disapprove a proposed pilot program if he/she determines the use of the ADF, under the terms and conditions of the Stage 2 program as proposed, poses an unacceptable risk to the community(ies) in which the program is proposed to be conducted, or its risks substantially outweigh the putative benefits of using the ADF;

(D) No approval of a Stage 2 program shall be effective without an approved Executive Order (EO) executed between the Executive Officer and the applicant(s). The EO shall include terms and conditions that the applicant must meet in order to provide the ADF fuel in California during the term of the EO. The terms and conditions shall be based on the information specified in (1)(A)-(G) above, as well as require the following:

1. any additional information requested in writing by the Executive Officer to fill in data gaps that may have been identified during the application process;

2. additional toxicity and other testing the Executive Officer determines is necessary and appropriate to better characterize any substance in the ADF;

3. substantial progress in working in good faith with the original equipment/engine manufacturers of the engines involved in the EO, consensus standards organizations (e.g., ASTM), regulatory agencies, and other interested parties toward developing a consensus set of fuel specifications for the ADF. These efforts must culminate in adoption of consensus standards by the end of the Stage 2 EO.

(4) Operation under a Stage 2 EO

(A) For the duration of the EO, the applicant must meet all the terms and conditions specified therein;

(B) The Executive Officer may terminate or modify a EO, with 30 days written notice to the applicant(s), for failure of the applicant(s) to comply with any of the terms and conditions of the EO, failure to comply with any other applicable provision in this subarticle, or for good cause. Good cause includes, but is not limited to, a determination by the Executive Officer that the information submitted in the application was inaccurate or incomplete and that the use of the ADF, under the terms and conditions of the approved Stage 2 program, may pose an unacceptable risk to the community in which the Stage 2 program is being conducted, or its risks substantially outweigh the putative benefits of using the ADF;

(C) In the event an applicant cannot complete an approved Stage 2 program within the allotted time, the applicant(s) may request a 1 year extension, renewable up to four times. The Executive Officer may provide additional extensions due to delays in completion of a multimedia evaluation, adoption of the applicable consensus standards, or for other good cause;

(D) Upon successful completion of the Stage 2 program, the applicant(s) may sell, offer for sale, or supply an ADF intended for use in motor vehicles in California pursuant to either Stage 3A or 3B, whichever applies, as specified in section 2293.5(c) or (d) below.

(5) Multimedia Evaluation and Determination of Potential Adverse Emissions Impacts

(A) Pursuant to the approved Stage 2 EO, Health and Safety Code section 43830.8, and the Multimedia Evaluation Guidance Document, the applicant shall conduct the prescribed multimedia evaluation under direction from ARB staff;

(B) The multimedia evaluation shall identify and evaluate any significant adverse impact on public health or the environment, including air, water, or soil, that may result from the production, use, or disposal of the ADF, relative to an appropriate baseline identified by the multimedia working group, under Stage 2, 3A, and 3B;

(C) In addition to determining any significant impacts, the multimedia assessment shall also include an evaluation of potential strategies that may reduce or eliminate each of the significant impacts identified;

(D) Approval of a multimedia evaluation shall be subject to the provisions of Health and Safety Code section 43830.8;

If the findings from the multimedia evaluation indicates a statistically significant increase in any criteria, toxic, or other air pollutant from the use of an ADF in a motor vehicle, compared to the appropriate baseline, the Executive Officer shall determine whether there is a level below which the use of a candidate ADF or a candidate ADF blend would avoid a detrimental impact on ambient pollutant.

(6) Completion of Stage 2

A person operating under a Stage 2 EO may qualify for commercial sales of the ADF under subsection (c) for Stage 3A or subsection (d) for Stage 3B if the Executive Office determines in writing that the person has successfully completed the requirements of Stage 2. To successfully complete Stage 2, the applicant must meet all the following requirements:

(A) Comply with all requirements specified in the approved Stage 2 EO;

(B) Adopt consensus standards applicable to the ADF;

(C) Obtain approval of at least 75 percent of compression ignition engine original equipment manufacturers for which the ADF is expected or intended to be used. Such approval must represent approval of the ADF blend levels expected or intended to be used in those engines;

(D) Identify appropriate fuel specifications or in-use requirements for the ADF identified as part of the multimedia evaluation conducted according to the provisions of this article;

(E) Obtain a written determination by the Executive Officer that all the above requirements have been met.

In the event the Executive Officer makes a determination of potential adverse emissions impacts under (5)(E), the Executive Officer shall post notice on the ARB website of his/her intent to initiate an evaluation to determine if the use of an ADF or ADF blends would lead to adverse emissions impacts considering the existence of offsetting factors, and if so develop and establish appropriate fuel specifications and/or in-use requirements to be added to section 2293.6 or 2293.7 as appropriate. Upon completion of that evaluation, all persons subject to Stage 2 for an ADF shall be subject to the provisions of Stage 3A.

(c) Stage 3A: Commercial Sales Subject to In-use requirements

In the event the Executive Officer has determined that a candidate ADF or candidate ADF blend has potential adverse emissions impacts, the Executive Officer shall direct ARB staff to conduct an evaluation to consider the effects of offsetting factors and the resultant impact that the use of the candidate ADF will have on criteria, toxic, or other air pollutants and resultant effect on air quality:

(1) If the Executive Officer determines that no adverse emissions impact will occur as a result of the use of a candidate ADF or candidate ADF blend, in consideration of offsetting factors, the candidate ADF shall then be subject to the provisions of Stage 3B of this regulation.

(2) If the Executive Officer finds that after considering the use of offsetting factors, the use of a candidate ADF or candidate ADF blend would result in adverse emissions impacts, then the Executive Officer shall determine conditions of ADF use including, but not limited to appropriate fuel specifications and/or in-use requirements to preclude adverse emission impacts. Conditions of use may consider, but are not limited to, the effect of ADF feedstocks, the region of ADF use, or any seasonal effects relative to emissions impacts on air quality mandates;

(3) If the Executive Officer finds appropriate fuel specifications and/or in-use requirements that would eliminate or reduce the adverse air quality impacts found in 2293.5(c)(1), then the Executive Officer will direct staff to initiate a rulemaking process to establish those standards under this subarticle.

(d) Stage 3B: Commercial Sales Not Subject to In-use Requirements

If the Executive Officer has determined that there are no potential adverse emissions impacts in accordance with 2293.5(b)(5)(E), or that there would be no adverse emissions impacts in accordance with 2293.5(c)(1) for an ADF or ADF blend, no additional conditions or sales restrictions are required under this article for that ADF or ADF blend. For an ADF that is subject to this provision, the fuel provider shall report to the Executive Officer the following information on a quarterly basis for any such ADF or ADF blend the fuel provider sold, offered for sale, or supplied for use in California:

(1) The volume of ADF blendstock, if applicable;

(2) the volume of ADF neat fuel, if applicable;

(3) the volume of ADF/CARB diesel blend, if applicable; and

(4) any other appropriate information deemed appropriate.

For purposes of this provision, the fuel provider may use information submitted to the ARB through the Low Carbon Fuel Standard Reporting Tool (LRT), as appropriate.

Note: Authority cited: Sections 39600, 39601, 39667, 43013, 43018, and 43101, Health and Safety Code; and *Western Oil and Gas Ass'n. v. Orange County Air Pollution Control District*, 14 Cal. 3d 411, 121 Cal.Rptr. 249 (1975). Reference: Sections 39000, 39001, 39002, 39003, 39010, 39500, 39515, 40000, 43000, 43016, 43018, 43026, 43101, 43830.8, and 43865, Health and Safety Code: and *Western Oil and Gas Ass'n. v. Orange County Air Pollution Control District*, 14 Cal. 3d 411, 121 Cal.Rptr. 249 (1975).

## §2293.6. In-use Requirements for Specific ADFs subject to Stage 3A.

ADFs which have been determined to have adverse emissions impacts after accounting for offsetting factors shall have a sub-section under this section listing appropriate in-use requirements including pollutant emissions control trigger levels.

(a) Biodiesel Provisions

This section includes specific provisions applicable to the use of biodiesel in the State

(1) Phase-in period for biodiesel

Starting January 1, 2016, any person who produces, imports, blends, sells, or offers for sale or supply any biodiesel, shall be subject to the reporting requirements of Stage 3A, pursuant to 2293.8(b).

Starting January 1, 2018 any person who produces, imports, blends, sells, or offers for sale or supply any biodiesel in California, shall be subject to pollutant control levels under sub section (a)(2) of this section.

(2) Pollutant Control Level

Table A.1 below shows fuel quality requirements for biodiesel blends depending on feedstock saturation and time of year. Biodiesel blends above the pollutant control level for NOx emissions are required to employ one of the in-use requirements for biodiesel listed in Appendix 1.

Table A.1. Pollutant Control Level for NOx

| Feedstock Saturation | Time of Year | NOx Control Level |
|---|---|---|
| Low Saturation | Apr 1 to Oct 31 | B5, 5 volume percent biodiesel |
| | Nov 1 to Mar 31 | B10, 10 volume percent biodiesel |
| High Saturation | Jan 1 to Dec 31 | B10, 10 volume percent biodiesel |

(3) Biodiesel saturation level:

Table A.2 below shows the requirements for determination of saturation level for biodiesel feedstocks. The following documents are hereby incorporated by reference:

(A) ASTM D613-14, "Standard Test Method for Cetane Number of Diesel Fuel Oil (2010)."

(B) ASTM D6890-13be1, "Standard Test Method for Determination of Ignition Delay and Derived Cetane Number (DCN) of Diesel Fuel Oils by Combustion in a Constant Volume Chamber (2013)."

Table A.2 Biodiesel Saturation Level

| Biodiesel Saturation Level | Unadditized Cetane Number | Test Method |
|---|---|---|
| Low Saturation | ≤56 | ASTM D613-14; or ASTM D6890-13be1 |
| High Saturation | ≥56 | ASTM D613-14; or ASTM D6890-13be1 |

(4) Sunset of Biodiesel Blend Fuel Quality for NOx Control

NOx Control requirements under 2293.6(a)(2) for biodiesel blends up to B20 will no longer be required *when the following conditions are met:*

(A) When the vehicle miles travelled (VMT) by NTDE heavy-duty vehicles in California reaches 90 percent of total VMTs by the California heavy-duty diesel vehicle fleet, the NOx Control requirements under 2293.6(a)(2) for biodiesel blends will no longer be required. The portion of VMTs in California represented by NTDEs shall be determined using the most current ARB mobile source emission inventory based on EmFAC.

(5) Exemption from In-Use Requirements

(A) Any person may request an in-use requirement exemption from section 2293.6(a)(2) by submitting an application to the Executive Officer containing all the information required under section 2293.6(a)(5)(C) and (D)

(B) For purposes of this subsection, "In-Use Requirement Exemption" means an exemption from fuel requirements described under the in-use requirements stipulated in section 2293.6(a)(2) up to B20 blends, for biodiesel use in fleets that do not result in increased NOx emissions relative to the same fleet operated with CARB diesel.

(C) Before an exemption can be granted, the following demonstrations must be made:

1. Fueling facility has a centralized, secure fueling area, or uses another secure method of fueling,

2. Subject vehicle fleet under exemption consist of at least 90 percent in aggregate of either: Light or Medium duty diesel vehicles (GVWR ≤14,500lbs), or Heavy duty diesel vehicles equipped with New Technology Diesel Engines (NTDEs). The aggregation of this provision shall be weighted according to each vehicle's rated maximum horsepower.

3. Subject fleet fueling facility has procedures or protocols in place to reasonably preclude mis-fueling from other vehicles which have not received an exemption in accordance with this subsection.

(D) In order for an exemption to be granted, the applicant must submit an application containing the following:

1. The name, title, address and telephone number of the person(s) requesting an exemption from whom further information may be requested; and

2. Type of exemption being sought, either NTDE exemption or Light/medium duty exemption; and

3. Type of facility being requested for exemption, either public retail refueling facility, private fueling facility; and

4. For public retail fueling facility, applicant must include information, data, surveys, or other proof, that demonstrates that the customer base being serviced under the exemption will consist in aggregate of 90 percent of Light or Medium duty diesel vehicles (GVWR ≤14,500lbs), in combination with Heavy duty diesel vehicles equipped with New Technology Diesel Engines (NTDE).

(E) Within 20 days upon receipt of an application for an application, the executive officer shall advise the applicant in writing either that the application is complete or that specified information is required to make it complete. Within 15 days of submittal of additional information, the executive officer shall advise the applicant in writing that the information submitted makes the application complete or that specified additional information is still required to make application complete. Within 20 days after an application has been deemed complete, the executive office shall grant or deny an application.

(F) An exemption shall be granted by the executive officer upon successful demonstration of subparagraph (5)(C). The exemption shall be granted in the form of an executive order which shall sunset in accordance with 2293.6(a)(4).

(6) In-Use Requirement Program Review

(A) On or before December 31, 2019, ARB staff will conduct a program review of biodiesel in-use requirements to determine the efficacy of in-use requirements under section 2293.6(a)(2). In conducting the program review, staff will consider the effects of offsetting factors, in addition to any other factors that may affect NOx emissions stemming from biodiesel use in motor vehicles.

Note: Authority cited: Sections 39600, 39601, 39667, 43013, 43018, and 43101, Health and Safety Code; and *Western Oil and Gas Ass'n. v. Orange County Air Pollution Control District*, 14 Cal. 3d 411, 121 Cal.Rptr. 249 (1975). Reference: Sections 39000, 39001, 39002, 39003, 39010, 39500, 39515, 40000, 43000, 43016, 43018, 43026, 43101, and 43865, Health and Safety Code: and *Western Oil and Gas Ass'n. v. Orange County Air Pollution Control District*, 14 Cal. 3d 411, 121 Cal.Rptr. 249 (1975).

## §2293.7. Specifications for Alternative Diesel Fuels

Unless more stringent specifications are required for any ADF that is sold, offered for sale, supplied for use in California, produced, or imported into California must meet the following specifications:

(a) Specifications for Biodiesel.

(1) Biodiesel Blendstock or Neat Fuel (B100).

(A) The following documents are hereby incorporated by reference:

1. ASTM D287-12b, "Standard Test Method for API Gravity of Crude Petroleum and Petroleum Products (Hydrometer Method) (2012)."

2. ASTM D5453-93, "Standard Test Method for Determination of Total Sulfur in Light Hydrocarbons, Spark Ignition Engine Fuel, Diesel Engine Fuel, and Engine Oil by Ultraviolet Fluorescence (1993)."

Table A.3. Fuel Specifications for B100

| **Property** | **Value** | **Test Method** |
|---|---|---|
| Unadditized Cetane Number | ≥47 | ASTM D613-14 or ASTM D6890-13be1 |
| API Gravity | ≥27 degrees API | ASTM D287-12b |
| Sulfur | ≤15 ppm | ASTM D5453-93 |

(2) *Biodiesel Blends.* The fuel specifications promulgated by the California Department of Food and Agriculture in 4 CCR sections 4140-4148, 4200, and 4202-4205 shall apply to any biodiesel blend.

Note: Authority cited: Sections 39600, 39601, 39667, 43013, 43018, and 43101, Health and Safety Code; and *Western Oil and Gas Ass'n. v. Orange County Air Pollution Control District*, 14 Cal. 3d 411, 121 Cal.Rptr. 249 (1975). Reference: Sections 39000, 39001, 39002, 39003, 39010, 39500, 40000, 43000, 43016, 43018, 43026, 43101, 43865, Health and Safety Code: and *Western Oil and Gas Ass'n. v. Orange County Air Pollution Control District*, 14 Cal. 3d 411, 121 Cal.Rptr. 249 (1975).

## §2293.8. Reporting and Recordkeeping.

(a) Sampling

(1) For reporting of fuel properties as required by the EO, an applicable sampling methodology set forth in 13 CCR section 2293.5 shall be used.

(b) Reporting

(1) For Stages 1 and 2

A person operating under a Stage 1 or Stage 2 EO must submit quarterly reports to the Executive Officer throughout the term of the EO. Each report shall include the following:

(A) The volume of ADF and ADF blend offered, supplied, or sold during each quarter;

(B) Results of a specified number of representative samples, for fuel properties by test methods specified in the EO;

(C) Progress made toward completing the terms of the EO;

(D) Any changes or updates to information submitted during the application process regarding the beneficial or adverse impacts of the ADF in California.

(2) For Stage 3A

Except as provided in this paragraph, a person operating within Stage 3A must submit quarterly reports to the Executive Officer. Each report shall include the following:

(A) The volume of ADF and ADF blend offered, supplied, or sold during each month;

(B) Results of a specified number of representative samples, for fuel properties by test methods specified in the EO;

(C) The volume of other applicable quantity of the in use requirements used during each month; and

(D) The blend rate of in use requirements used during each month, if applicable.

(3) For Stage 3B

A person operating within Stage 3B must submit quarterly reports to the Executive Officer, with each report specifying the volume of ADF sold, supplied, or offered for sale in California during each month. In addition, the monthly reports shall contain results of a specified number of representative samples, for fuel properties by test methods as otherwise specified in the EO under 2293.5(b)(4)(A).

(c) Recordkeeping

(1) The ADF producer shall maintain, for two years from the date of each sampling, records showing the sample date, product sampled, container or other vessel sampled, final blend volume, and the results of the fuel properties by the proscribed test methods.

(2) The ADF importer shall maintain, for two years from the date of each sampling, records showing the sample date, product sampled, container or other vessel sampled, final blend volume, and the results of the fuel properties by the proscribed test methods.

(3) Biodiesel Recordkeeping Requirements on or after January 1, 2016

(A) Producers shall maintain records regarding:
- Volume of total monthly B100 production supplied to California by facility,
- Volume of biodiesel produced for California by feedstock,
- Volume of biodiesel blends sold,
- Product transfer documentation for B100 including volume sold, CI pathway,
- Transaction invoices provided to downstream customers, including direct sales to fleets
- Volume of biodiesel or biodiesel blends sold under exemption from in-use requirements pursuant to 2293.6(5)

(B) Importers shall maintain records regarding:
- Total volume of B100 or biodiesel blends imported into California by source
- Volume of biodiesel produced for California by feedstock
- Product transfer documentation for B100 including volume sold, CI pathway,
- Transaction invoices provided to downstream customers, including direct sales to fleets

(C) Blenders shall maintain records pertaining to:
- Volume of biodiesel blends by blend level, including but not limited to B5, B10, B20, B100
- Volume of each biodiesel blend level recorded as either high saturation or low saturation; any mix of both high and low saturation will be recorded as low saturation.
-Volume of B5 blend level include any blend between B1 to B5.
- Product transfer documentation provided to downstream customers

(D) Distributors shall maintain records pertaining to:
- Product transfer documentation which indicates volume sold, CI pathway,

(E) Retailers
- Product transfer documentation which indicates volume sold, CI pathway
- Copy of any exemptions provided pursuant to subparagraph 2293.6(a)(5)

(4) Biodiesel Recordkeeping Requirements on or after January 1, 2018

(A) Producers shall also maintain records regarding:
- Volume of B100 that has been produced in accordance with in-use requirements in Appendix 1, including method of NOx control

(B) Importers shall maintain records regarding:
- Total volume of B100 or biodiesel blends imported into California by source including volumes sold that have been treated for NOx control per in-use requirements in Appendix 1 (if applicable) and method of NOx control

(C) Blenders shall maintain records
- Statements on invoices indicating NOx control for each transaction of B100 or biodiesel blend as described in Appendix 1

(D) Distributors
- Statements on invoices indicating that B100 or biodiesel blend contains NOx control and the type of NOx control, as described in Appendix 1

(E) Retailers
- Statements on invoices indicating that B100 or biodiesel blend contains NOx control and the type of NOx control, as described in Appendix 1

Note: Authority cited: Sections 39600, 39601, 39667, 43013, 43018, and 43101, Health and Safety Code; and *Western Oil and Gas Ass'n. v. Orange County Air Pollution Control District*, 14 Cal. 3d 411, 121 Cal.Rptr. 249 (1975). Reference: Sections 39000, 39001, 39002, 39003, 39010, 39500, 39515, 40000, 43000, 43016, 43018, 43026, 43101, and 43865, Health and Safety Code: and *Western Oil and Gas Ass'n. v. Orange County Air Pollution Control District*, 14 Cal. 3d 411, 121 Cal.Rptr. 249 (1975).

## §2293.9. Severability.

Each part of this subarticle shall be deemed severable, and in the event that any part of this subarticle is held to be invalid, the remainder of this subarticle shall continue in full force and effect.

Note: Authority cited: Sections 39600, 39601, 39667, 43013, 43018, and 43101, Health and Safety Code; and *Western Oil and Gas Ass'n. v. Orange County Air Pollution Control District*, 14 Cal. 3d 411, 121 Cal.Rptr. 249 (1975). Reference: Sections 39000, 39001, 39002, 39003, 39010, 39500, 40000, 43000, 43016, 43018, 43101, and 43865, Health and Safety Code: and *Western Oil and Gas Ass'n. v. Orange County Air Pollution Control District*, 14 Cal. 3d 411, 121 Cal.Rptr. 249 (1975).

## Subarticle 3. Ancillary Provisions

### §~~2293~~2294. Equivalent Test Methods.

~~(a)~~ Whenever ~~sections 2292.1 thru 2292.7 provide for~~ this article requires the use of a specified test method, another test method may be used following a determination by the Executive Officer that the other test method produces results equivalent to the results obtained with the specified method.

Note: Authority cited: Sections 39600, 39601, 39667, 43013, 43018, and 43101, Health and Safety Code; and *Western Oil and Gas Ass'n. v. Orange County Air Pollution Control District*, 14 Cal. 3d 411, 121 Cal.Rptr. 249 (1975). Reference: Sections 39000, 39001, 39002, 39003, 39010, 39500, 40000, 43000, 43016, 43018 and 43101, Health and Safety Code: and *Western Oil and Gas Ass'n. v. Orange County Air Pollution Control District*, 14 Cal. 3d 411, 121 Cal.Rptr. 249 (1975).

### §~~2293.5~~2295. Exemptions for Alternative Motor Vehicle Fuel Used in Test Programs.

The E~~e~~xecutive ~~o~~Officer shall consider and grant test program exemptions from the requirements of this Article in accordance with section 2259.

Note: Authority cited: Sections 39600, 39601, 39667, 43013, 43018, and 43101, Health and Safety Code; and *Western Oil and Gas Ass'n. v. Orange County Air Pollution Control District*, 14 Cal. 3d 411, 121 Cal.Rptr. 249 (1975). Reference: Sections 39000, 39001, 39002, 39003, 39010, 39500, 40000, 43000, 43016, 43018 and 43101, Health and Safety Code: and *Western Oil and Gas Ass'n. v. Orange County Air Pollution Control District*, 14 Cal. 3d 411, 121 Cal.Rptr. 249 (1975).

### *Appendix 1. In-use Requirements for Pollutant Emissions Control*

A person subject to the Stage 3A in use requirements (section 2293.5(c)) may meet the in-use requirements imposed above the Pollutant Control Trigger Level by implementing any of the following in-use requirements as applicable, either alone or in combination:

Additives approved for NOx emission control purposes, an ADF-CARB diesel blend certified as emissions equivalent to CARB diesel or better, a neat ADF finished fuel certified as emissions equivalent to CARB diesel or better, or other options certified by the Executive Officer for this purpose.

(a) Biodiesel:

(1) Approved Emissions Equivalent Additives:

The following list shows the additive and required amounts by saturation and blend level:

(A) Di-tert-butyl peroxide (DTBP): Biodiesel blends above the NOx emission control trigger level that contain DTBP by volume in the amounts specified in the table below meet the in-use requirements for biodiesel.

Table A.5: DTBP NOx Control Blend Level

| **Biodiesel Saturation Level** | **Biodiesel Blend Level** | **Required level of DTBP (volume percent of blend)** |
|---|---|---|
| Low Saturation | >B5 to <B10 | 0.5 minimum |
| | B10 to <B15 | 0.75 minimum |
| | B15 to B20 | 1.0 minimum |
| High Saturation | B10 to <B15 | 0.25 minimum |
| | B15 to B20 | 0.5 minimum |

(B) [Reserved]

(2) Certification of Alternative Diesel Fuels Resulting in Emissions Equivalence with Diesel

(A) The Executive Officer, upon application of any producer or importer, may certify alternative diesel fuel formulations or additives in accordance with (a)(2) of this appendix. The applicant shall initially submit a proposed test protocol to the Executive Officer. The proposed test protocol shall include: (A) the identity of the entity proposed to conduct the tests described in (a)(2)(F) of this appendix; (B) test procedures consistent with the requirements of (a)(2) of this appendix; (C) test data showing that the fuel to be used as the Reference CARB Diesel satisfies the specifications identified in (a)(2)(E) of this appendix; (D) reasonably adequate quality assurance and quality control procedures; and (E) notification of any outlier identification and exclusion procedure that will be used, and a demonstration that any such procedure meets generally accepted statistical principles.

Within 20 business days of receipt of a proposed test protocol, the Executive Officer shall advise the applicant in writing either that it is complete or that specified additional information is required to make it complete. Within 15 business days of submittal of additional information, the Executive Officer shall advise the applicant in writing either that the information submitted makes the proposed test protocol complete or that specified additional information is still required to make it complete. Within 20 business days after the proposed test protocol is deemed complete, the Executive Officer shall either approve the test protocol as consistent with this (a)(2) of this appendix or advise the applicant in writing of the changes necessary to make the test protocol consistent with (a)(3) of this appendix. Any notification of approval of the test protocol shall include the name, telephone number, and address of the Executive Officer's designee to receive notifications pursuant to (a)(2)(F) of this appendix. The tests shall not be conducted until the protocol is approved by the Executive Officer.

Upon completion of the tests, the applicant may submit an application for certification to the Executive Officer. The application shall include the approved test protocol, all of the test data, a copy of the complete test log prepared in accordance with (a)(2)(F) of this appendix, a demonstration that the candidate fuel meets the requirements for certification set forth in (a)(2)(C) of this appendix, and such other information as the Executive Officer may reasonably require.

Within 20 business days of receipt of an application, the Executive Officer shall advise the applicant in writing either that it is complete or that specified additional information is required to make it complete. Within 15 business days of submittal of additional information, the Executive Officer shall advise the applicant in writing either that the information submitted makes the application complete or that specified additional information is still required to make it complete. Within 20 business days after the application is deemed complete, the Executive Officer shall grant or deny the application. Any denial shall be accompanied by a written statement of the reasons for denial.

(B) The candidate fuel.

The candidate fuel to be used in the comparative testing described in (a)(2)(F) of this appendix shall be one of the following:

1. ADF formulation: The candidate fuel shall be the fuel blendstock or fuel blend that the applicant is attempting to certify. If the applicant is attempting to certify a fuel blend, that blend shall consist of the fuel blendstock blended to 20 percent with the Reference CARB Diesel. The applicant shall report all of the candidate fuel properties under (a)(3)(C) of this appendix for the candidate fuel.

2. Biodiesel additives: The candidate fuel shall be a mixture of the additive to be certified at the concentration specified by the applicant and the biodiesel additive certification fuel specified in (a)(3)(D) of this appendix. If the additive to be certified is meant to be used in B20 fuel blends, the candidate fuel shall be a mixture of the additive to be certified at the concentration specified by the applicant and the biodiesel additive certification fuel specified in (a)(3)(D) of this appendix blended to 20 volume percent biodiesel content with the Reference CARB Diesel. The applicant shall report all of the candidate fuel properties under (a)(3)(C) of this appendix for both the certification fuel without the additive, and the candidate fuel.

(C) Candidate fuel properties.

1. The applicant shall report all of the properties listed below for the candidate fuel. The candidate fuel shall be representative of the fuel that the applicant will produce commercially, and shall not contain streams or feedstocks that will not be used in the commercial fuel that the applicant intends to sell. If the executive officer determines that the candidate fuel contains streams or feedstocks that will not be used in the commercial fuel, this will be grounds for rejection of the application.

2. The following documents are incorporated by reference:

a. ASTM D5186-03, "Standard Test Method for Determination of the Aromatic Content and Polynuclear Aromatic Content of Diesel Fuels and Aviation Turbine Fuels By Supercritical Fluid Chromatography (2009)."

b. ASTM D4629-12, "Standard Test Method for Trace Nitrogen in Liquid Petroleum Hydrocarbons by Syringe/Inlet Oxidative Combustion and Chemiluminescence Detection (2012)."

c. ASTM D445-14e2, "Standard Test Method for Kinematic Viscosity of Transparent and Opaque Liquids (and Calculation of Dynamic Viscosity) (2012)."

d. ASTM D93-13e1, "Standard Test Methods for Flash Point by Pensky-Martens Closed Cup Tester (2013)."

e. ASTM D86-12, "Standard Test Method for Distillation of Petroleum Products at Atmospheric Pressure (2012)."

f. EN 14103:2011, "Fat and oil derivatives. Fatty acid methyl esters (FAME). Determination of ester and linolenic acid methyl ester contents (2011)."

Table A.7: Candidate fuel properties

| **Property** | **Test Method** |
|---|---|
| Sulfur Content | ASTM D5453-93 |
| Aromatic Hydrocarbon Content, Volume % | ASTM D5186-03(2009) |
| Polycyclic Aromatic Content, Weight % | ASTM D5186-03(2009) |
| Nitrogen Content | ASTM D4629-12 |
| Unadditized Cetane Number | ASTM D613-14 or ASTM D6890-13be1 |
| API Gravity | ASTM D287-12b |
| Viscosity at 40°C, cSt | ASTM D445-14e2 |
| Flash Point, °F, minimum | ASTM D93-13e1 |
| Distillation, °F | ASTM D86-12 |
| Initial Boiling Point | |
| 10 % Recovered | |
| 50 % Recovered | |
| 90 % Recovered | |
| End Point | |
| FAME Content % | EN14103:2011 |

(D) *Biodiesel additive certification fuel.*
The biodiesel additive certification fuel shall be a biodiesel (fatty acid methyl ester) produced by transesterification of virgin soybean oil with the following properties.

Table A.8: Additive certification fuel blendstock properties

| Property | Test Method | Fuel Specifications |
|---|---|---|
| Sulfur Content | ASTM D5453-93 | 15 ppm maximum |
| Nitrogen Content | ASTM D4629-12 | 10 ppm maximum |
| Unadditized Cetane Number | ASTM D613-14 or ASTM D6890-13be1 | 47-50 |
| API Gravity | ASTM D287-12b | 27 – 33 |
| Viscosity at 40°C, cSt | ASTM D445-14e2 | 2.0 – 4.1 |
| Flash Point, °F, minimum | ASTM D93-13e1 | 266 |
| Distillation, °F | ASTM D86-12 | |
| 90 % Recovered | | 620-680 |
| FAME Content % | EN 14103:2011 | Report |

(E) *The Reference CARB Diesel.*
The Reference CARB Diesel used in the comparative testing described in (a)(2)(F) of this appendix shall be produced from straight-run California diesel fuel by a hydrodearomatization process and shall have the characteristics set forth below under "Reference Fuel Specifications" (the listed ASTM methods are incorporated herein by reference):

Table A.9: Reference Fuel Specifications

| Property | Test Method | Fuel Specifications |
|---|---|---|
| Sulfur Content | ASTM D5453-93 | 15 ppm maximum |
| Aromatic Hydrocarbon Content, Volume % | ASTM D5186-03(2009) | 10 % maximum |
| Polycyclic Aromatic Content, Weight % | ASTM D5186-03(2009) | 10 % maximum |
| Nitrogen Content | ASTM D4629-12 | 10 ppm maximum |
| Unadditized Cetane Number | ASTM D613-14 or ASTM D6890-13be1 | 48 minimum |
| API Gravity | ASTM D287-12b | 33 – 39 |
| Viscosity at 40°C, cSt | ASTM D445-14e2 | 2.0 – 4.1 |
| Flash Point, °F, minimum | ASTM D93-13e1 | 130 |
| Distillation, °F | ASTM D86-12 | |
| Initial Boiling Point | | 340 – 420 |
| 10 % Recovered | | 400 – 490 |
| 50 % Recovered | | 470 – 560 |
| 90 % Recovered | | 550 – 610 |
| End Point | | 580 – 660 |

(F) *Emissions testing.*

1. Exhaust emission tests using the candidate fuel and the reference fuel shall be conducted in accordance with the "California Exhaust Emission Standards and Test Procedures for 1985 and Subsequent Model Heavy-Duty Diesel-Powered Engines and Vehicles," as incorporated by reference in Title 13, California Code of Regulations, Section 1956.8(b). The tests shall be performed using a Detroit Diesel Corporation Series 60 engine, through December 31, 2017, or a 2004-2006 model-year, Cummins ISM370 engine having a nominal torque rating of 1450 ft-lb and a nominal power output of 360 to 380 hp, and produced between January 2004 and December 2006, inclusive, starting January 1, 2015, or, if the Executive Officer determines that the 2004-2006 Cummins ISM370 is no longer representative of the pre-2007 model-year, heavy duty diesel engine fleet, another engine found by the Executive Officer to be representative of such engines. A determination by the Executive Officer that an engine is no longer representative shall not affect the certification of a diesel fuel formulation based on prior tests using that engine pursuant to a protocol approved by the Executive Officer.

2. The comparative testing shall be conducted by a party or parties that are mutually agreed upon by the Executive Officer and the applicant. The applicant shall be responsible for all costs of the comparative testing.

3. The applicant shall use one of the following test sequences:

a. If both cold start and hot start exhaust emission tests are conducted, a minimum of five exhaust emission tests shall be performed on the engine with each fuel, using either of the following sequences, where "R" is the Reference CARB Diesel and "C" is the candidate fuel: RC RC RC RC RC (and continuing in the same order). or RC CR RC CR RC (and continuing in the same order).

The engine mapping procedures and a conditioning transient cycle shall be conducted with the Reference CARB Diesel before each cold start procedure using the Reference CARB Diesel. The reference cycle used for the candidate fuel shall be the same cycle as that used for the fuel preceding it.

b. If only hot start exhaust emission tests are conducted, one of the following test sequences shall be used throughout the testing, where “R” is the Reference CARB Diesel and “C” is the candidate fuel:

Alternative 1: RC CR RC CR (continuing in the same order for a given calendar day; a minimum of twenty individual exhaust emission tests must be completed with each fuel)

Alternative 2: RR CC RR CC (continuing in the same order for a given calendar day; a minimum of twenty individual exhaust emission tests must be completed with each fuel)

Alternative 3: RRR CCC RRR CCC (continuing in the same order for a given calendar day; a minimum of twenty-one individual exhaust emission tests must be completed with each fuel)

For all alternatives, an equal number of tests shall be conducted using the Reference CARB Diesel and the candidate fuel on any given calendar day. At the beginning of each calendar day, the sequence of testing shall begin with the fuel that was tested at the end of the preceding day. The engine mapping procedures and a conditioning transient cycle shall be conducted after every fuel change and/or at the beginning of each day. The reference cycle generated from the Reference CARB Diesel for the first test shall be used for all subsequent tests.

For alternatives 2 and 3, each paired or triplicate series of individual tests shall be averaged to obtain a single value which would be used in the calculations conducted pursuant to (a)(3)(G) of this appendix.

4. The applicant shall submit a test schedule to the Executive Officer at least one week prior to commencement of the tests. The test schedule shall identify the days on which the tests will be conducted, and shall provide for conducting the test consecutively without substantial interruptions other than those resulting from the normal hours of operations at the test facility. The Executive Officer shall be permitted to observe any tests. The party conducting the testing shall maintain a test log which identifies all tests conducted, all engine mapping procedures, all physical modifications to or operational tests of the engine, all recalibrations or other changes to the test instruments, and all interruptions between tests and the reason for each such interruption. The party conducting the tests or the applicant shall notify the Executive Officer by telephone and in writing of any unscheduled interruption resulting in a test delay of 48 hours or more, and of the reason for such delay. Prior to restarting the test, the applicant or person conducting the tests shall provide the Executive Officer with a revised schedule for the remaining tests. All tests conducted in accordance with the test schedule, other than any tests rejected in accordance with an outlier identification and exclusion procedure included in the approved test protocol, shall be included in the comparison of emissions pursuant to (a)(3)(G) of this appendix.

5. In each test of a fuel, exhaust emissions of oxides of nitrogen (NOx) and particulate matter (PM) shall be measured.

(G)The average emissions during testing with the candidate fuel shall be compared to the average emissions during testing with the Reference CARB Diesel, applying one-sided Student's t statistics as set forth in Snedecor and Cochran, *Statistical Methods* (7th ed.), page 91, Iowa State University Press, 1980, which is incorporated herein by reference. The Executive Officer shall issue a certification pursuant to this paragraph only if he or she makes all of the determinations set forth in (a)(3)(G) below, after applying the criteria of (a)(3)(G) of this appendix.

1. The average individual emissions of NOx and PM, respectively, during testing with the candidate fuel do not exceed the average individual emissions of NOx and PM, respectively, during testing with the Reference CARB Diesel.

2. Use of any additive identified pursuant to (a)(2)(B) of this appendix in heavy-duty engines will not increase emissions of noxious or toxic substances which would not be emitted by such engines operating without the additive. In addition, cellular tests on the particulate emissions from heavy-duty engines will not show greater harm for mutagenicity, inflammation, DNA damage, or oxidative stress with the use of any such additive than would occur with such engines operating without the additive.

3. In order for the determinations of (a)(2)(G) of this appendix to be made, for each referenced pollutant the candidate fuel shall satisfy the following relationship:

$$\bar{x}_C < \bar{x}_R + \delta - S_P \times \sqrt{\frac{2}{n}} \times t(a, 2n-2)$$

Where:

| | |
|---|---|
| $\bar{x}_C$ = | Average emissions during testing with the candidate fuel |
| $\bar{x}_R$ = | Average emissions during testing with the Reference CARB Diesel |
| $\delta$ = | tolerance level equal to 1 percent of $\bar{x}_R$ NOx, 2 percent of $\bar{x}_R$ for PM. |
| $S_p$ = | Pooled standard deviation |
| t (a, 2n-2) = | The one-sided upper percentage point of t distribution with a = 0.15 and 2n-2 degrees of freedom |
| n = | Number of tests of candidate fuel and Reference CARB Diesel |

(H) If the Executive Officer finds that a candidate fuel has been properly tested in accordance with (a)(2)(F) of this appendix, and makes the determinations specified in (a)(2)(G) of this appendix, then he or she shall issue an Executive Order certifying the alternative diesel fuel or additive formulation represented by the candidate fuel. The Executive Order shall identify all of the characteristics of the candidate fuel determined pursuant to (a)(2)(C) of this appendix. The Executive Order shall provide that the certified alternative diesel fuel formulation has the following specifications: [1] a sulfur content, total aromatic hydrocarbon content, polycyclic aromatic hydrocarbon content, and nitrogen content not exceeding that of the candidate fuel, [2] a

cetane number and API gravity not less than that of the candidate fuel, [3] any additional fuel specification required under (a)(3) of this appendix, and [4] presence of all additives that were contained in the candidate fuel, in a concentration not less than in the candidate fuel, except for an additive demonstrated by the applicant to have the sole effect of increasing cetane number. Additionally the Executive Order shall contain a table mirroring the table in Appendix 1 (a)(1)(A) listing the required concentration of additive at each 5 percent interval of blend level, if applicable. All such characteristics shall be determined in accordance with the test methods identified in (a)(2)(C) of this appendix. The Executive Order shall assign an identification name to the specific certified biodiesel fuel formulation.

(I) In-use testing.

1. If the executive officer determines that a commercially available biodiesel fuel blend meets all of the specifications of a certified biodiesel fuel formulation set forth in an Executive Order issued pursuant to (a)(2)(H) of this appendix, but does not meet the criteria of (a)(2)(G) of this appendix when tested in accordance with (a)(2)(F), the Executive Officer shall modify the Executive Order as is necessary to assure that biodiesel fuel blends sold commercially pursuant to the certification will meet the criteria set forth in (a)(2)(G). The modifications to the order may include additional specifications or conditions, or a provision making the order inapplicable to specified biodiesel fuel producers.

2. The Executive Officer shall not modify a prior Executive Order without the consent of the applicant and of the producer of the commercially available biodiesel fuel blend found not to meet the criteria, unless the applicant and producer are first afforded an opportunity for a hearing in accordance with Title 17, California Code of Regulations, Part III, Chapter 1, Subchapter 1, Article 4 (commencing with Section 60040). If the Executive Officer determines that a producer would be unable to comply with this regulation as a direct result of an order modification pursuant to this subsection, the Executive Officer may delay the effective date of such modification for such period of time as is necessary to permit the producer to come into compliance in the exercise of all reasonable diligence.

(b) [Reserved]

# APPENDIX B

## Members of the Multimedia Working Group

February 2015

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# APPENDIX C

## Air Resources Board: Impact Assessment of Biodiesel on Exhaust Emissions from Compression Ignition Engines

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**State of California**
**Air Resources Board**

# Impact Assessment of Biodiesel on Exhaust Emissions from Compression Ignition Engines

February 2015

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# 1. INTRODUCTION

The staff of the Air Resources Board (ARB or Board) intends to establish new motor vehicle fuel specifications for biodiesel as part of the proposed regulation on the commercialization of alternative diesel fuels (ADFs). The ADF regulation[1] is intended to provide a framework for low carbon diesel fuel substitutes to enter the commercial market in California, while mitigating any potential environmental or public health impacts. The first ADF that will be regulated under the proposed regulation is biodiesel.

Health and Safety Code (HSC) section 43830.8 requires a multimedia evaluation to be conducted and reviewed by the California Environmental Policy Council (CEPC) before new fuel specifications are established. A "multimedia evaluation" is the identification and evaluation of any significant adverse impact on public health or the environment, including air, water, and soil, that may result from the production, use, or disposal of the motor vehicle fuel that may be used to meet the state board's motor vehicle fuel specifications.[2]

This report provides staff's assessment of the emissions data and air quality impact information obtained during the biodiesel multimedia evaluation and staff's overall conclusions and recommendations to the CEPC. Staff's assessment is based on the data and information provided for the biodiesel multimedia evaluation, including the University of California (UC) researchers' multimedia reports (Final Tier I, Tier II and Tier III Reports); the "*CARB Assessment of the Emissions from the Use of Biodiesel as a Motor Vehicle Fuel in California*" (ARB Emissions Study)[3] by UC Riverside from emissions testing conducted at the College of Engineering – Center for Environmental Research and Technology (CE-CERT) and ARB emissions test facilities in Stockton and El Monte, California; and the "*CARB Comprehensive B5/B10 Blends Heavy-Duty Engine Dynamometer Testing*" (B5/B10 Study)[4] by ARB, UC Riverside, and UC Davis.

## A. Multimedia Evaluation of Biodiesel

Pursuant to HSC section 43830.8, researchers from UC Davis and UC Berkeley conducted the multimedia evaluation of biodiesel compared to diesel meeting ARB motor vehicle fuel specifications (CARB diesel). After each tier of the evaluation process, the UC researchers submitted a tier report for review and approval by the Multimedia Working Group (MMWG). The final reports are listed below:

- California Biodiesel Multimedia Evaluation Final Tier I Report (Final Tier I Report)[5]

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[1] Air Resources Board. *Proposed Regulation on the Commercialization of New Alternative Diesel Fuels Staff Report: Initial Statement of Reasons*. January 2, 2015. Appendix A: Proposed Regulation Order.
[2] California Air Pollution Control Laws. Health and Safety Code, Division 26, Part 5, Chapter 4, Section 43830.8(b).
[3] Durbin, T.D. et al. *CARB Assessment of the Emissions from the Use of Biodiesel as a Motor Vehicle Fuel in California "Biodiesel Characterization and NOx Mitigation Study."* October 2011.
[4] Karavalakis, G. et al. *CARB Comprehensive B5/B10 Blends Heavy-Duty Engine Dynamometer Testing.* June 2014.
[5] Ginn, T.R. et al. *California Biodiesel Multimedia Evaluation Final Tier I Report*, September 2009.

- California Biodiesel Multimedia Evaluation Final Tier II Report (Final Tier II Report)[6]
- California Biodiesel Multimedia Evaluation Final Tier III Report (Final Tier III Report or Biodiesel Final Report)[7]

During Tier I of the biodiesel evaluation, the UC researchers completed a detailed review of biodiesel, evaluated potential impacts, and determined key knowledge gaps. Upon completion of Tier I, the overall scope of the biodiesel evaluation was established. The knowledge gaps identified in Tier I necessitated further study, testing, and a more detailed impact assessment of biodiesel in Tier II. The biodiesel Tier II risk assessment design included various test plans and studies to fill in key knowledge gaps identified in Tier I. Tier III began with the implementation of the Tier II risk assessment protocols and concluded with the formal submittal of the Biodiesel Final Report.

Based on the biodiesel multimedia evaluation and the information provided in the Tier I, Tier II, and Tier III reports by UC Davis and UC Berkeley, ARB staff determined that with in-use requirements biodiesel, as specified in the multimedia evaluation and proposed regulation, does not pose a significant adverse impact on public health or the environment from potential air quality impacts.

### B. ARB Emissions Testing Program

The comprehensive ARB Emissions Study and subsequent B5/B10 Study were conducted to determine emissions impacts of biodiesel and various fuel blends compared to CARB diesel.

Table 1 summarizes the test matrix covered in the ARB Emission Study. In general, the results from the ARB Emissions Study found that most emissions from biodiesel are reduced from CARB diesel, including particulate matter (PM), carbon monoxide (CO), total hydrocarbons (THC), and most toxic species. However, oxides of nitrogen (NOx) emissions were found to increase at certain biodiesel blends. The results of this study apply specifically to heavy-duty vehicles that do not use post-exhaust NOx emissions control.

[6] Ginn, T.R. et al. *California Biodiesel Multimedia Evaluation Final Tier II Report*, January 2012.
[7] Ginn, T.R. et al. *California Biodiesel Multimedia Evaluation Final Tier III Report*, June 2014.

**Table 1. Summary of Testing Done by ARB and UC Riverside**

| Application | Engine | Feedstocks | Test Cycles |
|---|---|---|---|
| On-road chassis | Caterpillar C15<br>Cummins ISM<br>DDC MBE4000<br>Cummins ISX | Animal<br>Soy<br>Renewable Diesel<br>GTL | UDDS<br>FTP<br>40mph Cruise<br>50mph Cruise |
| On-road HD engine | Cummins ISM<br>DDC MBE4000 | Animal<br>Soy | UDDS<br>FTP |
| Non-road engine | John Deere 4084<br>Kubota TRU | Animal<br>Soy | ISO 8178-4 |

The B5/B10 Study expands on the ARB Emissions Study to provide more comprehensive information on the emissions impacts from lower biodiesel blends. Two engines were used for this test program, including a 2006 model year Cummins ISM engine and a 1991 Detroit Diesel Corporation (DDC) Series 60 Engine.

In general, criteria pollutants and ozone precursors including PM, NOx, CO, and THC were measured for soy and animal B5 and B10 biodiesel blends. Consistent with the results from the ARB Emissions Study, statistically significant reductions of PM, CO, and THC were found. NOx emissions results showed statistically significant increases.[8]

[8] Karavalakis, G. et al. *CARB Comprehensive B5/B10 Blends Heavy-Duty Engine Dynamometer Testing.* June 2014. viii.

## 2. BIODIESEL

Biodiesel is defined as a fuel composed of mono-alkyl esters of long chain fatty acids derived from vegetable oils or animal fats and meeting ASTM International standard D6751. Pure biodiesel contains no petroleum but can be blended with petroleum diesel to create a biodiesel blend. In this report, CARB diesel blended with 10 volume percent (vol%), 20 vol% or 50 vol% biodiesel is denoted as B10, B20 or B50, respectively. Pure biodiesel is denoted as B100.[9]

To produce biodiesel, a feedstock undergoes an esterification reaction with methanol and a catalyst to produce methyl esters, which compose biodiesel, also known as Fatty Acid Methyl Esters (FAME). Primary biodiesel feedstocks expected to be used in California include soybean oil, palm oil, corn oil, yellow grease, animal tallow, trap (brown) grease, canola oil, and safflower oil.[10]

Biodiesel feedstocks are classified by their fatty acid profile; the fatty acid composition greatly influences a fuel's characteristics, as esters of different fatty acids have different physical and chemical properties. Generally, the quality of the fuel is dependent on the quality and fatty-acid composition of the feedstock, the production process, and post-production handling. Biodiesel blends up to B5 must meet ASTM D975 standards. ASTM has also established ASTM D7467 for blends of B6 to B20, and ASTM D6751-12 for B100.[11]

### A. Production

The process used to convert virgin oils or animal fats into biodiesel is called transesterification and involves mixing the oil or fat with alcohol and a catalyst, usually lye. Transesterification can be used to convert either plant oil or animal fats to biodiesel.

Raw vegetable and animal oils consist primarily of triacylglycerides, commonly known as triglycerides. Structurally, triglycerides are composed of three fatty acids attached to a glycerol molecule. Though these oils can be directly used in diesel engines, engine manufacturers generally discourage this practice, as their use can cause engine problems. This is primarily because combustion of raw oils form engine deposits, with carbon residue and plugging in engine injector nozzles, piston rings, and lubricating oil. This happens due to polymerization of the triglycerides in the raw oils as the fuel is combusted. Converting the raw oils into a form of esters or biodiesel prevents these issues.

Before transesterification is conducted, the raw oils and fats are filtered and pretreated to remove water and contaminants. Water in the feedstock leads to the formation of

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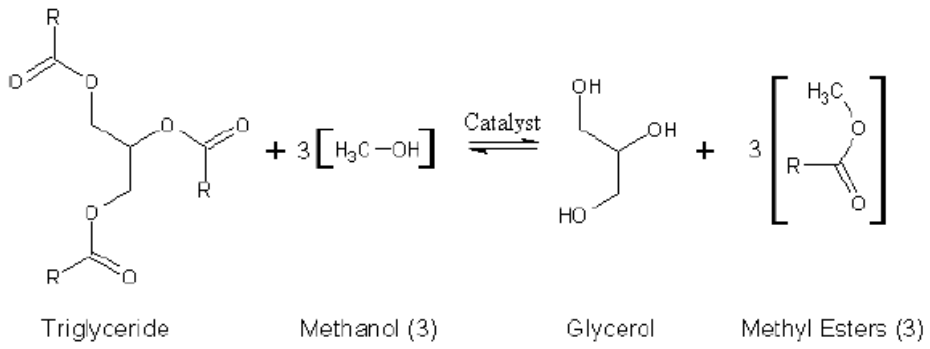
[9] Air Resources Board. *Proposed Regulation on the Commercialization of New Alternative Diesel Fuels Staff Report: Initial Statement of Reasons*. October 23, 2013, 17.
[10] Ginn, T.R. et al. *California Biodiesel Multimedia Evaluation Final Tier III Report*. June 2014, I-1.
[11] Ginn, T.R. et al. *California Biodiesel Multimedia Evaluation Final Tier III Report*. June 2014, I-1,I-2.

soaps, which are an undesirable by-product, reduces the yield of biodiesel, and makes the separation of glycerin in the products more difficult.

As shown in Figure 1, transesterification involves reacting triglyceride oils with alcohol (usually methanol) in the presence of a catalyst (usually lye) in a simple closed reactor system at low temperature and pressure. The products of the transesterification reaction are FAME and glycerin as a co-product. After transesterification, a majority of the alcohol is removed from the glycerin and recycled back into the system to continue the process. The biodiesel from the process is purified and washed to remove any residual catalyst and soaps. The glycerin from transesterification can be purified and sold to the pharmaceutical or cosmetic industry to be processed into lotions and creams.[12]

**Figure 1. Transesterfication Reaction**




There are two basic conversion routes for FAME production, base and direct acid catalyzed transesterification. The base catalyzed option tends to be the most economical for virgin oil feedstocks and as such is most commonly used to produce esters on a commercial scale. The processing equipment operates at relatively lower temperatures and pressures. The process has high conversion rates (around 98%) to FAME with low reaction times without producing intermediate compounds.

Acid catalyzed transesterification is expected to be the preferred method for conversion of waste oils, since it is less sensitive to free fatty acids in the feedstock. This conversion method seems to be more economical than base catalyzed transesterification of waste oils, because an extra pretreatment step is required to remove fatty acid impurities before the base catalyzed process. For base transesterification the fatty acid content of feedstocks must generally be less than four percent. Acid catalyzed transesterification has not been optimized for commercial scale production.

[12] California Air Resources Board, *Staff Report: Initial Statement of Reasons for Proposed Rulemaking, Proposed Regulation on the Commercialization of New Alternative Diesel Fuels.* October 23, 2013, 27.

In base catalyzed transesterification, a strong base of sodium hydroxide or potassium hydroxide is usually used as the catalyst for the reaction. In the acid catalyzed process, sulfuric acid is usually used as the catalyst. For the base catalyzed process the molar ratio of methanol to oil is about 6:1, while for an acid catalyzed process the ratio is about 50:1.

The purity and the yield of biodiesel from transesterification is affected by the molar ratio of glycerides to alcohol, the type of catalyst, the reaction time, the reaction temperature, the amount of free fatty acids, and the amount of water present in the feedstock. Both purity and yield affect the amount of cleanup that must be performed on the finished product.[13]

### B. Feedstocks

In the U.S., there are many potential plant oil feedstocks that can be used including soybean, peanut, canola, cottonseed and corn oil. Most of the world's production of biodiesel comes from plant oils such as soybean, rapeseed (canola), and palm oil. About 55 percent of U.S. biodiesel was made from soybean oil feedstocks in 2012.[14]

Biodiesel can also be made from waste feedstocks such as waste vegetable oil and tallow. These feedstocks are wastes, so there are no greenhouse gas (GHG) emissions due to land use change associated with these feedstocks. Biodiesel from wastes is sometimes referred to as advanced biodiesel in order to differentiate it from crop-based biodiesel because of its lower carbon intensity.[15]

### C. Fuel Quality

According to the Coordinating Research Council, Inc. Report No. AVFL-17, *Investigation of Biodistillates as Potential Blendstocks for Transportation Fuels, released in June 2009, "*One of the biggest concerns of the biodiesel industry is the quality of finished fuels being used in the marketplace. The use of poor quality fuels can lead (and has led) to field problems and customer complaints, which reduce public confidence and jeopardize the future of the industry. Steps to address these concerns have been taken in recent years by adoption (or modification) of ASTM D6751 (for B100) and D7467 (for B6-B20), and by development of the BQ-9000 Quality Management System. Fuel quality surveys have indicated that problems with blending control and off-spec products were common in the past. However, it appears that with more stringent fuel specifications and increasing producer experience, the overall quality of biodiesel in the marketplace is improving."

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[13] California Air Resources Board, *Staff Report: Initial Statement of Reasons for Proposed Rulemaking, Proposed Regulation on the Commercialization of New Alternative Diesel Fuels.* October 23, 2013, 28.
[14] U.S. Department of Energy, *Annual Energy Outlook 2012 with Projection to 2035,* June 2012.
[15] California Air Resources Board, *Staff Report: Initial Statement of Reasons for Proposed Rulemaking, Proposed Regulation on the Commercialization of New Alternative Diesel Fuels.* October 23, 2013, 28.

Ensuring oxidative stability of biodiesel in the marketplace is a major product quality concern. Due to the complex degradation pathways involved, no single test method is fully able to assess fuel stability in all circumstances. One of the most widely utilized test methods is the Rancimat oxidative stability test (EN 14112), which is based upon detection of volatile, secondary oxidation products that result from reaction of biodiesel with oxygen at elevated temperature. The Rancimat test was incorporated in 2007 in the ASTM standard specifications for B100. This test was originally developed as an indicator of vegetable oil storage stability, but is also regarded as a suitable means to assess storage stability of biodiesel and its blends. (Another oxidation stability test, prEN 15751, has been provisionally accepted.)

For many users, low temperature operability is the greatest biodiesel concern, particularly during cold seasons of the year. Just as with conventional diesel fuel, precautions must be taken to ensure satisfactory low temperature operability of biodiesel and its blends. These concerns are often greater with biodiesel, due to its higher cloud point and pour point compared to petroleum diesel. Poor low temperature operability may be exhibited in several ways, but principally by filter plugging due to wax formation, and engine starving due to reduced fuel flow.[16]

As with fuel stability, there is no single best test to assess low temperature operability. U.S. fuel standards do not include explicit specifications for low temperature operability for conventional diesel or biodiesel (or blends of the two). However, the fuel seller is generally required to give an indication of low temperature operability by reporting the cloud point of the fuel. Also, a cold-soak filterability standard test method for B100 is under development by ASTM. Beginning in 2008, ASTM D6751 required test method 'Annex A1' to assess cold soak filterability of B100 intended for blending with diesel.

Poor low temperature operability is usually caused by long-chain saturated fatty acid esters present in biodiesel. In general, the longer the carbon chain, the higher the melting point, and poorer the low temperature operability. The presence of carbon-carbon double bonds significantly lowers the melting point of a molecule (hydrocarbon or fatty acid alkyl ester). Therefore, to a certain degree, a trade-off exists between fuel stability and low temperature operability. With increasing degree of unsaturation, stability decreases but low temperature operability improves.

In large part, the fatty acid composition of the fats and oil precursors to biodiesel dictate the low temperature operability of the final fuels. Feedstocks with highly saturated fatty acid structures (such as palm oil and tallow) produce biodiesels with poor operability; whereas feedstocks with highly unsaturated fatty acid structures (such as rapeseed and safflower oil) have better operability. Proper choice of feedstocks is critical to providing a finished biodiesel fuel having acceptable low temperature operability.[17]

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[16] California Air Resources Board, *Staff Report: Initial Statement of Reasons for Proposed Rulemaking, Proposed Regulation on the Commercialization of New Alternative Diesel Fuels.* October 23, 2013, 29.
[17] California Air Resources Board, *Staff Report: Initial Statement of Reasons for Proposed Rulemaking, Proposed Regulation on the Commercialization of New Alternative Diesel Fuels.* October 23, 2013, 29.

Other approaches that are helpful in particular circumstances include the following:

- Blending with petroleum diesel;
- Use of commercial petroleum diesel additives;
- Use of new cold flow improver (CFI) additives for biodiesel;
- Use of higher alcohols (including ethanol) for transesterification; or
- Crystallization fractionation (wax removal).[18]

Water solubility and water contamination are other issues of concern. At room temperature, water is very slightly soluble in conventional diesel fuel (< 100 ppm), but has significant solubility in B100 (up to 1200 ppm). Water solubility in B20 is intermediate between these two extremes. The generally higher water levels in biodiesel can exacerbate problems with corrosion, wear, suspension of solids, and microbial growth. When dealing with biodiesel, extra 'housekeeping' precautions may be necessary to remove excess water and sediment. In particular, this is required when first introducing biodiesel into tanks previously used for conventional diesel, as accumulated water and sediment may become dispersed and plug filters under these conditions.[19]

### D. Availability

According to Biodiesel Magazine, as of February 2015 there are 145 operational commercial biodiesel production plants in the U.S. with a total production capacity of about 2.6 billion gallons. There are about 11 major plants in California with annual production capacities varying between 1 to 36 million gallons. The total biodiesel production capacity in California is about 106 million gallons per year.[20] This compares to total conventional diesel production of about 3.5 billion gallons per year.

According to the Low Carbon Fuel Standard (LCFS) Reporting Tool, California biodiesel production facilities produced about 27 million gallons of biodiesel in 2013. Staff has communicated with many of the stations that sell biodiesel as well as the major terminal operators in the state, and has found that the vast majority of the biodiesel currently being sold in California and expected to be sold in the future is sold as blends of B5 or less.[21]

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[18] California Air Resources Board, *Staff Report: Initial Statement of Reasons for Proposed Rulemaking, Proposed Regulation on the Commercialization of New Alternative Diesel Fuels.* October 23, 2013, 29, 30.
[19] Coordinating Research Council, Inc.,Report No. AVFL-17, Alpharetta, Georgia, pp. 16-18, *Investigation of Biodistillates as Potential Blendstocks for Transportation Fuels*, June 2009.
[20] Biodiesel Magazine, Biodiesel Plants. *USA Plants*, California. Last Modified February 4, 2015.
[21] California Air Resources Board, *Staff Report: Initial Statement of Reasons for Proposed Rulemaking, Proposed Regulation on the Commercialization of New Alternative Diesel Fuels.* October 23, 2013, 30.

# 3. EXHAUST EMISSIONS

Emissions testing were performed to characterize regulated emissions, including PM, NOx, CO and THC, and various unregulated toxic emissions in heavy duty engines.

## A. Emissions Testing

### i. ARB Emissions Study

Emissions testing under the ARB Emissions Study program were conducted on two engines and three vehicles. Test fuels included five primary fuels including various biodiesel blends (B5, B20, and B50) to comprise the full test matrix. Two biodiesel feedstocks were used, including one soy-based and one animal-based biodiesel fuel. These fuels were selected to provide a range of properties that are representative of typical feedstocks representing different characteristics of biodiesel in terms of cetane number and degree of saturation.[22]

The ARB Emissions Study included both engine testing and chassis testing. Engine dynamometer testing was conducted at UC Riverside's CE-CERT and chassis dynamometer testing was conducted at ARB's Heavy-Duty Engine Emissions Testing Laboratory in Los Angeles. Test results were straight averages of the difference between biodiesel and CARB diesel emissions.

#### a. Engine Dynamometer Testing

Engine testing focused primarily on standard emissions, including CO, THC, NOx, PM, and carbon dioxide ($CO_2$). Each fuel blend was tested six times, and each test yielded THC, CO, NOx, PM, $CO_2$, and brake specific fuel consumption (BSFC) measurements.

Test engines included typical six cylinder, in-line, direct injection, turbocharged, heavy-duty diesel engines. The following test engines were used:

- 2006 Cummins ISM 370
- 2007 Detroit Diesel Corporation (DDC) MBE4000

The engines were selected from two model year categories; 2002-2006 and 2007-2009. The 2002-2006 engines were estimated to represent an important contribution to the emissions inventory from the present through 2017. The 2007-2009 model year engine represented the latest technology that was available at the time of testing.[23]

Engine specifications are listed in Table 2.

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[22] Durbin, T.D. et al. *CARB Assessment of the Emissions from the Use of Biodiesel as a Motor Vehicle Fuel in California "Biodiesel Characterization and NOx Mitigation Study."* October 2011, xxv.
[23] Durbin, T.D. et al. *CARB Assessment of the Emissions from the Use of Biodiesel as a Motor Vehicle Fuel in California "Biodiesel Characterization and NOx Mitigation Study."* October 2011, 5-6.

**Table 2. Engine Dynamometer Engine Specifications**

| | | |
|---|---|---|
| Engine Manufacturer | Cummins | Detroit Diesel Corp. |
| Engine Model | ISM 370 | MBE4000 |
| Model Year | 2006 | 2007 |
| Engine Type | In-line 6 cylinder 4 stroke | 7DDXH12.8DJA |
| Displacement | 10.8 liters | 12.8 |
| Power Rating | 385 hp @ 1800 rpm | 410 hp @ 1900 rpm |
| Fuel Type | Diesel | Diesel |
| Induction | Turbocharger with charge air cooler | Turbocharger with after cooler |

The following test cycles were used:

- U.S. EPA Heavy duty Federal Test Procedure (FTP)
- Urban Dynamometer Driving Schedule (UDDS) modified for engine dynamometer
- CARB Heavy Heavy-Duty Diesel Truck (HHDDT) 50 mph Cruise cycle modified for engine dynamometer

b. Chassis Dynamometer Testing

Chassis dynamometer testing focused primarily on toxic pollutants. The primary test fuels were the same as those used for the engine testing, including the CARB baseline diesel, and the soy-based and animal-based biodiesels. These fuels were obtained from the same batches of fuel used for engine testing and blended at the same time.

Four vehicles were tested for the chassis dynamometer testing, as follows:

- 2006 International Truck equipped with a 2006 11 liter Cummins ISM engine.
- 2008 Freightliner Truck equipped with a 2007 MBE4000 engine.
- 2000 Freightliner Truck equipped with a 2000 Caterpillar C-15 engine.
- Kenworth model T800 truck equipped with a 2010 Cummins ISX15 engine.

The first two vehicles were equipped with the same engines used in the engine testing. The Cummins ISX15 was not included in the evaluation because the data analyses of the results were not completed and not included in the report.

The following test cycles were used:

- UDDS
- CARB HHDDT 50 mph Cruise cycle

The biodiesel blends were tested at the 20 vol%, 50 vol% and 100 vol% blend levels because the typically greater variability for the chassis testing would make it difficult to identify trends for the lower 5 vol% to 10 vol% blend levels. Each fuel blend was tested six times on the UDDS and three times on the 50 mph cruise cycle. Each test yielded measurements for the pollutants listed in Table 3.

**Table 3. Chassis Dynamometer Emissions Measurements**

| Analyte | Collection Media | Analysis |
|---|---|---|
| THC | Modal, Bag | FID |
| NMHC | Modal, Bag | FID |
| NOx, $NO_2$ | Modal, Bag | Chemiluminescence |
| CO, $CO_2$ | Modal, Bag | NDIR |
| BTEX | Tedlar Bags | GC-FID |
| Carbonyls | 2,4-DNPH cartridges | HPLC |
| PM Mass | Teflon 47mm (Teflo) | Gravimetric |
| Organic/Elemental Carbon | Quartz fiber filter 47mm | Thermo/Optical Carbon Analysis |
| Elements | Teflon filter | ICP-MS |
| PAH | Teflon Filter/PUF/XAD | GC-MS |
| $N_2O$ | Tedlar Bags | FTIR |

ii. <u>B5/B10 Study</u>

The B5/B10 Study expands on the ARB Emissions Study to provide more comprehensive information on the emissions impacts from lower biodiesel blends. The test fuels included the baseline CARB diesel fuel and the B5 and B10 biodiesel fuels with both animal-based and soy-based biodiesel feedstock. The CARB diesel fuel was the blendstock used for the B5 and B10 fuels, and the baseline fuel to which the biodiesel fuels were compared.

Engine dynamometer emissions testing was conducted on two heavy-duty on-road engines, including a 2006 model year Cummins ISM engine and a 1991 DDC Series 60 engine. Testing was conducted using three test cycles, including the FTP, UDDS, and

Supplemental Emissions Test (SET). The SET cycle is a 13-mode, steady state engine dynamometer test cycle.

For all tests, standard emissions measurements were made including NOx, PM, THC, CO, and $CO^2$. Staff's analysis focused primarily on NOx emissions for lower biodiesel blends compared to CARB diesel.[24]

### B. Results

Emissions measurements for regulated emissions and selected unregulated toxic emissions were obtained from testing. The results below are from the ARB Emissions Study and subsequent B5/B10 Study.[25,26]

#### i. ARB Emissions Study

##### a. Air Emissions

The emissions measurements for the engine testing under the ARB Emissions Study focused primarily on regulated emissions, including PM, NOx, THC, CO, and $CO_2$. More extensive testing, including toxics analyses, was completed for the chassis testing.

Average PM emissions results showed consistent and significant reductions for all biodiesel blends, with the magnitude of reductions increasing with blend level. For the 2006 Cummins engine over the FTP test cycle, PM reductions for soy-based biodiesel were approximately 6% for B5, 25% for B20, and 58% for B100. For animal-based biodiesel, PM reductions ranged from 19% for B20 to 64% for B100.

Average NOx emissions showed trends of increasing NOx emissions with increasing biodiesel blend level. Soy-based biodiesel blends showed a higher increase in NOx emissions for essentially all blend levels and test cycles compared to animal-based biodiesel blends. For soy-based biodiesel over the FTP cycle, results for the 2006 Cummins engine showed NOx increases of 2.2% for B5, 6.6% for B20, and 27% for B100. Animal-based biodiesel results showed NOx increases of 1.5% for B20 to 14% for B100. For the 2007 MBE4000 engine, NOx increases were greater than those of the 2006 engine for nearly all biodiesel blends and test cycles.

Average THC emissions for the 2006 Cummins showed consistent and significant reductions for all biodiesel blends, with the magnitude of reductions increasing with blend level. For soy-based biodiesel over the FTP cycle, THC emissions showed 6%

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[24] Karavalakis, G. et al. *CARB Comprehensive B5/B10 Blends Heavy-Duty Engine Dynamometer Testing.* June 2014.
[25] Durbin. T.D. et al, *CARB Assessment of the Emissions from the Use of Biodiesel as a Motor Vehicle Fuel in California, "Biodiesel Characterization and NOx Mitigation Study."* October 2011.
[26] Karavalakis, G. et al. *CARB Comprehensive B5/B10 Blends Heavy-Duty Engine Dynamometer Testing.* June 2014.

reductions for B10, 11% for B20, and 63% for B100. For animal-based biodiesel, THC reductions ranged from 13% for B20 to 71% for B100.
Average CO emissions also showed consistent and significant reductions for animal-based biodiesel, ranging from 7% for B5, 14% for B20, and 27% for B100. For soy-based biodiesel, CO trends were less consistent with some results not statistically significant.

Average tailpipe $CO_2$ emissions showed a slight increase for the higher biodiesel blends. For the 2006 Cummins engine, the increase ranged from about 1% to 4%, with increases being statistically significant for the B100 fuels for all tests, the B50 fuel for the cruise cycles, and other testing combinations. For the 2007 MBE 4000 engine, only the B100 showed consistent and statistically significant increases for the different cycles, ranging from 1% to 5%.

The biodiesel blends showed an increase in fuel consumption with increasing biodiesel blends. The fuel consumption differences were generally greater for the soy-based biodiesel in comparison with animal-based biodiesel for the 2006 Cummins engine, but not for the 2007 MBE 4000 engine. For the 2006 Cummins engine, changes in fuel consumption for soy-based biodiesel blends ranged from 1.4% to 1.8% for B20 and 6.8% to 9.8% for B100. Animal-based biodiesel blends ranged from no statistical difference to 2.6% for B20 and 4.4% to 6.7% for B100. For the 2007 MBE4000 engine, the differences in fuel consumption ranged from no change to 2.5% for B50 and lower blends, while the increases for B100 blends ranged from 5.6% to 8.3%.

Additionally, on a federal level, a U.S. EPA biodiesel exhaust emissions study found beneficial impacts associated with biodiesel use. Tailpipe emissions from heavy-duty engines were compiled and analyzed. Compared to federal diesel, data showed approximately 10% to 20% emissions reductions of PM, CO and HC from biodiesel blends of B20 and approximately 45% to 65% range reductions from B100.[27]

Tables 4, 5, 6, and 7 provide the test results from the engine dynamometer testing. Chassis dynamometer test results were comparable.

[27] U.S. Environmental Protection Agency, *A Comprehensive Analysis of Biodiesel Impacts on Exhaust Emissions*, EPA420-P-02-001, October 2002.

## Table 4. Summary of Average Emissions Results for Soy-Biodiesel Blends (2006 Cummins ISM)*

| | | THC | | | CO | | | NOx | | | PM | | | CO2 | | | BSFC | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cycle | Fuel | Avg g/bhp-hr | % Diff | P Value | Avg g/bhp-hr | % Diff | P Value | Avg g/bhp-hr | % Diff | P Value | Avg g/bhp-hr | % Diff | P Value | Avg g/bhp-hr | % Diff | P Value | Avg g/bhp-hr | % Diff | P Value |
| UDDS | *CARB* | 0.830 | | | 2.116 | | | 5.868 | | | 0.065 | | | 828.4 | | | 0.085 | | |
| | *B20* | 0.727 | -12% | 0.000 | 2.215 | 5% | 0.115 | 6.107 | 4.1% | 0.002 | 0.050 | -24% | 0.002 | 834.7 | 0.8% | 0.448 | 0.086 | 1.8% | 0.093 |
| | *B50* | 0.601 | -28% | 0.000 | 2.662 | 26% | 0.000 | 6.444 | 9.8% | 0.000 | 0.046 | -30% | 0.000 | 848.9 | 2.5% | 0.055 | 0.089 | 5.1% | 0.001 |
| | *B100* | 0.376 | -55% | 0.000 | 3.419 | 62% | 0.000 | 6.890 | 17.4% | 0.000 | 0.044 | -33% | 0.000 | 863.1 | 4.2% | 0.003 | 0.093 | 9.8% | 0.000 |
| FTP | *CARB* | 0.309 | | | 0.747 | | | 2.012 | | | 0.081 | | | 624.9 | | | 0.064 | | |
| | *B5* | | -1% | 0.087 | | -1% | 0.471 | | 2.2% | 0.000 | | -6% | 0.000 | | 0.1% | 0.816 | | 0.3% | 0.228 |
| | *B10* | | -6% | 0.000 | | -2% | 0.171 | | 2.6% | 0.000 | | -17% | 0.000 | | -0.1% | 0.569 | | 0.3% | 0.167 |
| | *B20* | 0.275 | -11% | 0.000 | 0.724 | -3% | 0.078 | 2.145 | 6.6% | 0.000 | 0.061 | -25% | 0.000 | 627.2 | 0.4% | 0.309 | 0.064 | 1.4% | 0.001 |
| | *B50* | 0.219 | -29% | 0.000 | 0.720 | -4% | 0.038 | 2.278 | 13.2% | 0.000 | 0.044 | -46% | 0.000 | 628.2 | 0.5% | 0.159 | 0.066 | 3.1% | 0.000 |
| | *B100* | 0.115 | -63% | 0.000 | 0.770 | 3% | 0.163 | 2.547 | 26.6% | 0.000 | 0.034 | -58% | 0.000 | 634.0 | 1.5% | 0.007 | 0.068 | 6.8% | 0.000 |
| 40 mph Cruise | *CARB* | 0.247 | | | 0.599 | | | 2.030 | | | 0.049 | | | 572.6 | | | 0.058 | | |
| | *B5* | 0.249 | -1% | 0.573 | 0.615 | 2% | 0.427 | 2.062 | 1.7% | 0.135 | 0.045 | -6% | 0.101 | 582.8 | 1.7% | 0.085 | 0.059 | 1.9% | 0.065 |
| | *B20* | 0.207 | -16% | 0.000 | 0.582 | -3% | 0.160 | 2.109 | 3.9% | 0.000 | 0.036 | -26% | 0.000 | 577.4 | 0.8% | 0.056 | 0.059 | 1.8% | 0.001 |
| | *B50* | 0.158 | -36% | 0.000 | 0.599 | 0% | 0.986 | 2.214 | 9.1% | 0.000 | 0.026 | -48% | 0.000 | 580.0 | 1.3% | 0.053 | 0.060 | 3.8% | 0.000 |
| | *B100* | 0.075 | -70% | 0.000 | 0.602 | 0% | 0.868 | 2.454 | 20.9% | 0.000 | 0.015 | -69% | 0.000 | 589.9 | 3.0% | 0.000 | 0.063 | 8.4% | 0.000 |
| 50 mph Cruise | *CARB* | 0.185 | | | 0.471 | | | 1.733 | | | 1.733 | | | 544.8 | | | 0.055 | | |
| | *B5* | 0.183 | -2% | 0.222 | 0.478 | 1% | 0.649 | 1.727 | -1.1% | 0.588 | 0.051 | -5% | 0.036 | 544.9 | 0.0% | 0.959 | 0.056 | 0.3% | 0.690 |
| | *B20* | 0.164 | -12% | 0.000 | 0.462 | -2% | 0.330 | 1.741 | 0.5% | 0.800 | 1.741 | -18% | 0.000 | 547.8 | 0.6% | 0.227 | 0.056 | 1.6% | 0.002 |
| | *B50* | 0.128 | -31% | 0.000 | 0.442 | -6% | 0.002 | 1.842 | 6.3% | 0.001 | 0.031 | -43% | 0.000 | 551.4 | 1.2% | 0.008 | 0.057 | 3.8% | 0.000 |
| | *B100* | 0.059 | -68% | 0.000 | 0.404 | -14% | 0.000 | 2.050 | 18.3% | 0.000 | 0.027 | -50% | 0.000 | 558.9 | 2.6% | 0.000 | 0.060 | 8.0% | 0.000 |

** **Bold** percentage differences are considered statistically significant (p≤ 0.05. 95% confidence level)*

## Table 5. Summary of Average Emissions Results for Soy-Biodiesel Blends (2007 MBE4000)*

| | | THC | | | CO | | | NOx | | | PM | | | CO2 | | | BSFC | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cycle | Fuel | Avg g/bhp-hr | % Diff | P Value | Avg g/bhp-hr | % Diff | P Value | Avg g/bhp-hr | % Diff | P Value | Avg g/bhp-hr | % Diff | P Value | Avg g/bhp-hr | % Diff | P Value | Avg g/bhp-hr | % Diff | P Value |
| UDDS | *CARB* | 0.023 | | | 0.022 | | | 2.378 | | | 0.004 | | | 730.031 | | | 0.074 | | |
| | *B20* | 0.021 | -11% | 0.770 | 0.008 | -62% | 0.453 | 2.482 | 4.4% | 0.005 | 0 | -94% | 0.187 | 730.195 | 0.0% | 0.971 | 0.075 | 1.0% | 0.121 |
| | *B50* | 0.030 | 27% | 0.400 | -0.003 | -111% | 0.154 | 2.743 | 15.3% | 0.000 | 0.004 | 9% | 0.874 | 736.776 | 0.9% | 0.334 | 0.076 | 2.5% | 0.083 |
| | *B100* | 0.019 | -18% | 0.683 | 0.007 | -67% | 0.491 | 3.249 | 36.6% | 0.000 | 0.002 | -37% | 0.470 | 766.186 | 5.0% | 0.000 | 0.08 | 8.3% | 0.000 |
| FTP | *CARB* | 0.004 | | | 0.081 | | | 1.285 | | | 0.001 | | | 578.891 | | | 0.059 | | |
| | *B5* | 0.006 | 38% | 0.005 | 0.061 | -20% | 0.135 | 1.307 | 0.9% | 0.007 | 0 | -61% | 0.096 | 580.317 | 0.0% | 0.398 | 0.059 | 0.3% | 0.113 |
| | *B20* | 0.006 | 33% | 0.005 | 0.091 | -13% | 0.534 | 1.361 | 5.9% | 0.000 | 0.001 | -4% | 0.944 | 578.65 | 0.0% | 0.909 | 0.059 | 1.0% | 0.016 |
| | *B50* | 0.006 | 25% | 0.018 | 0.040 | -50% | 0.031 | 1.481 | 15.3% | 0.000 | 0.001 | 58% | 0.216 | 579.867 | 0.2% | 0.722 | 0.06 | 1.7% | 0.034 |
| | *B100* | 0.005 | 20% | 0.081 | 0.021 | -74% | 0.002 | 1.774 | 38.1% | 0.000 | 0.001 | 64% | 0.403 | 592.639 | 2.4% | 0.000 | 0.062 | 5.6% | 0.000 |
| 50 mph Cruise | *CARB* | 0.003 | | | 0.015 | | | 1.21 | | | 0.001 | | | 505.763 | | | 0.051 | | |
| | *B20* | 31.151 | -5% | 0.801 | 0.003 | -6% | 0.809 | 0.014 | 6.9% | 0.000 | 1.293 | -19% | 0.746 | 508.004 | 0.4% | 0.249 | 0.052 | 1.5% | 0.002 |
| | *B50* | 0.003 | -20% | 0.430 | 0.010 | -33% | 0.302 | 1.43 | 18.2% | 0.000 | 0.001 | 2% | 0.970 | 507.535 | 0.4% | 0.548 | 0.052 | 1.9% | 0.081 |
| | *B100* | 0.003 | -13% | 0.594 | 0.012 | -21% | 0.508 | 1.78 | 47.1% | 0.000 | 0 | -100% | 0.704 | 518.93 | 2.6% | 0.000 | 0.054 | 5.9% | 0.000 |

** **Bold** percentage differences are considered statistically significant (p≤ 0.05. 95% confidence level)*

## Table 6. Summary of Average Emissions Results for Animal-Biodiesel Blends (2006 Cummins ISM)*

| | | THC | | | CO | | | NOx | | | PM | | | CO2 | | | BSFC | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cycle | Fuel | Avg g/bhp-hr | % Diff | P Value | Avg g/bhp-hr | % Diff | P Value | Avg g/bhp-hr | % Diff | P Value | Avg g/bhp-hr | % Diff | P Value | Avg g/bhp-hr | % Diff | P Value | Avg g/bhp-hr | % Diff | P Value |
| UDDS | *CARB* | 0.799 | | | 2.052 | | | 6.010 | | | 0.065 | | | 841.3 | | | 0.086 | | |
| | *B20* | 0.670 | -16% | 0.000 | 1.842 | -10% | 0.000 | 5.923 | -1.5% | 0.376 | 5.923 | -10% | 0.009 | 836.3 | -0.1% | 0.640 | 0.087 | 1.2% | 0.404 |
| | *B50* | 0.495 | -38% | 0.000 | 1.800 | -12% | 0.000 | 6.018 | 0.1% | 0.935 | 6.018 | -24% | 0.001 | 851.1 | 1.2% | 0.201 | 0.089 | 3.1% | 0.005 |
| | *B100* | 0.214 | -73% | 0.000 | 1.634 | -20% | 0.000 | 6.127 | 1.9% | 0.243 | 6.127 | -31% | 0.000 | 862.4 | 2.5% | 0.016 | 0.092 | 6.7% | 0.000 |
| FTP | *CARB* | 0.303 | | | 0.712 | | | 2.075 | | | 0.076 | | | 627.5 | | | 0.064 | | |
| | *B5* | 0.295 | -3% | 0.011 | 0.686 | -4% | 0.008 | 2.089 | 0.3% | 0.298 | 0.070 | -9% | 0.000 | 624.7 | -0.3% | 0.191 | 0.067 | 2.9% | 0.031 |
| | *B20* | 0.263 | -13% | 0.000 | 0.665 | -7% | 0.000 | 2.106 | 1.5% | 0.000 | 0.062 | -19% | 0.000 | 628.2 | 0.1% | 0.733 | 0.065 | 1.4% | 0.145 |
| | *B50* | 0.194 | -36% | 0.000 | 0.609 | -14% | 0.000 | 2.208 | 6.4% | 0.000 | 0.044 | -42% | 0.000 | 630.4 | 0.4% | 0.117 | 0.066 | 1.8% | 0.038 |
| | *B100* | 0.087 | -71% | 0.000 | 0.522 | -27% | 0.000 | 2.368 | 14.1% | 0.000 | 0.027 | -64% | 0.000 | 632.1 | 0.7% | 0.018 | 0.067 | 4.4% | 0.001 |
| 50 mph Cruise | *CARB* | 0.180 | | | 0.469 | | | 1.788 | | | 0.056 | | | 544.7 | | | 0.056 | | |
| | *B20* | 0.155 | -14% | 0.000 | 0.437 | -7% | 0.003 | 1.748 | -2.3% | 0.151 | 0.047 | -16% | 0.000 | 548.7 | 0.7% | 0.170 | 0.057 | 2.6% | 0.010 |
| | *B50* | 0.114 | -37% | 0.000 | 0.426 | -9% | 0.066 | 1.802 | 0.8% | 0.588 | 0.036 | -35% | 0.000 | 552.8 | 1.5% | 0.014 | 0.058 | 3.5% | 0.000 |
| | *B100* | 0.049 | -73% | 0.000 | 0.354 | -25% | 0.000 | 1.883 | 5.3% | 0.000 | 0.023 | -59% | 0.000 | 553.1 | 1.6% | 0.008 | 0.059 | 5.9% | 0.000 |

* ***Bold*** *percentage differences are considered statistically significant (p≤ 0.05. 95% confidence level)*

## Table 7. Summary of Average Emissions Results for Animal-Biodiesel Blends (2007 MBE4000)*

| | | THC | | | CO | | | NOx | | | PM | | | CO2 | | | BSFC | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cycle | Fuel | Avg g/bhp-hr | % Diff | P Value | Avg g/bhp-hr | % Diff | P Value | Avg g/bhp-hr | % Diff | P Value | Avg g/bhp-hr | % Diff | P Value | Avg g/bhp-hr | % Diff | P Value | Avg g/bhp-hr | % Diff | P Value |
| UDDS | *CARB* | 0.026 | | | 0.013 | | | 2.414 | | | 0 | | | 733.64 | | | 0.074 | | |
| | *B20* | 0.034 | 33% | 0.000 | 0.016 | 18% | 0.003 | 2.454 | 1.6% | 0.000 | 0 | 224% | 0.779 | 733.891 | 0.0% | 0.000 | 0.075 | 0.2% | 0.000 |
| | *B50* | 0.03 | 8% | 0.695 | -0.003 | -16% | 0.875 | 2.743 | 7.3% | 0.000 | 0.004 | 285% | 0.219 | 736.776 | 1.0% | 0.024 | 0.076 | 1.2% | 0.008 |
| | *B100* | 0.027 | 6% | 0.755 | 0.028 | 109% | 0.238 | 2.801 | 16.0% | 0.000 | 0.004 | 1043% | 0.000 | 745.008 | 1.5% | 0.009 | 0.08 | 8.1% | 0.000 |
| FTP | *CARB* | 0.005 | | | 0.084 | | | 1.29 | | | 0 | | | 581.328 | | | 0.059 | | |
| | *B5* | 0.006 | 13% | 0.612 | 0.072 | -11% | 0.202 | 1.314 | 1.3% | 0.000 | 0 | -32% | 0.553 | 584.678 | 0.3% | 0.007 | 0.059 | 0.5% | 0.001 |
| | *B20* | 0.006 | 13% | 0.376 | 0.082 | -3% | 0.841 | 1.354 | 5.0% | 0.000 | 0 | -40% | 0.341 | 581.70 | 0.1% | 0.743 | 0.059 | 0.3% | 0.182 |
| | *B50* | 0.028 | -13% | 0.568 | 0.011 | -39% | 0.040 | 2.592 | 12.1% | 0.000 | 0.001 | 15% | 0.757 | 740.725 | 0.2% | 0.391 | 0.075 | 0.4% | 0.069 |
| | *B100* | 0.006 | 5% | 0.756 | 0.023 | -73% | 0.000 | 1.669 | 29% | 0.000 | 0 | -24% | 0.611 | 590.872 | 1.6% | 0.000 | 0.064 | 8% | 0.000 |
| 50 mph Cruise | *CARB* | 0.003 | | | 0.018 | | | 1.224 | | | 0.001 | | | 508.127 | | | 0.052 | | |
| | *B20* | 0.004 | 17% | 0.425 | 0.017 | -7% | 0.733 | 1.297 | 5.9% | 0.000 | 0 | -49% | 0.143 | 508.356 | 0.0% | 0.837 | 0.052 | 0.2% | 0.301 |
| | *B50* | 0.003 | -13% | 0.448 | 0.012 | -36% | 0.144 | 1.424 | 16.3% | 0.000 | 0 | -58% | 0.103 | 510.231 | 0.4% | 0.150 | 0.052 | 0.6% | 0.036 |
| | *B100* | 0.003 | 3% | 0.905 | 0.008 | -55% | 0.027 | 1.706 | 39.4% | 0.000 | 0 | -39% | 0.237 | 514.60 | 1.3% | 0.002 | 0.056 | 7.8% | 0.000 |

* ***Bold*** *percentage differences are considered statistically significant (p≤ 0.05. 95% confidence level)*

b. Toxic Air Contaminants

ARB identified diesel PM as a toxic air contaminant in 1998, and determined that diesel PM accounts for about 70% of the toxic risk from all identified toxic air contaminants. As previously stated, test results showed that the use of biodiesel reduces PM emissions with increasing blend levels.

Other toxic emissions tests were conducted for various carbonyls, volatile organic compounds (VOCs), and polycyclic aromatic hydrocarbons (PAHs). Overall, results show decreases in most PAHs and VOCs. Carbonyl emissions did not show consistent trends between different fuels. Genotoxicity assays were also performed and results showed either reduced toxicity compared to CARB diesel or no difference in toxicity.

The VOC emissions measured for the chassis testing included benzene, toluene, ethyelbenzene, 1,3-butadiene, *m-/p*-xylene, and *o*-xylene. The VOC emissions typically showed trends for the higher biodiesel blend levels, with emissions for biodiesel being lower than CARB diesel. Generally, the reductions in aromatic VOCs were consistent with the reduction in aromatics in the fuel. For lower biodiesel blend levels, the differences with CARB diesel were typically not significant.[28]

PAH emissions in the particulate and vapor phase were measured during chassis testing. The most abundant PAHs measured were naphthalene, 2-methylnaphthalene, and 1-methylnaphthalene. In all fuels and blends, the emissions of these three compounds accounted for approximately 70% to 80% of all PAHs measured. The results for the 2000 Caterpillar C-15 vehicle showed statistically significant reductions in naphthalene as a function of increasing blend level. More specifically, the reductions in soy B100 and animal B100 relative to CARB diesel were 52% and 44%, respectively. Results also showed statistically significant reductions in 1-Methylnaphthalene as a function of increasing biodiesel blend level for the 2000 Caterpillar C-15. Reductions in soy B100 and animal B100 relative to CARB diesel were 46% and 67%, respectively.[29]

Carbonyl emissions results showed general reductions for most species, including formaldehyde, acetaldehyde, acetone, and m-tolualdehyde. Test results showed that formaldehyde and acetaldehyde were the most prominent carbonyls. Acetone emissions were also prominent for the 2000 Caterpillar C-15. Overall, carbonyl emissions did not show consistent trends as a function of biodiesel blend level.[30]

Genotoxicity analyses were also conducted under the CARB Emissions Study. Mutagen emissions generally decreased as a function of increasing biodiesel blend level. Mutagen emissions were tested with tester strains TA98 or TA100. For particle

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[28] Durbin. T.D. et al, *CARB Assessment of the Emissions from the Use of Biodiesel as a Motor Vehicle Fuel in California, "Biodiesel Characterization and NOx Mitigation Study."* Oct 2011, Li, 234.
[29] Durbin. T.D. et al, *CARB Assessment of the Emissions from the Use of Biodiesel as a Motor Vehicle Fuel in California, "Biodiesel Characterization and NOx Mitigation Study."* Oct 2011. 193, 194, 202, 203.
[30] Durbin. T.D. et al, *CARB Assessment of the Emissions from the Use of Biodiesel as a Motor Vehicle Fuel in California, "Biodiesel Characterization and NOx Mitigation Study."* Oct 2011, 164,169,170.

samples tested in TA98, results showed that mutagen emissions decrease with increasing levels of soy biodiesel. For animal biodiesel, results showed an increase in emissions compared to CARB diesel. Similar trends were seen in tester strain TA100.[31]

c. Greenhouse Gas Emissions

GHG emissions are primarily $CO_2$, methane ($CH_4$), nitrous oxide ($N_2O$), and hydrofluorocarbons.[32] GHG emissions from the use of fuels are primarily $CO_2$.[33] The ARB Emissions Study included emissions measurements for $CO_2$. As previously stated, average tailpipe $CO_2$ emissions showed a slight increase for higher biodiesel blends. However, this measured increase in $CO_2$ emissions does not necessarily suggest that the fuels lead to an overall increase in carbon emissions. Most THC and CO convert to $CO_2$ in the atmosphere, so total $CO_2$ produced by the biodiesel combustion process is determined by direct $CO_2$ emissions, as well as THC and CO.

The fate of most fatty acids in plants or animals is metabolism by animals or microorganism to produce energy and $CO_2$. Production of biodiesel fuel reduces the amount of $CO_2$ produced by energy metabolism. Combustion of the fatty acid moiety of biodiesel produces an amount of $CO_2$ that is approximately equal this reduction in $CO_2$ production. The presence of methanol esterified to fatty acids may lead to a small increase in $CO_2$ releases from production and use of biodiesel fuel. However, the net increase in $CO_2$ releases from production and use of biodiesel is far less than net $CO_2$ releases from production and use of an equivalent amount of petroleum-based diesel.

Life cycle GHG emissions include emissions associated with the production, transportation, and use of a fuel in a motor vehicle. The life cycle analysis (LCA) of a fuel includes direct emissions from producing, transporting, and using the fuel, as well as other indirect effects, including land use change. Depending on the fuel, GHG emissions from each step of the life cycle can include $CO_2$, $CH_4$, $N_2O$, and other GHG contributors. The "carbon intensity" of a fuel represents the equivalent amount of $CO_2$ emitted from each stage of the fuel's life cycle and is expressed in terms of grams of $CO_2$ equivalent per megajoule ($gCO_2e/MJ$).[34]

In contrast, end-of-pipe or tailpipe emissions only include exhaust emissions associated with the use of a fuel in an internal combustion engine.[35] Tailpipe $CO_2$ emissions are only one component in determining a fuel's life cycle carbon emissions. Therefore, the

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[31] Durbin. T.D. et al, *CARB Assessment of the Emissions from the Use of Biodiesel as a Motor Vehicle Fuel in California, "Biodiesel Characterization and NOx Mitigation Study."* October 2011. Liii, 215

[32] Air Resources Board. *Staff Report: Initial Statement of Reasons for Proposed Rulemaking, Public Hearing to Consider Adoption of Regulations to Control Greenhouse Gas Emissions from Motor Vehicles*. August 6, 2004, i.

[33] Air Resources Board. *Proposed Re-Adoption of the Low Carbon Fuel Standard*. *Staff Report: Initial Statement of Reasons*. December 2014, ES-2.

[34] Air Resources Board. *Proposed Re-Adoption of the Low Carbon Fuel Standard. Staff Report: Initial Statement of Reasons.* December 2014.

[35] Air Resources Board. *Proposed Regulation to Implement the Low Carbon Fuel Standard. Staff Report: Initial Statement of Reasons.* 2009, IV-12.

measured increase in $CO_2$ emissions may not necessarily lead to an overall increase in carbon emissions. An increase in $CO_2$ reflects more complete combustion, and is an expected result of decreased THC and CO emissions.

Under the ARB Emissions Study, biodiesel blends also showed an increase in average brake specific fuel consumption (BSFC) with increasing levels of biodiesel. This is consistent with expectations based on the lower energy density of biodiesel. The changes in fuel consumption for soy-based biodiesel blends for the 2006 Cummins engine range from 1.4% to 1.8% for B20 and 6.8% to 9.8% for B100. The changes in fuel consumption for animal-based biodiesel blends for the 2006 Cummins engine range from no statistical difference to 2.6% for B20 and 4.4% to 6.7% for B100.

For an alternative fuel, the determination of GHG emissions impact is the result of a full LCA of the fuel. For biodiesel, the outcome of the analysis is greatly dependent on the feedstock source. The LCA of biodiesel under the Low Carbon Fuel Standard (LCFS) showed reductions in GHG emissions of about 15% to 95% depending on feedstock source.[36] The LCFS has six LCA pathways that were developed for biodiesel. Table 8 shows the CI values of diesel and biodiesel in the LCFS.[37]

**Table 8. Carbon Intensity Values for Biodiesel Compared to CARB Diesel**

| **Fuel and Pathway Description** | **Direct CI** ($gCO_2e/MJ$) | **Indirect CI** ($gCO_2e/MJ$) | **Total CI** ($gCO_2e/MJ$) |
|---|---|---|---|
| Diesel – ULSD based on the average crude oil supplied to CA refineries and average CA refinery efficiencies | 98.03 | 0 | 98.03 |
| Biodiesel – Conversion of waste oils (used cooking oil) to biodiesel where “cooking” is required | 15.84 | 0 | 15.84 |
| Biodiesel – Conversion of waste oils (used cooking oil) to biodiesel where “cooking” is not required | 11.76 | 0 | 11.76 |
| Biodiesel – Conversion of Midwest soybeans to biodiesel | 21.25 | 62 | 83.25 |
| Biodiesel – Conversion of waste oils (used cooking oil) to biodiesel where “cooking” is required. Fuel produced in the Midwest. | 18.72 | 0 | 18.72 |
| Biodiesel – Conversion of waste oils (used cooking oil) to biodiesel where “cooking” is not required. Fuel produced in the Midwest. | 13.83 | 0 | 13.83 |
| Biodiesel – Conversion of corn oil, extracted from distillers grains prior to the drying processes, to biodiesel. | 4.00 | 0 | 4.00 |

[36] Air Resources Board, LCFS Carbon Intensity Lookup Table, December 2012.
[37] California Air Resources Board, *LCFS Carbon Intensity Lookup Table*, December 2012. http://www.arb.ca.gov/fuels/lcfs/lu_tables_11282012.pdf (accessed February 12, 2015).

d. Ozone Precursors

Ozone is produced by photochemical reactions. Its precursor components are primarily the result of road traffic. Unlike many of the other GHGs, ozone is a short-lived gas that is found in regionally varying concentrations. Nevertheless, it is the third most important anthropogenic GHG behind $CO_2$ and $CH_4$.[38]

Both THC and NOx emissions determine ozone concentrations. As previously stated, THC emissions showed consistent and significant reductions with the magnitude of the reductions increasing with blend level. However, NOx was found to increase at certain biodiesel blend levels.

The results of both the ARB Emissions Study and B5/B10 Study apply specifically to heavy-duty vehicles that do not use post-exhaust NOx emissions control. Therefore, the results of this study should not be extended to New Technology Diesel Engines (NTDEs) or light-duty and medium-duty vehicles.

Engines that meet the latest emission standards through the use of Selective Catalytic Reduction (SCR) systems have been shown to have no significant difference in NOx emissions based on the fuel used. A study conducted by the National Renewable Energy Laboratory looked at two Cummins ISL engines that were equipped with SCR and found that NOx emissions control eliminates fuel effects on NOx, even for B100.[39]

Light-duty and medium-duty vehicles have similarly been found not to experience increases in NOx due to the use of biodiesel. For example, a study performed on three light-duty vehicles using different biodiesel blends found no significant and consistent pattern in NOx emissions based on blend levels across the different engines, blends, and cycles.[40]

ii. <u>B5/B10 Study</u>

a. General Results

Criteria pollutants and ozone precursors including PM, NOx, CO, and THC were measured for soy and animal B5 and B10 biodiesel blends. Consistent with the results from the ARB Emissions Study, test results showed statistically significant reductions of PM, CO, and THC. NOx emissions results showed statistically significant increases and are the focus of staff's analysis. Chapter 3 of the B5/B10 Study Final Report[41] provides detailed results.

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[38] Air Resources Board. Proposed Rulemaking, *Public Hearing to Consider Adoption of Regulations to Control Greenhouse Gas Emissions from Motor Vehicles*. August 6, 2004, i.
[39] Lammert et al., *Effect of B20 and Low Aromatic Diesel on transit Bus NOx emissions Over Driving Cycles with a Range of Kinetic Intensity*, SAE Int. J Fuels Lubr., 5(3):2012
[40] Air Resources Board. *Proposed Regulation on the Commercialization of New Alternative Diesel Fuels Staff Report: Initial Statement of Reasons*. January 2, 2015. Page 45.
[41] Durbin. T.D. et al, *CARB Assessment of the Emissions from the Use of Biodiesel as a Motor Vehicle Fuel in California, "Biodiesel Characterization and NOx Mitigation Study."* October 2011. Chapter 3.

b. NOx Emissions

For the 2006 Cummins ISM engine over the FTP cycle, NOx emissions results showed a statistically significant increase of 1.0% and 1.9% for the soy B5 and B10 blends, respectively. For the UDDS cycle, only the soy B10 blend showed a statistically significant increase of 3.6% compared to the CARB diesel fuel, but did not show statistically significant differences for the soy B5 blend.

For the 1991 DDC Series 60 engine over the FTP and UDDS cycles, soy B5 emissions results showed a statistically significant increase of 1.0% and 3.2%, respectively. Similarly, soy B10 emissions results showed a statistically significant increase of 1.5% and 1.3% over the FTP and SET cycles, respectively. The animal B5 blend did not show any statistically significant differences for any of the three cycles. The animal B10 blend showed a statistically significant increase of 0.7% for the FPT, but did not show statistically significant differences for the other cycles.[42]

[42] Durbin. T.D. et al, *CARB Assessment of the Emissions from the Use of Biodiesel as a Motor Vehicle Fuel in California, "Biodiesel Characterization and NOx Mitigation Study."* October 2011. Page 7.

# 4. SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

In this chapter, ARB staff provides the multimedia evaluation air quality assessment and emissions impact summary, conclusions, and recommendations.

## A. Summary

ARB staff completed an air quality assessment of biodiesel fuel. The evaluation includes a description of the biodiesel emissions test program and impact analysis on criteria pollutants, toxic air contaminants, and ozone precursors. The complete report is provided in Appendix C.

Staff's assessment is based on the data and information provided for the biodiesel multimedia evaluation, including the UC researchers' multimedia reports (Final Tier I, Tier II and Tier III reports); the ARB Emissions Study[43] by UC Riverside from emissions testing conducted at CE-CERT and ARB emissions test facilities in Stockton and El Monte, California; and the B5/B10 Study[44] by ARB, UC Riverside, and UC Davis. The B5/B10 Study expands on the ARB Emissions Study to provide more comprehensive information on the emissions of lower biodiesel blends.

As part of the ARB Emissions Study and subsequent B5/B10 Study, emissions testing were conducted on biodiesel (B100) and various biodiesel blends (B5, B10, B20, B50) with CARB diesel as the baseline fuel. For the ARB Emissions Study, test fuels include five primary fuels that were subsequently blended at various levels to comprise the full test matrix. Two biodiesel feedstocks were used for testing, including one soy-based and one animal-based biodiesel fuel. These fuels were selected to provide a range of properties that are representative of typical feedstocks representing different characteristics of biodiesel in terms of cetane number and degree of saturation.[45]

The ARB Emissions Study included both engine testing and chassis testing. Engine testing was performed on a 2006 Cummins ISM engine and a 2007 MBE4000 engine. Chassis testing was performed on the following test vehicles:

- 2006 International Truck equipped with a 2006 Cummins ISM engine
- 2008 Freightliner Truck equipped with a 2007 MBE4000 engine
- 2000 Freightliner Truck equipped with a 2000 Caterpillar C-15 engine
- Kenworth model T800 truck equipped with a 2010 Cummins ISX engine

Test engines included typical six cylinder, in-line, direct injection, turbocharged, heavy-duty diesel engines. The engines were selected from two model year categories;

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[43] Durbin, T.D. et al. *CARB Assessment of the Emissions from the Use of Biodiesel as a Motor Vehicle Fuel in California "Biodiesel Characterization and NOx Mitigation Study."* October 2011.
[44] Karavalakis, G. et al. *CARB Comprehensive B5/B10 Blends Heavy-Duty Engine Dynamometer Testing.* June 2014.
[45] Durbin, T.D. et al. *CARB Assessment of the Emissions from the Use of Biodiesel as a Motor Vehicle Fuel in California "Biodiesel Characterization and NOx Mitigation Study."* October 2011, xxv.

2002-2006 and 2007-2009. The 2002-2006 engines were estimated to represent an important contribution to the emissions inventory from the present through 2017. The 2007-2009 model year engine represented the latest technology that was available at the time of testing.[46]

1. Air Emissions

The emissions measurements for the engine testing under the ARB Emissions Study focused primarily on regulated emissions, including PM, NOx, THC, CO, and $CO_2$. More extensive testing, including toxics analyses, was completed for the chassis testing.

Average PM emissions results showed consistent and significant reductions for all biodiesel blends, with the magnitude of reductions increasing with blend level. For the 2006 Cummins engine over the Federal Test Procedure (FTP) test cycle, PM reductions for soy-based biodiesel were approximately 6% for B5, 25% for B20, and 58% for B100. For animal-based biodiesel, PM reductions ranged from 19% for B20 to 64% for B100.

Average NOx emissions showed trends of increasing NOx emissions with increasing biodiesel blend level. Soy-based biodiesel blends showed a higher increase in NOx emissions for essentially all blend levels and test cycles compared to animal-based biodiesel blends. For soy-based biodiesel over the FTP cycle, results for the 2006 Cummins engine showed NOx increases of 2.2% for B5, 6.6% for B20, and 27% for B100. Animal-based biodiesel results showed NOx increases of 1.5% for B20 to 14% for B100. For the 2007 MBE4000 engine, NOx increases were greater than those of the 2006 engine for nearly all biodiesel blends and test cycles.

Average THC emissions for the 2006 Cummins showed consistent and significant reductions for all biodiesel blends, with the magnitude of reductions increasing with blend level. For soy-based biodiesel over the FTP cycle, THC emissions showed 6% reductions for B10, 11% for B20, and 63% for B100. For animal-based biodiesel, THC reductions ranged from 13% for B20 to 71% for B100.

Average CO emissions also showed consistent and significant reductions for animal-based biodiesel, ranging from 7% for B5, 14% for B20, and 27% for B100. For soy-based biodiesel, CO trends were less consistent with some results not statistically significant.

Average tailpipe $CO_2$ emissions showed a slight increase for the higher biodiesel blends. For the 2006 Cummins engine, the increase ranged from about 1% to 4%, with increases being statistically significant for the B100 fuels for all tests, the B50 fuel for the cruise cycles, and other testing combinations. For the 2007 MBE 4000 engine, only the B100 showed consistent and statistically significant increases for the different cycles, ranging from 1% to 5%.

[46] Durbin, T.D. et al. *CARB Assessment of the Emissions from the Use of Biodiesel as a Motor Vehicle Fuel in California "Biodiesel Characterization and NOx Mitigation Study."* October 2011, 5-6.

The biodiesel blends showed an increase in fuel consumption with increasing biodiesel blends. The fuel consumption differences were generally greater for the soy-based biodiesel in comparison with animal-based biodiesel for the 2006 Cummins engine, but not for the 2007 MBE 4000 engine. For the 2006 Cummins engine, changes in fuel consumption for soy-based biodiesel blends ranged from 1.4% to 1.8% for B20 and 6.8% to 9.8% for B100. Animal-based biodiesel blends ranged from no statistical difference to 2.6% for B20 and 4.4% to 6.7% for B100. For the 2007 MBE4000 engine, the differences in fuel consumption ranged from no change to 2.5% for B50 and lower blends, while the increases for B100 blends ranged from 5.6% to 8.3%.

Additionally, on a federal level, a U.S. EPA biodiesel exhaust emissions study found beneficial impacts associated with biodiesel use. Tailpipe emissions from heavy-duty engines were compiled and analyzed. Compared to federal diesel, data showed approximately 10% to 20% emissions reductions of PM, CO and HC from biodiesel blends of B20 and approximately 45% to 65% range reductions from B100.[47]

### 2. Toxic Air Contaminants

ARB identified diesel PM as a toxic air contaminant in 1998, and determined that diesel PM accounts for about 70% of the toxic risk from all identified toxic air contaminants. As previously stated, test results showed that the use of biodiesel reduces PM emissions with increasing blend levels.

Other toxic emissions tests were conducted for various carbonyls, VOCs, and polycyclic PAHs. Overall, results show decreases in most PAHs and VOCs. Carbonyl emissions did not show consistent trends between different fuels. Genotoxicity assays were also performed and results showed either reduced toxicity compared to CARB diesel or no difference in toxicity.

### 3. Greenhouse Gas Emissions

GHG emissions are primarily $CO_2$, $CH_4$, $N_2O$, and hydrofluorocarbons.[48] GHG emissions from the use of fuels are primarily $CO_2$.[49] The ARB Emissions Study included emissions measurements for $CO_2$. As previously stated, average tailpipe $CO_2$ emissions showed a slight increase for higher biodiesel blends. However, this measured increase in $CO_2$ emissions does not necessarily suggest that the fuels lead to an overall increase in carbon emissions. Most THC and CO convert to $CO_2$ in the atmosphere, so total $CO_2$ produced by the biodiesel combustion process is determined by direct $CO_2$ emissions, as well as THC and CO.

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[47] U.S. Environmental Protection Agency, *A Comprehensive Analysis of Biodiesel Impacts on Exhaust Emissions*, EPA420-P-02-001, October 2002.

[48] Air Resources Board. *Staff Report: Initial Statement of Reasons for Proposed Rulemaking, Public Hearing to Consider Adoption of Regulations to Control Greenhouse Gas Emissions from Motor Vehicles*. August 6, 2004, i.

[49] Air Resources Board. *Proposed Re-Adoption of the Low Carbon Fuel Standard*. *Staff Report: Initial Statement of Reasons*. December 2014, ES-2.

The fate of most fatty acids in plants or animals is metabolism by animals or microorganism to produce energy and $CO_2$. Production of biodiesel fuel reduces the amount of $CO_2$ produced by energy metabolism. Combustion of the fatty acid moiety of biodiesel produces an amount of $CO_2$ that is approximately equal this reduction in $CO_2$ production. The presence of methanol esterified to fatty acids may lead to a small increase in $CO_2$ releases from production and use of biodiesel fuel. However, the net increase in $CO_2$ releases from production and use of biodiesel is far less than net $CO_2$ releases from production and use of an equivalent amount of petroleum-based diesel.

Life cycle GHG emissions include emissions associated with the production, transportation, and use of a fuel in a motor vehicle. The life cycle analysis (LCA) of a fuel includes direct emissions from producing, transporting, and using the fuel, as well as other indirect effects, including land use change. Depending on the fuel, GHG emissions from each step of the life cycle can include $CO_2$, $CH_4$, $N_2O$, and other GHG contributors. The "carbon intensity" of a fuel represents the equivalent amount of $CO_2$ emitted from each stage of the fuel's life cycle and is expressed in terms of grams of $CO_2$ equivalent per megajoule ($gCO_2e/MJ$).[50]

In contrast, end-of-pipe or tailpipe emissions only include exhaust emissions associated with the use of a fuel in an internal combustion engine.[51] Tailpipe $CO_2$ emissions are only one component in determining a fuel's life cycle carbon emissions. Therefore, the measured increase in $CO_2$ emissions may not necessarily lead to an overall increase in carbon emissions. An increase in $CO_2$ reflects more complete combustion, and is an expected result of decreased THC and CO emissions.

Under the ARB Emissions Study, biodiesel blends also showed an increase in average BSFC with increasing levels of biodiesel. This is consistent with expectations based on the lower energy density of biodiesel. The changes in fuel consumption for soy-based biodiesel blends for the 2006 Cummins engine range from 1.4% to 1.8% for B20 and 6.8% to 9.8% for B100. The changes in fuel consumption for animal-based biodiesel blends for the 2006 Cummins engine range from no statistical difference to 2.6% for B20 and 4.4% to 6.7% for B100.

For an alternative fuel, the determination of GHG emissions impact is the result of a full LCA of the fuel. For biodiesel, the outcome of the analysis is greatly dependent on the feedstock source. The LCA of biodiesel under the Low Carbon Fuel Standard showed reductions in GHG emissions of about 15% to 95% depending on feedstock source.[52]

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[50] Air Resources Board. *Proposed Re-Adoption of the Low Carbon Fuel Standard. Staff Report: Initial Statement of Reasons.* December 2014.
[51] Air Resources Board. *Proposed Regulation to Implement the Low Carbon Fuel Standard. Staff Report: Initial Statement of Reasons.* 2009, IV-12.
[52] Air Resources Board, LCFS Carbon Intensity Lookup Table, December 2012.

4. Ozone Precursors

Ozone is produced by photochemical reactions. Its precursor components are primarily the result of road traffic. Unlike many of the other GHGs, ozone is a short-lived gas that is found in regionally varying concentrations. Nevertheless, it is the third most important anthropogenic GHG behind $CO_2$ and $CH_4$.[53]

Both THC and NOx emissions determine ozone concentrations. As previously stated, THC emissions showed consistent and significant reductions with the magnitude of the reductions increasing with blend level. However, NOx was found to increase at certain biodiesel blend levels.

The results of both the ARB Emissions Study and B5/B10 Study apply specifically to heavy-duty vehicles that do not use post-exhaust NOx emissions control. Therefore, the results of this study should not be extended to New Technology Diesel Engines (NTDEs) or light-duty and medium-duty vehicles.

Engines that meet the latest emission standards through the use of Selective Catalytic Reduction (SCR) systems have been shown to have no significant difference in NOx emissions based on the fuel used. A study conducted by the National Renewable Energy Laboratory looked at two Cummins ISL engines that were equipped with SCR and found that NOx emissions control eliminates fuel effects on NOx, even for B100.[54]

Light-duty and medium-duty vehicles have similarly been found not to experience increases in NOx due to the use of biodiesel. For example, a study performed on three light-duty vehicles using different biodiesel blends found no significant and consistent pattern in NOx emissions based on blend levels across the different engines, blends, and cycles.[55]

### B. Conclusions

Based on a relative comparison between biodiesel and CARB diesel, staff concludes that with in-use requirements biodiesel, as specified in the multimedia evaluation and proposed regulation, does not pose a significant adverse impact on public health or the environment from potential air quality impacts.

Staff also makes the following general conclusions:

- Biodiesel reduces PM emissions in diesel exhaust.

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[53] Air Resources Board. Proposed Rulemaking, *Public Hearing to Consider Adoption of Regulations to Control Greenhouse Gas Emissions from Motor Vehicles*. August 6, 2004, i.
[54] Lammert et al., *Effect of B20 and Low Aromatic Diesel on transit Bus NOx emissions Over Driving Cycles with a Range of Kinetic Intensity*, SAE Int. J Fuels Lubr., 5(3):2012
[55] Air Resources Board. *Proposed Regulation on the Commercialization of New Alternative Diesel Fuels Staff Report: Initial Statement of Reasons*. January 2, 2015. Page 45.

- Biodiesel reduces emissions and health risk from PM in diesel exhaust, a toxic air contaminant identified by ARB.
- Biodiesel reduces CO emissions in diesel exhaust.
- Biodiesel reduces THC emissions in diesel exhaust.
- Biodiesel at certain blend levels increases NOx emissions in diesel exhaust.
- In consideration of NOx, in-use requirements incorporated in the proposed ADF regulation will not result in any significant adverse impacts from biodiesel use.

In general, studies have found environmental benefits associated with biodiesel use as compared to use of CARB diesel fuel. Biodiesel is considered a low carbon fuel and supports GHG emission reductions. Biodiesel emits less CO, PM, THC, and air toxics than CARB diesel.

### C. Recommendations

Based on the air quality assessment and evaluation of emissions impacts from the use of biodiesel, ARB staff recommends that the CEPC find that the use of biodiesel, as described in the multimedia evaluation and the proposed regulation, does not pose a significant adverse impact on public health or the environment from potential air quality impacts, relative to CARB diesel fuel.

## 5. REFERENCES

Note: References are listed according to the corresponding footnote in the staff report. For references available online, electronic links have been provided. References used more than once are indicated as a duplicate (e.g., "Same as Footnote 2"), excluding specific page numbers, and are listed to maintain the order and numbering of the footnotes in the report.


1. Air Resources Board. *Proposed Regulation on the Commercialization of New Alternative Diesel Fuels Staff Report: Initial Statement of Reasons*. Appendix A: Proposed Regulation Order. January 2, 2015. http://www.arb.ca.gov/regact/2015/adf2015/adf15isor.pdf.

2. California Air Pollution Control Laws. Health and Safety Code, Division 26, Part 5, Chapter 4, Section 43830.8. http://www.arb.ca.gov/bluebook/bb11/hea/hea-43830_8.htm (accessed February 23, 2015).

3. Durbin, T.D. et al. *CARB Assessment of the Emissions from the Use of Biodiesel as a Motor Vehicle Fuel in California "Biodiesel Characterization and NOx Mitigation Study."* October 2011. http://www.arb.ca.gov/fuels/diesel/altdiesel/20111013_CARB%20Final%20Biodiesel%20Report.pdf (accessed February 23, 2015).

4. Karavalakis, G. et al. *CARB Comprehensive B5/B10 Blends Heavy-Duty Engine Dynamometer Testing.* June 2014. http://www.arb.ca.gov/fuels/diesel/altdiesel/20140630CARBStudyB5_B10.pdf (accessed February 23, 2015).

5. Ginn, T.R. et al. *California Biodiesel Multimedia Evaluation Final Tier I Report*, September 2009. http://www.arb.ca.gov/fuels/multimedia/meetings/Biodiesel_FinalTierIReport_Sep2009_110413.pdf (accessed November 4, 2013).

6. Ginn, T.R. et al. *California Biodiesel Multimedia Evaluation Final Tier II Report*, January 2012. http://www.arb.ca.gov/fuels/multimedia/meetings/Biodiesel_FinalTierII_Jan2012_110413.pdf (accessed November 4, 2013).

7. Ginn, T.R. et al. *California Biodiesel Multimedia Evaluation Final Tier III Report*, June 2014. http://www.arb.ca.gov/fuels/multimedia/meetings/Biodiesel_FinalReport_Jun2014.pdf (accessed February 24, 2015).

8. Same as Footnote 4.

9. Air Resources Board. *Proposed Regulation on the Commercialization of New Alternative Diesel Fuels Staff Report: Initial Statement of Reasons*. October 23, 2013. http://www.arb.ca.gov/regact/2013/adf2013/adf2013isor.pdf (accessed February 24, 2015).

10. Same as Footnote 7.

11. Same as Footnote 7.

12. Same as Footnote 9.

13. Same as Footnote 9.

14. U.S. Department of Energy, *Annual Energy Outlook 2012 Projection to* 2035, June 2012. http://www.eia.gov/forecasts/aeo/pdf/0383(2012).pdf (accessed February 24, 2015).

15. Same as Footnote 9.

16. Same as Footnote 9.

17. Same as Footnote 9.

18. Same as Footnote 9.

19. Coordinating Research Council, Inc., Report No. AVFL-17, Alpharetta, Georgia, pp. 16-18, *Investigation of Biodistillates as Potential Blendstocks for Transportation Fuels*, June 2009. http://crcao.org/reports/recentstudies2009/AVFL-17/AVFL-17%20Final%20Report%20June%202009.pdf (accessed February 24, 2015).

20. Biodiesel Magazine, Biodiesel Plants*. USA Plants*, California. Last Modified February 4, 2015. http://www.biodieselmagazine.com/plants/listplants/USA/page:1/sort:state/direction:asc (Accessed February 24, 2015).

21. Same as Footnote 9.

22. Same as Footnote 3.

23. Same as Footnote 3.

24. Same as Footnote 4.

25. Same as Footnote 3.

26. Same as Footnote 4.

27. U.S. Environmental Protection Agency, *A Comprehensive Analysis of Biodiesel Impacts on Exhaust Emissions*, Draft Technical Report. EPA420-P-02-001, October 2002. http://www.epa.gov/otaq/models/analysis/biodsl/p02001.pdf (accessed February 23, 2015).

28. Same as Footnote 3.

29. Same as Footnote 3.

30. Same as Footnote 3.

31. Same as Footnote 3.

32. Air Resources Board*. Staff Report: Initial Statement of Reasons for Proposed Rulemaking, Public Hearing to Consider Adoption of Regulations to Control Greenhouse Gas Emissions from Motor Vehicles*. August 6, 2004. http://www.arb.ca.gov/regact/grnhsgas/isor.pdf (accessed February 23, 2015).

33. Air Resources Board. *Proposed Re-Adoption of the Low Carbon Fuel Standard. Staff Report: Initial Statement of Reasons*. December 2014. http://www.arb.ca.gov/regact/2015/lcfs2015/lcfs15isor.pdf (accessed February 23, 2015).

34. Same as Footnote 33.

35. Air Resources Board. *Proposed Regulation to Implement the Low Carbon Fuel Standard. Staff Report: Initial Statement of Reasons.* March 2009. http://www.arb.ca.gov/regact/2009/lcfs09/lcfsisor1.pdf (accessed February 23, 2015).

36. Air Resources Board, *LCFS Carbon Intensity Lookup Table*, December 2012. http://www.arb.ca.gov/fuels/lcfs/lu_tables_11282012.pdf (accessed February 24, 2015).

37. Same as Footnote 36.

38. Same as Footnote 32.

39. Lammert et al. National Renewable Energy Laboratory. *Effect of B20 and Low Aromatic Diesel on transit Bus NOx emissions Over Driving Cycles with a Range of Kinetic Intensity*, SAE Int. 2012-01-1984. Published September 2012. http://www.nrel.gov/docs/fy13osti/55672.pdf (accessed February 23, 2015).

40. Same as Footnote 1.

41. Same as Footnote 3.

42. Same as Footnote 3.

43. Same as Footnote 3.

44. Same as Footnote 4.

45. Same as Footnote 3.

46. Same as Footnote 3.

47. Same as Footnote 27.

48. Same as Footnote 32.

49. Same as Footnote 33.

50. Same as Footnote 33.

51. Same as Footnote 35.

52. Same as Footnote 36.

53. Same as Footnote 32.

54. Same as Footnote 39.

55. Same as Footnote 1.

# APPENDIX D

## State Water Resources Control Board: Biodiesel Multimedia Evaluation

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CALIFORNIA
Water Boards

EDMUND G. BROWN JR.
GOVERNOR

MATTHEW RODRIQUEZ
SECRETARY FOR
ENVIRONMENTAL PROTECTION

**State Water Resources Control Board**

**TO:** Floyd Vergara
Chief, Alternative Fuels Branch
California Air Resources Board

**FROM:** Shahla Dargahi Farahnak, P.E.
Assistant Deputy Director
**Division of Water Quality**

**DATE:** August 26, 2013

**SUBJECT:** STAFF COMMENTS AND RECOMMENDATIONS ON BIODIESEL AND RENEWABLE DIESEL APPLICATION FOR MULTIMEDIA WORKING GROUP REVIEW

State Water Resources Control Board (State Water Board) staff have completed its review of the Biodiesel January 2009 Tier I Report, February 2012 Tier II Report and May 2013 Tier III Report. State Water Board staff have also completed its review of the Renewable Diesel September 2011 Tier I Report and April 2012 Tier III Report.

This memo transmits State Water Board staff comments and recommendations on the above mentioned Tier III reports.

If you have any questions regarding staff recommendations, please contact Laura Fisher, Chief of the UST Leak Prevention Unit at (916) 341- 5870 or laura.fisher@waterboards.ca.gov.

Attachments (2)

cc: Mr. Kevin L. Graves, Manager
UST and Site Cleanup Program
State Water Resources Control Board

Ms. Laura S. Fisher, Chief
UST Leak Prevention and
Office of Tank Tester Licensing
State Water Resources Control Board

Attachment #1

State Water Resources Control Board Staff Comments
Biodiesel Multimedia Evaluation

Below are comments on the California Biodiesel Multimedia Evaluation, May 2013, Tier III Report, prepared by the University of California, Davis, and the University of California, Berkeley.

## Background

State Water Resources Control Board (State Water Board) staff has reviewed the University of California, Davis and the University of California, Berkeley, Tier I, Tier II, and Tier III Reports. The multimedia evaluation and review of environmental impacts is specific to the difference between biodiesel and to California Air Resources Board (CARB) diesel.

Biodiesel is an alternative diesel derived from biological sources. To create biodiesel a biological feedstock is reacted with alcohol and a catalyst to produce Fatty Acid Methyl Ester and the byproduct glycerin. Fatty Acid Methyl Ester also known as biodiesel can be blended with CARB diesel; B100 refers to pure biodiesel, B20 refers to a blend of 20% pure biodiesel and 80% CARB diesel, and so on.

## Water Impacts

Based on a relative comparison between biodiesel and CARB diesel, as substantiated in the multimedia evaluation, State Water Board staff concludes:

- Aquatic toxicity screening with unadditized and additized biodiesel and biodiesel blends showed an increase in toxicity to subsets of screening species compared to CARB diesel.
- Water allocation and agricultural impacts associated with the growing of feedstocks used in the production of biodiesel were not considered as part of the multimedia evaluation. A supplemental multimedia review may need to be performed in the future to evaluate any agricultural and water resource impacts if feedstocks are to be grown in California.

## UST Material Compatibility and Leak Detection

Material compatibility testing has demonstrated that biodiesel and biodiesel blends are incompatible with various products commonly used in California's existing underground storage tank (UST) infrastructure. Incompatibility increases the risk of unauthorized releases, therefore material selection in UST equipment and leak detection technology is important to prevent releases. Material compatibility and leak detection functionality with a stored substance is a requirement of the UST laws and regulations, and verified by the local permitting agency with the UST owner or operator. Recently revised UST regulations allow the storage of substances not certified as compatible by an independent testing organization, typically Underwriters Laboratories (UL), if the manufacturer of the components provides affirmative statements of compatibility. This option however is limited to double-walled UST's. UL's current certification status of

biodiesel blends only includes blends up to B5. Therefore biodiesel blends up to B5 can be stored in both single or double-walled petroleum approved USTs. Blends above B5 may be stored in double-walled petroleum USTs when the manufacturer provides affirmative statements of compatibility.

Biodegradability and Fate/Transport

Multimedia evaluation identifies that unadditized biodiesel and biodiesel blends consistently show increased biodegradation as compared to CARB diesel, and that additized biodiesel and biodiesel blends can result in decreased biodegradation. These biodegradability scenarios are influenced by the additives used and biodiesel blend concentration.

Waste Discharge From Manufacturing

Chemicals used in the production and byproducts are required to comply with hazardous waste laws and regulations. No significant areas of concern have been identified by staff when comparing the waste streams of biodiesel to CARB diesel.

Conclusion and Recommendations

State Water Board staff concludes that given the information provided by University of California, Davis, and the University of California, Berkeley, there are minimal additional risks to beneficial uses of California waters posed by biodiesel than that posed by CARB diesel alone. State Water Board staff supports the multimedia evaluation of biodiesel which meets the ASTM fuel specifications and the finding of no significant adverse impacts on public health or the environment with the recommendations provided in the Biodiesel Multimedia Evaluation Staff Report.

As identified in the California Biodiesel Multimedia Evaluation Report, Tier III, the potential scope of any unanticipated impacts is difficult to determine due to the limited funding and time of the multimedia evaluation. Unanticipated risks and problems that may occur as full scale use of biodiesel becomes common will need to be addressed as they occur.

This recommendation is contingent upon biodiesel and biodiesel blends meeting the ASTM fuel specifications and using the same additives described in the California Biodiesel Diesel Multimedia Evaluation.

Attachment #2

# State Water Resources Control Board Staff Comments
# Renewable Diesel Multimedia Evaluation

Below are comments on the California Renewable Diesel Multimedia Evaluation, April 2012, Tier III Report, prepared by the University of California, Davis, and the University of California, Berkeley.

## Background

State Water Resources Control Board (State Water Board) staff has reviewed the University of California, Davis and the University of California, Berkeley, Tier I and Tier III Reports. The multimedia evaluation and review of environmental impacts is specific to the difference between renewable diesel and California Air Resources Board (CARB) diesel.

Renewable diesel is an alternative diesel derived from non-petroleum sources. Renewable diesel is free of ester compounds and has a chemical composition that is almost identical to petroleum based diesel. To produce renewable diesel, a feedstock is converted into diesel fuel through a catalytic treatment that adds hydrogen. Hydrogenated-derived renewable diesel is then refined, typically at existing oil refineries. Renewable diesel can be blended with CARB diesel to create various renewable diesel blends.

## Water Impacts

Aquatic toxicity was considered by comparing renewable diesel and CARB diesel. State Water Board staff reviewed the data comparing the effects of renewable diesel and CARB diesel when exposed to a series of aquatic toxicity tests. No significant changes in aquatic toxicity were identified by the multimedia study.

## UST Material Compatibility and Leak Detection

California statutes require that underground storage tank (UST) systems be compatible with the substance stored, and that leak detection equipment be able to function appropriately with the substance stored. The multimedia evaluation indicates that renewable diesel is chemically comparable to CARB diesel, therefore differences in compatibility and leak detection are not anticipated.

## Biodegradability and Fate/Transport

University of California, Davis, and University of California, Berkeley, provided data on the impacts of fate and transport properties of renewable diesel as compared to the CARB diesel. Fate and transport, as well as biodegradability, are not expected to be significantly different given the similar chemical composition of renewable diesel and CARB diesel.

## Waste Discharge From Manufacturing

Chemicals used in, and byproducts created by, the production are required to comply with hazardous waste laws and regulations. No significant areas of concern have been identified when comparing the waste streams of renewable diesel to CARB diesel.

## Conclusion and Recommendations

State Water Board staff concludes that given the information provided by University of California, Davis, and University of California, Berkeley, and the similarities of renewable diesel and CARB diesel, there are minimal additional risks to beneficial uses of California waters posed by renewable diesel than that posed by CARB diesel alone. State Water Board staff supports the multimedia evaluation of ASTM D975 renewable diesel and a finding of no significant adverse impacts on public health or the environment with the recommendations provided in the Renewable Diesel Multimedia Evaluation Staff Report.

As identified in the California Renewable Diesel Multimedia Evaluation Report, Tier III, the potential scope of any unanticipated impacts is difficult to determine due to the limited funding and time of the multimedia evaluation. Unanticipated risks and problems that may occur as full scale use of renewable diesel becomes common will need to be addressed as they occur.

This recommendation is contingent upon renewable diesel meeting the ASTM D975 fuel specifications, being chemically indistinguishable from CARB diesel, and using the same additives described in the California Renewable Diesel Evaluation.

# APPENDIX E

## Office of Health Hazard Assessment: The Potential for Oxidant-Mediated Toxicity of Biodiesel versus Petroleum Diesel Exhausts

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# MEMORANDUM

## THE POTENTIAL FOR OXIDANT-MEDIATED TOXICITY OF BIODIESEL VERSUS PETROLEUM DIESEL EXHAUSTS


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**February 2, 2015**

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# Office of Environmental Health Hazard Assessment

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## MEMORANDUM

**DATE:** February 2, 2015

**TO:** Floyd V. Vergara, Chief
Industrial Strategies Division
Air Resources Board

**FROM:** Page Painter, M.D., Ph.D., Senior Toxicologist
Office of Environmental Health Hazard Assessment

**VIA:** Allan Hirsch, Chief Deputy Director
Office of Environmental Health Hazard Assessment

**SUBJECT:** THE POTENTIAL FOR OXIDANT-MEDIATED TOXICITY OF BIODIESEL (BD) VERSUS PETROLEUM DIESEL (PD) EXHAUSTS

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On November 15, 2014, the Office of Environmental Health Hazard Assessment (OEHHA) received a memorandum from Floyd Vergara, Chief, Industrial Strategies Division, California Air Resources Board (CARB), requesting assistance in evaluating the comment, made by a peer reviewer, that "biodiesel exhaust may be causing increased effects of oxidative stress, as compared to human exposure to conventional CARB diesel." The peer reviewer, Dr. Stephen Nesnow, was a member of an independent expert panel that reviewed "Staff Report: Multimedia Evaluation of Biodiesel (California Environmental Protection Agency, 2013)" as required by California Health and Safety Code section 57004 prior to adoption of a fuel specification regulation for biodiesel. Another peer reviewer, Dr. Paul White, also cited evidence that combustion emissions (CE) from engines using biodiesel (BD) appear to be more toxic than CE from engines using petroleum diesel (PD) in eliciting oxidative stress responses in laboratory animals or animal cells. CARB also asked OEHHA to review new biodiesel studies published after the inception of the initial multi-media working group (MMWG) review. Subsequent to the memo, CARB additionally asked that OEHHA review possible inflammation effects that could be associated with biodiesel exposure.

In order to address these issues OEHHA carried out a review of a number of relevant biodiesel toxicity studies. The appendix of this memorandum contains short summaries

of the studies identified during our initial work on BD (which began in January 2013) and of studies published from January 1, 2013 to November 12, 2014, including Hawley *et al.* (2014), which was appended to your November 2013 memo.

As regards the original peer review comments regarding potential for oxidative stress, Dr. Nesnow noted that recent toxicology studies were indicating that exposure to BD CE could induce increased levels of inflammation and oxidative stress relative to PD. We agree that a number of these studies find that BD CE is more potent than PD CE in eliciting responses associated with inflammation and oxidative stress in cell-based assays and *in vivo*. OEHHA notes that in most studies, the toxicity per mass unit of particulate matter (PM) is evaluated, and the smaller mass of PM generally associated with biodiesel is not included in the comparison. At this time, it is not clear whether BD combustion emissions are more potent than PD combustion emissions when compared using doses based on PM emissions per mile (or per horsepower hour). Nonetheless, the results of these reports are important to consider and raise concern regarding the relative reactivity in biological systems of the many components associated with PM from biodiesel and conventional diesel.

The data from recent *in vitro* and *in vivo* animal studies indicate that BD CE exposure can induce enhanced inflammatory responses relative to PD particulate when measured on a mass basis. This may be offset by BD's lower mass emissions of PM and other constituents. However, the recent research increases concern regarding health impacts associated with an inflammatory response. Further research is warranted to determine whether the increased toxicity of BD CE, at least related to oxidative stress and the inflammatory response, might outweigh the beneficial reduction of particulate mass (and associated toxicity) that would result from the use of biodiesel. Of note, volatile constituents of combustion emissions vary by fuel type and are likely involved in the oxidative stress and inflammatory responses measured in these studies.

In conclusion, OEHHA cannot determine with certainty whether replacing PD by BD or BD-PD blends for on-road motor vehicle use will reduce adverse human health impacts attributable to oxidative stress and inflammation from toxic chemicals in diesel-engine emissions. The emissions of toxic substances that result in oxidative stress and inflammation (and other cellular damage leading to a variety of health outcomes) from both BD combustion and PD combustion depend on many factors including the type of engine used in the test, the workload protocol, the source of the BD used in the test, and the type of PD (e.g., CARB diesel, low S diesel, high S diesel, etc). For example, several studies showed increases in carbonyls in the emissions with certain BD fuels while a few showed decreases. Application of oxidative catalysts also affected the emissions of organic compounds including aldehydes. Most studies indicated decreases in polycyclic aromatic hydrocarbon (PAH) emissions with BD versus PD combustion. The types of published studies evaluating potential toxicity of BD versus PD emissions include both *in vitro* protocols (exposing cells), and *in vivo* animal

exposures. Extrapolation, particularly from *in vitro* studies to human health effects, has limitations because physiological conditions of cells exposed *in vitro* can be very different from physiological conditions of cells exposed *in vivo*. Finally, the mix of toxic chemicals in exhaust, which varies as noted above by fuel, engine, and operating conditions, affects the overall toxicity of engine exhaust in ways that cannot be fully characterized. The most useful comparisons of the toxicity of combustion emissions for CARB's purposes would be to compare emissions from CARB diesel-fueled engines to BD-fueled engines under conditions (workload patterns) typical of heavy duty diesel use in California. Except for the CARB-sponsored study by Durbin *et al.* (2011), the toxicity studies mostly do not look at CARB diesel, use a variety of engines, use different BD fuels, and employed different workload patterns than may be typical of heavy-duty engines in California.

Some, but not all, of the more recent studies published in 2013 and 2014 raise concerns that use of BD will result in increased emissions of substances that induce oxidative stress and/or indicators of inflammation. While some studies indicate combustion emissions from BD are more potent than combustion emissions from PD in producing these particular effects when compared on a per µg of PM basis, the reduced mass of PM produced per unit of engine work from BD may offset the increase in potency per µg of PM. Thus, a comparison of oxidative stress, or other toxicity, induced in a biological system per µg of PM emission needs to be placed in context of decreased overall emissions. Another complication is the role of non-PM constituents in induction of oxidative stress. These volatile components of emissions have not necessarily been measured in the *in vitro* and *in vivo* toxicology studies; rather, PM is usually used as the exposure metric. Thus, the responses cannot be evaluated in terms of exposure to particular volatile or semi-volatile components. Further, it must be noted that oxidative stress may be just one of the mechanisms involved in the toxicity of diesel exhaust emissions, which include respiratory and cardiovascular health effects, immunotoxicity, and carcinogenicity.

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## APPENDIX A

**Review Notes for Studies that Compare Health Impacts of Biodiesel Combustion Exhaust to those of Petroleum Diesel Exhaust**

Bünger *et al.* (2001)

Bünger *et al.* (2001) compared engine emissions from a diesel engine manufactured to power a tractor (Fendt 306 LSA) fueled with either PD (characteristics unspecified) or BD consisting of rapeseed oil methyl ester (RME). The emissions testing was performed using a chassis dynamometer and a heavy-duty 13-mode test cycle (ECE R39). The use of emissions controls with the test engine was not noted, and the semivolatile (SV) phase of the engine exhaust was not sampled. Total PM mass was higher in RME-fueled engine exhaust compared to PD-fueled engine exhaust on a g/hour basis. However, "insoluble" particulate matter" (presumably elemental carbon) was lower in RME-fueled engine exhaust compared to PD-fueled engine exhaust.

Exhaust PM extract cytotoxicity was assayed in the mouse fibroblast cell line L929 using a neutral red exclusion cytotoxicity assay. RME engine exhaust PM extracts from an idling engine was approximately four-fold more toxic than PD engine exhaust PM extracts. However, the toxicity from PM extracts of both fuel types was equivalent at full power testing. PM extract mutagenicity was tested using the *Salmonella typhimurium* mutagenicity assay. PD PM extracts demonstrated increased mutagenicity in test strains TA98 and TA100 (approximately four-fold and two-fold, respectively, in the presence and absence of metabolic activation provided by rat liver microsomes) compared to RME PM extracts.

Bünger *et al.* (2007)

Bünger *et al.* (2007) studied the comparative mutagenicity of exhaust extracts from EN590 petroleum (< 10 ppm sulfur) diesel-fueled engines and RME-fueled engines using the *Salmonella typhimurium* mutagenicity assay. The test engine was a heavy duty truck diesel (Mercedes-Benz OM 906 LA). The emissions testing were performed using an engine dynamometer and the 13-mode European Stationary Cycle (ESC) protocol. The use of emissions controls with the test engine was not reported. The SV phase of the engine exhaust was sampled. No significant difference in SV phase extract mutagenicity was observed between EN590 CE and RME-fueled engine CE in test strains TA98 and TA100, in the presence or in the absence of rat liver microsomes (S9). Additionally, no significant difference in PM extract mutagenicity was observed between EN590 and RME-fueled engines in test strains TA98 without S9 and TA100 with S9. Small but statistically significant increases in

RME PM extract mutagenicity were noted in TA98 with S9 (approximately two-fold) and TA100 without S9 (approximately 50%).

Liu *et al.* (2008)

Liu *et al.* (2008) evaluated *in vitro* cytotoxicity induced by PM and SV exhaust extracts generated from a QC 495 generator (manufacturer unspecified) without emissions controls fueled by either PD (retail blend, sulfur content unspecified), palm fatty acid methyl ester (PME), or blends of PD and PME (10, 30, 50 or 75% PME). Cytotoxicity was assayed using either the Microtox test or the MTT assay in BEAS-2B human bronchial epithelial cells. The Microtox test measures the bioluminescence of the marine bacterium *V. fischeri*. A reduction in bioluminescence is associated with a reduction in cell viability. The MTT assay is a colorimetric assay for measuring the activity of cellular enzymes that reduce the tetrazolium dye, MTT. A loss of reductive activity is associated with a loss of cell viability.

PM output from the test engine fueled with PME blends was greater than PM output from the test engine fueled with PD (four-fold higher for 100% PME) on a g/kWhr basis. Additionally, the soluble organic fraction (SOF) of PM from the PME blends was 27–53% higher than from PD exhaust PM. On a toxicity/exhaust volume basis, the Microtox test results indicated that the PM extract-induced toxicity was approximately equivalent for PD and all PME blends, but the SV extract-induced toxicity was increased for all PME blends (two-fold for B100) compared to PD. However, on a toxicity/extract weight basis, PD exhaust PM extract was approximately 5-fold more toxic than PM exhaust extracts for all the PME blends. No difference in toxicity was noted in the MTT assay between PD exhaust extract and 100% PME exhaust extracts for both the PM and SV exhaust phases on a toxicity/extract weight basis.

Murtonen *et al.* (2009)

Murtonen *et al.* (2009) compared engine emissions from a truck (Scania DT 12 11 420, Variant L01) diesel engine fueled with EN590 or RME. The emissions testing was performed using an engine dynamometer following the Braunschweig testing cycle protocol. Emission outputs were expressed in units of weight/power output (e.g. mg/kW-hr). The exhaust gas was sampled for aldehyde content, but otherwise the SV phase of the engine exhaust was not sampled.

In the absence of a diesel oxidation catalyst (DOC)/particulate oxidation catalyst (POC) emission control device, PM and PAH output from the Scania engine run on RME was increased (43% and 6%, respectively) compared to operation on EN590. However, a decrease (42%) was noted for mutagenicity in *Salmonella typhimurium* (strain TA98) treated with RME-fueled engine PM extract in the absence of metabolic activation compared to PM extract from a EN590-fueled engine. A small increase in formaldehyde (FA) emissions (11%) but not acetaldehyde emissions was noted in the RME-fueled engine exhaust compared to the EN590-fueled engine.

In the presence of a DOC/POC, PM output from the Scania engine run on RME was reduced (23%) compared to operation on EN590. No change was noted for PAHs in the RME-fueled engine exhaust compared to the EN590-fueled engine. No mutagenicity was noted in *Salmonella typhimurium* (strain TA98) treated with RME-fueled engine PM extract in the absence of metabolic activation, and mutagenicity from PM extract from EN590 CE was described by the authors as "minor" (93% reduction compared to a non-DOC/POC equipped-engine). Aldehydes and ketones could not be measured in the DOC/POC output due to analytical interference.

Karavalakis *et al.* (2009)

Karavalakis *et al.* (2009) evaluated emissions from a 1998 Toyota diesel automobile engine fueled with EN590 PD or fueled with blends of PD and RME containing 5%, 10%, or 20% RME or fueled with blends of PD and PME containing 5%, 10%, or 20% PME. No emissions controls were used, and the test cycles used were the New European Driving Cycle (NEDC) and the Athens Driving Cycle (ADC). Carbonyl and PAH exhaust content was expressed in units of mg/km and µg/km, respectively. Use of the biodiesel blends resulted in generally decreased PAH and nitro-PAH exhaust content compared to EN590. Changes in carbonyl emissions were variable, depending on fuel blend and test cycle (Table 1).

