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In vitro dioxin-like potencies of HO- and MeO-PBDEs and inter-species sensitivity variation in birds

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ABSTRACT

Due to their bioaccumulative properties, hydroxylated and methoxylated polybrominated diphenyl ethers (HO-/MeO-PBDEs) may pose ecological risks to wild life, including birds. However, their toxicity potencies in avian species are largely unknown. In the present study, an avian AHR1 luciferase reporter gene (LRG) assay with luciferase probes from chicken, pheasant and quail was used to test activations of avian aryl hydrocarbon receptor (AHR)-mediated pathways by 19 HO- or MeO-PBDEs in different avian species. Species-specific relative potencies (RePs) of HO-/MeO-PBDEs to tetrachlorodibenzo-p-dioxin (TCDD) and relative sensitivities of various species to each chemical were estimated. The results indicated that the ReP of the most potent HO-/MeO-PBDEs, 5-Cl-6-HO-BDE-47, was 7.8×10^{-4} for chicken, 1.1×10^{-2} for pheasant, and 1.7×10^{-1} for quail comparing to TCDD. In addition, it was found that avian species with the greatest sensitivity to TCDD did not always have the greatest sensitivity to HO-/MeO-PBDEs and vice versa. This study contributed to filling in the knowledge gap regarding the dioxin-like activity of HO-/MeO-PBDEs in birds, and provided beneficial information for the prioritization of HO-/MeO-PBDEs for further research.

Capsule abstract: HO-/MeO-PBDEs activate avian AHR-mediated pathways in a congener- and species-specific manner. 5-Cl-6-HO-BDE-47 was the most potent among the nineteen HO-/MeO-PBDEs tested.

1. Introduction

Hydroxylated and methoxylated polybrominated diphenyl ethers (HO-/MeO-PBDEs) are analogs of PBDEs which have been used as flame retardants and are widely distributed in the environment. However, HO-/MeO-PBDEs have been detected at concentrations even greater than those of PBDEs (Covaci et al., 2011) in various environmental media, such as sediment (Kelly et al., 2008a; Zhang et al., 2012), surface water, and precipitation in Ontario (Ueno et al., 2008), and in various living organisms, including algae (Malmvärn et al., 2005), mussels (Malmvärn et al., 2005), fish (Marsh et al., 2003a), birds (Jaspers et al., 2013; Nordlof et al., 2010; Olsson et al., 2000; Verreault et al., 2005), marine mammals (Haglund et al., 1997), and polar bears (Verreault et al., 2005), as well as in human blood (Qiu et al., 2009). Concentrations of MeO-PBDEs 100-fold greater than those of PBDEs have been measured in animals, such as the Arctic and North Atlantic whale (McKinney et al., 2006b; Teuten et al., 2005). Some HO-PBDEs are more potent than their postulated precursor PBDEs and corresponding MeO-PBDEs for several toxicological endpoints, including endocrine disorders, neurotoxicity (Canton et al., 2008; Dingemans et al., 2008; Meerts et al., 2000, 2001; Mercado-Feliciano and Bigsby, 2008), cytotoxic effects, and genotoxicity (Su et al., 2014). Conversely, MeO-PBDEs have greater effects than PBDEs or HO-PBDEs on steroidogenesis in the H295R human adrenocortical carcinoma cell line (He et al., 2008). Our previous studies have demonstrated that HO-/MeO-PBDEs could activate a wide array of molecular mechanisms in *in vitro* assays, including interacting thyroid hormone receptor (ThR), estrogen receptor (ER), and androgen receptor (AR) (Liu et al., 2011; Hu et al., 2011). Furthermore, HO-PBDEs were shown to be more cytotoxic than the parent PBDEs and MeO-PBDEs, and could activate “organic acid/oxoacid/carboxylic acid metabolic process” pathways in E.coli (Su et al., 2012a, 2012b, 2014). However, the AHR-mediated pathway was

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recently identified to be the most sensitive molecular pathway in hepatic cells at low concentration exposure of chemicals (Zhang et al., 2016). These results suggested that AHR-mediated effects might be a very important aspect in the toxicological assessment of HO-/MeO-PBDEs.

While it is still unclear what proportion of HO- and MeO-PBDEs in the environment derives from natural sources rather than from the metabolic transformation of PBDEs, the potential bioaccumulation of these compounds *via* water and food has caused concern regarding their potential adverse effects in humans and wildlife (Lacorte and Ikonomou, 2009; Wan et al., 2009). Animal experiments (rat, mouse, bird, and fish) and *in vitro* studies with human liver microsomes have demonstrated the transformation of some PBDEs by cytochrome P450 enzymes (Hakk and Letcher, 2003; Malmberg et al., 2005; Munschy et al., 2010; Qiu et al., 2007; Stapleton et al., 2009; Wan et al., 2010) and the interconversion of certain HO-PBDEs and MeO-PBDEs (Wan et al., 2010). Both HO- and MeO-PBDEs activate the mammalian aryl hydrocarbon receptor (AHR) and induce dioxin-like effects (Su et al., 2012a, 2012b). However, the AHR-mediated effects of HO- or MeO-PBDEs in birds have yet to be determined.

Because of high detection frequency of HO-/MeO-PBDE in wild avian species (Jaspers et al., 2013; Nordlof et al., 2010; Olsson et al., 2000; Verreault et al., 2005), it is important to understand the potential effects of these chemicals and inter-species sensitivity variation to birds. According to some recent reports, birds can be classified into three main groups of sensitivity to dioxin-like compounds (DLCs): chicken (*Gallus gallus domesticus*)-like (type 1), ring-necked pheasant (*Phasianus colchicus*)-like (type 2), or Japanese quail (*Coturnix japonica*)-like (type 3) (Farmahin et al., 2012, 2013; Head et al., 2008). AHR1-mediated avian species-specific dioxin-like effects of chemicals and relative sensitivities (ReS) among avian species can be predicted using a recently developed avian AHR1 luciferase report gene (LRG) assay with AHR1

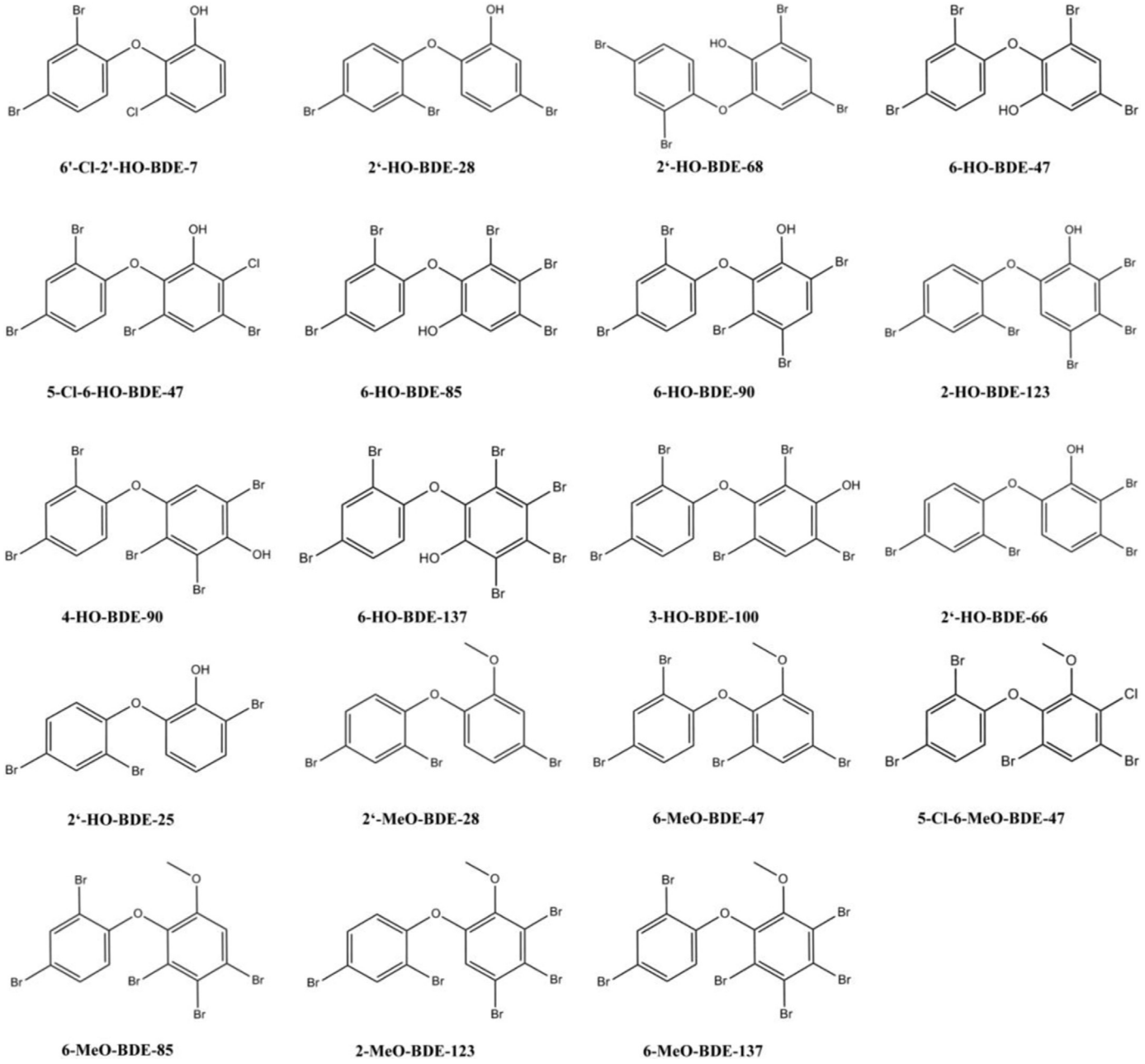

Fig. 1. Structures of the 19 HO-/MeO- polybrominated diphenyl ethers (PBDEs) tested in this study.

constructs from chicken, ring-necked pheasant and Japanese quail (Manning et al., 2012; Zhang et al., 2013). Thus, in the present study, the dioxin-like potencies of 19 HO-/MeO-PBDE congeners were tested using the avian AHR1-LRG assay. Avian species-specific relative potency (ReP) and ReS values for the tested HO-/MeO-PBDEs were determined. ReP values from the avian AHR1s-LRG assay were also compared with those derived using H4IIE-*luc* cell assay previously (Su et al., 2012a, 2012b).

