

**Annual Report**

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**Research to Determine the  
Accumulation of Organic Constituents  
and Heavy Metals from Petroleum-  
Impacted Sediments by, Marine  
Detritivores of the Alaskan Outer  
Continental Shelf**

**Contract No. 2311102778**

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To the  
National Oceanic & Atmospheric  
Administration, Boulder, Colorado

**April 1977**

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RESEARCH TO DETERMINE THE ACCUMULATION  
OF ORGANIC CONSTITUENTS AND HEAVY METALS  
FROM PETROLEUM-IMPACTED SEDIMENTS BY MARINE  
DETRITIVORES OF THE ALASKAN OUTER CONTINENTAL SHELF

by

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ANNUAL REPORT  
to the  
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## PREFACE

For the past ten months, individuals in the Battelle Marine Research Laboratory at Sequim, Washington, have been studying the bioavailability of petroleum hydrocarbons and trace metals from petroleum-impacted sediments. Since our study is relevant to petroleum development of the Alaskan Outer Continental Shelf, Prudhoe Bay Crude oil was used as a test oil. Our test animals were cold-water species of the Pacific Northwest, similar to those that may be found on the Alaskan shelf. The results of our preliminary experiments suggested productive avenues for further experimentation. Long-term studies are now in progress. Results on studies regarding uptake of naphthalenes by *Macoma inquinata* have been submitted for publication in the journal *Environmental Pollution*.

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## ABSTRACT

Experiments were conducted to examine the bioavailability of petroleum hydrocarbons and trace metals from petroleum-impacted marine sediments. The feasibility of using bivalve condition index as an indicator of stress due to petroleum exposure was also tested. Prudhoe Bay Crude was the test oil in **all** experiments.

When simultaneously exposed to 600  $\mu\text{g/g}$  oil in sediment for 40 days in the field, detectable levels of hydrocarbons ( $\sim 10$   $\mu\text{g/g}$  combined **aliphatic**, and **di-** and **tri-aromatic**) were present in two deposit-feeding species, *Phascolosoma agassizii* and *Macoma inquinata*, but not in *Protothaca staminea*, a filter-feeder. These results suggest that mode of feeding is a determinate factor in the availability of sediment-sorbed hydrocarbons to benthic animals. Tissue magnification of hydrocarbon concentrations was not observed.

Additional short-term experiments with  **$^{14}\text{C}$ -labeled** specific aromatic hydrocarbons in the laboratory indicated that ingestion of contaminated sediment resulted in negligible uptake of 2-methyl naphthalene by *Macoma inquinata*. **Methylnaphthalene** released from sediment to seawater appeared to be the primary contributor to tissue concentrations of this compound. Uptake of  **$^{14}\text{C}$ -phenanthrene**, **-dimethylbenzanthracene**, and **-benzo(a)pyrene**, however, exhibited components which could be attributed to both direct uptake from sediment and uptake from seawater. Magnification factors showed that hydrocarbons were concentrated from seawater but not from sediment. Long-term exposure indicated that uptake of  **$^{14}\text{C}$ -benzo(a)pyrene** by *M. inquinata* was **linear** for at least 6 weeks. **No** indication of a **steady-state** tissue concentration was observed.

Condition index of *Macoma inquinata* was sensitive to stress, with a significant reduction observed under unfavorable field conditions, as opposed to the laboratory. Condition index was reduced, but **not significantly**, in clams exposed to oil-contaminated sediment.

Compared to sediment concentrations, nickel, copper, zinc, and manganese were elevated in *Phascolosoma agassizii*, and nickel, zinc, and selenium in *Macoma inquinata*. Other compounds were present at levels similar to or lower than those of sediment. Exposure to oil-contaminated sediment did not appear to affect trace metals content of either species. Individual variation of trace metals content in *M. inquinata* was relatively low. Coefficient of variation for all elements ranged from 5 to 20%.

## INTRODUCTION

With increasing petroleum utilization and transport, there has been a concomitant increase in the amount of petroleum hydrocarbons that enter the marine environment. Charter *et al.* (1973) estimated that the total influx of petroleum to the oceans exceeds  $3 \times 10^6$  tons per year. Numerous studies have now been conducted on interactions between oil-contaminated seawater and marine organisms. Considerable information is available on the toxicity, uptake and deputation, metabolism, and physiological effects of these compounds (Anderson *et al.*, 1974; Neff *et al.*, 1976a; Malins, 1977; Anderson, 1977). Although it is known that hydrocarbon levels are elevated in marine sediments in the vicinity of petroleum inputs such as oil spills (Blumer *et al.*, 1970; Gilfillan *et al.*, 1976), sewage effluents (Barrington and Quinn, 1973), and refinery operations (Wharfe, 1975), little is known about the effects of oil-contaminated sediments on **benthic** organisms. Shaw *et al.* (1976) reported increased mortalities of clams *Macoma balthica* exposed to oiled sediment, while Rossi (1977) and Anderson *et al.* (1977) found little or no uptake of **naphthalenes** from oil-contaminated mud or detritus by a **polychaete**. Furthermore, there is no information regarding interactions between marine organisms and trace metals present in oil.

Our study has been concerned with the **bioavailability** of petroleum hydrocarbons and trace **metals** from petroleum-contaminated marine sediments using diverse experimental approaches. Two species have been emphasized as test organisms: a **detritivorous clam** *Macoma inquinata* and a sediment-ingesting **sipunculid** *Phascolosoma agassizii*. An initial attempt to rear the **polychaete** *Neanthes arenaceodentata* as a laboratory test organism was unsuccessful due to temperature limitations and, therefore, terminated. Exposures utilized sand and detritus (both from natural sources) and laboratory and **field** conditions. Several analytical techniques were employed to quantify **hydrocarbons** in animal tissues and sediment: ultraviolet and infrared **spectrophotometry**, gas chromatography, and liquid scintillation **spectrometry**. Trace

metals were analyzed by x-ray fluorescence or neutron activation analysis.

To date, we have conducted experiments to examine the following: (1) comparison of **bioavailability** of petroleum hydrocarbons from sediment in **benthic** deposit- and filter-feeders; (2) uptake of specific aromatic carbons from sediment in short-term experiments, differentiating between the relative importance of uptake from sediment versus seawater; (3) long-term uptake of specific hydrocarbons from sediment; (4) condition index of oil-exposed clams, and (5) uptake of trace metals from oil-contaminated sediment. The results are presented in this report. **Prudhoe** Bay Crude oil was the test oil in all experiments.

INFLUENCE OF FEEDING TYPE ON BIOAVAILABILITY  
OF PETROLEUM HYDROCARBONS FROM SEDIMENT

Benthic organisms are represented by species which exhibit diverse feeding modes. When considering the problem of uptake of material from sediment, it is reasonable to presume that organisms which feed directly on sediment or detritus would have a greater opportunity for accumulation from sediment than species which do not. We tested this hypothesis by exposing filter-feeding, detritus-feeding, and sediment-ingesting species to oil-contaminated sediment, then analyzing the organisms for tissue hydrocarbon concentrations. The clams *Protothaca staminea* and *Macoma inquinata* and sipunculid *Phascolosoma agassizii* were chosen as test species representative of the respective feeding modes listed above.

Exposures were conducted in sediment chambers described by Anderson *et al.* (1977), consisting of fiberglass boxes divided into three compartments, each with a fiberglass mesh bottom. Each compartment contained oil-contaminated sediment (initial concentration 622  $\mu\text{g/g}$  total hydrocarbons) prepared by a mixing technique (Anderson *et al.*, 1977) and one of the three test species. The exposure chambers were placed in the intertidal zone of Sequim Bay. The fiberglass screens on the bottoms of sediment boxes allowed percolation of seawater through the experimental sediment at low tide. To date, samples from 40-day exposures have been collected and analyzed.

Tissues were extracted and analyzed by gas chromatography for aliphatic hydrocarbons ( $\text{C}_{12}$  to  $\text{C}_{29}$ ), methylnaphthalenes, dimethylnaphthalenes, trimethyl naphthalenes, and total triaromatics (Warner, 1976). Two to three animals were pooled for each analysis. The results are summarized in Table 1.

