ANALYTICAL METHODOLOGY DEVELOPMENT FOR TOXAPHENE BY HIGH RESOLUTION GAS CHROMATOGRAPHY / HIGH RESOLUTION MASS SPECTROMETRY WITH ELECTRON CAPTURE NEGATIVE IONIZATION

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Introduction

Toxaphene is a broad-spectrum insecticide, was one of the most heavily used agricultural chemicals on a global scale, especially against pests in cotton field and vegetable farms. Basically, commercial production of toxaphene involves the reaction of camphene, chlorine activated by ultraviolet radiation and certain catalysts to yield chlorinated camphene with chlorine content of 67 to 69% by weight. Environmental hazards and increasing public concerns associated with toxaphene are reviewed[1]. By 1974, cumulative world use of toxaphene was, estimated as 450,000 metric tons. Production of toxaphene declined from 1973 to 1980; however, annual consumption in 1980 was estimated as 105,000 tons, thus qualifying toxaphene as one of the most heavily utilized agricultural chemicals. Toxaphene is extremely persistent in soil and waters, with documented half times of 9 to 11 years. Toxaphene is especially hazardous to non-target marine and freshwater organisms, with death recorded at ambient water concentrations substantially below 10 ng/mL, and adverse effects observed on growth, reproduction, and metabolism at water concentrations between 0.05 and 0.3 ng/mL.

The theoretical number of toxaphene isomer [e.g., $C_{10}H_{18-x}Cl_x$ (X=1-18)] yields about 32,768 isomers after the reaction of chlorinated -bornane, -bornene and camphene. This product is a relatively stable which, composed of a mixture of structurally similar compounds and isomers. In technical toxaphene, the chlorinated bornane alone contributed about 4096 isomers. Among them, only >670 isomers are considered to be major toxaphenes. Information on chemical properties, fate and effects of the remaining components of toxaphene is missing or incomplete[2]. Analysis of toxaphene is challenging and needed to be updated with high sensitive, selective and reliable method. Particularly, very recent reports documented that electron capture negative ionization (ECNI) seems to best suited method for toxaphene determination at trace levels. Considering those credits, in present investigation, we developed a method for toxaphene analysis by high resolution gas chromatography / high resolution mass spectrometry (HRGC/HRMS) with ENCI and eventually, environmental air and fish samples were tested.

Materials and Methods *Standard*:

Technical toxaphene "namely; chlorinated bornane, bornene and camphene" (a mixture of several toxaphene congeners) was purchased from SUPELCO while, individual toxaphene congeners e.g., 2-endo,3-exo,5-endo,6-exo,8,8,10,10-Octachlorobornane (Parlar #26), 2-endo,3-exo,5-endo,6-exo,8,8,9,10,10-Nonachlorobornane (Parlar #50) and 2,2,5,5,8,9,9,10,10-Nonachloro -bornane (Parlar #62) were purchased from Promochem, Germany. The individual Parlar #26, #50 and #62 standards were mixed and diluted 0.4, 2, 10, 25, 50 and 250 pg/μL for calibration solution.

Analysis: There are some representative studies have put forward the toxaphene analysis by GC/ECD[3], HRGC/LRMS[4]. In this study, analysis was conducted with Thermoelectron Finnigan MAT95XL by electron impact (EI) method. The EI mass spectrum results showed complicated fragmentation and therefore, ENCI was chosen. Tomy[5] and his co-workers have already been conducted toxaphene analysis using HRGC/HRMS-ECNI. The result of SCAN analysis by ECNI in low resolution showed the specific mass spectrum, and monitor ion of Parlar #26 and #50 in SIM analysis, $[M\text{-}Cl]^-$ was selected and for Parlar #62, $[M\text{-}HCl\text{-}Cl]^-$ was selected. The $^{13}C_{10}$-trans-chlordane was used as internal standard. The calibration solution was evaluated for relative response factor (RRF) and relative standard deviation (RSD). The overall HRGC/HRMS program for toxaphene analysis has been listed in Table 1.

Table 1. HRGC/HRMS analytical conditions and programming

GC		HP6890 Series GC System (Agilent)	
	Injector	7683 Series Injector (Agilent)	
	Auto sampler	7683 Series Auto Sampler (Agilent)	
	Column	DB-5MS (J&W, 30m × 0.25mm (i.d.) [0.1μm])	
		HT8-PCB (SGE, 60m × 0.25mm (i.d.))	
	Injector Temp.	120°C-(100°C/min)-300°C(45min)	
	Column Temp.	120°C-(20°C/min)-230°C(40min)	
	He flow ratio	1.0mL/min	
	Injection	On-Column injection	
	Injection Volume	2μL	
MS		MAT95XL (Thermoelectron/Finnigan)	
	Ionization method	EI	ECNI
	Reagent gas	-	Methane or Isobutane
	Ionization Volt.	40V	90V
	Trap current	450μA	250μA
	Accel. Volt.	5kV	-5kV
	Interface Temp.	230°C	230°C
	Ion source Temp.	300°C	130°C
	Resolution	M/ΔM>10,000 (10% valley)	

