A Descriptive Evaluation of the Effects of No. 2 Fuel Oil on the Tissues of the Soft Shell Clam, *Mya arenaria* L.

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Introduction

Bivalve molluscs have been found to accumulate petroleum and petrochemical derivatives. BLUMER, et al (1970) described hydrocarbon uptake by oysters and scallops following a fuel oil spill. ZITKO (1971), LEE, et al. (1972), STEGEMAN and TEAL (1973), VAUGHAN (1973), and NEFF and ANDERSON (1975) have reported the occurrence and uptake of petroleum hydrocarbons by bivalve molluscs. A mechanism by which soft shell clams may accumulate oil was described by STAINKEN (1975).

The effects of petroleum oils and derivatives on bivalves are varied. Some of the effects reported have described alterations in oxygen consumption, carbon budgets, larval development, behavior, filtration rates, mortality and biochemical effects. The histological effects of petrochemicals are also varied. Deleterious effects of oil on bivalve tissue structure have been reported by LAROCHE (1972), CLARK, et al. (1974) and GARDNER, et al. (1975). Histological aberrations in bivalves have been reported by BARRY, et al. (1971), JEFFRIES (1972), and BARRY and YEVICH (1975). The aberrations were believed to be due to pollution effects. In contrast, VAUGHAN (1973) found few effects of oils on bivalves.

Reports on the effects of petroleum oils are often conflicting. Some of the studies were field studies and exposure concentrations were unknown. The reported effects of petroleum oil exposure have ranged from extensive to relatively none. Bivalves in the environment are frequently exposed to single spill or chronic discharges. This study was therefore performed to experimentally determine the effects of subacute concentrations of No. 2 fuel oil on the soft shell clam.

A No. 2 fuel oil was chosen for study because it is commonly shipped in coastal waters, used in coastal industrial installations, and has already been involved in a well documented spill (BLUMER, et al. 1970). A winter temperature (4°C) was chosen because spills are more likely to occur during the inclement winter weather. In the event of a spill during the colder months, it is probable that much of the oil would be dispersed and emulsified in the water column through turbulent wave action. The clams were therefore exposed 28 days to oil initially added in an emulsified form to simulate a potential naturally occurring condition of chronic exposure.

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Materials and Method

The No. 2 fuel oil was supplied by the U. S. Environmental Protection Agency, Industrial Waste Treatment Laboratory, Edison, N. J. The specific gravity of the oil was 2.40 centistokes. The oil was composed of 14% aromatics and 86% nonaromatics according to ASTM method No. D2549-68. Oil-in-water emulsions were ultrasonically prepared according to a procedure developed by GRUENFELD and BEHM (1973).

Clams for the experiments were collected from Sequine Point, Staten Island, N. Y. Young clams with a mean shell length of 25 mm were utilized because young bivalves tend to have greater filtration rates than those of older bivalves. The clams were acclimated to the experimental conditions for a duration of 6 days before the emulsions were added.

An exposure period of 28 days to No. 2 fuel oil emulsions having concentrations of 10, 50 and 100 ppm was utilized. Four 20 gallon aquaria containing 60 liters of filtered sea water/aquaria (salinity = 20‰) were employed. The sea water was collected from Sandy Hook Bay and filtered through a coarse plankton net. One aquaria served as a control and each of the remaining aquaria received either 10, 50 or 100 ppm of oil emulsion. The water was continuously aerated and the temperature was maintained at 4°C. Sampling for hydrocarbon content of water and clams was performed every 7 days. The hydrocarbon content of the water was determined by the method of GRUENFELD (1972). Complete results from these experiments will be published at a later date.

At the beginning of the experiment, just before addition of the emulsions (Time 0), five clams were fixed in Davidson's fixative (SHAW and BATTLE, 1957). At the end of the 28 day exposure period, ten clams from each concentration and the control tank were removed for histological examination. The clams sampled represented 10% of the experimental population. Five of the ten clams randomly removed from each concentration were fixed in Davidson's fixative and five were fixed in cold 10% acetate buffered neutral formalin, pH 7. The two fixatives were employed to determine whether alterations in tissue structure were fixation artifacts. The results indicated that artifacts did not occur.

