Published in final edited form as:
Toxicol Appl Pharmacol. 2011 November 1; 256(3): 281–289. doi:10.1016/j.taap.2011.01.005.

Is the PentaBDE Replacement, Tris (1,3-dichloro-2-propyl) Phosphate (TDCPP), a Developmental Neurotoxicant? Studies in PC12 Cells

Laura V. Dishaw[a], **Christina M. Powers**[b], **Ian T. Ryde**[a], **Simon C. Roberts**[a], **Frederic J. Seidler**[b], **Theodore A. Slotkin**[b], and **Heather M. Stapleton**[a]

[a] Nicholas School of the Environment, Duke University, Durham, NC 27708, USA

[b] Department of Pharmacology & Cancer Biology, Duke University, Durham, NC 27710, USA

Abstract

Organophosphate flame retardants (OPFRs) are used as replacements for the commercial PentaBDE mixture that was phased out in 2004. OPFRs are ubiquitous in the environment and detected at high concentrations in residential dust, suggesting widespread human exposure. OPFRs are structurally similar to neurotoxic organophosphate pesticides, raising concerns about exposure and toxicity to humans. This study evaluated the neurotoxicity of tris (1,3-dichloro-2-propyl) phosphate (TDCPP) compared to the organophosphate pesticide, chlorpyrifos (CPF), a known developmental neurotoxicant. We also tested the neurotoxicity of three structurally similar OPFRs, tris (2-chloroethyl) phosphate (TCEP), tris (1-chloropropyl) phosphate (TCPP), and tris (1,3-dibromopropyl) phosphate (TDBPP), and 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47), a major component of PentaBDE. Using undifferentiated and differentiating PC12 cells, changes in DNA synthesis, oxidative stress, differentiation into dopaminergic or cholinergic neurophenotypes, cell number, cell growth and neurite growth were assessed. TDCPP displayed concentration-dependent neurotoxicity, often with effects equivalent to or greater than equimolar concentrations of CPF. TDCPP inhibited DNA synthesis, and all OPFRs decreased cell number, and altered neurodifferentiation. Although TDCPP elevated oxidative stress, there was no adverse effect on cell viability or growth. TDCPP and TDBPP promoted differentiation into both neuronal phenotypes, while TCEP and TCPP promoted only the cholinergic phenotype. BDE-47 had no effect on cell number, cell growth or neurite growth. Our results demonstrate that different OPFRs show divergent effects on neurodifferentiation, suggesting the participation of multiple mechanisms of toxicity. Additionally, these data suggest that OPFRs may affect neurodevelopment with similar or greater potency compared to known and suspected neurotoxicants.

Address correspondence to: Heather Stapleton, PhD, Nicholas School of the Environment, Duke University, Box 90328, LSRC A220, Durham, NC 27708, Phone: 919–613–8717, Fax: 919–684–8741 heather.stapleton@duke.edu.

Conflicts of interest

TAS has provided expert witness testimony in the past three years at the behest of the following law firms: The Calwell Practice (Charleston WV), Weltchek Mallahan & Weltchek (Lutherville MD), Finnegan Henderson Farabow Garrett & Dunner (Washington DC), Carter Law (Peoria IL), Gutglass Erickson Bonville & Larson (Madison WI), The Killino Firm (Philadelphia PA), Alexander Hawes (San Jose, CA) and the Shanahan Law Group (Raleigh NC).

Keywords

Flame Retardants; Organophosphate; TDCPP; Neurotoxicity; PC12 Cells

Introduction

For several decades, a variety of flame retardant (FR) additives have been applied to polymers and resins found in commercial products, including electronics, furniture, and textiles (Blum *et al.*, 1978; IPCS, 1998; Alaee *et al.*, 2003; Sjodin *et al.*, 2003; Stapleton *et al.*, 2009b). FRs serve to slow the combustion of treated materials and are applied to meet state and federal flammability standards. Polybrominated diphenyl ethers (PBDEs), particularly the commercial mixture known as PentaBDE, were the primary FRs used in polyurethane foam found in furniture manufactured in North America prior to 2004 (Hale *et al.*, 2003). Studies have demonstrated that, over time, PBDEs leach out of treated products, typically accumulating in household dust and resulting in both human and environmental exposures (Hale *et al.*, 2003; Sjodin *et al.*, 2003; Jones-Otazo *et al.*, 2005; Johnson *et al.*, 2010). Additionally, PBDEs are both bioaccumulative and persistent in the environment (de Wit, 2002; Sjodin *et al.*, 2003; Stapleton *et al.*, 2004; Stapleton *et al.*, 2008b), increasing concerns about human health. Elevated PentaBDE body burdens in humans are associated with effects on circulating hormone levels, fecundability, impaired neurodevelopment, and an increased incidence of adverse birth outcomes such as decreased weight and cryptorchidism (Chao *et al.*, 2007; Main *et al.*, 2007; Turyk *et al.*, 2008; Meeker *et al.*, 2009; Harley *et al.*, 2010; Herbstman *et al.*, 2010). In response to the growing body of literature reporting bioaccumulation, persistence, and toxicity, U.S. production of PentaBDE ended in 2004 and its use in commercial products was phased out (Tullo, 2003). Since that time, manufacturers have turned increasingly to alternative FR chemicals to meet flammability standards (Stapleton *et al.*, 2008a; Stapleton *et al.*, 2009b). However, there is no legal requirement to disclose information regarding the chemical identity of FR formulations or the types of products to which they are applied.

We recently identified tris (1,3-dichloro-2-propyl) phosphate (TDCPP), an organophosphate (OP)-based FR (OPFR), as one of the primary FRs now found in polyurethane foam used in furniture (Stapleton *et al.*, 2009b). TDCPP has been detected in U.S. and Japanese dust samples at levels that are similar to PBDEs (Stapleton *et al.*, 2009b; Takigami *et al.*, 2009), suggesting that it has either been used for decades or is a primary PentaBDE replacement. Due to its persistence in the environment (Marklund *et al.*, 2005b; Marklund *et al.*, 2005a; Reemtsma *et al.*, 2008) and abundance in residential dust samples (Stapleton *et al.*, 2009a; Takigami *et al.*, 2009), TDCPP exposure is likely to mimic that of PBDEs. Furthermore, these findings suggest that, on a daily basis, the majority of the U.S. population is exposed to TDCPP and other OPFRs currently on the market.

OPFRs have been in use since the late 1970s (Reemtsma *et al.*, 2008) and as early as the 1980s were known to accumulate in human adipose tissue (Lebel and Williams, 1983; Lebel *et al.*, 1989) and seminal fluid (Hudec *et al.*, 1981). Recently, elevated levels of OPFRs, including TDCPP, in house dust were associated with altered hormone levels and decreased sperm quality in men (Meeker and Stapleton, 2010). The primary route of FR exposure is believed to be inhalation or inadvertent ingestion of contaminated dust (Jones-Otazo *et al.*, 2005; Lorber, 2008). Current models estimate that children ages 1–5 ingest anywhere from 2-10x the amount of dust as an adult, indicating that children are likely have the highest exposures to FR chemicals (U.S.EPA, 2008). Given their structural similarity to OP pesticides, such as chlorpyrifos (CPF), OPFRs may also act as developmental neurotoxicants (Figure 1). Importantly, the OP pesticides, including CPF, exert many neurodevelopmental

effects through mechanisms unrelated to acute toxicity via cholinesterase inhibition (Pope, 1999; Slotkin, 2005), indicating that OPFRs may exert similar neurodevelopmental actions.

