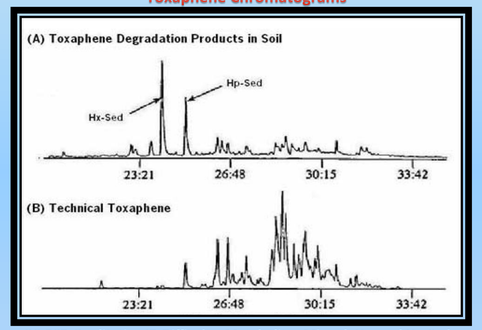
SW-846 Method 8276: MULTI-LAB VALIDATION OF TOXAPHENE AND TOXAPHENE CONGENERS IN FISH

Abstract No. 194

Toxaphene has been historically analyzed along with other organochloride pesticides by Method 8081, using gas chromatography with fused-silica, open tubular, capillary columns and electron capture detectors (ECD) or electrolytic conductivity detectors (ELCD). Several limitations were found: unable to measure congeners or breakdown products; interference from other organochloride pesticides; and insufficient sensitivity of the method.

A new method was developed for better detection and quantitation of technical toxaphene and 8 environmentally significant toxaphene congeners in groundwater, soil, sediment, and fish tissue using gas chromatography negative ion chemical ionization mass spectrometry (GC-NICI/MS). The new Method 8276 is posted online. (http://www.epa.gov/osw/hazard/testmethods/pdfs/8276.pdf)

Toxaphene Degrades: GC-ECD/EPA Method 8081 Toxaphene Chromatograms



GC-ECD Qualitative ID Difficult – Metabolites Missed: Hx- and Hp-Sed

The New Approach

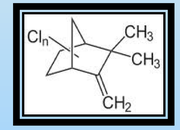
Gas Chromatography with Negative Ion and Chemical Ionization/Mass Spectrometry (GC-NICI/MS) Analysis Theory

1) High energy electrons interact with moderating gas (e.g., CH4)
2) Low energy thermal electrons are produced
   e^{**-} (150 eV) + CH4 (moderating gas) → e^{*-} thermal electrons (2 eV)
3) Thermal electrons interact with toxaphene congeners (M) producing negative species
   $e^{*-} + M \rightarrow M^{*-}$

New Method 8276: Toxaphene and Toxaphene Congeners Using Gas Chromatography with Negative Ion and Chemical Ionization Mass Spectrometry (GC-NICI/MS)

COMPOUND	CAS Registry No
Technical Toxaphene	8001-35-2
Toxaphene Congeners	
2-exo,3-endo,6-exo,8,9,10-Hexachlorobornane (Hx- Sed)	57981-29-0
2-endo,3-exo,5-endo,6-exo,8,9,10-Heptachlorobornane (Hp-Sed)	70649-42-2
2-endo,3-exo,5-endo,6-exo,8,8,10,10-Octachlorobornane (P26)	142534-71-2
2-endo,3-exo,5-endo,6-exo,8,9,10,10-Octachlorobornane (P40)	166021-27-8
2-exo,3-endo,5-exo,8,9,9,10,10-Octachlorobornane (P41)	165820-16-6
2-exo,5,5,8,9,9,10,10-Octachlorobornane (P44)	165820-17-7
2-endo,3-exo,5-endo,6-exo,8,8,9,10,10-Nonachlorobornane (P50)	6680-80-8
2,2,5,5,8,9,9,10,10-Nonachlorobornane (P62)	154159-06-5

Toxaphene Ring



Validation Study Approach

Phase I: Initial Demonstration of Proficiency (IDP)
- Analysis of spiked solvents with known concentrations of toxaphene and toxaphene congeners

Completed in December 2008

Phase II: Validation for Groundwater, Soil and Sediment
- Analysis of varying levels of toxaphene and toxaphene congeners in extracts of groundwater, soil, and sediment

Completed in November 2009

Phase III: Validation for Fish Tissue
- Development of a protocol for extraction and cleanup of fish tissue
- Preparation and verification of study samples
- Analysis of toxaphene and toxaphene congeners in fish extracts

Completed in March 2012

Phase III Validation Study

The EPA Region 4 Laboratory completed all extraction, cleanup, concentration, and spiking as necessary for sample matrices.

With the exception of the referee chemist, none of the laboratory participants knew the concentrations of the prepared laboratory QC samples and fish extracts.