All fixation was done in a refrigerator. After 24 hours, clams fixed in Davidson's were transferred to cold 70% ethanol. All fixed material was stored in a refrigerator until further processing. Prior to embedding, the formalin fixed animals were washed 1½ hours in running tap water. All tissues were dehydrated and brought to paraffin utilizing an Autotechnicon. Final infiltration was accomplished employing a vacuum infiltrator for 15 minutes at 13-15 inches Hg. The paraffin embedded clams were oriented to cut beginning at the anterior pedal opening. Serial cross sections were cut at 6 and 7 microns to the level of the

heart and kidney. Sections were mounted according to the procedure of LUNA (1968). The sections were stained with Harris-Lillie hematoxylin (Fisher Scientific) and 0.5% Eosin Y in 95% ethanol.

Several cross sections of the visceral mass, stomach and pallium of each clam from each experimental group were stained for mucosubstances and necrotic tissue. Mucosubstances were stained using the aldehyde fuchsin-alcian blue method of LUNA (1968) and azure A/eosin B was employed for necrotic tissues (GRIMSTONE and SKAER, 1972).

One section of the visceral mass and stomach from each clam from each experimental group was stained by a modification of McManus's method for glycogen, the Periodic Acid - Schiff reaction or PAS (LUNA, 1968). The modifications were made from procedures described by LUNA (1968) and LILLIE (1965). To check the specificity of the PAS reaction for glycogen, sections were treated in an amylase solution (Diastase, Sigma chemical Co.) at a concentration of 0.1 g/100 ml in distilled water, pH 6.8 for one hour.

Results

Throughout the oil exposure period, all clams in the control and oil exposed groups seemed to remain in good condition. After the addition of the oil emulsions, the concentrations of oil in the water column decreased rapidly. The actual hydrocarbon concentrations are listed in Table 1.

Table 1. Hydrocarbon concentration (ppm) in the water column during the 28 day exposure period.

	Tank #	Time 0*	Week 1	Week 2	Week 3	Week 4
Control	1	0	0	0	0	0
10 ppm	2	4.5	1.31	0.56	0.37	0
50 ppm	3	43.72	1.04	0.71	0.37	0.29
100 ppm	4	60.71	1.52	0.78	0.32	0.46

* The Time 0 sample measurement was made two hours after addition of the emulsified oil.

Several factors were probably responsible for the gradual depletion of oil from the water. Much of the oil was apparently removed from the water column by the mucociliary feeding and ejection mechanisms of the clams. Large masses of mucus were

ejected from the clams and were accumulated on the cooling coils. Subsequent chemical analysis revealed a large content of oil in the mucus. After 28 days, a sample of the mucus from the 100 ppm aquaria was found to contain 833 micrograms of hydrocarbons. Mass spectrometric analysis demonstrated that these hydrocarbons were mostly dimethyl and trimethyl naphthalenes and paraffins in the C-14 and C-15 regions (STAINKEN, 1975).

Harris's Hematoxylin was used to examine the general morphology of clams. Radical tissue aberrations were not observed. A gradation of tissue effects was apparent. The clams exposed to 100 ppm exhibited the largest number of anomalies from the controls. The pallial muscle appeared edematous in four clams, similar to that described by PAULEY and SPARKS (1965, 1966). There were more leukocytes in the pallial blood sinuses of 100 ppm exposed clams than controls, with occasional leukocyte nests as described by PAULEY and CHENG (1968) occurring in the pallial blood sinuses. The leukocytes often formed a band underlying the mantle epithelium similar to that illustrated by DES VOIGNE and SPARKS (1968). In some clams, the anterior adductor muscle was midly edematous and infiltrated with leukocytes. The area between the mantle membrane and the cell layer next to the shell was also edematous and contained many leukocytes in some clams. In one clam, they formed a plug in the hemocoel of part of the foot. In seven clams, the style sac, intestine and diverticula appeared very vacuolar and the diverticula appeared much reduced in size. There was a small loss of chromatophilic material at the top of the gill filaments in several clams exposed to 100 ppm oil.

At 50 ppm, the effects were less marked, except in the diverticula and intestine. The diverticula of seven clams were shrunken in size. The diverticula epithelium appeared almost cuboidal instead of the normal columnar epithelium. Portions of the intestinal mucosa appeared to be sloughing into the lumen which was not prevalent in the controls. The intestine, style sac and diverticula were abnormally vacuolar in appearance. In one clam, a few diverticula near the gonad appeared necrotic and undergoing resorbtion. The pallial muscle was edematous in one clam. Several clams had more leukocytes and leukocyte nests in the blood sinuses below the inner pallial epithelium than the controls. Only one clam had more than normal leukocytes and leukocyte nests between the mantle epithelium and the cell layer next to the shell. In one clam, a portion of the gill had a pavement of leukocytes along the lining of the blood sinuses next to the central water tube.