Table 1. Carbonyl emissions changes from biodiesel-fueled engine exhaust compared to EN590 petroleum diesel-fueled engine exhaust (adapted from Karavalakis *et al.*, 2009)

| | Test Cycle | NEDC | | | ADC | | |
|---|---|---|---|---|---|---|---|
| | Fuel | PME 5 | PME 10 | PME 20 | PME 5 | PME 10 | PME 20 |
| Formaldehyde | | 17% | 54% | 63% | 16% | 24% | 53% |
| Acetaldehyde | | -14% | -53% | -64% | -4% | -24% | -31% |
| | Fuel | RME 5 | RME 10 | RME 20 | RME 5 | RME 10 | RME 20 |
| Formaldehyde | | 25% | 121% | 157% | -29% | 269% | 350% |
| Acetaldehyde | | -79% | -85% | -90% | 560% | 1740% | 2150% |
| Acrolein/acetone | | -15% | -81% | 95% | -7% | -18% | -22% |

RME: rapeseed oil methyl ester PME: palm fatty acid methyl ester

He *et al.* (2009)

He *et al.* (2009) compared carbonyl emissions from a light duty diesel engine (FAW-WDEW 4CK) fueled with either PD (350 ppm sulfur) or soy-derived methyl ester (SME) containing 160 ppm sulfur. Test runs were done on an engine dynamometer using the ISO 8178 Type C1 8-mode steady state cycle.

Total carbonyl concentrations were significantly increased in the SME-fueled engine exhaust compared to the PD-fueled engine exhaust. The authors stated that biodiesel exhaust specific reactivity (ozone forming potential) was lower than that of the PD exhaust, primarily because of the lowered formaldehyde content in the SME CE.

Yuan *et al.* (2009)

Yuan *et al.* (2009) evaluated carbonyl exhaust emissions from a Mitsubishi 6D14 heavy-duty diesel engine. The fuels used in the study included PD (source unspecified), 100% PME (P100) and a 20% PME 80% PD blend (denoted P20). The sulfur content of these fuels was not specified. An engine dynamometer was used in the study, the test cycle employed was the US HDD Transient Cycle, and the use of emissions controls was not noted in the study description. P20 and P100

CE contained 3.28% and 14.5% more carbonyl compounds, respectively, (measured on a mg/bhp-hr basis) than CE from PD. Increased formaldehyde and acetaldehyde emissions were also noted for P20 (10 and 39%, respectively) and P100 (16.8 and 61.8% respectively) compared to PD CE.

Jalava *et al.* (2010)

Jalava *et al.* (2010) compared exhaust toxicities from a small industrial diesel engine (Kubota D1105-T) fueled with EN590 or RME using an ISO C1 steady-state test cycle. The SV phase of the engine exhaust was not sampled.

PM output (mg/kW-hr) from the RME-fueled engine was 26% less in the absence of a DOC/POC and was 10% less in the presence of a DOC/POC compared to the EN590-fueled engine. Particulate-phase total and genotoxic PAH levels were substantially lower in RME-fueled engine exhaust compared to EN590-fueled engine exhaust (66% and 83% lower, respectively; expressed as ng/mg PM) in the absence of a DOC/POC. RME-fueled engine emissions demonstrated moderately reduced total particulate-phase PAH emissions (31%) and genotoxic particulate-phase PAH emissions (11%) compared to emissions from the EN590-fueled engine in the presence of a DOC/POC.

The effects of engine exhaust PM extracts on cytotoxicity and apoptosis were tested in vitro using the mouse macrophage RAW264.7 cell line at exposure levels of 0, 50, 150 and 300 µg/ml. PM extract-induced cytotoxicity was measured by the MTT-test. Apoptosis was determined by using a flow cytometry assay to evaluate propidium iodide (PI)-stained cells. No significant differences in either cytotoxicity or apoptosis were noted in the mouse macrophage cell line RAW264.7 when exposed *in vitro* to PM from the test engine fueled with RME compared to PM from the test engine fueled with EN590 with or without use of a DOC/POC.

The effects of RME- and EN590-fueled engine PM on MIP-2 and TNF-$\alpha$ (cytokines that mediate inflammation) release were studied using mouse macrophage RAW264.7 cells *in vitro*. There was no significant difference in release of either cytokine between the fuel types in the presence or absence of a DOC/POC.

No significant difference in DNA damage (COMET assay) or reactive oxygen species (ROS) production in mouse macrophage RAW264.7 cells treated *in vitro* with RME-fueled engine PM compared to cells treated with EN590-fueled engine PM was noted in the presence or absence of a DOC/POC.

Karavalakis *et al.* (2010)

Karavalakis *et al.* (2010) studied the effects of BD10 derived from different methyl ester feedstocks on DE PAH, nitro-PAH and oxy-PAH content. The PD used for blending was EN590. The BDs tested were SME, UFOME, PME, SUME and RME. The test vehicle was a Euro 3 compliant Renault Laguna equipped with a 1.9 liter diesel engine. The engine used exhaust gas recirculation (EGR) and a DOC. NEDC and Artemis test cycles were used.

High molecular weight PAH output in BD-fueled exhaust varied considerably compared to PD-fueled exhaust (either increased or decreased) depending on BD feedstock and test cycle. The authors stated "nitro-PAHs were found to reduce with biodiesel whereas oxy-PAH emissions presented important increases with the biodiesel blends".

Ratcliff *et al.* (2010).

The comparative emissions characterization of a 2002 model year heavy-duty diesel engine (Cummins ISB, 5.9 L) equipped with a DOC/POC and run on ultralow-sulfur diesel (sulfur < 15 ppm) or SME (20 or 100%) was evaluated by Ratcliff *et al.* (2010). An engine dynamometer and AVL 8-mode test cycle were used in the study.

Acrolein levels were reduced in CE from both B20 and B100 fuels compared to PD, and formaldehyde was reduced in CE from BD fuels in the presence but not absence of a POC. Concentrations of other carbonyl compounds in CE were similar for all three fuels. PAH exhaust content was also generally reduced by use of B20 or B100, with the exception of naphthalene, which was increased in B100 exhaust but not B20 exhaust compared to PD exhaust.

Fontaras *et al.* (2010)

Fontaras *et al.* (2010) compared carbonyl concentrations in CE from several 10% BD-90% PD blends (B10 blends) in a Euro 3-compliant DOC-equipped Renault Laguna 1.9 liter displacement auto engine tested on a chassis dynamometer using NEDC and Artemis test cycles. The biodiesel types tested were PME, RME, SME, used frying-oil methyl ester (UFOME) and sunflower oil-derived methyl ester (SuME).

Compared to EN590-fuel exhaust, total carbonyl concentrations in B10 exhaust (expressed as mg/km) were generally increased with PME and RME blend use, remained unchanged with SuME blend use, and decreased with UFOME and SME blend use across the several test cycles. It should be noted that the total carbonyl exhaust concentrations for UFOME10 use were reported to decrease relative to EN590 use in this study, in contrast to the results reported in the Karavalakis (2010) study.

Brito *et al.* (2010)

Brito *et al.* (2010) studied effects of CE from a Branco BD2500 CFE diesel engine in male Balb/c mice. One group of mice was exposed to ambient air. A second group was exposed to CE produced when the engine was fueled with soy oil ethyl ester BD (denoted BD100). A third group was exposed to CE produced when the engine was fueled with a Brazilian PD composed of 97% PD (containing up to 500 ppm sulfur) and 3% BD, and a fourth group was exposed to CE produced when the engine was fueled with a blend of 50% BD and 50% PD (denoted BD50). Mice were exposed for one hour to CE diluted with filtered air in an attempt to achieve the target concentration, 550 $\mu g/m^3$ PM, in the exposure chamber. The average concentrations of PM during the exposure period were 556 $\mu g/m^3$, 552 $\mu g/m^3$ and 550 $\mu g/m^3$ for PD exhaust, BD50 exhaust and BD100 exhaust, respectively. The concentration of polycyclic aromatic hydrocarbons was significantly higher in PM from PD combustion than it was in PM from BD50 combustion or BD100 combustion.

An electrocardiogram (ECG) was obtained from anesthetized mice in each exposure group shortly before exposure and at 30 min and 60 min after exposure. The ECGs were analyzed for parameters of heart rate variability. The low frequency (LF) component of heart rate variability analysis in mice exposed to CE from BD100 was significantly greater than that of mice exposed to PD emission or that of mice exposed to ambient air (control mice).

Analysis of blood obtained 24 hours after exposure found a significantly higher concentration of platelets in mice exposed to BD100 emissions than in mice exposed to PD emissions or to ambient air.

In every case where an adverse impact in mice appears to be greater in animals exposed to BD emissions than it is in animals exposed to PD emissions, the concentration of PM was nearly the same for the two different exposure groups. Therefore, it appears that the PD CE may have been diluted with a larger volume of air to reach the target PM concentration than that used to dilute BD PM because PD CE generally has a higher concentration of PM than BD CE has. This would be

problematic in that the gaseous and semi-volatile components could have been diluted more in the PD CE than in the BD CE. This study does not compare the impact of PM produced by an engine fueled with PD that performs a specified quantity of work with the impact of PM from the same engine performing the same quantity of work while burning BD.

Karavalakis *et al.* (2011)

Karavalakis et al. (2011) analyzed emissions from a Euro 4 compliant passenger car (2007 Hyundai i-10) fueled with PD, BD10, BD20 or BD30. The PD used was EN590: 2009 (ultra-low sulfur), the BDs tested were soy-palm blend methyl ester (SMEP), animal fat-derived methyl ester (AFME), UFOME and olive oil methyl ester (OME), and the engine was equipped with a DOC. The test cycles used were NEDC and Artemis.

The authors reported increased carbonyl concentrations (e.g. formaldehyde, acrolein) from engines fueled with the BD blends tested compared to PD. Additionally, this increase was higher in the oxidized biodiesel blends (OME, UFOME), with the greatest increase seen with use of UFOME.

Total exhaust PAH, nitro-PAH and oxy-PAH content tended to be reduced in the non-oxidized BD blend-fueled engine exhaust compared to the PD-fueled engine exhaust. However, the oxidized biodiesel blends (OME, UFOME) tended to produce exhaust with equivalent or greater PAH, nitro-PAH and oxy-PAH exhaust content compared to PD-fueled engine exhaust, with the greatest increase seen with use of UFOME.

Hemmingsen *et al.* (2011)

Hemmingsen *et al.* (2011) studied the toxicity profiles of PM extracts from the exhaust of two light-duty automobile diesel engines (an Audi 1.9 liter TDI and a Peugeot 1.6 HDI engine) that were not equipped with diesel particle filters. The Audi engine met Euro2 emission standards, and the Peugeot engine met Euro4 emission standards. The dynamometer used was a Horiba Schenck eddy current dynamometer, but the test cycle used and dynamometer configuration (engine or chassis) were not stated by the authors. The fuels used were an ultralow-sulfur reference petroleum diesel (D100), 20% RME (RME20) and 20% AFME (AFME20) blends.

DNA damage was measured in human alveolar basal epithelial A549 cells, mRNA levels of CCL2 and IL8 (inflammatory cytokines) in acute monocytic leukemia THP-1

cells, and expression of ICAM-1 and VCAM-1 (involved in recruitment of inflammatory immune cells) in human umbilical cord endothelial cells (HUVECs). Cytotoxicity and production of ROS (oxidation of dichlorofluorescin diacetate) were studied in all cell types.

The PAH content in Euro2 AFME20-fueled engine exhaust PM was generally reduced compared to PD-fueled engine exhaust PM. Cytotoxicity in the THP-1 and A549 cell lines used appeared to be increased for Euro2 AFME20-fueled engines. However, cytotoxicity was not increased across all cell lines for Euro2 RME20-fueled and Euro4 AFME20-fueled and RME20-fueled engines compared to CE from Euro2 and Euro4 PD-fueled engines. PM from both the Euro2 and Euro4 engines fueled with either RME20 or AFME20 did not increase ROS production, DNA damage or CCL2, IL8, ICAM-1 or VCAM-1 mRNA expression relative to PM from PD fueled engines.

Song *et al.* (2011)

Song *et al.* (2011) compared exhaust PM PAH content from a Cummins ISBe4 Euro4 diesel engine fueled with either local PD (sulfur content 26 ppm) or a cottonseed oil-derived biodiesel (esterification not specified). Testing was performed using an engine dynamometer with the 13-mode ESC test cycle. The exhaust SV phase was not sampled.

The PAH content of exhaust PM was approximately equal for both fuels, and a combined cancer potency estimate for the exhaust PM derived using U.S. Environmental Protection Agency Toxic Equivalent Factor (TEF) was less for the B100 fuel PM than the potency estimate for PD PM.

Durbin *et al.* (2011)

CARB sponsored a biodiesel- and renewable diesel emissions study using several different types of diesel engine and emission controls (Durbin *et al,* 2011). Additional study data regarding acrolein and other aldehyde emissions were provided in Cahill and Okamoto (2013). A CARB-certified ultralow sulfur diesel (ULSD) fuel was the baseline for testing. The CARB fuel was obtained from a California refinery. Two biodiesel feedstocks were utilized for testing: a soy-based (S100) and an animal-based (A100) biodiesel fuel. Particulate phase and vapor phase exhaust emission samples were obtained from a model year 2000 Caterpillar C-15 engine and a model year 2007 Detroit Diesel MBE4000 equipped with a diesel

particulate filter. Both engines were tested on a chassis dynamometer using an Urban Dynamometer Driving Schedule (UDDS).

For all three heavy-duty diesel engines, the amount of oxides of nitrogen ($NO_x$) per highway mile in CE was significantly greater in most tests when biodiesel or biodiesel blends were used compared to the amount of $NO_x$ emitted when PD was used. However, the mass of PM emitted per highway mile was significantly less in most tests using BD or BD blend fuels than the amount emitted when PD was used. Emissions of the carcinogens benzene ethyl benzene and naphthalene were less when BD fuels were used. Emissions of PAHs, nitro PAHs and oxy PAHs in PM were less when BD fuels or BD-PD blend fuels were used, compared to emissions when PD was used. However, emissions of 1,2 naphthoquinone, a chemical that is mutagenic and that induces oxidative stress, were greater when BD fuels or BD-PD blend fuels were used, compared to emissions when PD was used.

Carbonyl emissions did not show consistent trends as a function of biodiesel blend level. However, acrolein emissions were significantly increased in the S50 and S100 Caterpillar C-15/UDDS test cycle exhaust compared to ULSD. Acrolein emissions were slightly increased in the A50 and A100 Caterpillar C-15/UDDS test cycle exhaust compared to ULSD. Acrolein emissions for the MBE4000 engine fueled with S50 or S100 were not significantly different from the ULSD-fueled engine emissions. A50 and A100 were not similarly tested in this engine.

*Salmonella typhimurium* test strains TA98 and TA100 were exposed to emissions samples from an engine run on either ULSD fuel, S20, S50, S100, A20, A50 or A100 in the presence or absence of metabolic activation provided by rat liver S9. For the Caterpillar C-15 engine, particulate phase exhaust mutagenicity decreased as the percentage of biodiesel in the engine fuel increased in both test strains with or without S9 with the exception of the A20 blend, which demonstrated increased mutagenicity in both test strains ± S9. Vapor phase exhaust mutagenicity was not significantly increased by soy-based biodiesel exhaust in both test strains ± S9. Vapor phase exhaust mutagenicity decreased as the percentage of animal-based biodiesel in the engine fuel increased in both test strains ± S9 with the exception of A20 in TA100 ± S9 and A50 in TA100 without S9.

For the Detroit Diesel MBE4000 engine, particulate-phase exhaust mutagenicity was similar for ULSD and all soy-based biodiesel blends in both test strains ± S9 with the exception of the S100 blend, which demonstrated decreased mutagenicity in TA100 - S9. Vapor phase exhaust mutagenicity was not significantly increased by soy-based biodiesel exhaust in both test strains - S9 and in TA98 + S9 compared to ULSD exhaust. Vapor phase exhaust mutagenicity appeared to decrease with increasing soy-based biodiesel concentration in TA100 + S9.

Human U937 monocytic cells were exposed to particulate-phase engine exhaust extract obtained from the Caterpillar C-15 engine under the conditions described above and evaluated for induction of DNA damage using the COMET assay. No increase in DNA damage was induced by exhaust from a soy-based or animal–based biodiesel or biodiesel blend-fueled engine PM extract.

Particulate phase exhaust extract from soy-based and animal-based biodiesel and biodiesel blend-fueled engines did not increase the release of interleukin 8 (IL-8; a cytokine mediator of inflammation) from a human U937 macrophage cell line or cyclooxygenase 2 (COX-2; an inflammation mediator) from a human NCI-H441 bronchiolar Clara cell line compared to particulate-phase exhaust extract from the corresponding ULSD-fueled engines.

Topinka *et al.* (2012) studied exhaust emissions from 1) a Cummins ISBe4 fueled with either PD, fuel-grade rapeseed oil (FRO) or BD100 (RME) and operated on a modified ESC test cycle; 2) a Cummins ISBe4 fueled with either PD or FRO and operated on the WHSC test cycle; 3) a Zetor 1505 engine fueled with PD or FRO and operated on the NRSC test cycle. EN590 was the PD used in this study, and the engines were not equipped with emissions control devices.

Exhaust PM was filtered, and the filters were extracted with dichloromethane. The resulting extract was analyzed for PAH content. Additionally, calf thymus DNA was treated with exhaust extract in the presence and absence of rat liver microsomal S9 fraction then analyzed for DNA adducts using a $^{32}$P-postlabeling assay. The sampling protocol would not be expected to capture PAHs contained in the semivolatile phase of the exhaust.

The relationship of PAH and c-PAH (a PAH subset with cancer potency values) levels (expressed as µg/kWh) in exhaust from the Cummins engine run on the modified ESC test cycle was PD > FRO > RME. In contrast, PAH and c-PAH levels in exhaust from the Cummins engine tested using the WHSC test cycle were slightly and substantially higher, respectively, when run on FRO compared to PD. The Zetor 1505 engine exhaust PAH levels were slightly higher when run on PD compared to FRO, but c-PAH levels were substantially higher when run on FRO compared to PD. Evaluation of the PAH and c-PAH levels were complicated by the lack of statistical analysis for this data set.

DNA adduct induction was generally consistently higher for PD compared to FRO for both engines under all test cycles in the presence or absence of metabolic activation when expressed either as adducts/mg exhaust PM, or as adducts/kWh engine output. The one exception to this was the Zetor 1505 exhaust, where adducts expressed as adducts/kWh in the absence of S9 was essential the same for both fuels.

Adduct levels were higher for RME-fueled exhaust than PD-fueled exhaust in the presence but not absence of S9 when expressed as adducts/mg exhaust PM. In contrast, adduct levels were higher in both the presence and absence of S9 for PD compared to FRO when expressed as adducts/kWh.

Jalava *et al.* (2012)

Jalava *et al.* (2012) compared exhaust toxicities from a 2005 model year Scania heavy-duty diesel engine fueled with EN590 or RME using an Braunschweig test cycle (Murtonen *et al.*, 2009). This engine was not equipped with a DOC/POC for RME operation, and the SV phase of the engine exhaust was not sampled.

Particulate-phase total PAHs (expressed as ng/mg PM) were reduced in B30-fueled and B100-fueled engine exhaust (13 and 49%, respectively). Particulate-phase genotoxic PAHs (WHO/IPCS 1998 definition) were reduced in B100-fueled engine exhaust (11%) but slightly increased (11%) in B30-fueled engine exhaust compared to EN590-fueled engine exhaust.

The effects of engine exhaust PM extracts on cytotoxicity, apoptosis, inflammatory cytokine release, DNA damage and ROS production were studied using the methodology described for Jalava *et al.* (2010). No significant difference was noted for cytotoxicity, apoptosis, inflammatory cytokine release, DNA damage or ROS production between RME-fueled and EN590-fueled engine exhaust extract.

Agarwal *et al.* (2013)

Agarwal *et al.* (2-13) compared CE PM from a Tata Sagari DICOR 3 l diesel engine fueled with PD to CE PM from the same engine fueled with a blend of 20% biodiesel and 80% PD (B20). The engine was operated at dynamometer-produced loads ranging from 0% to 100% of the maximum load. The authors do not include statistical analysis of data in this paper. However, the mass of PM in PD CE does not appear to be significantly different from the mass of PM in B20 CE. The PAH and toxicity equivalent-weighted PAH content of particles in PD CE appears to be significantly higher than that in B20 CE.

Gerlofs-Nijland *et al.* (2013)

Gerlofs-Nijland *et al.* (2013) evaluated toxicity of PM in CE from a 2 l Peugeot HDi engine equipped with a diesel particle filter (DPF) and DOC. The engine was operated following combinations of two test protocols designed to simulate engine load conditions on urban roads and two protocols designed to simulate load patterns on rural roads. PM in CE was collected when the engine was fueled with commercial European PD and operated with or without a diesel particle filter/diesel oxidizing catalyst (DPF/DOC). In separate tests using the same dynamometer cycles, the engine was fueled with a blend of 59% PD and 50% BD comprised of RME. The oxidizing potential of PM was assessed by measuring the rate of oxidation of dithiothreitol and the rate of oxidation of ascorbate. When expressed as the rate of reduction per mass of PM multiplied by mass of PM per highway mile, BD50 CE PM has less oxidizing potential than PD CE PM.

BEAS-2B cells (a cell line derived from human bronchial epithelial cells) were used in assays for cytotoxicity and pro-inflammatory response. Cell death was evaluated by measuring the fraction of cells containing propidium iodide as a function of the mass per ml of PM in the cell culture medium. When operated without a DPF/DOC, BD50 CE PM was more cytotoxic and induced a larger release of interleukin-6 (a pro-inflammatory cytokine) than PD CE PM. When operated with a DPF/DOC, the PM from both PD and BD-fueled engines was more toxic than PM in CE obtained when the engine was not equipped with a DPF/DOC.

Steiner *et al.* (2013)

Steiner *et al.* (2013) compared effects in cultured cells of CE from an Opel Astra X20DTL diesel engine fueled with pure RME biodiesel (BD100), low-sulfur (<10ppm) PD or a blend of 20+% RME and 80% PD (BD20). All post-combustion treatment devices were removed from the engine. Diesel exhaust (DE) was diluted 10-fold with filtered air before entering the exposure chamber. Cultures of human cells were exposed to CE for either 2 hours or 6 hours. The lactate dehydrogenase (LDH) concentration in culture medium, an indicator of cytotoxicity, was significantly higher in cultures exposed for 6 hours to BD CE than it was in cultures exposed for 6 hours to PD CE. Exposure to CE reduced intracellular concentrations of reduced glutathione, and the reduction was significantly greater in cells exposed for 2 hours to PD CE than it was in cells exposed for 2 hours to BD CE. Measurement of expression of genes that are indicators of inflammation or oxidative stress did not find any significant differences between cultures exposed to BD CE and cultures exposed to PD CE.

Bakeas and Karavalakis (2013)

Bakeas and Karavalakis (2013) reported emissions data including carbonyl and PAH concentrations in exhaust from a Euro 4 compliant SUV (2007 Hyundai Santa Fe, 2.2 liter diesel engine, DOC equipped). The engine was fueled with either PD (EN590:2009 + A1:2010; ultra-low sulfur), or BD (SME10 and SME30). The test cycles used were NEDC and Artemis.

The authors stated that "most carbonyl compound emissions increased with biodiesel, with the exception of aromatic aldehydes". However, the increases in total carbonyl emissions seen with the BD10 and BD30 blends were not large, and it is unclear as to whether those increases were statistically significant.

Total PAH and oxy-PAH emissions from BD-fueled exhaust were generally either similar or reduced compared to PD-fueled exhaust, depending on the test cycle. However, total nitro-PAH emissions were similar for both PD and BD. Additionally, the authors calculated a total TEF value for each fuel-test cycle combination. A TEF was calculated from the emissions concentration and carcinogenic potency for each individual PAH for which a carcinogenic potency was available. The TEFs for the individual PAHs were then summed. The TEFs for the BD10 and BD30 blends were generally similar to or greater than the corresponding TEF for PD.

Yanamala *et al.* (2013)

Yanamala *et al.* (2013) compared the effects of PM from an Isuzu C230 diesel engine fueled with petroleum-derived ULSD to those of CE from the same engine fueled with corn oil methyl ester BD. The diesel engine was equipped with a DOC and was operated using the ISO 8-mode test cycle. C5178Bl/5 mice were exposed to aqueous suspensions of diesel PM or to pure water by forced aspiration. Mice in the low-dose group were given suspensions of PM containing 9 μg total carbon and high-dose mice were given suspension of PM containing 18 μg carbon. The response of mice to PM was evaluated by analyzing bronchoalveolar lavage (BAL) fluid removed from bronchi 24 hours, 7 days or 28 days after exposure. Animals were sacrificed at these times, and lung tissue homogenates were analyzed for markers of tissue injury and inflammation.

Examination of BAL fluid for white blood cells revealed a significant increase in the number of polymorphonuclear (PMN) cells (indicators of inflammation) 24 hours after exposure and a significant increase in the number of macrophages 7 days after exposure for both BD PM and PD PM. The increase in PMN cells at the high dose at

24 hours was significantly greater in mice exposed to BD PM than it was in mice exposed to PD PM. The increase in macrophages (indicator of inflammation) at 7 days was also significantly greater in mice exposed to the high dose of BD PM than it was in mice exposed to the high dose of PD PM.

The concentration of protein in BAL fluid (an indicator of tissue injury) was significantly increased 24 hours after exposure to both BD and PD PM and was significantly higher in mice exposed to the high dose of BD PM than it was in mice exposed to the high dose of PD PM. The concentration of another indicator of cell injury, LDH, in BAL fluid was significantly elevated 7 days after exposure to either BD or PD PM and was significantly higher in fluid from mice exposed to the high dose of BD PM than it was in mice exposed to the high dose of PD PM. The concentration of a third indicator of tissue injury, myeloperoxidase (MPO), in BAL fluid was significantly increased 24 hours after exposure to both BD and PD PM and was significantly higher in mice exposed to the low and high dose of BD PM than it was in mice exposed to the low and high dose of PD PM.

In samples obtained 24 hours, 7 days and 28 days after exposure, the level of 4-hydroxynonenal (4HNE), an indicator of oxidative stress, was significantly higher in homogenates of lung from mice exposed to the high dose of BD PM than it was in homogenates from mice exposed to the high dose of PD PM. In homogenates obtained 28 days after exposure, the levels of protein carbonyls, another indicator of oxidative stress, were significantly higher in samples from mice exposed to the high dose of BD PM than they were in samples from mice exposed to the high dose of PD PM. The levels were also significantly higher in samples from mice exposed to the low dose of BD PM than they were in samples from mice exposed to the low dose of PD PM.

Shvedova *et al.* (2013)

Shvedova *et al.* (2013) exposed BALB/c female mice to CE from a 6.4 HP Yanmar L70 V diesel engine fueled with either BD comprised of fatty acid methyl ester from soy oil or PD. The CE was diluted with filtered air to achieve target PM concentrations of approximately 50, 150, or 500 $\mu g/m^3$, and mice were exposed by inhalation to the diluted exhaust for 4 hours per day, 5 days per week for 4 weeks. At the end of the exposure period, samples of lung and liver from mice exposed to CE or to filtered air were examined for LDH, glutathione (GSH), 4HNE, myeloperoxidase (MPO), tumor necrosis factor-α (TNF-α), interferon γ (IFN-γ), interleukin 6 (IL-6), IL-10, IL-12p70 and monocyte chemoattractant protein 1 (MCP-1). Both BD emissions and PD emissions caused tissue damage and inflammation. Elevations of lactate dehydrogenase in lung and liver were elevated to similar extents above control with exposure to both PD and

BD emissions, indicating cell damage. Statistically elevated increases in several markers of damage and inflammation occurred with both PD and BD in lung and liver. However, in this study overall, at the same PM concentration, BD CE produced more oxidative stress (increase in 4HNE and oxidized proteins) and increased inflammation (elevated myeloperoxidase) by most measures than did PD CE. It should be noted that the statistics presented are for the exposures to PD or BD compared to controls, and not reported for comparisons of PD to BD. It is apparent from the data, however, that for some measurements, only BD induced significant changes in the various measures of oxidative stress and inflammation relative to controls and not PD exposure. In a few instances, D100 exposure produced greater changes than BD exposure (IFN-γ in lung), or equivalent changes (IL-10 in lung, TNF-α in lung and liver).

It should be noted that PM was used as a metric of exposure to CE in this study. This does not mean that the semivolatile/gas phase components were not responsible for the oxidative stress induction, and it is not possible to know what the difference in exposure of the animals to these other exhaust constituents was between BD and PD exposed animals in this study.

Fukagawa *et al.* (2013)

Fukagawa *et al.* (2013) compared CE PM from a 1.9 liter Volkswagen diesel fueled with ULSD PD to CE PM from the same engine fueled with a blend of 20% soy biodiesel and 80% PD (B20 blend). The engine was operated following a 9-mode steadystate cycle. The mass of PM produced during the cycle using PD was more than twice the mass of PM produced using B20.

Cultured BEAS-2B cells, a cell line derived from human bronchial epithelial cells and cultured macrophages derived from THP-1 cells, a human monocyte cell line, were exposed to PM from PD CE or B20 CE at concentrations of 10 μg/ml or 20 μg/ml. Analysis of culture medium for cytokines revealed a significantly higher level of granulocyte colony stimulating factor (G-CSF) in medium of THP-1 cells exposed to10 μg/ml B20 PM compared to the level in the medium of cells exposed to 10 μg/ml PD PM. In the medium of BEAS-2B cells, there was a significantly higher level of interleukin-8 (IL-8) in medium of cells exposed to 20 μg/ml B20 PM compared to the level in the medium of cells exposed to 20 μg/ml PD PM.

Female C57BL/6 mice were exposed to approximately 84 μg of B0 or B20 particulate via oropharyngeal (OP) aspiration. The mice were sacrificed after three days on study. It is not clear from the article whether the mice received one particle treatment or three particle treatments. The bronchoalveolar lavage fluid (BALF) from mice exposed to B20 PM had significantly higher levels of G-CSF and Interferon-μ-inducible protein 10

(IP-10) than did the fluid from mice exposed to PD PM. Lung tissue of mice exposed to B20 PM also had significantly higher levels of G-CSF than did lung tissue from mice exposed to PD PM. However, if the mice were exposed to PM produced for a specified amount of work performed by the engine, the mice exposed to B20 PM would receive less than one-half the dose of PM given to the PD PM group, and the response in the B20 PM-exposed group would not be significantly greater than the response in the PD PM-exposed group.

Bhavaraju *et al.* (2014)

Bhavaraju *et al.* (2014) studied the toxicity of CE PM from a 2002 300 hp Cummins ISB diesel engine equipped with a DPF/DOC. The engine was operated using low-sulfur petroleum diesel (PD) or a blend of 80% PD and 20% BD (B20) under conditions where the temperature of the DOC was not high enough to achieve catalytic oxidation. PM was removed from the DPF/DOC and suspended in culture medium. Suspensions of macrophages obtained from Kyoto Wistar rats by broncho-alveolar lavage were incubated for 24 hours with PM at concentrations of 1 μg/ml, 10 μg/ml, or 100 μg/ml. The level of cyclooxygenase-2, an indicator of inflammation, was significantly increased in macrophages incubated with 100 μg/ml B20 PM or PD PM. The level of migration inhibitory protein-2, another indicator of inflammation, was significantly increased in macrophages incubated with 100 μg/ml PD PM, but not with 100 μg/ml B20 PM.

Mullins *et al.* (2014)

Mullins *et al.* (2014) evaluated effects of biodiesel (rapeseed methyl ester) and ultralow-sulfur diesel (ULSD) CE from a 3.9 L Isuzu 4BDT-1 engine. Cultured 10KT cells and cultured NuLi-1 cells were exposed to CE diluted 10-fold with filtered air or to filtered air alone (controls). Compared with controls, apoptosis was increased and cell viability was decreased for both TK10 and NuLi-1 cell cultures exposed to CE. CE from ULSD increased apoptosis and decreased viability more than CE from biodiesel. In both TK10 cells and NuLi-1 cells, the content of IL-6 and the content of IL-8 measured 24-hr after exposure were significantly higher for both ULSD CE and biodiesel CE than for controls. The IL-6 content was higher in both TK10 and NuLi-1 cells exposed to biodiesel CE than it was in cells exposed to ULSD CE. However, the content of IL-8 was lower in TK10 cells exposed to biodiesel CE but higher in NuKi-1 cells exposed to biodiesel CE when compared to the content of IL-8 in cells exposed to ULSD CE.

Westphal *et al.* (2014)

Westphal *et al.* (2014) evaluated CE from a 6.37 L Mercedes Benz OM 906 LA diesel engine. PD meeting EN590 standards and three fuels derived from plant oils, hydrotreated vegetable oil renewable diesel (HVORD), RME and jetropha methyl ester (JME) were combusted while the engine was operated using the ESC. When HVORD was used as diesel fuel, the quantity of CO in CE per kWh was similar to the amount of CO in CE when PD was used. The amount of NOx in CE from HVORD was approximately 15% less than the amount produced by PD combustion, while the amount of NOx produced by RME or JME combustion was greater than that produced by PD combustion. The amount of PM per kWh produced by HVORD combustion was approximately 8% less than that produced by PD combustion, and PM in CE from RME or JME combustion was approximately 35% less than PM from PD combustion. However, the soluble organic fraction (SOF) was greater in PM from RME combustion or JME combustion than it was in PM from PD combustion. The amount of PAHs in toluene extracts of PM from HVORD combustion was much less than the amount of PAHs extracted from PM produced by PD combustion. However the amount of the acetaldehyde, formaldehyde and acrolein produced by HVORD combustion was greater than the amount produced from PD combustion.

Toluene extracts of PM and condensates of CE were tested in the Salmonella typhimurium mutagenicity test using strain TA98 and strain TA100. All tests were performed with S9 (rat liver microsomes) and without S9. In both strains TA98 and TA100, the extract of PM from HVORD combustion produced many more mutations than did the extract of PM from PD combustion both in the presence or in the absence of S9This is in contrast to the results reported in Durbin *et al.* (2011). These investigators observed that extracts of PM or extracts of condensates from combustion of HVORD in a 2000 Caterpillar C15 diesel engine did not produce more mutations in either strain TA98 or strain TA100 than were produced by extracts of PM or condensates from the same engine fueled with PD.

Ballesteros *et al.* (2014)

Ballesteros *et al.* (2014) compared the carbonyl content of CE from a Nissan Euro 2M1-Bk 1.994 l diesel engine operated using the New European Driving Cycle protocol. Carbonyls (aldehydes and ketones) bound to PM were removed by heating and were combined with gas-phase carbonyls before being analyzed. The engine was operated with and without a DOC. CE from the engine fueled with BD derived from animal fat were compared with CE from the engine fueled with Rapsol, a commercial PD, and with CE from the engine fueled with a blend of 50% BD and 50% PD (B50). The total carbonyl content in CE increased with the BD content. However this increasing trend

did not occur for all carbonyls that were quantified. For example, the amount of acrolein in CE was highest when the engine was fueled with PD. This last result contrasts with that of Westphal where emissions from a PD-fueled engine contained less acrolein than the same engine burning a biodiesel fuel (HVORD).

Zhang and Balasubramanian (2014)

Zhang and Balasubramanian (2014) compared CE from a Yanmar Corporation L70AE 0.296 l diesel engine fueled with low-sulfur (<50 ppm) PD to blends of PD and BD comprised of palm oil methyl ester. The engine was connected to a generator and operated at 25%, 50% or 75% of maximum load. The mass of PM per kWh pf engine work decreased with increasing percentage of BD in the blend of fuel. The total particle-phase PAH per kWh also decreased with increasing percentage BD in the blend as did the total benzo[*a*]pyrene (BaP) equivalents per kWh. The soluble organic fraction of PM increased with the percentage of BD in the blend.

Hawley *et al.* (2014)

Hawley *et al.* (2014) compared the toxicity of CE from a John Deere 4045H PowerTech Plus diesel engine using BD fuel to the toxicity of PD CE from the same engine using PD fuel. The engine was operated at a workload of 107 BHP (75% of maximum power) and was equipped with a post-combustion diesel particulate filter and oxidizing catalyst (DPF/OC) that could be removed. Normal human bronchial epithelial (NHBE) cells were exposed to CE or to filtered air in this study. The NHBE cells were obtained from two human volunteers (donors) and grown on polycarbonate membrane filters placed in culture medium. Exposure to substances in CE PM was facilitated by a 4 kV electrical field gradient.

Toxicity to the NHBE cells was quantified by measuring relative amounts of mRNA from the gene coding for heme oxygenase 1 (HO-1) and mRNA from the gene for cytochrome p450 1a1 (CYP1a1). The former gene is induced by chemicals causing oxidative stress and the latter is induced by PAHs and certain other toxic organic substances.

Concentrations of PM, CO, $CO_2$, NO and $NO_2$ were determined in undiluted CE. The measurements were divided by 20 to estimate the air concentration to which NHBE cells were exposed. The concentrations of PM, organic carbon (OC) in PM and elemental carbon (EC) are listed in Table 2. When the engine was operated without a DPF/OC, these levels were much higher in CE from PD combustion than they were in

CE from BD combustion. When the engine was operated with a DPF/OC, these emissions were reduced more than 90%.

Table 3 lists the concentrations of CO, $CO_2$, gaseous hydrocarbons (HC), NO and $NO_2$ in CE. When the engine was operated without a DPF/OC, the CO concentration was higher in CE from PD combustion than it was in CE from BD combustion. When the engine was operated with a DPF/OC, the CO concentrations were reduced more than 95%. When the engine used PD, the DPF/OC reduced NO by approximately 40% but increased $NO_2$. The data are consistent with conversion of NO to $NO_2$ by the oxidizing catalyst. When BD was used, there was a large standard error in the data for NO when the DPF/OC was not used. Nonetheless, the data are consistent with conversion of NO to $NO_2$ by the oxidizing catalyst.

Levels of HO-1 mRNA and CYP1a1 mRNA were measured in NHBE cell cultures exposed to DE or filtered air for 5, 20 or 60 min. Levels were higher in NHBE cells exposed for 60 minutes to CE from BD than from PD, and levels were higher for both PD and BD when the engine was operated with an DPF/OC. However, none of these differences in mRNA levels was reported to be statistically significant. In cells exposed for 69 min to CE from PD or BD, levels of mRNA were found to be statistically significantly increased above levels in cells exposed to filtered air for 60 min.

The study found a reduction of PM, CO and organic carbon emissions when BD is used instead of PD. These reductions were enhanced when the engine used in the study was equipped with a DPF/OC. Given these large reductions in emissions, it is surprising that induction of HO-1 and CYP1a1 by CE is not reduced when the engine is equipped with a DPF/OC. The authors ascribed the effect to increased NO2 concentrations, as well as, the residual ultra-fine particle fraction and organic carbon content of the filtered exhaust.

In summary, Hawley *et al.* (2014) exposed normal human bronchial epithelial cells to whole CE from ultralow-sulfur PD and commercial grade BD. Cellular oxidative stress was measured by looking at heme oxygenase 1 (HO-1) mRNA transcript induction. No significant differences in HO-1 levels were found when the unfiltered PD and BD exhausts were compared, or between the filtered CE from the two fuel types.[1] On its own, this study indicates that—at least under one set of specified conditions, e.g., one set of fuel types, engine type, control technology, duty cycle, and toxicity test—BD CE appears to produce equivalent levels of oxidative stress compared to PD.

[1] Although not immediately relevant to the issue addressed in this memorandum, it should be noted that the filtered exhaust from both fuels showed a significant HO-1 increase relative to unfiltered exhaust. This may have been due to the fact that DPF/DOC treated exhausts contained significantly more $NO_2$ and contained residual, partially oxidized organic carbon content.

**Table 2. Carbon Content of Emissions in Each Exhaust Treatment**

| Exhaust Treatment | PM (g/m3) | OC (µg/m3) | EC (µg/m3) |
|---|---|---|---|
| Petrodiesel, DPF− | 850.3 (± 125.9) | 186.7 (± 88.2) | 595.8 (± 2.5) |
| Petrodiesel, DPF+ | 35.3 (± 6.6) | 8.8 (± 11.0) | 6.2 (± 5.7) |
| Biodiesel, DPF− | 235.6 (± 8.8) | 93.5 (± 13.3) | 65.9 (± 7.2) |
| Biodiesel, DPF+ | 21.7 (± 1.4) | 7.7 (± 8.8) | 2.1 (± 2.5) |

*Note.* Values are averages, and one standard deviation is shown in parentheses. OC and EC content represent the organic and elemental carbon content.

**Table 3. Gaseous Pollutant Concentrations During Cell Exposures**

| Exhaust Treatment | CO (ppm) | $CO_2$ (ppm) | HC (ppm) | NO (ppm) | $NO_2$ (ppm) |
|---|---|---|---|---|---|
| Petrodiesel, DPF− | 5.7 (± 0.50) | 0.31 (± 0.017) | 3.1 (± 0.048 | 19.0 (± 0.411) | 2.30 (± 0.368) |
| Petrodiesel, DPF+ | 0.13 (± 0.015) | 0.30 (± 0.0022) | 0.42 (± 0.029 | 10.1 (± 0.197) | 10.1 (± 0.0088) |
| Biodiesel, DPF− | 3.4 (± 0.036) | 0.26 (± 0.05) | 2.0 (± 0.0077) | 11.7 (± 9.60) | 1.41 (± 1.67) |
| Biodiesel, DPF+ | 0.08 (± 0.001) | 0.29 (± 0.0051) | 0.26 (± 0.055) | 13.0 (± 0.628) | 9.55 (± 0.534) |

*Note.* Values are averages; one standard deviation is shown in parentheses. with the percentage of BD in the blend.

**References**

Agarwal AK, Gupta T, *et al.* (2013). Assessment of toxic potential of primary and secondary particulates/aerosols from biodiesel vis-à-vis mineral diesel fuelled engine. Inhalation Toxicology, 25:325-332.

Alonso JR, Cardellach F, *et al.* Carbon monoxide specifically inhibits cytochrome c oxidase of human mitochondrial respiratory chain. (2003). Pharmacology and Toxicology, 93:142-146.

Bakeas EB, Karavalakis G. Regulated, carbonyl and polycyclic aromatic hydrocarbon emissions from a light-duty vehicle fueled with diesel and biodiesel blends. Environ Sci Process Impacts. 2013 15(2): 412-422.

Ballesteros R, Guillén-Flores J, *et al.* (2014). Carbonyl emission and toxicity profile of diesel blends with an animal-fat biodiesel and a tire pyrolysis liquid fuel. Chemosphere, 96:155-166.

Bhavaraju L, Shannahan J, *et al.* (2014). Acute cardiovascular and inflammatory toxicity induced by inhalation of diesel and biodiesel exhaust particles. Chemosphere, 104:126-133.

Brito JM, Belotti L, *et al.* (2010). Acute cardiovascular and inflammatory toxicity induced by inhalation of diesel and biodiesel exhaust particles. Toxicological Sciences, 116:67-78.

Bünger J, Krahl J, *et al.* (2000). Cytotoxic and mutagenic effects, particle size and concentration analysis of diesel engine emissions using biodiesel and petrol diesel as fuel. Archives of Toxicology, 74:490-498.

Bünger J, Krahl J, *et al.* (2007). Strong mutagenic effects of diesel engine emissions using vegetable oil as fuel. Archives of Toxicology, 81:599-603.

Cahill TM and Okamoto RA. (2013). Emissions of acrolein and other aldehydes from biodiesel-fueled heavy-duty vehicles. Environmental Science and Technology, 46:8382-8388.

California Air Resources Board (CARB). (2011). CARB Assessment of the emissions from the use of biodiesel as a motor vehicle fuel in California. "Biodiesel Characterization and NOx Mitigation Study". Final Report. Available from:< http://www.arb.ca.gov/fuels/diesel/altdiesel/20111013_CARB%20Final%20Biodiesel%20Report.pdf>.

California Environmental Protection Agency. (2013). Staff Report: Multimedia evaluation of biodiesel. Available from: <http://www.arb.ca.gov/fuels/multimedia/meetings/BiodieselStaffReport_Nov2013.pdf>.
Carrillo A, Monreal Y, *et al.* (2004). Transcription regulation of TNF-α-early response genes by poly(ADP-ribose) polymerase-1 in murine heart endothelial cells. Nucleic Acids Research, 32:757-766.

Cool BL, Foretz M, *et al.* (2008). AMP-activated protein kinase inhibits transforming growth factor-beta-induced Smad3-dependent transcription and myofibroblast transdifferentiation. Journal of Biological Chemistry, 283:10461-10469.

Fontaras G, Karavalakis G, *et al.* (2010). Effects of low concentration biodiesel blends application on modern passenger cars. Part 2: Impact on carbonyl compound emissions. Environmental Pollution, 158:2496-2503.

Gerlofs-Nijland ME, Totlandsdal AI, *et al.* (2013). Cell toxicity and oxidative potential of engine exhaust particles: impact of using particulate filter or biodiesel fuel blend. Environmental Science and Technology, 47:5931-5938.

Hawley B, L'Orange C, *et al.* (2014). Oxidative stress and aromatic hydrocarbon response of human bronchial epithelial cells exposed to petro- or biodiesel exhaust treated with a diesel particulate filter. Toxicological Sciences, 141:505-514.

He C, Ge Y, *et al.* (2009). Comparison of carbonyl compounds emissions from diesel engine fueled with biodiesel and diesel. Atmospheric Environment, 43:3657-3661.

Jalava PI, Aakko-Saksa P, *et al.* (2012). Toxicological properties of emission particles from heavy duty engines powered by conventional and bio-based diesel fuels and compressed natural gas. Particle and Fibre Toxicology, 9:37-50.

Jalava PI, Tapanainen M, *et al.* (2010). Toxicological effects of emission particles from fossil- and biodiesel-fueled diesel engine with and without DOC/POC catalytic converter. Inhalation Toxicology, 2:48-58.

Karavalakis G, Bakeas E, *et al.* (2010). Influence of oxidized biodiesel blends on regulated and unregulated emissions from a diesel passenger car. Environmental Science and Technology, 44:5306-5310.

Karavalakis G, Boutsika V, *et al.* (2011). Biodiesel emissions profile in modern diesel vehicles. Part 2: Effect of biodiesel origin on carbonyl, PAH, nitro-PAH and oxy-PAH emissions. Science of the Total Environment, 409:738-747.

Karavalakis G, Stournas S, *et al.* (2009). Light vehicle regulated and unregulated emissions from different biodiesels. Science and the Total Environment, 407:3338-3346.

Karthikeyan S, Thomson EM, *et al.* (2013). Nitrogen dioxide and ultrafine particles dominate the biological effects of inhaled diesel exhaust treated by a catalyzed diesel particulate filter. Toxicological Sciences, 135:437-450.

Liu YY, Lin TC, *et al.* (2008). Biological toxicities of emissions from an unmodified engine fueled with diesel and biodiesel blend. Journal of Environmental Science and Health, Part A. Toxic/Hazardous Substances and Environmental Engineering, 43:1735-1743.

Martínez-Romero R, Cañuelo A, *et al.* (2012). Nitric oxide modulates hypoxia-inducible factor-1 and poly(ADP-ribose) polymerase-1 cross talk in response to hypobaric hypoxia. Journal of Applied Physiology, 12:816-823.

Mullins BJ, Kicic A, *et al.* (2014). Biodiesel exhaust-induced cytotoxicity and proinflammatory mediator production in human airway epithelial cells. Environmental Toxicology. doi: 10.1002/tox.22020. [Epub ahead of print]

Murtonen T, Aakko-Saksa P, *et al.* (2009). Emissions with heavy-duty diesel engines and vehicles using FAME, HVO and GTL fuels with and without DOC+POC after treatment. SAE International Journal of Fuels and Lubricants, 2:147-166.

Ratcliff MA, Dane AJ, *et al.* (2010). Diesel particle filter and fuel effects on heavy-duty diesel engine emissions. Environmental Science and Technology, 44:8343-8349.

Sarti P, Forte E, *et al.* (2012). Cytochrome c oxidase and nitric oxide in action: molecular mechanisms and pathophysiological implications. Biochimica et Biophysica Acta, 1817:610-619.

Shvedova AA, Yanamala N, *et al.* (2013). Oxidative stress, inflammatory biomarkers, and toxicity in mouse lung and liver after inhalation exposure to 100% biodiesel or petroleum diesel emissions. Journal of Toxicology and Environmental Health, Part A, 76:907-921.

Song WW, He KB, *et al.* (2011). Emissions of EC, OC, and PAHs from cottonseed oil biodiesel in a heavy-duty diesel engine. Environmental Science & Technology, 45:6683-6689.

Steiner S, Czerwinski J, *et al.* (2013). Comparison of the toxicity of diesel exhaust produced by bio- and fossil diesel combustion in human lung cells in vitro. Atmospheric Environment, 81:380-388.

Topinka J, Milcova A, Schmuczerova J, Mazac M, Pechout M, Vojtisek-Lom M. Genotoxic potential of organic extracts from particle emissions of diesel and rapeseed oil powered engines. Toxicol Lett. 2012 212(1): 11-17.

University of California, Davis and Berkeley. (2013). California Biodiesel Multimedia Evaluation Final Tier III Report. Available from: <http://www.arb.ca.gov/fuels/multimedia/meetings/Biodiesel_FinalReport_May2013_101113.pdf>.

Westphal GA, Krahl J, *et al.* (2013). Combustion of hydrotreated vegetable oil and jatropha methyl ester in a heavy duty engine: emissions and bacterial mutagenicity. Environmental Science and Technology, 47:6038-6046.

Yuan CS, Lin YC, *et al.* (2009). Reducing carbonyl emissions from a heavy-duty diesel engine at US transient cycle test by use of paraffinic/biodiesel blends. Atmospheric Environment, 43:6175-6181.

Zhang ZH and Balasubramanian R. (2014). Physicochemical and toxicological characteristics of particulate matter emitted from a non-road diesel engine: comparative evaluation of biodiesel-diesel and butanol-diesel blends. Journal of Hazardous Materials, 264:395-402.

# APPENDIX F

## Department of Toxic Substances Control: Recommendation on Proposed Biodiesel

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# Department of Toxic Substances Control

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## MEMORANDUM

TO: Aubrey Gonzalez
Air Resources Engineer
Substances Evaluation Section
California Air Resource Board (CARB)

FROM: Donn Diebert, PE
Chief, Policy Implementation Unit
Policy and Program Support Division
Hazardous Waste Management Program

DATE: August 21, 2013

SUBJECT: RECOMMENDATION ON PROPOSED BIODIESEL

---

Summary of Evaluation

The Department of Toxic Substances Control (DTSC) staff evaluated impacts of biodiesel (during its manufacture, use, transport ,and disposal in California) to human health and the environment. Specifically, DTSC's evaluation focused on (1) hazardous waste generation during production, use/storage, and disposal of biodiesel and biodiesel blends; and (2) impacts on the fate and transport of biodiesel/biodiesel blends in subsurface soil from unauthorized spills/releases.

Biodiesel production occurs when certain biomass feedstock reacts with an alcohol in the presence of a catalyst. The biomass feedstock could be vegetable oils, animal fats, or waste greases. Feedstocks expected to be used in California are: soybean, palm, canola, and safflower oils; yellow grease; animal tallow; algae; and trap grease. Unlike California Air Resource Board (CARB) diesel, biodiesel is composed of methyl esters of long chain fatty acid. It does not contain aromatic hydrocarbon or sulfur. Biodiesel can be mixed with CARB diesel (e.g., B20 is 20% biodiesel and 80% CARB diesel) or used as a pure product (e.g., B100). When compared with CARB diesel, biodiesel appears to reduce certain engine exhaust emission pollutants, such as PM, CO, HC, and PAHs[1] .

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[1] PM: Particulate Matter; CO: Carbon Monoxide; HC: Hydrocarbons; PAHs: Polycyclic Aromatic Hydrocarbons

Biodiel's chemical composition depends on the feedstock's fatty acid content that has a significant bearing on final biodiesel characteristics. Generally, biodiesel is more acidic and reactive to certain plastics, some rubbers, and metals than is CARB diesel.

Based on the feedstock, hazardous waste can be generated during biodiesel production, storage, distribution, and use. Potential hazardous wastes from production include, but are not limited to: hexane, ethanol, methanol, potassium/sodium hydroxides, sulfuric acid, and phosphoric acid. Furthermore, biodiesel and production chemical releases into ground surface and subsurface environments are expected because of ruptures or leaks to above and below ground storage tanks; production equipment; piping and fittings; and/or transport, delivery, and spills during use. Large feedstock extraction will probably occur out of state, but could include the possibility of in-state manufacture. Such release scenarios should be evaluated on a case-by-case basis. Mitigation strategies should be established to avoid such spills, limit the migration of biodiesel and biodiesel blends, and comply with the appropriate hazardous waste management standards.

We understand the impracticality of a complete evaluation/testing of all types of biodiesel blends and potential additives. Tests were conducted using pure biodiesel, CARB diesel, and biodiesel blends. Additionally, tests were done with two additives (Kathon FP-1.5 as a biocide, and Bioextend-30 as an antioxidant). The results of the experiments were reported in the Tier II report. When comparing the data of CARB diesel to that of biodiesel, the biodiesel has the following characteristics:

1. Biodiesel aerobically biodegrades more readily.
2. Biodiesel with the Bioextend-30 (an antioxidant) preliminarily has a higher aquatic toxicity for the small subset of tested species.
3. Biodiesel, in general, has no significant difference in vadose zone infiltration rate. Biodiel's infiltration rate from animal fat appeared to be similar to CARB diesel; however, biodiesel left a noticeable increase in the residual's in vertical dimension and spread less extensive horizontally.

Based on the tests performed, biodiesel appears to react differently in the environment than does CARB diesel . The assumption made was that additives used in the tests would be the baseline for bringing biodiesel to market. As biodiesel or biodiesel blends are brought into the market, distribution chain monitoring would occur, and that information presented to the Multi Media Work Group (MMWG). Follow-up from unforeseen releases or impacts would be addressed by the affected agencies. If new or different additives from those tested are proposed for use, appropriate evaluation through the MMWG process should occur.

DTSC Conclusions and Recommendations

DTSC supports the biodiesel Multimedia evaluation based on reduction of certain air emissions and under the following conditions:

1) Any hazardous substances and hazardous waste used in production, storage, and transportation of biodiesel will be handled in compliance with applicable California laws and regulations.

2) If new or different additives from those tested are proposed for use with biodiesel and biodiesel blends, then conduct the MMWG process for an appropriate environmental impact evaluation. Additionally, new or different additives should also be registered with the U.S. EPA pursuant to Title 40, California Code of Federal Regulations (CFR), Part 79.

If there is any indication of a significant risk to human health or the environment, then MMWG will review the use of biodiesel or biodiesel blends for appropriate action.

cc: Li Tang
Hazardous Substances Engineer
Program Implementation Unit
Hazardous Waste Management Program

# APPENDIX G

## California Biodiesel Multimedia Evaluation
## Final Tier III Report by UC Davis and UC Berkeley

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# California Biodiesel Multimedia Evaluation

## Tier III Report

**Prepared**

**By**

**The University of California, Davis**
**The University of California, Berkeley**

**For the**

**California Environmental Protection Agency**
**Multimedia Working Group**

## FINAL

**June 2014**

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**Table 1.1. Summary of the recommended multimedia risk assessment process.**

**Figure 1.1. Multimedia evaluation process flow chart**

# Executive Summary

In general, the State of California needs information that will allow an informed decision as to the relative risk posed by any newly proposed fuel or fuel additive to the State's resources, human health and the environment. The purpose of this multimedia assessment is to provide the State of California information that will allow an informed decision as to the relative impacts posed by biodiesel to the State's resources, human health, and environment. Biodiesel is a name for an alternative diesel-substitute fuel, derived from biological sources (such as vegetable oils or tallow), which can be used in unmodified diesel-engine vehicles. In this document, "B100" refers to pure biodiesel; "B20" refers to a blend of 20% biodiesel and 80% petroleum diesel, and so on; and "biodiesel" refers generically to B100.

Because biodiesel blends are new fuels, the California Air Resources Board (CARB) must provide a "multimedia assessment" of their potential impacts before adopting new fuel specifications (as required by California Health and Safety Code, Section 43830.8). Further, the "California Air Resources Board cannot adopt any regulation establishing a motor vehicle fuel specification unless a multimedia evaluation is conducted to determine whether the regulation will cause a significant adverse impact on the public health or environment" (California Senate Bill 140, 2007). Further, as required by Section 43830.8 California Health and Safety Code (2011), before adopting new fuel specifications, the CARB is required to submit a completed multimedia assessment to the California Environmental Policy Council for final review and approval.

The multimedia risk assessment evaluation includes three components or tiers each designed to provide input to the next stage of the decision-making process. The tiered approach also enables structured peer review to take place between stages of the evaluation.

Pure biodiesel contains no petroleum, but it can be blended with petroleum diesel to create a biodiesel blend. To create biodiesel, a vegetable oil or animal fat is subjected to a chemical reaction known as transesterification. In this reaction, a feedstock (either oil or fat) is reacted with alcohol in the presence of a catalyst to produce glycerin as a byproduct and methyl esters, which compose biodiesel, also know as Fatty Acid Methyl Ester or FAME.

This report summarizes the results of Biodiesel Tier I (UC, 2009; Appendix III-A) and Tier II studies (UC, 2012; Appendix III-B; Durbin, et al., 2011) along with interpretations and conclusions from these studies regarding the suitability of biodiesel as a motor-vehicle fuel in California.

It must be recognized that the multimedia impact assessment is a process and not a product. Research on and use of biodiesel blends continues to grow and so this report is only a snapshot of the state of understanding as of this writing. Life-cycle approaches to emerging fuel options are often difficult to apply and may be burdened by uncertainty such that these studies become more informative as fuel technologies mature and are deployed. A life-cycle impact assessment is a contingent process, based on scenarios that will be modified as new knowledge is acquired,

and is not intended to make firm predictions. The uncertainties identified will inform decision-makers regarding:

- investments to improve the knowledge base,
- formulation of processes used to collect and manage new information,
- formulation of processes to evaluate and communicate uncertainty, and
- adjustment of the risk assessment process to mitigate the practical impact of uncertainty on decision-making.

The combination of various biomass sources used to make biodiesel, the variability these sources introduces into the evaluation of biodiesel performance, along with the large number of possible additive combinations, makes a systematic evaluation of biodiesel multimedia impact impossible at this time. Once the industry and market is more developed, additional more complete multimedia evaluations may be possible. The information currently available indicates:

**Biodiesel Has Beneficial Characteristics That Will Help California Meet Low Carbon and Renewable Fuel Goals:**

- Biodiesel is considered a low carbon fuel (with various approved biodiesel pathway carbon intensity values between 4.0 and 83.25, compared to CARB diesel 98.03) which helps meet the State's low carbon fuel goals.
- Biodiesel recycles carbon sequestered by the plant or animal feedstock materials used to make biodiesel.
- Biodiesel that meets the specific biodiesel definition and standards approved by ASTM International (formerly American Society for Testing and Materials) D6751-12 is compatible with existing diesel engines for B5 through B20. Some original equipment manufacturers also support B100.
- Air emissions studies have confirmed that biodiesel combustion emissions contain less hydrocarbons, carbon monoxide, and polycyclic aromatic hydrocarbons (PAHs) than CARB ULSD. B100 does not contain sulfur or aromatic hydrocarbons.
- Aerobic biodegradation of biodiesel is faster and more extensive than that of ULSD across a range of fuel blends.
- In general, tests show that life-cycle pollutant emissions from B100 biodiesel are considerably less toxic than life-cycle pollutant emissions from diesel fuel derived entierly from petroleum. Formulations of mixed biodiesel-diesel fuel such as B20 or B50 have shown results consistent with the calculated diluent effect of the percentage of biodiesel fuel on the total toxicity of the mixture.

**Issues of ongoing concern:**

***Resource Impacts and Sustainability***

- This report does not address direct and indirect environmental, ecological, and health impacts associated with biomass production—such as changes in land use and the possible net gain in carbon emissions due to feedstock cultivation. In general, as global

human populations grow, food used as a fuel is not sustainable. More sustainable sources of biodiesel feedstocks are encouraged such as yellow, or brown grease, tallow or oils from algae. However, the Low Carbon Fuel Standard (LCFS) regulation addresses land use and carbon intensity through fuel pathway analyses (title 17, California Code of Regulations (CCR) section 95480, et. seq.).

- Agricultural impacts to ecological receptors and water resources is a potential concern during the growing of plants used to make biodiesel. Currently most biodiesel feedstocks are produced outside the State of California. The environmental impacts from the increased use of fertilizers and water and land resources may be significant if the production of plant oils to supply biodiesel diesel feedstocks increases in the State. These factors, while not explicitly considered in the biodiesel Tier I, Tier II and Tier III evaluations, could become potentially important impacts to California as the biofuels industry expands.

***Air Impacts***

- In general, the effects of biodiesel on toxic air pollutant emissions appear to be favorable relative to standard petroleum diesel. But the current absence of industry standards for feedstocks, fuel formulation, and additives makes it problematic to assess the potential toxicity of all biodiesel uses as a generic fuel.
- Tier I literature review of biodiesel emissions indicate that, relative to standard petroleum diesel, there is a potential but still uncertain improvement in emissions profiles during combustion with reduced particulate-matter (PM), hydrocarbons, and CO emissions and with indications that $NO_x$ emissions may increase for certain biodiesel blends. Biodiesel Tier II air emission studies conducted by UC and CARB, using modern diesel engines, confirm previous studies findings regarding the decreased release of PM, CO, and total hydrocarbon (THC) emissions and the increased release of nitrogen oxides during biodiesel combustion for some blends, B20 or higher. Nitrogen oxides, which, in addition to their association with potential adverse health effects, have been identified as an ozone precursor.
- Approximately 80-95% of diesel exhaust particulate matter (DEPM) mass from standard diesel combustions consists of PM2.5 (a new fine particulate matter standard), and within that is a subgroup of ultrafine particles (UFPs) with an aerodynamic diameter of less than or equal to 0.1 micron. UFPs account for ~1-20% of the DEPM mass and 50-90% of the total number of particles. In spite of the observed overall drop in PM2.5 emissions in biodiesel blends, there is some uncertainty that that a drop in total PM mass may not necessarily equate with an overall reduction in the number of UFP emitted from combustion. This is an issue of national interest and more testing would be required to fully address it.
- Tier II Air Emissions test results show a general trend in decreased emissions in formaldehyde, but overall carbonyl emissions did not show any consistent trends between test fuels (Durbin et al., 2011). How expected formaldehyde emissions relate to cumulative exposures and disease burden is not clear since much of the current exposure to formaldehyde in California is attributable to indoor sources. If formaldehyde emission

increases are real, then formaldehyde emissions from biodiesel may contribute to the current chronic California recommended exposure limit (REL) for formaldehyde of 2 ppb.

- Aldehyde emissions overall are on par or reduced for biodiesel blends with respect to CARB ULSD, with possibly the exception of acrolein. A CARB study identifed increases in acrolein emissions up to a factor of 2 for soy biodiesel blends in particular.
- The type of biodiesel feedstock and conventional petroleum diesel (used for blending) can influence these emissions. The $NO_x$ emissions from biodiesel fuels increased with percent biodiesel blended and vary considerably with biodiesel feedstock used. Biodiesel containing feedstocks high in polyunsaturated fatty acids emit a greater percentage of $NO_x$ than biodiesel high in saturated fatty acids.
- The CARB Air Emissions study was conducted to provide an important assessment of potential impacts of biodiesel use in California and provides a basis for the development of fuel specifications and regulations. This study also makes an important contribution to the scientific knowledge of the impacts of biodiesel with CARB diesel in heavy-duty engines.

***Additives***

- As with air emissions, it should be recognized that, due to the large number of fuel formulations along with the resources and cost required to evaluate each formulation, it is not feasible to assess all combinations of engine types and fuel formulations. This is especially the case with additives, since the number of additive and feedstock combinations could be very large. So it will be important in future assessments to target a smaller set of archetypal and informative combinations of engines and fuel formulations. The Air Emissions studies evaluated two additives both for $NO_x$ reduction. Neat biodiesel fuels were also additized with a stability additive to help provide sufficient stability against oxidation throughout the program (Durbin et al, 2011). Effects of other additives such as biocides and cold flow enhancers may be necessary if these are planned for use. Additional additives for $NO_x$ reduction may also need to be tested prior to widespread use i.e. urea.
- California low-aromatics and -sulfur diesel-fuel formulations require the addition of cetane enhancers to achieve required emissions reductions. These additives are anticipated to be used in biodiesel blends as well. Further reducing the aromatics also can reduce lubricity and most California diesel includes a lubricity additive. Further, when diesel is distributed by pipeline, the operator may inject corrosion inhibiting and/or drag reducing additives. A typical additive package may contain: a detergent/dispersant, one or more stabilizing additives, a cetane number improver, a low temperature operability additive (flow improver or pour point reducer), and a biocide. Each refiner or marketer is likely to use a different package of additives and a different treat rate. The specific chemical composition of the additives used by various biodiesel manufactures is typically not specified and the environmental impact of these additives is not well described. The impact from releases of associated additives and production chemicals not yet characterized could be of concern unless state guidelines restrict additves to those already in use and/or already characterized.

- However, in the case of B20, it is reasonable to assume that most of the additives used in biodiesel are currently used in CARB ULSD and would continue to be used with no substantive difference in environmental impact due to additives. If this is the case, then new studies on multimedia transport and impact from these ULSD additives would not be necessary under the confirmation that the impacts of additives in CARB ULSD are either acceptable or at least well-characterized.

- ***Quality Assurance and Quality Control***
  - The biodiesel industry and market is still evolving. Biodiesel production is largely boutique in nature, industry oversight and enforcement of quality assurance standards are still being developed but are not well supervised and are voluntary. Biodiesel that does not meet minimum quality standards can have significant impacts on engine performance. However to meet LCFS volumes requires large scale production with the economy of scale to ensure adequate biodiesel fuel quality. Industry already recognizes this through development of BQ9000 program. Production at this scale could alliviate QA/QC concerns that arise from small producers.
  - Bulk and component properties of biodiesel beyond those included in ASTM International D6751 for fuel use are still largely unknown due to the variability in feedstocks and processing techniques. Identification of major chemical components and individual studies may elucidate which ones are causing the largest impacts to the environment.
  - The variety of fuel sources and additives make for complex oversight and create uncertainty regarding environmental impacts and quality of the fuel delivered to the customer. While it appears that different strategies will provide mitigation for different engines, the specific response varies from engine to engine. Further, while various studies have been performed determining properties of biodiesel, these may vary significantly from the feedstocks and additives used in California.

***Materials Compatibility***

- Because materials compatibility issues with tanks and piping particularly seals material penetration is a concern, storage and distribution systems should be selected with a goal of mitigating any compatibility issues. For example, biodiesel may not compatible with brass, bronze, copper, lead, tin and zinc as these metals can initiate oxidation and sediment production. Nitrile rubber compounds, polypropylene, polyvinyl, and Tygon are particularly vulnerable to biodiesel degradation. Biodiesel has higher solvency properties than petroleum diesel fuel and can act as a mild solvent. It can dissolve the residual sediments in diesel storage tanks and engine fuel tanks. These dissolved sediments can harm fuel systems as they plug fuel filters. Since biodiesel can react with some metals and polymers, it is critical that the material of tanks, hoses, gaskets, and other parts that may come in contact with biodiesel, are compatible with the fuel's properties.
- Biodiesel is susceptible to chemical changes during long-term storage. Fuel aging and oxidation by atmospheric oxygen can lead to increased acid numbers (which can increase corrosion), increased fuel viscosity, and the formation of gums and sediments. Storage

stability of biodiesel is also affected by the presence of water within the tank. Water can cause hydrolytic degradation of the fuel, contribute to microbial growth in the fuel, and can cause corrosion of fuel systems and storage tanks.

- It is important to mitigate issues with materials compatibility by ensuring biodiesel storage and distribution involves only compatible materials. Additional risk mitigation may be provided via secondary containment systems and leak detection systems.

***Toxicity***

- Assessing the aquatic toxicity of biodiesel is a priority in California for a variety of reasons. First, due to the potential for biodiesel to be used and transported in areas surrounding both freshwater and saltwater, toxicity information relevant to species present in each of these environments are necessary. Second, studies evaluating toxicity in the literature did not always use the feedstocks most likely used in California. It was shown, however, that different feedstocks had widely different effects on toxicity. Third, the possibility of additives may also create differences in the toxicity of biodiesel used in California rather than the biodiesel used in previous studies. Lastly, the reference petroleum diesel used in the toxicity experiments in the literature is different than the petroleum diesel currently sold for use in the State of California.
- The greatest difficulty in determining the exact human and ecological toxicity of biodiesel fuels is that biodiesel fuel is not a defined chemical formulation or a defined mixture of components, but can be formulated from any of a very large number of feedstocks with different chemical components. It is also difficult to exhaustively test all of the organisms that may be exposed to biodiesel during use or after a spill. As a result, representative species are often selected to make generalized statements of toxicity.
- The evaluation of toxicity of various biodiesel blends is an ongoing research topic. Recent literature identifies particular aspects of comparative toxicity of ULSD vs. different biodiesel blends. It has been found that rapeseed-based methyl-ester biodiesel had a lower mutagenic potency than petroleum diesel and the study attributed this to lower emissions of polycyclic aromatic compounds. The authors also found a higher toxicity that was speculated to be due to increased carbonyl compounds and unburned fuel that reduced the benefits of the lower emissions of solid particulate matter and mutagens from the rapeseed biodiesel. This is congruent with the identification of acrolein as an increased emittant with biodiesel fuels combustion.
- Microtox tests comparing a range of biodiesel blends with petroleum diesel indicates that the exhaust TUVs (toxicity unit per liter exhaust sampled, TU/L-exhaust, where TU is equal to $100/EC_{50}$, control-corrected) in the semi-volatile emisssions were 3 to 5 times those of the particulate emissions. Particulates from 100% petroleum diesel had the highest unit toxicity, TUW (toxicity unit per g soluble organic fraction of particulate, TU/g particle SOF) of all of the other biodiesel blends. Additionally, biodiesel was found to be more toxic than diesel because it "promoted cardiovascular alterations as well as pulmonary and systemic inflammation."
- Other researchers found that 20 % soy biodiesel (Soy B20) effectively reduced the emissions of PAHs; furthermore, the unit mass cytotoxicity of ultrafine particles and

nano-particles in the emissions was also lowered (by an average of 52.6%). The authors conclude that soybean biodiesel (S20) can be used as an alternative fuel to petroleum diesel to reduce the hazards of emissions from diesel engines to human health.

- Aquatic toxicity screening with biodiesel blends during Biodiesel Tier II studies by UC Davis, using California ULSD, exhibited somewhat increased toxicity to subsets of screened species compared to ULSD. Both B100 biofuels and their B20 mixtures caused variable effects on algae cell growth, water flea survival and reproduction and abalone shell development. Except for algae, screening with the antioxidant-additized B20 fuels consistently resulted in greater toxicity than was detected with the unadditized B20 fuels, suggesting that conducting screening for a less toxic additive may be warranted in blends above B20 with additives not yet tested for aquatic toxicity.

***Transport and Fate***

- There is a potential for releases to water and soil from from leaks and spills during fueling and vehicle use as well as atmospheric deposition from combustion. The transport and fate of a fuel in the environment is dependent on the multimedia transport properties of its constituent and additive chemicals. The properties are used to determine the equilibrium distribution of biodiesel and ULSD between different environmental compartments. The difficulty with biodiesel and ULSD are their chemical properties are not the same between samples. Production may occur from a wide variety of different feedstocks and lead to widely different key properties.
- Through analysis of the primary release scenarios, a bulk release of biodiesel from a storage tank or during transport may introduce significant quantities into the subsurface environment. Non-aqueous phase liquids that are highly mobile in the subsurface with low solubility can be very difficult to clean up and may cause additional risk to down gradient water sources. The exact compositions for both ULSD and biodiesel vary with the source and production factors. Aqueous solubility of biodiesel and diesel are widely variable on a constituent basis. Solubility is typically characterized in terms of pure chemicals and then scaled through partitioning relations for a multicomponent chemical. Due to biodiesel's polarity, it may be expected to be on average somewhat more soluble in water than diesel. Use of additives that have not been tested may enhance mobility, solubility or persistence in the subsurface leading to contamination of groundwater.
- Small-scale laboratory infiltration experiments in two-dimensional sandboxes were used to visualize the relative rates of biodiesel infiltration, redistribution, and lens formation on the water table in comparison to that of ULSD. Digital photography was used to record images of fuel behavior in side-by-side tests of biodiesel blend and ULSD. The experiments found that Soy B100, Soy B20, as well as AF B20, do not exhibit any significant differences among the four temporal metrics used to time the infiltration and lens formation, nor among the qualitative unsaturated zone residual or lens shape at steady state, compared to the same metrics for ULSD.
- Several studies have been performed on the biodegradation of biodiesel blends. Due to the structure of biodiesel compared to ULSD, biodiesel is more readily biodegradable in general. There are numerous bacterial groups indigenous to aquatic systems and soils that are capable of biodegrading biodiesel and petroleum hydrocarbons and this contributes to

the high potential for biodegradation in these media. Studies comparing various biodiesel components biodegradation to conventual petroleum diesel consistently found 85% to 88.5% degradation in 28 days compare to 26% degradation for conventual petroleum diesel. Biodiesel is also effective at promoting petroleum diesel degradation through co-metabolism. Additionally, biodegradation and solvency of biodiesel may enhance the biodegradation of pre-existing petroleum constituents. Biodegradation tests have also evaluated seed germination in contaminated soil and showed biodegradation to restore contaminated soil enough in four to six weeks to allow seed germination. The biological oxygen demand for biodiesel is much higher than for petroleum diesel. The chemical oxygen demand for diesel and biodiesel were very similar. With some minor variations among blends (soy vs. animal fat; additized vs. non-additized), the results indicate that the additives' effects are not significant on the biodegradation of biodiesel blends, and the blends tested are all more readily biodegradable than ULSD. In the event of a biodiesel blended with diesel spill, bacteria should consume biodiesel, and samples from the area of the spill can eventually be indistingishable from a comparible ULSD spill.

- While the results of the existing biodegradation experiments appear favorable for B100 and biodiesel blends with diesel, further evaluation is needed using the most up to date reference fuel for the state of California, CARB ULSD #2. In addition, due to various additive components not included in this multimedia assessment that may be necessary to improve fuel combustion properties, additional study of biodegradation is also needed to evaluate the impacts from the additives. Additives to prevent microbial growth in the fuel during storage and use may lead to significantly reduced biodegradation. Reducing biodegradation may lead to increased transport and mobility in the environment, especially in the subsurface where cleanup is especially difficult. Since biodiesel is a mild solvent, the solvency could potentially remobilize pre-existing chemical compounds in the area affected by a release.

***Waste Generation and Waste Management***

- In evaluating the production, distribution, and use of biodiesel (and other alternative diesel options) it is important for the multimedia assessment and the life-cycle assessment to identify where and what kind(s) of hazardous waste(s) may be generated.
- Similar to CARB diesel, biodiesel may be considered a waste if it is stored too long, is spilled, or becomes contaminated. Waste biodiesel that exhibits the hazardous waste characteristics of toxicity or ignitability may be classified as a hazardous waste. Biodiesel that is a hazardous waste and, potentially, environmental media that become contaminated with it may be subject to the hazardous waste management requirements in title 22 of the California Code or Regulations.
- Although there is evidence that biodiesel formulations are less toxic than standard diesel formulations, the storage stability of biodiesel is less than the standard ultra low sulfur diesel (ULSD). Degradation could be caused by temperature, oxidation, and/or material incompatibility; and some toxic components may be produced in the biodiesel.

- Once the sources, composition, and magnitude of waste streams from biodiesel fuel production, distribution, and use have been identified, there is a need to identify management approaches that could be applied to the identified hazardous waste streams.

# 1. Biodiesel Background Information

## 1.1. Introduction

In general, the State of California needs information that will allow an informed decision as to the relative risk posed by any newly proposed fuel or fuel additive to the State's resources, human health and the environment. The purpose of this multimedia assessment is to provide the State of California information that will allow an informed decision as to the relative impacts posed by biodiesel to the State's resources, human health, and environment. Biodiesel is a name for an alternative diesel-substitute fuel, comprising of fatty acid esters chemically derived from biological sources (such as vegetable oils or tallow), that can be used in unmodified diesel-engine vehicles. In this report the term "biodiesel" refers generally to any mixture of petroleum diesel and biomass-derived fatty acid esters; use the term B100 refers to pure biodiesel, the term B20 refers to blends involving 20 percent B100 and 80 percent petroleum diesel, and so on.

Because biodiesel blends are new fuels, the California Air Resources Board (CARB) must provide a "multimedia assessment" of their potential impacts before adopting new fuel specifications (as required by California Health and Safety Code, Section 43830.8). Further, the "California Air Resources Board cannot adopt any regulation establishing a motor vehicle fuel specification unless a multimedia evaluation is conducted to determine whether the regulation will cause a significant adverse impact on the public health or environment" (California Senate Bill 140, 2007). Further, as required by Section 43830.8 California Health and Safety Code (2011), before adopting new fuel specifications, the California Air Resources Board (CARB) is required to submit a completed multimedia assessment to the California Environmental Policy Council for final review and approval.

This report summarizes the results of Biodiesel Tier I (UC, 2009; Appendix III-A) and Tier II studies (UC, 2012; Appendix III-B; Durbin, et al., 2011) along with interpretations and conclusions from these studies regarding the suitability of biodiesel as a motor-vehicle fuel in California. Because this is a summary report, the reader is referred to the 2008 Guidance Document and the Biodiesel Tier I report (see Reference list) for specific citations and references supporting the finding summarized below. We begin here with a summary of the multimedia risk assessment process and how it was applied specifically to biodiesel. We then summarize Tier I and Tier II findings and provide our conclusions.

## 1.2. Background

B100 contains no petroleum, but it can be blended with petroleum diesel to create a biodiesel blend. To create biodiesel, a vegetable oil or animal fat is subjected to a chemical reaction known as transesterification. In this reaction, a feedstock (either oil or fat) is reacted with alcohol in the presence of a catalyst to produce glycerin as a byproduct and typically methyl esters, which compose biodiesel, also know as Fatty Acid Methyl Ester or FAME.

The multimedia risk assessment evaluation includes three components or tiers each designed to provide input to the next stage of the decision-making process. The tiered approach also enables

structured peer review to take place between stages of the evaluation. This process is summarized in Table 1.1 and illustrated in Figure 1.1.

The multimedia assessment process requires integration of information across different environmental media, different space and time scales, and different types of populations. New fuels or potential additives must be evaluated not only with regard to engine performance and emission requirements but also with consideration of health and environmental criteria involving air emissions and associated health risks, ozone formation potential, hazardous waste generation and management and surface and groundwater contamination resulting from production, distribution, and use.

The multimedia evaluation process begins with the applicant screening stage. This is a preliminary review by the CalEPA MMWG to assess the proposed fuel plausibility and/or feasibility. The purpose of this tier is to screen out any proposals that are not worth pursuing even to Tier I. For example, ideas that clearly violate basic concepts of scientific feasibility—mass balance, the laws of thermodynamics, etc., or ideas that appear to be the work of a team with no financial or technical resources to move forward on the concept.

Tier II follows the work plan developed during Tier I to draft a risk assessment protocol report. During Tier III the risk assessment report is prepared providing recommendations from the overall executed multimedia risk assessment beginning with identification of knowledge gaps (Tier I), filling key knowledge gaps (Tier II), and drawing conclusions of relative risk.

**Table 1.1. Summary of the recommended multimedia risk assessment process.**

| | **Fuel Applicant** | **Multimedia Work Group Review** | **MMWG Consultation and Peer Review** |
|---|---|---|---|
| **Tier I** | Fuel Background Summary Report:<br>• Chemistry<br>• Release Scenarios<br>• Environmental behavior | Screens applicant and establishes key risk assessment elements and issues | Technical consultation during development of Tier I Experimental plan including identification of key risk assessment elements and issues |
| | Mutually agreed upon Experimental Plan for Tier II | | |
| **Tier II** | Experiments to evaluate key risk assessment elements | Draft Tier II Experimental Summary Report | Technical consultation and independent peer review of Tier II report |
| **Tier III** | Multimedia Risk Assessment Report | Prepare recommendations to the Environmental Policy Council based on Multimedia Risk Assessment Report | Independent peer review of Multimedia Risk Assessment report and MMWG recommendations |

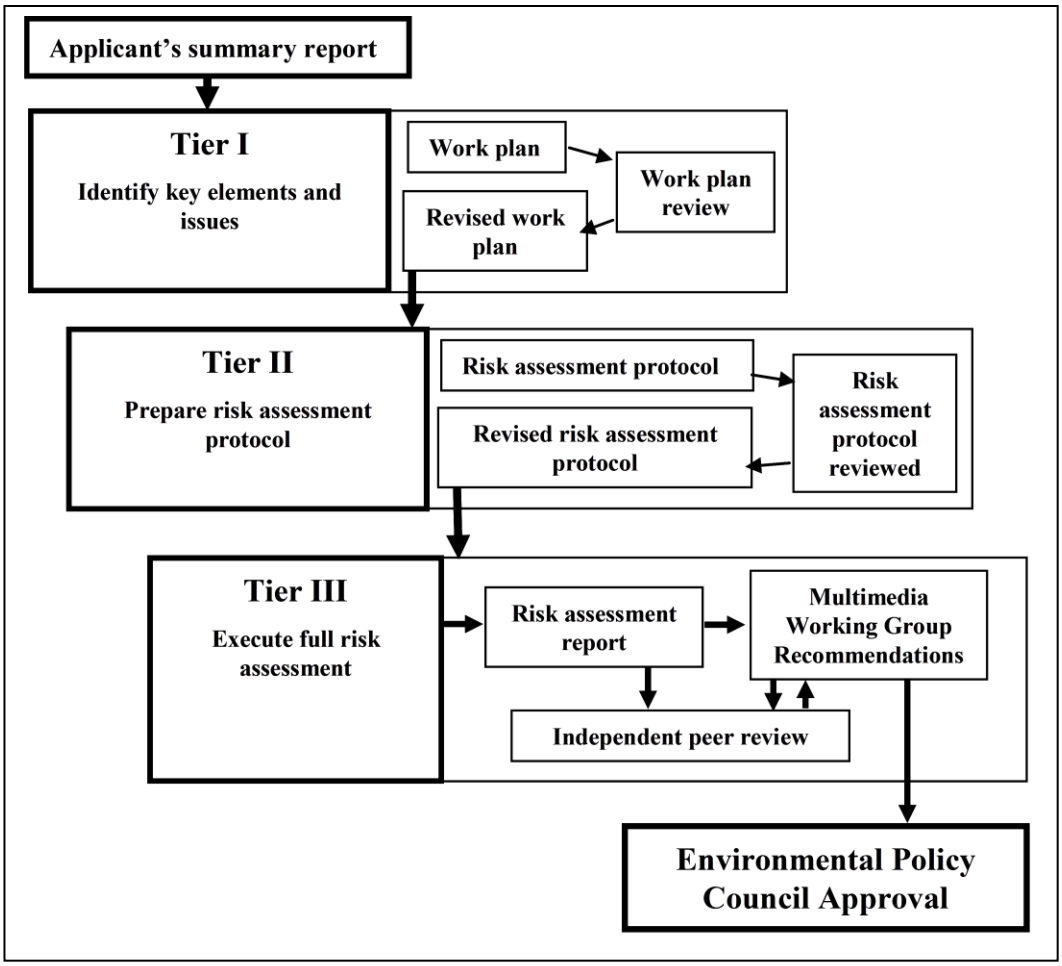


**Figure 1.1. Multimedia evaluation process flow chart**

Once a project has cleared the initial screening review, it moves in sequence through the next three Tiers. Tier I begins with the applicant bringing a summary report on the fuel to CalEPA and ends with the development of a work plan for the multimedia evaluation. A key goal of the Tier I report is to identify important knowledge gaps for a multimedia assessment and recommend approaches to address these gaps. This does not always involve additional experiments, but could include additional requests for information from the proponents of any new fuel to be used in California.

An important aspect of the applicant's Tier I summary report is an effort to assign measures of importance to all information—both available and missing. The Tier I Work Plan (Experimental Plan) is developed from important information gaps identified in the Tier I summary report. Due to time and funding limitations only the priority knowledge gaps are pursued in Tier II. The Tier I Work Plan identifies methods and/or experiments for estimating and/or measuring data needed to determine relative risk due to key knowledge gaps.

Using the work plan developed in Tier I, the Tier II report comprises further data collection and the execution of relative risk-assessment experiments to fill the key knowledge gaps. Tier II concludes with the preparation and MMWG review of a multimedia risk assessment experiment report that identifies the results of experiments for key uncertainties. The risk assessment experiment report should be based on the Tier I work plan and provide a comparison between the proposed fuel or fuel additive and the baseline fuel that the MMWG has agreed should be the basis for comparison in the work plan. Release scenarios of greatest interest will have been identified in the work plan based on the likelihood of adverse impact or occurrence.

During Tier III the risk assessment is executed and a report prepared providing the overall results of the executed multimedia risk assessment including information identified in Tier I and the results of experiments carried out in Tier II. The Tier III report is submitted to the MMWG for evaluation and preparation of recommendations to the Environmental Policy Council. Prior to submittal to the Environmental Policy Council, the submitted final multimedia risk assessment report as well as the MMWG recommendation will undergo independent external expert Tier III Peer Review.

This report is the Tier III report for biodiesel. The sections below provide the State of California information that will allow an informed decision as to the impacts posed by biodiesel to the State's resources, human health, and environment relative to CARB ULSD.

# 2. Summary of Biodiesel Tier I Findings[1]

Biodiesel and renewable diesel are names of alternative diesel-substitute fuels, derived from biological sources (such as vegetable oils or tallow), which can be used in unmodified diesel-engine vehicles. Biodiesel is defined as a fuel composed of mono-alkyl esters of long chain fatty acids derived from vegetable oils or animal fats. This report focuses on biodiesel derived from animal tallow and soybean oils. Currently, the majority of biological-source diesel fuels are fatty-acid methyl esters (FAME) produced through transesterification of non-petroleum oils. Biodiesel has been derived from a broad range of vegetable oils, recycled cooking greases or oils, animal fats and algal oils. It can be used as a pure fuel or as a blend with petroleum diesel, as biodiesel is miscible with petroleum diesel at all ratios. The most common blend is B20 (20% biodiesel mixed with 80% ultra-low sulfur diesel, ULSD). In this report, the word biodiesel refers to B100 that meets the specific biodiesel definition and standards approved by ASTM (American Society for Testing and Materials) D6751-12.

Preliminary tests of biodiesel emissions indicate that, relative to standard diesel, there is a potential but still uncertain improvement in emissions profiles during combustion with reduced particulate-matter (PM), hydrocarbons, and CO emissions with indications that $NO_x$ emissions may increase.

This report does not address direct and indirect environmental, ecological, and health impacts associated with biomass production—such as changes in land use and the possible net gain in carbon emissions due to feedstock cultivation.

## 2.1. Release Scenarios

Releases associated with the production, storage, distribution, and use of biodiesel can be regarded as normal (routine) or off-normal (unplanned but not necessarily unlikely). Different feedstock supplies and production processes may have different normal and off-normal releases and may affect different environmental media and human populations depending on geographic location.

Normal releases during the use of biodiesel include both the upstream feedstock production and fuel production emissions along with combustion tailpipe emissions, both to the air and to surface waters (in the case of marine use). The specific magnitude of these normal production and use releases within California are not yet well characterized and will remain difficult to quantify until more process-specific data become available and more engine/vehicle combustion tests are conducted.

There are several companies that are or will market biodiesel in California and elsewhere, but they have different production and marketing plans. A key issue for release scenarios upstream from the combustion stage is whether the vehicle is using pure or blended biodiesel and whether blending biodiesel stock will occur at a refinery or at a distribution facility.

[1] See Appendix III-A for the complete California Biodiesel Multimedia Evaluation Tier I Report.

Normal or routine releases during the production of biodiesel include:

- hexane or $CO_2$ released to the air during seed extraction from feed stocks such as rapeseed, cottonseed, sunflower, etc.,
- odors associated with waste biomass, and
- used process water discharges of various pH and trace-chemical composition.

Off-normal releases may include spills or leaks of bulk feedstock oil, production chemicals, such as methanol, hexane, acid, base, or blending stocks such as ULSD or B100, or finished B20 fuel. These off-normal releases may be the result of leak or rupture of:

- an above ground or underground storage tank and associated piping,
- a liquid transportation vehicle such as rail tank car, tanker truck, or tanker ship,
- a bulk fuel transport pipeline, or
- a tank storing unprocessed glycerin.

In the fuel-use stage of the biodiesel life cycle, the releases of greatest concern are emissions to air, but there are also potential releases to water and soil from atmospheric deposition and from leaks and spills during fueling and vehicle use. Several studies have determined that use of biodiesel (as B100 or a B20 blend) instead of conventional diesel may be expected to exhibit large reductions in hydrocarbons (HC), particulate matter (PM) and carbon monoxide (CO) emissions. The type of feedstock and conventional diesel (used for blending) can influence these emissions. The $NO_x$ emissions from biodiesel fuels increased with percent biodiesel blended and vary considerably with biodiesel feedstock used. Biodiesel containing feedstocks high in polyunsaturated fatty acids (FAMEs with double bonds in the carbon chain e.g., methyl oleate, methyl linolenate, and methyl linolate) emit a greater percentage of $NO_x$ than biodiesel high in saturated fatty acids.

## 2.2. Biodiesel Production, Storage, Distribution, and Use

To make biodiesel, a vegetable oil or animal fat is subjected to a chemical reaction known as transesterification. In this reaction, a feedstock (either oil or fat) is reacted with alcohol in the presence of a catalyst (acid or base) to produce glycerin and methyl esters (known as biodiesel).

Biodiesel is produced from a variety of feedstocks including: common vegetable oils (soybean, palm, rapeseed/canola, sunflower, safflower, algae, cottonseed, peanut), animal fats (usually tallow), and waste oils (used frying oils, trap grease). The greatest difference among feedstocks is the amount of free fatty acids (FFAs) that are associated with triglycerides. FFAs can form during the "recovery process for fats and oils, or when there is water present to break the glyceride-fatty acid bond. Hence, the FFA content is a significant measure of feedstock quality, because it indicates the degree of processing required" (Van Gerpen et al., 2004). Typically feedstocks high in FFAs contain greater levels of impurities and require a costly pretreatment to convert the FFAs to esters.

There are many steps involved in the production of biodiesel. As described above, two reactants, alcohol and oil, and a catalyst, are mixed in a reactor. Small production plants use batch reactors to mix the reactants while "larger plants (>4 million L/yr) use continuous flow processes involving continuous stirred-tank reactors (CSTR) or flow reactors". For a more complete

reaction with a lower alcohol requirement, the reactor step (where the reactants first enter the biodiesel production) can be divided into two component steps. After the reactor step, the next step involves separating the methyl esters and glycerin. Both products use a substantial amount of excess methanol. The methyl esters are then neutralized with acid (for base catalysis) to remove any residual catalyst and split any soap that may have formed during the reaction. Once biodiesel is processed into its usable form, it is then stored or distributed prior to use. Proper storage practices and materials are important to minimize the potential for unintended releases. Minimization of potential releases can be evaluated through testing material compatibility.

Material compatibility is an important property to consider during the storage and distribution of biodiesel. Since biodiesel can react with some metals and polymers, it is critical that the material of tanks, hoses, gaskets, and other parts that may come in contact with biodiesel, are compatible with the fuel's properties. Biodiesel is not compatible with brass, bronze, copper, lead, tin and zinc as these metals can initiate oxidation and sediment production. Nitrile rubber compounds, polypropylene, polyvinyl, and Tygon are particularly vulnerable to biodiesel. Material manufacturers guidelines or industry standard reports (e.g., NREL, 2009) should be consulted prior to biodiesel exposure of any material with unknown compatibility. Some compatible elastomers reported in NREL (2009) include nylon, teflon, and perfluoroelastomer. Biodiesel has higher solvency properties than petroleum diesel fuel and can act as a mild solvent. It can dissolve the residual sediments in diesel storage tanks and engine fuel tanks. These dissolved sediments can harm fuel systems as they plug fuel filters.

Biodiesel is susceptible to chemical changes during long-term storage. Fuel aging and oxidation by atmospheric oxygen can lead to increased acid numbers (which can increase corrosion), increased fuel viscosity, and the formation of gums and sediments. Storage stability of biodiesel is also affected by the presence of water within the tank. Water can cause hydrolytic degradation of the fuel, contribute to microbial growth in the fuel, and can cause corrosion of fuel systems and storage tanks.

In the fuel-use stage of the biodiesel life cycle, the releases of greatest concern are emissions to air, but there are also potential releases to water and soil from atmospheric deposition and from leaks and spills during fueling and vehicle use. The primary releases to air occur during the actual combustion process. There are also vapor emissions during fueling and liquid fuel spills.

Although considerable data are available on the effect of biodiesel on EPA-regulated pollutant emissions (i.e., HC, CO, $NO_x$, and particulates), most of these data were generated using older technology engines. Further, very little detailed exhaust characterization data on biodiesel exists beyond a small number of regulated pollutants. This raises a concern about the relevance of these data to newer engines. But recent studies with newer engines tend to confirm these earlier results and make clear factors that increase $NO_x$ emissions.

One of the key findings of the Tier-I biodiesel report regards emissions measurements. Given the wide variety of oils and fats that might be used to make biodiesel fuel, the actual emissions of PM and toxic air pollutants should be considered for each proposed formulation of biodiesel fuel to be used in California. According to the Tier I conclusions:

> "This situation requires a systematic and ongoing effort to assess emissions from diesel engines. But it should be recognized that, due to the large number of fuel formulations

along with the resources and cost required to evaluate each formulation, it is not feasible to assess all combinations of engine types and fuel formulations. This is especially the case with additives, since the number of additive and feedstock combinations could be very large. So it will be important in the Tier-II and Tier-III assessments to target a smaller set of archetypal and informative combinations of engines and fuel formulations."

## 2.3. Biodiesel Toxicity

The greatest difficulty in determining the human and ecological toxicity of biodiesel fuels is that biodiesel fuel is not a defined chemical formulation or a defined mixture of components, but can be formulated from any of a very large number of feedstocks with different chemical components. It is also difficult to exhaustively test all of the organisms that may be exposed to biodiesel during use or after a spill. As a result, representative species are often selected to make generalized statements of toxicity. These may vary on a regional basis or based on extensive toxicity databases for comparison purposes.

The Tier I report reviewed a number of animal and environmental toxicity studies in soil, water and air to gain insight about human and ecological toxicity. In most cases, tests show that B100 is considerably less toxic than pure diesel fuel. Formulations of mixed biodiesel-diesel fuel such as B20 or B50 have shown results consistent with the calculated diluent effect of the percentage of biodiesel fuel on the total toxicity of the mixture.

Insight on aquatic toxicity comes from acute short-term exposure of fish, water fleas, and green algae to a B100 water accommodated fraction. This study concluded that the No-Observed-Effect-Level (NOEL) was greater than 100 mg/L for all three species.

Several aquatic toxicity studies were found in the literature and reported in Tier I. These studies evaluated a variety of feedstocks for various different organisms. The University of Idaho studied the effects of rapeseed ethyl ester and soybean methyl ester biodiesels and 2-D Diesel on water fleas. The results showed diesel to be the most toxic but also showed that soybean methyl esters were more toxic than rapeseed ethyl esters.

Another study by Birchall et al (1995) examined the phytotoxicity of biodiesel. These toxicity experiments evaluated effects of biodiesel on macrophytes and showed biodiesel to be considerably less toxic than diesel for a wide array of different species of algae at all doses. Birchall et al (1995) also studied effects on rainbow trout, water fleas (Daphnia Magna), water snails, and various invertebrates. The results showed that diesel was more toxic to these organisms than biodiesel. Despite generally lower toxicity seen in the literature, spills to the environment can have detrimental effects on organism that may contact the spill. Wedel (1999) explains that organisms may become covered in oil after a spill leading to illness and death.

The evaluation of toxicity of various biodiesel blends is an ongoing research topic. Recent literature identifies particular aspects of comparative toxicity of ULSD vs. different biodiesel blends. Bunger et al. (2000) find that rapeseed-based methyl-ester biodiesel had a lower mutagenic potency than petroleum diesel and the study attributed this to lower emissions of polycyclic aromatic compounds. The authors also found a higher toxicity that reduced the benefits of the lower emissions of solid particulate matter and mutagens from the rapeseed biodiesel.

In Liu et al. (2008), conventional petroleum diesel and palm oil methyl esters (biodiesel) were blended in 6 ratios (0, 10, 30, 50, 75 and 100% of biodiesel by volume) and fed into an unmodified 4-stroke engine with a constant output power. This may correspond to uncontrolled and/or off-road use in California. The semi-volatile and particulate products in the exhaust were collected separately and their biological toxicities evaluated by both Microtox test and the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The Microtox test indicates that the TUVs (toxicity unit per liter exhaust sampled, TU/L-exhaust, where TU is equal to $100/EC_{50}$, control-corrected) in the semi-volatile emissions were 3 to 5 times those of the particulate emissions extracts. Particulates from the 100% petroleum diesel had the highest unit toxicity, TUW (toxicity unit per g soluble organic fraction of particulate, TU/g particle SOF) of all of the other biodiesel blends.

Brito et al. (2011) evaluated heartrate, heart rate (HR) variability, and blood pressure in mice after 1 hour exposure to petroeluem-diesel and biodiesel exhaust. B100 exhibited a decrease in the following emission parameters: mass, black carbon, metals, CO, polycyclic aromatic hydrocarbons, and volatile organic compounds compared with B50 and diesel. The root mean square of successive differences in heart beat interval increased with diesel compared with control, low frequency increased with diesel and B100 compared with control and HR increased with B100 compared with control. Biodiesel was found to be potentially more toxic than diesel because it "promoted cardiovascular alterations as well as pulmonary and systemic inflammation."

Tsai et al. (2011) evaluated the toxicity of Soy B20 relative to that of petroleum biodiesel. The authors found that Soy B20 effectively reduced the emissions of PAHs; furthermore, the unit mass cytotoxicity of ultrafine particles and nano-particles in the emissions was also lowered (by an average of 52.6%). The authors conclude that Soy B20 can be used as an alternative fuel to petroleum diesel to reduce the hazards of emissions from diesel engines to human health.

Song et al. (2011) examines elemental carbon (EC), organic carbon (OC), and polycyclic aromatic hydrocarbons (PAHs) emissions from cottonseed oil biodiesel (CSO-B100). "Relative to normal diesel fuel, CSO-B100 reduced EC emissions by 64% (+/-16%). The bulk of EC emitted from CSO-B100 was in the fine particle mode (<1.4 pm), which is similar to normal diesel. OC was found in all size ranges, whereas emissions of OC(1.4-2.5) were proportionately higher in OC(2.5) from CSO-B100 than from diesel. The CSO-B100 emission factors derived from this study are significantly lower, even without aftertreatment, than the China-4 emission standards established in Beijing and Euro-IV diesel engine standards. The toxic equivalency factors (TEFs) for CSO-B100 was half the TEFs of diesel, which suggests that PAHs emitted from CSO-B100 may be less toxic."

Assessing the aquatic toxicity of biodiesel is a priority in California for a variety of reasons. First, due to the potential for biodiesel to be used and transported in areas surrounding both freshwater and saltwater, toxicity information relevant to species present in each of these environments are necessary. Second, studies evaluating toxicity in the literature did not always use the feedstocks most likely used in California. It was shown, however, that different feedstocks had widely different effects on toxicity. Third, the possibility of additives may also create differences in the toxicity of biodiesel used in California rather than the biodiesel used in

previous studies. Lastly, the reference petroleum diesel used in the toxicity experiments in the literature is different than the petroleum diesel currently sold for use in the State of California.

## 2.4. Biodiesel Air Emissions

In general, the effects of biodiesel on toxic air pollutant emissions appear to be favorable relative to standard diesel. But the current absence of industry standards for feedstocks, fuel formulation, and additives makes it problematic to assess the potential toxicity of biodiesel as a generic fuel. As a prerequisite to Tier II, it will be necessary to provide the criteria used to select and define biodiesel formulations to be studied in depth. Relative to petroleum diesel emissions from engine combustion, biodiesel emissions have been shown to contain less particulate matter, hydrocarbons, carbon monoxide, and polycyclic aromatic hydrocarbons (PAHs). However, available measurements indicate that the combustion of biodiesel in a diesel engine can increase the release in nitrogen oxides, which, in addition to their association with potential health effects, have been identified as an ozone precursor. Despite the reduction in the total mass of particulate matter, it is not clear whether or by how much the shift in PM and toxic air pollutant composition changes in terms of chemistry and toxicity potential. It should be noted that approximately 80-95% of diesel exhaust particulate matter (DEPM) mass consists of PM2.5 (a new fine particulate matter standard), and within this range is a subgroup of ultrafine particles (UFPs) with an aerodynamic diameter of less than or equal to 0.1 micron. UFPs account for ~1-20% of the DEPM mass and 50-90% of the total number of particles. Given the current literature demonstrating the health effects of UFPs, we note that there is a need to consider measuring UFP levels as part of the toxicity assessment for biodiesel. Such measurements can address the concern that a drop in total PM mass does not necessarily equate to a drop in UFP number. Finally, the observation that formaldehyde increases significantly with biodiesel combustion indicates a need to address this issue in future efforts. At a minimum, modeling should be used to assess how expected formaldehyde emissions relate to air-basin exposures. This will determine if formaldehyde emissions from biodiesel will become an issue in light of the current chronic California recommended exposure limit (REL) for formaldehyde of 2 ppb.

## 2.5. Transport and Fate

The transport and fate of a fuel in the environment is dependent on the multimedia transport properties of its constituent and additive chemicals. For the multimedia evaluation of biodiesel, the purpose is to identify potential impacts and compare them to its reference fuel, Ultra-Low Sulfur Diesel (ULSD), in order to make a judgment of relative multimedia risk. Fate and transport of biodiesel blends requires chemical properties information about the chemical components for both biodiesel and ULSD. The properties are used to determine the equilibrium distribution of biodiesel and ULSD between different environmental compartments. The difficulty with biodiesel and ULSD is that their chemical properties are not the same between samples. Production may occur from a wide variety of different feedstocks and lead to widely different key properties.

Some of the basic properties needed to describe the chemical fate of biodiesel are listed below. These are necessary for both ULSD and biodiesel in order to make a comparison of their relative multimedia risk.

- Partitioning coefficients to different multimedia pairs (i.e. air to water or fuel to solids)
- Physical properties: density, viscosity, interfacial tension
- Chemical properties: solubility, vapor pressure, composition

Biodiesel is composed of alkyl esters of long chain fatty acids. ULSD is composed of aliphatic and aromatic hydrocarbons with trace amounts of nitrogen, sulfur, and other elemental additives. The exact compositions for both ULSD and biodiesel vary with the source and production factors. Aqueous solubility of biodiesel and diesel are widely variable on a constituent basis. Solubility is typically characterized in terms of pure chemicals and then scaled through partitioning relations for a multicomponent chemical. Solubility of biodiesel and ULSD bulk fuel phases were not found in the scientific literature during the Tier I evaluation. A more accurate composition of the fuels could lead to individual components that have solubility information available in the literature. Due to biodiesel's polarity, it may be expected to be on average 15 to 25 % more soluble in water than diesel. Octanol-water partition coefficients of B100 were not available in the literature. In addition, vapor pressure information for biodiesel was not available for Tier I.

Several studies have been performed on the biodegradation of biodiesel. Due to the structure of biodiesel compared to ULSD, biodiesel is more readily biodegradable. Studies by Zhang et al (1998) compare various biodiesel components to Philip's 2-D Diesel. All biodiesels performed consistently with 85% to 88.5% degradation in 28 days compare to 26% degradation by diesel. Biodiesel is also effective at promoting diesel degradation through cometabolism. Biodegradation tests have also evaluated seed germination in contaminated soil and showed biodegradation to restore contaminated soil enough in four to six weeks to allow seed germination. Stolz et al (1995) performed both aerobic and anaerobic biodegradation in microcosms. Biodiesel from this study was not compared to diesel. Lapinskiene and Martinkus (2007) measured biodegradation in an argon atmosphere. Over 60 days, biodegradation was about twice as high for biodiesel than diesel. Knothe et al (2005) studied the chemical and biological oxygen demand of biodiesel and 2-D diesel. The biological oxygen demand for biodiesel is much higher than for diesel. The chemical oxygen demand for diesel and biodiesel were very similar. While the results of the existing biodegradation experiments appear favorable for biodiesel and biodiesel blends with diesel, further evaluation is needed using the most up to date reference fuel for the state of California, California Air Resources Board ULSD #2. In addition, due to various additive components that may be necessary to improve fuel combustion properties, additional study of biodegradation is also needed to evaluate the impacts from the additives. Additives to prevent microbial growth in the fuel during storage and use may lead to significantly reduced biodegradation. Reducing biodegradation may lead to increased transport and mobility in the environment, especially in the subsurface where cleanup is especially difficult. Since biodiesel has mild solvency, biodegradation can also serve as a way to facilitate increased biodegradation in preexisting contamination sources. Additionally, if biodegradation were to be reduced due to additives or other reasons, the solvency could remobilize potentially toxic contaminants in soil or water.

Studies regarding the subsurface fate and transport of biodiesel were not found in the Tier I literature review. The majority of the fate and transport information available in the scientific literature is associated with releases to air as a result of fuel combustion. Through analysis of the

primary release scenarios, a bulk release of biodiesel from a storage tank or during transport may introduce significant quantities to the subsurface environment. The movement and transfer of the bulk biodiesel phase to the soil and groundwater need additional study in order to characterize the relative risk. Non-aqueous phase liquids that are highly mobile in the subsurface with low solubility can be very difficult to clean up and may cause additional risk to down gradient water sources. Analysis of composition and properties of the biodiesel components are also needed to provide additional information about the movement of biodiesel through the subsurface compared to ULSD. Literature on biodiesel estimates biodiesel aqueous solubility to be very low. Fuels that are relatively immiscible can be difficult to clean up in the subsurface environment. In addition, toxic components may be soluble enough to reduce water quality below acceptable standards. The movement of biodiesel through the environment is very important to evaluating the impacts to groundwater down gradient of a spill site. Use of additives may enhance mobility, solubility or persistence in the subsurface leading to contamination of groundwater resources and contamination in drinking water wells.

Even when releases of biodiesel would not cause significantly greater impacts to the environment, human health, or water resources relative to CARB ULSD, the impact from releases of associated additives and production chemicals could be of concern. The specific chemical composition of the additives used by various biodiesel manufactuers is typically not specified and the environmental impact of these additives is not well described.

California low aromatics and sulfur diesel fuel formulations require cetane enhancers to achieve required emissions reductions. Further reducing the aromatics also can reduce lubricity and most California diesel includes a lubricity additive. Further, when diesel is distributed by pipeline, the operator may inject corrosion inhibiting and/or drag reducing additives. A typical additive package may contain: a detergent/dispersant, one or more stabilizing additives, a cetane number improver, a low temperature operability additive (flow improver or pour point reducer), and a biocide. Each refiner or marketer is likely to use a different package of additives and a different treat rate.

In the case of B20, it is reasonable to assume that most of the additives used in biodiesel are currently used in CARB ULSD and would continue to be used with no substantive difference in environmental impact due to additives. If this is the case, then new studies on multimedia transport and impact from these ULSD additives would not be necessary under the confirmation that the impacts of additives in CARB ULSD are either acceptable or at least well-characterized. However, when the additives used in biodiesel are different from those in ULSD with regard to composition and/or quantity, then a multimedia transport and impact assessment will be needed to determine the magnitude and significance of these additives.

## 2.6. Biodiesel Tier I Findings

The paragraphs below summarize the key findings of the biodiesel Tier I report.

### 2.6.1. Additives impacts.

To provide a stable, useful, and reliable fuel, additive chemicals will need to be introduced into almost all biodiesel blends. These additives will be required to control oxidation, corrosion, foaming, cold temperature flow properties, biodegradation, water separation, and $NO_x$ formation.

The specific chemicals and amounts used have not been well-defined for the emerging industry in California. A careful evaluation of the possible chemicals would be beneficial to California and may lead to a "recommended list" or "acceptable list" that would minimize the uncertainty of future impacts as industry standards are developed.

### 2.6.2. Subsurface fate and transport properties.

The impacts of leaks and spills of biodiesel fuel product during transport, storage, and distribution have not been addressed. This is an important issue for California. Because the chemical composition of biodiesel differs significantly from that of petroleum diesel, it is expected that infiltration, redistribution, and lens formation on water tables will differ for the two fuels, leading potentially to significant differences in relative impacts to groundwater quality. Properties governing these processes are density, viscosity, and interfacial tensions. Component (including additive) solubility into the water phase ultimately governs water quality and so inter-phase solubilization of individual components also needs to be identified. To address these issues requires experiments with conventional soil column tests that will establish relative transport behaviors among different fuel compositions and for site-specific analyses. But the relevance of these results for state-wide assessments should be considered along with the value of full-scale comparative field tests with releases into the groundwater, or into the vadose zone just above the groundwater table.

### 2.6.3. Biodegradation in soils and aquifers.

The anticipated use of biocides in B20 fuels may affect the biodegradation potential for biodiesel released into the environment. The impact of biocides added to blended biodiesel may reduce the biodegradation of biodiesel and other petroleum-based fuels leaked or spilled into the subsurface. Since subsurface biodegradation can play an important role in the remediation of fuel spills and leaks, an understanding of the fate of biodiesel with biocide additive is needed.

B100 without additives may be more biodegradable than ULSD and may be preferentially metabolized by subsurface microbes. The interaction of B100 with existing gasoline or conventional diesel previously released into the subsurface needs to be examined more closely.

### 2.6.4. Production and storage releases.

In addition to impacts from released B100 or blended B20 biodiesel, increased production and associated feedstock processing may involve impacts from released reactants and by-products. There are potential impacts to California's air and water during the large-scale industrial operations use to extract seed oils. These impacts may result from air emissions of solvents used to extract the seed oil (e.g., hexane) and from leaking tanks containing chemicals to process the plant oils into biodiesel. There is also the issue of occupational exposures. Finally, UST material compatibility must be addressed: owner/operators are required by state health and safety codes to demonstrate material compatibility prior to storage of biodiesel. The impacts during seed extraction will become more of an issue for California as in-state production of plant-derived oils increases and may require further study. Currently, the possible impacts during seed extraction will be minimal in California since it is anticipated that most of the seed oils will be derived from soy grown and extracted out-of-state.

Among the most important current production reactants are: methanol, generic acid or base catalysts, feedstock oils, and post-processing water. As the biodiesel industry matures, release scenarios developed in this report need to be refined and prioritized.

### 2.6.5. Additional air-emission studies.

There are not yet sufficient data to assert that the use of biodiesel will reduce the emissions of criteria and toxic air pollutants. Although considerable data are available on the effect of biodiesel on EPA-regulated pollutants (i.e., HC, CO, $NO_x$, and particulates), most of these data were generated using older technology engines. Further, very little detailed exhaust characterization data on biodiesel exist beyond a small number of regulated pollutants. Planned emission testing is based on newly blended B20 fuel stocks with only an anti-oxidant added and purged with nitrogen. B5, B50 and B100 will also be tested. The anti-oxidant and nitrogen purge are needed because the fuel used for the planned testing will be at least six month old-which is at the maximum recommended storage time for biodiesel. We note that these fuel mixes may not necessarily represent the general storage conditions expected throughout California. It is well established that fuel-handling practices have an important influence on engine performance and combustion emissions. Additionally, the impact of various additives on combustion air emissions needs to be evaluated. Given the wide variety of oils and fats that might be used to make biodiesel fuel and the potential additives, the actual emissions of PM and toxic air pollutants will have to be determined for each proposed formulation of biodiesel fuel to be used in California. This situation demands a systematic and ongoing effort to assess emissions from diesel engines. In particular, there is a need for more controlled combustion studies to assess how the spectrum of toxic air pollutants, such as the spectrum of PAHs, will shift both in terms of volatile and particle-bound fraction but also in terms of any changes in toxic equivalency.

### 2.6.6. Waste generation and waste management

Similar to CARB diesel, biodiesel may be considered a waste if it is stored too long, is spilled, or becomes contaminated. Waste biodiesel that exhibits the hazardous waste characteristics of toxicity or ignitability may be classified as a hazardous waste. Biodiesel that is a hazardous waste and, potentially, environmental media that become contaminated with it may be subject to the hazardous waste management requirements in title 22 of the California Code or Regulations.

### 2.6.7. Life cycle impacts.

Only differences in emissions inventories were considered during recent studies comparing life cycle inventories (LCI) of biodiesel to petroleum diesel. Differences in health and environmental impacts associated with these LCI differences need to be evaluated. Additionally, current LCI studies have been limited to only soybean oil feedstocks. It is well established that different feedstocks can have an important influence on life cycle emissions. Information is needed for other feedstocks as well as for renewable diesel.

### 2.6.8. Priority list of biodiesel formulations.

Because the number of potential feedstocks, the number of fuel blends, and the number of additive choices and mixes makes for an unmanageable suite of permutations of cases for consideration, it is critical to identify the priority feedstocks, fuel blends, and additives requiring

study for our impacts assessment. Not specifically addressed in this Tier I evaluation are the environmental impacts from the increased use of fertilizers, water and land resources as the production of plant oils increases in the State. These factors may be some of the most important eventual impacts to California as the biofuels industry expands. More sustainable sources of biodiesel such as yellow or brown grease may be preferable and should be encouraged.

# 3. Summary of Biodiesel Tier II Findings[2]

This section reports on the results of experimental activities performed to address and rank knowledge gaps in Tier II of the California multimedia risk assessment of biodiesel blends, as identified in the Tier I assessment of biodiesel as an alternative fuel in California (UC, 2009) and as outlined in the plan for these experiments (Ginn et al., 2009). These experimental investigations include study of toxicity, transport in porous media, and aerobic biodegradation (UC, 2012). Further testing (solubility, materials compatibility) identified in the Tier II plan were not pursued as a result of time and funding limitations.

Additionally, a Tier II Biodiesel Air Emissions Characterization and $NO_x$ Mitigation Study was coordinated by California Air Resources Board (CARB) in conjunction with researchers from the University of California Riverside (UCR), the University of California Davis (UCD), and others including Arizona State University (ASU). The results of this study are reported in Durbin, et al., 2011.

The summary and results of each of the toxicity, transport in porous media, and aerobic biodegradation experimental suites as well as the air emission studies are as follows.

## 3.1. Aquatic Toxicity Tests

A series of aquatic toxicity tests were conducted on the seven fuel types including ultra-low sulfur diesel (ULSD), neat 100% biofuels derived from animal fat (AF B100) and soy (Soy B100) feed stocks as well as 80% ULSD:20% (w/w) mixtures of the two biofuels (AF B20 and Soy B20) and two additized B20 mixtures (AF B20A and Soy 20A) amended with an antioxidant, Bioextend (Eastman Company). Other additives, such as biocides and $NO_x$ reducers, that may be required to enhance biodiesel useability in California were not pursued due to time and funding limitations as well as unclear guidance on the mixing ratios. The chronic toxicity test species included three freshwater organisms including a green alga (*Selenastrum capricornutum*), an invertebrate (water flea, *Ceriodaphnia dubia*), and a fish (fathead minnow, *Pimephales promelas*), along with three estuarine organisms including a mollusk (red abalone, Haliotis rufescens), an invertebrate (mysid shrimp, *Mysidopsis bahia*) and a fish (topsmelt, *Atherinops affinis*). The water accommodated fraction (WAF) of each fuel was prepared by the slow-stir method and tested using a control and six concentrations of WAF (1, 5, 10, 25, 50, and 100%). The tests closely followed published USEPA protocols with regard to quality assurance (QA) including statistical evaluation of test endpoints, monitoring of water quality conditions in test solutions, and protocol control performance requirements. Statistical evaluation of test results included determination of the no-observable-effect-concentration (NOEC), lowest-observable-effect-concentration (LOEC), Effects Concentration (EC25 and EC50) for each test protocol endpoint. Sensitivity of the test organisms to the fuels was evaluated by comparing toxic units (TUs; 100/EC25. For example if 25% of the population shows effects at 50WAF, then the TU is 100/50=2. On the other hand if 25% of the population shows effects at 1WAF, then

[2] See Appendix III-B for complete California Biodiesel Multimedia Evaluation Tier II Report on Aquatic Toxicity, Biodegradation, and Subsurface Transport Experiments.

the TU is 100/1=100. This way, TU is an increasing measure of toxicity). Each of the tests met all protocol QA requirements and tests that were repeated to assess consistency, closely matched the results of the original test. Results of the tests varied widely depending on fuel type and test species. Tests with ULSD only detected effects on mysid growth (1.0 TU) and water flea reproduction (1.8 TU). None of the AF or Soy B100 fuels or their B20 mixtures without antioxidant additive produced detectable effects on mysid, topsmelt or fathead minnow endpoints. However, both B100 biofuels and their B20 mixtures caused variable effects on algae cell growth (5 - 21.3 TU), water flea survival and reproduction (<1 - 21.3 TU) and abalone shell development (3.0 - 35.5 TU). Except for algae, tests with the additized B20 fuels consistently resulted in substantially greater toxicity than was detected with the unadditized B20 fuels, suggesting that conducting screening for a less toxic additive may be warranted.

The Lawrence Berkeley National Laboratory (LBNL) Environmental Energy Technologies Division provided chemical analyses of the biodiesel/diesel components present in the WAFs prepared in a similar manner to those used during toxicity testing. Sample chemical analyses were not taken during toxicity testing.

LBNL developed and applied a stir bar sorptive extraction (SBSE) method followed by thermal desorption gas chromatography/mass spectrometry (TD-GCMS) analysis to identify and quantify the chemical composition of the aqueous-phase solutions for four different biofuels and ULSD under four different WAF preparations. Insufficient ULSD sample volume led to an analysis of the four biofuels under four WAF preparations, for a total of 16 analyses.

The fuels analyzed included all the biodiesel mixtures used during toxicity testing (AF B100, Soy B100, AF B20, Soy B20). Since unadditized ULSD was not available, all the resulting fuel mixtures were additized. In addition, the same four salinity and temperature conditions used during the toxicity testing were used during the preparation of the WAFs eventually analyzed.

The chemical analyses did not unambiguously reveal any causative compound for the toxicity, and further testing is required to confirm the identity of compounds or combination of compounds responsible for the toxic response in additized B20 fuels.

## 3.2. Infiltration Experiments

Small-scale laboratory infiltration experiments in two-dimensional sandboxes were done to visualize the relative rates of biodiesel infiltration, redistribution, and lens formation on the water table in comparison to that of ULSD. Experimental design involved unsaturated sand as model porous media with ~10cm vertical infiltration of fuels to the saturated zone. Experiments were performed in triplicate for Animal Fat and Soybean based biodiesel additized with Bioextend antioxidant, including pure (B100a) and blended (B20a) biodiesel formulations. As a control, unadditized AF B100 was also tested and it showed similar behavior to additized AF B100. Digital photography was used to record images of fuel behavior in side-by-side tests of biodiesel blend and ULSD. Experiments in each of the four blends (AF B100a, AF B20a, Soy B100a, and Soy B20a) were run to effective steady-state lens formation on the top of the saturated zone (water table) that involved durations ranging from 1.5 to 2 hours, with on average 24 photographs taken per experiment, generating 288 images. (24 snapshots in time x 4 fuel blends x 3 replicates). The experiments found that Soy B100, Soy B20, as well as AF B20, do not

exhibit any significant differences among the four temporal metrics used to time the infiltration and lens formation, nor among the qualitative unsaturated zone residual or lens shape at steady state, compared to the same metrics for ULSD. However, while the AF B100 blend exhibited mostly the same values of the infiltration timing metrics as ULSD, it showed noticeable increases in the amount of residual that occurred in the unsaturated zone, and it resulted in final lens geometry that was thicker in vertical dimension and less extensive in horizontal dimension than the ULSD lens. This behavior is consistent with the physical properties of animal fat biodiesel that include higher viscosity and interfacial tension than ULSD.

## 3.3. Biodegradation Experiments

Microcosm experiments were conducted to assess the aerobic aqueous biodegradation potential for solutions in contact with biodiesel fuels, relative to ULSD. Fuels mixtures used were AF B100, AF 20, Soy B100, Soy 20, and ULSD. These fuel blends were used as source phases and tested with antioxidant only (Bioextend), biocide only (Kathon FP1.5), and with both additives together, with ULSD tested for comparison. Experiments were done in batch (250ml) with 2g of soil inoculum added to 190ml of stock solution with addition of 5 μL of test fuel as substrate. Experiments were performed in a respirometer in which the $CO_2$ production in microcosms was measured during the experiment for duration of 28-30 days. Control experiments using sterilized inoculated solution with substrate were done to examine whether the test substrate is degraded abiotically and to test the adsorption of test substrate onto glass and or inoculum material. Controls with inoculum but no fuel also were prepared to test for $CO_2$ production by microorganisms in absence of substrate. Results show enhanced $CO_2$ production for all biodiesel blends and all additive combinations relative to that for ULSD. With some minor variations among blends (soy vs. animal fat; additized vs. non-additized), the results indicate that the additives effects are not significant on the biodegradation of biodiesel blends, and the blends tested are all more readily biodegradable than ULSD.

## 3.4. Air Emissions Characterization and $NO_x$ Mitigation Study

One of the key findings of the Tier-I biodiesel report regards absence of data on air emissions measurements. Given the wide variety of oils and fats that might be used to make biodiesel fuel, Tier I recommends that the actual emissions of PMs and toxic air pollutants should be considered for each proposed formulation of biodiesel fuel to be used in California. According to the Tier I conclusions: "This situation requires a systematic and ongoing effort to assess emissions from diesel engines. It should be recognized that, due to the large number of fuel formulations along with the resources and cost required to evaluate each formulation, it is not feasible to assess all combinations of engine types and fuel formulations."

To better characterize the emissions impacts of biodiesel under a variety of conditions, CARB conducted a comprehensive air emissions study of biodiesel fuels compared to CARB diesel. As part of Biodiesel Tier II experimental activities, a Biodiesel Air Emissions Characterization and $NO_x$ Mitigation Study was coordinated by California Air Resources Board (CARB) in conjunction with researchers from the University of California Riverside (UCR), the University of California Davis (UCD), and others including Arizona State University (ASU). The results of this study are reported in Durbin, et al., 2011.

The goal of the study was to understand and, to the extent possible, mitigate any impact that biodiesel has on NOx emissions from diesel engines. However, to assure a representative assessment, Tier II used a broad range of fuel source/engine/blend/test-cycle conditions to brachet expected operational uses. Fuels were selected to provide a range of properties that are representative of typical feedstocks, but also to have feedstocks representing different characteristics of biodiesel in terms of cetane number and degree of saturation. The CARB air emissions study also looked at the impact of biodiesel on toxic emissions. In summary the tests included:

- Test fuels included five primary fuels subsequently blended at various levels to comprise the full test matrix. The testing included a basline CARB ultralow sulfur diesel fuel, two biodiesel feedstocks (one soy-based and one animal-based) tested on blend levels of B5, B20, B50, and B100, a biomass-to-liquid (BTL) or renewable diesel, and a gas-to-liquid (GTL) diesel fuel tested at 20%, 50%, and 100% blend levels.
- Testing for this program was conducted on a wide range of engines from heavy-duty on-highway engines, off-road engines, and heavy-duty engines. The full test matrix included testing on two heavy-duty enignes, four heavy-duty vehicles, and two off-road engines. For the on-highway engine and chassis dynamometer testing, several test cycles were also utilized to evaluate the impact of biodiesel on emissions under different operating conditions and loads.
- Emissions measurements for the heavy-duty engine dynamometer test and the off-road engine tests focused primarily on standard emissions, including THC, CO, $NO_x$, and particulates.
- To address the Tier I recommendation for additional studies of exhaust emissions for a larger suite of possible air pollutants, more extensive testing was conducted for the heavy-duty chassis dynamometer testing, including regulated emissions, real-time PM analysis, and sampling for exhaust composition, toxicity, and health effects. For PM compositions, analyses were done for organic and elemental carbon, ions, and elements. Toxic analyses included PAHs, nitro-PAHs, and oxy-PAHs, VOCs, and carbonyls. The health effects analyses include mutagenicity, oxidative stress, inflammation, and DNA damage. The results of these studies are described in detail in the Tier II report.

## 3.5. Biodiesel Tier II Findings

### 3.5.1. Knowledge Gaps Addressed During Tier II Experimental Investigations

- Tested biodiesel blends exhibit somewhat increased toxicity to subsets of tested species compared to ULSD, and additized blends increase this toxicity for a smaller subset of tested species. Future testing addressing the potential toxicity of additives not a part of this multimedia assessment, including chemical analysis of exposure medium, may be needed.
- Biodiesel fuel blends show similar infiltration and lens formation to ULSD in unsaturated sandy porous media, with AF B100 exhibiting greater residual in the vadose zone and less spreading of fuel lens on the water table, consistent with increased viscosity and

interfacial tension of this fuel. Additional work relating to new additives may be needed as well as chemical analyses.

- Aerobic biodegradation of biodiesel is faster and more extensive than that of ULSD across a range of fuel blends and included biocide additives. Anaerobic biodegradation may also need additional work relating specifically to fuel/additive/blend combinations as the biodiesel industry in California matures.
- Heavy-duty chassis on-road modern engine results showed a consistent trend of increasing $NO_x$ emissions with increasing biodiesel blend level. The magnitude of the effects differed between the biodiesel feedstocks. The soy-based biodiesel blends showed a higher increase in $NO_x$ emissions for essentially all blend levels and test cycles in comparison with the animal-based biodiesel blends. $NO_x$ emissions were found to increase based on engine load and cycle power
- Overall, PM, THC, and CO emissions showed consistent reductions for most biodiesel blend level and cycle combinations. Reductions in aromatic VOCs were consistent with the reduction in aromatics in the fuel. For THC in the 2007 MBE4000 engine, soy biodiesel exhibited a statistically significant increase compared to CARB ULSD.
- $CO_2$ emissions for soy and animal fat biodiesel were also seen to experience slight increases for blends with higher blending ratios in the 2006 Cummins engine and only B100 experienced this in the MBE4000 engine. Several results mentioned statistically insignificant data. These may need additional analysis to make a judgment if there are differences between biodiesel and ULSD
- Fuel consumption was also seen to increase with increasing blending ratios for biodiesel. This is consistent with estimates of biodiesel having lower energy density than ULSD
- Blends of 15% renewable diesel or gas to liquid (GTL) diesel were proved successful in mitigating $NO_x$ for a B5 soy blend, giving a formulation more comparable to what might be implemented with the low carbon fuel standard. A 1% di tertiary butyl peroxide (DTBP) additive blend was found to fully mitigate the $NO_x$ impacts for a B20 and B10 soy biodiesel, while 2-ethylhexyl nitrate (2-EHN) blends had little impact on improving $NO_x$ emissions. Other $NO_x$ emissions strategies may need additional testing to determine their effectiveness.
- Mutagen emissions and chromosomal damage were not different from ULSD.

### 3.5.2. Biodiesel Tier II Remaining Experimental Uncertainties

- Additional testing addressing the potential toxicity of additives including chemical analysis of exposure medium is needed.
- Of the three groups of additives only blends with antioxidants, and biocidal additives (biodegradation experiments only) were studied. Cold flow additives were not studied in any of the performed experiments. The impact of cold flow additives on aquatic toxicity and biodegradation needs to be studied.
- Infiltration experiments with biocidal and cold flow additives were not performed. Additional test may be needed as those additives may have different impact on the biodiesel infiltration.

# 4. Tier III Conclusions

Through a review of the current knowledge on biodiesel production, use, and environmental impacts, this report provides an assessment to aid the CalEPA Multimedia Working Group in formulating recommendations to the California Environmental Policy Council regarding the consequences of increased use of biodiesel in California.

It must be recognized that the multimedia impact assessment is a process and not a product. Life-cycle approaches to emerging fuel options are often difficult to apply and may be burdened by uncertainty such that these studies become more informative as fuel technologies mature and are deployed. It is important to realize that much is unknown about the full implantation an emerging transportation fuel system and will remain uncertain until the full system is created. Adaptive decision-making refers to learning by doing. A life-cycle impact assessment is a contingent process, based on scenarios that will be modified as new knowledge is acquired, and is not intended to make firm predictions.

The uncertainties identified will inform decision-makers regarding:

- investments to improve the knowledge base,
- formulation of processes used to collect and manage new information,
- formulation of processes to evaluate and communicate uncertainty, and
- adjustment of the risk assessment process to mitigate the practical impact of uncertainty on decision-making.

The combination of various biomass sources used to make biodiesel, the variability these sources introduces into the evaluation of biodiesel performance, along with the large number of possible additive combinations, makes a systematic evaluation of biodiesel multimedia impact impossible at this time. Once the industry and market is more developed, additional more complete multimedia evaluations may be possible. The information currently available indicates:

## 4.1. Biodiesel Has Beneficial Characteristics That Will Help Meet California Low Carbon and Renewable Fuel Goals

- Biodiesel is considered a low carbon fuel (with various approved biodiesel pathway carbon intensity values between 4.0 and 83.25, compared to CARB diesel 98.03) which helps meet the State's low carbon fuel goals.
- Biodiesel helps the US meet renewable fuel goals.
- Biodiesel recycles carbon sequestered by the plant or animal feedstock materials used to make biodiesel.[3]
- Biodiesel that meets the specific biodiesel definition and standards approved by ASTM International (formerly American Society for Testing and Materials) D6751-12 is

[3] See Tier I, Section 2 – Production of Biodiesel

compatible with existing diesel engines for B5 through B20.[4] Some original equipment manufacturers also support B100.

- Air emissions studies have confirmed that biodiesel combustion emissions contain less hydrocarbons, carbon monoxide, and polycyclic aromatic hydrocarbons (PAHs) than CARB ULSD. B100 does not contain sulfur or aromatic hydrocarbons.[5]
- Aerobic biodegradation of biodiesel is faster and more extensive than that of ULSD across a range of fuel blends.[6]
- In general, tests show that life-cycle pollutant emissions from B100 biodiesel are considerably less toxic than life-cycle pollutant emissions from diesel fuel derived entierly from petroleum. Formulations of mixed biodiesel-diesel fuel such as B20 or B50 have shown results consistent with the calculated diluent effect of the percentage of biodiesel fuel on the total toxicity of the mixture.[7]

## 4.2. Issues of Ongoing Concern

The Tier II report addressed the knowledge gaps that were identified in Tier I has uncertainties with high priority. Here we summarize remaining uncertainties and consider options and benefits for addressing these uncertainties.

### 4.2.1. Sustainability

This report does not address direct and indirect environmental, ecological, and health impacts associated with biomass production—such as changes in land use and the possible net gain in carbon emissions due to feedstock cultivation. In general, as global human populations grow, food used as a fuel is not sustainable. More sustainable sources of biodiesel feedstocks are encouraged such as yellow or brown grease, tallow or oils from algae. However, the Low Carbon Fuel Standard (LCFS) regulation addresses land use and carbon intensity through fuel pathway analyses (title 17, California Code of Regulations (CCR) section 95480, et. seq.).

### 4.2.2. Resource Impacts[8]

Agricultural impacts to ecological receptors and water resources is of concern during the growing of plants used to make biodiesel. Currently most of the biodiesel feedstocks are produced outside the State of California. The environmental impacts from the increased use of fertilizers and water and land resources may be significant if the production of plant oils to supply biodiesel feedstocks increases in the State. These factors, while not explicitly considered in the Tier I, Tier II and Tier III evaluations, could become potentially important to California as the biofuels industry expands.

[4] See Tier I, Section 2.3
[5] See Tier I, Section 4.4
[6] See Tier I, Section 6.4
[7] See Tier I, Sections 6.4 and 7.
[8] See Tier I, Sections 8, 9.

### 4.2.3. Air Emissions[9]

In general, the effects of biodiesel on toxic air pollutant emissions appear to be favorable relative to standard petroleum diesel. But the current absence of industry standards for feedstocks, fuel formulation, and additives makes it problematic to assess the potential toxicity of all biodiesel uses as a generic fuel.

Tier I literature review of biodiesel emissions indicate that, relative to standard petroleum diesel, there is a potential but still uncertain improvement in emissions profiles during combustion with reduced particulate-matter (PM), hydrocarbons, and CO emissions and with indications that $NO_x$ emissions may increase for certain biodiesel blends.

Biodiesel Tier II air emission studies conducted by UC and CARB, using modern diesel engines, confirm previous studies findings regarding the decreased release of PM, CO, and THC, and the increased release of nitrogen oxides during biodiesel combustion for some blends, B20 or higher. Nitrogen oxides, which, in addition to their association with potential adverse health effects, have been identified as an ozone precursor.

Approximately 80-95% of diesel exhaust particulate matter (DEPM) mass from standard diesel combustions consists of PM2.5 (a new fine particulate matter standard), and within that is a subgroup of ultrafine particles (UFPs) with an aerodynamic diameter of less than or equal to 0.1 micron. UFPs account for ~1-20% of the DEPM mass and 50-90% of the total number of particles. In spite of the observed overall drop in PM2.5 emissions in biodiesel blends, there is some uncertainty that that a drop in total PM mass may not necessarily equate with an overall reduction in the number of UFP emitted from combustion. This is an issue of national interest and more testing would be required to fully address it.

Tier II Air Emissions test results show a general trend in drecreased emissions in formaldehyde, overall, carbonyl emissions did not show any consistent trends between test fuels (Durbin et al., 2011). How expected formaldehyde emissions relate to cumulative exposures and disease burden is not clear since much of the current exposure to formaldehyde in California is attributable to indoor sources. If formaldehyde emission increases are real, then formaldehyde emissions from biodiesel may contribute to the current chronic California recommended exposure limit (REL) for formaldehyde of 2 ppb.

Aldehyde emissions overall are on par or reduced for biodiesel blends with respect to CARB ULSD, with possibly the exception of acrolein. The study by Cahill and Okamoto (2012) identify increases in acrolein emissions up to a factor of 2 for soy biodiesel blends in particular.

The type of biodiesel feedstock and conventional petroleum diesel (used for blending) can influence these emissions. The $NO_x$ emissions from biodiesel fuels increased with percent biodiesel blended and vary considerably with biodiesel feedstock used. Biodiesel containing feedstocks high in polyunsaturated fatty acids emit a greater percentage of $NO_x$ than biodiesel high in saturated fatty acids.

The CARB Air Emissions study was conducted to provide an important assessment of potential impacts of biodiesel use in California and provides a basis for the development of fuel

[9] See Tier I, Sections 4.4, 7.3, and 9.

specifications and regulations. This study also makes an important contribution to the scientific knowledge of the impacts of biodiesel with CARB diesel in heavy-duty engines.

### 4.2.4. Additives[10]

As with air emissions, it should be recognized that, due to the large number of fuel formulations along with the resources and cost required to evaluate each formulation, it is not feasible to assess all combinations of engine types and fuel formulations. This is especially the case with additives, since the number of additive and feedstock combinations could be very large. It may be important in future assessments to target a smaller set of archetypal and informative combinations of engines and fuel formulations. The Air Emissions studies evaluated two additives both for $NO_x$ reduction. Neat biodiesel fuels were also additized with a stability additive to help provide sufficient stability against oxidation throughout the program (Durbin et al, 2011).

The specific chemical composition of the additives used by various biodiesel manufactures is typically not specified and the environmental impact of these additives is not well described. Some examples of expected or potential fuel blend-additive combinations are as follows. California low-aromatics and -sulfur diesel-fuel formulations require the addition of cetane enhancers to achieve required emissions reductions. These additives are anticipated to be used in biodiesel blends as well. Further reducing the aromatics also can reduce lubricity and most California diesel includes a lubricity additive. Further, when diesel is distributed by pipeline, the operator may inject corrosion inhibiting and/or drag reducing additives. A typical additive package may contain: a detergent/dispersant, one or more stabilizing additives, a cetane number improver, a low temperature operability additive (flow improver or pour point reducer), and a biocide. Each refiner or marketer is likely to use a different package of additives and a different treat rate. Effects of other additives such as biocides and cold flow enhancers may be necessary if these are planned for use. Additional additives for $NO_x$ reduction may also need to be tested prior to widespread use (i.e., urea). Future testing addressing the potential toxicity of additives not yet tested including chemical analysis of exposure medium is needed.

Even when releases of biodiesel would not cause significantly greater impacts to the environment, human health, or water resources relative to CARB ULSD, the impact from releases of associated additives and production chemicals could be of concern unless state guidelines restrict additves to those already in use and/or already characterized.

However, in the case of B20, it is reasonable to assume that most of the additives used in biodiesel are currently used in CARB ULSD and would continue to be used with no substantive difference in environmental impact due to additives. If this is the case, then new studies on multimedia transport and impact from these ULSD additives would not be necessary under the confirmation that the impacts of additives in CARB ULSD are either acceptable or at least well-characterized.

[10] See Tier I, Sections 4.3 and 9.

### 4.2.5. Quality Assurance and Quality Control[11]

The biodiesel industry and market is still evolving. Biodiesel production is still largely boutique in nature, industry oversight and enforcement of quality assurance standards are still being developed but are not well supervised and are voluntary. Biodiesel that does not meet minimum quality standards can have significant impacts on engine performance. However to meet LCFS volumes requires large scale production with the economy of scale to ensure adequate biodiesel fuel quality. Industry already recognizes this through the development of the voluntary BQ9000 program (http://www.bq-9000.org/). Production at this scale could alliviate QA/QC concerns that arise from small producers.

Bulk and component properties of biodiesel beyond those included in ASTM International D6751 for fuel use are still largely unknown due to the variability in feedstocks and processing techniques. Identification of major chemical components and individual studies may elucidate which ones are causing the largest impacts to the environment.

The variety of fuel sources and additives make for complex oversight and create uncertainty regarding environmental impacts and quality of the fuel delivered to the customer. While it appears that different strategies will provide mitigation for different engines, the specific response varies from engine to engine. Further, while various studies have been performed determining properties of biodiesel, these may vary significantly from the feedstocks and additives used in California.

### 4.2.6. Materials Compatibility[12]

Because materials compatibility issues with tanks and piping particularly seals material penetration is a concern, storage and distribution systems should be selected with a goal of mitigating any compatibility issues. For example, biodiesel may not compatible with brass, bronze, copper, lead, tin and zinc as these metals can initiate oxidation and sediment production. Nitrile rubber compounds, polypropylene, polyvinyl, and Tygon are particularly vulnerable to biodiesel degradation. Material manufacturers guidelines or industry standard reports (e.g., NREL, 2009) should be consulted prior to biodiesel exposure of any material with unknown compatibility. Some compatible elastomers reported in NREL (2009) include nylon, teflon, and perfluoroelastomer. Biodiesel has higher solvency properties than petroleum diesel fuel and can act as a mild solvent. It can dissolve the residual sediments in diesel storage tanks and engine fuel tanks. These dissolved sediments can harm fuel systems as they plug fuel filters. Since biodiesel can react with some metals and polymers, it is critical that the material of tanks, hoses, gaskets, and other parts that may come in contact with biodiesel, are compatible with the fuel's properties.

Biodiesel is susceptible to chemical changes during long-term storage. Fuel aging and oxidation by atmospheric oxygen can lead to increased acid numbers (which can increase corrosion), increased fuel viscosity, and the formation of gums and sediments. Storage stability of biodiesel is also affected by the presence of water within the tank. Water can cause hydrolytic degradation

[11] See Tier I Sections 2, 3, and 4.
[12] See Tier I Section 3.

of the fuel, contribute to microbial growth in the fuel, and can cause corrosion of fuel systems and storage tanks.

It is important to mitigate issues with materials compatibility by ensuring biodiesel storage and distribution involves only compatible materials. Additional risk mitigation may be provided via secondary containment systems and leak detection systems.

### 4.2.7. Toxicity[13]

The greatest difficulty in determining the exact human and ecological toxicity of biodiesel fuels is that biodiesel fuel is not a defined chemical formulation or a defined mixture of components, but can be formulated from any of a very large number of feedstocks with different chemical components. It is also difficult to exhaustively test all of the organisms that may be exposed to biodiesel during use or after a spill. The experimental requirements to confirm potential aquatic toxicity are complex. As a result, representative species are often selected to make generalized statements of toxicity.

Assessing the aquatic toxicity of biodiesel is a priority in California for a variety of reasons. First, due to the potential for biodiesel to be used and transported in areas surrounding both freshwater and saltwater, toxicity information relevant to species present in each of these environments are necessary. Second, studies evaluating toxicity in the literature did not always use the feedstocks most likely used in California. It was shown, however, that different feedstocks had widely different effects on toxicity. Third, the possibility of additives may also create differences in the toxicity of biodiesel used in California rather than the biodiesel used in previous studies. Lastly, the reference petroleum diesel used in the toxicity experiments in the literature is different than the petroleum diesel currently sold for use in the State of California.

Biodiesel blends tested for aquatic toxicity during Biodiesel Tier II studies by UC Davis, using California ULSD, exhibited somewhat increased toxicity to subsets of tested species compared to ULSD, and antioxidant-additized blends increase this toxicity for a smaller subset of tested species.

### 4.2.8. Transport and Fate[14]

There is a potential for releases to water and soil from from leaks and spills during fueling and vehicle use as well as atmospheric deposition from combustion. The transport and fate of a fuel in the environment is dependent on the multimedia transport properties of its constituent and additive chemicals. The properties are used to determine the equilibrium distribution of biodiesel and ULSD between different environmental compartments. The difficulty with biodiesel and ULSD are their chemical properties are not the same between samples. Production may occur from a wide variety of different feedstocks and lead to widely different key properties.

