SURVEY OF TOXAPHENE LEVELS

IN

GEORGIA ESTUARIES

by

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INTRODUCTION

During the 1970-1971 contract period between The University of Georgia Marine Institute and Hercules, Inc., research was initiated to focus on the role of toxaphene contamination in estuarine ecology. Different approaches were utilized during this time. Laboratory procedures were implemented or modified as outlined in Wilson, 1969. Static bioassays were conducted using estuarine organisms collected from the Duplin estuary marsh adjacent to Sapelo Island, Georgia.

Data collected and processed during the period from 1 August 1970 to 31 July 1971 are included in this report. An effort has been made to cover each portion of the research with explanations of methodology and results. This procedure deviates from the normal introduction, methods, results and discussion to provide the reader with a coherent summary of the research findings. The sections are subdivided in the following broad categories: (1) environmental toxaphene residue results (except sediment); (2) sediment analysis; (3) bioassays; (4) trawl data; and (5) a summary of findings related to the problems of toxaphene contamination in the salt marsh. All tables referred to in the text are presented in the Appendix. Chromatograms used in the toxaphene analysis are not reproduced in this report. The originals are on file at the Marine Institute on Sapelo Island, and interested persons may contact the authors for further information.

Acknowledgements

Thanks are extended to Hercules, Inc., for making this research work possible. The investigators are especially indebted to Mr. Charles Dunn, Synthetics Division, Wilmington, Mr. Clell Tyler and Mr. Millard Dusenbury of the Brunswick operations, Hercules, Inc. for their financial support and encouragement of this research. Appreciation is also extended to the staff of The University of Georgia Marine Institute, particularly to Captain B. J. Rouse and Mr. T. Walker who operated the research vessels. Recognition is also made of the support of the National Science Foundation - NSF Kit Jones Grant (GD-27249) which provided ship time in support of this research project. Bioassay research was conducted with the assistance of Mr. Mallory S. May III, Brunswick Junior College.

SAMPLING METHODS AND LABORATORY PROCEDURE

Collections began within the ten quadrats selected by the use of random coordinates (Figure 1). In this report quadrats 26 and 34 will be considered as one (#26/34) since trawls were usually made continuously through these two quadrats. An additional collection site was established in quadrat #33 at the toll bridge on the Torras Causeway as this was the closest point to Terry Creek where oysters were available. In March, 1971, a collection site was also established in Mackay River at its junction with Back River (Figure 2). Collections were also made at miscellaneous stations which are identified by quadrat numbers in this report for the sake of convenience. Samples for toxaphene analysis were taken from collections for species diversity study. The following is a description of the field collection methods utilized for different species along with a statement of the laboratory preparation executed prior to extraction of pesticides and the analytical results.

The group considered herein as "small crabs" includes Panopeus, Sesarma and Uca crabs. These crabs were caught on the marsh surface by hand or dug out of their burrows with a small spade. After being transported to the laboratory in jars, the crabs were ground in a blender and an aliquot of this tissue was taken using wet weight as a basis for quantitation of pesticide content after extraction.

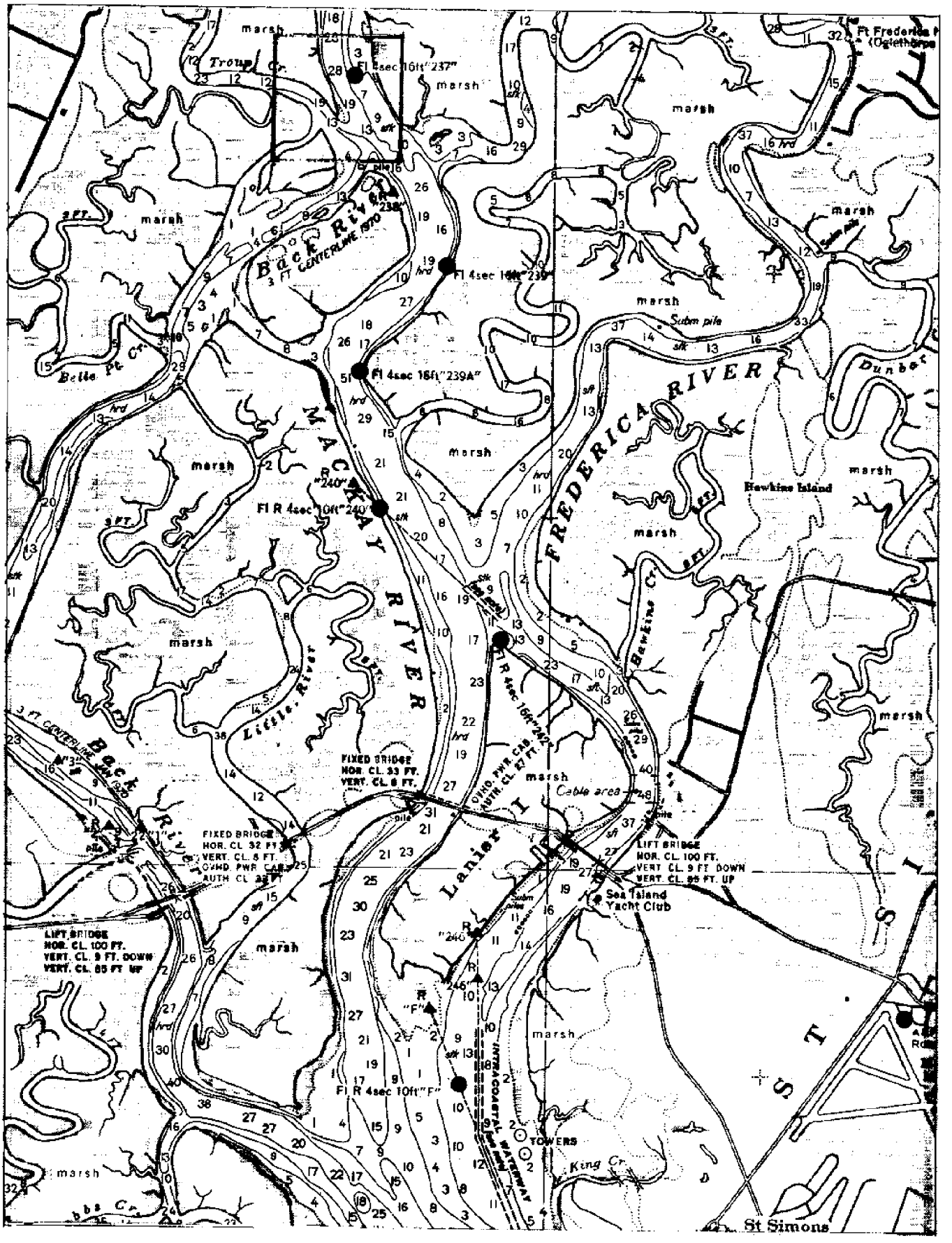

Figure 2. Study area showing Mackay River collection station.

