California Biodiesel Multimedia Evaluation

Tier III Report

Prepared

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For the

California Environmental Protection Agency
Multimedia Working Group

FINAL

June 2014

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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: • Chemistry • Release Scenarios • 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

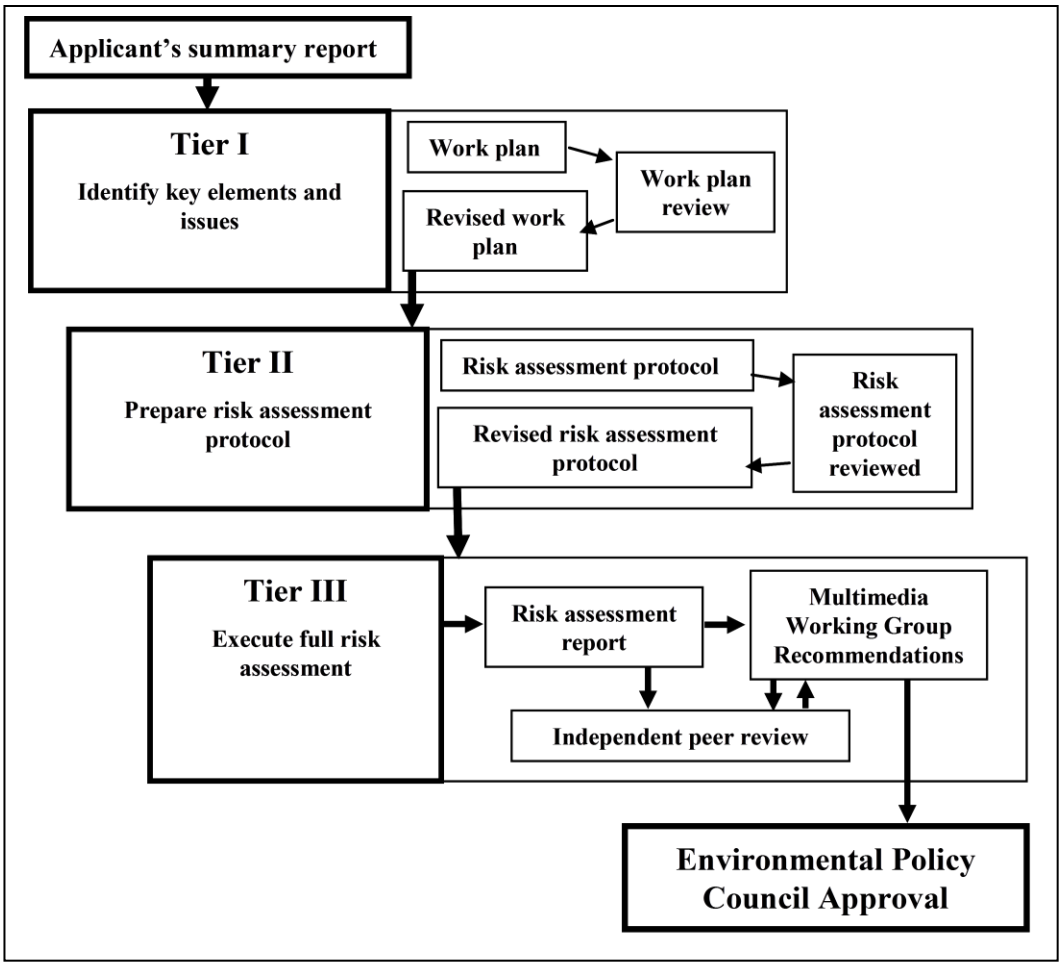

Figure 1.1. Multimedia evaluation process flow chart

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

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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,

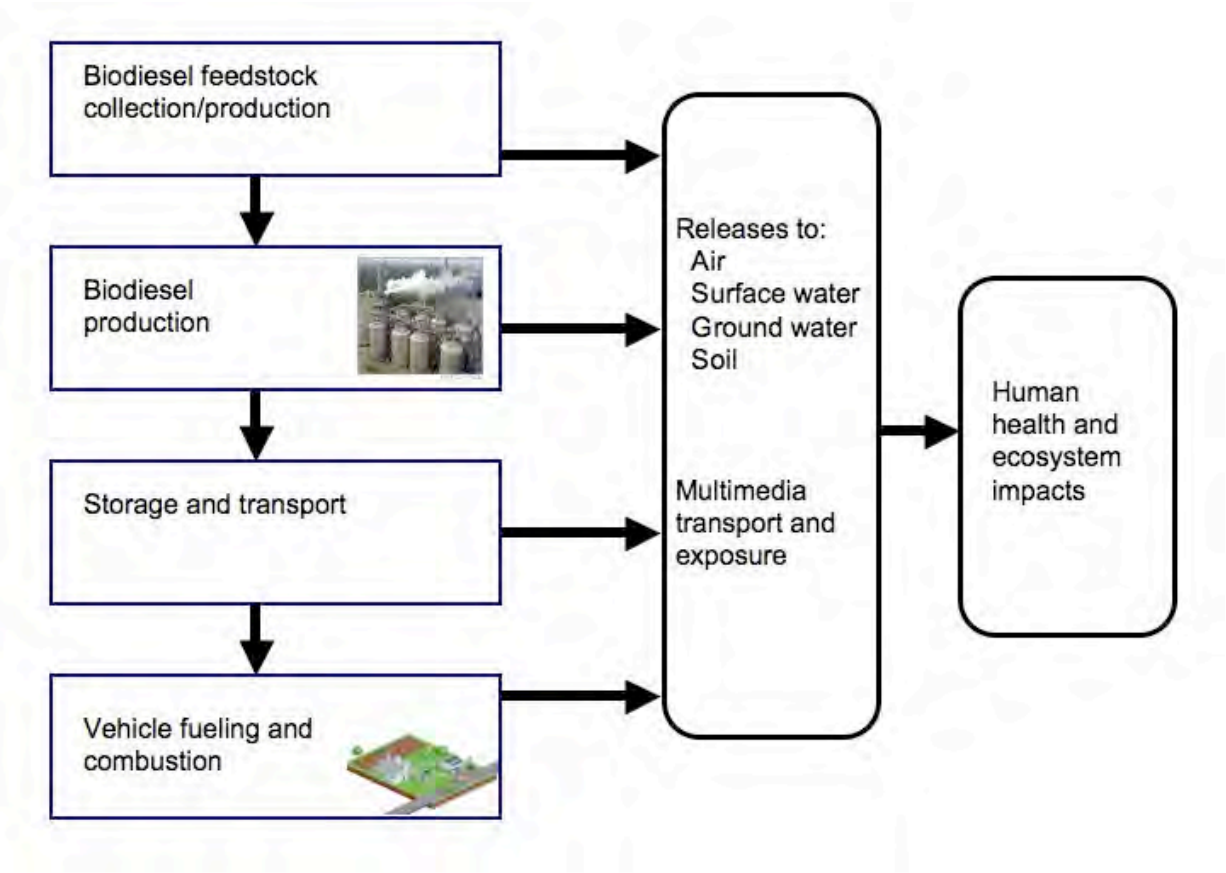
- Summary of the expected environmental behavior (transport and fate conceptual models associated with release scenarios) of proposed fuel or fuel components that may be released, and
- Comparison of physical, chemical, and toxic properties of the fuel or additive components to appropriate agreed upon 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*.

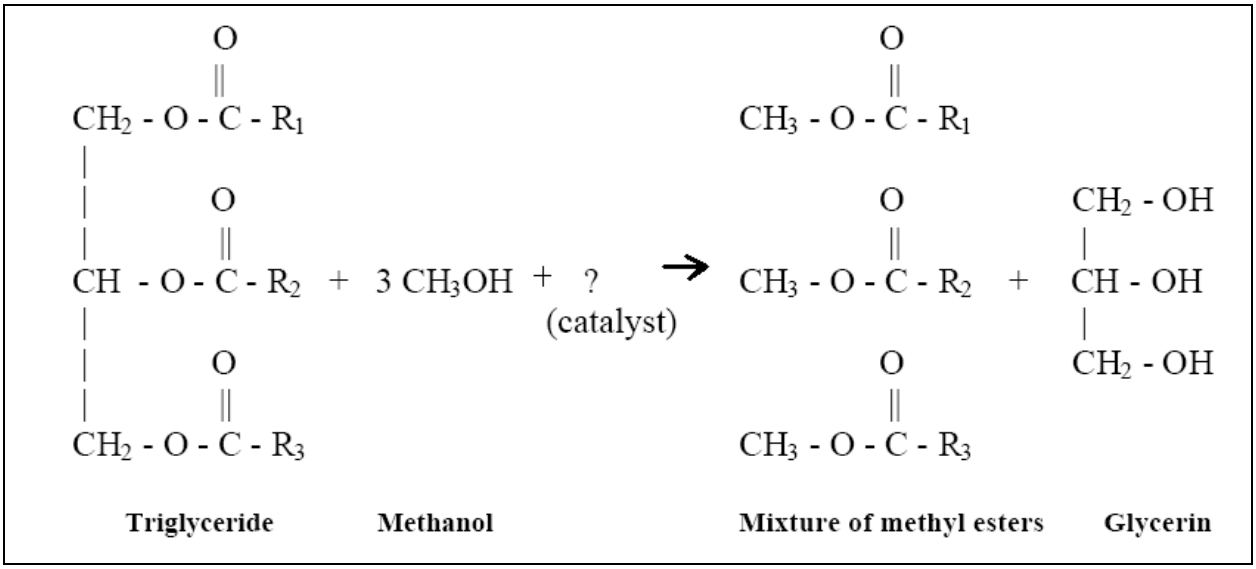


*Figure from Van Gerpen (2004)

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*.

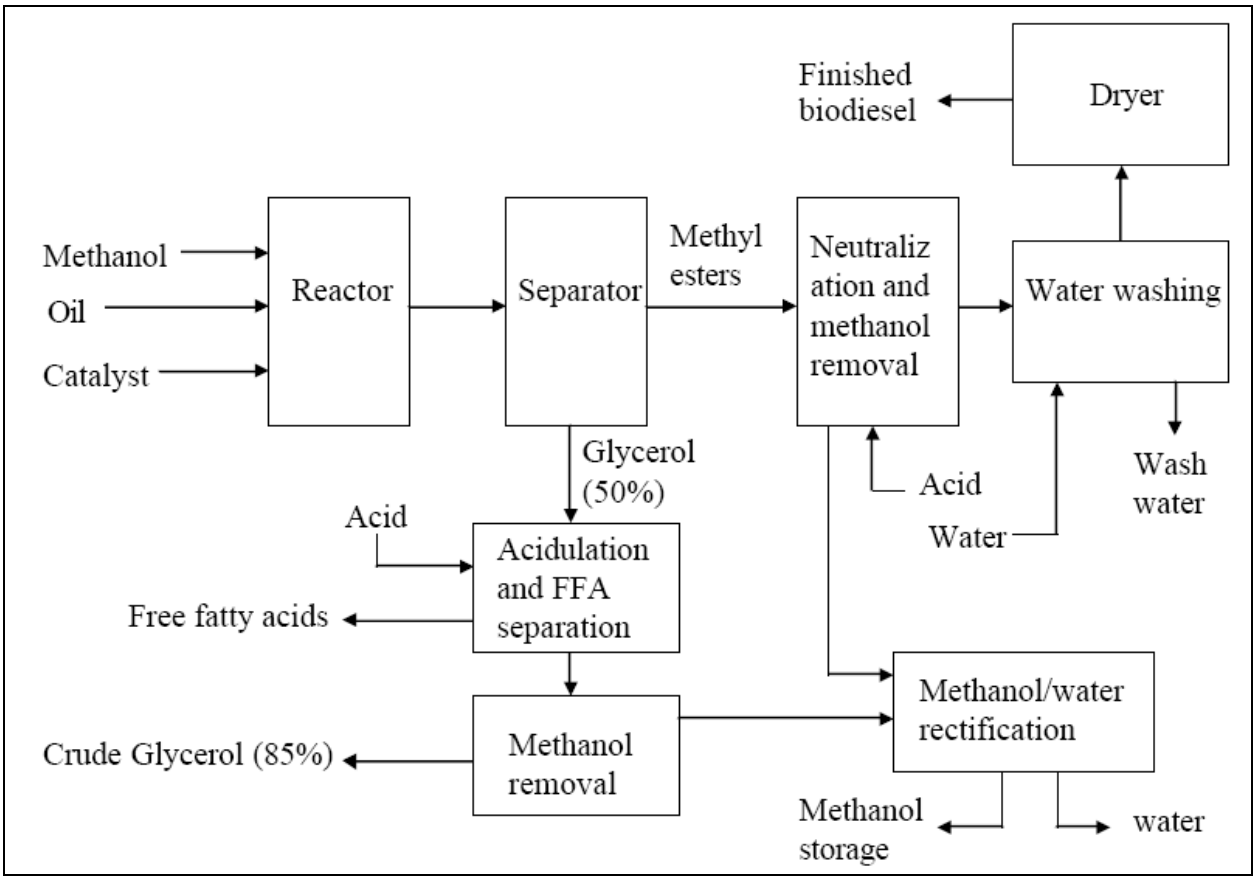
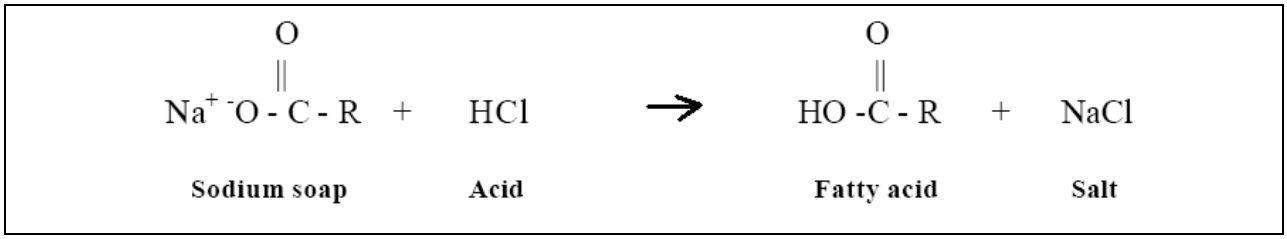


*Figure from Knothe et al. (2005).

