Innovative Application of Fluoro Tagging To Trace Airborne Particulate and Gas-Phase Polybrominated Diphenyl Ether Exposures

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Polybrominated diphenyl ethers (PBDEs) are flame retardants applied as coatings to many consumer products, including household items. PBDEs are released and produce airborne vapors and dusts. Inhalation of particle-phase and/or gas-phase PBDEs is therefore a major route of exposure. In an attempt to mimic realistic airborne exposures, actual uptake, and deposition of particles and vapors, we prepared and characterized particles for future animal exposure studies. To trace the particles in environmental and biological systems, we employed fluoro tagging. We synthesized, characterized, and employed three PBDE congeners, 35, 47, and 99, and five fluoro-substituted PBDEs (F-PBDEs), 17-F5′, 25-F5′, 28-F3′, 35-F5′, 47-F3, and 99-F3′, for this study. The PBDE congeners were selected because they are commonly found in house dust. For that reason, we coated spherical silica particles of 3 μm and C_{18} endcapped silica as representative and inert support materials, with 20, 30, and 40% PBDEs. We determined the particle size distributions by aerodynamic particle size spectrometry and the morphology by scanning electron microscopy. The suitability of the fluoro-tagged tracers to mimic their corresponding parent PBDEs was investigated by extraction studies from spiked blood serum. Our study is of fundamental importance to the development of xenobiotic tracers for monitoring routes of human exposure to PBDEs and understanding uptake of PBDEs from particles and vapors.

Introduction

Polybrominated diphenyl ethers (PBDEs)[1] are incorporated into plastics, foams, and textiles to provide consumer products with flame retardant properties (*1*). They may be released as fine particles and/or partition into the air because of their semivolatile nature (*2*). The concentrations of many persistent organic pollutants, such as polychlorinated biphenyls (PCBs), polychlorinated dibenzo dioxins (PCDDs), and pesticides are decreasing, whereas those of PBDEs are on the rise (*3−6*). PBDEs are distributed globally and are detected in most environmental media including air (*7, 8*), water (*9*), soil (*10*), sediment (*11, 12*), and biota (*3, 9, 13*). They are also found in human tissue, breast milk, and blood (*14−18*) and have been associated with endocrine disruption (*19*) and reproductive (*20*), developmental (*21*), and neurotoxicity (*22*). Recent in vitro studies have shown that hydroxylated metabolites of PBDEs bind with high affinity to the thyroid hormone transport protein, transthyretin (*23, 24*), and to the thyroid hormone receptors (*24, 25*). Only the fully brominated technical DecaPBDE has been tested for its carcinogenic potential, and these tests have shown some evidence of carcinogenicity in animals (*26*).

Despite their potential to promote adverse health effects, little is known about the relative importance of different routes by which humans are exposed to PBDEs. The main route of exposure is sometimes ingestion, such as through consumption of fish that have accumulated PBDEs in their fatty tissue (*27, 28*). Alternatively, the predominate route of exposure may be inhalation or dermal contact for particle-phase and/or gas-phase PBDEs in indoor (*29*) or occupational settings (e.g., computer/electronics repair, maintenance, or dismantling) (*30, 31*). Appropriate toxicology studies are complex to design, and control strategies that minimize exposures are difficult to craft, without a better understanding of the routes by which PBDEs enter the body.

Two key issues hamper our ability to evaluate routes of human exposure to PDBEs. First, toxicity tests are often conducted with a toxicant carried in a vehicle, such as dimethylsulfoxide (DMSO), dimethylformamide (DMF), or perfluoro surfactants like perfluorooctanoic acid (PFOA). Solvents can alter the biological uptake of toxins or PBDEs by functioning as membrane carriers (*32, 33*)

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[1] Abbreviations: APS, aerodynamic particle sizer; BZL, Ballschmitter−Zell−Luthe; ^{13}C NMR, carbon nuclear magnetic resonance spectroscopy; F-PBDE 17-F5′, 5′-fluoro-2,2′,4-tribromodiphenyl ether; F-PBDE 25-F5′, 5′-fluoro-2,3′,4-tribromodiphenyl ether; F-PBDE 28-F3′, 3′-fluoro-2,4,4′-tribromodiphenyl ether; F-PBDE 35-F5′, 5′-fluoro-3,3′,4-tribromodiphenyl ether; F-PBDE 47-F3, 3-fluoro-2,2′,4,4′-tetrabromodiphenyl ether; F-PBDE 99-F3′, 3′-fluoro-2,2′,4,4′,5-pentabromodiphenyl ether; F-PBDE, monofluoro-substituted polybrominated diphenyl ethers; F-tagged, fluoro-tagged or substituted; ^{1}H NMR, proton nuclear magnetic resonance spectroscopy; PBDE 35, 3,3′,4-tribromodiphenyl ether; PBDE 47, 2,2′,4,4′-tetrabromodiphenyl ether; PBDE 99, 2,2′,4,4′,5-pentabromodiphenyl ether; PBDEs, polybrominated diphenyl ethers; PCBs, polychlorinated biphenyls; PCDDs, polychlorinated dibenzo dioxins; SEM, scanning electron microscopy.
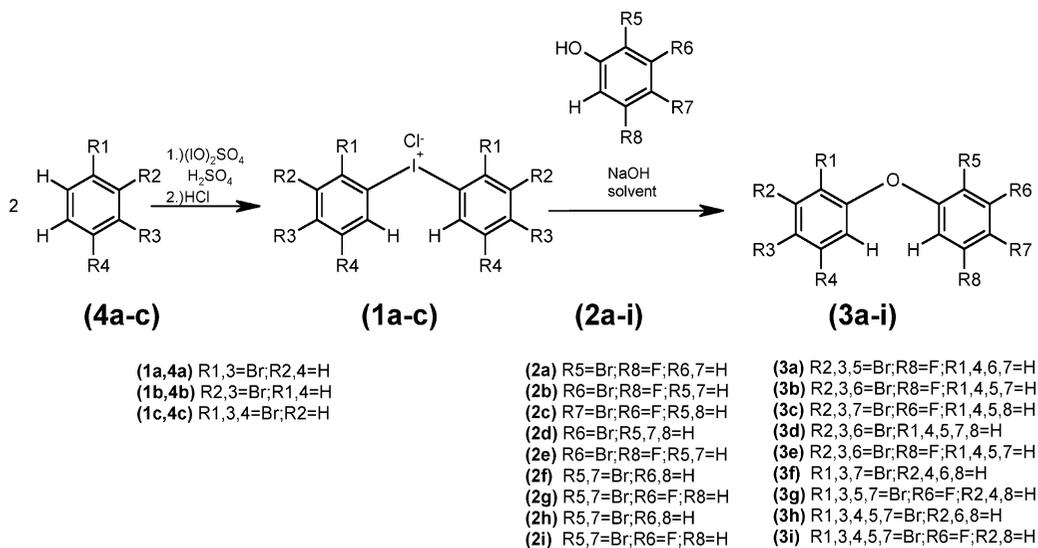
and have very little environmental relevance. However, PBDEs are often applied as coatings to a parent backbone material that may range from an inorganic material to an organic polymer (*34*). Thus, the size distribution, morphology, surface area, surface chemistry, particle reactivity, particle composition, and backbone material may influence the behavior of PBDEs and their biological uptake (*35*). Consistent with the message of the recent Toxicological Highlight (*36*) entitled "How Meaningful Are the Results of Nanotoxicology Studies in the Absence of Adequate Material Characterization", we characterized PBDEs prior to their application for in vivo and in vitro toxicological studies.