2. Materials and methods

2.1. Chemicals and solutions

Analogs of PBDE, including 13 HO-PBDEs and six MeO-PBDEs (Fig. 1), were synthesized in the Department of Biology and Chemistry of the City University of Hong Kong as described previously (Marsh et al., 2003b). The purities of the HO-/MeO-PBDEs were $>98\%$. No brominated dioxin and/or furans were generated during the synthesis, as confirmed by proton nuclear magnetic resonance, high-resolution gas chromatography high-resolution mass spectrometry, and electrospray liquid chromatography coupled with tandem mass spectrometry of the intermediates and end products (He et al., 2008; Su et al., 2012a, 2012b). Individual HO-/MeO-PBDEs were dissolved in dimethyl sulfoxide (DMSO; Sigma-Aldrich, St. Louis, MO, USA) and their concentrations in the stock solutions were confirmed using previously published methods (Su et al., 2012a, 2012b). The HO-/MeO-PBDE concentration range used in the LRG assays (0.05–20,000 nM) caused no cytotoxic effects, as determined previously in MTS cytotoxicity assays. Serial dilutions of tetrachlorodibenzo-*p*-dioxin (TCDD), used as a reference in the bioassays, were prepared from a stock solution in DMSO that had a concentration of 73.8 μg/ml according to a previously reported method (Herve et al., 2010).

2.2. COS-7 cell culture, transfection, and avian LRG assay

The methods used in cell culture, transfection, and the LRG assay are described elsewhere (Farmahin et al., 2012; Manning et al., 2012; Zhang et al., 2013). Chicken, ring-necked pheasant, and Japanese quail full-length AHR1 constructs were prepared at the Environment Canada Laboratories (Ottawa, Canada) using previously described methods (Farmahin et al., 2012; Manning et al., 2012; Zhang et al., 2013). Both a firefly luciferase reporter vector containing the common cormorant (*Phalacrocorax carbo*) CYP1A5 promoter region and a common cormorant ARNT1 vector were obtained from Dr. Hisato Iwata (Ehime University, Japan) (Lee et al., 2009; Yasui et al., 2007). African green monkey SV40-transfected kidney fibroblast cells (COS-7) were purchased from The Cell Bank of the Type Culture Collection of the Chinese Academy of Sciences. The cells were seeded in 96-well plates at a concentration of ~10,000 cells per well. Eighteen hours later, the cells in each well were transiently transfected with 6 μl of a transfection mixture consisting of ~50 ng of DNA and 0.2 μl of Fugene 6 transfection reagent (Promega) diluted in Opti-MEM (Invitrogen, Burlington, ON, Canada). The 50 ng of DNA was composed of 8 ng of the chicken, ring-necked pheasant, or Japanese quail AHR1 expression construct, 1.55 ng of cormorant ARNT 1, 7.5 ng of the CYP1A5 reporter construct, 0.75 ng of the *Renilla* luciferase vector, and 32.2 ng of salmon sperm DNA (Invitrogen). Five hours after transfection, the cells were dosed with DMSO (solvent control) or DMSO solutions containing either TCDD or the HO-/MeO-PBDEs. The final concentration of DMSO in the wells was 0.5%. A positive control (300 nM TCDD) was also included for each AHR1 construct tested in cells dosed with the different HO-/MeO-PBDEs. LRG activity was measured as luminescence using Dual-Glo luciferase assay kits (Promega) in a Synergy H4 Hybrid multi-mode microplate reader (BioTek Instruments) 20 h after dosing.

2.3. Avian LRG data analysis

For the avian LRG assay, triplicate concentration-response curves were obtained from three independent experiments for each AHR1 construct and HO-/MeO-PBDE treatment, each with four technical replicates per dilution of the individual HO-/MeO-PBDEs, TCDD, or DMSO. To eliminate variability resulting from differences in cell plating, pipetting inconsistencies, transfection efficiency, and toxicity, the luminescence values were expressed as the ratio of firefly luciferase units to *Renilla* luciferase units (Schagat et al., 2007). According to the protocol described in OECD guideline 455 (OECD, 2009), the data were expressed as a percentage of the positive control (PC) response. They were then imported into GraphPad (GraphPad Prism 5.0 software, San Diego, CA, USA) and fitted to a four-parameter logistic model (Head and Kennedy, 2007). A reliable half-maximal effective concentration (EC_{50}) could not be calculated when the concentration-response curve did not reach a maximum plateau or a plateau could not be estimated accurately by the use of curve fitting. In such cases, EC_{50} values for these curves are not presented and the greatest observed response rather than the maximal response is reported. EC_{50}, PC_{10}, PC_{20}, PC_{50}, PC_{80}, and maximal response values were determined for each replicate concentration-response curve by using logistic curve fitting. The values represent the mean obtained from three concentration-response curves ± the standard error.

2.4. H4IIE-luc assay data analysis

The background-corrected luciferase activities reflecting the responses to the tested HO-/MeO-PBDEs were obtained previously (Su et al., 2012a, 2012b) and re-processed in the present study. They were normalized to the percentage of the maximal response induced by TCDD and then imported into GraphPad (GraphPad Prism 5.0 software), where they were fitted to a four-parameter logistic model. Concentrations of HO-/MeO-PBDEs that elicited a response equal to 10, 20, 50, and 80% of the positive control response were referred to as PC_{10}, PC_{20}, PC_{50}, and PC_{80}, respectively. EC_{50}, PC_{10}, PC_{20}, PC_{50}, PC_{80}, and maximal response values were determined for each replicate concentration-response curve.

2.5. Calculation of ReS and ReP values

A detailed description of the calculation of the relative sensitivities of the avian constructs to AHR1 activation by DLCs is provided elsewhere (Zhang et al., 2013). Briefly, the ReS values were defined as: PC_{10} or EC_{50} of compound *X* in chicken AHR1 construct ÷ PC_{10} or EC_{50} of compound *X* in the AHR1 construct of interest. If no induction of avian LRG activity was detected, the EC_{50}-based ReS value was estimated by dividing the chicken value by the maximum concentration tested in the LRG assay.

ReP values were calculated according to the systematic framework proposed by Villeneuve et al. (2000), with modifications. In the absence of significant induction of LRG activity by a HO-/MeO-PBDE, a ReP_{EC50} value was estimated by dividing the EC_{50} value of TCDD by the maximum concentration of the HO-/MeO-PBDE tested. For each avian AHR1 construct, the relative potencies of the HO-/MeO-PBDEs compared to TCDD was defined as: EC_{50}, PC_{10}, PC_{20}, PC_{50}, or PC_{80} of TCDD in AHR1 construct *X* ÷ EC_{50}, PC_{10}, PC_{20}, PC_{50} or PC_{80} of the HO-/MeO-PBDE of interest in AHR1 construct *X*. As described previously, ReP_{EC50} was excluded from the ReP_{avg} calculation because it may overestimate potency

(Kennedy et al., 1996).

2.6. Statistical analysis

Normality was confirmed using the Kolmogorov–Smirnov test, and the homogeneity of variance using Levine's test. Significant differences between maximal response, $logEC_{50}$, $logPC_{10}$, $logPC_{20}$, $logPC_{50}$, and $logPC_{80}$ values for the different HO-/MeO-PBDEs or different avian AHR1 constructs were determined using a *t* test ($p \leq 0.05$) or a one-way ANOVA ($p \leq 0.05$) followed by Tukey's multiple comparison test ($p \leq 0.05$). Pairwise linear regression analyses were conducted between: (1) the log-transformed ReP_{avg} values of HO-/MeO-PBDEs derived from the chicken *vs*. the ring-necked pheasant *vs*. the Japanese quail construct and (2) the log-transformed avian AHR1s-LRG-ReP_{avg} values *vs*. the log-transformed H4IIE-*luc*-ReP_{avg} values of HO-/MeO-PBDEs.

