



Padilla Bay

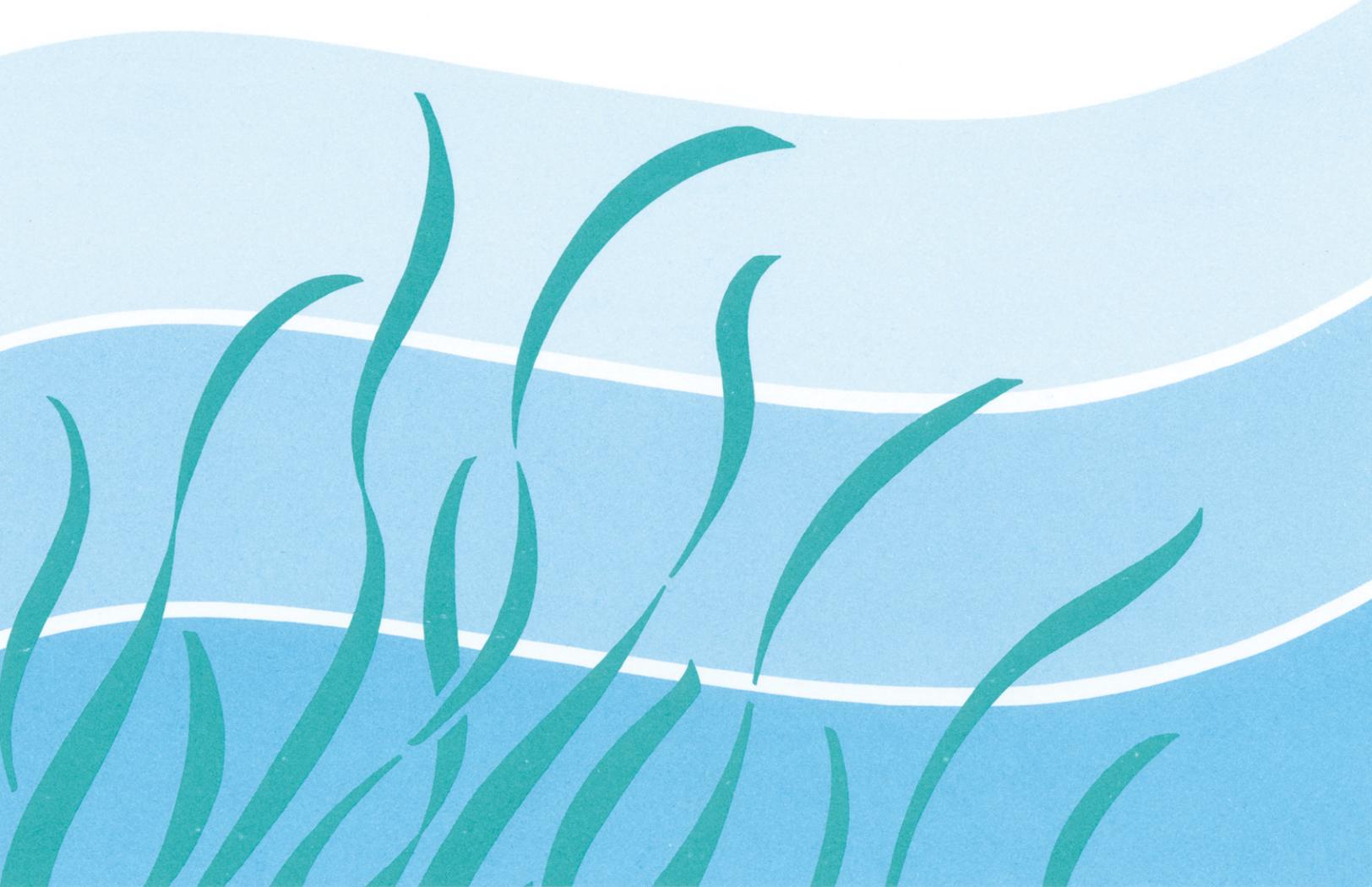
National Estuarine Research Reserve

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**POTENTIAL IMPACT OF AGRICULTURAL PESTICIDE
RUNOFF ON *ZOSTERA MARINA* AND *ZOSTERA JAPONICA*
(EELGRASS COMMUNITIES) IN PADILLA BAY, WASHINGTON**

J. Richard Mayer

1989



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PADILLA BAY AGRICULTURAL PESTICIDE STUDY

POTENTIAL IMPACT OF AGRICULTURAL PESTICIDE RUNOFF
ON ZOSTERA MARINA AND ZOSTERA JAPONICA (EELGRASS COMMUNITIES)

IN PADILLA BAY, WASHINGTON

Final Report

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Padilla Bay Agricultural Pesticide Study

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Padilla Bay Agricultural Pesticide Study

I Introduction

Agriculture today, in the United States, and in many other developed nations, reflects a range of new technologies that is a marvel to behold. Among these "green revolution" innovations is the development and application of chemical pesticides which have enabled farmers to better control the many kinds of animal and plant pests that can reduce crop yields, blemish the quality of agricultural products, or even decimate crops completely. Without doubt, pesticides have changed agricultural practices and productivity.

The widespread use and even dependence upon chemical pesticides has raised many questions regarding environmental contamination. The use of aldicarb and ethylene dibromide (EDB), as examples, is known to have caused groundwater contamination in many states. The ecological impact of DDT and the older chlorinated hydrocarbons is also well known because these persistent pesticides have had, and to some extent, are still having adverse effects on wildlife through food-chain bioaccumulation.

The development of newer types of pesticides, such as the organophosphates, has proven to be environmentally beneficial due to their relatively short half-lives and lessened potential to bioaccumulate along food chains. Nevertheless, their inherently greater toxicity can, in some cases, pose a significant hazard to chemical applicators, to farm workers, and to the environment.

This project was directed to this latter concern, the potential harm of pesticide runoff to Padilla Bay, the estuarine environment adjacent to the highly productive agricultural lands of the Skagit Valley in Washington State. It was felt by many interested observers that chemical pesticides might well be impacting the estuarine seagrass communities of Padilla Bay via transport first to the sloughs that drain farm lands and then to the Bay itself.

II The Importance of Padilla Bay

The Padilla Bay estuary, near Anacortes Washington, is comprised of 45 km² (4,500 hectares; 11,000 acres) of intertidal channelized mudflats which constitute substrate for both intertidal and subtidal eelgrasses (Webber et al. 1987). Padilla Bay is regarded as having one of the three most important and most extensive eelgrass communities in

the Pacific Northwest (Phillips 1984).

It has been estimated that Zostera marina and Zostera japonica occupy approximately 7,651 acres (3097 hectares) within the Padilla Bay National Estuarine Research Reserve (Webber et al. 1987). These seagrass meadows serve as the primary food resource and habitat for a large variety of aquatic organisms including benthic microalgae, macroalgae, epiphytes, epifauna, infauna, phytoplankton, zooplankton, herring, other small fish species, crabs, several salmonid species, ducks, brant, and geese (Thayer et al. 1975).

Lane (1980) reported that 60,000 to 70,000 black brant feed on eelgrass in Padilla Bay during their spring migration. In 1980 it was reported that 239 species of birds utilize the Padilla Bay eelgrass habitat (NOAA 1980). A colony of over 100 pairs of blue heron live in rookeries on Samish Island, just to the north of Padilla Bay. Their primary feeding ground is the Bay where they seek flatfish and sand crabs.

