88-e13

Segment No. 09-17-01
WA-17-0030

PORT TOWNSEND PEN-REARED SALMON MORTALITY: RESULTS OF SCREENING SURVEYS FOR TOXIC CHEMICALS IN TISSUES, SEDIMENTS, SEAWATER, AND EFFLUENTS OCTOBER - DECEMBER 1987

Art Johnson

Washington State Department of Ecology
Water Quality Investigations Section
Olympia, Washington 98504-6811

June 1988

ACKNOWLEDGMENTS

Blue Water Farm, Scan Am Fish Farms, and the National Marine Fisheries Service generously provided samples of Atlantic salmon for chemical analysis.

Drs. Michael Guerin, Bruce Tomkins, and Bob Ross of the Oak Ridge National Laboratory analyzed for munitions chemicals at no cost to Ecology.

Raleigh Farlow, EPA, gave valuable technical advice on sample analysis and quality assurance. Dave Terpening, also EPA, assisted with collection of salmon tissues and arranged for dioxin/furan analysis through an EPA contract laboratory.

Dale Norton, Ecology Water Quality Investigations Section, assisted with the collection of sediments samples and inspection of NUWES.

SUMMARY

Commercial attempts to pen-rear Atlantic salmon in Puget Sound's Port Townsend Bay in 1986 and 1987 experienced cumulative mortality of over 90 percent. Extensive examinations by pathologists at the Battelle Seqium laboratory led to the conclusion the fish died from liver disease caused by chronic exposure to a water-borne toxic chemical. Ecology responded to this potentially significant water quality problem by conducting chemical analyses of salmon tissues, bottom sediments, and seawater samples from Port Townsend Bay, and effluent samples from the Port Townsend Paper Corporation pulp mill at Glen Cove and the Naval Undersea Warfare Engineering Station sewage treatment plant on Indian Island. Chemicals analyzed included the EPA priority pollutants/hazardous substances list compounds, tetrachloro-dibenzodioxin, tetrachlorodibenzofuran, resin acids, munitions chemicals, and selected trace elements.

Results showed concentrations of metals, other trace elements, and chlorinated compounds in the tissues of affected fish collected from pens at Crane Point in Port Townsend Bay were similar to those in the same species reared at Ediz Hook, Cypress Island and Manchester. Nickel, gallium, selenium and copper concentrations were moderately elevated in the Crane Point fish. The significance of this finding is unknown. The level of chemical contamination in Port Townsend Bay sediments and seawater was generally low; no evidence was found of the unusual occurrence of toxic chemicals. Chemical analyses and limited bioassays of the pulp mill effluent and nearby sediments did not implicate the mill as the source of the problem. The Navy facility did not appear to be a significant source of contaminants to the bay.

Field studies are being initiated to determine if toxic conditions persist in the Port Townsend Bay and if other species are susceptible. Because the pulp mill is the only large discharge to the bay (approximately 12 million gallons per day) and because of reports in the literature that show liver damage resulting from laboratory exposure of rainbow trout to unbleached Kraft effluent constituents, there remains a concern that the mill effluent and liver disease could be related. Therefore, a long-term bioassay of the mill effluent will be conducted with Atlantic salmon to determine if this exposure produces the type of liver lesions observed in 1986 and 1987.

INTRODUCTION

On September 29, 1987, Water Quality Investigations received a request from John Pitts, Department of Agriculture, to investigate the cause of unusual mortality among Atlantic salmon (*Salmo salar*) being reared by Sea Farm Washington in Port Townsend Bay, Puget Sound. A meeting was subsequently held October 8 at the Ecology Southwest Regional Office where pathologist Michael Kent of the Battelle Marine Research Laboratory at Sequim Bay gave the following assessment of the problem (summarized from Kent [1987 draft]):

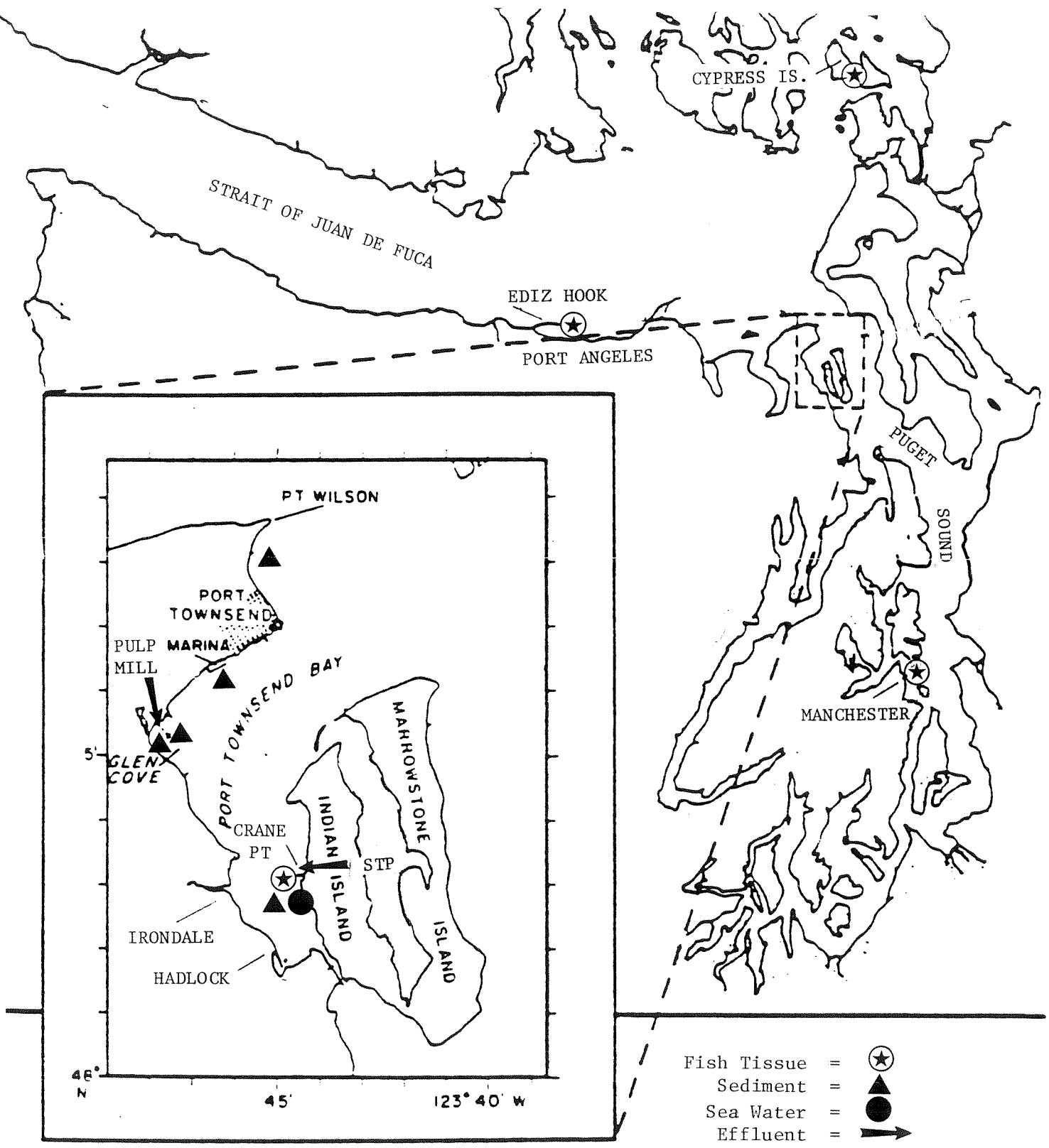
> One thousand Atlantic salmon smolts were introduced to commercial pens at Glen Cove on the west side of Port Townsend Bay in June, 1986; one month later the same grower introduced 15,000 smolts to pens on the opposite side of the bay at Crane Point (see figure). Excessive mortality began within a few months. By December 1986 only 82 of the Glen Cove fish remained alive. These were transferred to Crane Point where 9,000 fish still survived. By March 1987 less than 5% of the original Crane Point stock was alive.
>
> Diseased fish were "typically...dark-colored, emaciated and lethargic. Livers of fish with severe lesions were often atrophied, unusually opaque and, often, yellow-orange in color. ... Approximately 2 months after seawater introduction prominent diffuse hydropic degeneration and pyknosis (squat in shape) of hepatocytes (liver cells) was observed in moribund fish. As the disease progressed the livers of affected fish exhibited multifocal areas of regenerating hepatocytes intermixed throughout a necrotic parenchyma (dead tissue). Nuclear pleomorphism (variable sized nuclei) and hepatic megalocytosis (giant cells) were prominent in surviving fish... The lesions were consistent with toxicopathic changes, and bacterial and viral examinations revealed no infectious agent associated with the liver damage."

