POLYBROMINATED DIPHENYL ETHERS IN RATS AT MCMASTER UNIVERSITY ANIMAL QUARTERS AND EXPOSURE ASSESSMENT

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Introduction

Polybrominated diphenyl ethers (PBDEs) are flame retardants used in numerous household and commercial products. There are 209 congeners of PBDEs, varying in both number and position of bromination and they are produced industrially as mixtures designated penta-BDE, octa-BDE, and deca-BDE.

PBDEs administered to mice have induced permanent abnormalities in spontaneous motor behavior, learning, and memory that worsen with age[1]. PBDEs also disrupt thyroid function, which may explain their neurotoxicity[2]. Penta-BDE exposure results in decreased serum thyroid hormone levels in both rats and mice[2]. PBDE exposure inhibits estrogen sulfotransferase, leading to a net estrogenic effect[3]. Perinatal exposure of congener BDE 99 has led to reduced litter size and a delayed sensori-motor development[4]. Developmental effects in rats include delays in hardening of bones, bent limbs, and weight decreases due to exposure of deca-BDE . Penta-BDEs are immuno-suppressive and deca-BDE is a putative carcinogen[2].

PBDEs are ubiquitous environmental contaminants because they have been detected in sediments, biota, house dust, sewage sludge, air, water samples, and human and wildlife tissues[5]. They have become ubiquitous because of their widespread use in homes, offices, automobiles, and electronics[5]. Their lipophilicity and resistance to degradation make these compounds undesirable in the environment[6]. The combination of ubiquity and toxicity introduces a new and significant confounding factor in studies that use lab animals.

The effects of background environmental contamination on study outcomes are rarely considered. Even studies investigating PBDE effects have not always adjusted for the background levels in their controls. Certainly, studies investigating organohalogen effects have not considered PBDE contamination and subsequent interaction. Furthermore, we are not aware of any study which investigates sources of PBDE in laboratory animals. Accordingly, this report has three objectives: (1) To determine PBDE contamination of rats at the Central Animal Facility (CAF) of McMaster University (Hamilton, Ontario), (2) To determine whether adult or pup rats have higher PBDE exposure and (3) to investigate air and food as possible sources of exposure.

Materials and Methods:

Four adult and six pup rats were used in this investigation. The four adult rats were 42 ± 10 weeks old (mean ± SD) and all of the six pup rats were 7 weeks old. The animals were bred in the Central Animal Facility (CAF) at McMaster University. Approximately 0.5 g of epididymal tissue was collected at necropsy and samples were frozen. They were sent to the National Water Research Institute (Burlington, ON) where they were stored at -3ºC until extraction. Five air samples were collected using polyurethane foam and glass fibre filters to collect the gaseous and particulate phases of the air respectively. Air sampling was conducted on a 24 hour basis for five days using a flow rate of 0.2 m^3 of air/minute. Food samples of the rats (Harland Teklad 22/5 Rodent Diet) were also collected from CAF.

A mixture of seven ^{13}C-labelled BDEs (Wellington Laboratories, Guelph, Ontario) were used as a recovery standard. A ^{13}C-labelled BDE 138 (2,2',3,4,4',5'-hexabromodiphenyl ether) from Wellington Laboratories was used as an internal performance standard. All reagents and solvents were HPLC grade. Dichloromethane (DCM), hexane, toluene were purchased from Caledon Laboratories (Georgetown, ON). Sodium sulfate (Na_2SO_4) was purchased from ACP Chemicals (Montreal, QC), glass wool from Supelco (Bellefonte, PA), and silica from Caledon Laboratories (Georgetown, ON). Activated silica was prepared by firing silica gel 60 (100-200 µm or 70-150 mesh size) for 23 hours at 300ºC. A 44% (weight/weight) sulphuric acid/silica was prepared by mixing 560g of activated silica and with 25mL of concentrated sulphuric acid. Activated and acidified silica were stored in a desiccator until use.

