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ABSTRACT

Analysis of Chemical Contaminants in Tissue Samples from Two Species of Fish

by

Michael David Reive

Interaction of marine species with chemical pollutants within the marine ecosystem is extremely complex and difficult to interpret. The purpose of this thesis was to investigate the effect of an exposure of treated municipal wastewater on winter flounder (*Pseudopleuronectes americanus*) and striped bass (*Morone saxatilis*). Fish from two locations were exposed to different concentrations of wastewater. Half of the fish from each tank were sacrificed, and the remaining fish were given a depuration period in unpolluted water. Tissue samples were composited, soxhlet extracted and passed through a florisil cleanup column. Gas chromatography was used to analyze for hexachlorobenzene, hexachlorobutadiene, Aroclor 1254, bis-(ethylhexyl) phthalate, and di-n-octyl phthalate. The results show widespread distribution of the contaminant chemicals in the samples, typical of bioaccumulation and bioconcentration processes within the marine ecosystem. The levels of chemicals found in the samples were comparable to other studies of similar urban estuaries through out the United States.

ANALYSIS OF CHEMICAL CONTAMINANTS IN TISSUE SAMPLES FROM TWO SPECIES OF FISH

by
Michael David Reive

Thesis submitted to the Faculty of the Graduate School of
the New Jersey Institute of Technology in partial fulfillment of the
requirements for the degree of
Master of Science in Environmental Science
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1 INTRODUCTION

1.1 General

There are an abundant number of carcinogenic or possible carcinogenic compounds to be found in the marine ecosystems around the coastline of the United States. Statistics show that in the United States every 62 seconds a person dies of cancer. As cancer mortality rates climb, people become more health conscious especially in terms of what they eat. Sea food is an extremely healthy food, but not if it is contaminated with toxic chemicals. As aquaculture and marine fisheries become more and more important as a food resource the protection and management of our marine ecosystems are a great concern and we mismanage them at our peril.

In her perceptive book, "*Silent Spring*", Rachel Carson described how we have polluted the environment and thus threatened the survival of many forms of life, including our own. In the 1960's, her warnings echoed throughout the world and we learned to fear chemicals, such as toxic metals and chlorinated hydrocarbons.

Twenty years ago on April 22, 1970, the United States celebrated the first Earth Day with some 20 million citizens participating, the largest grassroots political demonstration ever. Earth Day 1970 was generally considered to be the event that marked the coming of age of the modern environmental movement. Today, two decades later, Earth Day 1990, which celebrates the anniversary of the first Earth Day, marks the global reach of environmentalism, as over 130 nations and upwards of 200 million people participate in a celebration on conservation of the planet. The United States, as well as the United Nations, have passed resolutions designating the 1990's as the decade of the environment.

Global environmental problems such as "The Greenhouse Effect and Global Warming", "Acid Rain", "Destruction of the Tropical Rain Forests", "The Hole in the Ozone Layer" and "Photochemical Smog" gain ever increasing publicity from the news media. However, one must not forget the huge burden of toxic chemicals that reaches the marine environment every year, and indeed their effect on the complex marine ecosystem.

Most major estuaries and harbors near heavily industrialized areas have been polluted with a myriad of toxic chemicals. This tremendous burden of toxic pollutants comes from many point and non-point sources. These include treated and non-treated municipal sewage, atmospheric fallout, urban runoff, spills and pipeline fractures, and numerous treated and non-treated industrial discharges. Once in the environment, the pollutants are subjected to transport and distribution processes within the marine ecosystem and become distributed in seawater, sediments, and organisms. Maximum concentrations of pollutants occur in the estuary and then tail off in open waters as dilution factors become more apparent. Chemical contaminants may be bioavailable to the organisms of the marine ecosystem, where they can be ingested or absorbed to become a part of the food chain. There, metabolism, excretion and bioaccumulation factors influence the final distribution of the chemicals within the marine ecosystem.

Our views of marine pollution are changing. We are just now beginning to fully appreciate the immensity and complexity of the problem. Today, there are an estimated 70,000 chemicals in common use and pesticides alone include some 1500 active ingredients (11). Many of these chemicals will eventually find their way into the marine ecosystem, adding to the environmental load. Many pollutants, such as polychlorinated biphenyls (PCBs), are highly resistant to chemical and biological

changes and thus can remain in the marine environment for years. Other organic compounds are readily transformed into a host of "new" chemical structures. Most of these products cannot be detected in marine samples, even with our most sophisticated analytical techniques. Thus, there are many toxic chemicals in the marine environment that currently remain undetected. This makes it very difficult to ascertain whether it is a large concentration of a particular compound that causing a given toxic effect, or whether it is in fact a minute, possibly undetectable, concentration of a highly potent toxic substance that is in fact causing a toxic response by the organism in question. Furthermore, chemical interactions can cause synergistic, antagonistic and potentiating effects, all of which make it virtually impossible to identify the most toxic chemicals.

Sediments serve as a repository for a large number of environmental contaminants, and because these chemicals accumulate over time periods of up to several years, sediment chemistry provides a temporal overview of pollution. Analysis of certain chemicals in certain sessile benthic biota also provide a means of assessing environmental contamination. In addition, such chemical analyses of fish generally reflect chemical contamination over a wider geographical area than is possible with either analysis of sediment or of sedimentary organisms. This is due in part to the mobility of fish which allows them to integrate chemical sources over a wide geographical area.

Evidence from field and laboratory studies has demonstrated a link between certain types of fish diseases and selected environmental contaminants. Thus, these fish diseases have been very useful in identifying pollution associated perturbations. Many idiopathic lesions of fish have a suspected chemical etiology because they are morphologically similar to lesions observed in laboratory rodents and fish exposed

to toxic or carcinogenic chemicals. Effects of exposure on fish can be estimated from biochemical responses which include alterations in biochemical, respiratory, and immune functions, as well as changes in population structure and developmental and structural abnormalities. Fish exposed to chlorinated organics may be induced to produce higher levels of enzymes capable of transforming many contaminants into more polar, but occasionally more toxic, metabolites. This makes it even more difficult for investigators to elucidate the etiology of disease among marine and esturine fishes in highly contaminated areas, because not only the toxicities of the chemicals in the ecosystem have to be understood, but also the toxicities of their metabolites. Furthermore, there may be subtle differences within a species of fish with respect to immunologic of metabolizing enzyme function. Fish from polluted regions may have built up tolerance to their polluted environment, whereas fish from more pristine locations may be more suceptible to higher levels of marine pollution.

Both the East and the West Coast of North America have areas of coastline that have particularly high levels of marine pollution. These areas have been pin-pointed, identified, and studied in great detail by investigators in order to gain a greater understanding of the aquatic pollution problems facing the United States.

Results of investigations of pollution-related problems in coastal waters near the four major population centers (San Diego, Los Angeles, San Francisco, and Seattle-Tacoma) of the United States West Coast revealed a variety of toxic chemicals in bottom sediments and in selected marine species (18). Associated with some of the most contaminated of these locations were a number of pathological conditions in bottom fish species (for example liver lesions or fin erosion) from San Diego Bay, the Los Angeles area, and from Commencement and Elliot Bays in

Puget Sound, Washington. The map in Figure 1. shows the sampling sites in selected urban and rural areas on the West Coast.

Liver lesions, including neoplasms, have been detected in one or more fish species from the Los Angeles area, San Francisco Bay, and several sites in Puget Sound (18). The extensive studies at 46 sampling sites in Puget Sound have yielded correlative evidence of a relationship between certain liver diseases in English sole (*Parophrys vetulus*) and concentrations of aromatic hydrocarbons in sediment and metabolites or aromatic compounds in fish bile. High body burdens of toxic chemicals and the presence of pathological conditions have been successfully used as indicators of adverse pollution effects on fish. However, histopathological conditions of fish tend to reflect the effects of chronic, long-term exposures to polluted environments, and provide little indication of acute effects which may be occurring. Moreover, polluted environments that are conducive to the induction of fish neoplasia may also be responsible for a host of serious and potentially unrecognized changes at both the organismal and ecosystem levels. Table 1. demonstrates the widespread diversity of chlorinated compounds than have been found in sediments and fish liver samples in similar marine environments to that of the Hudson-Raritan estuary.

Figure 2. shows liver concentrations of PCBs from selected sites on the West Coast. Liver concentrations of PCBs were highest in fish from the urban sites, with the highest mean concentrations in barred sand bass from the site in San Diego Bay (19,500 +/- 2100 ng/g) and in English sole from the Elliot Bay site (7600 +/- 2300 ng/g). Sediment concentrations of PCB's were also highest at these two sites.

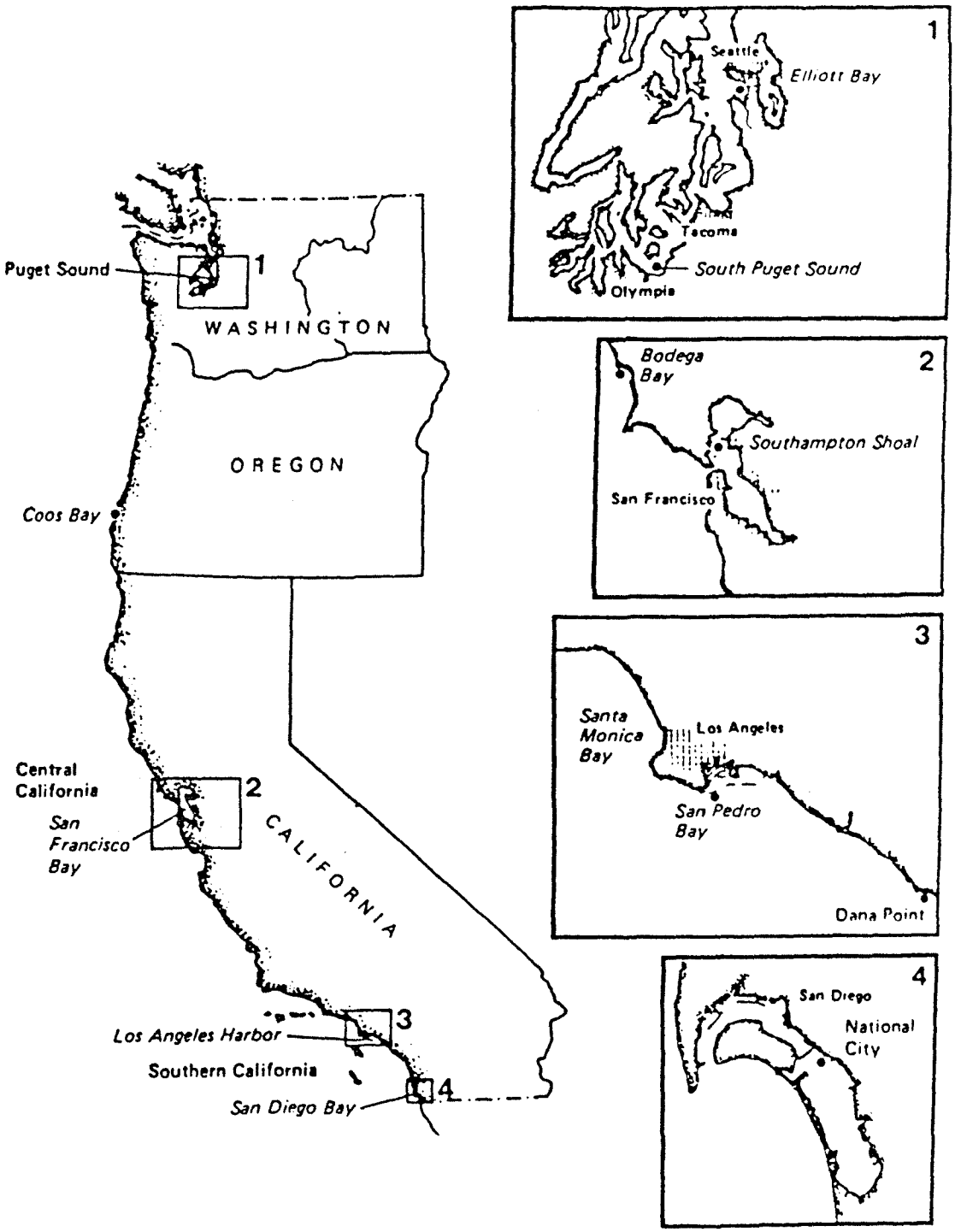


Figure 1. Map of selected sampling sites on the West Coast of the United States.