Kent concluded the cause of death was liver disease brought on by chronic exposure to a water-borne toxic chemical. His diagnosis of a chemical etiology was independently confirmed by pathologists at the NOAA Northwest and Alaska Fisheries Center and University of California. Rearing practices, feed, and genetics were ruled out as the cause because smolts from the same hatchery reared under identical cultural practices by the same grower at Ediz Hook in Port Angeles have not developed a single case of the disease. Phytoplankton toxins were also considered to be an unlikely source of the problem because of the persistence of the toxic effect and fact that other successful Atlantic salmon culture operations in Puget Sound and British Columbia routinely experience plankton blooms.

In 1987 the grower made a second attempt to raise Atlantic salmon in Port Townsend Bay, but at the Crane Point site only. Mortality and pathology repeated the pattern observed in 1986 (Kent, personal communication). That year Blue Water Farm began rearing coho salmon (*Oncorhynchus kisutch*) at pens in front of the Port Townsend marina. These fish did not develop the disease.

There is earlier evidence that the south end of Port Townsend Bay may be unsuited to salmon culture. DomSea placed the first pens at Crane Point in 1979 and stocked them with coho. Although some fish were ultimately harvested, the site was abandoned in 1980 due, in part, to plankton blooms (*Chaetoceros*), but also business decisions unrelated to fish health (G. Marquardt, personal communication). Scan Am Fish Farms then occupied the site between 1983 and 1985, attempting to raise coho and Donaldson trout (*Salmo gairdneri* hybrid). Although the mortality encountered was not of the magnitude experienced in 1986/1987, it was sufficient to cause the grower to quit the site. Low survival was attributed to poor water circulation (A. Landeroth, personal communication).

Potential sources of toxic chemicals to Port Townsend Bay, other than urban runoff and boat traffic typical of many Puget Sound embayments, are few. Two marine discharges are permitted under the National Pollution Discharge Elimination System (NPDES); the Port Townsend Paper Corporation pulp mill at Glen Cove



Location of samples collected for Port Townsend salmon mortality investigation.

(approximately 12 million gallons per day) and a sewage treatment plant discharge at Crane Point from the Naval Undersea Warfare Engineering Station (NUWES) on Indian Island (approximately 0.02 million gallons per day). The Port Townsend sewage treatment plant discharges to the Strait of Juan de Fuca.

The Navy stores munitions on Indian Island and has a demilling facility for removing the explosives from bombs. Historically there was ship building at Hadlock in the 1920s and an iron ore works at Irondale.

The occurrence of chemical contaminants and biological abnormalities in Port Townsend Bay has received little attention. Crecelius (1975) analyzed metals in a sediment sample east of Glen Cove; concentrations were not elevated. Until the present surveys, no data were available on organic toxicants in the bay. Kent (personal communication) examined the livers of several dozen Port Townsend Bay flatfish in 1987 and found no lesions.

Ecology did oyster larvae bioassays on a series of water samples collected from the bay in August 1985 as part of a receiving water survey of the pulp mill (Stinson, 1986). Percent mortality and abnormal larvae were elevated relative to control samples, with surface waters being more toxic than bottom waters. The reason for the adverse effects was not determined.

METHODS

In response to what appeared to be a potentially significant water quality problem in Port Townsend Bay, Ecology conducted a series of field surveys between October and December 1987 (Table 1) in an effort to identify the chemical suspected of causing the liver disease.

Sampling

Atlantic salmon were collected October 21 from among the surviving stock of 1987 fish at Crane Point and healthy fish at Ediz Hook. These fish had been put in the pens as smolts in May (Crane Point) and June (Ediz Hook) of 1987. Specimens were individually wrapped in aluminum foil, put in plastic bags, and placed on ice. Bile samples from fish being sacrificed for concurrent histopathological[1] examination by Battelle were collected in glass vials and iced. Samples were transported to the Ecology/EPA Environmental Laboratory in Manchester, Washington, the day following collection.

1 Histopathology refers to the application of light or electron microscopy to the study of tissue defects.

Table 1. Ecology screening surveys for toxic chemicals in Port Townsend Bay, October - December 1987

Survey Description	Date
Fish tissue collection at Crane Point and Ediz Hook pens	October 21, 1987
Bottom sediment samples from Port Townsend Bay	November 30, 1987
24-hour composite water sample from Crane Point pens	November 30 - December 1, 1987
Class II/biomonitoring inspection of Port Townsend Paper Corp.	December 1-2 , 1987
Inspection of Navy Indian Island facility (with EPA)	December 1, 1987
Fish tissue collections at Cypress Island and Manchester pens (reference areas)	December 16-17, 1987

Bile samples were frozen at Manchester on arrival. The fish were dissected on foil using stainless steel instruments cleaned with Liqui-Nox detergent and rinsed with dilute nitric acid, acetone, and de-ionized water. Muscle (skinless fillets) and liver tissues were composited from individual fish, homogenized in Waring blenders, and split into subsamples. Sample containers were glass jars with teflon-lined lids which had been pre-cleaned with 1:1 nitric acid, de-ionized water, and pesticide-grade methylene chloride (I-Chem, Hayward, CA). The tissues were frozen pending analysis.

Preliminary results from metals analysis of the fish tissues indicated a need for data on background concentrations of metals in Atlantic salmon. Therefore, fish of similar age were collected from commercial pens at Cypress Island in the San Juans and National Marine Fisheries Service pens at Manchester on December 16-17. Tissue samples were prepared as described above.

Port Townsend Bay bottom sediments were collected November 30 at four sites--one sample adjacent to the Crane Point pens, two samples in Glen Cove at the pulp mill effluent diffuser, and one sample each at the Port Townsend marina pens and southwest of Point Wilson. The Point Wilson site was considered a reference area removed from the influence of local sources in Port Townsend Bay. Samples were composites of two or three grabs collected with a van Veen sampler. The top 2 cm surface layer from the grabs was pooled, homogenized by stirring with stainless steel spoons in stainless steel beakers, and split into subsamples. Sample containers and cleaning procedures were as described above. The samples were transported on ice to the Manchester laboratory and refrigerated.

Seawater samples were collected at the Crane Point pens between November 30 and December 1. Most analyses were done on a 24-hour composite pumped from a depth of about 8 feet using an ISCO automatic sampler set to collect a 200 mL sample at 15-minute intervals. The sample was kept on ice during the compositing period. Volatile organics samples were surface grabs. The ISCO sampler had been previously cleaned by sequential rinses with detergent, de-ionized water, dilute nitric acid, de-ionized water, methylene chloride, and acetone. The seawater and effluent samples described below were transported on ice to the Manchester laboratory.

The pulp mill effluent was sampled December 1-2. Methods again consisted of a 24-hour ISCO composite with volatiles samples taken as grabs. The mill inspection was conducted by Don Kjosness, Industrial Section, and Don Reif, Water Quality Investigations. Kjosness has completed a report on the mill's compliance with NPDES permit limits (Kjosness, 1988). Reif will report the complete results of the inspection. The present report is confined to results of toxic chemical analyses and bioassays of the effluent.

Inspection of the naval station on Indian Island was done on December 1 with the assistance of Dave Ragsdale, EPA. Samples of the sewage treatment plant effluent, the only discharge permitted from this facility to Port Townsend Bay, were collected as

grabs. A tour of the island was given by NUWES personnel Thomas Cox, utility systems supervisor; Ronald Smith, engineering technician; and Donald Morris, fisheries biologist. Ecology also made a brief survey of the west shoreline of the island by boat.

Analysis

The chemical analyses and bioassays done for the Port Townsend surveys are summarized in Tables 2 and 3. Target chemicals included the EPA priority pollutant metals and other selected trace elements (22 elements); EPA organic priority pollutants and hazardous substances list compounds (approximately 125 compounds); resin acids and phenolic compounds characteristic of pulp mill effluents (16 compounds); and munitions chemicals (TNT and five other nitrogen-containing explosives or degradation products). Appendix A lists all organic compounds analyzed; Appendix B shows the detection limits achieved for these compounds.

The pulp mill effluent and Crane Point seawater samples were tested for mutagenic activity by the Ames test which measures reverse mutations of histidine-requiring bacteria (*Salmonella typhimurium*) to histidine independence as an indicator of genetic damage. Following standard Ecology procedures for industrial inspections, a suite of bioassays was performed on the pulp mill effluent and selected sediment samples. Test organisms exposed to the effluent were rainbow trout (*Salmo gairdneri*), oyster larvae (*Crassostrea gigas*) and a luminescent bacteria (*Photobacterium phosphoreum*). The last assay (trade name Microtox) measures changes in the bacteria's light output. Sediment samples at the pulp mill outfall and Point Wilson reference station were bioassayed using the amphipod *Rhepoxynius abronius*.

For some samples the decision to analyze for a specific chemical or group of chemicals was based on the environmental behavior of the chemical in question. Fish muscle and liver analyses, for example, were limited to selected organochlorine compounds and trace elements because other target chemicals do not accumulate significantly in these tissues. On the other hand, broad spectrum analyses were done for effluent and sediment samples collected in conjunction with the pulp mill inspection as this is standard Ecology practice.

