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Polybrominated Diphenyl Ethers (PBDEs) in Gammarids, Caddisflies, and Bed Sediments of the Lowland River Po

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Abstract Polybrominated diphenyl ethers (PBDEs) were investigated in sediments and invertebrates (gammarids and caddisflies) collected in the River Po, upstream and downstream from a polluted tributary. Besides a diffuse contamination by penta-BDE technical mixture, the river sediments identified the tributary as an important source to the main river of decabromodiphenyl ether (BDE-209), which peaked to 64 μg/g (OC) in the downstream stretch. At 10 km downstream from the tributary, a higher bioavailability was evident than at 22 km, and small gammarids accumulated at two/three times the levels of PBDEs found in large gammarids. The congener profiles of sediments and invertebrates were dominated by BDE-47, BDE-99 and BDE-209.

Keywords PBDEs · Echinogammarus · Hydropsychidae · BSAF

Polybrominated diphenyl ethers (PBDEs) are additive flame retardants that have been used in a variety of consumer products with increasing demand in the last decades (Covaci et al. 2005; La Guardia et al. 2006). Of the three technical mixtures produced commercially, deca-BDE has been the most widely used (>80% global demand of 2001). Even though it is the only one still exempt from regulations, there is increasing concern for the bioavailability of its major congener (decabromodiphenyl ether, BDE-209) to wildlife and the degradation potential of this congener to more toxic and bioaccumulative PBDEs (Stapleton et al. 2006; Xiang et al. 2007). As demonstrated in laboratory studies, benthic invertebrates can bioaccumulate PBDEs (Leppänen and Kukkonen 2004). Although these organisms should have a key role in the trophic transfer of these highly hydrophobic chemicals (Stapleton and Baker 2003; Law et al. 2006), the literature on PBDE levels in freshwater invertebrates is scarce, and previous studies have been mainly devoted to estuarine and marine environments (Boon et al. 2002; Burreau et al. 2006).

With this premise, our first general objective was to assess the major Italian watercourse, the River Po, for the levels of PBDEs in invertebrates and bed sediments. With this aim, two groups of samples (2003, 2005) were collected from its middle section where it receives the River Lambro, a tributary draining one of the most industrialized and densely populated sub-basins. Since gammarids and caddisflies were the dominant macroinvertebrates of the middle River Po, these two important taxa were used to investigate PBDE contamination in invertebrates. The second objective of this study was to provide more information on the distribution (fate) of PBDEs in a large riverine environment, and in this respect we examined how distance from sources and organism size may affect the bioaccumulation of PBDEs in invertebrates.

Materials and Methods

Recently, we determined the PBDEs and PCBs in 0+ cyprinids and sediments sampled in a parallel campaign of 2003 (Viganò et al. 2008). Within the same year, another

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study examined PAHs, PCBs and DDTs in gammarids from the same area (Viganò et al. 2007). The sediment sampling of 2003 was common to these two studies, and the PBDE contents are partially reported in the present study. We necessarily refer the reader to the above studies for many details of the present investigation.

Multi-plate samplers were used to collect macroinvertebrates at three sites along the River Po, located 5 km upstream and 10 and 22 km downstream from the confluence of the River Lambro. Gammaridae, identified as *Echinogammarus stammeri* Karaman, were the most abundant of the macroinvertebrates. In only 2003 they were analyzed after separation into small and large individuals (Viganò et al. 2007). Caddisflies, mainly represented by the family of Hydropsychidae, was the second most abundant taxon. In 2005, both gammarids and caddisflies were analyzed but without any size differentiation. In 2005, not enough caddisflies were collected at the 22 km site. Both the campaigns were undertaken in spring, after 4–5 months of stable low-flow conditions. As detailed in Viganò et al. (2007), sediments collected at the three sites of the River Po and within the River Lambro were freeze-dried and sieved. Only the fine fraction (<63 μm) was analyzed for PBDEs and organic carbon (OC) (Gaudette et al. 1974). Unless otherwise specified, PBDE concentrations in organisms and sediments will be discussed as lipid and OC normalized values, respectively.

