Publication No. 84-e44

WA-15-0020

CHEMICAL CONTAMINANTS IN CLAMS AND CRABS FROM EAGLE HARBOR, WASHINGTON STATE, WITH EMPHASIS ON POLYNUCLEAR AROMATIC HYDROCARBONS

October 1984

by:

Bill Yake
Joe Joy
Art Johnson

Water Quality Investigations Section
Washington State Department of Ecology

CHEMICAL CONTAMINANTS IN CLAMS AND CRABS FROM EAGLE HARBOR, WASHINGTON STATE: WITH EMPHASIS ON POLYNUCLEAR AROMATIC HYDROCARBONS

Introduction

In early 1984, the results of initial studies by the Northwest and Alaska Fisheries Center (National Oceanic and Atmospheric Administration, NOAA) in Eagle Harbor (Bainbridge Island) were transmitted to the federal Environmental Protection Agency (EPA, Region 10) and the Washington State Department of Ecology (WDOE). A letter dated March 19, 1984, from Dr. Donald Malins (NOAA) to Dr. Gary O'Neal (EPA) summarized findings from these studies including high concentrations of polynuclear aromatic hydrocarbons (PNAs) and pentachlorophenol (PCP) in Eagle Harbor sediments and a high incidence of pathological disorders in English sole collected in Eagle Harbor.

Although the data available at this time were not complete, particularly with regard to the public health implications of the preliminary findings, the environmental and public health agencies decided that it would be prudent to issue an advisory regarding consumption of fish and shellfish taken from Eagle Harbor. Therefore, the Bremerton-Kitsap County Health Department (BKCHD) issued an advisory on March 23, 1984, recommending that fish, crabs, and shellfish from Eagle Harbor not be consumed. Simultaneously, NOAA, EPA, and WDOE coordinated plans for investigations to collect data which would help address remaining environmental and public health questions.

Dr. Malins and his associates proceeded with the analysis of bottomfish tissues. One of the major purposes of this work was to quantify concentrations of PNAs and PNA metabolites in tissues including the edible muscle tissue of the English sole.

EPA collected and analyzed a number of surface sediment samples from Eagle Harbor. Both subtidal and intertidal sediments were collected, with the results intended to better define the extent and degree of surface sediment contamination in and near the harbor.

The Water Quality Investigations Section (WQIS) of WDOE was responsible for collecting, analyzing, and providing the initial interpretation of shellfish sample results from Eagle Harbor. This effort initially included only clams, but was later expanded to include crabs. The primary purpose of this work is to generate data which will help to provide a sound basis for the public health agencies to make decisions on advisories addressing consumption of Eagle Harbor crabs and clams.

This report presents the results of WDOE's Eagle Harbor shellfish analyses and compares concentrations found here to Food and Drug Administration (FDA) "action levels" where applicable. In addition, PNA concentrations in Eagle Harbor shellfish are compared to concentrations in samples collected at "control" locations as well as concentrations reported in the literature for shellfish in Puget Sound and other waters. Finally, PNA concentrations in Eagle Harbor shellfish are compared to concentrations reported in several other types of food.

These data will be reviewed by public health agencies including the BKCHD and the Washington State Department of Social and Health Services (DSHS) in their re-evaluation of the advisory for Eagle Harbor. Modifications to the original advisory will be issued, as necessary, by these agencies.

Methods

Site Selection. Site selection for the collection of clams and crabs was based on: (1) historical use of the location for shellfish collection by the public, and (2) the potential for contamination based on available information regarding contaminated sediments and other potential sources of contamination. A "control" station located some distance from Eagle Harbor was also selected for each survey.

Station locations were chosen after consulting Don Miles of the BKCHD, Al Scholz of the Washington State Department of Fisheries (WDF), Dan Tangarone of EPA, and several Bainbridge Island residents.

Figure 1 shows the locations of sampling sites. Numbers for the clam collection sites correspond to numbers assigned to intertidal sediment samples collected by EPA in their sediment sampling effort. There are a total of nine clam collection sites: six in Eagle Harbor, two just outside Eagle Harbor (one on the north side of Wing Spit, the other along the east shore of Wyckoff property just south of the entrance of Eagle Harbor), and one control site located at Point Blakely about 1.7 miles south of Eagle Harbor. These sites are described in Table 1.

Crab collection sites are also noted in Figure 1. Crabs were collected at four locations. Site A on the north shore of Eagle Harbor is a popular public crabbing area; Site B in the middle of the harbor is located where NOAA scientists found the highest concentrations of PNAs in sediment; and Site C which is also a crabbing area is located off the east-facing shore south of the harbor entrance near a location where contaminated seepage has been noted. The control (Station D) was placed in Rolling Bay. Rolling Bay was selected rather than Point Blakely because it represents better crab habitat and is a public crabbing area.

Sample Collection. Clam samples were collected on April 17 and 18, 1984, by Joe Joy and Art Johnson (WDOE, WQIS). A total of four clam species were represented in the collection. The number and type of clams collected at each site are given in Table 1. Butter- and steamer (littleneck) clams were selected preferentially wherever available. Horse clams were retained for analysis only when an insufficient number of butter- and steamer clams were available. Only undamaged clams were retained for analysis. These were rinsed in on-site sea water, placed in plastic bags, tagged, and stored on ice. All samples were transported within six hours to the WDOE/EPA Environmental Laboratory at Manchester, Washington, where they were refrigerated pending sample preparation.

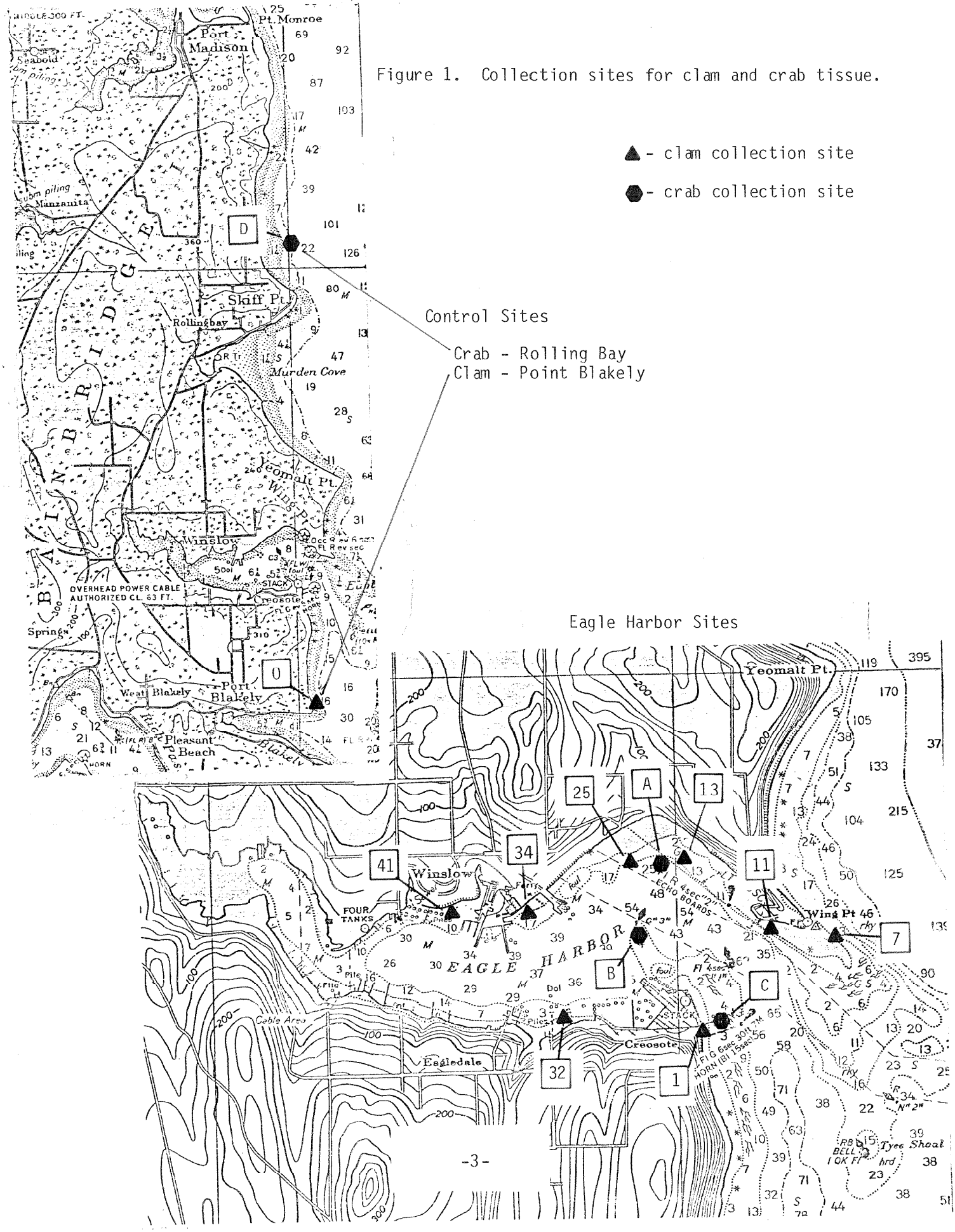

Figure 1. Collection sites for clam and crab tissue.

▲ - clam collection site

⬣ - crab collection site

Table 1. Data on clams taken from Eagle Harbor on April 17 and 18, 1984.

Station Number	Description	Collection Date	Total Shucked Sample Weight (grams)	Number Clams	Clan Species*			
					Butter	Native Littleneck	Japanese Littleneck	Horse
0	Control/Point Blakely	4/18/84	553	24	14	10	0	0
1	Wyckoff/East Shore	4/18/84	504	13	8	5	0	0
7	Wing Point Spit	4/17/84	517	29	5	24	0	0
11	Wing Point in front of lagoon	4/17/84	504	13	8	5	0	0
13	North Beach, directly north of Wyckoff	4/17/84	527	11	9	2	0	0
25	East of ferry terminal	4/17/84	433	15	13	2	0	0
32	West of Wyckoff log dump/south beach	4/17/84	184	22	1	7	3	11
34	Between Washington State Ferry terminal and maintenance facility	4/17/84	410	15	9	5	1	0
41	Winslow City Park	4/17/84	299	58	2	56	0	0

*Butter clam = Saxidomus giganteus
Native littleneck = Protothaca staminea
Japanese littleneck = Venerupis japonica
Horse clam = Tresus capax

Crab samples were collected between July 29 and 31, 1984, by Art Johnson, Dale Norton, and Bill Yake. Crabs were collected using pots baited with fish scraps. All of the crabs caught at the three stations in and near Eagle Harbor were Red Rock crabs (Cancer productus). Although several Dungeness crabs (Cancer magister) were caught at the Rolling Bay control site, only Red Cock crabs were retained for analysis so that all four samples would be as comparable as possible. The largest crabs obtained at each site were killed, wrapped in aluminum foil (previously rinsed with pesticide-grade acetone and methylene chloride), and placed on ice. Crabs were returned to the EPA/WDOE Manchester Laboratory on the day of collection and frozen pending sample preparation.

