Structure of a Persistent Heptachlorobornane in Toxaphene (B7-1000) Agrees with Molecular Model Predictions

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A Cl_7 component of technical toxaphene (CTT), previously detected in marine mammals and fish and referred to as "7-1", was isolated from contaminated estuarine sediment using preparative solid−liquid chromatography followed by reversed-phase HPLC. The structure of this compound, elucidated by GC/MS and ^{1}H NMR, was 2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,8,10-heptachlorobornane (hereafter referred to as B7-1000). This newly identified CTT eluted in the nonpolar fraction from silica and shares the alternating *endo*−*exo* chlorine substitution pattern with other relatively nonpolar, persistent congeners (e.g., B8-1413 and B9-1679). Based on ECNI-MS response, levels of B7-1000 in tissue samples of various higher organisms including humans were as high as 16% of B8-1413. Enantioselective determination of B7-1000 using a modified cyclodextrin chiral stationary phase (β-BSCD) resulted in enantiomer ratios that were depleted in adipose tissue of a marine bird (skua) and Weddell seal blubber (0.3 and 0.5, respectively), but not in elephant seal blubber (1.1). Elucidation of the structure of B7-1000 thus validates previous predictions of persistence based on structure−activity relationships, chromatographic properties, and molecular modeling.

Keywords: *Toxaphene; chlorinated bornanes; marine mammals; sediment; NMR*

INTRODUCTION

Toxaphene was the most heavily used organochlorine pesticides in the United States (*1*). Its global production was estimated at 1.3 million tons (*2*). Despite its ban in many countries over a decade ago, toxaphene residues are still found in the environment. Presently, compounds of technical toxaphene (CTTs) are major organochlorine contaminants in aquatic life worldwide (*3*). As a result, toxaphene is among the 16 substances for which production bans and "use according to agreed risk criteria" have been recommended (*4*).

In its unmodified form, toxaphene consists of several hundred CTTs, most of which are chlorobornanes. However, only a small subset of these are found in the environment (*5*). Species with highly specialized enzyme systems are able to transform most CTTs; thus, only a few hepta- to nonachlorobornanes have been found in higher mammals (*6*−*9*). Of the 11 CTTs detected by GC/ECNI-MS in blubber from five Antarctic seal species, 6 were identified by injection of single CTT standards (*9*), and another was subsequently isolated and identified (*10*).

The two major recalcitrant CTTs in marine mammals—B8-1413 and B9-1679—share the 2-*endo*,3-*exo*,5-*endo*,6-*exo* chlorine substitution pattern on the six-membered ring of the bornane skeleton (*9*, *11*−*14*). Together, B8-1413 and B9-1679 accounted for 50% or more of CTT residues in marine mammals (*9*, *12*), human blood (*7*), and monkey tissue (*8*). A heptachloro-CTT previously designated 7-1 was found at levels that were 14−55% of B8-1413 in Antarctic seals (*9*). More recently, this component was identified in estuarine sediment contaminated with toxaphene (*15*).

Several properties of 7-1 were gleaned from previous studies. It eluted prior to other persistent CTTs from silica using *n*-hexane as the eluent (*15*). Compared with other heptachlorobornanes, it had a very short retention time on nonpolar GC stationary phases (*16*). On the basis of these properties and molecular models of persistent chlorobornanes, two structural variants—2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,*x*,10-heptachlorobornane, where *x* is either 8 or 10—were predicted for 7-1 (*17*). Because the estuarine sediments in which it was most recently found were highly reducing, it was further suggested that 7-1 was a degradation product of 2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,8,10,10-octachlorobornane (B8-1413) (*15*).

The purpose of this study was to elucidate the structure of 7-1. Confirmation of its structure as one of the two predicted structural variants discussed previously would thus elevate our confidence in molecular models and structure-dependent activity relationships that predict/explain recalcitrance for toxaphene components in the environment.