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°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*.

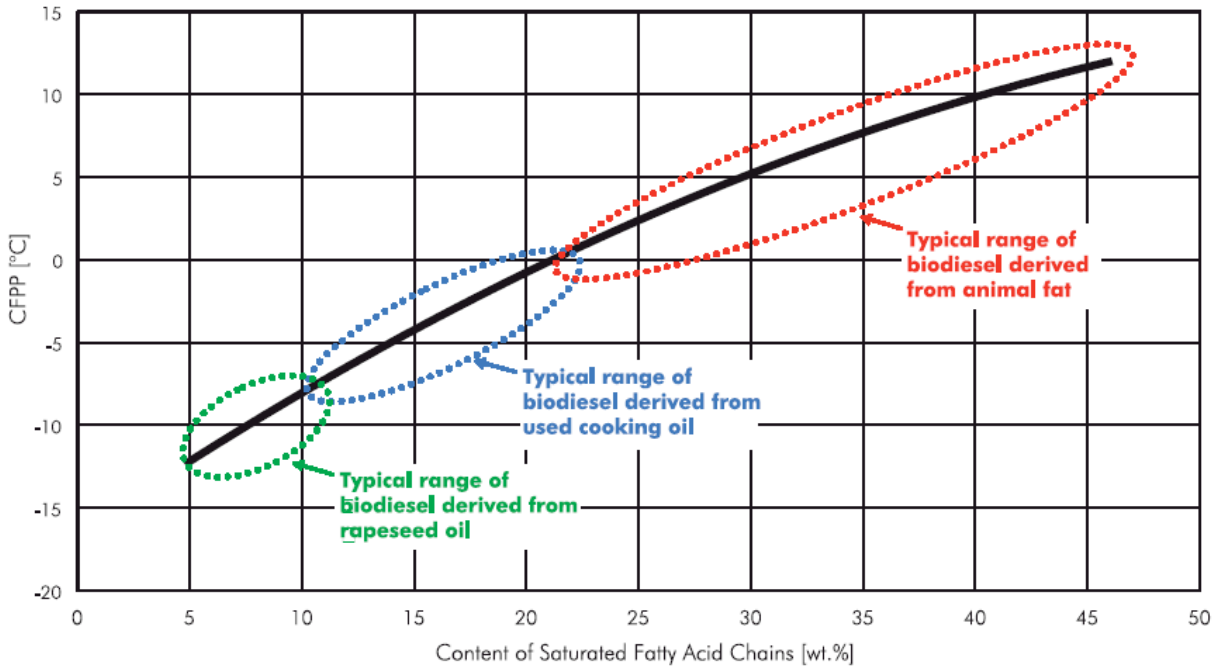


*Figure from Hilber et al. (2006).

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

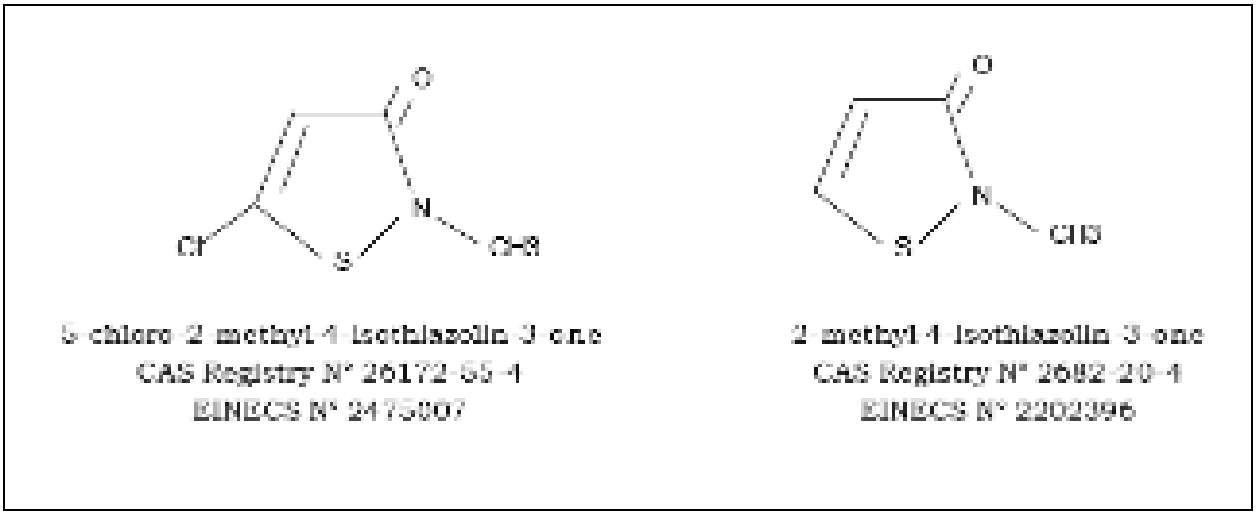
Fuel additives such as biocides can be added to the fuel to "destroy or inhibit the growth of fungi and bacteria which can grow at fuel-water interfaces to give high particulate concentrations in the fuel" (ASTM, 2012).

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*.

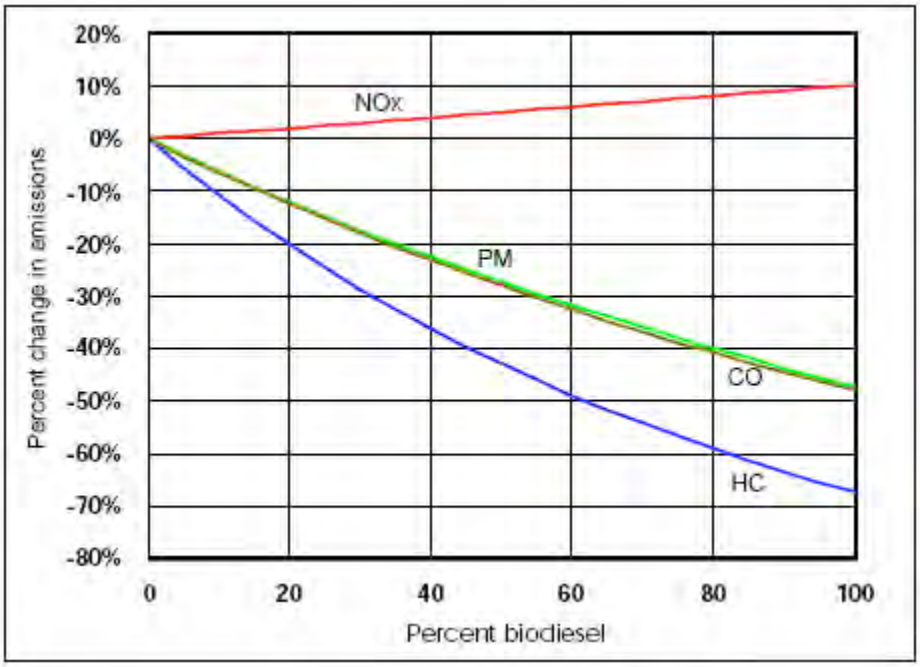


*Figure from USEPA (2002).

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.

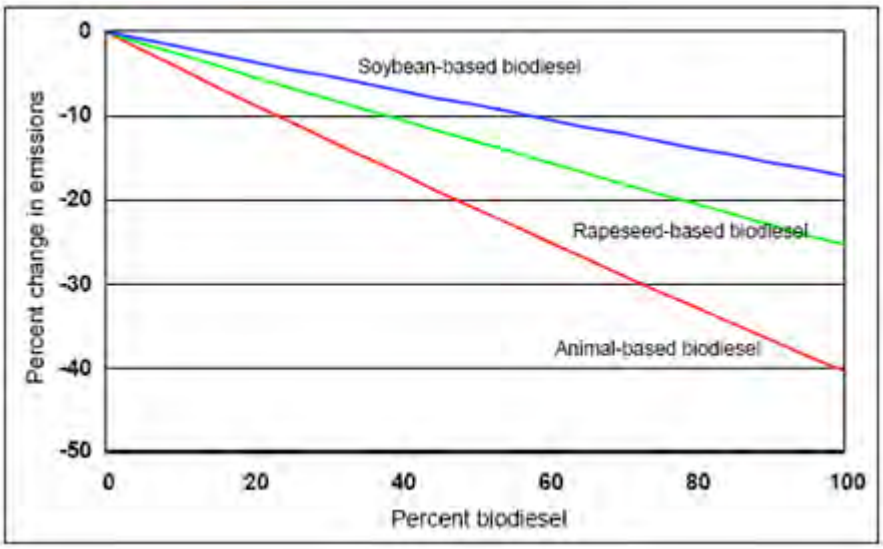


*Figure from USEPA (2002).

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*.

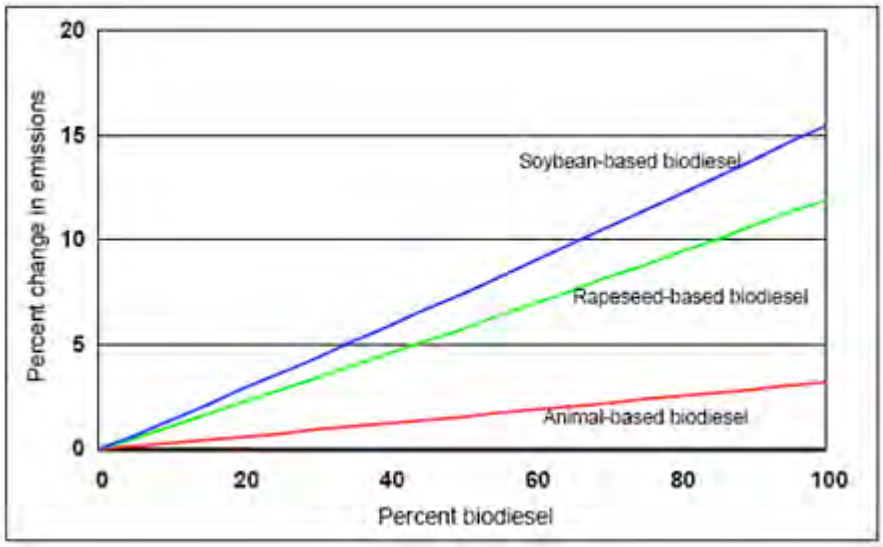


*Figure from USEPA (2002).

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*.

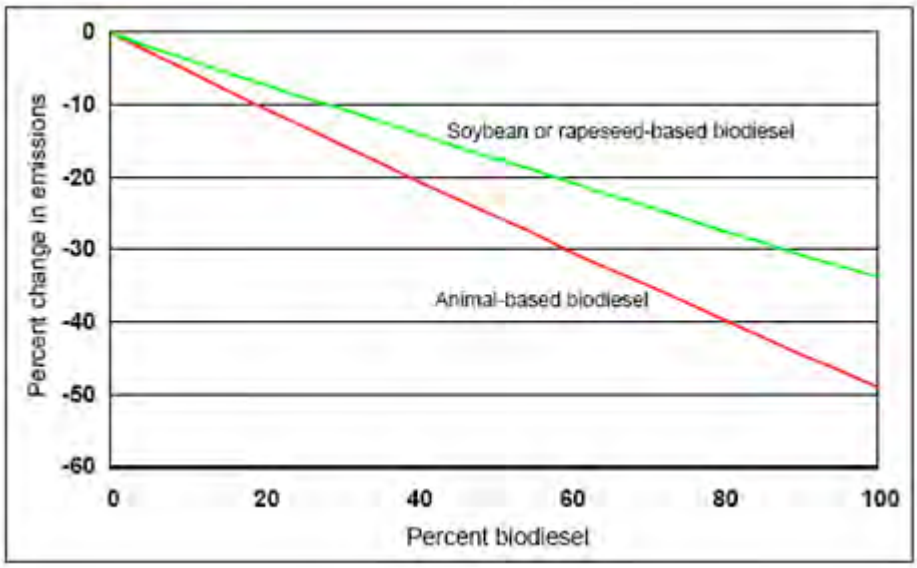


*Figure from USEPA (2002).

Figure 4.7. Regression model showing effect of oxygen on particulate matter*.

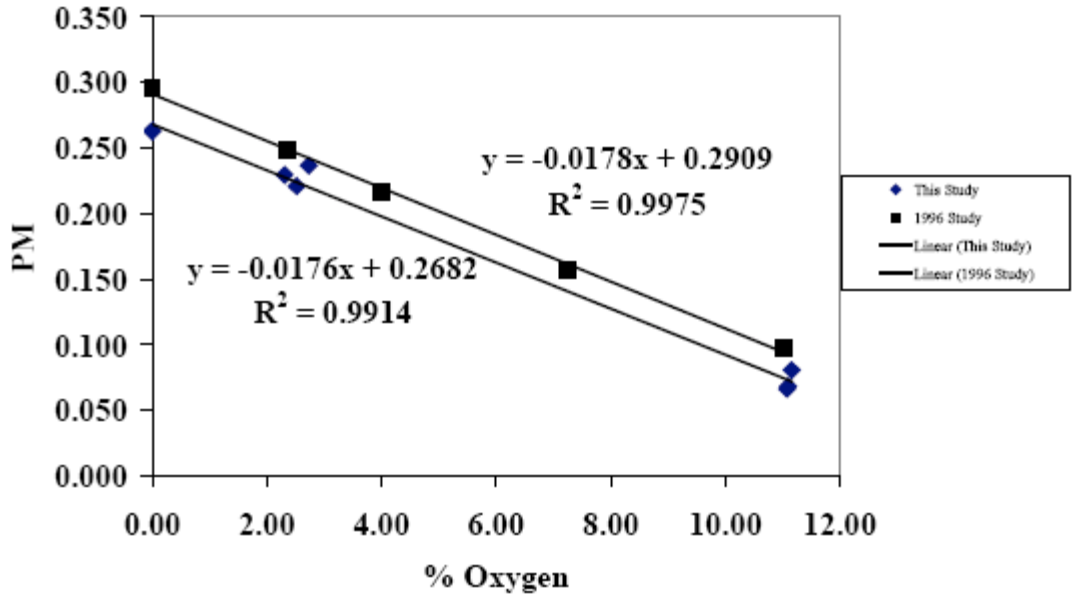


*Figure from Graboski et al. (2003).

