Enantioselective Analysis of a Heptachlorobornane Isolated from the Technical Product Melipax by Gas Chromatography/Mass Spectrometry

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A heptachloro **c**ompound of **t**echnical **t**oxaphene (CTT), recently isolated from the technical mixture Melipax, was enantioseparated by application of gas chromatography/mass spectrometry (GC/MS) with a chiral stationary phase. Using *tert*-butyldimethylsilylated β-cyclodextrin and two different ionization techniques, the first eluted enantiomer of 2-*exo*,3-*endo*,5-*exo*,9,9,10,10-heptachlorobornane (B7-1453) was found in significantly more abundance. Furthermore, the enantiomeric ratio (ER) of 1.26 ± 0.03 was reproduced after GC-ECD analysis on another chiral stationary phase, heptakis-(6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin. This confirms that B7-1453 was not present in racemic composition in the technical product. At present it is not clear if other technical products also contain B7-1453 and other compounds in nonracemic composition. Several synthesized CTT standards, however, showed racemic composition. An ER of 1.0 for B7-1453 was determined in a cod liver extract. Assuming that B7-1453 was present in nonracemic composition in the source of contamination, the ER of 1.0 in the cod liver would have been the result of a faster degradation of the first eluted enantiomer, which finally led to an ER of 1.0. If the source of contamination contained B7-1453 in racemic composition, the cod did not degrade B7-1453 enantioselectively. In this case, enantioselective analysis of CTTs in biota may be used to find the source of contamination.

Keywords: *Pesticide; toxaphene; gas chromatography; mass spectrometry; enantiomer separation*

INTRODUCTION

Enantioseparations continue to interest biochemists, natural product chemists, and pharmaceutical scientists (Nowotny, 1989). Capillary gas chromatography with chiral stationary phases (CSPs) was successfully applied to achieve enantioseparation of many compound classes for which it had not previously been possible (König, 1992). This technique also enabled the enantioseparation of chiral organochlorine pesticides in environmental samples at parts per billion to parts per trillion levels (Vetter and Schurig, 1997). Within the past few years, enantioselective biodegradation of α-hexachlorocyclohexane (Kallenborn et al., 1991), cyclodien pesticides (Buser et al., 1992), and even atropisomeric polychlorinated biphenyls (Glausch et al., 1994) was demonstrated in different biological matrices (Vetter and Schurig, 1997). The change in the enantiomeric ratio (ER) in a biological sample compared with a standard solution was easy to determine since these organochlorine compounds were applied as racemic mixtures (Vetter and Schurig, 1997). In the present study we produce evidence that this is not generally the case for compounds of technical toxaphene (CTTs).

Toxaphene is an organochlorine pesticide with one of the highest production rates in the world (Voldner and Li, 1995). Technical products such as Toxaphene, Melipax, and Strobane were distributed in several countries (Saleh, 1991). In scientific language, the term toxaphene is mostly used as a synonym of "the reaction product of the chlorination of technical camphene", and this covers all technical products. Presently, it is not possible to distinguish if toxaphene levels in the environment (e.g. the Arctic or the Antarctic) are residues of Toxaphene, Melipax, others, or mixtures of them.

Technical products are synthesized by the exhaustive chlorination of technical camphene. A Wagner−Meerwein rearrangement during the synthesis mainly leads to polychlorinated 1,7,7-trimethylbicyclo[2.2.1]heptanes (bornanes) (Casida et al., 1974). The technical toxaphene mixture consists of several hundred compounds that cannot be resolved even by application of high-resolution gas chromatography (Jansson and Wideqvist, 1983). Ninety-seven percent of the polychlorinated bornanes are chiral (Vetter, 1993). In biota, most of the CTTs are easily metabolized and only a few persistent CTTs accumulate in the adipose tissue of high trophic level biota (Casida et al., 1974).

Toxaphene was mutagenic in the Ames *Salmonella* test and highly carcinogenic in rats, mice, and, thus, also in man (Hooper et al., 1979; Saleh, 1991). Toxaphene may be one of the more toxic organochlorine insecticides to human beings (Saleh, 1991). In combination with other organochlorines, toxaphene may be estrogenic (Arnold et al., 1996). Single CTTs and technical toxaphene significantly varied in toxicity (Saleh, 1991). Furthermore, it was assumed that such a drastic biodegradation of chiral compounds will occur enantioselectively. It is not clear if the two enantiomeric forms of a chiral CTT have the same or different toxicities as was observed with pharmaceutical substances. Enantiopure CTT standards allowing such studies are presently not available since the CTTs resisted any enantioseparation by application of high-performance liquid chromatography (HPLC) with CSPs.

