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Enantioselective Determination of Chiral Toxaphene Congeners in Laying Hens and Eggs Using Multidimensional High-Resolution Gas Chromatography

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A total of 22 chiral toxaphene congeners were analyzed in organ tissues and eggs of laying hens after they had been fed with food spiked with technical toxaphene. For the analysis, multidimensional high-resolution gas chromatography using a chiral column coated with randomly silylated heptakis-(*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin, electron capture detection, and valveless "live column switching" technique was applied. The analytical results were additionally confirmed with mass spectral data, recorded in electron-capture negative ionization mode with selected-ion monitoring mass spectrometry. During both the feeding period of the laying hens with toxaphene-contaminated food (38 weeks, accumulation phase) and the following subsiding period without toxaphenes (another 14 weeks, decontamination phase), organs (liver, kidney, skin/fat), blood, meat, and eggs of the hens served as model matrices for toxaphene uptake. The enantiomeric ratios (ERs) of congeners 26, 31, 32, 40, 41, 42(a+b), 44, 50, and 62—known as the most important components of technical toxaphene occurring in the environment—could be analytically determined. Significant differences were observed with respect to their initial racemic ratios. On the basis of their chemical structures, the metabolic pathways of some congeners could be explained. Astonishingly, some of the toxaphenes applied as racemates could merely be found as single enantiomers at the end of the feeding program, for example, congener 32 in blood and meat samples or congener 44, especially in organ tissues, which showed ERs of zero or infinity. The findings of this study impressively emphasize that it is essential to isolate and analyze individual toxaphene enantiomers in food and biota tissues to be capable of evaluating their toxicity and metabolization more specifically.

KEYWORDS: Toxaphene; multidimensional gas chromatography; chiral metabolites; hens' organs; eggs

INTRODUCTION

Technical toxaphene is a complex mixture of at least 670 polychlorinated bicyclic terpenes consisting of more than 200 polychlorinated C10 terpenes (*1*, *2*). In agriculture, especially in cotton cultivation, it has been one of the most intensively used chlorinated insecticides in different parts of the world (*3*−*5*). Production of toxaphene in 1977 was nearly 40 million pounds. By 1982, when the U.S. Environmental Protection Agency (EPA) canceled most of its uses, toxaphene consumption according to an EPA fact sheet was reported to be 12 million pounds. Toxaphene is produced by exhaustive chlorination of camphene, resulting in a product that contains 67−69% of chlorine (*6*). Due to the lack of specificity and sensitivity during the chlorination process, the number of isomers increases with a nonuniform degree of substitution. Most of the congeners belong to the chlorobornane group, followed by chlorocamphenes and chlorobornenes (*7*). The fact that they are very persistent and detected in biota even from remote areas, for example, the polar regions (*8*, *9*), clearly points out the role of global environmental distribution mechanisms, which means that their release to the atmosphere by aerial and ground application, followed by their enrichment in ambient air and atmospheric long-range transport, is mainly influenced by the effects of weathering on the compounds (*10*−*13*). Much of the existing toxicity information is based on the racemic technical mixture, but this may be irrelevant for risk assessment of this banned organochlorine pesticide. Almost all chlorobornanes are racemates, and their two enantiomeric forms may have different biological properties. On the other hand, the metabolization of chlorobornanes can be stereoselective, and the uptake and extraction of (+)- and (−)-isomers may be very difficult (*14*). Therefore, the chiral analysis of racemates in biological systems can provide only one piece of information, but it is required for a better understanding of the metabolic behavior of such complex mixtures.

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High and risky toxaphene levels can still be found in aquatic food chains, for example, in that of fish from the northern Atlantic Ocean and the North Sea (*15*−*17*). These accumulations in fish are also due to the fact that ~1−1.3 million tons of toxaphene have been used as a piscicide in aquatic ecosystems, especially in Canada and the upper midwestern United States during the 1950s and for the next 40 years (*18*). Because toxaphene-contaminated wastes of the fish industry, especially entrails, are also partly used for the manufacture of chicken feed, it seems appropriate to investigate the behavior of congeners in the organisms of a chicken breed. In this work, therefore, laying hens were fed with toxaphene-contaminated diet to find out the enrichment and degradation rates of the congeners Parlar 11, 12, 15, 21, 25, 26, 31, 32, 40, 41, 42a, 42b, 44, 50, 56, and 62 in hens and eggs, which are the finally marketed products. In this way, the enantiomer-specific enrichment of some individual congeners and their metabolization in different sample matrices examined could be elucidated, and additionally, their structure−metabolization relationships.

MATERIALS AND METHODS

Reagents and Solvents. All chemicals and solvents were of analytical grade, but acetone, hexane, cyclohexane, dichloromethane, and ethyl acetate were additionally redistilled before use. Both toxaphene standards, the 22-component mixture and technical toxaphene (99% purity; 61.6% chlorine content), were purchased from Dr. Ehrenstorfer, Augsburg, Germany. Silica gel 60 (70−230 mesh) for column chromatography and sodium sulfate were obtained from Merck KGaA, Darmstadt (Germany).

Multidimensional High-Resolution Gas Chromatography−Electron Capture Detection (MD-HRGC-ECD). A Siemens (Karlsruhe, Germany) double-oven GC system (SiChromat 2-8) equipped with a Live-T piece for valveless heart-cut switching from precolumn to chiral column was used. Two ECD detectors were used, at 280 °C; ECD 1 monitored the time-dependent heart-cut switching on the precolumn, and ECD 2 was used for the accurate quantitation of separated enantiomers on the chiral main column. The ECD makeup gas was nitrogen at 50 mL/min. The injector was used in split/splitless (1 min splitless) mode, split ratio 1:10, at 230 °C. The injection volume was 1−2 μL, and the flow rate of the carrier gas, hydrogen, was 1.5 mL/min at the end of the two-column system. The achiral column was a DB5, 60 m × 0.32 mm i.d., 0.25 μm film thickness (J&W Scientific, Wesel, Germany), with a carrier gas prepressure of 15 MPa. The chiral column was a BGB-172 (20% BSCD), 30 m × 0.32 mm i.d., 0.20 μm film thickness (BGB-Analytic AG, Adliswil, Switzerland), with a carrier gas prepressure of 0.065 MPa. The temperature program of the achiral precolumn was as follows: 100 °C (15 min) raised to 250 °C (4 °C/min). The temperature programs used for the enantioselective separation on the chiral column are shown in **Table 1**.

HRGC-ECD Selective Ion Monitoring−Mass Spectrometry (HRGC-ECD-SIM-MS). A Hewlett-Packard 5890 series II gas chromatograph (Agilent, Waldbronn, Germany) was coupled with a Finnigan 8200 mass spectrometer (Bremen, Germany). The GC parameters were as follows: column, DB5, 30 m × 0.25 mm i.d., 0.25 μm film thickness (J&W Scientific, Wesel, Germany); temperature program, 50 °C (1 min) raised to 150 °C (70 °C/min, 5 min) and then to 260 °C (2 °C/min). The MS parameters were as follows: filament, 70 eV; reagent gas, CH_4; ion source pressure, 25×10^{-6} MPa; ion source temperature, 190 °C; scan, *m/z* 65−500, 1.2 s per scan; software, MASPEC Data System for MS-Windows (MSS, Manchester, U.K.), version 2.11, NIST Library.