Because of the cost of screening for a wide range of chemicals, sufficient numbers of samples could not be analyzed to determine if concentration differences between samples were statistically significant. Rather, the approach was to collect and analyze key samples--as composites and duplicates where possible--and examine the data for occurrence of unusual chemicals, substantial elevations above concentrations in reference areas, and concentration gradients in the sediments.

The accuracy of the data was assessed by analyzing standard reference materials, spiked samples, and blanks. Precision (agreement among repeated analysis of the same sample) was evaluated by analyzing duplicates. The accuracy and precision data are presented with the results. Chemical concentrations are expressed in terms of parts

Table 2. Chemicals analyzed for Port Townsend salmon mortality investigation, October-December, 1987.

Collection Site	Crane Point & Ediz Hook Pens	Cypress Is. & Manchester Pens	Crane Point & Ediz Hook Pens	Glen Cove Pulp Mill Outfall	Crane Point Pens	Port Townsend Marina Pens	Point Wilson	Crane Point Pens	Pulp Mill	NUWES
Sample Type	Tissue	Tissue	Bile	Sediment	Sediment	Sediment	Sediment	Seawater	Effluent	Effluent
Sample No.	8250-2854	8280;8281	--	8230;8231	8233	8234	8235	8232[a]	8240	8236
EPA Priority Pollutants/HSL Compounds:										
volatiles				+			+	+	+	+
semivolatiles				+	+	+	+	+	+	+
PCBs/OC pesticides	+			+	+	+	+	+	+	+
2,3,7,8-TCDD/TCDF	+									
metals[b]	+	+		+	+	+	+	+	+	+
cyanide									+	+
Other Toxicants:										
resin acids/guaiacols[c]/ catechols[c]			+	+	+	+	+	+	+	
munitions chemicals				+[d]	+	+[d]	+[d]	+		+
Bioassays:										
Ames test								+	+	
rainbow trout									+	
Microtox									+	
oyster larvae									+	
amphipod				+			+			

[a] replicate sample 8238 analyzed for volatiles only
[b] varies with sample type, see results
[c] recovery outside quality control limits; see text
[d] scan only, see Appendix B

Table 3. Analytical methods for Port Townsend salmon mortality investigation, October-December 1987.

Analysis	Method	Reference	Laboratory
	TISSUE		
As, Br, Ca, Cl, Cr, Cu, Ga, Fe, Pb, Mn, Ni, P, K, Rb, Se, S, Ag, As, Cd, Hg, Sb, Zn	energy dispersive x-ray fluorescence	Nielson and Sanders (1983)	Batelle Marine Research Laboratory Sequim, WA
Sn	atomic adsorption spectroscopy	in-house	Battelle Marine Research Laboratory Sequim, WA
organochlorine pesticides/PCBs	gas chromatography/electron capture detector, method 8080	EPA (1984b)	Analytical Resources Inc. Seattle, WA
dioxin/furan	gas chromatography/electron capture detector	EPA Duluth Laboratory method	Wright State, Dayton, OH
resin acid metabolites	gas chromatography/flame ionization detector/mass spectroscopy	Oikari, et al. (1982)	Battelle Marine Research Laboratory Sequim, WA
	SEDIMENT		
Ag, As, Be, Cd, Cr, Cu, Hg, Ni, Pb, Se, Sb, Tl, Zn	atomic adsorption spectroscopy	EPA Contract Lab. Program SOW No. 785, July 1985	Ecology/EPA Environmental Laboratory Manchester, WA
organochlorine pesticides/PCBs	gas chromatography/electron capture, method 608	EPA (1984b)	Analytical Resources Inc. Seattle, WA
acid-base/neutrals	gas chromatography/mass spectroscopy, method 625	"	"
volatiles	purge and trap/gas chromatography/ mass spectroscopy, method 624	"	"
resin acids, guaiacols, catechols	gas chromatography/mass spectroscopy	NCASI (1986)	Ecology/EPA Environmental Laboratory Manchester, WA
munitions chemicals	high pressure liquid chromatography	in-house	Oak Ridge National Laboratory Oak Ridge, TN
bioassay	Rhepoxynius abronius (amphipod) 10-day exposure	Swartz et al. (1985)	EVS Consultants, Seattle, WA
	WATER		
As, Cd, Cr, Hg, Pb	atomic adsorption spectroscopy	EPA Contract Lab Program, SOW No. 785, July 1985	Ecology/EPA Environmental Laboratory Manchester, WA
Ag, Be, Cu, Ni, Sb, Se, Tl, Zn	atomic adsorption spectroscopy	in-house	Battelle Marine Research Laboratory Sequim, WA
organochlorine pesticides/PCBs	gas chromatography/electron capture detector, method 608	EPA (1984a)	Analytical Resources, Inc. Seattle, WA
acid-base/neutrals	gas chromatography/mass spectroscopy, method 625	EPA (1984a)	Analytical Resources, Inc. Seattle, WA
volatiles	purge and trap/gas chromatography/ mass spectroscopy, method 624	EPA (1984a)	Analytical Resources, Inc. Seattle, WA
resin acids, guaiacols, catechols		NCASI (1986)	Ecology/EPA Environmental Laboratory Manchester, WA
munitions chemicals	high pressure liquid chromatography	in-house	Oak Ridge National Laboratory Oak Ridge, TN
cyanide	titrimetric, photometric	EPA (1979)	Ecology/EPA Environmental Laboratory Manchester, WA
bioassay	Salmonella mutagenicity assay	Ames, et al. (1975)	British Columbia Cancer Research Centre Carcinogen Testing Laboratory Vancouver, B.C.
"	Salmo gairdneri (rainbow trout) 4-day exposure	Ecology (1980)	Ecology/EPA Environmental Laboratory Manchester, WA
"	Microtox	Beckman operating manual	Ecology/EPA Environmental Laboratory
"	Crassostrea gigas (oyster larvae) 48-hour exposure, method E 724-80	ASTM (1980)	EVS Consultants, Seattle, WA

per million (ug/g) or parts per billion (ng/g or ug/L). Where a chemical was analyzed but not detected, the limit of detection is given accompanied by a "less than" (<) sign.

RESULTS AND DISCUSSION

Salmon Tissues

Table 4 shows the concentrations of metals and other trace elements in the Crane Point and Ediz Hook salmon tissues. The muscle samples were composite fillets from seven fish each; liver samples were composites from 12 fish (Crane Point) and 15 fish (Ediz Hook). With few exceptions, metals and other trace element concentrations in the Crane Point fish were very much like those at Ediz Hook. Analyses of duplicate samples of Crane Point muscle tissue were in close agreement except for lead. The between-sample differences in lead concentrations more likely reflect the amount of scales, skin, or bone fragments in the samples than environmental exposure. Lead is not generally considered to accumulate in the axial muscle of fish (Schmitt and Finger, 1987). Liver tissues from Crane Point and Ediz Hook had equal amounts of lead.

One metal, nickel, appeared substantially elevated (a factor of 5) in Crane Point muscle tissue. This result is unexpected as nickel has a low potential for bioaccumulation (Callahan, *et al.*, 1979). The significance of this finding is uncertain. Liver tissue from both sites showed no difference in nickel concentrations and high concentrations of nickel were not observed in Crane Point sediments (Table 8) or seawater (Table 12).

Gallium, selenium, and copper were marginally higher in the livers of the Crane Point fish (factors of 1.8, 1.7, and 1.9, respectively). Concentrations of these three elements in livers of the Ediz Hook fish were similar to those reared at Cypress Island and Manchester (Table 5), suggesting the Crane Point fish may have had above-normal levels of these elements. The aquatic toxicity of gallium has not been assessed, but selenium and copper are known to be toxic to fish and are generally considered to be chemicals of potential concern in the marine environment (Konasewich, *et al.*, 1982; O'Connor and Stanford, 1979). Selenium and copper are also, however, required as essential trace elements (Mertz, 1981). The fact that none of these elements were elevated in the sediment or water samples at Crane Point, coupled with the relatively small differences between Crane Point concentrations and those observed at Ediz Hook and reference areas makes the significance of this finding questionable.

Organochlorine compounds detected in the salmon tissues are shown in Table 6. Low and comparable concentrations of DDE and DDD (metabolites of DDT) and PCBs were measured in fish from both Crane Point and Ediz Hook. These are widespread contaminants of the marine environment. As shown in Table 7, the concentrations observed are in the middle to lower end of the range reported in free-living Puget Sound salmon. The U.S. Food and Drug Administration allows up to 5,000 ng/g and 2,000 ng/g of total DDT compounds and PCBs, respectively, in fish marketed commercially (FDA, 1985).

Table 4. Trace elements in Crane Point and Ediz Hook pen-reared Atlantic salmon collected October 21, 1987 (ug/g, wet).

