PCB - toxaphene group separation on silica for interference free quantitation of toxaphene residues in environmental samples by GC/ECD

Bernd Krock, Walter Vetter, and Bernd Luckas
Institut für Ernährung und Umwelt, Friedrich-Schiller-Universität Jena,
Dornburger Str. 25, D-07743 Jena, Germany

1. Introduction

Polychlorinated monoterpenes were first produced in the USA in 1945, and subsequently also in other countries. They were distributed under trade names such as Toxaphene®, Strobane®, Melipax® or Delicia Fribal® and used as pesticides[1]. Nevertheless in scientific language residues of polychlorinated monoterpenes in the environment were usually named „toxaphene". Therefore, toxaphene is both a trade mark and the trivial name for this kind of compounds. The global usage of toxaphene since 1950 was estimated to be more than 1.3 million tons[2]. Although toxaphene was banned in many western countries from the seventies on, it is still used in some third world countries particularly in cotton, soy bean and peanut plantations[3].
More than 670 compounds of technical toxaphene (CTTs) were identified in technical mixtures[4] and most of the CTTs were identified as polychlorinated bornanes with 5 to 12 chloro atoms[5]. Some of these CTTs are very persistent and accumulate in the lipophilic compartments of marine organisms, others are more or less metabolized[6].
In marine mammals 2 CTTs are dominating[7,8] and a recent study showed that 11 CTTs were abundant in different seal species and 6 of them could be assigned to structures using single CTT reference standards[9]. Fish are known to metabolize toxaphene less than marine mammals[10, 11]. Due to differences in the toxaphene patterns in technical mixtures and biota, toxaphene residue analysis requires the availability of appropriate CTT single standards[12].
Since the IUPAC-names of CTTs are very long and have led to misunderstandings in the past we chose the code names presented by Andrews and Vetter[13] and the Parlar numbers[14] in brackets.
In Germany, 3 toxaphene indicator congeners, B8-1413 (Parlar #26), B9-1679 (Parlar #50), and B9-1025 (Parlar #62), were selected to monitor CTT residues in fish and other foodstuffs[15].
Since GC/ECD was suggested as the detection method a pre-separation of PCBs is necessary for a precise quantitation of CTTs. The German Federal Institute for Health Protection of Consumers and Veterinary Medicine (BgVV) suggested the method of Specht and Tillkes[16] which was slightly modified by Fürst et al.[17] to separate aromatic from the more polar aliphatic organochlorines. This clean-up procedure is part of a multicomponental residue analysis procedure which also allows a group separation of phosphorus pesticides and carbamates[16].
Unfortunately, even the modified method did not elute all CTTs in one fraction. Particularly B8-1413 (Parlar #26) eluted into 2 fractions[15] and this major CTT had to be quantified in 2 fractions.
The aim of this work was to modify the method of Alder and Vieth[15] in order to achieve the following goals: a) all CTTs should elute into one fraction, b) the bulk of PCBs should be separated in order to exclude co-elutions with CTTs, c) the method should be suitable for routine residue analysis of CTTs, and d) the method should be compatible with standard analysis methods for organochlorine compounds.

2. Material and methods

Origin of samples
The herrings were fished in the Baltic Sea in November 1994 and gut by the Institut für Fischereiwesen Mecklenburg-Vorpommern in Born, Germany. The grey seal was found dead at the shore of Rügen, Baltic Sea, Germany and a blubber sample was kindly donated by Dr. K. Harder, Museum für Meereskunde und Fischerei, Stralsund, Germany.