The gas chromatographic enantioseparation of two persistent CTTs was recently obtained (Kallenborn et al., 1994; Buser and Müller, 1994a). In addition, several technical products of different manufacturers were

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Table 1. Chemical Names, Abbreviations, and Origins of CTT Standards

code name (Parlar no.)	chemical name (IUPAC), 2nd enantiomer	common abbrev	source (concn)
B7-515	2,2,5-*endo*,6-*exo*,8,9,10	toxicant B	Promochem, Wesel, Germany
(Parlar 32)	2-*exo*,3-*endo*,6,6,8,9,10		(5 ng/μL)
B7-1453	2-*exo*,3-*endo*,5-*exo*,9,9,10,10	TOX7	isolated from Melipax
(−)	3-*exo*,5-*endo*,6-*exo*,8,8,10,10		(several concentrations)
B8-1413	2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,8,10,10	T2	Dr. Ehrenstorfer, Augsburg, Germany
(Parlar 26)	2-*exo*,3-*endo*,5-*exo*,6-*endo*,9,9,10,10	TOX8	(10 ng/μL)
B9-1679	2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,8,9,10,10	T12; TOX9	Dr. Ehrenstorfer, Augsburg, Germany
(Parlar 50)	2-*exo*,3-*endo*,5-*exo*,6-*endo*,8,9,9,10,10	toxicant Ac	(10 ng/μL)
B8-1414	2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,9,10,10		Dr. Ehrenstorfer, Augsburg, Germany
(Parlar 40)	2-*exo*,3-*endo*,5-*exo*,6-*endo*,8,9,10,10		(1 ng/μL)
B8-1945	2-*exo*,3-*endo*,5-*exo*,8,9,9,10,10		Dr. Ehrenstorfer, Augsburg, Germany
(Parlar 41)	3-*exo*,5-*endo*,6-*exo*,8,8,9,10,10		(1 ng/μL)
B8-2229	2-*exo*,5,5,8,9,9,10,10		Dr. Ehrenstorfer, Augsburg, Germany
(Parlar 44)	3,3,6-*exo*,8,8,9,10,10		(1 ng/μL)
B9-1025	2,2,5,5,8,9,9,10,10		Dr. Ehrenstorfer, Augsburg, Germany
(Parlar 62)	3,3,6,6,8,8,9,10,10		(1 ng/μL)

investigated on a CSP using tandem mass spectrometry (MS/MS) in the *selective reaction monitoring* (SRM) mode (Buser and Müller, 1994b). It was reported that some chlorinated bornanes were present in racemic and others in nonracemic composition (Buser and Müller, 1994b). However, interferences from other compounds present in the complex technical mixtures cannot be excluded even by using MS/MS in the SRM mode. Nevertheless, if the educt of the synthesis, technical camphene, is nonracemic, the chlorination products may also retain nonracemic composition. Indeed, toxaphene products of different manufacturers showed a weak but significant levorotation in a chiroptical detector (Buser and Müller, 1994b). Recently, we isolated a persistent heptachlorobornane from Melipax (Krock et al., 1996). The acronym Melipax most likely goes back to the observation that the product was nontoxic to bees (honey bee = *Apis* ***melli***fera; ***pax*** = peace). Melipax was produced and distributed until 1990 in the former German Democratic Republic. Melipax accounted for ~5% of the global toxaphene production (Heinisch and Klein, 1994; Voldner and Li, 1995). The structure of the isolate was elucidated by ^{1}H NMR as 2-*exo*,3-*endo*,5-*exo*,9,9,10,10-heptachlorobornane (Krock et al., 1996). By application of the pure CTT and an appropriate CSP, we were able to establish the enantiomeric ratio of the isolate.

EXPERIMENTAL PROCEDURES

Gas Chromatography with Mass Spectrometry (GC/MS) and Electron Capture Detection (GC-ECD). GC/MS experiments were carried out on an HP 5989B MS engine connected to an HP 5890 II plus gas chromatograph (Hewlett-Packard, Waldbronn, Germany). Helium was used as the carrier gas. The samples were splitless injected (1.5 min) at 225 °C.

