

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

**Toxicopathic Conditions in Flatfish
Biliary FACs**

Composites of bile samples from English and rock sole captured for the Hylebos Waterway Fish Injury Studies were analyzed by BFLC with fluorescence detection. 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 Toxicopathic Conditions Study, the QCBatch identification for the analysis set is “HylPathol04”.

Method Blank

Results of HPLC analysis of the method blank for HylPathol04 showed fluorescence responses at BaP, NPH, and PHN wavelengths 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 (515%) 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 RSD for the analyses in HylPathol04 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 within the upper and lower control limits 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 1.7 to 23%, which is within the 50% RSD limit set in the SAP.

Toxicopathic Condition in Flatfish -- Bile Data

ID#	REPL	PHN	NPH	BaP	PHN/PROT	NPH/PROT	BaP/PROT	PROTEIN	QCBATCH
11th Street Bridge									
English sole									
94-ESB-ES-C01	1	41,000	120,000	1,000	34,200	100,000	833	1.2	HylPathol04
94-ESB-ES-C02	1	100,000	310,000	2,400	23,800	73,800	571	4.2	HylPathol04
94-ESB-ES-C03	1	54,000	160,000	1,800	45,000	133,300	1,500	1.2	HylPathol04
94-ESB-ES-C04	1	69,000	220,000	2,000	27,600	88,000	800	2.5	HylPathol04
94-ESB-ES-C05	1	60,000	190,000	1,500	16,200	51,400	405	3.7	HylPathol04
94-ESB-ES-C06	1	70,000	210,000	1,800	21,900	65,600	563	3.2	HylPathol04
For English sole from 11th Street		n = 6	n (protein) = 6	Protein ave: 2.7 ± 1.2 mg/ml					
AVE ± SD PHN (ng/g bile)		66,000 ± 18,000		AVE ± SD PHN/PROT (ng/mg protein)		28,100 ± 9,300			
AVE ± SD NPH (ng/g bile)		202,000 ± 59,000		AVE ± SD NPH/PROT (ng/mg protein)		85,000 ± 26,000			
AVE ± SD BaP (ng/g bile)		1,750 ± 430		AVE ± SD BaP/PROT (ng/mg protein)		780 ± 350			
Rock sole									
94-ESB-RS-C01	1	51,000	190,000	1,300	18,900	70,400	481	2.7	HylPathol04
94-ESB-RS-C02	1	60,000	200,000	2,100	27,300	90,900	955	2.2	HylPathol04
94-ESB-RS-C03	1	61,000	200,000	3,000	25,400	83,300	1,250	2.4	HylPathol04
94-ESB-RS-C03	2	59,000	190,000	3,500	24,600	79,200	1,458	2.4	HylPathol04
94-ESB-RS-C04	1	46,000	160,000	1,200	32,900	114,300	857	1.4	HylPathol04
94-ESB-RS-C05	1	31,000	120,000	700	14,800	57,100	324	2.1	HylPathol04
94-ESB-RS-C06	1	38,000	130,000	800	21,100	72,200	433	1.8	HylPathol04
For Rock sole from 11th Street		n = 7	n (protein) = 7	Protein ave: 2.1 ± 0.4 mg/ml					
AVE ± SD PHN (ng/g bile)		49,000 ± 11,000		AVE ± SD PHN/PROT (ng/mg protein)		23,600 ± 5,500			
AVE ± SD NPH (ng/g bile)		170,000 ± 31,000		AVE ± SD NPH/PROT (ng/mg protein)		81,000 ± 17,000			
AVE ± SD BaP (ng/g bile)		1,790 ± 1,020		AVE ± SD BaP/PROT (ng/mg protein)		820 ± 400			
Colvos Passage									
English sole									
94-FCL-ES-C01	1	10,000	36,000	300	5,900	21,200	182	1.7	HylPathol04
94-FCL-ES-C02	1	11,000	41,000	400	3,100	11,700	100	3.5	HylPathol04
94-FCL-ES-C03	1	10,000	37,000	300	4,800	17,600	152	2.1	HylPathol04
94-FCL-ES-C04	1	13,000	46,000	400	6,200	21,900	190	2.1	HylPathol04
94-FCL-ES-C05	1	19,000	79,000	500	4,200	17,600	116	4.5	HylPathol04
94-FCL-ES-C06	1	13,000	40,000	400	3,500	10,800	116	3.7	HylPathol04
For English sole from Colvos		n = 6	n (protein) = 6	Protein ave: 2.9 ± 1.0 mg/ml					
AVE ± SD PHN (ng/g bile)		13,000 ± 3,000		AVE ± SD PHN/PROT (ng/mg protein)		4,600 ± 1,100			
AVE ± SD NPH (ng/g bile)		47,000 ± 15,000		AVE ± SD NPH/PROT (ng/mg protein)		17,000 ± 4,000			
AVE ± SD BaP (ng/g bile)		390 ± 70		AVE ± SD BaP/PROT (ng/mg protein)		140 ± 30			
Rock sole									
94-CLP-RS-C01	1	17,000	63,000	600	6,800	25,200	252	2.5	HylPathol04
94-CLP-RS-C02	1	18,000	68,000	700	6,900	26,200	269	2.6	HylPathol04
94-CLP-RS-C03	1	23,000	91,000	400	6,800	26,800	124	3.4	HylPathol04
94-CLP-RS-C04	1	17,000	69,000	400	7,100	28,800	167	2.4	HylPathol04
94-CLP-RS-C05	1	11,000	47,000	400	4,600	19,600	179	2.4	HylPathol04
94-CLP-RS-C06	1	12,000	52,000	200	5,500	23,600	105	2.2	HylPathol04
94-CLP-RS-C06	2	13,000	55,000	300	5,900	25,000	145	2.2	HylPathol04
For Rock sole from Colvos		n = 7	n (protein) = 7	Protein ave: 2.5 ± 0.4 mg/ml					
AVE ± SD PHN (ng/g bile)		16,000 ± 4,000		AVE ± SD PHN/PROT (ng/mg protein)		6,200 ± 900			
AVE ± SD NPH (ng/g bile)		64,000 ± 14,000		AVE ± SD NPH/PROT (ng/mg protein)		25,000 ± 3,000			
AVE ± SD BaP (ng/g bile)		450 ± 150		AVE ± SD BaP/PROT (ng/mg protein)		180 ± 60			
Lower Turning Basin									
English sole									
94-LTB-ES-C01	1	110,000	280,000	2,300	20,800	52,800	434	5.3	HylPathol04
94-LTB-ES-C03	1	68,000	190,000	1,200	35,800	100,000	632	1.9	HylPathol04
94-LTB-ES-C04	1	90,000	230,000	1,500	28,100	71,900	469	3.2	HylPathol04
94-LTB-ES-C05	1	660,000	2,100,000	14,000	26,900	85,700	571	24.5	HylPathol04
94-LTB-ES-C06	1	92,000	240,000	1,600	17,400	45,300	302	5.3	HylPathol04
94-LTB-ES-C06	2	83,000	220,000	1,400	20,800	55,000	350	4.0	HylPathol04

