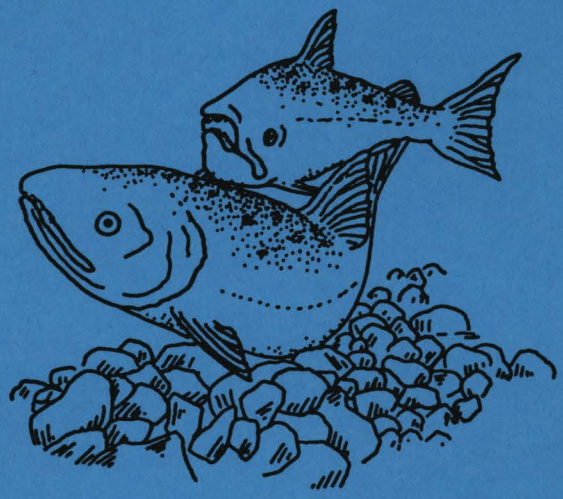
Cytochrome P-450 Induction of Pink Salmon (*Oncorhynchus gorbuscha*) Eggs and Larvae In Prince William Sound, Alaska: Effects of the *EXXON Valdez* Oil Spill

By
Michael Wiedmer

Technical Report No. 92-3



Alaska Department of Fish and Game
Habitat Division

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Table of Contents

List of Tables

List of Figures

List of Appendices

Abstract

The 24 March, 1989 *EXXON Valdez* spill in Prince William Sound and the Gulf Alaska oiled a minimum of 213 intertidal pink salmon spawning streams. Some stream sediments remained contaminated through spring 1992. To aid the development of anadromous fish stream clean-up recommendations, the Alaska Department of Fish and Game, Habitat Division, assessed the induction of Cytochrome P-450 in pre-emergent pink salmon eggs and alevins exposed to oil contaminated stream sediments. Cytochrome P-450 mediates the metabolism of xenobiotics such as polyaromatic hydrocarbons, which are constituents of North Slope crude oil. Elevated levels of P-450 indicate exposure to environmental pollutants. Between December 1989 and May 1991, pre-emergent pink salmon eggs and alevins were collected from four oiled and five unoiled sites in Prince William Sound and analyzed with a monoclonal antibody for P-450. Thirteen of sixteen alevin samples collected at oiled sites had elevated levels of P-450; no samples collected at unoiled sites had elevated levels of P-450. Up to eleven tissue types were induced in individual oiled samples; no tissue types were induced in unoiled samples. Regardless of contamination levels, no egg samples had elevated P-450 concentrations. P-450 induction persisted through the end of the study, spring 1991, indicating persistent chronic exposure to hydrocarbon contamination. Elevated levels of Cytochrome P-450 during salmon larval stages, periods of rapid cellular differentiation and organogenesis, suggest the potential for increased mutagenesis and lower future reproductive success.

Introduction

In the early morning hours of 24 March, 1989, the tanker *EXXON Valdez* struck Bligh Reef, 40 kilometers southwest of Valdez, Alaska, rapidly spilling 10.8 million gallons (257,000 barrels) of North Slope crude oil into the waters of Prince William Sound. In the days that followed, currents and winds carried the slick toward the islands of the western Sound and into the Gulf of Alaska.

At its maximum extent, oil reached beyond Chignik on the Alaska Peninsula, 800 km from Bligh Reef; oiling almost 2000 km of beaches. By the end of 1989, an estimated 17,000 bbls (6.6 %) of oil were recovered, 356 bbls (0.1 %) were burned, 77,100 bbls (30.0 %) evaporated, and the remaining 162,500 bbls (63%) were unaccounted for (Evans, pers. comm., 1991[1]). Much of the unaccounted oil washed ashore within the spill area's intertidal zone.

The State of Alaska, Departments of Environmental Conservation (DEC), Fish and Game (ADF&G), and Natural Resources (DNR) participated in the resultant spill response and clean-up. The ADF&G took a lead role in monitoring the clean-up of sensitive fish and wildlife habitats. In particular, the ADF&G was concerned with the adequacy of anadromous fish stream clean-up. Remaining oil, even after initial cleaning and weathering, might continue to impact salmon eggs and larvae developing within contaminated stream substrates.

Prince William Sound has a maritime climate, with a total annual precipitation ranging from 156 cm (Valdez) to 481 cm (Whittier) (AEIDC, 1989). The abundant rainfall and snowmelt drains from the rugged coastline through many small coastal streams. In many

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[1]L. J. Evans, Public Information Officer, DEC, Anchorage, Alaska

streams, high water velocities or falls prevent anadromous fish from ascending to upstream spawning habitats.

At least one anadromous species, pink salmon (*Oncorhynchus gorbuscha*), has responded to the relative unavailability of upstream habitat by spawning within the stream intertidal zone. Pink salmon are documented in over 900 streams in Prince William Sound (ADF&G, 1991), and spawn intertidally in most of these. Total annual Prince William Sound adult pink salmon wild stock production between 1978 and 1990 ranged from 4.0 to 21.0 million (Brady, 1990). Up to 75 % of this production comes from intertidal spawning habitats (Sharr, pers. comm., 1990[2]). In 1990, in addition to sport and subsistence catch, 12.3 million wild stock pink salmon were harvested by commercial fishermen, for an ex-vessel value of $ 11 million (Brady, 1990).

Surveys conducted by the ADF&G (Middleton et al., 1992) indicate approximately 106 intertidal pink salmon spawning streams within Prince William Sound oiled to some degree. At many streams, oil remained in stream substrates in and near spawning areas through the winter of 1989/1990. At several streams, oil remained through the winter of 1990/1991 and even into 1992.

In Prince William Sound, sexually mature pink salmon spawn deposit their eggs within the gravel and cobble substrates of intertidal and supratidal stream channels from late July through early October. Adults typically spend only a few days within the spawning channel, moving in and out of intertidal spawning areas with the tides. All adult pink salmon die soon after spawning.

The embryonic development within the interstitial spaces of stream substrates is dependent upon physical factors including temperature

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[2]S. Sharr, ADF&G, Commercial Fisheries Division, Cordova, Alaska.

and salinity. Constant surface stream flows are not necessary to insure survival; developing embryos are frequently maintained only by subsurface flows. Developing pink salmon embryos in the intertidal zone experience constant fluctuations of temperature and salinity. In western Prince William Sound, nearshore surface marine temperatures range from approximately 8° C in October to 3° C in December through March, and up to 7° C by May (AFK Hatchery, pers. comm., 1991[3]). Stream temperatures range from an average of 5.5° C in October to 0.5° C between December and May (Wiedmer, pers. observ., 1989, 1990). Autumn rains lower nearshore salinity to levels near 6 ppt in August and September. Freshwater runoff significantly decreases between late November and early March while air temperatures predominantly remain below freezing. Nearshore salinity reaches a maximum of 32 ppt in March (Cooney, pers. comm., 1991[4]).

Hatching typically occurs during December and January. In samples collected during the first week of December, the proportion of alevins to eggs ranged from 0 to 0.15. However, recently hatched alevins were observed as early as late September (Wiedmer, pers. observ., 1990). Fry emerge from spawning gravels between April and June. Emergence timing is temperature dependent. Fry incubating in warmer, lower intertidal zones outmigrate before fry incubating in cold, supratidal zones. During the 1990 outmigration, emergence from streams within the study area increased to a relatively constant rate during the last half of May (Sharp, pers. comm., 1990[5]). After emergence, pink fry feed in the nearshore zone for several months, gradually moving into deeper water as they mature (Scott and Crossman, 1973). Developing juveniles move into the

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[3]Armin F. Koernig Hatchery, Chenega, Alaska.

[4]Dr. T. Cooney, University of Alaska, Fairbanks; Institute of Marine Science.

[5]D. Sharp, ADF&G, Commercial Fisheries Division, Cordova, Alaska.

Gulf of Alaska, where they grow rapidly. They return to spawn in their natal streams approximately 16 months after emergence.

Fish are generally most sensitive to environmental pollutants as juveniles (McKim, 1977; Moles, et al., 1979). The environmental parameters to which juvenile fish are exposed also regulate their sensitivity to oil. Moles, et al. (1987), demonstrated that pre-emergent[6] pink salmon reared in a simulated tidal environment were more sensitive to the water soluble fraction (WSF) of Cook Inlet crude oil than pre-emergent pink salmon reared in fresh water. Oil-exposed juveniles in the intertidal regime had reduced yolk reserves and accumulated more hydrocarbons than juveniles in fresh water. Emergent pink salmon in seawater were twice as sensitive to Prudhoe Bay crude oil WSF than emergent pink salmon in fresh water (Moles, et al., 1979).

Malins, et al. (1977), found an inverse relation in salmon between hydrocarbon accumulation and rearing temperature. The 96 hour TLm (median tolerance limit) of pink salmon fry exposed to toluene was significantly lower at 4° C than at 12° C. The same trend was observed with the WSF of Cook Inlet Crude (Korn, et al., 1979).

Purpose and Rationale

The purpose of this study was to determine if *EXXON Valdez* oil residue in and near pink salmon spawning areas altered the physiological processes of developing pink salmon larvae. This information was critical for the ADF&G's oiled stream clean-up recommendations. Additional issues addressed by this study

[6]The term "pre-emergent" encompasses all developmental life stages occurring while the developing embryo remains within the interstitial spaces of the spawning substrate. By definition, the pre-emergent stage ends when the fry leaves the substrate and becomes free swimming. This stage approximately corresponds with the completion of yolk absorption (buttoning-up).

include: 1) development of linkages between observed oil in and near pink salmon spawning habitat and observed biological injuries as determined by other studies; and 2) an assessment of the field practicability of the Cytochrome P-450 immunohistochemical technique, which had not been previously used on a significant scale outside the laboratory.

Standard techniques for assessing biological injury to pre-emergent pink salmon are readily available. However, arguments that observed injuries are due to oil exposure are strengthened by demonstrating that injured fish have incorporated petroleum hydrocarbons and/or metabolites in their tissues. Direct measurement of parent hydrocarbons or metabolites in fish tissue (typically bile) is difficult (Malins, 1977), and the very small size of individual pre-emergent pink salmon and the relatively large sample sizes that must be collected to measure hydrocarbons precluded the approach in this study.

As the clean-up progressed, the ADF&G encountered increasing resistance to cleaning oiled pink salmon spawning areas. Technical advisors to spill managers implied that remaining oil was essentially inert and would not induce physiological changes in pre-emergent salmon. To determine if residual oil was being incorporated into developing salmon tissue, and thus raising concerns for biological injury, the ADF&G required a detectable marker that would reliably indicate exposure of individual pre-emergent pink salmon to petroleum hydrocarbons.

In recent years, Cytochrome P-450-dependent monooxygenase (MO) systems in fish have been validated as reliable markers of environmental contamination (Payne, 1976; Kurelec, et al., 1977; Stegeman, 1980; Lech, et al., 1982; Spies, et al., 1982; Stegeman, 1987). These enzymes, associated with the endoplasmic reticulum (Sivirajah, et al., 1977), mediate the oxidation of petroleum hydrocarbons and other xenobiotics. Cytochrome P-450 mediates the

conversion of hydrophobic parent compounds to more readily excreted hydrophilic metabolites (Figures 1 and 2).

Cytochrome P-450 is induced by environmental contaminants. In non-polluted environments, fish synthesize low levels of P-450. However, fish respond to increased levels of contamination by synthesizing increased enzyme concentrations. This increase in enzyme synthesis can be used as an indicator of exposure to environmental pollution.

Several pollutants can induce Cytochrome P-450 and its dependent monooxygenases (also called mixed-function-oxidases (MFOs): polychlorinated biphenyls (PCBs) and polyaromatic hydrocarbons (PAHs) are common inducers of the enzyme system. In areas of chronic industrial pollution, it is difficult to isolate specific inducing pollutants. However, Prince William Sound, particularly that portion impacted by the spill, was a largely pristine environment prior to March 1989.

In laboratory studies, fish ingesting PAH and PCB contaminated food (Spies, et al., 1982), or exposed to PAH or PCB water soluble fractions (Kurelec, et al., 1977; Binder and Stegeman, 1979; Binder and Stegeman, 1983; Binder, et al., 1985; Goksøyr and Solberg, 1987), or intraperitoneally injected with PAHs (Kloepper-Sams and Stegeman, 1989) showed a significant increase in inducible enzyme activity.

Studies of free-ranging fish exposed to petroleum contamination (Burns, 1975; Payne, 1976; Kurelec, et al., 1977) and to general industrial contamination (Spies, et al., 1982; Foureman, et al., 1983; Spies, et al., 1988) all demonstrated measurable increases in MO/Cytochrome P-450 activity.

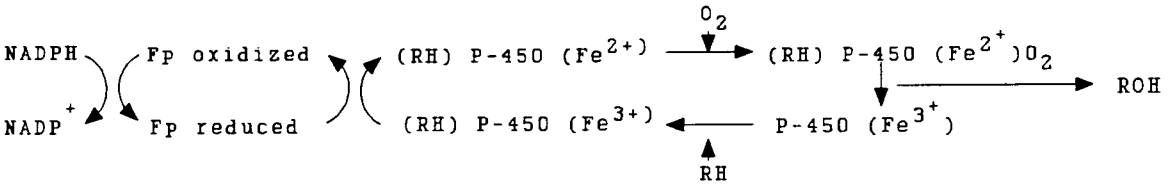


Figure 1. Scheme of hepatic microsomal P-450 MO system (From Lech et al., 1982).

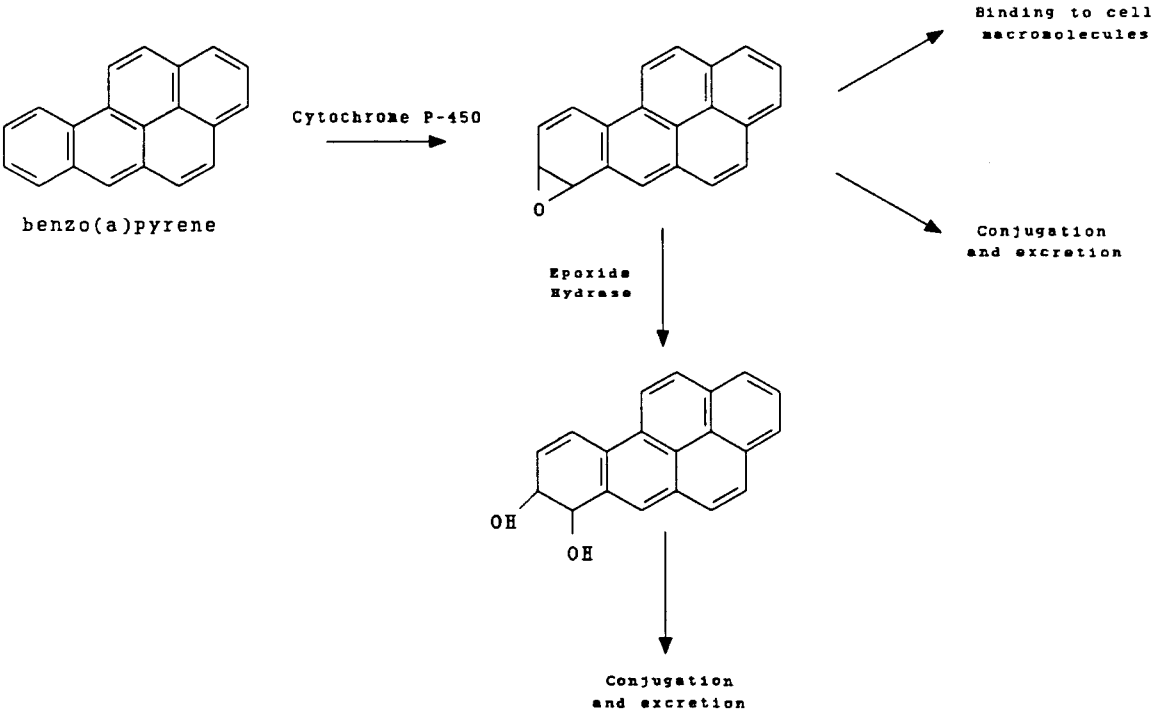


Figure 2. Simplified scheme for metabolism of benzo(α)pyrene by fish (Adapted from Stegeman, 1987).

While the correlation between exposure to contaminants and increases in Cytochrome P-450 is well documented, P-450 concentrations are known to fluctuate in healthy fish from non-polluted environments. Enzyme activity is dependent upon the sex, sexual maturity, age, and diet of the individual (Lech, et al., 1982; Stegeman, 1987). Embryonic (pre-feeding) forms of estuarine, marine, and freshwater fish are competent to respond to induction. Binder and Stegeman (1984) detected low enzyme activity in eggs of the estuarine killifish *Fundulus heteroclitus*. Yet twenty-four hours after hatching, larval enzyme activity increased 9-fold. Marine cod (*Gadus morhua*) larvae demonstrated marked increases in activity immediately after hatching (Goksøyr and Solberg, 1987). Freshwater brook trout (*Salvelinus fontinalis*) eggs and alevins also responded to inducers (Binder and Stegeman, 1983).

Various methods for assessing induction in fish have been proposed (Stegeman, 1987). However, two methods have particular relevance to the ADF&G's investigations. Aryl hydrocarbon hydroxylase (AHH, a specific MFO) is induced by PAHs. AHH is isolated typically from the hepatic microsomal fraction. AHH activity is determined by measuring the rate at which the isolated enzyme produces metabolites of an artificial substrate (e.g., radioactively labeled benzo(a)pyrene (^{3}H-BaP)). This method has the advantage of providing reasonably quantitative results.

