Exxon Valdez Oil Spill
State/Federal Natural Resource Damage Assessment Final Report

Fate and Toxicity of Spilled Oil from the *Exxon Valdez*

Subtidal Study Number 4
Final Report

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Study History: This study was originally undertaken in 1989 under the aegis of Air/Water Project Number 4. It was expanded in 1990 at the request of the NRDA Trustee Council, in response to specific recommendations from the Department of Justice's Peer Reviewers, and renamed Air/Water Project Number 6. In 1991, the study was again relabeled as Subtidal Study Number 4.

Abstract: Three separate papers are represented in this final report; Toxicity of intertidal and subtidal sediments contaminated by the *Exxon Valdez* oil spill; Comparative toxicities of polar and non-polar organic fractions from sediments affected by the *Exxon Valdez* oil spill in Prince William Sound, Alaska; and Fate of the oil spilled from the *T/V Exxon Valdez* in Prince William Sound, Alaska.

Key Words: *Ampelisca Abdita*, *Crassostrea gigas*, *Exxon Valdez*, oil fate, oil effects, Microtox, *Mytilus*, non-polar hydrocarbons, polar hydrocarbons, sediment toxicity, SOS Chromotest, toxicity

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EXECUTIVE SUMMARY

This study was originally undertaken in 1989 under the aegis of Air/Water Project number 4. It was expanded in 1990 at the request of the *Exxon Valdez* Oil Spill State/Federal Natural Resource Damage Assessment (NRDA)Trustee Council, in response to specific recommendations from the Department of Justice's Peer Reviewers, and renamed Air/Water Project number 6. In 1991, the study was again relabeled as Subtidal Project number 4. The present final report encompasses all the activities undertaken since 1989 under these various project designations. The study was designed to: a) determine the toxicity of oiled environmental samples, using standard toxicity tests; b) examine the extent to which any observed toxicity may be attributed to oxygenated, polar products in weathered oil (versus the parent hydrocarbons found in fresh crude); and c) promote the synthesis of data and information (generated largely by other projects) on the geographic distribution, weathering, and fate of the spilled *Exxon Valdez* oil (EVO). These major study topics are described in detail in separate sections of this report. This project has involved extensive collaboration of National Marine Fisheries Service (NMFS) scientists from the Environmental Conservation Division of Northwest Fisheries Science Center (NWFSC) and the Auke Bay Laboratory of the Alaska Fisheries Science Center, as well as projects carried out under contract to National Oceanic and Atmospheric Administration (NOAA) by Science Applications International Corporation (SAIC).

To satisfy objective (a), toxicity testing was carried out on sediment samples taken during the cruises of the *Fairweather* in 1989, the *Davidson* in 1990, and *The Big Valley* in 1991, using standard sediment toxicity bioassays (Microtox^{R} in 1989 and amphipod and bivalve larvae tests in 1990 and 1991). Petroleum hydrocarbons were analyzed by ultraviolet fluorescence spectroscopy on the samples collected in 1989 and 1990. Results indicate that some toxicity was still associated in 1990 with sediments from the lower intertidal zones of heavily oiled sites. While there were some indications of continued reduced intertidal toxicity in 1991, and of associated toxicity in shallow (0-6 m) subtidal sediments, no statistically significant differences between oiled and unoiled sites were demonstrated for intertidal sediments in 1991 or for subtidal sediments in either year.

Objective (b) was addressed through studies carried out by SAIC scientists under contract to NOAA, on the extent to which any toxicity present in oiled sediments and interstitial waters could be attributed to polar oxidation products (as opposed to parent hydrocarbons) in petroleum. A battery of toxicity tests was applied individually to chromatographically separated polar and non-polar fractions of the organic extracts from sediments and water from oiled and unoiled sites in Prince William Sound (PWS). Results indicated that although the overall toxicity of sediment and porewater extracts was generally low at the oiled site, it was consistently greater than that at the unoiled site, and a portion of that toxicity was attributed to the polar fractions of the extracts.

Objective (c) required a major synthesis function. Data and information on the distribution and fates of EVO need were assembled from a wide variety of sources, including

studies performed during the response phase of the spill, and damage assessment studies performed either with State-Federal or Exxon support. These data were interpreted in the light of previously existing information and models to derive best estimates for different processes affecting the fate of the spilled oil from the *Exxon Valdez*, and are presented here as an integrated mass balance through summer 1992.

OBJECTIVES

A. Determine whether contaminated sediments and related environmental samples were toxic to selected marine biota

B. At selected sites, document and quantify the occurrence of oxidized derivatives of EVO Valdez oil

C. Determine the extent to which any observed toxicity of oil-contaminated environmental samples would be attributable to oxidation products of petroleum

D. Construct a summary budget or "mass balance" summarizing the fate of the spilled oil

INTRODUCTION

The sediment toxicity surveys conducted under this project's objective (A) were initiated in 1989 under A/W Study number 4 as part of the multidisciplinary studies conducted aboard the NOAA vessel *Fairweather*. Other portions of this study were designed and undertaken in 1990 by NOAA at the request of the NRDA Trustee Council, partly in response to specific recommendations from the Department of Justice's Peer Reviewers. The project was originally designated A/W Study number 6, then redesignated Subtidal Study number 4. This report covers all activities undertaken under A/W4, A/W6, and S/T4. The study was designed to: a) demonstrate and quantify the toxicity of oiled environmental samples, using standard toxicity tests; b) determine the extent to which any observed toxicity may be attributed to oxygenated, polar products in weathered oil (versus the parent hydrocarbons found in fresh crude); and c) synthesize and summarize data and information (generated largely by other projects) on the geographic distribution, weathering, and ultimate fate of the petroleum spilled by the *Exxon Valdez*. These three objectives are addressed separately in the following three main sections of this report. This study was carried out in close coordination with Subtidal Studies Number 1 and 2, directed by the NMFS Auke Bay Laboratory and the Alaska Department of Environmental Conservation, respectively, and relied heavily also on collaboration with personnel of the NMFS Northwest Fisheries Science Center. Each of the following sections has received preliminary review by the individuals listed as coauthors.

All samples under this study were taken with careful adherence to Chain-of-Custody requirements. Chemical analyses reported here for intertidal and subtidal sediment samples were analyzed either by Dr. Peggy Krahn, NWFSC Environmental Conservation Division, or by Texas A&M University's Geochemical and Environmental Research Group, in accord with the QA/QC guidelines provided by the Technical Services 1 Analytical Committee. Methodologies for collection and analysis of intertidal and subtidal sediment samples are described in the relevant scientific sections of this report.

Toxicity of Intertidal and Subtidal Sediments Contaminated by the *Exxon Valdez* Oil Spill

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ABSTRACT

To estimate the potential toxicity of sediments oiled by the *Exxon Valdez* oil spill (EVOS), standardized toxicity tests were applied to intertidal (0 m) and subtidal (three to five depths ranging from 3 to 100 m) sediment samples taken from PWS and the nearby Gulf of Alaska (GOA) during 1989, 1990, and 1991. In 1989, the sediment toxicity was surveyed with Microtox^R at 43 (mostly spill-impacted) sites in PWS (18) and the GOA (25). Toxicity measured by Microtox^R in shallow subtidal (0-6 m) sediments showed a generally decreasing trend with increasing distance from the spill center. At the PWS sites, however, where the ranges of total hydrocarbon concentrations were greatest (at 0 and 3 m) and where those concentrations were related most certainly to EVO, the Microtox^R response did not show clear dose-response relations to ultraviolet fluorescence (UVF) signal. Laboratory tests confirmed the non-responsiveness of the Microtox^R test to EVO, and the test was not used further. In 1990 sediment toxicities at oiled and unoiled sites were compared (at 21 sites inside PWS and 8 outside) and 1991 (at 15 PWS sites) using a sediment elutriate test with larval oysters (*Crassostrea gigas*) and a whole sediment test with the amphipod *Ampelisca abdita*. The 1990 amphipod results indicated that: a) mortality was correlated with hydrocarbon concentrations in intertidal sediments, b) intertidal toxicity was substantially higher than subtidal toxicity, and c) mean toxicity of intertidal sediments at exposed sites was significantly higher than at reference sites. Mean mortalities in subtidal sediments (at 6, 20, and 40 m) were not significantly different between exposed and reference sites, although concentrations of total hydrocarbons were generally higher at the exposed sites down to 20 m. In 1991, toxicity was generally low at all depths at all sites. While some 1991 sediment samples (from 0, 6, and 20 m) caused mortalities of test organisms significantly greater than in controls, no significant differences in toxicity between oiled and reference sites were demonstrated with either amphipods or oyster larvae. Between 1990 and 1991, the relative toxicity of sediments from exposed sites (compared to reference sites) decreased for intertidal (0 m) sediments and may have increased slightly for shallow subtidal (6 m) sediments. These test results suggest that residual toxicity was still present during summer of 1990 in intertidal (0 m) sediments from some sites that were heavily oiled in 1989. By 1991, the toxicity of these sediments had diminished to background levels. These studies demonstrated no significant toxicity to test amphipods or oyster larvae that was clearly associated with EVO in subtidal sediments (6 to 40 m) during 1990-1991.

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INTRODUCTION

The 24 March 1989 grounding of the *Exxon Valdez* on Bligh Reef in PWS released approximately 35,500 metric tons (10.8 million gallons) of Prudhoe Bay crude oil (PBCO) into the Alaskan marine environment (Kelso and Kendziorek 1991; Maki 1991). Over the next several weeks much of this oil was distributed onto about 575 km (360 miles) of shoreline in PWS and about 1170 km (730 miles) in the GOA (Maki 1991). As a combined result of cleanup activities and storm-related wave action, this beached oil was subjected to removal and rigorous physical dispersion throughout the following summer and winter months, and a fraction of it migrated into adjacent shallow subtidal zones (O'Clair et al. 1993; Wolfe et al. 1993a, 1993b). As part of the State-Federal NRDA, a coordinated effort was designed to assess the distribution, persistence, and potential effects of petroleum in the lower intertidal and associated subtidal areas impacted by the EVOS. In this paper we report the results of standard toxicity test protocols, which were applied to assess the toxicity of sediments from the lower intertidal and shallow (from 0 to 100 m depths) subtidal zones during the first three years after the spill. Results from related studies on replicate sediment samples taken at the same times and stations described here are being published elsewhere on detailed hydrocarbon compositions and concentrations (O'Clair et al. 1993) and on microbial hydrocarbon degradation potentials (Braddock et al. 1993).

A very considerable body of literature on the toxicity of Alaskan crude oil to Arctic and subarctic marine invertebrates and fishes existed prior to the spill. Much of the early work in this area focused on the acute toxicities (generally 96 h exposures) of water-soluble fractions (WSF) of fresh Cook Inlet crude oil and PBCO to a variety of species and life stages of commercially or recreationally important Alaskan marine organisms. Data on the acute toxicities of crude oil to marine organisms of interest have been summarized by Brodersen et al. (1977), Craddock (1977), Moles et al. (1979), Rice et al. (1976, 1977, 1979, 1984), and NRC (1985). In general, LC-50's for the WSF of PBCO lie in the range of 0.1 to 2 mg L^{-1} for most organisms tested. Rice et al. (1981) demonstrated that the compositions of the water-soluble fractions of Cook Inlet and PBCOs were very similar both to one another and to that of the discharge from the ballast treatment facility at Valdez.

Sublethal effects of oil exposure have also been studied extensively, through the use of long-term exposures (e.g., up to 40 days) to WSF of Alaskan crude oil, or of prolonged exposure to oiled food or oiled sediments. Earlier work (which focused primarily on temperate organisms and crude oils from sources other than Alaska) was summarized by Anderson (1977), Johnson (1977), and Patten (1977). During the late 1970's and early 1980's, increased attention was given to arctic and subarctic organisms, especially relative to Alaskan and Canadian oils, and some of this more recent work has been reviewed by Rice et al. (1984), Rice (1985), Wolfe (1985), NRC (1985), and Karinen (1988). While it has been well documented that spilled petroleum constituents may persist for years in muddy sediments and continue to affect resident organisms (Mayo et al. 1978; Teal et al. 1978; Dauvin and Gentil 1990), relatively little work has been performed to characterize the threshold conditions for biological responses and toxicity caused by petroleum associated with fine-grained sediments.

In an effort to quantify the potential effects of the spilled EVO in the sediment environment, we applied recently developed standard sediment toxicity test protocols to intertidal and subtidal sediment samples from oiled and unoiled sites in PWS and the adjoining GOA. In this work we were primarily concerned with a) comparing sediment toxicity in relation to petroleum levels at oiled and unoiled sites; b) the potential transport of toxic petroleum constituents from the intertidal zone into adjacent subtidal sediments; and c) the changes in toxicity with time.

METHODS

Sampling Locations and Sediment Sampling

Sediment sampling was carried out from the NOAA Ship *Fairweather* between June 29 and August 22, 1989 (Table 1); from the NOAA Ship *Davidson* between June 25 and August 5, 1990 (Table 2); and from the charter vessel *The Big Valley* between June 15-25, 1991 (Table 2). All samples tested were composites of approximately equal amounts of surficial sediment, either from each of eight randomly located positions along a 30 m transect at 0, 3, 6, and 20 m depths, or from each of 3 Van Veen grabs from 40 and 100 m depths.

Surficial sediments (0-1 cm) were collected for hydrocarbon analysis by UVF and for toxicity testing by Microtox^{R} (1989 only) with a pre-cleaned non-contaminating spatula into precleaned 20 mL glass containers with teflon-lined caps. Two liters of surficial (0-2 cm) sediment were collected at each depth and site in 1990 and 1991 for toxicity testing with *Crassostrea* larvae and *Ampelisca*. These latter samples were kept cold (1-4°C) for up to eleven days before initiation of toxicity testing in Seattle, WA. In all years, separate replicate composite samples (i.e. from the same randomly located positions or grabs, but composited and homogenized separately) were taken concurrently from the same stations for chemical analysis by gas chromatography/mass spectrometry (GC/MS) and sediment grain-size analyses, and results are discussed elsewhere for many of those samples (O'Clair et al. 1993). The 1989 and 1990 sites, selected on the basis of preliminary information on distribution of floating and/or beached oil, encompassed the full geographic range of significant spill exposure (i.e. from Bligh Island in PWS to Katmai Bay on the Alaska Peninsula), whereas the 1991 sampling for toxicity testing was restricted to 8 "exposed" sites and 7 "reference" sites within PWS. The positions shown in Tables 1 and 2 are for the intertidal (mean low water- 0 m depth) sampling locations at each site; subtidal samples were taken at 3, 6, 20, 40 and 100 m depths located as near as possible to the intertidal locations. In 1989 samples from all depths were tested for toxicity, while in 1990 and 1991, only four depths (0, 6, 20, and 40 m) were so tested.

In 1989, samples were extracted and analyzed on board ship (SAIC 1989). A weighed portion (1-5 g) of sediment, mixed with an equal weight of anhydrous sodium sulfate, was

Table 1. Stations sampled in 1989 for toxicity testing with the Microtox[R] protocol.

Site No.	Site Name	Date Sampled	North Latitude	West Longitude
00	Kodiak Harbor	29-Jun	57°47.94'	152°22.20'
01	Fox Farm	01-Jul	59°58.43'	148°10.50'
02	Sawmill B. area	02-Jul	60°00.16'	147°58.90'
03	Shelter Bay	03-Jul	60°06.52'	147°58.90'
04	Iktua Bay	04-Jul	60°06.00'	147°59.70'
05	Mummy Island	05-Jul	60°17.26'	147°54.38'
06	Snug Harbor	06-Jul	60°14.38'	147°43.11'
07	Green Island	07-Jul	60°16.30'	147°26.30'
08	Bay of Isles	08-Jul	60°23.00'	147°44.90'
09	Smith Island	09-Jul	60°31.79'	147°20.75'
10	Cabin Bay	10-Jul	60°39.35'	147°26.30'
11	Columbia Bay	11-Jul	60°39.00'	147°01.40'
12	Northwest Bay	12-Jul	60°33.05'	147°34.70'
13	Disk Island	13-Jul	60°29.90'	147°39.50'
14	Herring Bay	14-Jul	60°25.90'	147°47.20'
15	Eshamy Bay	15-Jul	60°26.82'	147°58.50'
16	Sleepy Bay	16-Jul	60°04.14'	147°50.58'
17	Rocky Bay	17-Jul	60°20.20'	147°08.15'
18	Olsen Bay	18-Jul	60°45.13'	146°11.50'
19	Knowles Bay	19-Jul	60°40.68'	146°37.70'
20	Fox Island	25-Jul	59°56.20'	149°19.00'
21	Agnes Cove	26-Jul	59°46.00'	149°34.40'
22	Taroka Arm	27-Jul	59°37.54'	150°08.30'
23	Black Bay	28-Jul	59°32.12'	150°12.28'
24	McArthur Cove	29-Jul	59°26.60'	150°20.50'
25	Tonsina Bay	30-Jul	59°18.70'	150°55.00'
26	Gore Point	31-Jul	59°14.23'	150°58.79'
27	Port Dick	01-Aug	59°17.25'	151°08.75'
28	Windy Bay	02-Aug	59°13.84'	151°31.00'
29	Chugach Bay	03-Aug	59°11.20'	151°37.80'
30	Seldovia Bay	04-Aug	59°25.85'	151°44.30'
31	Ursus Cove	05-Aug	59°30.80'	153°45.40'
32	Amakdedori Beach	06-Aug	59°16.50'	154°07.80'
33	Douglas Beach	07-Aug	59°00.00'	153°29.50'
34	Ushagat Island	08-Aug	58°56.97'	152°17.61'
35	Andreon Bay	09-Aug	58°30.20'	152°25.10'
36	King Cove	14-Aug	58°11.03'	152°03.30'
37	Cape Douglas	15-Aug	58°50.54'	153°21.00'
38	Hallo Bay	16-Aug	58°27.48'	154°00.23'
39	Katmai Bay	17-Aug	57°58.50'	155°01.80'
40	Halibut Bay	18-Aug	57°21.47'	154°45.10'
41	Wide Bay	19-Aug	57°26.42'	156°15.12'
42	Chignik Bay	20-Aug	56°19.68'	158°25.40'
43	Ivanof Bay	21-Aug	55°52.12'	159°31.01'
44	Zachary Bay	22-Aug	55°19.55'	160°36.50'

Table 2. Names and Locations of Sites Sampled for Toxicity Testing and Chemical Analysis of Sediments in 1990 and/or 1991.

1990 Site No.	Name	Nominal Oiling Designation*	Latitude	Longitude	1991 Site No.
01	Olsen Bay	R	60° 44.80' N	146° 13.10' W	---**
02	Port Fidalgo	R	60° 50.20' N	146° 12.58' W	---
03	Smith Island	E	60° 31.80' N	147° 20.80' W	---
04	Zaikof Bay	R	60° 16.85' N	147° 02.10' W	15
05	Rocky Bay	R	60° 20.30' N	147° 08.20' W	14
06	West Bay	R	60° 51.80' N	146° 46.50' W	01
07	Herring Bay	E	60° 26.54' N	147° 47.13' W	05
08	Disk Island	E	60° 29.80' N	147° 39.70' W	03
09	Block Island	E	60° 31.70' N	147° 36.40' W	04
10	Northwest Bay	E	60° 33.10' N	147° 34.60' W	02
11	NE Knight Island	E	60° 26.35' N	147° 37.65' W	---
12	Bay of Isles	E	60° 22.80' N	147° 45.40' W	13
13	Green Island	E	60° 16.20' N	147° 26.10' W	---
14	MacLeod Harbor	R	59° 53.21' N	147° 45.80' W	10
15	Mooselips Bay	R	60° 12.55' N	147° 18.00' W	11
16	Snug Harbor	E	60° 14.25' N	147° 44.10' W	12
17	Chenega	E	60° 19.85' N	148° 00.45' W	07
18	Lower Herring Bay	R	60° 24.40' N	147° 47.80' W	06
19	Drier Bay	R	60° 19.20' N	147° 44.00' W	08
20	Sleepy Bay	E	60° 03.95' N	147° 50.35' W	09
21	Fox Farm	E	59° 58.40' N	148° 10.65' W	---
22	Sunny Cove	R	59° 56.20' N	149° 19.10' W	---
23	Agnes Cove	E	59° 46.05' N	149° 34.55' W	---
24	Black Bay	R	59° 32.61' N	150° 12.78' W	---
25	Chugach Bay	E	59° 11.16' N	151° 37.90' W	---
26	Tonsina Cove	E	59° 19.75' N	150° 54.90' W	---
27	Windy Bay	E	59° 13.85' N	151° 31.00' W	---
28	Hallo Bay	E	58° 27.45' N	154° 00.30' W	---
29	Katmai Bay	E	57° 54.50' N	155° 04.50' W	---

*R = reference site
E = exposed site.
**--- = Not sampled in 1991.

sonicated (Model UP400 ultrasonic probe, Sonicor Instrument Corporation) for 30 seconds with two successive 5 mL volumes of HPLC grade methylene chloride. The extracts were decanted through a filter containing about 2 g anhydrous sodium sulfate, and collected in a clean 25 mL graduated cylinder. Fluorescence was determined (Hitachi Model F-1200 fluorescence spectrophotometer) on diluted aliquots of these extracts, using excitation/emission wavelengths of 310/370 nm, and petroleum hydrocarbon concentration was estimated by comparison to a standard reference solution of EVO in methylene chloride.

In 1990, sediment samples were frozen (-80°C) on board ship and kept frozen till analyzed at the NWFSC in Seattle, WA (Krahn et al. 1991, 1993). Sediment (1.0 g) was mixed with sodium sulfate (10 g), activated copper (1 cc), and methylene chloride (20 mL) in a centrifuge tube. The tubes were placed in a sonic bath for 15 min, then centrifuged at 1500 rpm for 5 min; and the extracts were decanted into 50 mL concentrator tubes. Extraction was repeated twice more, using 10 mL of methylene chloride and 5 min sonication periods. The three extracts were combined, a polystyrene internal standard (100 uL; 50ug/uL) was added, the solution was concentrated by evaporation to about 4 mL. An aliquot (15 uL) was injected onto a high-performance liquid chromatography (HPLC) size-exclusion column and eluted with methylene chloride with a flow of 2.5 mL/min. Fluorescence was monitored at two different wavelength pairs, 260/380 nm (excitation/emission) and 290/335 nm, optimized for phenanthrene and naphthalenes plus dibenzothiophenes, respectively. Oil concentrations were estimated by comparison of chromatographic areas with those obtained with a standard reference solution of EVO.

GC/MS Analyses. Selected sediment samples from all three years were analyzed by GC/MS to provide confirmation and comparison for the UVF screening results. Methods used for sediment extraction, alumina/silica gel chromatographic cleanup of the extracts, and analysis by GC/MS have been described previously (Brooks et al. 1990; Krahn et al. 1993).

Toxicity Bioassays

Sediment samples were tested for toxicity using one or more of the standard bioassay protocols summarized in Table 3.

Microtox^R Assay. At least one of the replicate composite sediment samples obtained at each depth from each sampling site in 1989 was analyzed for sediment toxicity based on the inhibition of bioluminescence in *Photobacterium phosphoreum* (15 min Microtox^R assay). Organic extracts of the sediments were prepared and assayed for toxicity by the methods of Shiewe et al. (1985). The Microtox^R assay is rapid, simple, and inexpensive; and the bioassay results have generally correlated well in other studies with the results of other standard bioassays that use fish, amphipods or bivalve larvae as test organisms (Chang 1981, Williams et al. 1986, Giesy et al. 1988).

Table 3. Protocols used in this study to estimate sediment toxicity.

Test Medium	Test Organism & Endpoints Exposure Period	References
Organic Extracts (Microtox^{R}) of phosphorescence	*Photobacterium phosphoreum* 15 min inhibition	PSEP 1986 Shiewe et al. 1985
Whole Sediments	*Ampelisca abdita* (adults) 10 day (static) Survival	ASTM 1990a
Elutriates	*Crassostrea gigas* (larvae) 48 h (static) Survival & Development	ASTM 1990b

After the 1989 field survey, a laboratory study was performed to validate the ability of the Microtox^R assay to measure oil toxicity. Naturally weathered EVO was collected from PWS 11 days after the spill, and stored at 4°C. A sediment sample from Zachary Bay, Alaska, with undetectable concentrations of aromatic hydrocarbons (Krahn et al. 1991), was spiked with the weathered EVO and tested for toxicity with the Microtox^R assay. Portions (100 g) of sediment were amended with 0, 0.5, 5, 25, 100, or 500 mg of the weathered oil. The samples were mixed for 24 h on a modified rock tumbler and allowed to equilibrate for 72 h (all at 4°C). Samples were then extracted and tested using the Microtox^R procedure described above. The resultant concentrations of oil in sediment (0-5000 ppm) simulated the range of oil concentrations in sediment observed in 1989.

Toxicity Tests with Amphipods. The toxicity of test sediments to amphipods (*Ampelisca abdita*) was tested (SAIC 1990b, 1991) following the general procedures of Swartz et al. (1985) and Scott and Redmond (1990). Test organisms were collected in San Francisco Bay and shipped to Seattle by overnight courier. Five replicate 1 L beakers, each containing 175 mL of sediment and (approximately) 825 mL of filtered seawater from Alkai Point (Puget Sound, WA) were set up for each sediment sample. A sixth replicate container was used for daily measurement of dissolved oxygen, pH, salinity, and temperature. Clean control sediments were collected with the test organisms in San Francisco Bay, and (in 1991) reference sediments were collected also from two sites in Puget Sound (West Beach on Whidbey Island, and Samish Bay). Replicate control sediments were tested with each group of test sediments. Twenty amphipods were added to each container, and the containers were maintained at 20°C for 10 days with gentle aeration. At the end of the test period, live amphipods were enumerated, and the mean mortality of test organisms (corrected for control mortality) was determined for each sample.

Toxicity Tests with Oyster Larvae. Survival of oyster (*Crassostrea gigas*) larvae was tested in 48 h exposures to elutriates of test sediments (SAIC 1990b, 1991) following procedures described previously (Chapman and Morgan 1983; Chapman and Becker 1986). Sediment elutriates were prepared by vigorous mixing for 30 min of 200 g (wet weight) of test (or control) sediment into one liter of filtered, sterilized seawater obtained in Puget Sound from Alkai Point (1990) or Duwamish Head (1991). After the suspensions settled for 1 h, the supernatant elutriate was decanted, and the settled solids were discarded. Five test replicates, each containing approximately 980 mL of elutriate, were inoculated with 20,000-40,000 developing oyster embryos within 2 h of fertilization, for each sediment sample. After 48 h, three 10 mL aliquots from each test container were preserved in 5% buffered formalin. Normal and abnormal prodissoconch I larvae were counted, and the percent survival (normal survivors compared to initial density) and percent abnormality (abnormal survivors compared to total survivors) were calculated. Abnormality data were adjusted for the rates measured in control larvae that developed in control seawater.

RESULTS

Microtox^R Assays- 1989

Tables 4 and 5 present the means and standard deviations for petroleum hydrocarbon concentrations and Microtox^R EC-50s, respectively, from the 1989 surveys, by sampling region and depth. These means are depicted and compared graphically in Figure 1. At shallow depths (0-6 m), sediments from sites in PWS were more toxic on the average and had higher hydrocarbon concentrations than sediments from outside PWS. Shallow sediments from sites along the Kenai Peninsula exhibited toxicities and hydrocarbon concentrations very similar to those from Kodiak and the Alaska Peninsula (Fig. 1). At greater depths (40-100 m), PWS sites were not notably different from sites outside PWS, with respect either to Microtox^R response or to hydrocarbon concentration. At all depths except 40 m and 100 m, the UVF data showed a generally decreasing trend in hydrocarbon concentration with increasing distance from the spill site. This pattern was strongest in data from the 3 and 6 m depths. The Microtox^R EC-50s of sediment extracts from 3 to 20 m depths usually increased (i.e. were less toxic) as distance from the spill site increased.

Figure 2 depicts the Microtox^R and UVF results for two depths (0 and 3 m) at the PWS sites alone, where the total hydrocarbon concentrations exhibited the greatest range of values. Figure 2A includes an estimated EC-50 value (60 mg wet sediment/ mL of test extract) for the sediment sample from Rocky Bay, for which toxicity was too low to estimate a valid EC-50 at the standard test dilution. The hydrocarbon concentrations for the 0 m samples in PWS ranged over 5 orders of magnitude (Fig. 2A).

EC-50s could not be calculated for the four most heavily oiled samples, from Disk Island, Smith Island, NW Bay, and Herring Bay. Without those samples, there was no significant rank correlation between toxicity and hydrocarbon concentration, and the most toxic samples from within PWS were from Olsen Bay and Columbia Bay, areas that were not affected by the spill.

Figure 2B shows the same plot for the 3 m samples from PWS. The UVF response at this depth ranged over only three orders of magnitude. For this depth, most of the least toxic samples appear on the left with the more toxic ones generally toward the right, and there was a significant inverse rank correlation (Spearman, $p= 0.046$) between UVF and EC-50. However the toxicity was not dose-dependent, and there were curious outliers: the most toxic samples were from Olsen Bay, with Rocky Bay (only slightly oiled by the spill) a close second. Heavily oiled Sleepy Bay, by contrast, was the least toxic site.

At 6 m (Fig. 2C), UVF ranged over less than 2 orders of magnitude. The inverse Spearman rank correlation was also significant ($p= 0.040$) at this depth, and the most toxic samples (more so than at any other depth) occurred at two heavily oiled sites: NW Bay and Bay of Isles. Other heavily oiled sites (e.g. Snug Harbor, Smith Island, Herring Bay), however, exhibited toxicities similar to that at Olsen Bay.

Table 4. Total petroleum hydrocarbon concentrations (ppm) estimated by UVF in intertidal and subtidal Alaskan sediments from three regions within the EVOS zone in 1989.

Depth (m)	Mean	S.D.	Range	n
Prince William Sound (18 sites)				
0	841.30	2414.76	0.59-9800	17
3	22.70	46.07	1.4-190	18
6	8.83	12.21	1.5-50	18
20	11.70	19.79	1.4-87	18
40	6.36	3.54	1.1-12	18
100	5.85	3.54	0.16-11	17
Kenai Peninsula (14 sites)				
0	7.66	14.78	0.44-55	14
3	3.85	4.63	0.55-17	14
6	4.28	6.09	0.7-23	14
20	11.30	12.64	0.37-46	14
40	12.53	23.16	0.33-77	13
100	6.41	5.62	0.76-21	13
Kodiak and Shelikof Strait area (11 sites)				
0	7.25	13.26	0.40-37	11
3	1.11	0.72	0.34-2.6	11
6	2.83	4.28	0.4-15	11
20	4.77	6.97	0.34-19	11
40	4.65	7.41	0.26-26	11
100	4.49	6.86	0.94-20	7

Table 5. Mean Microtox[R] response (EC-50, in mg sediment/mL) by depth in intertidal and subtidal Alaskan sediments from three regions within the EVOS zone in 1989.

