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Attempt to unravel the composition of toxaphene by comprehensive two-dimensional gas chromatography with selective detection

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Abstract

Comprehensive two-dimensional gas chromatography (GC×GC) coupled with micro electron-capture and time-of-flight mass spectrometric (TOF-MS) detection has been used to analyse technical toxaphene. An HP-1×HT-8 column combination yielded highly structured chromatograms and revealed a complex mixture of over 1000 compounds what is significantly higher number than in any study before. The analysis of a mixture of 23 individual congeners and TOF-MS evaluation of technical toxaphene showed that the chromatogram is structured according to the number of chlorine substituents in a molecule. The nature of the compounds (bornane and camphene) does not appear to have any influence. The sum of the peak areas of all congeners in each group was calculated using laboratory-written software; based on these results, the composition of technical toxaphene as a function of the number of chlorine substituents was provisionally calculated and was found that hepta- and octachlorinated compounds represents 75% of the total toxaphene area.

Keywords: Gas chromatography, comprehensive two-dimensional; Toxaphene; Terpenes; Organochlorine compounds

1. Introduction

In the past few years, comprehensive two-dimensional GC, or GC×GC, has emerged as an extremely powerful separation technique. Much higher peak capacities can be obtained than in conventional multidimensional GC, or GC–GC, because each successive small fraction eluting from the conventional-size first-dimension column is subjected, in real time, to a second, orthogonal separation, on a relatively short (ca. 0.5 m) second-dimension column with different separation characteristics. In most instances, a non-polar first column is combined with a more polar second column, and the time span of each effluent fraction from the first column that is trapped, refocused and, next, transported to the second-dimension column, is on the order of 3–6 s. The final result is usually displayed as a two-dimensional contour plot.

The first problem that had to be solved when GC×GC was introduced [1] was designing a robust and user-friendly interface between the two columns. Without going into details, it may suffice here to say that, with the introduction of nearly comprehensive

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high-speed diaphragm valve modulator [2] and truly comprehensive modulators like thermal (SWEEPER) [3] and especially cryogenic modulators [4–6], this problem has been adequately solved. The other main challenge was to demonstrate the dramatic increase in peak capacity from, characteristically, n^1+n^2 for heartcut-type GC–GC, to $n^1\times n^2$ for GC×GC, where ^{1} and ^{2} indicate the first and second column, respectively. Initially, this was mainly done with a variety of petrochemical samples, with the clear emergence of separate groups or bands of alkanes, naphthenes and aromatics as an illustrative example [7]. More recently, the GC×GC analysis of other classes of compounds has been shown to be equally rewarding, e.g., essential oils [8], fatty acid methyl esters (FAMEs) [9] and polychlorinated biphenyls (PCBs) [10]. Finally, while initially essentially all work was done with flame ionisation detectors because only these detectors provided a sufficiently fast response for the 3–6-s second-dimension separations, very recently the successful use of, almost equally rapid, micro electron-capture detection and time-of-flight mass spectrometry has been reported [10–12].

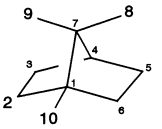
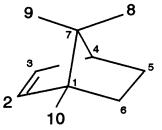
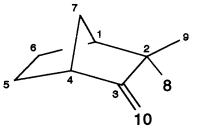
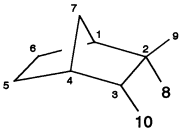
One further aspect which makes GC×GC especially attractive is the ordered structure of the two-dimensional chromatograms, which is observed when mixtures of related compounds, homologues or congeners are analysed. One good example are the series of chemical compounds present in petrochemical mixtures referred to above—other examples are the complex FAME mixtures typical of many fats and oils (ordering according to number of carbon atoms and double bonds) [9] and PCBs (ordering to number of chlorine atoms and their positions on the biphenyl ring) [10]. The distinct advantage is that, for unknown compounds, a structure can now be postulated on the basis of their position in the 2D-separation plane. While such an exercise can be performed fairly straightforwardly when a sufficient number of individual congeners, etc. is available—as is e.g., the case with PCBs—huge problems will be encountered when standards are not readily available, as is true for toxaphene.

Technical toxaphene is a complex mixture of polychlorinated monoterpenes produced by passing chlorine gas through a solution of technical-quality camphene (an isomerization product of α-pinene extracted from pine oils) in tetrachloromethane under UV irradiation. The resulting mixture contains 67–69% chlorine [13], corresponding with the empirical formula, $C_{10}H_{10}Cl_8$. The major constituents are chlorobornanes followed by chlorocamphenes, while chlorodihydrocamphenes and chlorobornenes (and bornadienes) are present as minor components [14–16]. The technical mixture also contains small amounts of other chlorinated hydrocarbons and non-chlorinated hydrocarbons [17]. The numbers of congeners that can, theoretically, be formed, and their carbon skeletons are shown in Table 1. In all probability, most of these compounds will not be present in the commercially available technical mixture, but the numbers quoted in the table may be considered a relevant reflection of the complexity that can be expected.

