SAMPLING AND ANALYSIS PLAN

FOR

KITIMAT ENVIRONMENTAL ASSESSMENT

Prepared By

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Project Background and Objectives

The ALCAN aluminum smelter is located at the head of Kitimat Arm in Kitimat, BC. Emissions generated by the production of aluminum at the smelter are a major anthropogenic source of polycyclic aromatic hydrocarbons (PAHs) at the site, and these substances have contaminated adjacent intertidal and subtidal areas. Several studies (Simpson et al. 1996, 1998; Paine et al. 1996) have been conducted to evaluate the extent of contamination in sediments in Kitimat Arm. Analyses have revealed extremely high concentrations of PAHs in sediments in the vicinity of the smelter and at nearby Hospital Beach, with typical values in the 10,000-100,000 ng/g dry wt range. These concentrations are well above those associated typically associated adverse effects in fish exposed to PAHs in urban sediments (Horness et al. 1998; Johnson 2000). However, there is some question as to whether comparable effects would be exhibited in organisms exposed to soot-associated PAHs such as those produced by the smelter, because of their limited bioavailability (Naes et al. 1999; Paine et al. 1996).

Because Kitimat Arm and the Kitimat fjord system are part of the historic fishing grounds of the Haisla Nation, the Haisla have been concerned for some time about potential impacts of the PAHs from the smelter on fisheries resources in the area. However, data on PAH uptake and biological effects in fish and shellfish from the site are limited. Clams (*Mya arenaria*) have been sampled from several beaches in the Kitimat fjord system (Simpson 1997). At Hospital Beach, which is relatively close to the smelter, total PAH concentrations in clam tissue were 5000-6000 ng/g dry wt. At Kitimat and Eurocan beaches, concentrations were approximately 1100 ng/g dry wt. At Kildala Beach, which is considered a relatively unimpacted reference area, concentrations were 83 ng/g dry wt. Analyses of bile from juvenile salmon (Cretney 1997) showed detectable concentrations of the PAH metabolites (measures as pyrene-1-glucoronide equivalents) in fish from the Yacht Basin and ALCAN Beach, near the smelter, and Kildala Arm. Concentrations were highest in the Yacht Basin and Alcan Beach areas. Hence, preliminary data suggest a potential for PAH exposure in organisms residing in areas impacted by the smelter.

The objective of the proposed study is to provide a more comprehensive characterization of PAH contamination and associated biological effects in representative fish from the Kitimat region. The Kitimat environmental assessment will document the current status of contaminant-associated injuries in fish indigenous to the Kitimat Arm and adjacent areas, quantify the extent of these injuries, and evaluate links between these injuries and chemical contaminants in this waterway, specifically PAH contamination associated with the ALCAN aluminum smelter. Evidence for evaluating links will include data on concentrations of selected chemical contaminants in sediments from sites

where fish are captured and in tissues and fluids of fish. In addition to PAHs, concentrations of other chemical contaminants, including selected chlorinated hydrocarbons (CHs) and metals will be monitored in fish tissues, and their potential impacts on fish health will be assessed.

This research will help to elucidate the spatial extent and severity of contamination from the smelter, the associated level of resource injury, and the type of remediation necessary to protect the health of marine organisms in the Kitimat area. Additionally, it will provide a strong basis for further understanding the linkage between PAH exposure and biological effects, which has previously been shown in other marine/estuarine areas.

The environmental assessment will consist of four primary components: 1) exposure assessment of juvenile salmon; 2) exposure assessment and hepatic disease in adult fish; and 3) assessment of reproductive function in adult flatfish; and 4) characterization of sediment PAH contamination at sites fish were collected and in subtidal nearshore areas throughout Kitimat Arm. These four components, together with more detailed background for each component, are described below:

I. Exposure Assessment of Juvenile Salmon

In studies conducted in the early 1990s, juvenile chinook salmon from urban waterways in Puget Sound (e.g., the Hylebos Waterway in Tacoma, and the Duwamish Waterway in Seattle) were found to have significantly elevated concentrations of PAHs, CHs or their derivatives in tissues, fluids or stomach contents (McCain et al. 1990; Varanasi et al. 1993; Stein et al. 1995; Stehr et al. 2000). Juvenile salmon from these waterways also had increased induction in liver of the PAH-metabolizing enzyme, cytochrome P450 1A (CYP1A), and higher levels of DNA damage, compared to juveniles from nonurban estuaries, and exhibited impaired immunocompetence and growth inhibition (Arkoosh et al. 1991, 2000; Varanasi et al. 1993; Stein et al. 1995; Casillas et al. 1999). Laboratory investigations have confirmed that immunocompetence of juvenile chinook salmon can be impaired by exposure to extracts of Duwamish Waterway or Hylebos Waterway sediments (Arkoosh et al. 1994, 2000), and that exposed fish show increased disease susceptibility in challenge trials with one of their natural pathogens, *Vibrio anguillarum.* Because the assessment of immunocompetence and growth is very expensive, we are proposing to initially examine exposure and early biomarkers (e.g. DNA damage and enzyme induction) in juvenile salmon from Kitimat. If significant exposure is found, then investigations to assess more serious biological effects may be considered if funds are available. Data linking specific classes of contaminants to impaired immunocompetence and reduced growth in salmonids is comparatively scarce,

but our recent results suggest that both PAHs and chlorinated hydrocarbons may be risk factors.

II. Exposure and Hepatic Disease in Adult Fish

In the early 1980s, elevated levels of a variety of toxic chemicals were found in tissues of English sole and rock sole from the urban sites in Puget Sound, such as Elliott Bay, Commencement Bay, and associated waterways (Malins et al. 1982, 1984). Many of these fish also had liver lesions, including tumors, which are associated with exposure to carcinogenic chemicals, especially high molecular weight PAHs (Myers et al. 1987, 1994a, Myers et al. 1991). To confirm the association between PAH exposure and the development of liver disease, Environmental Conservation Division (ECD) scientists conducted a long-term laboratory study in which a variety of unique degenerative and preneoplastic lesions were induced in English sole exposed to benzo[a]pyrene and a PAH-rich fraction of contaminants extracted from a contaminated sediment (Schiewe et al. 1991). More recently a suite of biochemical alterations has been demonstrated in a variety of fish species from several areas in Puget Sound with elevated concentrations of contaminants in sediment (Stein et al. 1992). Biochemical changes observed include increased concentrations of PAH metabolites in bile, induction of cytochrome P4501A (CYP1A), a major enzyme that metabolizes PAHs, and increased concentrations of PAH-DNA adducts in liver, an indicator of DNA damage. Measurements of these biological markers have been used to improve the assessment of contaminant exposure and early responses in indigenous fish (Krahn et al. 1986a; Stein et al. 1992; Collier et al. 1993a,b; Collier et al. 1995), and thus strengthen the link between exposure to toxic chemicals and the injuries described above. In studies conducted in Puget Sound, substantive correlations were found between the prevalences of a number of liver lesions and the levels of most of these biomarkers (Myers et al. 1998a,b). We have also conducted several laboratory studies to demonstrate the time- and dose-responsiveness of a number of these biomarkers (Collier and Varanasi, 1991; Stein et al. 1993). Additionally, using field data collected over a number of years, we have been able to estimate threshold concentrations of PAHs in sediments from urban sites that are associated with the development of liver lesions and DNA damage in English sole (Horness et al. 1998; Johnson 2000). This suite of endpoints will be applied to English sole populations from the Kitimat area to assess PAH-related injury in fish from sites impacted by the ALCAN smelter. The initial focus will be on documenting liver disease and PAH exposure, through measurement of biliary FACs; samples will be collected for CYP1A induction and DNA adducts, but held in reserve for future analyses. Samples of fish muscle tissue will also be collected to analyze for the presence of AHs, CHs, and metals.

III. Reproductive Function in Adult Flatfish

In an effort to assess other biological impacts of chemical contaminants on flatfish, the ECD developed a research program to evaluate the effects of these contaminants on the reproductive status of gonadally maturing adult female fish. Field studies showed that a significant proportion of female flatfish living in contaminated waterways of Puget Sound, such as the Duwamish Waterway and Eagle Harbor in Seattle, and Hylebos Waterway in Tacoma, exhibited reduced reproductive success (Johnson et al. 1988; Casillas et al. 1991; Johnson et al. 1999) compared to females from minimally contaminated sites. Another study suggested that female English sole from contaminated areas may not be making their spawning migrations at all (Collier et al. 1992). Among the reproductive effects observed were inhibition of oocyte development, inhibition of spawning, depressed plasma estradiol levels, reduced egg weight, increased larval abnormalities, and reduced viability of offspring. Again, similar to results for fish pathology, our data suggest that exposure to PAHs is a substantial risk factor in determining reduced reproductive success. Laboratory investigations have shown effects on circulating levels of sex hormones due to contaminant exposure, consistent with field results (Stein et al. 1991). More recently, indicators of reproductive function have been examined in male English sole. Preliminary data suggest that exposure to high concentrations of PAHs may alter plasma testosterone levels in males, but changes in gonadal growth comparable to those found in females have not been observed (Sol et al. 1998a).

