NEUROCHEMICAL EFFECTS OF PCBs: LIMITATIONS IN EXTRAPOLATION OF IN-VITRO EFFECTS TO WHOLE ANIMALS

Richard F. Seegal
Wadsworth Center, New York State Department of Health, and School of Public Health, The University at Albany, Box 509, Albany, NY 12201-0509, USA

1. Introduction

Polychlorinated biphenyls (PCBs) are members of a large class of persistent, widely-dispersed environmental contaminants known as halogenated aromatic hydrocarbons (HAHs) that include, in addition to PCBs, halogenated dioxins and dibenzofurans. HAHs, and in particular PCBs, alter behavior and neurochemical function in experimental animals [1,2], while epidemiological studies demonstrate a relationship between developmental exposure to PCBs and HAHs, and delays in physical development and deficits in behavior, including cognition [3,4].

Although earlier studies on the neurotoxicity of PCBs involved complex mixtures, more recent studies have shown that the structure of the PCB congener may influence its toxicity. Coplanar, and to a lesser extent, mono-*ortho*-coplanar congeners, interact with the aryl hydrocarbon (Ah) receptor, induce hepatic enzymes, alter immune function and induce structural teratogenic changes [5], while di-*ortho*-substituted, non-coplanar congeners do not. However, non-coplanar PCB congeners alter several aspects of nervous and hormonal function [6-8].

Much of the data demonstrating the differential toxicity of PCB congeners has been derived from *in-vitro* studies. Hepatoma cells have been used to determine the ability of coplanar congeners to interact with the Ah receptor and induce aryl hydrocarbon hydroxylase and cytochrome P-450s [9] while pheochromocytoma (PC12) cells [10] and cerebellar granule cells [11] have been used to investigate the neurotoxicity of structurally disparate PCB congeners. Although tissue-culture procedures have many advantages, we wish to describe limitations in the ability to extrapolate data derived from tissue-culture experiments to predict responses obtained in the whole animal. More specifically, we will compare the effects of exposure of PC12 cells to either individual PCB congeners or extracts of toxicants from Great Lakes salmon with results obtained from exposure of either the adult or developing rat to the same toxicants.

2. Methods

a. *In-vitro* Studies

Experimental procedures are described in detail in Seegal *et al.* [12]. Briefly, PC12 cells were grown in RPMI growth media supplemented with 10% horse serum and 5% fetal calf serum at 37°C in 95% O_2/5% CO_2 and seeded in 24-well culture plates. For the PCB congener experiments, cells were exposed to individual congeners in growth media containing 0.1-0.3% dimethylsulfoxide (DMSO) for 1-6 h. For studies describing the neurotoxicity of fish-borne contaminants, cells were exposed to

varying concentrations of florisil-column-fractionated, sulfuric-acid-treated hexane extracts of either Lake Ontario (LO) or Pacific Ocean (PO) salmon for 1 h. The extracts were dissolved in media containing 0.2% DMSO. Cells were harvested and cellular dopamine (DA) was determined by high-performance liquid chromatography (HPLC) with electrochemical detection. Neurochemical results are expressed as a percentage of vehicle control.

b. *In-vivo* Studies

For experiments describing the neurochemical effects of perinatal exposure to individual PCB congeners, rat dams were exposed from gestational day six through weaning to either the coplanar PCB congener #77 (3,4,3',4') at doses of 0.1 or 1 mg/(kg·day), or the non-coplanar congener #47 (2,4,2',4') at doses of 1, 10 or 20 mg/(kg·day). Male and female offspring were sacrificed on postnatal days 35, 60 or 90 and concentrations of DA were measured in the frontal cortex (FC) by HPLC. Results are expressed as ng DA per mg tissue.

For experiments describing the neurochemical effects of perinatal exposure to LO or Lake Huron (LH) salmon, female rats were fed diets containing either 5 or 20% lyophilized salmon for seventy days prior to mating. Exposure continued during pregnancy and weaning, and offspring were sacrificed when they were 90 days of age. DA concentrations were determined in the FC caudate nucleus (CN). Results are expressed as ng DA per mg tissue (FC) or ng DA per mg protein (CN); protein was determined by the method of Lowry *et al.* [13].

