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INVESTIGATING THE IMPACT OF DRILLING MUD AND ITS MAJOR COMPONENTS ON BIVALVE SPECIES OF GEORGES BANK

A Progress Report

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I. INTRODUCTION

The objective of this program has been to measure the response of commercially important marine bivalve molluscs to low levels of drilling muds. Because these materials are composed of several major components whose proportions vary between wells and with depth for a single well, the approach of this study has been to test the major components individually and then to test a "representative" synthetic mud and finally a used drilling mud from an offshore platform. In all but one of these tests the target organism has been the sea scallop, Placopecten magellanicus.

Preliminary range finding experiments were run to determine the ability of sea scallops to tolerate short term exposure to relatively high levels of six components: bentonite, attapulgite, barite, chrome lignosulfonate (CLS), plant starch, and Al stearate. From the results of these tests, dosage levels for the 28-day tests of sub-lethal stress were determined.

The following biological and chemical indices have been evaluated as indicators of environmental stress:

- 1) Visual inspection of selected tissue
- 2) Filtration rates of test organisms
- 3) Density and size of mucus cells on the gills and mantle edges
- 4) Chemical analyses of gills, mantle, fast and slow muscles, digestive gland, and kidney for several trace elements.

Since the most chemically active of the major ingredients appears to be CLS, the behavior of this component in sea water

suspensions has been studied and chromium concentrations are measured in all tissue samples from organisms exposed to CLS and to drilling mud mixtures.

During the balance of the first contract period, tests with the synthetic mixtures and with used drilling mud will be run with two other bivalves. Depuration experiments will assess whether these organisms can clear such materials and regain their pre-exposure appearance and behavior.

II. CHARACTERIZATION OF THE DRILLING MUD COMPONENTS

A. Solubility in sea water

Suspensions of each of the six drilling mud components obtained from Baroid Corporation were prepared using filtered Boston Harbor water. Initial tests were carried out at room temperature (20-22°C). To better simulate the conditions of the Georges Bank environment, additional tests were run in isothermal baths at either 10.0 or 15.0°C using Harbor water that had been evaporated to give a salinity between 32.2 and 33.0 ‰. Each suspension was stirred for 24 hours before an aliquot was filtered through a 0.45 µm porosity membrane.

The results of these tests are presented in Table 1. Solubility is expressed in the conventional concentration notation (g/L.) and also by "% insoluble material" because some of the components are themselves heterogeneous mixtures of water-soluble and insoluble components. For example, when suspensions of chrome lignosulfonate (CLS) were filtered first through a glass fiber prefilter and then a 0.45 µm porosity membrane, the residue on the prefilter was greenish yellow, but the membrane held a much smaller quantity of dark brown material. The results of further characterization of CLS are described below.

B. Quality of the produced water

Because of the considerable solubility of some of the organic components, their effects on water quality were also assessed. To evaluate immediate oxygen demand, the dissolved

TABLE 1
SOLUBILITY OF DRILLING MUD COMPONENTS IN SEAWATER

<u>Component</u>	<u>Temp. (°C)</u>	<u>Salinity (‰)</u>	<u>Weight of Component Added (g/L)</u>	<u>Filterable Solids (g/L)</u>	<u>Insoluble Material (%)</u>	<u>Solubility (g/L)</u>
Chrome ligno- sulfonate	10	32.2	5.0017	0.0870	1.7	>4.9
	15	32.2	5.0002	0.0706	1.4	>4.9
	RT*	30.0	4.7688	0.0686	1.4	>7.7
Barite	10	33.0	1.0007	0.8268	82.6	0.17
	15	33.0	1.0007	0.8256	82.5	0.18
	RT	30.0	0.9571	0.8581	89.7	0.10
Aluminum Stearate	10	33.0	1.0001	0.8334	83.3	0.17
	15	33.0	1.0018	0.7745	77.3	0.23
Plant Starch	10	33.0	1.0003	0.1270	12.7	0.87
	15	33.0	1.0021	0.1140	11.4	0.89
	RT	30.0	0.9537	0.0991	10.4	0.85
Bentonite	10	32.2	1.0012	0.8462	84.5	0.16
	RT	33.0	1.0014	0.8771	87.6	0.12
Attapulgate	10	33.0	1.0004	0.9992	99.9	<0.01
	15	33.0	1.0007	0.9340	93.3	0.06

oxygen (D.O.) concentrations of 1 g/L suspensions of the components (5 g/L of CLS) were measured several times during the first hour of mixing. D.O. levels did not drop more than 0.1 ppm in suspensions of either plant starch or aluminum stearate. The D.O. concentration of the 5 g/L suspension of CLS decreased from 6.0 ppm 5 minutes after mixing to 5.4 ppm 55 minutes later. The pH of the CLS suspension was 4.7 while that of Al stearate, at one-fifth the loading was 6.8 or 1.0 unit below the pH of the make-up seawater. While most marine fauna could tolerate the decreases in D.O. that were observed, a drop in pH of one unit or more would likely cause some stress and could prove fatal. The stock suspensions of the acidic components used in the 28-day experiments were neutralized with sodium carbonate before being added to the test tanks.

C. Studies of chrome lignosulfonate

Between January and August, 1978, Ms. Fanny Knox, a graduate student in the Department of Interdisciplinary Science at MIT, worked at the Aquarium Research laboratory. In her research she attempted to characterize the physical and chemical properties of a commercial CLS, "Q-Broxin" from Baroid Corporation, and to study the behavior of seawater suspensions of CLS. The results of this study are contained in Ms. Knox's M.S. thesis and are summarized in this section.

1. Physical and chemical properties

Q-Broxin is hygroscopic: samples stored in polyethylene bags for several weeks on a laboratory bench lost 8% of their weight after being dried overnight at 110°C. After aging at room temperature for two hours, the dried samples regained 1/4 of the weight lost on drying.

The average metallic composition of this material is 3% Cr, 1% Fe, 7% Na and 0.3% Ca w/w. The organic content of dried samples was determined indirectly by measuring the loss in weight after heating at 500°C for four hours. The ash constituted 25% of the original sample.

Dissolution of Q-Broxin samples always produced a brown solution with a yellow-green residue that was insoluble in distilled or saline water and in polar and non-polar organic solvents. This material made up 1.2% by weight of dry Q-Broxin and contained 5% Cr, 20% Fe, 7% Na and 0.8% Ca; the ash content was 79%. These values were the same for material collected from freshly mixed CLS in water, from a sample aged for three weeks at natural pH (3.5), and from seawater and distilled water solutions aged 2-8 days at pH 8. The residue was dissolved in hot concentrated nitric acid and also by treatment with strong base followed by strong (~6 M) acid. The resulting solution from the latter treatment was yellow-green. As its pH was raised, the solution turned green (pH 2.5), then red (pH 3.0), and at pH 6 a reddish-brown precipitate appeared.

As stated in section A, filtration of CLS suspensions through a glass fiber prefilter and then through a 0.45 μ m porosity membrane left a brown residue on the latter filter. The amount of filterable brown solid increased in seawater suspensions with aging, but decreased with time in distilled water suspensions. The material was mostly organic, containing less than 1% Fe and Cr and may be the product of an aggregation of organic polymers dissolved in media such as seawater where high ionic strength promotes gelling and colloid destabilization.

Q-Broxin is an acidic substance. Titration with base gave an equivalent weight of about 1200. However, the accuracy of this number is uncertain because of the slow rate at which the pH value of a CLS solution stabilized after the addition of base. The more slowly the titrant was added, the more of the titrant was required to produce a given increase in pH.

