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MOLECULAR MECHANISMS INVOLVED IN THE TOXIC EFFECTS OF POLYCHLORINATED BIPHENYLS (PCBs) AND BROMINATED FLAME RETARDANTS (BFRs)

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Exposure to polychlorinated biphenyls (PCBs) and brominated flame-retardants (BFRs) in human, primates, and rodents is accompanied by neurobehavioral changes. These involve adverse effects on both memory and learning and motor activity. There are also adverse effects observed on the endocrine and immune system. This review is restricted to our laboratory's recent findings of effects of these compounds on the nervous system and some molecular effects on the immune system. In the nervous system, data showed that PCBs and BFRs produce an effect on neurotransmitter transport mechanisms, in particular the neurotransmitter dopamine. It was demonstrated that this might explain the loss of dopamine in the brain seen after exposure to PCB. Further, it may explain the behavior of dopamine in preparations in vitro from brain tissue after exposure to PCB. Recently it was also reported that PCB and some BFRs induce formation of reactive oxygen species (ROS) in neurons. ROS act as messengers in the nervous system and may also be involved in cell death. In the case of PCB exposure, a correlation between ROS formation and death of neurons was found. In the immune system it was shown that PCBs and some of the BFRs induce formation of ROS in neutrophils (granulocytes). This takes place primarily through phosphorylation and subsequent activation of the NADPH oxidase. This production of ROS may have an adverse effect on the immune system.

Organohalogenated compounds like polychlorinated biphenyls (PCBs) and brominated flame retardants (BFRs) are widespread in nature and this has caused great concern. PCBs were first detected in the 1960s (Jensen, 1966) and since then the levels have increased, particularly in the Arctic. The use of PCBs has now been prohibited in most countries and there are signs that the levels of PCBs are beginning to decline in environmental samples. On the other hand, the environmental occurrence of other organohalogens such as BFRs, perfluorated hydrocarbons, and chlorinated alkanes is increasing. Several hundred thousand tons of BFRs are being produced each year, and a dramatic increase in the level of some of the BFRs in environmental samples has been observed during the last decade (de Wit, 2002). The PCBs and BFRs are extremely persistent and not easily degraded by metabolism. Further, they are

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lipophilic and tend to biomagnify in nature. The highest levels of these compounds are found in top predators such as polar bears, seals, and seagulls (Law et al., 2003). Of particular concern is the fact that the compounds are also detected in mother's milk (Meironyte et al.,1999; Landrigan et al., 2002; Schecter et al., 2003). This is a concern since early development in most species, including humans, seems to be a vulnerable period of exposure (Seegal, 1996).

There are several epidemiological studies in humans, particularly on children both after accidental exposure and after consuming contaminated food. The studies show that PCBs may affect neurological development and impair cognitive functions in human and animals (Tilson et al., 1990; Seegal, 1996). The two most recognized incidences are the accidents in Japan and Taiwan in 1968 and 1979 when 4000 people were exposed to cooking oil contaminated with thermally degraded PCB (Kuratsune et al., 1971; Hsu et al., 1985). The cooking oil also contained furans, dioxinlike compounds, which by some are claimed to be the major toxicants (Masuda, 1985). In these two cases children exposed to PCBs prenatally or during lactation displayed delayed motor development and cognitive defects (Seegal, 1996). There are also studies describing behavioral changes in children exposed to much lower concentrations through eating contaminated food (Rogan & Gladen, 1992; Jacobson & Jacobson, 1996). These studies concluded that for cognitive development of the children, exposure to PCB in utero seems to be more important than during lactation. Epidemiological studies may, however, be criticized since several additional unknown factors can be involved. In the Faroe Islands, fish and whale meat, which contain a mixture of, for example, PCBs and methylmercury, are an important part of the families' diet. Consumption of this diet by pregnant mothers was found to produce a negative effect on the neurobehavior of their children during early development (Schantz et al., 2003). These examples of epidemiological studies are strengthened by the results from several studies on feeding rats with PCBs. The most common findings after exposure during development of animals to PCBs are hyperactivity, and cognitive defects in learning and memory, while exposure during adulthood is correlated to decreased motor activity (Tilson et al., 1990; Seegal, 1996; Giesy & Kannan, 1998; Widholm et al., 2001). In addition, it was found that PCBs affect the so-called long-term potentiation, which is believed to be the biological substrate for memory (Altmann et al., 1995; Gilbert & Crofton, 1999). Widholm et al. (2001) exposed female rats to Arochlor 1254 (PCB mixture) at 6 mg/kg/d from gestational d 6 to d 21 and observed errors in discrimination and learning. In contrast, a recent study by Bushnell et al., (2002) only detected small behavioral effects after exposure of pregnant Long-Evans rats fed Aroclor 1254 at doses of 0, 1, or 6 mg/kg/d throughout gestation and nursing. The authors recognized that their findings contrast with those of many other studies, but were not able to explain the differences.

Less is known about the effect of BFRs on human behavior. In a series of studies, Eriksson and coworkers showed that some of the brominated diphenyl

ethers (PBDEs) and hexabromocyclododecane (HBCD) produce behavioral effects in mice when the compounds were injected during early development. Single doses of tetraBDE, pentaBDE, and hexaBDE were administered to mice at d 10 after gestation and produced an increase in spontaneous activity in the animals when tested after 2 and 4 mo (Eriksson et al., 2002; Viberg et al., 2003a, 2003b, 2004). Day 10 is the time when the nervous system in mice starts to make several new connections, and it is also the time when neurochemical parameters such as transmitter synthesis, storage, and release are rapidly increasing. It is to be expected that after exposure at d 10 to lipophilic BFRs a high concentration of the contaminants will remain in the brain for several days. It is therefore possible to expect that BFRs produce effects, at least during development, on brain neurochemistry, such as neurotransmitter transport, formation of reactive oxygen species (ROS), and cell signaling.

