

Exxon Valdez Oil Spill
Restoration Project Final Report

Assessment of the PAH Contamination of Populations of the
Forage Fish, Sand Lance (*Ammodytes hexapterus*), Inhabiting
Clean and Oil-impacted Sediments

Restoration Project 96163P
Final Report

Jack W. Anderson
Jennifer M. Jones

Columbia Analytical Services
6060 Corte del Cedro, Carlsbad, CA 92009

November 5, 1997

Assessment of the PAH Contamination of Populations of the
Forage Fish, Sand Lance (*Ammodytes hexapterus*), Inhabiting
Clean and Oil-impacted Sediments

Restoration Project 96163P
Final Report

Study History: Restoration Project 96163P was funded for one year in FY96. The project was discontinued when the study results indicated the lack of correlation between PAH and P450 RGS response for sediment samples.

Abstract: Samples were collected from Prince William Sound and were extracted by EPA methods (3540, 3550) to produce dichloromethane (DCM) extracts, and small aliquots of these were applied to human liver cancer cells (101L), which produce a luminescent enzyme (luciferase) if dioxins, furans, coplanar PCBs and polycyclic aromatic hydrocarbons (PAHs) are present. In this study, sediments were found to contain between 0.2 and 3.5 $\mu\text{g B(a)PEq/g}$. Levels of approximately 3 $\mu\text{g B(a)PEq/g}$ were found in sediments collected at Eleanor Island SE and Block Island NW. Many of the sand lance samples did not show any significant contamination, but other samples ranged from 2 to 16 $\mu\text{g B(a)PEq/g}$. A few samples of fish from Block Island NW contained 5.5 to 16.2 $\mu\text{g B(a)PEq/g}$, and one sample from East Bob Day Bay contained 3.3 $\mu\text{g B(a)PEq/g}$. There appeared to be significant contamination in some of the fish and sediment samples. The extracts were sent to the Auke Bay NOAA Laboratory for confirmation of the presence of PAHs. Analytical results demonstrated that the RGS assay had identified the two most contaminated sediment samples, but the fish tissues did not contain detectable amounts of PAHs. Apparently, the low level RGS responses observed in the fish extracts were from some unknown chemicals, not related to the *Exxon Valdez* oil spill.

Key Words: luciferase, polycyclic aromatic hydrocarbons, PAH, *Ammodytes*, sand lance, *Exxon Valdez* oil spill, Prince William Sound.

Project Data: *Description and format of data* -The data generated from this project include: P450 reporter gene system (RGS) assay of the triplicate luminometer readings for dichloromethane (DCM), dimethylsulfoxide (DMSO), and 2.0 ng/mL dioxin (TCDD), dry weight calculations of sediment samples, weight calculations of tissue samples, triplicate RLUs and calculated benzo[a]pyrene (B[a]P) equivalents, results of statistical analysis on B[a]PEqs from sand lance tissues, results of spiking study with B[a]P, analyses by the NOAA Auke Bay Laboratory of sediment samples, and comparison to P450 RGS data on a basis of B[a]P equivalents. *Custodian and availability of data* - The data can be obtained by contacting Dr. Jack Anderson, Columbia Analytical Services, 6060 Corte del Cedro, Carlsbad, CA 92009 (phone:

619-930-0417, e-mail: jander7927@aol.com), except the NOAA Auke Bay Laboratory for data of sediment samples, and comparison to P450 RGS can be obtained by contacting Jeff Short at Auke Bay Laboratory, 11305 Glacier Highway, Juneau, AK 99801 (phone 907-789-6065, e-mail jeff.short@noaa.gov).

Citation: Anderson, J.W. and J.M. Jones. 1997. Assessment of the PAH contamination of populations of the forage fish, sand lance (*Ammodytes hexapterus*), inhabiting clean and oil-impacted sediments, *Exxon Valdez Oil Spill Restoration Project Final Report (Restoration Project 96163P)*, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Office of Oil Spill Damage Assessment and Restoration, Juneau, Alaska.

**ASSESSMENT OF THE PAH CONTAMINATION
OF POPULATIONS OF THE FORAGE FISH,
SAND LANCE (*Ammodytes hexapterus*),
INHABITING CLEAN AND
OIL-IMPACTED SEDIMENTS**

PROJECT NUMBER 96163P

REVISED FINAL REPORT

TO THE

EXXON VALDEZ OIL SPILL TRUSTEE COUNCIL
RESTORATION OFFICE
ANCHORAGE, AK

AND

NOAA
OFFICE OF OIL SPILL
AUKE BAY, AK

FOR WORK ORDER 40ABNF601216

FROM

Jack W. Anderson and Jennifer M. Jones
Columbia Analytical Services
6060 Corte del Cedro, Carlsbad, CA 92009

November 5, 1997

ASSESSMENT OF THE PAH CONTAMINATION OF POPULATIONS OF THE
FORAGE FISH, SAND LANCE (*Ammodytes hexapterus*), INHABITING
CLEAN AND OIL-IMPACTED SEDIMENTS

ABSTRACT

APEX investigators collected samples of sand lance from Northwest Block Island, Southeast Eleanor Island, North Cabin Bay, and East Bob Day Bay. Sediments were collected from Southeast Eleanor Island, Northwest Block Island, MacPherson Isthmus, Fuel Cache Beach, MacPherson Narrows, and East Bob Day Bay. All samples were extracted by EPA methods (3540, 3550) to produce dichloromethane (DCM) extracts, and small aliquots of these were applied to human liver cancer cells (101L), which produce a luminescent enzyme (luciferase) if dioxins, furans, coplanar PCBs and polycyclic aromatic hydrocarbons (PAHs) are present. The amount of luciferase produced (light) is measured with a luminometer, and the intensity is a function of the concentrations and potency of the planar organic compounds present in the extract. The P450 RGS assay simulates the response of mammals and fish which may ingest contaminated *Ammodytes*. Tissue extracts were from composites of several whole fish, with the combined dry weight per sample of between 0.5 and 2.9 grams. Previous investigations with fish tissue collected in Southern California have detected levels of PAHs of from 0.3 to 4.0 μg of Benzo(a)pyrene Equivalents per g (ppm) in muscle, liver and ovary tissues. In this study, sediments were found to contain between 0.2 and 3.5 μg B(a)PEq/g. Levels of approximately 3 μg B(a)PEq/g were found in sediments collected at Eleanor Island SE and Block Island NW. Many of the sand lance samples did not show any significant contamination, but other samples ranged from 2 to 16 μg B(a)PEq/g. A few samples of fish from Block Island NW contained 5.5 to 16.2 μg B(a)PEq/g, and one sample from East Bob Day Bay contained 3.3 μg B(a)PEq/g. When the data are expressed as μg B(a)PEq/g lipid, values as high as 82 were observed. There appeared to be significant contamination in some of the fish and sediment samples. The extracts were sent to the Auke Bay NOAA Laboratory for confirmation of the presence of PAHs. Analytical results demonstrated that the RGS assay had identified the two most contaminated sediment samples, but the fish tissues did not contain detectable amounts of PAHs. Apparently, the low level RGS responses observed in the fish extracts were from some unknown chemicals, not related to the *Exxon Valdez* oil spill.

INTRODUCTION

Sand lance (*Ammodytes hexapterus*) are marine fish inhabiting the coastal Northeast Pacific. They are an important trophic link to tertiary fish and mammals in this ecosystem. When not foraging on zooplankton in the water column, these fish bury themselves in bottom sediments to avoid predation. Because of this, sand lance may be important indicators of sediment contamination, including that following the Exxon Valdez oil spill in Prince William Sound (PWS), Alaska in March of 1989.

Given the persistent nature of petroleum hydrocarbons in sediment, levels of PAH contamination in sediments collected from some sites in PWS even in 1996 may be

significant. Collier *et al* (1996) found that, in contrast to species in the littoral zone, nearshore benthic fish species in Prince William Sound showed continuing oil exposure through the first two field seasons after the spill, and there was some evidence of increased exposure even after more than two years. Sand lance, though preferring to burrow into clean sand, may be exposed to oil-impacted sediments in these shallow subtidal zones (Pinto *et al* 1984). Contaminant levels in tissue extracts of fish collected at these sites, as compared to those from sites where oiling did not occur, may indicate this exposure. In this study, sand lance as well as sediments were collected from several sites in PWS.