Pesticide-grade solvents and silica gel for column chromatography (63–200 mesh) were obtained from Sigma-Aldrich (Steinheim, Germany). Florisil, an adsorbent for chromatography (100–200 mesh), and anhydrous sodium sulfate were obtained from Fluka (Steinheim, Germany). Decabromodiphenyl (BB-209), decabromodiphenyl ether (BDE-209) and 2,2′,4,4′,6,6′-hexabromobiphenyl (BB-155) were purchased from AccuStandard (New Haven, CT, USA). An analytical standard solution (EO-5099) containing BDE congeners 1, 2, 3 (mono-BDEs), 7, 8, 10, 11, 12, 13, 15 (di-BDEs), 17, 25, 28, 30, 32, 33, 35, 37 (tri-BDEs), 47, 49, 66, 71, 75, 77, (tetra-BDEs), 85, 99, 100, 116, 118, 119, 126, (penta-BDEs), 138, 153, 154, 155, 166 (hexa-BDEs), 181, 183, 190 (hepta-BDEs), and a standard solution (EO-5101) containing [$^{13}C_{12}$]-BDE-77 and [$^{13}C_{12}$]-BDE-126 were purchased from Cambridge Isotope Laboratories (Andover, MS, USA). Samples were extracted and purified as described in Viganò et al. (2008). The major steps included automated Soxhlet extraction of freeze-dried invertebrates, sediments (0.5–1 g, d.w.) using a mixture of n-hexane:acetone (3:1 v/v), and clean-up using Florisil and deactivated silica gel column. The lipid content of invertebrates was determined gravimetrically from an aliquot of the Soxhlet extract brought to constant weight under a gentle flow of nitrogen. Analysis of PBDEs was performed using two different gas chromatographic methods as detailed in Binelli et al. (2006) and Viganò et al. (2008). Analysis of mono- to hepta-BDEs was performed using a gas chromatograph equipped with a Programmable Temperature Vaporizer injector coupled with an ion trap mass spectrometer. Congeners were separated by a Rtx-5MS capillary column, 60 m × 0.25 mm i.d., 0.25 μm film thickness (Restek, Bellefonte, PA, USA). Labeled BDE-77 and BDE-126 were used as injection internal standards. BDE-209 was determined on a GC system equipped with an on-column injector and coupled with an ECD detector using a CP-Sil 5 CB column, 7 m × 0.32 mm i.d., 0.25 μm film thickness (Varian, Palo Alto, CA, USA). Quantitative analysis was performed using BB-209 as an injection internal standard. To remove the lipid content and improve chromatographic performance prior to GC-ECD analysis, invertebrate extracts were treated with sulfuric acid. A confirmation analysis was undertaken to validate BDE-209 identification in positive samples using a more polar stationary phase, 14% phenyl-86% dimethyl polysiloxane CP-Sil 13 CB 7 m × 0.32 mm i.d., 0.25 μm film thickness (Varian, Palo Alto, CA, USA).

A recovery standard BB-155 was added to the samples prior to extraction (2 ng), and the mean recovery was 83 ± 11% (n = 16). As detailed in a previous study (Binelli et al. 2006), PBDE recoveries were evaluated using fish muscle tissue (BROC-1) and river sediment (BROC-2) provided as candidate reference materials by the Netherlands Institute for Fisheries Research (RIVO). Both results of sediment and muscle tissue showed good agreement with reference values, and the RSD was <20% for all congeners. Contrary to sediment, the candidate reference material for fish muscle was not certified for the BDE-209 concentration. In order to test the recovery of this congener in the biotic matrix of invertebrates, a freeze-dried gammarid sample (0.5 g) was spiked with 10 ng of BDE-209, which was left overnight to equilibrate and then analyzed. A mean recovery of 87 ± 13% (n = 4) was calculated. Using a signal-to-noise ratio of 10:1, the limits of quantification (LOQs) were estimated as 0.30 ng/g d.w for tri- and tetra-BDEs, 0.35 ng/g for penta- and hexa-BDEs, 0.45 ng/g for hepta-BDEs and 0.50 ng/g for deca-BDE. A procedural blank (n = 5) always showed levels lower than the LOQs of all PBDE congeners.

Results and Discussion

The sediments of the middle River Po showed high contamination levels of PBDEs, particularly in 2005 (Fig. 1a, b). In both years of sampling, the congener profiles were dominated by BDE-209 (>90% total PBDEs), whereas the four congeners BDE-47, -99, -100, and -153 were the major representatives of the lower brominated PBDEs.