The 10 ppm clams showed fewer effects of oil exposure than the 100 or 50 ppm clams. The diverticula were reduced in size and the diverticula, stomach and intestine were vacuolated in appearance. In two clams, a moderate number of leukocytes were observable in the pallial blood sinuses.

Cross sections of the clam visceral mass and pallium were stained with azure A/eosin B to demonstrate necrotic tissues. Major histological differences were not found between controls and oil exposed clams. The general effect of holding the clams for four weeks was an increase in vacuolization of the digestive diverticular and intestinal cells. The vacuolization was present in all groups. The diverticular cells of the Time 0 clams were distinct and contained few vacuoles. After four weeks, the diverticular cells of all clams appeared to contain many vacuoles and the cell membranes were often indistinct. However, this effect was exacerbated in the oil exposed clams compared to controls (Figure 1-3).

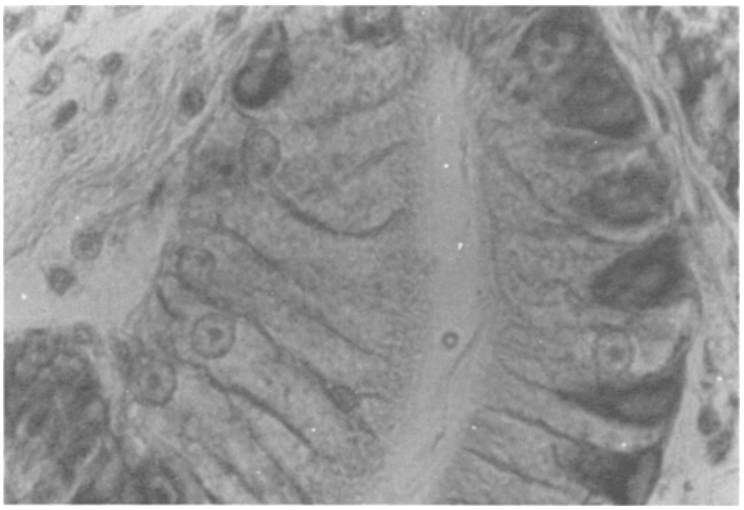

Figure 1. Section of the digestive diverticula. 100x. Stained Azure A/eoson B. Time 0.

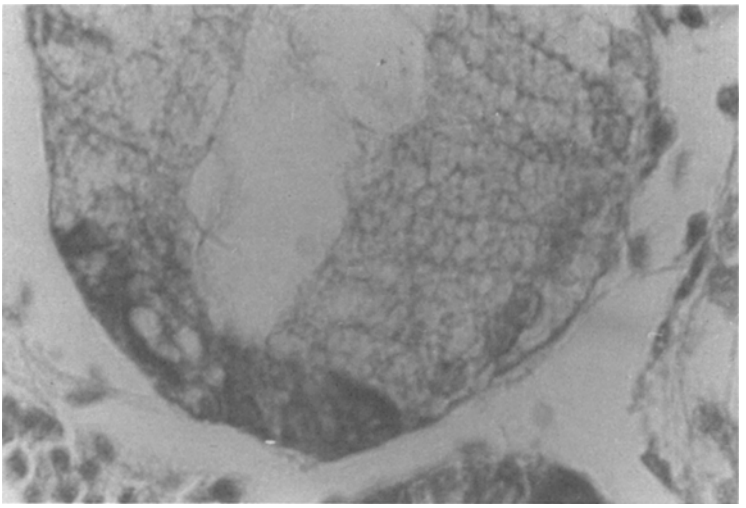

Figure 2. Section of the digestive diverticula. 100x. Stained Azure A/eosin B. Control.

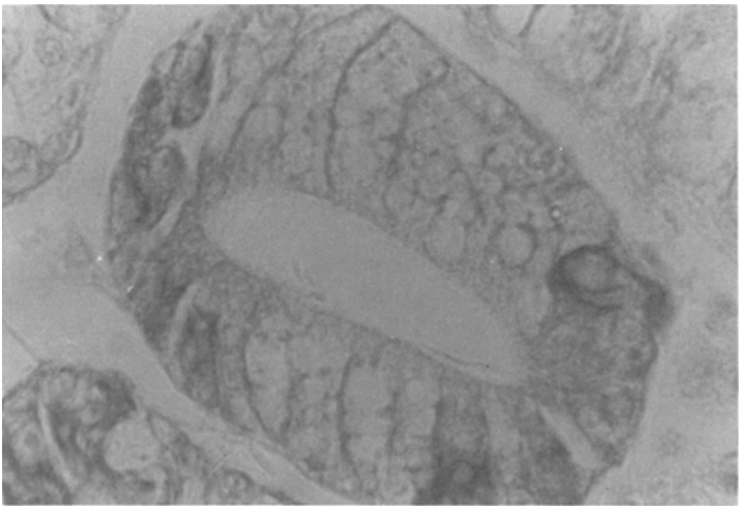

Figure 3. Section of the digestive diverticula. 100x. Stained Azure A/eosin B. 100 ppm exposed.