3. Results

a. Neurochemical Effects of Individual PCB Congeners

(i) *In-vitro* Effects

We exposed PC12 cells to more than fifty individual PCB congeners [10] and grouped them in terms of their ability to reduce cellular DA content, based on their EC_{50} values--that is the dose, expressed on a molar basis, required to reduce cellular DA content by 50%. In some cases, it was not possible to directly determine an EC_{50} and results are based on extrapolating a predicted EC_{50} from the existing dose-response data (*i.e.*, those cases in which EC_{50} values were greater than 200 μM). Representative congeners from each of the major subdivisions are presented below in Table I.

TABLE I. Representative EC_{50} Values (μM) for PCB Congener-Mediated Decreases in Cellular Dopamine Content Determined *In vitro* using Pheochromocytoma Cells in Culture

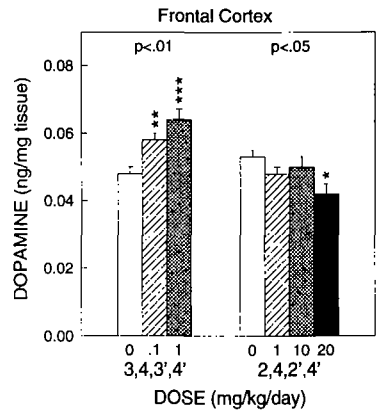
Di- and Tri-*Ortho* Congeners	EC_{50}	Mono-*Ortho* Congeners	EC_{50}	Non-*Ortho*, Mono-*Para* Congeners	EC_{50}	Non-*Ortho*, Di-*Para* Congeners	EC_{50}
2,2'	64	2	182	3,5,4'	310	4,4'	n.e.
2,4,6,2'	71	2,4,4'	196	4	335	3,4,3',4'	n.e.
2,5,2',5'	86			3,4'	410	3,4,5,3',4'	n.e.
2,5,2'	88						
2,4,2',4'	115						
2,4,6	150						
Mean	96	Mean	189	Mean	352		

n.e.=no effect observed

(ii) *In-vivo* Effects

Perinatal exposure of rats to the non-coplanar congener 2,4,2',4' significantly reduced DA concentrations in the FC (Fig. 1). In contrast, perinatal exposure to the coplanar congener 3,4,3',4' resulted in significant elevations in DA concentrations in the FC (Fig. 1) demonstrating that a coplanar congener--shown to be inactive in PC12 cells--may be a significant developmental neurotoxicant.

Figure 1



b. Neurochemical Effects of Fish-borne Contaminants

(i) *In-vitro* Effects

PC12 cells were exposed for 1 h to media containing the contaminants in: (i) a hexane-eluted florisil fraction (FRAC1) or (ii) a methylene chloride-eluted fraction (FRAC2) of the solvent extract of LO salmon at the concentrations listed in Table II. Only FRAC1 significantly reduced cellular DA content, demonstrating the usefulness of this procedure in separating a complex environmental mixture into toxicologically distinct fractions.

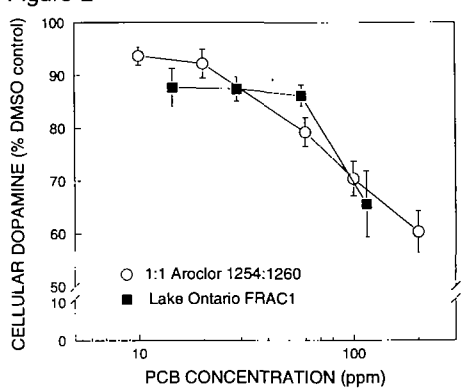
To determine whether the PCBs in FRAC1 of LO salmon were responsible for the reductions in cellular DA content, we exposed PC12 cells to varying concentrations of a 1:1 mixture of Aroclors 1254/1260 similar in its PCB congener makeup to the congeners found in LO salmon [14] and compared the reductions in cellular DA with that produced by exposure of the cells to differing amounts of the LO extract containing PCBs at the same concentration as found in the synthetic PCB mixture. The reductions in cellular DA content, induced by exposure to the Aroclor mixture, were identical to the reductions seen following exposure to the LO florisil fraction that contained similar concentrations of PCBs (Fig. 2), suggesting that the PCB congeners in the hexane-eluted florisil fraction of LO salmon were possibly responsible for these reductions.

