



Exposure to Flame Retardants in Commercial Aircraft

**National Air Transportation Center of Excellence for
Research in the Intermodal Transport Environment (RITE)
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3.0 BACKGROUND AND SIGNIFICANCE

Flame retardant chemicals represent a broad range of compounds used in a wide variety of consumer and commercial products, including those used on commercial aircraft, to slow the propagation of fire. Polybrominated diphenyl ethers (PBDEs) are one class of brominated flame retardants that have garnered increased attention from the health community over the past ten years. There are 209 congeners of PBDEs with groups of congeners associated with the three commercial products: pentaBDE, octaBDE and decaBDE. PBDEs are found in a wide range of consumer products at up to 20% by weight, from plastics in television set housings to foam cushions in chairs and carpet backing (Allen et al. 2008b). Their widespread use, combined with their lipophilic nature and persistence in the environment, led to their ubiquitous presence in food, on surfaces, and in the air of both outdoor and indoor environments (e.g., offices, homes, cars, aircraft). Further, unlike many familiar persistent organic compounds where diet is the primary route of exposure, the indoor environment has been implicated as a critical route of exposure (Allen et al. 2007; Jones-Otazo et al. 2005; Webster et al. 2005). Results of animal and *in vitro* studies suggest developmental reproductive and neurotoxic effects as well as endocrine disruption associated with exposure to these compounds (Birnbaum and Staskal 2004; Darnerud et al. 2001; McDonald 2002; Schreiber et al. 2009). Recent epidemiologic evidence suggests evidence of disruption of thyroid and sex hormone homeostasis (Chao et al. 2007; Hardell et al. 2006; Herbstman et al. 2010; Meeker et al. 2009; Meeker and Stapleton 2009).

The use of PBDE in commercial products is in the process of being discontinued, with the EU banning commercial products containing penta-, octa- and decaBDE. In the US, penta- and octaBDE were voluntarily phased out by the manufacturers in 2004, with a similar voluntary phaseout for decaBDE planned for 2012. Even with these phaseouts and bans, vast reservoirs of PBDEs exist in products that are intended to be used for decades (Harrad and Diamond 2006; USEPA 2006).

To protect the flying public and flight crew it is essential that materials within aircraft have flame retardant properties. Several newer and commercially important flame retardant chemicals are now being incorporated into products to replace PBDEs (Stapleton et al. 2008; Stapleton et al. 2009). These include brominated flame retardants (tetrabromobisphenol A [TBBPA] and hexabromocyclododecane [HBCD]), chlorinated phosphates (Tris(diCl-isopropyl)phosphate [TCDPP]), and brominated phthalates (2-

ethylhexyl-tetraBr-phthalate and Bis-(2-ethylhexyl)-tetraBr-phthalate [TBPH]). Toxicologic evidence suggests that these compounds may have important human health implications. For example, the chlorinated phosphate flame retardant being used today is similar to the brominated phosphate flame retardant used in children’s pajamas in the late 1970s which was found to be mutagenic (Gold et al. 1978).

Limited information is currently available regarding exposure to flame retardants on commercial aircraft. However, assessing exposure in this environment is gaining increased attention from researchers in this field. A poster presented at a conference on brominated flame retardants reported the single highest concentration of total PBDEs in dust anywhere in the world at that time was measured in lint collected from an airplane seat pocket (160,000 ng/g; n=1; (Gerecke 2007). The only peer-reviewed paper specific to commercial aircraft also reported concentrations of PBDEs in dust from aircraft that can exceed the highest dust concentrations found in homes (Figure 1) (Christiansson et al. 2008b).

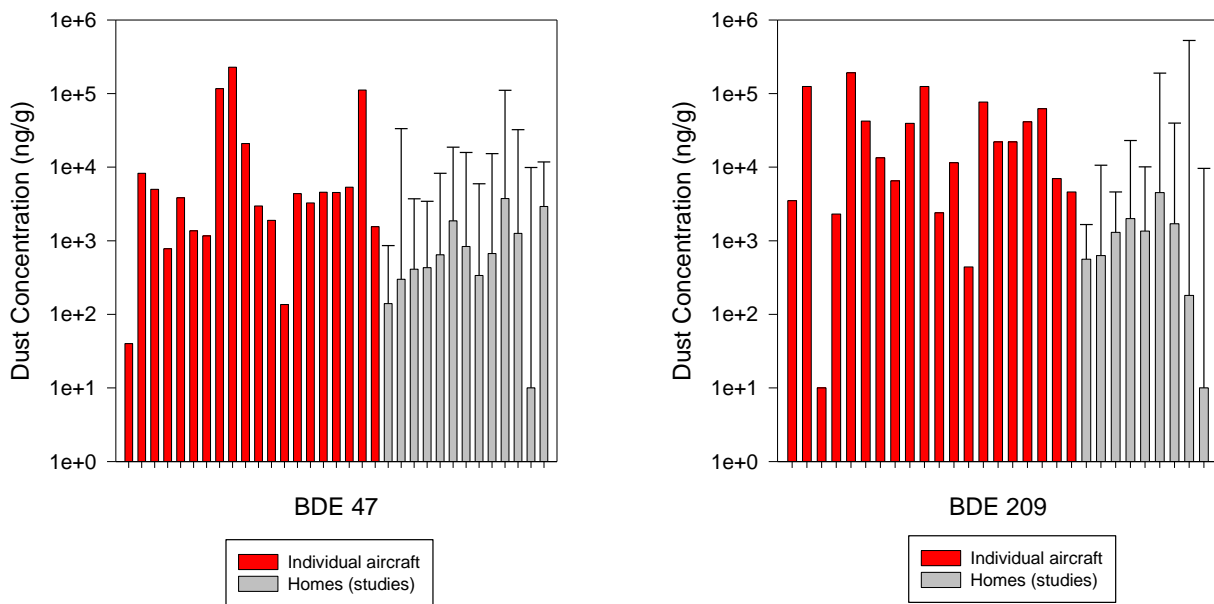


Figure 1. Concentrations of BDE47 and BDE209 in dust (ng/g) from 20 individual aircraft as reported by Christiansson et al. (2008), and the median and maximum concentrations from home studies conducted in 11 cities in North America (Allen et al. 2008a; Harrad et al. 2008; Ibarra et al. 2007; Rudel et al. 2003; Sjödin et al. 2008; Stapleton et al. 2005; Wilford et al. 2005; Wu et al. 2007; Zota et al. 2008).