A CLS solution stored at pH 8 formed a brown precipitate that contained 3.6% Cr and 11.6% Fe. These results indicate that the Fe and Cr in CLS may undergo slow hydrolysis likely involving hydrous oxide polymerization (olation) with the addition of base. Thus, some of the CLS in drilling muds, which are usually made alkaline with NaOH, may be present as lignosulfonate polymers highly cross-linked by the metals or as polymerized chromium and hydrous oxides incorporating lignosulfonate.

2. Molecular weight distribution

Gel filtration chromatography was used to determine the molecular weight distributions of the lignosulfonate and of the Fe- and Cr-carrying moieties of CLS. Chromatograms typical of those obtained with Sephadex G-100 resin for aged, slightly alkaline solutions and for fresh acidic solutions of CLS are shown in Figures 1 and 2. Every fifth point on each curve is marked for identification. In both curves, the maxima at fraction number 5 represents material eluting at the column void volume and so corresponds to a molecular size greater than $\sim 100,000$. The size distribution of dissolved organic matter in the two types of solution may be seen to be similar; however, there are striking differences in metal distributions. Much of the Cr and Fe in fresh, untreated CLS is associated with low ($<1000-4000$) molecular weight material. These results are consistent with those described in the previous section and further support the mechanism of slow elution of Cr and Fe in CLS solutions.

3. Interaction with EDTA

To test the strength and lability of the bonds between Fe and Cr and the lignosulfonate polymer, excess EDTA was added to freshly prepared and 3-week-old CLS solutions. The pH of the solutions was raised to 8 and then allowed to age 6-9 days before aliquots were eluted through Sephadex G-100. The resulting chromatograms are shown in Figures 3 and 4. The large maxima at V_T indicate that some of the Cr and much of the Fe that would

FIGURE 1

Chromatogram of a 2% FCLS Solution

Aged for 2 Days at pH 8

FIGURE 2

Chromatogram of a 2% FCLS Solution

Freshly Prepared at pH 4.2

FIGURE 3

Chromatogram of a 2% FCLS Solution
Freshly Prepared; Aged for 6 Days With EDTA

FIGURE 4

Chromatogram of a 2% FCLS Solution
Aged for 3 Weeks; Aged for 9 Days with EDTA

otherwise have been associated with high molecular weight lignosulfonate was present in a low molecular weight matrix, presumably as EDTA complexes. Less of the Cr was complexed in the sample aged for three weeks before EDTA was added, indicating that the reversal of olation may be a very slow process.

4. Interaction with clay minerals and thermal stability

To examine the interaction of CLS with clay minerals, three seawater suspensions containing 0.2 g/L Q-Broxin were prepared. One sample also contained 1.0 g/L attapulgite, another contained 1.0 g/L bentonite, and a third, with no clay added, served as a control. The samples were continuously stirred for 16 days; aliquots were taken periodically, centrifuged, and the dissolved levels of Cr, Fe, and lignosulfonate were measured. The results contained in Table 2 show that attapulgite adsorbs metals and lignosulfonate somewhat more rapidly than bentonite, although the proportion of the metals adsorbed onto the two clays after 16 days was about equal. Since Na and Ca forms of lignosulfonate have been found to be only slightly adsorbed by clays (McAtee and Smith, 1969), the adsorption of CLS is due to the presence of Fe and/or Cr. These results can be accounted for if the Cr and Fe adsorbed on the clay also retain some of their lignosulfonate ligands. Since more of the metals than the lignosulfonate are adsorbed, some of the latter may be displaced during the metal adsorption process, or only a fraction of the polymeric units contain Cr and Fe.

TABLE 2

COMPOSITION OF SUPERNATANTS OF CLS-CLAY SUSPENSIONS
(% of Initial Concentrations)

	<u>Ligno- sulfonate</u>	<u>Chromium</u>	<u>Iron</u>
<u>Attapulgite</u>			
2 min.	89	75	71
6 hours	79	53	41
1 day	72	46	33
2 days	70	38	28
5 days	68	32	23
9 days	66	24	18
12 days	64	23	18
16 days	62	18	16
<u>Bentonite</u>			
2 min.	96	84	72
6 hours	90	71	54
2 days	84	68	48
5 days	82	55	44
9 days	76	31	30
12 days	75	26	27
16 days	72	14	18

Since the temperature of the drilling fluid around the drill bit can reach 175°C, the effects of temperature on the stability of CLS in solution and on the interaction of CLS with attapulgite were examined. Two seawater-based solutions were prepared, one containing 0.2 g/L CLS and one containing 1.0 g/L attapulgite. The solutions were sampled just after mixing and then brought to boil, which took 30 min., on a combination stirring motor/hot plate. One minute after boiling, a second set of samples was taken, cooled in an ice bath, and then centrifuged. After 15 min. of boiling, a brown precipitate was observed in the CLS solution without clay. After 30 min. of boiling an additional set of samples was taken. The mixtures were allowed to cool, while being stirred, for an additional hour, when a final subsample was taken.

The results of the analyses of the supernatants are presented in Table 3. They show that at time B, after one minute of boiling but before the onset of precipitation, 50% of the lignosulfonate and over 95% of the Fe and Cr in CLS were adsorbed on attapulgite. That adsorption occurs much more rapidly at 100°C than at room temperature supports the interaction mechanism in which Cr and Fe undergo ligand exchange and serve as bridges between the clay lattice and lignosulfonate.

TABLE 3

COMPOSITION OF SUPERNATANTS OF HEATED SUSPENSIONS
(% of Initial Concentrations)

<u>CLS Only</u>	<u>Ligno-sulfonate</u>	<u>Chromium</u>	<u>Iron</u>
A	100	93	96
B	100	90	92
C	44	2.0	1.2
D	44	1.1	0.3

CLS and
Attapulgate

A	82	72	60
B	50	5.7	4.3
C	42	1.7	2.1
D	42	1.8	2.0

- A : Just after mixing
 B : After 1 minute of boiling
 C : After 30 minutes of boiling
 D : After 1 hour of cooling

III. PRELIMINARY TOXICITY STUDIES

To evaluate the acute toxicity of the non-clay components of drilling muds toward sea scallops, short term bioassays were run using the 7 test tanks in the NEA Research Laboratory. Suspensions each containing 1 g/L of one of the non-clay components were prepared with filtered sea water. Water temperatures were not closely controlled in these studies; however, room temperature was held between 16-18°C.

Five organisms were used in each test. To limit the build-up of waste products, the organisms were transferred into fresh test media at 48-hour intervals. No food culture was added to the tanks during testing.

The results of the tests are summarized in Table 4. The data show that the CLS and aluminum stearate suspensions were toxic to more than half the test populations within 24 hours. However, no deaths were observed in test populations exposed to 0.3 g/L suspensions of these two components.

TABLE 4

RESULTS OF SHORT-TERM BIOASSAYS WITH *PLACOPECTEN MAGELLANICUS*

Date	Time of Exposure	Component	Initial Loading (g/L)	Water Temperature (°C)		Cumulative Mortality (Out of 5)	
				Test	Control	Test	Control
1/10/78	0	CLS	1.0	16.5	17.5	0	0
1/11	22			15.1	16.3	3	0
1/12	48			15.5	15.5	3	0
1/13	72			16.8	15.9	5	0
1/11	0	CLS	0.3	11.5	11.5	0	0
1/13	48			16.3	16.0	0	0
1/16	120			16.3	16.0	0	0
1/17	144			15.8	15.5	0	0
1/10	0	Barite	1.0	17.3	17.5	0	0
1/11	24			16.3	16.3	0	0
1/12	48			15.5	15.5	0	0
1/13	72			16.0	15.9	0	0
1/23	0	Plant Starch	1.0	16.0	16.0	0	0
1/24	24			15.5	15.5	0	0
1/25	48			18.5	18.5	0	0
1/27	96			17.5	17.5	1	0
1/23	0	Al Stearate	1.0	16.0	16.0	0	0
1/24	24			15.5	15.5	4	0
1/30	0	Al Stearate	0.3	16.0	16.0	0	0
1/31	24			16.0	17.0	0	0
2/1	48			15.5	16.5	0	0
2/3	96			15.0	15.5	0	0

IV. METHODS FOR 28-DAY BIOASSAY

(See Table 5)

Deep sea scallops, Placopecten magellanicus, were collected by divers off the Maine coast and transported to the laboratory in insulated coolers chilled with sea water and ice. On arrival, the scallops were placed in running sea water aquaria for one week.

