Experimental Plan for Tier II Evaluation of Biodiesel
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Introduction

This document summarizes the plan for experiments to be performed at UC Davis 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 the Multimedia Working Group (MMWG) Tier I report. Biodiesel B100 is defined here as mono-alkyl ester-based non-petroleum derived diesel substitute meeting ASTM D6751-07be1 (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.). [Pyrolysis oil and "renewable diesel" are not addressed in this Tier II planning document.] In addition, biodiesel additives are available for a variety of purposes and these products may affect the behavior and impacts of biodiesel in the environment.

There are significant knowledge gaps pertaining to the fate, transport, biodegradation, and toxicity properties of Biodiesel in the environment, resulting from leaks and spills as identified in the Tier I report. To address these issues, a combination of experiments are proposed to investigate basic phenomena asssociated with environmental risk associated with unintended release of biodiesel into the environment. In all instances these experiments are intended to address *relative* risk as compared to that associated with ULSD. Because of time and funding limitations, this experimental package is designed to address highest priority knowledge gaps addressed in Tier I, and in a simplified and riskwise conservative fashion. This way maximum use is made of available time and resources in filling knowledge gaps, or corralling associated relative risk as below or above that associated with ULSD.

Knowledge Gaps Identified in Tier I

The Tier 1 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). Within the category "Additives impacts" are listed unknown impacts of cold flow, biocidal, and antioxidant additives, and in all cases priority is given to effects on human and ecosystem toxicity. These issues are partly addressed in the experimental plan described here as follows:

Knowledge Gap	Approach
○ Additives/Toxicity	Aquatic toxicity experiments
▪ cold flow	not tested
▪ biocidal, antioxidant	tested
○ Fate & transport	"Ant Farm" experiments
○ Biodegradation	Microcosm experiments
○ Release scenarios	Not tested
○ Air emissions studies	ongoing by CARB

also noted in Tier I and promoted to priority knowledge gap here is

○ Solubility, co-solvency	Equilibrium calculations, GCMS analyses

Budget and time constraints require restriction of the experimental investigation to incomplete treatment of the knowledge gaps identified, and so the plan scope covers the highest priority issues. Thus impacts of cold flow additive, and evaluation of release scenarios, are not evaluated in this Tier II study, while the remaining items (and additives) are the focus of the experiments planned. Aqueous solubility of fuel components and of additive components will also be studied. Toxicity studies are restricted to marine, estuarine, and freshwater characteristic systems (one invertebrate and one fish).

Blend and Additive Selection

Due to budget and time constraints, blend selection is also restricted to two feedstocks and two blend ratios as Biodiesel blends include primarily B20 and B100 as they represent highest expected use and maximum biodiesel samples respectively. Feedstocks include Soy and Animalfat, as they reflect high potential use and wide bracketing of dominant feedstock chemistry. Additives have been selected by criteria defined in Appendix I: in summary, antioxidant and biocide additives are hypothesized to be potentially involved with some frequency and to most likely to incur departures from ULSD behavior, so one representative additive from each category is selected in aquatic toxicity and biodegradation experiments. These feedstock and additive selections are also made in order to be consistent with ongoing CARB emissions testing.

Tier II Work Plan

Here the descriptions of each suite of experiments/calculations are defined, beginning with the Solubility preliminary. In each experimental section, the protocols designed for the relative risk assessment are summarized, the experimental permutations are listed, and the measured quantities are stated. Also, the volumes of ULSD, Soy and Animal based B100 and B20, approximately required for the experiments are listed.

Solubility

Many exposure paths and each of the three experimental areas (ant farms, aquatic toxicity, biodegradation) involve biodiesel/additive blend components partitioning to aqueous phase. Thus an understanding of component solubility is an important element in

quantifying relative risk. Chemically, weak dipole-induced dipole forces are responsible for the dissolution of oil-based compounds in water. This is due to distortions in the electron cloud surrounding the molecules due to the polarity created by the hydrogen in water (Silberberg et al. 2003). Solubility is also temperature dependent. Material Safety Data Sheets (MSDSs) for biodiesel and for component additives indicate solubility qualitatively and inconsistently. Thus we will pay attention to identification of solubilities both by calculations made tractable by simplifying assumptions, and by experimental identification of solutes by GCMS analyses.

