

**EROD activity and P4501A protein content in liver of yellow perch  
as biomarkers of PCBP exposure**

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by

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## **BACKGROUND**

Induction of the cytochrome P450 system by polyhalogenated aromatic hydrocarbons (PHAHs) has been experimentally documented in a variety of organisms as well as cell lines. This induction has been increasingly used in environmental monitoring as an indicator of exposure to PHAHs (Collier et al. 1992, Monosson and Stegeman, 1994, Courtenay et al. 1994). In fish as in other vertebrates, the PHAHs seem to have the same mode of action mediated by the cytosolic Ah receptor. Several studies have shown, however, that the induction may differ between various classes of vertebrates and the potency of induction vary among compounds. To evaluate the responsiveness of the cytochrome P450 system in yellow perch we measured EROD activity and P450 protein content in control fish group and in perch groups exposed to graded doses of 3,3',4,4',5-pentachlorobiphenyl (PCBP).

## **METHODS**

There were five groups of yellow perch: control no-treatment, control carrier (corn oil), and 10, 100 and 500  $\mu\text{g}$  PCBP/kg. Fish were given a single i.p. injection of PCBP administered in a volume of 2  $\mu\text{l/g}$  body weight. After the treatment each group of fish was held separately in a 150 gal tanks with flow-through aerated water at temperature 15°C. They were fed commercial pelleted fish food every other day. Four

weeks after the treatment fish were sacrificed, weighed, livers were excised. Mean individual fish weight as well as gonadosomatic index (GSI) and hepatosomatic index (HSI) were determined. These are given in Table 1.

EROD activity was measured in the microsomal fraction of the liver using 48-well plates and a Cytofluor 2300 fluorescence plate reader (Millipore). Reaction mixture contained 150  $\mu$ l of Tris buffer with added 2,67  $\mu$ M 7-ethoxyresorufin, 10  $\mu$ l of microsomal fraction (45  $\mu$ g protein), and 40  $\mu$ l of 5mM NADPH. Reactions were initiated by adding NADPH and the plates were placed in the Cytofluor for determination of fluorescence at 2 min intervals using 530 nm excitation and 590 nm emission filters. EROD activities were determined by comparing the rate of fluorescence change in the sample wells with the fluorescence of authentic resorufin standards (2-150 pmol/well). The results are presented in Tables 2 and 3 for females and males, respectively.

Measurement of P4501A protein content in the liver microsoms was performed by immunoblotting using modified method of Kloepper-Sams et al (1987). Microsomal proteins were separated on polyacryamide gels and electrophoretically transferred to nitrocellulose (Schleicher and Schuell). Monoclonal antibody (MAb) 1-12-3 to scup was used as the primary antibody; the secondary antibody was a goat anti-mouse IgG linked to alkaline phosphatase. The primary antibody is highly specific for CYP1A. Staining was performed using nitroblue tetrazolium (NBT) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP) (Sigma Chemical Co.).

The amount of stain was quantified by video imaging densitometry (Master Scan, Scanalytics/CSPI, Billerica, MA) and expressed as scup CYP1A equivalents. Microsomal protein was analyzed according to the Bradford method (Pierce Chemical Company). The P4501A data are shown in Tables 2 and 3.

## RESULTS

The research was intended to be performed on individuals of one gender, yellow perch males rather than on females in order to minimize the effect of other factors such as hormonal status, which is known to influence CYP1A indices. However, at the termination of the experiment it was realized that the females were more abundant than the males in each exposure group. Thus, samples were taken for analysis from all exposed individuals and the results were separated by gender. Statistical analysis was performed on females and males data. However, more weight has been given for the female results because of fewer males in each group. The results for males are included in this report but not discussed.

Mean individual body weight, relative liver and gonad weights were similar in all fish groups (Table 1). At the commencement of the exposure (end of January), the GSI was  $3.12 \pm 1.62$  and  $3.95 \pm 1.34$  in females and males, respectively. During the 4 weeks of the exposure the GSI increased markedly in all perch groups. There was no significant difference in the

females GSI among the experimental groups. However, the index was found to be lower in the PCBP treated females than in the control groups.