High-resolution GC has been used to study the composition of technical toxaphene. However, even with the separation performance then available, it is not possible at all to create an overall satisfactory separation. Typically, about 100 peaks show up in a GC–electron-capture detection (ECD) chromatogram of toxaphene. A better result is obtained by combining GC with another separation technique such as adsorption chromatography on silica [18] or active carbon [19], normal-phase LC [20], or GC–GC [21]. With these combined techniques, the number of compounds in technical toxaphene was estimated at, at least 177 [18], 246 [20], 300 [21], and 675 [19]. However, all the quoted methods were very time-consuming. For example, the 675-peak experiment required pre-fractionation into no less than 160 fractions with a subsequent 30-min GC analysis of each fraction.

With, on the one hand, the high complexity of toxaphene and, on the other hand, the impressive separation power of GC×GC in mind, we have made a first attempt to unravel the general composition of toxaphene by means of GC×GC. The selection of the stationary phases in the first- and second-dimension columns is one of the most important tasks when designing a GC×GC separation system. The main goal is not only to achieve a high separation efficiency but also to obtain ‘‘ordered’’ chromatograms. In a previous paper, we have demonstrated that the HP-1×HT-8 column combination delivered highly ordered chromatograms for PCBs based on the number of chlorine substituents of a compound

Table 1
Main classes of compounds present in toxaphene and their theoretically possible variety [14]

Class of compounds	Carbon skeleton	General formula	No. of congeners
Chlorinated bornanes		$C_{10}H_{18-x}Cl_x$	16 640
Chlorinated bornenes		$C_{10}H_{16-x}Cl_x$	n.c.[a]
Chlorinated camphenes		$C_{10}H_{16-x}Cl_x$	12 288
Chlorinated dihydrocamphenes		$C_{10}H_{18-x}Cl_x$	32 768

[a] Not calculated.

[10]. Although toxaphene is not as closely related to PCBs as is sometimes suggested (non-aromatic versus aromatic nature), this column combination, was evaluated for the analysis of toxaphene. ECD and time-of-flight mass spectrometry (TOF-MS) detection were used to obtain adequate selectivity.

2. Experimental

2.1. Reagents

A technical toxaphene mixture was obtained from Polyscience (Warrington, PA, USA). A standard mixture of 23 toxaphene constituents in cyclohexane (individual concentrations, 0.4 ng/μl) was purchased from Dr. Ehrenstorfer (Augsburg, Germany). The substitution patterns of the compounds present in the mixture according to IUPAC are given in Table 2. The well-known Parlar numbers and the eight-digit codes which provide essential structural information [22,23] are included in the table.

2.2. GC×GC–micro (μ) ECD

The GC×GC system was built from a HP 6890 (Hewlett-Packard, Wilmington, DE, USA) gas chromatograph equipped with a thermal modulator assembly (Zoex, Lincoln, NE, USA) consisting of a rotating slotted heater, a holder of the modulator tube, and a separate oven for temperature programming of the second-dimension column. Principles and working characteristics of the thermal sweeper modulator are extensively described in Ref. [3]. Helium gas (Hoek Loos, Schiedam, The Netherlands) with a purity of 99.999% was used as carrier gas through the GC×GC system at an inlet pressure of 50 p.s.i. The micro ECD (Hewlett-Packard) was operated at 300 °C, with 99.999% pure nitrogen (Hoek Loos) as make-up gas at a flow of 60 ml/min. One-μl samples were injected manually into a split/splitless inlet port (Hewlett-Packard) operated in the splitless mode at 260 °C; the purge time was 2 min. A 30-m×0.25-mm×0.25-μm HP-1 (100%-dimethylpolysiloxane) fused-silica column from Hew-

Table 2
Composition of 23-component standard mixture

Parlar no.	No. of Cl substituents	Acronym according to Wester et al. [22,23]	IUPAC name
11	6	C[032001]-(11)	2,2,3-*exo*,8,9,10-Hexachlorocamphene
12	6	C[021001]-(21)	2-*exo*,3-*endo*,8,8,9,10-Hexachlorocamphene
15	6	C[021011]-(11)	2-*exo*,3-*endo*,7,8,9,10-Hexachlorocamphene
21	7	B[30030]-(012)	2,2,5,5,9,10,10-Heptachlorobornane
25	7	C[032001]-(21)	2,2,3-*exo*,8,8,9,10-Heptachlorocamphene
26	8	B[12012]-(202)	2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,8,10,10-Octachlorobornane
31	8	C[032001]-(22)	2,2,3-*exo*,8,8,9,9,10-Octachlorocamphene
32	7	B[30012]-(111)	2,2,5-*endo*,6-*exo*,8,9,10-Heptachlorobornane
38	8	B[30030]-(022)	2,2,5,5,9,9,10,10-Octachlorobornane
39	8	B[32012]-(111)	2,2,3-*exo*,5-*endo*,6-*exo*,8,9,10-Octachlorobornane
40	8	B[12012]-(112)	2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,9,10,10-Octachlorobornane
41	8	B[21020]-(122)	2-*exo*,3-*endo*,5-*exo*,8,9,9,10,10-Octachlorobornane
42a	8	B[30012]-(211)	2,2,5-*endo*,6-*exo*,8,8,9,10-Octachlorobornane
42b	8	B[30012]-(121)	2,2,5-*endo*,6-*exo*,8,9,9,10-Octachlorobornane
44	8	B[20030]-(122)	2-*exo*,5,5,8,9,9,10,10-Octachlorobornane
50	9	B[12012]-(212)	2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,8,9,10,10-Nonachlorobornane
51	8	B[30030]-(112)	2,2,5,5,8,9,10,10-Octachlorobornane
56	9	B[30012]-(212)	2,2,5-*endo*,6-*exo*,8,8,9,10,10-Nonachlorobornane
58	9	B[32030]-(112)	2,2,3-*exo*,5,5,8,9,10,10-Nonachlorobornane
59	9	B[30012]-(122)	2,2,5-*endo*,6-*exo*,8,9,9,10,10-Nonachlorobornane
62	9	B[30030]-(122)	2,2,5,5,8,9,9,10,10-Nonachlorobornane
63	9	B[21022]-(212)	2-*exo*,3-*endo*,5-*exo*,6-*exo*,8,8,9,10,10-Nonachlorobornane
69	10	B[30032]-(122)	2,2,5,5,6-*exo*,8,9,9,10,10-Decachlorobornane