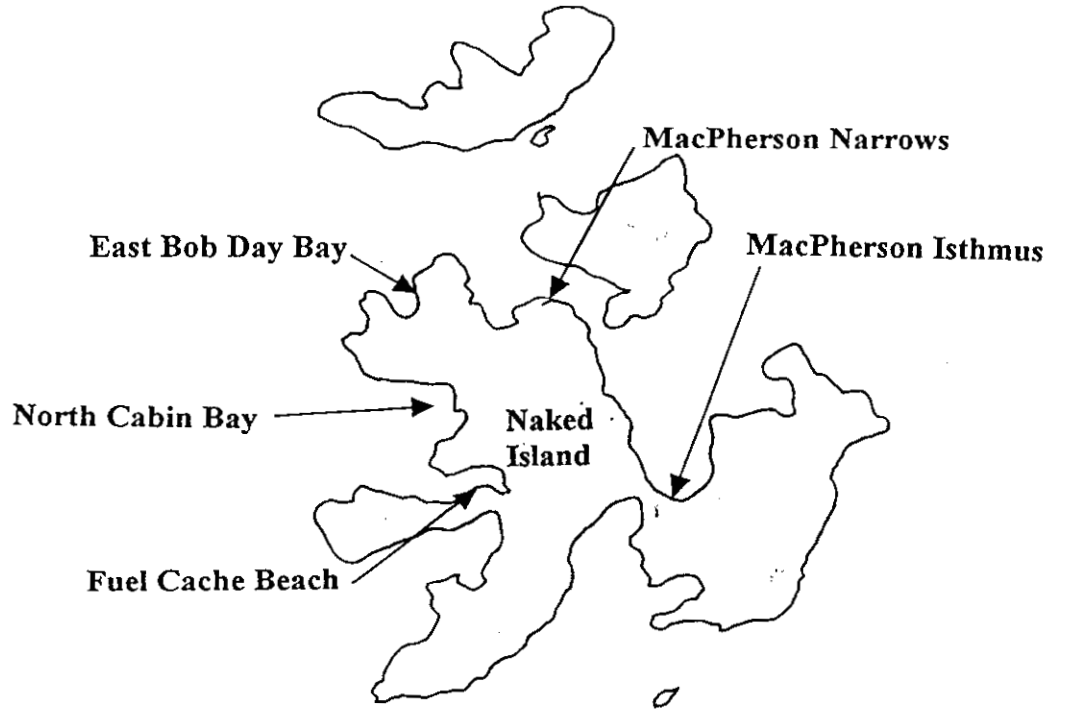
Induction of the cytochrome P450 system in fish in response to petroleum hydrocarbon contamination in sediments has been well characterized (Collier *et al*, 1995, 1996). These contaminants, including polycyclic aromatic hydrocarbons (PAHs) are known to bind to an intracellular cytosolic protein referred to as the Ah receptor. This complex is translocated to the nucleus of the cell, where it interacts with elements in the promoter of the CYP1A1 gene and causes transcription of the P450 enzyme system. While many of these enzymes are involved in metabolic detoxification pathways, epoxidation of benzo[a]pyrene by the P450 enzyme system produces a reactive metabolite, actually enhancing its carcinogenicity (Varanasi *et al* 1987).

To determine possible contamination which may be related to toxicity and carcinogenicity, extracts of sediments and fish tissues collected from PWS were analyzed by a P450 Reporter Gene System (RGS) (APHA 1996, ASTM 1997). This assay utilizes a human cell line, known as 101L, into which a plasmid has been stably integrated. This plasmid contains the CYP1A1 promoter linked to a reporter gene, firefly luciferase. When compounds that induce transcription of P450 by the CYP1A1 gene are present in these cells, the enzyme luciferase is produced. Luciferase acts on the substrate luciferin to produce light. P450 induction can therefore be measured by a simple assay that measures relative light units in a luminometer. In tissue extracts from mussels deployed in San Diego Bay, P450 RGS-estimated Benzo[a]Pyrene Equivalents were highly correlated to Toxic Equivalents (TEQs) based on chemical analytical data of these mussel tissues (Anderson *et al* 1997).

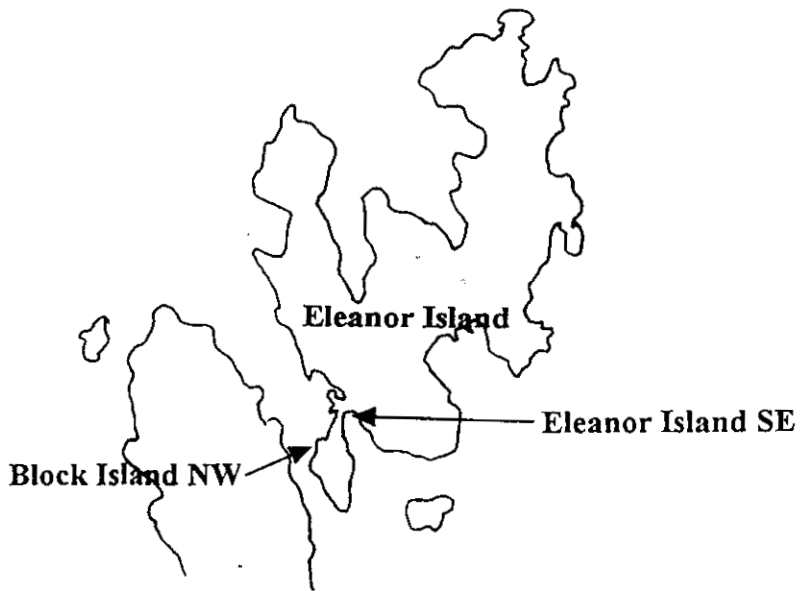
METHODS

Sediments

Approximately 40 grams of sediment from six stations in Prince William Sound (see map) were extracted by EPA method 3540 to produce 1 mL dichloromethane (DCM) extracts containing the extractable organic compounds. Two of these six stations were considered oiled, while the other four were thought to be relatively unoiled sites. Per cent solids were obtained for each sample.



**Prince William Sound,
Alaska**



Tissues

Sand lance were collected from six locations (two oiled and four unoiled) in PWS. Fish varied in length and mass, and some samples consisted of composites of four or five small fish. Because of this, samples were given a size classification, so that fish greater than 130 mm in standard length were assigned to Class A, from 100 mm to 130mm to Class B, and fish less than 100 mm in length, including composites of such fish, to Class C. Tissue samples were weighed and extracted as described above. Percent solids as well as percent lipids were determined for three samples, one from each of the three size classes.

Because of their flocculent lipid content, tissue extracts were centrifuged at 6000 rpm. The resulting supernatant was then decanted and saved. The lipid pellet was resuspended in fresh DCM, mixed, centrifuged again and the supernatants combined. In order to assure that no organic compounds were being harbored in the lipid pellet, one sample was spiked with the reference inducer TCDD and analyzed by the P450 RGS assay before and after several resuspensions in DCM. From this, it was determined that two resuspensions and centrifugations were sufficient to assure that little or no organic content of the sample was lost with the discarded lipid pellet.

Following this, tissue extracts were allowed to evaporate at room temperature to dryness, then taken up in 500 μ L of DMSO. DMSO is a more favorable solvent to the cells and the plastic tissue culture plates than DCM when addition of 10 to 20 μ L is necessary for optimum analysis of tissue samples. This exchange was unnecessary with sediment extracts since only 5 μ L of DCM was applied.

P450 RGS Analysis

The detailed methodology used in this study has been described elsewhere (Anderson *et al* 1995, 1996). Small portions of extracts were applied to approximately one million human liver cancer cells contained in three replicate wells with 2 mL of culture medium. After a 16-hour incubation, the cells were washed, lysed, the solution centrifuged, and 50 μ L of the supernatant was measured for luminescence. With each sample run, a solvent blank (using a volume of DCM or DMSO equal to the extract volume being tested) and a reference toxicant (2,3,7,8-dioxin at a concentration of 2 ng/mL) were applied to three replicate wells each. Fold induction of the solvent blank was set equal to 1, and the fold induction of each sample and the reference toxicant was determined by dividing the relative light units (RLUs) produced by that sample by the RLUs produced by the solvent blank.

In order to produce benzo[a]pyrene equivalents (B[a]PEq) for each sample, fold induction values were divided by 60, a value derived from previous studies showing that 1 μ g/mL (ppm) of B[a]P produces approximately a 60 fold induction in this P450 RGS assay. Further multiplication by a factor that brings the volume applied up to the total extract volume and then division by the dry weight (in grams) for both the sediment and tissue samples, and by the lipid weight of the tissues yielded B[a]P Eq values in μ g/g dry and μ g/g lipid, respectively.

Quality Assurance/Quality Control

After each test run, responses to the solvent blank and dioxin were compared to the those values in a control chart to assure that they were within two standard deviations of a running mean. Additional quality control procedures included spiking samples with benzo[a]pyrene, along with analysis of standard curves for both B[a]P and a quantified PAH mixture. The luminometer was calibrated using a luciferase standard curve of relative light units vs. enzyme concentration.

RESULTS

The results of this project are summarized in the following tables and figures and in Excel PC files on the enclosed disk.

Table 1 below shows the results of the seven different runs of the RGS assay, listing the mean of the triplicate luminometer readings for the dichloromethane (DCM), dimethylsulfoxide (DMSO), and 2.0 ng/mL dioxin (TCDD). In the first run, only sediment extracts in DCM were tested, so no DMSO blank was run. The tissue extracts, exchanged into DMSO, were run with a DMSO and usually a DCM blank, and the fold induction with both solvents is listed where applicable. All data points fell within 2 standard deviations of the means.

Table 1. Variability of Fold Induction in Multiple Runs.

DATE	DCM	DMSO	TCDD	FOLD (DCM)	FOLD (DMSO)
10/22/96	0.103	NA	10.706	103.9	NA
10/24/96	0.100	0.062	11.773	117.7	189.9
10/25/96	NA	0.055	10.001	NA	181.8
10/29/96	0.108	0.069	8.121	75.2	117.7
11/4/96	NA	0.060	10.663	NA	177.7
11/5/96	0.153	0.118	11.329	74.0	96.0
11/7/96	0.116	0.088	12.885	111.1	146.4
MEAN	0.116	0.075	10.783	96.4	151.6
S.D.	0.022	0.024	1.495	20.5	38.3
2S.D.	0.043	0.048	2.99	40.9	76.6
Upper C.L.	0.159	0.123	13.773	137.3	228.2
Lower C.L.	0.073	0.028	7.792	55.5	75.0
n	5	6	7	5	6

Table 2 lists the wet weights, percent solids, and subsequent dry weights of the six sediment samples as determined by the Columbia Analytical Services laboratory in Kelso, Washington.

Table 2. Dry Weight Calculations of Sediment Samples.

