

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

**Juvenile Salmon Injury**

**Cytochrome P4501A - measured as aryl hydrocarbon hydroxylase (AHH)  
Activity (Tables 1-3)**

**Microsomal Protein Standard Curve Calibration**

Both criteria of the QAP were met for data used in this calibration. Standard curves for each sample set had regression coefficient ( $r^2$ ) of  $\geq 0.990$  and each protein standard run in triplicate had RSD  $< 20\%$ .

**Method Blank**

Each sample set had a mean blank (calculated from 2 sets of duplicates) of 50 dpm  $^{14}\text{C}$  or less meeting the QAP guidelines.

**Performance Evaluation**

As stated in the QAP in order to meet an RSD of less than 30% for triplicate analyses of each sample, any outlying replicate was omitted from calculations of final AHH activity. In addition all values were greater than 50 pmol/mg microsomal protein/minute.

## Juvenile Salmon Injury

### Analyses of Cytochrome P4501A [measured as aryl hydrocarbon hydroxylase(AHH) Activity]

#### Table 1 Notes

##### **Microsomal Protein Standard Curve Calibration**

The Microsome # (column heading abbr. μsome #), Protein sample # (column heading abbr. prot. sample#) and Sample # are internal lab use numbers and for identification only. Column Final Liver Comp# is the official number assigned to each composited sample.

The number under Plate # column is the number assigned to each assay plate.

The concentrations of total protein were calculated using Bovine Albumin Serum (BSA) as the standard.

Column for Protein Standard concentrations (column abbr. Prot. Std. Conc. #) identify each standard concentration dilution used : 2.0, 4.0, 8.0, 16.0, 20.0, and 30.0 mg/ml, with all standards loaded on plate as triplicates (e.g. 2.0-1, 2.0-2, 2.0-3 etc).

The Prot. Std OD value column is the absorbance of the sample measured as Optical Density (OD) at a wavelength of 620nm.

Percent coefficient of variation ( column OD %CV is standard deviation (SD) of each triplicate analyses (reported in Prot. Std OD value) divided by mean of each triplicate, multiplied by 100.

Correlation Coefficient (column abbr. Corr. Coeff.) is generated from linear standard curve fit of mean CID values of protein standards.

TABLE 1: Protein Standard Curve Calibration

µsome #	Sample #	Final Liver Comp#	Plate #	Sample #	Prot. #	mg Prot. /ml	Protein Standard Curve			
							Prot. Std. Conc.#	Prot. Std. OD value	%CV (<20%)	OD
95012401	94 Chum PTH-C1	1	950124	A01	17.1	0.230	0.230	0.993		
95012402	94 Chum PTH-C2	2		A02	15.0	0.230	0.230			
95012403	94 Chum PTH-C3	3		A03	16.1	0.230	0.731			
95012404	94 Chum PTH-C4	4		A04	17.7	0.242				
95012405	94 Chum SKO-C1	5		A05	12.5	0.230				
95012406	94 Chum SKO-C2	6		A06	14.3	0.245	3.317			
95012407	94 Chum HYL(5/25)-C1	7		A07	12.1	0.284				
95012408	94 Chum HYL(5/25)-C2	8		A08	16.3	0.282				
95012409	94 Chum HYL(5/25)-C3	9		A09	14.4	0.278	1.086			
95012410	94 Chum HYL(6/1)-C1	10		A10	11.4	0.420				
95012411	94 Chum HYL(6/1)-C2	11		A11	13.8	0.431				
95012412	94 Chinook HYL(6/1)-C3	12		A12	11.9	0.435	1.81			
95012413	94 Chum HYL(6/2)-C4	13		A13	10.9	0.477				
95012414	94 Chum HYL(6/2)-C5	14		A14	11.0	0.473				
95012415	94 Chinook HYL(6/2)-C6	15		A15	14.1	0.448	3.37			
95012416	94 Chum HYL(6/8)-C1	16		A16	12.1	0.546				
95012417	94 Chum HYL(6/8)-C2	17		A17	13.8	0.580				
95012418	94 Chinook HYL(6/8)-C3	18		A18	9.8	0.604	5.051			
95012419	94 Chum HYL(6/9)-C4	19		A19	13.9					
95012420	94 Chum HYL(6/9)-C5	20		A20	14.1					
95012421	94 Chinook PSH(6/13)-C1	21		A21	13.6					
95012422	94 Chinook PSH(6/13)-C2	22		A22	14.4					
95012423	94 Chinook PSH(6/13)-C3	23		A23	14.8					
95012424	94 Chinook PSH(6/13)-C4	24		A24	12.5					
95012501	94 Chinook PSH(6/13)-C5	25	950125	A01	13.7	0.236	0.991			
95012502	94 Chum HYL(6/15)-C1	26		A02	18.9	0.236				
95012503	94 Chinook HYL(6/15)-C2	27		A03	13.3	0.239	0.731			
95012504	94 Chinook HYL(6/16)-C3	28		A04	13.4	0.242				
95012505	94 Chinook HYL(6/22)-C1	29		A05	18.4	0.245				
95012506	94 Chinook HYL(6/23)-C2	30		A06	15.1	0.244	0.628			
95012507	94 Chinook HYL(6/29)-C1	31		A07	14.4	0.293				

