A REVIEW OF THE NEUROTOXICITY OF NON-DIOXIN-LIKE POLYCHLORINATED BIPHENYLS

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It is perhaps both presumptuous and somewhat nostalgic for me to present research findings from both myself and others describing the changes in central nervous system (CNS) function that occur following exposure to non-dioxin-like polychlorinated biphenyls (PCBs) at the 2004 Dioxin meeting. Presumptuous because my presentation occurs during this meeting where the majority of the research is concerned with the consequences of exposure to dioxins—the most toxic substance produced by man—and nostalgic because, in 1992, I presented some of my earliest work demonstrating that non-dioxin-like PCBs were capable of significantly altering neurochemical function[1].

Much has changed in the intervening twelve years. Laboratories in the United States and Europe now routinely study and describe the neurotoxicological effects of developmental and *in vitro* exposure to non-dioxin-like PCBs. My task will be to briefly describe the highlights of that research and suggest additional approaches that will hopefully increase our ability to understand the mechanisms of action of non-dioxin-like PCBs on neuronal and behavioral function in populations of exposed humans.

Effects of PCB Exposure on Dopamine Function

The neurotransmitter system that has been most studied following exposure to PCBs is dopamine (DA). Early work by Seegal and co-workers demonstrated that exposure of adult rats to commercial mixtures of PCBs (Aroclor 1254, Aroclor 1260) significantly reduced concentrations of biogenic amines (DA, norepinephrine, serotonin)[2-4]. Similar studies conducted in adult non-human primates demonstrated reductions in basal ganglia and frontal cortical DA. These changes persisted for months after exposure ceased, even when serum levels of PCBs were significantly reduced due to metabolism and excretions[5]. In addition, significant reductions in the number of tyrosine hydroxylase (TH+) positive neurons in the substantia nigra of rodents and non-human primates exposed to PCBs[6,7]. Indeed, these results provided the biological basis for an ongoing epidemiological study of aging former capacitor workers who had been exposed to PCBs at such high levels that the geometric mean of their sera PCB concentrations was 300 ppb—a level 100-fold higher than seen in a control population[8].

We also demonstrated that non-dioxin-like PCBs significantly reduced DA concentrations in pheochromocytoma (PC12) cells[9]. Most importantly, these authors also demonstrated a strict structure-activity-relationship (SAR) in which lightly chlorinated non-dioxin-like congeners, but not dioxin-like congeners, reduced PC12 DA concentrations (see Table 1).

Table 1: Representative EC50 Values for PCB-Congener-Mediated Decreases in Cellular Dopamine
Content Determined *In vitro* Using Pheochromocytoma (PC12) Cells in Culture

Non-Dioxin-Like Congeners				Dioxin-Like Congeners			
Di-*Ortho*		Mono-*Ortho*		Mono-*Para*		Di-*Para*	
Congener	EC50	Congener	EC50	Congener	EC50	Congener	EC50
2,2'	64	2,4,6	150	3,5,4'	310	4,4'	no effect
2,4,6,2'	71	2	182	4	335	3,4,3',4'	no effect
2,5,2',5'	86	2,4,4'	196	3,4'	410	3,4,3',4',5'	no effect
2,5,2'	88						
2,4,2',4'	115						

Data selected from Table 1 in Shain *et al.*, *Toxicol. Appl. Pharmacol.* **111**, 33-45, 1991.

Developmental exposure of rodents to PCBs also alters DA function in the CNS. Surprisingly however, coplanar PCB congeners, shown to be inactive in tissue culture preparations, significantly elevate DA concentrations in the frontal cortex and basal ganglia of rats exposed during gestation and lactation[10]. These unexpected findings—that coplanar PCB congeners alter central DA function during development—provide evidence that, at least for these measures, coplanar congeners are true neuroteratogens. That is, the congeners are devoid of activity when administered to adult animals, but alter central neurochemical and behavioral function[11] when administered during critical periods of development. Subsequently, Seegal *et al.* have shown that these coplanar congeners, when administered either during development or to prepubertal rats, significantly elevate uterine weights demonstrating that either the congeners or their metabolites are estrogenic (see Table 2)[12] . Thus, exposure during critical periods of development to estrogenic-like PCB congeners is capable of altering central DA function and, perhaps, contributing to behavioral changes seen in rats exposed to coplanar congeners during development.

