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Isomer-Selective and Enantiomerselective Determination of DDT and Related Compounds Using Chiral High-Resolution Gas Chromatography/ Mass Spectrometry and Chiral High-Performance Liquid Chromatography

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The composition of technical DDT was investigated using achiral and chiral high-resolution gas chromatography (HRGC) and electron-ionization mass spectrometry (EI-MS). 2,4′-DDT and 2,4′-DDD, two important components of technical DDT, were enantiomerically resolved by chiral HRGC with silylated β-cyclodextrin and by chiral high-performance chromatography (HPLC) with permethylated γ-cyclodextrin as chiral selectors. The (+)- and (−)-enantiomers were assigned by chiral HPLC using chiroptical measurements. Enantiopure isolates were then used to identify these enantiomers in chiral HRGC analyses. Previous data indicated (+)- and (−)-2,4′-DDT to have *S*- and *R*-configuration, respectively, but the absolute configurations for (+)- and (−)-2,4′-DDD were hitherto unknown. They were now assigned via the reductive dechlorination of the individual 2,4′-DDT enantiomers which proceeded stereoselectively to the corresponding 2,4′-DDD enantiomers. The results showed (+)- and (−)-2,4′-DDD to have *R*- and *S*-configuration, respectively. The enantiomers of 2,4′-DDD thus have reversed signs of rotation for polarized light compared to the enantiomers of 2,4′-DDT with the same configuration. The enantiomer resolution of several additional chiral compounds in technical DDT is reported; enantiomeric ratios of ≈1.0 indicated all chiral compounds to be present as racemates in the technical and in the synthetic reference materials. We report the first enantioselective determinations of technical DDT; the methods presented should also be applicable to the analysis of environmental and biological samples.

DDT was one of the most important pesticides used in agriculture, forestry, and public health, but it was also probably one of the most controversial. DDT was first introduced in the 1940s, and production and usage increased to a maximum around 1960 (U.S. production in 1963, 80 500 tons).[1] The cumulative world production so far has exceeded 1 million tons. DDT was obviously valuable in combatting vector-transmitted deseases such as malaria with spectacular success, with millions of human deaths averted.[2] DDT is rather nontoxic to humans, but it is highly accumulating and persistent in the environment.[3,4] Following the discovery of its widespread occurrence, it was eventually banned in the United States in 1972, and since then in most industrialized countries. Nevertheless, DDT continues to be an important vector-control product in developing countries.[5]

In the technical production of DDT, chlorobenzene is condensed with 1,1,1-trichloroacetaldehyde (chloral) in the presence of concentrated sulfuric acid or chlorosulfonic acid to yield ≈70% 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (4,4′-DDT), up to 25% 1,1,1-trichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethane (2,4′-DDT), and other compounds.[4,6,7] 4,4′-DDT is the only isomer with insecticidal properties.

2,4′-DDT, the second major component in technical DDT, is estrogenically active in both avian and mammalian systems[8] and presumably is one of the compounds responsible for the eggshell thinning in predatory avian species. It was suggested to decrease the content of this poorly insecticidal isomer in technical DDT, thereby increasing the efficacy of the latter and reducing the amount of a known estrogen being deployed in the biosphere.[9] 1,1-Dichloro-2,2-bis(4-chlorophenyl)ethane (4,4′-DDD, also known as 4,4′-TDE) is an additional constituent of technical DDT but was also a pesticide itself.[10] The 2,4′-isomer has medicinal purposes,[11] and it is also an enzyme-inducing compound.[8]

The biodegradation of DDT is different under anaerobic and aerobic conditions. It has been extensively reviewed. Briefly, 1,1-dichloro-2,2-bis(4-chlorophenyl)ethene (4,4′-DDE), the principal stored DDT metabolite, and 4,4′-DDD are formed in anaerobic

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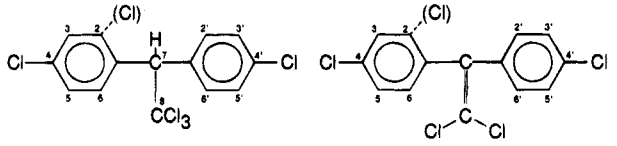
Chart 1. Structural Formulas of DDT Compounds Mentioned in the Text[a]



[a] Left: 4,4′- (and 2,4′-) DDT. For DDD, DDMS, and DDA, CCl_3 would be replaced by $CHCl_2$, CH_2Cl, and COOH, respectively. Right: 4,4′- (and 2,4′-) DDE.

reactions.[12] Aerobic reactions yield mainly bis(4-chlorophenyl)-acetic acid (4,4′-DDA), the principal urinary excretion product of DDT in mammals, and dichlorobenzophenone.[13-15]

DDT and its metabolites are still among the most prevalent environmental contaminants. The 2,4′-isomers of DDT and DDD are chiral and thus exist in two enantiomeric forms. As pointed out for other chiral contaminants, biotic processes (uptake, metabolism, excretion) may be different for enantiomers, whereas abiotic processes (chemical, photochemical, distribution, transport) will be the same.[16] Since 2,4′-DDT is a major chiral contaminant of technical DDT, changes in its enantiomeric ratios (ERs) (and those of other chiral contaminants) would point to biological degradation rather than degradation via abiotic pathways. So far, the environmental fate of the two enantiomers of 2,4′-DDT has not been fully delineated.

Detailed analyses of technical DDT and the enantioselective determination of chiral components are required to investigate the biological and environmental fate of this important pesticide. In this study, we report the first enantioselective analyses of technical DDT. We describe the enantiomeric resolution of 2,4′-DDT and 2,4′-DDD using chiral high-performance chromatography (HPLC) and of these and other chiral DDT components using chiral high-resolution gas chromatography (HRGC). Furthermore, the (+)- and (−)-enantiomers of 1,1-dichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethane (2,4′-DDD) and 2,4′-DDT were identified via chiroptical measurements and the absolute configurations of the 2,4′-DDD enantiomers assigned by chemical analogy to those known for the 2,4′-DDT enantiomers.

