

Tris (1,3-dichloro-isopropyl) phosphate, a PentaBDE Replacement: Detection in Consumer Products, Human Metabolism, and Neurodevelopmental Effects

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Introduction

Two of the three commercial polybrominated diphenyl ether (PBDE) flame retardant mixtures were voluntarily phased out by manufacturers and legislatively banned in different regions of the world. These two mixtures were collectively known as PentaBDE and OctaBDE based on their homologue compositions, and were primarily used in polyurethane foam (PUF) and ABS plastics, respectively. PentaBDE treated PUF was used in a variety of furniture and automobile products and had extensive use in North America. The heavy use of PentaBDE in PUF applications in North America is believed to be responsible for the high levels of PBDEs measured in indoor environments, and for the higher levels of PBDEs detected in human tissues from US and Canadian populations. With the phase-out of the PentaBDE mixture, alternate flame retardant mixtures are now being used, but little information is available on the chemicals currently being used in these applications, and their environmental levels and potential health effects. Manufacturers are often required to add chemical flame retardant additives to meet California Flammability Standard TB 117, or the Consumer Product Safety Commission Mattress Flammability Standard 16 CFR 1632. However, these manufacturers are not required to provide information on the types of chemicals used in these treatments, or the levels present in their products. This unfortunately hampers efforts to evaluate health risks from the use of these chemicals in consumer products.

In a recent study, we determined that tris (1,3-dichloro-isopropyl) phosphate (TDCPP) is a common use PentaBDE replacement in commercial products, including baby products, purchased in the United States and which contain PUF. TDCPP has recently been measured in indoor air and dust, suggesting it can leach out of consumer products similarly to PentaBDE (Stapleton et al., 2009). Concentrations of TDCPP in dust are similar to, or in some cases greater than, PBDE concentrations, particularly in automobile dust, and this data will be presented at BFR 2010 (Webster et al., 2010). Based on concentrations measured in indoor environments, human exposure is likely to occur, yet little is known about the health effects from exposure to TDCPP. Our objectives here were to evaluate the metabolism of TDCPP using *in vitro* techniques and evaluate potential human health effects using cell culture assays in order to provide more information on its potential metabolic fate and effects. This presentation will present an overview on our detection of TDCPP in specific products, results from our studies on the *in vitro* metabolism of TDCPP using pooled human hepatic S9 sub-cellular fractions, and results from our investigations into the neurodevelopmental toxicity of TDCPP using PC12 cells, a cell line derived from rat pheochromocytoma that provides a well-characterized model of neurodifferentiation.

Materials and Methods

Foam Analysis: Pieces of PUF were donated from friends and colleagues and were collected from in-use furniture products purchased in the United States between 2003-2009. The foam was analyzed for flame retardants at Duke University using gas chromatography mass spectrometry (GC/MS) as reported in Stapleton et al., 2009.

In Vitro Metabolism Studies: Pooled human liver S9 sub-cellular fractions were purchased from CellZDirect (Durham, NC) and used to investigate potential hepatic metabolism of tris (1,3-dichloro-isopropyl) phosphate (TDCPP). TDCPP was incubated in a phosphate based buffer with the necessary enzymatic co-factors for 90 minutes at 37 °C. After the incubation 1 mL of cold methanol was added to each step to stop all enzymatic reactions. Samples were then diluted with 2 mL deionized water and extracted using Varian Bond Elut Plexa (Varian Inc., Palo Alto, CA, USA). All extracts were reduced in volume and analyzed by GC/MS and LC/MS-MS. In some cases the extracts were derivatized with diazomethane for analysis by GC/MS. The rate of the parent chemical loss was measured and all extracts were screened for potential metabolites in both electron impact and negative chemical ionization modes.