Solvents and chemicals
All chemicals were of analytical grade. Perchloric acid (70%), acetic acid (100%), sulphuric acid (98%), anhydrous sodium sulphate, and silica gel 60 were obtained from E. Merck (Darmstadt, Germany), toluene from VEB Petrolchemisches Kombinat Schwedt (Schwedt, former GDR), and n-hexane for residue analysis from Promochem (Wesel, Germany). The CTT single standard compounds B8-1413 (Parlar #26), B9-1679 (Parlar #50), B8-531 (Parlar #39), B8-1414 (Parlar #40), B8-1945 (Parlar #41), B8-806/809 (Parlar #42) 1 ng/µl each were obtained from Dr. Ehrenstorfer (Augsburg, Germany). B7-1453 (TOX7) was formerly isolated from Melipax[18]. PCB-Mix I (PCB # 28, 52, 101, 138, 153, 180; 10 ng/µl each) was obtained from Dr. Ehrenstorfer and PCB #77, 105, 118, 126, 146, 156, 167, 169, 170, and 189 10 ng/µl each were obtained from Promochem (Wesel, Germany).

Sample clean-up
Samples were cleaned-up as described earlier[19]. In short, 93 g herring and 2 g seal blubber were homogenized in perchloric/acetic acid, the organochlorines were twice extracted with n-hexane and the fatty matrix was subsequently destroyed with sulphuric acid. Finally, the pesticide extract was purified on deactivated silica (30 % water). However, for separation of CTTs and PCBs any other clean-up will do as well.

Separation of CTTs and PCBs on activated silica
Silica was activated at 130°C over night. 8.0 g of the freshly activated silica were weighed and immediately slurred in n-hexane and poured into a 200 x 10 mm glass column equipped with a frit and a 50 mL solvent reservoir. Silica was topped with a 1 cm anhydrous sodium sulphate layer. Samples were evaporated to approximately 0.5 mL (n-hexane) and set on the column. The column was eluted with 48 mL n-hexane (fraction HX) for separation of PCBs and followed by 50 mL n-hexane/toluene 65 : 35 (v/v) (fraction HT) for CTTs.

Gas chromatography/electron capture negative ionization mass spectrometry (GC/ECNI-MS)
An HP 5890 Series II plus gas chromatograph (Hewlett-Packard, Ratingen, Germany) equipped with an HP 5989B mass spectrometer was employed for the identification of CTTs and PCBs. ECNI was carried out at an ion source temperature of 200°C and an ion source pressure of 1.6 mbar using methane as the reactant gas. The ion source was tuned for optimum performance with perfluoro-tributylamine (PFTBA) at m/z 264, 302, and 414. In the full scan mode mass spectra were recorded 12 min after injection within a mass range of m/z 50 - 500. The gas chromatographic conditions were as follows: separation on a 50 m x 0.25 mm i.d. fused silica capillary column coated with 0.25 µm CP-Sil 2 CB (Chrompack, Middelburg, The Netherlands), carrier gas: helium at 36.8 cm/sec flow velocity (constant flow); injection: 1 µL splitless; injector temperature: 250°C; transfer line: 250°C; temperature program: 80°C for 1 min, then 20°C/min to 180°C, 1 min, then 2°C/min to 240°C, 25°C/min to 275°C, 21.60 min (total run time: 60 min).

Gaschromatography/electron capture detector (GC/ECD)
An HP 5890 Series II gas chromatograph (Hewlett-Packard, Ratingen, Germany) equipped with an HP 7376 autosampler and two ECDs was used to control the fractionation of CTTs on silica. The gas chromatographic conditions were as follows: split after injector on two columns: 50 m x 0.25 mm i.d.