There is very limited human health and toxicity data available for TDCPP (Blum *et al.*, 1978; Hudec *et al.*, 1981; NRC, 2000; Honkakoski *et al.*, 2004; Meeker and Stapleton, 2010). This study was initiated to compare the effects of TDCPP to that of CPF, a known developmental neurotoxicant (Song *et al.*, 1998; Qiao *et al.*, 2001; Sledge *et al.*, 2009), using an *in vitro* model of neurodevelopment. Using the same experimental model, we then compared the neurotoxicity of TDCPP to that of three structurally similar OPFRs: tris (2-chloroethyl) phosphate (TCEP), tris (1-chloropropyl) phosphate (TCPP), and tris (1,3-dibromopropyl) phosphate (TDBPP). All three of these OPFRs have been or are currently used as flame retardant additives in consumer products. Finally, we compared the effects of TDCPP to 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47), a major component of the commercial PentaBDE mixture that has been found to bioaccumulate most readily in human tissues (Hale *et al.*, 2003; Sjodin *et al.*, 2008). Experiments were conducted in PC12 cells, a widely used *in vitro* model for neurotoxicity that has been shown to reproduce key mechanisms and features of *in vivo* developmental neurotoxicity of OP pesticides (Song *et al.*, 1998; Qiao *et al.*, 2001; Qiao *et al.*, 2005; Jameson *et al.*, 2006).

Materials and methods

All of the techniques used in this study have been reported previously (Song *et al.*, 1998; Qiao *et al.*, 2001; Qiao *et al.*, 2003; Qiao *et al.*, 2005; Jameson *et al.*, 2006), therefore only brief descriptions are provided here.

Cell cultures

Experiments were performed on cells that had undergone fewer than five passages. PC12 cells (American Type Culture Collection, 1721-CRL) obtained from the Duke Comprehensive Cancer Center (Durham, NC) were grown in RPMI-1640 medium (Sigma Chemical Co., St. Louis, MO). Medium was supplemented with 10% inactivated horse serum (Sigma), 5% fetal bovine serum (Sigma), and 50 μg/mL penicillin/streptomycin (Invitrogen, Carlsbad, CA). Cells were maintained at 37°C with 5% CO_2.

For studies in the undifferentiated state, the culture medium was changed 24 hr after seeding to include one of the following test agents: 50 μM CPF (98.8% purity; Chem Service, West Chester, PA) or 10, 20, or 50 μM TDCPP (99% purity as measured by GC-ECNI-MS; Chem Service). For studies in differentiating cells, the medium was changed 24 hr after seeding to include 50 ng/mL of 2.5S murine nerve growth factor (NGF; Promega, Madison, WI). The addition of NGF to the culture medium cues PC12 cells to exit the cell cycle and initiate phenotypic differentiation. All cultures were examined under a microscope to verify the subsequent outgrowth of neurites. In addition to the test agents used for experiments in undifferentiated cultures, the following chemicals were used for experiments with differentiating cell cultures: 10, 20, or 50 μM BDE-47 (100% Purity, AccuStandard, New Haven, CT), or 50 μM TCEP (97% purity; Sigma-Aldrich, St. Louis, MO), TCPP (96% purity; Pfaltz and Bauer, Waterbury, CT), or TDBPP (95.5% purity; Supelco Analytical, Bellefonte, PA). Test agents were added concurrently with the start of NGF treatment and cultures were maintained for up to 6 days. Medium renewal occurred at 48 hr intervals with the indicated agents included with every medium change.

Due to the limited water solubility of some of the test compounds, all were dissolved in dimethyl sulfoxide (DMSO). Controls included DMSO to match the final concentration achieved in the culture medium (0.1%). Previous studies have shown that this concentration of DMSO has no effect on PC12 cell replication or differentiation (Song *et al.*, 1998; Qiao *et*

al., 2001; Qiao *et al.*, 2003). The CPF concentration that was chosen for comparison with TDCPP is known to elicit a robust effect on cell replication and differentiation, but is below the threshold for outright cytotoxicity or loss of cell viability (Song *et al.*, 1998; Qiao *et al.*, 2001; Qiao *et al.*, 2003; Qiao *et al.*, 2005; Jameson *et al.*, 2006; Slotkin *et al.*, 2007a)

DNA and proteins

Cells were harvested, washed, and the DNA and protein fractions were isolated and analyzed as described previously (Song *et al.*, 1998; Slotkin *et al.*, 2007a). Changes in cell number, cell growth, and neurite growth were measured indirectly by calculating the total DNA content per dish (μg/dish), total protein to DNA (tP:DNA) ratio, and membrane to total protein (mP:tP) ratio, respectively. Neuronal cells contain a single nucleus, thus, the total DNA content per dish provides a measure of cell number. Similarly, although cell growth will increase the total protein per cell, the DNA per cell remains constant, so that the tP:DNA ratio provides an index of cell growth. If cell growth entails simply an increase in the perikaryon, then the mP:tP ratio will fall in parallel with the decline in the surface-to-volume ratio; however, when neurites are formed as a consequence of neurodifferentiation, this produces a specific *rise* in the mP:tP ratio.

Effects on DNA synthesis were assessed by measuring changes in the extent of ^{3}H-thymidine incorporation into the DNA fraction (Song *et al.*, 1998; Slotkin *et al.*, 2007a). To initiate this measurement, the medium was changed to include 1 μCi/mL of ^{3}H-thymidine (specific activity, 2 Ci/mmol; PerkinElmer Life and Analytical Sciences, Boston, MA) and the corresponding test agents. One hour later, the medium was aspirated, cells harvested, and the DNA and protein fractions isolated and quantified for radiolabel and total DNA content.

Oxidative stress

Oxidative stress was assessed through reaction with thiobarbituric acid to measure formation of malondialdehyde (MDA), a byproduct of lipid peroxidation (Qiao *et al.*, 2005). The MDA values were calculated relative to the DNA content to give the MDA concentration per cell.

Cell viability

Cell viability was assessed using a dye exclusion method where uptake of stain into the cytoplasm is indicative of dead or dying cells. The cell culture medium was changed to include trypan blue (Invitrogen; 1 vol per 2.5 vol medium). Cells were then examined for staining with a Zeiss Axio Observer (Carl Zeiss MicroImaging, Thornwood, NY) at 100× magnification. Scoring was performed independently by two blinded observers. An average of 100 cells per field was counted in two fields per culture.

Enzyme activities

Choline acetyltransferase (ChAT) and tyrosine hydroxylase (TH) assays were conducted according to published techniques (Lau *et al.*, 1988) to assess differentiation into the cholinergic or dopaminergic phenotypes, respectively. Briefly, radiolabeled enzyme substrates specific to the cholinergic (^{14}C-acetyl coenzyme A; specific activity, 60 mCi/mmol; PerkinElmer) and dopaminergic (^{14}C-λ-tyrosine; specific activity, 51.0 mCi/mmol isotopically diluted to 3.33 mCi/mmol, Moravek Biochemicals, Brea, CA) phenotypes were added to separate aliquots of culture homogenates. The enzyme products were then isolated and measured for radiolabel. Enzyme activities were calculated relative to the DNA content to give the activity per cell.

Data analysis

For TDCPP experiments assessing changes in DNA synthesis (24 hour exposure), cell number, cell growth, neurite growth, oxidative stress, cell viability (4 day exposure), and phenotypic differentiation (6 day exposure), measurements were performed on 8–10 cultures for each treatment, using one to two separate cell batches. For TDCPP experiments assessing changes in cell number, cell growth, and neurite growth (6 day exposure), measurements were performed on 15–23 cultures for each treatment, using three separate cell batches. We evaluated two analysis of variance (ANOVA) factors (treatment x cell batch) and found that the results did not vary significantly between different cell batches; therefore, results across different batches were normalized and combined for presentation. Comparisons between OPFRs were performed on 4–5 separate cultures from a single cell batch. BDE-47 studies were performed on 8–10 cultures for each treatment, using a single cell batch. The results are presented as the mean ± SEM. Comparisons between treatments were carried out by ANOVA followed by a post hoc Fisher's protected least significant difference. Significance was assumed at $p < 0.05$ (two-tailed).