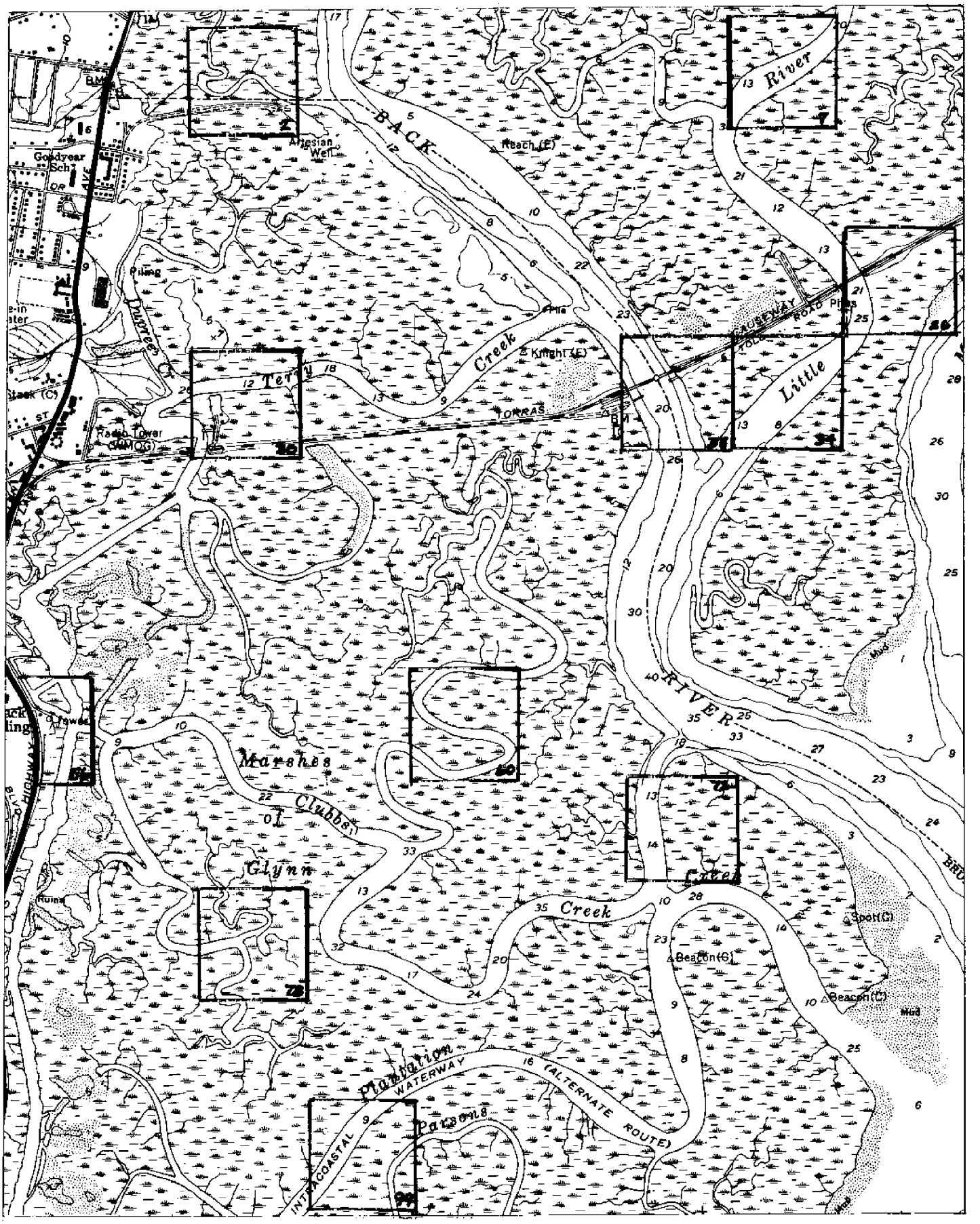

Figure 1. Study area, showing ten quadrats.

Sodium sulfate was used as a desiccant. Five samples of this type were analyzed and all were found to be free of chlorinated hydrocarbons.

Fin fish genera which were analyzed include: Anchoa, anchovy; Fundulus, mummichog; Mugil, mullet; Leiostomus, spot; Brevoortia, menhaden; Cynoscion, weakfish; Arius, sea catfish; Stellifer, star drum; Gobiosoma, naked goby; Bairdiella, yellowtail and croaker; Trichiurus, cutlassfish; Centropristis, rock sea bass; Paralichthys, flounder; and Rissola, striped cusk-eel. These were routinely caught in a ten foot otter trawl towed by the R/V Striker. All mullet samples were collected with a castnet. Small fish were weighed, frozen whole and then ground with sodium sulfate. In larger fish the liver and/or a fillet along the lateral line was removed, cut in small pieces, weighed, frozen and then ground with sodium sulfate. The amount of toxaphene was slightly higher in muscle tissue than in the liver of the same fish (Table 1). Fifty-five samples of fin fishes from the study area were analyzed and only two of the samples were found to be free of toxaphene.

Thirteen samples of shrimp (Penaeus) were analyzed for toxaphene content. The shrimp were collected in the otter trawl along with the fin fishes. Shrimp were frozen, cut into small pieces, weighed, refrozen and then ground in the osterizer using sodium sulfate as a drying agent. Often the heads (containing most of the digestive tract) were analyzed separately from the tails (muscle tissue).

Toxaphene occurred in the muscle at approximately one tenth of the concentration found in the heads (Table 2). Only three samples were found to be free of toxaphene.

Blue crabs (Callinectes) were also collected in the otter trawl. Muscle tissue was dissected, weighed, frozen and then ground with sodium sulfate. The gut samples included an aliquot of a homogenate prepared from the hepatopancreas and gametogenetic tissue (mixed with sodium sulfate as a desiccant). The crabs appear to have a higher concentration of toxaphene in the gut than in the muscle tissue. Five samples of crab samples were extracted for gas-liquid chromatographic detection; two of these were free of this compound.

Oyster samples (Crassostrea) were collected by hand from the shore or dredged with an oyster dredge. Eight to ten oysters were shucked into a jar and ground in the blender. An aliquot of this was mixed with sodium sulfate, frozen and then re-ground to the consistency of powder. The sample was then prepared for extraction. All of the twenty-one oyster samples analyzed contained toxaphene. One sample of ribbed muscle (Modiolus) (taken by hand from the mud surface) and blood ark (Arca) (caught in the oyster dredge) were treated in the same manner and found to contain toxaphene.

The salt-marsh periwinkle (Littorina) was collected from Spartina leaves or the mud surface. Two methods were used to prepare these for extraction.

