

June 5, 2006

Mr. Sail Ricks  
Protec Laboratory  
4300 FM2225  
Quitman, TX 75783

**DRAFT**

Re: Material Identification of BioPerformance Fuel Additive  
Chemir Analytical Job # 59833

Dear Mr. Ricks,

Per your request, we have partially completed your project. This is an interim report on the results to this date. We used Ultraviolet-visible Spectroscopy (UV-Vis), Pyrolysis Gas Chromatography-Mass Spectrometry (GC/MS), Nuclear Magnetic Resonance Spectroscopy (NMR), and Fourier Transform Infrared Spectroscopy (FT-IR)

#### SAMPLE LOG-IN

The sample was logged as follows:

SAMPLE DESCRIPTION	CHEMIR ANALYTICAL SAMPLE NUMBER
BioPerformance Fuel Biodegradable Enzyme Catalyst	561681

#### PROJECT OBJECTIVE

This project had three objectives:

1. Confirm the presence of protein in the sample.
2. Confirm the incorporation of naphthenate functional groups into the protein structure.
3. Identify the protein

#### ANALYSIS CONCLUSIONS

1. Protein was detected in the sample.
2. Naphthalene, a naphthenate or a related material was detected in the sample.
3. The protein will be further analyzed to try to identify the protein or enzyme.

#### ANALYSIS RESULTS AND DISCUSSION

##### Sample Preparation

Duplicate 10-gram aliquots of the sample were washed with toluene until all of the blue color was removed (approximately 10 times). The toluene has the effect of removing all hydrophobic materials (such as common solvents or naphthalene) from the sample. At this time, the "mothball-like" odor, presumably from naphthalene, was gone as well. The remaining material was yellow in color. The average percentage of mass remaining in the toluene-insoluble portion was approximately 0.2%.

Protein detection

Protein was determined using the Bradford Assay Kit (Pierce, #23236). This test is a general test for the presence of protein. No structural information is gained from the test. To a portion of washed material was added 1 mL of Bradford reagent. The mixture was allowed to incubate at room temperature for ten minutes. After the incubation, the solution was blue, indicating the presence of protein.

Determination of Naphthenate by NMR

Nuclear Magnetic Resonance Spectrometry (NMR) is an extremely useful method for material characterization. NMR is a physical phenomenon based upon the magnetic property of an atom's nucleus. NMR studies a magnetic nucleus (most commonly that of a hydrogen atom), by aligning it with a very powerful external magnetic field and perturbing this alignment using an electromagnetic pulse. The response to the perturbation is recorded, with each individual nucleus giving a response specific to its chemical, electronic, and spatial environment.

A portion of the washed sample was suspended in deuterated water, and analyzed by NMR. Naphthalene, naphthenates or any other naphthalene-related moiety would be expected to have a spectrum similar to naphthalene (CHART 1). More specifically, all of these compounds show a resonance signal between 7 and 8 ppm. According to the NMR data (CHART 2), the washed sample shows a very small signal at about 7.9 ppm. The very large signal at about 4.8 ppm is from deuterated water.

Identification of the Unknown Surfactant by GC/MS

In Gas Chromatography/Mass Spectrometry (GC/MS) GC resolves the sample components based on volatility, and MS detects and identifies the components. Sample components that interact less with the stationary phase spend less time in the chromatographic column. In MS, the resolved sample components are ionized and separated in a mass analyzer. The fragmentation pattern of a sample component and its computer library match enables sample identification.

Pyrolysis GC/MS was used for this sample. The sample is heated at 600°C in a quartz boat. The resulting materials are injected into a GC/MS instrument and analyzed. It is believed that in this case, any resulting naphthalene-like moieties will be removed from the protein, aiding in their detection. Attached in CHART 3, are the gas chromatograms and corresponding mass spectra from this analysis. The library match for the signal at 23.197 minutes indicates naphthalene, or azulene (a structurally related molecule). See the table below for a summary.

<b>Retention Time (min)</b>	<b>Library-matched compound</b>
6.7	3,3'-oxybis-1-propene
7.0	Isobutane
10.3	1-octene
10.7	3,3-dimethylhexane
13.9	Allyl methallyl ether
14.2	2,4-dimethylheptane
16.9	1,1-dimethyl-N,N'-diphenylsilanediamine
17.1	3,5-dimethyl-1-hexene
17.4	2-methyloctane
20.0	1-dodecane
20.3	Decane

21.6	1-isocyano-4-methylbenzene
22.7	1-dodecene
22.9	3,5-dimethyloctane
23.2	Azulene (or naphthalene)
25.2	1-dodecene
25.4	2,4-dimethylhexane
27.5	1-dodecene
27.7	2,7-dimethyloctane
29.7	1-hexadecene
29.8	2-methyl-decane
31.7	1-nonadecene
33.7	8-heptadecene
37.4	2-nonanone
38.3	n-hexadecanoic acid
40.7	2-nonadecanone
40.8	Undecanenitrile

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Identification of the protein

Identification of the protein material that was isolated from the sample is currently in progress. It will be performed on a GC/MS designed for this purpose or by gel electrophoresis.

INSTRUMENTATION

SCIENTIFIC INSTRUMENT	MANUFACTURER/MODEL	PURPOSE
Gas Chromatograph/Mass Spectrometer (GC/MS)	HP/5890 Series II GC/HP 5971 Mass Selective Detector	Identification of Sample Components
Pyroprobe	CDS Analytical/ Pyroprobe 2000	Polymer breakdown to monomeric units
Nuclear Magnetic Resonance Spectrometer (NMR)	Bruker/AC270	Identify Materials - Structural and Conformational Determination

CHARTS

Enclosed please find the following CHARTS generated during the analysis.

ENCLOSURE	DESCRIPTION
CHART 1	Standard NMR spectrum of naphthalene.
CHART 2	NMR spectrum of the sample, after washing with toluene.
CHART 3	Pyrolysis GC/MS chromatograms, spectra and associated library matches.