Results

TDCPP studies

Undifferentiated PC12 cells exposed to TDCPP for 24 hrs showed a significant concentration-dependent reduction in DNA synthesis, as indicated by decreased incorporation of ^{3}H-thymidine into the DNA fraction. The highest concentration of TDCPP produced an inhibitory effect on DNA synthesis equivalent to that seen with an equimolar concentration of CPF (Figure 2A). Following both 4 and 6 day exposures, differentiating cultures showed a progressive and significant reduction in DNA content, a measure of cell number, with increasing TDCPP concentrations (Figure 2B–C). Interestingly, the magnitude of the effect on cell number was increased significantly with exposure duration: cultures treated with 50 μM TDCPP showed a 22% decrease in DNA on day 4, whereas by day 6 this effect nearly doubled, with cultures showing a 39% decrease (ANOVA: treatment × time, $p = 0.001$). At both time points, TDCPP elicited a notably greater decrement in cell number when compared with an equal concentration of CPF (4 day: $p = 0.0016$; 6 day: $p < 0.0001$). Although exposure to TDCPP resulted in substantial reductions in cell number, it did not adversely affect the tP:DNA ratio, an index of cell growth. In fact, the tP:DNA ratio was significantly elevated in cultures treated with 50 μM TDCPP, suggesting an increase in cell growth (Figure 2D–E; 4 day: $p < 0.0001$; 6 day: $p = 0.021$).

To determine mechanisms underlying cell loss, we assessed the effects on oxidative stress and cell viability in differentiating cells following 4 day exposures. MDA, a byproduct of lipid peroxidation, was used as a measure of oxidative stress. Although there was no effect at the 10 or 20 μM concentrations, cultures treated with 50 μM TDCPP showed a 22% increase in lipid peroxidation ($p < 0.0001$; Figure 2F). Cells exposed to CPF also showed increased levels of MDA, however, they were significantly lower than that seen with the highest concentration of TDCPP ($p = 0.0162$). Interestingly, although lipid peroxidation was increased by CPF and 50 μM TDCPP we did not observe any significant decrements in cell viability, as measured by trypan blue exclusion, over the same exposure period (data not shown).

All concentrations of TDCPP promoted neurodifferentiation into both the dopaminergic and cholinergic phenotypes, as indicated by significant increases in TH ($p < 0.005$) and ChAT ($p < 0.01$) activity, respectively (Figure 3A–B). While the effect on the cholinergic phenotype was equivalent for all TDCPP exposure doses, when compared to the moderate and low concentrations, cultures treated with 50 μM TDCPP displayed significantly greater

elevations in TH activity ($p < 0.0001$). Consequently, for the high TDCPP concentration only, there was a net shift in cell fate favoring differentiation into the dopaminergic phenotype. In addition to expression of phenotype specific enzymes, the process of neurodifferentiation also entails neurite formation which is measured by the mP:tP ratio. Although TDCPP promoted phenotypic expression, there was no effect on the mP:tP ratio (data not shown).

OPFR comparison

The effects of 50 μM TDCPP, TCEP, TCPP and TDBPP on cell number, cell growth, and phenotypic expression were compared in differentiating PC12 cells after a 6 day exposure. Across all measures, the effects of TDBPP were indistinguishable from TDCPP (Fig 4A–C). All test chemicals elicited significant deficits in cell number ($p < 0.005$); however, the greatest effects were observed for TDCPP and TDBPP ($p < 0.0001$; Figure 4A). As with the TDCPP studies, OPFR treatments did not impair cell growth (data not shown) despite the profound effects on cell number. All four of the OPFRs tested promoted emergence of the cholinergic phenotype (Figure 4B; $p < 0.008$). Although TDCPP and TDBPP also robustly promoted emergence of the dopaminergic phenotype ($p < 0.0001$), TH activity was not affected by treatment with TCEP or TCPP (Figure 4C). Accordingly, TCEP and TCPP shifted differentiation in favor of the cholinergic phenotype.

BDE-47 studies

Changes in cell number, cell growth, and neurite growth were assessed in differentiating cultures following a 6 day exposure to 10, 20, or 50 μM BDE-47. 50 μM TDCPP was used as a positive control. Across all measures, no significant effect was observed regardless of the BDE-47 concentration (Figure 4D–F). Importantly, the lack of effect on DNA content and mP:tP ratio was statistically distinguishable from concurrently-run TDCPP samples ($p < 0.05$). Although there was no effect on cell growth, cultures treated with 50 μM BDE-47 showed an increase in the mP:tP ratio that approached significance ($p = 0.0501$).

Discussion

Our studies show that OPFRs have the potential to elicit developmental neurotoxicity in a manner similar to OP pesticides, a class of chemicals that are widely recognized to damage the developing brain (Song *et al.*, 1998; Qiao *et al.*, 2001; Sledge *et al.*, 2009). Importantly, we observed adverse effects in both the undifferentiated state and during neurodifferentiation, implying that the developing nervous system is likely to be vulnerable to disruption by OPFRs beginning in the earliest events of neural cell division and extending through later events of phenotype selection and formation of neural circuits. Notably, in the TDCPP studies, adverse effects were generally observed at concentrations less than or equivalent to those required to elicit the same effects with CPF.

Treatment with TDCPP resulted in rapid mitotic inhibition in undifferentiated cultures and profoundly reduced cell numbers during neurodifferentiation. Although TDCPP induced oxidative stress in PC12 cells, we did not observe any impairment of cell growth or loss of cell viability that would be expected if cell losses were being driven by cytotoxicity. Consequently, the present findings do not indicate that nonspecific cytotoxicity is the underlying mechanism for these effects. Rather, our results suggest that promotion of neurodifferentiation in the absence of NGF, the appropriate biochemical signal, is the critical mechanism for TDCPP toxicity and, potentially, all OPFRs tested here. Exposure to TDCPP enhanced TH and ChAT activity, hallmarks of phenotypic differentiation in PC12 cells. Since neurodifferentiation entails the loss of mitotic activity, this alone could explain the observed declines in DNA synthesis and resultant reductions in the number of cells

without compromising overall cell growth. Indeed, the increases in cell growth observed in the TDCPP studies are consistent with a promotional effect on neurodifferentiation, a process that also involves cell enlargement and neurite formation. There was no change in the mP:tP ratio despite the fact that TDCPP elicited an increase in cell growth, implying that there was an increase in neurite formation relative to controls. Had cell enlargement involved only the perikaryon, the mP:tP ratio would have fallen due to a decrease in the surface to volume ratio of the cell. This conclusion is further supported by the effects of TDCPP on cell and neurite growth in the BDE-47 studies; although the tP:DNA ratio was not increased significantly, the mP:tP ratio was significantly elevated, again indicating increased neurite extension.