Second, there are major deficiencies in the methodologies that are used to track particles in exposure studies to follow routes of PBDE uptake and distribution. This is a major challenge with the analytical tools available. Employing tracers is a strategy to address this. In tracer labeling, the compound of interest is tagged, facilitating its detection in exposure studies. A basic requirement is that the tagged analogue must resemble the compound of interest in terms of its chemical and physical properties but not be a normal constituent of the sample. Tags often include unusual isotopes, such as deuterated, ^{13}C, and radiolabeled analogues. Although ^{13}C and radioactive-labeled analogues are ideal tracers in some cases, they provide poor or no structural information. Mass selective detection (MS) and/ or radioactivity determinations are the only analytical approach to distinguish between isotopic and native compounds. These determinations are often uninformative. In addition, radiolabeled compounds are challenging to handle, their use is therefore restricted, and radioactive contamination of instrumentation is problematic.

Monofluorinated PBDEs (F-PBDEs) can act as tracers in environmental, toxicological, and pulmonary exposure studies aimed to monitor absorption, distribution, excretion, bioaccumulation, and magnification of PBDEs. F-PBDEs have a number of advantages in analytical identification and quantification (*37, 38*). Specific techniques can be applied to detect F-PBDEs, for example, MS and atomic emission detection (AED) for trace analysis. The utilization of F-PBDEs by means of MS, electron capture detection (ECD), or AED allows a simultaneous detection without separation by gas chromatography (GC) (*37, 38*). Application of ^{19}F nuclear magnetic resonance (NMR) spectroscopy allows characterization of chemical structures by chemical shift and spin coupling. In addition, F-PBDEs show very similar physicochemical behaviors as compared to their corresponding parent PBDEs and do not occur in the environment, which makes them ideal internal standards and tracers.

It is our hypothesis that defined dusts composed of PBDEs or dusts coated with PBDEs will best mimic environmentally relevant exposures. Moreover, we believe that F-tagging of these dusts will provide a useful tool for determining the relative importance of various potential exposure routes. Consequently, we synthesized and employed pairs of PBDEs/F-PBDEs and produced and characterized fine powders of these compounds as well as coatings of PBDEs/F-PBDEs pairs on polar and nonpolar C_{18} endcapped silica particles. F-PBDE/PBDE pairs were chosen on the basis of origin and toxicity. The nomenclature of F-PBDEs used herein follows the Ballschmitter−Zell−Luthe (BZL) numbering system (*37, 38*). 2,2′,4,4′-Tetrabromodiphenyl ether (PBDE 47) and 2,2′,4,4′,5-pentabromodiphenyl ether (PBDE 99) are the most detected congeners found in house dust (*39*). 3,3′,4-Tribromodiphenyl ether (PBDE 35) is one of the main tribromoPBDEs detected in breast milk and in human blood serum (*14−18*). The F-PBDEs, 5′-fluoro-3,3′,4-tribromodiphenyl ether (F-PBDE 35-

F5′), 3-fluoro-2,2′,4,4′-tetrabromodiphenyl ether (F-PBDE 47-F3), and 3′-fluoro-2,2′,4,4′,5-pentabromodiphenyl ether (F-PBDE 99-F3′), were utilized as tracers. In addition 5′-fluoro-2,2′,4-tribromodiphenyl ether (F-PBDE 17-F5′), 5′-fluoro-2,3′,4-tribromodiphenyl ether (F-PBDE 25-F5′), and 3′-fluoro-2,4,4′-tribromodiphenyl ether (F-PBDE 28-F3′) were used as surrogate standards. We characterized the particle size distributions using an aerodynamic particle sizer (APS) and the morphology by scanning electron microscopy (SEM).

To evaluate the concept of F-tagging, we investigated the discrimination of F-PBDE relative recoveries vs their corresponding parent PBDEs in blood serum spiked with F-PBDE/PBDE solution and coated silica gels. The employed workup procedure is based on a procedure for blood analysis of PCBs (*40*). The study presented here is of fundamental importance to develop particles of xenobiotic tracers to monitor and understand routes of human exposure to PBDEs.

Experimental Procedures

Materials. Compounds 1a and 1b. 2,2′,4,4′-Tetrabromodiphenyl iodonium chloride (**1a**))yield, 0.91 g; 86%) and 3,3′,4,4′-tetrabromodipenyl iodonium chloride (**1b**) (yield, 1.01 g; 95%) were synthesized according to published methods (*37, 38, 40*).

