

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

**Reproductive Toxicology in Flatfish
Biliary FACs**

Individual bile samples from English sole captured for the Hylebos Waterway Fish Injury Studies were analyzed by HFLC 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. The QCBatch identifications for the analyses sets are “HylRepro01” through “HylRepro05”.

Those samples used in the Reproductive Toxicology Interpretive Report are identified by “ReTox” under the INVEST# in the summary table. In addition, data from all samples listed in this case narrative are used with FAC data from the Toxicopathic Conditions Study, and the combined flatfish FAC data is summarized

in the Toxicopathic Conditions Interpretive Report. Therefore, the only revision in this case narrative is the inclusion of the additional FAC data of bile from male English sole, as well as additional data from English sole collected from Hylebos and Colvos waterways in months other than December and January. The inclusion of these additional data complete the overall flatfish Biliary FACs dataset discussed in the Toxicopathic Conditions Interpretive Report.

Method Blank

Results of HPLC analysis of the method blank for each of the QC Batches 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 ($\leq 15\%$) set in the quality control section (Table 4) of the SAP for each of the QC Batches.

Continuing Calibration Standards

The calibration standard was analyzed periodically throughout the sample sets. The relative standard deviations for the analyses in all three QC Batches were 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 to 28%, which is within the 50% RSD limit set in the SAP.

Reproductive Toxicology in Flatfish -- Bile Data

ID#	REPL	INVEST#	PHN	NPH	BaP	PHN/PROT	NPH/PROT	BaP/PROT	PROTEIN	QCBATCH
Colvos Passage-Dec										
English sole										
94-3505	1		21,000	90,000	500	7,000	30,000	163	3.0	HylRepro01
94-3506	1		20,000	120,000	600	8,300	50,000	229	2.4	HylRepro01
94-3507	1		16,000	85,000	600	11,400	60,700	450	1.4	HylRepro01
94-3508	1		42,000	240,000	900	12,000	68,600	269	3.5	HylRepro01
94-3508	2		44,000	280,000	1,000	12,600	80,000	286	3.5	HylRepro01
94-3509	1		34,000	170,000	1,000	4,200	21,000	123	8.1	HylRepro01
94-3510	1		20,000	110,000	400	6,300	34,400	122	3.2	HylRepro01
94-3511	1		19,000	120,000	300	11,200	70,600	165	1.7	HylRepro01
94-3512	1		29,000	190,000	400	9,100	59,400	119	3.2	HylRepro01
94-3513	1		22,000	130,000	800	9,600	56,500	343	2.3	HylRepro01
94-3514	1	ReTox	11,000	27,000	200	7,900	19,400	127	1.4	HylRepro04
94-3515	1	ReTox	10,000	30,000	100	10,500	29,700	128	1.0	HylRepro04
94-3516	1	ReTox	18,000	53,000	200	8,000	24,000	75	2.2	HylRepro04
94-3517	1		35,000	220,000	1,000	10,000	62,900	286	3.5	HylRepro01
94-3518	1	ReTox	7,000	26,000	100	12,400	44,000	138	0.6	HylRepro04
94-3518	2	ReTox	7,000	25,000	100	14,000	50,900	238	0.5	HylRepro04
94-3519	1		12,000	39,000	600	7,100	22,900	376	1.7	HylRepro01
94-3520	1	ReTox	12,000	36,000	200	5,100	15,700	99	2.3	HylRepro04
94-3521	1	ReTox	8,000	33,000	100	9,600	41,300	103	0.8	HylRepro04
94-3522	1		8,000	48,000	600	6,700	40,000	517	1.2	HylRepro01
94-3523	1	ReTox	10,000	32,000	100	9,000	29,100	134	1.1	HylRepro04
94-3524	1	ReTox	11,000	41,000	200	8,700	31,700	176	1.3	HylRepro04
94-3525	1	ReTox	39,000	142,000	500	9,000	32,900	119	4.3	HylRepro04
94-3526	1	ReTox	21,000	76,000	400	3,600	12,800	61	5.9	HylRepro04
94-3527	1	ReTox	7,000	23,000	100	7,700	25,100	127	0.9	HylRepro04
94-3528	1	ReTox	9,000	37,000	200	8,100	33,400	210	1.1	HylRepro04
94-3529	1	ReTox	16,000	54,000	200	6,100	20,800	86	2.6	HylRepro04
94-3529	2	ReTox	16,000	53,000	200	6,300	21,200	91	2.5	HylRepro04
94-3530	1	ReTox	25,000	145,000	300	11,500	65,900	132	2.2	HylRepro04
94-3531	1	ReTox	14,000	43,000	200	6,300	19,700	105	2.2	HylRepro04
94-3532	1	ReTox	19,000	73,000	400	5,800	22,800	119	3.2	HylRepro04
94-3533	1	ReTox	8,000	34,000	100	7,600	30,700	73	1.1	HylRepro04
94-3534	1	ReTox	15,000	50,000	200	7,700	26,100	127	1.9	HylRepro04
94-3535	1	ReTox	8,000	29,000	200	13,400	47,600	268	0.6	HylRepro04
94-3536	1	ReTox	55,000	190,000	800	11,100	38,000	157	5.0	HylRepro04
94-3537	1	ReTox	16,000	48,000	300	5,600	17,200	108	2.8	HylRepro04
94-3538	1	ReTox	10,000	34,000	100	16,500	55,900	217	0.6	HylRepro04
94-3539	1	ReTox	9,000	30,000	400	8,900	29,500	404	1.0	HylRepro04
94-3540	1	ReTox	8,000	28,000	400	5,000	16,500	235	1.7	HylRepro04
94-3541	1	ReTox	8,000	27,000	200	8,900	30,200	198	0.9	HylRepro04
94-3542	1	ReTox	33,000	101,000	400	9,900	30,600	126	3.3	HylRepro04
94-3543	1	ReTox	13,000	43,000	200	8,500	28,400	164	1.5	HylRepro04
94-3544	1	ReTox	10,000	33,000	300	5,200	17,300	165	1.9	HylRepro04
For English sole from Colvos			n = 43	n (protein) = 43	Protein ave: 2.3 ± 1.5 mg/ml					
AVE ± SD PHN (ng/g bile)			18,000 ± 11,500		AVE ± SD PHN/PROT (ng/mg protein)			8,700 ± 2,800		
AVE ± SD NPH (ng/g bile)			80,000 ± 65,000		AVE ± SD NPH/PROT (ng/mg protein)			36,000 ± 17,000		
AVE ± SD BaP (ng/g bile)			380 ± 260		AVE ± SD BaP/PROT (ng/mg protein)			190 ± 100		