In one method the snails were broken with a hammer and a wet weight of the tissue obtained; the sample was frozen and then ground in the blender with sodium sulfate. In the second method, whole snails were dried at 100°C for four to five days, broken with a hammer and the dried tissue separated and ground in a Wiley Mill (using a 40 micron sieve). A dry weight was then taken as a basis for analysis and the powdered snails were mixed with sodium sulfate. In the six samples prepared by this method (the second) no toxaphene was recovered. The seven samples prepared by the first method were all found to contain toxaphene. In one instance half of a collection of snails was held alive in the laboratory for twenty days and then analyzed by the first method. The amount of toxaphene recovered decreased 25% from the original analysis.

Of the thirty-one samples of Spartina alterniflora, the salt marsh cordgrass, collected for analysis, eighteen were found to be free of toxaphene. Collections of green grass were cut from standing growth with garden shears and placed in labeled bags for transport to the laboratory. The grass was cut into 6" lengths and each leaf was repeatedly washed with water and dried at 100°C for four to five days. Upon complete drying the grass was ground in the Wiley Mill and mixed with an appropriate amount of sodium sulfate. Dry weight was used in quantitation. (Other current research related to Spartina at the Marine Institute reveals that the dry weight is approximately 30% of the wet weight).

Samples of dead grass were taken from standing stems still attached to the root system. These were treated in the same manner as the green grass. In April, 1971, green and dead Spartina samples collected side by side revealed the dead grass contained more toxaphene than the green grass (Table 3). Two possible explanations are that 1.) the grass metabolized more toxaphene during the previous growing season than this season because there was more toxaphene in the water then; or 2.) the dead grass absorbs more toxaphene passively than the green grass actively metabolizes from the shallow sediments.

Birds which have been collected for analysis include the pied-billed grebe (Podilymbus), redwing blackbird (Agelaius), seaside sparrow (Ammospiza), long-billed marsh wren (Telmatodytes), dowitcher (Limnodromus), boat-tailed grackle (Cassidix), clapper rail (Sora) and scaup (Aythya).

In the large birds, the liver and breast muscle were analyzed separately. The tissue was weighed, (wet weight), cut up, frozen and ground with sodium sulfate. Small birds were skinned, weighed, frozen whole, chopped up, re-frozen and ground with sodium sulfate in the osterizer. Seven of fifteen samples contained toxaphene, eight contained DDE and eleven were contaminated with dieldrin.

Miscellaneous samples analyzed for toxaphene include: one diamond-back terrapin liver, a starfish (Asterias), a channelled whelk (Busycon) and some sea lettuce (Ulva). These samples were analyzed on a wet weight basis.

They were appropriately chopped, frozen and ground with sodium sulfate in preparation for extraction. Another sample type included water which was squeezed from wood chips collected on the bottom of Terry Creek. This interstitial water was filtered and then extracted.

Water samples were extracted as follows: one liter of water was shaken for two minutes with 100 ml of 6% ethyl ether in petroleum ether and the ether phase collected. This procedure was repeated twice and the ether phase concentrated and partitioned with acetonitrile. The acetonitrile was evaporated to dryness and the residue eluted from a Florisil column, concentrated and then injected into the gas chromatograph.

Extraction of tissue samples was carried out by the following procedure: the mixture of tissue and sodium sulfate was extracted for four hours with petroleum ether in a Soxhlet apparatus at a rate of one cycle every 6 or 7 minutes. The extract was concentrated and partitioned with acetonitrile; the acetonitrile evaporated to dryness and the residue eluted from a Florisil column. The extract was then identified by gas chromatographs equipped with electron capture detectors (tritium source). Column packing and operating parameters were as follows:

Columns: 5' x 1/8", glass, packed with 3% DC-200 on Gas Chrom Q, 80/100 mesh.

Temperature:

Detector	210°C
Injector	210°C
Oven	190°C

Carrier: prepurified nitrogen at a flow rate of 40 ml/minute.

Chart Speed: 30"/hour.

All calculations were made on the basis of 100% recovery and toxaphene concentrations stated in parts per million (ppm). Environmental samples with 0.25 to 0.15ppm were recorded as "trace" and concentrations of less than 0.15ppm were not distinguishable from zero. Water samples with 0.25 to 0.001ppm were reported as "trace" while water samples with less than 0.001ppm were not considered statistically significant.

COLLECTION STATIONS

The areas of collection are shown by Figures 1 and 2.

Mackay River: This station was established in March 1971 and oysters were collected on a monthly basis. Analysis of the samples revealed toxaphene in increasing amounts from March through June. This increase may possibly be indicative of the increasing proportion of gametogenetic tisuse to muscle tissue during these months instead of increasing amounts of toxaphene in the water. The amount of toxaphene decreased markedly in July, 1971. If the above hypothesis is true, this might indicate that the majority of the oysters had spawned by that time. All five oyster samples from this station contained toxpahene (Table 4).

Quadrat #2: This station is largely marsh surface area covered with Spartina and bordered by one small creek. It was necessary to navigate this creek in a small outboard and collections of fish had to be made with a castnet. Nine samples were taken from this quadrat and analyzed for toxaphene content.

Littorina, small crabs and green Spartina were free of toxaphene while dead Spartina, Fundulus and mullet were found to contain toxpahene in measurable amounts (Table 5), the mullet bearing the highest concentration of toxpahene (33 to 35 ppm).

Quadrat #29: Collections were made in Terry Creek for this quadrat. Forty-eight samples were taken for analysis by gas chromatography; six were found to be free of toxaphene, however, five of these were birds. The remaining clean sample was of small crabs. The sediments taken here and elsewhere will be discussed in a following separate section. Anchovies, Fundulus and Littorina samples all revealed high concentrations of toxaphene. The concentration in these organisms has generally decreased from July and August 1970 to June and July 1971, even though they are still high. The Spartina had a very high toxaphene concentration (18 to 98ppm) and the wood chips which abundantly line the bottom also contained large amounts of toxaphene. (The interstitial water pressed from these chips was filtered and then both filtrate and filter paper extracted for toxaphene. The great majority of the toxaphene was found on the filter paper while the filtrate was comparatively clean. This would indicate that little toxaphene actually dissolves in the water but rather the greater amount is carried on the particulate matter in the water). Analysis of birds from this quadrat mainly indicates that while birds are contaminated with toxpahene more have dieldrin and DDE in their tissues. Table 6 summarizes the data from this station.

Quadrats #26/34: This area is a portion of Little River through which a continuous trawl could be made. Nineteen samples from these quadrats were analyzed for toxaphene and six were found to contain none. The clean samples included estuarine water, small crabs, terrapin liver (though it contained dieldrin), green Spartina and dried Littorina. Toxaphene contaminated samples include all fin fishes analyzed, oyster, Littorina, starfish, blue crab, green and dead Spartina. Table 7 depicts the concentrations of toxaphene for the various samples considered.

Quadrat #33: This collection station at the Torras Causeway toll bridge was established in March 1971. The amounts of toxaphene fluctuated in the fourteen oyster samples taken here (Table 8). Toxaphene was determined in a blood ark from this station while Ulva (sea lettuce) collected here had no toxaphene.

