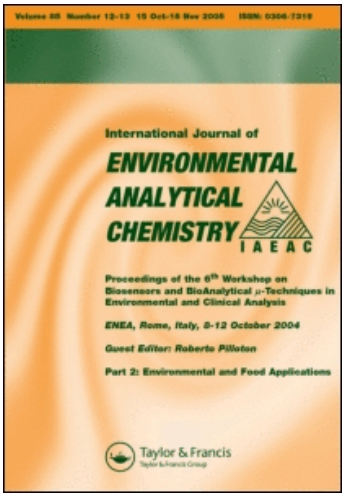

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TOXAPHENE (POLYCHLORINATED CAMPHENES) ANALYSIS IN HUMAN BLOOD

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A method for the determination of toxaphene in whole blood using gas chromatography (GC)-electron capture negative ion ECNI/MS and pure polychlorobornane congerners has been evaluated. Blood samples were extracted with hexane/acetone (9:1), extracts defatted with sulfuric acid, analytes fractionated on Carbopack-C/Florisil (or silica gel) SPE columns then concentrated to final volumes for GC-ECNI/MS analysis. For confirmation, interference from PCBs and other organochlorine pesticides were removed by nitration.

KEY WORDS: Blood, Toxaphene, ECNI/MS.

INTRODUCTION

Toxaphene (a broad spectrum organochlorine pesticide) was first manufactured in 1945 by chlorination of camphene to give thousands of components, consisting mainly of polychlorobornanes (CHBs, 76%), polychlorobornenes (18%), polychlorobornadienes (2%) containing from six to ten chlorine substituents[1-2]. Technical toxaphene has a chlorine content of 67–69%. Theoretically there are 32, 768 possible congeners for the bornane skeleton and 16, 458 possible congeners for Cl_6, Cl_7, Cl_8 and Cl_9 substituted bornanes[3]. In the early 1980's, toxaphene use was restricted in USA, Canada and other West European countries because of its toxicity, environmental persistence and bioaccumulating capabilities[1,3,5]. However, it is still being manufactured and heavily used in Africa as well as Pakistan, India, Egypt, Russia and Nicaragua[1,5].

Since the first introduction of Toxaphene in 1940's, the annual production levels of toxaphene consistently surpassed that of PCBs. It is one of the major environmental contaminants of concern in the Canadian Arctic food web[6], Great Lakes fish[7] and the Canadian East Coast[8]. Recently, some Toxaphene congeners (e.g. T_2 and T_{12})[6] have been reported in breast milk of Inuit women from Northern Quebec at concentration levels similar to DDE and PCB congener 153[9].

Currently, Toxaphene analysis and quantification from environmental samples are being performed with gas chromatography (GC)/electron capture detection (ECD), GC-high resolution mass spectrometry (HRMS) and GC-electron capture negative ion (ECNI)/MS techniques[6-11]. Several groups have reported that the composition and pattern of environmental samples is different from that of technical Toxaphene standard, which further complicates the problem of Toxaphene quantification[6-11]. The determination of

Toxaphene by GC-ECNI/MS has become the preferred method being both more sensitive and selective[10,11]. Taking into consideration the above concerns, we have used the individual chlorobornane congeners and ECNI/MS for monitoring Toxaphene levels in whole blood.