CAS ID#	Wet wt. (g)	%Solid	Dry wt. (g)
6369-1	40.2	96.7	38.9
5793-48	41.1	94.1	38.6
5793-49	40.7	79.3	32.3
5793-50	40.5	91.3	37.0
5793-51	40.5	94.2	38.1
5793-52	40.9	90.9	37.2

Table 3 lists the wet weights, percent solids, and percent lipids of the thirty-eight tissue samples also determined by the Columbia Analytical Services laboratory in Kelso, Washington. It also presents the subsequent dry weights and lipid weights of all tissue samples. The percent solids were based on analysis of two samples, both of which were found to be 28%. Percent lipids were based on analysis of three samples, one from each of the three fish size classes, and each sample was assigned a percent lipid value based on that classification, as explained in Methods.

Table 4 shows the triplicate luminometer readings in relative light units (RLUs), and the calculated fold induction and benzo[a]pyrene equivalents (B[a]PEq) for all samples. Fold induction was calculated as the mean RLU value of the sample divided by the mean RLU of the solvent blank (either DCM or DMSO). All values for % of the mean (coefficient of variation) are less than 20%, indicating little variation among the triplicates. B[a]PEq were calculated using the following formula:

$$B[a]PEq = (\text{fold}/60) * (V_e/V_a) / \text{wt}$$

where 60 = B[a]P conversion factor (see Methods)
V_e = total extract volume
V_a = volume of extract applied to cells
wt = dry or lipid weight of sample

This gives a B[a]PEq in µg per gram of either dry weight or lipid weight.

To summarize the data, Table 5 and Figures 1 and 2 present the B[a]PEq by sample, including the sample description and collection site.

Tissues

A sand lance tissue sample (CAS #6) from Eleanor Island SE was found to contain the highest amount of compounds that induce P450 RGS, calculated as 16 µg B[a]PEq per g dry weight and 82 µg B[a]PEq per g lipid weight. The majority of all tissue samples were lower in µg B[a]PEq per dry weight: 55% were < 2.0, 26% were between 2.0 and 5.0, and 18%, including the highest sample, were >5.0.

Table 3. Weight Calculations of Tissue Samples.

CAS I.D #	size class*	wet wt. (g)	%solid	dry wt. (g)	%lipid	lipid wt. (g)	CAS I.D #	size class*	wet wt. (g)	%solid	dry wt. (g)	%lipid	lipid wt. (g)
K5793-2	A	10.37	28	2.9	10.9	1.13	22	B	3.52	28	1.0	8.4	0.30
3	B	2.72	28	0.8	8.4	0.23	23	A	8.27	28	2.3	10.9	0.90
4	C	1.37	28	0.4	5.6	0.08	24	B	8.01	28	2.2	8.4	0.68
5	C	2.65	28	0.7	5.6	0.15	25	C	2.87	28	0.8	5.6	0.16
6	C	0.94	28	0.3	5.6	0.05	26	A	9.74	28	2.7	10.9	1.06
7	A	10.67	28	3.0	10.9	1.17	27	C	2.05	28	0.6	5.6	0.11
8	A	8.88	28	2.5	10.9	0.97	28	A	9.13	28	2.6	10.9	1.00
9	B	3.43	28	1.0	8.4	0.29	29	B	4.63	28	1.3	8.4	0.39
11	B	5.56	28	1.6	8.4	0.47	30	B	4.88	28	1.4	8.4	0.41
12	C	1.97	28	0.6	5.6	0.11	31	A	8.60	28	2.4	10.9	0.94
13	B	6.36	28	1.8	8.4	0.54	33	B	7.74	28	2.2	8.4	0.65
14	C	1.70	28	0.5	5.6	0.09	36	A	10.00	28	2.8	10.9	1.09
15	A	7.74	28	2.2	10.9	0.85	37	A	10.10	28	2.8	10.9	1.10
16	B	3.56	28	1.0	8.4	0.30	39	A	10.54	28	3.0	10.9	1.15
17	C	1.00	28	0.3	5.6	0.06	41	C	4.71	28	1.3	5.6	0.26
18	C	1.70	28	0.5	5.6	0.09	42	A	9.90	28	2.8	10.9	1.08
19	C	2.18	28	0.6	5.6	0.12	43	C	10.60	28	3.0	5.6	0.59
20	B	3.25	28	0.9	8.4	0.27	44	C	10.50	28	2.9	5.6	0.58
21	A	8.70	28	2.4	10.9	0.95	45	C	10.00	28	2.8	5.6	0.56

*Size classes:

A: Standard length ≥ 130 mm

B: Standard length between 100 and 130mm

C: Standard length ≤ 100 mm

Table 4. Triplicate RLUs and Calculated B[a]P Equivalents.

Test Date	CAS #	Solvent	Applied Volume (μL)	type*	RLU1	RLU2	RLU3	MEAN	S.D.	%MEAN	FOLD	B[a]P Eq. (μg/g dry)	B[a]P Eq. (μg/g lipid)
10/22/96	MB	DCM	5	NA	0.144	0.169	0.155	0.156	0.013	8.0	1.5	0.1	NA
10/22/96	6369-1	DCM	5	sed	4.068	3.783	3.375	3.742	0.348	9.3	36.3	3.1	NA
10/22/96	5793-48	DCM	5	sed	4.099	4.208	4.274	4.194	0.088	2.1	40.7	3.5	NA
10/22/96	49	DCM	5	sed-oiled	0.201	0.212	0.209	0.207	0.006	2.7	2.0	0.2	NA
10/22/96	50	DCM	5	sed	0.231	0.221	0.187	0.213	0.023	10.8	2.1	0.2	NA
10/22/96	51	DCM	5	sed-oiled	0.358	0.434	0.453	0.415	0.050	12.1	4.0	0.4	NA
10/22/96	52	DCM	5	sed	0.264	0.286	0.267	0.272	0.012	4.4	2.6	0.2	NA
10/22/96	43	DCM	10	SL-oiled	0.071	0.064	0.111	0.082	0.025	30.9	0.8	<1.1	<5.7
10/24/96	9	DMSO	10	SL	0.158	0.159	0.149	0.155	0.006	3.5	2.5	4.3	14.4
10/24/96	16	DMSO	10	SL-oiled	0.144	0.152	0.145	0.147	0.004	3.0	2.4	4.0	13.2
10/25/96	3	DMSO	10	SL	0.078	0.073	0.067	0.0727	0.006	7.6	1.3	2.9	9.6
10/25/96	4	DMSO	10	SL	0.074	0.071	0.068	0.071	0.003	4.2	1.3	5.6	28.3
10/25/96	5	DMSO	10	SL	0.097	0.101	0.101	0.0997	0.002	2.3	1.8	4.1	20.5
10/25/96	17	DMSO	10	SL-oiled	0.074	0.069	0.072	0.0717	0.003	3.5	1.3	7.8	39.1
10/25/96	18	DMSO	10	SL-oiled	0.116	0.125	0.111	0.1173	0.007	6.0	2.1	7.5	37.7
10/25/96	19	DMSO	10	SL-oiled	0.132	0.145	0.167	0.148	0.018	12.0	2.7	7.3	37.1
10/25/96	20	DMSO	10	SL-oiled	0.114	0.119	0.113	0.1153	0.003	2.8	2.1	3.8	12.8
10/25/96	22	DMSO	10	SL	0.070	0.072	0.061	0.0677	0.006	8.7	1.2	2.1	6.9
10/29/96	2	DMSO	5	SL	0.070	0.056	0.063	0.063	0.007	11.1	0.9	<1.43	<3.66
10/29/96	21	DMSO	5	SL-oiled	0.075	0.076	0.065	0.072	0.006	8.4	1.0	1.4	3.7
11/4/96	6	DMSO	5	SL	0.130	0.140	0.122	0.131	0.009	6.9	2.6	16.2	81.9
11/4/96	7	DMSO	5	SL	0.109	0.098	0.097	0.101	0.007	6.6	2.0	1.1	2.8
11/4/96	8	DMSO	5	SL	0.095	0.097	0.092	0.095	0.003	2.7	1.9	1.2	3.2
11/4/96	11	DMSO	5	SL	0.143	0.133	0.117	0.131	0.013	10.0	2.6	2.7	9.1
11/4/96	12	DMSO	5	SL	0.137	0.137	0.127	0.134	0.006	4.3	2.6	7.9	40.0
11/4/96	13	DMSO	5	SL	0.097	0.084	0.083	0.088	0.008	8.9	1.7	1.6	5.4
11/4/96	14	DMSO	5	SL	0.082	0.082	0.078	0.081	0.002	2.9	1.6	5.5	27.9
11/4/96	15	DMSO	5	SL	0.090	0.093	0.089	0.091	0.002	2.3	1.8	1.4	3.5
11/4/96	23	DMSO	5	SL	0.090	0.089	0.084	0.088	0.003	3.7	1.7	1.2	3.2
11/4/96	24	DMSO	5	SL	0.080	0.078	0.074	0.077	0.003	4.0	1.5	1.1	3.7
11/4/96	25	DMSO	5	SL	0.110	0.119	0.123	0.117	0.007	5.7	2.3	4.8	24.1
11/4/96	26	DMSO	5	SL	0.092	0.096	0.109	0.099	0.009	9.0	1.9	1.2	3.0
11/4/96	33	DMSO	5	SL	0.085	0.086	0.083	0.085	0.002	1.8	1.7	1.3	4.2
11/4/96	36	DMSO	5	SL	0.078	0.079	0.071	0.076	0.004	5.7	1.5	0.9	2.3
11/5/96	27	DMSO	5	SL	0.088	0.094	0.122	0.101	0.018	17.9	0.9	<1.22	<4.07
11/5/96	28	DMSO	5	SL	0.091	0.124	0.089	0.101	0.020	19.4	0.9	<1.22	<4.07
11/5/96	29	DMSO	5	SL	0.111	0.117	0.109	0.112	0.004	3.7	1.0	0.20	0.4
11/5/96	30	DMSO	5	SL	0.097	0.092	0.094	0.094	0.003	2.7	0.8	<1.22	<4.07
11/5/96	31	DMSO	5	SL	0.103	0.107	0.111	0.107	0.004	3.7	0.9	<1.22	<4.07
11/5/96	37	DMSO	5	SL	0.095	0.092	0.096	0.094	0.002	2.2	0.8	<1.22	<4.07
11/7/96	39	DMSO	5	SL	0.092	0.095	0.086	0.094	0.002	2.3	1.1	0.6	1.5
11/7/96	41	DMSO	5	SL	0.253	0.250	0.280	0.252	0.002	0.8	2.9	3.6	18.2
11/7/96	42	DMSO	5	SL	0.087	0.092	0.074	0.090	0.004	4.0	1.0	0.6	1.6
11/7/96	44	DMSO	5	SL	0.134	0.127	0.129	0.131	0.005	3.8	1.5	0.8	4.2
11/7/96	45	DMSO	5	SL-oiled	0.498	0.462	0.387	0.480	0.025	5.3	5.5	3.2	16.4

*type: SL=Sandlance tissue sample; SL-oiled= Sandlance tissue sample from oiled site;

sed= Sediment sample; sed-oiled = sediment sample from oiled site

Fold values in bold are <1.0, and are assigned B[a]PEq = < what a 1.0 fold would give.