Some of the basic properties needed to describe the chemical fate of biodiesel are listed below. These are necessary for both ULSD and biodiesel in order to make a comparison of their relative multimedia risk.

[13] See Tier I Sections 6.4, 7, and Tier II Section 3.1.
[14] See Tier I Section 6 and Tier II Section 3.

- Partitioning coefficients to different multimedia pairs (i.e. air to water or fuel to solids)
- Physical properties: density, viscosity, interfacial tension
- Chemical properties: solubility, vapor pressure, composition, biodegradation rates

Studies regarding the subsurface fate and transport of biodiesel were not found in the Tier I literature review. The majority of the fate and transport information available in the scientific literature is associated with releases to air as a result of fuel combustion.

Small-scale laboratory infiltration experiments in two-dimensional sandboxes were used to visualize the relative rates of biodiesel infiltration, redistribution, and lens formation on the water table in comparison to that of ULSD. The experiments found that Soy B100, Soy B20, as well as AF B20, do not exhibit any significant differences. Through analysis of the primary release scenarios, a bulk release of biodiesel from a storage tank or during transport may introduce significant quantities into the subsurface environment. The movement and transfer of the bulk biodiesel phase to the soil and groundwater may need additional study. Additional care may be needed when biodiesel is stored or transported in significant quantities.

Non-aqueous phase liquids that are highly mobile in the subsurface with low solubility can be very difficult to clean up and may cause additional risk to down gradient water sources. Fuels that are relatively immiscible can be difficult to clean up in the subsurface environment. The movement of biodiesel through the environment is very important to evaluating the impacts to groundwater down gradient of a spill site.

Use of additives may enhance mobility, solubility or persistence in the subsurface leading to contamination of groundwater resources. Analysis of composition and properties of the biodiesel additives not yet tested are also needed to provide additional information about the movement of biodiesel through the subsurface compared to ULSD. Solubility of biodiesel and ULSD bulk fuel phases were not found in the scientific literature during the Tier I evaluation. Literature on biodiesel estimates biodiesel aqueous solubility to be very low. Due to biodiesel's polarity, it may be expected to be on average 15 to 25 % more soluble in water than diesel. Octanol-water partition coefficients were not available in the literature.

Vapor pressure expresses the tendency of a substance to vaporize. At a given temperature, a substance with a higher vapor pressure will volitilize faster than a substance with a lower vapor pressure. Fuels with a high vapor pressure require vapor recovery systems in order to avoid vapor phase releases to the enviornment. The lower vapor pressure of biodiesel relative to ULSD should not require additional care for storage of biodiesel and biodiesel blends. In addition bulk phase releses would be anticipated to behave similar to ULSD in regard to offgassing of the contamination plume.

Biodiesel is composed of alkyl esters of long chain fatty acids. ULSD is composed of aliphatic and aromatic hydrocarbons with trace amounts of nitrogen, sulfur, and other elemental additives. The exact compositions for both ULSD and biodiesel vary with the source and production factors. Aqueous solubility of biodiesel and diesel are widely variable on a constituent basis. Solubility is typically characterized in terms of pure chemicals and then scaled through partitioning relations for a multicomponent chemical. Solubility of biodiesel and ULSD bulk fuel phases were not found in the scientific literature during the Tier I evaluation. A more accurate

composition of the fuels could lead to individual components that have solubility information available in the literature.

Several studies have been performed on the biodegradation of biodiesel. Due to the structure of biodiesel compared to ULSD, biodiesel is more readily biodegradable. Studies by Zhang et al (1998) compare various biodiesel components to Philip's 2-D petroleum Diesel. All biodiesels performed consistently with 85% to 88.5% degradation in 28 days compare to 26% degradation by petroleum diesel. Biodiesel is also effective at promoting petroleum diesel degradation through co-metabolism. Biodegradation tests have also evaluated seed germination in contaminated soil and showed biodegradation to restore contaminated soil enough in four to six weeks to allow seed germination. Stolz et al (1995) performed both aerobic and anaerobic biodegradation in microcosms. Biodiesel from this study was not compared to diesel. Lapinskiene and Martinkus (2007) measured biodegradation in an argon atmosphere. Over 60 days, biodegradation was about twice as high for biodiesel than diesel. Knothe et al (2005) studied the chemical and biological oxygen demand of biodiesel and 2-D diesel. The biological oxygen demand for biodiesel is much higher than for diesel. The chemical oxygen demand for diesel and biodiesel were very similar.

While the results of the existing biodegradation experiments appear favorable for biodiesel and biodiesel blends with diesel, further evaluation is needed using the most up to date reference fuel for the state of California, CARB ULSD #2. In addition, due to various additive components that may be necessary to improve fuel combustion properties, additional study of biodegradation is also needed to evaluate the impacts from the additives not included in this mulitmedia assessment. Additives to prevent microbial growth in the fuel during storage and use may lead to significantly reduced biodegradation. Reducing biodegradation may lead to increased transport and mobility in the environment, especially in the subsurface where cleanup is especially difficult. Since biodiesel has mild solvency, biodegradation can also serve as a way to facilitate increased biodegradation in preexisting contamination sources. Additionally, if biodegradation were to be reduced due to additives or other reasons, the solvency could remobilize potentially toxic contaminants in soil or water. These fate and transport concerns may be addressed with mitigating measures introduced in the rulemaking process.

With some minor variations among blends (soy vs. animal fat; additized vs. non-additized), the results indicate that the additives effects are not significant on the biodegradation of biodiesel blends, and the blends tested are all more readily biodegradable than ULSD.

### 4.2.9. Waste Generation and Waste Management[15]

In evaluating the production, distribution and use of biodiesel (and other alternative diesel options) it is to identify where and what kind(s) of hazardous waste(s) may be generated. For example, sodium hydroxide and potassium hydroxide may be used as base catalysts for producing fatty acid methyl esters (FAME) from fats and oils. Acids are also used as catalysts for converting free fatty acids to methyl esters. In the process of using those catalysts, corrosive hazardous wastes may be generated. Some solvents are applied in the production process as well.

[15] See Tier I, Sections 2.4 and 8.3.

Proper identification and management of the waste solvents are required to comply with hazardous waste laws and regulations. Although there is evidence that biodiesel formulations are less toxic than standard diesel formulations, the storage stability of biodiesel is less than the standard ultra low sulfur diesel (ULSD). Degradation could be caused by temperature, oxidation, and/or material incompatibility; and some toxic components may be produced in the biodiesel.

Once the sources, composition, and magnitude of waste streams from biodiesel fuel production, distribution, and use have been identified, there is a need to identify management approaches that could be applied to the identified hazardous waste streams.

# 5. Tier III References

ASTM 2012. American Society for Testing and Materials (ASTM). Standard D6751-12, "Standard Specification for Biodiesel Fuel Blend Stock (B100) for Middle Distillate Fuels," ASTM International, West Conshohocken, PA, 2012, www.astm.org.

Birchall, C., J. Newman, and M. Greaves. 1995. Degradation and Phytotoxicity of Biodiesel oil, Institute of Arable Crops Research, 1995.

Brito, J.M., Belotti, L., Toledo, A. C., Antonangelo, L., Silva, FS., Alvim, DS., Andre, PA., Saldiva, PHN., Rivero, DHRF., 2010. Acute Cardiovascular and Inflammatory Toxicity Induced by Inhalation of Diesel and Biodiesel Exhaust Particles, Toxicological Sciences 116(1): 67-78.

Bunger, J., Krahl, J., Baum, K., Schroder, O., Muller, M., Westphal, G., Ruhnau, P., Schulz, T.G., Hallier, E., 2000., Cytotoxic and mutagenic effects, particle size and concentration analysis of diesel engine emissions using biodiesel and petrol diesel as fuel, Archives of Toxicology, 74(8):490-498.

Cahill, T. M. and R. Okamoto. 2012. Emissions of acrolein and other aldehydes from biodiesel-fueled heavy-duty vehicles, Environ. Sci & Tech., Vol. 46(15):8382-8388.

CalEPA (California Environmental Protection Agency). 2008. Guidance Document and Recommendations on the Types of Scientific Information to be Submitted by Applicants for California Fuels Environmental Multimedia Evaluations.

CalEPA (California Environmental Protection Agency) Multimedia Working Group. 2009. California Biodiesel Multimedia Evaluation Tier I Final Report, prepared by the University of California, Davis and the University of California, Berkeley. September 2009.

California Health and Safety Code 2011: http://leginfo.ca.gov/cgi-bin/displaycode?section=hsc&group=43001-44000&file=43830-43835

Durbin, et al. 2011. CARB Assessment of the Emissions from the Use of Biodiesel as a Motor Vehicle Fuel in California, Biodiesel Characterization and NOx Mitigation Study. Final Report. October 2011.

Gangwar, J., Gupta, T., ; Gupta, S., Agarwal, AK., 2011. Emissions from diesel versus biodiesel fuel used in a CRDI SUV engine: PM mass and chemical composition, Inhalation Toxicology 23(8): 449-458.

Knothe, G., J.V. Gerpen, and J. Krahl. 2005. The Biodiesel Handbook, AOCS Press, Champaign, Illinois, 2005.

Lapinskiene, Asta, and Povilas Martinkus. 2007.Research on Anaerobic Biodegradation of Fats, Biodiesel Fuel and Diesel Fuel in Soil Medium. Environmental Research, Engineering and Management, 2007.No.1(39), P. 30-37.

Liu, Yu-Yin; Lin, Ta-Chang; Wang, Ying-Jan, Ho, W.-L., 2008. Biological toxicities of emissions from an unmodified engine fueled with diesel and biodiesel blend, J. Environmental

National Renewable Energy Laboratory NREL Technical Report TP-540-4367, Biodiesel Handling and Use Guide, 4th Edition. January 2009.

Science and Health Part A – Toxic/Hazardous Dubstances & Environmental Engineering 43(14): 1735-1743.

Song, WW., He, KB., Wang, JX., Wang, XT., Shi, XY., Yu, C., Chen, WM., Zheng, L., 2011. Emissions of EC, OC, and PAHs from Cottonseed Oil Biodiesel in a Heavy-Duty Diesel Engine, Environmental Science and Technology, 45(15):6683-6689.

Stolz, J., P. Follis, G. Floro, R. Donofrio, J. Buzzelli, and W. Griffin. 1995. Aerobic and Anaerobic biodegradation of the methyl esterified fatty acids of soy diesel in freshwater and soil environments. http://www.biodiesel.org/resources/reportsdatabase/reports/gen/19950101_gen-273.pdf

Tsai, JH., Huang, KL., Chiu, CH., Lin, CC., Kuo, WC., Lin, WY., Chaung, HC., Yang, TH., Chen, SJ., 2011. Particle-bound PAHs and Particle-extract-induced Cytotoxicity of Emission from a Diesel-generator Fuelled with Soy-biodiesel, Aerosol and Air Quality Research, 11(7): 822-836.

University of California (UC). 2009. California Biodiesel Multimedia Evaluation: Tier I Report (Final Draft). September, 2009.

University of California (UC). 2012. California Biodiesel Multimedia Evaluation: Tier II Report on Aquatic Toxicity, Biodegradation, and Subsurface Transport Experiments (Draft Final). September, 2012.

Van Gerpen, J., B. Shanks, R. Pruszko, D. Clements, and G. Knothe. 2004. Biodiesel Analytical Methods, National Renewable Energy Laboratory, NREL/SR-510-36240, July, 2004. http://www.nrel.gov/docs/fy04osti/36240.pdf.

Widegren, J. A. and T. J. Bruno, 2011. Vapor pressure measurements on saturated biodiesel fuel esters by the concatenated gas saturation method, Fuel, vol. 90(5):1833–1839.

Zhang, X., C. Peterson, D. Reece, G. Moller, and R. Haws. 1998. Biodegradability of Biodiesel in the Aquatic Environment, ASAE, 41(5): 1423 – 1430.

# 6. Tier III Appendices

## 6.1. Appendix III-A: California Biodiesel Multimedia Evaluation Tier I Final Report, September 2009

# California Biodiesel Multimedia Evaluation

## Tier I Report

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## FINAL

**September 2009**

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# Executive Summary

The purpose of this multimedia assessment is to provide the State of California information that will allow an informed decision as to the relative impacts posed by biodiesel to the State's resources, human health, and environment. Biodiesel and renewable diesel are names of alternative diesel-substitute fuels, derived from biological sources (such as vegetable oils or tallow), which can be used in unmodified diesel-engine vehicles. This report focuses on biodiesel.

## Background

Pure biodiesel contains no petroleum, but it can be blended with petroleum diesel to create a biodiesel blend. To create biodiesel, a vegetable oil or animal fat is subjected to a chemical reaction known as transesterification. In this reaction, a feedstock (either oil or fat) is reacted with alcohol in the presence of a catalyst to produce glycerin as a byproduct and methyl esters, which compose biodiesel, also know as Fatty Acid Methyl Ester or FAME.

Alternatives to transesterified biodiesel are appearing rapidly. Renewable diesel can be co-processed in existing refineries or be produced in stand-alone refineries* that use feedstocks similar to those used for biodiesel, but employ a refinery process such as hydro-treating to create a product that is chemically different from FAME but similar to the hydrocarbons that are constituents of petroleum diesel. Thus, transesterification is not involved, and the result is a non-ester renewable fuel. Thermal depolymerized diesel is another non-petroleum feedstock-based diesel that is produced through a hydrous-pyrolysis process that mimics the geological heat and pressures that naturally produce crude petroleum oil. Renewable diesel is the subject of another multimedia assessment, that is now in preparation.

Because biodiesel blends are new fuels, the California Air Resources Board (CARB) must provide a "multimedia assessment" of their potential impacts before adopting new fuel specifications (as required by California Health and Safety Code, Section 43830.8). Further, the "California Air Resources Board cannot adopt any regulation establishing a motor vehicle fuel specification unless a multimedia evaluation is conducted to determine whether the regulation will cause a significant adverse impact on the public health or environment" (California Senate Bill 140, 2007).

There are many steps involved in the transesterification production of biodiesel. Initially, the three components, alcohol, oil, and a catalyst, are mixed in a reactor. The next step involves separating the methyl esters and glycerin (a byproduct). The methyl esters are then neutralized with acid to remove any residual catalyst and to separate any soap that may have formed during the reaction. The mixture is washed with water and any alcohol is removed. The biodiesel may then be dried in a vacuum flash process that leaves a clear amber-yellow liquid with a viscosity similar to petroleum diesel. Some processes also distill the final product to remove undesirable impurities.

Primary biodiesel feedstocks expected to be used in California include: soybean oil, palm oil, yellow grease, animal tallow, trap (brown) grease, canola oil, safflower oil, and (perhaps in the future) algae. Biodiesel feedstocks are classified by their fatty acid profile; the fatty acid

*Stand-alone renewable diesel refineries are currently being commercialized. Federal regulations treat co-processed and stand-alone products differently.

composition greatly influences a fuel's characteristics, as esters of different fatty acids have different physical and chemical properties. Price, availability, origin, geography, and consistent quality generally dictate which feedstock biodiesel producers use.

Generally, the quality of the fuel is dependent on the quality and fatty-acid composition of the feedstock, the production process, and post-production handling. Pure biodiesel fuels when used as blendstocks, regardless of feedstock, must meet the ASTM D6751 standards. Biodiesel blends up to B5 must meet ASTM D975. ASTM has also established ASTM 7467 for blends of B6 to B20 and ASTM D6751-12 covers B100. Biodiesel blends that meet ASTM standards can be used in most modern engines without modifications, but some manufacturers recommend against using blends above B5.

## Study Approach and Results

This Tier I report is the first step in evaluating the cumulative health and ecological impacts from releases to air, surface water, groundwater and soil at all stages of the life cycle of bio-diesel blends: feedstock production/collection, fuel production, fuel storage and distribution, and fuel use. The potential impact posed by diesel is assessed relative to the ultra low sulfur diesel (ULSD) currently in use. This report does not aim to address the direct and indirect environmental, ecological, and health impacts that biodiesel production may have due to changes in land use and the possible net gain in carbon emissions due to feedstock cultivation.

Our focus in this multimedia evaluation is on a blend of 20% biodiesel with 80% standard ULSD (B20). B20 is the blend recommended by the biodiesel industry for use in on-site storage and distribution from fuel service stations. The transportation and storage of pure biodiesel (B100) as a distributed ingredient in on-site mixing are also considered. Biodiesel is typically transported via rail cars, tank trucks, and drums.

Material compatibility is an important property to consider during the storage and distribution of biodiesel. Since biodiesel can react with some metals and polymers, it is critical that the material of tanks, hoses, gaskets, and other parts that may come in contact with biodiesel, are compatible with the fuel's properties. Biodiesel is not compatible with brass, bronze, copper, lead, tin and zinc as these metals can initiate oxidation and sediment production. Nitrile rubber compounds, polypropylene, polyvinyl, and Tygon are particularly vulnerable to biodiesel. Biodiesel has higher solvency properties than petroleum diesel fuel and can act as a mild solvent. It can dissolve the residual sediments in diesel storage tanks and engine fuel tanks. These dissolved sediments can harm fuel systems as they plug fuel filters.

Biodiesel is susceptible to chemical changes during long-term storage. Fuel aging and oxidation by atmospheric oxygen can lead to increased acid numbers (which can increase corrosion), increased fuel viscosity, and the formation of gums and sediments. Storage stability of biodiesel is also affected by the presence of water within the tank. Water can cause hydrolytic degradation of the fuel, contribute to microbial growth in the fuel, and can cause corrosion of fuel systems and storage tanks.

There are a number of vehicle operability issues associated with the use of biodiesel blends. These include cold fuel flow, fuel foaming, water separation, and fuel oxidative stability. Cold flow problems can result in fuel system clogging. Fuel foaming can result in difficulty during tank filling and can potentially increase the possibility of fuel spills. Reduced water separation can result in water/fuel mixtures that can clog fuel systems and promote microbial growth that can also clog fuel systems. Oxidative degradation of biodiesel can result in injector deposits,

lacquer formation on fuel system components, and fuel systems corrosion. These impacts to the vehicle fuel system can result in reduced drive-ability, higher tailpipe emissions, and increased maintenance costs.

Chemical additives with a range of costs and effectiveness are commercially available to address the oxidative stability, cold-flow properties, microbial contamination, increased water affinity and increased $NO_x$ emissions of biodiesel. For example ultralow sulfur kerosene can be used with biodiesel blends to address flow problems in cold weather.

Releases associated with the production, storage and distribution, and use of biodiesel can be regarded as normal or off-normal. Different feedstocks and production processes may have different normal and off-normal releases and may affect different environmental media and human populations depending on geographic location. Normal or routine releases during the production of B100 may be:

- hexane or $CO_2$ released during seed-oil extraction residual oil washing.
- odors associated with waste biomass.
- methanol releases to air or water.
- used process water discharges of various pH.
- tailpipe emissions during combustion.

Normal releases during the use of biodiesel include combustion tailpipe emissions, both to the air and to surface waters in the case of marine use. The magnitude of these normal production and use releases within California is not clear yet.

Off-normal releases may include spills or leaks of bulk feedstock oil, production chemicals, such as methanol, hexane, acid, base, or blending stocks such as ULSD or B100, or finished B20 fuel. These off-normal releases may be the result of leak or rupture of:

- an above ground or underground storage tank and associated piping.
- a liquid transportation vehicle such as rail tank car, tanker truck, or tanker ship.
- a bulk fuel transport pipeline.
- tank storing unprocessed glycerin.

In the fuel-use stage of the biodiesel life cycle, the releases of greatest concern are emissions to air, but there are also potential releases to water and soil from atmospheric deposition and from leaks and spills during fueling and vehicle use. Several studies have determined that use of biodiesel (as B100 or a B20 blend) instead of conventional diesel may be expected to exhibit large reductions in hydrocarbons (HC), particulate matter (PM) and carbon monoxide (CO) emissions. The type of feedstock and conventional diesel (used for blending) can influence these emissions. The $NO_x$ emissions from biodiesel fuels increased with percent biodiesel blended and vary considerably with biodiesel feedstock used. Biodiesel containing feedstocks high in polyunsaturated fatty acids emit a greater percentage of $NO_x$ than biodiesel high in saturated fatty acids.

Deploying a multimedia assessment framework to understand potential impacts requires basic information about the chemical/physical properties of the substances under consideration. These properties describe how a substance will distribute itself among the major phases of the environment—air, water, and organic phases such as lipids and organic materials in soil. The

important multiphase transport properties identified include: chemical makeup of biodiesel diesel, solubility in water, sorption to solids, vapor pressure, and interfacial tensions. Here we summarize available information and knowledge gaps about these properties.

Biodegradation of hydrocarbons by microorganisms represents one of the primary mechanisms by which petroleum and diesel products are removed from the environment. The biodiesel biodegradation information evaluated in this report includes: biodegradability in aquatic environments, biodegradation in soil, biodegradation under aerobic and anaerobic conditions, and biological and chemical oxygen demand.

The greatest difficulty in determining the human and ecological toxicity of biodiesel fuels is that biodiesel fuel is not a defined chemical formulation or a defined mixture of components, but can be formulated from any of a very large number of feedstocks with different chemical components. In general, tests show that pure biodiesel is considerably less toxic than pure diesel fuel. Formulations of mixed biodiesel-diesel fuel such as B20 or B50 have shown results consistent with the calculated diluent effect of the percentage of biodiesel fuel on the total toxicity of the mixture.

Life cycle inventory (LCI) comparisons between biodiesel and petroleum diesel show that overall, B100 biodiesel yields 3.2 units of fuel product energy for every unit of fossil energy consumed in its life cycle. The fossil energy ratio of B20, however, is less than B100 with a ratio of 0.98 units. The LCI also included air pollutants: carbon monoxide (CO), nitrogen oxides ($NO_x$), particulate matter (PM), sulfur oxides ($SO_x$), non-methane hydrocarbons (NMHC), and toxic air pollutants. All tested pollutants, other than $NO_x$, hydrochloric acid (HCl), and total hydrocarbons (THC), show decreased emissions relative to petroleum diesel. A USDA/USDOE (1998) study of life-cycle impacts of biodiesel fuels determined that a bus using B100 derived from soy produces 32% less total particulate matter (TPM) and 35% less CO than does petroleum diesel for the same transportation service. This study also estimated biodiesel life cycle wastewater flows for B100 as almost 80% lower than petroleum diesel. But the study did not consider life-cycle water demands. A key issue for PM and toxic air pollutants from diesel and biodiesel alternatives is the need to address the shift in chemical composition of emissions as well as specific reductions/increases.

## Key Information Gaps and the Tier-II Sampling Plan

**1. Additives impacts.** To provide a stable useful, and reliable fuel, additive chemicals will need to be introduced into almost all biodiesel blends. These additives will be required to control oxidation, corrosion, foaming, cold temperature flow properties, biodegradation, water separation, and $NO_x$ formation. The specific chemicals and amounts used have not been well-defined for the emerging industry in California. A careful evaluation the possible chemicals would be beneficial to California and may lead to a “recommended list” or “acceptable list” that would minimize the uncertainty of future impacts as industry standards are developed.

The impact of various additives that may be used with biodiesel blends needs to be considered for releases to the air, water, and soils. Additives may affect fuel quality or storage stability in unintended ways. Because the properties of additives can potentially alter the characteristics of biodiesel, increasing its environmental and health risks, there is a need for additional tests on biodiesel with specific concentrations of additives. In particular it is necessary to assess the impact of

- cold flow property controllers on surface water- biodiesel interaction and on subsurface multiphase transport of biodiesel (see number 2 below).
- biocides and anti-oxidants on biodegradation (see number 3 below).
- all priority additives on human and ecosystem toxicity.

**2. Subsurface fate and transport properties**. The impacts of leaks and spills of biodiesel fuel product during transport, storage, and distribution have not been addressed. This is an important issue for California. Because the chemical composition of biodiesel differs significantly from that of petroleum diesel, it is expected that infiltration, redistribution, and lens formation on water tables will differ for the two fuels, leading potentially to significant differences in relative impacts to groundwater quality. Properties governing these processes are density, viscosity, and interfacial tensions. Component (including additive) solubility into the water phase ultimately governs water quality and so inter-phase solubilization of individual components also needs to be identified. To address these issues requires experiments with conventional soil column tests that will be used to establish relative transport behaviors among different fuel compositions and for site-specific analyses. But the relevance of these results for state-wide assessments should be considered along with the value of full-scale comparative field tests with releases into the groundwater, or into the vadose zone just above the groundwater table.

**3. Biodegradation in soils and aquifers**. The anticipated use of biocides in B20 fuels may affect the biodegradation potential for biodiesel released into the environment. The impact of biocides added to blended biodiesel may reduce the biodegradation of biodiesel and other petroleum-based fuels leaked or spilled into the subsurface. Since subsurface biodegradation can play an important role in the remediation of fuel spills and leaks, an understanding of the fate of biodiesel with biocide additive is needed.

Not all biodiesel fuel blends have been tested for degradation under aerobic and especially anaerobic conditions. To our knowledge, only one alternate electron acceptor, nitrate, has been tested; others such as sulfate and methanogenic conditions should be explored, because these are the primary available electron acceptors expected in the reducing environments expected in deeper soil contamination and in aquifers.

Pure biodiesel (B100) without additives may be more biodegradable than ULSD and may be preferentially metabolized by subsurface microbes. The interaction of B100 with existing gasoline or conventional diesel previously released into the subsurface needs to be examined more closely.

**4. Production and storage releases.** In addition to impacts from released B100 or blended B20 biodiesel, increased production and associated feedstock processing may involve impacts from released reactants and by-products. There are potential impacts to California's air and water during the large-scale industrial operations used to extract seed oils when these operations are carried out in California. These impacts may result from air emissions of solvents used to extract the seed oil (e.g., hexane) and from leaking tanks containing chemicals to process the plant oils into biodiesel. There is also the issue of occupational exposures. Finally, UST material compatibility must be addressed: owner/operators are required by state health and safety codes to demonstrate material compatibility prior to storage of biodiesel. The impacts during seed extraction will become more of an issue for California as in-state production of plant-derived oils increases and may require further study. Based on current projections, the possible impacts during seed extraction will be minimal in California since it is anticipated that most of the seed oils will be derived from soy grown and extracted out-of-state.

Among the most important current production reactants are: methanol, generic acid or base catalysts, feedstock oils, and post-processing water. As the biodiesel industry matures, release scenarios developed in this report need to be refined and prioritized.

**5. Additional air-emission studies**. There are not yet sufficient data to assert that the use of biodiesel will reduce the emissions of criteria and toxic air pollutants. Although considerable data is available on the effect of biodiesel on EPA-regulated pollutants (i.e., HC, CO, $NO_x$, and particulates), most of this data was generated using older technology engines. Further, very little detailed exhaust characterization data on biodiesel exists beyond a small number of regulated pollutants. Planned emission testing is based on newly blended B20 fuel stocks with only an anti-oxidant added and purged with nitrogen. B5, B50 and B100 will also be tested. The anti-oxidant and nitrogen purge are needed because the fuel used for the planned testing will be at least six month old-which is at the maximum recommended storage time for biodiesel. We note that these fuel mixes may not necessarily represent the general storage conditions expected throughout California. It is well established that fuel-handling practices have an important influence on engine performance and combustion emission. Additionally, the impact of various additives on combustion air emissions needs to be evaluated. Given the wide variety of oils and fats that might be used to make biodiesel fuel and the potential additives, the actual emissions of PMs and toxic air pollutants will have to be determined for each proposed formulation of biodiesel fuel to be used in California. This situation demands a systematic and ongoing effort to assess emissions from diesel engines. In particular, there is a need for more controlled combustion studies to assess how the spectrum of toxic air pollutants, such as the spectrum of PAHs, will shift both in terms of volatile and particle-bound fraction but also in terms of any changes in toxic equivalency.

**6. Tier II Sampling Plan.** Many of the data gaps identified above will be addressed in the sampling plan developed for the Tier II study and report. In preparing this report, we had a number of discussions with Cal-EPA staff, other academic researchers, and key stakeholders that provide important insight for developing the Tier-II sampling plan. First there is the issue of differences in emissions from different vehicle/engine classes such as on and off road vehicles. Where possible, this information should be included in the Tier two assessment. Next there are a number of issues of coordination among samples for different media and different objects. Samples used to test fate, transport, and toxicity should come from the same batch and be stored (aged) under the same conditions. When making comparisons of ULSD and biodiesel, all fuels should be subjected to the same tests. One example includes efforts to assure that fuel samples tested for water quality include the same types of fuel samples used by CARB for air emissions tests to evaluate the effects and toxicity of the antioxidant additive. A second example is that the biodiesel formulation used to approve underground storage tank components for material compatibility should be the same formulation used in material compatibility tests. FAME-derived-biodiesel samples used in fate, transport, and toxicity testing should represent the vegetable oil and the animal-fat feedstock most widely found in biodiesel marketed in California. Similarly, the California ULSD that is used in the comparison tests must represent formulations currently used in the State. Finally, samples used in fate studies should, where possible, represent both an ideal biodiesel composition that meets ASTM D6751 and ASTM D7467 specifications, as well as a more real-world example of fuel stored in an underground storage tank—containing water, peroxide and ammonia nitrogen levels attained through natural aging of biodiesel without antioxidants.

**7. Life Cycle Impacts.** Only differences in emissions inventories were considered during recent studies comparing life cycle inventories (LCI) of biodiesel to petroleum diesel. Differences in health and environmental impacts associated with these LCI differences needs to be evaluated. Additionally, current LCI studies have been limited to only soybean oil feedstocks. It is well established that different feedstocks can have an important influence on life cycle emissions. Information is needed for other feedstocks.

**8. Priority list of biodiesel formulations.** Because the number of potential feedstocks, the number of fuel blends, and the number of additive choices and mixes makes for an unmanageable suite of permutations of cases for consideration, it is critical to identify the priority feedstocks, fuel blends, and additives requiring study for our impacts assessment. Not specifically addressed in this Tier-I evaluation are the environmental impacts from the increased use of fertilizers and water and land resources as the production of plant oils increases in the State. These factors could become limiting as the biofuels industry expands. More sustainable sources of biodiesel such as yellow or brown grease may be preferable and should be encouraged to the extent that such feedstocks are available and can supply fuel quantities required by diesel engines in California.

Finally, a key goal of encouraging fuels such as biodiesel is to reduce California's carbon "footprint" as part of a global strategy. To consider only the environmental impacts to California and disregard the impacts that may be occurring nationally or internationally during the production of the biofuels feedstock that is used in California is short-sighted.

# 1. Biodiesel Background Information

## 1.1. Introduction

The purpose of this multimedia assessment is to provide the State of California information that will allow an informed decision as to the relative health and environmental impacts to the State's resources, human health and environment posed by the use biodiesel. Biodiesel and renewable diesel are the names of alternative diesel-equivalent fuels, derived from biological sources (such as vegetable oils or tallow), which can be used in unmodified diesel-engine vehicles.

Biodiesel is defined as a fuel composed of mono-alkyl esters of long chain fatty acids derived from vegetable oils or animal fats (NBB, 2007). Biodiesel has been derived from a broad range of vegetable oils, recycled cooking greases or oils, animal fats and algal oils. It can be used as a pure fuel or as a blend with petroleum diesel, as biodiesel is miscible with petroleum diesel at all ratios (Knothe et al., 2005). The most common blend is B20 (20% biodiesel mixed with 80% ultra-low sulfur diesel, ULSD). In this report, the word *biodiesel* refers to pure biodiesel (B100) and meets the specific biodiesel definition and standards approved by ASTM (American Society for Testing and Materials) D6751-12.

Pure biodiesel contains no petroleum, but it can be blended at any level with petroleum diesel to create a biodiesel blend. Alternatives to of biodiesel are also appearing rapidly. Renewable diesel (co-processed diesel) is another alternative fuel that has potential in California. Renewable diesel is comparable to biodiesel in that similar feedstocks are used in a traditional fractional distillation method that creates a chemically different product. Thermally depolymerized diesel is non-petroleum feedstock-based diesel that is produced through a hydrous pyrolysis process that mimics the geological heat and pressures that naturally produces crude petroleum oil.

Since biodiesel is a new fuel, the California Air Resources Board (CARB) must provide a "multimedia risk assessment" before adopting new fuel specifications (as required by California Health and Safety Code, Section 43830.8). Further, existing law states that the "California Air Resources Board cannot adopt any regulation establishing a motor vehicle fuel specification unless a multimedia evaluation is conducted to determine whether the regulation will cause a significant adverse impact on the public health or environment" (California Senate Bill 140, 2007).

As a result, the California Environmental Protection Agency (Cal-EPA) has initiated a program to assess the multimedia life-cycle impacts of biodiesel fuels used in California. This Tier I report is the first step in a three tier process evaluating the cumulative health and ecological impacts from releases to air, surface water, groundwater and soil at all stages of the biodiesel life cycle: production, storage and distribution, and use. The risk posed by biodiesel is assessed as a relative risk compared to ultra low sulfur diesel currently in use.

The goal of this Tier I report is to identify what is currently known about biodiesel along with a discussion of key uncertainties and data gaps, including:

- Physical, and chemical and environmental toxicity characteristics of the reference fuel, candidate fuel and additive components,
- Summary of all potential production, distribution, storage, and use release scenarios including a discussion of the most likely release scenarios,

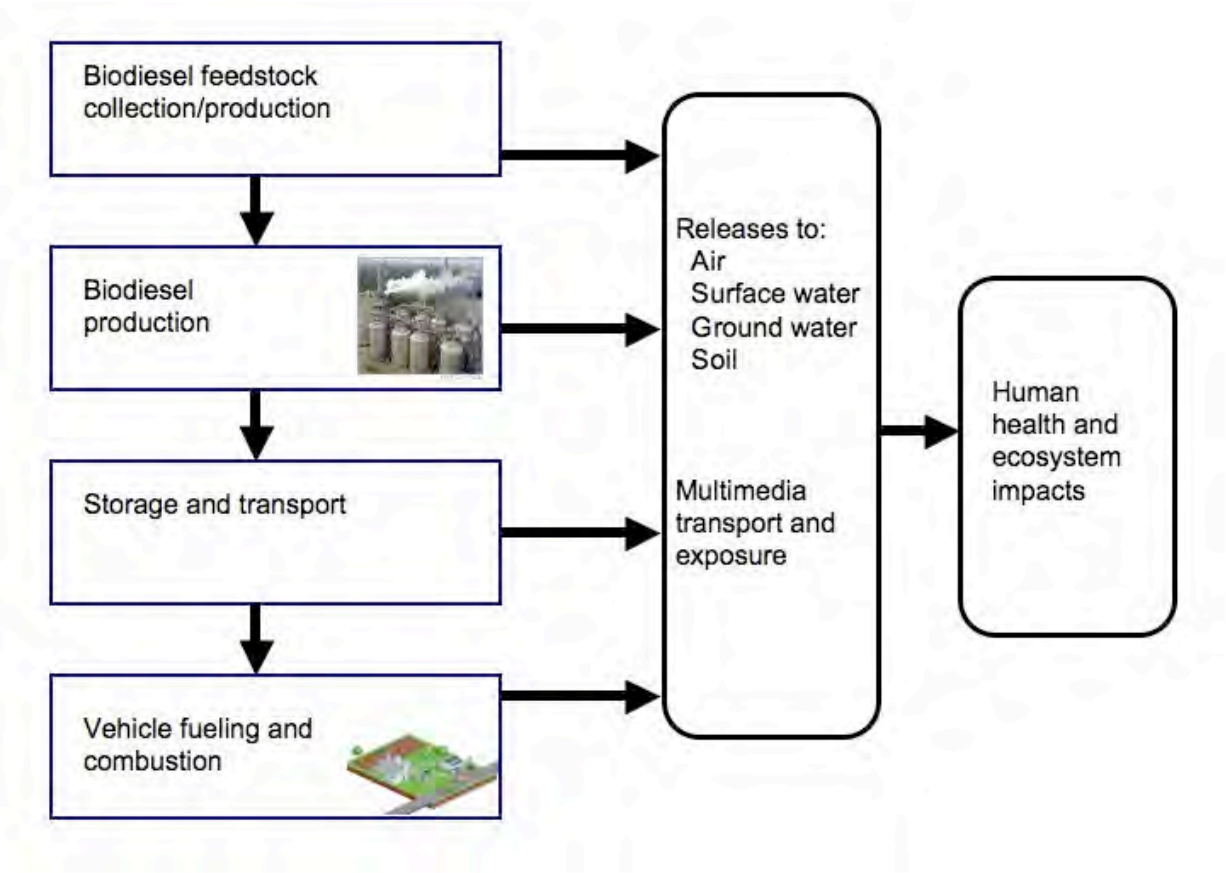
- Summary of the expected environmental behavior (transport and fate conceptual models associated with release scenarios) of proposed fuel or fuel components that may be released, and
- Comparison of physical, chemical, and toxic properties of the fuel or additive components to appropriate agreed upon control fuel or fuel components.

This report excludes the direct and indirect environmental, ecological, and health impacts associated with biomass production such as changes in land use and the possible net gain in carbon emissions due to feedstock cultivation. There is a scientific debate concerning the sustainability of wide scale energy conversion from fossil fuels to biofuels (Wang & Haq, 2008). Controversial findings suggest that the clearing of virgin rainforests and grasslands to make land for biofuel production will produce high initial "carbon debts" that are estimated to have decades or even centuries long pay-back periods due to the modest savings in carbon emissions from burning biofuels (Searchinger et al., 2008; UMN, 2008; The Wall Street Journal, 2008). Such issues have lead the European Union to propose a ban on certain biofuel sources such as palm oil from Southeast Asia due to associated deforestation and habitat loss, and due to non-sustainability of palm tree monoculture (Kantor, 2008; Rosenthal, 2007). Some end-users (e.g., Virgin Atlantic airlines) seek only sustainable sources of biofuels that are not produced in ways that compete for food grain production and/or add to deforestation (Clark, 2008). It is clear that the issue of sustainability and complete life cycle costs of biofuels are important aspects of the impacts biofuels will have. However, such evaluation is beyond the current scope that includes the risks associated with the production, transportation, storage, and use of biofuels and not the broader impacts of increased/decreased use of various raw feedstocks.

This Tier I report sets the stage for this multimedia assessment and follows the guidance set forth in the "Guidance Document and Recommendations on the Types of Scientific Information to be Submitted by Applicants for California Fuels Environmental Multimedia Evaluations" (2008).

During Tier II activities, a plan of action is developed to address these uncertainties and data gaps and conduct a life-cycle based assessment of the potential impacts biodiesel may have to the State's resources, human health and environment. During Tier III activities, the life cycle based assessment is performed and the results reported to California Environmental Policy Council. Figure 1.1 provides an overview of the life-cycle stages that we will be discussing in this report. We consider four major life stages—feedstock production/collection, biodiesel production, transport and storage, and fuel use (combustion).

**Figure 1.1. Generalized summary of biodiesel life cycle impacts.**




## 1.2. History

The first appearance and demonstration of an oil based diesel fuel was at the Paris Exhibition in 1900. The French Government requested the Otto Company to use Arachide (earth-nut or peanut) oil as a fuel in one of their small diesel engine cars. The demonstration went so well that few spectators were aware that the car was running on vegetable oil. This sparked interest in vegetable based fuels for many European countries. France, Italy, Belgium, the UK, and other countries with territories or colonies in Africa, saw potential for Arachide oil because it was readily abundant within the region (Knothe et al., 2005). It was thought that if Arachide could be used as a fuel, it would not only be economically feasible, but also result in a sustainable fuel source.

The use of crude vegetable oil as a fuel was explored until 1937 when Walton discovered that the viscosity of vegetable oil was too high for use in a combustion engine and that vegetable oil required a refining process to reduce its viscosity. In this process, oil was reacted with an alcohol to transform the triglycerides to esters with glycerin as a by product. Walton's work established the first research on biodiesel and a patent was granted for the "transformation of vegetable oils for their use as fuels" (Knothe et al., 2005).

World War II sparked an added interest in biodiesel. With limited oil supplies, many nations resorted to vegetable oil based fuels and used oil from locally grown plants to fuel large tankers and other war machinery (Knothe et al., 2005). Another wartime benefit of biodiesel was its byproduct, glycerin, used as a main ingredient for explosives. However, following the war, a steady supply of cheaper diesel became available again and the production of biodiesel was essentially curtailed.

The oil crisis in the 1970s and 1980s revived an interest in biodiesel. At that time, as the price of fuel doubled, it became clear that an alternative fuel was needed to reduce the nation's dependence on foreign oil. Early experiments on the production and use of biodiesel were conducted at the University of Idaho, University of Missouri, the National Renewable Energy Laboratory, and a handful of other institutions. In 1991, the first small-batch-process pilot plant was set up in Kansas City, Missouri to supply limited quantities of biodiesel. Today biodiesel continues to attract attention with the expectation that this renewable resource will provide environmental benefits with lower emissions (Kemp, 2006).

## 1.3. Legislative Incentives for Biodiesel

To reduce our nation's dependence on imported oil, Congress passed the Energy Policy Act of 1992 (EP Act). This Act required 75% of new vehicle purchases made by federal and state governments, and alternative fuel providers to be alternative fuel vehicles. Compliance was mandatory for these agencies that operated, leased, or controlled 50 or more light-weight vehicles. The alternative fuels on which these vehicles could run included: pure biodiesel (B100), blends of 85% or more of alcohol with gasoline, natural gas and liquid fuels domestically produced from natural gas, hydrogen, electricity, coal-derived liquid fuels, and liquefied petroleum gas (USDOE, 2005).

Energy Conservation and Reauthorization Act of 1998 amended and updated many elements of the 1992 EP Act. The 1998 amendment allowed "qualified fleets to use B20 in existing vehicles to generate alternative fuel vehicle purchase credits, with some limitations" (USDOE, 2006). This amendment significantly increased the use of B20 by government and alternative fuel provider fleets.

The Energy Policy Act of 2005 was signed into law in August 2005. This legislation supports the growth of the biodiesel industry. Consumer and business federal tax credits for biodiesel were extended to 2008 and the credits were provided to small agri-biodiesel producers. This legislation also requires a comprehensive two-year "analysis of impacts from biodiesel on engine operation for both existing and expected future diesel technologies, and provides recommendations for ensuring optimal emissions reductions and engine performance with biodiesel." (Federal Record, 2005).

In 2007, California Senator Christine Kehoe introduced Senate Bill 140 which, if passed will require all diesel sold in California to contain two percent renewable fuel. Two years after implementing this requirement, all diesel fuels will be required to contain at least five percent renewable fuels (California Senate Bill 140, 2007).

# 2. Production of Biodiesel

## 2.1. Biodiesel Production Chemistry

To make biodiesel, a vegetable oil or animal fat is subjected to a chemical reaction known as transesterification. In this reaction, a feedstock (either oil or fat) is reacted with alcohol in the presence of a catalyst to produce glycerin and methyl esters (known as biodiesel). This reaction is shown below.

**Figure 2.1. Transesterification Reaction*.**

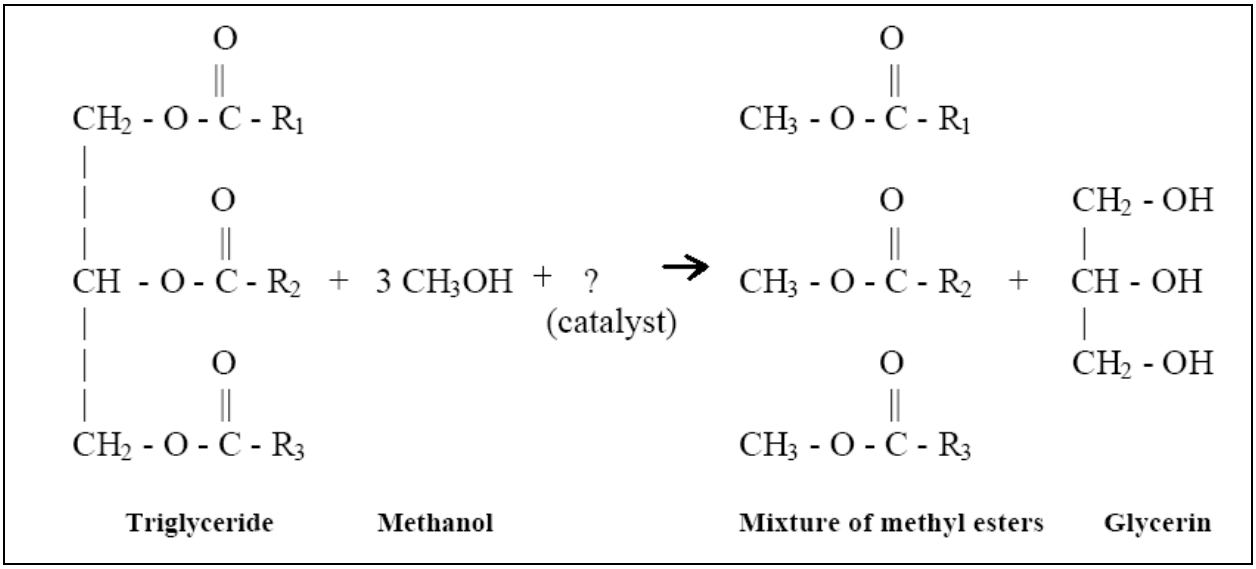



*Figure from Van Gerpen (2004)

In Figure 2.1, the $R_1$, $R_2$, and $R_3$ represent long-chain hydrocarbon fatty acid associated with the feedstock; these are largely palmitic, stearic, oleic, and linoleic acids from naturally occurring oils and fats (NBB, 2007). In theory, the transesterification reaction is reversible, however, "the back reaction does not occur or is negligible largely because the glycerin formed is not miscible with the product, leading to a two-phase system" (Knothe et al., 2005).

### 2.1.1. Biodiesel Transesterification Reactants

There are a variety of alternative reactants available for producing biodiesel. Reactants are selected based on economic considerations and on the chemical and physical properties of the feedstock.

*Alcohol*

Either methanol or ethanol can be used as a reactant alcohol in the transesterification process. Economic considerations generally dictate which one is selected because the chemical characteristics of biodiesels do not depend greatly on the particular alcohol used in this process (Van Gerpen et al., 2004). Compared with methanol, ethanol is currently more expensive and about 44% more ethanol is required for the transesterification reaction (Van Gerpen et al., 2004). But the alcohol recovery process is also an important factor in selecting between ethanol and methanol. Excess alcohol must be recovered to minimize operation costs and environmental

impacts. Although the stoichiometric molar ratio for alcohol to triglycerides is 3:1, a 6:1 molar ratio is typically used to ensure a total conversion of the fat or oil to its esters, leaving residual (unreacted) alcohol. Since methanol has a low boiling point, the unreacted alcohol can be removed fairly easily through distillation. Ethanol is more difficult to recover and return to the process because ethanol in water forms an azeotrope, i.e. a mixture for which there is little change in chemical composition through distillation (Van Gerpen et al., 2004).

*Triglyceride (Feedstock)*

Fats and oils (commonly referred to as the feedstock) are composed of organic compounds made up of three fatty acid units attached to a three-carbon backbone, known as "triglycerides" (Van Gerpen et al., 2004). Each feedstock has a different fatty acid unit. The properties of the final biodiesel product can vary greatly based on the composition of the unit. Feedstock is further discussed in Section 2.2.

*Feedstock extraction*

Primary biodiesel feedstocks expected to be used in California include: soybean oil, palm oil, yellow grease, animal tallow, trap (brown) grease, canola oil, safflower oil, and (perhaps in the future) algae. Once a feedstock is harvested, the oil-bearing component (such as seeds) must be crushed to extract the oil (which is then transesterified). Crushing facilities extract the oil in a two-step process. First, the biomass is pressed with expellers to extract 60 to 70% of the oil. Since the resulting product still contains between 14 and 20% oil, a second step is required. The product is sprayed with a solvent (typically hexane) and the oil, which is soluble in the solvent, is removed. The solvent is then distilled off, leaving crude oil.

While this oil extraction process is relatively simple, in California there are some additional challenges. Currently there are only two crushing facilities in the state: the J.G., Boswell Company in Corcoran and Adams Grain in Woodland (Kaffka, 2007). To support large-scale biodiesel production in California, additional facilities must be built.

The biggest challenge for increasing the number of oil extraction facilities in the state is obtaining the environmental permits for building them. Solvent losses to the atmosphere during several points in the production process range from 0.2 to 2.0 gallons per ton of biomass (seeds) processed. Since the solvent is composed of 100% volatile organic compounds, the process produces potentially harmful VOC releases. For example, hexane is classified under the Clean Air Act as a Hazardous air pollutant and emissions are highly restricted. With California's strict regulations, obtaining a permit to build a large VOC producing plant can be difficult.

*Catalyst*

Both acids and bases are used as catalysts in biodiesel production. The content of free fatty acids (FFAs) within the feedstock predominately determines the type of catalyst used in the transesterification process. For feedstocks with FFA levels around 1%, such as soybean oil, canola (rapeseed) oil, and higher grades of restaurant waste oils a base catalyst is used. Animal tallow, palm oil, and other feedstocks that are higher in FFA content (> 2%) can be treated either with an acid catalyst, or with a two-step process using an acid followed by a base catalyst. It is critical to use the appropriate catalyst as it can dictate the type of reaction that occurs. For example, when a base catalyst is mixed with a feedstock high in FFAs, the base catalyst will react with the FFAs to form soap and water (instead of biodiesel). This reaction happens "very fast and [goes] to completion before any esterification begins" (Van Gerpen et al., 2004).

Base catalysts are most commonly used to produce biodiesel. They provide many advantages including: relatively inexpensive production costs since only low temperatures and pressures are required; a high conversion rate (98% typically) with a short reaction time; no requirement for intermediate compounds to yield a direct conversion; minimal side reactions; and no need for exotic construction materials (NBB, 2007).

Base catalysts are typically sodium hydroxide, potassium hydroxide or other similar alkoxides with concentrations ranging from 0.3 to 1.5% based on the weight of oil (Knothe et al., 2005). An alkali catalyst yields fast reactions "with residence times from about 5 minutes to about 1 hour, depending on temperature, concentration, mixing and alcohol/triglyceride ratio" (Van Gerpen et al., 2004). Most commercial operations use sodium hydroxide (NaOH) because of its low cost, but some operations benefit from use of potassium hydroxide (KOH) because the potassium can precipitate as $K_3PO_4$, which can then be used as a fertilizer (when neutralized with phosphoric acid).

When an acid catalyst is used, a byproduct of the esterification reaction is water. The presence of water can create problems as it "reduces the yield of fatty acids to biodiesel and leaves too many fatty acids behind in the feedstock. Water also reduces yields in the transesterification step" (Van Gerpen et al., 2004) and result in additional treatment costs. Biodiesel producers must take precautions that the water from the acid catalyst is removed prior to the addition of a base catalyst.

Triglycerides and FFAs require different processes for biodiesel production. Acids (such as $H_2SO_4$ or $H_3PO_4$) are very effective catalysts for converting FFAs to methyl esters. However, the "acid-catalyzed reaction of triglycerides and methanol is very slow, typically taking 2-3 days to reach completion" (Van Gerpen et al., 2004). To speed up the reaction, a two-step approach is typically used. First, the feedstock is pretreated with an acid catalyst to convert FFAs to esters and then an alkaline catalyst is used to convert the remaining triglycerides to methyl esters. The conversion of FFAs to esters during the first step prevents the formation of soap from the use of a base catalyst in the second step.

### 2.1.2. Managing the Transesterification Process

There are many steps involved in the production of biodiesel. As described above, two reactants, alcohol and oil, and a catalyst, are mixed in a reactor. Small production plants use batch reactors to mix the reactants while "larger plants (>4 million L/yr) use continuous flow processes involving continuous stirred-tank reactors (CSTR) or flow reactors" (Knothe et al., 2005). For a more complete reaction with a lower alcohol requirement, the reactor step (where the reactants first enter the biodiesel production) can be divided into two steps. First, approximately 80% of the methanol and catalyst are added to the first CSTR to react with the oil, then the excess glycerin is removed and the esters are transferred to a second CSTR, where the remaining 20% of the methanol and catalyst are added.

The next step involves separating the methyl esters and glycerin. Due to the "low solubility of glycerin in the esters, this separation generally occurs quickly and can be accomplished with either a settling tank or a centrifuge" (Knothe et al., 2005). Both products use a substantial amount of excess methanol; the methanol is not removed in this separation process because there is concern that the transesterification process may reverse without it.

The methyl esters are then neutralized with acid to remove any residual catalyst and split any soap that may have formed during the reaction. Figures 2.2 below illustrate the stages of the

transesterification process. Figure 2.3 shows the reaction of the soap with the acid to form FFA and water-soluble salts.

**Figure 2.2 Biodiesel transesterification process*.**

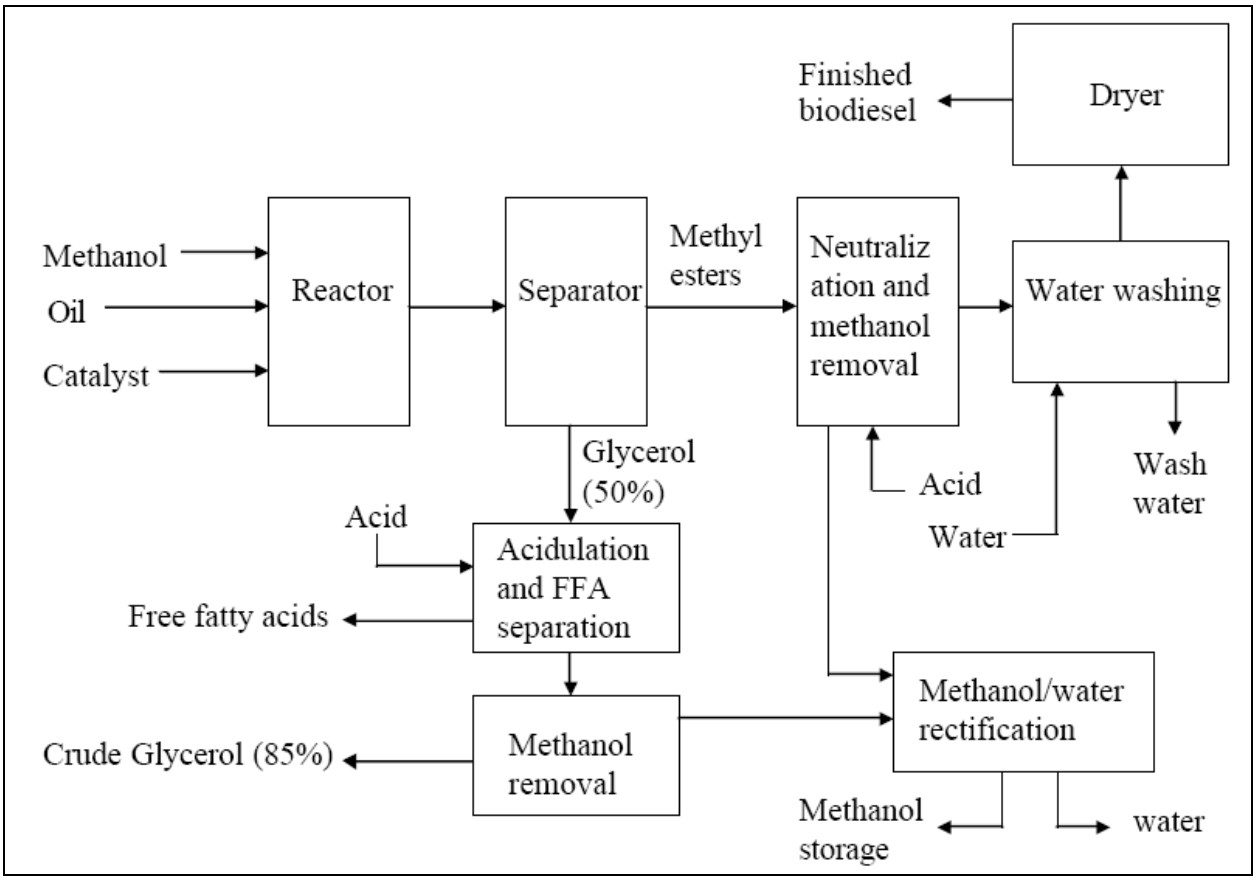
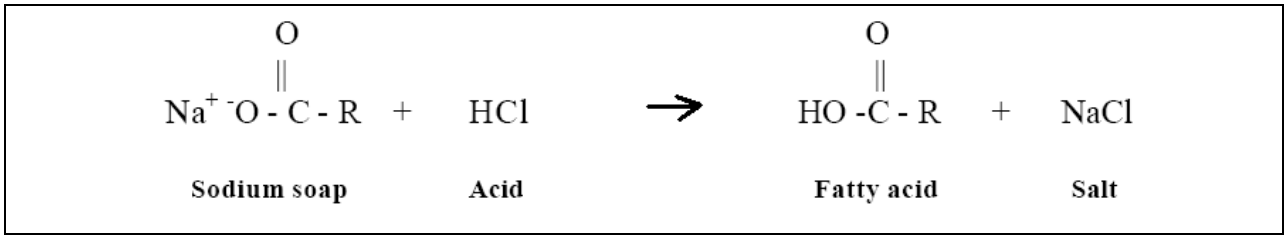



*Figure from Knothe et al. (2005).

**Figure 2.3. Glycerin refining using acid to produce fatty acids and salt.**




Methanol is also removed in the methyl esters neutralization step, since it passes through a methanol stripper that is "usually a vacuum flash process or a falling film evaporator" (Van Gerpen et al., 2004). This neutralization process reduces the water required for the washing and "minimizes the potential for emulsions to form when the wash water is added to the biodiesel." During the water washing, salts are removed and the FFAs remain. The biodiesel is then dried in a vacuum flash process; this leaves a clear amber-yellow liquid with a viscosity similar to petroleum diesel.

Figure 2.2 also shows that, after the glycerin (glycerol) is separated from the methyl esters, it still must be treated. The amount of glycerin actually leaving the separator is ~50% of the remaining product which also contains methanol, catalyst and soap. At this stage, the methanol content is so high that glycerin must be treated as a hazardous waste. To refine glycerin, acid must be added to split the soaps into FFA and salts (Figure 2.3). Since the FFAs are not soluble in glycerol, they can easily be removed and recycled. The last step is to remove methanol by using a vacuum flash process (or another type of evaporator). The resulting glycerin is about 85% pure and can be sold to glycerin refiners who can increase the purity, often as high as 99.5 – 99.7%.

## 2.2. Overview of Biodiesel Feedstocks

Biodiesel is produced from a variety of feedstocks including: common vegetable oils (soybean, palm, rapeseed/canola, sunflower, safflower, algae, cottonseed, peanut), animal fats (usually tallow), and waste oils (used frying oils, trap grease). The greatest difference among feedstocks is the amount of free fatty acids that are associated with triglycerides. FFAs can form during the "recovery process for fats and oils, or when there is water present to break the glyceride-fatty acid bond. Hence, the FFA content is a significant measure of feedstock quality, because it indicates the degree of processing required" (Van Gerpen et al., 2004). Typically feedstocks high in FFAs contain greater levels of impurities and require a costly pretreatment to convert the FFAs to esters.

According to the Biodiesel Council of California, "of the 75 million gallons of biodiesel produced in the United States [in 2006], only 6 million gallons were produced in California" (Krauter, 2006). This minimal production is due to the lack of feedstock available within the state. As of 2006, no California farmers were known to be growing feedstock for biodiesel production (Krauter, 2006). Instead, feedstock has to be transported from outside sources to California, increasing the overall costs of the fuel.

The future of wide-scale biodiesel production in California may be dependent on the capacity for local feedstock production. Because of California's arid climate, limited water resources, and fragile ecosystems, determining appropriate feedstock crops for cultivation is crucial. Feedstocks such as canola, sunflower, safflower, cotton, and Chinese tallow tree show potential. However, canola and rapeseed seem to show the greatest promise for wide-scale biodiesel production, as discussed below.

The four primary feedstocks--soybean, palm, yellow grease, and animal tallow--and the two alternatives (algae and trap grease) for multimedia review are described below. Additional crops that grow (or have potential to grow) in California and show promise as feedstocks with wide scale biodiesel production, are also discussed.

### 2.2.1. Primary Feedstocks for Multimedia Review

<u>*Soybean Oil*</u>

Soybean oil is the most popular biodiesel feedstock in the United States. Approximately 75% of the virgin plant-based feedstock used in biodiesel production is soy oil (Van Gerpen, 2004). This is largely because soybeans are a major domestic crop with massive production and infrastructure and tend to be the least expensive vegetable oil available in the U.S.

The composition of soy oil is relatively constant with few contaminants (including water) affecting biodiesel production. Its low FFA level (less than one percent) simplifies the refining

process. Since soybean oil is composed primarily of unsaturated fatty acids, it has beneficial cold flow properties. However, it also has a lower performance as a fuel compared to other plant oils.

Currently, the primary challenge for the use of soy oil as a biodiesel feedstock is agricultural competition. With the high demand for ethanol, many farmers are shifting their focus to corn. Generally, as the price and demand for corn increase, the production of soy decreases; USDA reported that "soybean ending stocks for 2007-2008 are projected to be 320 million bushels, or nearly 50 percent lower than the 2006-2007 stocks." Similarly, prices for soy oil are expected to increase with demand. The average "soybean prices for 2007-2008 are projected at $6.65 to $7.65 per bushel, up 15 cents on both ends of the range" (Sioux City Journal, 2007).

*Palm Oil*

Palm oil is grown primarily in tropical or subtropical areas such as Malaysia and Indonesia. It is characterized by high concentrations of medium-chain saturated (palmitic acid) and monosaturated (oleic acid) fatty acids. One of its greatest advantages as a biofuel feedstock is high oil yield (Kemp, 2006). Palm plantations "typically produce about 610 gallons per acre of palm oil plantings, compared with 122 gallons per acre for rapeseed and 46 gallons per acre for soybeans"(Jessen, 2007). Also, the production costs of palm oil are low, providing a moderate world-market price compared to other edible vegetable oils.

Palm oil does have significant drawbacks. Its high levels of free fatty acids require an additional costly pretreatment. The oil also has a high saturated fatty acid content which corresponds with increased cold filter plugging and cloud point. This prevents the winter use of neat (100%) palm oil methyl esters in temperate climates (Mittelbach, 2004).

Issues of palm oil sustainability also are of concern. With the recent increased demand for feedstock, Indonesia and Malaysia, the world's top palm oil producers, are clear-cutting and burning forests to build palm plantations. This deforestation releases greenhouse gas emissions and threatens the rich biodiversity of the ecosystem (Jessen, 2007).

Greenhouse gas emissions from existing palm oil forests are also a concern. After the forests are destroyed, the lands are filled to make peat bogs where the palm oil trees can be grown. A four-year study conducted by the Wetlands International, Delft Hydraulics and Alterra Research Center of Wageningen University in Holland examined the carbon release from peat swamps in Indonesia and Malaysia in recent years. It was determined that on average, 600 million tons of carbon dioxide seep into the air each year from these peat bogs. It has been estimated that these carbon dioxide releases, combined with releases from burning of rain forests during clearing, equate to approximately 8% of the world's current carbon output from fossil fuels (Max, 2007).

To help efforts towards sustainability, a global, nonprofit organization known as the Roundtable on Sustainable Palm Oil (RSPO) was formed in April 2004. It is composed of 144 members who represent growers, processors, consumer goods companies, retailers and other non-governmental organizations. In November, 2005, the RSPO adopted eight criteria for sustainable palm oil production which include:

1. Commitment to transparency;
2. Compliance with applicable laws and regulations;
3. Commitment to long-term economic and financial viability;
4. Use of appropriate best practices by growers and millers;
5. Environmental responsibility and conservation of natural resources and biodiversity;

6. Responsible consideration of employees, individuals and communities affected by growers and mills;
7. Responsible development of new plantings;
8. Commitment to continuous improvement in key areas of activity.

*Yellow Grease*

Yellow grease consists of waste vegetable oils (WVO) such as soy, peanut, canola, and sunflower that are recycled from industrial cooking, franchise cooking operations, or other large scale cooking projects. It is estimated that recycling and processing waste oils can generate over 2.75 billion pounds of yellow grease annually (Kemp, 2006). Since yellow grease is a waste product, it is relatively inexpensive and available in all regions.

The FFA level ranges from 1% to 20%, with the median approximately 10%. "The low end of this range corresponds to an oil recently used in cooking and the high end is an oil that may have been stored for a considerable time before it is processed" (Van Gerpen et al., 2004). Yellow grease requires pre-treatment with an acid catalyst before transesterification can take place (Canacki and Van Gerpen, 2001).

With any WVO, there will always be variability in the quality of the oil. During the cooking process, varying amounts of water, solids and other impurities can be incorporated into the oil. These contaminants can cause difficulties in the transesterification process. This can be one of the greatest hurdles for producers since "having an oil supply of consistent quality is possibly the most important step in manufacturing biodiesel and eliminating excessive waste due to rejected batches" (Kemp, 2006).

A drawback to yellow grease is that it contains substantial quantities of saturated oils that exhibit cold flow problems. Yellow grease is generally solid at room temperature, and requires preheating to maintain it in fluid phase. The biodiesel made from yellow grease feedstock therefore tends to gel in cold climates, plugging fuel filters and fuel injection systems (Kemp, 2006). It is possible to improve the cold flow performance if producers use WVOs that are produced from unsaturated canola or soy oil. Another possible disadvantage for the use of yellow grease is the competition for this feedstock as an additive in animal feed. Competition could cause a price increase in the WVO.

*Trap (Brown) Grease*

Trap grease (also known as brown grease) is the oil that is recovered from the bottom of commercial frying systems and from grease traps. Typically restaurants install grease traps as part of a discharge system to collect the grease that is washed down the drain. The trap collects grease before it enters the sewer, where it can congeal on the pipe walls and restrict flow. Restaurants normally pay to have these traps emptied and for the grease to be disposed of. Since the grease currently has no other market value, its cost is extremely low.

Converting trap grease to biodiesel can be a daunting process. It is highly variable in composition, containing an assortment of fats, oils, greases, food particles, dirt, water and anything else that washes down the drain. This grease requires "dewatering, filtering, grit removal, and may also require deodorization and bleaching prior to use" (Van Gerpen et al., 2004).

Trap grease also has extremely high levels of FFAs ranging from 40 to 100%. The extensive pretreatment that is required can lead to a costly production. Another hurdle is odor control. Modifications to the production process must be made to adapt to the very volatile feedstock.

*Animal Tallow*

Animal tallow is a triglyceride material that is recovered by a rendering process, where the animal residues are cooked and the fat is recovered as it rises to the surface. Since it is a waste by-product, it is relatively inexpensive, sustainable, and is available locally. Rendered animal fats typically have a FFA content between 5% and 30%, mostly depending on the time of year. "In winter, when animals carcasses cool quickly with little decomposition, the FFA will be low. In hot summers, the FFA can be quite high" (Van Gerpen et al., 2004).

Since the tallow content is high in saturated fatty acids, it is generally semi-solid or solid at room temperature (Van Gerpen et al., 2004). This saturated fat produces good engine efficiency and subsequently reduces exhaust emissions (Hilber et al., 2007). Biodiesel derived from animal fats also exhibits high cetane numbers and good oxidation stability. However, the high levels of FFAs can pose some drawbacks. Animal tallow has a high melting point that can lead to precipitation and poor engine performance in cold weather.

*Canola*

Canola was developed through conventional plant breeding with rapeseed. To improve the characteristics of rapeseed, breeders created cultivars with reduced levels of erucic acid and glucosinolates. The end product, canola, is now widely grown in Canada, with some production in the United States. North Dakota is the leading state in the production of canola and typically grows approximately 90% of the total U.S. canola.

While there is little experience with canola in California, much may be learned from Australia's success in cultivating the crop. The climate where canola is grown in Australia is similar to the California Central Valley from Bakersfield to Redding (Kaffka, 2007). Canola is considered to be a relatively drought tolerant crop that typically requires around 18 inches of water a year (under Australian conditions) (Johnson, 2007). California's similar climate and the crop's relatively low water requirement suggest that canola could be widely produced within the state. Steve Kaffka, a University of California Cooperative Extension agronomist, is conducting a UC study on the conditions required to grow canola efficiently in California. As part of the study, trial canola varieties have been planted in Chico, Davis, the West Side Field Station and the Imperial Valley.

Canola oil shows promise as a feedstock; it has properties similar to soy oil with a composition primarily of unsaturated fatty acids and a low content of FFAs. The oil yield of canola, however, is much higher than soy; the seed contains 45% oil. Canola feedstock can produce fuel with beneficial cold flow properties; its 24$^{o}$F CFPP is superior to almost all other feedstocks (soy is 28 degree CFPP, palm is 55 degree CFPP, and tallow is 58 degree CFPP) (Kotrba, 2007). On the negative side, canola does have a higher risk for oxidation and it exhibits poor storage stability.

*Safflower*

Safflower is the most common oil seed produced in California. It can tolerate extreme weather conditions and is considered a low input and drought tolerant crop. Little fertilizer is needed as the crop is extremely deep rooted and can recover lost nutrients. In fact, an advantageous trait of the crop is its ability to aggressively recover nitrogen from the soil. Safflower could also be

beneficial to the California agricultural industry as tests have shown that safflower can tolerate irrigation with saline water (Kaffka, 2007).

There are two types of safflower varietals--those high in monounsaturated fatty acid (oleic) and those high in polyunsaturated fatty acid (linoleic). In both varietals, the fatty acid profiles are so similar that they possess the same characteristics: poor storage stability, low cetane number and good cold flow characteristics. Another advantage of safflower is that its seed has a high oil concentration of 42 to 48%.

*Algae*

Algae is a single-celled organism that contains a relatively large volume of plant oil. According to the National Renewable Energy Laboratory, there are more than 300 algae strains demonstrating potential for use as fuel feedstock and some contain more than 50 percent oil. While there are multiple species of algae, all generally share similar fatty acid profiles. The majority of fatty acids present are oleic (36%), palmitic (15%), stearic (11%), and linoleic (7.4%). There are other saturated and monounsaturated fatty acids that can be present but they typically represent less than 5% each of the total fatty acids (Van Gerpen et al., 2004). Algae's high level of saturated and monounsaturated fatty acid content provides for optimal fuel quality.

High-oil algae species have been studied since 1978 by the US Department of Energy as part of its biodiesel fuels research. Intensive production of algae can lead to production of as much as 10,000 gallons of feedstock per acre per year (Kram, 2007). Algae is sensitive to temperature fluctuations, pH, atmospheric levels of $CO_2$, and competition between promising strains and less prolific strains of algae. If limitations associated with a narrow range of growing and harvesting conditions can be addressed, "enough algae-based biodiesel can be produced each year to power the current US fleet of vehicles (140 billion gallons or 500 billion liters) using a mere 9.5 million acres [3.8 million hectares] of cultivation space" (Cox, 2006). This acreage is minimal compared to the 3 billion acres of farmland required to produce the same amount of oil from soybeans. Competition with food production is also reduced due to minimized use of farmland.

Air pollutants can be reduced with the production of algae. Since algae thrives on high concentrations of carbon dioxide and nitrogen oxide, atmospheric emissions from power plants can be used to feed the algae. This creates a sustainable energy system that could "enable a power plant to meet emerging state regulations for both $CO_2$ reduction and renewable power generation" (Cox, 2006).

### 2.2.2. Feedstock Characteristics

Biodiesel feedstocks are classified based on fatty acid profile (Table 2.2); the fatty acid composition greatly influences a fuel's characteristics, as different esters of fatty acids have different physical and chemical properties. The carbon chain length and the degree of saturation of the raw material can affect key properties including--pour point, cloud point, cetane number, viscosity, and storage stability. Typical values of fatty acid compositions of different feedstocks are shown below. The numbers describing each acid indicate the number of carbon atoms in the chain, followed by the number of unsaturated carbon-carbon bonds in the chain. For example, Oleic acid, 18:1, has 18 carbon atoms and one unsaturated bond.

A saturated oil or fat has no double bonds; instead, all of its carbon atoms (in the fatty acid portion) are bound to two hydrogen atoms (except at one end, which has 3 H atoms attached). This allows the chains of fatty acids to tightly align together, resulting in solidification at higher

temperatures. Conversely, unsaturated fatty acids contain carbon-to-carbon double bonds and will exhibit lower cloud points as they resist solidification at low temperatures.

**Table 2.2. Fatty acid percentages in biodiesel feedstocks*.**

| | Myristic 14:0 | Palmitic 16:0 | Stearic 18:0 | Oleic 18:1 | Linoleic 18:2 | Linolenic 18:3 | Arachidic 20:0 | Erucic 22:1 |
|---|---|---|---|---|---|---|---|---|
| **Soybean** | | 6-10 | 2-5 | 20-30 | 50-60 | 5-11 | | |
| **Corn** | 1-2 | 8-12 | 2-5 | 19-49 | 34-62 | trace | | |
| **Hi linoleic Safflower** | | 5.9 | 1.5 | 8.8 | 83.8 | | | |
| **Hi Oleic Safflower** | | 4.8 | 1.4 | 74.1 | 19.7 | | | |
| **Hi Oleic Rapeseed** | | 4.3 | 1.3 | 59.9 | 21.1 | 13.2 | | |
| **Hi Erucic Rapeseed** | | 3 | 0.8 | 13.1 | 14.1 | 9.7 | 7.4 | 50.7 |
| **Tallow** | 3-6 | 24-30 | 20-25 | 37-43 | 2-3 | | | |
| **Yellow Grease** | 1.3 | 17.4 | 12.4 | 54.7 | 8 | 0.7 | 0.3 | 0.5 |

*Data from Van Gerpen et al. (2004) and Van Gerpen (2004).

Feedstock such as animal tallow and some recycled vegetable oils are high in saturated fatty acids, whereas oils including soy and canola are composed of unsaturated fatty acids. The degree of saturation is indicated by the iodine number of the oil. Iodine is used to break any double bonds as it attaches itself to each carbon atom that holds together the double bonds. The value of the iodine number corresponds to the degree of saturation; feedstock with a high composition of unsaturated fatty acids will demonstrate a high iodine value (since more iodine is needed to break apart the double bonds).

The cetane number of biodiesel is positively affected by an increased amount of saturated fatty acids. Biodiesel fuels with low iodine numbers (composed of saturated fatty acids) exhibit greater efficiency and maintain high cetane numbers. However, "the greater the fraction of saturated fatty acids, the poorer the cold weather performance" tends to be. (Van Gerpen et al., 2004) While the cold flow properties are better for biodiesel with high iodine numbers, the cetane number is low (indicating poor performance), and there is poor storage stability (as there is a higher risk for oxidation). The correlation between iodine and cetane number can be seen in Table 2.3.

**Table 2.3. Comparison of iodine number and cetane number for different types of biodiesel*.**

| | Rapeseed/ Canola Oil | Soybean Oil | Palm Oil | Lard | Tallow |
|---|---|---|---|---|---|
| **Iodine Number** | 110 - 115 | 125 - 140 | 44 - 58 | 60 - 70 | 50 – 60 |
| **Cetane Number** | 58 | 53 | 65 | 65 | 75 |

*Data from Helber et al. (2006).

### 2.2.3. Feedstock Economic Considerations

Price, availability, origin, geography, and consistent quality generally dictate which feedstock biodiesel producers use. Because the cost of feedstock constitutes between 70 and 85% of the overall cost of biodiesel production (Knothe et al., 2005), there is a strong incentive to purchase the cheapest feedstock that is in close proximity to the production facility. Before development of infrastructure involving long-term dependence on a particular feedstock, it is important to consider if it is sustainable, has potential expansion for growth, and has consistent properties. Table 2.4 below summarizes properties typical of the different major kinds of feedstocks.

**Table 2.4. Economic considerations of biodiesel feedstocks*.**

| Biodiesel Feedstock | Cost/Unit | Supply/Growth Flexibility^ | Content/Quality Variability | Degree of Pretreatment Required |
|---|---|---|---|---|
| Virgin plant based feedstocks (e.g. soy, palm, canola, corn, cottonseed, sunflower, etc.) | Moderate to high | Supply can be expanded | Low variability | Modest |
| Virgin animal based feedstocks (e.g. lard, tallow, chicken fat, fish oil) | Moderate | Fixed (dependent on meat, poultry, fish, demand and processing) | Low to moderate variability | Modest to High |
| Recycled feedstock (e.g. yellow grease from frying or brown grease) | Low | Fixed (dependent on restaurant, fried activity) | High variability | High |

*Data from Hilber et al. (2006).
^Ability to expand total supply in response to price increases from demand shifts

## 2.3. Biodiesel Standardization and Fuel Quality

Generally, the quality of a biodiesel fuel is dependent on the quality and fatty acid composition of the feedstock, the production process, and post-production parameters (Knothe et al., 2005). Pure biodiesel fuels, regardless of feedstock, must meet the ASTM D6751 standards (Table 2.5). Because there are so many varying factors in the production of biodiesel, it is critical that these specifications are met. Biodiesel blends that meet ASTM standards (ASTM 2009a; ASTM 2009b; ASTM 2012) can be used in most "modern engines without modifications while maintaining the engine's durability and reliability" (Van Gerpen et al., 2004), While B100 can be used in some engines, a considerable amount of experience exists with B20 blends, and some manufacturers recommend against using blends above B5. Moreover, the language of ASTM D6751 notes in many places that its specifications are for B100 used in blending such that it provides "satisfactory vehicle performance".

Thus, the fuels reviewed in this multimedia risk assessment will be B20 (20% ASTM D6751 biodiesel mixed with 80% ASTM D975 ultra low sulfur diesel according to ASTM D7467-09a specifications). Our focus on B20 is designed to target the composition of fuel most frequently involved in on-site storage and distribution from fuel service stations. We will also consider transportation and storage of B100 as a distributed ingredient in on-site mixing.

**Table 2.5. ASTM D6751-12. Specifications for biodiesel (B100)*.**

| Biodiesel Property | ASTM Method* | Limits | Units |
|---|---|---|---|
| *Calcium and Magnesium, combined* | EN 14538 | 5 max. | ppm (ug/g) |
| *Flash Point, cup* | D 93 | 93 min. | Degrees, C |
| *Alcohol Control (One of the following must be met)* | | | |
| *1. Methanol Content* | EN 14110 | 0.2 max. | % volume |
| *2. Flash Point* | D 93 | 130 min. | Degrees, C |
| *Water and Sediment* | D 2709 | 0.05 max. | % volume |
| *Kinematic Viscosity, 40 C* | D 445 | 1.9 – 6.0 | $mm^2$/sec |
| *Sulfated Ash* | D 874 | 0.02 max. | % mass |
| *Copper Strip Corrosion* | D 130 | No. 3 max. | |
| *Cetane Number* | D 613 | 47 min. | |
| *Cloud Point* | D 2500 | Report | Degrees, C |
| *Carbon Residue, 100% sample* | D 4530* | 0.05 max. | % mass |
| *Acid Number* | D 664 | 0.50 max. | mg KOH/g |
| *Cold soak filterability* | Annex A1 | 360 max | seconds |
| *Free Glycerin* | D 6584 | 0.020 max. | % mass |
| *Total Glycerin* | D 6584 | 0.240 max | % mass |
| *Phosphorus Content* | D 4951 | 0.001 max. | % mass |
| *Distillation Temperature, T90 AET* | D 1160 | 360 max. | Degrees, C |
| *Sodium and Potassium, combined* | EN 14538 | 5 max | ppm |
| *Oxidation Stability* | EN 15751 | 3 min. | hours |

* ASTM D6751-12

To ensure compatibility with diesel engines, the transesterification reaction must be run to completion and without accumulation of byproducts. If there is any remaining glycerin, catalyst, alcohol, or FFAs in the biodiesel, operational problems can occur. The ASTM D6751 standard "prescribes the required properties of biodiesel fuel at the time and place of delivery" unless other agreements were arranged between purchaser and supplier. (ASTM, 2012) All biodiesel produced for commercial sale must be registered with the United States Environmental Protection Agency under 40 CFR Part 79.

The ASTM D6751-12 "Standard Specification for Biodiesel Fuel Blend Stock (B100) for Middle Distillate Fuels" identifies the parameters that pure "diesel (B100) Grades S15 and S500 for use as a blend component with middle distillate fuels" (ASTM, 2012). These specifications, shown below, prescribe the required properties of the fuel as existing at the time and place of the sale.

### 2.3.1. Biodiesel Fuel Energy Content

The energy content of a fuel can greatly influence fuel economy, torque, and horsepower. Compared to diesel, the energy content of biodiesel (measured in Btu/gal) is slightly lower. The EPA reported that the average energy content of biodiesel is dependent upon the type of feedstock. Testing revealed that "rapeseed and soybean-based biodiesels cannot be distinguished from one another, but that the animal-based biodiesels can be distinguished from plant-based biodiesels (at a 99% confidence level)" (USEPA, 2002). This is clearly indicated by the results shown below (Table 2.6).

**Table 2.6. Average energy content of 100% biodiesel*.**

| | Average net Btu/gal |
|---|---|
| All biodiesels | 118,296 |
| Animal-based | 115,720 |
| Rapeseed/canola-based | 119,208 |
| Soybean-based | 119,224 |
| Rapeseed or soybean-based | 119,216 |

*Table from EPA (2002).

The energy content of conventional diesel fuel is 129,500 Btu/gal. Animal based biodiesel and plant-based biodiesel contain 10.6% and 7.9% less energy, respectively, than diesel.

## 2.4. Waste Generation and Waste Management

Biodiesel may be considered a waste if it is stored too long, is spilled, or becomes contaminated. Waste biodiesel that exhibits the hazardous waste characteristics of toxicity or ignitability may be classified as a hazardous waste. Biodiesel that is a hazardous waste and, potentially, environmental media that become contaminated with it may be subject to the hazardous waste management requirements in title 22 of the California Code or Regulations.

In evaluating the production of biodiesel (and other alternative diesel options) it is important for the multimedia assessment and the life-cycle assessment to identify where and what kind(s) of hazardous waste(s) may be generated. For example, sodium hydroxide and potassium hydroxide may be used as base catalysts for producing fatty acid methyl esters (FAME) from fats and oils. Acids are also used as catalysts for converting free fatty acids to methyl esters. In the process of using those catalysts, corrosive hazardous wastes may be generated. Some solvents are applied in the production process as well. Proper identification and management of the waste solvents are required to comply with hazardous waste laws and regulations. Although biodiesel formulations are less toxic than standard diesel formulations, the storage stability of biodiesel is less than the standard ultra low sulfur diesel (ULSD). Degradation could be caused by temperature, oxidation, and/or material incompatibility; and some toxic components may be produced in the biodiesel. Thus, further studies are required to determine:

a) if the leaked or spilled Biodiesel after oxidation and degradation contains any hazardous substances; and
b) if the Biodiesel product, stored beyond the recommended six-month term, becomes a hazardous waste.

These issues must be addressed in the Tier II and Tier III assessments.

Once the sources, composition, and magnitude of waste streams from biodiesel fuel production have been identified, there is a need to identify management approaches that could be applied to the identified hazardous waste streams. When generated hazardous wastes are identified, the appropriate waste management approach, such as treatment, storage, and disposal should be identified and described in the Tier II and Tier III reports. Among the waste management strategies considered, priority should be given to available alternatives for hazardous waste reduction and pollution prevention. To address these and other hazardous-waste issues, the Tier II and Tier III reports will include a section that provides a work plan to specify the hazardous waste storage, transportation, treatment, disposal, waste reduction, and emergency planning for the biodiesel life cycle.

# 3. Storage and Distribution of Biodiesel

## 3.1. Material Compatibility

Material compatibility is an important property to consider. Since biodiesel can react with some metals and polymers, it is critical that the material of tanks, hoses, gaskets, and other parts that may come in contact with biodiesel, are compatible with the fuel's properties. When biodiesel is exposed to incompatible materials, it can degrade, soften, or seep through them (USDOE, 2006). As discussed above, biodiesel is not compatible with brass, bronze, copper, lead, tin and zinc as these metals can initiate oxidation and sediment production. Biodiesel can also have compatibility issues with some polymers. Table 3.1 shows different polymer's compatibility relative to diesel. Nitrile rubber compounds, polypropylene, polyvinyl, and Tygon are particularly vulnerable to biodiesel.

**Table 3.1. Biodiesel materials compatibility.**

**Biodiesel Material Compatibility Table**

| Polymer | Relative to Standard Diesel |
|---|---|
| Fluorosilicon | Hardness little change, swell +7% |
| Nitrile | Hardness –20%, swell +18% |
| Nylon 6/6 | Little Change |
| Polypropylene | Hardness –10%, swell +8-15% |
| Polyurethane | Hardness little change, swell +6% |
| Polyvinyl | Much worse |
| Teflon | Little change |
| Tygon | Worse |
| Viton A401-C | Little change |
| Viton GFLT | Little change |

**Table from Van Gerpen, 2004**

Vehicles manufactured before 1993 may have issues with incompatible seals, gaskets and adhesives as they were made from natural and nitrile rubber (Van Gerpen, 2004) that prohibit the use of biodiesel blends. But most engines produced after 1994 are potentially compatible with biodiesel (B20); however, "the user should consult the equipment manufacturer or owner's manual regarding the suitability of using biodiesel (B100) or biodiesel blends in a particular engine" (ASTM, 2012). Since biodiesel is considered a "hazardous substance", UST owner/operators are required by state health and safety codes to demonstrate material compatibility prior to storage of biodiesel (SWRCB, 2008).

Materials such as "Teflon, Viton, and Nylon have very little reaction to biodiesel and can be used to update incompatible equipment" (NBB, 2007). However, some grades of these materials have shown compatibility issues with different blends of biodiesel and the manufacturer of the material should be contacted to determine the most suitable grade. Additional acceptable materials for non contaminated biodiesel (pure B100) storage tanks are: aluminum, steel, and fluorinated polyethylene or polypropylene. However there have been cases of biodiesel oxidizing

to form peroxides that corroded steel tanks. Contamination by water or sulfur also has the potential to weaken materials commonly used in UST (Hodam, 2008).

Assuring materials compatibility requires the compilation of both comparative corrosion tests and elastomers tests. Comparative corrosion tests should include copper and steel strip tests for all samples. Elastomers should be tested for tensile strength, hardness, and swelling for at least 1000 hours of exposure at 60 F. The elastomer samples to be used will be determined in consultation with Cal-EPA staff as part of the Tier-II effort.

## 3.2. Biodiesel Solvency

Biodiesel has higher solvency properties than diesel fuel and can act as a mild solvent. It can dissolve the residual sediments in diesel storage tanks and engine fuel tanks. These dissolved sediments can harm fuel systems as they plug fuel filters. Solvency decreases with a decreasing percentage of biodiesel. Pure biodiesel (B100) exhibits the greatest solvency effects; whereas typically "20% or less blends of biodiesel in diesel will nearly completely dilute the solvency effect" (Van Gerpen, 2004).

To avoid such problems with solvency, the USDOE recommends that users "clean the tanks and anywhere in the fuel system where sediments or deposits may occur before filling with B100" (USDOE, 2006). After the cleaning process, it is important to remove all excess water, as this can affect fuel quality (as discussed previously). When switching a vehicle to biodiesel, it is recommended to change the fuel filters several times after the switch as some sediments could clog the filters causing operation problems.

## 3.3. Storage Stability

Biodiesel is susceptible to chemical changes during long-term storage. Fuel aging and oxidation by atmospheric oxygen can lead to increased acid numbers, increased fuel viscosity, and the formation of gums and sediments. Storage stability refers to the ability of the fuel to resist chemical changes during long-term storage. While storage stability is an important parameter, the ASTM biodiesel standards have not yet established "stability tests that ensure satisfactory long-term storage of biodiesel (B100)" (ASTM, 2012). There are data that suggest that common 100 hour fuel exposure tests may not be adequate as drastic changes in material properties can occur at times between 300 and 1000 hours of exposure with biodiesel (Hodam, 2008). Due to the lack of information on storage stability, the National Biodiesel Board recommends no longer than a six-month storage life for B100 biodiesel (NBB, 2007). As discussed below, the chemical reactivity of esters (biodiesel) depends on the fatty acid profile, fuel additives, temperature, metals, and the presence of water and natural antioxidants.

### 3.3.1. Biodiesel Oxidation

Biodiesel oxidative stability is affected by exposure to air, sunlight, and elevated temperatures. Sunlight will accelerate the oxidation of fatty esters through a photo-oxidation process "whereby oxygen directly attacks the olefinic (double-bonded) carbons" (Southwest Research Institute, 2005). This can cause fuel degradation, which consequently can alter the fuel's quality. High storage temperatures can also accelerate fuel degradation. Thus, ASTM D6751 recommends underground or isothermal storage to avoid extreme temperatures, with limited exposure of headspace to atmospheric oxygen. Fixed roof tanks should be kept full to limit oxygen supply and tank breathing (ASTM, 2012).

Much of the fuel's oxidation behavior can be characterized by its fatty acid profile. The lower the level of saturation in an ester, the more susceptible it will be to oxidation. The presence of double bonds within a fatty acid leads to autoxidation; the rate at which autoxidation proceeds depends on the "number and position of the double bonds" (Knothe et al., 2005). Fatty acid compounds that are composed of adjacent allylic (double-bonded) carbons contain pi bonds, where the *p* orbitals overlap and electrons are shared throughout the carbon chain (Kemp and Vellacio, 1980). This electron sharing leads to greater atomic forces, creating a weak hydrogen bond. When hydrogen is removed "oxygen rapidly attacks and a hydroperoxide is ultimately formed" (SRI, 2005). Hydroperoxides then decompose and interact to form "numerous secondary oxidation products including aldehydes, alcohols, shorter chain carboxylic acids, and higher molecular weight oligomers often called polymers" (SRI, 2005). These secondary products of the oxidation process cause the fuel to eventually deteriorate.

Oils high in polyunsaturated fatty acids are most susceptible to autoxidation. As a rule, saturated fatty acids (such as 16:0 or 18:0) are stable; but as the presence of double bonds (between carbon atoms) increases (for example from 18:1 to 18:2 to 18:3), the oxidative stability decreases (these ratios describe the number of carbon atoms and double bonds in the fatty acid chain such that "18:2" refers to a fatty acid chain comprised of eighteen carbon atoms and that there exist two double bonds in the chain). This was verified in an early study that "measured the relative rate of oxidation for the methyl esters of oleic (18:1), linoleic (18:2), and linolenic (18:3) acids to be 1:12:25" (SRI, 2005). Soybean oil and canola (rapeseed) are the feedstocks highest in linoleic and linolenic acid and most prone to oxidation.

Metals are known to catalyze oxidation reactions of biodiesel. Copper, iron, and other transition metals, "act as a Lewis acid to catalyze polymerization of polyunsaturated hydrocarbon molecules in biodiesel" (Kenreck, 2007). These metals may be present if corrosion occurs in the manufacturing process. Free fatty acids can also adversely affect the oxidative stability of biodiesel as they may degrade or cause corrosion and thermal instability. (Kenreck, 2007).

### 3.3.2. Residual Water

While biodiesel is generally considered to be insoluble in water, it can actually contain as much as 1500 ppm of dissolved water (Van Gerpen et al., 2004). Storage stability of biodiesel is also affected by the presence of water within the tank. Water can enter fuel tanks through vents and seals as humidity in the air where it either condenses or dissolves into the fuel. According to Van Gerpen et al. (1996), virtually all diesel fuel storage tanks can be assumed to contain some water. Further, blending biodiesel into ULSD reduces the water separation capability of the fuel and the formation of water/fuel mixtures (Quigley, 2007). Water can cause hydrolytic degradation of the fuel, contribute to microbial growth in the fuel, and can cause corrosion of fuel systems and tanks.

The presence of water within the biodiesel can cause corrosion of fuel tanks and engine fuel system components. The most direct form of corrosion is rust, "but water can become acidic with time and the resulting acid corrosion can attack storage tanks" (Wedel, 1999). Hydrolytic degradation can also occur if concentrations of water are present within the tank. Substances such as "mono- and diglycerides (intermediates in the transesterification reaction) or glycerol can emulsify water" (Knothe et al., 2005).

Condensed water in a fuel tank can support the growth of bacteria and mold that use the hydrocarbons in the biodiesel as a food source. These "hydrocarbon-degrading bacteria and molds will grow as a film or slime in the tank and accumulate as sediment" (Wedel, 1999).

The control of water is primarily a housekeeping issue (i.e. keeping storage tanks clean) and a problem frequently addressed by using fuel filters (Sunny Beaver of Yokayo Biofuels [B100 distributor], personal communications). Additives may also be used to address residual water problems.

## 3.4. Distribution and Blending of Biodiesel

Biodiesel is typically transported via rail cars, tank trucks and drums; the choice of vessel depends on the quantity of biodiesel being transferred and the cold flow properties of the fuel. Due to biodiesel's poor cold flow properties, it is recommended to ship the fuel by the following means in cold climates: hot biodiesel in tank cars that are rapidly delivered, solidified biodiesel in tank cars that are equipped with steam coils, 20% biodiesel blends with winterized diesel, or 50% biodiesel blends with diesel No. 1 (Van Gerpen, 2004).

Transportation vessels must be composed of materials that are compatible with biodiesel. Seals, gaskets, and adhesives present in the transfer system should also be compatible with biodiesel. If the vessels have been previously used to transport diesel, they should be cleaned and dried prior to biodiesel transport, due to biodiesel's high solvency properties (as mentioned previously). Given the potential contact hazards of biodiesel, "it is recommended that PVC-coated gloves as well as safety glasses or goggles be used when handling biodiesel" (Van Gerpen, 2004).

There are various regulations in place for biodiesel transport and biodiesel plants. The Clean Water Act (CWA) and the Oil Pollution Act (OPA) "outline various requirements that must be met in order to comply with regulations" (Van Gerpen, 2004). Under these acts, there is no distinction between petroleum oils, vegetable oils, and animal fats, as they share common physical properties and produce similar environmental effects.

With the amendment of the OPA in 2002, the National Oil and Hazardous Substances Pollution Contingency Plan (NCP) was introduced. This legislation requires "owners or operators of vessels and certain facilities that pose a serious threat to the environment to prepare facility response plans" (Van Gerpen, 2004). Greater contingency planning can reduce spills during transportation and at the plant.

In 2002, the EPA published a Spill Prevention Control and Countermeasure (SPCC) rule at Title 40 of the Code of Federal Regulations, Part 112 (40 CFR 112) to ensure that facilities put in place containment and other countermeasures that would prevent oil spills. While each SPCC is unique to the facility, all should clearly address: "operating procedures that prevent oil spills, control measures installed to prevent a spill from reaching navigable waters, and countermeasures to contain, clean up, and mitigate the effects of an oil spill that reaches navigable waters" (Van Gerpen, 2004).

# 4. Use of Biodiesel

## 4.1. Vehicle Operability Issues

There are a number of vehicle operability issues associated with the use of biodiesel blends. These include cold fuel flow, fuel foaming, water separation, and fuel oxidative stability (Taracha, 2006; Quigley, 2007). Cold flow can result in fuel system clogging. Fuel foaming can result in difficulty during tank filling and can potentially increase the possibility of fuel spills. Reduced water separation can result in water/fuel mixtures that can clog fuel systems and promote microbial growth that can also clog fuel systems. Oxidative degradation of biodiesel can result in injector deposits, and lacquer formation, and fuel systems corrosion. These impacts to the vehicle fuel system can result in reduced drive-ability, higher tailpipe emissions, and increased maintenance costs.

## 4.2. Biodiesel Cold Flow Characteristics

One of the greatest challenges associated with the use of biodiesel is cold flow behavior. In cold temperatures, biodiesel can start to freeze or gel. If the fuel begins to gel, "it can clog filters or can eventually become too thick to pump from the fuel tank to the engine" (USDOE, 2006). Biodiesel's behavior in cold climates can depend on the fatty acid profile and the amount and types of impurities within the fuel (Pradhan et al., 2007). Several cold flow properties are commonly used to classify cold flow performance: cloud point, pour point, and cold filter plugging point (CFPP).

The cloud point is the temperature at which crystals begin to precipitate from the fuel, giving it an appearance as if wax was forming. As the temperature decreases, the biodiesel reaches its pour point; this is the lowest temperature at which the fuel can still flow, before the crystals start to gel. To determine cold flow characteristics in a more precise and reliable manner, the cold filter plugging point can be evaluated. CFPP is the lowest temperature at which the fuel can pass through a standard test filter under standard conditions.

As the content of saturated fatty acids increases, so does the cold filter plugging point. Saturated fatty acids are comprised of single bonds and can easily form highly regular crystalline structures; this results in crystallization at high temperatures. Typical relationships between the content of saturated fatty acids in biodiesel (without additives) and the corresponding CFPP value are displayed in Figure 4.1.

## 4.3. Use of Additives

Chemical additives are commercially available to address the oxidative stability, cold-flow properties, microbial contamination, increased water affinity and increased $NO_x$ emissions of biodiesel. The varying properties of the fuel, based on the feedstock variety and fragmented nature of the new industry, result in a lack of consistency in the current use of additives. However, as larger companies enter the market and standards are further developed, more uniform practices regarding the use of additives can be expected.

**Figure 4.1. Relation between content of saturated fatty acids in biodiesel (without additives) and its CFPP value*.**

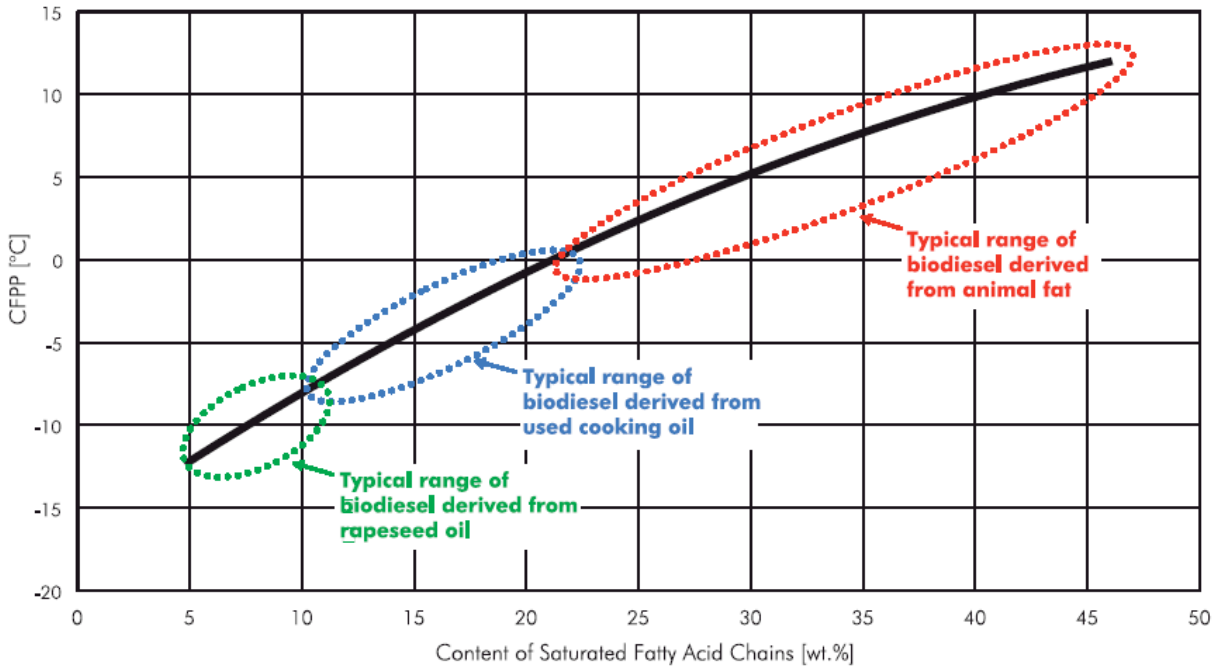



*Figure from Hilber et al. (2006).

### 4.3.1. Antioxidants

Oxidation of biodiesel results in the formation of hydroperoxides, which in turn induce free-radical chain reactions that lead to decomposition into low-molecular weight, highly oxidized species including aldehydes, ketones, formic acids and acetic acids (Albermarle Corp., 2005).

Vegetable oils (such as soybean oil) typically contain naturally occurring antioxidants (tocopherols, i.e., vitamin E) and provide some protection against oxidation. Antioxidants contain a highly-labile hydrogen that is easily extracted, acting as a free radical. This resulting antioxidant free radical is "either stable or further reacts to form a stable molecule that does not contribute to the chain oxidation process" (SRI, 2005).

Natural antioxidants can be added to biodiesel to improve the fuel's antioxidant behavior. However, during the feedstock processing for biodiesel derived from plant oil (where the seeds are bleached, deodorized and the oil is distilled) the natural antioxidants may be removed. Recent studies have shown that synthetic antioxidants may actually be more effective than natural antioxidants (SRI, 2005). The efficiency and necessary quantity of antioxidants are strongly dependent on the feedstock and biodiesel production technology (Lacoste et al., 2003). These additives have different effects on biodiesel, depending on the feedstock; however, fuel properties such as viscosity, cold-filter plugging point, density, and others, are not affected by the antioxidants (Knothe et al., 2005).

A category of compounds referred to as "hindered phenols" are frequently employed, which react very rapidly with free radicals because of the low energy required to remove the hydrogen

located alpha to the double bonds. The free radical generated does not initiate oxidation because of resonance stabilization.

Several antioxidants consisting of hindered phenols are commonly referenced in biodiesel lab tests and used in commercial products (Ribiero et al., 2007). These include:

- Tertiary butylhydroquinone (TBHQ)
- Butylated hydroxyanisole (BHA)
- Butylated hydroxytoluene (BHT; 2,6-di-tert-butyl-4-mehylphenol)
- Pyrogallol (PY; 1,2,3-trihydroxybenzene)
- n-Propyl gallate (PG; 3,4,5-trihydroxybenzoic acid propyl ester)

In recent antioxidant "evaluations involving biodiesel methyl esters, TBHQ was frequently found to be the best overall performer" (SRI, 2005).

The additive manufacturers' Material Safety Data Sheets (MSDSs) and product literature list several of these and other hindered phenols as components in a number of commercial biodiesel antioxidant products (Appendix I-B). Given that the biodiesel oxidation process begins immediately, additive manufacturers recommend to biodiesel suppliers to blend in antioxidants as far upstream in the delivery process as possible (ASTM, 2012; Bill Silzle of Lubrizol Corp., personal communication).

### 4.3.2. Cold-Flow Enhancement

The traditional cold weather treatment method for diesel is to blend in kerosene, a practice that is followed by some in the biodiesel industry (Sunny Beaver of Yokayo Biofuels [B100 distributor], personal communications). Commercial cold flow additives on the market contain proprietary components that are typically copolymers of ethylene and vinyl acetate or other olefin-ester copolymers (University of Idaho, 2005). A commonly used chemical for soybean feedstock biodiesel is Milan styrene ester, with Polymethacrylate and Ethylene vinyl acetate used for other feedstocks, such as rapeseed (Bill Silzle of Lubrizol Corp., personal communication). A review of manufacturer MSDSs listed in Table I-B-3 of Appendix I-B reveals instances of esters, but also components such as naphtha and toluene.

Currently cold-flow additives apparently provide unsatisfactory results with B100 in the United States. According to a Department of Energy Biodiesel Handling and Use Guideline document (D.O.E., 2006): "B100 in the United States cannot be effectively managed with current cold flow additives like some petro-diesel fuels or European rapeseed oil based biodiesel. The U.S. biodiesel oils and fats contain concentrations of saturated compounds that are too high for most additives to be effective. Cold flow additive effectiveness can also change dramatically depending on the exact type of biodiesel and the processing it has undergone."

### 4.3.3. Biocides

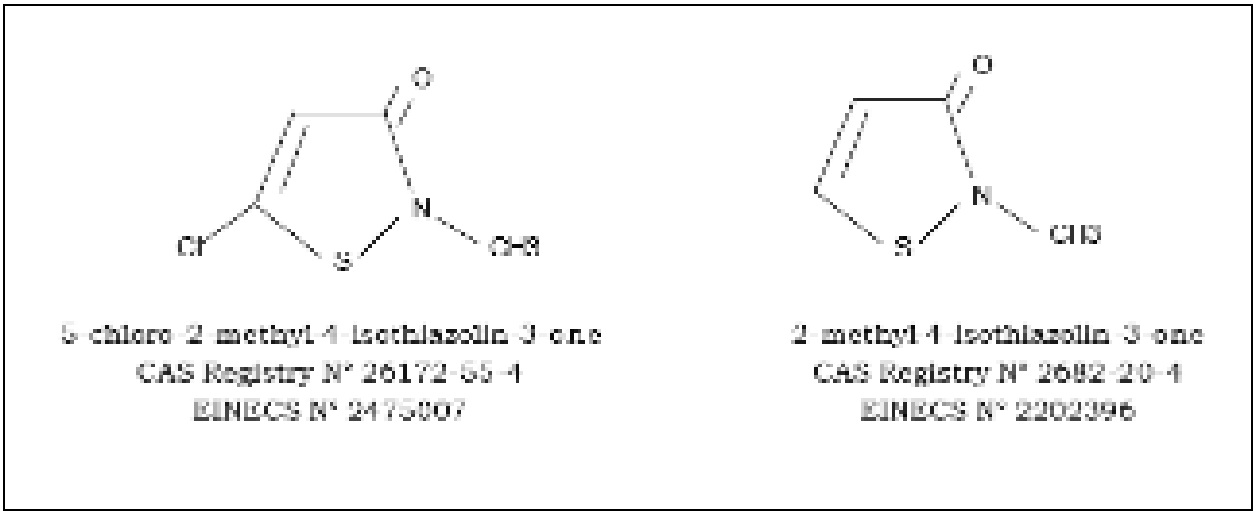
Fuel additives such as biocides can be added to the fuel to "destroy or inhibit the growth of fungi and bacteria which can grow at fuel-water interfaces to give high particulate concentrations in the fuel" (ASTM, 2012).

Additives used to control microbes are generally water-soluble and migrate into any water found in the fuel storage tank. Given the biocides mix with the water, the same biocides used in petroleum based diesel fuel systems are used with biodiesel. Biocides are too expensive to be widely deployed upstream in the distribution process, and there is an added concern of creating

microbial resistance, so biocides are typically used on an "as-needed" basis in the distribution chain wherever and whenever microbial contamination is detected as a problem (Howard Cheznow, Fuel Quality Systems Corp., personal communication).

The market-leading biocide is manufactured by the large chemical company Rohm and Haas Corporation and is sold under the product name of Kathon FP 1.5. The active ingredients in the Kathon product, isothiazols, are shown in Figure 4.2 and listed in Appendix I-B, Table I-B-4.

**Figure 4.2. Rohm and Haas Kathon FP 1.5 Biocide*.**




*Source: Rohm and Haas (1999).

Other common fuel biocide chemicals are methylene bisthiocyanate (MBT) and nitromorphalines (Howard Cheznow, Fuel Quality Systems Corp., personal communications). MBT is often used as a biocide in water treatment plants, paper mills, and other industrial processes involving water. Carbamates also appear in MSDSs of some commercial biocides listed in Table I-B-4 of Appendix I-B.

An environmental issue for biocides involves the treatment and disposal of biocide-containing effluent drained from the storage tanks. The Rohm Haas literature discusses this process and proper deactivation, which involves the use of sodium metabisulphate or sodium bisulphate (Rohm and Haas, 1999).

### 4.3.4. Cetane Enhancers and NOx Reduction Additives

A frequently used indicator of diesel fuel quality is its cetane number. This number is a measure of a fuel's ignition delay. It measures the time period between the start of injection and start of combustion (ignition) of the fuel. The cetane numbers for biodiesel are generally higher than for standard diesel, ranging from 48-65 and 40-55 respectively (D.O.E., 2006). Increases in cetane numbers reduce $NO_x$ emissions, so there remains a motivation to maximize this value in biodiesel fuels (Ribiero et al., 2007). However, NOx emissions and their link to centane number is a complicated issue that must be addressed in the Tier III report

Cetane enhancers are commonly based on 2-ethylhexyl nitrates (Bill Silzle of Lubrizol Corp., personal communication). It is also a component in the Oryx Energy International's $NO_x$

reduction additive, which passed an official emissions testing required by the Texas Commission on Environmental Quality for entry into the market (Irwin, 2007). Another product passing the Texas requirements for biodiesel blends was Viscon, which lists on its MSDS a Polyisolbutylene polymer, describe the company's website as a "high molecular weight pure hydrocarbon polymer," (Viscon, 2008). According to a Brazilian report, Oleochemical carbonates are finding increasing interest in commercial biodiesel applications as cetane number enhancers (Ribiera et al. 2007).

Clean Diesel Technologies in Connecticut provides a $NO_x$ reduction solution consisting of a urea-injection system, which injects urea (or ammonia) into the exhaust gas of the operating engine, reducing $NO_x$ to elemental nitrogen and water vapor. They claim that at typical exhaust temperatures, the reduction of $NO_x$ emissions is between 70% and 90% (Clean Diesel Technologies, 2008).

A report was issued in 2007 by McMinnville Energy System on the results of a stationary biodiesel test involving a large bore Caterpillar power generator in a grid-connected electricity generating application. Funded by the Department of Energy, the American Public Power Association, the National Biodiesel Board, and The Tennessee Soybean Promotion Association, it demonstrated a 96.6% reduction in $NO_x$ emissions from a B100-powered Caterpillar generator using a catalytic converter process that required no ammonia or urea (McMinnville Energy System, 2007).

### 4.3.5. Water Dispersants

There are two general categories of additives used to deal with water in the tanks of vehicles. A *demulsifying* agent extracts water out of suspension within fuel, which allows it to be separated from the fuel by the fuel filter. An *emulsifying* agent works by surrounding water molecules with additive molecules, holding them in suspension and making them small enough that they pass harmlessly through the fuel system.

Little information is available specifically on the chemical composition of commercial demulsifiers/emulsifiers, as they tend to be components of multi-purpose additives. A research paper from the Russian Research Institute of Oilfield Chemistry claims that current demulsifiers primarily consist of "non-ionic surfactants, such as alkylene oxide block copolymers" (Solodov et al., 2005). This corresponds to the Biofuels Systems Group FTG Fuel Treatment product, which lists a "non-ionic surfactant" (Alcohol Ethoxylate) as an ingredient in its MSDS.

### 4.3.6. Anti-Foaming Agents

Although an issue with biodiesel blends, foaming does not appear to be a serious issue with B100 biodiesel (Quigley, 2007). Anti-foaming agents are typically silicon based (Bill Silzle of Lubrizol Corp., personal communication). The German additive manufacturer, Degussa, states in a 2007 product brochure that its diesel (and B5 biodiesel blend) anti-foaming product contains "organosilicone technology" (Degusa, 2008).

## 4.4. Potential Impacts During Fuel Use

In the fuel-use stage of the biodiesel life cycle, the releases of greatest concern are emissions to air, but there are also potential releases to water and soil from atmospheric deposition and from leaks and spills during fueling and vehicle use. The primary releases to air occur during the actual combustion process. There are also vapor emissions during fueling and liquid fuel spills.

### 4.4.1. Biodiesel Impact on Air Quality

Because of the importance of the combustion emissions, the focus below is on air-quality impacts of biodiesel relative to extant diesel fuels. Several studies have determined that use of biodiesel (as a neat fuel or as a blend with petroleum-derived fuel) instead of conventional diesel may be expected to exhibit large reductions in hydrocarbons (HC), particulate matter (PM) and carbon monoxide (CO) emissions. Fuel properties, such as cetane number and oxygen content, are attributed to biodiesel's emission advantages. Since biodiesel (B100) contains approximately 11% oxygen by weight (Graboski et al., 2003), the fuel is able to burn more completely, resulting in fewer unburned fuel emissions.

But studies to date indicate that biodiesel use may have little impact on reducing emissions of $NO_x$ and some toxic air pollutants and could lead to increasing emissions of these pollutants. Because $NO_x$ emissions have a large impact on ambient ozone concentrations, an effective control strategy for reducing ozone is to decrease $NO_x$ emissions. Since there are very few ways of reducing $NO_x$ emissions from a broad range of combustion sources, any small increase in $NO_x$ from biodiesel could affect the California State Implementation Plan. This means that the significance of any increases of $NO_x$ emissions must be discussed and evaluated in some detail in both the Tier II and Tier III multimedia impact reports. In addition, the question of whether the health benefits of decreases in CO, PAHs, and PM are out-weighed by the increases in NOx, ozone, and volatiles should be addressed in the Tier II or Tier III report.

### 4.4.2. Exhaust Emissions

The US Environmental Protection Agency (USEPA) conducted a comprehensive analysis of the emission impacts of biodiesel fuel (EPA, 2002). Previous studies were reviewed and data from 39 out of 80 studies were retained for the EPA analysis. It is important to note that the available data only included tests on heavy-duty diesel vehicles (HDDV) designed for highway use. No predictions could be made concerning the impacts of biodiesel emissions from light-duty vehicles or diesel-powered off-road equipment. The database was also limited in that 98% of the tests were performed on engines with a model year of 1997 or earlier. These engines were not equipped with exhaust gas recirculation (EGR), $NO_x$ absorbers, or PM traps. However, the EPA has "no reason to believe that biodiesel will have substantially different impacts on emissions" for engines lacking this equipment (USEPA, 2002). There is also concern that much of the data used in the 2002 EPA come from a single study that tested many biodiesel samples but used only one engine. This could limit the ability to asses engine-dependent factors and provides incentive for addition emissions testing during Tier II studies.

The USEPA 2002 investigation "made use of statistical regression analysis to correlate the concentration of biodiesel in conventional diesel fuel with changes in regulated and unregulated pollutants" (USEPA, 2002). The results from the USEPA 2002 study are shown below (Figure 4.3). Particulate matter (PM), carbon monoxide (CO), and hydrocarbons (HC) were significantly reduced with increasing concentrations of biodiesel.

Similar emission results for HDDV were estimated in a study entitled "Impacts of biodiesel fuels on air quality and human health" conducted by ENVIRON International Corporation (Morris et al., 2003). These results are summarized in Table 4.1.

**Figure 4.3. Average emission impacts of biodiesel for heavy-duty highway engines*.**

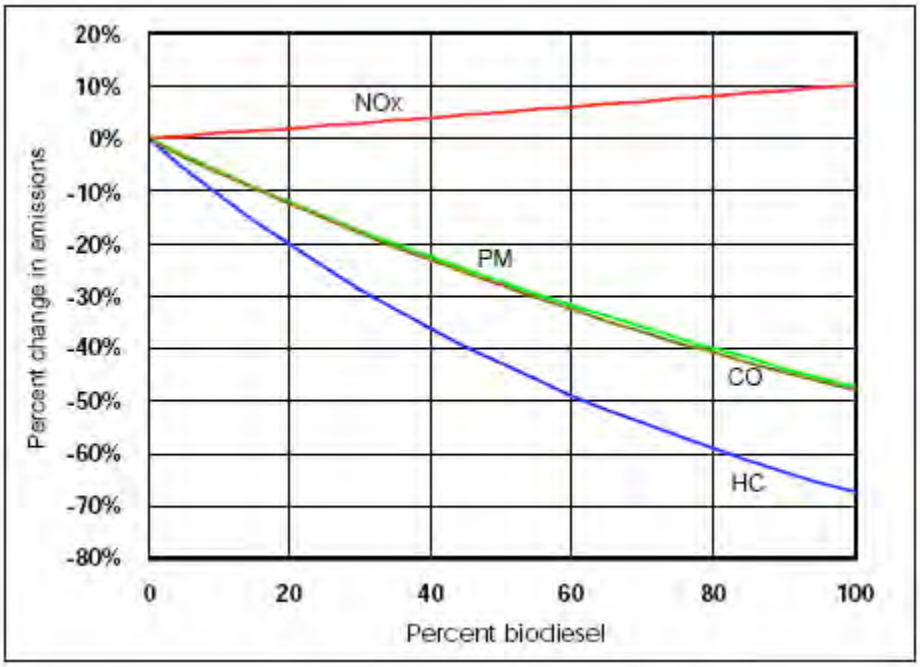



*Figure from USEPA (2002).

**Table 4.1. Overall average change in mass emission effects due to use of biodiesel fuels in heavy-duty highway vehicles compared to standard diesel fuel*.**

| Biodiesel Fuel | NOx | PM | CO | VOC | $SO_2$ |
|---|---|---|---|---|---|
| B20 | +2.4% | -8.9% | -13.1% | -17.9% | -20% |
| B100 | +13.2% | -55.3% | -42.7% | -63.2% | -100% |

*Table from Morris et al. (2003a).

Both studies reveal that the use of biodiesel reduces the emissions of four pollutants regulated by the EPA—PM, CO, HC, and sulfur dioxide ($SO_2$)—but increases slightly nitrogen oxides emissions.

The USEPA 2002 analysis indicates that various components of biodiesel fuel can influence the pollutant emissions. While the engine model year did not impact emissions, the type of feedstock and the type of conventional diesel used for blending did influence the emission. The feedstocks used within the study were divided into three categories: soybean, rapeseed/canola, and animal fat. Type of feedstock affected $NO_x$, PM, and CO emissions but not hydrocarbons emissions.

Carbon monoxide is generated from a variety of combustion processes including industrial sources, household heating, and motor vehicles. Typically 90% of CO emissions in urban areas

come from on-road motor vehicles (Mansell et al., 2003). The CO emissions are dependent on feedstocks, as can be seen in Figure 4.4; however, the USEPA 2002 and other similar reports provided no explanation for this trend.

**Figure 4.4. Biodiesel feedstock effect on CO emissions.**

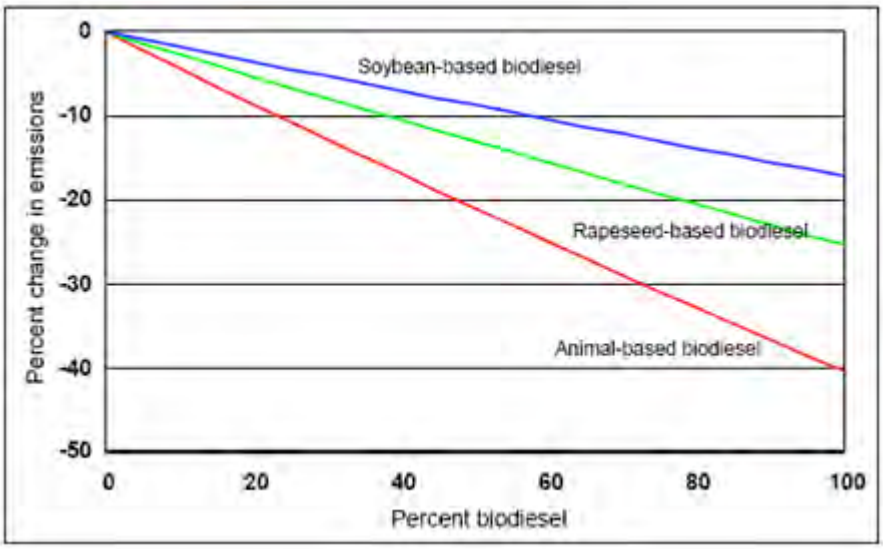



*Figure from USEPA (2002).

The $NO_x$ emissions from biodiesel fuels increased with percent biodiesel in the fuel mix and varied considerably with biodiesel feedstock because the fuel's chemistry can greatly affect emissions. For a fixed percent of biodiesel, biodiesel containing feedstocks high in polyunsaturated fatty acids emit a greater percentage of $NO_x$ than biodiesel high in saturated fatty acids. As illustrated in Figure 4.5, highly unsaturated fuels, such as soybean-based and rapeseed-based biodiesel, produce higher $NO_x$ emissions than do saturated animal-based fuels.

Fuels made from very highly unsaturated feedstocks, such as linseed oil, can have few double bonds and low cetane numbers. A fuel with a low cetane number can cause excessive ignition delay and poor combustion performance, resulting in higher $NO_x$ emissions (Graboski et al., 2003). A study conducted by Colorado Institute for Fuels and Engine Research determined that biodiesels with cetane numbers greater than about 68 are expected to produce $NO_x$ emissions equal to or less than diesel (Graboski et al., 2003).

The length of the fatty-acid carbon chain can also affect $NO_x$ emissions. The shorter chain hydrocarbons, those below C12, produce higher $NO_x$ emissions. The shorter chain esters have densities greater than longer chain esters, but the mechanism(s) by which $NO_x$ emissions increase with the shortening of hydrocarbon chain lengths remains unknown (Graboski et al., 2003).

As can be seen in Figure 4.6, PM emissions are reduced significantly with the use of biodiesel. Unlike for $NO_x$, particulate matter emissions are dependent not on the molecular structure of the biodiesel, but also on the sulfur and oxygen content of the fuel. Lower sulfur content reduces PM emissions. A higher oxygen content generally allows a fuel to burn more completely, resulting in fewer unburned or partially burned PM emissions (Graboski et al., 2003). Thus the more oxygen contained within a fuel, the lower the PM emissions released.

**Figure 4.5. Biodiesel feedstock effect on $NO_x$ emissions*.**

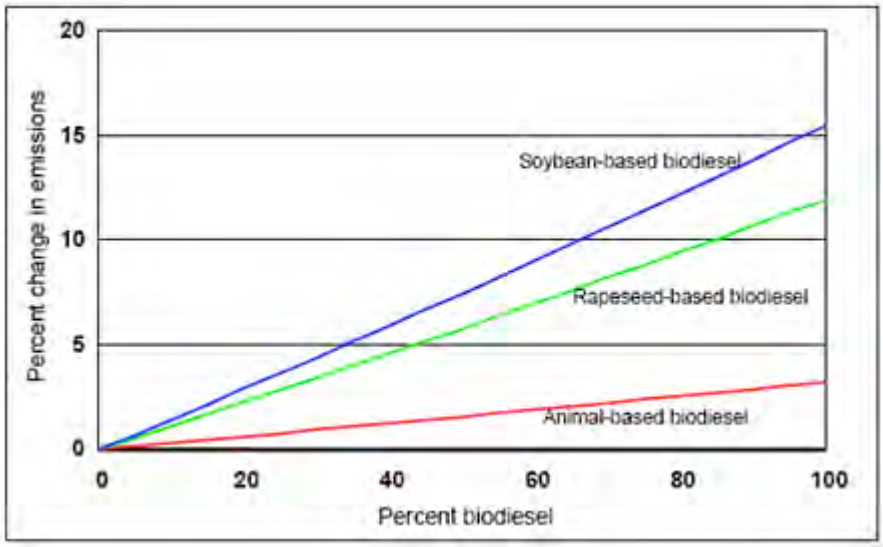



*Figure from USEPA (2002).

The study conducted by Graboski et al. (2003) at the Colorado Institute for Fuels and Engine Research confirms this relationship, as indicated in Figure 4.7. In the USEPA 2002 study, the lower PM emissions from animal-based biodiesel relative to plant-based biodiesel is attributable to slightly more oxygen in the animal-based formulation.

**Figure 4.6. Biodiesel feedstock effect on PM emissions*.**

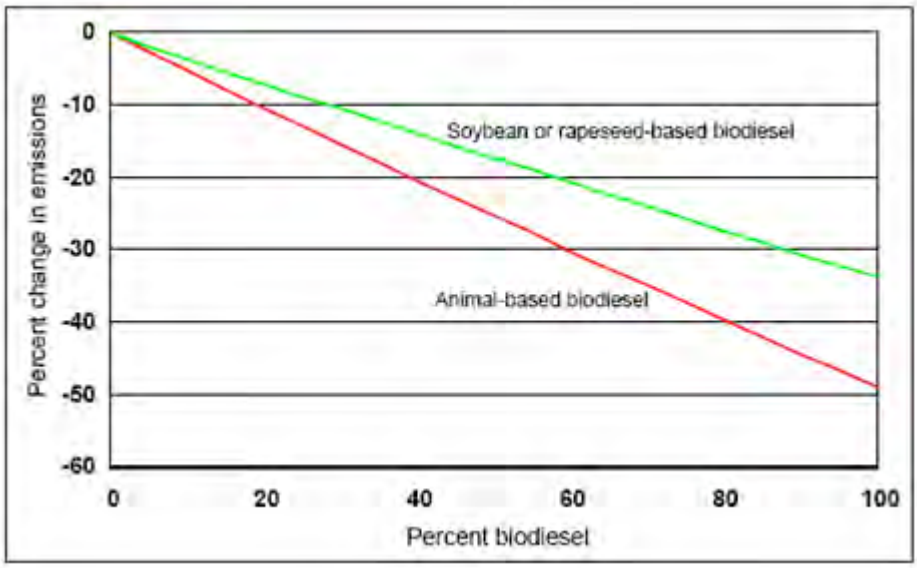



*Figure from USEPA (2002).

**Figure 4.7. Regression model showing effect of oxygen on particulate matter*.**

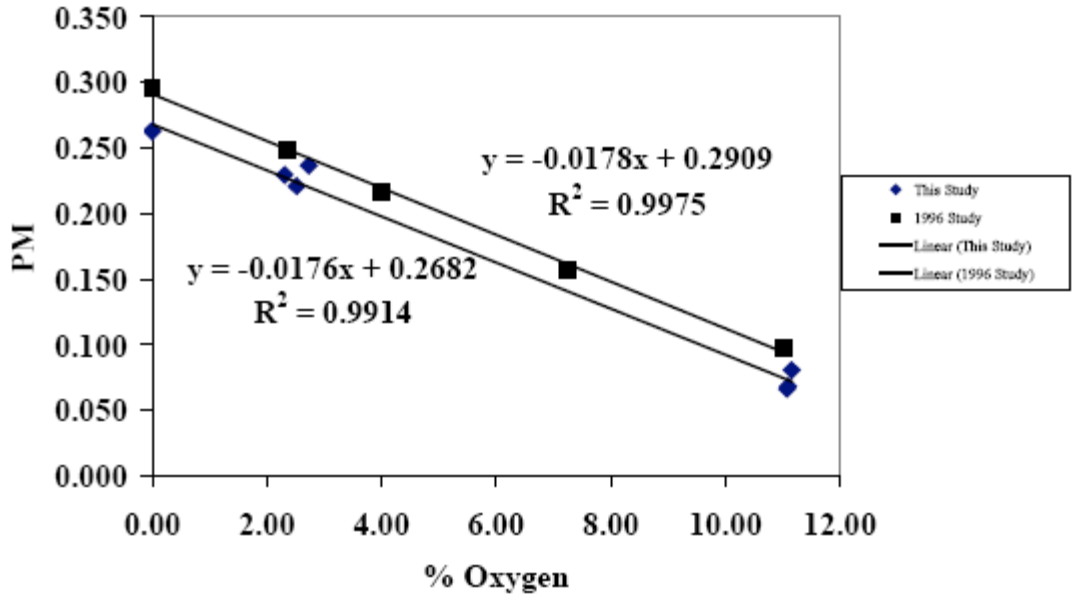



*Figure from Graboski et al. (2003).

### 4.4.3. Effects on Ambient CO and PM Concentrations

CO and PM are criteria air pollutants that are regulated under the 1990 Clean AIR Act. The Clean Air Act specifies that all regions of the US comply with EPA-specified National Ambient Air Quality Standards (NAAQS), which specify threshold concentrations that CO and PM.

While some studies have focused specifically on how biodiesel use impacts exhaust emissions from HDDV, researchers at the ENVIRON International Corporation considered more generally how biodiesel use can impact ambient air quality (Mansell et al., 2003). Two NAAQS have been established for CO: a one-hour standard of 35 ppm and an eight-hour standard of 9 ppm. Typically 90% of "area-wide CO emissions in congested urban areas come from on-road motor vehicles" (Mansell et al., 2003). One-hour and eight-hour CO concentrations were estimated in the Las Vegas Valley for standard diesel and B20 with scenarios of 50% and 100% penetration in the HDDV. The results (Table 4.2) indicate that biodiesel does reduce peak CO concentrations; however, these reductions are quite small, ranging from 0.01 to 0.03 ppm (< 0.2%).

Biodiesel effects on ambient concentrations of PM in the South Coast (Los Angles regions) Air Basin (SoCAB) have also been investigated. The NAAQS for $PM_{10}$ (particulate matter of 10 μm or less) consists of an annual standard of 50 $\mu g/m^3$ and a 24-hour average standard of 150 $\mu g/m^3$ (Morris and Jia, 2003a). Additionally, $PM_{2.5}$, a new fine particulate matter standard, should not exceed annual and 24-hour average thresholds of 15 and 65 $\mu g/m^3$, respectively.

Morris and Jia (2003a) assessed separately the effects of biodiesel for particulate sulfate, nitrate, ammonium, elemental carbon (EC), organic carbon (OC), other fine particulate, course matter, total $PM_{10}$ mass, total $PM_{2.5}$ mass, and exposure to $PM_{10}$ and $PM_{2.5}$. The maximum increases and decreases in PM concentrations for each of the PM species listed above are shown in Table 4.3.

**Table 4.2. Peak estimated 1-hour and 8-hour carbon monoxide (CO) concentrations in the Las Vegas Valley, 100% B20, and 50% B20 emission scenarios and the differences in CO concentrations between the biodiesel fuel scenarios and the standard diesel base case*.**

| | Std. Diesel | 50% B20 | | 100% B20 | |
|---|---|---|---|---|---|
| Episode | Peak (ppm) | Peak (ppm) | Difference (ppm) | Peak (ppm) | Difference (ppm) |
| 1-Hour CO Dec 8-9 | 17.90 | 17.89 | -0.01 | 17.87 | -0.02 |
| 8-Hour CO Dec 8-9 | 9.39 | 9.38 | -0.01 | 9.37 | -0.02 |
| 1-Hour CO Dec 19-20 | 18.38 | 18.36 | -0.02 | 18.35 | -0.03 |
| 8-Hour CO Dec 19-20 | 13.73 | 13.72 | -0.01 | 13.71 | -0.02 |

*Table from Mansell et al. (2003).

**Table 4.3. Estimated maximum increases and decreases in particulate matter (PM) concentrations ($\mu g/m^3$) in the Southern California air basin due to a 100% penetration of B20 biodiesel in the heavy-duty diesel vehicle fleet*.**

| | Annual Average | | Maximum 24-Hour Average | |
|---|---|---|---|---|
| PM Species | Maximum Increase | Maximum Decrease | Maximum Increase | Maximum Decrease |
| Sulfate | 0.00 | -0.03 | 0.00 | -0.07 |
| Nitrate | +0.04 | -0.09 | +0.58 | -1.12 |
| Ammonium | +0.01 | -0.03 | +0.15 | -0.34 |
| EC | 0.00 | -0.06 | 0.00 | -0.10 |
| OC | 0.00 | -0.15 | 0.00 | -0.27 |
| Other PFIN | 0.00 | -0.01 | 0.00 | -0.01 |
| Other PCRS | 0.00 | -0.01 | 0.00 | -0.01 |
| $PM_{10}$ Mass | +0.04 | -0.31 | +0.62 | -1.61 |
| $PM_{2.5}$ Mass | +0.04 | -0.30 | +0.62 | -1.61 |

*Table from Morris and Jia (2003a).

Nitrate and ammonium exhibit both increases and decreases associated with use of B20 fuel. Since the SoCAB is already high in $NO_x$, a precursor to ammonium nitrate, these species can act as major contributors to PM. The decrease in nitrate occurred in "more populated portions of SoCAB, whereas the increases occurred east of the SoCAB in the desert" (Morris et al., 2003a). Despite these small increases, the results indicate that the $PM_{10}$ for annual and 24-hour exceedances are reduced 4% and 7%, respectively, in biodiesel compared to standard diesel fuel.

### 4.4.4. Effect on Ozone

Ozone is formed in the atmosphere through "complex reactions involving Volatile Organic Compounds (VOC) and oxides of Nitrogen ($NO_x$) in the presence of sunlight" (Morris et al., 2003a). In some air basins, $NO_x$ levels will impact ambient ozone concentrations, but this relationship is location specific. In cases where $NO_x$ levels are the limiting factor for ozone formation, the most effective control strategy for reducing ozone would be to decrease $NO_x$ emissions.

The threshold values for NAAQS 1-hour and 8-hour ozone concentrations are 0.12 ppm (124 ppb) and 0.08 ppm (84 ppb), respectively. An ozone air quality modeling study for biodiesel was

conducted by ENVIRON International Corporation (Morris et al., 2003b). The effect of biodiesel fuel use on urban and regional ozone air quality was evaluated in Southern California (SoCAB), Lake Michigan, and the Northeast Corridor. The results revealed small increases and decreases in peak daily maximum 1-hour and 8-hour ozone concentrations for B20 with 100% and 50% penetration in the HDDV (Table 4.4 and Table 4.5). Because increasing $NO_x$ is expected to increase ozone levels, these results seem inconsistent with the relatively large increase of $NO_x$ from B20. But ozone formation depends on both VOC and $NO_x$ levels. It appears that ozone levels remain low in Morris et al. (2003) results due to reductions of VOC (see Table 4.1) that compensate for increases in $NO_x$ emissions.

Since the maximum ozone increase is well below 1 ppb, "the use of biodiesel fuel is estimated to have no measurable adverse impact on 1-hour and 8-hour ozone attainment in Southern California and the Eastern United States" (Morris et al., 2003b). In fact, based on the results from the SoCAB region, the use of biodiesel can result in small but potentially measurable beneficial impacts on ozone attainment, because the daily maximum ozone concentrations were reduced by approximately 1 ppb.

**Table 4.4. Maximum increases and decreases in daily maximum 1-hour ozone concentrations in three air basins*.**

| Date | 50% B20 Biodiesel (ppb) | | 100% B20 Biodiesel (ppb) | |
|---|---|---|---|---|
| | Max Increase | Max Decrease | Max Increase | Max Decrease |
| **2007 Lake Michigan Domain** | | | | |
| July 11, 1995 | +0.03 | -0.16 | +0.05 | -0.33 |
| July 12, 1995 | +0.07 | -0.10 | +0.09 | -0.19 |
| July 13, 1995 | +0.05 | -0.12 | +0.09 | -0.24 |
| July 14, 1995 | +0.07 | -0.09 | +0.09 | -0.18 |
| July 15, 1995 | +0.04 | -0.26 | +0.08 | -0.53 |
| **2007 Northeast Corridor Domain** | | | | |
| July 11, 1995 | +0.06 | -0.08 | +0.11 | -0.14 |
| July 12, 1995 | +0.07 | -0.12 | +0.13 | -0.25 |
| July 13, 1995 | +0.12 | -0.06 | +0.15 | -0.07 |
| July 14, 1995 | +0.15 | -0.04 | +0.20 | -0.09 |
| July 15, 1995 | +0.10 | -0.10 | +0.18 | -0.20 |
| **1997 South Coast Air Basin Domain** | | | | |
| August 4, 1997 | +0.09 | -0.48 | +0.17 | -0.95 |
| August 5, 1997 | +0.10 | -0.56 | +0.19 | -1.11 |
| August 6, 1995 | +0.11 | -0.60 | +0.22 | -1.20 |
| August 7, 1995 | +0.13 | -0.49 | +0.26 | -0.98 |

*Table from Morris et al. (2003b).

**Table 4.5. Maximum increases and decreases in daily maximum 8-hour ozone concentrations in three air basins*.**

| Date | 50% B20 Biodiesel (ppb) | | 100% B20 Biodiesel (ppb) | |
|---|---|---|---|---|
| | Max Increase | Max Decrease | Max Increase | Max Decrease |
| **2007 Lake Michigan Domain** | | | | |
| July 11, 1995 | +0.02 | -0.14 | +0.04 | -0.28 |
| July 12, 1995 | +0.03 | -0.09 | +0.07 | -0.17 |
| July 13, 1995 | +0.04 | -0.11 | +0.09 | -0.21 |
| July 14, 1995 | +0.03 | -0.09 | +0.07 | -0.18 |
| July 15, 1995 | +0.03 | -0.20 | +0.07 | -0.40 |
| **2007 Northeast Corridor Domain** | | | | |
| July 11, 1995 | +0.05 | -0.07 | +0.10 | -0.13 |
| July 12, 1995 | +0.05 | -0.10 | +0.11 | -0.20 |
| July 13, 1995 | +0.07 | -0.04 | +0.12 | -0.07 |
| July 14, 1995 | +0.07 | -0.04 | +0.14 | -0.07 |
| July 15, 1995 | +0.06 | -0.04 | +0.15 | -0.08 |
| **1997 South Coast Air Basin Domain** | | | | |
| August 4, 1997 | +0.06 | -0.34 | +0.12 | -0.68 |
| August 5, 1997 | +0.07 | -0.39 | +0.15 | -0.77 |
| August 6, 1995 | +0.08 | -0.48 | +0.15 | -0.96 |
| August 7, 1995 | +0.08 | -0.42 | +0.15 | -0.83 |

*Table from Morris et al. (2003b).

### 4.4.5. Effect on Toxic Air Pollutants

The class of air contaminants referred to as toxic air pollutants or "air toxics" contains numerous chemical compounds with widely varying sources, environmental fate, exposure pathways, and health outcomes. Over 40 chemical compounds in diesel exhaust have been listed as toxic air pollutants based on carcinogenicity and exhaust from diesel engines account for a significant fraction of the total added cancer risk in outdoor air from all hazardous air pollutants combined (Morris and Jia, 2003). There have been a number of studies comparing toxic air pollutant emissions, particularly polycyclic aromatic hydrocarbons (PAHs), from conventional diesel and biodiesel. In the paragraphs below we review key issues that can be drawn from these studies.

In the early 1990s, Kado et al. (1996) in collaboration with the University of Idaho, the Montana Department of Environmental Quality, and the U.S. Department of Energy, investigated the concentrations of PAHs in the exhaust from four different fuels tested in a 1995 Dodge 3/4 ton pickup truck with a Cummins B (5.9 L, Turbo diesel). The four tested fuels include: 1) 100% ethyl ester of rapeseed oil (REE), 2) 100% diesel 2-D low sulfur fuel, 3) 20% REE + 80% diesel, and 4) 50% REE + 50% diesel. For a catalyst-equipped engine, they found volatile PAHs such as phenanthrene, fluoranthene, and pyrene remained at an approximately equivalent emission rate (g/mile) independent of the REE content in the fuel (ranging from 100% diesel to 100% REE). In addition, the more chemically reactive PAHs [for example, benzo(a)pyrene] were emitted at greater levels for the pure REE and some of the blended REE fuels than in emissions from 100% diesel fuel.

Turrio-Baldassarri et al. (2004) compared the chemical (and toxicological) characteristics of emissions from an urban bus engine fueled with standard diesel and a biodiesel blend. Exhaust gases were produced by a turbocharged EURO 2 heavy-duty diesel engine, operating in steady-state conditions on the European test 13 mode cycle (ECE R49). They studied both regulated and unregulated pollutants, including PAHs and nitrated derivatives of PAHs (nitro-PAHs), carbonyl

compounds, and light aromatic hydrocarbons. The also evaluated the size distribution of PM. They found that the use of biodiesel blend seems to result in small reductions of emissions of most of the aromatic and PAH compounds. But they noted that these differences were not statistically significant. They found formaldehyde to have a statistically significant increase (18%) in emissions from the biodiesel blend. Their electron microscopy analysis indicated that PM for both fuels has the same chemical composition, morphology, shape and granulometric spectrum, with most of the particles in the range 0.06–0.3 µm.

Lin et al. (2006) investigated PAH and regulated pollutant emissions from heavy-duty diesel engines (HDDEs) fueled with premium diesel fuel (PDF), palm-biodiesel–PDF blends and paraffinic–palm-biodiesel blends in brand-new (zero mileage) engines as the engines accumulated miles. Their results indicate that while the emissions of THC and CO increased with operation time, the emissions of $NO_x$ and PAHs decreased with operation time between 0 and 300 h (18,000 km). They also found that palm-biodiesel–PDF blends or paraffinic–palm-biodiesel blends in place of PDF in HDDEs reduced the emissions of PM by 6.11to 26.8%, total PAHs by 43.0 to 90.2% and total benzo(a)pyrene toxic equivalents (BaPeq) by 63.1 to 89.6%.

Yang et al. (2007) carried out an 80,000-km durability test on two engines using diesel and biodiesel (methyl ester of waste cooking oil) blend (B20) to evaluate emissions resulting from the use of biodiesel. They measured emissions of regulated air pollutants, including CO, HC, $NO_x$, and PM, and PAHs at 20,000-km intervals. At 0 km, HC, CO and PM emission levels were lower for the B20 engine than those for diesel. After running for 20,000 km and longer, emissions of these pollutants were higher. However, the deterioration coefficients for these regulated air pollutants were not statistically higher than 1.0, implying that the emission factors do not increase significantly after 80,000 km of driving. Yang et al. (2007) also found that total (gaseous+particulate phase) PAH emission levels for both B20 and diesel decreased as the driving mileage accumulated. However, for the engine using B20 fuel, particulate PAH emissions increased as engine mileage increased. The average total PAH emission factors were 1097 and 1437 mg $(\text{bhp h})^{-1}$ [bhp h = brake horsepower hour] for B20 and diesel, respectively. For B20, the benzo[a]pyrene equivalent emission factors were 0.77, 0.24, 0.20, 7.48, 5.43 and 14.1 mg $(\text{bhp h})^{-1}$ for 2-, 3-, 4-, 5-, 6-ringed and total PAHs.

Acrolein is an unsaturated aldehyde that is both a primary pollutant and a secondary oxidation product of vehicle emissions. Comparative acrolein and other aldehyde emissions were recently measured (Cahill and Okamoto, 2012) via chassis dynamometer from two heavy-duty trucks under both city and cruising drive scenarios, with CARB ULSD, soy biodiesel, animal fat biodiesel, and renewable diesel fuels. The biodiesels were used in both neat and 50-50 mixes with ULSD. Acrolein emissions from soy blends were found to roughly double the emissions from ULSD and acrolein emissions from animal fat blends were ~25% - 50% higher than those from ULSD.