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.

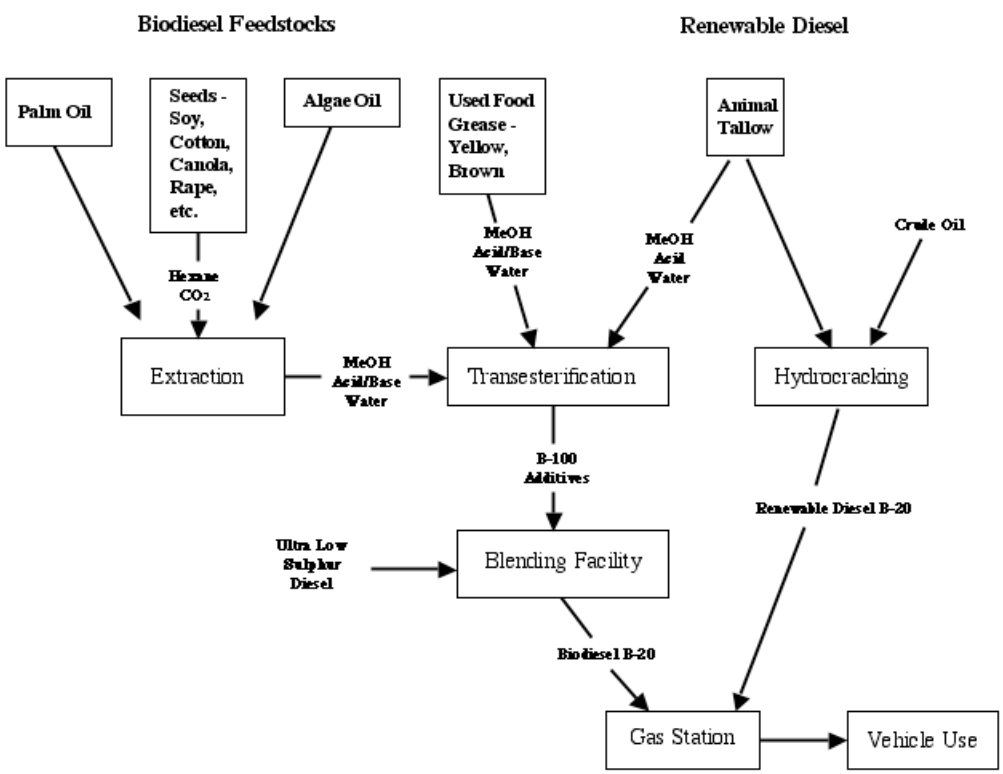
5. Release Scenarios

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.

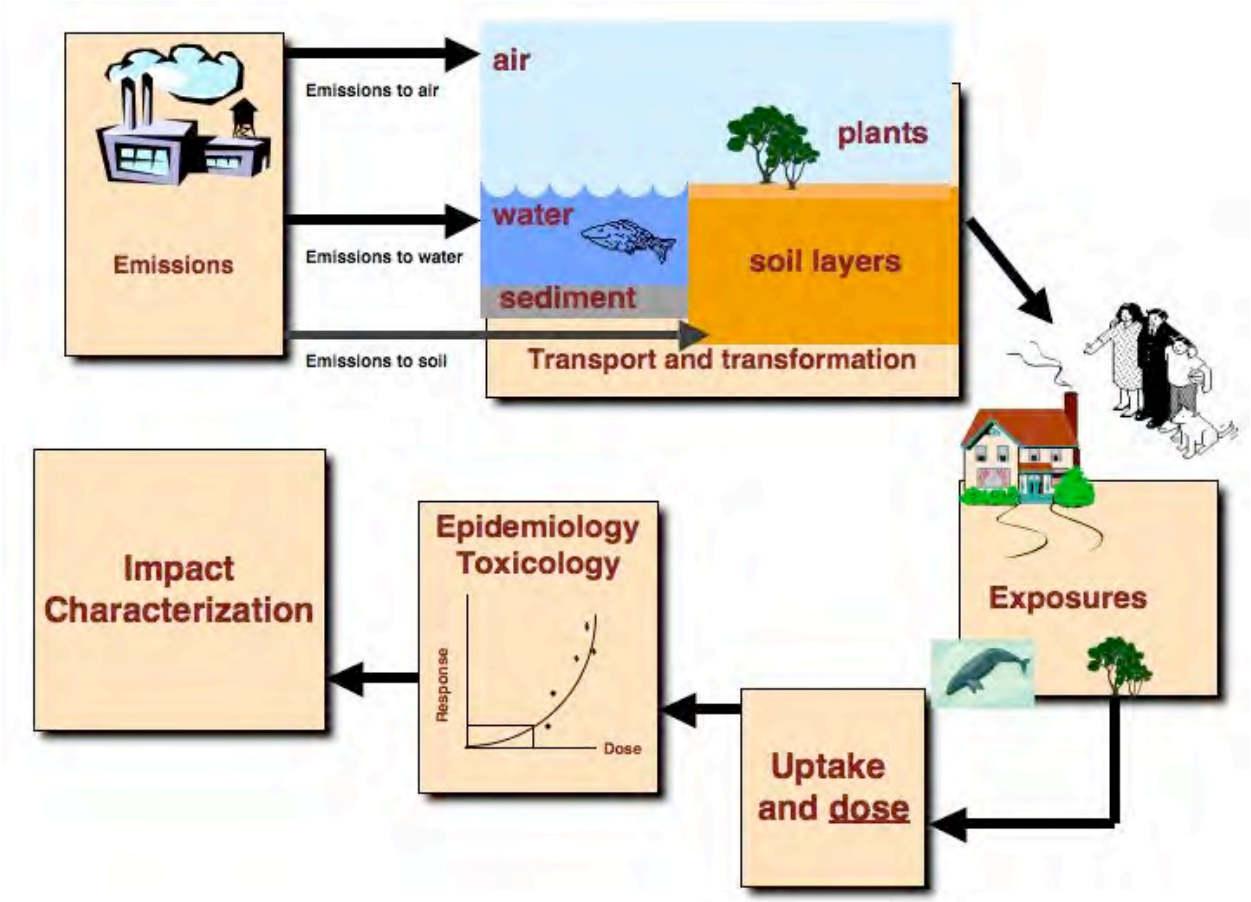


Figure 6.1. A conceptual illustration how pollutant emissions from each life-stage of biodiesel enter air, water, and or soil, undergo multimedia transport into exposure media, followed by contact with humans and ecosystems.

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).

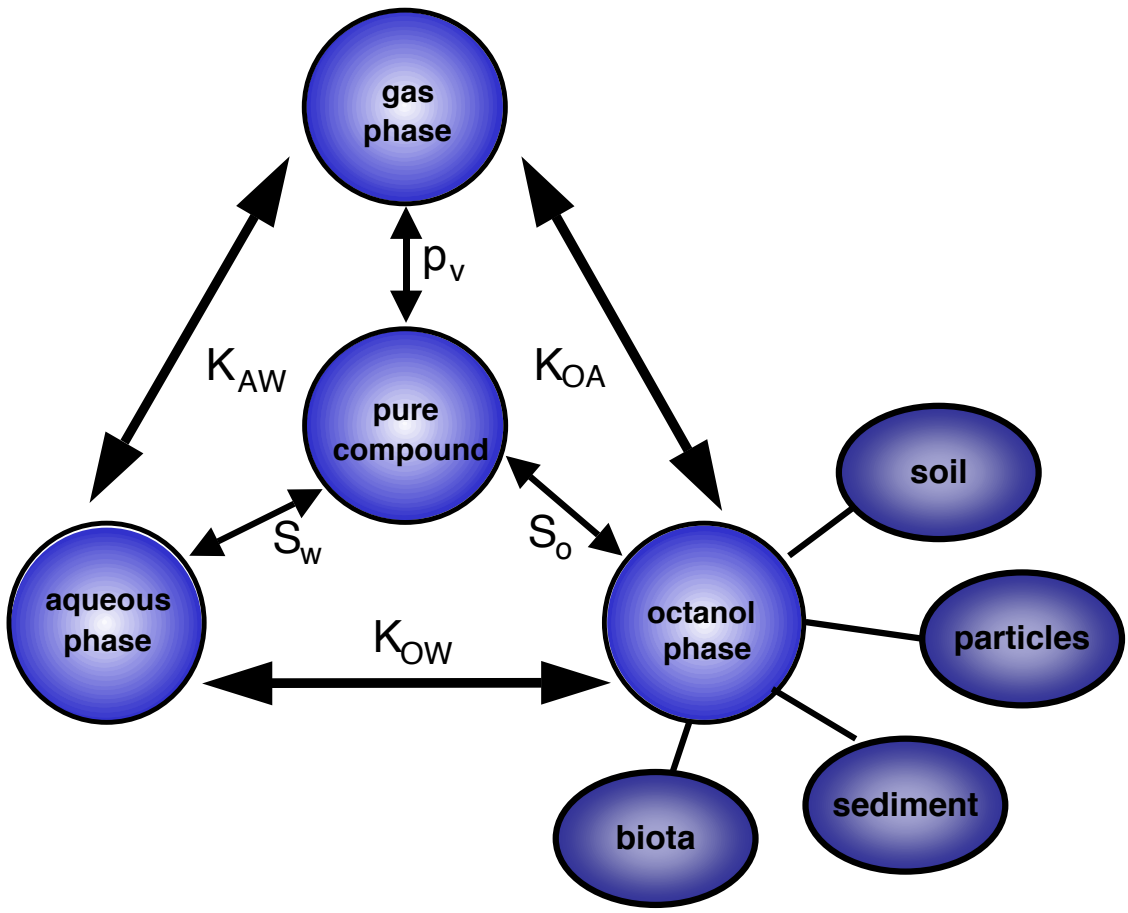


Figure 6.2. Phase distribution equilibria involving pure phase partition coefficients (water solubility S_W, solubility in octanol S_O and vapor pressure p_V) showing the links for describing environmental phase partitioning (K_{AW} air/water partition coefficient, K_{OW} octanol/water partition coefficient, 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° 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)*.

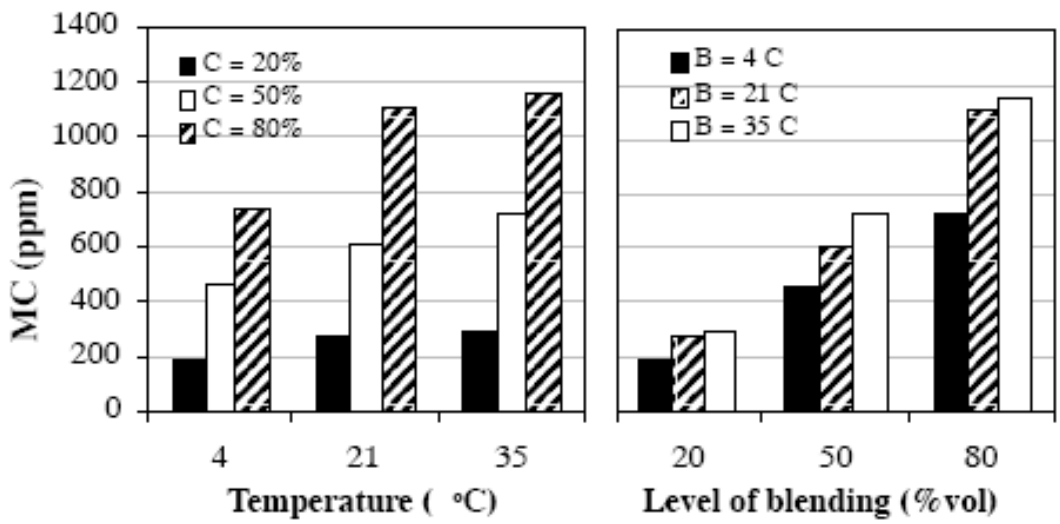


*Figure from He et al. (2007)

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*.

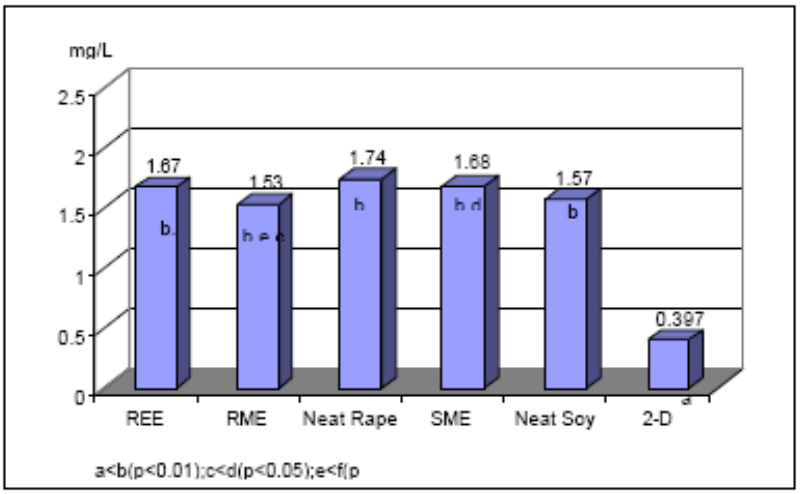


*Figure from Knothe et al. (2005)

Figure 6.5. COD values for biodiesel and diesel*.

