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Congener and Enantioselective Analysis of Toxaphene in Sediment and Food Web of a Contaminated Estuarine Wetland

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Enantioselective gas chromatography with electron capture negative ion mass spectrometry (GC/ECNI-MS) was employed to investigate the fate of toxaphene residues in contaminated sediment (source) and biota (receptor) representing several levels of an aquatic food web. Samples were collected from an estuarine marsh that was impacted by discharge from a former toxaphene plant. Several penta- to nonachlorobornanes were identified and enantiomerically resolved on a chiral stationary phase. Additional peak confirmation was obtained after fine fractionation of extracts on silica. Previously identified congeners including B6-923 (Hx-Sed), B7-515 (P-32), B7-1001 (Hp-Sed), B8-806/9 (P-42), B8-1412, B8-1413 (P-26), B8-2229 (P-44), B9-1679 (P-50), and B9-2206 (P-63) were detected, as were several unknown penta- and heptachlorobornanes. The majority of prominent congeners were essentially racemic in marsh sediment (maximum deviation 15%). In contrast, several congeners as well as several unknown hepta-CTTs were enantioselectively depleted in lower food web organisms; the enantiomer ratios (ERs) for B6-923 and B7-1001 in small fish (*Fundulus* sp.) were ∼2 and 0.6, respectively. These significant alterations of the ERs prove that even low-trophic level biota is able to degrade toxaphene components to some degree. In higher level predators, the ERs for B7-1001 were closer to racemic. Irrespective of trophic level, little or no shift in the ERs for B8-1413 (P-26) and B9-1679 (P-50), two recalcitrant chlorobornanes in mammals, relative to sediment were observed. These results indicate that biochemical transformations within the scope of the estuarine food web studied likely act to alter the residue pattern and the ultimate fate of toxaphene.

Introduction

Toxaphene, a nonsystemic organochlorine pesticide with a large global inventory, is ranked 32nd on the ATSDR list of hazardous pollutants (*1*) and is among the 11 critical pollutants selected for detailed source, transport, and remedial action studies in the Great Lakes region (*2*). Technical toxaphene products (e.g. Toxaphene, Melipax, Strobane) consist of several hundred bicyclic components, most of which are chlorobornanes (CHBs) (see Figure 1) (*2*). Species with highly developed enzyme systems (e.g. marine mammals, birds and some fish) at or near the top of marine food webs are able to degrade most toxaphene components, also referred to as components of technical toxaphene or CTTs. As a result, only a few CTTs—mostly hepta- through nonachloro-homologues—accumulate in fatty tissues of mammals and fish (*3*−*5*).

Under anaerobic conditions, reductive dechlorination of toxaphene leads to a shift toward earlier eluting toxaphene residues as evidenced in gas chromatograms of various anaerobic media (e.g. soil, sediment and sewage sludge) (*6, 7*). Furthermore, the reductive dechlorination process for CHBs is thought to occur at carbons with geminal chlorine substituents, with one of the geminal chlorine atoms replaced by hydrogen. The carbon atoms C-1, C-4, and C-7 on the bornane skeleton (see Figure 1) are typically free of chlorine substitution (*8, 9*). Thus, a maximum of seven chlorine atoms can remain on the bornane skeleton if the Cl/H-exchange at geminal positions occurs quantitatively (*10*). The reductive dechlorination of CTTs with geminal hydrogens on one or two secondary carbons would thus result in hexa- and pentachlorobornanes. Recently, Maruya et al. detected two pentachlorobornanes in toxaphene-contaminated estuarine marsh sediment (*11*). B6-923 (Hx-Sed) and B7-1001 (Hp-Sed), compounds that were earlier identified by Miskimmin et al. (*6*) and isolated by Stern et al. (*12*) were the most abundant CHBs in these sediments, whereas the higher chlorinated CTTs were lower in abundance and in some cases were not detected. Fingerling et al. also identified B6-923 as a degradation product in soil (*13*). B6-923 is likely derived from higher CTTs such as B7-515 (P-32) and B8-806/809 (P-42) (*12, 13*) and B7-1001 from B8-1412, B8-1414 (P-40), and B9-1679 (P-50) (*10*).

Since most CTTs are chiral (*14*), enantioselective studies are well suited for investigating the environmental fate of toxaphene, especially where the residue pool resides in highly reducing sedimentary environments. Enantioselective gas chromatography in residue analysis is, however, more complicated than achiral chromatography. First, chiral stationary phase (CSPs) are shorter in length than typical capillary columns; at the same time, the successful separation of enantiomer pairs adds an additional signal to be resolved. Second, enantiomer separations are typically performed isothermally to maximize chiral resolution (*15*). Lastly, coeluting components may alter the correct ER of target components even when the interferents have different SIM masses (*16*). Such artifacts may be avoided, however, by improvement of the chromatographic separation of the components either by MDGC (*17*) or liquid chromatographic fractionation prior to CSP-GC.

In this study, sediment, crustaceans, and fish representing a simple food web were collected from an estuarine wetland that was heavily impacted by residues of toxaphene. These samples were analyzed by enantioselective GC-MS to (i) provide additional confirmation of the identity of individual toxaphene residues and (ii) determine whether enantiomer ratios of prominent congeners were altered in biota relative to sediment (source) and also relative to food web level.

Materials and Methods

Sample Collection and Food Web Description. Sediment and biological samples were collected within 1 km of a former toxaphene plant from the Terry/Dupree Creek salt marsh in Brunswick, GA, during 1997−1998. Species collected included

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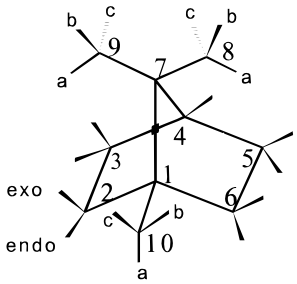

FIGURE 1. Bornane structure showing carbon numbering and substituent orientation. Letters represent conformations on primary carbons, *exo*/*endo* refers to orientation on secondary carbons.