Sample Preparation. Clam samples were prepared on April 20, 1984, two to three days after collection. The clams were shucked; rinsed with distilled, de-ionized water; and placed in pre-weighed glass jars with teflon lids. All soft tissues of the butter- and steamer (littleneck clams) were retained for analysis; however, only the neck, mantle, and foot of the horse clams were used. Thus, these samples represent commonly eaten clam tissues; and, at all but one station (32), represent whole (soft tissue) clam samples. The total weight of each sample was determined (Table 1) and samples refrigerated pending analysis.

Crab samples were prepared within two days of collection. Each crab was weighed, carapace width measured, and sex recorded. All of this information is presented in Table 2. The crabs were then dissected on solvent-rinsed aluminum foil. Muscle and hepatopancreas tissues were removed with stainless steel scissors, forceps, and scalpels, and placed in pre-weighed one-pint glass jars with teflon lids. The total weight of each sample was determined (Table 2) and samples re-frozen to await analysis.

To prevent cross-contamination of samples, all instruments used to prepare these samples were cleaned between processing each sample. The cleaning procedure consisted of: (a) washing with detergent, rinsing with tap water, then rinsing three times with distilled-deionized water, (b) rinsing twice with acetone, (c) rinsing twice with methylene chloride, and (d) drying for at least ten minutes at 100°C.

Glass jars with teflon lids were used to store prepared samples. These jars were cleaned using the following procedure: (a) washing with hot water and detergent, (b) rinsing with tap water, (c) rinsing with distilled water, (d) drying overnight at 350°C, (e) rinsing with pesticide-grade acetone, (f) rinsing with pesticide-grade methylene chloride, and (g) air-drying.

Sample Digestion, Extraction, and Analysis. Tissue analyses were conducted at two laboratories--the WDOE/EPA Environmental Laboratory at Manchester, Washington, and NOAA's Northwest and Alaska Fisheries Center at Montlake (Seattle, Washington). The anlayses performed at each laboratory are given in Table 3.

Table 2. Data on Red Rock crabs (*Cancer productus*) collected from Eagle Harbor between July 26 and July 31, 1984.

Station Designation	Station Location	Size, Weight, and Sex Data: Carapace Width (mm)*	Total Weight (g)	Sex	Muscle Sample: Laboratory Number	Muscle Sample: Weight (g)	Hepatopancreas Sample: Laboratory Number	Hepatopancreas Sample: Weight (g)
A	Along north shore of Eagle Harbor, mid-way betwen ferry dock and Wing Point	150	492	M				
		140	365	M	31506	157.1	31507	60.5
		160	562	M				
B	Middle of Eagle Harbor at Black Can #5, north of Wyckoff	160	550	M				
		137	325	F				
		112	183	F				
		120	196	F	31510	223.3		
		130	273	F				
		123	250	F				
		117	190	F				
							31512	151.2
		180	649	M				
		140	375	M				
		130	218	F				
		120	169	F	31511	218.4		
		130	244	F				
		130	190	F				
		130	238	F				
C	East shore of Wyckoff near end of Milwaukee dock	155	576	M				
		151	360	M				
		132	322	M				
		145	395	M	31513	222.9	31514	122.0
		147	387	M				
		103	152	F				
		149	317	F				
D	Rolling Bay (control)	111	164	M				
		118	194	F				
		141	360	M	31515	146.9	31516	88.7
		126	192	F				
		133	320	M				
		140	335	M				

* = 1 inch = 25.4 mm

Table 3. Analyses performed on Eagle Harbor tissue samples.

Sample Type	Clams		Crabs	
Tissue	(see text)		Muscle	Hepatopancreas
Laboratory	WDOE/EPA	NOAA	WDOE/EPA	WDOE/EPA
Analyses				
Metals	X			
PCBs	X			
PNAs	X	X	X	X
Chlorinated phenols	X		X	X
Percent lipids	X	X	X	X
Percent solids	X	X	X	X

The initial analyses of clam tissues conducted at the WDOE/EPA laboratory included determination of metals and PCB concentrations. Methods used for the digestion, extraction, and analyses of metals and PCBs have previously been detailed in Gahler et al., 1982.

Percent solids and lipids were determined for all samples. Solids were determined using method 160.3 (EPA, 1979). Lipids were determined by liquid extractions of a tissue subsample. The subsamples were each extracted three times in petroleum ether. The extract was subsequently dried and the lipid content determined gravimetrically (EPA, 1980).

Analysis for PNAs and chlorinated phenols in these samples required some modifications in techniques previously used by the WDOE/EPA laboratory. These changes were necessary both because of the nature of tissue samples (matrix effects and the presence of numerous organic compounds in tissues) and the relatively low detection limits required. These modifications were incorporated after discussions between WDOE/EPA and NOAA laboratory personnel.

The method developed and used at the WDOE/EPA Manchester laboratory is included in the appendix. The major steps in the extraction and analysis are shown in Figure 2.

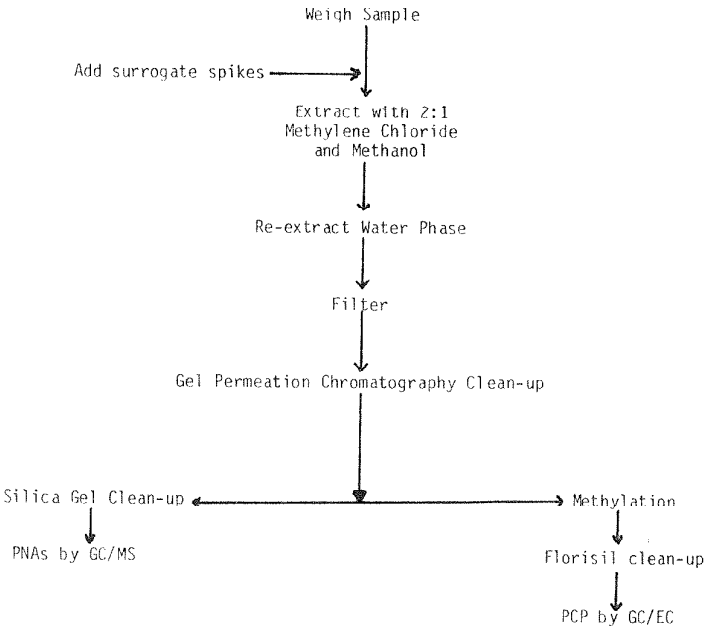

Figure 2. WDOE/EPA Manchester analytical scheme for PNA and PCP in tissue.

The methods used by the NOAA laboratory for tissue extraction and subsequent analysis for PNAs were slight modifications of those described by Malins et al. (1980). The methods used are described in detail by MacLeod et al. (1984, in press).

Four of the clam tissue samples were split for PNA analysis by both the WDOE/EPA laboratory and the NOAA laboratory. In comparing the results, it is important to keep in mind several differences between the laboratories and their procedures:

1. The WDOE/EPA laboratory used gas chromatographic/mass spectral (GC/MS) analysis to detect and quantify individual PNAs, while the NOAA laboratory used gas chromatographic/flame ionization detection (GC/FID) for this purpose.

2. Extraction of PNAs from tissues and subsequent analysis involves a number of painstaking procedures. Differences in experience, technique, and analytical equipment make comparison of results from different laboratories somewhat difficult and should be considered when interpreting results.

3. Both laboratories add specific deuterated compounds (for instance, D-10 pyrene) early in the extraction procedure to determine the efficiency with which this compound is recovered during extration and analysis. Although there are some differences between the laboratories in how this is done (WDOE/EPA calls these deuterated compounds "surrogate spikes" and adds them directly to the homogenated tissue in the flask prior to solvent addition, while NOAA calls them "internal standards" and adds them to the homogenated tissue in the flask just after solvent addition), the procedures appear to be essentially equivalent. The major differences between the laboratories appear to be the degree of recovery obtained and whether or not the final results are corrected for any losses which may occur in the extraction/analysis process.

   Table 4 summarizes the recoveries obtained by each laboratory for their surrogate spikes (internal standards).

Table 4. Percent recovery of surrogate spikes or internal standards. mean percent and (range).

Sample Type	Clam		Crab	
Tissue Type	(see text)		Muscle	Hepatopancreas
Laboratory	WDOE/EPA	NOAA	WDOE/EPA	WDOE/EPA
Deuterated Compound				
D-8 naphthalene		86(//-92)		
D-10 pyrene	31(17-44)	95(88-100)	72(50-89)	76(59-102)
D-12 perylene		101(99-103)	72(29-97)	74(65-90)

It is clear that the NOAA laboratory was able to achieve consistently higher recoveries than the WDOE/EPA laboratory. WDOE/EPA laboratory recoveries for the clam samples were noticeably lower than those obtained by NOAA for the same samples. This is not unexpected when considering that this was the first time the WDOE/EPA laboratory had used this particular analytical procedure.

A second significant difference between the laboratories is that while the NOAA laboratory uses their internal standard recovery to adjust their final reported results, the WDOE/EPA laboratory does not. The disparity in recovery rates coupled with this reporting difference is probably largely responsible for the fact that WDOE/EPA's reported clam tissue PNA concentrations are consistently lower than NOAA's.

Results and Discussion

Clam Tissue

Metals. The concentrations of seven metals (As, Cd, Cr, Pb, Hg, Ni, and Zn) in clam tissues are summarized in Table 5. These concentrations are reported on both a wet-weight (Table 5A) and dry-weight (Table 5B) basis. In general, metals concentrations in clams from the control location are equivalent to metals concentrations in clams taken from Eagle Harbor. There is some indication that lead and mercury concentrations may be slightly elevated in some of the Eagle Harbor samples.

Table 6 compares metal concentrations in Eagle Harbor clam tissue to concentrations reported in other Puget Sound clams and bivalves. In all cases, metals concentrations in Eagle Harbor clams appear to be within the range of previously reported values. In addition, these concentrations are compared to FDA guidelines and "action levels" for edible tissues of fish and shellfish. In the case of the three metals for which guidelines or "action levels" exist, concentrations in Eagle Harbor clam tissue are well below these criteria.

Polychlorinated Biphenyls (PCBs). PCB concentrations in clam tissue are given on a wet-weight basis in Table 7A, and on a dry-weight basis on Table 7B. These concentrations are quite low, ranging from less than 10 to 28 ug/Kg (ppb) wet weight. As noted in Table 6, these concentrations are about 1 percent of the FDA action level for edible fish tissue. They are also at the lower end of the range of PCB concentrations reported in clam tissues from seven rural and urbanized embayments (Malins et al., 1980). The dry-weight concentrations of PCBs in Eagle Harbor clams (<54 to 155 ppb) correspond well to concentrations (24 to 160 ppb) reported by Malins et al. (1982) for clams from reference areas. It should be noted, however, that the clams analyzed by Malins et al. (1980, 1982) were different species than those collected in the Eagle Harbor area.