The objective of the proposed study in Kitimat is to assess PAH exposure and associated alterations in reproductive function in flatfish from Kitimat Arm and reference areas in the vicinity. Although English sole would be the ideal target species for this research, it may not feasible to assess their reproductive status as part of this field effort because they typically spawn in winter. Instead, we propose to examine reproductive function in yellowfin sole, which spawn during the summer and will be undergoing gonadal development during the period when we plan to sample flatfish in Kitimat. We have not previously conducted a detailed reproductive toxicology study with this species, so their sensitivity to reproductive injury in comparison to English sole is not known. However, we have some laboratory and field data to suggest that PAH exposure may suppress plasma steroid concentrations in yellowfin sole (Johnson et al. 1995; Sol et al. 2000), so consider it a suitable target species for this study. Because this is a preliminary study, the major emphasis will be on obtaining data on gonadal development only, and exposure assessment will initially be limited. Histopathology, determination of somatic indices, and analyses of otoliths in all male and female fish will be completed as part of this project. Plasma and bile samples will be also collected, along with muscle and liver tissue for chemical analyses, and archived for possible future analysis.

IV. Sediment Contamination Characterization

In order to establish links between PAH exposure and effects in resident biota, and to better identify sources of exposure, it is important to have adequate information on the levels and spatial extent of PAH contamination in sediments within Kitimat Arm and at sites where resident fish were collected. Accordingly, in conjunction with fish sampling, sediment samples will be collected along trawl tracks at the fish sampling sites. Additionally, in order to characterize the spatial extent of contamination in and around Kitimat Arm, intensive systematic sediment sampling will be conducted in nearshore areas where bottomfish reside.

Descriptions and scheduling of tasks

General

Juvenile Salmonids. Juvenile chinook salmon and coho salmon (Kemano Village only) were collected for exposure assessment studies in the spring of 2000. Fish were collected using a seine deployed from beaches or docks. Tissues collected for these studies included liver, stomach contents and bile for measurements of organic contaminants (CHs, PAHs, and their derivatives); and liver for measurements of biomarkers, e.g. DNA adducts and CYP1A. Juvenile salmon were sampled from Kitimat Arm, Kildala Estuary, and Kitlope from May 12-20. Fish were collected from seven sites: five sites from the upper harbor (Hospital Beach, Eurocan Beach, ALCAN Inner Harbor, Wathisto Creek, and Minette Bay), one site in Kildala Arm, and one site at Kemano Village. An attempt was made to collect fish from Kitlope, but they could not be obtained. Juvenile salmon were also sampled from three hatchery stocks: Upper Kitimat, Lower Kitimat, and Kildala. A second group of fish was sampled from Kildala Estuary and ALCAN Inner Harbor in Kitimat Arm on June 15-16. The number of fish and samples collected are summarized in Appendix 1, Table 1.

Thirty-nine composites of bile and thirty-eight composites of stomach contents were collected during the course of the study. For the May sampling, this included three bile and three stomach composites from the Upper Kitimat River Hatchery, three bile and two stomach composites from the Lower Kitimat Hatchery, three bile and three stomach composites from the Kildala Hatchery, three bile and three stomach composites each from Hospital Beach, ALCAN Inner Harbor, Wathisto Creek, Minette Bay, and Kildala Estuary, three bile and four stomach composites from Eurocan Beach, and six bile (4 chinook, 2 coho) and five stomach (4 chinook, 1 coho) composites from Kemano Village. In June,

three bile and three stomach composites each were collected from Kildala Estuary and ALCAN Inner Harbor. Additionally, thirty-eight composites for whole body chemistry, and thirty-eight composites of liver for CYP1A analyses, thirty-eight composites of liver DNA adduct analyses, and ten composites for lipid analysis were collected from the sites listed above.

In September-November 2000, all bile samples will be analyzed for AH metabolites (Krahn et al. 1986), and stomach contents composites will be analyzed for AHs, CHs, and retene (an indicator of exposure to pulp mill effluent) using gas chromatography / mass spectrometry (GS / MS) as described in Sloan et al. (1993). This method of analysis provides accurate quantitation of a number of individual AH and CH analytes (see Appendix 2). Additionally a sample of fish food obtained from the hatchery will be analyzed for AHs and CHs by GC / MS (Sloan et al. 1993). Other samples will be archived for possible future analysis.

Toxicopathic Conditions in Flatfish. English sole were collected for assessment of histopathological conditions of the liver, kidney, and gonad, and measurements of organic contaminants and selected metals (muscle only) in liver, muscle, and bile, DNA adducts in liver, and CYP1A in liver. Otoliths were also collected for age determination. Fish were sampled in June of 2000 by otter trawl at six sites --- four sites in Kitimat Arm (Hospital Beach, Eurocan, Kitamaat Village, and along the path of the ALCAN plume south of ALCAN on the west side of Kitimat Arm), one site in Kildala Arm, and one site at Kitlope. Approximately thirty to fifty English sole were collected at each site, for a total of 246 fish (See Appendix 1; Table 2).

In September-November 2000, all bile samples will be analyzed for AH metabolites (Krahn et al. 1986), and muscle samples from four sites (Hospital Beach, Eurocan, Kildala, and Kitlope) will be analyzed for AHs and CHs (Sloan et al. 1993), metals (Meador et al. 1994), and dioxins. Dioxin analyses will be conducted by Axys Labs under a separate contract, and are not included in the budget outlined under this SAP. Tissues from all fish will be examined microscopically for histopathological conditions (Myers et al. 1987), and ages of all fish will be determined from otoliths. Other samples will be archived for possible future analysis.

Reproductive Toxicology in Flatfish. Adult male and female yellowfin sole were collected by otter trawl from the R / V Harold W. Streeter. Sampling was conducted in June 2000, in conjunction with sampling for the toxicopathic injury study. During this season, vitellogensis and spermatogenesis normally occur in this species. A total of 193 yellowfin sole were collected, 120 males and 73 females (See Table 2). Males (18-21 per site) were obtained at Hospital Beach,

Eurocan, KITAMAAT Village, the ALCAN plume, and Kitlope. Complete collections of females (20 per site) were obtained only at Hospital Beach, Eurocan, and Kildala Arm. Smaller number of females (2-9 per site) were collected at the other sampling sites. In addition, because it was noted that some English sole were beginning to undergo gonadal maturation, extra female English sole (7-13 per site) were collected from the Hospital Beach, Eurocan, Kitamaat Village, ALCAN Plume, and Kildala Arm sampling sites for reproductive injury evaluation. Otoliths were collected for age determination. Blood samples were collected for measurement of reproductive steroid hormones (estradiol and 11-ketotestosterone) and vitellogenin. Liver and gonad were collected for histopathological examination and staging of gonadal development. Liver, muscle, and bile were collected for measurement of contaminant exposure.

In September-November 2000, tissue samples from all fish will be examined microscopically for reproductive stage and pathological conditions in the liver and ovary (Johnson et al. 1991; Myers et al. 1987), and ages of all fish will be determined from otoliths. Other samples will be archived for possible future analysis.

Sediment Characterization. Sediment samples were collected using a Van-Veen grab along trawl tracks at flatfish sites. Grab samples were collected at three stations per site, at the beginning, middle, and end of the trawl tracks. A total of twenty-six sediment samples were collected: three stations per site at the six sites where flatfish were collected (Hospital Beach, Eurocan, Kitamaat Village, the ALCAN plume, Kildala Arm, and Kitlope); and one station per site at the sites where chinook salmon where collected (Hospital Beach, Eurocan Beach, ALCAN Inner Harbor, Wathlsto Creek, Minette Bay, and Kildala Estuary). Sediments were also collected from two stations off the marina at Kemano, where coho salmon were collected. Locations of sediment collection sites are listed in Appendix 1. Additionally, in order to characterize the spatial extent of contamination in and around Kitimat Arm, intensive sediment sampling was conducted in nearshore areas along the ALCAN plume area, Hospital Beach, ALCAN Inner Harbor and Eurocan, and in the vicinity of Kitimat Village. Samples were collected systematically between the 30 and 250 foot depth contours. A total of 140 sediment samples were collected. Latitudes, longitudes and depths at sites where sediments were collected are shown in Appendix 1, Table 3.

In September-November 2000, sediment samples from sites where fish were collected will be analyzed for AHs using the high performance liquid chromatography (HPLC)/fluorescence method of Krahn et. al (1991). This method provides a rapid semi-quantitative estimate of AH levels. Grain size

and total organic carbon (TOC) analyses will also be performed on these samples by an outside laboratory (e.g., Columbia Analytical of Kelso, WA). Other samples will be archived for possible future analysis.

Data Analysis and Products

The data from these injury assessment investigations will be placed in an integrated data management system. Statistical tests will be performed 1) to evaluate relationships between contaminant exposure and biological effects, 2) to compare values from fish captured in the Kitimat Arm with values for fish captured in reference areas, and 3) to compare concentrations of contaminants in liver tissue, prevalences of liver lesions and biomarker responses with similar parameters measured in previous studies.