Depth (m)	Mean	S.D.	Range	n
Prince William Sound (18 sites)				
0	12.48	11.39	1.4-38	13*
3	5.44	6.22	1.0-29	18
6	5.78	7.28	0.53-28	18
20	9.76	11.34	0.53-47	18
40	18.62	15.68	1.2-64	18
100	20.86	19.66	0.81-75	17
Kenai Peninsula (14 sites)				
0	24.64	11.39	13-49	11*
3	15.19	10.98	1.8-38	14
6	14.32	8.93	2.3-29	14
20	11.90	8.92	1.5-26	14
40	16.58	9.48	0.72-29	13
100	17.27	10.89	1.2-34	12
Kodiak and Shelikof Strait area (11 sites)				
0	18.41	10.23	5.0-35	11
3	19.45	12.84	2.8-45	11
6	16.23	8.67	4.7-31	11
20	18.68	10.33	3.9-38	11
40	20.79	13.96	2.9-50	10
100	16.30	11.41	3.2-31	7

*In four PWS samples, precipitation of oil precluded valid determination of EC-50s; while in four other samples, toxicity was too low to estimate EC-50s at the dilutions used. See text.

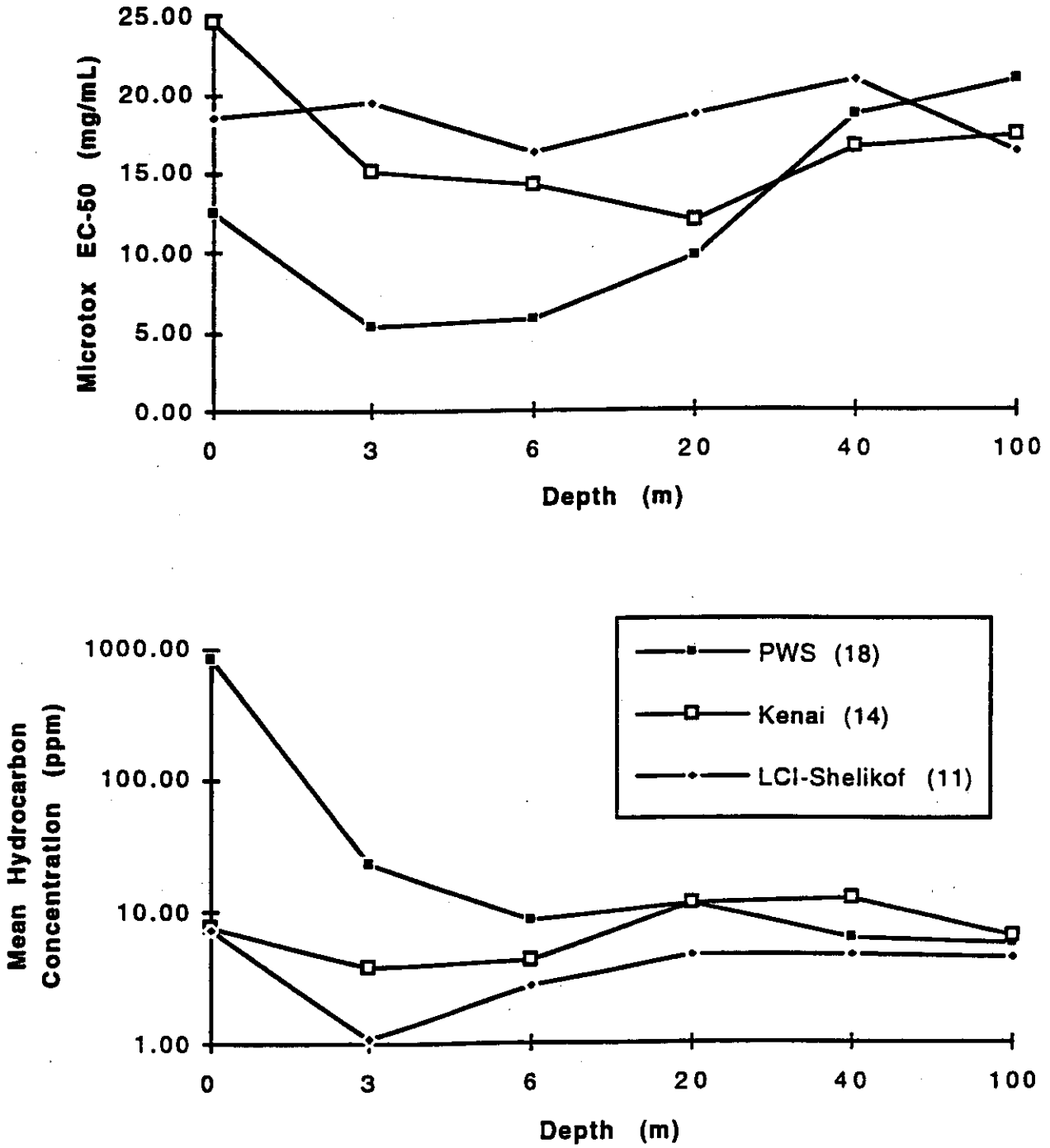

Figure 1. Mean Microtox[R] EC-50 (top), in mg/mL, and mean hydrocarbon concentrations (bottom) estimated by UVF, for sediments from different regions and depths in 1989. See Tables 4 and 5 for estimates of variance.

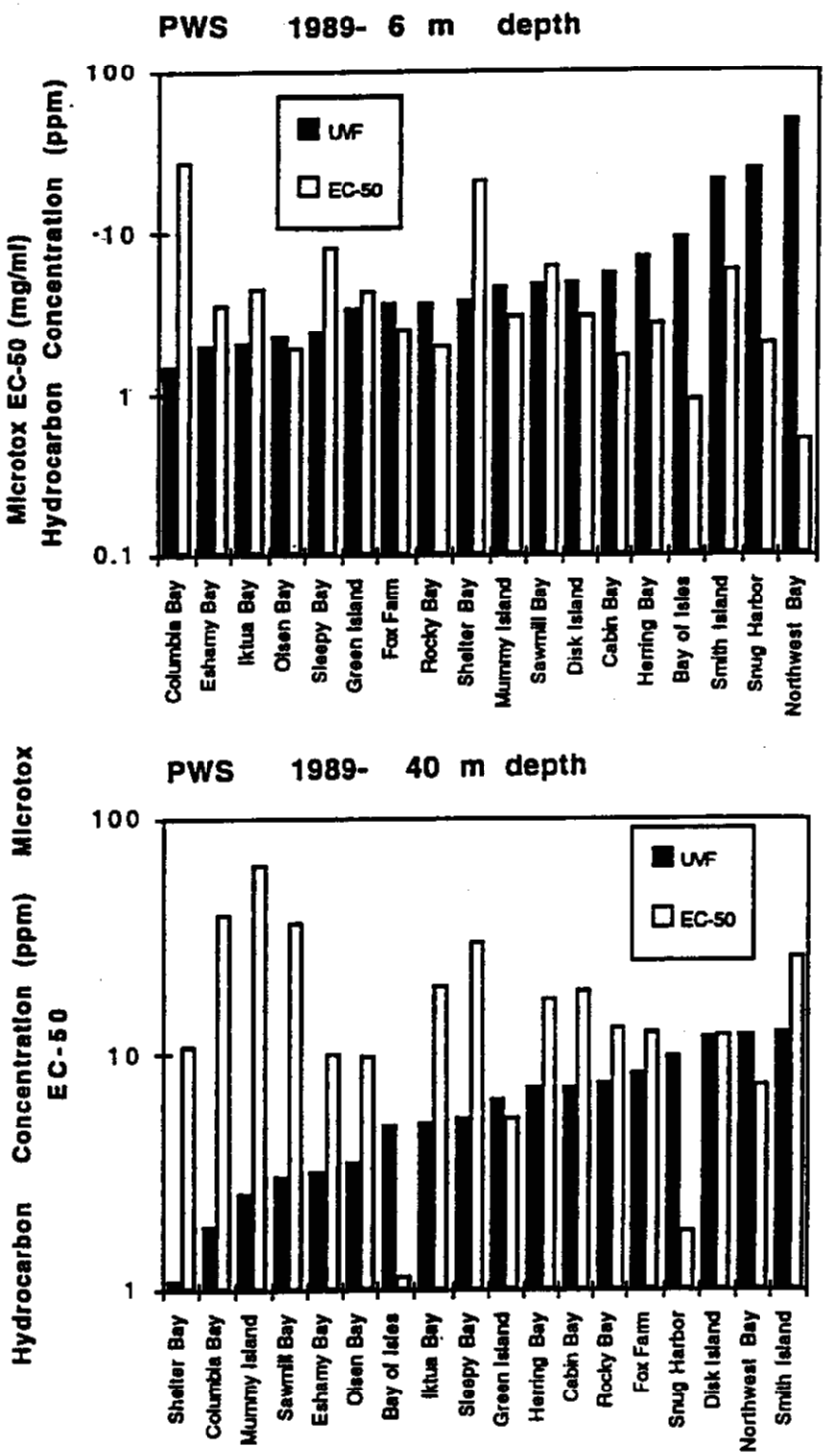

Figure 2. Concentrations of petroleum hydrocarbons, estimated by UVF, and Microtox^{R} response (EC-50, in mg/mL) for sediment samples from PWS sites at 0 m [A] and 3 m [B] depths. For each depth sites are arranged in order of ascending hydrocarbon concentration.

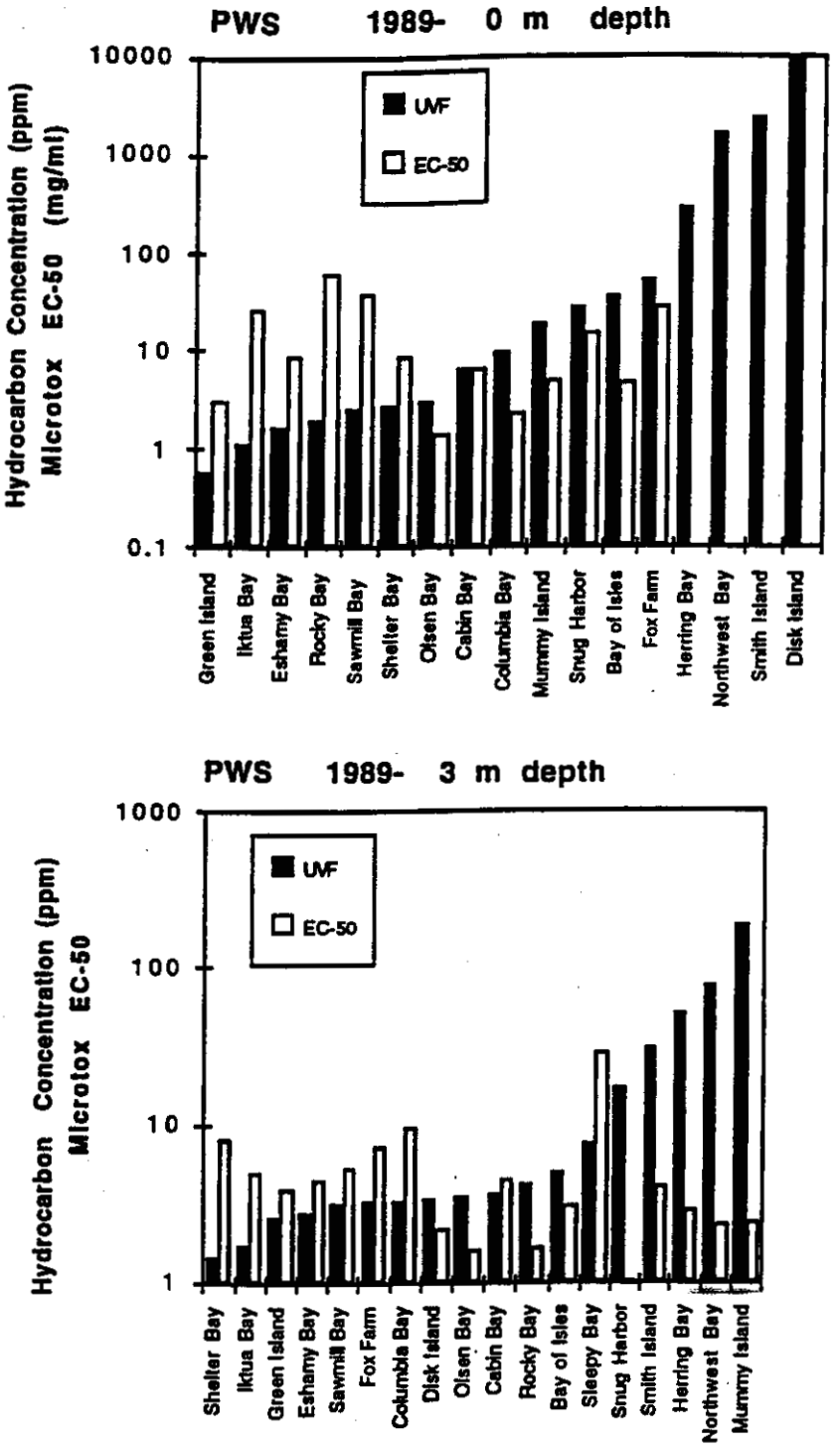

Figure 2 (Continued). Concentrations of petroleum hydrocarbons, estimated by UVF, and Microtox^{R} response (EC-50, in mg/mL) for sediments from PWS sites at 6 m [C] and 40 m [D] depths. At each depth sites are arranged in order of ascending hydrocarbon concentration.

At 40 m (Fig. 2D), the UVF spanned just one order of magnitude, and there was no relationship between toxicity and hydrocarbon concentration. Although the most toxic 40 m samples were at Bay of Isles and Snug Harbor (two sites that were heavily oiled intertidally), the EC-50s for these samples were in the same range (1-3 mg/mL) as many of the samples from the 6 m depth (Fig. 2B), and the total petroleum hydrocarbons at these stations were not notably different from others at this depth.

The laboratory tests of the Microtox^R procedure with oil-amended sediments showed no relationship between EC-50 and the concentration of EVO in the sediments, over a four order-of-magnitude range in concentration (Table 6). This unexpected finding caused us to abandon the Microtox^R assay in the 1990 and 1991 surveys of sediment toxicity.

Patterns of Hydrocarbon Distribution with Depth: 1989-1990

Both hydrocarbon concentrations and Microtox^R EC-50s varied considerably with depth and exhibited quite different patterns with depth at different stations. Three qualitative distributional patterns of UVF signal with depth were tentatively identified from analyses in both 1989 and 1990 (Fig. 3): A) moderate to very high intertidal values, usually associated with intertidal oiling, with decreasing values at depth; B) a usually variable maximum value at 20-40 m, with lower values both intertidally and at greater depth; and C) variable low values in the intertidal and shallow subtidal samples, usually increasing to a maximum at 40-100 m. These three qualitative patterns are illustrated (using 1990 data) in Figure 3, and the stations exhibiting the different patterns are identified in Tables 7-9. Because the 1989 UVF and 1990 HPLC/UVF methods were different, the 1989 and 1990 values are not directly comparable, but the maximum values for each site pattern are given in Tables 7-9 to permit comparison of relative concentrations among sites in either year.

Stations exhibiting pattern A consistently represented stations that showed strong evidence of significant intertidal oiling and that may have received either coincident or consequent subtidal exposure. Most of these sites were in PWS (Table 7), but a few were in the Kenai or Alaska Peninsula areas (Table 9). All of the sites exhibiting pattern A in 1990 also exhibited the HPLC elution pattern characteristic of PBCO (Krahn et al. 1993). Many of the GOA sites that exhibited pattern A in 1989 showed shifts either to pattern B or C in 1990 (Table 9). Sites exhibiting pattern B generally showed near minimal UVF values at 0 m (but samples from Green Island, Sleepy Bay, and Chugach Bay exhibited unusually high variability in intertidal replicates), and with two exceptions (MacLeod Harbor 1990 and Agnes Cove 1989 and 1990), showed indications of Prudhoe Bay oil in subtidal samples. With the exception of MacLeod Harbor (1990, pattern B), all sites designated as unoiled (or only slightly oiled) reference sites consistently exhibited pattern C and did not show the characteristic PBCO pattern on HPLC elution (Tables 7 and 8).

Table 6. Results of Microtox[R] toxicity measurements on sediments amended with increasing amounts of PBCO collected from PWS 11 days after the EVOS.

Oil Concentration (ug oil/g sediment)	EC_{50a} (mg sediment equivalents)
5000	4.6 (3.9-5.5)
1000	3.7 (3.2-4.4)
250	2.7 (2.2-3.4)
50	2.0 (1.5-2.6)
5	2.9 (2.5-3.3)
0	1.7 (1.5-2.4)

Results are reported as the amount of sediment equivalents in the reaction mixture that would decrease the light output by 50% relative to a control. A lower EC-50 value indicates greater toxicity to the photobacteria. Values in parentheses represent lower and upper 95% confidence intervals for the EC-50 value.

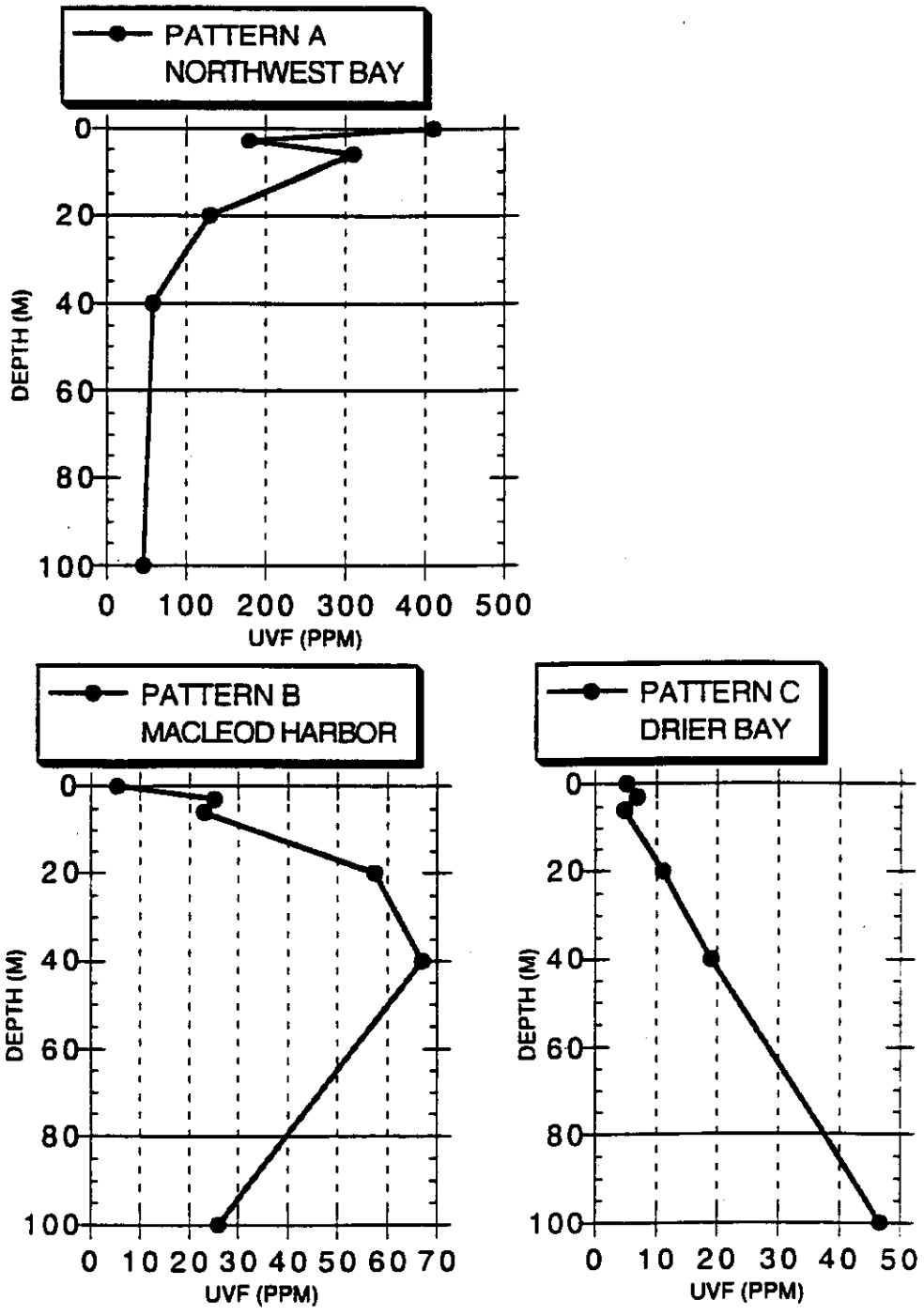

Figure 3. Typical patterns of distribution with depth of hydrocarbons measured by UVF spectrometry.

Table 7. Patterns of Hydrocarbon Distribution (see Fig. 3) at sites in PWS, 1989-1990.

Site type	Site Name	Pattern A 1989 Max	Pattern A 1990 Max	Pattern B 1989 Max	Pattern B 1990 Max
X*	Herring Bay	289.5	970•		
X*	Northwest Bay	1589	410•		
X*	Snug Harbor	27.42	100•		
X*	Disk Island	9842	53•		
X	NE Knight Island	NS	51•		
X*	Chenega	NS	41•		
X	Fox Farm	51.01	29•		
X*	Bay of Isles	35.31	26•		
X	Smith Island	2420	23•		
X*	Block Island			NS	210•
X*	Sleepy Bay			86.73	76.7•
R*	MacLeod Harbor			NS	67

X=exposed
R=reference
NS= no sample
*Sites sampled also in 1991
•=Weathered PBCO pattern on HPLC elution (Krahn et al. 1993).
Values in ppm for the depth with the highest concentration at each site
(UVF: 1989 Analyses by SAIC; 1990 Analyses by Krahn et al.)

Table 8. Patterns of hydrocarbon distribution at sites in PWS, 1989-1990.

Site type	Site Name	Pattern B 1989 Max	Pattern C 1989 Max	Pattern C 1990 Max
X	Green Island	21.09		79•
R	Olsen Bay		4.99	54
R*	Drier Bay		NS	46.7
R*	Zaikof Bay		NS	33
R*	Rocky Bay		8.8	33
R*	West Bay		NS	30
R*	Mooselips Bay		NS	22
R*	Lower Herring Bay		NS	21
R	Port Fidalgo		NS	20

X=exposed
R=reference
NS= no sample
*Sites sampled also in 1991
•=Weathered PBCO pattern on HPLC elution (Krahn et al. 1993).
Values in ppm for the depth with the highest concentration at each site
(UVF: 1989 Analyses by SAIC; 1990 Analyses by Krahn et al.)

Table 9. Patterns of hydrocarbon distribution at sites in the GOA, 1989-1990.

	Pattern A		Pattern B	
Site Name	1989 Max	1990 Max	1989 Max	1990 Max
Tonsina Cove	55.07			37•
Windy Bay	21.67			62•
Agnes Cove			48.1	600

	Pattern A	Pattern B	Pattern C	Pattern C
	1989 Max	1989 Max	1989 Max	1990 Max
Sunny Cove		77.4		9.7•
Chugach Bay		46.06		20•
Black Bay			21.07	18•
Hallo Bay	30.47			17•
Katmai Bay	37.04			12•

•=Weathered PBCO pattern on HPLC elution (Krahn et al.1993)
Values in ppm for the depth with the highest concentration at each site (UVF: 1989 Analyses by SAIC; 1990 Analyses by Krahn et al.)

Amphipod and Oyster Bioassays: 1990-1991

Sampling in 1990 and 1991 was focused on the comparison of sets of selected "exposed" and "reference" sites in PWS (Table 10). Four reference sites were located on Montague Island, one on Bligh Island, and two on the west side of Knight Island. Some of these PWS reference sites, may have been subjected to minor oiling from the spill-- either by small amounts of floating oil early on, or by very low levels of dispersed oil in the water column over the following 18 months (Wolfe et al. 1993a, 1993b). West Bay and Green Island were sampled only in 1990; Zaikof only in 1991; the other sites were sampled both years. Sediment samples from 0, 6, 20, and 40 m were assayed by the amphipod and oyster larval tests, and the results are compared against UVF data from separate composite sediment samples taken concurrently.

Percent mortalities for *Ampelisca* and *Crassostrea* larvae exposed to sediments from the 0 m (intertidal) depths are shown in Figure 4, along with corresponding UVF hydrocarbon concentrations. The six reference sites are located on the left side of the figure; all had hydrocarbon concentrations of less than 5.1 ppm (Drier Bay), whereas the exposed sites ranged from 3.5 ppm (Green Island) to 26 ppm (Bay of Isles) to 970 ppm (Herring Bay). Mortality of control amphipods was high and variable in the 1990 tests (23 ± 12.1% S.D., 17 ± 17.9 %, and 28.8 ± 14.7% for the three test batches), and only the five most toxic exposed sites were significantly ($p = 0.05$) different from controls. Nonetheless the mean amphipod mortality at the 10 exposed sites was significantly greater (T-test; $p=0.02$) than that of the 6 reference sites (Table 11). However, the toxicity of the Herring Bay sample with the highest UVF signal (970 ppm oil) was similar (Fig. 4) to that of the West Bay sample with the lowest (at <1.0 ppm).

Control survival of *Crassostrea* larvae in reference seawater was only 70% in one test batch (compared to 88.3% and 98.0% in the other two) in 1990, but coefficients of relative variation (S.D.) were between ±6.1% and ±9.6% for all three control batches. The highest mortalities for *Crassostrea* larvae exposed to sediment elutriates from the 0 m depth were observed at West Bay and Rocky Bay, two reference sites with very low hydrocarbon levels and at Block Island (Fig. 4). There were no significant differences between the exposed and reference site means for *Crassostrea*, either at 0 m or any other depth (Table 12). Although there was a tendency towards greater mortality with increasing depth (both for the exposed group and for all sites taken together), these differences were not significant.

Figure 5 shows the mortalities for *Ampelisca* and *Crassostrea* larvae exposed to sediments from the 20 m depths. At this depth, mortality of *Ampelisca* was uniformly low, irrespective of hydrocarbon concentration, which ranged up to 120 ppm at Northwest bay. While a number of sites exhibited moderate 20 m sediment toxicity to *Crassostrea* larvae, this toxicity was independent of hydrocarbon signal. Except for *Ampelisca* at 0 m, the exposed site means were not significantly different from the reference site means for either test organism at any depth (Tables 11 and 12).

Table 10. Sites used for reference-versus-exposed paired comparisons of toxicity in PWS, 1990-1991.

Reference Sites	Exposed Sites
West Bay (1990 only)	Green Island (1990 only)
	Northeast Knight
Rocky Bay	Herring Bay
Mooselips Bay	Disk Island
MacLeod Harbor	Northwest Bay
Lower Herring Bay	Bay of Isles
Drier Bay	Snug Harbor
	Sleepy Bay
	Block Island
Zaikof Bay (1991 only)	Chenega Island

Table 11. Percent mortality of test amphipods in 1990 (Corrected for control mortality) at reference-versus-exposed Sites in PWS. (T-Test: N = 6 vs 10).

Depth	Reference		Exposed		Significance
(m)	Mean	S.D.	Mean	S.D.	Level
0	22.7	9.1	53.5	27.2	0.019
6	22.5	12.6	19.5	9.4	0.601
20	23.6	8.9	14.5	7.7	0.061
100	13.0	7.7	12.1	4.7	0.787

Table 12. Percent mortality of oyster larvae in 1990 (Corrected for control mortality) at reference-versus-exposed Sites in PWS (T-Test: N = 6 vs 10).

Depth	Reference		Exposed		Significance
(m)	Mean	S.D.	Mean	S.D.	Level
0	48.7	30.5	32.4	12.0	0.147
6	43.3	19.7	46.2	18.5	0.770
20	36.7	8.7	48.9	17.6	0.140
100	40.2	21.4	58.0	22.8	0.171

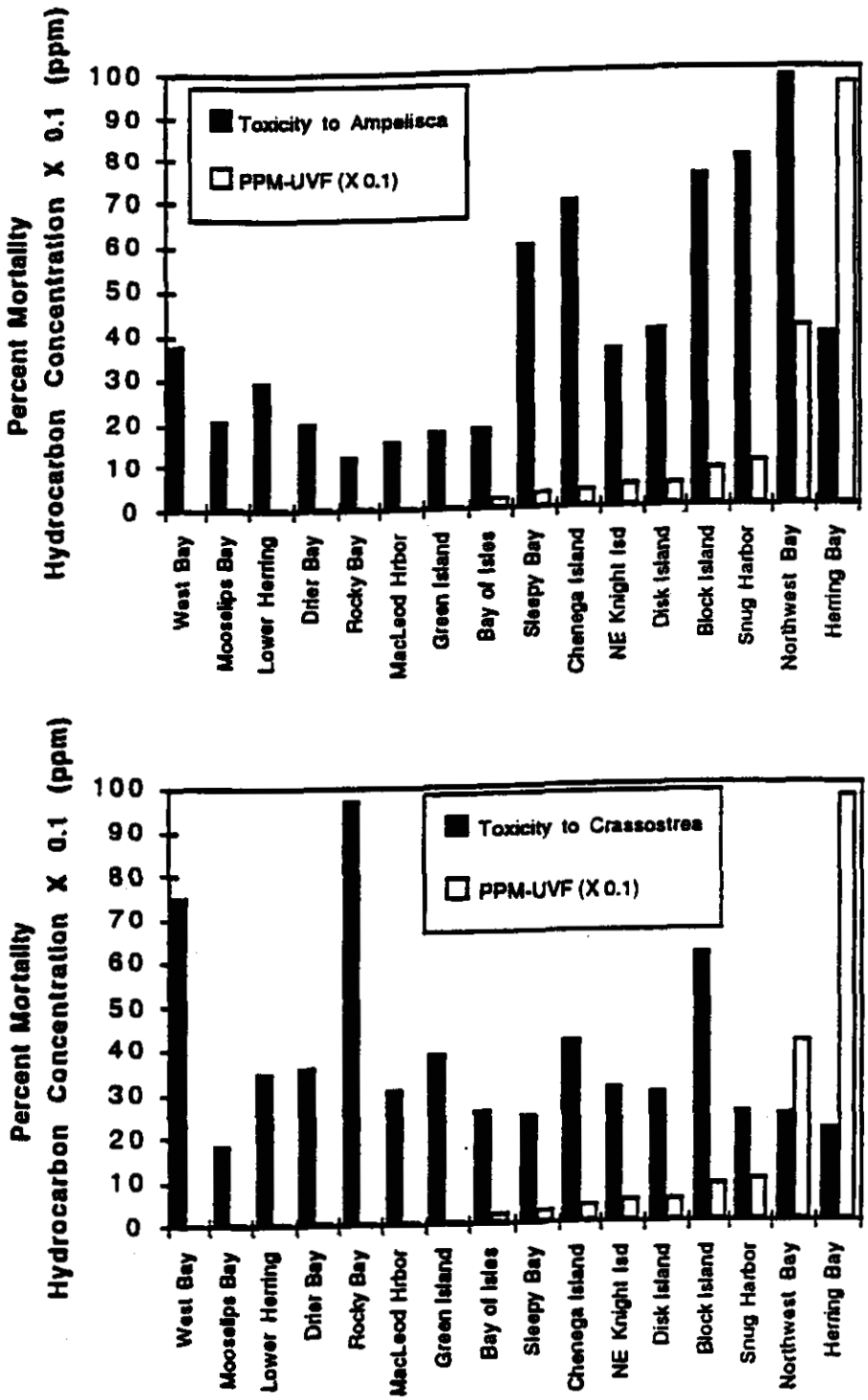

Figure 4. Hydrocarbon concentrations (ppm, by UVF) and toxicity of intertidal (0 m) sediment samples, collected in 1990. Toxicity is presented as percent mortality for *Ampelisca abdita* (top) and for *Crassostrea gigas* (bottom). Stations are arranged in ascending order of hydrocarbon concentration, with six reference sites (West Bay to MacLeod Harbor) at left and ten exposed sites (Green Island to Herring Bay) at right.