Sample Type	Muscle			Liver		NRCC Reference Material Dogfish Liver Tissue - 1		
Collection Site	Ediz Hook	Crane Point	Crane Point	Ediz Hook	Crane Point	Measured Value		Certified Value
Sample No.	8250	8252	8253 (dup.)	8251	8254	Rep #1	Rep #2	
No. Individuals	7	7	7	15	12			
Length Range (mm)[a]	208-209	260-325	260-325	137-309	260-325			
*silver	0.001	0.001	0.001	0.166	0.158	1.01	0.95	None
tin	0.008	<0.005	<0.005	0.042	0.007	0.052	0.053	None
*cadmium	<0.01	0.03	<0.01	<0.01	<0.01	4.14	4.62	4.18 ± 0.28
*antimony	0.01	0.01	0.01	0.01	0.01	0.06	0.06	None
*chromium	0.02	<0.01	<0.01	0.01	0.01	0.34	0.31	0.4 ± 0.07
*mercury	0.038	0.035	0.037	0.035	0.014	0.226	0.223	0.225 ± 0.037
gallium	0.14	0.12	0.12	0.98	1.75	0.53	0.54	None
*lead	0.06	0.56	0.13	0.02	0.02	1.65	1.01	1.36 ± 0.29
*selenium	0.30	0.30	0.23	3.28	5.52	7.38	7.60	7.34 ± 0.42
manganese	0.53	0.63	0.68	1.92	0.67	9.1	8.5	8.72 ± 0.53
*arsenic	0.7	0.7	0.8	0.7	0.9	9.9	10.6	10.1 ± 1.4
*nickel	0.2	0.99	1.04	0.01	0.01	0.25	0.21	0.26 ± 0.06
*copper	1.0	0.8	0.9	154.1	288.0	20.4	21.0	20.8 ± 1.2
rubidium	1.13	1.03	1.15	1.66	1.75	3.18	3.44	None
*zinc	4.7	3.6	3.7	24.9	30.3	90.4	90.3	92.5 ± 2.3
bromine	5	6	6	22	22	23	22	None
iron	6	4	5	232	169	810	857	712 ± 48
calcium	38	92	105	120	71	665	614	None
chlorine	555	640	728	2139	1990	6810	7060	None
sulphur	2277	2474	2654	2723	2951	12380	13100	None
phosphorus	2981	3298	3691	4322	3731	10400	10100	None
potassium	5529	5699	5973	4730	3746	10350	10480	None
% dry weight	24.04	24.25	24.28	24.01	21.08			

[a] fork length
*EPA priority pollutant metals
< = not detected at detection limit shown

Table 5. Trace elements in livers of Cypress Island and Manchester pen-reared Atlantic salmon collected December 16-17, 1987, compared to results for Crane Point and Ediz Hook (ug/g, wet).

Collection Site	Cypress Island	Cypress Island	Manchester	Ediz Hook	Crane Point
Sample No.	8280	8280 (dupl.)	8281	8251	8254
No. Individuals[a]	5	5	5	15	12
Length Range (mm)	373-410	373-410	350-409	137-309	260-325
*chromium	<2.9	<3.0	<2.7	<0.01	<0.01
gallium	1.13	0.85	0.80	0.98	1.75
*lead	<1.5	<1.2	<1.4	0.02	0.02
*selenium	3.77	3.80	2.79	3.28	5.52
manganese	1.30	1.28	0.79	1.92	0.67
*arsenic	0.61	0.67	0.38	0.7	0.9
*nickel	<0.77	<0.84	<0.72	0.01	0.01
*copper	173	173	126	154.1	288.0
rubidium	1.44	1.35	1.98	1.66	1.75
bromine	19.3	19.2	22.7	22	22
iron	104	102	74.1	232	169
calcium	54.7	56.2	91.6	120	71
chlorine	1630	1612	1776	2139	1990
sulphur	2893	2823	2764	2723	2951
phosphorus	3562	3601	3774	432	3731
potassium	3440	3388	3801	4730	3746
% dry weight	26.04	26.04	24.7	24.01	21.08

[a] fork length

*EPA priority pollutant metals

< = not detected at detection limit shown

Table 6. Organochlorines detected in Crane Point and Ediz Hook pen-reared Atlantic salmon collected October 21, 1987 (ng/g, wet).

Sample Type	Muscle				Liver	
Collection Site	Ediz Hook	Ediz Hook	Crane Point	Crane Point	Ediz Hook	Crane Point
Sample No.	8250	8250	8252	8253	8251	8254
No. Individuals[a]	7	7	7	7	15	12
Length Range (mm)	208-309	208-309	260-325	260-325	137-309	260-325
Pesticides/PCBs:						
p,p'-DDE	15	24	17	16	24	<60
p,p'-DDD	4	5	7	6	<20	<60
p,p'-DDT	<2	<2	<2	<2	<20	<60
PCB-1260	30B	35B	40B	35B	<200	<600
Surrogate spike recoveries:						
dibutylchlorendate	51%	78%	75%	64%	71%	69%
Dioxin/Furan:						
2,3,7,8-TCDD	NA[b]	NA	<0.0008	<0.0005	NA	<0.0005
2,3,7,8-TCDF	NA	NA	0.0013	0.0014	NA	0.0019
Matrix spike recoveries:						
2,3,7,8-TCDD	--	--	73%	--	--	--
2,3,7,8-TCDF	--	--	119%	--	--	--
% dry weight	23.7	--	24.1	23.8	23.9	20.3

[a] fork length

[b] not analyzed

< = not detected at detection limit shown

B = also detected in method blank

Table 7. Summary of DDT compounds and PCB concentrations measured in Puget Sound salmonids compared to results for Crane Point and Ediz Hook Atlantic salmon (ng/g, wet).

Species	Date	Location	Number of Samples	Concentration Range p,p'-DDE	p,p'-DDD	p,p'-DDT	PCBs	Reference
MUSCLE TISSUE								
Chinook and Coho	--	Elliott Bay	2	NA[a]	NA	NA	40-150[c]	Malins et al. (1982)
" " "	--	Commencement Bay	5	NA	NA	NA	22-57[c]	"
" " "	--	Reference areas	5	NA	NA	NA	29-100[c]	"
Chum	1973	"Puget Sound"	1 (composite)	6	ND[b]	ND	<40[d]	Stout and Beezhold (1981)
Pink	1973	"Puget Sound"	1 (composite)	9	ND	ND	<50[d]	"
Sockeye	1970	Ballard Locks	1 (composite)	15	10	15	NA[d]	"
Sockeye	1973	"Puget Sound"	1 (composite)	7	ND	ND	<40[d]	"
Chinook	1987	N. Puget Sound	9	4-42	<1-12	<1-10 est.	16-140[d]	EPA (unpublished data)
"	"	Everett Harbor	1	17	8	5	99[d]	"
"	"	Elliott Bay	9	1-34	<1-31	<1-18	29-170[d]	"
"	"	Commencement Bay	5	7-51	<1-23	<1-22	57-210[d]	"
Coho	"	Commencement Bay	2	9-29	3-22	4-28	69-93[d]	"
Atlantic Salmon	1987	Crane Point	2 (composites)	16-17	6-7	<2	35-40[e]	present study
" "	"	Ediz Hook	2 (composites)	15-24	4-5	<2	30-35[e]	"
LIVER TISSUE								
Chinook and Coho	--	Elliott Bay	2	NA	NA	NA	99-160[c]	Malins et al. (1982)
" " "	--	Commencement Bay	5	NA	NA	NA	48-190[c]	"
" " "	--	Reference areas	5	NA	NA	NA	41-150[c]	"
Chinook (juveniles)	1986	Duwamish Waterway	3 (composites)	NA	NA	NA	2000-3100[c]	McCain et al. (in prep)
" "	"	Nisqually River	2 (composites)	NA	NA	NA	780-1030[c]	"
Atlantic Salmon	1987	Crane Point	1 (composite)	<60	<60	<60	<600[e]	present study
" "	"	Ediz Hook	1 (composite)	24	<20	<20	<200[e]	"

[a] not analyzed

[b] not detected

[c] total PCBs

[d] PCB-1254

[e] PCB-1260

< = not detected at detection limit shown

2,3,7,8-Tetrachlorodibenzodioxin (TCDD) and a related compound, 2,3,7,8-tetrachlorodibenzofuran (TCDF), were analyzed in the Crane Point salmon only. TCDD was not detected, but extremely low concentrations of TCDF (0.0013-0.0019 ng/g; i.e., approximately 1 to 2 parts per trillion) were measured in both muscle and liver. Chlorinated furans are contaminants in PCB and polychlorinated phenols manufacture and produced in their combustion (Konasewich, *et al.*, 1982). TCDF and lower chlorinated dibenzofurans have been found, but not quantified, in sediment samples from central Puget Sound (Malins, *et al.*, 1980).

Concentrations of TCDF in the low parts trillion range are unlikely to have caused mortality in the Crane Point fish. TCDF was commonly detected in fish analyzed for EPA's National Dioxin Study; high concentrations are considered to be in the range of 0.02 - 0.05 ng/g or more (R. Hanson, personal communication). Recent analysis of limited numbers of Washington State freshwater and estuarine fish by EPA found 2,3,7,8-TCDF concentrations ranging from 0.0026 - 0.042 ng/g, the higher concentrations being in whole fish samples. TCDF has been shown to be less toxic to rainbow trout than TCDD (Mehrle, 1987) and is also considered less toxic than TCDD for purposes of human health risk assessment (EPA, 1987). FDA has advised that TCDD concentrations less than 25 parts per trillion (0.025 ng/g) in fish are little cause for human health concern (FDA, 1981).