Herring and smelt use the eelgrasses as spawning substrate. The Bay is also an important migratory route for juvenile Chinook salmon and an especially important rearing area for both Pink and Chum salmon. Other fish utilizing the Bay include English sole, Dover sole, rock sole, and starry flounder.

Marine mammals found in the Bay include harbor seals which utilize isolated sand and mud flats. As many as 150 seals have been seen on these isolated Bay islands at low tide.

III The Reason for a Padilla Bay Pesticide Study

Perhaps the most interesting series of events in the known history of Zostera marina is the "wasting disease" which occurred in the early 1930's. Eelgrass virtually disappeared from a large part of its range worldwide. On both sides of the Atlantic the decline was sudden and drastic. Originally attributed to a parasite, Labyrinthula, it has been postulated that the "wasting disease" was caused by a climatic temperature change (Rasmussen 1977). However, controversy continues and more recent research supports the view that recurrence of the eelgrass wasting disease in Maine, New Hampshire, and Massachusetts is due to a pathogenic strain of Labyrinthula (Short et al. 1987).

Many ecological changes occurred in the wake of this massive, worldwide decline of eelgrass: sandy beaches eroded, and most of the eelgrass-related fauna declined markedly. Phillips pointed out that among the populations most affected were scallops, clams, crabs, and water fowl. For example, black brant geese populations declined by as

much as 90 percent. Fish populations also declined but not as drastically (Phillips 1978). Significant recovery of eelgrass worldwide was not documented until after 1945 (Rasmussen 1977), and full recovery required 30 to 40 years.

In the 1960's another significant decline in eelgrass began, this time centered specifically in Chesapeake Bay; all submerged aquatic vegetation is affected, and the reduction of eelgrass may be greater now than its decline in the 1930's (Orth and Moore 1983, 1984 and 1986).

Interestingly, the decline in eelgrass was accompanied by a marked expansion, at least initially, of Myriophyllum spicatum known as Eurasian water milfoil. But by 1965 this species also had declined markedly.

Research into the causes of this more recent decline of seagrass meadows in Chesapeake Bay focused first on toxic chemicals such as industrial solvents and agricultural pesticides. The findings to date appear to have ruled out this possibility. Kemp stated that the likely impact of herbicide runoff from Chesapeake Bay's surrounding agricultural watershed is minimal. His experimental results, analyzed in terms of a numerical simulation model, showed that typical Bay concentrations of herbicides rarely exceeded 2 ug/L (ppb). These levels are known to be

non-toxic to seagrasses (Kemp et al. 1983).

Sheets and Lutz in 1969 studied the movement of herbicides in runoff water in two watersheds in southern Appalachia. Two pounds per acre each of 2,4-D, dicamba, 2,4,5-T, and picrolam were applied in selected areas. Water samples were collected and analyzed following rain events. The only herbicide detected in runoff water was 2,4-D, the highest concentration being 28 ppb (Sheets and Lutz 1969).

Earlier, Trichell had showed that dicamba was capable of precipitation-aided transport from sod plots treated with this herbicide. Observed levels of dicamba were 4.8 ppm (Trichell et al. 1968)

Both 2,4-D and dicamba, therefore, are known to be highly mobile and capable of watershed runoff. Additional data and literature supporting this generalization can be found in the U.S.D.A. Agricultural Handbook (USDA 1984).

A related study of Chesapeake Bay demonstrated that in 1980 ambient levels of atrazine, linuron, and treflan in the Bay and its tributaries averaged approximately 1 ug/L (ppb). Measured levels of herbicides in runoff from a defined agricultural watershed did not exceed 9 ug/L (ppb). The major pulse of herbicide transported to the estuary coincided with the first storm event following herbicide

application (Means et al. 1983).

Many investigators have studied the Chesapeake Bay problem. All of these studies point toward the likelihood that pesticide/herbicide transport to the Bay and its tributaries is not the cause of the ongoing decline in submerged aquatic vegetation. **The likelihood is that this massive vegetative decline has been caused by decreasing availability of light in the estuarine water column due to increased turbidity in the Bay's water.** It is believed that the increased turbidity in the waters of Chesapeake Bay has has been caused primarily by accelerated eutrophication which in turn has been stimulated by large additions of nutrients. The nutrients, for the most part, have come from sewage treatment plants (Orth 1977).

Padilla Bay, on the other hand, is a totally different estuarine system. Intensively cultivated farm lands lie adjacent to very large and singularly important eelgrass beds. It appears reasonable to assume that pesticide runoff in this ecosystem could adversely affect Zostera marina and Zostera japonica.

IV The Nature of the Padilla Bay Pesticide Project

Our study in the Skagit Valley was initiated in the spring of 1987. The participating agencies were Western Washington

University, the Padilla Bay National Estuarine Research Reserve, the Skagit County Conservation District, and Entranco Engineers Inc., Bellevue, Washington. This two-year research endeavor was funded with Referendum 39 funds through the Department of Ecology, State of Washington, Olympia Washington.

It was determined that the overall project would have three objectives:

(1) The development of a comprehensive annotated bibliography documenting contemporary scientific literature dealing with the effects of agricultural chemicals on submerged aquatic vegetation and associated aquatic communities. The result of this work is a 79-page report with more than 300 references.

(2) The creation of a freshwater budget reflecting seasonal rates and quantities of freshwater input to Padilla Bay from watersheds east of the Bay. The objective was to provide the capability of translating observed pesticide concentrations in Padilla Bay sloughs and in the Bay itself into actual loading rates. This work was carried out by Entranco Engineers.

(3) The conduct of a two-year investigation of pesticide runoff to Padilla Bay sloughs and to Padilla Bay itself based on a comprehensive sampling and analytical program. Fourteen agricultural pesticides were studied in both water and sediment samples collected from three Padilla Bay sloughs and the Bay itself.

V The Research Plan

We decided at the outset of this project that success in identifying pesticides being used in the Padilla Bay area would depend on close cooperation and communication with the agricultural community. Therefore a **Padilla Bay Agricultural Advisory Committee** was established with representation from all interested parties including farmers, chemical suppliers, the Skagit Conservation District, the Padilla Bay National Estuarine Research Reserve, and the Washington State University Extension Service. See Appendix I, "Padilla Bay Agricultural Advisory Committee."

Working with the Advisory Committee, an assessment was made of the principal pesticides used in the Skagit Valley agricultural areas. Thus, fourteen pesticides were

identified for study and therefore included in this survey: trifluralin, simazine, atrazine, diazinon, chlorthalonil, methamidophos, methyl parathion, parathion, dicamba, 2,4-D, PCNB, dinoseb, metribuzin, and terbutryn.

Figure 1 shows Padilla Bay, its three principal sloughs (Joe Leary, Big Indian, and Little Indian Sloughs), and our fifteen sampling stations - eleven on the three sloughs and four in the Bay itself. Two control sites were established: C1 on Thomas Creek and C2 on the Samish River. Sampling was carried out in the spring and summer of 1987 and in the spring and summer of 1988.

Each of the two spring sampling periods preceded the application of pesticides for that growing season and followed closely the occurrence of a major rain event.

Each of the two summer sampling periods followed pesticide applications and the first rainfall event after pesticide applications.