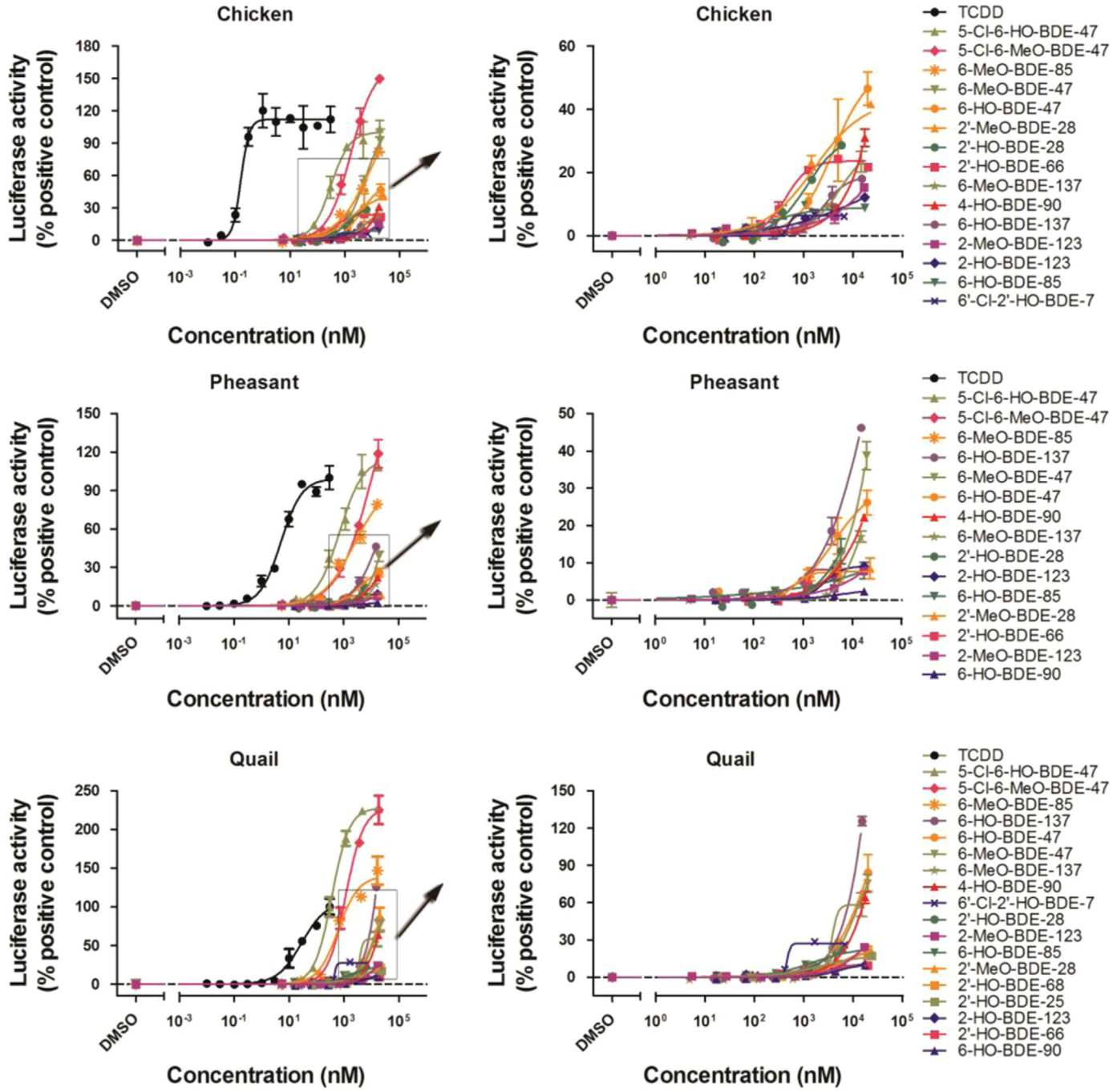

Fig. 2. Concentration-dependent effects of tetrachlorodibenzo-*p*-dioxin (TCDD) and HO-/MeO-polybrominated diphenyl ethers (PBDEs) on aryl hydrocarbon receptor 1 (AHR1)-mediated luciferase reporter gene (LRG) activity in COS-7 cells transfected with chicken, ring-necked pheasant, or Japanese quail AHR1 constructs. The data are presented as the percent response relative to that of the positive control (PC; 300 nM TCDD) for each avian construct. Concentration-response curves are presented for the HO-/MeO-PBDEs showing a significant ($p < 0.05$), concentration-dependent increase in LRG activity relative to the dimethyl sulfoxide response. Data points represent the mean, PC-normalized luciferase ratios obtained from three independent experiments, each with four technical replicates per concentration of HO-/MeO-PBDEs or TCDD. Bars represented standard error.

3. Results and discussion

3.1. Induction of luciferase reporter gene activity in COS-7 cells

Concentration-dependent effects of TCDD and HO-/MeO-PBDEs on LRG activity were determined in COS-7 cells transfected with the chicken, ring-necked pheasant, or Japanese quail AHR1 constructs (Fig. 2). The S-curves of TCDD reached a plateau at 300 nM TCDD for all three avian constructs, which demonstrated that 300 nM TCDD was the appropriate positive control. Among the 19 examined HO-/MeO-PBDEs, the following 15 induced significant LRG activity by the three avian constructs (Supplementary material Table S1): 2′-HO-BDE-28, 2′-HO-BDE-68, 6-HO-BDE-47, 5-Cl-6-HO-BDE-47, 6-HO-BDE-85, 2-HO-BDE-123, 4-HO-BDE-90, 6-HO-BDE-137, 2′-HO-BDE-66, 2′-MeO-BDE-28, 6-MeO-BDE-47, 5-Cl-6-MeO-BDE-47, 6-MeO-BDE-85, 2-MeO-BDE-123, and 6-MeO-BDE-137. Four PBDEs analogs (5-Cl-6-HO-BDE-47, 2′-HO-BDE-66, 6-HO-BDE-85, and 6′-Cl-2′-HO-BDE-7) induced a concentration-response curve that reached a plateau in chicken AHR1-transfected cells, three (2′-MeO-BDE-28, 2′-HO-BDE-66, and 2-HO-BDE-123) in ring-necked pheasant, and eight (5-Cl-6-HO-BDE-47, 5-Cl-6-MeO-BDE-47, 2′-HO-BDE-66, 6-MeO-BDE-85, 6-MeO-BDE-137, 2-HO-BDE-123, 6-HO-BDE-85, and 6′-Cl-2′-HO-BDE-7) in Japanese quail. The maximum observed responses to 5-Cl-6-HO-BDE-47 and 5-Cl-6-MeO-BDE-47 were greater than or equal to the responses to TCDD, as was the case for 2,3,7,8-tetrachlorodibenzofuran (Farmahin et al., 2012).

3.2. ReS of avian AHR1 constructs exposed to HO-/MeO-PBDEs

The ReS values indicated that the chicken AHR1 construct was the most sensitive to TCDD, as determined by the induction of luciferase activity (Table 1). The chicken AHR1 construct was 23-fold and 98-fold more sensitive than the ring-necked pheasant and Japanese quail AHR1 constructs. These results regarding interspecies sensitivity variation to TCDD were consistent with previous findings using the LRG assay (Farmahin et al., 2012, 2013; Manning et al., 2012; Zhang et al., 2013, 2014). The rank orders of the ReSs for the avian AHR1 constructs when exposed to HO-/MeO-PBDEs were not always consistent with those obtained following TCDD exposure (Table 1). For example, the AHR1 construct of Japanese quail was more sensitive than that of ring-necked pheasant to 6′-Cl-2′-HO-BDE-7, 2′-HO-BDE-28, 6-HO-BDE-47, 2′-HO-BDE-66, 6-MeO-BDE-47, and 5-Cl-6-MeO-BDE-47 and more sensitive than that of chicken in the case of 5-Cl-6-HO-BDE-47, 4-HO-BDE-90, 2-MeO-BDE-123, and 6-MeO-BDE-137. For 6-HO-BDE-137 and 6-MeO-BDE-85, the rank orders of the ReSs for the three avian AHR1 constructs were almost completely the opposite of those obtained for TCDD. These results are similar to previously reported findings regarding mono-*ortho* PCB congeners, including PCBs 105 and 118, and Aroclors 1260, 1016, and 1221 (Manning et al., 2013; Zhang et al., 2013). This result might be due to ligand-specific differences. Thus, differences in the amino acid sequences of avian AHR1s could cause conformational changes in the receptor-ligand complex, leading to differential co-activator recruitment and distinct interactions with xenobiotic response elements (Abnet et al., 1999; Zhou et al., 2003).