Neurodevelopmental assays: TDCPP was evaluated for potential developmental neurotoxic effects using a neuronotypic PC12 cell test battery previously validated against numerous toxicants. Methods for this assay have been previously published and are only briefly outlined here (Slotkin et al., 2008). In general, the approach is to examine relevant and sensitive endpoints for replicating cells and cells undergoing neurodifferentiation, which is initiated by adding nerve growth factor. In these assays, PC12 cells (American Type Culture Collection, 1721-CRL) were incubated in RPMI-1640 medium (Invitrogen, Calsbad, CA) supplemented with 5% fetal bovine serum (Sigma Chemical Co., St. Louis, MO), 10% inactivated horse serum (Sigma Chemical Co.), and 50 µg/mL penicillin-streptomycin (Invitrogen) with 5% CO₂ at 37°C. Test chemicals were first dissolved in DMSO at concentrations ranging from 0- 50 µM. Cell cultures were exposed to test chemicals 24 hours after seeding with a final concentration of 0.1% DMSO in the medium. After six days of exposure, cell cultures were harvested. DNA and protein content (measure of cell number and growth, respectively) were measured for each culture dish. The chlorinated organophosphate chlorpyrifos (CPF), a known developmental neurotoxicant, was used as a positive control at a concentration of 50 µM.

Results and Discussion

PUF Analysis: Twenty-seven different pieces of PUF were collected from in-use furniture items and from one sound proofing foam found in a laboratory grade sieve, purchased in the United States (U.S.) between 2003 and 2009. Of the 27 pieces tested, 16 were found to be treated with TDCPP in concentrations ranging from 1-5% by weight. PUF treated with TDCPP was found in chairs, couches, a pillow, foam from a nursery rocking chair, foam from a baby stroller, foam from a portable crib baby mattress and even foam from a laboratory sieve/shaker. A majority of these products were found to contain a label indicating that they meet California's flammability standard (Technical Bulletin 117) and contained labels indicating

that they were manufactured outside the U.S. This suggests that TDCPP is a common flame retardant in PUF imported into the U.S.

In Vitro Metabolism of TDCPP: In pooled human liver S9 sub-cellular fractions, TDCPP was metabolized at a rate of 10.44 pmoles/mg protein/hour. A paper by Lynn et al. (1981) found that TDCPP was fairly well metabolized in rats and one of the most prominent metabolites detected was bis (1,3-dichloroisopropyl) phosphate (BDCPP), which results from the cleavage of one ester group. BDCPP was positively identified in all the hepatic S9 fractions analyzed by GC/MS (Figure 1) based on comparison to a synthesized standard. However, formation rates of BDCPP cannot be made at this time due to an uncertain estimate of the purity of the BDCPP standard synthesized. Based on the peaks present in the S9 fractions, BDCPP does appear to be a significant metabolite. This observation is similar to *in vivo* exposure studies with rodents which identified BDCPP as a major metabolite (Lynn et al., 1981; Sasaki et al., 1984). With the formation of BDCPP from TDCPP, it is likely that 1,3-dichloropropanol (DCP) is also formed during this reaction, even though we did not specifically look for or identify DCP in these samples. BDCPP and DCP have been shown to both be toxic and mutagenic (Gold et al., 1978, Lynn et al., 1981). Therefore, human exposure to TDCPP would likely result in the formation of BDCPP and DCP in tissues. More work is needed to determine the levels of these metabolites in human urine and evaluate whether or not the levels present a health concern.

Neurodevelopmental PC12 Assays: PC12 cells were exposed to TDCPP at four different concentrations ranging from 0 to 50 μ M and 50 μ M chlorpyrifos (CPF) was used as a positive control. DNA was measured as an index of the number of cells; neurotypic cells contain a single nucleus, so the DNA content of the culture assesses the number of cells. The total protein/DNA Ratio was also assessed as an index of cell growth and the membrane/total protein ratio was used as a measure of the development of neuritic projections accompanying differentiation. In cells undergoing differentiation, a 6-day exposure to TDCPP exposure evoked a concentration-dependent decrease in DNA content relative to controls, with a magnitude equivalent to that seen with CPF (Figure 2). This suggests that TDCPP is likely to be as neurotoxic as CPF. In contrast, there was no impairment of cell growth based on the total protein/DNA ratio, nor was there any impairment of neurite outgrowth as monitored by the membrane/total protein ratio (data not shown). This suggests a specific targeting of either mitosis or apoptosis by TDCPP, rather than generalized cytotoxicity. Further experiments are being conducted to determine the underlying mechanisms by assessing effects on DNA and protein synthesis, indices of oxidative stress and cell differentiation, and potential effects

