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Functionality of Aryl Hydrocarbon Receptors (AhR1 and AhR2) of White Sturgeon (*Acipenser transmontanus*) and Implications for the Risk Assessment of Dioxin-like Compounds

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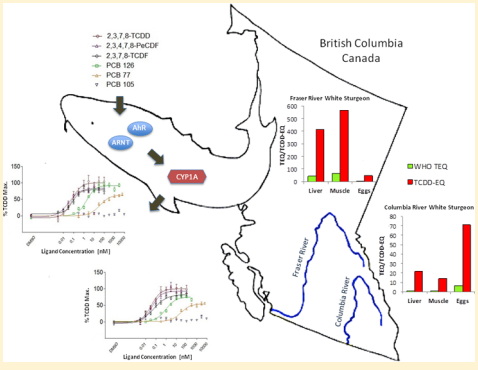
Supporting Information

ABSTRACT: Worldwide, populations of sturgeons are endangered, and it is hypothesized that anthropogenic chemicals, including dioxin-like compounds (DLCs), might be contributing to the observed declines in populations. DLCs elicit their toxic action through activation of the aryl hydrocarbon receptor (AhR), which is believed to regulate most, if not all, adverse effects associated with exposure to these chemicals. Currently, risk assessment of DLCs in fishes uses toxic equivalency factors (TEFs) developed for the World Health Organization (WHO) that are based on studies of embryo-lethality with salmonids. However, there is a lack of knowledge of the sensitivity of sturgeons to DLCs, and it is uncertain whether TEFs developed by the WHO are protective of these fishes. Sturgeons are evolutionarily distinct from salmonids, and the AhRs of sturgeons differ from those of salmonids. Therefore, this study investigated the sensitivity of white sturgeon (*Acipenser transmontanus*) to DLCs *in vitro* via the use of luciferase reporter gene assays using COS-7 cells transfected with AhR1 or AhR2 of white sturgeon. Specifically, activation and relative potencies (RePs) of 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin (TCDD), 2,3,4,7,8-pentachloro-dibenzofuran, 2,3,7,8-tetrachloro-dibenzofuran, 3,3′,4,4′,5-pentachlorobiphenyl, 3,3′,4,4′-tetrachlorobiphenyl, and 2,3,3′,4,4′-pentachlorobiphenyl were determined for each AhR. It was demonstrated that white sturgeon expresses AhR1s and AhR2s that are both activated by DLCs with EC_{50} values for 2,3,7,8-TCDD that are lower than those of any other AhR of vertebrates tested to date. Both AhRs of white sturgeon had RePs for polychlorinated dibenzofurans more similar to TEFs for birds, while RePs for polychlorinated biphenyls were most similar to TEFs for fishes. Measured concentrations of select DLCs in tissues of white sturgeon from British Columbia, Canada, were used to calculate toxic equivalents (TEQs) by use of TEFs for fishes used by the WHO and TCDD equivalents (TCDD-EQs) via the use of RePs for AhR2 of white sturgeon as determined by transfected COS-7 cells. TCDD-EQs calculated for endangered populations of white sturgeon were approximately 10-fold greater than TEQs and were within ranges known to cause adverse effects in other fishes, including other species of sturgeons. Therefore, TEFs used by the WHO might not adequately protect white sturgeon, illuminating the need for additional investigation into the sensitivity of these fish to DLCs.



1. INTRODUCTION

Most species of sturgeon (Acipenseridae) are endangered worldwide,[1] which has rendered these fishes of great interest in context with their susceptibility to anthropogenic stressors. There is particular concern about declines of populations of

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white sturgeon (*Acipenser transmontanus*) in the northwestern United States and British Columbia, Canada.[2] These declines have been attributed to several activities of humans, including overharvesting, alteration of habitats, and pollution.[3,4] Sturgeons are long-lived, and their sexual maturity is attained slowly.[5] They spawn intermittently, live in close association with sediments, and have a lipid content greater than that of numerous other fishes, which increases the likelihood of bioaccumulation of lipophilic pollutants.[5] Dioxin-like compounds (DLCs), which include polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and coplanar polychlorinated biphenyls (PCBs), are contaminants of particular concern with regard to sturgeons because of the ability of DLCs to bioaccumulate and because they can be persistent under certain conditions, such as those found in sediments. Some DLCs have been detected in white sturgeon at concentrations sufficient to warrant concern.[6−10] However, currently little is known about the sensitivity of sturgeons to these contaminants.