EXPERIMENTAL PROCEDURES

Reagents, Solvents, and Reference Standards. Chromatographic grade silica gel (60 mesh) used to fractionate sediment extracts was obtained from Merck (Darmstadt, Germany). Granular copper used to remove sulfur from sediment extracts was purchased from Fisher Scientific (Fair Lawn, NJ). Sulfuric and hydrochloric acids used in this study were of ACS reagent grade or better (Fisher Scientific). Gases used for GC analyses were of ultrahigh purity or better (>99.999%). Nitrogen gas used for blow down of organic extracts was of high purity or better (>99.99%). All organic solvents were of high purity (Optima grade, Fisher Scientific

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or equivalent). A solution of the technical product Camphechlor (10 ng/μL) was obtained from Promochem (Wesel, Germany). Standard solutions of 2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,8,10,10-octachlorobornane (B8-1413) (*18*), 2-*endo*,3-*exo*,5-*endo*,6-*exo*,-8,8,9,10,10-nonachlorobornane (B9-1679), 2-*exo*,5,5,8,9,9,10,10-octachlorobornane (B8-2229), 2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,9,10,10-octachlorobornane (B8-1414), 2-*exo*,3-*endo*,5-*exo*,8,9,9,10,10-octachlorobornane (B8-1945), and 2,2,5,5,8,9,9,10,10-nonachlorobornane (B9-1025) were from Dr. Ehrenstorfer (Augsburg, Germany). 2-*exo*,3-*endo*,5-*exo*,9,9,10,10-Heptachlorobornane (B7-1453) and 2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,8,9,10-octachlorobornane (B8-1412) were isolated from technical products and/or environmental samples (*10,19*).

Extraction and Isolation of B7-1000 from Contaminated Sediment. B7-1000 was extracted from toxaphene-contaminated sediment from the Terry/Dupree Creek estuarine marsh near Brunswick, GA. This marsh received discharge from a former toxaphene plant for more than three decades (~1948–1980). Four 250 g aliquots of wet sediment were extracted in 500 mL I-Chem glass jars with an equivolume mixture (200 mL total) of acetone and *n*-hexane. The jars were sealed with Teflon-lined screw caps and shaken overnight, and the liquid layer was decanted into a separatory funnel. The organic phase was partitioned into *n*-hexane by repeated, alternating rinses of *n*-hexane-washed water and *n*-hexane. Individual *n*-hexane extracts were transferred into a graduated glass tube, reduced to ~5 mL under a gentle stream of nitrogen, and treated with 5 mL of concentrated H_2SO_4. After vortexing, the *n*-hexane layer was removed with a glass Pasteur pipet. Each extract was then transferred into a 50 mL glass Erlenmeyer flask to which several grams of freshly activated granular Cu had been added to remove sulfur. The Cu was activated in concentrated HCl for 1 h followed by exhaustive rinses of acetone and *n*-hexane. The H_2SO_4 and Cu treatments were repeated as necessary until all extracts appeared to be colorless. The combined extracts contained ~1 mg of toxaphene. B7-1000 was identified as a heptachloro component in these samples (*15*).

To isolate B7-1000, the combined sediment extract was fractionated on 60 g of silica gel (60 mesh), activated at 130 °C for 16 h. Fractions of 25 mL of *n*-hexane were collected, with the majority of B7-1000 eluting in fraction 325–350 mL. By comparison, the bulk of B8-1413 eluted in a subsequent fraction (375–400 mL) but was also detected in fraction 325–350 mL. Fraction 325–350 mL was evaporated to dryness and redissolved in 500 μL of acetonitrile.

Two aliquots (200 μL each) of the B7-1000 fraction (325–350 mL) were injected into an automated reversed phase high-performance liquid chromatography (RP-HPLC) system (*10, 20*). After a delay of 2 min, fractions of 0.5 min were collected in 4 mL screw-cap glass vials. These fractions were analyzed according to the rapid method of Vetter et al. (*20*). Each HPLC fraction was amended with 0.5 mL of *n*-hexane, shaken for 30 s, and then left to separate for a few minutes before the organic layer was removed. This was repeated, and the *n*-hexane extracts were combined. One microliter of each extract was diluted 100-fold and analyzed for B7-1000 by gas chromatography (see below). The purity of B7-1000 in fraction 12.5–13.0 min was >90%. After two injections, fractions 12.0–12.5 min and 13.0–13.5 min (together containing ~40% of the level of B7-1000 in fraction 12.5–13.0 min) were evaporated and re-injected into the HPLC. The B7-1000-containing fraction from this HPLC run was combined with the original fraction 12.5–13.0 min and used in subsequent GC/MS and ^{1}H NMR investigations.