TABLE 1. Chlorinated compounds found in both sediment and in liver samples from fish collected at selected West Coast sites. Adapted from (11), (18).

Chlorinated Hydrocarbons

Hexachlorobenzene (HCB)	gamma-BHC (Lindane)
alpha-BHC	eptachlor
Hepatchlor epoxide	Aldrin
Dieldrin	Mirex
alpha-Chlordane	trans-Nonachlor
o,p'-DDE	p,p'-DDE
o,p'-DDD	p,p'-DDD
o,p'-DDT	p,p'-DDT
Polychlorinated biphenyls	**Chlorinated Butadienes**
Dichlorobiphenyls	Dichlorobutadienes
Trichlorobiphenyls	Trichlorobutadienes
Tetrachlorobiphenyls	Hexachlorobutadienes
Pentachlorobiphenyls	Heptachlorobutadiene
Hexachlorobiphenyls	
Octachlorobiphenyls	
Nonachlorobiphenyls	

The PCB concentrations in white croaker from the site in San Pedro Bay (4800 +/- 1000 ng/g) and starry flounder from the San Francisco Bay site (3800 +/- 1200 ng/g) were also relatively high compared to the respective reference sites (18).

Interspecies variation between different fish species may affect the type and prevalence of pathological conditions. Examples are interspecies variations such as mobility, diet, habitat requirements, life span, disease resistance, and the ability to metabolize xenobiotics and excrete their metabolic products (18). Furthermore, environmental factors such as the degree of contamination in the sediment, water column, and marine biota influence the potential exposure of the fish species to toxic chemicals. As a rule, pollution-related pathological conditions (for example liver lesions or fin erosion) are formed primarily in certain benthic fish species, in general, such species are exposed to higher levels of environmental contaminants (10).

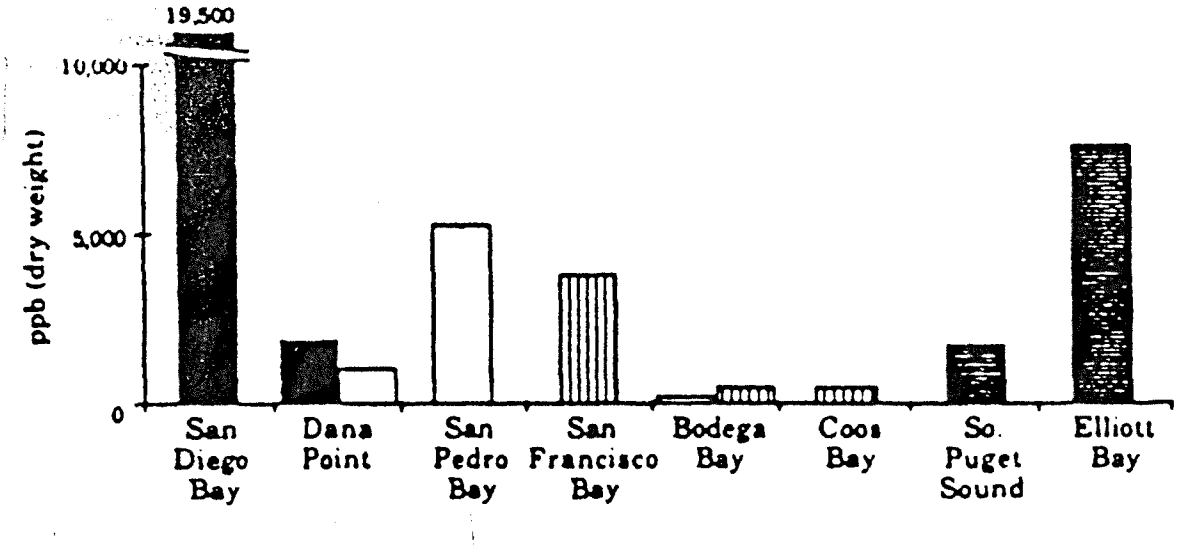


Figure 2. PCB concentrations in liver samples of various fish species from selected sites on the West Coast of the United States.

Histopathological conditions tend to reflect chronic, long-terms effects, and provide little indication of more acute effects which may be occurring in an ecosystem. Therefore, the presence of pathological conditions in fish is very likely an "early warning" indicator of toxic levels of environmental contaminants.

Figure 3. depicts the main sites on the East Coast of the United States where marine pollution is most prevalent which are in Boston, New Bedford, Providence, New York, Baltimore Harbor, and the Elizabeth River, Virginia. Surveys have shown high incidences of disease in fishes from New England, Long Island Sound, the Hudson River estuary/New York Bight and the Elizabeth River, Virginia.

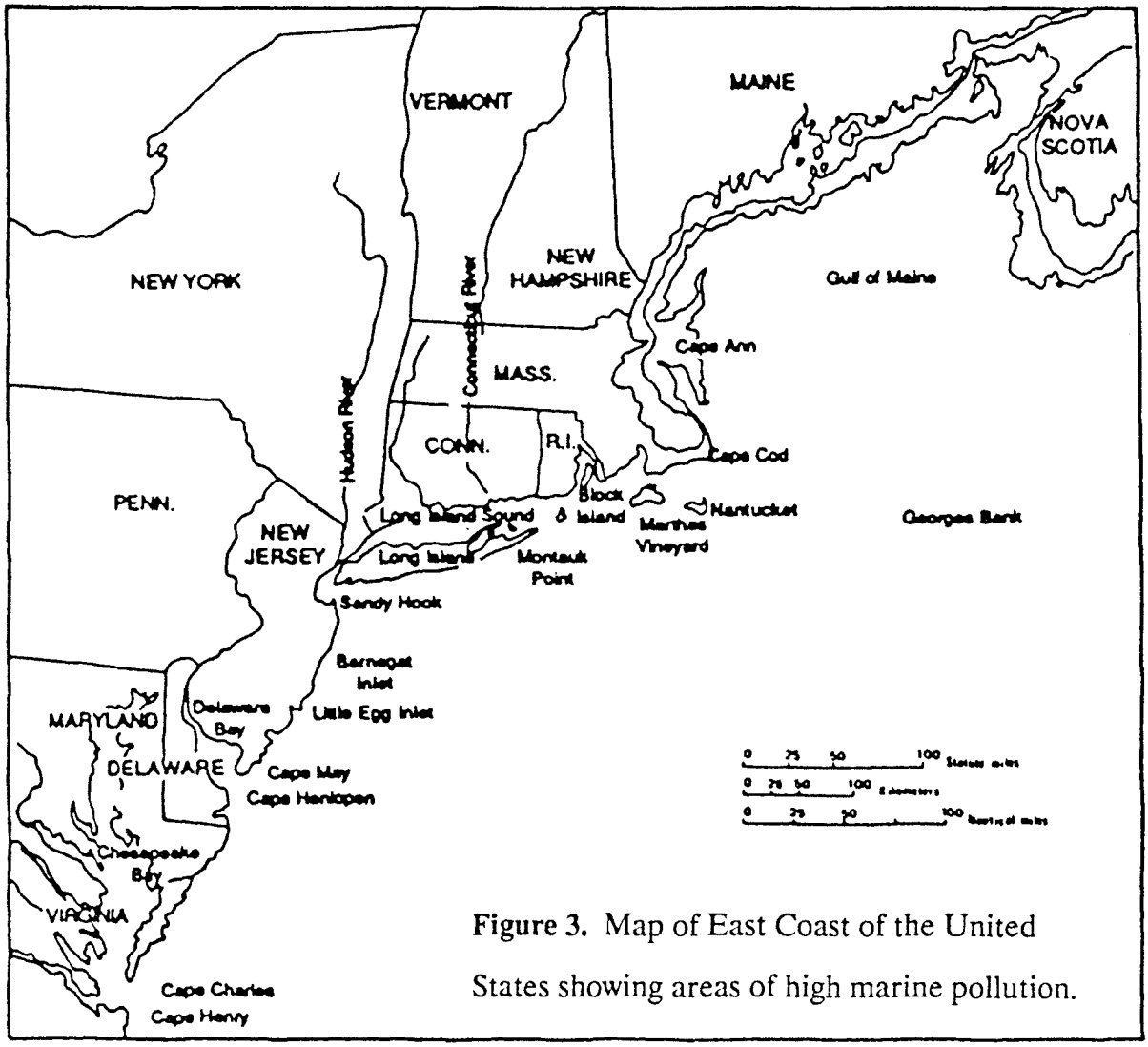


Figure 3. Map of East Coast of the United States showing areas of high marine pollution.

The most common diseases generally affect the liver. Neoplasia, including benign and malignant growths, have been documented for flat fish as well as for tomcod in the Hudson River estuary (20). Epidemiological studies and statistical analyses support the concept of an environmental etiology for disease in fishes from contaminated areas (20). Many pollutants such as PCBs are resistant to chemical and biological change and thus remain in the marine environment for years (11). An example of bioconcentration and bioaccumulation is that Malins et al (15) found that PCBs and hexachlorobenzene were consistently higher in livers of English sole than the sediments from which they were taken.

In the vicinity of the New York Bight PCBs ranged from 13-54 ppm in the skeletal muscle of striped bass from the Hudson river (20), and PCB concentrations in winter flounder ranged from 0.01 to 0.1 ug/g. Presumably these higher body burdens, compared to winter flounder sampled between the Gulf of Maine and the south side of Cape Cod, were due to the elevated PCB levels in the Hudson plume (20).

The feeling amongst current researchers (18) is that greater research efforts need to be expended to develop new bioassays and to better characterize existing bioassay systems. More investigations of cause-and-effect relationships between observed biological effects such as fish diseases and environmental contaminants should also be conducted. These investigations should include field studies as well as controlled laboratory studies.

A greater technical understanding of the relative potency of a chemical or chemical isomer in terms of toxicity would allow environmental risk assessors a greater degree of certainty on which to base their public policy recommendations.

Furthermore, in terms of environmental cleanup and pollution control of our esturine waters, the greater the understanding of which substances are the most toxic to the marine ecosystem, the more efficiently and effectively we can apply both research and development of environmental controls, and preventative environmental engineering controls, to ensure proper management of our marine resources. In addition, information on cause and effect relationships between biological perturbations and individual chemicals or groups of chemicals can help to implement source control and cleanup actions by management agencies.