Quadrat #56: Littorina, Spartina, ribbed mussel and estuarine water were collected for analysis at this location. Ten samples were extracted and three found to be free of toxaphene (green Spartina and Littorina). Table 9 shows the results of the toxaphene analyses from this station. An unusually high concentration of toxaphene was found in Littorina in July 1971.

Quadrat #60: Twenty-four samples from this branch of Clubbs Creek were analyzed. Five samples (Spartina and dried Littorina) were found free of toxaphene. Fin fishes, shrimp and one sample of Spartina from August make up the contaminated samples. Table 10 depicts a gradual decrease in amounts of toxaphene found here.

Quadrat #72: Near the mouth of Clubbs Creek, twenty samples were collected. Small crabs and Spartina (except Aug. 1970) were not contaminated with toxaphene. Fin fishes, shrimp and Littorina demonstrated measurable concentrations of toxaphene (Table 11). Anchovies, shrimp and Spartina all demonstrated a decrease of toxaphene concentrations during the study period.

Quadrat #78: Seventeen samples were collected from this location. Eight samples with no measurable toxaphene included tissues from blue crab, dried Littorina, whole shrimp and boat-tailed grackle. Spartina (green and dead), Fundulus, anchovies shrimp head and thorax, and mullet made up the contaminated samples. Fundulus, anchovies and Spartina all have significantly less toxaphene in June and July, 1971, compared to August and October, 1970, (Table 12).

Quadrat #99: Six of sixteen samples tested from Plantation Creek contained toxaphene. These six included anchovies, shrimp head and thorax, oysters and spot. The water, Spartina, shrimp abdomen, Littorina, cutlass fish and croaker had no measurable amounts of toxaphene (Table 13). These were the only fin fishes found between August 1970 and July 1971 at any station that did not contain toxaphene.

SEDIMENT STUDIES

The first sediment samples were collected in the Terry Creek study area in May 1969. One grab sample was taken from the mud surface at mean low water level at the junction of Dupree and Terry Creeks on the Northeast shore (Figure 1). This sample consisted of mud and ground wood particles. Laboratory analysis indicated a toxaphene concentration of over 4,200ppm. For confirmation, a portion of the sediment sample was analyzed at the University of Wisconsin Water Laboratory and found to contain 4,700ppm toxaphene. In addition, an aliquot of our extract was analyzed by the USDI Biological Laboratory, Gulf Breeze, Florida, which showed 4,600ppm toxaphene.

On July 16, 1969, another sediment grab sample taken at approximately the same area consisted primarily of mud. This sample contained 1,566ppm toxaphene. A sample taken at the mouth of Terry Creek (junction with Back River) on the same date contained 310.7ppm toxaphene.

Sediment samples were taken on the North shore of Terry Creek, 20 yards East of Dupree Creek, May 1, 1971, with a 45mm I.D. clear plastic core barrel. The core barrel, sharpened on the bottom end, was forced by hand 35cm into the sediment. The barrel was then filled with estuarine water, stoppered, withdrawn from the sediment, stoppered on the bottom and the excess water decanted off the top. The core barrel was returned to the laboratory and sliced into 5cm portions.

The sample from each 5cm tube was placed in a beaker and thoroughly mixed. A portion of this was spread thinly in a petri dish and allowed to dry at ambient temperature for six days. Samples for extraction ranged from 5 to 15 grams, dry sediment, depending on the depth of the sample. The toxaphene concentrations of these samples are found in Table 14. Toxaphene has a very high affinity for expanding lattice clays such as are found in marsh mud. This explains the relatively low concentration in the upper sand layer. The surface to 5cm sample chromatogram was almost identical to the technical grade toxaphene standard. The 10 to 15cm sample gave a good chromatogram for an environmental sample. The 15 to 20cm sample chromatogram showed a resemblance to toxaphene but was somewhat distorted. The 25 to 30cm sample chromatogram showed a tremendous build-up of peaks in the first part of the chromatogram. These chromatograms were quantitated as "a hydrocarbon resembling toxaphene".

Other sediment samples were collected with a 70 mm I. D. clear plastic core barrel on June 10, 1971. The larger core barrel created less internal friction and thus enabled us to collect a core sample of 80cm in depth. The samples were extracted from the core barrel in 10cm increments by forcing the sample out with a tightly fitting rubber plunger. Samples were extracted in the above described manner. This series of cores was taken at three locations: #1, North shore of Terry Creek,

50 yards East of the junction with Dupree Creek; #2, South shore of Terry Creek about one half the distance from the Hercules outfall to Back River; #3, South shore of Terry Creek 50 yards West of the junction with Back River (see Figure 1). All samples were taken at mean low water level.

The chromatograms from this series fo cores exhibited generally the same pattern of change with increasing depth as did those run on the smaller core. Table 16 indicates that toxaphene has a greater affinity for wood chips than for mud. This may or may not be true. The wood chips were introduced into the plant effluent ditch before the effluent reached the estuary and may have absorbed large quantities at this time. We have theories but no real explanation of the high toxaphene levels at the 30 to 50cm depth as shown in Table 17. The chromatograms of these two sub-samples more closely resembled technical grade toxaphene than the sub-samples collected above or below. This leads one to speculate that there had been an overturning of the sediments in this area. This is feasible since there are 1.) frequent mud slides and other disturbances caused by rain; 2.) daily trips by shrimp trawlers causing large wakes; and 3.) pleasure craft creating smaller wakes.

Two sediment samples were collected June 23, 1971, shortly after the termination of the U.S. Army Corps of Engineers' dredging of Terry Creek. The local news media quoted a figure of 10,000ppm toxaphene in the dredge spoil at the mouth of Terry Creek.

A composite from 10 points covering the spoil area (resultant from two days dredging operations) was split with Hercules, Inc., for analysis. Our analysis of the composite sample revealed 32.8ppm toxaphene while Hercules, Inc., found 30.6ppm toxaphene in the split sample. Another split sample was collected the same day from dragline spoil in Hercules, Inc.', wood yard at the new dock site. This was a dry, lumpy sample and not well mixed prior to splitting the sample with Hercules, Inc. Our analysis revealed 436.6ppm and Hercules' about 500.0ppm toxaphene. Surface sediment samples from two regular sampling stations, #34 and #78, contained 10.4ppm and 3.02ppm toxaphene respectively on the same date.

Other marsh mud sediment samples were collected from South End Creek on Sapelo Island. The sample was washed and sieved through a screen wire sieve to eliminate any shell and large plant material. Sixty grams, wet weight, of the slurry was placed into each of four beakers and three of these (B, C and D) spiked with technical toxaphene dissolved in acetone. Beaker A, without toxaphene, was kept open in the laboratory. Spiked sample C was covered with a vented glass cover and kept in full sunlight for 4 days. Spiked sample D was left in full sunlight for 4 days with no cover. All samples were then dried at ambient temperature in the laboratory for four additional days under identical conditions before being extracted. The samples at this time weighed approximately 25 grams each.