Dioxin-like compounds share structural similarities and bind with relatively great affinity to the aryl hydrocarbon receptor (AhR).[11] The AhR is a ligand-activated transcription factor in the Per-Arnt-Sim (PAS) family of proteins, which mediates the pleiotropic expression of a suite of genes and is believed to regulate most, if not all, adverse effects associated with exposure to DLCs.[12] In vertebrates, such effects can include hepatotoxicity, immune suppression, reproductive and endocrine impairment, teratogenicity, carcinogenicity, and loss of body mass.[13] It has been hypothesized that vertebrates underwent an ancient genome duplication event, which resulted in AhR1 and AhR2 clades.[14,15] Some fishes, such as salmonids, then underwent a second duplication event, which gave rise to multiple isoforms of AhR1 and AhR2.[14,15] The effects of DLCs have been shown to be mediated through AhR2, not AhR1, in all fishes studied to date,[16−19] while AhR1, not AhR2, drives effects of DLCs in birds.[20] White sturgeons express at least two forms of the AhR, AhR1 and AhR2, with AhR1 being most identical to the AhRs of tetrapods, such as birds, mammals, and amphibians, while AhR2 is most identical to AhR2s of other fishes.[21] Both AhR1 and AhR2 have similar levels of expression in target tissues of toxicity of DLCs and are upregulated following exposure to DLCs.[21,22] This has raised questions about the function of these AhRs and whether both have roles in mediating the toxicity of DLCs to sturgeons.

Currently, the assessment of risks posed by DLCs to fishes uses toxic equivalency factors (TEFs) developed by the World Health Organization (WHO) that are based largely on embryolethality studies with salmonids.[23] Sturgeons and some other ancient fishes, including sharks, rays, and skates, respond to exposure to DLCs through induction of cytochrome P4501A (CYP1A), which is consistent with responses of salmonids.[22,24−26] However, sturgeons and other ancient fishes are evolutionarily distinct from more modern fishes, such as salmonids, and because the sequence of amino acids of AhR1 and AhR2 of white sturgeon differ from those of salmonids, it is hypothesized that AhRs of white sturgeon might function differently.[21] This raises the question of whether TEFs currently suggested by the WHO for fishes ($TEF_{WHO\text{-}Fish}$) are adequately protective of white sturgeon.

The objective of this study was to investigate whether AhR1 and AhR2 of white sturgeon are activated by exposure to a suite of PCDDs, PCDFs, and coplanar PCBs. To determine this, a luciferase reporter gene (LRG) assay with COS-7 cells transfected with AhR1 or AhR2 of white sturgeon was used. Relative potencies (RePs) of selected DLCs were determined for each AhR of white sturgeon. RePs developed for white sturgeon in this study were compared against $TEF_{WHO\text{-}Fish}$ by use of measured concentrations of select DLCs in tissues from endangered populations of white sturgeon. This work supplements our current knowledge of evolutionary aspects of the AhR pathway among ancient fishes and allows for a better understanding of mechanisms by which sturgeons respond to DLCs, as well as a better understanding of the sensitivity of sturgeons to exposure to DLCs. This information could be essential in guiding more objective risk assessment of sturgeons to DLCs as part of ongoing conservation efforts worldwide.

2. MATERIALS AND METHODS

2.1. Chemicals. Stock solutions of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), 2,3,4,7,8-pentachloro-dibenzofuran (PeCDF), and 2,3,7,8-tetrachloro-dibenzofuran (TCDF) were prepared in dimethyl sulfoxide (DMSO) from >98% pure standards (Wellington Laboratories, Guelph, ON). Stock solutions of 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126), 3,3′,4,4′-tetrachlorobiphenyl (PCB 77), and 2,3,3′,4,4′-pentachlorobiphenyl (PCB 105) were prepared in DMSO from 100% pure standards (Chromographic Specialties, Brockville, ON). Nominal concentrations and purities of each stock solution were confirmed by use of high-resolution gas chromatography and mass spectrometry (GC−MS) according to U.S. EPA Method 1668.[27] Serial dilutions for each compound were made in DMSO on the basis of measured concentrations of stock solutions. Doses of 0.003−100 nM PCDDs and PCDFs and 0.01−9000 nM PCBs were used.

