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Reductive Debromination of Polybrominated Diphenyl Ethers in Anaerobic Sediment and a Biomimetic System

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Because of the bioaccumulation of penta- and tetra-polybrominated diphenyl ether (PBDE) flame retardants in biota, the environmental biotransformation of decabromodiphenyl ether (BDE-209) is of interest. BDE-209 accounts for more than 80% by mass of PBDE production and is the dominant PBDE in sediments. Most sediments are anaerobic and reports of microbial reductive dehalogenation of hydrophobic persistent organohalogen pollutants are numerous. Reductive debromination of BDE-209 in the environment could provide a significant source of lesser-brominated PBDEs to biota. Moreover, a recent study showed that BDE-209 debrominates in sewage sludge, and another demonstrated that some halorespiring bacteria will debrominate BDE-209. To determine whether reductive debromination of BDE-209 occurs in sediments, parallel experiments were conducted using anaerobic sediment microcosms and a cosolvent-enhanced biomimetic system. In the biomimetic system, reductive debromination occurred at rates corresponding to bromine substitution levels with a BDE-209 half-life of only 18 s compared with a half-life of almost 60 days for 2,2′,4,4′-tetrabromodiphenyl ether. In sediment, the measured debromination half-life of BDE-209 was well over a decade and was in good agreement with the predicted value obtained from the biomimetic experiment. Product congeners were predominantly double para-substituted. BDE-209 debrominated in sediment with a corresponding increase in nona-, octa-, hepta-, and hexa-PBDEs. Nine new PBDE congeners appeared in sediment from reductive debromination. Given the very large BDE-209 burden already in sediments globally, it is important to determine whether this transformation is a significant source of lesser-brominated PBDEs to the environment.

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Introduction

Annual global use of polybrominated diphenyl ether (PBDE) flame retardants is estimated to be more than 67 million kilograms (*1*). Three commercial mixtures of deca-, octa-, and penta-PBDE products are manufactured, but the octa- and penta- products are being phased out of production because of concerns about their increasing presence in the environment and potential toxicity. PBDEs are lipophilic and bioconcentrate through the trophic levels of ecosystem food chains. Since PBDEs are now found even in the Artic biosphere, they likely globally distribute via atmospheric transport. An intriguing indisputable fact is that decabromodiphenyl ether (BDE-209) accounts for ~83% of all PBDEs used (mass basis), yet the two dominant PBDE congeners detected in biota, including humans, are 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47) and 2,2′,4,4′,5-pentabromodiphenyl ether (BDE-99), which are contained in the penta-PBDE product. In contrast to biota, the PBDE congener distribution in sediment environments is substantially dominated by BDE-209 (*1*). Sediments are often anaerobic, and it is well-known that the persistent organo-halogen pollutants PCBs and, very likely, PBBs undergo reductive dehalogenation in sediments (*2–4*). Because of a recent report of BDE-209 debromination in anaerobic sewage sludge (*5*), it seems prudent to examine whether anaerobic reductive debromination of BDE-209 to less-brominated PBDEs such as BDE-99 and BDE-47 occurs in reducing sediments. If so, reductive debromination of BDE-209 could contribute some portion of the flux of PBDEs into the biosphere. Since sediments are a significant environmental receptor of PBDEs, knowledge about this potential biotransformation is an essential component of a global risk assessment regarding society's use of PBDE flame retardants. We expect PBDEs to slowly undergo microbial reductive debromination in anaerobic sediments.

The occurrence of photolytic and geochemical reductive debromination of BDE-209 has been proven (*6–9*). Gerecke et al. (*5*) recently reported on the reductive debromination of *meta*- and *para*-bromines of BDE-209 to octabromodiphenyl ether congeners in anaerobic mesophilic digester sludge which occurred over a 238 day incubation period. He et al. (*10*) demonstrated that known halorespiring anaerobic bacteria can debrominate BDE-209 and an octabromodiphenyl ether mixture. Although those experiments were conducted under pure culture conditions, which significantly enhance PBDE availability to the bacteria, it is noteworthy that identified debromination products included BDE-99, BDE-49, and BDE-47. Rayne et al. (*11*) detected complete debromination of 4,4′-dibromodiphenyl ether in anaerobic sediment. In contrast, Schaefer and Flaggs (*12*) did not observe any conclusive evidence of anaerobic debromination of BDE-209 or BDE-47 in sediments during a 32-week period. In an examination of PBDE distribution in the sediments of the Pearl River Delta and South China Sea, Mai et al. (*13*) detected octa-PBDE congeners that they could not attribute to commercial PBDE products and suggested that debromination of BDE-209 was a possible source. However, because of the extreme hydrophobicity (log $K_{ow} = 8.7$) (*14*), large size, and high MW of BDE-209, it would be expected that mass-transfer kinetics in sediments would be severely limited by low bioavailability. To date, no conclusive evidence of which we are aware regarding anaerobic reductive debromination of BDE-209 in sediments has yet been reported.

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Microbially mediated reductive dehalogenation is one of the most important routes for environmental transformation of persistent halogenated compounds. In addition to well established halo-respiration, gratuitous coreduction and biologically meditated abiotic dehalogenations also occur in the environment. The ability of cobalamins, such as coenzyme vitamin B_{12}, to mediate reductive dehalogenation of hydrophobic haloorganics abiotically in biomimetic laboratory studies is well-known (*15–17*). Cobalamins have been identified in cell extracts with dehalogenating ability, as well as in dehalogenase enzymes (*18, 19*). Cobalamin and porphyrin structures from decaying cells have been isolated from environmental samples. Thus, in the presence of low-potential electron donors, free cobalamins may abiotically mediate reductive dehalogenation reactions. The ubiquitous occurrence of vitamin B_{12} in anaerobic microorganisms, combined with its ability to mediate reductive dehalogenation reactions, suggests that a biomimetic experimental system using vitamin B_{12}, in parallel with sediment microcosm studies, provides an excellent experimental model for examination of the reductive dehalogenation of PBDE congeners. A similar type of biomimetic system was used by Gaul et al. (*20*) to study the debromination of the octa-PBDE product, DE-79.