EXPERIMENTAL SECTION

Materials and Reference Compounds. Synthetic racemic (±)-2,4′-DDT, (±)-2,4′-DDD, 4,4′-DDT, 4,4′-DDD, 4,4′-DDE, and 1,1-dichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethene (2,4′-DDE) were from Promochem (Wesel, Germany) (for structures, see Chart 1). Other chemicals (dimethyl sulfoxide, $NaBH_4$) were from Fluka (Buchs, Switzerland). Two samples of technical DDT were from Promochem and Maag (Dielsdorf, Switzerland), respectively. The latter sample was obtained in the 1950s and has been archived in Wädenswil, and it is now used for comparative analyses. Solutions of the reference compounds (1−10 ng/μL) and the technical products (100 ng/μL) in *n*-hexane were prepared and used in the HRGC analyses. Solutions at 0.1−1% in methanol were used for analysis by HPLC.

HRGC Mass Spectrometric Analyses. A VG Tribrid double focusing magnetic sector mass spectrometry (MS) instrument was used. The ion source was operated in the electron-impact mode (EI, 70 eV, 180 °C). Full-scan mass spectra (m/z 35−435; 1.16 s/scan; resolution $M/\Delta M = 500$) were recorded. Analyses were also carried out with selected-ion monitoring (SIM) for optimum resolution (0.5 s/scan), e.g., by using the ions at m/z 201, 235, 237, 246, 248, 316, 318, and 354 to detect the various compounds. A lock-mass of m/z 207.033 from the silicone bleed of the HRGC columns was used in SIM. Concentrations were determined from SIM analyses in comparison to those of known quantities of the reference compounds (where available) or using average response values. Enantiomeric compositions (ER values) were determined from peak area ratios in SIM analyses.[17] Retention indexes (RI) were calculated relative to the *n*-alkanes (C_{14}−C_{26}; RI 1400−2600) co-injected with the samples; linear interpolation was used in the temperature-programmed runs.

Achiral HRGC was carried out with a 25-m SE54 or a 60-m OV1701 fused silica (0.25 mm i.d.) HRGC column. Chiral HRGC was carried out with a 20-m or a 28-m OV1701/BS-β-CD fused silica column (0.25 mm i.d.) (BS-β-CD = *tert*-butyldimethylsilyl-β-cyclodextrin; 30% w/w) (see ref 18). These columns were temperature programmed as follows: 50 °C, 2 min isothermal, 20 °C/min to 160 °C, 3 °C/min to 240 °C. On-column injection (1−2 μL) at 50 °C was used to prevent thermal decomposition of DDT to DDE in a heated injector. Additionally, a 16-m OV1701/TB-β-DM column (TB-β-DM = 6-*tert*-butyldimethylsilyl-2,3-dimethyl-β-cyclodextrin; 30% w/w) was also used. In some analyses, these columns were temperature programmed as slow as 0.5 °C/min and with lower intermediate temperatures (80 °C) for optimum resolution. This resulted in retention times (t_R) as long as 3−4 h.

Chiral High-Performance Liquid Chromatography (HPLC) and Fractionation of DDT Compounds. Chiral reverse-phase HPLC was carried out with permethylated α-, β-, or γ-cyclodextrin (PM-α-CD, PM-β-CD, or PM-γ-CD) columns (all 200 × 4 mm, 5-μm particle size; Macherey-Nagel, Düren, Germany) operated with 0.8 mL/min 80% methanol/20% water and 0.5% triethylamine acetate (pH = 4.5) at room temperature. A Spectra-Physics (San Jose, CA) Model 8800 pump was used. Samples (5−20 μL of 0.1−1% solutions in methanol) were injected via a Rheodyne (Cotati, CA) Model 7125 injector and a 20-μL loop. Analyte detection was either by a Knauer (Berlin, Germany) variable wavelength UV detector set at 250 nm or with an IBZ (Hannover, FRG) polarimetric detector with a 300-μL cell. Optical rotation (OR) was monitored using both polychromatic and monochromatic light (interference filter, 589 ± 15 nm) as in a previous study; no difference in the sign of rotation for polarized light was observed in the two modes of operation. The 1% solutions were used to improve signal-to-noise ratios in these analyses.

Enantiopure fractions of 2,4′-DDT and 2,4′-DDD were collected from the PM-γ-CD column at the outlet of the UV detector while observing the UV absorbance, taking a time lag of ≈16 s between detection and elution into account. Approximately 100 μg of a racemate was injected. The analytes were recovered from the eluates (≈1 mL) by extraction with *n*-hexane (three portions, 1 mL each). After the extracts were dried over anhydrous Na_2SO_4, they were directly analyzed using chiral HRGC/MS.

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Reductive Dechlorination of Racemic and Enantiopure 2,4′-DDT. (±)-2,4′-DDT (32 mg) was dissolved in 1 mL of DMSO under slight warming. After cooling, this solution was added to 1 mL of DMSO containing 30 mg of $NaBH_4$ in a vial (see ref 19). After 1 h at room temperature, a 200-μL sample was removed, and the mixture was further reacted at 60 °C. After 1 and 2 h at this temperature, further samples were removed. The mixture was then left overnight at room temperature before a final sample was taken. All samples were immediately mixed with an equal volume of distilled water, carefully acidified with 0.5 M HCl to destroy excess $NaBH_4$ (*caution*: gas evolution), and then extracted with 1−2 mL of *n*-hexane. The extracts were washed 3−4 times with small amounts of distilled water until neutral, dried over anhydrous Na_2SO_4, and analyzed after suitable dilution with *n*-hexane.