2.2. Development of Expression Constructs for AhR1, AhR2, and ARNT2 of White Sturgeon. Full-length sequences of AhR1 and AhR2 of white sturgeon have been published previously.[21] ARNT2 of white sturgeon was acquired according to the methods described for the AhR by Doering et al.[21] In short, the full-length sequence was generated by paired end transcriptome sequencing by use of the *Illumina* HiSeq 2000 platform (*Illumina*, San Diego, CA) and cloned into vectors. A consensus nucleotide sequence was determined by aligning three or more replicated sequences. Expression constructs for AhR1, AhR2, and ARNT2 of white sturgeon were generated by use of the pENTR Directional TOPO entry vector kit (Invitrogen, Burlington, ON) and the pcDNA 3.2/V5-DEST gateway vector kit (Invitrogen) according to the protocol provided by the manufacturer. Primers used to amplify full-length AhR1, AhR2, and ARNT2 of white sturgeon for ligation into expression vectors were designed according to the protocol provided by the manufacturer (Invitrogen) and included a CACC 5′-overhang and the Kozak consensus sequence (CACCATGA) in the forward primer, with the stop codon being removed from the reverse primer (Table S1 of the Supporting Information). Expression constructs for AhR1, AhR2, and ARNT2 of white sturgeon were sequenced by the University of Calgary's University Core DNA Services (Calgary, AB), and products of expression constructs were synthesized by use of the TnT Quick-Coupled Reticulocyte Lysate System kit and FluoroTect $Green_{Lys}$ (Promega, Madison, WI) according to the protocol provided by the manufacturer. Bands were visualized by use of a Typhoon Trio imager (Molecular Dynamics, Sunnyvale, CA) to confirm the proper product size of each protein.

2.3. Transfection of COS-7 Cells, the Luciferase Reporter Gene (LRG) Assay, and AhR/ARNT Protein Expression. Culture of COS-7 cells, transfection of constructs, and the LRG assay were performed in 96-well plates according to methods described by Farmahin et al.,[28] with minor modifications. Optimized amounts of expression vectors transfected into cells were 8 ng of white sturgeon AhR1 or AhR2, 1.5 ng of white sturgeon ARNT2, 20 ng of rat CYP1A1 reporter construct[29,30] (donated by M. Denison, University of California, Davis, CA), and 0.75 ng of *Renilla* luciferase vector (Promega). The total amount of DNA that was transfected into cells was kept constant at 50 ng by addition of salmon sperm DNA (Invitrogen).

Western blot analysis was performed according to the methods described by Farmahin et al.[28] In brief, AhR1, AhR2, ARNT2, and β-actin protein concentrations in COS-7 cells were determined by use of the Bradford assay.[28] The anti-V5-HRP antibody (Invitrogen) was used for detecting V5-AhR1/AhR2 and V5-ARNT2, and anti-β-actin peroxidase (Sigma-Aldrich, Oakville, ON) was used as a loading control, both according to methods described by Farmahin et al.[28] Blots were visualized by enhanced chemiluminescence by use of a Typhoon Trio imager (Molecular Dynamics) to confirm expression of proteins in COS-7 cells.

2.4. Concentration−Response Curves and Statistical Analysis. Three concentration−response curves, each with four technical replicates per concentration of chemical, were obtained from three independent experiments for each combination of AhR and DLC. Response curves and effect concentrations (ECs) were developed by use of GraphPad Prism version 5.0 (San Diego, CA). Data were fit to a four-parameter logistic model. Lowest observed effect concentrations (LOECs) were defined as the first treatment dose that was statistically significant ($p \leq 0.05$) from the DMSO control treatment by use of analysis of variance (ANOVA) followed by Dunnett's test. The homogeneity of variance of each data set was determined by use of Levene's test. A logarithmic transformation was used whenever necessary to ensure homogeneity of variance. All data are shown as mean ± the standard error of the mean (SE).

2.5. Calculation of ReS and ReP Values. The relative sensitivity (ReS) and relative potency (ReP) were calculated by use of three points on the concentration−response curve according to methods described below, unless otherwise stated. The ReS between AhR1 and AhR2 of white sturgeon was calculated by use of the formula (eq 1)

$$\text{ReS} = \frac{\text{EC}_{XX}\ \text{of AhR1 or AhR2}}{\text{EC}_{XX}\ \text{of AhR2}} \quad (1)$$

where EC_{XX} of AhR1 or AhR2 is the average of the concentration to elicit a 20% (EC_{20}), 50% (EC_{50}), or 80% (EC_{80}) response in COS-7 cells transfected with AhR1 or AhR2 for selected DLCs.

ReP values were calculated by use of the formula (eq 2)

$$\text{ReP} = \frac{\text{EC}_{XX}\ \text{TCDD}}{\text{EC}_{XX}\ \text{DLC}} \quad (2)$$

where EC_{XX} is the average of the concentration to elicit EC_{20}, EC_{50}, and EC_{80} in COS-7 cells exposed to TCDD or the selected DLC.

