OCS Study
MMS 97-0023

Identification of Bioactive Compounds from Produced Water Discharge / Characterization of Organic Constituent Patterns at a Produced Water Discharge Site

Final Technical Summary

Final Study Report

U.S. Department of the Interior
Minerals Management Service
Pacific OCS Region

Identification of Bioactive Compounds from Produced Water Discharge / Characterization of Organic Constituent Patterns at a Produced Water Discharge Site

Final Technical Summary

Final Technical Report

Authors

Richard M. Higashi
A. Daniel Jones
Principal Investigators

Prepared under MMS Cooperative
Agreement No. 14-35-0001-30471
by
Southern California Educational Initiative
Marine Science Institute
University of California
Santa Barbara, CA 93106

U.S. Department of the Interior
Minerals Management Service
Pacific OCS Region

Camarillo
January 1997

Disclaimer

This report has been reviewed by the Pacific Outer Continental Shelf Region, Minerals Management Service, U.S. Department of the Interior and approved for publication. The opinions, findings, conclusions, or recommendations in this report are those of the author, and do not necessarily reflect the views and policies of the Minerals Management Service. Mention of trade names or commercial products does not constitute an endorsement or recommendation for use. This report has not been edited for conformity with Minerals Management Service editorial standards.

TABLE OF CONTENTS

FINAL TECHNICAL SUMMARY

STUDY TITLE: Identification of Bioactive Compounds from Produced Water Discharge / Characterization of Organic Constituent Patterns at a Produced Water Discharge Site

REPORT TITLE: Identification of Bioactive Compounds from Produced Water Discharge / Characterization of Organic Constituent Patterns at a Produced Water Discharge Site

CONTRACT NUMBER: MMS**:** 14-35-0001-30471

SPONSORING OCS REGION: Pacific

APPLICABLE PLANNING AREA(S): Southern and Central California

FISCAL YEAR(S) OF PROJECT FUNDING: 1992-93, 1993-94; 1994-95 (no-cost extension)

COMPLETION DATE OF REPORT: December, 1996

COSTS: FY 92-92: $66,008; **FY 93-94:** $64,846

PROJECT MANAGERS: [1*]R.M. Higashi and [2]A.D. Jones

AFFILIATION: [1]Bodega Marine Laboratory, [2]Facility for Advanced Instrumentation, University of California, Davis. *****Current affiliation: Crocker Nuclear Laboratory, Univ. of California, Davis

ADDRESS: [1]P.O. Box 247, Bodega Bay, CA 94923 and *****[2]University of California at Davis, Davis, CA 95616

PRINCIPAL INVESTIGATORS: R.M. Higashi and A.D. Jones

KEY WORDS: Santa Barbara Channel; produced water; hydrocarbons; sediment; sulfides; organosulfur; polysulfide; pyrolysis; humics; barium; transition metals; GCMS

BACKGROUND:

As produced water (PW) is a very complex mixture of diverse chemical categories consisting of hundreds of organic compounds [NRC, 1985; Boesch and Rabalais, 1987], the principal technical utility of this project is to significantly and systematically reduce the list of PW constituents that must be studied with regards to their fate and transport. There are many scientific works regarding the occurrence and fate of the organic components, but these are mostly limited to the petroleum hydrocarbons, which comprise only a portion of the total organic load of PW [NRC, 1985; Boesch and Rabalais, 1987]. The reasons for this self-imposed restriction are many, but among the likely reasons are the ease of analysis for hydrocarbons and the considerable body of literature regarding their biological effects. However, even with this restriction to just hydrocarbons, PW can harbor hundreds of compounds.

A less arbitrary means to reduce the complexity is to identify the compounds associated with biological effects. For example, we have successfully utilized a bioactivity-based fractionation of PW from a plant at Carpinteria, CA to characterize the constituent responsible for impairment of mussel embryo development [Higashi *et al*., 1993]; this may also relate to impairment of adult mussel reproductive development [Fan *et al*., 1993]. The identity of this constituent, Ba, is being verified in a series of laboratory experiments that investigate PW fractions and Ba chemistry as it relates to the symptoms and mechanisms of Ba toxicity in these biological systems; the latter is being conducted in conjunction with the project of Cherr and Fan [Schmitt, 1991]. Unfortunately, the discharge of PW from that source has ceased, making field verification impossible.

In order to study effects of PW in the field, Osenberg, *et al*. [Schmitt, 1991] have been studying the future PW discharge site near Point Conception, CA ("Gaviota") for the past three years, utilizing the Before-After Control-Impact Pair design (BACIP) for ecological studies. Although this PW plant at Gaviota is currently in operation by reinjection of PW into formations, information from the plant operator suggests that discharge of the PW into marine waters will not occur immediately. Thus, the present situation at Gaviota represents an outstanding opportunity to study organic constituents in the "Before" period of the BACIP study.

OBJECTIVES:

The goals of this project are to: (a) identify constituents of produced water (PW) responsible for various bioeffects of interest to SCEI researchers; and (b) estimate their relevance to an environmental situation by surveying the distribution of the bioactive constituents at the Carpinteria site. The scientific utility of goal (a) is to significantly reduce the list of chemicals to be studied, providing foci for other studies and greatly reducing analytical load. Goal (b) will provide basic data of potential interpretability to any case study research currently being conducted at the Carpinteria site.

These goals and research approaches (described in detail in the next section) address two targeted research areas for Environmental Studies in the Southern California Educational Initiative (SCEI). The goals, reformulated in terms of the Studies Framework, are as follows:

Fractionation of PW (addresses Studies Framework Issue #1: Fate and effects of produced water discharges in the nearshore environment)
Produced water will be analyzed in accordance with schemes we have previously applied in order to help establish causal linkages between specific PW constituents (or classes thereof) and bioeffects being studied by SCEI researchers. This will be accomplished by providing such fractions for bioeffects testing by collaborating SCEI researchers (e.g., Cherr and Fan, and Reed *et al*.).

Toxicant Identification (addresses Studies Framework Issue #2: Long-term effects of drilling discharges)
Our toxicant identification efforts will continue, currently directed by results of toxicity testing, which will expand as other bioeffects of PW are established in the laboratory and field. We will continue to archive PW samples through lyophilization (freeze-drying), and process samples according to our present scheme (Figure IA, and described below). The results of bioeffects testing will direct the choice of the chemical methods to be employed. As such, extensive analytical capability is required on a standby basis, as these substances may be expected to have very diverse

chemical structures and properties. A broad range of high-resolution analytical separation (capillary GCMS, HPLC, capillary electrophoresis) and detection systems (NMR, MS, ICP-AES, ICP-MS) will be brought to bear for the identification and early analysis of organic and inorganic substances. Once identified, the bioactive substances will be isolated or synthesized and integrated with toxicity testing (Cherr and Fan) as well as possibly other bioeffects testing (Reed, *et al*.- kelp zoospore settling tests).

DESCRIPTION:

The basic approach in this field study is chemical analysis of PW, site sediment, water column, and outplanted mussels, with the data to undergo BACIP analysis by Osenberg, *et al*. Due to logistical limitations, the study will focus on organosulfur and hydrophobic compounds, and rely heavily on high-resolution gas chromatographic (GC) and GC-mass spectrometric (GCMS) analyses. All sampling is conducted in coordination with the project of Osenberg, *et al*., originating from the Gaviota research site, as described elsewhere [Schmitt, 1991]. Samples are collected from field sites (near impact, far impact, and reference) semiannually, each site consisting of water column and outplanted mussels, each at two depths, and sediment sample. In addition, Gaviota PW samples are obtained twice per year in order to track trends in PW composition. Organic solvent extracts of these samples are analyzed by various GC methods. Most of the analytical data will consist of relative quantification of unknown compounds. Thus, an important analytical tool is GCMS, which can catalog structural information on these unknowns for future use and provide running checks on analytical efficiency via analysis of the deuterated standards. For example, mass spectra is used to ensure that all analytical data transferred to Osenberg, *et al*. consist of the same compounds (based on their GC retention time and mass spectra) throughout the study. Chemical identification of a given peak will commence upon the identification of that peak as related to biological effects in the field, as determined by the BACIP analyses. Another important feature of the project is the preservation of samples by unique means such as freeze-drying. This is vital if subsequent research indicates that certain compounds of biological relevance are not extracted efficiently by the original procedures.

SYNOPSIS OF MAJOR FINDINGS:

Major organic compounds found in PW were the expected hydrocarbons and alkylphenols, but there were also high levels of organosulfur compounds. In particular, thiocarboxylic acids and novel thiopyranones were found in abundance. Organopolysulfides were also identified, as were inorganic forms of sulfur, such as sulfides, thiosulfates, and polysulfides. Sulfide distribution among dissolved, particulate and colloidal phases were also determined. These findings are important to bioeffects due to the strong interaction of both organic and inorganic sulfur compounds with metal ions, as well as the possible bioactivity of the organosulfur compounds themselves.

Also found in Carpinteria PW was polysaccharidic material that is not cellulosic; this material was found in sediments prevailing downcurrent from the outfall, decreasing with distance. These substances may be useful PW markers and may affect microbial communities in the sediment, thereby altering microbial degradation of PW constituents.

Shell Ba content (normalized to Ca) of outplanted mussels showed a decreasing trend with distance from the Carpinteria outfall, but this was not true of all outplant experiments - some outplant

experiments showed no clear trend. This data, together with that from the project of Cherr & Fan, is being integrated into and analyzed by the project of Osenberg *et al*.

This research was completed under MMS Contract No. 14-35-0001-30761, Project #3, Title: Characterization of Organic Constituent Patterns at a Produced Water Site / Barium Relations to Bioeffects of Produced Water, Principal Investigators, Richard Higashi, A. Daniel Jones and Teresa Fan, FY 94-95, 95-96 (no cost).

STUDY PRODUCTS:

PUBLICATIONS

1993 Fan, T.W-M., T.D. Colmer, A.N. Lane, and R.M. Higashi. Determination of metabolites by ^{1}H NMR and GC: analysis for organic osmolytes in crude tissue extracts. *Analytical Biochemistry* **214**: 260-271.

1998 Witter, A.E., and A.D. Jones. Comparison of methods for inorganic sulfur speciation in a petroleum production effluent. *Environmental Toxicology and Chemistry* **17(11)**:2176-2184.

1998 Witter, A.E., S.A. Mabury, and A.D. Jones. Copper(II) complexation in northern California rice field waters: An investigation using differential pulse anodic and cathodic stripping voltammetry. *Science of the Total Environment* **212(1)**:21-37.

RESEARCH PRESENTATIONS

1995 Witter, A. E. and A. D. Jones, "A Comparison of Methods for Speciation of Sulfur in a Petroleum Production Effluent" *Abstracts of Papers of the American Chemical Society*, 211:, n.1-2, (1996): Abstract ENVR 91, 211th American Chemical Society National Meeting, New Orleans, Louisiana, USA, March 24-28, 1996.

1994 Higashi, R.M., T. W-M. Fan, and A.N. Lane. "Pyrolysis GC-MS and NMR studies of humics in contaminated sediments." 15th Annual Meeting of Society of Environmental Toxicology and Chemistry, Denver, CO, USA.

1994 Jones, A.D., A. E. Witter, and R. M. Higashi, "Petroleum-related Contaminants Near a Produced Water Discharge Site in the Santa Barbara Channel," presented as part of the Symposium on Marine Environmental Chemistry, *15th Annual Meeting of the Society for Environmental Toxicology and Chemistry*, Denver, CO, October 30-November 4, 1994 (Abstract 394).

1994 Witter, A. E. and A. D. Jones, "Measurement of Complexation Properties of Metal Ions by Polyelectrolytes using Ultrafiltration/GFAAS Methods," *15th Annual Meeting of the Society for Environmental Toxicology and Chemistry*, Denver, CO, October 30-November 4, 1994 (Abstract MB14).

1993 Cherr, G.N., G.D. Garman, R.M. Higashi, and M.C. Pillai. Effects of produced water on reproduction and development in marine organisms. 14th Annual Meeting of Society of Environmental Toxicology and Chemistry, Houston, TX, USA.

1993 Witter, A. E., A. D. Jones, and R. M. Higashi, "The Identification of Organosulfur Compounds in Produced Water by Mass Spectrometry," *Proceedings of the 41st ASMS Conference on Mass Spectrometry and Allied Topics*, San Francisco, CA, pp. 403a-403b.

1993 Witter, A. E., A. D. Jones, and R. M. Higashi, "Analysis of Organosulfur Compounds in Produced Waters using Mass Spectrometry," *14th Annual Meeting of the Society for Environmental Toxicology and Chemistry*, Houston, TX November 14-18, Abstract #444 (p. 126).

1993 Witter, A. E., A. D. Jones, and S. Mabury, "A Comparison of APCI and ESI Methods for the Quantitation of Small Molecules of Environmental Interest using LC/MS," *Proceedings of the 10th Montreux LC/MS Symposium at Cornell*, July 19-23, 1993, Ithaca, NY.