lett-Packard and a 1-m×0.1-mm×0.1-μm HT-8 (8% phenyl (equiv.) polycarborane-siloxane) from SGE International (Ringwood, Australia) were used as first- and second-dimension columns, respectively. A 7-cm×0.1-mm×3.5-μm 007-1 (100%-methylpolysiloxane) capillary column (Quadrex, Deerfield, IL, USA) was used as modulator tube. The head of the first column was connected directly to the injector and its outlet, via an 8-cm×0.1-mm I.D. fused-silica deactivated column (BGB Analytik, Adliswil, Switzerland), to the modulator tube. The head of the second-dimension column was connected to the modulator tube via a 6-cm×0.1-mm I.D. deactivated column and its end to the detector, also via a deactivated column (10-cm×0.1-mm I.D.). Micro and mini press-fits (Techrom, Purmerend, The Netherlands) were used for the connections. The slotted heater temperature was set 100 °C above the temperature of the first oven. The GC×GC operating programme, ver. 2.0z (Zoex) was used for the sweeper control and data acquisition. The modulation period was 6.5 s at a rotating speed of 0.15 rev/s, and with a pause time of 0.4 s. The data acquisition rate was 50 Hz. Transform software (Fortner Research, Sterling, VA, USA) was used for data visualisation and evaluation.

2.3. GC×GC–TOF-MS

The GC×GC system was built from a HP 6890 instrument (Hewlett-Packard) equipped with a longitudinally modulated cryogenic system (LMCS; Ph. Marriott, RMIT, Melbourne, Australia) consisting of a moving cryogenic CO_2 chamber and a separate oven for temperature programming of the second-dimension column. Principles and operation of the longitudinally modulated cryogenic system are extensively described in Ref. [4]. Helium (Hoek Loos) with a purity of 99.999% was used as carrier gas through the GC×GC system at a constant flow of 1.3 ml/min. One-μl samples were injected using an HP 7673 autosampler (Hewlett-Packard) into an Optic 2 programmable injector (ATAS, Veldhoven, The Netherlands) operated in the splitless mode at 260 °C; the purge time was 1 min. A Pegasus II time-of-flight mass spectrometer (LECO, St. Joseph,

MI, USA) was used as detector and was operated at a spectrum storage rate of 10 Hz, using a mass range of m/z 45–550, an ion source temperature of 200 °C, an ionisation energy of 70 eV, a transfer-line temperature of 300 °C and a multi-channel plate voltage of −1900 V. A 10 m×0.25-mm×0.25-μm DB-1 (100%-dimethylpolysiloxane) fused-silica column purchased from J&W Scientific (Folsom, CA, USA) and a 1-m×0.1-mm×0.1-μm HT-8 (8% phenyl (equiv.) polycarborane-siloxane) from SGE were used as the first- and second-dimension columns, respectively. The head of the first column was connected directly to the injector and its outlet, via a 12-cm×0.1-mm I.D. fused-silica deactivated column, to the second-dimension column. The outlet of the latter column was connected to the detector via an 18-cm×0.1-mm I.D. fused-silica deactivated column to allow the entire second column to be put into the separate oven. The columns were connected by means of a press-fit connector (Techrom). Data processing was performed using LECO Pegasus II software, version 1.10. For data transformation, evaluation and visualization, homemade software was used which enables the conversion of the raw data into a 2D array, to generate contour plots from this array and to perform, in combination with the Pegasus II software, mass spectra evaluation directly from the contour plots.

3. Results and discussion

3.1. GC×GC–μECD analysis of technical toxaphene

A typical GC×GC–μECD chromatogram is shown in Fig. 1. Because of the huge differences in concentration of the individual congeners in technical toxaphene, it is difficult to visualise major and minor peaks in the same contour plot. Therefore, Fig. 1 also contains an overlay by a so-called apex plot in which only the positions of the peak apexes are displayed. The overall improvement of the separation