Table 5. Summary of B[a]P Equivalents in APEX Samples.

CAS #	Sand Lance		B[a]PEq.*	B[a]PEq.*
K5793-	Description	Location	(µg/g dry)	(µg/g lipid)
2	SL	Block Island NW	<1.43	<3.66
3	SL	Block Island NW	2.9	9.6
4	SL	Block Island NW	5.6	28.3
5	SL	Block Island NW	4.1	20.5
6	SL	Block Island NW	16.2	81.9
7	SL	Block Island NW	1.1	2.8
8	SL	Block Island NW	1.2	3.2
9	SL-oiled	Block Island NW	4.3	14.4
11	SL	Block Island NW	2.7	9.1
12	SL	Block Island NW	7.9	40.0
13	SL	Block Island NW	1.6	5.4
14	SL	Block Island NW	5.5	27.9
15	SL	Block Island NW	1.4	3.5
16	SL-oiled	Block Island NW	4.0	13.2
17	SL-oiled	Block Island NW	7.8	39.1
18	SL-oiled	Block Island NW	7.5	37.7
19	SL-oiled	Block Island NW	7.3	37.1
20	SL-oiled	Block Island NW	3.8	12.8
21	SL-oiled	Block Island NW	1.4	3.7
22	SL	Eleanor Island SE	2.1	6.9
23	SL	Eleanor Island SE	1.2	3.2
24	SL	Eleanor Island SE	1.1	3.7
25	SL	Eleanor Island SE	4.8	24.1
26	SL	Eleanor Island SE	1.2	3.0
27	SL	Eleanor Island SE	<1.22	<4.07
28	SL	Eleanor Island SE	<1.22	<4.07
29	SL	Eleanor Island SE	1.2	4.1
30	SL	Eleanor Island SE	<1.22	<4.07
31	SL	Eleanor Island SE	<1.22	<4.07
33	SL	Eleanor Island SE	1.3	4.2
36	SL	Eleanor Island SE	0.9	2.3
37	SL	Eleanor Island SE	<1.22	<4.07
39	SL	Eleanor Island SE	0.6	1.5
41	Composite of SL #32,34,35,38,40	Eleanor Island SE	3.6	18.2
42	SL	Eleanor Island SE	0.6	1.6
43	SL-oiled	MacPherson Isthmus	<1.1	<5.7
44	SL	North Cabin Bay	0.8	4.2
45	SL-oiled	East Bob Day Bay	3.2	16.4
Sediment	Field Estimate	Location		
Blank	NA	NA	0.1	
K6369-1	sed	Eleanor Island SE	3.1	
K5793-48	sed	Block Island NW	3.5	
49	sed-oiled	MacPherson Isthmus	0.2	
50	sed	Fuel Cache Beach	0.2	
51	sed-oiled	East Bob Day Bay	0.4	
52	sed	MacPherson Narrows	0.2	

*Differences in detection limits result from variability among sample weight and fold induction values of blanks.

Figure 1. P450 RGS-estimated B[a]P Equivalents in Sand Lance Tissues from Prince William Sound, Alaska

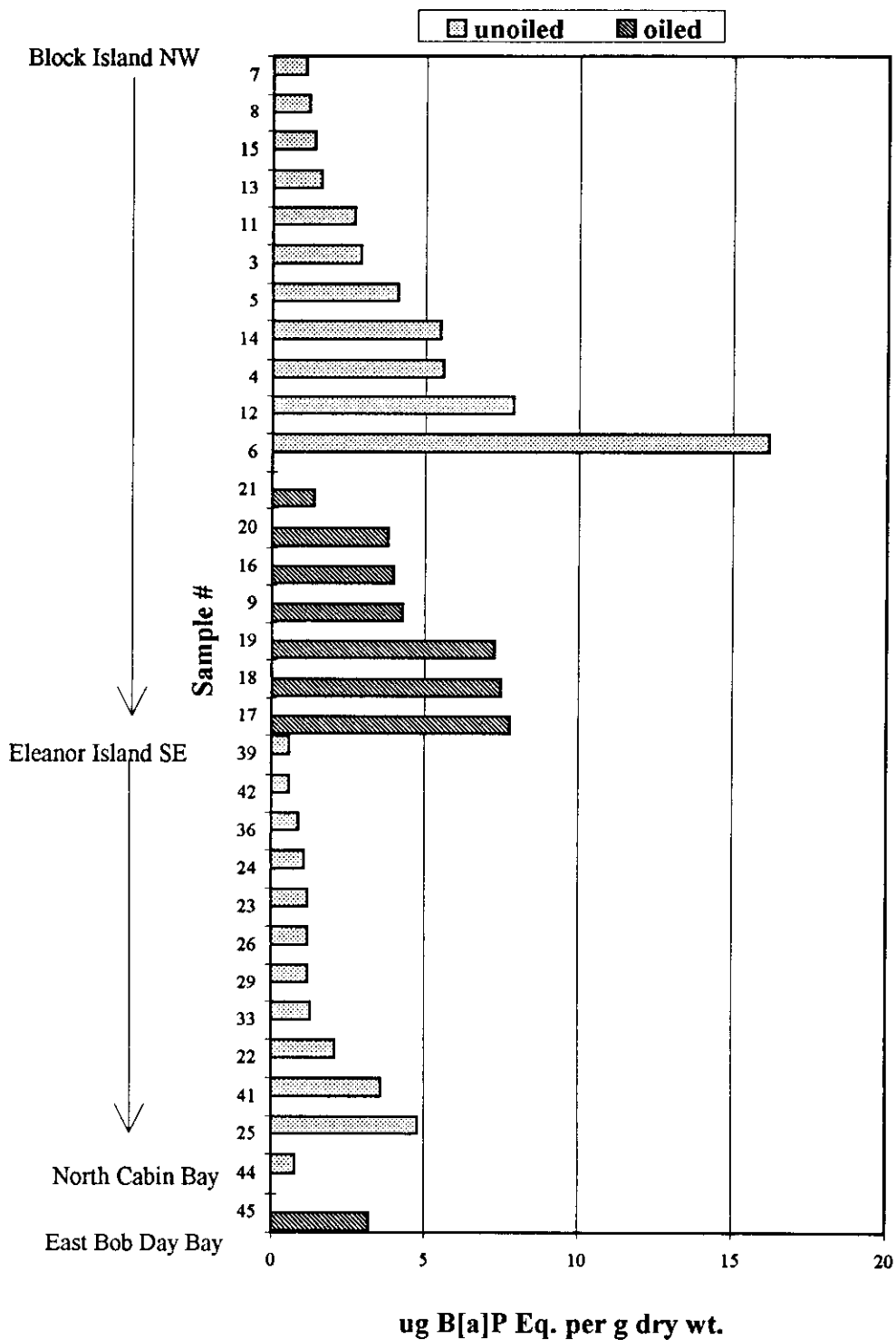
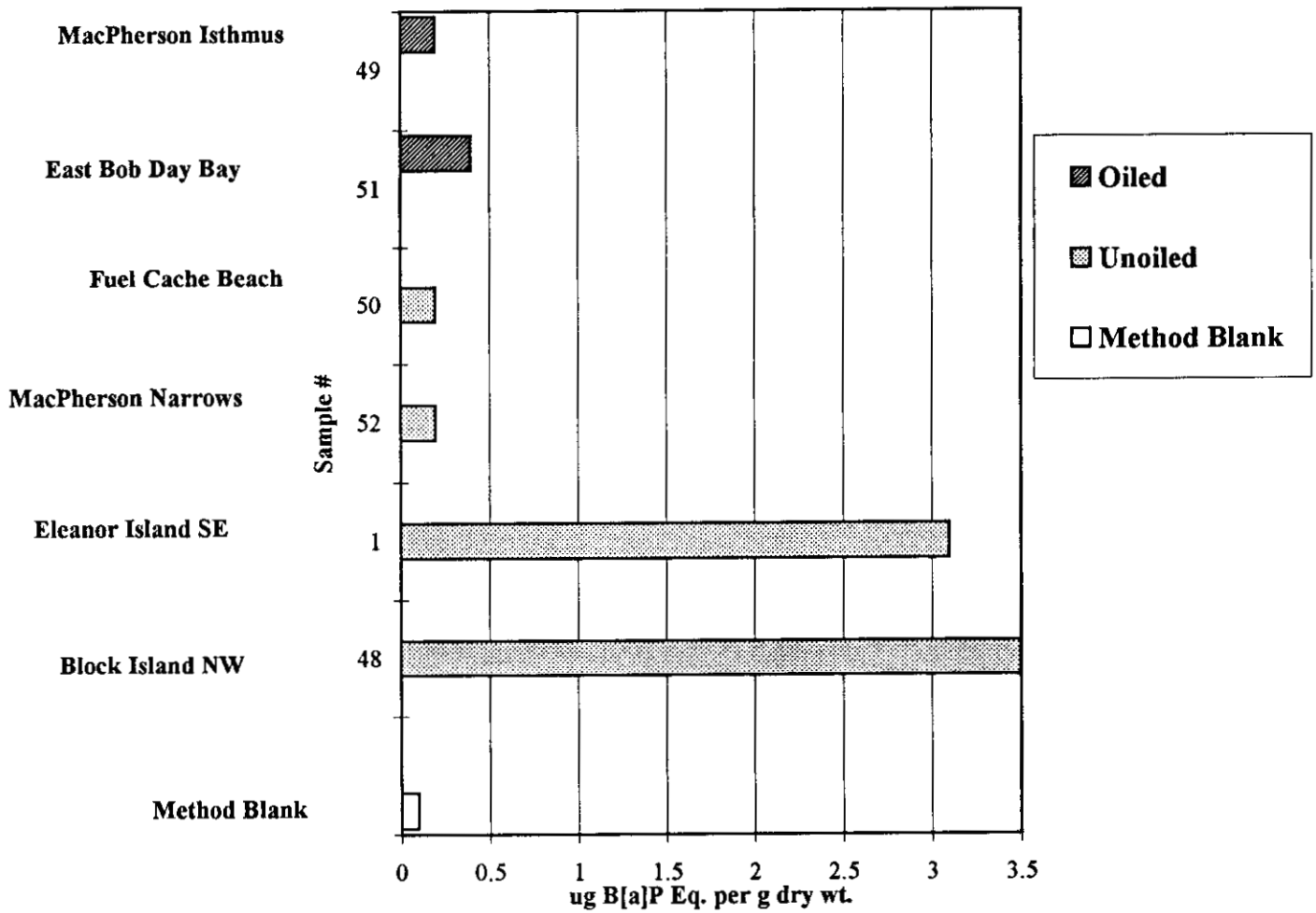