Characterization of Organic Constituent Patterns at a Produced Water Site / Barium Relations to Bioeffects of Produced Water

FINAL STUDY REPORT

I. GENERAL INTRODUCTION

IA. BACKGROUND AND PREVIOUS WORK

Produced water (PW) is a very complex mixture consisting of non-polar and polar organic substances, inorganic cations and anions, and combinations of these diverse chemical categories [NRC, 1985; Boesch and Rabalais, 1987]. PW is known to be an important source of hydrocarbon and metal pollution [Armstrong *et al*., 1979; Boehm, 1987; Neff, 1987], but little is currently known about the composition of other major categories of PW constituents (e.g., polar organic compounds and inorganic anions), and even less is known about the fate and transport of these substances in marine environments. For example, previous investigations of Carpinteria PW in one of our laboratories (A.D. Jones') have demonstrated the presence of alkyl phenols and polar toxicants such as phenol, as well as a complex mixture of over a thousand hydrocarbon substances. Clearly, the complexity of PW presents a formidable obstacle to understanding the distribution and effects of PW near discharge sites.

An attractive strategy for dealing with this obstacle is to reduce the complexity of the problem by first defining the properties of interest in PW. In the present case, the properties of chief interest are the various "bioeffects" of PW. This is the approach used for our past research into PW, and we are now at the later stages of generating a short list of substances that have toxicity to early life stages of molluscs [Higashi *et al*., 1993].

Briefly, the approach we have used is as follows, and is depicted in **Figure 1**. PW samples were collected into Teflon-capped glass bottles from a tap in the discharge pipe and shipped refrigerated to our laboratory with no headspace in the samples. We have separated PW samples (I) into volatile and nonvolatile (II) fractions by lyophilization (freeze-drying), then we subjected II to sequential organic solvent extraction that generated nonpolar (III, containing hydrocarbons), intermediate polar (IV, containing many other organic compounds such as phenolics), and water-only soluble (V, containing metal ions, polar organic, and anionic constituents) fractions. Further fractionation of III and IV were achieved using HPLC, while V was further extracted by an ion-exchange resin (Chelex resin) designed for trapping divalent ions (fraction VI), finally leaving a polar fraction (VII) that contains polar organic, inorganic anion, and other (non-polyvalent) cationic substances.

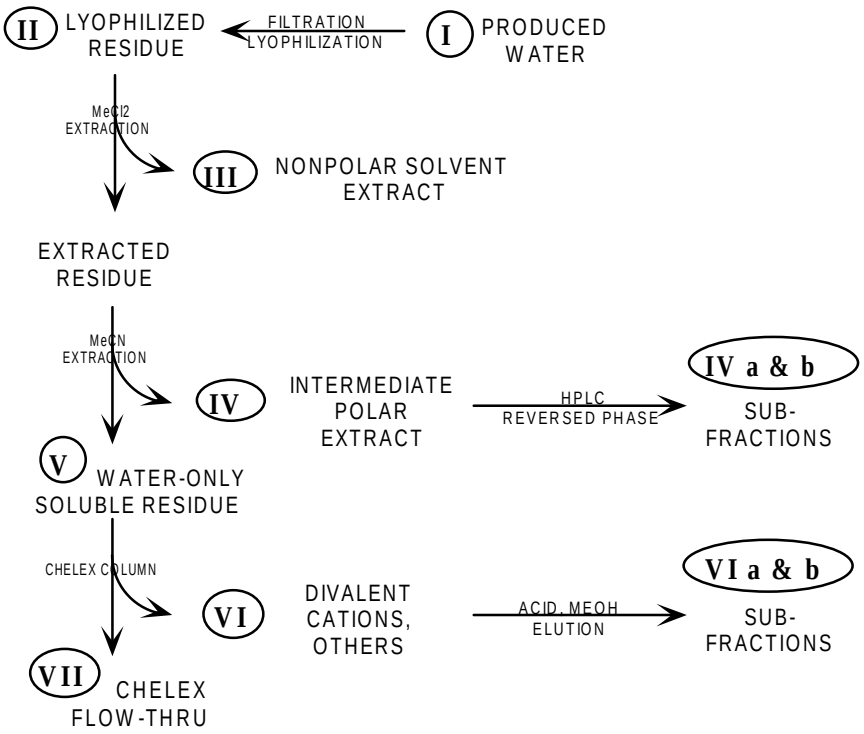

Figure1. Scheme for the fractionation of produced water [from Higashi, *et al*., 1993].

Table 1. Toxicity of Produced Water Fractions to Mussel Embryos.

	FRACTION	EC50 (%)
I	Whole PW	2.12
II	Lyophilized Residue of I	2.86
III	MeCl2 Extract of II	~15
IV	MeCN Extract of II	»20
IVa	Polar HPLC Fraction of IV	»20
IVb	Nonpolar HPLC Fraction of IV	»20
V	Water-only Soluble Residue of II	2.65
VIa	Chelex retained V, acid eluted	2.87
VIb	Chelex retained V, acidic MeOH eluted	4.63
VII	Chelex-Flow Thru of V	»20

Toxicity tests [Cherr *et al*., 1990] of all fractions have repeatably indicated that the majority of toxicity is removed by Chelex (compare V and VII in **Table 1**), but the elemental analysis by ICP-atomic emission spectroscopy that we performed on these fractions (**Table 2**) indicates that the more "classical" toxic elements sometimes present in Carpinteria PW (such as Cu, As, and Cr) are not consistently the culprits. In fact, among the transition metals we detected (Al, As, Cd, Co, Cu, Cr, Fe, Mn, Mo, Pb, Sn, Zn), none appeared (**Table 2**) to be of sufficiently high concentration - either alone or in additive combination - to fully account for the respective mussel toxicity (**Table**

1), discounting any synergistic effects [Higashi *et al.*, 1993]. The only elements that were consistently in high concentration were the alkali earth elements Ba and Sr, for which the chronic toxicity limits are not known for any aquatic system, to the best of our knowledge.

Table 2. Elemental Analysis of a Sample of Produced Water and its Fractions Directly Corresponding to **Table 1** (Sampled: July 30, 1991)

	Concentration (ppm)					
Element	I	II	IV	V	VIa	VII
Al	0.07					
As*	0.0067	0.0045	<0.001	<0.001		
Ba	13.0		1.6	18.0	12.0	0.004
Cd	<0.01					
Co	0.05		<0.01	0.04		
Cu	0.0044		<0.005	<0.005	0.012	<0.005
Cr	0.10		<0.075	0.08		
Fe	0.46					
Mn	0.45		0.03	0.05		
Mo	0.02		<0.02	0.08		
Pb	<0.10		<0.10	<0.10		
Sn	0.01					
Sr	13.0		<0.01	10.0	7.5	<0.01
Zn	0.05		0.06	0.10	0.015	<0.005

Blank spaces = not determined, usually due to toxicologically insignificant levels in the parent fraction.
* By hydride generator atomic absorption spectroscopy
Fractions III, IV a & b, and VI b are "organic" fractions and were not analyzed.

Further fractionation of the Chelex-trapped fraction has divided it into the expected metals and other divalent cations (VIa, eluted by aqueous acid), plus a polar organic fraction (VIb, eluted by acidic methanol). Fraction VIb was unexpected, as although Chelex has high affinity towards certain divalent cations such as metal ions, it is not known to trap organic compounds because of their generally neutral or anionic character. **Table 1** shows that the majority of toxicity was contained in VIa, but note that the chelex-organic fraction VIb also harbors some of the toxicity.

In summary, previous work indicated that petroleum hydrocarbons and transition metals in Carpinteria PW do not account for toxicity to mussel embryos. This result, if widely true of PW, suggests that analytical and modeling approaches traditionally used for PW research - that is, those that key on hydrocarbons and metals - may not be relevant to standard chronic toxicity indicators such as the mussel embryo system.

IB. PROJECT OBJECTIVES

The goals of this project are to: (a) identify constituents of produced water (PW) responsible for various bioeffects of interest to SCEI researchers; and (b) estimate their relevance to an environmental situation by surveying the distribution of the bioactive constituents at the Carpinteria site. The scientific utility of goal (a) is to significantly reduce the list of chemicals to be studied,

providing foci for other studies and greatly reducing analytical load. Goal (b) will provide basic data of potential interpretability to any case study research currently being conducted at the Carpinteria site.

These goals and research approaches (described in detail in the next section) address two targeted research areas for Environmental Studies in the Southern California Educational Initiative (SCEI). The goals, reformulated in terms of the Studies Framework, are as follows:

Fractionation of PW (addresses Studies Framework Issue #1: Fate and effects of produced water discharges in the nearshore environment)
Produced water will be analyzed in accordance with schemes we have previously applied in order to help establish causal linkages between specific PW constituents (or classes thereof) and bioeffects being studied by SCEI researchers. This will be accomplished by providing such fractions for bioeffects testing by collaborating SCEI researchers (e.g., Cherr &Fan, and Reed *et al.*).

Toxicant Identification (addresses Studies Framework Issue #2: Long-term effects of drilling discharges)
Our toxicant identification efforts will continue, currently directed by results of toxicity testing, which will expand as other bioeffects of PW are established in the laboratory and field. We will continue to archive PW samples through lyophilization (freeze-drying), and process samples according to our present scheme (**Figure 1**, and described below). The results of bioeffects testing will direct the choice of the chemical methods to be employed. As such, extensive analytical capability is required on a standby basis, as these substances may be expected to have very diverse chemical structures and properties. A broad range of high-resolution analytical separation (capillary GC-MS, HPLC, capillary electrophoresis) and detection systems (NMR, MS, ICP-AES, ICP-MS) will be brought to bear for the identification and early analysis of organic and inorganic substances. Once identified, the bioactive substances will be isolated or synthesized and integrated with toxicity testing (Cherr and Fan) as well as possibly other bioeffects testing (Reed, *et al*.- kelp zoospore settling tests).

IC. RELATION TO DECISION MAKING

This research is intended to provide entities, such as the Minerals Management Service, with information regarding the substance(s) present in the discharge that are bioactive in both the produced water (PW) discharge and environmental plume. This information, once verified for a PW given source and site, is consequential to at least three types of action that may be taken by the entity:

(1) The list may complement or modify an existing list of substances of environmental concern. This result can be significant because the identities of the substances (actually, their properties) should dictate the nature of further technical action by the entity.

(2) The lists may be used to determine monitoring strategy and, if warranted, PW source control options for those substance(s). For example, if the lists for the discharge and plume are identical, then only the discharge needs to be monitored; but if the lists differ substantially, then direct monitoring of the plume may also have to be instituted.

(3) The properties of chemicals on the lists may be used to estimate transport and fate of bioactive components for a given PW source and site, either through existing models or additional

investigations. As an example of this, if the identified chemical lists for the discharge do not correlate well with plume effects and concentrations, then transport and especially fate would be expected to be a major consideration in any scientific assessment of impact.

ID. PROPOSED APPROACH

GOAL (a): identify constituents of produced water responsible for various bioeffects of interest to SCEI researchers.

This proposal first seeks to extend our previous work [Higashi *et al*., 1993]. Based on the present system of mussel embryo toxicity tests (which will be performed under the proposed project of Cherr & Fan) we will expand the elemental as well as organic and inorganic anion analysis of fractions.

More specifically, this project will continue to pursue the detection of toxicants with the project of Cherr & Fan. According to the best present evidence, this would mean that the two candidates for sources of toxicity, Ba and Sr, will be obtained in pure salt form for toxicity confirmation and probed for their mechanism of action (see proposal of Cherr & Fan for details). We have also discussed experimental considerations with D. Reed for their work on the kelp zoospore swimming and settling effects. We also coordinated with the project of Cherr & Fan for exposure of individual adult bivalves for monitoring perturbations in gametogenesis by *in vivo* nuclear magnetic resonance (NMR). For these studies, we will begin by supplying the major fractions.

With regards to pursuing the toxic fraction VIa, analysis will be expanded to include other less common elements found in PW such as Ni and Ag. For identifying constituents causing other bioeffects, a very broad range of separation (capillary GC-MS, HPLC, capillary electrophoresis) and detection (NMR, MS, ICP-AES, ICP-MS) methods must be available on a standby basis. Details of methods to be used cannot be presented here because the bioactive substances may be of virtually any chemical class. As the full proposal describes, we have unrestricted access to these and other analytical techniques necessary to identify substances.

GOAL (b): estimate their relevance to an environmental situation by surveying the distribution of the bioactive constituents at the Carpinteria site.

We will develop methodology for analysis of the identified substances in field samples of water, sediment, and selected biota. Although specific methods cannot be discussed until identification is performed, the broad range of techniques just mentioned above will assist in the selection and development of more convenient analytical methods. Samples will be collected through the plume monitoring project of MacIntyre, *et al*. Alternatively, the project of Reed, *et al*. can provide the necessary water column samples, which should be sufficient to meet this objective. The purpose of this field survey is to estimate the extent of relevance of the identified substances to the discharge site. As previously stated, this project does NOT attempt to directly determine actual fate and transport of PW constituents in the field. The basic questions addressed by the field survey are: 1) are the identified chemicals present at all in the field, or are they degraded/transported from the system so rapidly that there is very little relevance of the substance to field bioeffects; 2) if present, are the concentrations high enough to account for bioeffects; and 3) if the concentrations are high enough, are they spatially relatable to the discharge?