Table 5. Metals in Eagle Harbor clam tissue.

A. Metals data reported on a wet-weight basis (ug/Kg, ppb).

Station Number	Control/ Point Blakely 0	Wyckoff/ East Shore 1	Wyckoff Log Dump 32	Winslow City Park 41	Between WSF Terminal & Maintenance 34	East of Terminal 25	North Beach Directly North of Wyckoff 13	Wing Point in Front of Lagoon 11	Wing Point Spit 7
Lab Number	16508	16507	16504	16506	16505	16503	16502	16501	16500
Metals									
Arsenic	3,700	2,300	1,500	2,600	3,100	3,300	3,800	4,400	2,500
Cadmium	110	140	140	290	110	90	80	130	290
Chromium	550	820	590	80	630	380	720	750	100
Lead	380	450	1,320	1,030	1,980	430	590	490	430
Mercury	12	18	31	68	54	22	34	27	10
Nickel	910	950	1,900	910	860	830	1,100	980	370
Zinc	12,300	14,300	12,700	14,000	14,400	13,400	14,600	15,400	14,000

B. Metals data reported on a dry-weight basis (ug/Kg, ppb).

Station Number	Control/ Point Blakely 0	Wyckoff/ East Shore 1	Wyckoff Log Dump 32	Winslow City Park 41	Between WSF Terminal & Maintenance 34	East of Terminal 25	North Beach Directly North of Wyckoff 13	Wing Point in Front of Lagoon 11	Wing Point Spit 7
Lab Number	16508	16507	16504	16506	16505	16503	16502	16501	16500
Metals									
Arsenic	21,300	12,570	8,740	486	17,600	18,300	21,400	25,200	14,130
Cadmium	630	760	820	2,070	620	500	450	740	1,640
Chromium	3,160	4,480	3,430	570	3,580	2,110	4,060	4,300	570
Lead	2,180	2,460	7,690	7,360	11,200	2,390	3,330	2,810	2,430
Mercury	69	98	181	486	307	122	192	155	57
Nickel	5,230	5,190	11,100	6,500	4,900	4,610	6,200	5,610	2,090
Zinc	70,700	78,100	74,000	100,000	81,800	74,400	82,400	88,200	79,100
Percent Lipids	1.4	1.2	1.3	0.8	1.0	1.1	1.2	1.0	1.2
Percent Solids	17.4	18.3	17.2	14.0	17.6	18.0	17.7	17.5	17.7

Table 6. Comparison of metals and PCBs in clam tissue to other Puget Sound studies and FDA "action levels" (mg/Kg, ppm wet weight, except as noted).

Source:	Olsen & Schell[1]						Dexter[2]	OMPA-2[3]	OMPA-1[4]		FDA	Present Study
Type of Clam:	Littleneck		Butter		Horse		"Bivalves"	Clams	"Clams"			
	Average	Range	Average	Range	Average	Range	Average	Range	Reference Area	Hylebos Duwamish		Range
Metals												
As		(0.3-2)		(1-7.5)		(1-2)	1.0					1.5-4.4
Cd	0.28	(0.15-0.40)	0.16	(<0.1-0.3)	0.20	(0.15-0.25)		0.12-0.32			0.5*	0.08-0.29
Cr								0.31-8.1				0.08-0.82
Pb	0.54	(0.25-0.8)	0.60	(0.2-1.0)	0.52	(0.25-0.6)	1.2	0.60-23			7.0*	0.38-2.0
Hg							0.032				1.0†	0.01-0.068
Ni								0.53-4.9				0.37-1.9
Zn	16.7	(11-25)	11.4	(6-20)	6.0	(5-7)	29	10.2-27.4				12.3-15.4
PCBs wet wt								0.002-0.18			2.0†	<0.010-0.028
dry wt								0.02-1.3	0.024-0.16	0.312-1.3		<0.054-0.155

[1]Olsen, S.J. & W.R. Schell, 1977. Baseline Study of Trace Heavy Metals in Biota of Puget Sound. METRO/U.W.

[2]Dexter, R.N., et al., 1981. A Summary of Knowledge of Puget Sound Related to Chemical Contaminants. NOAA-OMPA-13.

[3]Malins, D.C., et al., 1980. Chemical Contaminants in Central and Southern Puget Sound. NOAA-CMPA-2; Clams = Macoma nasuta, M. carlottensis, and Acila castrensis.

[4]Malins, D.C., et al., 1982. Chemical Contaminants and Abnormalities in Fish and Invertebrates from Puget Sound. NOAA-OMPA-19.

* = Unofficial guideline adapted from other types of food.

† = Action level for fish.

Chlorinated phenols (tetrachlorophenol and pentachlorophenol). Concentrations of tetrachlorophenol (TCP) and pentachlorophenol (PCP) in clam tissues are given in Tables 7A (wet weight) and 7B (dry weight). Concentrations in clams from the "control" site at Point Blakely were generally equivalent to concentrations in clams in and near Eagle Harbor.

It is somewhat difficult to place TCP and PCP concentrations in Eagle Harbor clams in perspective because few literature values are available for comparison. Murray et al. (1980) report PCP concentrations of 3.4 to 8.3 ppb (wet weight) in oysters from Galveston Bay, Texas. This compares to the 0.3 to 9.3 ppb concentrations reported in Eagle Harbor clams.

Based on available information, the reported PCP concentrations do not appear to imply significant human health effects. EPA (1980) cites an "upper limit for non-occupational daily exposure" to PCP at 0.03 mg/Kg or 2.1 mg/70 Kg person. To reach this exposure, a person would have to consume 225 Kg (about 500 pounds) daily of the Eagle Harbor clams with the highest PCP concentration (9.3 ppb, wet weight).

Polynuclear Aromatic Hydrocarbons (PNAs). The results of analyses for PNAs and other ringed organic compounds are given in Tables 7A (wet weight) and 7B (dry weight). In addition, an indication of the carcinogenic activity of several compounds is given in the left-hand margin.

Figure 3 graphically displays the concentrations of 2- and 3-ring PNAs, as well as the 4-, 5-, and 6-ring PNAs in clam tissue. In general, the concentrations of PNAs in clams in and near Eagle Harbor are substantially higher than those in clams taken from the control site at Point Blakely. The highest PNA concentrations were found in clams collected from the east-facing shore of Wyckoff Company (a pole and piling preserving plant) property on Bill Point south of the mouth of Eagle Harbor.

The distribution of PNAs in clam tissue shown in Figure 3 is generally consistent with what is known about sediment contamination, potential sources, and circulation patterns in and near Eagle Harbor. The only apparent anomaly in this pattern is the relatively low concentration of PNAs found in the clams collected at site #32 near the Wyckoff log rafting area. One potential explanation for this result may be related to the type of clams collected here and their preparation prior to analysis. As noted earlier, this was the only site where horse clams were retained for analysis. Half (11 of 22) of the clams in this sample were horse clams, and because they are generally much larger than individuals of other clam species, most of this sample consisted of horse clam tissue. As noted in the Methods section, only the foot, neck, and mantle of horse clams were retained for analysis. The low PNA results may reflect the fact that the internal organs (including digestive glands) of the horse clams were not analyzed. Further work would be required to test this hypothesis and determine if, in fact, the internal organs of horse clams (and other clam species) contain much higher concentrations of PNAs than other tissues like the foot, mantle, and neck.

Table 7A. PNAs, PCBs, and chlorinated phenols in Eagle Harbor clam tissue (ug/Kg, wet weight basis).

		Control/ Pt. Blakely		Wyckoff East Shore		Wyckoff Log Storage	City Park	Condos/ WS Ferries		E. of Terminal	North Shore Opp. Wyckoff		Wing Point Lagoon	Wing Point Spit
Station Number:		0		1		32	41	34		25	13		11	7
Laboratory Sample Number:		16508		16507		16504	16506	16505		16503	16502		16501	16500
Laboratory:		NOAA[1]	WDOE[2]	NOAA[1]	WDOE[2]	WDOE[2]	WDOE[2]	NOAA[1]	WDOE[2]	WDOE[2]	NOAA[1]	WDOE[2]	WDOE[2]	WDOE[2]
C.A.[a]	Polynuclear Aromatic Hydrocarbons													
	Priority Pollutant PNAs													
	Naphthalene	*	2u	26	6.6	2u	2u	6.4	2u	2u	6.1	2u	3.1	2u
	Acenaphthene	1.8	2u	130	55	2u	2.1	6.1	3.2	2u	5.4	2u	2u	2u
	Fluorene	7.9	2u	180	82	2u	2u	11	5.3	2u	11	5.3	5.7	2u
-	Phenanthrene	36	21	740	480	19	35	73	45	45	84	49	53	14
-	Anthracene	23	2u	130	67	2.2	2u	33	7.9	6.4	12	6.5	6.9	2u
-P/Co,M	Fluoranthene	63	35	970	560	43	90	200	130	130	210	130	120	26
-P/Co,M	Pyrene	47	29	920	430	26	66	200	99	81	230	85	81	19
†	Benzo(a)anthracene	10	2u	210	210	2u	22	35	31	28	46	31	32	2u
†	Chrysene	15	12	360	210	2u	28	120	46	44	I	46	39	2u
	Benzofluoranthenes	14	2u	120	120	2u	24	54	42	33	48	37	26	2u
†††,M	Benzo(a)pyrene	3.2	6u	50	45	6u	6u	14	11	6u	15	6u	6u	6u
†††	Dibenzo(a,h)anthracene	2.2	12u	8.6	12u	12u	12u	5.0	12u	12u	2.5	12u	12u	12u
†,M	Indeno(1,2,3-cd)pyrene	0.9u	12u	11	12u	12u	12u	6.9	12u	12u	6.3	12u	12u	12u
-P/Co,M	Benzo(g,h,i)perylene	2.5	12u	21	12u	12u	12u	7.9	12u	12u	8.4	12u	12u	12u
	Non-Priority Pollutant PNAs													
	1-methylnaphthalene	1.2		63				3.1			2.6			
	2-methylnaphthalene	*	2u	160	44	2u	2u	6.3	2u	2u	5.4	2u	2u	2u
	1,3-dimethylnaphthalene				75T									
	2,6-dimethylnaphthalene	9.0		79				2.8			3.1			
	2,3,5-trimethylnaphthalene	*		35				*			*			
	1-methylphenanthrene	I		97				I			28			
	2-methylphenanthrene				67T									
	3,6-dimethylphenanthrene	5.0		41				8.6			5.9			
	2-methylanthracene				110T									
	11H-benzo(a)fluoranthene				150T									
-	Benzo(e)pyrene	*		76				56			35			
	Perylene	*		15				4.1			3.5			
	Other Ringed Compounds													
	Biphenyl	1.0		35				1.2			1.3			
	Dibenzothiophene	1.2		71				3.3			4.3			
	Dibenzofuran	1.8	2u	120	62	2u	2u	3.5	2u	2u	4.5	2u	2u	2u
	Carbazole	1.0u		2.1u				1.2u			1.3u			
	PCBs													
	PCB-1254		10u		10u	10u	16		21	28		16	10u	10u
	Chlorinated Phenols													
	Tetrachlorophenol		3.6		10	3.1	5.8		12	5.6		0.2	3.6	3.3
	Pentachlorophenol		5.1		9.3	6.3	7.5		9.1	7.6		0.3	6.5	9.1
	Other Analyses													
	Percent Lipids		1.4%		1.2%	1.3%	0.8%		1.0%	1.1%		1.2%	1.0%	1.2%
	Percent Solids	16.5%	17.4%	16.5%	18.3%	17.0%	14.0%	18.0%	17.6%	18.0%	16.5%	17.7%	17.5%	17.7%

[1]Data reported in letter (8/10/84) from Donald Malins (NOAA, NMFS-Seattle) to Bill Yake (WDOE, WQIS-Olympia).

