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Determination of toxaphene in soil by electron-capture negative-ion mass spectrometry and capillary column gas chromatography

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Abstract

Although toxaphene is now limited in use, interest in its analysis continues because of its persistence and widespread atmospheric transport in the environment and its presence in many dump-sites all over the world. Top soil samples collected from a dump site were analyzed for toxaphene residues. Analyses were performed by wall-coated open tubular column gas chromatography in tandem with electron-capture negative-ion mass spectrometry. Since the concentrations of toxaphene residues were at mg/kg levels, the application of a mass spectrometer as a substance-selective detector has been applied. Advantages of this mode of real-time acquisition in continuous repetitive scanning of mass spectra has significant advantages in comparison to the selected-ion monitoring technique. An average R.S.D. of 10 % and recoveries of 90 to 109% were obtained. Levels down to 50 μg/kg are obtainable.

1. Introduction

Toxaphene presence in world-wide ecosystem requires establishing its unambiguous identification and quantitation in routine analytical laboratories. Interest in its quantitation continues because of its persistence especially in many dump-sites located in the United States, Russia, Egypt, Sudan, Ethiopia and Tanzania and other cotton producing countries [1].

Toxaphene is known to be toxic to various species of fish. Toxic effects in fish include decreased viability of ova of brook trout (*Salvelinus santinalis*) that have been exposed to toxaphene at ng/kg levels [2]. Evidence of long-range airborne transport of toxaphene has been documented by Bidleman and Olney [3].

The selection of analytical techniques for the measurement of toxaphene residues has received an every increasing attention because of its complex composition and wide-spread occurrence in various compartments of our ecosystem [1] especially it is used on cotton crops. Toxaphene represents a complex mixture of at least 700 polychlorinated bornane congeners with an average elemental composition of $C_{10}H_{10}Cl_8$. Due to its complex nature, the quantitation of toxaphene is difficult. Under gas chromatograph-

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ic conditions employing electron-capture detection (ECD), the toxaphene congeners often overlap each other, as well, organochlorine pesticides (OCs) interfere with quantitation, and there is no individual pure toxaphene congener commercially available for use as a primary standard.

In our previous report [4], we described the performance of gas chromatography–electron impact MS–selected-ion monitoring (SIM) and MS–SIM solid-probe programmable temperature techniques. The latter, especially provides a significant improvement of samples throughput, since the total *toxaphene residue* concentration is obtained in less than 5 min per sample. However, this methodology requires a ^{37}Cl-labelled toxaphene and preferably high-resolution mass spectrometer, since at low resolution results may be affected by interferences of other pesticides.

Current US EPA methodology (SW-846) [5] consists of electron impact mass spectrometry and high-resolution gas chromatography (HRGC)–ECD method (8270 and 8280), both of which are greatly affected by interferences such as polychlorinated biphenyls (PCBs) and chlordane [3]. Jannson and Wideqvist [6] and Swackhamer *et al.* [7] published an electron-capture negative ion HRGC–MS–SIM method that is selective for toxaphene in fish and milk samples.

The application of a mass spectrometer as a substance-selective detector in HRGC–MS is well recognized. The most widely used data acquisition technique is the continuous repetitive scanning of mass spectra. Each integer mass between the starting and ending masses is measured and recorded. The final mass spectrum illustrates a widely used data reduction process. Each point on the ordinate is the normalized sum of all the ion abundances in a single mass spectrum and each point on the abscissa represents a spectrum number of the electron-capture negative chemical ionization (EC-NCI) profile. This plot is referred as total ion current (TIC) profile. Since concentrations of toxaphene in samples were at mg/kg range, it was possible to perform both confirmation and quantitation of toxaphene in one run.

In our work, the electron capture negative ion HRGC–MS is extended to soil samples, which therefore suggests its applicability to other solid wastes. It is the purpose of this paper to present some of the concepts involved in the application of ECNCI HRGC–MS of the computer-controlled mass spectrometer as a substance-selective detector.

2. Experimental

2.1. Chemicals

Toxaphene and TCB reference standards were obtained from the US EPA Repository (Research Triangle Park, NS, USA). All solvents used for extraction and cleanup were purchased from Burdick & Jackson (Muskegon, MI, USA).