The assumptions of the calculation approach are as follows. When biodiesel is added to water each of the fatty acids will partition following Raoult's law, that the aqueous phase concentration is equal to the aqueous phase solubility of the constituent in equilibrium with the pure constituent phase, multiplied by the mole fraction of the constituent in the oil phase (e.g., Charbeneau 2000). In other words, the amount of a particular fatty acid methyl ester (FAME) that dissolves into the water is equal to the solubility of the pure phase FAME (a single FAME added to water) in water multiplied by the mole fraction of the FAME in biodiesel. Raoult's law implies the absence of cosolvency effects. This may not be a conservative assumption especially when additives are involved, some of which are completely soluble in water and may affect solubility of other components of biodiesel. It is assumed that the FAMEs are ideal mixtures and therefore the total volume of the mixture will be the sum of the volumes of the components added together (Charbeneau 2000), and that the additive adds no volume in the biodiesel. The activity of the solution is conservatively assumed to be equal to one since the concentration of FAMEs in water is expected to be small, or dilute. Preliminary calculations will be done for unadditized Soy B100, Soy B100 with Bioextend, and Soy B100 with Kathon FP1.5. The composition data for Animalfat biodiesel is as yet undetermined so it will not be used in these preliminary solubility calculations. The pure phase composition of Soy B100 is based on mass fractions of FAMEs determined in Zhang et al. (1998) and from data posted by the National Biodiesel Board. The solubilities of the FAMEs and additives will be determined based on the solubility experiments reported in Krop et al. (1997) and MSDS information. The MSDS for the additives have been supplied by the manufacturers.

The results of the solubility calculations are given in terms of a non-aqueous phase liquid (NAPL)-water partitioning coefficient. The partitioning coefficient represents the initial ratio of the concentration of FAMEs in the FAME phase to the concentration of FAMEs in the water phase. A large result indicates a low solubility of the FAME in water and a small number indicates a high solubility. As the composition of the Biodiesel changes with time due to biodegradation the partition coefficient will also change since the mole fraction of the FAMEs will change with time. The calculated partition coefficients will be compared to results from GCMS analyses. and both will be compared with literature values available (e.g., for rapeseed biodiesel, Birchall et al., 2002).

Experiment Descriptions

The following three suites of tests will be carried out at UC Davis.

1. "**Ant Farm**" 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. In addition to visual observation of patterns and mobility, aqueous samples will be collected for analyses for solutes including additives.
2. The biodegradability potential of various biodiesel recipes (summarized below) will be tested in microcosm batch under aerobic conditions. **Microcosm** study and CO_2 evaluation will be used to study the rates of biodiesel degradation.
3. **Aquatic toxicity** tests will be carried out to evaluate the relative toxicity of biodiesel blends potentially released to aquatic environments.

Table 1 below shows the summary experimental matrix reflecting the selection of different additive combinations (columns of Table 1) for testing with different fuel blends (rows of Table 1), 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. The additives selection discussion appears in the Appendix 1, and reflects again prioritization-based selection of representative additives from a list of multiple candidates based on expected impacts within the context of the limited scope of the experimental investigation.

Fuel Preparation			
ULSD	A, M, T		
Soy B100	M, T	M,T	M, T
Animal fat B100	M, T	M,T	M, T
Soy B20	M, T	M,T	M, T
Animal fat B20	M, T	M,T	M, T
Additives	Reference	Bioextend-30	Kathon FP 1.5
Additive Type	No Additive	Antioxidant	Biocide

Table 1. Tier II Testing Matrix: Experiment codes are: M Microcosm, A Ant Farm, T Aquatic Toxicity

Volumes summary. Volumes of ULSD, Animalfat B100 and B20, and Soy B100 and B20 are calculated per experimental suite below. Totals (rounded up) are 30L ULSD, 32L Animalfat B100, 32L Soy B100, 34L Soy B20, and 34L Animalfat B20. Kathon FP 1.5 will be added as needed. B20 will be mixed using available B100 and ULSD. Additinoally ant farm experiments will be done using cold-flow enhancer given time and resources.

1. Ant Farm

These tests are designed to explore relative migration and distribution of ULSD and biodiesel in the vadose zone. These tests will identify differences in fate and transport behavior of biodiesel formulations with respect to ULSD diesel. The testing chamber consists of glass walls for observation of the flow field. Sand, soil or other media is held within, and immersed in water (Figure 1). A basic representative medium

sand will be packed between two glass plates, with water saturating ~ half of the domain. The fuel sample is emplaced at the top and infiltration, redistribution, residual fuel formation, and lens formation on the water table is observed and recorded using digital photography and analyzed graphically. We plan several preliminary tests to determine the infiltration time through the unsaturated zone (see below), and will design the depth to saturated zone accordingly. Data will be relevant to determine the impact of a fuel spill or leak into the vadose zone environment.