The reactions were then carried out with (+)- and (−)-2,4′-DDT (≈50 μg each) isolated from the chiral HPLC (PM-γ-CD) system. The individual enantiomers were extracted from the HPLC eluates with *n*-hexane and then passed with 4 mL of *n*-hexane/methylene chloride (1:1) through small columns of silica (0.5 g of silica gel 60, from Merck, Darmstadt, Germany, in 5-mm-i.d. Pasteur pipettes) topped with anhydrous Na_2SO_4. The eluates were carefully brought to dryness, redissolved in 100 μL of DMSO, and combined with an equal volume of $NaBH_4$ solution. The mixtures were allowed to react for 14−40 h at room temperature. The extraction and cleanup were continued as for (±)-2,4′-DDT, and the formation of dechlorinated products was followed using chiral HRGC analysis.

RESULTS AND DISCUSSION

Achiral and Chiral Components in Technical DDT and Structural Considerations. The reaction of chlorobenzene with chloral leads to 4,4′-DDT as the major product and a number of additional components in a Bayer condensation.[6,7] 4,4′-DDT is achiral (prochiral; see Chart 1), but the two chlorophenyl groups are enantiotopic and thus may behave differently in a chiral environment. Metabolic action thus may affect the two groups differently, and the two enantiomers of the chiral metabolites need not be formed in equal amounts. This may be the case in situations such as ring hydroxylation leading to chiral hydroxy-DDT metabolites.[20,21]

There are six possible DDT isomers with bis(chlorophenyl) substitution: 2,2′-, 2,3′-, 2,4′-, 3,3′-, 3,4′-, and 4,4′-DDT. 2,4′-DDT, the second most abundant component in technical DDT, has an asymmetrically substituted C atom (C-7, see Chart 1) and is chiral; 2,3′- and 3,4′-DDT are also chiral, but we do not know to what level they are present in technical DDT. X-ray crystallographic analysis has revealed that (−)-2,4′-DDT has *R*-configuration,[22] and in Chart 2 we show the exact structures of both 2,4′-DDT enantiomers. Further components in technical DDT are 4,4′-DDD and 4,4′-DDE; both compounds are also metabolic products of 4,4′-DDT.[12−15] 4,4′-DDD is achiral. Semiempirical MNDO calculations (courtesy F. Müller-Plathe, Zurich) indicated energy barriers of 15−30 kJ M^{-1} for the hindered rotation about the C-7 to C-8 bond. These low-energy barriers between the achiral anti (trans) and the chiral gauche conformations are not expected to lead to stable stereoisomers of 4,4′-DDD at ambient temperatures. 2,4′-DDD is chiral; however, the two enantiomers have so far not been resolved, and their absolute configurations are hitherto unknown.

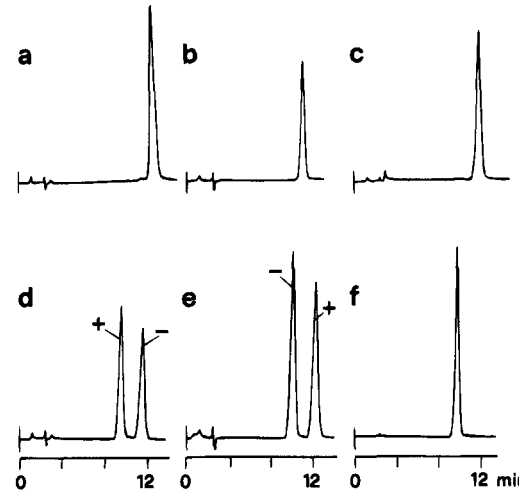
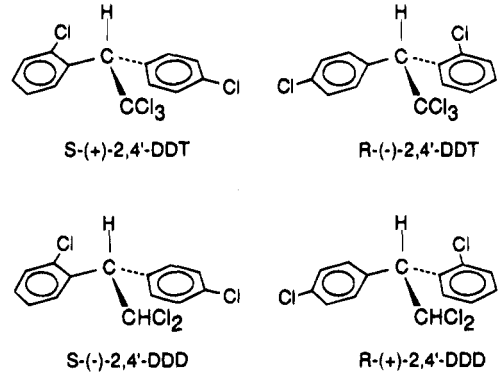


Figure 1. HPLC chromatograms (chiral immobilized PM-γ-CD column; UV detection) of (a) 4,4′-DDT, (b) 4,4′-DDD, (c) 4,4′-DDE, (d) (±)-2,4′-DDT, (e) (±)-2,4′-DDD, and (f) 2,4′-DDE. Note the enantiomer resolution of 2,4′-DDT and 2,4′-DDD (R = 4.4 and 4.7, respectively). The assignment of the (+)- and (−)-enantiomers is based on chiroptical measurements at 589 ± 15 nm.

Chart 2. Absolute Configurations of the (+)- and (−)-Enantiomers of 2,4-DDT and 2,4′-DDD



Separation and Isolation of 2,4′-DDD and 2,4′-DDT Enantiomers Using Chiral HPLC. The DDT reference compounds were analyzed on three chiral HPLC systems. The systems varied in the chiral selectors (PM-α-CD, PM-β-CD, and PM-γ-CD) but used the same mobile phase. Varied isomer and enantiomer resolution was observed. Whereas no enantiomer resolution was observed for (±)-2,4′-DDD and (±)-2,4′-DDT on the PM-β-CD column and only marginal resolution ($R \approx 0.1$) on PM-α-CD, both compounds were enantiomerically resolved ($R \approx$ 4−5) when the PM-γ-CD column was used (see Figure 1 and Table 1). Chiroptical detection indicated dextrorotation (D) for the earlier- and levorotation (L) for the later-eluted enantiomers of 2,4′-DDDT, whereas this sequence was reversed for the enantiomers of 2,4′-DDD (see Figure 1d,e).

Achiral and Chiral HRGC of DDT Reference Compounds. The 2,4′- and 4,4′-isomers of DDT, DDD, and DDE each showed a single peak when analyzed using achiral HRGC (25-m SE54 and 60-m OV1701; data not shown). The 2,4′-isomers eluted prior to the 4,4′-isomers, and the elution order was DDE < DDD < DDT.