In an effort to determine if the Crane Point location was influenced by the pulp mill effluent plume, bile samples from Crane Point and Ediz Hook Atlantic salmon and from coho at the Port Townsend marina pens were analyzed for the presence of resin acids and their metabolites. Resin acids, derived from oleoresin in softwood trees, are discharged in effluents from both chemical and mechanical pulping processes (Keith, 1976; Oikari and Holmbom, 1986). While resin acids tend not to accumulate in fish tissue, these compounds are transformed in the liver and excreted in the bile at concentrations up to 10,000 times the level in the ambient water (Oikari and Kunnamo--ojala, 1987).

Four resin acids--abietic, pimeric, dehydroabietic and isopimeric--were analyzed in salmon bile, but could not be detected in any of the samples. Recovery of internal standards, heptadecanoic and tricosanoic acids, averaged 96 percent and 74 percent, respectively. (See also results of sediment analysis for resin acids.)

Bottom Sediments

The metals concentrations measured in Port Townsend Bay sediments are shown in Table 8. Analysis of a standard reference material, National Bureau of Standards River Sediment, indicated the method did not recover antimony; other analyses appeared accurate.

Sediments at the Crane Point pens were not elevated in metals concentrations compared to sediments in other parts of the bay. Concentrations at Crane Point were similar to those at Point Wilson--considered a reference area for purposes of the

Table 8. Metals analysis of Port Townsend Bay sediment samples collected November 30, 1987 (ug/g, dry).

Location	Crane Point Pens	Glen Cove Pulp Mill Outfall		Port Townsend Marina Pens	Point Wilson	NBS Reference Material River Sediment 1645		
Depth (ft. @ MLLW)	80	47	49	48	45	Measured Value		Certified
Sample Number	8233	8230	8231	8234	8235	Rep. #1	Rep. #2	Value
% total organic carbon	1.1	4.9	5.2	2.6	1.8			
% fines	22.7	48.9	73.6	84.1	15.0			
% dry weight	52.6	36.0	35.9	38.3	73.2			
antimony	(<0.1)	(<0.1)	(<0.1)	(<0.1)	(<0.1)	0.8	0.5	51[a]
thallium	<0.1	<0.1	<0.1	<0.1	<0.1	0.6	0.9	1.44
cadmium	<0.02	0.47	0.13	<0.02	<0.02	11.6	10.8	10.2
selenium	<0.1	0.6	0.7	0.4	<0.1	1.2	0.9	1.5[a]
beryllium	0.02	0.12	0.18	0.23	0.07	0.68	0.55	None
mercury	0.02	0.05	0.04	0.02	0.02	NA[b]	NA	None
silver	0.09	0.21	0.17	0.13	<0.02	1.49	1.42	None
arsenic	3.0	5.9	5.6	5.4	2.9	52.5	54.5	66[a]
lead	4.6	13.8	13.5	14.2	0.5	679	653	714
copper	16.7	41.5	45.5	38.1	10.8	107	112	109
nickel	35.4	32.8	31.1	34.3	40.8	31.4	31.1	45.8
chromium	38.4	30.3	31.8	31.2	31.4	17000	17000	None
zinc	44.9	76.8	87.4	97.6	36.6	1600	1610	None

( ) reference material results indicate analysis not accurate

[a] not certified

[b] not analyzed

< = not detected at detection limit shown

Table 9. Acid-base/neutral compounds detected in Port Townsend Bay sediment samples collected November 30, 1987 (ng/g, dry).

Location	Crane Point Pens	Glen Cove Pulp Mill Outfall		Port Townsend Marina Pens	Point Wilson
Depth (ft. @ MLLW)	80	47	49	48	45
Sample No.	8233	8230	8231	8234	8235
% total organic carbon	1.1	4.9	5.2	2.6	1.8
% fines[a]	22.7	48.9	73.6	84.1	15.0
% dry weight	52.6	36.0	35.9	38.3	73.2
Polyaromatic Hydrocarbons:					
phenanthrene	43 est.	120	175	80 est.	15 est.
anthracene	<20	48 est.	57	<27	<14
fluoranthene	56 est.	230	420	150	22 est.
pyrene	58 est.	290	380	170	25 est.
benzo(a)anthracene	<50	210	180	120	<40
chrysene	<14	300	280	170	<10
benzo(b)fluoranthene & benzo(k)fluoranthene	44 est.	300	300	200 est.	17 est.
benzo(a)pyrene	<9.2	130	130	86	<6.7
Sum of PAH	250	1600	1900	990	110
Other Compounds:					
bis(2-ethylhexyl)phthalate	36 est.	42 est.	68 est.	47 est.	51 est.
phenol	<17	<50	<27	<24	320
4-methylphenol	<13	<35	<20	<18	48 est.
Surrogate Spike Recoveries:					
d5-nitrobenzene	82.8%	80.3%	52.9%	81.5%	84.8%
2-fluorobiphenyl	87.8%	92.6%	78.4%	91.0%	91.9%
d14-p-terphenyl	114%	135%	106%	109%	124%
d5-phenol	84.4%	88.3%	71.5%	83.6%	90.7%
2-fluorophenol	85.4%	86.2%	63.5%	85.8%	90.3%
2,4,6-tribromophenol	115%	98.4%	102%	111%	120%

[a] silt and clay (4-62 um)

< = not detected at detection limit shown

Table 10. Median concentrations of selected metals and organic compounds in Puget Sound sediments compared to Port Townsend sediments collected November 30, 1987.

	Urban Bays and Central Basin	Reference Areas	Port Townsend (range)
Metals (ug/g, dry):			
antimony	0.67	<0.1	
arsenic	11.0	5.6	5.9 - 2.9
cadmium	0.62	0.70	0.47 - <0.02
chromium	31.0	40.0	38.4 - 30.3
copper	55.0	33.0	45.5 - 10.8
lead	48.0	9.6	14.2 - 0.5
mercury	0.23	0.06	0.05 - 0.02
nickel	27.0	23.0	40.8 - 31.1
silver	0.38	0.23	0.21 - <0.02
zinc	100	76.0	87.4 - 36.6
Organics (ng/g, dry):			
phenanthrene	290	14	175 - 15
anthracene	110	6	57 - <14
fluoranthene	530	24	230 - 22
pyrene	630	22	380 - 25
benzo(a)anthracene	530	5.5	210 - <40
chrysene	420	10	300 - <10
benzo(b)fluoranthene & benzo(k)fluoranthene	710	18	300 - 17
benzo(a)pyrene	350	8.4	130 - <6.7
bis(2-ethylhexyl)phthalate	no data	no data	68 - 36
phenol	130	no data	326 - <17
4-methylphenol	180	no data	48 - <13

Source: Tetra Tech, Inc., 1986. User's Manual for Pollutants of Concern Matrix (August 15 Revision).

< = not detected at detection limit shown

Table 11. Resin acid analysis of Port Townsend Bay sediment samples collected November 30, 1987 (ng/g, dry).

Location	Crane Point Pens	Glen Cove Pulp Mill Outfall		Port Townsend Marina Pens	Point Wilson
Depth (ft @ MLLW)	80	47	49	48	45
Sample Number	8233	8230	8231	8234	8235
% total organic carbon	1.1	4.9	5.2	2.6	1.8
% fines[a]	22.7	48.9	73.6	84.1	15.0
% dry weight	52.6	36.0	35.9	38.3	73.2
Resin Acids:					
abietic acid	160 est.	4400	2400	1600	33 est.
neoabietic acid	(<270)	(<400)	(<450)	(<400)	(<200)
dehydroabietic acid	230 est.	3300	3200	1300	70 est.
dichlorodehydroabietic acid	<270	<400	<450	<400	<200
isopimaric acid	<200	1700	1700	660	<200
levopimaric acid	<270	<400	<450	<400	<200
sandaracopimaric acid	21 est.	400	970	160 est.	<200
palustric acid	<270	<400	<450	<400	<200
Sum of resin acids	410	9800	8300	3700	100
Surrogate Spike Recoveries:					
1,3-dihydroxybenzene-d6	- not recovered -				
2-naphthoic acid	51%	51%	54%	53%	51%
o-methylpodocarpic acid	52%	54%	52%	52%	48%

[a]silt and clay (4-62 um)

( ) matrix spike recovery low for this compound, see Appendix C.

< = not detected at detection limit shown

detection of 410 ng/g total resin acids at Crane Point suggests the mill discharge has influenced the sediments there--a distance of approximately three nautical miles. However, sediments at the Port Townsend marina pens again had higher levels of these compounds than Crane Point.