This technique requires isolated livers, which are difficult to collect from fish small as embryonic pink salmon and requires a large number of individual livers pooled into one sample. The activity of AHH degrades rapidly after death. To provide meaningful results, livers must be excised immediately *post mortem*, maintained in liquid nitrogen, and processed rapidly. While these conditions can be readily met in the laboratory, the realities imposed by winter field work in Prince William Sound make this method impractical. Additionally, as the technique requires bulk

homogenized tissue, it does not discriminate induction of specific tissues.

The other potential assay uses an immunohistochemical probe for Cytochrome P-450. In this method, a monoclonal antibody to Cytochrome P-450 is applied to thin sections prepared with standard histological techniques. The level of induction is determined by assessing the intensity of antibody binding and by the extent of tissues induced. While this technique does not provide the same degree of quantification, it is much less sensitive to sample degradation. Tissues preserved in formalin can be satisfactorily analyzed long after excision. Additionally, induction in individual tissues can be evaluated to provide information on the route of exposure and suggest organs susceptible to injury. For the purposes of this study, the immunohistochemical probe was selected as the best method.

Methods and materials

Pre-emergent pink salmon were collected from spawning gravels of nine Prince William Sound streams. Both eggs (embryos *sensu* Balon, 1975), and alevins (yolk sac and buttoned-up fry (eleutheroembryos *sensu* Balon, 1975)) were collected. Eggs averaged approximately 6-7 mm in diameter. Alevins averaged approximately 30 mm in length.

Between December 1989 and May 1991 (two brood years), pre-emergent pink salmon were collected from four oiled and five unoiled locations in southwestern Prince William Sound (Figure 3), the region most impacted by the *EXXON Valdez* spill. While some oil may have been observed in the general vicinity of the unoiled sites, the specific sample locations were isolated from any observable contamination. At the contaminated sites, oil deposition occurred primarily during late March and early April 1989. Exposure of

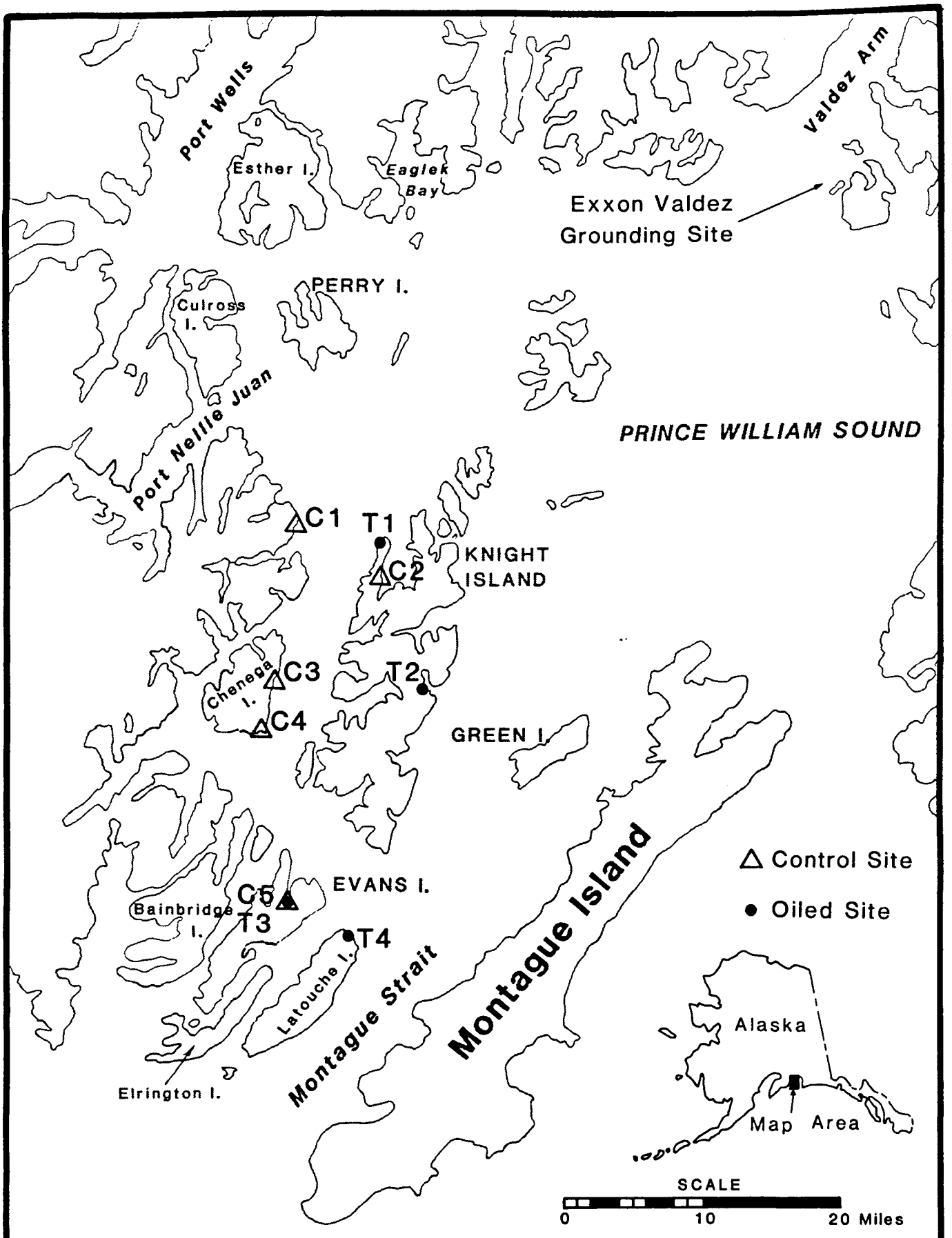


Figure 3. Pink salmon egg/fry collection site locations for the Cytochrome P-450 study, 1989-1991. (ADF&G, Habitat)

developing pink salmon embryos to hydrocarbons was due primarily to chronic leaching from contaminated sediments rather than reoiling from refloated oil.

Pre-emergent pink salmon were collected from intertidal spawning substrates during low tides. Because of freezing temperatures, hand shovels, rather than egg pumps, were used to extract specimens from spawning substrates. Excavated substrates were placed and agitated immediately upstream of a 0.7 m wide by 1.0 m high funnel net. Stream currents flushed the less dense eggs and alevins into the net. Specimens were separated from debris and immediately placed in hydrocarbon sampling jars containing 10% phosphate buffered formalin (Appendix I). Samples were labeled and processed under chain-of-custody procedures.

Formalin preserved specimens were sent to Woods Hole Oceanographic Institute (WHOI) for the analysis of Cytochrome P-450IA1 (a specific P-450) induction using the techniques of Smolowitz, et al. (1991). Six eggs/alevins constituted a sample. A total of thirty-three samples were analyzed. Fry and eggs were prepared using standard histopathological techniques. The localization and intensity of P-450IA1 was determined in sections prepared by standard histological methods, and stained with monoclonal antibody 1-12-3 and peroxidase-labeled second antibody. Tissue staining intensity and occurrence was determined for each egg or fry, and an overall, relative level of induction was determined for each sample (strong, moderate, mild, very mild, negative). Samples were analyzed blind; i.e. WHOI personnel did not know sample sources.

The experimental unit in this study was the stream. Egg/fry samples were pooled for each stream and stratified by time. The Mann/Whitney (Wilcoxon) two-sample test (Conover, 1980) was used to test for statistical differences between oiled and unoiled streams. Statistical tests were one-tailed and performed at the $\alpha = 0.10$

level. The null hypothesis for all tests was that oiled and unoiled streams were affected equally. Median induction scores (1 = negative, 2 = very mild, 3 = mild, 4 = moderate, 5 = severe) for each stream were calculated.

Representative sediment samples collected at each oiled site were analyzed for n-alkanes and isoprenoids with gas chromatography using flame ionization detection (GC/FID) and for aromatic hydrocarbons using gas chromatography with mass spectrometry (GC/MS). Samples were also fingerprinted (GC/MS) to verify that the oil source was North Slope crude.

At one oiled location, T1, the volume of bulk oil in the stream banks remaining 13 months after initial oiling was estimated. At twelve randomly selected points along the channel, oil surface thickness and depth of penetration were measured. At each point, substrate samples were collected for determination of bulk oil concentration. Bulk oil concentrations in the substrate samples were determined by a cold extraction procedure utilizing a sonicator and a CH_2Cl_2 cold extraction process (IOC, 1989; USEPA SW846 Method 3550, 1986).

Results

Cytochrome P-450 induction in pink salmon alevins collected in Prince William Sound ranged from undetectable in each unoiled sample to strong in fish collected (6/8/90) from Shelter Bay, a heavily contaminated site (Table 1). Samples from each oiled site showed some level of induction. Induction was observed in alevin collected at oiled sites between December 1989 and March 1991 (8 to 24 months after the initial spill).

No organs or tissues were induced in any unoiled samples. As many as eleven tissues were induced in samples from oiled streams. The

Table 1. Cytochrome P-450E induction analysis of pink salmon fry and eggs collected from streams in Prince William Sound, 1989-1991.

Sample No.	Date Collected	Location (Segment)	Stage	Oiled?	Induction	Tissue[1]
T89MW002V	12/8/89	Marsha Bay KN701B	egg	yes	negative	
T89MW003V	12/8/89	Marsha Bay KN701B	fry	yes	mild/mod	K_t
T89MW004V	12/9/89	Chenega Is. CH001A	egg	no[2]	negative	
T89MW005V	12/9/89	Chenega Is. CH001A	fry	no[2]	negative	
T90MW104V	5/25/90	Sleepy Bay LA018A	fry	yes	negative	
T90MW104VB	5/25/90	Sleepy Bay LA018A	fry	yes	very mild	VCC
T90MW105V	5/25/90	Sleepy Bay LA018A	fry	yes	very mild	VCC
T90MW106V	5/30/90	Marsha Bay KN701B	fry	yes	mild	VCC, $I_{m,c}$
T90MW108V	5/30/90	Herring Bay KN132B	fry	no[2]	negative	
T90MW110V	6/2/90	Loomis Ck. EB001A	fry	no[2]	negative	

Table 1. (Continued) Cytochrome P-450E induction analysis of pink salmon fry and eggs collected from streams in Prince William Sound, 1989-1991.

Sample No.	Date Collected	Location (Segment)	Stage	Oiled?	Induction	Tissue[1]
T90MW112V	6/8/90	Herring Pt. KN500B	fry	yes	moderate	G_e, Sk, VCC
T90MW115V	6/8/90	Shelter Bay EV025A	fry	yes	strong	G_e, $K_{t,c}$, H_e, B, PC, L, VCC, $I_{m,c}$, I_e
T90MW903V	9/7/90	Pt. Countess BP004A	eggs	yes	negative	
T90MW904V	9/7/90	Pt. Countess BP004A	eggs	yes	negative	
T90MW905V	9/7/90	Pt. Countess BP004A	eggs	no[2]	negative	
T90MW907V	9/25/90	Shelter Bay EV027A	eggs	no[2]	negative	
T90MW909V	9/25/90	Shelter Bay EV025A	eggs	yes	negative	
T90MW910V	9/25/90	Shelter Bay EV025A	eggs	yes	negative	
T90MW911	9/25/90	Shelter Bay EV025A	eggs	yes	negative	
T90MW914	9/25/90	Thumb Bay KN604A	eggs	no[2]	negative	

Table 1. (Continued) Cytochrome P-450E induction analysis of pink salmon fry and eggs collected from streams in Prince William Sound, 1989-1991.

Sample No.	Date Collected	Location (Segment)	Stage	Oiled?	Induction	Tissue[1]
N3000-91	3/13/91	Sleepy Bay LA018A	fry	yes	moderate	$G_{c,b}$, Y, $K_{c,}$ $K_{s,v}$, $H_{a,v}$,
T91MF001V	3/21/91	Marsha Bay KN701B	fry	yes	moderate	L_s, P, G_c, I_c, $K_{s,v}$
T91MF002V	3/21/91	Marsha Bay KN701B	fry	yes	mild	I_c, K_c
T91MF006V	3/21/91	Shelter Bay EV025A	fry	yes	mod/mild	Y, I_c, L_s, $K_{s,v}$
T91MF008V	3/21/91	Shelter Bay EV025A	fry	yes	moderate	I_c, $K_{s,v}$, G_b, $H_{a,v}$, L_h, Y
T91MF010V	3/21/91	Shelter Bay EV027A	fry	no[2]	negative	
T91MF012V	3/22/91	Kake Cove CH017A	fry	no[2]	negative	
T91MF014V	5/4/91	Sleepy Bay LA018A	fry	yes	moderate	G_c, P, $H_{a,v}$, $K_{s,v}$, K_c, Y, $L_{h,c}$
T91MF015V	5/4/91	Shelter Bay EV025A	fry	yes	mild	L_h, $K_{s,v}$
T91MF017V	5/4/91	Shelter Bay EV027A	fry	no[2]	negative	

Table 1. (Continued) Cytochrome P-450E induction analysis of pink salmon fry and eggs collected from streams in Prince William Sound, 1989-1991.

Sample No.	Date Collected	Location (Segment)	Stage	Oiled?	Induction	Tissue[a]
T91MF019V	5/5/91	Kake Cove CH017A	fry	no[b]	negative	
T91MF021V	5/14/91	Marsha Bay KN701B	fry	yes	negative	
T91MF023V	5/14/91	Marsha Bay KN701B	fry	yes	negative	

[a]Tissue types that immunochemically stained for cytochrome P-450E: B - brain vessel endothelium; $K_{t,e}$ - tubules of anterior kidney and endothelium; $K_{s,v}$ - kidney sinusoidal and vascular endothelium; K_c - kidney collecting duct; $I_{m,c}$ - midgut and cecal epithelium; I_e - anterior or posterior intestine enterocytes; $G_{b,e}$ - gill buds and epithelium; $H_{a,v}$ - heart atrial or ventricular endothelium; H_e - heart endothelium; L_c - liver central veins; $L_{h,s}$ - liver hepatocytes and sinusoidal endothelium; P - pharyngeal epithelium; PC - peritoneal connective tissue; Sk - skin; VCC - vertebral cord cartilage; Y - yolk sac endothelium.

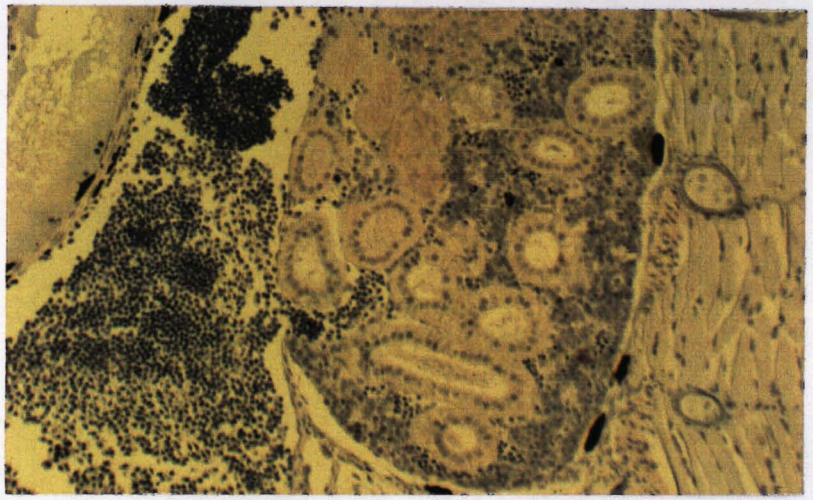
[b]While portions of the general stream area may have been initially oiled, the source of samples was upstream of the zone of contamination.

most commonly induced tissues were kidney sinusoidal and vascular endothelia, gill epithelia, yolk sac endothelia, vertebral cord foci, intestinal enterocytes, heart endothelia, and liver hepatocytes (Figures 4 - 7). Staining intensity and occurrence in each sample are listed in Appendix II. Because all tissues were not visible in sections from each individual fish, some induced tissues may have gone undetected. No statistical tests were performed on alevin samples collected December 1989 due to small sample size. However, examination of staining results indicate a difference in P-450 induction between oiled and unoiled sites (Figure 8). P-450 induction was significantly greater in oiled streams in May and June, 1990 ($P<0.067$) and March 1991 ($P<0.10$). No significant ($P<0.20$) induction was observed in May, 1991. There was no significant change in induction intensity between 1990 and 1991.

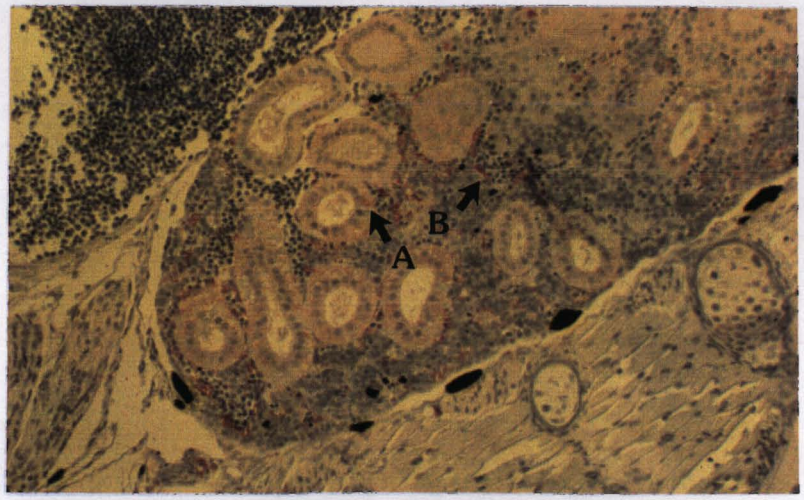
No eggs of any developmental stage collected from either control or oiled sites showed induction. Post-hatching alevins collected on the same date as eggs from an oiled location (T2) showed mild to moderate induction. Eighty-one percent (13 of 16) pink fry samples collected from oiled sites showed P-450 induction.