Because of this approach, any observed pesticide levels would represent a "worst case" scenario. In addition to the four complete sampling expeditions, we also conducted a special study of parathion runoff and/or aerial drift following a broadscale application of this chemical by air

in July 1987.

Sampling and analytical protocols were based on EPA and Tetra Tech published protocols (USEPA 1984; Tetra Tech 1986a,b,c). During each sampling expedition, both a water sample and a sediment sample were collected at each sampling station. As a part of quality control protocol, every tenth sample was collected in duplicate. In addition to water and sediment samples, four physical parameters, temperature, pH, conductivity, and dissolved oxygen, were determined at each site. See Appendix II, "Sampling Protocols" and Appendix III, "Project Analytical Protocols."

Analyses for these chemicals were based on gas chromatography utilizing a Hewlett Packard 5890A GC, two different 30-meter megabore (0.53 mm ID) fused silica columns (SPB-5 and Supelcowax-10) and electron capture detection. Pesticide confirmation and quantification were based on the Supelcowax-10 column. Pesticide analytical standards (obtained from EPA, Research Triangle Park in North Carolina) were prepared in hexane. Dicamba and 2,4-D were first methylated (diazomethane) and then included in the mix of standards.

VI Research Results

In both the spring of 1987 and the spring of 1988 (prior to pesticide application), the two sampling periods during which one might have expected to find little if any pesticide runoff, we were not able to demonstrate the presence of any of the fourteen pesticides under study in the water or in the sediments of the sloughs and the bay itself. See Appendix VI, "Pesticides Studied and Their Detection Limits. See Tables 1, 2, 6, and 7. This was not surprising because any residues that might have persisted following summer application of chemicals, would most likely have experienced runoff and/or weathering over the wet, rainy winter of western Washington. Furthermore, most of the pesticides under study are known to have relatively short half-lives of six to ten weeks.

The sampling expedition carried out in 1987: June 22 (slough sites) and July 7 (bay sites), followed a major rain event (0.45 inches on June 21). Dicamba was found in all water samples (all slough and bay sites) ranging from 10 to 170 ug/L (ppb). Dicamba was also found in four of the slough-sediment samples ranging from 2.1 - 17.1 ug/g (ppm). No dicamba was found in any of the bay sediments. See Tables 3, 4 and 5.

2,4-D was found in ten of the slough-water samples ranging from 0.14 - 1.3 ug/L (ppb). No 2,4-D was found associated with slough sediments and no 2,4-D was found in bay-water samples or bay-sediment samples. See Tables 3, 4 and 5.

The sampling expedition carried out in 1988: August 7 (slough sites) and August 9 (bay sites), followed two relatively minor rain events: 0.12 and 0.03 inches on August 5 and 6 respectively. The summer of 1988 was very dry, and these were the only rain events that occurred reasonably close to pesticide applications that year. No detectable levels of any of the pesticides under study could be demonstrated. See Tables 8 and 9.

In addition to the four broad-scale sampling efforts described above, a special study of the potential for parathion runoff and/or aerial drift was carried out. Parathion was applied aerially in the Skagit valley during the first week of July 1987 to control aphids on snow peas. Almost immediately following the spraying, a rain event took place. Analyses of water and sediment samples taken from the sloughs and the bay failed to disclose any detectable levels of parathion. See Table 10.

The overall results of the two-year pesticide-runoff study are summarized in Table 11.

VII Discussion of Results

Of the fourteen pesticides studied, only two were found in water and/or sediment samples through our sampling program. Those two were dicamba and 2,4-D, both of which are known to migrate across land surfaces (runoff) owing to precipitation events (Sheets and Lutz 1969; Trichell et al. 1968; USDA 1984).

It is important to point out that the Skagit County Department of Transportation uses dicamba (Banvel 720) to control roadside vegetation (Clark 1987); this could be the source of part of or all of the observed levels of dicamba in this study.

Dicamba, like 2,4-D, is selectively toxic toward perennial and annual broad-leaf weeds and brush. No literature was found dealing with the toxic effects of dicamba toward eelgrass species. Studies of algae, however, showed that some algal types reflected reduced growth rates when exposed to 10 ppm dicamba (Cullimore 1975). This level of exposure is 77 times the maximum level of dicamba found in Padilla Bay itself.

2,4-D is known to be toxic to eelgrass. Thomas (1968) has shown that eelgrass can be eradicated using Aqua Kleen (a

product of Amchem Products Inc. containing 20% 2,4-D). Eelgrass, at his experimental site (Prince Edward Island, Nova Scotia) was shown to be most sensitive to 2,4-D in late June. Assuming that the amounts of 2,4-D applied to the submerged eelgrass beds were dispersed in a water column averaging from 10 to 100 cm deep, the concentration of 2,4-D would be between 2.24 ppm and 22.4 ppm (Thomas 1968). Therefore the toxic level of 2,4-D (toxic to eelgrass) is from 1,700 to 17,000 times the highest level of 2,4-D we observed in Padilla Bay sloughs.

The overall result of this project is the finding that no ecologically significant levels of any of the fourteen pesticides studied were found in the water column of the sloughs of Padilla Bay or in the water column of the Bay itself during the two-year investigation. It could be argued that the higher levels of dicamba and 2,4-D found associated with some of the sediment samples are ecologically significant, but the sediments appear to be acting as a "sink" in these cases and the pesticides are relatively unavailable to the aquatic environment.

These results, therefore, support the view that runoff or transport of pesticides currently used in Skagit valley agricultural areas adjacent to Padilla Bay is not a problem.

Our findings can be summarized as follows:

- (1) Current agricultural practices in the Skagit valley adjacent to Padilla Bay reflect pesticide management practices which tend to protect nearby aquatic environments from chemical pesticide contamination.
- (2) The topography of the Skagit flatlands adjacent to Padilla Bay appears to minimize the potential for pesticide runoff.
- (3) The finding of dicamba and 2,4-D in several water and sediment samples is consistent with their known soil-surface transport behavior. The observed levels appear to be of no ecological significance.