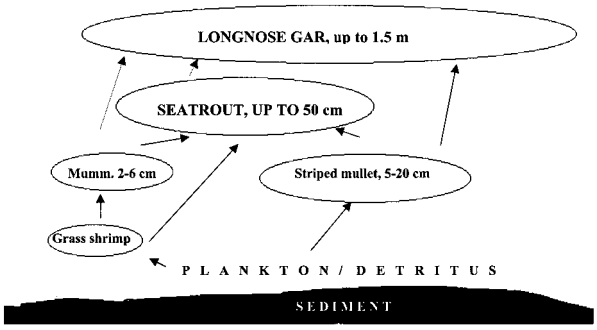

FIGURE 2. Simplified food web showing the relationships among species analyzed in this study.

grass shrimp (*Palaemonetes pugio*), mummichogs (*Fundulus heteroclitus*), striped mullet (*Mugil cephalus*), spotted seatrout (*Cynoscion nebulosus*), and longnose gar (*Lepisosteus osseus*). Surface sediment (*0−15 cm*), grass shrimp, and mummichogs were collected synoptically and composited for analysis as described in detail elsewhere (*18*). Mullet, seatrout, and gar were collected by gill net and/or hook and line during the same time period. Field samples were immediately placed in ice-filled coolers and then into a freezer upon return to the lab. Livers from seatrout and gar were excised from thawed specimens using solvent-rinsed stainless steel instruments. Skinned muscle fillets from three individual mullet were composited for analysis. Collectively, these organisms represent several levels of a typical estuarine food web of the southeastern U.S. (Figure 2). Grass shrimp are short-lived crustaceans (1−2 cm; 1 yr maximum) that feed on detritus and smaller zooplankton in shallow areas of the marsh. Grass shrimp are preyed upon by mummichogs, a minnow-like fish (2−6 cm; 1−2 yr maximum) with a limited home range in tidal creeks. Young striped mullet (5−20 cm; <1 yr) feed on plankton and spend their first year in the marsh. Mummichogs and young striped mullet are in turn preyed upon by seatrout, a year round inhabitant of the estuary (up to 50 cm; 4−5 kg; 7−8 yr maximum). Longnose gar are large (up to 1.5 m), opportunistic feeders that prefer small, mostly pelagic fish, and can live up to 20 years.

Sample Cleanup. Freeze-dried sediment and tissue samples were homogenized with Na_2SO_4 and extracted with 400 mL of CH_2Cl_2 in a glass Soxhlet apparatus for 16 h. Sediment extracts were then concentrated, chromatographed on silica gel, and Cu-treated for sulfur in accordance with previously published procedures (*11*). After concentration to ~10 mL, tissue extracts were allowed to evaporate overnight in a fume hood. The resulting residue was weighed, redissolved in *n*-hexane, and applied to a glass column packed with 18.0 g of Florisil, activated/deactivated as described previously (*19*). Three fractions were eluted with CTTs targeted in the first (~50 mL *n*-hexane) and second (150 mL *n*-hexane/CH_2Cl_2 (80:20, v:v)) fractions (*11*). These fractions were analyzed by GC/ECD. For enantioselective analysis, the CTT containing fractions were recombined and separated on 8 g of silica gel according to the method of Krock et al. (*20*). After elution with 48 mL *n*-hexane, the CTTs were eluted with 50 mL of *n*-hexane/ethyl acetate (90:10, v:v). For further peak confirmation, some extracts were fractionated on 8 g of silica (mobile phase: *n*-hexane). After 48 mL, 10 fractions of 5 mL each were collected. Further 25 or 50 mL were eluted with *n*-hexane/ethyl acetate (90:10, v:v). All fractions were analyzed by GC/MS.

Toxaphene Standards. Toxaphene single standards investigated in this study (Table 1) were purchased from Dr. Ehrenstorfer (Augsburg, Germany) or Promochem (Wesel, Germany) or were isolated from environmental samples. Throughout the paper, we use the AV-codes (*21*) as well as Parlar numbers (*22*) if available. Unknown CTTs were characterized by their GC retention time and LC elution profile (see below).

GC/MS Analysis. GC/ECNI-MS analyses (reactant gas: methane) were performed using a Hewlett-Packard 5890 gas chromatograph interfaced to a Hewlett-Packard 5989B mass spectrometer. After a solvent delay of 20 min, the following 10 ions were detected in parallel at 1.11 cycles/second: *m*/*z* 273/275 (pentachloro-CTTs), *m*/*z* 307/309 (hexachloro-CTTs), *m*/*z* 343/345 (heptachloro-CTTs), *m*/*z* 377/379 (octachloro-CTTs), and *m*/*z* 411/413 (nonachloro-CTTs). The ion source, quadrupole, and transfer line temperatures were 150, 100, and 250 °C, respectively. Splitless injections (1.5 min) were performed at 230 °C. The chiral stationary phase consisted of 25% randomly *tert*-butyldimethylsilylated β-cyclodextrin diluted in PS086 (β-BSCD) (*23*). The GC oven was programmed as follows: isothermal at 80 °C (4 min hold), 20 °C/min to 180 °C (15 min hold), 20 °C/min to 200 °C (25 min hold), and 20 °C/min to 230 °C (15 min hold).

GC/ECD Analysis. Extracts were analyzed on a Varian 3400CX gas chromatograph with a ^{63}Ni ECD. The injector and detector temperatures were 270 and 330 °C, respectively. The glass capillary column (DB-5, 30m × 0.25 mm × 0.25 μm; J&W Scientific, Folsom, CA) was programmed as follows: 120 °C (1 min hold); increase to 260 °C at 2 °C/min (15 min hold). Individual congener concentrations were computed from the average response of a 22-component mixture ("TM2", Dr. Ehrenstorfer) (five point calibration; $r^2 > 0.99$). Total toxaphene (ΣTOX) was estimated by summing the peak areas in a 30 min retention time window and applying an average response factor based on a dilution of a technical toxaphene product standard supplied by Hercules Inc. (*18*).

