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ALPHARETTA, GA 30022
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June 14, 2016
In reply, refer to: CRC Project No. DP-07-16-1

Dear Prospective Bidder:

The Coordinating Research Council, Inc. (CRC) invites you to submit a written proposal on a "Identification of Potential Parameters Causing Corrosion of Metallic Components in Diesel Underground Storage Tanks," as described in the attached Statement of Work, Exhibit A.

Please indicate via email by **June 28, 2016** whether or not you or your organization intends to submit a written proposal for the project. CRC will answer technical questions regarding the Request for Proposal if they are submitted in writing. CRC will then return written answers to all of the bidders, along with a copy of the original questions.

The CRC technical group composed of equipment, petroleum, and government representatives will evaluate your proposal. CRC reserves the right to accept or reject any or all proposals.

The reporting requirement will be text, data and charts to CRC in accordance with Exhibit A - Statement of Work. A Final Report documenting the results of the study will be published by CRC. The reporting requirement is described in more detail in the attachment entitled, "Reports" (Exhibit B).

The "Intellectual Property Rights Clause" (Exhibit C) and "Liability Clause" (Exhibit D) will be a part of the agreement, which may be executed as a result of this Request for Proposal solicitation.

The proposal must be submitted as two separate documents. The technical approach to the problem will be described in Part One and a cost breakdown that is priced by task will be described in Part Two. The cost proposal document should include all costs associated with conducting the proposed program.

CRC expects to negotiate either a cost reimbursable or a fixed price contract. Important selection factors to be taken into account are listed in Exhibit E. CRC evaluation procedures require the technical group to complete a thorough technical evaluation before considering costs. After developing a recommendation based on technical considerations, the costs are revealed and the recommendation is modified as needed.

Electronic copies of the separate technical and cost proposals should be submitted to:

Dr. Christopher J. Tennant
Coordinating Research Council, Inc.
5755 North Point Parkway, Ste. 265
Alpharetta, GA 30022

Phone: 678-795-0506, Ext. 105
Fax: 678-795-0509
E-mail: ctennant@crcao.org

The deadline for receipt of your proposal is **July 20, 2016**.

Sincerely,
Dr. Christopher J. Tennant
Deputy Director

EXHIBIT A

Identification of Potential Parameters Causing Corrosion of Metallic Components in Diesel Underground Storage Tanks

Statement of Work

Objective: Identify parameters that directly contribute to accelerated corrosion of metal parts and tank equipment in underground storage tank (UST) systems that are in ultra-low sulfur diesel service, including retail sales, fleet suppliers, and fuel storage for emergency power generation. The parameters to be evaluated will be based in large part on information obtained from studies sponsored by the Clean Diesel Fuel Alliance (CDFA)[1] and the U.S. EPA.[2]

Background: Accelerated rates of corrosion have been reported in underground storage tank equipment, dispensers and epoxy coated vehicle metal fuel tanks that used diesel fuel dispensed from commercial retail outlets and private bulk fleet storage facilities since 2007. Recent reports suggest that this corrosion also is affecting metal equipment in aboveground storage tanks (ASTs) storing diesel fuel. The timing coincides with the introduction of 15 ppm sulfur diesel -- S15 commonly known as ULSD -- and with increased blending of renewable fuels required by both the Energy Policy Act of 2005 and the Energy Security and Independence Act of 2007 (biodiesel in diesel and ethanol in gasoline). Other factors such as lower solubility of water in ULSD and the use of new and/or higher concentrations of additives such as lubricity and conductivity improvers were introduced about the same time. Other changes to fuel storage and distribution practices vary across companies and over time. The mid-2000s saw a general increase in the conversion of mid-grade gasoline tanks to diesel service.

CDFA and EPA have conducted field surveys investigating these corrosion phenomena by sampling and analyzing water, fuel, and vapor layers from USTs. The CDFA work also evaluated corrosion products from tanks and metallic equipment. Neither investigation yielded conclusive evidence of the corrosion mechanism(s) involved. However, the combination of study results and industry field experience suggest that Microbiologically Influenced Corrosion (MIC) linked to the production of low molecular weight organic (e.g., acetic and glyceric) acids is a leading mechanism of corrosion in USTs.[3]

