A New Semiautomatic Method Based on GPC/HPLC Techniques for GC-ECD and GC-MS Analysis of Toxaphene in Environmental Samples

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Besides the ubiquitous "classical" organohalogens such as DDT, DDE, hexachlorocyclohexanes (HCHs), dieldrin, polychlorinated biphenyls (PCBs), an additional group of lipophilic anthropogenic substances, the polychlorinated norbornanes, was detected in various environmental samples during the last decade due to advances in analytical methodology. Technical toxaphene, one of the most heavily used pesticide in the United States and other parts of the world, constits of an extremely complex mixture of several hundreds polychlorinated C_{10} terpenes predominately chlorinated bornanes with 5-12 chlorine atoms and an average elemental composition of $C_{10}H_{10}Cl_8$[1]. When DDT was banned in the early 70ies, toxaphene replaced DDT as major agricultural insecticide. From 1947 to 1985 more than $1{\cdot}10^6$ t have been primarily applied in cotton insect control, while other uses included soy beans, rape, vegetables plantations and control of external insects on livestock[2]. In the beginning of the 80ies many countries have introduced application restrictions for toxaphene in account to its persistence in the environment, toxicity toward some non-target organisms, and ability to accumulate in biota. Toxaphene concentrations in fish and marine mammals are often comparable to those of other organochlorine compounds such as PCBs and some DDT metabolites[3]. Many other typical cotton-growing countries including the former USSR, India, Mexico, and the former GDR have continued to use chlorinated bornanes in agriculture. In order to evaluate the extent and magnitude of the potential environmental threat of toxaphene, an accurate and reproducible analytical method is needed. Characterization of biological samples for toxaphene is complicated by several problems[5]:

1. The congeners of the pesticide mixture cannot be completely separated by high resolution gas chromatography (HRGC).
2. Other coextracted xenobiotics including PCBs, DDT and its homologues, and certain chlordane components which interfere seriously in the determination of the compounds of interest must be separated from toxaphene to allow accurate and reproducible identification and quantification.
3. Because of differing accumulation behaviour and decomposition rates of individual toxaphene congeners only a limited number of toxaphene compounds are found in the environmental samples compared to the industrial mixture[4].
4. The lack of pure single isomers standards required that quantification has to be carried out with the technical mixture as reference. Only recently it has become possible to quantify toxaphene residues in marine organisms by commercially available purely isolated chlorinated bornane derivatives[4].

Toxaphene congeners are masked by large quantities of PCBs which are present in samples from the North and Baltic Sea. Therefore a powerful clean-up needs to be carried out prior to chromatographic determination of toxaphene to eliminate or decrease interferences and natural lipophilic materials. Numerous techniques have been suggested for the removal of co-extractives e.g. gel-permeation chromatographic separation[6] and adsorption chromatography involving alumina[7], silicagel[4], and Florisil[8] microcolumns. These materials suffers from several disadvantages such as lot-to-lot variation, relatively poor column

efficiency, slow elution, and high consumption of solvents. In the last 10 years the possibilities of high performance liquid chromatography (HPLC) clean-up for the purpose of efficiently separating pesticides in environmental samples have been demonstrated by many applications[9,10]. This highly sophisticated analytical method offers the advantage of high reproducibility, low consumption of solvents, high efficiency, and higher sample loading capacity.
The intent of this work was to investigate the use of a simple normal-phase HPLC column for the efficient separation of toxaphene from PCBs and other usual organochlorine pesticides, with sufficient procedural simplicity for routine analytical application.
The presented method relies on an analytical procedure developed in our laboratory for the routine determination of PCBs and chlorinated pesticides in a wide range of environmental samples[11].