The available studies cited above indicate that biodiesel could reduce emissions of the measured toxic compounds, however these studies were conducted on engines that are now older and the results should be updated with studies on newer, more relevant engine technologies. There is also a need for more controlled combustion studies to assess how the spectrum of toxic air pollutants, in particular the spectrum of PAHs, will shift both in terms of volatile and particle-bound fraction but also in terms of any changes in toxic equivalency.

### 4.4.6. Summary Points on Biodiesel Emissions

Based on the discussions above, we note that, relative to petroleum diesel emissions from engine combustion, biodiesel emissions have been shown to contain less particulate matter, hydrocarbons, carbon monoxide, and polycyclic aromatic hydrocarbons (PAHs). However, available measurements indicate that the combustion of biodiesel in a diesel engine can increase the release in nitrogen oxides, which, in addition to their association with potential health effects, have been identified as an ozone precursor. Despite the reduction in a total mass of particulate matter, it is not clear whether or by how much the shift in PM and toxic air pollutant composition changes in terms of chemistry and toxicity potential. It should be noted that approximately 80-95% of diesel exhaust particulate matter (DEPM) mass consists of PM2.5, and within that is a subgroup of ultrafine particles (UFPs) with an aerodynamic diameter of less than or equal to 0.1 micron. UFPs account for ~1-20% of the DEPM mass and 50-90% of the total number of particles. It should be noted that the neither the data presented in Figures 4.3 and 4.6 nor the data from the corresponding references address the changes, if any, in UFP levels. Given the current literature demonstrating the health effects of UFPs, we note that there is a need to consider measuring UFP levels as part of the toxicity assessment for biodiesel. Such measurements can address the concern that that a drop in total PM mass does not necessarily equate to a drop in UFP number. Finally, the observation that formaldehyde increases significantly with biodiesel combustion indicates a need to address this issue in the Tier-II report. At a minimum, modeling should be used to assess how expected formaldehyde emissions relate to air-basin exposures. This will determine if formaldehyde emissions from biodiesel will become an issue in light of the current chronic California recommended exposure limit (REL) for formaldehyde of 2 ppb.

Although considerable data are available on the effect of biodiesel on EPA-regulated pollutants (i.e., HC, CO, $NO_x$, and particulates), most of these data were generated using older technology engines. Further, very little detailed exhaust characterization data on biodiesel exists beyond a small number regulated pollutants. This raises a concern about the relevance of these data to newer engines. But recent studies with newer engines tend to confirm these earlier results and make clear factors that increase $NO_x$ emissions. McCormick et al. (2006) for example tested emissions from entire vehicles (rather than engines) on a heavy-duty chassis dynamometer and found that the $NO_x$ impact of B20 varied with engine/vehicle technology and test cycle over the range -5.8% to +6.2%. Sze et al., (2007) used a series of paired (standard diesel/soy methyl-ester biodiesel blends) with transient cycles on a 2006 model year Cummins ISB compression ignition engine equipped with exhaust gas recirculation. Their results show statistically-significant differences in $NO_x$ emission for all fuel pairs with average $NO_x$ emissions due to biodiesel increased over each cycle, ranging from 0.9 to 6.6% and from 2.2 to 17.2% for the B20/B0 and B50/B0 fuel pairs, respectively. They also observed reductions in CO and PM in these tests. Eckerle et al. (2008) used a precisely-controlled single-cylinder diesel engine experiment to determine the impact of a 20% blend of soy methyl-ester biodiesel (B20) on $NO_x$ emissions and found that the magnitude and even direction of NOx effect changes with engine load, with higher duty cycle average power corresponding to a larger $NO_x$ increases.

Given the wide variety of oils and fats the might be used to make biodiesel fuel, the actual emissions of PMs and toxic air pollutants should be considered for each proposed formulation of biodiesel fuel to be used in California. This situation requires a systematic and ongoing effort to assess emissions from diesel engines. But it should be recognized that, due to the large number of fuel formulations along with the resources and cost required to evaluate each formulation, it is not feasible to assess all combinations of engine types and fuel formulations. This is especially

the case with additives, since the number of additive and feedstock combinations could be very large. So it will be important in the Tier-II and Tier-III assessments to target a smaller set of archetypal and informative combinations of engines and fuel formulations. For example, because the life-cycle impact assessment of biodiesel is a comparative evaluation between CARB diesel and biodiesel, the emissions assessment can be simplified if both CARB diesel and additives currently used in CARB are considered the baseline rather than just the CARB diesel alone.

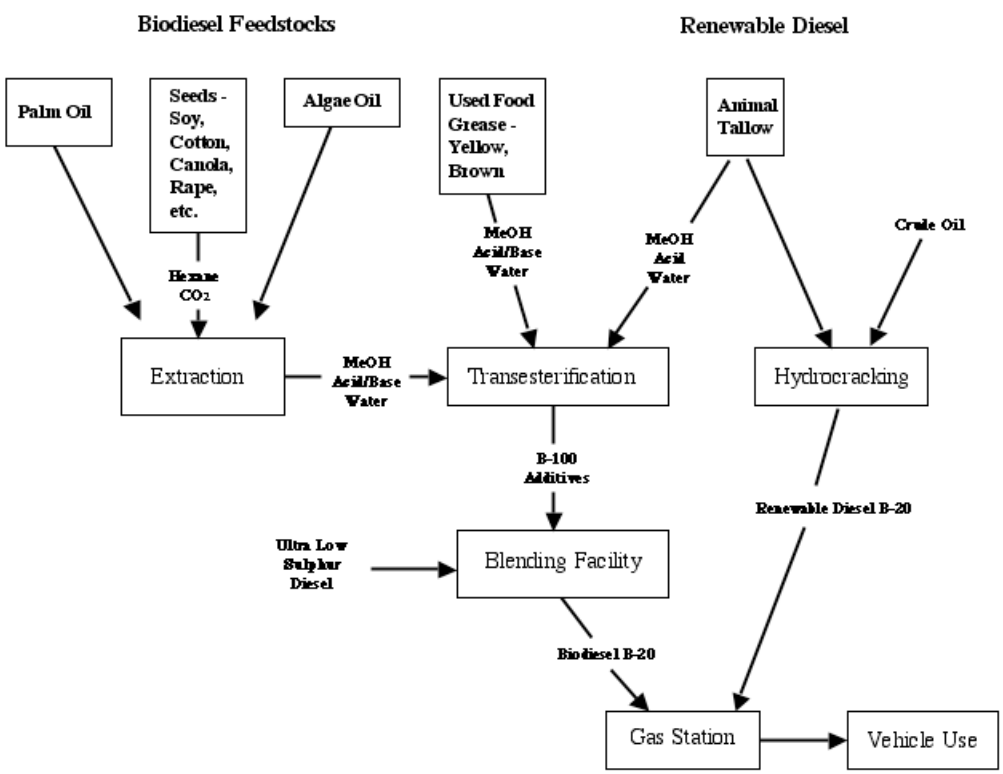
# 5. Release Scenarios

## 5.1. Defining Release Scenarios

One of big challenges in evaluating potential release scenarios associated with the use of biodiesel in California is the wide variety of feedstocks and different production processes that may be used. For the Tier I evaluation of release scenarios, our main focus is to identify releases that could have the greatest impact on the environment, human health, and important resources such as surface and ground waters.

As indicated previously in Section 2.2, production feedstocks can range from palm oil, to seed oils, to yellow or brown used greases, to animal tallow, to algae oil (Figure 5.1). Different groups of feedstocks use different catalysts depending on the amount of FFAs. Biodiesel from seed feedstocks require extraction using hexane or other chemical processes such as super-critical carbon dioxide. To address fuel stabilization and performance issues such as fuel oxidation, biodegradation, $NO_x$, residual water and sediments, and fuel pour point, a variety of additives may be required and stored in bulk at either a production facility or a blending facility. The storage of bulk feedstocks, processing chemicals, additives, and finished B100 and B20 will likely be accomplished using above ground storage tanks of various sizes.

**Figure 5.1. Biodiesel process flow chart. An alternative renewable diesel pathway is shown on the right.**



Further, the feedstocks will be widely distributed geographically and will use a variety of transportation means. Palm oil will likely arrive from distant global sources via tanker ship. Soy oil will likely arrive via rail tank car from the Midwestern United States. Yellow grease will be collected from a variety of sources within a city or region and transported by truck to a processing facility.

The higher density of fatty acid methyl esters compared to ULSD can cause heterogeneous biodiesel blends. Since many blenders rely on "splash blending" to mix B100 with ULSD there can be a tendency to form higher levels of pure diesel in the bottom of a tank than at the top. This, along with the fact that many distributors do not check the biodiesel content of the blends they receive from the blenders, can result in the distribution of biodiesel blends with B100 contents far from the advertised percentage (NREL 2005). One survey of B100 content in B20 blends found B100 levels ranging from 10% to 74%. In fact, only 10% of the samples taken for the survey met the standards of the US Department of Defense, a significant end-user of biofuels in the US (Reddy 2008).

It is also unclear where the final blending of the B100 with ULSD to make B20 will occur. It may occur at the B100 production facility or a centralized blending facility that collects B100 from a variety of feedstocks. The blending of a variety of feedstock B100 may be needed to create a standardized, fungible product. As the volume of biodiesel used increases, centralized blending facilities may need to locate near existing fuel pipelines that can import bulk ULSD and export B20 easily.

## 5.2. Normal Releases

Releases associated with the production, storage and distribution, and use of biodiesel can be regarded as normal (routine) or off-normal (unplanned but not necessarily unlikely). Different feedstocks and production processes may have different normal and off-normal releases and may affect different environmental media and human populations depending on geographic location.

Normal or routine releases during the production of B100 include:

- Hexane or $CO_2$ released to the air during seed extraction.
- Odors associated with waste biomass
- Methanol releases to air or water
- Used process water discharges of various pH and trace-chemical composition.

Normal releases during the use of biodiesel include combustion tailpipe emissions, both to the air and to surface waters in the case of marine use. The magnitude of these normal production and use releases within California is not clear yet.

## 5.3. Off-Normal Releases

Off-normal releases or unanticipated releases can occur primarily during the production, distribution and storage of B100 and B20. These off-normal releases may include spills or leaks of bulk feedstock oil, production chemicals, such as methanol, hexane, acid, base, or blending stocks such as ULSD or B100, or finished B20 fuel. These off-normal releases may be the result of leak or rupture of:

- an above ground or underground storage tank and associated piping,

- a liquid transportation vehicle such as rail tank car, tanker truck, or tanker ship.
- a bulk fuel transport pipeline

Even if releases of B100 or B20 would not cause significantly greater impacts to the environment, human health, or water resources when compared to ULSD, the impact of releases of associated additives and production chemicals is of concern. For example, small releases of normal diesel fuel typically naturally biodegrade in the environment. If a biocide additive in B100 or B20 inhibits this natural process, then environmental and resource impacts and costs of cleanup could increase significantly. Releases of $CO_2$ can impact both the environment and humans nearby. Releases of methanol into subsurface environments which are also contaminated with either or both biomass derived and petroleum based fuel oils may cause increased contamination to groundwater.

# 6. Environmental Transport and Fate of Biodiesel

## 6.1. A Multimedia Framework for Fate, Transport, and Exposure

A multimedia transport, fate and exposure assessment synthesizes information about partitioning, reaction, and multimedia-transport properties of a chemical in a representative or generic environment with information about exposed humans or wildlife to assess impacts, such as health risk. The multimedia impact characterization approach is illustrated in Figure 6.1. This assessment is typically carried out using models supplemented with limited sampling analyses. In the modeling approach for characterizing potential impacts, the environment is treated as a set of compartments that are homogeneous subsystems exchanging water, nutrients, and chemical contaminants with other adjacent compartments. A cumulative multi-pathway exposure assessment for humans relates contaminant concentrations in multiple environmental media to concentrations in the media with which a human population has contact (for example; personal air, tap water, foods, household dusts, and soils). The potential for harm is assessed either as the average daily intake or uptake rate or as time-averaged contact concentration.

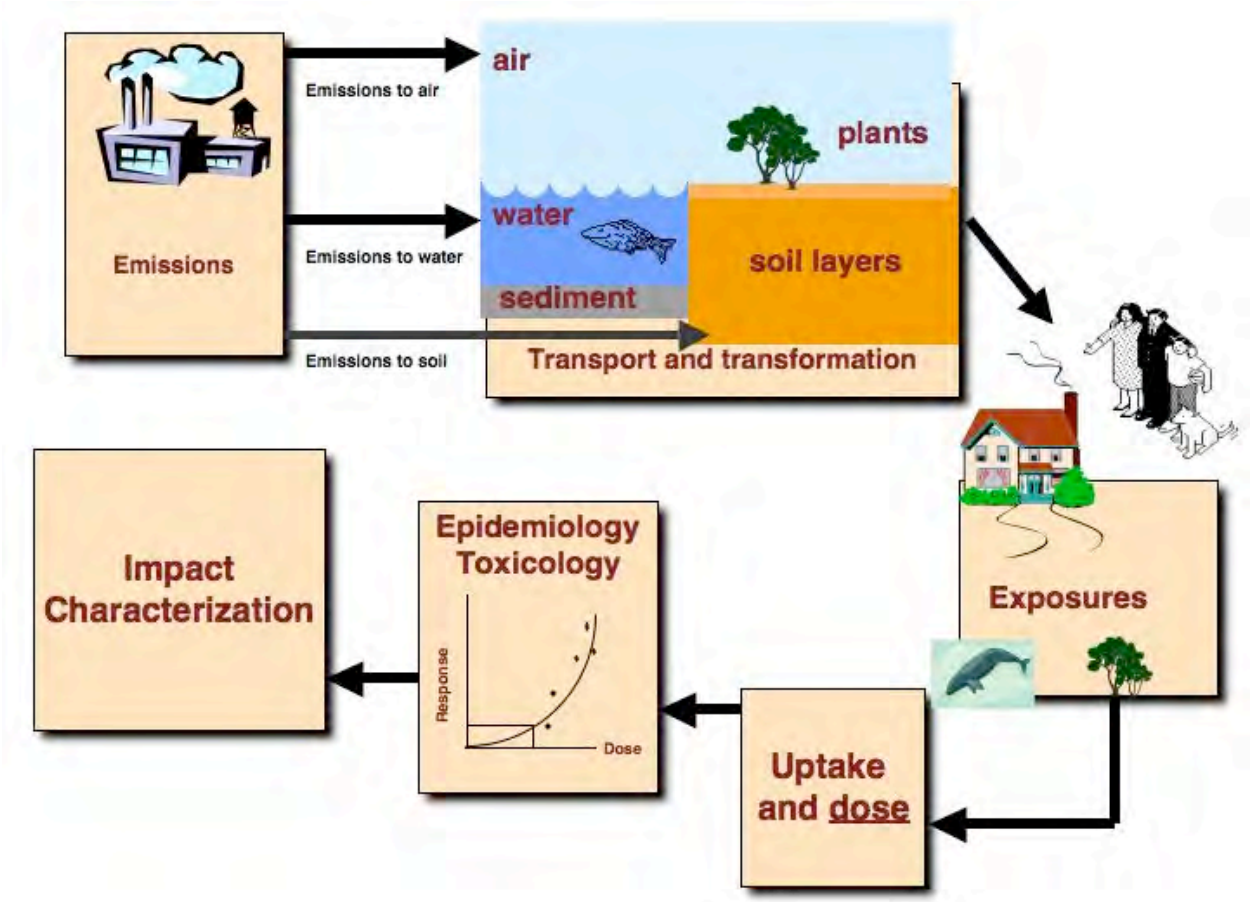



**Figure 6.1. A conceptual illustration how pollutant emissions from each life-stage of biodiesel enter air, water, and or soil, undergo multimedia transport into exposure media, followed by contact with humans and ecosystems.**

Multimedia contaminant fate and exposure models have been useful to decision makers because these models provide an appropriate quantitative framework to evaluate our understanding of the complex interactions between chemicals and the environment. The greatest challenge for multimedia models is to provide useful information without creating overwhelming demands for input data and producing outputs that cannot be evaluated. The multimedia modeler must struggle to avoid making a model that has more detail than can be accommodated by existing theory and data while also including sufficient fidelity to the real system to make reliable classifications about the source-to-dose relationships of environmental chemicals. In the early 1990s, the California Environmental Protection Agency adopted a multimedia approach for setting clean-soil goals through its CalTOX program (McKone, 1993), and the European Union adopted a multimedia multipathway framework for chemical risk assessment (RIVM, 1994.

Deploying a multimedia assessment framework requires basic chemical properties information about the substances under consideration. These properties include multiphase partitioning properties that describe how a substance will distribute itself among the major components of the environment—air, water, and organic phases such as lipids and organic materials in soil.

In the sections below, we identify the important multiphase transport properties needed for the multimedia impact assessment and summarize available information and information gaps. Where there are information gaps we consider how these gaps contribute to overall uncertainty and suggest approaches for increasing available information.

The greatest difficulty we can anticipate with determining the chemical properties of biodiesel fuels is that biodiesel fuel is not a defined chemical formulation or a defined mixture of components, but can be formulated from of any of a very large number of feedstocks with different chemical components.

## 6.2. Data Needs for Multimedia Transport

Multimedia transport and transformation models require three different types of input data. First they require chemical properties data. Next they require data describing the climate, soil, and hydrologic properties of the environment or landscape receiving the contaminants, and finally, information on emissions patterns and mode of entry (air, soil, water, groundwater). In this section we focus on data needs for the first of these, chemical properties.

The basic chemical properties needed to describe the environmental fate of a chemical, are those that define equilibrium distribution among the key environmental phases and those describing removal rates by biological or chemical degradation in air, water, and soil. Partitioning involves following media pairs:

- Air-Water
- Air-Atmospheric particulates (aerosols)
- Air-Soil
- Water-Solids (suspended solids, sediments and soil).
- Non-aqueous phase liquid (NAPL) and
- Pure phase

Degradation in air, water, and soil involve one or more of the following processes:

- Biodegradation
- Hydrolysis
- Photolysis
- Oxidation

For non-polar, non-ionizing organic substances a number of assumptions have been found to be reasonable for establishing partitioning among the primary environmental media. These are the air-water, $K_{AW}$; octanol-water, $K_{OW}$ and octanol-air, $K_{OA}$ partition coefficients. Other phase distributions can be derived from this basic set. For example, sorption to solids in air, water, soil, and sediments is dominated by partitioning or absorption into the organic fractions of these solids. $K_{OW}$ and $K_{OA}$ have been useful proxies for these solid-phase partition factors. These three partition coefficients, as well as those partition coefficients involving the pure substance phase (vapor pressure, solubility in water and solubility in octanol) are interrelated as shown in Figure 6.2 (Wania, 2001).

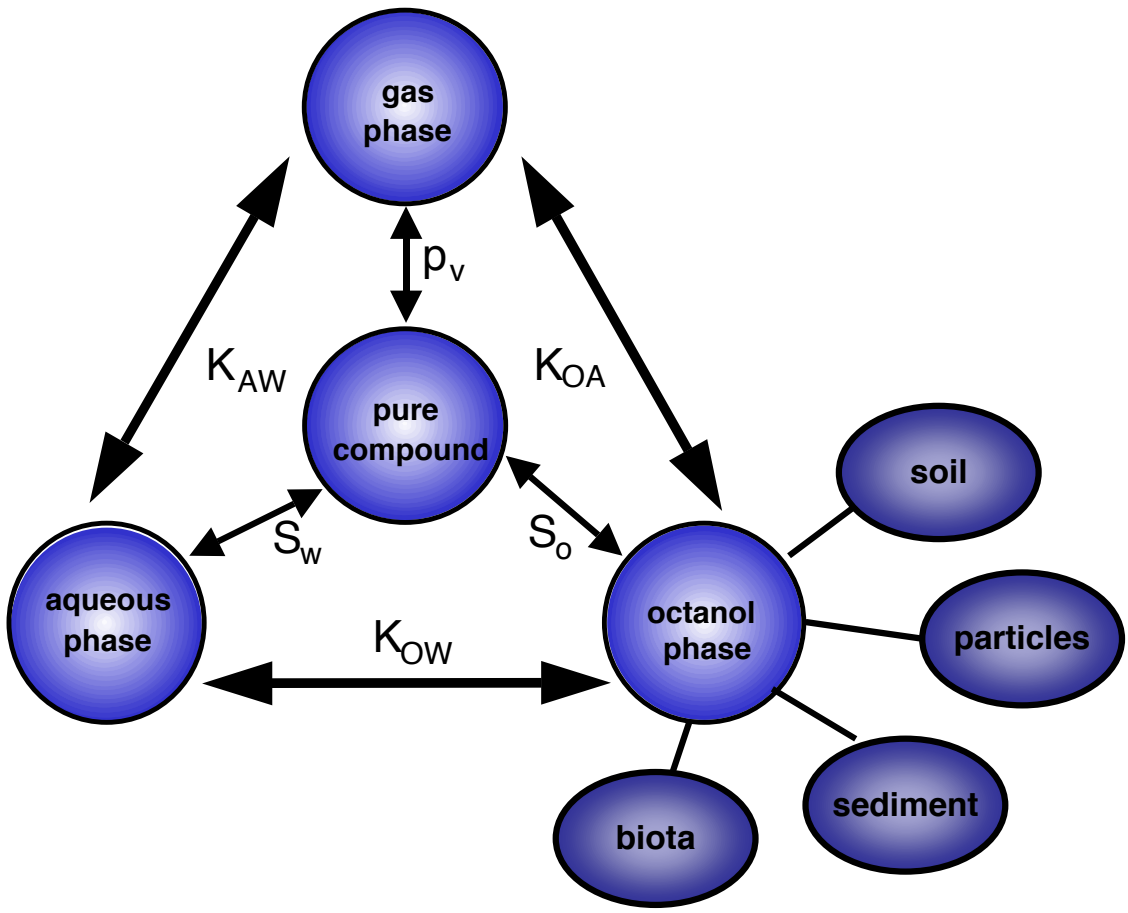



**Figure 6.2. Phase distribution equilibria involving pure phase partition coefficients (water solubility $S_W$, solubility in octanol $S_O$ and vapor pressure $p_V$) showing the links for describing environmental phase partitioning ($K_{AW}$ air/water partition coefficient, $K_{OW}$ octanol/water partition coefficient, $K_{OA}$ octanol/air partition coefficient). The octanol phase is used for soil, sediment, particles and biota).**

Figure 6.2 shows that the minimum data requirements for describing phase partitioning of non-polar organic substances are:

- Any two of the three partition coefficients $K_{AW}$, $K_{OW}$, and $K_{OA}$. The third can be estimated from the other two or:

- Any three of the six properties listed in the above figure as long as they do not form a triangle. (For example, if vapor pressure, water solubility and log $K_{OW}$ are known, the remaining partition coefficients can be deduced from these. However, if only vapor pressure, water solubility and log $K_{AW}$ are known, partitioning into organic solids cannot be established).

The approach outlined above for the non-polar substances may also be applicable to polar substances. Compounds that ionize in the aqueous phase can be treated as non-electrolytes by multiplying the partition coefficients $K_{AW}$ and $K_{OW}$ for the neutral species by the fraction that is undissociated, which in turn can be calculated from the dissociation constant of the compound pKA and the pH of ambient water. This approach assumes that the dissociated form does not volatilize and does not sorb to solids. This approach neglects, however, that the dissociated form might associate with the non-organic part of soil materials such as minerals. For many other compound classes, such as metals, surface active substances, extremely water insoluble or involatile substances, polymers, or biological macromolecules the environmental phase distribution can not be estimated using the octanol-based approach outlined for non-ionizing chemicals. For example, their sorption to solids is not necessarily dominated by simple phase partitioning into the organic fraction, but other mechanisms, such as specific surface sorption may become important. It may still be possible to describe them with the help of multimedia models, if the relevant environmental phase distributions can be quantified directly or if different estimation methods exist that allow the estimation of these phase distributions. However, it should be noted that multimedia models are meant to describe the environmental fate of chemicals that distribute into more than one environmental phase. For chemicals that occur predominantly in one medium of the environment, multimedia models help guide us to which components of the environment require the most detail in making an impact assessment. For example, a multimedia model will show that there we do not gain insight when estimating a characteristic atmospheric travel distance for a polymer or a biological macromolecule.

Reactivity information (i.e. degradation rates) for the various environmental compartments is required to carry out a multimedia assessment. The reaction rates in the various media can either be measured or estimated. Experimentally obtained rate data are preferred over estimated data. Accurate degradation rates are only needed for those compartments in which a significant part of the total chemical mass in the system resides. Because the question of how much of a chemical can be found in which environmental compartment strongly depends on the partitioning properties of the chemical, one way to estimate the presence of a chemical in the different compartments is based on its partition coefficients. It has been suggested that for compartments with less than 5% of the chemical mass present, as estimated from partitioning coefficients, a rough estimate of the degradation rate is sufficient (Woodfine et al. 2001).

## 6.3. Chemical Composition and Multimedia Transport Properties

The fate and transport of a fuel and its component chemicals in the environment depend on the properties of these constituent chemicals. The most important properties for established partition and transport in water and air phases are water solubility, vapor pressure, $K_{OW}$ and distributions coefficients. Component partitioning depends in turn on source product infiltration, redistribution, and lens formation on water tables in the subsurface. These multiphase transport processes are governed by viscosities, densities, and interfacial tensions of the aqueous and pure product phases. These properties may also depend on dissolved compounds. Both diesel and

biodiesel can contain tens and even hundreds of different compounds, each with different properties affecting their multimedia partitioning, mobility, and persistence.

### 6.3.1. Chemical Makeup of Biodiesel and Diesel

Unless we are considering B100, biodiesel fuels will consist of a mixture of standard diesel and biodiesel. Petroleum-based diesel fuel oils are mixtures of aliphatic (open chain and cyclic compounds that are similar to open chain compounds) and aromatic (benzene and compounds similar to benzene) petroleum hydrocarbons. In addition, they may contain small amounts of nitrogen, sulfur, and other elements as additives. The exact chemical composition (i.e., precise percentage of each constituent) of any particular diesel oil type can vary somewhat, depending on the petroleum source and other factors. Petroleum-based diesel fuels are distinguished from each other primarily by their boiling point ranges, and chemical additives. Bio-based diesel fuel consist of short chain alkyl (methyl or ethyl) esters along with performance and stability additives along with some aromatic hydrocarbons. Table 6.1 provides a summary of chemical properties data ranges for constituents of petroleum-based diesel (ATSDR, 1993). This table also illustrates the types of data that must be obtained for the ethyl and methyl esters and additives in different biodiesel formulations.

**Table 6.1 Physical and Chemical Properties of Fuel Oils (ATSDR, 1995)**

| Characteristic | Fuel oil no. 1 | Fuel oil no. 1-D | Fuel oil no. 2 | Fuel oil no. 2-D |
|---|---|---|---|---|
| Molecular weight | No data | No data | No data | No data |
| Color | Pale yellow[b]; Colorless to brown[c,d] | Colorless to brown[e] | Colorless to brown[e] | Colorless to brown[e] |
| Physical state | Liquid[e] | Liquid[e] | Liquid[e] | Liquid[e] |
| Melting point | -45.6°C[d] | -34°C[d] | -29°C[d] | 18°C[d] |
| Boiling point | 175–325°C[b]; 200–260°C[d] | 193–293°C[d] | 160–360°C[f] 282–338°C[d] | 282–338°C[d] |
| Density: | | | | |
| At 15°C | 0.810–0.9360 g/mL[c] | 0.810–0.9360 g/mL[c] | No data | No data |
| At 20°C | 0.80 g/mL[e,f] | No data | 0.8700–0.9500[e] | 0.8700–0.9500 g/mL[c] |
| Odor | Kerosene-like[e] | Kerosene-like[e] | Kerosene-like[e] | Kerosene-like[e] |
| Odor threshold (ppm) | 0.082[f]; 1[d] | 0.7[d] | No data | No data |
| Solubility | | | | |
| Water at 20°C | ≈5 mg/L[e] | ≈5 mg/L[e] | ≈5 mg/L[e] | ≈5 mg/L[e] |
| Organic solvent(s) | Miscible with other petroleum solvents[b] | No data | No data | No data |
| Partition coefficients: | | | | |
| Log $K_{ow}$ | 3.3–7.06[e] | 3.3–7.06[e] | 3.3–7.06[e] | 3.3–7.06[e] |
| Log $K_{oc}$ | 3.0–6.7[e] | 3.0–6.7[e] | 3.0–5.7[e] | 3.0–6.7[e] |
| Vapor pressure at 21°C | 2.12–26.4 mmHg[e] | 2.12–26.4 mmHg[e] | 2.12–26.4 mmHg[e] | 2.12–26.4 mmHg[e] |
| Henry's law constant at 20°C - atm-m$^3$/mol | 5.9 x $10^{-5}$–7.4[e] | 5.9 x $10^{-5}$–7.4[e] | 5.9 x $10^{-5}$–7.4[e] | 5.9 x $10^{-5}$–7.4[e] |

### 6.3.2. Solubility in Water

The solubility of biodiesel in water is an important characteristic as it can affect fuel quality and the environment (in the event of a fuel spill). Because biodiesel is a mixture of several chemicals, the solubility of biodiesel in water is compound specific, and will involve partitioning between multiple phases. One can also express an average solubility for biodiesel in water in terms of its

ability to absorb moisture. Due to biodiesel's unique oxygen-containing polar chemical structure of carboxyl groups, it is on average 15 to 25 times more soluble in water than diesel (He et al., 2007). Biodiesel absorbs 1,000 to 1,700 ppm (0.10 to 0.17%) moisture at temperatures of $4^o$ to $35^oC$. Tests have determined that the moisture content of biodiesel is not dependent on feedstock but is affected by temperature. As the temperature increases, "the moisture content increases at a rate of 22.2 ppm/$^oC$ which is more than 9 times higher than that of D-2 diesel" (He et al., 2007). The moisture absorbance is also affected by the level of blending (biodiesel/ D-2 diesel). Blending "creates a mixture with a lower capacity for moisture absorption." As can be seen in Figure 6.3, increasing concentrations of biodiesel within a mixture result in a greater level of moisture content.

**Figure 6.3. Statistical significance of temperature and level of blending on moisture content (MC)*.**

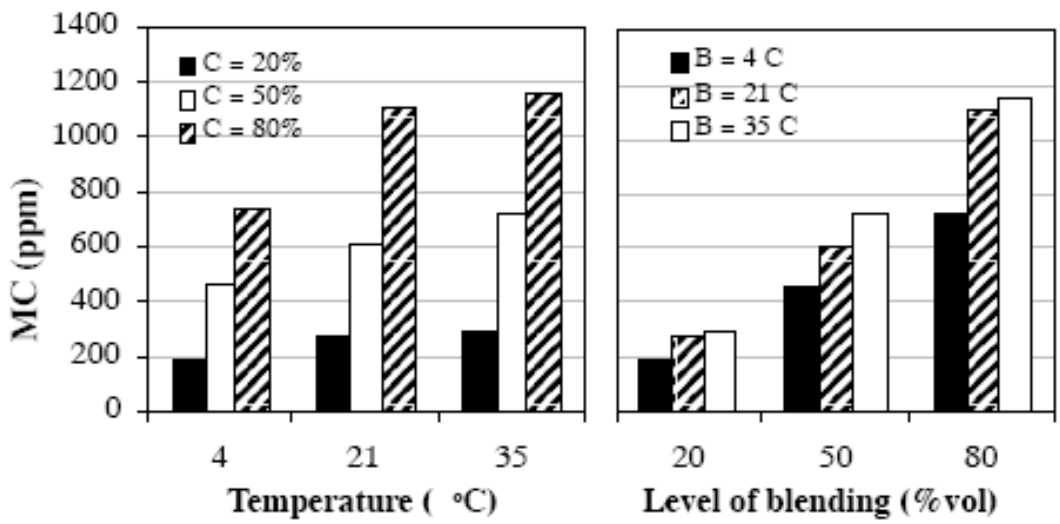



*Figure from He et al. (2007)

The high moisture content of biodiesel can cause problems because it makes the fuel susceptible to water accumulation and microbial growth. This can result in fuel deterioration during fuel handling, storage and transportation. In the event of a fuel spill, biodiesel will diffuse within natural water bodies (e.g., lakes, rivers and groundwater aquifers) faster on the average than diesel. This type of environmental dispersion will be a challenge to evaluate because it is ultimately compound specific and involves partitioning between multiple phases. This behavior will be problematic for cleaning up spills because it can be more difficult to remove the fuel from these water bodies.

### 6.3.3. Sorption to Solids

There are no reported measurements of the $K_{OW}$ of biodiesel fuel. There are few measurements of the air-particle, water-soil, or water-sediment partition coefficients. Because biodiesel is a mixture of several chemicals it may be necessary to assess the partition properties of each of the individual components to make an accurate assessment of the multimedia behavior of the different biodiesel formulations. In many cases it may be necessary to obtain direct measurements of the effective distribution coefficient (water-solid partitioning factor $K_D$) in sediments, soils, and aquifers.

### 6.3.4. Vapor Pressure

There are no reported measurements of the vapor pressure for biodiesel fuels, however there are some available data on individual chemicals in biofuels. Similarly, there are also no measurements of the air-water partition coefficient $K_{AW}$ for biodiesel. However, for the diesel component of B20 and other mixtures that include petroleum-based diesel, there are vapor pressure and $K_{AW}$ data available for the petroleum-based diesel components. Because biodiesel is a mixture of several chemicals it will be necessary to assess the vapor pressure and $K_{AW}$ of each of the individual components to make an accurate assessment of the multimedia behavior of the different biodiesel formulations.

## 6.4. Biodegradation of Biodiesel Components

As biodiesel becomes increasingly commercialized, its potential for persistence in the environment is an area of growing concern. Since petroleum spills have contributed significantly to environmental contamination in the past, it is essential to examine biodiesel's potential for biodegradation. The long-term persistence of the fuel in freshwater and marine aquatic environments, or in porous media such as soil and sediment, may be harmful to biota in these ecosystems.

Biodegradation of hydrocarbons by microorganisms "represent[s] one of the primary mechanisms by which petroleum and diesel products are removed from the environment" (Stolz et al., 1995). The simple chemical structures of biodiesel constituents make it more readily degradable than diesel. Microorganisms can easily break down the straight carbon chain structures of biodiesel under aerobic conditions via the beta-oxidation pathway. "Fatty acids are oxidized at the  carbon and degrade to acetic acid and a fatty acid with two fewer carbons" (Zhang et. al., 1998).. Diesel, in comparison, is not as readily biodegradable because of its chemical complexity. "Many components of [the fuel] are recalcitrant to microbial degradation" (Stolz, et al., 1995). The complex structure of diesel contains a mixture of "aliphatic cyclic hydrocarbons, polycyclic aromatic hydrocarbons (PAHs), and alkylbenzenes, as well as their derivatives such as toluene, xylenes, PCBs (phenyl and biphenyls), and so on" (Zhang et. al., 1998). Hydrocarbons are less susceptible to microbial degradation when oxygen is not available as a terminal electron acceptor.

### 6.4.1. Biodegradability in Aquatic Environments

The biodegradation potential of biodiesel and various biodiesel/diesel blends was investigated in aerobic shaker flask systems by Zhang et. al. (1998) at the University of Idaho. Mineral media were amended with a mixed microbial inoculum from soil, activated sewage and raw sewage. Carbon dioxide evolution was measured and compared to measurements by gas chromatography of the disappearance of biodiesel components. Six biodiesel fuels including neat rapeseed oil (NR), neat soybean oil (NS), rapeseed methyl ester (RME), rapeseed ethyl ester (REE), soybean methyl ester (SME), and soybean ethyl ester (SEE) were tested and the results were compared to those of Phillips 2-D reference diesel. The most common biodiesel used was REE, a biodiesel that is produced using ethanol as a catalyst and rapeseed as feedstock. Negligible differences between feedstocks were detected through statistical analysis, as they all degraded between 85.54 – 88.49% in 28 days. This percentage degradation is particularly high compared to diesel's 26.24% degradation (Zhang et al., 1998). The biodegradation rate of biodiesel was equivalent to that of dextrose (sugar).

Biodiesel can promote and accelerate the biodegradation of diesel through a process called cometabolism. Zhang et al. (1998) determined that diesel in a mixture degraded three times faster (56%) than diesel alone (16%) under aerobic conditions (as shown in Table 6.2). This "suggests that in the presence of REE, microorganisms use the fatty acids as an energy source to promote the degradation of diesel" (Zhang et. al., 1998).

**Table 6.2. Biodegradation of fuel in biodiesel mixture and diesel alone*.**

| Days | Diesel in mixture | Diesel Alone |
|---|---|---|
| 0 | 0 | 0 |
| 1 | 56.49% | 16.27% |
| 4 | 94.79% | 53.54% |

*Data from Zhang et al. (1998).

Differences in degradation patterns were also noted between pure biodiesel and biodiesel/diesel blends. After one day, REE 100 degraded 61.81% while in the same period of time a biodiesel/diesel mixture degraded 56.4%. This relatively small difference in degradation suggest that "microorganisms attacked the fatty acids in REE and alkane chains in the diesel at the same time and at the same rates instead of favoring the fatty acids only" (Zhang et. al., 1998).

Since microorganisms metabolize biodiesel and diesel at roughly the same rates, the more biodiesel present in a biodiesel/diesel mixture, the greater the rate of degradation. This can be seen in Table 6.3 below.

**Table 6.3. Percent degradation in different rapeseed ethyl ester (REE)/diesel mixtures*.**

| days | $CO_2$ evolution (%) | | | | |
|---|---|---|---|---|---|
| | REE100 | R80/d20 | R50/d50 | R20/d80 | d100 |
| 0 | 0% | 0% | 0% | 0% | 0% |
| 7 | 64.09% | 52.33% | 37.85% | 25.24% | 12.08% |
| 14 | 77.51% | 61.26% | 45.74% | 31.59% | 14.96% |
| 28 | 84.37% | 67.82% | 51.90% | 35.67% | 18.18% |

*Table from Zhang et al. (1998).

Because the presence of vegetable fatty acids increases the degradation rate of diesel, new biosolvents, based on vegetable methyl esters (similar to biodiesel), can be used as cleaning agents after a diesel spill. The California Department of Fish and Game recently licensed *CytoSol Biosolvent* (an oil similar to biodiesel) "as a shoreline cleaning agent to extract crude oil from shorelines and marshes after a spill" (Wedel, 1999).

### 6.4.2. Biodegradation in Soil

Five biodiesel fuels were also tested via gas chromatography for biodegradability in soil samples. Information on the type of soil and its associated physical and chemical properties was not indicated. In 28 days, the biodiesel fuels degraded 83 to 95%, with an average of 88%, while diesel (Phillips 2-D low sulfur diesel) only degraded 52%.

Seed germination is also used as an indicator of biodegradability and itself is an important criterion to consider in soils contaminated by fuels. The University of Idaho's study included an investigation of the seed germination rates in soil treated with diesel and the five biodiesel samples. In soil exposed to diesel, it took seven days longer for seeds to germinate than in the biodiesel treatments. The germination rate for biodiesel was initially slow; however, in week 3, after the biodiesel began to degrade, the seed germination rate increased (Knothe et al., 2005). After the sixth week, the germination rates reached 92 to 98%. These results demonstrate that "biodegradation can restore a biodiesel fuel-contaminated soil in 4 to 6 weeks to such a degree that it can support plant germination" (Knothe et al., 2005).

### 6.4.3. Biodegradation Under Aerobic and Anaerobic Conditions

Stolz et al. (1995) from Duquesne University studied the biodegradability of soy biodiesel under aerobic and anaerobic conditions (adding nitrate as an electron acceptor) in microcosms simulating freshwater and soil environments. The biodiesel completely degraded within 7 days under aerobic conditions and 14 days under anaerobic conditions. (No distinctions between degradation within aquatic and soil environments were specified). The presence of oxygen was a significant contributing factor to the rapid aerobic biodegradation. Biodiesel degraded at a slower rate under anaerobic conditions using alternative terminal electron acceptors to oxygen, such as nitrate, iron (Fe(III)), or sulfate (Stolz et al., 1995).

Lapinskiene and Martinkus (2007) measured the anaerobic biodegradation of fats, biodiesel fuel and diesel fuel in flooded soil samples under an argon atmosphere. Propionic acid fermentation was the major pathway for biodegradation of biodiesel fuel and fats, whereas butyric acid fermentation was important in diesel fuel samples. After 60 days of incubation, 47.9% of diesel fuel, 81.2 % of, biodiesel fuel, 78.6% of sunflower oil and 80% of beef grease was degraded.

### 6.4.4. Biological and Chemical Oxygen Demand

Biological oxygen demand ($BOD_5$) is a standardized method that can be used as a means to estimate the biodegradability of a chemical. It serves as a "relative measure of the amount of organic matter subject to microbially mediated oxidative processes present in biodiesel fuel" (Knothe et al., 2005). Chemical oxygen demand (COD) is a measure of the total oxidizable organic matter present in a sample. A study conducted by the University of Idaho measured $BOD_5$ and COD of biodiesel (Knothe et al., 2005).

While the biological oxygen demand of the six biodiesel fuels (same as listed above) did vary considerably, the magnitude of differences among the fuels was less than 10%. The biodegradability of all six biodiesel fuels are considered to be equivalent, relative to the difference in BOD between diesel and the biodiesel fuels which was substantially different (average 122% difference). The low $BOD_5$ values for Phillips 2-D diesel "indicates the presence of a much smaller amount of microbial biodegradable organic matter" (Knothe et al., 2005). It may also reflect the toxicity of this diesel fuel to microbes (Figure 6.4).

Since COD is a measure of oxidizable organic matter, it was expected that there would not be a significant difference between COD values for biodiesel or diesel. This was in fact the case and the results can be seen in Figure 6.5.

**Figure 6.4. $BOD_5$ values for biodiesel and diesel*.**

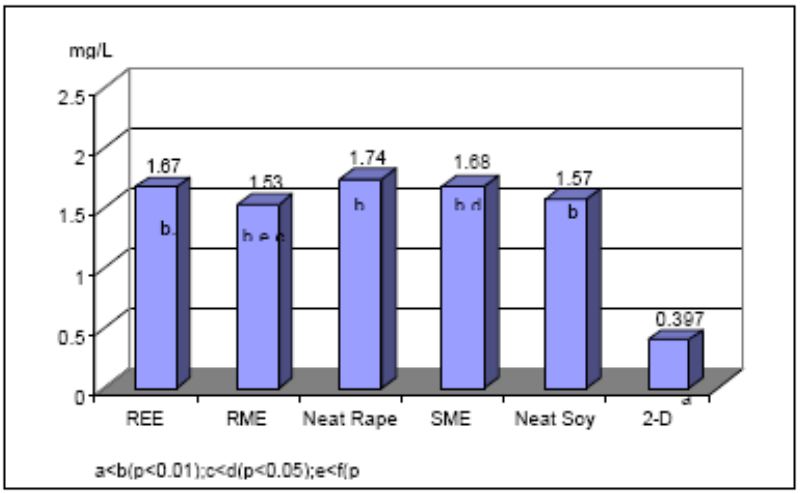



*Figure from Knothe et al. (2005)

**Figure 6.5. COD values for biodiesel and diesel*.**

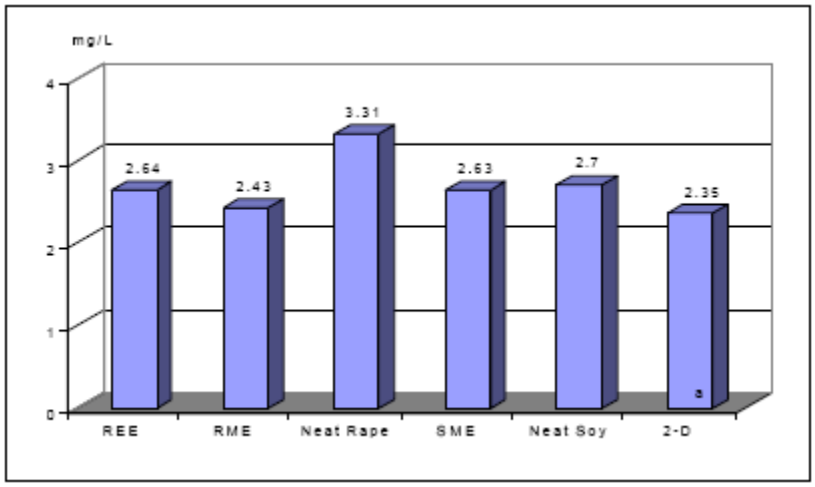



*Figure from Knothe et al. (2005).

# 7. Biodiesel Toxicity

## 7.1. Human and Ecological Risk Assessment

As with any new fuel formulation being introduced into California commerce in large quantities, we are concerned with the potential toxicity to humans and to the environment of biodiesel fuels. Estimating the toxicity requires that we follow a standard paradigm for risk assessment:

1. Hazard identification
2. Toxicity assessment
3. Evaluation of the potential for human and ecological exposure
4. Are there specific sensitive populations at risk of exposure to biodiesel fuel components?

The greatest difficulty we can anticipate with determining the human and ecological toxicity of biodiesel fuels is that biodiesel fuel is not a defined chemical formulation or a defined mixture of components, but can be formulated from of any of a very large number of feedstocks with different chemical components. It is beyond the scope of this study to attempt to consider all of these possibilities; we will analyze the toxicity of biodiesel formulations from one or two typical feedstocks in detail and then try to generalize when generalization is possible. Manufacture of biodiesel fuel may well occur, at least in part, in California, so we will have to consider potential releases of chemicals involved in synthesis and use of biodiesel during their transport and use, as well as their appropriate disposal, their combustion, and their degradation products. Extraction of oils from plants will generally require the use of organic solvents such as hexane, or saponification with alkaline methanol. Thus, we must consider potential adverse health effects and ecological damage related to release scenarios for organic solvents as well. Finally, there may be significant amounts of fuel additives added to biodiesel formulations; the toxicity of these compounds and their potential release products will also have to be considered. Significant routes of exposure that must be considered include oral, dermal, and inhalation. We anticipate that we will encounter significant data gaps at every stage of this process.

In general, tests show that pure biodiesel is considerably less toxic than pure diesel fuel. Formulations of mixed biodiesel-diesel fuel such as B20 or B50 have shown results consistent with the calculated diluent effect of the percentage of biodiesel fuel on the total toxicity of the mixture.

The evaluation of toxicity of various biodiesel blends is an ongoing research topic. Recent literature identifies particular aspects of comparative toxicity of ULSD vs. different biodiesel blends. Bunger et al. (2000) find that rapeseed-based methyl-ester biodiesel had a lower mutagenic potency than petroleum diesel and attributed this to lower emissions of polycyclic aromatic compounds. The authors also found a higher toxicity that was speculated to be due to increased carbonyl compounds and unburned fuel, that reduced the benefits of the lower emissions of solid particulate matter and mutagens from the rapeseed biodiesel. This is congruent with the identification of acrolein as an increased emittant with biodiesel fuels (Section 4.2.3).

In Liu et al. (2008), conventional diesel and palm oil methyl esters were blended in 6 ratios (0, 10, 30, 50, 75 and 100% of biodiesel by volume) and fed into an unmodified 4-stroke engine with a constant output power. The semi-volatile and particulate products in the exhaust were

collected separately and their biological toxicities evaluated by both Microtox test and the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The Microtox test indicates that the TUVs (toxicity unit per liter exhaust sampled, TU/L-exhaust) in the semi-volatile extracts were 3 to 5 times those of the particulate extracts. Diesel particulates had the highest unit toxicity, TUW (toxicity unit per g soluble organic fraction of particulate, TU/ g particle SOF) of all of the other biodiesel blends.

Brito et al. (2011) evaluated heartrate, heart rate variability, and blood pressure after 1 hour exposure to petroeluem and biodiesel exhaust. "B100 decreased the following emission parameters: mass, black carbon, metals, CO, polycyclic aromatic hydrocarbons, and volatile organic compounds compared with B50 and diesel; root mean square of successive differences in the heart beat interval increased with diesel ($p < 0.05$) compared with control; low frequency increased with diesel ($p < 0.01$) and B100 ($p < 0.05$) compared with control; HR increased with B100 ($p < 0.05$) compared with control; mean corpuscular volume increased with B100 compared with diesel ($p < 0.01$), B50, and control ($p < 0.001$); mean corpuscular hemoglobin concentration decreased with B100 compared with B50 ($p < 0.001$) and control ($p < 0.05$); leucocytes increased with B50 compared with diesel ($p < 0.05$); platelets increased with B100 compared with diesel and control ($p < 0.05$); reticulocytes increased with B50 compared with diesel, control ($p < 0.01$), and B100 ($p < 0.05$); metamyelocytes increased with B50 and B100 compared with diesel ($p < 0.05$); neutrophils increased with diesel and B50 compared with control ($p < 0.05$); and macrophages increased with diesel ($p < 0.01$), B50, and B100 ($p < 0.05$) compared with control. Biodiesel was more toxic than diesel because it promoted cardiovascular alterations as well as pulmonary and systemic inflammation."

Tsai et al. (2011) evaluated the toxicity of Soy B-20 relative to that of petroleum biodiesel. The authors found that Soy B-20 effectively reduced the emissions of PAHs; furthermore, the unit mass cytotoxicity of ultrafine particles and nano-particles in the emissions was also lowered (by an average of 52.6%). The authors conclude that soybean biodiesel (S20) can be used as an alternative fuel to petroleum diesel to reduce the hazards of emissions from diesel engines to human health.

Song et al. (2011) examines elemental carbon (EC), organic carbon (OC), and polycyclic aromatic hydrocarbons (PAHs) emissions from cottonseed oil biodiesel (CSO-B100). "Relative to normal diesel fuel, CSO-B100 reduced EC emissions by 64% (+/-16%). The bulk of EC emitted from CSO-B100 was in the fine particle mode (<1.4 pm), which is similar to normal diesel. OC was found in all size ranges, whereas emissions of OC(1.4-2.5) were proportionately higher in OC(2.5) from CSO-B100 than from diesel. The CSO-B100 emission factors derived from this study are significantly lower, even without aftertreatment, than the China-4 emission standards established in Beijing and Euro-IV diesel engine standards. The toxic equivalency factors (TEFs) for CSO-B100 was half the TEFs of diesel, which suggests that PAHs emitted from CSO-B100 may be less toxic."

## 7.2. Acute Oral and Acute Dermal Toxicity

Acute oral toxicity tests were conducted on albino rats and acute dermal toxicity tests were conducted on albino rabbits at the WIL Research Laboratories in Ashland, Ohio. Samples of biodiesel, biodiesel/diesel blends, and diesel (100% RME, 100% REE, 50% RME/50% 2-D, 50% REE/50% 2-D, 20% RME/80% 2-D, 20% REE/80% 2-D, and 100% 2-D) were administered (once) to rats via gastric intubation. No deaths were reported; however, the

"occurrences of clinical observations increased as the ratio of diesel fuel increased" (Knothe et al., 2005). The LD50 of each test substance was greater than 5 g/kg.

Biodiesel and diesel (100% RME, 100% REE, and 100% 2-D) were administered (once) dermally at a dose of 2 g/kg to the clipped, intact skin of albino rats. The rats were monitored for mortality, clinical observations, dermal findings, body weights and gross necropsy findings. It was reported that the 2 g/kg dose was a "No Observable Effect Level (NOEL) for systemic toxicity [via the dermal exposure route] under the conditions of this study for the three fuels tested" (Knothe et al., 2005). It was also noted that 100% REE was the least severe skin toxicant in the acute dermal toxicity study.

## 7.3. Toxic Air Pollutants and Human Health

The PM and HC emissions from diesel fuel combustion are suspected of causing cancer and other life threatening illnesses in exposed populations. The State of California and the U.S. EPA have identified diesel PM as a toxic compound. The use of biodiesel fuel has been reported to reduce total emissions of PM and associated toxics (Morris and Jia, 2003b). A review of the broader literature suggests that reduced emissions of PM10, PAHs, and nitro-PAHs can be anticipated with biodiesel formulations in comparison to diesel fuels (see section 4.4). However, as noted in section 4.4, the wide variety of oils and fats that might be used to make biodiesel fuel, makes the actual emissions of PM and toxic air contaminants uncertain. Moreover, the toxic profiles of the altered emissions also remain uncertain. Given this issue of different formulations of biodiesel probably having different emissions and toxicity profiles, it is not appropriate to accept any common wisdom or popular literature that suggests that biodiesel fuel has been thoroughly tested by the EPA and is "safe". Such issues can only be resolved with continued emissions and toxicity testing.

A number of recent studies have addressed some of the potential health impacts of emissions from biodiesel combustion. Bünger et al. (2000a, 2000b) compared the toxicity and mutagenicity of diesel exhaust particles (DEP) from standard diesel fuel (DF) with low-sulfur diesel fuel (LS-DF) rapeseed methyl esters (RME), and soybean methyl esters (SME). They found lower mutagenic potency of DEP from LS-DF, RME, and SME compared to DEP from DF but a higher toxicity from RME relative to DF. They estimate that the higher mutagenicity of DEP from DF is probably due to lower emissions of polycyclic aromatic compounds and that the higher toxicity of RME is probably caused by carbonyl compounds and unburned fuel. Finch et al. (2002) have measured the effects of subchronic inhalation exposure of rats to emissions from a diesel engine burning biodiesel fuel derived from soybean oil and found statistically significant, but minor and not consistently exposure-related, differences in body weight, nonpulmonary organ weights, serum chemistry, and glial fibrillary acidic protein in the brain. They found no significant exposure-related effects on survival, clinical signs, feed consumption, ocular toxicity, hematology, neurohistology, micronuclei in bone marrow, sister chromatid exchanges in peripheral blood lymphocytes, fertility, reproductive toxicity, or teratology. Finch et al. (2002) concluded that there are modest adverse effects at the highest exposure level, and none other than the expected physiological macrophage response to repeated particle exposure at the intermediate level. Ackland et al. (2007) compared the cellular effects of biodiesel emissions particulate matter (BDEP) and petroleum diesel emissions particulate matter (PDEP) using a human airway cell line. They found a lower induction of multinucleate cells for BDEP. Swanson et al., (2007) report that although the use of biodiesel fuel is favorably viewed, and there are suggestions that its exhaust emissions are less likely to present any risk to human health relative

to petroleum diesel emissions, the speculative nature of a reduction in health effects based on chemical composition of biodiesel exhaust needs to be followed up with more investigations in biologic systems.

According to the multiple air toxics exposure study (MATES-II) (SCAQMD, 2000), there are six compounds among currently identified toxic air contaminants (TACs) (diesel PM, four organic TACs—benzene, 1,3-butadiene, formaldehyde, and acetaldehyde—and hexavalent chromium) that account for over 90 percent of the risk associated with exposure to TACs in the South Coast Air Basin (SoCAB). Based on unit risk factors for these six compounds obtained from the Cal-EPA Office of Environmental Health Hazard Assessment's (OEHHA's) input to the toxic air contaminants identification process, Morris et al. (2003b) compared these TACs with B20 diesel particles by estimating the concentration leading to a one in a million risk of premature death due to long-term exposure. These concentrations are as follows:

- Benzene: $2.9 \times 10^{-5}$ $(\mu g/m^3)^{-1}$
- 1,3-Butadiene: $1.7 \times 10^{-4}$ $(\mu g/m^3)^{-1}$
- Acetaldehyde: $2.7 \times 10^{-6}$ $(\mu g/m^3)^{-1}$
- Formaldehyde: $6.0 \times 10^{-6}$ $(\mu g/m^3)^{-1}$
- Standard Diesel Particles: $3.0 \times 10^{-4}$ $(\mu g/m^3)^{-1}$
- B20 Diesel Particles: $2.85 \times 10^{-4}$ $(\mu g/m^3)^{-1}$

Based on the unit risk factors for these compounds, Morris et al. (2003b) estimated the unit risk factor if B20 diesel as being 0.95 that of standard diesel. They then estimated that the use of B20 fuel for the 50% and 100% HDDV fleet penetration scenarios would reduce the estimated risk of premature death due to air toxics in the SoCAB by approximately 2% and 5%, respectively.

Emissions of unregulated hazardous air pollutants (toxics) were investigated in an EPA study, "A Comprehensive Analysis of Biodiesel Impacts on Exhaust Emissions." The study focused on 11 mobile source air toxics that are significant contributors to toxic emission inventories. However, due to the limited nature of the data, the EPA considered the "conclusions regarding the effects of biodiesel on toxics as preliminary and only potentially indicative of the true effects" (EPA, 2002).

The Cal-EPA recognizes that risk ranking for biodiesel relative to other fuels requires consideration of toxic air pollutants that may be emitted from biodiesel beyond the six risk drivers listed above. In order to address this, a broader set of toxic air pollutants are considered here. Among the potential toxic air pollutants that should be considered in biodiesel risk comparison are polycyclic aromatic compounds (PAHs) and nitro-PAHs.

Semi-volatile organic compounds (SVOCs) such as PAHs are released to urban air from motor vehicles and from other combustion sources. In addition, these compounds may derive a large amount of their exposure from non-inhalation pathways, particularly ingestion through food. In a risk-ranking analysis for toxic air pollutants in indoor air, outdoor air and food, Loh (2005) estimated risks for a subset of key SVOCs based on total personal exposure. She divided this set of PAHs into two groups based on the EPA's weight-of-evidence classification for carcinogenic effects and the amount of available data from field studies. The first group includes benzo[a]anthracene, benzo[b]fluoranthene, benzo[a]pyrene, chrysene, dibenzo[a,h]anthracene, and indeno[1,2,3-cd]pyrene—compounds that are B2 carcinogens meaning there is sufficient animal evidence of carcinogenicity, but inadequate human evidence. The second group includes anthracene, benzo[g,h,i]perylene, phenanthrene, pyrene, fluoranthene, and naphthalene—

compounds that are classified as C or D carcinogens with limited information on potential carcinogenicity. Loh (2005) also provided toxic equivalency factors (TEFs) obtained from Cal-EPA and other sources. The TEF expresses the cancer potency of a given PAH relative to benzo[a]pyrene, which has a unit risk factor of 1.1 x $10^{-3}$ $(\mu g/m^3)^{-1}$ (ARB-OEHHA), 1994).

| Group 1 compounds and TEF | |
|---|---|
| benzo[a] anthracene | 0.1 |
| benzo[b] fluoranthene | 0.1 |
| benzo[a] pyrene | 1 |
| chrysene/ iso-chrysene | 0.001 |
| dibenz[a,h] anthracene | 1 |
| indeno (1,2,3-cd) pyrene | 0.1 |

| Group 2 compounds and TEF | |
|---|---|
| anthracene | 0.0005 |
| benzo[ghi] perylene | 0.02 |
| phenanthrene | 0.0005 |
| pyrene | 0.001 |
| fluoranthene | 0.05 |
| naphthalene | 0.03 |

In general, the effects of biodiesel on toxic air pollutants appear to be favorable. But the current absence of industry standards for feedstocks, fuel formulation, and additives makes it problematic to assess the potential toxicity of biodiesel as a generic fuel. As a prerequisite to Tier II, it will be necessary to provide the criteria used to select and define biodiesel formulations to be studied in depth.

## 7.4. Previous Toxicity Testing of Biodiesel Fuel Exhaust Emissions

A comprehensive toxicity study of the exhaust from a biodiesel fuel formulation, consisting of a 13-week subchronic inhalation study in F344 rats and additional tests, was reported by The Lovelace Respiratory Research Institute (LRRI, 2000). This report states that

> "No effects of biodiesel-exhaust-emission exposure were observed in a variety of endpoints including mortality, toxicity as revealed by detailed clinical observations, feed consumption, toxicity to the eyes, neurohistopathology, formation of micronuclei (MN) in bone marrow cells, sister chromatid exchanges (SCEs), fertility, reproductive toxicity, and teratology. Endpoints in which effects were caused by biodiesel-exhaust-emission exposure, with minor changes not deemed as biologically significant, included group mean body weights, non-pulmonary organ weights at necropsy, clinical chemistry, and glial fibrillary acidic protein (GFAP) in the brain. Weak mutagenicity in a bacterial mutagenicity assay was observed from extracts of both particulate and semi-volatile fractions of biodiesel-exhaust-emission fractions. Relative to total body weights, lung weights were increased in female rats in the high-level group compared to controls (0.52 vs. 0.49% of total body weight), and histopathological evaluation of a number of tissues revealed exposure-induced changes only in the lungs. Findings included the presence of particles in macrophages and macrophage hyperplasia; these findings were judged to be a normal physiologic response to exposure and

not a toxic reaction. Lesions included alveolar bronchiolarization, which was found only in rats in the high-level group, and alveolar histiocytosis, which was found in three of the four groups, but at slightly higher incidence in the high-level group. Based on these results, rats were adversely affected by exposure to high-level biodiesel exhaust emissions, the effect was greater in female rats than in males, and the no-adverse-effect-level for this study of inhaled biodiesel exhaust emissions was the intermediate level."

It should be noted that the exhaust from the biodiesel fuel tested was potentially harmful at high doses, and therefore that some testing of exhaust emissions will be required for different formulations of biodiesel fuel. At lower doses, hazard tests used to assess biofuels impact should be expanded, where feasible, to include endpoints that may presage asthma or other adverse effects on lung function--for example hyperreactive airways or changes in forced expiratory volume (FEV).

## 7.5. Aquatic Toxicity

Tests used to measure toxicity include measurements of LC50 (median lethal concentration), EC50 (median effective concentration), and IC50 (inhibition concentration). Comparative tests of all the fuel samples conducted for marine and freshwater conditions will also be used to determine the relative no observed adverse effects concentrations (NOAECs).

The University of Idaho exposed *Daphnia magna* to biodiesel fuels (and to the reference 2-D diesel) for 48 hours in static and flow-through environments. The mortality rates were measured at 24 and 48 hours and LC50s were calculated. In both static and flow through tests, "the rapeseed based fuels, REE and RME, displayed the highest EC50 values, signifying them to be less toxic than the other substances" (Knothe et al., 2005). Interestingly, soybean methyl esters (SME) were significantly more toxic than rapeseed biodiesel; however, the reasons for the differences are unknown. The biodiesel/diesel blends (REE 20/2-D 80 and REE 50/2-D 50) followed similar trends: the blend with the highest biodiesel percentage displayed characteristics similar to pure biodiesel. The blend with the least biodiesel (REE 20/2-D 80) produced results similar to diesel. Under static conditions, REE 20/2-D 80 was determined to be more toxic than 2-D diesel. These results can be seen below in Figure 7.1 and Figure 7.2.

The phytotoxicity of biodiesel oil was also studied at the Centre for Aquatic Plant Management. Birchall et al. (1995) examined the "comparative toxicity of diesel and biodiesel fuels to a range of aquatic species at doses which might result from spillages from boats." Single-species toxicity tests were used to assess the toxicity of biodiesel to algae, macrophytes and invertebrates.

Growth of all species of algae tested was reduced due to the impact of both diesel and biodiesel fuels. However, biodiesel was determined to be considerably less toxic than diesel to a diverse range of freshwater algal species at all doses. Macrophytes, such as *Myriophyllum spicatum* (water milfoil) and *Elodea canadensis* (Canadian pondweed), were also more susceptible to diesel. However, the floating plant, *Lemna minor* (duckweed) was equally affected by diesel and biodiesel; its growth rate was reduced 65% at the highest doses of both fuels. The growth of *Lemna minuta* was reduced by 60% from the highest dosage of biodiesel and the plant was killed by the same dosage of diesel.

**Figure 7.1. 48 hour static, non-renewal, *Daphnia magna* $EC_{50}$*.**

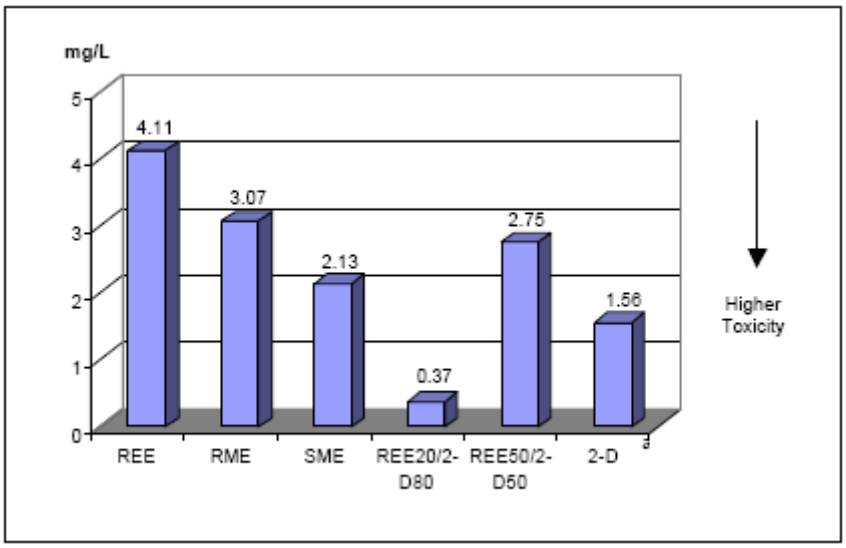



*Figure from Knothe et al. (2005).

**Figure 7.2. 48 hour flow-through, *Daphnia magna* $EC_{50}$*.**

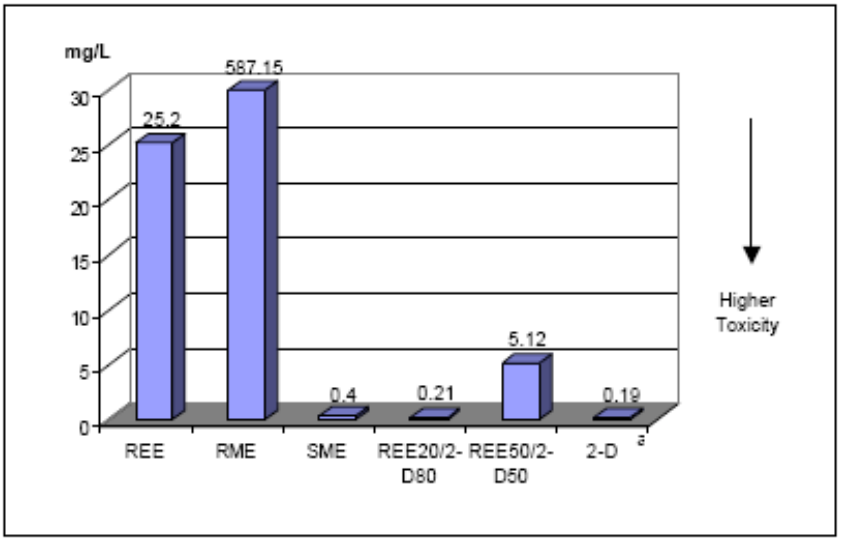



*Figure from Knothe et al. (2005).

The invertebrates tested were highly sensitive to diesel as they were "killed relatively quickly at all doses." *Daphnia magna* (water flea) and *Lymnea peregra* (water snail) were more tolerant to biodiesel with severe effects exhibited only at high doses. *Gammarus pulex* (water louse) was more sensitive with a mortality rate at relatively low biodiesel doses. Toxicity tests conducted on rainbow trout indicated that rainbow trout were more susceptible to diesel than biodiesel based on observations that the fish exposed to diesel exhibited greater weight loss, "more severe behavioral symptoms, loss of balance, muscular spasms and erratic fish and gill movements" (Birchall et al., 1995).

An indirect effect of biodiesel, through its behavior at the water surface, was also noted by Birchall et al. (1995). Biodiesel forms discrete globules on the water surface that "result in less interference with oxygen diffusion into the water, and with surface breathing or moving invertebrates" than would continuous distribution of the material. This globular distribution of the oil is much less harmful than the uniform "slicks" of diesel that cover the water's surface. The globules also aid in degradation as they allow biodiesel to "enter the water body more quickly than diesel."

While biodiesel is significantly less toxic than petroleum diesel, it can still cause harm to the environment in the event of a spill. Birds, mammals and fish can become coated with the oil, causing hypothermia, illness, or even death (Wedel, 1999). Biodiesel can also indirectly harm aquatic life as it can deplete oxygen during biodegradation.

## 7.6. Toxicity in Aerated Soil

Based on the results of a study conducted by Lapinskiene et. al. (2005), biodiesel is less toxic than diesel to soil microbial communities. The toxicity was evaluated by measuring the respiration of microorganisms and the activity of dehydrogenases in soil over a period of six days. Five concentrations (1, 3, 6, 9 and 12%) of diesel and biodiesel fuels were evaluated in the soil. The maximum material concentration of "12% was chosen because previous research established that at 24% of soil moisture, which corresponds to 60% of the soil's full water retention capacity, the maximum retention of diesel fuels and biodiesel fuels was 12% for both fuels" (Lapinskiene et al., 2005). Results of both assays indicated that "biodiesel fuel is non toxic at concentrations up to 12% (by weight) whereas that diesel exhibits toxic properties at concentrations higher than 3% (by weight)" (Lapinskiene et al., 2005).

# 8. Biodiesel Life Cycle Impacts

## 8.1. Life Cycle Assessment

The purpose of the life-cycle assessment (LCA) is to quantify and compare environmental flows resources and pollutants (to and from the environment) associated with both biodiesel and petroleum-based diesel, over the entire life cycle of the product. The flows of resources and pollutants provide a framework for assessing human health, environmental systems and resource impacts. LCA evaluates a broad range of requirements and impacts for technologies, industrial processes and products in order to determine their propensity to consume natural resources or generate pollution. The term "life cycle" refers to the need to include all stages of a process—raw material extraction, manufacturing, distribution, use and disposal including all intervening transportation steps—so as to provide a balanced and objective assessment of alternatives. An LCA includes three types of activities: (1) collecting life cycle inventory data on materials and energy flows and processes; (2) conducting a life-cycle impact assessment (LCIA) that provides characterization factors to compare the impacts of different product components; and (3) life-cycle management, which is the integration of all this information into a form that supports decision-making. A comprehensive LCA for biodiesel must address cumulative impacts to human health and the environment from all stages, impacts from alternative materials, and impacts from obtaining feedstocks and raw materials (Sheehan et al., 1998; Venturi, et al., 2003; Bemesson, et al., 2004; Nilsson and Hanson, 2004; Janulis,.2004). Figure 8.1 illustrates our approach for biodiesel LCA.

The focus of the Tier-II and Tier-III efforts is on the direct health and environmental impacts associated with pollutant emissions from biodiesel production and use. There are many other life-cycle issues that are of interest—including green-house-gas (GHG) emissions, water use, energy balance, land conversion, and competing uses for food crops. These are outside of the scope of this effort and are being addressed in great detail by other California programs—particularly the low-carbon fuel standard program. A list of reports currently available from this program is available at:
http://www.energy.ca.gov/low_carbon_fuel_standard/

The life-cycle of biodiesel fuels include the following stages

- Biomass production and preparation (for biodiesel derived from plant biomass)
- Oil extraction processes (for biodiesel derived from plant biomass)
- Collection of recycled oils and greases
- Biodiesel production (preparation and esterification)
- Refining the final product, B10, B20, B100 etc.
- Transportation, storage and distribution of biodiesel product
- End-use combustion

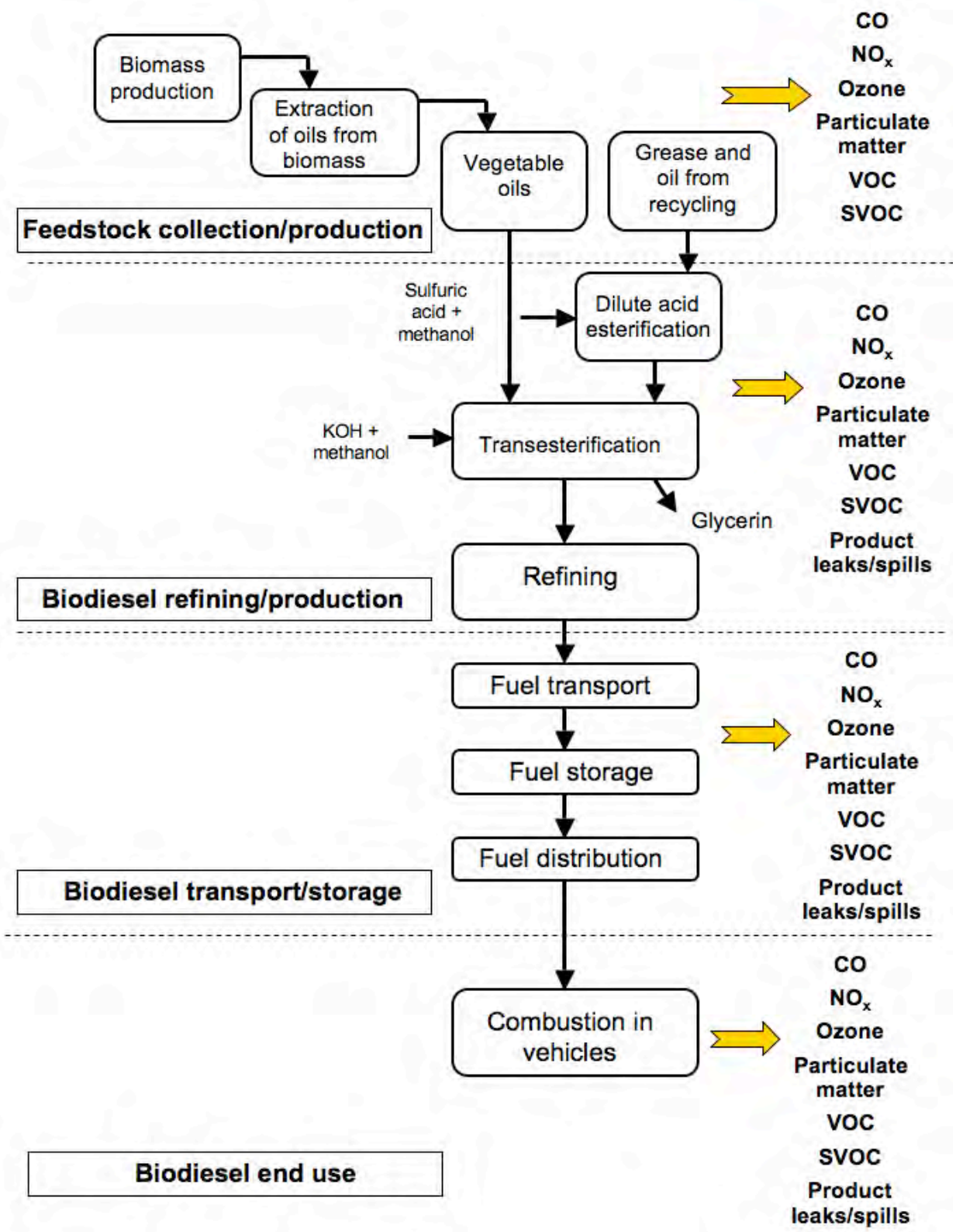



**Figure 8.1. An illustration of life stages and life-cycle impacts for biodiesel fuels.**

For each of these stages we must address emissions to the environment for the following pollutant categories

- Carbon monoxide (CO)
- Nitrogen oxides ($NO_x$)
- Ozone
- Particulate matter
- Volatile organic compounds (VOCs) such as benzene, formaldehyde, etc.
- Semi-volatile organic compounds (SVOCs) such as polycyclic aromatic hydrocarbons
- Metals
- Fuel product leaks and spills
- Hazardous wastes

In 1998, the U.S. Department of Agriculture and the U.S. Department of Energy sponsored a study on the life cycle of biodiesel and diesel fuels. life cycle inventories (LCIs) of both fuels allow a side-by-side comparison (USDA and USDOE, 1998). The biodiesel used in this case study was produced from soybeans and the diesel was characterized as "on-highway" low-sulfur diesel made from crude oil. The life cycles of both fuels were compared with the same "functional unit" that is based on the work (brake-horsepower hour (bhp-h)) the fuel provided to a bus engine.

In this study the LCIs quantified the total energy demands and the overall energy efficiencies of the process and products. Two types of energy efficiencies were determined: the fossil energy ratio and the life cycle energy ratio. The fossil energy ratio determines the degree to which a fuel is renewable. It is the ratio of the final fuel product energy to the amount of fossil energy required to make the fuel. While the life cycle energy is the ratio of fuel product energy to total primary energy.

The study determined that the life cycle energy demands of both fuels are essentially equivalent. The life cycle energy efficiencies of biodiesel and diesel are 80.55% and 83.28%, respectively. Lower biodiesel efficiency "reflect a slightly higher demand for process energy across the life cycle" (USDA and USDOE, 1998).

The production process of converting raw energy resources (petroleum or soybean oil) into fuels was almost equal in its efficiency for both fuels. However, since soy oil is biodiesel's largest raw resource and it is renewable, less fossil fuel (only 0.31 units) is required to make 1 unit of fuel. In contrast, diesel requires 1.2 units of fossil resources to produce 1 unit of fuel. Overall, "biodiesel yields 3.2 units of fuel product energy for every unit of fossil energy consumed in its life cycle" (USDA and USDOE, 1998). The fossil energy ratio of B20, however, is less than B100 with a ratio of 0.98 units.

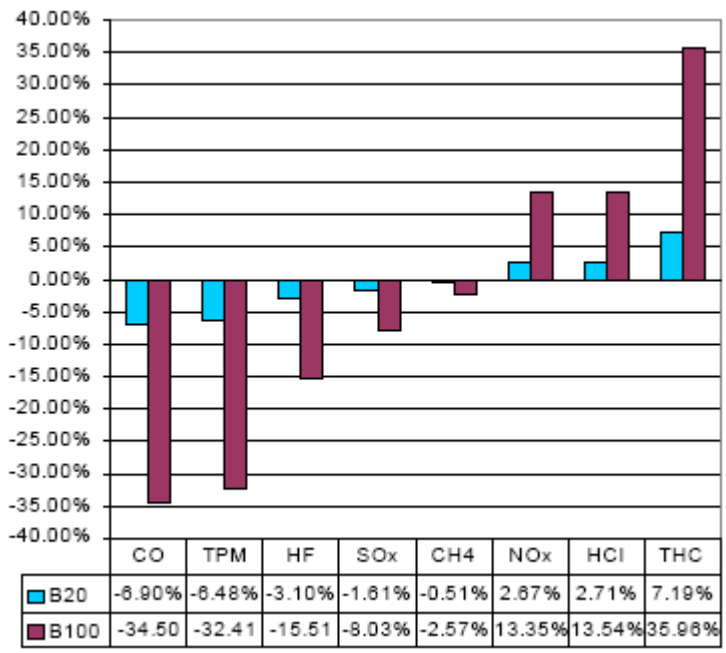
## 8.2. Emissions of Pollutants to Air

The LCI also included air pollutants: carbon monoxide (CO), nitrogen oxides ($NO_x$), particulate matter (PM), sulfur oxides ($SO_x$), and non-methane hydrocarbons (NMHC). All tested pollutants, other than $NO_x$, hydrochloric acid (HCl), and total hydrocarbons (THC), showed decreased emissions relative to diesel (Figure 8.2).

Biodiesel produces 32% less total particulate matter (TPM) and 35% less CO than does petroleum diesel in its typical life cycle. Of the TPM, the "PM10 emissions from an urban bus operating on biodiesel are 68% lower than those from an urban bus operating on petroleum diesel" (USDA and USDOE, 1998). $SO_x$ emissions also decreased 8.03% and 1.61% for B100 and B20 respectively. The amount of sulfur oxides emitted is a function of the sulfur content in the diesel fuel.

Both HCl and HF emissions occur as a result of coal combustion and electric power generation. These emissions are tracked because they may contribute to acidification in the environment. Since "HF levels drop with biodiesel in proportion to the amount of electricity consumed over the life cycle," a 15.51% reduction in emissions occurs (USDA and USDOE, 1998). The HCl biodiesel emissions, however, increase 13.54%. This is because there are "additional sources of HCl associated with the production and use of inorganic acids and bases used in the conversion step" (USDA and USDOE, 1998).

**Figure 8.2. Life cycle air emissions for B100 and B20 compared to petroleum diesel life cycle air emissions*.**




| | CO | TPM | HF | SOx | CH4 | NOx | HCl | THC |
|---|---|---|---|---|---|---|---|---|
| B20 | -6.90% | -6.48% | -3.10% | -1.61% | -0.51% | 2.67% | 2.71% | 7.19% |
| B100 | -34.50 | -32.41 | -15.51 | -8.03% | -2.57% | 13.35% | 13.54% | 35.96% |

*Figure from USDA and USDOE (1998).

Biodiesel's life cycle produces 35% more THC than does the diesel life cycle. Most of these emissions are produced during agricultural operations and soybean crushing. However, biodiesel's tailpipe emissions are 37% lower than diesel's emissions (these reductions were similar to those in the EPA study, discussed in Section V, Part A). Methane, $CH_4$, a greenhouse gas and a subset of THC emissions, produces 25% of the life cycle emissions of THC. For B100, $CH_4$ emissions were reduced by 2.57% relative to those from diesel.

## 8.3. Solid Waste and Emissions to Water

The USDA/USDOE study estimated biodiesel life cycle wastewater flows as almost 80% lower than petroleum diesel. The extraction of crude oil accounts for 78% of the total wastewater flow in diesel's life cycle, while only 12% is associated with the refinery process. The largest contributor to the wastewater flows of biodiesel come from soybean and oil processing (66%).

The life cycle assessments also include two classifications of solid waste: hazardous and nonhazardous. Biodiesel produces less hazardous waste than does diesel because it does not require a crude oil refining process. In contrast, almost all of diesel's hazardous solid waste is derived from the refining process. Agriculture accounts for 70% of biodiesel's hazardous waste, "but these flows are indirect charges against agriculture for hazardous waste flows associated with the production of diesel fuel and gasoline used on the farm" (USDA and USDOE, 1998). The total hazardous waste generation of diesel is 0.41g/bhp-h of engine work compared to 0.018g/bhp-h for biodiesel.

The nonhazardous waste generated within biodiesel's life cycle is 12.7g/bhp-h of engine work; this is largely attributed to the trash and trap metals that are removed from the soybeans after the crushing stage. Diesel's nonhazardous waste is significantly lower with only 2.8g/bhp-h of engine work. This waste is primarily generated in diesel's crude oil refining and extraction steps.

## 8.4. Life Cycle Inventory Information Gaps

The USDA/USDOE study provides a useful starting point of a biodiesel life-cycle impacts, but there are a number of limitations that must be addressed. First of all, the USDA/USDOE study considered only one type of biodiesel—that derived from soybeans. So there is a need to consider other types of biodiesel feedstock. The study considered only differences in emissions inventories, but did not address the differences in health and environmental impacts associated with these differences. There are computer models and databases that make this possible. The USDA/USDOE also did not address leaks and spills of fuel product during transport, storage, and distribution—an important issue for California.

# 9. Tier I Conclusions

Through a detailed review of the current knowledge on biodiesel use and impacts, this report provides a foundation for proceeding to a more detailed impact assessment for biodiesel use in California. A key focus of our review is identification of key knowledge gaps about physical, chemical, and toxicological properties of biodiesel governing environmental fate and transport and ecosystem and human health impacts. During this review, we discovered that there is a lack of information available on additives and their associated properties, both in mixture with biodiesel and alone. Therefore consideration of additive impacts leads the list of knowledge gaps by virtue of both the intrinsic impacts of the additives themselves, such as aquatic toxicity, and the coupled effects of additives on other properties of biodiesel fuels such as fate and transport and biodegradation. In the paragraphs below we summarize our key findings and conclusions. These issues set priorities for Tier II efforts.

**1. Additives impacts.** To provide a stable, useful, and reliable fuel, additive chemicals will need to be introduced into almost all biodiesel blends. These additives will be required to control oxidation, corrosion, foaming, cold temperature flow properties, biodegradation, water separation, and $NO_x$ formation. The specific chemicals and amounts used have not been well-defined for the emerging industry in California. A careful evaluation of the possible chemicals would be beneficial to California and may lead to a "recommended list" or "acceptable list" that would minimize the uncertainty of future impacts as industry standards are developed.

The impact of various additives that may be used with biodiesel blends needs to be considered for releases to the air, water, and soils. Additives may affect fuel quality or storage stability in unintended ways. Because the properties of additives can potentially alter the characteristics of biodiesel, increasing its environmental and health risks, there is a need for additional tests on biodiesel with specific concentrations of additives. In particular it is necessary to assess the impact of

- cold flow property controllers on surface water- biodiesel interaction and on subsurface multiphase transport of biodiesel (see number 2 below).
- biocides and anti-oxidants on biodegradation (see number 3 below).
- all priority additives on human and ecosystem toxicity.

**2. Subsurface fate and transport properties**. The impacts of leaks and spills of biodiesel fuel product during transport, storage, and distribution have not been addressed. This is an important issue for California. Because the chemical composition of biodiesel differs significantly from that of petroleum diesel, it is expected that infiltration, redistribution, and lens formation on water tables will differ for the two fuels, leading potentially to significant differences in relative impacts to groundwater quality. Properties governing these processes are density, viscosity, and interfacial tensions. Component (including additive) solubility into the water phase ultimately governs water quality and so inter-phase solubilization of individual components also needs to be identified. To address these issues requires experiments with conventional soil column tests that will establish relative transport behaviors among different fuel compositions and for site-specific analyses. But the relevance of these results for state-wide assessments should be considered along with the value of full-scale comparative field tests with releases into the groundwater, or into the vadose zone just above the groundwater table.

**3. Biodegradation in soils and aquifers**. The anticipated use of biocides in B20 fuels may affect the biodegradation potential for biodiesel released into the environment. The impact of biocides added to blended biodiesel may reduce the biodegradation of biodiesel and other petroleum-based fuels leaked or spilled into the subsurface. Since subsurface biodegradation can play an important role in the remediation of fuel spills and leaks, an understanding of the fate of biodiesel with biocide additive is needed.

Not all biodiesel or renewable-diesel fuel blends have been tested for degradation under aerobic and especially anaerobic conditions. To our knowledge, only one alternate electron acceptor, nitrate, has been tested; others such as sulfate and methanogenic conditions should be explored, because these are the primary available electron acceptors expected in the reducing environments expected in deeper soil contamination and in aquifers.

Pure biodiesel (B100) without additives may be more biodegradable than ULSD and may be preferentially metabolized by subsurface microbes. The interaction of B100 with existing gasoline or conventional diesel previously released into the subsurface needs to be examined more closely.

**4. Production and storage releases.** In addition to impacts from released B100 or blended B20 biodiesel, increased production and associated feedstock processing may involve impacts from released reactants and by-products. There are potential impacts to California's air and water during the large-scale industrial operations use to extract seed oils. These impacts may result from air emissions of solvents used to extract the seed oil (e.g., hexane) and from leaking tanks containing chemicals to process the plant oils into biodiesel. There is also the issue of occupational exposures. Finally, UST material compatibility must be addressed: owner/operators are required by state health and safety codes to demonstrate material compatibility prior to storage of biodiesel. The impacts during seed extraction will be become more of an issue for California as in-state production of plant-derived oils increases and may require further study. Currently, the possible impacts during seed extraction will be minimal in California since it is anticipated that most of the seed oils will be derived from soy grown and extracted out-of-state.

Among the most important current production reactants are: methanol, generic acid or base catalysts, feedstock oils, and post-processing water. As the biodiesel industry matures, release scenarios developed in this report need to be refined and prioritized.

**5. Additional air-emission studies**. There are not yet sufficient data to assert that the use of biodiesel will reduce the emissions of criteria and toxic air pollutants. Although considerable data are available on the effect of biodiesel on EPA-regulated pollutants (i.e., HC, CO, $NO_x$, and particulates), most of these data were generated using older technology engines. Further, very little detailed exhaust characterization data on biodiesel exist beyond a small number of regulated pollutants. Planned emission testing is based on newly blended B20 fuel stocks with only an anti-oxidant added and purged with nitrogen. B5, B50 and B100 will also be tested. The anti-oxidant and nitrogen purge are needed because the fuel used for the planned testing will be at least six month old-which is at the maximum recommended storage time for biodiesel. We note that these fuel mixes may not necessarily represent the general storage conditions expected throughout California. It is well established that fuel-handling practices have an important influence on engine performance and combustion emission. Additionally, the impact of various additives on combustion air emissions needs to be evaluated. Given the wide variety of oils and fats that might be used to make biodiesel fuel and the potential additives, the actual emissions of PMs and toxic air pollutants will have to be determined for each proposed formulation of

biodiesel fuel to be used in California. This situation demands a systematic and ongoing effort to assess emissions from diesel engines. In particular, there is a need for more controlled combustion studies to assess how the spectrum of toxic air pollutants, such as the spectrum of PAHs, will shift both in terms of volatile and particle-bound fraction but also in terms of any changes in toxic equivalency.

**6. Tier II Sampling Plan.** Many of the data gaps identified above will be addressed in the sampling plan developed for the Tier II study and report. In preparing this report, we had a number of discussions with Cal-EPA staff that provide important insight for developing the Tier-II sampling plan. First there is the issue of differences in emissions from different vehicle/engine classes such as on and off road vehicles. Where possible, this information should be included in the Tier two assessment. Next there are a number of issues of coordination among samples for different media and different objects. Samples used to test fate, transport, and toxicity should come from the same batch and be stored (aged) under the same conditions. When making comparisons of ULSD and biodiesel, all fuels should be subjected to the same tests. One example includes efforts to assure that fuel samples tested for water quality should include the same types of fuel samples used by CARB for air emissions tests to evaluate the effects and toxicity of the antioxidant additive. A second example is that the biodiesel formulation used to approve underground storage tank components for material compatibility should be the same formulation used in material compatibility tests. (The quantity of biodiesel required for this test at B20, B50, and B100 is probably on the order of one liter.) Fatty-acid-methyl-ester-derived biodiesel samples used in fate, transport, and toxicity testing should represent the vegetable oil feedstock most widely found in biodiesel marketed in California and the animal fat feedstock most widely found in biodiesel marketed in California. If the budget allows, we should also include the second most prevalent vegetable oil feedstock found in biodiesel marketed in California. Finally, samples used in fate studies should, where possible, represent both ideal conditions of biodiesel that meets ASTM D6751and ASTM D7467 specifications, as well as a more real-world example of fuel stored in an underground storage tank. These latter samples should contain 1.0% water at 100 ppm Cl salinity, peroxide levels of 150-200 ppm, and a total ammonia nitrogen (TAN) of 0.5 or greater. The peroxide and TAN levels should be attainable through natural aging of biodiesel without antioxidants. There will be a need to calculate quantities of each fuel, feedstock, blend, additive and water content, necessary to conduct all the planned sampling tests.

**7. Life Cycle Impacts.** Only differences in emissions inventories were considered during recent studies comparing life cycle inventories (LCI) of biodiesel to petroleum diesel. Differences in health and environmental impacts associated with these LCI differences needs to be evaluated. Additionally, current LCI studies have been limited to only soybean oil feedstocks. It is well established that different feedstocks can have an important influence on life cycle emissions. Information is needed for other feedstocks as well as for renewable diesel.

**8. Priority list of biodiesel formulations.** Because the number of potential feedstocks, the number of fuel blends, and the number of additive choices and mixes makes for an unmanageable suite of permutations of cases for consideration, it is critical to identify the priority feedstocks, fuel blends, and additives requiring study for our impacts assessment. Not specifically addressed in this Tier I evaluation are the environmental impacts from the increased use of fertilizers and water and land resources as the production of plant oils increases in the State. These factors may be some of the most important eventual impacts to California as the

biofuels industry expands. More sustainable sources of biodiesel such as yellow or brown grease may be preferable and should be encouraged.

Finally, a key goal of encouraging fuels such as biodiesel is to reduce California's carbon "footprint" as part of a global strategy. To consider only the environmental impacts to California and disregard the impacts that may be occurring nationally or internationally during the production of the biofuels feedstock that is used in California is short-sighted.

# 10. Tier I References

Ackland ML, Zou L, Freestone D, van de Waasenburg S, Michalczyk AA. 2007. Diesel exhaust particulate matter induces multinucleate cells and zinc transporter-dependent apoptosis in human airway cells. *Immunol Cell Biol*. 85(8): 617-622.

Albermarle Corporation. 2006. Solutions for improved biodiesel: Antioxidants for sustainable fuels. Product literature, 2006.

ARB-OEHHA. 1994. "Benzo[a]pyrene as a Toxic Air Contaminant." prepared by the staffs of the California Air Resources Board and the Office of Environmental Health Hazard-Assessment.

ASTM 2009a. . American Society for Testing and Materials (ASTM). Standard D7467-09a, "Standard Specification for Diesel Fuel Oil, Biodiesel Blend (B6 to B20)." ASTM International, West Conshohocken, PA, 2009, www.astm.org.

ASTM 2009b. American Society for Testing and Materials (ASTM). Standard D975-09b, "Standard Specification for Diesel Fuel Oils," ASTM International, West Conshohocken, PA, 2009, www.astm.org.

ASTM 2012. American Society for Testing and Materials (ASTM). Standard D6751-12, "Standard Specification for Biodiesel Fuel Blend Stock (B100) for Middle Distillate Fuels," ASTM International, West Conshohocken, PA, 2012, www.astm.org.

ATSDR (Agency for Toxic Substances and Disease Registry). 1995. Toxicological profile for fuel oils. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service.

Beaver, Sunny. 2008. Personal communication. Yokayo Biofuels, Ukiah, CA.

Bemesson, S., D. Nilsson, and P.-A. Hansson. 2004. A limited LCA comparing large- and small-scale production ofrape methyl ester (RME) under Swedish conditions. *Biomass & Bioenergy*, 26,545-559.

Birchall, C., J. Newman, and M. Greaves. 1995. Degradation and Phytotoxicity of Biodiesel oil, Institute of Arable Crops Research, 1995.

Biodiesel Magazine. 2006. "NREL, Chevron Consider Renewable Diesel Research", *Biodiesel Magazine*. December 2006.

Brito, J.M., Belotti, L., Toledo, A. C., Antonangelo, L., Silva, FS., Alvim, DS., Andre, PA., Saldiva, PHN., Rivero, DHRF., 2010. Acute Cardiovascular and Inflammatory Toxicity Induced by Inhalation of Diesel and Biodiesel Exhaust Particles, Toxicological Sciences 116(1): 67-78.

Bünger J, Krahl J, Baum K, Schröder O, Müller M, Westphal G, Ruhnau P, Schulz TG, Hallier E. 2000a. Cytotoxic and mutagenic effects, particle size and concentration analysis of diesel engine emissions using biodiesel and petrol diesel as fuel. *Arch Toxicol*. 74(8): 490-498.

Bünger J, Müller MM, Krahl J, Baum K, Weigel A, Hallier E, Schulz TG. 2000b. Mutagenicity of diesel exhaust particles from two fossil and two plant oil fuels. Mutagenesis 15(5): 391-397.

CalEPA (California Environmental Protection Agency). 2008. Guidance Document and Recommendations on the Types of Scientific Information to be Submitted by Applicants for California Fuels Environmental Multimedia Evaluations.

Cahill, T. M. and R. Okamoto. 2012. Emissions of acrolein and other aldehydes from biodiesel-fueled heavy-duty vehicles, *Environ. Sci & Tech.*, Vol. 46(15):8382-8388.

Canakci, M. and J. Van Gerpen, 2001. A Pilot Plant to Produce Biodiesel from High Free Fatty Acid Feedstocks, Proceedings, Amer. Soc. Agric. Engineers, 2001, paper number 016049, 2001.

Chesnow, Howard. 2008. Personal communication. Fuel Quality Systems Group. Corporation.

Chor H and D. Wilson. 2000. Improving the cold flow properties of biodiesel. A technical presentation given at the 91st American Oil Chemists' Society (AOCS) annual meeting, San Diego, CA, April 26, 2000. Lubrizol Corporation.

Clean Diesel Technologies. 2008.

Clark N. 2008. Virgin Atlantic Plans a Biofuel Flight, The New York Times, January 15, 2008.

Cox, C. 2006. "Is algae the new oil?", *Ode Magazine*, December 2006.

Degussa Corp. 2008. www.degusa.com

Fargione J, J. Hill, D. Tilman, S. Polasky, and P. Hawthorne. 2008. Land Clearing and the Biofuel Carbon Debt, *Science*, February 2008

Federal Record. 2005. One Hundred and Ninth Congress of the United States of America. Energy Policy Act of 2005,. City of Washington 2005. http://frwebgate.access.gpo.gov/cgi-bin/getdoc.cgi?dbname=109_cong_bills&docid=f:h6enr.txt.pdf

Finch GL, Hobbs CH, Blair LF, Barr EB, Hahn FF, Jaramillo RJ, Kubatko JE, March TH, White RK, Krone JR, Ménache MG, Nikula KJ, Mauderly JL, Van Gerpen J, Merceica MD, Zielinska B, Stankowski L, Burling K, Howell S. 2002. Effects of subchronic inhalation exposure of rats to emissions from a diesel engine burning soybean oil derived biodiesel fuel. *Inhal Toxicol*. 14(10): 1017-1048.

Graboski, M., R. McCormick, T. Alleman, and A. Herring. 2003. The Effect of Biodiesel Composition on Engine Composition on Engine Emissions from a DDC Series 60 Diesel Engine. Final Report, National Renewable Energy Laboratory, NREL/SR-510-31461, February, 2003.

He, B., J. Thompson, D. Routt, and J. Van Gerpen 2007. Moisture Absorption in Biodiesel and its Petro-Diesel Blends, *Applied Engineering in Agriculture*. 23(1): 71 – 76.

Hilber, T., E. AHN, M. Mittelbach, and E. Schmidt. 2007. "Animal Fats Perform Well in Biodiesel.", *Render Magazine*, February 2007.

Hodam R, 2008. Alternative Fuels: Ethanol and Biodiesel Compatibility: A Framework for Decision Making. California CUPA Forum, February, 2008.

Irwin, L. 2007. Additive Adjustment. Biodiesel Magazine, May 2007. www.biodieselmagazine.com

Janulis, P. 2004. Reduction of energy consumption in biodiesel fuel life cycle. *Renewable Energy*, 29, (6), 861-871.

Jessen, H.. 2007. Waving the Palm Branch, *Biodiesel Magazine*, February 2007. http://www.biodieselmagazine.com/article.jsp?article_id=1433

Johnson, B. 2007. Increasing Use of Biodiesel could Fuel Canola Explosion, *SeedQuest News*, California Farm Bureau Federation. March 2007.

Kado, NY, Okamoto, RA, Kuzmicky, PA (1996) Chemical and Bioassay Analyses of Diesel and Biodiesel Particulate Matter: Pilot Study, Final Report. Prepared for The Montana Department of Environmental Quality and the US Department of Energy by the Department of Environmental Toxicology, University of California, Davis, California 95616.

Kaffka, Stephen. 2007. Oral Communication. July 17, 2007.

Kantor J, 2008. Europe May Ban Imports of Some Biofuel Crops, The New York Times, January 15, 2008.

Kemp, D. and Frank V. 1980. Organic Chemistry, Worth Publishers, Inc. New York. 1980

Kemp, William. 2006. Biodiesel Basics and Beyond, Aztext Press, Ontario 2006.

California Senate Bill 140. 2007. Christine Kehoe. 2007 Senate Rules Committee. http://info.sen.ca.gov/pub/07-08/bill/sen/sb_0101-0150/sb_140_cfa_20070601_154941_sen_floor.html

Kenreck, G. 2007. Improving Biodiesel Stability with Fuel Additives, *Biodiesel Magazine,* February, 2007.

Knothe, G., J.V. Gerpen, and J. Krahl. 2005. The Biodiesel Handbook, AOCS Press, Champaign, Illinois, 2005.

Kotrba, Ron. 2007. Budding Opportunities in Canola, *Biodiesel Magazine*, February 2007.

Kram, Jerry. 2007. Algae on the Edge, *Biodiesel Magazine*, March 2007.

Krauter, Bob. 2006. Biodiesel Use Gaining more Converts in California, *Capital Press*, August 2006.

Lacoste, F., P. Bondioli, M. Mittelbach, J. Blassnegger, T. Brehmer, A. Frohlich, B. Dufrenoy, J. Fischer. 2003. Stability of Biodiesel, Summary Report, BLT, August, 2003

Lapinskiene, A., P. Martinkus, and V. Rebzdaite. 2005. Exo-toxicological studies of diesel and biodiesel fuels in aerated soil, *Environmental Pollution*, 142: 432 – 437.

Lapinskiene, Asta, and Povilas Martinkus. 2007.Research on Anaerobic Biodegradation of Fats, Biodiesel Fuel and Diesel Fuel in Soil Medium**.** Environmental Research, Engineering and Management, 2007.No.1(39), P. 30-37.

Leung, D., B. Koo, and Y. Guo, 2005. Degradation of biodiesel under different storage conditions, *Bioresource Technology*, 97: 250-256.

Lin, YC, Lee, WJ, Wu, TS, Wang, CT. 2006. Comparison of PAH and regulated harmful matter emissions from biodiesel blends and paraffinic fuel blends on engine accumulated mileage test, *Fuel*, **85**:2516–2523.

Liu, Yu-Yin; Lin, Ta-Chang; Wang, Ying-Jan, Ho, W.-L., 2008. Biological toxicities of emissions from an unmodified engine fueled with diesel and biodiesel blend, J. Environmental Science and Health Part A – Toxic/Hazardous Dubstances & Environmental Engineering 43(14): 1735-1743.

Loh, M. 2005. "Assessing Personal Exposures and Risks to Organic Hazardous Air Pollutants," PhD Dissertation, Harvard University School of Public Health.

LRRI (Lovelace Respiratory Research Institute). 2000. Final Report, Tier 2 Testing of Biodiesel Exhaust Emissions, Study Report Number FY98-056, May, 2000.

Mansell, G., R. Morris, and G. Wilson. 2003. Impact of Biodiesel Fuels on Air Quality and Human Health: Task 3 Report, Impact of Biodiesel Fuels on Ambient Carbon Monoxide Levels in the Las Vegas Nonattainment Area, National Renewable Energy Laboratory, NREL/SR-540-33796, May, 2003

Max, Arthur.2007. Palm Oil's Luster Fades on Biofuel Scene, *USA Today*. http://www.usatoday.com/tech/science/2007-04-01-palmoil_N.htm

McKone TE. 1993. CalTOX, a Multimedia Total-Exposure Model for Hazardous-Wastes Sites Part I: Executive Summary. Rep. UCRL-CR-111456PtI, Lawrence Livermore National Laboratory, Livermore, CA.

McMinnville Energy Systems. 2007. Project Report: Agricultural biofueled generation of electricity and the development of durable and efficient $NO_x$ reduction. www.mesystem.net

Mittelbach, M. and C. Remschmidt. 2004. Biodiesel: The Comprehensive Handbook, Martin Mittelbach, Austria.

Morris, R., A. Pollack, G. Mansell, C. Lindhjem, Y. Jia, and G. Wilson. 2003a. Impact of Biodiesel Fuels on Air Quality and Human Health, Summary Report, National Renewable Energy Laboratory, NREL/SR-540-33793, May, 2003.

Morris, R., G. Mansell, Y. Jia, and G. Wilson 2003b. Impact of Biodiesel on Air Quality and Human Health: Task 2 Report, The Impact of Biodiesel Fuels on Ozone Concentrations, National Renewable Energy Laboratory, NREL/SR-540-33795, May 2003.

Morris, R., and Y. Jia. 2003a. Impact of Biodiesel Fuels on Air Quality and Human Health: Task 4 Report, Impact of Biodiesel Fuel Use on PM, National Renewable Energy Laboratory, NREL/SR-540-33797, May, 2003.

Morris, R., and Y. Jia. 2003b. Impact of Biodiesel Fuels on Air Quality and Human Health: Task 5 Report, Air Toxics Modeling of the Effects of Biodiesel Fuel Use on Human Health in the South Coast Air Basin Region of Southern California, National Renewable Energy Laboratory, NREL/SR-540-33798, May, 2003.

Natl. Inst. Public Health Environ. Prot. 1994. Uniform system for the evaluation of substances (USES), version 1.0. Rep. VROM Distrib. 11144/150, RIVM; Minist. Hous., Spat. Plan. Environ. (VROM); Minist. Welf., Health, Cult. Aff. (WVC), The Hague, Netherlands.

NBB (National Biodiesel Board). 2007. Biodiesel Production and Quality. 2007 Fact Sheet12. http://www.biodiesel.org/pdf_files/fuelfactsheets/prod_quality.pdf

NREL (National Renewable Energy Laboratory ). 2005. Survey of the Quality and Stability of Biodiesel and Biodiesel Blends in the United States in 2004; Technical report NREL/TP-540-38836; National Renewable Energy Laboratory, 2005.

Pradhan, A., and D.S. Shrestha. 2007. Impacts of Some Common Impurities on Biodiesel Cloud Point, American Society of Agricultural and Biological Engineers, Michigan. http://asae.frymulti.com/abstract.asp?aid=23434&t=2

Quigley R. 2007. Biodiesel: The good, the bad, and the additives. Biofuels International, February 2007, pp. 70-72.

Reddy M, DeMello J, Carmichael C, Emily P, Li X, Arey JS, 2008. Determination of Biodiesel Blending Percentages Using Natural Abundance Radiocarbon Analysis: Testing the Accuracy of

Retail Biodiesel Blends. Environmental Science and Technology, February 2008. http://pubs.acs.org/cgi-bin/sample.cgi/esthag/asap/html/es071814j.html

Ribiero, NM, AC Pinto, CM Quintella, GO da Rocha, LSG Teixeira, LN Guariero, MC Rangel, MCC Veloso, MJC Rezende, RS Cruz, AM Oliviera, EA Torres, and JB Andrade. 2007. The role of additives for diesel and diesel blended (ethanol or biodiesel) fuels: A review. Energy and Fuels 21:2433-2445.

RIVM. 1994. *Uniform System for the Evaluation of Substances (Uses), Version 1.0. Rep. VROM Distribution No. 11144/150*, National Institute of Public Health and Environmental Protection (RIVM); Ministry of Housing, Spatial Planning and Environment (VROM); Ministry of Welfare, Health, and Cultural Affairs (WVC), The Hague, the Netherlands.

Rohm and Haas. 1999. Product literature, Publication number BIO.KFPI.5GENECAL.TB.

Rosenthal E, 2007. Once a Dream Fuel, Palm Oil May Be an Eco-Nightmare, The New York Times, January 31, 2007. http://www.nytimes.com/2007/01/31/business/worldbusiness/31biofuel.html?pagewanted=1

Searchinger T, Heimlich R, Houghton RA, Dong F, Elobeid A, Fabiosa J, Tokgoz S, Hayes D, Yu T, 2008. Use of U.S. Croplands for Biofuels Increases Greenhouse Gases Through Emissions from Land Use Change, Science, February 7, 2008.

Sheehan, J., Camobreco, V., Duffield, J., Graboski, M.S,. and Shapouri, H. 1998 H.; A life cycle inventory of biodiesel and petroleum diesel for use in an urban bus. National Renewable Energy Laboratory NRELlSR-580-24089.

Sheehan, J., T. Dunahay, J. Benemann, and P. Roessler. 1998. A Look Back at the U.S. Department of Energy's Aquatic Species Program – Biodiesel from Algae, National Renewable Energy Laboratory. http://www.nrel.gov/docs/legosti/fy98/24190.pdf

Sioux City Journal. 2007. Soybean Oil Prices put Squeeze on Biodiesel, June 12, 2007. http://www.siouxcityjournal.com/articles/2007/06/12/news/iowa/881238934ec50219862572f8000765ae.txt#

Sizle, Bill. 2008. Personal communication. Lubrizol Corporation.

Solodov VA, OA Varnavskaya, LK Khvatova, BR Kakruthdinov, NA Lebedev, IN Diyarov. 2005. Sorbitol-based demulsifying agents and their surfactant properties. Russian Journal of Applied Chemistry 78 (6): 944-949.

Song, WW., He, KB., Wang, JX., Wang, XT., Shi, XY., Yu, C., Chen, WM., Zheng, L., 2011. Emissions of EC, OC, and PAHs from Cottonseed Oil Biodiesel in a Heavy-Duty Diesel Engine, Environmental Science and Technology, 45(15):6683-6689.

South Coast Air Quality Management District (SCAQMD) 2000. Multiple air toxics exposure study (MATES-II) Final Report. March 2000. (2nd printing - July, 2000).

SRI (Southwest Research Institute). 2005. Characterization of Biodiesel Oxidation and Oxidation Products, National Renewable Energy Laboratory, NREL/TP-540-39096, November 2005.

Stolz, J., P. Follis, G. Floro, R. Donofrio, J. Buzzelli, and W. Griffin. 1995. Aerobic and Anaerobic biodegradation of the methyl esterified fatty acids of soy diesel in freshwater and soil environments. http://www.biodiesel.org/resources/reportsdatabase/reports/gen/19950101_gen-273.pdf

Swanson KJ, Madden MC, Ghio AJ. 2007. Biodiesel exhaust: the need for health effects research. *Environ Health Perspect.* 115(4): 496-499.

SWRCB, 2008. Memo to Local UST Regulatory Agencies and Interested Parties. State Water Resources Control Board, California Environmental Protection Agency, February 28th, 2008.

Taracha, J. 2006. Technology Issues and Trends: Biodiesel. ASPA Spring Meeting, June 2006. Lubrizol Corp.

Tsai, JH., Huang, KL., Chiu, CH., Lin, CC., Kuo, WC., Lin, WY., Chaung, HC., Yang, TH., Chen, SJ., 2011. Particle-bound PAHs and Particle-extract-induced Cytotoxicity of Emission from a Diesel-generator Fuelled with Soy-biodiesel, Aerosol and Air Quality Research, 11(7): 822-836.

Turrio-Baldassarria, L, Battistellia, CL, Contia, L, Crebellia, R., De Berardisa, B, Iamicelia, AL, Gambinob, M, Iannacconeb, S (2004) Emission comparison of urban bus engine fueled with diesel oil and 'biodiesel' blend, *Science of the Total Environment,* **327**:147–162.

The Wall Street Journal, 2008. Greenhouse Affect. February, 2008.

University of Idaho Department of Biological and Agricultural Engineering. 2005. Tech Note: Impact of additives on cold flow properties of biodiesel Fall/Winter 2005, volume 2, issue 2.

US (United States) DA (Department of Agriculture) and U.S. DOE (Department of Energy). 1998. An Overview of Biodiesel and Petroleum Diesel Life Cycles, National Renewable Energy Laboratory, NREL/TP-580-24772, May, 1998.

US EPA (United States Environmental Protection Agency). 2002. A Comprehensive Analysis of Biodiesel Impacts on Exhaust Emissions, Draft Technical Report EPA420-P-02-001, October 2002.

U.S. DOE (Department of Energy). 2006. Biodiesel handling and use guidelines, third edition, DOE/G0-102008-2358, September, 2006.

Van Gerpen, J. Biodiesel Production and Fuel Quality. University of Idaho. http://www.uidaho.edu/bioenergy/biodieselED/publication/01.pdf

Van Gerpen, J., B. Shanks, R. Pruszko, D. Clements, and G. Knothe. 2004. Biodiesel Analytical Methods, National Renewable Energy Laboratory, NREL/SR-510-36240, July, 2004. http://www.nrel.gov/docs/fy04osti/36240.pdf

Van Gerpen, J., E. Hammond, L. Johnson, S. Marley, L. Yu, I. Lee, and A. Monyem. 1996. Determining the Influence of Contaminants on Biodiesel Properties, Iowa State University.

Van Gerpen, Jon. 2004. Business Management for Biodiesel Producers, National Renewable Energy Laboratory, NREL/SR-510-36242, July, 2004. http://www1.eere.energy.gov/biomass/pdfs/36242.pdf

Venturi, P. and G. Venturi. 2003. Analysis of energy comparison for crops in European agricultural systems. *Biomass & Bioenergy*, 25, 235-255.

Viscom. 2008. www.viscomusa.com

Wang M, Haq Z, 2008. Response to February 7, 2008 Sciencexpress Article, Argonne National Laboratory, Transportation Technology R&D Center. http://www.transportation.anl.gov/media_center/news_stories/20080214_response.html

Wania F, Fate and Transport of Chemicals in the Environment. In: *Encylopedia of Physical Science and Technology*, Academic Press, San Diego CA, 2001.

Wedel, Randall. 1999. Technical Handbook for Marine Biodiesel, National Renewable Energy Laboratory, DE-AC36-83CH10093, April, 1999.

Woodfine DG, MacLeod M, Mackay D, Brimacombe JR. 2001. Development of Continental Scale Multimedia Contaminant Fate Models: Integrating GIS. *Environmental Science and Pollution Research* 8: 164-72.

Yang, HH, Chien, SM, Lo, MY, Lan, JCW, Lu, WC, Ku, YY. 2007 Effects of biodiesel on emissions of regulated air pollutants and polycyclic aromatic hydrocarbons under engine durability testing, *Atmospheric Environment* **41**:7232–7240.

Zhang, X., C. Peterson, D. Reece, G. Moller, and R. Haws. 1998. Biodegradability of Biodiesel in the Aquatic Environment, *ASAE*, 41(5): 1423 – 1430.

Zhang, Y., M.A. Dube, D.D. McLean, and M. Kates. 2003. Biodiesel Production From Waste Cooking Oil:1. Process Design and Technological Assessment. *Bioresource Technology* 89:1-16.

# 11. Tier I Appendices

## 11.1. Appendix I-A: Standard Property Descriptions for Biodiesel (B100) and Biodiesel Blends (B6 to B20)

The significance of the standard properties (described below) are based primarily on the commercial use of biodiesel in on-road and off-road diesel engine applications (ASTM, 2009; ASTM 2012).

*Calcium and Magnesium*

Biodiesel can contain small amounts of calcium and magnesium in the form of abrasive solids or soluble metallic soaps. "Abrasive solids can contribute to injector, fuel pump, piston, and ring wear, as well as engine deposits. Soluble metallic soaps have little effect on wear, but they may contribute to filter plugging and engine deposits".

*Sodium and Potassium*

While most of the excess catalyst is removed with the glycerol, some sodium and potassium may be present in the biodiesel as abrasive solids or soluble metallic soaps. This may contribute to piston, fuel pump, injector, and ring wear and also engine deposits. High levels of sodium and potassium can also cause increased back pressure and reduced service life. For biodiesel, the maximum allowable concentration of sodium and potassium should be 5ppm.

*Phosphorus Content*

With stricter emission controls, catalytic converters are becoming more common for diesel powered equipment. Phosphorus content must be limited as it can cause damage to catalytic converters. Biodiesel has been shown to have a low phosphorous content (below 1 ppm) which satisfies the national requirement of 10 ppm. However, biodiesel from other soureces may contain higher levels of phosphorous, so ASTM has set a standard of 0.001% mass.

*Flash Point*

This is the minimum temperature at which the fuel ignites on application of an ignition source; it has no direct relationship to engine performance but instead indicates the level of fire safety. The minimum flash point of biodiesel is much higher than diesel fuel and it "falls under the non-hazardous category under National Fire Protection Association codes." For biodiesel, the minimum flash point standard is set at 93$^{o}$C.

*Alcohol Control*

The levels of unreacted alcohol remaining in the biodiesel must be controlled. This can be done one of two ways: measuring the volume percent of methanol content directly or through a high flash point value. For biodiesel, the maximum methanol content is at 0.2% volume, otherwise, ASTM specifies a minimum flash point of 130$^{o}$C to control alcohol.

*Water and Sediment*

These are primarily considered as post-production parameters. While excess water can be contained in the biodiesel after production, the fuel most commonly comes into contact with water and sediment during storage.

Sediment "may consist of suspended rust and dirt particles or it may originate from the fuel as insoluble compounds formed during fuel oxidation" (Van Gerpen et al., 2004). These sediments can cause fuel filter plugging problems.

*Kinematic Viscosity*

It is important to designate "a minimum viscosity as there can be issues of power loss due to injection pump and injector leakage" when fuels with low viscosity are used. Likewise, a maximum viscosity must be met for "considerations involved in engine design, size, and characteristics of the injection system". Because blended biodiesel/diesel fuel can exhibit relatively high viscosities, the maximum viscosity for biodiesel (6.0 $mm^2/s$ at $40^oC$) is higher than the maximum for diesel fuels excluding Grade No. 4-D.

*Sulfated Ash*

The ash content describes the amount of inorganic contaminants such as abrasive solids, soluble metallic soaps, and residual catalysts. "These can contribute to injector, fuel pump, piston and ring wear, engine deposits", and filter plugging.

*Sulfur*

Limits have been placed on sulfur content for environmental reasons. B100 essentially contains no sulfur; the sulfur content in biodiesel blends is due to the diesel fuel. The limits for Grade S15 and Grade S500 indicate a limit of 15 ppm and 500 ppm of sulfur content, respectively. It is important to note that "other sulfur limits can apply in selected areas in the United States and in other countries" (ASTM, 2009). In California, the California Air and Resource Board has set the sulfur content for diesel fuels at 15 ppm or less.

*Copper Strip Corrosion*

This is a test to measure the presence of acids or sulfur-containing compounds in the fuel. A copper strip is immersed in the fuel to determine the level of corrosion that would occur if biodiesel came in contact with metals such as copper, brass, or bronze.

*Aromaticity*

This is and indication of the aromatics content of diesel fuel. Aromatic content of fuels over the specified level can have a negative impact on emissions.

*Cetane Number*

The cetane number is a measure of the ignition quality of the fuel. To obtain the highest fuel availability, the cetane number should be as low as possible; otherwise fuel will be ignited too quickly. For biodiesel fuels, a minimum cetane number of 47 is recommended.

*Cetane Index*

The Cetane Index is a limitation on the amount of high aromatic components in Grades S15 and S500.

*Cloud Point*

This is an important property as it "defines the temperature at which a cloud or haze of crystals appears in the fuel [and] relates to the temperature at which crystals begin to precipitate from the fuel" Petroleum based diesel fuel generally has a lower cloud point than biodiesel as it is not as susceptible to cold temperatures. There is currently no cloud point specification for biodiesel, although it does play a major role in cold weather operability.

*Carbon Residue*

Carbon residue is a measure of carbon depositing tendencies of a fuel oil when heated under prescribed conditions". This property is an approximation since it is not directly correlated with engine deposits. For biodiesel fuels Grades No. 1-D S15 and S500, the residue maximum is 0.05% mass.

*Acid Number*

The amount of free fatty acids within the biodiesel can be determined by the acid number. "Biodiesel with a high acid number has been shown to increase fueling system deposits and may increase the likelihood for corrosion." ASTM has a set a limit of 0.50 mg KOH per gram of biodiesel.

*Total and Free Glycerin*

Total glycerin refers to the "free glycerin and glycerin portion of any unreacted or partially reacted oil or fat. Low levels of total glycerin ensure the high conversion of the oil or fat into its mono-alkyl esters has taken place". Note that high levels of either mono-,di-, and triglycerids may cause injector deposits, filter plugging,and worsen cold weather operability. For B100 biodiesel a limit of 0.240% mass is required Within the finished B100, trace amounts of free glycerin can remain in small quantities as suspended droplets or dissolved into the fuel. If concentrations are high, the free glycerin can cause injector deposits and can clog fueling systems. The maximum free glycerin concentration is 0.02% mass for B100 biodiesel fuels.

*Distillation Temperature*

Distillation is a measure of the volatility of a fuel. "The fuel volatility requirements depend on engine design, size, nature of speed and load variations." Note that heavier fuels will provide the best fuel economy due to having greater heat content. This specification is incorporated in the ASTM as a "precaution to ensure the fuel has not been adulterated with high boiling contaminants." The maximum distillation temperature of biodiesel should be at 360$^{o}$C and is also the atmospheric equalivalent temperature for 90% recovery.

*Oxidation Stability*

Products of oxidation in biodiesel can cause fuel system malfunctions, deposits, and can lead to filter clogging. "Additives designed to retard the formation of acids and polymers can significantly improve the oxidation stability performance of biodiesel".

*Free Fatty Acids (FFAs)*

Free fatty acids are "any saturated or unsaturated monocarboxylic acids that occur naturally (as fats, oils or greases) but are not attached to glycerol backbones" (DOE, 2006). The greatest difference among feedstocks is the amount of free fatty acids associated with the triglycerides. The FFA content "affects the amount and extent of feedstock preparation necessary to use a particular reaction chemistry" (Van Gerpen et al., 2004).

*Saturation*

A fatty acid is saturated if it only contains single bonds. Unsaturated compounds can have double or triple bonds. (Discussed further in Section II, Part C this report).

*Microbial Contamination*

Uncontrolled microbial contamination in fuel systems can cause or contribute to a variety of problems, including increased corrosivity and decreased stability, filterability, and caloric value.

**References:**

ASTM 2009. ASTM Standard D7467-09a, "Standard Specification for Diesel Fuel Oil, Biodiesel Blen (B6 to B20)." ASTM International, West Conshohocken, PA, 2009, www.astm.org.

ASTM 2012. ASTM Standard D6751-09b, "Standard Specification for Biodiesel Fuel Blend Stock (B100) for Middle Distillate Fuels," ASTM International, West Conshohocken, PA, 2009, www.astm.org.

Van Gerpen, J., B. Shanks, R. Pruszko, D. Clements, and G. Knothe. 2004. Biodiesel Analytical Methods, National Renewable Energy Laboratory, NREL/SR-510-36240, July, 2004. http://www.nrel.gov/docs/fy04osti/36240.pdf

## 11.2. Appendix I-B: Biodiesel Additive Chemicals

**Figure I-B-1: Common Antioxidants**

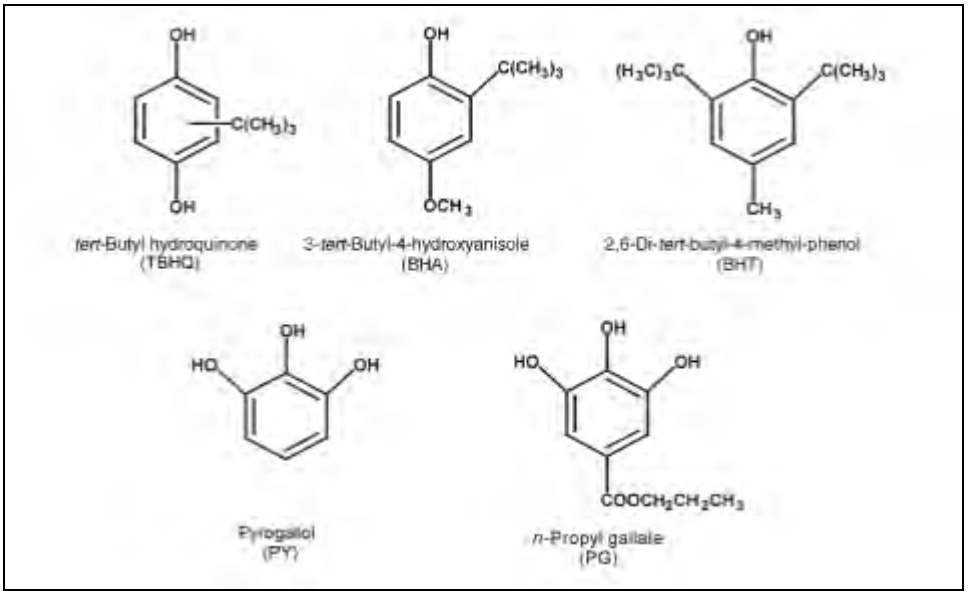
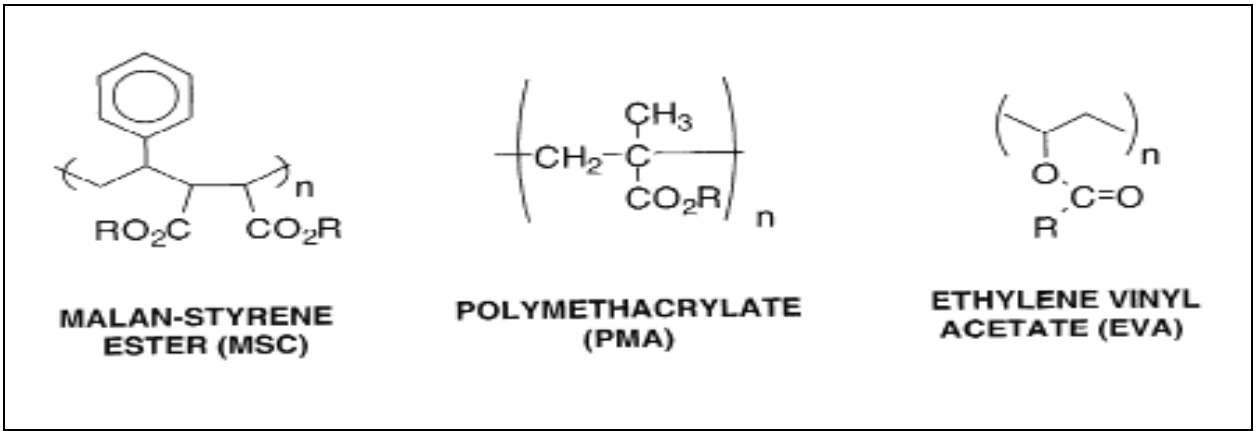



**Table I-B-1. Commercial Biodiesel Antioxidants***

| Manufacturer | Product Name | Chemical Components | % |
|---|---|---|---|
| Albemarle | Ethanox 4737 | 2,6 di-t-butylphenol<br>2,4,6 tri-tert butylphenol<br>2-tert-butylphenol<br>Phenol<br>Naptha<br>Petroleum | 52.5%<br>10.5%<br>7%<br>1.1%<br>25-30%<br>2% |
| Biofuel Systems | Baynox | 2,6 di-tert-butyl-4-methylphenol | 20% |
| Chemiphase | AllClear | Methyl Alcohol | 18-24% |
| Eastman Chemical | Bioextend30 | 2-tert-butylhydroquinone<br>Butyle acetate<br>Diethylene glycol monobutyl ether | 20%<br>30%<br>30% |
| Eastman Chemical | Tenox 21 | Tertiary butylhydroquinone | 20% |
| Lubrizol | 8471U | Butylated phenol | 70-79% |

*Source: Company MSDSs and Product Data Sheets

**Figure I-B-2. Lubrizol Corporation Cold Flow Additive Chemicals***




*Data from Chor et al. (2000). Lubrizol cold-flow additives are formulated for all diesel fuels and can be used with standard diesel and biodiesel formulations.

**Table I-B-3. Commercial Cold Flow Additives*.**

| Manufacturer | Product Name | Chemical Components | % |
|---|---|---|---|
| Biofuel Systems | Wintron XC30 | Toluene | 2% |
| Chemiphase | Coldflow 350 | Toluene | 2% |
| Hammonds | ColdFlo | Vinyl copolymer in hydrocarbon solvent<br>Naptha | N/A<br>40-70% |
| Lubrizol | FloZol502 | Copolymer Ester<br>Toluene | N/A<br>2% |
| Lubrizol | FlowZol503 | Naptha<br>Napthalene<br>Trimetheyl Benzene<br>Ethylbenzene<br>Alkylphenol<br>Xylene | 40-49%<br>4.4%<br>1.4.9%<br>1.6%<br>5-9.9%<br>6.4% |

*Source: Company MSDSs and Product Data Sheets

**Table I-B-4. Commercial Biocides.**

| Manufacturer | Product Name | Chemical Components | % |
|---|---|---|---|
| Chemiphase | AllKlear, FilterClear | Sodium dodecyclbenzene sulfonate | 2-32% |
| FPPF Chemical | Kill-Em | Disodium ethylenebisdithiocarbamate<br>Sodium dimethyldithiocarbamate<br>Ethylene thiourea | 15%<br>15%<br>1% |
| Hammonds | Biobor JF | Naptha<br>2,2-(1-methyltrimethylenedioxy)bis-(4-methyl-1,3,2 dioxyborinane; 2,2,oxybis(4,4,6-trimethyl-1,3,2-dioxaborinane)<br>[Substituted dioxaborinanes] | 4.5%<br>95% |
| Power Serve Products | Bio-Kleen | 4-(2-nitrobutyl)-morpholine<br>4,4, (2-ethyl-2-nitrotrimethylene)-dimorpholine<br>Methylene dimorpholine<br>Morpholine<br>1-Nitropropane | 76-85%<br>2-7%<br>3.9-6.5%<br>3-6%<br>.3-5.3% |
| Rohm and Haas | Kathon FP 1.5 | Magnesium nitrate<br>5-chloro-2-methyl-4-isothiazol-3-one<br>2-methyl-4-isothiazol-3-one | 1-2.5%<br>1-2.5%<br>To 1 mix |
| Star Brite Corp | Biodiesel Biocide | Sodium dimethyldithiocarbamate<br>Ethylenedimine<br>Dimethylamine<br>Ethylene thiourea<br>Nabam | 15-20.2%<br>0.0-0.75%<br>0.0-0.75<br>0-1.0%<br>15-20% |

**Table I-B-5. NOx Reduction**.

| Manufacturer | Product Name | Chemical Components | % |
|---|---|---|---|
| Clean Diesel Technologies | Aris2000 Injection system | Urea or Ammonia injected into exhaust | N/A |
| Oryxe | LED for biodiesel (and diesel) | 2-ethylhexyl nitrate<br>Toluene | 45% w/w<br>45-55 w/w |
| Viscon USA | Viscon | Polyisobutylene (Polyalphaolefin) Polymer | 5% |

## 11.3. Appendix I-C: Biodiesel Web Links

**Water Solubility**

- Moisture Absorption in Biodiesel and its Petro-Diesel Blends. http://www.webpages.uidaho.edu/~bhe/pdfs/MC.pdf
- Determining the Influence of Contaminants of Biodiesel Properties (has specific section on water solubility). http://www.nbb.org/resources/reportsdatabase/reports/gen/19960731_gen014.pdf
- Moisture Distribution in Biodiesel and its Fossil Diesel Blends. http://asae.frymulti.com/request.asp?JID=5&AID=21513&CID=por2006&T=2

**Toxicity**

- Acute Oral Toxicity Study of 100% REE in Albino Rats. http://www.biodiesel.org/resources/reportsdatabase/reports/gen/19960110_gen-220.pdf
- Acute Dermal Toxicity Study of 100% REE in Albino Rats. http://www.biodiesel.org/resources/reportsdatabase/reports/gen/19960110_gen-229.pdf
- Eco-toxicological Studies of Diesel and Biodiesel Fuels in Aerated Soil. http://www.sciencedirect.com/science?_ob=MImg&_imagekey=B6VB5-4HS3C1J-7-C&_cdi=5917&_user=4421&_orig=search&_coverDate=08%2F31%2F2006&_sk=998579996&view=c&wchp=dGLbVlb-zSkzk&md5=25221a1dcb50d96ee2131ef572acc00f&ie=/sdarticle.pdf
- Inhalation Toxicology: Effects of Subchronic Inhalation Exposure of Rats to Emissions From a Diesel Engine Burning Soy-oil Derived Biodiesel Fuel. http://www.informaworld.com/smpp/content~content=a713857050
- Mutagenic and Cytotoxic Effects of Exhaust Particulate Matter of Biodiesel Compared to Fossil Diesel Fuel. http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6T2D-3TDR7MP-2&_user=4421&_coverDate=07%2F08%2F1998&_rdoc=1&_fmt=&_orig=search&_sort=d&view=c&_acct=C000059598&_version=1&_urlVersion=0&_userid=4421&md5=556346ff8d6a7443b14cb75173ea45dc

**Aquatic Toxicity**

- Degradation and Phytotoxicity of Biodiesel Oil. http://www.biodiesel.org/resources/reportsdatabase/reports/mar/19950801_mar-008.pdf

**Air Quality and Human Health**

- A Comprehensive Analysis of Biodiesel Impacts on Exhaust Emissions, 2002. http://www.epa.gov/OMS/models/analysis/biodsl/p02001.pdf
- Impact of Biodiesel Fuels on Air Quality and Human Health: Summary Report Sept. 99 – Jan 2003. http://www.biodiesel.org/resources/reportsdatabase/reports/gen/20030501_gen-365.pdf
- Impact of Biodiesel Fuels on Air Quality and Human Health: Task 2. Impact of Biodiesel fuels on Ozone Concentrations. http://www.biodiesel.org/resources/reportsdatabase/reports/gen/20030501_gen-364.pdf
- Effect of Biodiesel Composition on Engine Emissions from a DDC Series 60 Diesel Engine. http://www.nrel.gov/docs/fy03osti/31461.pdf (by NREL 2003)

- Effect of Biodiesel Composition on $NO_x$ and PM from a DDC Series 60 Diesel Engine. http://www.biodiesel.com/images/emissions.pdf (by NREL 1999)
- Regulated Emissions from Biodiesel Tested in Heavy Duty Engines Meeting 2004 Emission Standards. http://www.nrel.gov/vehiclesandfuels/npbf/pdfs/37508.pdf
- Impact of Biodiesel Fuels on Air Quality and Human Health: Task 5 Report. Air Toxic modeling of the effects of biodiesel fuel use on human health in the south coast air basin region of Southern California 2003. http://www.nrel.gov/docs/fy03osti/33798.pdf
- Impact of Biodiesel Fuel on Air Quality and Human Health: Task 4 Report. Impact of Biodiesel fuel use on PM 2003. http://www.nrel.gov/docs/fy03osti/33797.pdf
- Impact of Biodiesel Fuel on Air Quality and Human Health: Task 3 Report. Impact of Biodiesel Fuels on Ambient Carbon Monoxide Levels in the Las Vegas Nonattainment Area 2003. http://www.nrel.gov/docs/fy03osti/33796.pdf
- $NO_x$ Solutions for Biodiesel. Final Report: Report 6 in Series of 6, 2003. http://www.nrel.gov/docs/fy03osti/31465.pdf
- Effects of Biodiesel Blends on Vehicle Emissions 2006. http://www.nrel.gov/vehiclesandfuels/npbf/pdfs/40554.pdf
- Bioassay Analysis of Particulate Matter from a Diesel Bus Engine Using Various Biodiesel Feedstock Fuels. Report 3 in a series of 6. http://www.nrel.gov/docs/fy03osti/31463.pdf
- $NO_x$ Reduction from Biodiesel Fuels. http://pubs.acs.org/cgi-bin/article.cgi/enfuem/2006/20/i01/pdf/ef050202m.pdf

**Biodegradability**

- Biodegradability of Biodiesel in the Aquatic Environment. http://www.canadianbioenergy.com/resources/Degradability_of_biodiesel_in_marine_environment.pdf
- Aerobic an Anaerobic Biodegradation of the Methyl Esterified Fatty Acids of Soy Diesel in Freshwater and Soil Environments. http://www.biodiesel.org/resources/reportsdatabase/reports/gen/19950101_gen-273.pdf
- Toxicity, Biodegradability, and Environmental Benefits of Biodiesel. http://www.biodiesel.org/resources/reportsdatabase/reports/mar/19940101_mar-002.pdf
- Biodegradability of Biodiesel fuel of Animal and Vegetable Origin. http://www3.interscience.wiley.com/cgi-bin/fulltext/114265170/PDFSTART
- The Effect of biodiesel on the rate of removal and weathering characteristics of crude oil within artificial sand columns. http://www.sciencedirect.com/science?_ob=MImg&_imagekey=B6VH4-3TB5W4J-3-1&_cdi=6056&_user=4421&_orig=search&_coverDate=12%2F31%2F1997&_sk=999959998&view=c&wchp=dGLzVlz-zSkWW&md5=d2bd2d70ff67724abadb0aac1f5407c6&ie=/sdarticle.pdf
- Biodegradability, $BOD_5$, COD, and Toxicity of Biodiesel Fuels. http://www.uidaho.edu/bioenergy/BiodieselEd/publication/04.pdf

**Storage/ Stability**

- Stability of Biodiesel: Used as a Fuel for Diesel Engines and Heating Systems. http://www.blt.bmlfuw.gv.at/BIOSTAB/download/BIOSTAB_Proceedings.pdf
- Stability of Biodiesel and Biodiesel Blends: Interim Report (by NREL 2006). http://www.nrel.gov/vehiclesandfuels/npbf/pdfs/39721.pdf
- Quantification and Improvements of the long term storage stability of biodiesel and biodiesel blends. http://www.biodiesel.org/resources/reportsdatabase/reports/gen/19971201_gen-022.pdf
- Oxidation Stability of Fatty Acid Methyl Esters. http://www.biodiesel.org/resources/reportsdatabase/reports/gen/19980611_gen-160.pdf
- Determination of Biodiesel Oxidation and Thermal Stability. http://www.biodiesel.org/resources/reportsdatabase/reports/gen/19970212_gen-230.pdf
- Characterization of Biodiesel Oxidation and Oxidation Products, 2005. http://www.nrel.gov/vehiclesandfuels/npbf/pdfs/39096.pdf
- Biodiesel Fuels: The Use of Soy Oil as a Blending Stock for Middle Distillate Petroleum Fuels (has section on storage). http://www.biodiesel.org/resources/reportsdatabase/reports/gen/20000701_gen-289.pdf
- Degradation of Biodiesel Under Different Storage Conditions http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6V24-4FWKM8F-2&_user=4421&_coverDate=01%2F31%2F2006&_rdoc=1&_fmt=&_orig=search&_sort=d&view=c&_acct=C000059598&_version=1&_urlVersion=0&_userid=4421&md5=7c2863e00abd845ca5cedf7bd8c48c4e
- The Effects of Fatty Acid Composition on Biodiesel Oxidative Stability. http://www3.interscience.wiley.com/cgi-bin/fulltext/109857530/PDFSTART

**Compatibility**

- Impact of Biodiesel on Fuel System Component Durability. http://www.nrel.gov/vehiclesandfuels/npbf/pdfs/39130.pdf
- Elastomer Compatibility Testing of Renewable Diesel Fuels, 2005. http://www.nrel.gov/vehiclesandfuels/npbf/pdfs/38834.pdf

**Life Cycle**

- An Overview of Biodiesel and Petroleum Diesel Life Cycles. http://www.biodiesel.org/resources/reportsdatabase/reports/gen/19980501-gen-203.pdf
- Biodiesel Energy Balance. http://www.uidaho.edu/bioenergy/NewsReleases/Biodiesel%20Energy%20Balance_v2a.pdf
- Energetic and Economic Feasibility Associated with the Production, Processing, and Conversion of Beef Tallow to a Sustainable Diesel Fuel. http://www.biodiesel.org/resources/reportsdatabase/reports/gen/20060601_gen373.pdf
- Exergy Analysis Applied to Biodiesel Production. http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6VDX-4MD469X-1&_user=4421&_coverDate=08%2F31%2F2007&_rdoc=1&_fmt=&_orig=search&_sort=d&view=c&_acct=C000059598&_version=1&_urlVersion=0&_userid=4421&md5=2ab63b26d47e3a484d69ee9302485ad6
- Environmental Impacts of Biodiesel Use. http://www.biodiesel.org/resources/reportsdatabase/reports/gen/19981001_gen-110.pdf

**General**

- Biodiesel Handbook. (An electronic copy of book that covers many of these topics above). http://www.chemlibnetbase.com/books/2240/1893997790_fm.pdf
- Sample Material Safety Data Sheet for Biodiesel, National Biodiesel Board, http://www.biodiesel.org/pdf_files/fuelfactsheets/MSDS.PDF
- Biodiesel Analytical Methods (Aug. 2002- Jan. 2004). http://www.nrel.gov/docs/fy04osti/36240.pdf
- Business Management for Biodiesel Producers (Aug. 2002- Jan. 2004). http://www.nrel.gov/docs/fy04osti/36242.pdf
- Biodiesel Cold Weather Blending Study. http://www.nrel.gov/vehiclesandfuels/npbf/pdfs/cftr_72805.pdf
- Production of Biodiesels from Multiple Feedstocks and Properties of Biodiesels and biodiesel/Diesel Blends. Final Report: Report 1 in a series of 6, 2003. http://www.nrel.gov/docs/fy03osti/31460.pdf
- US Biodiesel Overview, 1995. http://www.biodiesel.org/resources/reportsdatabase/reports/gen/19950101_gen-263.pdf
- Environmental Effect of Rapeseed Oil Ethyl Ester. http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6V4S-48YVSF4-5&_user=4421&_coverDate=12%2F31%2F2003&_fmt=full&_orig=search&_cdi=5766&view=c&_acct=C000059598&_version=1&_urlVersion=0&_userid=4421&md5=5bddf0f619d91e694ec64cbe8d42a29c&ref=full
- Assessing the Viability of Using Rape Methyl Ester (RME) As An Alternative to Mineral Diesel Fuel for Powering Road Vehicles in the UK (covers biodegradability and toxicity). http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6V1T-3VCVKSK-7&_user=4421&_coverDate=02%2F28%2F1998&_rdoc=1&_fmt=&_orig=search&_sort=d&view=c&_acct=C000059598&_version=1&_urlVersion=0&_userid=4421&md5=d4fe0729bea53b522f1b97702683779e#toc16

## 6.2. Appendix III-B: California Biodiesel Multimedia Evaluation Tier II Report on Aquatic Toxicity, Biodegradation, and Subsurface Transport Experimants, Final Report,

# California Biodiesel Multimedia Evaluation

## Tier II Report on Aquatic Toxicity, Biodegradation, and Subsurface Transport Experiments

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## FINAL

**January 2012**

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# Executive Summary

This document reports on the results of experimental activities performed to address and rank knowledge gaps in Tier II of the California multimedia risk assessment of biodiesel blends, as identified in the Tier I assessment of biodiesel as an alternative fuel in California (UC, 2009) and as outlined in the plan for these experiments (Ginn et al., 2009). These experimental investigations include study of toxicity, transport in porous media, and aerobic biodegradation. Further testing (solubility, materials compatibility) identified in the Tier II plan were not pursued as a result of time and funding limitations.