[2]Data reported in memorandum (8/8/84) from Dick Huntamer and Mike Schlender (Chemists, Manchester Laboratory) to Merley McCall and Bill Yake, "Analysis of Eagle Harbor shellfish for polynuclear aromatic hydrocarbons and pentachlorophenol."

T = Tentatively identified compound.
* = Present; however, also present in blank.
m = Present, but concentration below level of quantification.
u = Not detected at limit of detection.
I = Data not available due to interfering peak at the same retention time.

[a]Carcinogenic Activity: From Mix and Schaffer (1983) and Table 1.1 of Pucknat (1981), all routes of exposure.
- = Not carcinogenic
P/Co = promoter or cocarcinogen
M = Ames test mutagen
† = Carcinogenic
††† = Strongly carcinogenic

Table 7B. PNAs, PCBs, and chlorinated phenols in Eagle Harbor clam tissue (ug/Kg, dry weight basis).

	Station Number:	Control/ Pt. Blakely 0		Wyckoff East Shore 1		Wyckoff Log Storage 32	City Park 41	Condos/ WS Ferries 34		E. of Terminal 25	North Shore Opp. Wyckoff 13		Wing Point Lagoon 11	Wing Point Spit 7
	Laboratory Sample Number:	16508		16507		16504	16506	16505		16503	16502		16501	16500
	Laboratory:	NOAA[1]	WDOE[2]	NOAA[1]	WDOE[2]	WDOE[2]	WDOE[2]	NOAA[1]	WDOE[2]	WDOE[2]	NOAA[1]	WDOE[2]	WDOE[2]	WDOE[2]
C.A.[a]	**Polynuclear Aromatic Hydrocarbons**													
	Priority Pollutant PNAs													
	Naphthalene	*	11u	160	36	12u	14u	36	11u	11u	37	11u	18	11u
	Acenaphthene	11	11u	800	300	12u	15	34	18	11u	33	11u	11u	11u
	Fluorene	48	11u	1100	450	12u	14u	60	30	11u	69	30	33	11m
-	Phenanthrene	220	120	4500	2600	110	250	410	260	250	500	280	300	79
-	Anthracene	140	11u	800	370	13	14u	180	45	36	72	37	39	11u
-P/Co,M	Fluoranthene	380	200	5900	3100	250	640	1100	740	720	1300	730	690	150
-P/Co,M	Pyrene	610	170	5600	2300	150	470	1100	560	450	1400	480	460	110
†	Benzo(a)anthracene	62	11u	1300	1100	12u	160	190	180	160	280	180	180	11u
†	Chrysene	85	69	2200	1100	12u	200	690	260	240	I	260	220	11u
	Benzofluoranthenes	83	11u	710	660	12u	170	310	240	180	290	210	150	11u
†††,M	Benzo(a)pyrene	19	34u	350	250	35u	43u	76	63	33u	89	34u	34u	34u
†††	Dibenzo(a,h)anthracene	13	69u	52	66u	71u	86u	27	68u	67u	15	68u	69u	68u
†,M	Indeno(1,2,3-cd)pyrene	5.7u	69u	64	66u	71u	86u	38	68u	67u	38	68u	69u	68u
-P/Co,M	Benzo(g,h,i)perylene	15	69u	130	66u	71u	86u	44	68u	67u	51	68u	69u	68u
	Non-Priority Pollutant PNAs													
	1-methylnaphthalene	7.0		380				17			16			
	2-methylnaphthalene	*	11u	940	240	12u	14u	35	11u	11u	33	11u	11u	11u
	1,3-dimethylnaphthalene				420T									
	2,6-dimetnylnaphthalene	22		480				15			19			
	2,3,5-trimethylnaphthalene	*		210				*			*			
	1-methylphenanthrene	I		590				I			170			
	2-methylphenanthrene				370T									
	3,6-dimethylphenanthrene	30		250				48			36			
	2-methylanthracene				600T									
	11H-benzo(a)fluoranthene				820T									
-	Benzo(e)pyrene	*		460				310			210			
	Perylene	*		90				23			21			
	Other Ringed Compounds													
	Biphenyl	6.0		210				6.5			8.1			
	Dibenzothiophene	7.5		430				18			26			
	Dibenzofuran	11	11u	720	340	12u	14u	19	11u	11u	27	11u	11u	11u
	Carbazole	6.0u		13u				6.5u			8.1u			
	PCBs													
	PCB-1254		57u		54u	58u	114		119	155		90	57u	56u
	Chlorinated Phenols													
	Tetrachlorophenol		21		55	18	41		68	31		1.3	21	19
	Pentachlorophenol		29		51	37	54		52	42		1.7	37	51
	Other Analyses													
	Percent Lipids		1.4%		1.2%	1.3%	0.8%		1.0%	1.1%		1.2%	1.0%	1.2%
	Percent Solids	16.5%	17.4%	16.5%	18.3%	17.0%	14.0%	18.0%	17.6%	18.0%	16.5%	17.7%	17.5%	17.7%

[1]Data reported in letter (8/10/84) from Donald Malins (NOAA, NMFS-Seattle) to Bill Yake (WDOE, WQIS-Olympia).

[2]Data reported in memorandum (8/8/84) from Dick Huntamer and Mike Schlender (Chemists, Manchester Laboratory) to Merley McCall and Bill Yake, "Analysis of Eagle Harbor shellfish for polynuclear aromatic hydrocarbons and pentachlorophenol."

T = Tentatively identified compound.
* = Present; however, also present in blank.
m = Present, but concentration below level of quantification.
u = Not detected at limit of detection.
I = Data not available due to interfering peak at the same retention time.

[a]Carcinogenic Activity: From Mix and Schaffer (1983) and Table 1.1 of Pucknat (1981), all routes of exposure.
- = Not carcinogenic
P/Co = promoter or cocarcinogen
M = Ames test mutagen
† = Carcinogenic
††† = Strongly carcinogenic

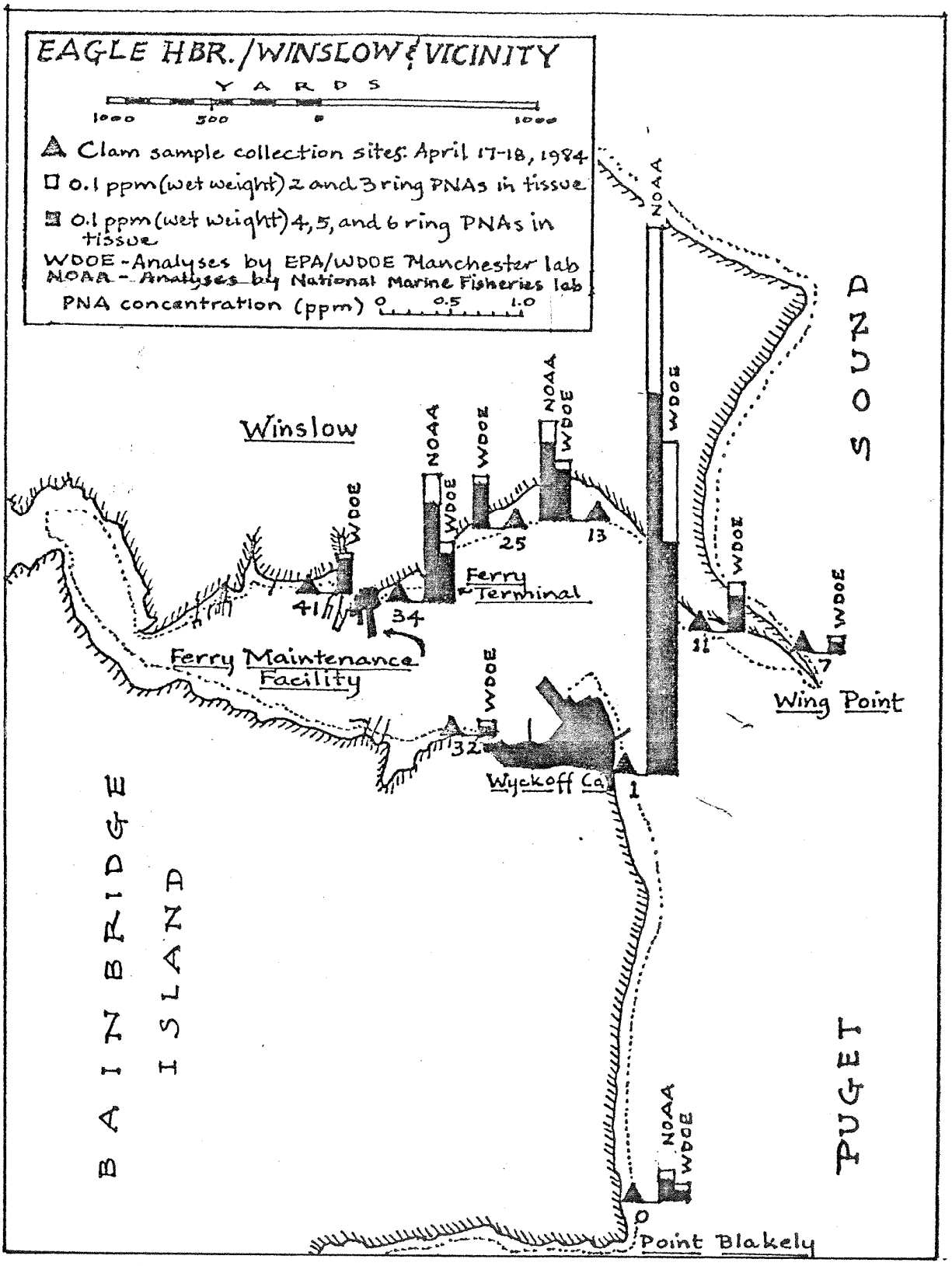

Figure 3. Concentrations of priority pollutant polynuclear aromatic hydrocarbons (PNAs) in the tissues of clams collected in and near Eagle Harbor, Washington.