2.2. Extraction

Extraction was accomplished according to standard methods using Polytron extractor [8]. The soil sample (1 g) was extracted with 50 ml CH_2Cl_2–acetone (1:1, v/v) in a 250-ml stainless-steel beaker. The sonicator was adjusted to pulsed operation and output control was set to full power and duty cycle to 50%. The sample was sonicated for 3 min. The beaker was lowered and the probe washed down into the beaker with CH_2Cl_2–acetone (10 ml). The extraction was repeated twice with fresh solvents. Combined extracts were dried through a Na_2SO_4 column. After filtration, 10 ml of isooctane was added to the filtrate, and the filtrate was evaporated on a rotary evaporator to 2–3 ml volume at 40°C.

2.3. Cleanup

A Florisil column was prepared by placing a charge of activated Florisil in a chromatographic column (30 × 1 cm I.D.) over a 1-cm layer of anhydrous Na_2SO_4 [2]. An amount of 5 g of 120–150 μm Florisil previously activated at 130°C for 16 h was added and topped with 1.5 cm layer of anhydrous Na_2SO_4. Each column was prewashed with 20 ml of *n*-hexane. When the solvent reached the top of the Na_2SO_4 layer,

the concentrate from the extraction step was quantitatively transferred onto the column and allowed to drain onto the bed of Florisil. The column walls were washed with a 10-ml portion of the 50 ml *n*-hexane–diethyl ether (94:6, v/v). When the solvent reached the top of the Florisil the remaining part of the eluent (40 ml) was gradually added to the column. The eluate was collected for further analysis. It contained aldrin, benzene hexachloride (BHC), chlordanes, DDD, heptachlor, DDE and DDT isomers, lindane, methoxychlor, mirex, PCBs and toxaphene. More polar compounds such as endosulfan, endrin, dieldrin and phthalates were removed from the column with 50 ml of hexane–diethyl ether (80:20) solution. The extract was filtered and concentrated just to dryness on a rotary evaporator. The Florisil column effluent was treated with 5 ml of cold sulfuric–fuming nitric acid (1:1) for 15 min at room temperature to remove DDT and its metabolities [8]. PCBs were removed from most of the pesticides by silica gel column chromatography [9].

About 20 g of silica gel (*e.g.* E. Merck silica gel 60) was placed in a 100-ml beaker and activated at 130°C for 16 h. Then, it was transferred to a 100-ml glass stoppered bottle. The silica gel column was prepared by plugging chromatographic column (30 × 1 cm I.D.) with glass wool, filling it with a 1 cm layer of anhydrous Na_2SO_4, 5 g of activated silica gel and topping it with a second 1 cm layer of anhydrous Na_2SO_4. The column was prewashed with 20 ml *n*-hexane. The sample extract was added to the column and rinsed with 5 ml *n*-hexane–diethyl ether (94:6). The remaining 35 ml of the solution was added and the effluent collected in a 125-ml round-bottom flask. This fraction contained the PCBs, hexachlorobenzene (HCB), aldrin, heptachlor, mirex and the *p,p'*-DDE. The second fraction eluate consisting of 40 ml *n*-hexane–diethyl ether (75:25) contained a small amount of *p,p'*-DDE, BHC isomers, DDT, chlordanes, nonachlor, heptachlor epoxide, methoxychlor and toxaphene. The eluate volumes were reduced on the Rotovap and the resulting residues adjusted to 1 ml with isooctane prior to their quantitation using HRGC–EC-NCI MS.

2.4. Gas chromatography–mass spectrometry

Mass spectra were measured with a Hewlett-Packard Engine 5989A GC–MS System and HP 5890 Series II gas chromatograph equipped with programmable split/splitless electronic pressure programming, which provides accurate and precise control of column head pressure, resulting in good retention time reproducibility. The inlet pressure can be constant, programmed, or set to maintain a desired column flow-rate. The oven contained a 25 m × 0.25 mm I.D. DB-5 wall-coated open tubular (WCOT) column [film thickness (d_f) – 0.25 μm]. Helium was used as the carrier gas at the linear velocity of 40 cm/s at 80°C. The initial temperature was held at 80°C for 1 min followed by a temperature programming rate of 20°C/min to 200°C, afterwards at 4°C/min to 280°C.