An input greater than 98% of point source pollutants and 13% of total fresh water input to the Hudson-Raritan estuary comes from municipal wastewater facilities. These wastewaters have a significant industrial as well as domestic input, and the chlorination process prior to discharge may enhance the overall toxicity of the wastewaters by creating compounds that were not previously present (21).

Polychlorinated biphenyls and phthalate esters are ubiquitous in the urbanized estuary environment in North America. However, little is known about the pharmacokinetics of a fish species' metabolic and excretion pathways in order to estimate the rate of recovery from an exposure to a pollutant waste stream, if indeed there is recovery at all. Fish exposed to toxic chemicals over a long period of time may acquire tolerance or develop stress to their exposure. Phthalate esters are abundant in the environment because of their widespread use as plasticizers in a broad range of products from the plastics industry.

An analysis of treated municipal wastewater from the Linden Roselle Sewerage Authority on 4/7/87 (22) gave a concentration of di-n-octyl phthalate of 30 ppb and a value of 810 ppb for bis(ethylhexyl) phthalate. Although not toxic in

very low concentrations little data exists on the bioconcentration or bioaccumulation of phthalate esters in the food chain or about toxicity which may result from the bioaccumulation of these compounds.

Malins et al (15) found that concentrations of PCBs and hexachlorobenzene were consistently higher in livers of English sole than in sediments from which they were taken. This strongly suggests that highly lipophilic refractory compounds are not only readily transferred from sediments to organisms but also appear to have a high potential for being transferred through food chains to higher forms of life. Malins et al (16) also found a possible relationship between toxic environmental chemicals and observed liver lesions, and Murchelano et al (19) suggested that PCBs are possible inducers of hepatomas in English sole and tomcod. O'Connor et al (20) found that winter flounder livers from Boston harbor were unique in comparison to winter flounder livers from other Northeast estuaries. The liver lesions from the Boston harbor fish were consistent with the action of a hepatotoxin.

The possible routes of exposure of fish to toxic chemicals include: consumption of benthic food organisms; uptake from sediment; and uptake from the water column. These routes of exposure might suggest that bottom dwelling or bottom feeding fish species are at a higher risk from exposure to toxic chemicals.

Tables 2-13 are examples of concentrations of chlorinated hydrocarbons that have been derived from other investigative studies. The results of the laboratory work will be compared to the data in these tables in the results and discussion section of this thesis.

Tables 2-4 are from the East coast, from the same location as was used in this thesis research. Tables 5-13 are from the West Coast of the United States from the Seattle and Los Angeles areas as shown in Figure 4. and Figure 5. respectively.

TABLE 2. Mean PCB concentrations in homogenized striped bass reference tissue from the New York Bight (ng/g dry weight). Adapted from (7).

Cl_3-Biphenyls	4300
Cl_4-Biphenyls	18000
Cl_5-Biphenyls	13000
Cl_6-Biphenyls	4400

TABLE 3. Mean PCB concentrations in winter flounder tissue and liver samples from Raritan Bay (mean values in ng/g dry weight). Adapted from (7).

	FLESH	**LIVER**
Cl_3-Biphenyls	76	1175
Cl_4-Biphenyls	183	4300
Cl_5-Biphenyls	83	4300
Cl_6-Biphenyls	46	5200
PCBs (Total)	433	14700

TABLE 4. Mean PCB concentrations in whole striped bass samples from the Hudson River (mean values in ng/g dry weight). Adapted from (7).

Cl_3-Biphenyls	4700
Cl_4-Biphenyls	17000
Cl_5-Biphenyls	10000
Cl_6-Biphenyls	3000
PCBs (Total)	35000

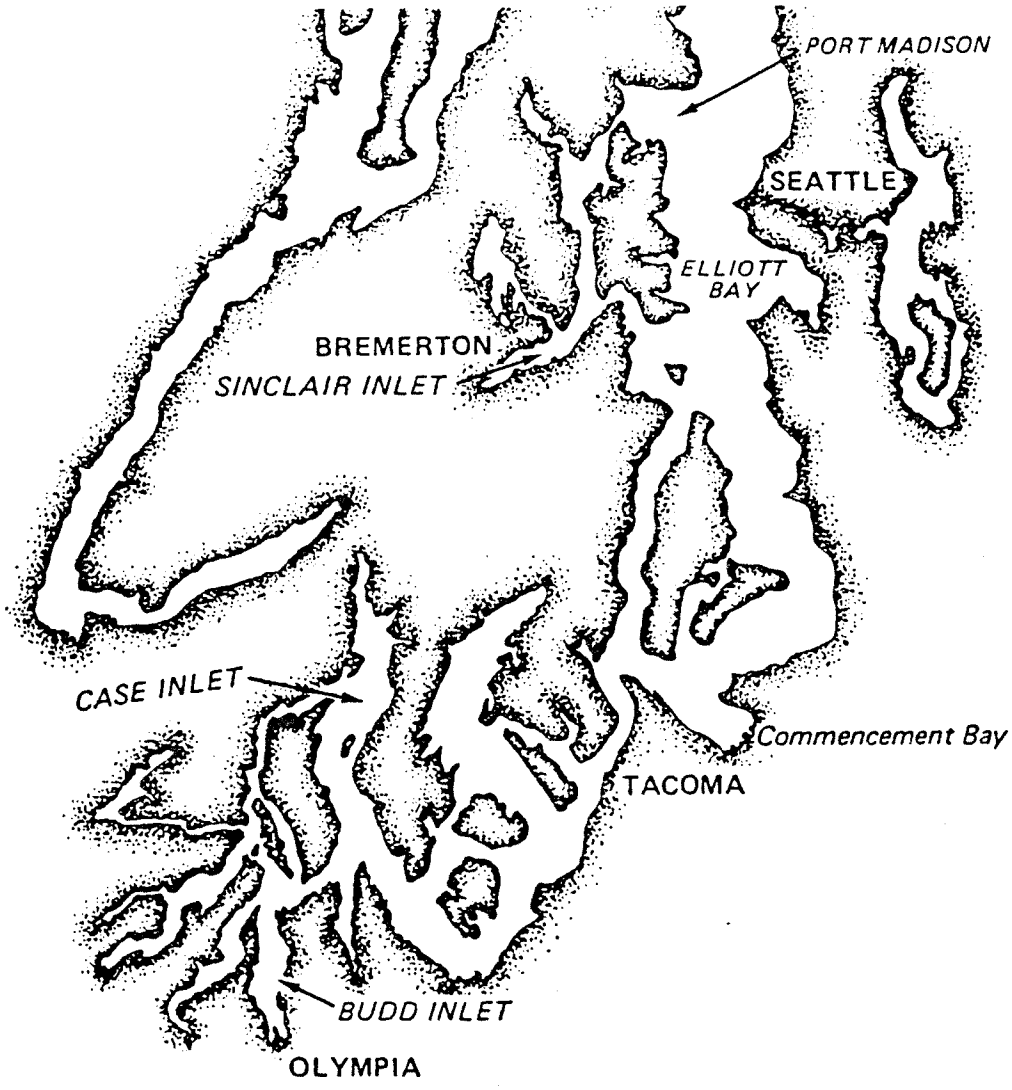


Figure 4. Selected sampling sites in Puget Sound in the Seattle and Tacoma area of Washington State, on the West Coast of the United States.

TABLE 5. Chlorinated hydrocarbon concentrations in individual English sole livers versus muscle tissue samples from Commencement Bay (ng/g dry weight). Adapted from (13).

	MUSCLE			LIVER		
	A	B	C	A	B	C
Hexachlorobenzene	50	200	40	1600	1400	400
Hexachlorobutadiene	10	300	70	1100	17000	800
PCBs (Total)	800	4600	4500	12000	2600	2600

TABLE 6. Mean chlorinated hydrocarbon concentrations in composited English sole fish liver samples from Elliot and Commencement Bays (ng/g dry weight). Adapted from (13).

	ELLIOT BAY	COMMENCEMENT BAY
Cl_2-Biphenyls	28	75
Cl_3-Biphenyls	390	100
Cl_4-Biphenyls	2200	1500
Cl_5-Biphenyls	3900	2600
Cl_6-Biphenyls	4500	4900
Cl_7-Biphenyls	2500	2500
Cl_8-Biphenyls	320	840
Cl_9-Biphenyls	14	130

TABLE 7. Summed PCBs from English sole livers samples from Elliot and Commencement Bays (ng/g dry weight). Adapted from (5).

	LIVER (Individual)	LIVER (Composite)
Elliot Bay	12000	35000
	13000	9200
		32000
Commencement Bay	16000	6100
	16000	20000
	10000	
	14000	

TABLE 8. Comparison of the concentration of PCBs (ng/g wet weight) in liver and muscle of sole, salmon, and cod samples from Elliot and Commencement Bays. Adapted from (5).

	English Sole Liver/Muscle	Salmon Liver/Muscle	Cod Liver/Muscle
Elliot Bay	9700/360	99/140	3300/38
	2100/270	160/150	4200/14
	12000/2100		
	16000/1100		
	6000/1300		
Commencement Bay	4400/700	63/57	2700/46
	1500/160	71/22	2700/31
	24000/610	190/43	2200/14
	3100/640	63/41	
	3900/850	48/42	

TABLE 9. Summed chlorinated butadienes (ng/g dry weight) in English sole liver samples from Elliot and Commencement Bays. Adapted from (5).

	LIVER (Individual)	LIVER (Composite)
Elliot Bay	3	3
		10
Commencement Bay	820	270
	1600	2900
	9100	
	1900	

TABLE 10. Summed Hexachlorobenzene (ng/g dry weight) in English sole liver samples from Elliot and Commencement Bays. Adapted from (5).

	LIVER (Individual)	LIVER (Composite)
Elliot Bay	10	20
	10	50
Commencement Bay	1100	270
	1300	2300
	3700	
	840	

TABLE 11. PCBs concentration in White Croaker liver samples from the Los Angeles area (see map in Figure 5). Adapted from (16).