Data acquired and analyzed will be used to address the following questions for each species:

1. Are fish exposed to PAHs in Kitimat Arm and adjacent areas?
2. Are other contaminants present in fish (e.g., chlorinated hydrocarbons, toxic metals) that could injure fishery resources?
3. What are the spatial extent and severity of exposure in relation to the ALCAN smelter?
4. Are the exposures believed to be sufficient to cause injury to the fish?
5. What proportion of the population is estimated to be injured by these contaminants?
6. What might be the overall effect of this injury on the population of each species?

Project Schedule and Costs

Fish and sediment sampling for the Kitimat Environmental Assessment were conducted in May-June 2000, and the first phase of sample analysis will take place between June and November of the same year, with data reporting by the end of the year. This phase of analysis will focus on measurement of contaminant concentrations in salmon, flatfish, and sediments from sites where fish were collected, and on fish histopathology, including pathology of ovaries and testes of reproductively maturing fish. An interpretive report should be completed by March, 2001. Scheduling of specific tasks for the four sections of the assessment is outlined in Table 1. A summary budget for the project is outlined in Table 2. The total cost for the proposed analyses is estimated at $134,577 US dollars.

Table 1. Scheduling of Specific Tasks for Kitimat Environmental Assessment

	Salmon investigations (contaminant exposure)	
Duration	**Activity**	**Numbers of fish and/or analyses**
April / May 2000	Seining for juvenile salmon (approximately 20 days)	Approximately 1600 fish
April / May 2000	Salmon necropsy and sample collection	Approximately 1600 fish
September-November, 2000	Chemical analysis of hatchery fish food	1 comp
September-November, 2000	Chemical analyses of composites of stomach contents and bile	38 comps / stomach, 39 comps / bile
September-November, 2000	Analyses of composites of liver for biomarkers of contaminant exposure	Archive for possible future analysis
December 2000	Data report	N / A
March 2001	Interpretive report	N / A

	Flatfish investigations (Contaminant exposure and toxicopathic conditions)	
Duration	**Activity**	**Numbers of fish and/or analyses**
June 2000	Flatfish collection aboard the RV Streeter (approximately 3 weeks)	246 fish
June 2000	Flatfish necropsy and sample collection	246 fish
September-November, 2000	Sectioning and staining of fixed tissues	246 samples
September-November, 2000	Histological examination of tissues	246 sections
September-November 2000	Chemical analyses of composites of bile for AH exposure (3 per site)	18 comps / bile
September-November 2000	Chemical analyses of composites of muscle for CHs and AHs (3 per site)	12 comps / muscle
September-November 2000	Chemical analyses (AH screening) of sediments from fish sampling sites	26 samples
September-November 2000	TOC / grain size analyses of sediments from fish sampling sites	26 samples
N / A	Chemical analyses of composites of liver for CHs (3 per site)	Archive for possible future analysis
N / A	Analyses of composites of liver for biomarkers of contaminant exposure	Archive for possible future analysis Archive
December 2000	Data report	N / A
March 2001	Interpretive report	N / A

	Flatfish investigations (reproductive toxicology)	
Duration	**Activity**	**Numbers of fish and/or analyses**
June 2000	Yellowfin and English sole collection aboard the RV Streeter (approximately 3 weeks)	Approximately 252 adult fish
June 2000	Yellowfin and English sole necropsy and sample collection	Approximately 252 adult fish
September-October, 2000	Sectioning and staining of fixed tissues (all fish)	Approximately 252 samples
September-November 2000	Histological examination of tissues (all fish)	Approximately 252 sections
September-November, 2000	Determination of somatic indices (all fish)	Approximately 252 samples
September-November, 2000	Chemical analyses of composites of bile (3 per site, yellowfin females)	Archive for possible future analysis
N / A	Analyses of blood plasma for estradiol (females)	Archive for possible future analysis
N / A	Analyses of blood plasma for 11-ketotestosterone (males)	Archive for possible future analysis
N / A	Analyses of blood plasma for vitellogenin (males and females)	Archive for possible future analysis
N / A	Chemical analyses of composites of liver and muscle (6 per site; 3 males and 3 females)	Archive for possible future analysis
December 2000	Data report	N / A
March 2001	Interpretive report	N / A

	Sediment Characterization (Contaminant concentration, TOC and grain size)	
Duration	**Activity**	**Numbers of samples and/or analyses**
June 2000	Sediment collection aboard the RV Streeter (approximately 3 weeks)	Approximately 166 samples
September-November 2000	Chemical analyses (AH screening) of sediments from fish sampling sites	26 samples
September-November 2000	TOC / grain size analyses of sediments from fish sampling sites	26 samples
N / A	Chemical analyses (AH screening) of sediments from additional sites throughout Kitimat Arm	Archive for possible future analysis (140 samples)
N / A	TOC / grain size analyses of sediments from additional sites throughout Kitimat Arm	Archive for possible future analysis (140 samples)
December 2000	Data report	N / A
March 2001	Interpretive report	N / A

Table 2. Budget Summary
Kitimat Environmental Assessment

Salmon analyses

# Analyses	Type of Analysis	Cost/sample	Total $
39	AH metabolites in bile	219	8541
38	*Stomach contents AHs & CHs (detai	1360	51680

Flatfish analyses

# Analyses	Type of Analysis	Cost/sample	Total $
18	AH metabolites in bile	219	3942
12	Muscle AHs & CHs (detailed)	1360	16320
12	Muscle metals	441	5292
498	Fish histopathology	76	37848

Sediment and fish food analyses

# Analyse	e of Analysis	Cost/sample	al $
26	Sediment AH screening	244	6344
26	Sediment TOC & grain size	125	3250
1	Fish food AHs & CHs (detailed)	1360	1360

TOTAL COST **134577**

Standard Operating Procedures

Sample Collection and Analyses for Kitimat Fish Injury Studies 2000: Juvenile Salmon Contaminant Exposure, Toxicopathic Conditions in Flatfish, Reproductive Toxicology of Flatfish, and Sediment Contamination Characterization

Field collections

Juvenile Salmon

Salmon are collected with beach seines generally following the procedures as described in the Puget Sound Protocols (PTI, 1990), and by Varanasi et al. (1993). Fish captured from estuarine sites are held alive in aerated seawater, and fish from hatcheries are maintained in aerated freshwater until necropsies can be conducted. Fish processing is done in laboratory facilities at the hatcheries.

Flatfish for Toxicopathic Conditions and Reproductive Toxicology Studies

Flatfish are collected by 25 foot SCCWRP otter trawl similar to that described in Puget Sound Protocols (PTI, 1990). The net is deployed from the 45 foot NWFSC research vessel Harold W. Streeter and towed for a duration of 5-15 minutes at a speed of 1.5-2.5 knots.

Fish are identified by trained fisheries biologists, and target species are placed in holding tanks on board the research vessel. Fish are maintained alive for no more than a few hours in tanks with flowing seawater until necropsies can be performed in the shipboard laboratory. By-catch is released.

Sediments

At fish collection sites, grab samples are taken at three stations located at the beginning, middle, and end of the fishing trawl tracks. For characterization of spatial patterns of sediment contamination, sediment sampling stations are systematically pre-selected using a grid system to achieve uniform spatial coverage. At each sampling station, surface sediment (top 2-3 cm) is collected with a modified Van Veen grab-sampler (0.1 m^2). One grab sample is taken at each station. If an acceptable sediment sample cannot be obtained after three attempts (e.g., because of rocky substrates or bottom debris), the site is abandoned. A surface skim (2-3 cm deep) is taken from each sampling, and

material is mixed and subdivided into one 4 ounce sample for chemical analysis and one 10 ounce sample for grain size and TOC determination.

Juvenile Salmon

At each site, or for every collection period, approximately 10-40 juvenile chinook salmon are sampled for each composite. The number of fish needed for a composite is dependent on the size of the fish. We attempt to collect 3 composites at each site for every sampling period; however the number of composites collected is dependent on the number of fish available.

Prior to necropsy, fish are weighed and measured for fork length. Bile, liver and stomach contents are collected as described in Varanasi et al. (1993), except that whole livers are collected and later subdivided for each type of analysis. Liver and stomach contents are composited into glass 20 ml vials previously rinsed with methylene chloride. Bile is composited into 4 ml vials containing glass limited-volume inserts. Bile, whole body, and stomach contents samples are maintained on ice during the necropsy procedure, then transferred to freezers for storage. Liver samples for DNA adduct and CYP1A analyses were frozen and stored in liquid nitrogen. Frozen bile, body, and stomach samples are shipped to Seattle via air on ice, while liver samples are shipped in a dry shipper charged with liquid nitrogen. Samples are distributed to the appropriate laboratories upon return to Seattle. Samples for organic chemical analyses are stored at -20° C, and samples for CYP1A and DNA adduct analyses are stored at -80° C until analyses are completed.