Sample: Environmental air sample was collected by using High volume air sampler with Glass fiber filter (GFF), Polyurethane foam (PUF) and Activated carbon filter (ACF). $^{13}C_{10}$-trans-chlordane was added to PUF before sampling. For biological sample, fish (Sea bass) purchased in commercial supermarkets was selected. Approximately 20 g of sample was used, and $^{13}C_{10}$-trans-chlordane was added prior to extraction.

Pretreatment: Schematic flow chart of toxaphene extraction and cleanup method for environmental air sample was summarized in Figure 1. In case of biological samples, Soxhlet extracted for 6-h using dichloromethane, rotary evaporated and fractionated exactly same procedure that adopted for air samples. In both sample matrixes, $^{13}C_{12}$-HxCB-138 used as syringe (injection recovery) spike. In order to establish a accurate cleanup methods, the toxaphene native standard was subjected into following cleanup methods; H_2SO_4-shaking, florisil column cleanup, 22% & 44%-H_2SO_4-silicagel cleanup, DMSO-hexane partitioning cleanup, silicagel column cleanup methods. The florisil was used to fractionation and eluted with 20% dichloromethane in hexane as first fraction was analyzed for Parlar #26, #50 and #62 for cleanup purpose of environmental air and biological sample (e.g., fish) due to good recovery without any matrix effect.

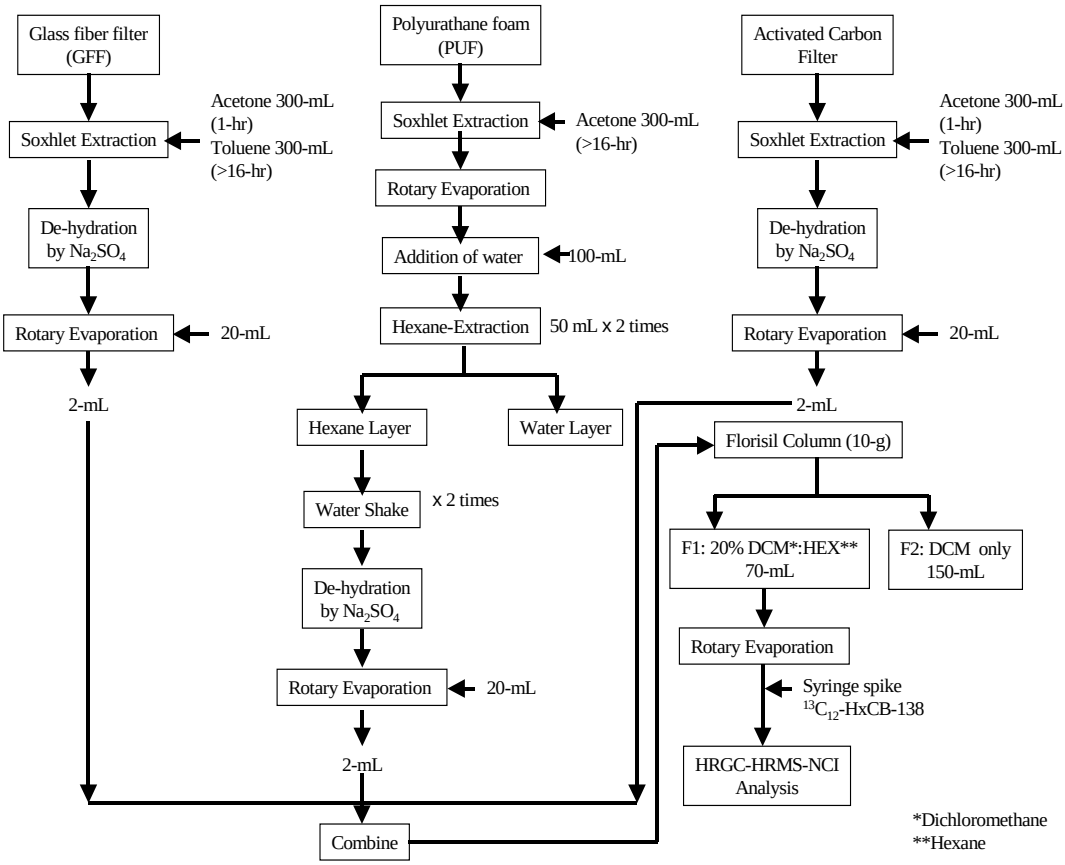

Figure 1. The schematic flow of cleanup procedure for toxaphene analysis in air sample

Results and Discussion

Calibration solution: Average RRF (n=3) was stable at higher concentrations, lowered at lower concentrations, especially for Parlar #62. Average RSD (%) of RRFs at all concentration levels was 5-7% for Parlar #26 and #50, while 12% for Parlar #62. These results comprehended the lower sensitivity of the latter in ultra trace analysis. The technical toxaphene standard was used to identify the retention times of other toxaphene congeners that could be comparable to individual Parlar #26, #50 and #62. The typical chromatogram of technical standard obtained was shown in Figure 2.