The main advantage of our approach is that the analysis will consist of the bioactive chemicals, that is, the analytical chemistry is driven by the potential bioeffects of interest. By keying on this relatively short list of substances, we will greatly reduce the analytical burden as compared with that of a general chemical survey, while simultaneously improving the relevance of the analyses to observed bioeffects. This was a major point of our original SCEI proposal, and it remains a major point of our presently proposed research program.

As already pointed out, much of the existing analytical data for hydrocarbons and transition metals apparently do not relate to bioeffects such as chronic toxicity to mussel embryos. Therefore, we will analyze for Ba, Sr, and expand to elements other than transition metals, as well as to anions and polar organic constituents, as appropriate. One possible result of this great difference in analytes is that the spatial distribution of our list of substances will depart from that of transition metals and petroleum hydrocarbons, due to the large differences in physical and chemical properties.

In addition to its use in the field survey, the development of analytical methods can assist in all phases of this study, with applications for other SCEI research (e.g., MacIntyre, *et al*. plume monitoring) and future MMS studies. Using mussel toxicity as an example, we now know that the Chelex resin is able to very efficiently extract the toxicant(s). Thus, we will use the resin columns to perform rapid, on-site extraction of PW for comparison with PW that is shipped and extracted two days later, thereby determining to what extent shipping and sample container material affects toxicant activity. In a similar application, these types of resins can be deployed at marine and freshwater field sites, either with a pump for short-term (hours) or in passive batch-mode for a continuous, longer-term (weeks) extraction. In fact, these types of "sampling" is so promising that we plan to deploy resin columns at the Carpinteria discharge site in conjunction with the project of MacIntyre, *et al*. Lastly, the Chelex resin can be used back in the laboratory to isolate and concentrate larger amounts of toxicant(s) from PW, releasing this major logistical constraint from the bioeffects testing of the projects by Cherr and Fan and Reed *et al*.

II. JULY 1992 to JUNE 1993

IIA. RATIONALE

This project has two interrelated objectives. The first is to extend our previous work [Higashi *et al*., 1993; Fan *et al*., 1993] on the identification of bioactive constituents of produced water (PW) under controlled laboratory conditions. The second is to provide information regarding the identity of PW organic constituents that are relatable to biological effects observed in the field. The overall goal of both objectives is to generate a "short list" of PW constituents that warrant further study with regards to their fate and transport in the environment. This list of compounds will likely differ from those used in most previous studies in that biological relevance is attached to the compounds. This list, if carefully validated, can eventually provide entities such as the Minerals Management Service with biologically relevant chemical markers for environmental monitoring and regulation.

As PW is a very complex mixture of diverse chemical categories consisting of hundreds of organic compounds [NRC, 1985; Boesch and Rabalais, 1987], the principal technical utility of this project is to significantly and systematically reduce the list of PW constituents that must be studied with regards to their fate and transport. There are many scientific works regarding the occurrence and fate of the organic components, but these are mostly limited to the petroleum hydrocarbons, which comprise only a portion of the total organic load of PW [NRC, 1985; Boesch and Rabalais, 1987].

The reasons for this self-imposed restriction are many, but among the likely reasons are the ease of analysis for hydrocarbons and the considerable body of literature regarding their biological effects. However, even with this restriction to just hydrocarbons, PW can harbor hundreds of compounds.

A less arbitrary means to reduce the complexity is to identify the compounds associated with biological effects. For example, we have successfully utilized a bioactivity-based fractionation of PW from a plant at Carpinteria, CA to characterize the constituent responsible for impairment of mussel embryo development [Higashi *et al*., 1993]; this may also relate to impairment of adult mussel reproductive development [Fan *et al*., 1993]. The identity of this constituent, Ba, is being verified in a series of laboratory experiments that investigate PW fractions and Ba chemistry as it relates to the symptoms and mechanisms of Ba toxicity in these biological systems; the latter is being conducted in conjunction with the project of Cherr and Fan [Schmitt, 1991]. Unfortunately, the discharge of PW from that source has ceased, making field verification impossible.

In order to study effects of PW in the field, Osenberg, *et al*. [Schmitt, 1991] have been studying the future PW discharge site near Point Conception, CA ("Gaviota") for the past three years, utilizing the Before-After Control-Impact Pair design (BACIP) for ecological studies. Although this PW plant at Gaviota is currently in operation by reinjection of PW into formations, information from the plant operator suggests that discharge of the PW into marine waters will not occur immediately. Thus, the present situation at Gaviota represents an outstanding opportunity to study organic constituents in the "Before" period of the BACIP study.

The basic approach in this field study is chemical analysis of PW, site sediment, water column, and outplanted mussels, with the data to undergo BACIP analysis by Osenberg, *et al*. Due to logistical limitations, the study will focus on hydrophobic compounds and rely heavily on high-resolution gas chromatographic (GC) and GC-mass spectrometric (GC-MS) analyses. All sampling is conducted in coordination with the project of Osenberg, *et al*., originating from the Gaviota research site, as described elsewhere [Schmitt, 1991]. Samples are collected from three sites (near impact, far impact, and reference) semiannually, each site consisting of water column and outplanted mussels, each at two depths, and sediment sample. In addition, Gaviota PW samples are obtained twice per year in order to track trends in PW composition. Organic solvent extracts of these samples are analyzed by various GC methods. Most of the analytical data will consist of relative quantification of unknown compounds. Thus, an important analytical tool is GC-MS, which can catalog structural information on these unknowns for future use and provide running checks on analytical efficiency via analysis of the deuterated standards. For example, mass spectra is used to ensure that all analytical data transferred to Osenberg, *et al*. consist of the same compounds (based on their GC retention time and mass spectra) throughout the study. Chemical identification of a given peak will commence upon the identification of that peak as related to biological effects in the field, as determined by the BACIP analyses. Another important feature of the project is the preservation of samples by unique means such as freeze-drying. This is vital if subsequent research indicates that certain compounds of biological relevance are not extracted efficiently by the original procedures.

IIB. METHODS

IIB.1 Organic Constituents of Gaviota Produced Water

Reagents: Dichloromethane, acetone, and hexane were Fisher Optima grade. Hydrochloric acid used for acidification was Fisher A.C.S. grade. Sodium sulfate (Fisher certified ACS grade,

anhydrous 10-60 mesh) was rinsed with dichloromethane prior to use. Deuterated *n*-hexadecane-d_{34} (1 mg/mL in dichloromethane, Cambridge Isotopes, Cambridge, MA) was used as an internal standard, and acenaphthene-d_{10} was used to calculate recovery (1 mg/mL in dichloromethane, Cambridge Isotopes). Mixed standards of C_6-C_{10} fatty acids (1 mg/mL in dichloromethane, Aldrich Chemicals, Milwaukee, WI), C_9-C_{22} alkanes (1 mg/mL in dichloromethane, Polyscience Corporation Analytical Standards), and C_{14}-C_{24} fatty acid methyl esters (167 µg/mL each in dichloromethane, H-104 FAME mixture, Alltech Associates, Inc., Deerfield, WI) were used for retention time and mass spectral comparisons. *O*-cresol (99%+, Aldrich Chem. Co.), *n*-decanoic acid (Aldrich Chem. Co.), dodecane (Polyscience Corporation), and 3,4-dihydro-6-methyl-2H-pyran-2-one, (97%, Aldrich Chemical Co.) (1 mg/mL each in dichloromethane) were used to generate a mean relative response factor used for quantifying produced water constituents.

Produced Water Extraction: Produced water samples were obtained every two weeks over a three-month period from the Gaviota oil processing facility and collected in 4-L acid cleaned Nalgene heavy-walled bottles. Bottles were cleaned following trace metal protocols (i.e., washed in detergent, and soaked in 6 N nitric acid (Fisher, Trace metal grade) for several weeks and rinsed in 18 mΩ water (Nanopure, Barnstead). All glassware was washed using detergent, followed by five rinses each of distilled water, acetone, dichloromethane, and hexane. Samples were collected without a headspace to minimize oxygen contact and constituent oxidation. 500-mL aliquots were withdrawn for organic analysis within a week of sampling, and poured into an 1800-mL beaker. Samples contained visible particulate matter that ranged in color from white to black, and the color of the solutions ranged from dark yellow to brown. A distilled water blank and reagent blank consisting of solvents used for produced water extraction were prepared at the same time as each sample. To each beaker, 100-µL of deuterated *n*-hexadecane-d_{34} internal standard solution was added. The pH was adjusted to pH 3.0 with concentrated HCl (Optima grade, Fisher Scientific). The acidifed produced water was transferred to a 1000-mL separatory funnel, and extracted four times with 25-mL aliquots of dichloromethane; this small volume of dichloromethane was used to minimize the loss of volatile target compounds during subsequent handling steps. The dichloromethane extract was passed through a glass filtration funnel containing ~5-g. pre-rinsed anhydrous sodium sulfate placed on Pyrex® wool (Corning Glass Works) to remove residual water. The four sequential extracts were combined in a 500-mL round bottomed flask, and evaporated under reduced pressure to ~5-mL in volume. The concentrated extract was transferred quantitatively to a concentrator tube, and further concentrated under a stream of nitrogen gas to a volume of ~2-mL. 1-mL was removed at this point for archiving. To the remaining 1-mL of extract, 50-µL of 1 mg/mL acenapthene-d_{10} standard was added. The sample was transferred to an amber vial and stored at 4°C until GCMS analysis.

Gas Chromatography/Mass Spectrometry of Produced Water Constituents: Mass spectrometric analyses were performed on a VG-Trio 2 quadrupole mass spectrometer (VG Masslab, Altrincham, UK), using a 30 m DB-1 capillary column (0.25 mm I.D., 0.25 µm film; J&W Scientific, Folsom, CA). Helium was used as carrier gas at a linear velocity of 30 cm/s. Splitless injections of 1-µL were made using a 0.75 min purge delay. Injector temperature was 285°C. The GC oven temperature was held at 40°C for 1 minute, then programmed from 40°C to 150°C at 20°C/min, 150°C to 300°C at 6°C/min, and held at 300°C for 10 minutes. Samples were ionized using 70 eV electron ionization and full scan mass spectra were acquired over the mass range from *m/z* 45 to 550

at 1 scan/sec. Peaks were identified by comparing retention times and mass spectra to 1) reference standards or 2) mass spectra from spectral libraries when suitable matches were available.

Further identifications were made by analyzing the same extracts described above on a 30 m DB-23 capillary column (0.25 mm I.D., 0.25 µm film, J&W Scientific, Folsom, CA). The DB-23 column was useful in identifying polar fatty acids without the need for methyl ester derivatization, and for enhancing separation of coeluting analytes on the DB-1 column. The GC oven temperature was held at 40°C for 2 minutes, then programmed from 40°C to 140°C at 20°C/min, and 140°C to 250°C at 6°C/min. The injector temperature was 250°C.

Quantification of Produced Water Constituents: Quantification of unknown constituents in produced water was accomplished using a mean relative response factor (RRF) calculated from the response of four compounds: *o*-cresol, *n*-decanoic acid, dodecane, and 3,4-dihydro-6-methyl-2H-pyran-2-one whose masses varied over the range 25-100 ng relative to a fixed mass (50 ng) of internal standard, *n*-hexadecane-d_{34}. We chose this quantification approach in lieu of individual calibration curves for each compound due to the complexity of produced water composition, and the lack of suitable reference standards for compounds known to be present in produced waters, including branched fatty acids and alkylated phenols. Reference compounds used in the RRF determination were chosen to represent the types of polar compound classes that are present in produced water. Relative response factors (RRFs) were calculated on a mass basis relative to the internal standard, yielding a mean value of 0.231± 0.067 which was used for quantitative analysis of all chromatographic peaks. Concentrations of unknown produced water constituents were calculated using the equation:

$$= \frac{\text{(area of unknown) *(concentration of internal standard added)}}{\text{(area of the internal standard)* RRF}}$$

IIB.2 Other Methods

Other methods used are as described previously [Higashi *et al*., 1993; Higashi and Crosby, 1994].

IIC. RESULTS & DISCUSSION

For the verification of Ba as a major toxicant from Carpinteria PW, we have initiated four lines of research. The first is to fractionate stored (freeze-dried) PW samples using the previous procedure [Higashi, *et al*., 1993] to verify that the toxic fraction - the "divalent cation fraction" - is consistently the toxic one. This has required large amounts of stored PW to be processed in analytical-scale amounts. We have also added Ni and Ag to the previous list of analytes (Al, As, Ba, Cd, Co, Cu, Cr, Fe, Mn, Mo, Pb, Sn, Sr, Zn) for greater confidence of the toxicant identity, and will continue to add to the analyte list. Finally, the biological uptake and effects research of Cherr and Fan are being coordinated with the above, which is described in their technical summary (MMS OCS 97-0024).

For the Gaviota site, samples of PW have been extracted using CH_2Cl_2 and analyzed by GC-MS in an effort to refine methods for analysis of organic constituents of PW. Our analytical results are in agreement with an earlier finding that Gaviota PW contains numerous sulfur-containing organic substances in addition to alkylphenols, but the mass spectra we obtained do not support the previous tentative identifications of the sulfur-containing compounds, which relied entirely upon computer-based library searches.