TABLE II. Concentrations of Contaminants Media from PC12 Cells Exposed to Lake Ontario Salmon FRAC1 and FRAC2

Contaminant	FRAC1	FRAC2
PCBs[1]	198	1.77
p,p'-DDE[1]	41	n.d.
Mirex[1]	14	n.d.
Hexachlorobenzene[1]	0.28	n.d.
PCDDs[2]	n.d.	1.35
PCDFs[2]	n.d.	1.02

[1]expressed as ppm
[2]expressed as ppb
n.d.=not detected

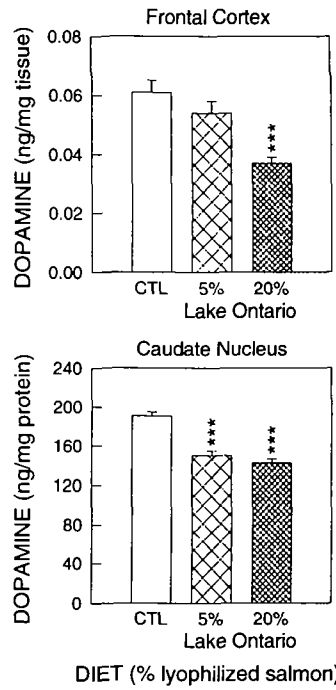
Figure 2



(ii) *In-vivo* Effects

Exposure of dams prior to gestation and during gestation and lactation to diets containing either 5 or 20% lyophilized LO or LH salmon resulted in significant reductions in DA concentrations in the FC and CN of exposed offspring tested at 90 days of age (Fig. 3).

Figure 3



On the basis of the PCB concentrations in the diets we calculated that the rats consumed as little as 17 µg/(kg·day) of PCBs, which is approximately 100 to 1,000 times lower than the amount of PCBs required to elicit similar neurochemical change [7]. The magnitude of the neurochemical changes seen following perinatal exposure to the diets containing lyophilized LO or LH salmon, combined with the low concentrations of PCBs found in the diets, strongly suggest that the neurochemical alterations are not due solely to the PCBs present in the diets but may involve other fish-borne neurotoxicants.

4. Discussion

The above data, describing the neurochemical effects of both *in-vitro* and *in-vivo* exposure to individual PCB congeners or mixtures of congeners derived from contaminated Great Lakes salmon, lead to conflicting interpretations.

First, using PC12 cells we demonstrated that only non-coplanar PCB congeners altered cellular DA content [10]. In contrast, developmental exposure of the rat to 3,4,3',4', which was inactive in PC12 cells, resulted in significant elevations in brain DA concentrations, suggesting that these latter congeners may be developmental neurotoxicants.

Secondly, both a synthetic mixture of PCB congeners and the hexane-eluted florisil fraction of contaminated Lake Ontario salmon, containing the same concentration of PCBs, induced similar decreases in PC12 cellular DA content. These results suggest that the PCB congeners in that fraction were responsible for the reductions in DA [14]. In contrast, developmental exposure of rats to diets containing either 5% or 20% lyophilized LO salmon, resulting in exposure to as little as 17 µg/(kg·day) of PCBs, significantly reduced brain DA concentrations. In order to induce significant alterations in either behavior or neurochemistry, experimenters employ PCB concentrations that range from 100 times greater than the PCB concentrations found in the lyophilized salmon diets [7]. These *in-vivo* results suggest that the neurotoxic actions of hexane extracts of putative fish-borne contaminants cannot be due solely to the PCBs present in the salmon.

What are the possible reasons for these discrepancies?

First, to the best of our knowledge, PC12 cells do not contain Ah receptors. Thus it is not surprising that coplanar congeners, whose actions require occupancy of the Ah receptor [15], would be inactive in PC12 cells.