Figure 2. P450 RGS-estimated B[a]P Equivalents in Sediments from Prince William Sound, Alaska



Sediments

A sediment extract from Block Island NW (CAS #48) was determined to have the highest level of B[a]PEq at 3.5 µg/g. Most of the sediment extracts (71%) had <1.0 µg B[a]PEq per g dry weight.

Statistical Analysis

A t-test was used to determine differences between the mean B[a]P Equivalency values of tissues from oiled and unoiled collection sites (Microsoft Excel 5.0). One data point from unoiled Eleanor Island SE (CAS #6) was not used in this analysis. This value was an extreme outlier, greater than three standard deviations above the mean B[a]PEq for unoiled samples. Using a two-tailed t-test with unequal variances, the mean B[a]PEq from the oiled sample set was found to be significantly greater than that from the unoiled set at the 95% confidence level ($p < 0.02$).

Table 6. Results of Statistical Analysis on B[a]PEqs from Sand Lance Tissues.

Variable	N	mean	S.D.
unoiled	22	2.43	2.0
oiled	8	4.93	2.3

$p < 0.021$

QA/QC

Figure 3 shows the dioxin control chart used to assure quality control of the P450 RGS assay. After every test run, the fold induction produced by dioxin at 2 ng/mL is entered into an Excel spreadsheet that adds that day's dioxin performance to obtain a running mean. From this, a confidence interval (shown on the chart by dashed horizontal lines at two standard deviations above and below the most recent value for the running mean) is determined and the test run is evaluated. All dioxin fold induction values obtained in this study (data points from 10/22 to 11/7) were within two standard deviations of the running mean, and were therefore acceptable.

In addition, a sediment extract method blank was tested along with the sediment samples. This extract yielded a 1.5 fold induction and 0.1 µg B[a]PEq per g dry weight (of sodium sulfate used in the extraction method). This gives a baseline value upon which B[a]PEqs calculated from true sediment samples can be analyzed.