Toxicopathic Condition in Flatfish -- Bile Data

ID#	REPL	PHN	NPH	BaP	PHN/PROT	NPH/PROT	BaP/PROT	PROTEIN	QCBATCH
For English sole from Lower Turning		n = 6	n (protein) = 6		Protein ave: 7.4 ± 7.8 mg/ml				
AVE ± SD PHN (ng/g bile)		184,000 ± 213,000	AVE ± SD PHN/PROT (ng/mg protein)		25,000 ± 6,100				
AVE ± SD NPH (ng/g bile)		543,000 ± 697,000	AVE ± SD NPH/PROT (ng/mg protein)		68,000 ± 19,000				
AVE ± SD BaP (ng/g bile)		3,670 ± 4,630	AVE ± SD BaP/PROT (ng/mg protein)		460 ± 120				
Upper Turning Basin									
English sole									
94-UTB-ES-C01	1	430,000	870,000	6,300	95,600	193,300	1,400	4.5	HyIPathol04
94-UTB-ES-C01	2	420,000	750,000	6,100	110,500	197,400	1,605	3.8	HyIPathol04
94-UTB-ES-C02	1	250,000	470,000	5,100	48,100	90,400	981	5.2	HyIPathol04
94-UTB-ES-C03	1	150,000	340,000	2,700	107,100	242,900	1,929	1.4	HyIPathol04
94-UTB-ES-C04	1	88,000	200,000	1,500	73,300	166,700	1,250	1.2	HyIPathol04
94-UTB-ES-C05	1	110,000	260,000	1,900	45,800	108,300	792	2.4	HyIPathol04
94-UTB-ES-C06	1	140,000	350,000	2,700	41,200	102,900	794	3.4	HyIPathol04
For English sole from Upper Turning		n = 7	n (protein) = 7		Protein ave: 3.1 ± 1.4 mg/ml				
AVE ± SD PHN (ng/g bile)		227,000 ± 134,000	AVE ± SD PHN/PROT (ng/mg protein)		74,500 ± 27,900				
AVE ± SD NPH (ng/g bile)		463,000 ± 235,000	AVE ± SD NPH/PROT (ng/mg protein)		157,000 ± 54,000				
AVE ± SD BaP (ng/g bile)		3,760 ± 1,870	AVE ± SD BaP/PROT (ng/mg protein)		1,250 ± 400				