Colvos Passage-Jan

English sole

94-3650	1	ReTox	27,000	120,000	500	10,400	46,200	188	2.6	HylRepro01
94-3651	1	ReTox	15,000	92,000	500	11,500	70,800	362	1.3	HylRepro01
94-3652	1	ReTox	16,000	53,000	600	7,000	23,000	278	2.3	HylRepro01
94-3653	1	ReTox	15,000	79,000	900	10,000	52,700	627	1.5	HylRepro01
94-3654	1	ReTox	22,000	130,000	700	12,900	76,500	406	1.7	HylRepro01
94-3654	2	ReTox	20,000	120,000	800	11,800	70,600	441	1.7	HylRepro01
94-3657	1	ReTox	12,000	28,000	200	17,500	39,800	276	0.7	HylRepro05
94-3659	1	ReTox	15,000	40,000	300	5,800	15,300	102	2.6	HylRepro05
94-3660	1	ReTox	10,000	32,000	200	7,400	24,300	162	1.3	HylRepro05
94-3661	1	ReTox	9,000	29,000	100	14,400	47,600	235	0.6	HylRepro05
94-3662	1	ReTox	9,000	29,000	100	6,600	20,500	91	1.4	HylRepro05
94-3662	2	ReTox	9,000	28,000	100	6,200	18,600	81	1.5	HylRepro05
94-3663	1	ReTox	12,000	38,000	200	3,500	11,000	62	3.5	HylRepro05
94-3664	1	ReTox	13,000	41,000	300	5,700	18,700	130	2.2	HylRepro05
94-3665	1		25,000	130,000	800	9,300	48,100	296	2.7	HylRepro01
94-3666	1	ReTox	17,000	54,000	600	6,500	20,700	225	2.6	HylRepro05
94-3668	1		29,000	190,000	900	7,800	51,400	246	3.7	HylRepro01

Reproductive Toxicology in Flatfish -- Bile Data

ID#	REPL	INVEST#	PHN	NPH	BaP	PHN/PROT	NPH/PROT	BaP/PROT	PROTEIN	QCBATCH
94-3669	1	ReTox	15,000	47,000	200	9,100	27,800	115	1.7	HylRepro05
94-3670	1	ReTox	21,000	110,000	400	23,300	122,200	489	0.9	HylRepro01
94-3672	1	ReTox	58,000	340,000	1,100	12,900	75,600	244	4.5	HylRepro01
94-3673	1		26,000	140,000	700	7,200	38,900	192	3.6	HylRepro01
94-3674	1		120,000	650,000	4,900	17,600	95,600	721	6.8	HylRepro01
94-3681	1	ReTox	10,000	34,000	200	4,700	15,300	73	2.2	HylRepro05
94-3682	1	ReTox	8,000	27,000	100	7,700	24,500	124	1.1	HylRepro05
94-3683	1	ReTox	17,000	56,000	800	14,200	46,500	663	1.2	HylRepro05
94-3684	1	ReTox	5,000	16,000	500	4,400	13,600	403	1.2	HylRepro05
94-3685	1	ReTox	13,000	39,000	200	5,100	15,700	71	2.5	HylRepro05
94-3686	1		19,000	120,000	700	12,700	80,000	480	1.5	HylRepro01
94-3687	1	ReTox	38,000	120,000	1,100	5,900	18,700	174	6.4	HylRepro05
94-3688	1	ReTox	9,000	25,000	200	10,500	27,600	269	0.9	HylRepro05
94-3689	1	ReTox	22,000	65,000	500	13,900	40,700	287	1.6	HylRepro05
94-3690	1	ReTox	11,000	38,000	100	9,400	31,800	118	1.2	HylRepro05
94-3691	1		29,000	170,000	900	8,800	51,500	264	3.3	HylRepro01
94-3691	2		34,000	230,000	1,100	10,300	69,700	333	3.3	HylRepro01