Studies in our laboratory investigated toxicological effects of PCBs and BFRs, and this article discusses the neurotoxicity of these groups of environmental toxins in light of our findings. Experiments were carried out on detached nerve terminals (synaptosomes) and synaptic vesicles isolated from brain tissue to study transport mechanisms of neurotransmitters. The synaptosomes are pinched off nerve endings, which can operate for hours in vitro and are used for studying neurotransmitter release, uptake, and synthesis. The synaptosome transport mechanisms are localized at the surface of the plasma membranes and are essential for the removal of neurotransmitters from the synaptic cleft. The uptake mechanism is ion and energy dependent. The vesicular transport mechanism is responsible for storing neurotransmitter for synaptic release, involves a proton pump, and has selective transport molecules different from those of the plasma membrane. Both transport systems are modulated by additional mechanisms such as protein phosphorylation. To study ROS formation and cell signaling, human neutrophils (granulocytes) and rat cerebellar granule cells were selected. Human granulocytes are both readily available and easy to isolate. These cells are powerful tools for investigation of induction of cell signaling pathways and potential immunotoxicological effects. Cerebellar granule cells are probably the primary neuron culture used most frequently in neurotoxicological studies, and they have all the signal systems and ion and receptor regulation found in nerve cells. They are isolated from rat pups 6-8 d old and represent the most abundant neuron type in the brain (Fonnum & Lock, 2004).

EFFECT OF PCBs AND BFRs ON NEUROTRANSMITTER TRANSPORT

It was established early that exposure to PCBs leads to a decrease in dopamine levels in different regions of the brain of rats and on nonhuman primates, and the effect was mainly due to the *ortho*-substituted PCBs (Seegal, 1996). The concentration of dopamine decreased in monkey brains exposed as adults to commercial PCB mixtures (0.8–3.2 mg/kg/d for 20 wk). A decrease was found at least 20 wk after exposure (Seegal, 1996). A pronounced effect

on dopamine levels was also reported in several in vitro studies using dopaminergic cell cultures (PC12 cells) or different brain preparations such as brain striatal slices (Angus & Contreras, 1994, 1996; Chishti et al., 1996). However, the precise mechanisms for the PCB-induced effect on dopamine remains unclear. Mariussen et al. (1999) showed that the vesicular uptake of dopamine was inhibited in vitro by low concentrations of PCBs (Table 1). The mechanism was shown to be a selective inhibition of the vesicular monoamine transporter, which is named VMAT-2. VMAT-2 is the common vesicle transporter for all amine neurotransmitters in brain (Peter et al., 1995). The function of VMAT-2 is to maintain a low level of dopamine, serotonin, and noradrenaline in the cytoplasm of the nerve terminal. It has been shown that the inhibition of VMAT-2 by ecstasy and amphetamines contributes to the neurodegeneration found with these abuse substances (Gainetdinov et al., 2002; Bogen et al., 2003). It is therefore possible that inhibition of this transport system would contribute to the neurotoxicological effects of PCBs. A structure–activity study revealed that only the *ortho*–chlorinated PCBs inhibited the dopamine uptake. Physicochemical properties such as absolute hardness and high lipophilicity of the PCBs were important for their function as inhibitors (Mariussen & Fonnum, 2001). An inhibition of the vesicular uptake of dopamine may explain the effects of PCBs on dopamine regulation in PC12 cells and striatal slices observed previously (Angus & Contreras, 1994, 1996; Chishti et al., 1996). Recently Bemis and Seegal (2004) concluded that VMAT-2 was an important factor responsible for the reduced dopamine level found in their experiment with synaptosomes exposed to PCBs. It was further shown that the *ortho*-substituted PCBs also inhibit uptake into nerve terminals through inhibition of the plasma membrane dopamine transporter (DAT) (Mariussen & Fonnum, 2001). In this case the most potent inhibitors were *ortho*-substituted PCBs with a low molecular size, for example, PCBs with less than six substituted chlorines. Dopamine reuptake to the terminals is regarded as essential for inactivating the effect of dopamine on the receptor and also for maintaining a low level of dopamine in the synapse. In fact, the level of dopamine in the brain is correlated to the number of DAT molecules (Gainetdinov et al., 2002). Imbalance between VMAT-2 and DAT inhibition or levels has in general been shown to

TABLE 1. Effect of PCBs and BFRs on Synaptosomal and Vesicular Uptake of Dopamine

PCB or BFR congener	Synaptosome IC50 (μ*M*)	Vesicle IC50 (μ*M*)
2,2′,4,5′-CB	8	11
3,3′,4,4′5-CB	≥50	≥50
2,2′,4,4′,5,5′-CB	≥50	14
2,2′,4,5′,6,-CB	15	7
PentaBDE	≥50	4
HBCD	4	3
TBBPA	9	3

Note. The data are taken from Mariussen et al. (2002) and Mariussen and Fonnum (2001, 2003).

have severe consequences for dopaminergic cells (Miller et al., 1999). Potent inhibition of VMAT-2 compared to DAT allows a high concentration of cytosolic dopamine to accumulate in the nerve terminal. This increases the possibility of autooxidation of dopamine and subsequent degeneration of dopaminergic structures (Hastings et al., 1996; Miller et al., 1999). The findings of PCBs on dopamine transport in vitro received support from in vivo experiments. Seegal and colleagues (2002) exposed rats to PCB over several days and measured the extracellular dopamine content by microdialysis. They showed that extracellular dopamine increased during the first days of exposure, followed by a reduction after 1 wk or more. The reduction was attributed to VMAT-2 inhibition (Seegal et al., 2002). Richardson and Miller (2004) exposed mice to a high single dose (500 mg/kg) of A1016 or A1260. They found a reduction in both DAT and VMAT-2. As suggested from the analysis of the properties of PCB inhibitor activity already described, they found that the low molecular size PCB mixture (A1016) inhibited preferentially DAT and the higher molecular size PCB mixture (A1260) inhibited VMAT-2. There has also been a report on a PCB-induced loss in the level of serotonin in brain after administration of PCBs (Tilson & Kodavanti, 1997). This is in general accordance with the inhibition of VMAT-2, which is involved in both dopamine and serotonin uptake into vesicles (Mariussen et al., 1999). So far there have been no reported effects of PCBs on dopamine receptors. The few studies performed have not suggested any changes (Roth-Harer et al., 2001; Mariussen & Fonnum, 2001).

A similar effect on the glutamate and GABA vesicular transporters was not found (Mariussen et al., 1999). However, low concentrations (≤ 5 μM) of *ortho*-PCBs partially inhibit the uptake of glutamate and GABA into synaptosomes (Mariussen & Fonnum, 2001). An increase in extracellular glutamate could play a role in excitotoxicity as discussed later and may be involved in neuronal cell death. It is difficult to explain why PCBs inhibit only partially the glutamate uptake. It is possible that PCBs only inhibit one of the glutamate transporters or that PCBs modulate the transporter molecules.