It is possible that the small degree of oxidative stress observed in the TDCPP studies may play a role in the observed outcomes. In PC12 cells, levels of oxidative stress that are insufficient to compromise cell viability are known to initiate neurodifferentiation in the absence of NGF (Katoh *et al.*, 1997). A number of recent studies have identified associations between elevations in biomarkers of oxidative stress and neurodevelopmental disorders (Ross *et al.*, 2003; Carter, 2006; James *et al.*, 2006; Wood *et al.*, 2009; Taurines *et al.*, 2010). Sajedl-Sulkowska et al. (2009) reported increased levels of oxidative stress and neurotrophin-3, a regulator of replication and differentiation during neurodevelopment, in autistic brains. Similarly, children diagnosed with attention deficit hyperactivity disorder were found to have elevated biomarkers of oxidative stress (Ross *et al.*, 2003)

It is important to note that differences were observed between the OP compounds, particularly with regard to the effects on neurodifferentiation. The greatest similarities were observed between compounds with similar halogenation substitution patterns, suggesting the presence of a structure activity relationship. Across all tested measures, the effects of TDCPP and TDBPP, which differ only by the type of halogen substituent, were nearly identical, with each promoting differentiation into both the cholinergic and dopaminergic phenotypes. TCEP and TCPP, however, which differ from TDCPP and TDBPP most notably by the number halogen substituents, showed distinct effects on neurodifferentiation; these OPFRs promoted emergence of the cholinergic phenotype without affecting dopaminergic expression. Thus, the results of these studies suggest that the difference in molecular size due to the presence of two different halogen atoms (i.e. bromine and chlorine) had little effect. Rather, differences in the halogen substitution patterns appear to be an important factor in determining the effect of OPFRs on phenotypic fate.

CPF, on the other hand, contains a phosphorus-sulfur bond with chlorine atoms attached to an ester-linked pyridine ring rather than an alkane chain, resulting in a distinct pattern of differentiation. In agreement with previous findings, CPF produced a shift in phenotypic expression in favor of the dopaminergic phenotype through promotion of TH combined with impairment of ChAT (Slotkin, 2005; Jameson *et al.*, 2006). CPF and other OP pesticides containing a phosphorus-sulfur bond undergo bioactivation by desulfuration via cytochrome P450 enzymes to form an oxon metabolite; these metabolites irreversibly bind to cholinesterase and, thus, are much more potent cholinesterase inhibitors (Poet *et al.*, 2003; Eaton *et al.*, 2008). Interestingly, however, when the developmental neurotoxicity of chlorpyrifos and its oxon-metabolite were compared, the parent compound was found to have a significantly greater effect, indicating that OPs are able to elicit effects through mechanisms independent of cholinesterase inhibition (Pope, 1999; Qiao *et al.*, 2001; Slotkin, 2005).

Some of the molecular mechanisms that drive differences in cell fate for OP pesticides are known. These include targeted activation and suppression of genes known to regulate critical neurotrophic factors involved in neurodevelopment, including the fibroblast growth

factor family, wingless, and frizzled (Slotkin *et al.*, 2007b; Slotkin *et al.*, 2008). It is possible that, like OP pesticides, exposure to OPFRs alters gene expression with certain targets being unique to specific compounds and structures (Slotkin *et al.*, 2007b; Slotkin *et al.*, 2008). Due to the similarities observed between the effects of TDCPP and CPF, the present findings suggest that neurotrophic factors are likely to be targeted by OPFRs as well. Changes in gene expression may dictate the observed effects on neurophenotypic fate, which in turn, could translate to disparities in behavioral or functional outcomes *in vivo.*

Regardless of the underlying mechanism, premature loss of neuronal cell replication, dysregulation of pathways involved in neurodifferentiation, and changes in phenotypic outcome that favor one neurotransmitter at the expense of another are likely to produce extensive miswiring of neuronal circuitry in the developing organism. Thus, if TDCPP elicits effects in the developing brain similar to those observed in the present studies, *in vivo* exposures will likely result in persistent cognitive and behavioral deficits akin to those seen with CPF. Importantly, all of the OPFRs tested in the present study elicited similar decrements in cell number and altered the phenotypic fate of differentiating cells. These data indicate that, as a whole, this class of compounds may be developmentally neurotoxic. Early brain development is characterized by periods of proliferation, migration, and differentiation, the timing of which are critical (Bondy and Campbell, 2005; Mueller and Wullimann, 2005). Disruption of these developmental stages, as seen here with OPFRs, may lead to irrevocable changes in brain function due to deficiencies in the number of neurons and altered neurodifferentiation.

Young children who are still undergoing brain maturation are expected to have the highest exposures to FR chemicals because of specific behaviors (crawling, hand-to-mouth contact) that elevate their intake of household dust (Bondy and Campbell, 2005; Jones-Otazo *et al.*, 2005; Lorber, 2008). A recent report by the EPA estimates that children ages 1–5 years ingest approximately 100–200 mg dust/day, whereas adults are predicted to ingest only 20–50 mg dust/day (U.S.EPA, 2008). Indeed, studies report that children living in California exhibit 2- to 10-fold higher concentrations of PBDEs when compared to the average American adult (Fischer *et al.*, 2006; Rose *et al.*, 2010). Both adolescent and adult rodent offspring that are perinatally exposed to PBDEs show slowed motor skill development (Branchi *et al.*, 2002) and changes in synaptic plasticity and long term potentiation in the hippocampus, a brain region involved in learning and memory (Dingemans *et al.*, 2007; Xing *et al.*, 2009). Correspondingly, neonatal administration of PBDEs in mice impaired adult performance on a Morris water maze, which assesses the ability to learn and recall the location of a hidden underwater platform (Viberg *et al.*, 2003; Viberg *et al.*, 2006). These effects were attributed to inhibition of the cholinergic system, which plays an important role in learning and memory, following developmental exposure to PBDEs (Viberg *et al.*, 2003). Although the exposure levels of the different PBDE congeners used in these studies varied, ranging from 0.45– 68 mg/kg, adverse neurological effects were often observed at doses >10 mg/kg. Recently, Herbstman et al. (2010) found similar relationships between PBDE exposure and neurodevelopmental deficits in humans; high concentrations of PBDEs in cord blood, an estimate of prenatal exposure, were associated with delayed motor development and lower IQ scores at several time points over a 72 month period.

Interestingly, despite the large body of literature showing adverse outcomes following developmental exposure to PBDEs, no significant effects were observed on measures of cell number, cell growth, or neurite growth following exposure to BDE-47. These findings suggest either that OPFRs are more potent neurotoxicants than PBDEs on these measures or their developmental neurotoxicity is elicited through mechanisms that were not evaluated in these studies. The pathways and products of metabolism are a critical aspect of toxicology. In rodent models, PBDEs are generally metabolized through cytochrome P450-mediated

oxidative reactions to form hydroxylated metabolites (Marsh *et al.*, 2006; Qiu *et al.*, 2007). Several studies by Dingemans *et al.* (2008; 2010a; 2010b) directly exposed PC12 cells to hydroxylated PBDE metabolites and found alterations in calcium homeostasis and neurotransmitter release at much lower concentrations than the parent compound, suggesting that these chemicals are bioactivated via oxidative metabolism. Importantly, cytochrome P450 enzymes are not normally expressed by PC12 cells (Mapoles *et al.*, 1993), which may explain the lack of effect observed in the present studies. Although metabolism of OPFRs have yet to be evaluated in humans, OP compounds are believed to be metabolized most readily by esterases (Costa *et al.*, 2005). Lynn *et al.* (1981) evaluated TDCPP metabolism in rats and identified bis (1,3-dichloro-2-propyl) phosphate (BDCPP) as the primary metabolite in urine. Aliquots of culture media from dishes treated with 50 μM TDCPP were taken immediately prior to media changes and analyzed with LC/MS-MS for the presence of BDCPP. We found significant formation of this compound (data not shown) therefore it is possible that the observed effects in the TDCPP studies were mediated by the BDCPP metabolite rather than the parent compound.