For English sole from Colvos

n = 34

n (protein) = 34

Protein ave: 2.3 ± 1.4 mg/ml

AVE ± SD PHN (ng/g bile)	21,500 ± 20,000	AVE ± SD PHN/PROT (ng/mg protein)	9,800 ± 4,300
AVE ± SD NPH (ng/g bile)	102,000 ± 118,000	AVE ± SD NPH/PROT (ng/mg protein)	43,000 ± 26,000
AVE ± SD BaP (ng/g bile)	630 ± 810	AVE ± SD BaP/PROT (ng/mg protein)	270 ± 170

Colvos Passage-Oct

English sole

94-3402	1		37,000	82,000	5,500	24,700	54,700	3,667	1.5	HylRepro02
94-3403	1		14,000	66,000	1,200	7,000	33,000	600	2.0	HylRepro02
94-3404	1		18,000	67,000	4,600	7,200	26,800	1,840	2.5	HylRepro02
94-3405	1		13,000	55,000	300	5,000	21,200	127	2.6	HylRepro02
94-3409	1		4,000	22,000	300	800	4,600	56	4.8	HylRepro02
94-3411	1		19,000	89,000	3,700	5,300	24,700	1,028	3.6	HylRepro02
94-3413	1		18,000	83,000	400	6,400	29,600	143	2.8	HylRepro02
94-3471	1		17,000	90,000	300	5,500	29,000	100	3.1	HylRepro02
94-3471	2		19,000	82,000	400	6,100	26,500	135	3.1	HylRepro02
94-3484	1		8,000	59,000	3,200	1,700	12,900	696	4.6	HylRepro02
94-3486	1		9,000	53,000	300	4,100	24,100	123	2.2	HylRepro02
94-3488	1		14,000	56,000	800	2,700	11,000	147	5.1	HylRepro02
94-3499	1		24,000	120,000	800	4,100	20,700	131	5.8	HylRepro02

For English sole from Colvos

n = 13

n (protein) = 13

Protein ave: 3.4 ± 1.3 mg/ml

AVE ± SD PHN (ng/g bile)	16,500 ± 7,900	AVE ± SD PHN/PROT (ng/mg protein)	6,200 ± 5,600
AVE ± SD NPH (ng/g bile)	71,000 ± 23,000	AVE ± SD NPH/PROT (ng/mg protein)	25,000 ± 12,000
AVE ± SD BaP (ng/g bile)	1,670 ± 1,800	AVE ± SD BaP/PROT (ng/mg protein)	680 ± 1,000

Hylebos-Dec

English sole

94-3546	1	ReTox	64,000	200,000	900	6,500	20,200	93	9.9	HylRepro01
94-3548	1	ReTox	25,000	72,000	600	19,200	55,400	485	1.3	HylRepro01
94-3550	1	ReTox	150,000	440,000	2,200	21,700	63,800	319	6.9	HylRepro01
94-3552	1	ReTox	89,000	270,000	1,100	20,200	61,400	250	4.4	HylRepro01
94-3552	2	ReTox	81,000	300,000	1,600	18,400	68,200	364	4.4	HylRepro01
94-3554	1	ReTox	50,000	165,000	800	23,800	78,700	380	2.1	HylRepro04
94-3556	1		26,000	100,000	700	13,000	50,000	360	2.0	HylRepro01
94-3558	1	ReTox	13,000	43,000	200	13,000	43,400	171	1.0	HylRepro04
94-3560	1	ReTox	41,000	113,000	400	45,100	125,500	496	0.9	HylRepro04
94-3562	1	ReTox	58,000	137,000	700	36,000	85,600	430	1.6	HylRepro04
94-3562	2	ReTox	56,000	134,000	700	32,900	78,700	400	1.7	HylRepro04
94-3566	1	ReTox	275,000	383,000	2,500	49,900	69,700	445	5.5	HylRepro04
94-3568	1	ReTox	32,000	88,000	500	40,300	110,600	658	0.8	HylRepro04
94-3569	1	ReTox	26,000	64,000	500	26,100	63,500	518	1.0	HylRepro04
94-3570	1		64,000	220,000	1,200	16,000	55,000	300	4.0	HylRepro01
94-3571	1		16,000	32,000	500	1,400	2,700	43	11.7	HylRepro01
94-3573	1		67,000	190,000	1,000	10,300	29,200	154	6.5	HylRepro01
94-3574	1	ReTox	220,000	383,000	2,800	73,400	127,800	928	3.0	HylRepro04
94-3576	1	ReTox	56,000	118,000	500	70,300	146,900	685	0.8	HylRepro04
94-3577	1	ReTox	125,000	180,000	1,400	37,900	54,500	419	3.3	HylRepro04
94-3580	1		150,000	380,000	3,300	24,200	61,300	532	6.2	HylRepro01
94-3581	1	ReTox	152,000	263,000	1,400	94,800	164,600	906	1.6	HylRepro04
94-3583	1		65,000	140,000	800	18,100	38,900	211	3.6	HylRepro01
94-3584	1		130,000	160,000	2,200	68,400	84,200	1,158	1.9	HylRepro01