HRGC/HRMS analysis: In HRGC/HRMS analysis, HpCB-185 was interfered with the Parlor #50 and thus modified method was developed. In earlier analysis, DB-5MS column (Table 1) was used, but HpCB-187 and 180 interfered with Parlar #50 and #62, respectively and therefore, HT8-PCB column (Table 1) was used. The result reveals that interference by PCBs was minimized with only a minor interference by HpCB-185 with Parlar #50. Effect of reagent gas such as iso-butane and methane prevailed that former revealed good intensity for Parlor #26 and #50, whereas, latter showed good intensity for Parlor #62. Methane gas was selected due to its low cost. Effect of injector and interface temperature (at 230^{o}C and 300^{o}C) was monitored. There was no discriminate differences were noticed at the selected temperature. Sensitivity was orders of magnitude greater for Parlor #26 and #50, than the Parlor #62. The overall monitoring ion ratio (m/z) was summarized in Table 2.

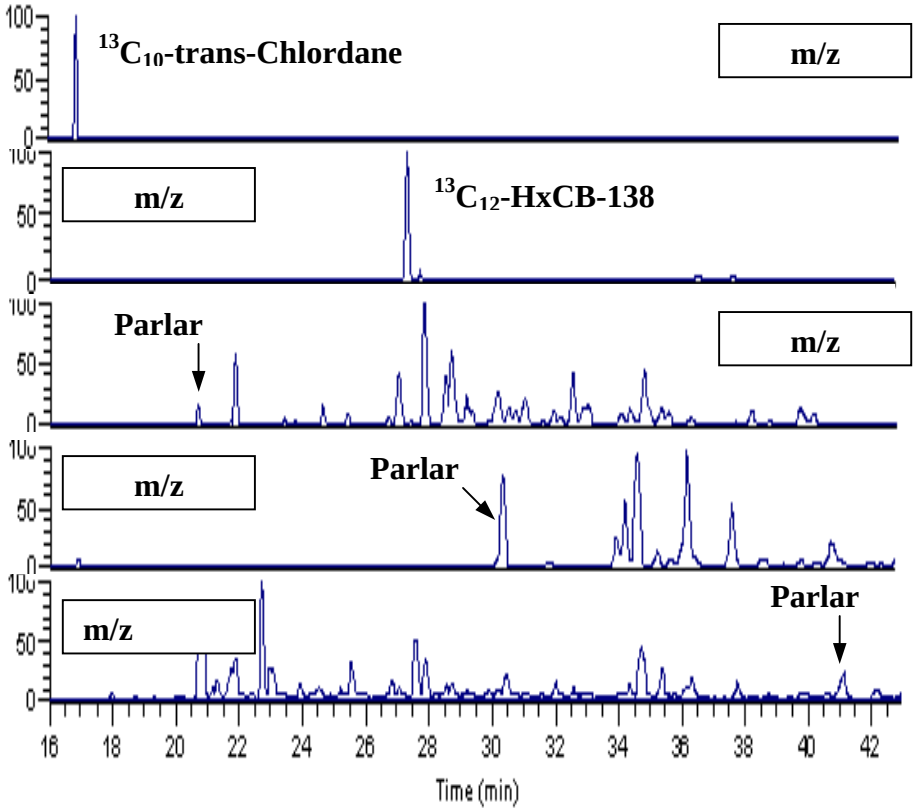

Figure 2. The typical chromatogram of technical toxaphene standard

Table 2. The mass (m/z) ion monitoring of Parlar-#26, 50 and 62.

