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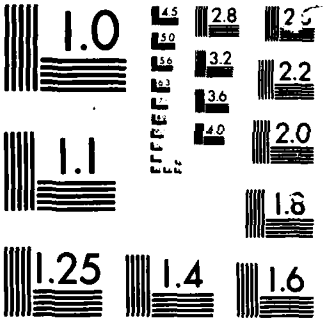

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ANALYTICAL METHODS FOR DETERMINING THE CONCENTRATION OF SOLVENT YELLOW 33, SOLVENT GREEN 3, SYNTHETIC - HC SMOKE COMBUSTION PRODUCTS AND DEGDN IN FRESHWATER

FINAL REPORT FOR PHASE I

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Laurel, MD 20707

Project Officer: Major John A. Kelly
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Analytical methods were developed for the determination of solvent yellow 33 [2-(2'-quinolinyl)-1,3-indandione], solvent green 3 (1,4-di-p-toluidinoanthraquinone), synthetic-HC (hexachloroethane) smoke combustion products and DEGDN (diethyleneglycol dinitrate) dissolved in freshwater. A reverse-phase high performance liquid chromatographic method was developed to determine levels of solvent yellow 33 (isocratic), solvent green 3 (gradient) and DEGDN (isocratic) using a deionized water:methanol

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Arsenic Chloride
Carbon tetrachloride
Tetrachloroethylene
Hexachloroethane
HCL

20. ABSTRACT (Cont'd)

mobile phase. A gradient was necessary for solvent green 3 because it is a 30:70 mixture of solvent yellow 33 and a green dye component. A combined gas chromatographic (electron capture) and atomic absorption spectrophotometric method was developed to measure the chlorinated organic (CCL_4, C_2Cl_4, C_2Cl_6 and C_6Cl_6) and metal (Al, Zn, Pb, Cd and As) concentrations, respectively, dissolved in freshwater from the synthetic-HC smoke combustion products mixture. A pentane extraction procedure was used to extract and concentrate the organics before analysis. Detection limits were determined to be 0.08 mg/L for both dyes and 0.286 mg/L for DEGDN. The detection limit for the chlorinated organics of the smoke combustion mixture is 0.01 mg/L. Detection limits for Al, Cd, Pb, As and Zn are 0.002, 0.0002, 0.0002, 0.003 and 0.00008 mg/L, respectively.

The solubility of the two dyes range from 0.09 to 0.17 mg/L, depending on temperature. Both dyes were found to be stable for 48 h, once dissolved in freshwater. Solubility of the organic components of the smoke combustion products mixture range from less than 0.01 mg/L for C_6Cl_6 to 3.15 mg/L for C_2Cl_4 at 22°C. Temperature (12°-22°C) has little effect on solubility. The solubility of the metals in the mixture range from 0.0014 mg/L for As to 19.2 mg/L for Zn at 22°C. The component concentrations of the mixture stay relatively constant for 24 h in a closed container but are unstable in an open container due to volatilization and precipitation. After 48 h in a closed container at 22°C, the chlorinated organic concentrations decrease by 40-50% while some of the metals decrease in concentration by as much as 70%. The mixture components are more stable at the lower test temperatures. DEGDN is soluble to 0.4 g/100 g water and is stable in freshwater for 48 h once dissolved.

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ANALYTICAL METHODS FOR DETERMINING THE CONCENTRATION OF SOLVENT YELLOW 33, SOLVENT GREEN 3, SYNTHETIC – HC SMOKE COMBUSTION PRODUCTS AND DEGDN IN FRESHWATER

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EXECUTIVE SUMMARY

The U.S. Army Medical Bioengineering Research and Development Laboratory (USAMBRDL) has expressed concern about the environmental fate and effects of smoke/obscurants and explosive propellants which are released to the environment during manufacturing activities and field deployment. The Health Effects Research Division of USAMBRDL was especially interested in the acute toxicity of four specific formulations to freshwater aquatic organisms: solvent yellow 33[2-(2'-quinolinyl)-1,3-indandione]; solvent green 3, a 30:70 mixture of solvent yellow 33 and a green dye component (1,4-di-p-toluidinoanthraquinone); synthetic-HC (hexachloroethane) smoke combustion products; the explosive propellant DEGDN (diethyleneglycol dinitrate). Prior to initiation of toxicity testing the following chemical studies were conducted: 1) analytical methods were developed for the measurement of the above compounds in freshwater; 2) the solubility of these compounds was determined at the three test temperatures to be used in later toxicity studies (12°, 17° and 22°C); 3) the stability of the dissolved phases of these compounds was determined at the same three temperatures.

A reverse-phase (C_{18} column) high performance liquid chromatographic method was developed to measure levels of solvent yellow 33 (isocratic) solvent green 3 (gradient) and DEGDN (isocratic) using a deionized-glass distilled water:methanol mobile phase. A 10% water:90% methanol mobile phase was used for the yellow dye while a 10 minute linear gradient from 10% water:90% methanol to 100% methanol was used for the green dye. A gradient was necessary for the solvent green 3 because it is a 30:70 mixture of solvent yellow 33 and a green dye component. A 30% water:70% methanol mobile phase was used for the DEGDN. Water samples for all three of these compounds could be filtered (0.45 μm) and injected directly onto the HPLC with no extra clean-up or concentration. At a 1 mL/min mobile phase flow rate the retention times for the solvent yellow 33, green dye component and DEGDN were approximately 6.9 min, 20.8 min and 5.8 min, respectively.

A combined gas chromatographic [packed column (OV-210 stationary phase), electron capture detector] and atomic absorption spectrophotometric method was developed to measure the chlorinated organics (CCL_4, C_2Cl_4, C_2Cl_6 and C_6Cl_6) and metals (Al, Zn, Pb, Cd and As) concentrations, respectively, dissolved in freshwater from the synthetic-HC smoke combustion products mixture. A pentane extraction method was used to concentrate the organics before analysis. The detection limits for the above compounds were as follows:

	Compound							
				Synthetic-HC				
	Solvent Yellow 33	Solvent green 3 green component	DEGDN	organics	Al	Cd and Pb	As	Zn
Detection limit (mg/L)	0.08	0.08	0.286	0.01	0.002	0.0002	0.003	0.00008

The solubility of the two dyes ranged from 0.09 to 0.17 mg/L, depending on temperature. Both dyes were found to be stable at 22°C for 48 h, once dissolved in freshwater. DEGDN is soluble to 0.4 g/100 g freshwater and is stable for 48 h at 22°C, once dissolved. The solubilities of CCl_4, C_2Cl_4, C_2Cl_6 and C_6Cl_6 were found to be 2.50 mg/L, 3.62 mg/L, 1.03 mg/L and less than 0.01 mg/L, respectively, at 22°C. The solubilities of Zn, Al, Pb, Cd and As were 19.2 mg/L, 0.047 mg/L, 0.003 mg/L, 0.02 mg/L and 0.0014 mg/L, respectively, at the same temperature. Solubilities of the synthetic-HC components were determined by adding known quantities to 15.5 L of freshwater, stirring for 24 h and sampling after a 6 h settling period. Temperature had little effect on the component solubilities. The component concentrations of the mixture stay relatively constant for 24 h in a closed container but are unstable in an open container due to volatilization of the organics and precipitation of the metals as pH returns to normal (approximately 7.9). After 48 h in a closed container at 22°C, the chlorinated organic concentrations decrease by 40-50% while some of the metals decrease in concentration by as much as 70% (Al). The major metal component of the mixture was zinc (19.2 mg/L), which decreased by 60% over 48 h in a closed chamber at 22°C. Loss of the compounds over time was less at the lower test temperatures (i.e., 22% loss of zinc in 48 h at 12°C; 26% loss of CCl_4 in 48 h at 12°C).

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SECTION 1

INTRODUCTION

The U.S. Army Medical Bioengineering Research and Development Laboratory (USAMBRDL) is concerned about the environmental fate and effects of smoke/obscurants and explosive propellants which are released to the environment during manufacturing activities and deployment in the field. The Health Effects Research Division of USAMBRDL has recently expressed interest in determining the acute toxicity of three smoke munition compounds: solvent yellow 33 [2-(2'-quinolinyl)-1,3-indandione], solvent green 3 (1,4-di-p-toluidinoanthraquinone), synthetic-HC (hexachloroethane) smoke combustion products and the explosive propellant DEGDN (diethyleneglycol dinitrate) to freshwater aquatic organisms. The solvent green 3 is actually a 30:70 mixture of solvent yellow 33 and a green dye component.

Solvent yellow 33 and solvent green 3 are components of a smoke munition. The green smoke munition contains a mixture of the green and yellow dyes. Synthetic-HC smoke combustion products are the combustion products of the M8 grenade, M5 smoke pot and M4A1 floating smoke pot. DEGDN has been chosen for use as a plasticizer replacement for nitroglycerin in the propellant mixtures for the 120 mm shell of the M1 Abrams tank. It is being manufactured and processed at Radford Army Amunition Plant, Radford, VA and Naval Ordinance Station, Indian Head, MD, which discharge to the New River and Potomac River, respectively.

Prior to the determination of the potential toxicity of these compounds to freshwater aquatic organisms, analytical methods had to be developed for monitoring the concentrations of the four test materials in water. In addition, solubility of the two dyes and the components of the synthetic-HC smoke combustion products had to be determined in freshwater at three toxicity test temperatures (12°, 17° and 22°C). Stability of all four test compounds also had to be determined at the three test temperatures in order to determine a testing protocol to be used in later acute toxicity tests (i.e. - static vs static-renewal test). This report addresses the analytical methods for monitoring the concentrations of the four materials in water.

SECTION 2

OBJECTIVES OF STUDY

1) To develop analytical methods for the measurement of solvent yellow 33 and solvent green 3, synthetic-HC smoke combustion products mixture and DEGDN in freshwater.

2) To determine the solubility of the above compounds at the three test temperatures to be used in later toxicity studies (12°, 17° and $22^{\circ}C$).

3) To determine the stability of the dissolved phases of the compounds at the same three test temperatures.

4) The purpose of 1), 2) and 3) above is to conduct acute toxicity testing with freshwater aquatic organisms.

SECTION 3

ANALYTICAL RESULTS AND DISCUSSION

3.1 Solvent Yellow 33 and Solvent Green 3

3.1.1 Test Materials

Solvent yellow 33 and solvent green 3 are components of a smoke munition. The chemical name of the yellow dye is 2-(2'-quinolinyl)-1,3-indandione. The chemical name of the green dye component of solvent green 3 is 1,4-di-p-toluidinoanthraquinone. Solvent green 3 is a dye mix which is approximately 30% solvent yellow 33 and 70% green dye component. Chemically, the dye materials were 93-95% pure with the major contaminants being the precursors used in synthesis or, in the case of solvent yellow 33, an apparent artifact of the synthesis process in which 3 instead of 2 molecules have combined (Henderson et al. 1984).

3.1.2 Analytical Methods

A detailed high performance liquid chromatographic (HPLC) protocol for determining concentrations of both dyes was developed for the USAMBRDL by the Inhalation Toxicology Research Institute (ITRI), Lovelace Biomedical and Environmental Research Institute (Henderson et al. 1984). This method dealt solely with the dyes dissolved in organic solvents but was easily modified in this study for use with aqueous samples.

The HPLC conditions used for separation of the components of the solvent yellow 33 were:

HPLC:	Waters Associates HPLC, dual M45 pumps with Model 680 gradient controller, Model 780 Data Module (integrator), U6K injector, Model 481 variable wavelength UV detector and Z-Module Radial Compression Column System.
Column:	Waters Radial-PAK, µBONDAPAC C_{18}
Standard Solvent:	Methanol
Mobile Phase:	10 % Deionized/glass distilled (DI) H_2O:90% CH_3OH
Method:	Isocratic
Flow Rate:	1 mL/min
Detector:	UV 254 nm
Injector Volume:	100 µL

The HPLC conditions used for separation of the components of the solvent green 3 were:

HPLC:	Same as above
Column:	Same as above
Standard Solvent:	Same as above
Mobile Phase:	DI H_2O:CH_3OH
Method:	Gradient-linear from 10:90 initial to 0:100 at 10 min. Hold at 0:100 to 32 min. Immediate at 32 min to 10:90 for 11 min.
Flow Rate:	1 mL/min
Detector:	UV 254 nm
Injection Volume:	100 µL

Standards of both dyes were made up in spectral grade methanol, both as technical grade and purified material. The purified material was received from the Lovelace Biomedical and Environmental Research Institute's Inhalation and Toxicology Research Institute (ITRI). Both a Varion Techtron Model 635 spectrophotometer (254 nm) and a Waters Associates HPLC with UV detector (254 nm) were used to analyze the standards directly. The HPLC was also used to analyze the technical dyes dissolved in water (100 µL unextracted sample). The solvent yellow 33 standard was made directly from the purified compound received from Lovelace ITRI. The solvent green 3 standard was a 50:50 mixture of purified yellow 33 and purified green 3 received from Lovelace ITRI.

The HPLC retention time (RT) for the major component of solvent yellow 33 ranged from 6.80 to 7.01 min, depending on whether a methanol standard or a water sample was injected. The retention time of the yellow component of the solvent green 3 was 6.80 to 7.01 min, while the retention time of the green component of this dye ranged from 20.69 to 21.0 min.

The detection limit for the spectrophotometric method is 0.1 mg/L for the solvent yellow 33 and the solvent green 3 in spectral grade methanol (Table 1). The detection limits were determined by injecting a decreasing series of standards into the HPLC until dye peaks were indistinguishable from background noise. Standard curves for the detection of these compounds both as the technical grade and the purified standards are given in Figure 1. The lowest linear correlation was 0.985 for the technical grade solvent yellow 33.