Tissue Samples. Tissue samples from eight species of fish, mammals, and birds were analyzed in this study. Harbor porpoise (*Phocoena phocoena*) samples from four sexually immature specimens (three male and one female) were collected in 1994 in Iceland. A blubber extract pooled from 11 harp seals (*Phoca sibirica*) collected in Spitsbergen (Arctic) in 1991 was also analyzed, as was blubber of Antarctic Weddell seals (*Leptonychotes weddelli*) collected in 1990 at Drescher Inlet in the Weddell Sea (72° 52′ S, 19° 25′ W). Samples from an elephant seal (*Mirounga leonina*) and a skua (*Catharacta maccormicki*) were collected in 1994 at the Jubany station in the Antarctic (62° 14′ 18″ S, 58° 40′ W). A human milk sample was obtained in 1994 from a Faeroese woman who regularly consumed whale blubber (*21*). Adipose tissue was taken from a mature (3.5-year-old) polar bear (*Ursus maritimus*), accidentally killed on June 24, 1993, 118.5 km north of Horn, Northwest Iceland (*22*). Extracts of cod liver oil (Baltic Sea) and of farmed salmon muscle (origin unknown) were also analyzed.

Tissue samples (except human milk and salmon fillet) were digested in acid. Organochlorines were partitioned into *n*-hexane by liquid–liquid extraction. The resulting organic extract was treated repeatedly with concentrated H_2SO_4, followed by adsorption chromatography on silica (*23*). PCBs and other aromatic organochlorines were separated from CTTs by an additional chromatographic step using 8 g of silica (*24, 25*). Extracts of human milk and salmon fillet (two fractions each sample, *n*-hexane and *n*-hexane/toluene, 65:35, v/v) were obtained from L. Alder (BgVV, Berlin, Germany) and were processed as described in Alder et al. (*26*). These extracts were recombined and refractionated using the 8 g silica procedure described above.

Gas Chromatography/Electron Capture Detection (GC/ECD). Analyses were performed on a Hewlett-Packard 5890 II gas chromatograph equipped with dual capillary columns and ^{63}Ni electron capture detectors (ECDs). The carrier and makeup gases (helium and nitrogen) were maintained at constant flow rates of 1.3 and 60 mL/min, respectively. The injector and detector temperatures were 230 and 300 °C, respectively. Two 50 m × 0.25 mm i.d. fused silica columns coated with 0.2 μm CP-Sil 2 or CP-Sil 8/20% C18 (Chrompack, Middelburg, The Netherlands) were installed in parallel in the GC oven. The GC oven program was as follows: 60 °C (1.5 min hold); ramp to 150 °C at 40 °C/min (5 min hold); ramp to 230 °C at 2 °C/min; and ramp to 270 °C at 5 °C/min (15 min hold).

Gas Chromatography/Mass Spectrometry (GC/MS). Electron capture negative ionization (ECNI) measurements were performed with a Hewlett-Packard 5890 Series II Plus GC/5989B MS engine system using previously published parameters (*16*). Two fused silica columns were used. The first was a 63 m × 0.25 mm i.d. column coated with 0.25 μm of CP-Sil 2 (Chrompack). This nonpolar phase was used to determine the elution order of CTTs in Camphechlor and tissue samples. In the selected ion monitoring (SIM) mode, we measured *m/z* 340.9, 342.9, 376.9, 378.9, 410.8, and 412.8 in groups of four ions in three time windows (*9*). The second was a 30 m × 0.25 mm i.d. column coated with 0.2 μm of 25% randomly *tert*-butyldimethylsilylated β-cyclodextrin (β-BSCD) diluted in PS086 (BGB Analytik, Adliswil, Switzerland). For this chiral stationary phase, we measured in SIM mode *m/z* 307, 309, 343, 345, 377, 378, 379, 380, 411, and 413 to cover the range from hexa- to nonachlorobornanes over the entire GC run.

Using β-BSCD, a chlordane component interfered with B7-1000 (*15*). This precluded the determination of accurate B7-1000/B8–1413 and B7-1000 enantiomer ratios. A partial solution was found by measuring the major isotopic peaks *m/z* 378 and 380 of the molecular ion, which in turn were interfered with by a dehydroheptachlor present in technical chlordane. The component with base peak at *m/z* 372 was tentatively identified as no. 40 in the chlordane analysis conducted by Dearth and Hites (*27*). However, *m/z* 378 and 380 are only minor ions of the dehydroheptachlor. Thus, we utilized *m/z* 380 to qualitatively distinguish B7-1000 from the interfering dehydroheptachlor in our human milk, polar bear, and fish samples.