The flow of methane reagent gas for chemical ionization was introduced via reagent gas flow controller and was optimized by employing the procedure for EC-NCI recommended by the manufacturer. The ion source pressure in EC-NCI mode was maintained at *ca.* 0.4 Torr (1 Torr = 133.3 Pa). The injector port and transfer line temperatures were maintained at 200 and 250°C, respectively. The ion source temperature was held at 150°C.

2.5. Quantitation

To quantitate ions by means of the mass chromatography, complete mass spectra from *m/z* 100 to 500 were scanned. The EC-NCI mass spectra are less complex than electron impact and chemical ionization spectra and exhibit only masses due to losses of Cl and HCl from the molecular ion. In the EC-NCI mass spectra, the most abundant ions were M^- of hexachloro bornanes and bornenes and the $[M - Cl]^-$ ions from hepta to decachloro congeners.

After running the total ion chromatogram (TIC) of a standard solution containing toxaphene the specific ions of chlorinated congener peaks were evaluated for impurities or contamination and compared with a real sample from a specific dump site. Only non-contaminated chro-

Table 1
Quantitation ions used for toxaphene in electron-capture negative-ion mass spectrometry

Congener group	M_r	Ion monitored	Quantitation ion
Hexachlorobornenes	340	M^-	342
Hexachlorobornanes	342	M^-	342
Heptachlorobornenes	374	$[M-Cl]^-$	343
Heptachlorobornanes	376	$[M-Cl]^-$	343
Octachlorobornenes	408	$[M-Cl]^-$	377
Octachlorobornanes	410	$[M-Cl]^-$	377
Nonachlorobornenes	442	$[M-Cl]^-$	413
Nonachlorobornanes	444	$[M-Cl]^-$	413
Decachlorobornenes	476	$[M-Cl]^-$	449
Decachlorobornanes	478	$[M-Cl]^-$	449

Mass chromatograms are shown in Figs. 1 and 2.

matographic peaks were selected for quantitation. Selected ions for monitoring toxaphene congeners are given in Table 1. Quantitation was performed relative to the internal standard 2,3,5,3′,4′-pentachloro biphenyl.

3. Results and discussion

The simplest type of process which leads to a negative ion mass spectrum under chemical ionization conditions occurs when the reagent gas acts simply as a buffer gas in producing a high yield of thermal electrons. These give rise to an electron capture mass spectrum of toxaphene moieties that possess a positive electron affinity, and this is the counterpart to the charge transfer mass spectrum in positive chemical ionization mode of operation.

The total ion chromatogram (TIC) shown in trace A (Fig. 1) of toxaphene standard scanned from m/z 200 to 500, illustrates complexity of the mixture of chlorinated bornanes and bornenes. It can be assumed that many chlorinated pesticides may overlap with toxaphene peaks and will obstruct determination of toxaphene.

The solid probe mass spectrum of the toxaphene standard shown in trace B (Fig. 1) indicates which ions should be selected for determination of toxaphene taking into account isotopic distribution of chlorine pattern and a correct abundances of different chlorine contributions according to the degree of chlorination. The specific ions selected for determination of toxaphene peaks are given in Table 1. The most abundant ions from a given isotopic cluster in Fig. 1B were selected to be the quantitation ions.

Fig. 2 shows the toxaphene mass chromatograms from the soil extract that can be used for the unambiguous determination of toxaphene and even for the homologue specific determination of its homologue specific patterns according to the chlorination of bornane/bornene moieties. A similar mass chromatogram (EC-NCI) can easily be obtained by pulling out individual masses from the TIC for the chlordane contamination which should be checked by monitoring masses at m/z values 237, 239; 264, 266; 300, 302; 334, and 336. Our samples were not contaminated with chlordane.

The use of EC-NCI HRGC–MS has provided information of the qualitative reliability, sensitivity, high selectivity and quantitative accuracy during analysis. There is no significant evidence for any contribution to chromatographic TIC trace by chemical degradation.