Quality Assurance/Quality Control (QA/QC). Provisions for QA/QC, including the analysis of procedural blanks, spiked reference sediment and tissue, and replicate samples, are outlined elsewhere (*11*, *18*). To summarize, the recovery of spiked technical toxaphene, individual chlorobornanes, and recovery surrogates (dibromooctafluorobiphenyl and α-HCH) was quantitative (80−100%) in all samples. Based on blank values and instrument sensitivity, nominal detection limits for individual congeners and ΣTOX were ~1 and 100 ng/g, respectively.

Two ions per molecule—a quantification and a confirmation ion—were recorded and integrated. The enantiomer ratio (ER) was deemed accurate when both ions varied less than 10%. ERs were rounded to five or zero for the second decimal digit. Five degrees of chlorination were monitored to ensure that no components were missed and to identify coeluting components that could result in artifacts (*16*). One sample of each species except striped mullet was fractionated on silica for elimination of possible coeluting organochlorine contaminants such as PCBs and for detection of low-abundant CTTs. One sample of each species was also reanalyzed several months after the original injection to check for long-term instrumental variability. No changes were

TABLE 1: Names, Structures, Chromatographic Parameters, and Source of CTTs Mentioned in this Study[a]

AV-code (*21*)	other names	IUPAC name	t_R [min]	silica fraction[b]	source of reference standard/ description of unknown CTTs
B7-???	7-1	not yet known	28.2/28.4	53−58 mL	Vetter et al. (*3*)
B5-???	Penta-1	not yet known	30.5	>98 mL	Maruya et al. (*11*)
C/E6-???	unsat. Hexa	not yet known	31.2/31.4	not determ	this study
B5-???	Penta-2	not yet known	31.3/31.7	>98 mL	Maruya et al. (*11*)
B7-1001	(Hp-Sed)	2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,9,10	36.3/37.0	88−93 mL	not available (isolate) (*12*)
B6-923	(Hx-Sed)	2-*exo*,3-*endo*,6-*exo,8,9,10*	38.3/38.6	>98 mL	not available (isolate) (*12, 13*)
B8-1412	8−3	2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,8,9,10	38.3	63−67 mL	not available (isolate) (*3, 27*)
B8-1413	(P-26)	2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,8,10,10	39.3/39.7	53−58 mL	Dr. Ehrenstorfer (*40*)
C/E7-???	unsat Hepta	not yet known	39.8/40.0	not determ	this study
B7-???	Hepta #A	not yet known	44.0/44.8	98−123 mL	this study
B7-???	Hepta #B	not yet known	46.2/46.7	98−123 mL	this study
B7-???	Hepta #C	not yet known	47.6/48.0	123−148 mL	this study
B7-???	Hepta #D	not yet known	50.5/50.9	123−148 mL	this study
B8-1945	(P-41)	2-*exo*,3-*endo*,5-*exo*,8,9,9,10,10	51:6/52:3	98−123 mL	Dr. Ehrenstorfer (*40*)
B7-515	(P-32)	2,2,5-*endo*,6-*exo,8,9,10*	51.9/52.1	123−148 mL	Dr. Ehrenstorfer (*40*)
B8-1414	(P-40)	2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,9,10,10	52:1/52:5	98−123 mL	Dr. Ehrenstorfer (*40*)
B9-1679	(P-50)	2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,8,9,10,10	52.9/53.2	68−73 mL	Dr. Ehrenstorfer (*40*)
B8-806	(P-42)	2,2,5-*endo*,6-*exo,8,8,9,10*	53:4[c]	88−93 mL	Dr. Ehrenstorfer (*40*)
B8-809	(P-42)	2,2,5-*endo*,6-*exo,8,9,9,10*	53:4[c]	88−93 mL	Dr. Ehrenstorfer (*40*)
B8-2229	(P-44)	2-*exo*,5,5,8,9,9,10,10	53.8/54.0	88−93 mL	Dr. Ehrenstorfer (*40*)
B9-2206	(P-63)	2-*exo*,3-*endo*,5-*exo*,6-*exo*,8,8,9,10,10	60.8/61.4	93−98 mL	Dr. Ehrenstorfer (*40*)

[a] Listing with increasing retention time of the first eluting peak, respectively. [b] CTTs distributed over several 5 mL fractions. The presented fraction represents the milliliter-range with highest abundance and purity of the respective component. [c] Only the later eluting, more abundant component in the reference standard was present in the samples.

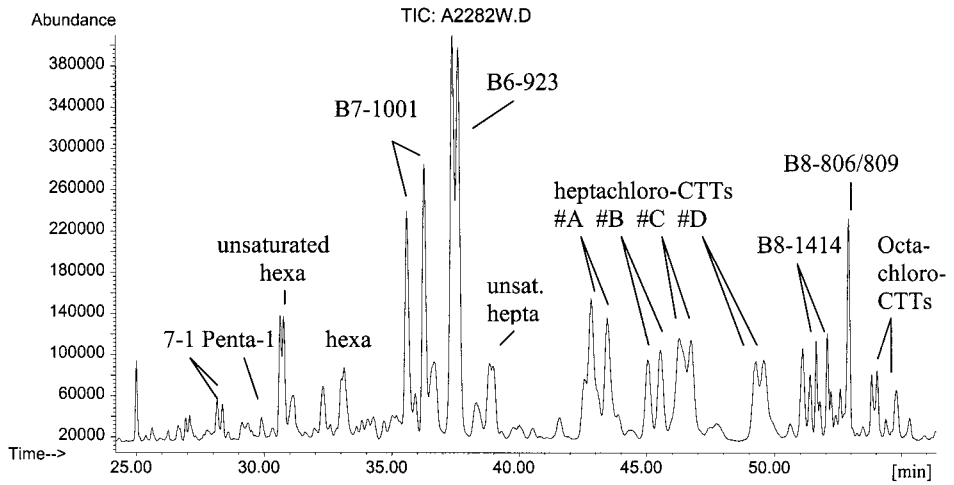

FIGURE 3. GC/ECNI-MS total ion chromatogram of Terry/Dupree Creek sediment extract using a chiral β-BSCD column showing several prominent toxaphene residues.

observed except for a shift toward shorter GC retention times due to aging of the GC column.