Flatfish

Fish are weighed (to the nearest gram) and measured (total length, to the nearest 1 mm), and otoliths are collected for age determination. Prior to necropsy, each fish is assigned a unique specimen number, and all sample containers are labeled with this number for sample tracking. Details of necropsy, histology sample collection and fixation, and chemistry sample collection and preservation are described in Stehr et al. (1993). Samples of liver, bile and muscle collected for organic chemical analysis are maintained on ice, then transferred to freezers for temporary storage at the end of each day. Samples of liver for CYP1A and DNA adducts are immediately placed in liquid nitrogen and stored there until transport to the Seattle laboratory. Frozen samples are shipped to Seattle via air on ice, and liquid nitrogen samples are shipped in a dry shipper charged with liquid nitrogen. Samples are distributed to the appropriate laboratories upon return to Seattle. Samples for organic chemical analyses are stored at -20° C, and samples for CYP1A and DNA adduct analyses are stored at -80° C until analyses are completed.

Toxicopathic Conditions Study: Typically at least thirty and up to sixty English sole are collected at each site. Fish are necropsied and tissues preserved according to standard procedures described in Stehr et al. (1993). Samples collected included otoliths for age determination, liver, kidney, spleen, and

gonad for histology; liver and muscle for organic contaminant and metals analyses, liver for CYP1A and DNA adducts; and bile for FACs.

Reproductive Toxicology Study: Up to twenty female and twenty male sole are collected at each site. Samples collected include otoliths for age determination; liver and gonad for histology and to determine gonadal maturation; liver and muscle for organic contaminant analyses; and bile for FACs. Blood is collected for plasma 17-β estradiol concentrations in female and plasma 11-ketotestosterone concentrations in males, and for determination of vitellogenin concentrations. Blood samples are taken with a heparinized syringe and centrifuged at 800 x g. Plasma is collected and samples are maintained on ice, then transferred to freezers for temporary storage at the end of each day. Weights of ovaries, liver, and gutted bodies are collected to determine gonadosomatic, hepatosomatic and condition indices. Sample collection procedures are described greater detail in Johnson et al. (1988, 1994) and Stehr et al. (1993). Samples are distributed to the appropriate laboratories upon return to Seattle. Samples for organic chemical analyses are stored at -20° C, and samples for CYP1A and DNA adduct analyses and plasma samples are stored at -80° C until analyses are completed.

Sample analyses

Juvenile Salmon Exposure Assessment

Organic chemical analyses (detailed analysis).

Fish stomach contents are analyzed for aromatic hydrocarbons (AHs) as well as CHs and pesticides, using the methods of Sloan et al. (1993). Retene concentrations will also be determined as an indicator of exposure to pulp mill effluent. Whole bodies and other tissues (e.g., liver, muscle) are analyzed for chlorinated hydrocarbons (CHs) and pesticides using the methods described by Sloan et al. (1993). Analytes measured are listed in Appendix 2, Table 2. Tissue or stomach contents are extracted by grinding tissue, sodium sulfate, dichloromethane, and surrogate standards with a Tekmar Tissumizer. Tissue extracts are filtered through silica-alumina and concentrated to 1 ml for further cleanup using size exclusion chromatography. The extract is concentrated and exchanged into hexane for analysis using GC/MS for aromatic hydrocarbons and GC with electron capture detection for chlorinated pesticides and hydrocarbons.

Biliary FACs.

FACs including benzo[a]pyrene (BaP), phenanthrene (PHN) and naphthalene (NPH) equivalents in bile are analyzed by HPLC according to the methods described by Krahn et al. (1986b) with the following modifications.

FACs in bile are analyzed on a Waters (Milford, MA) HPLC equipped with a Waters WISP™ model 715 automatic injector, and three Perkin Elmer (Norwalk, CT) model 40 fluorescence detectors connected in series and interfaced to a Waters Millenium™ data acquisition workstation. A 0.20- x 2-cm guard column containing Perisorb™ 30- to 44-µm reverse-phase C_{18} packing (Upchurch Scientific, Oak Harbor, WA) is used in series with a Perkin Elmer HC-ODS/PAH 10-µm (0.26- x 25-cm) reverse-phase analytical column (Figure 1).

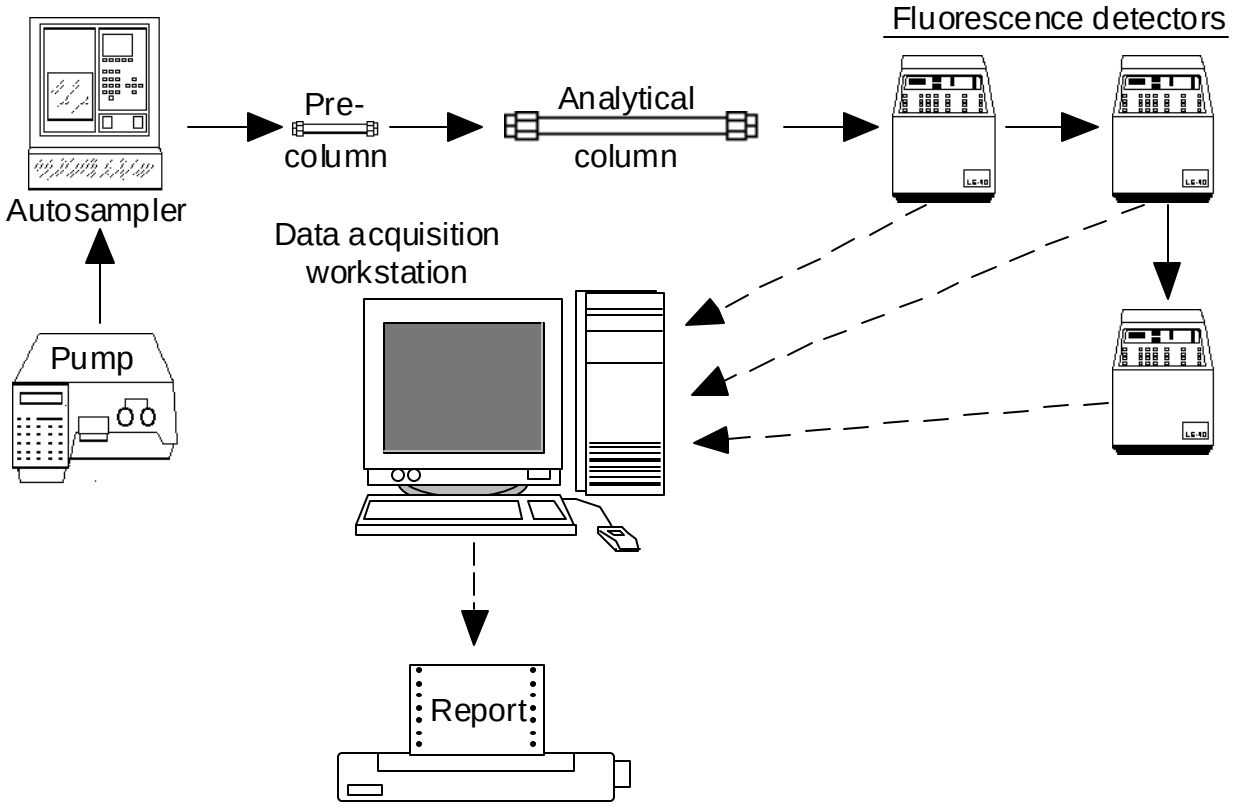

Figure 1. High performance liquid chromatography system for analyses of bile for fluorescent aromatic contaminants (solid lines indicate flow of mobile phase; dashed lines indicate electronic connections for data flow)

For each sample, 3-5 µL of thawed, untreated bile is injected onto the analytical column and eluted with an HPLC linear gradient (flow rate of 0.7 ml/min) beginning with 100% solvent A (water containing 5 ppm acetic acid) to a final composition of 100% solvent B (methanol) during a period of 15 min. After holding the mobile phase at 100% solvent B for 10 min, solvent conditions are returned to 100% solvent A during a period of 3 min. The system is then allowed to re-equilibrate for 10 min at 100% solvent A before the next sample is injected. The total run time, including running of the linear gradient and re-

equilibration of the system, is 38 min. All solvents are degassed with helium and the column temperature is held at 50°C.

Bile sample identification numbers, HPLC calibration standards (HPLC CS) and data from the bile reference pools for each analysis set are entered into a database in the Waters Millenium™2010 Chromatograpy Manager software (see below). For each set of samples, the HPLC CS, containing known concentrations of NPH, PHN and BaP, is analyzed at the beginning of the set and after every 6 or 7 samples. A quality assurance bile reference pool is analyzed near the beginning and end of each set analyzed by HPLC. Biliary FACs are monitored by fluorescence at excitation/emission (ex/em) wavelength pairs for NPH (ex/em 290/335 nm), PHN (ex/em 260/380 nm) and BaP (ex/em 380/430 nm). The total area for all peaks in the region of the chromatogram where FACs are known to elute (> 9 min) is integrated for each wavelength pair. Quantification of analytes is performed according to Krahn et al. (1986b). The concentrations (ng PAH equivalents per ml of bile or per mg biliary protein, wet weight) of FACs in the samples are calculated according to the response of each PAH in the HPLC CS. Biliary protein is measured according to Lowry et al. (1951). If the fluorescence response in a sample is sufficiently high that a detector response reaches its maximum (saturated), the sample is re-analyzed using a smaller injection volume.