Procedures

Thin, two-dimensional glass sand tanks will be built to visually compare the migration and distribution of biodiesel with that of ULSD in the unsaturated zone (Powers and McDowell, 2001 for similar experiments with ethanol). These experiments will provide information useful to assess the migration and distribution of ULSD diesel and biodiesel in the vadose zone at spill sites. Tanks will be constructed using glass with wooden spacers and compatible sealants, fixed with spring clamps. This simple design will allow dissembly, cleaning, and reassembly for inexpensive execution of the experiments, and small volumes of waste material generation. Potential vapor phase effects on the materials will affect only boundaries away from the hydraulic dynamic zone. Potential glass-fuel surface tension effects will be evaluated visually (see preliminary experiment notes below) and if necessary glass surface treatments will be used. Biodiesel sample and ULSD sample will be emplaced side by side instantaneously in small surface depressions in the porous media as initial conditions. Images will be collected using digital photography with controlled lighting (G. Redden, Idaho National Lab, personal communication) and used to evaluate relative infiltration rates and lens formation on water tables.

Permutations

Experiments will consider ULSD, Soy B100, B20, and Animalfat B100, and B20 with and without antioxidant additive. A total of ~50-200 mL of fuel biodiesel blend will be required for each experiment. An estimated 3ul of Solvent Red 26, hydrophobic, fat-soluble dye per liter fuel, will be added per 200 ml fuel to dye fuels for visualization (Cohen et al., 1992; Cohen and Mercer, 1993; Pankow and Cherry, 1996; Kram et al., 2001, Powers and McDowell, 2001). Volumes are as follows (experiments with triple replication) in Table 2.

	ULSD	Animalfat B100	Animalfat B20	Soy B100	Soy B20
Reference	200 mL				
antioxidant		200 ml two soils	200 ml	200 ml two soils	200 ml
unadditized		200 ml	200 ml	200 ml	200 ml
Totals	600mL	1800 ml	1200 ml	1800ml	1200ml

Table 2. Ant Farm experimental submatrix

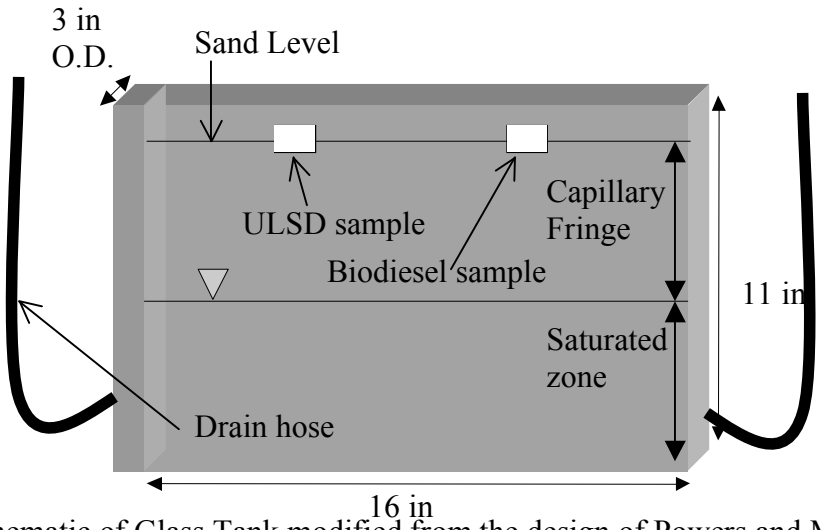

Figure 1. Schematic of Glass Tank modified from the design of Powers and McDonnell (2001).

Porous Media: A quantity of approximately 2 liters of each soil (medium sand, coarse sand) will be needed for each of the experiments, for a total quantity of 10 liters. Soil samples will be selected to bracket soil hydraulic properties, and we will use a medium sandy soil for all the permuations and a silt loam soil for comparisons with the B100 cases for both additives.

Design of Porous Media Selected:
Time and resources are limited to prevent representative sampling of different CA soil types which include essentially all types on the Soil Conservation Service soil texture triangle, also fractured rock and other subsurface materials. So first a basis for choice of which soil type to use is needed. Soil hydraulic properties, such as hydraulic conductivity or porosity, are a potential basis; however, any endmember pairs may not represent release scenario sites, nor do they pack the same in any flow cell, and repacking materials changes these properties significantly. An alternative basis would be "representative materials from a single candidate site"; but materials thus obtained are site-specific and unique and thus may not provide anything but site-specific results. Another alternative basis is "transport property in idealized but representative material" from which one chooses artificial sand and artificial clay for instance; however again the packing methods give different material properties even within a soil texture, also the experiments in these two materials are markedly different in ways that puts it out of scope for our work, e.g., transport in clay-loam is very slow. In view of these considerations and the time frame, we set as basis "an artifical material that makes for timely transport experiments, is reproducible, and generically representative of high-risk conditions" and we choose medium, and coarse, sand. This material has relatively well-known hydraulic properties that do not change significantly between packings. Thus the hydraulics are more controllable and the experiments more reproducible. Also we can see the water content through the glass. This allows us to use dyes and visual inspection.