EXPERIMENTAL

Toxaphene standard (Hercules Inc USA) was provided by Dr. P. Andrews of Food Research Division, Health Protection Branch. Toxaphene congeners namely, 2-exo, 3-endo, 5-exo, 6-endo,8,8,10,10-Cl_8 bornane (#1 or T2; Tox 8; Parlar 26)[6,12]; 2,2,5-endo, 6-exo,8,9,10-Cl_7 bornane (#2, TB)[12]; Cl_8 bornane (#3); 2-exo, 3-endo, 5-exo, 6-endo, 8,8,9,10,10-Cl_9 bornane (#4 or T12, Tox 9; Parlar 50); Cl_9 bornane (#5); 2,2,5-endo, 6-exo,8,8,9,10,10-Cl_9 bornane (#6); 2,2,5,5,8,8,9,10,10-Cl_9 bornane (#7 or Parlar 62); 2-exo,3-exo,5-endo,6-exo,8,8,9,10,10-Cl_9 bornane (#8); 2,2,3-exo,5-endo,6-exo, 8,8,9,10,10-Cl_{10} bornane (#9); 2,2,5,5,6-exo,8,8,9,10,10-Cl_{10} bornane (#10) and 2,2,3-exo, 5,5,8,8,9,10,10-Cl_{10} bornane (#11) were obtained as a mixture (61.8 pg/μl) from Prof. H. Parlar, University of Kassel, Germany). The organochlorine pesticides namely, *cis*-chlordane, *trans*-chlordane, *cis*-nonachlor were purchased from Accustandard, *trans*-nonachlor was a gift from Health Protection Branch and oxychlordane was obtained from Agriculture Canada. PCB congener $^{13}C_{12}$ 153 was purchased from Cambridge Isotope Laboratories and deca chlorobiphenyl (PCB # 209) was purchased from Aldrich. The organic solvents, hexane (BDH Omni Sol. and Anachemia), acetone (Burdick and Jackson), and dichloromethane (DCM) (Anachemia) were glass distilled and free from interfering residues as tested by GC-MSD after concentration from 15 mL to 50 μL. All glassware was washed twice with acetone, hexane and heated at 500°C for 20 h. Carbopack-C (60–80 mesh) was purchased from Supelco Canada. Octafluoronaphthalene (OFN) was supplied by Hewlett Packard. Nitric acid (70–71%, reagent) and sulfuric acid (reagent) were purchased from J.T. Baker.

Sample analysis was performed on Hewlett Packard mass spectrometer (MS-Engine 5989A) equipped with HP 5980 Series II gas chromatograph and a DB-5 column (30 m × 0.25 mm × 0.25 μm; J & W Scientific USA) connected directly into the ion source. Operating conditions were as follows: GC inlet: split/splitless, splitless injection (3 μL) with injector at 260°C. GC temperature ramp: 70°C, initial 2 min, 18°C/min to 230°C at 3 min, 3.5°C/min to 265°C, 13°C/min to 290°C and held for 5 min giving GC run time of 30.81 min. The MS was operated in the electron capture negative ion mode (ECNI) with methane reagent gas at source pressure of 1.9 torr, electron energy 200 ev, ion source temperature at 120°C and interface temperature at 280°C. The instrument was tuned for optimal conditions with perfluorotributylamine (PFTBA) at m/z 302, 452 and 633.

Whole blood was transferred into three centrifuge tubes (4 mL each) and per tube, 4 mL acetic acid was added. The mixtures were vortexed and allowed to stand for 10 min. Then, 4 mL (hexane/acetone; 9:1) was added to each tube, vortexed and centrifuged at 1800 rpm for 8 min. The upper layers were transferred to 15 mL centrifuge tubes. Extractions were repeated twice (2 × 4 mL) and each of the combined extracts were then defatted by vortexing with 1 mL sulfuric acid. Separated organic layers were concentrated to 0.5 mL using nitrogen gas. Fractionation was performed on Carbopack-C/silica gel (0.2 g/4 g) column topped with sodium sulfate (0.5 g). Each column was washed with 12 mL of dichloromethane and 12 mL of hexane. The concentrates were quantitatively transferred to the bed of the columns and allowed to drain onto the bed of Florisil. Each column was eluted with 14 mL hexane (fraction I) and then 20 mL 30% dichloromethane: hexane (fraction II). The fraction I and fraction II eluates were

concentrated to 0.3 mL using nitrogen gas, transferred to appropriately labelled microvials containing 20 μL of recovery standard ($^{13}C_{12}$ PCB 153, 1 ppb) and then made up to 40 μL for ECNI/MS analysis.

REMOVAL OF INTERFERENCES BY NITRATION

The second fraction from the silica gel column was evaporated to dryness in a glass centrifuge tube (15 mL) and 1 mL of nitration reagent (H_2SO_4-HNO_3; 1:1) was added[8]. After heating at 70°C for 1 h, the tube was cooled in an ice bath and 3 mL of chilled high purity water was added. The mixture was extracted with hexane (3 × 3 mL). The combined hexane extract was reduced to 0.2 mL using nitrogen gas and chromatographed on 1 g of Florisil (3% deactivation) SPE column having 0.4 g of sodium sulfate on top and bottom. The column was conditioned with 8 mL DCM followed by 8 mL hexane before loading the concentrate. The Toxaphene analytes were eluted with 10 mL of hexane. Then, hexane was concentrated to 40 μL before analysis by GC-ECNI/MS.

