Distribution and Biomagnification of Polychlorinated Biphenyls in the Benthic Community

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Figures and Tables

Introduction

Polychlorinated biphenyls (PCB) are a group of toxic aromatic chlorinated hydrocarbon pollutants related to DDT and presently found throughout marine and terrestrial environments. PCB was first acknowledged to be in the environment in 1966, when Jensen identified them as a group of compounds that had been interfering with pesticide analyses (Jensen 1966). Two years later, Risebrough and coworkers identified PCB in a number of organisms, mostly from the western U.S. (Risebrough et al. 1968). Since that time many studies have demonstrated the widespread occurrence of PCB in the environment. PCB has been reported in fish from all of the major waterways in the U.S. (Henderson et al. 1972), from the coastal areas of Nova Scotia (Zitko 1971), and from Tokyo Bay (Selikoff 1972); in fish, mussels, and birds from the Rhine River and Netherlands coastal area (Koeman et al. 1969); in seals near Scotland (Johnels 1970); and in Brown Pelican eggs from Panama and Adelie penguin eggs from Antarctica (Risebrough et al. 1968).

The sublethal and lethal effects of PCB in the part per billion range (nanograms PCB per gram of tissue) has been documented by several laboratory studies. Table 1 shows some of the adverse effects of PCB to several species of phytoplankton and crustaceans, a mollusc, and several species of fish when the PCB concentrations in the water are in the part per billion range. The fact that PCB is a mixture of several compounds makes determination of toxicity difficult. Several studies have documented the differential toxicity of individual PCB homologs

Table I

EFFECTS OF PCB ON AQUATIC ORGANISMS

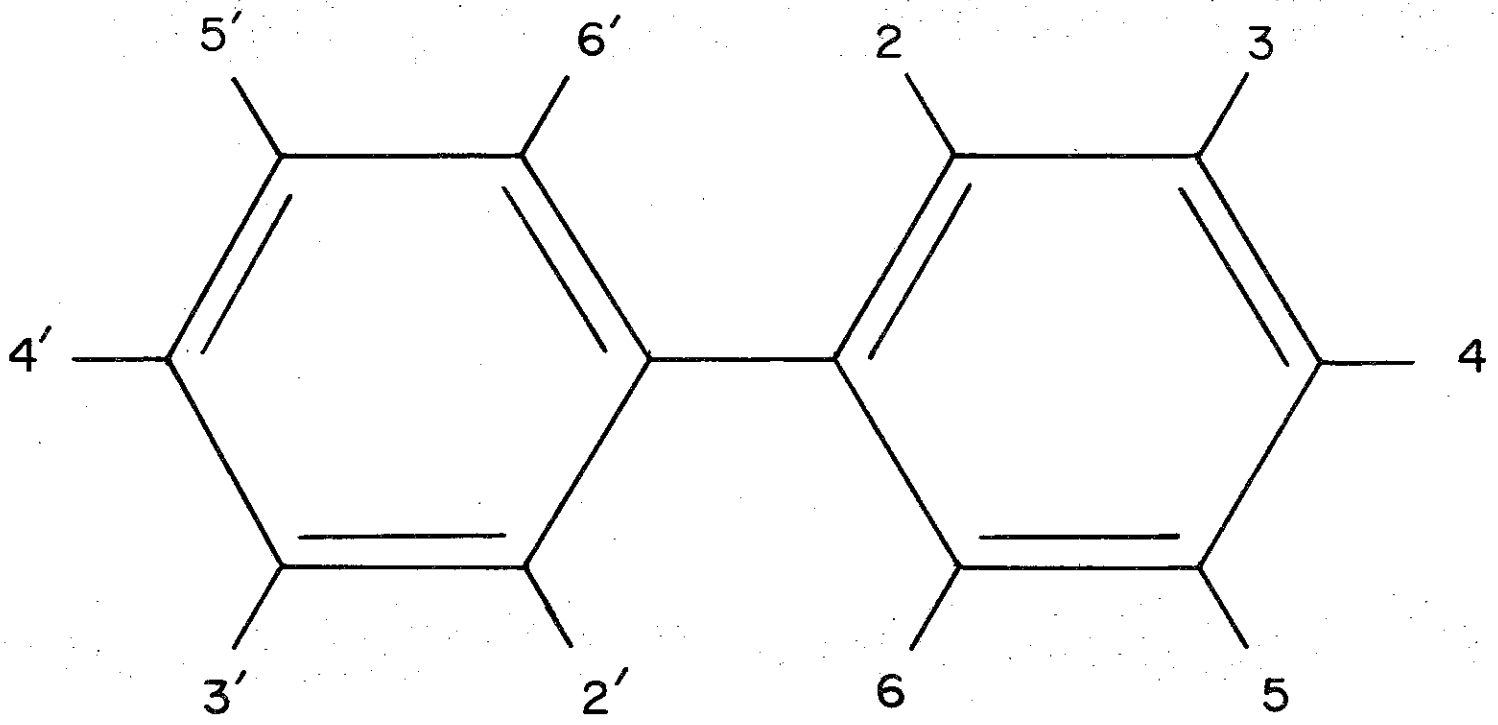
Species	Concentration in water (ppb)	Effect	Reference
1. Marine diatom			
(*Skeletonema costatum*)	10-100	inhibited growth	Mosser et al. (1972)
(*Cylindrotheca closterium*)	100	inhibited growth, chlorophyll & RNA levels reduced	Keil et al. (1971)
2. Amphipod (*Gammarus oceanicus*)	10	lethal threshold	Wildish (1970)
3. Juvenile pink shrimp (*Penaeus duoarum*)	5	72% mortality in 20 day exposure	Duke et al. (1970)
4. Oyster (*Crassostrea virginica*)	10	41% decrease in shell growth after 96 hr. exposure	Duke et al. (1970)
5. Pinfish (*Lagodon rhomboides*)	5	lethal threshold	Hansen et al. (1971)
6. Atlantic salmon (*Salmo parr*)	2000	lethal threshold	Zitko (1970)

(Villeneuve et al. 1971 & Lichtenstein et al. 1969). The presence of industrial impurities such as polychlorinated dibenzofurans has also been shown to affect the relative toxicity of PCB (Vos et al. 1970).

PCB is similar both in structure and behavior to DDT and its metabolites. The molecular composition of PCB allows as many as 210 different structural formations due to the possibility of different arrangements of chlorine atoms on the benzene rings of the PCB molecule. Figure 1 shows the PCB molecule with the positions for substitution by chlorine atoms. The particular characteristics of PCB: non-flammability, chemical stability, high dielectric constant, and plasticizing ability have found widespread use in industry and occasional use in agriculture since the late 1920's (Hammond 1972). Commercially, PCB is available as mixtures varying in chlorine content and are primarily used as dielectric fluids in capacitors and transformers and as hydraulic and heat transfer fluids. PCB has been used in sealants, adhesives, paints, and printing inks. They have also been recommended for use in pesticides, and have been reported to increase the longevity and toxicity of several groups of pesticides (Lichtenstein et al. 1969). Since September 1970, the Monsanto Chemical Company, the sole U.S. producer of PCB, has voluntarily restricted the use of PCB to "closed" systems.

There are no complete data concerning the rates of loss of PCB in the environment, but according to an estimate made in 1970, 15-25 thousand tons/year were released into the environment by vaporization and open burning of PCB-containing materials; 4-5 thousand tons/year were lost

Polychlorinated Biphenyl



(figure 1)

into fresh and coastal waters through leaks and disposal of transformer oils, hydraulic fluids, and lubricants; and 18 thousand tons/year were disposed of in dumps and landfills (Nisbet and Sarofim 1972). Once in the atmosphere PCB tends to adsorb onto particulate matter and is transported by wind currents. The major mechanism for PCB transport through the water is by adsorption to particulate matter in the water. Biological transport by aquatic organisms also affects the movement of PCB in the water (Selikoff 1972).