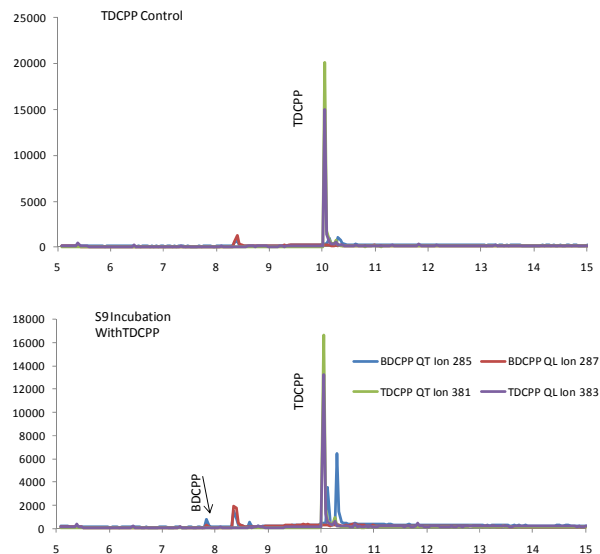


Figure 1. GC/EI-MS Chromatograms illustrating the detection of TDCPP And the metabolite BDCPP in human S9 incubations.

on the terminal neuronal phenotype (cholinergic vs. dopaminergic). Results from these experiments will be presented at the conference.

Our findings indicate that TDCPP is a major current use flame retardant found in consumer products likely to produce significant, human exposures, especially given that TDCPP is an additive flame retardant and that it has been measured in indoor air and dust. The concentrations detected in dust are similar to concentrations of PBDEs, so that TDCPP exposure may very well occur at the same levels as for PBDEs. However, unlike PBDEs, TDCPP is fairly well metabolized and may thus form more toxic metabolites in human tissues. The results from the developmental neurotoxicity assays suggest that TDCPP and/or its metabolites are potentially as neurotoxic as the organophosphate pesticide, chlorpyrifos, a compound whose use was curtailed specifically because of developmental neurotoxicity. Taken together this data raises concerns about children's exposure to this potential developmental neurotoxicant given its likely exposure to children via house dust.

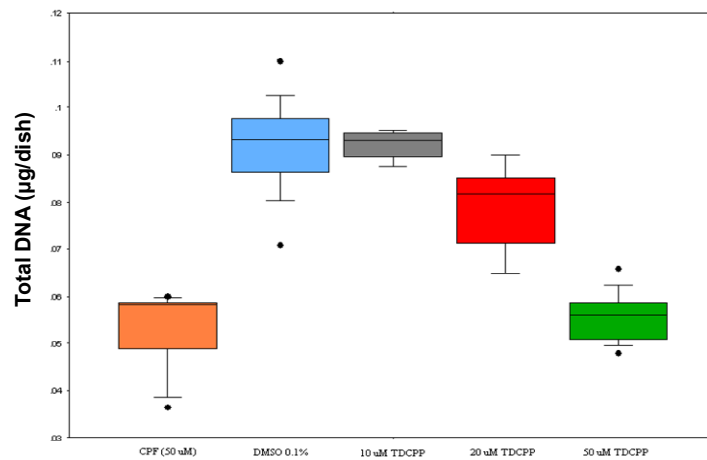


Figure 2. Effect of 50 µM Chlorpyrifos (CPF), 0.1% Dimethyl sulfoxide (DMSO), or 10, 20, 50 µM Tris (1,3-dichloro-isopropyl) phosphate (TDCPP) on DNA content of PC12 cultures exposed for 6 days. 50 µM CPF and 0.1% DMSO were used as a positive and negative control respectively.

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