2.6. Calculation of TEQ and TCDD-EQ. Published concentrations of TCDD, PeCDF, TCDF, PCB 126, PCB 77, and PCB 105 in liver, muscle, and eggs of white sturgeon from the Fraser River (n = 6) or upper Columbia River (n = 1) in British Columbia, Canada,[9,10] were used to calculate toxic equivalents (TEQs) and TCDD equivalents (TCDD-EQs) expressed as picograms of TCDD per gram of tissue by use of $\text{TEF}_{\text{WHO-Fish}}$[23] and RePs developed by use of responses in COS-7 cells transfected with AhR2 of white sturgeon, respectively. AhR2 was selected as TEQs and TCDD-EQs calculated by using AhR2 represented a more sensitive estimate of toxicity relative to those calculated using AhR1. Where concentrations from multiple individuals were available, the greatest concentration of each DLC was selected because of the limited number of individuals sampled and to be the most conservative by representing a worst-case scenario.

3. RESULTS

3.1. Concentration-Dependent Effects of TCDD, PeCDF, TCDF, PCB 126, PCB 77, and PCB 105. *3.1.1. Relative Sensitivity.* AhR1 and AhR2 of white sturgeon were activated in a concentration-dependent manner by exposure to TCDD, PeCDF, TCDF, PCB 126, and PCB 77 (Figure 1 and Table 1). However, concentrations of PCB 105 as great as 9000 nM did not activate either AhR (Figure 1 and Table 1). The sensitivity of AhR1 and AhR2 of white sturgeon to DLCs was approximately equal (Table 2).

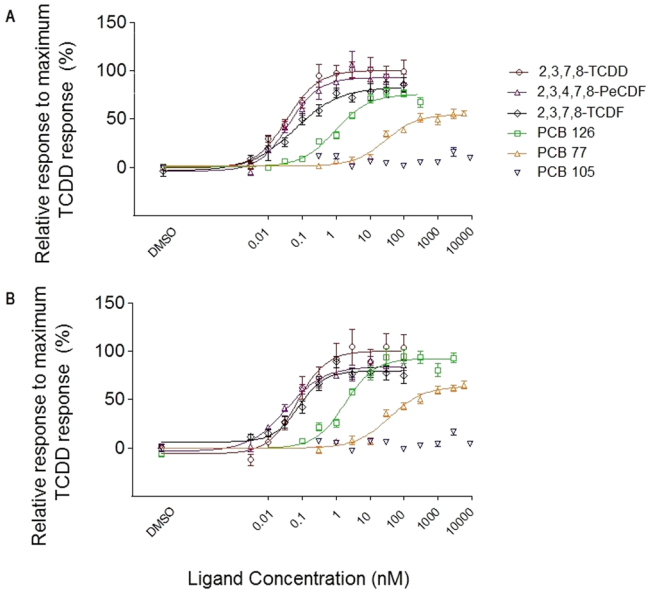

Figure 1. Responses of COS-7 cells transfected with AhR1 (A) or AhR2 (B) of white sturgeon following exposure to six dioxin-like compounds (DLCs). Dose−response curves of each DLC are presented as a percentage relative to the maximal response of TCDD. Data are presented as means ± SE based on three replicate assays conducted in quadruplicate.

3.1.2. Relative Potency. DLCs had chemical and receptor specific potencies in transfected COS-7 cells. TCDD and PeCDF were both the most potent DLCs with respect to AhR1 (Table 3). However, PeCDF was the most potent DLC with respect to AhR2 (Table 3). The order of potency for AhR1 based on ReP was TCDD = PeCDF > TCDF > PCB 126 > PCB 77 > PCB 105 (Table 1). The order of potency for AhR2 based on ReP was PeCDF > TCDD = TCDF > PCB 126 > PCB 77 > PCB 105 (Table 1). TCDD elicited the greatest

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Table 1. Calculated LOECs (nanomolar), ECs (nanomolar), and Maximal Responses Relative to the Maximal Response of TCDD (percent) for AhR1 and AhR2 of White Sturgeon[a]

	white sturgeon AhR1					white sturgeon AhR2				
	LOEC	EC_{20}	EC_{50}	EC_{80}	maximal response	LOEC	EC_{20}	EC_{50}	EC_{80}	maximal response
TCDD	0.01	0.0081 ± 0.001	0.036 ± 0.008	0.16 ± 0.05	100	0.03	0.018 ± 0.005	0.070 ± 0.02	0.28 ± 0.09	100
PeCDF	0.01	0.0097 ± 0.002	0.040 ± 0.01	0.16 ± 0.04	93	0.01	0.058 ± 0.002	0.034 ± 0.01	0.20 ± 0.08	84
TCDF	0.01	0.0073 ± 0.007	0.060 ± 0.02	0.49 ± 1	83	0.003	0.024 ± 0.02	0.079 ± 0.03	0.26 ± 0.1	79
PCB 126	0.1	0.19 ± 0.08	0.94 ± 0.1	4.7 ± 0.8	76	0.1	0.45 ± 0.2	1.8 ± 0.4	7.4 ± 1	92
PCB 77	1	6.5 ± 3	28 ± 2	124 ± 56	54	1	7.5 ± 3	38 ± 10	193 ± 67	64
PCB 105	–[b]	–[b]	–[b]	–[b]	<20	–[b]	–[b]	–[b]	–[b]	<20

[a]Standard errors of the mean are given. [b]Could not be calculated.