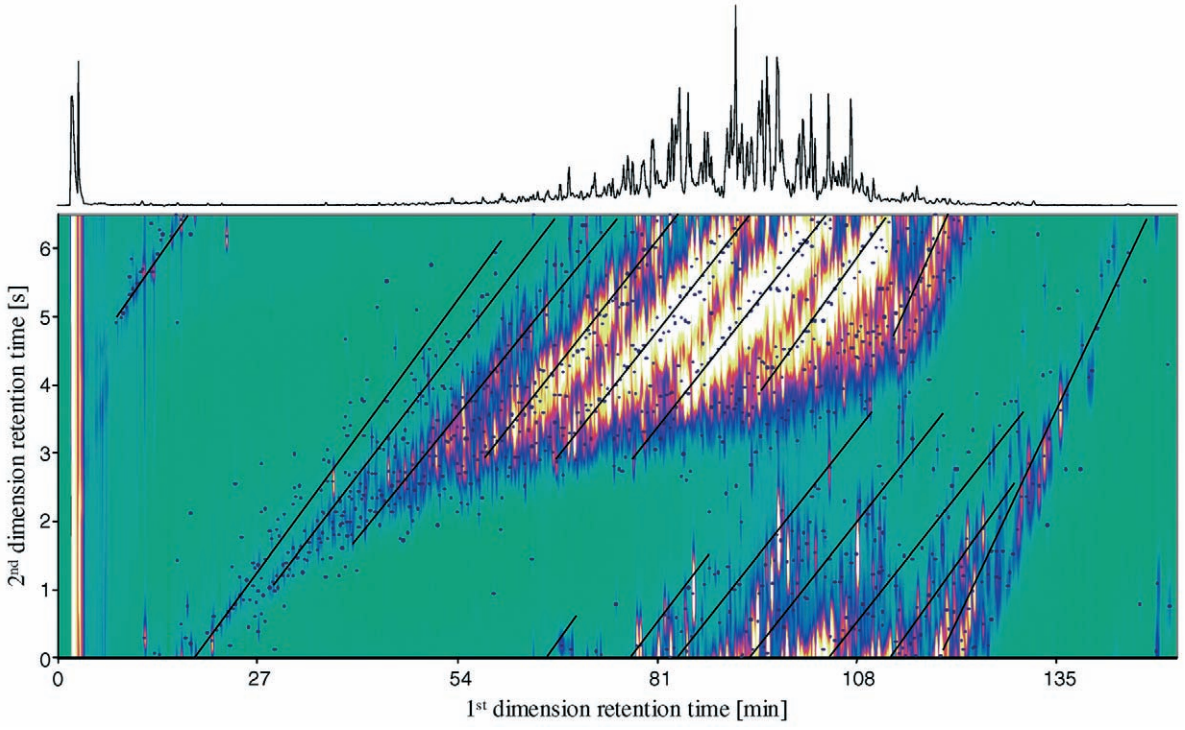


Fig. 1. GC×GC–μECD contour plot and overlaid apex plot (black dots) of technical toxaphene and reconstructed one-dimensional chromatogram. Temperature programme: 90 °C (2 min), at 5 °C/min to 110 °C, then at 1 °C/min to 220 °C (35 min) for the first oven, and 110 °C (2 min), at 5 °C/min to 130 °C, then at 1 °C/min to 240 °C (35 min) for the second oven.

of the mixture compared with the (reconstructed) 1D GC run shown at the top of the figure is impressive.

The HP-1 column used as first-dimension column has volatility-based selectivity, i.e., it will separate the toxaphene congeners by boiling point. As can be seen from Fig. 1, the toxaphene constituents elute in a broad temperature range—the first components elute at about 8 min, which corresponds to 112 °C, while the last components elute after 116 min, that is, at an elution temperature of 220 °C. The HT-8 column used in the second dimension has a polarity- and shape-based selectivity, and strong retention of the toxaphene congeners is observed. Although the second column was held at a temperature 20 °C higher than that of the first column during the entire temperature programme, wrap-around—indicated by second-dimension retention times being lower for later, than for earlier eluting peaks (see lower right-hand part of chromatogram)—of higher boiling congeners is observed. However, such wrap-around is no problem for either qualitative or quantitative analysis provided that later eluting peaks in the second dimension do not co-elute with earlier eluting peaks, which is true in this case.

As is indicated by the straight lines in Fig. 1, one can clearly observe an ordered structure of the chromatogram. There are several parallel groups of peaks except for one at the end of the chromatogram, which shows a distinctly different slope. This is caused by the fact that compounds comprised in this group elute during the final isothermal part of the run, while all other peaks elute during the temperature gradient. One suggestion could be to use a higher final temperature to avoid this phenomenon. However, with the sweeper modulator the maximum oven temperature is limited by the stability of the stationary phase in the modulator tube, where a ca. 100 °C higher temperature is applied than in the oven to sweep the trapped analytes into the second column.

The total analysis time was 140 min because a very slow temperature gradient of 1 °C/min was used. Steeper gradients of 2 and 3 °C/min were also tested; however, with these gradients a modulation into only one or two fractions per peak was achieved, while it is well-known that at least four modulations are required to maintain the first-dimension resolution [24] and, even more importantly, no order was observed anymore in the chromatogram. As can be seen from Fig. 1, the parallel groups of peaks are not completely separated from each other, and there is some co-elution between adjacent groups. This reflects the unusually high complexity of the toxaphene sample and the close structural relationship of the constituent compound classes (cf. Table 1). In addition, the distances between two groups are not constant through the chromatographic plane: they increase with increasing first-dimension elution times. In the lower left-hand part of the chromatogram it is, therefore, rather difficult to indicate groups ‘‘by line’’ and it may well be true that there are one or more additional (sub-)groups here. Based on earlier experiences with PCB separations [10], one may assume that, also here, separation occurs according to the number of chlorine substituents. In order to prove this, a number of pure standards should be analysed. At this time, the best option available is to use the commercial mixture of 23 congeners (cf. Table 2) and to apply mass spectrometric detection.