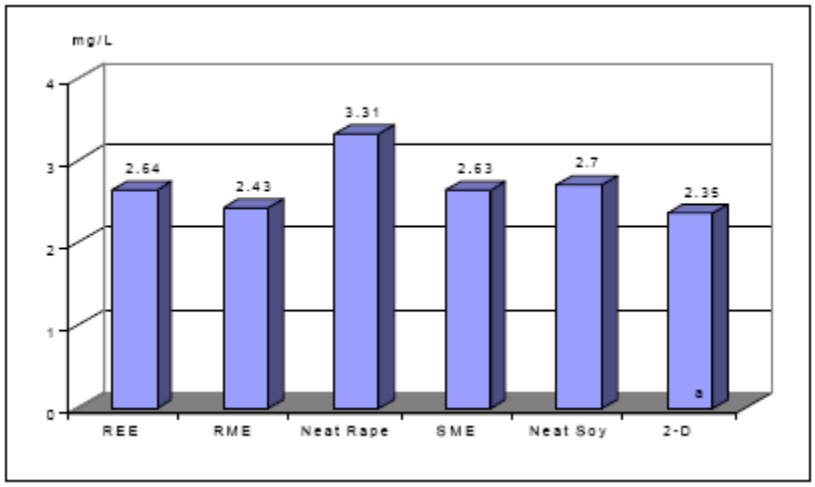


*Figure from Knothe et al. (2005).

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: 2.9×10^{-5} $(\mu g/m^3)^{-1}$
- 1,3-Butadiene: 1.7×10^{-4} $(\mu g/m^3)^{-1}$
- Acetaldehyde: 2.7×10^{-6} $(\mu g/m^3)^{-1}$
- Formaldehyde: 6.0×10^{-6} $(\mu g/m^3)^{-1}$
- Standard Diesel Particles: 3.0×10^{-4} $(\mu g/m^3)^{-1}$
- B20 Diesel Particles: 2.85×10^{-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}*.

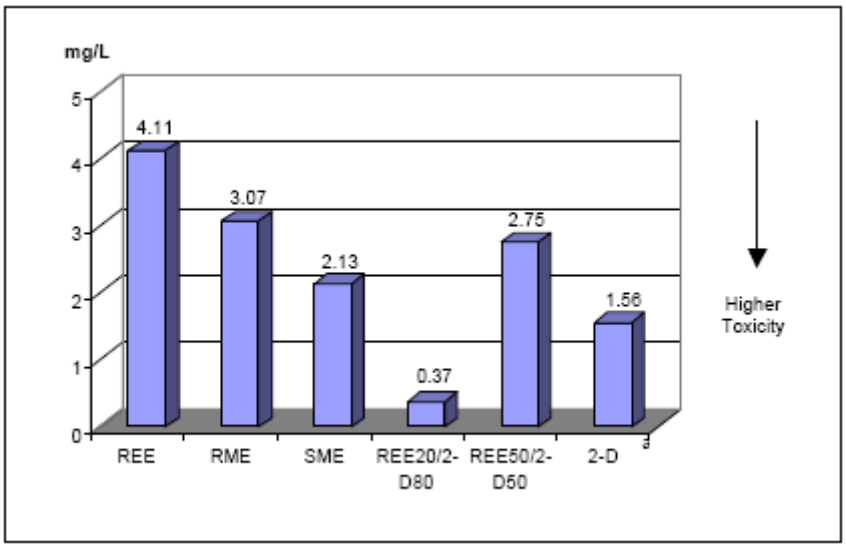


*Figure from Knothe et al. (2005).

Figure 7.2. 48 hour flow-through, *Daphnia magna* EC_{50}*.

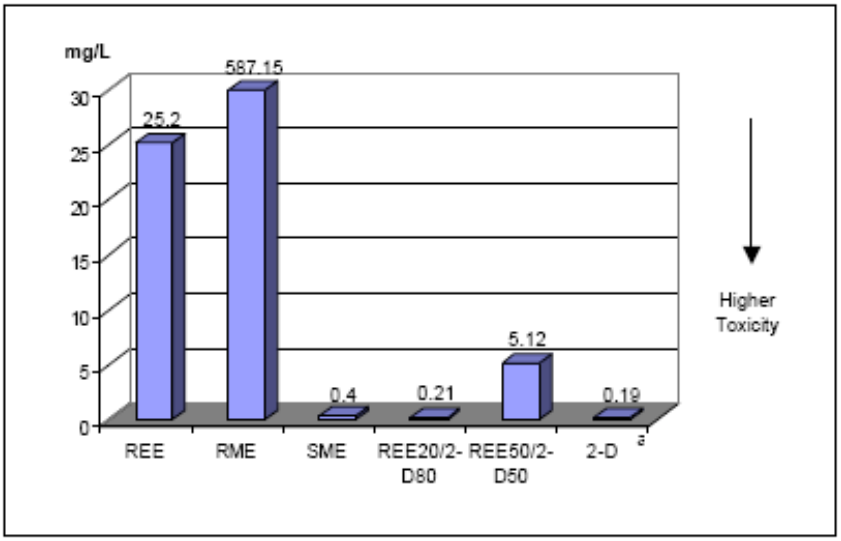


*Figure from Knothe et al. (2005).

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

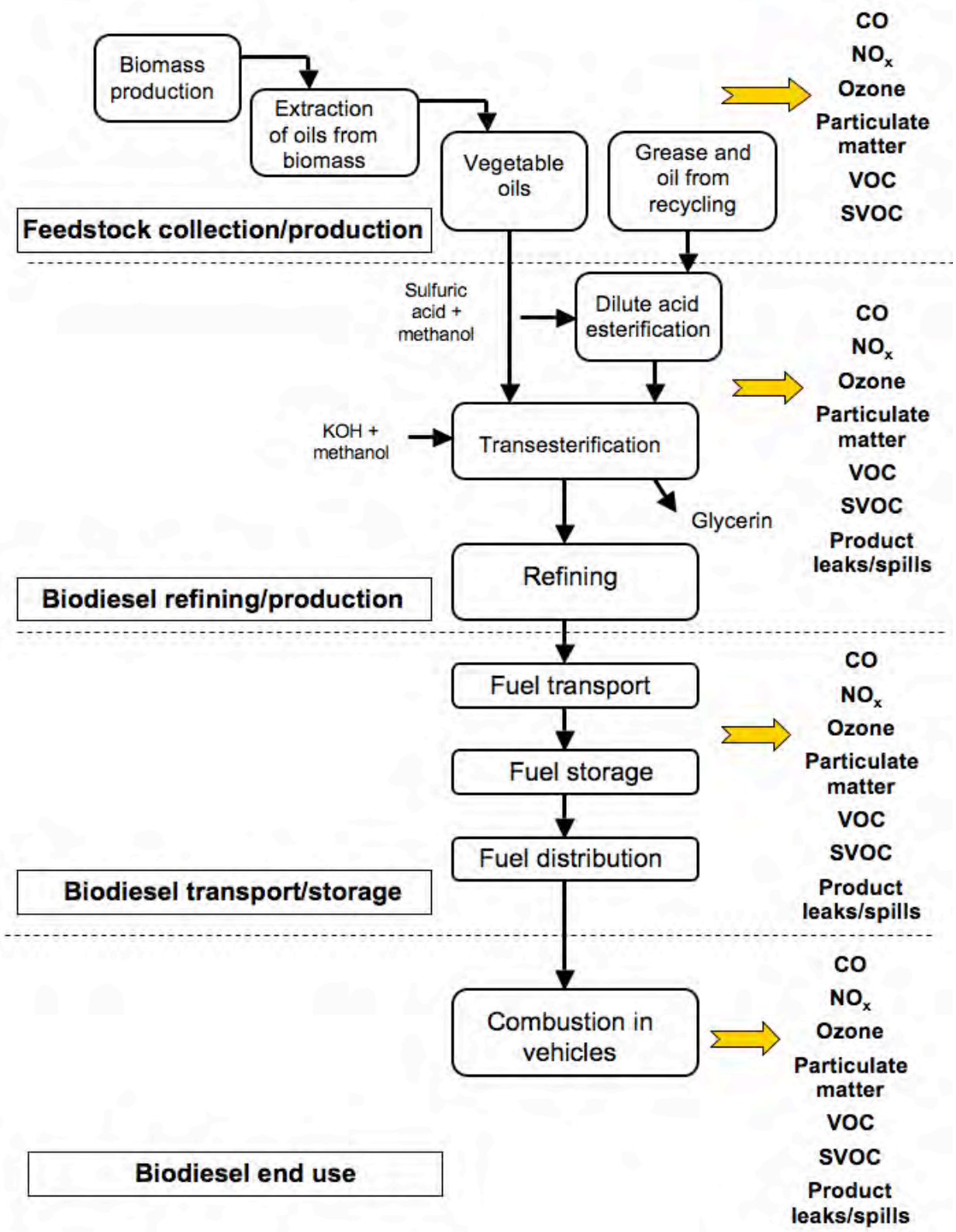


Figure 8.1. An illustration of life stages and life-cycle impacts for biodiesel fuels.

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.

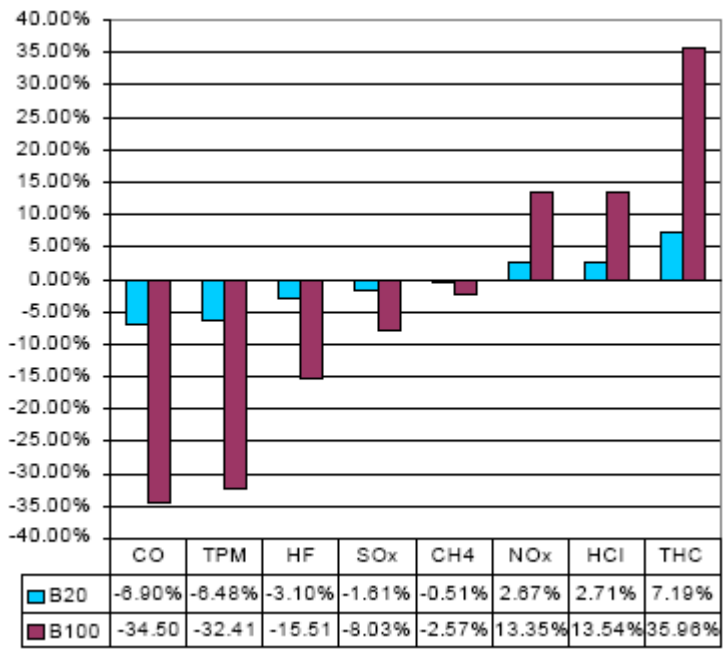
8.2. Emissions of Pollutants to Air

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.

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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°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°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°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:

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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.

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11.2. Appendix I-B: Biodiesel Additive Chemicals

Figure I-B-1: Common Antioxidants

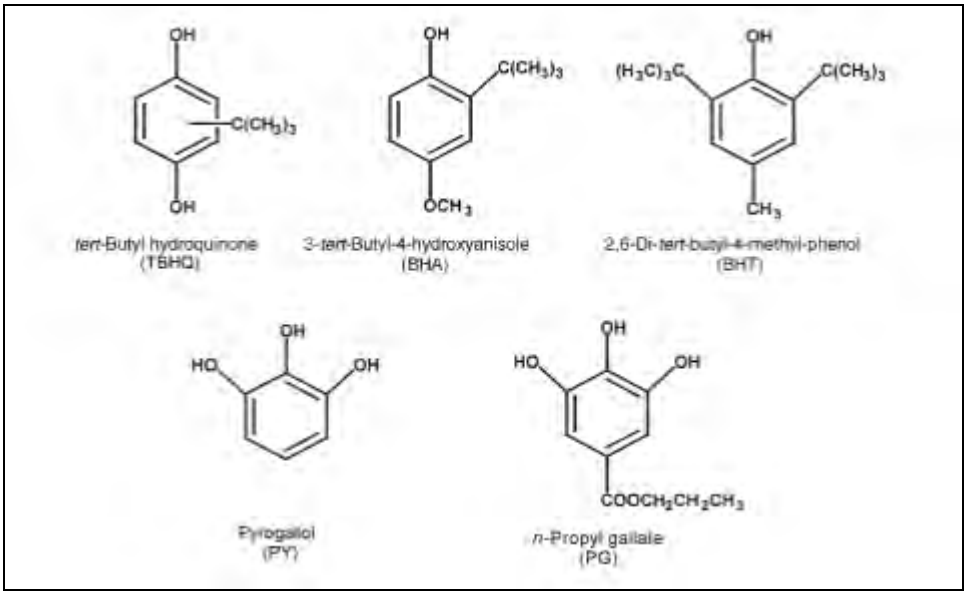
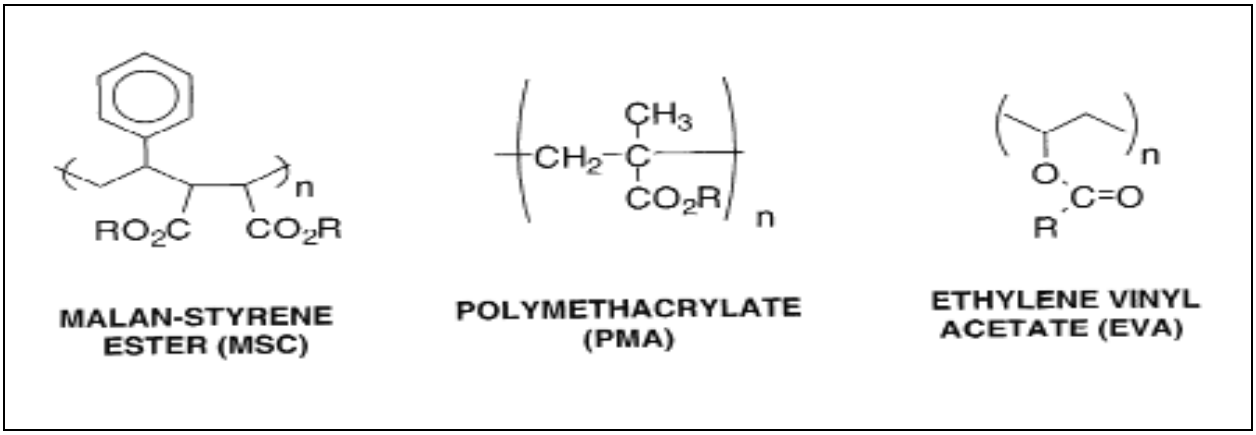