	Queensway Bay(1)	Cerritos Channel(2)	Reservation Point(4)	White Point(3)	Hyperion (5)
Cl_3-biphenyls	330	160	51	330	48
Cl_4-biphenyls	3700	3700	840	2000	150
Cl_5-biphenyls	4300	8800	2100	2800	240
Cl_6-biphenyls	2400	7900	1700	1600	290
Cl_7-biphenyls	1600	2300	420	540	120
Cl_8-biphenyls	220	440	110	150	34
Cl_9-biphenyls	77	130	36	60	15
PCBs (Total)	13000	23000	5300	7500	900

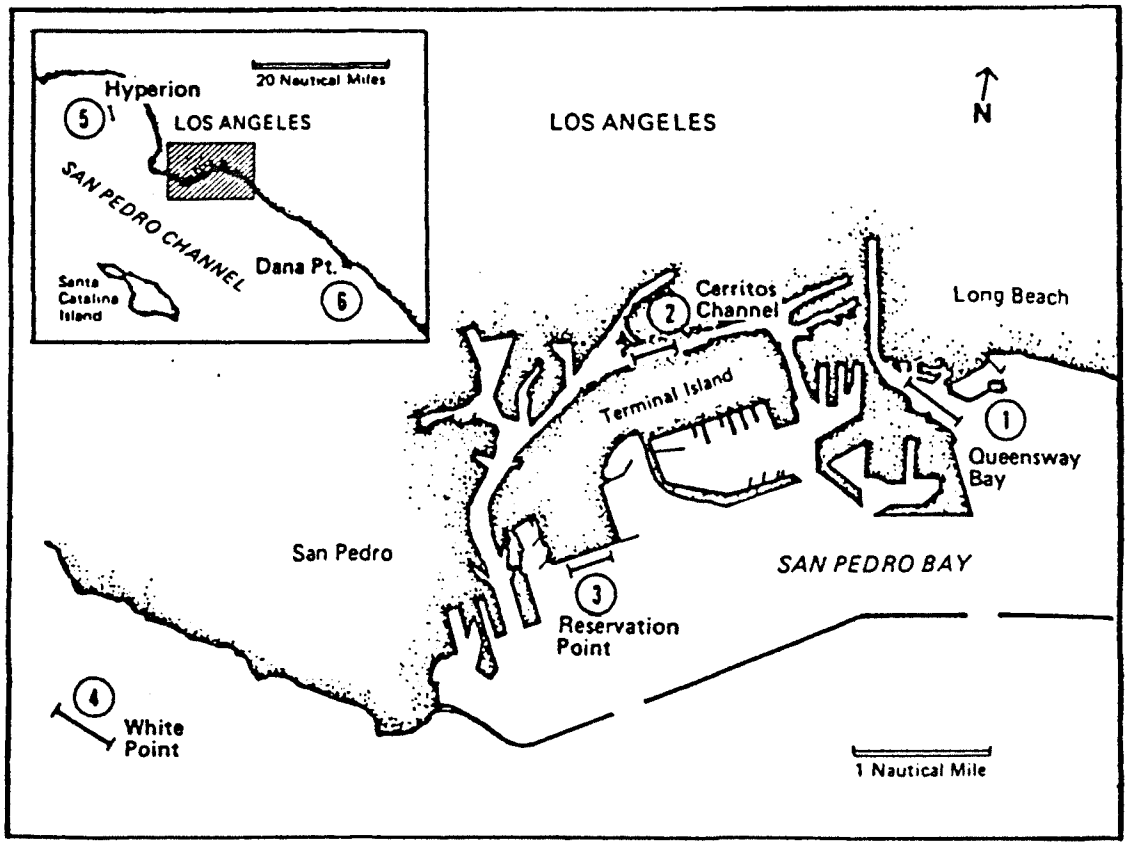


Figure 5. Selected sampling sites in the Los Angeles area of California State.

TABLE 12. Mean concentrations of PCBs, chlorinated butadienes (CBD), and hexachlorobenzene (HCB) in the liver and muscle of English sole from Elliot and Commencement Bays (ng/g dry weight). Adapted from (11).

	Elliot Bay		Commencement Bay	
	LIVER	MUSCLE	LIVER	MUSCLE
PCBs	39000	3400	47000	4800
CBD	1700	260	-	-
HCB	1100	170	35	8

TABLE 13. Concentrations of PCBs in English sole liver and composited muscle samples from Eagle Harbor. Adapted from (13).

	LIVER	MUSCLE
PCBs (Total)	1100	80

1.2 Experimental Design

The goal of this Master's thesis was to analyze composited fish liver samples from a controlled exposure of two species of fish from two distinct populations, to treated municipal wastewater. After an initial exposure, a further controlled depuration period of exposure to clean water, was given to half of the originally exposed fish. Additionally, two field collections of both liver and muscle from the same fish were analyzed. Note that these field collections were not exposed to treated municipal wastewater. The fish selected for the exposure and depuration study originated from both a polluted sampling location and from a relatively pristine sampling location.

The chemical pollutants selected were hexachlorobenzene, hexachlorobutadiene, Aroclor 1254, bis(ethylhexyl) phthalate, and di-n-octyl

phthalate. These chemicals were selected because of a combination of their widespread distribution in marine ecosystems, and their known or suspected toxicities. The chosen chemicals represent classes of compounds where a knowledge of their interaction within the marine ecosystem could enable help us predict the possible consequences, if no environmental controls are implemented to protect potentially sensitive areas or fish populations.

The experiment was designed to study the effect of various concentrations of treated municipal wastewater (TMW) on two species of fish, striped bass (*Morone saxatilis*) which is a pelagic fish and winter flounder (*Pseudopleuronectes americanus*) which is a benthic fish. Ten fish of each species were placed in each of 4 tanks. Each tank was filled with a predetermined dilution of TMW supplied by a proportional diluter. Two volume exchanges were carried out per day for each of the exposure tanks and the exposure time was 21 days. Table 14 below shows a summary of the fish exposure conditions.

TABLE 14. Summary of the fish exposure conditions during the exposure period (10/21/88 - 11/10/88).

EXPOSURE PERIOD Dose (V%)	Tank #1 0% TMW	Tank #2 1% TMW	Tank #3 5% TMW	Tank #3 10% TMW
No. of striped bass	10	10	10	10
No. of winter flounder	10	10	10	10
Total No. of Fish	20	20	20	20

After the exposure time, five fish of each species were removed from each tank. The total liver, muscle, and bile composite for each group of five fish were analyzed for the chemical pollutants. The fish composite (either liver, muscle or bile) that contained the highest level of chemical pollutants was selected for further study.

The remaining twenty fish were left in their respective tanks for a depuration time of 21 days during which no more pollution was introduced. This was a recovery or depuration period. Table 15 below shows a summary of the fish depuration conditions.

TABLE 15. Summary of the fish depuration conditions during the depuration period (11/11/88 - 12/01/88).

DEPURATION PERIOD Dose (V%)	Tank #1 0% TMW	Tank #2 1% TMW	Tank #3 5% TMW	Tank #4 10% TMW
No. of striped bass	5	5	5	5
No. of winter flounder	5	5	5	5
Total No. of Fish	10	10	10	10

The same analytical procedure, identical to the procedure used for the fish with no depuration time, was used for the remaining depurated fish. The data obtained can be compared with the baseline data for fish with no depuration time,

to give an indication of the rate the two species of fish clear the chemical pollutants from their system.

The sampling locations chosen were: Field Site - Raritan Bay in the Hudson-Raritan estuary; Reference Site - Shinnecock Bay, Long Island, NY as shown in Figure 6. The specimens were sacrificed on the boat by cervical section and frozen prior to soxhlet extraction, florisil cleanup, and gas chromatography analysis. The soxhlet extracted tissue samples were kindly provided by Dr. Peddrick Weis' laboratory at the New Jersey Medical School of the University of Medicine and Dentistry of New Jersey, Newark, NJ.

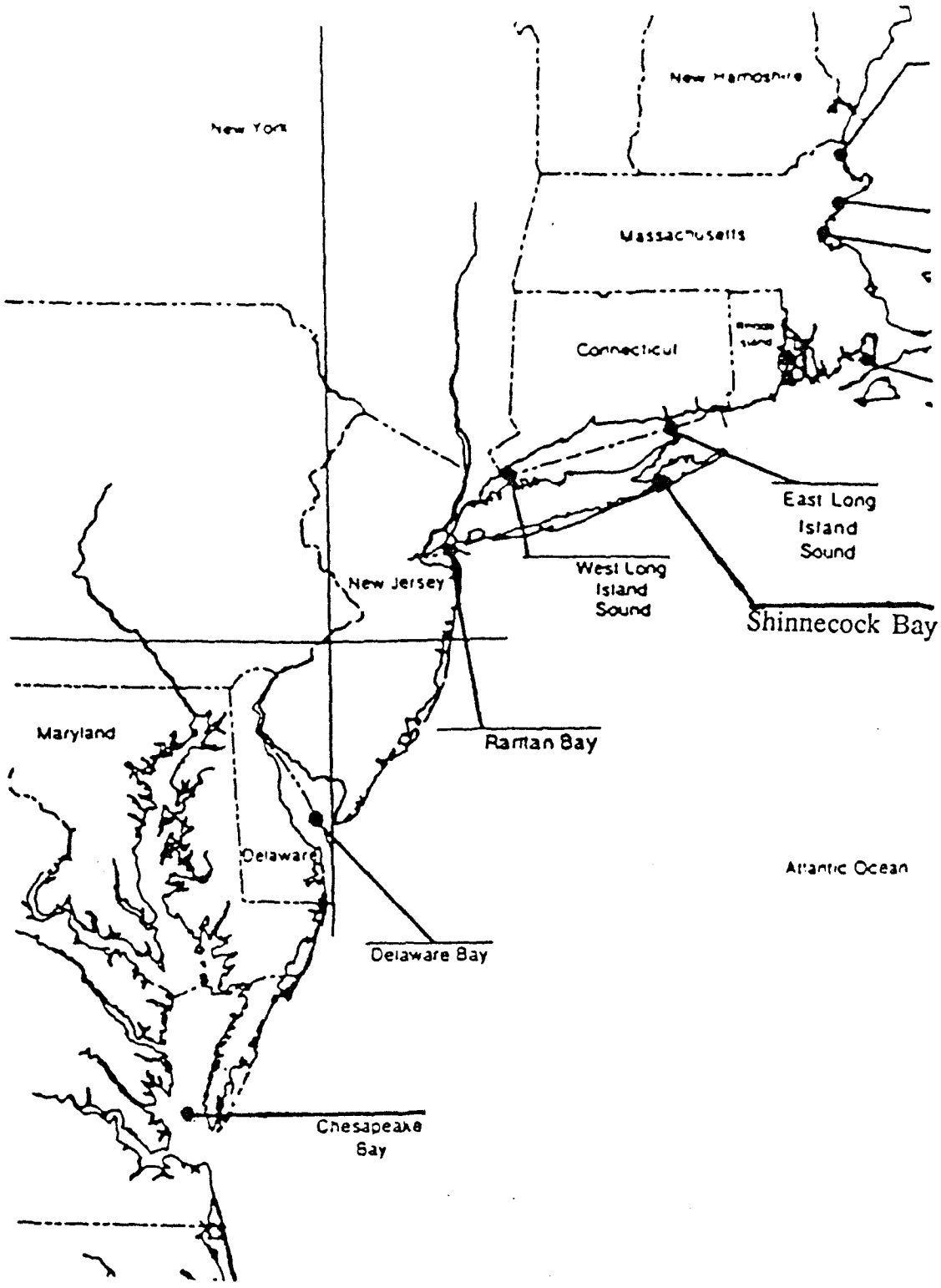


Figure 6. Map of the East Coast of the United States showing the sampling locations in Raritan Bay and Shinnecock Bay.

This thesis only covers composited liver samples that resulted from the exposure followed by depuration experiment of two species of fish from two distinct populations, as described earlier, plus an intraspecies comparison between two different tissue types (liver and muscle) from winter flounder. Some modifications were made from the original experimental design and are described below. Each composite that was analyzed consisted of between 5 and 8 pooled fish livers from the same species of fish. Young-of-the-year winter flounder aged between seven and eight months and five month old striped bass were used in the exposure and depuration study. Thus the amount of liver sample available for soxhlet extraction was minimal because of the small size of the young fish, therefore the number of fish in each pooled sample was different to that previously described because intergroup samples were pooled so that a large enough weight of liver tissue was available for extraction. In the case of the individual fish that were analyzed, nearly mature winter flounder were selected for analysis.

It must be pointed out here that many mishaps unfortunately occurred during the exposure and depuration period of this study due to technical difficulties. This resulted in data where it was not possible to make all the comparisons that were originally built into the experimental design. The list of samples in Table 16. indicates those samples that were available for florisil cleanup and gas chromatography analysis. This data will be presented in the results and discussion section of this thesis.