Packing Considerations: Emplacement of a sand requires decision on packing, i.e., wet vs. dry pluviation. Wet pluviation was determined to be advantageous over dry pluviation because it leads to less variability in unsaturated zone water contents and to water table elevations. It also allows us to establish easily reproducible antecedent moisture in the sand pack. The disadvantage of this is that the pack is looser than it is in a dry pluviation (put the sand in, then add water) because the terminal velocity of particles is much higher in air than in water (J. DeJong, UC Davis, personal communication) and the porosity is a little higher, but this is acceptable in our objective of relative risk assessment as it leads to faster transport in principle. We will also make several dry pluviation experiments for comparison, also to test two different "antecedent moisture conditions"

Preliminary Experiments.
A range of experiments were performed to establish experimental protocols for practical traveltimes, hydraulic integrity (no leaks), reproducibility, and anomalous effect control. The results of these experiments indicate use of a coarse sand with watertable at one-half the antfarm height (~5.5inches from base). This is achieved via wet pluviation of the sand into water-filled antfarms with subsequent drainage of water to establish water table height. Anomalous effects of concern include effect of the dye on hydrodynamics, and effect of the glass walls on the transport. Experiments were done with SoyB20 to examine dye vs. non-dye infiltration, and a representative image is in Figure 2, showing no perceptible difference. No replicates were performed. Experiments were done also using CARB#2 and Soy B20, with careful post-experiment excavation, in order to evaluate the role of the glass wall in infiltration, and representative images appear in Figure 3, showing no impact.

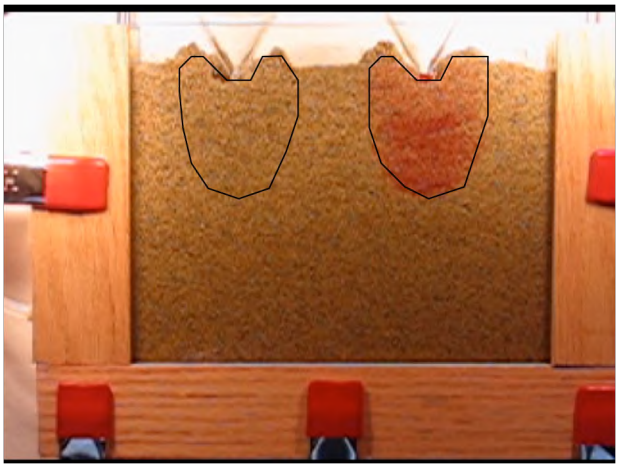

Figure 2. Infiltration of Soy B20 biodiesel with dye (right hand plume) and without dye (left hand plume), showing similar plume shapes. Identical behaviour was witnessed through 30 minutes observation ending with lens formation on water table.

Figure 3. Post-infiltration excavation of dual plumes of CARB#2 and Soy B20 biodiesel showing homogeneous plume structure between walls indicating absence of wall effects on infiltration.

2. Microcosm Biodegradation Experiments

Microcosm experiments are conducted to assess the relative (to petroleum diesel) aqueous biodegradation potential for solutions exposed to the test biodiesel fuels. Fuels derived from animal fat and soy feedstocks at B100 and B20 mixtures (with ULSD making up the complement) are used as source phases, and ULSD is also tested for comparison. The biodiesel blends will include either the antioxidant Bioextend-30, or both Bioextend-30 and the biocide Kathon FP1.5, at industry-specified amounts. The reference ULSD fuel will contain 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.

Approach

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, WHO, and the United Nations). The suite of microcosm experiments described here is designed based on the recommended OECD biodegradability test (OECD 2004). Based on the OECD recommendation, the microcosms comprise of mineral medium, bacterial inoculation from activated sludge from the aeration tank of a sewage treatment plant, and tested substrate. The bacterial inoculation of activated sludge was replaced with soil for better representation of environmental conditions for biodegradation of diesel and biodiesel.

Biological activity is measured through respiration; carbon compounds are broken down into CO_2 that can be quantified per EPA 560/6-82-003, PB82-233008. Thus the evolution of CO_2 as a result of microbial activities will be measured in our microcosms incubated at controlled temperature of 25 °C using a respirometer for the recommended 28 day test period.

