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

Toxicopathic conditions in Flatfish

Cytochrome P4501LA - 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 coefficent (r^2) of ≥0.990 and each protein standard run in triplicate had RSD < 20%.

Method Blank
Two sample sets had a mean blank (calculated from 2 sets of duplicates) of 50 dpm ^{14}C or less. In order to meet the QAP guidelines outlined in Table 4, one sample set with mean blank of > 50 dpm ^{14}C, was rerun. Previously unthawed microsome samples were used in the rerun set. The resulting mean dpm of blanks was less than 50 dpm ^{14}C for the rerun set, thus these data were used.

Performance Evaluation
Triplicate analyses of each sample met the criteria as stated in the QAP of less than 30% RSD, for all values > 50 pmol/mg microsomal protein/minute. Sample RS-CP-C3, which was < 50 pmol/mg microsomal protein/minute, had a SD of < 20 pmol/mg microsomal protein/minute, as outlined in Table 4 of the QAP.

Toxicopathic conditions in Flatfish

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 Sample # is species-internal site abbr. composite # (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 OD values of protein standards.

Sample Protein concentrations are given in column mg Prot, /ml and are generated by automated interpolation from the standard curve fit of the mean OD values of protein standards.

Table 1: Protein Standard Curve Calibration

Hylebos/NRDA Flatfish Pathology

µsome #	Sample #	Plate #	Prot. Sample#	mg Prot. /ml	Protein Standard Curve Prot. Std. Conc.#	Prot.Std OD value	OD %CV (<20%)	Corr. Coeff. (>0.95)
95060701	ES-UTB-C1	Data 06-16 0001	A01	21.3	2.0-1	0.225		0.994
95060702	ES-UTB-C2		A02	24.9	2.0-2	0.227		
95060703	ES-UTB-C3		A03	25.9	2.0-3	0.226	0.442	
95060704	ES-UTB-C4		A04	21.8	4.0-1	0.231		
95060705	ES-UTB-C5		A05	20.8	4.0-2	0.245		
95060706	ES-UTB-C6		A06	21.9	4.0-3	0.248	3.76	
95060707	ES-11th-C1		A07	22.4	8.0-1	0.3		
95060708	ES-11th-C2		A08	20.6	8.0-2	0.294		
95060709	ES-11th-C3		A09	18.1	8.0-3	0.299	1.08	
95060710	ES-11th-C4		A10	21.8	16.0-1	0.42		
95060711	ES-11th-C5		A11	22.6	16.0-2	0.413		
95060712	ES-11th-C6		A12	18.8	16.0-3	0.404	1.945	
95060901	RS-CP-C1		A13	18.5	20.0-1	0.456		
95060902	RS-CP-C2		A14	16.5	20.0-2	0.441		
95060903	RS-CP-C3		A15	16.7	20.0-3	0.463	2.48	
95060904	RS-CP-C4		A16	19.5	30.0-1	0.554		
95060905	RS-CP-C5		A17	22.3	30.0-2	0.518		
95060906	RS-CP-C6		A18	23.4	30.0-3	0.532	3.394	
95060907	ES-FC-C1		A19	21.0				
95060908	ES-FC-C2		A20	25.5				
95060909	ES-FC-C3		A21	25.9				Assay Date
95060910	ES-FC-C4		A22	21.1				6/16/95
95060911	ES-FC-C5		A23	25.4				
95060912	ES-FC-C6		A24	21.9				
95061201	ES-LTB-C1	Data 06-16 0002	A01	19.6	2.0-1	0.232		0.993
95061202	ES-LTB-C2		A02	24.8	2.0-2	0.232		
95061203	ES-LTB-C3		A03	27.4	2.0-3	0.231	0.253	
95061204	ES-LTB-C4		A04	26.5	4.0-1	0.243		
95061205	ES-LTB-C5		A05	24.5	4.0-2	0.242		
95061206	ES-LTB-C6		A06	27.3	4.0-3	0.241	0.414	
95061207	RS-11th-C1		A07	18.8	8.0-1	0.301		

Table 1: Protein Standard Curve Calibration

Hylebos/NRDA Flatfish Pathology

µsome #	Sample #	Plate #	Prot. Sample#	mg Prot. /ml	Protein Standard Curve Prot. Std. Conc.#	Prot.Std OD value	OD %CV (<20%)	Corr. Coeff. (>0.95)
95061208	RS-11th-C2		A08	23.4	8.0-2	0.293		
95061209	RS-11th-C3		A09	24.7	8.0-3	0.288	2.231	
95061210	RS-11th-C4		A10	19.0	16.0-1	0.4		
95061211	RS-11th-C5		A11	19.6	16.0-2	0.388		
95061212	RS-11th-C6		A12	20.7	16.0-3	0.416	3.5	
					20.0-1	0.456		
					20.0-2	0.459		
					20.0-3	0.437	2.647	
					30.0-1	0.532		
					30.0-2	0.519		**Assay Date**
					30.0-3	0.515	1.703	**6/16/95**

Toxicopathic conditions in Flatfish

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

Table 2 Notes

Method Blank - AHH Assay

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 Sample # is species-internal site abbr.-composite # (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 (µsome #) is the sample run as the blank for each set.