Since September 1972, an investigation has been underway to determine the concentration and distribution of PCB in phytoplankton, zooplankton, suspended particulate matter, water, and sediment in the Puget Sound, Washington (Pavlou et al. 1973). Few other studies have been done to examine the distribution of PCB in Puget Sound. In order to determine how PCB is distributed and where it is concentrated in the benthos of southern Puget Sound, we examined the mussel, Mytilus edulis, several species of bottom fish (cottids) including Leptocottus armatus and Oligocottus maculosus, and surface sediments throughout southern Puget Sound.

Factors determining the distribution of PCB within a community are numerous and complex, including such variables as size, age, sex, lipid content, diet, and habitat. Biomagnification, a mechanism through which organisms occupying higher trophic levels accumulate greater body burdens of a pollutant, may also be an important factor in determining the distribution of PCB within a community. It is not yet clear, however, that concentrations consistently increase as PCB passes through food

chains from lower to higher trophic levels. To determine the effects of biomagnification of PCB in a benthic community, we examined 11 different intertidal marine organisms from several different trophic levels within a food chain.

An important factor in determining the concentrations of PCB in an organism is the rate at which it accumulates and retains PCB from the environment. Accumulation and retention of PCB is affected by such factors as the organism's lipid content, morphology, and diet. We studied the mussel to determine how rates of uptake and retention affect the concentrations of PCB in this organism.

Field Procedures

Materials and Equipment

The materials required for sampling each site included a 4' x 40' beach seine; centigrade field thermometer; 4 oz collection jar; metal spatula; aluminum foil; stadia rod (12' marked in tenths of a foot); flagging tape; 100' cloth measuring tape; hand level; binoculars; plastic gloves; centimeter ruler; shovel; two 1' x 1' x 1' galvanized wire cages (½" mesh); insulated wire; and a compass.

Methods

Sample sites- All sites used in our study were surveyed to establish a reproducible study plot. A 50' x 100' area in the middle to lower zones of a beach was surveyed at each site using a hand held stadia rod and a hand level. A 100' baseline was established parallel to the low tide line. The 0,0 coordinate was established in the center of this baseline. Permanent reference points were established from surrounding landmarks or features for the baseline and 0,0 coordinate. Compass headings for the baseline and a perpendicular line passing through the 0,0 point were determined. Survey sites were marked at 20' intervals with flagging tape. Differences in altitude from the low tide mark (established by a tide chart) to the 0,0 coordinate were measured and recorded. From this information, the elevation at each of the marked points on the survey plot was measured and recorded.

Parameters such as wind direction and speed, weather conditions,

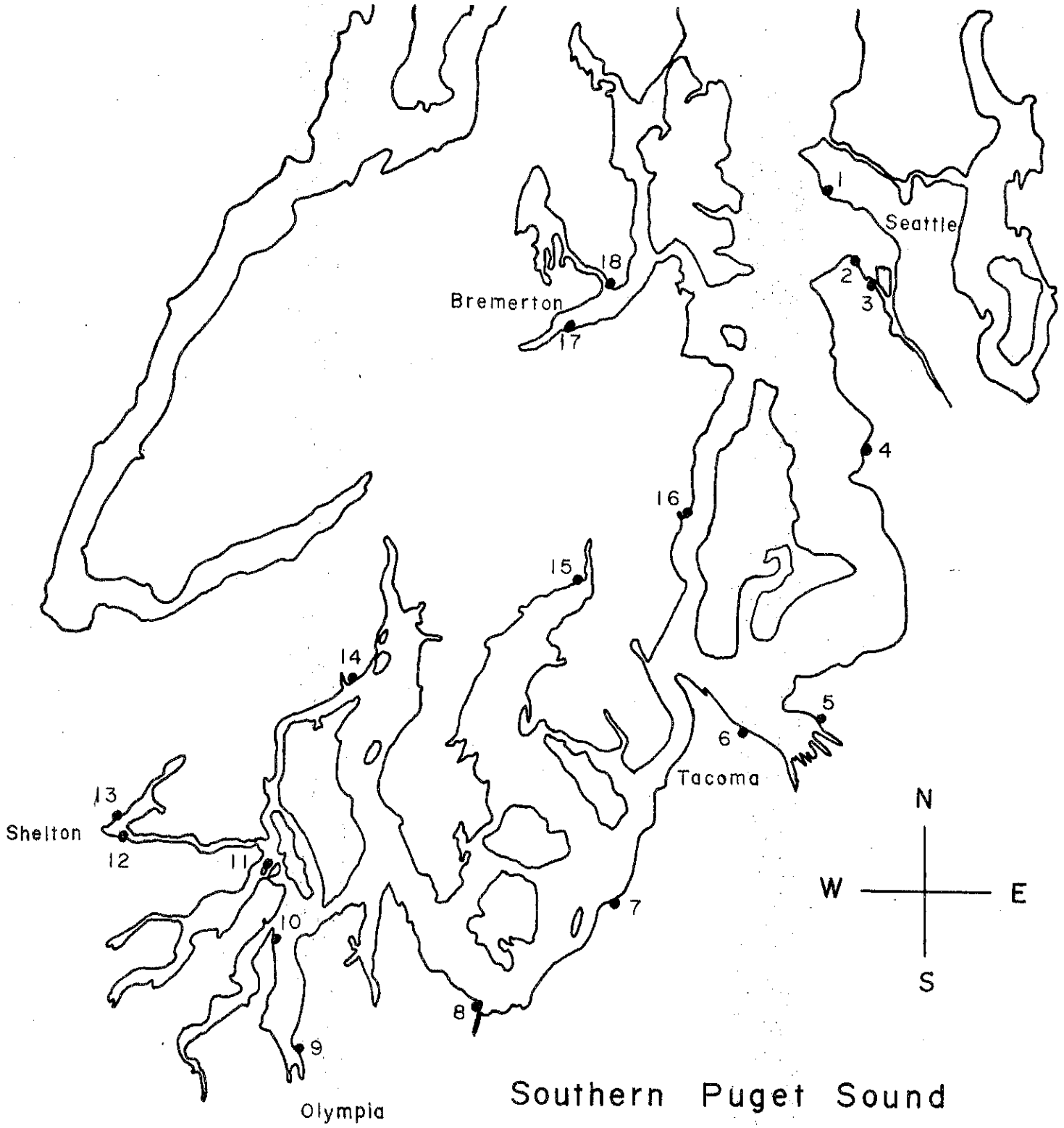
relative surface wave activity, and air and water temperature were recorded. (Air temperature was taken near the low tide level about 3' to 4' above the beach surface. Water was taken from approximately 6" under the surface and several feet past low tide level). The biological and physical zonation from high to low tide levels through our study area was described and substrate composition at each zone was recorded.

In order to gain an accurate representation of the distribution of PCB in mussels, cottids, and sediment, 18 sites were chosen throughout Puget Sound (see Fig. 2). In each area a site was chosen according to its accessibility and the occurrence of study organisms. Attention was given to the presence or lack of heavy industry, heavy shipping activity, and high population when choosing an area in which to select a site.

Samples were collected during the low tide period once during the summer to indicate PCB levels at each site. At each site, 15-25 mussels were collected from within the surveyed plot and wrapped in clean foil. Cottids were seined immediately offshore from the study site. 4 oz of the top 2 cm of sediment was taken using a metal spatula at the low tide mark for a given collection day and placed in a clean glass jar. All samples were handled with plastic gloves to prevent contamination and frozen within 1½ hours of collection.

To examine the possibility of biomagnification as a factor affecting the distribution of PCB among trophic levels in a benthic community, we sampled several intertidal species at different trophic levels from two sites, #6 in Tacoma and #12 in Shelton. Species sampled to represent

SITE LOCATIONS



Southern Puget Sound

(figure 2)

primary consumers at site #6 were the mussel, Mytilus edulis, and the limpet, Collisella strigatella. Hemigrapsus oregonensis, the shore crab, was sampled to represent a scavenger within a food chain. Thais lamellosa, the whelk, and Pisaster ochraceous, the starfish, represented the major secondary consumers within the community we examined.