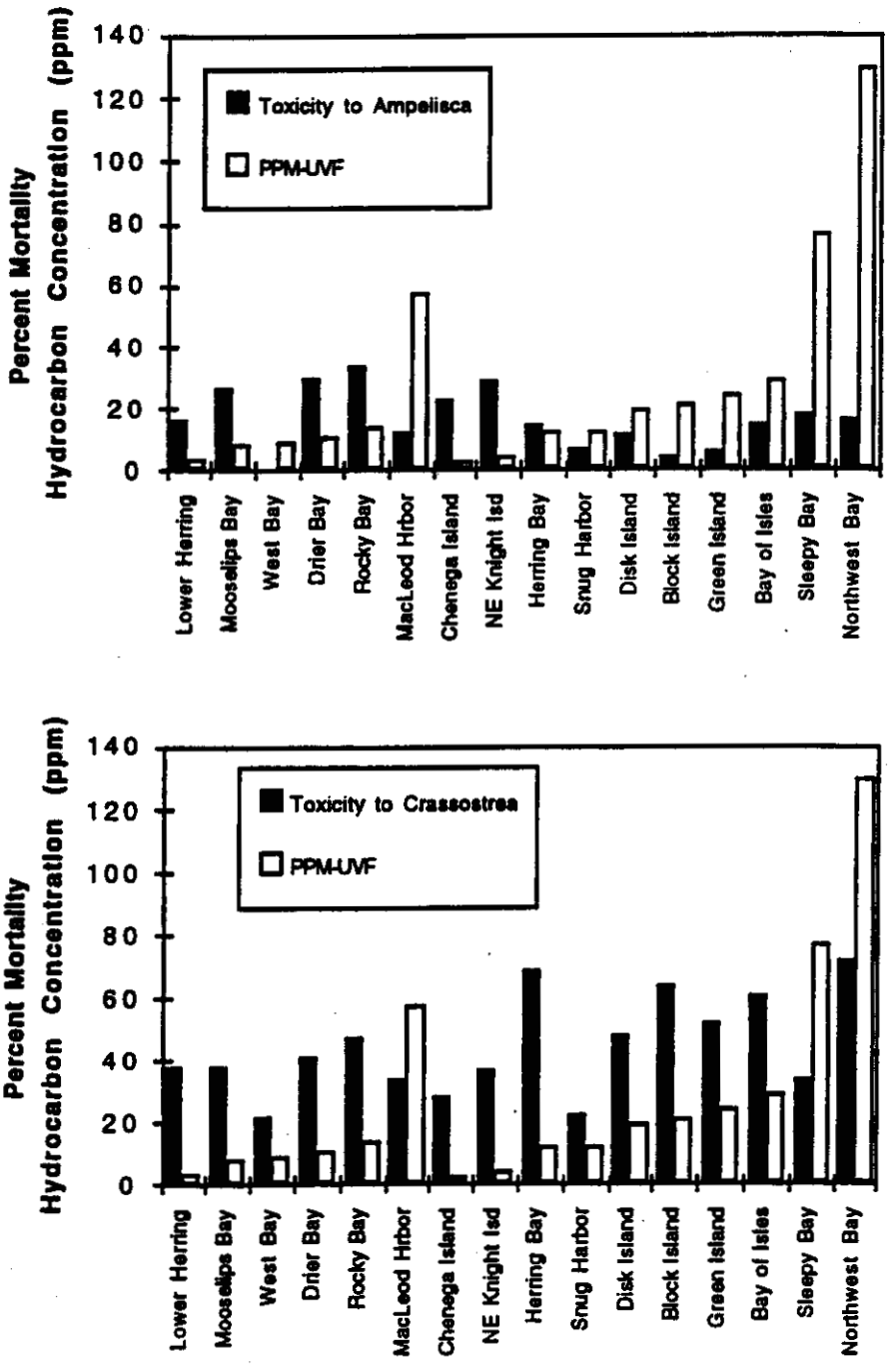

Figure 5. Hydrocarbon concentrations (ppm, by UVF) and toxicity of 20 m sediment samples, collected in 1990. Toxicity is presented as percent mortality for *Ampelisca abdita* (top) and for *Crassostrea gigas* (bottom). Stations are arranged in ascending order of hydrocarbon concentration, with six reference sites (West Bay to MacLeod Harbor) at left and ten exposed sites (Green Island to Herring Bay) at right.

The same sediment toxicity tests were performed again in 1991, but in this year control mortality in the *Ampelisca* test was lower and less variable than in 1990 (14.2 ± 6.6 %, 20.8 ± 13.2 %, and 12.5 ± 4.2% mortality in three test batches), resulting in a more valid test of relative toxicity among samples. Control mortality of *Crassostrea* larvae in reference seawater was also lower than in 1990; survival exceeded 85% in both test batches, with coefficients of relative variation (S.D.) between ±9.1% and ± 9.5%. Statistically significant mortalities (test vs. control) were observed with one or both test organisms for many of the sediment samples (Table 13). Figure 6 shows the 1991 control-corrected mortality data for *Ampelisca* and *Crassostrea* larvae at 0 m and 6 m depths at reference and exposed sites.

For some 1991 samples (Lower Herring Bay and Snug Harbor at 0 m, and MacLeod Harbor at 6 m), the quantity of available sediment was inadequate to conduct both tests, and no oyster data were generated. The other blank entries in Figure 6 represent zero percent mortalities. For both the *Crassostrea* and the *Ampelisca* tests, the threshold for statistical significance (relative to controls) in 1991 was at about 12-15 % mortality, depending on the control batch. Experience with a very large number of toxicity tests (Thursby, unpublished) has demonstrated, moreover, that mortalities in excess of 20% (survivals less than 80% of controls) are significant in about 95% of all tests. This "80% criterion" accounts for the potential variability of control sediments across test batches.

According to that criterion, sediments from 0 m exhibited toxicity at three exposed sites (Sleepy Bay, Northwest Bay and Bay of Isles-- all sites that had exhibited significant toxicity to *Ampelisca* the previous year), and at two reference sites (Mooselips Bay and more notably Drier Bay), which showed hits in one or both of the tests. Significant toxicity to *Ampelisca* was also found with the 6 m sediment samples from Snug Harbor and Drier Bay (Fig. 6). No significant toxicity in excess of 29 percent was seen for either test organism at either the 20 m or 100 m depths in 1991 There were no significant differences between mean toxicities at exposed and reference sites at any depth. The significant toxicity observed in sediments from shallow depths at Drier and Mooselips Bays remains unexplained.

Because of the large differences in control survival in 1990, the data sets are not directly comparable between years. Qualitative insights are possible, however, from comparisons of ratios of the mean *Ampelisca* toxicities for exposed and reference sites across years (Table 14). This approach suggests a decrease in the relative toxicity of exposed-to-reference sites between 1990 and 1991 at 0 m (from 3 to 1), and a possible increase (from 0.8 to 1.8) at the 6 m depth. This inference is consistent with an overall decline in toxicity, and a concomitant shift of toxicity from the intertidal towards shallow subtidal sediments.

DISCUSSION AND CONCLUSIONS

Similar UVF screening analysis methods have been used previously for investigations of oil spill impacts (e.g. at the *Argo Merchant* spill; Hoffman et al., 1979) and for monitoring programs involving discharges of petroleum hydrocarbon-containing constituents e.g. Georges

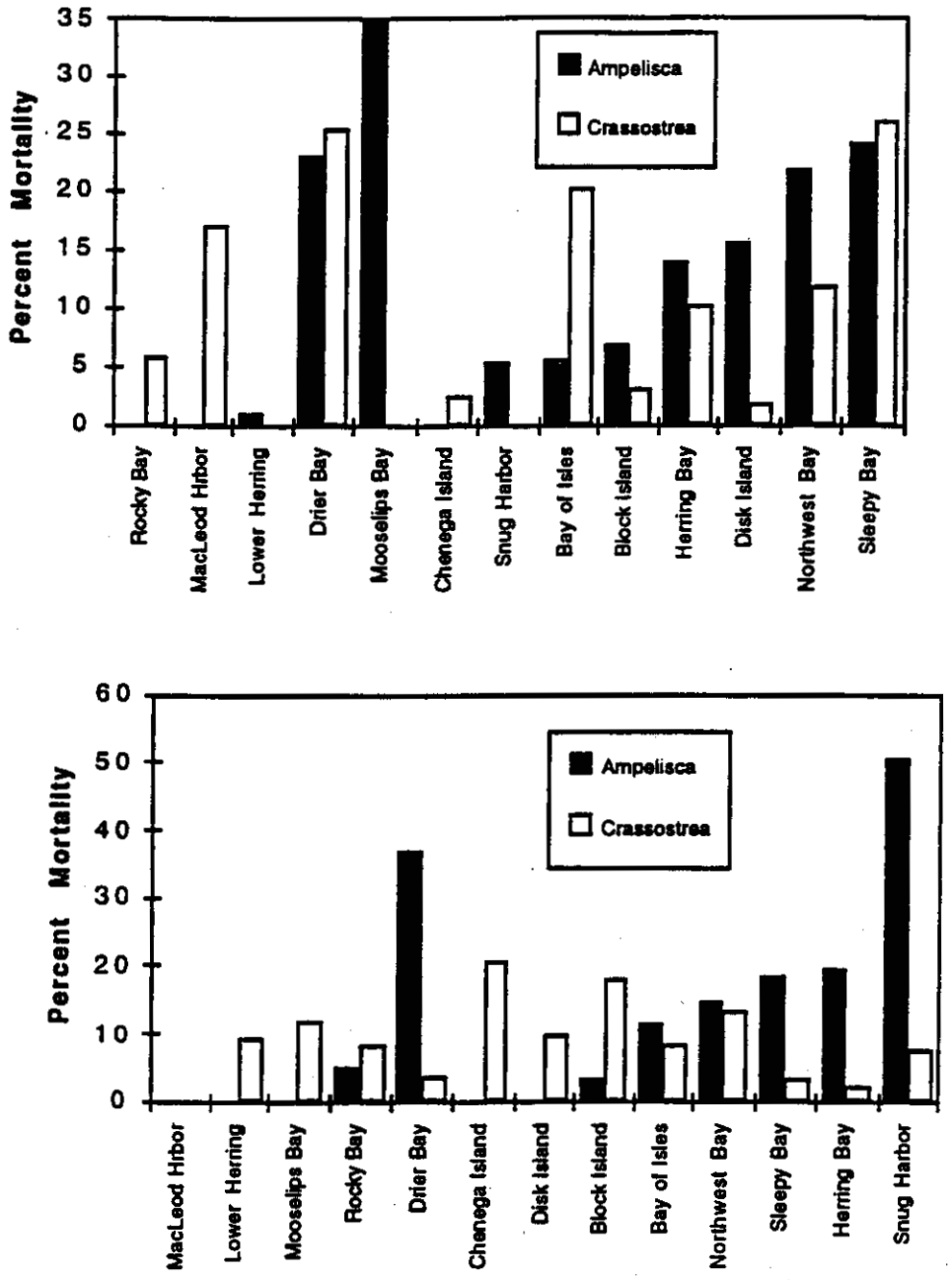

Figure 6. Percent mortality of adult *Ampelisca* and larval *Crassostrea* exposed to sediments collected in 1991 from 0 m (top) and 6 m (bottom) depths at five reference sites (MacLeod Harbor to Drier Bay at left of slide) and eight exposed sites in PWS. For Lower Herring Bay and Snug Harbor at 0 m and MacLeod Harbor at 6 m, the quantity of sediment was inadequate to conduct both tests, and no oyster data are available. The rest of the blanks represent actual zero percent mortalities.

Table 13. 1991 Sites significantly toxic relative to controls.

Station	Ref/ Exp	Depth (m) 0	6	20	100
Northwest Bay*	E	A	A	-	-
Snug Harbor*	E	-	A	A	-
Block Island*	E	-	A,C	C	-
Chenega Island*	E	C	C	-	-
Bay of Isles	E	C	A	-	-
Lower Herring Bay	R	-	-	-	-
Mooselips Bay	R	A	C	-	-
Drier Bay	R	A,C	A	A	-
Rocky Bay	R	-	C	-	-
MacLeod Harbor	R	C	-	-	-
Zaikof Bay	R	-	-	-	-

Significant (p= .05) Toxicity Relative to Controls
A= *Ampelisca*; C= *Crassotrea*
*Sites with significant intertidal toxicity to amphipods in 1990.

Table 14. Comparison of test amphipod mortalities, 1990 vs 1991, by depth (corrected for control mortality).

	Depth (m)			
	0	6	20	100
Reference sites (n = 5):				
1990	19.8	25.9	23.6	10.2
1991	11.8	8.40	4.98	1.12
Exposed sites (n = 8):				
1990	60.1	21.4	13.7	12.4
1991	11.7	14.8	5.58	0.96
Ratio of Exposed/Reference Means:				
1990	3.0	0.83	0.58	1.1
1991	0.99	1.8	1.1	0.86

Bank Monitoring Program; Phillips et al., 1987). These methods offer potential benefits related to sensitivity, speed, and low cost; however, the method is non-specific and only semi-quantitative, and the results may be difficult to interpret, especially when concentrations of the target oil are low relative to other fluorescing materials.

Gas chromatographic analyses for sediment samples taken concurrently with this study indicate that oil from the *Exxon Valdez* was generally absent in samples from 40 or 100 m depths and rarely occurred in appreciable quantities below 3m in 1989 (O'Clair et al. 1993). Other studies have shown that EVO was generally absent in offshore deep sediments of PWS, and that, along with pyrogenic and biogenic sources, many of the hydrocarbons in those sediments originate from coastal petroleum seeps at Katalla (east of the Copper River) (Kvenvolden et al. 1991, Carlson and Kvenvolden 1993, Page et al. 1994).

The HPLC/UVF methodology employed here was able to distinguish *Exxon Valdez* crude from this sometimes substantial background signal from other sources in deep (40 to 100 m) sediments (but not to quantify degrees of mixture) , and from an apparent diesel signal in some shallow bays outside the spill path, as verified by and correlated with GC/MS data (Krahn et al. 1993). The UVF screening approach appeared therefore to provide both a reliable indication of relative degree of contamination by fluorescent aromatic hydrocarbons of *Exxon Valdez* origin, particularly within the 0, 3, and 6 m depth strata, and a valid basis for relating hydrocarbon concentrations to measured sediment toxicities.

Estimates of most probable numbers (MPN) of hydrocarbon-degrading bacteria provided an independent measure of oil exposure in these sediment samples (SAIC 1990a, Braddock et al. 1992, 1993) Hydrocarbon-degrading bacteria were highly elevated in oil-exposed intertidal sediments in 1989, showing high correlation with the estimates of oil concentration (UVF) reported here. Numbers of hydrocarbon-degrading bacteria in subtidal sediments, however, indicated only sporadic contamination with spilled oil in 1989, and nearly all of those occurrences were outside of PWS (SAIC 1990a, Braddock et al. 1992, 1993). In subtidal samples, MPN was not correlated with the UVF values.

Microtox^{R} was used as the sole estimator of sediment toxicity in 1989. Microtox^{R} EC-50s did not show a clear dose-response relationship with hydrocarbon concentration, especially at high concentrations. Although toxicity was positively rank correlated with aromatic hydrocarbons (UVF), and was generally greatest at 0, 3, or 6 m depths at heavily oiled sites within PWS, the EC-50s measured at some sites known to have been either unoiled or only very lightly oiled by the spill (e.g. Olsen Bay, Rocky Bay, Chignik Bay) were about as low as at some of the most heavily oiled sites where the concentrations of total hydrocarbons were higher by 10 to 100-fold. The greatest differences in Microtox^{R} response between PWS samples and GOA samples (Fig. 1), as well as the strongest rank correlations of Microtox^{R} response with UVF signal, occurred in shallow (3-6 m) subtidal sediments. At 0 m, where the hydrocarbon concentrations exhibited the greatest range, commensurate differences in toxicity were not measured with Microtox^{R}. In deeper sediments (20-100 m), however, where EVO was usually very low or absent, toxicity was

still rank correlated with total hydrocarbons estimated by UVF. Taken together, these observations suggest a) that the Microtox^R test (with organic extracts) is basically sensitive to fluorescing aromatic constituents, but b) this sensitivity may be reduced in the presence of other oil constituents of fresh to moderately weathered oil, especially at high concentrations. Ho and Quinn (1993) presented Microtox^R data that exhibited strong rank correlations of response with polynuclear aromatic hydrocarbon (PAH) levels in the aromatic fractions of organic extracts of sediments. As reported here, the Microtox^R test was insensitive in direct laboratory tests to organic extracts of whole fresh or moderately weathered oil, even at very high hydrocarbon concentrations, although it gave a normal dose-response to a mixture of many of the PAH constituents of PBCO (Sol et al. 1992). For this reason, the Microtox^R test was not used in the subsequent surveys carried out in 1990 and 1991.

These observations lead us to speculate that relatively immobile, higher-molecular weight constituents of crude oil (perhaps the asphaltene/polar components), when present at high concentrations in a relatively fresh, unweathered form, may mask the bioavailability and toxicity of PAHs in the oil, at least as measured by Microtox^R with organic extracts. The full toxicity potential of the PAH may be manifested only after the oil has undergone some preliminary fractionation in the environment, e.g. through differential adsorption and partitioning processes. This hypothesis may warrant further examination in the future.

Most of the Microtox^R EC-50s reported here for oiled sediments from PWS are not remarkable, considering the extent of hydrocarbon contamination in some of the samples. The measured Microtox^R EC-50s in all samples at all depths ranged from 0.5 mg/mL [wet weight of sediments per unit volume of extract tested] (NW Bay at 6m) to 75 mg/mL. By contrast, a suite of coastal sediment samples taken recently along the length of Long Island Sound (Bricker et al. 1993) produced an EC-50 range from about 0.006 to 1.3 mg/mL. Based on Microtox^R results then, the sediments from representative coastal areas in Long Island Sound were about 50-to-100 times more toxic than those determined in 1989 for oiled subtidal sediments from PWS.

In 1990 and 1991, sediment toxicity was tested with *Ampelisca abdita* and *Crassostrea gigas*. In 1990, sediment toxicity was demonstrated only for sediments collected from 0 m depth at some of the more heavily oiled sites (NW Bay, Snug Harbor, Block Island, Chenega Island, and Sleepy Bay), which were significantly toxic (60 to 95 percent mortalities) to test *Ampelisca abdita*. At the 0 m depth, the mean sediment toxicity to *Ampelisca* for our 10 oiled sites in PWS was significantly higher than that for the six unoiled (or very slightly oiled) reference sites. However, samples from some other heavily oiled sites including Herring Bay, Disk Island, Northeast Knight Island, and Bay of Isles, exhibited relatively low toxicities to *Ampelisca*, comparable to those at sites that received no oil or were only very slightly oiled. No spill-related sediment toxicity to *Ampelisca* was demonstrated in 1990 samples from 6, 20, or 100 m, or to *Crassostrea* larvae in samples from any depth.

In 1991, we observed generally lower and less variable control mortality in the toxicity tests (compared to 1990), which enabled detection of smaller statistically significant differences

than in 1990. Although the mortality was lower in 1991, statistically significant toxicity (relative to controls) to *Ampelisca* and/or *Crassostrea* was indicated in 1991 in sediments from 0 and/or 6 m at several oiled sites (most notably Sleepy Bay, Snug Harbor, and Bay of Isles). Most of these statistically significant differences, however, occurred at relatively high survival rates; i.e. greater than 80 percent of controls. Repetitive tests with *Ampelisca* have demonstrated that the protocol will reliably detect 20 percent mortality (relative to controls) in 90 percent of the tests, and it has been suggested that *Ampelisca* test results should be judged significant only when survival of test animals is both statistically different from the batch controls and less than 80 percent (G. Thursby and K.J. Scott, unpublished). *Ampelisca* survival lower than 80 percent of controls was found at only three of eight oiled sites in 1991: Sleepy Bay and Northwest Bay at 0 m, and Snug Harbor at 6 m. In addition, *Crassostrea* survivals were lower than 80 percent of controls for 0 m samples from Sleepy Bay and Bay of Isles. Although mean mortality was similar in 1991 for exposed and reference sites (Table 14), mortality of test *Ampelisca* was less than 5% at three (0 m) or four (6 m) of the five reference sites, compared to only one (0 m, at Chenega Island) or three (6 m, at Chenega, Disk, and Block Islands) of the eight oiled sites. However, sediments from two of the five reference sites exhibited significant toxicities to *Ampelisca* (Drier Bay at 0 and 6 m and Mooselips Bay at 0 m) and/or *Crassostrea* (Drier Bay at 0 m), on the same order as the most toxic of the oiled sites. As a result, no statistical differences between oiled and unoiled sites were demonstrated in 1991.

Our results with *Ampelisca* conclusively demonstrated spill-related sediment toxicity in 1990 in the lower intertidal zones at some of the most heavily oiled sites. Results with all three toxicity tests in all three years demonstrate the general absence of any spill-related sediment toxicity at depths of 20 m or greater. Although the remaining results obtained for shallow sediments (i.e. with Microtox^{R} in 1989 and with *Ampelisca* and *Crassostrea* in 1990-1991) are ambiguous, they are consistent with a general pattern of declining sediment toxicity in the lower intertidal zone from 1989 through 1991, accompanied by associated sediment toxicity down to at least 6 m depth in adjacent subtidal zones. Low residual sediment toxicity was still measurable in 1991 at 0 m and 6 m at many of the most heavily oiled sites, although the presence of significant toxicity at two of the unoiled sites raises uncertainty about the spill-related causality. The decline in sediment toxicity at the 0 m depth between 1990 and 1991 may have been accompanied by a concomitant (small) increase in the toxicity of shallow (6-20 m) subtidal sediments in 1991. Overall, however, the measured chemical toxicity was low throughout the spill area in 1991; and it was not strongly correlated with oiling history at the sampling sites. The unexplained toxicity at unoiled sites (most notably at Drier Bay) was similar in magnitude to that at oiled sites. Except for the positive tests with *Ampelisca* in 1990 for samples from 0 m depth at some of the most heavily oiled sites, the levels of toxicity indicated by these tests (e.g. the subtidal results with Microtox^{R} in 1989, and with *Ampelisca* and *Crassostrea* in 1990 and 1991) were much lower than is typically associated with contaminated sites elsewhere in the United States.

Boehm et al. (1994) reported similar results from sediment toxicity tests conducted with the amphipod *Rhepoxynius abronius* in 1990-1991. Sediment toxicity was indicated for intertidal sediment samples from several heavily oiled sites in 1990, compared to very few in 1991. Some

of the observed toxicity was attributed to grain size differences. Significant differences between oiled and unoiled sites were found only for upper intertidal sediments from boulder/cobble beaches in 1990. Subtidal sediments (3 to 20 m) were not toxic to *Rhepoxynius* in either 1990 or 1991.

Chemical toxicity of the spilled oil was probably greatest during the first month or two of the spill when the more toxic aromatic constituents were more abundant. Much of the acute toxicity of oil is attributed directly to the content of soluble aromatic compounds (Neff et al. 1976). In short-term exposures, molar toxicity appears to increase with number of aromatic rings (i.e. benzene < naphthalene < phenanthrene), at least up through 3-ring compounds, and also with the extent of substitution (i.e. benzene < toluene < xylene < ethylbenzene, etc.) (Neff et al. 1976; Rice et al. 1977; Caldwell et al. 1977). The concentrations of monoaromatic hydrocarbons were largely depleted in the spilled EVO by evaporative processes during the first 2-3 days, before the oil slick was widely dispersed and spread onto shorelines by winds. Similarly the high winds that occurred during days 4-6 after the spill promoted the natural dispersion of oil into the water column (Wolfe et al. 1993a, 1993b), which probably also contributed to the early loss of some of the more toxic constituents from the floating oil that subsequently became beached. These processes undoubtedly reduced the effective toxicity substantially below that which would have occurred had the fresh oil been transported immediately into sheltered bays and shoreline sediments.

Other potential sources of spill-related toxicity, however, are the oxidized derivatives formed either by photo-oxidation or biodegradation of parent petroleum hydrocarbons. Such compounds have been found in harbor waters (Burns et al. 1990; Ehrhardt and Burns 1990) and in coastal waters contaminated by an oil spill (Ehrhardt and Burns 1993). Very limited information is available on the significance of either the polar constituents of crude oil or the intermediate oxidation products of petroleum hydrocarbons (whether from photooxidation or biodegradation) in terms of their potential for bioaccumulation and toxicity to resource organisms in the marine environment. Because these compounds have undergone preliminary oxidation and (sometimes) conjugation, they are more polar than their parent hydrocarbons, and would as a result generally be more subject to excretion or depuration, less subject to bioaccumulation, more susceptible to further oxidation (or biodegradation if accumulated), and more susceptible to dilution and dispersion in the water column.

To determine the contribution of polar constituents to the toxicity of sediments contaminated by EVO, Wolfe et al. (1993c, 1993d) compared the toxicities of polar and aromatic fractions of organic extracts from sediments from two sites in PWS. Large samples of sediment and pore water were collected in early September 1990 from the mid-intertidal zones at the Bay of Isles and Mooselips Bay sites. Methylene chloride extracts from these sediment and water samples were fractionated by liquid chromatography into aliphatic, aromatic and polar fractions, and the aromatic and polar fractions were tested for relative toxicity by a number of tests. In addition to the Microtox test and the bivalve larval mortality and development (with *Mytilus* instead of *Crassostrea*), the other tests were measures of genotoxicity. The samples from Bay of Isles (which

showed obvious signs of heavy oil, both to the eye and to the GC) were only slightly more toxic (usually a factor or 2X-5X) than the Mooselips Bay samples, which was not oiled (by GC) and which gave very low responses in all tests. However, for both sites, at the very high extract concentrations tested, the relative response to the polar and aromatic fractions were about the same in most tests. On the basis of these tests, it was concluded (Wolfe et al. 1993c, 1993d) that although the overall toxicity of the oil was low, at least a part of that toxicity was derived from polar constituents.

As with many marine oil spills, the initial shoreline oiling from the EVOS generally occurred primarily in the mid- to upper intertidal zones, where substantial mortalities occurred, through the cumulative effects of smothering, toxicity, and cleanup (especially high-pressure hot water) (Mearns and Shigenaka 1993). While intertidal infauna are clearly highly vulnerable to exposure by oiling, observations at past oil spills have demonstrated that in some cases the subtidal infauna can also be exposed and can suffer significant mortality and changes in population density (Cross et al. 1978; Cabioch et al. 1980; Beslier et al. 1980; Elmgren et al. 1980, 1983). Furthermore, Addy et al. (1978) and Dauvin and Gentil (1990) showed that chronic oil pollution could also have deleterious effects on benthic invertebrate assemblages in relatively deep offshore waters. It is possible that some of these changes may have been secondary ecological effects of sublethal responses to oil exposure. For example, feeding behavior and survival of polychaetes (*Abarenicola pacifica*) and survival and condition index of deposit-feeding clams (*Macoma inquinata*) were affected by prolonged exposure (11 days and 38-54 days, respectively) to PBCO concentrations in the range of 800-1200 ppm in sediments (Augenfeld 1980; Augenfeld et al. 1980). Observations of subtidal benthic effects after the oil spill in PWS are limited and the direct cause-effect mechanisms are not known (Jewett et al. 1993; Dean and Jewett 1993; Jewett and Dean 1993; Dean et al. 1993). The information presented here suggests that acute chemical toxicity could have been a causal factor in 1989 and 1990 for observed biological effects in the lower intertidal and associated shallow subtidal zones in areas of PWS that were heavily impacted by the EVOS.

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Comparative toxicities of polar and non-polar organic fractions from sediments affected by the *Exxon Valdez* oil spill in Prince William Sound, Alaska.

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ABSTRACT

Standardized tests were applied to aromatic and polar fractions of sediment extracts to determine whether polar constituents or oxidative degradation products contributed significantly to the toxicity of sediments oiled by the *Exxon Valdez* oil spill (EVOS). Intertidal sediment and porewater samples were collected in September 1990 from two heavily oiled sites and an unoiled site in Prince William Sound (PWS). Methylene chloride extracts from these samples were fractionated by liquid chromatography into aliphatic, aromatic and polar fractions, and the aromatic and polar fractions were tested for toxicity using the Microtox^{R} test, bivalve larval mortality and development (*Mytilus*); several measures of genotoxicity in *Mytilus*, including SOS Chromotest^{R}, anaphase aberrations and sister chromatid exchange; and survival, anaphase aberrations and teratogenicity in coho salmon (*Onchorhynchus kisutch*). Microtox^{R} and SOS Chromotest^{R} protocols were applied in a screening mode to all samples, whereas other tests were applied only to selected fractions from two sites. Samples from Bay of Isles (oiled) were consistently more toxic (but usually by only a factor of 2X-5X) than the Mooselips Bay (unoiled) samples, which gave very low responses in all tests. For both sites, however, responses to polar and aromatic fractions were about the same in most tests, suggesting that while the overall toxicity of the oil was low in these samples, at least part of that toxicity was derived from polar constituents. Compared to the parent hydrocarbons, polar oxidation products partition preferentially into porewater and are more rapidly diluted and dispersed in the water column. These results suggest that polar oxidation products of petroleum hydrocarbons pose little risk to marine organisms, except possibly for infauna continuously exposed to porewater in heavily oiled sediments. Independent surveys showed that sediment toxicity in PWS declined during 1989-1991 to near background levels, in accord with our previous understanding of oil weathering and toxicity.

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INTRODUCTION

The 24 March 1989 grounding of the *Exxon Valdez* in PWS released approximately 35,500 metric tons of Prudhoe Bay crude oil (PBCO) into the Alaskan marine environment (Kelso and Kendziorek 1991; Maki 1991). During the next several weeks this oil was widely distributed, eventually impacting about 575 km of shoreline in PWS and about 1170 km in the Gulf of Alaska (Maki 1991; Wolfe et al. 1993a, 1994a). Toxicity of sediments from lower intertidal and subtidal zones of selected oiled shorelines was surveyed each year from 1989 to 1991 in PWS and (1989 only) in the adjoining Gulf of Alaska (Wolfe et al. 1993b, 1994b). Compared to unoiled Alaskan sediments or to contaminated sediments from urban areas elsewhere in the United States, the toxicity of these oiled sediments was not remarkably high in any year, as determined by Microtox^{R} and by acute toxicity tests with amphipods and bivalve larvae. Nonetheless, low levels of toxicity were associated with heavily oiled sites in PWS in both 1989 and 1990, and this toxicity appeared to shift from lower intertidal sediments (0 m depth) into the shallow (3-6 m) subtidal zone during this time. While some oiled sites continued to exhibit significant toxicity, oiled and unoiled sites on average were indistinguishable from each other in 1991 (Wolfe et al. 1994b).