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Table 1. Retention Times (Retention Indexes, RI), Enantiomer Resolution (*R* Values), Enantiomer Elution Sequence, and Relative Responses (EI-SIM) of DDT Reference Compounds Using Chiral HPLC and Chiral HRGC/MS

	chiral HPLC[a,b] (PM-γ-CD)			chiral HRGC[b,d] (OV1701/BS-β-CD)			EI-SIM[e]	
compound	t_R (min)	*R*	OR[c]	RI	*R*	ER	rel respns	*m/z*
2,4′-DDE	9.8			2219			1.33 ± 0.01	246
4,4′-DDE	12.0			2309			1.00	246
R-(+)-2,4′-DDD[f]	12.3 (2)	4.7	D	2360 (1)	1.4	1.00 ± 0.02	1.53 ± 0.01	235
S-(−)-2,4′-DDD[f]	10.2 (1)		L	2366 (2)			1.53 ± 0.01	235
R-(−)-2,4′-DDT[g]	11.7 (2)	4.4	L	2475 (1)	2.8	1.00 ± 0.02	1.16 ± 0.01	235
S-(+)-2,4′-DDT[g]	9.6 (1)		D	2487 (2)			1.16 ± 0.01	235
4,4′-DDD	11.0			2517			1.52 ± 0.01	235
4,4′-DDT	13.0			2614			1.00	235

[a] Chiral silica/PM-γ-CD HPLC column. No enantiomer resolution with PM-β-CD, and only marginal resolution with PM-α-CD, see text. [b] Enantiomer elution sequence in parentheses: 1, first-eluted; 2, second-eluted enantiomer. [c] Optical rotation measured. [d] Chiral 28-m OV1701/BS-β-CD HRGC column. Temperature programming rate, 3 °C/min; intermediate temperature 160 °C. RI values relative to *n*-alkanes. [e] Responses relative to 4,4′-DDT (*m/z* 235; relative response, 1.00) and 4,4′-DDE (*m/z* 246; relative response, 1.00; relative response to 4,4′-DDT is 1.19 ± 0.02), respectively; average and range reported. [f] Enantiomer assignment from chiroptical data and ref 24. [g] Enantiomer assignment from this study, see text.

Different SIM chromatograms (e.g., at *m/z* 352, 354, and 356 for DDTs, and at *m/z* 318, 320, and 322 for DDDs) indicated signal coincidence and thus no resolution of isotopomers (isomers with different ^{35}Cl and ^{37}Cl contents), even at very slow temperature programming rates (0.5 °C/min). Partial resolution of isotopomers is commonly known for all-^{12}C and all-^{13}C isotopomers and deuterated analogues of other environmental contaminants. Apparently, the column efficiencies required for the resolution of ^{35}Cl and ^{37}Cl isotopomers are higher than those available from the columns used (200 000 theoretical plates for the 60-m OV1701 column).

However, when these reference compounds were analyzed using the chiral OV1701/BS-β-CD HRGC column, 2,4′-DDD and 2,4′-DDT were enantiomerically resolved (*R* = 1.4 and 2.8, respectively, see Figure 2), whereas the other compounds still eluted as single peaks. Despite the fact that 2,4′-DDD is eluting at a lower column temperature, its resolution is less than that of 2,4′-DDT eluting at a higher temperature. Both compounds showed ERs of ≈1.0, thus indicating racemic mixtures. In Table 1, we list the chromatographic data for the DDT reference compounds. Under similar conditions, a 16-m OV1701/TB-β-DM column enantiomerically resolved 2,4′-DDT but not 2,4′-DDD (data not shown).

Depending on the actual column temperature program, the order of elution of 4,4′-DDD and 2,4′-DDT changed. 4,4′-DDD was eluted either before or after both of the 2,4′-DDT enantiomers or right on top of one or the other (see Figure 2). It is clear that the exact elution orders need to be determined for a particular column and conditions, since there is obviously the possibility for interference. Enantiomer resolution generally increased with slower temperature programming (lower elution temperatures), but the enantiomer elution sequences were not changed. A reversal of elution sequences was discussed in previous studies[23,24] and was actually observed in some cases. The chromatograms in Figure 2 indicated no increase in peak width for 4,4′-DDD (compared to that of 4,4′-DDT) and thus gave no indication for the presence of additional conformational isomers. Increased peak widths are often observed in chiral HRGC for incompletely resolved enantiomers.

