SUPPLEMENTARY INFORMATION for:

Role of Pregnane X Receptor and Aryl Hydrocarbon Receptor in Transcriptional Regulation of *pxr*, *CYP2*, and *CYP3* Genes in Developing Zebrafish

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Running title: *pxr* and *CYP* genes in developing zebrafish

Table S1. Summary for *pxr*, *ahr2* and *CYP* gene expression in 72 hpf zebrafish eleutheroembryos treated with a morpholino blocking *pxr* translation (Pxr), a control morpholino (Ctl), or no morpholino (No), and subsequent exposure to pregnenolone (3 μM) or the vehicle DMSO (0.1%)

Exposure	Morpholino	*pxr*		*ahr2*		*CYP1A*		*CYP2AA1*		*CYP2AA12*		*CYP3A65*		*CYP3C1*	
DMSO	No	0.93 ± 0.14		0.90 ± 0.11		1.1 ± 0.31		0.91 ± 0.09		0.93 ± 0.16		1.1 ± 0.22		0.97 ± 0.09	
	Ctl	1.0		1.0		1.0		1.0		1.0		1.0		1.0	
	Pxr	0.53 ± 0.08		0.75 ± 0.11		0.77 ± 0.14		0.76 ± 0.31		1.1 ± 0.22		0.69 ± 0.18		0.98 ± 0.23	
Pregnenolone	No	2.5 ± 0.59		1.2 ± 0.1		1.7 ± 0.42		1.2 ± 0.19		1.3 ± 0.16		2.7 ± 0.73		1.5 ± 0.23	
	Ctl	3.0 ± 0.84		1.3 ± 0.28		1.2 ± 0.15		1.7 ± 0.44		1.7 ± 0.63		2.8 ± 0.67		1.5 ± 0.44	
	Pxr	1.3 ± 0.43	***	0.72 ± 0.08	***	0.86 ± 0.34		0.64 ± 0.42	***	1.1 ± 0.19	*	1.4 ± 0.28	**	1.1 ± 0.21	*

Data are normalized to the expression levels in the Ctl-MO + DMSO group and are shown as mean ± SD from four independent experiments with each determination made in at least duplicate. Significant decreases in the PN induction of expression of these genes were observed in the Pxr-MO group relative to the Ctl-MO group ($^{*}p < 0.01$, $^{**}p < 0.01$, $^{***}p < 0.001$). Note that there was no statistical difference in transcript levels of any of the genes examined between vehicle control group and Pxr-MO + pregnenolone group.

Table S2. Summary for *pxr*, *ahr2* and *CYP* gene expression in 96 hpf zebrafish eleutheroembryos treated with a morpholino blocking *ahr2* translation (Ahr2), a control morpholino (Ctl), or no morpholino (No), and subsequent exposure to PCB126 (5 nM) or the vehicle DMSO (0.02%)

Exposure	Morpholino	*pxr*	*ahr2*	*CYP1A*	*CYP2AA12*	*CYP3A65*	*CYP3C1*
DMSO	No	0.81	1.6	8.7	1.0	0.98	0.83
	Ctl	1.0	1.0	1.0	1.0	1.0	1.0
	Ahr2	0.78	2.2	0.63	0.93	0.88	0.96
PCB126	No	1.8	2.7	83	2.3	2.9	1.1
	Ctl	1.9	2.8	140	2.8	4.5	1.4
	Ahr2	1.1	3.0	18	1.5	1.6	0.73

Data are normalized to the expression levels in the Ctl-MO + DMSO group and are shown as mean of two replicates in a single experiment. cDNA samples collected in our previous studies (Jonsson et al., 2012) were used for quantification of *pxr*, *ahr2* and *CYP* genes. Note that the Ahr2-MO studies were repeated and transcript levels of two genes that were prominently induced by PCB126 in the first experiment, *CYP2AA12* and *CYP3A65*, were quantified. Induction of these two genes by PCB126 was blocked to the levels in the Ctl-MO + DMSO group, with significant reduction as compared to the levels in the Ctl-MO + PCB126 group (see Fig. 6).