The fairly consistent discrepancy between PNA concentrations reported by the WDOE/EPA laboratory and those reported by the NOAA laboratory are discussed in some detail in the Methods section. In the judgment of the writers, we recommend the reader use the conservative approach of accepting the NOAA results as closer approximations of reality and recognize that the actual concentrations of PNAs in the clam tissue samples analyzed by the WDOE/EPA laboratory may be approximately twice the values reported.

To place the PNA concentrations reported here in some perspective, tables have been generated which compare these concentrations to those reported in shellfish from Puget Sound and other locations (Tables 8 and 9) and concentrations in other foods with relatively high concentrations of PNAs (Table 9).

Although there is some difficulty in comparing PNA concentrations reported in clam tissues by various authors because of differences in species analyzed, sample preparation, and analytical techniques, some useful generalizations may be obtained from Table 8.

Concentrations for individual PNAs in clams in Eagle Harbor proper (i.e., NOAA results for sites 13 and 34) are at or near the upper end of the range of concentrations measured in clams from urbanized areas. Even the concentrations reported for the "control" site clams appear to be higher than many of the values reported in the literature, especially values reported for sites more remote from intense human activity.

PNA concentrations in the clams taken from site 1 (east shore of Wyckoff property) were substantially higher than concentrations commonly reported in the literature. Individual PNA concentrations ranged from about 2 to 15 times higher than the highest literature values summarized in Table 8.

Table 9 compares PNA levels in Eagle Harbor clams to concentrations reported for other types of shellfish and foods containing high concentrations of PNAs. It should be noted that data reported here represent the upper limit of PNA concentrations reported in food. For instance, Santodonato et al. (1981) estimate that the average concentration of benzo(a)pyrene (BaP) in foods is 0.1 to 1.0 ppb and that the concentration of total PNAs in food is 1 to 10 ppb. Using the NOAA data, this compares to a BaP concentration of about 15 ppb in Eagle Harbor clams (58 ppb in the clams off Wyckoff) and a total PNA concentration of about 800 ppb in Eagle Harbor clams (3900 ppb in the clams off Wyckoff). Thus, clams from the Eagle Harbor area contain BaP concentrations one to two orders of magnitude higher than the average concentrations in food, and total PNA concentrations two to three orders of magnitude higher.

The concentrations of PNAs in Eagle Harbor clams are generally near the upper end of the range of PNAs reported in PNA-contaminated foods. As noted in Table 9, these foods include smoked and charcoal-broiled meats and fish, as well as leafy vegetables and shellfish from contaminated environments.

Table 8. Comparison of polynuclear aromatic hydrocarbon concentrations (ug/Kg, ppb wet weight) in clams from the Eagle Harbor area to clams from other locations.

C.A.[a]		Great Barrier Reef[1]	Rhode Island[2]	NY Bight[3]	East Coast[4]	Coos Bay[5]	Puget Sound[6] CI	PM	SI	E1	E2	C3	C2	Eagle Harbor NOAA	Eagle Harbor WDOE
	Polynuclear Aromatic Hydrocarbons														
	Priority Pollutant PNAs														
	Naphthalene			0.4u-4.8			0.7u	5.4u	4.5u	N	11u	13	13u	6.1-26	2u-6.6
	Acenaphthene						1.5u	16u	4.5	27	46u	N	N	5.4-130	2u-55
	Fluorene						0.7u	1.8u	1.5u	11	2.8u	8.4	8	11-180	2u-82
-	Phenanthrene		0.2-1.7	0.7u-2.4u		9.4-162	N	N	12u	N	N	57	N	73-740	14-480
-	Anthracene	0.1u-3.2		0.4u-1.5u			0.7u	1.8u	6	21	5.6	18	18	12-130	2u-67
-P/Co,M	Fluoranthene	0.05-0.7	1.0-7.2	0.9u-9.5u		7.7-119	0.7u	7.2	42	208	14	110	45	200-970	26-560
-P/Co,M	Pyrene	0.1u-1.4	1.2-6.6	1u-3.8	3u-12	5.3-98.8	0.7u	11	N	157	71	180	45	200-920	19-430
†	Benzo(a)anthracene		0.1-0.4	1.3u-5.7	1u-1	2.0-71.5	2.2u	3.6u	68	160	39	100	22	35-210	2u-210
†	Chrysene	0.1u-1.4	0.3-0.9	0.7u-3.8		5.9-38.9	0.7u	3.6	54	102	11	69	26	35-360	2u-210
††	Benzo(b)fluoranthene					0.9-14.8									
-	Benzo(k)fluoranthene (Benzofluoranthenes)	0.003u-0.02				1.1-10.9	2.9u	5.4u	36	37	34	28	14u	48-120	2u-120
†††,M	Benzo(a)pyrene	0.01u-0.02		0.9u-4.8u	1u-0.3	2.3-11.4	0.7u	1.8u	18	37	14	35	3.2u	14-58	6u-45
†††	Dibenzo(a,h)anthracene					1.5-9.5								2.5-8.6	12u
†,M	Indeno(1,2,3-cd)pyrene					1.3-8.7	1.5u	1.8u	3u	5u	5.6u	1.4u	3.2u	6.3-11	12u
-P/Co,M	Benzo(g,h,i)perylene	0.02u-0.3				2.0-8.0								7.9-21	12u
	Non-Priority Pollutant PNAs														
	1-methylnaphthalene			0.4u-1.2			0.7u	1.8u	1.5u	4.8	2.8u	4.2	1.6u	2.6-63	
	2-methylnaphthalene			1u-12			0.7u	3.6u	7.5u	13u	7u	5.6	11u	5.4-160	2u-44
	1,3-dimethylnaphthalene														75T
	2,6-dimethylnaphthalene						0.7u	1.8	1.5u	9.6	2.8u	17	32	2.8-79	
	2,3,5-trimethylnaphthalene						0.7u	1.8u	1.5u	3u	2.8u	1.4u	3.2u	35	
	1-methylphenanthrene													28-97	
	2-methylphenanthrene														67T
	3,6-dimethylphenanthrene													5.9-41	
	2-methylanthracene														110T
	11H-benzo(a)fluoranthene														150T
-	Benzo(e)pyrene			0.9u-4.8u			1.5u	5.4	35	59.2	35	36	13u	35-76	
	Perylene	0.04u-0.08		1.1u-4.8u			1.5u	1.8u	17	5u	5.6u	9.8	3.2u	3.5-15	
	Other Ringed Compounds														
	Biphenyl			0.4u-1.5u			0.7u	1.8u	20	48	2.8u	7	3.2	1.2-35	
	Dibenzothiophene			1.1u-3u			1.5u	3.6u	3.0u	6.4u	5.6u	13	4.8u	3.3-71	
	Dibenzofuran													3.5-120	2u-62
	Carbazole													1.2u-2.1u	
	PCBs														
	PCB-1254			10-70*			1.8*	29*	170*	50*	183*	122*	55*		10u-28
	Chlorinated Phenols														
	Tetrachlorophenol														0.2-12
	Pentachlorophenol														0.3-9.3
	Other Analyses														
	Percent Lipids														0.8-1.4
	Percent Solids			15-33			7.3	18	15	16	14	14	16	16-18.5	14.0-18.3

[1]Smith, Bagg, and Bycroft (1984) Tridacna maxima from the Great Barrier Reef, Australia.

[2]Pruell, Hoffman and Quinn (1984) Mercenaria mercenaria from Rhode Island seafood stores.

[3]McLeod, Jr., et al. (1981) "Surf clams" from the New York Bight.

[4]Pancirov and Brown (1977) "clams" from Virginia, New Jersey, and Connecticut.

[5]Mix and Schaffer (1983a) Mya arenaria from Coos Bay, Oregon.

[6]Malins, et al. (1980) Macoma nasuta, M. carlottensis and Acila castrensis from: CI-Case Inlet, PM-Port Madison, SI-Sinclair Inlet, E1-Duwamish Waterway, E2-Seattle waterfront, C3-Commencement Bay waterways, C2-Hylebos Waterway.

N = Not quantified.
u = Not detected at limit of detection.
* = Total PCBs.
T = Tentatively identified compound.

[a]Carcinogenic Activity: From Mix and Schaeffer (1983) and Table 1.1 of Pucknat (1981), all routes of exposure.
- = Not carcinogenic
P/Co = promoter or cocarcinogen
M = Ames test mutagen
† = Carcinogenic
††, ††† = Strongly carcinogenic

Table 9. A comparison of polynuclear aromatic hydrocarbon (PNA) concentrations (ug/Kg, ppb wet weight) in various food items compared to values in clams taken from Eagle Harbor and vicinity.

C.A.[a]	Polynuclear Aromatic Hydrocarbons	Kale from Urban Environ.[1]	Kale from Urban Environ.[2]	Vegetable Oils and Margarine[3]	Smoked Fish[3]	Smoked Meats[3]	Charcoal Broiled Meat[3]	Smoked Ham[3]	Oysters from Georgia[4]	Upper Gulf of Thailand Shellfish[6]	Yaquina Bay Mussels[5]	Eagle Harbor Clams NOAA	Eagle Harbor Clams WDOE
	Priority Pollutant PNAs												
	Naphthalene											6.1-26	2u-6.6
	Acenaphthene											5.4-130	2u-55
	Fluorene				2.6-67					16.2, 16.3		11-180	2u-82
-	Phenanthrene		70-586	51**	4.1-52	17-104			0.1u-175			73-740	14-480
-	Anthracene		2.4-97.4	36**	1.5-26	0.7-20				4.4-6.7	45.9-284.3	12-130	2u-67
-P/Co,M	Fluoranthene	43-488	53.6-1,196	1.3-13	1.8-12	0.6-49	0.2-19.8	4.0-156	1.0-4.5			200-970	26-560
-P/Co,M	Pyrene	28.1-289	36.2-510	1.6-15	0.5u-6	0.5-42	0.1-19.1	2.0-161		470	10.7-242.2	200-920	19-430
†	Benzo(a)anthracene	21.1-209*	11.2-230	0.8-29.5	0.5-1.7	0.04-29	2.7, 31	0.5-33	0.05-0.6		15.4-142.3	35-210	2u-210
†	Chrysene		28.6-395	12**		0.6-9.6	0.3-25.4	3.0u-66			7.0-154.3	35-360	2u-210
††	Benzo(b)fluoranthene								0.05-0.3		47.6-137.6***		
-	Benzo(k)fluoranthene								0.05u-0.2		0.2-24.4		
	(Benzofluoranthenes)	26-65.1							0.05u-0.15		2.0u-22.5		
†††,M	Benzo(a)pyrene	4.2-15.6	0.9-48.5	0.2-8	0.2-6.6	0.04-10.5	2.6, 50.4	3.0-55	0.01u-0.5	1.0-8.1	0.8-33.1	48-120	2u-120
†††	Dibenzo(a,h)anthracene										0.5-12.5	14-58	6u-45
†,M	Indeno(1,2,3-cd)pyrene	5.8-15.2									0.2-9.4	2.5-8.6	12u
-P/Co,M	Benzo(g,h,i)perylene	7.6-16.0	1.2-46.4	0.6-4	0.2-2.4	0.04-6.7	0.9-14.9	1.4u-25			0.2-11.4	6.3-11	12u
												7.9-21	12u
	Non-Priority Pollutant PNAs												
	1-methylnaphthalene											2.6-63	
	2-methylnaphthalene											5.4-160	2u-44
	1,3-dimethylnaphthalene												75T
	2,6-dimethylnaphthalene											2.8-79	
	2,3,5-trimethylnaphthalene											35	
	1-methylphenanthrene											28-97	
	2-methylphenanthrene												67T
	3,6-dimethylphenanthrene											5.9-41	
	2-methylanthracene												110T
	11H-benzo(a)fluoranthene												150T
-	Benzo(e)pyrene	6.1-18.1	3.8-67.2	0.4-4	0.2-1.2	0.2-5.5	17.6	2.0u-26				35-76	
	Perylene	1.3-4.0	ND-7		0.4, 0.7	0.04-3.2						3.5-15	