Based upon new GC-MS analyses and chemical derivatizations, we have revised the identifications. The principal organic constituents extracted from Gaviota produced water are organosulfur compounds of intermediate polarity and are present in part-per-million concentrations. They appear to be thiopyranone derivatives, their isomers, and structurally related thiocarboxylic acids (**Figure 2**). We are in the process of isolating larger quantities of individual constituents for structure confirmation using other spectroscopic methods.

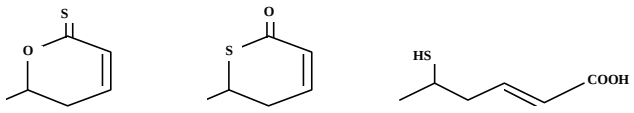

Figure 2. Tentative structures for major organosulfur compounds isolated from Gaviota produced water.

The thiopyranones are novel compounds not previously identified in any such systems, to our knowledge. Their formation might be attributed to reactions of inorganic sulfides and polysulfides with products of the oxidative degradation of aromatic hydrocarbons (**Figure 3**) and may be a common occurrence where these oxidation products encounter sulfide-rich waters. The chemical structures of these compounds suggest that they are reactive electrophilic compounds which might react with biological macromolecules, but at present the potential of these compounds to stimulate biological effects are unknown. We propose to synthesize and isolate several of the most abundant organosulfur compounds so that their bioeffects can be evaluated by collaborating SCEI researchers (e.g., projects of Cherr and Fan, Reed).

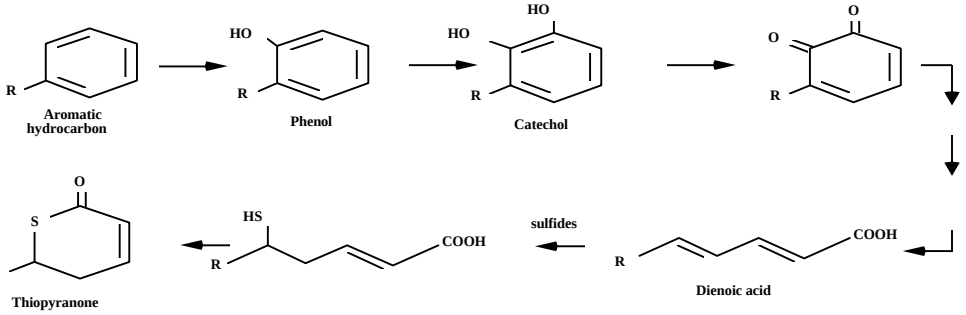

Figure 3. Proposed pathways for formation of organosulfur compounds from inorganic sulfides and products of oxidative degradation of aromatic hydrocarbons.

In addition to the efforts described above to characterize selected organic constituents and prepare fractions for bioactivity tests, GC and GCMS analyses are conducted to characterize the background (the "Before") organic substance profile in seawater, sediments, and mussels planted at several sites in the Gaviota area. A new analytical procedure has been developed to allow for simultaneous analysis for the PW constituents expected to be discharged and lipid composition of the mussels. The results from all of these analyses are being processed into a normalized format for incorporation into BACIP studies (project of Osenberg, *et al.*).

III. JULY 1993 to JUNE 1994

IIIA. RATIONALE

Macromolecular Organic Structures in Carpinteria PW and Sediments

As produced water (PW) is a natural water source rich in organic matter, it is possible that there are amorphous macromolecular constituents - loosely termed "humic substances" (HS) - present in considerable quantities. The interaction between HS and non-ionic contaminants carried by PW is critical to understanding the persistence and movement of both organic and inorganic compounds in the environment. Studies have shown reduced bioavailability of non-ionic contaminants in the presence of humics as a result of sorption, and increased persistence in the ecosystem [Senesi and Chen, 1989; Strek and Weber, 1982; Karickhoff, 1980]. Parallel with decreased availability, sorption of contaminants to particulate and colloidal organic matter may result in increased transport through air and water [Lara and Ernst, 1989].

However, as with most natural water, the presence and interaction of HS has not been investigated - and perhaps even avoided - due to the great difficulty in characterizing humic structures. HS are the product of reaction with a broad spectrum of chemical classes, including (but not limited to) amino acids, organic acids, lignin, polysaccharides, proteins, cutins, chitins, melanins, suberins, paraffinic macromolecules, and breakdown products of this list [Hayes, 1991]. HS are natural macromolelcular products that lack long-range order, contain a wide variety of functional groups which can be accessible or inaccessible for reaction, harbor hydrophobic and hydrophilic domains that can likewise be accessible or inaccessible, and tend to undergo conformational or chemical changes during isolation. HS genuinely rank among the most complex and least-understood substances in the world.

Despite their complexity, researchers in the 80's studied chemical degradation of HS under acid, oxidative, or reductive conditions, yielding useful information regarding their primary structures [Hayes, 1991]. However, this approach can also yield erroneous information as a result of extensive chemical modifications and rearrangements during the degradative processes; it also fails to provide information on the secondary or higher order structures, which is an essential element of characterization of any macromolecule, including HS [Hayes, 1991].

Over the past decade, advances in techniques such as nuclear magnetic resonance spectroscopy (NMR) and pyrolysis gas chromatography-mass spectrometry (py-GCMS) have enabled researchers to compile more comprehensive structural information on HS than was thought possible [Hayes, 1991]. Although NMR is considered the most frequently used and "definitive" analytical method for the study of HS [Malcolm and McCarthy, 1991], the parallel development of analytical pyrolysis (py), such as py-GCMS, has shown it to be an approach that is strongly complementary to NMR for the study of HS [e.g., Haider and Schulten, 1985; Schnitzer and Schulten, 1992]. Using these new methods, an explosion of information ensued on selected "reference" HS [*c.f.* Suffet and MacCarthy, 1989, and references cited therein] that is continuing today.

Therefore, in this year of investigation, we have examined the presence of HS - amounts and of what categories - that may be present in PW as well as in sediments at various distances from the Carpinteria outfall.

Gaviota Produced Water Characterization

Chemical characterization of the Gaviota PW continued this year, with focus on the possible presence of polysulfide compounds. Organic polysulfides are apparently common in marine sediments [Vairavamurthy and Mopper, 1990] but their existence is nevertheless generally unrecognized and readily misidentified as hydrogen sulfides.

IIIB. METHODS

IIB.1 Pyrolysis-GCMS

In pyrolysis-GCMS, a treated or even untreated sample is directly analyzed by heating rapidly to 400-1000°C under He gas to thermolytically fragment it. In the present analysis, we used approximately 0.1mg of lyophilized PW [Higashi *et al*., 1993] and sediments from Carpinteria directly with no further treatment, in a CDS Pyroprobe 120 resistive-coil pyrolysis unit, where the precision and rate of the temperature control is such that reproducible, quantitative results can be obtained. The He gas stream sweeps the fragments into a GCMS where it undergoes analysis.

The GC-MS was a Varian 3400 GC (Varian Instrument Co., Walnut Creek, CA, USA) outfitted with a 0.18 mm i.d. x 40 m length open tubular column with a 0.4 mm coat of DB-1 (polymethylsiloxane) (J&W Scientific, Folsom, CA, USA), interfaced with a line-of-site transfer line to a Finnegan ITD-806 mass spectrometer (Finnegan MAT, San Jose, CA, USA). GC parameters were: injector = 260ºC, split vent held closed for 1.5 min, then open at 15 ml/min, H2 carrier gas velocity = 40 cm/sec, column = 60ºC held for 2 min, ramped to 150ºC at 20ºC/min, then increased to 290ºC at 6ºC/min, transfer line = 290ºC. MS acquisition parameters were: manifold = 220ºC, electron energy = 70 eV, electron emission current = 10 μA, automatic gain control set at 45 amu, full scan acquisition from 46-650 m/z at a rate of three spectra/sec, which were averaged into one spectrum/sec. Mass calibration was by perfluorotributylamine, and a mass defect of 1.0 mmu/amu was applied to all spectra.

IIIB.2 Other Methods

Other methods used are as in previous years of this project, or as described previously [Higashi *et al*., 1993; Higashi and Crosby, 1994]

IIIC. RESULTS & DISCUSSION

In the past year, we have implemented a class of analytical technique that is capable of obtaining profiles of organic matter in sediment, in particular the ones traditionally recalcitrant to analysis such as the macromolecular, non-soluble humic substances (HS). The relevance of HS is covered in the Rational section. We used “analytical pyrolysis”, which consists of an instrument that pyrolyzes solid samples in a quartz tube, interfaced for analysis by GC-MS. The principal initial use is in "fingerprinting" of "total organic" profiles of effluents as well as of sediments. Analysis of archived sediments from the Carpinteria produced water (PW) discharge site (no longer active) appear to reflect some of the organic pattern of produced water from the Carpinteria plant (**Figure 4**).

Not surprisingly, pyrolytic markers of PW decreased with increasing distance from the outfall (**Figure 4**). However, many of these markers were suggestive of polysaccharidic origin, not solely of hydrocarbons. The more “traditional” HS markers were present only at a low level. The polysaccharides were primarily indicated by the presence of certain alkylfurans. Since the classical

pyrolysis linkages indicative of cellulosic (*beta*-1,4) materials were not present, these saccharides were probably not from autotrophs, possibly bacterial in origin, and most likely degraded from their original structures.

Such macromolecular components would not be readily detected in PW using conventional analytical approaches, and in this case, appears to comprise a significant portion of the organic matter in Carpinteria PW, as well as in Carpinteria sediments. These types of substances can strongly influence the microbial activity and community structure of the impacted sediments, in ways that will be dramatically different from that predicted by a hydrocarbon-based discharge. We were not able to find any literature reference regarding the nature such polysaccharides in PW or related sediments.

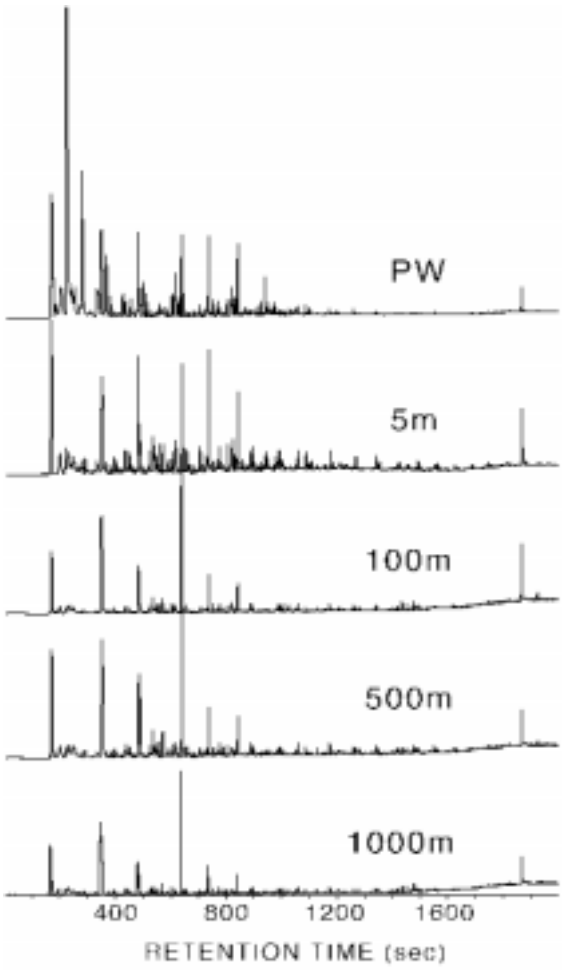

Figure 4. Pyrolysis-GCMS total ion chromatograms of sediments from the Carpinteria produced water discharge site, which is now inactive. The distances indicated are distances prevailing downcurrent from the outfall. There are numerous thermolytic markers present in PW that are reflected in the sediments, and these markers are depleted with distance. Many of these are of course hydrocarbon-based, but surprisingly, some of the largest markers appear to be polysaccharidic in origin. If so, such a composition might strongly affect the microbial degradation and community structure of impacted sediments in ways quite different from those predicted by a hydrocarbon-dominated composition.

Another interesting aspect of pyrolysis GCMS analysis is shown in **Figure 5**. Van Loon *et al*. [1991] have shown that SO_2 evolution from pyrolysis of high and low molecular weight compounds followed different time courses, thus being distinguished from each other in pyrolysis of river whole

sediments. However, in marine sediments, the high sulfide content can interfere with this type of analysis, as seen in **Figure 5**. But this figure also shows that sulfonylated humic substances, sulfides, and a common marine sediment mercaptan, 3-mecaptopropionate, can be distinguished from each other by the combination of SO_2 and CS_2 thermolytic evolution. Thus, if we employ techniques better suited for these highly volatile compound analyses, we may be able to estimate sulfur content due to high and low molecular weight organic compounds, directly from whole sediment.

Because of the types of analyses illustrated above, pyrolysis is rapidly emerging as a premier analytical tool in the soil sciences, but it is essentially unknown with regards to sediment applications. As with much of our past analytical developments, this method was selected because it is relatively non-specific in its analysis; we expect that analytical pyrolysis will continue to bear out the concept that non-specific analysis is important to understanding environmental chemistry.