Petroleum markers in samples from each oiled site verify that the hydrocarbon source of observed oil residue was North Slope Crude (GERG, 1991). Aromatic hydrocarbon concentrations of sediment samples collected at each oiled site are presented in Table 2. Concentrations of total aromatic hydrocarbons ranged from 13 μg/g to 877 μg/g. In all samples, 2-3 ring aromatics predominated, although the 2-3 ring:4-5 ring ratio decreased over time. Thirteen months after the initial spill (early May), the total petroleum hydrocarbon concentration retained in sediments of the intertidal channel banks of site T1 averaged 3.8 g oil/kg sediment (Table 3).

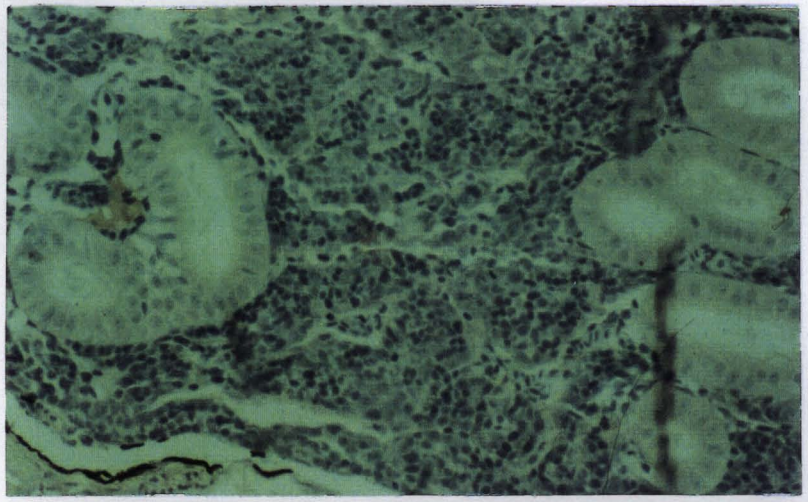
Figure 4. Distribution of Cytochrome P-450IA1 in pink salmon alevin kidney.



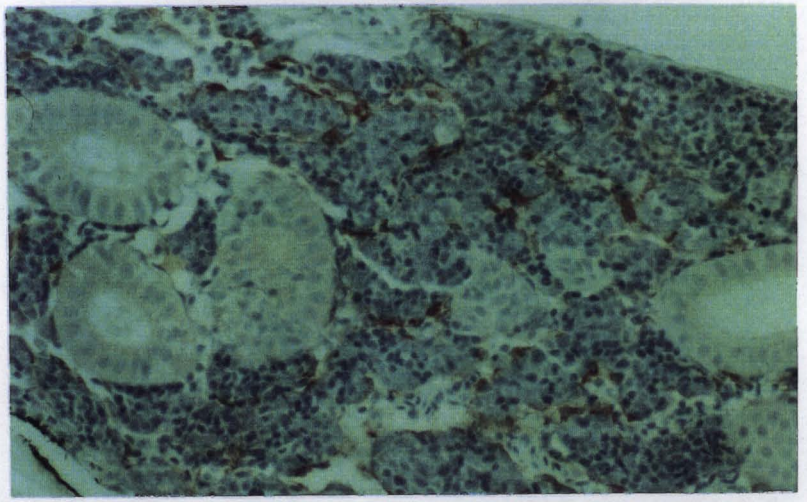
A. Kidney, control for B. Nonspecific antibody substituted for specific antibody in immunochemical staining procedure. No specific staining present (100x).



B. Kidney. Positive and specific staining in tubular epithelium (A) and hematopoietic vascular endothelium (B) in fish from oiled stream (100x).



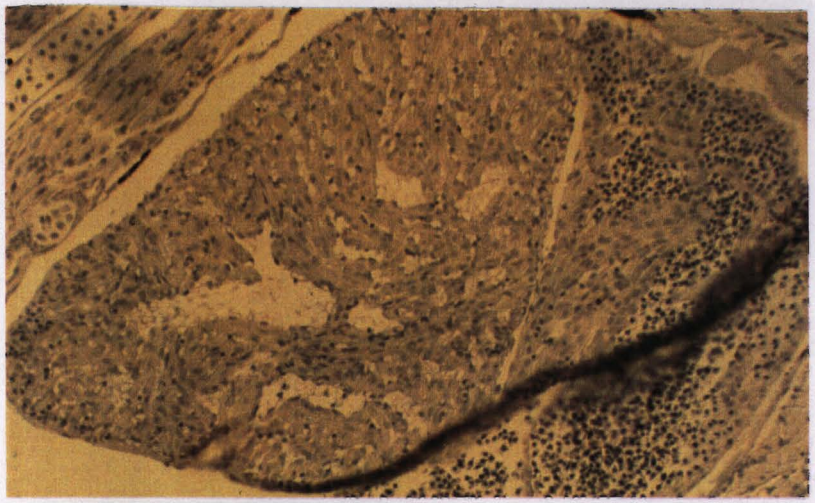
C. Kidney, control for D. Nonspecific antibody substituted for specific antibody in immunochemical staining procedure. No specific staining present (250x).



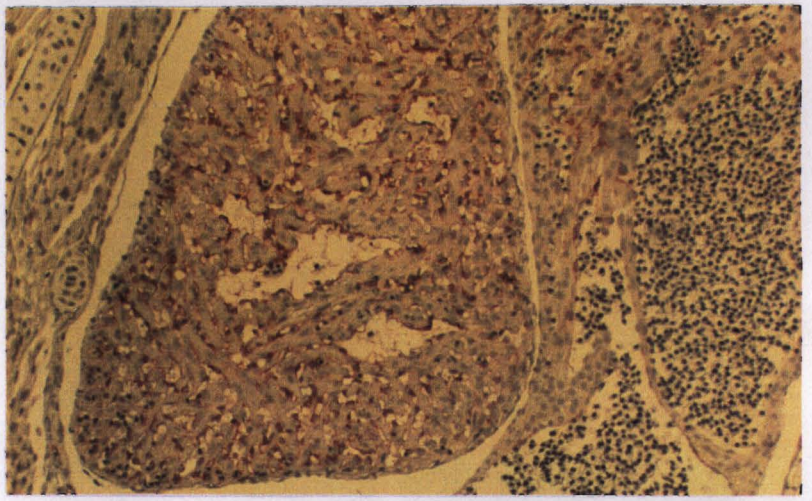
D. Kidney. Positive and specific staining in hematopoietic vascular endothelium in fish from oiled stream (250x).

Microphotographs by R. Smolowitz.

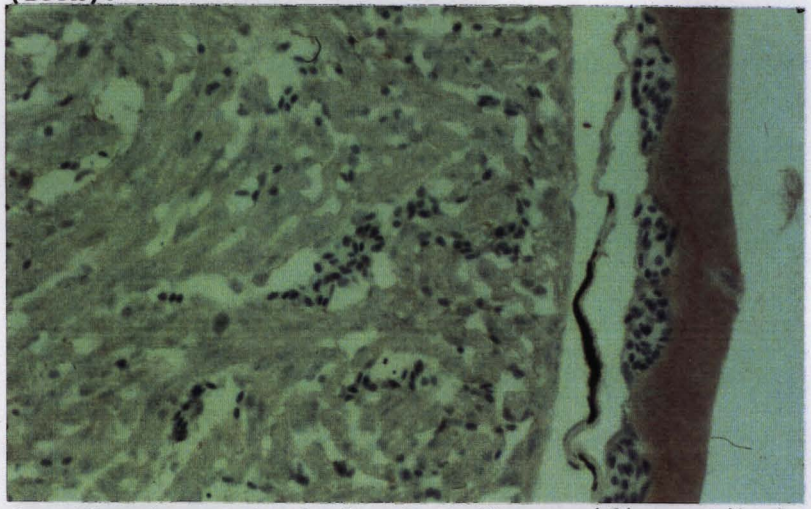
Figure 5. Distribution of Cytochrome P-450IA1 in pink salmon alevin heart.



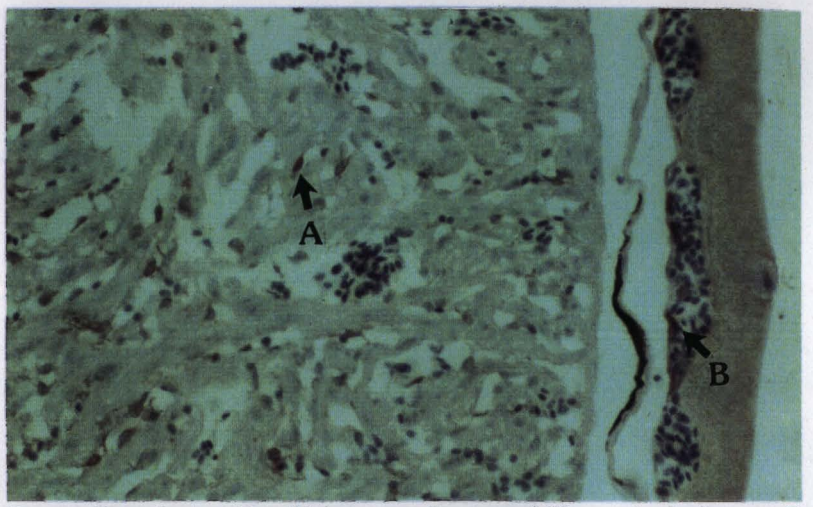
A. Heart, control for B. Nonspecific antibody substituted for specific antibody in immunochemical staining procedure. No specific staining present (100x).



B. Heart. Positive and specific staining of endocardium in fish from oiled stream (100x).



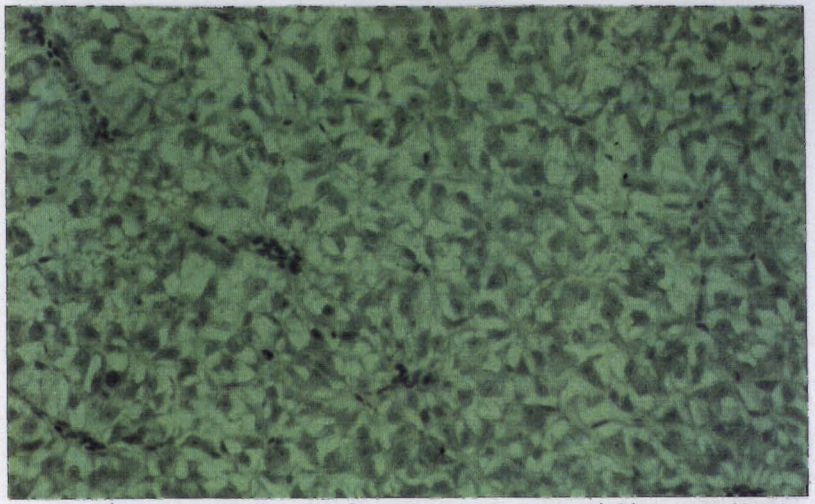
C. Heart, control for D. Nonspecific antibody substituted for specific antibody in immunochemical staining procedure. No specific staining present (250x).



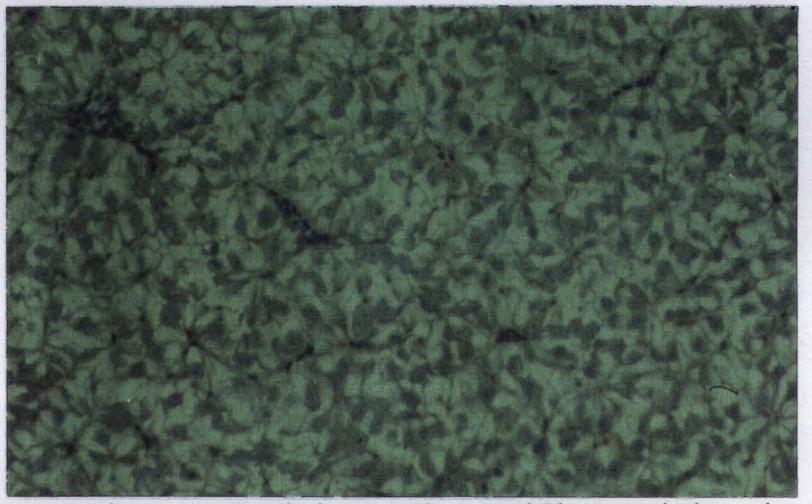
D. Heart. Positive and specific staining of endocardium (A) and vascular endothelium (B) in fish from oiled stream (250x).

Microphotographs by R. Smolowitz.

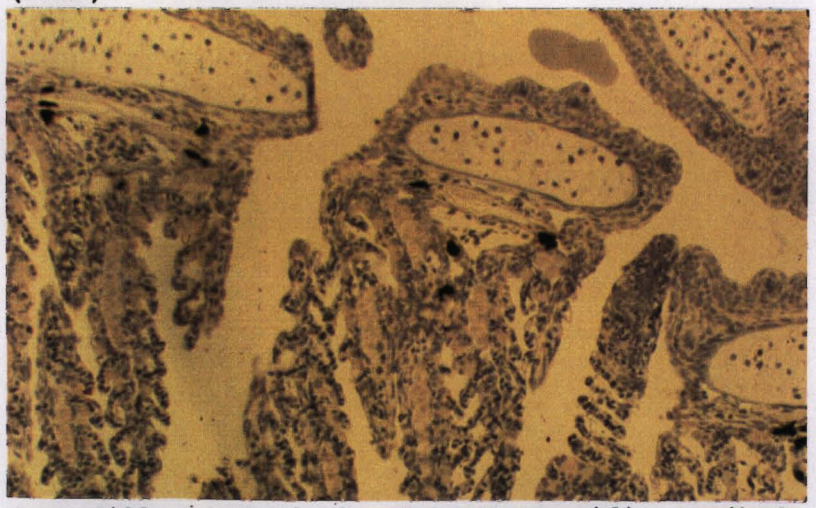
Figure 6. Distribution of Cytochrome P-450IA1 in pink salmon alevin liver and gill.



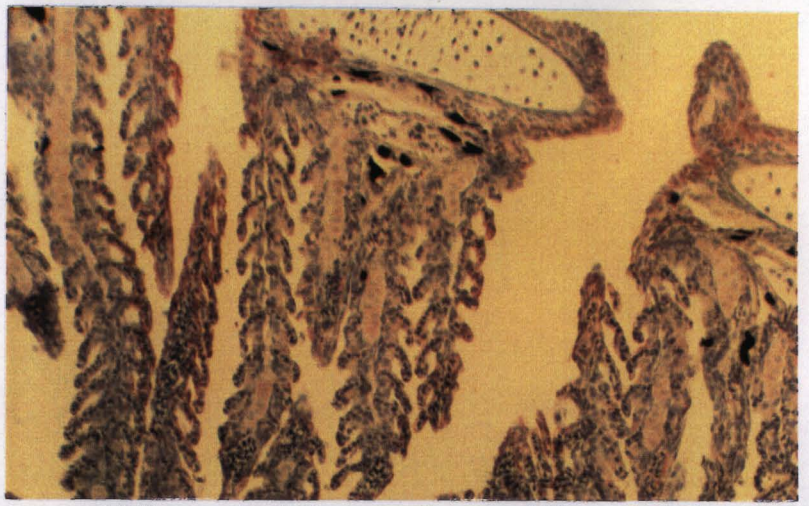
A. Liver, control for B. Nonspecific antibody substituted for specific antibody in immunological staining procedure. No specific staining present (250x).



B. Liver. Positive and specific staining in hepatocyte canalicular borders in fish from oiled stream (250x).



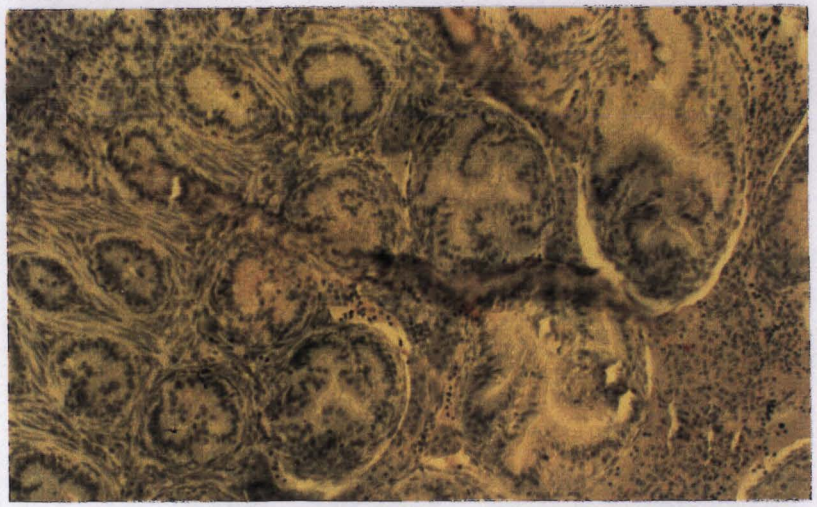
C. Gill, control for D. Nonspecific antibody substituted for specific antibody in immunological staining procedure. No specific staining present (250x).