Table 2. Relative Sensitivities (ReS) of AhR1 Relative to AhR2 of White Sturgeon to Selected Dioxin-like Compounds

	TCDD	PeCDF	TCDF	PCB 126	PCB 77	PCB 105
white sturgeon AhR1	1.8	1.4	0.7	1.1	1.2	–[a]
white sturgeon AhR2	1.0	1.0	1.0	1.0	1.0	–[a]

[a]Could not be calculated.

maximal response to both AhR1 and AhR2 (Figure 1 and Table 1).

RePs developed for white sturgeon based on responses in COS-7 cells transfected with AhR1 or AhR2 were distinct from those based on responses in COS-7 cells transfected with AhRs of other vertebrates or $TEF_{WHO\text{-}Fish}$, which is based on mortalities of embryos of salmonids (Table 3). Both AhR1 and AhR2 of white sturgeon had RePs for PeCDF and TCDF more similar to $TEF_{WHO\text{-}Bird}$, while AhR1 and AhR2 of white sturgeon had RePs for PCBs most similar to $TEF_{WHO\text{-}Fish}$ (Table 3).

3.2. Comparison of TEQs in White Sturgeon. A comparison of TCDD-EQ developed from RePs derived by use of COS-7 cells transfected with AhR2 of white sturgeon (this study) and TEQs developed from $TEF_{WHO\text{-}Fish}$ to exposure data previously reported for white sturgeon[9,10] showed that the greatest contributions to TCDD-EQs and TEQs were observed for TCDD and TCDF in liver, muscle, and eggs of white sturgeon collected from the Fraser River and the upper Columbia River (Table 4). On the basis of RePs for AhR2 of white sturgeon derived from the study presented here, TCDD-EQs for the six DLCs were approximately 10-fold greater in liver, muscle, and eggs from white sturgeon from the Fraser River and upper Columbia River relative to TEQs developed by use of $TEF_{WHO\text{-}Fish}$ (Table 4).

4. DISCUSSION

4.1. Relative Sensitivity of White Sturgeon AhR1 and AhR2 Compared to the Sensitivity of Those of Other Species. As a first step in characterizing the sensitivity of white sturgeon to DLCs, this study investigated the *in vitro* sensitivity of AhR1 and AhR2 of white sturgeon to several prototypic DLCs. Both AhR1 and AhR2 of white sturgeon were activated by exposure to DLCs and had EC_{50}s for TCDD less than those of any other AhR tested to date with values of 0.036 and 0.070 nM, respectively. By comparison, EC_{50}s of other fishes derived for TCDD with AhR1- and AhR2-transfected COS-7 cells

Table 3. Relative Potencies (RePs) of Selected Dioxin-like Compounds (DLCs) with Respect to AhRs of White Sturgeon Compared to AhRs of Other Vertebrates

	TCDD	PeCDF	TCDF	PCB 126	PCB 77	PCB 105
white sturgeon AhR1	1.0	1.0	0.4	0.04	0.001	–[h]
red seabream AhR1[a]	1.0	1.5	2.5	–[h]	NA[i]	NA[i]
chicken AhR1[b]	1.0	1.0	1.0	0.07	0.002	0.00003
human AhR[c]	1.0	0.01	0.04	0.01	0.0001	–[h]
white sturgeon AhR2	1.0	1.3	1.0	0.04	0.002	–[h]
rainbow trout AhR2α[c]	1.0	0.2	0.5	0.2	0.01	–[h]
zebrafish AhR2[c]	1.0	0.4	1.6	–[h]	–[h]	–[h]
red seabream AhR2[a]	1.0	0.9	1.5	0.009	NA[i]	NA[i]
embryos of pallid sturgeon[d]	1.0	NA[i]	NA[i]	0.08	NA[i]	NA[i]
embryos of shovelnose sturgeon[d]	1.0	NA[i]	NA[i]	0.07	NA[i]	NA[i]
embryos of rainbow trout[e]	1.0	0.3	0.03	0.005	0.0002	–[h]
$TEF_{WHO\text{-}Fish}$[f]	1.0	0.5	0.05	0.005	0.0001	<0.000005
$TEF_{WHO\text{-}Bird}$[f]	1.0	1.0	1.0	0.1	0.05	0.0001
$TEF_{WHO\text{-}Mammal}$[g]	1.0	0.3	0.1	0.1	0.0001	0.00003

