Toxaphene residues in sediment and biota from a highly polluted area: congener- and enantioselective analysis

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Introduction

Toxaphene, a non-systemic organochlorine pesticide with a range field of applications, is ranked thirty second on the ATSDR list of hazardous pollutants [1]. Toxaphene is also among the eleven 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 [2]. Species with highly developed enzyme systems (e.g. marine mammals, birds and in some fish) at or near the top of marine food webs are able to degrade most of the components in these technical mixtures. As a result, only a few hepta- to nonachlorobornane congeners accumulate in fatty tissues.

In contrast, the reductive dechlorination of toxaphene leads to a shift towards earlier eluting components of technical toxaphene (CTTs), as evidenced in gas chromatograms of various anaerobic media (e.g. soil, sediment and sewage sludge). A single hexa- and a heptachlorobornane (B6-923 and B7-1001) were the predominant CTTs in such samples while the highly chlorinated CTTs were greatly reduced, in some cases to nondetectable levels [3]. Consequently, toxaphene weathered under anaerobic conditions may exhibit a lower intrinsic potential for bioaccu-mulation. Fisk and coworkers calculated bioconcentration factors (BCFs) that were <1 for B6-923 and B7-1001 in fish [4], suggesting that higher animals are able to eliminate these and other hexa- and heptachlorobornanes. Since most CTTs are chiral [5], enantioselective studies are well suited for investigating the environmental fate of toxaphene, especially where the residue pool resides in highly reducing environments. Sediment, crustaceans and fish representing a simple estuarine food chain were collected from a highly polluted wetland and analyzed with this technique.

Material 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, Georgia, USA. Species collected during our 1997-98 surveys included grass shrimp (*Palaemonetes pugio*), mummichogs (*Fundulus heteroclitus*), striped mullet (*Mugil cephalus*), *s*potted seatrout (*Cynoscion nebulosus*) and alligator gar (*Lepisosteus spatula*). These organisms represent several levels of a typical estuarine food web. Grass shrimp are short-lived crustaceans (1-2 cm; 1 yr. max) that live and feed on detritus and smaller zooplankton in shallow areas of salt marshes. Grass shrimp are eaten by small fish such as mummichogs, a minnow-like fish (2-6 cm; 1-2 yr. max.) that has a limited home range in shallow 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 food for seatrout, a year round inhabitant of the estuary (up to 50 cm; 4-5 kg; 7-8 yr. max). Alligator gar are long (up to 1m) slender fish that are opportunistic feeders and are little-changed from early times.

GC/MS parameters. GC/ECNI-MS analyses were performed with a Hewlett-Packard 5989B system. After a solvent delay of 20 min, the following 10 ions were detected in parallel at 1.11 cycles/sec: 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 and quadrupole temperatures were 150°C and 100°C, respectively. Splitless injections (1.5 min) were performed at 230°C. The chiral stationary phase consisted of 25% randomly *tert*.-butyldimethylsilylated ß-cyclodextrin diluted in PS086 (ß-BSCD) [6]. The GC oven was heated with the following program: 80°C (4 min), then at 20°C/min to 180°C (15 min), at 20°C/min to 200°C (25 min) and at 20°C/min to 230°C (15 min).

Sample clean-up. Tissues samples were wet homogenized with Na_2SO_4 and extracted with 400 mL CH_2Cl_2 in a glass Soxhlet apparatus for 16 h. After concentration to ~10 mL, tissue extracts were allowed to evaporate overnight in a fume hood. The resulting residue was weighed, re-dissolved in n-hexane and applied to a glass column packed with 18.0 g of Florisil, activated/ deactivated as described previously [7]. Three fractions were eluted from the Florisil with CTTs targeted in the first (~50 mL n-hexane) and second (150 mL n-hexane/CH_2Cl_2 (80:20, v:v)) fractions [7]. The CTT containing fractions were recombined and separated on 8 g silica gel according to the method of Krock et al. [8]. After elution with 48 mL n-hexane, the CTTs were eluted with 50 mL n-hexane/ethyl acetate (90:10, v:v).