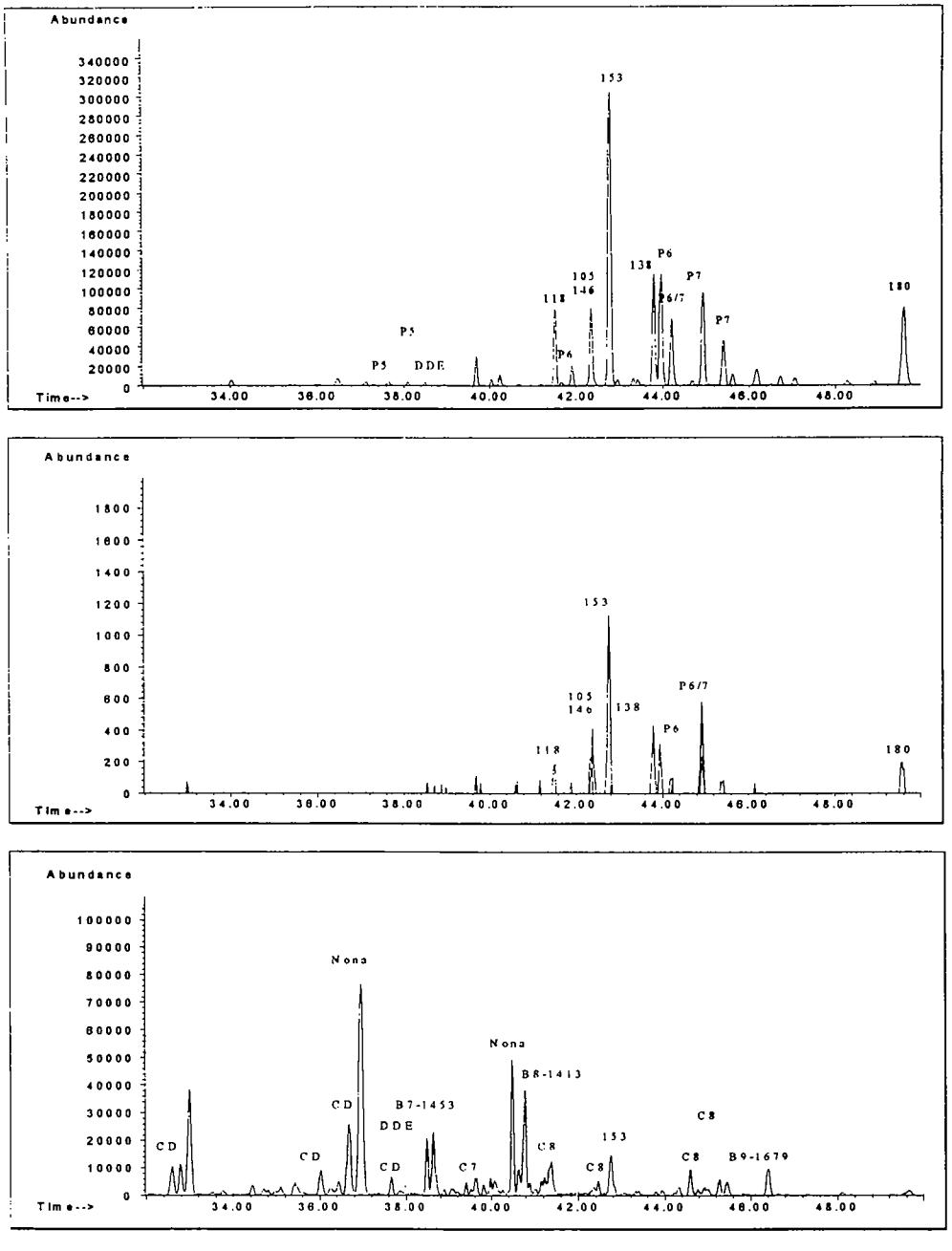

Figure 1 GC/ECNI-MS chromatograms of a grey seal sample from the Baltic Sea
above: Fraction HX, total ion chromatogram
middle: Fraction HT, ion tracks: 256, 292, 326, 260, 394, and 430 m/z
down: Fraction HT, total ion chromatogram

fused silica capillary columns each, coated with 0.25 µm CP-Sil 19 CB and 0.25 µm CP-Sil 8C/20% C18 CB respectively (both: Chrompack, Middelburg, The Netherlands), carrier gas: nitrogen at 0.8 bar head pressure, injection: 1 µL splitless, injector temperature: 250°C, detector temperature: 300°C, temperature program: 75°C for 1.5 min, then 15°C/min to 180°C, 4 min, then 2°C/min to 240°C, 1 min, then 20°C/min to 275°C, 25 min (total run time: 70.25 min).