Figure 3. P450 RGS TCDD (2 ng/mL) Control Chart

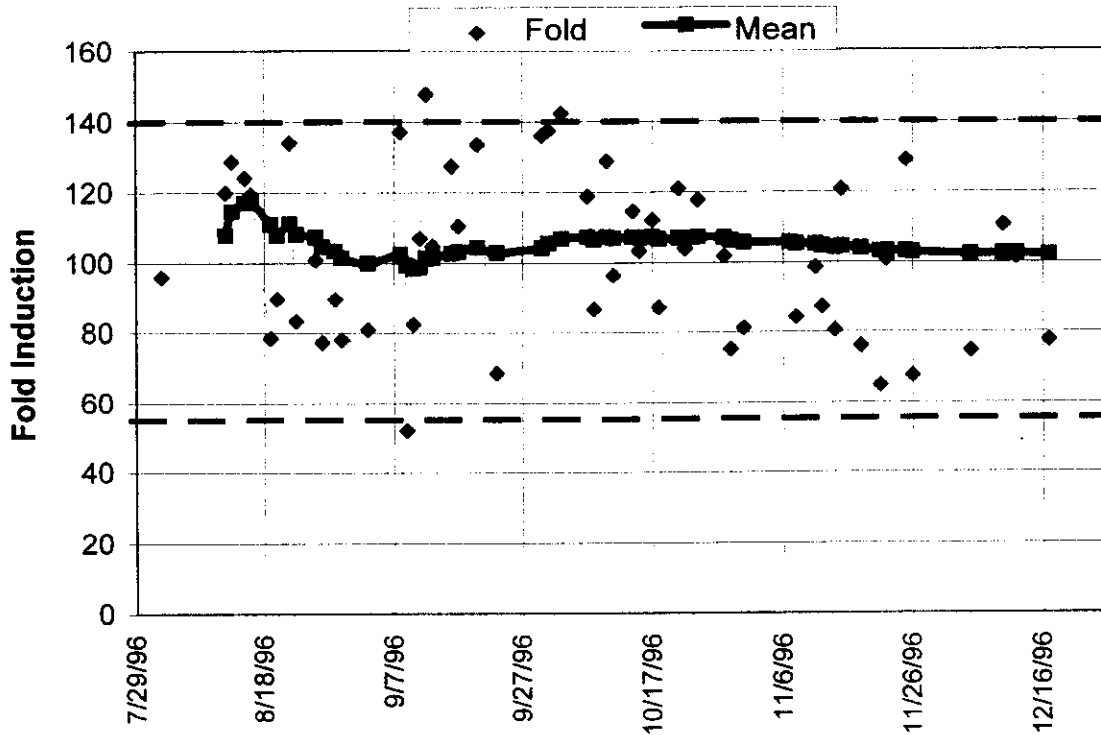


Figure 4. P450RGS Induction from Benzo[a]Pyrene

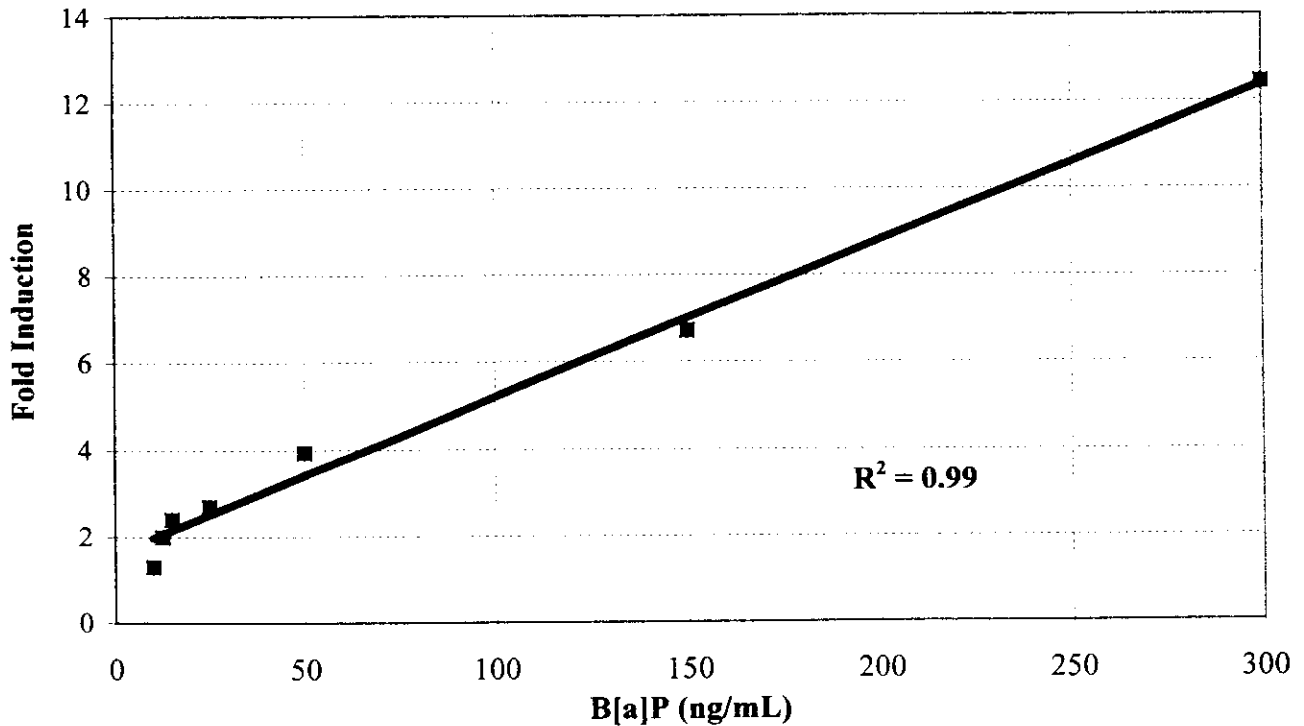


Figure 5. P450 RGS Induction from PAH Mixture

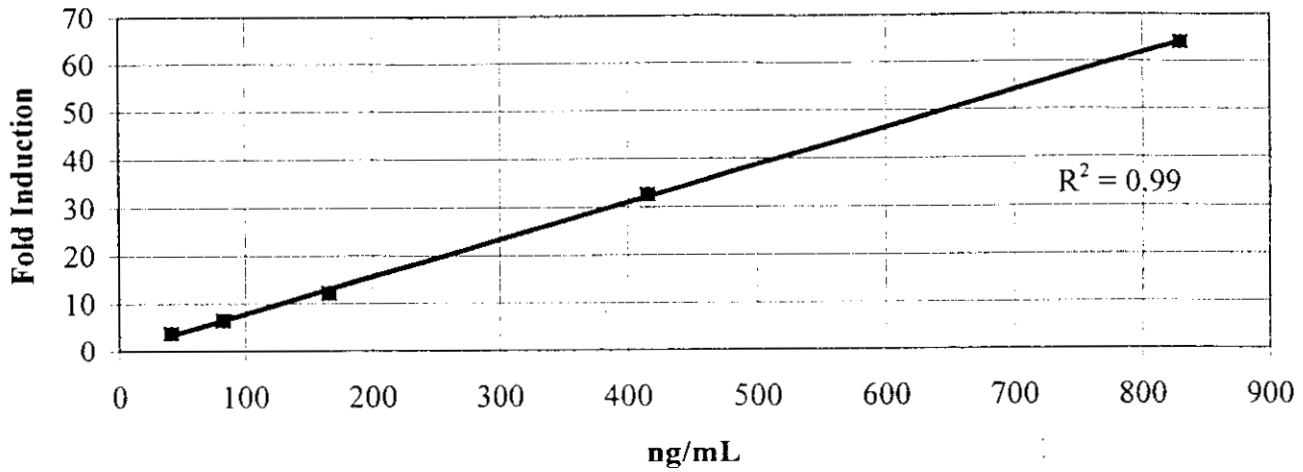


Figure 6. Luciferase Standard Curve (11/6/96)

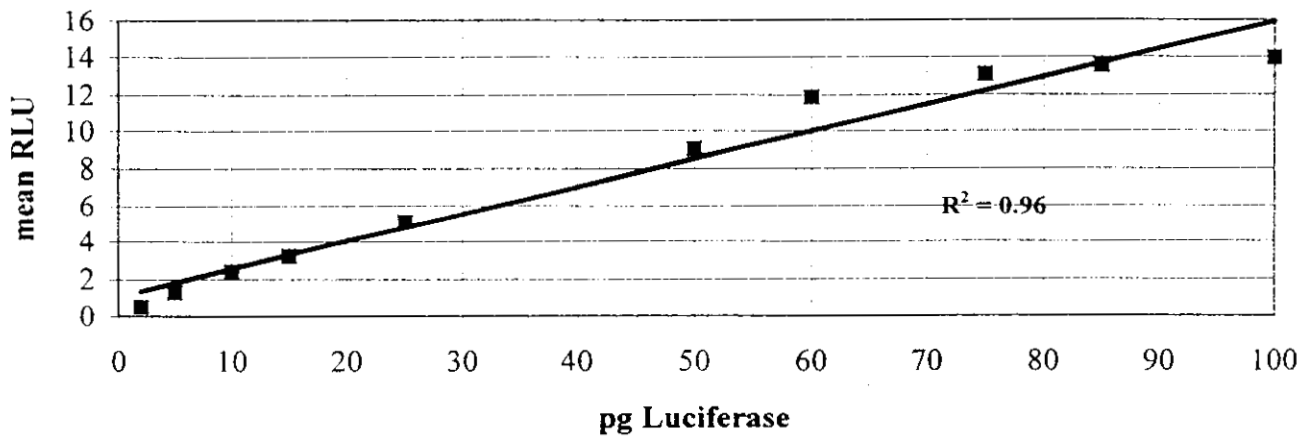
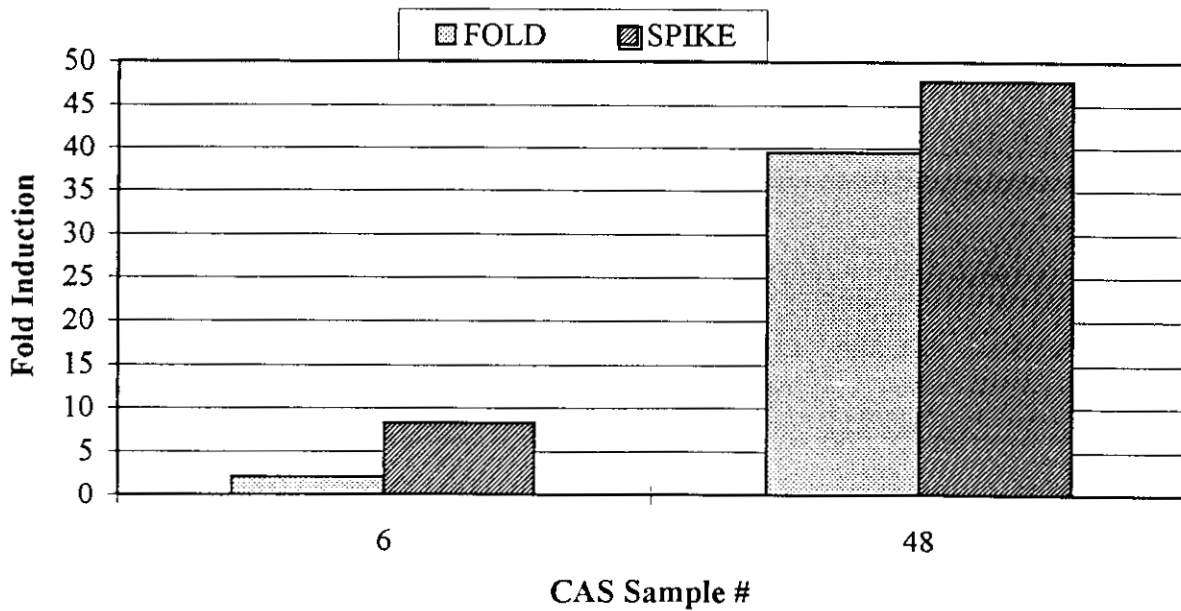


Figure 7. P450 RGS Fold Induction Response to Spike with 300 ng/mL B[a]P



Standard curves for Benzo[a]Pyrene and a commercially obtained PAH mixture are shown in Figures 4 and 5, respectively. Along with this, a luciferase standard curve (Figure 6) shows the lower detection limit of the luminometer and the strong linear correlation between RLUs and enzyme concentration, indicating the sensitivity and precision of the instrument.

Results from the spiking study are shown in Table 7 and Figure 7. Three samples were spiked: the sediment sample showing the highest induction (CAS #48) and two tissue samples (CAS #6 and CAS #36). Sample #48 and # 6 were spiked with 300 ng/mL B[a]P, while sample #36 (not shown in Figure 7) was spiked with only 50 ng/mL B[a]P (see Table 7).

Table 7. Results of Spiking Study with B[a]P.

CAS ID#	B[a]P Spike (ng/mL)	FOLD Sample alone	FOLD B[a]P spike	FOLD Sample + spike
5793-6	300	1.5	9.4	8.2
36	50	0.7	3.0	1.8
48	300	38.2	9.4	47.8

In cases where the fold induction of samples is ≤ 1.0 (equal or less than that of the solvent blank), as with many of the sand lance tissue extracts in this study, the B[a]P EQ are expressed as < what would result from a 1.0 fold induction. From previous studies using very low concentrations of 2,3,7,8-dioxin, the lower endpoint of detection with the P450 RGS assay is approximately 0.001 ng/mL, yielding between 1 and 2-fold. From the luciferase standard curve (Figure 6), detection of as little as 5 picograms of luciferase is possible. Preliminary studies with a PAH mixture (Figure 5) indicate that at least 40 ng/mL yields a reproducible detection of between 2 and 4-fold, and B[a]P concentrations as low as 10 ng/mL have been detected (Figure 4). Other individual PAH compounds, particularly benzo[k]fluoranthene, have even lower detection limits.

Analytical Chemistry of Extracts

Investigators Marie Larsen, Jeff Short, and Stan Rice, of the Auke Bay NOAA laboratory, graciously analyzed extracts of the sand lance and sediments used in our investigation (data in Appendix). The tissue extracts from composites of several whole fish, with the combined dry weights per sample of between 0.5 and 2.9 grams, were not found to contain detectable amounts of the higher molecular weight PAHs. These 4- to 6-ring aromatic compounds are those that would be expected to produce the P450 RGS responses observed. We recognized that any measured concentrations would be quite low, as the RGS responses were just above background. Apparently, the induction of CYP1A1 observed was from uncharacterized compounds present in the tissues. Previous investigations with fish tissue collected in Southern California have detected levels of contaminants from 0.3 to 4.0 μg of Benzo(a)pyrene Equivalents per g (ppm) in muscle, liver and ovary tissues. It was later determined that PCBs and not PAHs were responsible for the P450 RGS induction observed in these samples.