Table 2: PCB-Congener-Induced Alterations in Uterine Wet Weight—A Measure of Estrogenicity—Determined In Prepubertal Female Rats Tested at the Maximum Tolerated Dose
(Percent of Control Uterine Wet Weight to Body Weight Ratio)

Non-Dioxin-Like Congener	Dose	% of Control	Dioxin-Like Congener	Dose	% of Control
2,4,2',4'	32 mg/kg	91	3,4,3',4'	27 mg/kg	166
			3,4,5,3',4'	400 µg/kg	170

Non-Dioxin-Like PCB Congener Effects on Regulation of Intra-Neuronal Calcium

One of the most active areas of PCB research concerns the effects of non-dioxin-like PCB congeners on regulation of neuronal calcium. The reason for this emphasis is the central role that calcium plays in regulating neuronal function including influencing: (i) exocytosis of neurotransmitter upon nerve terminal depolarization[13]; (ii) second messenger systems, including cAMP and protein kinases[14], (iii) neuronal growth and synaptogenesis[15] and (iv) formation of ROS and disruption of mitochondrial function[16]. There are two posited mechanisms by which non-dioxin-like congeners alter intra-neuronal calcium concentrations.

Firstly, researchers at the USEPA, used cultured rat cerebellar granule cells and demonstrated that a non-dioxin-like congener (2,2'-dichlorobiphenyl), at high micromolar concentrations, elevated cytosolic calcium concentrations and decreased uptake of radio-labeled calcium into cerebellar granule cell mitochondria and microsomes[17]. The same group also demonstrated that non-dioxin-like congeners increased phorbol ester binding to PKCCa measure of activation and translocation of protein kinase C in rat cerebellar granule cells[18]. Indeed, in that manuscript, Kodavanti *et al.* also confirmed the SAR reported by Shain, Bush and Seegal[9]. The similarity of the SARs between the two studies suggest that, despite the highly different measures employed, non-dioxin-like PCB congeners alter signal transduction pathways that influence both intra and inter-neuronal signaling pathways. The mechanisms responsible for the elevations in intracellular calcium were further investigated by Shafer *et al.* who demonstrated that non-dioxin-like congeners increased IP_3 binding to receptors on cerebellar granule cell microsomes and either enhanced release of microsomal calcium stores or further inhibited cytosolic calcium sequestration[19].

The second mechanism by which non-dioxin-like PCB congeners can influence intracellular calcium concentrations has been investigated by Pessah and coworkers. These investigators demonstrated that low micromolar concentrations of non-dioxin-like congeners increased calcium concentrations in microsomes from skeletal, cardiac and brain tissue[20,21] by a ryanodine receptor (RyR) dependent mechanism that involves receptor activation and an increase in the open probability of the ryanodine channel[22]. In addition, Wong and Pessah demonstrated that non-dioxin-like PCB congeners dose dependently both induced release and inhibited uptake of calcium from sarcoplasmic/endoplasmic reticulum by a immunophilin-based mechanism (*i.e.*, rapamycin and FK506 disassociated the immunophilin FKBP12 from the RyR, resulting in a significant reduction in the ability of non-dioxin-like congeners to elevate intra-cellular and intra-organelle calcium concentrations)[23].

Although there is evidence that both IP_3 and RyR mechanisms play a role in the non-dioxin-like congener-induced elevations in intracellular calcium concentrations, the much lower concentrations of non-dioxin-like congeners (as low as 400 nanomolar) needed to alter calcium regulation by a RyR- dependent mechanism strongly suggests that this is most likely the initial, and perhaps, most important event leading to alterations in intracellular calcium concentrations.

