TEMPERATURE AFFECTS THE ENANTIOSELECTIVITY OF ELIMINATION OF THE TOXAPHENE COMPOUND B6-923

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Introduction

Toxaphene is a multicomponent organohalogen insecticide which is transformed to a large degree in the environment[1]. Whereas aquatic sediments contain mainly penta- to heptachlorobornanes, only a few hepta- to nonachlorobornanes resist degradation in fish[1]. In a recent study we observed that fish (*Fundulus* sp.) contaminated with weathered toxaphene eliminated 2-exo,3-endo,6-exo,8,9,10-hexachlorobornane (B6-923) within 60 days and did so with high enantioselectivity[2]. Using first order kinetics, we determined the half life of B6-923 to be 7 days, and also found that the less stable B6-923 enantiomer was eliminated twice as fast as the more stable enantiomer[2]. This study was conducted during the warmer months (water temperature range: 22-28 °C; mean = 25 °C). In the present study, we repeated the elimination experiment again with naturally contaminated fish, but this time during the colder months (water temperature range: 10-20 °C; mean = 15 °C) to investigate the influence of temperature on the individual elimination rates of B6-923 enantiomers.

Materials and Methods

Mummichogs (*Fundulus* sp.), a small, non-migratory fish commonly found in coastal Western Atlantic estuaries, were collected using baited minnow traps from the cooling water discharge canal of a former toxaphene manufacturing facility near Brunswick, Georgia, USA. These fish were transported to the lab in coolers filled with aerated site water. Control fish with little or no toxaphene residues were collected from a reference site. Naturally contaminated (2 tanks) and control fish (1 tank) were kept in 0.3 m^3 fiberglass tanks supplied with a continuous flow of filtered seawater with undetectable levels of toxaphene. Water temperature, pH and salinity in each tank were monitored daily. Fish were maintained for 60 days on a diet of commercial fish food (TetraMin). Two to three individual fish were collected from each tank 0, 3, 11, 20 and 64 days into the elimination phase. Individual weight and length measurements were taken before each fish was placed in a solvent rinsed glass vial and stored at –20 °C. Individual (whole) fish were Soxhlet-extracted and cleaned up using Florisil column chromatography[3]. B6-923 was enriched in the 125-150 ml hexane fraction eluting from 8 g silica[4].

GC/ECNI-MS analysis

GC/MS in the electron-capture negative ion mode was performed with a Hewlett-Packard HP5890 series II gas chromatograph interfaced to a 5989 mass spectrometer. The chiral stationary phase installed in the GC oven consisted of *tert.*-butyldimethylsilylated b-cyclodextrin (BGB Analytik, Adliswil, Switzerland). In the selected ion monitoring mode, two m/z values each were recorded for penta- to nonachlorobornanes[4]. Congener-specific (22 components) and total toxaphene concentrations were estimated using GC-ECD[5].

Results and discussion

To account for growth dilution during the experiment and also to correct for expected variations in toxaphene concentrations of individual fish at the start of the experiment, we adjusted time dependent concentrations of B6-923 by multiplying the measured concentration of B6-923 at time t ($C_{B6\text{-}923,\ meas}$) by the ratio of the mean concentration of 2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,8,9,10,10-nonachlorobornane (B9-1679) at time 0 ($C_{B9\text{-}1679,\ to}$) and the measured concentration of B9-1679 at time t ($C_{B9\text{-}1679,\ t}$). ($C_{B6\text{-}923,t}$ = $C_{B6\text{-}923,\ t,\ meas} * [C_{B9\text{-}1679,\ to}/C_{B9\text{-}1679,\ t}]$)^2. Our justification for this adjustment is based on previous reports that B9-1679 (Figure 1) is the most persistent nonachlorobornane in fish and mammals [6,7], with a reported >1 yr half life in salmonids[8], and the fact that fish increased in size with time during both experiments as expected (data not shown).

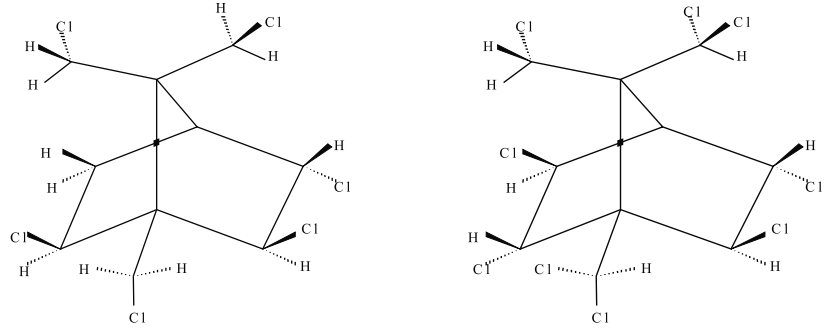

Figure 1. structures of B6-923 (left) and B9-1679 (right).

We then applied a simple, first order kinetic model to fit the elimination data (Table 1) and to calculate the half-life of B6-923 at 25 °C[2], and again at 15 °C (this study). Fish in the 15 °C experiment demonstrated a significantly slower elimination of B6-923 compared with the 25 °C experiment. This was also found for other congeners and total toxaphene (data not shown).

The enantiomer ratio (ER) at the start of the 15 °C experiment was higher than the corresponding value for the 25 °C experiment (1.6 vs. 1.3, Table 1). This may be due to the different seasons during which fish were captured from the contaminated tidal creek[2]. Due to the low resolution of B6-923 on the b-BSCD chiral stationary phase (CSP) — the only CSP known to resolve the enantiomers of this compound — along with the rapidity of elimination (Figure 2), we determined ERs using chromatographic peak heights (not areas). It is readily apparent that the second eluting enantiomer of B6-923 ("E2") was eliminated much faster than the first eluting enantiomer ("E1").