The rat adipose was homogenized with 5x weight Na_2SO_4 before being extracted. All samples were extracted by Soxhlet for 24 hours with DCM. DCM was chosen because it is a good solvent for dissolving the hydrophobic PBDEs. Soxhlet sample extracts were slowly passed through an Allihn funnel half filled with Na_2SO_4 to remove any water. Samples were concentrated using a rotary evaporator at 35-40ºC to about 5mL. They were transferred to 15mL test tubes and further concentrated to 1mL done under nitrogen, heated at (40ºC). Extracts, except the air samples, were passed through gel permeation columns to separate PBDEs from lipids. Gel permeation columns were pre-washed with 100mL 1:1 DCM/hexane. The first elution of 100mL 1:1 DCM/hexane was collected into pre-weighed beakers for lipid analysis. The next elution of 150mL 1:1 DCM/hexane contained the PBDEs. Samples were again concentrated using a rotary evaporator, transferred and further concentrated under nitrogen. All samples were then passed through silica columns for further clean-up. Silica columns were packed, from bottom to top, with glass wool, Na_2SO_4 (1cm), activated silica gel (4g), 44% acidified silica gel (4g), and Na_2SO_4 (1cm). First fraction of 63mL hexane and second fraction of 130mL 1:1 DCM/hexane was collected. Samples were concentrated using a rotary evaporator, transferred, and concentrated under nitrogen until near dryness. Samples were spiked with 20µL ^{13}C PBDE 138 and transferred to conical vials to be analyzed by gas chromatography-high resolution mass spectrometer (GC-HRMS).

The GC-HRMS in electron ionization (EI) mode was used in the quantification of the PBDEs. GC-HRMS was carried out on a Micromass Ultima HRMS in selected ion mode (SIM) at a mass resolution of 10,000 following separation on an Agilent 6890 GC using a 15 m DB5-HT column (J& W Scientific, 0.25mm X 0.10um). The GC column was maintained at 100°C for 2 minutes, then ramped at 25°C/min to 250°C, ramped at 1.5°C/min to 260°C and then at 25°C/min to 325°C and held for 7 minutes. Helium was used as the carrier gas in constant pressure mode. Source and injection temperatures were maintained at 280 °C. Quantification was performed using isotope dilution methods using ^{13}C-PBDEs. Peaks that matched the retention times and the isotopic ratio of the primary and secondary ions of the compounds in the standards were quantified. The mass spectrometer was operated in SIM mode using a total of 9 function groups to analyze the suite of PBDE congeners.

To prevent contamination, glassware was soaked in basic liquid detergent from EMB Chemicals (Gibbstown, NJ), rinsed three-times with tap water, three-times with distilled water, twice with methanol, twice with DCM, and fired in oven at (230ºC) overnight. Aluminium foil was used to wrap cleaned glassware. No plastic materials were used in order to avoid PBDE contamination. All transferring of samples was done quantitatively by rinsing all glassware with three aliquots of 1mL hexane. Blanks were run through the entire extraction and clean-up procedures to assess background levels of PBDEs in the lab which will help to quantify levels of PBDEs in samples.

Statistical Analysis:
The statistical analyses were performed with SPSS software, version 15.0 for Windows. Data for pups and adults were tested by the Student t-test. Statistical differences were considered significant when $p < 0.05$.

Results:
BDE 47, 99, 100, 153, 154, and 209 were found in both the adult and pup adipose. Other congeners were analyzed for but were given a value of zero either because they were under the limit of detection and quantification of the GC-HRMS or their concentrations were lower than the blanks. Concentrations are reported in pg/g lipid weight (lw). Since samples were pure adipose, they are assumed to be 100% lipid. The mean concentration of all PBDE congeners in pups was 6882 ± 1189 pg/g lw (mean ± SD, n=6) and 5632 ± 874 pg/g lipid (n=4) for the adult rats (See Table 1). The congeners with the largest concentrations were BDE 47 (2900 ± 2300 pg/g lw [mean ± SD]), 99 (1300 ± 1200 pg/g lw), and 209 (1300 ± 900 pg/g lw). The mean concentration for BDE 209 appeared higher for the adult rats (1800 ± 800 pg/g lw) than the pups (1000 ± 900 pg/g lw) but this was not significant ($p = 0.217$). For the lower molecular weight BDE congeners 47, 99, 100, and 153, there was no statistically significant difference between the levels of contamination in the pups and adults ($p = 0.329$). No significant difference was observed for BDE 154.