The work described here was undertaken to determine whether toxicity associated with oiled sediments in PWS was attributable in part to oxidized degradation products of petroleum. Earlier work demonstrated that photo-oxidation products generated in confined systems by irradiation of petroleum or refined petroleum products can enhance toxicity to test organisms (Lacaze and Villedon de Naide 1976, Larson et al. 1977, Malins et al. 1985). Oxygenated petroleum derivatives have been documented in inshore waters of Bermuda (Burns et al. 1990; Ehrhardt and Burns 1990) and in coastal water contaminated by the 1992 Persian Gulf oil spill (Ehrhardt and Burns 1993). Because such compounds are not regularly measured in oil-contaminated environments, it is important to understand their contri-bution to the toxicity manifested after an oil spill, to avoid underestimates of toxicity potential based on parent oil alone. This paper describes results of toxicity tests on the aromatic and polar fractions from extracts of sediments and porewater collected in 1990 at oiled and unoiled sites in PWS.

METHODS

Sampling Locations and Sample Extraction

Intertidal sediments and interstitial pore water were collected from an unoiled reference site at Mooselips Bay (60°12.45' N, 147°17.90' W), and two heavily oiled sites on Knight Island: Bay of Isles (60°22.90' N, 147°42.75' W) and Herring Bay (60°27.25' N, 147°42.65' W) (Fig. 1). These three sites were sampled respectively on September 11, 12, and 13, 1990.

Intertidal sediments were collected from depths up to 10 cm by scooping (with pre-cleaned metal utensils) fine-grained material, into 500-ml, "certified clean" (I-Chem^{R}) glass jars. Two samples (40 jars each, ~20 kg total weight) were collected at each location. Samples were

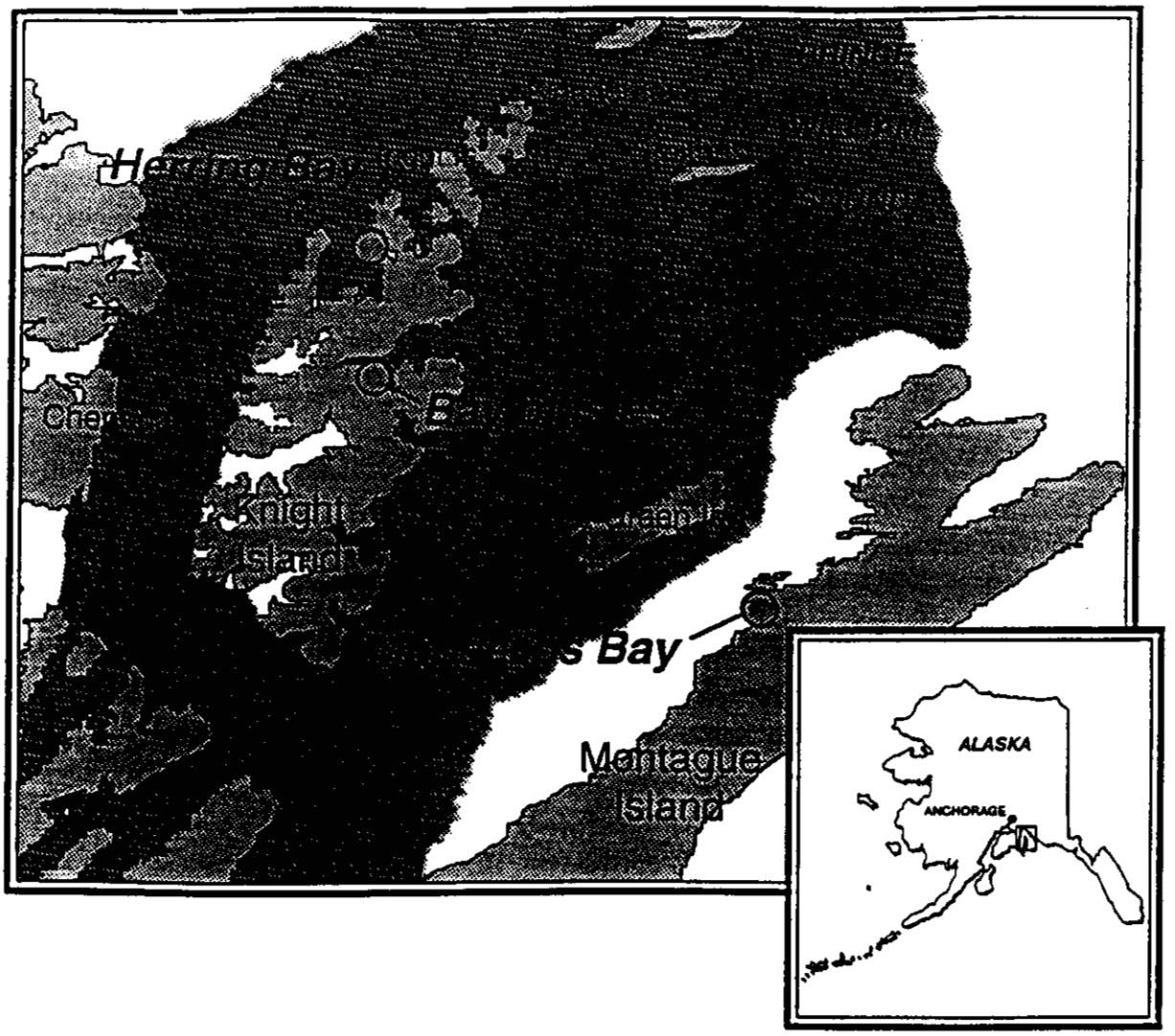

Figure 1. Location of Sampling Sites for intertidal sediments and pore-water in PWS, Alaska. The shaded area represents the approximate extent of floating oil from the EVOS.

frozen within 12 h of collection and maintained frozen until extracted at the SAIC laboratory facilities in San Diego, California. Sediments were sequentially extracted in 500-g batches, using 400 mL methanol and then three 400-mL volumes of a 1:1 (v:v) mixture of methylene chloride-ethyl acetate. Combined extracts from different batches within a sample were concentrated to ~250 mL by evaporation over a water bath at 90-100°C in a round-bottom flask fitted with a Snyder column.

Interstitial pore water was collected during receding tidal cycles from areas of ~144 m^2 at each intertidal location. Two samples (180 L each) were pumped from several excavations (15-30 cm deep) at each location into clean 20 L glass carboys. Samples were acidified in the field to pH 1-2 with HCl, and extracted on board ship. Five hundred (500) mL of 1:1 (v:v) methylene chloride-ethyl acetate and 200 mL of methylene chloride were added to each carboy and mechanically agitated for 2 min with a stainless steel stirring device. Following phase separation, the solvent layer was transferred through stainless steel and teflon tubing to a one-liter separatory funnel, using nitrogen gas overpressure; and each carboy was re-extracted twice more with 500 mL of 1:1 (v:v) methylene chloride-ethyl acetate. Extracts were combined and transferred to clean 4-liter glass bottles for transport to the Kasitsna Bay field laboratory (operated for National Oceanic and Atmospheric Administration (NOAA) by the University of Alaska), where extracts were concentrated (at 80-100°C) in one-liter flasks fitted with Snyder columns to ~250-500 mL. Concentrated pore water extracts were shipped (4°C, dark) to the Science Applications International Corporation (SAIC) laboratory in one-liter, "certified clean" (I-Chem^R) glass bottles.

Chemical Fractionation and Solvent Exchange

Solvent extracts from sediment and water samples were separated into aliphatic, aromatic, and polar fractions by liquid chromatography (LC) on silica gel (70-140 micron, Sigma Chemical Co., St. Louis; column dimensions of 19 mm I.D. x 200 mm). About one mL of extract, representing less than 0.5 g extracted residue weight, was loaded onto the LC column; the aliphatics (F1) eluted with 30 mL hexane; the aromatics (F2) eluted with 45 mL hexane:benzene (1:1, v:v); and the polar constituents (F3) eluted (sequentially) with 80 mL methylene chloride/methanol (1:1, v:v), 80 mL methanol, and 80 mL methanol/ethyl acetate (9:1, v:v), which were then recombined. Elution patterns were checked with suites of analytical standards (aliphatics from nC-12 to nC-32, and aromatics from naphthalene to benzo[ghi]perylene) to confirm that the desired LC separations were being achieved.

Prior to toxicity testing, fractions were solvent-exchanged into dimethyl sulfoxide (DMSO). A 30 mL aliquot of the fraction (F2 or F3) was concen-trated to 1-5 mL by evaporation at room temperature under a stream of ultrapure N_2, brought back to 30 mL with DMSO, re-evaporated under N_2, and replaced again with DMSO. The DMSO solutions were then subjected to toxicity testing.

Sample extracts containing substantial quantities of oil routinely formed two-phase systems (i.e. oil/DMSO) upon partitioning with DMSO. Preliminary screening tests showed that

not all toxic components partitioned from the oil into the DMSO. To ensure that maximum solubilization into DMSO was achieved for toxicity testing and to avoid toxicity losses, various dilutions of sample extracts were prepared directly during the DMSO exchange step. That is, dilutions were prepared by exchanging different volumes of extract fractions into set volumes of DMSO (and not by serial dilution of a single initial DMSO solution). Toxicity information was estimated from a range in the dilution series that gave linear dose-responses relative to the quantity of sample extract that was partitioned against the DMSO.

Toxicity Testing

Microtox^{R} is a commercially available (Microbics Corp., Carlsbad, CA) bioassay based on the inhibition of bacterial luminescence (*Photobacterium phosphoreum*) (Chang et al 1981; Shiewe et al. 1985). Prior to testing, extracts (at various initial dilutions) in DMSO were further diluted with 2% saline solution to a final DMSO concentration of 2%. For testing, 500 uL of 2% saline solution was dispensed into each cuvette followed by 10 uL of bacterial suspension. After 15 min an initial light reading was recorded and 500 uL of the diluted sample was added. Final light readings were recorded after a 15 min exposure period. The final DMSO concentration (1%) produced no light diminution in controls. Each sample dilution was run in triplicate, and a negative control containing 1% DMSO in 2% saline was tested along with each sample. Sample responses were corrected for blank response, and the effective concentration of extract yielding a 50% decrease in luminescence was calculated using either the trimmed Spearman-Karber method (Hamilton et al. 1977), or probit analysis.

The SOS Chromotest^{R} is a commercially available (Orgenics Corp., Israel) bioassay kit used to screen test samples for genotoxic activity. The test uses a strain of *Escherichia coli* (PQ37), genetically engineered to lack the enzyme beta-galactosidase, but in which the operon for that enzyme is linked to the cell's DNA repair mechanism (the "SOS" system). When DNA damage occurs, the SOS system is activated, initiating production of beta-galactosidase, which is measured colorimetrically in the assay (Quillardet and Hofnung 1985; Quillardet et al. 1985). Viability of the test organism was checked by assays of alkaline phosphatase activity. Sample extracts in DMSO were diluted with DMSO/saline to yield a final DMSO concentration of 10% prior to testing. Genotoxic activity was calculated (Dayan et al. 1987) as an SOS Induction Factor (SOSIF). Dayan et al. (1987) suggested that a SOSIF >1.0 indicates genotoxicity, but we used more conservative criteria in this study, requiring that: (a) the SOSIF must be statistically different from the DMSO control; (b) the SOSIF must be >1.3; and (c) the observed response must be dose-dependant. Dunnet's procedure (Zar 1984) was applied to SOSIF values to determine statistical differences.

Toxicity was also assayed using 48 h static exposures of larval bivalve mollusks (Chapman and Morgan 1983; ASTM 1990; USEPA/ACOE 1991), with % normal shell development (to the prodissoconch I stage) and % survival as endpoints. Blue mussels (*Mytilus edulis*) were induced to spawn by raising water temperature from 15° to 20°C, and fertilization was accomplished in a clean 1 L beaker within 2 h. Embryo density was determined on two 1.0 mL aliquots of each of five 1:99 dilutions of the homogeneous suspension. Embryos were inoculated into test vessels

with a calibrated automatic pipette. Fractions were tested in 1% DMSO in sterile, filtered (0.22um) seawater adjusted with deionized water to 28 ppt salinity. After 48 h exposure, two 10 mL aliquots were removed from test vessels and preserved in 5% formalin-rose bengal; and normal (completely developed, straight-hinged, "D"-shaped prodissoconch I stage) and abnormal larvae were counted. Mortality data are expressed as the ratio of the number of surviving larvae in test samples to that in seawater-DMSO controls. Abnormality is expressed as % abnormal larvae, based on the total number recovered for each replicate. Statistics were performed on arcsin/square root-transformed data, using multiple paired t-tests.

Incidences of anaphase aberrations and sister chromatid exchange (SCE) were also determined in the exposed mussel larvae. Beginning 12 h after fertilization, early trochophore larvae were exposed to the extract fractions in 1% DMSO. After 12 h exposure, larvae were concentrated by centrifugation, fixed (for 1 h) in Carnoy's solution (methanol/acetic acid), and then placed in 2% aceto-orcein stain for at least 15 min. Four slides were prepared from each replicate using a standard squash method, and sealed with clear nail polish. For each replicate, 100 anaphase cells were counted for aberrations (Hose 1985), including stray chromosomes, lagging chromosomes, acentric fragments, attached fragments, unequal distribution of chromosomes, translocation bridges, side-arm bridges, and multipolar spindle formations.

For SCE counts, 10^{-5} M bromo-deoxyuridine was added to the exposure medium and colchicine was added to halt metaphase after 10.5 hr of exposure. Larvae were collected on 10-um mesh screens, then exposed successively to 2:1, 1:1, and 1:2 mixtures of seawater and 0.6% KCl for 10 min each, and fixed twice in methanol:acetic acid (3:1) solution. After centrifugation, excess fixative was decanted and 60% acetic acid was added to disaggregate the larvae. Larvae were pipetted onto a microscope slide, squashed with a second slide, then air dried and stained for 10 min with 33258 Hoechst stain in McIlvaine's buffer at pH 8. The slides were exposed to ultraviolet light for 60 min, rinsed in tap water, air-dried, and stained in 4% Giensa for 10 min before final rinsing, drying, and mounting. Ten second-division cells from each of two slides were scored for SCE under 1250X magnification for three replicates. Statistical analyses for SCE and anaphase aberrations were similar to those used for larval survival.

Fertilized eggs of coho salmon (*Onchorhynchus kisutch*) were exposed for 24 h (beginning 72 h after fertilization) to extract fractions to determine mortality, teratogenic and genotoxic effects. Following exposure, dead eggs were culled from test solutions and scored, and 25 eggs per treatment were placed in 5% buffered formalin for anaphase aberration scoring, using procedures similar to those for bivalves (Hose 1985). An additional 25 eggs per treatment were rinsed three times with deionized water, and returned to glass dishes (covered with sterile gauze) randomly distributed in Heath trays in a recirculating hatchery system. Eggs were checked periodically for development to the "eyed" stage, and scored weekly for survival. Dead eggs were recorded and removed. Six weeks after fertilization, the eggs were placed in partitioned Heath trays to accommodate emerging sac fry and monitored for hatching success. By nine weeks after fertilization, 90% of the control fish had absorbed their yolk sacs, and the test was terminated. Replicates were scored for final mortality, and the embryos were placed in 10% buffered formalin for subsequent teratogenic analysis. The embryos were scored for gross anomalies likely to

preclude survival under natural conditions: stunted growth, spine deformities, fin deformities, aberrant optical lobes, protrusive neoplasms, and coagulated yolk (Birge et al. 1983). The numbers of terata were noted per surviving embryo, and statistical analyses (multiple two-sample t-tests) were performed on arcsin/square root-transformed data.

Chemical Analyses and Characterizations

To check on completeness of the LC separations and provide preliminary characterization of the oil constituents present in the samples, the aliphatic, aromatic, and polar fractions were analyzed on a Hewlett Packard 5890 gas chromatograph (FID-GC) equipped with flame ionization detector and a Model 7673A automatic sampler. The column (0.32 mm x 30 m) had a DB-5 stationary phase with a film thickness of 0.25 um, and the flow rate of carrier gas was 2.0-2.5 mL min^{-1}. Following an initial 5-min period at 45°C, temperature was programmed to increase at 3.5°C min^{-1} to 280°C. Some fractions were also subjected to liquid chromatography and particle beam mass spectrometry (LC/PB-MS) (Miles et al. 1992, Doerge et al. 1993). The LC/MS measurements represented an experimental effort to detect and identify oxygenated polar metabolites (e.g. hydroxy and quinone derivatives) of the parent polynuclear aromatic hydrocarbons (PAH) in the oiled samples. Portions of selected fractions were also sent to Drs. Ed. Overton (Louisiana State University), and Manfred Ehrhardt (University of Kiel) for more detailed GC-MS analysis and comparison with previously identified mass spectra from petroleum oxidation products.

RESULTS

Chromatograms are illustrated in Figures 2-3 for the three fractions of one porewater sample each from Herring Bay (FOX WA), Mooselips Bay (LIPS WB), and Bay of Isles (BAY WA), respectively. Chromatograms for the fractions from one sediment sample (BAY SA) from Bay of Isles are shown in Figure 3. Repeating series of n-alkanes (along with pristane and phytane) were present in the aliphatic fractions of all samples (both water and sediments) from Herring Bay and Bay of Isles. Aliphatic fractions from these locations (Figs. 2a, 3a,d) include nC_{12} through nC_{32}, while the aromatic fractions (Figs. 2b, 3b,e) include 2- to 6-ring PAHs, from sub-stituted naphthalenes through benzo(ghi)perylene, reflecting moderately weathered residual oil. Standards demonstrated that neither aliphatics nor PAH were eluting with the polar fraction. Furthermore, comparison of chromatographic profiles for the aliphatic (F1), aromatic (F2), and polar (F3) fractions for the replicate samples from Bay of Isles and Herring Bay (Figs. 2-3) indicated that prominent FID-GC peaks in polar factions were not observed in corresponding aromatic or aliphatic fractions. Appropriate separation of the aliphatic, aromatic, and polar constituents into their respective fractions was therefore confirmed.

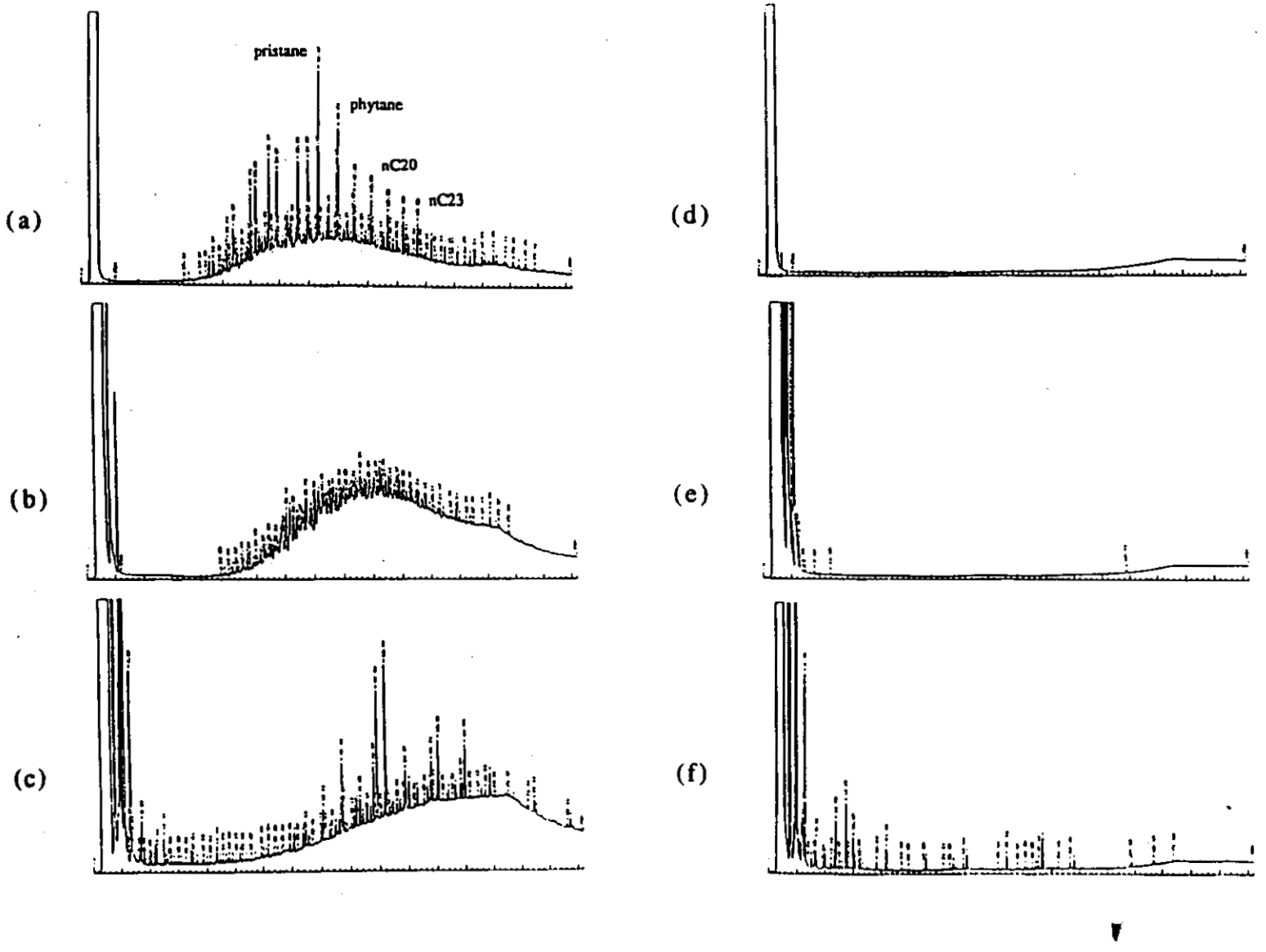

Figure 2. Chromatograms (GC/FID) for porewater extracts from Herring Bay (Sample FOX WA, chromatograms a-c) and Mooselips Bay (LIPS WB; d0f). The top charts in each series (a + d) are the aliphatic fractions; the middle (b + e) are the aromatic fractions; and the lower (c + f) are the polar fraction.

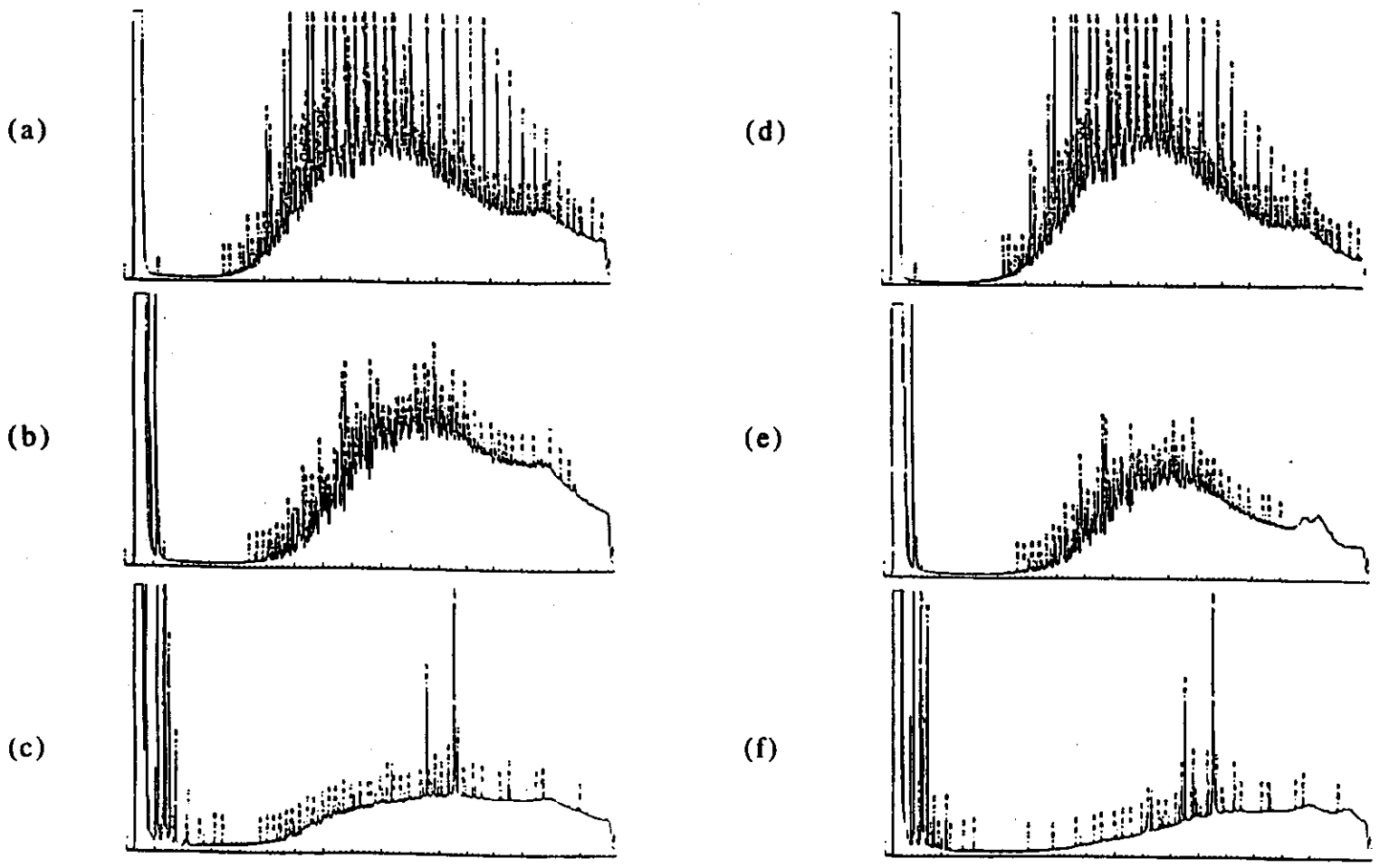

Figure 3. Chromatograms (GC/FID) for extracts of porewater (Sample BAY WA, chromatograms a-c) and sediments from Bay of Isles (BAY SA; d-f). The top charts in each series (a + d) are the aliphatic fractions; the middle (b + e) are the aromatic fractions; and the lower (c + f) are the polar fractions.

In sharp contrast to samples from oiled sites, the aliphatic and aromatic fractions from Mooselips Bay porewater (Fig. 2) exhibited nearly flat baselines, similar to traces obtained with water method blanks. The Mooselips Bay polar fraction (F3) exhibited several peaks, but these were many fewer in number and much lower in concentration than those in polar fractions from oiled sites (Compare Fig. 2f with Figs. 2c and 3c).

All samples were screened with Microtox^R and SOS Chromotest^R, whereas only selected fractions were submitted to the other tests. Table 1 shows test results for various fractions and Table 2 shows statistically significant differences among selected samples and fractions. Microtox^R results indicate that Bay of Isles samples were generally more toxic than corresponding samples from Mooselips Bay, while Herring Bay (FOX) exhibited intermediate toxicity. Samples BAY SBF3 and LIPS WBF2 were modest exceptions to this rule. While the toxicity of polar fractions (F3) was much lower than F2 for Mooselips Bay water, F2 and F3 gave notably similar results for water samples from Bay of Isles (Table 1).

At the highest concentration tested (0.9%), four fractions, all from Bay of Isles (BAY SAF2, SAF3, SBF3, and WAF2) met our SOS Chromotest^R criteria for genotoxicity, with an induction factor (SOSIF) >1.3. The SOSIFs for these same four samples also exceeded 1.3 at the 0.45% dilution, but fell below that level at the 0.09% dilution. At the 0.9% dilution, the SOSIF for sample BAY WBF3 also approached 1.3. Both aromatic and polar fractions were represented in these genotoxic samples, and relative genotoxicity between F2 and F3 was mixed. The polar fraction from Bay of Isles (BAY WAF3), however, inhibited the activities of both beta-galactosidase and alkaline phosphatase, indicating acute toxicity without significant genotoxicity.

Toxicity data for bivalve larvae (survival, development, and genotoxicity) are presented only for the 0.1% dilutions of each fraction (Tables 1 and 2). Differences between Bay of Isles and Mooselips Bay samples were also discernible at the 1% dilutions, but generally not at 0.01% and 0.001%. At 1%, all Bay of Isles fractions except BAY SBF2 (77.9%) caused frequencies of 93-100% abnormal larvae. Except for LIPS WAF2, the Mooselips Bay fractions (1% dilution) elicited frequencies of 11-19% abnormality. LIPS WAF2 caused high mortality, and the few surviving larvae were all abnormal.

Larval mortality was usually higher for Bay of Isles fractions than for corresponding Mooselips Bay fractions, except for the comparison of F2 fractions from sediment samples (BAY SAF2 and BAY SBF2 versus LIPS S1F2), where the mortality for the LIPS sample was slightly higher than that for the BAY samples (Table 1). In all cases where differences were statistically significant, larval abnormality was greater for Bay of Isles samples than for Mooselips Bay samples (Table 2). At lower test concen-trations (0.01% and 0.001%), mortality was low and variable, without any consistency of direction among samples, and abnormality was consistently low, with few statistically significant differences between samples.

Both anaphase aberrations and SCE exhibited statistically greater responses in bivalve larvae for Bay of Isles samples than for Mooselips Bay (Tables 1 and 2). The principal types of

Table 1. Summary of Test Results from Polar (F3) and Aromatic (F2) Fractions of Organic Extracts of Replicate Sediments (SA, SB) and Pore Water (WA, WB) from Bay of Isles (BAY) and Mooselips Bay (LIPS). A hyphen (-) indicates the sample was not tested for that endpoint.

	Microtox	SOSIF	Bivalve (0.1%)				Salmonids (0.1% fraction)		
Sample	EC-50 (%)	(0.9% fraction)	Mortality (%)	Abnormal (%)	Anaphase Abberation (%)	SCE (# per chromosome)	Mortality (%)	Terata (# per fish)	Anaphase Abberation (%)
BAY SA F2	0.25	2.24	14.8	57.7	18.2	0.232	11.1	0.68	35
BAY SA F3	0.22	1.58	25.6	92.8	25.3	**	8.0	0.71	41
BAY SB F2	0.50	1.19	16.2	16.5	-	-	17.3	0.43	33
BAY SB F3	1.44	1.33	29.3	77.6	-	-	8.0	0.38	43
BAY WA F2	0.67	1.66	28.6	99.5	21.0	0.217	2.7	0.59	32
BAY WA F3	0.26	0.366	28.3	33.8	25.6	0.286	8.0	0.59	30
BAY WB F2	0.09	0.855	32.0	22.8	-	-	12.0	0.84	-
BAY WB F3	0.23	1.27	17.5	96.3	-	-	10.7	0.08	-
LIPS S1 F2	1.03	1.11	21.5	18.9	7.3	0.154	6.7	0.13	32
LIPS S1 F3	1.38	1.17	15.8	20.0	11.6	0.218	6.7	0.11	30
LIPS S2 F2	2.45	0.962	-	-	-	-	-	-	-
LIPS S2 F3	1.45	1.062	-	-	-	-	-	-	-
LIPS WA F2	1.03	0.891	14.8	17.4	10.6	0.182	10.8	0.32	30
LIPS WA F3	5.8	0.963	11.1	15.5	14.2	0.204	8.0	0.41	30
LIPS WB F2	0.73	0.964	-	-	-	-	-	-	-
LIPS WB F3	5.23	1.037	-	-	-	-	-	-	-
FOX SB F2	0.54	1.049	-	-	-	-	-	-	-
FOX SB F3	0.99	0.792	-	-	-	-	-	-	-
FOX WB F2	0.12	1.070	-	-	-	-	-	-	-
FOX WB F3	1.14	0.751	-	-	-	-	-	-	-
FOX MB F2	0.12	0.827	32.7	20.0	-	0.220	10.7	0.63	-
FOX MB F3	1.14	0.898	15.2	15.1	-	0.228	17.3	0.49	-
MB269 F2	0.16	0.841	14.2	26.0	-	-	17.3	0.15	-
MB269 F3	6.65	0.83	15.9	26.3	-	-	5.3	0.53	-

Table 2. Statistical differences between fractions at 0.1% dilution. Entries identify the samples that were significantly more toxic at p=0.05, unless noted otherwise.