Compound 1c. 2,2′,4,4′,5,5′-Heaxabromodipenyl iodonium chloride (**1c**) was synthesized with modifications of the procedure. Fuming sulfuric acid (20%, SO_3 free, 1 mL) and fuming nitric acid (100%, 0.15 mL) were added to iodine (0.161 g, 0.64 mmol), into a round-bottom flask at room temperature. The reaction mixture was stirred at 70−80 °C for 2 h. The obtained iodyl sulfate was diluted in sulfuric acid (5 mL). Under vigorous stirring and cooling to 5 °C, 1.0 g of 1,2,4-tribromobenzene (3.2 mmol) was slowly added to the iodyl sulfate $(IO)_2SO_4$. The reaction mixture was stirred at room temperature overnight. After this, the blend was cooled to 0 °C, and ice (3.5 g) was added carefully in small portions (CAUTION: exothermic reaction!). After the blend was left to stand for 15 h, the aqueous solution was filtered under aspirator pressure and the crude product was washed with water and ether (5 mL). The crude product was dissolved in methanol (50 mL) and crystallized as the chloride salt by addition of concentrated hydrochloric (0.2 mL) acid dissolved in 1 mL of methanol. In addition, the solvent volume was reduced to 10 mL. The mixture was maintained for 2 h to precipitate 2,2′,4,4′,5,5′-hexadiphenyliodonium chloride. The product was filtered under aspirator pressure and washed with ether (3 × 2 mL). The yield was 0.49 g and 0.62 mmol (49%).

2,4-Dibromo-3-fluorophenol (2c). A 2.55 g (9.55 mmol) amount of aluminum(III)-bromide was diluted in 3.97 g (14.8 mmol) of bromine under nitrogen atmosphere and stirring. A 1.50 g (9.55 mmol) amount of 5-fluoro-2-nitrophenol was added portion wise (CAUTION: HBr was produced). After all of the compounds were added, the mixture was kept overnight. The remaining bromine and hydrogen bromide were removed by a gentle stream of nitrogen. Ice was added under stirring. The obtained precipitation was filtered under reduced pressure. The yield was (crude) 3.0 g and 9.55 mmol (99%). The obtained crude 6-nitro-3-fluoro-2,4-dibromophenol (**2a**) was dissolved in 100 mL of ethanol:water (1:1, v:v). A 16.6 g (160 mmol) amount of sodium hydrosulfite was added, and the reaction mixture was stirred at room temperature for 2 h. Ethanol was evaporated under reduced pressure, and the precipitate was filtered under aspirated pressure. The yield was (crude) 1.9 g and 6.69 mmol (70%). 6-Amino-3-fluoro-2,4-dibromophenol (**2b**) was dissolved

(4a-c)   **(1a-c)**   **(2a-i)**   **(3a-i)**

(1a,4a) R1,3=Br;R2,4=H
(1b,4b) R2,3=Br;R1,4=H
(1c,4c) R1,3,4=Br;R2=H

(2a) R5=Br;R8=F;R6,7=H
(2b) R6=Br;R8=F;R5,7=H
(2c) R7=Br;R6=F;R5,8=H
(2d) R6=Br;R5,7,8=H
(2e) R6=Br;R8=F;R5,7=H
(2f) R5,7=Br;R6,8=H
(2g) R5,7=Br;R6=F;R8=H
(2h) R5,7=Br;R6,8=H
(2i) R5,7=Br;R6=F;R8=H

(3a) R2,3,5=Br;R8=F;R1,4,6,7=H
(3b) R2,3,6=Br;R8=F;R1,4,5,7=H
(3c) R2,3,7=Br;R6=F;R1,4,5,8=H
(3d) R2,3,6=Br;R1,4,5,7,8=H
(3e) R2,3,6=Br;R8=F;R1,4,5,7=H
(3f) R1,3,7=Br;R2,4,6,8=H
(3g) R1,3,5,7=Br;R6=F;R2,4,8=H
(3h) R1,3,4,5,7=Br;R2,6,8=H
(3i) R1,3,4,5,7=Br;R6=F;R2,8=H

Figure 1. Synthesis of the diphenyliodonium salts (**1a−c**) from bromobenzenes (**4a−c**) and the synthesis of F-PBDEs and PBDEs (**3a−i**) by nucleophilic aromatic substitution of the diphenyliodonium salts (**1a−c**) with bromophenols (**2a−i**).

in 15 mL of dioxane and 5 mL of hydrochloric acid (20%) and cooled to 0 °C. A solution of sodium nitrite (1.32 g, 20.1 mmol) in 5 mL of water was added dropwise over 30 min under stirring and maintained for 30 min at 0 °C. Twenty milliliters of H_2PO_3 was added, and the reaction mixture was warmed slowly to 45 °C while stirring overnight. The product (**2c**) was extracted with methylene chloride and dried over magnesium sulfate, and the solvent was evaporated under reduced pressure. The crude product was purified by flash column chromatography with silica gel as the stationary phase and hexanes:ethyl acetate (9:1) as the mobile phase [yield, 0.65 g and 2.41 mol (35%); overall yield, 0.65 g and 2.41 mmol (25.2%)].

General Procedure for Nucleophilic Substitution of the Iodyl Salts by the Phenols on the Example F-PBDE 17-F5′ (3a). 2,2′,4,4′-Tetrabromodiphenyl iodonium chloride (0.5 g, 0.79 mmol) and 2-bromo-5-fluorophenol (0.30 g, 1.58 mmol) were diluted in 15 mL of dimethyl formamide (DMF). Sodium hydroxide (0.03 g, 0.8 mmol) was added, and the mixture was stirred and refluxed overnight. After the reaction was complete, the mixture was filtered by employing a flash column with silica gel and was washed with *n*-hexane to remove the remaining DMF and inorganic impurities. Purification by flash column chromatography with silica gel and *n*-hexane resulted in 0.29 g and 0.68 mmol (86%), GC-MS purity (99%).