EXPERIMENTAL

Experiments were carried out with solutions in n-hexane of several classes of compounds:

(a) Technical mixture of toxaphene and purely chlorinated bornane derivatives (Parlar No. 26,32,50,62,69)

(b) PCB congeners (No. 28,52,101,138,153,180) and Clophen A 50

(c) Pesticides (α,β,γ-HCH, hexachlorobenzene (HCB), pentachlorobenzene (PCBz), heptachlor (HC), heptachlor epoxide (HE), dieldrin, aldrin, octachlorostyrene (OCS), pp'-DDE, pp'-DDD, pp'-DDT, oxychlordane, cis/trans-chlordane, cis/trans-nonachlor)

Pesticide solutions were eluted on a stainless-steel column (120 x 4 mm I.D.) packed with the following materials, respectively:

- Hypersil silica gel, 5 µm
- Lichrospher Si 60, 5 µm
- Lichrospher NH_2, 5 µm
- Lichrospher CN, 5 µm
- Nucleosil NO_2, 5 µm

n-Hexane was used as the mobile phase at a flow-rate of 1 ml/min. The eluate was collected in fractions and analysed by GC-electron-capture detection (ECD).

The present method was applied to herring gull egg samples which were collected from the North and Baltic Sea by the German Environmental Specimen Banking. The samples were mixed with anhydrous sodium sulfate/seasand to form a free flowing product which is extracted with n-hexane/acetone in an extraction column. The majority of the lipid and biogenic material was removed from the raw extract using size exclusion chromatography. Afterwards the collected pesticide fraction were precleaned by a HPLC step to eliminate the major part of polar impurities, that might be present. More details about the applied procedures are given in[11]. Then, the pesticide fraction was concentrated to about 2 ml in a Kuderna-Danish (K.-D.) concentrator for further normal-phase liquid-chromatographic fractionation. The HPLC-system constits of a semipreparative column (Lichrospher Si 60, 125 x 8 mm I.D.) and a precolumn (30 x 4 mm I.D.) filled with the same material. Two HPLC fractions were obtained using n-hexane (0-26 ml) at a flow-rate of 2 ml/min and 30% dichloromethane in n-hexane (26-62 ml) at a flow-rate of 4 ml/min. Finally the column was back-flushed for cleaning with 100% dichloromethane. The first eluate contained PCBs and lower polarity chlorinated pesticides (HCB, PCBz, HC, aldrin, pp'-DDE, OCS). The second fraction contained toxaphene and the more polar chlorinated pesticides (α,β,γ-HCH, HE, dieldrin, chlordane compounds, pp'-DDD, pp'-DDT, and little amounts of pp'-DDE). The fractions were reduced in volume with a K.-D. concentrator for gas chromatographic analysis.
GC analysis was performed with the following temperature programme: 60°C (0 min) - 10°C/min - 180°C (1 min) - 4°C/min - 250°C (0 min) - 10°C/min - 280°C (14 min), in the splitless injection mode and at an injection temperature of 230°C.

RESULTS AND DISCUSSION

Silica gel column chromatography has been reported to remove PCBs from most of the pesticides[8]. We investigated several normal-phase column materials of different polarity with n-hexane as mobile phase. Our aim was to completely separate toxaphene from PCBs and other organics in a relatively small range of elution volume. Therefore, we chose a column material that provides high capacity factors in terms of the compounds of interest and rapidely elutes PCBs congeners. As can be seen from Figure 1, Hypersil silicagel (surface: 170 m^2/g) is less suitable for the separation of PCBs from toxaphene in comparison to Lichrospher Si 60 (surface: 650 m^2/g) on account to the lower surface.