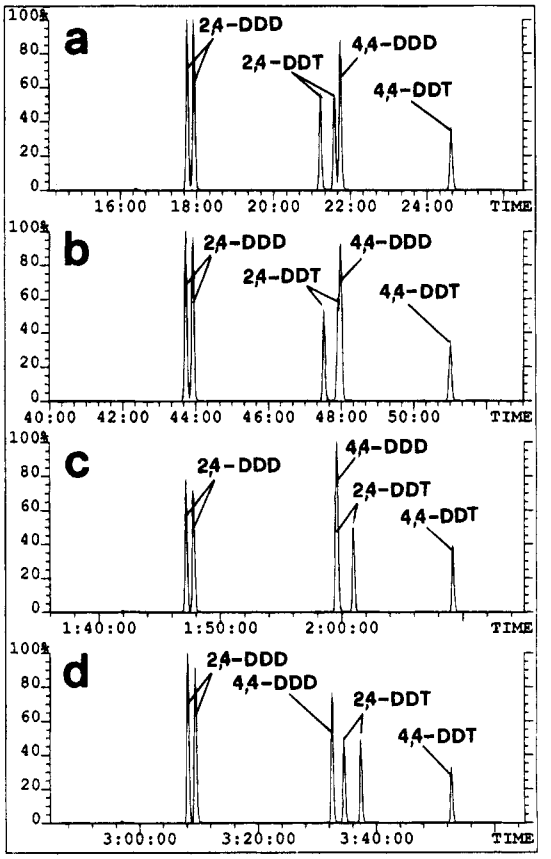

Figure 2. EI-SIM chromatograms (*m/z* 235) showing elution of 4,4′-DDD, 4,4′-DDT, and the enantiomers of 2,4′-DDD and 2,4′-DDT, analyzed using the chiral 20-m OV1701/BS-β-CD HRGC column: (a) intermediate temperature 160 °C, 3 °C/min; (b) intermediate temperature 80 °C, 3 °C/min; (c) intermediate temperature 80 °C, 1 °C/min; and (d) intermediate temperature 80 °C, 0.5 °C/min; for other conditions, see text. Note the changing elution order of 4,4′-DDD with respect to the 2,4′-DDT enantiomers. Enantiomer resolutions (*R*) for 2,4′-DDD and 2,4′-DDT were 1.39 and 2.78 (a), 1.45 and 2.81 (b), 1.69 and 4.17 (c), and 1.76 and 4.06 (d), respectively. Enantiomer elution sequence, see text. Time scale, h:min:s.

Enantiomer Assignment Using Chiral HRGC. HPLC isolates from the first- and second-eluted enantiomers and reanalysis by chiral HRGC showed the isolates to be virtually enantiopure (>99%), as shown by the EI-SIM chromatograms in Figure 3. The isolates allowed the unambiguous assignment of

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Table 2. Components and Composition of Technical DDT

compound	RI[a]	EI-MS data[b] $M^{\bullet+}$	EI-MS data[b] $(M-R)^+$	wt % of total mixture[c] Maag	wt % of total mixture[c] Promochem
4,4′-DDT	2614	352 (5)	235 (2)	79.0 ± 0.2	79.0 ± 1.0
4,4′-DDD	2517	318 (4)	235 (2)	2.1 ± 0.2	1.2 ± 0.2
X6 (2)[d]	2496, 2507	352 (5)	235 (2)	0.8 ± 0.05	0.7 ± 0.05
2,4′-DDT (2)[d]	2475, 2487	352 (5)	235 (2)	15.6 ± 0.3	17.5 ± 0.5
X5	2467	352 (5)	235 (2)	0.7 ± 0.05	0.75 ± 0.05
X4 (2)[d]	2391, 2406	318 (4)	201 (1)	0.6 ± 0.05	<0.03
X3 (2)[d]	2373, 2376	352 (5)	235 (2)	0.2 ± 0.05	0.2 ± 0.03
2,4′-DDD (2)[d]	2360, 2366	318 (4)	235 (2)	0.6 ± 0.05	0.35 ± 0.05
4,4′-DDE	2309	316 (4)	246 (2)[e]	0.2 ± 0.05	0.2 ± 0.05
X2	2302	unknown	201 (1)	0.1 ± 0.05	<0.02
X1 (2)[d]	2267, 2276	284 (3)	201 (1)	0.2 ± 0.05	<0.03

[a] Retention indexes on OV1701/BS-β-CD; RIs of both enantiomers listed, if applicable. [b] MS data, *m/z* values of $M^{\bullet+}$ and $(M-R)^+$, respectively; number of Cl's in parentheses. [c] EI-SIM data relative to reference compounds, if available; average and range of two replicate determinations listed. [d] Summed concentrations of both enantiomers. [e] $(M-Cl_2)^{\bullet+}$ ion.

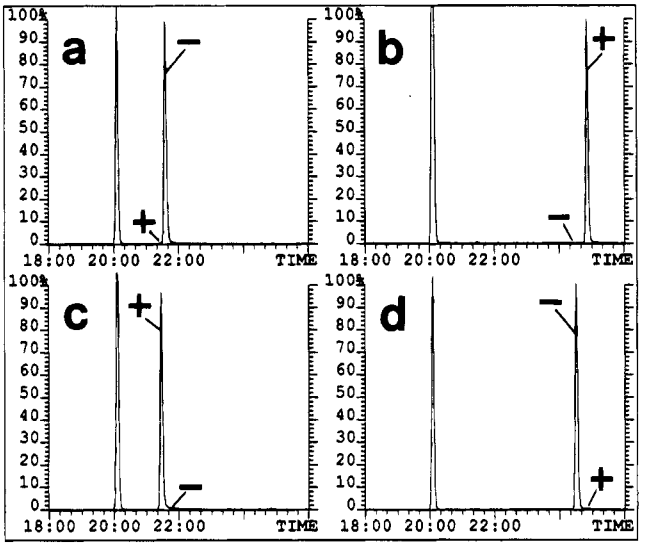

Figure 3. EI-SIM chromatograms (*m/z* 248) showing elution of the (+)- and (−)-enantiomers of 2,4′-DDD and 2,4′-DDT isolated from the chiral PM-γ-CD HPLC column and analyzed using the chiral 20-m OV1701/BS-β-CD HRGC column: (a) HPLC fraction 1 with (−)-2,4′-DDD, (b) HPLC fraction 1 with (+)-2,4′-DDT, (c) HPLC fraction 2 with (+)-2,4′-DDD, and (d) HPLC fraction 2 with (−)-2,4′-DDT. 4,4-DDE added as an internal standard to each sample (t_R = 20.1 min). Note that enantiomeric purities are >99%. Time scale, min:s.