The MS method scanned for penta- to nonachlorobornanes for the entire chromatographic run (i.e. no time windows were run). For each homologue (Cl_5 to Cl_9), two abundant isotopic masses of the $[M - Cl]^-$ fragment ion were monitored (*10, 11*). This is in contrast to methods that monitor the expected molecular ions for chlorobornanes with six or less Cl (*24, 25*). Molecular ions, however, are not observed for some hexachlorobornanes using ECNI-MS (*13*). Consequently, some penta- and hexachloro CTTs would not be detected if only molecular ions are monitored.

Results and Discussion

Estimated Concentrations, General Profiles, and Enantiomer Separation of Toxaphene Residues. Several toxaphene residue components were prominent in both sediment and tissue extracts. Although the overall CTT profile appears to be "weathered" relative to unmodified technical toxaphene, sediment extracts contained up to ~100 components, many of which were present at low ECNI-MS abundance (Figure 3). However, B6-923 (Hx-Sed) and B7-1001 (Hp-Sed) were the most abundant residues of toxaphene in all samples, sediment and biota alike (Figure 3, Table 2), supporting the theory that reductive dechlorination at carbons with geminal chlorine substituents (one chlorine is substituted by hydrogen) is the major transformation pathway of CHBs. B7-515 (P-32 or Toxicant B) and B8-806/809 (P-42 or Toxicant A), two major CTTs in technical mixtures, were also abundant in sediment from Terry/Dupree Creek. The presence of these likely parent components of B6-923 indicates that reductive dechlorination has not occurred quantitatively in these sediments. This may be due to the high toxaphene levels (*11*) and/or a deficiency of suitable dechlorinating chemical equivalents and/or microorganisms.

Of the >40 nonachloro components identified in technical toxaphene (*26*), only B9-1679 (P-50) and B9-2206 (P-63) were abundant in these sediments. Several unknown penta- to octachloro CTTs were also present in these extracts. Two pentachlorobornanes ("Penta-1", see Figure 3, and "Penta 2", see Table 1) were found earlier by Maruya et al. (*11*). Using chiral GC/ECNI-MS, the resolved enantiomers of

TABLE 2: Individual Congener and Estimated Total Toxaphene (ΣTOX) Levels in Sediment and Biota[a] from Terry/Dupree Creek[c]

congener	sediment ($n = 2$)	grass shrimp ($n = 2$)	mummichogs ($n = 3$)	mullet ($n = 1$)	seatrout ($n = 2$)	gar ($n = 1$)
B6-923	250 ± 30	39 ± 7.3	200 ± 27	38	930 ± 180	460
B7-1001	260 ± 31	39 ± 4.3	180 ± 22	36	760 ± 200	510
B8-1413 (P-26)[b]	<=130 ± 11	<=35 ± 7.8	<=70 ± 19	<=21	<=360 ± 110	<=210
?/B7-515 (P-31/32)	40 ± 6.2	8.3 ± 1.6	12 ± 2.3	4.4	66 ± 12	31
B8-1414/1945 (P-40/41)	58 ± 8.5	11 ± 2.2	27 ± 4.2	5.9	95 ± 25	47
B8-806/809 (P-42)	57 ± 8.6	15 ± 2.5	24 ± 4.9	6.9	110 ± 24	32
B8-2229 (P-44)	150 ± 43	39 ± 7.0	71 ± 13	12	220 ± 63	130
B9-1679 (P-50)[b]	<=81 ± 9.4	24 ± 3.5	36 ± 5.6	5.1	100 ± 35	90
B9-2206 (P-63)	7.0 ± 0.82	<1.0	2.1 ± 0.48	<1.0	5.6 ± 2.7	<1.0
ΣTOX	7300 ± 260	1400 ± 90	4500 ± 1200	990	20000 ± 3300	7300

[a] Whole body (shrimp, mummichogs); muscle (mullet); liver (seatrout, gar). [b] <= values for B8-1413 (P-26) and B9-1679 (P-50) are maximum due to coeluting interferences. [c] Values ($x \pm \sigma$) are in ng/g (dry wt for sediment; wet wt for tissue).