3.3. RePs of the HO-/MeO-PBDEs in the avian AHR1 constructs

The rank orders of the potencies of HO-/MeO-PBDEs were generally similar for the chicken, ring-necked pheasant, and Japanese quail AHR1 constructs (Fig. 3, Table 2). Linear regressions of the log-transformed ReP_{avg} values of HO-/MeO-PBDEs derived from the assays with the avian AHR1s-LRG showed their mutual correlation (Supplementary material Fig. S1). However, the potency order of 2′-MeO-BDE-28 and 2′-HO-BDE-66 for the chicken construct and of 6′-Cl-2′-HO-BDE-7 and 6-HO-BDE-85 for the Japanese quail construct differed from that obtained with the other two avian AHR1 constructs (Table 2). In addition, the ReP_{avg} values of the HO-/MeO-PBDEs differed for the different avian AHR1 constructs. Thus, the ReP_{avg} values of all tested HO-/MeO-PBDEs were 3- to 31- and 3- to 526- fold greater for the AHR1 constructs of ring-necked pheasant and Japanese quail than for the AHR1 construct of chicken. As discussed in other studies, this might have been due to differences in the AHR1 amino acid sequence among the various avian species, which would potentially lead to AHR1 binding cavities of different sizes or differences in ligand-receptor conformation (Abnet et al., 1999; Farmahin et al., 2013; Zhou et al., 2003).

Table 1
ReS (relative sensitivity) values for the chicken, ring-necked pheasant, and Japanese quail AHR1 (aryl hydrocarbon receptor 1) constructs exposed to tetrachlorodibenzo-p-dioxin (TCDD) or HO-/MeO-PBDEs (polybrominated diphenyl ethers).

	Compound	ReS	AHR1 construct		
			Chicken	Pheasant	Quail
TCDD	TCDD	ReS_{EC50}	1.0[a]	0.029[a]	0.0063[b]
		ReS_{PC10}	1.0[a]	0.095[b]	0.028[c]
12A	5-Cl-6-HO-BDE-47	ReS_{EC50}	1.0[a]	NC	0.84[a]
		ReS_{PC10}	1.0[ab]	0.77[b]	1.4[a]
12B	5-Cl-6-MeO-BDE-47	ReS_{EC50}	1.0	NC	NC
		ReS_{PC10}	1.0[a]	0.70[a]	0.97[a]
4B	2′-MeO-BDE-28	ReS_{EC50}	1.0	NC	NC
		ReS_{PC10}	1.0[a]	N/A	0.090[b]
19A	2′-HO-BDE-66	ReS_{EC50}	1.0[a]	0.35[b]	0.76[a]
		ReS_{PC10}	1.0	N/A	N/A
13B	6-MeO-BDE-85	ReS_{EC50}	1.0	NC	NC
		ReS_{PC10}	1.0[a]	1.7[b]	3.6[c]
4A	2′-HO-BDE-28	ReS_{EC50}	1.0	NC	NC
		ReS_{PC10}	1.0[a]	0.15[c]	0.38[b]
8B	6-MeO-BDE-47	ReS_{EC50}	1.0	NC	NC
		ReS_{PC10}	1.0[a]	0.12[c]	0.24[b]
8A	6-HO-BDE-47	ReS_{EC50}	1.0	NC	NC
		ReS_{PC10}	1.0[a]	0.57[b]	0.65[b]
17A	6-HO-BDE-137	ReS_{EC50}	1.0	NC	NC
		ReS_{PC10}	1.0[a]	1.5[b]	1.4[b]
17B	6-MeO-BDE-137	ReS_{EC50}	1.0	NC	NC
		ReS_{PC10}	1.0[a]	0.37[b]	1.2[a]
16A	4-HO-BDE-90	ReS_{EC50}	1.0	NC	NC
		ReS_{PC10}	1.0[a]	0.91[a]	1.6[b]
15B	2-MeO-BDE-123	ReS_{EC50}	1.0	NC	NC
		ReS_{PC10}	1.0[a]	N/A	1.5[a]
15A	2-HO-BDE-123	ReS_{EC50}	1.0	NC	NC
		ReS_{PC10}	1.0[a]	N/A	0.58[a]
7A	2′-HO-BDE-68	ReS_{EC50}	1.0	NC	NC
		ReS_{PC10}	1.0	N/A	N/A
13A	6-HO-BDE-85	ReS_{EC50}	1.0[a]	NC	0.14[b]
		ReS_{PC10}	1.0	N/A	N/A
18A	3-HO-BDE-100	ReS_{EC50}	1.0	NC	NC
		ReS_{PC10}	1.0	N/A	N/A
3A	6′-Cl-2′-HO-BDE-7	ReS_{EC50}	1.0[a]	< 0.019	1.0[a]
		ReS_{PC10}	1.0	N/A	N/A
14A	6-HO-BDE-90	ReS_{EC50}	1.0	NC	NC
		ReS_{PC10}	1.0	N/A	N/A
20A	2′-HO-BDE-25	ReS_{EC50}	1.0	NC	NC
		ReS_{PC10}	1.0	N/A	N/A

Superscript letters indicate significant differences in the EC_{50} or PC_{10} values between AHR1 constructs ($p < 0.05$) for a given treatment.
NC: Not calculated because the EC_{50} value was not available.
N/A: Not calculated because the PC10 value was not available

3.4. Comparison of the ReP values derived from the avian AHR1-LRG and H4IIE-luc assays

The ReP_{avg} values of HO-/MeO-PBDEs derived from the H4IIE-*luc* assays were as much as four orders of magnitude lower than

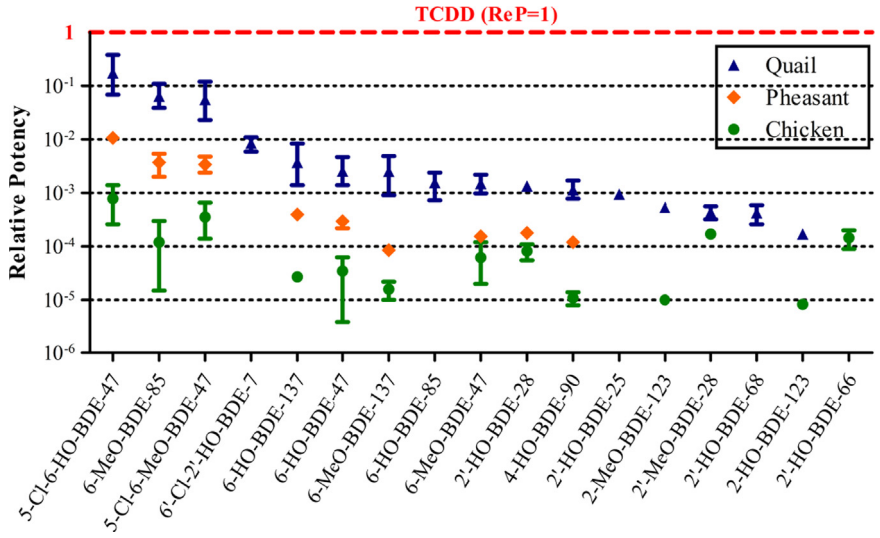

Fig. 3. Relative potency (ReP) of HO-/MeO-polybrominated diphenyl ethers (PBDEs) relative to tetrachlorodibenzo-*p*-dioxin (TCDD) determined in chicken, ring-necked pheasant, and Japanese quail aryl hydrocarbon receptor 1 (AHR1) constructs. The average ReP (ReP_{avg}) values were sorted to show the ranking of different chemicals. Bars represent the range of ReP values calculated for each compound (ReP_{range}), and the dots represent the ReP_{avg} values. The red dotted line with ReP-coordinate equal to 1 represents the TCDD-ReP value.

those obtained using the avian AHR1-LRG assays (Table 2 and Supplementary material Table S2). This result is in general agreement with previous findings that the ReP values of dioxins determined in avian *in vivo* and *in vitro* bioassays were up to four orders of magnitude higher than the values obtained in H4IIE-*luc* assays (Table S3 and S4). Because of species specificity, high efficiency and non-animal consumption, the avian AHR1-LRG assay would offer a more reasonable approach than rat H4IIE-luc assay to assess the AHR-mediated effects of DLCs on birds relative to rat H4IIE-luc assay, which had been applied to predict effects on birds previously (Frank et al., 2001; Lam et al., 2008).

3.5. Significance and research directions ahead

The results showed that the potencies for some of the HO-/MeO-PBDEs were comparable to some PCBs. 5-Cl-6-HO-BDE-47, 5-Cl-6-MeO-BDE-47, and 2′-MeO-BDE-28 might be more potent AHR1 agonizts than OctaCDD/F and most dioxin-like PCBs by comparing ReP_{avg} values of HO-/MeO-PBDEs determined using the chicken AHR1 construct with the WHO-TEFs (toxic equivalency factors) for birds which were developed primarily from chicken toxicity data (Van den Berg et al., 1998). In addition, given that the TCDD-TEQ (toxicity equivalence) approach considers only PCDD/Fs and dioxin-like PCBs without involving potential DLCs, such as HO-/MeO-PBDEs, thus, our results indicated that the ecological risk of HO-/MeO-PBDEs have been overlooked given their ubiquitous distribution in the environment.