Substrate blank is reaction mixture without substrate (^{14}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 hyroxylase (AHH) Assay Hylebos/NRDA Flatfish Pathology

AHH Assay Date	usome #	AHH #	Sample #	Site Name	AHH Set Blk (µsome#)	Substrate Blk (DPM2)	Substrate Blk (DPM1)	Boiled Blk (DPM1)	Boiled Blk (DPM2)
6/14/95	95060701	6/14/95-1	ES-UTB-C1	Upper Turning Basin	95060703	36	36	41	27
	95060702	6/14/95-2	ES-UTB-C2	Upper Turning Basin					
	95060703	6/14/95-3	ES-UTB-C3	Upper Turning Basin					
	95060704	6/14/95-4	ES-UTB-C4	Upper Turning Basin					
	95060705	6/14/95-5	ES-UTB-C5	Upper Turning Basin					
	95060706	6/14/95-6	ES-UTB-C6	Upper Turning Basin					
	95060707	6/14/95-7	ES-11th-C1	11th Street Bridge					
	95060708	6/14/95-8	ES-11th-C2	11th Street Bridge					
	95060709	6/14/95-9	ES-11th-C3	11th Street Bridge					
	95060710	6/14/95-10	ES-11th-C4	11th Street Bridge					
	95060711	6/14/95-11	ES-11th-C5	11th Street Bridge					
	95060712	6/14/95-12	ES-11th-C6	11th Street Bridge					
6/26/95	95060901	6/26/95-1	RS-CP-C1	Colvos Passage	95060902	57	67	97	83
	95060902	6/26/95-2	RS-CP-C2	Colvos Passage					
	95060903	6/26/95-3	RS-CP-C3	Colvos Passage					
	95060904	6/26/95-4	RS-CP-C4	Colvos Passage					
	95060905	6/26/95-5	RS-CP-C5	Colvos Passage					
	95060906	6/26/95-6	RS-CP-C6	Colvos Passage					
	95060907	6/26/95-7	ES-FC-C1	Colvos Passage					
	95060908	6/26/95-8	ES-FC-C2	Colvos Passage					
	95060909	6/26/95-9	ES-FC-C3	Colvos Passage					
	95060910	6/26/95-10	ES-FC-C4	Colvos Passage					
	95060911	6/26/95-11	ES-FC-C5	Colvos Passage					
	95060912	6/26/95-12	ES-FC-C6	Colvos Passage					
6/28/95	95061201	6/28/95-1	ES-LTB-C1	Lower Turning Basin	95061203	41	35	29	34
	95061202	6/28/95-2	ES-LTB-C2	Lower Turning Basin					
	95061203	6/28/95-3	ES-LTB-C3	Lower Turning Basin					
	95061204	6/28/95-4	ES-LTB-C4	Lower Turning Basin					
	95061205	6/28/95-5	ES-LTB-C5	Lower Turning Basin					
	95061206	6/28/95-6	ES-LTB-C6	Lower Turning Basin					
	95061207	6/28/95-7	RS-11th-C1	11th Street Bridge					

AHH Assay Set Blk
Avg DPM 34

AHH Assay Set Blk
Avg DPM 76*

AHH Assay Set Blk
Avg DPM 35

* Avg >50 dpm : Set Rerun

Table 2: Method Blank - Aryl hydrocarbon hyroxylase (AHH) Assay Hylebos/NRDA Flatfish Pathology