The RGS assay indicated that two sediment samples (Figure 2), collected at Eleanor Island SE and Block Island NW contained low but detectable amounts of PAHs (3.1 to 3.5 $\mu\text{g B(a)PEq/g}$). Chemical analyses by the Auke Bay scientists demonstrated that the content of high molecular weight PAH in these two sediment samples were 100 and 208 ng/g, respectively (Table 8). The other two sediment samples analyzed by NOAA (Fuel Cache Bay and MacPherson Narrows) showed no detectable levels of any PAH, which agrees with the baseline values reported for the P450 RGS assay. Using the Toxic Equivalency Factors (TEFs) for specific PAHs generated in our laboratory, the analytical data for the two samples were converted to estimated Toxic Equivalency values (in $\mu\text{g B(a)PEq/g}$). These values for Chemically derived benzo[a]pyrene equivalents (7.3 and 2.4 ng/g) compare very favorably (correlation of 0.86) with the estimates of toxic equivalency produced earlier by the RGS assay. The absolute concentrations of the calculated toxic equivalents are, however, three orders of magnitude lower than the observed RGS responses. The chemical composition of the sediments reported are unusual in that two key compounds often present in oiled sediments (benzo[a]pyrene and benzo[k]fluoranthene) were not observed. Perhaps these and other inducing compounds were present at levels just below the detection limits.

Table 8. Analyses by the Auke Bay NOAA Laboratory of Sediment Samples, and Comparison to P450 RGS Data on a Basis of Benzo[a]pyrene Equivalents

Sediment Samples					Calculated Chem. B[a]PEq				
	5793-48 BI NW	-50 Fuel C B	-52 MacP Nar	6369-1 El I SE (#47)					
Estimated Condition	unoiled	unoiled	unoiled	unoiled	RGS TEF	5793-48	-50	-52	6369-1 (#47)
Benzo[a]anthracene	12.97	0	0	0	0.4	5.2	0.0	0.0	0.0
Benzo[a]pyrene	0	0	0	0	1	0.0	0.0	0.0	0.0
Benzo[b]fluorathene	54.45	0	0	23.38	3	163.4	0.0	0.0	70.1
Benzo[g,h,i]perylene	24.21	0	0	16.09	0.02	0.5	0.0	0.0	0.3
Benzo[k]fluoranthene	0	0	0	0	25	0.0	0.0	0.0	0.0
Dibenzo[a,h]anthracene	10.68	0	0	0	4	42.7	0.0	0.0	0.0
Chrysene	93.96	0	0	61.06	0.4	37.6	0.0	0.0	24.4
Ideno[1,2,3-cd]pyrene	11.47	0	0	0	3	34.4	0.0	0.0	0.0
T HMW PAHs(ng/mL)	207.74	0	0	100.53					
RGS B[a]PEq ($\mu\text{g/g}$)	3.51	0.19	0.24	3.12					
Chem TEQ ($\mu\text{g/mL}$)	0.28	0	0	0.09		283.7	0	0	94.9
Sample Dry Weight (g)	38.6	37.0	37.2	38.9					
Chem B[a]PEq (ng/g)	7.3	0.0	0.0	2.4					
Correlation	0.86								

DISCUSSION AND CONCLUSIONS

The induction of luciferase production in the 101L cell line used in the P450 RGS assay, as measured by relative light units of cell lysates, indicates that the applied sample contains chemicals that act upon the *CYP1A1* gene and cause transcription of cytochrome P450. This response is known to be associated with human and aquatic organism health effects, including carcinogenesis. These inducing chemicals include PAHs, PCBs, and dioxins, and are present, likely as mixtures, as environmental contaminants. As a result of the *Exxon Valdez* oil spill, Prince William Sound sediments are known to have significant levels of primarily PAH contamination. While this contamination can be measured directly using chemical analysis (GC/MS, HPLC, etc.) of sediment and pore water, this is a tedious and costly process, and the impact of the quantified chemicals on wildlife remain unclear. The P450 RGS analysis is a valid and useful, as well as quick and inexpensive, screening tool to assess the potential of contaminated sediment to have deleterious health effects on humans and wildlife via the Ah-mediated pathway.

In addition, analysis of tissue extracts by P450 RGS enables a further investigation into exposure and food web transfer of contaminants. While PAHs are metabolized by fish, many invertebrates accumulate these chemicals, leading to the transfer of much higher levels to organisms that ingest them.

This study investigated the potential for tissue extracts of sand lance, a fish that may be particularly susceptible to sediment-associated contamination during burrowing, to induce cytochrome P450. Such induction would presumably occur in organisms ingesting sand lance tissue. The results of this study indicate that the induction potential of these tissue samples was not necessarily correlated with the estimated level of oiling in the collection areas. For example, the strongest-inducing tissue sample came from an area that was presumed to be relatively clean. It must be noted here, however, that this particular sample extract (CAS #6) came from the tissue sample of lowest mass, contributing to its higher B[a]PEq value. Given that only five of the thirty-eight tissue samples analyzed were collected from oiled sites, the implications of this are unclear.

Sediment analyses also yielded results inconsistent with assumptions of oiling levels in collection sites. The two extracts that produced the strongest induction were from presumably clean sites. Obviously, there are many factors, including fish movement as well as contaminant patchiness, contributing to the difficulties in interpreting these findings.

In this study, the main focus was on investigating the potential of the P450 RGS assay as a screening tool for detecting PAH contamination in both fish tissues and sediments in a wide area, such as Prince William Sound. In previous studies, P450 RGS-estimated B[a]PEq have been found to be highly correlated with calculated toxic equivalency values determined from chemical analysis of extracts.

While we feel that sand lance can be important indicators of sediment contamination, including that following an oil spill, it is apparent that by 1996 no residual contamination from the 1989 *Exxon Valdez* oil spill is present in their tissues. Very low levels of sediment contamination were identified in two samples by the P450 RGS assay, and this finding has now been confirmed by detailed chemical analyses.

The P450 Reporter Gene System has demonstrated the ability to determine which of many sediment samples should be further evaluated for petroleum contamination. In the case of RGS responses to tissue extracts, we do not know what substances induced the measured responses. The relatively low level of response observed does not warrant the type of research effort that would be required to evaluate the chemistry of these extracts.

REFERENCES

- Anderson, J.W., S.S. Rossi, R.H. Tukey, T. Vu, and L.C. Quattrochi. 1995. A biomarker, 450RGS, for assessing the potential toxicity of organic compounds in environmental samples. *Environ. Toxicol. Chem.* 14:1159-1169.
- Anderson, J.W., K. Bothner, T. Vu, and R.H. Tukey. 1996. Using a biomarker (P450 RGS) test method in environmental samples. In G.K. Ostrander, ed., Techniques in Aquatic Toxicology. Lewis Publishers, Boca Raton, FL. pp. 277-286.
- Anderson, J.W., K. Bothner, J. Means, D. McMillin, T. Vu, and R. Tukey. 1997. Correlation of CYP1A1 induction, as measured by the P450 RGS biomarker assay, with Benzo[a]Pyrene equivalents (B[a]PTEQs) in extracts of mussels deployed at various sites in San Diego Bay. To be published as part of a special issue of *Marine Environmental Research* on biomarkers in deployed mussels.
- APHA. 1996. P450 Reporter Gene Response to Dioxin-like Organics. Method 8070, In: Standard Methods for the Examination of Water and Wastewater, 19th Edition Supplement, pp. 24-25, American Public Health Association, Washington D.C.
- ASTM 1997. Standard Guide E 1853 - 96 for Measuring the Presence of Planar Organic Compounds which Induce CYP1A, Reporter Gene Test Systems, pp. 1392-1397 In: Volume 11.05, Biological Effects and Environmental Fate, 1997 Annual Book of ASTM Standards, American Society for Testing and Materials, West Conshohocken, PA.
- Collier, T.K., B.F. Anulacion, J.E. Stein, A. Goksoyr, and U. Varanasi. 1995. A field evaluation of Cytochrome P4501A as a biomarker of contaminant exposure in three species of flatfish. *Environ. Toxicol. Chem.* 14:143-152.
- Collier, T.K., C.A. Krones, M.M. Krahn, J.E. Stein, S.Chan, and U. Varanasi. 1996. Petroleum exposure and associated biochemical effects in subtidal fish after the

Exxon Valdez oil spill. In S.D. Rice, R.B. Spies, D.A. Wolfe, and B.A. Wright, eds., Proceedings of the Exxon Valdez Oil Spill Symposium, American Fisheries Society Symposium 18, Bethesda, MD. pp. 671-683.

Pinto, J.M., W.H. Pearson, and J.W. Anderson. 1984. Sediment preferences and oil contamination in the Pacific sand lance *Ammodytes hexapterus*. *Marine Biology* 83:193-204.

Varanasi, U., J.E. Stein, M. Nishimoto, W.L. Reichert, and T.K. Collier. 1987. Chemical carcinogenesis in feral fish: Uptake, activation, and detoxification of organic xenobiotics. *Environ. Health Perspect.* 71:155-170.

APPENDIX

April 2, 1997

This spreadsheet contains the hydrocarbon data for the 4 sediment extracts sent to ABL by Jack Anderson.