TABLE 1. ABSORBANCE (254 nm) FOR SOLVENT YELLOW 33 AND SOLVENT GREEN 3 AT VARYING CONCENTRATIONS USING VARION SPECTROPHOTOMETER[a]

Dye	Chemical Grade	Concentration (mg/L)	Absorbance at 254 nm
Solvent Yellow 33	Lovelace Std.	0[b]	0
		1.12	0.064
		2.8	0.168
		5.6	0.320
		8.4	0.460
		11.2	0.625
	Technical	0[c]	0
		1.050	0.069
		2.625	0.152
		5.250	0.250
		7.875	0.520
		10.5	0.610
Solvent Green 3	Lovelace Std.	0[d]	0
		1.15	0.084
		2.875	0.221
		5.75	0.394
		8.625	0.620
		11.5	0.822
	Technical	0[e]	0
		1.08	0.076
		2.70	0.204
		5.4	0.380
		8.1	0.561
		10.8	0.721

a. The values presented in this table were used to draw the curves depicted in Figure 1.
b. $r = 0.9996$ $y = -0.1447 + 18.2306\ (x)$ x = absorbance
c. $r = 0.9847$ $y = 0.3093 + 16.0858\ (x)$ x = absorbance
d. $r = 0.9992$ $y = -0.0542 + 14.0920\ (x)$ x = absorbance
e. $r = 0.9995$ $y = -0.2504 + 15.1039\ (x)$ x = absorbance

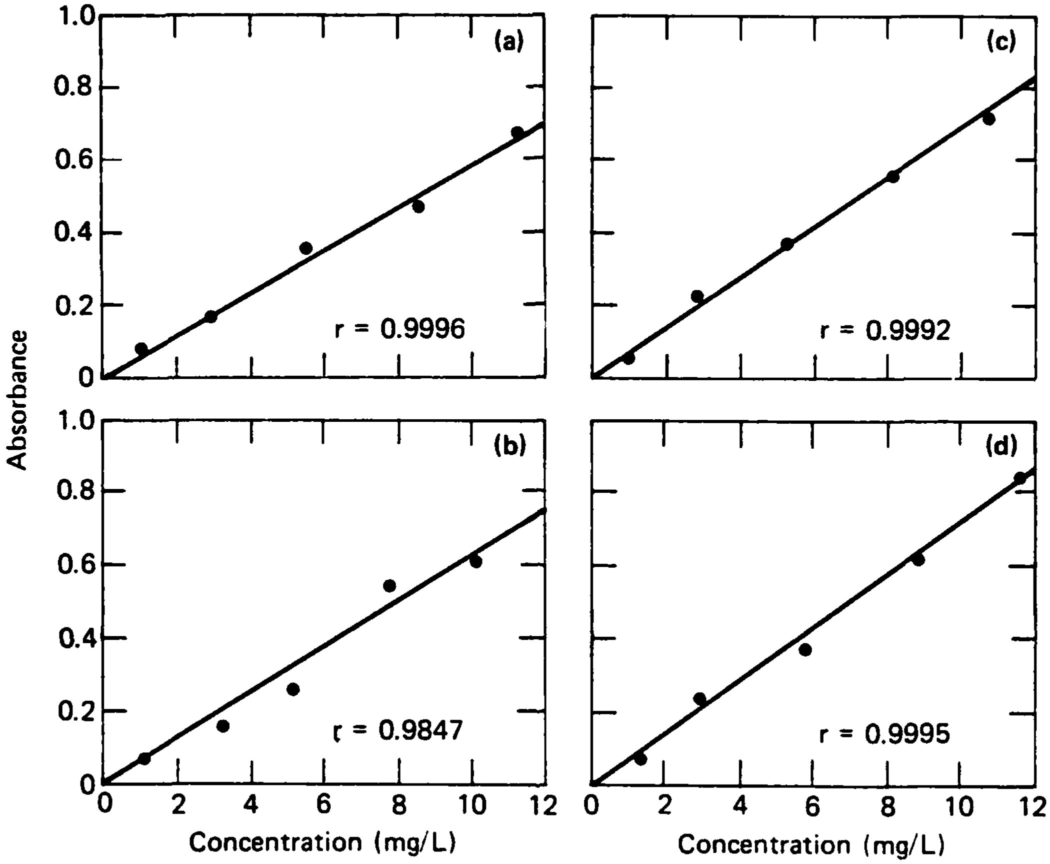

Fig. 1 The linear correlation between absorbance (254 nm) and concentration of the solvent yellow 33 and solvent green 3 using the Varion spectrophotometer; (a) Lovelace purified solvent yellow 33, (b) Technical solvent yellow 33, (c) Lovelace purified solvent green 3, (d) Technical solvent green 3.

The detection limit for the HPLC method for solvent yellow 33 and solvent green 3 in spectral grade methanol is 0.08 mg/L (Figure 2). The linear correlation between area under the curve (AUC) and compound concentration for the yellow and green components of the technical solvent green 3 are presented in Table 2 and Figure 3 (A B). The Lovelace ITRI purified solvent green 3 standard curve is presented in Figure 3C. There was no yellow component present in the Lovelace ITRI purified solvent green 3. The linear correlations for solvent yellow 33 are shown in Figure 4, with the values used to generate the curves presented in Table 3. These standard curves generated from the HPLC data were used for the determinations of dye concentrations in diluent water during the solubility and stability studies.

In order for a peak to be considered as a contaminant and not a background problem in the HPLC method, it must meet three criteria: (1) the contaminant must increase in AUC as the total concentration of the dye increases; (2) the contaminant must have a reproducible retention time and (3) the area under the curve must be greater than 20,000. The solvent yellow 33 technical grade material and the Lovelace ITRI purified standard have one minor contaminant that elutes from the column at 8.42 min and meets these criteria (Figure 5). Note the noise and the negative injection peak on all three chromatograms. The technical grade solvent green 3 contains a solvent yellow 33 component and a green component which elutes at approximately 21 min. (Figure 6A). In addition, the contaminant associated with the solvent yellow 33 in Figure 5 is also present in the solvent green 3, along with at least 3 other minor contaminants. The Lovelace ITRI purified solvent green 3 contained all the minor contaminants mentioned above; they were not present in the solvent yellow 33 peak (Figure 6B).

The dyes were found to be stable in methanol when stored at 4°C in the dark. A 2.8 mg/L yellow standard was found to remain stable over a 3-week period. The average concentration determined by HPLC at the end of 3 weeks was 2.82 mg/L (±0.03 S.D., N=7). Solvent green 3 concentrations in a 1.40 mg/L yellow and 1.28 mg/L green standard mixture averaged 1.41 yellow (±0.01 S.D., N=7) and 1.35 mg/L green (±0.09 S.D., N=7) over the same period.

The accuracy and precision of the HPLC method for these dyes was excellent, even at dye concentrations as low as 0.10 mg/L. For example, a 2.8 mg/L known sample of solvent yellow 33 analyzed nine separate times, yielded a mean value of 2.83 mg/L with a S.D. of only 0.03. At its 22°C solubility limit, the solvent yellow 33 samples yielded a mean aqueous concentration of 0.17 mg/L (± 0.01 S.D., n=4). A known solvent green dye 3 sample containing 1.40 mg/L solvent yellow 33 and 1.28 mg/L green component was analyzed 9 times and yielded concentrations of 1.41 mg/L (± 0.08 mg/L S.D.) solvent yellow 33 and 1.35 mg/L (± 0.09 mg/L S.D.) green component.

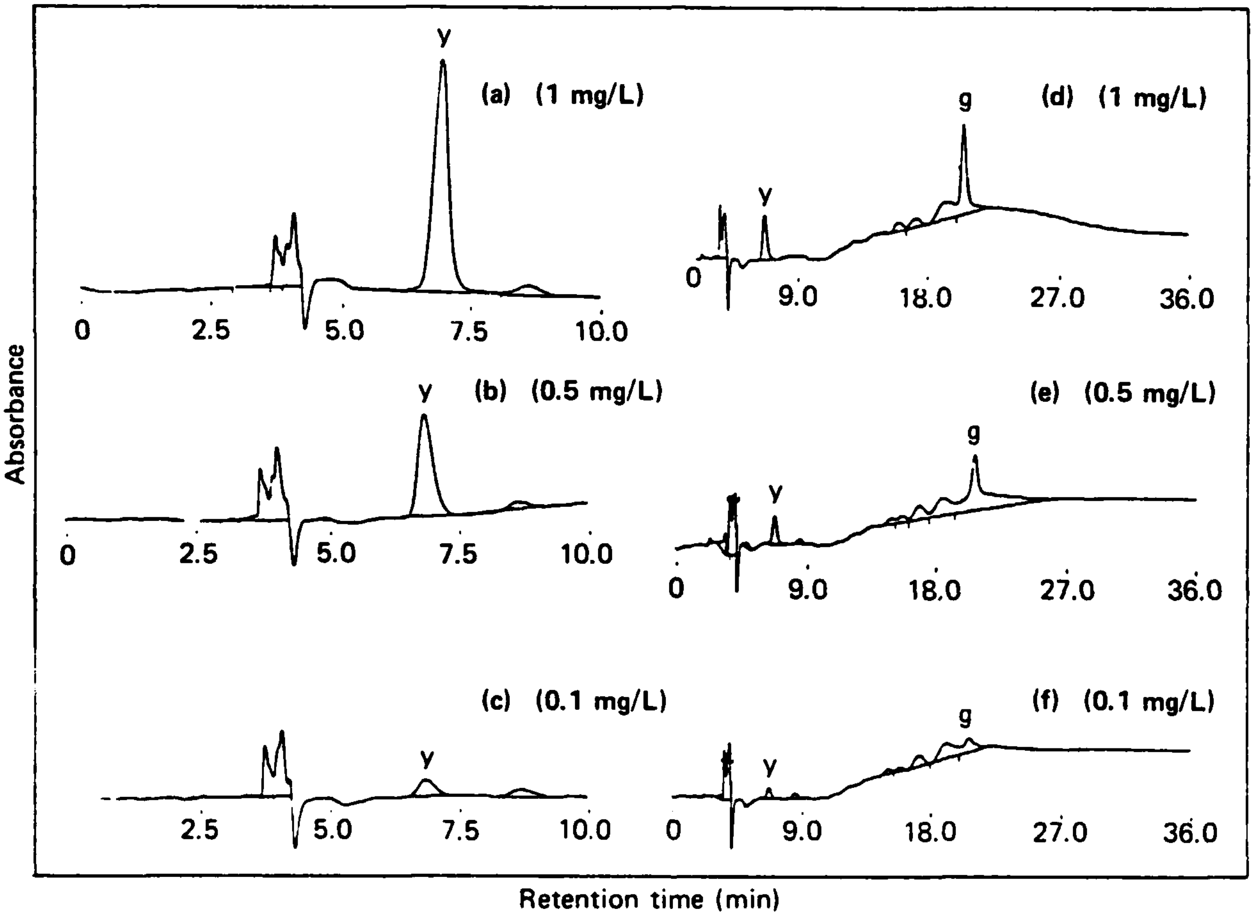

Fig. 2 HPLC chromatograms of technical grade solvent yellow 33 (a,b,c) and solvent green 3 (d,e,f). Note: The lower case (y) stands for solvent yellow 33 and the lower case (g) stands for solvent green 3.

TABLE 2. AREA UNDER THE CURVE (AUC) FOR ABSORBANCE (254 nm) FOR THE TECHNICAL GRADE SOLVENT GREEN 3 AND THE PURE SOLVENT GREEN 3 SUPPLIED BY LOVELACE (HPLC)[a].

Dye	Chemical Grade	Chemical	Concentration (mg/L)	AUC at 254 nm
Solvent Green 3	Technical	Yellow 33	1.08[b]	109,327
			2.7	266,986
			5.4	530,517
			8.1	835,446
			10.8	1,109,490
		Green 3	1.08[c]	441,009
			2.70	856,366
			5.4	1,466,248
			8.1	2,272,093
			10.8	2,969,206
	Lovelace Std.	Green 3	1.15[d]	593,462
			2.875	1,343,988
			5.75	2,382,966
			8.625	3,657,710
			11.55	4,950,859

[a] The values presented in this table were used to draw the curves depicted in Figure 3.

[b] The linear correlation $r = 0.9996$ and the equation of the line $Y = 0.11889 + 0.00000965\ (x)$ where x = AUC

[c] The linear correlation $r = 0.9991$ and the equation of the line $Y = -0.50843 + 0.00000382\ (x)$ where x = AUC.

[d] The linear correlation $r = 0.9992$ and the equation of the line $Y = -0.22132 + 0.00000240\ (x)$ where x = AUC.

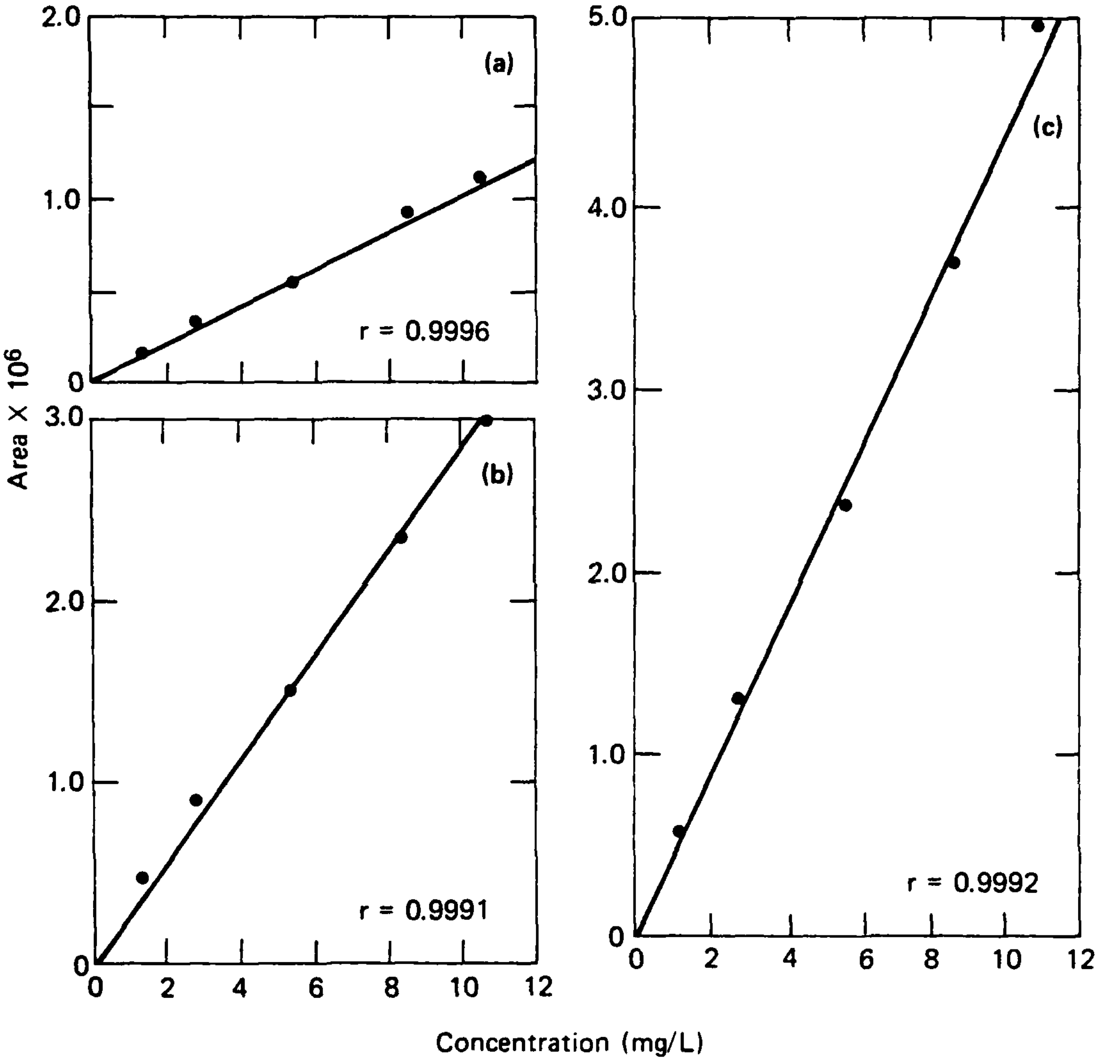

Fig. 3 The linear correlation between area of absorbance (254 nm) and concentration using the Waters HPLC; (a) Technical solvent green 3 - yellow component, (b) Technical solvent green 3 - green component, (c) Lovelace purified solvent green 3.