Non-Dioxin-Like PCB Inhibit Monoamine Transporters and Induce Oxidative Stress

A biologically plausible model for the reductions in DA concentrations and the number of TH+ neurons reported above has been suggested by Fonnum and co-workers and elaborated on by Bemis and Seegal[24]. Fonnum and co-workers demonstrated that non-dioxin-like PCB congeners, but not coplanar PCB congeners, inhibit uptake of labeled DA by both the dopamine transporter (DAT) and the vesicular monoamine transporter (VMAT)[25]. These transporters are responsible, respectively, for the uptake of newly released DA from the synaptic cleft to the cytosol and uptake of DA from the neuronal cytosol into the synaptic vesicle. In turn, reductions in the ability to adequately regulate DA storage may reduce DA synthesis by activating synthesis inhibiting autoreceptors; lead to the formation of reactive oxygen species (ROS) by increasing free concentrations of cytosolic DA and ultimately result in neuronal dysfunction and cell death.

The relative importance of PCB-induced inhibition of DAT versus VMAT, on regulation of synaptosomal DA concentrations, was recently reported by Bemis and Seegal[24]. In that study they demonstrated that the VMAT was found to play a much larger role than the DAT in regulating tissue DA after PCB exposure. Furthermore, elevations in free cytosolic DA lead to formation of hydrogen peroxide and reactive intermediate metabolites of DA[26] and subsequent neuronal dysfunction. Hence, the observations of reductions in the number of TH+ neurons in the substantia nigra of PCB exposed non-human primates may reflect cell death mediated initially by increases in free cytosolic DA.

ROS formation following exposure to non-dioxin-like PCB congeners is not limited to DA-containing cells. Ganey and co-workers have demonstrated that exposure of neutrophils to either Aroclor 1242 (which contains many lightly chlorinated non-dioxin-like congeners) or the non-dioxin-like congener 2,4,2',4'-tetrachlorobiphenyl inhibited Cu/Zn superoxide dismutase activity resulting in significant elevations in superoxide anion production[27]. Finally, Robertson and co-workers have shown that non-dioxin-like congeners, susceptible to metabolism to semi-quinone intermediates, react with oxygen to form quinones and superoxide anion[28].

What=s Needed?

A neurologist with whom I work closely, and whose speciality is movement disorders, frequently states that >everything works in the test tube, almost nothing works in the clinic=. A similar statement can be made when referring to understanding the putative mechanisms of action of PCBs. Using only *in vitro* techniques, questions have been, and continue to be raised, concerning the relevance of these findings to understanding the actions of these contaminants in altering complex behaviors, including learning and memory. In my estimation an important goal that has not been adequately addressed is the need to increase the ability to extrapolate from simpler to more complex neuronal systems.

A second area that needs to be, in my opinion, further addressed is an emphasis on studies of the neurotoxicity of complex mixtures. Many current studies use either Aroclor mixtures or individual PCB congeners, both of which are rarely or never found in the environment. Instead, I suggest using either environmentally relevant mixtures of PCB congeners or mixtures of PCBs and other toxicants present in contaminated food products that constitute the major vector for exposure. An outstanding example of the use of environmentally relevant mixtures of PCB congeners is the study of Hmong and Laotian emigres in Green Bay, Wisconsin (NIEHS Centers for Children=s Environmental Health and Disease Prevention Research; FRIENDS Childrens= Environmental

Health Center at the University of Illinois at Urbana/Champaign. In this multi-institutional study, laboratory-based investigator use a mixture of PCB congeners nearly identical to that found in fish caught and consumed by those recent emigres. This reasoned approach increases the ability to relate laboratory-based findings to those obtained in concurrent epidemiological studies.

Finally, I suggest that more effort be devoted to the study of the neurological effects of PCBs and other environmental and occupational neurotoxicants in aging human populations. Have we not devoted sufficient resources and time to the study of the developmental consequences of these toxicants? Aging >baby boomers= constitute a large and growing portion of the population of the United States and many European countries. The consequences of either current or past exposure to neurotoxicants and their putative interactions with aging processes demand further investigation.

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