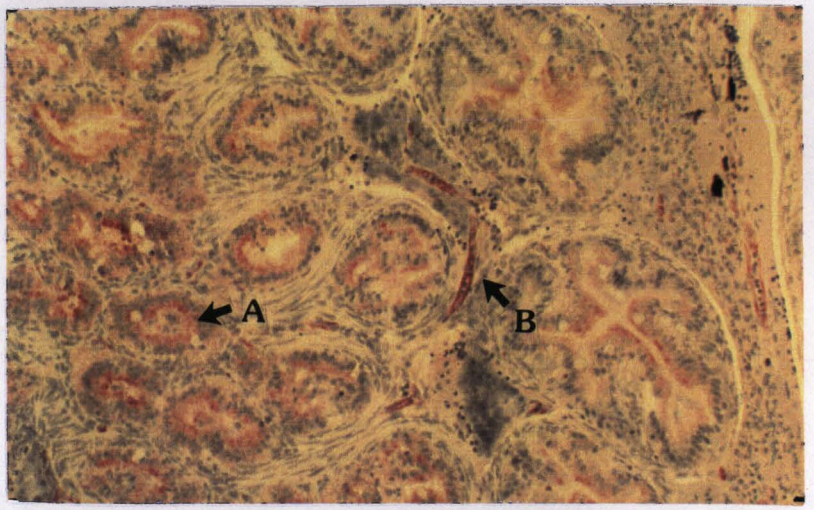
D. Gill. Positive and specific staining of epithelium in fish from oiled stream (250x).

Microphotographs by R. Smolowitz.

Figure 7. Distribution of Cytochrome P-450IA1 in pink salmon alevin pyloric ceca.



A. Ceca, control for B. Nonspecific antibody substituted for specific antibody in immunological staining procedure. No specific staining present (250x).



B. Ceca. Positive and specific staining in epithelium (A) and vascular endothelium (B) in fish from oiled stream (250x).

Microphotographs by R. Smolowitz.

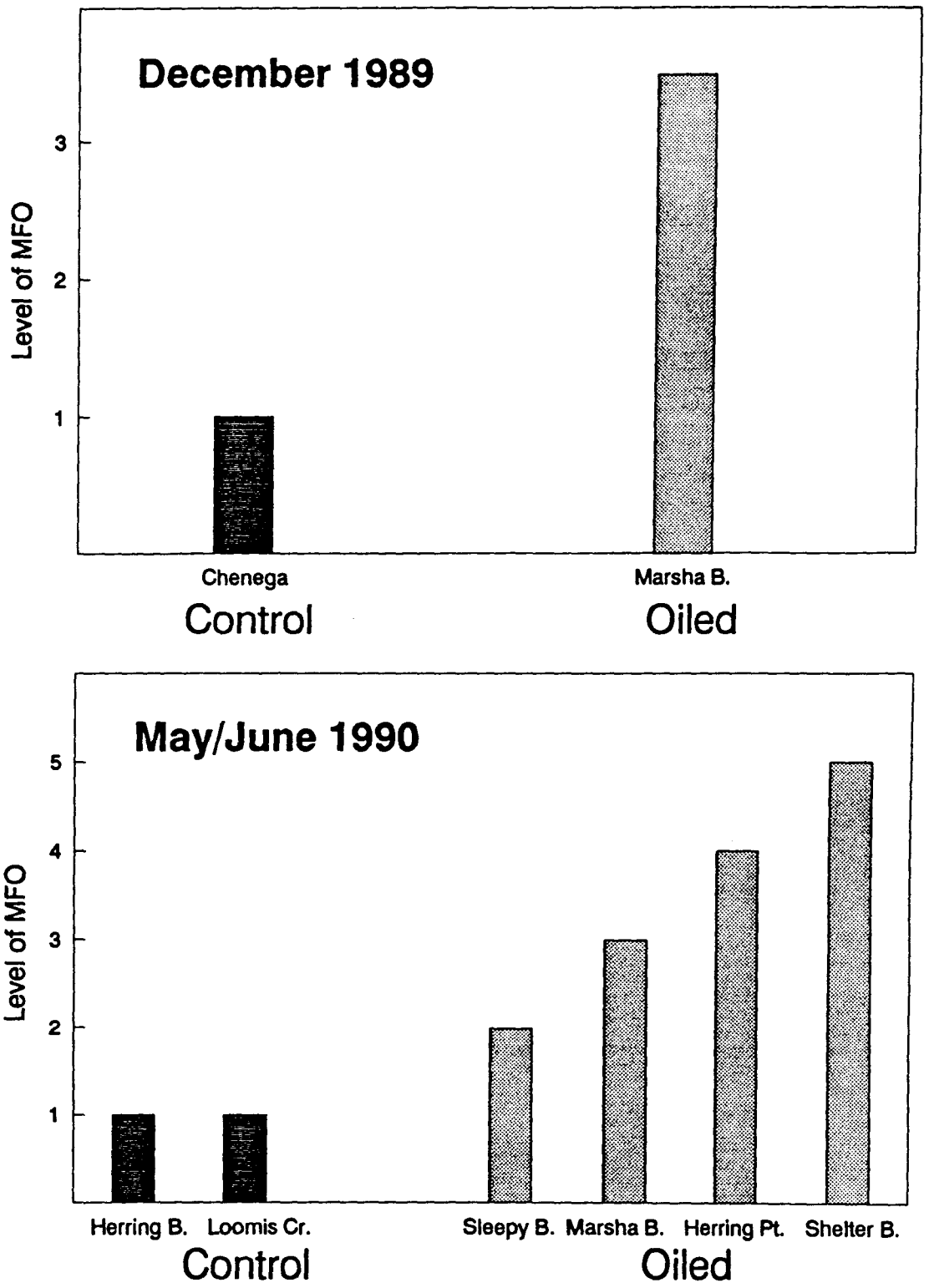


Figure 8. Cytochrome P-450 induction in pink salmon alevin samples collected in December, 1989 and May/June 1990 from unoiled and oiled streams in Prince William Sound Alaska. Induction scores: 1 = negative, 2 = very mild, 3 = mild, 4 = moderate, 5 = severe.

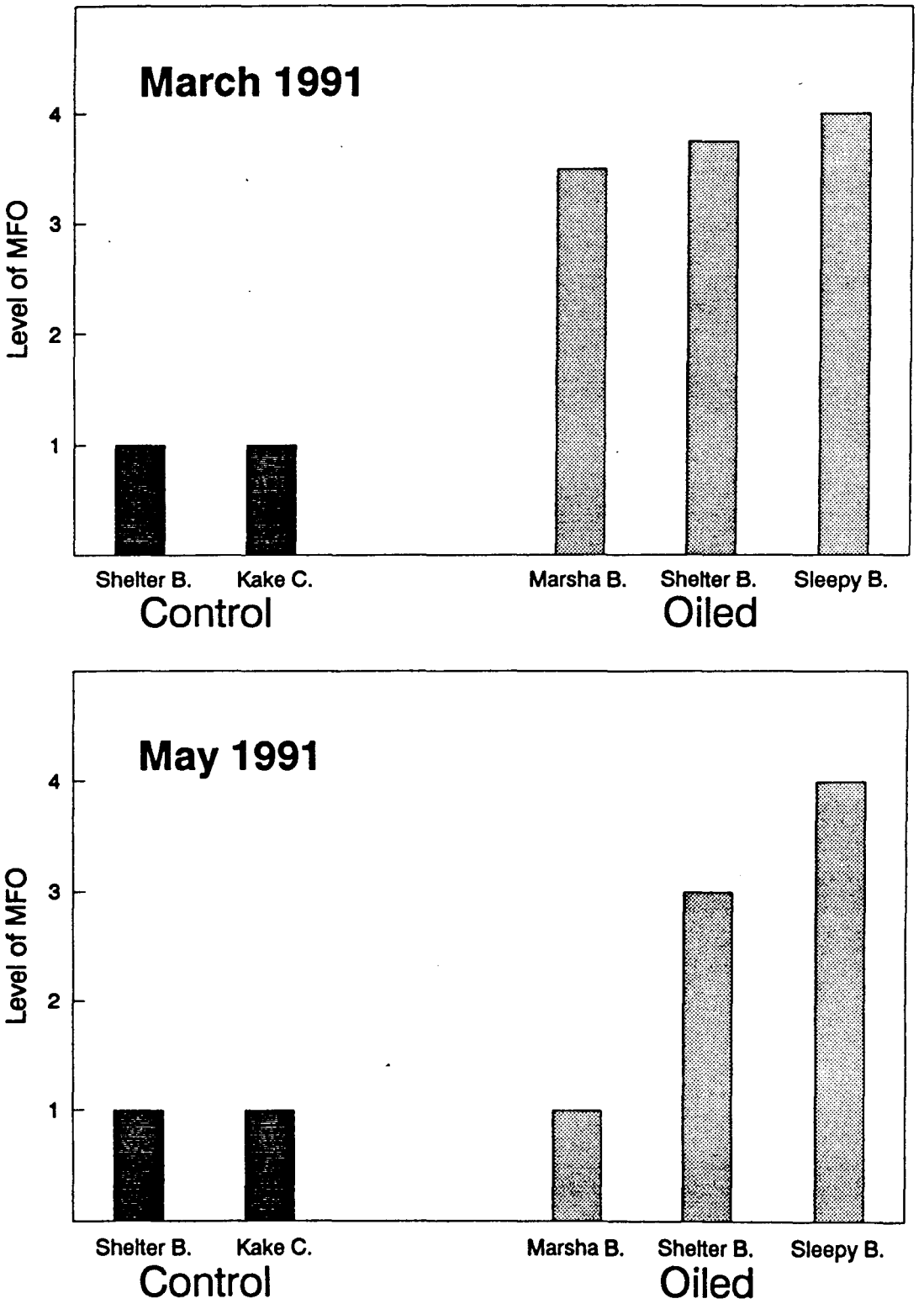


Figure 9. Cytochrome P-450 induction in pink salmon alevin samples collected in March and May 1991 from unoiled and oiled streams in Prince William Sound Alaska. Induction scores: 1 = negative, 2 = very mild, 3 = mild, 4 = moderate, 5 = severe.

Table 2. Aromatic hydrocarbon concentrations (ng/g) in intertidal substrates. See Figure 3 for locations of sites T1 through T4.

	T1	T2			T3		T4		
Compound	5/12/90	8/16/89	10/20/89	6/2/90	9/8/89	11/7/89	8/20/89	10/22/89	6/2/90
Naphthalene	9	9	150	151	8	158	22	619	4
C1-Napthalenes	30	958	12247	279	66	3435	118	26345	26
C2-Napthalenes	62	3584	39892	1726	468	17855	640	64265	80
C3-Napthalenes	285	8025	75940	12354	5101	46529	5064	104990	347
C4-Napthalenes	1540	5717	48946	23414	6456	34102	9282	60670	549
Biphenyl	6	225	3840	142	17	1212	34	5505	10
Acenapthalene	1	44	65	24	1	38	2	53	2
Acenaphtene	2	60	738	66	16	369	22	1234	2
Fluorene	10	454	6544	171	72	2990	216	6525	11
C1-Fluorenes	83	2004	23593	3708	1785	13311	3122	24447	142
C2-Fluorenes	1166	3852	39627	14426	4911	22501	9841	37583	528
C3-Fluorenes	1833	3978	36728	19838	4696	21464	10643	34280	743
Phenanthrene	19	1467	19382	606	103	11548	297	26230	66
Anthracene	6	7	193	131	21	98	22	290	1
C1-Phen_anthr	113	5097	49597	7990	3230	32741	5134	69618	490
C2-Phen_anthr	2055	6355	55428	27146	7349	37129	13770	73791	1409
C3-Phen_anthr	2506	3376	32347	25155	4532	22876	8423	39667	1185
C4-Phen_anthr	1317	1299	13239	13415	1816	9705	3664	17061	601
Dibenzothio	35	1286	16620	889	175	9973	398	23562	58

Table 2 (continued). Aromatic hydrocarbon concentrations (ng/g) in intertidal substrates. See Figure 3 for location of sites T1 through T4.

	T1	T2			T3		T4		
Compound	5/12/90	8/16/89	10/20/89	6/2/90	9/8/89	11/7/89	8/20/89	10/22/89	6/2/90
C1-Diben	170	4621	43033	9177	3796	28594	5770	59774	506
C2-Diben	3658	8258	70869	6930	9894	48115	19577	93636	2034
C3-Diben	4224	6193	54852	38558	7910	35446	15305	68659	2014
Fluoranthene	61	136	1413	753	176	965	299	1916	31
Pyrene	115	161	1475	1291	224	1077	457	2066	53
C1-Fluoran_pyr	0	754	7670	7260	1198	5053	2125	10347	303
Benanthracene	36	82	262	342	79	355	72	554	15
Chrysene	394	798	5190	4994	753	3849	1062	4740	254
C1-Chrysenes	547	855	6713	7115	825	4983	1160	6298	325
C2-Chrysenes	571	689	6311	7394	670	5147	990	5663	344
C3-Chrysenes	237	225	1804	2580	267	1949	381	1983	125
C4-Chrysenes	186	171	1845	2168	168	1617	355	1877	123
Ben*b*fluoran	36	52	405	484	51	344	82	485	23
Ben*k*fluoran	11	24	90	58	16	171	18	202	2
Benepyrene	88	104	981	1236	103	852	151	1070	60
Benapyrene	13	23	77	84	16	125	25	136	19
Perylene	24	38	210	250	17	100	38	211	6
I123cdanthra	2	4	92	115	0	166	10	43	5

Table 2 (continued). Aromatic hydrocarbon concentrations (ng/g) in intertidal substrates. See Figure 3 for location of sites T1 through T4.

	T1	T2			T3		T4		
Compound	5/12/90	8/16/89	10/20/89	6/2/90	9/8/89	11/7/89	8/20/89	10/22/89	6/2/90
Dbahanthra	7	7	87	147	11	243	11	196	3
Bghiperylene	25	20	181	185	31	232	22	210	18
2-methylnaph	16	433	5862	16	34	1594	66	13205	16
1-methylnaph	15	524	6385	10	32	1841	52	13140	10
2,6-dimethnaph	28	1731	27079	54	264	10061	335	38930	54
2,3,5 trimetna	83	2598	34913	156	2264	18172	2100	39419	156
1-methylphen	35	1797	17716	107	1267	11560	2360	20633	107
Tot. aromatics	21 µg/g	71 µg/g	679 µg/g	243 µg/g	67 µg/g	427 µg/g	119 µg/g	877 µg/g	13 µg/g
2-3 ring arom.	19 µg/g	67 µg/g	644 µg/g	206 µg/g	62 µg/g	400 µg/g	111 µg/g	839 µg/g	11 µg/g
4-5 ring arom.	2 µg/g	4 µg/g	35 µg/g	36 µg/g	5 µg/g	27 µg/g	7 µg/g	38 µg/g	2 µg/g

GERG, 1990

Table 3. Measurements of bulk oil remaining in sediments of stream channel at Site T1 thirteen months after initial oiling (5/12/90).

Station	Oil thickness (m)	Oil penetration (m)	mg oil/kg sed[a].	mg oil/l sed[b].	gal oil[c]/m^3 sed.
1	0.00001	0.12	695	1529	0.493867
2	0.00001	0.1	1577	3469.4	1.1206162
3	0.00001	0.025	2402	5284.4	1.7068612
4	0.00001	0.03	3310	7282	2.352086
5	0.00001	0.12	1855	4081	1.318163
6	0.00001	0.04	7100	15620	5.04526
7	0.00001	0.03	2809	6179.8	1.9960754
8	0.00001	0.03	5463	12018.6	3.8820078
9	0.00001	0.03	3281	7218.2	2.3314786
10	0.00001	0.025	3605	7931	2.561713
11	0.00001	0.03	2590	5698	1.840454
12	0.00001	0.03	10914	24010.8	7.7554884
AVE.	0.00001	0.05083333333	3800.083	8360.183	2.700339
ST. DEV.	2.3748406e-13	0.03818813079	1799.164	6207.616	2.00506

[a]Gravimetrically determined (GGC, 1990) except for sample 6 (GERG, 1990)

[b]Assumes an average sediment density of 2.2 kg/l.

[c]Assumes an average oil density of 0.8 kg/l.

Discussion

This study demonstrates that pre-emergent pink salmon alevins exposed to petroleum contaminated sediments up to 26 months after the initial oiling of the intertidal zone were incorporating and physiologically responding to polyaromatic hydrocarbons. Studies of induced estuarine killifish (*Fundulus heteroclitus*) indicate that P-450 content drops to basal levels 3 weeks after removal of the inducer, although this rate may be temperature dependent (Kloepper-Sams and Stegeman, 1989). Clearly, two years after the spill, oil residues contaminating intertidal substrates were not biologically inert.