The objectives of this study were to determine whether BDE-209, BDE-99, and BDE-47 undergo reductive debromination in anaerobic sediments and in a cosolvent enhanced biomimetic system using vitamin B_{12} as an electron-transfer mediator. The cosolvent enhanced biomimetic system eliminates the bioavailability component that confounds the sediment experiments. Comparison of the relative reaction kinetics obtained from the biomimetic system to those obtained in sediments will provide some insight into the influence of bioavailability on debromination kinetics in sediments. Our hypotheses were that (1) PBDEs would undergo reductive debromination reactions in the biomimetic system at rates corresponding to the number of bromine substitutions, such that BDE-209 would debrominate faster than BDE-99 and BDE-99 faster than BDE-47, and (2) PBDEs would undergo reductive debromination in sediments, but with retarded debromination rates because of their strong adsorption to organic matter. Finally, comparison of results between the two experimental systems will yield information about the relative influence of adsorption and mass transfer limitations in sediments on debromination kinetics.

Materials and Methods

Chemicals. BDE-209, -99, and -47, for spiking microcosm sediments and the internal standard decabromobiphenyl were purchased from Accustandard (New Haven, CT). A calibration and check standard was constructed from a mixture of PBDE congeners (EO-5113) purchased from Cambridge Isotope Laboratory, Inc. (Andover, MA), and from individual congeners purchased from Wellington Laboratories (Guelph, Ontario, Canada) and Accustandard (see Table S1, Supporting Information). Vitamin B_{12} was obtained from Sigma Chemical Co. (St. Louis, MO).

Experimental Setup. *Sediment Microcosms.* Sediment containing no detectable PBDEs was collected from Celery Bog Park, West Lafayette, IN, and was characterized by Turf Diagnostics and Design (Linwood, KS) as loam sediment. Sediment (pH 6.3) contained 16.4% organic carbon that was measured by a Carlo Erba 1108 elemental analyzer. To avoid the addition of large volumes of toluene to the microcosms, a highly concentrated spiking sediment was prepared. A toluene solution containing 3.5 mg of BDE-209, BDE-99, or BDE-47 was mixed with 10 g of air-dried, sieved (at 2 mm to remove roots, macro-organic fragments, and large sand grains) sediment. After the solvent was volatilized, the dry sediments were crushed, homogenized, and then blended with 70 g of sieved wet sediments (50% moisture) to yield a final concentration of approximately 5.0 μg/g in all sets of "new" microcosms. The final concentration of BDE-209 in one set of "old" microcosms was 0.3 μg/g. The sediment was transferred to 125 mL serum bottles, and 50 mL of phosphate buffer (100 mg/L NH_4Cl, 500 mg/L KH_2PO_4, 2.0 g/L K_2HPO_4) was added to each. Microcosms were fed with 50 μL of methanol and 25 mg of dextrose to provide an organic electron donor and to ensure anaerobic conditions. Dextrose is rapidly biodegradable by numerous and diverse microorganisms, while methanol is easily catabolized by certain methanogenic bacteria. In combination, dextrose and methanol promote the rapid onset of anaerobic and methanogenic conditions without the addition of exogenous reducing chemicals. The serum bottles were sealed with Teflon-coated stoppers and aluminum crimp caps, were shaken vigorously after the addition of the substrate, and were then incubated statically in the dark at room temperature (22 °C). Controls were prepared identically and were autoclaved three times for one hour on three consecutive days. Methane gas produced by the live anaerobic incubations was measured and released by inserting a needle with a glass-barreled syringe through the stopper.

Extraction. Subsamples of sediment incubations were taken for extraction of PBDEs. Disposable 10.0 mL borosilicate glass pipets (tips cutoff) were fitted to an automatic pipetor. The bottles were shaken; the stoppers were removed, and 5 mL of sample slurry was removed by pipet. Then the bottles were recapped. It was experimentally verified by comparison of individual samples to the mean of all samples that each subsample contained a distribution of PBDEs that was representative of the whole sample. Subsamples were pipeted into 50 mL precleaned beakers and were placed into a constant-temperature cabinet (General Signal BLUE-M drying oven) at 40 °C until they were dry (16–24 h). The dried sediment samples were then individually weighed and placed into precleaned test tubes.Toluene (1.0 mL) and the internal recovery standard (IRS) (decabromobiphenyl, 50 μg AccuStandard) were then added by syringe. The test tubes were then capped with PTFE-lined solid screw caps and vortexed for 60 s, sonicated for 10.0 min, then subsequently centrifuged for 10.0 min at 5000 rpm in an International Equipment Co. (IEC Centra-8) centrifuge. The organic phase was then removed with a pipet and placed into a separate precleaned test tube. This sequence was repeated two more times for a total of three extractions. Three drops of sulfuric acid were added to the combined toluene extracts, and they were shaken to degrade non-PBDE organics. The sulfuric acid and oxidized organics were extracted twice with 10 mL of 2% NaCl solution. Final cleanup of the toluene fraction was completed by filtration of the solvent through a column (Pasteur pipet) containing ~80% 100–200 mesh florisil (to remove extracted polar organics) and 20% reduced copper powder (to remove sulfides). The PBDEs were eluted from the cleanup column with toluene. After filtration, the samples were blown down to dryness under a gentle stream of N_2 gas in a warm (35 °C) water bath. Exactly 1.0 mL of toluene was added, and the test tubes were sonicated for ~10.0 min to ensure that all of the PBDEs were dissolved into the toluene. The samples were then transferred to amber GC autosampler vials. Finally, 10 μg of 1,2,3,4-tetrachlorobenzene was added as an absolute recovery standard. PBDEs in the samples were analyzed using GC-ECD and GC-MS spectrometer. Yields of BDE-209 from spike recovery experiments were 82.9% (±7.5, $n = 6$).