Similar to the findings of elevated concentrations in dust from aircraft, flame retardant concentrations in air samples from aircraft can also be significantly higher than homes and worksites. In preliminary work associated with ACER, air samples previously collected (n=22) from Airline B were analyzed for two congeners of polybrominated diphenyl ethers (PBDEs). The highest PBDE concentrations from this analysis were found to be up to several orders of magnitude greater than air concentrations measured for occupationally-exposed workers and typical levels found in U.S. homes. (Figure 2; median and maximum concentrations).

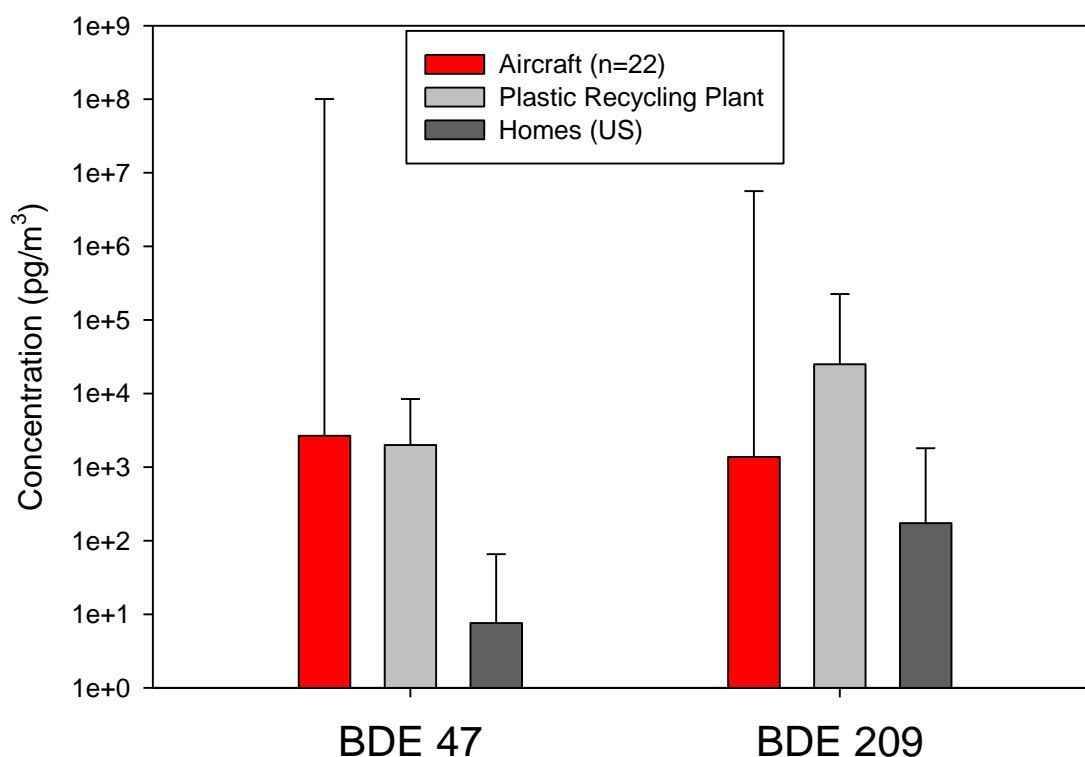


Figure 2. Comparison median and maximum PBDE concentrations (pg/m³) in air samples collected from ACER aircraft, a plastics recycling plant (Sjödin et al. 2001), and homes in the U.S (Allen et al. 2007).

Additionally, Christiansson et al. found that while body burdens of PBDEs in air travelers were similar to levels measured in the general population, the post-flight body burdens were higher than pre-flight body burdens suggesting travel-related exposure (Christiansson et al. 2008b). Recent evidence suggests an increased prevalence of thyroid disease in flight attendants. Increased levels of brominated flame retardants in women’s blood were recently associated with longer time to pregnancy (Harley et al.

2010). Flight crew's potential for prolonged elevated exposures to these compounds which have thyroid hormone effects makes understanding flight crew body burdens a critical research need.

The limited exposure data currently available suggest that aircraft may be a potentially relevant exposure environment for flame retardant chemicals. Additional research is needed to identify exposure-relevant flame retardants and fully elucidate the source-environment-receptor exposure pathways specific to commercial aircraft. In addition, finding elevated concentrations of flame retardants in aircraft air and dust indicates that these chemicals are migrating from their source products. It is not known if the loss of the flame retardant chemicals in the source products presents a safety issue due to loss of flammability protection. This study aims to address these knowledge gaps using a three-phased approach and drawing on the expertise of several ACER members and FAA technical experts.

The research described in this report, conducted in 2010-2011, aims to determine if flame retardant chemicals on aircraft pose a health risk to passengers and/or flight crew by addressing the knowledge gaps related to assessing potential exposure to flame retardants in commercial aircraft. The objectives of this study have been designed to specifically address the critical research needs related to assessing exposure to flame retardants in aircraft:

- 1) To identify flame retardants in commercial aircraft, conduct preliminary inhalation exposure estimates using previously collected samples, and develop ACER/RITE analytical capabilities
- 2) To assess source and exposure concentrations in relevant media in representative commercial aircraft.
- 3) To explore the potential associations between exposure (as measured by body burden) and early markers of thyroid function in flight crew.

4.0 METHODS AND RESULTS - 2011

To address knowledge gaps regarding exposures to PBDEs on aircraft, the FAA initiated a two-year research project designed to enhance our understanding of exposures to flame retardants (FRs) on aircraft. During that phase of the research, we collected in-flight air samples onboard aircraft, dust samples on aircraft and initiated a study of PBDE concentrations in the serum from flight attendants.