Our studies raise concerns that OPFRs, which are now being used increasingly as PentaBDE replacements, may be neurotoxicants. TDBPP was commonly used to treat children's sleepwear prior to 1977; its use was discontinued following findings that it was mutagenic, carcinogenic, and mutagenic metabolites were detected in children's urine (Blum and Ames, 1977; Blum *et al.*, 1978; Gold *et al.*, 1978). TDCPP became the primary replacement for TDBPP in children's pajamas, despite data indicating that it too acted as a weak mutagen (Gold *et al.*, 1978). The use of TDCPP in children's sleepwear was eventually discontinued (Lynn *et al.*, 1981), yet the levels measured in U.S. house dust (Stapleton *et al.*, 2009b) indicate that exposure in children continues. With respect to decrements in cell number and altered phenotypic differentiation, however, the effects of TDCPP and TDBPP are identical. Similarly, TCEP is recognized as a carcinogen by both the World Health Organization and the State of California and its use has been largely replaced by TDCPP and TCPP (IPCS, 1998; Stapleton *et al.*, 2009a). The results of this study point to the importance of endpoints other than mutagenesis and carcinogenesis in evaluating the toxicity of OPFRs, especially in light of evidence showing that children have the highest exposures. Furthermore, the possibility of *in utero* developmental effects as a result of maternal exposure has yet to be fully explored. Although it is unclear to what extent the chemicals tested here cross the placental barrier, Ahmed et al. (1993) reported accumulation of low levels of the OPFR, tri-o-cresyl phosphate, in mouse fetal brain and spinal cord following a single maternal exposure. Clearly, the potential for prenatal OPFR exposure should not be dismissed. Indeed, we observed profound effects on neural cell replication and neurodifferentiation, processes that are critical to early neurodevelopment.

The *in vitro* PC12 cell model chosen for these experiments provides a number of advantages and disadvantages in evaluating the neurotoxicity of potential toxicants. We were able to assess the direct effects of toxicants on neuronal cell replication, growth, and differentiation in the absence of many of the confounding variables found *in vivo*, such as stress, nutrition or confounds involving maternal factors. Also, in PC12 cells, differentiation is triggered by the addition of NGF to the culture media, allowing examination of effects at specific stages of neurodevelopment, beginning at cell replication and extending though differentiation; these types of determinations require coordinated and uniform differentiation of the cell population, a situation that cannot be modeled *in vivo*. *In vitro* models, however, are limited in that they do not to account for many factors that can affect exposure and toxicity *in vivo*. PC12 cultures do not reproduce the complex three dimensional architecture of the mammalian brain and, thus, cannot detect toxicity that is mediated by cell-to-cell interactions. Also, *in vitro* exposures are relatively short, lasting for hours to days, while *in vivo* exposures may extend throughout the course of neurodevelopment. Furthermore, as a

transformed cell line, PC12 cells are resistant to the effects of chemicals, generally requiring a higher concentrations to elicit an effect than would be needed *in vivo*. Notably, these limitations are likely to result in an *underestimation* of neurotoxicity, reinforcing the importance of the present results.

Due to the ubiquitous nature of OPFRs in both indoor and outdoor environments (Marklund *et al.*, 2003; Reemtsma *et al.*, 2008; Stapleton *et al.*, 2009b), it is likely that the general population and, particularly, children are subject to chronic exposure. Although the present work represents a significant step forward in our understanding of the potential toxicity of TDCPP and other OPFRs, more work is necessary to further characterize the effects this class of chemicals will have on development. Additional studies are needed to identify potential OPFR gene targets and determine whether these mechanisms can predict similarities and differences between the effects of different OPFRs. Furthermore, since the present data suggest that there may be an even greater sensitivity to OPFRs *in utero*, an examination of the extent of placental transfer and bioaccumulation in fetal tissues is warranted.

Acknowledgments

This work was supported by R01 ES016099 and P30 ES011961 from the National Institute of Environmental Health Sciences (NIEHS).

Abbreviations

ANOVA	Analysis of variance
BDE-47	2,2′,4,4′-tetrabromodiphenyl ether
BDCPP	Bis (1,3-dichloropropyl) phosphate
ChAT	Choline acetyltransferase
CPF	Chlorpyrifos
DMSO	Dimethyl sulfoxide
FR	Flame retardant
MDA	Malondialdehyde
mP	tP, Membrane to total protein ratio
NGF	Nerve growth factor
OP	Organophosphate
OPFR	Organophosphate flame retardant
PBDE	Polybrominated diphenyl ether
PentaBDE	Pentabrominated diphenyl ether
TCEP	Tris (1-chloro-2-propyl) phosphate
TCPP	Tris (2-chloroethyl) phosphate
TDBPP	Tris (1,3-dibromo-2-propyl) phosphate
TDCPP	Tris (1,3-dichloro-2-propyl) phosphate
TH	Tyrosine hydroxylase
TPP	Triphenyl phosphate

tP DNA, Total protein to DNA ratio

References

Ahmed AE, Jacob S, Soliman S, Ahmed N, Osman K, Loh JP, Romero N. Whole-Body Autoradiographic Disposition, Elimination and Placental Transport of C-14 Tri-O-Cresyl Phosphate in Mice. J Appl Toxicol. 1993; 13:259–267. [PubMed: 8376726]

Alaee M, Arias P, Sjodin A, Bergman A. An overview of commercially used brominated flame retardants, their applications, their use patterns in different countries/regions and possible modes of release. Environment International. 2003; 29:683–689. [PubMed: 12850087]

Blum A, Ames BN. Flame-Retardant Additives as Possible Cancer Hazards. Science. 1977; 195:17–23. [PubMed: 831254]

Blum A, Gold MD, Ames BN, Kenyon C, Jones FR, Hett EA, Dougherty RC, Horning EC, Dzidic I, Carroll DI, Stillwell RN, Thenot JP. Children Absorb Tris-BP Flame-Retardant from Sleepwear - Urine Contains Mutagenic Metabolite, 2,3-Dibromopropanol. Science. 1978; 201:1020–1023. [PubMed: 684422]

Bondy SC, Campbell A. Developmental neurotoxicology. Journal of Neuroscience Research. 2005; 81:605–612. [PubMed: 16035107]

Branchi I, Alleva E, Costa LG. Effects of perinatal exposure to a polybrominated diphenyl ether (PBDE 99) on mouse neurobehavioural development. NeuroToxicology. 2002; 23:375–384. [PubMed: 12387364]

Carter CJ. Schizophrenia susceptibility genes converge on interlinked pathways related to glutamatergic transmission and long-term potentiation, oxidative stress and oligodendrocyte viability. Schizophrenia Research. 2006; 86:1–14. [PubMed: 16842972]

Chao HR, Wang SL, Lee WJ, Wang YF, Papke O. Levels of polybrominated diphenyl ethers (PBDEs) in breast milk from central Taiwan and their relation to infant birth outcome and maternal menstruation effects. Environment International. 2007; 33:239–245. [PubMed: 17079016]

Costa LG, Cole TB, Vitalone A, Furlong CE. Measurement of paraoxonase (PON1) status as a potential biomarker of susceptibility to organophosphate toxicity. Clinica Chimica Acta. 2005; 352:37–47.

de Wit CA. An overview of brominated flame retardants in the environment. Chemosphere. 2002; 46:583–624. [PubMed: 11999784]

Dingemans MML, de Groot A, van Kleef R, Bergman A, van den Berg M, Vijverberg HPM, Westerink RHS. Hydroxylation increases the neurotoxic potential of BDE-47 to affect exocytosis and calcium homeostasis in PC12 cells. Environmental Health Perspectives. 2008; 116:637–643. [PubMed: 18470311]

Dingemans MML, Heusinkveld HJ, Bergman A, van den Berg M, Westerink RHS. Bromination Pattern of Hydroxylated Metabolites of BDE-47 Affects Their Potency to Release Calcium from Intracellular Stores in PC12 Cells. Environmental Health Perspectives. 2010a; 118:519–525. [PubMed: 20368133]

Dingemans MML, Ramakers GMJ, Gardoni F, van Kleef RGDM, Bergman Ãk, Di Luca M, Van den Berg M, Westerink RHS, Vijverberg HPM. Neonatal Exposure to Brominated Flame Retardant BDE-47 Reduces Long-Term Potentiation and Postsynaptic Protein Levels in Mouse Hippocampus. Environmental Health Perspectives. 2007:865–870. Superintendent of Documents. [PubMed: 17589592]