The data indicate that *Macoma inquinata* and *Phascolosoma agassizii* accumulated both aliphatic and aromatic hydrocarbons, while *Protothaca staminea* did not, thus confirming our original theory that benthic animals

TABLE 1. Petroleum hydrocarbon concentrations of *Phascolosoma agassizii*, *Macoma inquinata*, and *Protothaca staminea* exposed to oil-contaminated sediment for 40 days in the field.

Species	Treatment	Hydrocarbon concentrations (µg/g wet weight)					
		C <sub>12</sub> -C <sub>29</sub>	methylnaph	dimethyl naph	trimethylnaph	triaromatics	total aromatics
<i>P. agassizii</i>	control	<0.10	<0.01	<0.01	<0.01	<0.10	<0.15
	exposed	1.90	0.23	0.60	0.95	2.25	4.03
	exposed	0.73	0.01	0.15	0*44	0.77	1.36
<i>M. inquinata</i>	control	<0.10	<0.01	<0.01	<0.01	<0.10	<0.15
	exposed	0.69	0.06	0.89	0.90	1.90	3.75
<i>P. staminea</i>	exposed	<0.10	<0.01	<0.01	<0.01	<0.10	<0.15

which feed on oil-contaminated sediment or detritus are more likely to accumulate hydrocarbons than filter-feeders. Total **naphthalenes** and **total triaromatics** were accumulated to essentially equivalent concentrations in both *P. agassizii* and *M. inquinata*. Relative concentrations of **n-alkanes** were higher in *P. agassizii* than in *M. inquinata*. The significance of this elevation in *P. agassizii* is not presently known, although it may reflect differences associated with ingestion or differential absorption across membranes or **epithelia** in the two species. It should be noted that the **n-alkane** concentrations which we detected in the two species are considerably lower than the 622  $\mu\text{g/g}$  total hydrocarbons (measured by IR) initially present in exposure sediment. Thus, the levels present in test organisms did not indicate **biomagnification**. Mortalities did not occur at the 40-day sampling interval.

These results may have particular relevance to studies concerning environmental monitoring as well as those directly concerned with **bioavailability** of hydrocarbons from sediment. Selection of test species which are representative of a single feeding type, such as filter-feeding, may overlook uptake from contaminated sources not available to those species. When uptake of compounds from sediment is considered to be a cause of tissue contamination, those species that ingest sediment or detritus would be desirable organisms to monitor. Our preliminary results indicate that species which coexist in similar habitats with different feeding modes can exhibit different uptake of petroleum hydrocarbons from sediment.



UPTAKE OF  $^{14}\text{C}$ -LABELED AROMATIC HYDROCARBONS  
BY *MACOMA INQUINATA*  
IN SHORT-TERM EXPERIMENTS

Studies by Shaw *et al.* (1976), Rossi (1977), and Anderson *et al.* (1977) suggest that detritus-feeding organisms take up little or no hydrocarbons from oil-contaminated sediment. Additional work is required on this subject, and, with the exception of Rossi (1977) and Anderson *et al.* (1977) who measured naphthalenes levels, individual hydrocarbons have not been examined.

Our initial efforts consisted of short-term (1 week) experiments to survey the relative uptake of various aromatic hydrocarbons from oil-contaminated sediments. The objective was to screen several compounds in an attempt to identify those which may have greater significance with respect to bioavailability from marine sediments. We selected *Macoma inquinata* as a test species, since preliminary observations indicated that this clam is an active detritus-feeder. The test compounds were 2-methylnaphthalene, phenanthrene, dimethylbenzanthracene, and benzo(a)-pyrene.

Clams were collected from intertidal regions of Sequim Bay, Washington, and held at the Marine Research Laboratory of Battelle-Northwest, Sequim, Washington. Holding tanks contained raw, flowing seawater of about 10°C and 30 ‰ and sediment obtained from the vicinity of the clams' natural habitat.

Detrital material which settles out of our flowing seawater system was collected and filtered onto No. 42 Whatman filter paper. Fifteen grams were weighed and suspended in approximately 30 ml seawater. Ten  $\mu\text{Ci}$  of the appropriate  $^{14}\text{C}$ -labeled hydrocarbon and 0.033 ml Prudhoe Bay Crude oil dissolved together in 1 ml ethyl ether were added to the suspended detritus, mixed thoroughly by shaking, then filtered onto No. 42 Whatman filter paper.

The contaminated detritus was used in exposures. Stock solutions of  $^{14}\text{C}$ -hydrocarbons were tested for radioisotope purity by thin-layer chromatography and auto-radiography. Measurements by infrared **spectrophotometry** (IR) indicated approximately 2,000  $\mu\text{g/g}$  total hydrocarbons in the detritus.

Since oil-contaminated sediments can release hydrocarbons to the surrounding water, it was necessary to consider the possibility of uptake of **solubilized**, as well as sediment-bound, hydrocarbons. Therefore, some clams were placed on the bottom of exposure aquaria containing the contaminated detritus, while others were placed in a nylon-mesh (**Nitex**) basket suspended in the water **column** above the detritus. The first group fed directly on the detritus and the latter served as a control for uptake from the water. Seven-day exposures were conducted in all-glass aquaria containing detritus and 3  $\ell$  of  $0.45\mu$  filtered seawater. At the end of exposure, some individuals from the bottom and suspended basket were removed for immediate extraction, **while** the remainder were transferred to clean seawater for a 24-h gut purging period. Rossi (1977) has demonstrated in the **polychaete** *Neanthes arenaceodentata*, a deposit-feeder, that hydrocarbons associated with gut contents can contribute to an apparent uptake. Since **Hylleberg** and **Gallucci** (1975) reported that turnover of ingested material by *Macoma nasuta* required 3 to 9 h, we considered that 24 h would be an adequate deputation period **for** *M. inquinata*.

Actual uptake from sediment, i.e., the amount of hydrocarbon ingested and present in clam tissue at the end of the exposure period, can be **calculated** as follows:

$$\text{Actual uptake} = \text{Concentration in clams on bottom} - \text{concentration due to seawater uptake} - \text{concentration in gut contents} + \text{concentration lost from tissue during gut purging.}$$

If uptake **is** primarily due to absorption of **solubilized** hydrocarbons, then the **value** for actual uptake would be essentially zero or negative.

Seawater samples were taken prior to the addition of clams and at 1, **2**, 4 and 7 days. Detritus was sampled initially and at 7 days. All samples were analyzed by liquid scintillation spectrometry and corrected for quench.

Detritus was extracted in hexane or by the method of Warner (1976). Tissue was extracted by the method of Warner (1976).

Concentrations of individual aromatic hydrocarbons in the experimental aquaria were not stable during the course of exposure and decreased as indicated in Tables 2 to 5 and summarized in Table 6. Half-times increased from 1.2 to 5.2 days (Table 6) as the aromatic ring numbers increased from 2 in 2-methylnaphthalene to 5 in benzo(a)pyrene. The percent of radioactivity initially released from the detritus to seawater decreased as the size of the molecules increased (Table 6). These results are consistent with observations that solubilities of hydrocarbons decrease with increased molecular weight and aromatic ring numbers.

Data on the uptake of the four individual hydrocarbons are presented in Tables 2 to 5 and summarized in Table 7. There was no measurable uptake of 2-methylnaphthalene via ingestion of sediment. Concentrations in clams could be accounted for by uptake of solubilized methyl naphthalenes. With phenanthrene, dimethyl benzanthracene, and benzo(a)pyrene, a fraction of the total uptake could be accounted for as uptake and assimilation from contaminated sediments. The values for the three compounds cannot be compared directly due to the differences in initial exposure concentrations. However, comparison of the values with other parameters provided some interesting relationships. With the exception of 2-methylnaphthalene, concentrations of the hydrocarbons taken up from sediment were reasonably similar (within one order of magnitude) to concentrations taken up from seawater. Thus, the contribution of each source to the tissue concentration was approximately the same. Magnification factors, however, indicated that hydrocarbons in seawater were more readily available than those in sediment. Values for sediment (= actual uptake/geometric mean concentration in sediment) ranged from 0.039 to 0.057, while those for seawater (= uptake from seawater/geometric mean concentration in seawater) ranged from 3.2 to 420; the latter values represented increases of 2 to 4 orders of magnitude. When compared to the former, magnification factors for uptake from seawater also suggested a correlation between uptake and molecular weight or number of aromatic rings since larger molecular-weight compounds were magnified to a greater extent. Sediment magnification factors did not reflect such a trend.