Permutations

Table 3 shows a listing of this set of experiments. Experimental suites will be done for the following permutation factors:
- Feedstock (soy, animalfat),
- blend (100% biodiesel, 20% biodiesel),
- additive package (Bioextend-30; Bioextend-30 and Kathon FP1.5).

With the additional suite for ULSD there are 11 total suites.

	ULSD	Animal fat B100	Animal fat B20	Soy B100	Soy B20
Reference	X				
antioxidant		X	X	X	X
antioxidant and biocide		X	X	X	X
No additives		X	X	X	X

Table 3. Microcosm biodegradation experimental submatrix

For each one of these 11 suites, a set of experimental treatments with replicates will be performed. The different treatments and number of replicates and flasks for one such suite are shown in Table 4.

Procedures per Experimental Suite

The microcosms are prepared using a 250 mL flask that consists of 200 ml mineral medium, 2 g soil as bacterial inoculums and an amount of test substrate that is equivalent of a nominal concentration of 25 ppm The mineral medium contains KH_2PO_4, K_2HPO_4, $NaHPO_4$, NH_4Cl, $CaC_{l2}.H2O$, $MgSO_4$, and $FeCl_3.6H_2O$ (OECD 2004).

Description	**Content**			**# of Rep.**	**# of Microcosm**
	Substrate	*Inoculum*	*Mineral*		
Test suspension	X	X	X	3	3x11 = 33
Inoculum blank		X	X	3	3
Abiotic +Adsorption control	X Sterilized	X Sterilized	X Sterilized	1	1x11 = 11
			TOTAL Microcosms:		47

Table 4. Single Suite Microcosm Experiment: Treatment and Controls

For each treatment, an abiotic sterile control will be prepared. This control contains sterilized inoculated solution with substrate to examine whether the test substrate is degraded abiotically, also to test the adsorption of test substrate onto glass. Three replicates of inoculum blank (no substrate) will also be prepared. The inoculum blank is to examine if there is any CO_2 production by microorganisms in absence of substrate. Substrate tests and inoculums blank control will be prepared in 3 replicates. The sterile controls will only have 1 replicate.

Assessing Biological Activity

The CO_2 production in microcosms will be measured every 10 hours using the respirometer (Columbus Instruments) during the experiment. At the beginning and completion of the study, subsamples of liquid will be removed, preserved and sent to an analytical lab for GC-MS analysis of the fuel and additive constituents. The change of fuel constituent concentration during the test will be correlated with the CO_2 evolution to determine the biodegrability of different test fuels in each microcosm. The additive concentrations in the solution will be correlated with the biodegradation extent to evaluate the impact of the additive on aqueous biodegradation.

Analyses

At the beginning and completion of the study, subsamples of liquid will be removed. The organic compounds of samples will be extracted using the aqueous-aqueous extraction method according to the EPA Method 3510c. Extractions will be sent to Lawrence Berkeley National Laboratory for GC-MS analysis of key biodiesel

compounds. All data will be analyzed by comparison of results for each biodiesel to those for ULSD.

3. Aquatic Toxicity Testing

Toxicity experiments will be conducted to evaluate the toxicity of biodiesels that could be discharged into aquatic environments. Biodiesel unintentionally spilled during transport or storage could enter fresh or marine waters resulting in toxicity to any level of the aquatic food chain. To investigate this potential B100 and B20 will be tested with and without antioxidant additive, and given time and resources, also with biocide additive.

Procedures

Testing the toxicity of the biodiesels relative to ULSD will be performed by AQUA-Science (Davis, CA). Toxicity testing will be performed according to Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms, 1st Edition, August 1995 (EPA 600/R-95-136), Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms, 3rd Edition, October 2002 (EPA 821-R-02-014), and Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, 4th Edition, October 2002 (EPA 821-R-02-013).