Biomimetic Experiment with Vitamin B_{12}. In Teflon-capped glass vials, 0.03 mM BDE-209, -99, or -47 was mixed with 5.0 mM titanium citrate and 0.2 mM vitamin B_{12} in 0.33 M TRIZMA buffer solution (pH 7.4) containing tetrahydro-

furan (33%, v/v). Titanium citrate is an electron-donating reducing agent and was prepared as described by Zehnder and Wuhrmann (*21*). The final total volume was 31 mL, and the calculated redox potential was approximately −400 mV. The experimental parameters were chosen so that pH and reducing conditions were similar to those in methanogenic sediments and debromination would follow pseudo-first-order kinetics (i.e., the concentration of titanium citrate and vitamin B_{12} was sufficiently larger than the PBDEs such that reaction of the titanium did not significantly alter its concentration relative to the PBDE). The control contained the same concentrations as above except no titanium citrate was added. All experiments (dark, in triplicate) were performed in an anaerobic chamber. Throughout the experiments, lasting up to 15 days of reaction, 1 mL of aliquots were subsampled from each reaction vial and combined with 2 mL of toluene, followed by 10 mL of 2% NaCl solution. After vortexing, sonication, and centrifugation of the mixture, the toluene layer was collected and prepared as described above, except no sulfuric acid was added. All debromination products were identified and quantified using GC-ECD and GC-MS spectrometers.

Analysis. A Hewlett-Packard (HP) 5890 Series II Gas Chromatograph (GC) equipped with a ^{63}Ni electron-capture detector (ECD) and an HP 7673 autosampler were used for quantitative analysis of PBDEs. To reduce on-column decomposition of BDE-209, injections were direct on-column to a short (50 mm × 0.53mmID) precolumn, with the injection port set to track the oven program. The capillary column used was a RESTEK Rtx-5MS with the following parameters: 30 m (length) × 0.25 mm (i.d.) and 0.25 μm (film thickness). Helium at 25 psi was used as the carrier gas. The oven temperature program began at 150 °C, was held for 3 min, ramped at 4 °C/min to 245 °C, then immediately ramped at 2 °C/min to 275 °C, and held for 118 min, for a total run time of 160 min. The detector temperature was 320 °C with nitrogen makeup gas. Carrier and makeup gas were conditioned with a Supelco high-temperature gas purifier. Debromination product identification was based on matching retention times of PBDE standards (Table S1) to retention times of product peaks (±0.005). Further confirmation of product identity was completed for congeners with available standards by GC-MS. Unknown congeners were assigned to homologue groups based on relative retention times obtained from the literature (*6, 22*). Quantification for congeners contained in the calibration mixture was done by comparison of the relative response factor to the internal standard. Other congeners were quantified by calculating an average homologue group response factor from known congeners. Calibration curves (6 point) were obtained by injection of standard solutions containing (0.04–10.6 μM) PBDEs. Quantification for congeners contained in the calibration mixture was done by comparing the relative response factor to the internal standard. Other congeners were quantified by calculation of an average homologue group response factor from known congeners. The GC-ECD detection limit of PBDE congeners was 15–50 pg (BDE-209 to BDE-1), and the quantification limit from sediment extractions was 0.03–0.16 μg/g (BDE-209 to BDE-8).

Results are presented as a mole fraction distribution of PBDEs for all experiments where the total moles of PBDE in live/reactive samples were equivalent to control samples at the time of analysis.

A Thermo Finnigan Trace GC 2000 with a PolarisQ ion-trap gas chromatograph/mass spectrometer (GC/MS) equipped with a PTV inlet and autosampler was used to identify the molecular masses of compounds of interest in the sediment microcosm samples. A Shimadzu QP5050A quadrupole GC/MS system running in SIMS mode and interfaced with a GC17A gas chromatograph was used to identify products from the Vitamin B_{12} biomimetic samples. The capillary column used in both systems was a 30 m × 0.25 mm 0.25μm RESTEK Rtx-5MS. The oven temperature program began at 150 °C was held for 2 min, ramped at 10 °C/min to 290 °C, and was held for 74 min. The injector/inlet temperature program followed the oven temperature program. Helium was used as the carrier gas at a constant flow rate of 1.5 mL/min. One microliter injections were made under positive electron impact ionization utilizing full-scan mode from 50 to 1000 atomic mass units for the Finnigan system and 50–900 AMU for the Shimadzu system.