It does not seem that anyone has investigated the effect of BFRs on the levels of neurotransmitters in the brain. However, the findings of Branchi et al. (2002) revealing neurobehavioral effects after exposure of animals to BFRs indicate that these compounds may affect neurochemical parameters. Two of the frequently used BFRs, hexabromocyclodecane (HBCD) and tetrabromobisphenol-A (TBBPA), where shown to inhibit the plasma membrane uptake of glutamate and dopamine and the vesicular uptake of dopamine (Table 1; Mariussen & Fonnum, 2003). The effective concentrations were similar to those observed for the PCBs. The commercial pentaBDE mixture (DE-71, Great Lakes), which resembles the congener pattern of PBDEs found in environment, inhibited dopamine uptake into vesicles, but had only minor effects on neurotransmitter uptake into synaptosomes. Commercial mixtures of octaBDE and decaBDE had no effect on transmitter uptake, either in synaptosome or in synaptic vesicles (Mariussen & Fonnum, 2003). In the future the effects of these compounds on other neurotransmitter parameters in brain need to be investigated.

EFFECTS OF PCBs AND BFRs ON CALCIUM IN NEUTROPHILS AND BRAIN PREPARATIONS

It was reported that PCBs influence calcium homeostasis in different subcellular fractions such as synaptosomes, mitochondria and microsomes, and cerebellar granule cells. The effects of PCBs on calcium homeostasis in neurons were thoroughly investigated and were suggested to be an important factor involved in the neurotoxicity of PCBs (Tilson & Kodavanti, 1997; Wong et al., 1997; Mundy et al., 1999; Inglefield & Shafer, 2000). Calcium is involved in a range of cellular processes, including neurotransmitter release, protein phosphorylation, and formation of ROS. It is therefore crucial for the cell to maintain Ca^{2+} homeostasis. The perturbation of Ca^{2+} homeostasis in human granulocytes exposed to PCBs was investigated (Voie & Fonnum, 1998). *Ortho*-substituted PCB congeners increased intracellular free calcium, $[Ca^{2+}]_i$, in a concentration-dependent manner. The increase in $[Ca^{2+}]_i$ was inversely proportional to the total surface area of the *ortho*-substituted congeners. The effect of PCB congeners was dependent on external Ca^{2+}, and the calcium level was reduced by addition of the phospholipase C (PLC) inhibitor U-72133. It was suggested that PCB activated PLC-induced release of Ca^{2+} from intracellular stores by inositol phosphate generation followed by a subsequent activation of Ca^{2+} release activated Ca^{2+} channels (CRAC) in the plasma membrane. *Ortho*-substituted PCB congeners stimulate $[Ca^{2+}]_i$ elevation in human granulocytes, and this could in part account for the effects of PCBs on ROS formation. Kodavanti and coworkers showed that PCBs induce a calcium-dependent increase in inositol phosphate accumulation and suggested it to be an important factor involved in the neurotoxicity of PCBs (Kodavanti et al., 1994; Shafer et al., 1996).

Less is known about the effects of BFRs on the calcium homeostasis. The pentaBDE mixtures were found to induce influx of calcium homeostasis in a range of different cells types ranging from astrocytes to macrophages (Wiegand et al., 2001). In our laboratory both granulocytes and cerebellar granule cells were exposed to the brominated flame retardant TBBPA and a significant increase in intracellular calcium was found. Cells exposed to TBBPA in calcium-free buffer containing EGTA also induced a small but significant increase in intracellular calcium. This indicates that TBBPA, in addition to uptake of extracellular calcium, also elevates cytosolic free calcium through release from intracellular compartments (Reistad et al., 2005). These findings suggest that TBBPA may activate a series of calcium-dependent enzyme reactions and influence calcium-dependent processes in the cell, such as formation of oxidative stress, as discussed later.

EFFECTS OF PCBs AND BFRs ON FORMATION OF REACTIVE OXYGEN SPECIES AND CELL SIGNALING

Human neutrophil granulocytes play a key role in host defenses against invading pathogens and are major effectors of the acute inflammatory reactions.

Activation of these cells during an immune response leads to formation of reactive oxygen species used to kill microorganisms. The importance of ROS formation is demonstrated by chronic granulomatous disease (CGD), which leads to early death due to lack of ROS production (Babior, 1999). A possible threat to the cellular homeostasis arises from these reactive oxygen species, as they are known to be involved in cellular signaling and gene regulation (Finkel, 1998; Allen & Tresini, 2000). In addition to the direct threat to cells and tissue, Koner et al. (1997) showed a possible connection between free radical formation and immune suppression in the rabbit. Formation of ROS in granulocytes may be induced by different mechanisms, of which activation of the NADPH oxidase is the most important. The neutrophil NADPH oxidase is a multicomponent membrane-bound enzyme that catalyzes NADPH-dependent reduction of oxygen to superoxide radical ($O_2^{\cdot -}$), which may be converted to hydrogen peroxide (H_2O_2), peroxynitrite ($OONO^-$), hypochlorite (HOCl), and hydroxyl radical ($OH^{\cdot}$) (Babior, 1999). Several pathways may activate the NADPH oxidase, of which mitogen-activated protein kinase (MAPK) and PKC seem to be important (Figure 1). The MAPKs are major information pathways from the cell surface to the nucleus. ROS formation can be measured by a series of fluorescence and chemiluminescence methods (Myhre et al., 2003). The dichlorofluorescin (DCF) assay is an attractive and sensitive method as an overall index for oxidative stress in biological systems. It is reported to detect several types of reactive molecules such as H_2O_2 in the presence of cellular peroxidases, $OONO^-$ and $OH^{\cdot}$, but has no sensitivity toward the superoxide anion radical. Lucigenin is a sensitive probe for the detection of ($O_2^{\cdot -}$). Luminol is a chemiluminescence method measuring HOCl. All are frequently used to demonstrate activation of respiratory burst in granulocytes (Myhre et al., 2003). The DCF and luminol assays are primarily indicators of intracellular formation of ROS, whereas the lucigenin assay primarily measures extracellular ROS (Caldefie-Chezet et al., 2002).