Samples taken from the Shelton site representing primary consumers were the barnacle, Balanus glandula; several clams: Protothaca staminea, Veneripus japonica, and Saxidomus giganteus; the cockle, Clinocardium nutalli, and M. edulis. H. oregonensis was also sampled at this site to represent a scavenger. T. lamellosa and Polinices lewisii, the moon snail, were sampled to represent secondary consumers within a food chain.

Sampling procedures were essentially the same with clams, cockles, and moon snails collected at the lower tidal zone using a shovel.

To examine rates of uptake and retention within a marine organism, we designed a study involving the exchange of caged mussels between areas of high and low PCB concentrations. We chose to study the mussel due to the ease of maintenance and collection of this organism. To establish rates of PCB uptake, approximately 100 mussels collected from site #14, an area of relatively low PCB concentration, were placed in a wire cage and transferred to site #3, an area of relatively high PCB concentration. This procedure in reverse was followed to establish rates of retention of PCB. Cages containing mussels native to each site were also set up at sites #3 and #14 to serve as controls. Each cage was wired shut and anchored to a permanent feature such as a rock or piling at a level

where mussels naturally occurred. Samples were collected from all four cages at 1, 2, 3, and 7 weeks.

Laboratory Procedures

Materials and Equipment

Burdick and Jackson Laboratories supplied glass-distilled hexane, acetone, and 2,2,4-trimethylpentane. J.T. Baker Chemical Co. supplied concentrated sulfuric acid, copper turnings, and sodium bicarbonate. Celite was obtained from the Kensington Scientific Corp.; fuming sulfuric acid from Mallinckrodt Chemical Works; and BFM solution, Mixture F (2 vols. 70% perchloric acid in 3 vols. glacial acetic acid), from G.F. Smith Chemical Co.. The Monsanto Chemical Co. supplied the polychlorinated biphenyls Aroclor 1242, Aroclor 1254, and Aroclor 1260.

PCB was analyzed on a Hewlett-Packard 5700A electron-capture gas chromatograph (Ni^{63} detector) on line with a Hewlett-Packard 3380A Integrator and a Hewlett-Packard 7123A chart recorder. Calculations were performed on a Hewlett-Packard 2000C computer. Other miscellaneous equipment included a Unimetrics Corp. #5010RM 10 µl syringe; Precision Sampling Corp. #120025 50 µl syringe; Mettler H72 balance; Ohaus Dial-O-Gram balance; Perkin-Elmer AD-2 Autobalance; Corning PC-35 hot plate; Waring blendor; Büchi Rotavapor-R rotary evaporator; Knotes tube heater; VWR-MSE GT-2 centrifuge; Nikon Model S-KE II microscope, and a Thermovac Industries freeze-dryer.

Methods

(1) Biological specimens- Measurements of the sample were recorded, and a sufficient number of whole specimens to give 10 - 20 g of tissue

were accurately weighed into a beaker. Molluscs were shucked prior to analysis. Approximately 2 ml of BFM solution (2 vols. 70% perchloric acid in 3 vols. glacial acetic acid) was added for every gram of sample present. A blank, consisting of approximately 40 ml of BFM solution, was analyzed every time a series of 5 - 8 tissue samples were analyzed.

The sample was digested by heating it on a steam bath for 4 - 6 hours. After cooling, the volume was doubled with distilled water. The sample was transferred to a separatory funnel and extracted with approximately 3 x 20 ml glass-distilled hexane. The combined extracts were transferred to a mixing cylinder and shaken to insure uniform mixing. For lipid determination, approximately 15 ml of the extract was poured into a beaker and allowed to evaporate to dryness. The beaker was reweighed, and the difference between the two weights was used to calculate the lipid weight of the sample.

Approximately 10 ml of the hexane extract was transferred to a centrifuge tube and shaken for 3 minutes with 5 - 10 grains of copper turnings (previously extracted in hexane). The copper turnings were removed and 1 - 2 ml of concentrated sulfuric acid was added to the hexane extract. The centrifuge tube was again shaken for one minute, then centrifuged at approximately 3000 rpm for 10 minutes. The sulfuric acid portion was frozen with dry ice, and the hexane layer was removed and concentrated to an appropriate volume using a Kontes tube heater or rotary evaporator prior to gas chromatographic analysis.

(2) Sediment- Samples were freeze-dried for approximately 48 hours.

The composition of the sediment was determined through visual and microscopic analysis (see Appendix A). An accurately weighed portion of freeze-dried sediment was transferred to a fritted glass extraction thimble and soxhlet-extracted with approximately 120 ml glass-distilled hexane for 12 - 18 hours. A blank consisting of approximately 150 ml hexane was carried through the same procedures as the sediment every time a series of 6 - 8 sediment samples were extracted. Sulfur was removed from the hexane extracts by one of the following two methods.

(a) A celite-sulfuric acid mixture was prepared by adding 21 ml each of fuming sulfuric and concnetrated sulfuric acids to approximately 70 g celite. The celite was previously extracted with hexane for 12 hours, then activated at 100C for several hours prior to use. Enough hexane was added to make a slurry, and the mixture was thoroughly stirred. Two-thirds of the volume of a fritted glass extraction thimble was packed with this slurry, and an appropriate volume of hexane extract was added to the thimble while it was in the soxhlet extractor. The thimble containing the slurry was soxhlet-extracted with hexane for 1½ hours, then removed from the extractor. Approximately 20 ml of a saturated solution of sodium bicarbonate was added to the hexane extract, and the resulting mixture was refluxed for 1½ hours. The aqueous portion was frozen, and the hexane extract was removed and concentrated to an appropriate volume prior to gas chromatographic analysis.

(b) The hexane extract was concentrated, then transferred to

a centrifuge tube and shaken with 1 - 2 ml concentrated sulfuric acid for one minute, then centrifuged at approximately 3000 rpm for 10 minutes. The sulfuric acid portion was frozen with dry ice, and the hexane layer was transferred to a 15 ml vial containing 50 - 100 grains cleaned copper turnings.

(3) Glassware cleaning- All of the glassware used in sample analysis was cleaned by washing in hot soapy water, followed sequentially by a tapwater rinse, chromic acid rinse, tapwater rinse, distilled water rinse, dry, acetone rinse, dry, and hexane rinse. The glassware was then baked at 260C for 12 hours, then capped with aluminum foil. The glassware involved in the soxhlet-extraction of sediment samples was cleaned by washing in hot soapy water, followed by a tapwater rinse and distilled water rinse. After drying, the glassware was pre-extracted with a 1:1 acetone-hexane mixture for at least 12 hours before use.

(4) Sample analysis- A Hewlett-Packard 5700A electron-capture gas chromatograph (Ni^{63} detector) coupled to a Hewlett-Packard 3380A Integrator was used to quantitatively determine the amount of PCB present in each sample. From 4 - 40 µl of sample was injected onto a coiled 6' glass column packed with 1" 33% NaOH/KOH on Gas Chrom Q 80/100 mesh followed by 10% DC-200 on Gas Chrom Q 80/100 mesh maintained at the following operating parameters: oven temperature 225C; detector temperature 300C; carrier gas 60 ml/min 95% argon-methane.

Two PCB standards were prepared by mixing equal quantities of Monsanto Aroclor 1242 (Lot #KB-03-410), Aroclor 1254 (Lot #KB-01-604), and Aroclor 1260 in enough glass-distilled 2,2,4-trimethylpentane to yield concentrations

of 300 µg/l and 60 µg/l. A PCB standard was injected after every three samples injected.

(5) Quantification- We determined the amount of PCB present in our samples through identification and quantification of the individual PCB homologs. Twenty-two different homolog and homolog pairs were identified in the PCB standard by comparing their peak profiles with those published by Webb and McCall (1973). Figure 3 shows a chromatogram of the PCB standard with the 22 numbered peaks. The names of the homologs and homolog pairs corresponding to each peak are given in Appendix B.