Physiological systems of pre-emergent pink salmon alevins have developed sufficiently to synthesize Cytochrome P-450 in response to oil contamination. Not only are hepatic tissues induced, but at least 11 tissues of 9 organ systems are also inductively competent at these developmental stages. Induction of the vascular endothelium of kidney, brain, heart, and ceca suggests a response to blood-borne xenobiotics. These results correspond well with reports of P-450IA1 localization in other fish species (Smolowitz, et al., 1991). Induction of gill epithelium may indicate exposure to oil *via* water. However, gill epithelia were also induced when fish were exposed to xenobiotics *via* intraperitoneal injection (Smolowitz, et al., 1991), indicating the route of exposure may involve passage of PAHs from the blood. Induction of cecal endothelium was observed by Smolowitz, et al. (1991), in fish not exposed to food-borne xenobiotics, but to intraperitoneal injection. They suggest that inducers entered the gut with the bile, causing induction in mucosal tissue. Induction of the canalicular borders of liver hepatocytes also suggest bile as the carrier of xenobiotics. However, Malins (pers. comm., 1991[7])

[7]Dr. D. Malins, Pacific Northwest Research Foundation, Seattle, Washington.

cautions that fish have poorly developed lacteal systems and little is known about lymphatic transport of xenobiotics; therefore, differentiation between blood and bile exposure is tentative.

Elevated P-450 levels were not detected in eggs; even those collected at the same time and location as post-hatching alevins with elevated P-450 levels. This suggests that either eggs do not incorporte PAHs (e.g., the chorion is a barrier to PAH absorption) and/or that P-450 synthesis in response to PAH exposure is much reduced prior to hatching. The latter interpretation is consistent with other findings (Binder and Stegeman, 1984; Goksøyr and Solberg, 1987).

As a result of MO mediated metabolism, toxicity of PAHs greatly increases (Nebert and Gelboin, 1968; Diamond and Clark, 1970; Malaveille, et al., 1975; Wood, et al., 1976). The products of MO mediated metabolism bind to the cellular macromolecules RNA (Blobstein, et al., 1976) and DNA (Glover and Sims, 1968; Gelboin, et al., 1969; Bogdan and Chmielewicz, 1973; Sims, et al., 1974) via arene oxide intermediates (Jerina and Daly, 1974). The degree of metabolite binding is positively correlated to carcinogenic (Brookes and Lawley, 1964) and mutagenic (Jerina and Daly, 1974) potential. These observations indicate that induced enzyme systems in pre-emergent pink salmon from oiled streams may create an increased potential for mutagenesis and carcinogenesis (Stegeman and Lech, 1991).

Rainbow trout (*Oncorhynchus mykiss*) and carp (*Cyprinus carpio*) exposed to xenobiotics demonstrated an inverse correlation between MO activity and androgen, estrogen, and corticoid concentrations (Sivirajah, et al., 1977). Oil exposed male Atlantic salmon (*Salmo salar*) demonstrated an inverse correlation between MO activity and plasma androgen concentration, leading to decreased testicular development (Truscott, et al., 1983). Spies, et al. (1988), in studies of free-ranging flounder (*Platichthys stellatus*) in San

Francisco Bay, found an inverse correlation between MO activity and parameters of female reproductive success (e.g., decreased proportion of viable eggs, decreased fertilization success, decreased embryological success).

These observations suggest that the reproductive potential of salmon developing in contaminated sediments may be diminished. The fish of this study were exposed to oil during their most dynamic developmental period; a period of rapid cellular differentiation and organogenesis. Pink salmon are semelparous, they reproduce but once. Factors with deleterious effects on the initial organogenesis of gonadal tissue will directly effect life time reproductive success. Changes in hormonal concentrations during the early life stages of oil-exposed fish may decrease parameters of reproductive potential such as egg number, size, and viability.

Analyses of sediment samples indicate that intertidal pink salmon spawning areas throughout the spill zone were contaminated by North Slope crude oil. The current study demonstrates that pink salmon alevins in some heavily impacted streams incorporated this oil into various tissues more than two years after the initial spill and that the oil and its metabolites induced detectable physiological changes. These results can provide valuable links between the *EXXON Valdez* oil spill and any biological injuries in pink salmon observed in other studies.

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Appendix I

Buffered Neutral Formalin Solution.

37 - 40 % formalin	100.0 ml
Distilled water	900.0 ml
Sodium Phosphate monobasic	4.0 g
Sodium Phosphate dibasic (anhydrous)	6.5 g

Source: Luna, 1968

Appendix II Summary of Cytochrome P-450 induction in pink salmon eggs and fry collected in Prince William Sound, Alaska, 1989-1991.

SAMPLE ID DATE	SEGMENT STREAM#	LIFESTAGE	OILING	GENERAL RESULTS	SLIDE	C/T	ORGAN	TISSUE	STAIN	OCCURRENCE	COMMENTS
N3000-91 03/17/91	LA018 A 2264016780	SAC FRY	YES	MODERATE	1A1	1/1	KIDNEY	COLLECTING DUCT	NEGATIVE		
N3000-91 03/17/91	LA018 A 2264016780	SAC FRY	YES	MODERATE	1A1	1/1	YOLK SAC	ENDOTHELIUM	MILD	RARE	
N3000-91 03/17/91	LA018 A 2264016780	SAC FRY	YES	MODERATE	1A1	1/1	GILL	EPITHELIUM	MILD	MULTIFOCAL	
N3000-91 03/17/91	LA018 A 2264016780	SAC FRY	YES	MODERATE	1A1	1/1	GILL	BUDS	NEGATIVE		
N3000-91 03/17/91	LA018 A 2264016780	SAC FRY	YES	MODERATE	1A1	1/1	HEART	ATRIAL ENDOTHELIUM	MILD	RARE	
N3000-91 03/17/91	LA018 A 2264016780	SAC FRY	YES	MODERATE	1A1	1/1	HEART	VENTRICULAR ENDOTHELIUM	NEGATIVE		
N3000-91 03/17/91	LA018 A 2264016780	SAC FRY	YES	MODERATE	1A1	1/1	KIDNEY	SINUSOIDAL ENDOTHELIUM	MODERATE	DIFFUSE	
N3000-91 03/17/91	LA018 A 2264016780	SAC FRY	YES	MODERATE	1A1	1/1	KIDNEY	VASCULAR ENDOTHELIUM	MODERATE	MULTIFOCAL	
N3000-91 03/17/91	LA018 A 2264016780	SAC FRY	YES	MODERATE	1A2	1/1	KIDNEY	COLLECTING DUCT	VERY MILD	MULTIFOCAL	
N3000-91 03/17/91	LA018 A 2264016780	SAC FRY	YES	MODERATE	1A2	1/1	YOLK SAC	ENDOTHELIUM	MILD	MULTIFOCAL	
N3000-91 03/17/91	LA018 A 2264016780	SAC FRY	YES	MODERATE	1A2	1/1	GILL	EPITHELIUM	MILD	RARE	
N3000-91 03/17/91	LA018 A 2264016780	SAC FRY	YES	MODERATE	1A2	1/1	GILL	BUDS	NEGATIVE		

Appendix II (Continued) Summary of Cytochrome P-450 induction in pink salmon eggs and fry collected in Prince William Sound, Alaska, 1989-1991.

SAMPLE ID DATE	SEGMENT STREAM#	LIFESTAGE	OILING	GENERAL RESULTS	SLIDE	C/T	ORGAN	TISSUE	STAIN	OCCURRENCE	COMMENTS
N3000-91 03/17/91	LA018 A 2264016780	SAC FRY	YES	MODERATE	1A2	1/1	HEART	ATRIAL ENDOTHELIUM	NE		CELLS NOT SEEN
N3000-91 03/17/91	LA018 A 2264016780	SAC FRY	YES	MODERATE	1A2	1/1	HEART	VENTRICULAR ENDOTHELIUM	NE		CELLS NOT SEEN
N3000-91 03/17/91	LA018 A 2264016780	SAC FRY	YES	MODERATE	1A2	1/1	KIDNEY	SINUSOIDAL ENDOTHELIUM	MODERATE	DIFFUSE	
N3000-91 03/17/91	LA018 A 2264016780	SAC FRY	YES	MODERATE	1A2	1/1	KIDNEY	VASCULAR ENDOTHELIUM	MODERATE	MULTIFOCAL	
N3000-91 03/17/91	LA018 A 2264016780	SAC FRY	YES	MODERATE	2A1	1/1	KIDNEY	COLLECTING DUCT	NEGATIVE		
N3000-91 03/17/91	LA018 A 2264016780	SAC FRY	YES	MODERATE	2A1	1/1	YOLK SAC	ENDOTHELIUM	NEGATIVE		
N3000-91 03/17/91	LA018 A 2264016780	SAC FRY	YES	MODERATE	2A1	1/1	GILL	EPITHELIUM	MILD	RARE	
N3000-91 03/17/91	LA018 A 2264016780	SAC FRY	YES	MODERATE	2A1	1/1	GILL	BUDS	NEGATIVE		
N3000-91 03/17/91	LA018 A 2264016780	SAC FRY	YES	MODERATE	2A1	1/1	HEART	ATRIAL ENDOTHELIUM	NE		CELLS NOT SEEN
N3000-91 03/17/91	LA018 A 2264016780	SAC FRY	YES	MODERATE	2A1	1/1	HEART	VENTRICULAR ENDOTHELIUM	NE		CELLS NOT SEEN
N3000-91 03/17/91	LA018 A 2264016780	SAC FRY	YES	MODERATE	2A1	1/1	KIDNEY	SINUSOIDAL ENDOTHELIUM	MILD	MULTIFOCAL	
N3000-91 03/17/91	LA018 A 2264016780	SAC FRY	YES	MODERATE	2A1	1/1	KIDNEY	VASCULAR ENDOTHELIUM	NE		CELLS NOT SEEN
N3000-91 03/17/91	LA018 A 2264016780	SAC FRY	YES	MODERATE	2A2	1/1	KIDNEY	COLLECTING DUCT	NEGATIVE		

Appendix II (Continued) Summary of Cytochrome P-450 induction in pink salmon eggs and fry collected in Prince William Sound, Alaska, 1989-1991.

SAMPLE ID DATE	SEGMENT STREAM#	LIFESTAGE	OILING	GENERAL RESULTS	SLIDE	C/T	ORGAN	TISSUE	STAIN	OCCURRENCE	COMMENTS
N3000-91 03/17/91	LA018 A 2264016780	SAC FRY	YES	MODERATE	2A2	1/1	YOLK SAC	ENDOTHELIUM	NEGATIVE		
N3000-91 03/17/91	LA018 A 2264016780	SAC FRY	YES	MODERATE	2A2	1/1	GILL	EPITHELIUM	MILD	MULTIFOCAL	
N3000-91 03/17/91	LA018 A 2264016780	SAC FRY	YES	MODERATE	2A2	1/1	GILL	BUDS	MILD	RARE	
N3000-91 03/17/91	LA018 A 2264016780	SAC FRY	YES	MODERATE	2A2	1/1	HEART	ATRIAL ENDOTHELIUM	MILD	MULTIFOCAL	
N3000-91 03/17/91	LA018 A 2264016780	SAC FRY	YES	MODERATE	2A2	1/1	HEART	VENTRICULAR ENDOTHELIUM	MILD	MULTIFOCAL	
N3000-91 03/17/91	LA018 A 2264016780	SAC FRY	YES	MODERATE	2A2	1/1	KIDNEY	SINUSOIDAL ENDOTHELIUM	MILD	DIFFUSE	
N3000-91 03/17/91	LA018 A 2264016780	SAC FRY	YES	MODERATE	2A2	1/1	KIDNEY	VASCULAR ENDOTHELIUM	NE		CELLS NOT SEEN
N3000-91 03/17/91	LA018 A 2264016780	SAC FRY	YES	MODERATE	3A1	1/1	KIDNEY	COLLECTING DUCT	NEGATIVE		
N3000-91 03/17/91	LA018 A 2264016780	SAC FRY	YES	MODERATE	3A1	1/1	YOLK SAC	ENDOTHELIUM	MILD	RARE	
N3000-91 03/17/91	LA018 A 2264016780	SAC FRY	YES	MODERATE	3A1	1/1	GILL	EPITHELIUM	NE		CELLS NOT SEEN
N3000-91 03/17/91	LA018 A 2264016780	SAC FRY	YES	MODERATE	3A1	1/1	GILL	BUDS	NE		CELLS NOT SEEN
N3000-91 03/17/91	LA018 A 2264016780	SAC FRY	YES	MODERATE	3A1	1/1	HEART	ATRIAL ENDOTHELIUM	NE		CELLS NOT SEEN
N3000-91 03/17/91	LA018 A 2264016780	SAC FRY	YES	MODERATE	3A1	1/1	HEART	VENTRICULAR ENDOTHELIUM	NEGATIVE		

Appendix II (Continued) Summary of Cytochrome P-450 induction in pink salmon eggs and fry collected in Prince William Sound, Alaska, 1989-1991.

SAMPLE ID DATE	SEGMENT STREAM#	LIFESTAGE	OILING	GENERAL RESULTS	SLIDE	C/T	ORGAN	TISSUE	STAIN	OCCURRENCE	COMMENTS
N3000-91 03/17/91	LA018 A 2264016780	SAC FRY	YES	MODERATE	3A1	1/1	KIDNEY	SINUSOIDAL ENDOTHELIUM	MILD	DIFFUSE	
N3000-91 03/17/91	LA018 A 2264016780	SAC FRY	YES	MODERATE	3A1	1/1	KIDNEY	VASCULAR ENDOTHELIUM	MODERATE	DIFFUSE	
N3000-91 03/17/91	LA018 A 2264016780	SAC FRY	YES	MODERATE	3A2	1/1	KIDNEY	COLLECTING DUCT	NEGATIVE		
N3000-91 03/17/91	LA018 A 2264016780	SAC FRY	YES	MODERATE	3A2	1/1	YOLK SAC	ENDOTHELIUM	MILD	MULTIFOCAL	
N3000-91 03/17/91	LA018 A 2264016780	SAC FRY	YES	MODERATE	3A2	1/1	GILL	EPITHELIUM	NEGATIVE		
N3000-91 03/17/91	LA018 A 2264016780	SAC FRY	YES	MODERATE	3A2	1/1	GILL	BUDS	NEGATIVE		
N3000-91 03/17/91	LA018 A 2264016780	SAC FRY	YES	MODERATE	3A2	1/1	HEART	ATRIAL ENDOTHELIUM	NEGATIVE		
N3000-91 03/17/91	LA018 A 2264016780	SAC FRY	YES	MODERATE	3A2	1/1	HEART	VENTRICULAR ENDOTHELIUM	MILD	RARE	
N3000-91 03/17/91	LA018 A 2264016780	SAC FRY	YES	MODERATE	3A2	1/1	KIDNEY	SINUSOIDAL ENDOTHELIUM	MODERATE	DIFFUSE	
N3000-91 03/17/91	LA018 A 2264016780	SAC FRY	YES	MODERATE	3A2	1/1	KIDNEY	VASCULAR ENDOTHELIUM	MODERATE	DIFFUSE	
T89MW002V 12/08/89	KN701 B 2263016840	EGGS	YES	NEGATIVE		/			NEGATIVE		NO SPECIFIC STAINING DETECTED.
T89MW003V 12/08/89	KN701 B 2263016840	SAC FRY	YES	MILD/ MODERATE	A1	2/2			NEGATIVE		NO KIDNEY PRESENT IN SECTIONS.
T89MW003V 12/08/89	KN701 B 2263016840	SAC FRY	YES	MILD/ MODERATE	A2	1/2	ANTERIOR KIDNEY	TUBULES	MILD		OTHER FISH, NO TUBULES SEEN IN ANTERIOR KIDNEY, BUT TUBULES WERE SEEN IN POSTERIOR KIDNEY.

Appendix II (Continued) Summary of Cytochrome P-450 induction in pink salmon eggs and fry collected in Prince William Sound, Alaska, 1989-1991.

SAMPLE ID DATE	SEGMENT STREAM#	LIFESTAGE	OILING	GENERAL RESULTS	SLIDE	C/T	ORGAN	TISSUE	STAIN	OCCURRENCE	COMMENTS
T89MW003V 12/08/89	KN701 B 2263016840	SAC FRY	YES	MILD/ MODERATE	A3	1/2	ANTERIOR KIDNEY	TUBULES	MODERATE		MODERATE STAINING OF 2 CROSS SECTIONS. OTHER FISH, NO KIDNEY PRESENT.
T89MW004V 12/09/89	CH001 A 2262016280	EGGS	NO	NEGATIVE		/			NEGATIVE		NO SPECIFIC STAINING DETECTED.
T89MW005V 12/09/89	CH001 A 2262016280	SAC FRY	NO	NEGATIVE	A1	2/2			NEGATIVE		NO SPECIFIC STAINING SEEN IN ANY ORGANS. NO HEAD KIDNEY TUBULES PRESENT IN SECTIONS.
T89MW005V 12/09/89	CH001 A 2262016280	SAC FRY	NO	NEGATIVE	A2	2/2	HEAD KIDNEY	TUBULES	NEGATIVE		NO SPECIFIC STAINING SEEN ON ANY ORGANS. HEAD KIDNEY TUBULES PRESENT IN BOTH FISH.
T89MW005V 12/09/89	CH001 A 2262016280	SAC FRY	NO	NEGATIVE	A3	2/2			NEGATIVE		NO STAINING OF ANY TISSUES. NO KIDNEY PRESENT IN SECTIONS.
T90MW104V 05/25/90	LA018 A 2264016780	BUTTONED UP FRY	YES	NEGATIVE		6/6			NEGATIVE		NO SPECIFIC STAINING DETECTED.
T90MW104VB 05/25/90	LA018 A 2264016780	BUTTONED UP FRY	YES	VERY MILD		5/6	VERTEBRAL CORD	CARTILAGE	MODERATE		36 FOCI.
T90MW105V 05/25/90	LA018 A 2264016780	BUTTONED UP FRY	YES	VERY MILD		3/6	VERTEBRAL CORD	CARTILAGE	MODERATE		13 FOCI.
T90MW106V 05/30/90	KN701 B 2263016840	BUTTONED UP FRY	YES	MILD		5/6	VERTEBRAL CORD	CARTILAGE	MODERATE		38 FOCI.
T90MW106V 05/30/90	KN701 B 2263016840	BUTTONED UP FRY	YES	MILD		1/6	MIDGUT & CAECA	EPITHELIUM	MODERATE	MULTIFOCAL	
T90MW108V 05/30/90	KN132 B 2261016982	BUTTONED UP FRY	NO	NEGATIVE		6/6			NEGATIVE		NO SPECIFIC STAINING DETECTED.
T90MW110V 06/02/90	EB001 A 2253015060	BUTTONED UP FRY	NO	NEGATIVE		6/6			NEGATIVE		NO SPECIFIC STAINING DETECTED.
T90MW112V 06/08/90	KN500B B 2261016996	BUTTONED UP FRY	YES	MODERATE		1/6		SKIN	POSITIVE		

Appendix II (Continued) Summary of Cytochrome P-450 induction in pink salmon eggs and fry collected in Prince William Sound, Alaska, 1989-1991.