Test Animals and Toxaphene Application. Seventy-two laying hens of the breed Lohmann were included in the feeding trials. At the beginning of this experimental series, the hens were 22 weeks old (so-called pullets) and separated into several groups (**Table 2**). The feeding was carried out over a period of 38 weeks, and technical toxaphene doses in air-dried and non-silo-stored animal fodder (Legehennen-Allein, chicken feed from the Institute of Animal Science and Animal Behavior, Federal Agricultural Research Center Neustadt-Mariensee, Germany) amounted to 0 mg/kg for the control group to 0.5, 1, and 5 mg/kg for the experimental groups. One weight percent of soya oil was used as additive to spike the fodder homogeneously with the different chlorinated camphene amounts every 4 weeks. Preliminary trials have ensured that the soya oil used for mixing was toxaphene-free; its PCB content, which could possibly contaminate the fodder during mixing, was nearly irrelevant.

Table 1. Retention Time (RT) Windows on the Achiral BD5 Column Used for the Preseparation of Toxaphene Congeners and the Corresponding Temperature Programs Applied for the Enantioselective Separation on the Chiral BGB-172 Column

congener	RT, achiral colum (min)	temperature program, chiral column
11	30.6–30.9	100 °C (25 min) to 250 °C (3 °C/min)
12	30.9–31.2	100 °C (25 min) to 250 °C (3 °C/min)
15	31.7–32.1	100 °C (25 min) to 250 °C (3 °C/min)
21	32.8–33	100 °C (25 min) to 250 °C (3 °C/min)
25	33.7–34.1	100 °C (25 min) to 250 °C (3 °C/min)
26	34.1–34.3	100 °C (25 min) to 250 °C (1 °C/min)
31+32	35.1–35.4	100 °C (25 min) to 250 °C (2 °C/min)
38	36.2–36.6	100 °C (25 min) to 250 °C (3 °C/min)
39	36.7–36.9	100 °C (25 min) to 250 °C (3 °C/min)
40+41	37–37.2	100 °C (25 min) to 250 °C (2 °C/min)
42	37.2–37.4	100 °C (25 min) to 250 °C (2.5 °C/min)
44	37.5–37.7	100 °C (25 min) to 250 °C (1.5 °C/min)
50	38.5–38.8	100 °C (25 min) to 250 °C (2 °C/min)
51	38.8–39.1	100 °C (25 min) to 250 °C (3 °C/min)
56	39.9–40.2	100 °C (25 min) to 250 °C (2.5 °C/min)
58	40.3–40.6	100 °C (25 min) to 250 °C (3 °C/min)
59	40.6–40.9	100 °C (25 min) to 250 °C (3 °C/min)
62	41.4–41.9	100 °C (25 min) to 250 °C (2 °C/min)
63	41.9–42.3	100 °C (25 min) to 250 °C (3 °C/min)
69	46.4–46.7	100 °C (25 min) to 250 °C (3 °C/min)

As a basis of calculation, a feed requirement of 0.13 kg per animal and day was assumed, plus an add-on of 5%. Therefore, 1 kg of soya oil was treated with 25 mL aliquots of differently premixed toxaphene/isooctane solutions. These premixtures (B−D) were prepared as follows: B, 50 mg of technical toxaphene in 25 mL of isooctane plus 1 kg of soya oil; C, 100 mg of technical toxaphene in 25 mL of isooctane plus 1 kg of soya oil; and D, 500 mg of technical toxaphene in 25 mL of isooctane plus 1 kg of soya oil. Finally, these spiked premixtures were added to 99 kg of the hen's feed, which was thoroughly mixed and homogenized.

The test animals were divided into groups A−E, and the feeding was partitioned into two phases.

The actual feeding phase consisted of (i) group A (9 animals), uncontaminated fodder; (ii) group B (9 animals), fodder contaminated with 0.5 ppm of toxaphene; (iii) group C (9 animals), fodder contaminated with 1 ppm of toxaphene; (iv) group D1 (9 animals), fodder contaminated with 5 ppm of toxaphene; and (v) group E (18 animals), uncontaminated fodder.

The subsiding feeding phase comprised (vi) group D2 (9 animals), fodder contaminated with 5 ppm of toxaphene during the actual feeding phase and afterward uncontaminated.

After 18, 28, and 38 weeks of application, three animals were slaughtered each and their organs, muscle meat, skin, fat, and blood tissues, as well as the yolk of their weekly sampled eggs, were analyzed for toxaphene contamination. From the three animals of each group, 1:1 mixtures of the thigh and breast muscles and the fatty matrix of stomach and entrails, as well as their organs (liver, kidney, and skin) and yolk samples from the egg pool, were prepared, homogenized, and stored at −50 °C until analysis. All slaughtering dates for the different test groups are shown in **Table 2**.

Extraction of the Samples. Amounts of 2.5 g of meat, 2.5 g of fat, 2 g of liver, 4 g of skin, 2 g of kidney, 6 g of blood, and 4 g of egg yolk were taken and mixed with water-free sodium sulfate (1:4). These mixtures were mortared and then homogenized and Soxhlet-extracted for 4 h with cyclohexane/dichloromethane (2:1). Prior to each experiment, the Soxhlet thimbles were cleaned in an alkaline solution for 2

Table 2. Slaughtering Times and Feeding Program of the Experimental Hens

group	total no. of animals	no. of anmials slaughtered								
		feeding phase (spiked fodder)			subsiding feeding phase (unspiked fodder)					
		18 weeks	28 weeks	38 weeks	39 weeks	40 weeks	41 weeks	43 weeks	46 weeks	54 weeks
A and E[a]	27	3	3	3	3	3	3	3	3	3
B[b]	9	3	3	3						
C[c]	9	3	3	3						
D1 and D2[d]	27	3	3	3	3	3	3	3	3	3

[a] Control groups, received unspiked fodder. [b] Received fodder spiked with 0.5 mg/kg technical toxaphene. [c] Received fodder spiked with 1.0 mg/kg. [d] Received fodder spiked with 5 mg/kg technical toxaphene.

h. The sample extracts were vacuum-evaporated in a water bath (40 °C) and dried until constant weight to determine the fat contents.

Sulfuric Acid Cleanup. The sample purification (elimination of interfering analytes from the matrix) was accomplished with sulfuric acid according to the cleanup procedure suggested by Xu et al. (*19*). For this purpose, the sample extract was mixed with hexane (30 mL/g of fat) plus 15 mL of concentrated sulfuric acid and allowed to stand without shaking until complete separation of the two phases. The acidic phase was rejected and the whole procedure repeated five times. Thereafter, the remaining combined extract was again five times shaken out with 15 mL of concentrated sulfuric acid until the lower phase showed no coloring anymore, and each acidic phase was discarded. Then the sample was shaken with 15 mL aliquots of distilled water until neutral pH. Finally, the purified sample extract was dried with 10 g of anhydrous sodium sulfate.

Column Chromatographic Fractionation. During the acid cleanup described above, the toxaphene congeners were separated, taken up, and collected in hexane. However, due to other interfering chlorinated compounds, a further purification step was necessary and carried out by column chromatographic fractionation on silica gel 60 (70−230 mesh) according principally to the method of Mueller (*20*). After removal of the sodium sulfate by filtration, the sample was vacuum-evaporated to 1 mL at 40 °C. The column (30 cm in length; 1 cm i.d.) was packed with 4.3 g of wet and slurried silica gel, which had been preconditioned by heating for 48 h at 140 °C and deactivated with 5% water. Then the silica gel-filled column was pre-eluted with 20−30 mL of hexane. After application of the evaporated sample extract, the sample vial was rinsed two times with 0.5 mL of hexane, which was also transferred to the column. The first-eluting solvent (hexane) was replaced after 13 mL (sample extract volume was included) by 9 mL of hexane/dichloromethane (2:1). Together with the 3 mL portion of hexane, already contained in the wet sorbent layer, a total elution volume of 25 mL resulted. The elution velocity hereby was adjusted to 7 mL/min, which corresponds to a nitrogen excess pressure in the system of ∼0.03 MPa, and guarantees high and constant reproducibility rates. The toxaphene congeners were eluted in the fraction from 18 to 25 mL. The columns had to be cleaned and refilled for each analysis with new silica gel to exclude possible contaminations by the afore-purified samples.