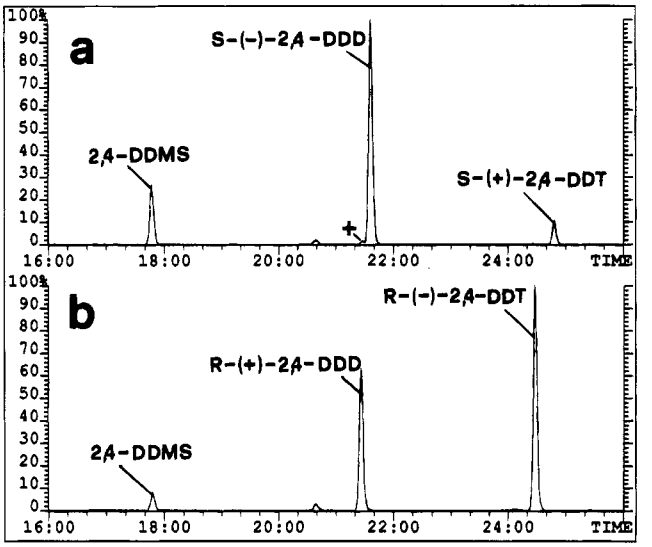

Figure 4. EI-SIM chromatograms (*m/z* 235) of $NaBH_4$ reaction products of the individual 2,4′-DDT enantiomers, analyzed using the 28-m OV1701/BS-β-CD HRGC column. (a) Reaction of (+)-2,4′-DDT yielding (−)-2,4′-DDD and 2,4′-DDMS. (b) Reaction of (−)-2,4′-DDT yielding (+)-2,4′-DDD and 2,4′-DDMS. Note the presence of unreacted 2,4′-DDT enantiomers in both chromatograms. Time scale, min:s.

these enantiomers using chiral HRGC. For 2,4′-DDT (absolute configurations known), the *R*-(−)-enantiomer was earlier and the *S*-(+)-enantiomer later eluted. For 2,4′-DDD, the elution order was reversed, with the (+)-enantiomer earlier and the (−)-enantiomer later eluted (see Table 1). The chromatograms indicated little if any enantiomerization during HRGC analysis.

Absolute Configuration of the 2,4′-DDD Enantiomers. The absolute configurations of the 2,4′-DDD enantiomers were hitherto unknown, but we were able to deduce these configurations via chemical analogy to the 2,4′-DDT enantiomers. In an initial experiment with (±)-2,4′-DDT, the reduction with $NaBH_4$ caused reductive dechlorination of the CCl_3 group, leading to 2,4′-DDD ($CHCl_2$ group) and 1-chloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethane (2,4′-DDMS; CH_2Cl group), leaving the chlorophenyl groups unaffected. The reaction was then repeated with isolates of the individual 2,4′-DDT enantiomers and shown to proceed stereoselectively. Reduction of the later-eluted *S*-(+)-2,4′-DDT led to the later-eluted (−)-enantiomer of 2,4′-DDD (see Figure 4a), which therefore has *S*-configuration. Correspondingly, the earlier-eluted *R*-(−)-2,4′-DDT led to the earlier-eluted (+)-2,4′-DDD, which therefore has *R*-configuration (see Figure 4b). Both 2,4′-DDT enantiomers also gave 2,4′-DDMS [$M^{\bullet+}$, *m/z* 284; $(M-R)^+$, *m/z* 235; RI 2217], but the two enantiomers were not resolved on OV1701/BS-β-CD. They were, however, later resolved on an OV1701/TB-β-DM column (data not shown). The above reactions established that the enantiomer elution sequences for 2,4′-DDT as well as for 2,4′-DDD were the same (*R*- prior to the *S*-) when the OV1701/BS-β-CD HRGC column was used (see Table 2).

EI Mass Spectra of DDT Compounds. The EI mass spectra of the DDT compounds have been described.[25] Briefly, all the 2,2-diphenylethane derivatives show weak to medium intensity molecular ions [$M^{\bullet+}$, *m/z* 284 (Cl_3), 318 (Cl_4), and 352 (Cl_5) for DDMS, DDD, and DDT, respectively] and higher mass fragment

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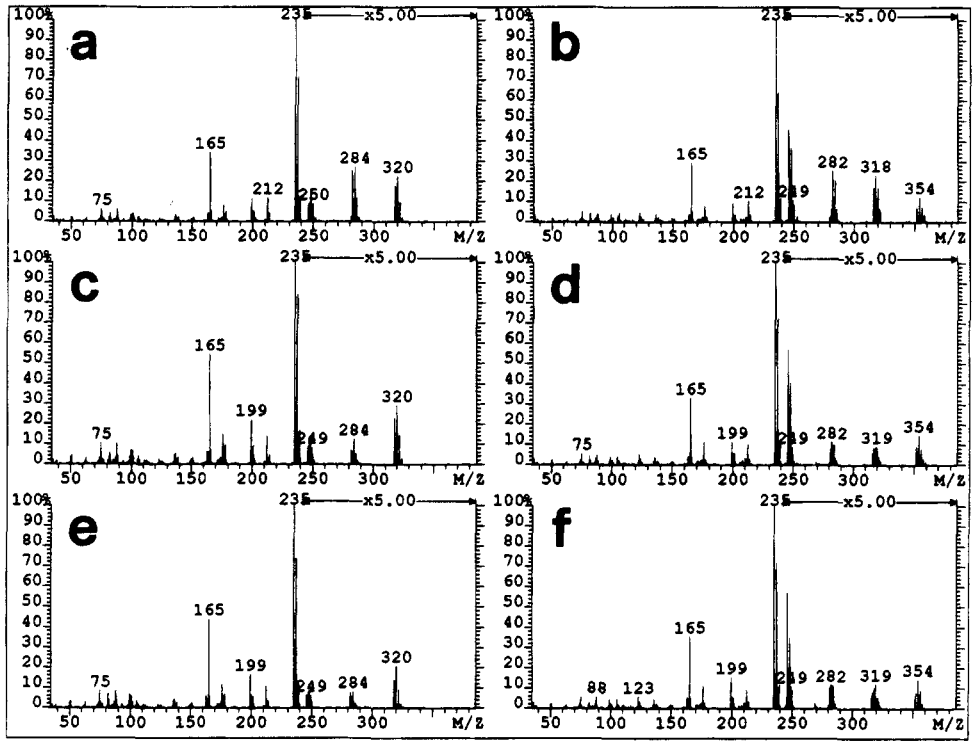