Additionally, a Tier II Biodiesel Air Emissions Characterization and $NO_x$ Mitigation Study was coordinated by California Air Resources Board (CARB) in conjunction with researchers from the University of California Riverside (UCR), the University of California Davis (UCD), and others including Arizona State University (ASU). The results of this study are reported in Durbin, et al., 2011.

The summary and results of each of the toxicity, transport in porous media, and aerobic biodegradation experimental suites are as follows.

## Aquatic Toxicity Tests

A series of aquatic toxicity tests were conducted on the seven fuel types including ultra-low sulfur diesel (ULSD), neat 100% biofuels derived from animal fat (AF B-100) and soy (Soy B-100) feed stocks as well as 80% ULSD:20% (w/w) mixtures of the two biofuels (AF B-20 and Soy B-20) and two B-20 mixtures amended with an antioxidant additive (AF B-20A and Soy B-20A). The chronic toxicity test species included three freshwater organisms including a green alga (*Selenastrum capricornutum*), an invertebrate (water flea, *Ceriodaphnia dubia*), and a fish (fathead minnow, *Pimephales promelas*), along with three estuarine organisms including a mollusk (red abalone, *Haliotis rufescens*), an invertebrate (mysid shrimp, *Mysidopsis bahia*) and a fish (topsmelt, *Atherinops affinis*). The water accommodated fraction (WAF) of each fuel was prepared by the slow-stir method and tested using a control and six concentrations of WAF (1, 5, 10, 25, 50, and 100%). The tests closely followed published USEPA protocols with regard to quality assurance (QA) including statistical evaluation of test endpoints, monitoring of water quality conditions in test solutions, and protocol control performance requirements. Statistical evaluation of test results included determination of the no-observable-effect-concentration (NOEC), lowest-observable-effect-concentration (LOEC), Effects Concentration ($EC_{25}$ and $EC_{50}$) for each test protocol endpoint. Sensitivity of the test organisms to the fuels was evaluated by comparing toxic units (TUs; $100/EC_{25}$, For example if 25% of the population shows effects at 50WAF, then the TU is 100/50=2. On the other hand if 25% of the population shows effects at 1WAF, then the TU is 100/1=100. This way, TU is an increasing measure of toxicity). Each of the tests met all protocol QA requirements and tests that were repeated to assess consistency, closely matched the results of the original test. Results of the tests varied widely depending on fuel type and test species. Tests with ULSD only detected effects on mysid growth (1.0 TU) and water flea reproduction (1.8 TU). None of the AF or Soy B-100 fuels or their B-20 mixtures without antioxidant additive produced detectable effects on mysid, topsmelt or fathead minnow endpoints. However, both B-100 biofuels and their B-20 mixtures caused variable effects on algae cell growth (5 - 21.3 TU), water flea survival and reproduction (<1 - 21.3 TU) and abalone shell development (3.0 - 35.5 TU). Except for algae, tests with the additized B-20 fuels

consistently resulted in substantially greater toxicity than was detected with the unadditized B-20 fuels, suggesting that conducting screening for a less toxic additive may be warranted.

The Lawrence Berkeley National Laboratory (LBNL) Environmental Energy Technologies Division provided chemical analyses of the biodiesel/diesel components present in the WAFs prepared in a similar manner to those used during toxicity testing. Sample chemical analyses were not taken during toxicity testing.

LBNL developed and applied a stir bar sorptive extraction (SBSE) method followed by thermal desorption gas chromatography/mass spectrometry (TD-GCMS) analysis to identify and quantify the chemical composition of the aqueous-phase solutions for four different biofuels and ULSD under four different WAF preparations. Insufficient ULSD sample volume led to an analysis of the four biofuels under four WAF preparations, for a total of 16 analyses.

The fuels analyzed included all the biodiesel mixtures used during toxicity testing (AF B-100, Soy B-100, AF B-20, Soy B-20). Since unadditized ULSD was not available, all the resulting fuel mixtures were additized. In addition, the same four salinity and temperature conditions used during the toxicity testing were used during the preparation of the WAFs eventually analyzed.

The chemical analyses did not unambiguously reveal any causative compound for the toxicity, and further testing is required to confirm the identity of compounds or combination of compounds responsible for the toxic response.

## Infiltration Experiments

Small-scale laboratory infiltration experiments in two-dimensional sandboxes were done to visualize the relative rates of biodiesel infiltration, redistribution, and lens formation on the water table in comparison to that of ULSD. Experimental design involved unsaturated sand as model porous media with ~20cm vertical infiltration of fuels to the saturated zone. Experiments were performed in triplicate for Animal Fat and Soybean based biodiesel, including pure (B-100) and blended (B-20) biodiesel formulations. As a control, AF B-100 with antioxidant was also tested and it showed similar behavior to unadditized AF B-100. Digital photography was used to record images of fuel behavior in side-by-side tests of biodiesel blend and ULSD. Experiments in each of the four blends (AF B-100, AF B-20, Soy B-100, and Soy B-20) were run to effective steady-state lens formation on the top of the saturated zone (water table) that involved durations ranging from 1.5 to 2 hours, with on average 24 photographs taken per experiment, generating 288 images. (24 snapshots in time x 4 fuel blends x 3 replicates). The experiments found that Soy B-100, Soy B-20, as well as AF B-20, do not exhibit any significant differences among the four temporal metrics used to time the infiltration and lens formation, nor among the qualitative unsaturated zone residual or lens shape at steady state, compared to the same metrics for ULSD. However while the AF B-100 percent blend exhibited mostly the same values of the infiltration timing metrics as ULSD, it showed noticeable increases in the amount of residual that occurred in the unsaturated zone, and it resulted in final lens geometry that was thicker in vertical dimension and less extensive in horizontal dimension than the ULSD lens. This behavior is consistent with the physical properties of animal fate biodiesel that include higher viscosity and interfacial tension than ULSD.

## Biodegradation Experiments

Microcosm experiments were conducted to assess the aerobic aqueous biodegradation potential for solutions in contact with biodiesel fuels, relative to ULSD. Fuels mixtures used were AF B-

100, AF B-20, Soy B-100, Soy B-20, and ULSD. These mixtures were used as source phases with and without antioxidant and biocide additives, with ULSD tested for comparison. Experiments were done in batch (250ml) with 2g of soil inoculum added to 190ml of stock solution with addition of 5 μL of test fuel as substrate. Experiments were performed in a respirometer in which the CO2 production in microcosms was measured during the experiment for duration of 28-30 days. Control experiments using sterilized inoculated solution with substrate were done to examine whether the test substrate is degraded abiotically and to test the adsorption of test substrate onto glass and or inoculum material. Controls with inoculum but no fuel also were prepared to test for CO2 production by microorganisms in absence of substrate. Results show enhanced CO2 production for all biodiesel blends and all additive combinations relative to that for ULSD. With some minor variations among blends (soy vs. animal fat; additized vs. non-additized), the results indicate that the additives effects are not significant on the biodegradation of biodiesel blends, and the blends tested are all more readily biodegrable than ULSD.

## Biodiesel Tier II Summary

Experimental investigations address the knowledge gaps as follows:

- Tested biodiesel blends exhibit somewhat increased toxicity to subsets of tested species compared to ULSD, and additized blends increase this toxicity for a smaller subset of tested species. Future testing addressing the potential toxicity of additives including chemical analysis of exposure medium may be needed.
- Biodiesel fuel blends show similar infiltration and lens formation to ULSD in unsaturated sandy porous media, with AF B-100 exhibiting greater residual in the vadose zone and less spreading of fuel lens on subsurface water table, consistent with increased viscosity and interfacial tension of this fuel.
- Aerobic biodegradation of biodiesel is faster and more extensive than that of ULSD across a range of fuel blends and included additives.

## Remaining Tier II Uncertainties

- Additional testing addressing the potential toxicity of additives including chemical analysis of exposure medium is needed.
- Of the three groups of additives only blends with antioxidants, and biocidal additives (biodegradation experiments only) were studied. Cold flow additives were not studied in any of the performed experiments. The impact of cold flow additives on aquatic toxicity and biodegradation needs to be studied.
- Infiltration experiments with biocidal and cold flow additives were not performed. Additional test may be needed as those additives may have different impact on the biodiesel infiltration.

# 1. Background

This document summarizes the results of experiments performed at Davis and Lawrence Berkeley National Laboratory (LBNL) as part of the Tier II Multimedia Risk Assessment of Biodiesel for the State of California. Existing research on the topic has been collected in UC (2009), the Multimedia Working Group (MMWG) Tier I report (referred to henceforth at the "Tier I report")[1], and the plan for these experiments is found in the "Experimental Plan for Tier II Evaluation of Biodiesel," (Ginn et al., 2010)[2] referred to henceforth at the "Tier II Plan"). Biodiesel B-100 is defined here as a mono-alkyl ester-based non-petroleum derived diesel substitute meeting ASTM D6751-12 (Standard Specification for Biodiesel Fuel Blend Stock (B100) for Middle Distillate Fuels). Biodiesel blends B50, B20, B5 also referred to as "biodiesel" are mixtures of B100 with California Air Resources Board Ultra Low-Sulfur Diesel #2 (ULSD) in indicated proportions, by volume. Biodiesel studied here is primarily fatty-acid methyl esters (FAMEs) resulting from the trans-esterification of oils derived from animal fats or vegetable/seed oils or other feedstocks, and may include residual reactants and products of the transesterification (e.g., methanol, water, etc.)

The purpose of the experiments performed is to fill knowledge gaps pertaining to the fate, transport, biodegradation, and toxicity properties of biodiesel occurring in the environment due to unintended precombustion releases.

| Knowledge Gap | Approach |
|---|---|
| Toxicity | Aquatic toxicity experiments |
| unadditized | tested |
| cold flow additive | not tested |
| biocidal additive | not tested |
| antioxidant additive | tested |
| Fate & transport | "Ant Farm" experiments |
| Biodegradation | Microcosm experiments |
| unadditized | tested |
| cold flow additive | not tested |
| biocidal additive | tested |
| antioxidant additive | tested |
| Release scenarios | not tested |
| Air emissions studies | ongoing by CARB |
| Solubility | not tested |
| Materials Compatibility | not tested |

In all instances the experiments are intended to address *relative* risk as compared to that associated with ULSD. Because of time and funding limitations, the experiments performed are designed to address the highest priority knowledge gaps identified in Tier I and outlined in the

[1] http://www.arb.ca.gov/fuels/diesel/altdiesel/090910biodiesel-tier1-final.pdf
[2] www.arb.ca.gov/fuels/multimedia/031209TierIIrev.pdf

Tier II plan, and in a simplified and riskwise conservative fashion. The Tier I study identified as high priority knowledge gaps, Additives impacts, Subsurface fate & transport properties, Biodegradation in soils and aquifers, production and storage release scenarios, complete air emissions studies (Tier I Report, pages 75, 76). These issues are partly addressed in the experimental plan described here as follows:

Budget and time constraints required restriction of the experimental investigation to incomplete treatment of the knowledge gaps identified, and so the experiments cover the highest priority issues. Thus impacts of cold flow additive, evaluation of release scenarios, aqueous solubility, and materials compatibility are not evaluated in this Tier II study. Toxicity studies are restricted to marine, esturine, and freshwater toxicity.

Additionally, a Tier II Biodiesel Air Emissions Characterization and $NO_x$ Mitigation Study was coordinated by California Air Resources Board (CARB) in conjunction with researchers from the University of California Riverside (UCR), the University of California Davis (UCD), and others including Arizona State University (ASU). The results of this study are reported in Durbin, et al., 2011.

# 2. Tier II Experimental Descriptions

Blend selection is restricted to two feedstocks and two blend ratios, B-20 and B-100, as these represent the highest expected use and maximum biodiesel samples respectively. Feedstocks include Soy and Animal fat, as they reflect high potential use and wide bracketing of dominant feedstock chemistry. Additives have been selected by criteria defined in Appendix I of the Tier I report: in summary, antioxidant and biocide additives are hypothesized as those most likely to incur departures from ULSD behavior, so one representative additive from each category is selected. These feedstock and additive selections are also made in order to be consistent with ongoing CARB emissions testing.

The following three suites of tests have been carried out.

1. Aquatic **toxicity** tests were carried out to evaluate the relative toxicity of biodiesel blends potentially released to aquatic environments. Chemical analyses of separately prepared water accomodated fractions was performed in an attempt to identify the chemical compounds associated toxic responses.
2. Sandbox **infiltration** tests are a visual method for studying fluid transport through unsaturated two-dimensional porous media to contact with a saturated zone resulting in lens formation at the unsaturated-saturated interface.
3. Microcosm study and $CO_2$ evaluation were used to study the rates of biodiesel **biodegradation** under aerobic conditions by soil microbes.

Table 1 shows the experimental matrix reflecting the selection of different additive combinations (columns) for testing with different fuel blends (rows), in experimental suites labeled by letter with identifications in the caption. The selection reflects prioritization of particular additives for association with higher risk impacts such as biocides impacting biodegradation as described in the Appendix 1 of the Tier II Plan.

**Table 1. Tier II Testing Matrix:**

| **Fuel Preparation** | | | |
|---|---|---|---|
| ULSD | T, I, B[a] | | |
| Soy B-100 | T, B | I, B, A | B |
| Animal fat B-100 | T, I, B | I, B, A | B |
| Soy B-20 | T, B | T, I, B, A | B |
| Animal fat B-20 | T, B | T, I, B, A | B |
| **Additives** | Reference | Bioextend-30 | Kathon FP 1.5, Bioextend-30 |
| **Additive Type** | No Additive | Antioxidant | Biocide and Antioxidant |

[a] Experimental codes are T = Toxicity, A = Analyses, I = Infiltration, B = Biodegradation.

The experimental details for each of the three experimental suites, Aquatic Toxicity with Chemical Analyses, Infiltration, and Biodegradation, are presented in the Appendices A and B, C, and D, respectively. These sections include particulars of experimental design, experimental permutations (fuel blends/additives, experimental conditions) tested, execution of experiments,

and results. Conclusions of the experiments are presented here in terms of the relevance to the filling of the knowledge gaps identified in the Tier II plan of the California multimedia risk assessment for biodiesel.

# 3. Tier II Results and Conclusions

## 3.1. Aquatic Toxicity Experiments

Aquatic toxicity testing involved ULSD compared to Soy and Animal Fat (AF) B-20 and B-100 unadditized fuels, and Soy and AF B-20 with an antioxidant additive. Tests involved three freshwater organisms (green alga, fathead minnow larvae, and water flea) and three estuarine/marine organisms (red abalone, mysid shrimp, and topsmelt fish). Toxicity endpoints for each species are detailed in Appendix II-A. Toxicity metric in each case includes both the 25 and 50% Effects Concentrations ($EC_{25}$, $EC_{50}$) as reported in Appendix II-A. For instance, $EC_{25}$ is the relative concentration in percent of substrate (relative to equilibrium solubility concentration of a given fuel in aqueous phase) at which 25 percent of the test species population exhibits an effect. Also reported are Toxicity Units, "TU," defined as the quantity $100/EC_{25}$. Thus, if one-quarter of a population shows an effect only at the 100% concentration (that corresponding to equilibrium solubility) then the TU value = 100/100 = 1. If however one-quarter of a population exhibits an effect at the concentration equal to 1% of the equilibrium solubility concentration, then the TUc value = 100/1 = 100. Each fuel/species combination tested involved identical solute preparation, standardized to create an experimentally defined "equilibrium solute concentration" resulting from timed exposure of an aqueous phase to the ULSD or biodiesel blend. Details are given in Appendix II-A. The results are as follows.

- ULSD produced relatively low but detectable toxicity on mysid growth (1.0 TUc) and water flea reproduction (1.8 TUc). No toxicity (< 1.0 TUc) was detected with any of the other species tested.
- Neither of the unadditized Animal Fat or Soy biodiesel test materials produced detectable toxicity to the mysid, topsmelt or fathead minnow.
- Animal Fat B-100, Soy B-100 and their B-20 mixtures caused toxicity to algae cell growth, water flea survival and/or reproduction, and abalone shell development.
- Tests that were repeated for confirmation produced similar results as the original test.
- Except for algae, the additized biodiesel B-20 test materials were substantially more toxic than the corresponding unadditized material. Figure 1 illustrates the frequency and magnitude of the toxic response to the additized AF B20a and Soy B20a exposures, as Toxicity Unit (TU) response for all species and all endpoints except for that of Green Algae that showed a different trend (reduced toxicity with additive). Note that the vertical axis is on a logarithmic scale for TU. Maximum toxicity was achieved for all species (except for that of Green Algae) in their exposure to AF and/or Soy B20a (with additive). This toxicity was pronounced (greater than or equal to 50 TU) for *C. dubia* and Abalone.

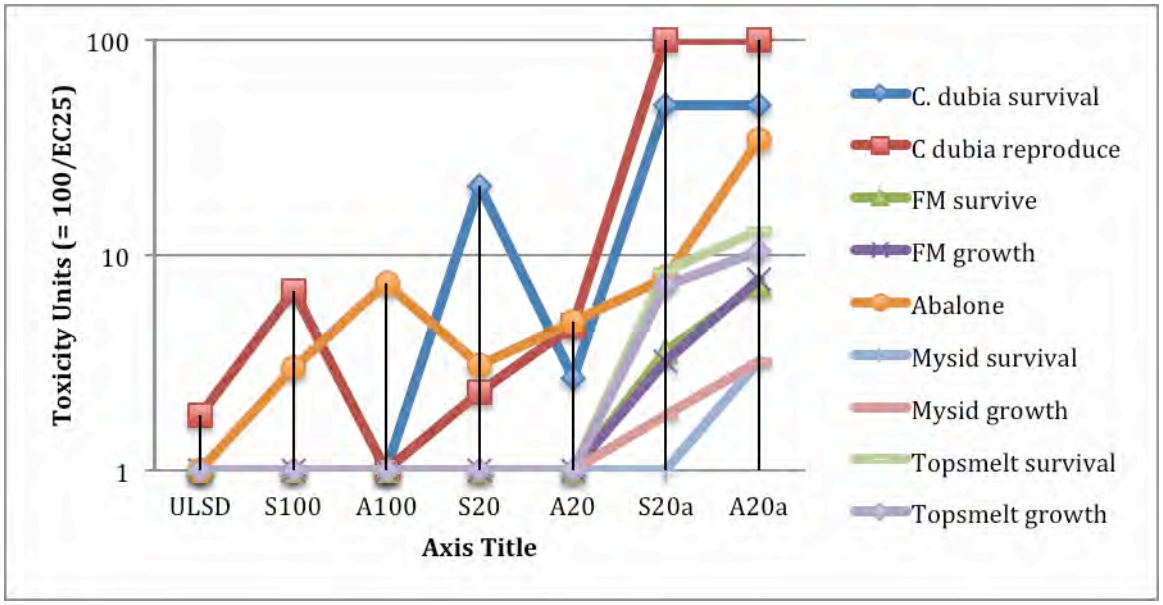



**Figure 1. Toxicity scores (as Toxicity Units, = 100/ $EC_{25}$), for the different endpoints (e.g., survival, reproduction, growth) of 5 of the 6 species tested, as a function of fuel blend to which species was exposed. The graph is absent Green Algae that showed different behavior than the trend observed here.**

## 3.2. Chemical Analyses of Selected Water Accomodated Fractions

The LBNL Environmental Energy Technologies Division provided chemical analyses of the biodiesel/diesel components present in the WAFs prepared in a similar manner to those used during toxicity testing. Samples for chemical analysis were not taken during toxicity testing. LBNL developed and applied a stir bar sorptive extraction (SBSE) method followed by thermal desorption gas chromatography/mass spectrometry (TD-GCMS) analysis to identify and quantify the chemical composition of the aqueous-phase solutions for four different biofuels and ULSD under four different WAF preparations. Insufficient ULSD sample volume led to an analysis of the four biofuels under four WAF preparations, for a total of 16 analyses.

The fuels analyzed included all the biodiesel mixtures used during toxicity testing (AF B-100, Soy B-100, AF B-20, Soy B-20). Since unadditized ULSD was not available, all the resulting fuel mixtures were additized. As noted above the most toxic cases for *all* species with the exception of Green Algae corresponded to exposure to 20% blends with additive. Therefore we analyzed the four WAFs after exposure to AF B20a and Soy B20a. To also evaluate occurrence of additive in the 100% biofuel cases we analyzed the four WAFs after exposure to AF B100a and Soy B100a as well.

In addition, the same four salinity and temperature conditions used during the toxicity testing were used during the preparation of the WAFs eventually analyzed. Conditions used (mixing temperature and salinity) of these solutions are given in Table II-B-1 of Appendix II-B.

The measured chemical concentrations for each of the fuel WAFs are listed in Tables B8 – B11 of Appendix II-B for Soy-B100a, Soy-B20a, AF-B100a and AF-B20a, respectively. The antioxidant fuel additives acetic acid, butyl ester and 1,4-Benzenediol, 2-(1,1-dimethylethyl), also known as TBHQ, were identified in the majority of the samples. However, the

concentrations were highly variable. We presume that the addition of the additive to the original fuel was consistent so the variability was likely due to either the WAF mixing conditions or the extraction conditions. The additive butyl acetate was lowest in the WAF-04 sample, which had the highest salinity so the solubility may be affected by pH. Without further testing one cannot rule out the extraction as a source of the variability for either of the measured additives.

Despite the variability, the concentrations of acetic acid butyl ester additive do in fact increase in all four WAFs from Soy B20a exposures to AF B20a exposures, and this is consistent with the increase in toxicity for the majority of species/endpoints between Soy B20a and AF B20a exposures (see Figure 1, right-hand side). However the measured concentrations of this additive are generally below 50 ug/l, whereas the concentrations associated with toxicities (EC50) reported for various species in the Materials Safety Data Sheet for this compound are in the 10's-100's of mg/l range. TBHQ did not appear increasing from Soy B20a to AF b20a exposed WAFs and concentrations overall were rather low.

The only other compounds exhibiting increased concentrations associated with Soy B20a to AF B20a WAFs include some petroleum diesel compounds and some FAMEs, both at low or suspect concentrations. Both of the animal fat biofuel WAF-01 (low salinity) mixtures (AF B-100, AF B-20) had significantly higher concentrations of FAMEs and the Soy-B100 WAF-01 also had somewhat elevated FAME. Sample contamination was suspected in the form of oil droplets present in the AF-B100 WAF-01 (greyed out values in Table 10) but this was not noticed in the other WAF-01 samples. Comparing the average results for the duplicate AF-B20 WAF-01measurements to the previous measurement used in the range finding pre-experiment calibration found that the later measurements seem to have been contaminated with FAME. Both the initial measurement from the range finding and the average of the replicate measurements are reported in Table 11 but the results with high FAME are likely due to contamination. The low level of FAME in the Soy-B20 WAF_01 rules out contamination in the source water used to mix the WAF. Further testing would be needed to determine if the mixing conditions used for the WAF_01 samples resulted in elevated FAME in the Soy-B100 relative to the Soy-B20 or if the difference was due to contamination during mixing.

Only one alkane (2,2,3,3-tetramethyl-Butane) was measured in the WAFs and it was also detected at elevated levels in the blanks, including the HPLC water and in the direct analysis. The fact that the alkane was in the diluted fuel which was not extracted with stir-bar indicates that the methanol used in the dilution may have been the source.

In summary, the chemical analyses failed to identify unequivocally a source of the toxicity observed. Hypotheses that may explain the observations include a co-solvency effect associated with a compound in the Soy B20a and AF B20a exposed WAFs that facilitates higher aqueous concentration of a petroleum diesel compound, enhanced (cross-) toxicity associated with the acetic acid, butyl ester additive in combination with another (or more) FAME or petroleum diesel component. Further toxicity experiments that include chemical analysis of exposure media may be useful.

## 3.3. Infiltration Experiments

Small-scale laboratory infiltration experiments in two-dimensional sandboxes with glass walls to allow visualization of dyed fuels were completed to allow observation of the relative rates of biodiesel infiltration, redistribution, and lens formation on the water table in comparison to that

of ULSD. These experiments were performed at UC Davis in the lab of Professor T. R. Ginn and involved various preliminary experiments to establish standard procedures, and these are detailed in Appendix II-C. Experiments involved unsaturated sand as model porous media with ~20cm vertical domain of unsaturated zone above the saturated level of the sand. Dyed fuel samples (a biodiesel blend and a ULSD sample) of identical volumes were simultaneously emplaced in divots in the sand surface at the top of the sandbox, and time-lapse digital photography was used to record infiltration of this ponded source fuel, redistribution and residual formation in the unsaturated zone, and lens formation on the top of the saturated zone. Experiments were performed in triplicate for animal fat and soybean based biodiesel, including pure (B-100) and blended (B-20) biodiesel formulations (as well as animal fat B-100 with antioxidant additive as a control). Experiments in each of the four blends (AF B-20, AF B-100, Soy B-20, and Soy B-100) were run to effective steady-state lens formation on the top of the saturated zone (water table) and involved durations ranging from 1.5 to 2 hours, with on average 24 photographs taken per experiment. A complete description of the experiments and a complete catalogue of the images is contained in Hatch (2010), a summary form of which comprises Appendix II-C.

Visual analyses of these images was done to evaluate four separate time metrics defined in order to time the progress of the infiltration, redistribution, and formation of the lens of biodiesel on the saturated zone surface at the steady-state. These metrics are characteristic times for: elimination of ponded fuel, plume separation from surface, initial commencement of lens spreading on water table, steady-state lens formation on water table. In addition the qualitative characteristics of quantity of residual fuel appearing in the unsaturated zone and of lens shape after steady-state are reported. The experiments show that

- The antioxidant additive did not affect the infiltration of AF B-100
- Soy biodiesel blends at both 20 and 100 percent, as well as the AF 20 percent blend, do not exhibit any significant differences among the four temporal metrics or among the qualitative residual or lens shape metrics compared to ULSD.
- Animal fat 100 percent blend exhibited similar values of the temporal metrics as ULSD, but it showed noticeable increases in the amount of residual that occurred in the unsaturated zone, and it resulted in final lens geometry that was thicker in vertical dimension and less extensive in horizontal dimension than the ULSD lens.

This behavior is consistent with the physical properties of animal fate biodiesel that has higher viscosity and interfacial tension than ULSD. These differences become significantly more pronounced at temperatures below 20 degrees Celsius.

## 3.4. Biodegradation Experiments

Aerobic biodegradation is a primary path for natural remediation of unintentional releases of fuel compounds. Although anaerobic conditions may make up a larger fraction of the environmental domain in which fuels may occur, aerobic conditions are typically encountered first in releases, and are selected in the Tier II plan as the highest priority knowledge gap for natural remediation of biodiesel. In order to investigate the relative rates of aerobic biodegradation of biodiesel blends and ULSD, microcosm experiments were conducted in laboratory setting with 250ml batch reactors. Fuels derived from animal fat and soy feedstocks at B-100 and B-20 mixtures (with ULSD making up the complement) were used as source phases, with ULSD tested for comparison. The biodiesel blends included either no additives, an antioxidant additive, or both an

antioxidant and a biocide additive, at manufacturer-specified concentrations, while the reference ULSD fuel contained no additives. This experimental approach is designed intentionally as a conservative evaluation of the differences in biodegradation potential between petroleum and biomass-derived diesels. Each batch reactor includes190 ml of prepared solution, 2g soil (Yolo, silty-loam) as bacterial inoculum and addition of 5μL of test fuel as substrate. Experiments were performed in a respirometer in which the CO2 production in microcosms was measured during the experiment for duration of 28-30 days. Control experiments using sterilized inoculated solution with substrate were done to examine whether the test substrate is degraded abiotically and to test the adsorption of test substrate onto glass and or inoculum material. Controls with inoculum but no fuel also were prepared to test for CO2 production by microorganisms in absence of substrate. Conclusions are as follows.

- Controls reveal no CO2 production in the absence of fuel substrate
- Controls reveal no CO2 production in the absence of soil inoculum
- Respironmeter data show enhanced CO2 production for all biodiesel blends relative to that for ULSD.
- Additives do not impart a significant effect on the aerobic biodegradation of biodiesel blends

## 3.5. Biodiesel Tier II Summary

Experimental investigations address the knowledge gaps as follows:

- Tested biodiesel blends exhibit somewhat increased toxicity to subsets of tested species compared to ULSD, and additized blends increase this toxicity for a smaller subset of tested species.
- Biodiesel fuel blends show similar infiltration and lens formation to ULSD in unsaturated sandy porous media, with AF B-100 exhibiting greater residual in the vadose zone and less spreading of fuel lens on subsurface water table, consistent with increased viscosity and interfacial tension of this fuel.
- Aerobic biodegradation of biodiesel is faster and more extensive than that of ULSD across a range of fuel blends and included additives.

## 3.6. Remaining Tier II Uncertainties

- Additional testing addressing the potential toxicity of additives including chemical analysis of exposure medium is needed.
- Of the three groups of additives only blends with antioxidants, and biocidal additives (biodegradation experiments only) were studied. Cold flow additives were not studied in any of the performed experiments. The impact of cold flow additives on aquatic toxicity and biodegradation needs to be studied.
- Infiltration experiments with biocidal and cold flow additives were not performed. Additional test may be needed as those additives may have different impact on the biodiesel infiltration.

# 4. Tier II References

Durbin, et al. 2011. CARB Assessment of the Emissions from the Use of Biodiesel as a Motor Vehicle Fuel in California, Biodiesel Characterization and NOx Mitigation Study. Final Report. October 2011.

Ginn, T. R., M. B. Johnson, J.A. Last, K.M. Scow, L. Rastagarzadeh, T. Hatch, P. L'Amoreaux, V. Nino. R. Okamoto, R. Hodam. 2009. Experimental Plan for Tier II Evaluation of Biodiesel 1st revision, 9 March 2009, Prepared for the California Environmental Protection Agency Multimedia Working Group, 22 pp.

Hatch, T. 2010. Biodiesel Relative Risk: A Qualitative Approach to Determining the Environmental Fate of Animal Fat and Soy Biodiesels through a Direct Experimental Comparison with ULSD and Screening Model Simulations using HSSM. Thesis submitted in partial satisfaction of the requirements for the degree of Master of Science, Department of Civil and Environmental Engineering, University of California, Davis.

University of California (UC). 2009. California Biodiesel Multimedia Assessment Tier I Report (Final Draft). Prepared for the California Environmental Protection Agency Multimedia Working Group, by the University of California, Davis and the University of California, Berkeley. Sept. 2009. 95 pp.

# 5. Tier II Appendices

# 6. Appendix II-A: Toxicity of Biodiesel Blends And ULSD to Selected Freshwater and Marine/Estuarine Organisms

## Background

Biodiesel is a fuel composed of monoakyl esters of long chain fatty acids derived from biological sources such as animal fat or vegetable oils. It can be used as a pure fuel or as a blend with petroleum diesel, since it is miscible with diesel at all ratios. The most common blend is B20 (20% biodiesel with 80% ultra-low sulfur diesel, ULSD). Since biodiesel is a new fuel, the California air Resources Board must provide a "multimedia risk assessment". As a result, the California Environmental Protection Agency has initiated a 3-tier program conducted by UC Davis and UC Berkeley to assess the multimedia life-cycle impacts, including ecological effects, of biodiesel fuels used in California. One of the data gaps identified by the Tier I assessment (1) is the paucity of aquatic toxicity information on the most common biofuels, from soy and animal feedstocks, along with their most common blend and additive. The impact of biodiesel is assessed as a relative risk compared with ULSD. Accordingly, AQUA-Science was retained by UC Davis (Dr. Michael Johnson, Director of the Ecosystems Analysis Laboratory) to conduct aquatic toxicity testing using a suite of three freshwater and three estuarine/marine organisms. The test organisms are phylogenetically diverse and have published USEPA aquatic toxicity protocols available. AQUA-Science has over 30 years experience in conducting these test protocols and is certified by the Environmental Laboratory Accreditation Program (ELAP; Certificate No. 2205) to conduct chronic toxicity tests with all six organisms selected for this study.

## Methods and Materials

### Source and Preparation of Biodiesel Test Solutions

The test materials included seven fuel types, including ultra-low sulfur diesel (ULSD), neat biofuels derived from animal fat (AF B-100) and soy (Soy B-100) feedstocks, 80% ULSD:20% (w/w) mixtures of the two biofuels (AF B-20 and Soy B-20), as well as the two B-20 mixtures amended with an antioxidant additive (AF B-20A and Soy B-20A). The test materials were provided by CA Air Resources Board (c/o R. Okamoto) and collected by T. Ginn/UC Davis and . stored in 1-gallon or 1-quart glass amber bottles in the dark at 20 °C with minimal headspace. Samples transferred to the AQUASCI lab were stored in original containers in the dark at 4°C until the water accommodated fractions (WAFs) were prepared. WAFs of the test materials were prepared using a low mixing energy procedure that eliminates the entrainment of particulate oil in the water column and prevents emulsification (2, 3, 4). The test materials were added to the top of a 2-gallon glass aspirator bottle containing the appropriate toxicity test dilution water at a 1:10 fuel-water ratio. The bottle was capped with aluminum foil and stirred using a magnetic stirrer at low speed (~120 rpm using a stir bar of 1.5 cm L x 0.5 cm diameter) without vortex formation. Mixing was conducted at the toxicity test protocol temperature for 18 hours followed by a 2-hour settling period to allow re-coalescence and surfacing of bulk oil particles. The WAF was carefully removed by siphon and stored at toxicity test protocol temperature until use within 24 hours of preparation. Samples of each WAF (100 mL) were taken immediately after

preparation and from the highest concentration in the toxicity test after 24 hours or at test termination (as appropriate) for analytical chemistry. The fuels and mixtures tested in this study are shown in Table II-A-1.

**Table II-A-1. Fuels used in the Aquatic Toxicity testing**

| ***Fuel Type***[a] | ***Code*** |
|---|---|
| 100% Ultra-Low Sulfur Diesel | ULSD |
| 100% Soy Biodiesel | Soy B-100 |
| 20% Soy Biodiesel + 80% ULSD (w/w) | Soy B-20 |
| 20% Soy + 80% ULSD (w/w) amended with additive[b] | Soy B-20A |
| 100% Animal Fat Biodiesel | AF B-100 |
| 20% Animal Fat Biodiesel + 80% ULSD (w/w) | AF B-20 |
| 20% Animal Fat + 80% ULSD (w/w) amended with additive | AF B-20A |

a Soy and Animal Fat refer to the feed stocks for the fuel
b The additive was Eastman BIOEXTEND™ 30 antioxidant

**Aquatic Toxicity Tests**

The suite of aquatic test organisms tested in this study included both freshwater and estuarine/marine species comprising a wide phylogenetic diversity. Freshwater organisms included a green alga (*Selenastrum capricornutum*), a larval fish (fathead minnow, *Pimephales promelas*), and an invertebrate (water flea, *Ceriodaphnia dubia*). These species constitute the USEPA three-species test series that is employed extensively throughout the U.S. to evaluate the toxicity of discharges (treated effluents and storm waters), as well as chemicals that may enter ambient freshwaters (5). The estuarine/marine organisms included a mollusk (red abalone, *Haliotis rufescens*), an invertebrate (mysid shrimp, *Mysidopsis bahia*), and a fish (topsmelt, *Atherinops affinis*). The abalone and topsmelt are species recommended by USEPA when tests are used in assessment of toxicity of effluents and chemicals discharged to West Coast estuarine and marine waters (6), while the mysid shrimp is a standard estuarine/marine species recommended by USEPA (7) for use in toxicity tests with discharges into all estuarine receiving waters. A summary of the test protocol conditions are shown in Table II-A-2.

For continuity, each of the toxicity tests were conducted using the same dilution series: Control (laboratory dilution water amended to protocol specifications), 1, 5, 10, 25, 50 and 100% WAF for each fuel and mixture. Some tests were randomly repeated to check for reproducibility.

**Table II-A-2. Summary of Aquatic Chronic Toxicity Test Protocol Conditions**

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

*Green Algae Chronic Test Procedures*

The 96-hour algae (*S. capricornutum*) toxicity tests were conducted in 4 replicates of 125-mL flasks containing 50-mL of test sample filtrate (0.45 µm). A fifth replicate was used as a surrogate for daily water quality measurements. The flasks, containing algal assay media with EDTA, were inoculated with 1 x $10^4$ cells/mL of a 2-4 day-old culture of *S. capricornutum* (University of Texas Algae Type Collection, Austin, TX) in log phase growth. A sixth replicate was tested without algae inoculate to confirm that indigenous algae were not present. This replicate was also used as a sample blank. Flasks were placed on a shaker table (100 rpm) in an environmental chamber at 25 °C ± 1 °C with continuous lighting (400 ± 40 fc) and were randomized twice daily. After the 96-hour test period, the absorbance was measured with a spectrophotometer at 750 nm (Model DR2800, Hach Co., Loveland, CO). The absorbance units were corrected to cell number using a calibration curve as follows:

cell number = (absorbance units @ 750 nm x 13.026) - 0.0328 ($R^2$ = 0.9995)

Using this conversion, the test was acceptable if the mean algal density in the control flasks was greater than or equal to 1 x $10^5$ cells/mL and the coefficient of variation in the control replicates was ≤20%.

*Water Flea Chronic Test Procedures*

Water flea *(C. dubia*) neonates (< 24 hours old) were obtained from in-house cultures maintained in reverse osmosis- and granular carbon-treated well water amended with dry salts to USEPA moderately hard (EPAHM) specifications. Tests were conducted in 20 mL glass scintillation vials containing 18 mL of test solution, which was renewed daily. There were ten vials per concentration with one *C. dubia* per vial. EPAMH was used as dilution water. Tests were conducted in an environmental chamber at 25 ± 1 °C with a photoperiod of 16 hours light:8 hours dark. Organisms were fed a mixture of green algae (*S. capricornutum*); University of Texas Algae Type Collection; Austin, TX), blended trout food (Silvercup, Murray, UT), and organic alfalfa obtained locally. Mortality and reproduction endpoints and water quality

parameters were monitored daily. The test was terminated after ≥60% of the controls had delivered three broods. The test protocol requires 80% survival and a minimum of 15 neonates per female in the control.

*Fathead Minnow Chronic Test Procedures*

Fathead minnows (*P. promelas*; < 24 hours old) were obtained from AQUA-Tox Inc. (Hot Springs, AK) via overnight air freight. Exposures were conducted in 500 mL glass beakers containing 200 mL of sample using 10 fish per replicate with 4 replicates per concentration, in a temperature-controlled room at 25 ± 1 °C with a photoperiod of 16 hours light:8 hours dark. Dilution water was reverse osmosis- and granular carbon-treated well water amended with dry salts to EPAMH specifications. Fish were fed *Artemia* sp. nauplii twice daily. Test solutions were renewed and mortality was noted daily. At test termination, fish were killed by immersion in anesthetic (MS-222), pooled by replicate, dried for 6 hours at 100 °C and weighed to an accuracy of 0.01 mg using an electronic balance (Denver Instrument Co., Denver, CO). The test protocol requires a minimum of 80% survival and a minimum weight of 0.25 mg/fish in the control.

*Red Abalone Chronic Test Procedures*

Gravid red abalone (*H. rufescens)* were obtained from The Cultured Abalone (Goleta, CA) and acclimated in a recirculating seawater system for ≥48 hours prior to testing. Test samples were brought to protocol salinity (34 ± 2 ppt), using hypersaline brine (HSB) prepared by freezing high quality seawater. Dilution water was EPAMH water amended with HSB to 34 ± 2 ppt. Four male and female abalone were induced to spawn using a hydrogen peroxide solution and gametes were collected separately. Sperm and eggs were combined and 2000 embryos were used for each replicate with five replicates per concentration. Tests were conducted in an environmental chamber at 15 ± 1 °C with a light intensity of 10 $\mu E/m^2/sec$ and a photoperiod of 16 hours light:8 hours dark. After 48 hours, embryos were removed from the replicates, washed with seawater, placed in 20-mL labeled glass vials, and terminated by addition of 750 µL of 37% formalin to each replicate. One hundred embryos from each replicate were examined microscopically and scored for normal shell development. The protocol acceptability requirement is ≥80% normal shell development in the control.

*Mysid Chronic Test Procedures*

Mysids (*M. bahia*; 7 days old at test initiation) were obtained from Aquatic Bio Systems, Inc. (Fort Collins, CP) via overnight air freight. Mysids were acclimated in EPAHM water amended with dry sea salts (Instant Ocean™, www.marinedepot.com) to 20-30 ± 2 ppt. Testing was conducted in an environmental chamber at 25 ± 1 °C using a 16 hours light:8 hours dark photoperiod. Test containers were 400 mL plastic beakers containing 250 mL of test solution using eight replicates containing five mysids for each test concentration. Mysids were fed *Artemia* sp. nauplii twice daily. Test solutions were renewed by 80% water replacement and mortality was noted daily. At test termination, mysids were anesthetized in an ice bath, grouped by replicate, dried at 100 °C for 6 hours and weighed to 0.01 mg using an electronic balance (Denver Instrument Co., Denver, CO). The protocol control performance requirements are ≥80% survival and a minimum weight of 0.20 mg/mysid.

*Topsmelt Chronic Test Procedures*

Larval topsmelt (*A. affinis*; 9-12 days old) were obtained from Aquatic Bio Systems, Inc. (Fort Collins, CO) via overnight air freight. Fish were acclimated in EPAMH water amended with dry sea salts to 25 ± 3 ppt. Testing was conducted in an environmental chamber at 20 ± 1 °C using a 16 hours light:8 hours dark photoperiod. Test containers were 600 mL plastic beakers containing 200 mL of test solution using five replicates containing five fish for each test concentration. Fish were fed *Artemia* sp. nauplii twice daily. Test solutions were renewed and mortality was noted daily. At test termination, fish were anesthetized (MS-222), grouped by replicate, dried at 100 °C for 6 hours and weighed to 0.01 mg using an electronic balance (Denver Instrument Co., Denver, CO). The protocol control performance requirements are ≥80% survival and a minimum weight of 0.85 mg/fish.

### Water Quality Measurements

Water quality measurements including temperature, dissolved oxygen (D.O.), pH, alkalinity, hardness, and conductivity or salinity were made on freshly prepared samples. Temperature, D.O. and pH were measured in 24-hour solutions from sample change-out. Temperature was measured in initial and daily test solutions at change-out with a calibrated digital thermometer (Central Co., Friendswood, TX), and was continuously recorded in the environmental chambers using a Dickson circular chart recorder (Model ICT855, Addison, IL). Water quality instrumentation included dissolved oxygen (YSI Model 550A, Yellow Springs, OH), pH (Beckman 240, Fulton, CO), and conductivity (WTW Model 330, Ft. Myers, FL) meters. Alkalinity (Hach Model AL-DT) and hardness (Hach HA-DT) were measured with Hach colorimetric tests (Hach Co., Loveland, CO).

### Test Endpoint Determination

Test endpoint calculations were performed using a computer program (ToxCalc v. 5.2.23, TidePool Scientific, McKinleyville, CA) and the results are reported in terms of four metrics, per species-endpoint combination. The metrics are: no-observable-effect-concentration (NOEC), the highest concentration that did not produced statistically significant effects compared with the control; lowest-observable-effect-concentration (LOEC), the lowest concentration that produced a statistically significant effect compared with the control; effects concentration affecting 25% of the test population ($EC_{25}$); effects concentration affecting 50% of the test population ($EC_{50}$); and toxic units (TU) defined as the reciprocal of the $EC_{25}$ x 100. The percent minimum significant difference (PMSD) is the smallest difference between the control and another test treatment that can be determined as statistically different in a given test. Therefore, PMSD is a measure of test sensitivity that is dependent upon the within-test variability. Each of the statistical outputs was checked against the test raw data by the Laboratory Quality Assurance Manager.

## Results and Discussion

Chronic toxicity test results for ULSD and the two biofuels and mixtures are presented by test species.

### Algae Chronic Toxicity Test Results

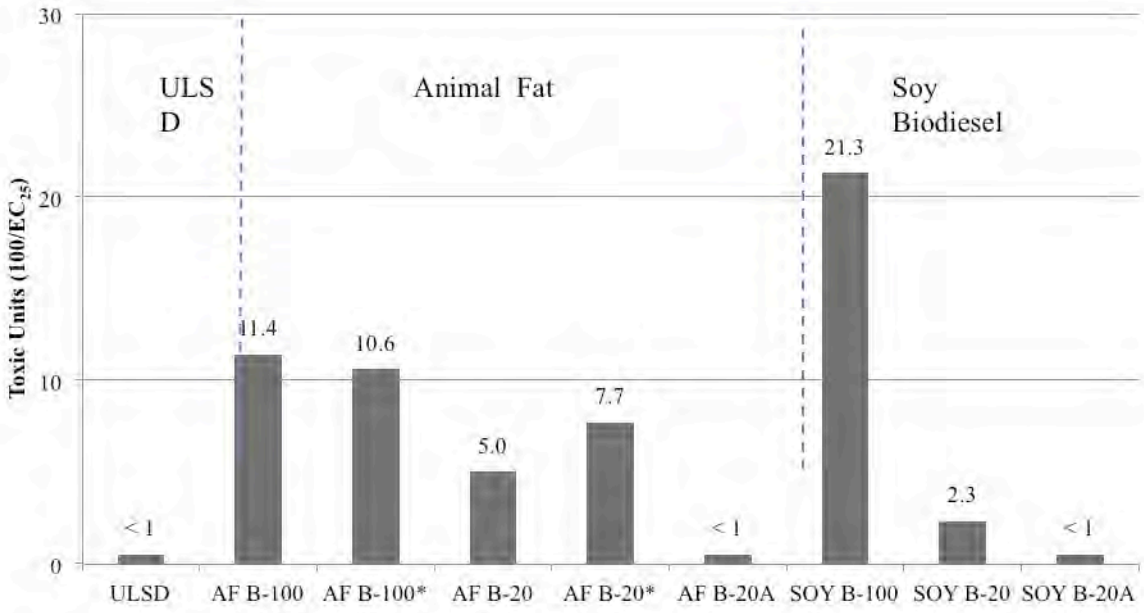
Table II-A-3 and Figure II-A-1 summarize results of the biofuel toxicity tests with green algae.

**Table II-A-3. Summary of Biodiesel Toxicity Tests with Green Algae (*S. capricornutum*)**

| *Fuel Type* | *Values are % WAF* | | | | *Toxic Units* | *PMSD* |
|---|---|---|---|---|---|---|
| | *NOEC[a] (%)* | *LOEC (%)* | *$EC_{25}$ (%)* | *$EC_{50}$ (%)* | *(100/$EC_{25}$)* | *(%)* |
| ULSD | 100 | 100 | > 100 | > 100 | **< 1.0** | 12.1 |
| AF B-100 | 1 | 5 | 8.8 | 26.1 | **11.4** | 9.3 |
| AF B-100[a] | 5 | 10 | 9.3 | 21.9 | **10.8** | 6.6 |
| AF B-20 | 5 | 10 | 13.0 | 28.9 | **7.7** | 6.2 |
| AF B-20[c] | 1 | 5 | 20.1 | > 100 | **5.0** | 6.4 |
| AF B-20A | 50 | 100 | > 100 | > 100 | **< 1.0** | 6.8 |
| Soy B-100 | 1 | 5 | 4.7 | 9.3 | **21.3** | 5.3 |
| Soy B-20 | 5 | 10 | 44.1 | 75.5 | **2.3** | 8.9 |
| Soy B-20A | 25 | 50 | > 100 | > 100 | **< 1.0** | 14.2 |
| Soy B-20A[a] | 50 | 100 | > 100 | > 100 | **< 1.0** | 9.1 |

[a] No-observable-effect-concentration
[b] Lowest-observable-effect-concentration
[c] Repeat test

**Figure II-A-1.Chronic Toxicity of Ultra-Low Sulfur Diesel (ULSD) and Biodiesel to Green**




* repeat test

ULSD did not produce a detectable reduction in algal cell growth, e.g., the NOEC=100%. Two tests conducted with AF B-100 resulted in TUc values of 11.4 and 10.6, while two tests conducted with AF B-20 demonstrated less toxicity with values of 5.0 and 7.7. Toxicity tests with the Soy biodiesel resulted in 21.3 TUc for the Soy B-100 and 2.3 TUc for the Soy B-20. The Soy B-20A and the AF B-20A mixtures with the additive did not exhibit toxicity, which was surprising given the increased toxicity imparted by the additive in toxicity tests with all of the other species. Additional tests with the additive and with the B-20 mixtures coupled with the analytical chemistry results would be required to elucidate the causes of these results.

**Water Flea (*C. dubia*) Chronic Toxicity Test Results**

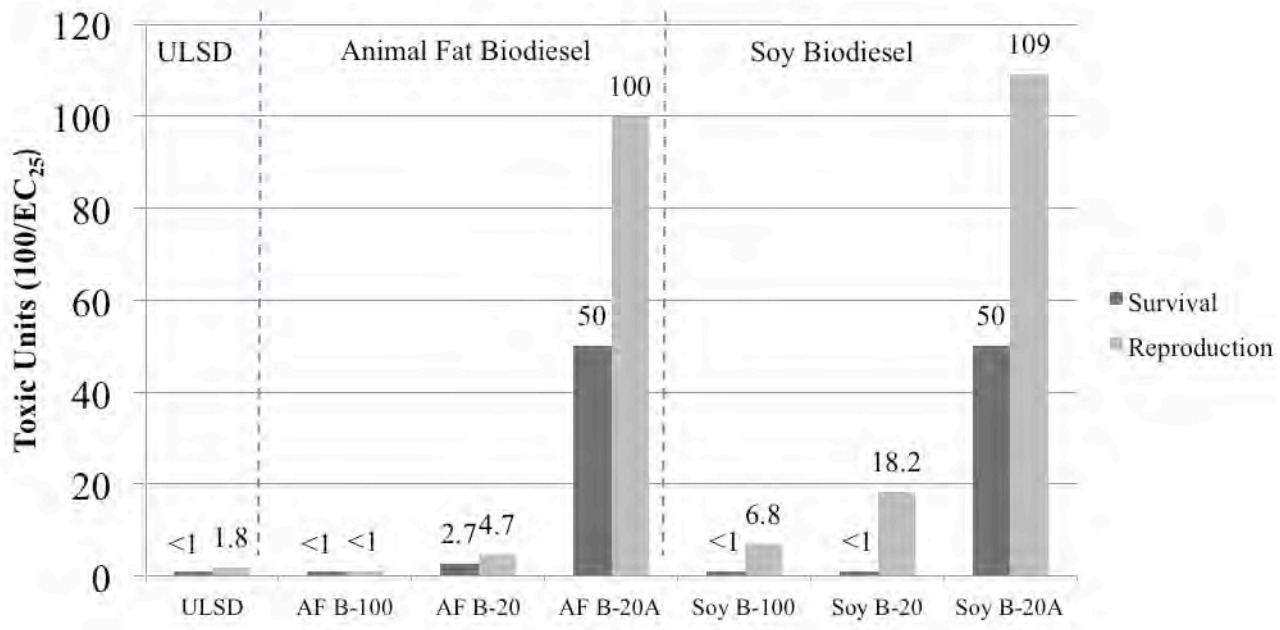
Table II-A-4 and Figure II-A-2 summarize results of the biofuel toxicity tests with *C. dubia*. The raw data for this test series is found in Section A2.

**Table II-A-4. Summary of Biodiesel Toxicity Tests with Water Flea (*C. dubia*)**

| *Fuel Type* | *Values are % WAF* | | | | | *Toxic Units* | *PMSD* |
|---|---|---|---|---|---|---|---|
| | *Test Endpoint* | *NOEC (%)* | *LOEC (%)* | *$EC_{25}$ (%)* | *$EC_{50}$ (%)* | *($100/EC_{25}$)* | *(%)* |
| ULSD | Survival | 100 | > 100 | > 100 | > 100 | **< 1** | 7.9 |
| | Reproduction | 25 | 50 | 54.5 | 71.9 | **1.8** | 22.6 |
| AF B-100 | Survival | 100 | > 100 | > 100 | > 100 | **< 1** | 19.6 |
| | Reproduction | 100 | > 100 | > 100 | > 100 | **< 1** | 22.7 |
| AF B-20 | Survival | 25 | 50 | 37.5 | > 50 | **2.7** | 16.3 |
| | Reproduction | 10 | 25 | 21.2 | 34.8 | **4.7** | 17.8 |
| AF B-20A | Survival | 1 | 5 | 2.0 | 3.0 | **50** | a |
| | Reproduction | < 1 | < 1 | 1.0 | 2.4 | **100** | 18.1 |
| Soy B-100 | Survival | 100 | > 100 | > 100 | > 100 | **< 1** | 19.2 |
| | Reproduction | 5 | 10 | 14.7 | 31.8 | **6.8** | 10.6 |
| Soy B-20 | Survival | 1 | 5 | 4.7 | 9.3 | **21** | 5.3 |
| | Reproduction | 5 | 10 | 44.1 | 75.5 | **2.3** | 8.9 |
| Soy B-20A | Survival | 1 | 5 | 2.0 | 3.0 | **50** | 6.5 |
| | Reproduction | 1 | 5 | 0.9 | 2.5 | **111** | 17.8 |

a Cannot be determined

**Figure II-A-2.Chronic Toxicity of Ultra-Low Sulfur Diesel (ULSD) and Biodiesel to *C. dubia* Survival and Reproduction.**




ULSD produced no effects on water flea survival and relatively low toxicity (1.8 TUc) on reproduction. Similarly, the AF B-100 resulted in no toxicity to both endpoints (< 1 TUc), while the AF B-20 resulted in moderate toxicity to both survival (2.7 TUc) and reproduction (4.7 TUc), which, interestingly, was greater than the toxicity of either of the two individual components (ULSD and AF B-100) that comprise the mixture. A similar pattern was seen with the soy biodiesel materials for the reproductive endpoint. Neither Soy B-100 nor B-20 exhibited effects on survival. Soy B-100 exhibited 6.8 TUc, while Soy B-20 exhibited 18.2 TUc on reproduction. There are obvious interactions between USLD and both biodiesel materials that would require additional toxicity tests on the mixtures to elucidate. Very high toxicity (50 to >100 TUc) was observed on survival and reproduction with both B-20A mixtures (containing additive). Dose-response curves associated with both tests were extremely steep (a large effect resulted from a very small increase in the additive concentration), which suggests that the additive affected a very sensitive and possibly specific receptor in the organisms. Toxicity screening of other additive chemicals to identify less toxic alternatives for use in biodiesel appears warranted.

**Fathead Minnow Chronic Toxicity Test Results**

Table II-A-5 and Figure II-A-3 summarize results of the biofuel toxicity tests with fathead minnow. The raw data for this test series is found in Section 3.

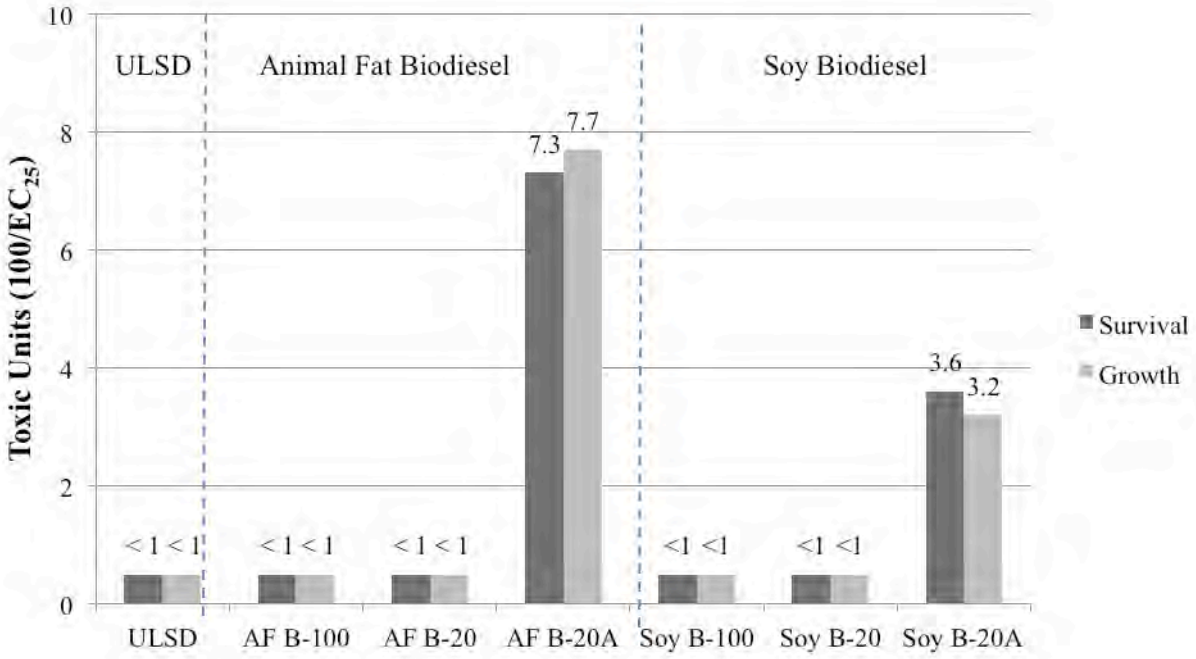
The fathead minnow survival and growth endpoints were unaffected by ULSD, AF B-100, AF B-20, Soy B-100 and Soy B-20. However, both biodiesel B-20A mixtures resulted in toxicity to both endpoints. AF B-20A exhibited moderately greater toxicity (7.3 TUc and 7.7 TUc) than did the Soy B-20A (3.6 TUc and 3.2 TUc) to the survival and reproduction endpoints, respectively.

**Table II-A-5. Summary of Biodiesel Toxicity Tests with Fathead Minnow (*P. promelas*)**

| *Fuel Type* | *Test Endpoint* | *Values are % WAF* NOEC (%) | LOEC (%) | $EC_{25}$ (%) | $EC_{50}$ (%) | *Toxic Units* ($100/EC_{25}$) | *PMSD* (%) |
|---|---|---|---|---|---|---|---|
| ULSD | Survival | 100 | > 100 | > 100 | > 100 | **< 1** | 3.8 |
| | Growth | 100 | > 100 | > 100 | > 100 | **< 1** | 14.4 |
| AF B-100 | Survival | 100 | 100 | > 100 | > 100 | **< 1** | 3.8 |
| | Growth | 25 | 50 | > 100 | > 100 | **< 1** | 8.7 |
| AF B-20 | Survival | 100 | > 100 | > 100 | > 100 | **< 1** | a |
| | Growth | 100 | > 100 | > 100 | > 100 | **< 1** | 12.4 |
| AF B-20[a] | Survival | 100 | > 100 | > 100 | > 100 | **< 1** | a |
| | Growth | 100 | > 100 | > 100 | > 100 | **< 1** | 10.7 |
| AF B-20A | Survival | 10 | 25 | 13.7 | 17.4 | **7.3** | 2.5 |
| | Growth | 10 | 25 | 13.0 | 17.0 | **7.7** | 11.0 |
| Soy B-100 | Survival | 100 | > 100 | > 100 | > 100 | **< 1** | 2.0 |
| | Growth | 100 | > 100 | > 100 | > 100 | **< 1** | 13.2 |
| Soy B-20 | Survival | 100 | > 100 | > 100 | > 100 | **< 1** | b |
| | Growth | 100 | > 100 | > 100 | > 100 | **< 1** | 10.7 |
| Soy B-20A | Survival | 10 | 25 | 27.9 | 35.3 | **3.6** | 2.3 |
| | Growth | 10 | > 10 | 30.9 | 37.3 | **3.2** | 11.7 |

a PMSD could not be determined
b Repeat test

**Figure II-A-3.Chronic Toxicity of Ultra-Low Sulfur Diesel (ULSD) and Biodiesel to Fathead Minnow Survival and Growth**



**Abalone Chronic Toxicity Test Results**

Table II-A-6 and Figure II-A-4 summarize results of the biofuel toxicity tests with abalone. The raw data for this test series is found in Section A4.

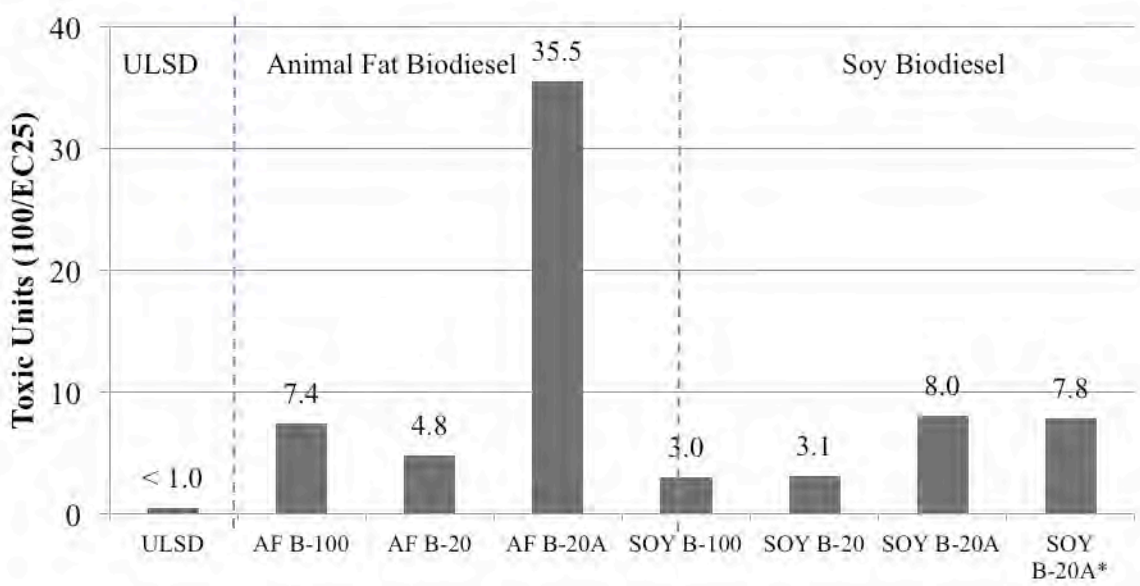
No effects on abalone shell development were detected with ULSD. AF B-100 exhibited somewhat higher toxicity than the Soy B-100 (7.4 TUc and 3.0 TUc, respectively), while the AF B-20 and Soy B-20 mixtures had similar or slightly less toxicity as their respective B-100 fuels (4.8 and 3.1 TUc, respectively), as expected. The additive substantially increased the toxicity of both B-20 mixtures: AF B-20A exhibited 34.5 TUc, a 7-fold increase, while two Soy B-20A tests detected 7.7 TUc and 8.1 TUc, approximately a 3-fold increase.

**Table II-A-6. Summary of Biodiesel Toxicity Tests with Abalone (*H. rufescens*)**

| *Fuel Type* | *Values are % WAF* | | | | *Toxic Units (100/EC$_{25}$)* | *PMSD (%)* |
|---|---|---|---|---|---|---|
| | *NOEC (%)* | *LOEC (%)* | *EC$_{25}$ (%)* | *EC$_{50}$ (%)* | | |
| ULSD | 1 | 5 | > 100 | > 100 | **< 1.0** | 4.0 |
| AF B-100 | 10 | 25 | 13.5 | 17.4 | **7.4** | 3.0 |
| AF B-20 | 10 | 25 | 20.6 | 31.0 | **4.9** | 4.6 |
| AF B-20A | 1 | 5 | 2.9 | 5.1 | **34.5** | 4.0 |
| Soy B-100 | 25 | 50 | 33.1 | 42.7 | **3.0** | 4.0 |
| Soy B-20 | 10 | 25 | 32.0 | 41.2 | **3.1** | 4.5 |
| Soy B-20A | < 1 | 1 | 13.0 | 17.0 | **7.7** | 3.5 |
| Soy B-20[a] | 5 | 10 | 12.3 | 16.5 | **8.1** | 4.2 |

a Repeat test

**Figure II-A-4.Chronic Toxicity of Ultra-Low Sulfur Diesel (ULSD) and Biodiesel to Abalone Shell Development**



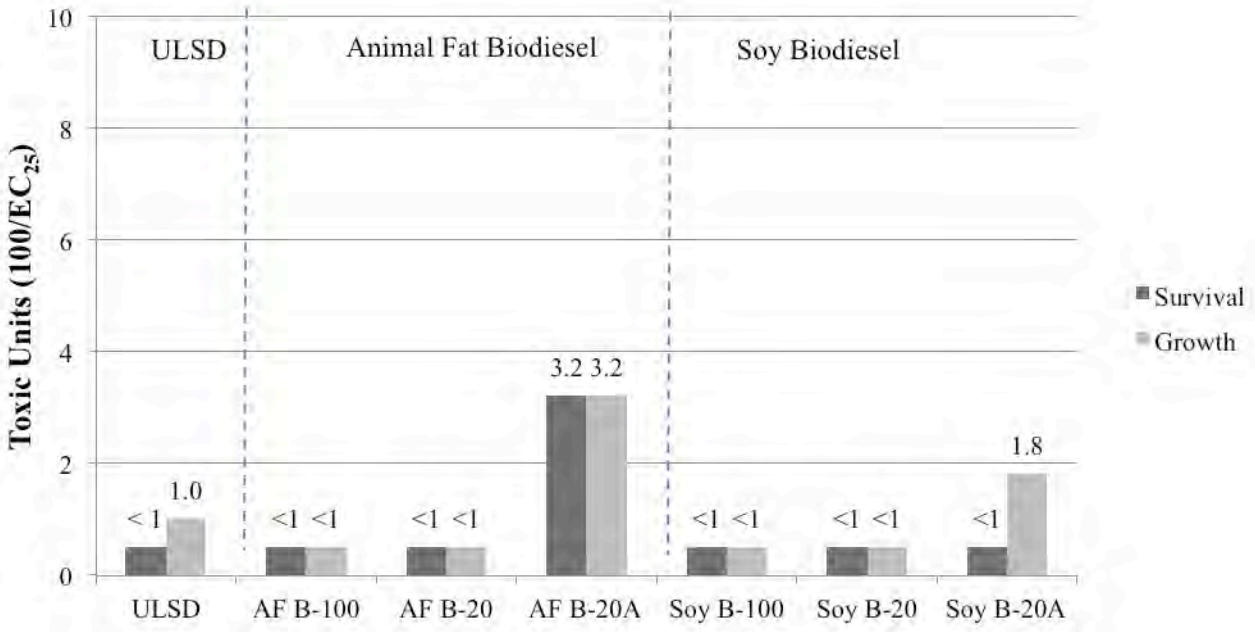
**Mysid Chronic Toxicity Test Results**

Table II-A-7 and Figure II-A-5 summarize results of the biofuel toxicity tests with mysid. The raw data for this test series is found in Section A5.

**Table II-A-7. Summary of Biodiesel Toxicity Tests with Mysid (*M. bahia*)**

| *Fuel Type* | *Test Endpoint* | *Values are % WAF* | | | | *Toxic Units* | *PMSD* |
|---|---|---|---|---|---|---|---|
| | | *NOEC (%)* | *LOEC (%)* | *$EC_{25}$ (%)* | *$EC_{50}$ (%)* | *(100/$EC_{25}$)* | *(%)* |
| ULSD | Survival | 100 | > 100 | > 100 | > 100 | **< 1** | 3.4 |
| | Growth | 50 | 100 | 99.0 | > 100 | **1.0** | 14.3 |
| AF B-100 | Survival | 100 | > 100 | > 100 | > 100 | **< 1** | 3.2 |
| | Growth | 100 | > 100 | > 100 | > 100 | **< 1** | 17.1 |
| AF B-20 | Survival | 100 | > 100 | > 100 | > 100 | **< 1** | 4.0 |
| | Growth | 50 | 100 | > 100 | > 100 | **< 1** | 16.4 |
| AF B-20A | Survival | 25 | 50 | 31.5 | 39.6 | **3.2** | 10.0 |
| | Growth | 25 | 50 | 31.4 | 39.6 | **3.2** | 18.6 |
| Soy B-100 | Survival | 100 | > 100 | > 100 | > 100 | **< 1** | 4.4 |
| | Growth | 100 | > 100 | > 100 | > 100 | **< 1** | 13.1 |
| Soy B-20 | Survival | 100 | > 100 | > 100 | > 100 | **< 1** | 3.8 |
| | Growth | 100 | > 100 | > 100 | > 100 | **< 1** | 11.4 |
| Soy B-20A | Survival | 100 | >100 | > 100 | > 100 | **< 1** | 15.2 |
| | Growth | 25 | 50 | 56.9 | > 100 | **1.8** | 19.1 |

**Figure II-A-5. Toxicity of Ultra-Low Sulfur Diesel (ULSD) and Biodiesel to Mysid Survival and Growth**



Effects on the mysid survival and growth endpoints were either absent or very low (< 1 or 1.0 TUc) for the USLD, and all biofuels and mixtures tested except those containing additive. The AF B-20A exhibited 3.2 TUc to both endpoints, while the Soy B-20A produced 1.8 TUc to the growth endpoint.

**Topsmelt Chronic Toxicity Test Results**

Table II-A-8 and Figure II-A-6 summarize results of the biofuel toxicity tests with topsmelt. The raw data for this test series is found in Section A6.

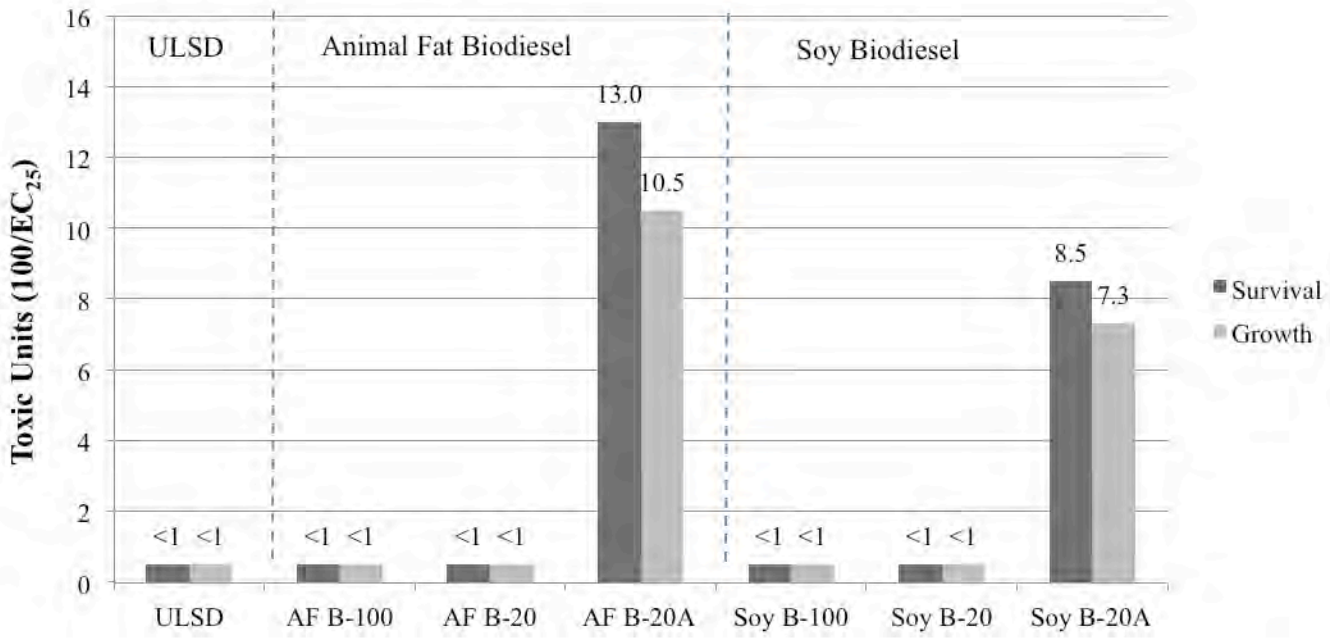
No effects on either survival or growth were detected with ULSD or either of the biofuels and mixtures that did not contain the additive. The AF B-20A test detected 13.0 TUc on survival and 10.5 TUc on growth, while the Soy B-20A test detected slightly less toxicity with 8.5 TUc on survival and 7.3 TUc on growth.

**Table II-A-8. Summary of Biodiesel Toxicity Tests with Topsmelt (*A. affinis*)**

| *Fuel Type* | *Test Endpoint* | *Values are % WAF* | | | | *Toxic Units* | *PMSD* |
|---|---|---|---|---|---|---|---|
| | | *NOEC (%)* | *LOEC (%)* | *$EC_{25}$ (%)* | *$EC_{50}$ (%)* | *(100/$EC_{25}$)* | *(%)* |
| ULSD | Survival | 100 | > 100 | > 100 | > 100 | **< 1** | 11.5 |
| | Growth | 100 | > 100 | > 100 | > 100 | **< 1** | 18.4 |
| AF B-100 | Survival | 100 | > 100 | > 100 | > 100 | **< 1** | 3.2 |
| | Growth | 100 | > 100 | > 100 | > 100 | **< 1** | 16.1 |
| AF B-20 | Survival | 100 | > 100 | > 100 | > 100 | **< 1** | 3.1 |
| | Growth | 100 | > 100 | > 100 | > 100 | **< 1** | 12.5 |
| AF B-20A | Survival | 5 | 10 | 7.7 | 11.2 | **13.0** | 15.3 |
| | Growth | 5 | 10 | 9.5 | 14.6 | **10.5** | 15.2 |
| Soy B-100 | Survival | 100 | > 100 | > 100 | > 100 | **< 1** | a |
| | Growth | 100 | > 100 | > 100 | > 100 | **< 1** | 16.0 |
| Soy B-20 | Survival | 100 | > 100 | > 100 | > 100 | **< 1** | a |
| | Growth | 100 | > 100 | > 100 | > 100 | **< 1** | 11.0 |
| Soy B-20A | Survival | 5 | 10 | 11.8 | 16.2 | **8.5** | 6.9 |
| | Growth | 10 | 25 | 13.7 | 17.5 | **7.3** | 15.7 |

a PMSD could not be determined

**Figure II-A-6. Chronic Toxicity of Ultra-Low Sulfur Diesel (ULSD) and Biodiesel to Topsmelt Survival and Growth**




## Conclusions

- ULSD produced relatively low but detectable toxicity on mysid growth (1.0 TUc) and water flea reproduction (1.8 TUc). No toxicity (< 1.0 TUc) was detected with any of the other species tested.
- Neither of the unadditized Animal Fat or Soy biodiesel test materials produced detectable toxicity to the mysid, topsmelt or fathead minnow.
- Animal Fat B-100, Soy B-100 and their B-20 mixtures caused toxicity to algae cell growth, water flea survival and/or reproduction, and abalone shell development
- Except for algae, the additized biodiesel B-20 test materials were substantially more toxic than the corresponding unadditized material.
- Tests that were repeated for confirmation produced similar results as the original test.
- Analytical chemistry information is needed on the fuel samples collected during the study to elucidate the chemical causes of toxicity and to provide information on the stability of WAF components during the toxicity tests. Appendix II-B provides such information for the WAF made with additized biodiesel blends AF-B100, AF-B20, Soy-B100, and Soy-B20. The results are only partly conclusive, as more work is needed to refine the WAF preparation and techinques. See main body of report and Appendic B for summary conclusions.

## References

ASTM 2012. American Society for Testing and Materials (ASTM). Standard D6751-12, "Standard Specification for Biodiesel Fuel Blend Stock (B100) for Middle Distillate Fuels," ASTM International, West Conshohocken, PA, 2012, www.astm.org.

Aurand D, Kucklick JH (Eds). 1995. Proceedings of the Third Meeting of the Chemical Response to Oil Spills Ecological Effects Research Forum. Marine Spill Response Corporation. Washington D.C. MSRC Technical Report Series 95-018. 69pp.

Blenkinsopp, S, Boileau, Kyle D, Sergy G, Fingas M. 1996. How to Prepare Water Accommodated Fractions from Petroleum Hyrdocarbons for Use in Aquatic Toxicity Testing: The Basis: Proceedings of the 19th Arctic Marine Oil Spill Program (AMOSP) Technical Seminar. *Environ. Canada*. Ottawa, Ontario. Pp515-528.

Schluep M, Imboden DM, Galli R, Zeyer J. 2001. Mechanisms Affecting the Dissolution of Non-Aqueous Phase Liquids into the Aqueous Phase in Slow-Stirring Batch Systems. *Environ Toxicol Chem*. 20:3 pp 459-466.

University of California. 2009. California Biodiesel Multimedia Assessment, Tier I Report (Final Draft). Prepared for the California Environmental Protection Agency Multimedia Working Group. Prepared by the University of California, Davis and the University of California, Berkeley. Sept. 2008. 95 pp.

US EPA. 1995. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms. August 1995. USEPA 600/R-95/136.

US EPA. 2002a. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. Fourth Edition. October 2002. EPA 821-R-02-013.

US EPA. 2002b. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms. October 2002. USEPA 821-R-02-014.

# 7. Appendix II-B: Chemical Analysis of the Water Accommodated Fractions of Biofuels Using Stir Bar Sorptive Extraction

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# ABSTRACT

Biofuels are diesel-equivalent fuels derived from the transesterification of the triglycerides that come from animal- or plant-based biological sources. The resulting fatty acid methyl esters (FAME) can be used in their pure form or mixed with additives and different proportions of diesel to prepare fuel formulations. Biofuels have a number of potential advantages over petroleum-based fuels. For example, biofuels come from renewable sources, may produce lower net greenhouse gas emissions, and have been shown to readily degrade in the environment. However, information about the activity of biodiesel when released into the environment is limited, in particular, its fate in aquatic systems and its effects on aquatic organisms. Biofuel formulations are complex mixtures containing a large number of aliphatic and aromatic hydrocarbons and fatty acid methyl esters. When biofuel comes into contact with water, the solubility and partition coefficients of the individual chemical constituents in the fuels and the salinity and temperature of the water dictate the ultimate composition of the biofuel chemicals in the aqueous phase. It is the aqueous phase composition that is most relevant to aquatic toxicity tests and chemical fate studies.

In this project, we prepare aqueous phase solutions of biofuel formulations for conditions (temperature and salinity) representing four different ecosystems. The aqueous solutions, referred to as water accommodating fractions (WAF), were prepared to represent different ecosystems for standard toxicity test protocols, varying both the salinity of the water and the mixing temperature. We develop and apply a stir bar sorptive extraction (SBSE) method followed by thermal desorption gas chromatography/mass spectrometry (TD-GCMS) analysis to identify and quantify the composition of the aqueous-phase solutions for four different biofuels. The fuels include animal- and plant-based biofuels in pure 100% biodiesel (B100) and 80% diesel/20% biodiesel (B20) formulations.

Although the composition of the fuels are dominated by aliphatic hydrocarbons and/or fatty acid methyl esters, the composition of the WAF was typically dominated by branched aromatics including alky-benzenes, alkyl-indenes/indanes and alkyl-naphthalenes. WAF composition and concentrations are reported for the different fuels and mixing scenarios and the effects of salinity and temperature are discussed.

# INTRODUCTION

The world's current dependence on fossil fuels presents inherent dangers and concerns. Given that the sources of petroleum currently being exploited today are naturally finite, research into alternative sources of fuel is increasing rapidly. Biodiesel has emerged as a a potentially important new fuel in an ongoing effort to transition from the use of petroleum-based fuels to renewable fuels. Biodiesels are diesel-equivalent fuels made from methanol transesterification of triglycerides derived from biological sources (Demirbas, 2009). Common biological sources include plant-based oils like soybean, sunflower, rapeseed, canola, and cotton, as well as animal fats and lard (Singh & Singh, 2010). Aside from the fact that it can be made from renewable sources, biodiesel also boasts a number of environmentally friendly attributes not shared with petroleum diesel, such as biodegradability (DeMello et al., 2007; Prince et al, 2008), as well as being carbon neutral and helping to decrease net greenhouse gas emissions (Coronado et al., 2009). In addition, some researchers have studied the potential of biodiesel as a bioremediation agent in helping to clean up oil spills (Fernandez-Alvarez et al, 2007). However, information on the aquatic environmental fate and toxicity of biodiesel is limited.

Leme et al. (2011) found that both diesel and biodiesel blends have cytotoxic effects on human cells, something they attributed to the presence of polycyclic aromatic hydrocarbons (PAHs). Researchers at the University of California, Davis are studying the environmental fate, biodegradability and aquatic toxicity of biofuel blends in support of the California multimedia risk assessment of biodiesel blends (Ginn et al., 2009; UC, 2009). Common to all of these studies is the need for knowledge of the composition and concentration of biofuel constituents in the aqueous phase solutions being tested.

Analysts have used gas chromatography/mass spectrometry (GC/MS) to identify chemicals present in various sample matrices. However, when dealing with organic compounds present in trace amounts, an extraction and enrichment step needs to occur before chromatographic separation. In recent studies, scientists have relied on the use of solvent extractions followed by a pre-concentration step to prepare samples for GC/MS analysis (Deasi, et al., 2010; Hansen, et al., 2011; Rodrigues, et al., 2010). However, traditional solvent extractions contain several drawbacks, such as being labor intensive, expensive, as well as producing high amounts of organic chemical waste (Sabik, Jeannot, & Rondeau, 2000). Solid-phase extraction (SPE) is an alternative that uses less organic solvents and has been used to successfully analyse WAFs (Lewis, Pook, & Galloway, 2008; Gonzalez-Doncel, Gonzalez, Fernandez-Torija, Navas, & Tarazona, 2008), however both solvent extraction and SPE are best suited for semi-volatile compounds due to the differences in boiling points that the analytes and the solvents must possess and the need for solvent evaporation prior to analysis (Roy, Vuillemin, & Guyomarch, 2005).

An alternative solvent free method for extracting organic compounds from aqueous solutions is stir-bar sorptive extraction (SBSE) followed by thermal desorption and GCMS analysis (Baltussen, Sandra, David, & Cramers, 1999). SBSE exploits a compound's hydrophilic and hydrophobic interactions with a polydimethylsiloxane (PDMS) coating on a glass covered stir-bar that is thermally desorbed and cryofocused directly into the GC inlet providing a simple and highly sensitive method for sampling organic chemicals in water. In an earlier phase of this project, we optimized conditions for analyzing water-accommodated fractions of biofuel using SBSE (McCreary Jr., 2010). We expand on that work here and apply the method to 16 different

fuel/WAF mixtures representing the range of biofuels and aquatic ecosystems. The goal of this study is to identify and quantify biodiesel constituents in WAF mixtures prepared with soy- and animal fat biofuels in B100 and B20 formulations. The WAF mixtures were prepared with temperatures and salinity representing fresh-, estuarine- and sea-water that are relevant to standard aquatic toxicity studies (see Appendix II-A) performed as part of the Tier II multimedia risk assessment for biofuels (Ginn et al., 2010).

# MATERIALS AND METHODS

## Materials

The biofuel used in this study was collected by University of California Davis researchers directly from storage barrels at the California Air Resources Board storage facility in Sacramento, CA (Stockton facility). The fuels include 100% animal fat biofuel (AF-B100), 100% soy biofuel (Soy-B100) and blends prepared with 20% biofuel to 80% ultra-low sulfur diesel (w/w) resulting in an AF-B20 and Soy-B20. All fuels were labeled indicating that the fuels included additives. The headspace in the storage barrels had been purged with nitrogen. The fuel was transferred directly from the storage barrels to 1-gallon amber glass jars filled to the top to minimize headspace in the jars and delivered to LBNL for testing. The jars were stored at room temperature and fuel was used within 1 week of receiving.

The water mixtures that were used to prepare WAF were prepared by Aquasci, Inc. (Davis, CA) and were used during toxicity testing. The samples to be analyzed were collected by UCD researchers during the toxicity testing for delivery to LBNL. The different salinity test waters used during the toxicity tests were prepared as described in Ginn et.al (2011). The fresh water was reverse osmosis and granular carbon filtered well water with dry salts added to achieve USEPA moderately hard (EPAMH) specifications. The EPAMH water was further amended with either dry salts (25 ppt) or hyper-saline brine (33 ppt) to prepare estuarine and marine waters, respectively. The waters were stored in 1-gallon polyethylene jugs and delivered to LBNL along with the test fuels.

Chromatography, Pesticide Residue Analysis, and Spectrophotometry-grade methanol (Burdick & Jackson, Muskegon, MI) was used in this study. An internal standard was prepared using deuterium labeled dimethyl phthalate in methanol (100 ng/µL, AccuStandard, New Haven, CT). Extractions were carried out with 10 mm glass covered magnetic stir bars coated with a 0.5 mm layer of polydimethylsiloxane (PDMS), commercially sold under the name Twister™ (Gerstel, Mulheim a/d Ruhr, Germany). Before initial use, the stir-bars were conditioned in dedicated 6 mm diameter glass thermal desorption tubes at 300°C for 2 hours in a tube conditioning oven (TC2, Gerstel, Mulheim a/d Ruhr, Germany) under a constant flow of Helium 100 mL/min). After conditioning and between uses, the stir-bars were stored in the thermal desorption tube sealed in poly propylene tubes with Teflon end caps.

## Preparation of Water Accommodated Fraction (WAF)

The WAF was prepared according to a low-energy mixing procedure (Singer, et al., 2000; Schluep, Imboden, Galli, & Zeyer, 2001) that was developed to prevent oil/water emulsification or oil droplets from getting into the water phase. Mixing temperatures and salinities for the different WAF are outlined in **Error! Reference source not found.**II-B-1.

The WAFs were prepared in clean 250 ml beakers. A small piece of Teflon tubing was fitted with a luer attachment and connected to the wall of each beaker so that the bottom of the tube rested near the bottom of the beaker and the luer fitting extended above the edge of the beaker. The tube apparatus allowed for the removal of the aqueous phase by syringe, after the WAF was prepared, without disturbing the organic (fuel) layer on the surface. For mixing, the test water (200 mL) was added to each beaker along with a small magnetic stir bar (approximately 2 cm long). The fuel (20 mL) was then added to the surface of the water by pipetting gently down the side of the beaker to prevent mixing of the fuel and water. The mouth of the beaker was covered tightly with a piece of foil to limit volatilization of the fuel components during preparation of the WAF. The fuel/water solution was stirred at 120 rpm for 18 hours in a temperature controlled environment set to the appropriate temperature. After the 18 hour stirring period, the beakers were removed from the temperature controlled environment and allowed to sit at room temperature for 2 hours.

The WAF was removed from the beaker by syringe using the Teflon tubing. The first 10 mL of water was transferred to waste. This removed water in the tubing. The remainder of the aqueous layer was then transferred from the beaker, being careful not to disturb the fuel layer. The WAF samples were stored in detergent washed, 250 mL amber glass jars with Teflon-lined caps at room temperature until extraction. **Error! Reference source not found.** summarizes the mixing conditions for each fuel/water combination.

## Stir Bar Sorptive Extraction (SBSE)

Range finding experiments were run as part of the method development. The range finding experiments included 1) direct injections of fuels in water followed by SBSE and 2) mixing samples with increasing amounts of WAF (0 mL, 1mL, 10 mL and 20 mL) diluted in a final volume of 40 mL water. The results found that the composition of the WAF was significantly different from the direct fuel spikes and that a 10 mL aliquot of WAF provided good detection of fuel constituents across all fuels without over-loading the analytical instrument.

WAF samples were prepared for extraction by first transferring 10 mL of each WAF from the glass jars to 40 mL glass screw-top vials. Methanol (4 mL) was added to the WAF to achieve a final concentration of 10% MeOH in the final extract volume (Leon, Alvarez, Cobollo, Munoz, & Valor, 2003; Prieto, et al., 2010). The internal standard was added to the vial and the contents were topped off with HPLC water to eliminate headspace resulting in a total extract volume of 40 mL. A preconditioned stir-bar was added to each sample and the vials were capped and stirred for four hours at 1500 rpm at room temperature. After extraction, the stir-bars were removed from the sample solutions using a Kimwipe covered magnet. The stir-bars were rinsed with HPLC water, dried on a clean Kimwipe, and returned to the thermal desorption tube for chemical analysis.

## Analytical Instrumentation

Stir-bars were thermally desorbed using a thermodesorption auto-sampler (Model TDSA2; Gerstel), a thermodesorption oven (Model TDS3, Gerstel) and a cooled injection system (Model CIS4; Gerstel). The cooled injection system was fitted with a glass-bead-packed glass liner. Stir-bar desorption was run in splitless mode at a starting temperature of 25 $^{o}$C with a 0.5 minute delay followed by a 60 $^{o}$C/min ramp to 300 $^{o}$C and a 2 minute hold time with the transfer line temperature at 290 $^{o}$C and the desorption flow at 20 mL/min (solvent vent mode). The cryogenic trap was held at -100 $^{o}$C throughout desorption and then heated within 0.2 minutes to 290 $^{o}$C at a

rate of 12 $^{o}$C/s, followed by a 2.3 minute hold time then a second temperature ramp to 300 $^{o}$C at a rate of 1 $^{o}$C/s and held for 2.9 minutes. The inlet was in solvent vent mode throughout desorption until 0.00 minutes (start of injection) then flow was changed to 6.0 mL/min from 0.0 to 3.0 minutes resulting in a 5:1 split injection. After injection (3.0 minutes), the vent flow was returned to 20 mL/min to purge the inlet during the secondary temperature ramp period. Compounds were resolved on a GC (Series 6890Plus; Agilent Technologies) equipped with a 30 meter long by 0.25 mm diameter HP-5 capillary column with 0.25 μm film thickness. The initial oven temperature was 10 $^{o}$C held for 5.0 minutes then ramped to 200 $^{o}$C at 5 $^{o}$C/min then to 280 $^{o}$C at 8 $^{o}$C/min holding for 5 minutes. The helium flow through the column was constant at 1.2 mL/min (initial pressure 49.5 kPa, 39 cm/sec). The resolved analytes were detected using electron impact MS (5973; Agilent Technologies) operated in scan mode with mass range from 34.0 to 500 amu. The MS temperature settings were 260 $^{o}$C, 230 $^{o}$C and 150 $^{o}$C for the transfer line, MS source and MS quad, respectively.

## Identification and Quantification of WAF Constituents

The large numbers of compounds in diesel and biofuel samples make it impractical to identify and quantify all the compounds using retention times and calibration curves that are based on pure standards. In this section, we describe a semi-quantitative approach for the GCMS analysis to identify and quantify compounds using a mass spectral library search and a modified toluene equivalent mass calibration. Toluene equivalent mass has long been used in reporting total volatile organic compounds (TVOC) (Hodgson, 1995). To use toluene equivalent mass for individual compounds, the peaks in the total ion chromatogram (TIC) must be well resolved so that the area under the chromatographic response for the specific compound can be related to the mass of toluene using a toluene response factor. However, for complex chromatograms that have large numbers of unresolved or partially resolved peaks, identifying the area under the TIC that is related to a specific chemical is more difficult. For these chemicals, it is better to use a dominant and/or unique fragment ion chromatogram in the mass spectra, referred to here as the extracted ion chromatogram (EIC).

To identify target compounds for the analytical method we first analyzed a 1000:1 dilution of each fuel in MeOH directly injected (2 μL) into the instrument with the analysis conditions described above except that a Gerstel septumless sampling head with 5:1 split was used to introduce sample onto the column. Each of the four fuels was analyzed in this way to determine their composition. Next, the 1000:1 dilution for each fuel was spiked into 40 mL of EPAMH water amended with 10% MeOH and extracted by SBSE (as part of the range finding experiment). Both the AF-B100 and Soy-B100 had a small number of dominant fatty acid methyl esters (FAME) but the AF-B100 had a larger number of minor FAME. Both neat fuels had been mixed with the same stock diesel so we concluded that the AF-B20 sample provided the widest variety of target chemicals for developing the method. The AF-B20 WAF created in the EPAMH water was extracted using the SBSE to identify the chemical composition of the WAF and to determine the relationship between EIC for individual chemicals and the response factor for toluene.

We identified 127 chemicals in the AF-B20 WAF using a mass spectral library search with the NIST08 database. For each chemical, we recorded both the EIC and the TIC. The chemicals in the WAF SBSE were assigned to one of five categories including 1) alkyl-benzene, 2) alkyl-naphthalene, 3) FAME, 4) alkane and 5) other. For each chemical (x), where we were able to

determine both an EIC and TIC, we calculated the $EIC_x/TIC_x$ ratio. For chemicals that were not well resolved and the TIC could not be determined, we assigned them the average ratio for the particular chemical category.

Specific chemicals were selected as surrogates for the different chemical categories and then a calibration was prepared by spiking the surrogate compounds into water for SBSE analysis. The surrogate compounds and their concentrations are listed in Table II-B-33. We assume that the TIC response factor (instrument response per unit mass of chemical) for the surrogate compounds is equal to the TIC response for all chemicals in the surrogate class. With this assumption, the average response factor for each surrogate category ($EI_s$) was normalized to the individual chemicals ($EI_x$) by

$$EI_s \times \frac{TI}{EI_s} \times \frac{EI_x}{TI} = EI_x$$

The $EI_x$ values were then entered into the calibration table within the ChemStation® software for each concentration in the quantification method and the relative response factor determined by forcing the two point calibration curve through zero.

# RESULTS AND DISCUSSION

## Composition of the raw fuels

Chromatograms from the raw fuel analysis are shown in Figure II-B-1. The large peaks starting at about 2600 seconds are the FAME and the smaller peaks that show up earlier in the in the B20 chromatograms are from the diesel fuel. The major FAME peaks include the hexadecanoic acid methyl ester and isomers of octadecanoic acid methyl ester. Although the AF-B100 was also dominated by two major FAME peaks, there were a larger number of minor FAMEs (lower carbon number) in the animal fat biofuel than in the soy biofuel. This can be seen by the relative size of the major FAME peaks in the two B100 chromatograms in Figure II-B-1. The diesel fuel chromatogram is shown in Figure II-B-2. Diesel fuel consists of approximately 75% saturated hydrocarbons and 25% aromatic hydrocarbons (ATSDR 1995), which was consistent with our analysis.

The direct spike of the 1000:1 MeOH:biofuel dilution (v:v) into EPAMH water followed by SBSE extraction resulted in a similar chemical fingerprint with the FAME and saturated hydrocarbons dominating the chromatogram and the aromatic hydrocarbons making up a smaller fraction of the measured chemicals. A 4 μL spike was added to each of the three salinity waters defined in Table II-B-1 and analyzed by SBSE along with an HPLC water blank. The results are shown in the overlay in Figure II-B-3. The large evenly spaced peaks in the figure are siloxanes from the stir-bar coating and are not included in the quantification method. The saturated hydrocarbons were also excluded from the quantification method because saturated hydrocarbons are not present in WAF as discussed later but we did quantify the FAME and aromatic hydrocarbon fraction in the spiked samples to evaluate the precision of the SBSE method. The precision of the internal standard was 13% (coefficient of variation of the three spike samples) without a clear trend in response of internal standard with changes in water salinity. However, the sum of the aromatic hydrocarbon and FAME chemicals in the 40 mL water spiked with 4 μL of the 1000:1 dilution of AF-B20 did show a decreasing trend as a function of increasing salinity. The EPAMH water concentration after the spike was 0.3 ppm

(sum of aromatics and FAMEs) while the highest salinity water had a concentration of 0.2 ppm representing a drop of approximately 2% in concentration with each unit increase in salinity ($r^2$ = 0.99). It was not clear why the increasing salinity would reduce the capacity of the stir-bar but future work should consider bringing the pH to neutral in saline waters prior to extraction. Nevertheless, a 2% variation in the spike samples is a reasonable precision for the SBSE of biofuel in water.

## Composition of the WAF

After evaluating the fuel composition using direct injections, and the SBSE efficiency using spiked water samples, a range finding experiment was performed using increasing fractions of the AF-B20 WAF in EPAMH water diluted with HPLC water (final volume 40 mL). The resulting chromatograms for the dilutions are shown in Figure II-B-4. A 10 mL dilution of WAF in 40 mL final aqueous phase volume was determined to be appropriate for the SBSE analysis. An important observation with the WAF, compared to the direct fuel analysis and the analysis of fuel spiked in water is that the chemical composition in the WAF was dominated by aromatic hydrocarbons (alkyl-benzene, alkyl-indene/indane and alkyl-naphthalene). The saturated hydrocarbons and the FAME in the direct fuel and the spiked fuel were either not present in the WAF or at very low concentrations. This is highlighted in Figure II-B-5 that zooms in on the region of the chromatogram where FAME elutes and overlays the chromatograms from the direct injection, the spike and the WAF for AF-B20.

The 50% dilution AF-B20 WAF chromatogram was used to identify the initial set of target compounds in the WAFs. The mass spectra from each peak were used to search in the NIST08 Mass Spectral Database using the ChemStation® Enhanced Data Analysis software. After constructing the initial target chemical list using the AF-B20 chromatogram, the spiked fuel extract was used to identify lower concentration FAME peaks. The other WAF samples were then carefully screened using the target compound list and any additional peaks not identified previously were added to the target compound list. The final list of compounds found in the soy and animal fat biofuel WAF are given in Table II-B-4. It is important to note that the library search cannot distinguish between chemical isomers so we included chromatographic retention time in Table II-B-4 to facilitate future identification using pure standards. Also listed in Table II-B-44 are the ratios for the mass spectral fragment ion or extracted ion for the individual chemical (EI) and the total ion for the chemical (TI) which was used in the quantification method to normalize the response of the individual chemicals to that of the surrogate compounds (Table II-B-3) used in the calibration.

## Precision of SBSE measurements

Sixteen WAF mixtures plus three water blanks from the test waters were each analyzed one time by SBSE. The AF-B100 and AF-B20 WAF were analyzed a second time to characterize the repeatability of the analysis. The precision of the internal standard was assessed across all analyses and the results are shown for the different WAF mixing conditions and the different fuels in Table II-B-5. The overall precision of the internal standard (n = 21) was 30%. We did not find the same trend in the internal standard response in the WAF samples that we found in the spiked samples. In this case, the EPAMH water (WAF_01) tended to have the lower internal standard response. The WAF_01 samples also had a higher coefficient of variation across all measurements and the AF-B100 WAF_01 had particularly poor precision (CV = 43%). On inspection, we found that the AF-B100 WAF_01 sample had oil droplets in the WAF indicating

contamination with raw fuel. The raw fuel contamination results in excessively high instrument response for a large number of chemicals that can reduce the detector response for the internal standard, particularly when large amounts of co-eluting compounds are present. The coefficient of variation for the AF-B100 samples drops from 43% to 20% when we exclude the contaminated AF-B100 WAF_01 samples.

The duplicate SBSE analysis for the AF-B100 and AF-B20 WAFs were used to assess precision of the measurements. The results for each chemical (ng) from the duplicate samples were first used to estimate the relative precision of the measurements. If relative precision (difference between measurements divided by the average of the measurements) is low then it always indicates that precision is good but if the relative precision is high, then it is important to check the absolute precision. Often, when the concentration measured is exceedingly low then a very small difference in replicate measurements can result in a large relative precision variability. We excluded all values that had absolute precision less than 20 ng and the resulting precision is listed for all compounds in each sample pair in Table II-B-6. The median precision across all sample pairs was approximately 15%.

## Composition and quantification of blank source waters

The three test waters and an HPLC grade blank water were analyzed as 36 mL of water with 4 mL of MeOH to determine blank concentrations. The concentrations of each compound in the blank water are listed in Table II-B-7. When there were two or more water blanks that had detectable levels of a given chemical, we calculated three times the blank level (listed in the last column of Table II-B-7) and subtracted that from the subsequent measurements. If only one of the water samples had detectible levels of a compound, then we subtracted that value from the subsequent results. The first blank water (EPAMH or W_01) and the HPLC water both had slightly elevated levels of a number of hydrocarbons and FAME which may indicate instrument carry-over because both these samples were run in series after either a spike sample or after a WAF sample. The other two blank waters were run in series after the first blank and these had very low levels of hydrocarbon and FAME. Even with the possible carry-over between analyses, the chemical concentrations measured in the blank waters were low compared to the actual samples so no additional troubleshooting was done to determine the source of chemicals in the blank waters.

## Quantification of WAF Constituents

The measured chemical concentrations for each of the fuel WAFs are listed in Tables II-B-8 thru II-B-11 for Soy-B100, Soy-B20, AF-B100 and AF-B20, respectively. Both of the animal fat biofuel WAF_01 mixtures had significantly higher concentrations of FAMEs and the Soy-B100 also had somewhat elevated FAME. We already noted contamination in the form of oil droplets present in the AF-B100 WAF_01 (greyed out values in Table II-B-10) but we did not notice visible oil droplets in the other WAF_01 samples. Comparing the average results for the duplicate AF-B20 WAF_01measurements to the previous measurement used in the range finding experiment found that the later measurements seem to have been contaminated with FAME. Both the initial measurement from the range finding and the average of the replicate measurements are reported in Table II-B-11 but the results with high FAME are likely due to contamination. The low level of FAME in the Soy-B20 WAF_01 rules out contamination in the source water used to mix the WAF. Further testing would be needed to determine if the mixing

conditions used for the WAF_01 samples resulted in elevated FAME in the Soy-B100 relative to the Soy-B20 or if the difference was due to contamination during mixing.

Only one alkane (2,2,3,3-tetramethyl- Butane) was measured in the WAF and it was also detected at elevated levels in the blanks, including the HPLC water and in the direct analysis. The fact that the alkane was in the diluted fuel which was not extracted using a stir-bar indicates that the methanol used in the dilution may have been the source. The antioxidant fuel additives acetic acid, butyl ester (synonym – butyl acetate) and 1,4-Benzenediol, 2-(1,1-dimethylethyl) (synonym – *tert*-Butylhydroquinone, TBHQ) were also identified in the majority of the samples. However, the concentrations were highly variable. We can assume that the addition of the additive to the original fuel was consistent so the variability was likely due to either the WAF mixing conditions or the extraction conditions. The butyl acetate was lowest in the WAF_04 which had the highest salinity so the solubility may be affected by pH but without further testing we cannot rule out the extraction as a source of the variability for either of the measured additives.

The overall trend in concentrations of the aromatic hydrocarbons indicates that the salinity and the temperature may both have an effect on the solubility of the aromatic hydrocarbons in the fuels. In particular, the highest salinity water had the lowest concentration for FAME, aromatic hydrocarbons and the additives. The lowest salinity water had the highest and most variable FAME concentrations. Additional measurements are needed to characterize the temperature and salinity effect on solubility.

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# REFERENCES

Agency for Toxic Substances and Disease Registry (ATSDR). 1995. Toxicological profile for fuel oils. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service

Baltussen, E., Sandra, P., David, F., & Cramers, C. 1999. Stir bar sorptive extraction (SBSE), a novel extraction technique for aqueous samples: theory and principles. *Journal of Microcolumn Separations, 11*(10), 737-747.

Coronado, C. R., Andrade de Carvalho Jr., J., & Silveira, J. L. 2009. Biodiesel CO2 emissions: a comparison with the main fuels in the Brazilian market. *Fuel Processing Technology, 90*(2), 204-211.

Deasi, S. R., Verlecar, X. N., Ansari, Z. A., Jagtap, T. G., Sarkar, A., Vashistha, D., et al. 2010. Evaluation of genotoxic responses of Chaetoceros tenuissimus and Skeletonema costatum to water accommodated fraction of petroleum hydrocarbons as biomarker of exposure. *Water Research, 44*, 2235-2244.

DeMello, J. A., Carmichael, C. A., Peacock, E. E., Nelson, R. K., Arey, J. S., & Reddy, C. M. 2007. Biodegradation and environmental behavior of diesel mixtures. *Marine Pollution Bulletin, 54*, 894-904.

Demirbas, A. 2009. Progress and recent trends in biodiesel fuels. *Energy Conversion and Management, 50*(1), 14-34.

Fernandez-Alvarez, P., Villa, J., Garrido, J. M., Grifoll, M., Feijoo, G., & Lema, J. M. 2007. Evaluation of biodiesel as bioremediation agent for the treatment of the shore affected by the heavy oil spill of the Prestige. *Journal of Hazardous Materials, 147*(3), 914-922.

Ginn, T. R., M. B. Johnson, J.A. Last, K.M. Scow, L. Rastagarzadeh, T. Hatch, P. L'Amoreaux, V. Nino. R. Okamoto, R. Hodam. 2009. Experimental Plan for Tier II Evaluation of Biodiesel 1st revision 9 March 2009, Prepared for the California Environmental Protection Agency Multimedia Working Group, 22 pp.

Ginn, T.R., T. Hatch, J. Miller, K. M. Scow, A. Epple, M. B. Johnson, L. Rastagarzadeh, T. Schetrit, T. Barkouki, V. Nino. 2010. DRAFT - MULTIMEDIA RISK ASSESSMENT TIER II COMPLETION REPORT: BIODIESEL, Prepared for the California Environmental Protection Agency Multimedia Working Group, 41 pp. http://www.arb.ca.gov/fuels/multimedia/biodieseltierii_dftfinal.pdf

Gonzalez-Doncel, M., Gonzalez, L., Fernandez-Torija, C., Navas, J. M., & Tarazona, J. V. 2008. Toxic effects of an oil spill on fish early life stages may not be exclusively associated to PAHs: Studies with Prestige oil and medaka (Oryzias latipes). *Aquatic Toxicology, 87*, 280-288.

Hansen, B. H., Altin, D., Rørvik, S. F., Øverjordet, I. B., Olsen, A. J., & Nordtug, T. 2011. Comparative study on acute effects of water accommodated fractions of an artificially weathered crude oil on Calanus finmarchicus and Calanus glacialis (Crustacea: Copepoda). *Science of the Total Environmnet, 409*, 704-709.

Hodgson, A.T. 1995. A Review and a Limited Comparison of Methods for Measuring Total Volatile Organic Compounds in Indoor Air. *Indoor Air, 5,* 247-257.

Leme, D. M. 2011. Cytotoxicity of water-soluble fraction from biodiesel and its diesel blends to human cell lines. *Ecotoxicol. Environ. Saf.*, doi:10.1016/j.ecoenv.2011.08.012.

Leon, V. M., Alvarez, B., Cobollo, M. A., Munoz, S., & Valor, I. 2003. Analysis of 35 priority semivolatile compounds in water by stir bar sorptive extraction-thermal desorption-gas chromatography-mass spectrometry I. Method optimisation. *Journal of Chromatography A*(999), 91-101.

Lewis, C., Pook, C., & Galloway, T. 2008. Reproductive toxicity of the water accommodated fraction (WAF) of crude oil in polychaetes Arenicola marina (L.) and Nereis virens (Sars). *Aquatic Toxicity, 90*, 73-81.

McCreary Jr., R. 2010. *Method optimization of stir bar sorptive extraction-thermal desorption-gas chromatography/mass spectroscopy for analysis of biodiesel in fresh water and sea water.* Unpublished Manuscript, Lawrence Berkeley National Laboratory, Berkeley, CA.

Prieto, A., Basauri, O., Rodil, R., Usobiaga, A., Fernandez, L. A., Etxebarria, N., et al. 2010. Stir-bar sorptive extraction: A view on method optimization, novel applications, limitations, and potential solutions. *Journal of Chromatography A*(1217), 2642-2666.

Prince, R. C., Haitmanek, C., & Lee, C. C. 2008. The primary aerobic biodegradation of biodiesel b20. *Chemosphere, 71*, 1446-1451.

Rodrigues, R. V., Miranda-Filho, K. C., Gusmão, E. P., Moreira, C. B., Romano, L. A., & Sampaio, L. A. 2010. Deleterious effects of water-soluble fraction of petroleum, diesel and gasoline on marine pejerrey Odontesthes argentinensis larvae. *Science of the Total Environment, 408*, 2054-2059.

Roy, G., Vuillemin, R., & Guyomarch, J. 2005. On-site determination of polynuclear aromatic hydrocarbons in seawater by stir bar sorptive extraction (SBSE) and thermal desorption GC-MS. *Talanta, 66*, 540-546.

Sabik, H., Jeannot, R., & Rondeau, B. 2000. Multiresidue methods using solid-phase extraction techniques for monitoring priority pesticides, including triazines and degradation products, in ground and surface waters. *Journal of Chromatography A, 885*, 217-236.

Schluep, M., Imboden, D. M., Galli, R., & Zeyer, J. 2001. Mechanisms affecting the dissolution of nonaqueous phase liquids into the aqueous phase of slow-stirrin batch systems. *Environmental Toxicology and Chemistry, 20*(3), 459-466.

Singer, M. M., Aurand, D., Bragin, G. E., Clark, J. R., Coelho, G. M., Sowby, M. L., et al. 2000. Standardization of the preparation and quantitation of water-accommodated fractions of petroleum for toxicity testing. *Marine Pullution Bulleton, 40*(11), 1007-1016.

Singh, S. P., & Singh, D. 2010. Biodiesel production through the use of different sources and characterization of oils and their esters as the substitute diesel: a review. *Renewable and Sustaiable Energy Reviews, 14*(1), 200-216.

University of California (UC). 2008. California Biodiesel Multimedia Assessment Tier I Report (Final Draft), prepared for the California Environmental Protection Agency Multimedia

Working Group, by the University of California, Davis and the University of California, Berkeley. Sept. 2008. 95 pp.

**Table II-B-1: Stock water and mixing temperature for preparing representative WAF for toxicity assays**

| Water Mix | Organism | Base Water | Mixing Temp ( °C) | Salinity (ppt) |
|---|---|---|---|---|
| W_01 | Cerio/Alg/FM | EPAMH[2] | 25 | 0 |
| W_02[1] | Mysid | EPAMH + DS[3] | 26 | 25 |
| W_03[1] | Top smelt | EPAMH + DS | 20 | 25 |
| W_04 | Abalone | EPAMH + HB[4] | 15 | 33 |

[1] The water used to prepare W_02 and W_03 are from the same initial salinity mixture but the WAF is prepared under different temperature as indicated by "Mixing Temp". [2]EPAMH is moderately hard reconstituted water based on USEPA specifications. [3]DS is dry salts. [4]HB is hyper saline brine

**Table II-B-2: Mixing volumes for preparation of WAF**

| | Fuel (mL) All samples include additives | | | | Water (mL) and Mixing Temperature (C) [see Table 1 for details] | | | |
|---|---|---|---|---|---|---|---|---|
| SampleName | $SoyB_{100}$ | $AFB_{100}$ | $SoyB_{20}$ | $AFB_{20}$ | W_01 | W_02 | W_03 | W_04 |
| Soy-$B_{100}$_01 | 20 | | | | 200 | | | |
| Soy-$B_{100}$_02 | 20 | | | | | 200 | | |
| Soy-$B_{100}$_03 | 20 | | | | | | 200 | |
| Soy-$B_{100}$_04 | 20 | | | | | | | 200 |
| AF-$B_{100}$_01 | | 20 | | | 200 | | | |
| AF-$B_{100}$_02 | | 20 | | | | 200 | | |
| AF-$B_{100}$_03 | | 20 | | | | | 200 | |
| AF-$B_{100}$_04 | | 20 | | | | | | 200 |
| Soy-$B_{20}$_01 | | | 20 | | 200 | | | |
| Soy-$B_{20}$_02 | | | 20 | | | 200 | | |
| Soy-$B_{20}$_03 | | | 20 | | | | 200 | |
| Soy-$B_{20}$_04 | | | 20 | | | | | 200 |
| AF-$B_{20}$_01 | | | | 20 | 200 | | | |
| AF-$B_{20}$_02 | | | | 20 | | 200 | | |
| AF-$B_{20}$_03 | | | | 20 | | | 200 | |
| AF-$B_{20}$_04 | | | | 20 | | | | 200 |
| Blank_01 | | | | | 200 | | | |
| Blank_02-03 | | | | | | 200 | 200 | |
| Blank_04 | | | | | | | | 200 |

**Table II-B-3: Surrogate compounds in standard mixes used to quantify samples**

| | Class | Surrogate | Calibration concentrations | |
|---|---|---|---|---|
| | | | Low (ppb) | High (ppb) |
| 1 | Mono-aromatic | *o*-Xylene | 5.0 | 39.7 |
| | | *m/p*-Xylene | 5.1 | 40.7 |
| | | 1,2,4-trimethylbenzene | 5.2 | 41.8 |
| | | 1,2,3-trimethylbenzene | 5.2 | 41.8 |
| 2 | Poly-aromatic | Naphthalene | 4.8 | 38.0 |
| 3 | FAME | methyl- Palmitate | 10 | 50 |
| | | methyl- Oleate & Linolenate | 10 | 50 |
| | | methyl- Stearate | 10 | 50 |
| 4 | Alkanes | *n*-Undecane | 5.1 | 41.0 |
| | | *n*-Dodecane | 5.1 | 41.2 |

**Table II-B-4: Target chemical identified in Biofuel WAF and Extracted Ion / Total Ion Ratios**

| Compound Name | Retention Time (min) | Chemical Class | EI/TI[1] |
|---|---|---|---|
| Dimethyl phthalate-3,4,5,6-d 4 | 33.25 | ISTD | 0.25 |
| Benzene | 6.29 | mono-aromatic | 0.69 |
| Butane, 2,2,3,3-tetramethyl- | 7.19 | alkane | 0.52 |
| Toluene | 10.92 | mono-aromatic | 0.47 |
| Acetic acid, butyl ester | 13.15 | ester | 0.38 |
| Ethylbenzene | 14.79 | mono-aromatic | 0.47 |
| m-Xylene | 15.17 | mono-aromatic | 0.38 |
| p-Xylene | 15.22 | mono-aromatic | 0.37 |
| o-Xylene | 16.03 | mono-aromatic | 0.39 |
| Oxime-, methoxy-phenyl- | 16.45 | mono-aromatic | 0.47 |
| Benzene, (1-methylethyl)- | 17.16 | mono-aromatic | 0.48 |
| Hexanoic acid, methyl ester | 17.29 | FAME | 0.31 |
| Benzene, propyl- | 18.26 | mono-aromatic | 0.52 |
| Benzene, 1-ethyl-2-methyl- | 18.55 | mono-aromatic | 0.44 |
| Benzene, 1-ethyl-4-methyl- | 18.66 | mono-aromatic | 0.45 |
| Benzene, 1,2,3-trimethyl- | 18.86 | mono-aromatic | 0.38[2] |
| Benzene, 1,3,5-trimethyl- | 19.15 | mono-aromatic | 0.44 |
| Benzene, 1,2,3-trimethyl- | 19.74 | mono-aromatic | 0.39 |
| Benzene, (1-methylpropyl)- | 20.19 | mono-aromatic | 0.48 |
| Benzene, 1-methyl-2-(1-methylethyl)- | 20.57 | mono-aromatic | 0.44 |
| Benzene, 1,2,4-trimethyl- | 20.66 | mono-aromatic | 0.41 |
| Benzene, 1-methyl-2-(1-methylethyl)- | 20.75 | mono-aromatic | 0.34 |
| Indane | 21.15 | indane[3] | 0.38 |
| Benzene, 1,3-diethyl- | 21.49 | mono-aromatic | 0.25 |
| Benzene, 1-methyl-3-propyl- | 21.61 | mono-aromatic | 0.45 |
| Benzene, 1,2,3,4-tetramethyl- | 21.79 | mono-aromatic | 0.27 |
| Benzene, 1-methyl-4-(1-methylethyl)- | 21.83 | mono-aromatic | 0.40 |
| Benzene, 1-methyl-4-propyl- | 22.08 | mono-aromatic | 0.48 |
| Benzene, 2-ethyl-1,4-dimethyl- | 22.42 | mono-aromatic | 0.41 |
| Benzene, 1-ethyl-2,4-dimethyl- | 22.52 | mono-aromatic | 0.46 |
| Benzene, 4-ethenyl-1,2-dimethyl- | 22.66 | mono-aromatic | 0.34 |
| Benzene, 1-ethyl-2,4-dimethyl- | 22.72 | mono-aromatic | 0.41 |
| Benzene, 1-ethenyl-4-ethyl- | 22.80 | mono-aromatic | 0.40 |
| Benzene, 1-ethyl-2,4-dimethyl- | 22.86 | mono-aromatic | 0.41 |
| Benzene, 1-methyl-4-(1-methylpropyl)- | 23.22 | mono-aromatic | 0.30 |
| Benzene, 1-ethyl-2,3-dimethyl- | 23.34 | mono-aromatic | 0.37 |
| Benzene, 1-methyl-4-(1-methylpropyl)- | 23.49 | mono-aromatic | 0.39 |
| Benzene, 1,2,3,4-tetramethyl- | 23.75 | mono-aromatic | 0.38 |
| Benzene, 1,2,3,4-tetramethyl- | 23.86 | mono-aromatic | 0.39 |
| Benzene, 1-methyl-4-(1-methylpropyl)- | 24.28 | mono-aromatic | 0.41 |
| Benzene, (2-methyl-1-butenyl)- | 24.44 | mono-aromatic | 0.38 |
| Indan, 1-methyl- | 24.54 | indane | 0.27 |
| 1H-Indene, 2,3-dihydro-4-methyl- | 24.81 | indene[3] | 0.38 |
| Benzene, 1,2,4,5-tetramethyl- | 24.85 | mono-aromatic | 0.38 |
| Benzene, 1-methyl-4-(1-methylpropyl)- | 25.03 | mono-aromatic | 0.38 |
| Benzene, 1-methyl-4-(1-methylpropyl)- | 25.19 | mono-aromatic | 0.38 |

| Compound Name | Retention Time (min) | Chemical Class | EI/TI[1] |
|---|---|---|---|
| Naphthalene, 1,2,3,4-tetrahydro- | 25.27 | poly-aromatic | 0.26 |
| Benzene, 2-ethyl-1,3-dimethyl- | 25.49 | mono-aromatic | 0.51 |
| 1H-Indene,2,3-dihydro-2,2-dimethyl- | 25.89 | indene | 0.38* |
| 1H-Indene,2,3-dihydro-2,2-dimethyl- | 25.97 | indene | 0.34 |
| Naphthalene | 26.10 | poly-aromatic | 0.21* |
| 1H-Indene, 2,3-dihydro-1,6-dimethyl- | 26.22 | indene | 0.38* |
| Benzene, 1,3-dimethyl-5-(1-methylethyl) | 26.48 | mono-aromatic | 0.38* |
| Benzene, 1,3-dimethyl-5-(1-methylethyl) | 26.74 | mono-aromatic | 0.41 |
| Naphthalene, 1,2,3,4-tetrahydro-2-methy | 26.89 | poly-aromatic | 0.22 |
| Naphthalene, 1,2,3,4-tetrahydro-1-methy | 27.10 | poly-aromatic | 0.33 |
| Bicyclo[4.2.1]nona-2,4,7-triene, 7-ethyl | 27.28 | poly-aromatic | 0.21 |
| Benzocycloheptene | 27.68 | mono-aromatic | 0.38* |
| 1H-Indene, 2,3-dihydro-4,7-dimethyl- | 27.80 | indene | 0.34 |
| 1H-Indene, 2,3-dihydro-4,7-dimethyl- | 28.15 | indene | 0.32 |
| Naphthalene, 1,2,3,4-tetrahydro-6-methyl | 28.34 | poly-aromatic | 0.21* |
| Naphthalene, 1,2,3,4-tetrahydro-1,5-dimethyl | 28.53 | poly-aromatic | 0.21 |
| Phenol, 2-(2-methyl-2-propenyl)- | 28.60 | mono-aromatic | 0.21 |
| 1H-Indene, 2,3-dihydro-4,7-dimethyl- | 28.70 | indene | 0.36 |
| Naphthalene, 1,2,3,4-tetrahydro-1,5-dimethyl | 28.91 | poly-aromatic | 0.21* |
| Naphthalene, 1,2,3,4-tetrahydro-5-methyl | 29.05 | poly-aromatic | 0.21* |
| 1H-Indene, 2,3-dihydro-1,4,7-trimethyl- | 29.19 | indene | 0.29 |
| Naphthalene, 1-methyl- | 29.30 | poly-aromatic | 0.31 |
| Decanoic acid, methyl ester | 29.70 | FAME | 0.11* |
| Naphthalene, 1-methyl- | 29.74 | poly-aromatic | 0.21* |
| Naphthalene, 1,2,3,4-tetrahydro-1,4-dimethyl | 29.84 | poly-aromatic | 0.14 |
| 1H-Indene, 2,3-dihydro-1,1,5-trimethyl- | 29.98 | indene | 0.34 |
| Naphthalene, 2-ethyl-1,2,3,4-tetrahydro | 30.14 | poly-aromatic | 0.13 |
| Naphthalene, 1,2,3,4-tetrahydro-1,5-dimethyl | 30.25 | poly-aromatic | 0.14 |
| Naphthalene, 1,2,3,4-tetrahydro-1,4-dimethyl | 30.36 | poly-aromatic | 0.15 |
| (1,4-Dimethylpent-2-enyl)benzene | 30.40 | mono-aromatic | 0.12 |
| Naphthalene, 1,2,3,4-tetrahydro-1,4-dimethyl | 30.47 | poly-aromatic | 0.16 |
| Naphthalene, 1,2,3,4-tetrahydro-1,5-dimethyl | 30.69 | poly-aromatic | 0.32 |
| Ethanol, 2-(2-butoxyethoxy)-, acetate | 30.80 | glycolether | 0.11* |
| Naphthalene, 5-ethyl-1,2,3,4-tetrahydro | 30.85 | poly-aromatic | 0.21* |
| Methyl 4-oxododecanoate | 31.08 | FAME | 0.11 |
| Naphthalene, 5-ethyl-1,2,3,4-tetrahydro | 31.19 | poly-aromatic | 0.20 |
| Acenaphthylene, 1,2,2a,3,4,5-hexahydro- | 31.38 | poly-aromatic | 0.21* |
| Biphenyl | 31.58 | poly-aromatic | 0.40 |
| Naphthalene, 1,2,3,4-tetrahydro-5,7-dimethyl | 31.74 | poly-aromatic | 0.28 |
| Naphthalene, 1,2,3,4-tetrahydro-5,7-dimethyl | 31.83 | poly-aromatic | 0.23 |
| Diphenylmethane | 31.95 | poly-aromatic | 0.18 |
| Naphthalene, 1,4-dimethyl- | 32.27 | poly-aromatic | 0.21* |
| Naphthalene, 1,2,3,4-tetrahydro-6,7-dimethyl | 32.51 | poly-aromatic | 0.21* |
| Naphthalene, 1,7-dimethyl- | 32.58 | poly-aromatic | 0.21* |
| Naphthalene, 1,2,3,4-tetrahydro-5,6-dimethyl | 32.66 | poly-aromatic | 0.21* |
| Naphthalene, 2,6-dimethyl- | 32.71 | poly-aromatic | 0.21* |
| Nonanoic acid, 9-oxo-, methyl ester | 32.81 | poly-aromatic | 0.10 |
| Naphthalene, 1,2,3,4-tetrahydro-2,5,8-t | 32.93 | poly-aromatic | 0.23 |

| Compound Name | Retention Time (min) | Chemical Class | EI/TI[1] |
|---|---|---|---|
| Naphthalene, 1,2,3,4-tetrahydro-2,5,8-t | 33.07 | poly-aromatic | 0.11 |
| Naphthalene, 1,2,3,4-tetrahydro-2,5,8-t | 33.19 | poly-aromatic | 0.21* |
| Naphthalene, 1,2-dimethyl- | 33.49 | poly-aromatic | 0.24 |
| Naphthalene, 1,2,3,4-tetrahydro-1,4,6-t | 33.64 | poly-aromatic | 0.14 |
| 1,4-Dimethyl-2-cyclopentylbenzene | 33.95 | mono-aromatic | 0.18 |
| 1,1'-Biphenyl, 4-methyl- | 34.26 | poly-aromatic | 0.25 |
| 1,1'-Biphenyl, 4-methyl- | 34.51 | poly-aromatic | 0.17 |
| Dodecanoic acid, methyl ester | 34.83 | FAME | 0.21 |
| Naphthalene, 1,4,6-trimethyl- | 35.14 | poly-aromatic | 0.21* |
| Naphthalene, 1,6,7-trimethyl- | 35.19 | poly-aromatic | 0.21* |
| Naphthalene, 1,6,7-trimethyl- | 35.37 | poly-aromatic | 0.23 |
| Nonanedioic acid, dimethyl ester | 35.51 | FAME | 0.08 |
| Naphthalene, 2,3,6-trimethyl- | 35.71 | poly-aromatic | 0.23 |
| 1,4-Benzenediol, 2-(1,1-dimethylethyl)- | 35.80 | mono-aromatic | 0.15 |
| 4,4'-Dimethylbiphenyl | 36.77 | poly-aromatic | 0.21* |
| Naphthalene, 1-(2-propenyl)- | 37.04 | poly-aromatic | 0.15 |
| Methyl myristoleate | 39.18 | FAME | 0.07 |
| Methyl tetradecanoate | 39.45 | FAME | 0.19 |
| 4,4'-Dimethylbiphenyl | 39.50 | poly-aromatic | 0.21* |
| Pentadecanoic acid, methyl ester | 41.58 | FAME | 0.20 |
| Naphthalene, 1,2,3,4,4a,5,8,8a-octahydro | 42.04 | poly-aromatic | 0.09 |
| 9-Hexadecenoic acid, methyl ester, (Z)- | 43.15 | FAME | 0.05 |
| Methyl palmitoleate | 43.22 | FAME | 0.03 |
| Hexadecanoic acid, methyl ester | 43.70 | FAME | 0.16 |
| cis-10-Heptadecenoic acid, methyl ester | 44.97 | FAME | 0.04 |
| Heptadecanoic acid, methyl ester | 45.34 | FAME | 0.17 |
| 9,12-Octadecadienoic acid (Z,Z)-, methy ester | 46.45 | FAME | 0.07 |
| Octadecenoic acid, ME (Isomers #3-4) | 46.60 | FAME | 0.04 |
| Octadecanoic acid, ME(Isomer #5) | 46.59 | FAME | 0.03 |
| Octadecanoic acid, methyl ester | 46.91 | FAME | 0.15 |
| Pyrene, 4,5-dihydro- | 46.36 | poly-aromatic | 0.21* |
| Pyrene | 47.34 | poly-aromatic | 0.21* |
| cis-11,14-Eicosadienoic acid, methyl ester | 49.05 | FAME | 0.04 |

[1]The extracted ion to total ion ratio is used to convert the chemical response of the surrogate compounds (listed in Table 3) to response for the specific chemical in Table 4.
[2]Where a well resolved peak was not achieved and we could not determine TIC for a given compound, the average EI/TI ratio (listed with a * superscript) for the class of chemicals was used.
[3]When a TIC could not be measured for indane or indene, the average EI/TI ratio for the mono-aromatic was used.