The achiral CP-Sil 2 column (50 m length, 0.25 mm internal diameter, and 0.25 μm film thickness) was from Chrompack, Middelburg, The Netherlands. This particularly nonpolar phase consists of a chemically bonded and cross-linked (4%) silicon-containing high molecular weight hydrocarbon similar to squalane (Estel et al., 1995). The following temperature program was applied: 80 °C (1 min), 15 °C/min to 160 °C (1 min), 2 °C/min to 255 °C, 15 °C/min to 290 °C (21.67 min); the total run time was 80 min.

Enantioseparations of CTTs were carried out with a 30 m fused silica capillary column, coated with 0.2 μm 25% *tert*-butyldimethylsilylated β-cyclodextrin in 85% dimethyl, 15% diphenyl polysiloxane (BGB Analytik, Adliswil, Switzerland), hereafter abbreviated β-BSCD. The GC temperature program started at 120 °C (2 min); then the temperature was raised at 15 °C/min to 190 °C (2 min), at 2 °C/min to 210 °C (40 min), and at 10 °C/min to 240 °C (13.33 min). The total run time was 75 min.

Electron capture negative ionization mass spectrometry (ECNI-MS) experiments were performed with methane as reagent gas. The ion source was set at 150 °C and the quadrupole at 100 °C. The system was optimized by manual tuning using *m*/*z* 312, 414, and 464 of perfluorotributylamine (PFTBA). The methane pressure was 1.6 mbar. In the SIM mode the following time windows were run: 35−47.5 min, heptachloro CTTs (*m*/*z* 340.9, 342.9) and octachloro CTTs (*m*/*z* 376.9, 378.9, 380.9); 47.5−80 min, octachloro CTTs (*m*/*z* 376.9, 378.9); and nonachloro CTTs (*m*/*z* 410.8, 412.8, 412.9). Dwell time was 80 ms each (2.56 cycles/s).

Electron ionization mass spectrometry (EI-MS) was used to study the enantiomeric ratio of B7-1453. In the SIM mode *m*/*z* 161, 259, 293, 295, and 297 were recorded at a dwell time of 60 ms. The electron multiplier voltage was 2400 V. All other parameters were identical with ECNI.

Enantioseparation of B7-1453 was also performed by GC-ECD. The 20 m fused silica capillary column, coated with 0.15 μm 35% heptakis(6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin in OV 1701 (85% methyl, 7% phenyl 7% cyanopropyl, 1% vinyl polysiloxane) was purchased from M. Müller (Eidgenössische Forschungsanstalt Wädenswil, Switzerland) and is hereafter abbreviated β-TBDM. The GC temperature program started at 120 °C (2 min); then the temperature was raised at 15 °C/min to 140 °C (150 min) and at 20 °C/min to 220 °C (10 min). The total run time was 167 min.

Standards. Table 1 lists chemical names, abbreviations, and origins of CTT standards. Instead of the unwieldy chemical names we used code names developed for all polychlorinated bornanes (Andrews and Vetter, 1995). A major recalcitrant heptachloro CTT in biota, B7-1453, was recently isolated both from Melipax (VEB Fahlberg-List, Magdeburg, former GDR) and from Baltic cod liver. The isolation procedure was reported in detail (Krock et al., 1996). Every step of the isolation from the technical product was checked in parallel by isolation of the compound from Baltic cod livers. In brief, 50 g of silica was eluted with *n*-hexane. HPLC on silica and *n*-hexane as the eluent was then applied. The next step was reversed phase HPLC with acetonitrile/water as the eluent. Finally, 50 g of silica was eluted with *n*-hexane. ^{1}H-NMR structure elucidation of B7-1453 gave 2-*exo*,3-*endo*,5-*exo*,9,9,10,10-heptachlorobornane (Krock et al., 1996).

RESULTS AND DISCUSSION

Figure 1 shows the structure of B7-1453, which was recently identified as a major recalcitrant CTT in different marine mammals from the Antarctic as well as fish and other foodstuffs (Vetter et al., 1997a; Krock et al., 1997). B7-1453 contains the structural elements of CTTs leading to stability in the environment (Vetter et al., 1997a). Substitution of one hydrogen by chlorine leads to B8-1413 (Parlar 26), and addition of a further chloro substituent leads to B9-1679 (Parlar 50) (see Table 1). These two CTTs are two major recalcitrant CTTs in environmental samples (Stern et al., 1992;

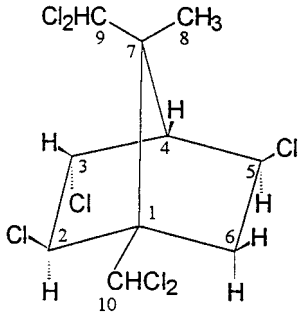

Figure 1. Structure of B7-1453 (2-*exo*,3-*endo*,5-*exo*,9,9,10,10-heptachlorobornane).