Improved injection techniques permit electronic pressure programming. This means that column flow-rate can be maintained at a constant rate during temperature programming run, or it can be programmed as an additional parameter to achieve the required quick sample transfer onto WCOT column, better separation and shorter run times. We applied pressure programming

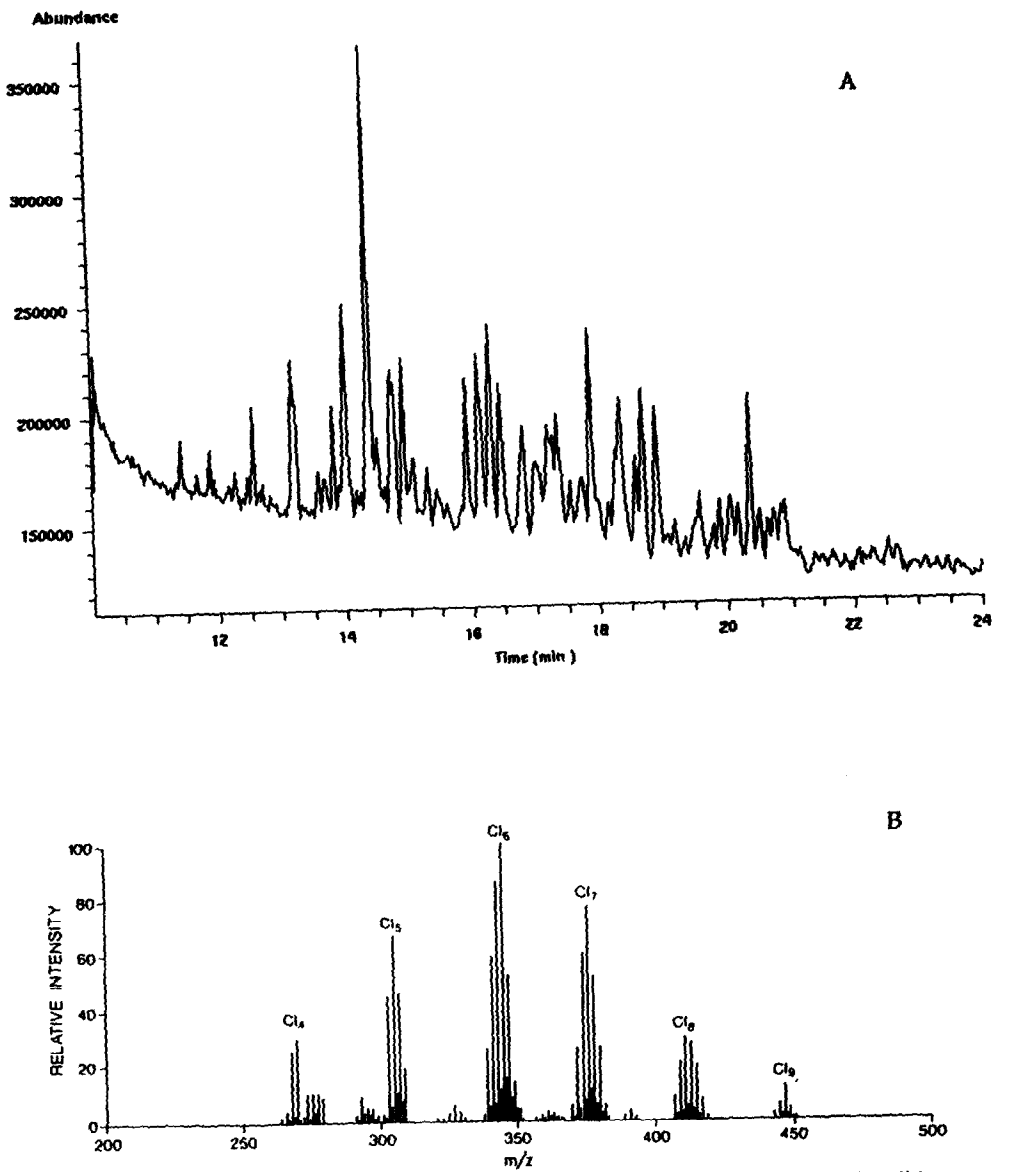

Fig. 1. (A) The total ion chromatogram of toxaphene (100 μg/kg) scanned from m/z 200 to 500 (conditions are given in the text). (B) Methane electron-capture negative-ion mass spectrum of toxaphene obtained via solid-probe inlet.