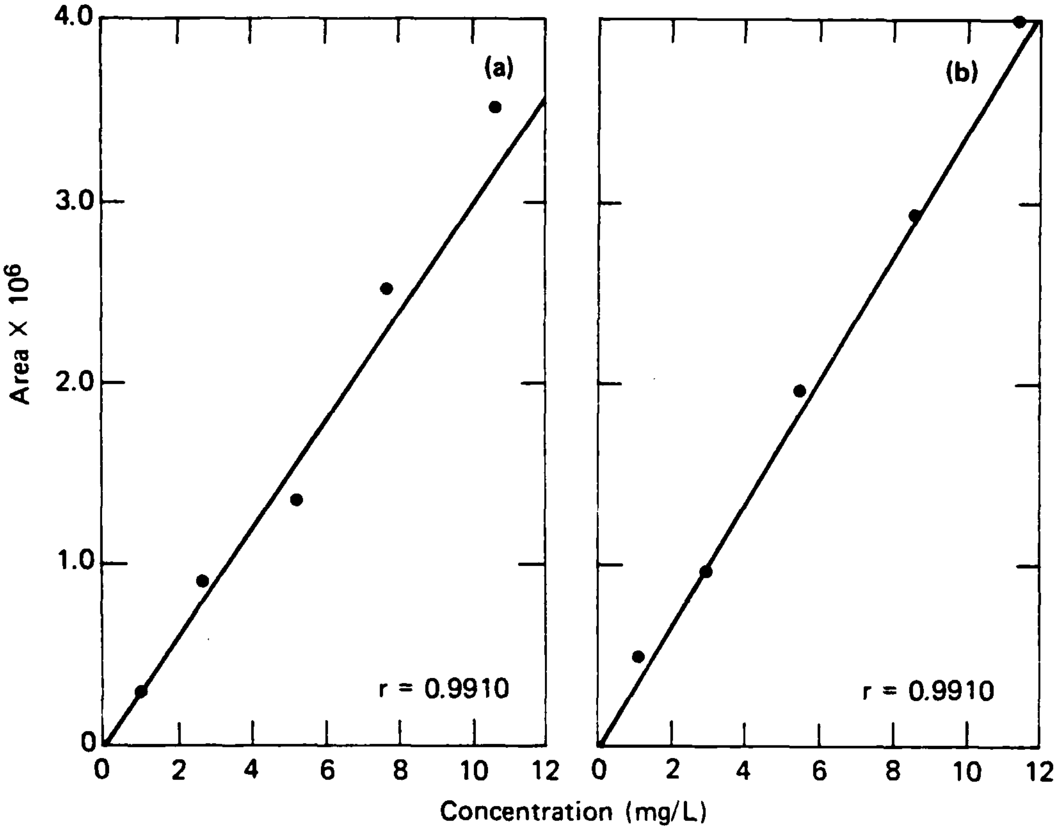

Fig. 4 The linear correlation between area of absorbance (254 nm) and concentration using the Waters HPLC; (a) Technical solvent yellow 33, (b) Lovelace purified solvent yellow 33.

TABLE 3. AREA UNDER THE CURVE (AUC) FOR ABSORBANCE (254 nm) FOR THE TECHNICAL GRADE SOLVENT YELLOW 33 AND THE PURE SOLVENT YELLOW 33 SUPPLIED BY LOVELACE.[a]

Dye	Chemical Grade	Chemical	Concentration (mg/L)	AUC at 254 nm	Retention Time (min)
Solvent Yellow 33	Technical	Yellow 33	1.050	339,446[b]	6.794
			2.625	865,478	6.787
			5.250	1,340,647	6.786
			7.875	2,540,194	6.797
			10.5	3,474,687	6.801
	Lovelace Std.	Yellow 33	1.12	428,044[c]	6.792
			2.8	997,541	6.792
			5.6	1,973,327	6.793
			8.4	2,849,803	6.796
			11.2	3,989,594	6.787

[a] The values presented in this table were used to draw the curves depicted in Figure 4.

[b] The linear correlation r = 0.9910 and the equation of the line $Y = 0.37072 + 0.00002973 (x)$ where x = AUC.

[c] The linear correlation r = 0.9910 and the equation of the line $Y = 0.03614 + 0.000002862 (x)$, where x = AUC.

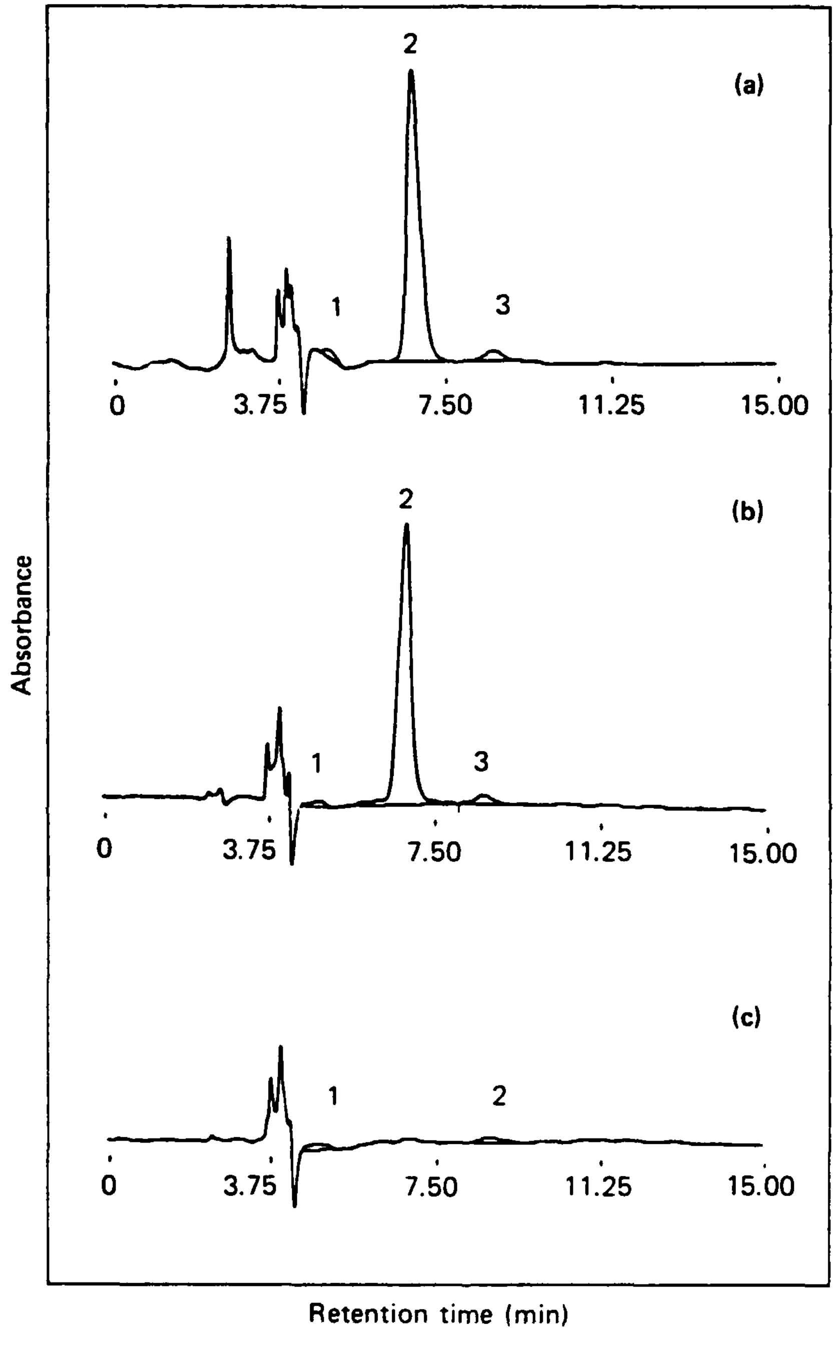

Fig. 5 Chromatograms (HPLC) of solvent yellow 33 and contaminants. (a) 1 mg/L Technical solvent yellow 33 with three peaks; (1) retention time (RT) = 4.90, area under the curve (AUC) = 8,662, (2) RT = 6.83, AUC = 422,616, (3) RT = 8.643, AUC = 18,854. (b) 1.12 mg/L Lovelace purified solvent yellow 33 with three peaks; (1) RT = 4.93, AUC = 12,499, (2) RT = 6.79, AUC = 428,044, (3) RT = 8.62, AUC = 20,516. (c) HPLC grade MEOH with two peaks; (1) RT = 4.97, AUC = 15,947, (2) RT = 8.70, AUC = 13,590.

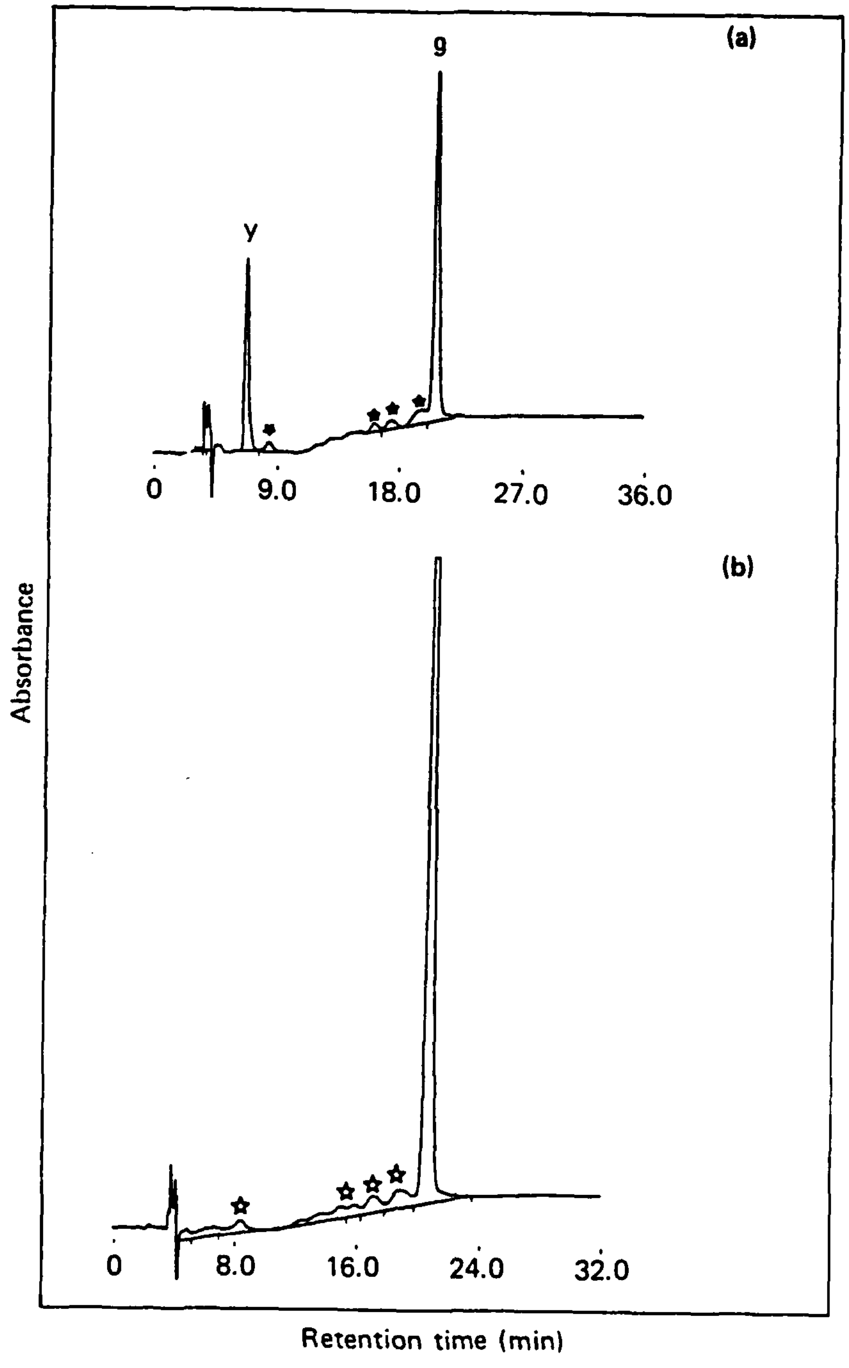

Fig. 6 Comparison of HPLC chromatograms of Technical solvent green 3 with the Lovelace purified solvent green 3; (a) 5.0 mg/L Technical solvent green 3 with yellow 33 component (y) and green 3 component (g) indicated, (b) 5.7 mg/L Lovelace purified solvent green 3. Note the absence of the yellow component in the purified dye. The stars indicate minor contaminants shared by the two formulations.