The *in vitro* assay results presented here contribute to filling in the knowledge gap regarding the dioxin-like activity of HO-/MeO-PBDEs in birds and inter-avian species differences in sensitivity to these compounds. In addition, 5-Cl-6-MeO-BDE-47, 6-MeO-BDE-85, 6-HO-BDE-47, 6-MeO-BDE-47, 6-HO-BDE-137, 2′-MeO-BDE-28, 6-MeO-BDE-137, 2-MeO-BDE-123 and 2-HO-BDE-123 were identified as priority HO-/MeO-PBDEs for future *in vivo* toxicity assessment and avian risk assessment based on potencies among these 3 avian species and concentrations in wild birds and birds' eggs (Jaspers et al., 2013; Kelly et al., 2008b; Liu et al., 2010; McKinney et al., 2006a; Nordlof et al., 2010; Verreault et al., 2005). Further *in vivo* study should be carried out to validate and assess the *in vivo* toxicity of HO-/MeO-PBDEs with high AHR activation potencies. It should also be investigated whether the combined effects of HO-/MeO-PBDEs and dioxins are dose or concentration additive, and whether HO-/MeO-PBDEs as AHR agonizts have non-AHR mediated effects that might increase their dioxin-like potencies. In addition, whether HO-/MeO-PBDEs have any other toxicity mechanisms, for example endocrine disruption or genotoxicity in birds, might be other research interests ahead.

4. Conclusions

Among the 19 tested HO-/MeO-PBDEs, 15 ones have the potential to activate avian AHR1-mediated molecular toxicological pathways. Despite differences in the avian species-specific RePs of the HO-/MeO-PBDEs, their potency rank orders were generally similar. In addition, some HO-/MeO-PBDEs (5-Cl-6-HO-BDE-47, 5-Cl-6-MeO-BDE-47, and 2′-MeO-BDE-28) were more potent AHR1 agonizts than OctaCDD/F and most dioxin-like PCBs. The ReS values indicated that the sensitivity rank orders of the avian AHR1 constructs for HO-/MeO-PBDEs were not always the same as for TCDD. The data presented here contributed to filling in the knowledge gap regarding the dioxin-like activity of HO-/MeO-PBDEs in birds, and indicated priority HO-/MeO-PBDEs for further testing.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.ecoenv.2015.12.040.

Table 2
Relative potency (ReP) of HO-/MeO-polybrominated diphenyl ethers (PBDEs) in the chicken, ring-necked pheasant and Japanese quail aryl hydrocarbon receptor 1 (AHR1) constructs.

AHR1	Compound	ReP_{EC50}	ReP_{PC10}	ReP_{PC20}	ReP_{PC50}	ReP_{PC80}	ReP_{avg}	ReP range
Chicken	TCDD	1.0	1.0	1.0	1.0	1.0	1.0	1.0–1.0
	5-Cl-6-HO-BDE-47	5.4×10^{-4}	1.4×10^{-3}	9.7×10^{-4}	5.1×10^{-4}	2.6×10^{-4}	7.8×10^{-4}	2.6×10^{-4}–1.4×10^{-3}
	5-Cl-6-MeO-BDE-47	NC	6.6×10^{-4}	4.1×10^{-4}	2.1×10^{-4}	1.4×10^{-4}	3.5×10^{-4}	1.4×10^{-4}–6.6×10^{-4}
	2′-MeO-BDE-28	NC	1.7×10^{-4}	NE	NE	NE	1.7×10^{-4}	
	2′-HO-BDE-66	3.8×10^{-4}	2.0×10^{-4}	9.0×10^{-5}	NE	NE	1.5×10^{-4}	9.0×10^{-5}–2.0×10^{-4}
	6-MeO-BDE-85	NC	3.0×10^{-4}	1.3×10^{-4}	3.6×10^{-5}	1.5×10^{-5}	1.2×10^{-4}	1.5×10^{-5}–3.0×10^{-4}
	2′-HO-BDE-28	NC	1.1×10^{-4}	5.5×10^{-5}	NE	NE	8.5×10^{-5}	5.5×10^{-5}–1.1×10^{-4}
	6-MeO-BDE-47	NC	1.2×10^{-4}	7.3×10^{-5}	3.5×10^{-5}	2.0×10^{-5}	6.1×10^{-5}	2.0×10^{-5}–1.2×10^{-4}
	6-HO-BDE-47	NC	6.3×10^{-5}	3.7×10^{-5}	3.9×10^{-6}	NE	3.5×10^{-5}	3.9×10^{-6}–6.3×10^{-5}
	6-HO-BDE-137	NC	2.7×10^{-5}	NE	NE	NE	2.7×10^{-5}	
	6-MeO-BDE-137	NC	2.2×10^{-5}	1.0×10^{-5}	NE	NE	1.6×10^{-5}	1.0×10^{-5}–2.2×10^{-5}
	4-HO-BDE-90	NC	1.4×10^{-5}	8.8×10^{-6}	NE	NE	1.1×10^{-5}	8.8×10^{-6}–1.4×10^{-5}
	2-MeO-BDE-123	NC	1.0×10^{-5}	NE	NE	NE	1.0×10^{-5}	
	2-HO-BDE-123	NC	8.3×10^{-6}	NE	NE	NE	8.3×10^{-6}	
	2′-HO-BDE-68	NC	NE	NE	NE	NE	NA	NA
	6-HO-BDE-85	7.4×10^{-4}	NE	NE	NE	NE	NA	NA
	3-HO-BDE-100	$<9.3\times10^{-6}$	NE	NE	NE	NE	NA	NA
	6′-Cl-2′-HO-BDE-7	3.3×10^{-4}	NE	NE	NE	NE	NA	NA
	6-HO-BDE-90	$<9.3\times10^{-6}$	NE	NE	NE	NE	NA	NA
	2′-HO-BDE-25	$<6.8\times10^{-6}$	NE	NE	NE	NE	NA	NA
Pheasant	TCDD	1.0	1.0	1.0	1.0	1.0	1.0	1.0–1.0
	5-Cl-6-HO-BDE-47	NC	1.1×10^{-2}	1.1×10^{-2}	9.9×10^{-3}	1.1×10^{-2}	1.1×10^{-2}	9.9×10^{-3}–1.1×10^{-2}
	5-Cl-6-MeO-BDE-47	NC	4.8×10^{-3}	3.4×10^{-3}	2.4×10^{-3}	3.0×10^{-3}	3.4×10^{-3}	2.4×10^{-3}–4.8×10^{-3}
	2′-MeO-BDE-28	8.7×10^{-3}	NE	NE	NE	NE	NA	NA
	2′-HO-BDE-66	4.5×10^{-3}	NE	NE	NE	NE	NA	NA
	6-MeO-BDE-85	NC	5.4×10^{-3}	3.8×10^{-3}	2.0×10^{-3}	NE	3.7×10^{-3}	2.0×10^{-3}–5.4×10^{-3}
	2′-HO-BDE-28	NC	1.8×10^{-4}	NE	NE	NE	1.8×10^{-4}	
	6-MeO-BDE-47	NC	1.5×10^{-4}	1.6×10^{-4}	NE	NE	1.6×10^{-4}	1.5×10^{-4}–1.6×10^{-4}
	6-HO-BDE-47	NC	3.7×10^{-4}	2.2×10^{-4}	NE	NE	3.0×10^{-4}	2.2×10^{-4}–3.7×10^{-4}
	6-HO-BDE-137	NC	4.2×10^{-4}	3.7×10^{-4}	NE	NE	4.0×10^{-4}	3.7×10^{-4}–4.2×10^{-4}
	6-MeO-BDE-137	NC	8.6×10^{-5}	NE	NE	NE	8.6×10^{-5}	
	4-HO-BDE-90	NC	1.3×10^{-4}	1.1×10^{-4}	NE	NE	1.2×10^{-4}	1.1×10^{-4}–1.3×10^{-4}
	2-MeO-BDE-123	NC	NE	NE	NE	NE	NA	NA
	2-HO-BDE-123	2.2×10^{-3}	NE	NE	NE	NE	NA	NA
	2′-HO-BDE-68	NC	NE	NE	NE	NE	NA	NA
	6-HO-BDE-85	NC	NE	NE	NE	NE	NA	NA
	3-HO-BDE-100	NC	NE	NE	NE	NE	NA	NA
	6′-Cl-2′-HO-BDE-7	NC	NE	NE	NE	NE	NA	NA
	6-HO-BDE-90	NC	NE	NE	NE	NE	NA	NA
	2′-HO-BDE-25	NC	NE	NE	NE	NE	NA	NA
Quail	TCDD	1.0	1.0	1.0	1.0	1.0	1.0	1.0∼1.0
	5-Cl-6-HO-BDE-47	7.1×10^{-2}	6.9×10^{-2}	9.0×10^{-2}	1.6×10^{-1}	3.8×10^{-1}	1.7×10^{-1}	6.9×10^{-2}–3.8×10^{-1}
	5-Cl-6-MeO-BDE-47	2.2×10^{-2}	2.3×10^{-2}	2.8×10^{-2}	5.1×10^{-2}	1.2×10^{-1}	5.5×10^{-2}	2.3×10^{-2}–1.2×10^{-1}
	2′-MeO-BDE-28	NC	5.6×10^{-4}	3.2×10^{-4}	NE	NE	4.4×10^{-4}	3.2×10^{-4}–5.6×10^{-4}
	2′-HO-BDE-66	4.5×10^{-2}	NE	NE	NE	NE	NA	NA
	6-MeO-BDE-85	4.1×10^{-2}	3.9×10^{-2}	4.4×10^{-2}	6.2×10^{-2}	1.1×10^{-1}	6.4×10^{-2}	3.9×10^{-2}–1.1×10^{-1}
	2′-HO-BDE-28	NC	1.5×10^{-3}	1.2×10^{-3}	NE	NE	1.3×10^{-3}	1.2×10^{-3}–1.5×10^{-3}
	6-MeO-BDE-47	NC	9.9×10^{-4}	1.3×10^{-3}	2.2×10^{-3}	NE	1.5×10^{-3}	9.9×10^{-4}–2.2×10^{-3}
	6-HO-BDE-47	NC	1.4×10^{-3}	1.7×10^{-3}	2.4×10^{-3}	4.7×10^{-3}	2.5×10^{-3}	1.4×10^{-3}–4.7×10^{-3}
	6-HO-BDE-137	NC	1.4×10^{-3}	1.8×10^{-3}	3.3×10^{-3}	8.4×10^{-3}	3.7×10^{-3}	1.4×10^{-3}–8.4×10^{-3}
	6-MeO-BDE-137	7.1×10^{-3}	9.1×10^{-4}	1.8×10^{-3}	4.9×10^{-3}	NE	2.5×10^{-3}	9.1×10^{-4}–4.9×10^{-3}
	4-HO-BDE-90	NC	7.8×10^{-4}	9.7×10^{-4}	1.7×10^{-3}	NE	1.1×10^{-3}	7.8×10^{-4}–1.7×10^{-3}
	2-MeO-BDE-123	NC	5.6×10^{-4}	5.2×10^{-4}	NE	NE	5.4×10^{-4}	5.2×10^{-4}–5.6×10^{-4}
	2-HO-BDE-123	1.6×10^{-2}	1.7×10^{-4}	NE	NE	NE	1.7×10^{-4}	
	2′-HO-BDE-68	NC	5.9×10^{-4}	2.6×10^{-4}	NE	NE	4.2×10^{-4}	2.6×10^{-4}–5.9×10^{-4}
	6-HO-BDE-85	1.7×10^{-2}	2.4×10^{-3}	7.3×10^{-4}	NE	NE	1.6×10^{-3}	7.3×10^{-4}–2.4×10^{-3}
	3-HO-BDE-100	$<9.3\times10^{-6}$	NE	NE	NE	NE	NA	NA
	6′-Cl-2′-HO-BDE-7	5.4×10^{-2}	5.9×10^{-3}	1.1×10^{-2}	NE	NE	8.6×10^{-3}	5.9×10^{-3}–1.1×10^{-2}
	6-HO-BDE-90	NC	NE	NE	NE	NE	NA	NA
	2′-HO-BDE-25	NC	9.5×10^{-4}	NE	NE	NE	9.5×10^{-4}	