The CYP1A protein content and EROD activity were found to be induced in females exposed to 10 ppb PCBP (Table 2). In females treated with a dose of 100 ppb PCBP both CYP1A protein content and EROD activity were inhibited. The highest dose tested, 500 ppb did not effect EROD activity, however, CYP1A protein content was found to be decreased in comparison to control groups. ANOVA analysis showed, however, that the differences among the groups were not significant ( $p=0.09$ , Appendix I-1) because of high individual variability. Fisher's PLSD test indicated significant difference between 10 ppb and 100 ppb dosed females (Appendix I-2) as well as between 100 ppb dosed and untreated females ( $p=0.051$ ).

TABLE 1. Mean individual fish weight and HSI and GSI indexes.

Fish group/ Treatment	Fish weight, g, Mean $\pm$ SD	HSI (%) Mean $\pm$ SD	GSI (%) Mean $\pm$ SD
Untreated	72.95 $\pm$ 9.6	1.84 $\pm$ 0.37	4.95 $\pm$ 1.71
Carrier	70.05 $\pm$ 12.9	1.67 $\pm$ 0.41	5.67 $\pm$ 2.01
10 $\mu$ g/kg	71.9 $\pm$ 13.4	1.84 $\pm$ 0.30	3.29 $\pm$ 1.73
100 $\mu$ g/kg	76.3 $\pm$ 13.1	2.03 $\pm$ 0.43	4.82 $\pm$ 1.77
500 $\mu$ g/kg	75.4 $\pm$ 13.8	1.65 $\pm$ 0.27	4.97 $\pm$ 1.45

TABLE 2. EROD activity and P4501A protein in yellow perch females.

Treatment	Microsomal Protein Content (mg/ml)	EROD Activity (pmol/min/mg)	P4501A (pmol/mg)
Untreated	5.95 $\pm$ 1.21	63.17 $\pm$ 22.07	6.80 $\pm$ 1.33
Carrier	6.12 $\pm$ 0.56	52.74 $\pm$ 30.62	6.21 $\pm$ 0.71
10 ppb PCBP	5.45 $\pm$ 0.60	75.58 $\pm$ 22.82	15.33 $\pm$ 3.02
100 ppb PCBP	5.78 $\pm$ 1.22	34.80 $\pm$ 12.5	5.26 $\pm$ 0.03
500 ppb PCBP	6.27 $\pm$ 0.78	52.52 $\pm$ 16.69	5.83 $\pm$ 0.34

Table 3. EROD activity and P4501A protein in yellow perch males.

Treatment	EROD Activity (pmol/min/mg)	P4501A (pmol/mg)
Untreated	178.0 ± 0.1	51.2 ± 9.7
Carrier	143.7	46.3
10 ppb PCBP	105.0 ± 32.2	32.2 ± 8.3
100 ppb PCBP	125.9 ± 15.7	9.5 ± 3.0
500 ppb PCBP	194.0 ± 35.3	18.9 ± 1.9

APPENDIX I - 1

**ANOVA Table for pmol/min-mg**

Split By: sex

Cell: f

	DF	Sum of Squares	Mean Square	F-Value	P-Value
treatment	4	5030.13	1257.53	2.24	.0938
Residual	25	14053.98	562.16		

Model II estimate of between component variance: 116.54

**Means Table for pmol/min-mg**

Effect: treatment

Split By: sex

Cell: f

	Count	Mean	Std. Dev.	Std. Err.
10 ppb PCB 126	6	75.59	24.98	10.20
100 ppb PCB 126	5	34.81	14.04	6.28
500 ppb PCB 126	7	52.52	18.03	6.81
carrier	5	52.75	34.60	15.47
unt	7	63.16	23.82	9.00