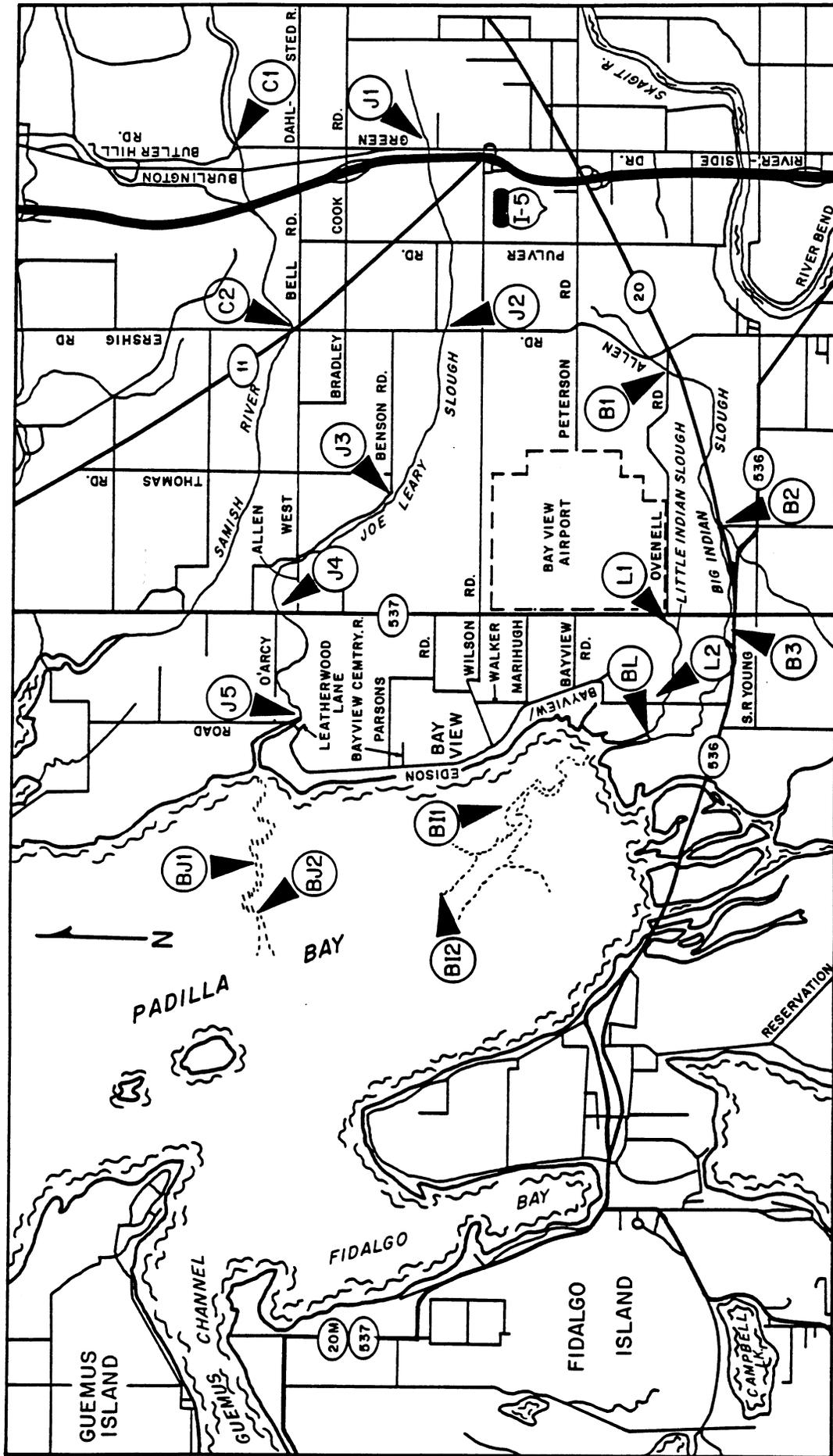


Figure 1. Sampling Stations on Padilla Bay and its Sloughs. Specific locations of sampling stations on Padilla Bay sloughs (Joe Leary, Big Indian, and Little Indian Sloughs) and on the Bay itself.

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Table 1. Slough and bay water samples collected on May 19, 25 and June 16, 1987. Padilla Bay Agricultural Pesticide Study, 1986-1988.

Sample	Date	Time	Tide ¹	Temp °C	Dissolved Oxygen mg/L	pH	Salinity o/oo	Analytical Result
J-1w	5/19	10:41	5.5	12.0	9.2	6.6	0.0	A ²
J-2w	5/19	10:19	5.8	12.0	5.6	6.7	0.0	A
J-3w	5/19	9:55	6.2	11.0	4.0	6.7	0.0	A
J-4w	5/19	9:36	6.3	11.0	3.6	6.8	0.0	A
J-5w	5/19	9:30	6.4	12.0	4.6	7.0	5.8	A
B-1w	5/19	11:07	5.1	13.5	5.9	6.6	0.0	A
B-2w	5/19	11:25	4.7	13.2	6.6	6.7	0.0	A
B-3w	5/19	11:37	4.5	13.0	5.7	6.7	0.0	A
L-1w	5/19	12:00	4.0	14.5	16.8	8.6	1.2	A
L-2w	5/19	12:15	3.6	18.0	4.8	7.2	10.0	A
BLw	5/19	12:45	3.0	17.0	8.4	7.8	27.2	A
C-1w	5/19	13:49	1.5	15.0	12.8	6.9	0.0	A
FBw	5/19	-	-	-	-	7.0	0.0	A
BI-1w	5/25	9:37	-0.2	15.0	10.4	8.1	29.5	A
BI-2w	5/25	10:00	-0.5	14.0	14.2	8.6	30.0	A
BJ-1w	6/16	14:08	-0.8	19.5	10.6	8.2	26.1	A
BJ-2w	6/16	14:48	-1.2	18.5	15.9	8.9	29.2	A

1 Adjusted for Padilla Bay (feet above chart datum)

2 "A" signifies that the gas chromatographic protocols employed in this study demonstrated that no detectable levels of any of the fourteen pesticides analyzed for were observed.

Table 2. Slough and bay sediment samples collected on May 19, 25 and June 16, 1987. Padilla Bay Agricultural Pesticide Study. 1986-1988.

Sample Code	Sample Date	Collection Time	% Loss on Drying	Analytical Result
J-1s	5/19	10:41	54.2	A ¹
J-2s	5/19	10:19	39.7	A
J-3s	5/19	9:55	68.8	A
J-4s	5/19	9:36	81.1	A
J-5s	5/19	9:30	69.1	A
B-1s	5/19	11:07	20.7	A
B-2s	5/19	11:25	32.2	A
B-3s	5/19	11:37	42.9	A
L-1s	5/19	12:00	81.8	A
L-2s	5/19	12:15	52.7	A
BLs	5/19	12:45	48.9	A
C-1s	5/19	13:49	68.2	A
BI-1s	5/25	9:37	30.3	A
BI-2s	5/25	10:00	21.9	A
BJ-1s	6/16	14:08	31.2	A
BJ-2s	6/16	14:48	33.7	A

1 "A" signifies that no pesticide was detected.

Table 3. Slough and bay water samples collected on June 22, 29, and July 7, 1987. Descriptive data. Padilla Bay Agricultural Pesticide Study, 1986-1988.

Sample	Date	Time	Tide ¹	Temp °C	Dissolved Oxygen mg/L	pH	Salinity o/oo
J-1w	6/22	15:23	6.2	16.2	2.3	6.8	0
J-2w	6/22	14:57	5.6	17.4	4.9	6.9	0
J-3w	6/22	14:43	5.2	14.6	4.9	6.8	0
J-4w	6/22	14:29	4.9	14.6	4.8	6.8	0
J-5w	6/22	14:20	4.7	17.0	7.8	7.1	0
B-1wa	6/22	15:38	6.4	17.2	7.4	6.8	0
B-1wb	6/22	15:38	6.4	17.2	7.4	6.8	0
B-2w	6/22	15:49	6.6	15.5	5.1	6.7	0
B-3w	6/22	15:56	6.7	14.7	5.6	6.9	0
L-1w	6/22	16:08	6.9	20.5	9.0	8.1	19.5
L-2w	6/22	16:03	6.8	22.0	7.8	7.9	23.5
BLw	6/22	16:18	7.0	17.8	9.2	8.1	31.0 ²
BI-1wa	6/29	13:46	-1.5	23.5	9.5	8.3	33.0 ²
BI-1wb	6/29	13:47	-1.5	23.5	9.6	8.3	33.0 ²
BI-2w	6/29	14:04	-1.4	21.5	12.8	8.6	34.0 ²
BJ-1w	7/7	11:15	.4	17.0	10.0	7.5	9.5
BJ-2w	7/7	10:55	0.0	16.0	11.6	8.2	34.0 ²
C-1w	6/22	15:07	5.8	13.3	10.2	7.8	0
FBw	6/22	-----	---	-	-	---	-

1 Adjusted for Padilla Bay (feet above chart datum)

2 These relatively high salinity values may be due to concentration by surface evaporation.

Table 4. Slough and bay water samples collected on June 22, 29 and July 7, 1987. Analytical Results. Padilla Bay Agricultural Pesticide Study, 1986-1988.