As stated above, fluorescence response data for HPLC chromatograms is acquired and processed with the Waters Millenium™ 2010 chromatography software package. Each tray of sample vials (bile samples, calibration standards and reference bile pools) analyzed by HPLC is assigned a unique Sample Set ID. After processing, the chromatograms and results of peak integration are printed for review to assure the quality of the results. After checking quality assurance criteria, an electronic copy of the data files is stored on disk and a hard copy of the data and results are printed for archival purposes. Specific details of the use of the acquisition software and workstation can be found in the operation manuals provided by Waters/Millipore Corporation.

CYP1A

Hepatic microsomes are prepared from frozen liver samples as described previously (Collier et al., 1995) except that microsomes are resuspended in 0.25 M sucrose made up in 80/20 v/v water/glycerol. The suspensions are frozen at -80°C until CYP1A assays are performed. Aryl hydrocarbon hydroxylase (AHH) activities are assayed in triplicate at 25° using ^{14}C-BaP as the primary substrate (Collier et al., 1995) .

DNA adducts

Hepatic xenobiotic DNA adducts are measured according to the methods of Reichert and French (1994).

Age determination

Fish age is determined to the nearest year by counting the number of clearly defined opaque zones of whole otoliths under a binocular dissecting microscope (Chilton and Beamish, 1982).

Histology

Livers, kidney and gonad are preserved in Deitrichs' fixative. Tissues are embedded in paraffin, sectioned, stained with hematoxylin and eosin and examined microscopically. Hepatic lesions are classified according to the criteria outlined in Myers et al. (1987), then grouped into the following categories: neoplasms (hepatocellular carcinoma, cholangiocellular carcinoma, adenoma, and cholangioma); foci of cellular alteration (eosinophilic foci, basophilic foci, clear cell foci), specific degeneration/ necrosis (nuclear pleomorphism and/or megalocytic hepatosis), hydropic vacuolation, and non-specific necrotic lesions. Gonad lesions and developmental stage are examined and classified as described Johnson et al. (1991) and Sol et al. (1998b).

Organic chemical Analyses.

Organic analyses on muscle tissue are conducted as described in the Juvenile Salmon Exposure Assessment section.

Metals Analyses

Metals analyses on muscle tissue are conducted as described in Meador et al. (1994). Tissue samples are dried and digested with nitric acid using a combination of convective and micro-wave heating. The digest is initially treated with hydrogen peroxide, then diluted with water. The digest is then analyzed using an atomic absorption spectrophotometer. Analytes measured include aluminum, silicon, iron, chromium, manganese, nickel, copper, zinc, arsenic, selenium, silver, cadmium, tin, antimony, mercury, and lead.

Biliary FACs Analyses

Biliary FACs are is measured as described in the Juvenile Salmon Exposure Assessment section.

Biochemical Analyses.

CYP1A and DNA adducts are measured as described in the Juvenile Salmon Exposure Assessment section.

Reproductive Toxicology

Plasma 17-β estradiol and 11-ketotestosterone Concentration.

A 1-3 mL blood sample is taken from each fish with a heparinized syringe. Blood samples are centrifuged at 800xg and plasma samples are collected, frozen, and stored at –80°C until analyses are conducted. Plasma 17-β estradiol and 11-ketotestosterone levels are determined by radioimmunoassay as described in Sower and Schreck (1982).

Plasma Vitellogenin.

A 1-3 mL blood sample is taken from each fish with a heparinized syringe. Blood samples are centrifuged at 800 x g, and a protease inhibitor (phenylmethylsulfonyl fluoride or aprotinin) is added to prevent breakdown of vitellogenin. Plasma is stored at -80°C until analyses are conducted. Plasma vitellogenin levels is measured by enzyme-linked immunosorbent assay (ELISA) as described in Lomax et al. 1998.

Histological Analyses.

Histological processing of tissues and examination of the liver for toxicopathic lesions is conducted as described above in the toxicological conditions study. The developmental stage of ovaries is determined and classified according to criteria described in Johnson et al. (1991). Ovaries are also examined for follicular atresia, hermaphroditism, ovarian macrophage aggregates, and other inflammatory lesions associated with oocyte resorption, including lymphoid or macrophage infiltrates, using criteria described in Johnson et al. (1991). The developmental stages of testes are determined and classified according to criteria described in Sol et al. (1998b), and testes are examined for inflammatory, necrotic, and proliferative lesions.

Determination of Somatic Indices.

Fish are weighed (to the nearest gram) and measured (fork length, to the nearest mm), and liver and gonads are excised and weighed (to the nearest gram). All other internal organs are then removed, and the animal is weighed (to the nearest gram) to determine gutted body weight.

Gonadosomatic index (GSI) (Nikolsky 1963, Shul-man 1974) is calculated according to the formula:

$$GSI = (\text{ovary weight (g)} / \text{gutted body weight (g)}) \times 100$$

Hepatosomatic index (HSI) (Nikolsky 1963, Shul-man 1974) is calculated according to the formula:

$$HSI = (\text{liver weight (g)} / \text{gutted body weight (g)}) \times 100$$

Condition factor. Because low body weight may be associated with suppressed ovarian development in adult female fish (Burton and Idler, 1987), a condition factor is determined for all sampled animals so the influence of emaciation on ovarian development can be distinguished from any potential effects of contaminant exposure. Condition factor (Ricker, 1975) is calculated using the formula:

$$\text{Condition factor} = \text{gutted body weight (g)} / \text{length}^3\,\text{(cm)}$$

Age Determination.

Otoliths are examined to determine age of the fish as described for the toxicopathic condition study.

Biliary FACs Analyses

Biliary FACs are measured as described in the Juvenile Salmon Exposure Assessment section.

Sediment Contamination Characterization

Organic Chemical Analyses of Sediments (AH Screening).

Sediments collected at fish collection sites are extracted by sonication and analyzed by high-performance liquid chromatography (HPLC) with fluorescence detection as described by Krahn et al. (1991). Briefly, sediment (2.0 g), sodium sulfate (10 g), activated copper (1 mL) and methylene chloride (20 mL) is mixed together in a centrifuge tube. The tubes are placed into a sonic bath for 15 min and then centrifuged at 1,500 rpm for 5 min. Each sample extract is decanted into a 50-mL concentrator tube. To each sediment sample, a 10-mL aliquot of methylene chloride is added, the sediment mixture is stirred and sonicated for 5 min and then centrifuged and decanted as before. This step is repeated and the three extracts are combined. The HPLC internal standard (polystyrene; 5,000 µg) is added to each sediment extract and each solution is concentrated by evaporation to ~4 mL.

A portion (15 µL) of the extract is injected on a size-exclusion HPLC column and eluted isocratically with methylene chloride (flow of 2.5 mL/min); the fluorescence is monitored at 260/380 nm (phenanthrene wavelengths; where phenanthrene, polystyrene, and other 2- 3-ring aromatic compounds fluoresce) and at 380/430 nm (where many 4- and 5-ring aromatic compounds, including BaP, fluoresce). The HPLC system is calibrated with dimethylnaphthalene to determine the beginning of the eluation of the fraction containing the aromatic compounds (retention times of >8.2 min for our HPLC system). Then, the chromatographic areas of the AC fraction are integrated at each set of wavelengths, and the PHN and BaP equivalents (µg/g, wet weight) are calculated by comparison to equivalent areas from the PHN and BaP standards.

Each set of sediment samples (13 field samples) for HPLC screening is accompanied by a method blank, a reference sediment material and a replicate of one of the samples. In addition, the HPLC system is calibrated daily and duplicate HPLC analyses are performed on approximately 10% of the samples.

Total organic carbon and grain size.

Sediment samples are analyzed for total organic carbon (TOC) and grain size by an outside laboratory (e.g., Columbia Analytical, Kelso, WA).