3.2. GC×GC–TOF-MS analysis of 23-congener standard mixture

In order to study the 2D retention characteristics and mass spectrometric fragmentation of the individual toxaphene components, the 23-congener standard mixture was analysed by GC×GC–TOF MS. For this part of the work, an LMCS modulation system was made available to us, which allowed a higher final programme temperature of 280 °C (there is no modulator tube in this set-up). The temperature gradient used was 1.5 °C/min; in addition, a shorter first-dimension column was utilised to allow elution of all analytes within 2 h, because that was the maximum run time available for unattended operation of the MS system. A GC×GC chromatogram together with the reconstructed 1D chromatogram is shown in Fig. 2. Under the selected conditions GC×GC completely resolved 19 of the 23 compounds present in the mixture and only the congener pairs P40/41 and P42a/42b were not separated. Significantly improved separation compared to 1D GC was achieved for the congener pairs P32/31, P51/50 and P41/42a/b and, also relevant, between toxaphene congeners such as P58, P59, P62 and P63 and

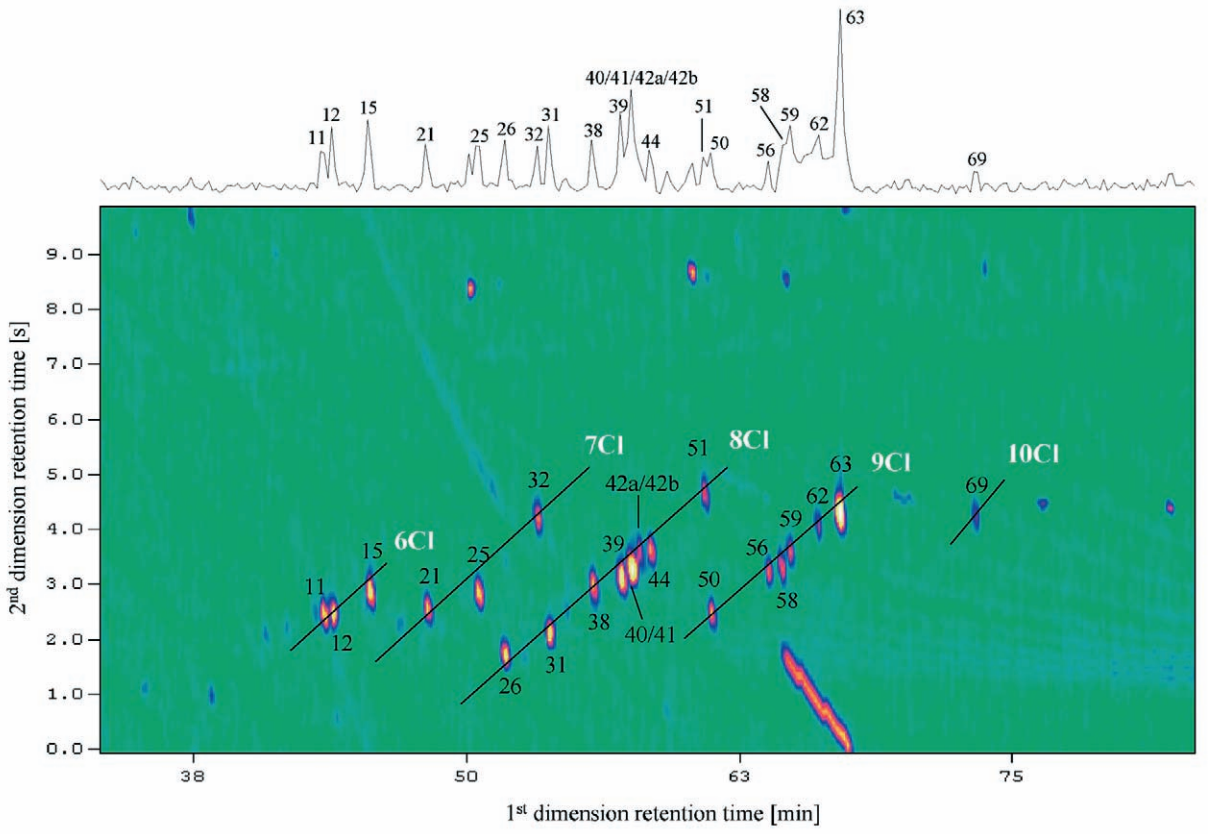


Fig. 2. Total-ion GC×GC–TOF-MS chromatogram and reconstructed 1D chromatogram of standard mixture of 23 toxaphene congeners. Temperature programme: 100 °C (2 min), at 10 °C/min to 130 °C, then at 1.5 °C/min to 280 °C (4 min) for the first oven, and 120 °C (2 min), at 10 °C/min to 150 °C, then at 1.5 °C/min to 300 °C (4 min) for the second oven.

impurities present in the standard mixture. Such improved separation is, of course, highly important when quantification is performed. The ordered structure of Fig. 2 fully confirms the assumption made above that each group of peaks comprises congeners with the same number of chlorine substituents. As regards compound classes, the mixture of standards contains five chlorinated camphenes (cf. Wester codes in Table 2) next to the chlorinated bornanes. Fig. 2 clearly shows that these two classes are not separated from each other; this is indicated by the results for the hexa-, hepta- and octa-substituted compounds, even through the hexachloro data are slightly less convincing because of the absence of bornanes from this series. To all probability, the position of the congeners within a group depends on both the substitution pattern on the six-membered ring and that of the methyl groups [25]. However, at the present time, no general conclusion can be drawn (as was possible for PCBs [10]), because the number of available congeners is too small.