Reproductive Toxicology in Flatfish -- Bile Data

ID#	REPL	INVEST#	PHN	NPH	BaP	PHN/PROT	NPH/PROT	BaP/PROT	PROTEIN	QCBATCH
94-3586	1	ReTox	28,000	67,000	600	21,200	51,900	425	1.3	HylRepro04
94-3586	2	ReTox	25,000	59,000	500	20,500	49,200	381	1.2	HylRepro04
94-3587	1	ReTox	25,000	63,000	300	41,500	105,800	448	0.6	HylRepro04
94-3588	1	ReTox	73,000	200,000	900	56,000	154,100	687	1.3	HylRepro04
94-3590	1	ReTox	418,000	655,000	4,300	144,200	226,000	1,496	2.9	HylRepro04
94-3591	1	ReTox	80,000	203,000	1,000	88,600	225,100	1,090	0.9	HylRepro04
94-3592	1	ReTox	79,000	215,000	1,000	30,300	82,700	383	2.6	HylRepro04
94-3593	1	ReTox	30,000	98,000	300	75,600	245,200	813	0.4	HylRepro04
94-3594	1	ReTox	72,000	230,000	1,000	40,000	127,800	556	1.8	HylRepro01

For English sole from Hylebos-Dec

n = 33

n (protein) = 33

Protein ave: 3.0±2.7 mg/ml

AVE ± SD PHN (ng/g bile)	86,600 ± 82,800	AVE ± SD PHN/PROT (ng/mg protein)	39,400 ± 30,100
AVE ± SD NPH (ng/g bile)	193,000 ± 134,000	AVE ± SD NPH/PROT (ng/mg protein)	91,000 ± 58,000
AVE ± SD BaP (ng/g bile)	1,160 ± 930	AVE ± SD BaP/PROT (ng/mg protein)	510 ± 310

Hylebos-Feb

English sole

94-3716	1		67,000	250,000	2,000	22,300	83,300	667	3.0	HylRepro02
94-3719	1		54,000	190,000	900	30,000	105,600	517	1.8	HylRepro02
94-3720	1		220,000	710,000	7,600	146,700	473,300	5,087	1.5	HylRepro02
94-3724	1		65,000	190,000	1,500	54,200	158,300	1,250	1.2	HylRepro02
94-3725	1		64,000	250,000	2,600	16,800	65,800	684	3.8	HylRepro02
94-3725	2		65,000	250,000	2,900	17,100	65,800	763	3.8	HylRepro02
94-3726	1		45,000	150,000	1,400	25,000	83,300	778	1.8	HylRepro02
94-3727	1		12,000	48,000	600	1,000	3,900	51	12.3	HylRepro02
94-3728	1		84,000	310,000	2,600	40,000	147,600	1,238	2.1	HylRepro02
94-3729	1		31,000	170,000	600	2,200	12,200	45	13.9	HylRepro02
94-3730	1		42,000	160,000	1,300	24,700	94,100	765	1.7	HylRepro02
94-3732	1		94,000	370,000	1,600	26,900	105,700	457	3.5	HylRepro02
94-3733	1		55,000	200,000	1,600	34,400	125,000	1,000	1.6	HylRepro02
94-3736	1		69,000	230,000	1,600	20,900	69,700	485	3.3	HylRepro03
94-3737	1		47,000	150,000	1,500	19,600	62,500	625	2.4	HylRepro03
94-3738	1		46,000	300,000	1,400	35,400	230,800	1,077	1.3	HylRepro03
94-3739	1		58,000	130,000	1,800	36,300	81,300	1,125	1.6	HylRepro03
94-3739	2		59,000	130,000	1,800	36,900	81,300	1,125	1.6	HylRepro03
94-3740	1		26,000	110,000	1,100	6,000	25,600	256	4.3	HylRepro03
94-3741	1		43,000	160,000	600	13,400	50,000	175	3.2	HylRepro03
94-3742	1		25,000	130,000	700	8,600	44,800	248	2.9	HylRepro03
94-3743	1		34,000	120,000	1,200	17,900	63,200	632	1.9	HylRepro03
94-3744	1		71,000	200,000	3,600	33,800	95,200	1,714	2.1	HylRepro03
94-3745	1		79,000	370,000	2,500	10,000	46,800	316	7.9	HylRepro03

For English sole from Hylebos-Feb

n = 24

n (protein) = 24

Protein ave: 3.5±3.2 mg/ml

AVE ± SD PHN (ng/g bile)	60,600 ± 38,400	AVE ± SD PHN/PROT (ng/mg protein)	28,300 ± 27,800
AVE ± SD NPH (ng/g bile)	220,000 ± 129,000	AVE ± SD NPH/PROT (ng/mg protein)	99,000 ± 92,000
AVE ± SD BaP (ng/g bile)	1,880 ± 1,410	AVE ± SD BaP/PROT (ng/mg protein)	880 ± 970