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APPENDIX 1. SAMPLE COLLECTION SUMMARY FOR KITIMAT ENVIRONMENTAL ASSESSMENT

TABLE 1. KITIMAT SALMON EXPOSURE SAMPLE COLLECTION SUMMARY

Date	Site Name / Hatchery	Bile Comp#	SC Comp#	PPL Comp#	AHH Comp#	Body Chem Comp #	Lipid det	Sed chem, TOC, Grain size
5/12/00	Upper Kitimat R., Hatchery	1 (23 indiv)	1 (10 indiv)	1 (15 indiv)	1(15 indiv)	1 (10 indiv, matched to SC	1 comp (10 indiv)	
5/12/00	Upper Kitimat R., Hatchery	2 (22 indiv)	2 (10 indiv)	2 (15 indiv)	2(15 indiv)	2 (10 indiv, matched to SC)		
5/12/00	Upper Kitimat R., Hatchery	3 (26 indiv)	3 (10 indiv)	3 (15 indiv)	3(15 indiv)	3 (10 indiv, matched to SC)		
5/12/00	Lower Kitimat R., Hatchery	1 (24 indiv)	1 (13 indiv)	1 (15 indiv)	1 (15 indiv)	1 (10 indiv, matched to SC	1 comp (10 indiv)	
5/12/00	Lower Kitimat R., Hatchery	2 (24 indiv)	2 (4 indiv)	2 (15 indiv)	2 (15 indiv)	2 (10 indiv)		
5/12/00	Lower Kitimat R., Hatchery	3 (22 indiv)		3 (15 indiv)	3 (15 indiv)	3 (10 indiv)		
5/12/00	Kildala R., Hatchery	1 (23 indiv)	1 (20 indiv)	1 (15 indiv)	1 (15 indiv)	1 (10 indiv)	1 comp (10 indiv)	
5/12/00	Kildala R., Hatchery	2 (26 indiv)	2 (12 indiv)	2 (15 indiv)	2 (15 indiv)	2 (10 indiv)		
5/12/00	Kildala R., Hatchery	3 (24 indiv)	3 (13 indiv)	3 (15 indiv)	3 (15 indiv)	3 (10 indiv)		
5/13/00	Hospital Beach	1 (25 indiv)	1 (25 indiv)	1 (15 indiv)	1 (15 indiv)	1 (10 indiv)	1 comp (10 indiv)	All
5/13/00	Hospital Beach	2 (24 indiv)	2 (32 indiv)	2 (15 indiv)	2 (15 indiv)	2 (10 indiv)		
5/13/00	Hospital Beach	3 (19 indiv)	3 (22 indiv)	3 (15 indiv)	3 (15 indiv)	3 (10 indiv)		
5/13,14,16/2000	Eurocan Beach	1 (22 indiv)	1 (10 indiv)	1 (10 indiv)	1 (18 indiv)	1 (10 indiv)	1 comp (10 indiv)	All
5/13,14,16/2000	Eurocan Beach	2 (32 indiv)	2 (31 indiv)	2 (10 indiv)	2 (15 indiv)	2 (10 indiv)		
5/13,14,16/2000	Eurocan Beach	3 (21 indiv)	3 (21 indiv)	3 (15 indiv)	3 (15 indiv)	3 (10 indiv)		
			4 (31 indiv)					
5/14/00	ALCAN Inner Harbor	1 (27 indiv)	1 (40 indiv)	1 (15 indiv)	1 (15 indiv)	1 (10 indiv)	1 comp (10 indiv)	All
5/14/00	ALCAN Inner Harbor	2 (28 indiv)	2 (37 indiv)	2 (15 indiv)	2 (15 indiv)	2 (10 indiv)		
5/14/00	ALCAN Inner Harbor	3 (29 indiv)	3 (37 indiv)	3 (15 indiv)	3 (15 indiv)	3 (9 indiv)		
5/15/00	Wathlsto Creek	1 (26 indiv)	1 (27 indiv)	1 (15 indiv)	1 (15 indiv)	1 (10 indiv)	1 comp (10 indiv)	All
5/15/00	Wathlsto Creek	2 (23 indiv)	2 (28 indiv)	2 (15 indiv)	2 (15 indiv)	2 (10 indiv)		
5/15/00	Wathlsto Creek	3 (24 indiv)	3 (27 indiv)	3 (15 indiv)	3 (15 indiv)	3 (10 indiv)		
5/15/00	Minette Bay	1 (23 indiv)	1 (30 indiv)	1 (15 indiv)	1 (15 indiv)	1 (10 indiv)	1 comp (10 indiv)	All
5/15/00	Minette Bay	2 (25 indiv)	2 (26 indiv)	2 (15 indiv)	2 (15 indiv)	2 (10 indiv)		
5/15/00	Minette Bay	3 (53 indiv)	3 (27 indiv)	3 (15 indiv)	3 (15 indiv)	3 (10 indiv)		
5/17/00	Kildala Estuary	1 (21 indiv)	1 (30 indiv)	1 (15 indiv)	1 (15 indiv)	1 (10 indiv)	1 comp (10 indiv)	All
5/17/00	Kildala Estuary	2 (30 indiv)	2 (30 indiv)	2 (15 indiv)	2 (15 indiv)	2 (10 indiv)		
5/17/00	Kildala Estuary	3 (25 indiv)	3 (25 indiv)	3 (15 indiv)	3 (15 indiv)	3 (10 indiv)		
5/19/00	Kemano Village*	1 (12 indiv)	1 (26 indiv)	1 (10 indiv)	1 (10 indiv)	1 (5 indiv)		
5/19/00	Kemano Village*	2 (27 indiv)		2 (10 indiv)	2 (10 indiv)	2 (4 indiv)		
5/18,19,20/2000	Kemano Village	1 (11 indiv)	1 (11 indiv)	1 (11 indiv)	1 (11 indiv)	1 (10 indiv)	1 comp (10 indiv)	All
5/18,19,20/2000	Kemano Village	2 (17 indiv)	2 (11 indiv)	2 (8 indiv)	2 (10 indiv)	2 (10 indiv)		
5/18,19,20/2000	Kemano Village	3 (13 indiv)	3 (8 indiv)	3 (7 indiv)	3 (10 indiv)	3 (10 indiv)		
5/18,19,20/2000	Kemano Village	4 (23 indiv)	4 (26 indiv)					
6/15/00	Kildala Estuary	1 (18 indiv)	1 (25 indiv)	1 (15 indiv)	1 (10 indiv)	1 (10 indiv)		
6/15/00	Kildala Estuary	2 (13 indiv)	2 (25 indiv)	2 (10 indiv)	2 (15 indiv)	2 (10 indiv)		
6/15/00	Kildala Estuary	3 (17 indiv)	3 (25 indiv)	3 (15 indiv)	3 (10 indiv)	3 (10 indiv)		
6/16/00	ALCAN Inner Harbor	1 (22 indiv)	1 (21 indiv)	1 (11 indiv)	1 (10 indiv)	1 (10 indiv)		
6/16/00	ALCAN Inner Harbor	2 (14 indiv)	2 (10 indiv)	2 (11 indiv)	2 (9 indiv)	2 (10 indiv)		
6/16/00	ALCAN Inner Harbor	3 (14 indiv)	3 (19 indiv)	3 (10 indiv)	3 (10 indiv)	3 (10 indiv)		

*coho salmon; all other samples are chinook salmon

TABLE 2. KITIMAT FLATFISH INJURY SAMPLE COLLECTION SUMMARY

Site		Total	Male	Female	Liver Chem	Bile	Histo	Muscle	Stom. Chem	Liver AHH	Liver PPL	Plasma
Hospital Beach												
	English sole	**52**	33	18	52	39	52	3 Comps	3 Comps	41	51	7
	Yellowfin sole/Repro	**40**	20	20	40	28	40		3 Comps		40	40
	English sole/Repro	**7**		7								
Eurocan												
	English sole	**53**	23	30	53	49	53	3 Comps	3 Comps	44	52	11
	Yellowfin sole/Repro	**41**	21	20	41	34	41		4 Comps		40	41
	English sole/Repro	**11**	1	10								
Kitamaat Village												
	English sole	**40**	10	30	40	38	40	3 Comps	3 Comps	37	40	13
	Yellowfin sole/Repro	**22**	20	2	22	20	22		3 Comps		22	22
	English sole/Repro	**13**	1	12								
Plume												
	English sole	**30**	12	18	30	29	30	3 Comps	3 Comps	30	30	14
	Yellowfin sole/Repro	**23**	21	2	23	21	23		2 Comps		23	23
	English sole/Repro	**14**	1	13								
Kildala Arm												
	English sole	**40**	8	32	40	36	40	3 Comps	3 Comps	40	40	10
	Yellowfin sole/Repro	**42**	19	20	42	37	42		2 Comps		42	42
	English sole/Repro	**10**	1	9								
Kitlope												
	English sole	**31**	18	11	30	24	29	3 Comps	3 Comps	28	31	0

TABLE 2. KITIMAT FLATFISH INJURY SAMPLE COLLECTION SUMMARY

Site	Total	Male	Female	Liver Chem	Bile	Histo	Muscle	Stom. Chem	Liver AHH	Liver PPL	Plasma
Yellowfin sole/Repro	**29**	19	9	29	16	29		3 Comps		28	28
English sole/Repro	**0**										

SEDIMENT COLLECTION - FISH SITES

			Analyses
Hospital Beach	**3 Stations**	**Trawl Track A, B,**	Chemistry and TOC , Grain Size
Eurocan	**3 Stations**	**Trawl Track A, B,**	Chemistry and TOC , Grain Size
Kitamaat Village	**3 Stations**	**Trawl Track A, B,**	Chemistry and TOC , Grain Size
Plume	**3 Stations**	**Trawl Track A, B,**	Chemistry and TOC , Grain Size
Kildala Arm	**3 Stations**	**Trawl Track A, B,**	Chemistry and TOC , Grain Size
Kitlope	**3 Stations**	**Trawl Track A, B,**	Chemistry and TOC , Grain Size
Kemano	**1 Station**	**Off Marina**	Chemistry and TOC , Grain Size