Electron ionization mass spectrometry (EI-MS) was performed using a Hewlett-Packard 5971 mass selective detector. In the full-scan mode, *m/z* 50–385 were recorded at a scan rate of 1.2 cycles/s. A 30 m × 0.25 mm i.d. fused silica column coated with 0.25 μm of permethylated β-cyclodextrin (Chirasil-Dex, Chrompack) was installed in the GC oven. Instrument conditions were as follows: 0.9 bar carrier gas (helium) head pressure; 1 μL splitless injection; injector temperature, 225

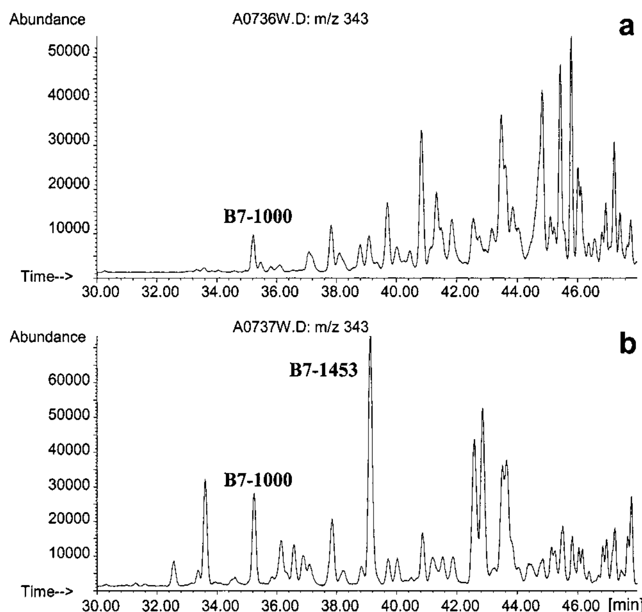

Figure 1. Detection of B7-1000 (7-1) in (a) technical toxaphene (camphechlor) and (b) harbor porpoise (*P. phocoena*) blubber. Ion trace *m/z* 343 confirms that B7-1000 is the first eluting heptachlorobornane in the technical product. Unlabeled peaks in (b) are not chlorobornanes due to wrong isotopic ratio of *m/z* 341 and 343.

°C; MS transfer line, 280 °C. The GC oven was programmed as follows: 75 °C (2 min hold); ramp to 140 °C at 20 °C/min (30 min hold); ramp to 230 °C at 20 °C/min (10 min hold) for a total run time of 49.75 min.

^{1}H NMR Spectroscopy. ^{1}H NMR measurements of the B7-1000 isolate were performed on a Bruker DRX 500 spectrometer (nominal frequency 500.13 MHz at 21 °C). All chemical shifts were referred relative to the solvent peak ($CDCl_3$) and recalculated with respect to TMS [$\delta(^1H) = 7.260$ ppm for $CDCl_3$]. The final digital resolution for the 1D spectra was 0.155 Hz. For resonance assignment and nuclear Overhauser enhancement (NOE), two-dimensional shift correlated (COSY) and phase-sensitive, time proportional phase incrementation spectra (NOESY) were recorded with a mixing time of 220 ms. The 2D data were collected in increments of 256 and 1024 for the t_1 and t_2 dimensions, respectively. For each t_1 value 256 (COSY) or 200 (NOESY) scans were recorded over a spectral width of 5040.32 Hz. Acquisition times of COSY and NOESY were 20.5 and 70.5 h, respectively.

RESULTS AND DISCUSSION

Our isolation of B7-1000 from sediment resulted in a solution that was of high purity as indicated by GC/ECD and GC/MS analyses (data not shown). Based on a mean ECD response factor computed from several early-eluting CTTs, the total isolated mass of B7-1000 was estimated at ~20 μg. Earlier studies demonstrated this amount to be sufficient for GC/MS as well as ^{1}H NMR characterization of a pure CTT (*10*, *13*).

Chromatographic Properties. B7-1000 was detected in technical toxaphene (Camphechlor) and blubber of a seal, that is, in top predators of marine food chains that accumulate only a few CTTs of particular persistence, using GC-ECNI-MS (Figure 1). Its presence in technical toxaphene confirms that B7-1000 is indeed present in technical products, although the possibility remains that it is also a metabolite of higher chlorinated homologues (*15*). It is noteworthy that B7-1000 is the first eluting heptachlorobornane in Camphechlor using