Identification of peaks in the environmental samples was achieved by comparing their relative retention times* and peak profiles with those of the PCB standard. A strong similarity in peak profile and relative retention time occurs after peak #6 between the chromatograms of our environmental samples (Figs. 4, 5, 6, 7, & 8) and the chromatogram of the PCB standard (Fig. 3).

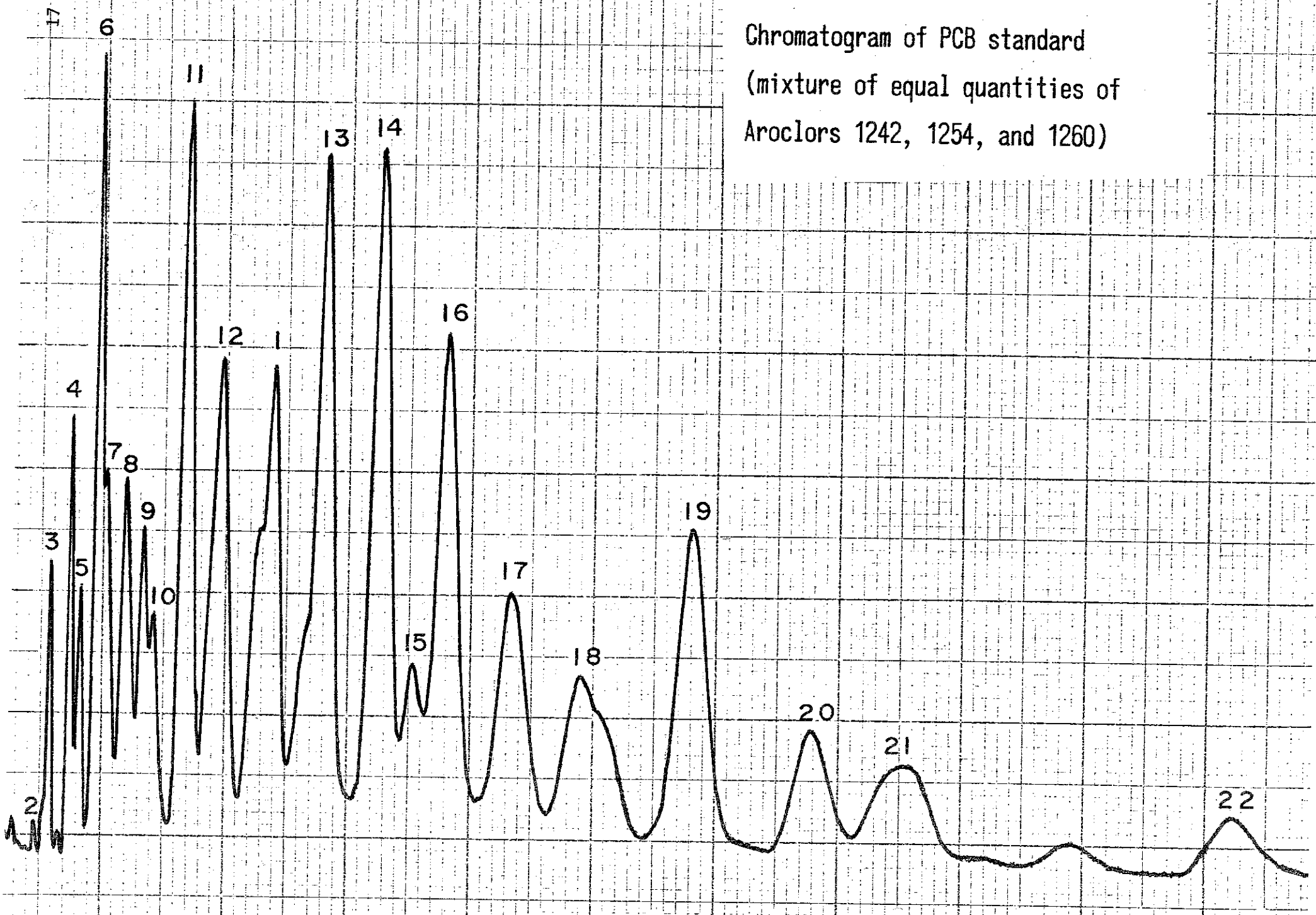
The mean weight percent of each peak in the PCB standard (Webb and McCall 1973) was determined and used to calculate the concentrations of PCB present in our samples (see Appendix B). A computer program was generated on a Hewlett-Packard 2000C time-shared BASIC system to do the calculations involved and compile the data. The nanograms of PCB and ppb wet weight were established for each homolog, as well as a total ppb wet weight for the major homologs appearing in our samples.

We established a method to estimate the amount of p,p' DDE in peak #1, which occurred in our environmental samples, interfering with the quantification of the PCB homologs occurring in that peak. The method

*2,4,5,2',4',5'-hexachlorobiphenyl = 1.000

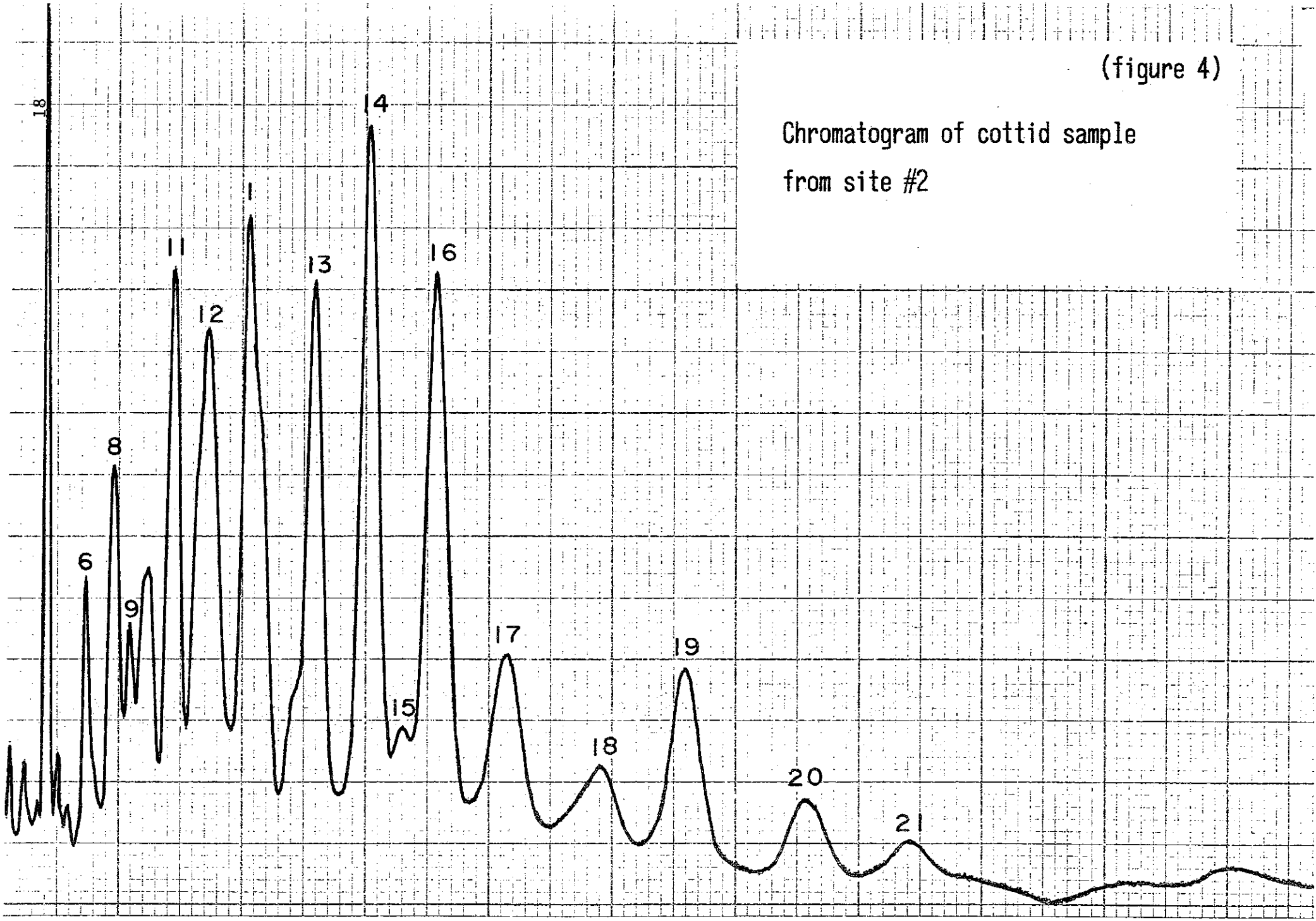
(figure 3)

