Sensitivity of Various Primary Avian Embryo Hepatocyte Cell Cultures to Cytochrome P4501A Induction by TCDD, TCDF and PCBs

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1. Introduction

Wild birds are exposed to complex mixtures of halogenated aromatic hydrocarbons (HAHs), including polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs). One goal of the research in our laboratory is to develop avian cell culture bioassays which allow determination of the sensitivity of different species of wild birds to the toxic effects of HAHs. Current research is focussed on (1) induction of cytochrome P4501A (CYP1A), a biochemical response that is associated with the toxicity of PCDDs, PCDFs, non-ortho substituted PCBs and mono-ortho substituted PCBs, and (2) porphyrin accumulation, a toxic effect caused by several HAHs in birds and mammals.

White Leghorn chicken embryo hepatocyte cultures are useful for studying the effects of HAHs on CYP1A gene expression and porphyrin accumulation[1,2]. We recently developed methods for measuring the catalytic activity of the CYP1A associated enzyme, ethoxyresorufin-O-deethylase (EROD), and porphyrin concentrations, in CEH cultures that are much less time-consuming than previous methods[3-5]. In the present study, we prepared primary hepatocyte cultures from various species of birds, and used the new EROD method to compare the sensitivity of the cultures to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), 2,3,7,8-tetrachlorodibenzofuran (TCDF), four non-ortho substituted PCBs and two mono-ortho substituted PCBs.

2. Materials and Methods

TCDD was kindly provided by Dr. J. Ryan, Health Canada (Ottawa, Ont.). TCDF and PCBs were from Ultra Scientific (Kingstown, RI). Chicken (White Leghorn, Turken, Dark Cornish, and Araucanas), Ring-Necked Pheasant, turkey (Naraganset), and duck (Pekin and Campbell) eggs were from local suppliers. Herring Gull eggs were collected from nests in northern Lake Superior, an area that contains relatively low concentrations of PCDDs, PCDFs and PCBs. Eggs were incubated in the laboratory until one day prior to hatching. Primary hepatocyte cultures were prepared in 48-well plates in serum-free Waymouth's medium that was supplemented with insulin (1 µg/ml) and thyroxine (1 µg/ml). After incubation for 24 hours at 37°C in an atmosphere of 95% air and 5% CO_2, the medium was removed and replaced with fresh medium. Dimethyl sulfoxide (DMSO) solutions of TCDD, TCDF or PCBs were added to the cultures. Cells were incubated for another 24 hours, the medium was removed, and plates were frozen on dry ice prior to transferring them to a freezer (-80°C). Plates were thawed, and EROD activity was determined with a fluorescence plate reader[3]. Total protein was determined using the fluorescamine assay[4].

3. Results and Discussion

Compounds that induced EROD caused dose-dependent increases in enzyme activity at low doses, which was followed by dose-dependent decreases in activity at higher doses (Fig. 1).

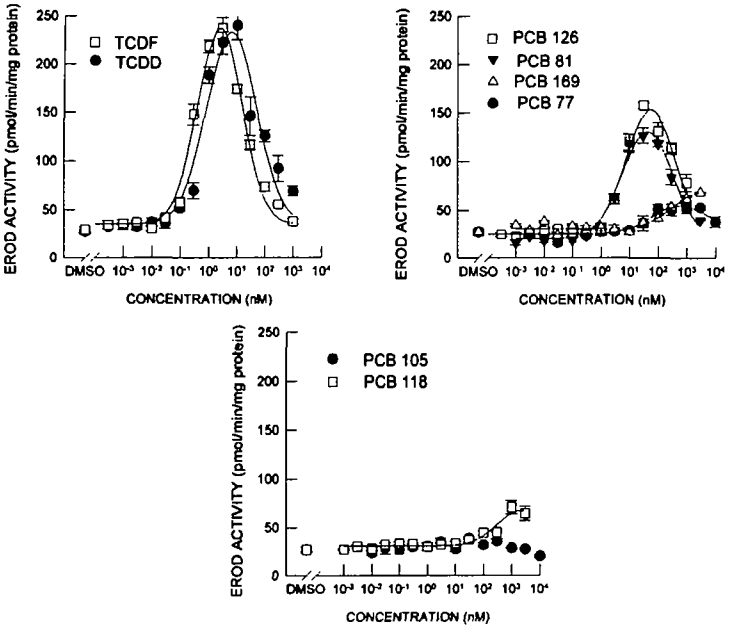

Figure 1. EROD dose response curves of TCDD, TCDF and PCBs (IUPAC nomenclature; structures are shown in Fig. 2) in Campbell Duck embryo hepatocyte cultures. Triplicates of each dose were administered.