An aldehyde Fuchsin - Alcian Blue 8GX stain was used to stain mucosubstances. Histological differences were not found between exposed and control groups along the intestines and the periphery of the visceral mass. There was a decrease in mucoid cells and staining intensities from two of the 100 ppm clams. The same effect occurred in one 50 ppm clam.

Clam sections were stained for glycogen according to the PAS technique. There was a gradation of effects of oil exposure in the digestive diverticular and intestinal cells. The 100 ppm clams had the least amount of PAS positive material (glycogen). Six of the 100 ppm clams had observable differences from the control clams. Generally, the diverticular cells decreased in size and contained much less PAS positive material. The diverticular cells of all 100 ppm clams appeared very vacuolar with few glycogen deposits. Several cells almost appeared amylase treated. The intestinal cells and basement membranes also contained less PAS positive material. Most of the diverticular cells appeared to be devoid of cytoplasm. The stomach mucosa of two of the 100 ppm clams contained less PAS positive material than did the controls. The gill filament tips and margins appeared to have a decrease in PAS positive material.

Generally, a pattern of cellular glycogen depletion was observed in 50 ppm clams similar to that described for 100 ppm clams. The diverticular cells were reduced in size, vacuolar in appearance and contained less PAS positive material than did the controls. The cells also appeared to be depleted of cytoplasm. The intestinal mucosa appeared to be sloughed into the lumen. The stomach mucosa of the 50 ppm clams also showed a depletion of PAS positive material as compared to controls.

Most digestive cells in the 10 ppm clams appeared normal. However, several clams diverticular and intestinal cells were depleted of PAS positive material and were more vacuolar in appearance than were the controls.

In the control clams, the bulk of the diverticular cells were full, round, and most of the cytoplasm was red. In the 10 ppm, 50 ppm and 100 ppm clams, the diverticular cells were more vacuolar and contained less cytoplasm than did those of the control group.

Discussion

Petroleum hydrocarbons are generally assumed to be carcinogenic, particularly the polycyclic aromatics. Reviews of the general carcinogenic effects of oil have been published by HEIDELBERGER (1970) and ZOBELL (1971). CLARK, et al. (1974) reported alterations in tissue structure of oysters and mussels exposed to outboard motor effluent. LAROCHE (1972), BARRY and YEVICH (1975) have reported a high incidence of gonadal tumors in soft shell clams exposed to oils. Hyperplastic germ cell

tumors were also present in the gills. BARRY, et al. (1971) reported a high incidence of hyperplasia of the gills and kidneys in soft shell clams from areas believed polluted. In contrast, VAUGHAN (1973) did not find evidence of histopathological change in oysters exposed to No. 2 fuel oil. There were some nonpathological changes evident in the epithelial layer of the inner mantle lobe, and it was suggested that oil restricted feeding activity.

The results of this study with Mya arenaria revealed that radical tissue changes did not occur after exposure to No. 2 fuel oil. It is possible, however, that either the very low concentration of oil present in the water column was not suffiecient to alter tissue structure (i.e. neoplasms), or the exposure time was not long enough. The hydrocarbon concentrations in the water column of each tank measured during the last 3 weeks of exposure varied from 1.52 to 0.29 ppm.

The general effects of subacute oil exposure can be characterized as a depletion of glycogen and generalized leukocytosis particularly evident in the blood sinuses of the pallium and mantle membrane. There was also an increase in vacuolization of the diverticula, stomach and intestines. The histological effects in Mya arenaria appeared to be dose dependent. The clams exposed to the initial 100 ppm oil emulsion had more frequent and noticeable histological differences from the controls. The depletion of glycogen and vacuolization may have been due to a suppression of feeding and consequent use of body reserved coupled with an altered respiratory rate. The increased vacuolization of oil-exposed clams may also represent inclusion and intracellular compartmentalization of hydrocarbons. The leukocytosis of the mantle blood sinuses beneath the inner epithelium probably represents an inflammation reaction with a migration of leukocytes into the affected areas.

Acknowledgement

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