Mode of Action of dioxin-like versus non-dioxin-like PCBs

Greet Schoeters[1], Linda Birnbaum[2]

[1]VITO (Flemish Institute for Technological research), Department of Environmental Toxicology, and University of Antwerp
[2]United States Environmental Protection Agency, Experimental Toxicology Division, National Health and Environmental Effects Research Laboratory,

Introduction:

Exposure of humans to polychlorinated biphenyls has been associated with different adverse effects such as immune impairment, changes in hormone levels, reproductive and neuropsychological changes and cancer. It is difficult to attribute the observed effects to either dioxin-like , non-dioxin-like PCBs or to both. All known human exposures are mixed, comprising dioxin and non-dioxin like PCB congeners as well as dioxins and furans. The purpose of this work was to evaluate, based on mechanistic data available in the open literature, whether non-dioxin like PCBs (NDL-PCBs) themselves may pose specific health risks.

It is clear that dioxin and NDL-PCBs differ in the spectrum of metabolizing enzymes they induce, but the mechanistic links to health of these biochemical changes remain unclear at the moment. NDL-PCBs also cause immunotoxicity and tumor promotion via different mechanisms than do dioxin-like PCBs. We focus on neurotoxicity which has been associated with developmental exposure to PCBs and which is considered as one of the most sensitive adverse health effects [1].

Methods:

To identify specific risks, we looked at 1) hazard or specific working mechanisms of individual congeners and 2) their potency relative to dioxin –like compounds. We examined *in vitro* studies using, single congeners and identified the biological endpoints which may be related to the neurotoxicity of PCBs, and then confirmed whether the *in vitro* findings were also observed in animal experiments .

Results and discussion:

Possible sites and mechanisms of neurotoxic action have been described for both non – dioxin and dioxin- like PCBs. We can distinguish direct effects of PCBs on neuronal cells and indirect effects on neuronal development and functioning through interference with hormone levels .

Direct neurochemical toxicity of non-dioxin –like PCBs: mechanistic studies *in vivo* and *in vitro* have shown that ortho substituted, non coplanar PCB congeners, having little or no dioxin like activity, can have direct effects on nervous tissue. We have identified at least four different mechanisms through which non-dioxin like PCBs can be neurotoxic.

Interference with signal transduction pathways: In vitro studies with neuronal cells and brain subcellular fractions showed that PCBs interfere with intracellular sequestration mechanisms of calcium and increase the activation of protein kinase C (PKC), thereby altering intracellular concentrations of calcium [2] . The most potent congeners (e.g., PCB4 , PCB52 , and PCB104) had multiple *ortho* chlorines, whereas congeners without *ortho*chlorines tended to have either no or lower activities [3].

These findings may be relevant for neurological functioning. Perturbations in calcium homeostasis have been shown to affect neuronal outgrowth and sustained increases in intracellular calcium have been associated with cell injury. The PKC signaling pathway has been implicated in the modulation of motoric behaviour as well as learning and memory. *In vivo* experiments in which Aroclor 1254 was administered perinatally were in line with the in vitro findings: perturbations in calcium homeostasis and changes in PKC activities were observed in the cerebellum and hippocampus of the post natal rat brain [4]

Neurotransmittersystems: One of the most important findings are the changes that are observed in the levels of dopamine. *In vitro* decreased cellular levels of dopamine are observed in cultured pheochromocytoma cells, which synthesize, store, release, and metabolize dopamine in a manner similar to the intact mammalian central nervous system[5] . Structure-activity studies of 50 PCB congeners in the pheochromocytoma *in vitro* system found that the most active congeners had at least two *ortho* chlorines (e.g., PCB50 , PCB52 and PCB48) and that congeners that were relatively strong Ah receptor agonists (e.g.,PCB77) were inactive or had minimal effects on dopamine levels [6]. The lower levels of dopamine have been attributed to decreased synthesis and uptake of dopamine in vesicles. Again, only the ortho-chlorinated biphenyls showed that activity while the non-ortho substituted congeners that were tested had no potency [7] . *In vivo* results confirmed *in vitro* findings. Reduced dopamine levels were observed *in vivo* in brain tissue from adult nonhuman primates (*Macaca nemestrina*) which were daily exposed to Aroclor 1016, for 20 weeks , only three PCB congeners were detected (PCB28 , PCB47 and PCB52), suggesting that nonplanar PCBs, which are poor Ah receptor agonists, may have been responsible for the effect [8]. In contrast to non-dioxin like PCBs, the dioxin-like PCB77 was shown to increase the dopamine concentrations in the frontal cortex and substantia nigra following perinatal exposure in rats [9] .

Changes in concentrations of biogenic amines in regions of the brain such as the basal forebrain and the hippocampus may affect brain functions such as learning and memory.

Also choline acetyltransferase (ChAT) levels were reduced in the hippocampus and fore brain of rat pups either prenatally or lactationally exposed to PCBs [10] . However this may be due to effects of PCBs on thyroid because cholinergic fibers are particularly sensitive to thyroid hormone deficiency.

*Apoptosis:*Apoptosis is essential for normal brain development. Perturbation of normal spatiotemporal patterns of apoptosis can cause persistent neural deficits.