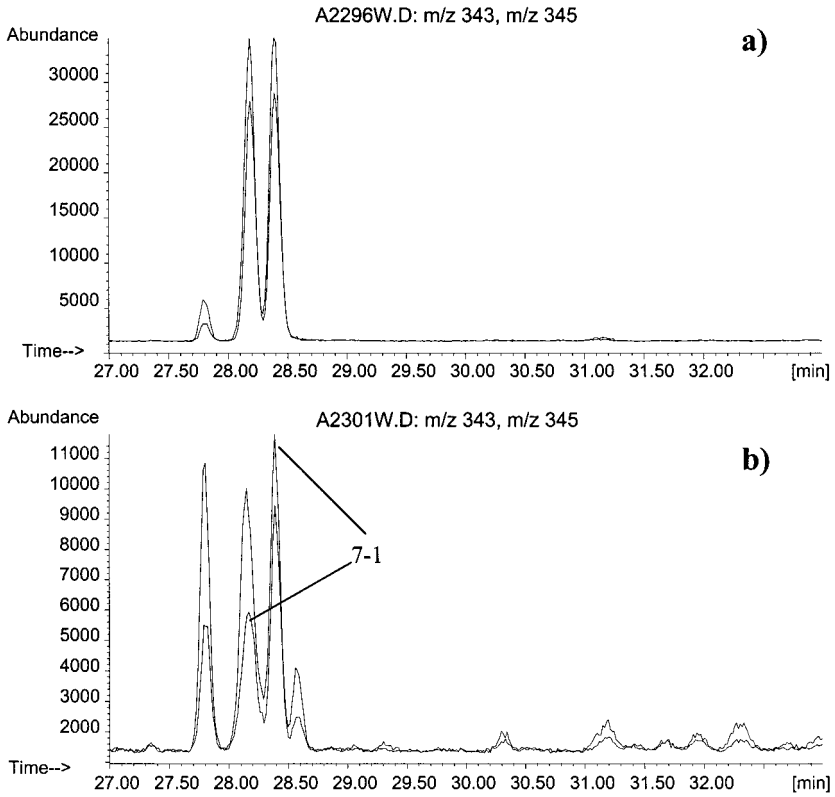

FIGURE 4. (a) Silica fraction 53−58 mL (7-1) of Terry/Dupree sediment extract and (b) Antarctic seal, unfractionated. Note that the earlier eluting enantiomer of 7-1 coelutes with a chlordane-related compound.

Penta-2 coeluted with a more abundant hexachloro component ("unsaturated hexa", Figure 3). In addition to B7-1001 and B7-515 (P-32), several other heptachloro CTTs were also abundant in sediment extracts ("7-1", components #A−#D in Figure 3). For octachloro components, B8-1412, a major toxaphene congener in marine mammals and birds (*27*), coeluted with the first enantiomer of B6-923. B8-1413 (P-26), a "recalcitrant" CTT in higher organisms (*28*), was found to be much lower in abundance than a coeluting heptachlorobornane. Furthermore, the second enantiomer of B9-1679 (P-50) coeluted with B8-806/809 (P-42), and both enantiomers of B8-2229 (P-44) coeluted with other unidentified octachlorobornanes. Unsaturated components (chlorobornenes or chlorocamphenes) were also detected although their enantiomers were only partly resolved (Figure 3). Hence, ERs for these components could not be determined. Neither B8-806/809 (P-42) nor Penta-1 were enantiomerically resolved on the β-BSCD column.

As previously demonstrated, PCBs can be separated from CTTs on 8 g of silica and elution with 48 mL of *n*-hexane (*20*). CTTs elute from silica after the PCBs, typically by using a higher polarity solvent. In this study, we extended the *n*-hexane elution of samples from the silica column to obtain a finer separation of prominent CTTs. Ten fractions of 5 mL each were collected and analyzed. The elution order of CTTs from silica is primarily a function of polarity. The more polar CTTs are retained on the silica column, whereas the nonpolar compounds are eluted early with *n*-hexane (*9*). It is noteworthy that nonpolar CHBs appear to be most stable in biota (*9*), suggesting that CTTs of higher polarity are more readily transformed by higher organisms. For example, B8-1413 (P-26) and B9-1679 (P-50), the two most abundant chlorobornanes in mammals, are among the earliest eluting CTTs on silica (Table 1). Other persistent CTTs such as B8-1412 and B8-2229 (P-44) also elute relatively early from silica (*20*). It follows that the identification of components in the first silica fraction may be important for the identification of persistent CTTs in biota.

The 53−58 mL hexane fraction of our sediment samples was dominated by B8-1413 (P-26) and a heptachlorobornane