**Table II-B-5. Precision of internal standard area response for different WAF and Fuels**

| | Count | Average Area | CV |
|---|---|---|---|
| WAF_01 | 6 | 6.93E+05 | 35% |
| WAF_02 | 6 | 1.19E+06 | 18% |
| WAF_03 | 6 | 1.26E+06 | 16% |
| WAF_04 | 6 | 1.38E+06 | 15% |
| Soy-B100 | 4 | 1.29E+06 | 25% |
| Soy-B20 | 4 | 1.28E+06 | 15% |
| AF-B100 | 8 | 1.11E+06 | 43% |
| AF-B100 (excluding WAF_01) | 6 | 1.33E+06 | 20% |
| AF-B20 | 8 | 9.96E+05 | 20% |
| blank test waters | 3 | 1.57E+06 | 17% |
| Overall | 21 | 1.18E+06 | 30% |

**Table II-B-6: Relative precision of sample pairs excluding pairs with absolute precision less than 10 ng**

| | AF-B100 sample pairs in WAF | | | | AF-B20 sample pairs in WAF | | | |
|---|---|---|---|---|---|---|---|---|
| Compound name | 01 | 02 | 03 | 04 | 01 | 02 | 03 | 04 |
| Benzene | 5% | | | | 5% | 9% | 19% | 17% |
| Butane, 2,2,3,3-tetramethyl- | 115 % | 33% | 56% | 21% | 69% | 76% | 87% | |
| Toluene | | | | | 26% | 0% | 49% | 10% |
| Acetic acid, butyl ester | 16% | 16% | 12% | 77% | 1% | 31% | 25% | 59% |
| Ethylbenzene | 8% | | | | 10% | 22% | 5% | 34% |
| m-Xylene | 5% | | | | 5% | 23% | 1% | 28% |
| p-Xylene | 4% | | | | 11% | 20% | 5% | 33% |
| o-Xylene | 3% | | | | 9% | 14% | 5% | 26% |
| Oxime-, methoxy-phenyl-_ | 8% | | | 17% | 5% | | 17% | 5% |
| Benzene, (1-methylethyl)- | | | | | 25% | 36% | 17% | 48% |
| Hexanoic acid, methyl ester | 23% | 54% | 29% | 73% | 12% | 38% | 36% | 66% |
| Benzene, propyl- | 1% | | | | 29% | 36% | 21% | 50% |
| Benzene, 1-ethyl-2-methyl- | 6% | | | | 25% | 27% | 14% | 41% |
| Benzene, 1-ethyl-4-methyl- | 8% | | | | 26% | 29% | 16% | 43% |
| Benzene, 1,2,3-trimethyl- | | | | | 24% | 28% | 14% | 40% |
| Benzene, 1,3,5-trimethyl- | 3% | | | | 23% | 24% | 12% | 36% |
| Benzene, 1,2,3-trimethyl- | 2% | | | | 21% | 21% | 10% | 34% |
| Benzene, (1-methylpropyl)- | | | | | | | 39% | |
| Benzene, 1-methyl-2-(1-methylethyl)- | | | | | 33% | 37% | 24% | 53% |
| Benzene, 1,2,4-trimethyl- | 3% | 10% | | | 20% | 15% | 8% | 28% |
| Benzene, 1-methyl-2-(1-methylethyl)- | | | | | 32% | 40% | 27% | 53% |
| Indane | 15% | 10% | | | 17% | 11% | 7% | 25% |
| Benzene, 1,3-diethyl- | | | | | 30% | 32% | 22% | 47% |
| Benzene, 1-methyl-3-propyl- | 13% | | | | 33% | 38% | 26% | 52% |
| Benzene, 1,2,3,4-tetramethyl- | 11% | 9% | | | 31% | 35% | 24% | 51% |
| Benzene, 1-methyl-4-(1-methylethyl)- | 8% | | | | 29% | 30% | 20% | 46% |
| Benzene, 1-methyl-4-propyl- | 3% | | | | 30% | 32% | 22% | 47% |
| Benzene, 2-ethyl-1,4-dimethyl- | 2% | | | | 26% | 25% | 17% | 41% |
| Benzene, 1-ethyl-2,4-dimethyl- | 4% | | | | 26% | 25% | 17% | 41% |
| Benzene, 4-ethenyl-1,2-dimethyl- | | | | | 24% | 21% | 14% | 35% |
| Benzene, 1-ethyl-2,4-dimethyl- | 7% | | | 3% | 25% | 25% | 17% | 41% |
| Benzene, 1-ethenyl-4-ethyl- | 10% | | | | 22% | 19% | 12% | 32% |
| Benzene, 1-ethyl-2,4-dimethyl- | | | | | 18% | 18% | 15% | 37% |
| Benzene, 1-methyl-4-(1-methylpropyl)- | 17% | | | | 32% | 44% | 32% | 62% |
| Benzene, 1-ethyl-2,3-dimethyl- | | | | | 22% | 18% | 13% | 34% |
| Benzene, 1-methyl-4-(1-methylpropyl)- | | | | | 30% | 44% | | 58% |
| Benzene, 1,2,3,4-tetramethyl- | 14% | | | | 22% | 18% | 13% | 34% |
| Benzene, 1,2,3,4-tetramethyl- | 4% | | | | 21% | 17% | 12% | 32% |
| Benzene, 1-methyl-4-(1-methylpropyl)- | | | | | 28% | | | 57% |
| Benzene, (2-methyl-1-butenyl)- | | | | | 25% | | | 55% |
| Indan, 1-methyl- | 14% | 101% | | | 18% | 14% | 9% | 26% |
| 1H-Indene, 2,3-dihydro-4-methyl- | 8% | 100% | | 7% | 18% | 10% | 7% | 23% |
| Benzene, 1,2,4,5-tetramethyl- | 7% | 107% | | | 19% | 13% | 9% | 26% |

| | AF-B100 sample pairs in WAF | | | | AF-B20 sample pairs in WAF | | | |
|---|---|---|---|---|---|---|---|---|
| Compound name | 01 | 02 | 03 | 04 | 01 | 02 | 03 | 04 |
| Benzene, 1-methyl-4-(1-methylpropyl)- | | | | | 26% | 32% | 22% | 47% |
| Benzene, 1-methyl-4-(1-methylpropyl)- | | | | | 19% | 30% | 22% | 48% |
| Naphthalene, 1,2,3,4-tetrahydro- | 9% | 102% | | | 17% | 10% | 6% | 20% |
| Benzene, 2-ethyl-1,3-dimethyl- | | | | | 24% | 30% | | 47% |
| 1H-Indene,2,3-dihydro-2,2-dimethyl- | 8% | 5% | 1% | 2% | 3% | 10% | 10% | 14% |
| 1H-Indene,2,3-dihydro-2,2-dimethyl- | | 112% | | | 21% | 19% | 13% | 34% |
| Naphthalene | | | 9% | 6% | 15% | 6% | 5% | 14% |
| 1H-Indene, 2,3-dihydro-1,6-dimethyl- | | | | | 20% | 17% | 12% | 31% |
| Benzene, 1,3-dimethyl-5-(1-methylethyl) | | | | 14% | 20% | 19% | 14% | 34% |
| Benzene, 1,3-dimethyl-5-(1-methylethyl) | | | | | 20% | 15% | 11% | |
| Naphthalene, 1,2,3,4-tetrahydro-2-methy | 17% | | | | 20% | 17% | 11% | 30% |
| Naphthalene, 1,2,3,4-tetrahydro-1-methy | 8% | | | | 18% | 13% | 9% | 25% |
| Bicyclo[4.2.1]nona-2,4,7-triene, 7-ethy | 11% | | | | 18% | 15% | 11% | 28% |
| Benzocycloheptene | | | | | 123% | 13% | 10% | 19% |
| 1H-Indene, 2,3-dihydro-4,7-dimethyl- | | 117% | | | 19% | 9% | 8% | 27% |
| 1H-Indene, 2,3-dihydro-4,7-dimethyl- | 15% | 120% | | | 15% | 8% | 7% | 22% |
| Naphthalene, 1,2,3,4-tetrahydro-6-methy | | 113% | | | 16% | 9% | 8% | 22% |
| Naphthalene, 1,2,3,4-tetrahydro-1,5-dim | | | | | 16% | | 15% | 42% |
| Phenol, 2-(2-methyl-2-propenyl)- | | | | | 14% | 11% | 8% | 21% |
| 1H-Indene, 2,3-dihydro-4,7-dimethyl- | | | | | 15% | 7% | 7% | 22% |
| Naphthalene, 1,2,3,4-tetrahydro-1,5-dim | | | | | 16% | 15% | 10% | |
| Naphthalene, 1,2,3,4-tetrahydro-5-methy | 18% | 115% | | | 15% | 6% | 6% | 18% |
| 1H-Indene, 2,3-dihydro-1,4,7-trimethyl- | | | | | 14% | 14% | 9% | 31% |
| Naphthalene, 1-methyl- | 15% | | | | 13% | 4% | 5% | 14% |
| Decanoic acid, methyl ester | 28% | | 1% | | 26% | 14% | | |
| Naphthalene, 1-methyl- | | | | | 13% | 2% | 6% | 14% |
| Naphthalene, 1,2,3,4-tetrahydro-1,4-dim | 30% | | | | 15% | 13% | 11% | 29% |
| 1H-Indene, 2,3-dihydro-1,1,5-trimethyl- | 12% | | | | 14% | 12% | 10% | 27% |
| Naphthalene, 2-ethyl-1,2,3,4-tetrahydro | | | | | 13% | 14% | 16% | |
| Naphthalene, 1,2,3,4-tetrahydro-1,5-dim | | | | | 14% | 15% | 17% | |
| Naphthalene, 1,2,3,4-tetrahydro-1,4-dim | | | | | 31% | 40% | 15% | 76% |
| (1,4-Dimethylpent-2-enyl)benzene | | | | | 5% | 7% | 10% | 25% |
| Naphthalene, 1,2,3,4-tetrahydro-1,4-dim | | | | | 23% | 13% | 19% | 30% |
| Naphthalene, 1,2,3,4-tetrahydro-1,5-dim | | | | | 11% | 7% | 8% | 26% |
| Ethanol, 2-(2-butoxyethoxy)-, acetate | 7% | 16% | 13% | 62% | 4% | 26% | 19% | 45% |
| Naphthalene, 5-ethyl-1,2,3,4-tetrahydro | | | | | 17% | 9% | 10% | 25% |
| Methyl 4-oxododecanoate | 13% | 24% | 20% | 50% | 7% | 26% | 24% | 38% |
| Naphthalene, 5-ethyl-1,2,3,4-tetrahydro | | | | | 10% | 6% | 2% | 34% |
| Acenaphthylene, 1,2,2a,3,4,5-hexahydro- | | | | | 12% | 6% | 9% | 19% |
| Biphenyl | 3% | | | | 13% | 3% | 4% | 14% |
| Naphthalene, 1,2,3,4-tetrahydro-5,7-dim | | | | | 11% | 5% | 6% | 22% |
| Naphthalene, 1,2,3,4-tetrahydro-5,7-dim | | | | | 10% | 5% | 8% | 23% |
| Diphenylmethane | | | | | 11% | 3% | 6% | 16% |
| Naphthalene, 1,4-dimethyl- | | | | | 9% | 2% | 6% | 13% |
| Naphthalene, 1,2,3,4-tetrahydro-6,7-dim | | | | | 11% | 6% | 7% | 18% |
| Naphthalene, 1,7-dimethyl- | | | | | 10% | 3% | 5% | 15% |
| Naphthalene, 1,2,3,4-tetrahydro-5,6-dim | | | | | 10% | 6% | 7% | 21% |
| Naphthalene, 2,6-dimethyl- | | | | | 9% | 4% | 6% | 17% |

| | AF-B100 sample pairs in WAF | | | | AF-B20 sample pairs in WAF | | | |
|---|---|---|---|---|---|---|---|---|
| Compound name | 01 | 02 | 03 | 04 | 01 | 02 | 03 | 04 |
| Nonanoic acid, 9-oxo-, methyl ester | 31% | 31% | 41% | | 1% | 51% | 51% | |
| Naphthalene, 1,2,3,4-tetrahydro-2,5,8-t | | | | | 2% | 9% | 16% | |
| Naphthalene, 1,2,3,4-tetrahydro-2,5,8-t | | | | | 7% | 8% | 10% | 26% |
| Naphthalene, 1,2,3,4-tetrahydro-2,5,8-t | | | | | 9% | 14% | 14% | |
| Naphthalene, 1,2-dimethyl- | | | | | 38% | 0% | 5% | 16% |
| Naphthalene, 1,2,3,4-tetrahydro-1,4,6-t | | | | | 3% | 4% | 12% | 28% |
| 1,4-Dimethyl-2-cyclopentylbenzene | | | | | 8% | 8% | 12% | 28% |
| 1,1'-Biphenyl, 4-methyl- | 5% | | | | 9% | 3% | 5% | 13% |
| 1,1'-Biphenyl, 4-methyl- | | | | | 8% | 2% | 5% | 14% |
| Dodecanoic acid, methyl ester | 46% | 16% | | | 13% | | | |
| Naphthalene, 1,4,6-trimethyl- | | | | | 9% | 4% | 7% | 14% |
| Naphthalene, 1,6,7-trimethyl- | | | | | 5% | 4% | 6% | 18% |
| Naphthalene, 1,6,7-trimethyl- | | | | | 5% | 5% | 5% | 16% |
| Nonanedioic acid, dimethyl ester | 9% | 34% | 35% | | 11% | 63% | 51% | |
| Naphthalene, 2,3,6-trimethyl- | | | | | 5% | 3% | 5% | 18% |
| 1,4-Benzenediol, 2-(1,1-dimethylethyl)- | 77% | 14% | 17% | 11% | 8% | 18% | 22% | 27% |
| 4,4'-Dimethylbiphenyl | | | | | 3% | 3% | 4% | 15% |
| Naphthalene, 1-(2-propenyl)- | | | | | 6% | 3% | 5% | 13% |
| Methyl myristoleate | 51% | | | | 7% | | | |
| Methyl tetradecanoate | 61% | | | | 6% | | | |
| 4,4'-Dimethylbiphenyl | | | | | 7% | 5% | 2% | 7% |
| Pentadecanoic acid, methyl ester | 56% | | | | 3% | | | |
| Naphthalene, 1,2,3,4,4a,5,8,8a-octahydr | 10% | 6% | 16% | | 4% | 32% | 27% | |
| 9-Hexadecenoic acid, methyl ester, (Z)- | 88% | | | | 5% | | | |
| Methyl palmitoleate | 59% | | | | 2% | | | |
| Hexadecanoic acid, methyl ester | 49% | 47% | 62% | 17% | 2% | 79% | 12% | 42% |
| cis-10-Heptadecenoic acid, methyl ester | 37% | | | | 2% | | | |
| Heptadecanoic acid, methyl ester | 36% | | | | 5% | | | |
| 9,12-Octadecadienoic acid (Z,Z)-, methy | 43% | 59% | | | 1% | | | |
| Octadecenoic acid, ME (Isomers #3-4) | | 57% | | 10% | 9% | | | |
| Octadecanoic acid, ME(Isomer #5) | | 36% | | | 107% | | | |
| Octadecanoic acid, methyl ester | | 53% | 74% | 17% | | 75% | | |
| Pyrene, 4,5-dihydro- | | | | | 2% | 5% | 2% | 13% |
| Pyrene | | | | | 5% | 5% | 2% | 6% |
| cis-11,14-Eicosadienoic acid, methyl ester | 44% | | | | 38% | | | |

**Table II-B-7: Blank concentrations (µg/L or ppb) of each compound in each water**

| Compound name | W_01 | W_02/03 | W_04 | HPLC | 3XSTDEV |
|---|---|---|---|---|---|
| Benzene | 0.02 | 0.02 | 0.02 | 0.06 | 0.05 |
| Butane, 2,2,3,3-tetramethyl- | 0.64 | 1.29 | 1.15 | 17.07 | 24.09 |
| Toluene | 0.08 | 0.03 | 0.03 | 0.20 | 0.23 |
| Acetic acid, butyl ester | | | | | |
| Ethylbenzene | 0.22 | | | 0.04 | 0.38 |
| m-Xylene | 0.49 | 0.03 | 0.03 | 0.07 | 0.68 |
| p-Xylene | 0.24 | | | 0.08 | 0.36 |
| o-Xylene | 0.31 | | | 0.05 | 0.56 |
| Oxime-, methoxy-phenyl-_ | 0.23 | 0.17 | 0.26 | 0.25 | 0.11 |
| Benzene, (1-methylethyl)- | 0.04 | | | | 0.04 |
| Hexanoic acid, methyl ester | | | | | |
| Benzene, propyl- | 0.26 | | | 0.02 | 0.50 |
| Benzene, 1-ethyl-2-methyl- | 0.76 | | | 0.07 | 1.46 |
| Benzene, 1-ethyl-4-methyl- | 0.47 | | | 0.04 | 0.92 |
| Benzene, 1,2,3-trimethyl- | 0.32 | | 0.10 | 0.06 | 0.42 |
| Benzene, 1,3,5-trimethyl- | 0.38 | | | 0.03 | 0.73 |
| Benzene, 1,2,3-trimethyl- | 1.31 | | 0.03 | 0.10 | 2.16 |
| Benzene, (1-methylpropyl)- | | | | | |
| Benzene, 1-methyl-2-(1-methylethyl)- | 0.07 | | | | 0.07 |
| Benzene, 1,2,4-trimethyl- | 0.31 | | | 0.03 | 0.59 |
| Benzene, 1-methyl-2-(1-methylethyl)- | 0.07 | | | 0.02 | 0.11 |
| Indane | 0.13 | | | 0.01 | 0.25 |
| Benzene, 1,3-diethyl- | 0.14 | | | | 0.14 |
| Benzene, 1-methyl-3-propyl- | 0.38 | | | 0.03 | 0.75 |
| Benzene, 1,2,3,4-tetramethyl- | 0.38 | | | 0.03 | 0.73 |
| Benzene, 1-methyl-4-(1-methylethyl)- | 0.34 | | | 0.03 | 0.67 |
| Benzene, 1-methyl-4-propyl- | 0.30 | | | 0.02 | 0.59 |
| Benzene, 2-ethyl-1,4-dimethyl- | 0.25 | | | 0.03 | 0.47 |
| Benzene, 1-ethyl-2,4-dimethyl- | 0.34 | | | 0.03 | 0.66 |
| Benzene, 4-ethenyl-1,2-dimethyl- | 0.07 | | | | 0.07 |
| Benzene, 1-ethyl-2,4-dimethyl- | 0.43 | | | 0.03 | 0.85 |
| Benzene, 1-ethenyl-4-ethyl- | 0.11 | | | 0.01 | 0.22 |
| Benzene, 1-ethyl-2,4-dimethyl- | 0.02 | | | | 0.02 |
| Benzene, 1-methyl-4-(1-methylpropyl)- | 0.24 | | | | 0.24 |
| Benzene, 1-ethyl-2,3-dimethyl- | 0.15 | | | | 0.15 |
| Benzene, 1-methyl-4-(1-methylpropyl)- | 0.09 | | | | 0.09 |
| Benzene, 1,2,3,4-tetramethyl- | 0.20 | | | | 0.20 |
| Benzene, 1,2,3,4-tetramethyl- | 0.27 | | | | 0.27 |
| Benzene, 1-methyl-4-(1-methylpropyl)- | 0.10 | | | | 0.10 |
| Benzene, (2-methyl-1-butenyl)- | 0.03 | | | | 0.03 |
| Indan, 1-methyl- | 0.39 | | | 0.02 | 0.78 |
| 1H-Indene, 2,3-dihydro-4-methyl- | 0.38 | | | 0.03 | 0.75 |
| Benzene, 1,2,4,5-tetramethyl- | 0.26 | | | 0.02 | 0.51 |
| Benzene, 1-methyl-4-(1-methylpropyl)- | 0.27 | | | 0.02 | 0.53 |
| Benzene, 1-methyl-4-(1-methylpropyl)- | 0.23 | | | 0.01 | 0.45 |
| Naphthalene, 1,2,3,4-tetrahydro- | 0.35 | | | 0.03 | 0.68 |

| Compound name | W_01 | W_02/03 | W_04 | HPLC | 3XSTDEV |
|---|---|---|---|---|---|
| Benzene, 2-ethyl-1,3-dimethyl- | 0.11 | | | | 0.11 |
| 1H-Indene,2,3-dihydro-2,2-dimethyl- | | | | | |
| 1H-Indene,2,3-dihydro-2,2-dimethyl- | 0.33 | | | | 0.33 |
| Naphthalene | 0.10 | 0.03 | | 0.05 | 0.10 |
| 1H-Indene, 2,3-dihydro-1,6-dimethyl- | 0.17 | | | | 0.17 |
| Benzene, 1,3-dimethyl-5-(1-methylethyl) | 0.07 | | | | 0.07 |
| Benzene, 1,3-dimethyl-5-(1-methylethyl) | 0.02 | | | | 0.02 |
| Naphthalene, 1,2,3,4-tetrahydro-2-methy | 0.23 | | | 0.01 | 0.46 |
| Naphthalene, 1,2,3,4-tetrahydro-1-methy | 0.10 | | | | 0.10 |
| Bicyclo[4.2.1]nona-2,4,7-triene, 7-ethy | 0.17 | | | | 0.17 |
| Benzocycloheptene | | | | | |
| 1H-Indene, 2,3-dihydro-4,7-dimethyl- | 0.23 | | | 0.01 | 0.47 |
| 1H-Indene, 2,3-dihydro-4,7-dimethyl- | 0.18 | | | | 0.18 |
| Naphthalene, 1,2,3,4-tetrahydro-6-methy | 0.54 | | | 0.05 | 1.05 |
| Naphthalene, 1,2,3,4-tetrahydro-1,5-dim | 0.18 | | | | 0.18 |
| Phenol, 2-(2-methyl-2-propenyl)- | 0.21 | | | | 0.21 |
| 1H-Indene, 2,3-dihydro-4,7-dimethyl- | 0.10 | | | | 0.10 |
| Naphthalene, 1,2,3,4-tetrahydro-1,5-dim | 0.03 | | | | 0.03 |
| Naphthalene, 1,2,3,4-tetrahydro-5-methy | 0.29 | | | 0.02 | 0.57 |
| 1H-Indene, 2,3-dihydro-1,4,7-trimethyl- | 0.08 | | | | 0.08 |
| Naphthalene, 1-methyl- | 0.14 | | | 0.03 | 0.23 |
| Decanoic acid, methyl ester | | | | | |
| Naphthalene, 1-methyl- | 0.07 | | | 0.03 | 0.08 |
| Naphthalene, 1,2,3,4-tetrahydro-1,4-dim | 0.55 | | | | 0.55 |
| 1H-Indene, 2,3-dihydro-1,1,5-trimethyl- | 0.16 | | | | 0.16 |
| Naphthalene, 2-ethyl-1,2,3,4-tetrahydro | | | | | |
| Naphthalene, 1,2,3,4-tetrahydro-1,5-dim | 0.04 | | | | 0.04 |
| Naphthalene, 1,2,3,4-tetrahydro-1,4-dim | 0.10 | | | | 0.10 |
| (1,4-Dimethylpent-2-enyl)benzene | | | | | |
| Naphthalene, 1,2,3,4-tetrahydro-1,4-dim | 0.11 | | | | 0.11 |
| Naphthalene, 1,2,3,4-tetrahydro-1,5-dim | 0.06 | | | | 0.06 |
| Ethanol, 2-(2-butoxyethoxy)-, acetate | | | | | |
| Naphthalene, 5-ethyl-1,2,3,4-tetrahydro | 0.17 | | | | 0.17 |
| Methyl 4-oxododecanoate | | | | | |
| Naphthalene, 5-ethyl-1,2,3,4-tetrahydro | 0.06 | | | | 0.06 |
| Acenaphthylene, 1,2,2a,3,4,5-hexahydro- | | | | | |
| Biphenyl | 0.24 | | | 0.04 | 0.44 |
| Naphthalene, 1,2,3,4-tetrahydro-5,7-dim | 0.25 | | | | 0.25 |
| Naphthalene, 1,2,3,4-tetrahydro-5,7-dim | 0.13 | | | | 0.13 |
| Diphenylmethane | 0.12 | | | | 0.12 |
| Naphthalene, 1,4-dimethyl- | 0.07 | | | | 0.07 |
| Naphthalene, 1,2,3,4-tetrahydro-6,7-dim | 0.10 | | | | 0.10 |
| Naphthalene, 1,7-dimethyl- | 0.10 | | | | 0.10 |
| Naphthalene, 1,2,3,4-tetrahydro-5,6-dim | 0.13 | | | | 0.13 |
| Naphthalene, 2,6-dimethyl- | 0.09 | | | | 0.09 |
| Nonanoic acid, 9-oxo-, methyl ester | | | | | |
| Naphthalene, 1,2,3,4-tetrahydro-2,5,8-t | 0.11 | | | | 0.11 |
| Naphthalene, 1,2,3,4-tetrahydro-2,5,8-t | 0.45 | | | | 0.45 |
| Naphthalene, 1,2,3,4-tetrahydro-2,5,8-t | 0.09 | | | | 0.09 |

| Compound name | W_01 | W_02/03 | W_04 | HPLC | 3XSTDEV |
|---|---|---|---|---|---|
| Naphthalene, 1,2-dimethyl- | | | | | |
| Naphthalene, 1,2,3,4-tetrahydro-1,4,6-t | 0.11 | | | | 0.11 |
| 1,4-Dimethyl-2-cyclopentylbenzene | 0.13 | | | | 0.13 |
| 1,1'-Biphenyl, 4-methyl- | 0.26 | | | 0.03 | 0.49 |
| 1,1'-Biphenyl, 4-methyl- | 0.18 | | | 0.04 | 0.29 |
| Dodecanoic acid, methyl ester | | | | | |
| Naphthalene, 1,4,6-trimethyl- | 0.08 | | | | 0.08 |
| Naphthalene, 1,6,7-trimethyl- | 0.07 | | | | 0.07 |
| Naphthalene, 1,6,7-trimethyl- | 0.08 | | | | 0.08 |
| Nonanedioic acid, dimethyl ester | | | | | |
| Naphthalene, 2,3,6-trimethyl- | 0.05 | | | | 0.05 |
| 1,4-Benzenediol, 2-(1,1-dimethylethyl)- | | | | | |
| 4,4'-Dimethylbiphenyl | 0.08 | | | | 0.08 |
| Naphthalene, 1-(2-propenyl)- | | | | | |
| Methyl myristoleate | | | | | |
| Methyl tetradecanoate | | | | | |
| 4,4'-Dimethylbiphenyl | | | | | |
| Pentadecanoic acid, methyl ester | | | | | |
| Naphthalene, 1,2,3,4,4a,5,8,8a-octahydr | | | | | |
| 9-Hexadecenoic acid, methyl ester, (Z)- | | | | | |
| Methyl palmitoleate | | | | | |
| Hexadecanoic acid, methyl ester | | | | 0.32 | 0.32 |
| cis-10-Heptadecenoic acid, methyl ester | | | | | |
| Heptadecanoic acid, methyl ester | | | | | |
| 9,12-Octadecadienoic acid (Z,Z)-, methy | | | | | |
| Octadecenoic acid, ME (Isomers #3-4) | | | | | |
| Octadecanoic acid, ME(Isomer #5) | | | | | |
| Octadecanoic acid, methyl ester | | | | 0.28 | 0.28 |
| Pyrene, 4,5-dihydro- | | | | | |
| Pyrene | | | | | |
| cis-11,14 Eicosadienoic acid, methyl ester | | | | | |

**Table II-B-8: Soy-B100 WAF concentrations (μg/L or ppb) with blank subtracted**

| Compound name | WAF_01 | WAF_02 | WAF_03 | WAF_04 |
|---|---|---|---|---|
| Benzene | 0.2 | 0.1 | 0.1 | 0.1 |
| Butane, 2,2,3,3-tetramethyl- | 24.5 | 7.0 | | |
| Toluene | 1.0 | 1.5 | 0.2 | |
| Acetic acid, butyl ester | 110.6 | 63.6 | 77.7 | 1.0 |
| Ethylbenzene | 0.3 | 1.1 | | |
| m-Xylene | 0.3 | 2.4 | | |
| p-Xylene | 0.1 | 1.1 | | |
| o-Xylene | 0.4 | 1.5 | | |
| Oxime-, methoxy-phenyl-_ | 0.8 | 0.4 | 0.3 | 0.5 |
| Benzene, (1-methylethyl)- | | | | |
| Hexanoic acid, methyl ester | 0.8 | 0.2 | | |
| Benzene, propyl- | | 0.4 | | |
| Benzene, 1-ethyl-2-methyl- | | 1.5 | | |
| Benzene, 1-ethyl-4-methyl- | | 0.8 | | |
| Benzene, 1,2,3-trimethyl- | 0.4 | 0.8 | | |
| Benzene, 1,3,5-trimethyl- | | 0.9 | | |
| Benzene, 1,2,3-trimethyl- | | 3.2 | | |
| Benzene, (1-methylpropyl)- | | | | |
| Benzene, 1-methyl-2-(1-methylethyl)- | | 0.1 | | |
| Benzene, 1,2,4-trimethyl- | | 0.9 | | |
| Benzene, 1-methyl-2-(1-methylethyl)- | | 0.1 | | |
| Indane | 0.1 | 0.5 | | |
| Benzene, 1,3-diethyl- | | 0.3 | | |
| Benzene, 1-methyl-3-propyl- | | 0.3 | | |
| Benzene, 1,2,3,4-tetramethyl- | | 0.3 | | |
| Benzene, 1-methyl-4-(1-methylethyl)- | | 0.4 | | |
| Benzene, 1-methyl-4-propyl- | | 0.3 | | |
| Benzene, 2-ethyl-1,4-dimethyl- | | 0.4 | | |
| Benzene, 1-ethyl-2,4-dimethyl- | | 0.4 | | |
| Benzene, 4-ethenyl-1,2-dimethyl- | | 0.2 | | |
| Benzene, 1-ethyl-2,4-dimethyl- | | 0.5 | | |
| Benzene, 1-ethenyl-4-ethyl- | | 0.3 | | |
| Benzene, 1-ethyl-2,4-dimethyl- | | 0.1 | 0.1 | |
| Benzene, 1-methyl-4-(1-methylpropyl)- | | 0.2 | | |
| Benzene, 1-ethyl-2,3-dimethyl- | | 0.4 | | |
| Benzene, 1-methyl-4-(1-methylpropyl)- | | 0.1 | | |
| Benzene, 1,2,3,4-tetramethyl- | | 0.5 | | |
| Benzene, 1,2,3,4-tetramethyl- | | 0.7 | | |
| Benzene, 1-methyl-4-(1-methylpropyl)- | | 0.1 | | |
| Benzene, (2-methyl-1-butenyl)- | | 0.3 | | |
| Indan, 1-methyl- | | 0.9 | | |
| 1H-Indene, 2,3-dihydro-4-methyl- | | 1.0 | | |
| Benzene, 1,2,4,5-tetramethyl- | | 0.5 | | |
| Benzene, 1-methyl-4-(1-methylpropyl)- | | 0.1 | | |
| Benzene, 1-methyl-4-(1-methylpropyl)- | | 0.0 | | |
| Naphthalene, 1,2,3,4-tetrahydro- | | 1.0 | | |

| Compound name | WAF_01 | WAF_02 | WAF_03 | WAF_04 |
|---|---|---|---|---|
| Benzene, 2-ethyl-1,3-dimethyl- | | 0.1 | | |
| 1H-Indene,2,3-dihydro-2,2-dimethyl- | 12.6 | 11.6 | 11.3 | 8.9 |
| 1H-Indene,2,3-dihydro-2,2-dimethyl- | 0.4 | 0.6 | 0.4 | 0.2 |
| Naphthalene | 0.1 | 0.4 | | 0.1 |
| 1H-Indene, 2,3-dihydro-1,6-dimethyl- | | 0.4 | | |
| Benzene, 1,3-dimethyl-5-(1-methylethyl) | | 0.2 | 0.1 | 0.2 |
| Benzene, 1,3-dimethyl-5-(1-methylethyl) | | 0.2 | | |
| Naphthalene, 1,2,3,4-tetrahydro-2-methy | | | | |
| Naphthalene, 1,2,3,4-tetrahydro-1-methy | | 0.3 | | |
| Bicyclo[4.2.1]nona-2,4,7-triene, 7-ethy | | 0.4 | | |
| Benzocycloheptene | | | | |
| 1H-Indene, 2,3-dihydro-4,7-dimethyl- | | 0.4 | | |
| 1H-Indene, 2,3-dihydro-4,7-dimethyl- | | 0.5 | | |
| Naphthalene, 1,2,3,4-tetrahydro-6-methy | | 1.0 | | |
| Naphthalene, 1,2,3,4-tetrahydro-1,5-dim | | 0.2 | | |
| Phenol, 2-(2-methyl-2-propenyl)- | | 0.2 | | |
| 1H-Indene, 2,3-dihydro-4,7-dimethyl- | | 0.3 | | |
| Naphthalene, 1,2,3,4-tetrahydro-1,5-dim | | 0.5 | | |
| Naphthalene, 1,2,3,4-tetrahydro-5-methy | | 0.6 | | |
| 1H-Indene, 2,3-dihydro-1,4,7-trimethyl- | | 0.2 | | |
| Naphthalene, 1-methyl- | | 0.5 | | |
| Decanoic acid, methyl ester | | | | |
| Naphthalene, 1-methyl- | | 0.3 | | |
| Naphthalene, 1,2,3,4-tetrahydro-1,4-dim | | 0.9 | | |
| 1H-Indene, 2,3-dihydro-1,1,5-trimethyl- | | 0.4 | | |
| Naphthalene, 2-ethyl-1,2,3,4-tetrahydro | | | | |
| Naphthalene, 1,2,3,4-tetrahydro-1,5-dim | | | | |
| Naphthalene, 1,2,3,4-tetrahydro-1,4-dim | | 0.3 | | |
| (1,4-Dimethylpent-2-enyl)benzene | | | | |
| Naphthalene, 1,2,3,4-tetrahydro-1,4-dim | | 0.3 | | |
| Naphthalene, 1,2,3,4-tetrahydro-1,5-dim | | 0.2 | | |
| Ethanol, 2-(2-butoxyethoxy)-, acetate | 34.7 | 21.0 | 27.8 | 1.3 |
| Naphthalene, 5-ethyl-1,2,3,4-tetrahydro | | 0.5 | | |
| Methyl 4-oxododecanoate | | | | |
| Naphthalene, 5-ethyl-1,2,3,4-tetrahydro | | | | |
| Acenaphthylene, 1,2,2a,3,4,5-hexahydro- | | | | |
| Biphenyl | | 0.8 | | |
| Naphthalene, 1,2,3,4-tetrahydro-5,7-dim | | 0.8 | | |
| Naphthalene, 1,2,3,4-tetrahydro-5,7-dim | | 0.3 | | |
| Diphenylmethane | | 0.4 | | |
| Naphthalene, 1,4-dimethyl- | | 0.3 | | |
| Naphthalene, 1,2,3,4-tetrahydro-6,7-dim | | 0.2 | | |
| Naphthalene, 1,7-dimethyl- | | 0.4 | | |
| Naphthalene, 1,2,3,4-tetrahydro-5,6-dim | | 0.3 | | |
| Naphthalene, 2,6-dimethyl- | | 0.3 | | |
| Nonanoic acid, 9-oxo-, methyl ester | | 1.1 | 0.9 | |
| Naphthalene, 1,2,3,4-tetrahydro-2,5,8-t | | | | |
| Naphthalene, 1,2,3,4-tetrahydro-2,5,8-t | | 1.0 | | |
| Naphthalene, 1,2,3,4-tetrahydro-2,5,8-t | | 0.1 | | |

| Compound name | WAF_01 | WAF_02 | WAF_03 | WAF_04 |
|---|---|---|---|---|
| Naphthalene, 1,2-dimethyl- | | | | |
| Naphthalene, 1,2,3,4-tetrahydro-1,4,6-t | | 0.1 | | |
| 1,4-Dimethyl-2-cyclopentylbenzene | | 0.2 | | |
| 1,1'-Biphenyl, 4-methyl- | | 0.7 | | |
| 1,1'-Biphenyl, 4-methyl- | | 0.4 | | |
| Dodecanoic acid, methyl ester | | | | |
| Naphthalene, 1,4,6-trimethyl- | | | | |
| Naphthalene, 1,6,7-trimethyl- | | 0.2 | | |
| Naphthalene, 1,6,7-trimethyl- | | 0.3 | | |
| Nonanedioic acid, dimethyl ester | 4.7 | 0.8 | 0.6 | |
| Naphthalene, 2,3,6-trimethyl- | | 0.2 | | |
| 1,4-Benzenediol, 2-(1,1-dimethylethyl)- | 53.3 | 14.4 | 12.6 | 16.4 |
| 4,4'-Dimethylbiphenyl | | 0.2 | | |
| Naphthalene, 1-(2-propenyl)- | | | | |
| Methyl myristoleate | | | | |
| Methyl tetradecanoate | 1.4 | | | |
| 4,4'-Dimethylbiphenyl | | | | |
| Pentadecanoic acid, methyl ester | | | | |
| Naphthalene, 1,2,3,4,4a,5,8,8a-octahydr | | | | |
| 9-Hexadecenoic acid, methyl ester, (Z)- | | | | |
| Methyl palmitoleate | | | | |
| Hexadecanoic acid, methyl ester | 127.1 | 2.4 | | 2.0 |
| cis-10-Heptadecenoic acid, methyl ester | | | | |
| Heptadecanoic acid, methyl ester | | | | |
| 9,12-Octadecadienoic acid (Z,Z)-, methy | 1094.6 | 73.0 | | |
| Octadecenoic acid, ME (Isomers #3-4) | 456.9 | 22.9 | | |
| Octadecanoic acid, ME(Isomer #5) | 23.8 | 0.4 | | |
| Octadecanoic acid, methyl ester | 59.8 | 0.9 | | 0.6 |
| Pyrene, 4,5-dihydro- | | | | |
| Pyrene | | 0.2 | | |
| cis-11,14 Eicosadienoic acid, methyl ester | | | | |

**Table II-B-9: Soy-B20 WAF concentrations (μg/L or ppb) with blank subtracted**

| Compound name | WAF_01 | WAF_02 | WAF_03 | WAF_04 |
|---|---|---|---|---|
| Benzene | 0.7 | 0.4 | 0.2 | 0.2 |
| Butane, 2,2,3,3-tetramethyl- | | | | |
| Toluene | 92.0 | 57.2 | 27.2 | 1.6 |
| Acetic acid, butyl ester | | 13.4 | 18.0 | |
| Ethylbenzene | 115.3 | 70.0 | 31.5 | |
| m-Xylene | 200.9 | 120.2 | 53.5 | 86.7 |
| p-Xylene | 83.7 | 50.4 | 22.5 | 22.7 |
| o-Xylene | 186.0 | 117.4 | 60.8 | 88.9 |
| Oxime-, methoxy-phenyl-_ | 0.5 | 0.8 | 0.8 | 0.7 |
| Benzene, (1-methylethyl)- | 16.2 | 8.4 | 3.4 | 1.7 |
| Hexanoic acid, methyl ester | | | | |
| Benzene, propyl- | 56.4 | 30.9 | 12.2 | 1.6 |
| Benzene, 1-ethyl-2-methyl- | 186.7 | 107.5 | 48.6 | 82.5 |
| Benzene, 1-ethyl-4-methyl- | 96.9 | 54.6 | 23.6 | 28.7 |
| Benzene, 1,2,3-trimethyl- | 64.8 | 36.9 | 16.9 | 30.8 |
| Benzene, 1,3,5-trimethyl- | 140.8 | 86.1 | 42.4 | 69.3 |
| Benzene, 1,2,3-trimethyl- | 319.2 | 190.9 | 96.2 | 149.0 |
| Benzene, (1-methylpropyl)- | 19.8 | | | |
| Benzene, 1-methyl-2-(1-methylethyl)- | 16.1 | 8.6 | 3.4 | 7.2 |
| Benzene, 1,2,4-trimethyl- | 131.2 | 83.7 | 46.8 | 68.5 |
| Benzene, 1-methyl-2-(1-methylethyl)- | 10.7 | 5.8 | 2.2 | 4.4 |
| Indane | 87.1 | 58.1 | 35.2 | 25.3 |
| Benzene, 1,3-diethyl- | 26.8 | 14.4 | 6.1 | 11.0 |
| Benzene, 1-methyl-3-propyl- | 49.3 | 25.5 | 9.7 | 18.2 |
| Benzene, 1,2,3,4-tetramethyl- | 47.3 | 24.5 | 9.3 | 10.1 |
| Benzene, 1-methyl-4-(1-methylethyl)- | 63.1 | 34.4 | 15.0 | 25.6 |
| Benzene, 1-methyl-4-propyl- | 56.5 | 31.6 | 13.3 | 24.5 |
| Benzene, 2-ethyl-1,4-dimethyl- | 54.4 | 31.1 | 14.9 | 25.5 |
| Benzene, 1-ethyl-2,4-dimethyl- | 58.3 | 32.9 | 15.5 | 26.7 |
| Benzene, 4-ethenyl-1,2-dimethyl- | 22.0 | 13.5 | 6.9 | 5.5 |
| Benzene, 1-ethyl-2,4-dimethyl- | 68.2 | 38.1 | 18.4 | 29.9 |
| Benzene, 1-ethenyl-4-ethyl- | 53.7 | 33.7 | 17.9 | 26.0 |
| Benzene, 1-ethyl-2,4-dimethyl- | 9.8 | 5.9 | 3.1 | 4.5 |
| Benzene, 1-methyl-4-(1-methylpropyl)- | 28.9 | 14.2 | 4.7 | 11.6 |
| Benzene, 1-ethyl-2,3-dimethyl- | 39.2 | 23.6 | 12.7 | 19.0 |
| Benzene, 1-methyl-4-(1-methylpropyl)- | 8.3 | 4.0 | 1.2 | 3.1 |
| Benzene, 1,2,3,4-tetramethyl- | 33.9 | 19.9 | 10.6 | 15.9 |
| Benzene, 1,2,3,4-tetramethyl- | 58.6 | 34.8 | 19.0 | 27.8 |
| Benzene, 1-methyl-4-(1-methylpropyl)- | 8.3 | 3.9 | 1.4 | 2.2 |
| Benzene, (2-methyl-1-butenyl)- | 6.2 | 3.6 | 1.6 | 2.8 |
| Indan, 1-methyl- | 103.5 | 63.8 | 37.8 | 50.0 |
| 1H-Indene, 2,3-dihydro-4-methyl- | 154.2 | 99.2 | 60.5 | 77.8 |
| Benzene, 1,2,4,5-tetramethyl- | 69.7 | 42.8 | 25.6 | 34.1 |
| Benzene, 1-methyl-4-(1-methylpropyl)- | 27.9 | 14.3 | 6.0 | 11.7 |
| Benzene, 1-methyl-4-(1-methylpropyl)- | 16.6 | 8.4 | 3.8 | 7.4 |
| Naphthalene, 1,2,3,4-tetrahydro- | 173.2 | 113.1 | 70.8 | 74.9 |

| Compound name | WAF_01 | WAF_02 | WAF_03 | WAF_04 |
|---|---|---|---|---|
| Benzene, 2-ethyl-1,3-dimethyl- | 8.4 | 4.1 | 1.9 | 3.3 |
| 1H-Indene,2,3-dihydro-2,2-dimethyl- | 2.1 | 2.6 | 2.4 | 0.1 |
| 1H-Indene,2,3-dihydro-2,2-dimethyl- | 63.2 | 36.8 | 19.8 | 28.7 |
| Naphthalene | 41.6 | 29.2 | 20.5 | 14.6 |
| 1H-Indene, 2,3-dihydro-1,6-dimethyl- | 41.3 | 24.6 | 13.6 | 19.1 |
| Benzene, 1,3-dimethyl-5-(1-methylethyl) | 9.7 | 5.1 | 2.9 | 4.3 |
| Benzene, 1,3-dimethyl-5-(1-methylethyl) | 5.3 | 3.0 | 1.8 | 2.5 |
| Naphthalene, 1,2,3,4-tetrahydro-2-methy | 43.8 | 26.0 | 14.4 | 19.2 |
| Naphthalene, 1,2,3,4-tetrahydro-1-methy | 38.2 | 23.9 | 14.4 | 18.5 |
| Bicyclo[4.2.1]nona-2,4,7-triene, 7-ethy | 36.1 | 21.0 | 12.9 | 16.4 |
| Benzocycloheptene | 1.2 | 0.7 | 0.4 | 0.6 |
| 1H-Indene, 2,3-dihydro-4,7-dimethyl- | 44.3 | 25.4 | 15.6 | 20.5 |
| 1H-Indene, 2,3-dihydro-4,7-dimethyl- | 46.5 | 28.1 | 17.9 | 22.0 |
| Naphthalene, 1,2,3,4-tetrahydro-6-methy | 105.0 | 62.9 | 39.5 | 48.8 |
| Naphthalene, 1,2,3,4-tetrahydro-1,5-dim | 9.7 | 4.7 | 2.3 | 4.3 |
| Phenol, 2-(2-methyl-2-propenyl)- | 10.3 | 5.9 | 3.8 | 4.7 |
| 1H-Indene, 2,3-dihydro-4,7-dimethyl- | 26.0 | 15.8 | 10.3 | 12.5 |
| Naphthalene, 1,2,3,4-tetrahydro-1,5-dim | 5.6 | 3.1 | 1.8 | 2.5 |
| Naphthalene, 1,2,3,4-tetrahydro-5-methy | 85.2 | 52.8 | 34.8 | 41.1 |
| 1H-Indene, 2,3-dihydro-1,4,7-trimethyl- | 13.5 | 7.5 | 4.7 | 6.0 |
| Naphthalene, 1-methyl- | 28.4 | 18.8 | 13.7 | 12.9 |
| Decanoic acid, methyl ester | 0.3 | 0.2 | | 0.1 |
| Naphthalene, 1-methyl- | 27.2 | 18.2 | 13.2 | 13.6 |
| Naphthalene, 1,2,3,4-tetrahydro-1,4-dim | 51.8 | 28.1 | 17.1 | 23.5 |
| 1H-Indene, 2,3-dihydro-1,1,5-trimethyl- | 27.4 | 12.0 | 7.4 | 9.9 |
| Naphthalene, 2-ethyl-1,2,3,4-tetrahydro | 4.1 | 2.5 | 1.6 | 2.0 |
| Naphthalene, 1,2,3,4-tetrahydro-1,5-dim | 5.4 | 3.0 | 1.9 | 2.7 |
| Naphthalene, 1,2,3,4-tetrahydro-1,4-dim | 13.5 | 14.4 | 9.2 | 10.9 |
| (1,4-Dimethylpent-2-enyl)benzene | 9.1 | 6.4 | 4.1 | 5.3 |
| Naphthalene, 1,2,3,4-tetrahydro-1,4-dim | 13.4 | 6.8 | 4.9 | 6.5 |
| Naphthalene, 1,2,3,4-tetrahydro-1,5-dim | 14.4 | 8.5 | 5.6 | 6.9 |
| Ethanol, 2-(2-butoxyethoxy)-, acetate | 6.8 | 5.1 | 8.2 | |
| Naphthalene, 5-ethyl-1,2,3,4-tetrahydro | 18.4 | 10.1 | 6.5 | 8.2 |
| Methyl 4-oxododecanoate | | | | |
| Naphthalene, 5-ethyl-1,2,3,4-tetrahydro | 8.6 | 5.6 | 3.3 | 4.9 |
| Acenaphthylene, 1,2,2a,3,4,5-hexahydro- | 3.4 | 2.0 | 1.3 | 1.6 |
| Biphenyl | 54.6 | 34.7 | 26.6 | 21.6 |
| Naphthalene, 1,2,3,4-tetrahydro-5,7-dim | 30.3 | 17.1 | 11.7 | 14.1 |
| Naphthalene, 1,2,3,4-tetrahydro-5,7-dim | 13.6 | 7.8 | 5.4 | 6.6 |
| Diphenylmethane | 24.6 | 14.8 | 10.4 | 10.8 |
| Naphthalene, 1,4-dimethyl- | 7.8 | 4.5 | 3.3 | 3.5 |
| Naphthalene, 1,2,3,4-tetrahydro-6,7-dim | 14.2 | 8.1 | 5.6 | 6.9 |
| Naphthalene, 1,7-dimethyl- | 19.0 | 11.8 | 8.5 | 8.6 |
| Naphthalene, 1,2,3,4-tetrahydro-5,6-dim | 17.2 | 9.5 | 6.9 | 8.0 |
| Naphthalene, 2,6-dimethyl- | 12.9 | 7.9 | 5.8 | 5.8 |
| Nonanoic acid, 9-oxo-, methyl ester | | | | |
| Naphthalene, 1,2,3,4-tetrahydro-2,5,8-t | 5.5 | 2.9 | 2.0 | 2.9 |
| Naphthalene, 1,2,3,4-tetrahydro-2,5,8-t | 25.7 | 13.3 | 9.4 | 13.0 |
| Naphthalene, 1,2,3,4-tetrahydro-2,5,8-t | 3.4 | 1.6 | 1.1 | 1.6 |

| Compound name | WAF_01 | WAF_02 | WAF_03 | WAF_04 |
|---|---|---|---|---|
| Naphthalene, 1,2-dimethyl- | 2.7 | 1.7 | 1.2 | 1.2 |
| Naphthalene, 1,2,3,4-tetrahydro-1,4,6-t | 8.5 | 4.2 | 3.1 | 4.6 |
| 1,4-Dimethyl-2-cyclopentylbenzene | 7.9 | 4.0 | 2.9 | 4.0 |
| 1,1'-Biphenyl, 4-methyl- | 33.0 | 19.7 | 15.0 | 13.8 |
| 1,1'-Biphenyl, 4-methyl- | 18.8 | 11.1 | 8.7 | 6.7 |
| Dodecanoic acid, methyl ester | | | | |
| Naphthalene, 1,4,6-trimethyl- | 3.4 | 2.3 | 1.7 | 1.6 |
| Naphthalene, 1,6,7-trimethyl- | 6.9 | 3.8 | 3.0 | 3.1 |
| Naphthalene, 1,6,7-trimethyl- | 6.8 | 3.8 | 2.9 | 2.9 |
| Nonanedioic acid, dimethyl ester | | | | |
| Naphthalene, 2,3,6-trimethyl- | 6.3 | 3.6 | 2.7 | 2.9 |
| 1,4-Benzenediol, 2-(1,1-dimethylethyl)- | 18.4 | 4.7 | 4.6 | 7.7 |
| 4,4'-Dimethylbiphenyl | 4.5 | 2.5 | 2.1 | 2.1 |
| Naphthalene, 1-(2-propenyl)- | 9.1 | 5.8 | 4.4 | 4.1 |
| Methyl myristoleate | | | | |
| Methyl tetradecanoate | | | | |
| 4,4'-Dimethylbiphenyl | 3.4 | 2.2 | 1.6 | 1.7 |
| Pentadecanoic acid, methyl ester | | | | |
| Naphthalene, 1,2,3,4,4a,5,8,8a-octahydr | | | | |
| 9-Hexadecenoic acid, methyl ester, (Z)- | | | | |
| Methyl palmitoleate | | | | |
| Hexadecanoic acid, methyl ester | | | | |
| cis-10-Heptadecenoic acid, methyl ester | | | | |
| Heptadecanoic acid, methyl ester | | | | |
| 9,12-Octadecadienoic acid (Z,Z)-, methy | 13.1 | | | |
| Octadecenoic acid, ME (Isomers #3-4) | 8.3 | | | |
| Octadecanoic acid, ME(Isomer #5) | | | | |
| Octadecanoic acid, methyl ester | 0.4 | | | |
| Pyrene, 4,5-dihydro- | 1.5 | 0.8 | 0.6 | 0.7 |
| Pyrene | 4.6 | 3.0 | 2.1 | 2.3 |
| cis-11,14 Eicosadienoic acid, methyl ester | | | | |

**Table II-B-10: AF-B100 WAF concentrations (µg/L or ppb) with blank subtracted**

| Compound name | WAF_01[1] | WAF_02 | WAF_03 | WAF_04 |
|---|---|---|---|---|
| Benzene | 0.5 | 0.2 | 0.2 | 0.3 |
| Butane, 2,2,3,3-tetramethyl- | 3.2 | | | |
| Toluene | 0.6 | 0.1 | 0.0 | 0.1 |
| Acetic acid, butyl ester | 40.2 | 56.4 | 69.1 | 3.1 |
| Ethylbenzene | 0.4 | | | |
| m-Xylene | 0.8 | | | |
| p-Xylene | 0.3 | | | |
| o-Xylene | 0.5 | | | |
| Oxime-, methoxy-phenyl-_ | 2.1 | 0.7 | 0.8 | 0.6 |
| Benzene, (1-methylethyl)- | | | | |
| Hexanoic acid, methyl ester | 50.5 | 21.7 | 25.7 | 1.6 |
| Benzene, propyl- | 0.2 | | | |
| Benzene, 1-ethyl-2-methyl- | 0.3 | | | |
| Benzene, 1-ethyl-4-methyl- | 0.0 | | | |
| Benzene, 1,2,3-trimethyl- | 0.2 | 0.0 | | |
| Benzene, 1,3,5-trimethyl- | 0.4 | | | |
| Benzene, 1,2,3-trimethyl- | 1.0 | | | |
| Benzene, (1-methylpropyl)- | | 0.0 | | |
| Benzene, 1-methyl-2-(1-methylethyl)- | | | | |
| Benzene, 1,2,4-trimethyl- | 0.5 | | | |
| Benzene, 1-methyl-2-(1-methylethyl)- | 0.1 | | | |
| Indane | 0.3 | | | |
| Benzene, 1,3-diethyl- | 0.1 | | | |
| Benzene, 1-methyl-3-propyl- | 0.4 | | | |
| Benzene, 1,2,3,4-tetramethyl- | 2.5 | 0.3 | 0.0 | 0.0 |
| Benzene, 1-methyl-4-(1-methylethyl)- | 0.5 | | | |
| Benzene, 1-methyl-4-propyl- | 0.3 | | | |
| Benzene, 2-ethyl-1,4-dimethyl- | 0.3 | | | |
| Benzene, 1-ethyl-2,4-dimethyl- | 0.3 | | | |
| Benzene, 4-ethenyl-1,2-dimethyl- | | | | |
| Benzene, 1-ethyl-2,4-dimethyl- | 0.3 | | | |
| Benzene, 1-ethenyl-4-ethyl- | 0.3 | 0.0 | | |
| Benzene, 1-ethyl-2,4-dimethyl- | | | | |
| Benzene, 1-methyl-4-(1-methylpropyl)- | 0.8 | 0.1 | | |
| Benzene, 1-ethyl-2,3-dimethyl- | | 0.1 | | |
| Benzene, 1-methyl-4-(1-methylpropyl)- | | | | |
| Benzene, 1,2,3,4-tetramethyl- | 0.3 | 0.1 | | |
| Benzene, 1,2,3,4-tetramethyl- | 0.6 | 0.3 | | |
| Benzene, 1-methyl-4-(1-methylpropyl)- | 0.1 | | | |
| Benzene, (2-methyl-1-butenyl)- | | | | |
| Indan, 1-methyl- | 0.5 | 0.1 | | |
| 1H-Indene, 2,3-dihydro-4-methyl- | 1.1 | 0.6 | | |
| Benzene, 1,2,4,5-tetramethyl- | 0.5 | 0.3 | | |
| Benzene, 1-methyl-4-(1-methylpropyl)- | 0.5 | | | |
| Benzene, 1-methyl-4-(1-methylpropyl)- | 0.4 | | | |
| Naphthalene, 1,2,3,4-tetrahydro- | 1.2 | 0.7 | | |

| Compound name | WAF_01[1] | WAF_02 | WAF_03 | WAF_04 |
|---|---|---|---|---|
| Benzene, 2-ethyl-1,3-dimethyl- | 0.3 | 0.1 | | |
| 1H-Indene,2,3-dihydro-2,2-dimethyl- | 7.1 | 10.4 | 9.5 | 9.0 |
| 1H-Indene,2,3-dihydro-2,2-dimethyl- | 1.0 | 0.5 | | 0.1 |
| Naphthalene | 0.3 | 0.3 | 0.1 | 0.1 |
| 1H-Indene, 2,3-dihydro-1,6-dimethyl- | 0.2 | 0.4 | | |
| Benzene, 1,3-dimethyl-5-(1-methylethyl) | 0.2 | | | 0.1 |
| Benzene, 1,3-dimethyl-5-(1-methylethyl) | | | | |
| Naphthalene, 1,2,3,4-tetrahydro-2-methy | 0.6 | 0.2 | | |
| Naphthalene, 1,2,3,4-tetrahydro-1-methy | 0.5 | 0.4 | | |
| Bicyclo[4.2.1]nona-2,4,7-triene, 7-ethy | 0.6 | 0.3 | | |
| Benzocycloheptene | | | | |
| 1H-Indene, 2,3-dihydro-4,7-dimethyl- | 0.6 | 0.3 | | |
| 1H-Indene, 2,3-dihydro-4,7-dimethyl- | 0.8 | 0.5 | | |
| Naphthalene, 1,2,3,4-tetrahydro-6-methy | 1.4 | 0.7 | | |
| Naphthalene, 1,2,3,4-tetrahydro-1,5-dim | 0.0 | | | |
| Phenol, 2-(2-methyl-2-propenyl)- | | | | |
| 1H-Indene, 2,3-dihydro-4,7-dimethyl- | 0.1 | 0.2 | | |
| Naphthalene, 1,2,3,4-tetrahydro-1,5-dim | | | | |
| Naphthalene, 1,2,3,4-tetrahydro-5-methy | 1.1 | 0.7 | | |
| 1H-Indene, 2,3-dihydro-1,4,7-trimethyl- | 0.1 | 0.1 | | |
| Naphthalene, 1-methyl- | 0.3 | 0.1 | | |
| Decanoic acid, methyl ester | 197.9 | 0.3 | 0.2 | |
| Naphthalene, 1-methyl- | | 0.1 | | |
| Naphthalene, 1,2,3,4-tetrahydro-1,4-dim | 2.0 | 0.5 | | |
| 1H-Indene, 2,3-dihydro-1,1,5-trimethyl- | 0.8 | 0.2 | | |
| Naphthalene, 2-ethyl-1,2,3,4-tetrahydro | 0.1 | 0.3 | 0.7 | |
| Naphthalene, 1,2,3,4-tetrahydro-1,5-dim | | | | |
| Naphthalene, 1,2,3,4-tetrahydro-1,4-dim | | 0.2 | | |
| (1,4-Dimethylpent-2-enyl)benzene | | | | |
| Naphthalene, 1,2,3,4-tetrahydro-1,4-dim | | 0.1 | | |
| Naphthalene, 1,2,3,4-tetrahydro-1,5-dim | 0.3 | 0.2 | | |
| Ethanol, 2-(2-butoxyethoxy)-, acetate | 28.2 | 25.2 | 28.3 | 4.2 |
| Naphthalene, 5-ethyl-1,2,3,4-tetrahydro | 0.8 | 0.2 | | |
| Methyl 4-oxododecanoate | 17.4 | 10.8 | 10.7 | 1.8 |
| Naphthalene, 5-ethyl-1,2,3,4-tetrahydro | | | | |
| Acenaphthylene, 1,2,2a,3,4,5-hexahydro- | | | | |
| Biphenyl | 0.4 | | | |
| Naphthalene, 1,2,3,4-tetrahydro-5,7-dim | 0.9 | 0.2 | | |
| Naphthalene, 1,2,3,4-tetrahydro-5,7-dim | 0.1 | | | |
| Diphenylmethane | 0.1 | 0.0 | | |
| Naphthalene, 1,4-dimethyl- | | | | |
| Naphthalene, 1,2,3,4-tetrahydro-6,7-dim | | 0.0 | | |
| Naphthalene, 1,7-dimethyl- | | 0.0 | | |
| Naphthalene, 1,2,3,4-tetrahydro-5,6-dim | | 0.0 | | |
| Naphthalene, 2,6-dimethyl- | | | | |
| Nonanoic acid, 9-oxo-, methyl ester | 20.1 | 21.7 | 26.6 | |
| Naphthalene, 1,2,3,4-tetrahydro-2,5,8-t | 0.1 | | | |
| Naphthalene, 1,2,3,4-tetrahydro-2,5,8-t | 0.4 | 0.1 | | |
| Naphthalene, 1,2,3,4-tetrahydro-2,5,8-t | | | | |

| Compound name | WAF_01[1] | WAF_02 | WAF_03 | WAF_04 |
|---|---|---|---|---|
| Naphthalene, 1,2-dimethyl- | | | | |
| Naphthalene, 1,2,3,4-tetrahydro-1,4,6-t | 0.1 | | | |
| 1,4-Dimethyl-2-cyclopentylbenzene | | | | |
| 1,1'-Biphenyl, 4-methyl- | 0.5 | | | |
| 1,1'-Biphenyl, 4-methyl- | 0.0 | | | |
| Dodecanoic acid, methyl ester | 980.4 | 1.3 | 0.9 | |
| Naphthalene, 1,4,6-trimethyl- | | | | |
| Naphthalene, 1,6,7-trimethyl- | | | | |
| Naphthalene, 1,6,7-trimethyl- | | | | |
| Nonanedioic acid, dimethyl ester | 53.3 | 34.9 | 34.3 | |
| Naphthalene, 2,3,6-trimethyl- | | | | |
| 1,4-Benzenediol, 2-(1,1-dimethylethyl)- | 17.7 | 4.9 | 5.1 | 17.9 |
| 4,4'-Dimethylbiphenyl | | | | |
| Naphthalene, 1-(2-propenyl)- | | | | |
| Methyl myristoleate | 736.7 | 0.2 | 2.1 | |
| Methyl tetradecanoate | 10145.0 | 2.6 | 1.0 | |
| 4,4'-Dimethylbiphenyl | 0.4 | | | |
| Pentadecanoic acid, methyl ester | 865.0 | | | |
| Naphthalene, 1,2,3,4,4a,5,8,8a-octahydr | 12.2 | 13.7 | 12.9 | |
| 9-Hexadecenoic acid, methyl ester, (Z)- | 974.7 | | | |
| Methyl palmitoleate | 14445.3 | | | |
| Hexadecanoic acid, methyl ester | 89326.2 | 16.6 | 6.4 | 8.8 |
| cis-10-Heptadecenoic acid, methyl ester | 2223.3 | | | |
| Heptadecanoic acid, methyl ester | 2567.7 | | | 0.2 |
| 9,12-Octadecadienoic acid (Z,Z)-, methy | 55098.9 | 9.4 | 1.8 | |
| Octadecenoic acid, ME (Isomers #3-4) | | 21.7 | 4.8 | 2.9 |
| Octadecanoic acid, ME(Isomer #5) | | 8.9 | 4.4 | 1.4 |
| Octadecanoic acid, methyl ester | | 6.1 | 1.6 | 2.3 |
| Pyrene, 4,5-dihydro- | 2.3 | | | |
| Pyrene | | | | |
| cis-11,14 Eicosadienoic acid, methyl ester | 3058.2 | | | |

[1] The AF-B20 WAF_01 had visible oil droplets on the surface of the WAF before extraction indicating that the water had been contaminated during mixing so the excessively high levels of FAME in this sample are not valid.

**Table II-B-11: AF-B20 WAF concentrations (µg/L or ppb) with blank subtracted**

| Compound name | WAF_01[1] | WAF_01[2] | WAF_02 | WAF_03 | WAF_04 |
|---|---|---|---|---|---|
| Benzene | 0.7 | 9.0 | 0.2 | 0.2 | 0.3 |
| Butane, 2,2,3,3-tetramethyl- | | 54.0 | | | |
| Toluene | 59.9 | 341.3 | 28.2 | 23.4 | 40.5 |
| Acetic acid, butyl ester | 68.1 | 55.6 | 20.6 | 26.1 | 8.3 |
| Ethylbenzene | 81.0 | 223.0 | 36.7 | 35.3 | 49.3 |
| m-Xylene | 154.4 | 375.1 | 65.6 | 63.8 | 85.6 |
| p-Xylene | 60.0 | 151.6 | 26.8 | 25.9 | 35.0 |
| o-Xylene | 155.5 | 339.3 | 75.1 | 69.7 | 81.4 |
| Oxime-, methoxy-phenyl-_ | 1.9 | 1.0 | 0.9 | 0.8 | 0.7 |
| Benzene, (1-methylethyl)- | 10.6 | 23.7 | 3.9 | 4.2 | 5.9 |
| Hexanoic acid, methyl ester | 28.8 | 14.4 | 3.7 | 4.6 | 2.0 |
| Benzene, propyl- | 37.8 | 80.2 | 13.9 | 15.3 | 19.8 |
| Benzene, 1-ethyl-2-methyl- | 145.2 | 273.3 | 56.9 | 61.5 | 70.5 |
| Benzene, 1-ethyl-4-methyl- | 72.1 | 136.8 | 27.9 | 30.0 | 35.2 |
| Benzene, 1,2,3-trimethyl- | 51.7 | 90.7 | 20.5 | 21.3 | 23.6 |
| Benzene, 1,3,5-trimethyl- | 119.8 | 208.3 | 51.9 | 52.6 | 55.5 |
| Benzene, 1,2,3-trimethyl- | 284.8 | 467.4 | 122.5 | 124.2 | 125.6 |
| Benzene, (1-methylpropyl)- | 8.0 | 21.2 | 1.2 | 3.8 | 1.8 |
| Benzene, 1-methyl-2-(1-methylethyl)- | 12.1 | 20.5 | 3.9 | 4.6 | 5.3 |
| Benzene, 1,2,4-trimethyl- | 130.6 | 198.8 | 61.8 | 59.1 | 55.6 |
| Benzene, 1-methyl-2-(1-methylethyl)- | 8.0 | 13.7 | 2.5 | 3.0 | 3.5 |
| Indane | 92.0 | 137.5 | 46.9 | 43.6 | 39.2 |
| Benzene, 1,3-diethyl- | 21.3 | 34.1 | 7.2 | 8.3 | 9.1 |
| Benzene, 1-methyl-3-propyl- | 37.1 | 60.8 | 11.4 | 13.5 | 15.4 |
| Benzene, 1,2,3,4-tetramethyl- | 37.5 | 59.4 | 11.8 | 13.5 | 15.3 |
| Benzene, 1-methyl-4-(1-methylethyl)- | 52.9 | 81.1 | 18.1 | 20.4 | 21.4 |
| Benzene, 1-methyl-4-propyl- | 46.2 | 73.5 | 16.3 | 17.9 | 19.1 |
| Benzene, 2-ethyl-1,4-dimethyl- | 48.8 | 71.8 | 18.7 | 19.8 | 19.7 |
| Benzene, 1-ethyl-2,4-dimethyl- | 51.6 | 75.9 | 19.6 | 20.8 | 20.4 |
| Benzene, 4-ethenyl-1,2-dimethyl- | 20.6 | 30.0 | 9.2 | 9.0 | 8.5 |
| Benzene, 1-ethyl-2,4-dimethyl- | 61.3 | 87.4 | 23.1 | 24.8 | 24.1 |
| Benzene, 1-ethenyl-4-ethyl- | 52.5 | 76.9 | 23.7 | 23.3 | 21.5 |
| Benzene, 1-ethyl-2,4-dimethyl- | 9.0 | 12.7 | 3.9 | 4.0 | 3.7 |
| Benzene, 1-methyl-4-(1-methylpropyl)- | 22.2 | 33.0 | 5.5 | 6.9 | 8.4 |
| Benzene, 1-ethyl-2,3-dimethyl- | 37.9 | 53.1 | 16.5 | 16.5 | 15.1 |
| Benzene, 1-methyl-4-(1-methylpropyl)- | 6.6 | 9.5 | 1.5 | 1.9 | 2.4 |
| Benzene, 1,2,3,4-tetramethyl- | 33.4 | 44.8 | 14.2 | 14.2 | 12.6 |
| Benzene, 1,2,3,4-tetramethyl- | 58.2 | 77.5 | 25.3 | 25.1 | 22.1 |
| Benzene, 1-methyl-4-(1-methylpropyl)- | 6.9 | 9.2 | 1.7 | 2.1 | 2.4 |
| Benzene, (2-methyl-1-butenyl)- | 5.5 | 8.0 | 2.0 | 2.1 | 4.4 |
| Indan, 1-methyl- | 108.7 | 142.6 | 51.6 | 49.0 | 42.1 |
| 1H-Indene, 2,3-dihydro-4-methyl- | 170.5 | 223.8 | 84.4 | 77.6 | 66.3 |
| Benzene, 1,2,4,5-tetramethyl- | 74.7 | 96.4 | 34.6 | 32.9 | 28.3 |
| Benzene, 1-methyl-4-(1-methylpropyl)- | 24.8 | 32.4 | 7.5 | 8.5 | 8.6 |
| Benzene, 1-methyl-4-(1-methylpropyl)- | 15.5 | 19.2 | 4.8 | 5.4 | 5.5 |
| Naphthalene, 1,2,3,4-tetrahydro- | 194.4 | 254.3 | 99.8 | 90.9 | 76.5 |

| Compound name | WAF_01[1] | WAF_01[2] | WAF_02 | WAF_03 | WAF_04 |
|---|---|---|---|---|---|
| Benzene, 2-ethyl-1,3-dimethyl- | 7.7 | 9.6 | 2.3 | 2.6 | 2.6 |
| 1H-Indene,2,3-dihydro-2,2-dimethyl- | 4.3 | 8.0 | 5.8 | 4.9 | 2.6 |
| 1H-Indene,2,3-dihydro-2,2-dimethyl- | 62.6 | 81.8 | 26.3 | 26.3 | 23.0 |
| Naphthalene | 50.9 | 66.7 | 28.0 | 24.1 | 19.8 |
| 1H-Indene, 2,3-dihydro-1,6-dimethyl- | 42.0 | 54.1 | 18.5 | 18.0 | 15.5 |
| Benzene, 1,3-dimethyl-5-(1-methylethyl) | 9.4 | 12.2 | 3.7 | 3.8 | 3.3 |
| Benzene, 1,3-dimethyl-5-(1-methylethyl) | 5.3 | 6.8 | 2.3 | 2.3 | 2.0 |
| Naphthalene, 1,2,3,4-tetrahydro-2-methy | 43.7 | 57.3 | 19.5 | 18.6 | 15.9 |
| Naphthalene, 1,2,3,4-tetrahydro-1-methy | 41.5 | 52.4 | 19.6 | 18.4 | 15.4 |
| Bicyclo[4.2.1]nona-2,4,7-triene, 7-ethy | 38.1 | 46.0 | 17.0 | 16.4 | 13.9 |
| Benzocycloheptene | 8.9 | 1.7 | 0.7 | 0.6 | 0.5 |
| 1H-Indene, 2,3-dihydro-4,7-dimethyl- | 47.9 | 57.0 | 21.3 | 20.4 | 17.0 |
| 1H-Indene, 2,3-dihydro-4,7-dimethyl- | 50.5 | 61.4 | 24.3 | 22.7 | 18.0 |
| Naphthalene, 1,2,3,4-tetrahydro-6-methy | 113.6 | 137.5 | 54.1 | 50.5 | 40.5 |
| Naphthalene, 1,2,3,4-tetrahydro-1,5-dim | 9.8 | 10.6 | 2.8 | 3.2 | 3.0 |
| Phenol, 2-(2-methyl-2-propenyl)- | 11.2 | 13.2 | 5.1 | 4.8 | 3.9 |
| 1H-Indene, 2,3-dihydro-4,7-dimethyl- | 29.0 | 34.5 | 13.9 | 13.0 | 10.3 |
| Naphthalene, 1,2,3,4-tetrahydro-1,5-dim | 5.7 | 7.1 | 2.4 | 2.4 | 2.0 |
| Naphthalene, 1,2,3,4-tetrahydro-5-methy | 96.5 | 117.2 | 49.1 | 44.3 | 34.8 |
| 1H-Indene, 2,3-dihydro-1,4,7-trimethyl- | 13.9 | 18.5 | 6.1 | 5.9 | 4.9 |
| Naphthalene, 1-methyl- | 34.0 | 44.5 | 18.5 | 15.8 | 12.1 |
| Decanoic acid, methyl ester | 9.9 | 0.7 | 0.2 | 0.1 | 0.1 |
| Naphthalene, 1-methyl- | 32.8 | 41.7 | 18.4 | 15.4 | 11.8 |
| Naphthalene, 1,2,3,4-tetrahydro-1,4-dim | 53.7 | 65.1 | 22.2 | 22.2 | 18.0 |
| 1H-Indene, 2,3-dihydro-1,1,5-trimethyl- | 22.1 | 27.6 | 9.9 | 9.6 | 7.8 |
| Naphthalene, 2-ethyl-1,2,3,4-tetrahydro | 5.3 | 5.9 | 2.4 | 2.3 | 1.6 |
| Naphthalene, 1,2,3,4-tetrahydro-1,5-dim | 5.9 | 6.6 | 2.7 | 2.6 | 2.1 |
| Naphthalene, 1,2,3,4-tetrahydro-1,4-dim | 19.6 | 15.8 | 8.8 | 11.4 | 7.1 |
| (1,4-Dimethylpent-2-enyl)benzene | 10.2 | 10.8 | 5.3 | 5.2 | 4.2 |
| Naphthalene, 1,2,3,4-tetrahydro-1,4-dim | 13.9 | 16.8 | 6.4 | 5.8 | 4.9 |
| Naphthalene, 1,2,3,4-tetrahydro-1,5-dim | 15.4 | 19.7 | 7.6 | 7.2 | 5.5 |
| Ethanol, 2-(2-butoxyethoxy)-, acetate | 20.8 | 31.2 | 12.9 | 17.6 | 7.9 |
| Naphthalene, 5-ethyl-1,2,3,4-tetrahydro | 18.8 | 22.3 | 8.5 | 8.5 | 6.6 |
| Methyl 4-oxododecanoate | 9.7 | 10.7 | 3.5 | 4.3 | 2.0 |
| Naphthalene, 5-ethyl-1,2,3,4-tetrahydro | 8.6 | 10.3 | 4.9 | 4.4 | 3.4 |
| Acenaphthylene, 1,2,2a,3,4,5-hexahydro- | 3.8 | 4.7 | 1.9 | 1.8 | 1.4 |
| Biphenyl | 64.9 | 83.1 | 36.0 | 30.4 | 22.9 |
| Naphthalene, 1,2,3,4-tetrahydro-5,7-dim | 32.3 | 40.2 | 15.5 | 14.5 | 11.0 |
| Naphthalene, 1,2,3,4-tetrahydro-5,7-dim | 15.0 | 18.0 | 7.0 | 6.6 | 5.0 |
| Diphenylmethane | 27.5 | 35.7 | 14.4 | 12.6 | 9.2 |
| Naphthalene, 1,4-dimethyl- | 8.8 | 11.2 | 4.4 | 3.9 | 2.8 |
| Naphthalene, 1,2,3,4-tetrahydro-6,7-dim | 15.6 | 19.1 | 7.6 | 7.0 | 5.4 |
| Naphthalene, 1,7-dimethyl- | 21.6 | 28.3 | 11.7 | 9.9 | 7.2 |
| Naphthalene, 1,2,3,4-tetrahydro-5,6-dim | 18.4 | 21.7 | 9.0 | 8.4 | 6.4 |
| Naphthalene, 2,6-dimethyl- | 14.7 | 19.3 | 7.8 | 6.7 | 4.9 |
| Nonanoic acid, 9-oxo-, methyl ester | 22.2 | 22.9 | 5.8 | 6.7 | |
| Naphthalene, 1,2,3,4-tetrahydro-2,5,8-t | 5.6 | 6.5 | 2.4 | 2.5 | 1.9 |
| Naphthalene, 1,2,3,4-tetrahydro-2,5,8-t | 27.0 | 32.5 | 11.6 | 11.7 | 9.0 |
| Naphthalene, 1,2,3,4-tetrahydro-2,5,8-t | 3.6 | 3.7 | 1.3 | 1.3 | 1.0 |

| Compound name | WAF_01[1] | WAF_01[2] | WAF_02 | WAF_03 | WAF_04 |
|---|---|---|---|---|---|
| Naphthalene, 1,2-dimethyl- | 4.6 | 4.0 | 1.8 | 1.5 | 1.1 |
| Naphthalene, 1,2,3,4-tetrahydro-1,4,6-t | 8.4 | 9.7 | 3.7 | 3.6 | 2.8 |
| 1,4-Dimethyl-2-cyclopentylbenzene | 8.0 | 9.5 | 3.7 | 3.5 | 2.8 |
| 1,1'-Biphenyl, 4-methyl- | 37.8 | 50.0 | 20.7 | 17.1 | 12.4 |
| 1,1'-Biphenyl, 4-methyl- | 22.1 | 28.5 | 11.8 | 9.8 | 7.2 |
| Dodecanoic acid, methyl ester | 81.8 | 1.4 | 0.2 | 0.3 | |
| Naphthalene, 1,4,6-trimethyl- | 4.0 | 5.4 | 2.4 | 1.9 | 1.4 |
| Naphthalene, 1,6,7-trimethyl- | 7.5 | 9.5 | 3.9 | 3.3 | 2.5 |
| Naphthalene, 1,6,7-trimethyl- | 7.5 | 9.4 | 3.8 | 3.2 | 2.4 |
| Nonanedioic acid, dimethyl ester | 42.8 | 27.4 | 5.0 | 7.3 | 1.4 |
| Naphthalene, 2,3,6-trimethyl- | 6.8 | 9.1 | 3.8 | 3.2 | 2.3 |
| 1,4-Benzenediol, 2-(1,1-dimethylethyl)- | 15.3 | 12.0 | 5.0 | 5.4 | 7.2 |
| 4,4'-Dimethylbiphenyl | 5.2 | 6.6 | 2.7 | 2.3 | 1.7 |
| Naphthalene, 1-(2-propenyl)- | 10.4 | 14.4 | 6.4 | 5.1 | 3.7 |
| Methyl myristoleate | 60.1[3] | | | | |
| Methyl tetradecanoate | 484.2 | | | | |
| 4,4'-Dimethylbiphenyl | 4.1 | 4.5 | 2.4 | 1.9 | 1.4 |
| Pentadecanoic acid, methyl ester | 27.9 | | | | |
| Naphthalene, 1,2,3,4,4a,5,8,8a-octahydr | 10.5 | 12.1 | 3.9 | 5.4 | |
| 9-Hexadecenoic acid, methyl ester, (Z)- | 468.3 | 0.3 | | | |
| Methyl palmitoleate | 556.3 | | | | |
| Hexadecanoic acid, methyl ester | 5981.3 | 0.7 | 5.5 | 2.6 | 2.9 |
| cis-10-Heptadecenoic acid, methyl ester | 95.3 | 4.2 | | | |
| Heptadecanoic acid, methyl ester | 121.9 | 0.6 | | | |
| 9,12-Octadecadienoic acid (Z,Z)-, methy | 3094.6 | | 1.1 | | |
| Octadecenoic acid, ME (Isomers #3-4) | 9907.0 | | 5.2 | | |
| Octadecanoic acid, ME(Isomer #5) | 6118.5 | | 0.2 | | |
| Octadecanoic acid, methyl ester | 3699.3 | 0.6 | 2.8 | 0.8 | 1.2 |
| Pyrene, 4,5-dihydro- | 1.5 | 1.9 | 1.1 | 0.8 | 0.5 |
| Pyrene | 4.3 | 7.4 | 3.5 | 2.6 | 1.8 |
| cis-11,14 Eicosadienoic acid, methyl ester | 200.8 | | | | |

[1] The first column of WAF_01 results is the average of two measurements made of the same mixture subsequent to the initial range finding experiment. These values had excessive levels of FAME compared to the original measurements during the range finding experiment. [2] The results from the range finding experiment are reported here. [3] the values in the box are likely from contamination of the WAF with fresh B-100 fuel.

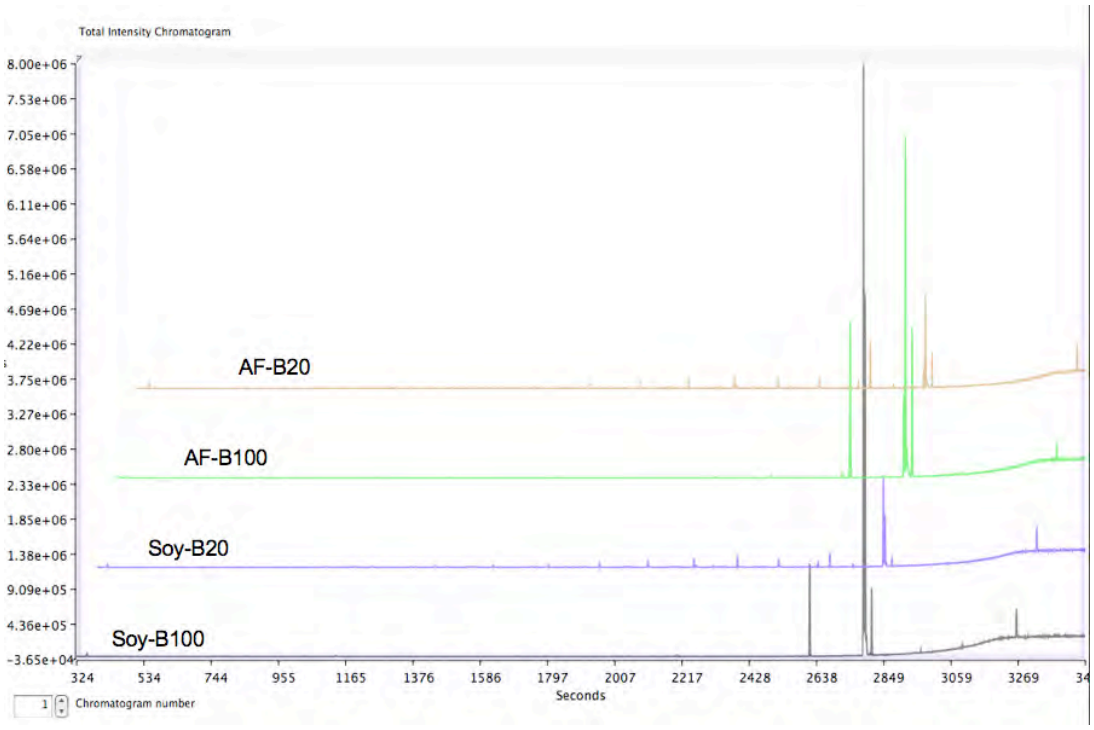


**Figure II-B-1. Overlay total ion chromatogram of 1:1000 (v/v) dilution of each neat fuel in MeOH injected (2 µL) with 5:1 split.**

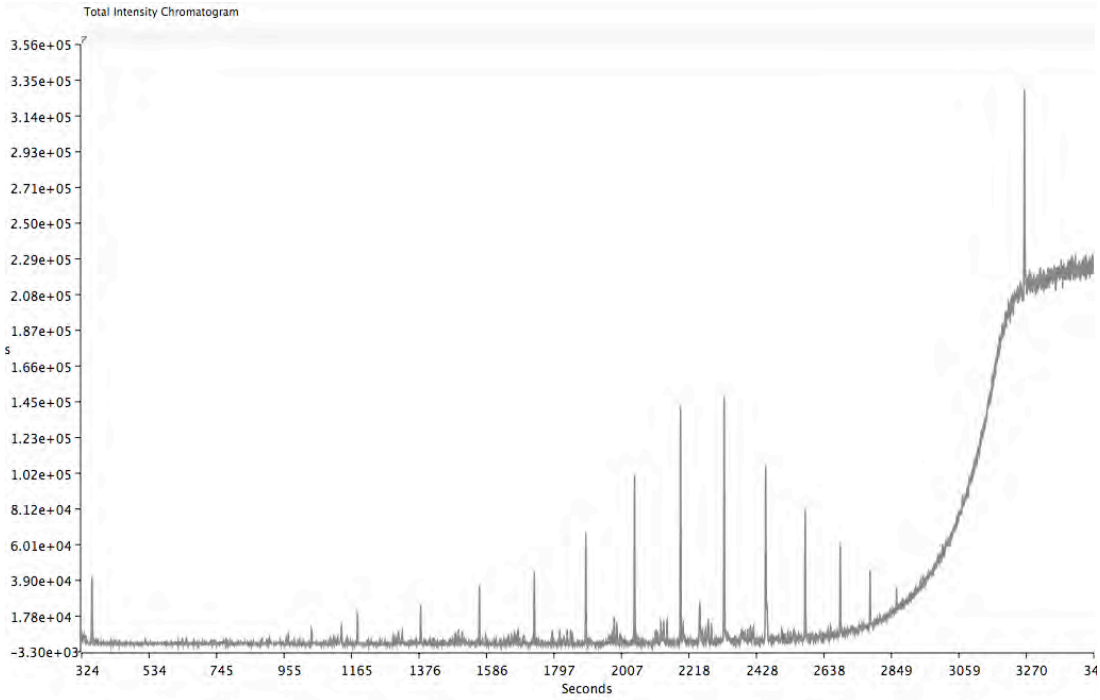


**Figure II-B-2. CARB Diesel total ion chromatogram of 1:1000 (v/v) dilution of neat fuel in MeOH injected (2 µL) with 5:1 split.**

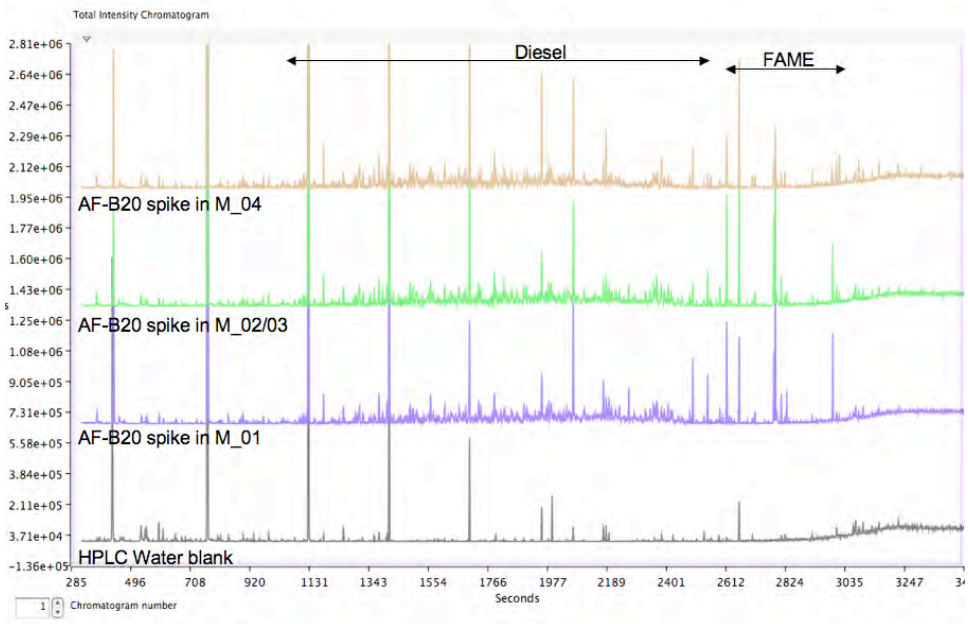


**Figure II-B-3. Overlay of 1:1000 (v/v) dilution of neat AF-B20 fuel in MeOH spiked (4 µL) in each of the test waters (40 mL). The HPLC water blank is 40 mL of the water used as makeup volume in the WAF analysis. The large evenly spaced peaks are siloxanes from the stir-bar coating and are not quantified in the method.**

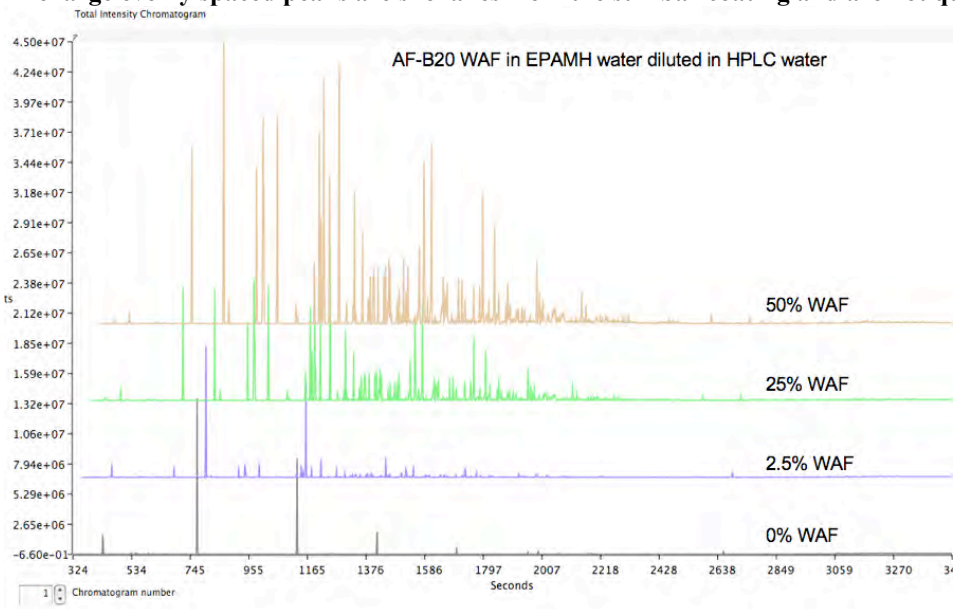


**Figure II-B-4. Range finding experiment with increasing fractions of AF-B20 WAF_01 in HPLC water. The optimal dilution for the SBSE analysis was identified as 25% WAF in HPLC water.**

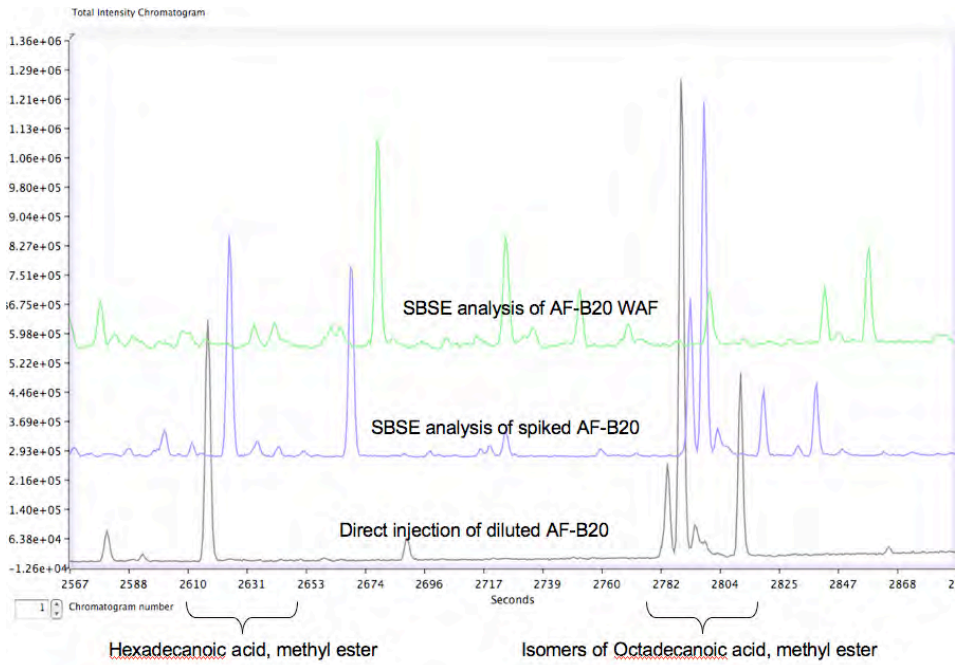


**Figure II-B-5. Zoomed overlay in the region of the chromatogram where FAME elutes showing the presence of hexadecanoic acid methyl ester and the isomers of octadecanoic acid methyl ester in the raw fuel and in the spiked water but significantly reduced or absent in the WAF. The peak eluting at about 2660 seconds in the SBSE chromatograms is a siloxane from the stir bar and not part of the WAF or fuel.**

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# 8. Appendix II-C: Relative Rates Of Infiltration Of Biodiesel Blends And ULSD In Laboratory-Scale Sandboxes

As part of a multimedia risk assessment of biodiesel, the relative risks associated witih infiltration into the subsurface and eventual fate and transport processes affecting groundwater were identified as a priority knowledge gap (UC, 2009; Ginn et al., 2009). To address this knowledge gap, small-scale "sandbox" infiltration experiments, were performed in order to simulate and evaluate the qualitative impacts of biodiesel fate and mobility in the subsurface compared directly to Ultra Low Sulfur Diesel (ULSD). For the purpose of the study two feedstocks were used: Animal Fat and Soybean Oil. Experiments were run with a pure fuel (B100) and a blended fuel (B20) for both feedstocks in a relative setting to afford relative assessmnt of the differences in fuel infiltration into unsaturated porous media, redistribution within the unsaturated zone, and eventual lens formation on the saturated surface.

Biodiesel is made up of multiple fatty-acid methyl esters (FAMEs), all of which have densities lighter than water. The resulting light non-aqueous phase liquid (LNAPL) is expected to float on water and thus to form lens geometries upon infiltration to a ground water table. As LNAPLs infiltrate into the subsurface after a spill, capillary forces cause some of the LNAPL to remain trapped in the pores above the water table. Once the main front of the plume reaches the water table it will start ponding within the capillary fringe just above the water table. The geometry of this lens is important to groundwater contamination because it is from the associated LNAPL/groundwater table interface that soluble components partition into the water phase. With enough LNAPL ponding, the weight of the lens can displace some water from the beneath the lens. As the groundwater flows beneath the lens, more LNAPL is free to partition into the water phase.

## METHODS AND MATERIALS

### Source and Preparation of Biodiesel Test Solutions

Infiltration experiments were carried out for 5 different biodiesels blends, including three fuels derived from animal fat and two derived from soybean oil. For both animal fat and soy feedstocks, a pure sample (B-100) and a blended sample (B-20, with ULSD as the blend) all additized with the antioxidant Bioextend as per manufacture's suggestion was evaluated. The fuels were provided by CA Air Resources Board (c/o R. Okamoto) and collected by T. Ginn/UC Davis and stored in 1-gallon or 1-quart glass amber bottles in the dark at 20 °C with minimal headspace. Each of these four fuel blends were compared in triplicate experiments to CARB #2 ULSD. An additional unadditized animal fat B100 was also tested in triplicate to see if there were any noticeable effects on infiltration induced by the additive itself. The resulting suite of experiments is given in Table II-C-1.

**Table II-C-1. Suite of blends studied in the sandbox infiltration experiments**.

| Sandbox Experimental Matrix | | | | | | |
|---|---|---|---|---|---|---|
| | | | Additization | | | |
| Type | Feedstock | Totals | None | | Bioextend | |
| | | | # | Quantity | # | Quantity |
| B100 | Animal-fat | 6 | 3 | 50 mL/test | 3 | 50 mL/test |
| | | | | | | |
| B100 | Soy | 3 | | | 3 | 50 mL/test |
| | | | | | | |
| B20 | Animal-fat | 3 | | | 3 | 50 mL/test |
| | | | | | | |
| B20 | Soy | 3 | | | 3 | 50 mL/test |
| | | | | | | |
| ULSD | petroleum | 15 | 15 | 50 mL/test | | |
| | | | | | | |

**Note:** Tests will include side by side comparison between ULSD and Biodiesel within the same antfarm for consistency of sand compaction.

## Sandbox Design

The objective of the sandbox design is to allow visualization of infiltrating fuels in side-by-side (biodiesel blend vs. ULSD) plumes introduced simultaneously. This calls for small-scale infiltration domains in unsaturated porous media in two dimensions. The overall design of the sandbox is similar to commonly known vertical glass sandboxes known as "ant-farms." The design criteria for the fate and transport experiments were that it be of a scale where we could run side-by-side tests within the same apparatus to compare the biodiesel and ULSD. Sandbox design targets also easy assembly/disassembly and cleaning for use in multiple experiments with watertight conditions and with hose assembly to allow control of the elevation of the water table within the sandbox. It also needed to be made of non-reactive materials that would last long enough to complete all the experiments while exposed to the ULSD and biodiesel. The preliminary experiments and design testing details pertaining to these and other aspects of the sandboxes are described in detail in Hatch (2010). Only summary aspects of the medium selected, the fuel dye, and the photographic set up are presented here.

In order to provide a standardized medium for comparative assessment of fuel behavior, a uniform medium to coarse sand was selected for the model porous medium since it is easily replicated for future experiments and it would provide a relatively high hydraulic conductivity for infiltration of the fuels thus reducing the experiment run time while representing a high-risk environment for groundwater contamination. Thus for the experiments, Cemex #30 sandblasting

sand was used as the porous media. It was readily available in the local hardware store and provided a size range based on the #30 sieve size.

In order to perform a direct comparison of the fate and transport of biodiesel to ULSD it was necessary that they be done simultaneously. It was also important for the plumes to be far enough apart so that they would not meet and interact prior to reaching the water table.

To accommodate digital photography of the dual infiltrating plumes, a sandbox design was developed using wood to build a three-sided frame, 16 inches by 11 inches. The frame was used to separate two glass walls of same dimension (Figure II-C-1). Glass is used instead of plexiglass in order to maintain a consistent refractive index in the presence of potentially reactive fuels after replicate use of the sandbox. Clamps are used to hold the sandbox together as these afford ready reassembly. The frame includes internal sealant on the wood components, watertight seals, and hoses with ports in the side panels to allow control of the water table elevation.

**Figure II-C-1: Sandbox in photo booth**

Diesel fuel and biodiesel are not clearly visible compared to water in porous media. To render all fuel blends visible, 0.15 ml of a hydrophobic fuel dye (Solvent Red 26, Kinder Morgan, Inc.) used to dye diesel fuel for agricultural and off road applications was added to the 50 ml fuel samples. Preliminary experiments were done as controls to investigate the impact of this dye concentration on fuel transport effects and none were found (Hatch, 2010).

Digital photography was used to capture time-series of images of the side-by-side dyed fuel infiltration, redistribution, residual formation in the vadose zone, and lens formation on the water table. Each experiment was run for a duration of up to 2.0 hours (until steady state was reached). A mobile photo booth was designed following advice of George Redden of Idaho National Laboratory, an expert in digial photograph of experiments involving flow in porous media. This booth (Figure II-C-1) involves consistent placement of the sandbox, a black velvet drape with fasteners to eliminate external light, and internal lamps placed at angles to the sandbox's outer facing glass window in order to provide controlled lighting without glare. A camera is placed on a tripod within a sealed window of the drape with remote control to allow the experimentalist to take photos at specified times without touching the experimental apparatus.

Visual analyses of the images was done to evaluate four separate time metrics defined in order to time the progress of the infiltration, redistribution, and formation of the lens of biodiesel on the saturated zone surface at the steady-state. These metrics are characteristic times for: elimination of ponded fuel, plume separation from surface, initial commencement of lens spreading on water table, steady-state lens formation on water table. In addition the qualitative characteristics of quantity of residual fuel appearing in the unsaturated zone and of lens shape after steady-state are reported.

## RESULTS AND DISCUSSION

Figure II-C-2 shows the final images for two example fuels, Soy B-20 and Animal Fat B-100. These are selected to reflect the main result of the experiments, that with the exception of Animal Fat B-100, the biodiesel blends do not behave significantly differently from ULSD formation and mobility of the biodiesel in a qualitative fashion for groundwater contamination. The left-hand panel shows Soy B-20 (with ULSD) and the similarity between the biodiesel and petroleum diesel fuel behavior here is representative of that observed in all fuel blends except for Animal Fat B-100, that shows a greater residual and thicker lens formation than ULSD, as shown in the right-hand panel. The behavior of the additized Animal Fat B-100 was very similar to that of the unadditized Animal Fat B-100.

The four time metrics are shown respectively for each experiment in Figures II-C-3, C-4, C-5, and C-6, respectively. These figures show the characteristic times for each initial formation of the U-shaped plume underneath the ponded fuels, the time to separation of the fuel from the surface, the time for initial lens spreading on the water table, and the time for complete lens formation on the water table. These figures reflect identical behavior for each test fuel vs. ULSD in all cases with one minor difference seen for Soy B-100 in Figure II-C-3. The images themselves show the different qualitative behavior seen for Animal Fat B-100 (e.g., Figure II-C-2).

**Figure II-C-2. Example final digital images. Left panel: the triplicate images for Soy B-20 at 2 hours; the left hand plume is Soy B-20 and the right-hand plume is ULSD. Right pane: those for the Animal Fat B-100 at 2 hours; the left hand plume is Animal Fat B-100 and the right-hand plume is ULSD. Note the greater color density indicating increased residual of the Animal Fat B-100 in the vadose zone and the thicker lens formation on the water table, with respect to that of ULSD.**

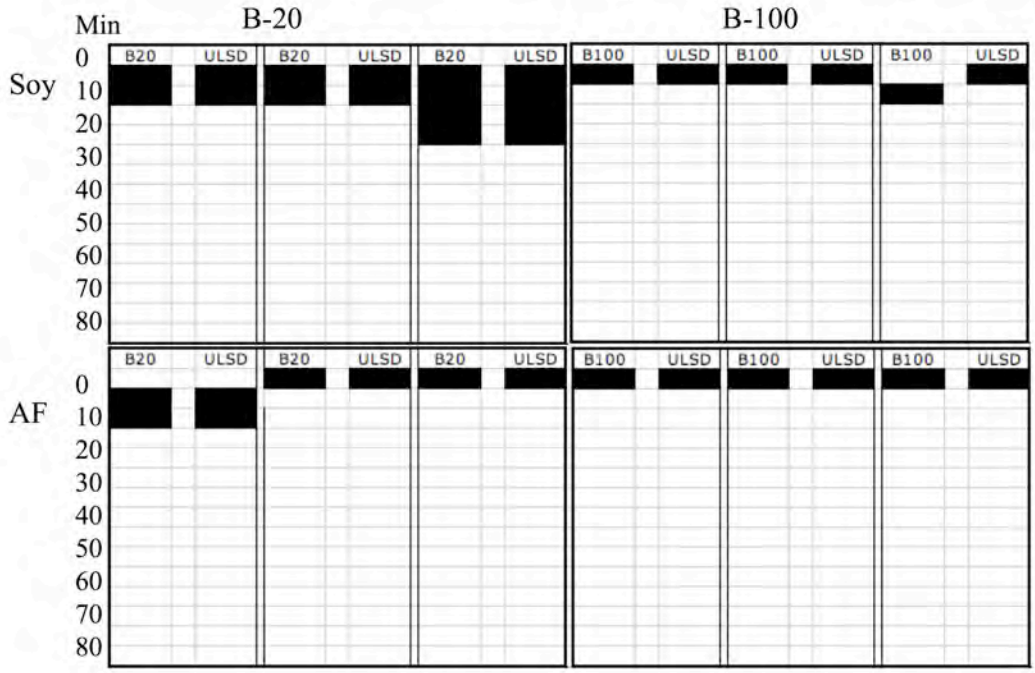


**Figure II-C-3: Characteristic times to formation of the U-shaped plume for each of the four blends (Soy B-20, Soy B-100, Animal Fat (AF) B-20, AF B-100) relative to ULSD in side-by-side comparison. The three columns per fuel blend show the results for each of the three replicates.**

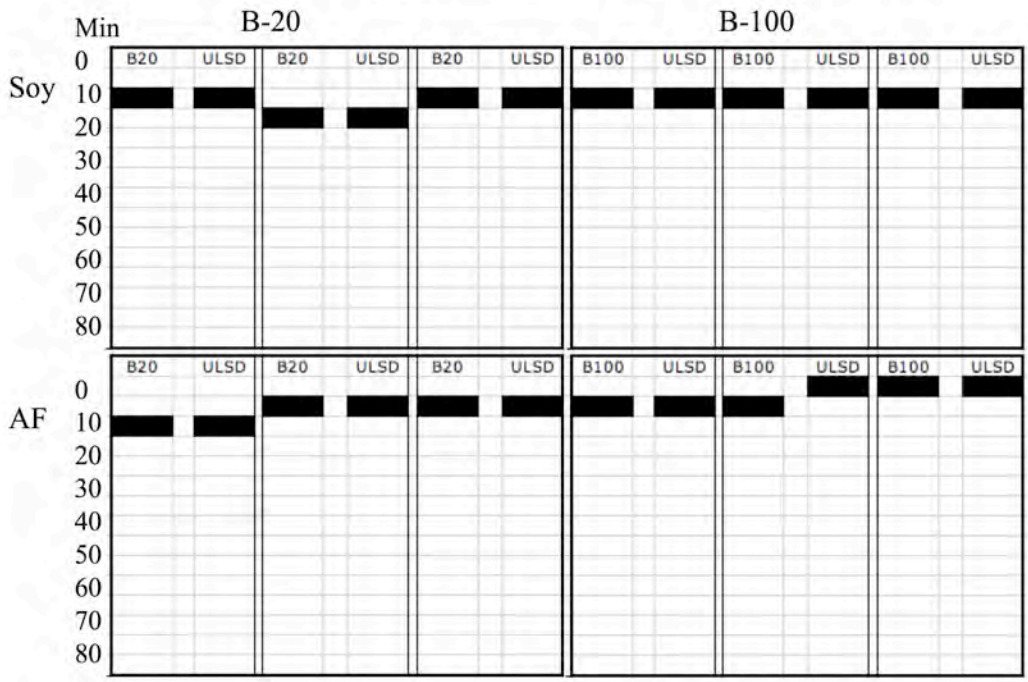


**Figure II-C-4: Characteristic times to plume plume separation from the sand surface for each of the four blends (Soy B-20, Soy B-100, Animal Fat (AF) B-20, AF B-100) relative to ULSD in side-by-side comparison. The three columns per fuel blend show the results for each of the three replicates.**

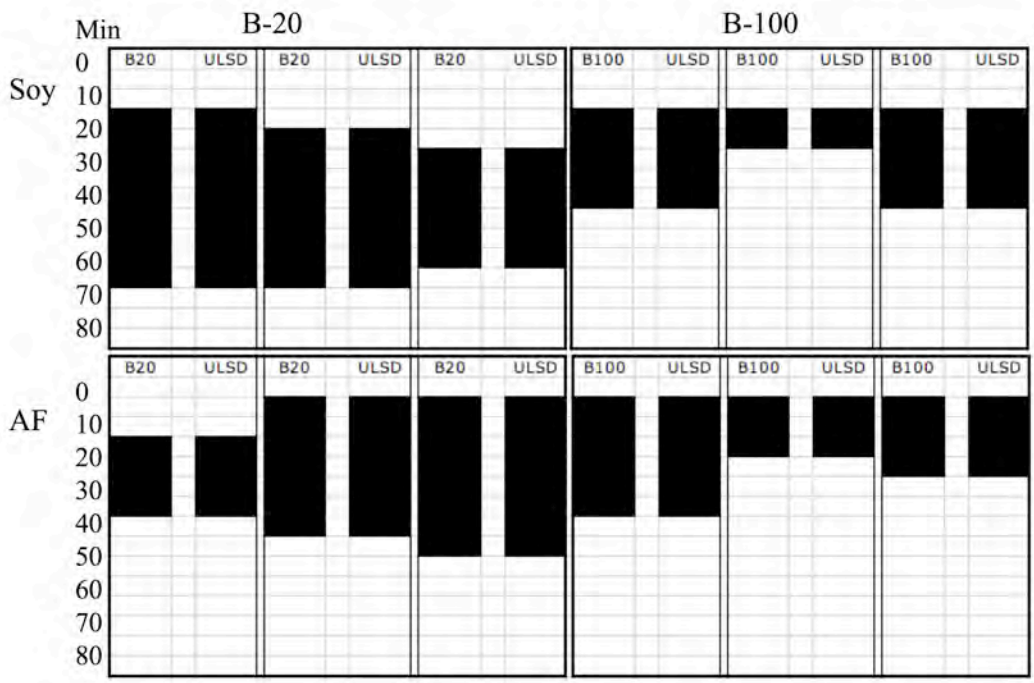


**Figure II-C-5: Characteristic times for commencement of lens spreading on the water table for each fuel (Soy B-20, Soy B-100, B-20, AF B-100) relative to ULSD in side-by-side comparison. The three columns per fuel blend show the results for each of the three replicates.**

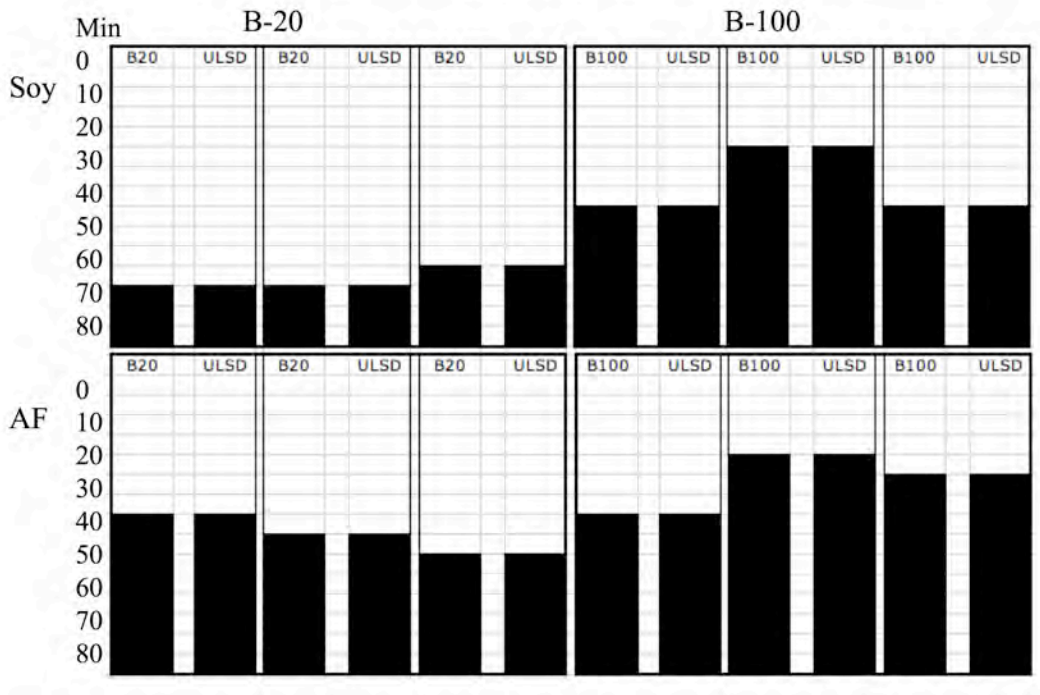


**Figure II-C-6: Characteristic times for lens formation on the water table for each of the four blends (Soy B-20, Soy B-100, AF B-20, AF B-100) relative to ULSD in side-by-side comparison. The three columns per fuel blend show the results for each of the three replicates.**

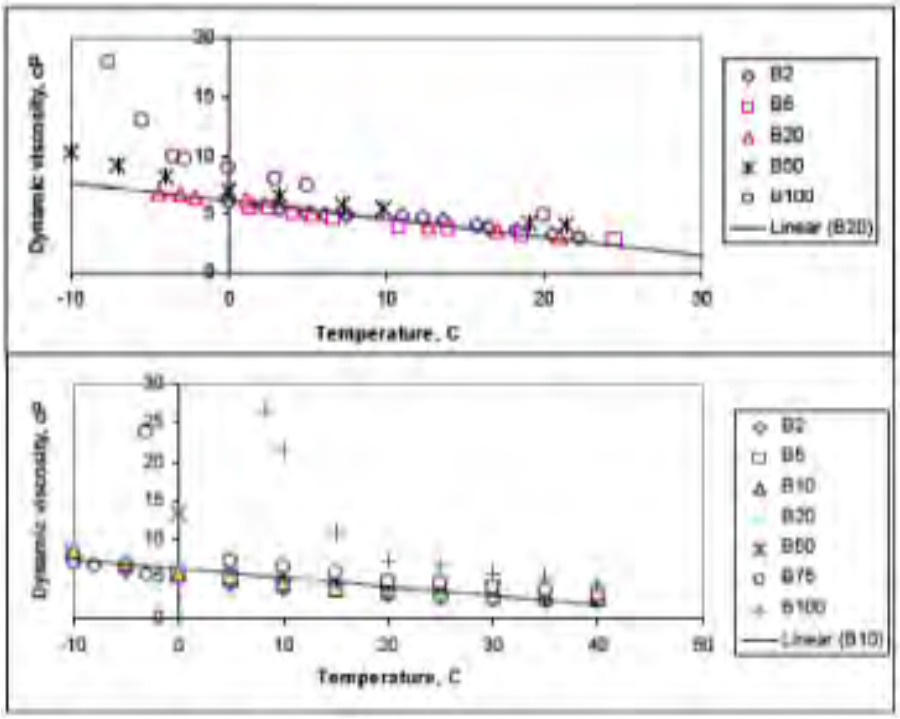


**Figure II-C-7. Biodiesel fuel blend (Soy, top; Animal fat, bottom) viscosities for a range of temperatures. Note relatively large increase in Animal Fat B-100 viscosity for temperatures below 20 degrees C. From Yang et al., 2008.**

## DISCUSSION

The increased residual and thicker form of the lens formed on the water table for the Animal Fat B-100 fuel may be ascribed to measureable physical propreties of the fuel. Yang et al. (2008) present data for a range of properties of animal fat and soy based biodiesel blends at different mixture fractions with petroleum diesel, from four states. An important distinguishing characteristic for Animal Fat biodiesel is an increased viscosity and interfacial tension. Figure B7 (from Yang et al., 2008) shows the viscosity values for different fuel blends as a function of temperature: note the enhanced viscosity for animal fat blends. The interfacial tensions reported by Yang et al. (2008) for biodiesel blends from Minnesota are 8.5/12.0 (mN/m) for Soy (B20/B100), and 15.0/19.5 AF (B20/B100), whereas the value for low-sulfur petroleum diesel is 7.4 mN/m. Increased values of these properties lead to increased residual and thicker lenses (e.g. Charbeneau, 2000; Weaver et al., 1994).

## CONCLUSIONS

- The antioxidant additive did not affect the infiltration of animal fat B-100
- Soy biodiesel blends at both 20 and 100 percent, as well as the animal fat 20 percent blend, do not exhibit any significant differences among the four temporal metrics or among the qualitative residual or lens shape metrics compared to ULSD.
- Animal fat 100 percent blend exhibited similar values of the temporal metrics as ULSD, but it showed noticeable increases in the amount of residual that occurred in the unsaturated zone, and it resulted in final lens geometry that was thicker in vertical dimension and less extensive in horizontal dimension than the ULSD lens.

  This behavior is consistent with the physical properties of animal fat based biodiesel that has higher viscosity and interfacial tension than ULSD. These differences become significantly more pronounced at temperatures below 20 degrees Celsius.

# REFERENCES

Charbeneau, Randall J. 2000. Groundwater Hydraulics and Pollutant Transport. Waveland Press, Inc. Copyright 2000.

Ginn, T. R., M. B. Johnson, J.A. Last, K.M. Scow, L. Rastagarzadeh, T. Hatch, P. L'Amoreaux, V. Nino. R. Okamoto, R. Hodam. 2009. Experimental Plan for Tier II Evaluation of Biodiesel 1st revision 9 March 2009, Prepared for the California Environmental Protection Agency Multimedia Working Group, 22 pp.

Hatch, T. 2010. Biodiesel Relative Risk: A Qualitative Approach to Determining the Environmental Fate of Animal Fat and Soy Biodiesels through a Direct Experimental Comparison with ULSD and Screening Model Simulations using HSSM, Thesis submitted in partial satisfaction of the requirements for the degree of Master of Science, Department of Civil and Environmental Engineering, University of California, Davis.

University of California (UC). 2009. California Biodiesel Multimedia Assessment Tier I Report (Final Draft), prepared for the California Environmental Protection Agency Multimedia Working Group, by the University of California, Davis and the University of California, Berkeley. Sept. 2009. 95 pp.

Weaver, J. W., Charbeneau, R. J., and Lien, B. K. 1994. "The Hydrocarbon Spill Screening Model (HSSM)," Volume 1: User's Guide, United States Environmental Protection Agency, EPA/600/R-94/039 a, April 1994.

Yang, C., S. Lee, S. Larsen, M. Madsen. 2008. ULSD/Biodiesel blend and its effect on fuel/ water separation, Amer. Filtration & Separation Soc. Annual Conf., May 19-22 (2008), Valley Forge, PA.

# 9. Appendix II-D: Relative Rates Of Aerobic Biodegradation Of Biodiesel Blends And ULSD

Microcosm experiments were conducted to assess the aerobic aqueous biodegradation potential (relative to that of petroleum diesel) for solutions exposed to the test biodiesel fuels. Ultra low sulfur diesel (ULSD) was used as the benchmark. Fuels derived from animal fat and soy feedstocks were tested as source phases as received (B100) or blended with ULSD to a B20 mixture (20% biodiesel). The biodiesel blends were tested in three forms: unamended, amended (at industry specified amounts) with the antioxidant Bioextend-30, and amended with both Bioextend-30 and the biocide Kathon FP1.5. The reference ULSD fuel contained no additives. This suite of experiments is designed for a risk wise conservative simplified examination of the differences in biodegradation potential between petroleum and biomass-derived diesels.

The requirements for biodegradation testing of new chemicals vary widely among agencies, both in the US and internationally. The most extensive set of biodegradability tests are published by the OECD (a consortium of European agencies, the European Economic Community, the World Health Organization (WHO), and the United Nations). We followed the suite of microcosm experiments described here is designed based on the modified recommended OECD biodegradability test (OECD 2004). The OECD recommended, that microcosms be comprised of mineral salts medium, tested substrate, and bacterial inoculation using activated sludge from the aeration tank of a sewage treatment plant. In our microcosm experiments, we inoculated with soil rather than activated sludge for better representation of environmental conditions for biodegradation of spills of diesel and biodiesel.

Biological activity was assessed by measuring products of  measured through respiration. Under aerobic biodegradation, carbon compounds are transformed to biomass and $CO_2$ and the latter can be quantified by standard methods (per EPA 560/6-82-003, PB82-233008). Thus the evolution of $CO_2$ from biodegradation of the substrates as a result of microbial activity was measured in our microcosms using a respirometer (Columbus Instrument, Columbus, OH). Microcosms were incubated at controlled temperature of 25 °C for the recommended 28-30 days test period.

## METHODS AND MATERIALS

### Fuel Sample and Microcosm Preparation

The test materials included thirteen fuel types, including ultra-low sulfur diesel (ULSD), neat biofuels derived from animal fat (AF B-100) and soy (Soy B-100) feedstocks, 80% ULSD:20% (w/w) mixtures of the two biofuels (AF B-20 and Soy B-20): each of these four biodiesel blends was tested in the three forms, unadditized, additized with an antioxidant (Bioextend) and additized with both the antioxidant and a biocide (as per manufacturer's specifications). The fuels were provided by CA Air Resources Board (c/o R. Okamoto) and collected by T. Ginn/UC Davis and stored in 1-gallon or 1-quart glass amber bottles in the dark at 20 °C with minimal headspace.  The full suite of fuels tested is listed in Table II-D-1 below.

The microcosms were prepared using a 250 mL flask that consists of 190 ml mineral medium, 2g soil (Yolo, silty-loam) as bacterial inoculum and addition of 5µL of test fuel as substrate- using micro pipette- that was roughly equivalent of a nominal concentration of 25 ppm (effective massic mass density if the fuel were to be dissolved) for each fuel test. The mineral medium contained the OECD-recommended nutrients $KH_2PO_4$, $K_2HPO_4$, $NaHPO_4$, $NH_4Cl$, $CaCl_2.H2O$, $MgSO_4$, and $FeCl_3.6H_2O$ (OECD 2004). Each treatment microcosm was prepared in three

replicates. For each treatment, one abiotic sterile control was prepared using addition of 1% sodium azide. This control was to examine whether the test substrate is degradable in the absence of microorganisms. Three replicates of inoculum blank (no fuel substrate) were also prepared. The inoculums blank was to examine if there is any $CO_2$ production by microorganisms in the absence of fuel substrate.

**Table II-D-1: Arrangement of fuel types and their abbreviation for each set of respirometer experiment.**

| Experiment | Fuel Type | |
|---|---|---|
| | Description | Abbreviation |
| #1 | Diesel | ULSD |
| | Soy biodiesel 20% blend + bioextend | Soy B-20 A |
| | Animal fat biodiesel 20% blend + bioextend | AF B-20 A |
| | Soy biodiesel 20% blend -no additives | Soy B-20 |
| #2 | Diesel | ULSD |
| | Soy biodiesel 100% - no additives | Soy B-100 |
| | Animal fat biodiesel 20% blend - no additives | AF B-20 |
| | Animal fat biodiesel 100% - no additives | AF B-100 |
| #3 | Diesel | ULSD |
| | Soy biodiesel 20% blend + bioextend + biocide | Soy B-20 AA |
| | Animal fat biodiesel 20% blend + bioextend + biocide | AF B-20 AA |
| | Soy biodiesel 100% + bioextend + biocide | Soy B-100 AA |
| #4 | Diesel | ULSD |
| | Animal fat biodiesel 100% + bioextend + biocide | AF B-100 AA |
| | Animal fat biodiesel 100% + bioextend | AF B-100 |
| | Soy biodiesel 100% + bioextend | Soy B-100 |

## Assessing Biological Activity

The $CO_2$ production in microcosms was automatically measured using a respirometer during the experiment. The carbon content of each fuel was determined by combustion/gas chromatography (Costech ECS4010 elemental analyzer). The carbon content of each fuel type measured by combustion/gas chromatography was reported as percent carbon by weight (percent gram of carbon per gram of fuel). The carbon content of 5uL, initial fuel test in each microcosm, was calculated using percent carbon content and density of each fuel.

The carbon content of each microcosm is correlated with the accumulated CO2 production to compare the potential biodegradability of each fuel test in regard to diesel.

## Respirometer

Aerobic biodegradation of diesel and biodiesel in microcosms was studied monitoring the respiration of microorganisms as indicated by $CO_2$ production. Respiration of the microcosms was measured using a Micro-Oxymax closed circuit respirometer (Columbus Instrument, Columbus, OH). The respirometer was equipped with a single beam, nondispersive, infrared $CO_2$ detector with a range of 0 to 0.8%. The headspace in the microcosms was refreshed with air when $CO_2$ concentrations exceeded ± 0.5%. $CO_2$ measurements were taken every 8-10 hours. The respirometer has 20 chambers (Figure II-D-1) and each experiment comprised of 4 sets of fuel test and 1 set of control blank (no substrate) microcosms. At each experiment diesel fuel was one of the sets for comparison with other test fuels. Table II-D-1 shows the arrangement of each experiment and code used for each fuel type. The duration of each experiment was 28-30 days.

**Figure II-D-1: Respirometer equipment used for aerobic biodegradation monitoring in 29-day tests.**

For each microcosm, the total initial carbon was compared to the cumulative carbon evolved as CO2 production. The fraction of initial carbon evolved as CO2 was taken as a measure of the biodegradability of each fuel.

## Fuel Carbon Content

Carbon content of each fuel type was determined using combustion/gas chromatography (Costech ECS4010 elemental analyzer).

### RESULTS AND DISCUSSION

#### Initial Carbon Content of Fuel Blends

Initial carbon contents for the fuels tested are shown in Table II-D-2. Because each microcosm receives 5 mL of fuel substrate, the initial carbon is calculated as the mass fraction of carbon in the fuel times the volumetric mass density times 5mL. The volumetric mass densities (data not

shown) range from 0.86 to 1.02g/mL, and the resulting initial carbon contents (last column of Table II-D-2) range from 3.78 to 4.15 for the biofuel blends compared to 4.54 for the ULSD.

**Table II-D-2: Carbon content of the 12 biodiesel blends and one petroleum diesel tested**.

| Fuel type | | % Carbon by weight | g C/mL Fuel | Initial **C** content in microcosm (mg) |
|---|---|---|---|---|
| AB100 | AF B-100 | 84.7 | 0.81 | 4.066 |
| | AF B-100 A | 76.8 | 0.81 | 4.032 |
| | AF B-100 AA | 74.9 | 0.76 | 3.782 |
| SB100 | Soy B-100 | 78 | 0.79 | 3.939 |
| | Soy B-100 A | 77 | 0.81 | 4.043 |
| | Soy B-100 AA | 77.2 | 0.77 | 3.860 |
| AB20 | AF B-20 | 84.6 | 0.83 | 4.145 |
| | AF B-20 A | 84.2 | 0.78 | 3.915 |
| | AF B-20 AA | 85.9 | 0.79 | 3.951 |
| SB20 | Soy B-20 | 84.2 | 0.80 | 4.000 |
| | Soy B-20 A | 84.1 | 0.78 | 3.911 |
| | Soy B-20 AA | 71.6 | 0.67 | 3.365 |
| ULSD | | 88.1 | 0.91 | 4.537 |

## Biodegradation Results: CO2 production over time for all fuels

Assuming accumulated $CO_2$ in each microcosm is a result of utilizing the fuel carbons by microorganisms aerobically, the total carbon consumption in each microcosm was calculated using the stoichiometry of Equation D1.

$$C + O_2 \rightarrow CO_2 \qquad \text{Equation D1}$$

Sterile (no biological activity) and blank (no fuel substrate) microcosms showed no $CO_2$ production. Lack of $CO_2$ production in these controls indicates that any $CO_2$ production in test microcosms is a result of microbial activities and not due to chemical reactions.

The percent degradation of each fuel type was calculated based on the initial carbon content and total carbon oxidation (Table II-D-3). In Experiment number 4, the amount of utilized carbon was measured more than initial carbon content due to malfunction of respirometer during the experimental period.

**Table II-D-3 – Percent degradation of different fuel types**

| Experiment | Fuel Type | Accumulated CO2 (mg) | Equivalent oxidized carbon (mg) | Percent degradation |
|---|---|---|---|---|
| #1 | ULSD | 7.87 | 2.15 | 47.40 |
| | Soy B-20 A | 10.23 | 2.80 | 71.48 |
| | AF B-20 A | 11.24 | 3.07 | 78.43 |
| | Soy B-20 | 13.53 | 3.70 | 92.40 |
| #2 | ULSD | 6.37 | 1.74 | 38.36 |
| | Soy B-100 | 9.04 | 2.47 | 62.70 |
| | AF B-20 | 8.83 | 2.41 | 58.18 |
| | AF B-100 | 11.31 | 3.09 | 75.99 |
| #3 | ULSD | 7.43 | 2.03 | 44.74 |
| | Soy B-20 AA | 10.30 | 2.81 | 83.65 |
| | AF B-20 AA | 9.55 | 2.61 | 66.02 |
| | Soy B-100 AA | 9.30 | 2.54 | 65.80 |
| #4 | ULSD | 10.78 | 2.95 | 64.92 |
| | AF B-100 AA | 18.86 | 5.15 | 136.26 |
| | AF B-100 A | 21.89 | 5.98 | 148.32 |
| | Soy B-100 A | 18.56 | 5.07 | 125.42 |

The mild slowing of the Animal Fat blends may be due to product or other inhibition process. Another potential explanation is that the degrable fraction component in Animal Fat biodiesel is different from that in Soy blends, and more limited. Interestingly the 20% biodiesel blends appear to induce greater CO2 production than the 100% biodiesel fules. Unfortunately the identity of the degraded fraction component is unknown. Further study would involve chemical analyses of the samples selected from various points in time during the biodegradation, to identify degraded and undegraded fractions.

Figure II-D-2 shows the time-dependent accumulation of CO2 in experimental suites 1, 2, and 3, for each fuel tested. These data show a small lag time (20-60 hours) followed by linear to mildly-decreasing accumulation rates with all biodiesel blends exhibiting faster degradation in all cases than ULSD. Animal fat blends generally show a more rapid production of CO2 at early time, that is followed by a slowing of production so that Soy blend CO2 production in some cases reaches the same cumulative CO2 production.

Figure II-D-3 shows a comparison of percent of carbon biodegradation with the different fuel types in microcosm respirometry at the end of the experiments. These results reflect the mixed degrability of Animal Fat vs. Soy biodiesel blends observed at the end of the ~29-day experiments shown in Figure II-D-2.

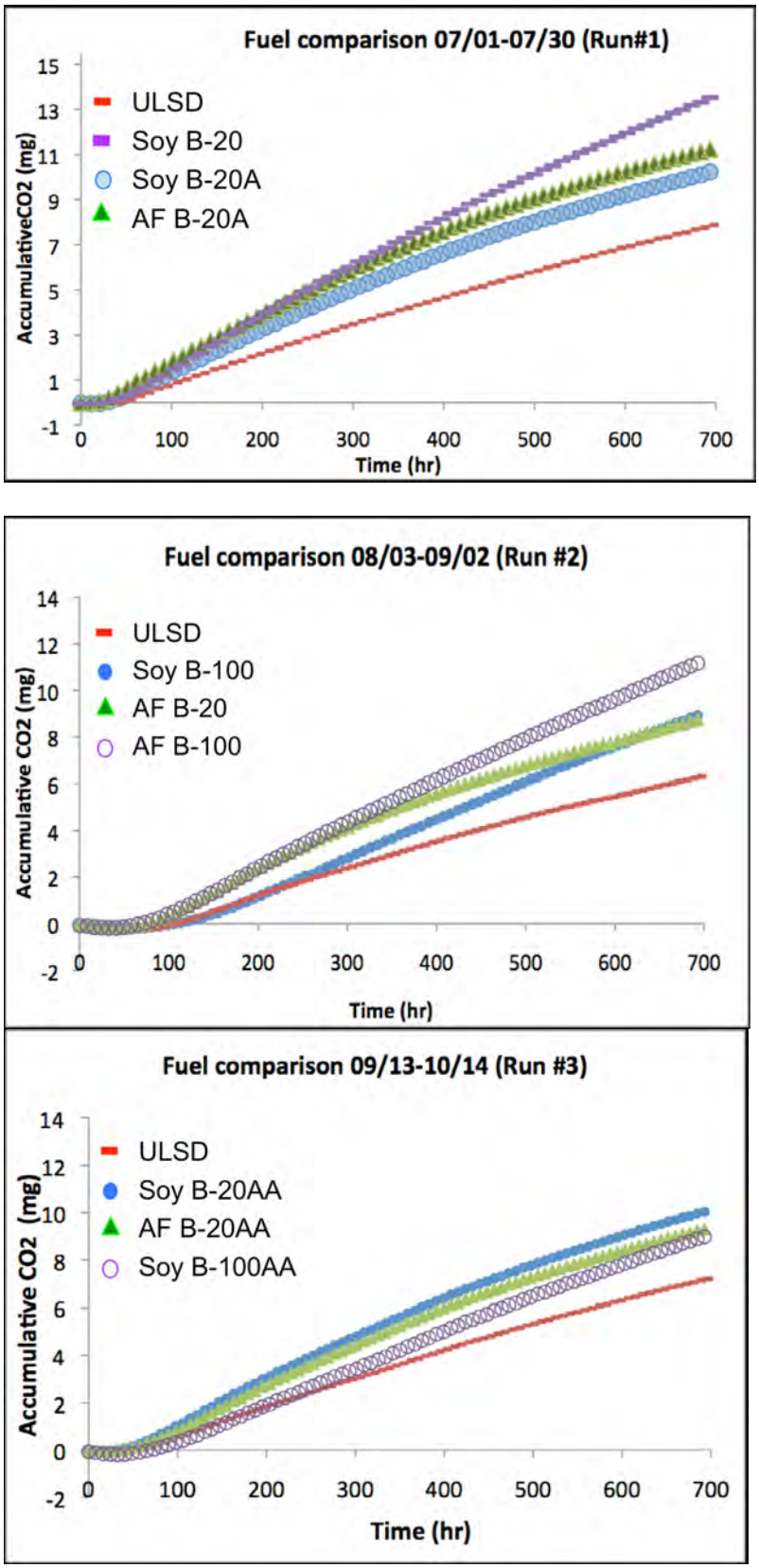


Figure II-D-2. Respirometry data on CO2 production in experimental suites #1 (top), #2 (middle) and #3 (bottom).

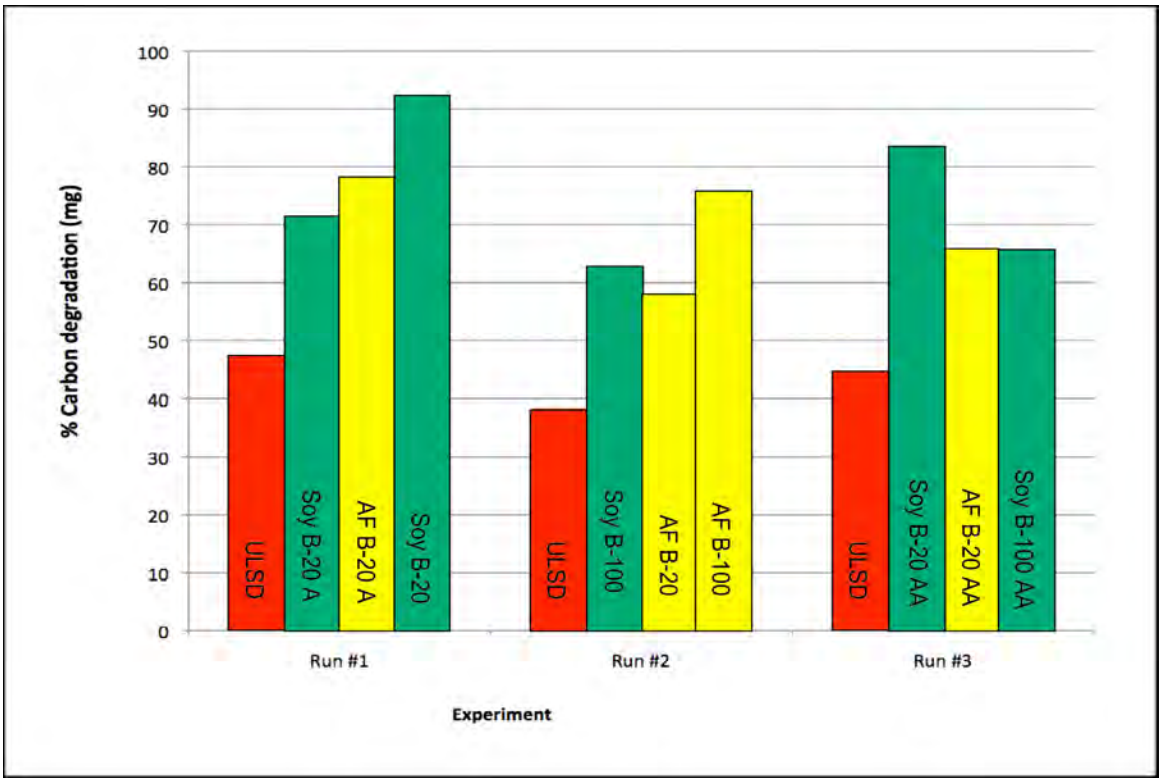


Figure II-D-3: Comparison of percent of carbon biodegradation with different fuel types in microcosm respirometry at the end of the experiments.

The primary implications of these results are that the biodiesel blends of all types and all additive cases are significantly more biodegrable than CARB ULSD#2. Mild variations in rate are seen in the transient data, most clearly the decline in CO2 production rate for Animal Fat blends. Sample chemical analyses would be required to identify organic fractions associated with the degrable and non-degrable fractions. Further study could include different soil inocula, different temperatures, and different moisture contents to represent soil conditions. In our tests only respiration was measured and more information may be obtained by identifying microbial growth in terms of cell number or protein.

## CONCLUSIONS

- All biodiesel blends are more readily degraded than the reference ULSD#2
- Additives do not exhibit any clear impact on biodiesel biodegrability
- The 20% biodiesel blends appear to be somewhat more susceptible to degradation than 100% blends.

## REFERENCES

Birchall, C., J. R. Newman, M. P. 2002. Greaves, Degradation and Phytotoxicity of biodiesel oil. Technical report under contract ref: CSA 2614, Centre for Aquatic Plant Management, Reading, UK, 2002.

EPA-560/6-82-003, PB82-233008. 1982. Test guidelines: chemical fate – aerobic aquatic biodegradation.

European Commission. 1996. Technical Guidance Document in support of Commission Directive 93/67/EEC on Risk Assessment for New Notified Substances and Commission Regulation (EC) No 1488/94 on Risk Assessment for Existing Substances, second ed. ISBN: 92-827- 8012-0. 2003[3].

[3] http://ecb.jrc.it/Documents/TECHNICAL_GUIDANCE_DOCUMENT/EDITION_2/tgdpart2_2ed.pdf.

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EPA-560/6-82-003, PB82-233008. 1982. Test guidelines: chemical fate – aerobic aquatic biodegradation.

European Commission. 1996. Technical Guidance Document in support of Commission Directive 93/67/EEC on Risk Assessment for New Notified Substances and Commission Regulation (EC) No 1488/94 on Risk Assessment for Existing Substances, second ed. ISBN: 92-827- 8012-0. 2003[3].

[3] http://ecb.jrc.it/Documents/TECHNICAL_GUIDANCE_DOCUMENT/EDITION_2/tgdpart2_2ed.pdf.

# APPENDIX H

## Requests for External Peer Review of the Biodiesel Multimedia Evaluation

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# Air Resources Board

**Mary D. Nichols, Chairman**
1001 I Street • P.O. Box 2815
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**Matthew Rodriquez**
*Secretary for*
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**Edmund G. Brown Jr.**
*Governor*


TO: Gerald W. Bowes, Ph.D.
Manager, California Environmental Protection Agency
Scientific Peer Review Program
Office of Research, Planning and Performance

FROM: Floyd V. Vergara, Esq., P.E. *Original Signed*
Assistant Chief, Mobile Source Control Division
(Formerly Chief, Alternative Fuels Branch)

DATE: November 19, 2013

SUBJECT: REQUEST FOR EXTERNAL PEER REVIEWERS FOR THE MULTIMEDIA WORKING GROUP'S ASSESSMENT OF THE BIODIESEL AND RENEWABLE DIESEL MULTIMEDIA EVALUATIONS

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In accordance with Health and Safety Code (H&SC) sections 43830.8 and 57004, the California Air Resources Board (ARB) staff requests external peer reviewers for two staff reports entitled, "*Staff Report: Multimedia Evaluation of Biodiesel*" (Biodiesel Staff Report) and "*Staff Report: Multimedia Evaluation of Renewable Diesel"* (Renewable Diesel Staff Report), which were authored by the Multimedia Working Group (MMWG). The MMWG is composed of representatives from various California Environmental Protection Agency organizations.

The staff reports consist of the MMWG's assessment of the biodiesel and renewable diesel multimedia evaluations conducted by researchers at the University of California (UC), Berkeley, and UC Davis, and the MMWG's analysis of potential significant adverse impacts on public health and the environment.

For this peer review, we suggest that the reviewers have expertise in environmental and multimedia impacts analysis, including: (1) air quality; (2) surface and ground water quality; (3) public health, and (4) soil impacts and hazardous waste. We estimate that six reviewers would be sufficient to cover all needed areas of expertise.

Peer review comments will be addressed by the MMWG in the staff reports, and the MMWG's summary and recommendations will be finalized and submitted to the California Environmental Policy Council (CEPC or Council) to complete the multimedia evaluation. The CEPC consists of the following Council members: Secretary for Environmental Protection, Chairman of ARB, Director of the Office of Environmental

Health Hazard Assessment (OEHHA), Chairman of the State Water Resources Control Board (SWRCB), Director of the Department of Toxic Substances Control (DTSC), Director of the Department of Pesticide Regulation, and Director of the Department of Resources Recycling and Recovery.

The CEPC will determine whether the use of biodiesel and renewable diesel fuel will cause a significant adverse impact on public health or the environment. Before fuel specifications are established, a multimedia evaluation must be conducted pursuant to H&SC section 43830.8. Pending completion of the biodiesel and renewable diesel multimedia evaluations, ARB staff intends to establish fuel quality specifications for biodiesel and renewable diesel fuel.

The following attachments are enclosed:

1. Attachment 1 - Plain English Summary of the Biodiesel Multimedia Evaluation and Renewable Diesel Multimedia Evaluation
2. Attachment 2 - Description of Scientific Conclusions to be Addressed by Peer Reviewers
3. Attachment 3 - List of Participants
4. Attachment 4 - References

The staff reports prepared by the MMWG and other supporting documentation will be ready for review by November 20, 2013. Staff requests that the peer review be completed and comments from the reviewers be received by **December 23, 2013.**

If you should have questions regarding this request, please contact Ms. Aubrey Gonzalez, Air Resources Engineer, Substance Evaluation Section at (916) 324-3334 or via email at agonzale@arb.ca.gov. Thank you for your time and consideration of this request.

Attachments (4)

cc: Aubrey Gonzalez
Air Resources Engineer
Substance Evaluation Section

Jim Aguila, Manager
Substance Evaluation Section

## ATTACHMENT 1

## Plain English Summary of the Biodiesel Multimedia Evaluation and Renewable Diesel Multimedia Evaluation

The Multimedia Working Group (MMWG) prepared two staff reports, one for the multimedia evaluation of biodiesel and the other for the multimedia evaluation of renewable diesel. The complete titles of each of these reports are provided below:

1. Staff Report: Multimedia Evaluation of Biodiesel including 10 appendices (Biodiesel Staff Report)
2. Staff Report: Multimedia Evaluation of Renewable Diesel including 10 appendices (Renewable Diesel Staff Report)

The staff reports consist of the MMWG's assessment of the biodiesel and renewable diesel multimedia evaluations conducted by researchers at the University of California (UC), Berkeley, and UC Davis, and the MMWG's analysis of potential significant adverse impacts on public health and the environment.

The MMWG conclusions and recommendations in the staff reports are primarily based on the results of the multimedia evaluation and information provided in the UC researchers' final reports entitled, "California Biodiesel Multimedia Evaluation Final Tier III Report" (Biodiesel Final Tier III Report) and "California Renewable Diesel Multimedia Evaluation Final Tier III Report" (Renewable Diesel Final Tier III Report).

### Biodiesel Multimedia Evaluation

"Biodiesel" is composed of mono-alkyl esters of long chain fatty acids derived from vegetable oils or animal fats and meets the specifications set forth by ASTM International standard D6751.

The MMWG completed their assessment of the biodiesel multimedia evaluation and potential impacts on public health and the environment. The evaluation is a relative comparison between biodiesel fuel and diesel fuel meeting Air Resources Board (ARB) motor vehicle diesel fuel specifications (CARB diesel).

Based on the results of the biodiesel multimedia evaluation and the information provided in the UC's Biodiesel Final Tier III Report, the MMWG makes the overall conclusion that biodiesel specifically evaluated within the scope of the evaluation will not cause a significant adverse impact on public health or the environment.

### Renewable Diesel Multimedia Evaluation

"Renewable diesel" is produced from non-petroleum renewable resources and is not a mono-alkyl ester. Renewable diesel consists solely of hydrocarbons and meets ARB motor vehicle fuel specifications under title 13, California Code of Regulations, section 2281 et seq.

The MMWG completed their assessment of the renewable diesel multimedia evaluation and potential impacts on public health and the environment. The evaluation is a relative comparison between renewable diesel and CARB diesel.

Based on the results of the multimedia evaluation and the information provided in the UC's Renewable Diesel Final Tier III Report, the MMWG makes the overall conclusion that renewable diesel specifically evaluated within the scope of the evaluation will not cause a significant adverse impact on public health or the environment.
Hard copies of the MMWG Biodiesel Staff Report and Renewable Diesel Staff Report, including the UC Biodiesel Final Tier III Report and Renewable Diesel Final Tier III Report, will be provided. Also, all references cited in each of the staff reports will be provided electronically on a compact disk.

## ATTACHMENT 2

### Description of Scientific Conclusions to be Addressed by Peer Reviewers

**The statutory mandate for external scientific peer review (H&SC section 57004) states that the reviewer's responsibility is to determine whether the scientific basis or portion of the proposed rule is based upon sound scientific knowledge, methods, and practices.**

**We request your review to allow you to make this determination for each of the following conclusions that constitute the scientific basis of the staff reports. An explanatory statement is provided for each conclusion to focus the review.**

**For those work products which are not proposed rules, as is the case here, reviewers must measure the quality of the product with respect to the same exacting standard as if it was subject to H&SC section 57004.**

The following conclusions are based on information provided in the Multimedia Working Group's (MMWG's) staff reports:

1. Staff Report: Multimedia Evaluation of Biodiesel including 10 appendices (Biodiesel Staff Report)
2. Staff Report: Multimedia Evaluation of Renewable Diesel including 10 appendices (Renewable Diesel Staff Report)

Biodiesel and renewable diesel are defined in Attachment 1.

### 1. Biodiesel

**The MMWG concludes that the use of biodiesel fuel in California, as specified in the biodiesel multimedia evaluation, does not pose a significant adverse impact on public health or the environment relative to diesel fuel meeting Air Resources Board (ARB) motor vehicle diesel fuel specifications (CARB diesel).**

Based on the results of the biodiesel multimedia evaluation and the information provided in the University of California (UC) final report, "California Biodiesel Multimedia Evaluation Final Tier III Report" (Ginn, T.R., *et al.*, May 2013), the MMWG makes the overall conclusion that biodiesel specifically evaluated within the scope of the biodiesel multimedia evaluation will not cause a significant adverse impact on public health or the environment relative to CARB diesel. The MMWG based their conclusion on each individual agency's assessment of the biodiesel multimedia evaluation. (Biodiesel Staff Report, Chapter 3)

a. **Air Emissions Evaluation. Air Resources Board (ARB) staff concludes that the use of biodiesel does not pose a significant adverse impact on public**

**health or the environment from potential air quality impacts.** ARB staff completed a comparative air quality assessment of biodiesel fuel relative to CARB diesel. ARB staff made conclusions based on their assessment of various emissions test results and air quality data, including criteria pollutants, toxic air contaminants, ozone precursors, and greenhouse gas emissions data. (Biodiesel Staff Report, Chapters 2 and 3)

b. **Water Evaluation. State Water Resources Control Board (SWRCB) staff concludes that there are minimal additional risks to beneficial uses of California waters posed by biodiesel than that posed by CARB diesel alone.** SWRCB staff completed an evaluation of potential surface water and groundwater impacts from biodiesel fuel and made conclusions based on their assessment of potential water impacts and materials compatibility, functionality, and fate and transport information. (Biodiesel Staff Report, Chapter 2 and 3)

c. **Public Health Evaluation. Office of Environmental Health Hazard Assessment (OEHHA) staff concludes that the substitution of biodiesel for CARB diesel reduces the rate of addition of carbon dioxide to the atmosphere and reduces the amount of particulate matter (PM), benzene, ethyl benzene, and polycyclic aromatic hydrocarbons (PAHs) released into the atmosphere, but may increase emissions of oxides of nitrogen ($NO_x$) and acrolein for certain blends.** OEHHA staff evaluated potential human health impacts from the use of biodiesel and made conclusions based on their analysis of potential impacts on atmospheric carbon dioxide and combustion emissions results. (Biodiesel Staff Report, Chapter 2 and 3)

d. **Soil and Hazardous Waste Evaluation. Department of Toxic Substances Control (DTSC) staff concludes that biodiesel aerobically biodegrades more readily than CARB diesel, has potentially higher aquatic toxicity for a small subset of tested species, and generally has no significant difference in vadose zone infiltration rate.** DTSC staff evaluated impacts of biodiesel to human health and the environment and made conclusions based on their evaluation of screening aquatic toxicity testing, hazardous waste generation during the production, use, storage, and disposal of biodiesel and biodiesel blends, and potential impacts on the fate and transport of biodiesel fuel in the subsurface soil from unauthorized spills or releases. (Biodiesel Staff Report, Chapter 2 and 3)

## 2. Renewable Diesel

**The MMWG concludes that the use of renewable diesel fuel in California, as specified in the renewable diesel multimedia evaluation, does not pose a significant adverse impact on public health or the environment relative to CARB diesel.**

Based on the results of the renewable diesel multimedia evaluation and the information provided in the UC final report, "California Renewable Diesel Multimedia Evaluation Final Tier III Report" (McKone, T.E. *et al.*, April 2012), the MMWG makes the overall conclusion that renewable diesel specifically evaluated within the scope of the renewable diesel multimedia evaluation will not cause a significant adverse impact on public health or the environment relative to CARB diesel. The MMWG based their conclusion on each individual agency's assessment of the multimedia evaluation. (Renewable Diesel Staff Report, Chapter 3)

a. **Air Emissions Evaluation. ARB staff concludes that the use of renewable diesel does not pose a significant adverse impact on public health or the environment from potential air quality impacts**. ARB staff completed a comparative air quality assessment and impacts analysis of renewable diesel fuel relative to CARB diesel. ARB staff made conclusions based on their assessment of various emissions test results and air quality data, including criteria pollutants, toxic air contaminants, and greenhouse gas emissions data. (Renewable Diesel Staff Report, Chapter 2 and 3)

b. **Water Evaluation. SWRCB staff concludes that there are minimal additional risks to beneficial uses of California waters posed by renewable diesel than that posed by CARB diesel alone.** SWRCB staff completed an evaluation of potential surface water and groundwater impacts from renewable diesel and made conclusions based on their assessment of potential water impacts and material compatibility, functionality, and fate and transport information. (Renewable Diesel Staff Report, Chapter 2 and 3)

c. **Public Health Evaluation. OEHHA staff concludes that PM, benzene, ethyl benzene, and toluene in combustion emissions from diesel engines using hydrotreated vegetable oil renewable diesel are significantly lower than combustion emissions using CARB diesel.** OEHHA staff evaluated potential human health impacts from the use of renewable diesel and made conclusions based on their analysis of toxicity testing data and combustion emissions results. (Renewable Diesel Staff Report, Chapter 2 and 3)

d. **Soil and Hazardous Waste Evaluation. DTSC staff concludes that renewable diesel is free of ester compounds and has low aromatic content. The chemical compositions of renewable diesel are almost identical to that of CARB diesel. Therefore, the impacts on human health and the environment in case of a spill to soil, groundwater, and surface waters would be expected to be similar to those of CARB diesel.** DTSC staff assessed potential impacts to human health and the environment from the production and use of renewable diesel compared to CARB diesel, and made conclusions based on their analysis of hazardous waste generation during the production, use, and storage of renewable diesel in California and cleanup of

contaminated sites in case of unauthorized spills or releases. (Renewable Diesel Staff Report, Chapter 2 and 3)

## 3. MMWG's Recommendations to the California Environmental Policy Council

**The MMWG recommends that the California Environmental Policy Council (CEPC) find that the use of biodiesel and renewable diesel, as specified in the respective multimedia evaluations, does not pose a significant adverse impact on public health or the environment.** Based on the MMWG's conclusions in Chapter 3 of the Biodiesel Staff Report and the Renewable Diesel Staff Report, the MMWG proposes recommendations to the CEPC. (Biodiesel Staff Report and Renewable Diesel Staff Report, Chapter 4)

## 4. Big Picture

Reviewers are not limited to addressing only the specific conclusions presented above, and are asked to contemplate the following questions:

(a) In reading the staff report and supporting documentation, are there any additional scientific issues that are part of the scientific basis or conclusion of the multimedia evaluation not described above? If so, please provide further comments.

(b) Taken as a whole, are the conclusions and scientific portions of the multimedia evaluation based upon sound scientific knowledge, methods, and practices?

Reviewers should note that in some instances, the conclusions may rely on the professional judgment where the scientific data may be less than ideal. In these situations, every effort was made to ensure that the data was scientifically defensible.

The proceeding guidance will ensure that reviewers have an opportunity to comment on all aspects of the scientific basis of the multimedia evaluation of the proposed fuels. At the same time, reviewers also should recognize that the Board has a legal obligation to consider and respond to all feedback on the scientific portions of the multimedia evaluation. Because of this obligation, reviewers are encouraged to focus feedback on scientific issues that are relevant to the central regulatory elements being proposed.

## ATTACHMENT 3

## List of Participants*

### Principal Investigators, Authors, Researchers, and Students Involved in the Biodiesel and Renewable Diesel Multimedia Evaluation

Principal Investigators and Authors of the Multimedia Evaluation (MME) Final Reports

| | |
|---|---|
| Thomas McKone | University of California, Berkeley |
| David Rice | University of California, Berkeley consultant<br>Lawrence Livermore National Laboratory (retired) |
| Timothy Ginn | University of California, Davis |
| Tyler Hatch | University of California, Davis |

Test Program Researchers and Authors of MME Tier II Associated Reports

| | |
|---|---|
| Kate Scow | University of California, Davis |
| Michael Johnson | University of California, Davis (retired) |
| Jeffrey Miller | University of California, Davis |
| Eric LaBolle | University of California, Davis |
| Jerry Last | University of California, Davis |
| Randy Maddalena | University of California, Berkeley |
| Thomas Durbin | University of California, Riverside |

Students Involved in the Multimedia Evaluation Process

| | |
|---|---|
| Tomer Schetrit | University of California, Davis |
| Vanessa Nino | University of California, Davis |
| Amande Epple | University of California, Davis |
| Tammer Barkouki | University of California, Davis |
| Idy Lui | University of California, Davis |
| Shima Motlagh | University of California, Davis |
| Laleh Rastegarzadeh | University of California, Davis |
| Josue Villagomez | University of California, Davis |

Note: None of the University of California principal investigators, authors, researchers, nor students involved in the biodiesel and renewable multimedia evaluations participated in the development of ARB's proposed rulemaking to establish fuel quality specifications for biodiesel and renewable diesel fuel.

**Members of the Multimedia Workgroup**

| | |
|---|---|
| Aubrey Gonzalez | Air Resources Board |
| Alexander Mitchell | Air Resources Board |
| Stephen d'Esterhazy | Air Resources Board |
| Susie Chung | Air Resources Board |
| Jim Aguila | Air Resources Board |
| Floyd Vergara | Air Resources Board |
| Jim Guthrie | Air Resources Board |
| Mark Schuy | Air Resources Board |
| Patrick Wong | Air Resources Board |
| Russel Hansen | State Water Resources Control Board |
| Laura Fisher | State Water Resources Control Board |
| Shahla Farahnak | State Water Resources Control Board |
| Li Tang | Department of Toxic Substances Control |
| Adriana Ortegon | Department of Toxic Substances Control |
| Andre Algazi | Department of Toxic Substances Control |
| Donn Diebert | Department of Toxic Substances Control |
| Page Painter | Office of Environmental Health Hazard Assessment |
| Hristo Hristov | Office of Environmental Health Hazard Assessment |
| John Budroe | Office of Environmental Health Hazard Assessment |

* No person may serve as an external scientific peer reviewer for the scientific portion of the multimedia evaluation if that person participated in the development of the scientific basis or scientific portion of the multimedia evaluation.

## ATTACHMENT 4

## References

All references cited in the Biodiesel Staff Report and the Renewable Diesel Staff Report will be provided on a compact disk. For references available online, electronic links will also be provided in the staff reports.

# Air Resources Board

**Mary D. Nichols, Chairman**
1001 I Street • P.O. Box 2815
Sacramento, California 95812 • www.arb.ca.gov

**Matthew Rodriquez**
*Secretary for*
*Environmental Protection*

**Edmund G. Brown Jr.**
*Governor*


TO: Gerald W. Bowes, Ph.D., Manager
Cal/EPA Scientific Peer Review Program

FROM: Jim M. Aguila, Chief
Program Planning and Management Branch

DATE: January 21, 2015

SUBJECT: REQUEST FOR SUPPLEMENTAL EXTERNAL PEER REVIEW OF THE MULTIMEDIA WORKING GROUP'S ASSESSMENT OF THE BIODIESEL MULTIMEDIA EVALUATION

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By way of this memorandum, California Air Resources Board (ARB) staff requests supplemental external peer review of the revised staff report entitled, "*Staff Report: Multimedia Evaluation of Biodiesel*" (Biodiesel Staff Report) prepared by the Multimedia Working Group (MMWG). An initial peer review of the previous version of this report, dated November 2013, was completed in February 2014.

The revised Biodiesel Staff Report, dated December 2014, consists of proposed modifications to the MMWG's assessment of the biodiesel multimedia evaluation. Staff's proposed modifications include updates to the air quality and public health evaluations from staff's assessment of new biodiesel studies and publications, and revisions that address peer review comments from the initial peer review.

For this supplemental review, we suggest that peer reviewers have expertise in air quality and public health impacts. We anticipate that a minimum of two reviewers for each topic would be adequate. Also, due to the extensive peer review completed in February 2014, and the numerous reports and sources included in that review, consideration should be given to those who performed the initial review.

Peer review comments will be addressed by the MMWG in the final staff report, and the MMWG's summary and recommendations will be finalized and submitted to the California Environmental Policy Council to complete the multimedia evaluation. Pending completion of the multimedia evaluation, ARB staff intends to establish fuel quality specifications for biodiesel in support of the proposed Alternative Diesel Fuel regulation.