4.1 Inhalation Exposure Assessment

4.1.1 Methods

Air Sampling

A total of 59 air samples were collected from 2009 to 2010, each from a different aircraft. Integrated samples were collected for the period of 10,000 feet ascending to 10,000 feet descending on all flights. A sampling manifold split the flows from the pumps to obtain the target flow for each sample type (additional air samples were collected for other analytes; results for those analytes are not reported here). Flows rates ranged from 1.5 to 8.6 liters per minute depending on flow requirements of other sample types collected concurrently. Flows for each sample were measured using a TSI model 4146 volumetric flow meter (Shoreview, MN) and recorded at sample set up and take down and periodically checked during the flight. Samples were collected on two types of sorbent samplers, both of which consisted of two sections of polyurethane foam (PUF) and XAD-2 sorbent: commercially-available tubes for low-volume sampling (SKC 226-143) and samplers prepared in-house using 5 g of pre-cleaned XAD-2 (Supelco) sandwiched between two sections of pre-cleaned PUF (Supelco, one half PUF plug per sampler), packed in a freshly-muffled glass cartridge (URG). The sorbent tubes were shipped and maintained chilled after field spiking with deuterated surrogates until sample extraction.

Samplers were spiked with surrogate recovery standards (SRSs) before transferring to either a Soxhlet extractor or Accelerated Solvent Extractor (ASE, Dionex) cell. Samplers were extracted in dichloromethane (DCM) or 1:1 hexane:DCM for Soxhlet and ASE extraction, respectively. Extracts were solvent-exchanged into methyl-t-butyl ether (MTBE) and concentrated to 100 μ L. The QC samples

included solvent method blanks, matrix blanks and matrix spikes (10 ng each analyte). BDE 126 and $^{13}\text{C}_{12}$ BDE 209 were added to the matrix as surrogate recovery standards (SRSs; 15 to 40 ng each, depending on analyte and sample set) just prior to extraction to assess method performance on a sample-by-sample basis, and to provide correction for analytical losses, with BDE 126 as SRS for the PentaBDE and OctaBDE congeners, and $^{13}\text{C}_{12}$ BDE 209 for BDE 209. Dibromobiphenyl was added to the extract just prior to analysis as the internal standard (IS). Samples were analyzed using GC/MS in the multiple ion detection mode using negative chemical ionization with methane reagent gas. The GC column (DB-5, 15 m, 0.25 mm id, 0.10 μm film thickness) was programmed from 100-300 °C at 30 °C/min. Quantification was performed using the internal standard method, against a 5 point calibration curve, covering 2 to 150 ng/mL (plus a zero level standard; range for BDE 209 was 4 to 300 ng/mL), that was analyzed with each set. Calibration curves were generated using a linear least squares method. Analyte concentrations in samples (including blanks and spikes) were corrected by the corresponding SRS recovery value; then, analyte concentrations in field samples and spiked samples were corrected by average SRS-corrected blank values.

Quality Assurance/Quality Control

QA/QC samples included field blanks, matrix blanks and field duplicates. SRS recoveries for BDE 126 were higher for Soxhlet and longer-duration ASE extraction method (average of 89%) compared to a shorter-duration ASE method (average of 24%). For $^{13}\text{C}_{12}$ BDE 209, recoveries were higher with Soxhlet extraction (76% on average) compared to either ASE extraction duration (37% and 16% for long and short-duration ASE, respectively). Blanks for commercially-prepared samplers were consistently higher than for laboratory-prepared samples by approximately a factor of 10, with average values of 2 and 0.3 ng/sampler, respectively. It is likely that PDBEs were present in the polyurethane foam in the sampling media. Sample results were corrected for contamination of sampling media. Analyte recoveries in matrix spike samples were similar to matched SRS recoveries, confirming that SRS spike recoveries provide a reasonable estimate of extraction efficiency. Further research is needed to determine the joint effects of the amount of co-extracted material from the sampler matrix; extraction method, solvent, and duration/time; and source/lot of sampler material on analyte recoveries. Variability in the matrix spike recoveries demonstrate that it is critical to include $^{13}\text{C}_{12}$ BDE 209 SRSs since it appears that BDE 209 recovery is impacted by the variables listed above and possibly others. Instrument limits of detection are low, approximately 0.2 ng/mL, so method detection limits (MDLs) are driven by background

contamination of the samplers, which is best characterized from field blanks. MDLs were defined as three times the standard deviation of field blanks. On average, MDLs for PentaBDE, OctaBDE, and DecaBDE congeners were 1.0 (n = 5 sample batches), 3.6 (n = 3), and 1.1 (n = 5) ng/sampler, respectively. Note that MDLs were determined for each sampler batch/extraction method. Analysis of duplicate pairs indicated good detect v. non-detect agreement (90%) and precision (median coefficient of variation = 13%).

Daily Dose Calculations

Average daily dose (ADD) was calculated using a similar methodology employed by the U.S. Environmental Protection Agency (EPA) to derive 'Public Health Levels' for PCBs in schools (USEPA 1997, 2010). ADDs for aircraft and non-aircraft exposures were calculated using the formulas and assumptions presented in Table 1, weighted by the time spent in each environment. Aircraft exposures totaled 1000 hours per year to simulate a typical flight attendant duty schedule (Schechter et al. 2011). PBDE exposure concentrations were obtained from data from this study and the peer-reviewed literature (Allen et al. 2007; Allen et al. 2008a; CARB. California Air Resources Board 2005; Christiansson et al. 2008a; Lorber 2008). ADDs were calculated using 95th percentile estimates of inhalation rate, dust ingestion and median exposure concentrations for non-aircraft and aircraft exposures. A high estimate of ADDs was then calculated by substituting median aircraft exposure concentrations with 95th percentile values.