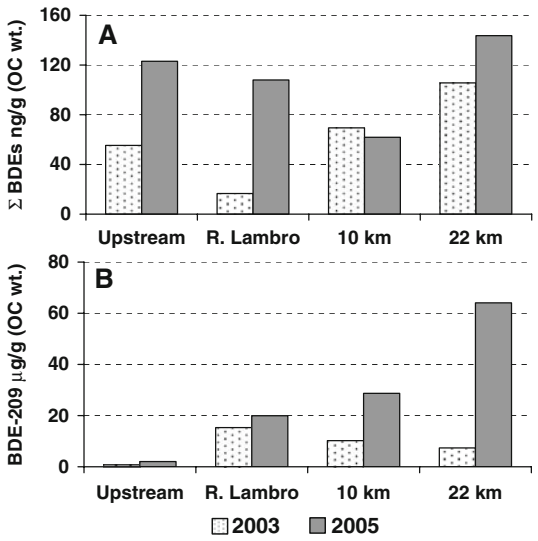

Fig. 1 Levels of lower brominated PBDEs (**a**) (ng/g organic carbon) and BDE-209 (**b**) (µg/g OC) in the fine fraction (<63 µm) of bed sediments collected along the River Po upstream, 10 km and 22 km downstream of the confluence of the River Lambro, and within the Lambro tributary. Sediments were collected in 2003 (dotted bars) and 2005 (grey bars)

Minor occasional contributions from BDE-49, -66, and -154 were also found. The sum of the lower brominated congeners was 55 ng/g and 106 ng/g in 2003, and 123 ng/g and 144 ng/g in 2005, upstream and 22 km downstream of the tributary, respectively (Fig. 1a). Characterized by higher and similar concentrations of BDE-47 and -99, this group of congeners is typical of technical penta-BDE formulation (La Guardia et al. 2006). Sediments were of limited help in identifying the River Lambro as a source of the penta-BDE mixture. Rather, a widespread contamination seemed to be evident for the middle River Po, with levels comparable to other moderately polluted river sites (Law et al. 2006; Xiang et al. 2007). Conversely, the River Lambro is such an important source of BDE-209 that its concentration in downstream River Po sediments was more than one order of magnitude higher (Fig. 1b). At the same two sites mentioned above, we found concentrations of BDE-209 of 846 ng/g and 7,353 ng/g in 2003, and 2,010 ng/g and 64,042 ng/g in 2005. These levels and those found at the mouth of the River Lambro (15,314 ng/g in 2003; 19,919 ng/g in 2005) are comparable to and even higher than those reported for other European rivers draining heavily industrialized and densely urbanized areas (de Boer et al. 2003; Sawal et al. 2005). There is no production of PBDEs in Italy (BSEF 2006), therefore the levels found in the River Po necessarily result from the manufacture, use and disposal of the many commercial products containing penta- and deca- formulations. The industrial applications of plastics (polymers) and textiles are the main emission sources of deca-BDE to the air and water (ECB 2004), and these industrial sectors are important in the Lambro watershed (Viganò et al. 2008). Particularly the concentrations of BDE-209 found in the downstream sediments show a marked increase from 2003 to 2005. Although we are comparing only two sets of results, the hypotheses of increasing inputs of BDE-209 would be consistent with the announced increase of EU consumptions of deca-BDE (ECB 2004) and with several other studies worldwide (Li et al. 2006 ; Xiang et al. 2007).