We have previously reported biphasic EROD dose-response curves in White Leghorn Chicken embryo hepatocytes[3,6], and other investigators have observed decreased EROD activity upon exposure to high levels of PCBs and PCDDs in several species, including mammals, birds, fish and cell cultures prepared from these organisms. In some situations, decreased EROD activity at high doses is likely caused by cytotoxicity or competitive inhibition of EROD activity by HAHs, but Hahn *et al.*[7] have suggested that cytotoxicity and/or enzyme inhibition may not provide a full explanation in all situations. Regardless of the mechanism(s) involved, dose-response data can be fitted to Gaussian curves, and curve parameters were used to obtain maximal responses and EC_{50s} (defined as the lower concentration of HAH where EROD activity is midway between basal and maximal activity). The EC_{50s} of TCDD, TCDF and PCB congeners are indicated in Table 1, and the relative sensitivities of the cultures to the compounds are illustrated in Figs. 2 and 3.

TCDD and TCDF were approximately equipotent in hepatocytes from most species, but TCDF was more potent than TCDD in hepatocytes from both species of ducks. Maximal EROD activity for all PCBs was lower than the maximal activity for TCDD, and EC_{50s} of PCBs were

higher than the EC_{50s} of TCDD. The non-ortho substituted PCBs, 81 and 126, gave similar dose-response curves in hepatocytes from all species, and were more potent than other PCBs.

Chicken embryo hepatocytes were considerably more sensitive to TCDD, TCDF, and non-ortho-substituted PCBs than were hepatocytes from other species. The rank order of sensitivity to TCDD, TCDF and non-ortho substituted PCBs was chicken > pheasant ≥ turkey ≥ duck ≥ Herring Gull.

			NON-ORTHO SUBSTITUTED PCBs				MONO-ORTHO SUBSTITUTED PCBs	
	TCDD	TCDF	PCB 126	PCB 81	PCB 77	PCB 169	PCB 118	PCB 105
W.L. CHICKEN	0.015 0.016 0.019 (0.017)	0.011 0.011 (0.011)	0.056 0.051 (0.054)	0.092	0.45 0.55 (0.50)	0.75 0.90 (0.82)	18 33 (26)	4.5
A. CHICKEN	0.048	0.053	0.17	N.D.	0.69	2.2	39	N.I.
T. CHICKEN	0.014	0.011	N.D.	N.D.	0.36	N.D.	70	N.I.
D.C. CHICKEN	0.040	0.025	0.21	N.D.	0.85	2.2	165	N.I.
PHEASANT	0.14 0.19 0.090 (0.14)	0.25 0.15 0.10 (0.16)	2.5	N.D.	12	18	8.0	N.I.
C. DUCK	0.77 0.50 0.58 (0.62)	0.30 0.29 0.26 (0.28)	3.4 5.5 (4.5)	3.8	40 21 (31)	317 224 (271)	222	N.I.
P. DUCK	1.8 2.0 (1.9)	0.48 0.48 (0.48)	15 11 (13)	3.9	84 268 (176)	294 408 (351)	N.I.	N.I.
TURKEY	0.59 0.66 (0.62)	0.81 0.77 (0.79)	5.1 5.0 (5.1)	7.1	8.9 14 (11)	21 27 (24)	37 27 (32)	28
H. GULL	1.3 0.59 0.76 (0.88)	1.1 0.88 (1.0)	13 19 (16)	N.D.	N.I.	13	N.I.	N.I.

Table 1. EROD EC_{50s} (nM) in primary hepatocyte cultures prepared from embryos of different species of birds. N.I., no induction; N.D. not done.