3.3. GC×GC–TOF-MS analysis of technical toxaphene

Finally, the technical toxaphene mixture was analysed by GC×GC–TOF MS. A full-scan chromatogram is shown in Fig. 3a. Two remarks should be made here. Firstly, although a higher sample concentration was used than for GC×GC–μECD, low-boiling compounds such as tetra- and lower substituted congeners were not observed. This may well be due to a lower sensitivity of TOF MS for these compounds. Secondly, in the right-hand part of

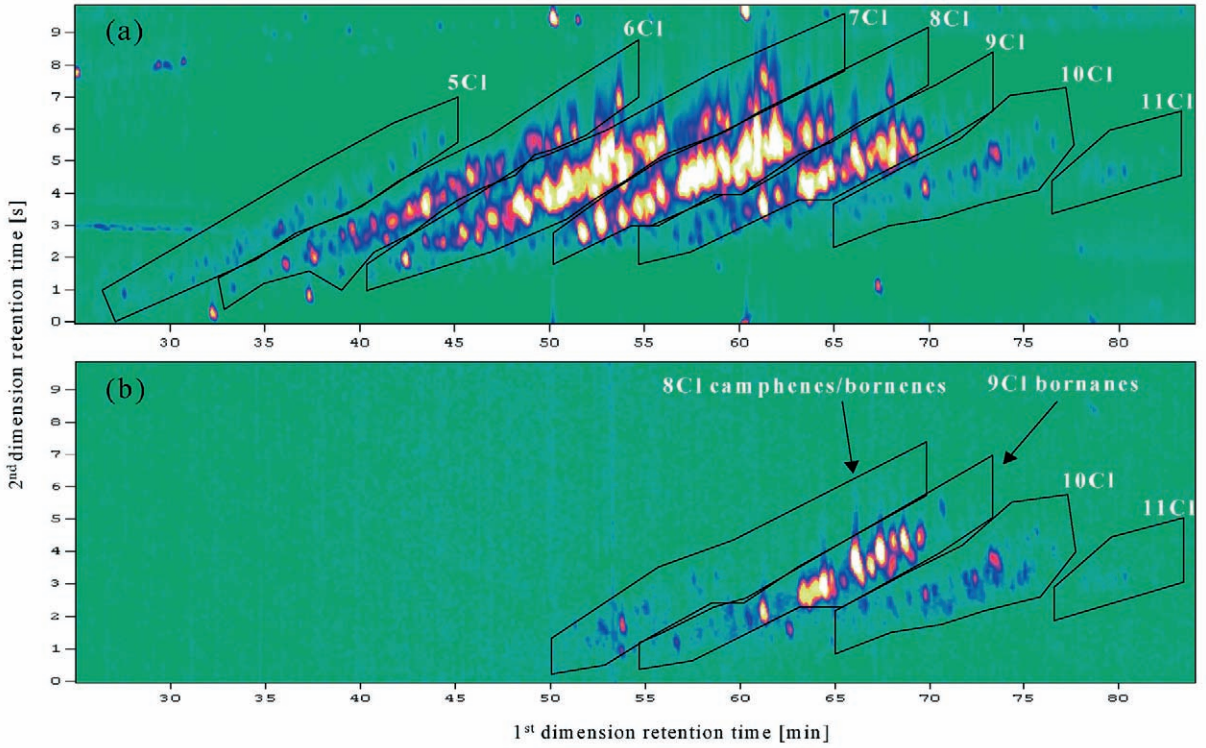


Fig. 3. GC×GC–TOF-MS chromatogram of technical toxaphene. (a) m/z 45–550 total ion chromatogram with polygons drawn by laboratory-written software to calculate sum of peak areas of individual congener groups, (b) extracted ion chromatogram for m/z 413. Temperature programme: 100 °C (2 min), at 10 °C/min to 130 °C, then at 1.5 °C/min to 280 °C (4 min) for the first oven, and 120 °C (2 min), at 10 °C/min to 150 °C, then at 1.5 °C/min to 300 °C (4 min) for the second oven.

the chromatogram, i.e., in the high-boiling region, searches for more highly substituted congeners showed a minor contribution of undecachlorinated congeners which are indicated in the figure and are seen to be part of the ''non-parallel group'' in the GC×GC–μECD chromatogram.