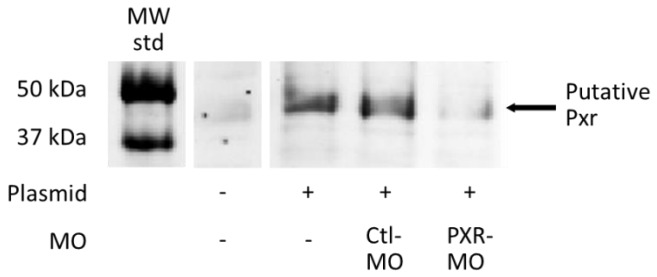

Fig. S1. Efficacy and specificity of morpholino (MO) against *pxr* assessed by *in vitro* transcription/translation system. Protein of zebrafish Pxr was synthesized by *in vitro* transcription/translation system with or without Pxr-MO or placebocontrol morpholino (Ctl-MO). The putative Pxr band was determined from the calculated molecular weight and the mobility of the *in vitro* synthesized protein relative to the molecular weight marker proteins, and is indicated by the arrow in the right margin.

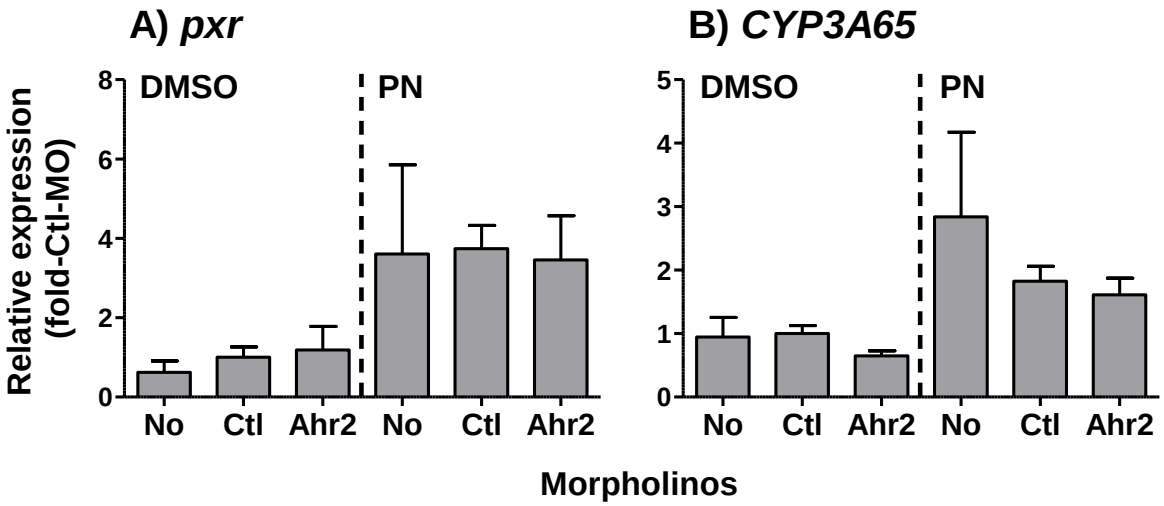

Fig. S2. Effect of Ahr2 morpholino (Ahr2-MO) treatment on the mRNA expression of *pxr* and *CYP3A65* in 72 hpf eleutheroembryos exposed to pregnenolone (PN; 3 μM) or vehicle (0.1% DMSO). Data are normalized to the expression levels in the control morpholino (Ctl-MO) + DMSO group and are shown as mean+SD of four replicates after combining data from two independent experiments. No significant suppression of PN induction of *pxr* or *CYP3A65* was observed in the Ahr2-MO group relative to the Ctl-MO group ($p > 0.05$), when statistical analysis was conducted by one-way ANOVA followed by Dunnett's multiple comparison test ($p > 0.05$).