Sample	Dicamba ¹ ug/L (ppb)	2,4-D ² ug/L (ppb)	Other Pesticides ug/L (ppb)
J-1w	160	1.1	A ³
J-2w	10	B ⁴	A
J-3w	110	0.31	A
J-4w	140	0.50	A
J-5w	120	0.16	A
B-1wa	170	1.3	A
B-1wb	90	0.14	A
B-2w	70	0.31	A
B-3w	60	0.24	A
L-1w	150	0.23	A
L-2w	130	B	A
BLw	50	0.25	A
BI-1wa	50	B	A
BI-1wb	130	B	A
BI-2w	130	B	A
BJ-1w	50	B	A
BJ-2w	80	0.10	A

- 1 The detection limit for dicamba is 6.1 ug/L.
 2 The detection limit for 2,4-D is 0.048 ug/L.
 3 "A" implies that none of the other pesticides under study (12) could be detected.
 4 "B" implies that this particular pesticide could not be detected.

Table 5. Slough and bay sediment samples collected on June 22, 29, and July 7, 1987. Descriptive data and analytical results. Padilla Bay Agricultural Pesticide Study, 1986-1988.

Sample	Date	Collection Time	Loss On Drying, %	Dicamba ¹ (ug/g dry weight)	Other Pesticides (ppm)
J-1s	6/22	15:23	49	A ²	A ²
J-2s	6/22	14:57	65	A	A
J-3s	6/22	14:43	60	A	A
J-4s	6/22	14:29	76	A	A
J-5s	6/22	14:20	42	A	A
B-1sa	6/22	15:38	41	9.5	A
B-1sb	6/22	15:38	39	2.1	A
B-2s	6/22	15:49	37	5.8	A
B-3s	6/22	15:56	42	A	A
L-1s	6/22	16:08	84	A	A
L-2s	6/22	16:03	54	17.1	A
BLs	6/22	16:18	59	A	A
BI-1sa	6/29	13:46	31	A	A
BI-1sb	6/29	13:47	33	A	A
BI-2s	6/29	14:04	23	A	A
BJ-1s	7/7	11:15	39	A	A
BJ-2s	7/7	10:55	32	A	A
C-1s	6/22	15:07	32	A	A

1 The only pesticide found in the sediments was dicamba.

2 "A" signifies that no pesticide was detected.

Table 6. Slough and bay water samples collected on April 13, 1988.
Padilla Bay Agricultural Pesticide Study, 1986-1988.

Sample	Date	Time	Tide ¹	Temp °C	Dissolved Oxygen mg/L	pH	Salinity o/oo	Analytical Result
J-1w	4/13	17:30	6.7	12.5	3.9	6.6	0	A ²
J-2w	4/13	17:20	6.78	12.0	5.4	6.7	0	A
J-3w	4/13	16:40	7.04	12.0	5.4	6.7	0	A
J-4w	4/13	16:20	7.14	11.0	7.5	6.7	0	A
J-5aw	4/13	16:05	7.18	12.0	10.2	6.8	0	A
J-5bw	4/13	16:05	7.18	12.0	10.2	6.8	0	A
B-1w	4/13	13:50	5.46	12.0	9.9	6.7	0	A
B-2w	4/13	14:08	5.86	12.0	8.8	6.7	0	A
B-3w	4/13	14:30	6.3	13.0	12.2	6.8	1	A
L-1w	4/13	15:10	6.9	13.7	12.8	6.8	0	A
L-2w	4/13	14:50	6.62	13.5	10.4	7.0	0	A
BLw	4/13	15:30	7.1	14.0	12.0	7.7	14	A
C-2w	4/13	16:50	6.98	11.0	11.6	7.1	0	A
FBw	4/13	-	-	-	-	6.9	0	A
BIN-1w	4/13	14:30	6.3	10.8	8.6	8.2	28	A
BIN-2w	4/13	14:40	6.46	11.2	9.0	8.3	27	A
BJL-1w	4/13	14:05	5.8	11.5	8.7	8.2	26	A
BJL-2aw	4/13	14:15	6.0	11.0	8.5	8.1	27	A
BJL-2bw	4/13	14:15	6.0	11.0	8.5	8.1	26	A

1 Adjusted for Padilla Bay (feet above chart datum)

2 "A" signifies that no pesticide was detected.

Table 7. Slough and bay sediment samples collected on April 13, 1988. Padilla Bay Agricultural Pesticide Study, 1986-1988.

Sample Code	Sample Date	Collection Time	Analytical Result
J-1w	4/13	17:30	A ¹
J-2w	4/13	17:20	A
J-3w	4/13	16:40	A
J-4w	4/13	16:20	A
J-5aw	4/13	16:05	A
J-5bw	4/13	16:05	A
B-1w	4/13	13:50	A
B-2w	4/13	14:08	A
B-3w	4/13	14:30	A
L-1w	4/13	15:10	A
L-2w	4/13	14:50	A
BLw	4/13	15:30	A
BIN-1w	4/13	14:30	A
BIN-2w	4/13	14:40	A
BJL-1w	4/13	14:05	A
BJL-2aw	4/13	14:15	A
BJL-2bw	4/13	14:15	A

1 "A" signifies that no pesticide was detected.

Table 8. Slough and bay water samples collected on August 7 and 9 1988. Padilla Bay Agricultural Pesticide Study, 1986-1988.

Sample	Date	Time	Tide ¹	Temp °C	Dissolved Oxygen mg/L	pH	Salinity o/oo	Analytical Result
J-1w	8/7	15:30	8.1	17.0	3.0	6.6	0.0	A ²
J-2w	8/7	15:53	8.1	18.1	3.8	6.7	0.0	A
J-3w	8/7	16:02	8.1	15.2	4.8	6.7	0.0	A
J-4w	8/7	16:15	8.05	16.0	5.0	6.9	0.0	A
J-5w	8/7	12:40	5.85	18.2	7.6	7.2	0.0	A
B-1wa	8/7	14:02	7.43	17.0	7.2	6.9	0.0	A
B-1wb	8/7	14:07	7.49	17.0	7.2	6.9	0.0	A
B-2w	8/7	13:50	7.3	17.2	5.7	6.7	0.0	A
B-3w	8/7	13:40	7.1	23.1	5.3	6.6	11.0	A
L-1w	8/7	13:30	6.9	23.3	8.1	8.0	24.0	A
L-2w	8/7	13:10	6.43	22.2	7.1	7.9	23.0	A
BLw	8/7	13:00	6.2	19.1	8.1	7.8	25.0	A
BI-1wa	8/9	16:20	6.8	12.5	8.5	8.1	27.0	A
BI-1wb	8/9	16:20	6.8	12.5	8.5	8.1	28.0	A
BI-2w	8/9	16:25	6.8	12.2	8.8	8.3	30.0	A
BJ-1w	8/9	15:59	6.8	13.0	9.4	8.2	29.0	A
BJ-2w	8/9	16:05	6.8	12.5	9.4	8.4	28.0	A
C-1w	8/7	16:30	8.0	17.2	9.7	7.7	0.0	A
FBw	8/7	-	-	-	-	7.1	0.0	A

1 Adjusted for Padilla Bay (feet above chart datum)

2 "A" signifies that no pesticide was detected.