Sample A gave a clean chromatogram which showed no contamination that would interfere with toxaphene analysis. Sample B, covered with plastic, was identical to technical grade toxaphene. Sample C, glass covered, showed very little change from Sample B. Sample D, open, showed a drastic change after only four days in open sunlight.

The results of this experiment indicate that the glass cover of Sample C filtered out ultra violet radiation which possibly destroys toxaphene. Sample D, exposed to full sunlight, indicates that toxaphene is rather quickly broken down (theoretically by the ultra violet light).

The beakers in the above experiment were 12cm deep and thus inhibited full sunlight on the entire sample for most of the day. Experiments will be initiated in low-sided petri dishes and exposed to open sunlight and to artificial ultra violet light for varying periods of time. Bioassays using marine organisms will be conducted in the near future to determine the toxicity of the photo-altered toxaphene.

SPECIES DIVERSITY

Trawl samples collected with a ten foot otter trawl (1-3/8" mesh) during the 1970-1971 study period were returned to the laboratory. After sorting, different species were wet-weighed and the total length of each fish was recorded. Field physical data collected simultaneously with the trawl included salinity, water temperature, tide stage, time of day, climate conditions, trawl duration and trawl identification number.

All trawl data was placed on key punch cards for later computer manipulation.

Species diversity indices have been considered by investigators to be an index of environmental quality of a given ecosystem. (Harrel and Dorris 1968; Dahlberg and Odum 1970). These investigators suggested that species diversity will decrease with increased contamination or pollution. The present study proposed that diversity of fin fish and economic epifaunal invertebrates could serve as one indicator of the effects of toxaphene and other contaminants in the salt marshes east of Brunswick, Georgia.

To date, three different species diversities have been calculated for the trawl data from this study.

For the "species richness" componentof diversity, Margalef (1958) suggests the following:

$$D_1 = (S-1)/\log N \qquad (1)$$

where D_1 = diversity, S = number of species, and N = number of individuals.

The second species diversity index used is according to Menhinick (1964) where:

$$D_2 = S/N \qquad (2)$$

here, S, and N have the same definition as before.

A third species diversity index, that of Odum et al. (1960) was used where:

$$D_3 = S * 1000/N$$

here too S and N have the same definition.

The results of species diversity indices for each different quadrat are summarized in Tables 18 - 20. The monthly values are contrasted to calculation of D_1, D_2, and D_3 from trawl samples taken in the Duplin estuary, Sapelo Island, Georgia. The results demonstrate that lowest diversity indices of all sample areas considered were found in quadrat #29 (Terry Creek) than any other location.

This data has not been statistically treated due to the need to collect additional seasonal information. At the conclusion of the second year of the current research contract, additional species diversity indices will be computed on data from the first and second years using the IBM 360-65. Computer programs are presently being written for computation of the additional following species diversity indices:

Shannon-Wiener diversity index $\overline{H}$

$$H = - \sum_{i=1}^{S} P_i \log P_i$$

where P_i is the proportion of individuals in the ith species. This index suggested by Margalef (1968) is a widely used index that increases as both the number of species and equability of species abundance increase.

Lloyd and Ghelardi's (1964) relative species abundance or equability index:

$$E = S^1/S$$

where S^1 is the number of species predicted by the "broken stick" model of MacArthur (1957) and S is the number of observed species.

As long as the type of data remain the same, Sanders (1968) claims his rarefaction method will allow comparison of unequal sample sizes which would result in the present study from unequal trawl lengths. The data from this three-year summary will also be compared by this technique.

A length frequency plot of each fish species would be meaningless at this time since no comparable data are available for comparison. Here too, it is more efficient and environmentally meaningful to compare the first and second year trawl data. Hence, further manipulation of the first year trawl data will be postponed until the end of the second contract year. All trawl data is available for inspection at the Marine Institute.

It is important to point out that many variables may effect the catch effort. It is anticipated that repeated monthly sampling during the three years of the contract will provide statistically significant environmental conclusions in spite of differences in trawl time, tide stage, salinity, water temperature, season of the year and climatic conditions. Once again, computer evaluation of the data will also most efficiently be conducted on at least two or three years' data.

BIOASSAY OF "TREATED EFFLUENT"

Hercules, Inc. presently is operating a pilot plant for the detoxification of its waste products from the toxaphene production process. The pilot plant is designed to reduce the toxicity of the effluent from the operations before it enters the estuarine waters of Terry Creek. Bioassays were designed to gain a preliminary indication of the toxicity of this treated effluent to selected estuarine organisms. Static bioassays were used for this preliminary research.

A freshly collected sample of effluent from the pilot plant was diluted with estuarine water collected from the ocean beach at Sapelo Island. The dilution of the effluent with estuarine and distilled waters resulted in salinities in the estuarine range (15 to 20 $^{o}/oo$). All static assays were conducted in one gallon rectangular glass aquaria with glass covers. Compressed air was used for aeration via an airstone. Comparisons between toxicity were made using "raw" ocean water, and membrane filtered (0.45μ pore size) ocean water but no significant differences were observed, consequently unaltered ocean water was used for all further dilutions.

A sample of the effluent used for each bioassay was analyzed for toxaphene and other chlorinated hydrocarbons prior to the assays. The effluent and dilution water were mixed, and placed in glass aquaria.

Estuarine organisms used in the bioassays were collected with a ten foot otter trawl. These estuarine fauna collected from the Duplin Estuary (adjacent to Sapelo Island) were placed in flowing seawater holding tanks in the laboratory for 48 hour acclimation prior to bioassays. (Exceptions to these conditions are stated below). Bioassays were conducted using the white shrimp (Penaeus setiferus); sea catfish (Arius felis); tonguefish (Symphurus plagiusa); and mullet (Mugil sp.).

The results of the bioassays are summarized in Tables 21 - 25. Absence of data indicated by a dash indicates that particular combination of factors was not tested. All concentrations of bioassay media are expressed in parts per billion (p.p.b.). This is the toxaphene concentration of the diluted effluent used for each static bioassay. The fractional expression used for each concentration and replicate is the number of living organisms at the end of the duration of assay over the total number of living organisms used at the outset of the assay. An example 3/5 indicates that initially five organisms were used in the assay and at the end of the exposure period three of the organisms were still living. Control assays used for each experiment consisted of ocean water and distilled water diluted to the same salinity as the assay test solutions; however, the control media contained no treated effluent.

The mean survival is tabulated as per cent of total number used surviving after exposure for a given time and at a certain concentration of treated effluent.