Compounds 3b, 3c, 3g, 3h, and 3i. F-PBDE 25-F5′ (**3b**) [yield, 0.215 g and 0.51 mmol (64%); purity, 99%], F-PBDE 28-F3′ (**3c**) [yield, 0.320 g and 0.75 mmol (95%); purity, 99%], F-PBDE 47-F3 (**3g**) [yield, 0.24 g and 0.48 mmol (39%); purity, 99%], PBDE 99, (**3h**) [yield, 0.21 g and 0.37 mmol (94%); purity, 99%], and F-PBDE 99-F5, (**3i**) [yield, 0.28 g and 0.48 mmol (50%); purity, 99%] were performed as described above.

Compound 3d. PBDE 35 (**3d**) was synthesized under modified conditions. 3,3′,4,4′-Tetrabromodiphenyl iodonium chloride (1.58 g, 2.5 mmol) and 3-bromophenol (0.43 g, 2.5 mmol) were diluted in 20 mL of water:dioxane (1:1, v:v). Sodium hydroxide (0.1 g, 2.5 mmol) was added, and the mixture was stirred and refluxed overnight. After the reaction was completed, it was extracted with diethyl ether (3 × 15 mL). The unified organic layers were dried over magnesium sulfate, and the solvent was evaporated under reduced pressure. The crude product was purified by flash column chromatography with silica gel and *n*-hexane [yield, 0.36 g and 0.88 mmol (35%); purity, 99%].

Compounds 3e and 3f. F-PBDE 35-F5′ (**3e**) [yield, 0.215 g and 0.51 mmol (65%); purity, 99%] and PBDE 47, (**3f**) [yield, 0.640 g and 1.31 mmol (83%); purity, 99%] synthesis was performed as described above for **3d**). For synthesis, see Figure 1; for ¹H, ¹³C, and MS characterization, see Tables S1−S5 of the Supporting Information. Silica gel (60 Å C:C 40−63 μm), silica gel (Nucleosil, 100 Å, 3 μm), and endcapped silica gel C_{18} (Nucleosil 100 Å, 3 μm) were from Fisher Scientific (Pittsburgh, PA).

Methods. Preparation and Characterization of Dust Composed Exclusively of PBDE/F-PBDE and Coated Spherical Silica Gel Particles with PBDE/F-PBDE. Preparation of Dust Composed Exclusively of PBDE/F-PBDE. Dusts composed exclusively of PBDEs were prepared by dissolving approximately 50 mg of the PBDE/F-PDBE in 0.5 mL of diethyl ether. A small volume (25 μL) of this solution was superimposed with a glass capillary onto a glass slide measuring 2.55 cm × 7.65 cm. The ether was evaporated, and the thin layers of PBDEs or F-PBDEs were scraped off with another glass slide to give the intended particles. Dusts of the individual congeners PBDEs 35, 47, and 99 and of the F-PBDEs 35-F5′, 47-F3, and 99-F3′ and a mixture were prepared according to this procedure.

Preparation of Dust Composed of Spherical Silica Gel Particles Coated with F-PBDE/PBDE. PBDEs have been broadly used to coat materials ranging from inorganic materials to organic polymers. To mimic organic and inorganic backbones while comparing the materials, we chose silica gel as a monodispersed and properly sized support material. Endcapped silica gel particles by C_{18} chains were applied to mimic a general organic backbone. The material properties of both backbones were identical in particle size range, mesh, porosity, and chemical composition. Because the organic matter was chemically bound to the particle, the organic component of the backbone should not function as a carrier for transport across the membrane. This was an additional benefit of this chosen approach. The size of the spherical silica gel particles was on average 3 μm in diameter. This was in the range of household dust particles that could contain PBDEs.

A stock solution was prepared with a total F-PBDE/PBDE concentration of 2 mg/mL, containing equal molar amounts of each congener PBDE 35, 47, and 99 and F-PBDE 35-F5′, 47-F3, and 99-F3′. A backbone of silica gel (Nucleosil, average diameter 3 μm, pore size 100 Å) was used as a support material,
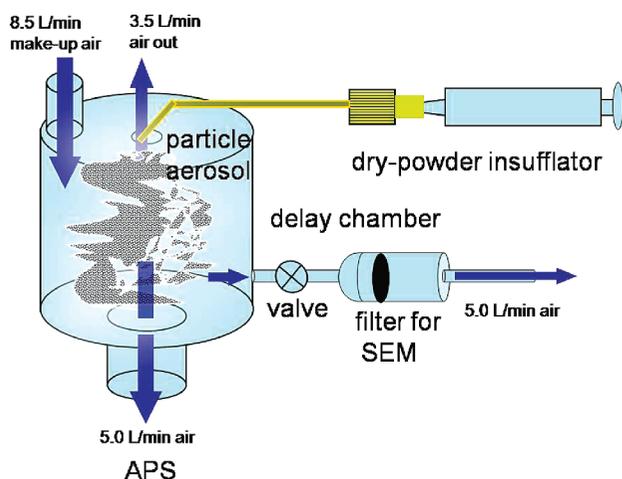
Figure 2. Set-up of the F-PBDE/PBDE particle measurements by APS and collections on a filter by aerosolization via a dry powder insufflation into a delay chamber. The blue arrows indicate the air flows in the system.

with C_{18} endcapped and original surface. Four different coating procedures for both support materials were applied to optimize the coating process: (A) Support material (40 mg), stock solution (5 mL), and diethyl ether (5 mL) as a solvent were mixed in a round-bottom flask, and the solvent was directly evaporated under reduced pressure in a rotary evaporator with tumbling at 30 rpm, without outside warming; (B) processed as above with outside warming to 36 °C; (C) incubation with diethylether and F-PBDE/PBDE mixture for 12 h prior to evaporation at 36 °C; and (D) incubation with only diethylether for 12 h and subsequent addition of the F-PBDE/PBDE mixture and evaporation at 36 °C. In all variations of the coating process, the silica gel particles were resuspended five times with 1.5 mL of diethyl ether, and the solvent was evaporated according to the descriptions of the individual procedures above. Following procedure B, particles of C_{18} endcapped and original silica gel with 20, 30, and 40% F-PBDE/PBDE coating were prepared [40 (20%), 35 (30%), or 30 mg (40%) of silica gel and 5.0 (20%), 7.5 (30%), or 10.0 mg (40%) of F-PBDE/PBDE mixture].