Crane Point Seawater

In light of the extensive metals analyses being done on fish tissue and sediments, metals analysis of the 24-hour composite water sample from the Crane Point pens was limited to antimony, thallium, selenium, beryllium, silver, copper, nickel, and zinc (Table 12). Concentrations of these metals were below method detection limits.[2]

Analyses for EPA organic priority pollutants/HSL compounds, resin acids, and munitions compounds failed to detect any of these contaminants in Crane Point seawater. However, two compounds not among the target analytes were tentatively identified in the acid-base/neutrals fraction--2-(2-butoxyethoxy) ethanol (estimated concentration 44 ug/L) and 2,4,6-trihydroxy-1,3,5-triazine (estimated concentration 2 ug/L). The former compound is a solvent (trade name poly-solv DB) with an aquatic toxicity of 100,000 - 10,000 ug/L (NIOSH, 1983). The latter compound is a selective herbicide--cyanuric acid. No toxicity data were found for this chemical, but herbicides in general exhibit toxic effects to fish at concentrations over 1,000 ug/L (Mayer and Ellersieck, 1986).

Results of Pulp Mill Inspection

The Port Townsend mill produces unbleached pulp by the Kraft process. The term unbleached signifies that chlorine and other bleaching agents are not used to remove residual lignin remaining after pulping. Effluent treatment consists of aerated lagoons.

Ecology's inspection showed the mill to be in compliance with NPDES permit limits for BOD (biochemical oxygen demand) and TSS (total suspended solids); the effluent

2 The Manchester laboratory initially reported a selenium concentration of 174 ug/L in this sample. Selenium was also detected in the pulp mill effluent and Navy sewage treatment plant discharge, 9 and 28 ug/L, respectively. Re-analysis of these samles by the Battelle Sequim laboratory found selenium to be below detection limits (0.85 - 0.77 ug/L) in all samples. A subsequent quality assurance review by Raleigh Farlow (EPA) and Steve Twiss (Ecology), which included analysis of Port Townsend Bay seawater samples collected by Dave Terpening (EPA) April 4, 1988, revealed possible positive interferences in Manchester's analysis of seawater for selenium and several other metals. Manchester data on these metals were therfore rejected.

Table 12. Metals analysis of Port Townsend water samples collected November 30 - December 2, 1987 (ug/L).

Location	Crane Point Pens	Pulp Mill Effluent	Navy Sanitary Discharge	NRCC Referrence Material North Atlantic Seawater (NASS-2)	
Date	Nov. 30 - Dec. 1	Dec 1 - 2	Dec 1		
Time	1230-1230[a]	1000-1000[a]	1045[b]	Measured	Certified
Sample Number	8232	8240	8236	Value	Value
antimony	<3.7	<2.4	<1.5	NA[c]	NA
thallium	<2.4	<0.55	<0.83	NA	NA
cadmium	NA	<0.2	<0.2	NA	NA
selenium	<0.85	<0.77	<0.77	NA	NA
beryllium	<0.007	<0.009	<0.007	NA	NA
mercury	NA	0.05	<0.05	NA	NA
silver	<0.04	0.02	<0.03	NA	NA
arsenic	NA	<1	10	NA	NA
lead	NA	<5	<5	NA	NA
copper	<0.31	6.3	9.0	<0.31	0.099 ± 0.010
nickel	<2.7	<4.9	<3.2	NA	NA
chromium	NA	<5	<5	NA	NA
zinc	<0.23	<0.04	10.1	0.23	0.159 + 0.028

[a] 24-hour composite

[b] grab

[c] not analyzed

< = not detected at detection limit shown

treatment system was found to be "operating satisfactorily" (Kjosness, 1988). The mill's technical superintendent has reported to Ecology that "the mill was operating in a completely normal mode" (Muehlethaler, 1988).

Metals analysis of the pulp mill effluent (Table 12) showed low concentrations of mercury (0.05 ug/L), silver (0.02 ug/L), and copper (6.3 ug/L) were detectable. Cyanide was also detected (8 ug/L). Cyanide is unstable in seawater (Crecelius, 1981). No organic compounds, including resin acids, guaiacols, and catechols, were detected in the mill effluent except for trace amounts of methylene chloride (estimated concentration 1.0-2.8 ug/L) and BEHP (estimated concentration 0.6-0.7 ug/L). These are both common laboratory contaminants encountered in low-level organics analysis of water. Methylene chloride was also used to clean the sampling equipment and was detected in blank samples.

Resin acids, quaiacols, and catechols are routinely reported to be present in Kraft mill effluents; resin acid concentrations are typically in the range of 50 - 2,500 ug/L (Keith, 1976; Oikari and Holmbom, 1986). Failure to detect resin acids in the Port Townsend effluent is most likely attributable to a shortcoming in the analysis of this particular sample, suggested by the fact that the surrogate spikes were not recovered (Appendix C). In the opinion of the analyst, this is probably the result of a poor extraction (D. Huntamer, personal communication). As previously noted, the quaiacols and catechols analyses done for all the Port Townsend survey samples failed to adequately recover these compounds. A second sample of the pulp mill effluent was collected April 5, 1988, and is currently being analyzed using an improved extraction procedure.

Results of NUWES Inspection

Discussion with NUWES personnel and a tour of Indian Island revealed no sources of contaminants to the bay of likely significance. The demilling facility is not large and is well inland. TNT is the only explosive reported to have been processed. Wastewater (red water) produced during removing explosives from bombs was reported to have been generated at this one site only and is recycled. The facility has no discharge to Port Townsend Bay. Systems for recovering and recycling wastewaters, including spills, appeared adequate.

The west shore of the island is largely undeveloped. Seepage to Port Townsend Bay from an old landfill at the north end of the island has been reported to contain elevated lead, chromium, and petroleum hydrocarbons (SCS Engineers, 1987 draft), but these are low-volume discharges. NUWES personnel indicated they were not aware of historical spills or unpermitted discharges to the bay, stormwater discharges from storage, and/or operations areas on the island.

Metals analysis of the sewage treatment plant effluent showed low concentrations of zinc (10 ug/L), arsenic (10 ug/L), and copper (9.0 ug/L) were detectable (Table 12). Organics analysis detected several haloforms (chloroform, bromodichloromethane, bromoform, and dibromochloromethane) in the range of 1.3 - 390 ug/L and toluene at

1.3 - 1.5 ug/L. These are common constituents of sewage treatment plant effluents (Feiler, 1980), the haloforms being by-products of chlorination. Munitions chemicals were not detected in the effluent.

Bioassays

Ames tests of the Crane Point seawater and pulp mill effluent samples were negative for mutagenic activity. This analysis was done, in part, because neoabietic acid, reported to be present in pulp mill effluents, has been shown to be mutagenic (Nestmann, *et al.*, 1980; Douglas, *et al.*, 1985). Analysis of extracts rather than whole water would improve the sensitivity of this test.

No mortalities occurred during 96-hour exposures of rainbow trout to a 65 percent concentration of the pulp mill effluent. There was also no evidence of toxicity in the Microtox assay of the effluent.

The mill effluent was toxic to Pacific oyster larvae. Oyster larvae are known to be particularly sensitive to pulp mill effluents (Cardwell, *et al.*, 1979 and references therein). The concentration of Port Townsend pulp mill effluent causing larval abnormalities in 50 percent of the test organisms (EC_{50}) in the 48-hour oyster larvae bioassay was estimated to be 3 percent (95 percent confidence limits were 2.2 - 4.6 percent). An LC_{50} (lethal concentration to 50 percent of the organisms) could not be calculated because the highest effluent concentration had less than 50 percent mortality.

Sediment bioassays were limited to 10-day exposures of the amphipod *Rhepoxynius abrnius* to samples from the pulp mill outfall and Point Wilson (five replicates per sample). The results (Table 13) showed a small but statistically significant difference in the number of amphipods surviving exposure to outfall sediments (means of 16.4 and 16.8 out of 20) relative to control sediments (mean of 19.8 out of 20). Sublethal effects, measured by avoidance of sediments and reburial in clean sediments at the end of the 10-day exposure period, were not observed.

CONCLUSIONS

Ecology's October-December, 1987 screening surveys for toxic chemicals in Port Townsend Bay did not identify the cause of Atlantic salmon mortality. Concentrations of metals, other trace elements, and chlorinated compounds in the tissues of affected fish collected from the Crane Point pens were similar to those in the same species reared at Ediz Hook, Cypress Island and Manchester. The level of contamination in Port Townsend Bay appeared to be generally low; no evidence was found of the unusual occurrence of toxic chemicals. Chemical analyses and limited bioassays of the pulp mill effluent and adjacent sediments did not implicate the mill as the source of the problem. The Navy facility did not appear to be a significant source of contaminants to Port Townsend Bay.

Table 13. Results of amphipod (Rhepoxyinus abronius) bioassays of selected Port Townsend Bay sediment samples collected November 30, 1987 (mean values ± S.D.).

Location	Glen Cove Pulp Mill Outfall		Point Wilson	Laboratory Control
Depth (ft. @ MLLW)	47	49	45	--
Sample Number	8230	8231	8235	--
Survival[a]	16.4 ± 2.6*	16.8 ± 1.8*	18.6 ± 0.5	19.8 ± 0.4
Avoidance[b]	1.1 ± 1.6	0.4 ± 0.6	0.4 ± 0.7	1.1 ± 1.0
Percent Reburial[c]	98.8	98.8	100	98.0

[a] n = 5; a value of 20.0 = 100 percent. Asterisks denote values significantly less than laboratory control sediments (West Beach, Whidbey Island) determined statistically using Dunnett's t-test (p=0.05).