Table 3. Kitimat sediment collection summary

Sediment Site Number	Collection Date	Depth (feet)	Latitude	Longitude
S001	6/6/00	34	53°59.910	128°41.600
S002	6/6/00	33	53°59.910	128°41.530
S003	6/6/00	25	53°59.920	128°41.490
S004	6/6/00	42	53°59.860	128°41.590
S005	6/6/00	42	53°59.870	128°41.550
S006	6/6/00	43	53°59.860	128°41.490
S007	6/6/00	41	53°59.860	128°41.480
S008	6/6/00	26	53°59.860	128°41.390
S009	6/6/00	25	53°59.860	128°41.340
S010	6/6/00		abandon site	3 tries
S011	6/6/00	49	53°59.820	128°41.440
S012	6/6/00	49	53°59.820	128°41.490
S013	6/6/00	45	53°59.820	128°41.440
S014	6/6/00	28	53°59.820	128°41.390
S015	6/6/00	52	53°59.820	128°41.330
S016	6/6/00	50	53°59.820	128°41.290
S017	6/6/00	46	53°59.820	128°41.030
S018	6/7/00	42	53°59.770	128°41.580
S019	6/7/00	48	53°59.770	128°41.540
S020	6/7/00	44	53°59.770	128°41.510
S021	6/7/00	43	53°59.770	128°41.440
S022	6/7/00	52	53°59.780	128°41.340
S023	6/7/00	52	53°59.760	128°41.300
S024	6/7/00	49	53°59.780	128°40.950
S025	6/17/00	38	53°59.760	128°41.480
S026	6/8/00	35	53°59.720	128°41.570
S027	6/8/00	42	53°59.720	128°41.540
S028	6/16/00	45	53°59.720	128°41.500
S029	6/8/00	29	53°59.710	128°41.440
S030	6/16/00	47	53°59.720	128°41.340
S031	6/16/00	52	53°59.720	128°41.300
S032	6/18/00	38	53°59.710	128°40.940
S033	6/18/00	37	53°59.710	128°40.910
S034	6/16/00	18	53°59.670	128°41.580
S035	6/16/00	44	53°59.660	128°41.540
S036	6/16/00	45	53°59.670	128°41.490
S037	6/16/00	44	53°59.670	128°41.440
S038	6/16/00	49	53°59.670	128°41.350
S039	6/16/00	56	53°59.670	128°41.290
S040	6/16/00	55	53°59.760	128°41.260
S041	6/18/00	37	53°59.760	128°40.950
S042	6/18/00	45	53°59.760	128°40.890
S043	6/16/00	46	53°59.760	128°41.540
S044	6/16/00	50	53°59.760	128°41.480
S045	6/16/00	49	53°59.760	128°41.430
S046	6/16/00	45	53°59.760	128°41.390

Sediment Site Number	Collection Date	Depth (feet)	Latitude	Longitude
S047	6/16/00	46	53°59.760	128°41.340
S048	6/16/00	58	53°59.620	128°41.290
S049	6/17/00	55	53°59.610	128°41.250
S050	6/18/00	16	53°59.630	128°41.000
S051	6/18/00	NA	53°59.620	128°40.940*estimate
S052	6/18/00	46	53°59.610	128°40.910
S053	6/17/00	48	53°59.560	128°41.530
S054	6/17/00	48	53°59.560	128°41.490
S055	6/17/00	52	53°59.560	128°41.440
S056	6/17/00	57	53°59.560	128°41.390
S057	6/17/00	88	53°59.560	128°41.340
S058	6/17/00	120	53°59.550	128°41.290
S059	6/17/00	110	53°59.560	128°41.230
S060	6/17/00	120	53°59.560	128°41.190
S061	6/17/00	130	53°59.560	128°41.140
S062	6/17/00	125	53°59.560	128°41.090
S063	6/17/00	125	53°59.560	128°41.030
S064	6/17/00	95	abandon site	no sample
S065	6/17/00	50	53°59.550	128°40.950
S066	6/17/00	55	53°59.550	128°40.900
S067	6/18/00	46	53°59.560	128°40.860
S068	6/18/00		abandon site	too shallow
S069	6/18/00	35	53°59.510	128°41.530
S070	6/18/00	69	53°59.510	128°41.470
S071	6/18/00	100	53°59.510	128°41.430
S072	6/18/00	113	53°59.520	128°41.400
S073	6/18/00	133	53°59.520	128°41.350
S074	6/18/00	154	53°59.510	128°41.290
S075	6/18/00	173	53°59.510	128°41.240
S076	6/18/00	190	53°59.510	128°41.190
S077	6/18/00	191	53°59.510	128°41.160
S078	6/18/00	190	53°59.510	128°41.120
S079	6/18/00	180	53°59.510	128°41.040
S080	6/18/00	190	53°59.510	128°41.000
S081	6/18/00		abandon site	too steep
S082	6/18/00	89	53°59.510	128°40.900
S083	6/18/00	95	53°59.510	128°40.840
S084	6/18/00	95	53°59.510	128°40.780
S085	6/18/00	70	53°59.520	128°40.740
S086	6/18/00		abandon site	too steep
S087	6/18/00	70	53°59.510	128°40.640
S088	6/18/00	85	53°59.510	128°40.590
S089	6/18/00	65	53°59.510	128°40.530
S090	6/18/00	80	53°59.510	128°40.490
S091	6/18/00	45	53°59.510	128°40.430
S092	6/20/00	40	53°59.460	128°41.540
S093	6/20/00	90	53°59.460	128°41.490
S094	6/20/00	135	53°59.460	128°41.440

Sediment Site Number	Collection Date	Depth (feet)	Latitude	Longitude
S095	6/20/00	175	53°59.460	128°41.390
S096	6/20/00	180	53°59.460	128°41.340
S097	6/20/00	200	53°59.460	128°41.290
S098	6/20/00	220	53°59.460	128°41.240
S099	6/20/00	235	53°59.460	128°41.190
S100	6/20/00	240	53°59.460	128°41.150
S101	6/20/00	230	53°59.460	128°41.100
S102	6/20/00	225	53°59.460	128°41.050
S103	6/20/00	236	53°59.460	128°41.000
S104	6/18/00	225	53°59.460	128°40.940
S105	6/18/00	180	53°59.460	128°40.890
S106	6/18/00	180	53°59.460	128°40.840
S107	6/18/00	180	53°59.460	128°40.790
S108	6/18/00	170	53°59.460	128°40.740
S109	6/18/00	170	53°59.460	128°40.690
S110	6/18/00	150	53°59.460	128°40.640
S111	6/18/00	150	53°59.460	128°40.590
S112	6/18/00	135	53°59.470	128°40.550
S113	6/18/00	130	53°59.460	128°40.480
S114	6/18/00	105	53°59.460	128°40.430
S115	6/21/00	90	53°59.450	128°40.390
S116	6/21/00	110	53°59.450	128°40.340
S117	6/21/00	86	53°59.450	128°40.270
S118	6/21/00	85	53°59.440	128°40.200
S119	6/20/00	35	53°59.410	128°41.540
S120	6/20/00	105	53°59.410	128°41.490
S121	6/20/00	150	53°59.410	128°41.440
S122	6/20/00	215	53°59.400	128°41.400
S123	6/20/00	240	53°59.410	128°41.330
S124	6/20/00	245	53°59.410	128°41.290
S125	6/20/00		abandon site	too deep
S126	6/20/00		abandon site	too deep
S127	6/20/00		abandon site	too deep
S128	6/20/00	36	53°59.360	128°41.540
S129	6/20/00	105	53°59.360	128°41.490
S130	6/20/00	185	53°59.360	128°41.450
S131	6/20/00	240	53°59.350	128°41.390
S132	6/20/00	30	53°59.310	128°41.540
S133	6/20/00	90	53°59.310	128°41.490
S134	6/21/00		abandon site	due to river current
S135	6/21/00		abandon site	due to river current
S136	6/21/00	120	53°59.320	128°39.650
S137	6/21/00	190	53°59.310	128°39.590
S138	6/21/00	206	53°59.310	128°39.540
S139	6/21/00	170	53°59.310	128°39.440
S140	6/21/00	135	53°59.310	128°39.390
S141	6/21/00	92	53°59.310	128°39.340
S142	6/21/00	45	53°59.310	128°39.290