	Bivalve (0.1% fraction)			Salmonids (0.1% fraction)	
Comparison	Abnormal	Anaphase Aberration	SCE	Terata	Anaphase Aberration
BAY vs LIPS					
SA F2 vs S1 F2	0	BAY	BAY	BAY	0
SB F2 vs S1 F2	0	-	-	0	-
SA F3 vs S1 F3	BAY	BAY	BAY*	BAY	0
SB F3 vs S1 F3	BAY	-	-	0	-
WA F2 vs WA F2	BAY	BAY	BAY	(BAY)	0
WB F2 vs WA F2	0	-	-	BAY	-
WA F3 vs WA F3	(BAY)	BAY	(BAY)	BAY	0
WB F3 vs WA F3	BAY	-	-	LIPS	-
F2 vs F3					
BAY SA	F3	0	F3*	0	0
BAY SB	F3	-	-	0	-
BAY WA	0	F3	F3	0	0
BAY WB	F3	-	-	F2	-
LIPS S1	0	0	F3	0	0
LIPS S2	0	0	0	0	0
SEDIMENT vs WATER					
BAY SA F2 vs WA F2	WA	0	0	(SA)	0
BAY SA F2 vs WB F2	0	-	-	0	-
BAY SB F2 vs WA F2	WA	-	-	0	-
BAY SB F2 vs WB F2	0	-	-	0	-
BAY SA F3 vs WA F3	SA	0	SA*	0	0
BAY SA F3 vs WB F3	0	-	-	SA	-
BAY SB F3 vs WA F3	SB	-	-	0	-
BAY SB F3 vs WB F3	WB	-	-	(SA)	-
LIPS S1 F2 vs WA F2	0	0	WA	WA	0
LIPS S1 F3 vs WA F3	0	0	0	WA	0

* BAY SA F3 judged toxic without statistical analysis
( ) = Probability between 0.05 and 0.10
0 = No significant difference
- = No data/samples not tested

anaphase aberrations observed were translocation bridges, side-arm bridges, and attached fragments (Hose 1985). The DMSO control, however, showed a significantly higher mean number of anaphase aberrations (15.8%) than the seawater control (7.0%). While all Bay of Isles samples were significantly higher than the seawater controls, no differences were detected between Mooselips Bay and seawater controls. BAY WAF3 was the only test fraction significantly different from the DMSO control. The number of sister chromatid exchanges was lowest in seawater (0.121 ± 0.007) and DMSO controls (0.134 ± 0.02) and highest in BAY WAF3 (0.286 ± 0.05). However, the BAY SAF3 fraction caused such heavy chromosome damage that SCE could not be quantified, and it was presumed that this sample was the most genotoxic for this endpoint. All fractions but the seawater control, LIPS S1F2 and FOX MBF2 (0.220 ± 0.063) exhibited significant ($p<0.05$) toxicity relative to the DMSO control.

Mortality of larval salmonids was low in freshwater (9.3%) and DMSO controls (10.7%), and was generally low in all test samples with no significant differences in mortality between any of the samples (Table 1). While the number of terata was higher in the in the DMSO control (0.25 per fish) than in the freshwater control (0.10 per fish), most of the Bay of Isles fractions elicited higher rates of teratogenicity. All but one of the significantly different paired comparisons were higher for Bay of Isles samples than for Mooselips Bay samples (Table 2). More than 90% of the observed teratogenic effects were stunted growth, bent spines, or irregular swelling of the optic lobes. The principal anaphase aberrations noted were translocation, side-arm bridges, and attached fragments; but the aberration rate was 24% in freshwater controls, and no significant differences were found between any of the fractions and the DMSO control (31%) (Table 2).

Preliminary analysis by LC/PB-MS of F3 fractions from Bay of Isles did not identify oxygenated derivatives of benzanthracene, chrysene, triphenylene, benzo[e]pyrene, or benzo[a]pyrene in either the sediment or water extracts, though these parent compounds were easily detected in the corresponding F2 fractions (Doerge et al. 1993). The sensitivity of LC/PB-MS was limited, however, especially for more volatile lower molecular weight constituents (PAH <4 rings), and oxygenated derivatives would probably not have been detected at concentrations much lower than the parent hydrocarbons. More detailed GC/MS analyses also failed to identify oxidized derivatives of petroleum hydrocarbons in the polar fractions of these sediment extracts (Pers. comm., M. Ehrhardt, University of Kiel). The F3 contained large numbers of aliphatic fatty acids, quite certainly of recent biogenic origin, along with a considerable amount of high-molecular weight material (possibly fulvic/humic acids, in part) that did not elute from the CP-Sil-8 CB (equivalent to SE54) column. This finding was similar to the results obtained with sediment and water samples from the Persian Gulf after the Gulf War Oil Spill, where, although UV fluorescence indicated petroleum-derived constituents in polar fractions, structural elucidation proved impossible (Ehrhardt and Burns 1993; Pers. comm., M. Ehrhardt). The actual sources of toxicity in the various chemical fractions from PWS therefore remain unidentified.

DISCUSSION OF PREVIOUS RELATED RESEARCH

The literature on the toxicity of Alaskan crude oil to Arctic and subarctic marine organisms is extensive (Anderson 1977; Rice 1985; Rice et al. 1976, 1977, 1979, 1984; NAS 1985; Wolfe 1985; Karinen 1988). Very little of this prior research was directed, however, toward the significance of either polar constituents of crude oil or intermediate oxidation products of petroleum hydrocarbons. Since these latter compounds have undergone preliminary oxidation and (sometimes) conjugation, they are more polar than their parent hydrocarbons, and would be expected generally to be less subject to bioaccumulation, more subject to excretion or depuration, more susceptible to further biodegradation, and more susceptible to dilution and dispersion in the water column (James and Kleinow 1994). Although the studies described in this paper suggest that such polar constituents may contribute an appreciable fraction of the residual total toxicity and mutagenicity associated with PBCO that has weathered *in situ* in intertidal sediments for 17 months, the total sediment toxicity measurable in most areas was already at a very low level by that time (Wolfe et al. 1993b, 1994b; Boehm et al. 1994).

Toxicity of Oil and its Constituents

Previous studies have shown that acute toxicity of oil diminishes as weathering progresses, and by the mid-1970's, it had been concluded that most oil toxicity was attributable directly to the more water-soluble aromatic compounds (Moore and Dwyer 1975; Neff et al. 1976; Rice et al. 1984). Interest existed at that time in which fractions of petroleum were most responsible for the toxicity observed in laboratory and field exposures to oil. In short-term exposures, molar toxicity appeared to increase with number of aromatic rings (i.e. benzene <naphthalene <phenanthrene), at least up through 3-ring compounds, and also with the extent of substitution (i.e. benzene <toluene <xylene <ethylbenzene, etc.) (Neff et al. 1976; Rice et al. 1977; Caldwell et al. 1977). In direct comparisons of various one- to three-ringed aromatics, the greatest toxicities (LC50-96 h) were associated with dimethylnaphthalene (Caldwell et al. 1977) or 1-methyl phenanthrene (Neff et al. 1976). Chrysene, benzo(a)pyrene, and dibenzanthracene were not lethal to the test organism *Neanthes arenaceodentata* at their limits of solubility in such short-term exposures (Neff et al. 1976). Based on the relative concentrations of low-molecular weight constituents in crude oil, it is generally accepted that acute toxicity effected by oil in the environment is derived mainly from mono- and di-nuclear aromatics. When a water-soluble fraction (WSF) of oil was simulated, however, by mixing the ten predominant aromatic hydrocarbons at the same concentrations and proportions found in a crude oil WSF, the toxicity of the resulting mixture was only 20-30% of the actual WSF, suggesting that either minor aromatic constituents, or other components, contribute significantly to the observed toxicity (Rice et al. 1984). Korn et al. (1985), exploring possible contributions of phenol and p-cresol to the toxicity of crude oil WSF, found that toxicity of phenol (and p-cresol) was about twice that of toluene but only one-fifth that of naphthalene. Because the concentrations of toluene and naphthalene were respectively about 50x and 2-7x greater than that of phenolic compounds in the WSF, however, they concluded that the phenols were not likely to be major contributors to WSF toxicity.

In a previous effort to determine which chemical constituents of PBCO exerted the greatest toxicity and mutagenicity, Warner et al. (1979) fractionated PBCO using a succession of solvent partitioning, gel permeation and adsorption chromatography. Toxicity of the resultant fractions was evaluated using the Ames bacterial mutagenicity test, a mammalian cell toxicity test, and a bioassay with mysid shrimp; and the results suggested that aromatic hydrocarbon fractions were most toxic and probably represented the greatest biological hazard. Although most other fractions showed no toxicity at the levels tested, one high-molecular-weight polar oil fraction (otherwise uncharacterized) was both toxic and slightly mutagenic.

In the marine environment petroleum is decomposed primarily through the processes of microbial biodegradation and photooxidation or auto-oxidation These processes are effective for oil in surface slicks, the water column, sediments, and the atmosphere (photooxidation of evaporated compounds). The extensive literature on hydrocarbon metabolism by microorganisms was recently summarized by Bartha and Atlas (1987). Although the paraffinic and aromatic fractions of petroleum are quite readily degradable, the polar fractions as well as most nitrogen- and sulfur-containing fractions are essentially nonbiodegradable (NAS 1985). The rate and final amount of biodegradation of any petroleum depend heavily on its composition and on specific abiotic environmental parameters. In general, the rate of petroleum biodegradation in marine waters or in surficial (1-2 cm) sediments is limited by the availability of inorganic nutrients and not by oxygen or temperature. Once oil penetrates into deeper sediments, however, oxygen may become the limiting factor for degradation of petroleum. High-molecular weight PAH may remain indefinitely in aquatic sediments, and significant sublethal effects to benthic organisms have been documented to accompany long-term persistence (Krebs and Burns, 1977; Jackson et al., 1989).

Products of photooxidation include fatty acids, alkylated naphthols, substituted 1- to 3-ring aromatic and heteroaromatic acids, and alkylated benzothiophene sulfoxides (Overton et al. 1979, 1980). Oxidized products of phenanthrene, including carbonyl, quinone, and carboxylic acid derivatives, were identified in seawater extracts after UV irradiation of a phenanthrene "slick" for 120 h (Malins et al. 1985). The asphaltene and resin fractions of crude oil appeared to inhibit production of photooxidized derivatives in these experiments; UV irradiation (120 h) of the separated aromatic/paraffinic fraction of PBCO caused a 20-fold increase in the partitioning of extractable organic material into underlying seawater and a 10-fold increase in seawater-soluble derivatives of ^{14}C- phenanthrene added before irradiation. About half the phenanthrene metabolites in seawater after UV irradiation were not methylene chloride extractable, indicating oxidation to highly water-soluble products (Malins et al. 1985).

Microbial biodegradation of alkanes, cycloalkanes, and mono-aromatics leads to the production of alcohols, aldehydes, and carboxylic acids that are generally of little concern from a toxicity standpoint. Highly condensed PAH, however, may be transformed by microbial metabolism to potential carcinogens or mutagens (James and Kleinow 1994). Benzo(a)pyrene and benzo(a)anthracene, for example, are oxidized by eucaryotic organisms (macroorganisms, yeasts and molds) to trans-dihydrodiols that are activated into oxides that are powerful mutagens. Procaryotic organisms (bacteria) oxidize the same compounds to cis-dihydrodiols

which undergo oxidative fission of the ring structure without passing through these mutagenic intermediates (Gibson 1977, Cerniglia and Heitkamp 1989).

Some metabolic products of PAH are demonstrated mutagens or carcinogens known to bind to DNA (Ahokas et al. 1979; Lech and Bend 1980; Varanasi et al. 1981). These same materials are associated with the prevalence of liver lesions, including neoplasms (Varanasi and Stein 1991). These intermediary metabolites are the inferred likely mediators of biological damage in tissues that bioaccumulated and metabolized the parent hydrocarbons (Lech and Bend 1980). Although it has been suggested that metabolites might contribute to the effects of hydrocarbon exposure through the food web (Carls 1987; James and Kleinow 1994), there is currently neither quantification nor even documentation of such an effect, nor is information available on the fate of hydrocarbon metabolites that are released into the environment. The data presented in this paper suggest that polar constituents do contribute part of the (albeit low) toxicity and genotoxicity associated with PBCO after more than one year of weathering in PWS sediments.

Asphaltenes and resins are heterogeneous, poorly characterized assortments of non-hydrocarbon compounds comprising respectively about 2% and 6% of PBCO (Clark and Brown 1977). Asphaltenes are tar constituents that are highly resistent to biodegradation, and are not generally considered to pose a risk of toxicity to marine organisms. Resins include polar and heterocyclic NSO compounds, such as phenols, cresols, thiophenes, dibenzothiophenes, pyridines, and pyrroles. Very little work has been published on these compounds, but some of them are likely to undergo biodegradation, and very broad suites of NSO compounds have been found in oil-contaminated marine environments (Wolfe et al. 1981; Krone et al. 1986). Like hydrocarbon metabolites, these compounds exhibit moderate water-solubilities and are subject to dispersion in the water column. Although some of these compounds might be toxic at high concentrations, no studies have focused specifically on the levels of toxicity exerted by these materials under spill conditions in the marine environment. Since these fractions were not distinguished or separated from other polar constituents in this study, no statements are possible on their contribution to the observed low levels of toxicity.

Uptake-Depuration, Metabolism, and Toxicity of Oxygenated Hydrocarbon Derivatives Relative to Parent Compounds

Bioavailability and bioaccumulation of hydrocarbons depend in large measure on whether the hydrocarbons are dissolved (or finely dispersed, or micro-colloidal) in the water column, adsorbed onto suspended particulate matter or sediments, or contained in food materials. The relative uptake for different hydrocarbons by organisms is related to hydrophobicity as reflected by octanol-water partition coefficients (Veith et al. 1979; Dunn 1980), as is their adsorption to sediments and suspended matter (Means et al. 1979, 1980; Karickhoff 1981; Wolfe 1987). Accumulation potential and retention generally increase with increasing molecular weight (i.e. with number of aromatic rings and with the degree of alkyl substitution on the rings) (Roubal et al. 1977; Melancon and Lech 1979; Rice et al. 1984). For the higher molecular weight PAH, however, this generalization may be obscured by their very low solubilities, associated slow

bioaccumulation rates, and by simultaneous metabolism within the organism.

Phenol, cresol and toluene were more effectively excreted across the gills by dolly varden char than were naphthalene, anthracene, and benzo[a]-pyrene; whereas metabolites of all of these compounds were excreted via the bile (Thomas and Rice 1981, 1982). The bile is a major route of excretion for hydrocarbon metabolites in fish (Collier et al. 1978, Varanasi and Gmur 1981). Most bile metabolites of naphthalene are in the form of conjugates, but some excretion of naphthols and dihydrodiols also occurs (Varanasi and Gmur 1981; Varanasi et al. 1981). Similar metabolites are excreted by crabs and shrimp (Lee et al. 1976; Sanborn and Malins 1980). Metabolic conversion rates in fish and crustacea must closely approximate the intake, since long-term accumulation of parent compounds does not occur. Prior exposure of Dolly Varden to naphthalene resulted in increased metabolism of orally-administered ^{14}C-naphthalene (Thomas and Rice 1985), reflecting the induction of cytochrome P450 by PAH (Stegeman et al. 1981, Lee et al. 1982). Prior exposure to toluene, however, did not induce accelerated metabolism of ^{14}C-toluene (Thomas and Rice 1985).

Both conjugated and nonconjugated metabolites occur also in muscle tissue (Varanasi and Gmur 1981; Thomas and Rice 1982). Consumption of such metabolites represents a potential mode of exposure to predatory macro-organisms, but only limited insight on the potential amounts of such metabolites in edible tissue is available from experimental results with labeled compounds. Twenty-four h after intragastric administration of ^{14}C-labeled naphthalene, anthracene, and benzo(a)pyrene to Dolly Varden char, 15%, 11%, and 7.5% of the respective radioactivities were found in muscle tissue. Of those amounts, only 5.1%, 3.3% and 8.8%, respectively, were associated with a polar metabolite fraction (as opposed to parent compound) (Thomas and Rice 1982). Similar studies could be used to estimate the relative proportions of metabolites and parent PAH in edible tissues (e.g. Varanasi et al. 1990).

Malins et al. (1985) compared a number of toxic responses to sea-water accommodated fractions (SWAF) of weathered and unweathered oils, including PBCO. Hatching success of English sole eggs was lower in unirradiated SWAF than in irradiated SWAF generated under low-flow conditions with fuel oil. When SWAFs of no. 2 fuel oil were produced in this agitated flow-through system, UV irradiation caused less than a 2-fold increase (over the unirradiated SWAF) in total extractable organic materials, and no differences were observed in mortalities of English sole embryos exposed for 48 h. When no. 2 fuel was irradiated under static conditions, however, the extractable organic material in the SWAF was enhanced about 23-fold (to 161 ppm), and mortality was substantial, with an apparent EC-50 of about 25 ppm. Preparation of SWAFs from PBCO under identical conditions, however, produced no differences between flow-through and static conditions either in levels of total extractable organic materials or in mortalities of English sole embryos. English sole larvae were more sensitive than the embryos, showing significant mortality after 48 h exposure to fuel oil SWAF (in the low-flow system). The toxicity to larvae of the non-irradiated SWAF was slightly greater than that of the irradiated SWAF. Mortalities of newly hatched surf smelt larvae exposed in the low-flow system were not different for irradiated oil, nonirradiated oil, and control (no oil) treatments. At sublethal levels of total hydrocarbon (0.25-0.35 ppm) in the unirradiated SWAF, however, the larvae showed

pronounced effects on swimming behavior. This effect diminished with time (probably due to loss of volatile hydrocarbons from the SWAF), and was reversible. Cytopathological examination of the affected larvae showed necrosis of sensory tissues and compression of muscle bundles. Larvae exposed to irradiated SWAF were apparently not so affected. These data suggest that the potentially toxic products of hydrocarbon oxidation are more readily dispersed in the water column than their parent hydrocarbons, thereby reducing bioavailability and toxicity potential. Malins et al. (1985) concluded that these studies provided no evidence that photooxidation would significantly enhance the toxicity of petroleum under most conditions in the marine environment.

CONCLUSIONS

The toxicity tests described here confirmed and extended the results of a survey that showed low residual sediment toxicity in 1990 in lower intertidal and shallow subtidal zones of heavily oiled sites in PWS (Wolfe et al. 1993b, 1994b). Fractions from Bay of Isles were consistently more toxic than corresponding fractions from Mooselips Bay for a variety of tests, including Microtox^{R}, SOS Chromotest^{R}, and abnormality, anaphase aberration, and sister chromatid exchange in bivalve larvae. However, samples from Bay of Isles (which showed obvious signs of heavy oiling) were only slightly more toxic (usually by a factor of 2X-5X) than the Mooselips Bay samples, which was not oiled and which gave very low responses in all tests.

The salmonid teratogenicity endpoint exhibited about the same sensitivity to oiled sediments (ability to distinguish Bay of Isles from Mooselips Bay samples) as the bivalve larval abnormality, anaphase aberration, and sister chromatid exchange endpoints, whereas the salmonid anaphase aberration was much less sensitive.

Toxicity of polar fractions from Bay of Isles was similar to, or higher than that of corresponding aromatic fractions. Polar fractions from porewater were much more toxic to Microtox^{R} at Bay of Isles than at Mooselips Bay, consistent with the observation that oxidized petroleum derivatives were more concentrated (relative to parent compounds) at oiled sites in porewater extracts than in sediment extracts (Pers. comm., M. Ehrhardt, University of Kiel). Although the overall toxicity of these samples was very low, we concluded that an appreciable part of that toxicity was attributable to polar constituents. No other patterns in relative response of corresponding sediment and water fractions were observed for any test or for either site.

Results from multi-year sediment toxicity surveys (Wolfe et al. 1993b, 1994b; Boehm et al. 1994) suggested that acute chemical toxicity (including that attributable to oxidized constituents) declined between 1989 and 1991, and shifted concomitantly from the lower intertidal zone (0 m) into shallow (3-6 m) nearshore sediments. The results are consistent with earlier published conclusions (Moore and Dwyer 1975; Neff et al. 1976; Rice et al. 1984) that the chemical toxicity of oil is associated primarily with the lower molecular weight aromatic compounds lost most readily through weathering. The most significant toxic effects probably occurred during the first days after the spill, when the most toxic aromatic constituents were

abundant (Wolfe et al 1993a, 1994a). Nonetheless, the low levels of toxicity and genotoxicity demonstrated here in relation to polar constituents and/or derivatives of oil cannot be dismissed entirely (Burns 1993). Our samples were collected approximately 17 mos after the spill, and the contribution of polar materials to the toxicity observed over that time period is unknown. Although the overall toxicity has been described, its origins are not known in detail; and for some organisms (e.g. intertidal infauna), prolonged exposure to polar materials in porewater could contribute significantly to the total. For practical purposes; however, the existing cumulative data bases on acute and sublethal oil toxicity (e.g. NAS 1985) appear appropriate for assessing relative sensitivities of marine species and estimating ranges of potential response to oil spills.

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Fate of the oil spilled from the T/V *Exxon Valdez* in Prince William Sound, Alaska

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ABSTRACT

This paper summarizes the overall fate of the 10.8 million gallons of oil spilled from the *Exxon Valdez*. Field observations integrated from several investigations have been compared with published information on spilled oil in the marine environment, to reconstruct the movements and transformations of the oil from the time of the spill through fall of 1992. The distribution of floating and beached oil during the first 6 weeks was accurately hindcast by the National Oceanic and Atmospheric Administration's (NOAA) On-Scene Spill Model (OSSM), which took into account real-time data for wind fields and currents moving the slick. The outputs of OSSM and of NOAA's Oil Weathering Model were used to estimate initial losses from the slick due to evaporation and dispersion into the water column. By April 30, 1989, about 20% of the spilled oil had evaporated, and another 20-25% had been dispersed naturally (mainly by wave action) into the water column. About 10% was transported out of Prince William Sound (PWS) as floating oil, and most of the remainder (approximately 40-45%) had beached within PWS. Much of the floating mousse that departed PWS was deposited on shorelines in the Kenai and Kodiak areas. About 2-4% of the spilled oil floated past Cape Douglas into the Shelikof Strait. The oil recovered by skimming operations in 1989 accounted for about 7-10% of the original spill volume. Cleanup operations on the beaches during the first four summers led to the recovery and disposal of approximately 31,000 tons of solid oily wastes, estimated to account for 5 to 8% of the original spilled oil. While evaporation was the dominant process in oil weathering during the first few days, biodegradation became a dominant process after the first month. Oil that was dispersed from the shorelines into the water column by storm action or by cleanup activities was flushed into the Gulf of Alaska (GOA) where it underwent dilution and degradation, with eventual settling of highly degraded residuals to the bottom. Coincident with the declines of intertidal oil, concentrations of oil and activity of hydrocarbon-

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degrading microbes increased in subtidal sediments, peaking by September 1990 at depths of 3-20 m. Sediment traps deployed at 10-20 m depths in PWS recovered oil-contaminated particulate material, verifying transport of oiled sediments from adjacent intertidal zones. While most of the oil originally beached had been removed by October 1992 through a combination of biodegradation, cleanup activities, and natural erosion into shallow nearshore sediments, some relatively unweathered oil still persisted in protected situations, e.g. deeply buried in some PWS beaches and in fine sediments under some mussel beds.

INTRODUCTION

Just after midnight on March 24, 1989, the 987-foot tank vessel *Exxon Valdez* ran hard aground on Bligh Reef in PWS, Alaska, releasing approximately 10.8 million gallons of North Slope crude oil in just a few hours and causing the largest oil spill in U.S. history. Over the next eight weeks, the oil was spread by winds and currents along about 1,750 km of shoreline, up to 750 km from the original spill site. The general events surrounding this spill have been described elsewhere (National Response Team 1989, Kelso and Kendziorek 1991, Maki 1991). In this paper we estimate the amounts of oil transported and transformed through various processes, and reconstruct the overall fate of the oil during the time from the spill through summer 1992.

Crude petroleum is an extremely complex mixture of thousands of organic compounds, ranging from methane to complex resins and asphaltenes, with unidentified chemical structures and molecular weights in excess of 20,000 (Clark and Brown 1977). A wide variety of physical, chemical, and biological processes begin almost immediately to transport and transform crude oil when it is introduced into the marine environment (NAS 1985, Clark and MacLeod 1977, Wolfe 1977, 1985, 1987, Jordan and Payne 1980, Payne and Phillips 1985) (Fig. 1). The petroleum spreads on the surface of the water and is advected by the water and wind. Different fractions of the oil may simultaneously undergo evaporation, dissolution, emulsification, dispersion, adsorptive processes with suspended particulate materials, and grounding on shorelines. Oil-contaminated sediments may be re-suspended or eroded from intertidal shorelines and transported into subtidal zones. During these transport and transformation processes, petroleum constituents also undergo oxidative decomposition, either through photochemical oxidation or biological metabolism. Collectively these processes contribute to the decomposition, or weathering, of the oil in the environment. We have examined these processes as they applied to the specific circumstances of the *Exxon Valdez* oil spill (EVOS) and affected the overall fate of the spilled oil. We present our best estimates for those various processes and construct an overall mass balance for the spilled oil.

Figure 2 summarizes the overall mass balance for the *Exxon Valdez* oil (EVO). It shows the time courses for several major components in the fate of the spilled oil, including: 1) the floating slick, 2) evaporation, 3) recovery of floating oil, 4) dispersion of oil into the water

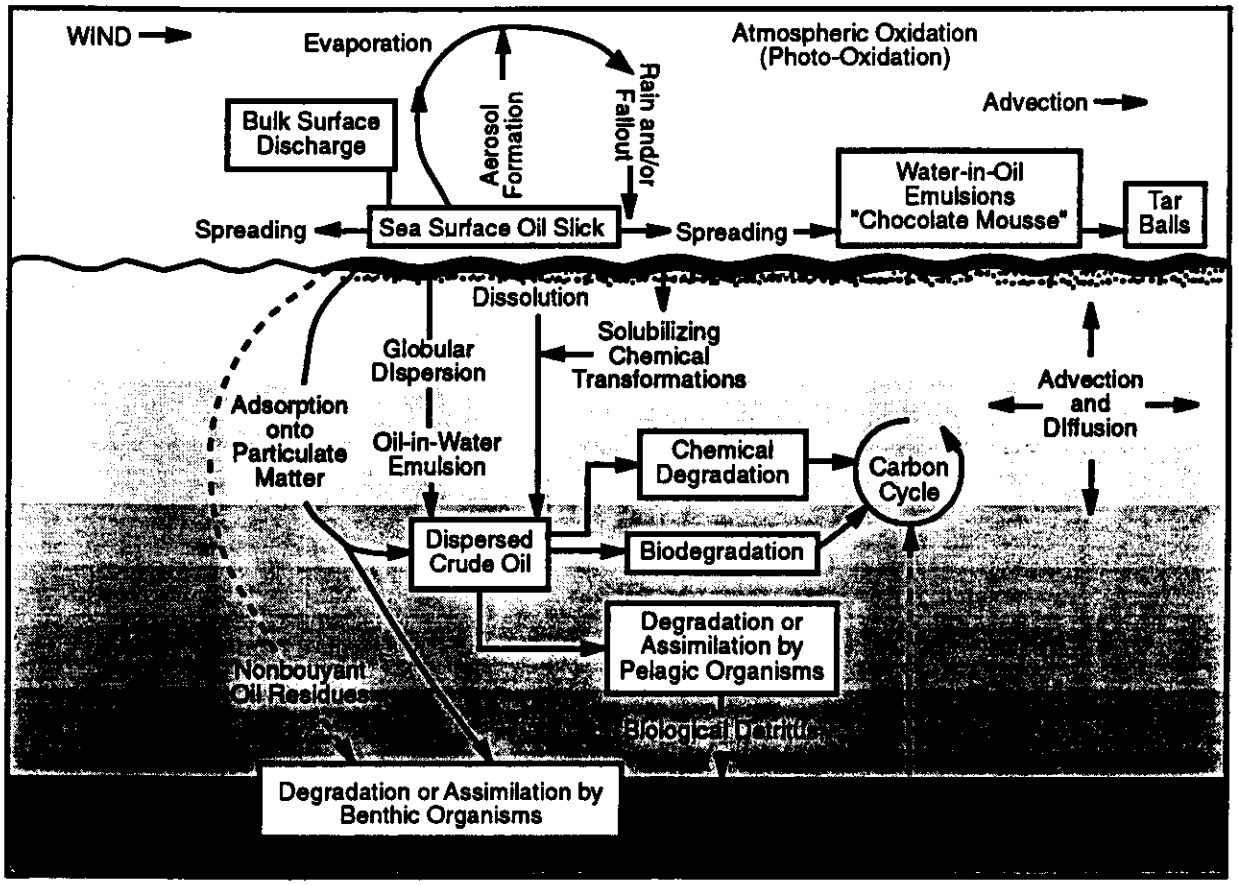

Figure 1. Processes affecting the distribution and transformation of crude oil spilled into the marine environment. Adapted from (NAS 1985).