SAMPLE ID DATE	SEGMENT STREAM#	LIFESTAGE	OILING	GENERAL RESULTS	SLIDE	C/T	ORGAN	TISSUE	STAIN	OCCURRENCE	COMMENTS
T90MW112V 06/08/90	KN500 B 2261016996	BUTTONED UP	YES	MODERATE		5/5	PHARYNX	PHARYNGEAL EPITHELIUM	MILD/ MODE	MULTIFOCAL	
T90MW112V 06/08/90	KN500 B 2261016996	BUTTONED UP	YES	MODERATE		6/6	GILL	EPITHELIAL CELLS	MILD/ MODE	MULTIFOCAL	
T90MW112V 06/08/90	KN500B B 2261016996	BUTTONED UP FRY	YES	MODERATE		4/6	VERTEBRAL CORD	CARTILAGE	MODERATE		15 FOCI.
T90MW115V 06/08/90	EV025 A 2264016613	BUTTONED UP FRY	YES	STRONG		1/6	BRAIN	VESSEL ENDOTHELIUM	MODERATE		
T90MW115V 06/08/90	EV025 A 2264016613	BUTTONED UP FRY	YES	STRONG		1/6		PERITONEAL CONNECTIVE TISSUE	MODERATE		
T90MW115V 06/08/90	EV025 A 2264016613	BUTTONED UP FRY	YES	STRONG		6/6	GILL	EPITHELIUM	MODERATE	MULTIFOCAL TO DIFFUSE	
T90MW115V 06/08/90	EV025 A 2264016613	BUTTONED UP FRY	YES	STRONG		5/5	KIDNEY	TUBULES	MILD		
T90MW115V 06/08/90	EV025 A 2264016613	BUTTONED UP FRY	YES	STRONG		1/6	GUT	VESSEL ENDOTHELIUM	MODERATE		
T90MW115V 06/08/90	EV025 A 2264016613	BUTTONED UP FRY	YES	STRONG		4/4	HEART	ENDOTHELIUM	MODERATE		
T90MW115V 06/08/90	EV025 A 2264016613	BUTTONED UP FRY	YES	STRONG		5/6	VERTEBRAL CORD	CARTILAGE	POSITIVE		17 FOCI TOTAL.
T90MW115V 06/08/90	EV025 A 2264016613	BUTTONED UP FRY	YES	STRONG		1/4	LIVER	VESSEL ENDOTHELIUM	MODERATE		
T90MW115V 06/08/90	EV025 A 2264016613	BUTTONED UP FRY	YES	STRONG		3/6	KIDNEY	ENDOTHELIUM	MILD/MODER		
T90MW115V 06/08/90	EV025 A 2264016613	BUTTONED UP FRY	YES	STRONG		4/4	LIVER	HEPATOCYTES	VERY MILD		

Appendix II (Continued) Summary of Cytochrome P-450 induction in pink salmon eggs and fry collected in Prince William Sound, Alaska, 1989-1991.

SAMPLE ID DATE	SEGMENT STREAM#	LIFESTAGE	OILING	GENERAL RESULTS	SLIDE	C/T	ORGAN	TISSUE	STAIN	OCCURRENCE	COMMENTS
T90MW115V 06/08/90	EV025 A 2264016613	BUTTONED UP FRY	YES	STRONG		2/6	MIDGUT & CAECA	EPITHELIUM	MODERATE		
T90MW115V 06/08/90	EV025 A 2264016613	BUTTONED UP FRY	YES	STRONG		3/6	MIDGUT & CAECA	EPITHELIUM	MILD		
T90MW115V 06/08/90	EV025 A 2264016613	BUTTONED UP FRY	YES	STRONG		1/6	MIDGUT & CAECA	EPITHELIUM	NEGATIVE		
T90MW115V 06/08/90	EV025 A 2264016613	BUTTONED UP	YES	STRONG		6/6	PHARYNX	PHARYNGEAL EPITHELIUM	MODERATE	MULTIFOCAL TO DIFFUSE	
T90MW903V 09/07/90	BP004 A 2262016397	EGGS	YES	NEGATIVE	ALL	/		ALL TISSUES	NEGATIVE		NEGATIVE FOR ANY SPECIFIC IMMUNOCHEMICAL STAINING. NO EMBRYOS SEEN.
T90MW904V 09/07/90	BP004 A 2262016397	EGGS	YES	NEGATIVE	ALL	/		ALL TISSUES	NEGATIVE		NEGATIVE FOR ANY SPECIFIC STAINING. NO EMBRYOS SEEN.
T90MW905V 09/07/90	BP004 A 2262016397	EGGS	NO*	NEGATIVE	ALL	/		ALL TISSUES	NEGATIVE		NEGATIVE FOR ANY SPECIFIC STAINING. NO EMBRYOS SEEN.
T90MW907V 09/25/90	EV027 A 2264016610	EGGS	NO*	NEGATIVE	ALL	/		ALL TISSUES	NEGATIVE		NEGATIVE FOR ANY SPECIFIC STAINING. SEVERAL EGGS DID NOT CONTAIN VIABLE (BEFORE FIXED) EMBRYOS.
T90MW909V 09/25/90	EV025 A 2264016613	EGGS	YES	NEGATIVE	ALL	/		ALL TISSUES	NEGATIVE		NEGATIVE FOR ANY SPECIFIC STAINING. SEVERAL EGGS DID NOT CONTAIN VIABLE (BEFORE FIXED) EMBRYOS.
T90MW910V 09/25/90	EV025 A 2264016613	EGGS	YES	NEGATIVE	ALL	/		ALL TISSUES	NEGATIVE		NEGATIVE FOR ANY SPECIFIC STAINING. SEVERAL EGGS DID NOT CONTAIN VIABLE (BEFORE FIXED) EMBRYOS.
T90MW911V 09/25/90	EV025 A 2264016613	EGGS	YES	NEGATIVE	ALL	/		ALL TISSUES	NEGATIVE		NEGATIVE FOR ANY SPECIFIC STAINING. SEVERAL EGGS DID NOT CONTAIN VIABLE (BEFORE FIXED) EGGS.
T90MW914V 09/25/90	KN604 A 2264016809	EGGS	NO*	NEGATIVE	ALL	/		ALL TISSUES	NEGATIVE		NEGATIVE FOR ANY SPECIFIC STAINING. SEVERAL EGGS DID NOT CONTAIN VIABLE (BEFORE FIXED) EMBRYOS.
T91MF001V 03/21/91	KN701 B 2263016840	SAC FRY	YES	MODERATE	1A1	1/1	KIDNEY	VASCULAR ENDOTHELIUM	NEGATIVE		

Appendix II (Continued) Summary of Cytochrome P-450 induction in pink salmon eggs and fry collected in Prince William Sound, Alaska, 1989-1991.

SAMPLE ID DATE	SEGMENT STREAM#	LIFESTAGE	OILING	GENERAL RESULTS	SLIDE	C/T	ORGAN	TISSUE	STAIN	OCCURRENCE	COMMENTS
T91MF001V 03/21/91	KN701 B 2263016840	SAC FRY	YES	MODERATE	1A1	1/1	LIVER	SINUSOIDAL ENDOTHELIUM	VERY MILD	RARE	
T91MF001V 03/21/91	KN701 B 2263016840	SAC FRY	YES	MODERATE	1A1	1/1	KIDNEY	SINUSOIDAL ENDOTHELIUM	MILD	RARE	
T91MF001V 03/21/91	KN701 B 2263016840	SAC FRY	YES	MODERATE	1A1	1/1	GILL	EPITHELIAL CELLS	MILD	RARE	
T91MF001V 03/21/91	KN701 B 2263016840	SAC FRY	YES	MODERATE	1A1	1/1	ANTERIOR INTESTINE	ENTEROCYTES	NEGATIVE		
T91MF001V 03/21/91	KN701 B 2263016840	SAC FRY	YES	MODERATE	1A1	1/1	PHARYNX	PHARYNGEAL EPITHELIUM	NEGATIVE		
T91MF001V 03/21/91	KN701 B 2263016840	SAC FRY	YES	MODERATE	1A2	1/1	GILL	EPITHELIAL CELLS	MILD	RARE	
T91MF001V 03/21/91	KN701 B 2263016840	SAC FRY	YES	MODERATE	1A2	1/1	LIVER	SINUSOIDAL ENDOTHELIUM	NEGATIVE		
T91MF001V 03/21/91	KN701 B 2263016840	SAC FRY	YES	MODERATE	1A2	1/1	ANTERIOR INTESTINE	ENTEROCYTES	NEGATIVE		
T91MF001V 03/21/91	KN701 B 2263016840	SAC FRY	YES	MODERATE	1A2	1/1	KIDNEY	VASCULAR ENDOTHELIUM	NEGATIVE		
T91MF001V 03/21/91	KN701 B 2263016840	SAC FRY	YES	MODERATE	1A2	1/1	PHARYNX	PHARYNGEAL EPITHELIUM	NEGATIVE		
T91MF001V 03/21/91	KN701 B 2263016840	SAC FRY	YES	MODERATE	1A2	1/1	KIDNEY	SINUSOIDAL ENDOTHELIUM	MILD	RARE	
T91MF001V 03/21/91	KN701 B 2263016840	SAC FRY	YES	MODERATE	2A1	1/1	GILL	EPITHELIAL CELLS	MILD	MULTIFOCAL	
T91MF001V 03/21/91	KN701 B 2263016840	SAC FRY	YES	MODERATE	2A1	1/1	LIVER	SINUSOIDAL ENDOTHELIUM	NEGATIVE		

Appendix II (Continued) Summary of Cytochrome P-450 induction in pink salmon eggs and fry collected in Prince William Sound, Alaska, 1989-1991.

SAMPLE ID DATE	SEGMENT STREAM#	LIFESTAGE	OILING	GENERAL RESULTS	SLIDE	C/T	ORGAN	TISSUE	STAIN	OCCURRENCE	COMMENTS
T91MF001V 03/21/91	KN701 B 2263016840	SAC FRY	YES	MODERATE	2A1	1/1	KIDNEY	SINUSOIDAL ENDOTHELIUM	NEGATIVE		
T91MF001V 03/21/91	KN701 B 2263016840	SAC FRY	YES	MODERATE	2A1	1/1	KIDNEY	VASCULAR ENDOTHELIUM	NEGATIVE		
T91MF001V 03/21/91	KN701 B 2263016840	SAC FRY	YES	MODERATE	2A1	1/1	PHARYNX	PHARYNGEAL EPITHELIUM	MILD	RARE	
T91MF001V 03/21/91	KN701 B 2263016840	SAC FRY	YES	MODERATE	2A1	1/1	ANTERIOR INTESTINE	ENTEROCYTES	NEGATIVE		
T91MF001V 03/21/91	KN701 B 2263016840	SAC FRY	YES	MODERATE	2A2	1/1	GILL	EPITHELIAL CELLS	MILD	MULTIFOCAL	
T91MF001V 03/21/91	KN701 B 2263016840	SAC FRY	YES	MODERATE	2A2	1/1	LIVER	SINUSOIDAL ENDOTHELIUM	NEGATIVE		
T91MF001V 03/21/91	KN701 B 2263016840	SAC FRY	YES	MODERATE	2A2	1/1	PHARYNX	PHARYNGEAL EPITHELIUM	MILD	MILD	
T91MF001V 03/21/91	KN701 B 2263016840	SAC FRY	YES	MODERATE	2A2	1/1	KIDNEY	VASCULAR ENDOTHELIUM	MILD	MULTIFOCAL	
T91MF001V 03/21/91	KN701 B 2263016840	SAC FRY	YES	MODERATE	2A2	1/1	ANTERIOR INTESTINE	ENTEROCYTES	VERY MILD	MULTIFOCAL	
T91MF001V 03/21/91	KN701 B 2263016840	SAC FRY	YES	MODERATE	2A2	1/1	KIDNEY	SINUSOIDAL ENDOTHELIUM	MILD	MULTIFOCAL	
T91MF001V 03/21/91	KN701 B 2263016840	SAC FRY	YES	MODERATE	3A1	1/1	KIDNEY	VASCULAR ENDOTHELIUM	NEGATIVE		
T91MF001V 03/21/91	KN701 B 2263016840	SAC FRY	YES	MODERATE	3A1	1/1	LIVER	SINUSOIDAL ENDOTHELIUM	NEGATIVE		
T91MF001V 03/21/91	KN701 B 2263016840	SAC FRY	YES	MODERATE	3A1	1/1	KIDNEY	SINUSOIDAL ENDOTHELIUM	VERY MILD	RARE	

Appendix II (Continued) Summary of Cytochrome P-450 induction in pink salmon eggs and fry collected in Prince William Sound, Alaska, 1989-1991.

SAMPLE ID DATE	SEGMENT STREAM#	LIFESTAGE	OILING	GENERAL RESULTS	SLIDE	C/T	ORGAN	TISSUE	STAIN	OCCURRENCE	COMMENTS
T91MF001V 03/21/91	KN701 B 2263016840	SAC FRY	YES	MODERATE	3A1	1/1	GILL	EPITHELIAL CELLS	VERY MILD	RARE	
T91MF001V 03/21/91	KN701 B 2263016840	SAC FRY	YES	MODERATE	3A1	1/1	ANTERIOR INTESTINE	ENTEROCYTES	MILD	RARE	
T91MF001V 03/21/91	KN701 B 2263016840	SAC FRY	YES	MODERATE	3A1	1/1	PHARYNX	PHARYNGEAL EPITHELIUM	VERY MILD	RARE	
T91MF001V 03/21/91	KN701 B 2263016840	SAC FRY	YES	MODERATE	3A2	1/1	GILL	EPITHELIAL CELLS	MILD	RARE	
T91MF001V 03/21/91	KN701 B 2263016840	SAC FRY	YES	MODERATE	3A2	1/1	LIVER	SINUSOIDAL ENDOTHELIUM	NEGATIVE		
T91MF001V 03/21/91	KN701 B 2263016840	SAC FRY	YES	MODERATE	3A2	1/1	ANTERIOR INTESTINE	ENTEROCYTES	NE		CELLS NOT SEEN
T91MF001V 03/21/91	KN701 B 2263016840	SAC FRY	YES	MODERATE	3A2	1/1	KIDNEY	VASCULAR ENDOTHELIUM	MILD	RARE	
T91MF001V 03/21/91	KN701 B 2263016840	SAC FRY	YES	MODERATE	3A2	1/1	PHARYNX	PHARYNGEAL EPITHELIUM	NEGATIVE		
T91MF001V 03/21/91	KN701 B 2263016840	SAC FRY	YES	MODERATE	3A2	1/1	KIDNEY	SINUSOIDAL ENDOTHELIUM	MILD	MULTIFOCAL	
T91MF002V 03/21/91	KN701 B 2263016840	SAC FRY	YES	MILD	1A1	1/1	KIDNEY	COLLECTING DUCT	NEGATIVE		
T91MF002V 03/21/91	KN701 B 2263016840	SAC FRY	YES	MILD	1A1	1/1	POSTERIOR INTESTINE	ENTEROCYTES	MILD	MULTIFOCAL	
T91MF002V 03/21/91	KN701 B 2263016840	SAC FRY	YES	MILD	1A1	1/1	ANTERIOR INTESTINE	ENTEROCYTES	MILD	DIFFUSE	
T91MF002V 03/21/91	KN701 B 2263016840	SAC FRY	YES	MILD	1A2	1/1	ANTERIOR INTESTINE	ENTEROCYTES	NE		CELLS NOT SEEN

Appendix II (Continued) Summary of Cytochrome P-450 induction in pink salmon eggs and fry collected in Prince William Sound, Alaska, 1989-1991.