3.1.3 Solubility in Diluent Freshwater

The HPLC method was used to determine solubility of technical grade solvent yellow 33 and solvent green 3 in diluent water (mean pH = 7.6; alkalinity = 156 mg/L as $CaCO_3$ and hardness = 180 mg/L as $CaCO_3$). The technical dyes were added to 300 mL of diluent water in glass beakers. At least two beakers were used for each dye at each test temperature (Table 4). The beakers were kept in a temperature controlled incubator and stirred for 24 h at 12, 17 and 22°C. Following stirring, the water was filtered to 0.45 µm and 100 µl was injected into the HPLC. The solubility of solvent yellow 33 ranged from 0.09 to 0.17 mg/L, depending on temperature (Table 4). The yellow component of solvent green 3 was soluble at concentrations ranging from 0.09 to 0.16 mg/L, again depending on temperature. There were no detectable levels of the green component of solvent green 3 at any test temperature, indicating a solubility of less than 0.08 mg/L. Chromatograms of solvent yellow 33 and solvent green 3 are shown in Figures 7 and 8, respectively, along with chromatograms of the diluent water. Note the noisy nature of the curves until approximately 6 min, with a subsequent smoothing except for the sample peaks. Also, note the baseline shift in Figure 8 which is due to the gradient solvent change used to analyze solvent green 3.

The low water solubility indicates that toxicity of the dissolved dyes at their solubility limit, without carrier solvent, will be minimal. For this reason we feel that the 0.08 mg/L detection limit with the HPLC is adequate. If these compounds are more toxic than expected, water samples will be concentrated to increase the sensitivity of the method. A Waters Associates C-18 Sep-Pak will be used to concentrate the dyes from the water, after which they will be eluted with methanol and injected into the HPLC.

3.1.4 Stability in Diluent Freshwater

Stability of the dissolved technical dyes in diluent water at 22°C was examined using the HPLC method. After the 24 h solubility experiment, the remaining dissolved dyes were held at 22°C and sampled after 24 and 48 h. Thus, the initial dissolved dye concentration in the stability study was the dissolved dye present at the end of the solubility study. Concentrations of the solvent yellow 33 and the yellow component of solvent green 3 were stable for 48 h in diluent water (Table 4). Since the green component of the solvent green 3 was not detectable after the 24 h solubility study, it was not possible to examine stability over time. Stability studies at test temperatures of 12 and 17°C were not performed since the yellow dye was found to be stable at the highest test temperature.

TABLE 4. SOLUBILITY AND STABILITY OF TECHNICAL DYE COMPONENTS

			Dissolved Concentration [mg/L ± S.D. (N)]		
Component	Dye	Temp (^{o}C)	24 h solubility	24 h stability	48 h stability
Yellow	Solvent Yellow 33	22	0.17 ± 0.01 (4)	0.17 ± 0.02 (4)	0.17 ± 0.02 (4)
		17	0.13 ± 0.02 (3)		
		12	0.09 ± 0.02 (2)		
	Solvent Green 3	22	0.16 ± 0.03 (4)	0.15 ± 0.04(4)	0.15 ± 0.04 (5)
		17	0.09 ± 0.02 (3)		
		12	0.09 ± 0.01 (2)		
Green	Solvent Green 3	22	ND*		
		17	ND		
		12	ND		

*ND = less than 0.08 mg/L detection limit.

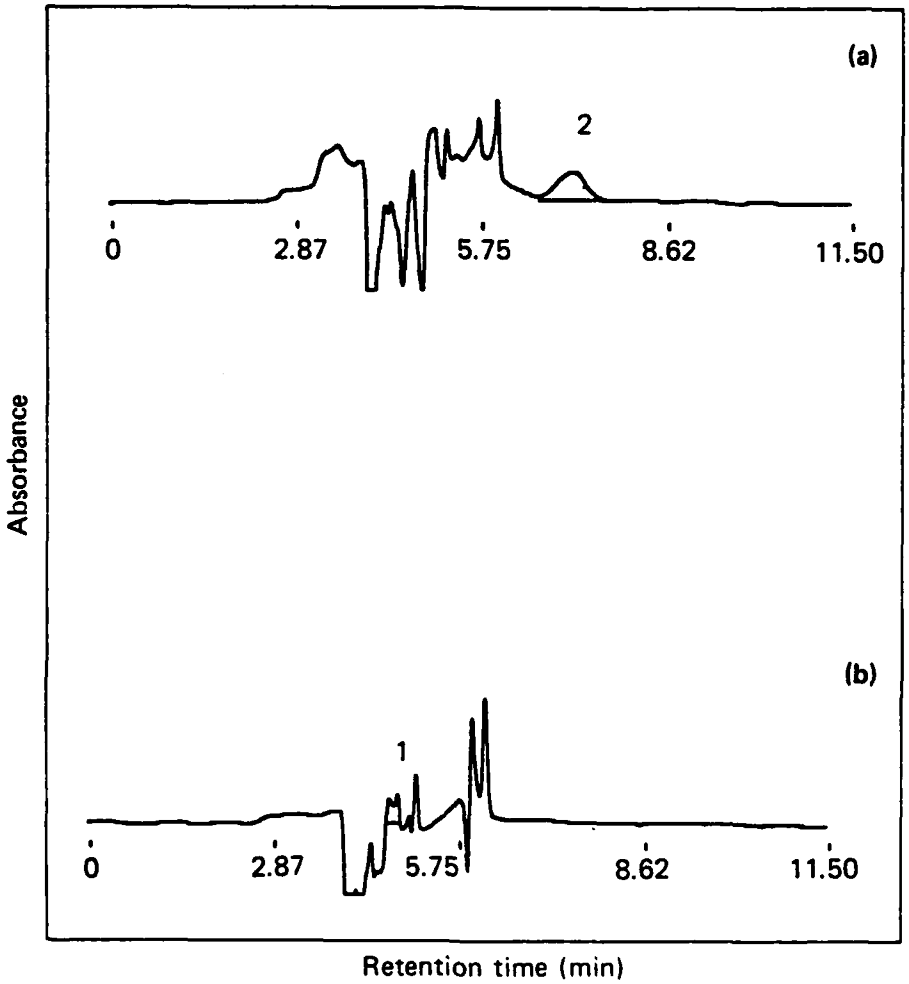

Fig. 7 Comparison of HPLC chromatograms of dissolved Technical solvent yellow 33 and control diluent water: (a) Technical solvent yellow 33 (yellow 33 component present at approximately 0.17 mg/L- peak 2), (b) diluent water only. Note the absence of peak 2.

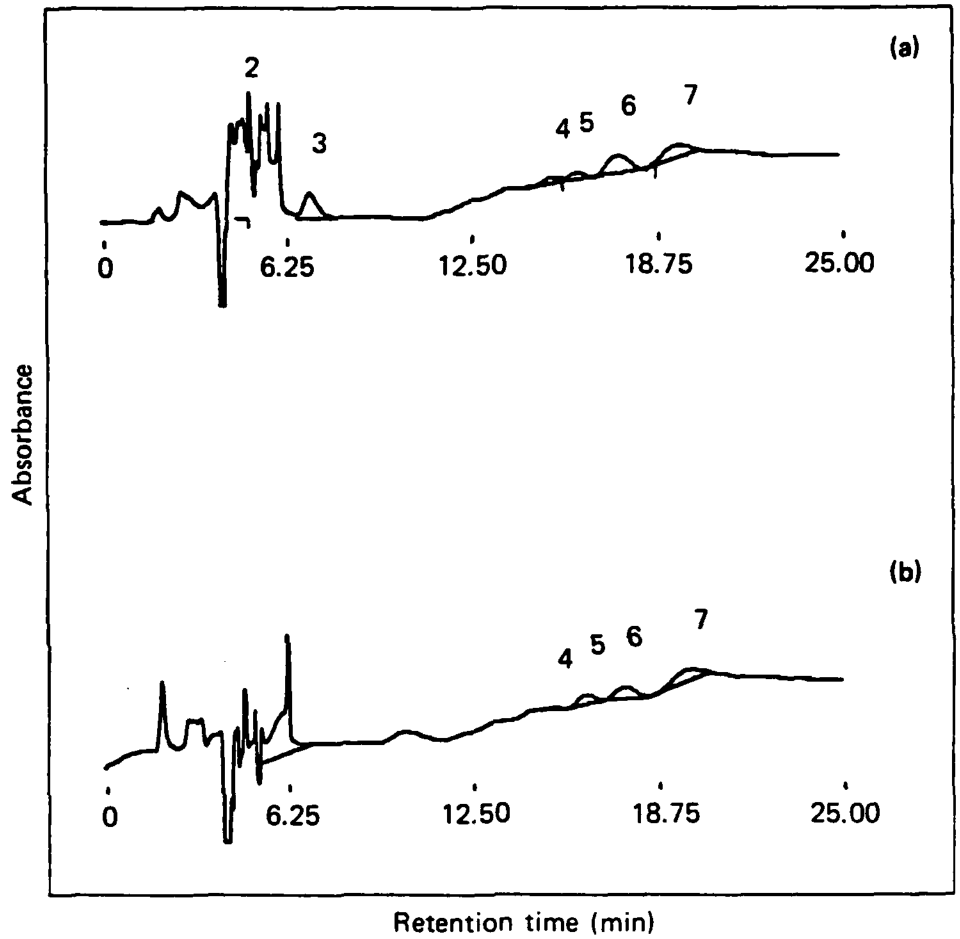

Fig. 8 Comparison of HPLC chromatograms of dissolved Technical solvent green 3 and control diluent water: (a) Technical solvent green 3 (solvent yellow 33 component present at a concentration of approximately 0.15 mg/L- peak 3; solvent green 3 component not detected at a retention time of 21 min), (b) diluent water only. Note the absence of the solvent yellow 33 component.

3.2 Synthetic-HC Smoke Combustion Products Mixture

3.2.1 Test Materials

Synthetic-HC smoke combustion products are a complex mixture of chlorinated organics, metals and HCL. Percent composition of this mixture is given in Table 5. It can be seen that the major constituent is $ZnCl_2$. Our intention was to study the dissolved fractions of these compounds in our freshwater dilution water.

3.2.2 Analytical Methods

The chlorinated organics analytical protocol was a modification of Standard Method 509A (APHA et al. 1985) for the analysis of organic pesticides. A pentane extraction procedure was used to remove the organics from the water prior to injection into a gas chromatograph equipped with an electron-capture detector. Aluminum, lead, cadmium and arsenic in the mixture were analyzed by EPA's atomic absorption spectrophotometric graphite furnace Methods 202.2, 239.2, 213.2 and 206.2, respectively (U.S. EPA 1983). Arsenic was also analyzed by EPA's atomic absorption spectrophotometric gaseous hydride Method 206.3 (U.S. EPA 1983). The zinc was analyzed by EPA's direct aspiration flame atomic absorption spectrophotometric Method 289.1 (U.S. EPA 1983).

HPLC grade organics (Burdick and Jackson, where possible) were used in this mixture. A Tracor Model 560 gas chromatograph with an electron capture detector and Spectra-Physics integrator was used to quantify the organochlorine compounds present in the mixture. A Perkin-Elmer 2380 Atomic Absorption Spectrophotometer was used to determine the metal constituents of the mixture.

Standards for the chlorinated organics were made by dissolving known quantities of the pure compounds in HPLC grade pentane (Burdick and Jackson). Since it was necessary to analyze all four organochlorines with a single injection, the standard contained all four organics. The standards for the metal analyses were certified atomic absorption standards from Fisher Scientific Company.

The gas chromatograph conditions used for separation of the organochlorine components of the synthetic-HC smoke combustion products mixture were:

Column:	Alltech Associates packed column. Gas Chrom Q support (mesh 100/120) with OV-210 stationary phase. Column is 6 ft. x 1/4 in. O.D. with 4 mm I.D.
Carrier Gas:	N_2 (48 cc/min)
Injector Temperature:	100°C
Detector:	Electron Capture
Detector Temperature:	350°C
Oven Program:	80°C initial-hold 1 min 15°C min to 160°C-hold 7 min cool to 80°C initial

TABLE 5. PERCENT COMPOSITION OF SYNTHETIC-HC SMOKE COMBUSTION PRODUCTS

HCl	1.828
CCl_4	5.515
C_2Cl_4	14.411
C_2Cl_6	4.480
C_6Cl_6	1.634
Al_2O_3	10.917
$ZnCl_2$	61.073
$PbCl_2$	0.089
$CdCl_2$	0.051
$AsCl_3$	0.001

Injection Volume: 1 µL
Recorder Chart Speed: 30 cm/h

The gas chromatograph retention times for the four organics were:

Carbontetrachloride (CCL_4)	57 sec or 0.95 min
Tetrachloroethylene (C_2Cl_4)	85 sec or 1.42 min
Hexachloroethane (C_2Cl_6)	190 sec or 3.17 min
Hexachlorobenzene (C_6Cl_6)	670 sec or 11.17 min

The linear correlations between GC peak area and concentration for the chlorinated organics in the synthetic-HC smoke combustion products mixture are shown in Figure 9. Notice that the regressions are based on a series of concentrations from 0.10 to 10.0 mg/L. It was found that at higher concentrations the detector response was not linear. For this reason, when the integrator indicated areas greater than that corresponding to 10.0 mg/L in a sample, the sample was diluted with Burdick and Jackson pentane. The detection limit for the GC method for each organochlorine standard in the synthetic-HC combustion products mixture was 0.10 mg/L (Figure 10). There was good separation and peak form for all four compounds in the standard. The integrator failed to detect these compounds at lower concentrations, even with the sensitivity set very close to background. Since water samples of 50 mL were concentrated to 5 mL in pentane when the actual solubility in diluent water experiments were conducted, the detection limit for the organics dissolved in water were 0.01 mg/L.

The accuracy and precision of the GC method was determined using known standards and unknown samples. Both the low and high concentration unknowns were prepared by an independent chemist. The low concentration unknown contained 0.23 mg/L CCl_4, 0.25 mg/L C_2Cl_4, 0.25 mg/L C_2Cl_6 and 0.25 mg/L C_6Cl_6, while the high concentration unknown contained 3.27 mg/L CCl_4, 3.35 mg/L C_2Cl_4, 3.35 mg/L C_2Cl_6 and 3.33 mg/L C_6Cl_6. After completion of a standard curve, these samples were analyzed using the GC methods described above. Four injections of the low concentration unknown yielded values of 0.22 mg/L (± 0.01 S.D.) CCl_4, 0.25 mg/L (± 0.01) C_2Cl_4, 0.24 mg/L (± 0.01) C_2Cl_6 and 0.26 mg/L (± 0.03) C_6Cl_6. An equal number of injections of the high concentration unknown yielded values of 3.37 mg/L (± 0.05 S.D.) CCl_4, 3.49 mg/L (± 0.06) C_2Cl_4, 3.48 mg/L (± 0.06) C_2Cl_6 and 3.27 mg/L (± 0.08) C_6Cl_6.

