APPENDIX C

CASE NARRATIVES

FOR

HYLEBOS WATERWAY FISH INJURY STUDIES

Data submitted to the Damage Assessment Office
February, 1997

Hylebos Waterway Fish Injury Studies
Individual Data and Quality Assurance Results
CASE NARRATIVE

Juvenile Salmon Injury
Biliary FACs

Composites of bile samples from salmon species captured for the Hylebos Waterway Fish Injury Studies were analyzed by HPLC with fluorescence detection in two separate sets. In addition to benzo(a)pyrene- (BaP) and naphthalene- (NPH) like compounds, fluorescence responses were also measured for phenanthrene- (PHN) like compounds. In accordance with the Sampling and Analysis Plan (SAP), analytical quality control (identified by QCBatch) was assessed with each analysis set. For the salmon studies, the QCBatch identification for the two analysis sets are "HylSal0l" and "HylSa102".

Method Blank

Results of HPLC analysis of the method blanks for both QCBatches showed fluorescence responses of BaP, NPH, and PHN met the following criteria: BaP, NPH, or PHN equivalents in the method blanks were less than 10% of concentrations in any bile sample analyzed in the same set.

Initial Calibration Standards

An initial calibration standard consisting of known concentrations of BaP, NPH, and PHN was analyzed in duplicate at the start of each sample set. The relative standard deviation (RSD) for each individual PAH for the two analyses met the criteria (-<15%) set in the quality control section (Table 4) of the SAP.

Continuing Calibration Standards

The calibration standard was analyzed periodically throughout the sample sets. The relative standard deviation for the analyses was within the 25% RSD limit set for each individual PAH (Table 4, SAP).

Bile Reference Material

A bile reference material was analyzed near the beginning and end of each sample set and compared to historical interlaboratory means of this reference material (see Quality Assurance section in SAP). The measured fluorescence responses of this bile sample were determined to be within the upper and lower control limit of the interlaboratory mean value for this reference bile (see Quality Assurance Results).

Replicates

Selected bile samples were analyzed in duplicate for every ten fish analyzed. The RSDs ranged from 0 to 11.4%, which is within the 50% RSD limit set in the SAP.