3. Results and discussion

A precise quantitation of CTTs by GC/ECD requires a separation from PCBs, which may occur in much higher amounts in environmental samples. Former experience in the isolation of organochlorines showed that columns filled with 50 g silica were suitable to separate compounds of even comparable polarity[18,20]. However, routine residue analysis requires an economical consumption of material and solvents. Since the analytical method according to Specht and Tillkes using 1 g silica deactivated with 1.5 % water did not yield all CTTs in one fraction, a compromise between the possible and the necessary had to be found. The goal of our investigations was to obtain all CTTs in one fraction. We found that fractionation of sample extracts on 8.0 g activated silica with 48 mL n-hexane and 50 mL n-hexane/toluene 65 : 35 (v/v) was such a compromise. The seven CTTs tested eluted in the order B8-1413 (Parlar #26), B7-1453 (TOX7), B9-1679 (Parlar #50), and finally together B8-531 (Parlar #39), B8-1414 (Parlar #40), B8-1945 (Parlar #41), and B8-806/809 (Parlar #42). Even in environmental samples with a more complex CTT composition B8-1413 always was the first eluting CTT which could be detected by GC/ECNI-MS. Therefore, B8-1413 can be used as an indicator congener for the optimization of the volumes of fraction HX and fraction HT on silica. The optimum volume of fraction HX was 48 mL n-hexane which did not elute CTTs even with high contaminated samples (see below). Testing the seven CTTs B8-1413 started to elute at 6 mL of fraction HT. However, if mixed with PCBs, CTTs elute significantly earlier, however, not before 48 mL of fraction HX. Under these conditions only small amounts of PCBs eluted together with the CTTs in fraction HT.
16 PCBs (#28, 52, 77, 101, 105, 118, 126, 138, 146, 153, 156, 167, 169, 170, 180, and 189) were chromatographed on 8.0 g activated silica. Five of them (#28, 52, 77, 105, and 126) smeared into fraction HT. According to this mainly lower chlorinated and non-ortho-chlorinated PCBs are longer retained on silica. However, lower chlorinated PCBs with up to 4 chloro atoms elute rather early from GC-columns and so merely interfer with CTTs, whereas non-ortho-substituted PCBs are found in very low concentrations in environmental samples and, therefore, do not interfer either. Since we noted that the presence of other organochlorines had a strong influence on the elution of CTTs, we chose two samples highly contaminated with organochlorines, i.e. herring and grey seal blubber, from the Baltic Sea, in order to validate this method. Fraction HX contained HCB, OCS, PCNs, DDE, and PCBs. Fraction HT contained the more polar chlorinated pesticides like HCHs, chlordanes, nonachlor, DDT-group, CTTs, and, traces of PCBs (see Figure 1). In fraction HT of the herring sample only PCB #105 was detected, whereas in fraction HT of the seal sample were found PCB # 105, 118, 138, 146, 153, 180, 3 unidentified hexachloro biphenyls, and 2 heptachloro biphenyls were detected in very low concentrations (see Figure 1). However, in GC/ECD chromatograms only traces of PCB 153 could be identified, while several CTTs could be identified.

4. Conclusions

The method presented allows a routine residue analysis of all CTTs, because of the separation from interfering PCBs by a silica column with economical use of silica and solvents. Furthermore, the higher amount of silica of our method in comparison to methods using only 1 g deactivated silica[15-17] gives a better reproducibility, because overloading of the column is prevailed. If a more complete separation of PCBs and CTTs is desired, this method can easily be changed by higher fillings of the column with silica and bigger elution volumina using B8-1413 as indicator congener for fractionation.