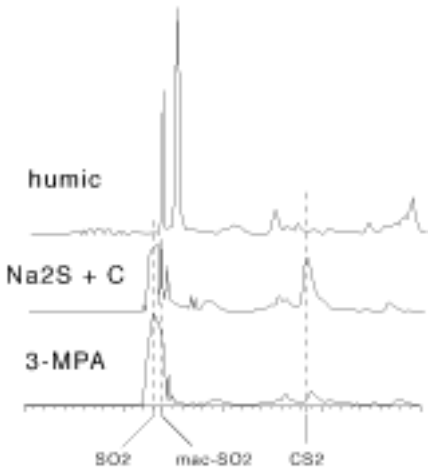

Figure 5. Pyrolysis GC-MS total ion chromatograms of isolated humic substance, sodium sulfide + carbonate, and 3-mercaptopropionate. This figure illustrates that the formation of SO_2 from various sources (confirmed by plotting m/z=64, data not shown) differ in formation rate (abscissa = time scale), that sulfide can interfere with the macromolecular SO_2 peak, and that CS_2 can be formed from sulfide but not from organic sources of SO_x. The latter means that CS_2 may be able to be used to subtract the sulfide interference.

For continuing analysis of PW samples from the Gaviota plant, delivery resumed in the Spring of 1995 after interruptions due to personnel changes at the oil processing facility. Produced water samples were collected every two weeks from March through June to establish temporal variability in produced water composition. A battery of analytical techniques (electrochemical, chromatographic, HPLC/spectrophotometry, and GCMS) were used to characterize organic constituents and inorganic sulfur compounds capable of reacting with organics and participate in the formation of organosulfur compounds.

All produced water samples contained high concentrations of sulfide (2-8 mM) which accounts for about 60% or more of the total sulfur in the produced water. Sulfide concentrations declined over time during the period, but salinity of the produced water samples increased during the same period, perhaps indicating dilution of produced water with brine at the treatment plant. Sampling procedures that remove sulfide via acidification and purging were found to perturb concentrations of

other inorganic sulfur species that are linked via coupled equilibria. HPLC methodologies were developed to explore whether non-extractable organic thiols are present in produced waters. Results documented the presence of other inorganic sulfur compounds such as polysulfides and thiosulfate (50-300 µM) but substantial concentrations of other organic thiols were not observed. Levels of sulfite were typically less than sulfide concentrations by about three orders of magnitude. Physical fractionation of produced water samples was performed to determine the distribution of sulfide among particulate, colloidal, and dissolved phases. Dissolved sulfide accounted for about 80% of total sulfide; particulates (> 0.45 µm) accounted for 10% and colloids accounted for about 10% of the sulfide. These distributions did not show appreciable change 6 hours after mixing produced water with seawater in the laboratory.

Levels of dichloromethane-extractable semi-volatile organic compounds in Gaviota produced waters were in the low µg/L range per component. Short chain alkylphenols were observed in addition to numerous compounds not previously identified. Some of these analytes were identified as heterocyclic organic polysulfides such as those shown below based upon matching with mass spectrum libraries. These compounds had not been detected in produced water samples collected before from either Gaviota or Carpinteria sites. Organosulfur compounds detected in these earlier samples (thiolactones) were not present in detectable amounts in the recent produced water samples.

Additional compounds tentatively identified as *N*,*N*-dimethylalkylamine surfactants were also present in µg/L concentrations. Numerous organic constituents have yet to be conclusively identified. These analyses have documented substantial variation in the organic constituents of produced water samples from the Gaviota facility. Levels of dissolved organic carbon were determined and found to be on the order of 100 mg/L. These levels are substantially greater than the total of semivolatile compounds detected and suggest the presence of significant amounts of polar non-extractable material. Acidification of produced water samples with hydrochloric acid led to formation of a colorless insoluble precipitate, suggesting the presence of an acidic polyelectrolyte (perhaps a treatment chemical) that may effect the bioavailability of trace elements present in produced water.

Figure 6. Organosulfur compounds detected in Gaviota produced waters during 1994-95, using GCMS.

Analysis of sediment samples for organic constituents continued, showing the presence of petroleum hydrocarbons in all sediments. Hydrocarbon patterns and concentrations showed no obvious correlations with location, and variability between samples collected from a single site were comparable to differences between sites. Absolute hydrocarbon concentrations were similar to those observed in Carpinteria sediments. Hydrocarbon patterns suggest that natural petroleum seeps and other biomass inputs are the source of much of this material. Several polycyclic aromatic

hydrocarbons (PAHs) including naphthalene and phenanthrene were detected in sediment extracts, but total PAH concentrations were typically less than 1 µg/kg dry weight. Normal and branched-chain alkanes (e.g., pristane) were present, with total n-alkane levels approximately 10-100 µg/kg.

Analyses of organic compounds in outplanted mussels and seawater samples also continued, yielding fatty acid profiles of mussels. These analyses did not detect the known semi-volatile constituents of produced water such as phenols or organosulfur compounds, but such results were expected given that discharges of produced water had either not begun or were very limited at Gaviota during the outplanting period.

Though sampling and analyses have ceased, current efforts are focused on tabulating the chemical analyses and identifying organic constituents of produced water that did not match entries in the mass spectrum library.

IV. JULY 1994 to JUNE 1995†

IVA. RATIONALE

In this year of work, we concentrated on further identifying a wide variety of chemical forms in the PW from Gaviota facility, including a number of inorganic sulfur forms. This is due to the fact that sulfur is a principal element in the Gaviota PW, and sulfur compounds are complex and often biologically active.

In our other main activity, we solved the problems regarding digestion of samples for Ba analysis, and analyzed new growth mussel shell samples from outplant experiments at Carpinteria 1990-93 conducted by Osenberg *et al*.

IVB. METHODS

IVB.1. Inorganic Constituents of Gaviota Produced Water

Reagents: All solutions were prepared using Nanopure water from a Barnstead purification system. All reagents were ACS grade and obtained from Fisher Scientific unless stated otherwise.

Sample Collection. Produced water samples were obtained from the Gaviota oil processing facility and were collected in 4-L acid cleaned Nalgene heavy-walled bottles. Bottles were cleaned following clean trace metal protocols (i.e., washed in detergent, soaked in 6 N trace-metal grade nitric acid (Fisher) for several weeks, and rinsed in nanopure water (Barnstead). Samples were collected without a headspace to minimize oxidation of reduced forms. Aliquots were withdrawn using a pipet for sulfide analysis within 24 hours of collection. Samples contained visible particulate matter that ranged in color from white to black, and the color of the solutions ranged from dark yellow to brown.

Differential Pulse Polarography (DPP) Analysis. Stock solutions of sulfide were prepared from reagent grade $Na_2S•9H_2O$ (Aldrich) and standardized using iodometric titrations. Thiosulfate standards were prepared from $Na_2S_2O_3•5H_2O$, and standardized against potassium iodate solution

---

† This work was completed on a no-cost extension for Project 22, MMS Contract No. 14-35-0001-30471 and with additional funding under MMS Contract No. 14-35-0001-30761, Project 3, *Characterization of Organic Constituent Patterns at a Produced Water Site / Barium Relations to Bioeffects of Produced Water*, Higashi, Jones and Fan, PIs.

(0.1011 N). Standard iodine solution was prepared using KI and elemental iodine, and standardized by titration with thiosulfate. Anhydrous sodium sulfite was used to make stock sulfite solutions.

A stock solution of 200 mM sodium acetate (Aldrich), adjusted to pH 6.0 with glacial acetic acid was used for sulfide analysis by DPP. Sulfide was also analyzed in 565 mM NaCl/4 mM $NaHCO_3$ adjusted to pH 8.0 with 100 mM NaOH using sampled DC polarography (SDC) because the salinity of produced waters can be significant. 100 mM sodium acetate adjusted to pH 4.5 with glacial acetic acid was used for sulfite and thiosulfate analyses.

Sulfide, thiosulfate, sulfite, and polysulfides were analyzed by DPP using an EG&G PARC model 394 polarographic analyzer system, with a model 303A static mercury drop electrode in the dropping mercury electrode (DME) mode. The working electrode was a "large" mercury drop (ca. 2.61 mm^2), and a platinum wire served as counter electrode. In order to avoid interference between sulfide and the EG&G Ag/AgCl reference electrode, the silver wire was sealed in a glass tube to prevent contact with solution. The reference electrode used instead was a saturated calomel electrode (SCE) separated from the analyte via a salt bridge filled with 565 mM sodium chloride solution. Electrolyte solutions were deoxygenated with filtered specialty grade (99.999%) helium at a flow rate of about 200 mL/minute for 4 minutes. A positive reservoir pressure of 15-20 kPa above ambient was maintained during DPP analyses.

Quantitation of sulfide in produced water was performed using two calibration methods. The first involved external calibration of sulfide in pH 6.0 100 mM sodium acetate using DPP. Sulfide in produced water was quantified from a 5 point external calibration curve generated in the following manner: 5.00 mL of 200 mM pH 6.0 acetate solution was pipetted into a glass cell. An appropriate volume of water was added using a Rainin pipettor (i.e., 5.00 mL, 4.95 mL, 4.90 mL, 4.85 mL, 4.80 mL and 4.75 mL), and the solution was purged for 4 minutes. When purging was complete, sulfide standard solution (~10 mM) was added to the cell to make a 10.00 mL total solution volume (100 mM final acetate concentration). The calibration range extended between 0 and 300 µM sulfide (final concentration). It was necessary to add sulfide stock solution after purging with helium to minimize volatilization of hydrogen sulfide from the matrix. We performed DPP with a 2 mV/S scan rate from -0.3 to -0.6 V, 50 mV pulse height and a 1-s drop time for the DME (all electrode potential are reported vs SCE). E_p for sulfide was measured in this matrix at -0.504 V. To determine the concentration of sulfide in produced water, 100 µL of produced water was pipetted into a deaerated cell containing a mixture of 5.00 mL buffer and 4.90 mL of water. Each produced water sample was analyzed in triplicate to determine a mean ± standard deviation, and sulfide concentrations were calculated from the calibration curve.

Sulfide Analysis using SDC Polarography. Sulfide was also quantified using sampled DC polarography and the method of standard additions. The electrochemical apparatus was the same as described above for the DPP method. We performed SDC polarography with a 2 mV/S scan rate from -0.4 V to -0.9 V using a drop time of 1 s. E_p for sulfide is -0.7 V in this matrix. Three standard additions of sulfide were made to this matrix, and sulfide concentrations were calculated using the equation:

$$C_a = \frac{i_1 * v * C_s}{i_2 * v + (i_2 - i_1) * V}$$

where: i_1= Stripping peak height for the sample

i_2= Stripping peak height for sample and spike
v= Volume of standard solution used for spike
V= Total volume of sample
C_S= Concentration of the sample used to spike
C_a= Concentration of the unknown in the sample

UV-Visible Spectrophotometry. Reactive sulfur nucleophiles were determined using the spectrophotometric method of Ellman [1959]. Phosphate buffers (100 mM) of pH 7.0 and pH 8.0 were made using sodium phosphate dibasic, and 85% phosphoric acid (HPLC grade). 5,5'-Dithiobis(2-nitrobenzoic acid) (DTNB) was obtained from Sigma. Sulfide standards used to generate the calibration curve (0-120 µM) were diluted from stock sulfide solution that had been standardized using iodometric titration.

Sulfide standards were derivatized according to published procedures reported by Ellman [1959]. Three aliquots at each standard concentration were derivatized and a mean ± standard deviation calculated. A standard curve was generated from absorbance measurements. Produced water was diluted in nanopure water to fall in the linear range of the calibration curve using estimates determined from the DPP method. A Shimadzu UV-2101PC spectrophotometer was used to measure absorbance at l = 412 nm and 25• C in quartz cuvettes.

Gravimetric Sulfate Determination. Precipitation by $BaCl_2$ addition was used to determine sulfate concentrations with drying of residue. For the gravimetric procedure, an aqueous solution of 100 g/L $BaCl_2$•$2H_2O$ was prepared, and sulfate was precipitated after acidification of the produced water to pH 2.0 with HCl according to reported procedures. Supor-450 0.45-µm membrane filters (Gelman Sciences) were used for filtration of $BaSO_4$ precipitate.

Iodometric Titration. Sulfide was determined in produced water using iodometric titration. Standards prepared for DPP analysis were also used in iodometric titrations. Standards were made fresh for each collection of produced water, and were restandardized every two days. Produced water (1.00 to 5.00 mL) was added to a 250 mL flask containing 10.00 mL standardized KI/I_2 solution and 10 mL of water. 2.00 mL of 6 M HCl was added. Titration of produced water was carried out with standardized thiosulfate solution to a colorless endpoint, as detected using starch indicator solution.