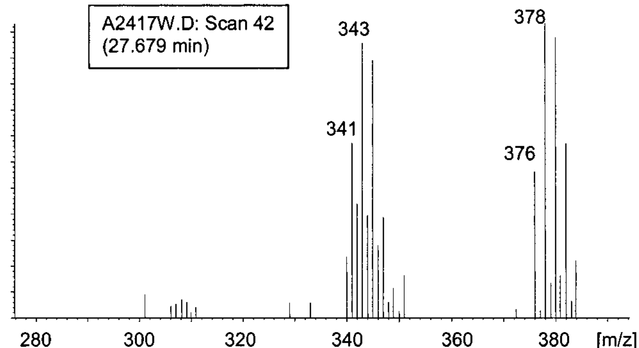

Figure 2. GC/ECNI mass spectrum of B7-1000.

nonpolar stationary GC phases (e.g., CP-Sil 2, 5, and 8). GC-ECNI-MS analysis of technical toxaphene is therefore a suitable method for identifying B7-1000. Early elution from the very nonpolar CP-Sil 2 phase is characteristic of CTTs with staggered *endo-exo-endo-exo* conformation on the six-membered ring (*16*). Like B7-1000, B8-1413 and B9-1679 share this structural feature and are the first eluting octa- and nonachlorobornanes in technical toxaphene, respectively. The staggered *endo-exo-endo-exo* conformation has also been found to be a predictor of persistence in the environment (*28*).

The "bridge-and-*exo*" rule (*29*), and the fact that all chlorobornanes have at least one chlorine substituent on C10 (*5*, *30*, *31*), limits the number of possible heptachlorobornane structures to three: (i) 8,8,10; (ii) 8,9,10; and (iii) 8,10,10 (*29*). A further differentiation can be made on the basis of the substitution pattern on C8 and C9. CTTs with a nonsymmetric distribution of chlorine atoms (structures i and iii above) elute earlier from GC columns than those with evenly distributed Cl atoms (*16*). Congener ii—B7-1001 or Hp-Sed—is symmetrical in this respect and elutes ~3 min after B7-1000 using standard GC conditions. The structural variants i and iii, which fulfill all previously described criteria that suggest environmental persistence (*17*), differ from B8-1413 by one chlorine atom on a primary bornane carbon (i.e. C8, C9, and C10).

Mass Spectral Characteristics. The full-scan ECNI mass spectrum of B7-1000 was dominated by the molecular ion starting at *m/z* 376 (Figure 2). This corresponds to the molecular formula $C_{10}H_{11}Cl_7$. Formation of the molecular ion is unusual for heptachlorobornanes, which usually exhibit highest abundance for the $[M - Cl]^-$ fragment ion (*32*). The $[M - Cl]^-$ fragment ion at *m/z* 341 was overlapped by the $[M - HCl]^-$ fragment ion, which accounted for ~40% of the former ion. This relatively high contribution of the $[M - HCl]^-$ fragment ion was also reported for B8-1413, providing further evidence as to the similarity of the two components.

In the EI-MS spectrum (Figure 3), the tropylium cation (*m/z* 159) is interfered with by the fragment ion $[C_7H_735Cl_2]^+$ (*m/z* 161). The abundance ratio between *m/z* 159 and 161 is <1, which is typical of heptachlorobornanes (*5*). Also, the molecular ion is not apparent, but the $[M - Cl]^+$ fragment ion at *m/z* 341 confirms that B7-1000 is a heptachloro-CTT. The abundant *m/z* 305 fragment ion corresponds to $[M - 71]^+$ or $[C_{10}H_{10}Cl_5]^+$. Additional losses of 36 u (corresponding to the loss of HCl) result in fragment ions $[C_{10}H_9Cl_4]^+$ (*m/z* 269), $[C_{10}H_8Cl_3]^+$ (*m/z* 233), and $[C_{10}H_7Cl_2]^+$ (*m/z* 197). Also prominent was the $[M - 83]^+$ or $[C_9H_{10}Cl_5]^+$ fragment

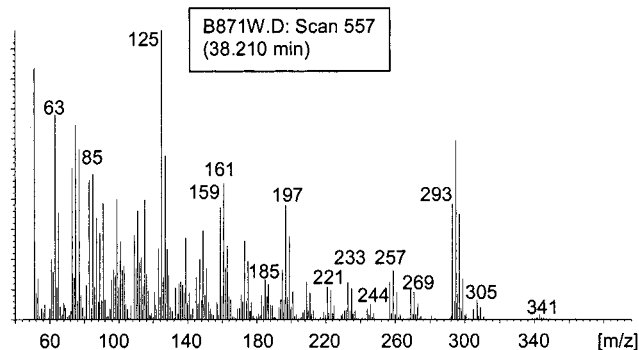

Figure 3. GC/EI mass spectrum of B7-1000.