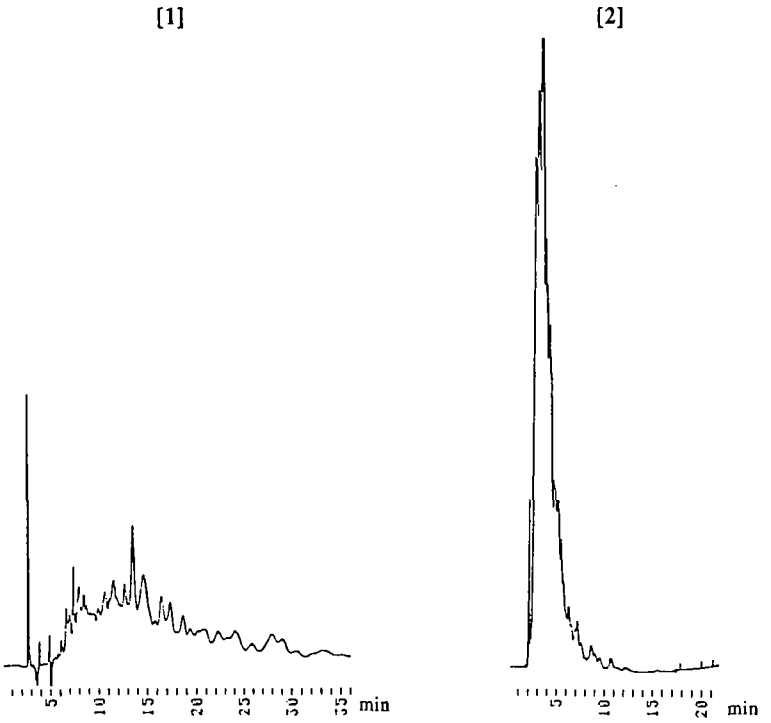

Figure 1: UVchromatogram (230 nm) of a technical toxaphene mixture separated on a Lichrospher Si 60 column (120 x 4 mm I.D.) [1] and a Hypersil column (120 x 4 mm I.D.) [2]. Mobile phase: n-hexane; flow-rate: 1 ml/min

Amino-, cyano- and nitro-modified stationary phases were not capable of completely separating PCBs from toxaphene because of their weak polarity. Under the experimental conditions described here, toxaphene is virtually totally retained for 26 ml n-hexane by Lichrospher Si 60 material, while PCBs are rapidely eluted in the first fraction. The "toxaphene" fraction also contains HCHs, chlordane components, dieldrin, pp'-DDD, pp'-DDT, and HE. These substances, however, do not generally interfere seriously in the final determination by capillary gaschromatography. Recoveries of all compounds investigated were between 90-100%. The separation by HPLC is very time-saving and runs automatically using an auto-sampler and a fraction collector. In addition, the column can be back-flushed for cleaning and multiple use purposes.

Herring gull eggs from the North and Baltic Sea, which are highly contaminated by PCBs, were purified as described before. Figure 2 shows the ECD chromatogram containing several signals of toxaphene.

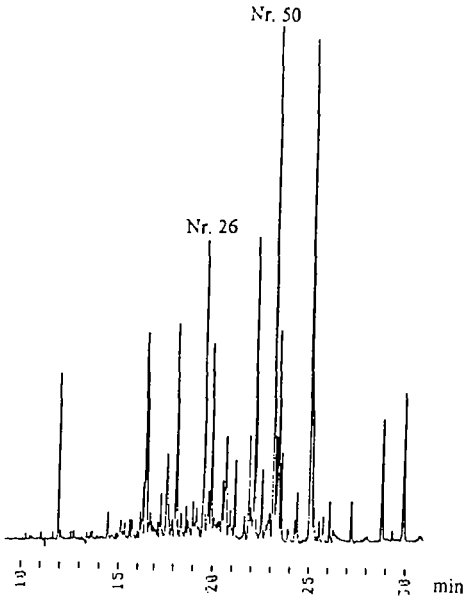

Figure 2: GC/ECD chromatogram of a herring gull egg extract from the Baltic Sea after clean-up by the presented method. (1) Parlar No. 26, (2) Parlar No. 50
Instrument: Carlo Erba HRGC 5160, fused silica capillary column DB-5 (0.32 mm x 30 m)
Carrier gas: H_2