Luckas et al., 1990). The structural similarity of these CTTs is also reflected in their chromatographic behavior. B7-1453 eluted from silica between the two persistent CTTs mentioned above. These early eluted CTTs are particularly nonpolar, while B7-515 (toxicant B) and B8-806/9 (toxicant A) eluted late from silica (Krock et al., 1997). B7-515 (toxicant B) and B8-806/9 (toxicant A) are two major CTTs in technical products (Khalifa et al., 1974; Turner et al., 1975) easily metabolized in biota (Alder and Vieth, 1996).

In contrast to biological samples, B7-1453 was only a minor CTT in technical mixtures. Identification of B7-1453 in technical mixtures without preseparation of the bulk of CTTs was impossible even with high-resolution gas chromatography (Krock et al., 1996). We estimated the amount of B7-1453 at <1% in Melipax. The mass chromatogram of the B7-1453 isolate from Melipax contained no significant impurities (see Figure 2a). This confirms our earlier findings on an HP-5 (95% dimethyl, 5% diphenyl polysiloxane) phase (Krock et al., 1996).

GC with CSPs was applied to establish the ER of B7-1453. Enantioseparation of CTTs was reported late compared with other chiral organochlorine pesticides such as α-HCH (König et al., 1989) and chlordane-related compounds (König et al., 1991). This is mainly due to the lack of suitable CSPs. In 1994, however, the first enantioseparations were obtained on β-BSCD (Kallenborn et al., 1994; Buser and Müller, 1994a,b). In a recent study some of us tested nine different CSPs and found that only β-BSCD enabled enantioseparation of the persistent B8-1413 (Vetter et al., 1997b). Additionally to CTTs, we enantioseparated α-HCH, oxychlordane, *cis*- and *trans*-chlordane, *o,p′*-DDT, *o,p′*-DDD, and several atropisomeric polychlorinated biphenyls (PCBs) on β-BSCD. This particular selectivity of β-BSCD has so far not been explained, but our results of the enantioseparation of atropisomeric PCBs are worthy of mention in this context. Stable atropisomeric PCBs have either a 2,3,6- or a 2,3,4,6-substituted ring (Kaiser, 1974). On permethylated β-cyclodextrin (β-PMCD) all 2,3,6-substituted PCB atropisomers were separated, but the more bulky 2,3,4,6-substituted PCBs resisted any enantioseparation on β-PMCD (Haglund and Wiberg, 1996). In a recent study, we also enantioseparated several 2,3,4,6-substituted PCB atropisomers on β-BSCD including PCB 144, which was not enantioseparated on other CSPs (Vetter et al., 1997c). So it is plausible that β-BSCD is particularly suited for the enantioseparation of larger organochlorine molecules such as CTTs.

The gas chromatographic enantioseparation of B7-1453 isolate from Melipax is shown in Figure 2b. The first eluted enantiomer was significantly more abundant. We applied gas chromatography/mass spectrometry using both electron ionization (EI) and ECNI techniques for an entire study of this phenomenon.