Characterization of Dusts by APS and SEM. As shown in Figure 2, a dry powder insufflator (model DP-4, Penn-Century, Inc., Philadelphia, PA) was used to aerosolize 0.5 mg of each compound into a cylindrical delay chamber (8 cm diameter × 12 cm height). This type of insufflator has been used to instill particles into rodents during exposure studies. Particle free air at 8.5 L/min was fed into the chamber to provide makeup air. The size distribution of the aerosolized dusts in the chamber was measured in triplicate with a APS (model 3321, TSI Inc., Shoreview, MN). The APS measures particle number concentration by aerodynamic diameter in 32 channel per decade from 0.5 to 20 μm aerodynamic diameter. Prior to conducting these measurements, an aerosol composed of 3 μm polystyrene latex spheres (Polysciences, Inc., Warrinton, PA) was measured to be within ±10% of their stated size to verify proper calibration of the APS.

To observe their morphology by SEM, a sample of the aerosolized particles in the chamber was collected onto a 0.4 μm polycarbonate filter (E0425-MB, SPI Supplies and Structure Probe, Inc., West Chester, PA) at a flow rate of 5 L/min for 30 s after injection. A portion of the filter was mounted on an SEM stub with double-sided, graphite tape, and the edge of the filter was painted with silver paint to promote conductivity. The surface of the sample was coated with a gold layer under an inert argon atmosphere by material beam for 2.5 min at 10 Torr

and 200 A. Images of the sample were taken with an SEM (Hitachi S-4800, Boston, MA) operated at an accelerating voltage of 1 kV.

Proof of Concept of the F-Tagging Tool for Particle Tracking. Sprague−Dawley rats were anesthetized and exsanguinated under an Institutional Animal Care and Use Committee (IACUC)-approved protocol. Pooled rat blood serum was spiked (66.7 mg F-PBDE/PBDE/g blood serum) with (i) F-PBDE/PBDE-coated silica gel particles directly and inverted for 120 min prior to extraction, (ii) F-PBDE/PBDE-coated C_{18}-end-capped silica gel particles and inverted for 120 min prior to extraction, (iii) F-PBDE/PBDE-coated silica gel particles pretreated with 1.0 mL of HCl (6 mol/L) and 6.0 mL of *n*-hexane and inverted for 120 min prior to extraction, and (iv) F-PBDE/PBDE-coated silica gel particles pretreated with 1.0 mL of HCl (6 mol/l) and 6.0 mL of *n*-hexane and inverted for 120 min prior to extraction. We normalized these values with previously dissolved stock F-PBDE/PBDEs in *n*-hexane. Recovery was above 99%. Each sample was separated equally into three centrifuge tubes (12 mL, tube 1) into which HCl_{aq} (1.0 mL, 6 M), 2-propanol (3.5 mL), and *n*-hexane:MTBE (3.5 mL, v:v, 1:1) were added. After each addition, the samples were mixed by a vortex mixer. The samples were inverted (5 min, 60 rpm) and centrifuged (1.380 G, 5 min). The organic layer was transferred (tube 2), and KCl_{aq} (4 mL, 1%) was added to it. The aqueous layer was extracted (tube 1) with *n*-hexane:MTBE (3.0 mL, v;v, 1:1), inverted (5 min), and centrifuged (5 min, 1.380 G), and the organic layer was transferred and unified (tube 2). The aqueous solution was extracted likewise, and the organic layers were unified (tube 2). The solvent was removed with a gentle stream of nitrogen on a water bath, till 0.5 mL remained. *n*-Hexane (6 mL) and H_2SO_4 (2 mL, concentrated) were added (tube 2), inverted (2 min, 60 rpm), and centrifuged (5 min, 1380 G), and the organic layer was transferred (tube 3). The aqueous layer (tube 2) was extracted with *n*-hexane (3 mL), inverted (2 min, 60 rpm), and centrifuged (5 min, 1.380 G), and the organic layers were again unified (tube 3). The solvent was evaporated as described above, and the remaining concentrate was transferred to a column [15 cm, 0.1 g of acidified silica gel (silica gel:H_2SO_4 2:1) in the bottom, 1 g of silica gel on the top] and eluted with *n*-hexane (10 mL). The solvent was evaporated, and the concentrate was transferred into a GC vial (1.5 mL). Analytical separation and quantification were carried out by GC-MS as described below. Comparisons of mean recoveries across experimental conditions were carried out with mixed linear regression models. Standard errors were calculated. Tests for mean differences were two-sided and were carried out with the SAS statistical software (Cary, NC).

Results

Synthesis of the PBDEs and F-PBDEs (3a−i). The nucleophilic substitution of the diaryl iodonium chlorides by phenols was used for the synthesis of all PBDE and F-PBDE congeners The nucleophilic substitution of the diaryl iodonium chlorides by phenols resulted in satisfactory yields for PBDEs 35 (**3d**), 47 (**3f**), and F-PBDEs 35-F5′ (**3e**). For the synthesis of all other model compounds, this procedure was modified by using DMF as the solvent and changing the molar ratios of the starting materials to iodonium salt:phenol molar ratios of 1:2. This resulted in yields of F-PBDE 17-F5′ (**3a**), 25-F5′ (**3b**), 28-F3′ (**3c**), 47-F3 (**3g**), 99 F-3′ (**3i**), and 99 (**3h**).