Contrary to bed sediments, gammarids and caddisflies indicated that the River Lambro is a net source to the River Po not only of BDE-209 but also of lower brominated PBDEs. Of the 37 congeners studied, 12 were found in downstream invertebrates, whereas a maximum of eight congeners were positively identified in sediments (BDE-17, -28, -71 and -85, excluded). This confirms previous observations, such as that a higher number of congeners is present either in biota compared to sediment, or in biota of increasing trophic position (Bragigand et al. 2006). As for sediments, BDE-47 and BDE-99 dominated the congener profile of lower brominated PBDEs in gammarids and caddisflies. Interestingly, the small gammarids of the 10 km site showed two or three times the levels of BDE-47 and BDE-99 found in large individuals of the same site (306 vs. 108 ng/g; 413 vs. 147 ng/g, respectively). They also showed higher concentrations of other congeners such as BDE-100 (50 vs. 7 ng/g) and -153 (38 vs. 18 ng/g) (Fig. 2). An analogous inverse dependence of bioaccumulation on gammarid size was observed for PCBs and DDTs and was explained in terms of faster uptake kinetics and higher feeding rates of smaller specimens, as well as their selective feeding on smaller and more contaminated detrital particles (Landrum et al. 2001; Viganò et al. 2007). The biota to sediment accumulation factors (BSAF) showed deviations from simple partitioning and also evident changes along the River Po. The BSAFs of the gammarids sampled at the 10 km site in 2003 were inversely related to the Log K_{ow} of congeners, both for small and large gammarids (Fig. 3). BDE-47 showed a higher BSAF than BDE-99, and also the highest value of all congeners. In small individuals, BSAFs peaked to 28 and 11 for BDE-47 and -99, respectively. Similar inverse relationships were found, for example, in a freshwater oligochaete (*Lumbriculus variegatus*) and two species of estuarine shrimps (Xiang et al. 2007). As far as biotransformation is not involved, these results are in agreement with the general trend of accumulation, so that beyond a maximum around Log K_{ow} 6–7, the uptake of most hydrophobic compounds decreases (Morrison et al. 1996; Fisk et al. 1998). Notably, the inverse relationship of the 10 km site becomes meaningless farthest downstream (22 km), where small and large gammarids showed low ($\leq$2) and no more distinguishable BSAFs, even if

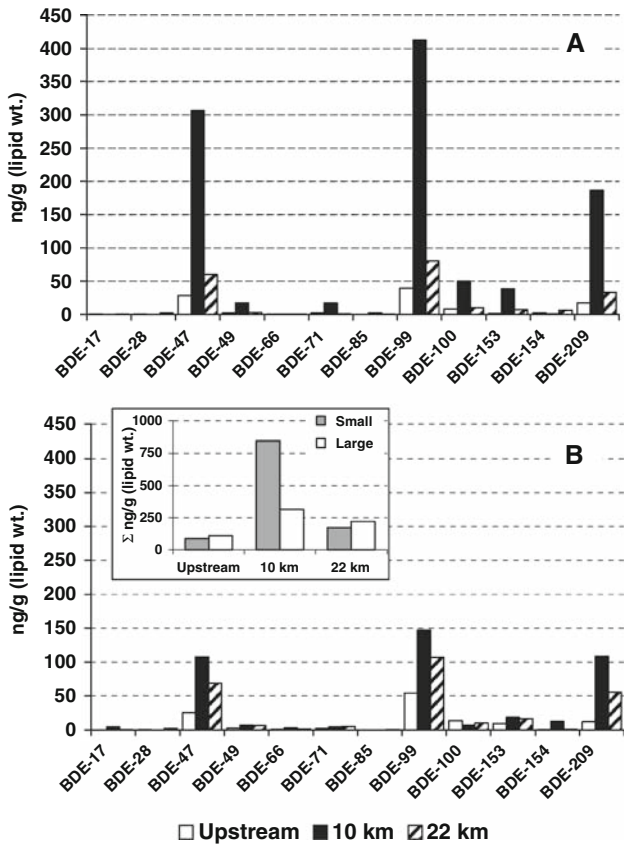

Fig. 2 PBDE congener profiles (ng/g lipid weight) in small (**a**) and large (**b**) *Echinogammarus stammeri* collected along the River Po, upstream and 10 km and 22 km downstream from the confluence of the River Lambro in the 2003 campaign. The small panel shows the sum of lower brominated PBDEs (BDE-209 excluded) determined in small and large gammarids

compared to upstream specimens (Fig. 3). As for related organochlorines, all of these results suggest that PBDE hydrophobicity mainly controls desorption and uptake, but they also suggest that contaminated particles, likely dependent on aging processes, progressively reduce the fraction of bioavailable PBDEs during their transport along the watercourse (Kukkonen et al. 2003; Viganò et al. 2006). Accordingly, (freshly) spiked sediments showed higher bioavailability of PBDEs to *L. variegatus* than composted biosolids (Ciparis and Hale 2005). The BSAFs of caddisflies, with values of 6.1 and 4.6 for BDE-47 and -99, were comparable to those of gammarids from the same 2005 campaign that showed values of 4.9 and 4.4, respectively. Also in 2005, the BSAFs calculated for gammarids confirmed the decrease with distance from sources. At 22 km, BDE-47 and -99 had BSAFs of 2.2 and 1.3, respectively. The control exerted by the hydrophobic properties of PBDEs over the accumulation process in invertebrates is further supported by the relationships depicted in Fig. 4a. The accumulation of lower PBDEs is directly related to the lipid content of invertebrates ($r^2 = 0.98$; $p = 0.0002$), although with marked differences between upstream and downstream bioavailabilities, as well as between the small gammarids of the 10 km site and the other invertebrate samples. The size dependent effect was evident only at the 10 km site, where bioavailability is expected to be higher (Viganò et al. 2007).