CRC believes that controlled laboratory testing is the next logical approach to addressing the unanswered questions generated by recent studies and surveys. A CRC Panel conducted a brainstorming exercise in the fall of 2015 to identify all of the potential causes of corrosion observed in the field and in the two aforementioned studies. The exercise led to the development of a lengthy list that pointed to a wide diversity of potential causes and parameters for investigation. The Panel members concluded that it was not practical to design a laboratory study that included <u>all</u> potential causes, parameters, considerations, combinations, and interactions, but that a manageable program could focus on those potential factors influencing or contributing to accelerated corrosion which showed a step change between the time periods before and after 2006, when ULSD was required. The replacement of LSD (Low Sulfur Diesel Fuel or S500) with ULSD, and common use of biodiesel (up to 5% by volume) were among the major step changes. These

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[1] Battelle Memorial Institute, *Corrosion in Systems Storing and Dispensing Ultra Low Sulfur Diesel (ULSD), Hypotheses Investigation*, Final Report, prepared for the Clean Diesel Fuel Alliance, September 5, 2012

[2] Battelle Memorial Institute, *Investigation of Corrosion Influencing Factors in Underground Storage Tanks with Ultra Low Sulfur Diesel Service*, final report in preparation for the US Environmental Protection Agency, expected release: mid-2016.

[3] See, for example, Little & Lee, 2007. *Microbiologically Influenced Corrosion*, Wiley. pp: 26, 29, 73, 82; 134.

and other factors (shown in the list below) such as additives, ethanol, and some combinations of them were considered to be relevant.

- Diesel Sulfur Content (i.e., Ultra-Low Sulfur Diesel Fuel vs Low Sulfur Diesel Fuel)
- Presence of biodiesel in the fuel
- Presence of a microbial population
- Presence of Glycerin
- Presence of Ethanol
- Presence of Lubricity additive
- Presence of Conductivity additive
- Cold Flow Improver content
- Presence of Corrosion Inhibitor
- Presence of free water
- Presence of Fiber Reinforced Polymer (FRP) material

The evaluation of all combinations of the factors listed above would require well over 1,000 tests and is not practical. A carefully designed factorial experiment (discussed in greater detail below) was drafted for the laboratory testing. If the laboratory tests are conducted properly, sufficient information regarding interactions among these factors should result.

Proposed Test Plan:

Abstract-Multiple (repeat) carbon steel coupons or spindles will be submerged in each sample and will extend from the bottom of the container (exposed to water and fuel) up through the headspace or vapor phase. Half of the containers also will contain an FRP coupon as specified in the fractional factorial design. Wide-mouthed clear glass bottles, fitted with lids, will be sized appropriately to provide sufficient corrosion coupon surface exposure (and to facilitate coupon sample handling) with 500-ml quantities of diesel utilized. Water will be added to some samples to simulate the scenario of a 10,000 gallon UST that contains a representative amount of water bottom while other samples will serve as “controls” with no water introduced. Various components and/or contaminants will be added to the samples and the impacts to corrosion on the metal parts will be measured over time. Corrosion will be measured by visual observation and weight gain/loss throughout the test period with the coupons “cleaned” at the end of the testing. Corrosion products will then be evaluated and the metal parts inspected after cleaning. The test specimens will be maintained under laboratory room temperature and humidity conditions inside of a closed container to minimize exposure to light sources. Observations will be conducted only once per week.

Project Details:

Base Fuel Samples-the test sample matrix will be developed from the following four primary base fuels:

- "Neat" ULSD (15 ppm sulfur max; meets D975; No Biodiesel; No Finished Fuel Additives)
- "Neat" LSDF (300 - 500 ppm sulfur); meets D975; No Biodiesel; No Finished Fuel Additives)
- B5 ULSD prepared by blending the "neat" ULSD with 5 vol% soy-based B100 blend stock compliant with ASTM D6751 specifications[4]
- B5 LSDF prepared by blending the "neat" LSDF with 5 vol% soy-based B100 blend stock compliant with D6751 specifications

These four base stocks will make up the primary fuels to which other components and/or contaminants will be added to fill out the test matrix. All four base stocks shall be evaluated "as is" under the following conditions:

- 500-ml quantities of fuel without added water
- 500-ml quantities of fuel with water (mixed with a mineral salts medium with approximately neutral pH to represent rainwater runoff) added at 1% of the total volume of fuel plus water (subject to verification and possible revision to be determined by the results of a preliminary study described in Appendix A).

It also is recognized that the sample microcosm size and configuration will likely have a significant impact on study results. The basis for maintaining a water to fuel ratio at ~1% is the assumption that polar organic compounds will partition from the fuel-phase into the aqueous-phase and that the consequent concentration of polar organics (flow-improver, corrosion inhibitor, etc.) in the aqueous phase will vary, based on the water to fuel ratio.