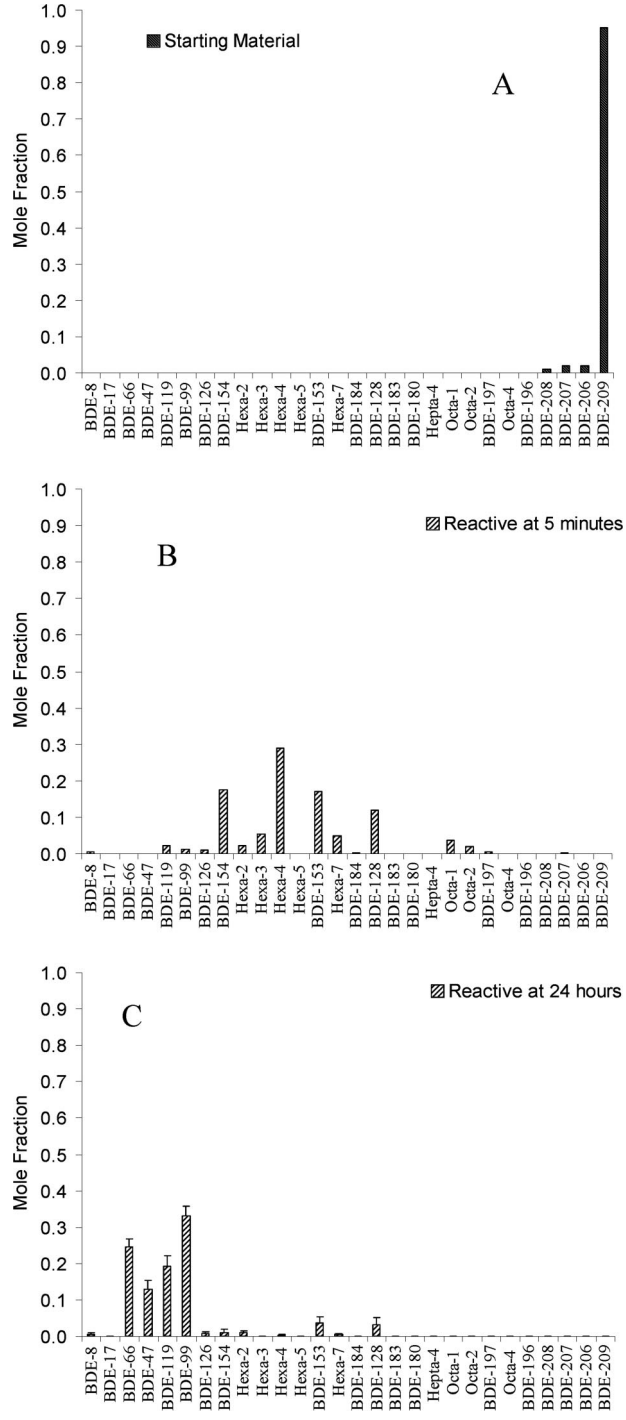

FIGURE 1. Reductive debromination of BDE-209 in a cosolvent enhanced biomimetic system. Starting material (A), reactive at 5 min (B), and reactive at 24 h (1C).

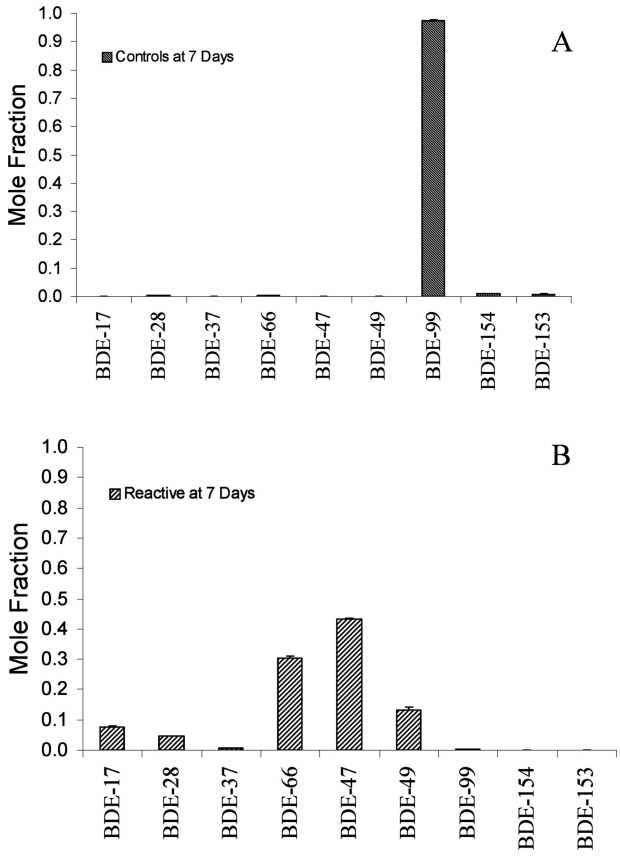

FIGURE 2. Reductive debromination of BDE-99 in a cosolvent-enhanced biomimetic system. Controls at 7 days (2A), reactive at 7 days (2B).

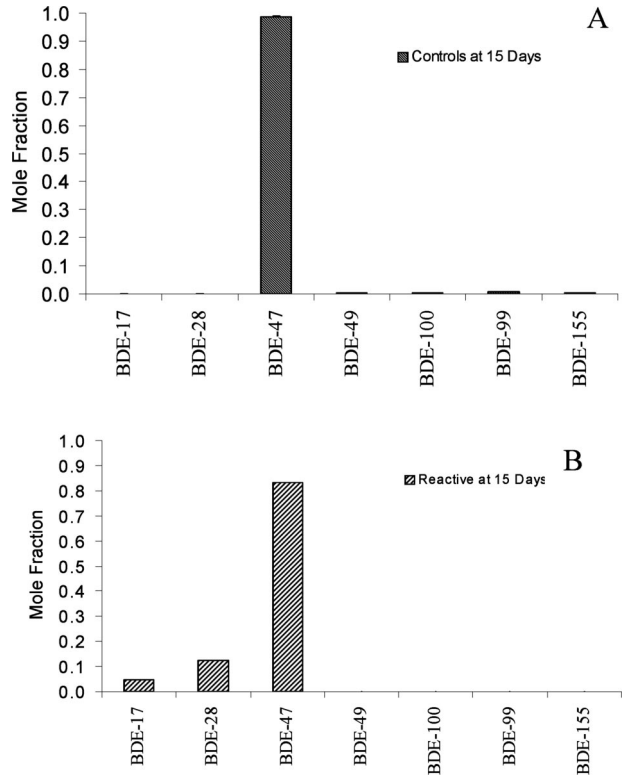

FIGURE 3. Reductive debromination of BDE-47 in a cosolvent-enhanced biomimetic system. Controls at 15 days (3A), reactive at 15 days (3B).