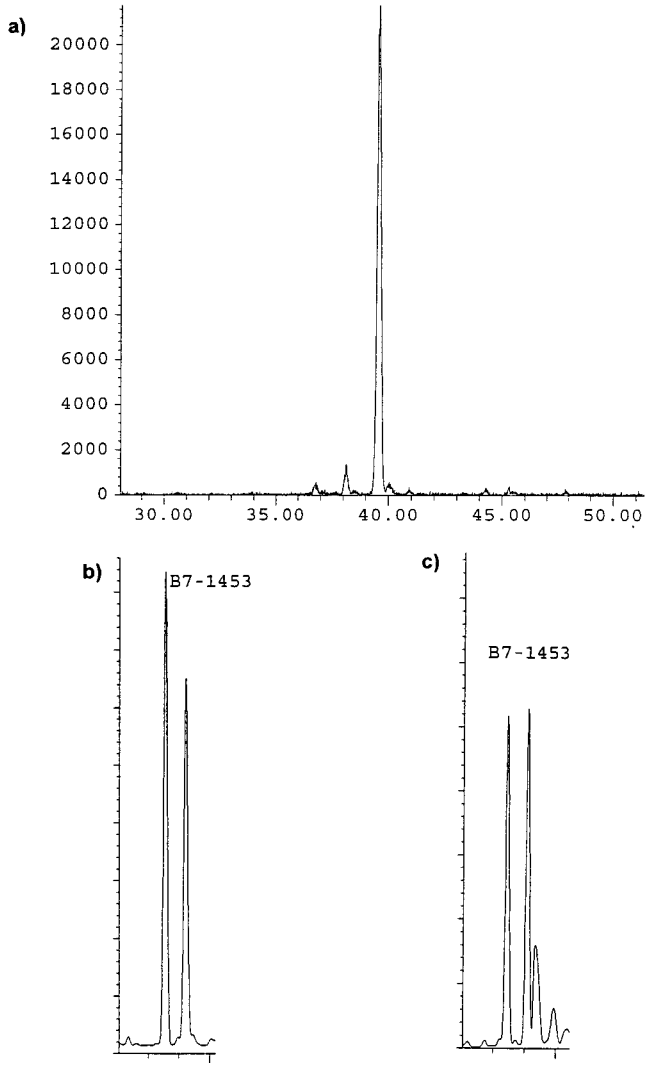

Figure 2. (a) GC/EI-MS mass fragmentograms of B7-1453 isolated from Melipax; (b) GC/ECNI-MS SIM chromatogram of the enantiomer separation of B7-1453 isolated from Melipax; (c) GC/ECNI-MS SIM chromatogram of the enantioseparation of B7-1453 isolated from cod liver oil.

Table 2. GC/ECNI-MS and GC/EI-MS Abundances of Significant Fragment Ions of B7-1453 and Calculated ERs

	1st eluted enantiomer (rel area)	2nd eluted enantiomer (rel area)	ER
GC/ECNI-MS Mass Fragment Ions			
m/z 341	10977973	8919518	1.23
m/z 343	21031684	16950866	1.24
m/z 377	475245	379792	1.25
m/z 379	967704	762116	1.27
m/z 381	875129	689651	1.27
Σ of all *m/z*			1.25
GC/EI-MS Mass Fragment Ions			
m/z 161	954674	775530	1.23
m/z 259	956418	749199	1.28
m/z 293	254518	195964	1.30
m/z 295	394896	310729	1.27
m/z 297	246791	191371	1.29
Σ of all *m/z*			1.26

Table 2 shows constant ERs for selective mass fragmentograms of both B7-1453 enantiomers after gas chromatographic enantioseparation on β-BSCD and ECNI-MS and EI-MS detection.

ECNI mass spectra of hepta-, octa-, and nonachlorobornanes are dominated by $M - Cl^-$ fragment ions, while the molecular ion and other fragment ions only show low abundance (Saleh, 1983; Swackhamer and

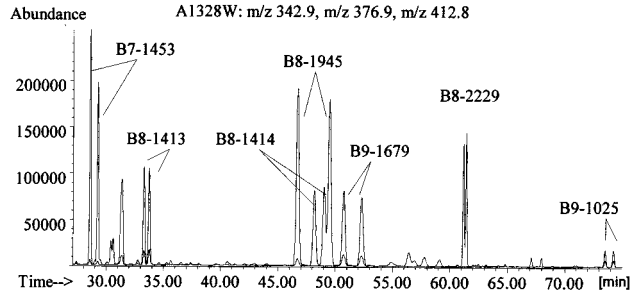

Figure 3. GC/EI-MS mass fragmentograms of selected CTTs.

Hites, 1987). In the SIM mode the two prominent isotopic peaks of the M − Cl^- ion cluster and three isotopic ions of typical octachloro mass fragments were recorded. The isotopic ratio *m/z* 341:343 of both enantiomers differed by <0.5% from the theoretical value. The isotopic ions *m/z* 377, 379, and 381 are typical masses of octachloro CTTs. However, the ratio of these ions was atypical of octachlorobornanes or octachlorocamphenes. These ions were identified as ^{13}C satellites of the molecular ion cluster of B7-1453. The relative peak abundances of these isotopic ^{13}C masses were <3% of the M − Cl mass fragments of heptachlorobornanes. From the ECNI data we deduced that the peaks at the retention time of B7-1453 enantiomers shown in Figure 2b are from plain heptachlorobornanes.