Figure 5. EI mass spectra of the 4,4′-isomers of DDD and DDT and the two enantiomers of the 2,4′-isomers, analyzed using the chiral 20-m OV1701/BS-β-CD HRGC column: (a) 4,4′-DDD ($M^{\bullet+}$, *m/z* 318, Cl_4), (b) 4,4′-DDT ($M^{\bullet+}$, *m/z* 352, Cl_5), (c) (+)-2,4′-DDD, (d) (−)-2,4′-DDT, (e) (−)-2,4′-DDD, and (f) (+)-2,4′-DDT. Note the mass spectral differences between isomers but the virtually identical mass spectra of the enantiomers of 2,4′-DDD and -DDT. Note also the vertical expansion (5×) in mass range *m/z* 240−385.

ions, with the typical clustering due to the ^{35}Cl and ^{37}Cl isotopes. The mass spectra are usually dominated by abundant $(M - R)^+$ type ions ($R = CCl_3$, $CHCl_2$, CH_2Cl, respectively) at *m/z* 235 for 2,2-bis(chlorophenyl) compounds such as DDT and DDD. The corresponding ion for the 2-phenyl-2-chlorophenyl analogues is at *m/z* 201. Further fragmentations are via loss of Cl, HCl, Cl_2, and consecutive losses from the $M^{\bullet+}$ and the $(M - R)^+$ ions. The mass spectra of the two isomers of DDE showed intense $M^{\bullet+}$ and major fragment ions $(M - Cl_2)^{\bullet+}$ at *m/z* 246.

The mass spectra of positional isomers showed only minor and quantitative differences, as shown in Figure 5 for the 2,4′- and 4,4′-isomers of DDD and DDT ($M^{\bullet+}$ − HCl ions more intense in the 2,4′-isomers). However, enantiomers have the same chemical and physical properties, and hence the mass spectra are truly identical. As also shown in Figure 5, the mass spectra of the (+)- and (−)-enantiomers of 2,4′-DDD and of 2,4′-DDT respectively are virtually identical.

The SIM responses of isomers varied to some degree (see relative response values in Table 1). However, the relative responses of enantiomers are identical, and therefore ERs are independent of detection mode. ERs can thus be determined without the availability of the individual enantiomers, as long as the enantiomers are sufficiently resolved and detection is selective (absence of interfering compounds). Enantiomer assignment, however, must be derived from (chiral) chromatographic data.

Analysis of Technical DDT Using Chiral HRGC and MS. Achiral HRGC analysis of the technical products showed the presence of 4,4′-DDT and 2,4′-DDT as well as a number of minor components including 4,4′- and 2,4′-DDD, particularly in the Maag sample from the 1950s. The analyses were then repeated using a chiral 28-m OV1701/BS-β-CD HRGC column. In Figure 6, we show combined EI-SIM chromatograms (*m/z* 201 and 235) for the two samples. These analyses confirmed the data from the achiral analyses and showed (beside 2,4′-DDT and 2,4′-DDD) four additional minor components (X1, X3, X4, and X6) enantiomerically resolved (see also Table 2). The ERs for all (chiral) compounds were ≈1.0 and indicated their presence as racemates in both samples. The compounds were tentatively identified by EI-MS as a 1,1-dichloro-2-phenyl-2-(chlorophenyl)ethane [X1, chiral; $M^{\bullet+}$, *m/z* 284; $(M - R)^+$, *m/z* 201], an unknown component [X2, achiral or enantiomerically unresolved; $M^{\bullet+}$, unknown; $(M - R)^+$, *m/z* 201], a minor DDT isomer [X3, chiral; $M^{\bullet+}$, *m/z* 352; $(M - R)^+$, *m/z* 235; 2,2-bis(chlorophenyl) or 2-phenyl-2-dichlorophenyl substitution], a 1,1,1-trichloro-2-phenyl-2-(chlorophenyl)-ethane [X4, chiral; $M^{\bullet+}$, *m/z* 318; $(M - R)^+$, *m/z* 201], a minor DDT isomer [X5, achiral or enantiomerically unresolved; $M^{\bullet+}$ *m/z* 352; $(M - R)^+$, *m/z* 235], and another minor DDT isomer [X6, chiral; $M^{\bullet+}$, *m/z* 352; $(M - R)^+$, *m/z* 235]. The actual isomerism of these compounds remained unknown. The 28-m OV1701/BS-β-CD column showed better resolution between *S*-(−)-2,4′-DDT and 4,4′-DDD than the 20-m column earlier used (compare to Figure 2). The analyses indicated the same content (79%) of 4,4′-DDT in the two samples but a larger number of contaminants in the Maag sample (see Table 2). In particular, the compounds X1, X2, and X4 with an $(M - R)^+$ ion of *m/z* 201 were missing in the other sample.

CONCLUSIONS

Chiral HRGC/EI-MS was used to analyze two samples of technical DDT. The samples contained 4,4′-DDT (79%), 2,4′-DDT (15−18%), and several minor components. The chiral OV1701/BS-β-CD HRGC columns enantiomerically resolved 2,4′-DDT, 2,4′-DDD, and four additional chiral DDT components. All chiral components showed ERs of ≈1.0, indicating the presence of