These preliminary results suggest toxicity to estuarine organisms at 8 p.p.b. toxaphene in the diluted effluent. No further conclusions can be drawn from these data. This work does suggest the urgent need for accurate flushing rates and hydrographic data on Terry Creek. In order to compute actual field dilutions of the toxaphene plant effluent, hydrographic data will be required to predict dilution factors during various tidal regimes.

FUTURE RESEARCH

The first year of the research has focused on several different facets of toxaphene contamination and estuarine ecology. During the second year, emphasis will shift from routine field collections to a more concentrated effort to "fill in" the data where additional observations seem necessary.

One new facet to be investigated will be sediment-grass relationships. Using marsh sediment, standard toxaphene will be mixed with the mud in varying concentrations. A subsample of this mud will be exposed to ultraviolet light for varying lengths of time. Following a recovery of the "UV light-treated toxaphene" a portion will be analyzed and another portion used for static bioassays. Another portion of the "labeled" mud will be used for growing Spartina (salt marsh cordgrass) under constant environmental conditions. This may enable quantification of the flux of toxaphene through Spartina. For a more quantitative approach to this, the use of ^{36}Cl labeled toxaphene may be provided by Hercules, Inc.

Another focal point during the second contract year will be the sediment concentrations of toxaphene in the marsh area under consideration. Duplicate cores will be taken in each of the quadrats. Also, if dredging is resumed in Terry Creek, we will have adequate background data on sediment concentrations of toxaphene.

In the field, more birds, insects and detritivores will be evaluated for toxaphene. Since the Spartina in the Terry Creek area (quadrat #29) appears to contain significant concentrations of toxaphene, this industrial contaminant will be employed to learn more about the estuarine detritus food web.

Several new staff members of the Marine Institute are already interested in looking into primary production, benthic respiration, community metabolism and the organic carbon budget of the marshes influenced by industrial contaminants contrasted to the more pristine marshes adjoining Sapelo Island, Further studies will probably be proposed during the next contract year to expand the present study in scope and duration. Efforts also may be directed toward separating out the effects of toxaphene contamination from other industrial contaminants.

The importance of the need for complete hydrographic studies in Terry Creek and surrounding areas can not be overemphasized. This data will facilitate all future calculations of effluent concentration and dilution. Several techniques for hydrographic examination of Terry Creek are available. Current research efforts utilizing remote sensing can be used to predict tidal inundation in the marsh. This coupled with thermal imagery can yield meaningful data in terms of the hydrography in the marsh watershed between Brunswick and St. Simons Island.

CONCLUSIONS

Based on the data reported herein, toxaphene concentration in fauna and flora of the Brunswick marsh area has decreased during the first contract year (1 July 1970 through June 1971). The following lists a summary of the research results:

1. Unique use of an industrial contaminant as an ecological label has been made in further quantification of the estuarine detritus food web.
2. Toxaphene has been found in significant quantities in Spartina alterniflora Loisel (salt marshcordgrass) in the marshes bordering Terry Creek.
3. Measurable quantities of toxaphene have been found in the salt marsh periwinkle (Littorina). This too suggests that Littorina may be involved in the Spartina detritus food web. There is no measurable toxaphene in these snails, however, if they are dried 4 to 5 days at 100° prior to toxaphene analysis.
4. Although the scientific literature has considered the fiddler crab (Uca) to be a detritivore, preliminary data suggests that fiddler crabs collected during this study contained no measurable quantity of toxaphene; consequently they may not be part of the Spartina detritus food web. This, however, requires further documentation.

5. Fin fish detritivores including killifish, mullet, menhaden and anchovies all were found to contain measurable quantities of toxaphene. This reconfirms their role in the Spartina detritus food web.
6. Toxaphene is concentrated in the white shrimp head and thorax (which contains the digestive system) by a factor of about ten times that found in the edible abdominal section.
7. The concentration of toxaphene in mullet is slightly higher in muscle tissue than it is in liver tissue.
8. Concerning marsh sediment samples, surface sediment chromatograms (for toxaphene analysis) more closely resemble technical grade toxaphene chromatograms than deeper core chromatograms. Although the sediment concentrations of toxaphene vary with distance away from Terry Creek, the surface mud chromatograms still closely resemble chromatograms of technical grade toxaphene.
9. Toxaphene concentrations in marsh surface sediment decrease with exposure to sunlight. It is suggested that ultra-violet radiation may be responsible for this degradation. Further research is suggested for confirmation.
10. Sediment samples from the short lived dredging operation in Terry Creek (June 1971) revealed toxaphene concentrations of 33ppm in the dredging spoil.

11. Based on analyses of water samples, toxaphene appears to be confined to the suspended material in the water and not dissolved in the estuarine water.
12. Preliminary static bioassays of selected estuarine fauna suggest that toxaphene concentrations greater than 8 p.p.b. in the diluted "treated effluent" may be toxic.
13. Species diversity in the various study quadrats more closely resembles species diversity of the Duplin estuary toward the end of the first contract year.

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APPENDIX

Quadrat #	Date	Type	Tox. ppm
60	Jul. '71	liver	6.28
"	"	muscle	7.65
2	"	liver	33.85
"	"	muscle	35.6

Table 1. Variation of toxaphene concentrations in mullet liver and muscle tissue.

Quadrat #	Date	Type	Tox. ppm.
72	Oct.'70	muscle	.64
"	"	heads	7.29
"	Jul.'71	muscle	Trace
"	"	heads	2.38
60	Oct.'70	muscle	1.30
"	"	heads	11.15
99	"	muscle	0.00
"	"	heads	2.83

Table 2. Variations of toxaphene concentrations in shrimp heads and muscle tissue. *

Quadrat #	Tox. concentration	
	green	dead
78	0.0	1.11
72	0.0	0.0
26/34	0.0	Trace
60	0.0	0.0
99	0.0	0.0
2	0.0	1.76
56	0.0	1.61

Table 3. Toxaphene concentrations in Spartina in April 1971.*

* "Trace" indicates less than .25ppm.

Date	PPM Toxaphene
Mar.'71	.69
Apr.'71	.71
May '71	1.60
Jun.'71	2.04
Jul.'71	Trace

Table 4. Toxaphene in oysters from Mackay River.*

*"Trace" indicates less than .25 ppm.

Date	Type	PPM Toxaphene
Aug.'70	small crabs	0.0
"	Spartina	2.45
Oct.'70	Littorina (dried)	0.0
Apr.'71	Spartina (green)	0.0
"	Spartina (dead)	1.76
Jun.'71	Spartina (green)	0.0
Jul.'71	Fundulus	10.4
"	mullet liver	33.85
"	mullet muscle	35.6

Table 5. Concentrations of toxaphene in samples from Quadrat #2.