Synthesis of Diaryl Iodonium Salts (1a−c). The preparation of the diaryl iodonium salts was based on the procedures reported by Luthe et al. (*37*) and Marsh et al. (*41*). The synthesis

F-Tagged Particular PBDEs

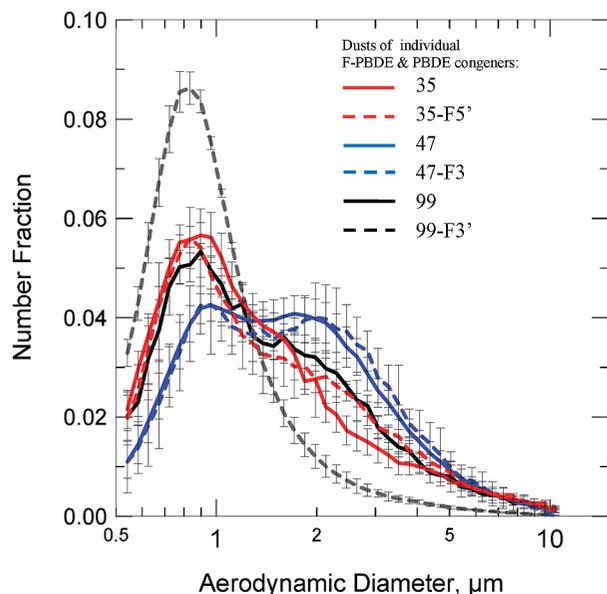*Chem. Res. Toxicol., Vol. 22, No. 1, 2009* **183**

Figure 3. Particle size distribution (μm) of pure dusts composed of congeners F-PBDEs 35-F5′ (**3d**), 47-F3 (**3g**), 99-F3′ (**3i**), PBDEs 35 (**3d**), 47 (**3f**), and 99 (**3h**).
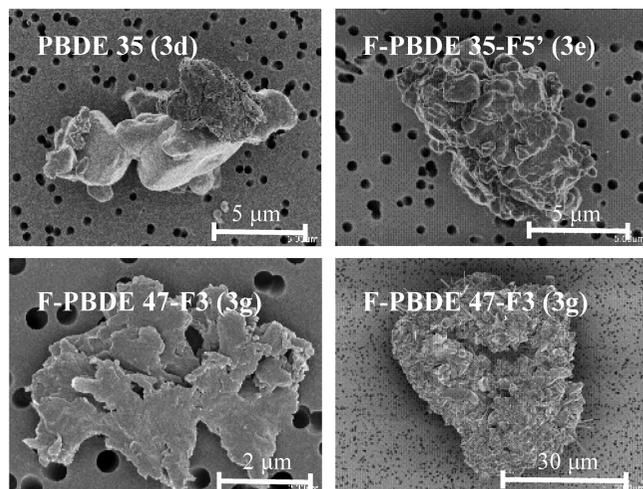

Figure 4. SEM of individual dust particles of F-PBDEs 35-F5′ (**3e**), 47-F3 (**3g**), and PBDE 35 (**3d**) exhibiting the wide range of morphology.

was conducted at lower temperature to prevent decomposition and was reported for the first time. We observed that the purities of the obtained products were higher, easing the workup procedure and the yields of the purified compounds.

Preparation and Characterization of PBDE/F-PBDE Dusts. PBDE/F-PBDE Dusts. Figure 3 shows the particle size distributions of the individual pure PBDEs and F-PBDEs dusts. The number median diameter (NMD) and geometric standard deviation (GSD, provided in parentheses) for the different powders, were as follows: PBDE 35, 1.2 μm (1.89); F-PBDE 35-F5′, 1.2 μm (1.97); PBDE 47, 1.6 μm (1.86); F-PBDE 47-F3, 1.6 μm (1.85); PBDE 99, 1.3 μm (1.90); F-PBDE 99F3′, 0.91 μm (1.57); and mixture, 1.2 μm (1.97). SEM images showed the broad size distributions and also revealed a wide variety in the morphology of the particles (Figure 4). Morphologies included flat particles, crystals, aggregates, and amorphous particles.

Dusts of F-PBDE/PBDE Coated Silica Gel Particles. Using procedure B, we coated both of the supporting materials with 20, 30, and 40% total amount of mixtures of PBDEs/F-PBDEs pairs 35, 47, and 99. Figure 5 shows the particle size distribu-
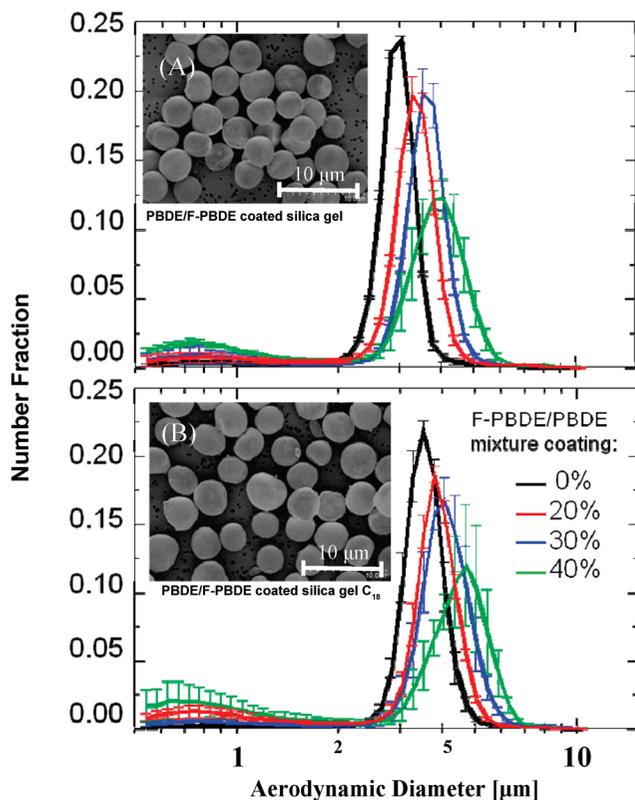
Figure 5. Number fraction of dusts composed of silica gel particles coated with 0, 20, 30, and 40% PBDE/F-PBDE pairs 35, 47, and 99: (A) nonsurface modified silica gel and (B) endcapped silica gel (100 Å pore size, 3 μm average particle size). The inset electron micrograph provides a representative image of the coated dust.

tions of the common and C_{18} endcapped silica gel particles with the equimolar PBDE/F-PBDE mixture and typical images, observed by SEMs. The NMD were as follows: 0% coating, 3.0 μm; 20%, 3.30 μm; 30%, 3.55 μm; and 40%, 3.68 μm for silica gel; and 0%, 3.51 μm; 20%, 3.72 μm; 30%, 3.99 μm; and 40%, 4.01 μm for the C_{18} chain endcapped silica gel particles. Particle size distributions showed not only an increased median with a higher percentage of coating but also an increased GSD, especially for 40% coating. Under these optimized conditions, coatings with percentages above 40% resulted in artifacts of crystals from PBDEs.