The atomic absorption spectrophotometric conditions for the graphite furnace (20 µL sample) analysis of aluminum, cadmium, lead and arsenic are given in Table 6. As stated earlier, analysis of zinc in the synthetic-HC combustion products mixture was done by direct aspiration flame spectroscopy. Detection limits for the metals in water are 0.002 mg/L for aluminum,

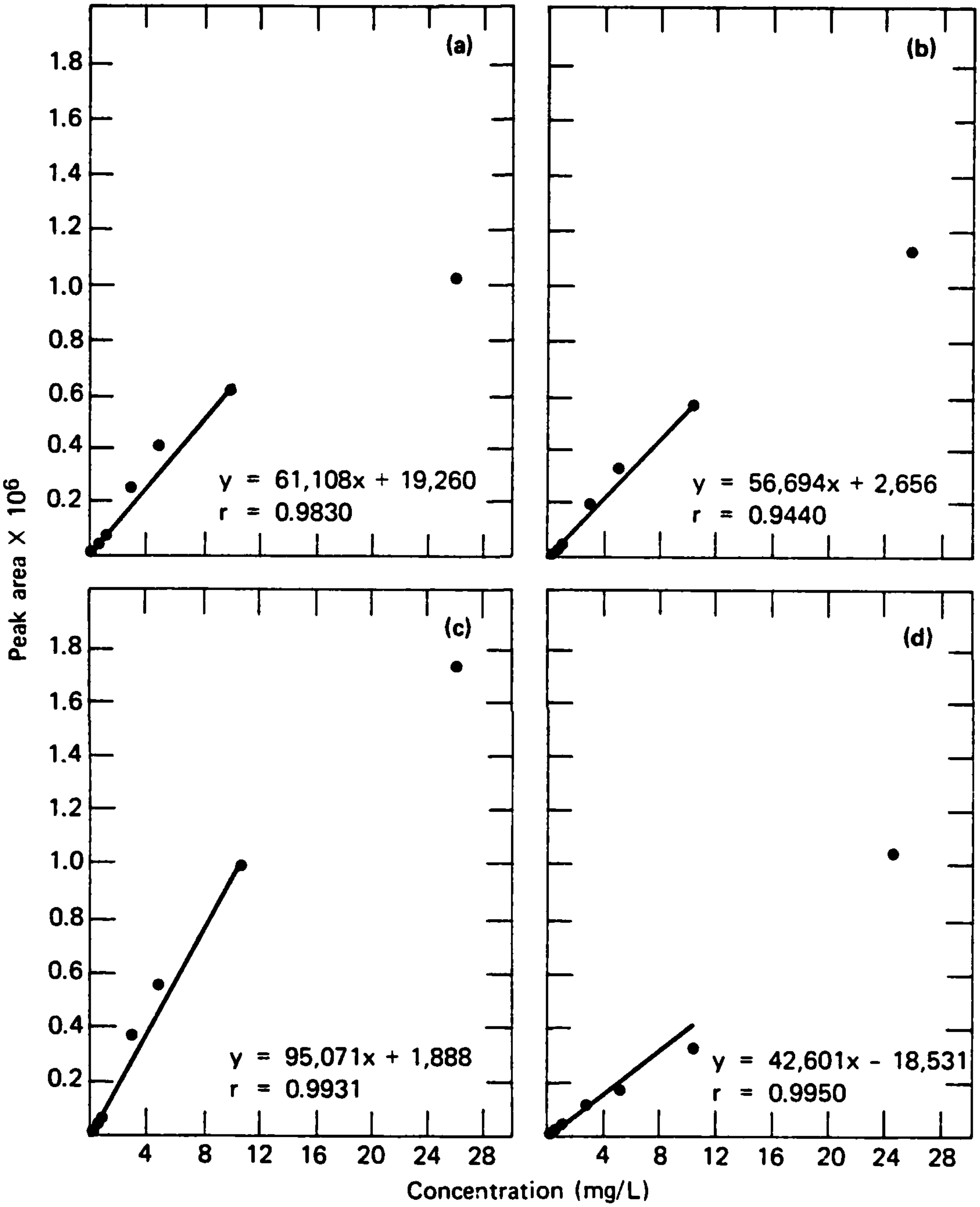

Fig. 9 The linear correlation between peak area and concentration for the chlorinated organics in the synthetic-HC smoke combustion products mixture using gas chromatography methods: (a) CCl_4, (b) C_2Cl_4, (c) C_2Cl_6, (d) C_6Cl_6. (Regressions based on concentrations from 0.1 to 10.0 mg/L.)

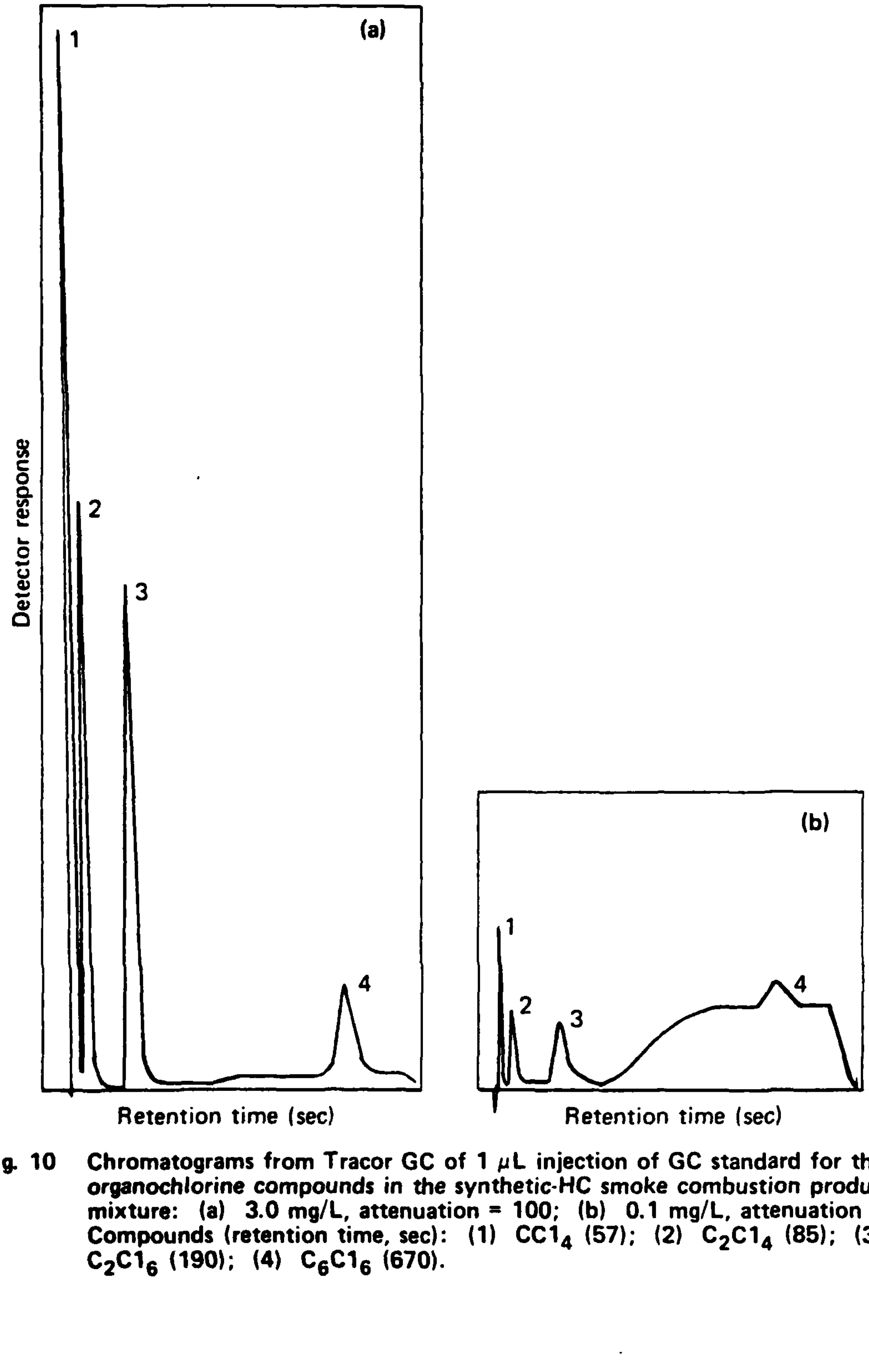

Fig. 10 Chromatograms from Tracor GC of 1 μL injection of GC standard for the *organochlorine compounds in the synthetic-HC smoke combustion products* mixture: (a) 3.0 mg/L, attenuation = 100; (b) 0.1 mg/L, attenuation = 10. Compounds (retention time, sec): (1) $CC1_4$ (57); (2) C_2C1_4 (85); (3) C_2C1_6 (190); (4) C_6C1_6 (670).

TABLE 6. GRAPHITE FURNACE CONDITIONS FOR ATOMIC ABSORPTION SPECTROSCOPY FOR ALUMINUM, CADMIUM, LEAD AND ARSENIC.

Step	Metal	Temp (°C)	Ramp Time (sec.)	Hold Time (sec.)
1 Dry	Al	120	20	20
	Cd	120	20	20
	Pb	120	20	20
	As	120	20	20
2 Char	Al	1700	5	15
	Cd	250	5	15
	Pb	500	5	15
	As	300	5	15
3 Atomize	Al	2400	0	5
	Cd	800	0	5
	Pb	1100	0	5
	As	1900	0	5
4 Clean	Al	2600	1	5
	Cd	2600	1	5
	Pb	2600	1	5
	As	2600	1	5

0.0002 mg/L for cadmium; 0.002 mg/L for lead and 0.003 mg/L for arsenic using the graphite furnace method, and 0.0002 mg/L for arsenic using the gaseous hydride method. The detection limit for zinc using flame spectroscopy is 0.00008 mg/L. The linear correlations between absorbance and metal concentrations for aluminum, cadmium, lead and arsenic analyses using the graphite furnace method are shown in Figure 11. The linear correlation for arsenic by the gaseous hydride method is shown in Figure 12.

The organic standard made in pentane was stable for at least 1 month when kept at 4°C in the dark in sealed volumetrics. A 10 mg/L standard analyzed immediately after preparation yielded average peak areas (± S. D.) of 603,124 (29,266) for CCl_4, 575,090 (29,797) for C_2Cl_4, 984,080 (67,025) for C_2Cl_6 and 372,105 (40,620) for C_6Cl_6. The same standard analyzed 30 days later yielded average peak areas of 587,667 (13,857) for CCl_4; 548,316 (18,513) for C_2Cl_4; 929,259 (13,449) for C_2Cl_6; and 350,571 (38,810) for C_6Cl_6. If the standards are used consistently over a week there can be a significant change due to evaporation. This can be avoided by dividing each standard into a number of separate GC vials which are used for only 2 or 3 days before discarding.

3.2.3 Solubility in Diluent Freshwater

The water-soluble fractions of the synthetic-HC smoke combustion products mixture were determined using a method similar to that used by Anderson *et al.* (1974) to determine the water-soluble fractions of crude and refined oils. Fifteen and one half liters of aerated diluent freshwater were placed in 22.5 L pyrex jars and kept in an incubator at one of three test temperatures (22, 17 or 12°C). The chlorinated organics (CCL_4, C_2Cl_4, C_2Cl_6, C_6Cl_6) metals (Al_2O_3, $ZnCl_2$, $PbCl_2$, $CdCl_2$, $AsCl_3$) and HCL were added in the proportions shown in Table 7. The percentages of each component were outlined in the initial proposal (Table 5). A stir bar was added, the jar was sealed and placed on a magnetic stir plate in an incubator at the test temperature. The stirring speed was adjusted so that a vortex extended approximately 25% of the distance to the bottom of the jar. After 24 h of stirring the jar was allowed to stand for 6 h to allow for any particulates to settle. Samples (50 mL) were then siphoned from mid-depth into a separatory funnel, to which 5 mL of HPLC grade Burdick and Jackson pentane was added. The funnel was shaken for 2 min., the layers allowed to separate and the bottom water layer drawn off for metals analysis. Concentrated nitric acid (100 µL) was added to the metal samples for storage. A clean pipette was used to transfer 1-2 mL of the top pentane layer to a GC bottle which was sealed (teflon cap) and stored at 4°C in the dark until GC analysis of the organics. GC conditions were described in Section 3.2.2. Every fourth sample analyzed was a known standard.

Each component of the synthetic-HC smoke combustion products mixture was added separately to the mixing jar except for $ZnCl_2$, $PbCl_2$, $CdCl_2$, $AsCl_3$ and HCL. A stock solution of these components was made in distilled water (61.07 g $ZnCl_2$, 89.22 mg $PbCl_2$, 51.00 mg $CdCl_2$, 0.97 mg $AsCl_3$ and 1.54 mL HCL).