Table 16. A list of tissue samples that were analyzed for selected chemical contaminants. Continued overleaf...
BL Background level striped bass liver from Raritan Bay; exposure to clean water for one week.
1 Reference winter flounder liver from Shinnecock Bay; 10% exposure to TMW for 3 weeks.
2D Combined winter flounder liver; 5% TMW exposure for 3 weeks, followed by a 3 week depuration in clean water.
3 Reference striped bass liver from Shinnecock Bay; 0% exposure to TMW for 3 weeks (control).
3D Reference winter flounder liver from Shinnecock Bay; 0% TMW exposure for 3 weeks, followed by a 3 week depuration in clean water (control).
4 Reference winter flounder liver from Shinnecock Bay; 5% exposure to TMW for 3 weeks.
4D Reference winter flounder liver from Shinnecock Bay; 5% exposure to TMW for 3 weeks, followed by a 3 week depuration in clean water.

Table 16. continued: A list of tissue samples that were analyzed for selected chemical contaminants.
9 Striped bass liver from Raritan Bay; 5% exposure to TMW for 3 weeks.
8D Striped bass liver from Raritan Bay; 5% exposure to TMW for 3 weeks followed by a 3 week depuration in clean water.
10 Striped bass liver from Raritan Bay; 1% exposure to TMW for 3 weeks.
10D Striped bass from Raritan Bay; 1% exposure to TMW for 3 weeks, followed by a 3 week depuration in clean water.
42M Winter flounder muscle from Raritan Bay. (Collection Date : November 1987).
42L Winter flounder liver from Raritan Bay. (Collection Date: November 1987).
47M Winter flounder muscle from Raritan Bay. (Collection Date: November 1987).
47M Winter flounder liver from Raritan Bay. (Collection Date: November 1987).

2 ANALYTICAL METHODOLOGY

2.1 Development of method

The method used for sample cleanup is based on EPA Test Methods for Evaluating Solid Waste: Method 3620 Florisil Column Cleanup (4). Adaptation is required so as to allow the combination of two methods, that is one method for chlorinated compound cleanup and one for phthalate ester cleanup. Thus a method is developed that will allow both classes of organic compound to be quantitated from a very small amount of fish liver or muscle sample, which has been soxhlet extracted with methylene chloride.

2.2 Scope and application

2.2.1 General: Extract purification.

Injection of extracts into a gas chromatograph can cause extraneous peaks, deterioration of peak resolution and column efficiency, and loss of detector sensitivity and can greatly shorten the lifetime of expensive columns.

2.2.2 Specific: Adsorption column chromatography.

Florisil is useful for separating analytes of a relatively narrow polarity range from extraneous, interfering peaks of a different polarity which may interfere with the analyte peaks on the GC/ECD.

2.3 Summary of method

Florisil is a magnesium silicate with acidic properties. It is used for general column chromatography as a cleanup procedure prior to sample analysis by gas chromatography.

The column is packed with florisil adsorbent, topped with a water adsorbent, and then loaded with the sample to be analyzed. Elution is effected with a suitable solvent(s) leaving the interfering compounds on the column. The eluate is then concentrated and analyzed by gas chromatography. Figure 7. is a flow chart that summarizes the analytical procedure.

2.4 Interferences

Analytical interferences may be caused by contaminants in solvents, reagents, glassware, and other processing hardware. All of these materials must be routinely demonstrated to be free of interferences, under conditions of the analysis, by running laboratory reagent blanks.

A reagent blank should be performed for the compounds of interest prior to the use of this method. The level of interferences must be below the method detection limit before this method is performed on actual samples.

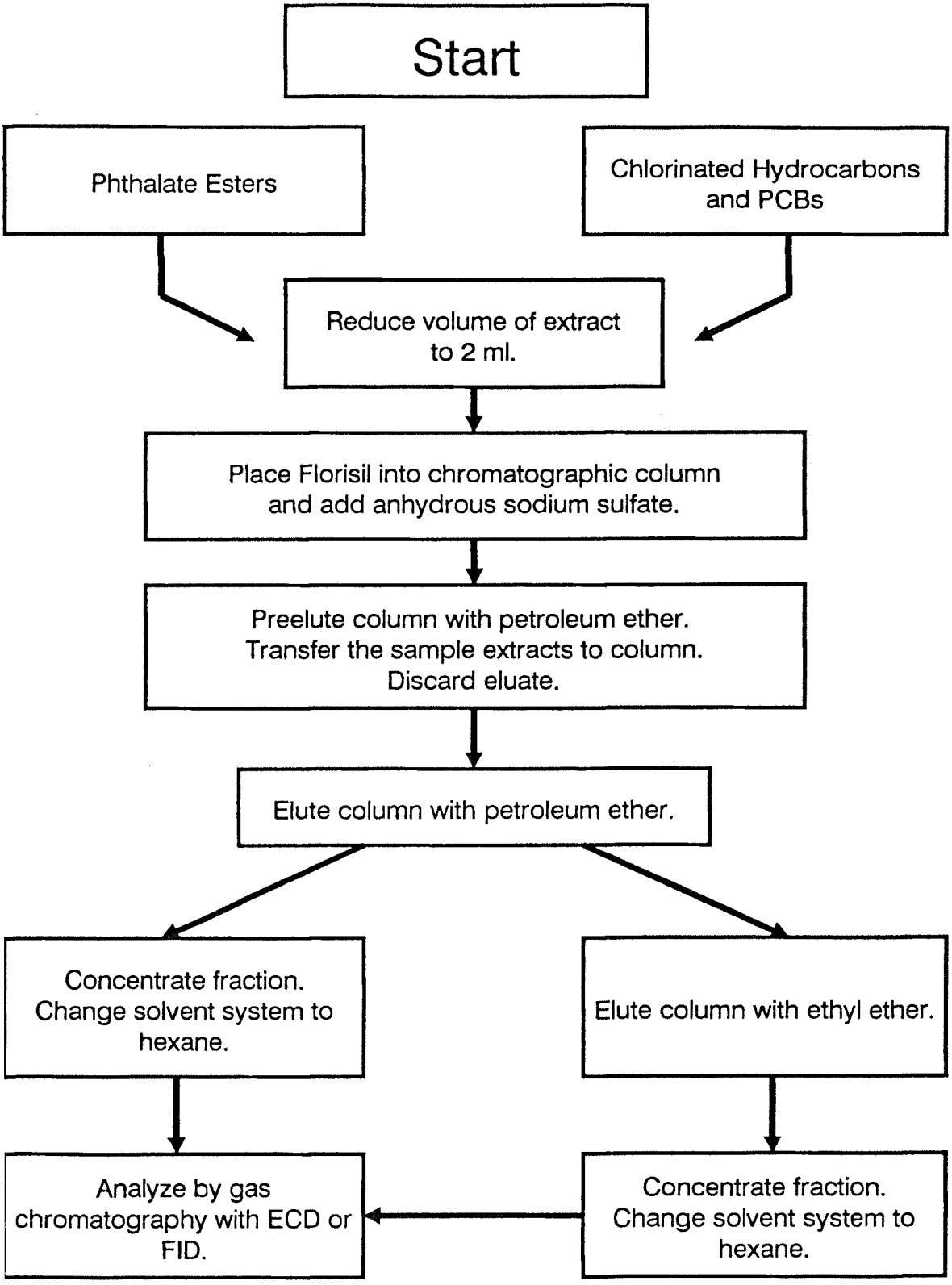


Figure 7. Flow chart summary of the analytical procedure.

2.5 Apparatus and materials

2.5.1 Equipment and glassware

(a) Gas Chromatograph fitted with a temperature programmer, electron capture detector (ECD), flame ionization detector (FID) a chart recorder and electronic integrator.

(b) Liquid Chromatographic Column 400mm x 22mm ID x 25mm OD (Supelco Cat. No. 6-4755) with Pyrex glass wool at bottom and a teflon stopcock.

NOTE: Fritted glass disks are difficult to decontaminate after highly contaminated extracts have passed through. Columns without frits may be purchased. Use a small pad of Pyrex glass wool to retain the adsorbent. Prewash the glass wool pad with 50 mL of acetone followed by 50 mL of elution solvent prior to packing the column with adsorbent.

(c) Kuderna-Danish (K-D) apparatus concentrator tube: 10-mL, graduated (Supelco Cat. No. 6-4706, Receiving vessel with 20mm screwthreads, screw top to prevent evaporation of extracts.

(d) Evaporation flask: 500 mL (Supelco Cat. No. 6-4706, Flask, 500 mL, 20mm screwthreads).

(e) Snyder column: Two-ball macro (Supelco Cat. No. 6-4724, Macro Snyder column with 20mm screwthreads, 2 ball, 170 mm)

(f) Muffle furnace suitable for activation of the florisil packing material prior to column cleanup.

(g) Reagent flask with ground glass stopper.

(h) Water bath: Heated, with concentric ring cover, capable of temperature control (+/- 5 °C. The water bath should be used in a hood.

(i) Teflon boiling chips: Solvent extracted, approximately 10/40 mesh.

2.5.2 Reagents (See Appendix for a complete list of reagents)

(a) Florisil: Pesticide residue (PR) grade (60/100 mesh) purchase activated at 1250 °F (677 °C), stored in glass containers with phenolic screw caps (Sigma Chemical Company, Lot 78F-0718).

(b) Sodium Sulfate (ACS): Granular, anhydrous purified by heating at 400 °C for 4 hours in a shallow tray (J.T.Baker 12-60 Mesh, Lot B39702).

(c) Solvents: Ethyl Ether; Acetone; Hexane; Methylene Chloride; Petroleum Ether (boiling range 30-60 °C): Pesticide quality or equivalent.

2.6 Experimental procedure

(a) Change the sample extract, typically dichloromethane soxhlet extract of tissue, to hexane prior to loading the column for the cleanup procedure.

(b) Activation of Florisil: for clean up of chlorinated hydrocarbons. Just before use, activate each batch at least 16 hours at 130 °C in a glass container loosely covered with aluminum foil.

(c) Place 12 g of Florisil into a 22 mm ID chromatographic column. Tap the column to settle the Florisil and add 1 to 2 cm of anhydrous sodium sulfate to the top.

(d) Preelute the column with 100 mL of petroleum ether. Discard the eluate and, just prior to exposure of the sodium sulfate layer to the air, add the sample extract and begin eluting the column with 200 mL of petroleum ether. Collect the eluate in a 500 mL K-D flask equipped with a 10 mL concentrator tube. This fraction should contain:

Hexachlorobenzene
Hexachlorobutadiene
PCB-1254

(e) Concentrate the fraction in the K-D flask, using hexane to prewet the column. When the apparatus is cool, remove the Snyder column and rinse the contents of the flask and its lower joint into the concentrator

tube with hexane. Adjust the final volume of the cleaned-up extract to whatever volume is required (1-10 mL), Analyze by gas chromatography.

(f) Change the eluting solvent to ethyl ether and further elute the florisil column with 100 mL of ethyl ether and collect the eluent in a 500 mL Kuderna-Danish flask equipped with a 10 mL concentrator tube. This fraction should contain all the phthalate esters:

Bis(ethylhexyl) phthalate

Di-n-octylphthalate

(g) Concentrate the fraction, using hexane to prewet the column. When the apparatus is cool, remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with hexane. Adjust the final volume of the cleaned-up extract to what ever volume is required (1-10 mL). Analyze by gas chromatography.