TABLE 2. Concentration of 2-methyl naphthalene in clams, detritus and seawater.

Sample	C o n c e n t r a t i o n	
Clams <sup>1</sup>	dpm/g	ppm 2-methylnaphthalene <sup>2</sup>
bottom	4,575.8 ± 1,944.8	0.052 ± 0.022
bottom depurated	6,392.7 ± 756.5	0.072 ± 0.009
suspended	4,282.3 *1,492.8	0.048 ± 0.017
suspended depurated	3,020.9 ± 1,198.5	0.034 ± 0.013
Detritus		
initial	2,074,882.0	23.30
7 days	33,201.5	0.37
Seawater		
initial	3,502.0	0.039
1 day	3,643.0	0.041
2 days	2,208.0	0.025
4 days	1,254.9	0.014
7 days	627.9	0.007

<sup>1</sup>Clams were exposed directly to detritus or suspended in the water column above the detritus. Depurated animals were transferred to clean seawater for 24 h.

<sup>2</sup>Concentration of 2-methyl naphthalene was calculated using the specific activity of stock <sup>14</sup>C-2-methylnaphthalene and a value of 2.48 mg/g 2-methylnaphthalene in Prudhoe Bay Crude oil. The latter value was kindly provided by R. M. Bean and R. G. Riley of the Environmental Chemistry Section, Battelle-Northwest, Richland, WA.

TABLE 3. Concentration of phenanthrene in clams, detritus and seawater.

Sample	Concentration	
<b>Clams<sup>1</sup></b>	<b>dpm/g</b>	<b>µg/g phenanthrene<sup>2</sup></b>
bottom	18,673.8 ± 11,839.0	0.133 ± 0.084
bottom depurated	<b>24,173.5 ± 12,127.9</b>	0.173 ± 0.087
suspended	5,303.7 ± 2,524.9	<b>0.038 ± 0.018</b>
suspended depurated	3,263.8 ± 960.8	0.023 ± 0.007
<b>Detritus</b>		
initial	<b>832,413.5 ± 17,618.2</b>	<b>5.973 ± 0.126</b>
7 days	68,284.7 ± 3,970.9	<b>0.490 ± 0.028</b>
<b>Seawater</b>		
initial	1,010.7	0.007
1 day	1,386.6	0.010
2 days	1,028.2	0.007
4 days	793.4	0.006
7 days	<b>516.7</b>	0.004

<sup>1</sup>**Clams** were exposed directly to detritus or suspended in the water column above the detritus. Depurated animals were transferred to clean seawater for 24 h.

<sup>2</sup>**Concentrations** of phenanthrene were calculated using 0.6 µg phenanthrene/g Prudhoe Bay Crude oil and the specific activity of stock <sup>14</sup>C-phenanthrene.

TABLE 4. Concentration of dimethyl benzanthracene in clams, detritus and seawater.

Sample	C o n c e n t r a t i o n	
<b>Clams<sup>1</sup></b>	<b>dpm/g</b>	<b>µg/g dimethyl-benzanthracene<sup>2</sup></b>
bottom	109,250.4 ± 573180.1	<b>1.181 ± 0.619</b>
bottom depurated	86,903.3 ± 7,421.2	0.939 ± 0.080
suspended	79,160.2 ± 9,845.4	0.856 ± 0.106
suspended depurated	59,389.8 ± 3,960.6	0.642 ± 0.043
<b>Detritus</b>		
initial	1,189,084.5	12.850
7 days	428,748.9	4.635
<b>Seawater</b>		
initial	970.0	0.010
1 day	255.0	0.002
2 days	272.0	0.003
4 days	360.4	0.004
7 days	58.7	0.001

<sup>1</sup>Clams were exposed directly to detritus or suspended in the water column above the detritus. **Depurated** animals were transferred to clean seawater for 24 h.

<sup>2</sup>Not corrected for amount originally present in Prudhoe Bay Crude oil since this value is not available.

TABLE 5. Concentration of benzo(a)pyrene in clams, detritus and seawater.

Sample	C o n c e n t r a t i o n	
<b>Clams<sup>1</sup></b>	<b>dpm/g</b>	<b>µg/g benzo[a] pyrene<sup>2</sup></b>
bottom	99,956.7 ± 22,850.9	0.222 ± 0.051
bottom depurated	40,771.1 ± 19,290.5	0.097 ± 0.043
suspended	16,451.6 ± 3,965.6	<b>0.037 ± 0.008</b>
suspended <b>depurated</b>	14,162.8* 5,427.3	0.032 ± 0.012
 Detritus		
initial	733,090	<b>1.63</b>
7 days	285,617	0.64
 Seawater		
initial	416.1	9.0X10 <sup>-4</sup>
<b>1</b> day	25.4	<b>5.6x10<sup>-5</sup></b>
2 days	20.7	4.6x10 <sup>-5</sup>
4 days	23.5	5.2x10 <sup>-5</sup>
7 days	19.1	4.3X10 <sup>-5</sup>

<sup>1</sup>Clams were exposed directly to detritus or suspended in the water column above the detritus. **Depurated** animals were transferred to clean seawater for 24 h

<sup>2</sup>Not corrected for amount originally present in Prudhoe Bay Crude oil since this **value** is not available.

TABLE 6. Half-times ( $t_{1/2}$ ) for specific hydrocarbons in oil-contaminated detritus, and percent of the total amount of hydrocarbon initially released from detritus to seawater.<sup>1</sup>

Compound	$t_{1/2}$ (days)	Percent of total hydrocarbons initially lost from sediment to seawater
2-methyl naphthal ene	1.2	33.7
phenanthrene	1.9	24.3
7,12-dimethylbenz[a]anthracene	4 . 7	16.3
benzo[a]pyrene	5.2	11.4

<sup>1</sup>Values calculated from levels of hydrocarbons in short-term uptake experiments (see tables 2 to 5),

TABLE 7. Summary of uptake of 2-methyl naphthalene, phenanthrene, dimethyl-benzanthracene, and benzo(a)pyrene from detritus and seawater.

Parameter	2-methyl-naphthalene	phenanthrene	dimethyl-benzanthracene	benzo a pyrene
Actual uptake from sediment <sup>1</sup> (µg/g)	0	0.096	0.297	0.059
Uptake from seawater (µg/g)	0.048	0.038	0.856	0.037
Sediment magnification factor <sup>2</sup>	0	0.056	0.039	0.057
Seawater magnification factor <sup>3</sup>	3.2	5.89	295	420

<sup>1</sup>Calculated as on page

<sup>2</sup>Sediment magnification factor = actual uptake/geometric mean concentration in sediment.

<sup>3</sup>Seawater magnification factor = uptake from seawater/geometric mean concentration in seawater.

There was no **biomagnification** of sediment hydrocarbons after 1 week exposure. The relative affinities of the individual hydrocarbons for **detrital** particles, tissue, and seawater undoubtedly played an important role in the partitioning into the three compartments.

Our results with 2-methyl **naphthalene** support the findings of Rossi (1977) that **naphthalenes** adsorbed to detritus are not available for uptake by detritus feeders; concentrations of **2-methylnaphthalene** by the clams in our study could be totally accounted for by uptake of **solubilized** molecules. Anderson *et al.* (1977) reported a low **level** of accumulation of **naphthalenes** in *Phascolosoma agassizii* exposed to oil-contaminated sand. The other compounds which we examined were taken up by the clams directly from detritus, but at a slow rate. **No** magnification could be demonstrated after 1 week exposure. Longer exposure periods are required for proper evaluation of this mode of uptake. Uptake of **solubilized** molecules also contributed a large portion to the tissue hydrocarbon burden. Although concentrations in seawater were extremely low compared to sediment concentrations, uptake from seawater was similar to that from the sediment. Magnification factors for seawater uptake were approximately 2 to 4 orders of magnitude greater than those for sediment uptake.