EI mass spectra of chlorinated bornanes exhibit complex fragmentation patterns (Saleh, 1983). If recorded with the same MS conditions, the EI fragmentation pattern, i.e. mass fragmentograms and the ratio of the respective signal abundances, is like the fingerprint of a CTT. In addition, the EI-MS fragmentation pattern must be identical for both enantiomers of a chiral CTT. In the SIM mode, we selected abundant mass fragments of the EI mass spectrum of B7-1453 (Krock et al., 1996). Interference of one peak of B7-1453 would have led to variations in the ER calculated for one or some of the recorded mass fragmentograms. The enantiomeric ratios calculated from the abundance of three major EI-MS fragments and two isotopic ions were constant for both enantiomers of B7-1453 (see Table 2). Using both ionization techniques, the ER of the two enantiomers was established as 1.26 ± 0.03.

GC-ECNI was used to study the enantioseparation of pure hepta-, octa-, and nonachlorobornane standards (for chemical names see Table 1) gained by photochlorination of low chlorinated camphenes (Burhenne et al., 1993; Hainzl et al., 1995; Nikiforov et al., 1995). Figure 3 shows the enantioseparation of a mixture of CTT standards including our B7-1453 isolate from Melipax. In contrast to B7-1453 the synthesized CTTs showed racemic composition. Deviations of the observed ER from the expected value of 1.0 were in general <0.05. Therefore, the experimental data of B7-1453 isolate exclude interferences and artifacts. In contrast to α-HCH and chlordane-related compounds, the enantioseparation of CTTs is very difficult. Until recently, CSPs consisting of β-BSCD only enentioseparated CTT congeners (Vetter and Schurig, 1997). However, recently we also enantioseparated several CTTs including B7-1453 on β-TBDM (Klobes et al., 1997). The enantiomeric ratio of B7-1453 on β-TBDM by GC-ECD was 1.25 and confirmed the enantiomeric ratio determined on β-BSCD with GC/MS. Therefore, the nonracemic composition of the B7-1453 isolate from Melipax is proven. Although several further chiral CTTs have been isolated from technical mixtures in the 1970s (Khalifa et al., 1974; Turner et al., 1975; Chandurkar et al., 1978; Anagnostopoulos, 1974), enantioseparation was not possible at that time. It is most likely that solutions of those isolates are no longer available (J. E. Casida, University of California, Berkeley, personal communication, 1990), and thus far, our isolate B7-1453 is the only pure compound which confirms the observation of Buser and Müller (1994b).

We also determined the enantiomeric ratio of B7-1453 in a Baltic cod liver extract. Enantioselective analysis of B7-1453 in cod livers was, however, impossible without preseparation of the bulk of organochlorine compounds due to interferences even in ECNI-SIM chromatograms. Therefore, we established the ER of B7-1453 after isolation of the compound in the same way as from Melipax. This cod liver isolate of B7-1453 showed an ER of 1.0 (see Figure 2c).

At present it is not possible to decide whether the nonracemic composition of B7-1453 observed in the product Melipax is also true for other technical toxaphene products or if this is true for other CTTs in any technical mixture. Assuming that B7-1453 was present in nonracemic composition in all technical products, the ER of 1.0 in the cod liver would have been the result of a faster degradation of the first eluted enantiomer, which finally led by accident to an ER of 1.0.

However, the hypothesis may be drawn that B7-1453 in cod livers originated from a technical product that contained B7-1453 in racemic composition. In such a case there would have been no enantioselective degradation of B7-1453 in cod. If some technical mixtures contain B7-1453 in racemic and some in nonracemic composition, enantioselective analysis may be used to find the source of contamination with CTTs in the environment.

Under existing conditions, interpretation of the ERs of CTTs in biological samples must be seen in a new light. The interpretation of ERs of CTTs in samples cannot simply be done by comparison of the ER with synthetic (racemic) CTT single standards. Furthermore, the initial steps of the toxaphene synthesis (Kwart, 1953; Kwart and Null, 1956; Jennings and Herschbach, 1965; Richey et al., 1965) including the stereochemical aspects of the synthesis should be reconsidered.

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