Chromatogram of PCB standard
(mixture of equal quantities of
Aroclors 1242, 1254, and 1260)



(figure 4)

Chromatogram of cottid sample from site #2



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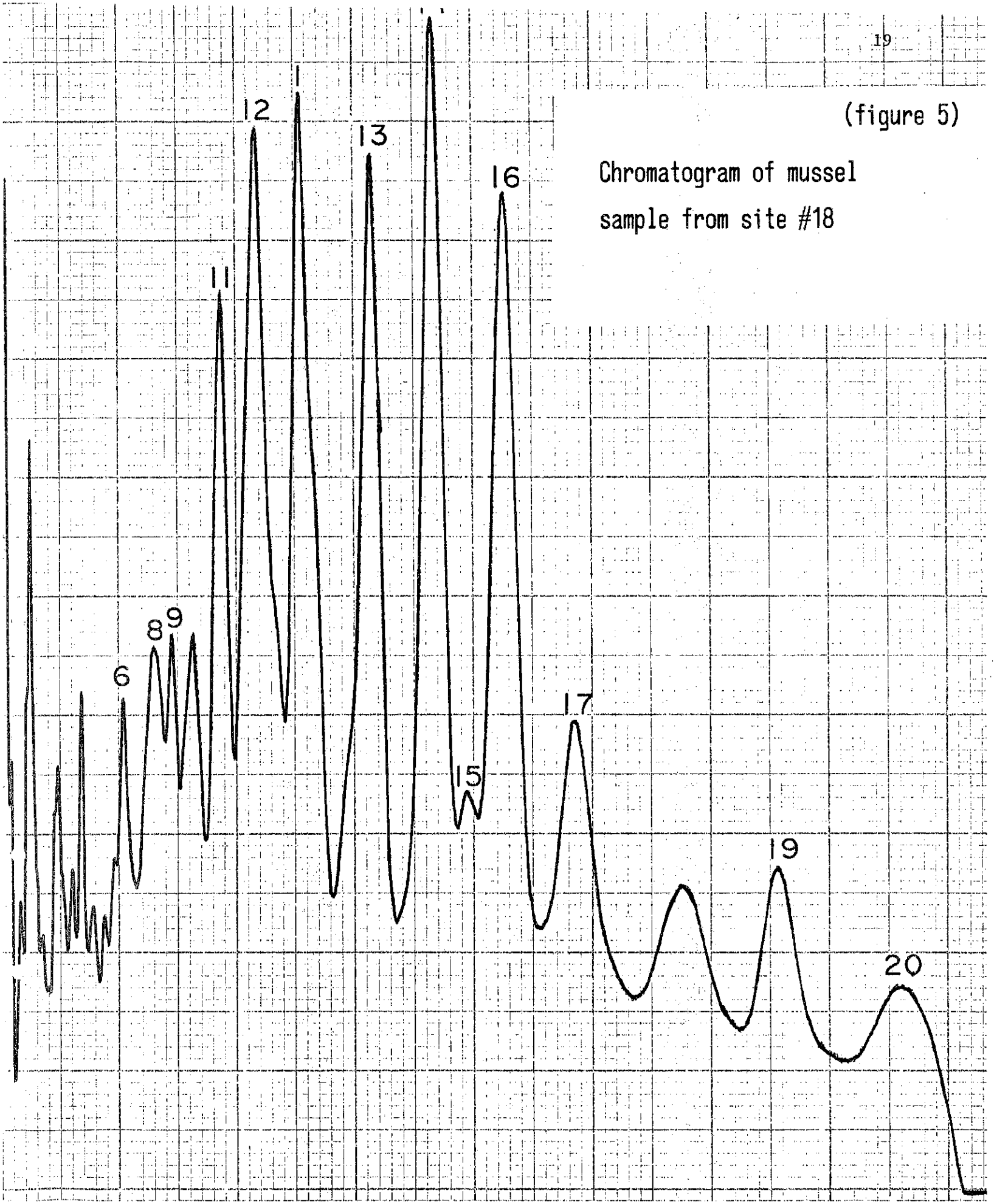
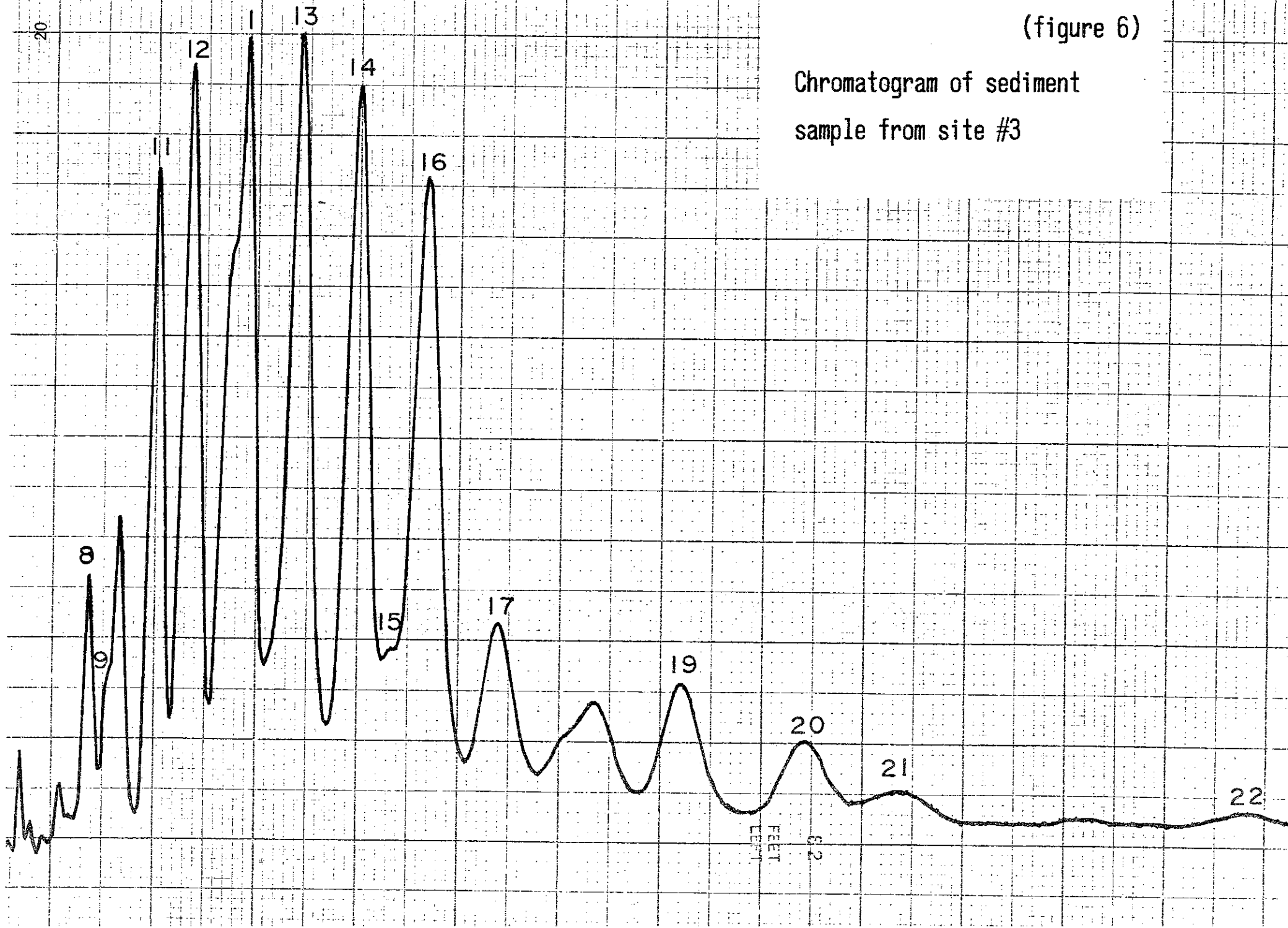
(figure 5)