NC: not calculated because the maximal response was not reached.
NE: not estimated because the maximum observed response was below 10%, 20%, 50%, or 80% of the positive control response.
NA: not available.

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Supplementary material

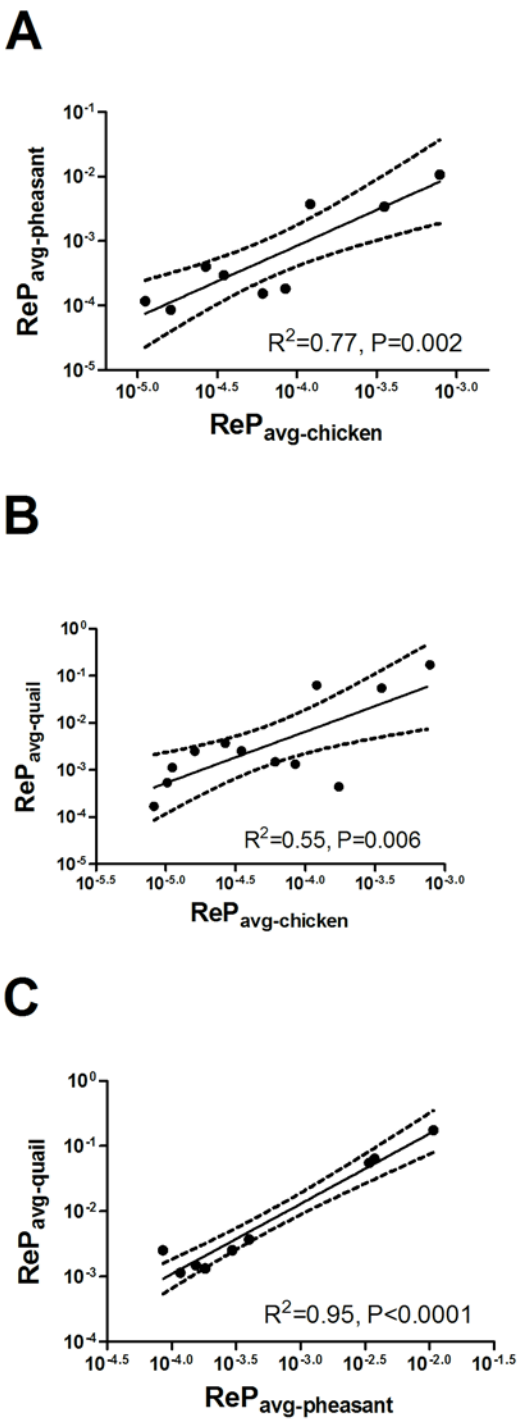

Figure S1. Linear regression comparing the log-transformed average relative potency (ReP_{avg}) values of OH-/MeO-PBDEs (polybrominated diphenyl ethers) determined in

avian aryl hydrocarbon receptor 1 (AHR1)-luciferase reporter gene assays.

Table S1. Endpoints for aryl hydrocarbon receptor 1 (AHR1)-mediated luciferase reporter gene activity in COS-7 cells transfected with chicken, ring-necked pheasant, or Japanese quail AHR1 constructs.

AHR1	Compound	EC_{50} ± SE (nM)	PC_{10} ± SE (nM)	PC_{20} ± SE (nM)	PC_{50} ± SE (nM)	PC_{80} ± SE (nM)	Max. response ± SE (% PC)
Chicken	TCDD	$1.61\times10^{-1} \pm 3.2\times10^{-3}$[a]	$7.34\times10^{-2} \pm 4.0\times10^{-3}$[a]	$9.67\times10^{-2} \pm 3.6\times10^{-3}$[a]	$1.53\times10^{-1} \pm 2.7\times10^{-3}$[a]	$2.30\times10^{-1} \pm 7.7\times10^{-3}$[a]	112 ± 12[b]
	5-Cl-6-HO-BDE-47	300 ± 5.3[b]	52.7 ± 7.5[a]	100 ± 11[a]	298 ± 21[a]	879 ± 84[a]	101 ± 7.5[bc]
	5-Cl-6-MeO-BDE-47	NC	112 ± 2.8[a]	238 ± 18[ab]	745 ± 115[a]	1614 ± 312[a]	150 ± 0.47[‡a]
	2'-MeO-BDE-28	NC	421 ± 48[a]	NE	NE	NE	42.3 ± 0.69[‡ef]
	2'-HO-BDE-66	428 ± 40[bc]	360 ± 35[a]	1072 ± 32.9[abc]	NE	NE	23.5 ± 0.92[ghi]
	6-MeO-BDE-85	NC	243 ± 30[a]	737 ± 60[abc]	4227 ± 148[a]	15757 ± 803.0[b]	82.0 ± 3.0[‡d]
	2'-HO-BDE-28	NC	643 ± 80[ab]	1757 ± 127[cd]	NE	NE	28.6 ± 0.43[‡fgh]
	6-MeO-BDE-47	NC	637 ± 21[ab]	1319 ± 76.6[bc]	4325 ± 524[a]	11677 ± 2877[b]	92.2 ± 11[‡cd]
	6-HO-BDE-47	NC	1167 ± 108[abc]	2585 ± 602[d]	39426 ± 4432[b]	NE	46.5 ± 5.3[‡e]
	6-HO-BDE-137	NC	2744 ± 566[bcd]	NE	NE	NE	18.0 ± 1.0[‡ghij]
	6-MeO-BDE-137	NC	3335 ± 369[cd]	9445 ± 358[e]	NE	NE	23.4 ± 3.2[‡ghi]
	4-HO-BDE-90	NC	5434 ± 516[de]	11035 ± 714.5[e]	NE	NE	31.0 ± 2.7[‡fg]
	2-MeO-BDE-123	NC	7170 ± 1345[ef]	NE	NE	NE	15.2 ± 0.70[‡hij]
	2-HO-BDE-123	NC	8904 ± 1779[f]	NE	NE	NE	12.1 ± 1.2[‡ij]
	2'-HO-BDE-68	NC	NE	NE	NE	NE	$3.78 \pm 2.9\times10^{-2}$[‡j]
	6-HO-BDE-85	217 ± 71[b]	NE	NE	NE	NE	8.74 ± 1.1[j]
	3-HO-BDE-100	> 17220.89	NE	NE	NE	NE	no induction
	6'-Cl-2'-HO-BDE-7	492 ± 107[c]	NE	NE	NE	NE	6.42 ± 0.32[j]