[a]RePs were calculated on the basis of an average of the minimal and maximal ReP of each DLC by use of luciferase reporter gene assays using COS-7 cells transfected with the respective AhR.[33] [b]RePs were calculated according to methods described by Villeneuve et al.[55] using luciferase reporter gene assays using COS-7 cells transfected with AhR1.[28,39] [c]RePs were calculated on the basis of EC_{50} values using luciferase reporter gene assays using COS-7 cells transfected with the respective AhR.[32] [d]RePs were calculated on the basis of LD_{50} values in embryos of pallid sturgeon and shovelnose sturgeon.[42] [e]RePs were calculated on the basis of LD_{50} values in embryos of rainbow trout.[56] [f]TEF values developed by the WHO.[23] [g]TEF value developed by the WHO.[57] [h]Could not be calculated. [i]Not analyzed in the referenced study.

Table 4. Maximal TEQs and TCDD-EQs of Liver, Muscle, and Eggs of Fraser River (A) and Upper Columbia River (B) White Sturgeon for Select DLCs Based on Maximal Measured Concentrations[9,10,a]

	(A) Fraser River White Sturgeon								
	liver			muscle			eggs		
	measured concn	TCDD-EQ	TEQ	measured concn	TCDD-EQ	TEQ	measured concn	TCDD-EQ	TEQ
TCDD	20.0	20.0	20.0	34.8	34.8	35.0	4.20	4.20	4.20
PeCDF	3.80	4.94	1.90	7.60	9.88	3.80	0.80	1.04	0.40
TCDF	390	390	19.5	520	520	26.0	42.6	42.6	2.10
PCB 126	7.80	0.312	0.390	10.7	0.428	0.054	1.80	0.072	0.009
PCB 77	59.0	0.118	0.006	62.9	0.126	0.006	7.0	0.014	0.001
PCB 105	9795	0.098	0.049	21337	0.213	0.110	2707	0.027	0.014
total		415	41.8		565	65.0		48.0	6.72
	(B) Upper Columbia River White Sturgeon								
	liver			muscle			eggs		
	measured concn	TCDD-EQ	TEQ	measured concn	TCDD-EQ	TEQ	measured concn	TCDD-EQ	TEQ
TCDD	0.146	0.146	0.150	0.172	0.172	0.170	2.37	2.37	2.40
PeCDF	0.276	0.359	0.140	0.166	0.216	0.083	0.550	0.719	0.280
TCDF	20.6	20.6	1.0	13.3	13.3	0.670	67.2	67.2	3.40
PCB 126	16.1	0.644	0.081	5.15	0.206	0.026	15.9	0.636	0.080
PCB 77	33.5	0.067	0.003	24.5	0.049	0.003	27.7	0.055	0.003
PCB 105	4680	0.047	0.023	3740	0.037	0.019	3750	0.038	0.019
Total		21.9	1.40		14.0	0.971		71.0	6.18

[a]TEQs calculated by use of $TEF_{WHO\text{-}Fish}$[23] and TCDD-EQs calculated by use of RePs developed by use of COS-7 cells transfected with AhR2 of white sturgeon (this study). The ReP for PCB 105 was set at 0.00001 on the basis of the absence of a response at up to 9000 nM. All concentrations are expressed in picograms per gram of wet weight.

ranged from 0.073 to 5.9 nM and from 0.1 to 1.9 nM, respectively.[31−38] It needs to be acknowledged, however, that there are some uncertainties with regard to the comparability of ECs derived by different studies with fish using COS-7 cells transfected with AhR1 or AhR2 and, thus, whether they allow for accurate prediction of relative sensitivity among species. This is mainly due to differences in the methods that were applied by these studies and which have been shown to affect ECs by >10-fold.[28] Although caution must be used when comparing EC_{50}s derived for other fishes, the EC_{50}s of chicken (*Gallus gallus*) described by Farmahin et al.[28] and Manning et al.[39] were derived by use of the same methods used in the study presented here and therefore can be compared directly. Chicken is the species of bird known to be most sensitive to TCDD with an LD_{50} to embryos of 210 pg/g of egg and an EC_{50} for AhR1 in transfected COS-7 cells of 0.22 nM.[28,40] On the basis of EC_{50}s, AhR1 and AhR2 of white sturgeon were more sensitive to TCDD, PeCDF, TCDF, PCB 126, and PCB 77 than AhR1 of chicken.[28,39] However, AhR1 of chicken was more sensitive to PCB 105, which did not show any significant response in white sturgeon.[39] The fact that white sturgeon have two AhRs with great sensitivity to PCDDs, PCDFs, and non-*ortho* PCBs might indicate that white sturgeon also have *in vivo* sensitivity to these compounds greater than those of most other vertebrates.