Dingemans MML, van den Berg M, Bergman A, Westerink RHS. Calcium-Related Processes Involved in the Inhibition of Depolarization-Evoked Calcium Increase by Hydroxylated PBDEs in PC12 Cells. Toxicological Sciences. 2010b; 114:302–309. [PubMed: 20044592]

Eaton DL, Daroff RB, Autrup H, Bridges J, Buffler P, Costa LG, Coyle J, McKhann G, Mobley WC, Nadel L, Neubert D, Schulte-Hermann R, Spencer PS. Review of the toxicology of chlorpyrifos with an emphasis on human exposure and neurodevelopment. Crit Rev Toxicol. 2008; 38(Suppl 2):1–125. [PubMed: 18726789]

Fischer D, Hooper K, Athanasiadou M, Athanassiadis I, Bergman A. Children show highest levels of polybrominated diphenyl ethers in a California family of four: a case study.(Children's Health). Environmental Health Perspectives. 2006; 114:1581, 1584. [PubMed: 17035146]

Gold MD, Blum A, Ames BN. Another Flame-Retardant, Tris (1,3-dichloro-2-propyl) phosphate, and Its Expected Metabolites are Mutagens. Science. 1978; 200:785–787. [PubMed: 347576]

Hale RC, Alaee M, Manchester-Neesvig JB, Stapleton HM, Ikonomou MG. Polybrominated diphenyl ether flame retardants in the North American environment. Environment International. 2003; 29:771–779. [PubMed: 12850095]

Harley KG, Marks AR, Chevrier J, Bradman A, Sjödin A, Eskenazi B. PBDE Concentrations in Women's Serum and Fecundability. Environ Health Perspect. 2010:118.

Herbstman JB, Sjodin A, Kurzon M, Lederman SA, Jones RS, Rauh V, Needham LL, Tang D, Niedzwiecki M, Wang RY, Perera F. Prenatal Exposure to PBDEs and Neurodevelopment. Environmental Health Perspectives. 2010; 118:712–719. [PubMed: 20056561]

Honkakoski P, Palvimo JJ, Penttilä L, Vepsäläinen J, Auriola S. Effects of triaryl phosphates on mouse and human nuclear receptors. Biochemical Pharmacology. 2004; 67:97–106. [PubMed: 14667932]

Hudec T, Thean J, Kuehl D, Dougherty RC. Tris (dichloropropyl) phosphate, a Mutagenic Flame Retardant: Frequent Occurrence in Human Seminal Plasma. Science. 1981; 211:951–952. [PubMed: 7466368]

IPCS. Flame Retardants: Tris (chloropropyl) phosphate and tris (2-chloroethyl) phosphate. World Health Organization; Geneva: 1998.

James SJ, Melnyk S, Jernigan S. Oxidative stress in children with autism: Metabolic biomarkers and genetic polymorphisms. NeuroToxicology. 2006; 27:893–894.

Jameson RR, Seidler FJ, Qiao D, Slotkin TA. Chlorpyrifos affects phenotypic outcomes in a model of mammalian neurodevelopment: Critical stages targeting differentiation in PC12 cells. Environmental Health Perspectives. 2006; 114:667–672. [PubMed: 16675418]

Johnson PI, Stapleton HM, Slodin A, Meeker JD. Relationships between Polybrominated Diphenyl Ether Concentrations in House Dust and Serum. Environ Sci Technol. 2010; 44:5627–5632. [PubMed: 20521814]

Jones-Otazo HA, Clarke JP, Diamond ML, Archbold JA, Ferguson G, Harner T, Richardson GM, Ryan JJ, Wilford B. Is house dust the missing exposure pathway for PBDEs? An analysis of the urban fate and human exposure to PBDEs. Environ Sci Technol. 2005; 39:5121–5130. [PubMed: 16082939]

Katoh S, Mitsui Y, Kitani K, Suzuki T. Hyperoxia induces the differentiated neuronal phenotype of PC12 cells by producing reactive oxygen species. Biochemical and Biophysical Research Communications. 1997; 241:347–351. [PubMed: 9425274]

Lau C, Seidler FJ, Cameron AM, Navarro HA, Bell JM, Bartolome J, Slotkin TA. Nutritional influences on adrenal chromaffin cell development: comparison with central neurons. Pediatric Research. 1988; 24:583–587. [PubMed: 2905035]

Lebel GL, Williams DT. Determination of Organic Phosphate Triesters in Human Adipose-tissue. Journal of the Association of Official Analytical Chemists. 1983; 66:691–699.

Lebel GL, Williams DT, Berard D. Triaryl Alkyl Phosphate Residues in Human Adipose Autopsy Samples from 6 Ontario Municipalities. Bulletin of Environmental Contamination and Toxicology. 1989; 43:225–230. [PubMed: 2775890]

Lorber M. Exposure of Americans to polybrominated diphenyl ethers. Journal of Exposure Science & Environmental Epidemiology. 2008; 18:2–19. [PubMed: 17426733]

Lynn RK, Wong K, Garvie-Gould C, Kennish JM. Disposition of the flame retardant, tris(1,3-dichloro-2-propyl) phosphate, in the rat. Drug Metabolism and Disposition. 1981; 9:434–441. [PubMed: 6117442]

Main KM, Kiviranta H, Virtanen HE, Sundqvist E, Tuomisto JT, Tuomisto J, Vartiainen T, Skakkebæk NE, Toppari J. Flame Retardants in Placenta and Breast Milk and Cryptorchidism in Newborn Boys. Environ Health Perspect. 2007:115. [PubMed: 18174959]

Mapoles J, Berthou F, Alexander A, Simon F, Menez J. Mammalian PC 12 cell genetically engineered for human cytochrome P450 2E1 expression. European Journal of Biochemistry. 1993; 214:735–745. [PubMed: 8391436]

Marklund A, Andersson B, Haglund P. Screening of organophosphorus compounds and their distribution in various indoor environments. Chemosphere. 2003; 53:1137–1146. [PubMed: 14512118]

Marklund A, Andersson B, Haglund P. Organophosphorus flame retardants and plasticizers in air from various indoor environments. Journal of Environmental Monitoring. 2005a; 7:814–819. [PubMed: 16049584]

Marklund A, Andersson B, Haglund P. Organophosphorus flame retardants and plasticizers in Swedish sewage treatment plants. Environ Sci Technol. 2005b; 39:7423–7429. [PubMed: 16245811]

Marsh G, Athanasiadou M, Athanassiadis I, Sandholm A. Identification of hydroxylated metabolites in 2,2 ′,4,4 ′-tetrabromodiphenyl ether exposed rats. Chemosphere. 2006; 63:690–697. [PubMed: 16213553]

Meeker JD, Johnson PI, Camann D, Hauser R. Polybrominated diphenyl ether (PBDE) concentrations in house dust are related to hormone levels in men. Science of the Total Environment. 2009; 407:3425–3429. [PubMed: 19211133]

Meeker JD, Stapleton HM. House Dust Concentrations of Organophosphate Flame Retardants in Relation to Hormone Levels and Semen Quality Parameters. Environ Health Perspect. 2010; 118:318–323. [PubMed: 20194068]

Mueller, T.; Wullimann, MF. Atlas of early zebrafish brain development : a tool for molecular neurogenetics. Elsevier; Boston: 2005.

NRC. Toxicological Risks of Selected Flame-Retardant Chemicals. National Academy Press; Washington, D.C: 2000. Tris (1,3-dichloropropyl-2) Phosphate; p. 358-386.