Results and Discussion

Two parallel experimental methods were used to study the fate of PBDEs under environmentally relevant reducing conditions. Three important congeners, BDE-209, BDE-99, and BDE-47 were used as starting material for experiments with both methods. BDE-209 is the single most widely produced and used PBDE congener by a wide margin, while BDE-99 and BDE-47 are the predominant congeners detected in the biota. Reductive debromination of BDE-209 could potentially produce BDE-99 and BDE-47, as well as other PBDE congeners. Using the cosolvent-enhanced biomimetic system, the debromination rate and the product distribution was monitored over the following times: 24 h for BDE-209, 7 days for BDE-99, and 15 days for BDE-47. Parallel sediment microcosm incubations were monitored for total PBDE concentration and for the appearance of debromination products. In the case of BDE-209, two microcosm sets were constructed: one "new" set was assembled about the same time as the BDE-99 and BDE-47 microcosms, and a second "old" set was assembled more than three years earlier.

Reductive Debromination of BDE-209 in a Biomimetic System. The BDE-209 parent starting material used in this experiment also contained small amounts of the three nonabromodiphenyl ethers, 2.0% BDE-206, 1.9% BDE-207, and 0.9% BDE-208 on a mole fraction basis (Figure 1A). The experiment was initiated by the addition of the titanium citrate reducing agent, and within five minutes, BDE-209 completely debrominated in the biomimetic system to more than a dozen daughter products, dominated by hexabromodiphenyl ether congeners (Figure 1B, see also Figure S1, Supporting Information). After 24 h, the debromination reaction pathways had converged to primarily two pentabromodiphenyl ether congeners, BDE-99 and BDE-119 (2,3′,4,4′,6-,), and two tetrabromodiphenyl ethers, BDE-47 and BDE-66 (2,3′,4,4′-,) (Figure 1C). It is noteworthy that all four of the major terminal congeners are double para-substituted, indicating that ortho and meta specificity dominates vitamin B_{12}-mediated debromination. The fate of BDE-99 and BDE-47 was investigated with new experiments initiated with BDE-99 or BDE-47 as the starting material.

Reductive Debromination of BDE-99 in a Biomimetic System. The parent BDE-99 material was greater than 97% pure (mole fraction) but contained small amounts of the hexabromodiphenyl ethers BDE-153 and BDE-154, which are also found in the commercial penta-product. After seven days the BDE-99 was almost completely debrominated. The daughter products were primarily three tetrabrominated congeners, BDE-47, BDE-66, and BDE-49 (2,2′,4,5′-), which converged by debromination to primarily two tribrominated congeners, BDE-28 (2,4,4′-) and BDE-17 (2,2′,4-), and a very small amount of BDE-37 (3,4,4′-) (Figure 2). In the first few hours of the experiment, before the appearance of any tribromodiphenyl ethers, BDE-47, BDE-66, and BDE-49 appeared in a mole ratio of about 5:4:1, respectively. Subsequently, BDE-17, BDE-28, and BDE-37 appeared in a mole ratio of about 3:2:0.15, respectively. Of the six tetra- and tri-BDE product congeners appearing from BDE-99, four were double para-substituted and accounted for more than 79% of the total.

Reductive Debromination BDE-47 in a Biomimetic System. The BDE-47 starting material used in the biomimetic experiment was 98% pure. After 15 days, more than 80% of the initial BDE-47 remained, with about 12% being transformed to BDE-28 and slightly less than 5% to BDE-17 (Figure 3). Since BDE-28 is the dominant product of vitamin B_{12}-mediated BDE-47 debromination, we can conclude that a significant portion of the BDE-17 appearing in the BDE-99 experiment goes through the BDE-49 intermediate.

The results indicate that BDE-47 has the lowest reactivity for debromination when compared to BDE-99 and -209. A comparison of the debromination kinetics of BDE-209, BDE-

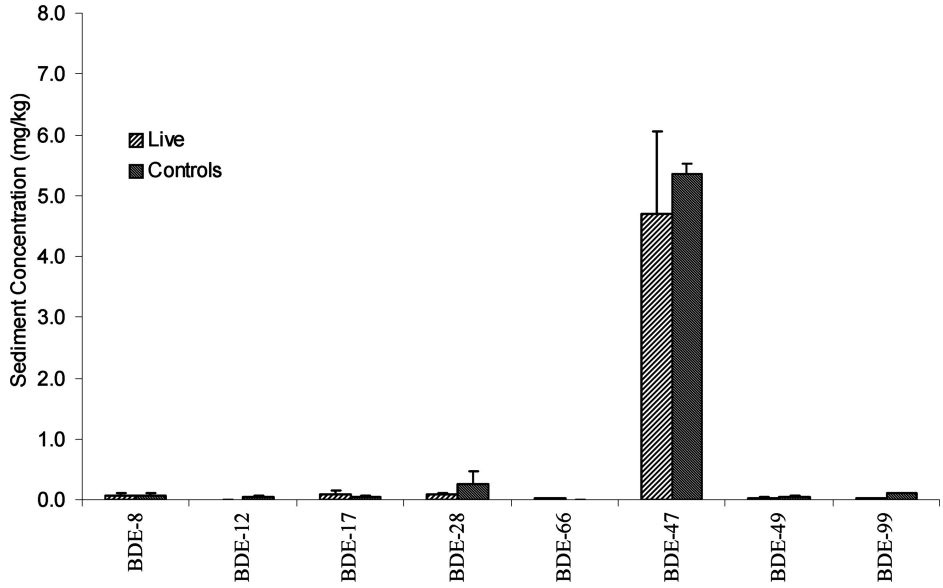

FIGURE 4. Concentration of BDE-47 after eight months incubation in anaerobic sediment microcosms.