Table 9. Slough and bay sediment samples collected on August 7 and 9, 1988. Descriptive data and analytical results. Padilla Bay Agricultural Pesticide Study, 1986-1988.

Sample	Collection Date	Time	Tide ¹	Pesticides ug/g (ppm)
J-1s	8/7	15:30	8.1	A ²
J-2s	8/7	15:53	8.1	A
J-3s	8/7	16:02	8.1	A
J-4s	8/7	16:15	8.05	A
J-5s	8/7	12:40	5.85	A
B-1sa	8/7	14:02	7.43	A
B-1sb	8/7	14:07	7.49	A
B-2s	8/7	13:50	7.3	A
B-3s	8/7	13:40	7.1	A
L-1s	8/7	13:30	6.9	A
L-2s	8/7	13:10	6.43	A
BLs	8/7	13:00	6.2	A
BI-1sa	8/9	16:20	6.8	A
BI-1sb	8/9	16:20	6.8	A
BI-2s	8/9	16:25	6.8	A
BJ-1s	8/9	15:59	6.8	A
BJ-2s	8/9	16:05	6.8	A
C-1s	8/7	16:30	8.0	A

1 Adjusted for Padilla Bay (feet above chart datum)

2 "A" implies that no pesticide was detected.

Table 10. Slough and bay water samples collected on July 6 and 7 1987 for analysis of parathion following aerial application of parathion to adjacent farm lands during the first week of July 1987. Padilla Bay Agricultural Pesticide Study, 1986-1988.

Sample	Date	Time	Temp °C	Dissolved Oxygen, mg/L	pH	Salinity o/oo	Analytical Result
J-5w	7/6	13:23	16.5	6.8	7.1	0.0	A ¹
B-3w	7/6	13:01	16.1	6.2	6.8	0.0	A
L-2w	7/6	13:11	20.2	11.6	8.1	32.2 ²	A
BJ-1w	7/7	11:15	17.0	10.0	7.5	9.5	A
BJ-2w	7/7	10:55	16.0	11.6	8.2	39.0 ²	A
C2	7/6	12:41	14.2	10.5	7.8	0.0	A

1 "A" signifies that the analytical method employed showed no detectable levels of parathion in the 10 ml concentrates from extracted sample filtrates. Similarly, negative results were obtained in studies of concentrates from filtered suspended matter. The detection limit was 0.91 ug/L (ppb). Analyses were carried out on a Perkin-Elmer Sigma 300 chromatograph utilizing a three-meter OV-210 packed column at 210 °C equipped with a flame photometric detector.

2 These relatively high salinity values may be due to concentration by surface evaporation.

Table 11. Summary of Analytical Results. Padilla Bay Agricultural Pesticide Study, 1986-1988.

YEAR OF STUDY:		1987		1988	
		SPRING	SUMMER	SPRING	SUMMER
PADILLA BAY SLOUGHS	WATER	NO PESTICIDE DETECTED	DICAMBA ALL SITES 10-170 ppb ----- 2,4-D 9 SITES 0.14-1.3ppb	NO PESTICIDE DETECTED	NO PESTICIDE DETECTED
	SEDIMENT	NO PESTICIDE DETECTED	DICAMBA ALL SITES 2.1-17.1ppm ----- NO 2,4-D FOUND	NO PESTICIDE DETECTED	NO PESTICIDE DETECTED
PADILLA BAY ESTUARY	WATER	NO PESTICIDE DETECTED	DICAMBA ALL SITES 50-130 ppb ----- NO 2,4-D FOUND	NO PESTICIDE DETECTED	NO PESTICIDE DETECTED
	SEDIMENT	NO PESTICIDE DETECTED	NO PESTICIDE DETECTED	NO PESTICIDE DETECTED	NO PESTICIDE DETECTED

Tyler C. Clark, First Chairman	Director, Skagit County Conservation District
Kraig Olasen, Second Chairman	Director, Skagit County Conservation District
Marvin Omdal	Dairy Farmer and Chairman Skagit Conservation District
Roger Knutzen	Row Crop Farmer and Aerial Pesticide Applicator
Ron Hawkins	Fieldman for Argichem, Inc
Stott Howard	Research Scientist, WSU Northwest Washington Research & Extension Unit
Lou Hiett	Fieldman Supervisor, Twin City Foods, Inc
Terry Stevens	Director, Padilla Bay National Estuarine Research Reserve
Richard Mayer	Professor of Environmental Studies, Western Washington University

The two-year study of agricultural pesticides in Padilla Bay sloughs and in the Bay itself is based upon periodic sampling in Joe Leary Slough, Big and Little Indian Sloughs, and the two eelgrass communities in the Bay.

Dates and times of sampling were coordinated with area farmers and chemical pesticide suppliers so as to assure that both "worst-case" and "best-case" scenarios will be assessed. Based on literature studies it is known that a worst case scenario (in terms of maximum levels of pesticide runoff to be observed) will occur within 24 hours of a storm event in the agricultural region being studied.

Eighteen sampling stations were established: twelve on the sloughs, two freshwater control sites (Thomas Creek and the Samish River) and four sites in the Bay, two near the mouth of Joe Leary Slough and two near the confluence of Big and Little Indian Sloughs. All four Bay sites are located in eelgrass communities. See Figure 1, "Sampling Stations".

Integrated water column samples were collected with a special custom-made water sampler based on a 2-meter copper pipe with a manually-controlled trap. One-liter water samples were collected in brown glass bottles previously cleaned with detergent, distilled water, and acetone. The bottles were sealed with aluminum foil and a screw cap.

Sediment samples, (about 100 g) obtained using a Peterson Dredge or a simple shovel, were collected in plastic containers.

All samples were cooled immediately in an insulated sample carrier containing frozen "blue ice." One duplicate water sample, one duplicate sediment sample, a control-site sample, and a field blank collected during each sampling period.

All samples were labeled in the field using permanent marker. The codes were: J = Joe Leary Slough; B = Big Indian Slough; L = Little Indian Slough; BL = the confluence of Big and Little Indian Slough; BJ1 (or BJL-1) = Padilla Bay eelgrass site nearest the mouth of Joe Leary Slough; BJ2 (or BJL-2) = the Padilla Bay eelgrass site to the west of BJ1; BI1 (or BIN-1) = the Padilla Bay bay eelgrass site nearest the mouth of Indian Slough; and BI2 (or BIN-2) = the Padilla Bay eelgrass site to the west of BI1; w = water sample; s = sediment sample;

Methylated samples, for the determination of phenoxy acids and dinoseb, were designated with a "-Me" or "-M." For example: BJ2w-Me. Duplicate samples carry an "a" and a "b" designation. For example: BJ2w-Me-a.