Disruption of calcium homeostasis might lead to formation of ROS and activation of the NADPH oxidase complex. In a series of experiments in our laboratory it was found that *ortho*-PCBs induce respiratory burst in neutrophils, measured by luminol chemiluminescence (Voie et al., 2000). The effect was linked to the previously discussed findings showing that *ortho*-PCBs elevate intracellular calcium in granulocytes. A thorough investigation of the mechanisms of the observed effect revealed an involvement of PKC, PLC and PLD (Figure 1). Both bisindolylmaleimide, which inhibits protein kinase C, and neomycin, which inhibits PLC, reduced the PCB-activated chemiluminescence. In addition, the increase of phospholipase D (PLD) activity, measured as the amount of [^{14}C] phosphatidylbutanol formed, may be related to the increase of ROS by PCB exposure. Tyrosine phosphorylation is involved in both PLC and PLD activation, and genistein, a nonselective tyrosine kinase inhibitor, reduced chemiluminescence in response to PCB. In conclusion, the results indicate that PCB-induced chemiluminescence in granulocytes is dependent on the activation of phospholipase D or phospholipase C by a

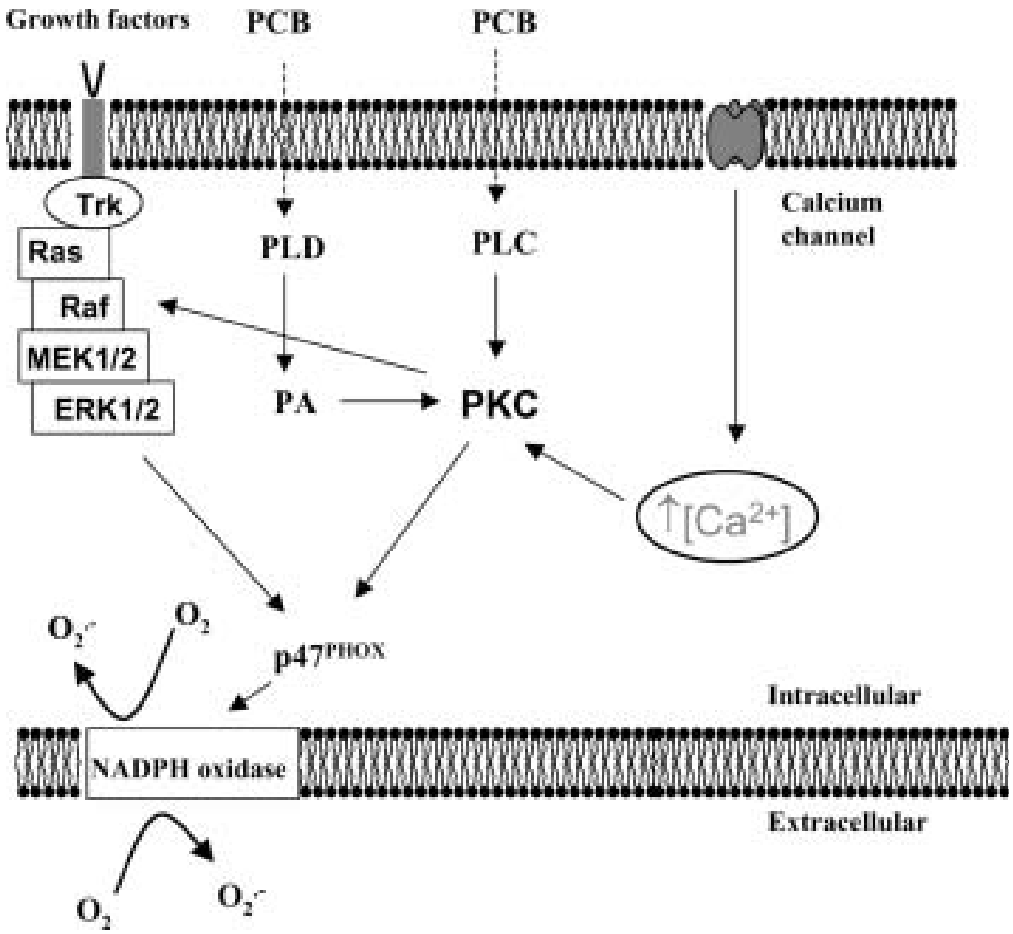

FIGURE 1. Pathways involved in ROS formation in neutrophil granulocytes. The mitogen-activated protein kinases (MAPKs) are major information pathways from the cell surface to the nucleus, activated by, for example, growth factors. The figure shows the signal pathways from phospholipase D (PLD), MAPK, and phospholipase C (PLC), which in turn activate protein kinase C (PKC) and ERK1/2. PKC and ERK1/2 will phosphorylate the $p47^{PHOX}$ subunit and thereby activate the NADPH oxidase to produce superoxide, which can be further transferred to other ROS.

tyrosine kinase-dependent mechanism, and PKC probably by a calcium-dependent mechanism prior to phosphorylation of the NADPH oxidase.

TBBPA, the most frequently used BFR, was recently also shown to induce respiratory burst in granulocytes by activation of ERK1/2 in the MAPkinase pathway and calcium dependent PKC. This was measured both by DCF fluorescence and lucigenin chemiluminescence. The ROS production in this case was partly dependent on extracellular calcium and inhibited by the MAPkinase inhibitor U0126, the PKC inhibitor bisindolylmaleimide, and the non-selective tyrosine kinase inhibitor erbstatin analog. The MAPkinase cascade and PKC probably act by phosphorylating the subunit $p47^{PHOX}$ of NADPH oxidase. The NADPH oxidase inhibitor diphenyleneiodonium (DPI) also inhibited ROS

formation, confirming the involvement of this multicomponent enzyme system (Reistad et al., 2005). The effect of TBBPA and the *ortho*-PCB 153 on respiratory burst was further shown to be additive when exposed to the cells in combination (Reistad et al., 2005). This is an important finding since human and animals are not exposed to single compounds but to mixtures of compounds.