The scallops were acclimated for two days in the test tanks prior to the experiment. A detailed description of the tanks and temperature controls is given in Appendix 1. During acclimation and during the experimental program, scallops were fed a daily ration of the marine flagellate Isochrysis galbana (15 μg chlorophyll/liter at 15°C; 1 μg chlorophyll/liter at 5°C). The algae were grown in modified F/2 media (without silicate or metals) under cool white fluorescent lights. The test suspension in each tank was changed every two days over the 28-day test period.

During the experiment any dead animals were removed, dissected, and the tissues saved for chemical or histological analysis. At the end of 28 days all scallops were removed, the shells were measured, and the test organisms allocated as follows.

(A) Filtration Rates

The relative filtration rates were estimated by the neutral red method of Ansell and Sivas (1973). Animals were placed in aquaria containing an 0.001% neutral red solution at

TABLE 5

SUMMARY OF BIOASSAY METHODS

15°C. After 2 hr, replicate 50 ml samples were taken from each experimental vessel. After acidifying to pH 6.5 with 0.1 N HCl, estimations of concentration were made at 520 nm in 1 cm cells in a Spectronic 600 spectrophotometer. The filtration rates were calculated by utilizing the differences between neutral red concentrations in experimental and control aquaria as described by Jorgensen (1943) and Willensen (1952). The formula used in the present studies was:

$$m = M \frac{\ln C_0 - \ln C_t}{t} - a$$

where m is the volume of water filtered in liters per hour; M is the volume of the suspension in liters; C_0 , C_t are the concentrations of the filtration medium (either particles or dyes) at the start and end of the experiment; and a is a correction factor derived from control data taken at the start and finish of an experiment, which accounts for changes in the sample beyond those brought about by the experimental organism. Following the filtration experiments, each animal was sacrificed, placed in a tared aluminum dish, dried at 100°C for 24 hrs, and weighed. These data allowed the expression of the relative filtration rates in relation to dry tissue weight, a less variable parameter than shell-size measurements or live weight determinations. Dry tissue weight also bears a more realistic relationship to the mass of actively metabolizing tissue which the organism must maintain under stressful conditions. Replicate filtration experiments were performed on animals from each exposure regimen. All of the scallops opened their valves and

formed a mantle veil immediately after being put in the filtration aquaria; they were actively pumping. Such activity must be observed at the outset of each experiment, for it must be assumed that the animals are constantly pumping order to estimate volume relationships -- a constraint of the method. The pumping rates must be considered as relative rates, for it has not been shown that sea scallops remove neutral red from solution at absolute efficiency.

(B) Visual Inspection of Tissues (All controls and experimental materials)

Shells were measured and then opened to permit a visual inspection of tissues prior to dissection. The types of observation included clogging of tissues with particulates and the general condition of tissues (see Table 2).

(C) Tissue Dissection

Samples of gills, mantle, fast and slow muscle, digestive gland, and kidney were dissected and preserved for chemical and histological analyses.

(D) Chemistry

Tissues for chemistry were frozen and later thawed, digested in boiling nitric and perchloric acids, and analyzed for Cr, Ba, Sr, Cd and Fe, by plasma emission spectroscopy.

(E) Histology

The remaining tissues were fixed in Bouin and Zenker fluids and embedded in polyester wax. The wax blocks were mounted

on a mandrel, sectioned on a microtome at 8-10 micrometers and mounted on glass slides without adhesive. The preparations were stained with celestine blue and with alcian blue stain specific for mucopolysaccharides, then dehydrated and mounted. The slides were studied using a compound microscope with 10X and 40X objectives.

Five slides were prepared from gill samples of organisms exposed to control and bioassay conditions. Five visual fields were chosen at random from each slide and 20 contiguous gill filament cross-sections were identified in each field, for a total of 125 cross-sections per slide. Each filament cross-section in a field was divided by classical anatomical landmarks into frontal, latero-frontal, lateral, and abfrontal areas. The presence of mucus goblet cells, stained with alcian blue stain, in each of the four areas of each cross-section was separately noted and enumerated. The observations per slide were made, therefore, according to the matrix:

experimental conditions x 5 fields x 25 cross sections
x 4 anatomical areas = 500 observations per slide.

Table 6 summarizes the experimental protocols of experiments from November 1977 through September 1978. The first three experiments tested the clay components of drilling muds -- attapulgite and bentonite -- at four concentrations and two temperatures. Experiments 4 and 5 tested chrome ligno-sulfonate and barium sulfate at four concentrations and two temperatures.

TABLE 6

SUMMARY OF EXPERIMENTAL PROTOCOL

<u>Expt.</u>	<u>Date</u>	<u>Material & Concentration (ppt)</u>		<u>Temp. (°C)</u>	<u>Organism</u>
1	9 Nov. 77- 7 Dec. 77	Attapulgate 3.0,1.0,0.3,0.1	Bentonite 3.0,1.0,0.3,0.1	15°	Placopecten magellanicus
2	20 Jan. 78- 6 Feb. 78	Attapulgate 3.0,1.0,.3,.1	Bentonite 3.0,1.0,.3,.1	5°	Placopecten magellanicus
3	20 Feb. 78- 20 Mar. 78	Attapulgate 3.0,1.0,.3,.1	Bentonite 3.0,1.0,.3,.1	5°	Placopecten magellanicus
4	27 Mar. 78- 26 Apr. 78	Chrome Lignosulfonate 1.0,0.3,0.1,0.03	Barium sulfate 3,1,0.3,0.1	5°	Placopecten magellanicus
5	12 May 78- 9 Jun. 78	Chrome Lignosulfonate 1.0,0.3,0.1,0.03	Barium sulfate 3,1,0.3,0.1	15°	Placopecten magellanicus
6	29 Jun. 78- 28 Jul. 78	Combined mud- Attapulgate 3.0,1.0,.3,.1	Combined mud- Bentonite 3.0,1.0,.3,.1	15°	Placopecten magellanicus
7	8 Aug. 78- 8 Sep. 78	Combined mud- Attapulgate 3.0,1.0,.3,.1	Combined mud- Bentonite 3.0,1.0,.3,.1	5°	Placopecten magellanicus
8	28 Sep. 78- 26 Oct. 78	Combined mud- Attapulgate 3.0,1.0,.3,.1	Combined mud- Bentonite 3.0,1.0,.3,.1	15°	Spisula solidissima

Aluminum stearate and plant starch were not tested individually; rather, these ingredients were added to mixtures of the major components. While seawater-based muds often contain aluminum stearate as a defoamer and plant starch to reduce fluid losses and to keep barite in suspension, these materials usually constitute only 0.1-0.3% of the total mud weight.

Based on some typical mud compositions contained in parts of an offshore drilling draft E.I.S. given us by Dr. Bruce Coons of A.P.I., the following weight ratios seemed appropriate.