As soon after field sampling as possible, all water samples (in glass) were refrigerated at close to 0° C until analyzed. All sediment samples (in plastic) were frozen and then thawed just prior to analysis.

In addition to sample collection the following physical parameters were measured: temperature, dissolved oxygen, salinity, and pH. The first two were determined in the field, while the latter two were determined in the lab.

A. Background

A defined set of analytical protocols has been established for this project. These are patterned after similar protocols developed and published by Tetra Tech and the USEPA (Tetra Tech. 1986a,b,c; USEPA 1984). In particular, sampling procedures (Tetra Tech. 1986a), and the analysis of pesticide residues in sediments (Tetra Tech. 1986b and 1986c) are based largely on these methods.

Published methods for multiresidue pesticide analysis leave much to be desired. On reviewing the work of Cessna and his colleagues (Cessna et al. 1985) and the recent book published by Chau et al. (1982), it is clear that many problems surround multiresidue analytical work. In our studies we recognized that a problem might occur in analyzing for dinoseb since it is a polar compound and could prove difficult to extract from water and sediment samples. Indeed, our early experimental work in trying to verify whether some of the published procedures would permit good recovery of dinoseb showed that very little dinoseb was recovered from water or sediment samples using conventional solvents such as hexane, methylene chloride or chloroform. Salting-out techniques also failed to produce good recoveries of dinoseb.

We found, and later confirmed, that using ether as an extraction solvent gave very good recoveries of dinoseb. This approach was discussed and later adopted after personal communication with Bob Rieck at E.P.A. Manchester (Rieck 1987). We knew also that good recovery of 2,4-D would depend on using ether. Therefore our experimental protocol for multiresidue analysis of the fourteen pesticides under study utilizes ether extractions - three extractions of an acidified water sample, and three more after making the sample alkaline.

This technique was verified by extracting a one-liter water sample spiked with four pesticides representing the types of pesticides under study. These four were parathion, dinoseb, atrazine, and 2,4-D. Good recoveries of all four were obtained.

The extraction procedure for pesticides in sediments is based for the most part on Tetra Tech procedures (Tetra Tech. 1986b and 1986c). Sonification, using 1:1 acetone - methylene chloride proved to be an effective method. This technique was verified using the same set of four pesticides. Sediment extracts and concentrates failed to yield good chromatograms due to extracted extraneous matter.

We solved this problem by using metallic mercury in a clean-up procedure. By shaking a sediment extract or concentrate vigorously with mercury, a variety of sulfur-containing contaminants are removed. We verified experimentally that none of the fourteen pesticides under study is affected by this treatment.

B. Determination of Physical Parameters

Temperature was determined using a calibrated mercury thermometer.

Dissolved Oxygen was determined using a calibrated Yellow Springs Model 54 DO Meter

pH was measured using an Orion Model 901 Ionanalyzer

Salinity was measured using a YSI Model 33 S-C-T Meter and/or an American Optical salinometer

Loss on drying (sediment samples) was determined by weighing a previously tared drying dish containing a weighed sediment sample after drying at 100° C until constant weight was achieved.

C. Protocol for Multiresidue Sample Extraction

NOTES: This procedure employs ether as an extraction solvent. Use great care in handling ether since it is flammable. Work in the hood at all times and have a fire blanket and a carbon dioxide extinguisher at hand. Keep hot plates and other electrical apparatus turned off during ether extractions and transfer to the KD apparatus.

Rinse each piece of glassware three times with small amounts of acetone prior to its use to eliminate contaminants.

Extraction Procedure

In each case a one-liter water sample is analyzed. Set up a filtration apparatus holding a 47 mm glass-fiber filter (e.g. Gelman Type A/E). Without shaking the bottle (which re-suspends sediments) filter the water sample until the quantity of filtered sample is 1000 ml. Save the remainder of the unfiltered water sample in the refrigerator for determination of pH and salinity.

Transfer the clear, filtered, 1000 ml sample to a 2-liter separatory funnel in the hood. Label the sep funnel with the sample number.

At this point make sure all electrical equipment (e.g. hot plate) is turned off.

Extract three times using pesticide-residue-grade ether (200 ml, 100 ml, and 100 ml). Use a clean 2-liter Erlenmeyer flask to collect the lower aqueous layer and a clean 1-liter erlenmeyer flask to collect the upper ether layer. Cover the ether extracts with aluminum foil. Label this flask with the sample number.

The aqueous layer from above is acidified to pH 2 with 20 drops of concentrated sulfuric acid. Test the pH with pH paper. Extract three times using three 100 ml portions of pesticide-residue-grade ether. As before, collect the lower aqueous layer and add the upper ether layer to that collected above.

The aqueous layer from above is made alkaline with conc NaOH to pH 10, and then ether-extracted three times: use 100 ml of ether each time. These ether extracts are added to the ether extracts above.

Note Do not turn on the hot plate-steam bath until the ether solution has been transferred to the KD and the Snyder column is in place.

The ether solution is transferred to a KD apparatus; As you transfer this solution, leave behind any obvious water layer that might be present. Rinse the flask once with ether and add to the ether solution in the KD again leaving behind any water layer. Label the KD with the sample number. Make sure the KD receiver has a boiling chip in it. Concentrate the ether solution, using a steam bath, to about 10 ml; it helps to wrap the "Erlenmeyer flask steam bath" and the Snyder column with aluminum foil.

As the ether concentrate approaches 10 ml, add 80 ml hexane to the KD in small portions waiting for the ether and/or hexane vapors to reappear at the top of the Snyder column before adding the next portion of hexane. Concentrate to a final volume of about 10 ml. Record the exact final volume.

Transfer the hexane solution (quantitative transfer is not necessary) to a 15 ml vial and label it with sampling station code, sample type, date of sample, and final volume. For example, J5 w 5/19/87 8.8 ml. Note the "w" is used to designate an extract from a water sample. If a GC scan is not to be run that day, place sample in the refrigerator.

D. Methylation Using Diazomethane

Note: Diazomethane is a very toxic gas; it is explosive on contact with scratched or broken glass. Therefore, work in the hood and treat diazomethane with respect.

1. This procedure uses a Wheaton macro-generator. Diazomethane is produced in the inner tube of the generator by slowly adding 5N NaOH dropwise to a water slurry of 1-methyl-3-nitro-1-nitrosoguanidine (available from Aldrich Chemical Co. Note: this chemical is a potent mutagen and is a cancer suspect agent.) The reaction is carried out in an ice-water bath. The pale yellow gas passes through a hole in the inner tube and falls toward the bottom of the outer tube of the generator which holds the sample to be methylated.
2. Using gloves, very carefully weigh out 170 mg of the above guanidine reagent and transfer into the inner tube of the Wheaton generator being careful to keep the hole of the inner tube pointing up. Add 0.5 ml of distilled water directly into the reagent. No reaction occurs at this point.
3. Add about 2 ml of the sample to be methylated into the outer tube of the generator. The exact volume is not important. Hexane is a superior solvent for this reaction, but other solvents, such as ether, work well also. A small teflon covered magnetic stirrer may be used to gently stir the sample as methylation occurs in the next step.
4. Clamp the outer tube containing the sample to a ring stand which has on its base a magnetic stirrer and an ice-water bath. Lower the tube into the bath, and place the inner generator tube in place. Use the special clamp provided so as to allow for release of undue pressure buildup in the generator. Screw on the cap and its teflon-lined septum.