PCBs were also shown to activate ROS formation in cerebellar granule cells. Several inhibitors of different signaling pathways were investigated to elucidate the mechanism of the observed effect (Figure 2). The ROS formation

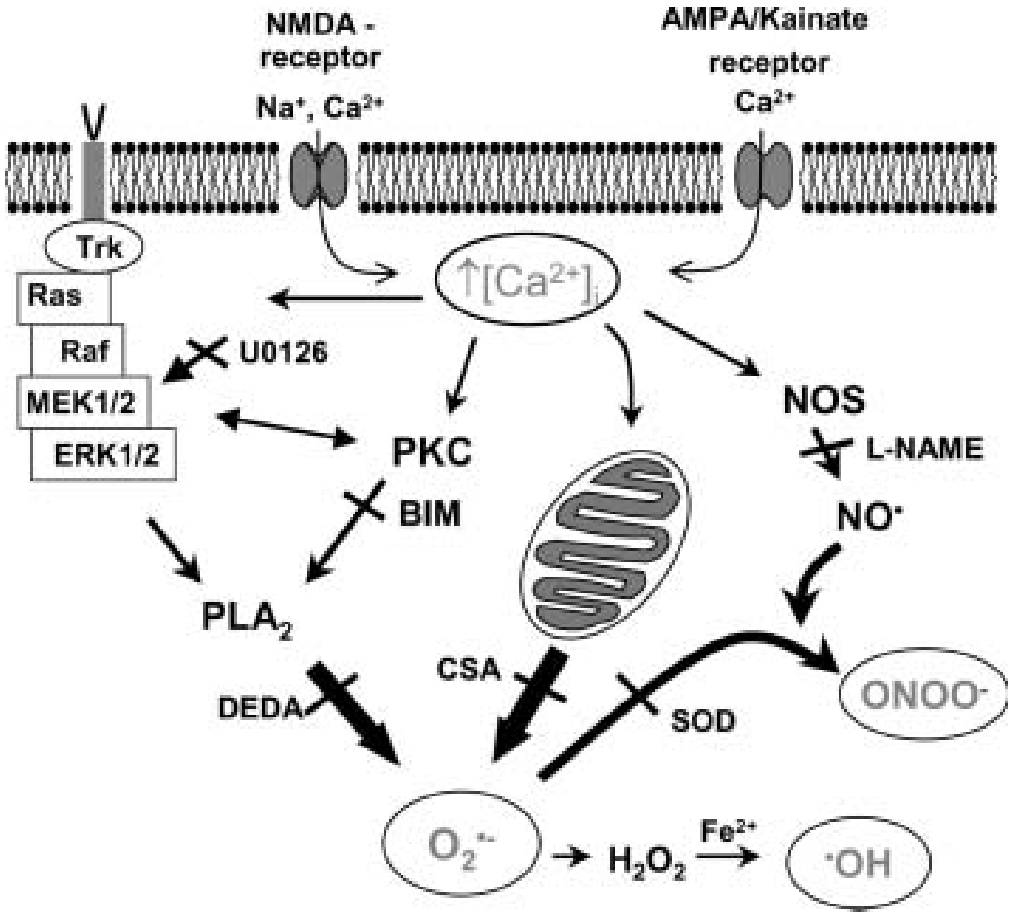

FIGURE 2. Signal pathway involved in formation of ROS in cerebellar granule cells. PCBs can activate glutamate receptors either directly or through the glutamate AMPA or NMDA (*N*-methyl D-aspartate) receptors. The NMDA receptor ion channel can allow calcium in high concentration to enter the cell. MK-801 can block this channel and partly prevent the calcium entry. Calcium can also enter through voltage-gated calcium channels and to a smaller extent through the AMPA receptor. Calcium may activate a series of signal pathway such as NO synthetase (NOS) or protein kinase C (PKC), and open the mitochondrial transition pore. Opening of the mitochondrial transition pore and activation of PKC and phospholipase 2 (PLA2) may lead to superoxide ($O_2^{\cdot -}$) production. The superoxide can undergo Fenton reaction to produce OH radical or react with NO to give peroxynitrite. CSA, cyclosporine A; BIM, bisindolylmaleimide; SOD, superoxide dismutase.

was inhibited by the NMDA-receptor antagonist MK-801, which blocks the calcium channel of this glutamate receptor (Mariussen et al., 2002) A possible connection between PCB exposure of cerebellar granule cells and increased response of glutamate receptor was also found by other investigators (Inglefield & Shafer, 2000; Gafni et al., 2004). Further, ROS formation was inhibited by PLA2 and NO-synthetase inhibitors, which are signaling pathways activated by calcium and glutamate receptors. In agreement, Kodavanti and Derr-Yellin (2002) found that *ortho*-PCBs activate PLA2 in cerebellar granule cells.

Exposure of cerebellar granule cells to *ortho*-PCBs also leads to cell death. Interestingly, the same compounds that were shown to inhibit ROS formation also protected the cells from cell death. In addition, the antioxidant vitamin E protected the cells, strengthening the view that ROS formation is involved in the observed effect (Figure. 3) (Mariussen et al., 2002). Further, a selection of the hydroxylated metabolites of PCBs induced ROS formation and cell death of cerebellar granule cells. In this respect it was interesting to note that *p*-hydroxylated PCB (two and three chloride substituted) induced elevated ROS formation both in granulocytes and in cerebellar granule cells, but only induced cell death at high concentrations evidenced by lactate dehydrogenase

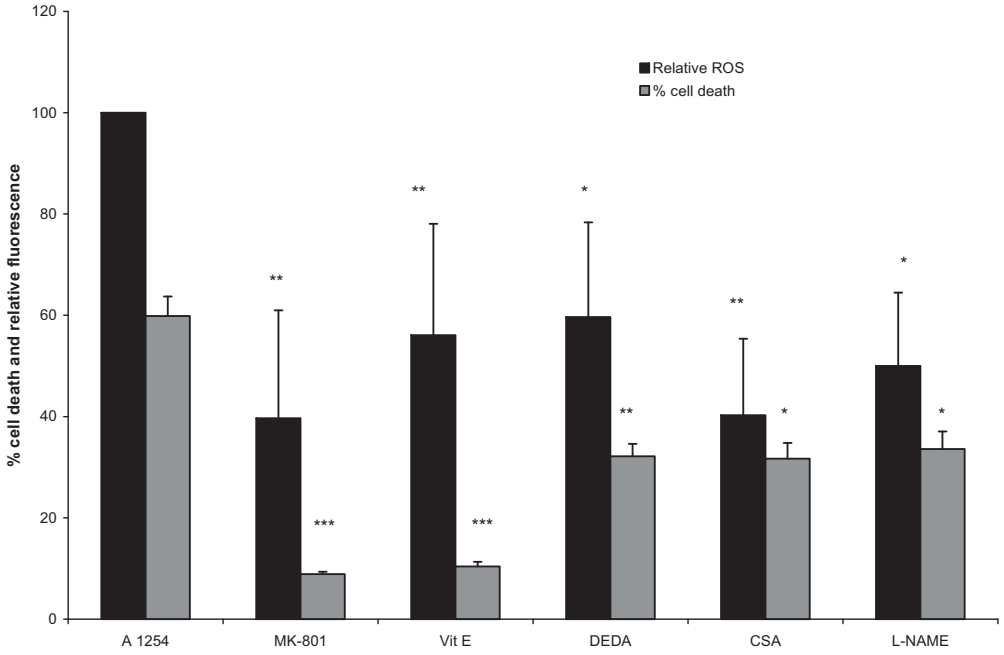

FIGURE 3. Effects of a series of inhibitors of ROS pathways on ROS production and cell death after exposure of the PCB mixture A1254 to cerebellar granule cells. For ROS the cells were exposed for 1 h; for cell death the cells were exposed for 6 h. MK-801, NMDA receptor antagonist; Vit E, vitamin E; CSA, cyclosporine; DEDA, PLA-2 inhibitor; NAME, NO synthetase inhibitor. The data are collected from Mariussen et al. (2002).