SAMPLE ID DATE	SEGMENT STREAM#	LIFESTAGE	OILING	GENERAL RESULTS	SLIDE	C/T	ORGAN	TISSUE	STAIN	OCCURRENCE	COMMENTS
T91MF002V 03/21/91	KN701 B 2263016840	SAC FRY	YES	MILD	1A2	1/1	KIDNEY	COLLECTING DUCT	NEGATIVE		
T91MF002V 03/21/91	KN701 B 2263016840	SAC FRY	YES	MILD	1A2	1/1	POSTERIOR INTESTINE	ENTEROCYTES	NEGATIVE		
T91MF002V 03/21/91	KN701 B 2263016840	SAC FRY	YES	MILD	2A1	1/1	KIDNEY	COLLECTING DUCT	NEGATIVE		
T91MF002V 03/21/91	KN701 B 2263016840	SAC FRY	YES	MILD	2A1	1/1	POSTERIOR INTESTINE	ENTEROCYTES	NEGATIVE		
T91MF002V 03/21/91	KN701 B 2263016840	SAC FRY	YES	MILD	2A1	1/1	ANTERIOR INTESTINE	ENTEROCYTES	NEGATIVE		
T91MF002V 03/21/91	KN701 B 2263016840	SAC FRY	YES	MILD	2A2	1/1	ANTERIOR INTESTINE	ENTEROCYTES	NE		CELLS NOT SEEN
T91MF002V 03/21/91	KN701 B 2263016840	SAC FRY	YES	MILD	2A2	1/1	POSTERIOR INTESTINE	ENTEROCYTES	NE		CELLS NOT SEEN
T91MF002V 03/21/91	KN701 B 2263016840	SAC FRY	YES	MILD	2A2	1/1	KIDNEY	COLLECTING DUCT	NEGATIVE		
T91MF002V 03/21/91	KN701 B 2263016840	SAC FRY	YES	MILD	3A1	1/1	KIDNEY	COLLECTING DUCT	NEGATIVE		
T91MF002V 03/21/91	KN701 B 2263016840	SAC FRY	YES	MILD	3A1	1/1	POSTERIOR INTESTINE	ENTEROCYTES	NE		CELLS NOT SEEN
T91MF002V 03/21/91	KN701 B 2263016840	SAC FRY	YES	MILD	3A1	1/1	ANTERIOR INTESTINE	ENTEROCYTES	NE		CELLS NOT SEEN
T91MF002V 03/21/91	KN701 B 2263016840	SAC FRY	YES	MILD	3A2	1/1	ANTERIOR INTESTINE	ENTEROCYTES	NEGATIVE		
T91MF002V 03/21/91	KN701 B 2263016840	SAC FRY	YES	MILD	3A2	1/1	KIDNEY	COLLECTING DUCT	VERY MILD	MULTIFOCAL	

Appendix II (Continued) Summary of Cytochrome P-450 induction in pink salmon eggs and fry collected in Prince William Sound, Alaska, 1989-1991.

SAMPLE ID DATE	SEGMENT STREAM#	LIFESTAGE	OILING	GENERAL RESULTS	SLIDE	C/T	ORGAN	TISSUE	STAIN	OCCURRENCE	COMMENTS
T91MF002V 03/21/91	KN701 B 2263016840	SAC FRY	YES	MILD	3A2	1/1	POSTERIOR INTESTINE	ENTEROCYTES	NEGATIVE		
T91MF006V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE/ MILD	1A1	1/1	LIVER	SINUSOIDAL ENDOTHELIUM	NEGATIVE		
T91MF006V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE/ MILD	1A1	1/1	KIDNEY	VASCULAR ENDOTHELIUM	MILD	RARE	
T91MF006V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE/ MILD	1A1	1/1	KIDNEY	SINUSOIDAL ENDOTHELIUM	MILD	RARE	
T91MF006V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE/ MILD	1A1	1/1	YOLK SAC	ENDOTHELIUM	NEGATIVE		
T91MF006V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE/ MILD	1A1	1/1	ANTERIOR INTESTINE	ENTEROCYTES	NEGATIVE		
T91MF006V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE/ MILD	1A2	1/1	KIDNEY	SINUSOIDAL ENDOTHELIUM	MILD	MULTIFOCAL	
T91MF006V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE/ MILD	1A2	1/1	LIVER	SINUSOIDAL ENDOTHELIUM	MILD	RARE	
T91MF006V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE/ MILD	1A2	1/1	ANTERIOR INTESTINE	ENTEROCYTES	NEGATIVE		
T91MF006V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE/ MILD	1A2	1/1	YOLK SAC	ENDOTHELIUM	NEGATIVE		
T91MF006V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE/ MILD	1A2	1/1	KIDNEY	VASCULAR ENDOTHELIUM	MILD	MULTIFOCAL	
T91MF006V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE/ MILD	2A1	1/1	LIVER	SINUSOIDAL ENDOTHELIUM	NEGATIVE		
T91MF006V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE/ MILD	2A1	1/1	ANTERIOR INTESTINE	ENTEROCYTES	NE		CELLS NOT SEEN

Appendix II (Continued) Summary of Cytochrome P-450 induction in pink salmon eggs and fry collected in Prince William Sound, Alaska, 1989-1991.

SAMPLE ID DATE	SEGMENT STREAM#	LIFESTAGE	OILING	GENERAL RESULTS	SLIDE	C/T	ORGAN	TISSUE	STAIN	OCCURRENCE	COMMENTS
T91MF006V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE/ MILD	2A1	1/1	YOLK SAC	ENDOTHELIUM	MILD	MULTIFOCAL	
T91MF006V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE/ MILD	2A1	1/1	KIDNEY	VASCULAR ENDOTHELIUM	MODERATE	MULTIFOCAL	
T91MF006V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE/ MILD	2A1	1/1	KIDNEY	SINUSOIDAL ENDOTHELIUM	MODERATE	MULTIFOCAL	
T91MF006V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE/ MILD	2A2	1/1	LIVER	SINUSOIDAL ENDOTHELIUM	NE		CELLS NOT SEEN
T91MF006V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE/ MILD	2A2	1/1	KIDNEY	VASCULAR ENDOTHELIUM	MODERATE	MULTIFOCAL	
T91MF006V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE/ MILD	2A2	1/1	YOLK SAC	ENDOTHELIUM	NEGATIVE		
T91MF006V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE/ MILD	2A2	1/1	KIDNEY	SINUSOIDAL ENDOTHELIUM	MODERATE	MULTIFOCAL	
T91MF006V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE/ MILD	2A2	1/1	ANTERIOR INTESTINE	ENTEROCYTES	NEGATIVE		
T91MF006V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE/ MILD	3A1	1/1	KIDNEY	SINUSOIDAL ENDOTHELIUM	VERY MILD	RARE	
T91MF006V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE/ MILD	3A1	1/1	ANTERIOR INTESTINE	ENTEROCYTES	NEGATIVE		
T91MF006V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE/ MILD	3A1	1/1	YOLK SAC	ENDOTHELIUM	NEGATIVE		
T91MF006V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE/ MILD	3A1	1/1	KIDNEY	VASCULAR ENDOTHELIUM	NEGATIVE		
T91MF006V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE/ MILD	3A1	1/1	LIVER	SINUSOIDAL ENDOTHELIUM	NEGATIVE		

Appendix II (Continued) Summary of Cytochrome P-450 induction in pink salmon eggs and fry collected in Prince William Sound, Alaska, 1989-1991.

SAMPLE ID DATE	SEGMENT STREAM#	LIFESTAGE	OILING	GENERAL RESULTS	SLIDE	C/T	ORGAN	TISSUE	STAIN	OCCURRENCE	COMMENTS
T91MF006V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE/ MILD	3A2	1/1	LIVER	SINUSOIDAL ENDOTHELIUM	NEGATIVE		
T91MF006V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE/ MILD	3A2	1/1	KIDNEY	VASCULAR ENDOTHELIUM	NEGATIVE		
T91MF006V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE/ MILD	3A2	1/1	YOLK SAC	ENDOTHELIUM	NEGATIVE		
T91MF006V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE/ MILD	3A2	1/1	KIDNEY	SINUSOIDAL ENDOTHELIUM	VERY MILD	MULTIFOCAL	
T91MF006V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE/ MILD	3A2	1/1	ANTERIOR INTESTINE	ENTEROCYTES	VERY MILD	RARE	
T91MF008V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE	1A1	1/1	YOLK SAC	ENDOTHELIUM	VERY MILD	RARE	
T91MF008V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE	1A1	1/1	HEART	VENTRICULAR ENDOTHELIUM	NE		CELLS NOT SEEN
T91MF008V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE	1A1	1/1	POSTERIOR INTESTINE	ENTEROCYTES	NEGATIVE		
T91MF008V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE	1A1	1/1	GILL	BUDS	NEGATIVE		
T91MF008V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE	1A1	1/1	HEART	ATRIAL ENDOTHELIUM	NE		CELLS NOT SEEN
T91MF008V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE	1A1	1/1	ANTERIOR INTESTINE	ENTEROCYTES	NEGATIVE		
T91MF008V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE	1A1	1/1	LIVER	HEPATOCYTES	VERY MILD	MULTIFOCAL	
T91MF008V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE	1A1	1/1	KIDNEY	SINUSOIDAL ENDOTHELIUM	MODERATE	MULTIFOCAL	

Appendix II (Continued) Summary of Cytochrome P-450 induction in pink salmon eggs and fry collected in Prince William Sound, Alaska, 1989-1991.

SAMPLE ID DATE	SEGMENT STREAM#	LIFESTAGE	OILING	GENERAL RESULTS	SLIDE	C/T	ORGAN	TISSUE	STAIN	OCCURRENCE	COMMENTS
T91MF008V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE	1A1	1/1	KIDNEY	VASCULAR ENDOTHELIUM	MODERATE	RARE	
T91MF008V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE	1A2	1/1	HEART	ATRIAL ENDOTHELIUM	NE		CELLS NOT SEEN
T91MF008V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE	1A2	1/1	ANTERIOR INTESTINE	ENTEROCYTES	VERY MILD	MULTIFOCAL	
T91MF008V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE	1A2	1/1	POSTERIOR INTESTINE	ENTEROCYTES	VERY MILD	RARE	
T91MF008V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE	1A2	1/1	GILL	BUDS	NEGATIVE		
T91MF008V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE	1A2	1/1	KIDNEY	VASCULAR ENDOTHELIUM	NE		CELLS NOT SEEN
T91MF008V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE	1A2	1/1	HEART	VENTRICULAR ENDOTHELIUM	NE		CELLS NOT SEEN
T91MF008V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE	1A2	1/1	LIVER	HEPATOCYTES	NE		CELLS NOT SEEN
T91MF008V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE	1A2	1/1	YOLK SAC	ENDOTHELIUM	MILD	RARE	
T91MF008V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE	1A2	1/1	KIDNEY	SINUSOIDAL ENDOTHELIUM	MILD	MULTIFOCAL	
T91MF008V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE	2A1	1/1	YOLK SAC	ENDOTHELIUM	MILD	RARE	
T91MF008V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE	2A1	1/1	ANTERIOR INTESTINE	ENTEROCYTES	NEGATIVE		
T91MF008V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE	2A1	1/1	KIDNEY	VASCULAR ENDOTHELIUM	NE		CELLS NOT SEEN

Appendix II (Continued) Summary of Cytochrome P-450 induction in pink salmon eggs and fry collected in Prince William Sound, Alaska, 1989-1991.

SAMPLE ID DATE	SEGMENT STREAM#	LIFESTAGE	OILING	GENERAL RESULTS	SLIDE	C/T	ORGAN	TISSUE	STAIN	OCCURRENCE	COMMENTS
T91MF008V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE	2A1	1/1	KIDNEY	SINUSOIDAL ENDOTHELIUM	MODERATE	MULTIFOCAL	
T91MF008V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE	2A1	1/1	HEART	ATRIAL ENDOTHELIUM	NEGATIVE		
T91MF008V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE	2A1	1/1	HEART	VENTRICULAR ENDOTHELIUM	NEGATIVE		
T91MF008V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE	2A1	1/1	POSTERIOR INTESTINE	ENTEROCYTES	NEGATIVE		
T91MF008V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE	2A1	1/1	GILL	BUDS	NE		CELLS NOT SEEN
T91MF008V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE	2A1	1/1	LIVER	HEPATOCYTES	NE		CELLS NOT SEEN
T91MF008V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE	2A2	1/1	KIDNEY	VASCULAR ENDOTHELIUM	NE		CELLS NOT SEEN
T91MF008V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE	2A2	1/1	KIDNEY	SINUSOIDAL ENDOTHELIUM	MODERATE	RARE	
T91MF008V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE	2A2	1/1	LIVER	HEPATOCYTES	NE		CELLS NOT SEEN
T91MF008V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE	2A2	1/1	GILL	BUDS	NEGATIVE		
T91MF008V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE	2A2	1/1	HEART	ATRIAL ENDOTHELIUM	MILD	MULTIFOCAL	
T91MF008V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE	2A2	1/1	ANTERIOR INTESTINE	ENTEROCYTES	NEGATIVE		
T91MF008V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE	2A2	1/1	POSTERIOR INTESTINE	ENTEROCYTES	NE		CELLS NOT SEEN

Appendix II (Continued) Summary of Cytochrome P-450 induction in pink salmon eggs and fry collected in Prince William Sound, Alaska, 1989-1991.

SAMPLE ID DATE	SEGMENT STREAM#	LIFESTAGE	OILING	GENERAL RESULTS	SLIDE	C/T	ORGAN	TISSUE	STAIN	OCCURRENCE	COMMENTS
T91MF008V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE	2A2	1/1	YOLK SAC	ENDOTHELIUM	NEGATIVE		
T91MF008V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE	2A2	1/1	HEART	VENTRICULAR ENDOTHELIUM	MILD	MULTIFOCAL	
T91MF008V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE	3A1	1/1	KIDNEY	SINUSOIDAL ENDOTHELIUM	VERY MILD	MULTIFOCAL	
T91MF008V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE	3A1	1/1	HEART	VENTRICULAR ENDOTHELIUM	NE		CELLS NOT SEEN
T91MF008V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE	3A1	1/1	LIVER	HEPATOCYTES	VERY MILD	DIFFUSE	
T91MF008V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE	3A1	1/1	YOLK SAC	ENDOTHELIUM	NEGATIVE		
T91MF008V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE	3A1	1/1	KIDNEY	VASCULAR ENDOTHELIUM	VERY MILD	RARE	
T91MF008V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE	3A1	1/1	ANTERIOR INTESTINE	ENTEROCYTES	NEGATIVE		
T91MF008V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE	3A1	1/1	POSTERIOR INTESTINE	ENTEROCYTES	VERY MILD	MULTIFOCAL	
T91MF008V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE	3A1	1/1	GILL	BUDS	NE		CELLS NOT SEEN
T91MF008V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE	3A1	1/1	HEART	ATRIAL ENDOTHELIUM	NE		CELLS NOT SEEN
T91MF008V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE	3A2	1/1	HEART	ATRIAL ENDOTHELIUM	NE		CELLS NOT SEEN
T91MF008V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE	3A2	1/1	HEART	VENTRICULAR ENDOTHELIUM	NE		CELLS NOT SEEN

Appendix II (Continued) Summary of Cytochrome P-450 induction in pink salmon eggs and fry collected in Prince William Sound, Alaska, 1989-1991.

SAMPLE ID DATE	SEGMENT STREAM#	LIFESTAGE	OILING	GENERAL RESULTS	SLIDE	C/T	ORGAN	TISSUE	STAIN	OCCURRENCE	COMMENTS
T91MF008V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE	3A2	1/1	LIVER	HEPATOCYTES	VERY MILD	DIFFUSE	
T91MF008V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE	3A2	1/1	KIDNEY	SINUSOIDAL ENDOTHELIUM	MILD	MULTIFOCAL	
T91MF008V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE	3A2	1/1	KIDNEY	VASCULAR ENDOTHELIUM	MILD	MULTIFOCAL	
T91MF008V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE	3A2	1/1	ANTERIOR INTESTINE	ENTEROCYTES	NE		CELLS NOT SEEN
T91MF008V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE	3A2	1/1	POSTERIOR INTESTINE	ENTEROCYTES	NEGATIVE		
T91MF008V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE	3A2	1/1	GILL	BUDS	MILD	RARE	
T91MF008V 03/21/91	EV025 A 2264016613	SAC FRY	YES	MODERATE	3A2	1/1	YOLK SAC	ENDOTHELIUM	VERY MILD	MULTIFOCAL	
T91MF010V 03/21/91	EV027 A 2264016610	SAC FRY	NO	NEGATIVE	ALL	6/6			NEGATIVE		ALL TISSUES NEGATIVE FOR ANY SPECIFIC STAIN.
T91MF012V 03/22/91	CH017 A 2262016262	SAC FRY	NO	NEGATIVE	ALL	6/6			NEGATIVE		ALL TISSUES NEGATIVE FOR ANY SPECIFIC STAIN.
T91MF014V 05/04/91	LA018 A 2264016780	BUTTONED UP	YES	MODERATE	1A1	1/1	GILL	EPITHELIAL CELLS	MODERATE	RARE	
T91MF014V 05/04/91	LA018 A 2264016780	BUTTONED UP	YES	MODERATE	1A1	1/1	LIVER	HEPATOCYTES	NEGATIVE		
T91MF014V 05/04/91	LA018 A 2264016780	BUTTONED UP	YES	MODERATE	1A1	1/1	PHARYNX	PHARYNGEAL EPITHELIUM	MILD	RARE	
T91MF014V 05/04/91	LA018 A 2264016780	BUTTONED UP	YES	MODERATE	1A1	1/1	HEART	ATRIAL ENDOTHELIUM	NE		CELLS NOT SEEN

Appendix II (Continued) Summary of Cytochrome P-450 induction in pink salmon eggs and fry collected in Prince William Sound, Alaska, 1989-1991.