Cleanup by Gel Permeation Chromatography (GPC) and Mini-Silica Gel Column Chromatography. For the quantitative determination of the technical toxaphene congeners by HRGC-MS analysis, the interfering matrix of the liver, kidney, meat, skin, and yolk samples was at first separated via GPC according to the method of Specht and Tillkes (*21*). The system consisted of a Biotronik HPLC pump BT 3020 (Eppendorf, Hamburg, Germany) combined with a sampling valve unit BT 3021: injection volume (sampling loop), 5 mL; column, 50 cm × 3.5 cm i.d., filled with Bio-Beads S-X3 (200−400 mesh); solvent, cyclohexane/ethyl acetate (1:1); pressure, 0.05 MPa.

To determine the elution bands of unwanted fat and wanted toxaphene components, a solution containing 29 μg/mL technical toxaphene standard in cyclohexane/ethyl acetate (1:1) was prepared, of which 5 mL was poured onto the GPC column. Because toxaphene congeners are only weakly absorbed at 280 nm and, therefore, poorly detected by the monitoring UV detector, the eluate was collected in several fractions, which were analyzed by HRGC-ECD, and the toxaphene congeners' elution band could be determined ranging from 120 to 240 mL.

For the following mini-silica-gel column chromatography cleanup step, the GPC eluate (evaporated to 1 mL and taken up in isooctane) was placed on a glass column filled with 1 g of silica gel. Prior to sample application, the silica gel was dried for 5 h at 130 °C, deactivated with 1.5% water, packed into the column, and pre-eluted with 10 mL of hexane. The separation of the interfering polychlorinated biphenyls (PCBs) from almost all of the organochlorine pesticides was achieved by pre-elution with 8 mL of hexane (eluate 0). Afterward, the organochlorines were eluted with 8 mL of hexane/toluene (65:35; eluate 1) and 8 mL of toluene (eluate 2). These two eluates were combined, vacuum-evaporated at 30 °C, and taken up in 10 mL of toluene. Residual levels of some other pesticides, for example, cyclodiene insecticides, can still be observed, but they did not interfere with toxaphene congeners during GC-ECD and GC-MS analysis.

Quantification of Toxaphenes. For the quantification by MD-HRGC-ECD, the peak area of each congener in the samples was compared to the corresponding one in the 22-component standard (**Figure 1**). For each of the 22 congeners, calibration curves were developed, including the concentrations of 6, 12, 24, 48, and 96 ng/mL. Each sample was injected three times to calculate the mean values and standard deviations. Additionally, a quantification of toxaphenes by HRGC-ECNI-SIM-MS analysis according to the method of Witte et al. (*22*) was carried out to confirm the obtained MD-HRGC-ECD results. As masses for quantification served the M, M−HCl, and M−Cl fragments and as standard again the 22-component mixture from Dr. Ehrenstorfer.

Influence of Cleanup Procedures on Toxaphene Recovery Rates in Samples of Laying Hens. To take into consideration the total percent loss of the individual congeners in the hens' samples during the different cleanup steps, which was observed to be extremely high by partial congener degradation during the sulfuric acid cleanup procedure, toxaphene-free organ and tissue control samples were mixed with sodium sulfate and spiked with technical toxaphene in concentrations of 0.5, 1, and 5 μg/mL, as well as the 22-component standard (0.1 μg/mL). Afterward, these spiked samples were homogenized for 5 min in an ultrasonic bath, worked up and analyzed as described above.

RESULTS

Determination of Toxaphene Recovery Rates in Samples of Laying Hens. The recovery rates are a simple but suitable tool to prove the applicability of a strongly matrix-dependent analytical method, especially when simulating the cleanup conditions of the real hen samples and covering high-to-trace amounts of the individual analytes to be determined in the spiked samples. High total recovery rates, ranging from 88.9 to 96.9% with standard deviations between 1.92 and 2.98%, could be found for both standards (the 22-component and technical toxaphene mixture) when the 22 individual structure-identified congeners were summed. The percentage levels showed a decreasing tendency with increasing initial toxaphene concentrations, which it was assumed was due to both lower extraction yields and higher losses of certain congeners during sulfuric acid cleanup. Nevertheless, the treatment with sulfuric acid is

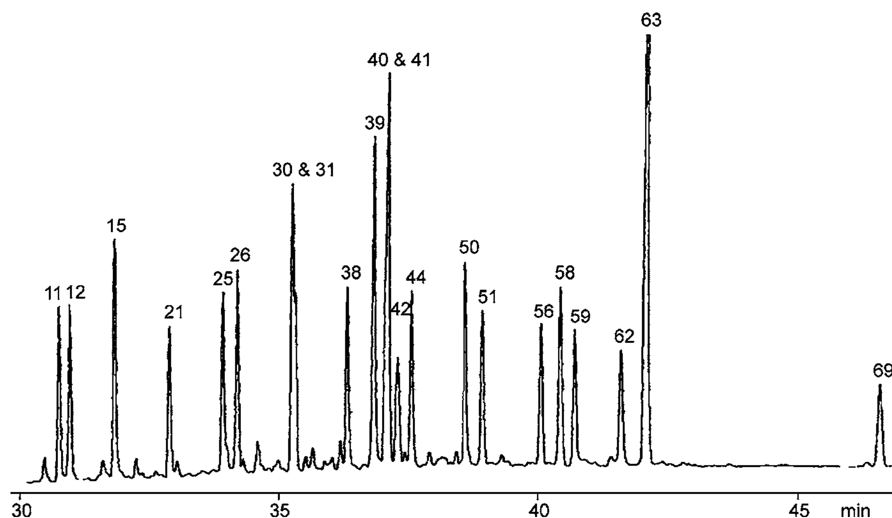

Figure 1. GC-ECD chromatogram of the 22-component toxaphene standard used.

Table 3. Retention Times (RT) of the Applied 22 Toxaphene Standard Congeners on the Achiral DB5 Capillary, as well as Their Calculated Relative Retention Times (RRTs) and Retention Time Indices (RRIs) in Relation to Parlar 26 and 50.

congener	RT (min)	RRT, congener 26[a]	RRT, congener 50[b]	RRI, ∑ congeners 26 + 50[c]
11	32.08	0.883	0.767	0.62
12	32.36	0.891	0.773	0.62
15	33.54	0.923	0.802	0.64
21	34.62	0.953	0.827	0.66
25	36.06	0.993	0.862	0.69
26	36.33	1.000	0.868	0.70
31+32	37.69	1.037	0.901	0.72
38	38.89	1.070	0.929	0.75
39	39.62	1.091	0.947	0.76
40+41	40.00	1.101	0.956	0.77
42	40.18	1.106	0.960	0.77
44	40.50	1.115	0.968	0.78
50	41.84	1.152	1.000	0.80
51	42.11	1.159	1.006	0.81
56	43.37	1.194	1.037	0.83
58	43.73	1.204	1.045	0.84
59	44.02	1.212	1.052	0.84
62	44.85	1.235	1.072	0.86
63	45.38	1.249	1.085	0.87
69	48.78	1.343	1.166	0.94

[a] $RRT_x = RT_x/(RT\ 26)$. [b] $RRT_x = RT_x/(RT\ 50)$. [c] $RRI_x = RT_x/(RT\ 26 + RT\ 50) \times 1.5$.

easy and less time-consuming and results in a better separation from the interfering matter. Further important arguments for its use are its low solvent consumption and low purchase and disposal costs.