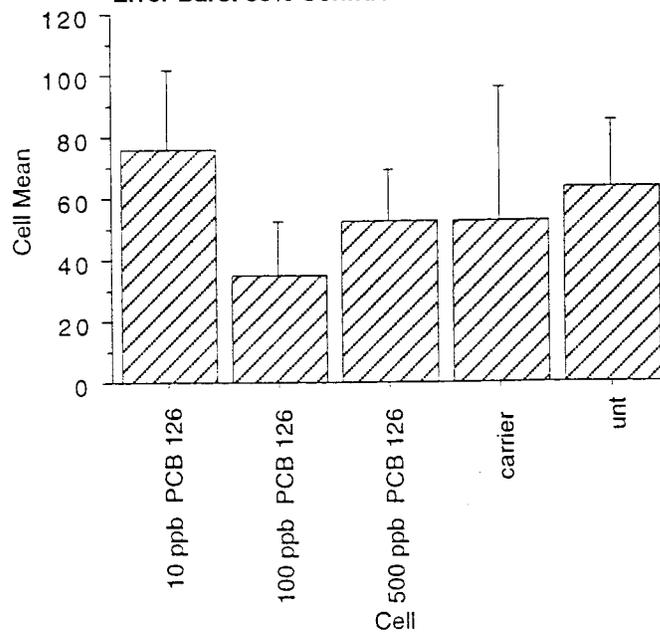
**Interaction Bar Plot for pmol/min-mg**

Effect: treatment

Split By: sex

Cell: f

Error Bars: 95% Confidence Interval



APPENDIX I - 2

Fisher's PLSD for pmol/min-mg

Effect: treatment

Significance Level: 5 %

Split By: sex

Cell: f

	Mean Diff.	Crit. Diff	P-Value	
10 ppb PCB 126, 100 ppb ...	40.78	29.57	.0088	S
10 ppb PCB 126, 500 ppb ...	23.07	27.17	.0926	
10 ppb PCB 126, carrier	22.84	29.57	.1242	
10 ppb PCB 126, unt	12.42	27.17	.3552	
100 ppb PCB 126, 500 ppb...	-17.71	28.59	.2137	
100 ppb PCB 126, carrier	-17.94	30.88	.2427	
100 ppb PCB 126, unt	-28.36	28.59	.0518	
500 ppb PCB 126, carrier	-.23	28.59	.9869	
500 ppb PCB 126, unt	-10.64	26.10	.4089	
carrier, unt	-10.41	28.59	.4602	

ANOVA Table for pmol/min-mg

Split By: sex

Cell: m

	DF	Sum of Squares	Mean Square	F-Value	P-Value
treatment	4	13989.56	3497.39	2.93	.1155
Residual	6	7150.28	1191.71		

Model II estimate of between component variance: 1102.71

Means Table for pmol/min-mg

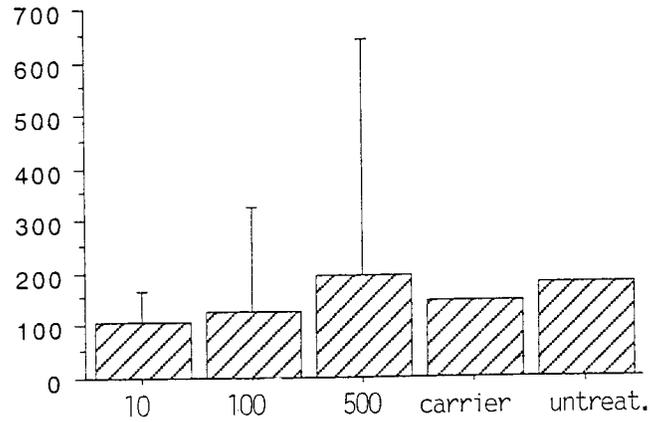
Effect: treatment

Split By: sex

Cell: m

	Count	Mean	Std. Dev.	Std. Err.
10 ppb PCB 126	4	105.04	37.23	18.62
100 ppb PCB 126	2	125.85	22.21	15.70
500 ppb PCB 126	2	194.00	49.98	35.34
carrier	1	143.71	•	•
unt	2	178.02	.17	.12