UPTAKE OF  $^{14}\text{C}$ -BENZO(a)PYRENE  
BY *MACOMA INQUINATA*  
IN A LONG-TERM EXPERIMENT

A portion of the uptake of  $^{14}\text{C}$ -benzo(a)pyrene from contaminated sediment by *Macoma inquinata* in our relatively short-term experiment could be ascribed to direct uptake from sediment and the remainder attributed to uptake of compounds released from detritus to the surrounding seawater. Uptake and release of benzo(a)pyrene from seawater has been reported for other marine bivalves (Lee *et al.*, 1972; Neff and Anderson, 1975; Dunn and Stich, 1976). Magnification factors of approximately 200 after 24 h exposure were similar to values reported earlier in this report for *M. inquinata*.

Benzo(a)pyrene concentrations in crude oils are about 1 to 2  $\mu\text{g/g}$  (Pancirov and Brown, 1975). About 6 tons of benzo(a)pyrene from oil, representing 0.1% of the benzo(a)pyrene from all sources, enters the marine environment annually (Suess, 1976). Although the contribution of benzo(a)pyrene in oil to the total environmental load is relatively small, its action as a potent carcinogen is justification for continued emphasis on this compound. Therefore, we examined long-term uptake of benzo(a)pyrene from sediment by *Macoma inquinata*. Since short-term experiments, already described, indicated a low level of accumulation of benzo(a)pyrene by *M. inquinata*, it was necessary to determine if prolonged exposure would also produce similar results.

Clams were collected in the intertidal region of Sequim Bay and held in the laboratory in flowing seawater of approximately 10°C and 30 ‰. Exposures were conducted in compartmentalized sediment trays already described. Each compartment was filled with 3 kg clean sand and placed in holding tanks with flowing seawater and a simulated diurnal tidal flux. Cement blocks held the trays at a level that prevented high tide from overflowing the upper edges of the sediment trays. Low tide completely drained

seawater from the trays through fiberglass mesh bottoms. Therefore, the only water flux in the exposure trays occurred through the tray bottoms as the trays drained and filled. Twenty clams were placed in each compartment. Six exposure and one control trays were prepared.

Contaminated detritus was prepared as described for short-term experiments using **<sup>14</sup>C-benzo(a)pyrene**. At high tide, approximately 25 g of suspended detritus was added to each compartment and allowed to settle on the surface of the sand containing clams. Clams and sediment were sampled at 3, 7, 14, 28, and 42 days of exposure. Each sampling period entailed removal of all clams and one sediment core from a compartment. Half the clams and the sediment core were extracted and analyzed immediately. The remaining clams were transferred to clean seawater in 24 h to allow purging of gut contents, then analyzed.

Clam tissue and sediment were extracted in **diethyl ether** by the method of Warner (1976), and an **aliquot** of the ether was counted for **<sup>14</sup>C-activity** by liquid scintillation **spectrometry**. All counts were corrected for background and quench. Sediment cores were mixed and divided into three portions prior to extraction. Sediments were **also** analyzed for total petroleum hydrocarbons by infrared spectrophotometry.

The initial concentration of **benzo(a)pyrene** in detritus was  $1.70 \times 10^6$  dpm/g, or  $3.79 \mu\text{g/g}$ . **During** the course of exposure, the detritus which had settled onto the surface of the sand penetrated into interstitial spaces as a result of the tidal fluxes. Since it was impossible to separate detritus from sand at sampling intervals after day 3, counts for core samples were used as a measure of **benzo(a)pyrene** content. For purposes of comparison, initial counts for detritus were corrected **to** account for the total sediment load (= detritus + sand), assuming uniform distribution of the detritus in sand. These values could then be directly compared to **values** for core samples.

Concentrations of **benzo(a)pyrene** and total petroleum hydrocarbons in sediment during the exposure period are presented in Table 8. Values were low and decreased over time with respective half-times of 17.33 days for **benzo(a)pyrene** and 23.1 days for **total** hydrocarbons. Analyses of random cores for vertical distribution of **benzo(a)pyrene** in sediment indicated

TABLE 8. Concentration of benzo(a)pyrene and total petroleum hydrocarbons in sediment during exposure to *Macoma inquinata*.

Time	Benzo(a)pyrene <sup>1</sup>		Total hydrocarbons
days	dpm/g	µg/g	µg/g
<b>initial<sup>3</sup></b>	12,611 ± 938	0.0282 ± 0.0021	20.2 ± 0.9
33	5,733 ± 789	0.0129 ± 0.0018	10.4 ± 1.0
7	1,873 ± 263	0.0042 ± 0.0006	15.8 ± 3.7
14	1,064 ± 105	0.0024 ± 0.0002	5.6 ± 1.1
28	1,808 ± 232	0.0041 ± 0.0005	4.2 ± 0.4
42	2,521 ± 835	0.0057 ± 0.0019	6.3 ± 1.0

<sup>1</sup>Analyzed by liquid scintillation spectrometry

<sup>2</sup>Analyzed by infrared spectrophotometry

<sup>3</sup>Calculated from concentrations in detritus and based on uniform distribution in total sediment

that concentrations were higher in the upper 3 cm of a 10-cm core by an approximate factor of 2. *Macoma inquinata* feeds at the sediment surface or at the upper portion of the sediment column; therefore, concentrations of **benzo(a)pyrene** in exposure sediment were highest in the active feeding region of this clam.

Uptake of <sup>14</sup>C-**benzo(a)pyrene** by *Macoma inquinata* is described in Figure 1. The rate of uptake was relatively **linear** during the exposure period and was on the order of 0.020 µg benzo(a)pyrene/g/day. There was no indication of an asymptote in the uptake curve. Therefore, continued uptake can be expected with extended exposure. At the end of the 42-day exposure, the mean tissue concentration of 1.04 µg/g was approximately 0.3 times the concentration in the initial detritus sample. Compared to core samples which represent total sediment concentrations, tissue magnification **levels** (= tissue concentration/sediment concentration) increased from 14.5 to 173.3 from day 3 to day 42.

There were no differences in **benzo(a)pyrene** concentrations in clams analyzed directly after removal from sediment or those analyzed after a 24-h deputation period. Our results indicate that there was considerable uptake of **benzo(a)pyrene** from contaminated detritus. Furthermore, there was no indication of an equilibrium in the tissue concentration with time. Continued exposure would, therefore, have resulted in continued uptake. Accumulation of <sup>14</sup>C-**benzo(a)pyrene** by clams occurred despite decreasing levels in sediment. Although all the **benzo(a)pyrene** in our exposure system was originally associated with contaminated detritus particles, uptake by clams may have occurred via mechanisms other than direct ingestion and assimilation of **benzo(a)pyrene** from sediment. We have already shown in short-term experiments, described earlier, that approximately 40% of **benzo(a)pyrene** taken up by clams exposed to contaminated detritus can be attributed to uptake of compounds released from detritus to surrounding seawater. Concentrations of **benzo(a)pyrene** in seawater in the present experiment were similar to those reported in the short-term experiments. Therefore, a similar proportion of the total uptake can probably be attributed to compounds released from sediment to seawater in the present experiment.