Chromatogram of mussel sample from site #18



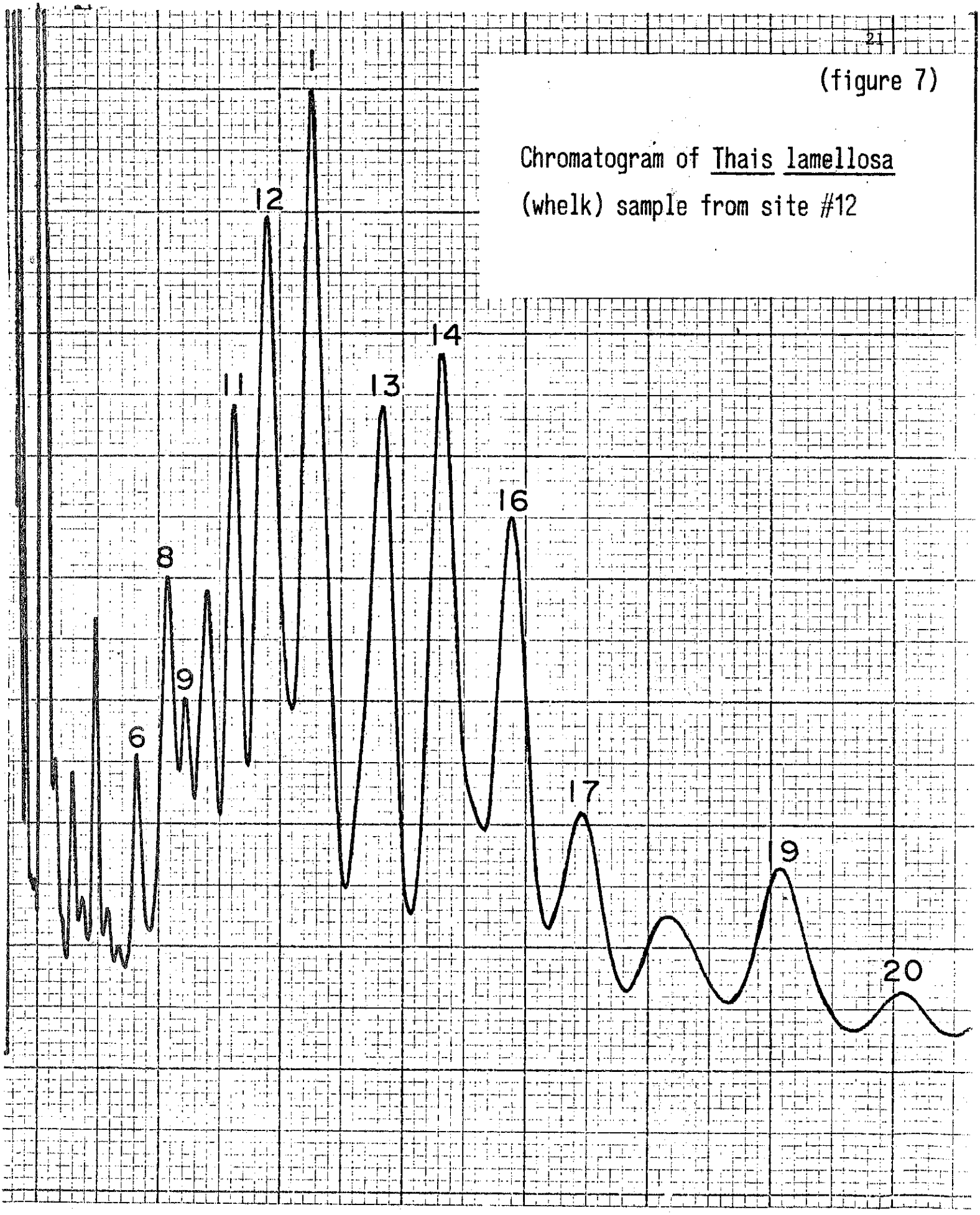
(figure 6)

Chromatogram of sediment sample from site #3



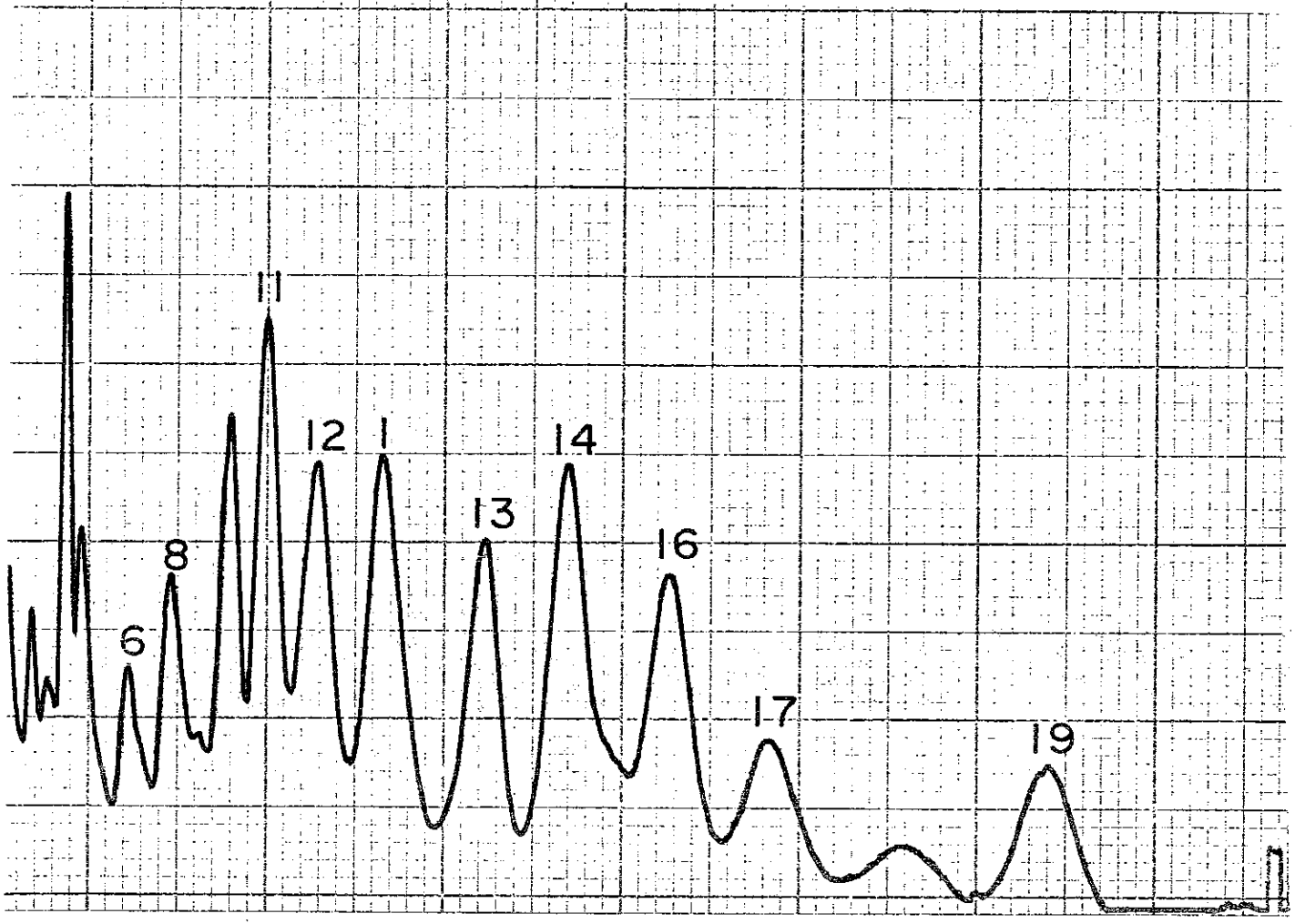
(figure 7)

Chromatogram of Thais lamellosa (whelk) sample from site #12



(figure 8)

Chromatogram of Hemigrapsus oregonensis (crab) sample from site #6



involves establishing a ratio between the peak interfered with and the surrounding two peaks in our standards, and then using this ratio to estimate the amount of PCB homologs present in the combined peak. The levels of p,p' DDE could then be extrapolated from the adjusted area of the peak. At the time of this writing, p,p' DDE concentrations have not been estimated for all of our samples, but preliminary findings indicate that the p,p' DDE concentrations constitute less than 5% of the PCB concentrations.