The following attachments are enclosed:

1. Attachment 1 - Plain English Summary of the Revised Biodiesel Multimedia Evaluation
2. Attachment 2 - Description of Scientific Conclusions to be Addressed by Peer Reviewers
3. Attachment 3 - List of Participants
4. Attachment 4 - References

The revised Biodiesel Staff Report and other supporting documentation will be ready for review by January 29, 2015. Staff requests that the peer review be completed and comments from the reviewers be received by **March 2, 2015.**

If you have questions regarding this request, please contact Aubrey Gonzalez, Air Resources Engineer, Substance Evaluation Section at (916) 324-3334 or by email at aubrey.gonzalez@arb.ca.gov.

Thank you for your time and consideration of this request.

Attachments (4)

cc: Aubrey Gonzalez, Air Resources Engineer
Substance Evaluation Section
Industrial Strategies Division

## ATTACHMENT 1

## Plain English Summary of the Revised Biodiesel Multimedia Evaluation

The Multimedia Working Group (MMWG) revised the staff report entitled "*Staff Report: Multimedia Evaluation of Biodiesel*" ((Biodiesel Staff Report). The revised Biodiesel Staff Report consists of proposed modifications to the MMWG's assessment of the biodiesel multimedia evaluation.

Staff's modifications include updates to the air quality and public health evaluations based on new biodiesel studies and publications, and revisions to the staff report based on the information and comments from the initial peer review. More specifically, the MMWG revised the Biodiesel Staff Report with the following modifications:

- Updated Air Resources Board (ARB) evaluation that includes new emissions data on lower biodiesel blends.
- Revised air quality impact summary and conclusions.
- Updated Office of Environmental Health Hazard Assessment (OEHHA) evaluation that includes additional health impacts studies.
- Revised public health impact summary and conclusions.
- Revised MMWG recommendations to the California Environmental Policy Council.

Air Quality

ARB staff completed a comparative air quality assessment of lower biodiesel blends relative to diesel fuel meeting ARB motor vehicle diesel fuel specifications (CARB diesel), updated their evaluation, revised the air quality impact summary, and made conclusions based on their assessment of new emissions test results and air quality data. Staff's revised conclusions are primarily based on the information provided in the University of California (UC), Riverside final report, entitled, "CARB Comprehensive B5/B10 Biodiesel Blends Heavy-Duty Engine Dynamometer Testing," and the February 2014 Biodiesel Peer Review Comments.

Based on staff's assessment, ARB staff concludes that with in-use requirements, biodiesel does not pose a significant adverse impact on public health or the environment from air quality impacts. ARB is currently conducting a rulemaking to establish in-use requirements for biodiesel use.

Public Health

OEHHA staff completed a review of scientific literature that compares the physical and chemical nature of combustion emissions from diesel engines fueled with biodiesel to the composition of combustion emissions from engines fueled with petroleum diesel. OEHHA staff updated their evaluation, revised the public health summary, and made conclusions based on their review of combustion emissions data.

Based on staff's scientific review, OEHHA staff concludes that replacing petroleum diesel with an energy-equivalent amount of biodiesel will decrease emissions of particulate matter (PM), benzene, and ethyl benzene but may increase emissions of oxides of nitrogen ($NO_x$). From studies comparing the biological impacts of biodiesel combustion emissions to those of petroleum diesel combustion emissions, OEHHA staff concludes that PM from biodiesel combustion emissions is more potent than PM from petroleum diesel combustion emissions in eliciting certain responses associated with inflammation and oxidative stress when biological responses per mass of PM are compared. However, in a study carried out at the University of California, Riverside and University of California, Davis, PM from combustion of soy-derived biodiesel is less potent in eliciting the responses associated with inflammation and oxidative stress than is PM in petroleum diesel combustion emissions when the comparison is made on a per mile basis. Staff's revised conclusions are based on several scientific publications and additional sources provided in the February 2014 Biodiesel Peer Review Comments.

## ATTACHMENT 2

## Description of Scientific Conclusions to be Addressed by Peer Reviewers

**The statutory mandate for external scientific peer review (H&SC section 57004) states that the reviewer's responsibility is to determine whether the scientific basis or portion of the proposed rule is based upon sound scientific knowledge, methods, and practices.**

**We request your review to allow you to make this determination for each of the following conclusions that constitute the scientific basis of the staff reports. An explanatory statement is provided for each conclusion to focus the review.**

**For those work products which are not proposed rules, as is the case here, reviewers must measure the quality of the product with respect to the same exacting standard as if it was subject to H&SC section 57004.**

The following conclusions are based on information provided in the revised staff report entitled, "*Staff Report: Multimedia Evaluation of Biodiesel*" (Biodiesel Staff Report) by the Multimedia Working Group (MMWG).

### 1. Air Emissions Evaluation

**Air Resources Board (ARB) staff concludes that with in-use requirements biodiesel does not pose a significant adverse impact on public health or the environment from potential air quality impacts.** ARB staff completed a comparative air quality assessment of lower biodiesel blends relative to diesel fuel meeting ARB motor vehicle diesel fuel specifications (CARB). ARB staff updated their evaluation, revised the air quality impact summary, and made conclusions based on their assessment of new emissions test results and air quality data.
(Revised Biodiesel Staff Report, Chapters 2 and 3)

### 2. Public Health Evaluation

**After reviewing scientific literature that compares the physical and chemical nature of combustion emissions from diesel engines fueled with biodiesel to the composition of combustion emissions from engines fueled with petroleum diesel, Office of Environmental Health Hazard Assessment (OEHHA) staff concludes that replacing petroleum diesel with an energy-equivalent amount of biodiesel will decrease emissions of particulate matter (PM), benzene, and ethyl benzene but may increase emissions of oxides of nitrogen ($NO_x$). From studies comparing the biological impacts of biodiesel combustion emissions to those of petroleum diesel combustion emissions, OEHHA staff concludes that PM from biodiesel combustion emissions is more potent than PM from petroleum diesel**

**combustion emissions in eliciting certain responses associated with inflammation and oxidative stress when biological responses per mass of PM are compared. However, in a study carried out at the University of California, Riverside and University of California, Davis, PM from combustion of soy-derived biodiesel is less potent in eliciting the responses associated with inflammation and oxidative stress than is PM in petroleum diesel combustion emissions when the comparison is made on a per mile basis.** OEHHA staff reviewed scientific literature that compares the physical and chemical nature of combustion emissions from diesel engines fueled with biodiesel to the composition of combustion emissions from engines fueled with petroleum diesel. OEHHA staff updated their evaluation, revised the public health summary, and made conclusions based on their review of combustion emissions data. (Revised Biodiesel Staff Report, Chapters 2 and 3)

## 3. Multimedia Working Group Recommendations

**The MMWG recommends that the California Environmental Policy Council (CEPC) find that the use of biodiesel, as specified in the biodiesel multimedia evaluation, does not pose a significant adverse impact on public health or the environment.** Based on the MMWG's conclusions in Chapter 3 of the revised Biodiesel Staff Report, the MMWG proposes recommendations to the CEPC. (Revised Biodiesel Staff Report, Chapter 4)

## 4. Big Picture

Reviewers are not limited to addressing only the specific conclusions presented above, and are asked to contemplate the following questions:

(a) In reading the staff report and supporting documentation, are there any additional scientific issues that are part of the scientific basis or conclusion of the multimedia evaluation not described above? If so, please provide further comments.

(b) Taken as a whole, are the conclusions and scientific portions of the multimedia evaluation based upon sound scientific knowledge, methods, and practices?

Reviewers should note that in some instances, the conclusions may rely on the professional judgment where the scientific data may be less than ideal. In these situations, every effort was made to ensure that the data was scientifically defensible.

The proceeding guidance will ensure that reviewers have an opportunity to comment on all aspects of the scientific basis of the biodiesel multimedia evaluation. At the same time, reviewers also should recognize that the Board has a legal obligation to consider and respond to all feedback on the scientific portions of the multimedia evaluation. Because of this obligation, reviewers are encouraged to focus feedback on scientific issues that are relevant to the central regulatory elements being proposed.

## ATTACHMENT 3

## List of Participants*

### Principal Investigators, Authors, Researchers, and Students Involved in the Biodiesel Multimedia Evaluation

Principal Investigators and Authors of the Multimedia Evaluation (MME) Final Reports

| | |
|---|---|
| Thomas McKone | University of California, Berkeley |
| David Rice | University of California, Berkeley consultant<br>Lawrence Livermore National Laboratory (retired) |
| Timothy Ginn | University of California, Davis |
| Tyler Hatch | University of California, Davis |

Test Program Researchers and Authors of MME Tier II Associated Reports

| | |
|---|---|
| Kate Scow | University of California, Davis |
| Michael Johnson | University of California, Davis (retired) |
| Jeffrey Miller | University of California, Davis |
| Eric LaBolle | University of California, Davis |
| Jerry Last | University of California, Davis |
| Randy Maddalena | University of California, Berkeley |
| Thomas Durbin | University of California, Riverside |
| Georgios Karavalakis | University of California, Riverside |
| Kent Johnson | University of California, Riverside |
| Maryam Hajbabaei | University of California, Riverside |

Students Involved in the Multimedia Evaluation Process

| | |
|---|---|
| Tomer Schetrit | University of California, Davis |
| Vanessa Nino | University of California, Davis |
| Amande Epple | University of California, Davis |
| Tammer Barkouki | University of California, Davis |
| Idy Lui | University of California, Davis |
| Shima Motlagh | University of California, Davis |
| Laleh Rastegarzadeh | University of California, Davis |
| Josue Villagomez | University of California, Davis |

Note: None of the University of California principal investigators, authors, researchers, nor students involved in the biodiesel multimedia evaluations participated in the development of ARB's proposed rulemaking to establish fuel quality specifications for biodiesel fuel.

**Members of the Multimedia Workgroup**

| | |
|---|---|
| Aubrey Gonzalez | Air Resources Board |
| Susie Chung | Air Resources Board |
| Alexander Mitchell | Air Resources Board |
| Stephen d'Esterhazy | Air Resources Board |
| Jim Aguila | Air Resources Board |
| Michael Waugh | Air Resources Board |
| Jim Guthrie | Air Resources Board |
| Mark Schuy | Air Resources Board |
| Patrick Wong | Air Resources Board |
| Floyd Vergara | Air Resources Board |
| Eric Morita | State Water Resources Control Board |
| Laura Fisher | State Water Resources Control Board |
| Kevin Graves | State Water Resources Control Board |
| Shahla Farahnak | State Water Resources Control Board |
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| Adriana Ortegon | Department of Toxic Substances Control |
| Andre Algazi | Department of Toxic Substances Control |
| Page Painter | Office of Environmental Health Hazard Assessment |
| John Budroe | Office of Environmental Health Hazard Assessment |
| Kenneth Kloc | Office of Environmental Health Hazard Assessment |
| Hristo Hristov | Office of Environmental Health Hazard Assessment |
| Melanie Marty | Office of Environmental Health Hazard Assessment |
| Allan Hirsch | Office of Environmental Health Hazard Assessment |

* No person may serve as an external scientific peer reviewer for the scientific portion of the multimedia evaluation if that person participated in the development of the scientific basis or scientific portion of the multimedia evaluation.

## ATTACHMENT 4

## References

All references cited in the revised Biodiesel Staff Report will be provided on a compact disk. For references available online, electronic links will also be provided in the staff report.

# APPENDIX I

## External Scientific Peer Reviews

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**Reviews of Biodiesel Assessment Reports and Supporting Documents (7)**

a) Edward J. Bouwer, Ph.D. - John Hopkins University
b) Tracey Holloway, Ph.D. - University of Wisconsin - Madison
c) An Li, Ph.D. - University of Illinois - Chicago
d) Stephen Nesnow, Ph.D. - Stephen Nesnow Consulting
e) Lisa A. Rodenburg, Ph.D. - Rutgers University
f) Paul White, Ph.D. - University of Ottawa
g) Xiusheng (Harrison) Yang, Ph.D. - University of Connecticut

Department of Geography and Environmental Engineering
Whiting School of Engineering

JOHNS HOPKINS UNIVERSITY

*Edward J. Bouwer (410) 516-7437; E-mail: bouwer@jhu.edu*

January 7, 2014

Gerald W. Bowes, Ph.D.
Manager, California Environmental Protection Agency
Scientific Peer Review Program
Office of Research, Planning, and Performance

Dear Dr. Bowes:

I have reviewed the **Staff Report: Multimedia Evaluation of Biodiesel** including 10 appendices. My expertise is microbial engineering that is applied to biodegradation of organic contaminants, transport and fate of bacteria in soil and aquifers, biofilm reactors, and contaminated sediments. I am providing external scientific peer review comments below mainly for the two sections on Water Evaluation and Soil and Hazardous Waste Evaluation.

**Water Evaluation**. Biodiesel is largely a mixture of fatty acid methyl esters (FAME). The FAME compounds tend to biodegrade at a faster rate than the compounds in CARB diesel. A general tendency is that liquid products from biomass are highly biodegradable under the proper conditions. For example, most liquid petroleum hydrocarbons (e.g., gasoline, diesel, jet fuel, oils, and FAME) can be biodegraded under aerobic conditions by many different species of bacteria. Several of these species of bacteria capable of petroleum hydrocarbon biodegradation are commonly found in rivers, lakes, and oceans and in the subsurface. Consequently, these liquid products tend not to persist for long periods when they are released to the environment. Furthermore, the vadose zone infiltration experiments showed that the vertical and horizontal extent of migration for biodiesel and CARB diesel were similar. The increased biodegradability of biodiesel in comparison to CARB diesel and similar transport properties means there is not an expected increase in risk from the use of biodiesel in comparison to CARB diesel when they come in contact with surface waters or groundwaters. I agree with the conclusion that there are likely to be minimal additional risks to the waters of California from the use of biodiesel.

The one factor that "clouds" the above conclusion is that additives are likely to be introduced in almost all biodiesel blends. These additives address issues of oxidation, corrosion, foaming, cold temperature flow properties, biodegradation during storage, and water separation. As long as the expectation holds that biodiesel will employ additives similar to those used currently in CARB diesel, then it follows that the health and environmental impacts of the two mixtures will be similar. If different additives are employed that might make the biodiesel mixture either more toxic or less biodegradable, then additional studies will need to be conducted to demonstrate the environmental health and safety of the biodiesel mixture planned for use.

**Soil and Hazardous Waste Evaluation**. Essentially, the same analysis provided for the Water Evaluation above applies for this topic. The enhanced biodegradability of biodiesel with FAME compounds in comparison to CARB diesel indicates that there will be less persistence of

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biodiesel in the subsurface following releases or spills. Consequently, there is not likely to be an increased risk to the environment with the use of biodiesel. The limited knowledge regarding the additives that will be used for biodiesel does add uncertainty to this conclusion. If such additives are different from the ones used for CARB diesel, then there is potential for the biodiesel mixture to behave differently in the environment, such as increased toxicity or reduced biodegradability. If different additives are used for biodiesel, then additional studies are recommended to properly document the new transport and fate properties.

In addition to the above comments for the major conclusions offered by the Staff Report, I provide following comments on specific sections of the report:

1. The Opening Glossary should contain CARB. The opening section does not define CARB diesel (page 4). CARB diesel is defined later in the report. If a reader starts with the opening section as I did, it will be confusing to not have a definition of CARB diesel up front. In Appendix G, the term "conventional petroleum diesel" or simply "petroleum diesel" is used. I suspect that CARB diesel and conventional petroleum diesel are terms for the same product. The broader community is likely to be more familiar with the term conventional petroleum diesel or petroleum diesel in comparison to CARB diesel.

2. Add CARB to the list of acronyms on page 8 of Appendix A. ARB is listed, but not CARB.

3. In Appendix F on page 2 of the 3-page memo from Donn Diebert, the opening sentence of the last paragraph is unclear. The sentence states that biodiesel appears to react differently in the environment than does CARB diesel. It is not clear if the three characteristics listed before the last paragraph are the main characteristics that are different. The opening sentence should provide more detail on the differences between biodiesel and CARB diesel by either referring to the characteristics listed or adding new characteristics that are most important for the differences. The knowledge about reaction differences is important for assessing the fate in the environment.

4. The bullet point at the top of page xi in Appendix G should mention that there are many bacterial groups indigenous to aquatic systems and soils that are capable of biodegrading biodiesel and petroleum hydrocarbons. This contributes to the high potential for biodegradation in these media.

5. On page 7 in the Appendix G report, there is a discussion that material compatibility is an important consideration. The second paragraph indicates some of the materials that are poorly compatible with biodiesel. It would be helpful to also provide examples of materials that are compatible with biodiesel. This will help educate the reader on materials to avoid along with those that are good to use. The same modifications are recommended to a similar discussion of materials compatibility that appears on page 25 in Appendix G. There is more discussion about materials compatibility in a section labeled "6. Tier III Appendices" within the

Appendix G tab on page I-26. More advice on compatible materials will be helpful. The impression given by this discussion is that most materials can be incompatible. It would be good to conclude with some suggested materials that can be used with high confidence.

6. As acknowledged thoroughly in the report, the presence of additives in the biodiesel is a source of uncertainty for the chemical and physical properties of the biodiesel. There is a statement in the last paragraph on page 24 of Appendix G that "it is reasonable to assume that most of the additives used in biodiesel are currently used in CARB ULSD". It would be helpful to provide some documentation that this assumption is true. The database might be limited, but are the current stocks of biodiesel using similar additives as CARB ULSD? Any evidence to support this statement will be helpful to support a conclusion that biodiesel is just as acceptable as CARB diesel.

7. In a section labeled "6. Tier III Appendices" within the Appendix G tab, there is a discussion of subsurface fate and transport properties on page I-5. The third sentence makes the statement that the composition of biodiesel differs significantly from that of petroleum diesel. Consequently, the behavior of the two liquids is likely to be different in the environment. It would be helpful if this discussion can include more specifics about the composition differences so it is easier to understand if the biodiesel might be more or less problematic. For example, we learn from the biodegradation studies that biodiesel is more biodegradable than petroleum biodiesel, so this is a positive attribute.

8. In a section labeled "6. Tier III Appendices" within the Appendix G tab, there is a discussion of biodegradation of biodiesel components on page I-55. Near the bottom of page I-55, the observed percentages for degradation are reported with 4 significant figures (e.g., 85.54% degradation). A batch biodegradation test cannot achieve this degree of precision, so it is recommended that the results be rounded to 2 or at most 3 significant figures. More detail needs to be provided for the results shown in Table 6.2 on page I-56 that are used to support the mechanism of co-metabolism. What is the ratio of diesel and biodiesel in the mixture? Was the total mass of diesel and biodiesel in the mixture the same as the mass of diesel alone? Without reporting the initial masses or concentrations, it is not clear from the reported data if more mass of diesel biodegraded in the presence of the biodiesel. Finally, there is a statement in the middle of page I-56 that microorganisms metabolize biodiesel and diesel at roughly the same rates. This statement is inconsistent with the reported data on page I-55 that after 28 days, the biodiesel exhibited 85 to 88% removal in comparison to 26% removal for petroleum diesel. The biodegradation rate for biodiesel is markedly faster than for petroleum diesel. The results shown in Table 6.3 also support this conclusion that biodegradation rates are faster for biodiesel.

9. Section 6.4.3 on page I-57 is called Biodegradation Under Aerobic and Anaerobic Conditions. It is better to explicitly mention the electron acceptor involved rather

than to call the conditions "anaerobic". For example, the situation with nitrate as the electron acceptor can be called nitrate respiration or denitrification. It is difficult to generalize from denitrification to all anaerobic conditions. The data on biodegradation for a wide range of anaerobic conditions are certainly sparse.

10. The Table II-D-3 on page II-84 within the Appendix G tab seems to arbitrarily show all of the data with two decimal points. This means that some of the percent degradation numbers are shown with 5 significant figures (e.g., 125.42). This level of precision is not possible for batch biodegradation testing, so the numbers should be reported with 2 or at most 3 significant figures. Furthermore, the last three tests have percent degradation values greater than 100%. The sources for this error should be discussed in the text.

11. Typos: Appendix G, page x: line 2 from the bottom: "compare" should be "compared". Same error on page 11, line 4 in the second full paragraph. On page 25 of Appendix G, "alliviate" should be "alleviate" in the second to last line of the first full paragraph. On page 27 of Appendix G, "volitilize" should be "volatilize". On page I-73 of Appendix G, "month" should be "months" in line 7 from the bottom. On page II-3 of Appendix G, "biodegrable" should be "biodegradable" in line 13 from the top. On page II-3 of Appendix G, "test" should be "tests" in line 2 from the bottom. On page II-17 of Appendix G, "without algae inoculate" should be "without an algae inoculum" in line 6 in the middle paragraph. On page II-20 of Appendix G, the caption for Figure II-A-1 is incomplete (i.e., it looks like some text is missing or is cutoff). On page II-32 of Appendix G, there is a statement about an error with the reference cited in the last line of text. The same error occurs on the next page II-33 in line 18 from the top. On page II-87 of Appendix G, "biodegrability" should be "biodegradability" in the second bullet in the middle of the page.

Sincerely,

Edward J. Bouwer, Ph.D.
Abel Wolman Professor of Environmental Engineering
Department Chair

*Review of*

"Staff Report: Multimedia Evaluation of Biodiesel"
Prepared by the Multimedia Working Group

*Tracey Holloway, Ph.D.*
*University of Wisconsin--Madison*

The California Air Resources Board (ARB) is proposing the development of new regulation for biodiesel. Biodiesel is considered a potentially desirable fuel alternative, given the lower carbon intensity relative to petroleum diesel fuel and possible other benefits. In this report, all conclusions about biodiesel are given relative to diesel fuel meeting ARB specifications, referred to in the report as "CARB diesel."

This review follows the topical areas of the MMWG report:

1. Biodiesel

Overall, the conclusions of the staff report are supported by the California Biodiesel Multimedia Evaluation (Final Tier I, II, and III reports) from researchers at UC Davis and UC Berkeley. In particular, the major conclusion that biodiesel use "does not pose a significant adverse impact on public health or the environment relative to diesel fuel" is in line with the findings of the Multimedia Evaluation.

The impacts of biodiesel relative to CARB diesel depend strongly on the percentage blend of petroleum diesel with biodiesel. However, the treatment of these categories and terminology is inconsistent through the report. For example:

- p. 4 introduces four categories of blending: B10, B20, B50, and B100 (where B10 = a 10% by volume blending of biodiesel with CARB diesel; B20, 20% blending, and so on).
- p. 8 report emissions for B5 blends, but not B10;
- p. 11 discusses B5 in the context of underground storage tanks (UST);
- Appendix A p. 4 defines only B5 and B20, as follows "(6) 'B5' means a biodiesel blend containing no more than five percent biodiesel by volume" and "(7) 'B20' means a biodiesel blend containing more than five and up to 20 percent biodiesel by volume." In this definition, both B10 and B20 would fall into the B20 category.
- Appendix A p. 5 defines "(8) 'CARB Diesel fuel' means ... which may be comingled with up to five (5) volume percent biodiesel..."Combining these definitions, B5 and CARB Diesel both have between 0 and 5 percent biodiesel by volume mixed with petroleum diesel meeting ARB standards.

The proposed regulation order and report would be improved by clearly defining the terms, especially clarifying whether B5 means a 5% blend of biodiesel, or a range from 0-5%

biodiesel, or some other range. Similarly, whether B20 means a 20% blend of biodiesel, or a range from 6-20% of biodiesel, or some other range.

a. Air Emissions Evaluation

The conclusion of "the use of biodiesel does not pose a significant adverse impact on public health or the environment from potential air quality impacts" is supported by the Multimedia Evaluation and discussion in the MMWG staff report. This conclusion is based on an analysis of criteria pollutant emissions (including ozone precursor emissions), toxic air emissions, and greenhouse gas emissions. Conclusions are drawn primarily from emission tests conducted at UC Riverside and at ARB test facilities. All types of emissions decrease except NOx, and even then only in heavy-duty vehicles that do not meet newer emissions standards.

Overall, the findings of the air emissions evaluation are well supported. However, the discussion of results could be improved in a few respects. These are noted below.

Section 1. (p. 7) is labeled "Criteria Pollutants." This section should begin with a discussion of what pollutants fall into this category, and which are evaluated here for biodiesel. Currently, this information is provided on p. 8, paragraph 2. However, this overview would be more helpful at the beginning of the section.

As written, Section 1 includes PM, nitrogen oxides ($NO_x$ = NO + $NO_2$), total hydrocarbons (THC), carbon monoxide (CO) and carbon dioxide ($CO_2$). However, THC and $CO_2$ are not criteria pollutants and do not belong in this section. $SO_2$ is a criteria pollutant that is not discussed here, but which may be reduced (per EPA[1]) by substituting biodiesel for petroleum diesel. $NO_x$ includes both the criteria pollutant $NO_2$ as well as NO, which is not a criteria pollutant. It would be helpful to know the size distribution of the PM emissions, for consistency with the criteria emissions categories of $PM_{2.5}$ and $PM_{10}$. Section 1 should report on all criteria pollutant emissions (or precursor emissions) in some way, and omit discussion of emissions that are not criteria pollutants.

Details are provided on the test vehicles used for emission tests (p. 7-8). It would be helpful to know how these were selected, and whether they are typical of the California vehicle fleet.

As noted, discussion of $CO_2$ emissions should be removed from Section 1, because $CO_2$ is not a criteria pollutant. It would fit more clearly in Section 4 (p. 10) on Greenhouse Gas Emissions. In addition, this section should be edited to clarify that the measured increase in $CO_2$ emissions does not suggest that biodiesel leads to a net increase in carbon emissions. It may be useful to note that a) end-of-pipe $CO_2$ emissions are only one component in determining a fuel's lifecycle carbon emissions (including uptake by feedstocks); b) an increase in $CO_2$ reflects more complete combustion, and is an expected result of decreased THC and CO emissions; c) the vast majority of THC and CO convert to $CO_2$ in the atmosphere, so the total $CO_2$ produced by the biodiesel combustion process is determined by direct $CO_2$ emissions, as well as THC and CO. As written, the discussion of $CO_2$ emissions could be misleading and a source of potential confusion.

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[1] http://nepis.epa.gov/Adobe/PDF/P1009IYE.pdf

Section 2 (p. 9) discusses "Toxic Air Contaminants." The discussion notes that the reduction in PM emissions would be expected to decrease toxic risk from diesel PM. This is a reasonable conclusion. In addition, some discussion should be included on the PM speciation from biodiesel versus petroleum diesel.

Section 3 (p. 9) discusses "Ozone Precursors." Because ozone is a criteria pollutant, this section would seem to be a better fit with Section 1 and/or follow directly afterward. For the benefit of readers unfamiliar with ozone chemistry, some brief comment should be added explaining that THC and $NO_x$ emissions determine ozone concentrations.

As written, Section 3 only discusses one ozone precursor: $NO_x$. At a minimum, it should include both THC and $NO_x$. Because THC is not a criteria pollutant, the discussion of THC from Section 1 would fit better here. Furthermore, the expected ozone impacts of THC reductions and $NO_x$ increases deserve some discussion. It may be beyond the scope of this report to comment on the expected ozone response to these competing precursor sensitivities. However, some qualitative comment would be helpful to frame the importance of the THC and $NO_x$ response to biodiesel.

Section 4 (p. 10) reports on Greenhouse Gas Emissions. This section would benefit from a number of changes. First, clarifying which greenhouse gas emissions have been evaluated - it appears only $CO_2$. As noted above, the $CO_2$ discussion from Section 1 should be moved to Section 4. The discussion notes an increase in fuel consumption due to the lower energy density of biodiesel. However, this analysis is of limited value, given that the fuel consumption impacts are given quantitatively, whereas the energy density changes are given only qualitatively. It would be helpful to include a more appropriate metric to compare the net $CO_2$ emissions from vehicle operation with CARB diesel versus biodiesel.

The difference between end-of-pipe emissions and life-cycle emissions should be more clearly defined in section 4. Overall, the paragraph (p. 10) discussing lifecycle emissions is unclear. It would benefit from more detail on what steps in the lifecycle were considered. In addition, it would be helpful to note that the 95% reduction in GHG emissions would arise from waste-oil feedstock use, whereas the 15% reduction in GHG emissions would arise from soybean production in the Midwestern U.S.

b. Water Evaluation

Overall, the MMWG conclusion that "there are minimal additional risks to beneficial uses of California waters posed by biodiesel" is well supported. However, the summary presentation of study findings could be clarified on a few points.

1. Water impacts (p. 11). There are two main impacts discussed in this section: aquatic toxicity, where there are results, and agricultural impacts, where there are no results from the current multimedia review. It would be helpful to break these two topics into separate paragraphs. More detail should be provided on the toxicity findings from the multimedia evaluation. Similarly, Section 3 would benefit from more detail clarifying issues related to biodegradability. Sections 2 and 4 seem to have an appropriate level of detail for the topic.

c. Public Health Evaluation

Overall, the public health evaluation seemed to be redundant with the air emissions evaluation, and lacking any specific discussion of health impacts. The public health conclusions are supported, in that Section 1 ("Combustion Emissions") summarizes the same changes in emissions presented in the Air Evaluation (p. 7-9). However, the report would be strengthened with a clearer discussion of health impacts.

At a minimum, the public health evaluation should address the conclusions on both air and water impacts in terms of health outcomes. For example, discussing the health outcomes of the PM reductions - both in terms of acute effects and toxicity - on exposed populations.

Section 2 (p. 13), entitled "Impact on Atmospheric Carbon Dioxide" does not have clear health linkages discussed. Overall, this explanation is unclear. Topically the material fits better in Section A4 where greenhouse emissions and lifecycle impacts are discussed.

d. Soil and Hazardous Waste Evaluation

Hazardous waste is outside the expertise of this reviewer. However, the discussion overall was clearly presented and seemed consistent with findings from the Multimedia Evaluation. It would seem appropriate, however, to define the term "vadose zone infiltration."

# Review Comments

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Submitted to Dr. Gerald W. Bowes (Gerald.Bowes@waterboards.ca.gov) on January 7, 2014

Document Reviewed: Staff Report: Multimedia Evaluation of **Biodiesel** by the Multimedia Working Group (MMWG), California Environmental Protection Agency (CEPA), November 2013

Topic/Area Reviewed: Surface and Ground Water Quality

The document reviewed here is a Staff Report prepared by MMWG of CEPA for the California Environmental Policy Council (CEPC), which will determine whether the proposed regulation on commercialization of new alternative diesel fuels poses significant adverse impact on public health or the environment. This is part of the process towards legally accepting and commercializing alternative diesel fuels in California.

The assignment to this reviewer is to help determine whether the scientific portions, particularly in the water quality section, of the MMWG Staff Report are based upon sound scientific knowledge, methods, and practice. The sections regarding water quality impacts were written based on the report from the State Water Resources Control Board (SWRCB) (Appendix D). The scientific knowledge is provided primarily in the Final Tiers I, II, and III Reports (Appendix G).

I have read the main Staff Report and its Appendices A, D, and G. I consider the tiered multimedia evaluation well designed, and the Tiers Reports (Appendix G) were well written. Tier I provided an excellent review of the key knowledge gaps through literature search, and presented a very good work plan. In Tier II, laboratory experiments were conducted in aquatic toxicity, transport in porous media, and aerobic biodegradation. CARB also conducted engine and chassis emission tests showing reductions for most air pollutants, demonstrating the major advantage of alternative diesel over petroleum based CARB diesel. All experimental results are highly valuable and the findings can be far-reaching, although some may be considered preliminary or screening in nature. However, constrains in time and budget prevented the experiments on materials compatibility and aqueous solubility; both of which are highly important to water quality impact evaluation. Tier III is a summary of all the work with qualitative risk assessment in some sections. A quantitative risk assessment and a full life cycle analysis may be difficult at this stage due to the lack of needed data. The Proposed Regulation Order (Appendix A) specifies the stages for commercializing new alternative diesel fuels; its implementation would further ensure that the impacts on the ecological environment and public health progressively change in a positive direction.

Provided below are my Overall comments, Comments on water quality impact assessment, and Document specific minor comments.

## Overall Comments

1. Within the scopes of my review and my expertise, I do not found major flaws in the scientific knowledge, methods, and practice presented in the main Staff Report and its Appendices A, D and G.

2. Large scale use of pure biodiesel (B100), as well as diesel blends with >20% B100, is considered premature at present, given the current knowledge gaps and uncertainties in several key areas.

3. In the main Staff Report, section I-C, I suggest summarizing the limitations of this multimedia evaluation immediately following the major MMWG conclusion on page 6. Some limitations are well described in the Tiers Reports, but are absent in the Staff Report. The limitations are different from the conditions in the Recommendations (page 17).

4. In contrast to the general positive impact on air quality due to reduced direct air emissions, the effects of switching to biodiesel on natural waters could be adverse and extensive. Most of the priority issues identified in Tier I Conclusions are related to water quality, including additives impacts, subsurface fate and transport, biodegradation, production and storage release. Unfortunately, these issues were not sufficiently investigated during Tier II experimental test stage. This leads to high uncertainty in making conclusions on the impact of using biodiesel on water quality. I consider this is the major weakness of this multimedia evaluation. Other regulations (such as the laws and regulations on underground storage tank and the hazardous waste, as mentioned in main Staff Report, section II-B) will help prevent water pollution; but they are not relevant to scientific assessment of biodiesel impact on surface and ground water quality.

5. Additives impacts remain a top concern. Additives, particularly those needed for biodiesel, are neither defined nor emphasized in the Proposed Regulation Order (Appendix A). Tier III assessment suggests no substantive change in additive impact in the case of B20, based on the expectation that most currently used additives would continue to be used (Tier III Report, page viii, 1st paragraph). Does this mean that no new additives will be used in new fuels covered by the proposed regulation? Given the needs of adding additives to biodiesel to control oxidation, corrosion, degradation, NOx formation and others as well as cetane value enhancement, there seems a disconnection between the findings of the Tiers conclusions and the proposed regulation.

6. The assessment of the supply and demand is not within the scope of this multimedia assessment. According to Hill et al. (2006), even dedicating all U.S. corn and soybean production to biofuels would meet only 12% of the gasoline and 6% of diesel demands in the country. Even with B20 or lower blends, whether all the available resources would meet the demand is unclear.

7. Biodiesel and renewable diesel were assessed separately. The advantages of each over the other were not quantitatively or qualitatively compared. According to UOP (2005), renewable diesel has a lower environmental impact than biodiesel and requires less capital investment to produce. This is in agreement with what I learned from reading the documents provided. However, I failed to find answers to the questions whether biodiesel is indeed needed and why biodiesel is being proposed as the first alternative diesel fuel in California, given the apparent advantages of the renewable diesel.

8. Tier-I Report, page I-20, is the only section about algae as a feedstock, and the discussion is highly positive. It is not clear what type of algae is relevant to biodiesel production. Given that California has long ocean shorelines, are there brackish water resources suitable for algae production? Are there any foreseen adverse impacts, besides the limitations associated with a narrow range of growing and harvesting conditions?

9. In the near future, the major feedstock could be soybeans grown in the US Midwest, where most adverse impact will occur. Although a complete evaluation of the impact outside California is beyond this work, a summary of available information on the impacts of the upstream processes (feedstock production, extraction, blending, etc.) on the environment and human health could have been included.

10. No occupational exposure and risk of any sort are included in this multimedia evaluation.

## Comments on Surface and Ground Water Quality Assessment

1. Please see my overall comments 4 and 5 above.
2. In the main Staff Report, the conclusion on water quality impact (page 16, part B) needs to be more specific. The current version is not consistent with summary section II-B, which indicates an increase in toxicity in part 1 and decreased biodegradation in part 3. It is not clear how the results summarized in section II-B lead to a conclusion of "minimal additional risks" in the Conclusions. In addition, the last several words should be changed from "public health or the environment" to "the quality of surface water and groundwater in California".
3. The incompatibility of biodiesel with underground storage tank (UST) as well as other infrastructure equipment calls for work plans needed in the cases of leaks into groundwater. Merely requiring affirmative statements of compatibility from biodiesel manufacturers and lead detection (main Staff Report, page 11, part 2) does not seem sufficient to ensure no adverse impact on groundwater.
4. Main Staff Report, page 11, part 4 indicates no significant areas of concerns when comparing biodiesel and CARB diesel with regard to waste discharges from manufacturing. This may not be correct because the manufacturing processes and chemical compositions are completely different between biodiesel and petroleum based CARB diesel, as detailed in the Tier I Report. The transportation and distribution may also differ between the two. Many chemicals are reviewed in Tier I Report, including acid and base as catalysts, various additives, etc. Not mentioned in Tier I Report and elsewhere is the possible incidental environmental release of glycerin, which is the major by-product of biodiesel production, and it is known to disrupt the microbial cleaning processes used in wastewater treatment (GAO, 2009) and has caused discharge problems (NYT, 2008).
5. NOx mitigating agent di-tert-butyl peroxide (DTBP) is the only additive included in Proposed Regulation Order (Appendix A, page 26). It is not clear whether this chemical has been used among the currently in-use additives added to CARB Diesel, or it is a new additive for new diesel fuels. Information on the basic physicochemical properties, environmental behavior, and the potential impacts of DTBP are not found in this multimedia evaluation.
6. Potentially positive impacts on water quality, if appropriate, could be mentioned somewhere in these documents. For example, plant feedstock production may help prevent soil erosion, remediate contaminated sites, build wetland and prairie, etc.

## Document Specific Minor Comments

<u>Main Report (19 pages)</u>

Table of Contents: I suggest changing II title from "Summary" to "Section Summaries" or "Summaries of Reports from Participating State Agencies", in order to avoid confusion with the summary of this Main Report.

Page 1, section A: There are three bulleted lines for air, water and wastes, respectively. It is not clear why public health is not included here. Risk assessment on the public health focuses on human, in contrast to those on environmental media. The same can be said for the bulleted lines in Page 2, section 2.

Page 5, section C: I suggest including one brief sentence on line 4 indicating that CARB diesel is conventional petroleum based ultra-low sulfur diesel, along with a brief time line. One or more references

should be helpful, directing readers to information on CARB diesel development and adoption, quantity of use in the state, its environmental and human health impacts, etc. This is especially helpful to stakeholders and interested parties who reside outside California and are unfamiliar with the phrase "CARB diesel".

Page 10, first paragraph, ending phrase: The words "and fuels" are confusing to me.

Appendix A – Proposed Regulation Order (36 page)

Page 4, (a), (1): If ADF means any non-CARB diesel fuel that does not consist solely of hydrocarbons, a question arises whether "renewable diesel" as defined in the 3-tier multimedia evaluation is an ADF. The renewable diesel, to my understanding, consists of predominantly hydrocarbons.

Page 5, (8): The definition for "CARB Diesel fuel" in this proposed regulation appears different from that for "CARB Diesel" used in the 3-tier multimedia evaluation. The former includes 5%v of FAME, while the latter is a pure ultra-low-sulfur diesel (ULSD) derived from petroleum.

Page 22, top lines: The definition of NBV is repeated.

Page 22, Table A.2. "Limit" column: The sign "≥" for both total aromatics and polycyclic aromatic hydrocarbons could be "≤".

Page 30, Table A.9, column "fuel Specifications", row 4 for PAHs w%: The 10% maximum seems incorrect for PAHs in a reference fuel. Please check.

Appendix D – SWRCB Submittal (5 pages)

Relevant to this review is Attachment #1 (2 pages).

Most part of Attachment #1 is the same as presented in the main Staff Report. Thus, same comments as explained above are applicable.

Appendix G – Final Tier III Report (31 pages)

Page vi, 9$^{th}$ line from bottom: There is an extra "that".

Page x, line 12: There is an extra "from".

Page x, line 21: Should the word "transport" be "transportation"?

Page 17, line 10 from bottom: Is "~10 cm" correct? Given in Tier II Report, page II-11, line 3 is "~20 cm".

Page 17, line 9 from bottom: "Bioextent" should be "Bioextent-30".

Page 18: At the end of section 3.2, it is helpful to add the environmental and remediation implications of the lens geometry from AF B100, as it is different from others.

Pages 21-29: Section 4 Conclusions has substantial overlap with the Executive Summary on pages iv – xi, therefore reads redundant.

Appendix G, Appendix – Tier I Report (94 pages)

I found this Report is of high quality. It is comprehensive and has sufficient details and depth. It is easy to read and has little redundancy.

A summary of the history and the current status of alternative diesels in California would be very helpful but is not found.

Page I-33, line 4 from bottom: The word "centane" should be "cetane", and a period is needed at the end.

Page I-39, first paragraph in 4.4.3, line 3: The word "that" might be "of".

Page I-39, line 3 from bottom: The word “course” could be “coarse”.

Appendix G, Appendix – Tier II Report (87 pages)

Page Pages II-29 to II-68 – Chemical Analysis: This work aimed at discovering the compounds responsible for the increased toxicity. It was a difficult task, and the methods using stir bar sorptive extraction (SBSE) and GCMS appear appropriate. From the multimedia evaluation perspective, the results from this work are considered by this reviewer as screening in nature. Future work is needed, and it would be more efficient to focus on the additives, based on Figure 1 (page II-9) and the toxicity test (page II-15 to II-28) which suggested strong impact of the additives on toxicity.

Page II-9, bottom two lines: I suggest rewriting the names of the antioxidant additives. They appear as 4 separate ones, but were just two. For acetic acid butyl ester, please delete the comma after acid, or simply change to butyl acetate. For 1,4-Benzenediol, 2-(1,1-dimethylethyl), the name tert-butylhydroquinone (TBHQ) is less confusing.

Page II-10, first two paragraphs: The results from GCMS for the additives are highly variable, resulting in high uncertainty or failure in identifying the source of increased toxicity. I doubt the SBSE extraction efficiencies for these two compounds, especially acetic acid butyl ester which has a log Kow of only 1.8 (EPI, 2013).

Page II-77, line 5: Figure B7 should be Figure II-C-7.

## Literature Cited

EPI, 2013. Estimation Program Interface (EPI) Suite. Version 4.11. U.S. Environmental Protection Agency. http://www.epa.gov/oppt/exposure/pubs/episuite.htm

GAO, 2009. Energy-Water Nexus: Many Uncertainties Remain about National and Regional Effects of Increased Biofuel Production on Water Resources. U.S. Government Accountability Office. GAO-10-116, Nov 30, 2009. http://www.gao.gov/new.items/d10116.pdf

Hill, J. et al. 2006. Environmental, economic, and energetic costs and benefits of biodiesel and ethanol biofuels. PNAS 103 (30), 11206–11210. (http://www.pnas.org/content/103/30/11206.abstract)

NRC, 2008. Water Implications of Biofuels Production in the United States. Committee on Water Implications of Biofuels Production in the United States, National Research Council. http://www.nap.edu/catalog/12039.html

NYT, 2008. Pollution Is Called a Byproduct of a ‘Clean’ Fuel. The New York Times. March 11, 2008. http://www.nytimes.com/2008/03/11/us/11biofuel.html?pagewanted=all&_r=0

UOP, 2005. Opportunities for Biorenewables in Oil Refineries. Final Technical Report. DE-FG36-05GO15085. Des Plaines, Illinois. http://www.osti.gov/scitech/biblio/861458

# Peer Review of Staff Report: Multimedia Evaluation of Biodiesel (Biodiesel Staff Report)

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1. Preface

The purpose of this document is to review The Staff Report: Multimedia Evaluation of Biodiesel to determine whether the scientific portions of the MMWG staff report is based upon "sound scientific knowledge, methods, and practices." The Staff Report: Multimedia Evaluation of Biodiesel is based on three previous documents California Biodiesel Multimedia Evaluation Final Tier I, II and III Reports that contain data and analyses from government reports, literature documents, and from reports of studies commissioned by the CARB.

2. General comments

Emissions from diesel fueled engines are a complex mixture consisting of both gaseous and particulate components. The gaseous phase contains ozone, sulfur oxides and the criteria pollutants, carbon monoxide, particulate matter, nitrogen dioxide and ozone. Many organic compounds are also present, such as acetaldehyde, acrolein, benzene, 1,3-butadiene, ethylbenzene, formaldehyde, polycyclic aromatic hydrocarbons and nitro-polycyclic aromatic hydrocarbons. Particulate matter, benzene, 1,3-butadiene, formaldehyde and benzo[a]pyrene are carcinogenic in experimental animals and are classified as human carcinogens and acetaldehyde, ethylbenzene and a number of other polycyclic aromatic hydrocarbons and nitro-polycyclic aromatic hydrocarbons have been classified as probably or possibly carcinogenic to humans by the International Agency for Research on Cancer (IARC, 2013). The particulate phase also contains trace metals such as lead, manganese arsenic and chromium and metals from the catalyst after treatment system, vanadium, copper and iron. Arsenic and arsenic inorganic compounds and chromium VI are classified as human carcinogens by IARC while lead and inorganic lead compounds as classified as probably or possibly carcinogenic to human, respectively by IARC. Moreover, diesel engine exhaust, diesel exhaust particles, diesel-exhaust condensates, and organic solvent extracts of diesel-engine exhaust were genotoxic. Increases in bulky DNA adducts were detected the lung tissues of rodents exposed to whole diesel exhaust and in workers exposed to diesel exhaust. In addition to lung cancer, diesel exhaust exposure in humans has been linked to lung inflammation, cardiovascular disease and cardiopulmonary disease (Madden et al., 2011).

The biological and toxicological information available for biodiesel emissions are very limited compared to the rich compendium available for diesel emissions and many of the biological and toxicological measures available for conventional diesel are not available for biodiesel. Therefore, surrogate measures need to be employed to make meaningful comparisons between the emission types. These measures include chemical and physical analyses of the biodiesel emissions and to a small extent some toxicological data on the biodiesel emissions.

The Staff Report bases the comparisons (chemical, physical and toxicological) of the biodiesel fuel emissions to those properties of CARB diesel emissions. The crux of the document's conclusion is that the selected parameters (chemical, physical and toxicological) examined were lower (with some exceptions) in emissions from engines fueled with biodiesel compared to CARB diesel with the exception of oxides of nitrogen and acrolein that had higher levels. Thus, the public health risk would not be greater than that already established for CARB diesel. The underlying premise is that lower levels of

specific emissions will equate to lower human health risk or adverse health effects. This premise is generally consistent with the quantitative results from many studies in animals and in human populations of each of the specific constituent within diesel exhaust emissions as well as studies in animals and human populations exposed to whole diesel exhaust emissions. Much of the data on emissions from the combustion of biodiesel is from quantitative chemical analysis and that is used to equate to lower toxic or adverse effects in exposed humans. The agents selected for comparison are from the group of EPA criteria pollutants and from selected VOCs commonly found in diesel exhaust and in ambient air. Each exhibits its own toxicity profile. There are few studies in whole animals exposed to complete exhaust emissions and a number of toxicological evaluations of the particulate matter and of organic extracts of particulates. There are no studies that I know of in humans exposed to complete exhaust emissions from biodiesel. Genotoxicity evaluations for the most part are based on organic extracts of particulates using bacterial tests for mutagenic activity; some evaluations were conducted with the vapor phase fraction. Some genotoxicity data in mammalian cells in culture are also available.

**The MMWG concludes that the use of biodiesel fuel in California, as specified in the biodiesel multimedia evaluation, does not pose a significant adverse impact on public health or the environment relative to diesel fuel meeting Air Resources Board (ARB) motor vehicle diesel fuel specifications (CARB diesel).**

Based on the results of the biodiesel multimedia evaluation and the information provided in the University of California (UC) final report, "California Biodiesel Multimedia Evaluation Final Tier III Report" (Ginn, T.R. *et al*., May 2013), the MMWG makes the overall conclusion that biodiesel specifically evaluated within the scope of the biodiesel multimedia evaluation will not cause a significant adverse impact on public health or the environment relative to CARB diesel. The MMWG based their conclusion on each individual agency's assessment of the biodiesel multimedia evaluation. (Biodiesel Staff Report, Chapter 3)

**Public Health Evaluation. Office of Environmental Health Hazard Assessment (OEHHA) staff concludes that the substitution of biodiesel for CARB diesel reduces the rate of addition of carbon dioxide to the atmosphere and reduces the amount of particulate matter (PM), benzene, ethyl benzene, and polycyclic aromatic hydrocarbons (PAHs) released into the atmosphere, but may increase emissions of oxides of nitrogen ($NO_x$) and acrolein for certain blends.** OEHHA staff evaluated potential human health impacts from the use of biodiesel and made conclusions based on their analysis of potential impacts on atmospheric carbon dioxide and combustion emissions results. (Biodiesel Staff Report, Chapter 2 and 3).

3. Peer review of the scientific issues

The basic premise of the conclusion: "that biodiesel specifically evaluated within the scope of the biodiesel multimedia evaluation will not cause a significant adverse impact on public health or the environment relative to CARB diesel" is based in large part on the measurements of the levels of key toxic components of emissions from biodiesel and CARB diesel and to a lesser degree on some toxicological measurements of these emissions.

Some of the issues of concern include: Are the metrics used to compare the levels and toxicity of individual or groups of pollutants of biodiesel to CARB diesel appropriate, relevant, specific, sensitive and accurate?; Are the CARB biodiesel results consistent with those reported by others in the literature?; Are all of most toxic components known to be present in diesel exhaust being measured in the CARB biodiesel studies?; Are the proportions of PAH and/or N-PAH the same?; Are the selected indicators of adverse human health accurate and comprehensive?; Are there additional markers that could be included?

Carbon dioxide, a major greenhouse gas is generated through the combustion of both diesel and biodiesel fuels. However, the plant feedstock used in the production of biomass fixes carbon dioxide from the atmosphere through photosynthesis thus recycling the carbon pool. This process does not occur using petroleum derived diesel fuel. There seems to be little difference in the levels of carbon dioxide exhaust emissions between biodiesel and diesel. However, the carbon dioxide released from biodiesel combustion is offset by the carbon dioxide incorporated in the plant feed stock. A National Renewable Energy Laboratory/U.S. Department of Energy life cycle study showed that the production and use of biodiesel fuel using urban buses, resulted in a 78.5% reduction in carbon dioxide emissions compared to carbon dioxide emissions from conventional diesel fuel emissions (NREL, 1998). This is within the range cited in the Report.

The Public Health Evaluation conclusion that the use of biodiesel compared to CARB diesel reduces the amount of particulate matter and polycyclic aromatic hydrocarbons released into the atmosphere, but may increase emissions of oxides of nitrogen ($NO_x$) is supported by studies described in the Staff Report based on the California Biodiesel Multimedia Evaluation Final Tier III Report, a number of government-conducted studies as well as studies reported in the open literature. The average emission impacts in terms of particulate matter levels of biodiesel compared to conventional diesel for heavy-duty highway engines were reduced by increasing levels of biodiesel, while oxides of nitrogen levels increased slightly (EPA, 2002). The literature on the levels of combustion emissions from biodiesel fueled engines compared to those from diesel fueled engines in the range of B5 to B100 was recently reviewed (Bünger et al., 2012). They reported that in most studies biodiesel emissions had lower levels of particulate matter compared to conventional diesel emissions and that the levels of many polycyclic aromatic hydrocarbons levels and some nitro polycyclic aromatic hydrocarbons were also lower in biodiesel emissions. They also reported that the levels of nitrogen oxides were increased in biodiesel emissions. Thus, the Public Health Evaluation conclusion that combustion emissions from biodiesel fueled engines compared to those from CARB diesel fueled engines leads to lower emissions of particulate matter and polycyclic aromatic hydrocarbons is consistent with the published literature as is the increase in nitrogen oxides. However, as pointed out in the report although there is a reduction in particulate matter emissions in biodiesel blends, “there is some uncertainty that that a drop in total PM mass may not necessarily equate with an overall reduction in the number of UFP emitted from combustion. This is an issue of national interest and more testing would be required to fully address it”. In fact, it has been recently reported that higher numbers of ultrafine particles (UFPs, < 100 nm) were emitted from a diesel engine combusting pure waste cooking oil biodiesel compared to ULSD supporting this concern (Betha and Balasubramanian, 2013).

The Public Health Evaluation conclusion that benzene levels are lower in emissions from biodiesel fueled vehicles compared to those vehicles using CARB diesel from the Durbin et al (2011) report is consistent with those measurements found in Magara-Gomez et al. (2012) and the NREL (1998) report.

Ethylbenzene has the potential to cause hepatic, CNS and renal damage. The Public Health Evaluation conclusion that ethylbenzene levels are lower in emissions from biodiesel fueled vehicles compared to those vehicles using CARB diesel from Durbin et al (2011) is supported by the results of a number of studies study including that of Magara-Gomez et al. (2012) who found decreases in ethylbenzene emission rates from biodiesel blends compared to ULSD.

There are several different conclusions regarding the levels of acrolein in the Staff Report and in the literature. In part C. Office of Environmental Health Hazard Assessment Evaluation, P 13 it states "In tests using a Caterpillar C-15 engine, the amount of acrolein was increased in emissions from combustion of B100 and B50 from both plant and animal sources when compared to the amount of acrolein in emissions from CARB diesel combustion." Under C. Conclusions on Public Health Impact, P 16, L5 it states "Limited emissions testing resulted in a non-statistical increase in acrolein for a higher B50 biodiesel blend level (i.e., confidence interval less than 95%). Furthermore, the statistical analysis for acrolein emissions results was compared to only one data point for the control sample". The first conclusion is likely based on the data from Cahill and Okamoto (2012) using a 2000 Caterpillar C-15 engine and a UDDS drive cycle. Cahill and Okamoto (2012) used N values of 2-3. The second conclusion is likely based on Figure 10-18 (P 174) in the Durbin et al (2011) report who 2000 Caterpillar C-15 engine and a UDDS drive and used N values of 1 and 2. The Bünger et al. (2012) analysis of papers comparing acrolein levels in B5, B10, B20 and 100% biodiesel emissions concludes shows general increased levels of acrolein in biodiesel emissions but the results are highly variable. My recommendation is to make one clear and consistent concluding statement regarding the levels of acrolein compared to CARB diesel a statement that encompasses the all of the available data.

The role of oxy-PAHs needs to be more fully described in the Staff Report. Durbin et al. (2011) states "The emission trends for Oxy-PAH emissions showed different trends for different compounds, with some compounds showing generally higher emissions in soy and animal-based biodiesels compared to CARB diesel, whereas others decreased in animal biodiesel and renewable diesel. For all toxic species, emission levels were significantly reduced in the DPF-equipped vehicle, and there were few fuel related trends." Oxy PAH levels were also increased in studies using methyl ester blends of vegetative and animal based oils compared to EN590 using a diesel passenger car (Karavalakis et al., 2009, 2011). One issue that has not been fully discussed is the apparent increase in the levels of 1,2-naphthoquinone as described in Durbin et al. (2011). This might be due to increased oxygen content of the ester-based biofuels. Inspection of the mean and standard deviation results of CARB animal and A100 levels in Figure 10-47 (Durbin et al., 2011) indicate that CARB animal and A100 levels of 1,2-naphthoquinone appears to be statistically significantly different. 1,2-Naphthoquinione is cytotoxic (Flowers-Geary et al., 1993) and genotoxic (Saeed et al., 2008) and 1,2-naphthoquinone and its analog 1,4-naphthoquinone each induce reactive oxygen species (ROS) (Thornalley et al., 1984 ). 1,4-Naphthoquinone, a component of particulates collected from road tunnel emissions is also cytotoxic and induced ROS and DNA damage in human lung epithelial cells, as did the road tunnel particles themselves (Shang et al., 2013). It is well

known that several ROS forms induce cytotoxicity and genotoxicity and the formation of ROS can lead to adverse health outcomes.

The toxicities of exhausts in rats from a biodiesel fueled engine were reported in by studies described in the California Biodiesel Multimedia Evaluation Final Tier III Report. "CARB diesel, biodiesel, and renewable diesel all induced inflammatory markers, such as COX-2 and IL-8 in human macrophages and the mucin related MUC5AC markers in Clara type cells, with the inflammatory markers higher in the 2000 Caterpillar C-15 engine vehicle than the 2007 MBE4000 engine vehicle. For the comet assay, at the limited dose levels tested, there was little increase of chromosomal damage (gross DNA damage) from the various fuels tested" (Durbin et al., 2011). The mutagenic activities of combustion emissions (as organic particulate extracts and some vapor phase fractions) from biodiesel fueled engines compared to those from conventional diesel fueled engines were reported in the California Biodiesel Multimedia Evaluation Final Tier III Report based in part on Durbin et al. (2011) and were also reviewed by Bünger et al. (2012). The available data indicate, with some exceptions, a general lowering of mutagenic activity based mainly on the data from bacterial assays which is consistent the lower levels of polycyclic aromatic hydrocarbons and nitro polycyclic aromatic hydrocarbons in the biodiesel emission extracts. It noted that in a recent study, organic extracts of particles from emissions of engines using rapeseed methyl ester and EN 590 fuels both produced DNA adduct levels to comparable extents in an acellular assay using calf thymus DNA both in the presence and absence of an exogenous metabolic activation system, suggesting equal genotoxic activities of the two extracts (Topinka et al., 2012). No increases in micronuclei in bone marrow or sister chromatid exchanges in peripheral blood lymphocytes were found in rats exposed by subchronic inhalation to emissions from a diesel engine burning soybean based biodiesel fuel (Finch, 2002).

There is an increasing body of new literature on inflammation, lung tissue damage, oxidative stress and oxidative damage where biodiesel emissions (particulate matter or complete emissions) have been shown to be more toxic than those from conventional diesel emissions. The results of many of these studies are summarized or quoted here. "Concentrations of inflammatory mediators (Interleukin-6, IL-6; Interferon-gamma-induced Protein 10, IP-10; Granulocyte stimulating factor, G-CSF) in the medium of B20-treated cells and in bronchioalveolar lavage fluid of mice exposed to B20 were ∼20−30% higher than control or B0 PM, suggesting that addition of biodiesel to diesel fuels will reduce PM emissions but not necessarily adverse health outcomes (Fukagawa et al., 2013)". Human bronchial BEAS-2B cells were exposed to particulate matter collected from diesel passenger vehicles with and without a diesel particulate filter using a rapeseed biodiesel (B50) blend or to diesel fuels. The particulate matter from the B50 blend induced increased cytotoxicity and IL-6 release in the cells compared to the diesel fuel per distance driven. These differences were observed irrespective of the use of a diesel particulate filter (Gerlofs-Nijland et al. 2013). Rat alveolar macrophages exposed to exhaust particles from heavy duty diesel engine combusting B20 biofuel resulted in an increased production of $PGE_2$ relative to particles from diesel fuel combustion (Bhavaraju et al., 2013). Mice were exposed by pharyngeal aspiration to diesel particulate matter collected from a diesel engine using biodiesel (NEXSOL BD-100) and ULSD. Biomarkers of tissue damage and inflammation were significantly elevated in the lungs of mice exposed to the biodiesel particulates. Inflammatory cytokines/chemokines/growth factors were up-regulated to

a greater extent and oxidatively modified proteins and 4-hydroxynonenal levels were increased by biodiesel particulates compared to diesel particulates (Yanamala et al., 2013). Mice were exposed by inhalation to combustion emissions of soy biodiesel (B100) and diesel. "B100 combustion emissions produced a significant accumulation of oxidatively modified proteins (carbonyls), an increase in 4-hydroxynonenal (4-HNE), a reduction of protein thiols, a depletion of antioxidant gluthatione (GSH), a dose-related rise in the levels of biomarkers of tissue damage (lactate dehydrogenase, LDH) in lungs, and inflammation (myeloperoxidase, MPO) in both lungs and liver. Significant differences in the levels of inflammatory cytokines interleukin (IL)-6, IL-10, IL-12p70, monocyte chemoattractant protein (MCP)-1, interferon (IFN) γ, and tumor necrosis factor (TNF)-α were detected in lungs and liver upon B100 and D100 complete emission exposures. Overall, the tissue damage, oxidative stress, inflammation, and cytokine response were more pronounced in mice exposed to biodiesel complete emissions" (Shvedova et al., 2013).

Overall, the Public Health Evaluation is generally supported by the data in the Staff Report with exceptions noted above.

3. **MMWG's Recommendations to the California Environmental Policy Council**

**The MMWG recommends that the California Environmental Policy Council (CEPC) find that the use of biodiesel and renewable diesel, as specified in the respective multimedia evaluations, does not pose a significant adverse impact on public health or the environment.** Based on the MMWG's conclusions in Chapter 3 of the Biodiesel Staff Report and the Renewable Diesel Staff Report, the MMWG proposes recommendations to the CEPC. (Biodiesel Staff Report and Renewable Diesel Staff Report, Chapter 4).

The MMWG conclusions "that the use of biodiesel fuel in California, as specified in the biodiesel multimedia evaluation, does not pose a significant adverse impact on public health or the environment relative to diesel fuel meeting Air Resources Board (ARB) motor vehicle diesel fuel specifications (CARB diesel)" is supported by many of the analytical chemical and biological measures of toxic components of emissions from CARB vs. biodiesel fueled engines as found in the Staff Report suggesting a lessened impact on public health, however, the recent toxicological data give me some concern that not enough studies have been conducted to unequivocally conclude that substitution of biodiesel for CARB diesel will not adversely affect public health and that the ARB should proceed with caution.

4. The Big Picture

In the holistic view, based on multiple lines of evidence from studies found in government reports and in the peer-reviewed literature is seem clear that the levels of a number of key constituent of emissions from the combustion of biodiesel fueled engines are lower than those measured in the emissions from conventional diesel fueled engines. These are carbon monoxide, total hydrocarbons, particulate matter and polycyclic aromatic hydrocarbons. Moreover the overall carbon dioxide levels released to the atmosphere are reduced due to recycling of the carbon dioxide by the vegetative feedstock. The levels of a number of VOCs (e.g. benzene, ethylbenzene) are also decreased. However increases in the emissions of nitrogen oxides and acrolein have been reported and there is an increase in the proportion of ultrafines in the particulate matter emissions. These results are in concert with a recent Health Canada and Environment Canada modeling study where the authors concluded: "Although modeling

and data limitations exist, the results of this study suggested that the use of biodiesel fuel blends compared to ULSD was expected to result in very minimal changes in air quality and health benefits/costs across Canada, and these were likely to diminish over time" (Rouleau et al., 2013). However, the levels of many of the constituents cited above have not been determined for the many different combinations of engine types (heavy and light duty) technology (old, new, catalyst type, test cycle and load), feed stock sources (plant and animal based) and mixture blends, therefore, some caution needs to be exercised in accepting these conclusions without further data on the most prevalent combinations. Decisions on the impact of the toxicity of emissions from the multitude of combinations should be revisited after more data is available and the recent toxicological data given weight in the current decision process.

In my opinion, the conclusions and scientific portions of the multimedia evaluation were based upon sound scientific knowledge, methods, and practices. The Report should be updated to incorporate the new chemical, physical and toxicological data now available.

**References:**

Betha R, Balasubramanian R. Emissions of particulate-bound elements from biodiesel and ultra low sulfur diesel: size distribution and risk assessment, Chemosphere. 2013 Jan;90(3):1005-15. doi: 10.1016/j.chemosphere.2012.07.052.

Bhavaraju L, Shannahan J, William A, McCormick R, McGee J, Kodavanti U, Madden M. Diesel and biodiesel exhaust particle effects on rat alveolar macrophages with in vitro exposure, Chemosphere. 2013 Nov 20. pii: S0045-6535(13)01540-3. doi: 10.1016/j.chemosphere.2013.10.080. [Epub ahead of print]

Bünger J, Krahl J, Schröder O, Schmidt L, Westphal GA. Potential hazards associated with combustion of bio-derived versus petroleum-derived diesel fuel. Crit Rev Toxicol. 2012 Oct;42(9):732-50.

Cahill TM, Okamoto RA. Emissions of acrolein and other aldehydes from biodiesel-fueled heavy-duty vehicles. Environ Sci Technol. 2012 Aug 7;46(15):8382-8.

Durbin TD, Miller JW, Johnson K, Hajbabaei M. CARB Assessment of the Emissions from the Use of Biodiesel as a Motor Vehicle Fuel in California "Biodiesel Characterization and NOx Mitigation Study" Final Report, 2011.

EPA, A Comprehensive Analysis of Biodiesel Impacts on Exhaust Emissions, US EPA, EPA420-P-02-001, October, 2002.

Finch GL, Hobbs CH, Blair LF, Barr EB, Hahn FF, Jaramillo RJ, Kubatko JE, March TH, White RK, Krone JR, Ménache MG, Nikula KJ, Mauderly JL, Van Gerpen J, Merceica MD, Zielinska B, Stankowski L, Burling K, Howell S. Effects of subchronic inhalation exposure of rats to emissions from a diesel engine burning soybean oil derived biodiesel fuel. Inhal Toxicol. 14(10): 1017-1048, 2002.

Flowers-Geary L, Harvey RG, Penning TM. Cytotoxicity of polycyclic aromatic hydrocarbon o-quinones in rat and human hepatoma cells, Chem Res Toxicol. 1993 May-Jun;6(3):252-60.

Fukagawa NK, Li M, Poynter ME, Palmer BC, Parker E, Kasumba J, Holmén BA. Soy Biodiesel and Petrodiesel Emissions Differ in Size, Chemical Composition and Stimulation of Inflammatory Responses in Cells and Animals, Environ Sci Technol. 2013 Oct 10. [Epub ahead of print]

Gerlofs-Nijland ME, Totlandsdal AI, Tzamkiozis T, Leseman DL, Samaras Z, Låg M, Schwarze P, Ntziachristos L, Cassee FR. Cell toxicity and oxidative potential of engine exhaust particles: impact of using particulate filter or biodiesel fuel blend. Environ Sci Technol. 2013 Jun 4;47(11):5931-8. doi: 10.1021/es305330y. Epub 2013 May 20.

IARC, International Agency for Research on Cancer, 2013, http://monographs.iarc.fr/ENG/Classification/index.php

Karavalakis G, Boutsika V, Stournas S, Bakeas E. Biodiesel emissions profile in modern diesel vehicles. Part 2: Effect of biodiesel origin on carbonyl, PAH, nitro-PAH and oxy-PAH emissions. Sci Total Environ. 2011 Jan 15;409(4):738-47. doi: 10.1016/j.scitotenv.2010.11.010.

Karavalakis G, Fontaras G, Ampatzoglou D, Kousoulidou M, Stournas S, Samaras Z, Bakeas E. Effects of low concentration biodiesel blends application on modern passenger cars. Part 3: impact on PAH, nitro-

PAH, and oxy-PAH emissions. Environ Pollut. 2010 May;158(5):1584-94. doi: 10.1016/j.envpol.2009.12.017.

Magara-Gomez KT, Olson MR, Okuda T, Walz K, Schauer, JJ. Sensitivity of hazardous air pollutant emissions to the combustion of blends of petroleum diesel and biodiesel fuel. Atmospheric Environment, 50, April 2012, P 307–313.

Madden MC, Bhavaraju L, Kodavanti UP. Toxicology of Biodiesel Combustion Products. In Biodiesel-Quality, Emissions and By-Products, G Montero and M Stoytcheva Eds), InTech, Chapter 13, p. 195-210, 2011.

NREL, National Renewable Energy Research Laboratory, Life Cycle Inventory of Biodiesel and Petroleum Diesel for Use in an Urban Bus. Final Report, 1998 NREL/SR-580-24089 UC Category 1503

Rouleau M, Egyed M, Taylor B, Chen J, Samaali M, Davignon D, Morneau D. Human health impacts of biodiesel use in on-road heavy duty diesel vehicles in Canada (2013) Environ. Sci. Technol. doi: 10.1021/es4023859).

Saeed M, Higginbotham S, Gaikwad N, Chakravarti D, Rogan E, Cavalieri E. Depurinating naphthalene-DNA adducts in mouse skin related to cancer initiation, Free Radic Biol Med. 2009 Oct 1;47(7):1075-81.

Shang Y, Fan L, Feng J, Lv S, Wu M, Li B, Zang YS. Genotoxic and inflammatory effects of organic extracts from traffic-related particulate matter in human lung epithelial A549 cells: the role of quinones, Toxicol In Vitro. 2013 Mar;27(2):922-31. doi: 10.1016/j.tiv.2013.01.008.

Shvedova AA, Yanamala N, Murray AR, Kisin ER, Khaliullin T, Hatfield MK, Tkach AV, Krantz QT, Nash D, King C, Ian Gilmour M, Gavett SH. Oxidative stress, inflammatory biomarkers, and toxicity in mouse lung and liver after inhalation exposure to 100% biodiesel or petroleum diesel emissions. J Toxicol Environ Health A. 2013;76(15):907-21. doi: 10.1080/15287394.2013.825217.

Thornalley PJ, Doherty MD, Smith MT, Bannister JV, Cohen GM. The formation of active oxygen species following activation of 1-naphthol, 1,2- and 1,4-naphthoquinone by rat liver microsomes, Chem Biol Interact. 1984 Feb;48(2):195-206.

Topinka J, Milcova A, Schmuczerova J, Mazac M, Pechout M, Vojtisek-Lom M. Genotoxic potential of organic extracts from particle emissions of diesel and rapeseed oil powered engines. Toxicol Lett. 2012 Jul 7;212(1):11-7. doi: 10.1016/j.toxlet.2012.04.017.

Yanamala N, Hatfield MK, Farcas MT, Schwegler-Berry D, Hummer JA, Shurin MR, Birch ME, Gutkin DW, Kisin E, Kagan VE, Bugarski AD, Shvedova AA. Biodiesel versus diesel exposure: enhanced pulmonary inflammation, oxidative stress, and differential morphological changes in the mouse lung, Toxicol Appl Pharmacol. 2013 Oct 15;272(2):373-83. doi: 10.1016/j.taap.2013.07.006.

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**External scientific peer review of the Multimedia Working Group's assessment of the biodiesel multimedia evaluation**

As reviewers we are specifically asked to evaluate the following statements:

A. **Air emissions evaluation. Air resources board staff concludes that the use of biodiesel does not pose a significant adverse impact on public health or the environment from potential air quality impacts.**
I find that this conclusion of the report is based on sound scientific knowledge, methods, and practices. While there were clearly gaps in this knowledge, these have been largely filled by the tier II investigations. These investigations showed that there are some tradeoffs associated with the air emissions from biodiesel. In particular, biodiesel appears to generate more NOx than regular diesel, however, these higher NOx emissions are offset by lower emissions of VOCs, such that the overall generation of ozone is about the same as for regular diesel. It is clear that biodiesel has significantly lower emissions of particulate matter, many hazardous air pollutants, and most VOCs. However, it should be noted that because there are so many possible sources of biodiesel, the emissions factors for all of these pollutants are likely to be highly variable. Thus although the reductions in emissions are statistically significant for the specific biodiesels investigated, they may not be significant for all biodiesels that may be sold in California. Nevertheless, it appears that there are no worrisome increases in emissions associated with biodiesel. The increase in production of NOx is small and not worrisome.

B. **Water evaluation. State water resources control board staff concludes that there are minimal additional risks to the beneficial uses of California waters posed by biodiesel than posed by CARB diesel alone.**
I find that this conclusion of the report is based on sound scientific knowledge, methods, and practices. Biodiesel has been demonstrated to be more biodegradable than regular diesel. In addition, the higher biodegradability of biodiesel augments the biodegradability of the regular diesel with which it is blended. The one area of concern for biodiesel is its tendency to act as a cosolvent and increase the solubility of other contaminants. This may be of concern in groundwater. However, it is probably of much

less concern than the cosolvent properties of ethanol with which gasoline is often blended.

C. **Public health evaluation. Office of environmental health hazard assessment staff concludes that the substitution of biodiesel for CARB diesel reduces the rate of emission of carbon dioxide to the atmosphere and reduces the amount of particulate matter, benzene, ethylbenzene, and polycyclic aromatic hydrocarbons released into the atmosphere, but may increase emissions of oxides of nitrogen and acrolein for certain blends.**
   I find that this conclusion of the report is based on sound scientific knowledge, methods, and practices. As noted above the increase NOx emissions are offset by the decrease VOC emissions and may lead to less overall ozone production. However, it should be noted that these tests were performed on a limited number of biodiesel blends. While it certainly appears that the overall trend for biodiesel is to produce less of many of these hazardous pollutants, additional types of biodiesel should probably be investigated.

D. **Soil and hazardous waste evaluation. Department of toxic substances control staff concludes that biodiesel aerobically degrades more readily than CARB diesel, has potentially higher aquatic toxicity for a small subset of tested species and generally has no significant difference in vadose zone infiltration rate.**
   I find that these conclusions of the report are based on sound scientific knowledge, methods, and practices. The higher aquatic toxicity of biodiesel may be related to its cosolvent properties, which increase the solubility and bioavailability of other toxic material within the diesel. However this property of the biodiesel also causes it to disperse more readily and because it is more biodegradable this means that spills of biodiesel may very well be less of a concern that spills of regular diesel.

In addition, as a reviewer I have been asked to evaluate the following statement:

**The MMWG recommends that the California environmental policy council find that the use of biodiesel and renewable diesel, as specified in the respective multimedia evaluations, does not pose a significant adverse impact on public health or the environment.**

I find that this conclusion is based on sound scientific knowledge, methods, and practices. The concerns related to biodiesel are more significant than those related to renewable diesel, because the chemical composition of biodiesel is demonstrably different from that of regular diesel. However the tier II assessment has filled and many of our knowledge gaps, and demonstrated that the air and water impacts of biodiesel are not likely to be any worse than those of regular diesel. The few negative impacts of biodiesel (increased NOx emissions) are more than offset by the positive qualities of biodiesel, which include

decreases in emissions of most hazardous air pollutants and decreased carbon dioxide emissions. In addition, biodiesel has been shown to be more readily biodegradable than regular diesel. Thus biodiesel may be regarded as a safer alternative to regular diesel. As opposed to renewable diesel, the use of biodiesel may require development of new types of additives, changes in materials used to store and transport biodiesel, and the building of new facilities to produce biodiesel. In those respects, the environmental impacts of biodiesel have more uncertainty associated with them.

As a reviewer, and also been asked whether there are additional scientific issues that are not described in the report. Several of the issues requiring further study are already mentioned in the report, including the effects of ultra fine particles, the possible cosolvent effects of biodiesel (which could mobilize contaminants that are, for example, sorbed to soils outside of leaking underground storage tanks), and the environmental impacts of additives. It is possible that new additives may have to be developed for use with biodiesel and these should be carefully vetted before being approved. The cosolvent effects could be positive in some cases, such as leading to greater dispersal of oil spills, for example. Perhaps it should be investigated whether biodiesel can be used as a dispersant for oil spills.

Other issues that should be investigated include synergistic effects of additives on the other properties of biodiesel. If the amount of additives to be used is a significant percentage of the amount of biodiesel, they will require their own life cycle and environmental impact assessment. Another issue raised by the report is the compatibility of various pipeline and tank materials with biodiesel. This is mentioned for example on page 25.

In comments below, I also note that sensitive populations should be taken into account when evaluating health effects of biodiesel. In particular people, especially children, who suffer from asthma or allergies may be at higher risk of health effects from biodiesel due to allergic response. Because biodiesel is derived from oils which themselves can cause allergic reactions, such as palm oil and soy oil, biodiesel has the potential to be an allergen. This is less of a concern with renewable diesel, because the chemical structure of renewable diesel has been shown to be so similar to that of regular diesel. However, it should be noted that regular diesel itself has been shown to cause allergic response.

**Additional comments.**

**If 6. Tier III appendices**

**California biodiesel multimedia evaluation tier one report**

Page I-3. This section focuses on some of the vehicle operability issues associated with the use of biodiesel blends. The impacts to a vehicle's fuel system can result in reduced reliability and increased maintenance costs. The next generation of environmental impact assessment for biodiesel should consider whether retrofitting of existing equipment or production of new vehicle equipment is going to require changes to engine design that could have environmental impacts. For example if the use of biodiesel would require, say, catalytic converters or other equipment that might contain platinum or other heavy metals, the production of those heavy metals has significant environmental impacts and should be considered in the assessment of biodiesel. (I am not suggesting that biodiesel will require catalytic converters, I'm only using them as an example of a technology that was designed to protect the environment but used a chemical—platinum--that has significant environmental impacts.) Page I-22 discusses the fact that most modern engines without modifications can run on biodiesel, however, there are impacts on the engine's durability and reliability. The next round of environmental assessment should consider whether more frequent vehicle replacement is going to be required. If so, the impact of all these new vehicles should be considered.

Page I-6. The report notes that the biodiesel used in many of the studies described in this report was at least six months old, which is the maximum recommended storage time for biodiesel. It might also be pointed out that this may represent a worst case scenario. Emissions of particulates, NOx, etc. are likely to be worse with this relatively old fuel.

Page I-13 describes the possible need to build new facilities for the processing or production of biodiesel. If such facilities are to be built, this will have a huge impact on a life cycle assessment of biodiesel. The next round of environmental impact assessment for biodiesel should consider these impacts and should try to estimate whether these facilities are going to be built, how many are going to be built, and what the environmental impacts of those facilities will be.

Page I-26 notes that acceptable materials for storage and transport of biodiesel include aluminum, steel, and fluorinated polyethylene or polypropylene. In particular, the fluorinated compounds are a big environmental problem and should be avoided at all costs. If increased use of biodiesel is going to require the use of these kinds of fluorinated compounds this could be a serious problem.

Page I-55. Typo about halfway down the page. "Fatty acids are oxidize at the _ carbon"

Page I-59 refers to specific sensitive populations at risk of exposure, yet I did not see anything in the report about this. Although asthma is mentioned as a possible problem

with biodiesel, this requires more discussion. Another important issue to investigate with regard to biodiesel is allergy. Many people are allergic to the raw oils such as palm oil or soy oil. Is there any reason to believe that the combustion products of biodiesels derived from these sources may cause an allergic response? People with known allergic responses are definitely a sensitive population that should be considered. Such allergies could be respiratory or dermal. There is some literature showing that regular diesel fuel is allergenic.

**7. Appendix II-B: chemical analysis of the water accommodated fractions of Bio fuels using stir bar sorptive extraction**

Page II-32 missing reference at bottom of page

Page II-33 another missing reference

Page II-83 Amount of diesel added to each microcosm is given as 5 mL, when it should be 5uL.

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## External Peer Review of "Multimedia Evaluation of Biodiesel"

***Re-statement of Objectives –***

External peer reviewers were instructed to evaluate the scientific portions of the Multimedia Working Group (MMWG) report and related documents to ensure that they are based on "sound scientific knowledge, methods and practices".

This review is primarily focussed on the *Public Health Evaluation* by the Office of Environmental Health Hazard Assessment (OEHHA), as well as additional components of the evaluation that relate to the toxicological hazards of biodiesel and biodiesel emissions (e.g., results of aquatic toxicity tests). The review encompasses the MMWG Staff Report "Multimedia Evaluation of Biodiesel", as well as the Tier I, Tier II and Tier III reports, and related documents (e.g., *Impact Assessment of Biodiesel on Exhaust Emissions from Compression Ignition Engines*).

***Recap of MMWG Conclusions to be addressed by Peer Reviewers (Biodiesel) –***

(1) ARB staff concludes that the use of biodiesel does not pose a significant adverse impact on public health or the environment from potential air quality impacts.
(2) SWRCB staff concludes that there are minimal additional risks to beneficial uses of California waters posed by biodiesel than that posed by CARB diesel alone.
(3) OEHHA staff concludes that the substitution of biodiesel for CARB diesel reduces the rate of addition of carbon dioxide to the atmosphere and reduces the amount of particulate matter (PM), benzene, ethyl benzene, and polycyclic aromatic hydrocarbons (PAHs) released into the atmosphere, but may increase emissions of oxides of nitrogen (NOx) and acrolein for certain blends.
(4) DTSC staff concludes that biodiesel aerobically degrades more readily than CARB diesel, has potentially higher aquatic toxicity for a small subset of tested species, and generally has no significant difference in vadose zone infiltration rate.

***Evaluation of MMWG Conclusions –***

Noteworthy shortcomings regarding the quality of the MMWG Evaluation of Biodiesel (i.e., the staff report), and the associated Tier I, II and III reports, **precluded effective scholarly evaluation of the aforementioned conclusions**. More specifically, in this reviewer's opinion, the MMWG evaluation of the available scientific information regarding the relative toxicological activity of biodiesel emissions is incomplete and superficial. **Consequently, it was necessary for this reviewer to collect, review and evaluate all publicly-available scientific information pertaining to the relative toxicological activity of biodiesel and petroleum diesel emissions**. The resulting review is provided in *Part II* of this document, and a detailed summary of the publicly-available scientific information is presented in a series of appended tables (i.e., Appendix I). *Part III* of this document comprises concluding remarks based on a thorough analysis of all publicly-available scientific information. *Part I* contains the more detailed peer review of the MMWG staff report and related documents.

Following a complete review of the available scientific information (i.e., Parts II and III of this document), including the information presented in the MMWG documents; this reviewer was able to render a professional, scholarly opinion regarding the MMWG conclusions. Noting that in some instances the available information may be incomplete and "less than ideal", ***this reviewer nonetheless supports the ARB and OEHHA conclusions listed above*** (i.e., 1 and 3). Although some of the published scientific information available to date shows enhanced toxicological activity for biodiesel emissions, relative to petroleum diesel, ***the weight of evidence supports the ARB and OEHHA conclusions***. With respect to the SWRBC and DTSC conclusions, this reviewer's analysis of the presented information did not reveal any problems or inconsistencies. However, it should be noted that this reviewer is not qualified to critically evaluate statements related to aerobic degradation or

vadose zone infiltration.

**Despite publicly-available scientific studies that have documented enhanced toxicological hazards for biodiesel emissions, the weight of evidence, in this reviewer's opinion, permits support of the MMWG's recommendations to the California Environmental Policy Council** (i.e., "that the use of biodiesel, as specified in the multimedia evaluations, does not pose a significant adverse impact on public health and the environment"). However, as noted below, **the WG must acknowledge studies that have documented enhanced toxicological hazards for biodiesel emissions; particularly those that noted effects generated under experimental conditions that have been linked to adverse effects in humans (e.g., inflammation).**

This reviewer certainly acknowledges that comprehensive statements regarding the relative toxicological hazards of biodiesel and conventional diesel emissions are hampered by variations in exhaust composition attributable to engine design, fuel formulations and blending rate, biodiesel source, aftertreatment, and test cycle; and moreover, that the available scientific data may indeed be "less than ideal". **Nonetheless, this evaluation of the WG conclusion can reasonably be regarded as an informed appraisal based on available information and professional judgement.**

Despite the aforementioned support of the MMWG conclusions, the MMWG is ***strongly*** encouraged to revise the reports such that they constitute a comprehensive, well balanced, scholarly evaluation of the available scientific information. The California H&SC states that multimedia evaluations "must be based on the best available scientific data"; and moreover, that the multimedia evaluation process must include a summary of the information available to date (i.e., "literature review") with identification of noteworthy knowledge gaps (Tier I). Although the Tier I report does identify some important knowledge gaps, it does not provide an acceptable scholarly summary of relevant toxicological information on biodiesel emissions available to date. **It is critical for the WG to effectively summarise all publicly-available evidence in order to effectively demonstrate that the risk of adverse health effects attributable to biodiesel emissions, or emissions of biodiesel-petroleum diesel blends, are similar or lower in comparison with conventional diesel emissions. Concurrently, it is also critical for the WG to acknowledge that a limited number of studies have documented enhanced toxicological hazards for biodiesel emissions; moreover, hazards related to pathophysiologic changes associated with an increased likelihood of human morbidity and mortality (e.g., pulmonary inflammation, oxidative stress, pulmonary tissue damage, cardiovascular irregularities).**

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## *Peer Review of MMWG Evaluation of Biodiesel–*

### *Part I – Peer Review of the MMWG Evaluation Staff Report and Related Documents*

It is this reviewer's understanding that the Tier I report, which constitutes the first step in "evaluating the cumulative health and ecological impacts from releases to air, surface water, groundwater and soil at all stages of the life cycle of biodiesel blends", should be based on the latest information available to date. In this regard, the Tier I review, and analysis of available information related to the toxicological hazards of biodiesel combustion emissions relative to conventional petroleum diesel combustion emissions, is incomplete and superficial, and, dare I say, naïve with respect to its analysis and interpretation. Moreover, in some instances the presentation of available information lacks the details required for the reader to understand and appreciate the relevant scientific publications (e.g., lack of units for air toxics emission rates). For example, the Tier I report states that the use of biodiesel is expected to contribute to large reductions in hydrocarbon (HC), particulate matter (PM) and carbon monoxide (CO) releases; however, the units upon which this statement is based are not provided (e.g., p. I-35). Although, as the report indicates, numerous studies have indeed documented noteworthy declines in key air toxics such as HC, CO and PM, for biodiesel and biodiesel blends relative to conventional diesel, the discussion in the Tier I report is superficial. For example, although the USEPA (2002) report on biodiesel exhaust emissions notes that B20 blends can be expected to contribute to average declines in PM, CO and HC emissions of 10.1%, 11.0% and 21.1%, respectively, relative to conventional diesel, it also notes that declines in emissions recorded in individual studies are dependent on engine design, fuel formulation and properties, and engine test cycle. Moreover, it is clear from the EPA report that declines in the aforementioned criteria air pollutants can be highly variable. For example, changes in HC emissions for B20 blends, relative to conventional diesel, can range from almost -100% to almost +100%. Variations in relative changes in PM and CO emission rates are somewhat lower (e.g., negligible to -60% for PM). Although this reviewer does acknowledge that there is strong evidence to support the assertion that biodiesel emissions rates for criteria air pollutants such as CO, PM and HCs are in fact reduced, the Tier I review and analysis of the available information is superficial. Similar statements can be made regarding other criteria air pollutants such as NOx, as well as air toxics such PAHs and aldehydes. Increased NOx has been clearly linked to an enhanced risk of human morbidly and mortality and the potential impacts on human health are only superficially acknowledged. It may be true that advanced emission control will offset the hazards associated with the increased NOx emissions rates; however, it is reasonable to expect the authors to present a more complete, quantitative evaluation.

From this reviewer's point of view, the most serious deficiencies in the MMWG evaluation of biodiesel relate to the incomplete review of publicly-available scientific information on the toxicological properties of biodiesel emissions relative to conventional diesel emissions. More specifically, in this reviewer's opinion, the review on pages I-42 and I-43 and I-59 to I-64 of the Tier I report is remarkably incomplete and superficial, and this superficial analysis is carried over into the Tier III report. Moreover, with respect to the organization of the Tier I report, it is unclear why information on the toxicological properties of emissions are introduced in Section 4 (Use of Biodiesel), and then discussed in more detail in Section 7 (Biodiesel Toxicity). Nevertheless, the combined information presented in both sections only reviews and discusses 7 of the roughly 45 scientific publications that have compared the toxicological properties of biodiesel and conventional diesel emissions. Moreover, the review is confusing for reader in the sense that it does not clearly distinguish between studies that examined fuels and fuel blends, and studies that examined combustion emissions. Furthermore, the discussion mixes up studies that conducted toxicological assessments based on measurements of air toxics emission rates with studies that assessed toxicological properties using *in vivo* and *in vitro* toxicity assessment tools. Finally, the presented

review of the pertinent scientific literature ignores critical information regarding the metric(s) used to express the magnitude of toxicological potency (i.e., potency unit). The units employed for quantitative evaluation of the results have a critical bearing on the relevance of the results for the assessment of human health risk. For example, the cited Turrio-Baldassarri et al (2004) study, which examined the mutagenic activity of organic extracts of PM from conventional diesel and B20 RME, compares mutagenic activity expressed per μg of extractable organic matter (EOM) and per unit of engine work (kWhr). The former unit is useful for studies that are interested in identifying the putative toxicants in combustion emissions, the latter, which requires information on EOM emission rates (e.g., μg per kWhr), is more useful for assessing the likelihood of post-emission adverse human effects. The Bunger et al study cited by the MMWG (i.e., 2000a), which examined the *cytotoxicity* of organic extracts of PM from conventional diesel and RME blends, notes that the potency of RME emissions, expressed per L of exhaust, is more pronounced. Interestingly, the MMWG review of the Bunger et al (2000) publications (i.e., 2000a and 2000b) only discusses *mutagenic* activity, and notes the reduction in potency associated with RME and SME emissions. In addition, the review, which is presented on page I-61, fails to acknowledge the units used for the potency comparison. Importantly, the Bunger et al (2000b) publication includes a comparative analysis of potency expressed per unit mass and per hour of engine operation. The latter unit is far more relevant for assessment of human risk. The noteworthy declines in the potency of biodiesel emissions described in the publication appear to be related to reduced potency of the PM, *as well as* the reductions in PM and PAH emission rates (e.g., g or μg per engine hour).

The superficial nature of the MMWG's review of the available information regarding the toxicological properties of biodiesel emissions relative to conventional diesel ***precludes an effective peer review of the WG's conclusions*****.** Moreover, the external peer review process provides the latitude to include any scientific information that is deemed to be pertinent to the review of the MMWG documents. Consequently, **it was necessary for this reviewer to collect and review all pertinent publicly-available scientific information**. This evaluation of available information is contained in *Part II* of this document; a review and analysis of the available literature pertaining to the relative toxicological properties of biodiesel and conventional diesel emissions.

It is certainly important to acknowledge that the Tier II analyses of biodiesel and renewable diesel emissions (i.e., the Durbin et al, 2011 report) constitutes a comprehensive comparative analysis of biodiesel and conventional diesel emissions. Nevertheless, it is also important to note that there are serious shortcomings in the Durbin et al report regarding the description of the methods employed for the toxicological analyses. Moreover, the concluding remarks and executive summary do not even comment on the relative ability of the emissions to induce inflammatory and oxidative stress responses. More specifically, the results presented indicate that extracts of biodiesel DEP from the 2000 model year engine without aftertreatment generally show a reduced ability to induce inflammatory signalling (COX-2, IL-8) or oxidative stress (HO-1), relative to conventional diesel, with noteworthy declines associated with NExBTL HVO blends. These results are never discussed in any meaningful way. Nevertheless, it is important to acknowledge the documented declines (i.e., in Durbin et al, 2011) in criteria air pollutants (e.g., PM, CO and HC) associated with biodiesel emissions; and moreover, the frequent observations of increasing reductions in emission rates for increases in blending rate. Similarly, it is important to acknowledge documented declines in biodiesel exhaust emission rates for air toxics such as PAHs, BTEX, and some carbonyl compounds, and the noteworthy increases in toxic aldehydes such as acrolein. Some researchers have suggested that compounds such as acrolein, which is a noteworthy irritant, may be responsible for documented increases in the toxicological activity of biodiesel emissions (Bunger et al, 2000; Bunger et al, 1998). Indeed, the increased risk of mucous membrane irritation in road maintenance workers exposed to

RME emissions, relative to conventional diesel, may be due to increased emissions rates of reactive aldehydes (Bunger et al, 2012[1]).

Although this peer review focussed primarily on toxicological hazards (i.e., health hazards) of biodiesel emissions, the information pertaining to the relative toxicological activity of biodiesel (i.e., the fuel) and conventional diesel were also reviewed. The Tier I report provides an effective overview of available information on the relative aquatic toxicity of biodiesels and conventional diesel; and moreover, identifies knowledge gaps that are effectively addressed in Tier II using well-established EPA methods. The conclusions of the MMWG, which state that the biodiesel blends "exhibit somewhat increased toxicity to subsets of tested species compared to ULSD" are supported by the data. Nevertheless, it is also important to note that the Tier I review of the literature is incomplete. For example, important studies by Leme et al 2012[2]) used water and soil spill simulations to show that soy-derived B100 yielded samples with an increased capacity to induce genetic damage in bacteria, mammalian cells and plants (i.e., *Allium cepa* root tips).

***Miscellaneous Editorial Comments –***
Although this review did not include detailed examination of spelling, grammar, or stylistic issues, a few of the more obvious problems are highlighted below.

Biodiesel Tier I, page I-4: The authors are reminded that it is critical to provide units when referring to changes in emission rates.

Biodiesel Tier I, page I-35: The authors often use statements such as "large reductions", but fail to qualify. How will the reader know what "large means". Is it 10% or 95%?

Biodiesel Tier I, page I-43: The authors refer to TEFs but fail to note what endpoint is being discussed. Presumably it's carcinogenic activity. Although some agencies use the term TEF to refer to carcinogenic activity relative to BaP, the authors are reminded that many readers will be more familiar with the terminology used the EPA's Integrated Risk Information System (IRIS) employs the acronym RPF (Relative Potency factor).

Biodiesel Tier I, page I-61: "…thoroughly tested by the EPA and is "safe". Where does this statement come from? The EPA is extremely reluctant to use adjectives such as "safe". The agency is far more likely to use statements such as "negligible increase in risk above background".

Biodiesel Tier I, section 7: Please pay attention to units!

Biodiesel Tier I, Section 7 (carried through to Tier III): Many vague statements need to be clarified. For example, "premature death" from what type(s) of effects? "More investigations in biological systems" – what systems? What endpoints? What route(s) of exposure?

Biodiesel Tier II, page II-32: Reference problem at bottom of page.

Throughout the Biodiesel Tier I, II and III reports: The quality of reproduced graphics (e.g., page II-77) is marginal. In some cases it is very difficult to make out the axes labels.

Durbin et al (2011), pages 222 and 224: "Marcophage" should be macrophage.

[1] Original source – Hasford, B. et al. 1997. Respiratory symptoms and lung function after exposure to exhaust from rapeseed oil in comparison to regular diesel. *Proceedings of the 9th International Conference on Occupational Respiratory Diseases: Advances in Prevention of Occupational Respiratory Diseases*. Elsevier, Kyoto, Japan, pp. 131-135.
[2] Leme et al. 2012. *J. Hazard Mat* **199-200**:343-349; Leme et al. 2012. *Chemosphere* **86**:512-520.

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***Part II – Review of Published Information on the Relative Toxicological Properties of Biodiesel and Petroleum Diesel Combustion Emissions***

## 1. INTRODUCTION – Toxicological Assessments of Vehicular Emissions for Comparisons of Petroleum-based Diesel, Biodiesel, Renewable Diesel, and Biodiesel or Renewable Diesel Blends

It is important for the MMWG to acknowledge that studies investigating the toxicological activity of diesel emissions can be conducted on diluted exhaust, exhaust particulate matter, filtered exhaust (i.e. gaseous portion), extracts of particulate matter, or concentrates of semi-volatile organics (i.e. SVOCs) adsorbed on a solid matrix (e.g. XAD resin). The nature of the toxicological assessment, and the units employed to express the observed responses, will influence the interpretation of the results in a human health contex.

The most sophisticated *in vivo* studies involve inhalation exposures whereby experimental animals are exposed to diluted vehicular emissions. Doses delivered via inhalation exposure are generally expressed as mg PM per $m^3$ in the exposure chamber, with additional information provided regarding the duration and frequency of the exposure. Other *in vivo* studies generally involve delivery of exhaust particulate suspensions or particulate extracts to the pulmonary system via intratracheal or intrapharyngeal instillation, or delivery of particulates or particulate extracts via oral gavage, dietary intake with food, topical treatment, or intraperitoneal (IP) injection. Intratracheal, intraperitoneal or dietary doses are generally expressed as total mg PM delivered/consumed or mg per kg body weight.

The majority of *in vitro* assessments of effects associated with vehicular emissions involve exposures of cells suspended in liquid medium, cells attached to solid culture surfaces (e.g. polystyrene), or cells imbedded in agar. More recently, it has become possible to hold cultured cells, including primary human cells or 3-dimensional tissue constructs, on semi permeable membranes and expose the cells at an air-liquid interface ([1-3]). However, such systems (e.g. VitroCell® or Cultex®) have rarely been employed to examine the toxicity of vehicular emissions (e.g., [4]). Thus, most *in vitro* assessments involve exposures to collected PM, organic extracts of PM, or concentrates of SVOCs. PM collection can present a substantial technical challenge, with most studies collecting PM on glass fibre filters. In some instances bulk, size-fractionated PM is collected using devices such as cascade impactors. In either case, preparation of PM extracts generally involves extraction of the "soluble organic fraction (SOF) using solvents such as dichloromethane (DCM), acetone, hexane, ethanol, methanol, or solvent mixtures. Extracts are generally exchanged with a bioassay-compatible solvent such as dimethyl sulfoxide (DMSO) before testing. SVOCs are generally collected by passing filtered exhaust over a solid adsorbent matrix (e.g. XAD resin) followed by solvent elution and concentration.

Concentration units employed for *in vitro* assessments of toxicological effects induced by vehicular emissions vary depending on the nature of the test article and the experimental system. Exposure concentrations for PM suspensions are generally expressed as mass of PM (µg or mg) per assay unit (e.g. agar plate or mL of culture medium). Exposure concentrations for organic extracts of PM are often expressed as µg of EOM, or µL of extract, per assay unit. Measures of EOM per unit mass of PM can be used to convert these concentration values into equivalent mg of PM per assay unit. In addition, measures of engine work, engine run time, fuel consumption, or distance travelled, can be employed to convert exposure concentrations to equivalent amounts of engine work in kWhr or hph, equivalent volume of fuel consumed, equivalent hour of engine operation, or equivalent distance travelled. Concentrations of SVOCs collected by adsorption on solid resins (e.g. XAD) are generally expressed as µg EOM per assay unit. With respect to the potential for adverse human effects, the potency of the sampled material (e.g., PM extracts or PM suspensions in effect per unit PM mass or EOM mass) must be considered in conjunction with the expected magnitude of the exposure.

Emission rates per unit engine work (e.g., g PM per brake hph) can be used to express results per unit of engine work, which can more readily be interpreted in a human health context.

## 2. Summary of Studies Comparing the Toxicological Properties of Diesel Engine Emissions for Biodiesel- or Renewable Diesel-fuelled Engines to Petroleum Diesel-fuelled Engines

Few studies have employed *in vivo* animal exposures to compare diesel emissions associated with engines or vehicles operated using petroleum-based fuels with emissions associated with biodiesels (i.e., FAMEs), other renewable fuels (e.g., HVO), or fuel blends. Only two studies examined effects elicited by diluted exhaust, and both studies noted evidence that biodiesel emissions can be associated with increased severity of toxicological responses. For example, Brito et al (2010) noted that SEE emissions are associated with increases in cardiovascular irregularities in Balb/c mice ([5]). Steiner et al (2013) employed an air-liquid interface system to demonstrate that diluted RME emissions (B100) induced increased cytotoxicity and oxidative stress in an *ex vivo* 3D human airway model, relative to petroleum diesel. The authors noted some decrease in inflammatory stress for biodiesel ([6]). In addition, Yanamala et al (2013) showed that pharyngeal aspiration of PM from corn-derived FAME induced increased pulmonary damage, oxidative stress and inflammation in C57BL/6 mice, relative to petroleum diesel PM ([7]). Importantly, the doses examined in the Yanamala et al study equate to human occupational exposures of 156.25 working days at an allowable MSHA concentration limit of 160 µg total carbon per $m^3$. With respect to carcinogenic hazard, a single study examined the emission rate of carcinogenic PAHs, expressed as total BaP equivalents, and concluded that the carcinogenic hazards of biodiesel emissions (source unspecified) are likely to be lower than petroleum-based diesel for PM-associated PAHs in primary and secondary aerosols ([8]).

Several *in vitro* studies have employed cultured animal cells to assess the toxicological activity of diesel PM suspensions. With respect to proinflammatory signalling, several studies have noted similar or reduced activity for biodiesel emissions, relative to petroleum diesel (expressed per unit mass of PM or kWhr). For example, Bhavaraju et al (2013), Hemmingsen et al (2011), and Ihalainen et al (2009) showed that exposures of several types of cells (e.g., rat alveolar macrophages, human pulmonary and endothelial cells) to biodiesel PM, including PM associated with RME, AFME and HVO, can contribute to modest declines in inflammatory signalling, relative to petroleum diesel PM ([9-11]). Similarly, with respect to cytotoxicity and/or cell death (i.e., apoptosis), several studies have shown similar or reduced activity for biodiesel emissions. Studies by Betha et al (2012), Bhavaraju et al (2013) and Ackland et al (2007) noted that cytotoxic responses to biodiesel-derived PM (e.g., waste cooking oil FAME) in human and rodent cells are similar or lower relative to petroleum diesel PM ([9, 12, 13]). Nevertheless, it should be noted that the Betha et al (2012) study noted greater cytotoxicity for the biodiesel PM (i.e., waste cooking oil FAME) for samples collected from high load tests. The same study also noted increased oxidative stress (GSH/GSSG ratio) associated with biodiesel PM collected under high load conditions. With respect to genotoxic activity, a single study noted similar or reduced responses for biodiesel PM (i.e., RME, AFME), relative to petroleum diesel ([10]). Finally, a single study noted that exposure of fresh rat alveolar macrophages to biodiesel PM resulted in an increase in macrophage activation (i.e., $PGE_2$ release), relative to petroleum diesel ([9]).

Several *in vitro* studies have employed cultured animal cells to assess the toxicological activity of organic extracts of diesel PM (i.e., SOF of collected PM). With respect to proinflammatory responses, several studies present fairly strong evidence that extracts of biodiesel PM (i.e., diesel exhaust particulates or DEP) have an enhanced ability to induce inflammatory signalling, relative to petroleum diesel. However, there is also evidence that extracts of RME DEP have a reduced ability to elicit inflammatory signalling. More specifically, two noteworthy studies showed that biodiesel DEP extracts have an enhanced ability to induce inflammatory signalling (i.e., IL-6 and IL-8 release) in

human BEAS-2B bronchial epithelial cells ([14, 15]). Importantly, the Gerlofs-Nijland et al study also examined PM emission rates and noted that reductions associated with fuel changes (i.e., from petroleum diesel to biodiesel) and/or aftertreatment (e.g., DPF) may not be sufficient to offset the increased hazard associated with biodiesel DEP SOF. Nevertheless, two studies by Jalava et al noted that the SOF from RME DEP had a reduced ability to induce inflammatory signalling (i.e., Tnf-α, Mip-2 release) in murine macrophages ([16, 17]). The same studies noted an increase in inflammatory signalling for HVO, relative to petroleum diesel. Extensive analyses by Durbin et al (2011) of DEPs and SVOCs from two heavy-duty engines revealed that extracts of biodiesel DEP from the 2000 model year engine without aftertreatment generally show a reduced ability to induce inflammatory signalling (COX-2, IL-8) or oxidative stress (HO-1), with noteworthy declines associated with NExBTL HVO blends ([18]). Although analyses of extracts of DEP emitted by the 2007 model year engine (SME and AFME blends only) with advanced aftertreatment (i.e., DOC/DPF combination) showed higher responses for extracts of biodiesel DEP, all responses were several orders of magnitude below those obtained for the aforementioned 2000 model year engine.

With respect to cytotoxicity and/or cell death, several studies have noted an increased response for SOF samples from biodiesel DEP and/or SVOC samples compared with petroleum diesel. For example, studies by Bunger et al (2000), Gerlofs-Nijland et al (2013) and Lui et al (2008) noted increased cytotoxicity in mouse fibroblasts, human BEAS-2B cells and luminescent bacteria, respectively, with the Bunger et al (DEP extract) and Lui et al (SVOC) results based on responses expressed per L of exhaust ([14, 19, 20]). Again, the Gerlofs-Nijland et al study noted that DEP reductions associated with fuel changes and/or aftertreatment may not be sufficient to offset the increased hazard of biodiesel DEP SOF. In contrast, in their analyses of mouse fibroblasts, murine macrophages and human BEAS-2B cells, studies by Bunger et al (1998), Jalava et al (2010), Jalava et al (2012) and Swanson et al (2009), respectively, documented little or no difference in the cytotoxic activity of SOF from biodiesel DEP, relative to petroleum diesel ([15-17, 21]). In addition, the study by Kooter et al (2011), of mouse macrophages exposed to DEP extracts, noted a decline in the cytotoxicity of biodiesel DEP extracts ([22]). The same study also noted no difference in oxidative stress (*Ho-1* expression) signalling between biodiesel DEP extracts and petroleum diesel DEP extracts. With respect to genotoxic activity, the aforementioned Jalava et al studies noted some reductions in the ability of SOF from biodiesel DEP to induce DNA strand breaks in murine macrophages, with the most pronounced decline, relative to petroleum diesel, associated with RME ([16, 17]).

A single study investigated the ability of extracts from biodiesel and conventional diesel DEP to damage naked DNA *in vitro*. More specifically, Topinka et al (2012) examined extracts of biodiesel (RME and RSO) DEP and conventional diesel DEP, and noted no appreciable fuel-related differences in ability to induce DNA adducts (i.e., per mg equivalent PM) ([23]).

## 3. Summary of Studies Comparing the Mutagenic Activity of Organic Extracts of Diesel Particulates from Biodiesel- or Renewable Diesel-fuelled Engines to Extracts of Diesel Particulates from Petroleum Diesel-fuelled Engines

A careful review of the literature revealed 27 studies that employed the Salmonella mutagenicity assay to compare the mutagenic activities of SOFs from biodiesel DEP and petroleum diesel DEP. Of these, 17 studies provide evidence that the SOF of biodiesel DEP is less potent relative to petroleum diesel. In contrast, 9 studies provide evidence of increased mutagenic activity for the SOF of biodiesel DEP. However, interpretation of the results requires scrutiny of the potency units employed to compare biodiesel and petroleum diesel derived samples. From a human hazard point of view, the most convincing studies compared mutagenic potency values expressed per unit distance (i.e. mile or km), per engine hour, per $m^3$ of exhaust, or per unit of engine work (i.e. kWhr or hph). Nine studies

noted that the potency of biodiesel DEP extracts is significantly lower in comparison to samples derived from conventional petroleum diesel DEP. For example, studies by Krahl et al. (2003) and Bunger et al (2006) revealed significant reductions in mutagenic potency (per engine hour) for RME or SME relative to conventional diesel, with the former study noting that potency values for conventional diesel were 4- to 5-fold higher than biodiesel ([24, 25]). Similar studies by Krahl et al (2005) and Westphal et al (2012) revealed significant reductions in mutagenic potency (per $m^3$ exhaust) for RME relative to conventional diesel, with the former study noting that extracts of conventional DEP yield samples with 2- to 8-fold higher potency than biodiesel ([26, 27]). The Wesphal et al (2012) study failed to reveal any fuel-related differences in the mutagenic activity of SVOC samples. Studies by Chase et al (2000), Bagley et al (1998), Kado and Kuzmicky (2003) and Rantanen et al (1993) revealed significant reductions in the mutagenic potency of DEP extracts, expressed per engine kWhr or hph, for biodiesel (i.e. SEE, SME, RME, CME, YGME, PLME, BTME) relative to conventional diesel ([28-31]). The Chase et al study also noted that SEE was associated with considerable reductions in the emission rates (per hph) of PM and PAHs. Moreover, the Rantanen et al (1993) study noted a correlation between mutagenic potency and PAH emission rates (both per kWhr). A study by Bunger et al (2000a) revealed significant reductions in the mutagenic potency, expressed per L of engine exhaust, of extracts from biodiesel DEP, compared with extracts from conventional diesel DEP ([19]). Interestingly, additional analyses showed higher PM emission rates for RME. Studies by Bunger et al (1998), Kado et al (1996), and Durbin et al (2011) revealed significant reductions in the mutagenic potency, expressed per engine mile or km, of extracts from biodiesel DEP compared with DEP from conventional diesel ([18, 21, 32]).

Several studies employed mutagenic potency values expressed per mg of DEP or per µg of EOM (extractable organic matter) to compare the mutagenic potency of extracts from biodiesel DEP and conventional diesel DEP. Studies by Bunger et al (2000b), Bunger et al (1998), Carraro et al (1997) and Kado et al (1996) noted that the mutagenic potency of extracts from biodiesel DEP is lower than extracts from conventional diesel DEP ([21, 32-34]).

Several studies failed to reveal any differences between the mutagenic potency of extracts of biodiesel DEP compared with conventional diesel DEP. For example, in their examination of DEP from several light- and heavy-duty vehicles, studies by Krahl et al failed to detect any differences between the mutagenic potency (per L exhaust) of emissions associated with diesel fuel, RME, or diesel/GTL/RME blends ([35, 36]). Similarly, in their study of emissions from a single cylinder research engine, Bunger et al (2000b) noted that the mutagenic potency (per engine hour) of DEP extracts are similar for conventional diesel, RME and SME ([33]). A study by Dorn and Zahoransky (2009) failed to detect mutagenic activity in extracts of DEP from conventional diesel or biodiesel ([37]). A study by Turrio-Baldassarri et al (2004) failed to detect any difference between the mutagenic potency, expressed per kWhr, of biodiesel (B20 RME) DEP extracts and extracts of DEP from conventional diesel ([38]).

In contrast to the aforementioned decreases in the mutagenic activity of extracts from biodiesel DEP, compared with DEP from conventional diesel, some studies have noted that the mutagenic potency of extracts from biodiesel DEP can be significantly greater than extracts from conventional diesel DEP. For example, studies by Bunger et al (2007) and Krahl et al (2007a, 2009b) noted increases in mutagenic potency (per L exhaust) for extracts of RME DEP in comparison with conventional diesel ([39-41]). Similarly, Kooter et al (2011) noted that the mutagenic potency (per µg PM) of extracts associated with biodiesel (source unspecified) is generally higher than conventional diesel ([22]). Of particular interest are studies that noted increased mutagenic potency for extracts of biodiesel DEP expressed per mg DEP or µg EOM (extractable organic matter), relative to extracts of DEP from

conventional diesel, but a reversal in relative potency when values are expressed per unit of engine work. For example, Rantanen et al (1993) noted that extracts of DEP from RME emissions were more mutagenic (per μg EOM) than extracts of DEP from conventional diesel; however, when expressed per unit of engine work (kWhr), the RME samples proved to be less mutagenic relative to conventional diesel ([31]). Similarly, the study by Kado and Kuzmicky (2003) noted that the potency of extracts of some biodiesel DEP samples (per mg PM) were higher than extracts of DEP from conventional diesel; however, when expressed per unit of engine work (hph), the biodiesel potency values are lower relative to conventional diesel ([30]).

Several of the aforementioned studies revealed lower emission rates of PM and/or PAHs and other PACs (e.g., nitro-PAHs and oxy-PAHs) for biodiesels and biodiesel blends in comparison with conventional diesel. For example, studies by Krahl et al (2005, 2007b), Schroder et al (2012), Turrio-Baldassarri et al (2004), Westphal et al (2012, 2013), Kooter et al (2011) and Carraro et al (1997) noted that biodiesel is associated with lower emission rates of PM, PAHs, oxy-PAHs, or nitro-PAHs ([22, 26, 27, 34, 35, 38, 42, 43]).

***Part III - Concluding Remarks***

Although comprehensive statements regarding the relative toxicological hazards of biodiesel and conventional diesel emissions are hindered by variations in exhaust composition attributable to engine design, fuel formulations and blending rate, biodiesel source, aftertreatment, and test cycle, the evidence generated to date suggests that the risk of adverse effects attributable to biodiesel emissions, or emissions of biodiesel-petroleum diesel blends, are similar or lower than conventional ULSD emissions. Nevertheless, it is critical to note that several studies have clearly documented enhanced toxicological hazards for biodiesel emissions; moreover, hazards related to pathophysiologic changes associated with an increased likelihood of human morbidity and mortality (e.g., pulmonary inflammation, oxidative stress, pulmonary tissue damage, cardiovascular irregularities). The most notable published studies include the *in vivo* murine studies of Brito et al (2010) and Yanamala et al (2013), and the air-liquid interface *ex vivo* study of Steiner et al (2013) that examined effects on 3D human airway epithelium constructs. In addition, several notable *in vitro* studies provide additional evidence suggesting the possibility of increased toxicological hazard for biodiesel emission. These include the *in vitro* DEP organic extract studies in human BEAS-2B bronchial epithelial cells by Swanson et al (2009) and Gerlofs-Nijland et al (2013), and the *in vitro* DEP extract studies in murine fibroblasts by Bunger et al (1998, 2000a) and Schroder et al (1999). Finally, several studies have shown that organic extracts from some biodiesel DEP have an enhanced ability, relative to extracts of conventional diesel DEP, to induce genetic damage and mutations that might be expected to increase the likelihood of cancer ([39-41]).

Several researchers have noted that the increased toxicological potency that has been observed for some biodiesel emissions may be associated with recorded increases in the emission rates of toxic aldehydes such as acrolein ([19, 21]). Indeed, the increased risk (i.e., OR = 1.3 to 2.2) of mucous membrane irritation in road maintenance workers exposed to RME emissions, relative to conventional diesel, may be due to increased emissions rates of reactive aldehydes ([45]). Moreover, the increased toxicological activity of biodiesel DEP may be associated with an increase in its soluble organic fraction (i.e., μg EOM per mg PM) ([7, 14, 20, 21, 25, 33]).

**Although critical examination of the available information presented in the previous sections does indeed indicate, in this reviewer's opinion, that the risk of adverse health effects attributable to biodiesel emissions, or emissions of biodiesel-petroleum diesel blends, is similar or lower relative to conventional diesel fuel emissions, it is critical for the MMWG to acknowledge that there are some studies that have documented enhanced toxicological hazards for biodiesel emissions; moreover, hazards that are mechanistically related to pathophysiologic changes associated with an increased likelihood of human morbidity and mortality (e.g., pulmonary inflammation, oxidative stress, pulmonary tissue damage, cardiovascular irregularities).** As indicated above, the most notable studies include the *in vivo* murine studies of Brito et al (2010) and Yanamala et al (2013), and the air-liquid interface *ex vivo* study of Steiner et al (2013), as well as several *in vitro* studies that provide additional evidence suggesting the possibility of increased toxicological hazard for biodiesel emissions. The latter includes studies by Swanson et al (2009) and Gerlofs-Nijland et al (2013) that examined the effects of DEP organic extracts on human BEAS-2B bronchial epithelial cells, and the *in vitro* DEP extract studies with murine fibroblasts conducted by Bunger et al (1998, 2000a) and Schroder et al (1999).

In this reviewer's opinion, the MMWG should also acknowledge studies which have shown that organic extracts from some biodiesel DEP have an enhanced ability, relative to extracts of conventional diesel DEP, to induce genetic damage and mutations that might be expected to increase the likelihood of cancer (e.g., Bunger et al, 2007; Krahl et al, 2007 and 2009).

Finally, in this reviewer's opinion, the MMWG should also acknowledge that several researchers have noted the increased SOF of biodiesel DEP, compared to conventional diesel PM; and moreover, suggested that the differences may be responsible for the observed differences in toxicological activity (Yanamala et al, 2013; Liu et al, 2008, Bunger et al, 1998, Bunger et al, 2006, Gerlofs-Nijland et al, 2013; Bunger et al, 2000).

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## APPENDIX I: Summary of Published Information Regarding the Relative Toxicological Properties of Biodiesel and Petroleum Diesel Emissions.

**Table 1. Summary of the published *in vivo* studies, or studies that estimated *in vivo* hazard using data on monitored toxics.**

| Engine | Fuels Examined | Exposure System | Endpoint(s) Examined | Results Obtained | Reference |
|---|---|---|---|---|---|
| Common rail direct injection 3.0L engine (Tata, Safari DICOR), photochemical reaction chamber for secondary aerosols, measurement of PM-bound PAHs. | DF, B20 (unspecified) | Conversion of PAHs to total BaP equivalents in ng/m$^3$. Used TEFs from Nisbet and Lagoy (1992) for relative carcinogenicity. | Total BaP equivalents (i.e., total carcinogenic PAH emission rate). | Total BaP equivalents in secondary aerosols higher than primary. B20 lower than DF for both primary and secondary aerosols. | 8 |
| Branco BD-2500 diesel generator. | "Metropolitan diesel" with 3% biodiesel, SEE B50 and SEE B100. | Adult male Balb/c mice exposed to diluted exhaust (550 µg/ m$^3$) for 1 hr. 12 animals per exposure group. | Heart rate, heart rate variability and blood pressure, before exposure and 30, 60 mins after. Blood, BAL and bone marrow examination 24 hr after exposure. | No differences in inflammatory cell infiltration between DF and biodiesel blends. Some indication that cardiovascular irregularities increased with biodiesel relative to DF. | 5 |
| Isuzu C240 2.369L with DOC, 4 steady state conditions, high volume DEP sampling system. | ULSD and corn-derived FAME. | C57BL/6 mice exposed to DEP via pharyngeal aspiration, 0, 9 and 18 µg total C per mouse as aqueous suspension, sacrifice 1, 7 and 28 days after exposure. | Pulmonary inflammation (by BAL counts & cytokine levels), oxidative stress (by-products of lipid peroxidation), and morphological changes (by histopathological assessment). | Significant elevation in inflammatory markers for FAME relative to ULSD, evidence of increased tissue damage and oxidative stress for FAME relative to ULSD, significant elevation in inflammatory cytokines, chemokines, growth factors for FAME, histological examination showed impaired clearance and retention of FAME particulates. | 7 |

**Table 2. Summary of the published *in vitro* studies in cultured animal cells**

| Engine | Fuels Examined | Exposure System | Endpoint(s) Examined | Results Obtained | Reference |
|---|---|---|---|---|---|
| 1979 1.6L Volkwagen Golf, ECE Euro 2 cycle, DEP collected on "filter papers". | DF, Biodiesel (unspecified) at B20, B40, B60, B80 B100. | A549 human alveolar adenocarcinoma cells exposed to 25 µg PM/mL for 5 days. | Induction of apoptosis (caspase III protein level, cytokeratin fragmentation) | Semi-quantitative analyses showed stronger induction of apoptosis by petroleum diesel, relative to biodiesel. | 13 |
| Yanmar single cylinder 296mL diesel generator, steady state at rated speed and 4 loads, DEP collected on Teflon® membranes and quartz filters. | ULSD, B100 and B50 (waste cooking oil). | A549 human alveolar adenocarcinoma cells directly exposed to PM on filters for 48 hr. | Cell viability and cytotoxicity, measured via production of fluorescent products, apoptosis as caspase III/VII, oxidative stress as GSH/GSSG ratio (Promega assays). | Cytotoxicity and oxidative stress higher for B100 relative to DF. Similar for apoptosis response. No significant difference between B100 and DF at lower engine loads, and largest difference at higher engine loads. | 12 |
| 2002 Cummins 5.9L engine (EPA 2004 certified) with common rail fuel injection, EGR, DOC and DPF, steady state operation. DEP collected by "back-flush" of DPF. | DF and B20 (unspecified) | Freshly isolated rat alveolar macrophages exposed to 100-500 µg PM/mL for 24 hr. | Cytotoxicity (LDH release), inflammatory signalling (*Cox-2*, *Mip-2* gene expression), and macrophage activation ($PGE_2$ release) | No difference in cytoxicity between DF and B20. Some increased inflammatory signalling for DF. Some increased macrophage activation for B20. | 9 |
| Fendt tractor, 13-mode ESC, DEP collected on Teflon®-coated GFFs, DCM Soxhlet extract. | DF and RME | L929 mouse fibroblasts exposed to solvent-exchanged extract (DMSO) in medium, 24 hr. | Cytotoxicity via Neutral Red uptake assay. | Reduction in cell viability more pronounced (at idling) for RME relative to DF (4-fold increase in potency expressed per L of exhaust). Difference not observed at rated power. RME yields higher particle emissions (g/hr). | 19, 46 |
| Volkswagen Vento 1.9L TDI with DOC, FTP-75, MVEG-A, and modified MVEG-A cycles. DEP collected on Teflon®-coated GFFs, DCM Soxhlet extract | DF and RME | L929 mouse fibroblasts exposed to solvent-exchanged extract (DMSO) in medium, 24 hr. | Cytotoxicity via Neutral Red uptake assay. | No significant difference between cytotoxic potency of RME and DF (based on relative concentration of extracts in culture medium). Slight increase in RME potency for FTP-75 only. | 21 |

| Engine | Fuels Examined | Exposure System | Endpoint(s) Examined | Results Obtained | Reference |
|---|---|---|---|---|---|
| Honda Accord (2.2L) 2.2i-CTDi (Euro4) with DOC and de-NOx, Peugeot (2.0L) 407 HDi with DOC and DPF, several composite driving cycles, DEP collected on Teflon®-coated GFFs, sonication MetOH extract. | DF, ULSD, RME | BEAS-2B bronchial epithelial cells exposed to DEP extracts suspended in culture medium, 24 hr, 0-200 µg equiv DEP per assay mL. | Cytotoxicity (necrosis, apoptosis) by flow cytometry, inflammatory stress via cytokine release (IL-6, IL-8). | On per mass basis, B50 significantly increased cytotoxicity and cytokine release. B50 and DPF both contribute to large reductions in PM emission rate. PM emission rate reduction for B50 may not be sufficient to compensate for increased potency on per mass basis. | 14 |
| Two light-duty diesel engines representing Euro2 and Euro4 standards. DEP collected on quartz filters. | ULSD, B20 RME, B20 AFME | A549 human alveolar adenocarcinoma cells, HUVEC cells, THP-1 cells exposed to 0.78–100µg PM/mL for 3 h. | DNA strand breaks in A549 cells by comet assay, and fpg-assisted comet assay, ICAM-1 and VCAM-1 expression in HUVEC cells, gene expression of *CCL-2* and *IL-8* in THP-1 cells. | All samples elicited concentration-related increases in DNA strand breaks and fpg-sensitive sites. RME B20 response lower than ULSD, AFME similar to diesel. With respect to *CCl-2* and *IL-8* expression, biodiesel responses similar or lower than DF. Levels of ICAM-1 and VACM-1 somewhat elevated for DF relative to biodiesel. | 10 |
| Kubota 1.123L D1105-T diesel engine (EPA Tier I), ISO C1 cycle, with or without DOC/POC, DEP collected using HVCI. | ULSD, HVO and RME | RAW264.7 mouse macrophage cells exposed to DEP suspension for 24 h | Production and release of proinflammatory cytokine TNF-α. | At 150 µg/mL decreased response for RME, relative to DF. HVO similar to DF. When based on per kW-hr exposures, reduced response for RME, especially with DOC/POC. Small reduction for HVO, relative to DF, without aftertreatment only. PM emission rates reduced for RME and HVO, relative to DF. Aftertreatment reduced PM emissions rates by 50-60%. | 11 |
| Kubota 1.123L D1105-T diesel engine (EPA Tier I), ISO C1 cycle, with or without DOC/POC, DEP collected using an HVCI with downstream polyurethane foam (PUF) and Teflon®-coated membrane, ultrasonic extraction with methanol. | ULSD, HVO and RME | RAW264.7 mouse macrophage cells exposed to 5–300µg/mL DEP extract and suspension of insoluble material for 24 h | DNA strand breaks by comet assay, proinflammatory cytokine production (Tnf-α, Mip-2), MTT reduction for cytotoxicity, apoptosis by flow cytometric analysis. | All samples yielded a significant concentration-related increase in cytotoxicity and DNA strand breaks. No difference in cytotoxicity across fuels types and aftertreatment. DOC/POC aftertreatment significantly reduced RME response only. ULSD and HVO elicited larger inflammatory response than RME. DOC/POC increased oxidative potential on a per mass basis; aftertreatment reduced PM emission rates by more than 50%. | 16 |

| Engine | Fuels Examined | Exposure System | Endpoint(s) Examined | Results Obtained | Reference |
|---|---|---|---|---|---|
| 2005 Scania 6-cylinder 11.7L Euro 4 engine with EGR, Braunschweig (bus) cycle, with or without DOC/POC (for LSDF and HVO 100 only), DEP collected on Teflon® filter, ultrasonic extraction with methanol. | LSDF, RME (B100 and B30), HVO (B100 and B30) | RAW264.7 mouse macrophage cells exposed to 15–300µg/mL DEP extract and suspension of insoluble material for 24 h | MTT reduction for cytotoxicity, proinflammatory cytokine production (Tnf-α, Mip-2), apoptosis, cell cycle and membrane permeability by flow cytometry. DNA strand breaks by comet assay. | Little differences in cytotoxicity across the fuels and aftertreatment conditions examined. Higher inflammatory response for HVO samples; lowest for RME. Little differences in apoptosis across conditions examined; some indication of higher levels for HVO. DOC/POC greatly reduced PM emission rate and PAH content of PM. | 17 |
| Six cylinder 12L Euro III truck, no DOC, with or without DPF, 13-mode ESC, DEP collected on Teflon®-coated GFFs, ethanol/DCM (1:1) sonication extract | DF, B100, B5, B10, B20, PPO | RAW264.7 mouse macrophage cells exposed to DEP extract for 24 h | Cytotoxicity via LDH release, oxidative stress as *Ho-1* gene expression. | Biodiesel blends and PPO elicited less cytotoxicity relative to DF; B100 significantly more cytotoxic (unit unknown). No differences in *HO-1* expression. Biodiesel associated with reductions in PM (g/kWh), PAHs and oxy-PAHs (µg/kWh). | 22 |
| Four cylinder 2.976L diesel generator, steady state, DEP collected on GFFs, SVOCs collected on XAD-16, Soxhlet extraction with DCM. | DF and palm-derived FAME (B10, B30, B50, B75, B100) | BEAS-2B bronchial epithelial cells exposed to DEP extracts for 24 hr. *Vibrio fischeri* exposed to DEP extracts for 5- and 15 mins (Microtox assay) | Bacterial cytotoxicity as reduction in bioluminescence, mammalian cell cytotoxicity as reduction of MTT. | Microtox TUs show sharp reduction for biodiesel blends when expressed per µg SOF, but increase for biodiesel, with maximum at B50, for SVOCs when expressed per unit volume of exhaust. Appears to be result of increased SOF emission rate (g per kW-hr or g per L fuel) for biodiesel. Some indication of reduction in emission rate of insoluble material for biodiesel. Some indication that SVOCs of biodiesel emissions more cytotoxic than diesel. | 20 |
| 1998 Opel Astra X20DTL (1.995L), continuous flow exposure system (air-liquid interface). | DF, RME (B20 and B100) | In vitro 3D human airway epithelial model, 2 or 6 hr exposures at low and high dilution. | Cytotoxicity as LDH release, oxidative stress as GSH, inflammatory response as TNF-α and IL-8, inflammation, necrosis, apoptosis and oxidative stress by gene expression (*HO-1*, *TNF*, *IL-8*, *CASP7*, *FAS*) | Some indication of enhanced cytotoxicity and oxidative stress for B100, pro-inflammatory responses weak relative to air control, some indication of reduced inflammatory response for B20. | 6 |

| Engine | Fuels Examined | Exposure System | Endpoint(s) Examined | Results Obtained | Reference |
|---|---|---|---|---|---|
| 1997 Caterpillar 3406E 14.6Lengine, EPA heavy-duty transient cycle, DEP collected on Teflon®-coated GFFs, DCM extract. | DF, SME, SEE | BEAS-2B bronchial epithelial cells exposed to DMSO solutions of DEP extracts for 24 hr (equiv µg DEP per assay mL). | Cell viability via LDH release and MTT reduction, inflammatory stress via cytokine release (IL-8, Il-6). | No consistent changes in cytotoxicity, induction of cytokine release significant higher for biodiesel, relative to DF (for SOF expressed on a per mass DEP basis). | 15 |
| 2000 Caterpillar C15 six cylinder 14.6L engine, 2007 MBE 4000 six cylinder 12.8L engine with EGR and DOC/DPF combination, chassis dynamometer UDDS and HHDDT, DEP collected on Teflon®-filters, PFE extraction with DCM followed b y DCM/Tol, SVOCs on PUF/XAD cartridges, DCM extraction. | CARB DF, SME and AFME blends, renewable (NExBTL HVO) | Human U937 macrophages and NCI-H441 Clara cell line (exposure details not provided) | Expression of oxidative and inflammatory stress markers (CYP1A1, COX-2, IL-8, HO-1, MUC5AC). Details not provided. DNA damage by comet. | For C15, some evidence of declines in oxidative stress and inflammatory responses (per engine mile) for biodiesels relative to DF. Strong declines in oxidative stress for HVO (R100). For MBE 4000 some evidence for increase in oxidative stress and inflammatory signalling (SME and AFME only). No appreciable changes in DNA damage (all blends). Nevertheless, some indication of declines for HVO and SME relative to DF, reverse for AFME. | 18 |

**Table 3. Summary of published *in vitro* analyses of naked DNA exposed to diesel exhaust particulate extract**

| Test Article | Fuels Examined | Exposure System | Endpoint(s) Examined | Results Obtained | Reference |
|---|---|---|---|---|---|
| 2003 4.5L Cummins ISBe4 engine and 2007 Zetor Euro 3 engine, ESC, WHSC and NRSC driving cycles. DEP collected with a high-volume sampler, DCM extract. | DF, RME (B100) and RSO | Incubation of Calf thymus DNA with DEP extract for 24 h with and without rat liver S9. | Frequency of stable, bulky DNA adducts by $^{32}$P-postlabelling. | Significant concentration-related increases in adduct frequency for all samples; higher responses with S9. Potency per mg PM similar for two engines, and similar across fuel types, diesel higher for WHSC. Similar potency trend per kWh. | [23] |

**Table 4. Summary of published results of Salmonella mutagenicity analyses of diesel exhaust particulate extracts**

| Test Article | Fuels Examined | Salmonella Strains[a]/Test Version | Results Obtained | Reference |
|---|---|---|---|---|
| DEP and SVOCs from a 1983 Caterpillar 7L heavy-duty engine with DOC, custom 16-mode cycle representing light- and heavy-duty operation. DEP collected on Teflon®-coated GFFs, SVOCs on XAD, DCM Soxhlet extract of DEP and XAD | LSDF and SME | TA98, TA100, TA98NR and TA98/1,8DNP$_6$, microsuspension preincubation version, Aroclor-induced rat liver S9 | Mutagenic potency, per kWh, greater for LSDF compared to SME. Potency far greater for DEP extracts than SVOC samples, and DOC resulted in over 50% reduction in mutagenic activity associated with DEP and SVOC. SME emissions showed lower TPM, and reduced PAHs and 1NP relative to LSFD. | 29 |
| DEP and exhaust condensate from a Mercedes-Benz Euro 3 6.37L, 6-cylinder engine, 13-mode ESC, Teflon®-coated GFFs, DCM Soxhlet extract of DEP | DF, RSO, RME, GTL | TA98 and TA100, standard plate-incorporation assay, PB/5,6BF-induced rat liver S9 | All samples elicited significant positive responses. Potency (per L exhaust) higher without S9 for TA100 only. DEP extracts for RSO and heated RSO fuels yielded the highest potency samples (9.7- to 59 fold greater than DF for TA98 and 5.4- to 22.3-fold for TA100). DEP extracts for RME also significant higher than DF. Condensate samples for RSO and heated RSO also significantly elevated relative to DF (up to 13.5-fold). | 39 |
| DEP from a Fraymann single cylinder engine, 5 load modes (0–85%), with and without DOC. Teflon®-coated GFFs, DCM Soxhlet extract | DF, LSDF, RME, SME | TA98 and TA100, standard plate-incorporation assay, PB/5,6BF-induced rat liver S9 | Mutagenic potency (per hr engine operation) generally lower for RME and SME, compared to DF or LSDF. Under partial load DOC generally led to reduced mutagenicity. Under heavy-duty conditions (rated power), DOC frequently led to increases in mutagenic activity. Without DOC, PM emission rate (g per hr) significantly higher for biodiesel relative to diesel (especially LSDF). Authors note this is likely attributable to higher SOF (g per hr) for biodiesel. | 25 |
| DEP from a Fraymann single cylinder engine, 5 load modes (0–85%), without DOC. Teflon®-coated GFFs, DCM Soxhlet extract | DF, LSDF, RME, SME | TA98 and TA100, standard plate-incorporation assay, PB/5,6BF-induced rat liver S9 | Mutagenic potency, per mg DEP, frequently higher without S9, and DF potency far greater (up to 10-fold) than RME or SME. No response on TA100 for RME and SME. Potency per engine hr yielded similar results and indicates that DF potency is higher at increased load and speed. PAH emissions per engine hr greatest for DF and SME; generally lower for RME. | 33 |

**Table 4. Summary of published results of Salmonella mutagenicity analyses of diesel exhaust particulate extracts**

| Test Article | Fuels Examined | Salmonella Strains[a]/Test Version | Results Obtained | Reference |
|---|---|---|---|---|
| DEP from a Fendt tractor, 13-mode ESC, Teflon®-coated GFFs, DCM Soxhlet extract | DF and RME | TA98 and TA100, standard plate-incorporation assay, PB/5,6BF-induced rat liver S9 | All samples elicited significant positive responses. Both fuels yielded samples more potent (per L exhaust) without S9. At rated power, RME potency far lower than DF. At idling, little difference between with and without S9, and RME potency far lower than DF. DF 2- to 8-fold higher response on TA98 and 2- to 3-fold higher on TA100. RME yields higher particle emissions (g/hr). | 19, 46 |
| DEP from a Volkwagen Vento 1.9L TDI with DOC, FTP-75, MVEG-A, and modified MVEG-A cycles. Teflon®-coated GFFs, DCM Soxhlet extract | DF and RME | TA98, TA97a, TA102, TA100, standard plate-incorporation assay, PB/5,6BF-induced rat liver S9 | Significant positive responses for DF and RME samples on TA98 and TA100, and potency (per mg DEP) generally higher without S9. Potency (per mg DEP) greater for DF compared to RME, particularly on TA98 (1.9- to 5.1-fold). Similar pattern for potency expressed per km. Potency generally higher for cycles that include a cold start (modified MVEG-A). | 21 |
| DEP from two light-duty (1.93L and 2.5L) engines with EGR, EUCD and FTP-75 cycles, with and without DPF, DOC or EGR modifications, DEP collected on Teflon®-coated GFFs, acetone sonication extract followed by separate acetone and benzene Soxhlet extractions | LSDF and biodiesel (unspecified) | TA98, TA100, standard plate-incorporation assay, unspecified S9 | Mutagenic activity (per µg DEP) highest on TA98 without S9. DPF increased potency (per µg DEP or per km) for the 1.93L engine, and decreased potency for the 2.5L engine. DPF dramatically reduced PM emissions per km. Greater engine stress elicited greater mutagenic activity. Biodiesel potency (per µg DEP) lower than reference LSDF, and Biodiesel emissions lower in PAHs and nitro-PAHs. Evaluation of EGR showed reduced potency (per µg DEP or per km) with EGR. | 34 |
| DEP and SVOCs from a 4.6L, 6-cylinder Caterpillar engine, EPA heavy-duty transient test cycle. DEP collected on Teflon®-coated GFF, DCM Soxhlet extract, SVOCs on PUF plugs, supercritical $CO_2$ extraction | DF, RME, HySEE HySEE50 blend (HySEE-hydrogenated soy ethyl ester) | TA98 and TA100, microsuspension preincubation version, Aroclor-induced rat liver S9 | Mutagenic potency of DEP extract (per hp-hr) higher without S9. HySEE potency lower than 50/50 blend with DF, which was lower than DF alone. SVOC samples from DF about 2-fold more mutagenic than HySEE. HySEE associated with considerable reductions in PM and PAH emission rates (per hp-hr). | 28 |
| DEP from 3 diesel engines, 1.686L, 4-cylinder light-duty, 10.8L, 6-cylinder heavy-duty with DPF and SCR, 10.52L, 6-cylinder, heavy-duty with DPF, DEP collected on GFF, DCM Soxhlet extract | DF and plant oils (peanut, rapeseed, soy, sunflower) | TA98, TA100, TA Mix, fluctuation assay (Xenometrics) | All samples in the range of the negative control with no evidence of differences in activity between the fuels. | 37 |

**Table 4. Summary of published results of Salmonella mutagenicity analyses of diesel exhaust particulate extracts**

| Test Article | Fuels Examined | Salmonella Strains[a]/Test Version | Results Obtained | Reference |
|---|---|---|---|---|
| DEP from a 1991 Detroit Diesel DDC Series 60, six cylinder 11.1L engine, heavy-duty transient cycle, DEP collected on Teflon®-coated GFFs, DCM sonication extract | DF, SME, CME, PLME, BTME, YGME (all B100) | TA98, microsuspension preincubation version, Aroclor-induced rat liver S9 | For cold start only, DF and CME more potent without S9. For hot start only, DF, SME and CME appreciably greater without S9. All others more potent with S9. For cold start, with S9, potency (per µg PM equiv) of biodiesel samples all higher than DF. Without S9, all samples except SME more potent than DF. For hot start all biodiesel potency values greater than DF. Mutagenicity emission rates (rev per hph) higher for DF compared with any of the biodiesels. PM emission rate for DF almost 4-fold greater than biodiesel rates. | 30 |
| DEP from a Cummins 5.9L, heavy-duty engine, EPA heavy-duty cycle, with or without DOC, DEP collected on Teflon®-coated GFF, DCM sonication extract | DF, B20 REE, B50 REE, B100 REE | TA98, microsuspension preincubation version, Aroclor-induced rat liver S9 | Mutagenic potency (per µg DEP) showed lowest mutagenicity for REE and highest for DF (with and without DOC). DOC contributed to increases in mutagenic potency per µg DEP. Similar potency pattern for potency expressed per mile. Higher potency with DOC. Some increase in 5- and 6-ring PAH emissions (µg per mile) for REE. | 32 |
| DEP and SVOCs from a Mercedes-Benz, 5.9L, 6-cylinder engine, 13-mode ESC, with and without DOC. DEP collected on Teflon®-coated GFFs, DCM Soxhlet extract, SVOCs from condensates. | 2 DFs, B100 RME, B20 RME | TA98 and TA100, standard plate-incorporation assay, PB/5,6BF-induced rat liver S9 | Mutagenic potency (unit not provided) uniformly higher without S9. Highest response for DF (reference fuel), with lowest for RME5 and RME. DOC further reduced activity of RME. No significant difference in potencies of SVOCs (per $m^3$), with complete elimination of activity by DOC. | 47 |
| DEP and SVOCs from a Mercedes-Benz, 6.37L, 6-cylinder engine, 13-mode ESC. DEP collected on Teflon®-coated GFFs, DCM Soxhlet extract, and condensates from gas phase collected at 50 °C | DF, RME, GTL, RSO, modified RSO | TA98 and TA100, standard plate-incorporation assay, PB/5,6BF-induced rat liver S9 | All samples yielded a positive response, and all potency values (per litre exhaust gas) unchanged or reduced upon addition of S9. DEP extract for RSO yielded the highest potency values (9.7- to 17-fold higher than DF on TA98 and 5.4- to 6.4-fold higher than DF on TA100). Modified RSO potency 2.4- to 3.5-fold higher than RSO. RSO condensate samples also yielded the highest potency values (up to 3-fold DF). Modified RSO 3- to 5-fold higher than RSO. Few differences between DEP extracts for DF, RME and GTL, although RME significantly greater than DF on TA98 with S9 and TA100 without S9. | 40, 41 |

**Table 4. Summary of published results of Salmonella mutagenicity analyses of diesel exhaust particulate extracts**

| Test Article | Fuels Examined | Salmonella Strains[a]/Test Version | Results Obtained | Reference |
|---|---|---|---|---|
| DEP and SVOCs from 3 heavy-duty diesel engines, Mercedes-Benz, 6.37L, 6-cylinder engine, MAN, 6.87L, 6-cylinder engine, AVL single-cylinder, 1.47L engine, 13-mode ESC, ETC, and rated power. DEP collected on Teflon®-coated GFFs, DCM Soxhlet extract, SVOCs from condensates. | DF, GTL, B100 RME, B20 RME | TA98 and TA100, standard plate-incorporation assay, PB/5,6BF-induced rat liver S9 | Mutagenic potency (unit not provided) uniformly higher without S9. For Mercedes engine GTL lowest activity followed by DF. RME similar to DF, but RME20 significantly elevated. For AVL and MAN engines, RME20 significantly elevated relative to DF, but RME lower than DF. For SVOCs from the MAN engine, DF potency greater than RME blends. For the Mercedes and MAN engines, PM emission rates (g/kWh) for RME about half of DF. | 48 |
| DEP from a Mercedes-Benz 6.37L, 6-cylinder and an IVECO 5.9L, 6-cylinder diesel test engine with SCR, 13-mode ESC. DEP collected on Teflon®-coated GFFs, DCM Soxhlet extract. | DF, RME, RSO, SMDS, B5 RME in SMDS, DF/RME/GTL blend. | TA98 and TA100, standard plate-incorporation assay, PB/5,6BF-induced rat liver S9 | Mutagenic potency values uniformly greater without S9. For the Mercedes engine, no significant difference in potency (per L exhaust gas) between DF, RME, SMDS and DF/RME/GTL blend. RO yielded significantly elevated potency (approximately 10-fold), also highest PM output in g/kWh. For the IVECO engine, SCR significantly reduced mutagenic potency, no difference between DF and RME, after 1000hrs SCR less effective. RME associated with reduced PM emissions (g/kWh). | 35, 36 |
| DEP from a Mercedes-Benz 6.37L, 6-cylinder engine, 13-mode ESC. DEP collected on Teflon®-coated GFFs, DCM Soxhlet extract. | Two DFs, RME, GTL, 4 FAME mixtures from soy, palm and rapeseed | TA98, standard plate-incorporation assay, PB/5,6BF-induced rat liver S9 | Mutagenic potency (per $m^3$) greater without S9 and highest for DF. RME potency less than half of DF potency. DEP emission rates lower (per kWh) for all FAMEs. | 26 |
| DEP from a Mercedes-Benz 4.25L, 4-cylinder engine, 13-mode ESC. DEP collected on Teflon®-coated GFFs, DCM Soxhlet extract. | DF, RME, LSDF, LSDF with high aromatic | TA98 and TA100, standard plate-incorporation assay, PB/5,6BF-induced rat liver S9 | Mutagenic potency (per engine hr) lowest for RME. DF 4- to 5-fold higher than RME, LSDF 2- to 3-fold higher. No significant difference with and without S9. DEP emission rates (per kWh) highest for DF. | 24 |
| DEP from a 12L 6 cylinder Euro III truck, no DOC, with or without DFP, 13-mode ESC, DEP collected on Teflon®-coated GFFs, ethanol/DCM (1:1) sonication extract | DF, B100, B5, B10, B20, PPO (pure plant oil) | TA98 and YG1024, YG1029. Standard plate incorporation version, Aroclor-induced rat liver S9 | No significant response in the presence of S9 for any sample. For TA98, significant response for B20 and PPO only. For YG1024, significant responses for B10, B100 and PPO only. Maximum responses on YG1024 for B100 and PPO (per µg PM). Biodiesel associated with reductions in PM (g/kWh), PAHs and oxy-PAHs (µg/kWh). | 22 |

**Table 4. Summary of published results of Salmonella mutagenicity analyses of diesel exhaust particulate extracts**

| Test Article | Fuels Examined | Salmonella Strains[a]/Test Version | Results Obtained | Reference |
|---|---|---|---|---|
| DEPs from four heavy-duty engines (8.5L, 6-cylinder, 7.4L, 6-cylinder and two 9.6L, cylinder), 13-mode ESC. DEP collected on Teflon®-coated GFF, DCM Soxhlet extract | DF, LSDF, 2 reformulated DFs, RME and RME30 | TA98, TA98NR, YG1021, standard plate incorporation assay, Aroclor-induced rat liver S9 | Mutagenic potency uniformly higher without S9. DF showed the highest mutagenic potency (per µg EOM), followed by LSDF reformulated DFs and RME. When expressed per kWh, RME potency lower than DF, but higher than other fuels (due to high EOM per unit mass). Good correlation between mutagenic potency per kWh and PAH emission per kWh. RME potency higher than predicted by PAH content. | 31 |
| DEP from Mercedes-Benz Euro III OM 906 6.37L six cylinder engine, ESC 13-mode test cycle, DEP collected on Teflon®-coated GFF, DCM Soxhlet extract. | DF, RME, LME, SME, PME, CME | TA98, TA100 with and without S9 (details not provided) | Responses higher without S9, and biodiesel responses (unit not provided) lower than DF. TA100 analyses of SME showed similar results relative to DF; B100 somewhat higher response. PM emission rates (g/kW-hr) lower for all biodiesels, relative to DF. PAH emissions for biodiesels far lower, relative to DF (rate not provided). | 42 |
| DEP from an IVECO Euro 2 7.8L, 6-cylinder heavy-duty engine, 13-mode ESC. Teflon®-coated GFFs, toluene ASE extract, SVOCs on PUFs, hexane/acetone (1:1) ASE extract, fractionated on silica into 5 fraction with increasing polarity. | DF and B20 RME | TA98, TA100, TA98, TA98/1,8DNP$_6$, YG1041 standard plate-incorporation assay, Aroclor-induced rat liver S9 | All samples elicited significant positive responses. Potency per mg EOM showed little difference between DF and B20 on either strain. Expression of potency per kWh did not show any difference between DF and B20. Fractionation showed 80–83% of the mutagenicity in fractions containing nitro-PAHs, dinitro-PAHs and oxygenated PAHs. B20 emissions contained slightly lower levels of PAHs. Subsequent study showed greater potency (per kWh) on YG1041 without S9 relative to TA98. | 38, 49 |
| DEP and SVOCs from a heavy-duty, 6-cylinder 6.4L Mercedes-Benz OM 906 LA Euro 3-compliant engine, with and without DOC, ESC. DEP collected on Teflon®-coated GFFs, DCM Soxhlet extract, SVOC on chilled surface. | Low-sulphur DF, RME, B5 RME in diesel | TA98, TA100 standard plate incorporation assay, PB/5,6BF-induced rat liver S9 | Mutagenic potency of DEP (per m$^3$ exhaust) modestly higher without S9. Without S9 potency highest for DF, and decreased for RME and 5% v/v RME. DOC contributed to modest reductions in potency without S9, and slight reductions with S9. DOC eliminated the mutagenic activity of SVOC. | 27 |

**Table 4. Summary of published results of Salmonella mutagenicity analyses of diesel exhaust particulate extracts**

| Test Article | Fuels Examined | Salmonella Strains[a]/Test Version | Results Obtained | Reference |
|---|---|---|---|---|
| DEP and SVOCs from a heavy-duty, 6-cylinder 6.4L Mercedes-Benz OM 906 LA Euro 3-compliant engine, ESC steady state cycle. DEP collected on Teflon®-coated GFFs, DCM Soxhlet extract, SVOC on chilled surface | DF, HVO, RME, JME | TA98, TA100 standard plate incorporation assay, with and without S9 (source not indicated) | Stronger responses for SVOC samples, relative to DEP extracts. SVOC samples and PM extracts for RME and JME elicited similar or greater responses on TA98 (unit not indicated), relative to DF. HVO responses much lower. RME and JME responses on TA100 substantial greater than DF. PM emission rates (g/kWhr) for RME and JME substantially lower than DF. HVO slightly lower. PAH emission rates (ng/test) substantially lower for biodiesels, relative to DF with HVO being the lowest. | 43 |
| 2000 Caterpillar C15 six cylinder 14.6L engine, 2007 MBE 4000 six cylinder 12.8L engine with EGR and DOC/DPF combination, chassis dynamometer UDDS and HHDDT, DEP collected on Teflon®-filters, PFE extraction with DCM followed b y DCM/Tol, SVOCs on PUF/XAD cartridges, DCM extraction. | CARB DF, SME and AFME blends, renewable (NExBTL HVO). | TA98, TA100, microsuspension preincubation version, rat liver S9 | C15 engine DEP extracts, for both TA98 and TA100, general decline in potency (per engine mile) with increasing concentrations of biodiesel. For SVOCs, appreciable decline for HVO only. For MBE4000 samples, appreciable decline in potency for SME blends only. | 18 |

[a]YG1021 – TA98 with plasmid pYG216, nitroreductase overproducing strain. YG1024 – TA98 with plasmid pYG219, O-acetyltransferase overproducing strain. YG1041 – TA98 with plasmid pYG233, nitroreductase and O-acetyl transferase overproducing strain. YG1026 – TA100 with plasmid pYG216, nitroreductase overproducing strain. YG1029 – TA100 with plasmid pYG219, O-acetyl transferase overproducing strain. YG1042 – TA100 with plasmid pYG233, nitroreductase and O-acetyl transferase overproducing strain.