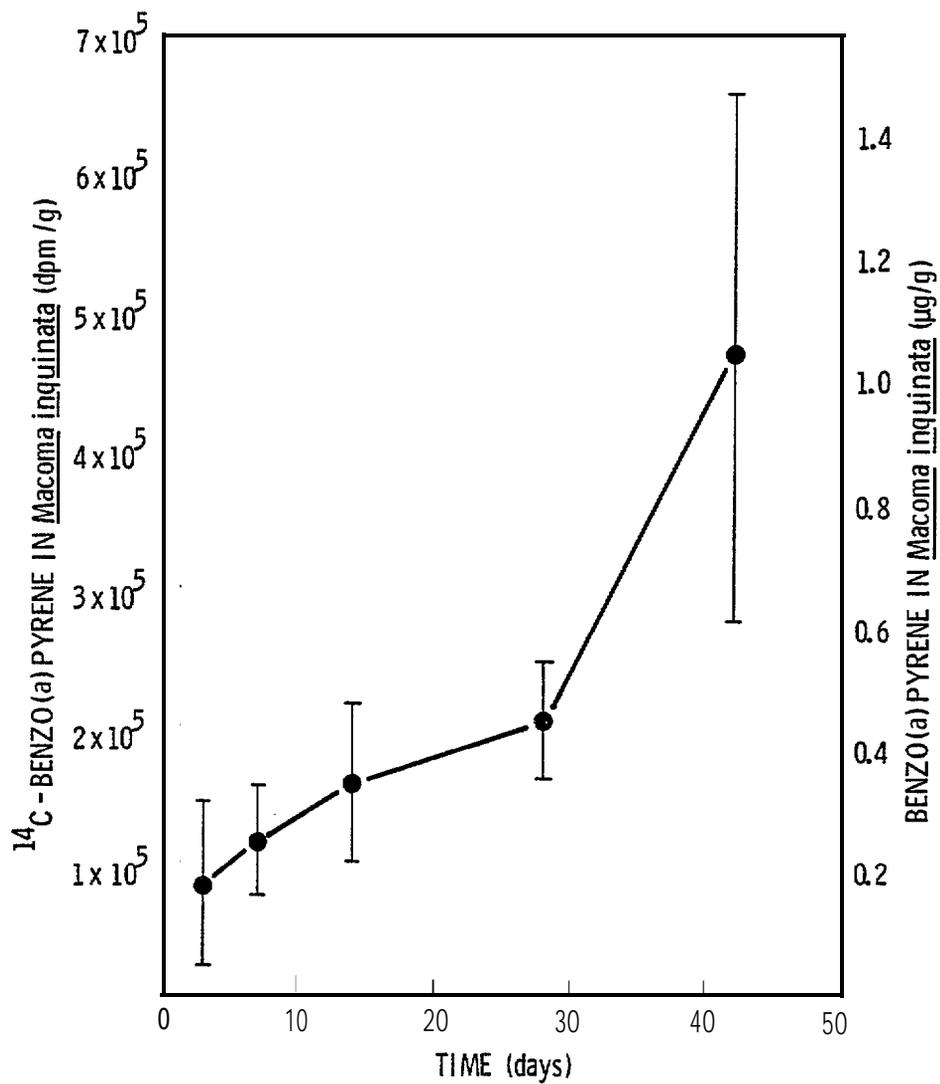


Fig. 1. Concentrations of  $^{14}\text{C}$ -benzo(a)pyrene in *Macoma inquinata* during exposure to sediment contaminated with  $^{14}\text{C}$ -benzo(a)pyrene and Prudhoe Bay Crude oil. Vertical bars indicate 1 standard deviation. (See Table 8 for hydrocarbon concentrations in exposure sediment. )

Although we are aware that  $^{14}\text{C}$  measured in our study may be associated with metabolized **benzo(a)pyrene** and not the parent compound, we did not attempt any metabolize separations at this time. Lee *et al.* (1972) and Dunn and Stich (1976), however, have provided evidence that marine molluscs are not capable of metabolizing benzo(a)pyrene. Metabolism of the compounds by sediment microbes and subsequent uptake of **benzo(a)pyrene** metabolizes by clams is still a possibility and should be tested in future experiments.

CONDITION INDEX OF *MACOMA INQUINATA*  
EXPOSED TO OIL-CONTAMINATED SEDIMENT  
IN THE FIELD AND LABORATORY

It is becoming increasingly evident that short-term acute toxicity bioassays are of limited use in the evaluation of petroleum pollution of the marine environment (Wilson, 1975). Chronic long-term exposures and examination of responses to sublethal concentrations of petroleum hydrocarbons are more useful **experimental** approaches to the study of this problem. Anderson (1977) reviewed the current literature relevant to sublethal effects of petroleum hydrocarbons on marine animals. With most of the biological parameters which have been examined, e.g., oxygen consumption, **osmoregulation**, breathing rate, coughing rate, the responses have varied depending on test species, oil concentration, or duration of exposure; and exposures have usually been relatively short, a few days or hours. Neff *et al.* (1976b) have also pointed out the variability of responses observed in numerous studies conducted in their laboratory. An exception to this trend appears to be inhibition of growth rate in marine animals exposed to petroleum hydrocarbons, an effect observed with several crustacean species (Neff *et al.*, 1976b; Anderson, 1977) and a **polychaetous annelid** (Rossi, 1976).

Energy balance is an integrated physiological parameter which is sensitive to stress in **bivalved molluscs** (Bayne, 1975; Bayne *et al.*, 1976). Bayne has shown that "scope for growth" (calculated from the basic energy equation  $\Delta W/\Delta t = Ab - R$ , where  $W$  = body weight,  $t$  = time,  $Ab$  = assimilation, and  $R$  = respiratory heat loss; all expressed as calories) in mussels *Mytilus edulis* is affected by temperature, food availability, salinity, and oxygen concentration. Using a similar approach, Gilfillan (1975) and Gilfillan *et al.* (1976) obtained a rough approximation of carbon flux in the clam *Mya arenaria*, and suggested **that** this process is sensitive to petroleum hydrocarbon exposure. A sustained alteration in energy balance should result in changes in biomass and, thus, a change in a parameter such as condition

index. Fluctuations in the condition of bivalved molluscs are considered normal (Trevallion, 1971; Ansell and Sivadas, 1973) and can be correlated to seasonal changes in nutrient storage and utilization. Periods of stress can lead to reductions in condition (Trevallion, 1971). Changes in condition index have been used as measures of relative health, nutritional state, and growth. An advantage in measurements such as scope for growth, carbon flux, and condition index when compared to incremental estimations of growth is that a net decrease in calories (or carbon or biomass), i.e., "negative growth," can be monitored. This aspect is especially useful when considering adult organisms which are approaching the asymptote of their growth curve.

Using the detritivorous clam *Macoma inquinata*, we tested the hypothesis that condition index is a sensitive indicator of stress from petroleum hydrocarbon exposure. In a coordinated laboratory and field experiment, we exposed *M. inquinata* to sediment contaminated with Prudhoe Bay Crude oil. Exposure lasted 2 months, and, at the end of that period, we determined condition index and hydrocarbon content of the clams.

Clams were collected from the intertidal region of Sequim Bay and held at the Battelle Marine Research Laboratory. Holding tanks contained raw, flowing seawater of approximately 10°C and 30 ‰. Clams were used in the experiment within 2 days of collection.

Sand from the upper intertidal region of Sequim Bay was passed through a 6-mm sieve and two portions of 30 kg each were weighed for use as control and exposure sediment. The latter was prepared as described by Anderson *et al.* (1977) using 70 ml Prudhoe Bay Crude oil and 30 kg sand. Briefly, a seawater emulsion of the oil was mixed with the sand in a fiberglass-lined cement mixer for 1 h, a duration previously determined to produce a homogeneous oil-sediment mixture.

Exposures were conducted in fiberglass trays divided into three equal compartments with a nylon mesh bottom. Roughly 5 kg sediment and 10 clams were placed in each compartment, i.e., 30 clams per tray. One tray was partially buried in the intertidal zone of Sequim Bay while another was submerged in a holding tank in the laboratory. Cement blocks were used to prop

the tray off the bottom of the tanks. The holding tanks contained flowing seawater with a simulated tidal flux which exposed the sediment trays when there was a negative tide in the field. The mesh on the bottoms of the trays allowed seawater to drain through the sediment at low tide. Control trays were prepared for laboratory and field exposures. Exposure began on November 22, 1976, and lasted 55 days.

Condition index was determined as

$$\frac{\text{ash-free dry weight}}{\text{length}^3} \times 1000 \text{ (de Wilde, 1975)}$$

Values varied from 6 to 14. High values indicate good condition, while low values poor condition.

Sediments were analyzed for total petroleum hydrocarbons at the beginning and end of exposure by infrared spectrophotometry. Clam tissues were analyzed for aliphatic and di- and tri-aromatic hydrocarbons by the method of Warner (1976). Clams were sampled at the end of exposure.