[1]PNA concentrations reported by Grimmer (1979) in kale from urban areas of West Germany and contaminated by air pollution.

[2]PNA concentrations reported by Hetteche (1971) in kale from urban areas.

[3]PNA values reported in Tables 5.5, 5.6, 5.8, and 5.9 of Pucknat (1981).

[4]PNA values reported by Lee et al. (1981) for Crassostrea virginica collected in various inlets of the Georgia coast.

[5]PNA values reported by Mix and Schaffer (1983b) for Mytilus edulis at two stations, over a period of a year (1979-1980) in Yaquina Bay, Oregon.

[6]PNA values reported by Hungspreungs, et al. (1984) for oysters (Ostrea plicatula), mussels (Perna viridis), and scallops (Amusium pleuronectes) from the upper gulf of Thailand.

*Benzo(a)anthracene + chrysene.

**Coconut oil value available only.

***Values from one station only.

ND = Not detected; detection limits not stated.

u = Not detected at limit of detection.

T = Tentatively identified compound.

[a]Carcinogenic Activity: From Mix and Schaffer (1983) and Table 1.1 of Pucknat (1981), all routes of exposure.

- = Not carcirogenic

P/Co = promoter or cocarcinogen

M = Ames test mutagen

† = Carcinogeric

††, ††† = Strongly carcinogenic

In summary, Eagle Harbor area clams appear, in general, to have PNA concentrations which match or exceed reported PNA concentrations in clams from urbanized areas. These concentrations also match or exceed PNA concentrations found in foods with high PNA concentrations.

Crab Tissue

Crabs were collected and analyzed after initial results were available from the analysis of clams. Based on these initial results, analysis of crab tissue was limited to PNAs and chlorinated phenols. As noted earlier, crab muscle and hepatopancreas tissues were analyzed separately. All of these analyses were performed by the WDOE/EPA laboratory in Manchester.

The results of these analyses are summarized in Table 10A (wet-weight basis) and Table 10B (dry-weight basis).

Chlorinated phenol results were generally equivalent to those for clams. TCP and PCP concentrations in crab muscle were at or near detection limits (0.5 to 1 ppb wet weight) while concentrations in hepatopancreas were in the 1-to-10-ppb wet weight range. Concentrations in crabs from the Rolling Bay control site were generally equivalent to concentrations in Eagle Harbor crabs. As noted earlier for clam tissues, the reported PCP concentrations in crab tissues do not appear to imply significant human health effects.

PNA results appear to have more significant implications. As noted in Table 4, the WDOE/EPA laboratory achieved substantially better recoveries for the deuterated compounds (surrogate spikes, internal standards) added to the crab tissue samples than with the previous clam samples. Thus although duplicate analyses were not performed by the NOAA laboratory, the results reported here are probably reasonable approximations of actual PNA concentrations in these crab tissues.

Figure 4 displays the total PNA data in graphical form. Several generalizations are apparent from this figure: (1) PNA concentrations in hepatopancreas are higher than those in the muscle of crabs collected at the same location, (2) PNA concentrations in tissues from crabs collected in and near Eagle Harbor are much higher than those in crabs collected from the Rolling Bay control site, and (3) the distribution of PNA concentrations in tissue of crabs collected in and near Eagle Harbor does not suggest any clear differentiation in crabs collected at different locations in and near the harbor. This latter observation may be due to the mobility of crabs and the possibility that collections at each of the sites sampled the same or intersecting populations of crabs.

It is interesting that a fairly large number (Table 2) of crabs were collected at Site B which was located as close as possible to the site at which Malins (1984a) found the highest concentrations of PNAs in sediments. These are sediments which displayed a high degree of toxicity to various organisms in six different bioassays (Malins, 1984b).

Table 10A. PNAs and chlorinated phenols in Eagle Harbor crab tissue (ug/Kg wet weight).

Station Number	North Shore Opposite Wyckoff		Middle of Eagle Harbor near Black Can Buoy #5			East Shore off Wyckoff Milwaukee Dock		Control Rolling Bay	
	A		B			C		D	
Laboratory Sample Number	31506	31507	31510	31511	31512	31513	31514	31515	31516
Tissue Type[1]	M	HP	M	M	HP	M	HP	M	HP
Polynuclear Aromatic Hydrocarbons									
Priority Pollutant PNAs									
Naphthalene	2.2	2.7	2.0u	12	12	2.4	8.8	2.0m	2.0u
Acenaphthene	4.2	11	3.1	3.2	54	3.4	30	4.0m	4.0u
Acenaphthylene	2.0u	8.0u	2.0u	2.0u	4.6	2.0u	2.6	2.0u	2.0u
Fluorene	7.7	8.2	6.6	8.8	30	2.0u	14	2.0m	2.0u
Phenanthrene	33	54	12	30	72	7.7	41	2.0u	2.0u
Anthracene	7.7	12	2.0u	6.0	33	2.0u	20	2.0u	2.0u
Fluoranthene	42	67	49	86	300	17	69	4.0m	13
Pyrene	26	38	19	19	110	11	45	2.0u	6.6
Benzo(a)anthracene	12	27	10	14	33	5.4	49	2.0m	6.4
Chrysene	14	50	6.6	14	100	5.4	54	4.0m	7.8
Benzofluoranthenes	53	100	4.0u	26	14	2.6	160	4.0m	4.0u
Benzo(a)pyrene	18	33	6.0u	6.4	6.0u	6.0m	37	6.0m	6.0u
Dibenzo(a,h)anthracere	10m	30m	10u	10m	10u	10u	73	10u	10u
Indeno(1,2,3-cd)pyrene	14	30m	5.0u	5.0m	10u	5.0u	92	5.0m	10u
Benzo(g,h,i)perylene	15	50m	10u	10m	10u	10u	90	10u	10u
Non-Priority Pollutant PNAs									
2-methylnaphthalene	1.3	2.0m	2.0u	3.2	4.6	2.0u	3.2	2.0u	2.0u
9-methyl 9H-fluorene					16T				
Other Ringed Compounds									
Dibenzofuran	7.0	6.9	4.2	15	26	2.0u	18	2.0u	2.0u
Chlorinated Phenols									
Tetrachlorophenol	0.5m	3.3	0.5u	0.5u	2.5	0.5m	1.3	1.0m	2.2
Pentachlorophenol	0.5m	10	0.5u	2.7	9.0	0.6	5.2	1.0	4.8
Other Analyses									
Percent lipids	0.03%	5.5%	0.04%	0.03%	2.54%	0.05%	1.28%	0.06%	1.40%
Percent solids	18.4%	20.9%	15.7%	19.9%	21.7%	21.5%	21.5%	16.4%	25.4%

[1]Tissue type: M = Muscle; HP = Hepatopancreas
T = Tentatively identified compound
m = Present, but at concentration below level of quantification
u = Not detected at limit of detection

Table 10B. PNAs and chlorinated phenols in Eagle Harbor crab tissue (ug/Kg dry weight).

Station Number	North Shore Opposite Wyckoff A		Middle of Eagle Harbor near Black Can Buoy #5 B			East Shore off Wyckoff Milwaukee Dock C		Control Rolling Bay D	
Laboratory Sample Number	31506	31507	31510	31511	31512	31513	31514	31515	31516
Tissue Type[1]	M	HP	M	M	HP	M	HP	M	HP
Polynuclear Aromatic Hydrocarbons									
Priority Pollutant PNAs									
Naphthalene	12	13	13u	60	55	11	41	12m	7.9u
Acenaphthene	23	53	20	16	250	16	140	24m	16u
Acenaphthylene	11u	38u	13u	10u	21	9.3u	12	12m	7.9u
Fluorene	42	39	42	44	140	9.3u	65	12m	7.9u
Phenanthrene	180	260	76	150	330	36	190	12u	7.9u
Anthracene	42	57	13u	30	150	9.3u	93	12u	7.9u
Fluoranthene	230	320	310	430	1400	79	320	24m	52
Pyrene	140	180	120	95	510	51	210	12u	26
Benzo(a)anthracene	65	130	64	70	150	25	230	12u	25
Chrysene	76	240	42	70	460	25	250	24m	31
Benzofluoranthenes	290	480	25u	130	65	12	740	24m	16u
Benzo(a)pyrene	97	160	38u	32	28u	28u	170	37m	24u
Dibenzo(a,h)anthracene	54u	140m	64u	50m	46u	46u	340	61u	39u
Indeno(1,2,3-cd)pyrene	76	140m	32u	25m	46u	23u	430	30m	39u
Benzo(g,h,i)perylene	81	240m	64u	50m	46u	46u	420	61u	39u
Non-Priority Pollutant PNAs									
2-methylnaphthalene	7.0	10u	13u	16	21	9.2u	15	12u	7.9u
9-methyl 9H-fluorene					74T				
Other Ringed Compounds									
Dibenzofuran	38	33	27	75	120	9.3u	84	12u	7.9u
Chlorinated Phenols									
Tetrachlorophenol	2.7m	16	3.2u	2.5u	12	2.3m	6.0	6.1m	8.7
Pentachlorophenol	2.7m	48	3.2u	2.5u	41	2.8	24	6.1	19
Other Analyses									
Percent lipids	0.03%	5.5%	0.04%	0.03%	2.54%	0.05%	1.28%	0.06%	1.40%
Percent solids	18.4%	20.9%	15.7%	19.9%	21.7%	21.5%	21.5%	16.4%	25.4%