BaSO ₄	1.50 parts
Clay	1.00
Chrome ligno-sulfonate	0.20
Al Stearate	.01
Plant Starch	.01

Translating these values directly into G/L gave a total loading of 2.7 g/L, or about the maximum employed for any one component in the earlier tests. Diluting this loading by factors of 3, 10 and 30 produced the usual 4-concentration experimental matrix. Attapulgate was used in one series and bentonite in the other.

V. RESULTS OF 28-DAY BIOASSAYS

A. Mortality

Dead scallops were removed from the tanks daily and a record kept of date of removal. Tables 7 through 13 summarize this data. Records of the mortality of test scallops during the first 10 days of the experiment (Tables 14-16) clearly show the effects of higher temperature at the high concentrations of mud components.

B. Visual Inspection of Tissues

The results of a visual inspection of tissues is summarized for experiments 6 and 7 (the synthetic muds with attapulgite or bentonite) in Tables 17-20. The observations fall into four categories: 1) disattachment of muscle and mantle from shell; 2) response of muscles to a probe; 3) presence of clay in mantle and gill; 4) condition of kidney.

Although the data are the results of subjective observation and the samples are small, stress is clearly evident in organisms exposed to the attapulgite-based synthetic muds at concentrations of 3.0 and 1.0 g/L and is present to a lesser degree at 0.3 g/L. The condition of scallops exposed to a concentration of 0.1 g/L is similar to that of the controls. In scallops exposed to combined muds and bentonite, similar effects are observed at the same concentration ranges, and clogging with clay was seen even at the lowest mud concentration.

TABLE 7

MORTALITY IN EXPERIMENT #1
(/8 in each tank)

Test Temp.: 15 °C.

DATE: 9 Nov. - 7 Dec. 1977

Test Material: ATTAPULGITE					BENTONITE				CONTROL
Dosage (g/L) :	3	1	.3	.1	3	1	.3	.1	
Exposure (Days)									
1									
2									
3									
4									
5	1				1			1	
6	1				2				
7	4	1			4				
8									
9	1	1							
10					1	1			
11	1								
12		1	1			2			
13									
14									
15									
16									
17									
18									
19									
20									
21	1								
22	2								
23									
24					1	1	1		
25									
26									
27					1				
28									

27

TABLE 8

MORTALITY IN EXPERIMENT #2
(/8 in each tank)

Test Temp.: 5 °C.

DATE: 20 Jan. - 6 Feb. 1978

Exposure (Days)	ATTAPULGITE				BENTONITE				CONTROL
	Dosage (g/L) :	3	.1	.3	.1	3	.1	.3	
1									
2									
3									
4						1			
5									
6									
7			1	1				1	
8									
9									
10									
11									
12									
13									
14									
15	2					1			
16						2			
17						1			
18						2			
19	Termination of experiment because of electrical power loss during blizzard								
20									
21									
22									
23									
24									
25									
26									
27									
28									

TABLE 9

MORTALITY IN EXPERIMENT # 3
(/8 in each tank)

Test Temp.: 5 °C.

DATE: 20 Feb. - 20 March 1978

Test Material:	ATTAPULGITE				BENTONITE				CONTROL
Dosage (g/L) :	3	1	.3	.1	3	1	.3	.1	
Exposure (Days)									
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									
13									
14					2				
15					1				
16					1				
17									
18									
19									
20					1				
21									
22									
23									
24									
25									
26									
27	1					1			
28									

29

TABLE 10

MORTALITY IN EXPERIMENT # 4
(/8 in each tank)

Test Temp.: 5 °C.

DATE: 27 March-26 April 1978

Test Material:	CHROME LIGNOSULFONATE				BARIUM SULPHATE				
Dosage (g/L) :	1	.3	.1	.03	3	1	.3	.1	CONTROL
Exposure (Days)									
1									
2									
3									
4									
5									
6									
7									
8	1								
9									
10									
11									
12									
13									
14									
15	1								
16	1								
17									
18									
19									
20	1								
21	1								
22									
23									
24									
25									
26									
27	1								
28									

30

TABLE 11

MORTALITY IN EXPERIMENT # 5
(/8 in each tank)

Test Temp.: 15°C.

DATE: 12 May - 9 June 1978

Test Material:	CHROME LIGNOSULFONATE				BARIUM SULPHATE				
Dosage (g/L) :	1	.3	.1	.03	3	1	.3	.1	CONTROL
Exposure (Days)									
1									
2									
3									
4									
5									
6									1
7						1	8		
8		4							
9		2							
10		2							
11		1				1			
12						1			
13									
14									
15									
16									
17					1				
18									
19									
20									
21									
22									
23									
24									
25									
26									
27									
28				1					31

TABLE 12

MORTALITY IN EXPERIMENT #6
(/8 in each tank)

Test Temp.: 15°C.

DATE: 29 June-28 July 1978

Test Material: SYNTHETIC MUD WITH ATTAPULGITE

SYNTHETIC MUD WITH BENTONITE

Dosage (g/L) : 3 1 .3 .1 3 1 .3 .1 CONTROL

Exposure
(Days)

1									
2									
3									
4							1		
5									
6									
7	5				1	1			
8	1								
9									
10									
11									
12		1							
13									
14									
15									
16		1							
17									
18									
19									
20		1							
21					2				
22			1						
23									
24	1	1							
25									
26	1	1							
27									
28									

TABLE 13

MORTALITY IN EXPERIMENT #7

(/8 in each tank)

Test Temp.: 5°C.

DATE: 8 Aug. - 8 Sept. 1978

Test Material: SYNTHETIC MUD WITH ATTAPULGITE

SYNTHETIC MUD WITH BENTONITE

Dosage (g/L) : 3 1 0.3 0.1

3 1 0.3 0.1

CONTROL

Exposure
(Days)1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28

1

1

33

TABLE 14

MORTALITY RATES (%) DURING FIRST 10 DAYS OF EXPERIMENTS 1-3

<u>Exp. #</u>	<u>Test Material & Temperature</u>	<u>Dosage</u>	<u>Mortality</u>
1	Attapulgate 15°C	3.0	100
		1.0	25
		0.3	0
		0.1	0
	Bentonite	3.0	100
		1.0	12
		0.3	0
		0.1	12
	Control	0	0
2	Attapulgate 5°C	3.0	0
		1.0	12
		0.3	12
		0.1	0
	Bentonite	3.0	12
		1.0	0
		0.3	0
		0.1	12
	Control	0	0
3	Attapulgate 5°C	3.0	0
		1.0	0
		0.3	0
		0.1	0
	Bentonite	3.0	0
		1.0	0
		0.3	0
		0.1	0
	Control	0	0

TABLE 15

MORTALITY RATES (%) DURING FIRST 10 DAYS OF EXPERIMENTS 4 & 5

<u>Exp. #</u>	<u>Test Material & Temperature</u>	<u>Dosage</u>	<u>Mortality</u>
4	Chrome Ligno- sulfonate 5°C	1.0	12
		0.3	0
		0.1	0
		0.03	0
	Barium Sulfate	3.0	0
		1.0	0
		0.3	0
		0.1	0
	Control		12
5	Chrome Ligno- sulfonate 15°C	1.0	100
		0.3	0
		0.1	0
		0.03	0
	Barium Sulfate	3.0	0
		1.0	12
		0.3	100
		0.1	12
	Control		0

TABLE 16

MORTALITY RATES (%) DURING FIRST 10 DAYS OF EXPERIMENTS 6 & 7

<u>Exp. #</u>	<u>Test Material & Temperature</u>	<u>Dosage</u>	<u>Mortality</u>
6	Synthetic Mud with Attapulgate 15°C	3.0	75
		1.0	0
		0.3	0
		0.1	0
	Synthetic Mud with Bentonite	3.0	12
		1.0	12
		0.3	12
		0.1	0
	Control		0
7	Synthetic Mud with Attapulgate 5°C	3.0	0
		1.0	0
		0.3	0
		0.1	0
	Synthetic Mud with Bentonite	3.0	0
		1.0	0
		0.3	0
		0.1	0
	Control		0