Thiosulfate, Sulfite, and Polysulfide Determination by DPP. Thiosulfate, polysulfides and sulfite were determined electrochemically in 100 mM acetate buffer, pH 4.5. For thiosulfate and sulfite determinations, 5.00 mL of produced water was pipetted into a 10 mL cell, along with 5.00 mL of pH 4.5 acetate buffer, (final concentration 50 mM). Differential pulse polarography was performed at a 2 mV/s scan rate from 0.0 V to -0.30 V with the DME. Pulse height was 50 mV and E_p was -0.12 V in this matrix. The starting potential for determination of sulfite was -0.50 V and the final potential was -0.80 V. E_p was -0.60 V in this matrix. Scan rate and pulse height were the same as for thiosulfate. Total polysulfide concentrations were determined according to the method of Luther *et al*. [1985]. Briefly, 10 mL of produced water was placed in a 50 mL polyethylene test tube, and diluted to 40 mL with 10 mM sodium sulfite. The tube was sealed under nitrogen and heated to 60ºC for two hours. After cooling, the sample was analyzed for thiosulfate by DPP as described above. Sulfite reacts quantitatively with polysulfide zero-valent sulfur, S(0), to form thiosulfate under these conditions. The difference between the value for thiosulfate before and after

heating with excess sulfite equals the total S(0) from polysulfides. A molar ratio of $5SO_3^{2-}$:$2S^0$ has been reported to ensure quantitative conversion of sulfite to thiosulfate [Farroha *et al*.,1984].

Thiosulfate and Sulfite Determination by HPLC. Thiosulfate and sulfite concentrations were measured using an adaptation of the method of Vairavamurthy and Mopper [1990]. Solvents used in preparation of reagent solutions, standards, and mobile phases were Optima grade (Fisher). The derivatization agent, 2,2'-dithiobis(5-nitropyridine) (DTNP) and the ion pairing agent tetrabutylammonium hydrogen sulfate (TBAHS) were obtained from Aldrich. A reversed-phase C18 column (250 mm x 4.6 mm, Adsorbosphere HS C18 5 µm, Alltech) was used to obtain HPLC separations via a 200-µL external loop. A guard column packed with 5-µm Spheri-5 RP-18 preceded the analytical column (Applied Biosystems). HPLC analyses were performed using a Varian 9010 pump equipped with a Varian 9050 uv-visible detector using spectrophotometric detection at l = 320 nm. HPLC separations were performed using gradient elution at a flow rate of 1 mL/min. The stronger eluent (B) was acetonitrile, and the weaker eluent (A) was 50 mM sodium acetate adjusted to pH 3.5 with 6 N HCl with 7.50 mM TBAHS added (final concentration). The gradient used was as follows: 1 minute hold at 10% B, followed by a multi-ramp program to 34% B at 9 minutes, 55% B at 23 minutes, 100% B at 28 minutes, and held at 100% B for an additional 7 minutes.

Stock thiosulfate and sulfite solutions from the DPP analysis were used to generate a six point external standard curve over the linear range 0-20 µM. Aliquots (1.00 mL) of sample or standard were derivatized by adding 50 µL of 10 mM DTNP in acetonitrile and 50 µL of 200 mM sodium acetate buffer, pH 6.0. The standard was centrifuged at ~2500 rpm during a 5 minute reaction period to remove insoluble particulates. Afterward, 200 µL of the supernatant was injected onto the HPLC. For produced water analysis, it was necessary to dilute the produced water ~50-fold to fall within the linear range of the HPLC calibration curve, because of the high ratio of excess sulfide to thiosulfate and sulfite. Sulfide reacts with and consumes derivatizing agent, thereby lowering derivatization efficiency for thiosulfate and sulfite.

Distribution of Sulfide among Dissolved, Particulate, and Colloidal Phases

Theory: Calculations for Sulfide Concentrations in Particulate, Colloidal, and Dissolved Phases Based on a Two-Step Sequential Filtration. Three fractions were defined according to their ability to pass through a series of filters of different pore sizes based on the mass balance equations of Hoffman et. al. [1981]. "Particulate sulfide" fraction was that portion of the sample retained on a 0.45-µm filter (>0.45-µm fraction). Filtrate (<0.45-µm fraction) was further separated into two size classes using ultrafiltration. "Colloidal sulfide" fraction was defined as the retentate fraction remaining after ultrafiltration through a 10,000 molecular weight cut-off (MWCO) filter (10K-0.45-µm fraction). "Dissolved sulfide" was defined as the filtrate fraction collected after ultrafiltration through the 10,000 MWCO ultrafilter (<10K fraction). The following mass balance equations were formulated to determine the concentration of sulfide in each fraction.

Definition of the system before fractionation: a solution of volume V, containing the following concentrations:

$[S_p]$ concentration of sulfide as particulates
$[S_c]$ concentration of sulfide as colloidal material
$[S_d]$ concentration of sulfide as dissolved material

After partial filtration of this solution (~ 50% of V through a 0.45 µm filter that retains 'particulates'), the volume of filtrate = V_1', and the fraction of the total volume accounted for by the filtrate is:

$$a_1 = V_1'/V;\ \text{the volume of retentate} = (1-a_1)*V$$

Filtration is conducted only to partial completion to minimize alterations in physical forms that may occur upon concentration of retained substances. At this stage, the following are concentrations of the various sulfide forms for the retentate (fraction 1), assuming that colloidal and dissolved sulfides are not retained by the filter:

$[S_p]_1 = [S_p]/(1-a_1)$
$[S_c]_1 = [S_c]$
$[S_d]_1 = [S_d]$

Concentrations of sulfide in the three forms in the filtrate (fraction 2) are:

$[S_p]_2 = 0$ (particulates do not pass through the filter)
$[S_c]_2 = [S_c]$
$[S_d]_2 = [S_d]$

The filtrate is subjected to partial ultrafiltration through a 10,000 MWCO ultrafilter, yielding a volume of ultrafiltrate = V_2', and the fraction of the original volume accounted for by the ultrafiltrate is

$$a_2 = V_2'/V_1' = V_2'/Va_1\ ;$$

At this stage, the following are concentrations of the various sulfide forms for the ultrafiltration retentate (fraction 3):

$[S_p]_3 = 0$
$[S_c]_3 = [S_c]_2/(1-a_2) = [S_c]/(1-a_2) = [S_c]/(1-(V_2'/Va_1))$
$[S_d]_3 = [S_d]$

Concentrations in the ultrafiltration filtrate (fraction 4) are:

$[S_p]_4 = 0$
$[S_c]_4 = 0$
$[S_d]_4 = [S_d]$

If sulfide is determined in fractions 1, 3, and 4, the following expressions describe total sulfide in each fraction:

$\mathbf{[S]_1} = [S_p]_1 + [S_c]_1 + [S_d]_1 = [S_p]/(1-a_1) + [S_c] + [S_d]$
$\mathbf{[S]_3} = [S_p]_3 + [S_c]_3 + [S_d]_3 = 0 + [S_c]/(1-a_2) + [S_d]$

$\mathbf{[S]_4} = [S_p]_4 + [S_c]_4 + [S_d]_4 = 0 + 0 + [S_d]$

Solving for the unknowns gives:

$[S_d] = \mathbf{[S]_4}$
$[S_c] = (1\text{-}a_2)(\mathbf{[S]_3} - [S]_d) = (1\text{-}a_2)(\mathbf{[S]_3} - \mathbf{[S]_4})$
$[S_p] = (1\text{-}a_1)(\mathbf{[S]_1} - (1\text{-}a_2)(\mathbf{[S]_3} - \mathbf{[S]_4}) - \mathbf{[S]_4})$

Sulfide was detected in fractions 1, 3, and 4 using iodometric titration. Iodometric titration allows for detection of sulfide, polysulfides, thiosulfate, and sulfite in produced water. Sulfate is inert to iodometric detection. Results from our earlier investigations revealed polysulfides, sulfite, and thiosulfate contributed <3% to the total reduced sulfur concentration; therefore, the primary sulfur species detected in produced water using iodometry is sulfide.

Sampling Procedure. Produced water was collected from the Gaviota oil processing facility in 4-L acid cleaned Nalgene heavy-walled bottles. High density polyethylene bottles (HDPE) were found to minimize sulfide volatilization relative to low density polyethylene bottles (LDPE). Bottles were cleaned following trace metal protocols (i.e., washed in detergent and soaked in 6 N trace-metal grade nitric acid (Fisher) for several weeks, followed by numerous rinses with 18 mΩ water (Nanopure, Barnstead). Samples were collected without a headspace to minimize oxygen contact and sulfide oxidation. Samples were analyzed for total sulfide within 24 hours of collection, and ultrafiltered within a week of collection. Samples were stored refrigerated at ~4°C and brought to room temperature (~25°C) before analysis.

Filtration and Ultrafiltration. 200-mL Amicon ultrafiltration cells (Model 8200, Amicon) and 62 mm Omega 10K membranes (Filtron, OM010062) were employed for ultrafiltration experiments. These filters consist of a modified polyether sulfone membrane with an approximate pore size of 0.1 nm. 0.45-µm Millipore Sterifil-D Disposable filter units (Millipore, SFHV047LS) were used for particulate removal and were disposed of after use. These filters consist of a polyvinylidene fluoride membrane with an approximate pore size of 450 nm. The filtration procedure was adapted from the method Hoffman *et. al.*[1981], developed for fractionation of dissolved organic carbon and metals. Duplicate 200-mL samples of produced water were vacuum filtered at a rate of 20 mL/min through separate 0.45-µm disposable Millipore filtration units. Samples were filtered to approximately one-half their initial volume. The volume of retentate (i.e., the >0.45-µm fraction) from filtration was measured in an acid-cleaned Nalgene graduated cylinder. After the volume was recorded, the retentate was transferred to a 125-mL acid-cleaned Nalgene bottle. The volume of remaining filtrate (~100-mL) was measured in a cleaned Nalgene graduated cylinder, and recorded. The filtrate was further filtered in the ultrafiltration cell, under positive N_2 pressure (10 psig) to half its initial volume (~50-mL). The ultrafiltrate (i.e., <10K fraction) was collected in an acid-cleaned Nalgene bottle and the volume was recorded. When the retentate had been reduced to 50% of its initial volume, the system was depressurized, and the retentate volume (the 10K-0.45-µm fraction) was recorded, and transferred to an acid cleaned Nalgene bottle. Three fractions of known volumes resulted from this fractionation scheme. Ultrafiltration membranes were cleaned by soaking in 18 mΩ water for several days, followed by filtration of 200-mL of 18 mΩ water through the filtration unit. Between analyses, ultrafiltration cells were soaked in dilute acid, then rinsed with 18 mΩ water and dried before analysis.

Reagents. All solutions were prepared with 18 mΩ water. Thiosulfate standards (Fisher Scientific, ACS grade) were prepared from $Na_2S_2O_3 \cdot 5H_2O$, and standardized against potassium iodate solution (0.1011 N, Fisher Scientific). Iodine solutions were prepared using KI (Fisher, certified ACS grade) and iodine (Fisher, certified ACS grade), and were standardized by titrating with standardized thiosulfate solution to a colorless endpoint as indicated by starch indicator solution.

Iodometric Titration. Sulfide was determined in produced water using iodometric titration [APHA, 1985]. All standards were made fresh for each collection of produced water, and were restandardized using iodometric titration every two days. Thiosulfate and iodine solutions were kept wrapped in aluminum foil to prevent light exposure. Produced water (1.00 to 5.00 mL) was added to a 250 mL flask containing 10.00 mL standardized I_2 solution and 10 mL of water. 2.00 mL of 6 M HCl was added. Titration of produced water was carried out with standardized thiosulfate solution to a colorless endpoint as detected using starch indicator solution.

TDS, DOC and ICP-AES Measurements. Conductivity and total dissolved solids (TDS) were measured using a Corning Model M10 hand-held probe (Corning). pH was measured using an Orion digital ionanalyzer/501 and a Fisher pH electrode after calibration with standard buffer solutions (Fisher). Dissolved organic carbon (DOC) was measured on 0.45-µm filtered, acidified samples by Dr. Edward Peltzer at the Woods Hole Oceanographic Institution in Woods Hole, MA using the method of Peltzer and Brewer [1993]. Salinity was measured using a hand-held refractometer (Atago).

Total metal measurements were determined by the UC Division of Agriculture and Natural Resources Analytical Laboratory, using inductively coupled plasma atomic emission spectroscopy (ICP-AES) and quantified using external calibration curves. 100-mL aliquots of produced water were acidified with 1-mL concentrated HCl (Optima grade, Fisher). NIST traceable standards were used to determine the response curve for each element.

IVB.2. Method for Ba/Ca analysis of Mussel Shells

Mussel shell fragments from the growing region was chipped off, dried, and pulverized into fine powders using a micro ball mill. Preliminary digestion of the shell powder (*ca*. 0 .5 g) was done in 6 ml of concentrated nitric acid in a Taylor tube overnight at room temperature followed by heated digestion at 50°C for 90 min, 100°C for 90 min, 140°C for 90 min (tube opening covered with a glass funnel for recondensing nitric acid vapor), 160°C for 90 min (with funnel removed), and 220°C until all nitric acid was evaporated. The residue was redissolved in 8 ml of 2% nitric acid and centrifuged at 4000 rpm to remove particulates. A parallel set of shell samples was spiked with 62.5 ppb Ba standard and processed identically as above.