[1]Tissue type: M = Muscle; HP = Hepatopancreas
T = Tentatively identified compound
m = Present, but concentration below level of quantification
u = Not detected at limit of detection

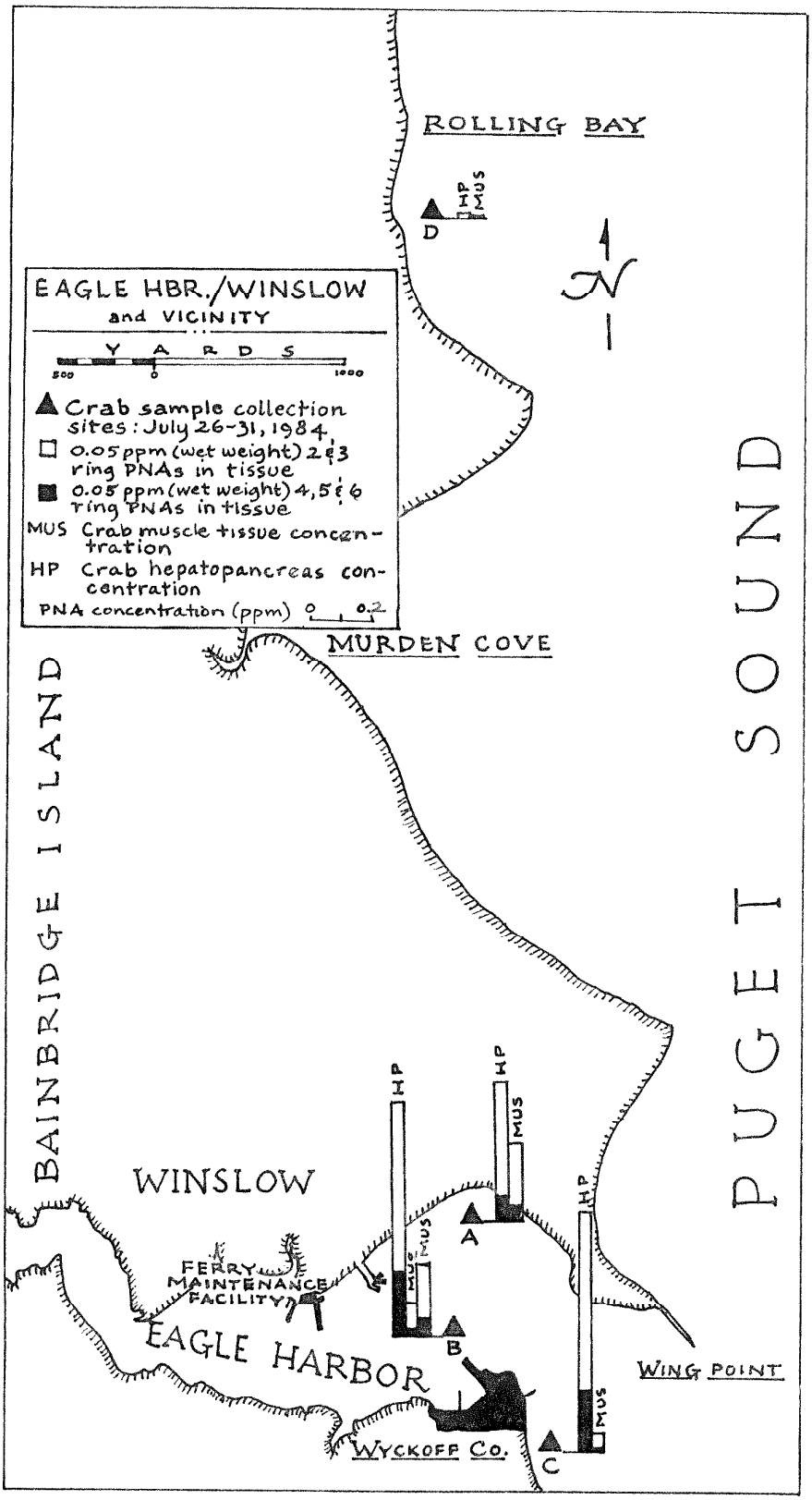

Figure 4. Concentrations of priority pollutant polynuclear aromatic hydrocarbons (PNAs) in crab (Cancer productus) muscle and hepatopancreas tissues.

PNA concentrations in crab tissues were generally lower than those found in clams but were within the same order of magnitude.

Relatively little information on PNA concentrations in crab tissue could be found in the literature. Table 11 summarizes the data that were available. Based on this information, it appears that PNA concentrations in Eagle Harbor are elevated; however, it is not possible to provide a good estimate of what concentrations one would expect in crabs from background locations or even other urbanized embayments in Puget Sound. Concentrations in Eagle Harbor crabs do appear to be generally higher than those reported in Elliot Bay (Romberg, personal communication, Table 11) and Commencement Bay (Barrick, personal communication) where concentrations of the higher-weight PNAs (3- to 6-ring) were not found at the 10 ppb detection limit.

Table 12 compares PNA concentrations in crab hepatopancreas to concentrations reported from six Puget Sound locations by Malins et al., 1980. Eagle Harbor crabs appear to have higher concentrations of PNAs in hepatopancreas than concentrations reported in crabs from these other locations. The differential is particularly marked for the higher-weight (4- to 6-ring) PNAs.

In comparing crab PNA concentrations to concentrations in other types of food, the reader is referred to the section discussing clam tissue results. Note that concentrations reported in crab hepatopancreas are generally equivalent to those reported for clam tissue, while crab muscle appears to contain PNA at concentrations approximately 10 to 40 percent of those reported in clams and crab hepatopancreas.

Summary and Conclusions

This paper reports the results of tissue analyses of clams and crabs collected in and near Eagle Harbor. The primary purpose of this work is to report data which will help provide a sound basis for public health agencies to make decisions on advisories addressing consumption of Eagle Harbor crabs and clams. To do this the concentrations of various compounds found in Eagle Harbor shellfish are compared to concentrations found at "control" sites, concentrations reported for similar tissues in other studies, and to other foods in the human diet. Observations and conclusions based on these data and subsequent comparisons include:

1. Trace metal and polychlorinated biphenyl (PCB) concentrations in Eagle Harbor clams are well within the range of concentrations which have been reported for clams and other shellfsh taken from Puget Sound. Although there is some indication that lead and mercury concentrations in some of the Eagle Harbor clam samples may be slightly elevated in comparison to the control site at Point Blakely, concentrations of these metals, as well as cadmium and PCB, are well below FDA guidelines, or "action levels," for edible fish and/or shellfish tissue.

Table 11. Comparison of polynuclear aromatic hydrocarbon concentrations (ug/Kg, ppb wet weight) in crab muscle tissue from Eagle Harbor to crabs from other locations.

Location Species	Chesapeake Bay[1] "Crabs"	Raritan Bay[1] "Crabs"	Puget Sound (Seattle METRO Area)[2] Richmond Beach	Carkeek Park	West Point	Alki Point	Denny Way	Present Study Control	Eagle Harbor
			"Rock and Dungeness Crabs"					Cancer	productus
Naphthalene			1.6u	1.6u	1.6u	1.6u	1.6u	2m	2u-2.4
Acenaphthene			3.0u	3.0u	3.0u	3.0u	120m	4m	3.1-4.2
Acenaphthalene			2.7u	2.7u	2.7u	2.7u	2.7u	2u	2u
Fluorene			3.4u	3.4u	3.4u	3.4u	120m	2m	2u-8.8
Phenanthrene			2.9u	2.9u	2.9u	2.9u	2.9u	2u	7.7-33
Anthracene			2.9u	2.9u	2.9u	2.9u	2.9u	2u	2u-7.7
Fluoranthene			7.6u	7.6u	7.6u	7.6u	74m	4m	17-86
Pyrene	0.2u	6	7.6u	7.6u	7.6u	7.6u	74m	2u	11-26
Benzo(a)anthracene	1.5u	2	18u	18u	18u	18u	18u	2m	5.4-14
Chrysene			18u	18u	18u	18u	1u8	4m	5.4-14
Benzofluoranthenes			24u	24u	24u	24u	24u	4m	2.6-53
Benzo(a)pyrene	0.5u	3	12u	12u	12u	12u	12u	6m	6u-18
Dibenzo(a,h)anthracene			34u	34u	34u	34u	34u	10u	10u-10m
Indeno(1,2,3-cd)pyrene			33u	33u	33u	33u	33u	5m	5u-14
Benzo(g,h,i)perylene			32u	32u	32u	32u	32u	10u	10u-15

[1]Pancirov and Brown (1977).

[2]Pat Romberg, Seattle METRO, personal communication.

m = Present, but concentration below level of quantification.

u = Not detected at limit of detection.

Table 12. Comparison of polynuclear aromatic hydrocarbon concentrations (ug/Kg, ppb wet weight) in crab hepatopancreas from Eagle Harbor area to crabs from other locations in Puget Sound.

	Puget Sound[1]						Present Study	
	CI	SI	BI	EI	C3	C2	Control	Eagle Harbor
Naphthalene	5.4u	4.6u	10u	33	55	7.8	2u	2.7-12
Acenaphthene	1.4u	1.2u	1.7u	160	67	34	4u	11-54
Acenaphthalene	1.4u	1.2u	1.7u	1.5u	1.5u	1.3u	2u	2.6-4.6
Fluorene	2.7	2.3	1.7u	66	1.5u	1.3u	2u	8.2-30
Phenanthrene	5.4	4.6	1.7u	63	29	65	2u	41-72
Anthracene	1.4	1.2	1.7u	18	32	35	2u	12-33
Fluoranthene	11	9.2	5.1u	9	8.7	13	13	67-300
Pyrene	22	18	1.7	6	12	9.1	6.6	38-110
Benzo(a)anthracene	19	16	3.4u	18	26	2.6u	6.4	27-49
Chrysene	5.4	4.6	1.7u	9u	12	1.3u	7.8	50-100
Benzofluoranthenes	1.4u	1.2u	1.7u	1.5u	1.5u	1.3u	4u	14-160
Benzo(a)pyrene	1.4u	1.2u	1.7u	6	1.5u	1.3u	6u	6u-37
Indeno(1,2,3-cd)pyrene	1.4u	2.3u	1.7u	1.5u	1.5u	1.3u	10u	10u-92

[1]Malins, et al. (1980): Crab hepatopancreas from: CI - Case Inlet, BI - Budd Inlet, SI - Sinclair Inlet, EI - Duwamish Waterway, C3 - Commencement Bay Waterways, and C2 - Hylebos Waterway.