TABLE 17

RESULTS OF VISUAL OBSERVATIONS FOR EXPERIMENT 6
(15°C)

<u>Bentonite-based Synthetic Mud (g/L)</u>					
<u>EFFECT</u>	3.0	1.0	0.3	0.1	Control
	<u>Yes/No</u>	<u>Yes/No</u>	<u>Yes/No</u>	<u>Yes/No</u>	<u>Yes/No</u>
1. Gaping	3/4	1/5	1/5	0/6	0/8
2. Mantle disattached from lower shell	0/7	0/6	1/5	0/6	0/8
3. Adductor disattached from lower shell	0/7	0/6	1/5	0/6	0/8
4. Mantle disattached from upper shell	0/7	0/6	1/5	0/6	0/8
5. Adductor disattached from upper shell	0/7	0/6	1/5	0/6	0/8
6. Mantle response to probe	3/4	1/5	1/5	0/6	0/8
7. Adductor response to probe	3/4	1/5	1/5	0/6	0/8
8. Smell	0/7	0/6	0/6	0/6	0/8
9. Mantle cavity clogged w/ "clay"	7/0	6/0	3/3	4/2	0/8
10. Mantle edge clogged w/ "clay"	4/3	4/2	1/5	1/5	0/8
11. Mantle surface w/ mucus	4/3	3/3	3/3	3/3	0/8
12. Gill clogged w/ "clay"	7/0	5/1	3/3	2/4	0/8
13. Gill frayed and tattered	6/1	1/5	1/5	0/6	0/8
14. Gill surface w/ mucus	5/2	3/3	3/3	3/3	0/8
15. Kidney texture	1/6	1/5	1/5	1/5	0/8
16. Kidney spotted	1/6	1/5	1/5	1/5	0/8
17. Kidney color	1/6	1/5	1/5	1/5	0/8

TABLE 18

RESULTS OF VISUAL OBSERVATIONS FOR EXPERIMENT 6

		<u>Attapulgitic-based Synthetic Mud (g/L)</u>				
		3.0	1.0	0.3	0.1	Control
<u>EFFECT</u>		<u>Yes/No</u>	<u>Yes/No</u>	<u>Yes/No</u>	<u>Yes/No</u>	<u>Yes/No</u>
1.	Gaping	8/0	4/3	1/5	0/6	0/8
2.	Mantle disattached from lower shell	5/3	3/4	1/5	0/6	0/8
3.	Adductor disattached from lower shell	0/8	0/7	1/6	0/6	0/8
4.	Mantle disattached from upper shell	4/4	2/5	1/6	0/6	0/8
5.	Adductor disattached from upper shell	0/8	0/7	1/6	0/6	0/8
6.	Mantle response to probe	0	2/5	5/1	6/0	8/0
7.	Adductor response to probe	0	0	5/1	6/0	8/0
8.	Smell	1/7	2/5	1/6	0/6	0/8
9.	Mantle cavity clogged w/ "clay"	8/0	3/4	1/6	0/6	0/8
10.	Mantle edge clogged w/ "clay"	8/0	3/4	1/5	0/6	0/8
11.	Mantle surface w/ mucus	8/0	3/4	1/5	0/6	0/8
12.	Gill clogged w/ "clay"	8/0	7/0	1/5	0/6	0/8
13.	Gill frayed and tattered	8/0	7/0	1/5	0/6	0/8
14.	Gill surface w/ mucus	8/0	7/0	1/5	0/6	0/8
15.	Kidney texture	0/8	1/6	0/6	0/6	0/8
16.	Kidney spotted	0/8	1/6	0/6	0/6	0/8
17.	Kidney color	0/8	1/6	0/6	0/6	0/8

TABLE 19

RESULTS OF VISUAL OBSERVATIONS FOR EXPERIMENT 7
(5°C)

<u>EFFECT</u>	<u>Attapulgitic-based Synthetic Mud (g/L)</u>					Control
	3.0	1.0	0.3	0.1		
	<u>Yes/No</u>	<u>Yes/No</u>	<u>Yes/No</u>	<u>Yes/No</u>		<u>Yes/No</u>
1. Gaping	0/8	1/7	0/8	0/8		0/8
2. Mantle disattached from lower shell	0/8	1/7	0/8	0/8		0/8
3. Adductor disattached from lower shell	0/8	0/8	0/8	0/8		0/8
4. Mantle disattached from upper shell	0/8	0/8	0/8	0/8		0/8
5. Adductor disattached from upper shell	0/8	0/8	0/8	0/8		0/8
6. Mantle response to probe	8/0	7/1	8/0	8/0		8/0
7. Adductor response to probe	8/0	7/1	8/0	8/0		8/0
8. Smell	8/0	7/1	0/8	0/8		0/8
9. Mantle cavity clogged w/ "clay"	5/3	2/6	0/8	0/8		0/8
10. Mantle edge clogged w/ "clay"	5/3	2/6	0/8	0/8		0/8
11. Mantle surface w/ mucus	5/3	2/6	0/8	0/8		0/8
12. Gill clogged w/ "clay"	5/3	2/6	0/8	0/8		0/8
13. Gill frayed and tattered	0/8	2/6	0/8	0/8		0/8
14. Gill surface w/ mucus	6/2	2/6	0/8	0/8		0/8
15. Kidney texture	8/0	7/1	8/0	8/0		8/0
16. Kidney spotted	0/8	7/1	8/0	8/0		0/8
17. Kidney color	-	-	-	-		-

TABLE 20

RESULTS OF VISUAL OBSERVATIONS FOR EXPERIMENT 7
(5°C)

<u>EFFECT</u>	<u>Bentonite-based Synthetic Mud (g/L)</u>				
	3.0	1.0	0.3	0.1	Control
	<u>Yes/No</u>	<u>Yes/No</u>	<u>Yes/No</u>	<u>Yes/No</u>	<u>Yes/No</u>
1. Gaping	1/7	0/7	0/7	0/7	0/8
2. Mantle disattached from lower shell	1/7	0/7	0/7	0/7	0/8
3. Adductor disattached from lower shell	1/7	0/7	0/7	0/7	0/8
4. Mantle disattached from upper shell	1/7	0/7	0/7	0/7	0/8
5. Adductor disattached from upper shell	1/7	0/7	0/7	0/7	0/8
6. Mantle response to probe	7/1	7/0	7/0	7/0	8/0
7. Adductor response to probe	7/1	7/0	7/0	7/0	8/0
8. Smell	1/7	0/7	0/7	0/7	0/8
9. Mantle cavity clogged w/ "clay"	3/5	0/7	1/6	0/7	0/8
10. Mantle edge clogged w/ "clay"	3/5	0/7	1/6	0/7	0/8
11. Mantle surface w/ mucus	3/5	0/7	1/6	0/7	0/8
12. Gill clogged w/ "clay"	3/5	0/7	1/6	0/7	0/8
13. Gill frayed and tattered	3/5	0/7	1/6	0/7	0/8
14. Gill surface w/ mucus	3/5	0/7	1/6	0/7	0/8
15. Kidney texture	3/5	0/7	1/6	0/7	0/8
16. Kidney spotted	7/1	7/0	6/1	7/0	8/0
17. Kidney color	1/7	0/7	1/6	0/7	0/8

C. Filtration Experiments

The results for 3 clearing rate studies are given in Tables 21 to 23. Table 21 shows the effects on clearing rate in Placopecten magellanicus held in attapulgite and bentonite. The animals exposed to attapulgite showed clearing rates greater than those of the controls throughout the exposure range. After exposure to bentonite, clearing rates increased for the lowest dosage, fell below controls at 0.3 g/L, and rose above control values for the remainder of the concentration range. Attapulgite at 15°C appears to induce higher clearing rates than does bentonite. Higher clearing rates require a greater expenditure of energy which over the long term would be more detrimental to the maintenance, growth, and reproductive capacity of the sea scallops.