Previous studies generally showed low/negligible levels of BDE-209 in invertebrates (Boon et al. 2002; de Boer et al. 2003; Burreau et al. 2006). However, as also observed in an ice amphipod (*Gammarus wilkitzkii*), important contributions of this congener (>50% total PBDEs) seem to be possible (Sørmo et al. 2006). Present results show that in 2003, BDE-209 was an important congener in gammarid profiles (ca. 16–26% total PBDEs) (Fig. 2). In 2005, however, it became the dominant congener both in gammarids (306 ng/g and 338 ng/g, at 10 and 22 km) and in caddisflies (325 ng/g) at the downstream

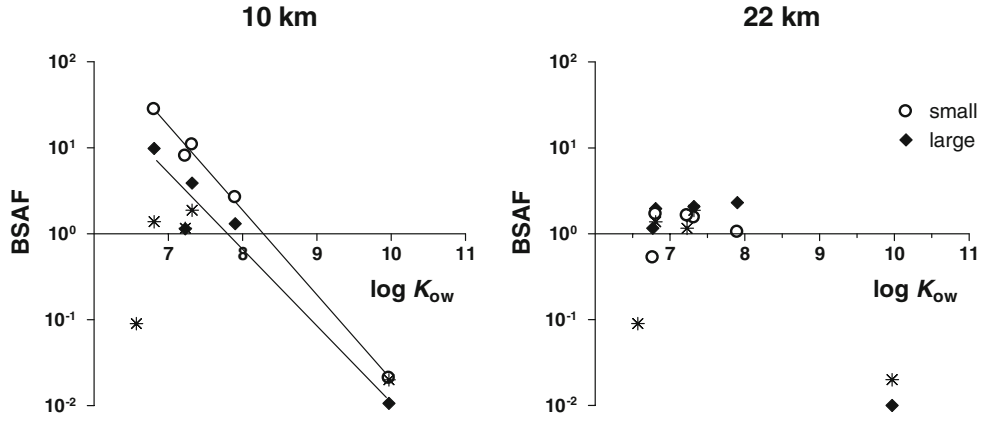

Fig. 3 Biota to sediment accumulation factors (BSAF) of PBDEs, for small (○) and large (◆) gammarids collected 10 km and 22 km downstream of the River Lambro. Both the graphs also show the BSAFs for small gammarids collected upstream (✳) of the tributary. For the 10 km site, the regression lines of BSAF versus congener Log K_{ow} are shown for small (Log BSAF = −Log K_{ow} + 8.3; $r^2 = 0.99$, $p = 0.0001$) and large (Log BSAF = −0.9 · Log K_{ow} + 7.8; $r^2 = 0.95$, $p = 0.005$) downstream specimens

