# A NEW GENERATION OF ENVIRONMENTALLY RELEVANT PCBs: STRUCTURE-ACTIVITY, MOLECULAR MECHANISMS, AND DEVELOPMENTAL NEUROTOXICITY

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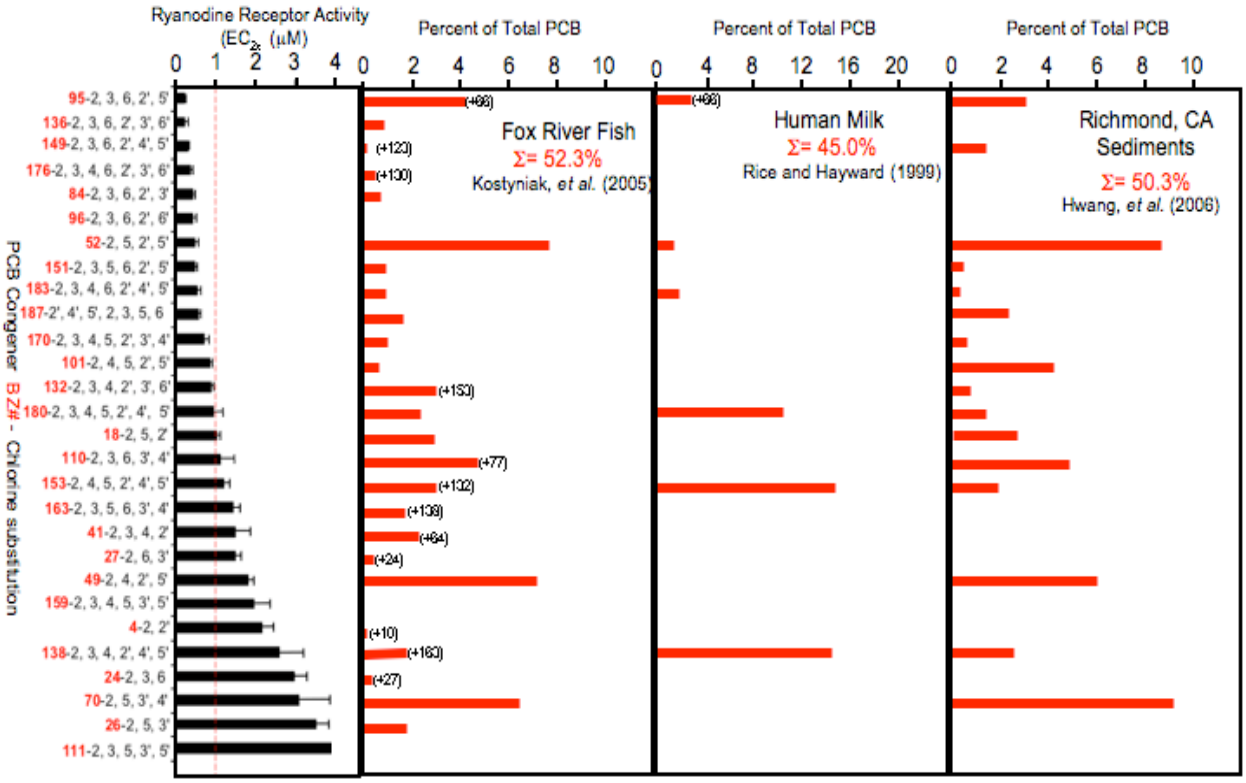
**PCBs Congeners of Current Concern to Human Health**

Certain dioxin-like polychlorinated biphenyl (PCB) congeners have been shown to cause toxicity similar to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), the most potent congener in the polychlorinated dibenzo-*p*-dioxin (PCDD) family. The toxicity responses include chloracne, wasting syndrome, increase incidence of soft tissue sarcomas, immunotoxicity, reproductive toxicity, developmental toxicity, disruption of endocrine pathways, hepatotoxicity, and thymic and splenic atrophy. These toxic responses might be mediated through a common pathway, which involves binding of HAHs to the Ah receptor. Among the structures examined, TCDD has been shown to have the highest affinity toward the Ah receptor (Denison *et al.,* 2003). As a result, relative toxicity of dioxin-like PCB, PCDD and polychlorinated dibenzofurans (PCDF) congeners have been ranked according to their relative binding affinity to the Ah receptor. World Health Organization held a meeting to derive consensus on toxicity equivalency factors (TEFs) for PCDDs, PCDFs and dioxin-like PCBs for risk assessment on human, fish and wildlife health (Van den Berg *et al.*, 1998). The TEF concept is based on a major assumption: at low dose, toxic equivalent (TEQ) concentration of HAHs in mixtures is additive, with no synergism nor antagonism among the individual congeners. However, several limitations on the TEF concepts have been identified (Safe *et al*., 2002). PCBs possessing two or more *ortho*-chlorine substitutions are very weak or completely lack AhR-binding activity. It is these *ortho*-substituted non-coplanar PCB that are potent sensitizers of ryanodine-sensitive $Ca^{2+}$ channels (ryanodine receptors; RyRs). Thus one significant limitation of the TEQ-based risk assessment is that it does not account for health risk factors contributed by exposures to persistent organic pollutants having non-coplanar structures. Figure 1 shows the structure-activity relationship for 28 *ortho*-substituted PCBs at the type 1 ryanodine receptor (RyR1). Plotted in Figure 1 is a selection of PCBs which double RyR1 channel activity at a final concentration <5 ~~mM~~ μM. The 2,3,6-Cl PCB configuration is most important for optimal recognition by the RyR1 complex and/or critical for sensitizing its activation. *para*-Substitution(s) diminish activity with *para*-chloro having higher potency than the corresponding *para*-hydroxy derivative. Addition of a more bulky *para*-methyl-sulfonyl group was found to eliminate activity toward RyR1 (Pessah *et al.*, 2006), indicating the importance of the para-positions in binding and sensitizing RyR1 channel activation.