2.7 Analysis by gas chromatography

A Varian 3700 Gas Chromatograph equipped with an Electron Capture Detector (ECD) was fitted with a Fused Silica Capillary Column. The column was an SPB-5, 30 m, 0.32 mm ID 0.25 um film thickness capillary column manufactured by Supelco (Cat. No. 2-4048).

The Instrumentation was set up according to the following set of parameters:

Injector Temperature: **250 °C.**

Initial Oven Temperature: **50 °C held for 5 mins.**

ECD Detector Temperature: **320 °C.**

Program Rate: **10 °C/min.**

Attenuator: **16**

ECD Range AMPS/MV: **10**

Output: **negative**

Splitter Flow: **155-160 cc/min.**

Helium (zero grade) Carrier Gas Flow Rate: **9 psig.**

Nitrogen (zero grade) Make-Up Gas: **30 cc/min.**

Soltec chart recorder (model 1242) speed: **30 mm/min.**

Integration System: **Labtech Chrom Computer Software.**

2.8 Quantitation

Quantitation is based on the precise match-up of retention times of the sample peak with the known retention time of the standard. The computer software allows the chromatogram to be expanded, thus allowing each individual peak to be assigned a retention time and a peak area. Furthermore, by matching up known retention times and peak areas which correspond to standard solutions of the target compounds, it is possible to calculate the analyte concentration in the biological samples.

The criteria for this match-up are as follows:

(a) The sample, and also the sample spike peaks, must lie within the average of the standard retention times, plus or minus the standard deviation of the standard retention times.

(b) Correct identification is also made based on the number, and magnitude of peak areas, of peaks around the specific standard retention time in question. This method is justified because of the variability that results from operator repeatability, in terms of sample injection, followed by activation of the temperature program run, which in turn is followed by activation of the data aquisition software. Furthermore, it should be noted that the lack of automation between the G.C. and the data system software results in a loss in precision, which will reflect in less accurate results. The following equation is used to calculate the amount of analyte in the biological samples:

$$\text{Quantity per gram of biological sample} = \frac{\text{D.F. [C (P.A. Spl. / P.A. Std.)]}}{\text{W}}$$

Where: **D.F.** is the dilution factor.

C is the amount of standard injected into G.C.

P.A. Spl. is the peak area of the sample.

P.A. Std. is the peak area of the standard.

W is the weight of the original biological sample.

2.9 Method validation

Appropriate use of data generated under the great range of analytical conditions encountered in environmental analysis of biological samples requires reliance on the quality control practices incorporated into the method.

The following quality control procedures were incorporated into the method in order to validate the resulting data:

2.9.1 Reagent and apparatus blank

A blank clean-up run is carried out where the conditions directly replicate a sample clean-up procedure. The significant difference is that 4 mL of reagent grade hexane is used as the blank sample in the procedure instead of the biological sample.

2.9.2 Spiked Recovery

A clean-up run is carried out where the conditions replicate a sample clean-up procedure. The significant difference is that 4 mL of reagent grade hexane is spiked with a known concentration of the target compounds, then this spiked mixture is analyzed using the method. The florisil column liquid chromatography, the Kuderna-Danish concentration, and the gas chromatographic analysis all remain exactly the same as when used in conjunction with biological samples.

2.9.3 Limits of Detection

The following variables all contribute to the amount of a compound that can be measured using this method.

(a) Detector Sensitivity
(b) Integrator Sensitivity
(c) Resolution of peaks
(d) Accuracy of injection volume

In order to achieve a cut off level on what concentrations of standards can accurately be reported using this method a calibration curve must be drawn with data from known concentrations of standard. More data points should be obtained near the limit of detection. Due to detector noise the gas chromatograph was not set at the highest sensitivity setting available.

3 RESULTS AND DISCUSSION

3.1 Polychlorinated biphenyl (PCB) and chlorinated hydrocarbon data.

Table 17 below shows the results obtained from analysis of 15 soxhelet extracted tissue samples. The data are given in concentrations of ng/g dry weight of fish tissue. The chemicals quantitated are: Hexachlorobenzene (HCB), Hexachlorobutadiene (HCBD), and Aroclor 1254.

TABLE 17. HCB[a], HCBD[b], and PCB-1254[c] data (ng/g dry weight).

Sample	HCB	HCBD	PCB-1254
BL (SB/HR)	ND	2600	2800
1 (WF/R/TMW-10%)	940	ND	ND
2D (WF/TMW-5%-D)	570	4700	850
3 (SB/R-C)	8400	17000	21000
3D (WF/C-D)	390	2400	610
4 (WF/R/TMW-5%)	ND	ND	3900
4D (WF/R/TMW-5%-D)	270	1900	580
9 (SB/HR/TMW-5%)	400	1400	250
8D (SB/HR/TMW-5%-D)	140	ND	650
10 (SB/HR/TMW-1%)	880	5400	280
10D (SB/HR/TMW-1%-D)	110	240	400
42-M (WF/HR)	340	900	60
42-L (WF/HR)	580	1700	140
47-M (WF/HR)	68	160	2000
47-L (WF/HR)	580	240	2300

a - hexachlorobenzene b - hexachlorobutadiene c - Aroclor 1254 SB - striped bass
WF - winter flounder R - reference C - control D - depurated HR- Hudson-Raritan
ND - not determined to be present above the limit of detection.

Figure 8. show a chromatogram of one of the samples after the florisil column cleanup procedure has been carried out. The characteristic polychlorinated biphenyl pattern occurs in the retention time range between 23 and 29 minutes. The sample shown is "4-D", a pooled reference winter flounder liver extract after exposure to 5% treated municipal wastewater and a period of depuration.

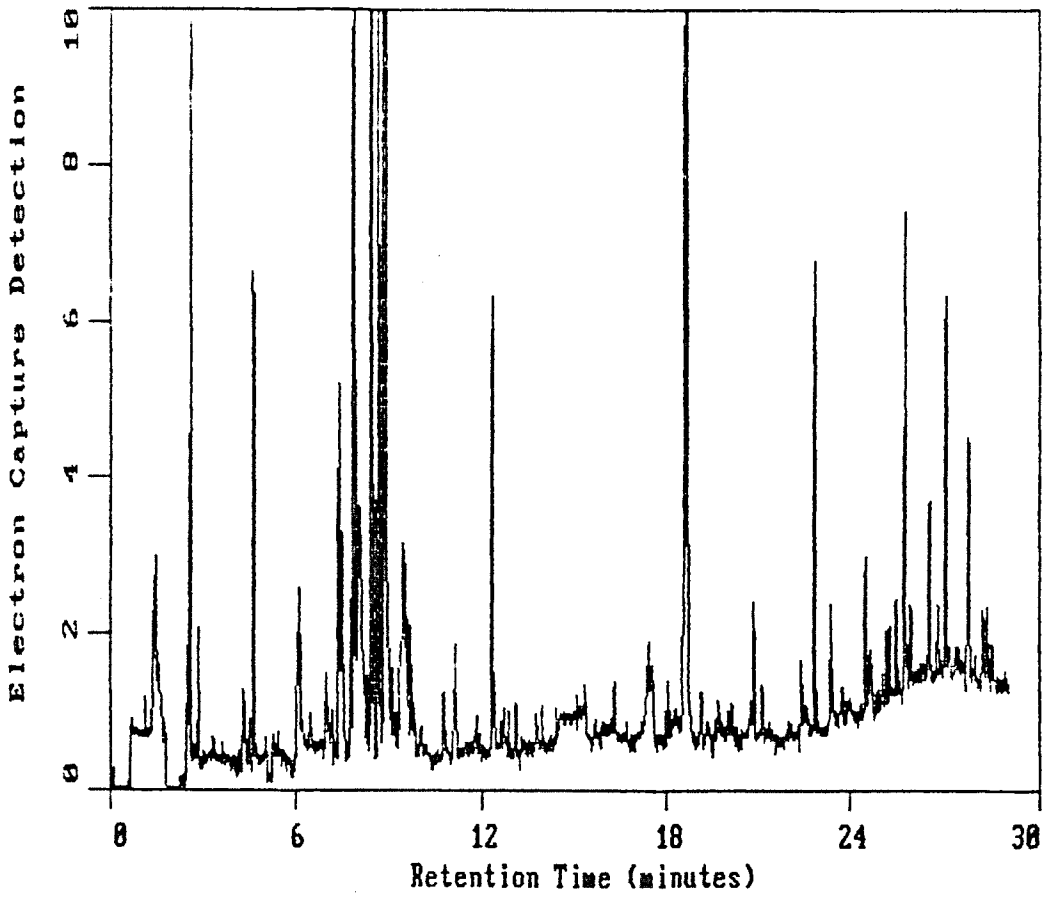


Figure 8. Gas chromatogram of a liver sample after florisil column cleanup.

Figure 9. shows the gas chromatogram of an Aroclor 1254 co-injected sample. The pattern of congener peaks unique to Aroclor 1254 are super imposed onto the chromatogram of sample "4-D" which is shown in Figure 8. The co-injected sample is "4-D", a pooled reference winter flounder liver extract after exposure to 5% treated municipal wastewater and a period of depuration.

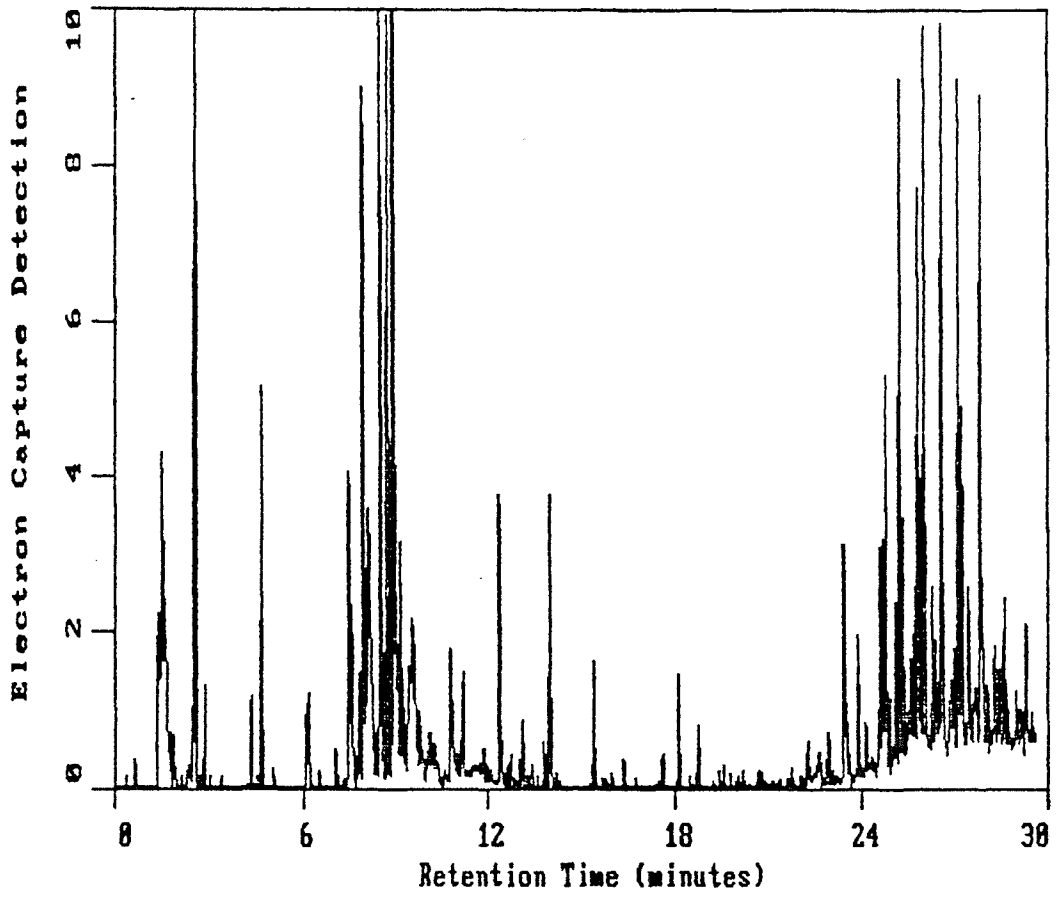


Figure 9. Gas chromatogram of an Aroclor 1254 co-injected liver sample after florisil column cleanup.