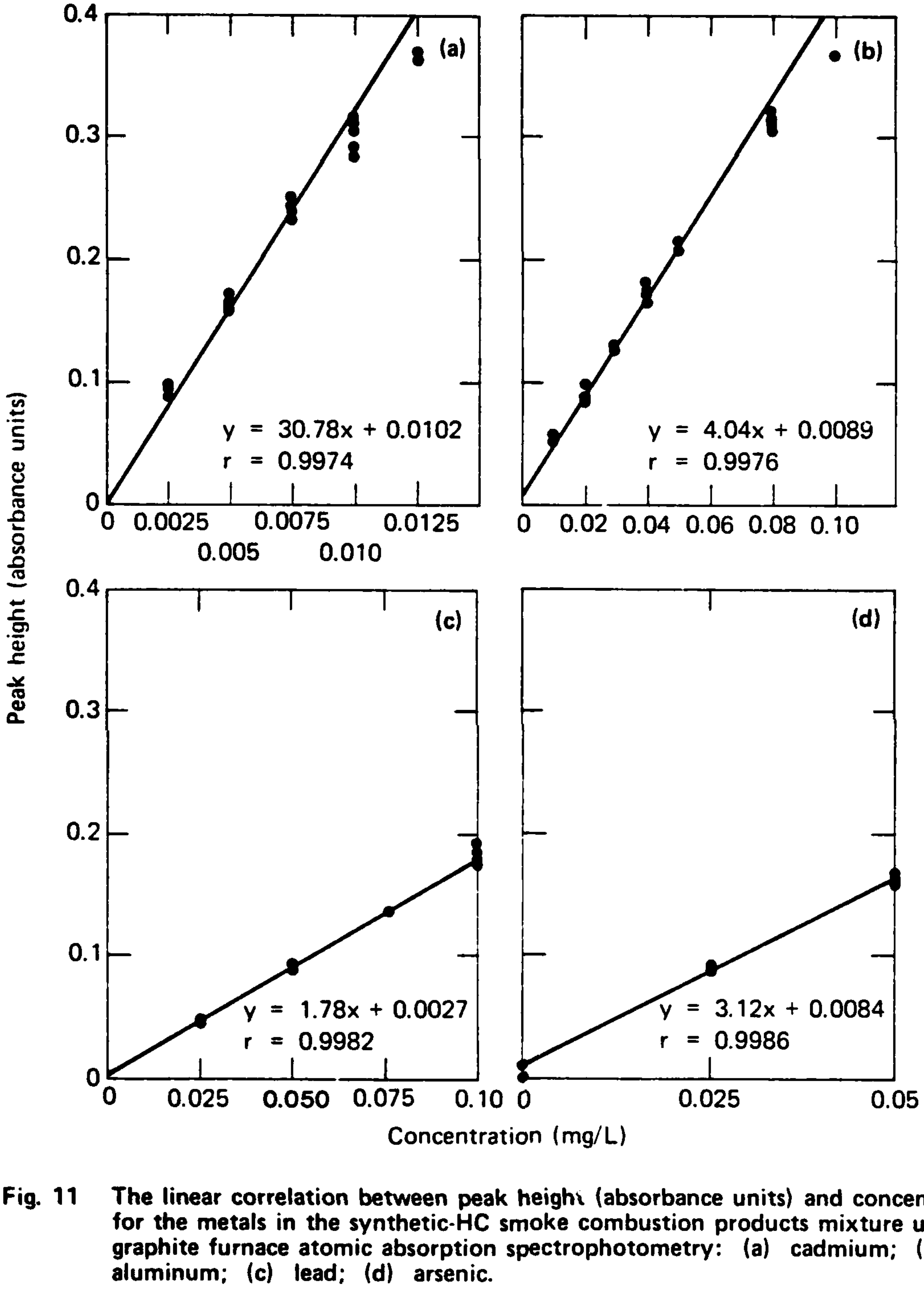

Fig. 11 The linear correlation between peak height (absorbance units) and concentration for the metals in the synthetic-HC smoke combustion products mixture using graphite furnace atomic absorption spectrophotometry: (a) cadmium; (b) aluminum; (c) lead; (d) arsenic.

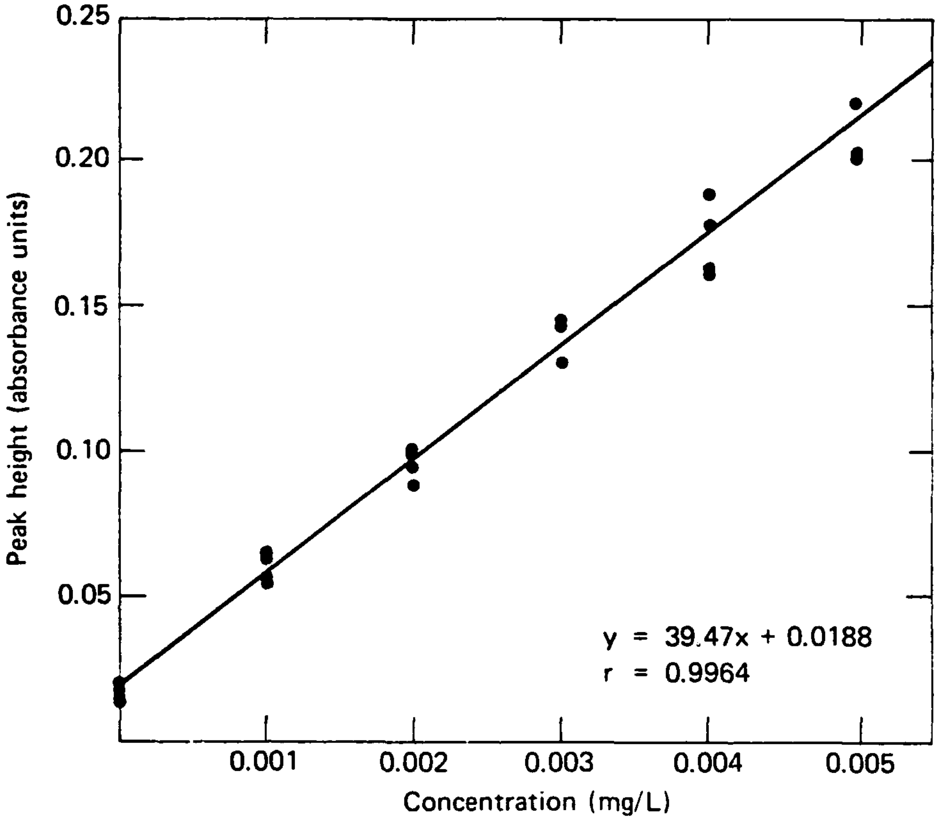

Fig. 12 The linear correlation between peak height (absorbance units) and concentration for arsenic analyzed by the gaseous hydride atomic absorption spectrophotometric method.

TABLE 7. QUANTITIES OF COMPONENTS OF THE SYNTHETIC-HC SMOKE COMBUSTION PRODUCTS MIXTURE ADDED TO 15.5 L DILUENT WATER DURING SOLUBILITY AND STABILITY STUDIES.

Component	Amount Added	Concentration (mg/L)	Percent of Total	
			Nominal	Actual
HCl	28.41 mg	1.83	1.828	1.828*
CCl_4	85.06 mg	5.49	5.515	5.503
C_2Cl_4	223.68 mg	14.43	14.411	14.471
C_2Cl_6	69.57 mg	4.49	4.48	4.501
C_6Cl_6	25.40 mg	1.64	1.634	1.643
Al_2O_3	169.3 mg	5.78 (Al)	10.917	10.953
$ZnCl_2$	946.63 mg	29.3 (Zn)	61.073	61.243
$PbCl_2$	1.38 mg	0.066 (Pb)	0.089	0.089
$CdCl_2$	0.79 mg	0.031 (Cd)	0.051	0.051
$AsCl_3$	0.02 mg	0.0005 (As)	0.001	0.001
Total	1,545.7 mg		99.999	100.283

*Nominal concentration only; HCl was not measured.

In order to make 15.5 L of the final mixture, 15.5 mL of this stock was added. The stock solution was stable at room temperature when stored in a dark bottle for two weeks. Initial measurements gave the following average concentrations (± S.D.): Zn-53.3 g/L (0.2); Pb-87.5 mg/L (0.5); Cd-46.0 mg/L (0.3) and As-2.2 mg/L (0.1) (by the gaseous hydride method). Note that the As concentration was about twice the nominal concentration which indicates a possible contamination problem or an analytical error. At the end of two weeks this same stock contained 58.8 g/L (0.2) Zn, 85.1 mg/L (0.4) Pb, and 45.4 (0.3) Cd, and 2.2 mg/L As (0.1) indicating its stability over time.

The pentane extraction method for the chlorinated organics was very efficient. An initial extraction of one water sample indicated the following concentrations: 2.08 mg/L CCl_4; 2.72 mg/L C_2Cl_4; 1.05 mg/L C_2Cl_6; <0.01 mg/L C_6Cl_6. An immediate 5 mL second extraction of the same sample yielded non-detectable levels (<0.01 mg/L) for all the organics. This indicates an extraction efficiency of approximately 99 percent. Concentrations of compounds in the following Tables and discussions are not corrected for recovery efficiency. A sample chromatogram for the chlorinated organics is shown in Figure 13. Note the good peak separation and shape. This sample represents 2.13 mg/L CCl_4, 3.15 mg/L C_2Cl_4, 0.80 mg/L C_2Cl_6 and 0.08 mg/L C_6Cl_6.

Precision of the chemical methods was good. Four water samples analyzed from the same mixing jar simultaneously gave a maximum standard deviation of 5 percent of the calculated concentration for all four organics (CCL_4, C_2Cl_4, C_2Cl_6, C_6Cl_6). For the metals, the standard deviation was 3.2 percent of the calculated value.

The dissolved concentrations of the components of the synthetic-HC smoke combustion products mixture after 24 h of mixing and 6 h of settling are presented in Table 8 for the three test temperatures. Table 9 indicates the percentages of the added components which were detected after the 24 h mixing and 6 h setting period. The solubility of the components was apparently not affected by temperature, with slightly more of each component dissolved at 12°C than at 22°C. In the closed mixing jar, the vapor pressure in the jar may have more effect on solubility than other factors. Note the high concentration of zinc in comparison to the other metals and organics. Also note that levels of hexachlorobenzene are below our detection limits (0.01 mg/L). There is a major anomaly concerning the solubility of C_2Cl_6 at 17°C. The solubility appears to be significantly less at this temperature than at either 22°C or 12°C. The pH of the diluent water was affected by the addition of the HCL and the metals. Initial pH prior to the addition of the components averaged 7.93 (± 0.07). The pH at the end of the 24 h mixing period averaged 6.89 (± 0.05). A 50% diluent water dilution of the 100% mixture yielded a slightly higher pH of 7.19.

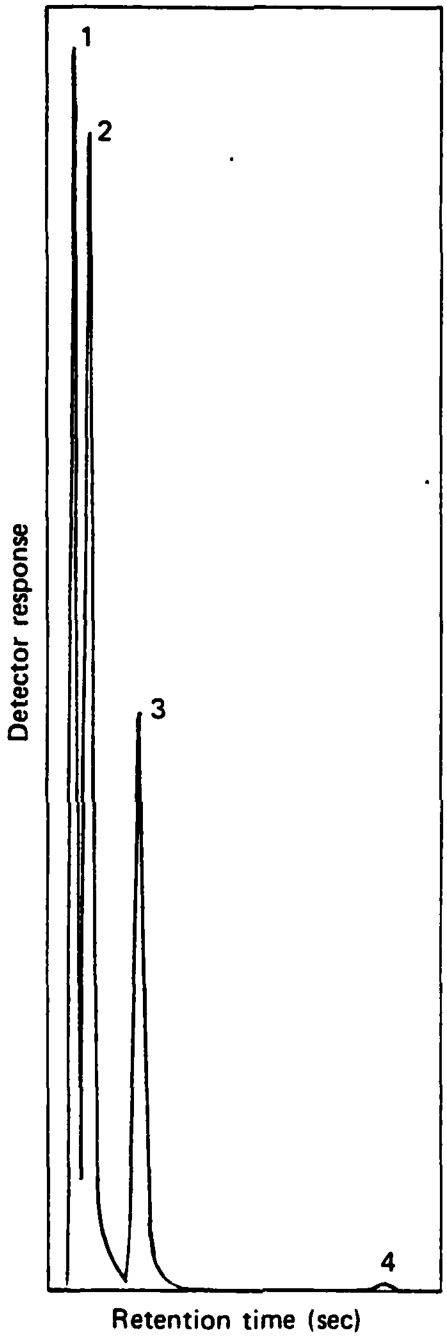

Fig. 13 Sample chromatogram of the four chlorinated organics in the synthetic-HC combustion products mixture following pentane extraction from diluent water (5 mL pentane : 50 mL water). This sample represents quantities of each compound after 24 h of mixing. Attenuation = 200: (1) $CC1_4$; (2) C_2C1_4; (3) C_2C1_6; (4) C_6C1_6 (very close to detection limit).

TABLE 8. SOLUBILITY OF COMPONENTS OF SYNTHETIC-HC SMOKE COMBUSTION PRODUCTS MIXTURE AT 22, 17 AND 12°C. INITIAL QUANTITIES ADDED TO 15.5 L DILUENT WATER ARE FROM TABLE 7.

Component Concentration (mg/L)

Sample Time	22°C								
	CCl_4	C_2Cl_4	C_2Cl_6	C_6Cl_6	Zn	Al	Pb	Cd	As[b]
1 h	0.637	0.804	0.275	ND[a]	22.0	0.019	0.015	0.021	ND
2 h	0.084	1.248	0.564	ND	23.0	0.037	0.018	0.022	ND
4 h	1.377	1.774	0.795	0.074	24.5	0.041	0.020	0.020	ND
6 h	1.653	2.177	0.955	ND	22.6	0.050	0.018	0.021	ND
8 h	1.864	2.310	0.955	ND	20.8	0.061	0.012	0.021	ND
10 h	2.077	2.722	1.050	ND	18.6	0.059	0.011	0.021	ND
12 h	2.162	2.808	1.074	0.092	23.6	0.057	0.015	0.021	ND
24 h	2.131	3.150	0.803	0.076	23.4	0.073	0.016	0.021	ND
24 h + 6 h									
-unfiltered	2.504	3.622	1.028	ND	19.2	0.047	0.003	0.020	ND
-filtered					19.1	0.023	ND	0.020	ND
-50% dilution	1.012	1.927	0.356	ND	8.9	0.025	ND	0.011	ND

	17°C								
	CCl_4	C_2Cl_4	C_2Cl_6	C_6Cl_6	Zn	Al	Pb	Cd	As
24 h + 6 h	2.725	3.326	0.329	ND	16.1	0.067	0.003	0.006	0.0014

	12°C								
	CCl_4	C_2Cl_4	C_2Cl_6	C_6Cl_6	Zn	Al	Pb	Cd	As
24 h + 6 h	2.721	4.237	1.228	ND	21.6	0.058	0.007	0.004	0.0014

[a] ND = non-detectable (<0.01 mg/L-C_6Cl_6; 0.002 mg/L-Pb; 0.0002 mg/L-As).

[b] A new quartz tube allowed for the detection of arsenic at 0.0002 mg/L by the gaseous hydride AA method at 17° and 12°C. This method was not available for analysis at the 22°C test temperature.

TABLE 9. PERCENTAGE OF EACH COMPONENT OF THE SYNTHETIC-HC SMOKE COMBUSTION PRODUCTS MIXTURE WHICH DISSOLVED AFTER 24 H OF MIXING AND 6 H OF SETTLING AT 22, 17 AND 12°C.