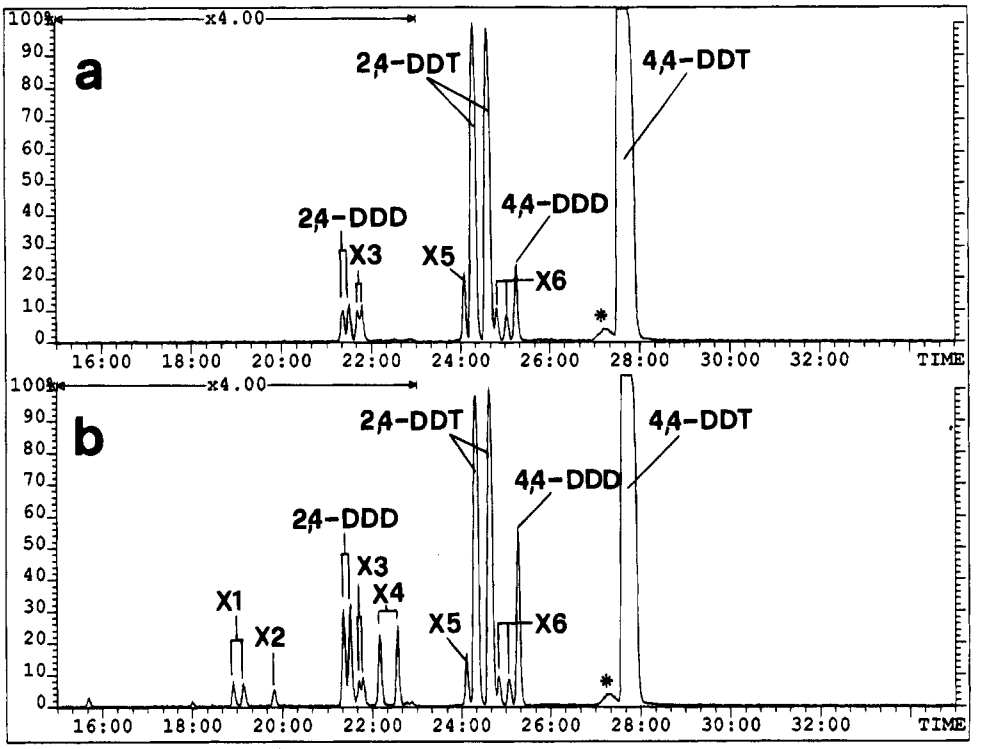

Figure 6. EI-SIM chromatograms (*m/z* 201, 235) showing elution of DDT-related compounds in (a) technical DDT, Promochem, and (b) technical DDT, Maag, analyzed using the 28-m OV1701/BS-β-CD HRGC column. Abbreviations, see text and Table 2. Artifact signals marked by asterisks. Time scale, min:s.

racemates. The data presented are for the first chiral analysis of technical DDT.

The reference compounds were also analyzed using chiral HPLC with three immobilized permethylated CDs. PM-γ-CD showed good enantiomer resolution for 2,4′-DDT and 2,4′-DDD. This allowed the isolation of these enantiomers in high purity and the assignment of the (+)- and (−)-enantiomers via chiroptical measurements. The isolates were then used to determine the enantiomer elution orders in chiral HRGC.

The absolute configurations of the 2,4′-DDD enantiomers, hitherto unknown, were determined by chemical analogy to those of the 2,4′-DDT enantiomers. Reductive ($NaBH_4$) dechlorination of the individual 2,4′-DDT enantiomers, for which the absolute configurations were known, led to one or the other 2,4′-DDD (and 2,4′-DDMS) enantiomer. The results indicated (+)-2,4′-DDD to have *R*-configuration and (−)-2,4′-DDD to have *S*-configuration, and thus to have reversed signs of rotation for polarized light compared to the enantiomers of 2,4′-DDT with the same configuration. This change in the direction of rotation must arise from a change in priority (polarizability, see ref 26) of the CCl_3 and $CHCl_2$ groups with respect to the chlorophenyl group. The priority sequence thus must be in an order such as CCl_3 > chlorophenyl > $CHCl_2$ > H. For both compounds, the enantiomer elution orders were *S* prior to *R* using the chiral HPLC (PM-γ-CD) system, and *R* prior to *S* using the chiral OV1701/BS-β-CD HRGC column. There seems to be a common principle in the chiral recognition of the 2,4′-DDD and 2,4′-DDT enantiomers, and it is different for the two different chiral selectors (systems).

The enantiotopic chlorophenyl substituents in 4,4′-DDT and 4,4′-DDD may behave differently in metabolic processes. Metabolites such as the hydroxyphenyl derivatives, important conversion products in bacteria and mammals,[20,21] are chiral, and their enantiomers need not be formed in equal amounts. In the present study, such metabolites were not investigated; however, they should be amenable to the chiral methods described.

Some adverse effects of technical DDT seem to be related to 2,4′-DDT. This compound reportedly binds to steroid hormone receptors and thus mimics the action of natural estrogens.[8] Significant levels of 2,4′-DDT have been detected in some species, particularly in fish.[27,28] Metabolic studies of 2,4′-DDT in mammals[29,30] have demonstrated that the ortho chloro ring was extensively metabolized in 2,4′-DDT, whereas the para chloro ring remained intact. These results would suggest that 2,4′-DDT is less resistant to metabolism than 4,4′-DDT and may explain why 2,4′-DDT is less abundant, particularly in warm-blooded organisms, than expected on the basis of its presence in the technical material.

The two enantiomers of 2,4′-DDT were reported to differ in estrogenic activity, with (−)-2,4′-DDT being far more estrogenically active.[31] In a preliminary study, we observed an ER of ≈0.8 for 2,4′-DDT in a sample of human adipose tissue (unpublished data). Similarily, an ER of ≈0.7 was found for 2,4′-DDT in cod liver oil.[32] Since in both these studies OV1701/BS-β-CD columns were used, we can now assign the less abundant, first-eluted

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enantiomer to *R*-(−)-2,4′-DDT. Our data thus indicate that the estrogenically more potent *R*-(−)-enantiomer is less abundant in human adipose tissue and in cod liver oil.

The question of whether 4,4′-DDT and the two enantiomers of 2,4′-DDT undergo different fates in the various environmental compartments remains unanswered. We feel, however, that the methods presented here should be valuable in investigating this important question. We recommend reanalysis of biota (wildlife) using the chiral analytical techniques outlined, possibly with previously achirally analyzed extracts. Enantiomeric compositions (ER values) of 2,4′-DDT and other chiral contaminants are independent of extraction and cleanup procedures (no optically active reagents involved) or partial chemical decomposition, and thus valid results should be obtained even if not fully adequate methods were previously used.

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