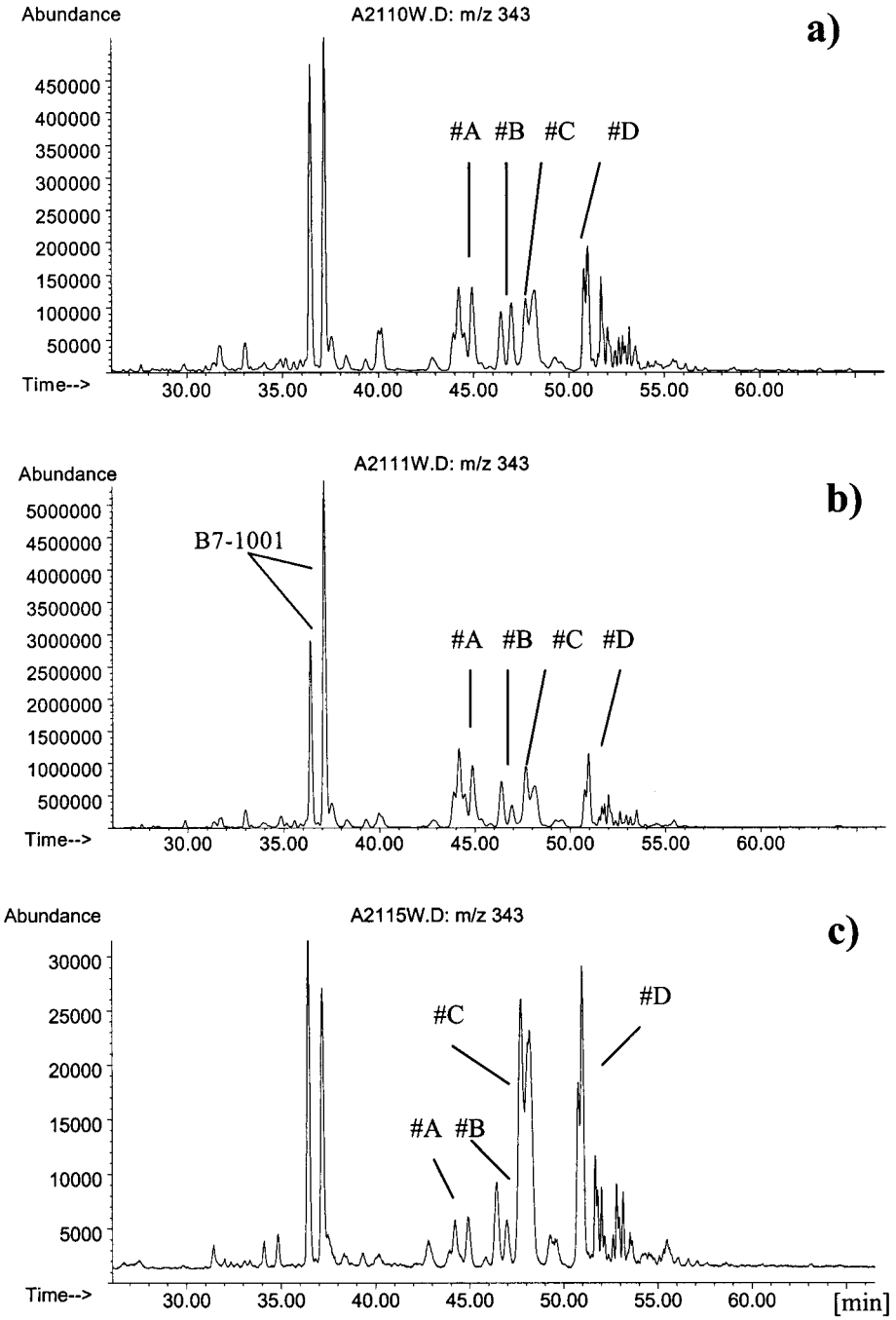

FIGURE 5. Enantioseparation of heptachlorobornanes in (a) sediment, (b) mummichogs (prey fish), and (c) longnose gar (predator fish).

(Figure 4a). The heptachlorobornane was labeled "7-1" owing to its earlier characterization in Antarctic seal samples (Figure 4b) (*3*). The early elution from silica and also from nonpolar stationary GC phases (e.g. 7-1 is the first heptachlorobornane in toxaphene eluting from CP-Sil 2 and DB-5) along with its apparent persistence in our samples suggests that the structure of 7-1 may be either 2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,8,-10-heptachlorobornane (B7-1000) or 2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,10,10-heptachlorobornane (B7-1002) (*9, 29*). B7-1000 and B7-1002 are formed from B8-1413 (P-26) by substitution of one chlorine by hydrogen at the geminal dichloro groups on C8 and C10, respectively. No metabolite of B8-1413 (P-26) has been identified in toxaphene-contaminated sediment thus far (*10*), and detection of 7-1 in our samples may be the first observation that B8-1413 (P-26) can be transformed in reducing environments. It is also noteworthy that most PCB/CTT separation methods distribute CTTs into two fractions based on polarity (*5*). Thus, 7-1 and B8-1413 (P-26) would be expected to elute primarily in the PCB fraction. This may explain why 7-1 has not been reported more frequently in environmental samples. Using DB-5-like columns, 7-1 eluted ~0.5 min after oxychlordane and 1.5 prior to *trans*-chlordane using GC/ECD (*27*). On the β-BSCD column, 7-1 is subject to interference from a chlordane-related component (Figure 4b). This chlordane component, however, was not detected in our samples.

Enantiomer Ratios (ERs). As reported previously, the ERs of B8-1413 (P-26) and B9-1679 (P-50), the most abundant CTTs in marine mammals and fish, are close to unity (*30, 31*). However, ERs of less stable congeners such as B8-2229 (P-44) and B8-1412 deviated significantly from racemic composition (*32*−*34*). Therefore, we might expect deviations

TABLE 3: Enantiomer Ratios of Selected Components in Sediment and Biota from Terry/Dupree Creek[f]

samples	Penta-2 (−)	B6-923 (−)	B7-1001 (−)	B8-1413 (P-26)	B9-2206 (P-63)
sediment 1	1.00	0.95	0.95	1.10[a]	1.00/1.00[a]
sediment 2	1.00	0.95	0.90/0.90[a]		
sediment 3	nd[b]	0.95	0.90		
mummichogs 1	0.85	2.35/2.40[d]	0.50/0.50[d]		1.05/1.05[a]
mummichogs 2	0.75	2.05/2.10[d]	0.70/0.70[d]		
mummichogs 3	0.95	2.10/1.85[c]	0.55/0.60[c]		
seatrout 1	1.00	1.55	1.10		
seatrout 2	0.95/0.95	1.45/1.30[d]	0.95/0.95[d]		
grass shrimp 1	1.00/1.40[d,e]	1.15	1.85	1.05[a]	1.00/1.00[a]
grass shrimp 2		1.20	1.80/1.90[a]		
striped mullet 1	1.30	1.55	0.55		
longnose gar 1	1.35/1.35[d]	1.60/1.60[d]	1.20/1.20[d]	1.10[a]	
longnose gar 2	1.30	1.60	1.15		

[a] Determined in silica fraction noted in Table 1. [b] Not determined. [c] Different sample cleanup. [d] Reanalyzed after several months. [e] 1.40 value likely high due to interference. [f] Values are rounded to zero or five at the second decimal digit.

from unity for ERs in our samples, particularly for those prominent CTTs that are not considered environmentally recalcitrant.

With our enantioselective method, we were able to separate the enantiomers of Penta-2, B6-923, 7-1, B7-1001, B7-515 (P-32), B8-1413 (P-26), B8-1414 (P-40), B8-1945 (P-41), B8-2229 (P-44), B9-1679 (P-50), and B9-2206 (P-63). Enantioseparation of these compounds also serves as confirmation of their presence in our samples. ERs could be established for Penta-2, B6-923, and B7-1001 in both sediment and tissue samples. ERs for 7-1, B8-1413 (P-26), B9-1679 (P-50), and B9-2206 (P-63) could be determined only after additional fractionation on silica (see above) for selected samples.