Hylebos-Jan

English sole

94-3594A	1	ReTox	66,000	181,000	1,500	66,200	181,300	1,483	1.0	HylRepro04
94-3595	1	ReTox	65,000	192,000	1,700	21,000	61,900	533	3.1	HylRepro04
94-3595	2	ReTox	66,000	193,000	1,700	21,200	62,300	553	3.1	HylRepro04
94-3596	1	ReTox	74,000	210,000	1,500	17,200	48,800	349	4.3	HylRepro01
94-3597	1		70,000	170,000	500	29,200	70,800	225	2.4	HylRepro01
94-3597	2		63,000	200,000	700	26,300	83,300	275	2.4	HylRepro01
94-3598	1	ReTox	25,000	68,000	300	9,900	27,400	138	2.5	HylRepro04
94-3599	1		87,000	430,000	2,400	6,000	29,900	167	14.4	HylRepro01
94-3600	1	ReTox	52,000	138,000	700	51,600	138,400	701	1.0	HylRepro04
94-3601	1		69,000	280,000	1,500	31,400	127,300	682	2.2	HylRepro01
94-3602	1	ReTox	30,000	114,000	400	25,200	95,100	295	1.2	HylRepro04
94-3603	1	ReTox	29,000	100,000	500	41,900	142,400	690	0.7	HylRepro04
94-3604	1	ReTox	34,000	78,000	400	38,200	87,100	456	0.9	HylRepro04
94-3605	1	ReTox	54,000	157,000	700	14,900	43,700	203	3.6	HylRepro04
94-3606	1		65,000	240,000	1,100	24,100	88,900	407	2.7	HylRepro01
94-3607	1	ReTox	54,000	154,000	1,200	49,000	139,800	1,058	1.1	HylRepro04
94-3608	1		56,000	180,000	1,600	26,700	85,700	762	2.1	HylRepro01
94-3615	1	ReTox	19,000	59,000	200	32,400	98,500	410	0.6	HylRepro04
94-3615	2	ReTox	18,000	55,000	200	35,700	109,600	462	0.5	HylRepro04
94-3616	1	ReTox	37,000	101,000	1,500	73,000	201,500	3,028	0.5	HylRepro04
94-3617	1	ReTox	102,000	408,000	1,600	48,600	194,100	770	2.1	HylRepro04

Reproductive Toxicology in Flatfish -- Bile Data

ID#	REPL	INVEST#	PHN	NPH	BaP	PHN/PROT	NPH/PROT	BaP/PROT	PROTEIN	QCBATCH
94-3618	1	ReTox	18,000	45,000	200	9,900	24,900	98	1.8	HylRepro05
94-3619	1		35,000	200,000	1,100	13,500	76,900	423	2.6	HylRepro01
94-3619	2		37,000	180,000	1,000	14,200	69,200	365	2.6	HylRepro01
94-3624	1		34,000	110,000	400	8,300	26,800	105	4.1	HylRepro01
94-3625	1		53,000	160,000	900	53,000	160,000	850	1.0	HylRepro01
94-3626	1		24,000	130,000	800	21,800	118,200	718	1.1	HylRepro01
94-3627	1		100,000	500,000	1,500	27,000	135,100	405	3.7	HylRepro01
94-3628	1	ReTox	42,000	131,000	500	27,700	87,100	353	1.5	HylRepro05
94-3629	1	ReTox	78,000	231,000	1,700	19,100	56,300	407	4.1	HylRepro05
94-3630	1	ReTox	34,000	94,000	600	14,600	40,800	252	2.3	HylRepro05
94-3630	2	ReTox	37,000	105,000	500	15,300	43,900	211	2.4	HylRepro05
94-3631	1	ReTox	28,000	80,000	400	35,600	99,700	501	0.8	HylRepro05
94-3632	1	ReTox	52,000	175,000	900	22,600	76,300	387	2.3	HylRepro05
94-3633	1	ReTox	117,000	264,000	1,400	64,800	146,800	789	1.8	HylRepro05
94-3634	1	ReTox	48,000	131,000	900	68,300	187,800	1,269	0.7	HylRepro05
94-3635	1	ReTox	33,000	105,000	500	20,600	65,800	311	1.6	HylRepro05
94-3636	1	ReTox	27,000	91,000	400	22,800	76,000	319	1.2	HylRepro05
94-3637	1	ReTox	34,000	92,000	400	42,500	115,600	496	0.8	HylRepro05
94-3639	1	ReTox	18,000	48,000	200	45,600	120,400	578	0.4	HylRepro05
94-3644	1	ReTox	70,000	196,000	1,000	1,400	4,000	21	49.0	HylRepro05
94-3644	2	ReTox	68,000	202,000	1,000	1,400	4,100	20	49.0	HylRepro05
94-3647	1	ReTox	41,000	118,000	500	17,800	51,400	221	2.3	HylRepro05
94-3648	1	ReTox	25,000	68,000	200	28,200	75,600	276	0.9	HylRepro05
94-3649	1	ReTox	30,000	86,000	400	27,600	77,900	393	1.1	HylRepro05