The behavior of clams in the laboratory indicated stress in oil-exposed individuals. During the exposure, 5 clams in the contaminated sediment came to the surface, a response previously reported for stressed *Macoma balthica* (de Wilde, 1975; Taylor *et al.*, 1977). Five clams in the contaminated sediment were also found dead and buried. In control sediment, no clams surfaced, and only one died during the experiment.

Field-exposed clams were not examined until the end of the experiment. At that time, it was evident that there was considerable scouring due to wave action at the experimental site. Some sediment in the trays had been washed away and partially replaced by new material. Dead or surfaced clams were not present in control or oil-exposed trays. However, pieces of broken shell from *M. inquinata* were present in the trays. Seven control and thirteen exposed clams were recovered.

Values for sediment hydrocarbon concentrations are summarized in Table 9. At the beginning of exposure, contaminated sediment contained  $1232.9 \pm 142 \mu\text{g/g}$  total petroleum hydrocarbons. After 55 days, hydrocarbon concentrations for sediment in the laboratory decreased to  $616.1 \pm 147 \mu\text{g/g}$ , 50% of the initial

TABLE 9. Concentrations and half-times for total petroleum hydrocarbons for sediment in condition experiment.

Treatment	Total hydrocarbons ( $\mu\text{g/g}$ )	$t_{1/2}$ (days)
Lab		
Control - initial	n.d. <sup>1</sup>	--
55 days	n.d.	--
Exposed - initial	1232.9 $\pm$ 142.0 (S. D.)	54.9
55 days	616.1 $\pm$ 147.0	
Field		
Control - initial	n.d.	--
55 days	n.d.	--
Exposed - initial	1232.9 $\pm$ 142.	14.4
55 days	87.8 $\pm$ 47.3	

<sup>1</sup>n.d. = not detectable

value, as opposed to  $87.8 \pm 47.3 \mu\text{g/g}$  in the field, 7% of the initial value. Respective half-times for the decreases in laboratory and field exposures were 54.9 days and 14.4 days. Control sediment contained undetectable levels, both initially and at 55 days.

Measurements of condition index indicated that differences caused by oil exposure were not statistically significant, although mean values for exposed clams were lower than for controls in both the laboratory and field (Table 10). Large individual variation obscured any possible effects due to oil exposure. On the other hand, condition index of animals transferred to the field were significantly lower than those held in the lab ( $p < 0.05$ ). It appears that conditions in the field were harsher than those created by oil exposure. Similar conclusions can be made by comparing total numbers of dead and unrecovered clams in laboratory and field situations. Shifting sand as a result of wave action can prevent settling and feeding and, thus, lead to reduced condition index (Trevallion, 1971). Such a process could easily explain our observations with *Macoma inquinata*. However, stress due to oil was evident in the laboratory experiment since 33% of exposed clams, as opposed to 3% of controls, either died or surfaced during the exposure.

Hydrocarbon analyses of clam tissue indicated a high level of contamination of exposed clams, particularly of those held in the laboratory (Table 11). Di- and tri-aromatic compounds accounted for most of the hydrocarbons in tissue.  $\text{C}_{12}\text{-C}_{29}$  n-alkanes were present at relatively low concentrations. The relatively high values in laboratory-exposed clams, compared to those in the field, were reflective of the loss rates of hydrocarbons from their respective exposure sediments. Sediment hydrocarbons decreased at a slower rate in the lab than in the field (Table 9). Compared to sediment concentrations, however, levels in clam tissue were relatively low.

Our inability to demonstrate a statistically significant reduction in condition index as a result of oil exposure should not yet be considered a lack of effect. The large variability among individuals (coefficient of variation ~20%) obscured the decrease in mean values of ~10% in both laboratory and field exposures. In order to demonstrate significant differences under such conditions, a sample size  $n$  of at least 65 per treatment is

TABLE 10. Condition index of *Macoma inquinata* exposed to oil-contaminated sediment in the laboratory and field for 55 days.

Treatment	Condition index <sup>1</sup>	Sample size
Lab		
control	11.5 ± 2.3 (S. D.)	20
exposed	10.3 ± 2.4	14
Field		
control	9.1 ± 2.2	7
exposed	8.1 ± 1.8	8

$$^1\text{condition index} = \frac{\text{ash-free dry weight}}{\text{Length}^3} \times 1,000$$

TABLE 11. Petroleum hydrocarbon concentrations of *Macoma inquinata* exposed to oil-contaminated sediment in the laboratory or field for 55 days.

Treatment	Hydrocarbon concentrations (µg/g wet weight)					
	C <sub>12</sub> -C <sub>28</sub>	methylnaph	dimethylnaph	trimethylnaph	triaromatics	total aromatics
Lab						
Control <sup>1</sup>	0.030	<0.001	<0.005	0.020	0.160	0.210
Exposed <sup>2</sup>	0.140	1.153	5.209	13.252	23.017	42.631
	0.422	<0.010	2.387	6.938	12.938	22.270
Field						
Control <sup>1</sup>	0.020	<0.005	<0.005	<(.)010	0.015	0.035
Exposed <sup>3</sup>	0.140	<0.050	0.022	0.016	0.523	0.561
	0.058	<0.010	0.031	0.057	0.401	0.489

<sup>1</sup>Total hydrocarbon concentrations in sediment measured by IR spectrophotometry were below detection limits.

<sup>2</sup>Initial hydrocarbon concentrations in sediment averaged 1232.9 ± 142 µg/g (n = 6). Fifty-five day concentrations averaged 616.1 ± 147.2 µg/g (n = 3).

<sup>3</sup>Initial hydrocarbon concentrations in sediment averaged 1232.9 ± 142 µg/g (n = 6). Fifty-five day concentrations averaged 87.8 ± 47.3 µg/g (n = 3).

3

required (Sokal and Rohlf, 1969). Future experiments will incorporate larger n in order to separate treatment from random effects. Meaningful sublethal effects of oil on marine organisms are yet to be adequately demonstrated. The general approach taken by Gilfillan (1975) and Gilfillan *et al.* (1976) in measuring carbon flux in oil-exposed *Mya arenaria* appears to be the most fruitful to date. Condition index, a parameter closely coupled to carbon flux and scope for growth, may be a sensitive sublethal measure of oil toxicity. Additional study is required to determine if such is the case.

BIODAVAILABILITY OF TRACE ELEMENTS  
FROM OIL-CONTAMINATED SEDIMENT

Numerous trace elements including heavy metals occur in crude oil at low concentrations (Shah *et al.*, 1970a, 1970b; Hitchon *et al.*, 1975). Little is known about the dynamics of petroleum-derived heavy metals in the marine environment. Accumulation of specific heavy metals from sediment by benthic organisms has been described in numerous studies (Bryan and Hummerstone, 1971, 1973a, 1973b; Renfro, 1973; Hess *et al.*, 1975; Luoma and Jenne, 1975; Renfro and Benayoun, 1975). The results, however, may not be directly applicable to petroleum-sediment-organism interactions since organic coatings on the surface of sediment particles can inhibit metals uptake (Luoma and Jenne, 1975). The presence of petroleum may interfere with the "normal" uptake kinetics of heavy metals from sediment. The low concentrations of trace metals in crude oil (e.g., 24  $\mu\text{g/g}$  in Prudhoe Bay Crude oil) suggest that the contribution of those metals to the environment will be very low. For example, sediment contaminated with 2,000  $\mu\text{g/g}$  Prudhoe Bay Crude will contain approximately 0.048  $\mu\text{g}$  total trace metals contributed by the oil, assuming that all the metals in oil remained in the sediment. Compared to the normal levels of trace metals in marine sediments, that from oil would be negligible. Feder *et al.* (1976) were not able to detect any changes in sediment metals content as a result of contamination by Prudhoe Bay Crude oil. However, experimental examination of the possible effects of oil on the uptake of trace metals is lacking. Bioavailability of petroleum-derived trace metals from oil-contaminated sediment or the effect of petroleum hydrocarbons on routine uptake of trace metals have not been reported. We have been investigating the uptake of trace metals from sediment contaminated with Prudhoe Bay Crude oil in two species of deposit-feeders: *Phascolosoma agassizii* and *Macoma inquinata*. Our findings to date are presented in this report.