The nitric digest was then subjected to inductively coupled plasma-atomic emission spectrometry (ICP-AES) for Ba and Ca. The emission wavelengths used for Ba and Ca was 455.4 and 315 nm, respectively. Other ICP measurement conditions included standard nebulizer, 17 mm torch observation height, nebulizer pressure 34-37.5 psi (adjusted daily with a Y standard), and 100 rpm pump rate. A calibration curve for Ba was composed of 0, 10, 50, 100, and 1000 ppb Ba standards while that for Ca consisted of 20, 100, and 400 ppm Ca standards. Each set of standards was run every 15 to 30 samples to check instrument drift with time; all standard curves had a linear correlation coefficient of > 0.9999.

IVB.3 Other Methods

Other methods used are as in previous years of this project, or as described previously [Higashi *et al*., 1993; Higashi and Crosby., 1994]

IVC. RESULTS & DISCUSSION

IVC.1. Gaviota Produced Water Characteristics

Produced water samples were obtained from the Gaviota processing facility on seven occasions from March to June 1995. Inorganic sulfur compounds were measured using an assortment of analytical techniques including iodometric titration of sulfide, differential pulse polarography (DPP) for determination of thiosulfate, polysulfides, and sulfite, and gas chromatography-mass spectrometry (GCMS) for determination of elemental sulfur. Sulfide levels in particular were high (millimolar concentrations) compared to concentrations known to elicit biological effects (micromolar to nanomolar). Polysulfides were the second most abundant soluble form of reduced sulfur, and were observed to play an important role in coupling equilibria between various reduced forms of sulfur (e.g., during purging of H_2S from solution).

Sample Collection Date	Sulfide by Iodometry (mM)	Thiosulfate by DPP (µM)	Polysulfides by DPP (µeq/L S(0))	Elemental sulfur by GC/MS (mg/L)	Sulfite by DPP (µM)
3/1/95	6.8	520	544	0.57	< LOD
3/21/95	8.1	96	516	42.4	< LOD
4/13/95	4.4	288	364	20.7	53
4/27/95	3.8	39	254	1.73	< LOD
5/11/95	2.8	160	238	3.58	21
5/31/95	1.2	145	212	1.89	< LOD
6/16/95	2.8	88	226	1.60	< LOD

> LOD = less than limit of detection

The distribution of sulfide among dissolved, particulate, and colloidal phases was investigated by using a sequential filtration/ultrafiltration scheme using a 0.45 µm filter to retain particles and a 10,000 molecular weight cut-off (MWCO) ultrafilter to retain colloids. Measurements found 85 ± 9% of the sulfide in produced water in the "dissolved" fraction; the "particulate" fraction contained 10 ± 7% sulfide, and the "colloidal" fraction accounted for 5 ± 3% of produced water sulfide. Mixing of produced water with comparable volumes of seawater did not caused marked differences in the distribution among these physical forms.

Additional water quality parameters including dissolved organic carbon (DOC), pH, salinity, sulfate concentration, and total dissolved solids were also measured. Sulfate concentrations varied over a wide range (45 - 1373 mM) and DOC levels were also high (117-440 mg/L). The overwhelming majority of organic matter measured in the DOC determinations consisted of polar substances not measured using CH_2Cl_2 extractions and GC-MS.

Collection Date	Sulfate (mM)	Dissolved Organic Carbon (mg/L)	Salinity (ppt)	Total Dissolved Solids (g/L)	pH
3/1/95	45	359	19.0	16.7	7.30
3/21/95	269	117	12.4	23.1	7.72
4/13/95	372	223	20.0	17.4	8.12
4/27/95	553	440	20.0	16.9	8.34
5/11/95	301	330	23.0	19.2	8.62
5/31/95	115	160	21.0	17.5	8.29
6/16/95	1373	133	20.0	16.3	8.29

Gaviota produced water samples were also analyzed for several trace elements using ICP-AES. Concentrations of barium ranged from about 1-6 ppm and were similar to Carpinteria produced water samples. Millimolar concentrations of calcium were also found. Concentrations of iron, aluminum, and chromium were low and barely above method limits of detection.

Collection Date	Ca (mM)	Fe (ppm)	Al (ppm)	Ba (ppm)	Cr (ppm)
3/1/95	21.3	0.13	<0.10	1.1	0.15
3/21/95	24.0	0.15	<0.10	3.8	0.15
4/13/95	22.9	0.15	<0.10	0.8	0.10
4/27/95	22.5	0.17	<0.10	1.6	0.15
5/11/95	27.6	0.15	<0.10	6.2	0.15
5/31/95	48.5	1.69	0.12	2.1	0.25
6/16/95	ND	ND	ND	ND	ND

ND = not determined

Produced water samples were extracted with CH_2Cl_2 and analyzed by GCMS without further clean-up. This approach allows for detection of a wide range of compounds varying from nonpolar hydrocarbons to moderately polar organics including many organic acids, phenols, and nitrogen-containing compounds. Most notable was the appearance of about 41 mg/L of several organic polysulfide heterocycles which appear to form from reactions of inorganic polysulfides with acetaldehyde (or biological precursors such as pyruvate or lactate). The transient appearance of these compounds points to substantial variability in levels of intermediate polarity organic compounds in produced waters and may indicate important contributions of microbial action in the formation of produced water constituents. Levels of elemental sulfur showed a sharp drop in the same produced water where the sulfur heterocycles were observed. Patterns of short chain fatty acids also exhibited substantial variability and lent further support to the notion of microbial mediation of produced water composition. Gaviota produced water samples also contained a series of phenols (total phenol concentrations ranged from 0.2-5.4 mg/L), solvent range aromatic hydrocarbons (benzene, toluene, and xylenes) in low mg/L concentrations, several nitrogen-containing compounds including some N,N-dimethylalkylamine surfactants, and two additional sulfur compounds identified as benzenemethanethiol and thiophene-3-carboxaldehyde (putative). Numerous additional minor constituents remain to be identified. Analyses of extracts of outplanted mussels did not yield detectable levels of the major identified organic constituents of produced water, as most of these compounds are expected to be either rapidly metabolized and not efficiently bioconcentrated.

Collection Date		**3-1-95**	**3-21-95**	**4-13-95**	**4-27-95**	**5-11-95**	**5-31-95**	**6-16-95**
Compound	**tr (min)**	**(mg/L)**	**(mg/L)**	**(mg/L)**	**(mg/L)**	**(mg/L)**	**(mg/L)**	**(mg/L)**
Miscellaneous Compounds								
Benzene	3:22	0.016	1.45	0.122	0.167	0.039	0.018	0.005
Toluene	4:15	0.068	3.39	0.494	0.651	0.009	0.038	0.046
Xylenes*	--	0.034	1.54	0.510	1.12	0.274	nd	nd
3-Thiophene carboxaldehyde	6:21	0.076	nd	0.393	nd	nd	nd	nd
Benzenemethanethiol	7:13	0.027	2.27	0.959	0.742	1.23	0.242	0.811
Polysulfide heterocycles*	--	nd	nd	nd	40.7	nd	nd	nd
MW 156	8:46	nd	nd	nd	nd	0.296	nd	0.229
MW 170	9:38	0.051	1.78	0.781	nd	1.07	nd	1.05
MW 184	11:36	nd	nd	nd	nd	0.441	nd	0.455
MW 167	12:05	nd	nd	nd	nd	nd	0.243	0.281
MW 192	12:34	nd	nd	nd	nd	nd	0.127	0.252
MW 192	14:49	0.032	1.18	nd	nd	0.384	0.244	nd
$C_{14}H_{13}N$	15:38	0.224	1.42	1.05	2.36	0.784	0.289	1.30
MW 192	15:54	nd	nd	nd	nd	nd	0.240	nd
Subtotal (mg/L)		**0.528**	**13.0**	**4.31**	**45.7**	**4.53**	**1.44**	**4.43**
Fatty Acids								
Butanoic	5:00	0.011	0.875	0.250	1.01	0.701	0.177	0.863
Branched C5	5:28	0.018	0.939	0.286	0.204	0.274	0.086	0.447
Pentanoic	5:58	0.009	0.612	0.339	1.37	0.499	0.178	0.891
Branched C6	6:35	0.057	2.20	0.809	nd	1.97	0.206	2.17
Hexanoic	6:47	0.011	0.875	0.220	1.25	nd	0.218	0.442
Branched C7	6:54	0.094	1.07	0.791	2.04	1.14	0.220	0.411
Branched C7	7:20	0.015	0.731	0.279	0.479	0.421	0.126	0.811
Heptanoic	7:38	0.027	0.668	0.386	nd	1.02	0.143	0.301
Subtotal (mg/L)		**0.242**	**7.97**	**3.36**	**6.35**	**6.03**	**1.35**	**6.34**
Phenols								
C1-Phenol	6:57	0.045	0.494	nd	nd	nd	0.328	nd
C1-Phenol	7:09	0.029	nd	nd	0.681	nd	nd	0.572
C2-Phenol	7:26	0.003	0.608	0.227	(0.127)	0.350	0.065	0.367
C2-Phenol	7:44	0.008	0.239	0.115	(0.793)	0.196	nd	0.618
C2-Phenol	7:52	0.012	0.485	0.214	(0.868)	0.272	0.310	0.129
C2-Phenol	8:03	0.009	0.987	0.425	(0.187)	0.288	0.035	0.222
C2-Phenol	8:09	0.014	0.679	0.142	0.240	0.174	0.099	1.17
C3-Phenol	8:18	0.030	1.05	0.449	0.330	0.463	0.324	nd
C2-Phenol	8:21	0.006	0.328	0.165	2.02	0.357	0.047	nd
C2-Phenol	8:35	0.004	0.444	0.182	0.179	0.187	0.025	nd
Subtotal (mg/L)		**0.160**	**5.31**	**1.92**	**5.43**	**2.29**	**1.23**	**3.08**

*Σ of analogs/isomers; nd = not detected; LOD ~1 µg/L; values in () are interpolated due to peak overlap

IVC.2. Mussel Shell Ba

Mussels outplanted at Carpinteria for three months were sampled for their new-growth shell material by the project of Osenberg *et al*. Ca was analyzed in addition to Ba in order to normalize to the carbonate shell mass, since shell may also contain non-carbonate material. The results of Ba/Ca for *Mytilus californianus* is shown in **Figure 7**, and that for *Mytilus edulis* is in **Figure 8**.

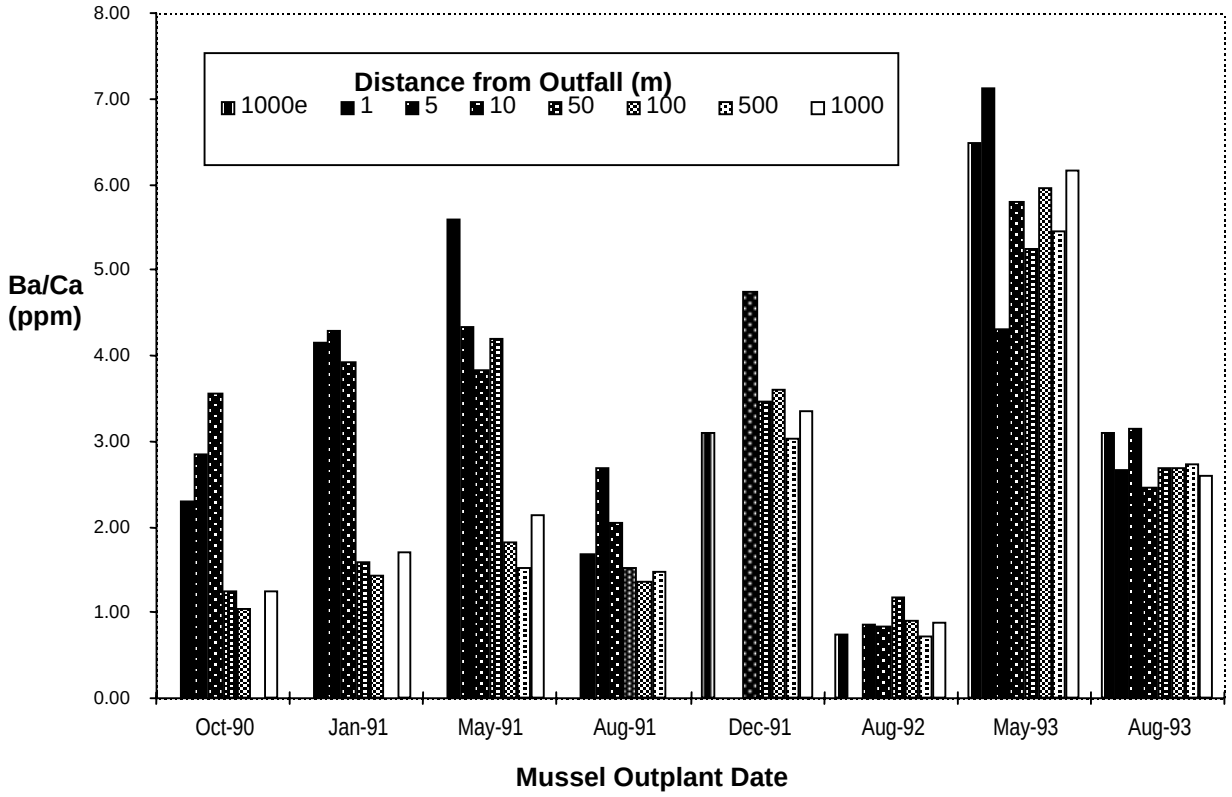

Figure 7. Ba concentrations (ppm) relative to Ca in new-growth shell material from *M. californianus* outplanted at the Carpinteria site. Eight outplant experiments, each at various distances from the PW outfall is plotted here. Exposure periods were 3 months.