Date	Type	PPM Toxaphene	Other
Jul. '70	Littorina (held alive)	43.9	
"	Spartina (green)	33.76	
"	Spartina (dead)	18.10	
"	Spartina (green)	29.9	
"	Littorina	61.9	
Aug. '70	water	.017	
"	Fundulus	73.83	
"	anchovy	236.36	
"	Spartina (green)	72.76	
Oct. '70	small crabs	0.0	
"	anchovy	172.1	
"	Fundulus	80.71	
Feb. '71	anchovy	21.39	
"	Fundulus	51.9	
"	filter paper	>100.0	
"	filtrate	.015	
"	filter paper	>100.0	
May '71	anchovy	18.07	
"	Fundulus	53.7	
Jun. '71	Fundulus	50.5	
"	anchovy	13.8	
"	Spartina	98.8	
Jul. '71	Littorina	23.25	
"	Willet breast		136.5ppb DDE 8.07ppb Dieldrin
"	Willet liver		29.8ppb Dieldrin 118.5ppb DDE
"	Redwing breast		73.1ppb DDE Trace Dieldrin
"	Redwing liver		272.5ppb DDE 32.5ppb Dieldrin
"	Seaside sparrows		72.4ppb DDE 21.95ppb Dieldrin
"	Long-billed wren	4.08	39.0ppb Dieldrin
"	Dowitcher breast	1.89	
"	Dowitcher liver	5.14	

Table 6. Concentrations of pesticides in samples from Quadrat #29.

Date	Type	PPM Toxaphene
Aug.'70	water	0.0
"	small crabs	0.0
"	Spartina (green)	1.1
Jan.'71	turtle liver	0.0
Feb.'71	oyster	1.94
"	seastar	1.56
"	naked gobi	2.95
Apr.'71	blue crab guts	3.1
"	" muscle	Trace
"	Spartina (green)	0.0
"	" (dead)	Trace
"	Littorina (dried)	0.0
May '71	star drum	5.3
Jun.'71	Spartina (green)	0.0
"	anchovy	3.1
Jul.'71	anchovy	4.34
"	spot	Trace
"	Littorina	Trace

Table 7. Concentrations of toxaphene in samples from Quadrats #26/34. "Trace" indicates less than .25ppm.

Date	Type	PPM Toxaphene
18 Mar.'71	oyster	2.27
"	blood ark	1.07
1 Apr.'71	oyster	2.84
8 Apr.'71	"	3.64
13 Apr.'71	"	3.80
22 Apr.'71	"	2.03
1 May '71	"	3.82
"	Ulva lactuca	0.00
4 May '71	oyster	3.36
13 May '71	"	5.60
21 May '71	"	2.59
25 May '71	"	1.17
2 Jun.'71	"	1.12
10 Jun.'71	"	2.06
23 Jun.'71	"	1.35
13 Jul.'71	"	1.15

Table 8. Concentrations of toxaphene in samples from Quadrat # 33.

Date	Type	PPM Toxaphene
Aug.'70	water	.015
"	Littorina	3.0
"	Spartina (green)	1.49
Oct.'70	Littorina	0.0
Apr.'71	Spartina (green)	0.0
"	Spartina (dead)	1.61
"	Modiolus	2.86
Jun.'71	Spartina	0.0
Jul.'71	Littorina	1.81
"	Littorina	16.74

Table 9. Concentrations of toxaphene in samples from Quadrat # 56.

Date	Type	PPM Toxaphene
Aug.'70	spot	46.73
"	Fundulus	4.11
"	mullet	35.42
"	shrimp	3.03
"	Spartina	.59
Oct.'70	Littorina (dried)	0.0
"	Fundulus	15.58
"	shrimp heads	11.15
"	shrimp muscle	1.3
"	anchovy	25.1
Apr.'71	anchovy	lost
"	Spartina	0.0
"	Spartina	0.0
May '71	croaker	1.97
Jun.'71	spot	1.37
"	Spartina	0.0
Jul.'71	spot	1.15
"	shrimp heads	trace
"	yellowtail	2.71
"	anchovy	1.02
"	Fundulus	2.48
"	mullet liver	6.28
"	mullet muscle	7.65

Table #10. Concentrations of toxaphene in samples from Quadrat #60. "Trace" indicates less than .25ppm.

Date	Type	PPM Toxaphene
Aug.'70	spot	26.2
"	anchovy	17.37
"	small crabs	0.0
"	Spartina	.61
Oct.'70	shrimp heads	7.29
"	shrimp muscle	.64
"	anchovy	10.24
Feb.'71	menhaden	1.27
"	weakfish	2.51
Apr.'71	Spartina	0.0
"	Spartina	0.0
May '71	sea catfish	trace
Jun.'71	star drum	.65
"	Spartina	0.0
Jul.'71	shrimp heads	2.38
"	shrimp muscle	trace
"	anchovy	2.11
"	small crabs	0.0
"	small crabs	0.0
"	Littorina	

Table #11. Concentrations of toxaphene in samples from Quadrat #72. ("Trace" indicates less than .25ppm.)

Date	Type	PPM Toxaphene
Aug.'70	Spartina	1.22
Oct.'70	Fundulus	7.6
"	anchovy	36.7
Apr.'71	Littorina (dried)	0.0
"	Spartina (green)	0.0
"	Spartina (dead)	1.11
May '71	blue crab gut	(Aroclor 1254)
"	blue crab muscle	"
Jun.'71	shrimp heads	1.8
"	Spartina	0.0
Jul.'71	anchovy	2.35
"	shrimp heads	0.0
"	mullet muscle	2.38
"	Fundulus	2.36
"	grackle	(DDE & Diel.)
"	grackle	"

Table #12. Concentrations of toxaphene in samples from Quadrat #78.

Date	Type	PPM Toxaphene
Aug.'70	water	0.0
"	anchovy	4.51
Oct.'70	Spartina	0.0
"	shrimp heads	2.83
"	shrimp muscle	0.0
Apr.'71	oyster	1.77
"	spot (muscle)	1.14
"	Spartina	0.0
"	Spartina	0.0
"	Littorina (dried)	0.0
May '71	anchovy	6.7
Jun.'71	anchovy	4.59
"	cutlass fish	0.0
"	Spartina	0.0
Jul.'71	croaker	0.0
"	shrimp heads	0.0

Table 13. Concentrations of toxaphene in samples from Quadrat #99.

Surf. to 5 cm	5 to 10 cm	10 to 15 cm	15 to 20 cm	20 to 25 cm	25 to 30 cm	30 to 35 cm
302.0 ppm	1,087.3 ppm	1,972.0 ppm	925.0 ppm	213.0 ppm	150.3 ppm	77.3 ppm
sand	sand wood chips	mud, sand wood chips	mud wood chips	mud, few wood chips	mud	mud

Table 14. Concentrations of toxaphene in core sample from the North shore of Terry Creek, 20 yards East of Dupree Creek, May 1, 1971.