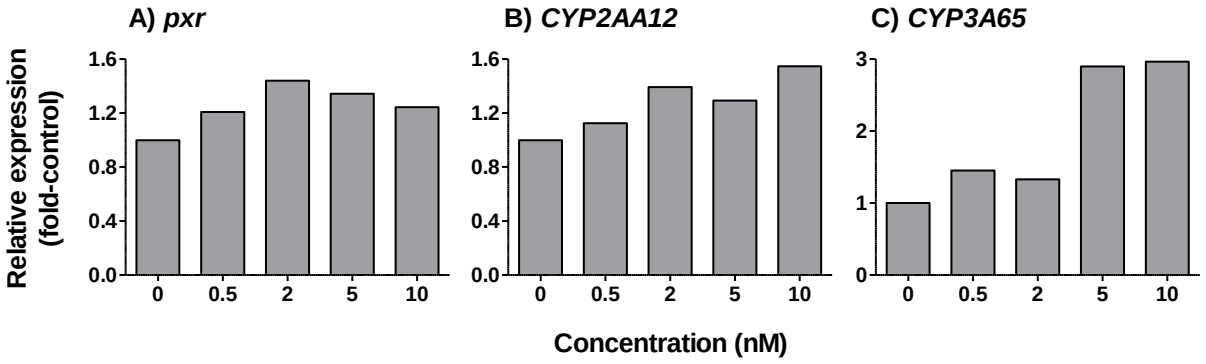

Fig. S3. Dose-response relationships for effects of PCB126 on the mRNA expression of *pxr* (A), *CYP2AA12* (B) and *CYP3A65* (C) in developing zebrafish (determined at 72 hpf). Embryos were exposed to carrier (0.02% DMSO) or differing concentrations of PCB126 (0.5, 2, 5 or 10 nM) for 24 h starting at 24 hpf. At 72 hpf, eleutheroembryos were sampled for quantitative real time PCR analysis. Relative expression (fold-control) was calculated by $E^{-\Delta\Delta Ct}$ using *ef1α* as a reference gene (Jonsson et al., 2007, 2012). $N = 2$ from a single experiment.

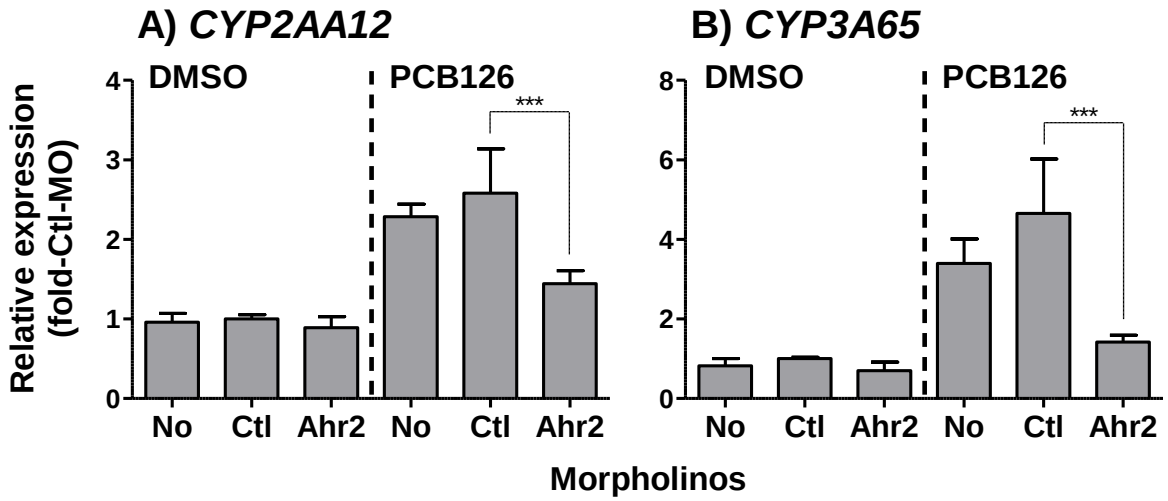

Fig. S4. Effect of Ahr2 morpholino (Ahr2-MO) treatment on the mRNA expression of *CYP2AA12* and *CYP3A65* in 96 hpf eleutheroembryos exposed to PCB126 (5 nM) or vehicle (0.02% DMSO). Data are normalized to the expression levels in the control morpholino (Ctl-MO) + DMSO group and are shown as mean+SD of four replicates after combining data from two independent experiments. Significant decreases in the PCB126 induction of expression of these genes were observed in the Ahr2-MO group relative to the Ctl-MO group ($^{***}p < 0.001$).