Table I-B-1. Commercial Biodiesel Antioxidants*

Manufacturer	Product Name	Chemical Components	%
Albemarle	Ethanox 4737	2,6 di-t-butylphenol 2,4,6 tri-tert butylphenol 2-tert-butylphenol Phenol Naptha Petroleum	52.5% 10.5% 7% 1.1% 25-30% 2%
Biofuel Systems	Baynox	2,6 di-tert-butyl-4-methylphenol	20%
Chemiphase	AllClear	Methyl Alcohol	18-24%
Eastman Chemical	Bioextend30	2-tert-butylhydroquinone Butyle acetate Diethylene glycol monobutyl ether	20% 30% 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 Naptha	N/A 40-70%
Lubrizol	FloZol502	Copolymer Ester Toluene	N/A 2%
Lubrizol	FlowZol503	Naptha Napthalene Trimetheyl Benzene Ethylbenzene Alkylphenol Xylene	40-49% 4.4% 1.4.9% 1.6% 5-9.9% 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 Sodium dimethyldithiocarbamate Ethylene thiourea	15% 15% 1%
Hammonds	Biobor JF	Naptha 2,2-(1-methyltrimethylenedioxy)bis-(4-methyl-1,3,2 dioxyborinane; 2,2,oxybis(4,4,6-trimethyl-1,3,2-dioxaborinane) [Substituted dioxaborinanes]	4.5% 95%
Power Serve Products	Bio-Kleen	4-(2-nitrobutyl)-morpholine 4,4, (2-ethyl-2-nitrotrimethylene)-dimorpholine Methylene dimorpholine Morpholine 1-Nitropropane	76-85% 2-7% 3.9-6.5% 3-6% .3-5.3%
Rohm and Haas	Kathon FP 1.5	Magnesium nitrate 5-chloro-2-methyl-4-isothiazol-3-one 2-methyl-4-isothiazol-3-one	1-2.5% 1-2.5% To 1 mix
Star Brite Corp	Biodiesel Biocide	Sodium dimethyldithiocarbamate Ethylenedimine Dimethylamine Ethylene thiourea Nabam	15-20.2% 0.0-0.75% 0.0-0.75 0-1.0% 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 Toluene	45% w/w 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
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- 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
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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
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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.

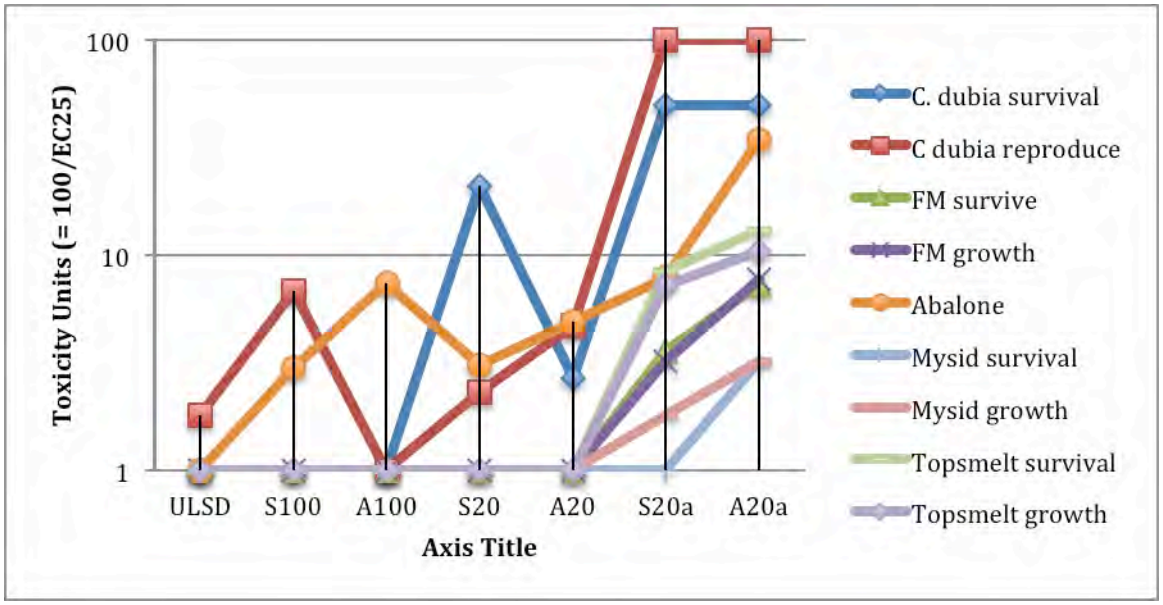

Figure 1. Toxicity scores (as Toxicity Units, = 100/ EC_{25}), for the different endpoints (e.g., survival, reproduction, growth) of 5 of the 6 species tested, as a function of fuel blend to which species was exposed. The graph is absent Green Algae that showed different behavior than the trend observed 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.

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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

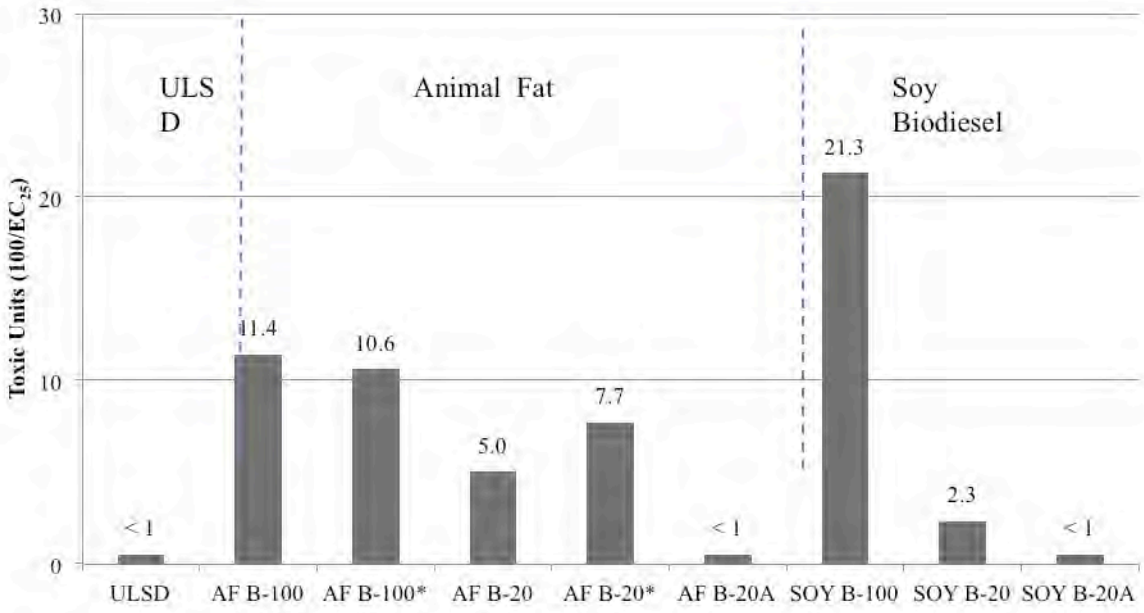
Table II-A-3 and Figure II-A-1 summarize results of the biofuel toxicity tests with green algae.

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

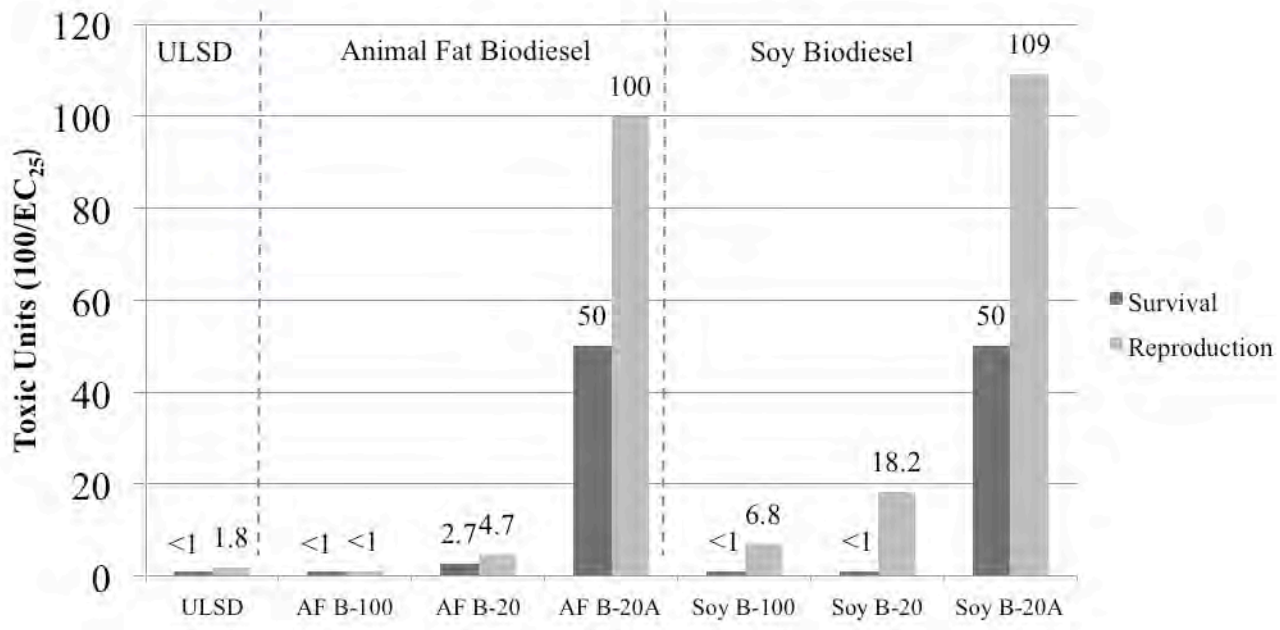
Table II-A-4 and Figure II-A-2 summarize results of the biofuel toxicity tests with *C. dubia*. The raw data for this test series is found in Section A2.

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.

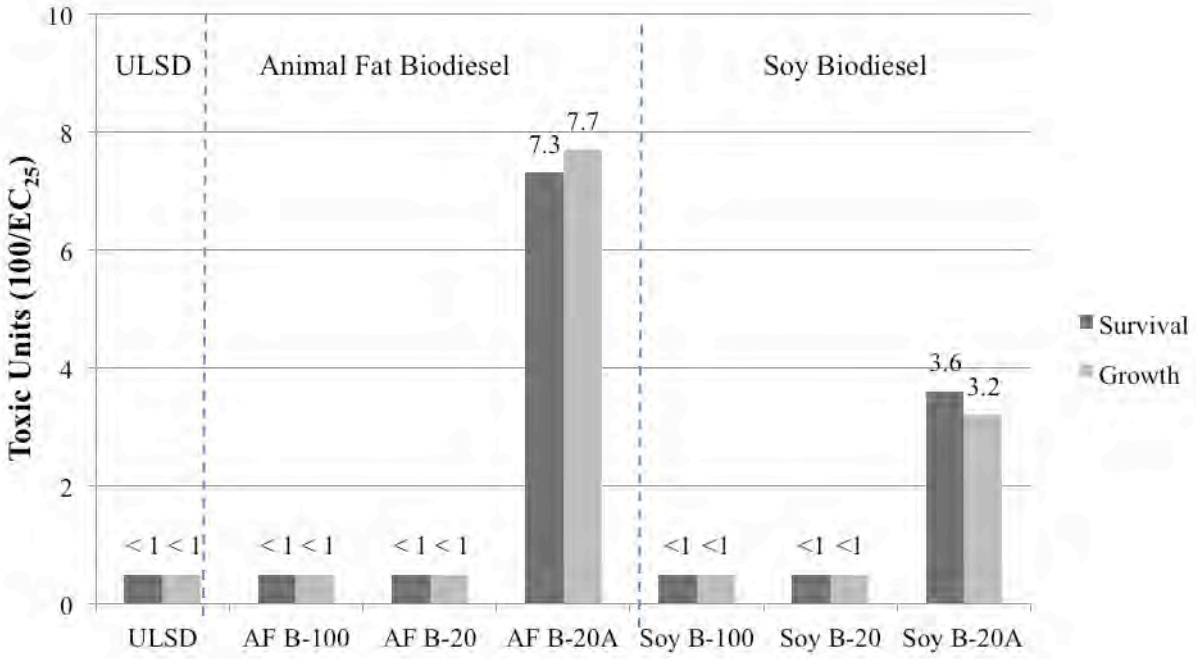
The fathead minnow survival and growth endpoints were unaffected by ULSD, AF B-100, AF B-20, Soy B-100 and Soy B-20. However, both biodiesel B-20A mixtures resulted in toxicity to both endpoints. AF B-20A exhibited moderately greater toxicity (7.3 TUc and 7.7 TUc) than did the Soy B-20A (3.6 TUc and 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.