Derived Inhalation Exposure Limits

Inhalation exposure limits were derived for congeners measured in this study with published RfDs (BDEs 47, 99, 209) ((USEPA 2008a, b, c). To derive the inhalation exposure limits, ADDs were calculated in the same manner described above for all exposure pathways excluding inhalation aboard aircraft using the high estimate of ADD. This ADD was then subtracted from the RfD for each congener. The difference represents the average daily dose that a worker could receive from inhalation of PBDEs aboard aircraft, accounting for all other exposure pathways, without the total dose exceeding the RfDs. To derive an inhalation exposure limit from this maximum inhalation ADD, we back-calculated the maximum exposure concentration using the same assumptions for inhalation exposure presented in Table 1.

Statistical Methods

Summary statistics and Spearman correlations were calculated using SAS Statistical Software, version 9.0 (Cary, NC), and graphs were produced using SigmaPlot graphing software (Systat Software Inc., San Jose, CA). Data less than the MDL were substituted with ½ MDL for calculating summary statistics. Bayesian Decision Analysis (BDA) was conducted using a method described by Hewett et al. and the American Industrial Hygiene Association (AIHA) (AIHA 2009; Hewett et al. 2006) and performed using IH Data Analyst Software (Exposure Assessment Solutions, Inc., Morgantown, WV). We assumed a non-informative prior and data satisfied log-normal distribution assumptions for BDA. The 95th percentile exposure values were calculated using log-probit regression to account for left censored data. Exposure categories were classified based on guidance on control banding from the American Industrial Hygiene Association (AIHA) (AIHA 2006, 2009).

4.1.2 Results

PBDEs in Aircraft Air

In-flight concentrations of PBDEs in air are presented in Table 1. BDE 47 was detected in 63% of samples with a median concentration of 1.3 ng/m³ (range: <0.04 – 20 ng/m³) and BDE 209 was detected in 42% of samples with a median concentration of <1.2 ng/m³ (range: ND – 2,100 ng/m³). BDE 99 and BDE 100 were detected in fewer than 30% of samples due to higher limits of detection as a result of background levels in the sampling media, with maximum concentrations of 41 and 9.4 ng/m³, respectively. BDE 183 was detected in 11 of 34 samples (median concentration = <1.3 ng/m³; maximum concentration = 98 ng/m³). In an analysis of detected values only, the concentrations of the congeners associated with the PentaBDE commercial product (BDEs 47, 99, 100) were moderately to strongly correlated (Spearman R: 0.72-0.93, p<0.01). There was suggestive evidence of a moderate correlation between BDE 47, BDE 99 and BDE 209 (Spearman R: 0.43, p=0.11; 0.65, p=0.06). BDE 183 and BDE 209 were also correlated (Spearman R: 0.76, p=0.01). A sufficient number of detected samples were not available for correlation analysis of the PentaBDE congeners and BDE 183.

Table 1. In-flight PBDE concentrations (ng/m³) in air

Congener	n	Pct Detect	Median	75th Pctile	95th Pctile	Max
BDE 47	59	63%	1.3	2.8	5.1	20
BDE 99	59	22%	<1.3	<11	27	41
BDE 100	59	29%	<0.4	0.8	6.3	9.4
BDE 183	34	32%	<1.3	25	82	98
BDE 209	59	42%	<1.2	5.3	39	2100

Daily Dose Estimates for Flight Crew

Total average daily dose (ADD) for non-aircraft exposures was calculated as 5.2 ng/kg-day for the five congeners. The non-aircraft exposure was dominated by dietary and dust ingestion, which accounted for over 95% of the non-aircraft dose. Average daily dose in aircraft, using median values for dust concentrations in aircraft (Christiansson et al. 2008a), median air concentrations from this study but high estimates of dust ingestion and inhalation rates (USEPA 1997), were calculated as 3.4 ng/kg-day. High estimates of average daily dose in aircraft, calculated using 95th percentile concentrations in aircraft from the same studies, was 61 ng/kg-day.

Derived Inhalation Exposure Limits

The calculated ADDs for BDEs 47, 99 and 209 do not exceed each congener's individual reference dose (Rfd) (EPA does not have a published RfD for BDE 100 or BDE 183). On a congener by congener basis, the calculated total ADDs from all non-aircraft and aircraft exposures, excluding the fraction from inhalation exposures onboard aircraft, were subtracted from EPA's RfDs for each congener. For the most health-protective scenario (i.e., highest estimates of background exposure), the remaining dose was 84 ng/kg-day, 73 ng/kg-day and 6981 ng/kg-day for BDE 47, 99, and 209, respectively. This translates into a

derived maximum inhalation exposure limit on aircraft of 2500 ng/m³, 2200 ng/m³ and 206,000 ng/m³, respectively, that a flight crew member could be exposed to without exceeding EPAs RfDs.

Likelihood Estimates for Exceeding Derived Inhalation Exposure Limits

The derived inhalation exposure limits are several orders of magnitude higher than the measured air concentrations in this study (Figure 3). The 95th percentile air concentrations in this study (Table 2) are less than 1% of the calculated maximum inhalation exposure limit. Based on guidance from the American Industrial Hygiene Association (AIHA), 95th percentile exposure concentrations that represent less than 10% of an OEL may be characterized as “Highly Controlled” (AIHA 2009). Results from Bayesian Decision Analysis indicate that the probability of 95th percentile air concentrations exceeding 1% of the exposure limit is zero for all congeners evaluated.