Accordingly, the contractor shall conduct a small, preliminary study (described more completely in Appendix A) prior to the main program in order to test the impact of water to fuel ratio on the concentration of lubricity additive, cold flow improver, corrosion inhibitor and conductivity additive in the aqueous-phase. The results will determine whether the microcosm design and configuration described above should be revised to address the impact of water to fuel ratios on corrosion test results.

Other test sample parameter details-Assuming that the preliminary study results validate the microcosm design and sample size conditions described above, the contractor shall prepare duplicates of the 500-ml aliquots of base fuel stocks to evaluate low and high levels of each of the ten factors listed in Table 1 below.

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[4] The contractor must verify that the soy-based biodiesel blend stock procured for the program is fresh since this product typically has a shelf life of about six months.

Table 1

Factors		Levels	
		Low*	**High**
1	Sulfur (ppm)	ULSD (15 ppm S max)	LSDF (300 – 500 ppm S)
2	Biodiesel (vol %)	0	5
3	Microbial Population	0	Microorganism selection and specifications for inoculating test microcosms to be determined per Appendix B and in consultation with CRC Panel)
4	Glycerin in water phase (ppm)	0	5,000
5	Ethanol in water phase (ppm)	0	10,000
6	Mono-acid Lubricity Additive	0	200
7	EVA Cold Flow Improver (CFI) Additive (ppm)	0	200
8	DSA-type Corrosion Inhibitor (ppm)	0	8 – 10
9	Conductivity Additive (ppm)	0	2 - 3
10	Water (vol %)	0	Per Appendix A
11	FRP Material	Absent	Present
*/A value of 0 signifies no intentional addition of this factor			

Note that the lubricity, cold flow improver, corrosion inhibitor and conductivity additives used in this study will have to be supplied through the Fuel Additive Technology Group (FATG) of the American Chemistry Council in order to blind the identity(ies) of the additive manufacturer(s). Likewise, a supply of replicate FRP and steel coupons will be provided in a blinded fashion through the Fiberglass Tank and Pipe Institute and the Steel Tank Institute, respectively. CRC will work with these organizations and with the contractor to facilitate these efforts.

Test Sample Design-As indicated above, a total of eleven factors that are believed to possibly influence corrosion will be evaluated in this laboratory study. These factors shall be evaluated using a 128 test sample screening design matrix as shown in Appendix C or one that is very similar. Note that the fractional factorial test design provided in Appendix C will permit a statistical estimation of the magnitude of the main effects for each of the eleven factors as well as the two factor interactions between the factors without confounding with other main effects and two factor interactions. There are a number of fractionation structures (other than that employed in Appendix C) which could be applied to the test matrix in terms of relating the different factors to each other. The final design will be determined in joint consultation between the contractor and CRC at the project kick-off. However, for budgeting purposes, bidders are requested to provide a quote that is based on a matrix design consisting of 128 samples. In addition, bidders should provide a cost quote on a "per sample" basis as a separate line item.

Test Sample Containers-Wide-mouthed clear glass bottles, fitted with lids, will be sized appropriately (based on the results of the preliminary experiment detailed in Appendix A) to provide sufficient corrosion coupon surface exposure (and to facilitate coupon sample handling) with 500-ml quantities of diesel utilized. The bidder should propose a protocol and/or sample lid design that permits observation and evaluation of the test coupons while minimizing loss of volatile material to the atmosphere during the prescribed inspections.

Test Specimens-Six repeat carbon steel coupons or spindles should be added to each sample container: one set of triplicate coupons for visual inspection and weight-loss determination, and one set of triplicate coupons for biofilm analysis. Microcosms with FRP exposure will contain one FRP coupon. All coupons will be submerged in each sample and will extend from the bottom of the container (exposed to water and fuel) up through the headspace or vapor phase. The apparatus for holding coupons will be the same whether an FRP coupon is included or not. Half of the containers will have an empty slot for the FRP coupon. Figure 1 provides an example that depicts how an array of test coupons can be fit into a 1L sample container.[5] Note that this figure is shown only for illustrative purposes and is not intended to represent a CRC endorsement of any specific experimental set-up.

Other Test Procedure Requirements-***ASTM G1-03(2011) Standard Practice for Preparing, Cleaning, and Evaluating Corrosion Test Specimens*** will be utilized in this project.

Each of the carbon steel test specimens will be visually inspected, photographed, and weighed prior to immersion in the test solutions.