Figure 7 reveals that *M. californianus*, in some outplant sessions, showed a clear decreasing trend in shell Ba/Ca levels with increasing distance from the outfall (Oct. '90, Jan. '91, May '91, Aug. '91, Dec '91), but at the other times did not show a trend at all. Note that, when sampled, site 1000m east of the outfall (vertical striped bars, seasonally prevailing upcurrent site) were comparable to 1000m west (white bars). Although there is no immediately obvious explanation to the results, the data is undergoing statistical tests by Osenberg *et al*., particularly in relation to Carpinteria discharge rates, prevailing current and environmental conditions, and biological parameters. This analysis may explain the trends observed in the Ba/Ca data.

Figure 8 shows that *M. edulis*, for the two outplant sessions, did not show a Ba/Ca trend in May '91, which was a time that *M. californianus* exhibited a strong trend (**Figure 7**), suggesting that *M. edulis* may resist the incorporation of Ba into their shells. Also note the Ba/Ca concentration

difference in the two figures. Again, Osenberg *et al*. is in the process of analyzing this data in relation to other factors.

This shell Ba/Ca data should also be compared against other interesting Ba data found in the final technical report of the project of Cherr & Fan.

Although subject to the data analysis conclusions of Osenberg *et al*., this data supports the hypothesis that, in the field, Ba is accumulated by *M. californianus* and recorded in their newly-grown shell material. Some of these trends are striking considering the high sulfate in seawater and dilution factors in the open coastal ocean.

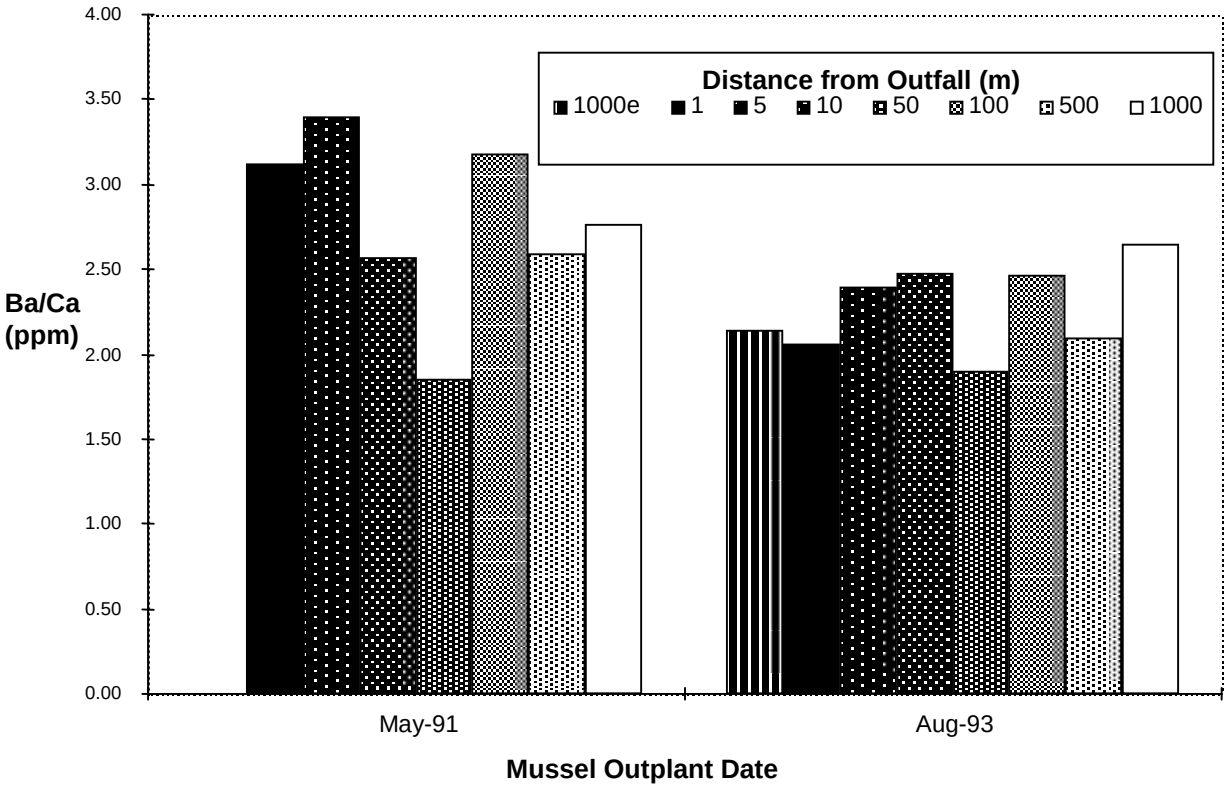

Figure 8. Ba concentrations (ppm) relative to Ca in new-growth shell material from *M. edulis* outplanted at the Carpinteria site. Two outplant experiments at various distances from the PW outfall is plotted here. Exposure periods were 3 months.

REFERENCES

APHA. 1985. *Standard Methods for the Examination of Water and Wastewater*, Port City Press, Baltimore, MD.

Armstrong, H.W., K. Fucik, J. Anderson, and J.M. Neff. 1979. Effects of oil field brine effluent on sediments and benthic organisms in Trinity Bay, Texas. *Mar. Environ. Res.* **2**:55-69.

Boehm, P.D. 1987 Transport and transformation processes regarding hydrocarbon and metal pollutants in offshore sedimentary environments. In: *Long-term environmental effects of offshore oil and gas development.* D.F. Boesch & N.N. Rabalais, eds., Elsevier Applied Science, New York, pp. 233-286.

Boesch, D.F. and N.N. Rabalais, eds. 1987 *Long-term Environmental Effects of Offshore Oil and Gas Development,* Elsevier Applied Science, New York, 708pp.

Cherr, G.N., J. Shoffner-McGee, and J.M. Shenker. 1990. Methods for assessing fertilization and embryonic/larval development in toxicity tests using the California mussel (*Mytilus californianus*). *Environmental Toxicology and Chemistry* **9**:1137-1145.

Ellman, G.L. 1959. *Arch. Biochem. Biophys.* **82**:70-77

Fan, T.W.-M., R.M. Higashi, G.N. Cherr, and M.C. Pillai. 1993. Use of noninvasive NMR spectroscopy and imaging for assessing produced water effects on mussel reproduction, In: *Produced Water: Technological/Environmental Issues and Solutions*, J.P. Ray, ed., Plenum Publishing, New York, pp. 403-414.

Farroha, S.M., A.E. Habbrough, M.N. Hicheal. 1984. Determination of milligram amounts of sulfur in hydrocarbons by constant current coulometry. *Anal. Chem.* **56**:1182-1183

Haider, K. and H-R. Schulten. 1985. Pyrolysis field ionization mass spectrometry of lignins, soil humic compounds, and whole soil. *J. Anal. Appl. Pyrolysis* **8**:317-331.

Hayes, M.H.B. 1991. Concepts of the origins, composition, and structures of humic substances. In: *Advances in Soil Organic Matter Research: The Impact on Agriculture and the Environment*, W.S. Wilson, ed., The Royal Society of Chemistry, Cambridge, pp. 3-22.

Higashi, R.M., G.N. Cherr, C.A. Bergens, and T.W-M. Fan. 1993. An approach to toxicant isolation from a produced water source in the Santa Barbara Channel, In: *Produced Water: Technological/Environmental Issues and Solutions*, J.P. Ray, ed., Plenum Publishing, New York, pp. 223-233.

Higashi, R.M. and D.G. Crosby. 1994. Environmental Degradation and Identification of Toxic Compounds of Petroleum and Associated Materials - Final Technical Report.

Hoffman, M.R., E.C. Yost, S.J. Eisenreich, W.J. Maier. 1981. Characterization of soluble and colloidal phase metal complexes in river water by ultrafiltration: a mass-balance approach. *Environ. Sci. Technol.* **15**:655-661

Karickhoff, S.W. 1980. Sorption Kinetics of Hydrophobic Pollutants in Natural Sediments. In: *Contaminants and Sediments*: Volume 2, R.A. Baker (ed.), Ann Arbor Science, Ann Arbor, Michigan.

Lara, R., W. Ernst. 1989 Interaction Between Polychlorinated Biphenyls and Marine Humic Substances. Determination of Association Coefficients. *Chemosphere* **19**:1655-1664.

Luther, G.W., A.E. Giblin, R. Varsolona. 1985. Polarographic analysis of sulfur species in marine porewaters. *Limnol. Oceanogr.* **30**:727-736

Malcolm, R.L. and P. McCarthy. 1991. The individuality of humic substances in diverse environments. In: *Advances in Soil Organic Matter Research: The Impact on Agriculture and the Environment*, W.S. Wilson, ed., The Royal Society of Chemistry, Cambridge, pp. 23-34.

National Research Council. 1985. *Oil in the Sea: Inputs, Fates, and Effects*. National Academy Press, Washington, D.C., 601pp.

Neff, J.M. 1987. Biological effects of drilling fluids, drill cuttings and produced waters. In: *Long-term environmental effects of offshore oil and gas development*. D.F. Boesch & N.N. Rabalais, eds., Elsevier Applied Science, New York, pp. 469- 538.

Peltzer, E.T. and P.G. Brewer. 1993. Some practical aspects of measuring DOC – sampling artifacts and analytical problems with marine samples. *Mar. Chem.* **41**:243-252

Schmitt, R.J., ed. 1991 Southern California Educational Initiative Program Overview: Year 2, Coastal Research Center, Marine Science Institute, University of California Santa Barbara, 174pp.

Schnitzer, M. and H.R. Schulten. 1992. The analysis of soil organic matter by pyrolysis-field ionization mass spectrometry. *Soil Sci. Soc. Am. J.* **56**:1811-1817.

Senesi, N., Y. Chen. 1989. Interaction of Toxic Organic Chemicals with Humic Substances. In: *Toxic Organic Chemicals in Porous media*, Z. Gerstl, Y. Chen, U. Mingelgrin, B. Yaron, (eds.) Springer-Verlag, New York.

Strek, H.J. and J.B. Weber. 1982. Behaviour of polychlorinated biphenyls (PCBs) in soils and plants. *Environmental Pollution* **A 28**:291-312.

Suffet, I.H., MacCarthy, P. (eds). 1989. *Aquatic Humic Substances, Influence on Fate and Treatment of Pollutants*, American Chemical Society, Washington, DC.

Vairavamurthy, A. and K. Mopper. 1990. Determination of sulfite and thiosulfite in aqueous samples including anoxic seawater by liquid chromatography after derivatization with 2,2'–dithiobis (5-nitropyridine). *Environ. Sci. Technol.* **24**:333-337.

van Loon, W.M.G.M.; J.J. Boon, and B. Degroot. 1991. Qualitative analysis of chlorolignins and lignosulphonates in pulp mill effluents entering the River Rhine using pyrolysis mass spectrometry and pyrolysis gas chromatography mass spectrometry. *Journal of Analytical and Applied Pyrolysis* **20**:275-302.

The Department of the Interior Mission

As the Nation's principal conservation agency, the Department of the Interior has responsibility for most of our nationally owned public lands and natural resources. This includes fostering sound use of our land and water resources; protecting our fish, wildlife, and biological diversity; preserving the environmental and cultural values of our national parks and historical places; and providing for the enjoyment of life through outdoor recreation. The Department assesses our energy and mineral resources and works to ensure that their development is in the best interests of all our people by encouraging stewardship and citizen participation in their care. The Department also has a major responsibility for American Indian reservation communities and for people who live in island territories under U.S. administration.

The Minerals Management Service Mission

As a bureau of the Department of the Interior, the Minerals Management Service's (MMS) primary responsibilities are to manage the mineral resources located on the Nation's Outer Continental Shelf (OCS), collect revenue from the Federal OCS and onshore Federal and Indian lands, and distribute those revenues.

Moreover, in working to meet its responsibilities, the **Offshore Minerals Management Program** administers the OCS competitive leasing program and oversees the safe and environmentally sound exploration and production of our Nation's offshore natural gas, oil and other mineral resources. The MMS **Royalty Management Program** meets its responsibilities by ensuring the efficient, timely and accurate collection and disbursement of revenue from mineral leasing and production due to Indian tribes and allottees, States and the U.S. Treasury.

The MMS strives to fulfill its responsibilities through the general guiding principles of: (1) being responsive to the public's concerns and interests by maintaining a dialogue with all potentially affected parties and (2) carrying out its programs with an emphasis on working to enhance the quality of life for all Americans by lending MMS assistance and expertise to economic development and environmental protection.