Determination of the Relative Response Factors (RRFs). The relative response factors of the 22 standard congeners, obtained with quantification data of the capillary columns used for MD-HRGC-ECD and HRGC-MS analysis, are summarized in **Table 3** (*23*). This calculation was necessary, because the absolute values differed extremely depending on the various equipment and working conditions applied. Therefore, the RRFs were calculated in relation to RRF = 1 of congener 50 [B9-1679; systematic code name according to Andrews and Vetter (*24*)], which is a major component in biota samples but only a minor one in the technical product (cf. **Table 3**). The table shows also the absolute retention times (RTs, analyzed on the achiral DB5 precolumn) and the relative retention times (RRTs), which were calculated in relation to both congener 50 (B9-1679) and congener 26 (B8-1413), as the most persistent congeners ubiquitously found in biota samples as well as in hens' organ and tissue samples. These are listed together with the relative retention time indices (RRIs), which were calculated by dividing the congeners' RTs by the sum of RTs of congeners 26 and 50. These values were low because of the long RTs of the two congeners and, therefore, were additionally multiplied by 1.5 (*25*). The above relative values were independent of the experimental conditions, and it was indispensable to compare the retention data from the different chromatographic systems used for the enantioselective analysis.

Enantioselective Determination of Chlorobornanes by MD-HRGC. Chiral toxaphene congeners can be classified into three different groups according to their enantiomer ratios (ERs); group 1, the racemic ratio of the enantiomers equals 1.0, which means that no enzymatic changes took place; group 2, ER differs slightly from 1.0, meaning that weak enzymatic changes occurred; and group 3, ER differs strongly from 1.0, concluding that distinct enzymatic changes occurred. The ER is directly obtained from the integration of the peaks derived from GC or NMR spectra by dividing the peak area E_1 of the first-eluting *S* enantiomer by that of the second-eluting *R* enantiomer (E_2), that is, ER = E_1/E_2 = *S*/*R* (*26*). Enantiomeric ratios around zero or nearly up to infinity (∞) imply that the *S* or *R* enantiomer had almost completely decomposed and that the corresponding enantiomer was enriched in the layers'samples.

Enantiomer Separation on *tert*-Butyldimethyl Silylated *b*-Cyclodextrin (b-BSCD). The results of the enantiomer separations in this study confirm the general statements that the separation's efficiency primarily depends on the kind of chiral phase and the pair of enantiomers to be separated. However, this above correlation is not clear without ambiguity. Therefore, the correct choice of a chiral phase for the optimal separation of chiral substances is still empirical and had to be done also in this study by trial-and-error. **Table 6** shows the ERs, the retention values (*R* values) of chiral separation, and the separation factors (α) of the 17 single compounds of technical toxaphene (CTTs), which could be enantioselectively separated by the used double-oven HRGC-ECD system with β-BSCD as chiral selector. As shown in **Figure 2**, some enantiomers of the 22-component standard overlap on the β-BSCD column. Due to the doubling of congener peaks on the chiral column, the total number of peaks amounts to 44, which increases of course the probability of such coelution effects. With the help of two(multi)-dimensional HRGC,

Table 4. Enantiomer Ratios (ERs), Chiral Resolution (*R* Values), and Separation Factors (α) of the 22-Component Toxaphene Standard Mixture on the Applied β-BSCD Capillary (No Enantioselective Separation Could Be Achieved for Parlar 38, 58, 59, 63, and 69)

congener	ER[a]	*R* value[b]	α[c]	enantiomer part (%) *S*	enantiomer part (%) *R*
11	0.987	4.75	1.009	49.7	58.375
12	0.98	1.44	1.003	49.5	50.5
15	0.96	1.22	1.003	48.9	51.1
21	1.15	2.01	1.004	53.4	46.6
25	1.06	5.7	1.012	51.6	48.4
26	1.00	0.6	1.002	50.1	49.9
31	1.04	6.4	1.015	50.6	49.4
32	1.00	0.7	1.002	49.9	50.1
39	1.02	0.5	1.001	50.5	49.5
40	0.90	2.2	1.007	47.2	52.8
41	0.81	3.2	1.004	44.6	55.4
42(a+b)[d]	2.65	1.64	1.004	72.6	27.4
44	1.03	0.8	1.002	50.7	49.3
50	1.03	1.00	1.002	50.7	49.3
51	0.95	0.86	1.002	48.7	51.3
56	0.96	0.7	1.001	48.9	51.1
62	1.1	1.0	1.002	50.4	49.6

[a] ER = *S*/*R*; *S* and *R* = peak areas of the first-eluting and the second-eluting enantiomers. [b] $R = (t_R - t_S)/(w_1 + w_2)$, where t_R and t_S are the retention times of *R* and *S* and w_1 and w_2 describe the peak widths at half peak height ($R = 0$ means no separation, $R = 1.0$ means 95% separation, $R > 1$ means complete separation). [c] α ($= t_R/t_S$). [d] ER value of both coeluting congeners 42(a+b) is presented as sum.

however, it was possible to transfer most of the racemates, which were completely separated on the achiral precolumn, onto the chiral column by heart-cut technique. A typical chromatogram of the 22-component toxaphene standard with lossless heart-cut transfer of congeners 26, 50, and 62 from the achiral to the chiral column and the corresponding chromatograms of the enantiomeric separation on the chiral capillary column are shown in **Figure 2**. The efficiency of the dual-column system (achiral DB 5/chiral β-BSCD) used for the determination of all standard congeners can be summarized as follows (cf. **Table 4**):

(i) In standard and hen samples, the enantiomers of the toxaphene congeners 11, 12, 15, 21, 25, 31, 40, 41, 42, 44, 50, and 62 were baseline-separated on the β-BSCD column. Due to coelution of congeners 40 and 41 as well as 42a and 42b on the achiral precolumn, the second-eluting enantiomer of congener 41 coincides with the first-eluting one of congener 40 because of identical retention times (merely three peaks on β-BSCD could be observed, which has been additionally proven and confirmed with a separate racemate of congener 40). Only one peak of the congeners 42a and 42b could be detected on the achiral precolumn, after a total transfer resulting in two peaks on the chiral column. When the 42a+b peak was partially cut and transferred from the achiral DB5 to the chiral β-BSCD column, always the same peak spectrum was obtained, which means that the enantiomers coeluted on both columns. Therefore, their ER value is presented as a sum (42a+b) in **Table 4**, and no clear statements about their enantiomeric separation can be made.

(ii) Enantiomers of congeners 26, 32, 39, 51, and 56 were not completely baseline-separated on β-BSCD, but sufficiently enough (cf. *R* and α values in **Table 4**) to calculate their ERs accurately, using the well-known GC standard integration method for partially overlapping peaks.