Table 1. ^{1}H NMR Data (Chemical Shifts, Coupling Constants, COSY and NOESY Correlations) of B7-1000 and B8-1413

B7-1000				B8−1413		
δ	M[a]	J[b] (Hz)	position[c]	δ	M[a]	J[b] (Hz)
6.68	s		H8 ($CHCl_2$)	6.90	q	0.6
			H10 ($CHCl_2$)	6.42	s	
5.12	dd	5.5; ∼1.2	H2-*exo*	5.29	dd	5.0; 0.9
4.66	m	5.5	H3-*endo*	4.73	d	5.0
4.56			H6-*endo*	4.80	d	4.7
4.56	m	(to 2.68)	H5-*exo*	4.66	dd	4.7; 4.6
4.19	dd	12.8	H10a (CH_2Cl)			
3.57	d	12.8	H10b (CH_2Cl)			
2.68	m	∼1.2 (to 4.56)	H4	2.72	dd	4.6; 0.9
1.89	s		H9 (CH_3)	1.88	d	0.6

[a] M = multiplicity; s = singlet; d = doublet; dd = doublet of doublet; m = multiplet (split signal due to several unresolved couplings), q = quartet. [b] J = coupling constant. [c] Position = location of the protons on bornane skeleton (see Figure 4 for carbon numbering).

ion (*m*/*z* 293), which confirms the presence of a $CHCl_2$ group. This fragment ion also loses HCl units to form $[C_9H_9Cl_4]^+$ (*m*/*z* 257), $[C_9H_8Cl_3]^+$ (*m*/*z* 221), and $[C_9H_7Cl_2]^+$ (*m*/*z* 185). An even-mass odd-electron ion is found at *m*/*z* 244, which corresponds with the composition $[C_8H_8Cl_4]^+$. Such fragment ions are characteristic of the retro-Diels−Alder reactions observed for chlorobornanes (*33*). The lack of fragments involving the losses of neutral fragments C_2HCl_3, C_2H_3Cl, C_2H_4, and C_2Cl_4 indicates a total of four Cl atoms on the six-membered ring, with two chlorines on each side.

Information on the chlorine distribution on primary bornane carbons is usually derived from fragment ions formed after elimination of the bridge (C7 to C9), for example, from the $[M - Cl - HCl]^+$ fragment ion at *m*/*z* 305. Chloro- and dichloromethyl groups on C8 would be apparent upon elimination of 76 and 110 u, respectively, resulting in *m*/*z* 229 and 195, respectively. Because of interferences (data not shown), we cannot determine the exact Cl substitution patterns on C8, C9, and C10 from these spectra. Nonetheless, the mass spectral characteristics of B7-1000 indicate a strong similarity to B8-1413.

^{1}H NMR. ^{1}H NMR as well as two-dimensional COSY and NOESY spectra were obtained for B7-1000 (see Table 1). Coupling partners were confirmed by the COSY experiment, whereas the NOESY experiment was recorded to elucidate space proximity of *exo*-protons in the six-membered ring to protons on the bridge.

In the ^{1}H NMR spectrum, no significant interference was found between 2.5 and 7 ppm, the range over which chlorobornane protons show resonance (*5*, *30*). Significant disturbances were observed between 0.8 and 2.0 ppm. A single signal with an intensity of three at 1.88 ppm could result from B7-1000 due to couplings identified in the COSY spectrum (see below). As C10 is generally substituted with at least one chlorine (*5*, *30*, *31*), the respective primary carbon must be on the bridge (C8 or C9). Furthermore, the lack of a coupling constant >1 Hz confirms the presence of three equivalent protons at C9 (*13*).

Three additional signals were assigned to protons on primary carbons. The signal at 6.68 ppm is in the typical range of $CHCl_2$ groups of chlorobornanes (*13*). The only geminal coupling constants at 12.8 Hz in the ^{1}H NMR spectrum of B7-1000 (between signals at 3.57 and 4.19 ppm) confirmed the presence of a chloromethyl group at C8 or C10 (*34*). Information on bridge (C7−C9) and bridgehead (C4) protons is derived from NOE experiments. Protons on the bridge show strong NOE effects on *exo*-protons associated with the six-membered ring (*13*). Because an NOE was observed between 6.68 and 4.56 ppm, the dichloromethyl group is located at the bridge, whereas the chloromethyl group is associated with C10.