RESULTS AND DISCUSSION

The analytical work-up is summarized in Figure 1. We developed the Carbopack-C/silica gel column methodology for the analysis of PCB congeners and organochlorine

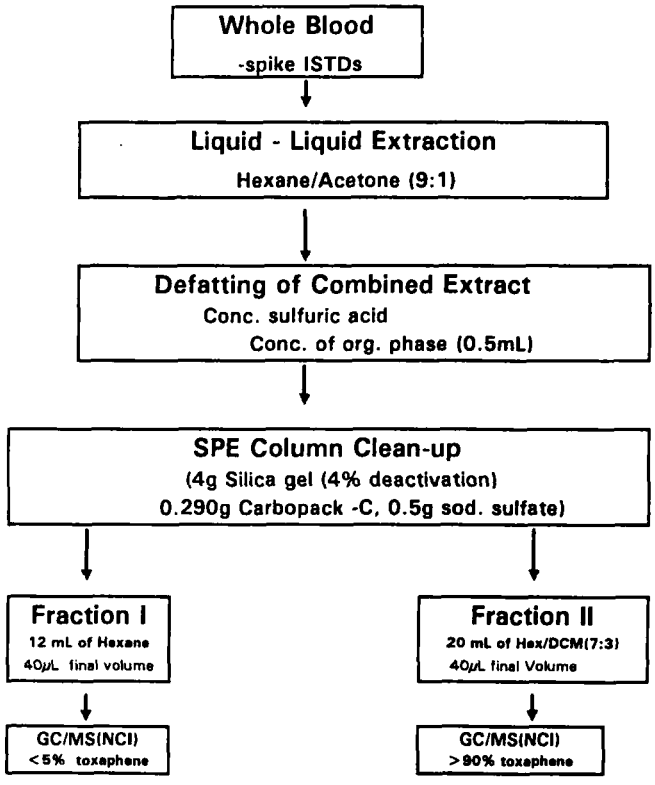

Figure 1 Flow chart for Toxaphene analysis.

pesticides from biological fluids[13]. A total ion chromatogram for Cl_7-Cl_{10} pure polychlorobornanes is shown in Figure 2a. The ions selected to monitor for PCCs include Cl_7 (340.7, 342.7); Cl_8 (376.7, 378.7); Cl_9 (410.7, 412.7) and Cl_{10} (446.7, 448.7), respectively. Figure 2b shows total ion chromatograms for Toxaphene congeners obtained from whole blood extract.

Due to their higher concentrations and retention times, PCBs, chlordanes and organochlorine pesticides interfere with the Toxaphene analysis of blood. It was