Component	Concentration If All Dissolved (mg/L)	Percentage Dissolved		
		$22^{\circ}C$	$17^{\circ}C$	$12^{\circ}C$
CCl_4	5.49	45.6	49.6	49.6
C_2Cl_4	14.43	25.1	23.0	29.4
C_2Cl_6	4.49	22.9	7.3	27.3
C_6Cl_6	1.64	ND[a]	ND	ND
Al	5.78	0.8	1.2	1.0
Zn	29.3	65.5	54.9	73.7
Pb	0.066	4.5	4.5	10.6
Cd	0.031	64.5	19.4	12.9
As	0.0005	ND	280.0[b]	280.0[b]

[a] ND = non-detectable (<0.01 mg/L C_6Cl_6; <0.003 mg/L As).

[b] Probably due to As contamination in manufacture of other metal chlorides. Small amount of As in $ZnCl_2$ could result in large concentration in stock since 61.07 g of $ZnCl_2$ were added to the stock solution. In fact, the stock concentration was determined to be 5.3 mg/L as $AsCl_3$ when only 1.0 mg/L was expected. Arsenic was not detected at 22°C because gaseous hydride method was not used for those samples.

3.2.4 Stability in Diluent Freshwater

The 24-h and 48-h stability of the components of the synthetic-HC smoke combustion products mixtures was determined at 22, 17 and 12°C. Both the 100% mixture and a 50% diluent water dilution of this mixture were tested to determine possible dilution effects on stability. An initial stability experiment indicated that the components were not very stable when tested in an open beaker. This was likely due to the volatility of some of the organics and by precipitation of the metals due to increases in pH of the mixture when the chambers were open to the atmosphere. This precipitation was evident after only a few hours. In order to study this effect, both open and closed containers were used to investigate stability of the mixtures. Immediately following the 24-h solubility experiments, 300 mL of the 100% mixture were siphoned into four beakers, two of which were covered. Four beakers of the 50% dilution mixture were prepared in the same way. These beakers were then placed in an incubator at the appropriate test temperature. Two open beakers and two closed beakers were sampled after 24-h and 48-h. The analytical methods used to determine the organics and metals were the same as used earlier.

The stability of the components of the synthetic-HC smoke combustion products mixture over 24 h and 48 h is presented in Table 10 (actual changes in concentration) and Table 11 (percentage remaining of initial). It is obvious that temperature has a dramatic effect on stability. It is also apparent that there is a greater loss of most of the components from the open beakers than from the covered beakers. The loss of the metal components appears to be pH related. All open beakers had a larger amount of particulates than the closed beakers after both 24 h and 48 h. The average pH of the 100% mixture-closed beakers was 6.97 (± 0.008 S.D.) after 24 hours and 7.04 (±0.07) after 48 h, while in the open beakers the average pH was 7.10 (±0.09) after 24-h and 7.24 (±0.06) after 48-h. The increased pH caused more precipitation of the metals, especially zinc and aluminum. The total amount of zinc and aluminum does not decrease over 48 h, only the concentration of the dissolved metals. For example, acidification of the 100% mixture 48 h sample caused the metals to redissolve. After acidification, the zinc concentration was 17.8 mg/L compared to an initial concentration of 19.2 mg/L, while the aluminum concentration was 0.045 mg/L compared to an initial concentration of 0.047 mg/L. The lack of values for arsenic from the 22°C solubility (Table 8) and stability (Table 10) experiments was due to a change in analytical methods after these experiments were conducted. The 22° samples were analyzed for arsenic by the graphite furnace method which has a higher detection limit (0.003 mg/L) than the gaseous hydride method used for the 17° and 12° samples (0.0002 mg/L). A functional quartz tube for the gaseous hydride method was not available during the earlier study. It is apparent from Table 10 that temperature does not have much effect on arsenic solubility and stability between 12° and 17°C, therefore we feel that the solubility and stability of the arsenic will be similar at 22°C.

TABLE 10. 24 H AND 48 H STABILITY OF COMPONENTS OF SYNTHETIC-HC SMOKE COMBUSTION PRODUCTS MIXTURE AT 22, 17 AND 12°C. INITIAL CONCENTRATIONS ARE THOSE FROM TABLE 8 AFTER 24 H + 6 H SETTLING-UNFILTERED.

Sample	Component Concentration (mg/L)								
	22°C								
	CCl_4	C_2Cl_4	C_2Cl_6	C_6Cl_6	Zn	Al	Pb	Cd	As[c]
Initial-100%	2.504	3.622	1.028	ND[a]	19.2	0.047	0.003	0.020	ND
-50%	1.012	1.927	0.356	ND	8.9	0.025	ND	0.011	ND
24 h - 100%									
closed[b] -	1.561	2.424	0.682	ND	18.0	0.015	ND	0.021	ND
open -	0.589	0.881	0.133	ND	6.9	0.012	ND	0.021	ND
24 h - 50%									
closed -	0.685	1.242	0.213	ND	7.7	0.014	ND	0.012	ND
open -	0.269	0.378	0.047	ND	3.0	0.012	ND	0.012	ND
48 h - 100%									
closed -	0.887	1.874	0.530	ND	7.9	0.015	ND	0.021	ND
open -	0.156	0.226	0.033	ND	6.3	0.016	ND	0.018	ND
48 h - 50%									
closed -	0.334	0.536	0.080	ND	3.9	0.013	ND	0.011	ND
open -	0.024	0.064	0.011	ND	1.7	0.014	ND	0.010	ND
	17°C								
Initial-100%	2.725	3.326	0.329	ND	16.1	0.067	0.003	0.006	0.0014
24 h - 100%									
closed -	2.185	2.708	0.256	ND	14.5	0.029	ND	0.007	0.0011
open -	1.337	1.653	0.077	ND	11.8	0.027	ND	0.007	0.0012
48 h - 100%									
closed -	1.726	2.397	0.176	ND	12.1	0.020	ND	0.006	0.0011
open -	0.503	0.642	0.026	ND	6.9	0.020	ND	0.006	0.0011
	12°C								
Initial-100%	2.721	4.237	1.228	ND	21.6	0.058	0.007	0.004	0.0014
24 h - 100%									
closed -	2.498	3.935	1.245	ND	19.1	0.026	ND	0.005	0.0009
open -	1.550	2.572	0.609	ND	14.8	0.023	ND	0.005	0.0012
48 h - 100%									
closed -	1.992	3.654	1.085	ND	16.8	0.013	ND	0.005	0.0014
open -	0.936	1.988	0.393	ND	13.1	0.014	ND	0.004	0.0011

[a] ND = non-detectable (<0.01 mg/L-C_6Cl_6; 0.002 mg/L-Pb; 0.0002 mg/L-As)

[b] Beaker covered.

[c] A new quartz tube allowed for the detection of arsenic at 0.0002 mg/L by the gaseous hydride AA method at 17° and 12°C. This method was not available for analysis at the 22°C test temperature.

TABLE 11. PERCENTAGE OF EACH COMPONENT OF THE SYNTHETIC-HC SMOKE COMBUSTION PRODUCTS MIXTURE REMAINING AFTER THE 24 H AND 48 H STABILITY STUDIES AT 22, 17 and 12°C.

Component	Temp °C	24 h				48 h			
		100%		50%		100%		50%	
		Closed	Open	Closed	Open	Closed	Open	Closed	Open
CCl_4	22	62.3	23.5	67.7	26.6	35.4	6.2	33.0	2.4
	17	80.2	49.1			63.3	18.5		
	12	91.8	57.0			73.2	34.4		
C_2Cl_4	22	66.9	24.3	64.5	19.6	51.7	6.2	27.8	3.3
	17	81.4	49.7			72.1	19.3		
	12	92.9	60.7			86.2	46.9		
C_2Cl_6	22	66.3	12.9	59.8	13.2	51.6	3.2	22.5	3.1
	17	77.8	23.4			53.5	7.9		
	12	101.4	49.6			88.4	32.0		
Zn	22	93.8	35.9	86.5	33.7	41.1	32.8	43.8	19.1
	17	90.1	73.3			75.2	42.9		
	12	88.4	68.5			77.8	60.6		
Al	22	31.9	25.5	56.0	48.0	31.9	34.0	52.0	56.0
	17	43.3	40.3			29.9	29.9		
	12	44.8	39.7			22.4	24.1		
Cd	22	105.0	105.0	109.1	109.1	105.0	90.0	100.0	90.9
	17	116.7	116.7			100.0	100.0		
	12	125.0	125.0			125.0	100.0		
As	22	b							
	17	78.6	85.7			78.6	78.6		
	12	64.3	85.7			100.0	78.6		
C_6Cl_6	ND[a] at all temperatures and treatments								
Pb	ND at all temperatures								

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[a] ND = non-detectable in samples, therefore no percentage calculated.

[b] No values for 22°C treatment. Gaseous hydride method not functional at the time these samples were analyzed.

3.3 Diethyleneglycol Dinitrate (DEGDN)

3.3.1 Test Materials

The propellant was obtained from the Naval Ordinance Station (NOS) Indian Head, MD. Upon manufacture, the pure DEGDN was dissolved in absolute ethanol for shipment. When dissolved in ethanol the propellant can be shipped as a flammable liquid rather than an explosive. No chemical stabilizers were used in the manufacture of the DEGDN. The DEGDN received was 2.86% by weight in absolute ethanol. The liquid was stored at room temperature, in the dark since DEGDN becomes less stable at lower temperatures (Basom, personal communication, 1985).

3.3.2 Analytical Methods

Initial review of available literature indicated that high performance liquid chromatography techniques were feasible for analysis of DEGDN and related nitrate esters (Juhasz and Doali 1977, Fariwar-Mohseni et al. 1979). The HPLC methods avoid the destruction of nitro compounds resulting from the temperature programming of gas chromatography (Holleman et al. 1983). The final HPLC method developed for our work is a modification of methods developed by Spanggord et al (1985) and Basom (personal comm. 1985) except that methanol is used in the mobile phase rather than acetonitrile. The stock material obtained from NOS contained 28.6 mg/mL DEGDN in absolute ethanol. The absolute ethanol was removed under vacuum and the pure DEGDN was resuspended in either filtered diluent water or ethanol for standard preparation. Both a Varian Techtron Model 635 spectrophotometer (215 and 254 nm) and a Waters Associates HPLC with a variable wavelenth UV detector (215 and 254 nm) were used to analyze for the DEGDN. The final HPLC conditions used for quantification of the DEGDN in water were:

HPLC:	Same as described in Section 3.1.2
Column:	Waters Radial-PAK, μBONDAPAK C_{18}
Standard Solvent:	Diluent freshwater
Mobile Phase:	30% DI H_2O:70% CH_3OH
Method:	Isocratic
Flow Rate:	1 mL/min
Detector:	UV 215 nm
Injection Volume:	100 μL

The HPLC retention time (RT) for the DEGDN in diluent water ranged from 4.738 to 4.781 min (N=12) with a 15% DI H_2O:85% methanol mobile phase. A 30% DI H_2O:70% methanol mobile phase was also tried in an effort to improve separation of the DEGDN peak from the solvent peak. This mobile phase yielded DEGDN retention times ranging from 5.814 to 5.857 min (N=8) and gave better separation from the solvent front (Figure 14). A diluent water blank yielded no peaks between 5.0 and 6.0 minutes with this mobile phase.

The UV detector wavelength of 215 nm was selected over the 254 nm wavelength because of better absorbance (i.e., greater sensitivity) by DEGDN at 215 nm versus 254 nm. At the 254 nm wavelength the DEGDN (28.6 mg/L ethanol) appears only as a shoulder on the ethanol absorbance peak, while at the 215 nm wavelength a distinct peak is evident (Figure 15). Consecutive injections of the same sample at the two wavelengths are shown in Figure 15. A similar type shoulder effect does not occur at the 215 nm wavelength until a 0.286 mg DEGDN/L sample is injected (Table 12).

The lowest concentration of DEGDN detected by the HPLC method using 100 µL injections of 0.45 µ filtered diluent was 0.286 mg/L (Table 13). This is the detection limit for this method without sample concentration or clean-up. There was good linearity between area under the curve (AUC) and the concentration of DEGDN over the range of concentrations tested (Figure 16). If sample concentration is found to be necessary once acute toxicity tests are initiated, a Waters C-18 Sep-Pak will be used.

Filtration of samples through a Waters Associates Sample Clarification Kit containing an HA 0.45 µ filter resulted in a 1% loss of DEGDN as determined by HPLC. Therefore, samples can be filtered immediately before injection into the HPLC without loss of the compound.

Because of the interference from other compounds present in the diluent water at 215 nm, the Varian Techtron Model 635 spectrophotometer could not be used for analysis of DEGDN with sufficient accuracy. The HPLC method allowed for separation of these contaminants by retention time.

The accuracy and precision of the HPLC method proved very good. A 28.6 mg/L standard in diluent water injected 9 separate times over a 1-week period yielded a measured concentration of 28.6 mg/L (± 0.2 S.D.). Initial range-finding toxicity tests indicated LC50 values for DEGDN ranging from 80 mg/L to 200 mg/L for invertebrates and fish. Thus, a 28.6 mg/L sample would represent the low end of the concentrations to be tested. At higher concentrations, the accuracy and precision were also good. A 180 mg DEGDN/L sample in diluent water was found to contain 183.3 mg/L (± 1.1 S.D., n=4).

3.3.3 Solubility in Diluent Freshwater

The solubility of DEGDN in water at 20° has been shown to be a 0.4 g/100 g (Lindner 1980). This solubility value was not verified in the present study.