with splitless injection using a single taper deactivated linear at 200°C to reduce possible decomposition of toxaphene labile components. The chromatograms indicated remarkable stability and no sign of decomposition with an excellent retention time stability. The initial program ramp from 80 p.s.i. (1 p.s.i. = 6894.76 Pa) was followed by a single downward ramp. We concluded that this type of injection is mandatory for obtaining correct quantitative results. Considering the possibility of dehydrochlorination or reductive dechlorination in a variety of biological systems, reduction would appear the less likely in this instance, since the soil was exposed to air

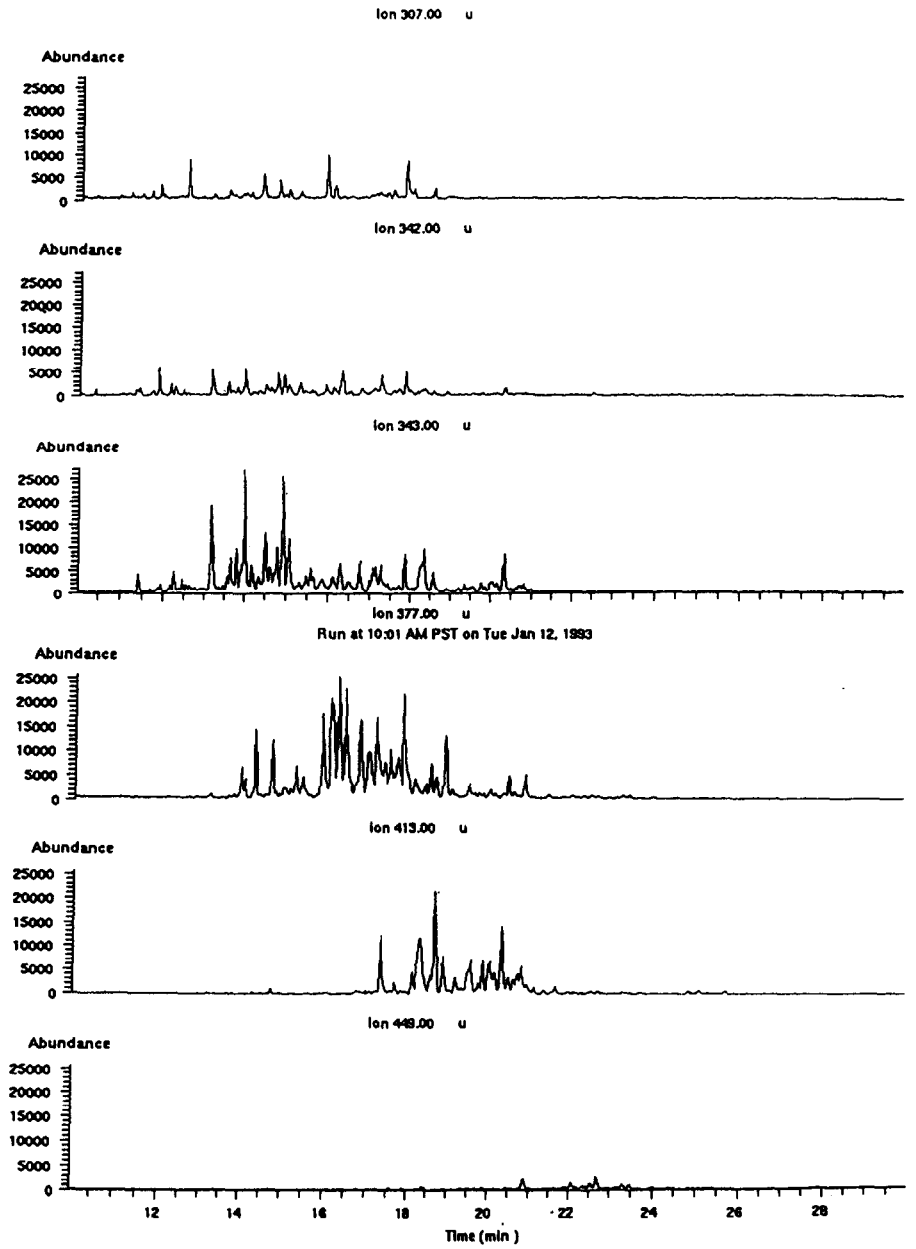

Fig. 2. Toxaphene mass chromatograms obtained from the TIC using specific masses selected from the solid-probe mass spectrum.

during the long period. However, no direct evidence was obtained on biodegradation since neither the components undergoing degradation nor metabolites were identified.