2. Concentrations of tetrachlorophenol (TCP) and pentachlorophenol (PCP) in clam, crab muscle, and crab hepatopancreas were generally equivalent between samples collected at the "control" sites and samples collected in and near Eagle Harbor. Few data were available reporting TCP or PCP concentrations in shellfish or other foods. Based on available information, the reported PCP concentrations do not appear to imply significant potential human health effects.

3. Concentrations of polynuclear aromatic hydrocarbons (PNAs) in clam, crab muscle, and crab hepatopancreas samples from in and near Eagle Harbor were substantially elevated with respect to concentrations at "control" locations. Concentrations were highest in clams collected from the east shore of Wyckoff Company property south of the entrance to Eagle Harbor. Tissues from clam samples collected within Eagle Harbor proper contained PNA concentrations which were at or near the upper end of the range of concentrations reported in clams from other urbanized embayments. PNA concentrations in clams from Site 1 (east shore of Wyckoff property) were substantially higher than values commonly reported in the literature.

   PNA concentrations in Eagle Harbor crab hepatopancreas were roughly equivalent to the concentrations found in Eagle Harbor clam tissue, while crab muscle concentrations were somewhat lower (about 10 to 40 percent of clam tissue concentrations).

4. PNA concentrations in Eagle Harbor clams are generally near the upper end of the range of PNAs reported in PNA-contaminated foods (including smoked and charcoal-broiled meats and fish, as well as leafy vegetables and shellfish from contaminated environments). These concentrations are one to three orders of magnitude higher than the average concentrations in the human diet.

5. The data for PNAs in clam tissue suggest the possibility that there may be some substantial difference between PNA concentrations in clam viscera and other tissues. This hypothesis is based on the observation that the PNA concentrations in clams collected at site 32 (west of Wyckoff log dump) were substantially lower than concentrations in clams collected at other Eagle Harbor locations. This was the only sample which predominantly contained horse clams which, in turn, were the only type of clams which were "cleaned" during sample preparation prior to analysis. The possibility of partitioning of PNAs in clam viscera should be pursued to determine if this hypothesis should be accepted or rejected.

REFERENCES

Barrick, R., 1984. Personal communication. Commencement Bay Superfund Project. Tetra Tech, Inc., Bellevue WA.

Dexter, R.N., D.E. Anderson, E.A. Quinlan, et al., 1981. A Summary of Knowledge of Puget Sound Related to Chemical Contaminants, National Oceanic and Atmospheric Administration Technical Memorandum OMPA-13, Boulder, CO. 435 pp.

EPA, 1979. Methods for Chemical Analysis of Water and Wastes. EPA-600/4-79-020. OMS4 ORD, Cincinnati, OH. 460 pp.

EPA, 1980. Manual of Analytical Methods for the Analysis of Pesticides in Humans and Environmental Samples. EPA-600/8-80-038.

Gahler, A.R., J.M. Cummins, J.N. Blazevich, et al., 1982. Chemical Contaminants in Edible, Non-Salmonid Fish and Crabs from Commencement Bay, Washington. EPA-910/9-82-093. U.S.EPA, Region 10, Seattle, WA. 115 pp.

Grimmer, G., 1979. "Sources and occurrence of polycyclic aromatic hydrocarbons," pg. 31 - in: Environmental Carcinogens: Selected Methods of Analysis, Vol. 3. edited by H. Egan, M. Castegnaro, P. Bogovski, H. Kunite, E. Walker, and W. Davis. International Agency for Research on Cancer, Lyon, France.

Hetteche, H.O., 1971. "Plant waxes as collectors of PCAH in the air of polluted areas," Staub. 31:72 as reported in Pucknat, 1981, on pg. 164.

Hungspreungs, M., S. Silpipat, C. Tonapong, R.F. Lee, H.L. Windom, and K.R. Tenore, 1984. "Heavy metals and polycyclic hydrocarbon compounds in benthic organisms of the upper Gulf of Thailand," Marine Poll. Bull. 15(6):213-218.

Lee, R.F., L. Lehsau, M. Madden, and W. Marsh, 1981. "Polycyclic aromatic hydrocarbons in oysters (Crassostrea virginica) from Georgia coastal waters, analyzed by high-pressure liquid chromatography," in: Proceedings of 1981 Oil Spill Conf., pg. 341-345, American Petroleum Institute, Washington, D.C.

MacLeod, W.D., Jr., L.S. Ramos, A.J. Friedman, et al., 1981. Analysis of Residual Chlorinated Hydrocarbons, Aromatic Hydrocarbons and Related Compounds in Selected Sources, Sinks, and Biota of the New York Bight. NOAA Technical Memorandum OMPA-6, 128 pp.

MacLeod, W.D., Jr., D.W. Brown, A.J. Freidman, et al., 1984. Standard Analytical Procedures of the NOAA National Analytical Facility, 1984-1985: Extractable Toxic Organic Compounds. 110 pp. (in press).

Malins D.C., B.B. McLain, D.W. Brown, et al., 1980. Chemical Contaminants and Biological Abnormalities in Central and Southern Puget Sound. NOAA Technical Memorandum OMPA-2, 295 pp.

Malins, D.C., 1984a. Letter of 3/19/84 to Dr. Gary O'Neal, U.S.EPA, Region 10. 8 pp.

Malins, D.C., 1984b. Letter of 8/7/84 to Dr. Gary O'Neal, U.S. EPA, Region 10. 10 pp.

Mix, M.C. and R.L. Schaffer, 1983a. "Concentrations of unsubstituted polycyclic aromatic hydrocarbons in softshell clams from Coos Bay, Oregon, U.S.A." Marine Poll. Bull. 14(3)94-97.

Mix, M.C. and R.L. Schaffer, 1983b. "Concentrations of unsubstituted polynuclear aromatic hydrocarbons in Bay mussels (Mytilus edulis) from Oregon, U.S.A." Marine Env. Res. 9:193-209.

Murray, H.F., G.S. Neff, Y. Hrung, and C.S. Giam, 1980. "Determination of benzo(a)pyrene, hexachlorobenzene, and pentachlorophenol in oysters from Galveston Bay, Texas." Bull. Env. Contam. & Toxicol. 25:663-667.

Olsen, S.J. and W.R. Schell, 1977. Base Line Study of Trace Heavy Metals in Biota of Puget Sound, University of Washington College of Fisheries and Municipality of Metropolitan Seattle, contract CR-2164, Seattle, WA. 66 pp.

Pancirov, R.J. and R.A. Brown, 1977. "Polynuclear aromatic hydrocarbons in marine tissues." Env. Sci. Tech. 11(10)989-992.

Pruell, R.J., E.J. Hoffman, and J.G. Quinn, 1984. "Total hydrocarbons, polycyclic aromatic hydrocarbons and synthetic organic compounds in the hardshell clam, Mercenaria mercenaria, purchased at commercial seafood stores." Mar. Env. Res. 11:163-181.

Pucknat, A.W., 1981. Health Impacts of Polynuclear Aromatic Hydrocarbons. Noyes Data Corp., Park Ridge, NJ.

Romberg, P., 1984. Personal communication. Seattle METRO TPPS Program.

Santodonato, J., P. Howard, and D. Baau, 1981. "Health ecological assessment of polynuclear aromatic hydrocarbons." Journ. Env. Path. & Toxicol. V. 5(1): p. 6.

Smith, J.D., J. Bagg, and B.M. Byeroft, 1984. "Polycyclic aromatic hydrocarbons in the clam, Tridacna maxima, from the Great Barrier Reef, Australia." Env. Sci. & Tech. 18(5):353-358.

APPENDIX

(Provided by Mike Schlender, WDOE/EPA Manchester laboratory)

PNAs AND PCP IN TISSUE

Range and Application of Method

The method described is specific for the quantification of polynuclear aromatic hydrocarbons (PNA) and pentachlorophenol (PCP) in biological tissues. This method involves liquid extraction, gel permeation/silica gel chromatography, and gas chromatographic electron capture (GC/EC) or gas chromatographic mass spectral (GC/MS) analysis.

Procedure

Tissue Extraction. Weigh approximately 100 grams of tissue homogenate into a 200 mL centrifuge tube and add appropriate surrogate internal standard compounds. Add 80 mL of methylene chloride in methanol (2:1) to the centrifuge-extraction vessel. With the aid of a Brinkman Polytron, grind the tissue for three minutes or until the liquid fully saturates the tissue. Centrifuge the mixture for two minutes at 1500 rev./min. Centrifugation yields three layers; an aqueous layer, a tissue layer, and an organic solvent layer. The aqueous and solvent layers as well as subsequent extraction vessels rinses, are filtered through a Whatman #1 filter paper and collected by vacuum filtration. After filtration, the tissue is replaced into the centrifuge tube and the extraction process is twice repeated, combining the extracts.

PCP Extraction. The tissue extract, wich typically contains water, is transferred to a 250 mL separatory funnel. The aqueous layer is retained in the funnel while the organic layer is collected in a 500 mL Kuderna-Danish (K-D) concentrator flask. The aqueous layer is acidified to a pH 2 and extracted three times with 50 mL portions of methylene chloride. Each 50 mL portion is passed thorugh a pre-washed glass wool plug and combined with the methylene chloride-methanol extract. The K-D boiling flask is then fitted with a three-ball Syner condensing column and the extract volume is reduced to approximately 10 mL on a steam bath.

Pre-column Cleanup. To remove tissue and particulate matter which may interfere with subsequent column chromatography, the concentrated extract is passed through a 10 micron pore-size teflon filter. The resulting filtrate is then fractionated according to molecular size using gel permeation (Bio-Beads) column chromatography (30 X 3.0 cm I.D. col.). The appropriate PNA/PCP fraction of methylene chloride eluent is collected from 50 to 110 minutes after sample application.

Silica Gel Separation. Isolation of the PNA components is accomplished using silica gel column chromatography. To prepare for separation, a 25 X 2 cm I.D. glass column is wet-packed with 10 grams of silica gel (activated at 130°C overnight). The sample is exchanged to cyclohexane and is applied to the head of the column. Aliphatic components are eluted from the sample with 25 mL of pentane and discarded. The PNA components are collected by eluting the sample with 25 mL of 40 percent methylene chloride in pentane. The PNA extract is then concentrated to 1.0 mL under a stream of Ultra pure nitrogen and subjected to gas chromatographic mass spectral analysis.

PCP Preparation. A portion of the organic extract after gel permeation separation is retained for PCP analysis. The extract is exchanged to diethyl-ether (ethanol free), and methyl esterification of the PCP is accomplished using EPA method 615 (Section 10.2.2). The extract is exchanged to iso-octane and column chromatographed with 5 mL of iso-octane on a 7.0 X 0.5 cm Florisil micro column. The iso-octane elutent is then concentrated to 1.0 mL and analyzed by GC/EC.