	6-HO-BDE-90	> 17220.89	NE	NE	NE	NE	no induction
	2'-HO-BDE-25	> 23646.36	NE	NE	NE	NE	no induction
Pheasant	TCDD	5.57 ± 0.50^{a}	$7.70\times10^{-1} \pm 6.2\times10^{-2a}$	1.58 ± 0.12^{a}	5.39 ± 0.32^{a}	17.8 ± 0.29^{a}	100 ± 9.2^{a}
	5-Cl-6-HO-BDE-47	NC	68.4 ± 10^{a}	150 ± 16^{a}	544 ± 21^{a}	1594 ± 149^{b}	$108 \pm 1.7^{‡a}$
	5-Cl-6-MeO-BDE-47	NC	160 ± 7.3^{a}	466 ± 16^{a}	2234 ± 52.5^{b}	5992 ± 153^{c}	$119 \pm 11^{‡a}$
	2'-MeO-BDE-28	641 ± 16^{b}	NE	NE	NE	NE	7.57 ± 0.64^{ef}
	2'-HO-BDE-66	1235 ± 32.2^{c}	NE	NE	NE	NE	8.24 ± 2.2^{ef}
	6-MeO-BDE-85	NC	142 ± 11^{a}	415 ± 46^{a}	2705 ± 306^{c}	NE	$79.3 \pm 0.70^{‡b}$
	2'-HO-BDE-28	NC	4218 ± 380^{c}	NE	NE	NE	$13.1 \pm 3.3^{‡ef}$
	6-MeO-BDE-47	NC	5112 ± 243^{d}	9970 ± 451^{d}	NE	NE	$38.7 \pm 3.8^{‡cd}$
	6-HO-BDE-47	NC	2062 ± 131^{b}	7291 ± 639^{c}	NE	NE	$26.1 \pm 3.3^{‡cde}$
	6-HO-BDE-137	NC	1817 ± 74.2^{b}	4237 ± 236^{b}	NE	NE	$46.2 \pm 1.0^{‡c}$
	6-MeO-BDE-137	NC	8960 ± 256^{e}	NE	NE	NE	$17.0 \pm 1.5^{‡ef}$
	4-HO-BDE-90	NC	6002 ± 287^{d}	14847 ± 46.68^{e}	NE	NE	$22.2 \pm 0.81^{‡def}$
	2-MeO-BDE-123	NC	NE	NE	NE	NE	$7.85 \pm 0.21^{‡ef}$
	2-HO-BDE-123	2595 ± 311^{d}	NE	NE	NE	NE	8.95 ± 1.8^{ef}
	2'-HO-BDE-68	NC	NE	NE	NE	NE	$2.40 \pm 0.31^{‡f}$
	6-HO-BDE-85	NC	NE	NE	NE	NE	$7.90 \pm 2.1^{‡ef}$
	3-HO-BDE-100	> 17220.89	NE	NE	NE	NE	no induction
	6'-Cl-2'-HO-BDE-7	> 26423.78	NE	NE	NE	NE	no induction
	6-HO-BDE-90	NC	NE	NE	NE	NE	$2.31 \pm 0.42^{‡f}$
	2'-HO-BDE-25	> 23646.36	NE	NE	NE	NE	no induction
Quail	TCDD	25.4 ± 4.0^{a}	2.60 ± 0.17^{a}	5.83 ± 0.32^{a}	22.8 ± 1.4^{a}	86.6 ± 6.6^{a}	100 ± 10^{cd}
	5-Cl-6-HO-BDE-47	358 ± 28^{ab}	38.0 ± 2.0^{a}	65.1 ± 3.7^{a}	142 ± 9.1^{a}	229 ± 16^{a}	227 ± 0.67^{a}
	5-Cl-6-MeO-BDE-47	1151 ± 98.7^{bc}	115 ± 12^{a}	205 ± 20^{a}	446 ± 38^{a}	727 ± 55^{a}	225 ± 18^{a}
	2'-MeO-BDE-28	NC	4677 ± 445^{b}	18040 ± 1049^{e}	NE	NE	$21.7 \pm 1.7^{‡efg}$

2'-HO-BDE-66	566 ± 104^{ab}	NE	NE	NE	NE	9.09 ± 1.5^{g}
6-MeO-BDE-85	615 ± 85^{ab}	67.7 ± 14^{a}	132 ± 14^{a}	371 ± 5.9^{a}	786 ± 42^{a}	140 ± 18^{b}
2'-HO-BDE-28	NC	1714 ± 6.44^{ab}	5045 ± 105^{bc}	NE	NE	21.4 ± 1.9^{‡efg}
6-MeO-BDE-47	NC	2619 ± 38.0^{ab}	4489 ± 134^{abc}	10562 ± 255^{d}	NE	75.5 ± 7.5^{‡d}
6-HO-BDE-47	NC	1802 ± 51.1^{ab}	3539 ± 51.3^{abc}	9641 ± 379^{d}	18408 ± 1647^{c}	84.0 ± 15^{‡cd}
6-HO-BDE-137	NC	1924 ± 139^{ab}	3330 ± 146^{abc}	6964 ± 172^{c}	10286 ± 202.3^{b}	126 ± 3.7^{‡bc}
6-MeO-BDE-137	3597 ± 41.4^{d}	2875 ± 29.1^{ab}	3280 ± 3.38^{abc}	4651 ± 138^{b}	NE	58.2 ± 9.5^{def}
4-HO-BDE-90	NC	3358 ± 103^{ab}	6009 ± 85.2^{c}	13545 ± 278.7^{e}	NE	64.3 ± 4.8^{‡de}
2-MeO-BDE-123	NC	4647 ± 161^{b}	11277 ± 290.4^{d}	NE	NE	24.2 ± 1.3^{‡efg}
2-HO-BDE-123	1607 ± 257^{c}	15480 ± 4480^{c}	NE	NE	NE	10.8 ± 1.6^{g}
2'-HO-BDE-68	NC	4410 ± 201^{b}	22842 ± 4562^{e}	NE	NE	19.6 ± 5.3^{‡fg}
6-HO-BDE-85	1519 ± 549^{c}	1095 ± 83.4^{ab}	7960 ± 1292^{cd}	NE	NE	21.9 ± 3.5^{efg}
3-HO-BDE-100	> 17220.89	NE	NE	NE	NE	no induction
6'-Cl-2'-HO-BDE-7	474 ± 7.34^{ab}	444 ± 2.4^{a}	511 ± 5.6^{ab}	NE	NE	29.4 ± 3.1^{efg}
6-HO-BDE-90	NC	NE	NE	NE	NE	9.58 ± 1.3^{‡g}
2'-HO-BDE-25	NC	2737 ± 165^{ab}	NE	NE	NE	17.2 ± 2.1^{‡fg}

EC, effective concentration; PC, positive control; SE, standard error; TCDD, tetrachlorodibenzo-*p*-dioxin; BDE, brominated diphenyl ether; NC, not calculated because a plateau was not reached; NE, not estimated because the maximum observed response was below 10%, 20%, 50%, or 80% of the PC response.

* EC_{50}, PC_{10}, PC_{20}, PC_{50}, PC_{80}, and maximal response values represent the average of three replicates ± SE obtained from three 96-well plates for each compound. PC_{10}, PC_{20}, PC_{50}, and PC_{80} values were not calculated if the maximum observed response was < 10% of that of the PC.

Superscript letters indicate significant differences among treatments ($p < 0.05$) for each AHR1 construct.

† Luciferase reporter gene activity values were normalized to the responses to the PC (300 nM TCDD). Maximal response values were obtained from the curve fit, unless otherwise indicated.

‡ A plateau was not reached. Values represent the highest determined response.