Test solutions will be prepared using the 'slow-stir' method (Schluep et al, 2001). Laboratory water and the test material are placed in a glass vessel at a 10:1 ratio, respectively, and stirred for 24 hours at the temperature specified in the toxicity test protocols. The solutions are allowed to stand for 2 hours and the diesel water-accommodated fraction (WAF) is carefully decanted off. Testing will be performed using a static renewal methodology where the test media is renewed on a schedule determined by the length of the test. Static renewal avoids potential problems of test material degradation, and dissolved oxygen depletion resulting from biological oxygen demand or chemical oxygen demand. Renewal can be accomplished by renewing the test solution or by transferring test organisms to a container of fresh test media. Testing will be performed using a control (laboratory water) and six predetermined concentrations of all test materials (1, 5, 10, 25, 50 and 100% WAF). Because the interest is in determining the toxicity of the biodiesel formulations relative to ULSD, test concentrations of the biodiesel treatments will be the same as for the ULSD treatment. Chronic tests will be performed on *Selenastrum capricornutum*, *Ceriodaphnia dubia*, *Pimephales promelas, Haliotis rufescens, Mysidopsis bahia,* and *Atherinops affinis*. The test duration for the latter five tests will allow a determination of both chronic and acute toxicity of the test chemicals at 24 or 48 hours. The *Selenastrum* test is a cell growth endpoint and does not provide a measure of acute toxicity.

Permutations

Initially, the toxicity tests will utilize ULSD, B20 Soy (S), B20 Animal fat (A), and the same stocks with biocide and antioxidant additives together (Table 5). If funding

permits, all B100 and B20 stocks will be tested separately with each of the additives. A total of 6L of fuel biodiesel blend will be required for each series of toxicity tests.

Test Species	Test Type	Test chemical				
		ULSD	B20S	B20S A+B[a]	B20A	B20A A+B
Green algae *(Selenastrum capricornutum)*	96-hr chronic cell growth	1L	1L	1L	1L	1L
Water flea *(Ceriodaphnia dubia)*	7-day acute and chronic (survival and reproduction)	1L	1L	1L	1L	1L
Fathead minnow *(Pimephales promelas)*	7-day acute and chronic (survival and growth)	1L	1L	1L	1L	1L
Red Abalone (*Haliotis rufescens*)	48-hr chronic (shell development)	1L	1L	1L	1L	1L
Mysid *(Mysidopsis bahia)*	7-day acute and chronic (survival and growth)	1L	1L	1L	1L	1L
Topsmelt (*Atherinops affinis*)	7-day acute and chronic (survival and growth)	1L	1L	1L	1L	1L
Totals		6L	6L	6L	6L	6L

A+B antioxidant and biocide

Table 5. Test fluids and volumes for toxicity testing. The first three species are freshwater, the bottom three species are estuarine or marine.

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Appendix I

Biodiesel Additive Recommendations for CARB Phase II Lifecycle Health Risk Analysis Lab Tests

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The purpose of this document is to recommend several additive products for inclusion in the CARB multi-media biodiesel lab tests, representative of additives that are likely to be commonly found in real-world biodiesel use. The primary selection criteria include products containing typical chemical components for the additive category, and products developed by major chemical manufacturers with capabilities for wide distribution and significant market volumes. Biodiesel additives include antioxidants, biocides, cold-flow enhancers and NOx reducers. The assumption is made that soy feedstock is used.

Information on manufacturing and the specific producer of the components in the chemical blend are included. Studies on acute and chronic toxicity have been analyzed, with the majority of data found in the National Library of Medicine's Hazardous Substance Databank, and the International Program on Chemical Safety's INCHEM database. Significant parameters include LD50's, LC50's, (the lethal dose and concentration respectively to kill on half of a test population) No Observed Adverse Effects Levels (NOAEL), and carcinogen potentials. When available, toxicity to specific organs or systems is included. Toxicity ratings from programs including the International Agency on Cancer Research (IARC), the National Institute for Occupational Health and Safety (NIOSH), the UC Berkeley Cancer Potency Database, United States Environmental Protection Agency, and California Proposition 65 are included when available.

Environmental fate and transport parameters include environmental half lives, e.g. the time for half the concentration of the substance in the soil water or atmosphere to be degraded or dispersed. The water/octanol partition coefficient is used to measure the relative solubility of a substance between water and a hydrocarbon; this is insightful on the partitioning of the substance in the environment. The solubility of a substance into water helps determine the mobility of a chemical in the environment. Vapor pressure, Henry's Constants and melting points are used to predict the phase changes of a substance in the natural environment.

Antioxidants

Antioxidants added to biodiesel to prevent oxidation by atmospheric oxygen. This process increases the acidity and viscosity, while decreasing combustion properties (UC Davis and UC Berkeley, 2008). It is probable that users will add an antioxidant blend to ensure fuel quality. The effects of these substances on human and environmental health must be assessed.

Biodiesel Recommendation: Eastman Chemical Corp. Bioextend30

Eastman Chemical is a major chemical manufacturer, Bioextend30 antioxidant is been formulated specifically for biodiesel. It contains a commonly used antioxidant - TBHQ.