Table 1: AV-codes, structures and source of the CTTs mentioned in the text

AV-code	(other names)	IUPAC name	source/description
B5-???	Penta-2	not yet known	Maruya et al. [9]
B6-923	(Hx-Sed)	2-*exo*,3-*endo*,6-*exo,8,9,10*	not available (isolate)[3]
B7-515	(P-32)	2,2,5-*endo*,6-*exo,8,9,10*	Dr. Ehrenstorfer [10]
B7-1001	(Hp-Sed)	2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,9,10	not available (isolate) [3]
B8-806	(P-42)	2,2,5-*endo*,6-*exo,8,8,9,10*	Dr. Ehrenstorfer [10]
B8-809	(P-42)	2,2,5-*endo*,6-*exo,8,9,9,10*	Dr. Ehrenstorfer [10]
B8-1412	(-)	2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,8,9,10	not available (isolate)[11]
B8-1413	(P-26)	2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,8,10,10	Dr.Ehrenstorfer [10]
B8-2229	(P-44)	2-*exo*,5,5,8,9,9,10,10	Dr.Ehrenstorfer[10]
B9-1679	(P-50)	2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,8,9,10,10	Dr. Ehrenstorfer [10]

Results and Discussion

From our earlier studies, it was found that the enantiomeric ratios (ERs) of the most abundant CTTs in marine mammals and fish, i.e. B8-1413 (P-26) and B9-1679 (P-50), were close to 1.0 [12]. However, the ER of less stable congeners such as B8-2229 (P-44) and B8-1412 deviated significantly from racemic composition [13][14]. Therefore, we assumed an enantioselective degradation of the major CTTs in biota living in environments where toxaphene is weathered under anaerobic conditions.

Reductive dechlorination of chlorobornanes with geminal chlorine substituents is thought to be the major degradation pathway under anaerobic conditions. In support of this theory, B6-923 (Hx-Sed) and B7-1001 (Hp-Sed) were among the most abundant chlorobornanes in Terry/Dupree Creek sediments; pentachlorobornanes ("Penta 2"; Table 1) were also detected [9].