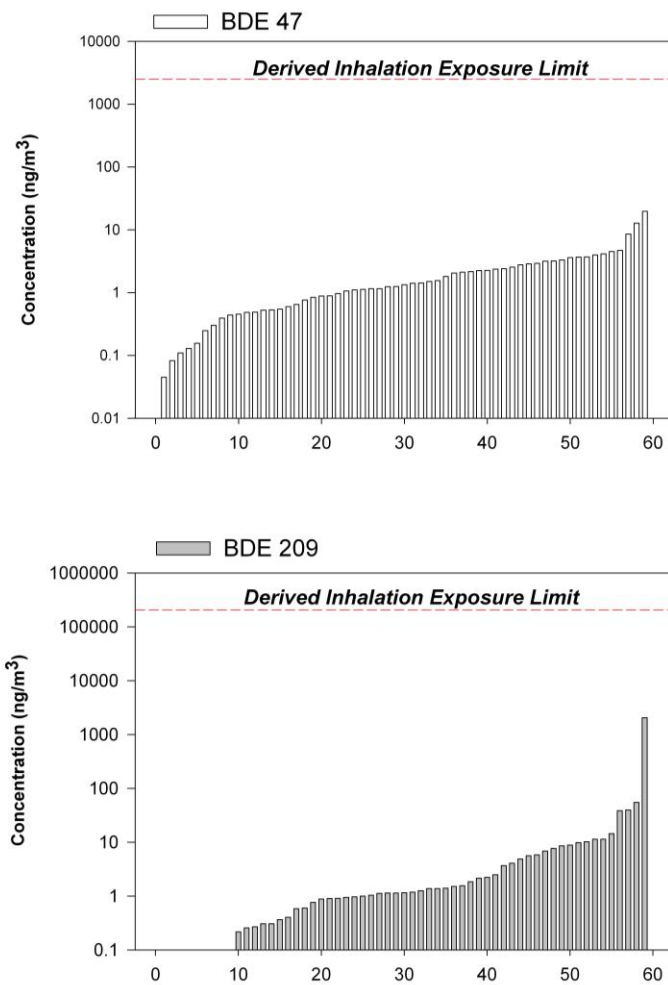


Figure 3. Distribution of measured BDE 47 and BDE 209 concentrations (ng/m³) in this study in relation to the derived inhalation exposure limit for flight crew

4.1.3 Development of ACER/RITE Analytical Capabilities

Overview

Two methods for analyzing BPDE flame retardants from dust, wipe and air samples to be collected from aircraft were evaluate using a Gas Chromatograph/Ion Trap Mass Spectrometer (GC/ITMS) in the Selective Ion Storage (SIS) mode. The first was to use Solid Phase Ion Extraction as the injection method and the second was to use Automated Pressure Sealed Vessel Solvent Extractions method. These were evaluated as they minimize the amount of material required and the amount of time require for extraction by trained chemists.

Instrumentation Sensitivity

Mass spectral considerations were used to improve sensitivities of the method using GC/ITMS in Selected Ion Storage (SIS) mode. A Saturn 2200 GC/MS ion trap mass spectrometer (Walnut Creek, CA) was used for analysis. The SIS mode has performed in these conditions: Target RIC of 21400 counts, Maximum ionization time of 65000usec, prescan ionization time of 100 usec, Background mass of 45 m/z, RF dump value of 650 m/z. and emission current of 30 uamps were used. Saturn GC/MS workstation version 6.6 software was used for data acquisition, integration, and quantification. The method detection limits for extraction of Sandy soils are presented below (Table 2).

Table 2. Method Detection Limits (ng/g of dust) with SPME			
Analyte	MDL	Analyte	MDL
PBDE 17	0.012	PBDE 154	0.077
PBDE 28	0.011	PBDE 153	0.055
PBDE 71	0.009	PBDE 138	0.238
PBDE 47	0.043	PBDE 183	0.228
PBDE 66	0.124	PBDE 190	0.570
PBDE 100	0.036	HBCD	0.524
PBDE 99	0.039	TBBPA	10.8
PBDE 85	0.151		

SPME Method

The automated Solid Phase Ion Extraction (SPME) method for PBDE analysis was evaluated for filter paper and dirt/dust using GC/ITMS in the SIS mode for the compounds listed in Table 3. The samples were prepared using two extraction methods. A headspace (HS)-SPME method was used for filter paper, house dust and soil samples. A SPME fiber holder was used in conjunction with a LEAP Combi PAL autosampler (Figure 4) to perform the extraction and injection into the GC/MS. In the initial experiments, analytes were spiked onto filter paper to simulate dust wipes and on to a sandy soil as a dust surrogate.

Table 3. Compounds evaluated for SPME analysis			
Haloethers	MW	Log Kow	MDLs (PPB)
4-bromophenyl phenyl ether	249.1	4.94	0.5
4-chlorophenyl phenyl ether	204.7	4.69	0.5
bis (2-chloroisopropyl) ether	171.1	2.39	1.0
bis(2-chloroethyl) ether	143.0	1.56	0.5



Figure 4. LEAP Combi PAL autosampler

SPME Method from Filter Paper

Two SPME methods were evaluated to optimize both extraction efficiency and maximize sensitivity. The methods were segregated for the smaller, lower boiling PBDEs and the larger PBDEs. PBDE 17 to 99 were done with method A while BDE 85 through TBBPA were analyzed with method B. The conditions for the SPME_Method A were: 65uM polydimethylsiloxane-divinylbenzene (PDMS/DVB)-coated SPME fiber, 0.3 to 0.5 g of filter, or soil in 20 mL vials, extraction time of 50 min, desorption time of 10 min, preinjection time of 2.58 min, incubation temperature of 135 °C, agitation speed of 350 rpm, agitation on time of 5 seconds, agitation off time of 2 seconds, vial penetration 25.0 mm, and injection penetration of 54.0 mm.

The conditions for SPME_Method B were: Green-7uM polydimethylsiloxane(PDMS)-coated SPME fiber, 0.3 to 0.5 g of filter, dust or soil in 20 mL vials, extraction time of 70 min, desorption time of 10 min, preinjection time of 2.58 min, incubation temperature of 185 °C, agitation speed of 350-500 rpm, agitation on time of 5 seconds, agitation off time of 2 seconds, vial penetration 25.0 mm, and injection penetration of 54.0 mm.

While the recoveries for the mid-weight compounds were acceptable they were poorer for the lightest and heaviest PBDEs and for HBCD and TBBPA (Figure 5). However, since a number of the

recoveries from filter paper were acceptable the approach was considered promising enough to continue to see if the conditions could be optimized and could be used for dust samples.