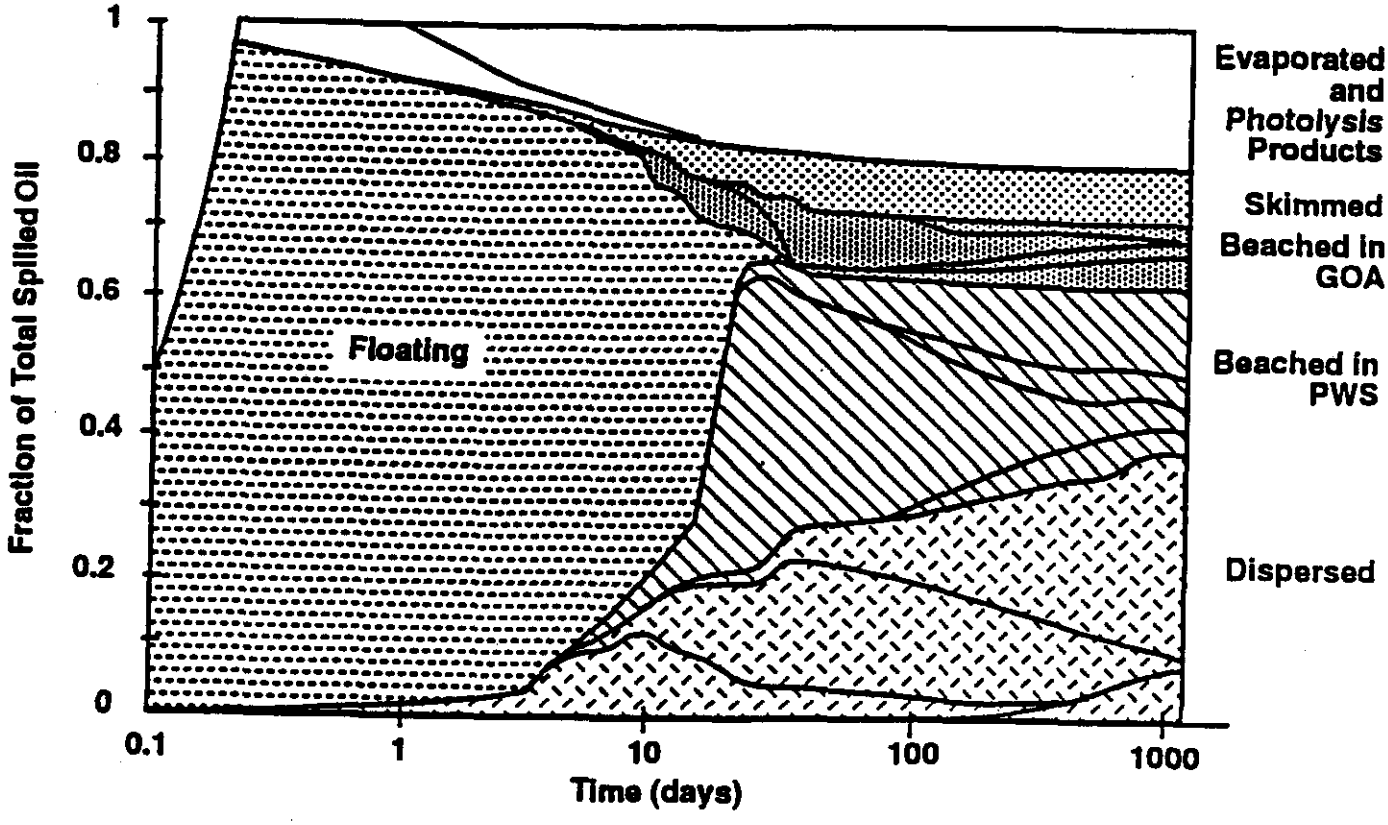

Figure 2. Fate of EVO: the overall mass balance from March 24, 1989 to October 1, 1992. Greater detail for the different shaded sections of this mass balance is provided in subsequent figures.

column, and 5) beaching and subsequent transformations. The logarithmic time scale on Figure 2 extends from about 2:30 AM on March 24, 1989 up to October 1, 1993. Most of the major components are divided into subcompartments that reflect the redistribution and transformation of the oil through time, and which are discussed in subsequent sections. The mass balance includes all processes thought to have significantly influenced the ultimate disposition of EVO in the Alaskan marine environment. For many of these processes, the estimates are based on considerable understanding and are supported by direct measurements or observations at the spill scene. For others, however, quantitative information is almost entirely lacking, and the values shown are the products of simulation models, or are reasoned estimates, determined by difference, used to illustrate the possible evolution in the quantities and location of spilled oil. The mass balance must be viewed as somewhat speculative, although great effort has been made to reconcile the estimates for various compartments and processes with one another to derive the most accurate possible "big picture" of the oil's fate. Supporting data and interpretations for the major components, including estimates of uncertainty, are discussed in the following sections, where supplemental illustrations provide greater detail, on the same time scale as Figure 2.

Distribution and movement of the surface slick

According to early accounts of the spill (National Response Team 1989, ADEC 1989), nearly all the spilled oil was released from the *Exxon Valdez* within five hours of the grounding at 12:04 AM on March 24. Final estimates (Exxon 1992) of the total spill volume converged on 10.84 million gallons (~258,000 barrels or ~35,500 metric tons). In Figure 2, the spill is depicted as complete 5 hours after the grounding of the ship, with 1/5 of the total spill volume being released each hour. Evaporation of the oil was appreciable even before all the oil had escaped from the ship.

For approximately six weeks after the spill, the distribution of floating oil was recorded almost daily by trained observers participating in surveillance overflights. When the spill occurred, and for most of the next three days, winds were mostly still (generally 5-10 knots) and the sea was calm in PWS. During this period the oil slick was concentrated in open water to the southwest of the grounded ship, spreading over an area of approximately 300 km^2. In the midafternoon of the third day (March 26), however, winds rose to 20-25 knots, and these winds were sustained (with gusts of 50 to 70 knots) over the next 3 days, moving the oil rapidly to the southwest, and driving it ashore on the beaches of Naked, Eleanor, Smith, Ingot, and Knight Islands. By March 30, the leading edge of the floating oil had passed through Montague Strait into the GOA. For the next three weeks, oil was repeatedly deposited, refloated, and re-deposited on affected shorelines in PWS as the local winds and tides shifted. During this period, floating oil continued to drift out of PWS into the GOA, where it floated in windrows and patches of mousse (viscous water-in-oil emulsion, containing up to 70% water), and grounded on many of the exposed headlands along the coast of the Kenai Peninsula. Gale force (40-70 knots) winds churned the GOA along the Kenai coast on April 9-10. The floating oil reached the Chugach and Barren Islands at the entrance to Lower Cook Inlet during April 18-19, entered the Shelikof Strait on April 24-25, and came ashore in Hallo Bay and Katmai Bay along the Alaska Peninsula on April 29-30. By May 3, minor quantities of oil had also been reported as far

southwest as Chignik. During the latter half of April, floating oil inside of PWS was reduced mostly to surface sheens, except in close association with heavily oiled shorelines, where in local situations winds and tides continued to lift the oil from beaches and shift it to other shorelines nearby. By May 1, very little oil remained floating; the more fluid oil fractions had seeped into coarse-textured (cobble) beaches, and the residual floating oil had formed water-in-oil emulsions (mousse) with relatively high viscosity and specific gravity and sticky surfaces, such that it adhered to the shoreline surfaces, and no longer was readily refloated by tidal, wave, and wind action. Galt et al. (1991) provide a detailed account of the local hydrology and meteorology as it affected the movements of floating oil in PWS and the GOA.

During the first 5-6 weeks the lighter components of the original crude oil were removed by evaporation and, to a much lesser extent, dissolution. These processes raise the specific gravity of the residual oil from 0.88 g/ml only to about 0.91 g/ml, and the oil remained extremely buoyant in seawater. Water also became emulsified into the floating oil to form mousse. Under turbulent conditions, the water content of floating oil can reach about 10% after 6 h, 17% after 12 h, 30% after one day, 50% after 2 days, 60% after 3 days, and 70% after about 5 days (Payne et al. 1983). Initially the larger particles of oil that are dispersed into the water column rise quickly to the surface, but as the specific gravity and water content rise, the dispersed droplets and globs of mousse may remain suspended in the water column for longer periods. Nonetheless, the oil retains a negative buoyancy, such that no appreciable sinking of the floating oil was likely during this initial period of the spill. The formation of mousse increases the effective volume of the floating oil by a factor of about three. Thus the floating oil that departed PWS represented a volume of material equivalent to three times its initial volume. As a result, the apparent volume in the downstream sectors of the spill trajectory was exaggerated relative to the proportion of oil that actually reached there.

Galt et al. (1991a, 1991b) used observational data and computational procedures to provide a quantitative hindcast estimate of the distribution of floating oil over time. In this hindcast, the distribution of the spilled oil was modeled using NOAA's OSSM, and the model output was reconciled with the oil distribution actually observed during overflights of the spill area. Losses from the slick to evaporation and dispersion, were calculated statistically to represent typical weathering characteristics for Prudhoe Bay crude oil (PBCO) under the prevailing weather conditions (Galt et al. 1991b, Payne et al. 1984). To minimize estimation errors due either to inaccurate trajectory estimates or identifications of floating oil in the field, the model output was compared frequently with observations recorded during overflights, and where appropriate, the model was reinitialized or adjusted to improve the fit to the field data. This hindcast provided the statistical estimates of the distribution of the floating (and initially beached) oil used in the present reconstruction of oil fate.

Evaporation

The primary factors controlling evaporation of oil from either a floating slick or from solution in the water column are: (1) the composition of the oil itself, with respect to the contents and vapor pressures of individual chemical constituents; (2) the area and thickness of the spill; (3) temperature; and 4) the wind speed across the surface of the slick (Mackay et al. 1983, Stiver and Mackay 1984, Stiver et al. 1989). Because PBCO contains such a poorly characterized, complex mixture of constituents, the overall course of oil evaporation cannot be determined practically with a component-specific approach, and "pseudo-component" approaches have been employed (Payne et al. 1983, 1984, 1991, Mackay et al. 1983, Reijnhart and Rose 1982).

Composition of PBCO in Relation to Oil Weathering

The composition of PBCO is summarized in Table 1 in terms of a series of successive distillation fractions (Payne et al. 1984). The composition of these "pseudo-component" fractions compares closely with published data (Table 2) on the chemical composition of PBCO (Clark and Brown 1977, Coleman et al. 1978), with the exception that the very light (natural gas) fractions may have been slightly underestimated. Extensive laboratory and field experience has demonstrated that the fractions containing alkanes up through n-C_{10} or n-C_{11} are lost quite rapidly through evaporation, whereas those fractions containing alkanes in the n-C_{12} to n-C_{16} range evaporate more slowly and are affected more predominantly by biodegradation processes (NAS 1985). The mass fractions represented in Table 1 by cut numbers 7 and below (~20% of the total mass) represent the oil most likely to evaporate under environmental conditions, while cut number 9 effectively represents a theoretical maximum (~30%) that might evaporate in the absence of competing processes (dispersion, skimming, and biodegradation).

Based on the "pseudo-component" fractions in Table 1, Payne et al. (1984) developed a weathering model that was used by Payne et al. (1991) to estimate the rates and amounts of evaporative and dispersive losses from the oil spilled by the *Exxon Valdez*. By merging predictions for an 8-knot wind during the first 3 days and a 20-knot wind during days 4-10, this process pointed towards asymptotes of about 0.20 and 0.23 (day 50-60) for the fractions evaporated and dispersed, respectively. Figure 3 illustrates the theoretical evaporative losses predicted by this model for different fractions under the high wind conditions of March 26-29, 1989. The resultant estimates are reflected in the evaporation component of Figure 2, and are in turn consistent with the amounts of floating oil that were estimated by the Galt et al. (1991a, 1991b) OSSM hindcast, and with the amounts reported to have been picked up by skimmers operating in PWS (ADEC 1992). This estimate for total evaporation is lower than previous estimates, but is nonetheless consistent with the match between the composition of PBCO (Table 1) and the composition of oil remaining in PWS a month after the spill (Payne et al. 1991), especially after the necessary adjustments are made for dispersion of a portion of the fresh oil into the water.

Table 1. Composition of the more Volatile Fractions of PBCO, in Decreasing Order of Volatility, by Distillation Cut. (Payne et al. 1984, Mackay et al. 1992). Fractions 1-7 contribute substantially to evaporative processes in the environment, while fractions 10-15 do not figure significantly in evaporative processes. B=benzene; T=toluene; X=xylene; N=naphthalene.

Cut no.	Boiling range (°C)	Specific gravity	Vol %	Cumulative vol %	Representative wt %	Constituents
1	<75	0.681	2.12	2.12	1.64	nC_{3-6}, B
2	75-100	0.711	2.63	4.75	3.76	nC_{6-7}, B, T
3	100-125	0.739	3.54	8.29	6.72	nC_{7-8}, T
4	125-150	0.768	3.64	11.93	9.86	nC_{8-9}, C_2B, X
5	150-175	0.777	3.74	15.67	13.16	nC_{9-11}, C_3B, indan
6	175-200	0.787	3.54	19.21	16.32	nC_{10-12}, $C_{4-5}B$, N
7	200-225	0.804	4.35	23.56	20.28	nC_{11-13}, $C_{5-6}B$, C_1N
8	225-250	0.822	4.85	28.41	24.80	nC_{13-15}, C_2N, biphenyl
9	250-275	0.836	5.06	33.47	29.60	nC_{14-16}, C_3N, acenaphthene
10	275-304	0.858	2.83	36.30	32.36	$nC_{>16}$, $>C_3N$, fluorene, phenanthrene
11	304-337	0.866	6.57	42.87	38.81	
12	337-363	0.882	6.88	49.75	45.69	anthracene (340°)
13	363-392	0.894	6.07	55.82	51.84	
14	392-421	0.903	7.48	63.30	59.50	pyrene (400°)
15	>>421	0.973	36.7	100.00	100.00	perylene (500°)

Table 2. Composition of PBCO, by Distillation Cut and major compound class (Clark and Brown 1977). All values volume percent, except for sulfur and nitrogen (weight percent).

	Total fraction	Paraffins	Naphthenes	Aromatics	Other	Sulfur	Nitrogen
Natural gas (<20°C)	3.08	3.08	0	0	0	0	0
Naptha fraction (20-205°C)	20.1	9.39	7.42	3.26	0	0.0028	0.004
Mid-Distillate (205-343°C)	24.6	2.19	3.54	(nd)	18.87[a]	0.084	0.0098
Wide-Cut Oils (343-565°C)	35	3.26	7.98	(nd)	23.77	0.367	0.056
Residuum (>565°C)	17.6	(nd)	(nd)	(nd)	13.4 4.1 [polar+insol]	0.405	0.120

[a]Includes aromatics and non-hydrocarbons

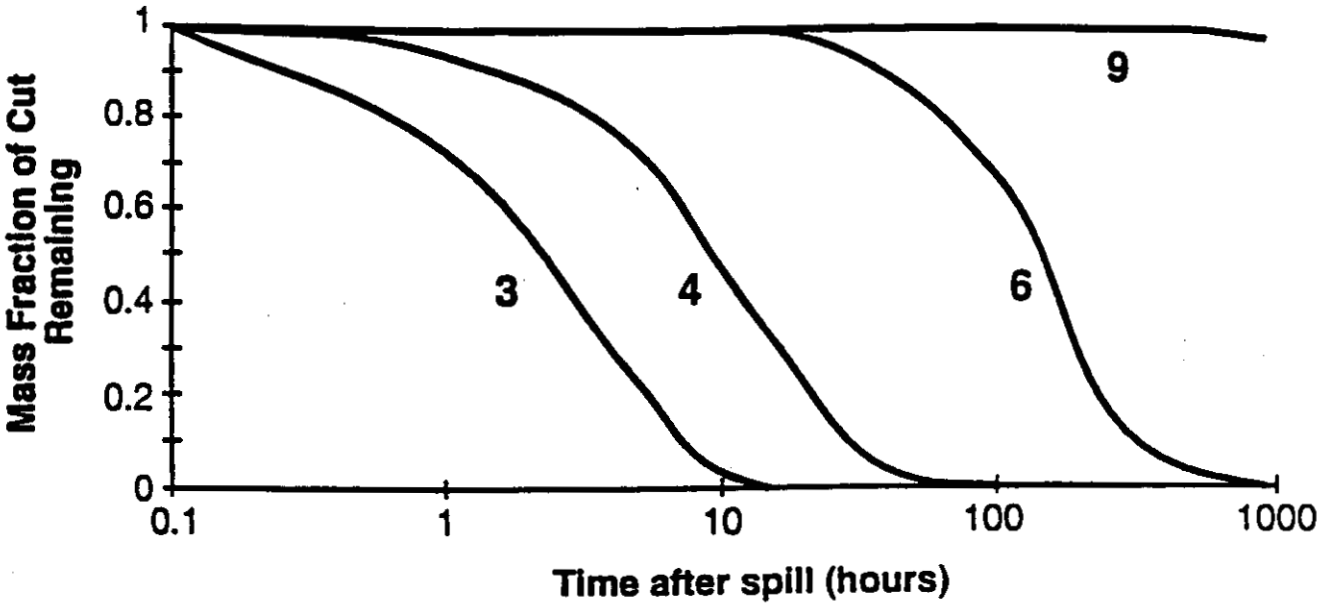

Figure 3. Predicted evaporation by distillation cut (Table 1) at 3.3°C and 20-knot windspeed. (Based on model described by Payne et al. (1984)).

Theoretical evaporation rates were calculated for selected volatile constituents from the oil slick as it floated in PWS (Hanna et al. 1991, Hanna and Drivas 1993). Benzene (Fig. 4), toluene, ethylbenzene, xylene, and hexane would exhibit about the same fractional evaporation rates. No direct measurements were made of the evaporation of these volatile constituents in PWS, but such extremely rapid losses of low-molecular-weight volatile hydrocarbons have been quantified from experimental releases of petroleum on the sea surface (McAuliffe 1977).

Distribution of Hydrocarbons in Air

Hanna et al. (1991) and Hanna and Drivas (1993) estimated the maximum concentrations for selected constituents that would occur in the air over the center of the oil slick at different times. For benzene, toluene, and octane, maximum concentrations would have occurred during the first hour of the spill, while maximum xylene concentrations would have occurred during hour 3. For benzene, with an initial concentration of 3.0 mg/g in fresh PBCO (Clark and Brown 1977), this maximum concentration would have been about 9 ppm. Benzene would account for approximately 5 percent of the total constituents within a similar volatility range.

Since the oil slick remained fairly compact for the first two days after the spill, it resembled a point source for the evaporated constituents to the atmosphere. The trajectory for this atmospheric component (for the first hour of the spill) passed over Naked Island, Eleanor Island, and Herring Bay on northern Knight Island (Hanna et al. 1991). The wind direction and speed remained approximately constant through the first 2.75 days of the spill, and the movement of atmospheric components would have followed essentially this same trajectory throughout this period (albeit with a wider track as the slick spread). After day 3, however, the slick was spread extensively and erratically around the islands of PWS, and as the oil was beached much of the oil settled into the interstices of coarse cobble beaches, where evaporation rates were slowed considerably below the theoretical rates applicable to floating oil. Throughout days 4-14 of the spill, the winds in PWS were consistently from the northeast (Galt et al. 1991a, 1991b, Hanna et al. 1991) and evaporated components would still have been transported generally to the southwest from the spill area. Atmospheric concentrations during this period were very much lower than during the initial 3 days due to the combined effects of the greater areal extent of the spill, the reduced volatility of the residual constituents, and the high winds that prevailed much of the time.

Photolysis of Hydrocarbons in Air

Once evaporated, petroleum hydrocarbons undergo rapid oxidation to photolysis products. Mackay et al. (1992) concluded from published studies that the half-lives for mono- and di-aromatic compounds in the vapor phase were generally less than one day. Based on these data, they suggested that all monoaromatics, along with indan, naphthalene, and substituted naphthalenes could be assigned a mean half-life in air of 17 h (or 1 day); while biphenyl, acenaphthene, fluorene, phenanthrene, and anthracene all exhibit effective half-lives of 55 h (or 2 days); and 4- and 5-ringed structures (pyrene to dibenzanthracene) all exhibit half-lives of about 170 h (or 7 days). In this scenario, the monoaromatic and naphthalenic compounds would be 90% degraded 3-4 days after they

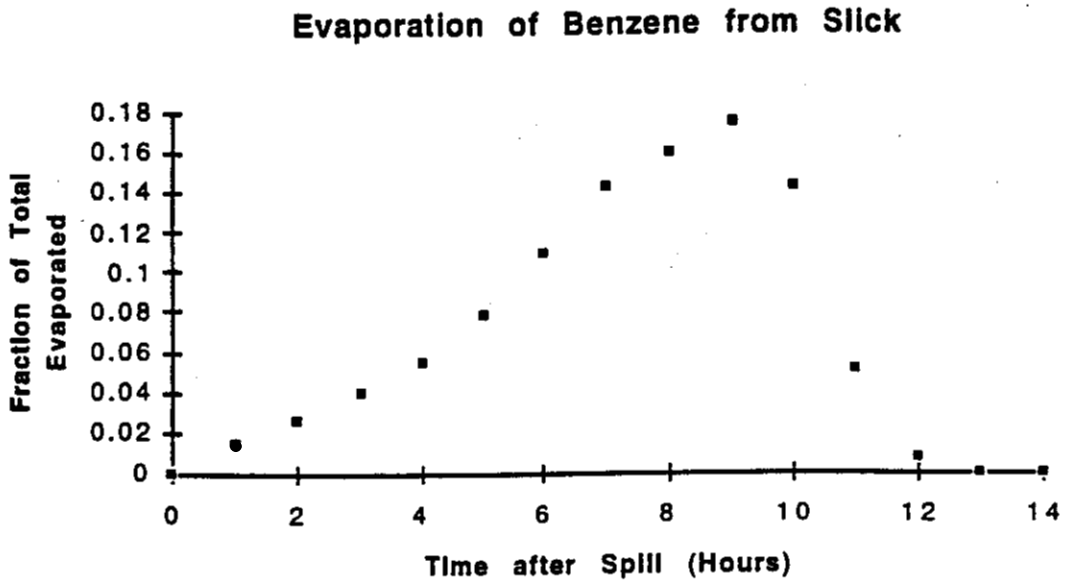

Figure 4. Hourly evaporation of benzene from surface slick, as fraction of total benzene present (Hanna et al. 1991). Benzene represents approximately 5 percent of the total oil within this range of volatility.

evaporated, and 99% degraded within a week. This decomposition is portrayed in Figure 2 by the division of the evaporated component into two sections, representing the initial parent compounds and resultant photolysis products, respectively.

Recovery or Destruction of Floating Oil

Cleanup activities after the spill included incineration of floating oil, skimming of floating oil (including oil refloated from oiled shorelines), and recovery of solid oily wastes (Carpenter et al. 1991). The estimates pertaining to floating oil are discussed below; solid wastes recovered from oiled shorelines are discussed later.

Test Burning of Floating Oil

During the evening of the second day of the spill (March 25), a small amount of spilled oil was combusted in an area about 10 km southwest of Bligh Island (Allen 1991). Lightly emulsified oil (estimated at 20-30 percent water) was concentrated and ignited within a section (138m in length) of floating Fire Boom^{R} towed between two boats. At an average oil depth of 13-18 cm, the holding capacity of the boom was estimated at 20-30 x 10^3 gal (75-110 x 10^3 L). Previously observed burn rates for thick layers of fresh crude oil suggested that approximately 15-30 x 10^3 gal could be consumed in the actual 45-minute period of intense burning. Based on this information, our best estimate (ADEC 1992) of the volume of oil consumed lies in the range of 15-25 x 10^3 gal, or about 0.14-0.23% of the original spill volume. This implied a combustion efficiency possibly as high as 98-99% for the oil in the boom.

Recovery of Skimmed Oil

During the first several weeks after the spill, floating oil was recovered by skimmers operating throughout PWS. This "emulsified oil and water from skimming activities was transported to Seattle or to Exxon's Baytown refinery for processing, oil recovery, and waste water treating" (Carpenter 1991). The National Response Team (1989) reported that as of 30 March, 7537 bbl had been recovered; by 8 April, the amount had grown to 17,000 bbl.; and on 20 April, nearly 41,062 bbl. of oil and water mixture, and 14,270 bbl. of oil were transferred to a barge alongside the *Exxon Valdez*. As of April 26, forty-two skimmers remained in operation. ADEC reported that 65,000 bbl. of oil-water emulsion were ultimately recovered for treatment (ADEC 1992).

Since the bulk of the oil was recovered 1-3 weeks after the spill, it contained a considerable amount of emulsified water. Payne et al. (1983) examined the rate of formation of this emulsion, or mousse, using PBCO in experimental wave tanks. After 5-6 days weathering time, the average water content of the emulsion was 50-60%, and the maximum was 70%. Three weeks after the EVOS, the measured water content of floating mousse from the vicinities of Eleanor Island and Knight Island was in the range of 45-57.8%, while a sample from Pt. Adam on the Kenai Peninsula contained 69.4% (Payne et al. 1991). The estimates of 18-22 x 10^3 bbl. of recovered oil (ADEC 1992) would

place the water content of the mixture at 66-72%. The mass balance (Fig. 2) depicts the amount recovered by skimmers at 8.3% of the original spilled oil (equivalent to 67% water content).

Dispersion and Dissolution in the Water Column

Most oil constituents have very low solubilities in water, and those with the greatest solubilities (low-molecular weight aromatics) also exhibit the greatest volatilities. As a result of the substantial amount of evaporation that occurred during the first 2.75 days, dissolution was considered an insignificant process after the EVOS. Oil enters the water column, however, primarily through the action of turbulence at the water's surface, which disperses tiny, discrete droplets of oil into suspension, and the amount that can be dispersed is appreciable (Payne 1984, Mackay et al. 1980). After initial dispersion, larger droplets of oil may refloat to the surface, but smaller droplets remain finely dispersed in the water column and are subject to the action of local currents and eddy diffusion. Dispersed oil exhibits the approximate composition of whole oil, without notable enhancement of the more soluble constituents (Short and Rounds 1993a).

Dispersion/Accommodation in the Water Column

Mackay et al. (1980) showed that the primary component of dispersion occurs in approximate proportion to the square of the wind velocity, which affects the depth to which oil is driven beneath the surface through wave action. Dispersion also occurs under non wave-breaking conditions, though at a much slower rate. Dispersion algorithms developed by Mackay et al. (1980) were adapted into an oil weathering model developed originally for NOAA, and validated with experimental data from wave tanks (Payne and Phillips 1985, Payne et al. 1984). This model was used to estimate the fraction of EVO dispersed into the water column after the spill.

For the first 2.75 days after the EVOS, winds were relatively light (5-10 knots), and dispersion was low relative to evaporation. During days 4 through 6, however, the average winds over the spill field were about 20 knots, and the oil slick encountered extreme turbulence both in the open waters of PWS and as it first came ashore in shallow coastal areas. Payne et al. (1984) estimated that concentrations on the order of 5-10 ppm could be generated in surface waters under a slick of fresh PBCO under sustained (24 h) wind conditions of 20-40 knots. According to the weathering model (Payne et al. 1984, 1991), approximately 3.5% of the total oil would have been dispersed 72 h after the spill under the influence of an 8-knot wind, whereas the amount dispersed after 3 days of sustained 20-knot winds would have been approximately 9.3%. For the mass balance (Fig. 2), the oil dispersed initially from the floating slick was estimated from these model runs by combining the estimated amount dispersed under 20-knot conditions during days 4-10 with the estimates obtained under 8-knot wind conditions for the first three days and for the period after day 10. This approach suggested an asymptote at about 23% (of total spill mass) for the dispersed oil at about 50 days.

Figure 5 shows the dispersion component of the mass balance by itself, illustrating clearly the initial slow rate of dispersion and the sharp effect of the sudden increase of wind on day 3. Most of

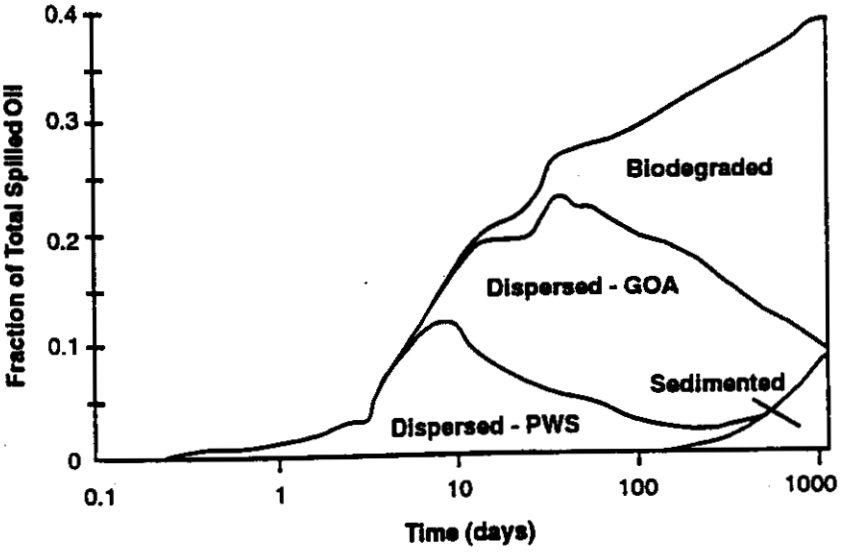

Figure 5. Formation, transport, and transformations of oil dispersed naturally from the floating slick and, through cleanup and storm wave action, from oiled shorelines.

this dispersion would have occurred inside of PWS before the floating slick began to exit through Montague Strait. Some of this dispersed oil undoubtedly became entrained in the coastal circulation patterns of PWS and was retained within the Sound longer than the main front of the slick. However, a substantial fraction of the dispersed oil (those portions entrained in the mainstream of Montague Strait and associated with the slick front itself) was probably departing the Sound at about the same time that measurements of oil in the water column began inside of PWS on March 31 or April 1. The amounts of petroleum hydrocarbons measured in the water column during early April can be reconciled approximately with this estimate of initial dispersion, through reasoned estimates of overall effective spill area and depth for the dispersed component (Table 3). For purposes of illustration, we assumed that the oil initially dispersed in PWS was transported into the GOA in proportion to the rates estimated by NOAA's OSSM for the floating oil. After May 1, the transfer of dispersed oil is shown with a half-clearance time of about one week for PWS (or an effective turnover time of 3-4 weeks).

The overall dispersion picture (Figs. 2 and 5) includes a second component for which there is no direct quantitative estimate and no available modeling tool. A portion of the oil that was initially beached was subsequently dispersed into the water column through the action of waves, high pressure cleanup activities on the shorelines, and winter storms. This dispersion is well documented through the analyses of elevated levels of petroleum hydrocarbons in nearshore waters during the summer and fall of 1989 (Short and Rounds 1993b, Neff 1990, Neff 1991a, Neff 1991b), and by photographs which show sometimes substantial plumes of suspended sediments flowing downstream from shoreline cleanup activities during 1989 (Mearns and Shigenaka 1993). Bragg and Yang (1993) suggested that this dispersion and resultant shoreline cleansing was enhanced through the process of clay-oil flocculation.

The shoreline dispersion component accounts for all the cumulative dispersion above 23% of the total spilled oil (Fig. 5). It was estimated (by difference) to remove about 15% of the total spilled oil or about 35% of the beached oil over the 3-year period after the spill. For illustration purposes, this process was estimated to start just beyond day 40, with about 2/3 of the total in the first summer and winter season. This estimate was balanced against other processes affecting the beached oil (biodegradation and erosion), to produce a residual beached fraction compatible with the progression of shoreline cleansing noted by shoreline assessment teams. The continued dispersion of oil from the beaches is supported by the persistence of oil bioaccumulation in caged mussels deployed at different depths and locations in PWS (Short and Rounds 1993a), and by the persistence of elevated aromatic metabolites in bile of fishes (Collier et al. 1993). Elevated petroleum hydrocarbons were measured in the tissues of caged mussels through the fall of 1989 into the spring of 1990 and in some areas into 1991 (Short and Rounds 1993a), attesting to the continued presence of low levels in the water column well after hydrocarbon concentrations had decreased below the detection limits for direct analysis of the water. Over this period, this oil must have been derived from material dispersed into the water column from the shorelines, through the combined effects of cleanup activities and natural wave action.

Table 3. Scenarios for the concentrations and compositions of EVO in the water column (dispersed-colloidal and/or dissolved) of PWS at different times after the spill, and fractions (weight percent) of the original spill represented by these amounts. References indicate actual measured concentrations in the ranges indicated.