The remaining five signals originated from protons located on the six-membered ring (C1−C6). Four signals showed no geminal couplings (>15 Hz) (*5*), fixing their locations at C2, C3, C5, and C6. The signal at 2.68 ppm was in the typical range for bridgehead protons (H4) (*5*), whereas the other four were between 4.56 and 5.12 ppm. The signal at 5.12 ppm shows a strong NOE to the three equivalent protons on C9, which is possible only if it is in the *exo*-orientation. This signal also couples ($J = 5.5$ Hz) to the signal at 4.66 ppm, which is typical of vicinal *anti*-couplings (*5*). Because there is no coupling to H4, H3 must be in the *exo*-position and H2 must be in the *endo*-position.

The two proton signals at 4.56 ppm exhibited nearly identical chemical shifts but were not completely resolved. This precluded the determination of exact coupling constants. Additional information, however, was derived from the COSY and NOESY spectra. Coupling to each other as well as coupling of one of the protons to H4 was confirmed in the COSY experiment. Therefore, these two protons must be located on C5 and C6, respectively. Because only *exo*-protons vicinal to the bridgehead at C4 couple with H4 (see above), the respective proton on C5 must be in the H5-*exo* orientation. This was confirmed by a strong NOE to proton H8. The lack of a large coupling (>8 Hz) excludes a vicinal syn-coupling (*5*), and, therefore, the second signal must be H6-*endo*. This interpretation requires a doublet of doublet for H5-*exo* and a doublet for H6-*endo*. The multiplet ∼4.56 ppm can be interpreted as such.

Comparing the ^{1}H NMR data for our isolate with that for B8-1413 confirms their structural similarities. Except for the additional proton H10 (the signal at 6.42 ppm is distributed in 3.57 and 4.19 ppm), the positions of all other protons were similar. In our isolate, Δppm ranged from 0.01 to 0.17 ppm. The largest deviation was observed for protons that were strongly influenced by the substituents on C10 (i.e., H2 and H6), whereas the more protected protons exhibited similar chemical shifts (i.e., H4, H3, and H5). This effect was observed for another structurally similar congener (B8-1412), which differs from B7-1000 by the addition of a Cl atom on C9. Moreover, B8-1412 showed chemical shifts similar to those of B7-1000 for the two protons on C10 (*10*).

In contrast to B8-1413 (*12*, *13*, *34*) and B8-1412 (*10*)

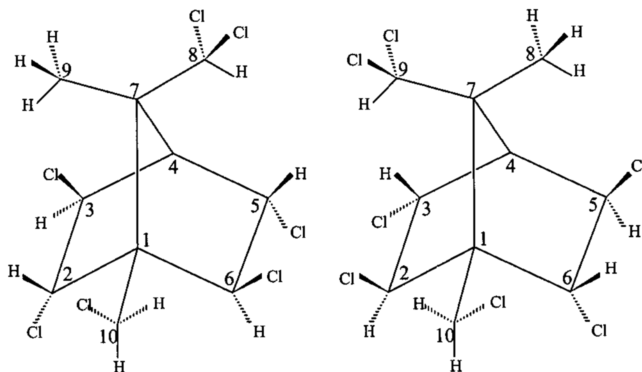

Figure 4. Structure of the enantiomers of B7-1000.

Table 2. ECNI-MS Signal Intensity of B7-1000 Relative to That of B8-1413 As Determined on a CP-Sil 2 Column

species	sample size	% of B8-1413 mean (range)
harbor porpoise, Iceland (*Phocoena phocoena*)	$n = 4$	9.2 (8.3−10.7)
harp seal, Spitsbergen (*Phoca sibirica*)	$n = 9$	10.0 (1 pool sample)
Weddell seals, Antarctic (*Leptonychotes weddelli*)	$n = 8$	16.2 (11.6−25.6)

the ^{1}H NMR spectra of B7-1000 showed no small coupling constants. This was likely due to the lower resolution in the present study. Although signal broadening was observed in several cases, we were not able to resolve small couplings. However, these determinations were not essential for structural assignment of our isolate.

Comparison with Molecular Model Predictions. Additional confirmation of our proposed structure can be derived from theoretical considerations. The proposed B7-1000 structure is in agreement with the "2-*exo* bridge rule" (*29*), which postulates that a dichloromethyl group on C8 requires an *exo*-chlorine on C6. Optimal conformations of chlorine atoms on primary carbons, using the "a,b,c" nomenclature of Frenzen et al. (*35*), are important for the stability of chlorobornanes (*29*). Although these conformations cannot be elucidated in detail by ^{1}H NMR analysis, the conformation rule allows one to predict the preferred conformation of any given structure. As we have determined that the three protons on C9 were equivalent for B7-1000 (see above), it follows that the 6-*exo* chlorine atom directs the chlorine atoms on C8 and C10 into the energetically preferred 8b, 8c, and 10c conformation (*17*).