Tables 22 and 23 show the effects of exposure to chrome lignosulfonate and barium sulfate on scallops held at 15°C and 5°C. At 15°C in chrome lignosulfonate, the clearing rate for scallops at 0.03 g/L is 12 ml/g/hr, rising to 21 ml/g/hr and falling to 9.5 ml/g/hr. At 5°C all rates are lower, but the same pattern may be observed with clearing rates falling substantially at the highest concentration (1.0 g/L). The concentration at which the greatest fall in clearing rate occurs is more than three times greater at 5°C than at 15°C (0.3 versus 1.0 g/L). Clearing rates of animals exposed to barium sulfate at 15°C were greater than for the controls at low dosages, but fell at the highest concentrations. At 5°C, there is a reduction in clearing rates

at 0.1 g/L, a rise at intermediate concentrations, and a fall below control levels at 3.0 g/L.

The data indicate that at 15°C, there may be compensatory depressions in clearing rates as the animals are exposed to low levels of drilling mud components (e.g., 0.03 g/L chrome lignosulfonate). The compensation may indicate an effort by the organism to reduce clearing rates in an attempt to limit the amount of contact it has with the mud component. At intermediate concentrations this strategy may not be tenable and so clearing activity accelerates in order to maintain clean respiratory surfaces. A reduction in clearing rates at the highest concentrations of CLS and barium sulfate may be either an adjustment to limit energy expenditure in order to save biochemical stores, or it may be an indication that the animal has exhausted its most accessible energy stores and is forced to continue clearing only slowly with less accessible energy sources.

To better understand the significance of these responses, metabolic studies such as measurements of oxygen uptake or the utilization of carbohydrate and lipid stores should be carried out. Such work would help understand the strategies adopted by animals exposed to various levels of mud to mobilize these energy stores and cope with the particulate perturbation.

TABLE 21

P. MAGELLANICUS CLEARING RATES AT 15°C

<u>Test Material</u>	<u>Dosage (g/L)</u>	<u>Clearing Rate (ml cleared per gram dry weight per hr)</u>
Attapulgate	0.1	35.1±0.9
	0.3	24.5±6.8
	1.0	36.0±7.9
	3.0	30.5±2.8
Bentonite	0.1	27.9±2
	0.3	14.9±0.7
	1.0	32.3±6.4
	3.0	24.8±9.2
Control		17.2±2.7

TABLE 22

P. MAGELLANICUS CLEARING RATES AT 15°C

<u>Test Material</u>	<u>Dosage (g/L)</u>	<u>Clearing Rate (ml cleared per gram dry weight per hr)</u>
Chrome Lignosulfonate	0.03	12.0±0.6
	0.10	21.0±1.0
	0.30	9.5±1.5
Barite	0.10	23.5±3.4
	1.00	16.0±2.0
	3.00	14.5±0.8
Control		18.0±4.0

TABLE 23

P. MAGELLANICUS CLEARING RATES AT 5°C

<u>Test Material</u>	<u>Dosage (g/L)</u>	<u>Clearing Rate (ml cleared per gram dry weight per hr)</u>
Chrome Lignosulfonate	0.03	4.5±1.1
	0.10	7.8±2.1
	0.30	7.9±0.7
	1.00	5.7±1.5
Barite	0.10	4.5±0.9
	0.30	14.5±4.7
	1.00	12.5±0.4
	3.00	8.0±1.4
Control		9.5±0.5

D. Histological Observations

To further assess the effects of exposure to the drilling mud components and synthetic mud mixtures, histological analysis of selected tissues have been carried out. The results reported describe cellular changes in several regions of the gills of P. magellanicus exposed to various levels of bentonite, attapulgite, barium sulfate and CLS.

Because they function in intimate contact with the external environment, the gills are susceptible to physical or chemical damage and to surface obstruction by high concentrations of particulate matter in the environment. Such damage and clumping or occlusion of the respiratory feeding surfaces would cause a major impairment to the function of these vital organs. With impaired function, the animals so afflicted could show reduced growth rates, reduced reproductive capacity, or increased mortality rates leading to a diminishing population.

Bivalve molluscs have two gills suspended close to the body at either side of the mantle cavity. Each gill is subdivided into two lamellae composed of minute parallel filaments. The filaments are tubular, blood-carrying structures, the walls of which are 1 cell-layer thick. The frontal surfaces of the filaments (thus, the gills) which first contact the inflowing water current bear ciliated cells which produce the water current and assist in retaining particles. To their side are the specialized frontolateral cilia which also trap particles. Mucus goblet cells are occasionally found on the frontal and frontolateral

surfaces under normal conditions, but only very rarely on the lateral and abfrontal aspects of the filaments. The cells produce and discharge mucous materials which form the mucus net to retain particles in the respiratory current.

The results from experiments 1-5 (Tables 24 to 27) show that as the concentration of particulate matter is increased, the incidence of mucus cells in the frontal area decreases. In the laterofrontal area, however, there seems to be a lower threshold concentration, 0.1 g/L attapulgite and 0.3 g/L bentonite, at which the frontolateral mucus cell occurrence resembles or surpasses that of controls. At higher doses the occurrence of mucus cells declines. The results for lateral and abfrontal mucus cell occurrence show an increase in mucus cell proliferation immediately upon addition of particles. Tissue structure observations reveal that as concentrations of particulate matter increase to 0.3 g/L attapulgite and 1.0 g/L bentonite, necrotic changes occur. The tissue changes are characterized by the placement of cell layers and broken cell membranes. These manifestations of dose impact are most outstanding in 1.0-3.0 g/L attapulgite; the effects shown for 1.0 g/L attapulgite are not approached with bentonite until it is used at a concentration of 3.0 g/L.

The results of the experiments to determine concentration effects of attapulgite and bentonite at 15°C are given in Tables 24 and 25. They show that at 0.1 g/L, the bentonite-exposed samples had reduced incidence of mucus cells, while at 1.0 g/L frontal incidence was only slightly lower than controls,

TABLE 24

P. MAGELLANICUS - MUCUS CELL OCCURRENCE IN VARIOUS ANATOMICAL AREAS OF GILLS
OF ANIMALS EXPOSED TO BENTONITE

<u>15°C</u>	<u>Frontal</u>		<u>Frontolateral</u>		<u>Lateral</u>		<u>Abfrontal</u>	
	<u>Mean</u>	<u>SD</u>	<u>Mean</u>	<u>SD</u>	<u>Mean</u>	<u>SD</u>	<u>Mean</u>	<u>SD</u>
Control	6.6 ± 1.1		10. ± 1.4		0.04 ± 0.01		0.12 ± 0.28	
Bentonite 3.0 ppt	-	-	2.0 ± 1.1		0.30 ± 0.61		0.12 ± 0.35	
Bentonite 1.0 ppt	5.6 ± 1.1		10. ± 2.		6.1 ± 1.7		1.3 ± 0.7	
Bentonite 0.1 ppt	4.4 ± 1.1		10. ± 2.		0.30 ± 0.38		0.10 ± 0.28	