SAMPLE ID DATE	SEGMENT STREAM#	LIFESTAGE	OILING	GENERAL RESULTS	SLIDE	C/T	ORGAN	TISSUE	STAIN	OCCURRENCE	COMMENTS
T91MF014V 05/04/91	LA018 A 2264016780	BUTTONED UP	YES	MODERATE	1A1	1/1	HEART	VENTRICULAR ENDOTHELIUM	MODERATE	MULTIFOCAL	
T91MF014V 05/04/91	LA018 A 2264016780	BUTTONED UP	YES	MODERATE	1A1	1/1	KIDNEY	COLLECTING DUCTS	MILD	MULTIFOCAL	
T91MF014V 05/04/91	LA018 A 2264016780	BUTTONED UP	YES	MODERATE	1A1	1/1	LIVER	CENTRAL VEINS	NEGATIVE		
T91MF014V 05/04/91	LA018 A 2264016780	BUTTONED UP	YES	MODERATE	1A1	1/1	KIDNEY	SINUSOIDAL ENDOTHELIUM	MODERATE	DIFFUSE	
T91MF014V 05/04/91	LA018 A 2264016780	BUTTONED UP	YES	MODERATE	1A1	1/1	KIDNEY	VASCULAR ENDOTHELIUM	MODERATE	MULTIFOCAL	
T91MF014V 05/04/91	LA018 A 2264016780	BUTTONED UP	YES	MODERATE	1A1	1/1	YOLK SAC	ENDOTHELIUM	MILD	RARE	
T91MF014V 05/04/91	LA018 A 2264016780	BUTTONED UP	YES	MODERATE	1A2	1/1	PHARYNX	PHARYNGEAL EPITHELIUM	MODERATE	RARE	
T91MF014V 05/04/91	LA018 A 2264016780	BUTTONED UP	YES	MODERATE	1A2	1/1	HEART	VENTRICULAR ENDOTHELIUM	NE		CELLS NOT SEEN
T91MF014V 05/04/91	LA018 A 2264016780	BUTTONED UP	YES	MODERATE	1A2	1/1	GILL	EPITHELIAL CELLS	MODERATE	MULTIFOCAL	
T91MF014V 05/04/91	LA018 A 2264016780	BUTTONED UP	YES	MODERATE	1A2	1/1	YOLK SAC	ENDOTHELIUM	MILD	RARE	
T91MF014V 05/04/91	LA018 A 2264016780	BUTTONED UP	YES	MODERATE	1A2	1/1	HEART	ATRIAL ENDOTHELIUM	NE		CELLS NOT SEEN
T91MF014V 05/04/91	LA018 A 2264016780	BUTTONED UP	YES	MODERATE	1A2	1/1	KIDNEY	VASCULAR ENDOTHELIUM	MILD	RARE	
T91MF014V 05/04/91	LA018 A 2264016780	BUTTONED UP	YES	MODERATE	1A2	1/1	LIVER	HEPATOCYTES	MILD	RARE	

Appendix II (Continued) Summary of Cytochrome P-450 induction in pink salmon eggs and fry collected in Prince William Sound, Alaska, 1989-1991.

SAMPLE ID DATE	SEGMENT STREAM#	LIFESTAGE	OILING	GENERAL RESULTS	SLIDE	C/T	ORGAN	TISSUE	STAIN	OCCURRENCE	COMMENTS
T91MF014V 05/04/91	LA018 A 2264016780	BUTTONED UP	YES	MODERATE	1A2	1/1	LIVER	CENTRAL VEINS	MILD	RARE	
T91MF014V 05/04/91	LA018 A 2264016780	BUTTONED UP	YES	MODERATE	1A2	1/1	KIDNEY	SINUSOIDAL ENDOTHELIUM	MODERATE	DIFFUSE	
T91MF014V 05/04/91	LA018 A 2264016780	BUTTONED UP	YES	MODERATE	1A2	1/1	KIDNEY	COLLECTING DUCTS	MILD	MULTIFOCAL	
T91MF014V 05/04/91	LA018 A 2264016780	BUTTONED UP	YES	MODERATE	2A1	1/1	KIDNEY	COLLECTING DUCTS	VERY MILD	MULTIFOCAL	
T91MF014V 05/04/91	LA018 A 2264016780	BUTTONED UP	YES	MODERATE	2A1	1/1	HEART	ATRIAL ENDOTHELIUM	NE		CELLS NOT SEEN
T91MF014V 05/04/91	LA018 A 2264016780	BUTTONED UP	YES	MODERATE	2A1	1/1	KIDNEY	VASCULAR ENDOTHELIUM	NE		CELLS NOT SEEN
T91MF014V 05/04/91	LA018 A 2264016780	BUTTONED UP	YES	MODERATE	2A1	1/1	LIVER	HEPATOCYTES	NE		CELLS NOT SEEN
T91MF014V 05/04/91	LA018 A 2264016780	BUTTONED UP	YES	MODERATE	2A1	1/1	LIVER	CENTRAL VEINS	NE		CELLS NOT SEEN
T91MF014V 05/04/91	LA018 A 2264016780	BUTTONED UP	YES	MODERATE	2A1	1/1	KIDNEY	SINUSOIDAL ENDOTHELIUM	MODERATE	DIFFUSE	
T91MF014V 05/04/91	LA018 A 2264016780	BUTTONED UP	YES	MODERATE	2A1	1/1	HEART	VENTRICULAR ENDOTHELIUM	NE		CELLS NOT SEEN
T91MF014V 05/04/91	LA018 A 2264016780	BUTTONED UP	YES	MODERATE	2A1	1/1	GILL	EPITHELIAL CELLS	MILD	RARE	
T91MF014V 05/04/91	LA018 A 2264016780	BUTTONED UP	YES	MODERATE	2A1	1/1	PHARYNX	PHARYNGEAL EPITHELIUM	NEGATIVE		
T91MF014V 05/04/91	LA018 A 2264016780	BUTTONED UP	YES	MODERATE	2A1	1/1	YOLK SAC	ENDOTHELIUM	NE		CELLS NOT SEEN

Appendix II (Continued) Summary of Cytochrome P-450 induction in pink salmon eggs and fry collected in Prince William Sound, Alaska, 1989-1991.

SAMPLE ID DATE	SEGMENT STREAM#	LIFESTAGE	OILING	GENERAL RESULTS	SLIDE	C/T	ORGAN	TISSUE	STAIN	OCCURRENCE	COMMENTS
T91MFO14V 05/04/91	LA018 A 2264016780	BUTTONED UP	YES	MODERATE	2A2	1/1	HEART	VENTRICULAR ENDOTHELIUM	NE		CELLS NOT SEEN
T91MFO14V 05/04/91	LA018 A 2264016780	BUTTONED UP	YES	MODERATE	2A2	1/1	LIVER	HEPATOCYTES	MODERATE	MULTIFOCAL	FOCALLY AT THE HILUS
T91MFO14V 05/04/91	LA018 A 2264016780	BUTTONED UP	YES	MODERATE	2A2	1/1	LIVER	HEPATOCYTES	VERY MILD	MULTIFOCAL	AT THE EDGES
T91MFO14V 05/04/91	LA018 A 2264016780	BUTTONED UP	YES	MODERATE	2A2	1/1	KIDNEY	VASCULAR ENDOTHELIUM	NE		CELLS NOT SEEN
T91MFO14V 05/04/91	LA018 A 2264016780	BUTTONED UP	YES	MODERATE	2A2	1/1	KIDNEY	COLLECTING DUCTS	VERY MILD	RARE	
T91MFO14V 05/04/91	LA018 A 2264016780	BUTTONED UP	YES	MODERATE	2A2	1/1	YOLK SAC	ENDOTHELIUM	MILD	MULTIFOCAL	
T91MFO14V 05/04/91	LA018 A 2264016780	BUTTONED UP	YES	MODERATE	2A2	1/1	HEART	ATRIAL ENDOTHELIUM	NE		CELLS NOT SEEN
T91MFO14V 05/04/91	LA018 A 2264016780	BUTTONED UP	YES	MODERATE	2A2	1/1	KIDNEY	SINUSOIDAL ENDOTHELIUM	MODERATE	MULTIFOCAL	
T91MFO14V 05/04/91	LA018 A 2264016780	BUTTONED UP	YES	MODERATE	2A2	1/1	GILL	EPITHELIAL CELLS	NEGATIVE		
T91MFO14V 05/04/91	LA018 A 2264016780	BUTTONED UP	YES	MODERATE	2A2	1/1	PHARYNX	PHARYNGEAL EPITHELIUM	MODERATE	RARE	
T91MFO14V 05/04/91	LA018 A 2264016780	BUTTONED UP	YES	MODERATE	2A2	1/1	LIVER	CENTRAL VEINS	NEGATIVE		
T91MFO14V 05/04/91	LA018 A 2264016780	BUTTONED UP	YES	MODERATE	3A1	1/1	KIDNEY	COLLECTING DUCTS	MILD	MULTIFOCAL	
T91MFO14V 05/04/91	LA018 A 2264016780	BUTTONED UP	YES	MODERATE	3A1	1/1	YOLK SAC	ENDOTHELIUM	MODERATE	MULTIFOCAL	

Appendix II (Continued) Summary of Cytochrome P-450 induction in pink salmon eggs and fry collected in Prince William Sound, Alaska, 1989-1991.

SAMPLE ID DATE	SEGMENT STREAM#	LIFESTAGE	OILING	GENERAL RESULTS	SLIDE	C/T	ORGAN	TISSUE	STAIN	OCCURRENCE	COMMENTS
T91MF014V 05/04/91	LA018 A 2264016780	BUTTONED UP	YES	MODERATE	3A1	1/1	LIVER	CENTRAL VEINS	NEGATIVE		
T91MF014V 05/04/91	LA018 A 2264016780	BUTTONED UP	YES	MODERATE	3A1	1/1	KIDNEY	VASCULAR ENDOTHELIUM	MILD	RARE	
T91MF014V 05/04/91	LA018 A 2264016780	BUTTONED UP	YES	MODERATE	3A1	1/1	HEART	VENTRICULAR ENDOTHELIUM	MILD	RARE	
T91MF014V 05/04/91	LA018 A 2264016780	BUTTONED UP	YES	MODERATE	3A1	1/1	LIVER	HEPATOCYTES	VERY MILD	MULTIFOCAL	
T91MF014V 05/04/91	LA018 A 2264016780	BUTTONED UP	YES	MODERATE	3A1	1/1	PHARYNX	PHARYNGEAL EPITHELIUM	MILD	MULTIFOCAL	
T91MF014V 05/04/91	LA018 A 2264016780	BUTTONED UP	YES	MODERATE	3A1	1/1	KIDNEY	SINUSOIDAL ENDOTHELIUM	MODERATE	DIFFUSE	
T91MF014V 05/04/91	LA018 A 2264016780	BUTTONED UP	YES	MODERATE	3A1	1/1	GILL	EPITHELIAL CELLS	NEGATIVE		
T91MF014V 05/04/91	LA018 A 2264016780	BUTTONED UP	YES	MODERATE	3A1	1/1	HEART	ATRIAL ENDOTHELIUM	MILD	MULTIFOCAL	
T91MF014V 05/04/91	LA018 A 2264016780	BUTTONED UP	YES	MODERATE	3A2	1/1	GILL	EPITHELIAL CELLS	MILD	RARE	
T91MF014V 05/04/91	LA018 A 2264016780	BUTTONED UP	YES	MODERATE	3A2	1/1	KIDNEY	SINUSOIDAL ENDOTHELIUM	MODERATE	MULTIFOCAL	
T91MF014V 05/04/91	LA018 A 2264016780	BUTTONED UP	YES	MODERATE	3A2	1/1	LIVER	HEPATOCYTES	VERY MILD	MULTIFOCAL	
T91MF014V 05/04/91	LA018 A 2264016780	BUTTONED UP	YES	MODERATE	3A2	1/1	LIVER	CENTRAL VEINS	NEGATIVE		
T91MF014V 05/04/91	LA018 A 2264016780	BUTTONED UP	YES	MODERATE	3A2	1/1	KIDNEY	VASCULAR ENDOTHELIUM	MILD	MULTIFOCAL	

Appendix II (Continued) Summary of Cytochrome P-450 induction in pink salmon eggs and fry collected in Prince William Sound, Alaska, 1989-1991.

SAMPLE ID DATE	SEGMENT STREAM#	LIFESTAGE	OILING	GENERAL RESULTS	SLIDE	C/T	ORGAN	TISSUE	STAIN	OCCURRENCE	COMMENTS
T91MF014V 05/04/91	LA018 A 2264016780	BUTTONED UP	YES	MODERATE	3A2	1/1	KIDNEY	COLLECTING DUCTS	MILD	MULTIFOCAL	
T91MF014V 05/04/91	LA018 A 2264016780	BUTTONED UP	YES	MODERATE	3A2	1/1	YOLK SAC	ENDOTHELIUM	VERY MILD	MULTIFOCAL	
T91MF014V 05/04/91	LA018 A 2264016780	BUTTONED UP	YES	MODERATE	3A2	1/1	PHARYNX	PHARYNGEAL EPITHELIUM	MILD	MULTIFOCAL	
T91MF014V 05/04/91	LA018 A 2264016780	BUTTONED UP	YES	MODERATE	3A2	1/1	HEART	ATRIAL ENDOTHELIUM	MILD	RARE	
T91MF014V 05/04/91	LA018 A 2264016780	BUTTONED UP	YES	MODERATE	3A2	1/1	HEART	VENTRICULAR ENDOTHELIUM	MILD	RARE	
T91MF015V 05/04/91	EV025 A 2264016613	BUTTONED UP	YES	MILD	1A1	1/1	KIDNEY	SINUSOIDAL ENDOTHELIUM	NEGATIVE		
T91MF015V 05/04/91	EV025 A 2264016613	BUTTONED UP	YES	MILD	1A1	1/1	LIVER	HEPATOCYTES	NEGATIVE		
T91MF015V 05/04/91	EV025 A 2264016613	BUTTONED UP	YES	MILD	1A1	1/1	KIDNEY	VASCULAR ENDOTHELIUM	NEGATIVE		
T91MF015V 05/04/91	EV025 A 2264016613	BUTTONED UP	YES	MILD	1A2	1/1	LIVER	HEPATOCYTES	NEGATIVE		
T91MF015V 05/04/91	EV025 A 2264016613	BUTTONED UP	YES	MILD	1A2	1/1	KIDNEY	VASCULAR ENDOTHELIUM	NEGATIVE		
T91MF015V 05/04/91	EV025 A 2264016613	BUTTONED UP	YES	MILD	1A2	1/1	KIDNEY	SINUSOIDAL ENDOTHELIUM	NEGATIVE		
T91MF015V 05/04/91	EV025 A 2264016613	BUTTONED UP	YES	MILD	2A1	1/1	KIDNEY	SINUSOIDAL ENDOTHELIUM	MILD	MULTIFOCAL	
T91MF015V 05/04/91	EV025 A 2264016613	BUTTONED UP	YES	MILD	2A1	1/1	LIVER	HEPATOCYTES	VERY MILD	MULTIFOCAL	

Appendix II (Continued) Summary of Cytochrome P-450 induction in pink salmon eggs and fry collected in Prince William Sound, Alaska, 1989-1991.

SAMPLE ID DATE	SEGMENT STREAM#	LIFESTAGE	OILING	GENERAL RESULTS	SLIDE	C/T	ORGAN	TISSUE	STAIN	OCCURRENCE	COMMENTS
T91MF015V 05/04/91	EV025 A 2264016613	BUTTONED UP	YES	MILD	2A1	1/1	KIDNEY	VASCULAR ENDOTHELIUM	MILD	MULTIFOCAL	
T91MF015V 05/04/91	EV025 A 2264016613	BUTTONED UP	YES	MILD	2A2	1/1	KIDNEY	VASCULAR ENDOTHELIUM	VERY MILD	RARE	
T91MF015V 05/04/91	EV025 A 2264016613	BUTTONED UP	YES	MILD	2A2	1/1	LIVER	HEPATOCYTES	NEGATIVE		
T91MF015V 05/04/91	EV025 A 2264016613	BUTTONED UP	YES	MILD	2A2	1/1	KIDNEY	SINUSOIDAL ENDOTHELIUM	VERY MILD	RARE	
T91MF017V 05/04/91	EV027 A 2264016610	BUTTONED UP	NO*	NEGATIVE	ALL	6/6		ALL TISSUES	NEGATIVE		ALL TISSUES WERE NEGATIVE FOR ANY SPECIFIC STAINING.
T91MF019V 05/05/91	CH017 A 2262016262	BUTTONED UP	NO*	NEGATIVE	ALL	6/6		ALL TISSUES	NEGATIVE		ALL TISSUES WERE NEGATIVE FOR ANY SPECIFIC STAINING.
T91MF021V 05/14/91	KN701 B 2263016840	BUTTONED UP	YES	NEGATIVE	ALL	6/6		ALL TISSUES	NEGATIVE		ALL TISSUES NEGATIVE FOR ANY SPECIFIC STAINING.
T91MF023V 05/14/91	KN701 B 2263016840	BUTTONED UP	YES	NEGATIVE	ALL	6/6		ALL TISSUES	NEGATIVE		ALL TISSUES NEGATIVE FOR ANY SPECIFIC STAINING.

* WHILE PORTIONS OF THE GENERAL STREAM AREA MAY HAVE BEEN INITIALLY OILED, THE SOURCE OF THE SAMPLE WAS UPSTREAM OF THE ZONE OF CONTAMINATION.

** SAMPLE COLLECTED FROM KITOI BAY FISH HATCHERY.