Proof of Concept of the F-Tagging Tool for Particle Tracing. Figure 6 exhibits the results of relative recoveries (Figure 5A) and relative standard deviations (SDs) (Figure 5B) of PBDE/F-PBDE pairs. The recovery in blood serum was above 99% for the diluted PBDEs/F-PBDEs. The highest recoveries in our experiment were found for the suspension in *n*-hexane for all PBDE/F/PBDE congeners with values around 15%. The second highest recoveries were found for the particle solutions in *n*-hexane and HCl (1.4−2.2% for silica coated gel and 2.5−3.7% for coated endcapped silica gel). The lowest recoveries were obtained by spiking with the coated particles directly (0.12−0.19% for silica coated gel and 0.04−0.07% for coated endcapped silica gel). While major differences in the relative recoveries occurred between the exposure procedures, the fluoro-tagged (F-tagged) analogues closely resembled the corresponding parent PBDEs. While the recovery from the C_{18} endcapped backbone was lower as compared with the silica gel, the finding becomes different after HCl treatment. The C_{18} chains functioned as a bonded liquid phase. The PBDEs/F-PBDEs were less stronger bound to the lipophilic C_{18} backbone. Figure 6B demonstrates the applicability of F-tagged analogues to serve
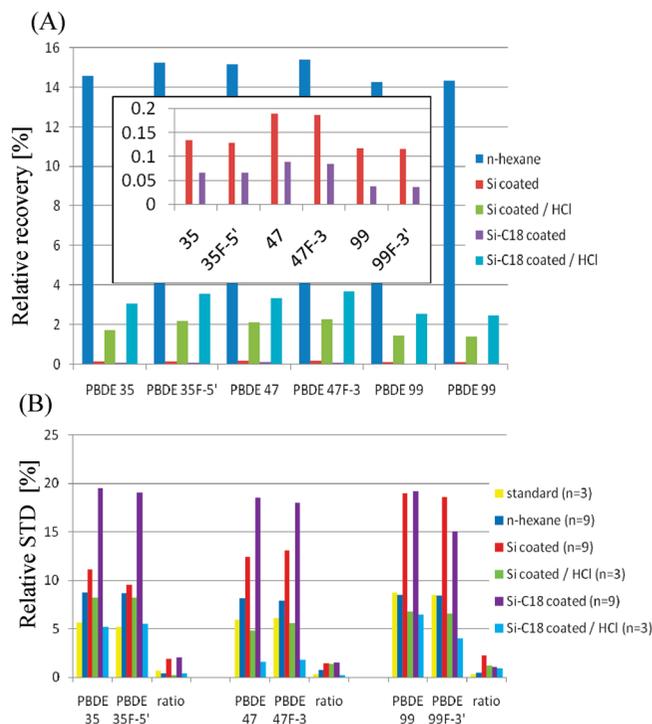
Figure 6. Relative recoveries (A) with insertion of the magnification and standard deviations (SDs) (B) of F-PBDEs 35-F5′ (**3d**), 47-F3 (**3g**), 99-F3′ (**3i**), PBDEs 35 (**3d**), 47 (**3f**), and 99 (**3h**) from pooled blood serum (Sprague−Dawley rat) carried out by liquid−liquid extraction. Spiking was carried out with coated particles and a solution in *n*-hexane and pretreatment with HCl.

as recovery standards for blood serum quantifications. The relative SD decreases for the investigated congeners from 5.2−8.7 down to 0.3−0.7% for injection triplets of the standard solutions. Additional investigations of the repeatability of the blood spiking extractions demonstrated that the SD decreases from 7.9−8.7 to 0.4−0.8% for a solution in *n*-hexane, 11.1−19.0 to 1.5−2.3% for coated silica particles, and 8.2−6.8 to 0.3−1.3% for HCl pretreated silica particles, 18.5−19.5 to 2.1−1.1% for coated C_{18} endcapped silica particles, and 6.4−5.2 to 0.9−0.3% for HCl pretreated particles. Irrespective of the high, medium, or low recoveries experimentally determined, the PBDE/F-PBDE recovery ratios are consistent within 0.3−2.3% SD.

Discussion

Synthesis of the PBDEs and F-PBDEs (3a−i). The advantages of nucleophilic substitution of the diaryl iodonium chlorides by phenols are the regioselectivity and the suppression of side reactions, resulting in high congener purity. Alternative pathways are the direct bromination of the diphenyl ether (*37*) and the Ullmann reaction (*37, 38, 40*). Both alternative pathways give product mixtures, which are difficult to separate. For the synthesis of all other model compounds, this procedure was modified by using DMF as the solvent and changing the molar ratios of the starting materials to iodonium salt:phenol molar ratios of 1:2. DMF exhibits a higher solubility for the diaryl iodonium salts and diminishes their decomposition. In addition, the excess of phenol suppresses the phenol substitution, originating from the decomposition of the iodonium salt. The synthesis and characterization of F-PBDE 47-F3 (**3g**), 99-F3′ (**3i**), 17-F5′ (**3a**), 25-F5′ (**3b**), and 35-F3′ (**3e**) are presented here for the first time.

Synthesis of Diaryl Iodonium Salts (1a−c). An excess of solvent was used to increase the solubility of the starting material at the lower reaction temperature, resulting in higher purities.

Synthesis of 2,4-Dibromo-3-fluorophenol (2g). The synthesis of 2,4-dibromo-3-fluorophenol (**2g**) is reported for the first time. The preparation via dibromination of 5-fluoro-2-nitrophenol (**4d**), followed by the reduction of the nitrogroup to the amino group, was chosen due to its high regioselectivity. A bromination of 3-fluorophenol would lead to different dibrominated isomers. A key condition was the excess of bromine. In the purification process, the di- and tribrominated products were easier to separate from each other than the mono- and dibrominated congeners. The diazonium salt was decomposed.