(LDH) release. These compounds are less lipophilic than the corresponding PCBs (Fonnum et al., 2004).

STRUCTURE–ACTIVITY RELATIONSHIP OF DIFFERENT SUBSTITUTED DIPHENYL COMPOUNDS

The substituted biphenyls include several hundred analogue compounds. Generally it was found that the noncoplanar PCBs induce neurotoxic effects, whereas the coplanar dioxinlike PCBs do not exert any effects. To elucidate in more detail the dependency of the substituted groups on a biphenyl, the effect of seven 2,2-substituted biphenyls on ROS and calcium homeostasis was investigated in granulocytes, and the effect on dopamine transport in synaptic vesicles and synaptosomes. Interestingly, only 2,2′ -chloro-, 2,2′-bromo-, and 2,2′-methylbiphenyl exerted effect toward all parameters and in a similar concentration range (5–50 μ*M*). The disubstituted 2,2′-fluoro biphenyl, which has a lower molecular size than the other halogenated biphenyls, had only effects on calcium influx at a very high concentration (50 μ*M*). On the other hand 2,2′-dihydroxy-, 2,2′-dinitro-, and 2,2′-dimethanol biphenyl did not show any effect toward any of the tested parameters (Mariussen et al., 2003). Important physicochemical parameters involved in the observed effects were lipophilicity, molecular size, and absolute hardness. A more thorough investigation of the polybrominated biphenyls revealed that the PBB analogues induced respiratory burst and calcium influx by a similar mechanism as observed for the PCBs (Kristoffersen et al., 2002).

CONCLUSIONS

There are several animal studies showing an adverse effect of PCB on memory, learning, and motor behavior, particularly during development. In agreement there are also several epidemiological studies showing adverse effects of PCBs on children during early development. In the latter cases it should be borne in mind that other lipophilic contaminants such as methylmercury can contribute to the findings. As reviewed, this has particularly been an issue at the Faroe Islands. Exposure of brain to high concentrations of *ortho*-substituted PCBs was accompanied by a decrease in the dopamine levels. Inhibition of the vesicular transporter VMAT-2 and the plasma membrane transporter DAT seems to be important in this respect. In particular, the ratio between VMAT-2 and DAT is important for the normal function of dopaminergic cells. Both *ortho*-substituted PCBs and some BFRs lead to increase of calcium in cells, and this change in calcium homeostasis activates several enzymes and signal systems in the neurons and neutrophils. One of these effects is the activation of ROS production both in neurons and in neutrophil granulocytes. In the case of PCBs there is a good correlation between ROS formation and cell death. Hydroxy metabolites of PCBs also lead to cell death of

cerebellar granule cells, but the link between ROS formation and cell death is less clear.

The ecological consequences of these findings are difficult to evaluate. The effect of ROS on phagocytic cells generally means that the cells were less active in killing microorganisms and therefore the individuals would be less able to counteract infections. Changes in dopamine in humans are usually associated for young people with hyperactivity and in older people with severe loss in dopamine with Parkinson's disease.

REFERENCES

Allen, R. G., and Tresini, M. 2000. Oxidative stress and gene regulation. *Free Radical Biol. Med.* 28:463–499.

Altmann, L., Weinand-Haerer, A., Lilienthal, H., and Wiegand, H. 1995. Maternal exposure to polychlorinated biphenyls inhibits long-term potentiation in the visual cortex of adult rats. *Neurosci Lett* 202:53–56.

Angus, W. G., and Contreras, M. L. 1994. The effects of Aroclor 1254 on undifferentiated and NGF-stimulated differentiating PC12 cells. *Neurotoxicology* 15:809–817.

Angus, W. G., and Contreras, M. L. 1996. Effects of polychlorinated biphenyls on dopamine release from PC12 cells. *Toxicol. Lett.* 89:191–199.

Babior, B. M. 1999. NADPH oxidase: An update. *Blood* 93:1464–1476.

Bemis, J. C., and Seegal, R. F. 2004. PCB-induced inhibition of the vesicular monoamine transporter predicts reductions in synaptosomal dopamine content. *Toxicol. Sci.* 80:288–295.

Bogen, I. L., Haug, K. H., Myhre, O., and Fonnum, F. 2003. Short- and long-term effects of MDMA ("ecstasy") on synaptosomal and vesicular uptake of neurotransmitters in vitro and ex vivo. *Neurochem. Int.* 43:393–400.

Branchi, I., Alleva, E., and Costa, L. G. 2002. Effects of perinatal exposure to a polybrominated diphenyl ether (PBDE 99) on mouse neurobehavioural development. *Neurotoxicology* 23:375–384.

Bushnell, P. J., Moser, V. C., MacPhail, R. C., Oshiro, W. M., Derr-Yellin, E. C., Phillips, P. M., and Kodavanti, P. R. 2002. Neurobehavioral assessments of rats perinatally exposed to a commercial mixture of polychlorinated biphenyls. *Toxicol. Sci.* 68:109–120.

Caldefie-Chezet, F., Walrand, S., Moinard, C., Tridon, A., Chassagne, J., and Vasson, M. P. 2002. Is the neutrophil reactive oxygen species production measured by luminol and lucigenin chemiluminescence intra or extracellular? Comparison with DCFH-DA flow cytometry and cytochrome *c* reduction. *Clin. Chim Acta.* 319:9–17.

Chishti, M. A., Fisher, J. P., and Seegal, R. F. 1996. Aroclors 1254 and 1260 reduce dopamine concentrations in rat striatal slices. *Neurotoxicology* 17:653–660.

de Wit, C. A. 2002. An overview of brominated flame retardants in the environment. *Chemosphere* 46:583–624.