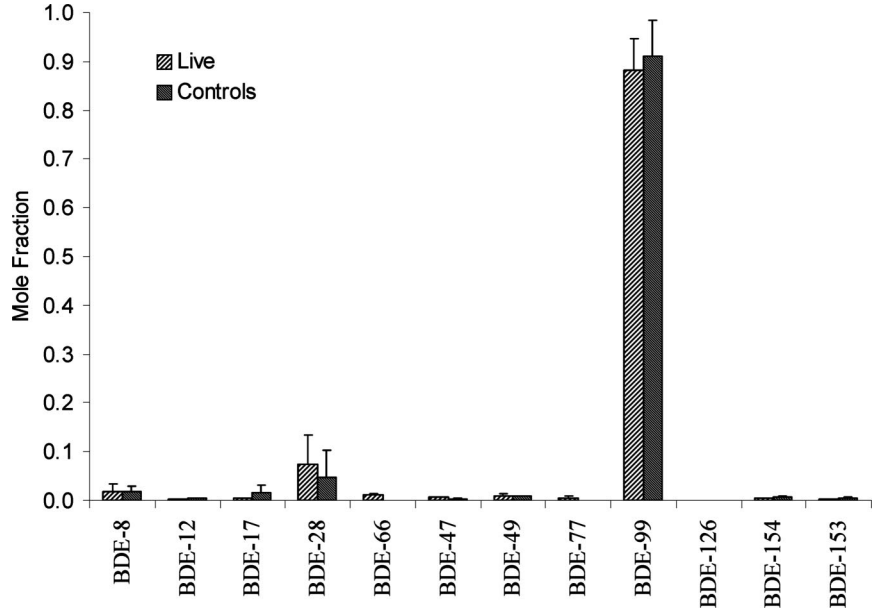

FIGURE 5. Mole fraction distribution of BDE-99 after eight months incubation in anaerobic sediment microcosms.

99, and BDE-47 is shown in Figure S2 (Supporting Information). The BDE-209 debromination half-life was 18 s, compared to a half-life of about 19.9 h for BDE-99, and almost 58 days for BDE-47. Clearly, the PBDE debromination rate was corresponding to the number of bromine substitutions, proving our first hypothesis. We have not studied a sufficient number of congeners to determine the influence of bromine substitution position on debromination kinetics.

Transformation of BDE-47 in Anaerobic Sediment Microcosms. A PBDE mole balance could not be closed for the BDE-47 microcosms; therefore, the data is presented as the absolute mass of PBDE congener/mass sediments (Figure 4) rather than a mole fraction distribution. After eight months, there was a high degree of variability among the eight live microcosm incubations containing BDE-47. In several of the microcosms, there was a decrease in the absolute BDE-47 concentration of more than 30% without a consistent concurrent increase of daughter debromination products, except for a slight increase in the concentration of BDE-17. This would indicate that there is likely a BDE-47 transformation process occurring other than reductive debromination. Complete debromination to diphenyl ether cannot be ruled out, although without detection of intermediate products it is an unlikely explanation. Complete debromination of PBB to biphenyl has been demonstrated (*23*). Since there are several reports of hydroxylated and methoxylated derivatives of tetra-BDEs occurring in biota (*24*, *25*) their appearance in sediment should be explored as well.

Transformation of BDE-99 in Anaerobic Sediment Microcosms. After eight months, the BDE-99 mole fraction in the anaerobic sediment microcosms decreased by more than three percent with a concurrent appearance of debromination products (Figure 5). A new congener, BDE-66, accounted for about one-third of the debromination products. BDE-28 accounted for approximately two-thirds of debromination products. Since the BDE-47 microcosms produced very little BDE-28, we hypothesize that BDE-99 debrominates to BDE-28 via a sequential, two-step debromination through BDE-66. In addition, although it is a small amount, the mole fraction of BDE-47 doubled.

Transformation of BDE-209 in Anaerobic Sediment Microcosms. Only a very slight decrease in the mole fraction