Figure 1

The test solutions containing the coupons will be stored in a dark, unlit chamber under laboratory room temperature and humidity conditions for the duration of the project. (Note that although temperature and humidity are not explicit factors being evaluated in this study, the contractor should nevertheless monitor and record these conditions throughout the test period and take steps to ensure that: (a) they are relatively constant, and (b) do not expose the test microcosms to the risk of excessive evaporative loss over the study timeframe. Exposure of the test materials to light (both artificial and natural) should be minimized during the investigation, limited to once per week during inspection.

A solution(s) of microbes will be cultured and confirmed active via ***D7687 Standard Test Method for Measurement of Cellular Adenosine Triphosphate in Fuel, Fuel/Water Mixtures, and Fuel-Associated Water with Sample Concentration by Filtration*** (preferred), or ***ASTM D7463 Standard Test Method for Adenosine Triphosphate (ATP) Content of Microorganisms in Fuel, Fuel/Water Mixtures, and Fuel Associated Water*** or another industry accepted technique. Portions of the broth will be added to the samples identified for microbial testing.

Inspection of Test Specimens-The carbon steel coupons will be visually inspected weekly throughout the project with photographs taken at each inspection. Differences in corrosion on the coupon regions exposed to the various media (water, fuel, and vapor) will be noted during the process. At the end of the fourth week, half of the coupons (3 of 6) will be removed, dried, and weighed. Photographs will be taken at that time. Once the rate of change in (and/or degree of) corrosion on the coupons appears to have slowed or

[5] Figure source: http://www.practical-sailor.com/issues/37_20/

reached a plateau, the testing will be terminated with the coupons removed, dried, scale/corrosion removed, weighed, and inspected for pitting.[6] The contractor shall then store the samples in a dark unlit enclosure for a period of up to 90 days while CRC evaluates the results of the initial 90 day test period. If corrosion does not appear to be evident at the end of the initial 90 day test period, then CRC may elect to ask the contractor to perform additional measurements at the end of the second 90 day time period. Bidders are therefore requested to provide a quote for a 90 day extension of the study as a separate line item in their cost proposals.

Corrosion materials will be removed from the coupons and will be analyzed to identify corrosion products and to assist in determining corrosion mechanisms. Bidders should specify both the protocol proposed for use in rinsing and removing the corrosion material from the coupons as well as the analytical technique(s) proposed for their subsequent evaluation. One suggested test method is ASTM G31-72, *Standard Practice for Laboratory Immersion Corrosion Testing of Metals.* Final determination of the test protocol(s) and method(s) will be made in consultation with CRC.

At the end of the program, the water and fuel contained in each sample will be analyzed and evaluated. Analyses will include pH, chloride and GC-MS for the water samples and GC-MS for the fuel samples with some also tested by either NACE TM-0172 or ASTM D7577.

All of the analytical tests prescribed above also shall be conducted at the start of the test program in order to provide a baseline for evaluating the results. In addition to the tests specified in the above narrative, Table 2 below lists the parameters that the contractor is expected – at a minimum - to monitor in order to assess the direct and interaction effects of the test factors over the duration of the laboratory study. Note that this list will be finalized by the contractor in consultation with CRC at the kick-off of the study.

[6] Note that the determination of a "plateau" is somewhat subjective. CRC can assist the contract lab by either on-site review and/or through photographs to help establish when the coupons should be removed for analysis.

Table 2

Parameter	Test Method[7]	Frequency
ATP ([cATP]) - fuel phase	ASTM D7687	Test Day = 0,30,60,90, end
ATP ([cATP]) - water phase	ASTM D7687	Test Day = 0,30,60,90, end
pH - water phase	ASTM D1293	Test Day = 0,30,60,90, end
Acidity/alkalinity - water phase	ASTM D1067	Test Day = 0,30,60,90, end
C_2-C_6 organic acids; Other unexpected compounds	GCMS (Marathon In-House Method)	Test Day = 0,30,60,90, end
Total Dissolved Solids	ASTM D5907	Test Day = 0,30,60,90, end
Surfactant production		
Water Separability	ASTM D1094	Test Day = 0,90, end
Genomic profile	SwRI In-House Method	Test Day = 0,90, end
Conductivity – fuel phase	ASTM D262	Test Day = 0,30,60,90,end
Fuel Stability	ASTM D7545	Test Day = 0,30,60.90, end
Test Chamber Temperature		Daily[8]

Phase 0 and Quality Assurance Plan – After conducting the partition coefficient evaluation described in Appendix A, but before initiating the main study described above, the contractor shall prepare a detailed Quality Assurance Program Plan (QAPP), depicting the procedures to be used for sample handling, testing and analysis, etc. in detail. As part of the QAPP, the contractor shall perform each step of the laboratory study described above with one sample/container on an accelerated timetable and under the on-site observation/participation of a technical representative of the CRC Project Panel. The purpose of this phase of the study is to verify capabilities, and (to the extent possible) assess, clarify and resolve any unforeseen issues that may have arisen with respect to the various elements of the test protocol. The specific conditions for this preliminary and accelerated test will be determined in consultation with CRC.