Table 5. Enantiomeric Ratios (ERs)[a] of Chiral Toxaphene Congeners Analyzed in Organs of Laying Hens Fed for 38 Weeks with Toxaphene-Spiked Fodder (Mean Values of Three Measurements)

		C (ppm) of congeners																	
		skin/fat			meat			liver			kidney			egg yolk			blood		
congener[b]	*C* (ppm) in feed	18 weeks	28 weeks	38 weeks	18 weeks	28 weeks	38 weeks	18 weeks	28 weeks	38 weeks	18 weeks	28 weeks	38 weeks	18 weeks	28 weeks	38 weeks	18 weeks	28 weeks	38 weeks
26	0.5	0.81	0.75	0.73	0.66	1.08	0.78	0.60	0.98	0.80	0.92	0.91	1.05	0.96	0.91	0.81	0.82	1.18	0.81
	1.0	0.78	0.72	0.72	0.78	0.72	0.72	0.49	0.60	0.59	0.70	0.72	0.75	0.80	0.76	0.77	0.96	0.92	0.91
	5.0	0.76	0.91	0.84	0.76	0.91	0.84	0.93	0.87	0.87	0.89	0.86	0.99	0.83	0.85	0.90	0.99	0.82	0.91
31	0.5	9.40	6.45	8.55	ND[d]	ND	ND	ND	ND	ND	ND	ND	ND	4.0	10.2	8.35	5.85	1.40	2.35
	1.0	17.0	24.8	20.0	ND	ND	ND	ND	ND	ND	ND	ND	ND	10.7	10.5	11.8	6.18	4.58	7.25
	5.0	14.4	18.0	13.9	ND	ND	ND	ND	ND	ND	ND	ND	ND	11.3	12.0	7.22	14.7	9.60	13.5
32	0.5	0.52	0.77	0.63	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.80	0.41	0.80	∞	1.03	0.96
	1.0	14.9	0.81	∞	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.42	0.50	0.57	0.80	1.20	∞
	5.0	2.39	1.04	1.0	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.51	0.53	0.89	0.91	1.59	∞
40	0.5	0.94	1.33	1.87	1.73	1.45	1.44	0.68	1.23	1.72	1.26	1.15	1.11	1.28	1.46	1.44	1.96	1.16	1.27
	1.0	1.56	1.83	0.80	1.62	1.78	1.17	0.89	1.68	1.46	0.97	1.23	0.83	1.90	1.35	1.21	2.47	1.95	2.55
	5.0	2.26	1.85	1.26	1.52	1.74	2.19	1.43	1.37	1.62	1.39	1.52	0.97	1.31	1.53	1.22	2.50	2.39	2.10
41	0.5	1.02	1.28	0.93	1.65	0.96	0.77	1.92	1.68	1.27	0.61	0.49	0.42	0.65	1.22	0.90	0.87	0.26	0.37
	1.0	6.54	7.10	1.48	1.06	1.15	0.79	1.06	1.15	0.79	0.56	0.73	0.47	1.10	1.38	1.41	1.18	0.71	1.15
	5.0	2.15	1.51	1.29	1.11	0.90	1.22	0.95	1.04	1.04	0.72	0.88	0.30	1.33	1.33	0.96	1.21	1.05	1.42
42(a+b)[c]	0.5	0.62	0.46	0.45	0.55	0.72	0.54	0.68	0.24	0.18	0.69	0.53	0.64	1.20	0.46	0.76	0.92	2.50	1.92
	1.0	0.16	0.19	0.57	0.82	1.03	0.50	0.40	0.18	0.45	1.12	0.47	0.77	0.55	0.54	0.59	0.82	1.04	0.72
	5.0	0.91	0.53	0.52	0.86	1.55	1.26	0.38	0.89	0.73	0.69	0.67	1.22	0.73	0.83	1.30	0.51	0.91	0.42
44	0.5	4.49	5.0	4.5	18.9	3.69	5.62	36.4	12.6	11.6	6.48	8.80	2.18	3.92	6.10	5.10	6.58	2.48	4.58
	1.0	43.1	19.6	6.14	2.14	1.88	6.47	∞	12.1	12.0	1.97	5.65	4.40	4.82	5.64	5.17	5.69	7.90	9.67
	5.0	6.60	9.1	63.0	5.68	4.64	4.74	7.10	10.7	10.3	7.64	9.16	6.49	5.56	5.62	4.40	8.16	9.49	10.4
50	0.5	1.23	1.19	1.15	1.61	1.10	1.08	2.25	2.10	1.16	1.40	1.10	0.82	1.25	1.40	1.27	1.51	1.40	1.54
	1.0	2.02	1.59	1.14	1.63	1.24	1.33	1.78	1.78	0.88	1.06	1.36	2.10	1.36	1.47	1.26	1.10	1.24	1.72
	5.0	1.47	1.49	1.52	1.26	1.65	1.60	2.13	1.30	1.34	1.93	2.60	1.06	1.47	1.59	1.36	1.67	1.95	1.36
62	0.5	9.61	10.2	9.63	14.7	5.75	9.5	8.04	31.2	11.9	6.34	6.58	9.37	7.40	12.9	11.5	4.72	2.06	2.64
	1.0	38.1	36.5	9.85	11.1	8.12	9.49	5.06	9.81	11.7	2.16	ND	ND	10.7	12.8	13.3	8.62	7.12	3.24
	5.0	4.69	11.2	12.5	10.7	12.1	16.7	37.1	13.1	13.0	10.9	12.5	9.85	10.4	11.4	9.0	8.58	8.68	13.0

[a] ER = *S*/*R*; pure or enantiomer-enriched reference materials were partly not available; therefore, the enantiomer elution sequence above is defined as follows: *S* = peak area of first-eluting enantiomer and *R* = that of second-eluting one; ER < 1, if *S* < *R*; ER > 1, if *S* > *R*; ER = 1 = racemate. [b] Congeners 11, 12, 15, 21, and 56 could not be detected in the organs; 25 was found in only the kidney (1.42, 2.25, and 2.60 ppm for 0.5, 1.0, and 5.0 ppm toxaphene-contaminated feed). [c] ER value of the both coeluting congeners 42a+b is presented as sum. [d] Not detected.

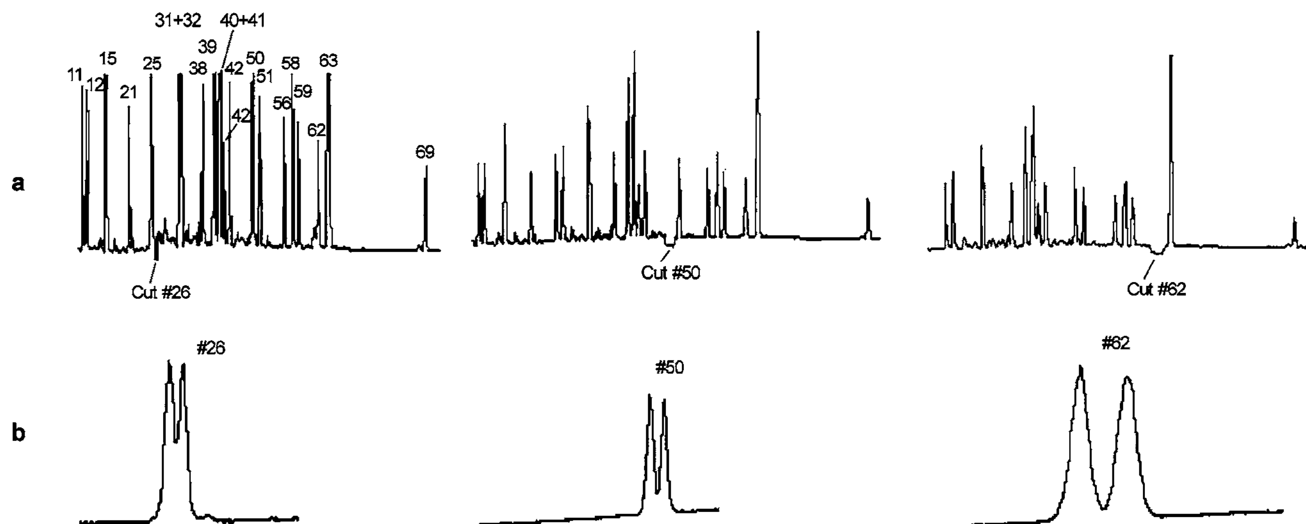

Figure 2. (**A**) Chromatograms of the 22-component standard on the achiral DB5 precolumn with heart-cutting of Parlar 26, 50, and 62; (**B**) chromatograms of the separated enantiomers on the chiral β-BSCD main column.