The sensitivity of AhRs *in vitro* might be an indicator of the sensitivity of fishes *in vivo* and could represent one method of predicting the relative sensitivity of endangered fishes, such as sturgeons, to DLCs by use of an *in vitro* approach.[41] EC_{50}s for TCDD in COS-7 cells transfected with AhR1 (0.073 nM) or AhR2 (0.51 nM) of red seabream (*Pagrus major*) were compared to EC_{50}s for upregulation of CYP1A in whole embryos exposed to TCDD (0.30−0.91 nM), with EC_{50}s for upregulation of CYP1A and activation of AhR2 being most similar, indicating that AhR2 activation might be predictive of responses in embryos.[33] However, little is known about the sensitivity of embryos of sturgeons to DLCs, and substantial variability exists in the available data. Embryo-lethality studies of pallid sturgeon (*Scaphirhynchus albus*) and shovelnose sturgeon (*Scaphirhynchus platorynchus*) determined these sturgeons to be the least sensitive fishes with LD_{50}s of 12000 and 13000 pg of TCDD/g of egg, respectively.[42] In contrast, an elevated incidence of malformations of embryos of shortnose sturgeon (*Acipenser brevirostrum*) and Atlantic sturgeon (*Acipenser oxyrinchus*) has been observed at concentrations as low as 50 pg of TCDD/g of egg.[43] However, LD_{50}s were not achieved at concentrations as great as 600 and 1450 pg of TCDD/g of egg for shortnose and Atlantic sturgeon, respectively.[43] In contrast, LD_{50}s of teleost fishes ranged from 65 to 2610 pg of TCDD/g of egg.[41] Evidence collected to date supports the hypothesis that there might be significant diversity in sensitivity to DLCs among sturgeons, with some species exhibiting great sensitivity to DLCs. On the basis of the greater sensitivities of both AhR1 and AhR2 *in vitro*, and the great inducibility of CYP1A *in vivo*,[22] it is hypothesized that the white sturgeon is sensitive to DLCs.

4.2. Relative Potency of Select Dioxin-like Compounds to AhR1 and AhR2 of White Sturgeon. On the basis of the results derived from COS-7 cells transfected with AhR1 or AhR2 of white sturgeon, it was found that PCDFs and non-*ortho* PCBs had greater potency relative to TCDD compared to the current $TEF_{WHO\text{-}Fish}$.[23] RePs determined for AhR2 of white sturgeon following exposure to PeCDF, TCDF, PCB 126, and PCB 77 were 3-, 20-, 8-, and 20-fold greater, respectively, than $TEF_{WHO\text{-}Fish}$.[23] However, neither AhR1 nor AhR2 exhibited a measurable response to mono-*ortho* PCB 105, which is consistent with what has been shown in all other fishes studied to date.[23,32] RePs derived for both AhR1 and AhR2 of white sturgeon were more similar to $TEF_{WHO\text{-}Bird}$ than to $TEF_{WHO\text{-}Fish}$.[23] However, there is uncertainty about how

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accurately *in vitro* activation in COS-7 cells transfected with AhR mirrors environmentally relevant end points *in vivo*. In birds, it has been demonstrated that activation of AhR1 in transfected COS-7 cells is predictive of effects of DLCs *in vivo* in a range of model and wild species.[28,39,44] Although fishes have not been studied to the same level of detail as birds, there is considerable similarity between RePs for PCDDs, PCDFs, and PCBs derived from activation of AhR2α of rainbow trout (*Oncorhynchus mykiss*) in transfected COS-7 cells and RePs derived from embryos of rainbow trout.[32] In embryos of pallid and shovelnose sturgeons exposed to serial doses of either TCDD or PCB 126, it was found that PCB 126 had RePs of 0.08 and 0.07, respectively, relative to TCDD compared to the RePs of 0.04 derived for both AhR1 and AhR2 of white sturgeon;[42] a difference of 2-fold compared to an 8-fold difference from the $TEF_{WHO\text{-}Fish}$ for PCB 126 of 0.005[23] (Table 3). Although *in vitro* systems do not consider differences in metabolism between congeners, fishes have been shown to metabolize DLCs more slowly than other vertebrates,[45] and therefore, on the basis of the similarity between *in vitro* and *in vivo* RePs in fishes, it appears that RePs that are derived from COS-7 cells transfected with AhR1 or AhR2 might be representative of environmentally relevant effects on embryos of sturgeons.