Eriksson, P., Viberg, H., Jakobsson, E., Orn, U., and Fredriksson, A. 2002. A brominated flame retardant, 2,2′,4,4′,5-pentabromodiphenyl ether: uptake, retention, and induction of neurobehavioral alterations in mice during a critical phase of neonatal brain development. *Toxicol. Sci.* 67:98–103.

Finkel, T. 1998. Oxygen radicals and signaling. *Curr. Opin. Cell Biol.* 10:248–253.

Fonnum, F., Dreiem, A., Lehmler, H. Y., Mariussen, E., Rykken, S., and Robertson, L. 2004. The effect of hydroxylated-PCB on DCF-fluorecence and cell death in cerebellar granule cells. *Organohalogens* 66:3120–3123.

Fonnum, F., and Lock, E. A. 2004. The contributions of excitotoxicity, glutathione depletion and DNA repair in chemically induced injury to neurones: Exemplified with toxic effects on cerebellar granule cells. *J. Neurochem.* 88:513–531.

Gafni, J., Wong, P. W., and Pessah, I. N. 2004. Non-coplanar 2,2′,3,5′,6-pentachlorobiphenyl (PCB 95) amplifies ionotropic glutamate receptor signaling in embryonic cerebellar granule neurons by a mechanism involving ryanodine receptors. *Toxicol. Sci.* 77:72–82.

Gainetdinov, R. R., Sotnikova, T. D., and Caron, M. G. 2002. Monoamine transporter pharmacology and mutant mice. *Trends Pharmacol. Sci.* 23:367–373.

Gilbert, M. E., and Crofton, K. M. 1999. Developmental exposure to a commercial PCB mixture (Aroclor 1254) produces a persistent impairment in long-term potentiation in the rat dentate gyrus in vivo. *Brain Res.* 850:87–95.

Giesy, J. P., and Kannan, K. 1998. Dioxin-like and non-dioxin-like toxic effects of pølychlorinated biphenyls (PCB): Implications for risk assesment. *Crit. Rev. Toxicol.* 28:511–569.

Hastings, T. G., Lewis, D. A., and Zigmond, M. J. 1996. Reactive dopamine metabolites and neurotoxicity: implications for Parkinson's disease. *Adv. Exp. Med. Biol.* 387:97–106.

Hsu, S. T., Ma, C. I., Hsu, S. K., Wu, S. S., Hsu, N. H., Yeh, C. C., and Wu, S. B. 1985. Discovery and epidemiology of PCB poisoning in Taiwan: A four-year followup. *Environ. Health Perspect.* 59:5–10.

Inglefield, J. R., and Shafer, T. J. 2000. Polychlorinated biphenyl-stimulation of Ca(2+) oscillations in developing neocortical cells: A role for excitatory transmitters and L-type voltage-sensitive Ca(2+) channels. *J. Pharmacol. Exp. Ther.* 295:105–113.

Jacobson, J. L., and Jacobson, S. W. 1996. Dose-response in perinatal exposure to polychlorinated biphenyls (PCBs): The Michigan and North Carolina cohort studies. *Toxicol. Ind. Health* 12:435–445.

Jensen, S. 1966. New Scientist: Report on a chemical hazard. 32:612.

Kodavanti, P. R., and Derr-Yellin, E. C. 2002. Differential effects of polybrominated diphenyl ethers and polychlorinated biphenyls on [^{3}H]arachidonic acid release in rat cerebellar granule neurons. *Toxicol Sci* 68:451–457.

Kodavanti, P. R., Shafer, T. J., Ward, T. R., Mundy, W. R., Freudenrich, T., Harry, G. J., and Tilson, H. A. 1994. Differential effects of polychlorinated biphenyl congeners on phosphoinositide hydrolysis and protein kinase C translocation in rat cerebellar granule cells. *Brain Res.* 662:75–82.

Koner, B. C., Banerjee, B. D., and Ray, A. 1997. Modulation of gamma glutamyl transpeptidase activity in lymphoid system by organochlorine pesticides in rats. *Indian J. Exp. Biol.* 35:1132–1134.

Kristoffersen, A., Voie, O. A., and Fonnum, F. 2002. Ortho-substituted polybrominated biphenyls activate respiratory burst in granulocytes from humans. *Toxicol. Lett.* 129:161–166.

Kuratsune, M., Yoshimura, T., Matsuzaka, J., and Yamaguchi, A. 1971. Yusho, a poisoning caused by rice oil contaminated with polychlorinated biphenyls. *HSMHA Health Rep.* 86:1083–1091.

Landrigan, P. J., Sonawane, B., Mattison, D., McCally, M., and Garg, A. 2002. Chemical contaminants in breast milk and their impacts on children's health: An overview. *Environ. Health Perspect.* 110:A313–A315.

Law, R. J., Alaee, M., Allchin, C. R., Boon, J. P., Lebeuf, M., Lepom, P., and Stern, G. A. 2003. Levels and trends of polybrominated diphenylethers and other brominated flame retardants in wildlife. *Environ. Int.* 29:757–770.

Mariussen, E., and Fonnum, F. 2001. The effect of polychlorinated biphenyls on the high affinity uptake of the neurotransmitters, dopamine, serotonin, glutamate and GABA, into rat brain synaptosomes. *Toxicology* 159:11–21.

Mariussen, E., and Fonnum, F. 2003. The effect of brominated flame retardants on neurotransmitter uptake into rat brain synaptosomes and vesicles. *Neurochem. Int.* 43:533–542.

Mariussen, E., Morch Andersen, J., and Fonnum, F. 1999. The effect of polychlorinated biphenyls on the uptake of dopamine and other neurotransmitters into rat brain synaptic vesicles. *Toxicol. Appl. Pharmacol.* 161:274–282.

Mariussen, E., Myhre, O., Reistad, T., and Fonnum, F. 2002. The polychlorinated biphenyl mixture aroclor 1254 induces death of rat cerebellar granule cells: The involvement of the *N*-methyl-D-aspartate receptor and reactive oxygen species. *Toxicol. Appl. Pharmacol.* 179:137–144.

Mariussen, E., Andersson, P. L., Olstrom, H., Voie, O. A., and Fonnum, F. 2003. The effect of various substituents in ortho position of biphenyls on respiratory burst, intracellular calcium elevation in human granulocytes and uptake of dopamine into brain synaptic vesicles and synaptosomes. *Environ. Toxicol. Pharmaco.* 14:43–50.