Table 6. Enantiomeric Ratios (ERs)[a] of Chiral Toxaphene Congeners Analyzed in Organs of Laying Hens Fed for 16 Weeks with Toxaphene-free Control Fodder (Mean Values of Three Measurements)

	C (ppm) in feed																							
	skin/fat				meat				liver				kidney				egg yolk				blood			
congener[b]	1 week	5 weeks	8 weeks	16 weeks	1 week	5 weeks	8 weeks	16 weeks	1 week	5 weeks	8 weeks	16 weeks	1 week	5 weeks	8 weeks	16 weeks	1 week	5 weeks	8 weeks	16 weeks	1 week	5 weeks	8 weeks	16 weeks
26	0.89	0.38	0.75	0.54	0.87	0.72	0.75	0.71	0.77	0.72	0.62	0.62	0.92	0.85	0.76	0.63	0.68	0.81	0.77	0.79	0.67	0.78	0.89	0.97
31	2.41	2.55	17.8	13.6	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	7.03	11.5	16.2	ND	8.09	3.42	3.28	1.9
32	1.14	1.43	∞	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.78	1.4	60.7	ND	0.29	1.1	1.07	1.1
40	4.24	9.07	1.34	0.73	2.18	0.98	1.12	1.12	1.49	1.22	1.13	0.99	1.05	0.89	0.65	0.69	6.6	2.35	3.86	1.07	1.84	1.38	1.43	1.11
41	0.32	0.35	5.01	3.39	1.76	1.48	2.31	1.82	2.71	1.74	4.53	2.67	0.46	1.04	0.97	1.55	0.26	3.7	2.76	3.72	0.98	0.68	0.76	0.26
42(a+b)	1.27	0.99	0.56	0.46	0.89	0.39	0.64	1.47	0.3	0.85	0.1	ND	0.85	0.61	1.79	1.05	1.25	0.84	0.75	0.46	0.89	1.29	1.34	2.72
44	3.09	5.1	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	3.2	∞	∞	∞	8.73	6.72	9.29	∞	12.2	10.9	11.9	1.76
50	0.14	0.68	1.26	2.07	1.73	1.01	1.85	1.82	1.92	1.8	1.83	1.94	2.1	1.34	1.63	2.0	1.46	1.99	2.07	2.12	1.54	1.47	1.75	1.82
62	5.81	5.9	58.1	∞	21.4	22.9	49.0	25.6	46.4	25.2	63.8	∞	10.3	20.4	10.6	38.5	13.1	14.8	20.5	∞	12.2	5.9	5.96	1.97

[a] For explanations see **Table 5**.

(iii) For congeners 38, 58, 59, 63, and 69, no separation of their enantiomers with the used chiral column was possible.

Toxaphene Contamination of Hen and Egg Samples. In an earlier study, the results of the hens' feeding program have been considered primarily under the aspects of toxaphene transfer from animal feed to laying hens and their eggs (*27*); the hens were fed toxaphene-dosed feed for 38 weeks and toxaphene-free feed for 16 weeks. In this work, we have focused specifically on the determination of the ERs of 16 structure-identified toxaphene congeners, which could be unambiguously analyzed in the different organ tissues and egg yolks of the laying hens after optimization of the experimental and analytical conditions as described before. The ERs ascertained by GC and GC-MS systems are summarized in **Tables 5** and **6**. The corresponding EF values are shown individually in **Figure 3**, which can be calculated from the ERs by the formula $EF = ER/(ER + 1)$. The graphical representation of EFs can be considered superior to that of ERs because they can always be plotted, even for ERs that reach infinity (∞). The EF values can only range from 0 to 1.0, with $EF = 0.5$ representing the racemic mixture. Generally, similar ER and EF tendencies could be observed during the feeding program, even at the highest toxaphene dosage.

DISCUSSION

ER and EF Values of Congeners during the Feeding Phase of Laying Hens. Toxaphene congeners 11, 12, 15, 21, and 56 could not be detected in the tissue, giblet, blood, and egg yolk samples of the layers (**Table 5**). Surprisingly, congener 25 was found only in kidney samples of the hens fed with 1 mg of toxaphene/g of animal feed. This result shows a >2-fold excess of the first-eluting enantiomer and, thus, indicates a combined effect of non-enantioselective exchange and/or processes, which should be interpreted with caution.

The ER and EF values of congeners 31, 32, 44 (B8-2229), and 62 in all analyzed samples, except for congener 32 in egg yolk, strongly deviated from the racemic ratio throughout the 38 weeks of the feeding program (**Table 5** and **Figure 3**). Furthermore, the presence of congeners 31 and 32 in liver, kidney, and meat samples could not be proven. On the contrary, the ERs of congener 26 (B8-1413) varied only between 0.49 and 1.18 and their corresponding EFs between 0.33 and 0.54 in all samples of the feeding series, which means that its enantiomers, known to be very persistent, were non-enantioselectively enriched in every organ or tissue of the layers and in their eggs' yolk. The enantiomers of congeners 40 and 41 (B8-1414/1945), together with those of congeners 26 (B8-1413) and 50 (B9-1679), belong to the group of congeners that were preferentially metabolized by the test hens. Both former