TBHQ

TBHQ was approved by the FDA as a food preservative in 1972, and has shown to be effective in preserving unsaturated oils. Its major use is as an antioxidant for oils, production is estimated at 2300 kilograms per year (National Library of Medicine, 2003). Chronic exposure to dust and vapors has been linked to vision problems. Acute expose can lead to GI tract and liver irritation, as well as jaundice. Hydroquinones exposure can cause headaches and dizziness (National Library of Medicine, 2003).

A No Observable Adverse Effects Level (NOAEL) has been established at 37.5 mg TBHQ per kilogram of bodyweight (Sheftel & Victor, 2000). In non human studies, a .5% dose in the diet of rats caused liver swelling after 6 days. Cellular toxicity was noted in bioassays of rat cells at a concentration of .5 millimoles per liter. Beagles were fed 0, 500, 1500, and 5000 mg TBHQ per kg of feed over a 117 week period. No organ damage was noted, although red blood cell counts were slightly lower in the 5000 mg group (Sheftel, 2000). DNA damage was observed in rat hepatocytes (liver cells) with doses of doses of 0.17 ug/ml. A 5000 mg TBHQ per kg feed dosage in hamsters did not induce tumor growth in stomach or bladder cells (National Library of Medicine, 2003).

The IARC states that "The agent (mixture or exposure circumstance) is not classifiable as to its carcinogenicity to humans. This category is used most commonly for agents, mixtures and exposure circumstances for which the evidence of carcinogenicity is inadequate in humans and inadequate or limited in experimental animals. Exceptionally, agents (mixtures) for which the evidence of carcinogenicity is inadequate in humans, but sufficient in experimental animals may be placed in this category when there is strong evidence that the mechanism of carcinogenicity in experimental animals does not operate in humans. Agents, mixtures and exposure circumstances that do not fall into any other group are also placed in this category (National Library of Medicine, 2003).

Butyl acetate

Butyl Acetate is used as an artificial fruit flavor in foods, approved by the FDA assuming that, "they consist of one or more of the following, used alone or in combination with flavoring substances and adjuvants generally recognized as safe in food, prior-sanctioned for such use, or regulated by an appropriate section in this part" (National Library of Medicine, 2005). It is used as a lacquer in film and plastics and a solvent in resins, fats and oils. As of 1993, 1.32378X10+8 kilograms were produced in the United States (National Library of Medicine, 2005).

The United States OSHA exposure limit is 150 ppm ambient concentration for a time weighted 8 hour period. Butyl Acetate is regulated under the Clean Water Act and CERCLA, with releases larger than 5000 pounds. In human studies, a 970 ppb ambient concentration caused throat irritation, while 1400 ppb caused ocular pain (Copestake & Malcolm, 2005). Acute exposure leads to central nervous system depression. Butyl acetate has not been considered a carcinogen by the IARC (National Library of Medicine, 2005)

Cats were exposed to a 3100 ppm concentration of airborne butyl acetate for 6 hours a day for six days. Respiratory irritation was noted in some of the animals,

although no deaths occurred. Bioassays on Salmonella Typhimurium hamster cells showed no mutagenic activity (Copestake & Malcolm, 2005).

Citric Acid

Citric acid is a common food additive for preservation, ph control and coloration. The FDA has classified it as Generally Recognized as Safe (GRAS.) Ingesting large amounts of the chemical have caused acidosis and hypocalcaemia in humans. No chronic toxicity has been noted.

Exposure to a 6% aerosol solution to guinea pigs caused coughing in the test subjects. A 15 mg/kg dosage in rats caused a 71% drop in blood pressure. Chronic toxicity has not been noted (National Library of Medicine, 20).

Diethylene Glycol Monobutyl Ether

Diethylene glycol monobutyl ether (DGME) is a solvent used in cleaners, lacquers, paints, and oils. It is allowed by the FDA as a food additive and as an inert component in pesticides. According to the Hazardous Substance Database, "2 mL/kg has produced cyanosis, tachypnea, and slight uremia (National Library of Medicine, 2007)." Bioassays on human cells at 5 and 10 millimolar concentrations did not yield cellular damage. DGME is not considered carcinogenic by the IARC (National Library of Medicine, 2007)

A study by the European Chemicals Bureau exposed rats to a DGME concentration of 117 ppb caused an increase in liver weight after 5 weeks. The authors determined an NOAEL of 39 ppb ambient concentrations. A six week exposure to 3.6 g/kg/day of DGME decreased body weights, enlarged spleens and hyperkeratosis (National Library of Medicine, 2007). A 60 day, 1g/kg/day dosage on rabbits yielded no reproductive toxicity or loss in fertility. Bioassays on hamster cells found no signs of genetic mutation. Neurotoxicity was not observed in a 13 week dosage of 2g/kg bodyweight in male and female rats.