The ions monitored for toxaphene are the $[M - Cl^{\bullet}]^{-}$ ions resulting from $C_{10}H_{12}Cl_x$ through $C_{10}H_8Cl_{10}$ compositions (bornanes and overlapping contributions from ions resulting

from bornenes. Internal standard, 2,4,5,3',4'-pentachlorobiphenyl having retention time of 14.38 min was monitored at m/z 326 for quantitation.

Table 2 provides results of analyses of selected soil samples for toxaphene. It can be seen even at different levels of the toxaphene concentration in soil samples between 800 and 1200 μg/g, that relative distribution among chlorine homologue classes did not differ significantly. A simple comparison between different levels provides an interesting observation. Differences in variation for hexachlorinated homologues are lower than 10%, and for heptachlorinated congeners less than 3%. Greater variations are observed for nona- and decachlorinated congeners (23.6 and 16%, respectively).

A similar laboratory experiment involving long-term exposure of soil samples containing approximately 25 μg/g toxaphene underwent 123 day treatments employing unique anaerobic dechlorination processes developed at Grace-Dearborn Environmental Engineering Group. Different treatment shows different kinetics of degradation as it can be seen in Fig. 3. A summary of these data indicates a modest degradation of toxaphene during this period.

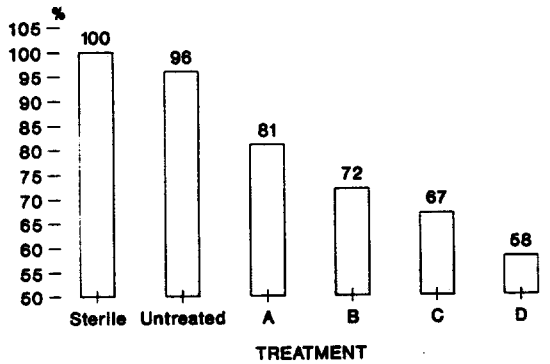

Fig. 3. A controlled experiment data showing biodegradation of toxaphene. Test duration 123 days.

4. Conclusions

The presented results demonstrate the feasibility of performing multicomponent quantitative analyses of toxaphene at the mg/kg–μg/kg level using a 1-g sample of soil by electron-capture negative-ion mass spectrometry after separation of toxaphene congeners by HRGC. The application of a mass spectrometer as a substance-selective detector shows advantages of the continuous repetitive scanning of mass spec-

Table 2
Quantitation of toxaphene in soil samples in mg/kg

Sample	Total tox.	6chloro-	7chloro-	8chloro-	9chloro-	10chloro-
1	716	171.8	250.6	229.1	57.3	7.2
2	908	217.9	317.8	290.5	72.6	9.2
3	842	168.4	269.4	286.3	88.4	9.5
4	728	174.7	254.8	232.9	58.2	7.4
5	891	222.7	311.8	294.0	58.0	4.5
6	1159	283.9	441.4	376.7	81.1	5.9
7	1070	235.4	342.4	353.1	117.7	21.4
8	821	180.6	262.7	270.9	90.3	16.5
9	1181	253.9	395.6	389.7	124.0	17.8
10	1233	272.3	419.2	394.6	135.6	11.3
11	668	147.0	233.8	217.1	66.8	3.3
12	940	188.0	300.8	319.8	94.0	37.6
13	772	154.4	254.8	254.9	84.8	23.1
14	1098	241.6	384.3	373.3	87.8	11.0
15	1950	448.5	702.0	624.0	146.2	29.3
16	1095	240.9	383.3	372.3	87.6	10.9

tra over selected ion monitoring. The EC-NCI mass spectra of toxaphene clearly indicates impurities that can be detected and corrected for and in SIM may provide false-positive data.

5. References

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