Surf. to 10 cm	10 to 20 cm	20 to 30 cm	30 to 40 cm	40 to 50 cm	50 to 60 cm	60 to 70 cm	70 to 80 cm
1,858.3 ppm	1,340.5 ppm	1,324.0 ppm	1,367.2 ppm	1,236.7 ppm	433.6 ppm	68.5 ppm	83.2 ppm
mud some chips	mud few chips	mud	mud	mud	mud	mud	mud

Table 15. Concentrations of toxaphene in core from location #1, North shore Terry Creek, 50 yards from junction with Dupree Creek, June 10, 1971.

Surf. to 10 cm	10 to 20 cm	20 to 30 cm	30 to 40 cm	40 to 50 cm	50 to 60 cm	60 to 70 cm
111.85 ppm	615.64 ppm	16.04 ppm	17.46 ppm	5.42 ppm	3.4 ppm	2.88 ppm
mud few chips	mud many chips	mud	mud	mud	mud	mud

Table 16. Concentrations of toxaphene in core from location #2, South shore of Terry Creek, $\frac{1}{2}$ the distance from Hercules outfall to Back River, June 10, 1971.

Surf. to 10 cm	10 to 20 cm	20 to 30 cm	30 to 40 cm	40 to 50cm	50 to 60 cm	60 to 70 cm	70 to 80 cm
35.5 ppm	35.47 ppm	21.9 ppm	70.65 ppm	79.8 ppm	21.0 ppm	18.5 ppm	5.27 ppm
mud	mud	mud	mud	mud	mud	mud	mud

Table 17. Concentrations of toxaphene in core from location #3, South shore of Terry Creek, 50 yards West of Back River, June 10, 1971.

Table 18. Species diversity index according to Menhinick (1964) for each quadrat and collecting data sampled. (D_2)

Date	Quadrat 029 Terry Creek	026/034	060	072	078	099	Duplin Estuary
25 Aug 1970	0.37	0.00	0.37	1.33	no sample	0.49	0.47
23 Oct 1970	0.39	0.68	1.21	1.25	0.60	1.00	0.72
5 Jan 1971	1.41	0.50	1.00	0.31	0.71	1.27	1.01
2 Feb 1971	0.14	0.65	no sample	0.83	no sample	no sample	0.81
2 Mar 1971	0.16	1.29	0.86	2.15	1.20	1.30	0.92
6 April 1971	0.39	1.55	0.77	1.58	0.44	1.22	0.56
27 April 1971	1.34	0.61	0.21	0.80	0.70	0.63	0.42
25 May 1971	0.81	1.70	0.86	1.04	0.70	1.51	1.81
23 June 1971	0.81	0.89	1.78	1.60	0.96	1.22	0.65

Table 19. Species diversity index according to Odum et al. (1960) for each quadrat and collecting date. (D_3)

Date	Quadrat 029 Terry Creek	026/034	060	072	078	099	Duplin Estuary
25 Aug 1970	68	1000	71	357	no sample	61	17
23 Oct 1970	52	158	163	312	120	250	47
5 Jan 1971	1000	250	1000	90	500	400	126
2 Feb 1971	7	38	no sample	53	no sample	no sample	83
2 March 1971	25	333	250	387	363	266	57
6 April 1971	76	400	200	500	200	500	35
27 April 1971	300	63	45	107	500	200	35
25 May 1971	333	363	250	155	500	571	466
23 June 1971	333	200	800	428	185	500	47

Table 20. Species diversity index according to Margalef (1958) for each quadrat and collecting date sampled. (D_1)

Date	Quadrat 029 Terry Creek	026/034	060	072	078	099	Duplin Estuary
25 Aug 1970	0.41	0.00	0.40	3.40	no sample	1.65	4.17
23 Oct 1970	1.14	1.56	4.59	3.32	1.43	2.49	4.22
5 Jan 1971	3.32	0.00	0.00	0.00	0.00	3.00	3.89
2 Feb 1971	0.75	4.06	no sample	5.03	no sample	no sample	3.53
2 March 1971	0.00	3.40	1.85	7.37	2.88	2.55	5.78
6 April 1971	0.76	4.25	0.92	4.00	0.00	2.57	3.33
27 April 1971	3.84	2.53	0.00	2.86	0.00	1.00	1.85
25 May 1971	1.28	5.21	1.85	3.63	0.00	3.54	5.10
23 June 1971	1.28	2.31	4.29	4.36	2.79	2.57	3.47

Table 21. Twenty-four hour bioassay of "treated effluent" using white shrimp (Penaeus setiferus)

Toxaphene concentration of diluted effluent	# of organisms living after 24 hours / # of organisms at beginning of assay			Mean expressed as % of survival
	Replicate 1	Replicate 2	Replicate 3	
control	4/5	5/5	4/5	86%
60ppb	0/5	0/4	0/5	0%
45ppb	0/5	0/5	--	0%
30ppb	0/5	0/5	--	0%
8ppb	1/5	3/5	--	40%
0.8ppb	4/5	5/5	5/5	93%
0.08ppb	4/5	5/5	3/5	80%

Table 22. Forty-eight hour bioassay of "treated effluent" using white shrimp (Penaeus setiferus)

Toxaphene concentration of diluted effluent	# of organisms living after 48 hours / # of organisms at beginning of assay			Mean expressed as % of survival
	Replicate 1	Replicate 2	Replicate 3	
control	3/4	3/4	1/3	63%
8ppb	3/4	1/4	2/4	50%
0.8ppb	2/4	3/4	3/4	66%
0.08ppb	3/3	3/4	2/4	66%
0.008ppb	3/4	3/3	3/4	81%

Table 23. One hundred twenty hour bioassay of "treated effluent" using sea catfish (Arius felis)

Toxaphene concentration of diluted effluent	# of organisms living after 120 hours / # of organisms at beginning of assay		Mean expressed as % of survival
	Replicate 1	Replicate 2	
control	2/2	2/2	100%
0.03ppb	0/4	2/2	33%
<0.01ppb	6/6	6/6	100%

Table 24. Ninety-six hour bioassay of "treated effluent" using tonguefish (Symphurus plagiusa)

Toxaphene concentration of diluted effluent	# of organisms living after 96 hours / # of organisms at beginning of assay		Mean expressed as % of survival
	Replicate 1	Replicate 2	
control	3/3	--	100%
1.7ppb	0/4	4/4	50%
0.4ppb	4/4	2/4	66%
	using sea catfish (Arius felis) for the same exposure		
control	2/2	--	100%
1.7ppb	3/4	--	75%
0.5ppb	2/2	2/2	100%

Table 25. One hundred sixty-eight hour bioassay of "treated effluent" using mullet (Mugil sp.)

Toxaphene concentration of diluted effluent	# of organisms living after 168 hours / # of organisms used in bioassay		Mean expressed as % of survival
	Replicate 1	Replicate 2	
control	3/3	--	100%
1.5ppb	0/2	0/3	0%
0.4ppb	3/3	2/3	83%