Poet TS, Wu H, Kousba AA, Timchalk C. In vitro rat hepatic and intestinal metabolism of the organophosphate pesticides chlorpyrifos and diazinon. Toxicological Sciences. 2003; 72:193–200. [PubMed: 12655035]

Pope CN. Organophosphorus pesticides: do they all have the same mechanism of toxicity? Journal of Toxicology and Environmental Health. 1999; 2:161–181. [PubMed: 10230392]

Qiao D, Seidler FJ, Slotkin TA. Developmental neurotoxicity of chlorpyrifos modeled in vitro: Comparative effects of metabolites and other cholinesterase inhibitors on DNA synthesis in PC12 and C6 cells. Environmental Health Perspectives. 2001; 109:909–913. [PubMed: 11673119]

Qiao D, Seidler FJ, Slotkin TA. Oxidative mechanisms contributing to the developmental neurotoxicity of nicotine and chlorpyrifos. Toxicology and Applied Pharmacology. 2005; 206:17–26. [PubMed: 15963341]

Qiao D, Seidler FJ, Violin JD, Slotkin TA. Nicotine is a developmental neurotoxicant and neuroprotectant: stage-selective inhibition of DNA synthesis coincident with shielding from effects of chlorpyrifos. Developmental Brain Research. 2003; 147:183–190. [PubMed: 14741763]

Qiu XH, Mercado-Feliciano M, Bigsby RM, Hites RA. Measurement of polybrominated diphenyl ethers and metabolites in mouse plasma after exposure to a commercial pentabromodiphenyl ether mixture. Environmental Health Perspectives. 2007; 115:1052–1058. [PubMed: 17637922]

Reemtsma T, Quintana J, Rodil R, García-López M, Rodríguez I. Organophosphorus flame retardants and plasticizers in water and air I. Occurrence and fate. Trends in Analytical Chemistry. 2008; 27:727–737.

Rose M, Bennett DH, Bergman A, Fangstrom B, Pessah IN, Hertz-Picciotto I. PBDEs in 2–5 Year-Old Children from California and Associations with Diet and Indoor Environment. Environ Sci Technol. 2010; 44:2648–2653. [PubMed: 20196589]

Ross BM, McKenzie I, Glen I, Bennett CPW. Increased levels of ethane, a non-invasive marker of n-3 fatty acid oxidation, in breath of children with attention deficit hyperactivity disorder. Nutritional Neuroscience. 2003; 6:277–281. [PubMed: 14609313]

Sajdel-Sulkowska EM, Xu M, Koibuchi N. Increase in Cerebellar Neurotrophin-3 and Oxidative Stress Markers in Autism. Cerebellum. 2009; 8:366–372. [PubMed: 19357934]

Sjodin A, Patterson DG, Bergman A. A review on human exposure to brominated flame retardants - particularly polybrominated diphenyl ethers. Environment International. 2003; 29:829–839. [PubMed: 12850099]

Sjodin A, Wong LY, Jones RS, Park A, Zhang Y, Hodge C, Dipietro E, McClure C, Turner W, Needham LL, Patterson DG. Serum concentrations of polybrominated diphenyl ethers (PBDEs)

and polyhrominated biphenyl (PBB) in the united states population: 2003–2004. Environ Sci Technol. 2008; 42:1377–1384. [PubMed: 18351120]

Sledge D, Yen J, Morton T, Dishaw L, Shuler K, Donerly S, Linney E, Levin E. A zebrafish model of the persisting neurobehavioral impairment caused by developmental chlorpyrifos exposure. Neurotoxicol Teratol. 2009; 31:242–242.

Slotkin, TA. Developmental neurotoxicity of organophosphates: a case study of chlorpyrifos. In: GRC, editor. Toxicity of Organophosphate and Carbamate Pesticides. Elsevier Academic Press; San Diego: 2005. p. 293-314.

Slotkin TA, MacKillop EA, Ryde IT, Tate CA, Seidler FJ. Screening for developmental neurotoxicity using PC12 cells: Comparisons of organophosphates with a carbamate, an organochlorine, and divalent nickel. Environmental Health Perspectives. 2007a; 115:93–101. [PubMed: 17366826]

Slotkin TA, Seidler FJ, Fumagalli F. Exposure to organophosphates reduces the expression of neurotrophic factors in neonatal rat brain regions: Similarities and differences in the effects of chlorpyrifos and diazinon on the fibroblast growth factor superfamily. Environmental Health Perspectives. 2007b; 115:909–916. [PubMed: 17589599]

Slotkin TA, Seidler FJ, Fumagalli F. Targeting of neurotrophic factors, their receptors, and signaling pathways in the developmental neurotoxicity of organophosphates in vivo and in vitro. Brain Res Bull. 2008; 76:424–438. [PubMed: 18502319]

Song X, Violin JD, Seidler FJ, Slotkin TA. Modeling the developmental neurotoxicity of chlorpyrifos in vitro: Macromolecule synthesis in PC12 cells. Toxicology and Applied Pharmacology. 1998; 151:182–191. [PubMed: 9705902]

Stapleton HM, Allen JG, Kelly SM, Konstantinov A, Klosterhaus S, Watkins D, McClean MD, Webster TF. Alternate and new brominated flame retardants detected in US house dust. Environ Sci Technol. 2008a; 42:6910–6916. [PubMed: 18853808]

Stapleton HM, Kelly SM, Pei R, Letcher RJ, Gunsch C. Metabolism of Polybrominated Diphenyl Ethers (PBDEs) by Human Hepatocytes in Vitro. Environmental Health Perspectives. 2009a; 117:197–202. [PubMed: 19270788]

Stapleton HM, Klosterhaus S, Eagle S, Fuh J, Meeker JD, Blum A, Webster TF. Detection of Organophosphate Flame Retardants in Furniture Foam and US House Dust. Environ Sci Technol. 2009b; 43:7490–7495. [PubMed: 19848166]

Stapleton HM, Letcher RJ, Li J, Baker JE. Dietary accumulation and metabolism of polybrominated diphenyl ethers by juvenile carp (Cyprinus carpio). Environ Toxicol Chem. 2004; 23:1939–1946. [PubMed: 15352483]

Stapleton HM, Sjodin A, Jones RS, Niehoser S, Zhang Y, Patterson DG. Serum levels of polyhrominated diphenyl ethers (PBDEs) in foam recyclers and carpet installers working in the united states. Environ Sci Technol. 2008b; 42:3453–3458. [PubMed: 18522133]

Takigami H, Suzuki G, Hirai Y, Ishikawa Y, Sunami M, Sakai S-i. Flame retardants in indoor dust and air of a hotel in Japan. Environment International. 2009; 35:688–693. [PubMed: 19185920]

Taurines R, Thome J, Duvigneau JC, Forbes-Robertson S, Yang LY, Klampfl K, Romanos J, Muller S, Gerlach M, Mehler-Wex C. Expression analyses of the mitochondrial complex I 75-kDa subunit in early onset schizophrenia and autism spectrum disorder: increased levels as a potential biomarker for early onset schizophrenia. Eur Child Adolesc Psych. 2010; 19:441–448.

Tullo, A. Chemical & Engineering News. American Chemical Society; 2003. Great Lakes to Phase Out Two Flame Retardants; p. 13

Turyk ME, Persky VW, Imm P, Knobeloch L, Chatterton R, Anderson HA. Hormone Disruption by PBDEs in Adult Male Sport Fish Consumers. Environmental Health Perspectives. 2008; 116:1635–1641. [PubMed: 19079713]

U.S.EPA. U. S. EPA. Child-Specific Exposure Factors Handbook (Final Report). U.S. Environmental Protection Agency; Washington, DC: 2008.