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## REFERENCES

1. Aufderheide M, Mohr U. CULTEX - A new system and technique for the cultivation and exposure of cells at the air/liquid interface. *Experimental and Toxicologic Pathology* 1999;51(6):489-90.

2. Aufderheide M, Mohr U. CULTEX - An alternative technique for cultivation and exposure of cells of the respiratory tract to airborne pollutants at the air/liquid interface. *Experimental and Toxicologic Pathology* 2000;52(3):265-70.

3. Aufderheide M, Knebel JW, Ritter D. An improved in vitro model for testing the pulmonary toxicity of complex mixtures such as cigarette smoke. *Experimental and Toxicologic Pathology* 2003;55(1):51-7.

4. Knebel JW, Ritter D, Aufderheide M. Exposure of human lung cells to native diesel motor exhaust - Development of an optimized in vitro test strategy. *Toxicology in Vitro* 2002;16(2):185-92.

5. Brito JM, Belotti L, Toledo AC, Antonangelo L, Silva FS, Alvim DS, et al. Acute cardiovascular and inflammatory toxicity induced by inhalation of diesel and biodiesel exhaust particles. *Toxicological Sciences* 2010;116:67-78.

6. Steiner S, Czerwinski J, Comte P, Popovicheva O, Kireeva E, Muller L, et al. Comparison of the toxicity of diesel exhaust produced by bio- and fossil diesel combustion in human lung cells invitro. *Atmos Environ* 2013;81:380-8.

7. Yanamala N, Hatfield MK, Farcas MT, Schwegler-Berry D, Hummer JA, Shurin MR, et al. Biodiesel versus diesel exposure: Enhanced pulmonary inflammation, oxidative stress, and differential morphological changes in the mouse lung. *Toxicol Appl Pharmacol* 2013;272:373-83.

8. Agarwal AK, Gupta T, Dixit N, Shukla PC. Assessment of toxic potential of primary and secondary particulates/ aerosols from biodiesel vis-á-vis mineral diesel fuelled engine. *Inhal Toxicol* 2013;25:325-32.

9. Bhavaraju L, Shannahan J, William A, McCormick R, McGee J, Kodavanti U, et al. Diesel and biodiesel exhaust particle effects on rat alveolar macrophages with in vitro exposure. *Chemosphere* 2013;.

10. Hemmingsen JG, Moller P, Nojgaard JK, Roursgaard M, Loft S. Oxidative stress, genotoxicity, and vascular cell adhesion molecule expression in cells exposed to particulate matter from combustion of conventional diesel and methyl ester biodiesel blends. *Environ Sci Technol* 2011; Oct 1;45:8545-51.

11. Ihalainen M, Kuuspalo K, Karunen T, Ruusunen P, Willman P, Tapanainen M, Jalava PI, Salonen RO, Pennanen AS, Happo M, Hirvonen M-R, Jokiniemi J. Biobased Diesel Fuels: Particulate Emissions and Their Inflammation Response. 13th ETH Conference on Combustion Generated Nanoparticles; 2009 June 22-24.

12. Betha R, Pavagadhi S, Sethu S, Hande MP, Balasubramanian R. Comparative in vitro cytotoxicity assessment of airborne particulate matter emitted from stationary engine fuelled with diesel and waste cooking oil-derived biodiesel. *Atmos Environ* 2012;61:23-9.

13. Ackland ML, Zou L, Freestone D, Van De Waasenburg S, Michalczyk AA. Diesel exhaust particulate matter induces multinucleate cells and zinc transporter-dependent apoptosis in human airway cells. *Immunol Cell Biol* 2007;85(8):617-22.

14. Gerlofs-Nijland M, Totlandsdal AI, Tzamkiozis T, Leseman DLAC, Samaras Z, Lag M, et al. Cell toxicity and oxidative potential of engine exhaust particles: Impact of using particulate filter or biodiesel fuel blend. *Environmental Science and Technology* 2013;47:5931-8.

15. Swanson KJ, Kado NY, Funk WE, Pleil JD, Madden MC, Ghio AJ. Release of the pro-inflammatory markers by BEAS-2B cells following in vitro exposure to biodiesel extracts. *Open Toxicology Journal* 2009;3:8-15.

16. Jalava PI, Tapanainen M, Kuuspalo K, Markkanen A, Hakulinen P, Happo MS, et al. Toxicological effects of emission particles from fossil- and biodiesel-fueled diesel engine with and without DOC/POC catalytic converter. *Inhal Toxicol* 2010; Dec;22 Suppl 2:48-58.

17. Jalava PI, Aakko-Saksa P, Murtonen T, Happo MS, Markkanen A, Yli-Pirila P, et al. Toxicological properties of emission particles from heavy duty engines powered by conventional and bio-based diesel fuels and compressed natural gas. *Particle and Fibre Toxicology* 2012;9.

18. Durbin TD, Miller JW, Johnson K, Hajbabaei M, Kado NY, Kobayashi R, et al. CARB Assessment of the

Emissions from the Use of Biodiesel as a Motor Vehicle Fuel in California. Biodiesel Characterization and NOx Mitigation Study. Final Report. Sacramento, CA: California Air Resources Board; 2011.

19. Bunger J, Krahl J, Baum K, Schroder O, Muller M, Westphal G, et al. Cytotoxic and mutagenic effects, particle size and concentration analysis of diesel engine emissions using biodiesel and petrol diesel as fuel. *Arch Toxicol* 2000; Oct;74:490-8.

20. Liu YY, Lin TC, Wang YJ, Ho WL. Biological toxicities of emissions from an unmodified engine fueled with diesel and biodiesel blend. *Journal of Environmental Science and Health - Part A Toxic/Hazardous Substances and Environmental Engineering* 2008;43:1735-43.

21. Bunger J, Krahl J, Franke HU, Munack A, Hallier E. Mutagenic and cytotoxic effects of exhaust particulate matter of biodiesel compared to fossil diesel fuel. *Mutat Res* 1998; Jul 8;415:13-23.

22. Kooter IM, van Vugt, M. A. T. M., Jedynska AD, Tromp PC, Houtzager MMG, Verbeek RP, et al. Toxicological characterization of diesel engine emissions using biodiesel and a closed soot filter. *Atmos Environ* 2011;45:1574-80.

23. Topinka J, Milcova A, Schmuczerova J, Mazac M, Pechout M, Vojtisek-Lom M. Genotoxic potential of organic extracts from particle emissions of diesel and rapeseed oil powered engines. *Toxicol Lett* 2012;212:11-7.

24. Krahl J, Munack A, Schröder O, Stein H, Bunger J. Influence of Biodiesel and Different Designed Diesel Fuels on the Exhaust Gas Emissions and Health Effects. Warrendale, PA: SAE International; 2003. Report No.: SAE Technical Paper Series 2003-01-3199.

25. Bunger J, Krahl J, Weigel A, Schroder O, Bruning T, Muller M, et al. Influence of fuel properties, nitrogen oxides, and exhaust treatment by an oxidation catalytic converter on the mutagenicity of diesel engine emissions. *Arch Toxicol* 2006; Aug;80:540-6.

26. Krahl J, Munack A, Schroder O, Stein H, Herbst L, Kaufmann A, et al. The Influence of Fuel Design on the Exhaust Gas Emissions and Health Effects. Warrendale, PA: SAE International; 2005. Report No.: SAE Technical Series 2005-01-3772.

27. Westphal GA, Krahl J, Munack A, Ruschel Y, Schroder O, Hallier E, et al. Mutagenicity of diesel engine exhaust is eliminated in the gas phase by an oxidation catalyst but only slightly reduced in the particle phase. *Environmental Science and Technology* 2012;46:6417-24.

28. Chase C, Peterson C, Lowe G, Mann P, Smith J, Kado N. A 322,000 kilometer (200,000 mile) Over the Road Test with HySEE Biodiesel in a Heavy Duty Truck. Warrendale, PA: SAE Iternational; 2000. Report No.: SAE Technical Paper Series 2000-01-2647.

29. Bagley ST, Gratz LD, Johnson JJ, McDonald JF. Effects of an oxidation catalytic converter and a biodiesel fuel on the chemical, mutagenic, and particle size characteristics of emissions from a diesel engine. *Environ Sci Technol* 1998;32:1183-91.

30. Kado N, Kuzmicky P. Bioassay Analyses of Particulate Matter from a Diesel Bus Engine Using Various Biodiesel Feedstock Fuels. Final Report. Report 3 in a Series of 6. Golden, CO: National Renewable Enegry Laboratory; 2003. Report No.: NREL/SR-510-31463.

31. Rantanen L, Mikkonen S, Nylund L, Kociba P, Lappi M, Nylund N. Effect of Fuel on the Regulated, Unregulated and Mutagenic Emissions of DI Diesel Engines. Warrendale, PA: SAE International; 1993. Report No.: SAE Technical Paper Series 932686.

32. Kado N, Okamoto R, Kuzmicky P. Chemical and Bioassay Analyses of Diesel and Biodiesel Particulate Matter: Pilot Study. Helena, MT: The Montana Department of Environmental Quality; 1996.

33. Bunger J, Muller MM, Krahl J, Baum K, Weigel A, Hallier E, et al. Mutagenicity of diesel exhaust particles from two fossil and two plant oil fuels. *Mutagenesis* 2000; Sep;15:391-7.

34. Carraro E, Locatelli AL, Ferrero C, Fea E, Gilli G. Biological activity of particle exhaust emissions from light-duty diesel engines. *J Environ Pathol Toxicol Oncol* 1997;16:101-9.

35. Krahl J, Munack A, Grope N, Ruschel Y, Schroder O, Bunger J. Biodiesel, rapeseed oil, gas-to-liquid, and a premium diesel fuel in heavy duty diesel engines: endurance, emissions and health effects. (Special Issue:

Celebrating the 60th birthday of Professor Mufit Bahadir.). *CLEAN - Soil, Air, Water* 2007;35:417-26.

36. Krahl J, Munack A, Ruschel Y, Schroder O, Schwarz S, Hofmann L, et al. Influence of the Phosphorus Content in Rapeseed Oil Methyl Esters During a 1000 Hours Endurance Test on the Function of a SCR-system Measured by Exhaust Gas Emissions and Health Effects. Warrendale, PA: SAE International; 2006. Report No.: SAE Technical Paper Series 2006-01-3282.

37. Dorn B, Zahoransky R. Non-Esterified Plant Oils as Fuel -Engine Characteristics, Emissions and Mutagenic effects of PM. Warrendale, PA: SAE International; 2009. Report No.: SAE Technical Paper Series 2009-24-0097.

38. Turrio-Baldassarri L, Battistelli CL, Conti L, Crebelli R, De Berardis B, Iamiceli AL, et al. Emission comparison of urban bus engine fueled with diesel oil and 'biodiesel' blend. *Sci Total Environ* 2004; Jul 5;327:147-62.

39. Bunger J, Krahl J, Munack A, Ruschel Y, Schroder O, Emmert B, et al. Strong mutagenic effects of diesel engine emissions using vegetable oil as fuel. *Arch Toxicol* 2007; Aug;81:599-603.

40. Krahl J, Munack A, Ruschel Y, Schroder O, Bunger J. Comparison of Emissions and Mutagenicity from Biodiesel, Vegetable Oil, GTL and Diesel Fuel. Warrendale, PA: SAE International; 2007. Report No.: SAE Technical Paper Series 2007-01-4042.

41. Krahl J, Knothe G, Munack A, Ruschel Y. Comparison of exhaust emissions and their mutagenicity from the combustion of biodiesel, vegetable oil, gas-to-liquid and petrodiesel fuels. *Fuel* 2009; 06;88:1064-9.

42. Emissions from diesel engines using fatty acid methyl esters from different vegetable oils as blends and pure fuel. 25th International Congress on Condition Monitoring and Diagnostic Engineering. Journal of Physics: Conference Series Volume 364; 2012IOP Publishing Ltd; .

43. Westphal GA, Krahl J, Munack A, Rosenkranz N, Schroder O, Schaak J, et al. Combustion of hydrotreated vegetable oil and jatropha methyl ester in a heavy duty engine: Emissions and bacterial mutagenicity. *Environmental Science and Technology* 2013;47:6038-46.

44. Emissions, ultra fine particles and health effects from exhaust gas of heavy-duty engines running on first and second generation alternative dieses fuels. ; 2007Technische Akademie Esslingen; p. 77-90.

45. Bunger J, Krahl J, Schroder O, Schmidt L, Westphal GA. Potential hazards associated with combustion of bio-derived versus petroleum-derived diesel fuel. *Crit Rev Toxicol* 2012;42:732-50.

46. Schröder O, Krahl J, Munack A, Bunger J. Environmental and Health Effects Caused by the Use of Biodiesel. Warrendale, PA: SAE International; 1999. Report No.: SAE Technical Paper Series 1999-01-3561.

47. Krahl J, Schroder O, Ruschel Y, Bünger J. Ultrafine particles from a heavy duty diesel engine running on rapeseed oil methyl ester. *SAE Int J Fuels Lubr* 2009;2(2):132-46.

48. Krahl J, Munack A, Ruschel Y, Schroder O, Bunger J. Exhaust Gas Emissions and Mutagenic Effects of Diesel Fuel, Biodiesel and Biodiesel Blends. Warrendale, PA: SAE International; 2008. Report No.: SAE Technical Paper Series 2008-01-2508.

49. Alsberg T, Stenberg U, Westerholm R, Strandell, M., Rannug, U., Sundvall A, Romert L, et al. Chemical and biological characterization of organic material from gasoline exhaust particles. *Environ Sci Technol* 1985;19:43-50.

Review of
Staff Report: Multimedia Evaluation of Biodiesel

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The staff report, prepared by the Multimedia Working Group (MMWG), provides an overall assessment of potential impacts on public health and the environment that may result from the production, use, and disposal of biodiesel, which is defined as a fuel composed on mono-alkyl esters of long chain fatty acids derived from vegetable oils or animal fats and meets the specifications set forth by ASTM International Standard D6751. The report concludes that biodiesel will not cause a significant adverse impact on public health or the environment. The conclusion was made largely based on the results of the "California Biodiesel Multimedia Evaluation Final Tier III Report" from the researchers at University of California. As requested, this reviewer provides the following assessment and determination of whether each of the conclusions that constitute the basis of the staff report is based on sound scientific knowledge, methods, and practices, and if additional issues need to be addressed.

## Overall Comments on the reports

The Staff Report is based on a cascade of studies conducted by University of California (UC) researchers. The PIs at UC are known scientists in the field. The evaluation procedure, as outlined in their final Tier III report, is sequential and logic. Literature cited in their reports is quite complete and up to date. Experiments were well designed and conducted. Data were carefully collected and analyzed. Therefore, it is fair to say that the UC final Tier III report and the Staff Report are based on sound scientific knowledge, methods, and practices. And consequently, the conclusions of the Staff Report are acceptable.

## Comments on specific conclusion statements

1. Air Emissions Evaluation. Air Resources Board (ARB) staff concludes that the use of biodiesel does not pose a significant adverse impact on public health 0r the environment from potential air quality impacts.

Based on engine and chassis emissions testing on multiple blends of biodiesel compared to the baseline California Air Resources Board (CARB) diesel fuel, the report concludes that for most of the criteria pollutants, toxic air contaminants, ozone precursors, and greenhouse gases, biodiesel blends could either reduce the emission into the atmosphere or impose only an insignificant adverse impact on air quality. This reviewer in general agrees with the findings of the evaluation studies that focused on the use of biodiesel. There might be a need to grow, storage, transport and process a large amount of biodiesel feedstock if portion of the biodiesel is produced with local resources. Further studies on the impact of these processes on air quality may be needed when large amount of biodiesel is used and produced in the state.

2. Water Evaluation. State Water Resources Control Board (SWRCB) staff concludes that there are minimal additional risks to beneficial uses of California waters by biodiesel than that posed by CARB diesel alone.

Water evaluation focused on aquatic toxicity and risks associated with fuel transport and storage (UST in particular). The report has concluded that biodiesel and biodiesel blends slightly increase the toxicity to subsets of screening species compared to CARB diesel, and that the adverse impact on public health and the environment is insignificant. Similar to the air emissions evaluation, the study does not include the effect of biodiesel production and distribution on water quality. Biodiesel is produced from biological feedstock, including plant and animal materials. Some are produced from community wastes (like “yellow grease”). It is not appropriate to assume that all biodiesel used in California will come from sources outside the state. If certain portion of the feedstock is from sources inside the state, or if certain portion of the production (conversion) process is done inside the state, an evaluation of the impact on water resources/quality by growing, transportation, storage, and conversion of large amount of biodiesel feedstock will be necessary.

3. Public Health Evaluation. Office of Environmental Health Hazard Assessment (OEHHA) staff concludes that the substitution of biodiesel for CARB diesel reduces the rate of carbon dioxide to the atmosphere and reduces the amount of particulate matter (PM), benzene, ethyl benzene, and polycyclic aromatic hydrocarbons (PAHs) released into the atmosphere, but may increase the emissions of oxides of nitrogen (NOx) and acrolein for certain blends.

Impact of biodiesel on public health was assessed by comparing the combustion emissions against that with petroleum based diesel fuels. Data show that there is a reduction in most of the primary pollutants from burning biodiesel, but a statistically significant increase in NOx. Since NOx is the main ingredient for ground level ozone, there should be a study on this secondary pollutant. Also, impact of feedstock collection, storage, transportation, and processing needs to be assessed if certain portion of the biodiesel is locally produced.

4. Soil and Hazardous Waste Evaluation. Department of Toxic Substances Control (DTSC) staff concludes that biodiesel aerobically biodegrades more readily than CARB diesel, has

potentially higher aquatic toxicity for a small subset of tested species, and generally has no significant difference in vadose zone infiltration rate.

Soil pollution and hazardous waste generation can occur during production, transportation, storage and use of biodiesel. It is very difficult to conduct a complete evaluation of the impact of biodiesel on soil and hazardous waste impact because there is a large variation in feedstock type, production method, composition (additives) and chemical properties (some of them are not yet known). The DTSC staff concludes that biodiesel is more readily to aerobically biodegrade, with higher potential aquatic toxicity for a small subset of tested species, and having a similar rate of vadose zone infiltration, compared to CARB diesel. However, the report also mentioned that biodiesel tends to move faster in the vertical than horizontal direction in subsurface soil, indicating a concern on potential deep soil and groundwater contamination. The transport and fate of chemicals in multimedia environment is also very strongly affected by meteorological and climatic variables. The studies conducted by UC researchers probably are sufficient for the purpose of impact evaluation. More research is called in the future for a better understanding of the multimedia transport and fate processes in biodiesel feedstock and fuel production, distribution, use and disposal.

Because the chemical composition of renewable diesel is similar to that of CARB diesel and renewable diesel has a lower content of aromatic hydrocarbons than CARB diesel, I agree with the DTSC staff on that the impacts on soil, surface water and groundwater of renewable diesel are similar to or less severe than that of CARB diesel. As pointed out by the DTSC Staff Report, the chemical composition and additives may vary with different feedstock and production processes. Large amount of biological feedstock also needs to be transported, stored, and processed should certain renewable diesel be produced locally. Therefore, additional studies may be needed in the future for regulatory purposes.

# APPENDIX J

## Multimedia Working Group Responses to Peer Review Comments

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**Multimedia Working Group Response**
**to Peer Review Comments**

The Multimedia Working Group (MMWG) appreciates the thorough written reviews submitted by the peer reviewers. The reviews and comments by the peer review panel have prompted the MMWG to further clarify and improve the "*Staff Report: Multimedia Evaluation of Biodiesel"* (Biodiesel Staff Report) in preparation for the MMWG's final submittal to the California Environmental Policy Council (CEPC).

In this appendix, each reviewer's comments are organized by topic and reproduced as submitted. The MMWG's corresponding response follows each comment. The MMWG includes staff from the Air Resources Control Board (ARB), Office of Environmental Health and Hazard Assessment (OEHHA), State Water Resources Control Board (SWRCB), and Department of Toxic Substances Control (DTSC). Based on the topic, the appropriate agency staff within the MMWG prepared a detailed response to each comment. The following format is used to present the reviewer's comments and the MWMG's responses:

| |
|---|
| Topic<br><br>[***Comment Number.***] **Comment:** [*Reviewer's Comment.*] (*Reviewer's last name, page number*)<br><br>**Response:** [*MMWG Agency's Response.*] (*Agency*) |

Similar comments with the same response are grouped together. Also, the citations included in the MMWG's responses are referenced as footnotes at the bottom of the page. Where applicable, the information provided in the responses has been incorporated in the Biodiesel Staff Report.

## Comments and Responses

Air Quality

**A-1.** **Comment:** Section 1. (p. 7) is labeled "Criteria Pollutants." This section should begin with a discussion of what pollutants fall into this category, and which are evaluated here for biodiesel. Currently, this information is provided on p. 8, paragraph 2.

As written, Section 1 includes PM, nitrogen oxides (NOx = NO + $NO_2$), total hydrocarbons (THC), carbon monoxide (CO) and carbon dioxide ($CO_2$). However, THC and $CO_2$ are not criteria pollutants and do not belong in this section. $SO_2$ is a criteria pollutant that is not discussed here, but which may be

reduced (per EPA1) by substituting biodiesel for petroleum diesel. NOx includes both the criteria pollutant $NO_2$ as well as NO, which is not a criteria pollutant. It would be helpful to know the size distribution of the PM emissions, for consistency with the criteria emissions categories of $PM_{2.5}$ and $PM_{10}$. Section 1 should report on all criteria pollutant emissions (or precursor emissions) in some way, and omit discussion of emissions that are not criteria pollutants. (Holloway, pg 2)

**Comment:** As noted, discussion of $CO_2$ emissions should be removed from Section 1, because $CO_2$ is not a criteria pollutant. It would fit more clearly in Section 4 (p. 10) on Greenhouse Gas Emissions. (Holloway, pg 2)

**Response:** The title of Section 1 in the Biodiesel Staff Report by the MMWG was revised from "Criteria Pollutants" to "Air Emissions." Not all air pollutants tested under the "*CARB Assessment of the Emissions from the Use of Biodiesel as a Motor Vehicle Fuel in California*" (ARB Emissions Study)[1] were criteria pollutants.

As defined in the proposed Alternative Diesel Fuel (ADF) regulation, a "criteria pollutant" is "any air pollutant for which a California ambient air quality standard (CAAQS) or national ambient air quality standard (NAAQS) has been established."[2] These air pollutants are called "criteria" pollutants because they are regulated by set standards and emission limits. California and national standards have been established for various pollutants, including particulate matter (PM), nitrogen dioxide ($NO_2$), sulfur dioxide ($SO_2$), ground-level ozone ($O_3$), CO, and lead.

The ARB Emissions Study focused primarily on regulated emissions, including nitrogen oxides (NOx), PM, CO, $CO_2$, and THC. The Biodiesel Staff Report was revised to include PM, CO, $CO_2$, and NO discussions in Section 1 (retitled to "Air Emissions"). Also, Section 3 ("Ozone Precursors") was switched with Section 4 ("Greenhouse Gas Emissions") so that general information on greenhouse gas emissions (GHG) is provided first. Additional data and information on life cycle and tailpipe $CO_2$ emissions were also added to revised Section 3 ("Greenhouse Gas Emissions," previously "Ozone Precursors") and NOx and THC results were added to revised Section 4 ("Ozone Precursors," previously "Greenhouse Gas Emissions"). (ARB)

**A-2.** **Comment:** Section 3 (p. 9) discusses "Ozone Precursors." Because ozone is a criteria pollutant, this section would seem to be a better fit with Section 1 and/or follow directly afterward. For the benefit of readers unfamiliar with ozone chemistry, some brief comment should be added explaining that THC and NOx emissions determine ozone concentrations. (Holloway, pg 3)

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[1] Durbin, T.D. et al. *CARB Assessment of the Emissions from the Use of Biodiesel as a Motor Vehicle Fuel in California "Biodiesel Characterization and NOx Mitigation Study."* October 2011.
[2] Air Resources Board. *Proposed Regulation on the Commercialization of New Alternative Diesel Fuels Staff Report: Initial Statement of Reasons*. January 2, 2015. Appendix A: Proposed Regulation Order. Page A-8.

**Response:** Section 4 ("Ozone Precursors") was revised to include an additional statement to clarify that both THC and NOx emissions determine ozone concentrations. Also, Section 1 (previously titled "Criteria Pollutants") was revised to "Air Emissions" and would, therefore, no longer fit under Section 1.

Lastly, as previously stated, Section 3 was switched with Section 4 so that the discussion of ozone precursors follows directly after GHG emissions. (ARB)

**A-3.** **Comment:** Section 4 (p. 10) reports on Greenhouse Gas Emissions. This section would benefit from a number of changes. First, clarifying which greenhouse gas emissions have been evaluated – it appears only $CO_2$. As noted above, the $CO_2$ discussion from Section 1 should be moved to Section 4. The discussion notes an increase in fuel consumption due to the lower energy density of biodiesel. However, this analysis is of limited value, given that the fuel consumption impacts are given quantitatively, whereas the energy density changes are given only qualitatively. It would be helpful to include a more appropriate metric to compare the net $CO_2$ emissions from vehicle operation with CARB diesel versus biodiesel. (Holloway, pg 3)

**Response:** Section 3 (previously Section 4) was revised to include more general information on GHG emissions. Section 3 now includes examples of GHG emissions and identifies $CO_2$ as one of the GHGs tested under the ARB Emissions Study. (ARB)

**A-4.** **Comment:** In addition, this section [Section 1] should be edited to clarify that the measured increase in $CO_2$ emissions does not suggest that biodiesel leads to a net increase in carbon emissions. It may be useful to note that a) end-of-pipe $CO_2$ emissions are only one component in determining a fuel's life cycle carbon emissions (including uptake by feedstocks); b) an increase in $CO_2$ reflects more complete combustion, and is an expected result of decreased THC and CO emissions; c) the vast majority of THC and CO more convert to $CO_2$ in the atmosphere, so the total $CO_2$ produced by the biodiesel combustion process is determined by direct $CO_2$ emissions, as well as THC and CO. As written, the discussion of $CO_2$ emissions could be misleading and a source of potential confusion. (Holloway, pg 2)

**Response:** As previously stated, Section 1 was retitled "Air Emissions" and revised to include an overview of ARB Emissions Study results for PM, NOx, THC, CO, and $CO_2$. Specific details on $CO_2$, as noted in the comment above, were also added to revised Section 3 ("Greenhouse Gas Emissions"), including clarification that an increase in direct tailpipe $CO_2$ emissions do not necessary reflect an overall net increase in carbon emissions. Further clarifications were also added after tailpipe $CO_2$ results were provided in the section.

End-of-pipe or tailpipe emissions include exhaust emissions associated with the use of a fuel in an internal combustion engine.[3] Tailpipe $CO_2$ emissions are only one component in determining a fuel's life cycle carbon emissions. As explained in the reviewer's comment, an increase in $CO_2$ tailpipe emissions does not necessarily lead to an overall increase in carbon emissions. An increase in $CO_2$ reflects more complete combustion and is an expected result of decreased THC and CO in tailpipe emissions. Also, secondary $CO_2$ formation is not accounted for in total life cycle carbon emissions. A life cycle analysis includes direct emissions associated with producing, transporting and using the fuel, and indirect effects such as emissions from land use changes. (ARB)

**A-5.** **Comment:** Section 2 (p. 9) discusses "Toxic Air Contaminants." The discussion notes that the reduction in PM emissions would be expected to decrease toxic risk from diesel PM. This is a reasonable conclusion. In addition, some discussion should be included on the PM speciation from biodiesel versus petroleum diesel. (Holloway, pg 3)

**Response:** As part of the ARB Emissions Study, real-time PM measurements were made to characterize size distribution and number concentration, particle length and diameter concentration, and particle-bound PAH concentration. The details of the data analysis, including the number of tests, driving runs, instruments used, and results are provided in the "*CARB Assessment of the Emissions from the Use of Biodiesel as a Motor Vehicle Fuel in California 'Biodiesel Characterization and NOx Mitigation Study*.'"[4] (ARB)

**A-6.** **Comment:** As written, Section 3 only discusses one ozone precursor: NOx. At a minimum, it should include both THC and NOx. Because THC is not a criteria pollutant, the discussion of THC from Section 1 would fit better here. Furthermore, the expected ozone impacts of THC reductions and NOx increases deserves some discussion. It may be beyond the scope of this report to comment on the expected ozone response to these competing precursor sensitivities. However, some qualitative comment would be helpful to frame the importance of the THC and NOx response to biodiesel. (Holloway, pg 3)

**Response:** Section 4 (previously Section 3) was revised to include a discussion of both NOx and THC and general information about ozone precursors. Also, as previously stated, Section 1 was revised to include THC. THC is now discussed in both revised sections. (ARB)

**A-7.** **Comment:** Details are provided on the test vehicles used for emission tests (p. 7-8). It would be helpful to know how these were selected, and whether they are typical of the California vehicle fleet. (Holloway, pg 2)

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[3] Air Resources Board. Proposed Regulation to Implement the Low Carbon Fuel Standard. Staff Report: Initial Statement of Reasons. 2009, IV-12.

[4] Durbin, T.D. et al. *CARB Assessment of the Emissions from the Use of Biodiesel as a Motor Vehicle Fuel in California "Biodiesel Characterization and NOx Mitigation Study."* October 2011, 27-28.

**Response:** Section A was revised to include additional information on the test engines and vehicles used for emissions testing. The ARB Emissions Study included both engine testing and chassis testing. Engine testing was performed on a 2006 Cummins ISM engine and a 2007 MBE4000 engine. Chassis testing was performed on the following test vehicles:

- 2006 International Truck equipped with a 2006 Cummins ISM engine
- 2008 Freightliner Truck equipped with a 2007 MBE4000 engine
- 2000 Freightliner Truck equipped with a 2000 Caterpillar C-15 engine
- Kenworth model T800 truck equipped with a 2010 Cummins ISX engine

Test engines included typical six cylinder, in-line, direct injection, turbocharged, heavy-duty diesel engines. The engines were selected from two model year categories; 2002-2006 and 2007-2009. The 2002-2006 engines were estimated to represent an important contribution to the emissions inventory from the present through 2017. The 2007-2009 model year engine represented the latest technology that was available at the time of testing.[5] (ARB)

**A-8. Comment:** The difference between end-of-pipe emissions and life-cycle emissions should be more clearly defined in section 4. Overall, the paragraph (p. 10) discussing life cycle emissions is unclear. It would benefit from more detail on what steps in the life cycle were considered. In addition, it would be helpful to note that the 95% reduction in GHG emissions would arise from waste-oil feedstock use, whereas the 15% reduction in GHG emissions would arise from soybean production in the Midwestern U.S. (Holloway, pg 3)

**Response:** Sections 3 ("Greenhouse Gas Emissions") and 4 ("Ozone Precursors") were revised to include more detailed information about life cycle emissions and analysis. Life cycle GHG emissions include emissions associated with the production, transportation, and use of a fuel in a motor vehicle. The life cycle analysis (LCA) of a fuel includes direct emissions from producing, transporting, and using the fuel, as well as other indirect effects, including land use change. Depending on the fuel, GHG emissions from each step of the life cycle can include $CO_2$, $CH_4$, $N_2O$, and other GHG contributors. The "carbon intensity" of a fuel represents the equivalent amount of $CO_2$ emitted from each stage of the fuel's life cycle and is expressed in terms of grams of $CO_2$ equivalent per megajoule ($gCO_2e/MJ$).[6]

The difference between tailpipe and life cycle emissions was also clarified in Section 3. End-of-pipe or tailpipe emissions only include exhaust emissions associated with the use of a fuel in an internal combustion engine.[7] Tailpipe $CO_2$ emissions are only one component in determining a fuel's life cycle carbon emissions. Therefore, the measured

[5] Durbin, T.D. et al. *CARB Assessment of the Emissions from the Use of Biodiesel as a Motor Vehicle Fuel in California "Biodiesel Characterization and NOx Mitigation Study."* October 2011, 5-6.
[6] Air Resources Board. *Proposed Re-Adoption of the Low Carbon Fuel Standard. Staff Report: Initial Statement of Reasons.* December 2014.
[7] Air Resources Board. *Proposed Regulation to Implement the Low Carbon Fuel Standard. Staff Report: Initial Statement of Reasons.* 2009, IV-12.

increase in $CO_2$ emissions may not necessarily lead to an overall increase in carbon emissions. An increase in $CO_2$ reflects more complete combustion, and is an expected result of decreased THC and CO emissions. (ARB)

**A-9. Comment:** This reviewer in general agrees with the findings of the evaluation studies that focused on the use of biodiesel. There might be a need to grow, storage, transport and process a large amount of biodiesel feedstock if portion of the biodiesel is produced with local resources. Further studies on the impact of these processes on air quality may be needed when large amount of biodiesel is used and produced in the state. (Yang, pg 2)

**Response:** In general, each agency's evaluation and resulting conclusions are based on the results of the multimedia evaluation and information provided in the "*California Biodiesel Multimedia Evaluation Tier I Final* Report" (Tier I Report),[8] "*California Biodiesel Multimedia Evaluation Tier II Report on Aquatic Toxicity, Biodegradation, and Subsurface Transport Experiments*" (Tier II Report),[9] and "*California Biodiesel Multimedia Evaluation Final Tier III* Report" (Tier III Report)[10] by the University of California (UC), Berkeley and UC Davis.

The purpose of the multimedia evaluation is to provide the information needed for the development of fuel regulations and to inform the overall rulemaking process. Under Health and Safety Code (HSC) section 43830.8(b), a multimedia evaluation is defined as "the identification and evaluation of any significant adverse impact on public health or the environment, including air, water, and soil, that may result from the production, use, or disposal of the motor vehicle fuel that may be used to meet the state board's motor vehicle fuel specifications."[11] Therefore, the primary focus of the multimedia evaluation is to determine the direct health and environmental impacts from biodiesel. As mentioned in the Tier I Report, other life cycle and indirect impacts may be of interest, including land use change and the production and use of raw feedstocks. These are outside the scope of this evaluation but are addressed under the LCFS program as part of the LCFS life cycle analysis.[12]

As part of the California Environmental Quality Act (CEQA), State and local agencies are required to identify any significant environmental impacts of their actions and to avoid or mitigate those impacts, if feasible, when new projects are proposed. Biodiesel production facilities have undergone the CEQA analysis and all future projects will undergo the same CEQA analysis. Also, as part of the rulemaking process for the proposed ADF regulation, an environmental analysis, including a full CEQA analysis, was completed. (ARB)

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[8] Ginn, T.R. et al. *California Biodiesel Multimedia Evaluation Tier I Report*, Sept 2009.
[9] Ginn, T.R. et al. *California Biodiesel Multimedia Evaluation Tier II Report on Aquatic Toxicity, Biodegradation, and Subsurface Transport Experiments*, Jan 2012.
[10] Ginn, T.R. et al. *California Biodiesel Multimedia Evaluation Final Tier III Report*, June 2014.
[11] California Air Pollution Control Laws. Health and Safety Code, Division 26, Part 5, Chapter 4, Section 43830.8(b).
[12] McKone, T.E. et al. *California Renewable Diesel Multimedia Evaluation Tier I Report*, Sept 2011. A-22, A-17, A-7.

**A-10. Comment:** Since NOx is the main ingredient for ground level ozone, there should be a study on this secondary pollutant. Also, impact of feedstock collection, storage, transportation, and processing needs to be assessed if certain portion of the biodiesel is locally produced. (Yang, pg 2)

**Response:** NOx emissions from biodiesel compared to CARB diesel were thoroughly evaluated as a significant part of both the ARB Emissions Study and B5/B10 Study. Section 4 (previously Section 3) provides a thorough discussion of ozone precursors, including NOx.

In regard to potential impacts of biodiesel feedstock collection, storage, transportation, and processing needs, as previously stated, the primary focus of a multimedia evaluation is to determine the direct health and environmental impact of a fuel. Other life cycle and indirect impacts may be of interest, including land use change and the production and use of raw feedstocks. These are outside the scope of the multimedia evaluation but are addressed under the LCFS program as part of the LCFS life cycle analysis.[13]

The LCFS life cycle analysis includes the direct emissions associated with producing, transporting, and using a fuel, as well as indirect effects such as land use change. For more information on the full life cycle analysis of biodiesel fuels produced from various feedstocks, please refer to the detailed fuel pathway documents posted on the *LCFS Fuel Pathways Documents* webpage.[14] (ARB)

<u>Public Health</u>

**B-1. Comment:** Overall, the public health evaluation seemed to be redundant with the air emissions evaluation, and lacking any specific discussion of health impacts. The public health conclusions are supported, in that Section 1 ("Combustion Emissions") summarizes the same changes in emissions presented in the Air Evaluation (p. 7-9). However, the report would be strengthened with a clearer discussion of health impacts.

At a minimum, the public health evaluation should address the conclusions on both air and water impacts in terms of health outcomes. For example, discussing the health outcomes of the PM reductions - both in terms of acute effects and toxicity - on exposed populations. (Holloway, pg 4)

**Response:** The purpose of the MMWG Staff Report is to provide a summary of the biodiesel multimedia evaluation (i.e., the Tier I, II & III reports) as well as to formulate conclusions and recommendations to the California Environmental Policy Council (CEPC) based on the evaluation. The MMWG was able to develop its conclusions and

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[13] McKone, T.E. et al. *California Renewable Diesel Multimedia Evaluation Tier I Report*, Sept 2011. A-22, A-17, A-7.

[14] Air Resources Board Low Carbon Fuel Standard Fuel Pathways Documents webpage: http://www.arb.ca.gov/fuels/lcfs/workgroups/workgroups.htm#pathways.

recommendations by comparing the relative emission levels and toxicities of air pollutants emitted by engines using biodiesel and diesel fuels. For example, the multimedia evaluation reported that benzene emissions from biodiesel (B100) combustion under several representative emission scenarios were significantly lower than those from CARB diesel combustion, implying that the cancer and non-cancer risks of exposure to benzene from the use of B100 would show a similar relationship. As such, a detailed discussion of the health outcomes of exposure to specific air pollutants was not required to carry out this analysis.

Regarding the impacts on human health from PM in diesel combustion emissions, there are Ambient Air Quality Standards for both short term (24 hr) and chronic (annualized average) exposure to PM2.5, based on dose-response analyses of the impact on cardiovascular mortality from many studies in the literature. Comparing the relative emissions of PM2.5 is reasonable from the standpoint of consistent effects observed across many studies in different geographic locations, populations, and mixes of pollutants. Having said that, the various adverse effects at the cellular and tissue level contributing to observed cardiovascular and respiratory morbidity and mortality (and other adverse health outcomes studied) in humans may depend on both the physical nature and chemical content of particles. There may be significant differences in physical nature of PM from biodiesel and PM from CARB diesel. There are clear differences in chemical composition of the complex mixture in the PM from these two sources. However, information on chemical species and toxicity to humans of the chemicals present in the PM is far from complete. As a consequence OEHHA scientists are only able to predict the magnitude of changes in human health effects that will result from replacing CARB diesel with biodiesel generally using what we know about the observed effects of PM. This is not to say there are not differences in sources of PM, but rather that we cannot determine differences in effect based on particle composition. (OEHHA)

**B-2.** **Comment:** Section 2 (p. 13), entitled "Impact on Atmospheric Carbon Dioxide" does not have clear health linkages discussed. Overall, this explanation is unclear. Topically the material fits better in Section A4 where greenhouse emissions and life cycle impacts are discussed. (Holloway, pg 4)

**Response:** The discussion of $CO_2$ emissions from biodiesel relative to diesel combustion was moved to section A-3 (previously A-4) of Chapter II of the Staff Report. (OEHHA)

**B-3.** **Comment:** Office of environmental health hazard assessment staff concludes that the substitution of biodiesel for CARB diesel reduces the rate of emission of carbon dioxide to the atmosphere and reduces the amount of particulate matter, benzene, ethylbenzene, and polycyclic aromatic hydrocarbons released into the atmosphere, but may increase emissions of oxides of nitrogen and acrolein for certain blends.

I find that this conclusion of the report is based on sound scientific knowledge, methods, and practices. As noted above the increase NOx emissions are offset by the decrease VOC emissions and may lead to less overall ozone production. However, it should be noted that these tests were performed on a limited number of biodiesel blends. While it certainly appears that the overall trend for biodiesel is to produce less of many of these hazardous pollutants, additional types of biodiesel should probably be investigated. (Rodenburg, pg 2)

**Response:** Staff's overall assessment was based on the data and information provided for the biodiesel multimedia evaluation, including the Final Tier I, Tier II, and Tier III Reports by UC Davis and UC Berkeley, and the ARB Emissions Study by ARB in conjunction with UC Riverside and UC Davis from emissions testing conducted at the College of Engineering – Center for Environmental Research and Technology, UC Riverside, and ARB emissions test facilities in Stockton and El Monte, California.[15]

As part of the ARB Emissions Study, emissions testing was conducted on biodiesel (B100) and various biodiesel blends (B5, B20, B50) compared to the baseline CARB diesel fuel. The test fuels for this program included five primary fuels that were subsequently blended at various levels to comprise the full matrix. Also, two biodiesel feedstocks were used for testing, including one soy-based and one animal-based biodiesel fuel. Although tests were performed on a limited number of biodiesel blends, these fuels were selected to provide a range of properties that are representative of typical feedstocks representing different characteristics of biodiesel fuel in California.

After the MMWG's release of the Biodiesel Staff Report in November 2013 and peer review, a follow-up study to the ARB Emissions Study was completed. The "*CARB Comprehensive B5/B10 Blends Heavy-Duty Engine Dynamometer Testing*" (B5/B10 Study)[16] by ARB, UC Riverside, and UC Davis expands on the ARB Emissions Study to provide more comprehensive information on the emission impacts of lower level B5 and B10 blends. The results of this study are included in the updated Staff Report as part of the biodiesel multimedia evaluation. The MMWG has considered this additional information in developing its overall conclusions and recommendations to the CEPC. (ARB)

**B-4.** **Comment:** Impact of biodiesel on public health was assessed by comparing the combustion emissions against that with petroleum based diesel fuels. Data show that there is a reduction in most of the primary pollutants from burning biodiesel, but a statistically significant increase in NOx. Since NOx is the main ingredient for ground level ozone, there should be a study on this secondary pollutant. Also, impact of feedstock collection, storage, transportation, and processing needs to be assessed if certain portion of the biodiesel is locally produced. (Yang, pg. 2)

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[15] Multimedia Working Group. *Staff Report: Multimedia Evaluation of Biodiesel.* November 2013, 7.
[16] Karavalakis, G. et al. *CARB Comprehensive B5/B10 Blends Heavy-Duty Engine Dynamometer Testing.* June 2014.

**Response:** The comprehensive ARB Emissions Study and subsequent B5/B10 Study were conducted to determine emissions impacts of biodiesel and biodiesel blends compared to CARB diesel. The B5/B10 Study expands on the ARB Emissions Study to provide more comprehensive information on potential NOx emissions impacts of lower biodiesel blends in CARB diesel fuel. The results of this study were reviewed in conjunction with the earlier ARB Emissions Study and other associated and related studies to evaluate the emissions impacts of biodiesel in the State.

Regarding potential life cycle impacts in the State, please also see response to comment A-9. (ARB)

**B-5. Comment:** The Public Health Evaluation conclusion that the use of biodiesel compared to CARB diesel reduces the amount of particulate matter and polycyclic aromatic hydrocarbons released into the atmosphere, but may increase emissions of oxides of nitrogen (NOx) is supported by studies described in the Staff Report based on the California Biodiesel Multimedia Evaluation Final Tier III Report, a number of government- conducted studies as well as studies reported in the open literature. The average emission impacts in terms of particulate matter levels of biodiesel compared to conventional diesel for heavy-duty highway engines were reduced by increasing levels of biodiesel, while oxides of nitrogen levels increased slightly (EPA, 2002). The literature on the levels of combustion emissions from biodiesel fueled engines compared to those from diesel fueled engines in the range of B5 to B100 was recently reviewed (Bünger et al., 2012). They reported that in most studies biodiesel emissions had lower levels of particulate matter compared to conventional diesel emissions and that the levels of many polycyclic aromatic hydrocarbons levels and some nitro polycyclic aromatic hydrocarbons were also lower in biodiesel emissions. They also reported that the levels of nitrogen oxides were increased in biodiesel emissions. Thus, the Public Health Evaluation conclusion that combustion emissions from biodiesel fueled engines compared to those from CARB diesel fueled engines leads to lower emissions of particulate matter and polycyclic aromatic hydrocarbons is consistent with the published literature as is the increase in nitrogen oxides. However, as pointed out in the report although there is a reduction in particulate matter emissions in biodiesel blends, “there is some uncertainty that a drop in total PM mass may not necessarily equate with an overall reduction in the number of UFP emitted from combustion. This is an issue of national interest and more testing would be required to fully address it”. In fact, it has been recently reported that higher numbers of ultrafine particles (UFPs, < 100 nm) were emitted from a diesel engine combusting pure waste cooking oil biodiesel compared to ULSD supporting this concern (Betha and Balasubramanian, 2013). (Nesnow, pg 4)

**Response:** OEHHA scientists agree that there is uncertainty in how PM from biodiesel combustion, including the number of ultrafine particles, differs from PM from CARB diesel combustion. Further, the ultrafine particle number likely differs depending on the type of diesel or biodiesel fuel used and the engine in which it is combusted. (OEHHA)

**B-6.** **Comment:** There are several different conclusions regarding the levels of acrolein in the Staff Report and in the literature. In part C. Office of Environmental Health Hazard Assessment Evaluation, P 13 it states "In tests using a Caterpillar C-15 engine, the amount of acrolein was increased in emissions from combustion of B100 and B50 from both plant and animal sources when compared to the amount of acrolein in emissions from CARB diesel combustion." Under C. Conclusions on Public Health Impact, P 16, L5 it states "Limited emissions testing resulted in a non-statistical increase in acrolein for a higher B50 biodiesel blend level (i.e., confidence interval less than 95%). Furthermore, the statistical analysis for acrolein emissions results was compared to only one data point for the control sample." The first conclusion is likely based on the data from Cahill and Okamoto (2012) using a 2000 Caterpillar C-15 engine and a UDDS drive cycle. Cahill and Okamoto (2012) used N values of 2-3. The second conclusion is likely based on Figure 10-18 (P 174) in the Durbin et al. (2011) report who 2000 Caterpillar C-15 engine and a UDDS drive and used N values of 1 and 2. The Bünger et al. (2012) analysis of papers comparing acrolein levels in B5, B10, B20 and 100% biodiesel emissions concludes shows general increased levels of acrolein in biodiesel emissions but the results are highly variable. My recommendation is to make one clear and consistent concluding statement regarding the levels. (Nesnow, pg 5)

**Response:** The increases in acrolein emissions from a biodiesel-fueled engine in Table 10-7 in the Durbin et al. report are larger (in comparison to acrolein emissions from the engine using petroleum diesel) than differences in emissions that are statistically significant in the same table. OEHHA scientists have requested the acrolein emission data from the authors in order to calculate p-values using Student's t test. The MMWG will continue to monitor and review this information for appropriate action.

Data on acrolein emissions from the Caterpillar C-15 engine obtained by a sampling method different from the method used to obtain data in Table 10-7 (DNPH cartridge method) are presented in Figure 10-18 and Table 10-9. The increase in acrolein concentration (using the mist chamber method) from soy biodiesel compared to acrolein from biodiesel is considered significant as is indicated in the text. (OEHHA)

**B-7.** **Comment:** The role of oxy-PAHs needs to be more fully described in the Staff Report. Durbin et al. (2011) states "The emission trends for Oxy-PAH emissions showed different trends for different compounds, with some compounds showing generally higher emissions in soy and animal-based biodiesels compared to CARB diesel, whereas others decreased in animal biodiesel and renewable diesel. For all toxic species, emission levels were significantly reduced in the DPF-equipped vehicle, and there were few fuel related trends." Oxy PAH levels were also increased in studies using methyl ester blends of vegetative and animal based oils compared to EN590 using a diesel passenger car (Karavalakis et al., 2009, 2011). One issue that has not been fully discussed is the apparent increase in the levels of 1,2-naphthoquinone as described in Durbin et al. (2011).

This might be due to increased oxygen content of the ester-based biofuels. Inspection of the mean and standard deviation results of CARB animal and A100 levels in Figure 10-47 (Durbin et al., 2011) indicate that CARB animal and A100 levels of 1,2-naphthoquinone appears to be statistically significantly different. 1,2-Naphthoquinione is cytotoxic (Flowers-Geary et al., 1993) and genotoxic (Saeed et al., 2008) and 1,2-naphthoquinone and its analog 1,4-naphthoquinone each induce reactive oxygen species (ROS) (Thornalley et al., 1984 ). 1,4-Naphthoquinone, a component of particulates collected from road tunnel emissions is also cytotoxic and induced ROS and DNA damage in human lung epithelial cells, as did the road tunnel particles themselves (Shang et al., 2013). It is well known that several ROS forms induce cytotoxicity and genotoxicity and the formation of ROS can lead to adverse health outcomes. (Nesnow, pg 5)

**Response:** OEHHA scientists agree that 1,2-naphthoquinone appears to be significantly higher in emissions from animal fat biodiesel compared with petroleum diesel. As noted by Dr. Nesnow, 1,2-naphthoquinone is cytotoxic and mutagenic. The information presented by the commenter has been used to support a statement of uncertainty regarding the determination of the relative toxicities of biodiesel emission particulate and petroleum diesel exhaust particulate. (OEHHA)

**B-8. Comment:** There is an increasing body of new literature on inflammation, lung tissue damage, oxidative stress and oxidative damage where biodiesel emissions (particulate matter or complete emissions) have been shown to be more toxic than those from conventional diesel emissions. The results of many of these studies are summarized or quoted here. "Concentrations of inflammatory mediators (Interleukin-6, IL-6; Interferon-gamma-induced Protein 10, IP-10; Granulocyte stimulating factor, G-CSF) in the medium of B20-treated cells and in bronchioalveolar lavage fluid of mice exposed to B20 were ~20−30% higher than control or B0 PM, suggesting that addition of biodiesel to diesel fuels will reduce PM emissions but not necessarily adverse health outcomes (Fukagawa et al., 2013)." Human bronchial BEAS-2B cells were exposed to particulate matter collected from diesel passenger vehicles with and without a diesel particulate filter using a rapeseed biodiesel (B50) blend or to diesel fuels. The particulate matter from the B50 blend induced increased cytotoxicity and IL-6 release in the cells compared to the diesel fuel per distance driven. These differences were observed irrespective of the use of a diesel particulate filter (Gerlofs-Nijland et al. 2013). Rat alveolar macrophages exposed to exhaust particles from heavy duty diesel engine combusting B20 biofuel resulted in an increased production of PGE2 relative to particles from diesel fuel combustion (Bhavaraju et al., 2013). Mice were exposed by pharyngeal aspiration to diesel particulate matter collected from a diesel engine using biodiesel (NEXSOL BD-100) and ULSD. Biomarkers of tissue damage and inflammation were significantly elevated in the lungs of mice exposed to the biodiesel particulates. Inflammatory cytokines/chemokines/growth factors were up-regulated to a greater extent and oxidatively modified proteins and 4-hydroxynonenal levels were increased by biodiesel particulates compared to diesel particulates (Yanamala et al., 2013).

Mice were exposed by inhalation to combustion emissions of soy biodiesel (B100) and diesel. "B100 combustion emissions produced a significant accumulation of oxidatively modified proteins (carbonyls), an increase in 4-hydroxynonenal (4-HNE), a reduction of protein thiols, a depletion of antioxidant gluthatione (GSH), a dose-related rise in the levels of biomarkers of tissue damage (lactate dehydrogenase, LDH) in lungs, and inflammation (myeloperoxidase, MPO) in both lungs and liver. Significant differences in the levels of inflammatory cytokines interleukin (IL)-6, IL-10, IL-12p70, monocyte chemoattractant protein (MCP)- 1, interferon (IFN) γ, and tumor necrosis factor (TNF)-α were detected in lungs and liver upon B100 and D100 complete emission exposures. Overall, the tissue damage, oxidative stress, inflammation, and cytokine response were more pronounced in mice exposed to biodiesel complete emissions" (Shvedova et al., 2013). (Nesnow, pg 6)

**Response:** In response to this comment, OEHHA conducted a follow-up evaluation of literature looking at possible associations between biodiesel, oxidative stress and inflammation. The evaluation is discussed in a memo to CARB dated February 2, 2015 that is included as Attachment 1 with this document.

Based on the evaluation, OEHHA agrees with the peer reviewer that a number of studies found that biodiesel combustion emissions are more potent on a mass basis than petroleum diesel combustion emissions in eliciting responses associated with inflammation and oxidative stress, both in cell-based assays and *in vivo*. However, this greater potency may be offset by biodiesel's lower mass emissions of PM and other constituents, which were not considered in the studies. Further research is warranted to determine whether the increased toxicity of biodiesel combustion emissions related to oxidative stress and the inflammatory response might outweigh the beneficial reduction of particulate mass (and associated toxicity) that would result from the use of biodiesel. OEHHA cannot determine with certainty at this time whether replacing petroleum diesel with biodiesel or biodiesel-petroleum diesel blends for on-road motor vehicle use will reduce adverse human health impacts attributable to oxidative stress and inflammation from diesel-engine emissions. (OEHHA)

**B-9.** **Comment:** It noted that in a recent study, organic extracts of particles from emissions of engines using rapeseed methyl ester and EN 590 fuels both produced DNA adduct levels to comparable extents in an acellular assay using calf thymus DNA both in the presence and absence of an exogenous metabolic activation system, suggesting equal genotoxic activities of the two extracts (Topinka et al., 2012). No increases in micronuclei in bone marrow or sister chromatid exchanges in peripheral blood lymphocytes were found in rats exposed by subchronic inhalation to emissions from a diesel engine burning soybean based biodiesel fuel (Finch, 2002). (Nesnow, pg 6)

**Response:** OEHHA has reviewed recently published studies comparing biodiesel emissions to petroleum diesel emissions (using diesel that does not meet CARB specifications). Biodiesel emissions are clearly toxic in these studies, and in some

toxicity tests PM from biodiesel emissions elicits a greater response than does PM from petroleum diesel. (OEHHA)

**B-10. Comment:** The MMWG conclusions "that the use of biodiesel fuel in California, as specified in the biodiesel multimedia evaluation, does not pose a significant adverse impact on public health or the environment relative to diesel fuel meeting Air Resources Board (ARB) motor vehicle diesel fuel specifications (CARB diesel)" is supported by many of the analytical chemical and biological measures of toxic components of emissions from CARB vs. biodiesel fueled engines as found in the Staff Report suggesting a lessened impact on public health, however, the recent toxicological data give me some concern that not enough studies have been conducted to unequivocally conclude that substitution of biodiesel for CARB diesel will not adversely affect public health and that the ARB should proceed with caution. (Nesnow, pg 7)

**Response:** Staff agrees with the commenter's point that several recent biodiesel emission particulate toxicity studies indicate the need to proceed with caution; the MMWG has revised its conclusions and recommendations accordingly. (OEHHA)

OEHHA conducted further review of more recent biodiesel studies published in 2013 and 2014. Please refer to Attachment 1 (complete OEHHA memorandum and appendix, dated February 2, 2015) for more information on studies reviewed.

Please also see response to comment B-8. (ARB)

**B-11. Comment:** Noteworthy shortcomings regarding the quality of the MMWG Evaluation of Biodiesel (i.e., the staff report), and the associated Tier I, II and III reports, precluded effective scholarly evaluation of the aforementioned conclusions. More specifically, in this reviewer's opinion, the MMWG evaluation of the available scientific information regarding the relative toxicological activity of biodiesel emissions is incomplete and superficial. Consequently, it was necessary for this reviewer to collect, review and evaluate all publicly-available scientific information pertaining to the relative toxicological activity of biodiesel and petroleum diesel emissions. (White, pg 1)

**Comment:** Noting that in some instances the available information may be incomplete and "less than ideal", this reviewer nonetheless supports the ARB and OEHHA conclusions listed above (i.e., 1 and 3). Although some of the published scientific information available to date shows enhanced toxicological activity for biodiesel emissions, relative to petroleum diesel, the weight of evidence supports the ARB and OEHHA conclusions. (White, pg 1)

**Response:** Staff thanks the reviewer for providing additional information on the toxicity of biodiesel emission particulates, some of which is based on publications that were unavailable to the authors of the Tier I, II, and III reports at the time of writing. The reviewer's comments and toxicological review are included as an appendix to the Staff

Report. Please refer to Appendix I. The MMWG has considered this additional information in formulating its conclusions and recommendations.

Please also see response to comments B-8 and B-10. (OEHHA)

**B-12. Comment:** Despite publicly-available scientific studies that have documented enhanced toxicological hazards for biodiesel emissions, the weight of evidence, in this reviewer's opinion, permits support of the MMWG's recommendations to the California Environmental Policy Council (i.e., "that the use of biodiesel, as specified in the multimedia evaluations, does not pose a significant adverse impact on public health and the environment"). However, as noted below, the WG must acknowledge studies that have documented enhanced toxicological hazards for biodiesel emissions; particularly those that noted effects generated under experimental conditions that have been linked to adverse effects in humans (e.g., inflammation). (White, pg 2)

**Comment:** It is critical for the WG to effectively summarise all publicly-available evidence in order to effectively demonstrate that the risk of adverse health effects attributable to biodiesel emissions, or emissions of biodiesel petroleum diesel blends, are similar or lower in comparison with conventional diesel emissions. Concurrently, it is also critical for the WG to acknowledge that a limited number of studies have documented enhanced toxicological hazards for biodiesel emissions; moreover, hazards related to pathophysiologic changes associated with an increased likelihood of human morbidity and mortality (e.g., pulmonary inflammation, oxidative stress, pulmonary tissue damage, cardiovascular irregularities). (White, pg 2)

**Comment:** From this reviewer's point of view, the most serious deficiencies in the MMWG evaluation of biodiesel relate to the incomplete review of publicly-available scientific information on the toxicological properties of biodiesel emissions relative to conventional diesel emissions. More specifically, in this reviewer's opinion, the review on pages I-42 and I-43 and I-59 to I-64 of the Tier I report is remarkably incomplete and superficial, and this superficial analysis is carried over into the Tier III report. (White, pg 3)

**Response:** The purpose of the MMWG Staff Report is to provide a summary of the biodiesel multimedia evaluation (i.e., the Tier I, II & III reports) as well as to formulate conclusions and recommendations to the CEPC based on the evaluation. The conclusions regarding impacts on human health that might result from replacing a petroleum diesel fuel by biodiesel fuel in California were based on critical review of studies comparing biodiesel emissions with CARB emissions. This is the most relevant comparison, as all diesel fuel sold in California must meet CARB specifications. While it might be ideal for this evaluation to include a comprehensive critical review of all studies comparing biodiesel emissions to petroleum emissions, such a review would require considerable resources and would be of only limited relevance for California.

Nonetheless, OEHHA carried out a review of a number of relevant toxicity studies. Please refer to Attachment 1 (complete OEHHA memorandum and appendix, dated February 2, 2015) for more information on the studies reviewed.

We agree with the commenter on the need to acknowledge recent evidence indicating that exposure to biodiesel emission particulates may produce enhanced adverse inflammatory effects in humans when compared to diesel PM. For example, biodiesel emission particulate studies such as the mouse lung instillation paper of Yanamala, *et al.* (2013) cited by the commenter, and the mouse inhalation study by Shvedova, *et al.* (2013)[17] both indicate that biodiesel emission particulate exposure may produce increased inflammation relative to petroleum diesel. In particular, Shvedova, et al. (2013) exposed mice, via inhalation, to several concentrations of particulate from soy-based biodiesel and diesel for four weeks (the lowest exposure concentration was 50 ug/m$^3$). They found relatively large differences in the concentration of inflammatory markers such as myeloperoxidase and pro-inflammatory interleukins. Biodiesel emission particulate was compared to diesel PM on a mass basis in this study, but the differences in response appeared in some cases to be large enough to negate expected reductions in PM mass obtained with soy-based biodiesel.

We have revised our conclusions to reflect the additional uncertainty arising from a consideration of these and other studies on biodiesel emission particulate-induced inflammation.

Please also see response to comments B-8 and B-10. (OEHHA)

**B-13. Comment:** Finally, the presented review of the pertinent scientific literature ignores critical information regarding the metric(s) used to express the magnitude of toxicological potency (i.e., potency unit). The units employed for quantitative evaluation of the results have a critical bearing on the relevance of the results for the assessment of human health risk. For example, the cited Turrio-Baldassarri et al (2004) study, which examined the mutagenic activity of organic extracts of PM from conventional diesel and B20 RME, compares mutagenic activity expressed per µg of extractable organic matter (EOM) and per unit of engine work (kWhr). The former unit is useful for studies that are interested in identifying the putative toxicants in combustion emissions, the latter, which requires information on EOM emission rates (e.g., µg per kWhr), is more useful for assessing the likelihood of post-emission adverse human effects. (White, pg 3-4)

**Response:** OEHHA staff agrees that comparisons of toxic effects of biodiesel emissions and diesel emissions should be made using units of toxic effect per horsepower hour (or other unit of work). (OEHHA)

[17] Shvedova AA, Yanamala N, Murray AR, Kisin ER, Khaliullin T, Hatfield MK, Tkach AV, Krantz QT, Nash D, King C, Gilmour MI, Gavett SH (2013) Oxidative Stress, Inflammatory Biomarkers, and Toxicity in Mouse Lung and Liver after Inhalation Exposure to 100% Biodiesel or Petroleum Diesel Emissions, Journal of Toxicology and Environmental Health, Part A Vol. 76, Iss. 15, 907-21.

**B-14. Comment:** It is certainly important to acknowledge that the Tier II analyses of biodiesel and renewable diesel emissions (i.e., the Durbin et al, 2011 report) constitutes a comprehensive comparative analysis of biodiesel and conventional diesel emissions. Nevertheless, it is also important to note that there are serious shortcomings in the Durbin et al report regarding the description of the methods employed for the toxicological analyses. Moreover, the concluding remarks and executive summary do not even comment on the relative ability of the emissions to induce inflammatory and oxidative stress responses. (White, pg 4)

**Response:** The Conclusion and Executive Summary of the Staff Report were revised accordingly. (OEHHA)

**B-15. Comment:** Few studies have employed in vivo animal exposures to compare diesel emissions associated with engines or vehicles operated using petroleum-based fuels with emissions associated with biodiesels (i.e., FAMEs), other renewable fuels (e.g., HVO), or fuel blends. Only two studies examined effects elicited by diluted exhaust, and both studies noted evidence that biodiesel emissions can be associated with increased severity of toxicological responses. For example, Brito et al (2010) noted that SEE emissions are associated with increases in cardiovascular irregularities in Balb/c mice (5). Steiner et al (2013) employed an air-liquid interface system to demonstrate that diluted RME emissions (B100) induced increased cytotoxicity and oxidative stress in an ex vivo 3D human airway model, relative to petroleum diesel. The authors noted some decrease in inflammatory stress for biodiesel (6). In addition, Yanamala et al (2013) showed that pharyngeal aspiration of PM from corn-derived FAME induced increased pulmonary damage, oxidative stress and inflammation in C57BL/6 mice, relative to petroleum diesel PM (7). Importantly, the doses examined in the Yanamala et al study equate to human occupational exposures of 156.25 working days at an allowable MSHA concentration limit of 160 µg total carbon per m3. With respect to carcinogenic hazard, a single study examined the emission rate of carcinogenic PAHs, expressed as total BaP equivalents, and concluded that the carcinogenic hazards of biodiesel emissions (source unspecified) are likely to be lower than petroleum-based diesel for PM-associated PAHs in primary and secondary aerosols. (White, pg 7)

**Response:** OEHHA appreciates this summary of results from published articles comparing toxicity of biodiesel emissions and petroleum diesel emissions. However, some of the results may be misleading because toxic effect per horsepower hour is not the basic unit used for comparisons. For example, in the Yanamala et al. (2013) study, toxicity per unit mass of PM from biodiesel emissions and petroleum diesel emissions is compared. OEHHA scientists do not agree with the commenter's statement, "Brito et al. (2010) noted that SEE emissions are associated with increases in cardiovascular irregularities in Balb/c mice." The pattern of heart beats (heart rate, heart rate variability measures) and blood pressure in mice exposed to diesel exhaust (not CARB diesel), a 50% mix of soybean ethyl esters and petroleum based diesel, 100% soybean ethyl

ester, or to filtered air was recorded in this study. Several statistical measures of variability were calculated from these recordings. Two of these statistical measures of variability were significantly increased in mice exposed to biodiesel emissions when compared to measures of variability from mice exposed to petroleum diesel exhaust. However, the study had a number of problematic issues. For example, the animals were anesthetized to make these measurements, which would change the parameters they were measuring. The effect of SEE100% was less than that of the 50% mixture for most of the measurements, and there was not a consistent pattern of measured changes in the cardiovascular function between the 50% mixture and diesel exposed groups. The diesel had to be diluted more than the SEE emissions to reach the same PM concentrations and thus the volatile component of the emissions was not comparable. These and other issues with the study design and reporting lead OEHHA to question the study results.

Please also see response to comments B-8 and B-10. (OEHHA)

**B-16. Comment:** No occupational exposure and risk of any sort are included in this multimedia evaluation. (Li, pg 3)

**Response:** At the request of ARB, OEHHA compared impacts of biodiesel combustion emissions with impacts of CARB diesel combustion emissions. Occupational exposure to diesel combustion emissions is likely to be higher than population exposure for workers in occupations utilizing diesel equipment (e.g., construction, mining, farming, trucking, etc.). The higher exposure rates experienced in occupational settings would result in greater health risks, but are not expected to produce a qualitatively different set of adverse effects than those seen in the general population. The conclusions of the Staff Report were based upon a comparison of the potential health risks from exposure to biodiesel and petroleum diesel exhaust. For example, the multimedia evaluation reported that benzene emissions from biodiesel (B100) combustion under several representative emission scenarios were significantly lower than those from CARB diesel combustion, implying that the cancer and non-cancer risks of exposure to benzene from the use of B100 would show a similar relationship. This reduced risk would be expected for occupationally exposed individuals as well as the general population.

Regarding workers involved in the production and transport of diesel fuel, the intent of the multimedia evaluation is to inform ARB's regulatory decisions by anticipating multimedia impacts, both to overall human health and the environment, from biodiesel fuels. The California Health and Safety Code defines the scope of the evaluation as follows:

> "At a minimum, the evaluation shall address impacts associated with all the following: (1) Emissions of air pollutants, including ozone forming compounds, particulate matter, toxic air contaminants, and greenhouse gases. (2) Contamination of surface water, groundwater, and soil. (3) Disposal or use of the byproducts and waste materials from the production of the fuel."

Although this does not rule out a more detailed assessment of the comparative occupational risks of exposure to biodiesel fuel versus petroleum diesel fuel, the MMWG has deemed this to be outside the scope of this evaluation. Current occupational-safety laws and regulations require employers to inform workers of chemicals present in biodiesel feedstock and products, hazards associated with those chemicals, and safety measures that workers should take to minimize their exposures and risks.

Chapter III, Section C ("Conclusions on Public Health Impact") of The Biodiesel Staff Report was revised to reflect the newer study results indicating a stronger inflammatory response to some biodiesel emissions.

C. Conclusions on Public Health Impact

Based upon the information presented in the biodiesel multimedia evaluation, the substitution of biodiesel for CARB diesel appears to reduce the rate of addition of carbon dioxide to the atmosphere and the amount of PM, benzene, ethyl benzene, and PAHs released into the atmosphere.

However biodiesel use may increase NOx emissions for certain blends. Further, biodiesel combustion may produce higher levels of some toxic constituents, such as 1,2-napthoquinone and acrolein, as well as a larger proportion of total particles as ultrafine particles relative to petroleum diesel.

The data from recent *in vitro* and *in vivo* animal studies indicates that biodiesel emission particulate exposure can induce enhanced oxidative stress and inflammatory responses relative to petroleum diesel particulate when measured on a mass basis. This may be offset by lower mass emissions of PM and other constituents. The study results are complicated by different types of biodiesel and petroleum diesel, as well as engine and workload protocols used to generate the exhaust for the exposures. The studies were not all comparing CARB diesel with specific biodiesel meeting CARB specifications. However, this research increases uncertainty regarding health impacts associated with oxidative stress and inflammation. Further research is warranted to determine whether the increased toxicity of biodiesel emission particulate observed in some studies might outweigh the beneficial reduction of particulate mass and other constituents such as PAHs (and associated toxicity) that would result from the use of biodiesel.

Switching from petroleum diesel to biodiesel is likely to reduce cancer risks since biodiesel emissions contains significantly less PM, PAHs and benzene. These are well characterized carcinogens, and the risk reduction is real. However, this benefit must be measured against a less certain increase of adverse health outcomes due to biodiesel emission particulate-induced oxidative stress and inflammation (e.g., possible exacerbation of asthma conditions, increased cardio-vascular episodes, and premature mortality). Increased NOx emissions from the use of biodiesel may also produce increased adverse respiratory and cardiovascular impacts, if not controlled.

In summary, the information currently available to OEHHA indicates a reduction in cancer risk from use of biodiesel, and a reduction in greenhouse gas emissions, which in itself is associated with myriad environmental and public health impacts. It is difficult to state with certainty that the use of biodiesel will decrease cardiovascular or respiratory health risks because of the uncertainty introduced by recent studies that provide evidence for increased oxidative stress and inflammatory response to biodiesel emissions relative to petroleum diesel particles on a mass basis. As noted above, the reduction in PM and other emissions may offset this potential increased inflammatory response. CEPC may want to emphasize in its determination the continued importance of emissions controls for biodiesel fueled engines, as has been the emphasis for petroleum diesel fueled engines. (OEHHA)

Water Quality

**C-1.** **Comment:** Overall, the MMWG conclusion that "there are minimal additional risks to beneficial uses of California waters posed by biodiesel" is well supported. However, the summary presentation of study findings could be clarified on a few points.

1. Water impacts (p. 11). There are two main impacts discussed in this section: aquatic toxicity, where there are results, and agricultural impacts, where there are no results from the current multimedia review. It would be helpful to break these two topics into separate paragraphs. More detail should be provided on the toxicity findings from the multimedia evaluation. Similarly, Section 3 would benefit from more detail clarifying issues related to biodegradability. Sections 2 and 4 seem to have an appropriate level of detail for the topic. (Holloway, pg 3)

**Response:** SWRCB staff concurs that the report could be improved by the suggested changes. (SWRCB)

**C-2.** **Comment:** In contrast to the general positive impact on air quality due to reduced direct air emissions, the effects of switching to biodiesel on natural waters could be adverse and extensive. Most of the priority issues identified in Tier I Conclusions are related to water quality, including additives impacts, subsurface fate and transport, biodegradation, production and storage release. Unfortunately, these issues were not sufficiently investigated during Tier II experimental test stage. This leads to high uncertainty in making conclusions on the impact of using biodiesel on water quality. I consider this is the major weakness of this multimedia evaluation. Other regulations (such as the laws and regulations on underground storage tank and the hazardous waste, as mentioned in main Staff Report, section II-B) will help prevent water pollution; but they are not relevant to scientific assessment of biodiesel impact on surface and ground water quality. (Li, pg 2)

**Comment:** I find that this conclusion of the report is based on sound scientific knowledge, methods, and practices. Biodiesel has been demonstrated to be

more biodegradable than regular diesel. In addition, the higher biodegradability of biodiesel augments the biodegradability of the regular diesel with which it is blended. The one area of concern for biodiesel is its tendency to act as a cosolvent and increase the solubility of other contaminants. This may be of concern in groundwater. However, it is probably of much less concern than the cosolvent properties of ethanol with which gasoline is often blended. (Rodenburg, pg 1)

**Response:** SWRCB staff concurs that there is some uncertainty with respect to subsurface, fate and transport and that focus remains on preventing water pollution. (SWRCB)

**C-3.** **Comment:** In the main Staff Report, the conclusion on water quality impact (page 16, part B) needs to be more specific. The current version is not consistent with summary section II-B, which indicates an increase in toxicity in part 1 and decreased biodegradation in part 3. It is not clear how the results summarized in section II-B lead to a conclusion of "minimal additional risks" in the Conclusions. In addition, the last several words should be changed from "public health or the environment" to "the quality of surface water and groundwater in California." (Li, pg 3)

**Response:** SWRCB staff concurs that the report could be improved by the suggested editorial changes.

**C-4.** **Comment:** The incompatibility of biodiesel with underground storage tank (UST) as well as other infrastructure equipment calls for work plans needed in the cases of leaks into groundwater. Merely requiring affirmative statements of compatibility from biodiesel manufacturers and leak detection (main Staff Report, page 11, part 2) does not seem sufficient to ensure no adverse impact on groundwater. (Li, pg 3)

**Response:** SWRCB staff disagrees with this comment, as the existing UST design, construction, and monitoring standards have been successful at preventing new releases. The existing standards not only require affirmative statements of material compatibility from manufacturers, but also require USTs be equipped with secondary containment, leak detection equipment, and tested on a periodic basis. These systems are designed to detect and contain releases while preventing contamination from entering the environment. (SWRCB)

**C-5.** **Comment:** Similar to the air emissions evaluation, the study does not include the effect of biodiesel production and distribution on water quality. (Yang, pg 2)

**Comment:** Biodiesel is produced from biological feedstock, including plant and animal materials. Some are produced from community wastes (like "yellow grease"). It is not appropriate to assume that all biodiesel used in California will come from sources outside the state. If certain portion of the feedstock is from

sources inside the state, or if certain portion of the production (conversion) process is done inside the state, an evaluation of the impact on water resources/quality by growing, transportation, storage, and conversion of large amount of biodiesel feedstock will be necessary. (Yang, pg 2)

**Comment:** Soil pollution and hazardous waste generation can occur during production, transportation, storage and use of biodiesel. It is very difficult to conduct a complete evaluation of the impact of biodiesel on soil and hazardous waste impact because there is a large variation in feedstock type, production method, composition (additives) and chemical properties (some of them are not yet known)…

The studies conducted by UC researchers probably are sufficient for the purpose of impact evaluation. More research is called in the future for a better understanding of the multimedia transport and fate processes in biodiesel feedstock and fuel production, distribution, use and disposal. (Yang, pg 3)

**Response:** The specific magnitude of the use of California's water resources on biodiesel was not well characterized by the researchers and remains difficult to quantify until more specific data become available. As identified in staff comments, additional study and procedures may need to be reviewed by the MMWG in the future. (SWRCB)

<u>Soil and Hazardous Waste</u>

**D-1.** **Comment:** In Appendix F on page 2 of the 3-page memo from Donn Diebert, the opening sentence of the last paragraph is unclear. The sentence states that biodiesel appears to react differently in the environment than does CARB diesel. It is not clear if the three characteristics listed before the last paragraph are the main characteristics that are different. The opening sentence should provide more detail on the differences between biodiesel and CARB diesel by either referring to the characteristics listed or adding new characteristics that are most important for the differences. The knowledge about reaction differences is important for assessing the fate in the environment. (Bouwer, pg 2)

**Response:** In Appendix F, DTSC comments:

> Based on the tests performed, biodiesel appears to react differently in the environment than does CARB diesel. The assumption made was that additives used in the tests would be the baseline for brining biodiesel to market.

The sentence was not based on biodiesel characteristics, but on the limited laboratory tests performed, UC Davis and UC Berkeley conducted three limited tests in laboratory scale for biodegradation, toxicity, and infiltration. The results of the tests were identified in the Tier II Report. For further clarification, see below.

1. Under an aerobic aqueous condition, biodegradation of B100 and B20 were compared with that of CARB diesel. The test also compared conditions with and without a biocide and antioxidant additive. The results showed that biodiesel biodegraded more rapidly than CARB diesel. The use of additives during the test did not significantly affect the biodiesel biodegradation rate.

2. An aquatic toxicity test for a small subset of biological species showed higher toxicity of biodiesel than that of CARB diesel when an antioxidant additive was used.

3. The two-dimensional sandbox test (with and without an antioxidant additive) exhibited no significant difference in infiltration rate in the vadose zone between biodiesel and CARB diesel. However, biodiesel left a notable increase in the residual in vertical dimension and appeared less extensive horizontally. It could be explained that some components in biodiesel have higher water solubility than the components in CARB diesel.

We understand the impracticality of a complete evaluation/testing of all types of biodiesel blends and all current additives. The aquatic toxicity test revealed that biodiesel behaved differently with CARB diesel when an additive existed. Other biodiesel characteristics may, or may not, cause differences. For example, no test has been conducted on the feedstock's variability to obtain biodiesel. The fatty acid composition of biodiesel feedstock varies with its source. Biodiesel's fatty acid content greatly influences its final chemical and physical properties. The use of additives and blending with CARB diesel can also change biodiesel's biological, chemical and physical characteristics. Since testing various feedstock and additive combinations would be a monumental undertaking, DTSC staff agree with the identified knowledge gaps in the final Tier III Report. As the industry matures, current additive packages relevant to the California market and determination of biodiesel's/CARB diesel blends' real life fate and transport behaviors are important to understand. (DTSC)

Specific fuel formulations and additives not included within the scope of the biodiesel multimedia evaluation will be reviewed by the MMWG for consideration of appropriate action. Please also see response to Comment E-8. (ARB)

**D-2. Comment:** Hazardous waste is outside the expertise of this reviewer. However, the discussion overall was clearly presented and seemed consistent with findings from the Multimedia Evaluation. It would seem appropriate, however, to define the term "vadose zone infiltration." (Holloway, pg 4)

**Response:** The vadose zone (i.e., unsaturated zone) is the part of Earth bounded by the land surface as the top boundary and below by the surface of the saturated zone (i.e., water table). The vadose zone is at atmospheric pressure. Water in the soil within the vadose zone, also called soil moisture, does not completely fill soil pore space. This is the void space between soil particles. Infiltration is the process by which a fluid when placed on the ground surface enters the vadose zone. The movement of water within

the vadose zone is different than water movement within the saturated zone. Other fluids placed on the ground surface will infiltrate slower or faster than water and will react differently as they migrate through the vadose zone either as a pure product or even as a solubilized product. Some contaminants can also be pushed by water through the vadose zone to the saturated zone. (DTSC)

**D-3.** **Comment:** Soil and hazardous waste evaluation. Department of toxic substances control staff concludes that biodiesel aerobically degrades more readily than CARB diesel, has potentially higher aquatic toxicity for a small subset of tested species and generally has no significant difference in vadose zone infiltration rate.

> I find that these conclusions of the report are based on sound scientific knowledge, methods, and practices. The higher aquatic toxicity of biodiesel may be related to its cosolvent properties, which increase the solubility and bioavailability of other toxic material within the diesel. However this property of the biodiesel also causes it to disperse more readily and because it is more biodegradable this means that spills of biodiesel may very well be less of a concern that spills of regular diesel. (Rodenburg, pg 2)

**Response:** DTSC staff agree with Dr. Rodenburg noted: biodiesel's co-solvent properties may "…increase the solubility and bioavailability of other toxic material within the diesel." (DTSC)

**D-4.** **Comment:** Page I-26 notes that acceptable materials for storage and transport of biodiesel include aluminum, steel, and fluorinated polyethylene or polypropylene. In particular, the fluorinated compounds are a big environmental problem and should be avoided at all costs. If increased use of biodiesel is going to require the use of these kinds of fluorinated compounds this could be a serious problem. (Rodenburg, pg 4)

**Response:** The Tier I Report dated December 2008 discussed biodiesel material compatibility. Page 31 of the Tier I Report states:

> Vehicles manufactured before 1993 may have issues with incompatible seals, gaskets and adhesives as they were made from natural and nitrile rubber (Van Gerpen, 2004). Most engines produced after 1994 are compatible with biodiesel (B20); however, "the user should consult the equipment manufacturer or owner's manual regarding the suitability of using biodiesel (B100) or biodiesel blends in a particular engine (ASTM, 2007).

Table 3.1 in the Tier I Report listed compatibility information of several potential gasket materials with biodiesel. Flourosilicon may not be a necessary choice for biodiesel. Staff agree that additional review is required if a potential gasket is recommended. (DTSC)

**D-5.** **Comment:** Soil and Hazardous Waste Evaluation. Department of Toxic Substances Control (DTSC) staff concludes that biodiesel aerobically biodegrades more readily than CARB diesel, has potentially higher aquatic toxicity for a small subset of tested species, and generally has no significant difference in vadose zone infiltration rate.

…More research is called in the future for a better understanding of the multimedia transport and fate processes in biodiesel feedstock and fuel production, distribution, use and disposal. (Yang, pg 2-3)

**Response:** DTSC staff agree with the reviewer about the need to gather more information on fate and transport in the future. (DTSC)

Multimedia Evaluation

**E-1.** **Comment:** The assessment of the supply and demand is not within the scope of this multimedia assessment. According to Hill et al. (2006), even dedicating all U.S. corn and soybean production to biofuels would meet only 12% of the gasoline and 6% of diesel demands in the country. Even with B20 or lower blends, whether all the available resources would meet the demand is unclear. (Li, pg 2)

**Response:** A multimedia evaluation focuses primarily on environmental and public health impacts in the State. The topics of supply and demand of biodiesel fuel, including feedstocks, are not within the scope of this evaluation.

As defined, a "multimedia evaluation" is the identification and evaluation of any significant adverse impact on public health or the environment, including air, water, and soil, that may result from the production, use, or disposal of the motor vehicle fuel that may be used to meet the state board's motor vehicle fuel specifications. [18]

In general, the purpose of the multimedia evaluation is to provide the information needed for the development of fuel regulations and inform the overall rulemaking process. (ARB)

**E-2.** **Comment:** In the near future, the major feedstock could be soybeans grown in the US Midwest, where most adverse impact will occur. Although a complete evaluation of the impact outside California is beyond this work, a summary of available information on the impacts of the upstream processes (feedstock production, extraction, blending, etc.) on the environment and human health could have been included. (Li, pg 2)

[18] California Air Pollution Control Laws. Health and Safety Code, Division 26, Part 5, Chapter 4, Section 43830.8(b).

**Response:** A multimedia evaluation focuses primarily on potential environmental and public health impacts in the State.[19] Therefore, out-of-State considerations and potential impacts were assessed but not thoroughly addressed as part of the multimedia evaluation.

Nonetheless, the UC Tier I, Tier II, and Tier III reports provide important information on upstream processes (production, storage, and distribution) for biodiesel throughout the life of the fuel, including potential impacts and other important considerations outside of California.

Please also see response to comment A-9. (ARB)

**E-3. Comment:** No occupational exposure and risk of any sort are included in this multimedia evaluation. (Li, pg 2)

**Response:** At the request of ARB, OEHHA compared impacts of biodiesel combustion emissions with impacts of CARB diesel combustion emissions. Occupational exposure to diesel combustion emissions is likely to be higher than population exposure for workers in occupations utilizing diesel equipment (e.g., construction, mining, farming, trucking, etc.). The higher exposure rates experienced in occupational settings would result in greater health risks, but are not expected to produce a qualitatively different set of adverse effects than those seen in the general population. The conclusions of the Staff Report were based upon a comparison of the potential health risks from exposure to biodiesel and petroleum diesel exhaust. For example, the multimedia evaluation reported that benzene emissions from biodiesel (B100) combustion under several representative emission scenarios were significantly lower than those from CARB diesel combustion, implying that the cancer and non-cancer risks of exposure to benzene from the use of B100 would show a similar relationship. This reduced risk would be expected for occupationally exposed individuals as well as the general population.

Regarding workers involved in the production and transport of diesel fuel, the intent of the multimedia evaluation is to inform CARB's regulatory decisions by anticipating multimedia impacts, both to overall human health and the environment, from biodiesel fuels. The California Health and Safety Code defines the scope of the evaluation as follows:

> "At a minimum, the evaluation shall address impacts associated with all the following: (1) Emissions of air pollutants, including ozone forming compounds, particulate matter, toxic air contaminants, and greenhouse gases. (2) Contamination of surface water, groundwater, and soil. (3) Disposal or use of the byproducts and waste materials from the production of the fuel."

Although this does not rule out a more detailed assessment of the comparative occupational risks of exposure to biodiesel fuel versus petroleum diesel fuel, the

[19] California Air Pollution Control Laws. Health and Safety Code, Division 26, Part 5, Chapter 4, Section 43830.8(b).

MMWG has deemed this to be outside the scope of this evaluation. Current occupational-safety laws and regulations require employers to inform workers of chemicals present in biodiesel feedstock and products, hazards associated with those chemicals, and safety measures that workers should take to minimize their exposures and risks. (OEHHA)

**E-4.** **Comment:** *(Tier 1 Report)* Page I-3. This section focuses on some of the vehicle operability issues associated with the use of biodiesel blends. The impacts to a vehicle's fuel system can result in reduced reliability and increased maintenance costs. The next generation of environmental impact assessment for biodiesel should consider whether retrofitting of existing equipment or production of new vehicle equipment is going to require changes to engine design that could have environmental impacts. For example if the use of biodiesel would require, say, catalytic converters or other equipment that might contain platinum or other heavy metals, the production of those heavy metals has significant environmental impacts and should be considered in the assessment of biodiesel. (I am not suggesting that biodiesel will require catalytic converters, I'm only using them as an example of a technology that was designed to protect the environment but used a chemical—platinum--that has significant environmental impacts.) Page I-22 discusses the fact that most modern engines without modifications can run on biodiesel, however, there are impacts on the engine's durability and reliability. The next round of environmental assessment should consider whether more frequent vehicle replacement is going to be required. If so, the impact of all these new vehicles should be considered. (Rodenburg, pg 4)

**Response:** A multimedia evaluation focuses primarily on potential environmental and public health impacts in the State.[20] Secondary or indirect impacts are not addressed as part of the evaluation. Therefore, potential indirect impacts on vehicle operability and vehicle turnover and replacement are not within the scope of the evaluation.

For the specific definition and scope of a multimedia evaluation, please see response to comment A-9. (ARB)

**E-5.** **Comment:** *(Tier 1 Report*) Page I-13 describes the possible need to build new facilities for the processing or production of biodiesel. If such facilities are to be built, this will have a huge impact on a life cycle assessment of biodiesel. The next round of environmental impact assessment for biodiesel should consider these impacts and should try to estimate whether these facilities are going to be built, how many are going to be built, and what the environmental impacts of those facilities will be. (Rodenburg, pg 4)

[20] California Air Pollution Control Laws. Health and Safety Code, Division 26, Part 5, Chapter 4, Section 43830.8(b).

**Response:** A multimedia evaluation focuses primarily on potential environmental and public health impacts in the State.[21] Secondary or indirect impacts are not addressed in the evaluation. Therefore, potential indirect impacts from the potential need to build new biodiesel facilities are not within the scope of the evaluation.

For the specific definition a multimedia evaluation, please see response to comment A-9. (ARB)

**E-6.** **Comment:** Page 1, section A: There are three bulleted lines for air, water and wastes, respectively. It is not clear why public health is not included here. Risk assessment on the public health focuses on human, in contrast to those on environmental media. The same can be said for the bulleted lines in Page 2, section 2. (Li, pg 3)

**Response:** At a minimum, HSC section 43830.8 requires the multimedia evaluation to address impacts associated with:

- Emissions of air pollutants;
- Contamination of surface, groundwater, and soil;
- Disposal or use of byproducts and waste materials from the productions of the fuel.

These three areas were specifically listed in the HSC. Therefore, the Staff Report lists these same areas accordingly.

However, it is important to note that based on the definition of a multimedia evaluation, it must include an evaluation of any significant adverse impact on public health and the environment. The biodiesel multimedia evaluation included a complete assessment of potential environmental and public health impacts. (ARB)

**E-7.** **Comment:** Biodiesel and renewable diesel were assessed separately. The advantages of each over the other were not quantitatively or qualitatively compared. According to UOP (2005), renewable diesel has a lower environmental impact than biodiesel and requires less capital investment to produce. This is in agreement with what I learned from reading the documents provided. However, I failed to find answers to the questions whether biodiesel is indeed needed and why biodiesel is being proposed as the first alternative diesel fuel in California, given the apparent advantages of the renewable diesel. (Li, pg 2)

**Response:** Each fuel subject to a multimedia evaluation is compared to the fuel it is displacing. Therefore, the baseline or reference fuel for biodiesel is CARB diesel. In general, a comparative analysis of a fuel to another fuel undergoing a multimedia evaluation is not within the scope of the evaluation. A justification of the general need in

[21] California Air Pollution Control Laws. Health and Safety Code, Division 26, Part 5, Chapter 4, Section 43830.8(b).

the State or why a specific fuel is being proposed for the development of fuel specifications is not within the scope of the evaluation.

The MMWG Staff Report was not revised in response to this comment, but the following background information is provided for further clarification on biodiesel and renewable diesel fuel within the proposed ADF regulation.

Consumption of diesel fuel substitutes, such as biodiesel and renewable diesel, is expected to increase due to a variety of policy incentives including the Renewable Fuel Standards, Low Carbon Fuels Standard, and potentially the continuance of federal blending tax credits. These fuels will help California meet its climate and petroleum reduction goals, provide fuel diversity, and contribute PM benefits. Thus, it is important to ensure that the full commercialization of these fuels do not increase air pollution or cause other environmental concerns. The proposed ADF regulation will ensure this by subjecting new ADFs to a rigorous, phased environmental review with specific terms and conditions. [22]

In general, the proposed ADF regulation would require an ADF to proceed through a three-stage process that evaluates the fuel for environmental impacts prior to use above a minimum threshold amount in California. As part of the evaluation process, the regulation establishes measures that apply to maintain current air quality protections. Many of the provisions in this regulation are already required under existing State law.[23]

In addition to governing the approval and use of future ADFs, the proposed ADF regulation would also explicitly identify biodiesel as the first ADF commercialized under this regulation. While renewable diesel is also an innovative diesel fuel replacement, it consists solely of hydrocarbons and is virtually indistinguishable from conventional diesel. Therefore, renewable diesel is not considered an ADF under the proposed regulation.[24] (ARB)

**E-8. Comment:** Additives impacts remain a top concern. Additives, particularly those needed for biodiesel, are neither defined nor emphasized in the Proposed Regulation Order (Appendix A). Tier III assessment suggests no substantive change in additive impact in the case of B20, based on the expectation that most currently used additives would continue to be used (Tier III Report, page viii, 1st paragraph). Does this mean that no new additives will be used in new fuels covered by the proposed regulation? Given the needs of adding additives to biodiesel to control oxidation, corrosion, degradation, NOx formation and others as well as cetane value enhancement, there seems a disconnection between the findings of the Tiers conclusions and the proposed regulation. (Li, pg 2)

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[22] Air Resources Board. *Proposed Regulation on the Commercialization of New Alternative Diesel Fuels Staff Report: Initial Statement of Reasons*. January 2, 2015, Page 13.
[23] Air Resources Board. *Proposed Regulation on the Commercialization of New Alternative Diesel Fuels Staff Report: Initial Statement of Reasons*. January 2, 2015, Page 12.
[24] Air Resources Board. *Proposed Regulation on the Commercialization of New Alternative Diesel Fuels Staff Report: Initial Statement of Reasons*. January 2, 2015. Pages 15, 52.

**Comment:** Several of the issues requiring further study are already mentioned in the report, including the effects of ultra fine particles, the possible cosolvent effects of biodiesel (which could mobilize contaminants that are, for example, sorbed to soils outside of leaking underground storage tanks), and the environmental impacts of additives. It is possible that new additives may have to be developed for use with biodiesel and these should be carefully vetted before being approved. (Rodenburg, pg 3)

**Response:** The only additives included in the proposed ADF regulation are those that were evaluated and tested as part of the ARB Emissions Study and biodiesel multimedia evaluation.[25,26] Furthermore, as stated as a condition to the recommendations by the MMWG, specific fuel formulations and additives not included within the scope of the multimedia evaluation must be reviewed for consideration of appropriate action.

Therefore, although the general statement from the UC report is loosely supported, the specific conditions and recommendations made by the MMWG (based on the testing and evaluation completed) strictly safeguard the overall purpose of the proposed ADF regulation to ensure that the full commercialization of the fuel does not increase air pollution or cause other environmental concerns.[27]

In general, the purpose of a multimedia evaluation is to provide the information needed for the development of fuel regulations and inform the overall rulemaking process. Therefore, no revisions were made to the MMWG Staff Report. (ARB)

**E-9.** **Comment:** As acknowledged thoroughly in the report, the presence of additives in the biodiesel is a source of uncertainty for the chemical and physical properties of the biodiesel. There is a statement in the last paragraph on page 24 of Appendix G that "it is reasonable to assume that most of the additives used in biodiesel are currently used in CARB ULSD." It would be helpful to provide some documentation that this assumption is true. The database might be limited, but are the current stocks of biodiesel using similar additives as CARB ULSD? Any evidence to support this statement will be helpful to support a conclusion that biodiesel is just as acceptable as CARB diesel. (Bouwer, pg 3)

**Comment:** The one factor that "clouds" the above conclusion is that additives are likely to be introduced in almost all biodiesel blends. These additives address issues of oxidation, corrosion, foaming, cold temperature flow properties, biodegradation during storage, and water separation. As long as the expectation holds that

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[25] Air Resources Board. *Proposed Regulation on the Commercialization of New Alternative Diesel Fuels Staff Report: Initial Statement of Reasons*. January 2, 2015. Appendix A: Proposed Regulation Order.
[26] Durbin, T.D. et al. *CARB Assessment of the Emissions from the Use of Biodiesel as a Motor Vehicle Fuel in California "Biodiesel Characterization and NOx Mitigation Study."* October 2011.
[27] Air Resources Board. *Proposed Regulation on the Commercialization of New Alternative Diesel Fuels Staff Report: Initial Statement of Reasons*. January 2, 2015. Page 13.

biodiesel will employ additives similar to those used currently in CARB diesel, then it follows that the health and environmental impacts of the two mixtures will be similar. If different additives are employed that might make the biodiesel mixture either more toxic or less biodegradable, then additional studies will need to be conducted to demonstrate the environmental health and safety of the biodiesel mixture planned for use. (Bouwer, pg 1)

**Response:** It is State Water Board staff's understanding that biodiesel and biodiesel blends will employ the same additives currently used in CARB diesel. State Water Board staff recommends that the ARB identify those additives and clearly state that additives used other than those identified are be evaluated separately by the MMWG. (SWRCB)

Please also see response to Comment E-8. (ARB)

**E-10. Comment:** NOx mitigating agent di-tert-butyl peroxide (DTBP) is the only additive included in Proposed Regulation Order (Appendix A, page 26). It is not clear whether this chemical has been used among the currently in-use additives added to CARB Diesel, or it is a new additive for new diesel fuels. Information on the basic physicochemical properties, environmental behavior, and the potential impacts of DTBP are not found in this multimedia evaluation. (Li, pg 3)

**Response:** Di-tert-butyl peroxide (DTBP) was evaluated as part of the ARB Emissions Study for NOx mitigation.[28] Based on the results of the study, a 1% DTBP additive blend was found to fully mitigate the NOx impacts of the candidate B20 and B10 soy biodiesel. 2-ethylehexyl nitrate (2-EHN) additive was also tested at a 1% level but results did not show any significant reductions in NOx. Therefore, 2-EHN was not included under the proposed ADF regulation.

Please also see response to Comment E-8. (ARB)

**E-11. Comment:** Large scale use of pure biodiesel (B100), as well as diesel blends with >20% B100, is considered premature at present, given the current knowledge gaps and uncertainties in several key areas. (Li, pg 2)

**Response:** ARB staff agrees with the reviewer that large scale use of higher biodiesel blends has not been reached.

Regarding general limitations of the multimedia evaluation, the UC Tier III Final Report clearly states the following: "It must be recognized that the multimedia impact assessment is a process and not a product. Life-cycle approaches to emerging fuel options are often difficult to apply and may be burdened by uncertainty such that these studies become more informative as fuel technologies mature and are deployed. It is important to realize that much is unknown about the full implantation and emerging

[28] Durbin, T.D. et al. *CARB Assessment of the Emissions from the Use of Biodiesel as a Motor Vehicle Fuel in California "Biodiesel Characterization and NOx Mitigation Study."* October 2011. Page xlviii.

transportation fuel system and will remain uncertain until the fully system is created."[29] (ARB)

Staff Report

**F-1.** **Comment:** The Opening Glossary should contain CARB. The opening section does not define CARB diesel (page 4). CARB diesel is defined later in the report. If a reader starts with the opening section as I did, it will be confusing to not have a definition of CARB diesel up front. In Appendix G, the term "conventional petroleum diesel" or simply "petroleum diesel" is used. I suspect that CARB diesel and conventional petroleum diesel are terms for the same product. The broader community is likely to be more familiar with the term conventional petroleum diesel or petroleum diesel in comparison to CARB diesel. (Bouwer, pg 2)

**Comment:** Add CARB to the list of acronyms on page 8 of Appendix A. ARB is listed, but not CARB. (Bouwer, pg 2)

**Response:** The Staff Report was revised to include "CARB diesel" in the Glossary. Staff also added the definition of CARB diesel to the Introduction (Chapter I, part C).

CARB diesel fuel meets ARB motor vehicle fuel specifications. The proposed ADF regulation defines "CARB diesel" as a light or middle distillate fuel that may be comingled with up to five (5) volume percent biodiesel and meets the definition and requirements for "diesel fuel" or "California non-vehicular diesel fuel" as specified in California Code of Regulation, title 13, section 2291 et seq.[30]

In the Tier I, Tier II, and Tier III reports by the UC researchers, "CARB diesel," "petroleum diesel," "conventional petroleum diesel," and "CARB Ultra Low Sulfur Diesel (ULSD)" are used interchangeably. (ARB)

**F-2.** **Comment:** The impacts of biodiesel relative to CARB diesel depend strongly on the percentage blend of petroleum diesel with biodiesel. However, the treatment of these categories and terminology is inconsistent through the report. For example:

- p. 4 introduces four categories of blending: B10, B20, B50, and B100 (where B10 = a 10% by volume blending of biodiesel with CARB diesel; B20, 20% blending, and so on).
- p. 8 report emissions for B5 blends, but not B10;
- p. 11 discusses B5 in the context of underground storage tanks (UST);

[29] Ginn, T.R. et al. *California Biodiesel Multimedia Evaluation Final Tier III Report*. June 2014, iv.
[30] Air Resources Board. *Proposed Regulation on the Commercialization of New Alternative Diesel Fuels Staff Report: Initial Statement of Reasons*. January 2, 2015. Appendix A: Proposed Regulation Order. Page A-8.

- Appendix A p. 4 defines only B5 and B20, as follows "(6) 'B5' means a biodiesel blend containing no more than five percent biodiesel by volume" and "(7) 'B20' means a biodiesel blend containing more than five and up to 20 percent biodiesel by volume." In this definition, both B10 and B20 would fall into the B20 category.
- Appendix A p. 5 defines "(8) 'CARB Diesel fuel' means ... which may be comingled with up to five (5) volume percent biodiesel..."Combining these definitions, B5 and CARB Diesel both have between 0 and 5 percent biodiesel by volume mixed with petroleum diesel meeting ARB standards.

(Holloway, pg 1)

**Response:** To clarify the terminology, the following definitions from the proposed ADF regulation are provided:

> "Biodiesel" means a fuel comprised of mono-alkyl esters of long chain fatty acids derived from vegetable oils or animal fats that is 99-100 percent biodiesel by volume (B99 or B100) and meets the specifications set for by ASTM International in the latest version of *Standard Specification for Biodiesel Fuel Blend Stock (B100) for Middle Distillate Fuels* D6751 contained in the ASTM publication entitled: Annual Book of ASTM Standards, Section 5, as defined in California Code of Regulations, title 4, section 4140(a).[31]
>
> "Biodiesel Blend" means biodiesel blended with petroleum-based CARB diesel fuel or non-ester renewable diesel.[32]
>
> "Blend Level" means the ratio of the alternative diesel fuel to the CARB diesel it is blended with, expressed as a percent by volume. The blend level may also be expressed a "AXX," where "A" represents the particular alternative diesel fuel and "XX" represents the percent by volume that alternative diesel fuel is present in the blend with CARB diesel (e.g., a 20 percent by volume biodiesel/CARB diesel blend is denoted as "B20."[33]
>
> "B5" means a biodiesel blend containing no more than five percent biodiesel by volume.[34]

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[31] Air Resources Board. *Proposed Regulation on the Commercialization of New Alternative Diesel Fuels Staff Report: Initial Statement of Reasons*. January 2, 2015. Appendix A: Proposed Regulation Order. Page A-7.
[32] Air Resources Board. *Proposed Regulation on the Commercialization of New Alternative Diesel Fuels Staff Report: Initial Statement of Reasons*. January 2, 2015. Appendix A: Proposed Regulation Order. Page A-7.
[33] Air Resources Board. *Proposed Regulation on the Commercialization of New Alternative Diesel Fuels Staff Report: Initial Statement of Reasons*. January 2, 2015. Appendix A: Proposed Regulation Order. Page A-7.
[34] Air Resources Board. *Proposed Regulation on the Commercialization of New Alternative Diesel Fuels Staff Report: Initial Statement of Reasons*. January 2, 2015. Appendix A: Proposed Regulation Order. Page A-7.

"B20" means a biodiesel blend containing more than five percent and no more than 20 percent biodiesel by volume.[35]

"CARB diesel" means a light or middle distillate fuel that may be comingled with up to five (5) volume percent biodiesel and meets the definition and requirements for "diesel fuel" or "California nonvehicular diesel fuel" as specified in California Code of Regulations, title 13, section 2281 et seq. "CARB diesel" may include: non-ester renewable diesel; gas-to-liquid fuels; Fischer-Tropsch diesel; diesel fuel produced from renewable crude; CARB diesel blended with additives specifically formulated to reduce emission of one or more criteria or toxic air contaminants relative to reference CARB diesel; and CARB diesel specifically formulated to reduce emissions of one or more criteria or toxic air contaminants relative to reference CARB diesel.[36]

Based on these definitions, biodiesel blend levels are characterized as follows:

- B5 = Biodiesel blend containing ≤ 5 vol% biodiesel
- B20 = Biodiesel blend containing > 5 vol% and ≤ 20 vol% biodiesel
- B100 = Biodiesel containing 99 – 100 vol% biodiesel

Diesel and biodiesel are regulated by multiple state agencies in California. The Division of Measurement Standards (DMS) adopted ASTM D6751 Standard Specification for Biodiesel Fuel Blend Stock (B100), ASTM D7467 Standard Specification for Diesel Fuel Oil, Biodiesel Blend (B6-B20), and ASTM D975, Standard Specification for Diesel Fuel Oils (up to B5). There are currently no ARB standards for biodiesel fuel.[37] For more information on other state regulations affecting biodiesel use in California, please refer to the Cal/EPA Fuels Guidance Document.[38] (ARB)

**F-3. Comment:** Page 5, section C: I suggest including one brief sentence on line 4 indicating that CARB diesel is conventional petroleum based ultra-low sulfur diesel, along with a brief time line. One or more references should be helpful, directing readers to information on CARB diesel development and adoption, quantity of use in the state, its environmental and human health impacts, etc. This is especially helpful to stakeholders and interested parties who reside outside California and are unfamiliar with the phrase "CARB diesel." (Li, pg 3)

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[35] Air Resources Board. *Proposed Regulation on the Commercialization of New Alternative Diesel Fuels Staff Report: Initial Statement of Reasons*. January 2, 2015. Appendix A: Proposed Regulation Order. Page A-8.

[36] Air Resources Board. *Proposed Regulation on the Commercialization of New Alternative Diesel Fuels Staff Report: Initial Statement of Reasons*. January 2, 2015. Appendix A: Proposed Regulation Order. Page A-8.

[37] Air Resources Board. *Proposed Regulation on the Commercialization of New Alternative Diesel Fuels Staff Report: Initial Statement of Reasons*. January 2, 2015, 26.

[38] California Environmental Protection Agency. *Cal/EPA Fuels Guidance Document. Version 1.0.* November 15, 2011. http://www.calepa.ca.gov/Biofuels/Guidance.pdf (accessed February 25, 2015).

**Response:** The Staff Report was revised to include the definition of CARB diesel in the Introduction (Chapter I, part C). The proposed ADF regulation defines "CARB diesel fuel" as a light or middle distillate fuel which may be comingled with up to five volume percent biodiesel, and meeting the definition and requirements for "diesel fuel" or "California non-vehicular diesel fuel" as specified in 13 CCR 2281 et seq.[39]

Please also see staff's response to Comment F-1 and F-2. (ARB)

**F-4.** **Comment:** In the main Staff Report, section I-C, I suggest summarizing the limitations of this multimedia evaluation immediately following the major MMWG conclusion on page 6. Some limitations are well described in the Tiers Reports, but are absent in the Staff Report. The limitations are different from the conditions in the Recommendations (page 17). (Li, pg 2)

**Response:** The purpose of the Staff Report is to provide a summary of the biodiesel multimedia evaluation (i.e., the Tier I, II, and III reports) and based on the evaluation provide the MMWG's overall conclusions and recommendations to the CEPC. The details of the evaluation, including the sources and specific limitations of the evaluation, are provided in the UC reports. The Final Tier III Report provides a comprehensive summary of the Tier I and Tier II findings, as well as the Tier III conclusions, where corresponding limitations are clearly explained.

The Staff Report was revised to include more details about the overall scope of the multimedia evaluation and purpose of the Staff Report in the Introduction (Chapter I). The report now states that the purpose and scope of the multimedia evaluation is to provide the information needed for the development of fuel regulations and inform the overall rulemaking process. For the proposed biodiesel specifications included as part of the ADF regulation, the MMWG prepared the Staff Report for submittal to the CEPC. The purpose of the Staff Report is to provide a summary of the multimedia evaluation and the MMWG's overall conclusions and recommendations to the CEPC based on the evaluation.

Regarding general limitations of the overall multimedia evaluation, please also see response to comment E-11. (ARB)

**F-5.** **Comment:** Table of Contents: I suggest changing II title from "Summary" to "Section Summaries" or "Summaries of Reports from Participating State Agencies," in order to avoid confusion with the summary of this Main Report. (Li, pg 3)

**Response:** The Staff Report was revised accordingly. The title of Chapter II "Summary" was changed to "Section Summaries" and the Table of Contents was updated. (ARB)

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[39] Air Resources Board. *Proposed Regulation on the Commercialization of New Alternative Diesel Fuels Staff Report: Initial Statement of Reasons*. January 2, 2015. Appendix A: Proposed Regulation Order, A-7.

**F-6. Comment:** Page 10, first paragraph, ending phrase: The words "and fuels" are confusing to me. (Li, pg 4)

**Response:** The Staff Report was revised to clarify the sentence.

The specific study referenced in the Staff Report: Initial Statement of Reasons (ISOR)[40] for the proposed ADF regulation was a study conducted by the National Renewable Energy Laboratory that looked at two Cummins ISL engines equipped with SCR systems.[41] Results showed that the use of SCR was effective at reducing NOx to near the detection limit on all duty cycles and fuels, including B100.[42] The overall objective of the study was to compare NOx emissions from transit buses to establish if there is a real-world NOx increase for transit bus duty cycles and engine calibrations. (ARB)

Source Reports

**G-1. Comment:** The bullet point at the top of page xi in Appendix G should mention that there are many bacterial groups indigenous to aquatic systems and soils that are capable of biodegrading biodiesel and petroleum hydrocarbons. This contributes to the high potential for biodegradation in these media. (Bouwer, pg 2)

**Response:** The Biodiesel Final Tier III Report was revised with information regarding bacterial groups capable of biodegrading biodiesel and petroleum hydrocarbons.

Several studies have been performed on the biodegradation of biodiesel blends. Due to the structure of biodiesel compared to ULSD, biodiesel is more readily biodegradable in general. There are numerous bacterial groups indigenous to aquatic systems and soils that are capable of biodegrading biodiesel and petroleum hydrocarbons and this contributes to the high potential for biodegradation in these media. (UC)

**G-2. Comment:** On page 7 in the Appendix G report, there is a discussion that material compatibility is an important consideration. The second paragraph indicates some of the materials that are poorly compatible with biodiesel. It would be helpful to also provide examples of materials that are compatible with biodiesel. This will help educate the reader on materials to avoid along with those that are good to use. The same modifications are recommended to a similar discussion of materials compatibility that appears on page 25 in Appendix G. There is more discussion about materials compatibility in a section labeled "6. Tier III Appendices" within the Appendix G tab on page I-26. More advice on compatible materials will be helpful. The impression given by this

[40] Air Resources Board. *Proposed Regulation on the Commercialization of New Alternative Diesel Fuels Staff Report: Initial Statement of Reasons*. January 2, 2015.
[41] Lammert et al., *Effect of B20 and Low Aromatic Diesel on transit Bus NOx emissions Over Driving Cycles with a Range of Kinetic Intensity*, SAE Int. J Fuels Lubr., 5(3):2012
[42] Air Resources Board. *Proposed Regulation on the Commercialization of New Alternative Diesel Fuels Staff Report: Initial Statement of Reasons*. January 2, 2015. Page 44.

discussion is that most materials can be incompatible. It would be good to conclude with some suggested materials that can be used with high confidence. (Bouwer, pg 2)

**Response:** The Biodiesel Final Tier III Report was revised to include additional guidance on materials compatibility and specific examples of compatible elastomers.

Material manufacturers guidelines or industry standard reports (e.g., NREL, 2009) should be consulted prior to biodiesel exposure of any material with unknown compatibility. Some compatible elastomers reported in NREL (2009) include nylon, teflon, and perfluoroelastomer.[43] (UC)

**G-3.** **Comment:** In a section labeled "6. Tier III Appendices" within the Appendix G tab, there is a discussion of subsurface fate and transport properties on page I-5. The third sentence makes the statement that the composition of biodiesel differs significantly from that of petroleum diesel. Consequently, the behavior of the two liquids is likely to be different in the environment. It would be helpful if this discussion can include more specifics about the composition differences so it is easier to understand if the biodiesel might be more or less problematic. For example, we learn from the biodegradation studies that biodiesel is more biodegradable than petroleum biodiesel, so this is a positive attribute. (Bouwer, pg 3)

**Response:** The passage noted is part of the Executive Summary of the Tier I report, where the knowledge gaps are identified. The knowledge gaps are filled in Tier II, where in particular the fate and transport properties of biodiesel are identified to be essentially similar to those of conventional diesel. This is reported in the Tier II and Tier III parts of the report. (UC)

**G-4.** **Comment:** More detail needs to be provided for the results shown in Table 6.2 on page I-56 that are used to support the mechanism of co-metabolism. What is the ration of diesel and biodiesel in the mixture? Was the total mass of diesel and biodiesel in the mixture the same as the mass of diesel alone? Without reporting the initial masses or concentrations, it is not clear from the reported data if more mass of diesel biodegraded in the presence of the biodiesel. (Bouwer, pg 3)

**Response:** The UC researchers provided additional details to the description of Table 6.2. Staff did not request the UC researchers to revise the Tier I report to include these additional details since these do not change the conclusions or recommendations by the MMWG. (ARB)

[43] NREL Technical Report TP-540-4367, "Biodiesel Handling and Use Guide," 4th Edition, January 2009, 55 pages.

**Table 6.2. Biodegradation of fuel in biodiesel mixture and diesel alone***

| Days | Diesel in Mixture | Diesel Alone |
|---|---|---|
| 0 | 0 | 0 |
| 1 | 56.49% | 16.27% |
| 4 | 94.79% | 53.54% |

*Data from Zhang *et al*.[44]

Biodiesel can promote and accelerate the biodegradation of diesel through a process called cometabolism. Zhang *et al.,* determined that diesel in a mixture degraded three times faster (56%) than diesel alone (16%) under aerobic conditions (as shown in Table 6.2 below). The table shows the percent degradation of the biodiesel fraction present in 50/50 by volume mixture with diesel (second column) or neat (third column). This "suggests that in the presence of rapeseed ethyl ester (REE), microorganisms use the fatty acids as an energy source to promote the degradation of diesel."[35]

Differences in degradation patterns were also noted between pure biodiesel and biodiesel/diesel blends. After one day, REE 100 degraded 61.81% while in the same period of time a biodiesel/diesel mixture degraded 56.4%. This relatively small difference in degradation suggest that "microorganisms attacked the fatty acids in REE and alkane chains in the diesel at the same time and at the same rates instead of favoring the fatty acids only."[35] (UC)

**G-5. Comment:** Page I-26 notes that acceptable materials for storage and transport of biodiesel include aluminum, steel and fluorinated polyethylene or polypropylene. In particular, the fluorinated compounds are a big environmental problem and should be avoided at all costs. If increased use of biodiesel is going to require the use of these kinds of fluorinated compounds this could be a serious problem. (Rodenburg, pg 4)

**Response:** The Tier I Report dated December 2008 discussed biodiesel material compatibility. Page 31 of the Tier I Report states:

Vehicles manufactured before 1993 may have issues with incompatible seals, gaskets and adhesives as they were made from natural and nitrile rubber (Van Gerpen, 2004). Most engines produced after 1994 are compatible with biodiesel (B20); however, "the user should consult the equipment manufacturer of owner's manual regarding the suitability of using biodiesel (B100) or biodiesel blends in a particular engine (ASTM, 2007).

Table 3.1 in the Tier I Report listed compatibility information of several potential gasket materials with biodiesel. Flurosilicon may not be a necessary choice for biodiesel. We agree that additional review is required if a potential gasket is recommended. (DTSC)

[44] Zhang, X., C. Peterson, D. Reece, G. Moller, and R. Haws. 1998. Biodegradability of Biodiesel in the Aquatic Environment, *ASAE*, 41(5): 1423 – 1430.

**G-6. Comment:** There is a statement in the middle of age I-56 that microorganisms metabolize biodiesel and diesel at roughly the same rates. This statement is inconsistent with the reported data on page I-55 that after 28 days, the biodiesel exhibited 85 to 88% removal in comparison to 26% removal for petroleum diesel. The biodegradation rate for biodiesel is markedly faster than for petroleum diesel. The results shown in table 6.3 also support this conclusion that biodegradation rates are faster for biodiesel. (Bouwer, pg 3)

**Response:** The wording on page I-56 erroneously implied biodiesel and diesel underwent degradation at the same rates. The intention was to relate the rate of degradation of pure biodiesel with that of the biodiesel fraction present in biodiesel-diesel blends, as consistent with the context of the foregoing citation of Zhang *et al*., 1998.[35] The phrase "metabolize biodiesel and diesel at roughly the same rates" should have been "metabolize biodiesel and biodiesel blended with diesel at roughly the same rates." (UC)

Staff did not request the UC researchers to revise the Tier I report to correct this error since this does not change the conclusions or recommendations by the MMWG. (ARB)

**G-7. Comment:** Section 6.4.3 on page I-57 is called Biodegradation Under Aerobic and Anaerobic Conditions. It is better to explicitly mention the electron acceptor involved rather than to call the conditions "anaerobic." For example, the situation with nitrate as the electron acceptor can be called nitrate respiration or denitrification. It is difficult to generalize from denitrification to all anaerobic conditions. The data on biodegradation for a wide range of anaerobic conditions are certainly sparse. (Bouwer, pg 3)

**Response:** The UC researchers clarified that this section summarizes one study involving nitrate reduction and one involving fermentation. Staff did not request the UC researchers to revise the Tier I report to include this clarification since this does not change the conclusions or recommendations by the MMWG. (ARB)

To clarify the comment the following information is provided for front of the passage: "Studies of anaerobic biodegradation of biodiesel are few. Here we summarize one study involving nitrate reduction and one study involving fermentation where the organic intermediates serve as electron acceptor." (UC)

**G-8. Comment:** Tier-I Report, page I-20, is the only section about algae as a feedstock, and the discussion is highly positive. It is not clear what type of algae is relevant to biodiesel production. Given that California has long ocean shorelines, are there brackish water resources suitable for algae production? Are there any foreseen adverse impacts, besides the limitations associated with a narrow range of growing and harvesting conditions? (Li, pg 2)

**Response:** The identification of economic and/or logistic feasibility of various feedstocks is a "moving target" and a topic with numerous aspects that are all unknown.

For instance, recent news on algae production involves land-based production plants with screens and this obviates questions regarding coastline. In the event of coastline-associated facilities, the process for production is not established so the risk analyses is premature and speculative. While the questions raised in the comment are worthwhile, they are well beyond the scope of this study. (UC)

**G-9. Comment:** *(Tier 1 Report)* Page I-59 refers to specific sensitive populations at risk of exposure, yet I did not see anything in the report about this. Although asthma is mentioned as a possible problem with biodiesel, this requires more discussion. Another important issue to investigate with regard to biodiesel is allergy. Many people are allergic to the raw oils such as palm oil or soy oil. Is there any reason to believe that the combustion products of biodiesels derived from these sources may cause an allergic response? People with known allergic responses are definitely a sensitive population that should be considered. Such allergies could be respiratory or dermal. There is some literature showing that regular diesel fuel is allergenic. (Rodenburg, pg 4)

**Response:** The *PeanutAllergy.com* web site indicates that "the way the peanuts are processed minimized or may even eliminate the risk. In order to manufacture the peanut oil used for both cooking and biofuels, much of the problematic peanut protein is filtered out. Still, some peanut oil products may still have enough protein to cause an allergic reaction", but, "…that it's probably not something worth worrying about. Even if there are trace amounts of peanut protein left in the oil, the way the fuel is used, including passing it through the engine, will degrade the remaining protein, rendering the oil safe for handling by those with allergies." Biofuel blended diesel fuel MSDS indicates that "Prolonged or repeated contact is not likely to cause significant skin irritation. Single acute exposure not expected to cause allergic response. Liquid may be absorbed through the skin in toxic amounts if large areas of skin are repeatedly exposed. Symptoms may include itching, irritation, pain and swelling." (UC)

**G-10. Comment:** *(Appendix G, Appendix – Tier II Report (87 pages))*
<u>Page II-10, first two paragraphs</u>: The results from GCMS for the additives are highly variable, resulting in high uncertainty or failure in identifying the source of increased toxicity. I doubt the SBSE extraction efficiencies for these two compounds, especially acetic acid butyl ester which has a log Kow of only 1.8 (EPI, 2013). (Li, pg 5)

**Response:** We agree with the comment. It is sensible that the extraction limitations may play a role, as well as other factors, and we recognize the limitations of the experiment as noted in the section. (UC)

**G-11. Comment:** In a section labeled "6. Tier III Appendices" within the Appendix G tab, there is a discussion of biodegradation of biodiesel components on page I-55. Near the bottom pf page I-55, the observed percentages for degradation are reported with 4 significant figures (e.g., 85.54% degradation). A batch biodegradation test cannot achieve this degree of precision, so it is

recommended that the results be rounded to 2 or at most 3 significant figures. (Bouwer, pg 3)

**Comment:** The table II-D-3 on page II-84 within the Appendix G tab seems to arbitrarily show all of the data with two decimal points. This means that some of the percent degradation numbers are shown with 5 significant figures (e.g., 125.42). This level of precision is not possible for batch biodegradation testing, so the numbers should be reported with 2 or at most 3 significant figures. Furthermore, the last three tests have percent degradation values greater than 100%. The sources for this error should be discussed in the text. (Bouwer, pg 4)

**Response:** Staff noted that the last three test results in Table II-D-3 on page II-84 exceeded 100 percent. Staff also noted the explanation researchers included on page II-83 stating, "In Experiment number 4, the amount of utilized carbon was measured more than initial carbon content due to malfunction of respirometer during the experimental period." However, staff did not request the UC researchers to revise the reports to correct these discrepancies since these do not change the conclusions or recommendations by the MMWG. (ARB)

**G-12. Comment:** Appendix G, page x: line 2 from the bottom: "compare" should be "compared. Some error on page 11, line 4 in the second full paragraph. On page 25 of Appendix G, "alliviate" should be "alleviate in the second to last line of the first full paragraph. On page 27 of Appendix G, "volitilize" should be "volatilize". (Bouwer, pg 4)

On page I-73 of Appendix G, "month" should be "months" in line 7 from the bottom. On page II-3 of Appendix G, "biodegrable" should be "biodegradable" in line 13 from the top. On page II-3 of Appendix G, "test" should be "tests" in line 2 from the bottom. On page II-17 of Appendix G, "without algae inoculate" should be "without an algae inoculum" in line 6 in the middle paragraph. On page II-20 of Appendix G, the caption for Figure II-A-1 is incomplete (e.g., it looks like some test is missing or is cutoff). On page Ii-32 of Appendix G, there is a statement about an error with the reference cited in the last line of text. The same error occurs on the next page II-33 in line 18 from the top. On page II-87 of Appendix G, "biodegrability" should be "biodegradability" in the second bullet in the middle of the page. (Bouwer, pg 4)

**Response:** Staff acknowledges these typographical and grammatical errors. Since these errors did not change the overall conclusions or recommendations by the MMWG, staff did not request the UC researchers to revise the Tier I, II, or III reports. (ARB)

**G-13. Comment:** *(Appendix G – Final Tier III Report (31 pages))*

<u>Page vi,</u> 9th line from bottom: There is an extra "that". Page x, line 12: There is an extra "from".

Page x, line 21: Should the word “transport” be “transportation”?

Page 17, line 10 from bottom: Is “~10 cm” correct? Given in Tier II Report, page II-11, line 3 is “~20 cm”.

Page 17, line 9 from bottom: “Bioextent” should be “Bioextent-30”.

Page 18: At the end of section 3.2, it is helpful to add the environmental and remediation implications of the lens geometry from AF B100, as it is different from others.

Pages 21-29: Section 4 Conclusions has substantial overlap with the Executive Summary on pages iv – xi, therefore reads redundant. (Li, pg 4)

**Comment:** *(Appendix G, Appendix – Tier I Report (94 pages))*

I found this Report is of high quality. It is comprehensive and has sufficient details and depth. It is easy to read and has little redundancy.

A summary of the history and the current status of alternative diesels in California would be very helpful but is not found.

Page I-33, line 4 from bottom: The word “centane” should be “cetane”, and a period is needed at the end. Page I-39, first paragraph in 4.4.3, line 3: The word “that” might be “of”.

Page I-39, line 3 from bottom: The word “course” could be “coarse”. (Li, pg 4)

**Comment:** Durbin et al (2011), pages 222 and 224: “Marcophage” should be macrophage. (White, pg 5)

**Response:** Staff acknowledges these typographical and grammatical errors. Since these errors did not change the overall conclusions or recommendations by the MMWG staff did not request the UC researchers to revise the reports. (ARB)

**G-14. Comment:** *(Tier 1 Report)* Page I-55. Typo about halfway down the page. “Fatty acids are oxidized at the ▫ carbon.” (Rodenburg, pg 4)

**Response:** When the Tier I word document was converted to a PDF format, it did not correctly convert the ‘β’ symbol and inadvertently displayed as an unrecognizable symbol. However, the prior sentence before the error symbol on page I-55 states, “Microorganisms can easily break down the straight carbon chain structures of biodiesel under aerobic conditions via the beta-oxidation pathway.” Staff did not request the UC researcher to revise the Tier I report to correct this error since it did not change the conclusions or recommendations by the MMWG. (ARB)

**G-15. Comment:** *(Appendix G, Appendix – Tier II Report (87 pages))*

Page Pages II-29 to II-68 – Chemical Analysis: This work aimed at discovering the compounds responsible for the increased toxicity. It was a difficult task, and the methods using stir bar sorptive extraction (SBSE) and GCMS appear appropriate. From the multimedia evaluation perspective, the results from this work are considered by this reviewer as screening in nature. Future work is needed, and it would be more efficient to focus on the additives, based on Figure 1 (page II-9) and the toxicity test (page II-15 to II-28) which suggested strong impact of the additives on toxicity.

Page II-9, bottom two lines: I suggest rewriting the names of the antioxidant additives. They appear as 4 separate ones, but were just two. For acetic acid butyl ester, please delete the comma after acid, or simply change to butyl acetate. For 1,4-Benzenediol, 2-(1,1-dimethylethyl), the name tert-butylhydroquinone (TBHQ) is less confusing.

Page II-77, line 5**:** Figure B7 should be Figure II-C-7.

(Li, pg 5)

**Response:** The Tier II Report (page II-29 to II-68 of Appendix II-B) presented and summarized how fuel composition changes in four different ecosystems varying in temperature and salinity. This experiment was performed to provide the aquatic environmental fate and toxicity of different biodiesel blends including some additives. As presented in Figure 1 (page II-9), there is an increased level of toxicity for fuels with additives. The UC researchers included a recommendation that analytical chemistry information is needed on fuel samples to elucidate the chemical causes of toxicity. As stated as a condition to the recommendations by the MMWG, specific fuel formulations and additives must be reviewed for consideration of appropriate action.

Staff also acknowledges the chemical convention suggestions and typographical errors. A subsequent section correctly notes acetic acid butyl ester without the comma and TBHQ is used instead of 1,4-Benzenediol, 2-(1,1-dimethylethyl). Staff did not request the UC researchers to revise the Tier II report to correct these errors since these do not change the conclusions or recommendations by the MMWG. (ARB)

**G-16. Comment:** *(Tier 1 Report)* Page I-6. The report notes that the biodiesel used in many of the studies described in this report was at least six months old, which is the maximum recommended storage time for biodiesel. It might also be pointed out that this may represent a worst case scenario. Emissions of particulates, NOx, etc. are likely to be worse with this relatively old fuel. (Rodenburg, pg 4)

**Response:** The shelf life of biodiesel may vary depending on feedstock and how it was stored. In general, the typical shelf life of biodiesel is about six months. It is noted in the report that the anti-oxidant and nitrogen purge were added to the fuel used for the

emission testing and accounted for the testing duration being more than six months to preserve the fuel quality. It is well established that fuel-handling practices have an important influence on engine performance and combustion emissions. All biodiesel referred to pure biodiesel (B100) in the report meets the specific biodiesel definition and standards approved by ASTM D6751-12. Also, biodiesel blends were mixed with B100 fuel meeting ASTM standards. (ARB)

**G-17. Comment:** (*Tier 2 Report* Appendix II-B: chemical analysis of the water accommodated fractions of Bio fuels using stir bar sorptive extraction)

Page II-32 missing reference at bottom of page
Page II-33 another missing reference
Page II-83 Amount of diesel added to each microcosm is given as 5 mL, when it should be 5uL. (Rodenburg, pg 4)

**Response:** Staff acknowledges these errors:

On page II-32 in Tier II report, the reference intended to be referred is Table II-B-1 on page II-42, *Stock water and mixing temperature for preparing representative WAF for toxicity assays*.

On page II-33, the intended reference is Table II-B-2 on page II-42, *Mixing volumes for preparation of WAF*.

On page II-83, 5 uL is the correct amount added as described in *Fuel Sample and Microcosm Preparation* and *Assessing Biological Activity* section.

Staff did not request the UC researchers to revise the Tier II Report to correct these errors since it did not change the overall conclusions or recommendations by the MMWG. (ARB)

**G-18. Comment:** Biodiesel Tier I, page I-4: The authors are reminded that it is critical to provide units when referring to changes in emission rates. (White, pg 5)

**Comment:** Biodiesel Tier I, section 7: Please pay attention to units! (White, pg 5)

**Response:** Staff acknowledges the importance of units. However, staff did not request the UC researchers to revise the Tier I report since it did not change the conclusions or recommendations by the MMWG. (ARB)

**G-19. Comment:** Biodiesel Tier I, page I-35: The authors often use statements such as “large reductions”, but fail to qualify. How will the reader know what “large means.” Is it 10% or 95%? (White, pg 5)

**Response:** Staff acknowledges this comment and recognizes that the amount or range of reductions may vary depending on the various studies and testing parameters for which the evaluation is based. Some reductions are quantified in the following section (Section 4.4.2) and includes results from a U.S. EPA emissions study on biodiesel. Average emissions reductions are shown in Figure 4.3 (Average emission impacts of biodiesel for heavy-duty highway engines) and quantified in Table 4.1 (Overall average change in mass emission effects due to use of biodiesel fuels in heavy-duty highway vehicles compared to standard diesel fuel). Staff did not request the UC researchers to revise the Tier I report since it did not change the conclusions or recommendations by the MMWG. (ARB)

**G-20. Comment:** Biodiesel Tier I, page I-43: The authors refer to TEFs but fail to note what endpoint is being discussed. Presumably it's carcinogenic activity. Although some agencies use the term TEF to refer to carcinogenic activity relative to BaP, the authors are reminded that many readers will be more familiar with the terminology used the EPA's Integrated Risk Information System (IRIS) employs the acronym RPF (Relative Potency factor). (White, pg 5)

**Response:** Staff acknowledges this comment and believes that the carcinogenic endpoint is inferred in the first paragraph of the section (Section 4.4.5 on page I-42), which states:

> "Over 40 chemical compounds in diesel exhaust have been listed as toxic air pollutants based on carcinogenicity and exhaust from diesel engines account for a significant fraction of the total added cancer risk in outdoor air from all hazardous air pollutants combined (Morris and Jia, 2003). There have been a number of studies comparing toxic air pollutant emissions, particularly polycyclic aromatic hydrocarbons (PAHs), from conventional diesel and biodiesel. In the paragraphs below we review key issues that can be drawn from these studies."

Staff did not request the UC researchers to revise the Tier I Report since the MMWG conclusions or recommendations did not change. (ARB)

**G-21. Comment:** Biodiesel Tier I, page I-61: "…thoroughly tested by the EPA and is "safe". Where does this statement come from? The EPA is extremely reluctant to use adjectives such as "safe." The agency is far more likely to use statements such as "negligible increase in risk above background." (White, pg 5)

**Response:** Staff acknowledges this comment and believes that the intent of the sentence, as quoted by the reviewer on page I-61 (Section 7.3.), is clarified within context of the rest of the paragraph, as follows:

> "A review of the broader literature suggests that reduced emissions of PM10, PAHs, and nitro-PAHs can be anticipated with biodiesel formulations in comparison to diesel fuels (see section 4.4). However, as noted in section 4.4, the wide variety of oils and fats that might be used to make biodiesel fuel,

makes the actual emissions of PM and toxic air contaminants uncertain. Moreover, the toxic profiles of the altered emissions also remain uncertain. Given this issue of different formulations of biodiesel probably having different emissions and toxicity profiles, it is not appropriate to accept any common wisdom or popular literature that suggests that biodiesel fuel has been thoroughly tested by the EPA and is "safe." Such issues can only be resolved with continued emission and toxicity testing."

Staff did not request the UC researchers to revise the Tier I Report with additional information since the MMWG conclusions or recommendations did not change. (ARB)

**G-22. Comment:** Biodiesel Tier I, Section 7 (carried through to Tier III): Many vague statements need to be clarified. For example, "premature death" from what type(s) of effects? "More investigations in biological systems" – what systems? What endpoints? What route(s) of exposure? (White, pg 5)

**Response:** Staff acknowledges this comment and recognizes that the amount of detail provided in the report may vary depending on the specific source or publication referenced. Since all sources are clearly referenced in the report, staff may access and review complete references for further details and information. Accordingly, staff did not request the UC researchers to revise the Tier I Report. (ARB)

**G-23. Comment:** Biodiesel Tier II, page II-32: Reference problem at bottom of page. (White, pg 5)

**Response:** The reference in the report is to Table II-B-1 on page II-42. The sentence is currently missing the word "Table" and should read as follows: "Mixing temperatures and salinities for the different WAF are outlined in Table II-B-1." With this understanding and no change to the MMWG's overall conclusions and recommendations, staff did not request the UC researchers to revise the report. (ARB)

**G-24. Comment:** Throughout the Biodiesel Tier I, II and III reports: The quality of reproduced graphics (e.g., page II-77) is marginal. In some cases it is very difficult to make out the axes labels. (White, pg 5)

**Response:** Staff acknowledges this comment but did not request the UC researchers to revise the report with improved graphics. Staff notes that the electronic files of these reports may be easier to view. (ARB)

<u>Proposed Regulation</u>

**H-1. Comment:** *(Next five comments on Appendix A – Proposed Regulation Order (36 page)) Page* 4, (a), (1): If ADF means any non-CARB diesel fuel that does not consist solely of hydrocarbons, a question arises whether "renewable diesel" as defined in the 3-tier multimedia evaluation is an ADF. The renewable diesel, to my understanding, consists of predominantly hydrocarbons. (Li, pg 4)

**Response:** The first ADF under the proposed regulation is biodiesel. While renewable diesel is also an innovative diesel fuel replacement, it consists solely of hydrocarbons and is virtually indistinguishable from conventional diesel. Therefore, renewable diesel is not considered an ADF under the proposed regulation.[45]

Please also see response to comment F-2. (ARB)

**H-2. Comment:** Page 5, (8): The definition for "CARB Diesel fuel" in this proposed regulation appears different from that for "CARB Diesel" used in the 3-tier multimedia evaluation. The former includes 5%v of FAME, while the latter is a pure ultra-low-sulfur diesel (ULSD) derived from petroleum. (Li, pg 4)

**Response:** The Staff Report was revised to include "CARB diesel" in the Glossary. Staff also added the definition of CARB diesel to the Introduction (Chapter I, part C).

The biodiesel multimedia evaluation is a relative comparison between biodiesel fuel and diesel fuel meeting ARB motor vehicle fuel specifications (CARB diesel). The proposed ADF regulation defines "CARB diesel fuel" as a light or middle distillate fuel which may be comingled with up to five volume percent biodiesel, and meeting the definition and requirements for "diesel fuel" or "California non-vehicular diesel fuel" as specified in 13 CCR 2281 et seq.[46]

In the Tier I, Tier II, and Tier III reports by the UC researchers, "CARB diesel," "petroleum diesel," "conventional petroleum diesel," and "CARB Ultra Low Sulfur Diesel (ULSD)" are used interchangeably.

Please also see response to comment F-2. (ARB)

**H-3. Comment:** Page 22, top lines: The definition of NBV is repeated. (Li, pg 4)

**Response:** The proposed regulation has been revised. Please see the Proposed Regulation Order[47] for the complete regulation. (ARB)

**H-4. Comment:** Page 22, (Proposed Regulation) Table A.2. "Limit" column: The sign "≥" for both total aromatics and polycyclic aromatic hydrocarbons could be "≤". (Li, pg 4)

**Response:** The proposed regulation has been revised. Please see the Proposed Regulation Order[48] for the complete regulation. (ARB)

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[45] Air Resources Board. *Proposed Regulation on the Commercialization of New Alternative Diesel Fuels Staff Report: Initial Statement of Reasons*. October 23, 2013, ES-1, 1.
[46] Air Resources Board. *Proposed Regulation on the Commercialization of New Alternative Diesel Fuels Staff Report: Initial Statement of Reasons*. January 2, 2015. Appendix A: Proposed Regulation Order.
[47] Air Resources Board. *Proposed Regulation on the Commercialization of New Alternative Diesel Fuels Staff Report: Initial Statement of Reasons*. January 2, 2015. Appendix A: Proposed Regulation Order.
[48] Air Resources Board. *Proposed Regulation on the Commercialization of New Alternative Diesel Fuels Staff Report: Initial Statement of Reasons*. January 2, 2015. Appendix A: Proposed Regulation Order.

**H-5. Comment:** Page 30, Table A.9, column “fuel Specifications”, row 4 for PAHs w%: The 10% maximum seems incorrect for PAHs in a reference fuel. Please check. (Li, pg 4)

**Response:** The proposed regulation has been revised. Please see the Proposed Regulation Order[49] for the complete regulation. (ARB)

[49] Air Resources Board. *Proposed Regulation on the Commercialization of New Alternative Diesel Fuels Staff Report: Initial Statement of Reasons*. January 2, 2015. Appendix A: Proposed Regulation Order.