Table S2. Relative potency (ReP) values of OH-/MeO-polybrominated diphenyl ethers (PBDEs) derived from H4IIE-luc assays[‡]

Compound	ReP_{EC50}	ReP_{PC10}	ReP_{PC20}	ReP_{PC50}	ReP_{PC80}	ReP_{avg}	ReP range
TCDD	1.0	1.0	1.0	1.0	1.0	1.0	1.0~1.0
5-Cl-6-HO-BDE-47	NC	1.0×10^{-5}	6.6×10^{-6}	4.2×10^{-6}	5.1×10^{-6}	6.5×10^{-6}	4.2×10^{-6}~1.0×10^{-5}
5-Cl-6-MeO-BDE-47	3.2×10^{-6}	1.3×10^{-6}	1.3×10^{-6}	8.5×10^{-7}	NE	1.1×10^{-6}	8.5×10^{-7}~1.3×10^{-6}
2'-MeO-BDE-28	NC	3.8×10^{-8}	2.7×10^{-8}	NE	NE	3.2×10^{-8}	2.7×10^{-8}~3.8×10^{-8}
2'-HO-BDE-66	NC	5.2×10^{-8}	4.5×10^{-8}	NE	NE	4.8×10^{-8}	4.5×10^{-8}~5.2×10^{-8}
6-MeO-BDE-85	NC	1.7×10^{-6}	6.3×10^{-7}	NE	NE	1.2×10^{-6}	6.3×10^{-7}~1.7×10^{-6}
2'-HO-BDE-28	NC	3.9×10^{-8}	NE	NE	NE	3.9×10^{-8}	
6-MeO-BDE-47	NC	3.3×10^{-8}	NE	NE	NE	3.3×10^{-8}	
6-HO-BDE-47	NC	9.4×10^{-7}	1.0×10^{-6}	NE	NE	9.8×10^{-7}	9.4×10^{-7}~1.0×10^{-6}
6-HO-BDE-137	NC	1.3×10^{-6}	9.4×10^{-7}	6.5×10^{-7}	NE	9.7×10^{-7}	6.5×10^{-7}~1.3×10^{-6}
6-MeO-BDE-137	NC	8.0×10^{-8}	5.7×10^{-8}	NE	NE	6.9×10^{-8}	5.7×10^{-8}~8.0×10^{-8}
4-HO-BDE-90	NC	4.8×10^{-8}	NE	NE	NE	4.8×10^{-8}	
2-MeO-BDE-123	NC	NE	NE	NE	NE	NA	NA
2-HO-BDE-123	NC	9.2×10^{-8}	5.2×10^{-8}	NE	NE	7.2×10^{-8}	5.2×10^{-8}~9.2×10^{-8}
2'-HO-BDE-68	NC	NE	NE	NE	NE	NA	NA
6-HO-BDE-85	1.7×10^{-5}	5.0×10^{-6}	4.2×10^{-6}	NE	NE	4.6×10^{-6}	4.2×10^{-6}~5.0×10^{-6}
3-HO-BDE-100	NC	2.4×10^{-8}	NE	NE	NE	2.4×10^{-8}	
6'-Cl-2'-HO-BDE-7	NC	3.7×10^{-8}	NE	NE	NE	3.7×10^{-8}	
6-HO-BDE-90	NC	NE	NE	NE	NE	NA	NA
2'-HO-BDE-25	NC	NE	NE	NE	NE	NA	NA

[‡] The original background-corrected luciferase activities were obtained from Su et al., 2012, and they were re-processed again in the present study to maintain method consistency between the H4IIE-luc assay and avian AHR1 LRG assays.

TCDD, tetrachlorodibenzo-*p*-dioxin; NC, not calculated because the maximal response was not reached; NE, not estimated because the maximum observed response was below 10%, 20%, 50%, or 80% of the positive control response; NA, ReP estimates were not available to calculate the value

Table S3. Relative potency (ReP) values of several dioxins derived from different bioassays of three different bird species.

Species	Compound	LD	LRG	Primary hepatocyte cell culture			$ReP_{bioassay\text{-}avg}^{\eta}$
				EROD	CYP1A4	CYP1A5	
Chicken	TCDD	1.0	1.0	1.0	1.0	1.0	1.0
	2,3,4,7,8-PeCDF$^{\alpha}$	0.75-1.1‡	0.5-1.1§	0.9	0.7	2	1.1
	PCB 126$^{\beta}$	0.19†	0.074$^{\Phi}$	0.36	0.12	0.12	0.15
	PCB 77$^{\beta}$	0.022†	$2.1\times10^{-3\Phi}$	0.035	1.1×10^{-3}	4.6×10^{-4}	0.012
	PCB 105$^{\beta}$	$9.6\times10^{-5\dagger}$	$3.0\times10^{-5\Phi}$	3.0×10^{-5}	3.6×10^{-5}	9.1×10^{-5}	5.7×10^{-5}
	PCB 118$^{\beta}$	$2.7\times10^{-5\dagger}$	$6.9\times10^{-5\Phi}$	4.1×10^{-4}	6.3×10^{-4}	5.2×10^{-4}	3.3×10^{-4}
Pheasant	TCDD	1.0	1.0	1.0	1.0	1.0	1.0
	2,3,4,7,8-PeCDF$^{\alpha}$	2.6-13‡	3-4§	3	2	N/A	4.1
	PCB 126$^{\beta}$	0.047$^{\dagger\kappa}$	0.037$^{\Phi}$	0.022	N/A	N/A	0.035
	PCB 77$^{\beta}$	$1.3\times10^{-3\dagger\lambda}$	$1.0\times10^{-3\Phi}$	8.5×10^{-4}	N/A	N/A	1.1×10^{-3}
	PCB 105$^{\beta}$	N/A	$< 1.0\times10^{-4\Phi}$	3.3×10^{-5}	N/A	N/A	3.3×10^{-5}
	PCB 118$^{\beta}$	N/A	$< 1.0\times10^{-4\Phi}$	9.2×10^{-5}	N/A	N/A	9.2×10^{-5}
Quail	TCDD	1.0	1.0	1.0	1.0	1.0	1.0
	2,3,4,7,8-PeCDF$^{\alpha}$	3.3-11‡	13-26§	13	8	17	13
	PCB 126$^{\beta}$	0.066$^{\dagger\zeta}$	0.32$^{\Phi}$	0.016	2.7×10^{-3}	1.6×10^{-3}	0.081
	PCB 77$^{\beta}$	N/A	$4.8\times10^{-3\Phi}$	1.0×10^{-3}	3.0×10^{-5}	4.1×10^{-5}	1.5×10^{-3}
	PCB 105$^{\beta}$	N/A	$< 3.3\times10^{-3\Phi}$	0.011	3.0×10^{-4}	1.4×10^{-3}	0.037$^{\delta}$
	PCB 118$^{\beta}$	N/A	$3.3\times10^{-3\Phi}$	3.0×10^{-3}	1.2×10^{-4}	4.3×10^{-4}	1.7×10^{-3}

LD, lethal dose; LRG, luciferase reporter gene; EROD, ethoxyresorufin O-deethylase; CYP1A4 and 1A5, cytochrome P4501A4 and 1A5; ReP, relative potency.; TCDD, tetrachlorodibenzo-*p*-dioxin; PCB, polychlorinated biphenyl; PeCDF, pentachlorodibenzofuran; N/A, not available.

$^{\alpha}$ ReP values taken from Farmahin et al.(Farmahin et al., 2012)

$^{\beta}$ ReP values taken from Manning et al.(Manning et al., 2012, 2013)

$^{\eta}$ The arithmetic average ReP values of LD, LRG, EROD, CYP1A4, and CYP1A5.

‡ $ReP_{LD20\text{-}80}$

§ $ReP_{PC20\text{-}80}$

† ReP_{LD50}

$^{\Phi}$ ReP_{avg}

$^{\kappa}$ The LD_{50} value of PCB 126 for bobwhite quail (2G, the same subtype as ring-necked pheasant) was used as a substitute for that of ring-necked pheasant (2G) because the value for the latter was not available.

$^{\lambda}$ The LD_{50} value of PCB 77 for wild turkey (2A, the same type as ring-necked pheasant) was used as a substitute for that of ring-necked pheasant (2G) because the latter value was not available.

$^{\xi}$ The average LD_{50} value of PCB 126 for American kestrel and common tern (3B), the same type as Japanese quail) was used as a substitute for that of Japanese quail (3A) because the value for the latter was not available. The LD_{50} values of TCDD and PCB 126 for double-crested cormorant (3B) were used to calculate the ReP_{LD50} value as a substitute for that of Japanese quail (3A). The data in the table are the average of these substitutions.

$^{\delta}$ The average of ReP_{CYP1A4} and ReP_{CYP1A5} was assigned to the ReP_{LRG} to calculate the $ReP_{bioassay\text{-}avg}$ because the ReP_{CYP1A4} and ReP_{CYP1A5} were in the range of the ReP_{LRG} ($< 3.3\times10^{-3}$).

Table S4. Relative average potency values determined in bioassays ($ReP_{bioassay\text{-}avg}$) of the effects of typical dioxin-like compounds on birds vs. the values derived from H4IIE-*luc* assays

Compound	Chicken	Ring-necked pheasant	Japanese quail	H4IIE-*luc*[‡]
TCDD	1.0	1.0	1.0	1.0
2,3,4,7,8-PeCDF	1.1	4.1	13	0.69
PCB 126	0.15	0.035	0.081	0.017
PCB 77	0.012	1.1×10^{-3}	1.5×10^{-3}	7.1×10^{-4}
PCB 105	5.7×10^{-5}	3.3×10^{-5}	0.037	$< 1.0\times10^{-6}$
PCB 118	3.3×10^{-4}	9.2×10^{-5}	1.7×10^{-3}	$< 1.0\times10^{-6}$

TCDD; tetrachlorodibenzo-*p*-dioxin; PeCDF, pentachlorodibenzofuran; PCB, polychlorinated biphenyl.

[‡] ReP values taken from Sanderson et al. (Sanderson et al., 1996)

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