AHH Assay Date	usome #	AHH #	Sample #	Site Name	AHH Set Blk (µsome#)	Substrate Blk (DPM2)	Substrate Blk (DPM1)	Boiled Blk (DPM1)	Boiled Blk (DPM2)
	95061208	6/28/95-8	RS-11th-C2	11th Street Bridge					
	95061209	6/28/95-9	RS-11th-C3	11th Street Bridge					
	95061210	6/28/95-10	RS-11th-C4	11th Street Bridge					
	95061211	6/28/95-11	RS-11th-C5	11th Street Bridge					
	95061212	6/28/95-12	RS-11th-C6	11th Street Bridge					
10/27/95	95060901	10/27/95-1	RS-CP-C1	Colvos Passage	95060902	24	23	49	29
	95060902	10/27/95-2	RS-CP-C2	Colvos Passage					
	95060903	10/27/95-3	RS-CP-C3	Colvos Passage					
	95060904	10/27/95-4	RS-CP-C4	Colvos Passage					
	95060905	10/27/95-5	RS-CP-C5	Colvos Passage					
	95060906	10/27/95-6	RS-CP-C6	Colvos Passage					
	95060907	10/27/95-7	ES-FC-C1	Colvos Passage					
	95060908	10/27/95-8	ES-FC-C2	Colvos Passage					
	95060909	10/27/95-9	ES-FC-C3	Colvos Passage					
	95060910	10/27/95-10	ES-FC-C4	Colvos Passage					
	95060911	10/27/95-11	ES-FC-C5	Colvos Passage					
	95060912	10/27/95-12	ES-FC-C6	Colvos Passage					

RERUN OF 6/26/95 set

AHH Assay Set Blk	
Avg DPM	31

Toxicopathic conditions in Flatfish

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

Table 3 Notes

Performance Evaluation Final AHH Activity-

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 Sample # is species-internal site abbr.-composite (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 DPM2, 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 protein 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 - Aryl hyrdocarbon hyrdoxylase (AHH) Activity Calculations Hylebos/NRDA Flatfish Pathology

AHH Assay Date	usome #	AHH #	Sample #	Site Name	AHH Set Blk	mg Prot. /mg	AHH DPM1	AHH DPM2	AHH DPM3	AHH DPM Avg	DPM SD	%CV of DPM (<30%)	Avg AHH minus Set Blk	Final AHH Activity
6/14/95	95060701	6/14/95-1	ES-UTB-C1	Upper Turning Basin	34	21.3	2921	2868	2722	2837	103	4	2803	895
	95060702	6/14/95-2	ES-UTB-C2	Upper Turning Basin		24.9	3080	2982	3041	3034	49	2	3000	820
	95060703	6/14/95-3	ES-UTB-C3	Upper Turning Basin		25.9	3940	4050	3976	3989	56	1	3955	1039
	95060704	6/14/95-4	ES-UTB-C4	Upper Turning Basin		21.8	3095	3271	3212	3193	90	3	3159	986
	95060705	6/14/95-5	ES-UTB-C5	Upper Turning Basin		20.8	3554	3370	3242	3389	157	5	3355	1097
	95060706	6/14/95-6	ES-UTB-C6	Upper Turning Basin		21.9	2905	2679	2729	2771	119	4	2737	850
	95060707	6/14/95-7	ES-11th-C1	11th Street Bridge		22.4	2619	2715	2657	2664	48	2	2630	799
	95060708	6/14/95-8	ES-11th-C2	11th Street Bridge		20.6	3564	3758	3571	3631	110	3	3597	1188
	95060709	6/14/95-9	ES-11th-C3	11th Street Bridge		18.1	2319	2488	2372	2393	86	4	2359	887
	95060710	6/14/95-10	ES-11th-C4	11th Street Bridge		21.8	2338	2318	2367	2341	25	1	2307	720
	95060711	6/14/95-11	ES-11th-C5	11th Street Bridge		22.6	2575	2500	2626	2567	63	2	2533	762
	95060712	6/14/95-12	ES-11th-C6	11th Street Bridge		18.8	2013	1982	2004	2000	16	1	1966	711
6/26/95	95060901	6/26/95-1	RS-CP-C1	Colvos Passage	76	18.5	256	264	270	263	7	3	187	69
	95060902	6/26/95-2	RS-CP-C2	Colvos Passage		16.5	394	417	380	397	19	5	321	132
	95060903	6/26/95-3	RS-CP-C3	Colvos Passage		16.7	186	180	183	183	3	2	107	44
	95060904	6/26/95-4	RS-CP-C4	Colvos Passage		19.5	614	626	617	619	6	1	543	189
	95060905	6/26/95-5	RS-CP-C5	Colvos Passage		22.3	458	437	482	459	23	5	383	117
	95060906	6/26/95-6	RS-CP-C6	Colvos Passage		23.4	633	617	598	616	18	3	540	157
	95060907	6/26/95-7	ES-FC-C1	Colvos Passage		21.0	684	755	711	717	36	5	641	208
	95060908	6/26/95-8	ES-FC-C2	Colvos Passage		25.5	767	707	771	748	36	5	672	179
	95060909	6/26/95-9	ES-FC-C3	Colvos Passage		25.9	910	1044	999	984	68	7	908	239
	95060910	6/26/95-10	ES-FC-C4	Colvos Passage		21.1	819	853	837	836	17	2	760	245
	95060911	6/26/95-11	ES-FC-C5	Colvos Passage		25.4	653	658	642	651	8	1	575	154
	95060912	6/26/95-12	ES-FC-C6	Colvos Passage		21.9	828	863	874	855	24	3	779	242
6/28/95	95061201	6/28/95-1	ES-LTB-C1	Lower Turning Basin	35	19.6	2440	2417	2417	2425	13	1	2390	829
	95061202	6/28/95-2	ES-LTB-C2	Lower Turning Basin		24.8	3860	3981	3426	3756	292	8	3721	1021
	95061203	6/28/95-3	ES-LTB-C3	Lower Turning Basin		27.4	4716	4546	4580	4614	90	2	4579	1137
	95061204	6/28/95-4	ES-LTB-C4	Lower Turning Basin		26.5	3884	3849	3931	3888	41	1	3853	989
	95061205	6/28/95-5	ES-LTB-C5	Lower Turning Basin		24.5	3102	3219	3138	3153	60	2	3118	866
	95061206	6/28/95-6	ES-LTB-C6	Lower Turning Basin		27.3	4475	4393	4175	4348	155	4	4313	1075
	95061207	6/28/95-7	RS-11th-C1	11th Street Bridge		18.8	2680	2806	2671	2719	75	3	2684	971
	95061208	6/28/95-8	RS-11th-C2	11th Street Bridge		23.4	4266	4300	4145	4237	81	2	4202	1222
	95061209	6/28/95-9	RS-11th-C3	11th Street Bridge		24.7	4086	4078	3791	3985	168	4	3950	1088
	95061210	6/28/95-10	RS-11th-C4	11th Street Bridge		19.0	3023	2680	2912	2872	175	6	2837	1016
	95061211	6/28/95-11	RS-11th-C5	11th Street Bridge		19.6	2570	2631	2632	2611	36	1	2576	894
	95061212	6/28/95-12	RS-11th-C6	11th Street Bridge		20.7	2378	2757	2679	2605	200	8	2570	844