Sipunculids *Phascolosoma agassizii* and clams *Macoma inquinata* were collected from intertidal regions of Sequim Bay and held at the Battelle

Marine Research Laboratory. Holding tanks contained raw, flowing seawater of approximately 10°C and 30 ‰.

Experiments were of two general designs. First, individuals of *Phascolosoma agassizii* were exposed to oil-contaminated sediment (~2000 µg/g) prepared by mixing sand and oil as described in previous sections. Exposures were conducted in sediment trays immersed in holding tanks containing flowing seawater. Animals were sampled after 1, 7 and 14 days of exposure and 14 days deputation. Whole animals or tissue homogenates were dried and analyzed by x-ray fluorescence. Secondly, clams *Macoma inquinata* were exposed to oil-contaminated detritus as described previously for experiments on short-term uptake of aromatic hydrocarbons. Exposures lasted for 2 weeks. Fifteen g of oil-contaminated detritus (2000 µg/g) were supplied initially and on the seventh day of exposure. Clams were sampled at the end of exposure, allowed to deplete gut contents for 24 h, then dried. Tissue from 10 clams were pooled and analyzed by x-ray fluorescence.

A separate experiment was conducted to determine natural variability of trace metals in *Macoma inquinata*. Immediately following collection, 100 clams were placed in clean, flowing seawater for 5 days to allow deputation of gut contents. Following the deputation period, the clams were dried and analyzed for trace metals by x-ray fluorescence. Ten samples of 9 to 10 clams each were analyzed.

Two samples of Prudhoe Bay Crude oil were analyzed by neutron activation analysis. Concentrations of trace elements in Prudhoe Bay Crude oil are presented in Table 12. Only four compounds, vanadium, cobalt, zinc, and bromine, were present at levels above detection limits. The total trace elements concentration was approximately 24 µg/g.

Several difficulties arose in the experiment to assess uptake of trace metals by *Phascolosoma agassizii*. Primary among these was the fact that exposure sediment became anaerobic in the early stages of exposure, and exposed worms crawled onto the sediment surface. Therefore, worms may have been releasing to seawater rather than taking up metals from sediment during the exposure period. Such observations may account for the decreasing levels of compounds such as chromium, manganese, and nickel during exposure. Most

TABLE 12. Trace element concentrations in Prudhoe Bay Crude oil. Samples represent oil from two different barrels and were analyzed by neutron activation analysis.

Element	Concentration (µg/g)	
	Sample 1	Sample 2
Na	<0.06	0.097
<b>Mg</b>	<30	<33
Al	<0.5	<0.5
Cl	<1	<b>0.95</b>
K	<4	<1.4
Sc	<0.001	<0.007
V	20.9	18.0
<b>Cr</b>	<0.21	<b>&lt;0.15</b>
<b>Mn</b>	<b>&lt;0.04</b>	<0.02
Fe	<1.6	<1.7
Co	0.018	0.017
<b>Cu</b>	<5	<3
<b>Zn</b>	0.31	0.31
As	<b>&lt;0.03</b>	<0.01
Se	--	<b>&lt;0.3</b>
Br	5.73	2.75
Rb	<0.06	<0.08
In	<b>&lt;0.005</b>	<b>&lt;0.003</b>
<b>Sb</b>	<0.002	<0.002
<b>Cs</b>	<b>&lt;0.002</b>	<b>&lt;0.001</b>
Ba	<23	<8
La	<b>&lt;0.01</b>	<b>&lt;0.01</b>
Sm	<b>&lt;0.002</b>	<b>&lt;0.001</b>
Eu	<0.001	<b>&lt;0.001</b>
Tb	<0.007	<b>&lt;0.006</b>
Ta	<b>&lt;0.04</b>	--
Hg	<b>&lt;0.03</b>	<0.03
Th	<0.008	<0.006

were present at levels below those of the sediment, although a few such as nickel, copper, zinc, and manganese, were elevated compared to sediment concentrations. No discernible differences were observed between control and exposed groups. Values for aluminum, silicon, and titanium can be attributed to metals associated with sediment in the gut (Table 13).

Levels of trace metals in *Macoma inquinata* (Table 14) were generally similar to those of *Phascolosoma agassizii* with a few exceptions: concentrations of chromium, manganese, iron, and nickel were substantially lower in *M. inquinata* and occurred despite the fact that metals concentrations in the respective sediment of the two species showed considerable variation. Nickel, zinc, and selenium were magnified in clam tissue, compared to sediment levels. Oil exposure did not affect metals concentrations in *M. inquinata*. With the exception of titanium and lead, individual variability was relatively low for all metals (Table 15) (combined coefficient of variation =  $12.3 \pm 5.5$  So.). Titanium was most likely to be associated with sediment remaining in the gut and would therefore be more likely to exhibit a degree of inconsistency. Concentrations of lead approached detection limits of the technique. Only three samples possessed measurable quantities of lead. Only values for chromium and manganese in the previous experiment did not fall within the 95% confidence limits determined in this study.

Our results, to date, suggest that petroleum hydrocarbons have little effect on trace metals concentrations of *Phascolosoma agassizii* or *Macoma inquinata*. However, additional work is obviously required before more definitive statements can be made. The use of neutron-activated oil and sediment samples will aid in our future studies on this subject.

TABLE 13. Uptake of trace elements by *Phascolosoma agassizii* exposed to oil-contaminated sediment.

Treatment	Concentration ( $\mu\text{g/g}$ dry weight)									
	Al	Si	P	S	Cl	K	Ca	Ti	V	Cr
<b>Control</b>										
sediment	60,500	35,500	-	-	-	8,200	29,400	4,550	33	690
sediment	60,700	<b>37,200</b>	-	-	-	8,500	30,100	4,810	61	703
animals from field	7,600	3,150	1,905	8,710	43,490	6,706	6,208	204	7.8	42
animals held in lab in sediment	5,070	2,716	1,606	9,953	38,650	7,600	2,344	242	6.0	<b>29</b>
animals held in lab 2 days out of sediment	5,800	1,310	395	10,500	49,700	6,820	3,480	153	<b>&lt;6</b>	45
<b>Exposed</b>										
1 day - whole animals	5,000	437	1,536	1,300	43,400	7,350	1,850	100	<b>&lt;5</b>	14
1 day - homogenate	3,400	1,300	790	9,490	32,034	6,230	2,326	145	<b>&lt;6</b>	142
1 day - homogenate	2,000	1,224	<b>1,680</b>	9,180	40,633	7,100	2,684	210	9.8	94
7 days - homogenate	19,450	1,584	2,408	1,140	46,190	9,174	3,986	128	6	47
14 days - homogenate	12,630	1,636	1,216	1,150	49,320	<b>9,478</b>	2,323	<b>83</b>	<b>&lt;4</b>	29
14 days + 14 days deputati on - homogenate	20,990	<b>1,673</b>	<b>&lt;727</b>	13,300	92,050	10,230	3,112	58	<b>&lt;4</b>	15
<b>Control</b>										
sediment	443	17,900	<b>&lt;30</b>	<b>&lt;2</b>	<b>&lt;3</b>	14	5.5	1.5	3.9	
sediment	442	17,700	<b>&lt;30</b>	<b>&lt;2</b>	<b>&lt;3</b>	12	2.0	0.8	3.5	
animals from field	1,120	4,647	20	18	132	<b>3.4</b>	6.4	9.4	837	
animals held in lab in sediment	452	4,256	24	9.2	109	8.0	5.9	12	<b>751</b>	
animals held in lab 2 days out of sediment	1,316	3,994	22	15	158	3.8	9.3	10	920	
<b>Exposed</b>										
1 day - whole animals	180	2,824	19	20	154	3.1	5.5	10.5	822	
1 day - homogenate	1,087	3,673	49	21	251	4.5	15.5	13.1	1,026	
1 day - homogenate	1,385	3,947	44	19	185	4.2	19	12	814	
7 days - homogenate	598	3,494	31	12	145	<b>&lt;.3</b>	9	12	712	
14 days - homogenate	273	2,629	13	12	80	<b>&lt;.5</b>	<b>&lt;1</b>	12	<b>696</b>	
14 days + 14 days deputati on - homogenate	123	<b>2,241</b>	9	13	112	<b>&lt;.5</b>	<b>&lt;1</b>	11	810	

TABLE 14. Uptake of trace elements by *Macoma inquinata* exposed to oil-contaminated detritus (~2000 µg/g total hydrocarbons).