5. References

1) Saleh M.A. (1991): Toxaphene: chemistry, biochemistry, toxicity and environmental fate. Rev. Environ. Cont. Toxicol. 118, 1-85
2) Voldner E.C. and Y.F. Li (1995): Global usage of selected persistent organochlorines. Sci. Total Environ. 160/161, 201-210
3) Bidleman T.F., M.T. Zaranski, and M.D. Walla (1988): Toxaphene: usage aerial transport and deposition. *In:* Schmidtke N.W. (Ed.): Toxic Contamination in Large Lakes, Vol.I, Lewis Publishers, Chelsea, pp 257-284
4) Jansson B. and U. Wideqvist (1983): Analysis of toxaphene (PCC) and chlordane in biological samples by NCI mass spectrometry. Intern. J. Environ. Anal. Chem. 13, 309-321
5) Saleh M.A. (1983): Capillary gaschromatography-electron impact and chemical ionization mass spectrometry of toxaphene. J. Agric. Food Chem. 31, 748-751
6) Casida J.E., R.I. Holmstead, S. Khalifa, J.R. Knox, T. Ohsawa, K.J. Palmer, and R.Y. Wong (1974): Toxaphene insecticide: a complex biodegradable mixture. Science 183, 520-521
7) Luckas B., W. Vetter, P. Fischer, G. Heidemann, and J. Plötz (1990): Characteristic chlorinated hydrocarbon patterns in the blubber of seals from different marine regions. Chemosphere 21, 13-19
8) Stern G.A., D.C.G. Muir, C.A. Ford, N.P. Grift, E. Dewailly, T.F. Bidleman, and M.D. Walla (1992): Isolation and identification of two major recalcitrant toxaphene congeners in aquatic biota. Environ. Sci. Technol. 26, 1838-1840
9) Vetter W., B. Krock, and B. Luckas (1995): Congener specific determination of compounds of technical toxaphene (CTTs) in different Antarctic seal species, submitted
10) Musial C.J. and H.F. Uthe (1983) Widespread occurrance of the pesticide toxaphene in Canadian east coast marine fish. Intern. J. Environ. Anal. Chem. 14, 117-124
11) Parlar H. (1991): Chlorierte Bornan-Derivate - eine neue Klasse umweltrelevanter Chemikalien. Nachr. Chem. Tech. Lab. 39, 26-37
12) Burhenne J., D. Hainzl, L. Xu, B. Vieth, L. Alder, and H. Parlar (1993): Preparation and structure of high-chlorinated bornane derivatives for the quantification of toxaphene residues in environmental samples. Fresenius J. Anal. Chem. 346, 779-785
13) Andrews, P., W. Vetter (1995): A systematic nomenclature system for toxaphene congeners. Part 1: chlorinated bornanes. Chemosphere 31(8), 3879-3886
14) Parlar, H., D. Angerhöfer, M. Coelhan, L. Kimmel (1995): HRGC and HRGC-ECNI determination of toxaphene residues in fish with a new 22 components standard. Organohalogen Compounds 26, 357-362
15) Alder L. and B. Vieth (1996): A congener-specific method for the quantification of camphechlor (toxaphene) residues in fish and other foodstuffs. Fresenius J. Anal. Chem. 354, 81-92
16) Specht W. and M. Tillkes (1985): Gaschromatographische Bestimmung von Rückständen an Pflanzenbehandlungsmitteln nach Clean-up über Gel-Chromatographie und Mini-Kieselgelsäulen-Chromatographie. Fresenius Z. Anal. Chem. 322, 443-455
17) Fürst P., C. Fürst, and W. Groebel (1989): Zur Analytik polychlorierter Terpene in fetthaltigen Lebensmitteln am Beispiel von Fischölen. Dtsche. Lebensm. Rdschau. 85/9, 273-279
18) Krock B., W. Vetter, and B. Luckas (1996): Structure elucidation of a main heptachloro congener of toxaphene in marine organisms after isolation from Melipax. Chemosphere, in press
19) Vetter W., C. Natzeck, B. Luckas, G. Heidemann, B. Kiabi, and M. Karami (1995): Chlorinated hydrocarbons in the blubber of a seal (Phoca caspica) from the Caspian Sea. Chemosphere 30, 1685-1696
20) Vetter W. and B. Luckas (1995): Synthesis, isolation, and chromatography of perdeuterated α-1,2,3,4,5,6-hexachlorocyclohexane. J. High Resol. Chromatogr. 18, 643-646