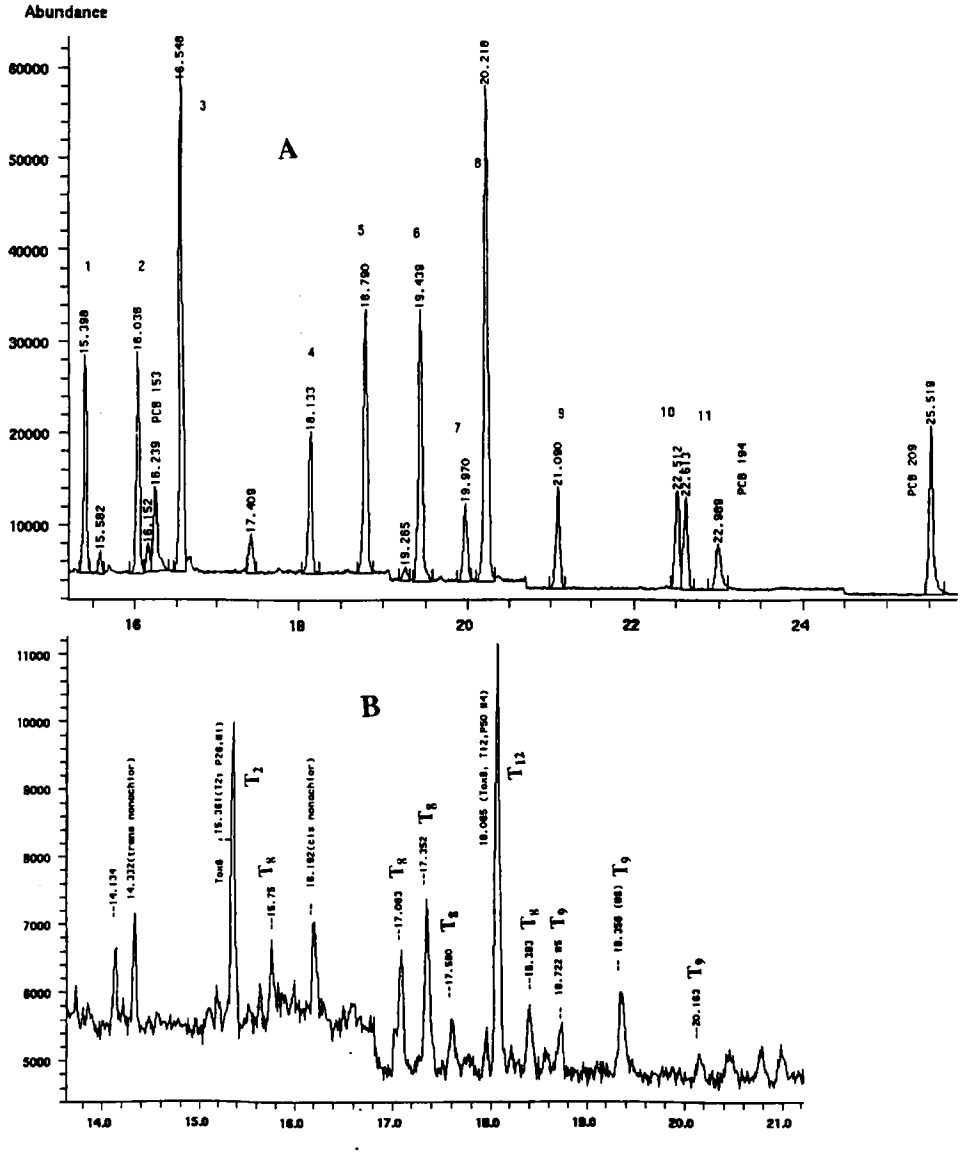

Figure 2 (A) Total ion chromatogram (TIC) of Parlar's toxaphene congeners by GC-MS/ECNI-SIM (B) TIC of whole blood extract by GC-MS/ECNI-SIM.

observed that the small amount of oxygen in the ion source formed $[M\text{-}Cl+O]^-$ and $[M\text{-}H+O]^-$ fragments of PCBs (hexa & hepta) which gave the same m/z ratios as Toxaphene congeners (Cl_7 to Cl_9) in ECNI/MS[11]. However, this problem could be eliminated by careful monitoring of mass spectrometer operations and running check samples of OFN