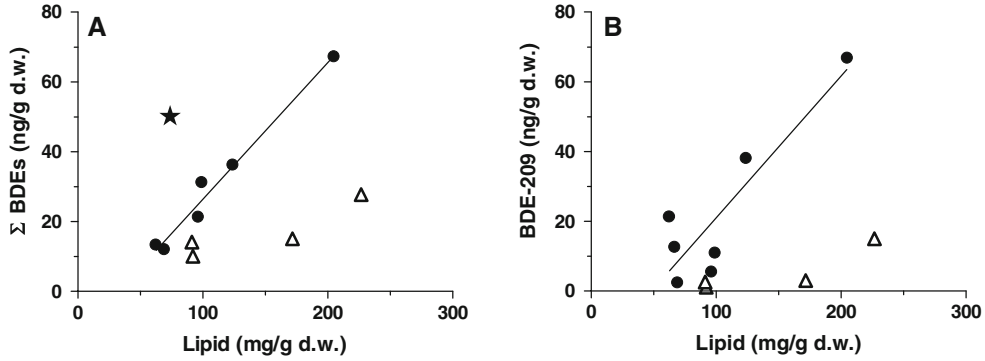

Fig. 4 Relationships between the concentrations of lower brominated PBDEs (**a**) and BDE-209 (**b**) in invertebrates and their lipid contents. Both upstream (Δ) and downstream (●) data are plotted, but only significant downstream regression lines are depicted. The level of lower PBDEs in 10-km small gammarids (★) was excluded from the regression line (A). (ΣPBDEs = 0.4 · lipid + 32.6, $r^2 = 0.98$, $p = 0.0002$; BDE-209 = 0.4 · lipid + 48.3, $r^2 = 0.79$, $p = 0.007$)

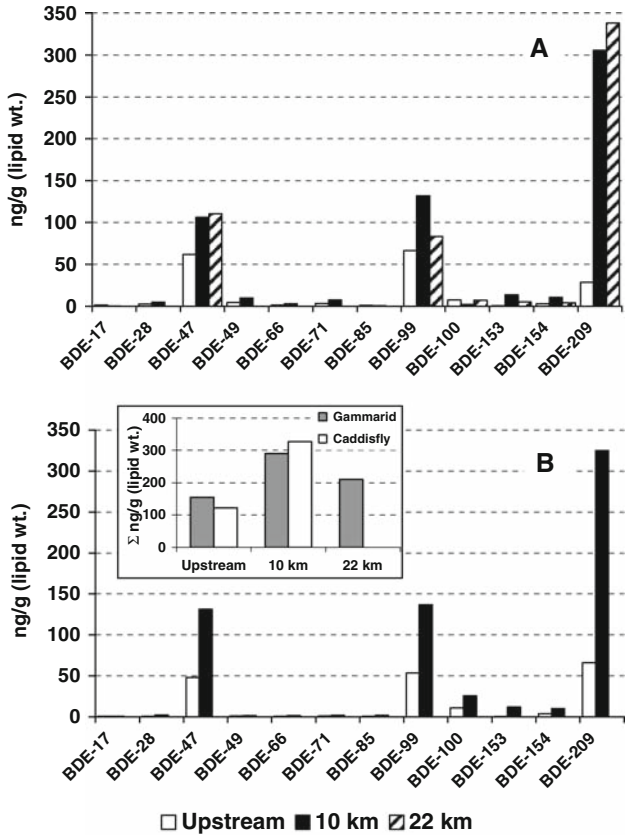

Fig. 5 Congener profiles of PBDEs (ng/g lipid weight) in gammarids (**a**) and caddisflies (**b**) collected along the River Po in the 2005 campaign, upstream and 10 and 22 km downstream from the confluence of the River Lambro. The small panel shows the sum of lower PBDEs (BDE-209 excluded) determined in gammarids and caddisflies

stretch (Fig. 5), accounting for 52% and 50% of total PBDEs, respectively (10 km site). These results are similar to those of two estuarine shrimp species (60% and 66.7%), although BDE-209 was analyzed only in edible soft tissues (Xiang et al. 2007). This aspect could be important, because in previous studies, BDE-209 was generally analyzed in whole-body homogenates of invertebrates, and this prevented the identification of a real uptake process. In addition, gut clearance after invertebrate capture was not allowed in the present study, so we cannot exclude the contribution of gut contents to the levels of BDE-209. However, when all gammarids and caddisflies were considered, BDE-209 showed a linear dependence on the lipid content (Fig. 4b), similar to that described for the lower brominated congeners. Again this dependence was observed downstream of the River Lambro ($r^2 = 0.79$; $p = 0.007$), whereas upstream of it, the slope of the regression line did not deviate significantly from zero ($r^2 = 0.74$; $p = 0.14$). These results do not support a leading role of ingested material; conversely, they suggest that a real uptake of BDE-209 can take place in gammarids and caddisflies. Beside this, the BSAF of BDE-209 in these invertebrates was always very low and essentially in the range of 0.01–0.02. Comparable results were observed in two species of estuarine shrimp (Xiang et al. 2007) or in caddisflies exposed to other very hydrophobic chemicals, such as 2,3,7,8-tetrachlorodibenzofuran and octachlorodibenzo-*p*-dioxin (Pastershank et al. 1999). In caddisflies, bioconcentration and cuticle adsorption were shown to be secondary uptake pathways to the ingestion of contaminated particles.

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