[b] Number of amphipods on the surface per jar per day (out of a maximum of 20.0).

[c] At the end of the ten-day exposure, the surviving amphipods were transferred to clean sediment and the number of individuals able to rebury after one hour recorded.

FOLLOW-UP STUDIES

Ecology is continuing to investigate the salmon mortality problem during the summer of 1988. At the agency's request, Battelle has designed studies to answer the following questions:

1. Are the waters of south Port Townsend Bay still toxic to Atlantic salmon and are other fish species susceptible to the disease? To answer this question Atlantic salmon, chinook salmon (*Oncorhynchus tshawytscha*), and Shiner perch (*Cymatogaster aggregata*) will be introduced to a pen at Crane Point in early June. Monthly samples of liver and other organs will be fixed and processed for histopathological examination.

2. Does the toxic effect extend to other parts of the bay? Atlantic salmon will be reared at the Port Townsend marina pens and examined monthly as described above.

3. Does exposure to the Port Townsend Paper Corp. effluent produce liver lesions of the type observed in 1986 and 1987? As already shown, the screening surveys conducted in 1987 did not implicate the pulp mill as the cause of salmon mortality. However, there remains a concern that the mill discharge and liver disease could be related. This is based on the fact that the mill is the only large discharge to the bay and that unbleached Kraft effluent constituents have been reported to have adverse effects on the liver of rainbow trout (Oikari and Nakari, 1982; Oikari, *et al.*, 1981). Rainbow trout are the same genus, *Salmo*, as Atlantic salmon.

Therefore, a bioassay of the mill's effluent will be conducted at the Battelle Seqium laboratory concurrently with the field studies. Atlantic salmon smolts will be exposed to effluent concentrations of 30 percent, 10 percent, 1 percent and 0 percent in seawater; each exposure will be done in duplicate. The bioassay will be static with weekly renewal of effluent/seawater mixtures. Histopathological examination of tissue samples will be done monthly.

The above studies will be funded by Ecology and are expected to run four months. Water Quality Investigations will do limited chemical analyses of effluents and seawater to aid in interpreting the bioassay and field exposure results. The final report on this work is expected by December 1988. All histological slides will be reviewed by an independent expert.

REFERENCES

American Society for Testing and Materials, 1980. Standard practice for conducting static acute toxicity tests with larvae of four species of bivalve molluscs. Philadelpha, PA.

Ames, B.N., J. McCann, and F. Yamasaki, 1975. Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian microsome mutagenicity test. Mutat. Res. 31: 347.

Callahan, M.A., M.W. Slimak, N.W. Gabel, I.P. Moy, C.F. Fowler, J.R. Freed, P. Jennings, R.L. Durfee, F.C. Whitmore, B. Maestri, W.R. Mabey, B.R. Holt, and C. Gould, 1979. Water-related fate of 129 priority pollutants, Volume I. EPA-440/ 4-79-029a.

Cardwell, R.D., C.E. Woelke, M.I. Carr, and E.W. Sanborn, 1979. Toxic substances and water quality effects on larval marine organisms. State of Washington, Dept. Fisheries, Tech. Rept. No. 45.

Crecelius, E., 1981. Letter to R. Tomlinson, Seattle METRO, Seattle, WA.

Crecelius, E.A., M.H. Bothner, and R. Carpenter, 1975. Geochemistry of arsenic, antimony, mercury, and related elements in sediments of Puget Sound. Environ. Sci. Technol. 9:325-333.

Douglas, G.R., E.R. Nestmann;, A.B. McKague, R.H.C. San, E.G-.H. Lee, Y.W. Liu-Lee, and D.J. Kowbel, 1985. Determination of potential hazard from pulp and paper mills: mutagenicity and chemical analysis. pp. 151-164 in H.F. Stich (ed) Carcinogens and Mutagens in the Environment. Vol. V. CRC Press, Inc., Boca Raton, FA.

Ecology, 1980. Biological testing methods. July 1981 revision. DOE 80-12.

EPA, 1984a. Guidelines establishing test procedures for the analysis of pollutants under the Clean Water Act; final rule and interim rule and proposed rule. Fed. Reg. 49 (209): 1-210.

EPA, 1984b. Test methods for evaluating solid waste.

EPA, 1987. Interim procedures for estimating risks associated with exposures to mixtures of chlorinated dibenzo-p-dioxins and -dibenzofurans. EPA/625/3-87/012.

Food and Drug Administration, 1981. Correspondence from FDA Commissioner A.H. Hall to governor of Michigan, W.G. Millikan. August 26, 1981.

Food and Drug Administration, 1985. Action levels for poisonous or deleterious substances in human food and animal feed. Center for Food Safety and Applied Nutrition, Washington, DC.

Keith, L.H., 1976. Identification of organic compounds in unbleached treated Kraft pulp mill wastewaters. Environ. Sci. Technol. 10(6): 555-564.

Kent, M.L., M.S. Myers, D.E. Hinton, W.D. Eaton, and R.A. Elston, 1987 (draft). Suspected toxicopathic hepatic necrosis and megalocytosis in pen-reared Atlantic salmon (*Salmo salar*) in Puget Sound, Washington, USA. Battelle Marine Research Laboratory, Sequim, WA.

Kjosness, D., 1988. Letter and attachments to E.T. Muchlethaler, Port Townsend Paper Corp., February 17, 1988.

Konasewich, D.E., P.M. Chapman, E. Gerencher, G. Vigers, and N. Treloar, 1982. Effects, pathways, processes and transformation of Puget Sound contaminants of concern. NOAA Tech. Memo. OMPA-20.

Malins, D.C., B.W. McCain, D.W. Brown, A.K. Sparks, and H.O. Hodgins, 1980. Chemical contaminants and biological abnormalities in central and southern Puget Sound. NOAA Technical Memo. OMPA-2.

Malins, D.C., B.W. McCain, D.W. Brown, A.K. Sparks, H.O. Hodgins, and S-L Chan, 1982. Chemical contaminants and abnormalities in fish and invertebrates from Puget Sound. NOAA Technical Memorandum OMPA-19.

Mayer, F.L. and M.R. Ellersieck, 1986. Manual of acute toxicity: interpretation and data base for 410 chemicals and 66 species of freshwater animals. U.S. Fish and Wildlife Service. Resource Pub. 160.

McCain, B.B., D.C. Malins, M.M. Krahn, D.W. Brown, W.D. Gronlund, L.K. Moore, and S-L Chan, 1988 (in press). Uptake of aromatic and chlorinated hydrocarbons by juvenile chinook salmon (*Oncorhynchus tshawytscha*) in an urban estuary. Proceedings of First Annual Meeting on Puget Sound Research, Seattle, WA. March 18-19, 1988.

Mehrle, P.M., 1987 (draft). Toxicity and bioconcentration of 2,3,7,8-tetrachlorodibenzo-dioxin and 2,3,7,8-tetrachlorodibenzofuran in rainbow trout. USFWS. Columbia, MO.

Mertz, W., 1981. The essential trace elements. Science 213: 1332-1338.

METRO, 1980. Unpubl. data cited in Konasewich, *et al.* (1982)

METRO, 1984. TPPS technical report C1: presence, distribution and fate of toxicants in Puget Sound and Lake Washington. Report No. 6A.

Muchlethaler, E.T., 1988. Letter to G. Houck, Ecology, February 5, 1988.

National Council for Air and Stream Improvement, 1986. Procedures for the analysis of resin and fatty acids in pulp mill effluents. Tech. Bull. 501. New York, NY.

National Institute of Occupational Health and Safety, 1983. Registry of toxic effects of chemical substances. Cincinnati, OH.

Nestmann, E.R., E.G.-H. Lee, T.I. Matula, G.R. Douglas, and J.C. Mueller, 1980. Mutagenicity of constituents identified in pulp and paper mill effluents using the *Salmonella*/mammalian microsome assay. Mutat. Res. 79: 203-212.

Nielson, K.K. and R.W. Sanders, 1983. Multielement analysis of unweighed biological and geological samples using backscatter and fundamental parameters. Advan. X-ray Anal. 26: 385-390.

O'Conner, J.S. and H.M. Standford (eds), 1979. Chemical pollutants of the New York Bight; priorities for research. NOAA, Boulder, CO.

Oikari, A., B-E Lonn, M. Castren, T. Nakari, B. Snickars-Nikinmaa, H. Bister, and E. Virtanen, 1983. Toxicological effects of dehydroabietic acid (DHAA) on the trout, *Salmo gairdneri* Richardson, in freshwater. Water Res. 17: 81-89.

Oikari, A. and B. Holmbom, 1986. Assessment of water contamination by chlorophenolics and resin acid with the aid of fish bile metabolites. pp 252-267 in Poston, T.M. and R. Purdy (eds) Aquatic Toxicology and Environmental Fate: Ninth Volume. ASTM STP 921.