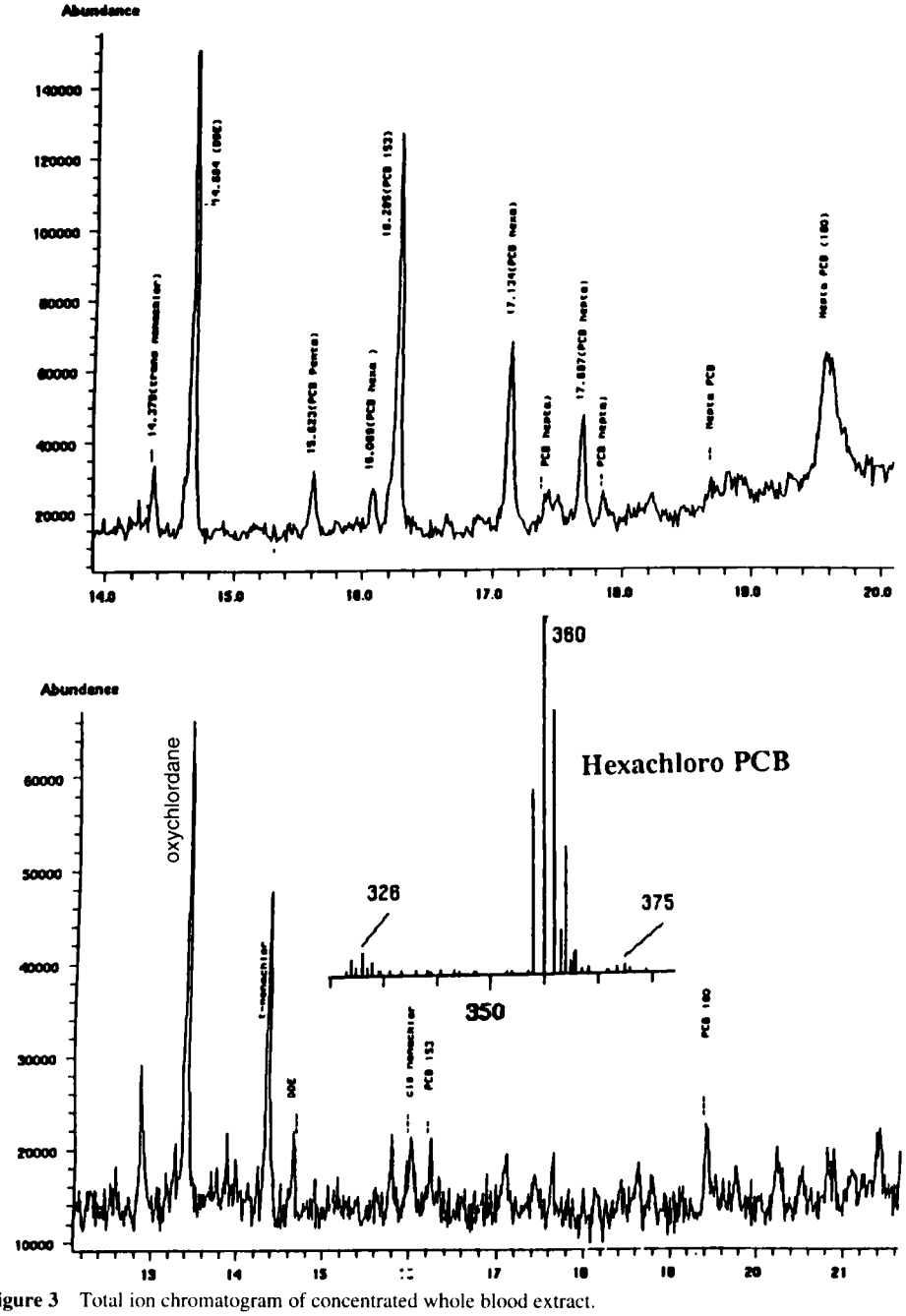

Figure 3 Total ion chromatogram of concentrated whole blood extract.

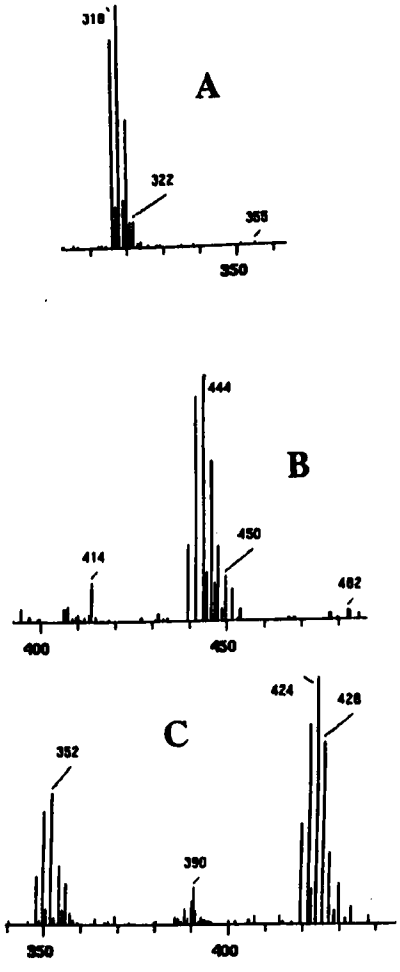

Figure 4 Mass Spectra (ECNI) of selected peaks from concentrated whole blood extract. a: DDE; b: Nonachlor; c: oxychlordane.