The separate visualisation of each group present in the mixture can be achieved by selecting the proper extracted ion traces of the GC×GC–TOF MS record. Straightforward use of this visualisation presupposes the presence of unique m/z values for each group—a condition which is not always fulfilled. The electron ionisation (EI) mass spectra of the major component class—bornane congeners—are characterised by complex fragmentation patterns. For bornanes, molecular ions are normally absent and major fragment ions in the high mass range are produced by the sequential loss of a combination of Cl, HCl, $CHCl_2$ and/or CH_2Cl (Fig. 4a). Therefore, $[M–Cl]^+$ ions are used for the selective monitoring of bornanes. For the present study, this implies that m/z 275, 309, 343, 377, 413, 447 and 481 were used to detect bornanes with 5 up to 11 chlorine substituents. However, one should be aware of some complications caused by the presence of camphenes, the second major compound class in the toxaphene mixture, and so-called ''mass leakage'' or ''cross-over'' problems [26]. These are exemplified in Fig. 3b which shows the extracted ion chromatogram of m/z 413, the $[M–Cl]^+$ ion of nonachlorobornanes. Because in contrast with bornane spectra, EI mass spectra of camphenes are characterised by the presence of the molecular ion (Fig. 4b), the octachlorocamphenes also showed up in Fig. 3b. Moreover, it was reported that the third major compound class in the toxaphene mixture, bornenes, cannot be easily distinguished from camphenes by electron impact MS [27]. Therefore, it is very likely that

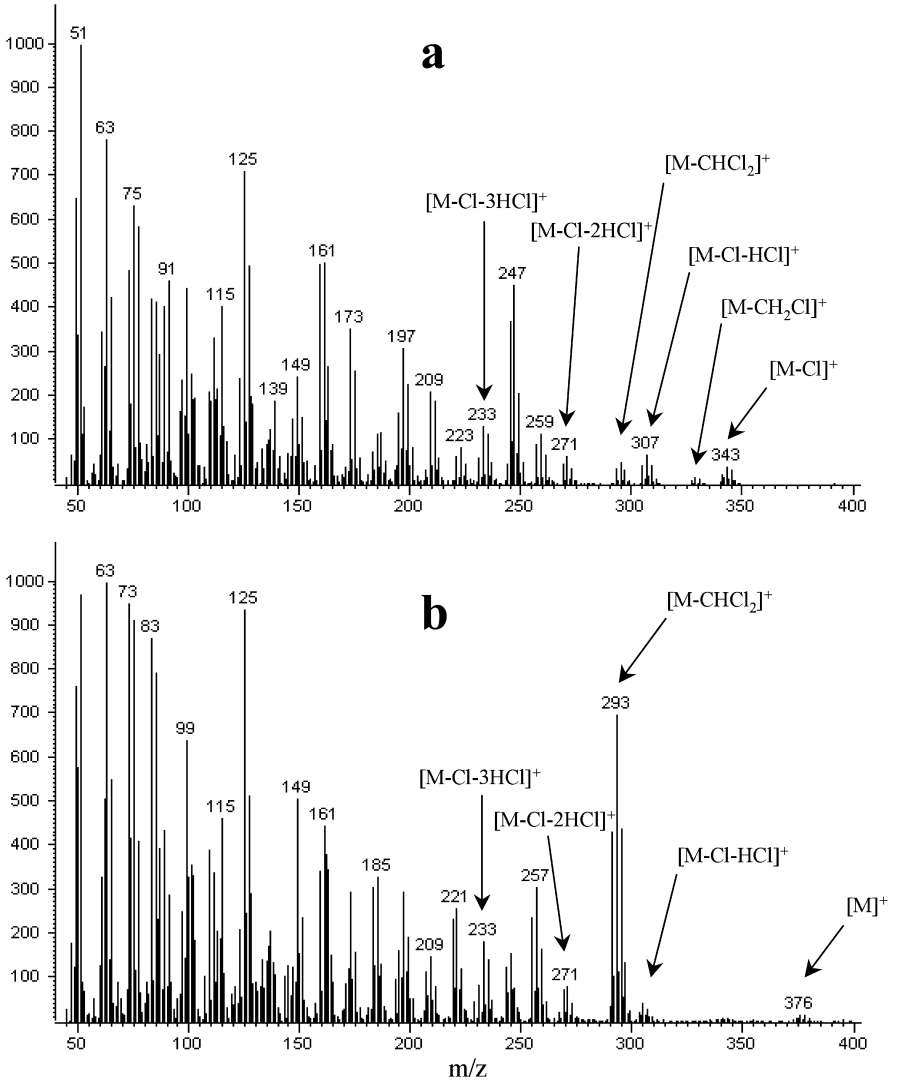


Fig. 4. Electron-ionisation TOF mass spectra of (a) heptachlorinated bornane (P21), and (b) heptachlorinated camphene (P25).

octachlorobornenes also showed up in the same group with octachlorocamphenes. However, the $[M-Cl]^+$ have a very low abundance in camphene spectra and likely also in bornene spectra. Therefore, in Fig. 3b, the cluster of nonachlorinated compounds represents only nonachlorobornanes. It has to be added here, that only a few chlorocamphenes and their mass spectra are known, and it is not sure that all congeners will form molecular ions.

Next to the nonachlorinated bornane and octachlorinated camphene/bornene clusters, two more groups showed up in Fig. 3b: deca- and undecachlorinated compounds. This is due to isotope peaks from the $[M-Cl-HCl]^+$ and $[M-Cl-2HCl]^+$ ions, the ''mass leakage'' referred to above. Because these fragments are present in spectra of both chemical classes, the groups 10Cl and 11Cl in Fig. 3b contain both compound classes. Such selective ion mass chromatograms were visualized for all $[M-Cl]^+$ masses and the same behaviour was observed in all instances. Always one group of lower chlorinated camphenes/bornenes, one selected group of bornanes and all higher chlorinated groups were present in the chromatograms for these ions. This underlines

the statement made above that, with the present GC×GC set-up, the toxaphene congeners are separated into groups with the same number of chlorine atoms in the molecule. It has to be added that technical toxaphene also contains minor amounts of dihydrocamphenes, which further complicates the overall picture. This topic was not studied here, because no standards were available.