Assay date  
1/26/95

**TABLE 1: Protein Standard Curve Calibration**

Hylebos/NRDA Juvenile Flatfish Survey

μsome #	Sample #	Final Liver Comp#	Plate #	Prot. Sample #	mg Prot. /ml	Protein Standard Curve				
						Prot. Std. Conc.#	Prot.Std OD value	%CV (<20%)	OD	Corr. Coeff. (>0.95)
95012508	94 Chinook NisH(5/9)-C1	32		A08	18.2	8.0-2	0.295			
95012509	94 Chinook NisH(5/13)-C2	33		A09	18.5	8.0-3	0.289	1.045		
95012510	94 Chinook NisH(5/13)-C3	34		A10	17.8	16.0-1	0.419			
95012511	94 Chinook NisE(5/18)-C1	35		A11	18.8	16.0-2	0.437			
95012512	94 Chinook NisE(5/20)-C2	36		A12	21.1	16.0-3	0.445	3.073		
95012513	94 Chinook NisE(5/31)-C3	37		A13	16.3	20.0-1	0.461			
						20.0-2	0.463			
						20.0-3	0.482	2.474		
						30.0-1	0.533			
						30.0-2	0.569			
						30.0-3	0.581	4.455		
									Assay date 1/26/95	

## Juvenile Salmon Injury

### Analyses of Cytochrome P4501A [measured as aryl hydrocarbon hydroxylase(AHH) Activity]

#### Table 2 Notes

##### **Method Blank - AHH Assay**

The numbers under columns Microsome # (column abbr. μsome #), AHH # and Sample # are internal lab use numbers and for identification only. Column Final Liver Comp# is the official number assigned to each composited sample.

The AHH Assay Date column is the day sample set was run. The column AHH Set Blk (μome #) is the sample run as the blank for each set.

Substrate blank is reaction mixture without substrate ( <sup>14</sup>C-Benzo[a]Pyrene ) added. Boiled blank is reaction mixture with an aliquot of boiled microsome sample. Both blank sets run as duplicates (i.e. DPM1 and DPM2). All 4 blanks are then averaged to give AHH Assay Set Blk Avg DPM.

Table 2 : Method Blank - Aryl hydrocarbon hydroxylase (AHH) Assay

AHH Assay Date	Assay #	AHH #	Sample #	Final Liver Comp#	AHH Set Blk (µsome#)	Substrate Blk (DPM1)	Substrate Blk (DPM2)	Boiled Blk (DPM1)	Boiled Blk (DPM2)
1/31/95	95012401	1/31/95-1	94 Chum PTH-C1	1	95012401	47	48	41	50
	95012402	1/31/95-2	94 Chum PTH-C2	2					
	95012403	1/31/95-3	94 Chum PTH-C3	3					
	95012404	1/31/95-4	94 Chum PTH-C4	4					
	95012405	1/31/95-5	94 Chum SKO-C1	5					
	95012406	1/31/95-6	94 Chum SKO-C2	6					
	95012407	1/31/95-7	94 Chum HYL(5/25)-C1	7					
	95012408	1/31/95-8	94 Chum HYL(5/25)-C2	8					
	95012409	1/31/95-9	94 Chum HYL(5/25)-C3	9					
	95012410	1/31/95-10	94 Chum HYL(6/1)-C1	10					
	95012411	1/31/95-11	94 Chum HYL(6/1)-C2	11					
	95012412	1/31/95-12	94 Chinook HYL(6/1)-C3	12					
	95012413	1/31/95-13	94 Chum HYL(6/2)-C4	13					
	95012414	1/31/95-14	94 Chum HYL(6/2)-C5	14					
	95012415	1/31/95-15	94 Chinook HYL(6/2)-C6	15					
	95012416	1/31/95-16	94 Chum HYL(6/8)-C1	16					
	95012417	1/31/95-17	94 Chum HYL(6/8)-C2	17					
	95012418	1/31/95-18	94 Chinook HYL(6/8)-C3	18					
	95012419	1/31/95-19	94 Chum HYL(6/9)-C4	19					
	95012420	1/31/95-20	94 Chum HYL(6/9)-C5	20					
	95012421	1/31/95-21	94 Chinook PSH(6/13)-C1	21					
	95012422	1/31/95-22	94 Chinook PSH(6/13)-C2	22					
	95012423	1/31/95-23	94 Chinook PSH(6/13)-C3	23					
	95012424	1/31/95-24	94 Chinook PSH(6/13)-C4	24					
2/1/95	95012501	2/01/95-1	94 Chinook PSH(6/13)-C5	25	95012501	45	48	49	53
	95012502	2/01/95-2	94 Chum HYL(6/15)-C1	26					
	95012503	2/01/95-3	94 Chinook HYL(6/15)-C2	27					
	95012504	2/01/95-4	94 Chinook HYL(6/16)-C3	28					
	95012505	2/01/95-5	94 Chinook HYL(6/22)-C1	29					
	95012506	2/01/95-6	94 Chinook HYL(6/23)-C2	30					
	95012507	2/01/95-7	94 Chinook HYL(6/29)-C1	31					
<b>AHH Assay Set Blk</b>						<b>47</b>	<b>48</b>	<b>41</b>	<b>50</b>
<b>Avg DPM</b>						<b>46</b>			
<b>AHH Assay Set Blk</b>						<b>45</b>	<b>48</b>	<b>49</b>	<b>53</b>
<b>Avg DPM</b>						<b>49</b>			