Sediment. In Terry/Dupree Creek sediment, Penta-2 was racemic (*11*). B6-923 was not baseline resolved, and the resultant ER (0.95) may be considered as unity (i.e. racemic) since the later eluting enantiomer is usually prone to misinterpretation for partially resolved peaks. However, B7-1001 showed a slight excess of the later eluting enantiomer (Figures 3 and 5). This was confirmed by analysis of silica fraction 53−58 mL (Table 2). The nonracemic distribution of B7-1001 was observed to be more pronounced in sediment from a toxaphene-treated Canadian lake (10). In sediment cores from this same lake, changes with time were found for the ER of B7-1001 (10), suggesting that sediment microorganisms may be responsible for the enantioselective transformation of CTTs.

The ER of B9-1679 (P-50), eluting in the 63−67 mL fraction, was determined to be 1.15. No coelution was observed which was supported by the agreement between the ER based on the TIC and that of the two ions of the $[M\text{-}Cl]^-$ fragment. The ERs of B8-1413 (P-26) and 7-1, determined in the 53−57 mL fraction, were 1.10 and 1.00, respectively. Generally, the enantioselective depletion of CTTs in sediment was subtle at most.

Tissue. ERs for tissue samples ranged from 0.5 to greater than 2.0 (Table 3). ERs for lower trophic level species (e.g. grass shrimp, mummichogs) exhibited the largest deviations from racemic composition. This is in contrast to a simple construct/prediction where lower food web items would mirror the source (sediment) more closely and that larger deviations in ERs would be expected in species at higher food web levels.

The ERs for B7-1001 in mummichogs (Figure 5b) and striped mullet were ∼0.5, whereas in grass shrimp the same parameter was ∼1.8. In both cases, the one enantiomer exceeded the other by >40%. Typically, ER shifts become more pronounced as one moves up the food web due to increased specialization of enzyme systems in higher organisms. However, the ERs for B7-1001 in the higher level organisms in our study (seatrout and gar) were 1.10/1.00 and 1.20, respectively (Figure 5c), which is closer to our sediment ER (Figure 5a). Compared with the ER in the forage fish (∼0.5), the change was, however, remarkable. If one assumes that the major diet of these predators is the smaller fish, the much larger, expected ER shift in predators could be moderated by the opposite ER trend in their primary food. On the other hand, the difference in the ER of the two seatrout samples (see Table 3) may be attributed simply to a difference in food preference/selection. If seatrout #1 fed more on grass shrimp than seatrout #2, the higher ER of B7-1001 in seatrout #1 becomes more plausible.

The first eluting enantiomer of B6-923 was more abundant in all investigated species indicating biologically mediated transformation of this major congener. The highest ER was observed in mummichogs. This finding was consistent with the decrease in relative abundance of both B6-923 and B7-1001 in recent toxaphene residue elimination experiments (*35*). These forage species also depleted the first eluting enantiomer of Penta-2, whereas mullet and longnose gar accumulated higher levels of the first eluting enantiomer.

The ion trace representing heptachlorobornanes (m/z 343) in sediment, mummichogs, and longnose gar as extracted from the total ion current further illustrates the enantioselective nature of toxaphene residues in our samples (Figure 5). In addition to B7-1001, eight signals from four pairs of enantiomers of unknown heptachloro CTTs (#A−#D) were prominent in all samples (*36*). Information on which of the eight signals are pairs of enantiomers was derived from fractionating experiments on silica. These findings are in agreement with those of Muir et al. who recently detected five abundant unknown heptachlorobornanes in sediment from lakes in the Canadian Arctic (*37*). The shift of the ER from sediment to mummichogs was significant for #A−#D. Note also that #C and #D were enriched relative to #A and #B in longnose gar (Figure 5c). Since these heptachloro CTTs were prominent in all samples, elucidation of their structures would significantly contribute to the understanding of the fate of toxaphene in reducing environments. For hexachloro CTTs, the later eluting enantiomer of "unsaturated hexa" (Figure 3) was almost completely removed from mummichogs (data not shown), indicating that this component is metabolized by this species. In a recent study that correlated the thermodynamical stability of CTTs derived from molecular modeling with persistence in biota, a low stability of chlorobornenes was predicted (*29*).

Figure 6 shows the octachlorobornanes at higher retention times including the resolved enantiomers of B8-1414 (P-40) and B8-1945 (P-41). The later eluting enantiomer of B8-1414 (P-40) was strongly depleted in mummichogs and longnose gar, whereas the ER of B8-1945 (P-41) was little changed. Nonracemic composition of these CTTs in biological samples