Viberg H, Fredriksson A, Jakobsson E, Orn U, Eriksson P. Neurobehavioral Derangements in Adult Mice Receiving Decabrominated Diphenyl Ether (PBDE 209) during a Defined Period of Neonatal Brain Development. Toxicol Sci. 2003; 76:112–120. [PubMed: 12915714]

Viberg H, Johansson N, Fredriksson A, Eriksson J, Marsh G, Eriksson P. Neonatal Exposure to Higher Brominated Diphenyl Ethers, Hepta-, Octa-, or Nonabromodiphenyl Ether, Impairs Spontaneous

Behavior and Learning and Memory Functions of Adult Mice. Toxicol Sci. 2006; 92:211–218. [PubMed: 16611620]

Wood SJ, Yucel M, Pantelis C, Berk M. Neurobiology of Schizophrenia Spectrum Disorders: The Role of Oxidative Stress. Annals Academy of Medicine Singapore. 2009; 38:396–401.

Xing TR, Chen L, Tao YA, Wang M, Chen JT, Ruan DY. Effects of Decabrominated Diphenyl Ether (PBDE 209) Exposure at Different Developmental Periods on Synaptic Plasticity in the Dentate Gyrus of Adult Rats In Vivo. Toxicological Sciences. 2009; 110:401–410. [PubMed: 19535737]

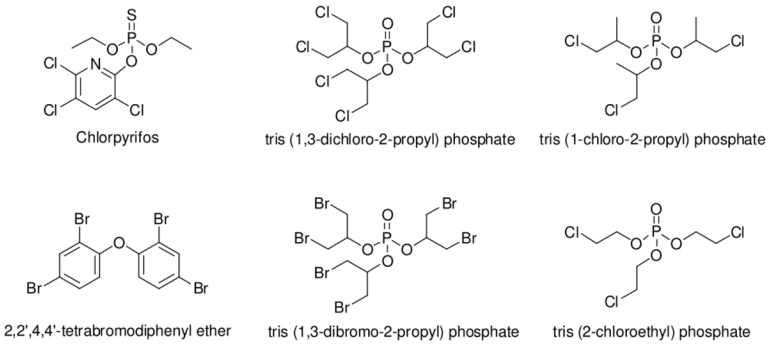

Figure 1.
Structures of chemicals tested in this study.

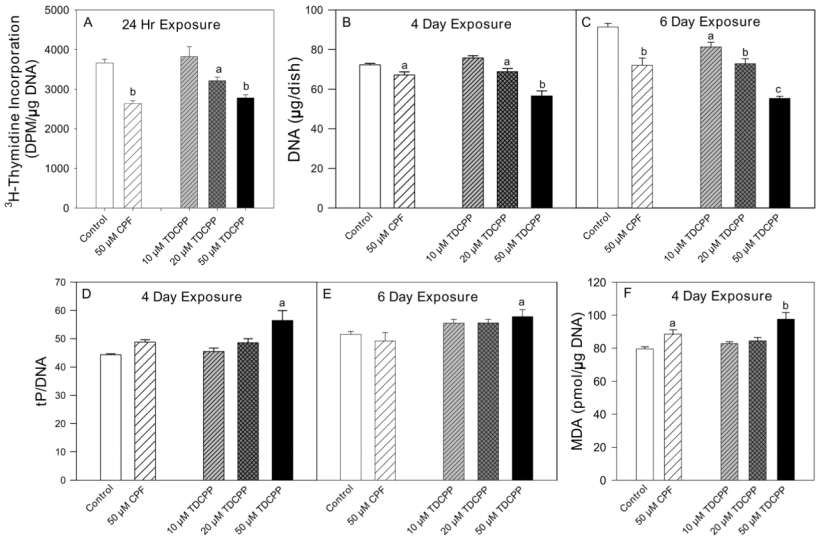

Figure 2.

Effects of TDCPP in undifferentiated and differentiating PC12 cells. (A) DNA synthesis in undifferentiated cells after a 24 hr exposure; (B–F), evaluations in differentiating cells of cell number (B–C) and cell growth (D–E) after 4 and 6 day exposures, and oxidative stress (F) after a 4 day exposure. Data represent means ± SEM (24 hour and 4 day exposures: n = 8–10; 6 day exposure: n = 15–23). ANOVA shows a significant main effect of treatment for all panels ($p < 0.001$ for A–D, F; $p < 0.05$ for E). Letters indicate significant difference from control. Bars with different letters indicate a significant difference from each other.

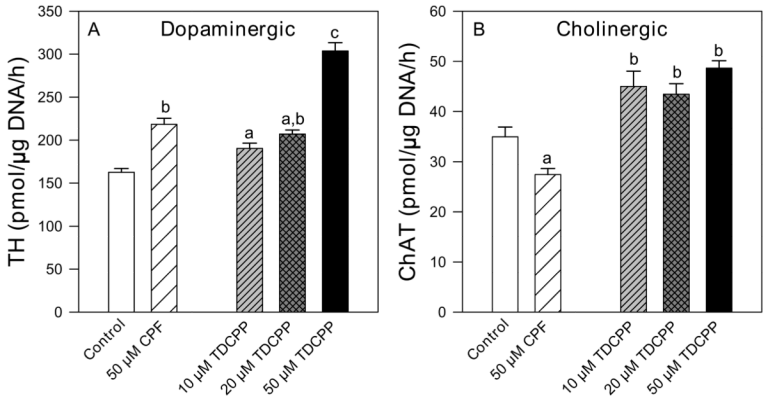

Figure 3.

Effects of TDCPP on phenotypic differentiation. (A) Dopaminergic phenotype and (B) cholinergic phenotype after a 6 day exposure. Data represent means ± SEM (n = 8–10). For both measures, ANOVA indicates a main effect of treatment ($p < 0.0001$). Letters indicate significant difference from control. Bars with different letters indicate a significant difference from each other.

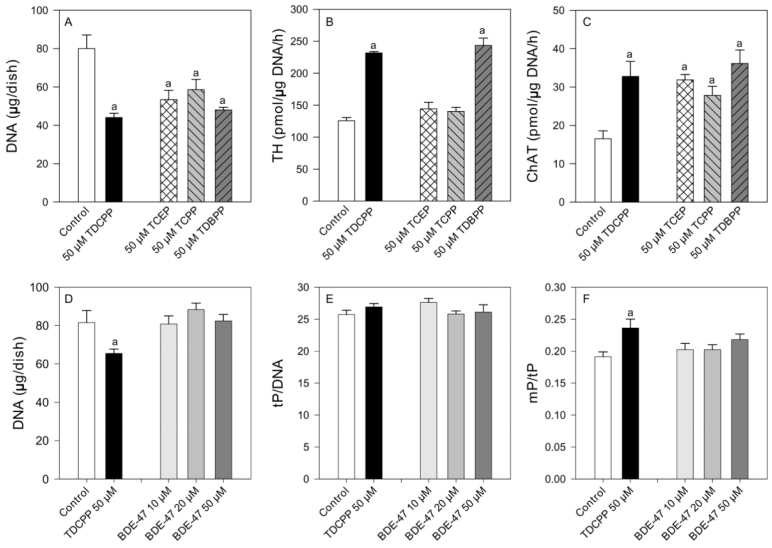

Figure 4.

Effects of OPFRs and BDE-47 on differentiating PC12 cells. (A) Cell number, (B) dopaminergic phenotype, and (C) cholinergic phenotype after a 6 day exposure to TCEP, TCPP, and TDBPP. (D) Cell number, (E) cell growth, and (F) neurite growth after a 6 day exposure to BDE-47. Data represent means ± SEM (OPFR comparison: n = 4–5; BDE-47 Studies: n = 8–10). For all measures, ANOVA indicates a main effect of treatment ($p < 0.001$). Letters indicate significant difference from control. Bars with different letters indicate a significant difference from each other.