and PCB 153. The presence of heptachlor epoxide, chlordanes, DDE as well as PCBs was confirmed by running full scan spectra of concentrates of the whole blood extracts (Figures 3 and 4). The concentration levels of the above pollutants were much higher compared to toxaphene levels in this particular sample extract so it was not possible to obtain a full scan spectra for Toxaphene peaks. However, the Toxaphene chromatograms free from these interference pesticides and PCBs were obtained by nitration (experimental section). During our investigations we observed that preseparation of PCBs from Toxaphene can be obtained using a Carbopack-C/Silica Gel (0.2 g/3.8 g, 4% deactivation) column and with an elution of 12–15 mL hexane (fraction I, PCBs & OCs) and 20–25 mL of hexane/dichloromethane (7:3; fraction II, Toxaphene).

PCBs and organochlorine interferences can be removed by converting them into more polar nitro compounds. Only Toxaphene and *cis*, *trans*-nonachlor remained after the work-up. Total Toxaphene values obtained were between 162–174 pg/mL, whereas the amount of T2 and T12 congeners account for 90% of the total value. Figures 5 and 6

peaks identified as common PCCs in both a whole blood extract and a technical Toxaphene standard. After checking in the selected ion monitoring mode, the content of the microvial was further reduced to 10 μL. We then injected 3 μL of this concentrate in the full scan mode for the confirmation of Toxaphene congeners. Thus we further confirmed the presence of the major congeners namely, T_2 (#1 (Parlar 26) or Tox 8) as well as T_{12} (#4 (Parlar 50) or Tox 9) by obtaining their full scan spectra. The confirmation of the other minor Toxaphene congeners requires the use of at least 100 mL of whole blood. This work is in progress.