In summary, the structure of our CTT isolate is 2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,8,10-heptachlorobornane (Figure 4), or B7-1000 using the systematic AV codes (*18*). As predicted, B7-1000 is similar in structure to B8-1413 except for the lack of a single proton on C10 (*15*).

B7-1000 in Environmental Samples. Quantitation of B7-1000 in our tissue samples was not possible due to lack of a quantitative standard and the fact that ECNI-MS response factors can vary significantly for chlorobornanes. Preliminary results suggest, however, that the ratios of the ECD and ECNI-MS response of B7-1000 and B8-1413 were similar. Estimated levels of B7-1000 relative to B8-1413, using ECNI-MS abundances of m/z 343 for B7-1000 relative to m/z 377 for B8-1413, suggested that B7-1000 accounted for ~10−15% of the ECNI abundance of B8-1413 (Table 2).

Like 97% of all 32768 theoretically possible chlorobornanes (*36*), B7-1000 is chiral. GC enantioseparation of several CTTs, including B7-1000, was recently achieved using the β-BSCD phase (*15*). To avoid coelutions, m/z 378 and 380 were used to establish the ratio of the first- to the second-eluting enantiomer (direction of light rotation unknown). Enantioseparation of B7-1000 without interference from other compounds was obtained for elephant and Weddell seal blubber as well as for skua. Elephant seal blubber showed an ER of 1.1, which is close to racemic, but the Weddell seal and skua samples exhibited depleted ERs of 0.5 and 0.3, respectively (Figure 5). Nonracemic ERs have also been observed for several other CTTs (e.g., B8-1412, B8-2229, B8-1945, B8-1414, and B9-1025) that are prominent in higher organisms (*37*), but never for B8-1413 and B9-1679. Thus, ERs have been used to distinguish between persistent (B8-1413 and B9-1679, ER ≈ 1) and degradable CTTs. Our data suggest B7-1000 is at least partially degradable. It is noteworthy that elephant seals were recently described as having lower metabolic power compared with several other seal species (*38*).

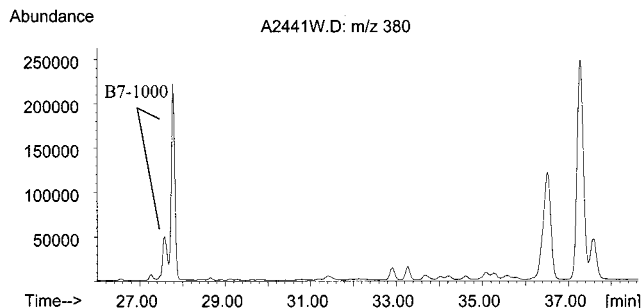

Figure 5. Enantioseparation of B7-1000 in skua adipose tissue.

It is curious that B7-1000 has not been mentioned more frequently in the literature. There is strong evidence that B7-1000 was detected by Buser and Müller (*39*) in Antarctic penguin tissue (visible as the peak at t_R ~11.6 min). However, another GC program utilized by these investigators included a solvent delay of 12 min that may have prevented its detection in other tissue samples (e.g., seal and herring). Another possibility for the lack of widespread detection of this CTT is monitoring too narrow a mass range for m/z 343. Yet another reason is the relative nonpolarity, discussed previously, of B7-1000. As preseparation of PCBs is frequently performed before quantification of CTTs, B7-1000 may partially or totally elute in the PCB fraction. Therefore, it is unwise to analyze environmental samples for B7-1000 (i) without checking the PCB fraction or (ii) by ECD without MS confirmation.

Our results, past and present, indicate that B7-1000 is detectable in many types of environmental samples. It has been identified in Camphechlor and was also suspected to be a metabolite of higher chlorinated bornanes in reducing sediments (*15*). Elucidation of its structure is incrementally important in understanding the environmental fate and persistence of toxaphene residues. This is underscored by the fact that the structure of B7-1000 was previously predicted by molecular modeling. The list of environmentally relevant CTTs that have been isolated or are available as reference standards continues to grow, allowing for quantification of toxaphene residues on a congener-specific basis. As none of the commercially available heptachlorobornanes to date are found regularly in higher organisms, the availability of B7-1000 in stan-

dard form would further contribute to the advancement of toxaphene analysis.

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