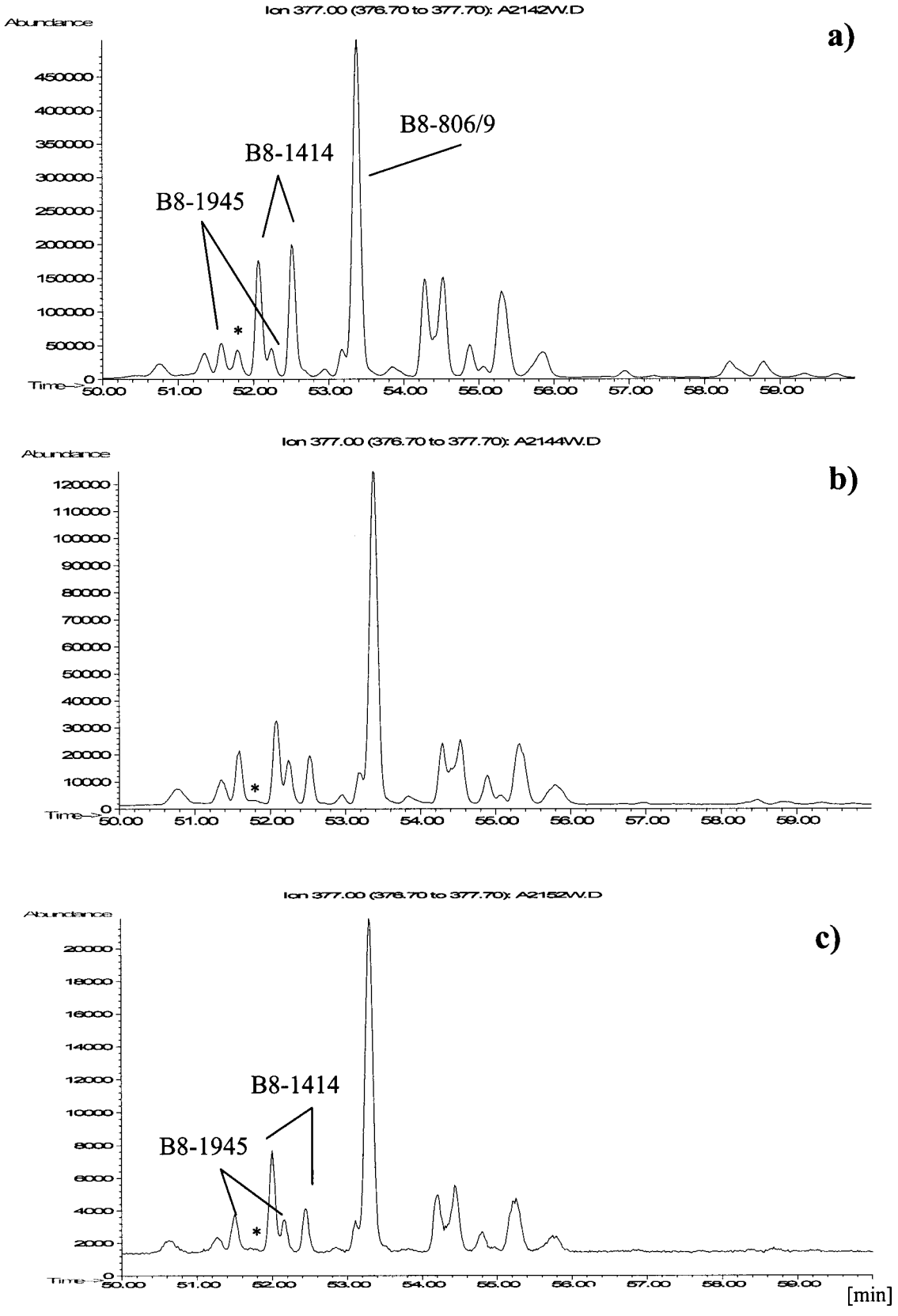

FIGURE 6. Enantioseparation of octachlorobornanes (medium retention times) in (a) sediment, (b) mummichogs, and (c) longnose gar. Note that the compound in sediment denoted by the asterisk in (a) is quantitatively eliminated in fish.

has recently been reported in samples from the Great Lakes (*38*). It is important to note that while changes in enantiomer ratios across food web organisms suggest biochemical transformation of a component, racemic or near racemic enantiomer proportions do not necessarily rule out the presence of such transformations. Chiral recognition by enzyme systems appears to be an individual event for each component. Like enzyme systems, CSPs often resolve one

enantiomers but not the other.

Implications of Enantioselective Information on the Fate of Toxaphene Residues. The distribution of CTTs in contaminated media, including marsh sediments which are considered the chronic source of toxaphene residues at our study site, clearly indicate a weathered profile that is skewed toward lower chlorinated homologues. It follows that these contaminants may thus exhibit a lower intrinsic potential for bioaccumulation relative to technical toxaphene. For example, Fisk et al. calculated biomagnification factors (BMFs) that were <1 for B6-923 and B7-1001 in fish (*39*), suggesting that higher animals are able to eliminate these and other hexa- and heptachlorobornanes. Thus, upon removal of the contaminant source, one might expect these lower homologues to disappear more rapidly in biota than other, more persistent congeners such as B8-1413 (P-26) and B9-1679 (P-50). The species represented in this study can be thought of in terms of a simple food web (Figure 2), which is typical of the Terry/Dupree Creek marsh and of southeastern U.S. coastal marshes in general. Clearly, this simplified food web does not demonstrate the complexities in time and space of food web dynamics. Also, the species represented in this study varied in size, age, and stage of development/maturity. The smallest species (grass shrimp) were ~1 cm in length, and as a result, whole individuals were pooled for analysis (see Materials and Methods). Moreover, different tissues/organs from a small sample size of individual larger fish (muscle from mullet, livers from seatrout and gar) were analyzed. Thus, some caution must be made when comparing averaged (pooled) ERs with single point (large fish) ERs.

We hesitate to interpret these results in detail, since it is not clear if alteration of the ER of Penta-2, B6-923, and B7-1001 in fish is a consequence of metabolism and/or formation during degradation of higher chlorinated components. However, our enantioselective analysis demonstrates clearly that nonracemic proportions of several residues of toxaphene exist in environmental compartments within the contaminated Terry/Dupree Creek marsh. Furthermore, the shifts in ERs across species indicates that biochemical and/or ecological processes are important in terms of tracking the fate of toxaphene contamination. It was also evident that all species were able to degrade/eliminate lesser-chlorinated compounds to some extent.

The alterations in ERs for penta- through octachlorobornanes described herein clearly demonstrate the utility of enantioselective investigations at multiple levels of the local food web. Moreover, the reported deviations of tissue ER relative to sediment (original source) indicate that these species, including the lower trophic fish, are able to transform several CTTs enantioselectively. This finding has important implications for the reduction of human health and ecological risks associated with this and other heavily contaminated sites. It may lead to additional information as to the metabolic and elimination capabilities of fish and other biota in Terry/Dupree Creek, particularly for the most abundant, lesser-chlorinated CTTs such as B6-923 and B7-1001. Controlled uptake and elimination studies may help determine the relative importance of passive elimination versus biochemical metabolism of these persistent and potentially toxic contaminants. In terms of contaminated sediment remediation and risk reduction, enantioselective analysis can help distinguish between abiotic and microbially mediated transformation of toxaphene residues under the reducing conditions of the contaminated salt marsh environment. Long-term monitoring of environmental contamination using enantioselective analysis can serve to better understand the natural attenuation processes that act to remediate high levels of contamination over time.

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