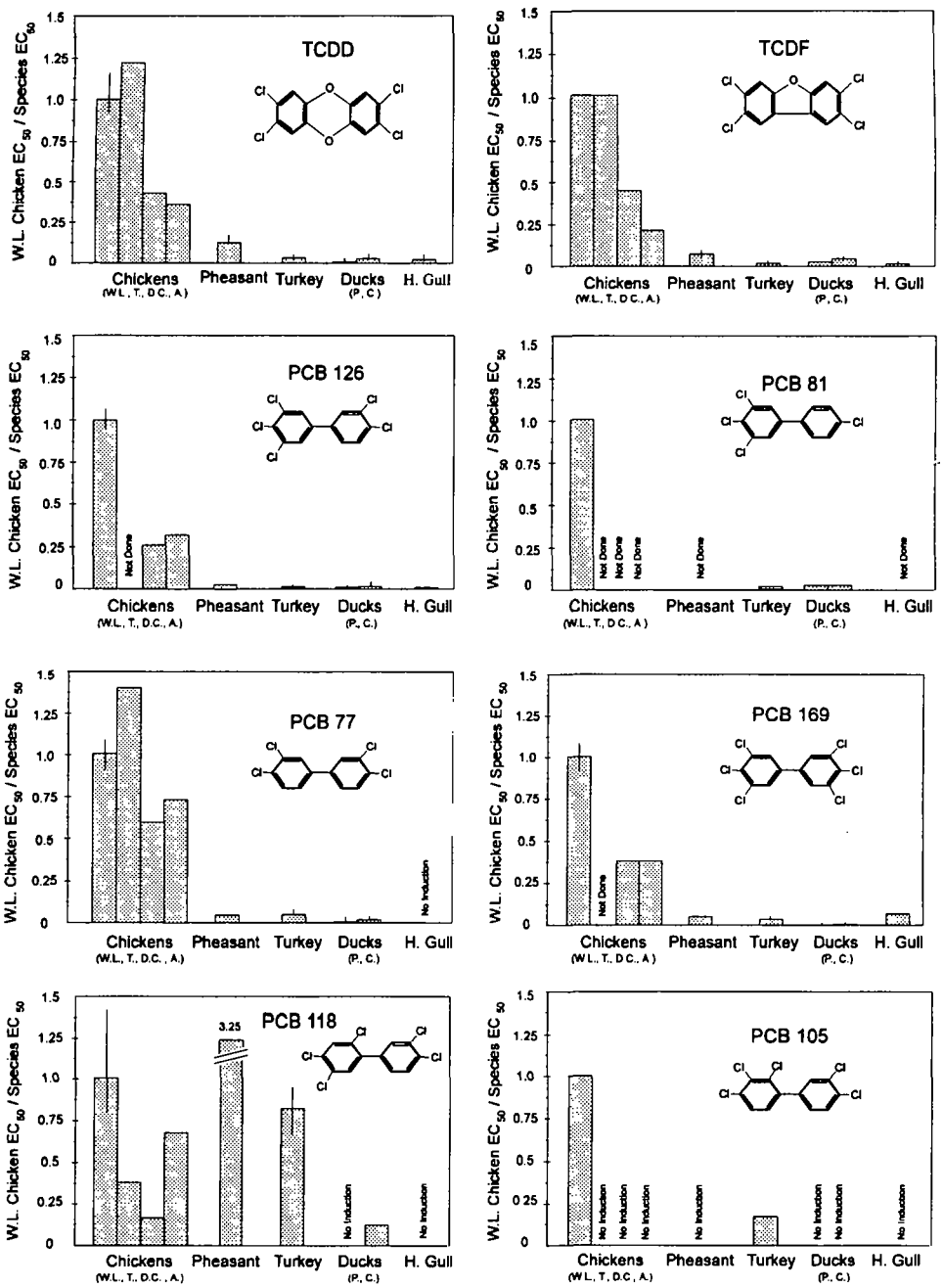

Figure 2. Relative sensitivity of primary avian hepatocyte cultures to TCDD, TCDF and PCBs. Bars represent the range of response in situations where two or more plates were used per compound.