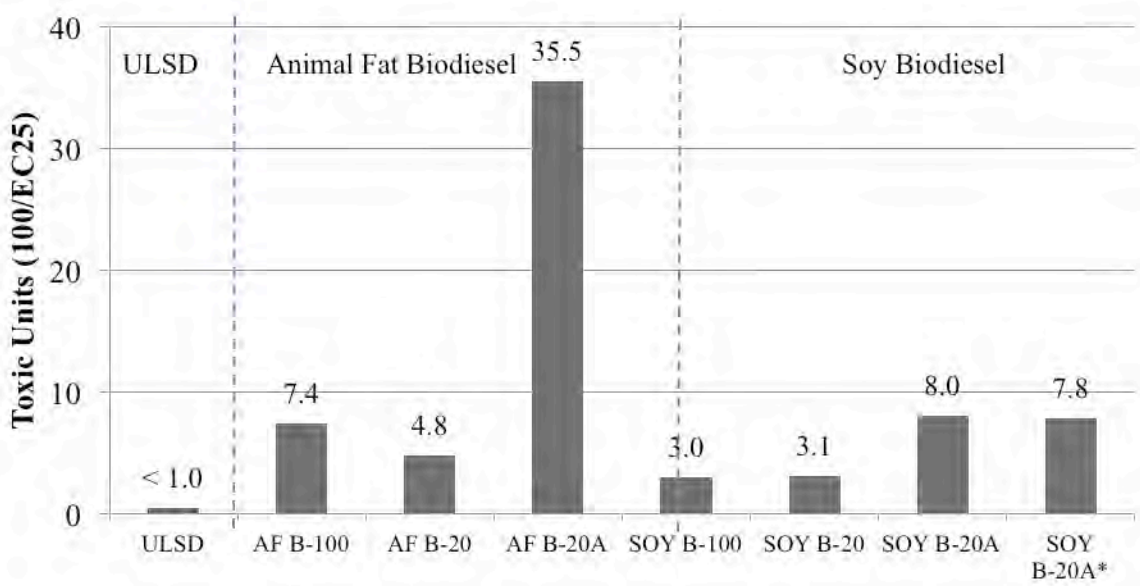
No effects on abalone shell development were detected with ULSD. AF B-100 exhibited somewhat higher toxicity than the Soy B-100 (7.4 TUc and 3.0 TUc, respectively), while the AF B-20 and Soy B-20 mixtures had similar or slightly less toxicity as their respective B-100 fuels (4.8 and 3.1 TUc, respectively), 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



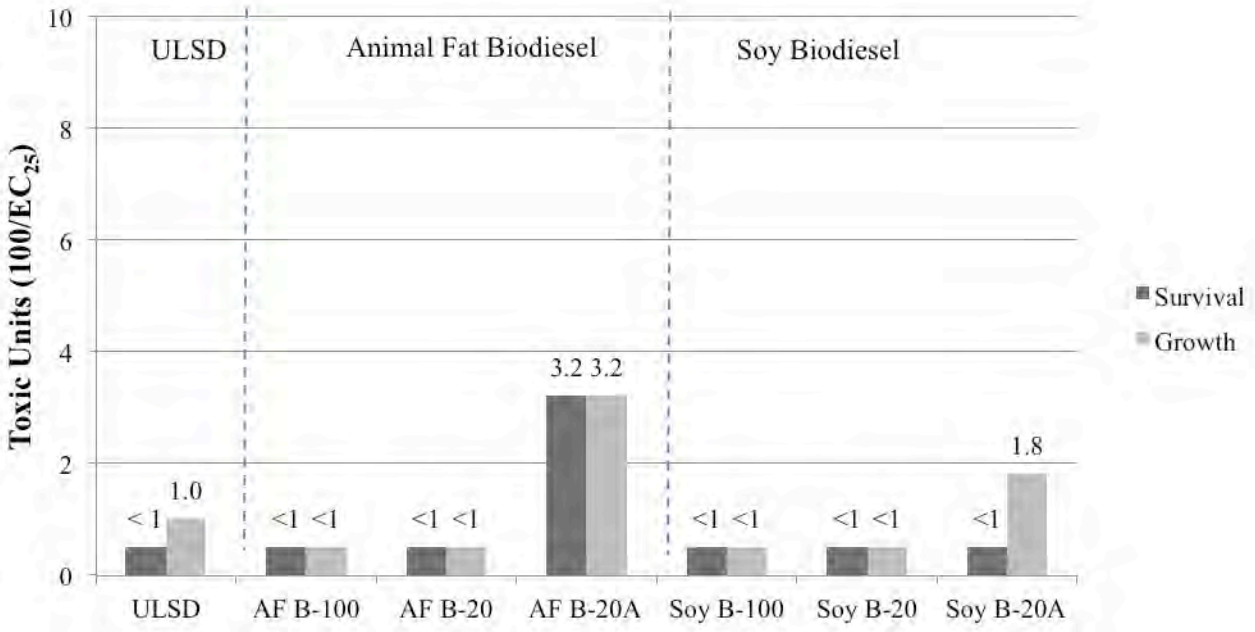
Mysid Chronic Toxicity Test Results

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.

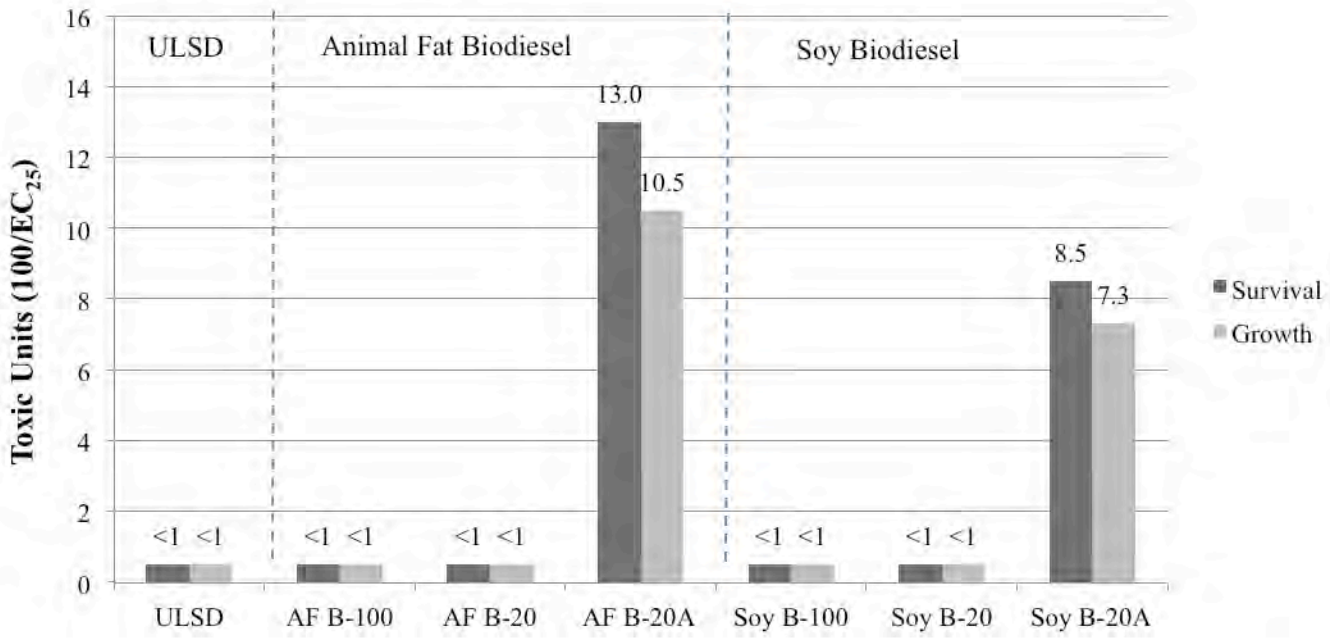
No effects on either survival or growth were detected with ULSD or either of the biofuels and mixtures that did not contain the additive. The AF B-20A test detected 13.0 TUc on survival and 10.5 TUc on growth, while the Soy B-20A test detected slightly less toxicity with 8.5 TUc on survival and 7.3 TUc 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.

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7. Appendix II-B: Chemical Analysis of the Water Accommodated Fractions of Biofuels Using Stir Bar Sorptive Extraction

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This work was sponsored by the Assistant Secretary for Energy Efficiency and Renewable Energy, Office of Building Technology, State, and Community Programs, of the U.S. Department of Energy under Contract No. DE-AC02-05CH11231. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the US DOE.

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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.

Acknowledgments

This work was conducted with support from the California Air Resources Board and the University of California Berkeley through a subcontract to LBNL. The project was conducted as part of the Faculty and Student Teams (FaST) internship program, which is a collaboration between the Department of Energy's Office of Science Workforce Development for Teachers and Scientists and the National Science Foundation. The FaST program is managed at Lawrence Berkeley National Laboratory by the Center for Science and Engineering Education. The work was sponsored by the Assistant Secretary for Energy Efficiency and Renewable Energy, Office of Building Technology, State, and Community Programs, of the U.S. Department of Energy under Contract No. DE-AC02-05CH11231.

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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.

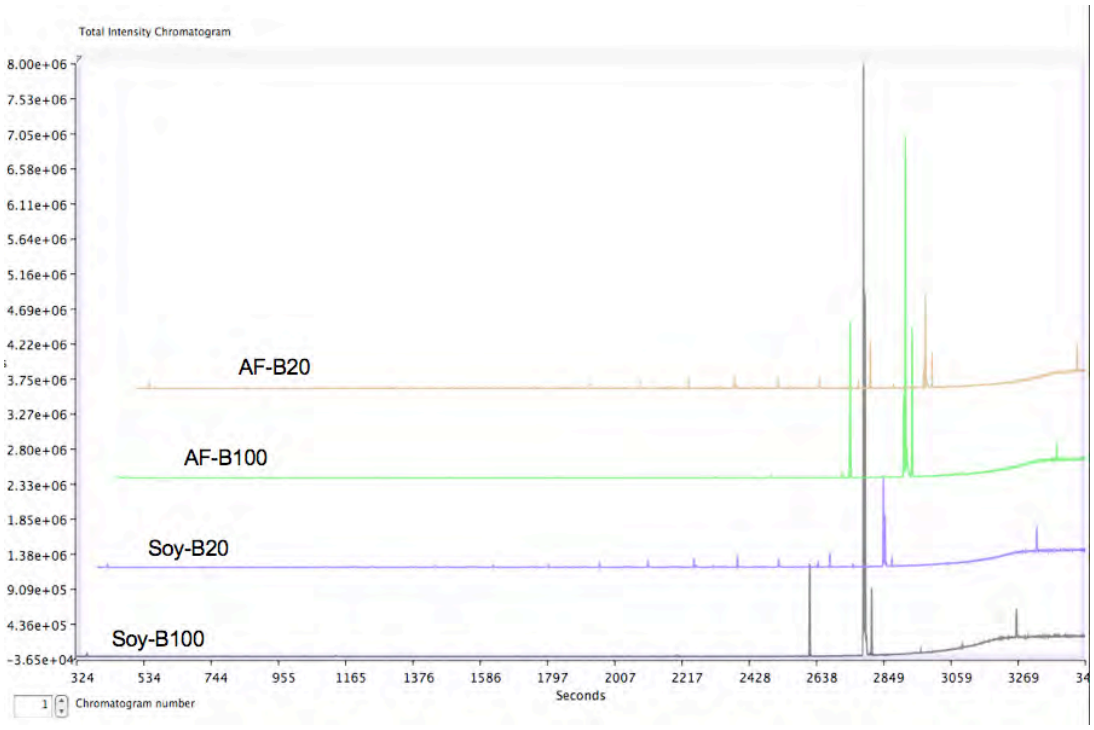

Figure II-B-1. Overlay total ion chromatogram of 1:1000 (v/v) dilution of each neat fuel in MeOH injected (2 µL) with 5:1 split.

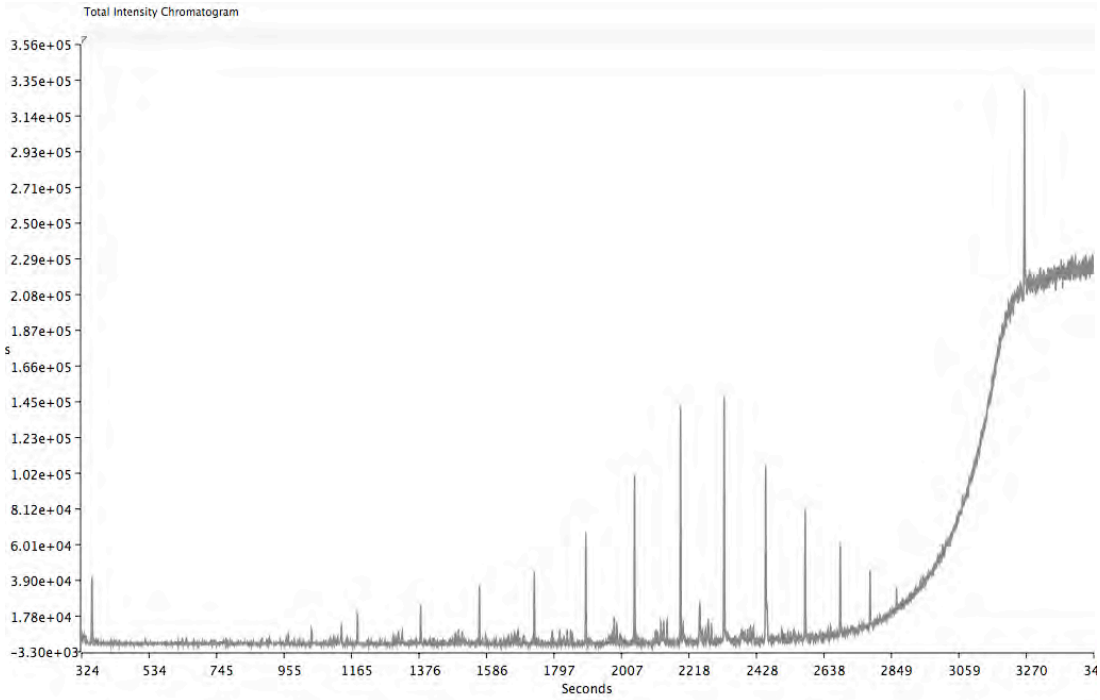

Figure II-B-2. CARB Diesel total ion chromatogram of 1:1000 (v/v) dilution of neat fuel in MeOH injected (2 µL) with 5:1 split.