• Outlier of triplicate

Table 3: Performance Evaluation - Aryl hyrdocarbon hyrdoxylase (AHH) Activity Calculations Hylebos/NRDA Flatfish Pathology

AHH Assay Date	usome #	AHH #	Sample #	Site Name	AHH Set Blk	mg Prot. /mg	AHH DPM1	AHH DPM2	AHH DPM3	AHH DPM Avg	DPM SD	%CV of DPM (<30%)	Avg AHH minus Set Blk	Final AHH Activity
10/27/95	95060901	10/27/95-1	RS-CP-C1	Colvos Passage	31	18.5	264	293	274	277	14.7	5	246	77
	95060902	10/27/95-2	RS-CP-C2	Colvos Passage		16.5	502	533	469	501.3	32	6	470	166
RERUN of	95060903	10/27/95-3	RS-CP-C3	Colvos Passage		16.7	150	171	179	166.7	15	9	136	47
6/26/95	95060904	10/27/95-4	RS-CP-C4	Colvos Passage		19.5	773	773	761	769	6.93	1	738	220
set	95060905	10/27/95-5	RS-CP-C5	Colvos Passage		22.3	566	518	532	538.7	24.7	5	508	132
	95060906	10/27/95-6	RS-CP-C6	Colvos Passage		23.4	691	783	826	766.7	69	9	736	183
	95060907	10/27/95-7	ES-FC-C1	Colvos Passage		21.0	747	825	652	741.3	86.6	12	710	197
	95060908	10/27/95-8	ES-FC-C2	Colvos Passage		25.5	1025	884	944	951	70.8	7	920	210
	95060909	10/27/95-9	ES-FC-C3	Colvos Passage		25.9	1003	963	1105	1024	73.2	7	993	223
	95060910	10/27/95-10	ES-FC-C4	Colvos Passage		21.1	936	956	1056	982.7	64.3	7	952	262
	95060911	10/27/95-11	ES-FC-C5	Colvos Passage		25.4	730	640	660	676.7	47.3	7	646	148
	95060912	10/27/95-12	ES-FC-C6	Colvos Passage		21.9	750	806	864	806.7	57	7	776	206

• Outlier of triplicate