Quality Assurance Bile Results – Toxicopathic Conditions In Flatfish

	PHN	NPH	BaP	REPL	QCBatch
HylPathol04					
Bile Reference Material					
	49,645	104,640	430	3-34	HylPathol04
	48,208	102,557	403	3-34	HylPathol04
	56,354	115,251	488	3-34	HylPathol04
For Bile Reference n = 3	AVE ± SD PHN (ng/g bile)		51,402 ± 3,550		RSD = 6.9%
	AVE ± SD NPH (ng/g bile)		107,483 ± 5,558		RSD = 5.2%
	AVE ± SD BaP (ng/g bile)		440 ± 35		RSD = 8.1%
Blank					
	153	1,536	1		HylPathol04
For Blank n = 1	AVE ± SD PHN (ng/g bile)		153 ± 0		RSD = 0.0%
	AVE ± SD NPH (ng/g bile)		1,536 ± 0		RSD = 0.0%
	AVE ± SD BaP (ng/g bile)		1 ± 0		RSD = 0.0%
Continuing Calibration					
	5,760	15,431	97		HylPathol04
	5,696	15,570	109		HylPathol04
	6,591	17,643	102		HylPathol04
	5,712	15,723	110		HylPathol04
	5,717	15,551	97		HylPathol04
	5,750	15,389	96		HylPathol04
	6,459	16,793	89		HylPathol04
For Continuing n = 7	AVE ± SD PHN (ng/g bile)		5,955 ± 363		RSD = 6.1%
	AVE ± SD NPH (ng/g bile)		16,014 ± 801		RSD = 5.0%
	AVE ± SD BaP (ng/g bile)		100 ± 7		RSD = 7.0%
Initial Calibration Standard					
	5,892	15,316	98		HylPathol04
	6,422	16,585	102		HylPathol04
For Initial Calibration n = 2	AVE ± SD PHN (ng/g bile)		6,157 ± 265		RSD = 4.3%
	AVE ± SD NPH (ng/g bile)		15,951 ± 635		RSD = 4.0%
	AVE ± SD BaP (ng/g bile)		100 ± 2		RSD = 2.0%

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

 Compositing Scheme of Bile Sampled for Toxicopathic Conditions in Flatfish Study.

Site	Species	Composite ID	Bile Samples in Composite
Hylebos Upper Turning Basin	English sole	94-UTB-ES-C01	94.3002, 3003, 3015, 3016, 3034
		94-UTB-ES-C02	94.3039, 3046, 3048, 3049, 3053
		94-UTB-ES-C03	94.3001, 3005, 3006, 3007, 3008
		94-UTB-ES-C04	94.3010, 3011, 3012, 3013, 3014
		94-UTB-ES-C05	94.3017, 3018, 3019, 3020, 3021
		94-UTB-ES-C06	94.3022, 3023, 3024, 3025, 3026
Hylebos Lower Turning Basin	English sole	94-LTB-ES-C01	94.3063, 3064, 3068, 3072, 3081
		94-LTB-ES-C03	94.3065, 3066, 3069, 3070, 3071
		94-LTB-ES-C04	94.3073, 3074, 3075, 3076, 3077
		94-LTB-ES-C05	94.3078, 3083, 3086, 3087, 3088
		94-LTB-ES-C06	94.3089, 3092, 3093, 3094, 3095
Hylebos 11th St. Bridge	English sole	94-ESB-ES-C01	94.3121, 3123, 3124, 3126, 3131
		94-ESB-ES-C02	94.3132, 3139, 3145, 3146, 3149
		94-ESB-ES-C03	94.3122, 3128, 3130, 3133, 3134
		94-ESB-ES-C04	94.3136, 3142, 3175, 3176, 3177
		94-ESB-ES-C05	94.3179, 3180, 3182, 3183, 3184
		94-ESB-ES-C06	94.3185, 3186, 3187, 3188, 3189
Colvos Passage	English sole	94-FCL-ES-C01	94.3325, 3326, 3329, 3330, 3339
		94-FCL-ES-C02	94.3311, 3312, 3314, 3315, 3316
		94-FCL-ES-C03	94.3317, 3318, 3319, 3333, 3335
		94-FCL-ES-C04	94.3321, 3322, 3323, 3324, 3327
		94-FCL-ES-C05	94.3331, 3332, 3336, 3337, 3338
		94-FCL-ES-C06	94.3341, 3342, 3342, 3344, 3345
Hylebos 11th St. Bridge	Rock sole	94-ESB-RS-C01	94.3152, 3153, 3155, 3156, 3157
		94-ESB-RS-C02	94.3158, 3159, 3160, 3162, 3163
		94-ESB-RS-C03	94.3151, 3164, 3165, 3166, 3167
		94-ESB-RS-C04	94.3169, 3170, 3171, 3172, 3174
		94-ESB-RS-C05	94.3206, 3207, 3208, 3209, 3210
		94-ESB-RS-C06	94.3211, 3212, 3213, 3214, 3215
Colvos Passage	Rock sole	94-CLP-RS-C01	94.3416, 3417, 3421, 3423, 3424
		94-CLP-RS-C02	94.3425, 3426, 3427, 3428, 3429
		94-CLP-RS-C03	94.3418, 3419, 3420, 3422, 3430
		94-CLP-RS-C04	94.3431, 3432, 3433, 3435, 3436
		94-CLP-RS-C05	94.3437, 3438, 3439, 3440, 3442
		94-CLP-RS-C06	94.3443, 3444, 3446, 3447, 3448