Preparation and Characterization of PBDE/F-PBDE Dusts. PBDE/F-PBDE Dusts. The NMDs were similar in that they ranged between 1 and 2 μm. However, the APS has reduced counting efficiency for particles smaller than 0.8 μm. Consequently, the actual NMD is likely to be smaller than that reported here because there may have been uncounted particles that were smaller than 0.8 μm. The fact that the size distributions were quite broad indicates that the powders were composed of a range of particles. This finding is consistent with the mechanical process that was used to prepare these powders. SEM images confirmed the broad size distributions and wide variety in the morphology of the particles. The observed size distributions of these pure powders may have implications for toxicity studies. Particle size and morphology will likely influence the route of exposure, uptake, solubility, and toxicity of the PBDEs. Thus, one would be unable to attribute toxic effects of a discrete size and particle morphology that elicit an adverse effect in a toxicity study. Additionally, the fact that these pure dusts are not associated with a backbone material probably renders them unlike a common environmental exposure in an indoor or occupational setting.

Dusts of F-PBDE/PBDE Coated Silica Gel Particles. Because of the fact that PBDEs are used as coating materials, we chose to coat defined spherical particles with F-PBDEs and PBDEs to gain the study materials for our ongoing biological studies. Four different coating procedures were applied to optimize the coating process under mechanical mixture with a rotavapor setup. Procedure B gave satisfactory results for our study. Under these optimized conditions, coatings with percentages above 40% resulted in artifacts of crystals from PBDEs. The comparison of the two backbones showed that the adhesion of PBDEs and F-PBDEs was stronger with common silica gel. The endcapped material resulted in particles with somewhat greasy properties. This is due to the weak interactions of the C_{18} chains with the PBDEs and F-PBDEs, leading to inhibition of a crystallization of the coating material on the surface. Comparing the particles of F-PBDEs/PBDEs with the coated spherical silica gel, a tremendous increase in the quality of the particles was observed. The particle size distribution was reduced to less than 2 μm and followed a symmetrical log-normal distribution. The morphology was comparable for all particles, equally spherical and crystalline on the surface of the silica gel and amorphous on the C_{18} endcapped silica gel. These uniformly coated F-PBDE/PBDE particles are ideal test probes to investigate pulmonary exposure to particle bound PBDEs. In addition, this particle material allows us to validate the F-tagging concepts for monitoring, tracing, and probing particular matter.

Proof of Concept of the F-Tagging Tool for Particle Tracing. It is our hypothesis that F-tagging is a tool to trace particles. We introduce F-PBDEs as model compounds to prove the hypothesis. F-PBDEs could function as tracers in environ-

mental, toxicological, and pulmonary exposure studies aimed at monitoring absorption, distribution, excretion, bioaccumulation, and magnification of PBDEs. From previous studies utilizing F-PBDEs (*37, 38*) or F-tagged PCBs (*42, 43*) and polyaromatic hydrocarbons (*44*), we know that monofluoro-substituted aromatic compounds closely resemble their corresponding parent compounds from a chemical and physical point of view. However, this is the first time that F-tagging is employed to trace particles instead of single compounds. To investigate the effect of the solubility of solid particles vs homogeneous solutions, we spiked pooled rat blood serum with (i,ii) F-PBDE/PBDE coated silica gel and C_{18}-endcapped silica gel particles and inverted prior to extraction and (iii, iv) F-PBDE/PBDE coated silica gel and C_{18}-endcapped silica gel particles pretreated with HCl and *n*-hexane and inverted prior to extraction. We normalized these values with prior dissolved stock F-PBDE/PBDEs in *n*-hexane. The employed workup procedure is based on a procedure for blood analysis of PCBs (*40*). One should note that standard operation conditions were used with no further attempt to optimize, because it was the mutual similarity of PBDE/F-PBDE pair data—generally required for monitoring and mimicking purposes—not the completeness of the extraction method, that was of interest. In this case, a low recovery yields more interesting data. Analytical separation and quantification were carried out by GC-MS.

While major differences in the relative recoveries occurred between the exposure procedures, the F-tagged analogues closely resembled the corresponding parent PBDEs. In general, the recovery in blood was low due to the high lipophilicity of the serum. However, recoveries were higher with solutions in *n*-hexane as compared with particles, showing that the PBDEs/F-PBDEs adhered well to the backbones. While the recovery from the C_{18} endcapped backbone was lower as compared with the silica gel, the finding becomes different after HCl treatment. The C_{18} chains functioned as a bonded liquid phase. The PBDEs/F-PBDEs were less strongly bound to the lipophilic C_{18} backbone. With HCl treatment, the partitioning between the *n*-hexane and C_{18}-phase changed. From chromatographic procedures, it is known that the C_{18} chains fold together while exposed to low pH levels. This results in a decreased solubility for the PBDEs/F-PBDEs and release into the *n*-hexane solution. Figure 6B demonstrates the applicability of F-tagged analogues to serve as recovery standards for blood serum quantifications. Preliminary in vivo studies seem to confirm these findings. F-PBDE/PBDE pairs were detected in similar concentrations in blood serum taken from rats after pulmonary instillation of F-PBDE/PBDE-coated particles. The general outcome of the comparison is that F-PBDEs appear to be well-suited for use as tracers in fate studies of PBDE particle exposure in biological systems. How meaningful toxicological studies are depends on the correct characterization of the xenobiotic and its correct administration (*36*). This is especially the case for dusts. Particle size, distribution, morphology, composition, surface area, and chemistry and particle reactivity in solution are all important factors. PBDEs are released as fine particles and/or partition into the air. It is fundamental for toxicological and environmental studies investigating routes of exposure and the toxicity of PBDEs to accurately characterize the particulate matter.

Supporting Information Available: Additional experimental procedures, tables of ^{1}H NMR chemical shifts and couplings, and tables of *m/z* values and relative abundances of ions. This material is available free of charge via the Internet at http://pubs.acs.org.

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