Secondly, PC12 cells are limited in their ability to metabolize PCBs. Shain (personal communication) has shown that the reductions in cellular DA concentrations following exposure to the most potent

non-coplanar congener 2,2', are due to the parent congener. Mammals, on the other hand, readily metabolize PCB congeners [16], and metabolites of PCBs (especially hydroxylated biphenyls) accumulate in the fetus, are estrogenic [17], and alter binding of tetra-iodothyronine (T4) to transthyretin, the major T4 transporter in the rat [18]. Thus, the limited ability of PC12 cells to metabolize PCB congeners may also contribute to differences in results vis à vis those obtained in the whole animal.

Thirdly, as suggested above, PCBs may act as 'endocrine disrupters' altering gonadal and thyroid hormone function in the rat [8,19]. In turn, alterations in these hormones, particularly during development, can alter neurochemistry [20,21]. Given the lack of steroid or thyroid hormone receptors in PC12 cells [22], this test system would appear to be a poor choice to investigate possible PCB/hormone effects on neurochemical function.

Finally, although we have not presented the data here, PC12 cells are insensitive to exposure to fish-borne contaminants including DDE, mirex, methylmercury and chlordane pesticides [14]. However, exposure of the developing rat to these contaminants can alter both behavior and neurochemistry [23].

The relative inability to extrapolate neurochemical effects of PCB exposure, obtained in tissue culture, to the whole animal is not restricted to biogenic amine neurotransmitters or PC12 cells. Kodavanti and Tilson, using cerebellar granule cells, examined changes in protein kinase C (PKC) and intracellular Ca^{2+} concentrations, and demonstrated a structure-activity relationship remarkably similar to that seen in PC12 cells for DA inhibition (*i.e.*, only non-coplanar PCB congeners were active). However, following developmental exposure of the rat to the coplanar congener, 3,4,5,3',4', these investigators observed significant alterations in PKC and intracellular Ca^{2+} (personal communication).

In conclusion, despite many advantages, the use of tissue-culture procedures to determine the neurotoxicity of individual PCB congeners and complex mixtures of environmental contaminants may lead to results that are contrary to those seen in the developing organism. Differences in rates of metabolism and the presence or absence of Ah receptors may play important roles in limiting the ability to extrapolate from the less complex *in-vitro* system to the more complex *in-vivo* preparation. These limitations should be kept in mind when examining data derived from tissue-culture systems that are used to define mechanisms of action and determine the risk of human exposure to environmental contaminants.

ACKNOWLEDGEMENT: Research support for this work was provided in part by GLPF grant FG291-1185 and NIH grant ES04913 to Richard F. Seegal.

5. References

[1] Seegal, R.F., B. Bush and K.O. Brosch (1991): Comparison of effects of Aroclors 1016 and 1260 on nonhuman primate catecholamine function. Toxicology 66, 145-163.
[2] Schantz, S.L., E.D. Levin and R.E. Bowman (1991): Long-term neurobehavioral effects of perinatal polychlorinated biphenyl (PCB) exposure in monkeys. Environ.Toxicol.Chem. 10, 747-756.
[3] Rogan, W.J., B.C. Gladen, K.L. Hung, S.L. Koong, L.Y. Shih, J.S. Taylor, Y.C. Wu, D. Yang, N.B. Rogan and C.C. Hsu (1988): Congenital poisoning by polychlorinated biphenyls and their contaminants in Taiwan. Science 241, 334-336.
[4] Jacobson, J.L., S.W. Jacobson and H.E.B. Humphrey (1990): Effects of in utero exposure to polychlorinated biphenyls and related contaminants on cognitive functioning in young children. J.Pediatr. 116, 38-45.