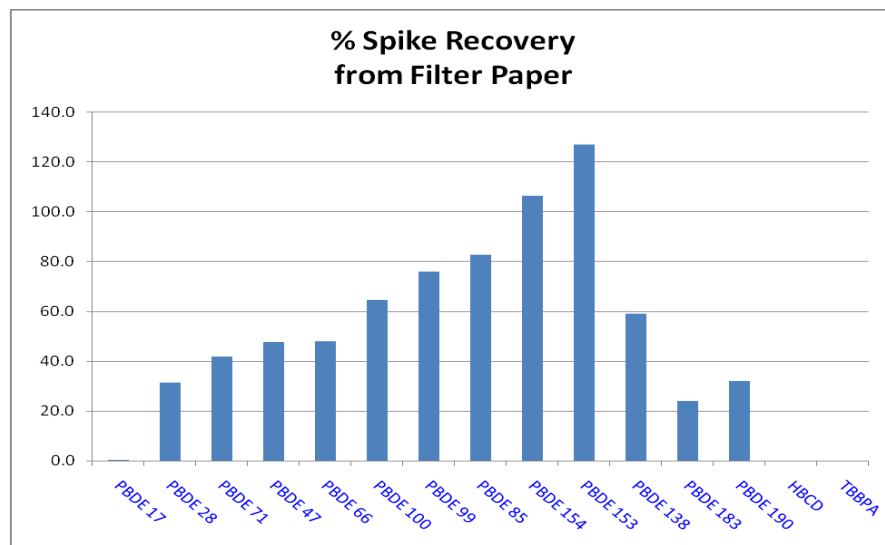


Figure 5. Recovery of flame retardants from filter paper

SPME Method from Soil/Dust

The recovery from soil/dust was more difficult to improve. The equilibrium that defines the partition between fiber and sample matrix was not significantly driven to the fiber within normal operating parameters. Very high temperatures > 250 °C and long deposition times > 200 min provided little improvement in recovery. The soil/dust recoveries did not reach a high enough level to continue with SPME methods (Figure 6). It was judged that at least a 40% minimum recovery would be required to use stable isotopic internal standards to compensate for sub 100% recoveries. Thus, the SPME method was deemed not likely to provide adequate analytical results for use so another extraction and analysis protocol was evaluated.

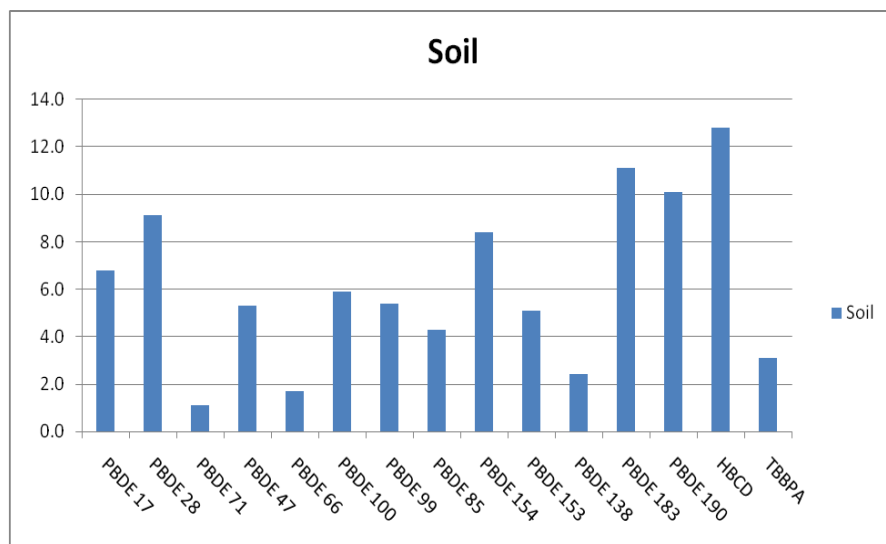


Figure 6. Recovery of flame retardants from soil/dust

Automated Liquid Extraction

An Automated Pressure Sealed Vessel Solvent Extraction (PVSE) method for PBDE analysis in house dust using programmable temperature vaporization large volume injection and GC/ITMSMSMS was evaluated.

Pressure Sealed Vessel Solvent Extraction

The conditions for the PVSE method were: 0.3 to 0.5 g of house dust in pressurized 20 mL vials, 4 mL of methylene chloride as a extraction solvent, extraction time of 20 min, incubation temperature of 100 °C, agitation speed of 600 rpm, agitation on time of 5 seconds, agitation off time of 2 seconds.

GC/ITMSMSMS Conditions

The Saturn 2200 GC/MS system described for the SPME evaluation was also used this analysis. The injector temperature program started at 100°C and held in a splitless mode for 1.5 min, the temperature was rated from 100°C–280°C at 100 °C/min and held for 15min. Other GC/MS parameters and conditions were maintained as described above. The two principal differences between the SPME and

PVSE GC/MS protocols were the use of a shorter column (quicker elution without loss of complete separation) and an MS/MS/MS mode to increase the signal to noise ratio.

A triple stage Mass Spectrometry (MS/MS/MS) method was performed as a detection tool. The primary procedure for MS³ is the same as MS/MS, only an MS³ experiment allows repeated isolation and CID using either resonant or non-resonant excitation. The optimized MS² and MS³ parameters for each PBDE are summarized in Table 4a and 4b.

Table 4a. Optimized MS/MS parameters for the analysis of PBDE standard.

Segment Time (min.)	Precursor 1 st Product Mass (m/z)	Excitation Storage Levels (m/z)	Excitation Amplitude (Volts)
8.00 –13.00	247.0	109.0	0.20
13.00 –16.60	327.0	144.0	0.40
16.60 – 20.22	407.0	180.0	0.30
20.22 – 24.60	487.0	215.0	0.30
24.60 – 35.58	564.0	250.0	1.40

* The isolation window in m/z is 5.0 for all and their waveform type is resonant.

* Target RIC of 21400 counts, Maximum ionization time of 65000usec. Prescan ionization time of 1500 usec, Background mass of 45 m/z, RF dump value of 650 m/z. and emission current of 30 uamps

Table 4b. Optimized MS/MS/MS parameters for the analysis of PBDE standard.