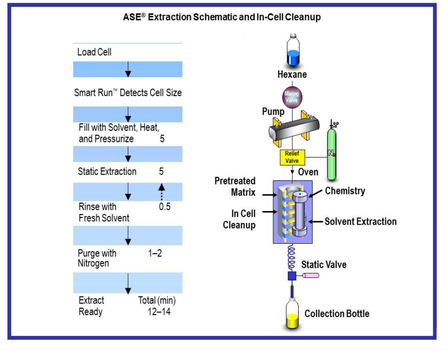
Each laboratory was responsible for conforming to study instructions and the QC parameters recommended in Method 8276 (March 2010, Revision 0).

Eight participants at five laboratories participated in the study.

Method 8276 Phase III Study Design

Method 8276 Phase III Study Design								
Sample Matrices	Surrogates (pg/µL)		Internal Std (pg/µL)	Technical Toxaphene Spike Conc. (pg/µL)	Congener Spike Conc. (pg/µL)	Min No. of both Analyses	Total Min No. of Analyses	Total No. of Vials
	PCB 209	ε-HCH	PCB 204					
Calibration Stds	20-160	50-400	50	50-400	1-500	1	13	13
Second-source Std	100	250	50	150	NA	2	2	1
ORS			50	NA	NA	1	1	1
MB	100	250	50	NA	NA	2	4	1
LCS1	100	250	50	Unk	Unk	2	4	2
Source Fish Matrix	100	250	50	NA	NA	2	4	1
LCS2	100	250	50	Unk	Unk	2	4	2
MS/MSD	100	250	50	Unk	Unk	2	8	4
Terry Creek 1	100	250	50	NA	NA	2	4	2
Terry Creek 2	100	250	50	NA	NA	2	4	2
Total							48	29

Fish Sample Extraction/Cleanup Procedure



Samples were extracted in hexane in an Accelerated Solvent Extractor (ASE) using a modified version of Method 3545A with a sample mass of 10 g of fish tissue (or Ottawa sand for control samples). In-cell cleanup was done in the ASE extraction cell using activated, acidified (30% H2SO4) silica gel per Method 3665A and Method 3630C. These extracts were further subjected to cleanup using a Florisil® cartridge (1% deactivated with deionized water) approach according to Method 3620C. The sample solvent was hexane throughout the process and was concentrated to a final extract volume of 1 mL.

PHASE III MULTI-LABORATORY VALIDATION DATA USING FISH SAMPLES WITH EXTRACTS SUBJECTED TO CLEANUP

Toxaphene				
ID	No. of Participants	Grand Mean (µg/kg)	Std. Deviation (µg/kg)	Reproducibility % RSD
TC 1	8	7030	1170	16.7
TC 2	8	650	112	17.2
Hx-Sed				
ID	No. of Participants	Grand Mean (µg/kg)	Std. Deviation (µg/kg)	Reproducibility % RSD
TC1	8	160	10.7	6.65
TC2	8	10.8	0.84	7.75
Hp-Sed				
ID	No. of Participants	Grand Mean (µg/kg)	Std. Deviation (µg/kg)	Reproducibility % RSD
TC1	8	184	10.3	5.59
TC2	8	15.1	0.963	6.37
P26				
ID	No. of Participants	Grand Mean (µg/kg)	Std. Deviation (µg/kg)	Reproducibility % RSD
TC1	8	16.0	0.738	4.62
TC2	8	2.32	0.063	2.71
P40				
ID	No. of Participants	Grand Mean (µg/kg)	Std. Deviation (µg/kg)	Reproducibility % RSD
TC1	8	21.8	2.05	9.37
TC2	8	2.49	0.114	4.57
P41				
ID	No. of Participants	Grand Mean (µg/kg)	Std. Deviation (µg/kg)	Reproducibility % RSD
TC1	8	8.93	0.322	3.60
TC2	8	1.05	0.027	2.53
P44				
ID	No. of Participants	Grand Mean (µg/kg)	Std. Deviation (µg/kg)	Reproducibility % RSD
TC1	8	9.53	0.693	7.28
TC2	8	1.34	0.099	7.40
P50				
ID	No. of Participants	Grand Mean (µg/kg)	Std. Deviation (µg/kg)	Reproducibility % RSD
TC1	8	16.5	1.20	7.25
TC2	8	2.57	0.125	4.87
P62				
ID	No. of Participants	Grand Mean (µg/kg)	Std. Deviation (µg/kg)	Reproducibility % RSD
TC1	8	22.3	7.01	31.5
TC2	8	3.07	1.02	33.3

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