3.2 Phthalate ester data.

Table 18. shows the results obtained from analysis of 15 soxhelet extracted tissue samples. The data are given in concentrations of ng/g dry weight of fish tissue. The chemicals quantitated are: bis(ethylhexyl) phthalate and di-n-octyl phthalate.

TABLE 18. Bis(ethylhexyl) phthalate and di-n-octyl phthalate data.

Sample		Bis(ethylhexyl)[a]	Di-n-Octyl[b]
BL	(SB/HR)	1600	990
1	(WF/R/TMW-10%)	2800	ND
2D	(WF/TMW-5%-D)	3700	ND
3	(SB/R-C)	ND	ND
3D	(WF/C-D)	70	81
4	(WF/R/TMW-5%)	8800	ND
4D	(WF/R/TMW-5%-D)	420	350
9	(SB/HR/TMW-5%)	490	ND
8D	(SB/HR/TMW-5%-D)	130	220
10	(SB/HR/TMW-1%)	ND	490
10D	(SB/HR/TMW-1%-D)	140	ND
42-M	(WF/HR)	13	18
42-L	(WF/HR)	40	ND
47-M	(WF/HR)	ND	ND
47-L	(WF/HR)	35	ND

a is bis(ethylhexyl) phthalate **b** is di-n-octyl phthalate SB - striped bass

WF - winter flounder R - reference C - control D - depurated HR- Hudson-Raritan

ND - not determined to be present above the limit of detection.

3.3 Spiked recovery data.

The result of the method recovery of a spiked sample of Aroclor 1254 was that 76% of the spiked sample was recovered when quantitated using the method described in the analytical methodology section of this thesis. Since the method is designed for chlorinated hydrocarbon cleanup, and was verified for Aroclor 1254 a spiked recovery was not run for hexachlorobenzene or hexachlorobutadiene.

Unfortunately, the method recovery for the spiked sample of phthalate esters, which was run concurrently with the Aroclor 1254 spiked recovery, is not available due to problems with the integration software. From the chart recording of the chromatogram, the retention times of the phthalate ester peaks match exactly with the retention times of the standards. Furthermore, the peaks are of significant peak height (the peaks go off scale) and peak area to conclude, with a high degree of certainty, that there is significant recovery of the spiked phthalates, although the exact percentage figure cannot be quantitated.

3.4 Method blank data.

The result of the method blank where, only hexane was used as the sample, was that no peaks appeared in the chromatogram apart from the solvent peaks and extraneous peaks that appeared in every gas chromatogram. These peaks are probably due to laboratory contamination, septum bleed, and column bleed. Note that any small contamination in any of the solvents or glassware will be concentrated throughout the cleanup procedure and will show up as large peaks in the chromatogram, especially when detected by the extremely sensitive electron capture detector.

3.5 Limits of detection

The detection limits calibration graphs for the electron capture detector (ECD) and the flame ionization detector (FID) are shown in Figure 10. and Figure 11 respectively. The ECD calibration graph is curved especially at concentrations below 50 ppb. The computerized integration system fails to integrate peaks below 5 ppb even if the more sensitive chart recorder can still distinguish peaks above the background noise. The detection limit for Aroclor 1254 at the gas chromatograph sensitivity setting chosen of the sample runs is 5 ppb (Figure 10).

The FID calibration graph is plotted for peak height instead of peak area. This is because the integrated peaks areas were unreliable at lower concentrations of standard solutions of the phthalate esters. The best estimate of the limit of detection for the phthalate esters is about 10 ng/gram.

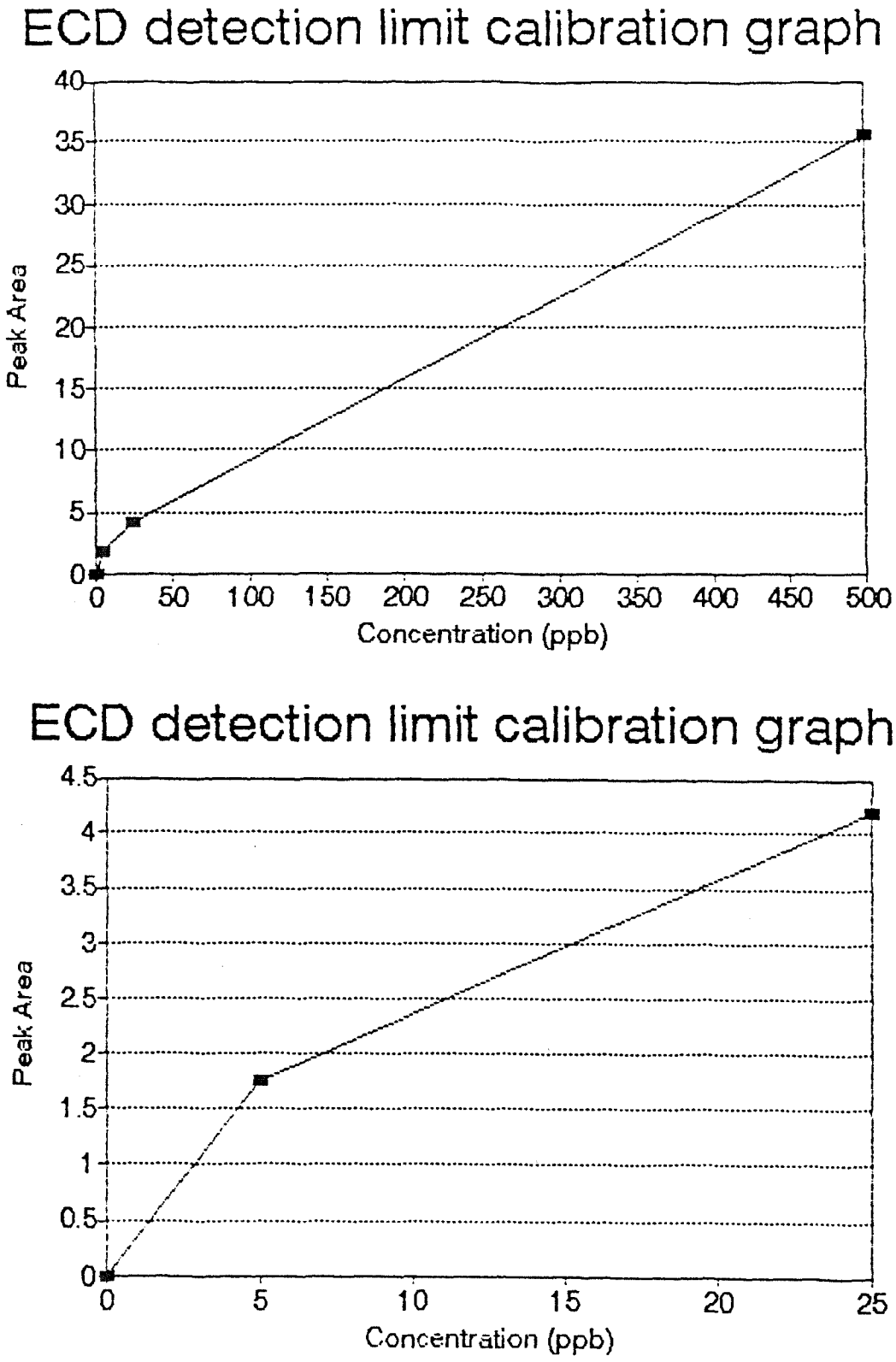
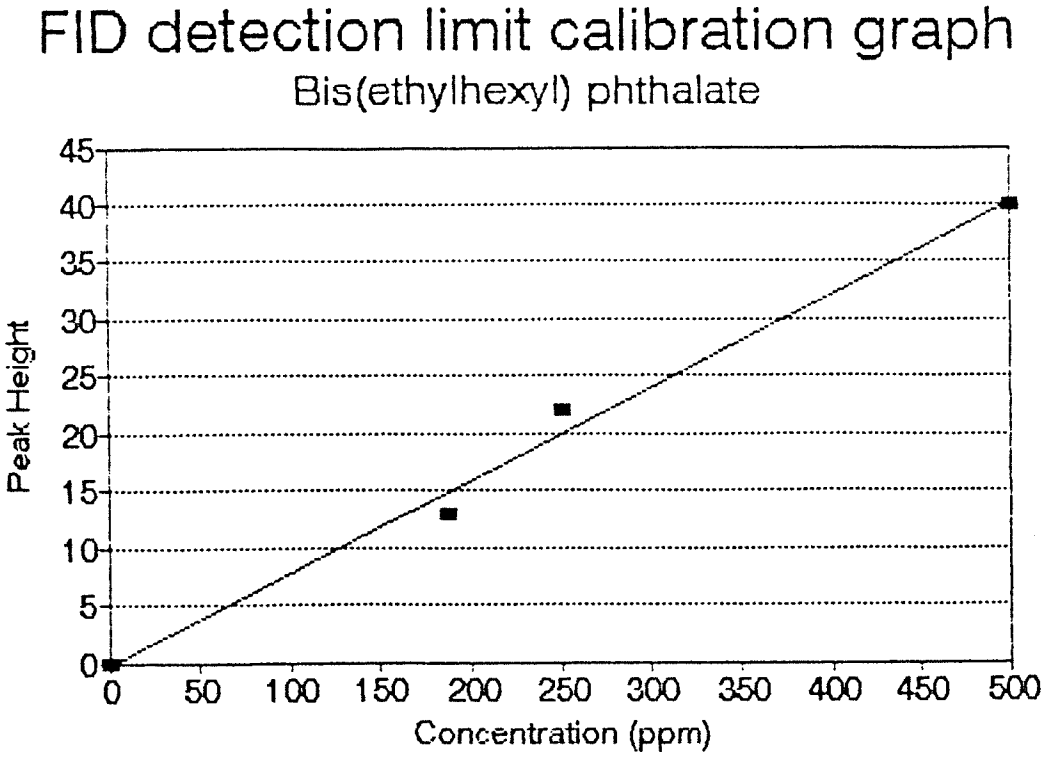


Figure 10. Detection limit calibration graph of the electron capture detector.