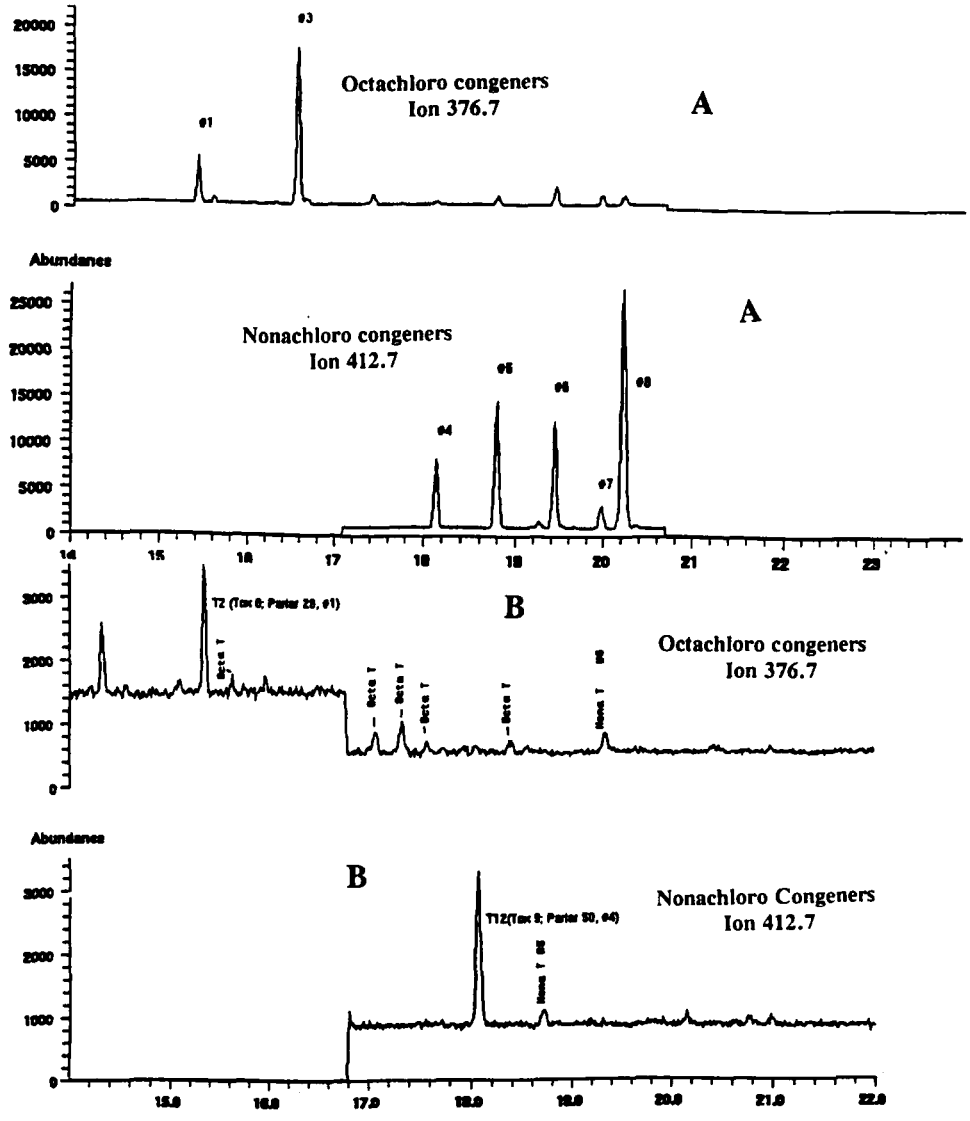

Figure 5 (A) Selected ions (m/z 376.7 and 412.7) of Parlar's mixture of congeners. (B) Selected ions (m/z 376.7 and 412.7) from whole blood extract.

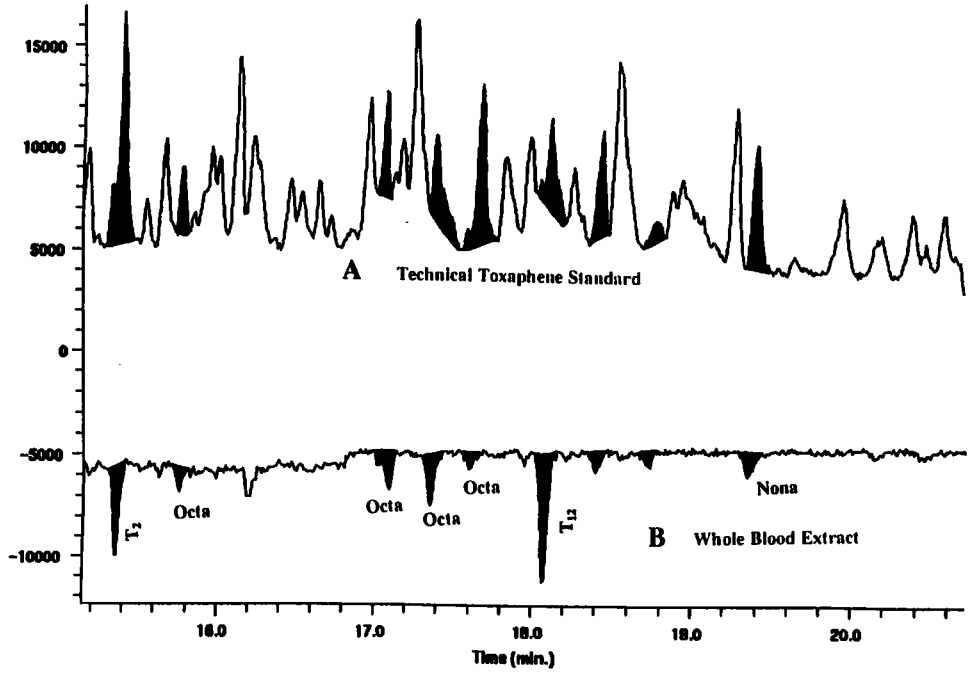

Figure 6 Total ion chromatograms of technical toxaphene standard (A) and whole blood extract (B). Peaks indentified PCCs in A and B are black.

CONCLUSIONS

Toxaphene residues in whole blood can be quantified by using pure congeners (available from Prof. H. Parlar, Germany) and ECNI/MS technique. The congeners T2 and T12 are the two major components (there are eight other minor components) in whole blood.

Acknowledgements

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