For English sole from Hylebos-Jan

n = 45

n (protein) = 45

Protein ave: 4.3±9.9 mg/ml

AVE ± SD PHN (ng/g bile) 49,300 ± 23,800

AVE ± SD PHN/PROT (ng/mg protein) 29,200 ± 17,500

AVE ± SD NPH (ng/g bile) 161,000 ± 96,000

AVE ± SD NPH/PROT (ng/mg protein) 90,000 ± 49,000

AVE ± SD BaP (ng/g bile) 870 ± 530

AVE ± SD BaP/PROT (ng/mg protein) 520 ± 480

Hylebos-Oct

English sole

94-3365	1		88,000	210,000	1,300	51,800	123,500	765	1.7	HylRepro02
94-3366	1		190,000	330,000	4,700	37,300	64,700	922	5.1	HylRepro02
94-3366	2		190,000	340,000	6,100	37,300	66,700	1,196	5.1	HylRepro02
94-3369	1		260,000	580,000	9,400	70,300	156,800	2,541	3.7	HylRepro02
94-3372	1		150,000	360,000	2,600	26,800	64,300	464	5.6	HylRepro02
94-3375	1		87,000	190,000	1,400	79,100	172,700	1,273	1.1	HylRepro02
94-3378	1		210,000	400,000	4,300	61,800	117,600	1,265	3.4	HylRepro02
94-3380	1		180,000	490,000	4,600	78,300	213,000	2,000	2.3	HylRepro02
94-3382	1		19,000	100,000	600	15,800	83,300	492	1.2	HylRepro02
94-3385	1		120,000	380,000	3,200	21,400	67,900	571	5.6	HylRepro02
94-3388	1		85,000	310,000	1,400	24,300	88,600	400	3.5	HylRepro02
94-3391	1		330,000	730,000	12,000	80,500	178,000	2,927	4.1	HylRepro02
94-3394	1		690,000	1,300,000	28,000	143,800	270,800	5,417	4.8	HylRepro02
94-3401	1		170,000	580,000	6,500	21,500	73,400	823	7.9	HylRepro02
94-3401	2		170,000	580,000	6,700	21,500	73,400	848	7.9	HylRepro02

For English sole from Hylebos-Oct

n = 15

n (protein) = 15

Protein ave: 4.2±2.1 mg/ml

AVE ± SD PHN (ng/g bile) 195,900 ± 151,200

AVE ± SD PHN/PROT (ng/mg protein) 51,400 ± 33,700

AVE ± SD NPH (ng/g bile) 459,000 ± 278,000

AVE ± SD NPH/PROT (ng/mg protein) 121,000 ± 62,000

AVE ± SD BaP (ng/g bile) 6,050 ± 6,150

AVE ± SD BaP/PROT (ng/mg protein) 1,460 ± 1,290

Hylebos-Sept

English sole

94-3271	1		29,000	130,000	600	3,200	14,100	70	9.2	HylRepro01
94-3272	1		93,000	330,000	2,800	10,700	37,900	322	8.7	HylRepro01
94-3273	1		39,000	160,000	1,100	39,000	160,000	1,100	1.0	HylRepro01
94-3274	1		47,000	170,000	1,000	14,200	51,500	303	3.3	HylRepro01
94-3275	1		200,000	720,000	5,100	40,800	146,900	1,041	4.9	HylRepro01
94-3276	1		170,000	470,000	1,800	60,700	167,900	571	2.8	HylRepro01
94-3277	1		26,000	120,000	800	9,300	42,900	300	2.8	HylRepro01
94-3278	1		29,000	73,000	300	13,800	34,800	143	2.1	HylRepro02
94-3280	1		91,000	190,000	1,500	10,500	21,800	172	8.7	HylRepro02
94-3281	1		220,000	680,000	4,200	47,800	147,800	913	4.6	HylRepro02
94-3282	1		140,000	310,000	3,800	63,600	140,900	1,727	2.2	HylRepro02
94-3282	2		150,000	300,000	4,200	68,200	136,400	1,909	2.2	HylRepro02
94-3283	1		93,000	230,000	1,000	30,000	74,200	323	3.1	HylRepro02
94-3284	1		170,000	500,000	2,100	29,300	86,200	362	5.8	HylRepro02
94-3285	1		180,000	530,000	3,300	46,200	135,900	846	3.9	HylRepro02

Reproductive Toxicology in Flatfish -- Bile Data

ID#	REPL	INVEST#	PHN	NPH	BaP	PHN/PROT	NPH/PROT	BaP/PROT	PROTEIN	QCBATCH
94-3286	1		110,000	320,000	1,800	28,200	82,100	462	3.9	HylRepro02
94-3287	1		240,000	620,000	5,300	50,000	129,200	1,104	4.8	HylRepro02
94-3288	1		180,000	530,000	2,400	69,200	203,800	923	2.6	HylRepro02
94-3289	1		48,000	180,000	900	15,000	56,300	294	3.2	HylRepro02
94-3290	1		230,000	660,000	2,100	54,800	157,100	500	4.2	HylRepro02
94-3291	1		210,000	540,000	4,700	63,600	163,600	1,424	3.3	HylRepro02
94-3291	2		220,000	560,000	4,600	66,700	169,700	1,394	3.3	HylRepro02
94-3292	1		250,000	830,000	5,200	31,600	105,100	658	7.9	HylRepro02
94-3295	1		66,000	130,000	1,500	94,300	185,700	2,143	0.7	HylRepro02
94-3299	1		250,000	730,000	3,400	48,100	140,400	654	5.2	HylRepro02
94-3304	1		340,000	800,000	10,000	54,000	127,000	1,587	6.3	HylRepro02
94-3305	1		740,000	2,200,000	21,000	50,000	148,600	1,419	14.8	HylRepro02
94-3308	1		200,000	580,000	5,200	29,900	86,600	776	6.7	HylRepro02