Masuda, Y. 1985. Health status of Japanese and Taiwanese after exposure to contaminated rice oil. *Environ Health Perspect* 60:321–325.

Meironyte, D., Noren, K., and Bergman, A. 1999. Analysis of polybrominated diphenyl ethers in Swedish human milk. A time-related trend study. 1972–1997. *J. Toxicol. Environ. Health A.* 58:329–341.

Miller, G. W., Gainetdinov, R. R., Levey, A. I., and Caron, M. G. 1999. Dopamine transporters and neuronal injury. *Trends Pharmacol. Sci.* 20:424–429.

Mundy, W. R., Shafer, T. J., Tilson, H. A., and Kodavanti, P. R. 1999. Extracellular calcium is required for the polychlorinated biphenyl-induced increase of intracellular free calcium levels in cerebellar granule cell culture. *Toxicology* 136:27–39.

Myhre, O., Andersen, J. M., Aarnes, H., and Fonnum, F. 2003. Evaluation of the probes 2′,7′–dichlorofluorescin diacetate, luminol, and lucigenin as indicators of reactive species formation. *Biochem. Pharmacol.* 65:1575–1582.

Peter, D., Liu, Y., Sternini, C., de Giorgio, R., Brecha, N., and Edwards, R. H. 1995. Differential expression of two vesicular monoamine transporters. *J. Neurosci.* 15:6179–6188.

Reistad, T., Mariussen, E., and Fonnum, F. 2005. The effect of a brominated flame retardant, tetrabromobisphenol-A, on free radical formation in hum, an neutrophil granulocytes: The involvement of the MAP kinase pathway and protein kinase C. Toxicol. Sci. in press. 83:89–100.

Richardson, J. R., and Miller, G. W. 2004. Acute exposure to Aroclor 1016 or 1260 differentially affects dopamine transporter and vesicular monoamine transporter 2 levels. *Toxicol. Lett.* 148:29–40.

Rogan, W. J., and Gladen, B. C. 1992. Neurotoxicology of PCBs and related compounds. *Neurotoxicology* 13:27–35.

Roth-Harer, A., Lilienthal, H., Bubser, M., Kronthaler, U., Schmidt, W., Winterhoff, H., and Winneke, G. 2001. Neurotransmitter concentrations and binding at dopamine receptors in rats after maternal exposure to 3,4,3′,4′-tetrachlorobiphenyl: the role of reduced thyroid hormone concentrations. *Environ. Toxicol. Pharmacol.* 9:103–115.

Schantz, S. L., Widholm, J. J., and Rice, D. C. 2003. Effects of PCB exposure on neuropsychological function in children. *Environ. Health Perspect.* 111:357–576.

Schecter, A., Pavuk, M., Papke, O., Ryan, J. J., Birnbaum, L., and Rosen, R. 2003. Polybrominated diphenyl ethers (PBDEs) in U.S. mothers' milk. *Environ. Health Perspect.* 111:1723–1729.

Seegal, R. F. 1996. Epidemiological and laboratory evidence of PCB-induced neurotoxicity. *Crit. Rev. Toxicol.* 26:709–737.

Seegal, R. F., Okoniewski, R. J., Brosch, K. O., and Bemis, J. C. 2002. Polychlorinated biphenyls alter extraneuronal but not tissue dopamine concentrations in adult rat striatum: An in vivo microdialysis study. *Environ. Health Perspect.* 110:1113–1117.

Shafer, T. J., Mundy, W. R., Tilson, H. A., and Kodavanti, P. R. 1996. Disruption of inositol phosphate accumulation in cerebellar granule cells by polychlorinated biphenyls: A consequence of altered Ca^{2+} homeostasis. *Toxicol. Appl. Pharmacol.* 141:448–455.

Tilson, H. A., and Kodavanti, P. R. 1997. Neurochemical effects of polychlorinated biphenyls: An overview and identification of research needs. *Neurotoxicology* 18:727–743.

Tilson, H. A., Jacobson, J. L., and Rogan, W. J. 1990. Polychlorinated biphenyls and the developing nervous system: Cross-species comparisons. *Neurotoxicol. Teratol.* 12:239–248.

Viberg, H., Fredriksson, A., and Eriksson, P. 2003a. Neonatal exposure to polybrominated diphenyl ether (PBDE 153) disrupts spontaneous behaviour, impairs learning and memory, and decreases hippocampal cholinergic receptors in adult mice. *Toxicol. Appl. Pharmacol.* 192:95–106.

Viberg, H., Fredriksson, A., Jakobsson, E., Orn, U., and Eriksson, P. 2003b. Neurobehavioral derangements in adult mice receiving decabrominated diphenyl ether (PBDE 209) during a defined period of neonatal brain development. *Toxicol. Sci.* 76:112–120.

Viberg, H., Fredriksson, A., and Eriksson, P. 2004. Investigations of strain and/or gender differences in developmental neurotoxic effects of polybrominated diphenyl ethers in mice. *Toxicol. Sci.* 81:344–353.

Voie, O. A., and Fonnum, F. 1998. Ortho substituted polychlorinated biphenyls elevate intracellular [Ca^{2+}] in human granulocytes. *Environ. Toxico. Pharmacol.* 5:105–112.

Voie, O. A., Tysklind, M., Andersson, P. L., and Fonnum, F. 2000. Activation of respiratory burst in human granulocytes by polychlorinated biphenyls: A structure–activity study. *Toxicol. Appl. Pharmacol.* 167:118–124.

Widholm, J. J., Clarkson, G. B., Strupp, B. J., Crofton, K. M., Seegal, R. F., and Schantz, S. L. 2001. Spatial reversal learning in Aroclor 1254-exposed rats: Sex-specific deficits in associative ability and inhibitory control. *Toxicol. Appl. Pharmacol.* 174:188–198.

Wiegand, H., Desaiah, D., Dehnhardt, M., and Smolnikar, K. 2001. Polyhalogenated hydrocarbons induced perturbation of intracellular calcium homeostasis: From astrocytes to human macrophages. *Organohalogen Compounds* 57:182–184.

Wong, P. W., Brackney, W. R., and Pessah, I. N. 1997. Ortho-substituted polychlorinated biphenyls alter microsomal calcium transport by direct interaction with ryanodine receptors of mammalian brain. *J. Biol. Chem*. 272:15145–15153.