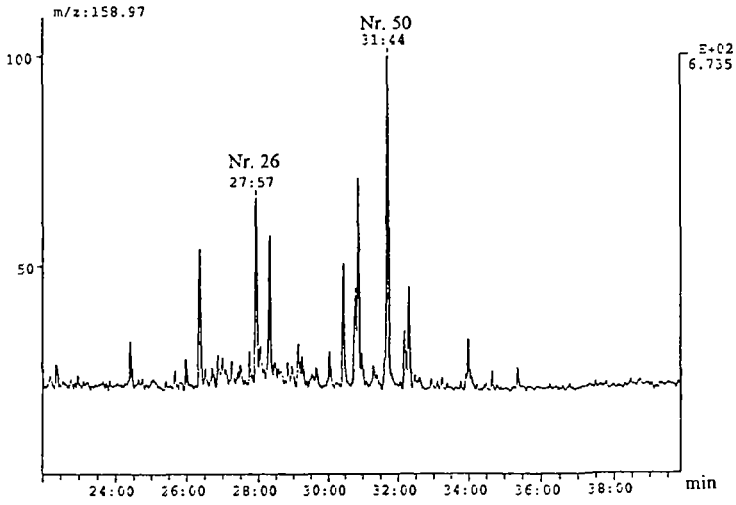

Figure 3: GC-MS/SIM (m/z:158.97) chromatogram of a herring gull egg extract from the Baltic Sea after clean-up by the presented method. (1) Parlar No. 26, (2) Parlar No. 50
Instrument: Finnigan 8230 (EI mode: 70 eV).
Fused silica capillary column: DB-5-MS (0.25 mm x 60 m); Carrier gas: He
Interface temperature: 260°C; R: 3000

Octachlorobornane Parlar No. 26 and nonachlorobornane Parlar No. 50 were identified with the help of purely isolated chlorinated bornane derivatives and the use of GC columns of different selectivity. It is known that both compounds are the most abundant toxaphene congeners in marine mammals and fish[12]. Toxaphene congeners in peaks measured by GC with ECD were ascertained by the use of GC with a high resolution sector field MS in the SIM-EI mode monitoring the signal representative of the toxaphene compounds (m/z:158.97) (see Fig. 3). Furthermore, the identity of the toxaphene residues was confirmed by negative chemical-ionization MS (MS/NCI) in the full-scan mode, which is much more sensitive and selective than EIMS.

In addition to Parlar No. 26 and 50, three signals of octachlorobornanes and one heptachlorobornane identified by mass spectra constitute the major proportion of the total toxaphene residues observed by GC-MS/NCI analysis of herring gull egg extracts (see Fig. 4).