For English sole from Hylebos-Sept

n = 28

n (protein) = 28

Protein ave: 4.7 ± 3.0 mg/ml

AVE ± SD PHN (ng/g bile) 170,000 ± 137,000

AVE ± SD PHN/PROT (ng/mg protein) 40,800 ± 22,500

AVE ± SD NPH (ng/g bile) 485,000 ± 401,000

AVE ± SD NPH/PROT (ng/mg protein) 113,000 ± 53,000

AVE ± SD BaP (ng/g bile) 3,630 ± 3,940

AVE ± SD BaP/PROT (ng/mg protein) 840 ± 560

Quality Assurance Bile Results – Reproductive Toxicology In Flatfish

	PHN	NPH	BaP	REPL	QCBatch
HylRepro01					
Bile Reference Material					
	50,620	92,327	394	3-34	HylRepro01
	47,243	99,650	400	3-34	HylRepro01
For Bile Reference	AVE ± SD PHN (ng/g bile)		48,932 ± 1,689	RSD = 3.5%	
n = 2	AVE ± SD NPH (ng/g bile)		95,989 ± 3,662	RSD = 3.8%	
	AVE ± SD BaP (ng/g bile)		397 ± 3	RSD = 0.8%	
Blank					
	110	240	7	HylRepro01	
For Blank	AVE ± SD PHN (ng/g bile)		110 ± 0	RSD = 0.0%	
n = 1	AVE ± SD NPH (ng/g bile)		240 ± 0	RSD = 0.0%	
	AVE ± SD BaP (ng/g bile)		7 ± 0	RSD = 0.0%	
Continuing Calibration					
	6,086	15,701	98	HylRepro01	
	5,844	15,784	98	HylRepro01	
	6,348	15,818	98	HylRepro01	
	5,902	15,778	102	HylRepro01	
	6,345	15,651	99	HylRepro01	
	6,121	15,816	98	HylRepro01	
	6,071	15,803	99	HylRepro01	
	5,918	15,753	101	HylRepro01	
	5,925	15,543	102	HylRepro01	
For Continuing	AVE ± SD PHN (ng/g bile)		6,062 ± 176	RSD = 2.9%	
n = 9	AVE ± SD NPH (ng/g bile)		15,739 ± 87	RSD = 0.6%	
	AVE ± SD BaP (ng/g bile)		99 ± 2	RSD = 1.6%	
Initial Calibration Standard					
	5,722	16,227	99	HylRepro01	
	6,273	15,839	101	HylRepro01	

Bile Reference Material (Historical/Interlab.):

	NPH	PHN	BaP
\bar{X}	99,000	48,000	420
SD	11,000	4,800	110
UCL	121,000	57,200	640
LCL	77,000	38,800	200

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

Quality Assurance Bile Results – Reproductive Toxicology In Flatfish

	PHN	NPH	BaP	REPL	QCBatch
For Initial Calibration	AVE ± SD PHN (ng/g bile)		5,998 ± 276		RSD = 4.6%
n=2	AVE ± SD NPH (ng/g bile)		16,033 ± 194		RSD = 1.2%
	AVE ± SD BaP (ng/g bile)		100 ± 1		RSD = 1.0%

HylRepro02

Bile Reference Material

	46,835	104,685	523	334	HylRepro02
	51,180	101,255	473	334	HylRepro02
For Bile Reference	AVE ± SD PHN (ng/g bile)		49,008 ± 2,173		RSD = 4.4%
n=2	AVE ± SD NPH (ng/g bile)		102,970 ± 1,715		RSD = 1.7%
	AVE ± SD BaP (ng/g bile)		498 ± 25		RSD = 5.0%

Blank

	140	550	0		HylRepro02
For Blank	AVE ± SD PHN (ng/g bile)		140 ± 0		RSD = 0.0%
n=1	AVE ± SD NPH (ng/g bile)		550 ± 0		RSD = 0.0%
	AVE ± SD BaP (ng/g bile)		0 ± 0		RSD = ?

Continuing Calibration

	6,165	16,200	104		HylRepro02
	6,326	15,764	100		HylRepro02
	6,146	16,036	102		HylRepro02
	6,057	15,962	100		HylRepro02
	5,962	16,014	101		HylRepro02
	5,765	16,047	95		HylRepro02
	6,102	16,189	97		HylRepro02
	5,828	16,065	98		HylRepro02
	6,235	16,393	100		HylRepro02
For Continuing	AVE ± SD PHN (ng/g bile)		6,065 ± 174		RSD = 2.9%
n=9	AVE ± SD NPH (ng/g bile)		16,074 ± 165		RSD = 1.0%
	AVE ± SD BaP (ng/g bile)		100 ± 3		RSD = 2.5%

Initial Calibration Standard

	5,991	15,977	100		HylRepro02
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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 – Reproductive Toxicology In Flatfish

	PHN	NPH	BaP	REPL	QC Batch
	5,996	16,080	100		HylRepro02

For Initial Calibration	AVE ± SD PHN (ng/g bile)	5,994 ± 3	RSD = 0.0%
n = 2	AVE ± SD NPH (ng/g bile)	16,029 ± 52	RSD = 0.3%
	AVE ± SD BaP (ng/g bile)	100 ± 0	RSD = 0.0%

HylRepro03**Bile Reference Material**

	46,693	86,828	402	334	HylRepro03
	49,414	97,316	558	334	HylRepro03

For Bile Reference	AVE ± SD PHN (ng/g bile)	48,054 ± 1,361	RSD = 2.8%
n = 2	AVE ± SD NPH (ng/g bile)	92,072 ± 5,244	RSD = 5.7%
	AVE ± SD BaP (ng/g bile)	480 ± 78	RSD = 16.3%

Blank

	425	825	1	HylRepro03
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For Blank	AVE ± SD PHN (ng/g bile)	425 ± 0	RSD = 0.0%
n = 1	AVE ± SD NPH (ng/g bile)	825 ± 0	RSD = 0.0%
	AVE ± SD BaP (ng/g bile)	1 ± 0	RSD = 0.0%