At the conclusion of Phase 0, the contractor shall deliver a report to CRC that details the QAPP and any recommended test protocol changes resulting from this effort. The CRC Panel, at its discretion, will then either: (a) authorize the contractor to proceed with the main test program as originally proposed, or (b) request that the test protocol be revised in response to observations and recommendations arising from the Phase 0 effort and QAPP.

Bidders should cost this program element (Phase 0 and QAPP) as a separate line item in their proposals.

[7] The contractor should use the most current published version of the listed test method.
[8] The contractor should periodically take readings every 4 hours to determine diurnal temperature changes in the space where the microcosms are being stored.

Deliverables:

The following are the proposed deliverables for this laboratory study:

1. Project plan to complete deliverables with proposed due dates (Microsoft Word)
2. Monthly progress reports (Microsoft Word)
3. Quality Assurance Program Plan depicting detailed procedures to be used for sample handling, testing and analysis, etc. (Microsoft Word)
4. Sample Analysis (Microsoft Word)
5. Sample Summary (Microsoft Excel)
6. First Draft Report (Microsoft Word)
7. Second Draft Report (Microsoft Word)
8. Final Report (Microsoft Word)

APPENDIX A

PARTITION COEFFICIENT EVALUATION

Microcosm size and configuration is likely to have a significant impact on the study results. Two conflicting goals have been articulated:

1) Keep microcosm size small (~1L) in order to facilitate logistics; and
2) Maintain water to fuel ratios @ ~1% to simulate ratios observed in fuel storage tanks.

The basis for goal #2 is the assumption that polar organic compounds will partition from the fuel-phase into the aqueous-phase and that the consequent concentration of polar organics (flow-improver, corrosion inhibitor, etc.) in the aqueous phase will vary, based on the water to fuel ratio.

The contractor shall conduct a small, preliminary study to test the impact of water to fuel ratio on the concentration of lubricity additive, cold flow improver, corrosion inhibitor and conductivity additive in the aqueous-phase.[9] In order to simplify the effort, only two of the four control fuel samples are to be tested: "Neat" ULSD and the B5-ULSD. Both will be blended with the full suite of seven additives/contaminants specified in the Scope of Work for the main body of the test program.

At T= 0 hours and at T = 24 hours, the contractor shall test and analyze for Total Dissolved Solids (TDS) and individual component concentrations. TDS and individual component concentrations in the aqueous phase will then be plotted as functions of water to fuel ratio (or %water v/v of total fluid).

<u>Materials:</u>

Fuels – ULSD, LSDF, B5-ULSD & B5-LSDF; 10L ea.
Synthetic bottoms-water – 1,000 mL: Dissolve 0.5g synthetic seawater salts into 1,000mL distilled or deionized water (DIW)
Separatory funnels, 1L – 80 (tests can be run in batches to reduce the total number of separatory funnels needed)
Standard laboratory glassware and supplies
Requisite analytical instrumentation

<u>Protocol (summary):</u>

1. Per the Table below, dispense fully additized ULSD into each of 20 1L separatory funnels. Repeat for ULSD-B5.
2. Per Table below, add synthetic bottoms-water to create 1%, 5%, 10% and 20% (v/v) water in fuel.
3. Stopper funnel and shake vigorously for 30 seconds.
4. Let sit for 24 hours.

[9] Through interaction with the Fuel Additive Technology Group (FATG) of the American Chemistry Council (ACC), the CRC Project Panel will provide guidance to the contractor on generally accepted literature values and other assumptions concerning the water phase partitioning of common additives that may help to facilitate this evaluation.

5. Drain aqueous phase into appropriate sample vial.
6. Test aqueous-phase for TDS, each additive component and total petroleum hydrocarbon (TPH).
7. Plot each #6 component as function of % water in fuel.

Separatory Funnel Contents

% Water	Vol Fuel (mL)	Vol Water (mL)
1	495	5
5	475	25
10	450	50
20	400	100

Interpretation

If the slopes of the respective plots are all 1±0.1, then the water to fuel ratios are unlikely to affect corrosion test results.