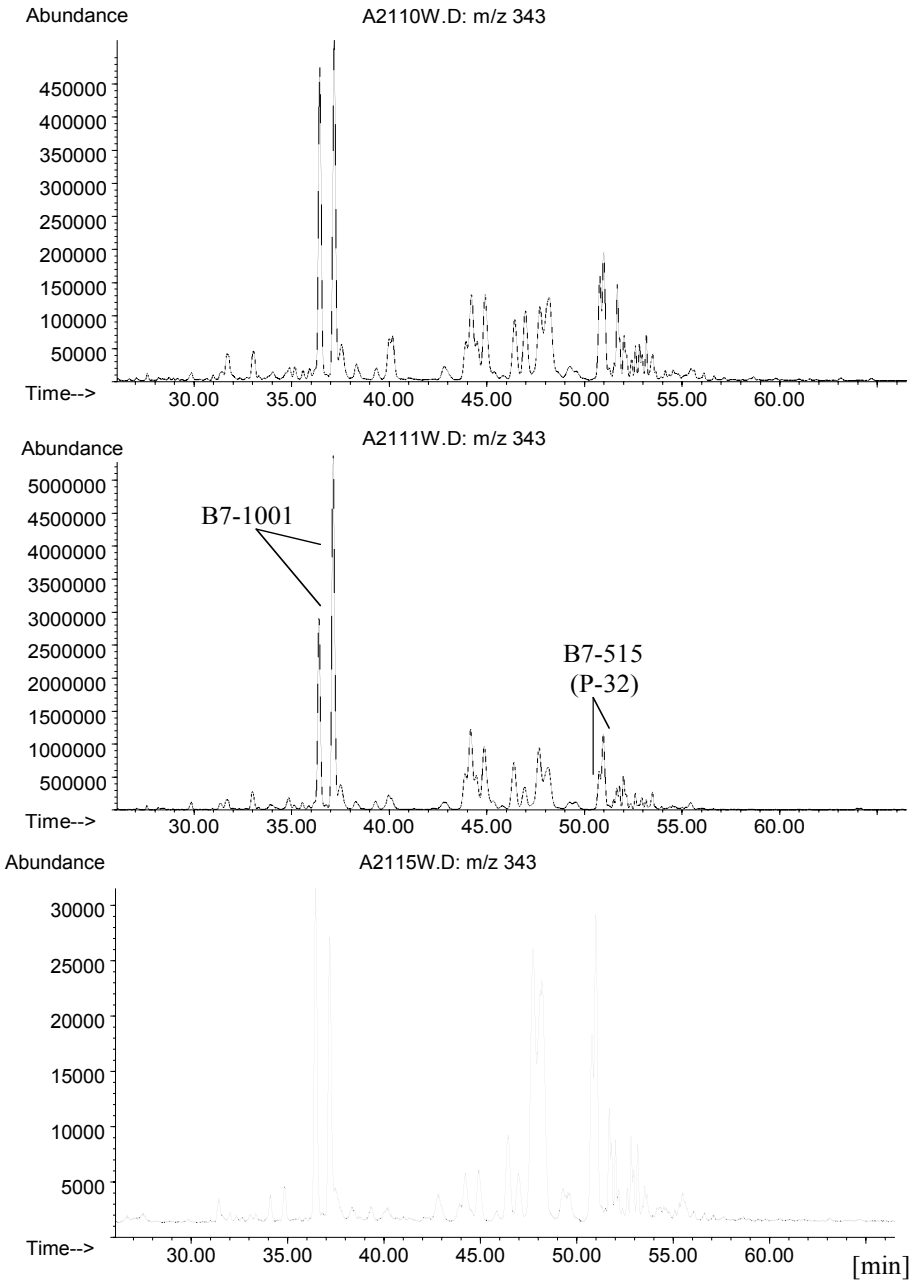

Figure 1. Enantioseparation of heptachlorobornanes in (a) sediment; (b) mummichogs (prey fish); and (c) alligator gar (predator fish) from Terry/Dupree Creek, GA, USA.

High levels of two major CTTs, B7-515 (P-32 or Toxicant B) and B8-806/809 (P-42 or Toxicant A), were also found. B8-806/809 was not enantioresolved using the ß-BSCD column, thus, we focused this report on the enantioselective fate of Penta-2, B6-923, B7-1001 and B7-515 (P-32). While B6-923 and Penta-2 were racemic in sediments [9], B7-1001 and B7-515 (P-32) showed a slight excess of the later eluting enantiomer (Figure 1a). These findings are in agreement with those for sediment samples from Hanson Lake (Canada) [15]. In biological samples, significant shifts in the ERs were noted in all four CTTs monitored. Surprisingly, lower trophic level fish exhibited ERs that were significantly changed relative to sediment; e.g., the ER of B7-1001 in mummichogs and striped mullet was < 0.5 (Figure 1b). Penta-2, B7-515 (P-32) and B6-923 also showed significant relative shifts in the ER. Interestingly, the earlier eluting enantiomer of Penta-2 was more abundant in striped mullet but less abundant in mummichogs. Typically, ERs shifts become more pronounced as one moves up the food web. However, the ERs for B7-1001 in seatrout and alligator gar were 1.1 and 1.2, respectively. This represents only a slight shift towards the earlier eluting enantiomer (Figure 1c) and a reversal of the ER relative to the lower trophic level fish. However, if one assumes that the major diet of these predators are these smaller fish, the much larger, expected ER shift in predators could be moderated by the opposite ER trend in their primary food. A similar trend, although less pronounced, was observed for B6-923. These results clearly demonstrate that an enantioselective investigation of multiple levels of local food webs is necessary to elucidate alterations of the ER for toxaphene residues. However, the reported deviations of tissue ER relative to sediment (original source) confirm that these species, including the lower trophic fish, are able to degrade these components. This finding has important implications for the reduction of human health and ecological risks associated with this and other heavily contaminated sites.

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