Segment Time (min)	Precursor 1 st Product Mass (m/z)	Excitation Storage Levels (m/z)	Excitation Amplitude (Volts)
8.00 –13.00	247.0	109.0	0.80
	139.0	61.1	0.40
13.00 –16.50	327.0	144.0	1.00
	220.0	96.9	0.40
16.50 – 20.22	407.0	180.0	0.90
	298.0	131.0	0.40
20.22 – 24.60	487.0	215.0	3.40
	376.0	165.9	0.40
24.60 – 36.00	564.0	250.0	4.00
	456.0	201.3	0.40

* Target RIC of 21400 counts, Maximum ionization time of 65000usec. Prescan ionization time of 1500 usec, Background mass of 45 m/z, RF dump value of 650 m/z. and emission current of 30 uamps

PBDE Recoveries from Dust for the PVSE Method

The overall recoveries and detection limits of the PVSE method were acceptable for use with labeled isotopes to correct for individual compounds (Table 5). Further optimization and comparison to a standard extraction method is on-going.

Table 5. Recoveries and Method Detection Limits (ng/g of dust) with PVSE					
Analyte	% Recovery	MDL (ng/g)	Analyte	% Recovery	MDL (ng/g)
PBDE 17	72.9	0.20	PBDE 85	62.4	0.30
PBDE 28	38.9	0.26	PBDE 154	39.4	0.03
PBDE 71	15.9	1.32	PBDE 153	67.0	0.06
PBDE 47	67.6	0.20	PBDE 138	66.9	0.22
PBDE 66	23.9	0.66	PBDE 183	86.5	0.03
PBDE 100	116	0.17	PBDE 190	97.1	0.06
PBDE 99	300*	0.22			

*PBDE 99 was present in the dust but the recovery has not been corrected for the background level yet, so it exceeds 100%.

4.2 Aircraft Characterization and Exposure Assessment

4.2.1 Methods

Dust Sampling

Dust samples (n=40) were collected on aircraft using a standardized collection protocol based on previously published methods (Allen et al. 2008a; Rudel et al. 2003). Briefly, a cellulose extraction thimble is fit into a vacuum crevice tool made of Teflon and secured using a rubber O-ring. The sampling tool is then connected to a canister vacuum and researchers collect dust from a pre-defined surface area by drawing the crevice tool across the top of all surfaces. After dust collection, the thimbles are wrapped in aluminum foil, sealed in polyethylene zip bags and stored at -4 °C. Equipment is cleaned in a 1% solution of detergent and hot water between sample collection to prevent cross contamination.

Analytic Methods

Dust samples were sieved to less than 500 µm to remove large debris. Sieving was performed using an ASTM certified metal sieve. Samples were analyzed for the broad range of flame retardants listed in Table 6 according to previously published methods (Stapleton et al. 2005; Stapleton et al. 2009)

Category	Analyte
Polybrominated diphenyl ethers (PBDEs)	BDE 47
	BDE 99
	BDE 100
	BDE 209
Tris Halogenated Phosphates	Tris(1,3-diCl-isopropyl)phosphate (TCDDPP)
	Tris(2-Cl-ethyl)phosphate
	Tris(2,3-diBr-propyl) phosphate
High-use brominated flame retardants (BFRs)	tetraBromo bisphenol A (TBBPA)
	Hexabromocyclododecane (HBCD)
Firemaster 550 compounds	2-ethylhexyl-tetraBr-benzoate (EHTBB)
	Bis-(2-ethylhexyl)-tetraBr-phthalate (BEHTBP)

Source Identification by XRF

Bromine concentration in products, as determined by x-ray fluorescence, have been previously shown to be a useful indicator of PBDE concentration (Allen et al. 2008b). To identify materials on aircraft that were likely to contain PBDEs, we used a portable XRF analyzer (Innov-X, Woburn, MA) to quantify bromine concentrations in materials aboard 20 aircraft. Over 200 measurements were recorded (between 5 and 23 samples were collected per aircraft).

HEPA Filters

Onboard HEPA filters were collected from numerous aircraft (~100) in segments approximately 10cm by 10cm. A relatively small portion of the filter was previously extracted for analysis for TCPS and metals; the portions that remain are archived at Kansas State University and the University of Medicine and Dentistry of New Jersey (UMDNJ). These samples represent different aircraft models and loading factors. An initial screening of all filters for bromine levels was done using XRF to determine which filters contain the highest bromine levels and therefore most likely to contain the highest quantities of brominated flame retardants and to ensure that the used filters have higher bromine levels than the filter material prior to conducting the detailed chemical analyses.

Filter punches were received at room temperature and stored in a freezer until analysis. Keddeg (5418) and Donaldson (5229) filters were used in an initial evaluation of the planned extraction method (used previously on dust samples). For each filter type, 2 punches (duplicates) and one matrix spike (spikes ranged from 10-25 ng) were extracted by sonication in hexane:dichloromethane (DCM). Precipitates formed when the samples were concentrated, more so for the Keddeg filter samples. Some extracts were milky even after centrifuging. Due to the precipitates, a sample clean-up step using acid silica was used. The high-use brominated flame retardants and Firemaster 550 compounds are acid labile and not expected to withstand the clean-up step, but were included in the analyte list.

Filters were extracted by sonicating in 12 mL of 1:1 hexane:DCM for 15 minutes. A 10-mL aliquot was removed and fresh hexane:DCM was added to the filter vial and sonicated for another 15 minutes. The two extract volumes were combined in a flask containing 1 g acid silica and were shaken for one hour at 75 RPM. The extract was decanted through a small plug of muffled glass wool, and the silica was

rinsed three times, with shaking for 10min each, with 10-mL of hexane:DCM. The final extract was concentrated under nitrogen with mild heating (45°C and 55°C) to concentrate the initial extract and solvent exchange into hexane for a final volume of 1 mL. Table 3 presents the surrogate recovery spike compounds and approximate amounts.