If one or more slopes varies from 1 by ≥50% it is very likely that water to fuel ratios will have a significant impact on corrosion test results. Microcosm design (200mL to 300mL fuel over 50mL to 100mL water in 1L bottles) will have to be revised.

If one or more slopes vary from 1 by >10% but <50%, then CRC will want to discuss the return on investment versus incremental costs of creating microcosms that have:

a. Sufficient water for requisite testing (≥2 cm of each corrosion coupon immersed in aqueous-phase, ≥2cm of each corrosion coupon exposed to vapor-phase and≥2cm of each corrosion coupon exposed to fuel-phase); and
b. Water volume ≈ 1% of total volume.

APPENDIX B

MICROCOSM DEVELOPMENT

Purpose:

Microorganisms must become acclimated to the conditions of fuel over water systems before they will thrive. Before testing the impact of microbial communities on test microcosms, a robust challenge community must be developed. This Appendix provides instructions for developing challenge communities to be used to inoculate test microcosms employed in this project.

Materials and Reagents:

- Jars, wide-mouth, glass, screw-cap, 1L; 16
- Fuels – ULSD, LSDF, B5-ULSD & B5-LSDF; 2 L ea.
- Mesh, wire, 250ga., 10cm x 10cm sections; 8
- Synthetic bottoms-water – 800 mL: Dissolve 0.4g synthetic seawater salts into 800mL distilled or deionized water (DIW).
- *Rid-X® Septic System Concentrated Treatment Powder*, Reckitt Benckiser LLC. – 0.25g/primary (1° microcosm) OR:
- High bioburden (≥10,000pg cATP/mL, or comparably bioburden as determined by another test method) microbially contaminated, ULSD (or B5-ULSD) bottoms-water – 5 mL/1°microcosm.

Protocol:

For each fuel grade, prepare 1° microcosms:

Add 100 mL synthetic bottoms-water to each of four 1L glass jars.

Add either 0.25g Rid-X or 5.0mL of microbially contaminated bottoms-water.

Add 500mL fuel:

Add one of the fuel grades to two of the eight jars.

Repeat for each remaining fuel grade.

Screw caps onto jars.

Shake each jar vigorously for 30 sec.

Let stand for 30 min to permit phase separation and Rid-X population rehydration.

Draw 1mL sample for testing by ASTM D7687.

Test for ATP-biomass by ASTM D7687.

Place in 35°C incubator.

After 1-week, draw 1 mL bottoms-water sample from each microcosm.

Test for ATP-biomass by D7684.

If Week-1 cATP concentration is not >10x the initial concentration, incubate for a second week and re-test.

If Week-2 cATP concentration is not >10x the initial concentration, reinoculate with an additional 0.25g Rid-X or 5.0mL of microbially contaminated bottoms-water. Repeat previous two steps for up to 4-weeks.

If cATP concentration is ≥10x the initial concentration, prepare °2 microcosms:

Add 90 mL synthetic bottoms-water to 1L glass jar.

Place wire mesh over mouth.

Use pipette to transfer 10mL of high ATP-biomass bottoms-water from °1 microcosm. Pipette fluid through mesh in order to separate Rid-X inert solids from biomass.

Discard used mesh in accordance with local regulations.

Add 500 mL of the same grade as used for the 1° microcosm from which the inoculum was transferred.

Shake vigorously for 30 sec.

Incubate for 1-week @ 35°C.

Repeat the same biomass checks as used for the 1° microcosms.

When bottoms-water ATP-biomass is ≥ 100,000 pg cATP/mL, test fuel-phase ATP-biomass of 5mL sample drawn 1cm to 2 cm above the fuel-water interface.

Microcosm population is ready for use as test microcosm inoculum when fuel-phase ATP-biomass is ≥10,000 pg cATP/mL. This can take 1-week to 4-weeks.

When setting up test microcosms, inoculate test microcosms with bottoms-water from 2° microcosm of the same fuel grade. Inoculum volume should be ≈0.1x the total bottoms-water volume (see Appendix A).

APPENDIX C

TEST MATRIX DESIGN

One example of a 128 test sample screening design matrix is shown in the first tab of the worksheet file *available for download along with this request for proposals.* The second tab of the worksheet presents the information from the first tab in table format, for ease of viewing, as shown in the following two pages of this Appendix.

As stated in the main body of this Statement of Work, there are a number of fractionation structures (other than that employed in this Appendix) which could be applied to the test matrix in terms of relating the different factors to each other. The final design will be determined in consultation with CRC at the project kick-off. However, for budgeting purposes, the contractor should plan on a matrix design consisting of 128 samples.