4.3. Application to Risk Assessment. Previous studies have shown that COS-7 cells transfected with AhR1s are predictive of *in vivo* sensitivity of birds to DLCs.[28] Assuming that the greater sensitivity of white sturgeon to some PCDFs relative to TCDD as determined by COS-7 cells transfected with AhR1 or AhR2 is similarly predictive of *in vivo* sensitivity, this would have significant implications for the assessment of risk to populations of this species. Several DLCs were detected in tissues and eggs of adult white sturgeon from the Fraser River and upper Columbia River in British Columbia, Canada, with PCDFs having among the greatest concentrations.[9,10] On the basis of concentrations of TCDD, PeCDF, TCDF, PCB 126, PCB 77, and PCB 105 in individuals collected from the Fraser River, TEQs calculated by use of $TEF_{WHO\text{-}Fish}$ were 65.0 and 41.8 pg of TCDD/g of wet weight in muscle and liver, respectively, whereas TEQs of the fishes collected in the upper Columbia River were 0.971 and 1.40 pg of TCDD/g of wet weight in muscle and liver, respectively (Table 4). TEQs calculated for eggs of white sturgeon were 6.18 and 6.70 pg of TCDD/g of egg wet weight for fishes from the Fraser River and upper Columbia River, respectively. Although there is no consensus about whether TEQs within these ranges represent a significant concern,[46−51] adverse effects have been observed in some fishes at concentrations that are significantly less.[52] In adult rainbow trout exposed to environmentally relevant concentrations of TCDD via the diet for 300 days, the most sensitive end points measured were survival of adult females and effects on behavior, both of which occurred at LOECs of 0.22 pg of TCDD/g of wet weight in liver and 0.21 pg of TCDD/g of wet weight in muscle.[52] However, early life stages of fishes, such as embryos, are known to be among the most sensitive to DLCs.[47] TEQs for eggs of white sturgeon exceed the LOEC of 0.3 pg of TCDD/g of egg wet weight that was observed in one study following maternal transfer of TCDD to embryos of rainbow trout.[52] However, other studies had LOECs for embryos of salmonids ranging from 15 to 34 pg of TCDD/g of egg wet weight.[48,50]

TCDD-EQs derived from responses in COS-7 cells transfected with AhR2 of white sturgeon were approximately 10-fold greater than TEQs derived from $TEF_{WHO\text{-}Fish}$ in liver, muscle, and eggs from fishes from the Fraser River and upper Columbia River (Table 4A). On the basis of RePs, concentrations of TCDD-EQs in muscle and liver of white sturgeon from the Fraser River were 565 and 415 pg of TCDD/g of wet weight, respectively, whereas TCDD-EQs in muscle and liver of the white sturgeon collected from the upper Columbia River were 14.0 and 21.9 pg of TCDD/g of wet weight, respectively (Table 4). These concentrations of TCDD-EQs significantly exceed concentrations shown in several studies to cause adverse effects in fishes[52−54] and are likely to have some chronic impacts on white sturgeon from these rivers. TCDD-EQs in eggs were 48.0 and 71.0 pg of TCDD/g of egg wet weight in white sturgeon collected in the Fraser River and upper Columbia River, respectively (Table 4B). These concentrations significantly exceed effect concentrations for several fishes,[49−52] including shortnose, Atlantic, pallid, and shovelnose sturgeons.[42,43]

In conclusion, this study demonstrates that white sturgeon express two distinct AhR proteins, AhR1 and AhR2, that are responsive to exposure to DLCs. More importantly, the EC_{50}s derived in this study for 2,3,7,8-TCDD were less than those previously reported for any other AhR of vertebrates tested to date. These unique and sensitive patterns of response mediated by AhRs of white sturgeon might be indicative of greater sensitivity of white sturgeon to some DLCs relative to other fishes, in particular PCDFs. On the basis of RePs developed for TCDD, PeCDF, TCDF, PCB 126, PCB 77, and PCB 105 by use of COS-7 cells transfected with AhR2 of white sturgeon, it appears $TEF_{WHO\text{-}Fish}$ might not accurately predict the risk of DLCs to endangered populations of white sturgeon. Because numerous species of sturgeons are endangered and can have elevated levels of exposure to mixtures of DLCs, future research should investigate whether RePs derived using COS-7 cells transfected with AhR1 or AhR2 accurately represent RePs derived by use of *in vivo* end points of biological relevance such as embryo-lethality or other environmentally relevant end points and establish the relative sensitivity of white sturgeons to DLCs compared to salmonids. The development of sturgeon specific RePs could be essential for objective risk assessments of endangered sturgeons worldwide.

■ ASSOCIATED CONTENT

Supporting Information

Accession numbers of white sturgeon genes used to design oligonucleotide primers used in producing expression constructs (Table S1). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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