Gently stir, and, using a 1-ml syringe, start adding 0.6 ml of 5N NaOH dropwise. Do this in a hood with the safety glass door pulled down so as to cover your face. Make sure the inner generator tube is not connected too tightly to the outer tube and that pressure buildup will be able to dislodge it somewhat.

5. The first few drops of NaOH should be added very slowly. After about 0.1 ml has been added, the rate of addition can be increased. The entire 0.6 ml of NaOH can be added in about one to two minutes. You may see excess yellow-green diazomethane appear in the sample.

6. After the NaOH addition, let the solution stand in the ice-water bath for fifteen minutes. Remove the apparatus from the ice bath and allow to come to room temperature. Unscrew the top cap, remove the inner generator tube, and pour its contents into a hazardous waste collection bottle. Wash the tube in the hood, and leave it in the hood until it is to be cleaned. Using dry nitrogen gas, and working in the hood, gently blow the excess diazomethane from the outer tube. You may see a quick loss in color at this point. The methylated sample can now be transferred to a sample bottle using a Pasteur pipet. Note that one should not allow a glass pipet to make contact with diazomethane; at this point in the procedure, however, the diazomethane is no longer present.
7. Allow both the inner and outer generator tubes to lie in the hood for some time before removing them from the hood so as to assure that no diazomethane will be escape from the hood area.

E. The Chromatography

There are two possible approaches to obtaining the needed chromatography. The first is to run samples (under the conditions noted below) prior to methylation, and then methylate using either boron trifluoride - methanol or diazomethane; the methylated sample is then run (same chromatographic conditions).

The second approach is to methylate a portion of the sample preferably in hexane, and obtain the chromatography.

At the start of each series of GC scans, run one or more standard mixes of pesticides of interest. An alternative to this is to run a specially prepared standard mix of 14 pesticides. This will be a methylated sample.

Obtain a GC scan by injecting 1 ul of sample. Use the solvent flush technique. The GC conditions are: 180 °C oven, 30 kPa helium head pressure, electron capture detector at 350 °C using nitrogen make-up gas, 30 m SPB-5 fused silica column (0.53 mm id), on-column injection.

After obtaining a satisfactory GC scan, return the sample to the refrigerator. If a methylation reaction is to be run at this time, remove a 1.5 ml subsample of the hexane sample for methylation and archive the remainder of the sample in the freezer. Carry out the methylation using the methylation protocol.

The chromatography of the methyl esters is carried out using a thermal GC program: 180 C for 12 minutes; 10 C/minute temperature rise to 210 C; hold at 210 C for 45 minutes. Total time is 60 minutes. Allow a 7-minute instrumental equilibration time between analytical runs.

Inject 1.0 ul of sample using the solvent flush technique and on-column injection. Mark the chromatogram as in the following example: Padilla Bay Study / 5/19/87; J5 s-Me 1.3 ml; 1.0 ul injected; thermal program.

Run the standard pesticide mix containing the phenoxy acid esters to create a one-point calibration curve. Report the presence of any phenoxy acids as the acid and in terms of ug/g (ppm).

Note: For Water Samples - Analytical results were calculated as follows:

$$Y = \frac{A \times B \times C \times D \times 1000}{E \times F \times G}$$

where, Y = pesticide concentration in ug/L (ppb)
 A = sample peak height
 B = concentration of standard
 C = volume of standard injected (ul)
 D = volume of sample KD concentrate (ml)
 E = standard peak height
 F = volume of sample injected (ul)
 G = volume of water sample extracted (ml)

Note: For Sediment Samples - Analytical results were calculated as follows:

$$Y = \frac{A \times B \times C \times D \times 100}{E \times F \times G \times H}$$

where, Y = pesticide concentration in ug/g (ppm)
 A = sample peak height
 B = concentration of standard
 C = volume of standard injected (ul)
 D = volume of sample in KD concentrate
 E = standard peak height
 F = volume of sample injected (ul)
 G = sediment sample wet weight
 H = 100 - percent loss on drying

Appendix IV Quality Control

Quality Control in this study is based on generally recognized and accepted procedures regarded as "Good Laboratory Practice" as well as the following considerations:

1. All laboratory procedures and protocols are documented.
2. All sampling containers are glass, pre-cleaned with detergent, water, distilled water, and acetone rinsed three times.
3. All sampling containers are pre-labeled with unambiguous sampling-site codes.
4. Sampling is carried out with reference to a carefully documented sampling-site map. See Figure 1. All samples are chilled immediately in the field upon collection and later placed in the laboratory refrigerator.
5. Field blanks are employed and analyzed as needed.
6. One duplicate water sample and one duplicate sediment sample are collected as a part of each sample set and analyzed.
7. All glassware used in extraction and concentration work is pre-cleaned with detergent, water, distilled water, and rinsed with acetone three times.
8. Analytical standards are run on the GC just prior to each set of samples. All instrumental parameters are carefully monitored, especially the helium carrier gas head pressure which is held at 30 kPa at all times. Oven temperature is controlled automatically.
9. An internal standard is added to each water and sediment sample to determine percent recoveries. Initially, malathion was selected due to its similarity to parathion and because of a very desirable, non-interfering retention time. However, we soon found that the mercury clean-up procedure removed malathion due to its -S- bonding.
10. A control site is used against which to compare analytical results from experimental sites. The control site used for the first data set was Thomas Creek (Site C1, Figure 1). Since this creek's flow is intermittent and may not be representative of uncontaminated Padilla Bay region fresh surface waters, we chose a second control site on the Samish River (Site C2, Figure 1).

NOTE: See Figure 1 for Sampling Sites

CODESET

Sample Sites and Sample Codes. Padilla Bay
Agricultural Pesticide Study, 1986-1988.

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<u>Code</u>	<u>Description</u>
J	Joe Leary Slough sites: J1-J5
B	Big Indian Slough sites: B1-B3
L	Little Indian Slough sites: L1 & L2
BI or BIN	Bay sites BI1 & BI2 near the mouth of the Indian sloughs
BJ or BJL	Bay sites 1 & 2 near the mouth of Joe Leary slough
BL	Site of confluence of Big & Little Indian sloughs
FB	Field blank
C1	Thomas Creek Control site
C2	Samish River Control site
w	water sample
s	sediment sample
d	duplicate sample

<u>PESTICIDE</u>	<u>DETECTION LIMITS</u>	
	<u>WATER, ug/L</u> <u>(ppb)</u>	<u>SEDIMENTS, ug/Kg</u> <u>(ppb)</u>
TRIFLURALIN	0.02	1.9
SIMIZINE	0.63	62.7
ATRAZINE	0.49	48.8
DIAZINON	0.18	18.1
CHLORTHALONIL	0.15	15.4
METHAMIDOPHOS	10.8	1080
METHYL PARATHION	0.03	2.8
PARATHION	0.06	6.4
DICAMBA*	6.10	610
2,4-D*	0.10	4.8
PCNB	0.01	0.93
DINOSEB	0.11	11.3
METRIBUZIN	0.01	1.34
TERBUTRYN	5.76	576

* The phenoxyacid herbicides were analyzed as their respective methyl esters but reported as the free acid.