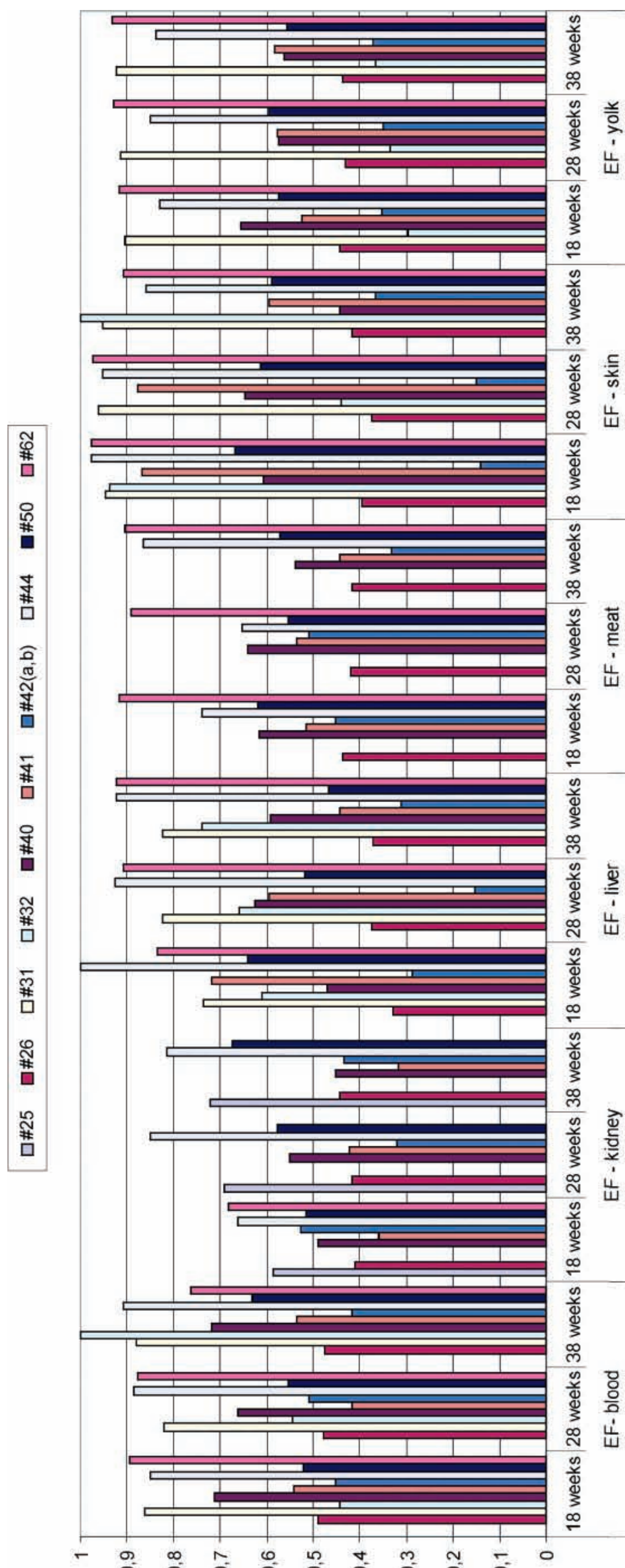

Figure 3. Enantiomeric fractions (EFs) of toxaphene congeners in hens' organs during the initial feeding phase with 1.0 ppm of toxaphene.

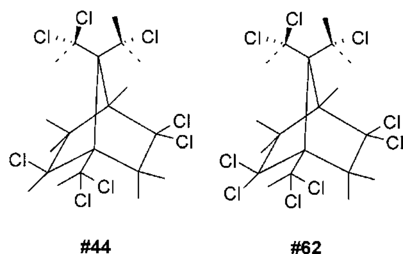

Figure 4. Chemical structures of toxaphene congeners 44 and 62.

congeners have recently been found in muscle and liver tissues of the Arctic fox and in many other Arctic species (*28*, *29*).

All EF values of congeners 44 and 62 strongly deviated from the racemic composition (EF = 0.5), especially in the hens' liver samples (**Figure 3**). The liver is well-known as a detoxification organ for xenobiotics, and the enrichment of the first-eluting enantiomers of both congeners is the result of one or more stereoselective degradation and/or separation processes. From a comparison of the structures of congeners 44 and 62, it can be recognized that both differ only in terms of the chlorine atom at position 2 (**Figure 4**). The probability that congener 44 dechlorinates to congener 62 is increased, because almost all ERs and EFs of the two compounds are nonracemic. This affirms the theory that an unsubstituted C atom within the ring favors the dechlorination of the neighbored one. We further observed that the toxaphene congeners 26 and 50 in meat, skin, blood, and yolk samples after 18, 28, and 38 weeks of feeding were scarcely metabolized, whereas the highest deviations of congener 50 occurred in kidney and liver microsomal fractions, which is attributable to its stereospecific metabolization and enzymatic transport processes.

ER Values of Congeners during the Subsiding Phase of the Laying Hens. During the subsiding period from the 39th to the 54th week, the hens were fed toxaphene-free feed. Congeners 44 and 62 showed strongly nonracemic ERs in the different samples of the layers slaughtered after 1, 5, 8, and 16 weeks of decontamination (**Table 6**). These deviations were clearly >1 or have gone to infinity for the ERs, particularly in meat, liver, and kidney samples, which means that the first-eluted enantiomer was always more highly enriched than the second-eluting one. A similar enrichment of the first enantiomer of congener 31 could be observed in yolk and skin samples. This new enrichment effect (ER ≠ 1) of congeners 44 and 62 supports the assumption that both are dechlorinated.

The enantiomers of congeners 31 and 32 could not be determined in liver, kidney, and meat using the β-BSCD column for analysis, whereas the enantiomeric excess (ee, as the peak area of enantiomer 1 divided by the sum of the peak areas of both enantiomers 1 and 2 multiplied by 100) of congener 32 in yolk, skin, and blood samples amounted to 100, 60.7, and 52.3%, respectively. Therefore, it can be suspected that metabolization of these compounds in both liver and kidney does not play an important role. To a certain extent, congener 26 is peculiar, because all of the ERs found were below 1 (**Table 6**), which means that no serious changes of the initial racemic mixtures took place. The ERs measured for congener 50 in the sample extracts ranged from 0.14 to 2.12. With consideration of the ER values of congeners 26 and 50, it can be supposed that the methyl group on position 9 seems to influence the stability of these bornanes (**Figure 5**), because congener 26 shows ERs only slightly deviating from 1, which is not the case for congener 50. Usually, it can be expected that a chlorine substitution would hinder an attack on this methyl group, but the opposite is the case. Under these conditions, congener 26 seems to be much more stable than congener 50.

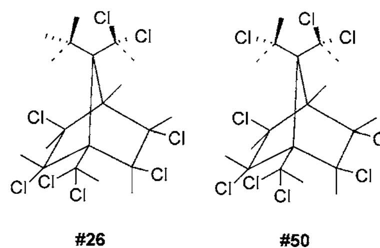

Figure 5. Chemical structures of toxaphene congeners 26 and 50.

Generally, it can be deduced from the ER ratios of the technical toxaphene congeners that metabolization of nearly all members in this mixture is possible. Toxicant B (congener 26) seems to be the most stable congener, followed by congener 50. The coeluting congeners 42a and 42b (or possibly one of them) were preferentially metabolized compared to congeners 26 and 50. Congener 31, which showed varying ERs, and congener 32, with an almost racemic ratio of the antipodes, were found in skin, blood, and yolk samples, evidencing for both completely different degradation behaviors. All of these facts emphasize that a reproducible isolation and individual analysis of different toxaphene congeners in biota samples is an essential precondition and the only way to evaluate their toxicity adequately.

SAFETY

When working with applied toxaphenes, one should carefully consult their safety data sheets to ensure that regulations are complied with.

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Supporting Information Available: Description of the nomenclature of all applied toxaphene congeners including their chemical structures. This material is available free of charge via the Internet at http://pubs.acs.org.

LITERATURE CITED

(1) Vetter, W.; Oehme, M. Toxaphene, analysis and environmental fate. In New Types of Persistent Halogenated compounds, chapter 9. In *Handbook Environmental Chemistry, Anthropogenic Compounds*; Hutzinger, O., Paasivirta, J., Eds.; Springer: Heidelberg, Germany, 2000; Vol. 3, pp 237−287.

(2) Casida, J. E.; Holmstead, R. L.; Khalifa, S.; Knox, J. R.; Ohsawa, T.; Palmer, K. J.; Wong, R. Y. Toxaphene insecticide—complex biodegradable mixture. *Science* **1974**, *4124* (183), 520−521.