The concentrations of PCB in our samples reflects the sum of the PCB homologs present in peaks #6-9, #11-14, #16, #18, and #19. Values for peaks #2-5 and #10 were not included because either they did not occur in the sample or their peak profiles and relative retention times did not correspond to those in the PCB standard. Peak #1 was excluded because of interference due to p,p' DDE. Peaks #15, #18, and #20-22 were often not accurately integrated by the Hewlett-Packard Integrator and were thus excluded to insure consistency among levels reported at different sites and for purposes of comparison. It should be understood that the PCB levels in our samples thus tend to reflect the minimum amount of the actual PCB concentrations present.

Using the above methods, three cottid samples "spiked" with 30 µg of the PCB standard yielded an average 90% recovery, and a sediment sample "spiked" with 15 µg of the PCB standard yielded close to 100% recovery.

Results and Discussion

The concentrations of PCB in cottid, mussel, and sediment samples taken from each of the 18 sites in southern Puget Sound are shown in Tables 2 - 4. The highest levels of PCB in cottids, mussels, and sediment occurred at site #3 at the mouth of the Duwamish River, 1/2 mile downstream from an area where PCB was spilled when a transformer tipped over in September 1974, releasing over 200 gallons of PCB containing fluid into the river (De Yonge 1974). The lowest levels in cottids and sediment occurred at site #14, and the lowest levels in mussels occurred at site #15.

The PCB levels in our cottid samples are probably influenced in a large part by the PCB levels in the sediment, as cottids have a tendency to forage for food in the sand or mud (Jones 1962). A study done in California on chlorinated hydrocarbons shows a strong correlation between sediment concentrations and concentrations in bottom fish (Young et al. 1975). Our data tend to support this conclusion. The range of PCB in cottids and sediment varies greatly throughout southern Puget Sound, ranging from 21-840 ppb in cottids and 0.65-330 ppb in sediment, which suggests that PCB accumulates in certain areas and is not evenly distributed throughout the sediment in southern Puget Sound.

A distinctive finding from our data is that the levels of PCB in environmental samples are positively correlated to the degree of industrialization, shipping and human population. Highest levels of

Table 2

CONCENTRATION OF PCB IN COTTIDS FROM SOUTHERN PUGET SOUND

Site No.	Average length (cm.)	Sample size	Concentration (ppb wet wt.)
1	9	2	65
2	6	6*	470*
3	10	2	840
4	4	7	180
5	7	3**	200**
6	24	1	500
7	15	1	100
8	10	2	130
9	13	1	56
10	15	1	66
11	20	1	29
12	8	3	29
13	12	1	62
14	12	1	21
15	13	1	29
16	21	1	63
17	9	2	160
18	9	2	190

*average of 5 values **average of 4 values

Table 3

CONCENTRATION OF PCB IN MUSSELS FROM SOUTHERN PUGET SOUND

Site No.	Average length (cm.)	Sample size	Concentration (ppb wet wt.)
1	4.8	7	85
2	4.6	7*	95*
3	4.7	6	210
4	5.0	4	31
5	4.3	7*	72*
6	5.1	6	38
7	4.2	6	16
8	5.4	6	50
9	4.2	6	27
10	5.9	4	30
11	4.5	6	14
12	4.9	8	24
13	4.5	7	11
14	4.7	5	16
15	—	—	10
16	4.5	6	11
17	5.1	5	14
18	4.7	6	40

*average of five values

Table 4

CONCENTRATION OF PCB IN SEDIMENT FROM SOUTHERN PUGET SOUND

Site No.	Elevation (ft. above sea level)	Sediment type (see Appendix B)	Concentration (ppb, dry wt.)
1	-0.8	MS, a	1.8
2	-1.8*	P,G,MS, S, C, b	70*
3	-0.8	FS, S, C	330
4	-2.3	MS	<1.0
5	-0.8*	MS, S, C	6.3*
6	-2.1	MS, S, C	17
7	-2.3	MS, CS, C, S	1.5
8	-1.6	MS, S, C	2.6
9	——	MS, CS	1.1
10	-1.4	MS, S, C	2.1
11	-1.5	P, G, CS, MS	0.70
12	-1.4	MS, S, C, a	1.5
13	-1.7	FS, S, C, a	3.1
14	-1.8	CS, VCS, c	0.65
15	-1.6	CS, FS, S, C	5.7
16	-1.8	MS, S, C	1.8
17	-1.7	FS, S, C	10
18	-0.9	P, G, MS, CS, b	7.3

*average of five values

PCB were found at areas of high industrialization, shipping and population, specifically at sites #2 and #3 in Seattle and sites #5 and #6 in Tacoma. Moderate levels were found in areas of moderate industry and population and heavy shipping, namely sites #17 and #18 in Bremerton. Lower levels occurred at sites #9 and #10 in Olympia and sites #12 and #13 in Shelton, which have relatively lower populations and light industry and shipping. The lowest levels occurred at sites #11, #14, and #15, which are the furthest removed from heavy industry and shipping. The moderately high levels at site #8 are probably influenced by the fact that it is located at the mouth of a river delta, which may serve as a depository for any PCB leached into the Nisqually River, which empties into this delta. Moderately high levels of PCB at sites #1, #4, and #7 are probably due to their proximity to Seattle and Tacoma. Our findings that high PCB levels are generally found at or near highly populated areas with heavy industry and shipping is supported by a study done in Escambia Bay, Florida (Duke et al. 1970). Levels of PCB in fish, blue crabs, and sediment decreased rapidly as distance from an industrial source increased.

The concentrations of PCB in the intertidal organisms collected from sites #6 and #12 are given in Tables 5 and 6. They generally tended to support the hypothesis that biomagnification may play an important role in determining the distribution of PCB among different trophic levels. Levels in secondary consumers from site #6 were 3 to 9 times higher than those in primary consumers (Table 5), and levels in secondary consumers from site #12 were from 2 to 22 times higher than those in primary consumers

Table 5

PCB CONCENTRATIONS IN MARINE ORGANISMS FROM DIFFERENT TROPHIC LEVELS

Trophic Level	Species	No. in sample	Concentration in organism at site #6 (ppb wet wt.)
Secondary consumers	Thais lamellosa (whelk)	4	290
	Pisaster ochraceous (starfish)	1	130
Primary consumers	Mytilus edulis (mussel)	4	36
	Collisella strigatella (limpet)	12	46
Scavenger	Hemigrapsus oregonensis (shore crab)	4	32

Table 6

PCB CONCENTRATIONS IN MARINE ORGANISMS FROM DIFFERENT TROPHIC LEVELS

Trophic Level	Species	No. in sample	Concentration in organism at site #12 (ppb wet wt.)
Secondary consumers	Thais lamellosa (whelk)	3	97
	Polinices lewisii (moon snail)	1	8.2
Primary consumers	Balanus glandula (barnacles)	51	5.1
	Mytilus edulis (mussel)	5	6.7
	Protothaca staminea (littleneck clam)	3	4.8
	Veneripus japonica (littleneck clam)	3	3.6
	Saxidomus giganteus (butterclam)	1	0.6
	Clinocardium nutallii (cockle)	1	5.3
Scavengers	Hemigrapsus oregonensis (shore crab)	10	4.8

(Table 6). Separate studies done by Christopher Dlugokenski and Karen Oakley in our laboratories document the possibility of further biomagnification in trophic levels higher than those that we studied. For example, truecod from Commencement Bay in Tacoma had from 1.6 to 3.6 ppm of PCB in their livers. Pigeon guillemot eggs (Cepphus columba) taken from Elliot Bay in Seattle near site #1 had levels of 16.6 and 20.6 ppm of PCB, reflecting a 300 fold increase over levels in cottids taken from the same area. A study done in the Baltic Sea substantiates our findings that PCB concentrations are markedly higher in organisms occupying higher trophic levels. PCB concentrations increased by a factor of 10 to 500 times between primary and secondary consumers (Jensen et al. 1969).