[5] Safe, S.H. (1994): Polychlorinated biphenyls (PCBs): Environmental impact, biochemical and toxic responses, and implications for risk assessment. CRC Crit.Rev.Toxicol. 24, 87-149.
[6] Seegal, R.F., B. Bush and W. Shain (1990): Lightly chlorinated ortho-substituted PCB congeners decrease dopamine in nonhuman primate brain and in tissue culture. Toxicol.Appl.Pharmacol. 106, 136-144.
[7] Brouwer, A., U.G. Ahlborg, M. van den Berg, R.F. Seegal, *et al.* (1995): Functional aspects of developmental toxicity of polyhalogenated aromatic hydrocarbons in experimental animals and human infants. Eur.J.Pharmacol. 293, 1-40.
[8] Ness, D.K., S.L. Schantz, J. Mostaghian and L.G. Hansen (1993): Effects of perinatal exposure to specific PCB congeners on thyroid hormone concentrations and thyroid histology in the rat. Toxicol.Lett. 68, 311-323.
[9] Parkinson, A., L. Robertson, L. Safe and S. Safe (1980): Polychlorinated biphenyls as inducers of hepatic microsomal enzymes: Structure-activity rules. Chem.Biol.Interact. 30, 271-285.
[10] Shain, W., B. Bush and R.F. Seegal (1991): Neurotoxicity of polychlorinated biphenyls: Structure-activity relationship of individual congeners. Toxicol.Appl.Pharmacol. 111, 33-42.
[11] Kodavanti, P.R.S., T.R. Ward, J.D. McKinney and H.A. Tilson (1996): Inhibition of microsomal and mitochondrial Ca^{2+}-sequestration in rat cerebellum by polychlorinated biphenyl mixtures and congeners: Structure-activity relationships. Arch.Toxicol. 70, 150-157.
[12] Seegal, R.F., K.O. Brosch, B. Bush, M. Ritz and W. Shain (1989): Effects of Aroclor 1254 on dopamine and norepinephrine concentrations in pheochromocytoma (PC-12) cells. Neurotoxicology 10, 757-764.
[13] Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall (1951): Protein measurement with the Folin phenol reagent. J.Biol.Chem. 193, 265-275.
[14] Seegal, R.F., B. Bush, P. O'Keefe and W. Shain (1995): In vitro neurotoxicity of Great Lakes fish-borne contaminants. Great Lakes Res.Rev. 1, 31-35.
[15] Poland, A., E. Glover and A.S. Kende (1976): A stereospecific, high affinity binding of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin by hepatic cytosol: Evidence that the binding species is the receptor for induction of aryl hydrocarbon hydroxylase. J.Biol.Chem. 251, 4936-4946.
[16] Sundstrom, G., O. Hutzinger and S. Safe (1976): The metabolism of chlorobiphenyls - A review. Chemosphere 5, 267-298.
[17] Korach, K.S., P. Sarver, K. Chae, J.A. Mclachlan and J.D. McKinney (1988): Estrogen receptor-binding activity of polychlorinated hydroxybiphenyls: conformationally restricted structural probes. Mol.Pharmacol. 33, 120-126.
[18] Morse, D.C., E. Klasson Wehler, M. van de Pas, A.T.H.J. de Bie, P.J. van Bladeren and A. Brouwer (1995): Metabolism and biochemical effects of 3,3',4,4'-tetrachlorobiphenyl in pregnant and fetal rats. Chem.Biol.Interact. 95, 41-56.
[19] Jansen, H.T., P.S. Cooke, J. Porcelli, T.-C. Liu and L.G. Hansen (1993): Estrogenic and antiestrogenic actions of PCBs in the female rat: in vitro and in vivo studies. Reprod.Toxicol. 7, 237-248.
[20] Vaccari, A., Z.L. Rossetti, G. de Montis, E. Stefanini, E. Martino and G.L. Gessa (1990): Neonatal hypothyroidism induces striatal dopaminergic dysfunction. Neuroscience 35, 699-706.
[21] Simerly, R.B. (1989): Hormonal control of the development and regulation of tyrosine hydroxylase expression within a sexually dimorphic population of dopaminergic cells in the hypothalamus. Mol.Brain Res. 6, 297-310.
[22] Lustig, R.H., P. Hua, W. Yu, F.J. Ahmad and P.W. Baas (1994): An in vitro model for the effects of estrogen on neurons employing estrogen receptor-transfected PC12 cells. J.Neurosci. 14, 3945-3957.
[23] Cuomo, V., L. Ambrosi, Z. Annau, R. Cagiano, N. Brunello and G. Racagni (1984): Behavioural and neurochemical changes in offspring of rats exposed to methyl mercury during gestation. Neurobehav.Toxicol.Teratol. 6, 249-254.