	M+2-HCl-Cl	M+4-HCl-Cl	M+2-Cl	M+4-Cl	M+6-Cl
Parlar #26			376.8573	378.8544	
Parlar #50				412.8154	414.8124
Parlar #62	374.8416	376.8387		412.8154	414.8124

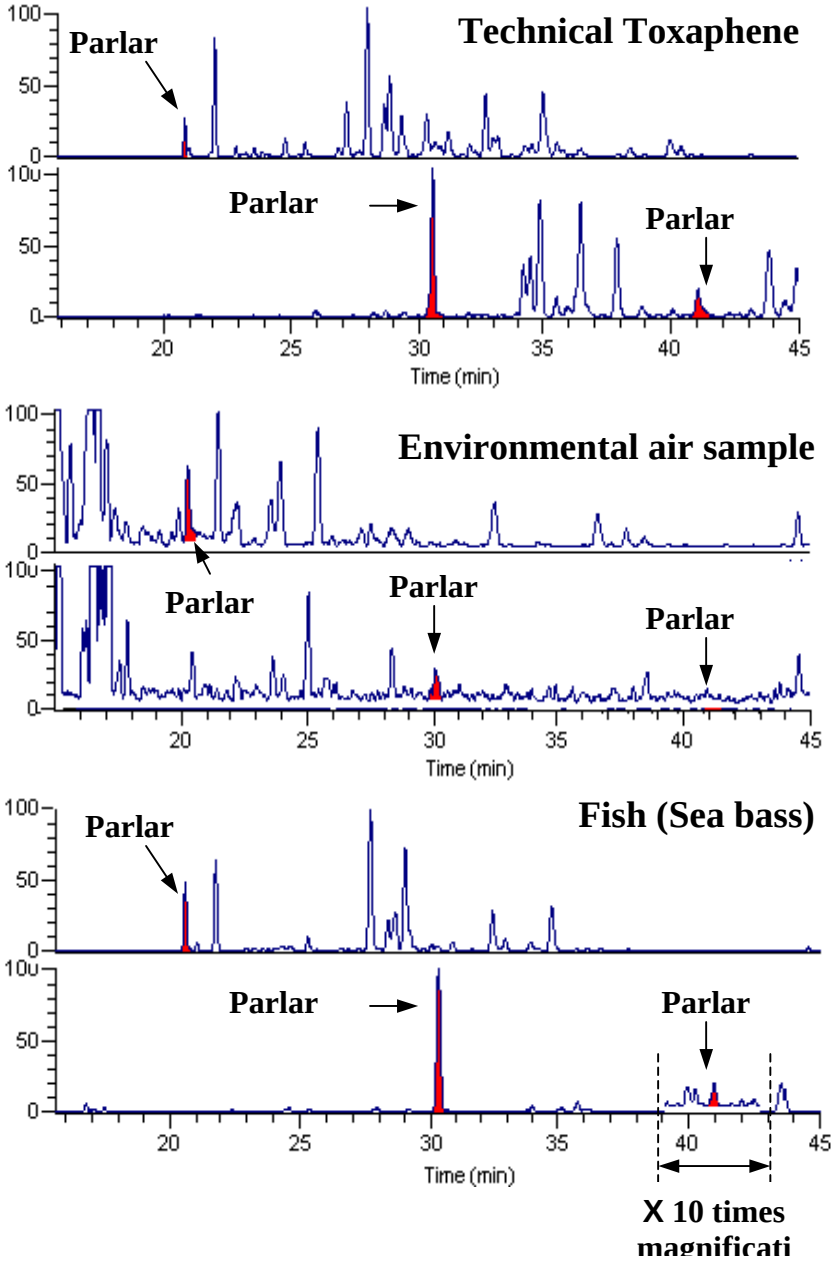

Figure 3. The typical chromatogram for the air and fish sample

Analytical Results: The results indicate the ultra trace analysis of toxaphene congeners with the recovery of (100-104%) in air and (93-95%) biological sample. The typical chromatogram for the air and biological sample was plotted in Figures 3.

In Japan, technical toxaphene has not been used as insecticide. However, the concentrations of Parlar #26, #50 and #62 in air sample was 0.21, 0.053 and <0.5 pg/m^3, respectively. While, concentrations of Parlar-#26, #50 and #62 in sea bass sample were 140, 210 and 80 pg/g, respectively. Compare to technical toxaphene standard chromatogram, the other congeners were detected, these results suggested the possibility of global pollution through air and of the accumulation in ecosystem. Sum concentrations of 3 congeners in this study were much lesser than 100's of fish species from USA[6]. However, these studies reports total toxaphene concentrations that determined by GC/ECD and HRGC/LRMS. Takazawa[7] and his co-workers reviewed analytical results of Parlar #26, #50, #62 and other toxaphene congeners collected over global terms. They also compiled toxaphene concentrations in various biological and abiotic matrices. Tomy[5] and his co-workers reported parts per trillion order of Parlar #26 (B8-1413), #50 (B9-1679) and #62 (B9-1025) in catfish collected from Detroit River and those levels were similar to that our findings in sea bass.

In case of HpCB-185 interfere with Parlar #50 in HRGC/HRMS-ECNI analysis, Parlor #50 can be fractionated by florisil through eluting 70mL of 10% dichloromethane in hexane after eluting 40mL of hexane. Further research is under the way with ultra trace analysis of several toxaphene congeners in HRGC/HRMS-ECNI.

Literature Cited

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