Sediment Site Number	Collection Date	Depth (feet)	Latitude	Longitude
S143	6/20/00	32	53°59.260	128°41.590
S144	6/20/00	65	53°59.260	128°41.540
S145	6/20/00	112	53°59.260	128°41.480
S146	6/21/00	195	53°59.260	128°39.430
S147	6/21/00	136	53°59.260	128°39.390
S148	6/21/00	80	53°59.260	128°39.340
S149	6/20/00		abandon site	on beach
S150	6/20/00	80	53°59.200	128°41.640
S151	6/20/00	100	53°59.200	128°41.590
S152	6/20/00	125	53°59.210	128°41.530
S153	6/20/00	172	53°59.210	128°41.490
S154	6/21/00	154	53°59.210	128°39.440
S155	6/21/00	155	53°59.210	128°39.390
S156	6/21/00	100	53°59.210	128°39.340
S157	6/20/00		abandon site	too close to beach
S158	6/20/00	90	53°59.160	128°41.680
S159	6/20/00	140	53°59.160	128°41.640
S160	6/20/00	180	53°59.160	128°41.590
S161	6/21/00	200	53°59.160	128°39.390
S162	6/21/00	145	53°59.160	128°39.340
S163	6/21/00	69	53°59.150	128°39.290
S164	6/20/00	85	53°59.100	128°41.740
S165	6/20/00	150	53°59.100	128°41.690
S166	6/21/00	180	53°59.110	128°39.340
S167	6/21/00	140	53°59.110	128°39.290
S168	6/21/00		abandon site	too close to rock
S169	6/20/00		abandon site	marina on site
S170	6/20/00	70	53°59.060	128°41.770
S171	6/20/00	170	53°59.060	128°41.730
S172	6/21/00	125	53°59.060	128°39.290
S173	6/21/00	60	53°59.040	128°39.250
S174	6/21/00		abandon site	3 tries
S175	6/22/00		abandon site	marina on site
S176	6/22/00		abandon site	marina on site
S177	6/22/00	130	53°59.000	128°41.790
S178	6/10/00	150	53°59.010	128°39.300
S179	6/10/00	71	53°59.000	128°39.250
S180	6/10/00	55	53°59.000	128°39.190
S181	6/10/00		abandon site	3 tries
S182	6/22/00	40	53°59.930	128°41.840
S183	6/22/00	100	53°59.950	128°41.790
S184	6/13/00	170	abandon site	3 tries
S185	6/13/00	180	53°58.950	128°39.240
S186	6/13/00	125	53°58.950	128°39.200
S187	6/13/00	70	53°58.950	128°39.140
S188	6/13/00	41	53°58.950	128°39.090
S189	6/22/00	40	53°58.900	128°41.840
S190	6/22/00	105	53°58.900	128°41.790

Sediment Site Number	Collection Date	Depth (feet)	Latitude	Longitude
S191	6/13/00	220	53°58.910	128°39.280
S192	6/13/00	190	53°58.910	128°39.240
S193	6/13/00	110	53°58.920	128°39.190
S194	6/13/00	75	53°58.910	128°39.160
S195	**see S199**	**see S199**	**see S199**	**see S199**
S196	6/22/00	20	53°58.850	128°41.890
S197	6/22/00	65	53°58.850	128°41.890
S198	6/22/00	150	53°58.850	128°41.890
S199	6/13/00	141	53°58.850	128°41.890
S200	6/13/00	100	abandon site	3 tries
S201	6/13/00	58	53°58.860	128°39.200
S202	6/22/00	30	53°58.800	128°41.890
S203	6/22/00	85	53°58.800	128°41.830
S204	6/13/00	90	53°58.790	128°39.330
S205	6/13/00		abandon site	too close to beach
S206	6/22/00	30	53°58.760	128°41.890
S207	6/22/00	105	53°58.750	128°41.840
S208	6/13/00	141	53°58.740	128°39.380
S209	6/13/00	70	abandon site	too steep
S210	6/22/00	25	53°58.700	128°41.880
S211	6/22/00	120	53°58.700	128°41.840
S212	6/22/00	50	53°58.650	128°41.880
S213	6/22/00	135	53°58.640	128°41.820
S214	6/22/00		abandon site	too close to beach
S215	6/22/00		abandon site	too steep
S216	6/22/00		abandon site	too close to beach
S217	6/22/00		abandon site	too steep
Kitlpe A	6/3/00	160	53°15.930	127°54.520
Kitlope B	6/3/00	178	53°15.140	127°54.420
Kitlope C	6/3/00	150	53°15.280	127°54.360
Kemano A	6/4/00	100	58°28.760	128°07.550
Kemano B	6/4/00	103	58°28.760	128°07.610
Hospital Beach A	6/6/00	215	53°59.130	128°41.570
Hospital Beach B	6/6/00	145	53°59.450	128°41.520
Hospital Beach C	6/6/00	35	53°59.790	128°41.510
Kitimat Village A	6/8/00	156	53°58.310	128°39.300
Kitimat Village B	6/8/00	157	53°58.460	128°39.440
Kitimat Village C	6/8/00	155	53°58.630	128°39.450
Kildala Arm A	6/15/00	205	53°49.970	128°31.390
Kildala Arm B	6/15/00	200	53°49.950	128°31.050
Kildala Arm C	6/15/00	185	53°49.870	128°30.730
Eurocan A	6/16/00	155	53°59.380	128°40.300
Eurocan B	6/16/00	145	53°59.460	128°40.650
Eurocan C	6/16/00	49	53°59.610	128°40.910
Plume A	6/22/00	95	53°54.030	128°46.720
Plume B	6/22/00	130	53°53.910	128°46.890
Plume C	6/22/00	120	53°53.750	128°46.990

APPENDIX 2. SUMMARY OF ANALYTICAL METHODS AND LIST OF ANALYTES

Table 1A. Summary of Analytical Methods for Assessment of Exposure to Juvenile Salmon.

Sample type	Analysis	Reference
Whole bodies	AHs, CHs, pesticides	Sloan et al. 1993
Stomach contents	AHs, CHs, pesticides	Sloan et al. 1993
Bile	AH metabolites	Krahn et al. 1986

Table 1B. Summary of Analytical Methods for Toxicological Conditions in Flatfish.

Sample type	Analysis	Reference
Liver, muscle	CHs, pesticides (detailed) CYP1A DNA adducts	Sloan et al. 1993 Collier et al. 1995 Reichert et al. 1994
Bile	AH metabolites	Krahn et al. 1986
Liver, kidney, spleen, gonad	Histopathology	Liver lesions classified after Myers et al. (1987). Ovarian stages and lesions classified after Johnson et al. (1991). Testis stages and lesions classified after Sol et al. (1998)
Otoliths	Age determination	Chilton and Beamish 1982

Table 1C. Summary of Analytical Methods for Reproductive Toxicology in Flatfish.

Sample type	Analysis	Reference
Liver	CHs, pesticides if biological effects detected	Krahn et al. 1994 (screening); Sloan et al. 1993 (detailed)
Bile	AH Metabolites	Krahn et al. 1986
Liver, gonad	Histopathology	Liver lesions classified after Myers et al. (1987). Ovarian stages and lesions classified after Johnson et al. (1991). Testis stages and lesions classified after Sol et al. (1998)
Otoliths	Age determination	Chilton and Beamish 1982
Ovary, liver and gutted body weight	Gonadosomatic index Hepatosomatic index Condition index	Johnson et al. (1991)

Blood plasma	plasma E2, 11-KT levels, and vitellogenin levels	Sower and Schreck (1982); Lomax et al. (1998).

Table 1. Cont.

Table 1D. Summary of Analytical Methods for Sediment Contaminant Characterization

Sample type	Analysis	Reference
Sediment	AH screening	Krahn et al. 1991
Sediment	TOC and grain size	
Sediment	AHs, CHs, pesticides (detailed)	Sloan et al. 1993

Table 2. List of Analytes

Detailed Organic Analyses (stomach contents for juvenile salmon, salmon hatchery food, and flatfish muscle samples)

Low molecular weight AHs
Naphthalene
Acenaphthylene
Fluorene
Anthracene
Acenaphthene
Phenanthrene
Retene (1-methyl-7-isopropyl phenanthrene) (indicator of exposure to pulp mill effluent)
2-Methylnaphthalene
LAH [sum of low molecular weight AHs]

High molecular weight AHs
Fluoranthene
Pyrene
Chrysene
Benzo(*a*)pyrene
Dibenz(*ah*)anthracene
Benz(*a*)anthracene
Benzo(*b*+*k*)fluoranthenes
Ideno(1,2,3-*cd*)pyrene
Benzo(*ghi*)perylene
HAHs [sum of high molecular weight AHs]

PCBs (stomach contents, muscle)
PCB congeners (Nos. 18, 28, 44, 52, 66, 101, 118, 128, 138, 153, 170, 180, 187, 195, 206, 209)

DDTs (stomach contents, muscle)
p,p'-DDE
p,p'-DDT
p,p'-DDD

Pesticides (stomach contents, muscle)
Aldrin
Chlordane [sum of α and γ]

Heptachlor
Dieldrin
Lindane
Hexachlorobenzene

Table 2 Cont.

Metals
(muscle tissue from flatfish)

Aluminum
Iron
Chromium
Manganese
Nickel
Copper
Zinc
Arsenic
Selenium
Silver
Cadmium
Tin
Mercury
Lead