APPENDIX C (Continued)

Water	Sulfur	Biodiesel	Cold Flow Improver	Lubricity	Ethanol	Conductivity Additive	Glycerin	Corrosion Inhibitor	Microbes	FRP
yes	ULSD	none	no	no	no	no	no	no	yes	no
							yes	yes	no	no
					yes	yes	no	no	yes	yes
							yes	yes	no	yes
				yes	no	yes	no	yes	no	no
							yes	no	yes	no
					yes	no	no	yes	no	yes
							yes	no	yes	yes
			yes	no	no	no	no	yes	yes	yes
							yes	no	no	yes
					yes	yes	no	yes	yes	no
							yes	no	no	no
				yes	no	yes	no	no	no	yes
							yes	yes	yes	yes
					yes	no	no	no	no	no
							yes	yes	yes	no
		5%	no	no	no	yes	no	yes	yes	yes
							yes	no	no	yes
					yes	no	no	yes	yes	no
							yes	no	no	no
				yes	no	no	no	no	no	yes
							yes	yes	yes	yes
					yes	yes	no	no	no	no
							yes	yes	yes	no
			yes	no	no	yes	no	no	yes	no
							yes	yes	no	no
					yes	no	no	no	yes	yes
							yes	yes	no	yes
				yes	no	no	no	yes	no	no
							yes	no	yes	no
					yes	yes	no	yes	no	yes
							yes	no	yes	yes
	LSDF	none	no	no	no	yes	no	no	no	no
							yes	yes	yes	no
					yes	no	no	no	no	yes
							yes	yes	yes	yes
				yes	no	no	no	yes	yes	no
							yes	no	no	no
					yes	yes	no	yes	yes	yes
							yes	no	no	yes
			yes	no	no	yes	no	yes	no	yes
							yes	no	yes	yes
					yes	no	no	yes	no	no
							yes	no	yes	no
				yes	no	no	no	no	yes	yes
							yes	yes	no	yes
					yes	yes	no	no	yes	no
							yes	yes	no	no
		5%	no	no	no	no	no	yes	no	yes
							yes	no	yes	yes
					yes	yes	no	yes	no	no
							yes	no	yes	no
				yes	no	yes	no	no	yes	yes
							yes	yes	no	yes
					yes	no	no	no	yes	no
							yes	yes	no	no
			yes	no	no	no	no	no	no	no
							yes	yes	yes	no
					yes	yes	no	no	no	yes
							yes	yes	yes	yes
				yes	no	yes	no	yes	yes	no
							yes	no	no	no
					yes	no	no	yes	yes	yes
							yes	no	no	yes

APPENDIX C (Continued)

Water	Sulfur	Biodiesel	Cold Flow Improver	Lubricity	Ethanol	Conductivity Additive	Glycerin	Corrosion Inhibitor	Microbes	FRP
no	ULSD	none	no	no	no	yes	no	yes	no	yes
							yes	no	yes	yes
					yes	no	no	yes	no	no
							yes	no	yes	no
				yes	no	no	no	no	yes	yes
							yes	yes	no	yes
					yes	yes	no	no	yes	no
							yes	yes	no	no
			yes	no	no	yes	no	no	no	no
							yes	yes	yes	no
					yes	no	no	no	no	yes
							yes	yes	yes	yes
				yes	no	no	no	yes	yes	no
							yes	no	no	no
					yes	yes	no	yes	yes	yes
							yes	no	no	yes
		5%	no	no	no	no	no	no	no	no
							yes	yes	yes	no
					yes	yes	no	no	no	yes
							yes	yes	yes	yes
				yes	no	yes	no	yes	yes	no
							yes	no	no	no
					yes	no	no	yes	yes	yes
							yes	no	no	yes
			yes	no	no	no	no	yes	no	yes
							yes	no	yes	yes
					yes	yes	no	yes	no	no
							yes	no	yes	no
				yes	no	yes	no	no	yes	yes
							yes	yes	no	yes
					yes	no	no	no	yes	no
							yes	yes	no	no
	LSDF	none	no	no	no	no	no	yes	yes	yes
							yes	no	no	yes
					yes	yes	no	yes	yes	no
							yes	no	no	no
				yes	no	yes	no	no	no	yes
							yes	yes	yes	yes
					yes	no	no	no	no	no
							yes	yes	yes	no
			yes	no	no	no	no	no	yes	no
							yes	yes	no	no
					yes	yes	no	no	yes	yes
							yes	yes	no	yes
				yes	no	yes	no	yes	no	no
							yes	no	yes	no
					yes	no	no	yes	no	yes
							yes	no	yes	yes
		5%	no	no	no	yes	no	no	yes	no
							yes	yes	no	no
					yes	no	no	no	yes	yes
							yes	yes	no	yes
				yes	no	no	no	yes	no	no
							yes	no	yes	no
					yes	yes	no	yes	no	yes
							yes	no	yes	yes
			yes	no	no	yes	no	yes	yes	yes
							yes	no	no	yes
					yes	no	no	yes	yes	no
							yes	no	no	no
				yes	no	no	no	no	no	yes
							yes	yes	yes	yes
					yes	yes	no	no	no	no
							yes	yes	yes	no