(3) Bidleman, T. F.; Jantunen, L. M. M.; Wiberg, K.; Harner, T.; Brice, K. A.; Su, K.; Falconer, R. L.; Leone, A. D.; Aigner, E. J.; Parkhurst, W. J. Soil as a source of atmospheric heptachlor epoxide. *Environ. Sci. Technol.* **1998**, *32* , 1546−1548.

(4) Kosubová, P.; Grabic, R.; Holoubek, I. Toxaphene and other chlorinated pestizides in the Czech Mountain and lowland ecosystems. *Fresenius' Environ. Bull.* **2005**, *14* (3), 160−166.

(5) Thron, K. U.; Bruhn, R.; McLachlan, M. S. The influence of age, sex, body-condition, and region on the levels of PBDEs and toxaphene in harbour porpoises from European waters. *Fresenius' Environ. Bull.* **2004**, *13* (2), 146−155.

(6) Parlar, H.; Nitz, S.; Gaeb, S.; Korte, F. Contribution to structure of toxaphene components—spectroscopic studies on chlorinated bornane derivates. *J. Agric. Food Chem.* **1977**, *25*, 68−72.

(7) Kimmel, L.; Angerhoefer, D.; Gill, U.; Coelhan, M.; Parlar, H. HRGCECD and HRGC-ECNI−SIM-HRMS quantification of toxaphene residues by six environmentally relevant chlorobornanes as standard. *Chemosphere* **1998**, *37*, 549−558.

(8) Norstrom, R. J.; Simon, M.; Muir, D. C. G.; Schweinsburg, R. E. Organochlorine contaminants in Arctic marine food chains—identification, geographical distribution and temporal trends in polar bears. *Environ. Sci. Technol.* **1988**, *22*, 1063−1071.

(9) Ekici, P.; Schulz-Jander, D.; Parlar, P. A short comment on the direct and indirect photolysis of toxaphene under tropospheric conditions. *Fresenius' Environ. Bull.* **2005**, *14* (3), 248−250.

(10) Bidleman, T. F.; Wideqvist, U.; Jansson, B.; Soderlund, R. Organochlorine pesticides and polychlorinated bihenyls in the atmosphere of southern Sweden. *Atmos. Environ.* **1987**, *21* (3), 641−654.

(11) Oehme, M.; Mano, S. The long-range transport of organic pollutants to the artic. *Fresenius' Z. Anal. Chem.* **1984**, *319* (2), 141−146.

(12) Atlas, E.; Giam, C. S. Global transport of organic pollutants—ambient concentrations in the remote marine atmosphere. *Science* **1981**, *211* (4478), 163−165.

(13) McHugh, B.; Glynn, D.; Nixon, E.; McGovern, E. *The Occurrence and Risk Assessment of the Pesticide Toxaphene in Fish from Irish Waters*; Marine Environment and Health Series 12; Marine Institute, Marine Environment and Food Safety Services: Abbotstown, Dublin, Ireland, 2003; ISSN 1649-0053, http://www.marine.ie/publicationsandlibrary/.

(14) Wiberg, K. Enantiospecific analysis and environmental behavior of chiral persistent organic pollutants (POPs). In *Enantiomers Are Non-super-imposable Mirror Images of Each Other*; Sigurdsson, B., Ed.; 2001; www.diva-portal.org/diva/getDocument?urn_nbn_se_umu_diva-9−1__fulltext.pdf.

(15) Herzke, D.; Gabrielsen, G. W.; Evenset, A.; Burkow, I. C. Polychlorinated camphenes (toxaphenes), polybrominated diphenyl ethers and other halogenated organic pollutants in glaucous gull (*Larus hyperboreus*) from Svalbard and Bjornoya (Bear Island). *Environ. Pollut.* **2003**, *121*, 293−300.

(16) Foreid, S.; Rundberget, T.; Severinsen, T.; Wiig, O.; Skaare, J. U. Determination of toxaphenes in fish and marine mammals. *Chemosphere* **2000**, *41* (4), 521−528.

(17) Kosubova, P.; Grabic, R.; Holoubek, I. (HR)GC-MS/MS analysis of toxaphene congeners in various matrices from the Czech environment. *Fresenius' Environ. Bull.* **2003**, *12* (11), 1303−1308.

(18) Voldner, E. C.; Li, Y. F. Global usage of toxaphene. *Chemosphere* **1993**, *27* (10), 2073−2078.

(19) Xu, L.; Hainzl, D.; Burhenne, J.; Parlar, H. HRGC-ECD and HRGC-NICI-SIM quantification of toxaphene residues in selected marine organism by environmentally relevant chlorobornanes as standard. *Chemosphere* **1994**, *28* (2), 237−243.

(20) Mueller, R. Bestimmung von Toxaphen- und PCB-Rueckstaenden in ausgewaehlten Nahrungsmitteln und Umweltproben. Dissertation, Technical University of Munich, 1998.

(21) Specht, W.; Tillkes, M. Gas-chromatic determination of pesticide-residues after clean-up by gel-permeation chromatography and mini-silicagel-column chromatography 5. Clean-up of foods and feeds of vegetable and animal origin for multiresidue analysis of fat-soluble and water-soluble pesticides. *Fresenius' Z. Anal. Chem*. **1985**, *322* (5), 443−455.

(22) Witte, J.; Buthe, A.; Ternes, W. Congener-specific analysis of toxaphene in eggs of seabirds from Germany by HRGC-NCI-MS using a carborane-siloxane copolymer phase (HT-8). *Chemosphere* **2000**, *41* (4), 529−539.

(23) Gantzer, C. J.; Wackett, L. P. Reductive dechlorination catalyzed by bacterial transition-metal coenzymes. *Environ. Sci. Technol.* **1991**, *25* (4), 715−722.

(24) Andrews, P.; Vetter, W. A systematic nomenclature system for toxaphene congeners. I. Chlorinated bornanes. *Chemosphere* **1995**, *31*, 3879−3886.

(25) Vetter, W.; Klobes, U.; Krock, B.; Luckas, B. Congener-specific separation of compounds of technical toxaphene on a nonpolar CP-Sil 2 phase. *J. Microcolumn Sep.* **1997**, *9*, 29−36.

(26) Kallenborn, R.; Huhnerfuss, H. Chiral xenobiotics in the environment. In *Chiral Environmental Pollutants Trace, Analysis and Ecotoxicology, 17*; Kallenborn, R., Huhnerfuss, H., Eds.; Springer: Berlin, Germany, 2001; pp 62−73.

(27) Kaltenecker, M.; Schwind, K.-H.; Uberschar, K.-H.; Hecht, H.; Petz, M. Transfer of toxaphene from animal feed to laying hens and their eggs—first results of a withdrawal study. *Organohalogen Compounds* **1998**, *35*, 281−286.

(28) Hoekstra, P. F.; Braune, B. M.; O'Hara, T. M.; Elkin, B.; Solomon, K. R.; Muir, D. C. G. Organochlorine contaminant and stable isotope profiles in Arctic fox (*Alopex lagopus*) from the Alaskan and Canadian Arctic. *Environ. Pollut.* **2003**, *122* (3), 423−433.

(29) Hoekstra, P. F.; O'Hara, T. M.; Pallant, S. J.; Solomon, K. R.; Muir, D. C. G. Bioaccumulation of organochlorine contaminants in bowhead whales (*Balaena mysticetus*) from Barrow, Alaska. *Arch. Environ. Contam. Toxicol.* **2002**, *42* (4), 497−507.

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