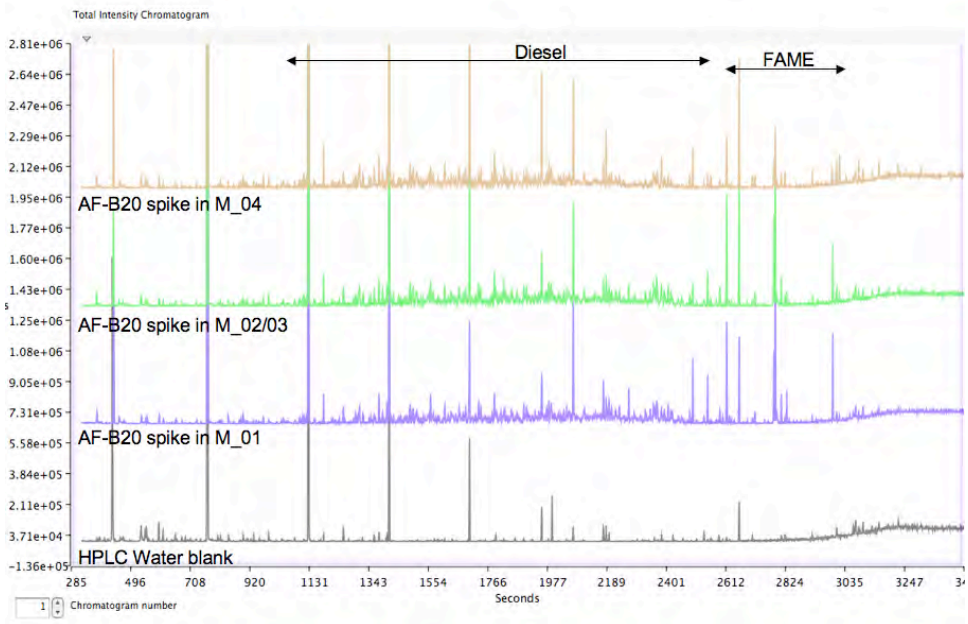

Figure II-B-3. Overlay of 1:1000 (v/v) dilution of neat AF-B20 fuel in MeOH spiked (4 µL) in each of the test waters (40 mL). The HPLC water blank is 40 mL of the water used as makeup volume in the WAF analysis. The large evenly spaced peaks are siloxanes from the stir-bar coating and are not quantified in the method.

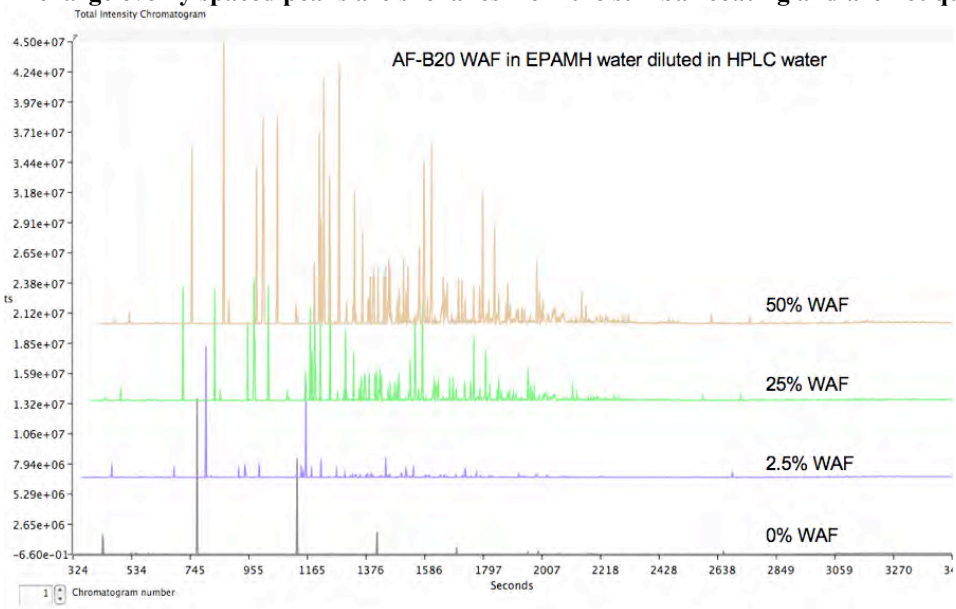

Figure II-B-4. Range finding experiment with increasing fractions of AF-B20 WAF_01 in HPLC water. The optimal dilution for the SBSE analysis was identified as 25% WAF in HPLC water.

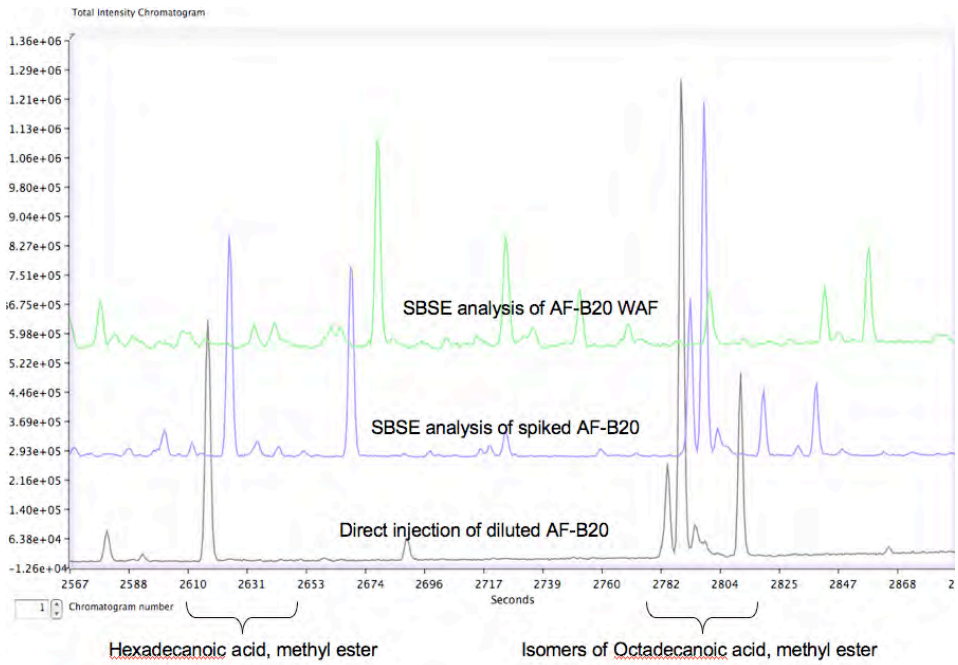

Figure II-B-5. Zoomed overlay in the region of the chromatogram where FAME elutes showing the presence of hexadecanoic acid methyl ester and the isomers of octadecanoic acid methyl ester in the raw fuel and in the spiked water but significantly reduced or absent in the WAF. The peak eluting at about 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.

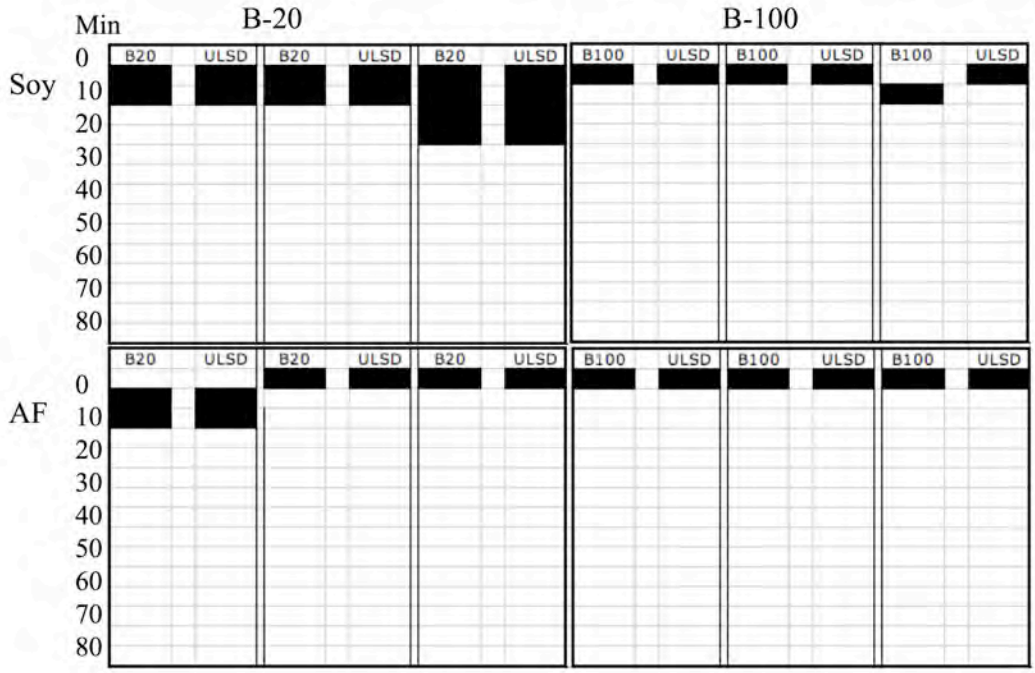

Figure II-C-3: Characteristic times to formation of the U-shaped plume for each of the four blends (Soy B-20, Soy B-100, Animal Fat (AF) B-20, AF B-100) relative to ULSD in side-by-side comparison. The three columns per fuel blend show the results for each of the three replicates.

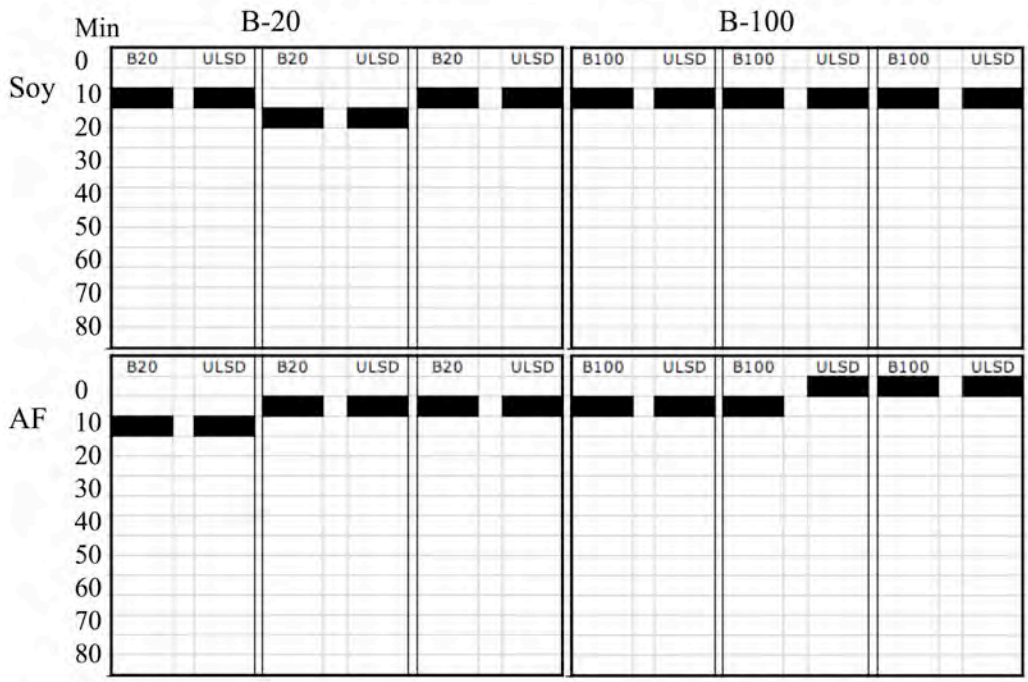

Figure II-C-4: Characteristic times to plume plume separation from the sand surface for each of the four blends (Soy B-20, Soy B-100, Animal Fat (AF) B-20, AF B-100) relative to ULSD in side-by-side comparison. The three columns per fuel blend show the results for each of the three replicates.