The final extract was spiked with internal standard and transferred to a GC vial insert. This extract was analyzed using negative chemical ionization (NCI) GC/MS in multiple ion detection (MID) mode for BDEs using a DB-5 GC column (15m, 0.25 mm inner diameter (id), 0.10 µm film thickness, Agilent), with the GC oven temperature programmed from 100 to 200°C at 10°C/minute (min) and then 200 to 300°C at 30°C/min.

The GC-MS was calibrated by analyzing calibration standards interspersed with the samples in each sample analysis set. Calibration curves for each analyte and SRS were generated; the correlation coefficient (r^2) was ≥ 0.98 for each curve. Most of the calibration curves were linear and forced through the origin. The calibration standards were 0, 2, 10, 50, 100, and 250 ng/mL for all compounds except for BDE 183, which had the following calibration standards: 0, 4, 20, 100, 200, and 500 ng/mL. The calibration curve for $^{13}C^{12}$ BDE 209 included standards at 0, 54, 95, 200, 325, and 650 ng/mL. Analyte concentrations were calculated based on relative response (analyte area/IS area) and applied to the appropriate calibration curve. It was discovered after the filter samples had been analyzed that the stock mix of native BDEs for the calibration curve contained $^{13}C^{12}$ BDE 209 rather than unlabelled BDE 209. As a result, the instrument response to $^{13}C^{12}$ BDE 209 was applied for quantification of native BDE 209. This is an entirely appropriate way to handle this situation, as the ionization efficiencies are not affected by the addition of the labeled carbon.

4.2.2 Results

Dust, Filters and Source Identification by XRF

Only preliminary results for dust, filters and source concentrations aboard aircraft have been received from the laboratory and/or analyzed as of the date of this report. Final results are expected in mid-2012, and will be described in the Technical Report for 2013.

4.3 Flight Crew Body Burden and Relationship to Early Markers of Thyroid Effect

4.3.1 Methods

Overview

In the pilot phase of this project, we recruited 10 flight attendants through contact information supplied by the American Flight Attendant's union (AFA). Inclusion criteria were flight attendants working a non-stop flight route from Boston within the next two months that is no less than 3 hours. These criteria allowed us to track exposures before and after flight duty (arrival city) that are connected to a specific aircraft and includes moderate exposure times. We collected samples from the flight attendant at the airport hub location before report to duty and at the hotel in the arrival city.

Serum

Venous blood samples were collected and analyzed for several PBDEs (BDE 47, BDE 99, BDE 100, BDE 209) and represent an estimate of total absorbed dose. Briefly, two 10-ml Vacutainer red-top vials of blood were drawn from each participant by trained phlebotomists. The blood was drawn into evacuated plain tubes. The tubes were centrifuged and serum transferred to pre-washed glass bottles and kept at -20°C until analysis. From one vial, a 5-ml aliquot of serum was analyzed for PBDEs. Briefly, the serum samples will be extracted using a solid phase extraction cartridge (SPE), followed by an additional SPE cleanup step, methylation, and analysis using NCI GC/MS in the MID mode. The second 5-ml aliquot was used for analysis of thyroid hormones.

Hand-wipes

Hand wipes were collected at the end of the flight according to the method described and validated by Stapleton et al (2008). Technicians collect samples using a sterile gauze immersed in 3.0mL of isopropyl alcohol and wiped the entire surface of the hand (1 sample per hand, 2 samples per person, one field blank). Samples were stored in pre-cleaned 50 mL glass centrifuge tubes for sample extraction and analysis of PBDE congeners at the lab.

Human Subjects

The study protocol involved the testing of human subjects and therefore research protocols are subject to review by an Institutional Review Board. All protocols were approved by the Harvard IRB before research is initiated.

4.3.2 Results

Laboratory results for serum samples, hand-wipe samples and thyroid hormone were received in late 2011. The data are currently undergoing quality assurance reviews. Data analysis will be conducted in 2012 and results will be reported in the Technical Report for 2013.

5.0 FUTURE RESEARCH

5.1 Inhalation Exposure

The inhalation exposure concentrations described in this report were measured at cruising altitude, a time when aircraft ventilation rates are maximal and can exceed 15 air changes per hour. Ventilation rates while aircraft are on the ground are lower and therefore exposure concentrations in the aircraft could be higher during this part of travel. Future work should evaluate maintenance crew exposures because of the expected higher exposure concentrations while aircraft are on the ground and potential for longer exposure durations.

5.2 Characterizing Dust Exposure

Preliminary analysis of dust concentrations aboard aircraft indicate that dust is an important route of exposure. Increasing the sample size beyond the 20 aircraft in this study and including dust from additional aircraft manufacturers is warranted. Further characterization of this exposure route is also necessary, including evaluating dust from different locations in the aircraft that are relevant to exposure (e.g., seats v. floors v. galley) and the form of the PBDE compounds in dust (e.g., attached to abraded plastic or fine particles) which can be determined using scanning electron microscopy.

5.3 Understanding the Impact of Flame Retardant Migration from Source Products on Material Flammability

PBDEs are additive flame retardants (ie, not bound to the materials they are used in) and therefore migrate from the source materials into the environment. Evidence of this is the high concentrations of PBDEs in aircraft dust. What is not known is if the migration of flame retardant chemicals from the source materials causes a reduced flammability protection of that source material. Flammability testing, evaluating flame retardant emission rates, and characterizing the degree of loss of flame retardants in source material over time is warranted.

5.4 Alternate Flame Retardants

The PentaBDE and OctaBDE commercial products have been phased out, and DecaBDE is scheduled to be phased out in the coming year. Despite these phaseouts, aircraft will continue to be a source of PBDE exposure for many years due to the long usage life of the materials that flame retardants are incorporated into. As PBDEs are being replaced, however, exposure to a new class of compounds has already begun. Toxicological evidence suggests that many of these compounds, for example Tris(1,3-diCl-isopropyl)phosphate (TCDPP), could have potentially important health impacts. Examining exposure to these “new” flame retardants, and characterizing their changing use and exposure patterns over time on aircraft, is therefore a critical research need.

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