<u>5°C</u>								
Control	4.8 ± 1.1		7.1 ± 1.2		0.04 ± 0.01		0.04 ± 0.01	
Bentonite 3.0 ppt	0.04 ± 0.01		0.5 ± 0.7		0.16 ± 0.54		0.04 ± 0.01	
Bentonite 1.0 ppt	0.40 ± 0.38		1.2 ± 0.4		-	-	0.08 ± 0.25	
Bentonite 0.3 ppt	1.1 ± 0.7		8.3 ± 1.3		0.60 ± 0.54		0.24 ± 0.37	

TABLE 25

P. MAGELLANICUS - MUCUS CELL OCCURRENCE IN VARIOUS ANATOMICAL AREAS OF GILLS
OF ANIMALS EXPOSED TO ATTAPULGITE

	<u>Frontal</u>		<u>Frontolateral</u>		<u>Lateral</u>		<u>Abfrontal</u>	
<u>15°C</u>	<u>Mean</u>	<u>SD</u>	<u>Mean</u>	<u>SD</u>	<u>Mean</u>	<u>SD</u>	<u>Mean</u>	<u>SD</u>
Control	6.6 ± 1.1		10. ± 1.		0.04 ± 0.01		0.12 ± 0.28	
Attapulgate 3.0 ppt	-		-		-		-	
Attapulgate 1.0 ppt	-		-		3.0 ± 1.4		0.28 ± 0.38	

<u>5°C</u>								
Control	4.8 ± 1.1		7.1 ± 1.2		0.04 ± 0.01		0.040 ± 0.01	
Attapulgate 3.0 ppt	-		-		-		-	
Attapulgate 1.0 ppt	0.28 ± 0.47		1.4 ± 0.7		0.1 ± 0.8		0.20 ± 0.45	
Attapulgate 0.3 ppt	1.5 ± 0.8		5.3 ± 1.5		0.8 ± 0.7		0.28 ± 0.38	
Attapulgate 0.1 ppt	2.8 ± 1.2		8 ± 2		0.44 ± 0.45		0.28 ± 0.51	

TABLE 26

P. MAGELLANICUS - MUCUS CELL OCCURRENCE IN VARIOUS ANATOMICAL AREAS OF GILLS
OF ANIMALS EXPOSED TO CHROME LIGNOSULFONATE

	<u>Frontal</u>		<u>Frontolateral</u>		<u>Lateral</u>		<u>Abfrontal</u>	
<u>15°C</u>	<u>Mean</u>	<u>SD</u>	<u>Mean</u>	<u>SD</u>	<u>Mean</u>	<u>SD</u>	<u>Mean</u>	<u>SD</u>
Control	4.8 ± 0.9		10 ± 2		0.11 ± 0.32		0.11 ± 0.32	
Chrome Lignosulfonate 1.0 ppt	-		-		-		-	
Chrome Lignosulfonate 0.3 ppt	2.2 ± 0.9		4.2 ± 1.1		0.11 ± 0.32		-	
Chrome Lignosulfonate 0.1 ppt	5.3 ± 1.2		9 ± 2		0.3 ± 0.5		0.11 ± 0.32	
Chrome Lignosulfonate 0.03 ppt	4.6 ± 1.0		8 ± 2		0.3 ± 0.5		0.6 ± 1.0	
<hr/>								
<u>5°C</u>								
Control	3.6 ± 0.8		11.5 ± 1.6		-		-	
Chrome Lignosulfonate 1.0 ppt	-		-		-		-	
Chrome Lignosulfonate 0.1 ppt	6.2 ± 1.1		9.8 ± 1.4		1.3 ± 1.4		0.9 ± 0.9	
Chrome Lignosulfonate 0.03 ppt	7.38 ± 0.07		9.3 ± 0.8		-		-	

TABLE 27

P. MAGELLANICUS - MUCUS CELL OCCURRENCE IN VARIOUS ANATOMICAL AREAS OF GILLS
OF ANIMALS EXPOSED TO BARIUM SULFATE

	<u>Frontal</u>		<u>Frontolateral</u>		<u>Lateral</u>		<u>Abfrontal</u>	
<u>15°C</u>	<u>Mean</u>	<u>SD</u>	<u>Mean</u>	<u>SD</u>	<u>Mean</u>	<u>SD</u>	<u>Mean</u>	<u>SD</u>
Control	4.81 ± 0.939		10 ± 2		0.1 ± 0.3		0.11 ± 0.32	
Barium Sulfate 3.0 ppt	4.3 ± 0.8		13 ± 1		2.2 ± 1.1		1.9 ± 1.0	
Barium Sulfate 1.0 ppt	3.7 ± 0.8		6 ± 2		0.6 ± 0.9		1.6 ± 1.1	
Barium Sulfate 0.3 ppt	6.7 ± 1.4		9 ± 2		1.3 ± 1.0		2.6 ± 1.2	
Barium Sulfate 0.1 ppt	4.0 ± 0.9		7 ± 2		0.14 ± 0.48		0.9 ± 1.1	
<hr/>								
<u>5°C</u>								
Control	3.5 ± 0.8		11.8 ± 1.6		-		-	
Barium Sulfate 3.0 ppt	2.0 ± 1.1		6.2 ± 1.7		-		-	
Barium Sulfate 1.0 ppt	2.2 ± 1.2		6.4 ± 1.5		0.9 ± 1.6		0.4 ± 0.9	
Barium Sulfate 0.3 ppt	4.2 ± 1.2		6.0 ± 1.6		0.3 ± 0.7		0.5 ± 0.9	
Barium Sulfate 0.1 ppt	3.1 ± 0.7		8.2 ± 1.4		-		-	

while frontolaterals had elevated numbers in comparison to controls. The lateral cells proliferated at 0.1 g/L bentonite and to a very large 6.16 in 1.0 g/L bentonite; at 3.0 g/L bentonite, the lateral incidence had dropped. The findings for abfrontal cells was nearly as for lateral aspects; the lateral and the abfrontal occurrences increased. At 3.0 g/L attapulgit, the sections consisted of completely disrupted cells, with only occasional cell ghosts or broken membranes visible through the microscope.

E. Trace Elements in Scallop Tissues

The tissues from organisms exposed to Q-Broxin at 5°C and 15°C and to the bentonite-based synthetic mud at 15°C have been analyzed for Cr and several other elements. The results of these measurements are contained in Tables 28 and 29.

The data in Table 28 show that scallops exposed to solutions of Q-Broxin accumulate chromium. Elevated levels of the element were found in the fast and slow muscles as well as in those organs exposed directly to the Q-Broxin solutions: the gill, mantle and digestive gland.

Concentrations of the element in muscle tissues indicates that the animal is assimilating the element in addition to pumping it through its digestive system.

Organisms exposed to a mixture of mud components including Q-Broxin also showed an increase in Cr levels in several target tissues including the fast muscle and kidney. These results seem to indicate that, despite being associated with a fine-grained

TABLE 28

CHROMIUM* IN TISSUES OF P. MAGELLANICUS EXPOSED TO Q-BROXIN

<u>Temp.</u>	<u>Q-Broxin Loading (g/L)</u>	<u>Digestive Gland (mg/g)</u>	<u>Fast Muscle (µg/g)</u>	<u>Gill (mg/g)</u>	<u>Kidney (µg/g)</u>	<u>Mantle (µg/g)</u>	<u>Slow Muscle</u>
5°C	1.0	0.19	57	5.6	27	84	15
	0.3	0.29	7	0.22	23	25	3.2
	0.1	0.10	5	0.15	11	10	2.0
	0.03	0.05	1	0.16	11	11	1.8
15°C	1.0**	0.18	8	0.11	24	32	1.5
	0.3	0.42	7	0.34	18	27	6.6
	0.1	0.33	3	0.22	46	33	3.5
	0.03	0.02	2	0.14	43	19	0.4

* Concentration values in Tables 28 and 29 are based on dry tissue weights.