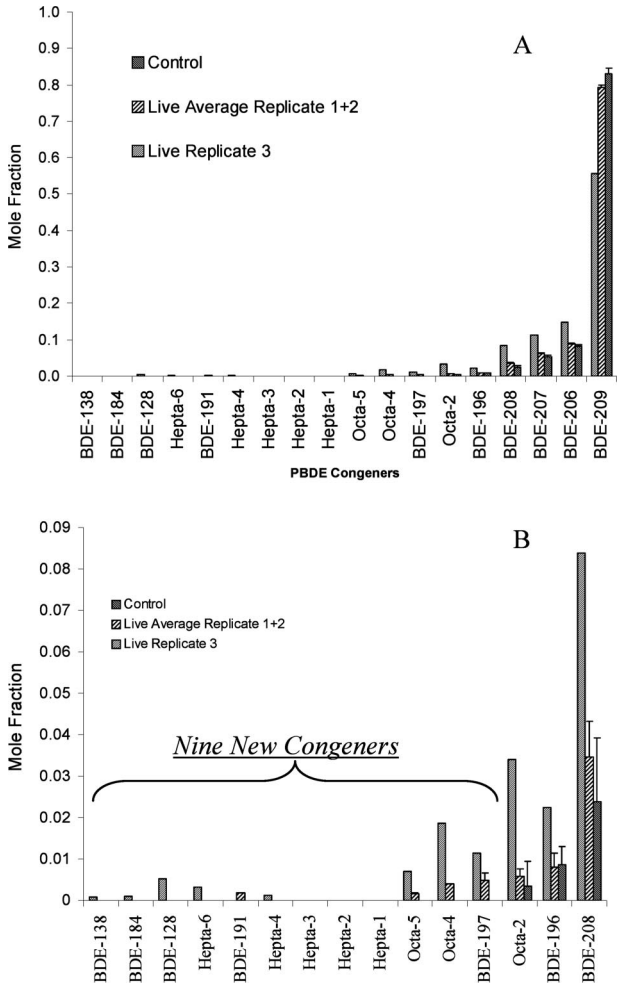

FIGURE 6. Mole fraction distribution of BDE-209 after 3.5 years of incubation in anaerobic sediment microcosms. All congeners are shown in panel A, and only congeners with less than 10% mole fraction are shown in panel B.

of BDE-209 with a concurrent increase in the three non-abromodiphenyl ethers was measured in the new sediment microcosm set after 10 months (Figure S3, Supporting Information). However, debromination was more extensive in older BDE-209 microcosms after 3.5 years of incubation. One replicate of older microcosms exhibited debromination that was substantially more extensive than in the other two. Therefore, data for replicates one and two are shown averaged together and replicate three is shown separately. Significant debromination of BDE-209 was observed in all three replicates along with the concurrent increase in a number of daughter congeners (Figure 6A). We observed a statistically significant increase in the mole fraction of all three non-abromodiphenyl ethers. This is in contrast to Gerecke et al. (*5*) who observed only para -debromination to BDE-208 and meta-debromination to BDE-207, but no ortho-debromination to BDE-206. A view of product congeners representing less than 10% mole fraction clearly illustrates the distinct difference of replicate three from the other two (Figure 6B). It should also be noted that nine new congeners that were not present in the starting parent material appeared. Furthermore, the total sum of all debromination products was greater than the starting fraction of non-BDE-209 PBDE congeners, showing that debromination of BDE-209 in sediment must have contributed to the appearance of lesser-brominated congeners. Moreover, the three positively identified hexa- (BDE-128, BDE-138) and heptabromodiphenyl ether products (BDE-184) are all double para-substituted, indicating that debromination specificity in sediments is very similar to that in the biomimetic system.

The half-life of BDE-209 in the six live microcosms ranged from 6 to 50 years with an average of just over 14 years. One can examine how well the cosolvent-enhanced biomimetic system debromination rate compares to the measured experimental sorption-constrained BDE-209 debromination rate in the sediment microcosms using eq 1 (*26*). Dividing the biomimetic rate ($k_{B_{12}}$) by a term that accounts for sorption to organic matter, we can estimate an effective observed rate in sediment for comparison

$$k_{\text{eff}} \approx \frac{k_{B_{12}}}{1 + K_p \dfrac{\text{mass (kg)}}{\text{vol (L)}}} \quad (1)$$

We can estimate the soil partition coefficient K_p with eq 2

$$K_p = f_{oc} K_{oc} \quad (2)$$

where f_{oc} was measured (0.164), and K_{oc} is obtained from eq 3 (*27*)

$$\log K_{oc} = 0.82 \log K_{ow} + 0.14 \quad (3)$$

In the sediment microcosms, the soil mass (kg) to solution volume (L) was approximately 1.4, yielding a calculated k_{eff} equal to 0.284 year^{-1}. The experimental value derived from a regression of the pseudo-first-order rate measured from six live BDE-209 sediment microcosms was 0.049 year^{-1} (range was 0.014–0.12 year^{-1}). Given that the experimental sediment values range over an order of magnitude and the calculated k_{eff} is just outside the upper range of these values, eq 1 gives a reasonable approximation of the influence of sorption on reaction kinetics. This approximation suggests that debromination kinetics of BDE-209 in sediments are reduced by a factor of 10^6−10^7 because of partitioning and mass-transfer constraints, and this is in agreement with our experimental results.

Only Gerecke et al. (*5*) and He et al. (*10*) have previously observed microbial reductive debromination of BDE-209. In the experiments with anaerobic digester sludge, product congeners were limited to two nonabromodiphenyl ethers (BDE-207, and 208) and six octabromodiphenyl ethers (*5*). Pure cultures of *Sulfurospirillum multivorans* debrominated BDE-209 to unidentified hepta- through octa-BDEs (*10*). In our 3.5 year anaerobic sediment incubations, there was more extensive BDE-209 debromination to hexabromodiphenyl ethers. The absence of ortho debromination of BDE-209 to BDE-206 in anaerobic digester sludge (*5*) and no observed debromination of BDE-209 at all by *Dehalococcoides* sp. (*10*) suggests there is still much to be learned about microbial debromination specificity. Since anaerobic digester sludge, sediments, and soil are receptors for PBDEs, further research into their fate is justified. The finding that BDE-99 also undergoes slow debromination to tetra- and tribromodiphenyl ether congeners in sediment, strongly indicates that anaerobic reductive debromination of BDE-209 could be responsible for some portion of the flux of lower molecular weight PBDEs into the biosphere. The biomimetic experiments clearly demonstrate that BDE-209, BDE-99, and to some extent BDE-47 are highly amenable to reductive debromination transformation. However, in aquatic environments, there are competing influences of hydrophobicity and reactivity, and therefore, significant retardation in the rate of debromination.