Time period (days)	Effective Spill Area (m^2) or Volume	Petroleum component	Concentration (ug/L) (~ppb)	Fraction of spilled oil (wt %)	References
OFFSHORE					
(24-26 Mar)	$3x10^8$	BTEX	0.8-1.32[a]	0.0034	
(27-30 Mar)	$10x10^8$	PHC	800[b]	23	
(31 Mar -4 Apr)	$2x10^9$[c]	tPAH	1-5	0.056-0.28	Short and Rounds 1993b
		PHC[d]	67-335	4-19	
(14 Apr -4 May)	$2x10^9$[c]	tPAH	0.1	0.0056	Short and Rounds 1993b
		PHC[d]	1-6	0.056-0.34	
NEARSHORE					
(31 Mar -4 Apr)	$7x10^7(m^3)$[e]	VOA[f]	1.5	0.0003	Neff 1991a, 1991b
		tPAH	4	0.0008	Short and Rounds 1993b
		PHC	80-240	0.016-0.040	

[a]BTEX concentrations estimated as 2% of original oil, with 91% evaporated at 62 h, and 3.1% dispersed (from NOAA model) uniformly into upper 3-5 m of water column over spill area.
[b]Estimated concentrations from 23% dispersed fraction (NOAA model): equivalent to 12.2 ug PAH/L with uniform dispersion (by 20-25 knot winds) to 10-m depth over effective spill area.
[c]Effective spill area as of 2 April, 1989: PWS and adjacent GOA.
[d]Mass fractions estimated from measured values, assuming uniform dispersion to 10-m depth over effective spill area
[e]Mass fractions estimated as dissolved uniformly to 5-m depth in a 100-m wide band off heavily (75.6 km) and moderately (64.4 km) oiled shorelines in PWS.
[f]VOA (volatile organic analytes- Neff 1991).

Measurements of Dispersed and Dissolved Hydrocarbons in PWS

Data for petroleum hydrocarbon concentrations in the water column in the spill area include dissolved and finely dispersed (colloidal) material, but generally exclude samples in which there was obvious particulate oil, i.e. large droplets, or mousse (Payne et al. 1991, Short and Rounds 1993b, Neff 1991a, 1991b). The earliest sampling was on March 30 (Allen 1991) and March 31 (Short and Round 1993) in areas where slicks were present or had been recently observed. Table 3 summarizes some of these observations, and compares them in time and space to the estimates derived from the NOAA model for total spill fraction dispersed. The measurements underlying Table 3 are discussed below in some detail.

Neff's (1991a, 1991b) analyses showed that high quantities of oil were accommodated into the water column, especially in nearshore samples from areas where the oil made initial landfalls. In samples that did not contain particulate mousse material, concentrations ranged upward to 1 ppm petroleum hydrocarbons (PHC) analyzed by gas chromatography. Water samples were also analyzed by infrared (IR) spectrophotometry for total petroleum hydrocarbons (TPH). Of 506 water samples analyzed, 18 samples exceeded 1 ppm and 4 exceeded 3 ppm TPH. In a few samples exhibiting contamination with mousse material, much higher concentrations were observed: 459 ppm PHC and 1700 ppm PHC (Snug Harbor and Northwest Bay, in April); 2.2-2800 ppm PHC in 10 surface samples (Morning Cove on Ragged Island, Kenai Peninsula, during an August storm); and 435 ppm PHC (unidentified PWS shoreline site). Although these values were much greater than typical concentrations and most likely represented samples with temporarily dispersed mousse particles, they illustrate the substantial capacity for dispersion in the water column, and demonstrate that the concentrations necessary to have achieved the 23% dispersed fraction were actually observed in PWS.

Polynuclear Aromatic Hydrocarbons (PAH). Neff (1991a, 1991b) reported total PAH data (as the sum of 37 aromatic components from naphthalene to benzo[g,h,i]perylene) from offshore sites in PWS for seven sampling periods in 1989 and for one in 1990. Total aqueous PAH concentrations ranged from below the detection limit (0.01 ppb) to 7 ppb. Highest concentrations occurred in mid- to late April and all 5 samples (out of 798) that exceeded 1 ppb were found in surface water samples. Of the total number of offshore samples, fifty-two (6.5%) contained PAH at concentrations in the range of 0.2-1.0 ppb. The highest concentrations occurred in bays and near islands where the oil made its early landfalls. Average concentrations in surface and subsurface waters appeared to peak during mid April, but many of the samples taken in early April were reportedly taken in front of the spill (or were not reported because of contamination problems), and the peak concentrations most probably occurred earlier. After the survey in early July, mean concentrations were uniformly low (0.03-0.06 ppb). At nearshore sites concentrations of PAH were somewhat higher than at offshore sites (Neff 1991a, 1991b), with 16 samples (of 391 total, or about 4%) in the 1-30 ppb range, and 47 samples (12%) in the 0.2-1.0 ppb range. Highest concentrations were observed in June in Northwest Bay. In nearshore samples modestly elevated PAH concentrations were associated with shoreline treatment activities; otherwise concentrations generally declined throughout summer 1989.

Neff's (1991a, 1991b) results for PHC in these same samples are useful for estimating the total amounts of EVO incorporated into the water column along with the PAH. At the offshore PWS sites, PHC concentrations corresponded generally with the PAH concentrations. Twenty-three of 778 (~3%) offshore water samples from PWS contained PHC in excess of 100 ppb (ranging up to 1000 ppb), while 60 (7.7%) were in the range of 50-100 ppb, and the balance were less than the method detection limit of 50 ppb. At nearshore sites, 23 of 381 samples (6%) exceeded 100 ppb, while 44 (11.5%) were in the range of 50-100 ppb.

The PAH concentration in the spilled oil has been estimated at about 1.5 ng PAH/100 ng oil (=Wt %) (Short and Rounds 1993a). Using this metric, one would expect PHC values on the order of 67X the PAH values for whole oil dispersed into seawater. The distribution of PHC values measured by Neff (1991a, 1991b) appears to be somewhat higher than predicted on this basis from his corresponding PAH distribution. If dissolution were a significant process, one would expect the PAHs to be enhanced in the water column relative to other PHCs, because PAHs exhibit greater solubilities than most other petroleum constituents. This situation would lead to a ratio of less than 67X for PHC to PAH. Short and Rounds (1993b) found this situation in only a few of their samples indicating that dispersion was the dominant process (as inferred also from Neff's data).

Short and Rounds (1993b) reported that PAH concentrations ranged up to 6.24 +/- 0.63 ug/L (~ppb) in the water column at Snug Harbor, and ranged from 1.26 +/- 0.40 to 4.72 +/- 1.18 ug/L near other heavily oiled beaches (including Northwest Bay, Herring Bay, southeast Eleanor Island, north shore of Smith Island, and Bay of Isles). Concentrations of PAH were also elevated (about 1 ug/L) at offshore sites throughout much of Montague Strait during March 31 to April 4. Concentrations at 5m were generally somewhat lower that those in surface (1 m) samples. Except for sites where beach cleanup activities were actively underway, water column concentrations of hydrocarbons decreased (generally by a factor of 2 or more) between April 1 and April 15, and had declined further (again by factors of more than 2) before the final sampling three weeks later. As was the case with Neff's (1991a, 1991b) water samples, aliphatic hydrocarbons (C_{10}-C_{30}) were present in many of these samples, verifying that the samples contained colloidal oil droplets.

Table 3 shows that the pattern of analytical results (Short and Rounds 1993b, Neff 1991a, 1991b) is reasonably consistent with the theoretical estimates based on spill areas or on model outputs, as included in the mass balance. The modeled estimates for total dispersed hydrocarbons during March 27-30 are consistent with the concentrations of PHC measured a few days later, and are in the range of 67X the maximum concentrations of total PAH measured during March 31-April 4, if uniform dispersion to depths of 10 m is assumed over the spill area. Dispersed hydrocarbons were probably more highly concentrated in the region of the slick front as it departed PWS, but those amounts would have been missed by these initial sampling efforts. By the time sampling was initiated inside of PWS, a large portion of the water mass containing dispersed oil was already departing the sound. Also, water samples containing obvious mousse particles were selected against in the sampling described here. These categories of unmeasured dispersed oil could easily account for the imperfect correspondences between the measured concentrations and the modeled estimates of total dispersed amounts (Table 3). Furthermore, many of the one- to three-ringed aromatic hydrocarbons, along with the normal alkanes, typically undergo photolysis and degradation in the water column with half-lives of 1-3 weeks (Mackay

et al. 1992), thereby further reducing the estimated hydrocarbon concentrations from the initial loading of dispersed petroleum. Direct observation of dispersed oil in deep water is lacking, but anecdotal evidence is provided by observations of oil exposure in rockfish found dead in PWS immediately after the spill (Hoffman et al. 1993).

Dissolved Hydrocarbons: Volatile Organic Compounds. Neff (1991a, 1991b) measured volatile organic analytes (VOA) in water samples collected at 35 "offshore" sites and 51 nearshore oiled sites in PWS between March 31 and mid-October 1989. Highest VOA concentrations were found in early and mid-April, but many of the samples on the first cruise were contaminated by sampling gear and the results were rejected. In mid-April, seven offshore water samples (all suspected to have been contaminated by sampling gear) showed VOA concentrations between 5 and 25 ppb, and several more (about 7.2% of all samples) were in the range of 1-5 ppb. By the end of April, average concentrations of VOA at offshore sites in PWS had returned to background levels (about 0.2 ppb). Concentrations of VOA at nearshore sites in PWS showed overall ranges and distributions of values very similar to those seen at offshore sites, with 57/349, or 16%, of the total number of samples in the 1-5 ppb range, and 6 samples in the range of 5-16 ppb. All of these most elevated samples, however, occurred during surveys in June through October (not in April-May), and the incidence of observations in the 1-5 ppb range was also greater during these later sampling periods. While these elevated concentrations observed 3-6 months after the spill in PWS might have been associated with near-shore cleanup activities, this observation suggests that other (non-spill) hydrocarbon sources could also have been involved. Button et al. (1992) report that toluene and xylene are at least partly of natural origin, occurring regularly in the fjord waters of Resurrection Bay at concentrations on the order of 0.13 and 0.05 ug/L, respectively.

Neff (1991a) concluded that aromatic hydrocarbons reached maximum concentrations in the water column by early-to-mid April, and had returned to near background levels by June. Considering their very high volatility, however, it is most probable that the maximum concentrations of dissolved monoaromatics had already occurred prior to Neff's first sampling for water column constituents. Hanna et al. (1991) calculated that essentially all the benzene, toluene, and xylenes would have evaporated from the slick during the first 2 days of the spill. The NOAA weathering model (Payne et al. 1991) also indicates elimination of these compounds by hour 10-12 of the spill (Fig. 3). Table 3 shows one estimate of the amount of aromatic constituents potentially accommodated into the water column during the first few days after the spill, as explained below. The total monoaromatic concentration in the naphtha fraction of the original PBCO (20-205°C, which includes essentially all of the benzene, toluene, xylene, and ethyl benzene and part of the substituted monoaromatics up to C_4-C_5) is around 3.2% by weight with the BTEX representing about 2% (Clark and Brown 1977). According to the weathering model (Payne et al. 1984, 1991), approximately 9% of the distillation cuts containing BTEX would have remained 62 h after the spill, and 3.1% of that amount would have been dispersed into the water column. Uniform dispersion into the upper 3-5 m of the water column would have produced concentrations of BTEX in the range of 0.8-1.2 ug/L (Table 3).

Photolysis and Biodegradation in the Water Column

Constituents dispersed or dissolved in the water column may: (a) evaporate into the atmosphere, (b) undergo photolysis/oxidation in the water column, © undergo biodegradation in the water column, (d) be adsorbed onto suspended particulate sediments and detrital material subject to sinking, or (e) be sorbed (bioaccumulated) into living cells (plankton) and tissues of macroorganisms. For the more soluble constituents (mono- and di-aromatics) evaporation is by far the most rapid removal process. Photolysis or chemical oxidation of these compounds in the water column would be confined to the upper layers of water and is relatively slow compared to biodegradation by microorganisms. Nonetheless, the ultimate fate of most of the dispersed oil in the water column is microbial biodegradation, with coincident settling to the bottom sediment of the partially biodegraded or unbiodegradable residuals.

For the monoaromatic hydrocarbons, the small quantities dissolved or dispersed during the first hours of the spill would be subject to evaporation from the water column. The half-life for volatilization of dissolved benzene from a water depth of one meter (at about 25°C) has been estimated at 2.7 h to 4.81 h. Half-lives are similar (3 to 7 h) for toluene, ethylbenzene, xylene, isopropyl benzene, and for naphthalene and biphenyl (Mackay 1992). Evaporation rates would be lower at the temperatures (3-4°C) in PWS, leading to longer effective half-lives. If VOA was injected through turbulent dispersion of whole oil droplets deeper than one meter into the water column, these half-lives would also underestimate residence times in the water column.

According to the OSSM hindcast (Galt 1991a, 1991b) almost all of the dispersed oil would have been swept by the Alaska coastal current into and through the Shelikof Strait and along the Alaska Peninsula. Less than 1% of the oil (floating and/or dispersed) would have passed southeast of Kodiak into the GOA. During this advection process, the finely dispersed oil would have been undergoing rapid biodegradation, without significant limitation due to oxygen, nitrogen, or phosphorus. Direct measurements of biodegradation, however, are very limited. About 12 days after the spill, toluene biodegradation potential was elevated (estimated at >100-fold) in water samples taken in coastal waters near Knight Island and in open surface and subsurface (5 m) waters of the GOA about 5 km outside of Resurrection Bay, but not in water from either Port Valdez or the head of Resurrection Bay (Button 1992). Elevated rates based on C_{14}-naphthalene and hexadecane were also detected extensively in porewater samples collected in 1989 in association with sediments from oiled shorelines (Braddock et al. 1992). Past studies (Leahy and Colwell 1990) indicate that saturated constituents of petroleum are degraded most rapidly, followed by light aromatics and finally high-molecular aromatics. The asphaltenes and resins in petroleum are generally recalcitrant to biodegradation, though evidence for co-oxidation has been observed under some circumstances (Bertrand et al. 1983, Rontani et al. 1985).

Mackay et al. (1992) give half-lives for photochemical and microbial degradation of aromatics in water (exclusive of volatilization). Benzene has a half-life of about 170 h (~1 week; range: 100-300 h) in water; and Mackay suggests that all the other monoaromatics can be assigned a mean half-life

(temperature not specified) of about 550 h (~3 weeks; range: 300-1000 h) in water. Analogous estimates for PAHs follow:

- indan, naphthalene, substituted naphthalenes, and biphenyl: 170 h or 1 wk; (range: 100-300 h)
- acenaphthene, fluorene, phenanthrene, and anthracene: 550 h or 3 wks; (range: 300-1000 h)
- 4- and 5-ringed structures (pyrene to dibenzanthracene): 1700 h or about 2 mos; (range: 1000-3000 h)

Non-volatile constituents that are only very slightly soluble in water, such as the 4- and 5-ringed PAHs (solubility ranges from pyrene, at 0.132 ppm, to dibenz[a,c]anthracene, at 0.0016 ppm), exhibit slower biodegradation rates, and a greater tendency to sorb onto living or dead particulate matter, compared with smaller aromatic structures. Log K_{ow} ranges from 2.13 for benzene, to 3.18 for xylene, 3.37 for naphthalene and 5.00 for Tri-methyl naphthalene, to 4.18 for fluorene, 5.18 for pyrene, and 6.75 for dibenz[a,c]anthracene. Many of the higher molecular weight aromatics and related compounds probably become adsorbed onto suspended particulate matter and sedimented into the coastal shelf or deep ocean prior to their complete biodegradation. Similarly, some products of incomplete oxidation, which may appear during the early stages of biodegradation and photooxidation (Button et al. 1992), might also be resistant to further degradation. Such compounds were likely to become adsorbed and settle to the bottom, along with the residual refractory asphaltene and resin constituents of crude petroleum, or (depending upon their solubilities) they might persist in an extremely dilute state in the water column. While the residual biodegradation products consist largely of carbon dioxide and water, some partial biodegradation products may persist at very low concentrations in the water column, indistinguishable from other "natural" dissolved organic material in seawater.

Biodegradation is represented in the mass balance (Figs. 2 and 5) by a decreasing exponential algorithm based on oil composition and published biodegradation rates for hydrocarbons: 40% of the oil was diminished with a half-life of 50 days, 40% with a half-life of 400 days, and the balance with a very long half-life of 10,000 days. According to literature values (Mackay et al. 1992), these rates would be somewhat slow for aromatic compounds in the water column, but fast for those in sediments. Thus, we have begged the question of exactly where and how fast the hydrocarbons biodegrade as they slowly settle from the water column. Approximately 20% of the oil originally dispersed is probably now widely distributed in deep sediments mainly in the GOA (Fig. 5). The inability to identify EVO signatures in deep sediments (Kvenvolden et al. 1991, Carlson and Kvenvolden 1993, O'Clair et al. 1993, Page et al. 1993), indicates that such material is very highly weathered and generally indistinguishable from other naturally occurring organic compounds, including petrogenic hydrocarbons, or is present only in very low concentrations.

Transport and Transformations of Beached Oil

Between April and August 1989, the extent of shoreline oiling was determined by Shoreline Cleanup Assessment Teams (SCAT) as a basis for identifying priorities for cleanup efforts. Oiling was categorized as heavy, moderate, light, or very light in three different areas, PWS, Kenai/Cook Inlet, and Kodiak/Shelikof. Overall some 5190 km of shoreline were characterized as having been oiled to some degree (Michel and Hayes 1991), with 1260 km in PWS, 820 km in Kenai/Cook Inlet, and 3110 km in the Kodiak/Shelikof area. Of these regional totals, 600, 195, and 115 km, respectively, were

characterized as moderately to heavily oiled. In the Kodiak/Shelikof area, 2670 km were only "very lightly" oiled. A second survey was conducted in September/October 1989, at the end of the first summer's cleanup efforts, and the total lengths of oiled beaches for the same 3 areas was reported as 576 km, 258 km, and 75 km, respectively, with 136 km, 22 km, and 2.1 km in the moderately to heavily oiled categories. Results of followup surveys in 1990-1992 are reported by ADEC (1992). While these shoreline assessments do not represent quantitative estimates of the amounts of oil present, they do support independent evaluations of both the extent of oiling and the temporal changes ensuing in the intertidal zone (Jahns et al. 1991, Koons and Jahns 1993).

The most reliable estimates of the initial quantities and distribution of oil beached throughout the spill zone are those provided by OSSM (Galt et al. 1991a, 1991b) at the time (about 1 May 1989) when prediction of floating oil trajectories ceased to be conducted for response purposes. At that time, approximately 41% of the spilled oil was estimated to remain "on the beaches" in PWS (north of latitude 59.95 °N); 5.2% was beached on the Kenai Peninsula (east of longitude 152°W); about 1.8% of the spilled oil had floated past Cape Douglas into the Shelikof Straits (where much of it had already beached); while another 2% remained floating in the Kenai sector, still to come ashore either in that sector or in the Kodiak/LCI/Alaska Peninsula sector. These estimates are thought to represent the distribution of beached oil fairly accurately (as of May 1), even though OSSM did not directly take into account the amount of oil being picked up inside of PWS by skimmers. Comparison of OSSM's assumptions about the weathering losses of the crude oil in this spill with the more detailed estimates provided by the NOAA oil weathering model (Payne et al. 1984) suggest that the sum of evaporation and dispersion was overestimated in the OSSM hindcast by an amount (8.5%) approximately equal to that recovered by skimmers. Since the OSSM hindcast focuses on the floating oil, however, and was based on several model runs, each re-initialized with data from actual observations during the period before May 1, the output is believed to reflect the actual distributions of floating oil (including the effect of skimmers) fairly well.

By far the bulk of the floating oil that left PWS was transported by currents and winds along the coast, where it ultimately beached on the headlands and fiords of the Kenai Peninsula, the Barren Islands, the shores around the mouth of Lower Cook Inlet, the northeast- and north-facing shores of Shuyak and Afognak Islands and the shores of the Alaska Peninsula facing eastward and northeastward along the Shelikof Strait. Only very minor amounts passed to the south of Kodiak with some beaching of tarballs along the shores of Chiniak Bay and in the vicinity of Kodiak harbor. By the time the oil reached the GOA, nearly all of the evaporation (probably >95%) and dispersion (>90%) had already occurred, and a good first approximation is that all the residual floating oil eventually was beached. Since a portion of the estimated 2% that remained floating in the Kenai area as of May 1 was no doubt carried subsequently into the Shelikof Strait, the best estimate for the amount of oil ultimately beached in the Kenai area lies between 5 and 7% of the total spilled oil, and that for the Shelikof Strait area lies between 2 and 4%.

Figure 2 illustrates these estimates for the initial beaching of the floating oil separately for PWS and the GOA. After about April 18 the OSSM hindcast showed near-cessation of transport of floating oil out of PWS. This was represented in the budget as the effective grounding (and associated retention) of the residual floating oil within PWS soon after this time. The oil that remained floating in the Kenai

and Lower Cook-Shelikof areas were similarly assumed to have grounded and become temporarily immobilized within those areas during the periods April 24-30 and April 29-May 5, respectively.

Once beached, the spilled oil was subjected to many simultaneous processes, including:

- repeated refloatation and beaching as a result of tides and changes in wind direction;
- burial within the porous gravel/cobble substrate of the beaches (as a result of both the initial seeping into the substrate and of subsequent storm-induced changes in beach profiles); recovery through cleanup activities;
- dispersion of oil droplets or particles into the water column by high-pressure cleanup activities and wave action (discussed previously);
- weathering through a combination of biodegradation (both natural and enhanced by bioremediation activities), photo-oxidation, and continued (very minor) evaporation; and
- dispersion and erosion (of oil-contaminated sediments) into the adjacent subtidal zones.

Figure 6 (an expansion of Fig. 2) illustrates our estimates for the major processes (other than dispersion) that affected the disposition or fate of the oil left on the beaches of PWS, including solid waste removal, biodegradation, and erosion to shallow subtidal sediments. These estimates, as explained in the following sections, are based primarily on studies carried out by NOAA as part of the Spill Response Activity (Michel and Hayes 1991, 1992, Michel et al. 1991) or as part of the NRDA process (Short and Rounds 1993a, Short and Rounds 1993b, O'Clair et al. 1993, Sale et al. 1993) at selected sites in PWS.

Shoreline Oiling In PWS

Michel et al. (1991) reported that surface oil contamination was greatest on the upper one-third to one-half of the intertidal zone, regardless of the shoreline type. There were very few occasions of visible oil in the lower intertidal zone, although the extensive hot-water flushing transported contaminated sediments into this zone during treatment. Surface oil in PWS occurred mostly as a coating on rock surfaces ranging in thickness from a stain to several millimeters; outside PWS the emulsified oil formed mousse patches and patties as well. On rocky substrates, the oil tended to pool in crevices and at the base of boulders. On finer-grained sediments the oil penetrated into the surface sediments, forming soft asphalt pavements. Michel et al. (1991) estimated the extent of surface oil coverage monthly over the winder of 89-90 at 18 stations in PWS: at the most exposed stations mean % oil coverage decreased regularly each month (between September 89 to March 90) to about 20% of the initial level; on intermittently exposed shorelines showed a reduction only to about 50% of the initial level of surface oiling. TPH analyses of surficial sediments suggested reductions of 90%, 70% and 70% respectively, for the 3 shoreline types. Subsurface oil, however, was much more persistent. Where large amounts of oil came ashore on exposed cobble/boulder beaches, it penetrated to depths greater than one meter, though the average depth of penetration was about 50 cm. Penetration was greatest at the high-tide berm and along stream banks, which are the most permeable parts of the beach, and where porosity changes very little with depth. At other locations (i.e. mostly lower elevations) on a beach, grain size

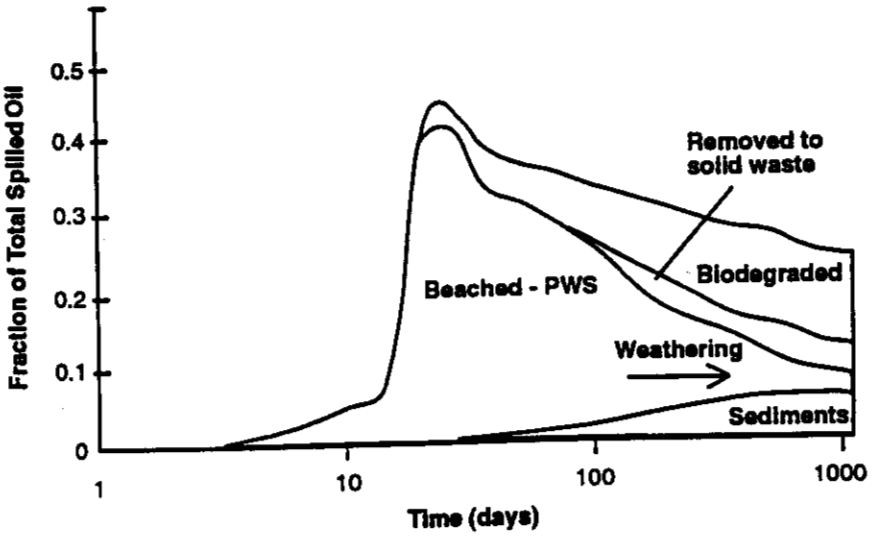

Figure 6. Fate of EVO beached inside of PWS, exclusive of oil dispersed through the action of winter storms and cleanup activities.

distribution changes significantly with depth: the cobbles on the surface are underlain by a mixture of pebbles and granule. In these finer-grained sediments, the oil penetrated and occurred at concentrations in the range of 0.5 to 3% by volume. Michel et al. (1991) estimated that the overwinter reduction of subsurface oil in the active high-tide berm was 90% or more, whereas in the stable central platform of the beaches, the depth of active sediment reworking was usually less than 25 cm. At greater depths, removal of oil was very low. TPH analyses of subsurface sediments indicated average reductions of about 40% at depths of 25-45 cm. On the basis of these data, they estimated that average removal of subsurface oil during the 1989-1990 storm season was about 55%.

Shoreline surveys were repeated at the NOAA stations in January and August 1991 (Michel and Hayes 1992). During summer of 1991 the high tide berms at several of the sites were mechanically relocated into the mid- to lower intertidal zones to facilitate removal of surface and subsurface oil from the high tide zone and from the substrate. This process was very effective for the removal of subsurface oil from the high tide berms in 1991. Sites with fairly impermeable sediments also showed substantial diminution of oil levels from 1990 to August 1991. At sites where the oil had penetrated deeply into the central platform of boulder/cobble beaches, however, oil persisted in both amount and composition almost unchanged from the previous (1990) fall. At Sleepy Bay, the subsurface oil had largely disappeared after extensive mechanical removal during summer 1991.

Recovery and Disposal of Solid Oily Wastes

Carpenter et al. (1991) described the characteristics and quantities of solid wastes recovered during cleanup operations by Exxon contractors in 1989. In PWS, where shoreline treatments relied heavily on water washing techniques, solid wastes consisted mainly of oiled sorbent materials and shoreline debris. In the GOA area, by contrast, the wastes consisted primarily of oily sand and gravel gathered concomitantly from the shoreline with mousse patties and tarballs. The wastes were bagged in plastic and transported to shore facilities in Valdez and Seward, where they were sorted and stored for transhipment or incineration. In Seward and also at Anchorage, wastes were repackaged, after shredding and addition of absorbent materials, for landfill disposal.

Approximately 2600 tons of oily solid wastes were incinerated (Carpenter et al. 1991) either in one of five small onshore incinerators (2100 tons total) or in a barge-mounted silo hearth incinerator (500 tons). An additional 22,400 tons of solid wastes (6,500 tons processed at Seward, 15,900 at Anchorage), along with 8,000 tons of added absorbent, was processed for disposal in an industrial waste landfill in Arlington, Oregon. No further information was available on the origin of the wastes by region, or relative amounts of different waste categories, or the quantities of oil contained in these wastes (ADEC 1992, and Pers. comm.: October 1992, L.J. Evans, CACI, Anchorage, AK; R. Mastracchio, Exxon, Houston, TX).

During the 1990 and 1991 field seasons, respectively, additional amounts of approximately 5,000 and 600 tons of oily solid wastes were similarly processed for landfill disposal in Oregon (ADEC 1992). Also during 1990, residents of local communities in the spill area collected oiled sediments and debris; approximately 219 tons were collected from within PWS for shipment to the Oregon disposal site, and approximately 16 tons from Kodiak (ADEC 1992). These 1990 and 1991 wastes consisted largely of

oiled beach sediments in the coarse sand to gravel size range (Pers. comm.: L.J. Evans, CACI; Ray Morris, Oil Spill Info. Center, Anchorage, AK).

Approximately 100 tons of oiled beach sediment were also collected for disposal in 1992. The 1992 wastes were treated under partial vacuum in a rotary kiln at 800-850°F to remove the oil so that the residual gravel and soil could be disposed directly into the municipal landfill at Palmer, Alaska. TPHs in the 1992 oiled sediments were extracted and analyzed (by EPA Standard Method 418.1). Five separate batches of wastes showed initial concentrations of 50,000; 32,200; 37,200; 47,000; and 60,000 ppm (dry wt. basis), for an average of about 4.5% TPH. The TPH content of the calcined sediment was in the range of 10 ppm, and the recovered oil was fed in a continuous flow from the condenser back into the burners of the kiln (Pers. comm., Ray Morris, Anchorage, AK).

Using 4.5% TPH as a minimum estimate of oil content for all four years (1989-1992) gives the following minimal annual amounts and fractions (wt %) of total oil spilled: 1125t (2.9%), 225t (0.58%), 27t (0.07%), and 4.5t (0.011%), for a cumulative total of 3.561 wt%. It is reasonable to hypothesize, however, that the oil content of sediments recovered in earlier years (especially in 1989) was greater than 4.5%. For depiction in the mass balance, we assumed simply that the average oil content of the solid wastes recovered in 1989 was in the range of 7.5% (and 4.5% in the other 3 years), giving a cumulative total of 5.5 (wt%) for the oil recovered. That amount was allocated for illustration purposes with 70% inside of PWS (Fig. 6) and 30% outside (Fig. 7). If instead we speculate that: (a) the wastes processed at Seward in 1989 (6500 tons) were collected outside of PWS (i.e. along the coasts of Kodiak and the Kenai and Alaska peninsulas); (b) much of the oil gathered from these areas was in the form of discrete patches of mousse, with a total oil content of 20%; (c) the wastes that were incinerated (2600 tons) also had an oil content of 20%; and (d) the balance of the 1989 wastes had an oil content of 10%, we arrive at an estimate of 3410 tons of oil recovered in 1989, or 8.75% (wt%). Combining these assumptions with the 4.5% oil content for the other three years leads to a maximum estimate of 3666.5 tons recovered, or 9.41 wt %, with 1300 tons, or 3.33 wt% from outside of PWS in 1989. The actual amount recovered most probably lies between 5% and 8% of the original spill mass.