TABLE 1. CONCENTRATION OF BDE CONGENERS 47, 99, 100, 153, 154, 209 IN ADULT AND PUP RATS. BDE CONGENER CONCENTRATION (PG/G LIPID WEIGHT, MEAN ± SD)							
	47	99	100	153	154	209	Sum
Adult(n=4)	2000 ± 2000	900 ± 300	500 ± 200	200 ± 100	90 ± 80	1800 ± 800	6000 ± 2000
Pups (n=6)	3000 ± 3000	1000 ± 2000	600 ± 200	300 ± 400	90 ± 80	1000 ± 900	7000 ± 4000
p-value	0.589	0.473	0.321	0.357	0.919	0.217	0.589

BDE congeners 47, 99, and 100 were detected in both food and air samples. Other congeners were analyzed for but were given a value of zero either because they were under the limit of detection and quantification of the GC-HRMS or their concentrations were lower than the blanks. The concentration of PBDE in air was calculated as pg/m^3 and food was calculated as pg/g lw (which is 5% of sample weight according to composition index).The total mean concentration from food was 350 ± 90 pg/g wet weight (lipid = 5.0%, mean ± SD, n=5). The total mean concentration for air was 800 ± 40 pg/g wet weight (mean ± SD, n=10), after adding concentrations from the gaseous and particulate phases of air (See Table 2).

TABLE 2. CONCENTRATION OF BDE CONGENER 47, 99, 100 IN FOOD AND AIR SAMPLES. BDE CONGENER CONCENTRATION (MEAN ± SD)				
	47	99	100	Sum
Food (n=5, pg/g wet weight, lipid = 5.0%)	700 ± 300	400 ± 100	100 ± 40	1200 ± 400
Air: Gaseous + Foam (n=5, pg / m^3)	2000 ± 600	300 ± 300	80 ± 50	2300 ± 900

PBDE exposure from air is limited to the rate of which air enters the lungs of the rats. The UFAW Handbook on the Care & Management of Laboratory Animals report lab rats report a respiratory rate of 92.5 breaths/min and tidal volume of 1.6mL for lab rats. Adjusting for the rat's air flow produces an exposure of 500 ± 200 pg PBDEs over 24 hours. PDDE exposure from food is measured based on the diet of 26g per day from the UFAW Handbook and results in an exposure of 30000 ± 200 pg PBDEs daily (See Figure 1).