As a result, the ER rapidly increased from a mean initial value of 1.6 to 3.2 after 3 days, and to 7.3 after 11 days (Table 1). Surprisingly, the ER increased more rapidly than during the 25 °C experiment, however, this may be due to the greater recalcitrance of E1. This can also be seen from the chromatogram at day 64 (Figure 2). While E2 was barely detectable (S/N<3), E1 was still present at an S/N rate of ~10. This is in contrast to the findings of the previous study at higher water temperature where both enantiomers of B6-923 were below the limit of detection after 60 days.

Table 1. Time dependent concentration (normalized to B9-1679) and enantiomer ratios (ER) of B6-923 in fish at 15 °C and 25 °C.

Time (d)	25 °C[2]		15 °C (this study)	
	[ng/g]	ER	[ng/g]	ER
0	830	1.3	1220	1.65(1.85;1.65;1.45)
3	360	1.6	580	3.2
7	260	2.6		
11			200	7.3
14	120	6		
20			150	
28	110	>100		
41			120	
60	nd[1]			
64			120	>100

[1] nd – not detected

From these results we conclude that

(i) Overall elimination rates of B6-923 are temperature dependent

(ii) Enantiomer-specific elimination rates are disproportionally different at 15 °C than at 25 °C, with a more pronounced selective elimination of the less stable enantiomer (E2) at the lower temperature

(iii) Temperature affects elimination rate and enantioselectivity

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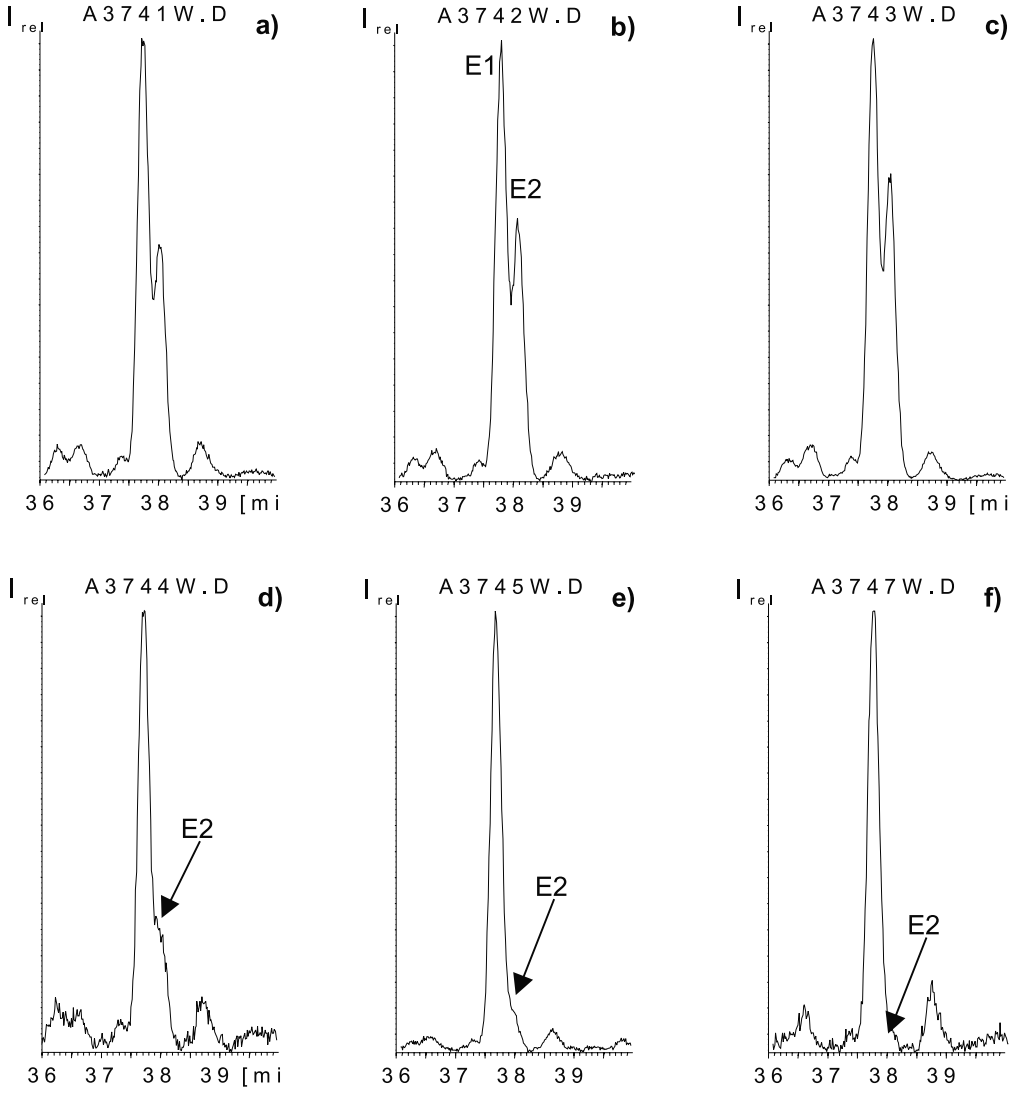

Figure 2. GC/ECNI total ion chromatogram showing enantioseparation of B6-923 using b-BSCD column at various times throughout the elimination study. (a,b,c) three different samples at t = 0 days; (d) sample at t = 3 days; (e) sample at t = 11 days; (f) sample at t = 64 days (end of experiment).