The concentrations of PCB in mussels exchanged between sites #3 and #14 are given in Tables 7 and 8. PCB levels in mussels transplanted from site #14 (an area of relatively low PCB concentration) to site #3 (an area of relatively high PCB concentration) show a relatively rapid increase, reaching control levels by the second week (Table 7). PCB levels in mussels transplanted from site #3 to site #14 did not show a smooth decrease, but seem to indicate that mussels lose PCB to the environment, reaching control levels by the seventh week (Table 8). Since mussels flush large volumes of water through their bodies daily (Wilbur 1966), it seems likely that this is the major mechanism through which they accumulate and lose PCB in the environment.

The levels of PCB that we found in our samples are comparable to

Table 7

UPTAKE OF PCB BY MUSSELS

Time	Concentration in mussels transferred from site #14 to site #3 (ppb wet wt.)	Concentration in control mussels at site #3 (ppb wet wt.)
0 week		95
1 week	31	160
2 weeks	210	150
3 weeks	240	160
7 weeks	160	200

Table 8

RETENTION OF PCB BY MUSSELS

Time	Concentration in mussels transferred from site #3 to site #14 (ppb wet wt.)	Concentration in control mussels at site #14 (ppb wet wt.)
0 week		———
1 week	130	9
2 weeks	10	22
3 weeks	100	10
7 weeks	7	11

those found in similar organisms from other coastal and estuarine systems throughout the world. Average PCB concentrations in mussels and herring from sites along the coast of Sweden were 30 ppb and 270 ppb, respectively (Jensen et al. 1969). In the Irish coastal system, mussels were reported to have from 50 to 500 ppb and herring contained from 10 to 2000 ppb of PCB (Holgate 1970). Mussel samples from the New Brunswick coast and herring from the Bay of Fundy contained 140 and 540 ppb, respectively (Zitko 1971). PCB levels in mussels from San Francisco Bay were reported to be between 30 and 60 ppb (Risebrough and Schmidt 1975).

Conclusions

The relative similarity in global PCB levels found in coastal and estuarine environments throughout the ecosystem suggests that the material is ubiquitous in its distribution and consistent in behavior once it is released into the environment. The observance of the distribution of PCB and its accumulation in the biota of the Puget Sound reveals significantly higher levels consistently occurring near areas of heavy industrialization and high population with rapid decrease in levels as distance from source increases.

The significance of PCB levels observed in this study with regard to potential adverse effects in the environment is difficult to ascertain. Preliminary findings of others indicate PCB to be toxic to some marine organisms at part per billion levels in the water. Based on this data, concentrations found in most of southern Puget Sound probably do not approach recognized toxicity levels. However, it should be taken into consideration that research regarding toxicity at part per billion levels has been restricted to few species. Very little is known about sublethal effects. Therefore it is difficult to predict adequately the potential effects throughout a highly diversified ecosystem such as exists in the Puget Sound. Further studies involving nearly all aspects of PCB and its interaction with the environment are necessary to accurately evaluate the significance of present levels of the pollutant in the world ecosystem.

Appendix A

COMPOSITION OF PCB STANDARD

Peak No.	Mean Weight Percent	Homolog Name
2	0.97	1- chlorobiphenyl
3	3.77	2- chlorobiphenyl
4	3.67	2- & 3- chlorobiphenyl
5	2.03	3- chlorobiphenyl
6*	3.83	3- chlorobiphenyl
7*	3.70	3- chlorobiphenyl
8*	5.00	4- chlorobiphenyl
9*	3.23	3- & 4- chlorobiphenyl
10	2.33	4- chlorobiphenyl
11*	8.73	4- & 5- chlorobiphenyl
12*	9.43	4- & 5- chlorobiphenyl
1**	9.57	5- & 6- chlorobiphenyl
13*	10.73	5- & 6- chlorobiphenyl
14*	8.50	5- & 6- chlorobiphenyl
15	2.07	6- & 7- chlorobiphenyl
16*	6.93	6- chlorobiphenyl
17*	3.70	6- & 7- chlorobiphenyl
18	3.60	6- & 7- chlorobiphenyl
19*	3.67	7- chlorobiphenyl
20	1.40	7- chlorobiphenyl
21	1.33	8- chlorobiphenyl
22	0.50	8- chlorobiphenyl

* peaks used in quantifying our samples

** area of p,p' DDE overlap

The following calculations were used to determine the amount of PCB present in our samples.

The response factor (R.F.) for a given peak W in the PCB standard was calculated by multiplying the mean weight percent of peak W by the total nanograms of PCB injected to give the nanograms of PCB represented by peak W, then dividing this figure by the area of peak W (equation 1).*

$$(1)\quad (\text{R.F. for peak W}) = \frac{(\text{mean wt. \% of peak W})(\text{total ng. of PCB injected})}{(\text{area of peak W})}$$

The response factors for each of the peaks in a continous series of PCB standards were averaged and used to calculate the nanograms of PCB represented by the corresponding peaks in environmental samples injected within the same time period. For example, the averaged response factor for peak W would be multiplied by the corresponding area for peak w in the environmental sample to give the nanograms of PCB represented by peak w (equation 2).

$$(2)\quad (\text{ng. of PCB represented by peak w}) = (\text{R.F. for peak W})(\text{area of peak w})$$

The sum of the nanograms represented by each peak in an environmental sample is then determined to give the total amount of PCB present in the injected sample. This figure is used to calculate the parts per billion (ng./g.) concentration of PCB present according to equation 3.

$$(3)\quad x = \frac{(n)(h)(1000)}{(i)(g)}$$

n = total amount of PCB in sample injected (ng)
h = total vol. of hexane used in extraction (ml)
i = injection volume (µl)
g = wet wt. of sample (g)
x = concentration of PCB (ppb)

*area of peak determined by Hewlett-Packard Integrator

Appendix B

CHARACTERIZATION OF SEDIMENT SAMPLES

The composition of sediment samples taken from southern Puget was determined by the following method (Wentworth 1922). After freeze-drying, particle size was determined by both visual and microscopic analysis and the approximate percent composition of shell pieces was determined. The following table lists the classifications used in determining particle size.

Grade limits (diameter)	Name	Abbreviations used in Table 4
Above 256 mm	Boulder	B
256 - 64 mm	Cobble	Co
64 - 4 mm	Pebble	P
4 - 2 mm	Granule	G
2 - 1 mm	Very Coarse Sand	VCS
1 - 0.5 mm	Coarse Sand	CS
0.5 - 0.25 mm	Medium Sand	MS
0.25 - 0.125 mm	Fine Sand	FS
0.125 - 0.063 mm	Very Fine Sand	VFS
0.063 - 0.004 mm	Silt	S
Below 0.004 mm	Clay	C

The percent composition of shell pieces was broken down into the following categories.

Percent composition of shell pieces	Abbreviations used in Table 4
less than 30%	a
30% - 60%	b
greater than 90%	c

No attempt was made to determine the individual percentages of the different particle sizes occurring in a sediment sample, and only those particle sizes comprising a significant portion of the samples were listed in Table 4.

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