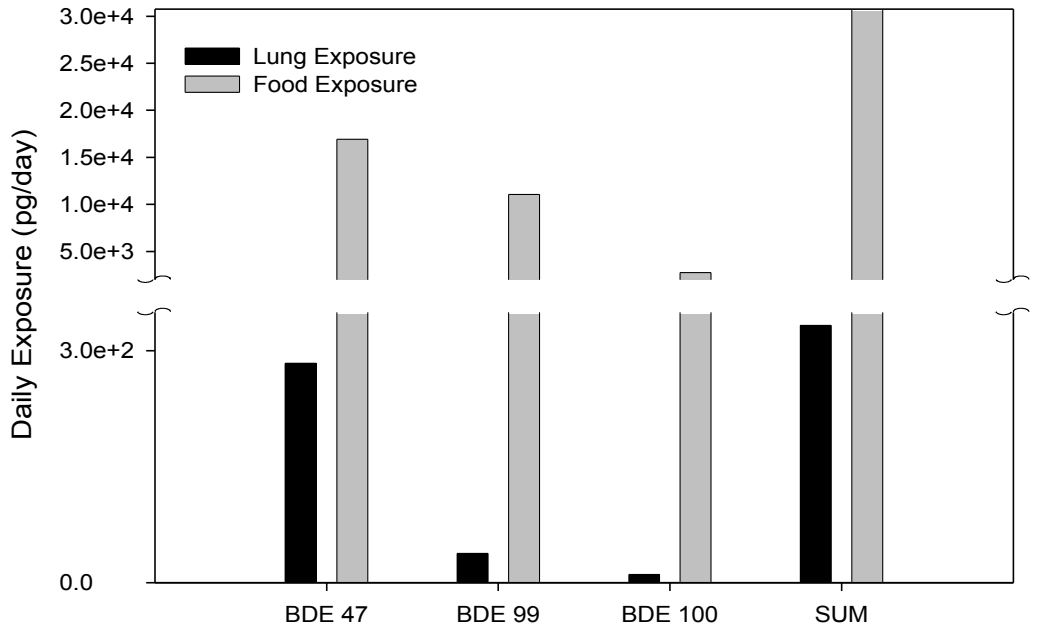

Figure 1. Daily exposure of PBDEs to rats via lung and food intake

Discussion:
Inadvertent PBDE exposure of lab animals maybe a significant confounder in studies. BDE 47 was the most abundant congener (2700 ± 2300 pg/g lw [mean ± SD]) while BDE 99 (1200 ± 1200 pg/g) and BDE 209 (1400 ± 900 pg/g) were also abundant. High levels of the lower weight PBDEs are troublesome because of their high toxicity compared to higher weight congeners[7-9]. BDE-209 is also problematic as it has been tested for carcinogenicity and has shown statistically significant increases in hepatocellular (liver) carcinomas and marginal increases in thyroid follicular cell carcinomas in mice[2]. Although there is no conclusive data on the minimum level of PBDEs that lead to ill-effects in rats, a single-dose exposure of 0.8 mg / kg of BDE 99 has led to neurobehavioral alterations in post-natal mice[10]. Our study indicates that the rats are exposed to approximately 30 ng from their diet and 500 pg from the air. Since there is not an established threshold at which PBDEs cause physiologic impact, it is difficult to determine how much this background contamination has an effect on experimental data though this exposure is several orders of magnitude lower that levels known to cause effects. However, interaction of these PBDEs with other organohalogens may contribute to effects[11].

The difference between the PBDE concentrations between adult and pup rats maybe due to several factors. Darnerud and Risberg[2] have demonstrated lactational transfer of PBDEs to neonatal mice. Since PBDEs are lipophilic, they tend to be concentrated in milk fat, thus lactation may be a significant source of PBDEs. Furthermore, pups have a reduced ability to excrete BDE 47, which raise the concentration of this congener compared to the adult rats[12].

Both air and diet have been shown to contain PBDEs which indicate that they are both possible sources of contamination. Adjusting for the daily exposure in both sources, it is probable that food is the primary source of exposure (Figure 1). It is however difficult to determine how much contamination can be attributed to food or air exposure since uptake may not equal exposure. Although only lower weight congeners of 47, 99, and 100 were found in abundance in the air and food, the higher congeners are likely present. In our extractions high blank levels meant that the blank-adjusted values for the higher congeners resulted in a value of zero. Finally it may be that air and food are not the only sources of PBDEs and other materials such as bedding located in the animal cages, should be investigated in order to discover all possible exposures.

A limitation of this study includes the small sample size which limits statistical power. The low number of samples also reduced our ability to analyze intra-sample variation. More investigation and analysis is needed of contamination of other types of lab animals and their exposure in different academic or institutional animal quarters. Nonetheless, our work does show that background PBDE contamination of lab animals should not be ignored and that further investigation is needed to determine the impact of these typically unmeasured contaminants on experimental animals.

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