Continued use and release of PBDEs to the environment will undoubtedly result in their uptake and bioconcentration in humans and biota. Many nations are restricting or eliminating the use of the penta-PBDE product because the lower-brominated congeners are demonstrably more mobile

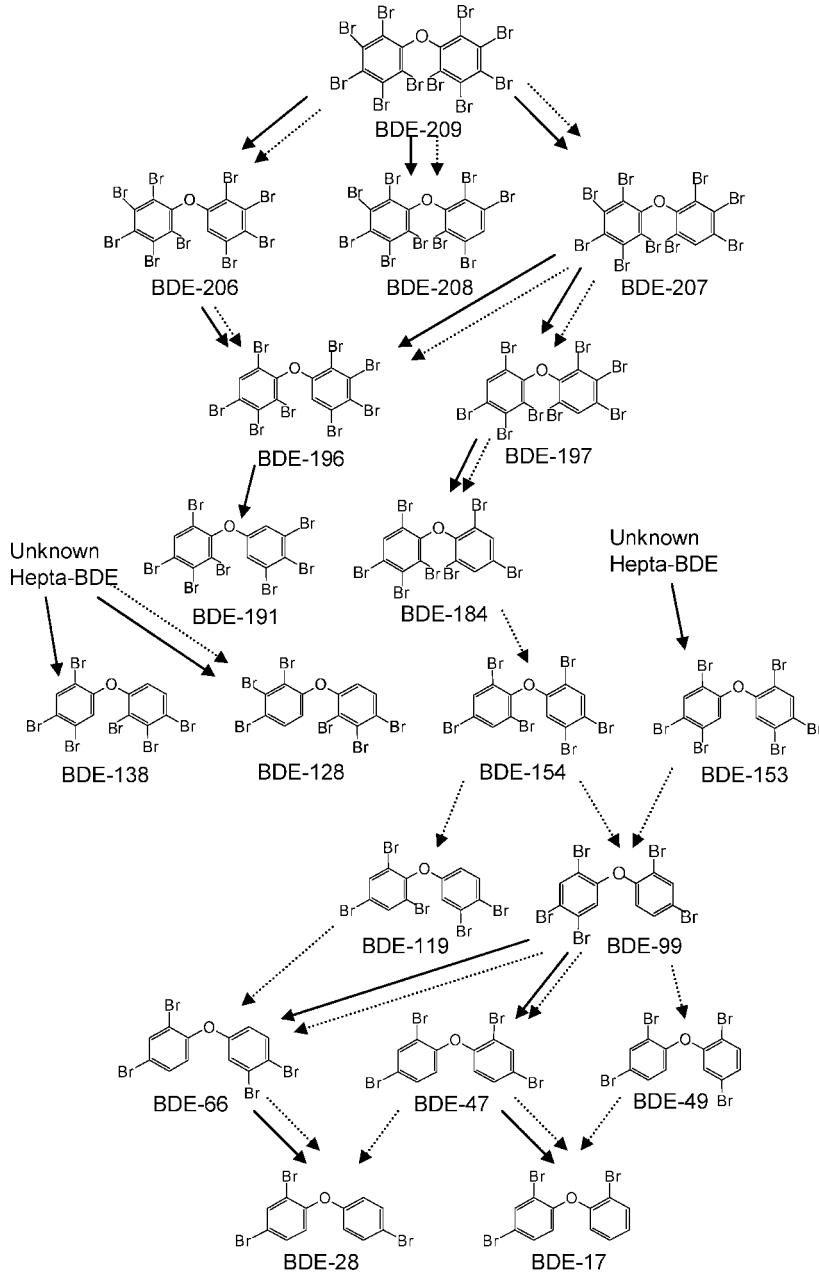

FIGURE 7. Synthesis of major debromination pathways for PBDEs derived from both sediment microcosms and biomimetic results. Only positively identified PBDE congeners are shown. Solid arrows show debrominations observed in sediments and dashed arrows show biomimetic debrominations.

in the environment than BDE-209. However, a major environmental receptor for BDE-209 is sediment, where it is the dominant PBDE congener (*1*), and it is highly likely to undergo reductive debromination under anaerobic conditions. A synthesis of the BDE-209 debromination sequence constructed from the combined results of our sediment microcosm experiments and biomimetic experiments strongly indicates that BDE-209 can undergo reductive debromination to lower mass PBDE congeners that are of greater concern to human and environmental health (Figure 7). Zhu and Hites (*28*) recently estimated that approximately 95 t of BDE-209 reside in Great Lakes sediments. Even at the slow debromination rates measured in our microcosms, the sediment burden of BDE-209 in the Great Lakes could produce on the order of metric tons per year of lower brominated PBDE congeners via reductive debromination. The results from this study will provide an important contribution to the body of knowledge that is needed to assess the risk of continued use of BDE-209.

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Supporting Information Available

Table of PBDE standards used in this study (Table S1), a sequence of GC traces from one replicate of an experiment showing reductive debromination of BDE-209 in the biomimetic system (Figure S1), a plot showing the relative debromination kinetics of BDE-209, BDE-99, and BDE-47 (Figure S2), and a graph of the mole fraction distribution of BDE-209 in sediments after 10 months is available (Figure

S3). This information is available free of charge via the Internet at http://pubs.acs.org.

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