Table 2 : Method Blank - Aryl hydrocarbon hydroxylase (AHH) Assay Hylebos/NRDA Juvenile Salmon Survey

AHH Assay Date	usome #	AHH #	Sample #	Final Liver Comp#	AHH Set Blk (μsome#)	Substrate Blk (DPM1)	Substrate Blk (DPM2)	Boiled Blk (DPM1)	Boiled Blk (DPM2)
	95012508	2/01/95-8	94 Chinook NisH(5/9)-C1	32					
	95012509	2/01/95-9	94 Chinook NisH(5/13)-C2	33					
	95012510	2/01/95-10	94 Chinook NisH(5/13)-C3	34					
	95012511	2/01/95-11	94 Chinook NisE(5/18)-C1	35					
	95012512	2/01/95-12	94 Chinook NisE(5/20)-C2	36					
	95012513	2/01/95-13	94 Chinook NisE(5/31)-C3	37					

## Juvenile Salmon Injury

### Analyses of Cytochrome P4501A [measured as aryl hydrocarbon hydroxylase(AHH) Activity]

#### Table 3 Notes

##### **Performance Evaluation Final - AHH Activity**

Microsome # (column abbr. µsome #), AHH # and Sample # are internal lab use numbers and for identification only. Column Final Liver Comp# is the official number assigned to each composited sample.

The AHH Assay Date column is the day sample set was run. The AHH Set Blk (µsome #) is the sample run as the blank for each set.

Samples are run in triplicate. The percent coefficient of variation (%CV of DPM column) is the standard deviation (value reported in column DPM SD) of each triplicate (reported in columns :AHH DPM1, AHH PM2, AHH DPM3) divided by mean of each triplicate (column AHH DPM Avg) multiplied by 100.

Triplicate sample outlier indicated by •, was not used in final calculation to meet criteria of %CV < 30%.

The column Avg AHH minus set Blk, is AHH DPM Avg, minus the value calculated in **Table 2 AHH set Blk**) for each set.

Final AHH Activity is normalized for total microsomal prote in i.e. “Avg AHH minus set Blk” divided by “mg prot/ ml’ (from **Table 1**).

Final units for AHH activity are picomoles BaP metabolized per minute per mg microsomal protein.



Table 3 : Performance Evaluation Final - Aryl hydrocarbon hydroxylase (AHH) Activity Calculations Hylebos/NRDA Juvenile Salmon Survey