Bioextend30 Chemical Composition:

Butyl acetate	123-86-4	30%
diethylene glycol monobutyl ether	112-34-5	30%
2-tert-butylhydroquinone	1948-33-0	20%
citric acid	77-92-9	5%

Treatment concentration: 400ppm pre-blended in the B100 biodiesel

Biocide

Bacteria, fungi and other microbes consume oils as a source of energy through biological oxidation. This leads to a lower energy content of the fuel as well as fouling of storage tanks and engine parts.

Recommended Biocide: Rohm and Haas Corp. Kathon FP1.5

Rohm and Haas is a major chemical manufacturer, Kathon FP 1.5 is a large production biocide, and isothiazol is a widely used biocide in numerous industries involving water equipment processes and fuels. "Kathon" is a blend of 5-chloro-2-methyl-2H-isothiazol-

3-one and 2-Methyl-4-isothiazolin-3-one in a 3:1 ratio, commonly refered to as Methylisothiazolinone. (United States Environmental Protection Agency, 1996)

Toxicity Information
Methylisothiazolinone

Short term studies on rats found that exposure to methylisothiazolinone would decrease body weight gain, and water consumption. These effects were noted in oral dosage at rates between 2.4 and 24.7 mg/kg/day as well as the inhalation of a 2.64 ppb concentration (United States Environmental Protection Agency, 1996). Exposure over a 2 year period induced hyperplasia and hyperkeratosis in rat stomachs; no malignant tumors were found. Chromosomal damage in mouse bone marrow did not occur in the ingestion of up to 30 mg/kg. In bioassays, 0.0005 μl/plate dosages caused mutanogenic activity (United States Environmental Protection Agency, 1996). Overall, the substance has been determined to non-carcinogenic by the EPA.

Methylisothiazolinone is miscible in water; 96 hour toxicity tests on rainbow trout determined an LC50 of .19 ppm. It is less toxic to birds; the LC50 for mallard ducks was 717 ppm. For freshwater invertibrates, the Maximum Allowable Toxicant Concentration has been set between .1 and .18 ppm (EPA.) In the agency's risk assessment, purging of underground storage tanks would result in "minimal to no exposure (EPA.)"

Magnesium Nitrate

Acute exposure to dust can lead to eye and mucous gland irritation as well as depression of the central nervous system. Magnesium nitrate has no been linked to cancer in human or animal studies. According to Sheftel, a .5-5 g/kg is a probable leathal dose in humans (National Library of Medicine, 2003).

Magnesium Chloride

Magnesium chloride is a component in disinfectants, textiles, ceramics and fire retardants. Acute magnesium poisoning is known cause nausea, hypotension, and depression of the central nervous system. The IARC does not consider magnesium chloride a carcinogen, and no studies have identified risk from chronic exposure. (National Library of Medicine, 2008)

In studies on rats, cardiac arrest occurred following the injection of a 25 millimolar (2.38 g / liter) solution of magnesium chloride. Chronic dosage of 2.5 g per kilogram of feed in rats yielded no tumor growth; however a slight decrease in bodyweight gain occurred (National Library of Medicine, 2008).

Dipropylene Glycol

Dipropylene Glycol (DPG) is used as a plasticizer, solvent in plastics and inks, as well as antifreeze solutions (SIDS.) It is allowed as an inert ingredient in pesticides under FIFRA (National Library of Medicine, 2005). The chemical is not highly toxic to humans; the lethal dose for adults is estimated to 1 pint. An NOAEL has been estimated at 1.2 g/kg/day. Large doses are expected to cause liver and kidney damage, corresponding to 80 g/L dosages in rats (HSDB.) A 5.9 ml/kg bodyweight injections was given to dogs, causing some central nervous system depression. Concentrations of 5 g/L

was found to be toxic to fish, and 3.1 g/L harmed amphibians (SIDS.) No DNA damage has been seen in bioassays (SIDS.)

Kathon FP 1.5

Magnesium nitrate	10377-60-3	1-2.5%
5-chloro-2-methyl-2H-isothiazol-3-one	26172-55-4	1-3.0%
2-Methyl-4-isothiazolin-3-one	2682-20-4	0.3 - 0.4%
Magnesium Chloride	7786-30-3	1.00%
Dipropylene glycol (Mixed isomers)	25265-71-8	88 - 90.0%
Water		6.00%

Treatment concentration: 100ppm in final mixture regardless of diesel/biodiesel ratio

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