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**STUDY TITLE:** Studying and Verifying the Use of Chemical Biomarkers for Identifying and Quantitating Oil Residues in the Environment

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KEY WORDS: Biomarkers; oil weathering; self-normalizing fingerprint indexes; oil degradation; tarballs; bioaccumulation; GC/MS

**BACKGROUND:** Petroleum released into the marine environment undergoes varying degrees of environmental weathering that affect its composition. As weathering proceeds, certain groups of oil constituents are lost in a predictable sequence. However, biomarkers are petroleum components that remain detectable and relatively unchanged in oil residues even after natural environmental weathering. They are also typically resistant to biodegradation and are therefore, useful as chemical markers. Biomarker fingerprinting by gas chromatography/mass spectrometry (GC/MS) and the calculation of eight different source-normalizing fingerprint indexes (SFI) may be necessary for environmental samples in which identification of spilled oil is difficult due to weathering of target compounds.

**OBJECTIVE(S):** The objective of this research is to refine the use of biomarkers as tools for oil spill identification and assessment, and to verify the analytical approach utilized by the Louisiana State University, Institute for Environmental Studies, Response

and Chemical Assessment Team (LSU IES/RCAT) with both laboratory and field evaluation studies.

**DESCRIPTION:** The research was divided into 4 sub-divisions: preliminary stages; a water degradation experiment; a bioaccumulation study; and classification and identification of tarballs collected from three areas of southwest Louisiana. The preliminary stages included a literature search and GC/MS method development. A comprehensive literature review of environmental and petroleum industry/geological journals was conducted; and a total of 180 pertinent articles out of over 300 titles were acquired and reviewed. The instrument methodology involved the development of an improved GC/MS analytical method that included newly identified biomarker families in addition to those compounds currently used for oil spill response.

The water degradation experiment evaluated the changes in the composition of Eugene Island crude oil, after exposure to nine natural waters collected across the country, with or without additional nutrients. Biolog™ analyses were performed in conjunction with the water degradation experiment to determine whether significantly different bacterial communities were present in the various waters. The final stage of the water degradation experiment examined the stability of the SFI for the pre- and post-weathered reference oil after 28-days of laboratory-simulated weathering.

The bioaccumulation experiment examined biomarker up-take in bivalves exposed to oil of known biomarker composition from samples collected recently from Prince William Sound, Alaska. Theoretically, biomarkers are not significantly altered by microbial degradation; hence, there is a possibility that these lipid soluble materials will accumulate in marine organisms and could be used as an indicator of possible exposure during oil spills. The results of this task will establish a time frame in which biomarker compounds may be detected in bivalves since the samples were collected ten years after the T/V *Exxon Valdez* incident.

Classification and identification of tarballs collected from three areas along the southwest coast of Louisiana was carried out at several different levels. A total of 88 tarballs were collected and described based on seven different morphological descriptions and eight weathering classifications. Visual comparisons were also carried out for each biomarker fingerprint. As a result of the visual comparison, three of the SFI were chosen for scatterplot analysis due to the fact that the indexes selected were relatively unaffected by weathering and laboratory variations.

**SIGNIFICANT CONCLUSIONS:** The biomarker compounds that provide the best tool for oil spill identification, both visually and statistically, are C-3 dibenzothiophene, C-3 phenanthrene, C-3 DBT peak area/C-3 Phen peak area, and Norhopane/Hopane. These compounds, as demonstrated by the water degradation experiment, remain stable even after weathering and will distinguish one oil source from another when other biomarkers are lost (i.e. nC-17/pristane and nC-18/phytane). GC/MS analysis and visual examination should still include all eight compounds because, when combined, they provide information about the extent of weathering and biodegradation from their

respective fingerprints. Visual examinations of the fingerprints can easily distinguish one type of oil or weathering stage from another based on the pattern of the fingerprint alone.

**STUDY RESULTS:** One part of the water degradation experiment was to assess the effects of microorganism populations present in different water sources in two ways: Biolog™ microplate analyses and percent reduction of total target aromatic hydrocarbons (TTAH). The organisms in the different water samples had widely varying metabolic capabilities; however, the results from the Biolog™ analyses and the %TTAH calculated from the GC/MS data did not correlate. This is due to several factors: differing carbon sources; differing nutrient substrates; and differing dominant microbial communities.

The uptake of biomarkers, and in general polycyclic aromatic hydrocarbons (PAHs), was examined in bivalves exposed to oil from the T/V *Exxon Valdez*. The analysis provided important information about the uptake and utilization of petroleum hydrocarbons by filter feeding organisms after 10 years of exposure. Overall, the biomarker and PAH concentration for both mussel and clam tissues were low. Unfortunately, a baseline concentration of biomarker compounds has not been established for biological samples from the beginning of the T/V *Exxon Valdez* incident.

The classification and identification of tarball samples collected in 1998 suggests that the majority of the tarballs collected were from mid- to heavy-range crude oil sources. Of the 70 tarballs extracted and analyzed, four matches were found through visual comparison and double SFI scatterplots. Since the distribution of sources was extensive, it is believed that the tarballs collected represent oil contamination from small petroleum and/or bilge waste discharge.

**STUDY PRODUCT(S):** Ashton, B.M.; East, R.S.; Walsh, M.M.; Miles, M.S., Overton, E.B. 2000. Studying and Verifying the Use of Chemical Biomarkers for Identifying and Quantitating Oil Residues in the Environment. A final report for the U.S. Department of the Interior, Minerals Management Service Gulf of Mexico Region, New Orleans, LA. Contract No. 14-35-0001-30660-19933. OCS Study MMS 2000-086. 77 pp.

Ashton, B.M.; East, R.S.; Walsh, M.M.; Miles, M.S. 2000. Studying and Verifying the Use of Chemical Biomarkers for Identifying and Quantitating Oil Residues in the Environment. Poster presentation at the Clean Gulf Conference, November 13-15, New Orleans, LA.

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