Apoptosis has been observed in primary cultures of rat hippocampal neurons subsequent to activation of the ryanodine receptor and increased production of reactive oxygen species. Coplanar PCB 77 was not able to induce apoptosis in any of the cultures [11] Formation of reactive oxygen species has also been observed in cultured rat cerebellar granule cells exposed to Aroclor 1254 ,

Aroclor 1242 or PCB153. Cell death and increased ROS formation was mediated through the N-methyl-D aspartate receptor [12]

*Arachidonic acid release:*PCB mixtures such as Aroclors 1016 and 1254 have been shown to stimulate release of arachidonic acid by cerebellar granule cells *in vitro*, in a concentration-dependent manner through mediation of phospholipase A(2) activity. The latter activity has been associated with learning and memory, and arachidonic acid has been identified as a second messenger involved in synaptic plasticity [13] .

PCBs affect brain functioning and development through the neuro-endocrine axes

Interference of PCBs with hormones such as sex-steroids, thyroid hormones and with retinoic acid has been described . The effects may be due to PCB regulation of CYP oxygenases that activate or deactivate different endogenous steroid hormones, to interference of PCBs with the hormone receptor or with the hormone transport protein.

Using *in vitro* cellular assays, both estrogenic activity as well as anti-estrogenic activity have been observed for NDL-PCBs and hydroxylated metabolites (HO-PCBs) of lower chlorinated non-planar PCBs. Structure activity relationships were complex and differed from one assay to another [14] . *In vivo* animal studies, using single congeners, confirmed the *in vitro* findings. Increases in uterine weight, changes in estrogen and progesterone receptors were observed after exposure to non-dioxin like PCBs and hydroxylated PCBs. However, dioxin like PCBs showed similar changes and were more potent : LOAEL in rats of 0.016 mg PCB126 /kg.day versus a LOAEL of 8 mg PCB18 /kg.day for the same endpoint [15 16] .

Recent *in vitro* findings suggest that NDL- PCBs may also interfere with the binding of testosterone with the androgen receptor at non – cytotoxic doses. 30% (by weight) of the **PCBs** in human milk are androgen antagonists (**PCBs** 66, 74, 105, and 118) and a further 25% are partial antagonists (P**CBs** 138, 153, and 156) [17] . *In vivo*, effects on male reproductivity have been observed with different Arochlor mixtures. But also dioxin-like PCB77 lowers testosterone levels in WISTAR rats with a LOAEL of 0.1 mg/kg.day [18].

The effects of PCBs on thyroid hormone status appear to involve Ah-receptor mediated actions as well as actions that appear to be independent of the Ah receptor. NDL-PCBs and HO-PCBs may affect thyroid hormone status by inhibiting the binding of T4 to transthyretin, which is an important transport protein for both T4 and T3 [19] ,some HO-PCBs are potent inhibitors of thyroid hormone sulfation [20] . DL-PCBs may affect the concentration of thyroid hormones mainly by enhancing the metabolism of thyroid hormones, through activation of phase II enzyme UDP-glucuronosyltransferase, which is co-induced with CYP1A1 [21] . *In vitro* results were in line with *in vivo* findings showing reduced T4 levels after exposure to NDL-PCBs, HO-PCBs and sulfonated PCBs. However DL-PCBs were more potent: PCB126 induced reduced levels of T4 with a LOAEL of 0.25 µg/kg.day following exposure of pregnant rats [22]. Changes in plasma levels of TT4,FT4,TT3 may be related to hypothyroidism in fetal and early prenatal life which may result in profound effects on the developing brain including hearing deficits [23]

PCBs have also been shown to decrease the aromatase activity in the brain of newborn male rats after maternal exposure of rats to a PCBmixture reconstituted according to the congener pattern found in human breast milk [24] . During critical developmental periods, changes in aromatase activity may result in morphological and functional changes in several brain regions, altering sex-dependent neurobehaviour.

Conclusion:

Dioxin and non-dioxin like PCBs affect directly neuronal cells but through different mechanisms. Non-dioxin like PCBs have been shown to reduce dopamine neurotransmitter levels , this was not seen with DL-PCBs. Interference with calcium homeostasis, cytotoxicity associated with increased production of reactive oxygen species, apoptosis and enhanced arachidonic acid release were observed with both DL-PCBs and NDL-PCBs . However NDL-PCBs were more potent if tested in the same systems.

Both dioxin and non-dioxin like PCBs, as well as some hydroxymetabolites were shown to interfere with endocrine systems (steroids, retinoic acid, thyroid). They can act on multiple endpoints and through these mechanisms impair neurological development and functioning. DL-PCBs act through a different mechanism than NDL-PCBs, with DL- PCBs more potent than NDL-PCBs .

In vivo animal studies showed that PCB interference with thyroid function is one of the most sensitive endpoints which may affect neurobehaviour.

Risk of NDL-PCBs to induce adverse neurodevelopmental effects thus depends on the concentration of the congeners in the brain tissue relative to the concentration of DL-PCBs, taking into account that the latter are more potent. The importance of PCB induced changes in dopamine levels needs further exploration , as disfunctioning dopamine systems may be associated to specific behaviour changes such as attention-deficit/hyperactivity disorder in man [25]

(This abstract does not reflect Agency policy.)

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