Shoreline Treatment, Bioremediation, and Biodegradation

Field-testing of nutrient-enhanced bioremediation was conducted at two test sites on Knight Island over the summer of 1989, and bioremediation was carried out on about 70 miles of heavily oiled shorelines in PWS in both 1989 and 1990 (Chianelli et al. 1991, Pritchard and Costa 1991). The technique selected for general use involved the application of an oleophilic fertilizer (Inipol EAP22™), either alone or in combination with a granular slow-release fertilizer (Customblen™). Shaker flask and column flow studies with these products in the laboratory demonstrated substantial enhancement of biodegradation over unfertilized background rates (Bragg et al. 1990, Tabak et al. 1991). Field trials in 1989 also suggested initial enhancement of biodegradation rates of surficial oil at fertilized sites relative to control sites. After 2-3 weeks the surface of the treated beach (at Passage Cove) was visibly cleaner. However high rates of biodegradation occurred also on the control beaches, and there were no

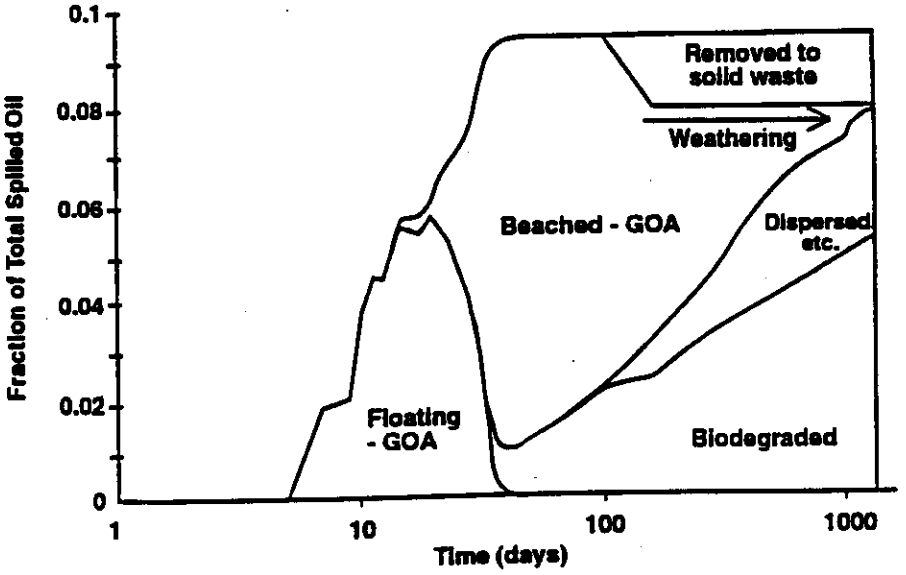

Figure 7. Fate of EVO floating and beached in the GOA. See text for explanation of various compartments and estimates.

consistent statistical differences in the measured rates of change in pristane/phytane ratios, alkane/hopane ratios, or total residual hydrocarbons in the sediments (Pritchard and Costa 1991).

Further examination of data from 1990 fertilizer applications at three sites on Knight Island confirmed that the rates of natural background biodegradation during the first year after the spill differed greatly among locations and also between surficial and buried oil (Bragg et al. 1993). Considering all data, the mean annual (for the first year after the spill) fractional loss due to background biodegradation of oil in intertidal sediments was 28 percent for surface oil and 12 percent for subsurface oil. Multiple regression techniques were used to analyze changes in hydrocarbon/hopane ratios in relation to polar content of the residual oil and nitrogen loading relative to the oil content in the sediments. Results from this approach suggested that on average, for the one-month period following fertilization, the addition of fertilizers enhanced biodegradation over background rates by factors of 3.75 (based on total extractable hydrocarbons) to 5.2 (based on total gas-chromatographically detectable hydrocarbons). As biodegradation proceeds, the content of refractory polar constituents increases and concurrently the rate of further biodegradation diminishes and is no longer limited by nutrients (Bragg et al. 1993). In a related study, fertilizer additions resulted in higher mineralization potentials for hexadecane and phenanthrene, and increased numbers of hydrocarbon-degrading microbes at treated sites compared to controls (Lindstrom et al. 1991), also suggesting enhancement over natural hydrocarbon biodegradation rates. Quantification of both the natural biodegradation rates and the potential enhancement by shoreline bioremediation remains elusive because of the extreme heterogeneity of oil distribution in shoreline sediments and the extreme complexity of petroleum biodegradation itself (being a composite of widely different rates on a mixture of hydrocarbon substrates). The problem is further compounded because other (physical) removal processes are simultaneously acting on the petroleum constituents. Based on changes in the ratios of other hydrocarbons to (17a, 21B-hopane), Prince et al. (1991) estimated that the natural rate of oil biodegradation on the more heavily oiled shorelines of PWS during 1989-1990 was about 2.2 g oil/kg sediment/ yr for surficial sediments, and about half that at 30 cm depth. Analogous figures reported by Bragg et al. (1993) ranged from 0.6 to 2.8 g oil/kg dry sediment for surficial sediments and from 0.2 to 3.0 for subsurface sediments.

Using the values reported by Prince et al. (1991) [2.2 + 1.7 + 1.1 g oil/kg (dry)/yr] for biodegradation at the 0-10 cm, 10-20 cm, and 20-30 cm depth intervals, respectively in a crude scaling exercise, we estimated that about 14% of the total spilled oil might have biodegraded on the 140 km (as of fall 1989) of moderately-to-heavily oiled shoreline in PWS between April 1, 1989 and September 1, 1990 (assuming homogeneous distribution of oil over a 20-m shoreline width, a specific gravity (including a dry-to-wet weight conversion) of 2 $kg \cdot L^{-1}$ for "sediment", and 75% of the annual biodegradation during the period April through August).

Based on temporal trends of PAH in intertidal sediments at several PWS sites, Boehm et al. (1994) estimated environmental half-lives for the removal of these constituents of EVO. Half-lives for PAH for the period May 1989 to August 1990 ranged from 2.0 months in upper intertidal sediments to 3.8 months in the lower intertidal. During this period, loss rates due to biodegradative weathering was no doubt supplemented considerably by physical removal processes. For the period August 1990 to August 1991, when both the relative influence of physical removal and the proportion of lower

molecular weight PAH had diminished, the estimated PAH half-lives were 7.4 months in the upper intertidal and 16 months in the lower.

Mackay et al. (1992) give half-lives in sediments of 1700 h (2 mos) for biphenyl; 5500 hrs (8 mos) for indan to trimethyl naphthalenes; 17,000 h (2 yrs) for acenaphthalene to anthracene; and 55,000 h (6 yrs) for pyrene to dibenzanthracene. These rates are substantially slower than for the same compound classes in water, reflecting the limiting nutrient or oxygen concentrations that are typically encountered in sediments. As noted previously, biodegradation rates for saturated petroleum constituents generally are more rapid than for the aromatics (Leahy and Colwell 1990).

To estimate and illustrate in-situ biodegradation of oil in beach sediments for purposes of the mass balance (Fig. 6), we used a three-component exponential function similar to that used for biodegradation of dispersed oil, with 40% of the oil diminished by a half-life of 100 days, 40% with a half-life of 1000 days, and the balance with a long half-life of 10,000 days. This function is reasonably consistent with published literature on PBCO composition and hydrocarbon biodegradation rates, and with the specific observations and measurements from PWS, discussed above.

Transport of Oil to Subtidal Sediments

Three distinct processes probably contributed to the transport of EVO to subtidal sediments in PWS and the GOA: (a) adsorption of dispersed and/or dissolved oil onto suspended particulate matter which would later settle to the bottom; (b) incorporation of sediments, e.g. in the surf zone, into floating masses of mousse, leading to sinking; and (c) erosion --either through natural processes or through cleanup activities-- of oiled sediments from the intertidal beach face into the nearshore subtidal zone. During the first several months after the spill, the first two of these processes are believed to have been inconsequential in terms of oil mass balance, because of (a) the very low concentrations of suspended particulate material throughout the spill area, (b) the relative scarcity of fine sediments on the beaches, especially in PWS and along the Kenai coast, and (c) the very low specific gravity of PBCO at this stage in the weathering process (Table 1). Over a period of months to years, however, related downward transport mechanisms probably accompanied biodegradation processes acting on the dispersed oil that was convected out of the spill area into the GOA. Highly weathered residual products of microbial (and planktonic) biodegradation most probably became associated with suspended detritus and particulate matter (such as zooplankton fecal pellets and exuviae) which slowly settle from the water column and are deposited in offshore sediments.

Inside of PWS, however, shoreline erosion and dispersion processes (process c, above) did transport EVO from the intertidal zone into shallow subtidal sediments (O'Clair et al. 1993, Page et al. 1993, Sale et al. 1993), and this process is therefore reflected in the mass balance (Fig. 6). While the actual mass transport of petroleum constituents from beaches to shallow subtidal sediments has not been quantified, the amount shown in Figure 6 was estimated, along with shoreline dispersion, to be consistent with observed oil loss rates on intermittently exposed and sheltered shore-lines, estimated (Michel et al. 1991) to be about 70% over winter, 1989-90. However, the overwinter loss rates observed on exposed high-energy beaches were about 90% during 1989-1990 (Koons and Jahns 1993), and our estimate for the cumulative losses due to dispersion, biodegradation, and transfer to subtidal

sediments might be somewhat low. In either case, relatively undegraded oil still persisted in some protected situations at the end of summer 1992, e.g. in the central platforms of some highly oiled cobble beaches (Roberts et al. 1993) and in the fine sediments underlying some mussel beds (Babcock et al. 1993). Except for these special situations, however, the oil still residing at the end of 1992 either on the beaches or in shallow subtidal sediments consisted largely of highly weathered refractory material.

During shoreline cleanup in many areas of PWS, high-pressure water was used to flush oil from the intertidal zone into adjacent boomed areas of open water where it could be recovered by absorbents or skimmers. During this process, considerable amounts of suspended sediments were also dislodged from the beaches, producing visible plumes trailing away from the beaches being treated (Mearns and Shigenaka 1993). These oil-contaminated sediments probably settled out mostly in shallow areas near the treated beaches, (although more finely divided sediments could have been carried some distance from the site and deposited in deeper waters). This washing process undoubtedly also contributed simultaneously to the re-entrainment (dispersion) of beached oil directly into the water column where it could not be recovered by absorbents or skimmers. Both of these transport processes most probably occurred also as the direct result of storm-induced wave action on the beaches, especially over the wintertime (Michel and Hayes 1991, Michel et al. 1991). The presence of elevated hydrocarbon levels in nearshore waters adjacent to beach cleanup activities in 1989 was recorded by Neff (1990, 1991a, 1991b). The petroleum hydrocarbons bioaccumulated by caged mussels (Short and Rounds 1993a), especially during 1990-1991, probably also arose from re-entrainment of beached oil into the water column.

In conjunction with the survey of oil distribution in subtidal sediments, subtidal sediment traps were deployed during 1990-1992 at a series of oiled and unoiled sites in PWS (Sale et al. 1993). In 1990 trapped particles and subtidal sediments collected adjacent to the traps exhibited elevated petroleum hydrocarbon levels at sites associated with oiled beaches. Highest levels of petroleum hydrocarbons captured overwinter 1991-1992 were found at east Northwest Bay, Sleepy Bay, and Snug Harbor, with lowest levels at Eshamy Bay, Stockdale Harbor, and Port Fidalgo, showing a clear association with the degree of oil impact on adjacent beaches. At locations with heavily oiled shorelines, significant quantities of oil were transported into shallow subtidal depths (O'Clair et al. 1993). At Sleepy Bay, for example, hydrocarbon concentrations at the 3-m depth peaked in September 1989, and at 6-m and 20-m depths in November 1989. In Northwest Bay and Herring Bay, hydrocarbon concentrations at depths of 6-20 m peaked between September 1989 and September 1990. During 1989-1992, EVO was generally not detected (or was present only in extremely low concentrations) in sediments at depths of 40 m and 100 m (O'Clair et al. 1993).

Deep (mostly 200-400 m) sediment samples were taken in May 1989 and in May-June, 1990 from 15 (1989) and 11 (1990) sites along the spill trajectory in PWS (around Naked and Knight Islands and in Montague Strait), and analyzed for petroleum hydrocarbons (Kvenvolden et al. 1991, Rapp et al. 1990). Additional shallow (mostly 18-50 m) samples were taken in 1990 near heavily oiled shorelines, including Northwest Bay, Herring Bay, Sleepy Bay, Smith Island, and off the northeastern shores of Storey and Naked Islands). Except for very slight (inconclusive) enrichment of aliphatics and aromatics in one sample from the north end of Latouche Passage, there was no indication of recent PBCO contamination in any of the deep sediment samples in 1989. In 1990, 11 of 13 shallow-water sediment

samples showed evidence of slight contamination with EVO. Except for C23/C30 ratios, which were consistently enhanced in 1990, the 1990 deep sediment samples exhibited molecular ratios remarkably similar to those observed in 1989, indicating lack of contamination from the spill (Kvenvolden et al. 1991).

Hydrocarbons in the deep sediments of PWS exhibit a gas chromatography/mass spectrometry (GC/MS) compositional pattern characteristic of the coastal oil seeps in the vicinity of Katalla and Cape Yakataga in the eastern GOA (Page et al. 1993). These authors also verified, however, the presence of *Exxon Valdez* crude oil in shallow subtidal sediments adjacent to heavily oiled shorelines in PWS in 1989. By 1991 these crude oil residues were highly degraded and sporadically distributed.

Shoreline Oiling Outside of PWS

The oil that reached the shores of the Kenai/Kodiak region and the Alaska Peninsula had a character very different from that beached within PWS. The oil had already been afloat for at least 7-8 days before it left PWS, and what had been in PWS a fairly continuous slick of liquid oil was breaking up into streamers and patches of floating mousse, which would eventually become stranded as discrete (albeit large in some areas) patches instead of blanketing entire beach surfaces with a flowing fluid. This stranded mousse did not penetrate into shorelines nearly to the extent that the oil did in PWS, and as a result, was much more amenable to physical removal and cleanup. In the areas of heaviest shoreline oiling along the coasts of the Kenai and Alaska peninsulas, extensive cleanup activities were carried out during June-July 1989, and by early August, many of these beaches appeared generally clean with only sparsely distributed small tarballs and mousse patties. The effectiveness of natural and human beach-cleaning processes in this area was reflected in the drastic reduction in the lengths of moderately and heavily contaminated beach recorded by the shoreline survey parties (Michel and Hayes 1991) for the Kodiak/Shelikof area between the initial (spring-summer)1989 survey (116 km) and the fall 1989 survey (2.1 km). These shoreline characterizations provide the most quantitative indicator of the rates of change in the magnitude and extent of shoreline oiling for this area.

Figure 7 illustrates estimates of fate for that portion of the mass balance representing the transfer of floating oil from PWS to the adjacent GOA, and its subsequent beaching and redistribution (analogous to Fig. 6 for PWS). Biodegradation is represented in Figure 7 for the GOA by the same rate function used for PWS. Shoreline dispersion and erosional transport to subtidal sediments are not distinguished from each other, and probably occurred in this area almost exclusively as a result of natural processes. As noted above, oil that beached along the Kenai and Alaska Peninsulas disappeared very rapidly in the first year after the spill (ADEC 1992, Michel and Hayes 1992, Michel et al. 1991, Gundlach et al. 1991).

Summary Mass Balance for the Spill

In the preceding sections we have presented and discussed the field observations, data, model outputs and theoretical considerations underlying our provisional mass balance (Figs. 2 and 5-7). Table 4 lists our estimates for the various rates and/or compartments included in the overall mass balance, and the likely ranges for these values. As seen in Table 4, the estimates are extremely uncertain for biodegradation rates (and therefore the amounts biodegraded both in the water column and in intertidal and subtidal sediments), along with the estimated amounts transported to subtidal sediments (either through erosion from the intertidal zone or through settling of refractory residual constituents from the water column). These processes, while well-documented to have occurred, could not be quantified directly over the full range of conditions applicable to the spill.

The numerical values presented for these and other processes do not represent quantitative measures of the fate of EVO, but they do provide reasonable (based on qualitative observations and literature values) and internally consistent estimates of both their absolute and relative magnitudes. Most constituents of the EVO will be transformed ultimately (through biodegradation and photooxidation) into carbon dioxide and water. Although some more refractory residual constituents of petroleum (containing, for example, high molecular weight polynuclear aromatic hydrocarbons, resins, and asphaltenes) may persist indefinitely, these constituents will generally be mixed with, and usually indistinguishable from, other petroleum sources and naturally occurring hydrocarbon residues (e.g. seep petroleum, combustion products, and biogenic organic materials).

The various spilled oil components discussed above have been recombined to produce a summary mass balance (Fig. 8) as of October 1, 1992. The cumulative fractions lost to, or remaining in, the various compartments as of October 1992 are listed in Table 5. Ranges are given for each component to make clear the limitations recognized for these estimates.

In summary then, we estimate that as of October 1992 about 20% of the spilled oil had evaporated and undergone photolysis in the atmosphere; about 50% had biodegraded either in-situ on the beaches or in the water column; about 14% was recovered or disposed; less than 1% remained in the water column (except as biodegradation products); 2% remained on intertidal shorelines (both within and outside of PWS-- but a very large proportion of this was in the form of highly weathered, biologically inert residuals); and about 12% in subtidal sediments, mostly in the GOA and again mostly as highly weathered residuals. While we believe that these estimates are a reasonable approximation of the overall fate of the oil spilled from the *Exxon Valdez*, we will continue to review additional information as it comes to our attention.

Comparison with Observations from other Oil Spills

The concentrations of oil measured in the water column after the EVOS were similar to, or only slightly lower than, those found after many other spills. The highest spill concentrations ever observed were at the subsurface blowout of Ixtoc I, where concentrations of VOA reached 400 ug/L (Brooks et al. 1981) and PAH concentrations ranged from 2860 ug/L in the immediate vicinity of the well to about

Table 4. Values and Basis for Calculation of the Estimates Included in the Mass Balance for the *Exxon Valdez* Spilled Oil

Component & Estimate	Source/Basis	Minimum	Maximum
Evaporation 0.20	Payne et al. (1984) model; PBCO compositional data; field-observed oil composition	0.18	0.25
Dispersion (Initial Slick) 0.23	Payne et al. (1984) model; Galt et al. (1991a) OSSM model; Short and Rounds (1993b) & Neff (1991a, 1991b) field data	0.20	0.30
Dispersion (Shoreline) 0.15	No direct data: Inferred from shoreline cleanup rates (Michel and Hayes 1992) and bioaccumulation by mussels (Short and Rounds 1993a)	0.10	0.20
Floating/ Beached Oil Distribution	Galt et al. (1991a) OSSM model; Slick Overflight & Beached Oil observations		
0.41 PWS		0.35	0.45
0.07 KEN		0.05	0.09
0.02 LC/SS		0.018	0.04
Skimmed Oil 0.083	Carpenter et al. (1991) & ADEC (1992) data; 67% water-in-oil composition	0.08	0.10
Burned Oil 0.0014	Carpenter et al. (1991) & ADEC (1992) data;	0.0012	0.0025
Recovered Solid Waste 0.055	Carpenter et al . (1991) & ADEC (1992) data;	0.04	0.09
70% PWS	(no data examined on	50%	75%
30% GOA	geographic distribution)	25%	50%
Biodegradation of Beached oil 0.13 PWS 0.06 GOA	40% @ $T_{1/2}=10^2$d, 40% @ $T_{1/2}=10^3$d, 20% @ $T_{1/2}=10^4$d; (Mackay et al. 1992, Michel and Hayes 1992 Bragg et al. 1993, Prince et al. 1991, Braddock et al. 1993)	40% (of oil initially beached)	80%
Biodegradation of Dispersed Oil 0.30	40% @ $T_{1/2}=50$d; 40% @ $T_{1/2}=400$d; 20% @ $T_{1/2}=10^4$d; (Mackay et al. 1992, Braddock et al. 1993);	60% (of dispersed oil)	90%
Erosion/Transport to Nearshore 0.045	Gradual accumulation (fall- winter '89) to 55% of residual beached-(no data). (O'Clair et al. 1993, Michel and Hayes 1992, Sale et al. 1993)	20% (of total Subtidal residual biodegraded beached material)	70%
Settling to Off- shore Subtidal 0.08	Dispersed residual oil (no data); Oil composition data (Clark and MacLeod 1977 Mackay et a. 1983, Coleman et al. 1978)	80% (of total unbiodegraded dispersed fraction)	100%

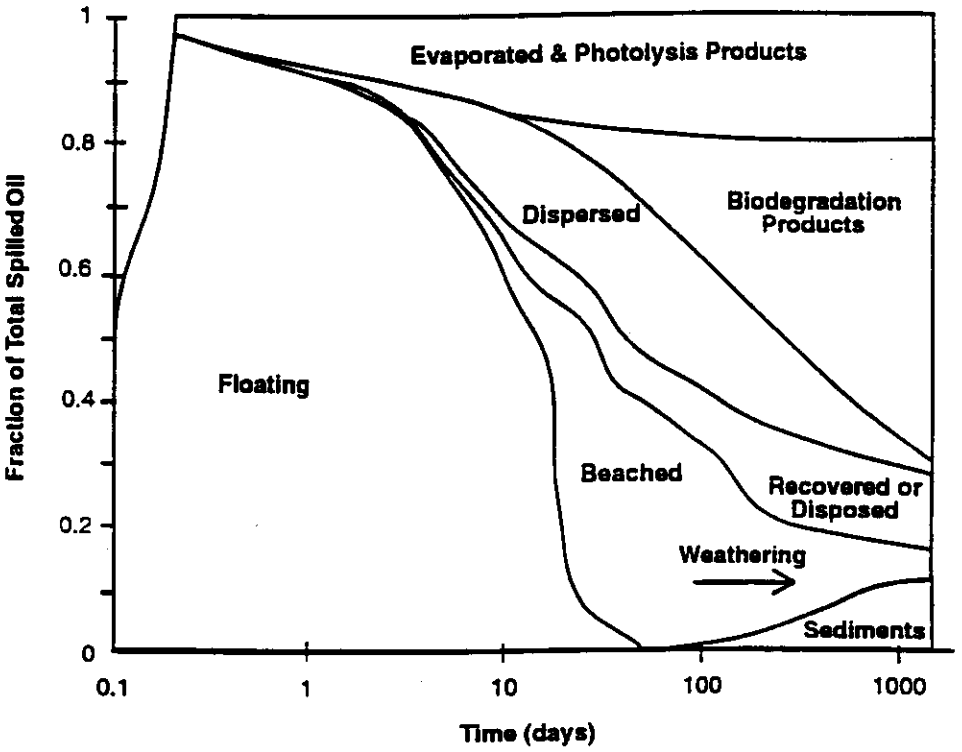

Figure 8. Overall fate of EVO, recombining and summarizing the compartments illustrated previously in Figures 2 and 5-7.

Table 5. Provisional summary mass balance (as of Fall 1992)

Compartment	Estimate	Minimum	Maximum
Subtidal Sediments*	0.13	0.05	0.15
Floating	0.0	0.00	0
Beached*	0.02	0.001	0.04
Recovered/disposed	0.14	0.12	0.19
Dispersed*	0.01	0	0.03
Aqueous Biodegradation & Photolysis Products	0.50	0.4	0.6
Atmospheric Photolysis Products	0.20	0.18	0.25
Total	1	0.75	1.26

*Primarily (>90% in offshore sediments; >60% in nearshore sub-tidal and beached) as highly weathered refractory residuals.

5 ug/L at a distance of 40 km (Boehm and Fiest 1980). After the *Argo Merchant* spill, PAH concentrations remained elevated (up to 50 ng/L for individual PAHs) in the water column for several months (Boehm et al. 1979), similar to the 1.0 ug/L total PAH observed in PWS during the first month after the spill. After the *Amoco Cadiz* spill, concentrations of TPHs (analyzed spectrofluorometrically) ranged up to 138 ug/L in subsurface coastal waters (Marchand and Caprais 1981), and up to 500 ug/L in the enclosed estuarine waters of Aber Wrac'h (Calder and Boehm 1981). These values are comparable to the 5-6 ug/l total PAH (equivalent to about 350 ug/L total PHC) measured in coastal situations in PWS 1-2 months after the EVOS. These spill situations were all characterized by conditions of extreme turbulence at the time and place of the spill, which would have driven fresh oil deep into the water column. At the EVOS, by contrast, the weather was relatively calm for nearly 3 days after the spill, allowing about 10% of the oil to evaporate with only modest mixing. This difference may have contributed to the somewhat lower concentrations of petroleum hydrocarbons in the water column at the EVOS. Another factor, however, may have been the rapid dilution and flushing of dispersed oil from PWS that occurred in the period between March 27 and early April. Dispersion rates were probably at their maximum during March 27-30, and a portion of the oil dispersed in Montague Strait during that period would have been carried out of PWS with the main front of the oil slick on March 30-31. The dispersed oil that was carried initially into the semi-enclosed bays of Eleanor and Knight (and other) islands would also have been continually flushed by wind-driven and tidal currents back into the mainstream PWS circulation pattern where it would be diluted and soon carried out of the sound, while the floating (and/or beached) oil was being retained in the bays as a result of wind and shoreline interactions.

In March, 1978 the supertanker *Amoco Cadiz* grounded offshore Portsall, in Brittany, France and lost 223,000 metric tons (more than 6X the amount of oil lost in PWS) of light Arabian and Iranian crude oil into the southern end of the English Channel. The fate of the oil spilled from the *Amoco Cadiz* was estimated by Gundlach et al. (1983), and our estimates for the fate of the EVO are compared briefly here with those estimates. These earlier estimates did not make use of trajectory model hindcasts or weathering model estimates, but were reconstructed mainly from chemical compositional data obtained from water and sediment samples at the spill scene. Of the total oil lost from the *Amoco Cadiz*, evaporation was estimated to account for 30%, dispersion into the water column for 13.5% (including about 4.5% that was biodegraded soon after release), grounding on shore for 28%, transport to subtidal sediments for 8%, while 20.5% was unaccounted for. A higher proportion of the floating oil from the *Amoco Cadiz* was transported into open offshore waters where it disappeared at sea through some combination of dispersion, dilution, biodegradation, and sinking. While the fraction of total oil unaccounted for was considered most likely to consist of surface slicks and tarballs (Gundlach et al. 1983), it now seems probable that much of this material was also dispersed into the water column where it soon underwent substantial biodegradation with ultimate settling of refractory residuals into the offshore sediments. The estimate of the *Amoco Cadiz* oil evaporated was not based on detailed compositional data for the original cargo oil itself, but was a coarse estimate (the mean of a range of 20-40%, based on prior laboratory observations, compositional data on weathered oil samples, and general knowledge of oil composition at the time). The 30% value may have represented a modest overestimate of the total fraction evaporated, since biodegradation becomes a more dominant factor in the loss of oil after the initial loss of materials lower than the C_{12}-C_{14} range of molecular weight. The high wave energies associated with the Brittany coast were thought to be important in redistributing the oil as a

finely dispersed emulsion in the water column, and in maintaining optimal conditions (adequate supplies of oxygen, nitrogen, and phosphorus) for high rates of biodegradation. No estimates were available for the total amounts of oil cleaned up from the Brittany shorelines.

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STATUS OF INJURY ASSESSMENT

OBJECTIVE A

The toxicity bioassay results show that residual toxicity was still associated, in summer 1991, with the *Exxon Valdez* oil in lower intertidal and adjacent shallow subtidal sediments at several of the more heavily oiled sites in Prince William Sound. However, the differences between mean toxicities at oiled and unoiled sites were smaller in 1991 than in 1990 (and not significantly different from each other), consistent with the decreasing levels of petroleum hydrocarbons in the sediments (as measured by UVF and GC/MS). The finding of significant toxicity at some of our nominal reference sites was unexpected and remains unexplained.

OBJECTIVES B & C

Results from the battery of toxicity tests on the polar and nonpolar fractions from interstitial water and sediments taken in September 1990 at the heavily oiled Bay of Isles site and the unoiled Mooselips Bay site consistently indicated higher levels of toxicity at the oiled site, but these responses were elicited only at very high extract concentrations. That is, none of the tests indicated high levels of sediment toxicity compared with coastal sites typically considered "polluted". At both sites, however, the polar fraction usually elicited toxic responses as great as, or slightly greater than, those associated with the non-polar fractions of the extracts. We concluded therefore, that although the toxicity of the residual *Exxon Valdez* oil was low in intertidal and shallow subtidal sediments, a portion of that toxicity was derived from polar constituents. Those constituents have not been identified chemically.

APPENDIX A

Contract Research Reports Received under this Project.

Burns, K.A. 1991. Report on Project to Assess the Feasibility of Detecting Oxidation Products of Petroleum Hydrocarbons in Bivalves from Prince William Sound. Report Submitted to NOAA Ocean Assessments Division, Rockville, MD. 22 pp. + Appendices.

SAIC 1989. Screening Analysis for Petroleum Hydrocarbons in Sediments and Sediment Pore Waters by Use of Ultra-Violet Fluorescence Spectrophotometry for *Exxon Valdez* Damage Assessment. Final Report. NOAA Contract No. 50-DSNC-8-00141, Task No. 55-DSNC-9-00017. Science Applications International Corp., Everett, Washington. December 22, 1989. 17 pp + Tables, Figures, and Appendices.

SAIC 1990. Bioassay of toxicity associated with intertidal and subtidal sediments contaminated by the *Exxon Valdez* oil spill. Final Report. NOAA Contract No. 50-DSNC-8-00141, Task No. 55-DSNC-0-00008. Science Applications International Corp., Bothell, Washington 98011. November 12, 1990. 53 pp + Appendices.

SAIC 1991. Bioassay of toxicity associated with intertidal and subtidal sediments contaminated by the *Exxon Valdez* oil spill: Second Year (1991). Final Report. NOAA Contract No. 50-DSNC-1-00107. Science Applications International Corp., Bothell, Washington 98011. October, 1991. 26 pp + Appendices.

SAIC 1992. Determination of toxicity and genotoxicity associated with polar degradation products of petroleum in intertidal and subtidal sediments contaminated by the *Exxon Valdez* oil spill. Final Report. NOAA Contract No. 50-DSNC-8-00141, Task Order 55-DSNC-0-00013. Science Applications International Corp., Narragansett, Rhode Island 02882. August 20, 1992. 38 pp + Figures + Tables +Appendices.

SAIC and University of Alaska Water Research Center 1990. Microbial Hydrocarbon Degradation in Sediments Impacted by the *Exxon Valdez* Oil Spill. Final Report. NOAA Contract No. 50-DSNC-8-00141, Task No. 55-DSNC-9-00016. Science Applications International Corp., Everett, Washington. February 1990. 32 pp + Appendices.

APPENDIX A (Cont'd.)
Previous Reports Submitted by this Project and its Predecessors.

Wolfe, D. A. 1990. Air/Water Study Number 4. Petroleum Exposure and Injury to Infaunal Resources. Draft Preliminary Status Report. January 12, 1990. National Oceanic & Atmospheric Administration, Ocean Assessments Division, Rockville, Maryland. 28 pp.

Wolfe, D. A. 1990. Air/Water Study Number 6. Fate and Toxicity of Spilled Oil from the *Exxon Valdez*. Draft Preliminary Status Report. November 7, 1990. National Oceanic & Atmospheric Administration, Ocean Assessments Division, Rockville, Maryland. 18 pp.

Wolfe, D. A. 1991. Subtidal Study Number 4. Fate and Toxicity of Spilled Oil from the *Exxon Valdez*. Draft Preliminary Status Report. November 22, 1991. National Oceanic & Atmospheric Administration, Ocean Assessments Division, Rockville, Maryland. 33 pp.