EXHIBIT B

REPORTS

MONTHLY TECHNICAL PROGRESS REPORTS

The contractor shall submit a monthly technical progress report covering work accomplished during each calendar month of the contract performance. An electronic Microsoft® Word compatible file (<1 MB) of the monthly technical progress report shall be distributed by the contractor within ten (10) calendar days after the end of each reporting period. The report shall contain a description of overall progress, plus a separate description for each task or other logical segment of work on which effort was expended during the reporting period.

FINAL REPORT

The contractor shall submit to or distribute for CRC an electronic (Microsoft Word) copy transmittable via email) of a rough draft of a final report within thirty (30) days after completion of the technical effort specified in the contract. The report shall document, in detail, the test program and all of the work performed under the contract. The report shall include tables, graphs, diagrams, curves, sketches, photographs and drawings in sufficient detail to comprehensively explain the test program and results achieved under the contract. The report shall be complete in itself and contain no reference, directly or indirectly, to the monthly report(s).

Within thirty (30) days after receipt of the approved draft copy of the final report, the contractor shall make the requested changes and deliver to CRC thirty (30) hardcopies including a reproducible master copy of the final report. The final report shall also be submitted as electronic copies in a pdf and Microsoft Word file format. The final report may be prepared using the contractor’s standard format, acknowledging author and sponsors. An outside CRC cover page will be provided by CRC. The electronic copy will be made available for posting on the CRC website.

EXHIBIT C

INTELLECTUAL PROPERTY RIGHTS

Title to all inventions, improvements, and data, hereinafter, collectively referred to as ("Inventions"), whether or not patentable, resulting from the performance of work under this Agreement shall be assigned to CRC. Contractor X shall promptly disclose to CRC any Invention which is made or conceived by Contractor X, its employees, agents, or representatives, either alone or jointly with others, during the term of this agreement, which result from the performance of work under this agreement, or are a result of confidential information provided to Contractor X by CRC or its Participants. Contractor X agrees to assign to CRC the entire right, title, and interest in and to any and all such Inventions, and to execute and cause its employees or representatives to execute such documents as may be required to file applications and to obtain patents covering such Inventions in CRC's name or in the name of CRC's Participants or nominees. At CRC's expense, Contractor X shall provide reasonable assistance to CRC or its designee in obtaining patents on such Inventions.

To the extent that a CRC member makes available any of its intellectual property (including but not limited to patents, patent applications, copyrighted material, trade secrets, or trademarks) to Contractor X, Contractor X shall have only a limited license to such intellectual property for the sole purpose of performing work pursuant to this Agreement and shall have no other right or license, express or implied, or by estoppel. To the extent a CRC member contributes materials, tangible items, or information for use in the project, Contractor X acknowledges that it obtains only the right to use the materials, items, or information supplied for the purposes of performing the work provided for in this Agreement, and obtains no rights to copy, distribute, disclose, make, use, sell or offer to sell such materials or items outside of the performance of this Agreement.

EXHIBIT D

LIABILITY

It is agreed and understood that _____________ is acting as an independent contractor in the performance of any and all work hereunder and, as such, has control over the performance of such work. _______________ agrees to indemnify and defend CRC from and against any and all liabilities, claims, and expenses incident thereto (including, for example, reasonable attorneys' fees) which CRC may hereafter incur, become responsible for or pay out as a result of death or bodily injury to any person or destruction or damage to any property, caused, in whole or in part, by __________'s performance of, or failure to perform, the work hereunder or any other act of omission in connection therewith.

EXHIBIT E

PROPOSAL EVALUATION CRITERIA

1) Merits of proposed technical approach.
2) Previous performance on related research studies.
3) Personnel available for proposed study – related experience.
4) Timeliness of study completion.
5) Cost.