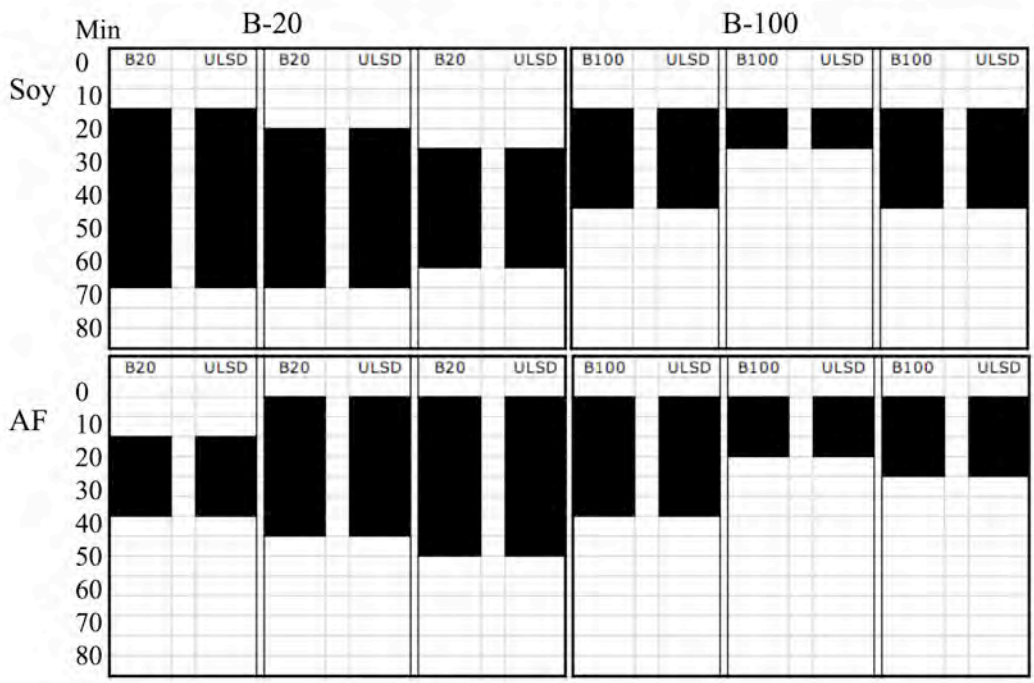

Figure II-C-5: Characteristic times for commencement of lens spreading on the water table for each fuel (Soy B-20, Soy B-100, B-20, AF B-100) relative to ULSD in side-by-side comparison. The three columns per fuel blend show the results for each of the three replicates.

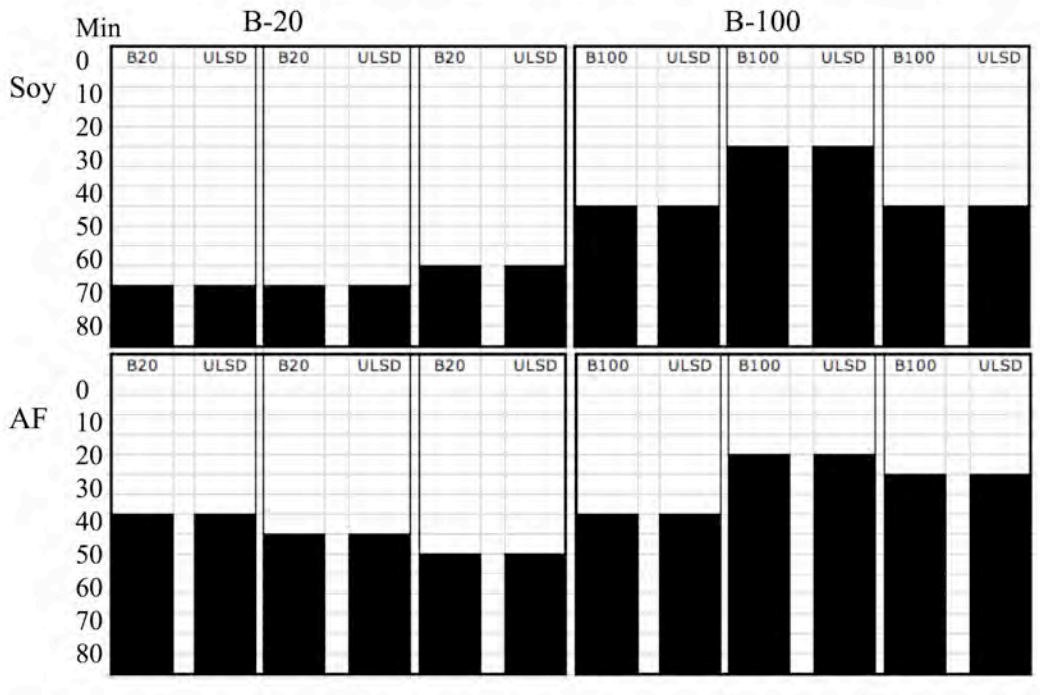

Figure II-C-6: Characteristic times for lens formation on the water table for each of the four blends (Soy B-20, Soy B-100, AF B-20, AF B-100) relative to ULSD in side-by-side comparison. The three columns per fuel blend show the results for each of the three replicates.

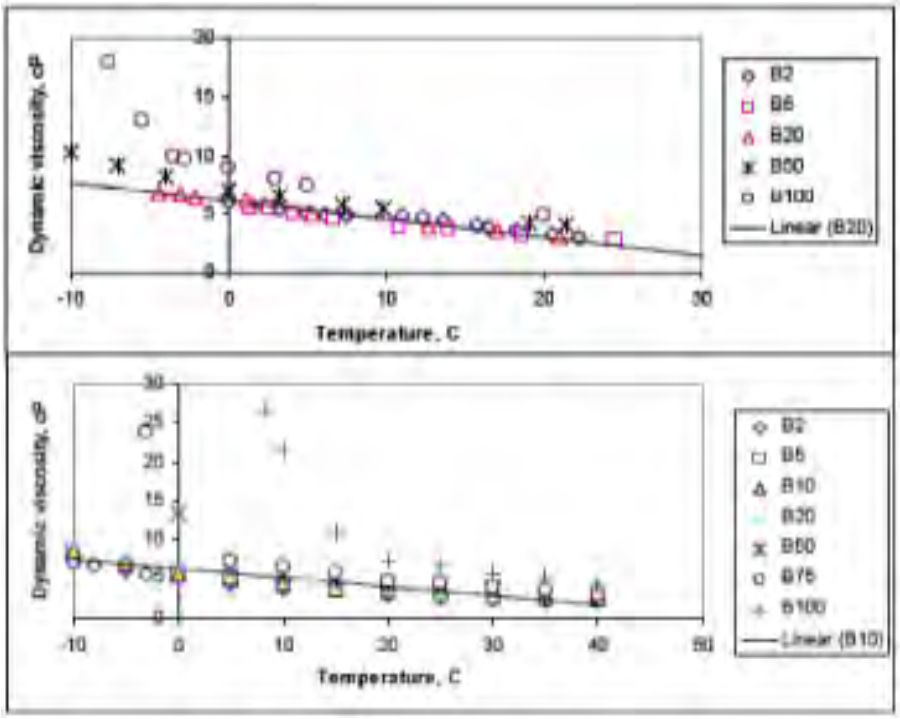

Figure II-C-7. Biodiesel fuel blend (Soy, top; Animal fat, bottom) viscosities for a range of temperatures. Note relatively large increase in Animal Fat B-100 viscosity for temperatures below 20 degrees C. From Yang et al., 2008.

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.

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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.

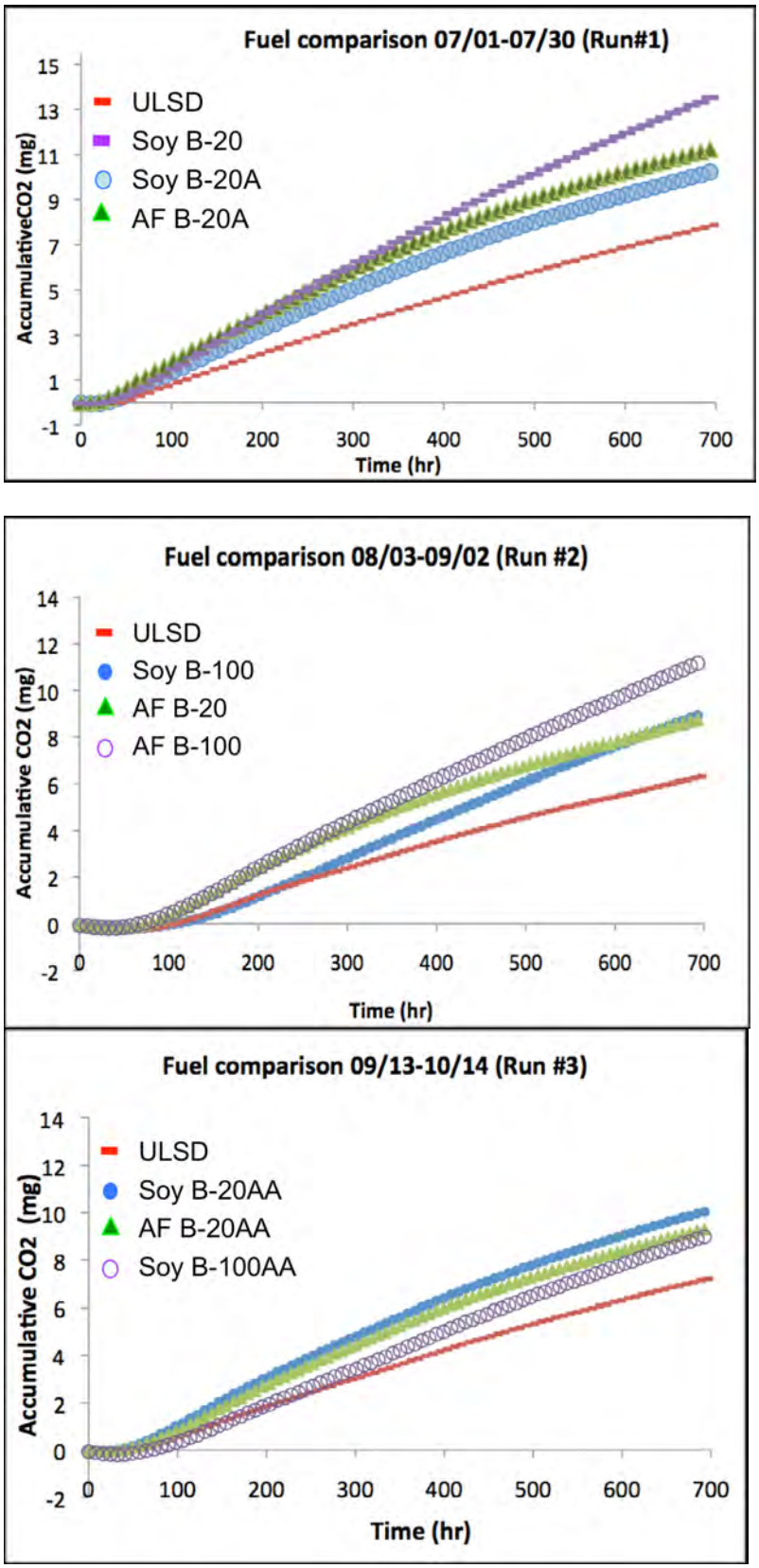

Figure II-D-2. Respirometry data on CO2 production in experimental suites #1 (top), #2 (middle) and #3 (bottom).

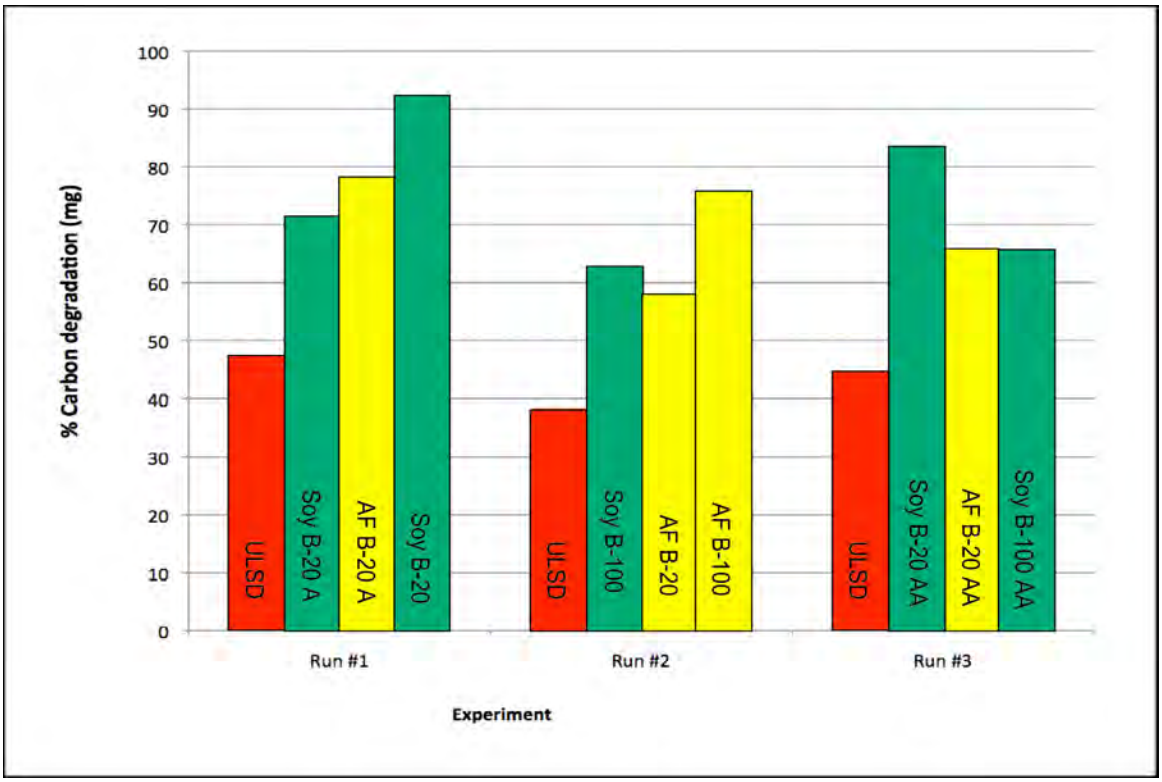

Figure II-D-3: Comparison of percent of carbon biodegradation with different fuel types in microcosm respirometry at the end of the experiments.

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.

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[3] http://ecb.jrc.it/Documents/TECHNICAL_GUIDANCE_DOCUMENT/EDITION_2/tgdpart2_2ed.pdf.

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