These data are calculated in total ng in the extract because no sample weight was reported. These data have been filtered and the data has been edited according to these filters.

	jd = 5793-48	5793-50	5793-52	6369-1
qcbatch =	R03037	R03037	R03037	R03037
drywt =	0	0	0	0
wetwt =	1	1	1	1
matrix =	SEDIMENT	SEDIMENT	SEDIMENT	SEDIMENT

Surrogate Recoveries (%)

NAPHTHALENE d-8	42.68	42.58	47.12	62.07
ACENAPHTHENE d-10	60.08	59.13	60.16	78.68
PHENANTHRENE d-10	70.07	79.47	74.92	86.00
ANTHRACENE d-10	93.13	86.70	88.74	104.26
BENZ-a-ANTHRACENE d-12	111.96	78.65	76.16	121.95
CHRYSENE d-12	69.74	62.35	78.67	93.13
BENZO-a-PYRENE d-12	52.40	45.59	41.99	77.81
PERYLENE d-12	51.85	47.67	43.31	71.04

Analytes

	(ng)			
naphthalene	0.00	0.00	0.00	0.00
2-methylnaphthalene	0.00	0.00	0.00	0.00
1-methylnaphthalene	0.00	0.00	0.00	0.00
2,6-dimethylnaphthalene	0.00	0.00	0.00	0.00
C-2 naphthalenes	0.00	0.00	0.00	0.00
2,3,5-trimethylnaphthalene	0.00	0.00	0.00	0.00
C-3 naphthalenes	0.00	0.00	0.00	0.00
C-4 naphthalenes	0.00	0.00	0.00	0.00
biphenyl	0.00	0.00	0.00	0.00
acenaphthylene	0.00	0.00	0.00	0.00
acenaphthene	0.00	0.00	0.00	0.00
fluorene	0.00	0.00	0.00	0.00
C-1 fluorenes	0.00	0.00	0.00	0.00
C-2 fluorenes	0.00	0.00	0.00	0.00
C-3 fluorenes	0.00	0.00	0.00	0.00
dibenzothiophene	0.00	0.00	0.00	0.00
C-1 dibenzothiophenes	0.00	0.00	0.00	0.00
C-2 dibenzothiophenes	147.00	0.00	0.00	120.31
C-3 dibenzothiophenes	434.86	0.00	0.00	401.19
phenanthrene	0.00	0.00	0.00	0.00
1-methylphenanthrene	0.00	0.00	0.00	0.00
C-1 phenanthrenes/anthracenes	42.38	0.00	0.00	0.00
C-2 phenanthrenes/anthracenes	260.49	0.00	0.00	238.13
C-3 phenanthrenes/anthracenes	615.06	0.00	0.00	646.48
C-4 phenanthrenes/anthracenes	272.89	0.00	0.00	310.95
anthracene	0.00	0.00	0.00	0.00
fluoranthene	58.30	0.00	0.00	0.00
pyrene	45.27	0.00	0.00	0.00
C-1 fluoranthenes/pyrenes	53.85	0.00	0.00	34.17
benz-a-anthracene	12.97	0.00	0.00	0.00
chrysene	93.96	0.00	0.00	61.06
C-1 chrysenes	160.21	0.00	0.00	144.09
C-2 chrysenes	109.76	0.00	0.00	158.13
C-3 chrysenes	114.51	0.00	0.00	147.78
C-4 chrysenes	0.00	0.00	0.00	0.00
benzo-b-fluoranthene	51.45	0.00	0.00	23.38
benzo-k-fluoranthene	0.00	0.00	0.00	0.00
benzo-e-pyrene	84.51	0.00	0.00	47.53
benzo-a-pyrene	0.00	0.00	0.00	0.00
perylene	12.15	0.00	0.00	0.00
Indeno-123-cd-pyrene	11.47	0.00	0.00	0.00
dibenzo-a,h-anthracene	10.68	0.00	0.00	0.00
benzo-g,h,i-perylene	27.21	0.00	0.00	16.09

March 14, 1997

This worksheet contains the aromatic hydrocarbon data results from the analysis of Sandlance tissues in DMSO. These samples were sent to ABL from Jack Anderson of Columbia Analytical Services.

Internal QA samples

We have no sample weights so the data are reported in ng. These data have been filtered according to a tissue mdL and the data below detection limits have been edited.

id =	5793-9	5793-16	5793-17	5793-18	5793-19	5793-20	5793-23	5793-24	5793-26	5793-29	5793-36	5793-39	5793-45	AREF	BREF	MBLK	NISTARO	NISTARO
qcbatch =	R02247	R02247	R02247	R02247	R02247	R02247	R02247	R02247	R02247	R02247	R02247	R02247	R02247	R02247	R02247	R02247	R02247	R02247
drywt =	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
wetwt =	1	1	1	1	1	1	1	1	1	1	1	1	1	10	10	10	1	1
matrix =	SANDLANC													OCTISSUE	OCTISSUE	QCBLANK	QCCALI	QCCALI
submatrix =	DMSO	DMSO	DMSO	DMSO	DMSO	DMSO	DMSO	DMSO	DMSO	DMSO	DMSO	DMSO	DMSO					

Surrogate Recoveries (%)

NAPHTHALENE d-8	72.78	69.90	68.59	68.07	66.06	70.47	76.61	76.60	72.22	75.33	76.43	69.17	60.26	73.87	71.18	57.36	101.12	100.99
ACENAPHTHENE d-10	83.93	76.09	75.44	74.47	73.66	75.74	83.09	83.00	79.68	83.00	88.68	80.98	89.90	82.30	80.29	69.97	100.00	100.72
PHENANTHRENE d-10	102.78	91.76	90.85	90.75	87.41	89.41	84.94	87.84	95.00	98.37	106.28	95.05	107.14	96.71	96.36	39.25	99.34	98.27
ANTHRACENE d-10	102.83	103.87	99.72	100.03	98.24	98.62	102.29	106.19	108.25	107.32	108.85	96.76	111.38	95.45	106.19	85.29	96.63	89.33
BENZ-a-ANTHRACENE	96.71	83.71	86.03	87.83	80.51	82.42	93.29	102.49	104.76	104.86	109.91	97.80	111.04	86.23	109.59	74.48	89.31	95.79
CHRYSENE d-12	93.66	80.77	78.03	81.60	75.72	78.74	86.03	96.24	91.30	95.49	104.47	94.13	108.38	93.12	102.64	91.92	94.30	100.42
BENZO-a-PYRENE d-12	68.58	55.09	52.84	58.76	53.38	56.80	64.19	76.65	74.87	75.56	91.48	79.91	99.23	71.74	90.18	48.43	83.55	97.23
PERYLENE d-12	72.15	60.11	57.44	62.92	66.53	60.96	68.17	80.68	76.41	78.65	93.81	82.07	101.57	77.30	93.64	58.76	85.64	96.08

Analyte Concentrations (ng)

naphthalene	0.00	28.85	33.06	28.00	35.25	39.29	52.97	68.21	71.24	49.16	45.49	66.76	40.69	47.56	47.21	0.00	342.06	344.51
2-methylnaphthalene	0.00	0.00	32.97	20.37	24.16	27.47	50.31	77.78	69.28	48.22	41.46	58.97	49.89	48.08	48.91	0.00	390.59	388.94
1-methylnaphthalene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	296.42	402.08
2,6-dimethylnaphthalene	7.55	0.00	10.44	7.63	9.72	10.17	16.32	27.42	21.71	14.76	14.29	20.74	19.86	40.92	40.62	0.00	359.37	364.32
C-2 naphthalenes	23.77	14.97	28.33	21.72	33.60	30.45	48.38	83.40	67.10	47.30	40.83	68.89	62.60	41.46	41.43	0.00	326.76	366.11
2,3,5-trimethylnaphthalene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	328.96	324.47
C-3 naphthalenes	0.00	0.00	0.00	0.00	8.01	0.00	16.70	29.59	27.33	19.57	23.75	33.50	38.40	40.81	39.68	0.00	326.96	325.80
C-4 naphthalenes	0.00	0.00	0.00	0.00	0.00	0.00	9.46	21.25	39.93	17.09	38.10	29.00	7.35	0.00	0.00	0.00	9.42	8.42
biphenyl	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	351.95	351.04
acenaphthylene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	37.74	38.24	0.00	345.46	348.68
acenaphthene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	43.33	43.47	0.00	360.12	382.20
fluorene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	44.36	44.74	0.00	348.63	355.51
C-1 fluorenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	16.78	0.00	0.00	0.00	22.57	22.59
C-2 fluorenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	16.35	16.35
C-3 fluorenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	16.35	16.35
dibenzothiophene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	407.67	412.65
C-1 dibenzothiophenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-31.28	-31.28
C-2 dibenzothiophenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-31.28	-31.28
C-3 dibenzothiophenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-31.28	-31.28
phenanthrene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	11.44	38.19	37.37	249.18	350.68
1-methylphenanthrene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	38.83	37.82	0.00	360.73	346.00
C-1 phenanthrenes/anth	0.00	0.00	0.00	22.94	31.51	27.23	0.00	28.94	41.96	32.67	28.06	22.16	39.32	38.83	38.10	0.00	353.27	348.20
C-2 phenanthrenes/anth	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C-3 phenanthrenes/anth	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C-4 phenanthrenes/anth	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
anthracene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	40.80	41.02	0.00	385.89	390.36
fluoranthene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	32.82	32.62	0.00	291.89	290.14
pyrene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	32.42	32.18	0.00	290.77	286.79
C-1 fluoranthenes/pyrene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
benz-a-anthracene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	18.68	19.02	0.00	171.58	171.31
chrysene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	37.92	37.23	0.00	349.71	345.16
C-1 chrysenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C-2 chrysenes	0.00	0.00	0.00	0.00	0.00	0.00	13.07	16.80	0.00	0.00	0.00	14.16	0.00	0.00	0.00	0.00	0.00	0.00
C-3 chrysenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C-4 chrysenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
benzo-b-fluoranthene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	278.44	257.07
benzo-k-fluoranthene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	33.10	30.51	0.00	277.15	267.80
benzo-e-pyrene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	36.57	32.59	0.00	307.07	279.98
benzo-a-pyrene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	38.58	37.17	0.00	342.65	335.16
perylene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	40.61	38.60	0.00	358.75	360.24
indeno-123-cd-pyrene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	24.41	27.67	0.00	272.59	298.12
dibenzo-a,h-anthracene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	16.00	22.40	0.00	208.12	238.51
benzo-g,h,i-perylene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	243.86	251.32

NMFS ALKE BAY

04/03/97 THU 08:20 FAX 19077896094