Juvenile Salmon Injury -- Bile Data

ID#	REPL	PHN	NPH	BaP	PHN/PROT	NPH/PROT	BaP/PROT	PROTEIN	QCBATCH
Hylebos									
Chinook									
HY94.097 C12	1	180,000	830,000	4,600	36,000	166,000	920	5.0	HylSal01
HY94.112 C15	1	100,000	490,000	2,400	6,600	32,200	158	15.2	HylSal01
HY94.158 C18	1	64,000	330,000	1,500	18,800	97,100	441	3.4	HylSal01
HY94.176 C18	1	110,000	530,000	2,900	33,300	160,600	879	3.3	HylSal01
HY94.210 C27	1	240,000	1,100,000	6,200	68,600	314,300	1,771	3.5	HylSal02
HY94.211 C27	1	75,000	350,000	2,000	20,800	97,200	556	3.6	HylSal02
HY94.214 C28	1	80,000	380,000	2,000	7,600	36,200	190	10.5	HylSal02
HY94.214 C28	2	89,000	340,000	1,700	8,500	32,400	162	10.5	HylSal02
HY94.260 C29	1	135,000	670,000	2,900	6,100	30,500	132	22.0	HylSal02
HY94.265 C30	1	76,000	360,000	2,000	19,500	92,300	513	3.9	HylSal02
HY94.270 C31	1	44,000	210,000	1,200	11,300	53,800	308	3.9	HylSal02
For Chinook from Hylebos	n = 11	n (protein) = 11		Protein ave: 7.7±5.9 mg/ml					
AVE ± SD PHN (ng/g bile)	108,000 ± 55,000			AVE ± SD PHN/PROT (ng/mg protein)	21,600 ± 17,800				
AVE ± SD NPH (ng/g bile)	510,000 ± 250,000			AVE ± SD NPH/PROT (ng/mg protein)	101,000 ± 82,000				
AVE ± SD BaP (ng/g bile)	2,670 ± 1,420			AVE ± SD BaP/PROT (ng/mg protein)	550 ± 470				
Chum									
HY94.070 C07	1	80,000	290,000	2,200	6,400	23,200	176	12.5	HylSal01
HY94.071 C08	1	81,000	330,000	2,400	3,800	15,600	113	21.2	HylSal01
HY94.072 C08	1	97,000	390,000	2,700	4,700	18,900	131	20.6	HylSal01
HY94.073 C09	1	120,000	440,000	3,500	5,000	18,300	145	24.1	HylSal01
HY94.074 C07	1	110,000	440,000	3,300	21,600	86,300	647	5.1	HylSal01
HY94.095 C10	1	51,000	210,000	1,700	2,400	10,000	81	21.1	HylSal01
HY94.096 C11	1	130,000	470,000	3,800	4,600	16,500	134	28.4	HylSal01
HY94.110 C13	1	35,000	140,000	1,300	2,400	9,500	88	14.8	HylSal01
HY94.111 C14	1	80,000	300,000	2,500	3,800	14,200	118	21.2	HylSal01
HY94.156 C17	1	54,000	240,000	1,400	3,900	17,500	102	13.7	HylSal01
HY94.157 C16	1	81,000	360,000	2,100	4,600	20,500	119	17.6	HylSal01
HY94.174 C19	1	98,000	410,000	2,500	5,200	21,900	134	18.7	HylSal01
HY94.175 C20	1	55,000	220,000	1,400	3,800	15,400	98	14.3	HylSal01
HY94.208 C26	1	77,000	330,000	2,100	6,000	25,600	163	12.9	HylSal02
HY94.212 C26	1	67,000	290,000	1,900	10,600	46,000	302	6.3	HylSal02
HY94.213 C26	1	27,000	140,000	700	3,400	17,500	86	8.0	HylSal02
For Chum from Hylebos	n = 16	n (protein) = 16		Protein ave: 16.3±6.3 mg/ml					
AVE ± SD PHN (ng/g bile)	78,000 ± 28,000			AVE ± SD PHN/PROT (ng/mg protein)	5,800 ± 4,500				
AVE ± SD NPH (ng/g bile)	310,000 ± 100,000			AVE ± SD NPH/PROT (ng/mg protein)	24,000 ± 18,000				
AVE ± SD BaP (ng/g bile)	2,220 ± 820			AVE ± SD BaP/PROT (ng/mg protein)	160 ± 130				
Nisqually Estuary									
Chinook									
HY94.141 C35	1	12,000	82,000	500	3,200	22,200	122	3.7	HylSal01
HY94.142 C36	1	13,000	90,000	500	4,100	28,100	163	3.2	HylSal01
HY94.143 C37	1	14,000	100,000	700	5,000	35,700	239	2.8	HylSal01
For Chinook from Nisqually	n = 3	n (protein) = 3		Protein ave: 3.2±0.4 mg/ml					
AVE ± SD PHN (ng/g bile)	13,000 ± 1,000			AVE ± SD PHN/PROT (ng/mg protein)	4,100 ± 700				
AVE ± SD NPH (ng/g bile)	90,000 ± 10,000			AVE ± SD NPH/PROT (ng/mg protein)	29,000 ± 6,000				
AVE ± SD BaP (ng/g bile)	550 ± 90			AVE ± SD BaP/PROT (ng/mg protein)	170 ± 50				
Nisqually Hatchery									
Chinook									
HY94.144 C32	1	15,000	100,000	800	2,100	14,300	119	7.0	HylSal01
HY94.145 C33	1	16,000	110,000	1,000	3,300	22,900	208	4.8	HylSal01
HY94.146 C34	1	18,000	120,000	1,200	3,600	24,000	240	5.0	HylSal01
HY94.146 C34	2	19,000	110,000	1,200	3,800	22,000	240	5.0	HylSal01
For Chinook from Nisqually	n = 4	n (protein) = 4		Protein ave: 5.5±0.9 mg/ml					
AVE ± SD PHN (ng/g bile)	17,000 ± 2,000			AVE ± SD PHN/PROT (ng/mg protein)	3,200 ± 600				
AVE ± SD NPH (ng/g bile)	110,000 ± 10,000			AVE ± SD NPH/PROT (ng/mg protein)	21,000 ± 4,000				
AVE ± SD BaP (ng/g bile)	1,060 ± 150			AVE ± SD BaP/PROT (ng/mg protein)	200 ± 50				