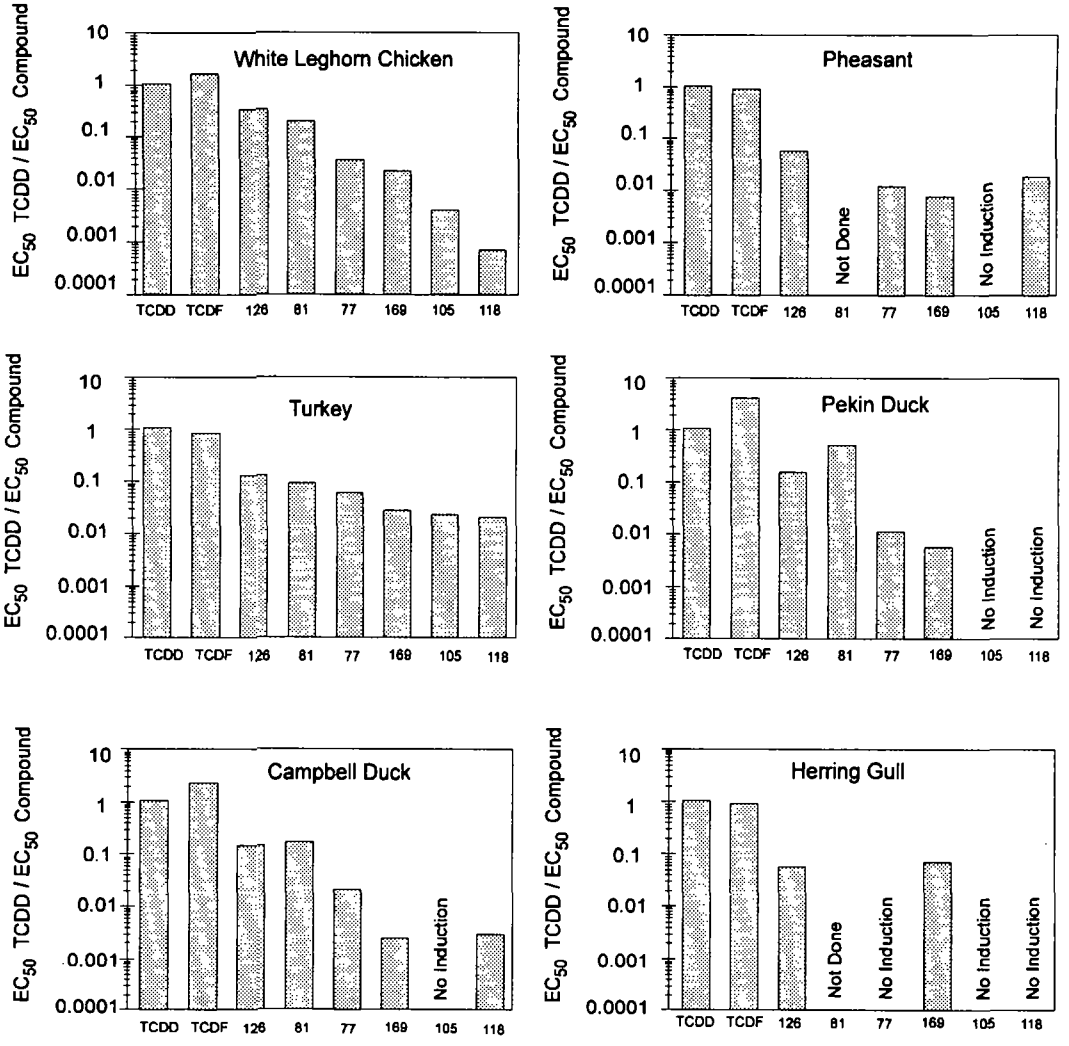

Figure 3. Relative sensitivity of primary avian hepatocyte cultures to TCDD, TCDF and PCBs.

There were large interspecies differences in sensitivity to the mono-ortho substituted PCBs, 105 and 118, relative to TCDD. For example, in White Leghorn Chicken embryo hepatocytes, PCB 118 was approximately 1000 times less potent than TCDD; in Pekin Duck and Herring Gull hepatocytes, no induction by PCB 118 was observed. In contrast, PCB 118 was approximately 50 times less potent than TCDD in Naraganset Turkey and Ring-Necked Pheasant embryo hepatocytes (Table 1, Fig. 3). PCB 105 induced EROD in White Leghorn Chicken embryo hepatocytes (approximately 250 times less potent than TCDD) and Naraganset Turkey embryo hepatocytes (50 times less potent than TCDD). PCB 105 was non-inducing in hepatocytes from other species.

Efforts are underway to attempt to develop internationally accepted toxic equivalency factors (TEFs) for PCBs, and induction of CYP1A is one of the endpoints that is used in these evaluations. Most studies have shown that the mono-ortho substituted PCBs 105 and 118 are much less toxic than TCDD, and TEFs of 0.0001 to 0.001 have been recommended. The results of the present study indicate that these PCBs are considerably more potent inducers of EROD activity in primary hepatocyte cultures prepared from some species of birds than they are in mammalian and fish hepatocyte cultures. Studies are currently underway in our laboratory to determine if interspecies differences in avian hepatocyte cultures are predictive of differences in interspecies differences in biochemical and toxic effects in ovo.

4. References

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