Interaction Bar Plot for pmol/min-mg  
 Effect: treatment  
 Split By: sex  
 Cell: m  
 Error Bars: 95% Confidence Interval



Cell

Fisher's PLSD for pmol/min-mg  
 Effect: treatment  
 Significance Level: 5 %  
 Split By: sex  
 Cell: m

	Mean Diff.	Crit. Diff	P-Value
10 ppb PCB 126, 100 ppb ...	-20.81	73.15	.5125
10 ppb PCB 126, 500 ppb ...	-88.96	73.15	.0248 S
10 ppb PCB 126, carrier	-38.66	94.44	.3551
10 ppb PCB 126, unt	-72.97	73.15	.0504
100 ppb PCB 126, 500 ppb...	-68.15	84.47	.0958
100 ppb PCB 126, carrier	-17.85	103.45	.6875
100 ppb PCB 126, unt	-52.16	84.47	.1815
500 ppb PCB 126, carrier	50.29	103.45	.2792
500 ppb PCB 126, unt	15.98	84.47	.6597
carrier, unt	-34.31	103.45	.4481

**ANOVA Table for pmol/min-mg**

	DF	Sum of Squares	Mean Square	F-Value	P-Value
sex	1	58264.552	58264.552	56.492	<.0001
Residual	39	40223.953	1031.383		

Model II estimate of between component variance: 3555.394

**Means Table for pmol/min-mg**

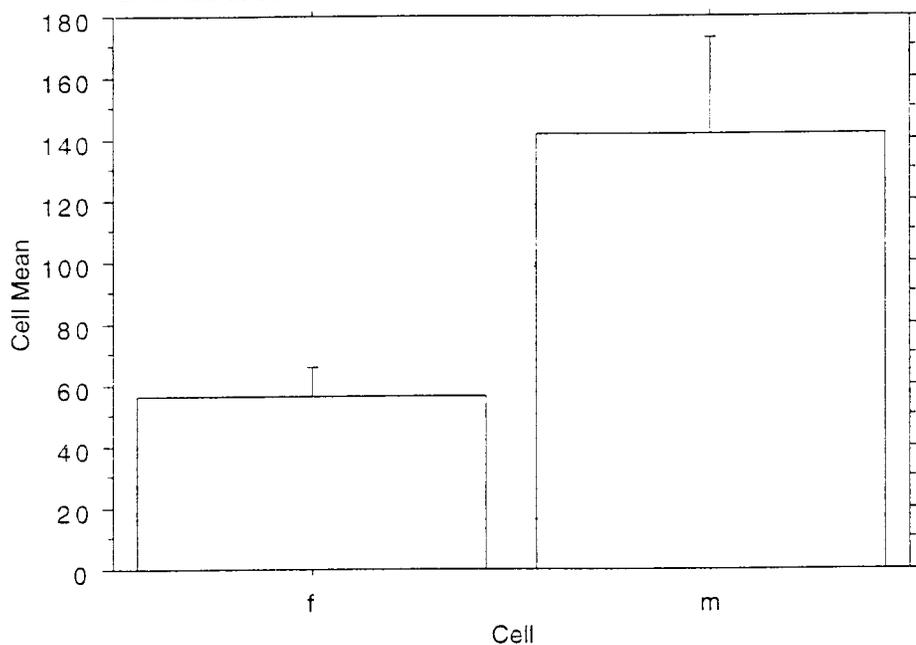
Effect: sex

	Count	Mean	Std. Dev.	Std. Err.
f	30	56.703	25.653	4.684
m	11	141.785	45.978	13.863

**Interaction Bar Plot for pmol/min-mg**

Effect: sex

Error Bars: 95% Confidence Interval



**Fisher's PLSD for pmol/min-mg**

Effect: sex

Significance Level: 5 %

	Mean Diff.	Crit. Diff	P-Value	
f, m	-85.082	22.897	<.0001	S