Juvenile Salmon Injury -- Bile Data

ID#	REPL	PHN	NPH	BaP	PHN/PROT	NPH/PROT	BaP/PROT	PROTEIN	QCBATCH
Puyallup State Hatchery									
Chinook									
HY94.203 C21	1	23,000	150,000	1,200	5,300	34,900	279	4.3	HylSal01
HY94.204 C22	1	20,000	130,000	1,100	4,300	28,300	239	4.6	HylSal01
HY94.204 C22	2	22,000	120,000	1,100	4,800	26,100	239	4.6	HylSal01
HY94.205 C23	1	24,000	150,000	1,300	4,300	26,800	232	5.6	HylSal01
HY94.206 C24	1	33,000	180,000	1,500	5,800	31,600	263	5.7	HylSal01
HY94.207 C25	1	23,000	140,000	1,200	3,300	20,300	174	6.9	HylSal01
For Chinook from Puyallup State		n = 6	n (protein) = 6		Protein ave: 5.3±0.9 mg/ml				
AVE ± SD PHN (ng/g bile)		24,000 ± 4,000			AVE ± SD PHN/PROT (ng/mg protein)		4,600 ± 800		
AVE ± SD NPH (ng/g bile)		150,000 ± 20,000			AVE ± SD NPH/PROT (ng/mg protein)		28,000 ± 5,000		
AVE ± SD BaP (ng/g bile)		1,230 ± 140			AVE ± SD BaP/PROT (ng/mg protein)		240 ± 30		
Puyallup Tribal Hatchery									
Chum									
HY94.062 C01	1	6,000	48,000	400	800	6,900	54	7.0	HylSal01
HY94.063 C03	1	7,000	41,000	400	1,000	5,900	60	7.0	HylSal01
HY94.064 C02	1	8,000	55,000	500	1,100	7,300	68	7.5	HylSal01
HY94.065 C04	1	11,000	79,000	700	5,500	39,500	365	2.0	HylSal01
For Chum from Puyallup Tribal		n = 4	n (protein) = 4		Protein ave: 5.9±2.2 mg/ml				
AVE ± SD PHN (ng/g bile)		8,000 ± 2,000			AVE ± SD PHN/PROT (ng/mg protein)		2,100 ± 2,000		
AVE ± SD NPH (ng/g bile)		60,000 ± 10,000			AVE ± SD NPH/PROT (ng/mg protein)		15,000 ± 14,000		
AVE ± SD BaP (ng/g bile)		510 ± 140			AVE ± SD BaP/PROT (ng/mg protein)		140 ± 130		
Skokomish Estuary									
Chum									
HY94.066 C05	1	18,000	100,000	900	1,700	9,200	86	10.9	HylSal01
HY94.067 C05	1	8,000	49,000	300	1,300	8,600	56	5.7	HylSal01
HY94.068 C06	1	10,000	68,000	400	3,800	26,200	150	2.6	HylSal01
HY94.069 C06	1	8,000	54,000	300	1,300	8,600	54	6.3	HylSal01
For Chum from Skokomish		n = 4	n (protein) = 4		Protein ave: 6.4±3.0 mg/ml				
AVE ± SD PHN (ng/g bile)		11,000 ± 4,000			AVE ± SD PHN/PROT (ng/mg protein)		2,000 ± 1,100		
AVE ± SD NPH (ng/g bile)		70,000 ± 20,000			AVE ± SD NPH/PROT (ng/mg protein)		13,000 ± 8,000		
AVE ± SD BaP (ng/g bile)		500 ± 260			AVE ± SD BaP/PROT (ng/mg protein)		90 ± 40		

Quality Assurance Bile Results -- Juvenile Salmon Injury

HylSal01

Bile Reference Material

PHN	NPH	BaP	REPL	QCBatch
50,217	98,411	390	3-33	HylSal01
51,569	100,575	424	3-33	HylSal01