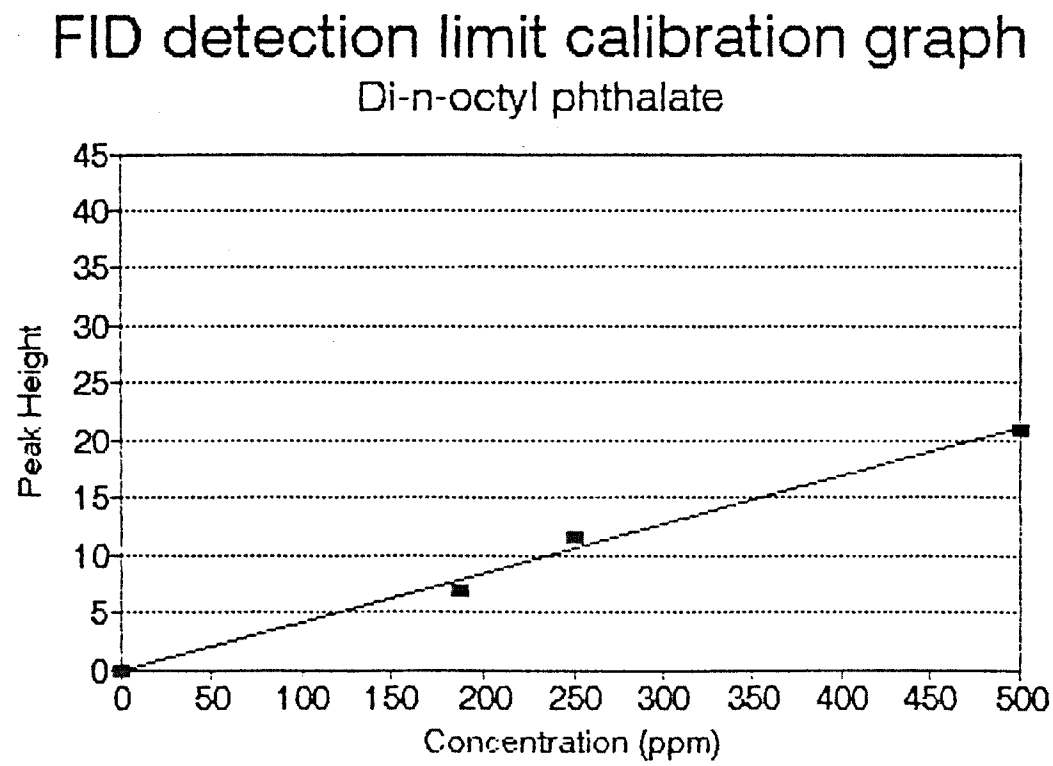


Figure 11. Detection limit calibration graph for the flame ionization detector.

3.6 Tissue sample results and discussion.

Since it is clear that there are not enough data to discuss the full merits of the designed experiment, the data will be compared wherever possible to data from the same exposure tank and sampling location. Furthermore all data, where applicable, will be compared with the results from other investigators around the coast of the United States. The tables of data for comparison purposes are in the introduction section of this thesis along with their respective source references. It should be noted that these data tables were adapted from previously published data.

The background level ("BL") Striped bass liver sample from the Hudson Raritan estuary shows bioconcentration of phthalate esters, and a high concentration of hexachlorobutadiene (HCBD), which are similar to reported findings (5). The Aroclor 1254 concentration is consistent with other reported findings from similar urban estuaries (13). The reference winter flounder ("1") from Shinnecock Bay showed little bioconcentration effects in response to the 10% treated municipal wastewater exposure except for hexachlorobenzene (HCB) which is present in a relatively small concentration. This may be due in part to the treated municipal wastewater exposure, but could also be due to previous environmental exposures and bioconcentration affects, although the young of the year have not been accumulating pollutants for very long. Furthermore, since the batch of treated municipal wastewater used in this experiment appears to be relatively toxic, fatality occurred to all the winter flounder in the 10% tank, a more likely explanation is that the fish uptake the pollutants from the treated municipal wastewater. Environmental bioconcentration is probably the reason for the high bis(ethylhexyl) phthalate concentration when compared to the other exposed and depurated liver samples from the experiment. It should be noted that the striped bass exposed to 10% treated municipal wastewater became anerexic, and the winter flounder from

the Hudson-Raritan died from their exposure to the 10% treated municipal wastewater.

The reference striped bass and winter flounder ("3" and "3D") from Shinnecock Bay show a highly polluted striped bass composite except for phthalate esters. Interestingly, when compared to the literature data, HCBD appears to be an order of magnitude too high. This is almost certainly an accumulating error caused by the very small weight of liver that was soxhelet extracted, the error being multiplied throughout the experimental procedure. The winter flounder shows levels consistent with an exposure to a relatively clean environment with the exception of a high level for HCBD.

The groups of winter flounder from Shinnecock Bay ("2D", "4", "4D") show relatively low concentrations of HCB, which is consistent with less polluted marine ecosystems. HCBD concentrations are higher in two of the samples ("2D" and "4D") which may be indicative of the treated municipal wastewater exposure, but could be attributable to previous environmental exposure. Aroclor 1254 concentration levels show variation consistent with other published works, where the variability may be due to one very exposed fish within a pool of fish each with a relatively low levels of pollutants.

Striped Bass from the Hudson-Raritan estuary ("9" and "8D") show moderate levels of the selected chemicals, which is consistent with pelagic fish from more polluted urban estuaries. In addition, striped bass from the Hudson-Raritan estuary ("10" and "10D") show moderate levels of the selected chemicals and the concentration of HCBD is fairly high in comparison to other samples in this study.

Overall the results from the exposure and depuration study indicate concentrations of selected contaminants similar to those previously documented by other investigators as shown in tables in the introduction to this thesis. It is difficult to assess from the available data the overall effect of exposure to treated municipal wastewater or the depuration period. The data from the experiment however does in general show contaminant concentrations consistent with fish species habitat behavior, for example benthic feeders compared with pelagic feeders, or the geographical area the fish was caught in terms of the degree of pollution. This is consistent with the findings of other investigative studies.

The comparison of winter flounder muscle and liver ("42-M", "42-L", "47-M", "47-L") from the Hudson-Raritan estuary clearly show that elevated levels of chlorinated hydrocarbons occur in the liver. This is consistent with other reported data from similar investigations (8) and is also comparable to other published data as shown in the adapted data tables in the introduction section of this thesis. The winter flounder "47" shows typical tissue concentrations of hexachlorobenzene in a liver : muscle ratio of approximately 5 : 1. This ratio is not however consistent with hexachlorobutadiene and Aroclor 1254 which show similar levels in both liver and muscle. Winter flounder "42" shows similar levels of the chlorinated compounds in both liver and muscle, but with a trend towards higher concentrations in the liver as would be expected.

The phthalate ester data was very low and is probably due to the samples being from a single fish, as compared to pooled samples from a number of fish, and there is likely to be only a small amount of the phthalate esters present in each sample. This would result in the phthalate esters being below the detection limits of the detector because the phthalate esters were detected using a flame ionization

detector, a much less sensitive detector than an electron capture detector. From the data it appears that the winter flounder bioaccumulate more of the phthalate esters than the striped bass. The reproducibility of the results in terms of the integrated areas are probably no better than an order of magnitude. This is because of the variability from injection to injection and also the poor accuracy of the integration system. If a greater weight of fish liver tissue had been available for soxhelet extraction, there would have been a greater probability of the target compounds being present in levels above the detection limit. In actuality this is difficult to achieve because of the small weight of liver available from young of the year fish. Also, all of the pooled fish liver tissue was not available for this analytical determination because some was used for determination of other contaminants, for example benzo[a]pyrene and heavy metals, such as cadmium and mercury.

4 CONCLUSION

Better interpretation of the experimental results would have been possible if a better knowledge of the chemical composition of the treated municipal wastewater was known. For example, if elevated levels of hexachlorobutadiene (HCBD) were found then one could possibly attribute high levels of HCBD in the liver samples to the treated municipal wastewater exposure, and not to the bioconcentration of natural exposures in the marine ecosystem. Further controlled exposure studies could elucidate this possibility.

Phthalate esters, HCB, HCBD, and Aroclor 1254 have been proved to have a widespread distribution in the biological samples, due to the bioaccumulation processes within the marine environment, and to exposure uptake from treated municipal wastewater.

Assessing the amount of previous exposure and uptake of pollutants, was difficult with the amount of data that was available, but in general pollutant levels in older fish should be higher than young of the year fish. However, since the precise geographical and habitat behavior of the fish were not controlled, and unfortunately some data from the original experimental design are not available, it is difficult to conclude whether the pollutants came from exposure to treated municipal wastewater or from natural exposures in the wild.

A recommendation for future controlled laboratory studies, similar to the research attempted in this thesis, would be to set up the same exposure followed by depuration study only, this time, to use hatchery reared fish which have had a minimal previous exposure to chemical contaminants. A greater number of fish

would be used in order to obtain sufficient extractable pooled liver sample so the resulting analytical data is above the detection limits, particularly with respect to the flame ionization detector. These fish would then be exposed to treated municipal wastewater or even treated municipal wastewater spiked with chemicals that are of particular interest in the study. Fish food could also be spiked with chemicals in order to achieve a controlled exposure to certain chemicals. Individual and pooled tissue samples would give statistically worthwhile results and there would be no errors resulting from the use of fish which have previously been exposed to a myriad of toxic chemicals, even for a short time interval, prior to the exposure and depuration periods of the experiment. However, it should be noted that an experiment, as described above, does not reflect a real world situation and is not without limitations. For example no account is taken into consideration by the experimental design of: fish migration and spawning characteristics; the depth of the water column that the fish feeds and resides in; and natural resistance to bioaccumulation of toxic chemicals. For example, the degree in which the P-450 mixed function oxidase enzyme system in the liver has been induced by the chlorinated compounds, in order to metabolize them into more polar, but sometimes more toxic, hydrophilic compounds, which are more readily excreted by the kidneys (23). What the experiment would show, however, are bioconcentration and bioaccumulation of toxic chemicals, and a fish species metabolic and excretion ability, over a period of time. Information gained from such controlled exposure experiments could be used in predictive database modeling studies in order to allow better management of persistent chemicals in the marine environment, and also to identify compounds that are known to cause stress in certain fish species which could ultimately be passed along the food chain, eventually resulting in human exposure to toxic chemicals.

APPENDIX

Chemicals used in the sample preparation prior to analysis by Gas Chromatography:

Acetone: Burdick & Jackson
Capillary GC/GC-MS Solvent
Boiling Point: 56.3 ^{o}C
Lot: AU110
Water (%): 0.28
CAS: 67-64-1

Ethyl Ether: Burdick & Jackson
High Purity Solvent
Boiling Point: 34.6 ^{o}C
Lot: AV13822
UV Cutoff (nm): 208
Water (%): 0.005
Contains 2% (v/v) ethanol as preservative.
CAS: 60-29-7

Hexane: Burdick & Jackson
Capillary GC/GC-MS Solvent
Boiling Point 68.7 ^{o}C
Lot: AU429
Water (%): 0.003
CAS: 110-54-3

Methanol: Burdick & Jackson
High Purity Solvent
Lot: AU419
Water (%):0.006
UV Cutoff (nm): 203
CAS: 67-56-1

Petroleum Ether: Burdick & Jackson
Capillary GC/GC-MS Solvent
Boiling Range 30-60 ^{o}C
Lot: AV973
Water (%): 0.006
CAS: 8032-32-4

Florisil: A registered tradename of the Floridin Company.
Sigma Chemical Company
No. F-9127
Magnesium silicate activated at 1250 ^{o}F
Mesh: 60-100/PR
Lot: 78F-0718

Sodium Sulfate: J.T. Baker Inc.
Granular, (12-60 Mesh)
Lot: B39702
Hexane Extractables: < 0.00002 %

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