Continuing Calibration

	5,494	16,164	98	HylRepro03
	5,833	16,154	100	HylRepro03
	6,008	15,288	98	HylRepro03
	5,924	15,845	99	HylRepro03

For Continuing	AVE ± SD PHN (ng/g bile)	5,815 ± 195	RSD = 3.4%
n = 4	AVE ± SD NPH (ng/g bile)	15,863 ± 356	RSD = 2.2%
	AVE ± SD BaP (ng/g bile)	99 ± 1	RSD = 0.8%

Initial Calibration Standard

	5,932	15,985	99	HylRepro03
	6,053	16,078	101	HylRepro03

Bile Reference Material (Historical/Interlab.):

	NPH	PHN	BaP
\bar{X}	99,000	48,000	420
SD	11,000	4,800	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 – Reproductive Toxicology In Flatfish

	PHN	NPH	BaP	REPL	QCBatch
For Initial Calibration	AVE ± SD PHN (ng/g bile)		5,993 ± 61		RSD = 1.0%
n = 2	AVE ± SD NPH (ng/g bile)		16,032 ± 47		RSD = 0.3%
	AVE ± SD BaP (ng/g bile)		100 ± 1		RSD = 1.0%

HylRepro04

Bile Reference Material

	49,727	99,693	392	3-34	HylRepro04
	54,905	107,279	472	3-34	HylRepro04
For Bile Reference	AVE ± SD PHN (ng/g bile)		52,316 ± 2,589		RSD = 4.9%
n = 2	AVE ± SD NPH (ng/g bile)		103,486 ± 3,793		RSD = 3.7%
	AVE ± SD BaP (ng/g bile)		432 ± 40		RSD = 9.3%

Blank

	16	37	8		HylRepro04
For Blank	AVE ± SD PHN (ng/g bile)		16 ± 0		RSD = 0.0%
n = 1	AVE ± SD NPH (ng/g bile)		37 ± 0		RSD = 0.0%
	AVE ± SD BaP (ng/g bile)		8 ± 0		RSD = 0.0%

Continuing Calibration

	6,107	16,168	101		HylRepro04
	6,050	16,353	100		HylRepro04
	6,517	17,508	109		HylRepro04
	6,053	16,312	102		HylRepro04
	7,039	19,138	110		HylRepro04
	6,809	17,768	121		HylRepro04
	5,511	17,222	87		HylRepro04
	5,901	16,362	96		HylRepro04

For Continuing	AVE ± SD PHN (ng/g bile)		6,248 ± 471		RSD = 7.5%
n = 8	AVE ± SD NPH (ng/g bile)		17,104 ± 960		RSD = 5.6%
	AVE ± SD BaP (ng/g bile)		103 ± 10		RSD = 9.2%

Initial Calibration Standard

	6,175	16,368	104		HylRepro04
	5,969	15,932	99		HylRepro04

Bile Reference Material (Historical/Interlab.):

	NPH	PHN	BaP
\bar{X}	99,000	48,000	420
SD	11,000	4,800	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 – Reproductive Toxicology In Flatfish

	PHN	NPH	BaP	REPL	QCBatch
For Initial Calibration	AVE ± SD PHN (ng/g bile)		6,072 ± 103		RSD = 1.7%
n = 2	AVE ± SD NPH (ng/g bile)		16,150 ± 218		RSD = 1.3%
	AVE ± SD BaP (ng/g bile)		102 ± 3		RSD = 2.5%

HylRepro05

Bile Reference Material

	48,181	96,364	375	3-34	HylRepro05
	51,846	105,287	444	3-34	HylRepro05

For Bile Reference	AVE ± SD PHN (ng/g bile)		50,014 ± 1,833		RSD = 3.7%
n = 2	AVE ± SD NPH (ng/g bile)		100,826 ± 4,462		RSD = 4.4%
	AVE ± SD BaP (ng/g bile)		410 ± 35		RSD = 8.4%

Blank

	0	0	11		HylRepro05
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For Blank	AVE ± SD PHN (ng/g bile)		0 ± 0		RSD = ?
n = 1	AVE ± SD NPH (ng/g bile)		0 ± 0		RSD = ?
	AVE ± SD BaP (ng/g bile)		11 ± 0		RSD = 0.0%

Continuing Calibration

	5,909	16,829	90		HylRepro05
	6,159	22,454	102		HylRepro05
	5,859	17,390	96		HylRepro05
	6,177	17,212	101		HylRepro05

For Continuing	AVE ± SD PHN (ng/g bile)		6,026 ± 143		RSD = 2.4%
n = 4	AVE ± SD NPH (ng/g bile)		18,471 ± 2,308		RSD = 12.5%
	AVE ± SD BaP (ng/g bile)		97 ± 5		RSD = 4.9%

Initial Calibration Standard

	6,189	16,038	101		HylRepro05
	5,998	15,879	102		HylRepro05

Bile Reference Material (Historical/Interlab.):

	NPH	PHN	BaP
\bar{X}	99,000	48,000	420
SD	11,000	4,800	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 -- Reproductive Toxicology In Flatfish

	PHN	NPH	BaP	REPL	QCBatch
For Initial Calibration	AVE ± SD PHN (ng/g bile)		6,094 ± 96		RSD = 1.6%
n = 2	AVE ± SD NPH (ng/g bile)		15,959 ± 80		RSD = 0.5%
	AVE ± SD BaP (ng/g bile)		102 ± 1		RSD = 0.5%

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