Congener profiles have been elucidated for several environmental and tissue samples. In fact, it is the more lightly chlorinated *ortho*-chloroinated congeners that currently predominate in biological and environmental samples (Hansen 1998). The composition of three published PCB profiles for which RyR1 channel structure-activity data are available are summarized in Figure 1. It is important to note that the most potent PCB congeners known to sensitize RyR1 channels are

among the most abundant congeners found in human milk (Rice and Hayward, 1999), game fish (Kostyniak et al 2005), and San Francisco Bay sediments (Hwang *et al.,* 2006).




Interestingly the total composition of RyR1-active congeners ranges from at least 45 to 52 percent of the total PCBs in these samples (Fig 1). Thus *ortho*-substituted PCB congeners collectively represent a significant component of PCB mixtures found in environmental samples and biological tissues. It is therefore necessary to develop new approaches for risk assessment of *ortho*-substituted PCB congeners based on critical mechanisms responsible for initiating dysfunction leading to toxicity. This is especially important since several epidemiological studies have indicated that environmental exposures to PCBs during pre- and post-natal development impairs cognitive and behavioral functions in children (Safe 1994; Jacobson and Jacobson, 1996; Schantz *et al.,* 2003) and adults (Schantz *et al.,* 2001).

**Developmental Neurotoxicity of Non-coplanar PCBs**

Because of their common occurrence in biological and environmental samples and their high RyR activities, we studied PCB 95 and PCB 170 for their ability to alter synaptic transmission in the hippocampal slice preparation. Field excitatory postsynaptic potentials (*f*EPSP) were evoked by single pulse stimulation of Schaffer Collateral/commissural fibers at striatum radiatum of the CA1 region, hippocampal slice. Following exposure to 100 nM PCB 170, time-dependent changes in the slope and amplitude of *f*EPSP were seen, with phases of enhancement and depression. To investigate the contribution of inhibitory neurons in the actions of PCB 170, hippocampal slices were pre-treated with the GABAa receptor antagonist,

picrotoxin (PTX, 100 ~~nM~~). Pre-treatment with PTX resulted in negligible change in EPSP slope elicited by single pulse stimuli. Importantly PCB170 (1-100 nM) introduced in the presence of GABAa block produced sustained facilitation of synaptic transmission (>250% of initial EPSP slope) and pronounced after potential discharges. By contrast, perfusion of ACSF containing 10 nM PCB 95 alone produced a sustained increased *f*EPSP slope reaching 200% of baseline that was not further enhanced the inclusion of PTX. These effects of PCB 95 were blocked by the RyR antagonist dantrolene.

Based on these electrophysiological results we investigated whether PCB 95 alters seizure threshold *in vivo* using two models; flurothyl (bis-2,2,2-triflurothyl ether) induced seizures and pentylenetetrazole (PTZ) - induced kindling. Lactating dams were exposed to PCB 95 (oral 1 mg/kg/day) between PND 0 and PND21. Offspring were tested for onset time for myoclonic jerk and tonic-clonic seizure in response to flurothyl application (20 ml/min) to the test chamber. Onset time of myoclonic jerk was significantly shorter ($p<0.05$) in PCB95 exposed animals. PTZ (30 mg/kg, i.p. every 48 hrs) and rats were scored according to their seizure response (0~5) for 30 min after the PTZ challenge. Animals exposed to PCB 95 kindled significantly faster than control the group ($P<0.05$ on day 5). Altered seizure susceptibility was tightly correlated with elevated activity of RyRs in microsomal preparations from treated animals. These results provide evidence that lactational exposure to non-coplanar PCB can significantly alter hippocampal excitability and seizure thresholds, and implicate RyRs in the process. Considering the important role contributed by RyRs within the peripheral and central nervous systems (Pessah and Wong, 2003), and the potency of environmentally prelevant PCBs toward sensitizing their functions, RyR activity could serve as a biomarker of exposure and potential risk. The data also suggest that individuals possessing impairments in GABAergic signaling may be especially susceptible to excitotoxicity of non-coplanar PCBs.

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