Treatment	Concentration (µg/g dry weight)						
	Cl	K	Ca	Ti	V	Cr	Mn
<b>Control</b>							
Initial seal.	16,800	<b>11,900</b>	21,100	4,690	80	<b>145</b>	668
2-wk seal.	<b>16,400</b>	<b>11,600</b>	<b>20,900</b>	<b>4,610</b>	<b>109</b>	<b>101</b>	706
Initial tissue	52,900	3,000	?, 970	21	<4	<b>&lt;2</b>	<b>11</b>
2-wk tissue	55,200	3,900	1,590	29	<4	2.7	<b>12</b>
2-wk tissue	<b>51,100</b>	2,500	2,030	55	<4	5.6	19
<b>Exposed</b>							
Initial seal.	15,400	<b>11,100</b>	<b>19,600</b>	4,460	84	126	682
<b>2-wk seal.</b>	16,600	11,400	20,400	4,500	73	97	706
2-wk tissue	55,500	<b>13,200</b>	1,870	42	<4	5.5	14
	Fe	Ni	Cu	Zn	Se	Pb	As
<b>Control</b>							
Initial seal.	38,800	<b>&lt;36</b>	31	85	1.3	10	9.2
2-wk seal.	38,600	<32	34	92	7.4	12	13.1
Initial tissue	284	4.2	7.7	<b>199</b>	3.1	<1.2	11
2-wk tissue	370	3.5	9.1	210	3.1	<b>&lt;1.3</b>	<b>11</b>
2-wk tissue	516	4.9	10.3	202	2.7	<1.3	11
<b>Exposed</b>							
Initial seal.	37,400	<43	<b>31</b>	84	<b>1.5</b>	10	<b>10.1</b>
2-wk seal.	<b>38,100</b>	<33	35	90	1.3	13	10.1
2-wk tissue	535	3.3	7.5	163	2.9	2.1	8.2

TABLE 15. Analysis of trace elements in *Macoma inquinata* by x-ray fluorescence. Estimation of sample variability.

Element	Sample size <sup>1</sup>	Concentration ( $\mu\text{g/g}$ ) $\bar{x} \pm 2 \text{ S.E.}$
P	10	4,651 $\pm$ 686
s	10	15,374 $\pm$ 591
cl	10	53,859 $\pm$ 3,695
K	10	13,504 $\pm$ 245
<b>Ca</b>	<b>10</b>	2,003 $\pm$ 140
<b>Ti</b>	10	23.7 $\pm$ 9.5
v	3	3.58 $\pm$ 0.45
<b>Cr</b>	5	3.92 $\pm$ 0.60
Mn	10	9.136 $\pm$ 1.043
Fe	10	315.2 $\pm$ 37.3
co	4	2.497 $\pm$ 0.442
Ni	<b>10</b>	3.282 $\pm$ 0.391
<b>Cu</b>	<b>10</b>	<b>8.108</b> $\pm$ 0.374
<b>Zn</b>	10	195.2 $\pm$ 12.5
Ga	<b>10</b>	n.d. <sup>2</sup>
<b>Hg</b>	10	n.d.
Se	10	<b>3.177</b> $\pm$ 0.188
Pb	3	0.815 $\pm$ 0.680
As	<b>10</b>	10.319 $\pm$ 0.368
<b>Br</b>	10	262.5 $\pm$ <b>17.8</b>
Rb	10	n.d.
<b>Sr</b>	10	29.59 $\pm$ 2.46

<sup>1</sup>In sample size <10, the remaining samples (= 10-n) were below detection limits. Nine to ten clams comprised a single sample.

2nd. = not detectable.



## DISCUSSION AND CONCLUSIONS

The data presented in this report suggest that petroleum hydrocarbons impacted in marine sediments **are** available for uptake by certain types of **benthic** organisms at relatively low levels. Filter-feeding clams did not possess detectable levels of hydrocarbons when exposed up **to 40** days to oil-contaminated sediment. Simultaneously exposed deposit-feeding **sipunculids** and clams, however, possessed **levels** of approximately 10  $\mu\text{g/g}$  combined **aliphatic** and di-, **tri-aromatic** hydrocarbons. Exposure sediment had approximately 600  $\mu\text{g/g}$  total hydrocarbons.

Experiments with  $^{14}\text{C}$ -specific aromatic hydrocarbons and the **detritivore** *Macoma inquinata* also indicated a low level of uptake. Moreover, hydrocarbons released from sediment to surrounding seawater contributed significantly to tissue hydrocarbon levels. Ingestion of contaminated sediment was not the sole, or probably major, mode of uptake. The description "low **level** of uptake" should not be confused with insignificant uptake since long-term exposure to  $^{14}\text{C}$ -benzo(a)pyrene has indicated otherwise. Increases in tissue radioactivity were linear up to 42 days in *inquinata* exposed to  $^{14}\text{C}$ -benzo(a)pyrene with no indication of steady-state levels. Prolonged exposure of **benzo(a)pyrene** to the clams, which apparently do not possess metabolic pathways to degrade the compound (Lee *et al.*, 1972), can result in relatively high tissue levels, given a sufficient period of exposure. Furthermore, it should be pointed out that the contaminated sediment contained Prudhoe Bay Crude oil ( $\sim 2000$   $\mu\text{g/g}$  initially), as well as the  $^{14}\text{C}$ -aromatic hydrocarbon in each experiment. Therefore, a petroleum hydrocarbon "matrix" existed in the sediment which possibly affected the uptake of  $^{14}\text{C}$ -compound. Hydrocarbon-hydrocarbon interactions can be expected to play **an important role** in the **bioavailability** of specific hydrocarbons from **petroleum-contaminated** sediment. The nature of such interactions, however, is not known.

Efforts to correlate changes in condition in *Macoma inquinata* as an estimation of stress due to exposure to oil-contaminated sediment were not **wholly** successful. Individual variation obscured an apparent reduction in condition of oil-exposed clams.

Concentrations of trace metals in **sipunculids** or clams exposed to **oil**-contaminated sand or detritus were not different from **levels** determined for animals in clean sediment. It appears that uptake of trace metals is not affected by oil exposure. However, it is still premature for definitive statements. We plan additional experiments to test this hypothesis. Individual variation of trace metals in *Macoma inquinata* was relatively low (coefficient of variation 5-20%) with the exception of elements such as titanium which was abundant in sediment or lead which was present at levels approaching detection limits.

Experiments similar to those described in this report will be continued since they represent productive approaches to the study of **bioavailability** of sediment-impacted petroleum hydrocarbons and trace metals. Examination of the role of metabolism of hydrocarbons will be of high priority in future experiments, particularly in those which employ **<sup>14</sup>C-labeled** hydrocarbons. Degradation of the compounds in the sediment and test animals is a major consideration. Examination of condition index and other indices of stress will also be continued with reference to the petroleum-in-sediment problem. Experiments with the **bioavailability** of trace metals **will** incorporate exposure of organisms to neutron-activated sediment or Prudhoe Bay Crude oil. This approach will enhance enormously the detectable limits of metals in exposed animals and **also** allow us to measure flux of the metals.

**Disclaimer:** The brand names are used to assist the reader in evaluating the experiments, but do not imply endorsement by **Battelle** Laboratories.

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APPENDIX A

PAPERS SUBMITTED FOR PUBLICATION CONCERNING  
WORK CONDUCTED ON THE PRESENT CONTRACT

Roesijadi, G., D. L. Woodruff, J. W. Anderson. Bioavailability of naphthalenes from marine sediments artificially contaminated with Prudhoe Bay Crude oil. Submitted to *Environmental Pollution* 1977.