Assay Date	usome #	AHH #	Sample #	Final Liver Comp#	AHH Set /ml	mg prot. /ml	AHH DPM			AHH DPM Avg	SD	DPM (<30%)	%CV of DPM	Avg AHH minus Set Blk	Final AHH Activity
							DPM1	DPM2	DPM3						
1/31/95	95012401	1/31/95-1	94 Chum PTH-C1	1	46	17.1	99	104	86	96	9	10	50	20	
	95012402	1/31/95-2	94 Chum PTH-C2	2		15.0	113	121	124	119	6	5	73	33	
	95012403	1/31/95-3	94 Chum PTH-C3	3		16.1	160	150	136	149	12	8	103	43	
	95012404	1/31/95-4	94 Chum PTH-C4	4		17.7	115	137	124	125	11	9	79	30	
	95012405	1/31/95-5	94 Chum SKO-C1	5		12.5	83	94	78	85	8	10	39	21	
	95012406	1/31/95-6	94 Chum SKO-C2	6		14.3	87	108	104	100	11	11	54	26	
	95012407	1/31/95-7	94 Chum HYL(5/25)-C1	7		12.1	178	175	152	168	14	8	122	69	
	95012408	1/31/95-8	94 Chum HYL(5/25)-C2	8		16.3	343	351	309	334	22	7	288	120	
	95012409	1/31/95-9	94 Chum HYL(5/25)-C3	9		14.4	202	226	246	225	22	10	179	84	
	95012410	1/31/95-10	94 Chum HYL(6/1)-C1	10		11.4	167	165	147	160	11	7	114	68	
	95012411	1/31/95-11	94 Chum HYL(6/1)-C2	11		13.8	172	222	221	205	29	14	159	78	
	95012412	1/31/95-12	94 Chinook HYL(6/1)-C3	12		11.9	213	222	180	205	22	11	159	91	
	95012413	1/31/95-13	94 Chum HYL(6/2)-C4	13		10.9	148	154	120	141	18	13	95	59	
	95012414	1/31/95-14	94 Chum HYL(6/2)-C5	14		11.0	91	102	88	94	7	8	48	29	
	95012415	1/31/95-15	94 Chinook HYL(6/2)-C6	15		14.1	222	229	265	239	23	10	193	93	
	95012416	1/31/95-16	94 Chum HYL(6/8)-C1	16		12.1	129	150	92	140	15	11	94	53	
	95012417	1/31/95-17	94 Chum HYL(6/8)-C2	17		13.8	225	246	231	234	11	5	188	93	
	95012418	1/31/95-18	94 Chinook HYL(6/8)-C3	18		9.8	107	113	90	103	12	12	57	40	
	95012419	1/31/95-19	94 Chum HYL(6/9)-C4	19		13.9	256	287	299	281	22	8	235	115	
	95012420	1/31/95-20	94 Chum HYL(6/9)-C5	20		14.1	306	285	269	287	19	6	241	116	
	95012421	1/31/95-21	94 Chinook PSH(6/13)-C1	21		13.6	94	95	101	97	4	4	51	25	
	95012422	1/31/95-22	94 Chinook PSH(6/13)-C2	22		14.4	164	92	75	84	12	14	38	18	
	95012423	1/31/95-23	94 Chinook PSH(6/13)-C3	23		14.8	112	113	111	112	1	1	66	30	
	95012424	1/31/95-24	94 Chinook PSH(6/13)-C4	24		12.5	87	85	81	84	3	4	38	21	
2/1/95	95012501	2/01/95-1	94 Chinook PSH(6/13)-C5	25	49	13.7	99	112	104	105	7	6	59	29	
	95012502	2/01/95-2	94 Chum HYL(6/15)-C1	26		18.9	573	528	488	530	43	8	484	174	
	95012503	2/01/95-3	94 Chinook HYL(6/15)-C2	27		13.3	257	232	221	237	18	8	191	98	
	95012504	2/01/95-4	94 Chinook HYL(6/16)-C3	28		13.4	324	318	277	306	26	8	260	132	
	95012505	2/01/95-5	94 Chinook HYL(6/22)-C1	29		18.4	527	521	424	491	58	12	445	164	
	95012506	2/01/95-6	94 Chinook HYL(6/23)-C2	30		15.1	341	288	324	318	27	9	272	122	

• Outlier of triplicate

Table 3 : Performance Evaluation Final - Aryl hydrocarbon hydroxylase (AHH) Activity Calculations Hylebos/NRDA Juvenile Salmon Survey

Assay Date	usome #	AHH #	Sample #	Final Liver Comp#	AHH Set Blk	mg /ml	prot. /ml	AHH DPM			AHH DPM			%CV of DPM (<30%)	Avg minus Set Blk	Final AHH Activity
								DPM1	DPM2	DPM3	Avg	SD	DPM			
	95012507	2/01/95-7	94 Chinook HYL(6/29)-C1	31			14.4	141	116	124	127	13	10	81	38	
	95012508	2/01/95-8	94 Chinook NisH(5/9)-C1	32			18.2	415	409	380	401	19	5	355	133	
	95012509	2/01/95-9	94 Chinook NisH(5/13)-C2	33			18.5	343	342	320	335	13	4	286	105	
	95012510	2/01/95-10	94 Chinook NisH(5/13)-C3	34			17.8	415	422	361	399	33	8	350	134	
	95012511	2/01/95-11	94 Chinook NisE(5/18)-C1	35			18.8	277	287	230	265	30	11	216	78	
	95012512	2/01/95-12	94 Chinook NisE(5/20)-C2	36			21.1	307	310	261	293	27	9	244	79	
	95012513	2/01/95-13	94 Chinook NisE(5/31)-C3	37			16.3	181	185	181	182	2	1	133	56	

• Outlier of triplicate