** Data are for tissues blended from seven organisms, all of which died within 1 week of the start of the test.

TABLE 29

TRACE ELEMENTS IN TISSUES OF P. MAGELLANICUS EXPOSED TO
BENTONITE-BASED SYNTHETIC MUD AT 15°C

<u>Tissue</u>	<u>Dosage</u>	<u>Ba</u>	<u>Fe</u>	<u>Cd</u>	<u>Cr</u>
Kidney	3.0	.80 mg/g	.68 mg/g	.27 mg/g	36 µg/g
	1.0	<.004	.67	.30	23
	0.3	<.007	.69	.25	14
	0.1	<.006	.65	.32	9
Digestion Gland	3.0	.34 mg/g	1.3 mg/g	.36 mg/g	.59 µg/g
	1.0	.28	2.0	.48	.46
	0.3	.12	1.7	.40	.38
	0.1	.23	.9	.41	.22
Gill	3.0	.15 mg/g	.17 mg/g	4.6 µg/g	65 µg/g
	1.0	.30	.13	3.2	62
	0.3	.17	.10	3.0	40
	0.1	.26	.09	3.4	28
Mantle	3.0	.57 mg/g	190 µg/g	<3.3 µg/g	33 µg/g
	1.0	.52	60	6.0	17
	0.3	.39	52	3.9	14
	0.1	.09	52	3.6	7.2
Slow Muscle	3.0	240 µg/g	24 µg/g	<1.8 µg/g	2.4 µg/g
	1.0	25	14	<1.8	1.8
	0.3	4.5	22	<2.1	2.1
	0.1	< 1.5	< 1.5	<2.1	1.2
Fast Muscle	3.0	110 µg/g	36 µg/g	< .9 µg/g	2.6 µg/g
	1.0	45	34	1.5	2.0
	0.3	22	27	<1.8	2.7
	0.1	34	19	<2.1	.9

particulate phase, Cr is still assimilated by the scallop.

Ba, Fe and Cd were also measured in the latter set of tissues: Ba was present in the test material as BaSO_4 ; Q-Broxin is 1% Fe, and Cd was believed to be a possible contaminant in the mixture. The data in Table 29 indicate that Cd is not enriched in the target tissues as a result of exposure to the mixed mud. Both Ba and Fe were assimilated as indicated by the elevated levels of these elements in the muscles and, in the case of Ba, in the kidneys of animals exposed to the higher dosages of the synthetic mud. The lack of change in Fe concentrations in kidney tissues with increased dosage rates may mean that the animal does not view this essential element as an undesirable constituent which it must purge from its body, even when it is present at elevated levels.

VI. CONCLUSIONS

At suspended solid loadings approaching 3 g/L, drilling muds, especially those containing attapulgite as the principal clay mineral, are acutely toxic to P. magellanicus. Mortality was significantly higher under summer conditions: 15°C water and relatively high food loading. The reason for the greater toxicity of attapulgite versus bentonite, either alone or in mud mixtures, was not established. There exist considerable differences in primary structure, degree of hydration and/or cation exchange capacity between the two minerals.

Visual inspection proved to be a surprisingly precise index of environmental stress. The most useful types of response were muscular attachment to the shell, condition of the kidney, presence of clay in various organs, and conditions of the gills. The control samples and those exposed to 0.1 g of synthetic mud/L were usually similar; however, the appearance of animals exposed to higher dosages changed significantly with dosage.

Filtration rate has not been the precise and predictable indicator of stress that had been hoped at the start of the study. In most experiments, low levels of mud components apparently promoted filtration, perhaps as the animals attempted to clear particulate matter from their gills. At the highest loadings animals under the most stress exhibited a decrease in clearing rate as their energy stores were depleted. However, the significance in these trends in individual experiments is often doubtful because of the relatively large uncertainties in the

measurements. More precise measurements would require more test organisms than are now being used and more uniformity in their size.

Another effect of exposure to drilling mud components is a change in mucus cell proliferation and mucus production on the gills. With increasing concentrations of mud components, mucus is produced to assist in cleaning the gill surfaces, transporting particles, and producing pseudofeces. Increased mucus production places demands on the metabolism and energy reserves of the animals. The energy stores held by the animals for normal maintenance, growth and reproduction are then consumed under the stress of the particulates exposed. The utilization of these energy reserves for the immediate survival of the individual may have long term detrimental effects on reproduction and recruitment.

Organisms assimilate some of the elements present at high concentrations in drilling muds: Fe, Cr and Ba. The extent to which these elements are taken up appears to be a function of the total elemental loading, which is mostly in a particulate form. Presumably dissolved Fe, Cr and Ba concentrations are not a direct function of mud loading -- a presumption that is being checked by analyses of test tank seawater as this report is being written. Of these three elements, only Fe is not found at elevated levels in the kidneys of animals exposed to the highest loadings of synthetic mud, indicating the Fe does appear to the organisms to be an undesirable element. The length of

time during which the Cr and Ba content of the kidney remains high after exposure to drilling mud will soon be measured in a series of depuration experiments.

VII. REFERENCES

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VIII. APPENDIX

The Suspended Sediments Seawater Aquarium System

The suspended sediments seawater aquarium system consists of four basic components (Fig. A1):

- (1) a seawater treatment facility,
- (2) an aquarium tank-disperser assembly (seawater),
- (3) a temperature control/regulator assembly (fresh water,
- (4) a thermal exchanger unit at which 2 and 3 interface.

In addition to the aquarium system and seawater treatment facility a unit has been built in which thick sediment-seawater slurries may be mixed. The job is a particularly dirty one, so it will be performed outside the laboratory area, the mixed slurries being pumped to the laboratory for storage and eventual use.

Seawater Treatment

The laboratory system was designed to study turbidity effects on marine animals. Water treatment, for our purposes, means removing naturally occurring particulates from the general laboratory supply and killing the bacteria in the filtered stream. The intent of these operations is to provide particulate-free water for making up experimental turbidity loads and insuring a low rate of water-quality degradation by bacteria during an experiment.

Aquarium-Disperser Assembly

The part of the installation in which marine organisms are exposed to various experimental turbidity loads is the aquarium-disperser assembly. It consists of 16 helmet-shaped fiberglass aquarium tanks (a

Fig. A1. Diagram of the suspended sediments seawater aquarium system: 1, treated seawater supply; 2, aquarium-disperser assembly; 3, thermal exchanger; 4, electronic temperature control-regulator.

single unit is pictured in Fig. 1). A disperser head is integral to each of the aquaria. In addition, a pump is associated with each aquarium-disperser assembly. Each of the 16 assemblies is a discrete aquarium unit containing its own turbidity load. No intermingling of the contents of neighboring aquaria can take place.

The pump in each assembly serves a dual purpose. It strips seawater-slurry from the aquarium and returns the mixture to the tank by way of a thermal exchanger and the disperser head. In this manner the pump not only maintains a homogeneous suspension of particulate matter within the aquarium but also creates an interface with the temperature control regulation circuit.

Thermal Exchanger

The thermal exchanger is of a simple tube-in-shell construction. The fluid contents of the aquarium-disperser assemblies pass down tubes which are surrounded by temperature-regulated fresh water flowing in the shell. During the passage, the seawater-slurries attain the temperature of the exchanger water.

Temperature Control/Regulator

The temperature of the fresh water flowing through the thermal exchanger (and, therefore, in the seawater-slurries in the aquaria) is governed by the temperature control/regulator assembly. It consists of reservoirs for hot- and cold-tempered fresh water, an electronic control device and automatic demand valve units.

The heart of the system is the control device; in essence a small solid-state logic system. It acts upon signals from the exchanger supply circuit