Finally, one important conclusion stands out here: even the first stage of unravelling the overall composition of a mixture as complex as technical toxaphene requires the use of GC×GC, and of mass spectrometric detection, to visualize the strongly overlapping bands of the toxaphene congeners substituted with different numbers of chlorine atoms.

3.4. Composition of toxaphene

One interesting challenge is to reveal how many congeners are ''at least'' present in toxaphene. In this study, the number of individual peaks that showed up in the GC×GC–μECD chromatogram (cf. Fig. 1), was found to be 1010. This is a considerable improvement compared to the numbers reported in earlier studies, which ranged between 200 and 675 (cf. Section 1). The difference becomes even clearer when we consider that it took only 2–3 h to obtain the GC×GC chromatogram, while more than 80 h were required for the 675-peak assessment. For our evaluation, the μECD was preferred to the TOF MS chromatogram because a more efficient first-dimension column was used in the former instance and also, of course, because of the distinctly higher sensitivity for toxaphene congeners already referred to above. Unfortunately, until now no software is available which enables the automated counting of individual peaks in the 2D plane. Therefore, counting was done manually from the 2D plane visualized by Transform software. Each local maximum higher than five times the signal-to-noise ratio was considered as a peak. Localizing the individual 3D-peak apexes allowed us to construct a so-called apex plot of the GC×GC–μECD run which is shown as an overlay in Fig. 1. One should add that, in the full-scan GC×GC–TOF MS chromatogram of Fig. 3a, several peaks were visible in the top and bottom parts of the chromatogram which obviously belonged to none of the chlorinated-congener groups. On the basis of their mass spectra, these peaks were identified as minor contaminants present in the solvent used, isooctane, such as phthalates and other esters. Of course, they were not included in the total peak count.

The ordered structure of the GC×GC chromatograms enable to read directly from the 2D picture the number of congener groups with different degrees of chlorination present in the mixture. The range of chlorination found in GC×GC with TOF MS was 5 to 11 substituents per molecule. The maximum agrees with the upper limit of chlorination degree proposed by Vetter and Scherer [28]. The minimum degree of chlorination, on the other hand, reflects a lack of TOF MS detectability because at least two more congener groups are observed in the GC×GC–μECD chromatogram. They comprise tetra- and tri-substituted toxaphene constituents.

Finally, an attempt was made to estimate the weight percentage of the individual penta- to undeca-substituted congener groups in technical toxaphene. For this purpose, home-made software that can handle the LECO TOF MS data files was created. This software enables to draw manually polygons around the peak groups of interest and to calculate the sum of the areas of all peaks appearing within each polygon. The polygons for all groups present in the GC×GC–TOF MS chromatogram are shown in Fig. 3a. The results, which are presented in Table 3, were compared with those given by Saleh [17], who used GC–MS. The mutual differences are seen not to be too large, with hepta- and octa-substituted con-

Table 3
Mass percentage of di- to undeca-substituted congener groups in technical toxaphene

No. of Cl substituents	Mass percentage by:	
	GC×GC–TOF-MS	GC–MS [17]
2		2.5
3		0.2
4		0.4
5	1.1	3.7
6	8.5	14
7	33	26
8	42	38
9	14	7.8
10	1.3	2.5
11	0.1	0.6

geners representing 60–70% of the total mass in both cases. However, it is also true that degree of chlorination is found to be higher with the present technique (or for the present sample) than in Saleh's study. Hexa- to nonachlorinated compounds are seen to represent some 97% of the total toxaphene peak area based on the GC×GC–TOF MS data, as against 85% based on the GC–MS data. Although in our study lower chlorinated congeners were not visible (cf. above), knowledge regarding the synthesis of toxaphene [14] suggests that the contribution of these lower chlorinated compounds will not significantly alter the calculated percentages of the Table 3.

4. Conclusions

The use of comprehensive two-dimensional GC substantially improves the quality of toxaphene analysis, not only compared to 1D GC but also compared to two-dimensional separations such as LC–GC or heart-cut GC–GC. Over 1000 congeners could be distinguished in technical toxaphene after a run which took less than 2–3 h (although we have to admit that, partly because of the software problems referred to above, the evaluation of the 2D chromatogram took 5 days). The HP-1×HT-8 column combination provides a separation of toxaphene components into groups according to the number of chlorine substituents, while there apparently is very little, if any, dependence on the class of components (bornanes or camphenes). Hexa- to nonachlorinated compounds were found to be the major components of toxaphene and represent some 97% of the total toxaphene mass. This satisfactorily agrees with earlier findings reported in the literature, which were based on GC–MS using a set of selected m/z values. At the same time, it is evident that much more research will be required to unravel the composition of toxaphene in more detail. However, we are convinced that, if pure standards of more individual congeners become available, the present technology will be up to the task of solving the analytical problems.

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