Oikari, A., E. Anas, G. Kruzynski, and B. Holmbom, 1984. Free and conjugated resin acids in the bile of rainbow trout, *Salmo gairdneri*. Bull. Environ. Contam. Toxicol. 33: 233-240.

Oikari, A. and T. Kunnamo-ojala, 1987. Tracing of xenobiotic contamination in water with the aid of fish bile metabolites: a field study with caged rainbow trout (*Salmo gairdneri*). Aquatic Toxicol. 9: 327-341.

Oikari, A. and T. Nakari, 1982. Kraft pulp mill effluent components cause liver dysfunction in trout. Bull. Environ. Contam. Toxicol. 28: 266-270.

Schmitt, C.J. and S.E. Finger, 1987. The effects of sample preparation on measured concentrations of eight elements in edible tissues of fish from streams contaminated by lead mining. Arch. Environ. Contam. Toxicol. 16: 185-207.

SCS Engineers, 1987 (draft). Current situation report: Naval Undersea Warfare Station, Keyport and Indian Island, Washington. Bellevue, WA.

Stinson, M., 1986. Port Townsend Paper Mill oyster bioassay results. Ecology memorandum to S. Prescott.

Stout, V.F. and F.L. Beezhold, 1981. Chlorinated hydrocarbon levels in fishes and shellfishes of the northeastern Pacific Ocean, including the Hawaiian Islands. Mar. Fish. Rev. 43(1).

Swartz, R.C., W.A. DeBen, J.K.P. Jones, J.O. Lamberson, F.A. Cole, 1985. Phoxocephalid amphipod assay for marine sediment toxicity. pp. 284-307 in: R.C. Cardwell, R.Purdy, and R.C. Bahner (eds) Aquatic Toxicology and Hazard Assessment: Seventh Symposium. ASTM STP 854. Amer. Soc. Testing and Materials, Philadelphia, PA.

Tetra Tech, Inc., 1986. User's manual for the pollutant of concern matrix. Bellevue, WA.

Appendix A. Organic compounds analyzed for Port Townsend salmon mortality investigation.

Volatiles	Acid-Base/Neutrals		Resin Acids, Guaiacols, Catechols
Chloromethane	Phenol	Anthracene	Abietic acid
Bromomethane	bis(2-Chloroethyl)ether	Di-n-Butylphthalate	Neoabietic acid
Vinyl chloride	2-Chlorophenol	Fluoranthene	Dehydroabietic acid
Chloroethane	1,3-Dichlorobenzene	Pyrene	Dichlorodehydroabietic acid
Methylene chloride	1,4-Dichlorobenzene	Butylbenzylphthalate	Isopimaric acid
Acetone	Benzyl alcohol	3,3'-Dichlorobenzidine	Pimaric acid
Carbon disulfide	1,2-Dichlorobenzene	Benzo(a)anthracene	Levopimaric acid
1,1-Dichloroethene	2-Methylphenol	bis(2-Ethylhexyl)pthalate	Sandaracopimaric acid
1,1-Dichloroethane	bis(2-Chloroisopropyl)ether	Chrysene	Palustric acid
trans-1,2-Dichloroethene	4-Methylphenol	Di-n-Octylphthalate	Guaiacol
Chloroform	N-Nitroso-di-n-propylamine	Benzo(b)fluoranthene &	4,5-Dichloroguaiacol
1,2-Dichloroethane	Hexachloroethane	Benzo(k)fluoranthene	4,5,6-Trichloroguaiacol
2-Butanone	Nitrobenzene	Benzo(a)pyrene	Tetrachloroguaiacol
1,1,1-Trichloroethane	Isophorone	Indeno(1,2,3 cd)pyrene	4-Chlorocatechol
Carbon tetrachloride	2 Nitrophenol	Dibenzo(a,l)anthracene	4,5-Dichlorocatechol
Vinyl acetate	2,4-Dimethylphenol	Benzo(g,h,i)peylene	3,4,5-Trichlorocatechol
Bromodichloromethane	Benzoic acid		Tetrachlorocatechol
1,2-Dichloropropane	bis(2-Chloroethoxy)methane		
trans-1,3-Dichloropropene	2,4-Dichlorophenol		
Trichloroethene	1,2,4-Trichlorobenzene		
Dibromochloromethane	Naphthalene		
1,1,2-Trichloroethane	4-Chloroaniline		Munitions Chemicals
Benzene	Hexachlorobutadiene		2,4,6-Trinitrotoluene
cis-1,3-Dichloropropene	4-Chloro-3-methylphenol	PCBs/OC Pesticides	2,4-Dinitrotoluene
2-Chloroethylvinylether	2-Methylnaphthalene	alpha-BHC	2,6-Dinitrotoluene
Bromoform	Hexachlorocyclopentadiene	beta-BHC	Tetryl
4 Methyl 2 pentanone	2,4,6-Trichlorophenol	delta-BHC	Hexahydro-1,3,5-trinitro-1,3,5-triazine
2-Hexanone	2,4,5 Trichlorophenol	gamma-BHC (Lindane)	Cyclotetramethylenetetranitramine
Tetrachloroethene	2-Chloronaphthalene	Heptachlor	
1,1,2,2-Tetrachloroethane	2-Nitroaniline	Aldrin	
Toluene	Dimethylphthalate	Heptachlor epoxide	
Chlorobenzene	Acenaphthylene	Endosulfan I	
Ethylbenzene	3-Nitroaniline	Dieldrin	
Styrene	Guaiacol	p,p'-DDE	
Total Xylenes	Acenaphthene	Endrin	
	2,4-Dinitrophenol	Endosulfan II	
	4-Nitrophenol	p,p'-DDD	
	Dibenzofuran	Endosulfan sulfate	
	2,4-Dinitrotoluene	p,p'-DDT	
	2,6-Dinitrotoluene	Methoxychlor	
	Diethylphthalate	Endrin aldehyde	
	4-Chlorophenyl phenylether	Chlordane	
	Fluorene	Toxaphene	
	4-Nitroaniline	PCB-1016	
	4,6-Dinitro-2-methylphenol	PCB-1242	
	N-Nitrosodiphenylamine(1)	PCB-1248	
	4-Bromophenyl-phenylether	PCB-1254	
	Hexachlorobenzene	PCB-1260	
	Pentachlorophenol		
	Phenanthrene		

Appendix B. Detection limits for organic compounds analyzed in samples collected during Port Townsend salmon mortality investigation, October-December, 1987 (ppb).

	Matrix				
Analysis	Muscle Tissue	Liver Tissue	Bile	Sediment	Water
volatiles	NA[a]	NA	NA	0.2-11	0.2-3.5
acid-base/neutrals	NA	NA	NA	2.3-390	0.1-1.6
PCBs	20	200-600	NA	100-240	2
OC pesticides	1-4[b]	10-60[b]	NA	5-48[b]	0.1-0.4[b]
2,3,7,8-TCDD	0.0005-0.0008	0.0005	NA	NA	NA
cyanide	NA	NA	NA	NA	5
resin acids	NA	NA	90-17,900	200-450	10-20
munitions chemicals	NA	NA	NA	300-600[c]	40-80[c]

[a] not analyzed

[b] toxaphene detection limits were 200 ppb (muscle), 2000-6000 ppb (liver), 1000-2400 ppb (sediment), and 20 ppb (water)

[c] Extracts from sediment samples 8230, 8231, 8233, 8234, 8235; seawater sample 8232; and NUWES effluent 8236 were also screened for organonitrogen compounds at the Ecoloyg/EPA Manchester laboratory by GC/FID. No compounds were detected down to a detection limit of 8 ppb (sediment) or 1 ppb (water).

Appendix C. Resin acid, guaiacol and catechol spike and spike duplicate recoveries for Port Townsend samples collected November 30 - December 2, 1987 (percent).

Matrix	Sediment		Seawater		Effluent	
Sample Number	8230Y	8230Z	8232Y	8232Z	8240Y	8240Z
Matrix Spike Compounds:						
isopimaric acid	100	107	73	73	69	85
levopimaric acid	51	42	53	53	52	37
dehydroabietic acid	162	153	77	76	75	97
abietic acid	82	63	78	96	17	80
neoabietic acid	not recovered		49	60	3.8	16
dichlorodehydroabietic acid	69	69	116	100	117	109
chlorocatechol/guaiacol*	2	17	2.3	0.1	4.4	0.2
dichlorocatechol/guaicol*	16	62	21	4.3	25	4.2
trichlorocatechol/guaiacol*	82	79	76	49	79	75
tetrachlorocatechol/guaiacol*	79	69	89	95	89	90
Surrogate Spike Compounds:						
1,3-dihydroxybenzene-d6	- - - - - - - - not recovered - - - - - - - -					
2-naphthoic acid	99	95	94	99	89	96
o-methylpodocarpic acid	91	90	80	73	72	93

*Analysis did not distinguish between guaiacols and catechols.