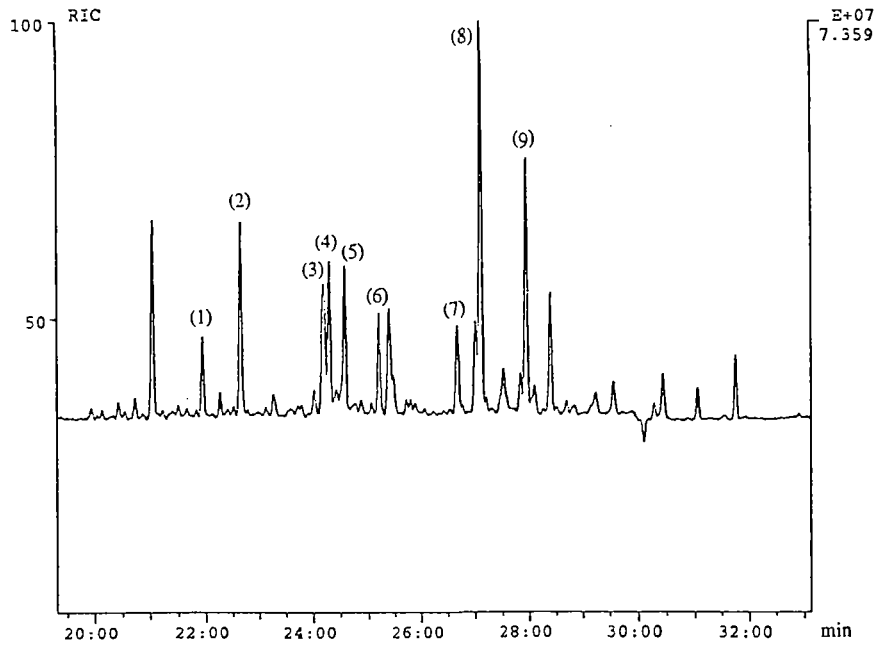

Figure 4: GC-MS/NCI total ion current chromatogram of a herring gull egg extract from the Baltic Sea after clean-up by the presented method.
(1) cis-chlordane, (2) trans-nonachlor, (3) Parlar No. 26, (4) heptachlorobornane, (5) octachlorobornane, (6) cis-nonachlor, (7) octachlorobornane, (8) octachlorobornane, (9) Parlar No. 50
Instrument: Finnigan SSQ 7000 MS; CI gas: CH_4; source temperature: 150°C
Fused silica capillary column: DB-5-MS (0.25 mm x 30 m); Carrier gas: He

CONCLUSION

This study shows that a simple HPLC silicagel column employed with the described elution system is well suited for separating large excess of interfering compounds from toxaphene for the purpose of accurate analysis by GC-ECD and GC-MS. The clean-up procedure presented here is reproducible and efficient.

Investigations on the application of the method to environmental samples have indicated the versalitity of the column.

REFERENCES

1. Saleh M. A. Toxaphene: Chemistry, biochemistry, toxicity and enviromental fate
   Rev. Environ. Contam. Toxicol. (1991):118:1-66
2. Bidleman T.F., Zaranski M.T., Walla M.D. Toxaphene: Usage, arial transport and deposition,
   in N.W. Schmitke (Ed.), Toxic Contamination in Large Lakes, Volume I, Lewis Publishers, Chelsa, pp
   257-284
3. Parlar H. Chlorierte Borman-Derivate - eine neue Klasse umweltrelevanter Chemikalien
   Nachr. Chem. Tech. Lab. (1991);39:26-37
4. Xu L., Hainzel D., Burhenne J., Parlar H. HRGC-ECD and HRGC-NCI/SIM quantification of
   toxaphene residues in selected marine organism by environmentally relevant chlorobornanes as
   standard
   Chemosphere (1994);28:237-243
5. Ribick M.A., Dubay G.R., Petty J.D., Stalling D.L., Schmitt C.J. Toxaphene residues in fish:
   Identification, quantification, and confirmation at part per billion levels
   Environ. Sci. Technol. (1982);16:310-318
6. Brumley W.C., Brownrigg C.M. Determination of toxaphene in soil by electron-capture negative ion
   mass spectrometry after fractionation by high-performance gel permeation chromatography
   J. Chromatogr. (1993);633:177-183
7. Atuma S.S., Jensen S., Mowrer J., Oera U. Separation of lipophilic substances in environmental
   samples with special references to toxaphene
   Intern. J. Environ. Anal. Chem. (1982);24:213-225
8. Onuska F.I., Terry K.A. Quantitative high resolution gas chromatography and mass spectrometry of
   toxaphene residues in fish samples. J. Chromatogr. (1989);471:161-171
9. Petrick G., Schulz J.C., Duinker J.C. Clean-up of environmental samples by high-performance liquid
   chromatography for analysis of organochlorine compounds by gas chromatography with electron-
   capture detection. J. Chromatogr. (1988);435:241-248
10. Schantz M.M., Parris R.M., Kurz J., Ballschmiter S.A. Comparison of methods for the gas-
    chromatographic determination of PCB congeners and chlorinated pesticides in marine reference
    materials. Fresenius J. Anal. Chem. (1993);346:766-778
11. Oxynos K., Schmitzer H., Duerbeck H.W., Kettrup A. Specimen Banking, Springer Verlag, (1992)
    Berlin Heidelberg
12. Vetter W., Luckas B., Oehme M. Isolation and purification of the two main toxaphene congeners in
    marine organisms. Chemosphere (1992);25:1643-1652