For Bile Reference	AVE ± SD PHN (ng/g bile)	50,893 ± 676	RSD = 1.3%
n = 2	AVE ± SD NPH (ng/g bile)	99,493 ± 1,082	RSD = 1.1%
	AVE ± SD BaP (ng/g bile)	407 ± 17	RSD = 4.2%

Blank

PHN	NPH	BaP	REPL	QCBatch
119	653	0		HylSal01

For Blank	AVE ± SD PHN (ng/g bile)	119 ± 0	RSD = 0.0%
n = 1	AVE ± SD NPH (ng/g bile)	653 ± 0	RSD = 0.0%
	AVE ± SD BaP (ng/g bile)	0 ± 0	RSD = ?

Continuing Calibration

6,123	16,135	98	HylSal01
6,115	16,100	104	HylSal01
5,950	15,684	112	HylSal01
5,949	15,708	102	HylSal01

For Continuing	AVE ± SD PHN (ng/g bile)	6,034 ± 85	RSD = 1.4%
n = 4	AVE ± SD NPH (ng/g bile)	15,907 ± 211	RSD = 1.3%
	AVE ± SD BaP (ng/g bile)	104 ± 5	RSD = 4.9%

Initial Calibration Standard

6,058	16,100	101	HylSal01
6,008	15,915	104	HylSal01

For Initial Calibration	AVE ± SD PHN (ng/g bile)	6,033 ± 25	RSD = 0.4%
n = 2	AVE ± SD NPH (ng/g bile)	16,008 ± 93	RSD = 0.6%
	AVE ± SD BaP (ng/g bile)	103 ± 2	RSD = 1.5%

HylSal02

Bile Reference Material (Historical/Interlab.):

	NPH	PHN	BaP
$\bar{X}$	99,000	48,000	420
SD	11,000	4,600	110
UCL	121,000	57,200	640
LCL	77,000	39,800	200

$\bar{X}$ = Mean, SD = Standard Deviation,
UCL = Upper Control Limit, LCL = Lower Control Limit

Quality Assurance Bile Results -- Juvenile Salmon Injury

	PHN	NPH	BaP	REPL	QCBatch
Bile Reference Material					
	51,028	103,843	359	3-33	HylSal02
	50,716	101,018	433	3-33	HylSal02
For Bile Reference	AVE ± SD PHN (ng/g bile)		50,872 ± 156		RSD = 0.3%
n = 2	AVE ± SD NPH (ng/g bile)		102,431 ± 1,413		RSD = 1.4%
	AVE ± SD BaP (ng/g bile)		396 ± 37		RSD = 9.3%
Blank					
	83	737	0		HylSal02
For Blank	AVE ± SD PHN (ng/g bile)		83 ± 0		RSD = 0.0%
n = 1	AVE ± SD NPH (ng/g bile)		737 ± 0		RSD = 0.0%
	AVE ± SD BaP (ng/g bile)		0 ± 0		RSD = ?
Continuing Calibration					
	6,067	16,113	102		HylSal02
	6,065	16,005	111		HylSal02
	5,998	16,357	97		HylSal02
For Continuing	AVE ± SD PHN (ng/g bile)		6,043 ± 32		RSD = 0.5%
n = 3	AVE ± SD NPH (ng/g bile)		16,158 ± 147		RSD = 0.9%
	AVE ± SD BaP (ng/g bile)		103 ± 6		RSD = 5.6%
Initial Calibration Standard					
	6,040	15,941	101		HylSal02
	6,016	16,043	101		HylSal02
For Initial Calibration	AVE ± SD PHN (ng/g bile)		6,028 ± 12		RSD = 0.2%
n = 2	AVE ± SD NPH (ng/g bile)		15,992 ± 51		RSD = 0.3%
	AVE ± SD BaP (ng/g bile)		101 ± 0		RSD = 0.0%

Bile Reference Material (Historical/Interlab.):

	NPH	PHN	BaP
$\bar{X}$	99,000	48,000	420
SD	11,000	4,600	110
UCL	121,000	57,200	640
LCL	77,000	39,800	200

$\bar{X}$ = Mean, SD = Standard Deviation,
UCL = Upper Control Limit, LCL = Lower Control Limit