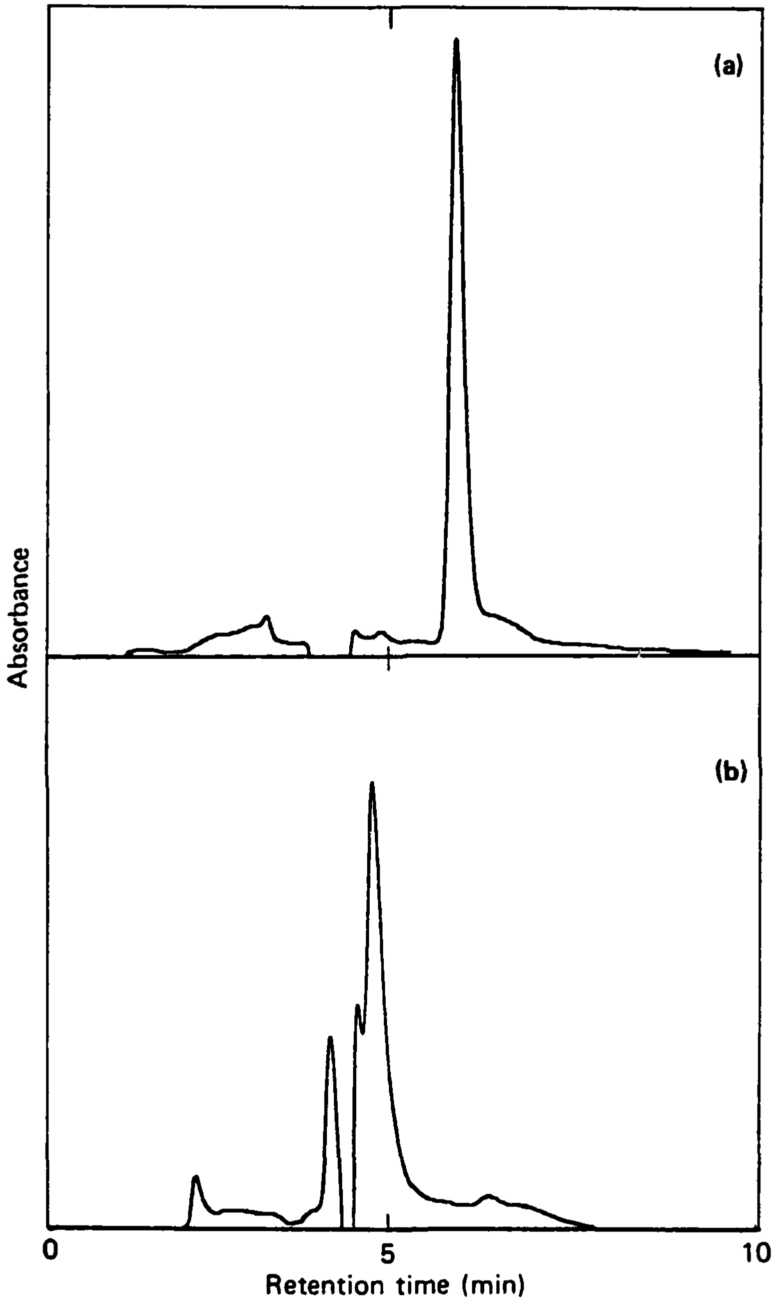

Fig. 14 Difference in retention time for DEGDN when using a 30% DI H_2O: 70% MEOH mobile phase (a) (RT = 5.855 min) versus a 15% DI H_2O: 85% MEOH mobile phase (b) (RT = 4.742 min). DEGDN concentration was 286 mg/L diluent water. Solvent front occurs from 4.123 - 4.502 min.

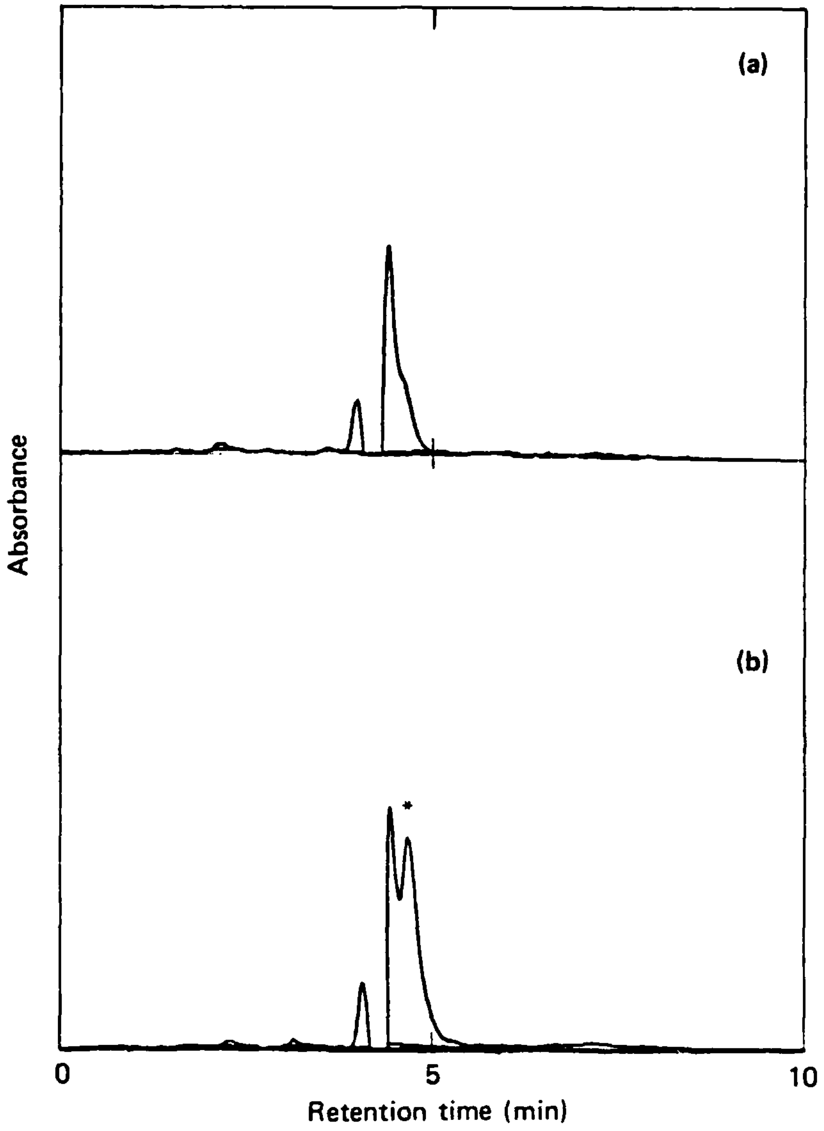

Fig. 15 Difference in absorbance of DEGDN at 254 nm (a) and 215 nm (b) for the same sample (28.6 mg DEGDN/L ethanol). The mobile phase was 15% DI H_2O : 85% MEOH. Note: The retention time is 4.772 min and the DEGDN appears as a shoulder peak at 254 nm while a distinct peak (*) is visible at 215 nm. The injection volume was 5 μL.

TABLE 12. AREA UNDER THE CURVE (AUC) FOR ABSORBANCE AT 215 NM AND 254 NM FOR DIETHYLENEGLYCOL DINITRATE (DEGDN) IN ETHANOL AT VARIOUS CONCENTRATIONS[a]

Chemical	Concentration (mg/L)	AUC 215 nm	AUC 254 nm
DEGDN	28,600	34,382,364	1,866,158
	2,860	10,165,695	245,934
	286	1,516,221	50,694
	28.6	545,143[b]	c
	2.86	189,844	ND[d]
	0.286	c	ND
	0.028	ND	ND

[a] The HPLC conditions were as follows: 5 µL injected, 15% DI H_2O: 85% MEOH, DEGDN dissolved in ethanol, 1 mL/min flow rate, and C_{18} column.

[b] The DEGDN appears as a double peak at the solvent front; see Figure 2B.

[c] AUC not calculated by integrator because only a shoulder is observed at the solvent front.

[d] ND = non-detectable.

TABLE 13. AREA UNDER THE CURVE (AUC) FOR ABSORBANCE (215 nm) FOR DIETHYLENEGLYCOL DINITRATE (DEGDN) IN DILUENT WATER AT VARIOUS CONCENTRATIONS[a]

Chemical	Concentration[b] (mg/L)	Retention Time (min)	AUC
DEGDN	286	5.870	17,881,762
	143	5.849	10,819,196
	71.5	5.847	6,174,506
	28.6	5.822	2,097,518
	2.86	5.827	271,103
	0.715	5.856	171,430
	0.286	5.830	72,093
	0.028	-	ND

[a] The following is the linear equation and correlation coefficient for all concentrations:

$y = 63{,}745x + 502{,}025$; $r = 0.993$

for concentrations at or below 143 mg/L:

$y = 76{,}591x + 115{,}112$; $r = 0.998$

[b] DEGDN was dissolved in diluent water and 100 μL samples were injected into the HPLC with a 30% DI H_2O:70% MEOH.

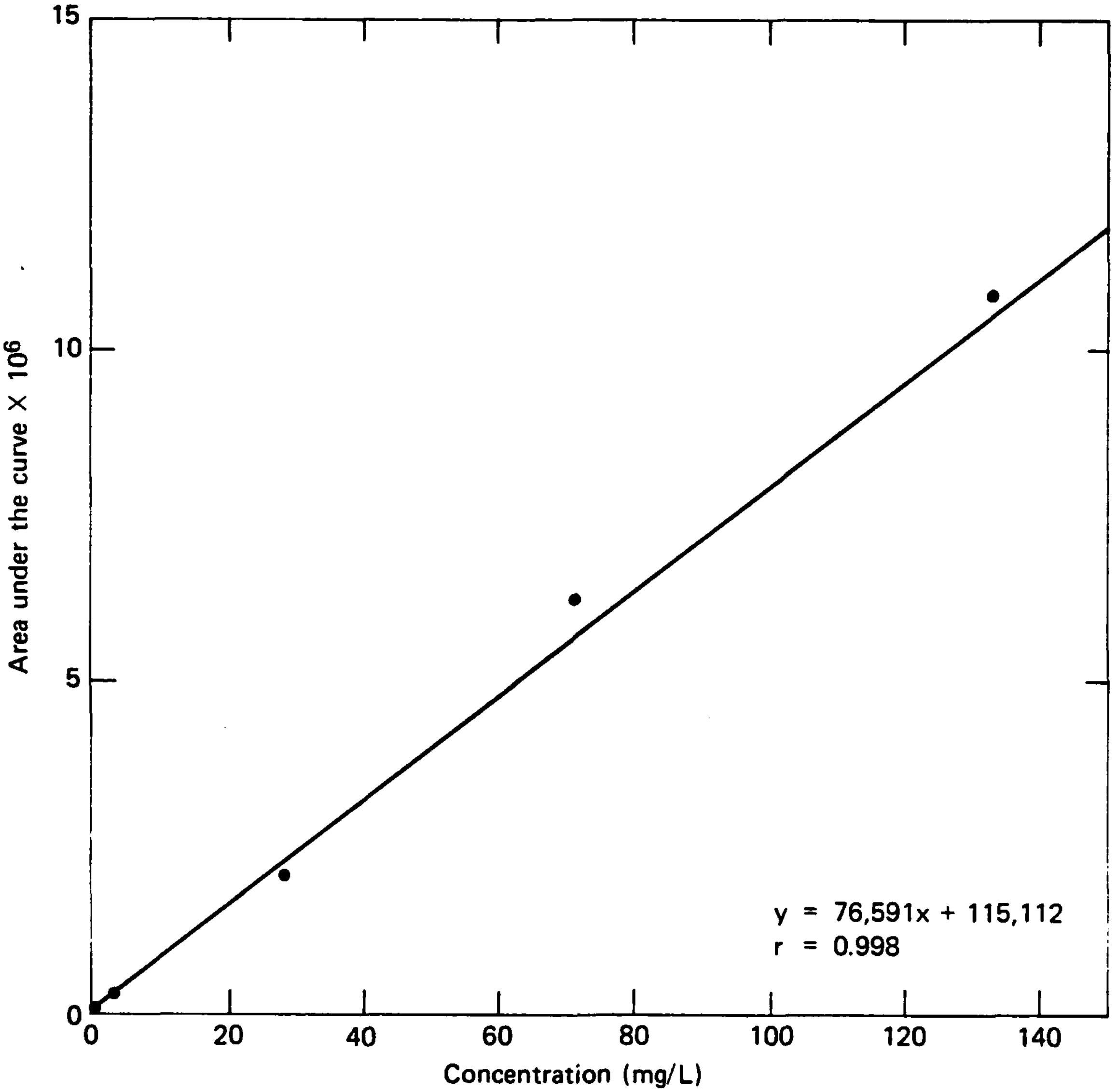

Fig. 16 The linear correlation between absorbance (215 nm) and concentration of DEGDN dissolved in diluent water using the HPLC. Injection volume was 100 μL.

3.3.4 Stability in Diluent Freshwater

Stability of the DEGDN in diluent water at 22°C was examined using the HPLC method. DEGDN at 28.6 mg/L was stable in diluent water for 48 h (Table 14 and Figure 17). Because of a material present in the diluent water which elutes just after DEGDN, peak height rather than AUC gives a better estimation for concentration in samples when the contaminant shoulder peak is not picked up as a peak by the integrator. For example, in the chromatogram presented in Figure 17(a), the integrator did not pick up the shoulder peak (peak 2) and therefore integrated a greater area under the curve than in chromatograms (b) and (c). If one examines only peak height (PH) in Table 14, it can be shown that the greatest change in concentration over time was only 6%. Since DEGDN appeared stable over 48 h in diluent water at 22°C, stability was not determined at the lower test temperatures (12° and 17°C).

Since DEGDN appears stable in diluent water, we believe DEGDN standards can be prepared in capped GC vials for use in toxicity tests. This decision was made for three reasons: 1) initial work with standards in ethanol indicated a rapid vaporization of ethanol and a subsequent change in DEGDN standard concentration once the teflon septum on the standard vial was pierced; 2) would allow for exact matching of retention times on chromatograms (i.e. both standards and test samples injected in same solvent-diluent water and 3) no ethanol absorbance peak interference with DEGDN (Figure 14A versus 15B).

TABLE 14. STABILITY OF DIETHYLENEGLYCOL DINITRATE (DEGDN) DISSOLVED IN DILUENT WATER FOR 6, 24 and 48 HOURS AS AREA UNDER THE CURVE (AUC) AND PEAK HEIGHT (PH)[a]

Treatment		Time (h) 6	24	48
10 mL Capped Vial	AUC	2,267,287	2,380,777	3,955,380[b,c]
	PH[d]	6.20	6.28	6.49
300 mL Covered Beaker				
Replicate				
A	AUC	2,277,828	2,407,911	2,640,846
	PH	6.15	6.38	6.38
B	AUC	2,234,860	2,677,596	2,886,446
	PH	6.10	6.32	6.49
C	AUC	2,283,810	2,353,639	2,877,956
	PH	6.15	6.29	6.45

[a] The HPLC conditions were as follows: 100 µL injected, wavelength 215 nm, mobile phase 30% DI H_2O:70% methanol, 1 mL/min flow rate and C_{18} column.

[b] The relative increase in AUC is due to the incorporation of a diluent water contamination peak by the integrator.

[c] The concentration of DEGDN was 28.6 mg/L.

[d] Peak height (PH) was measured in centimeters and the largest delta change was a 6% increase.

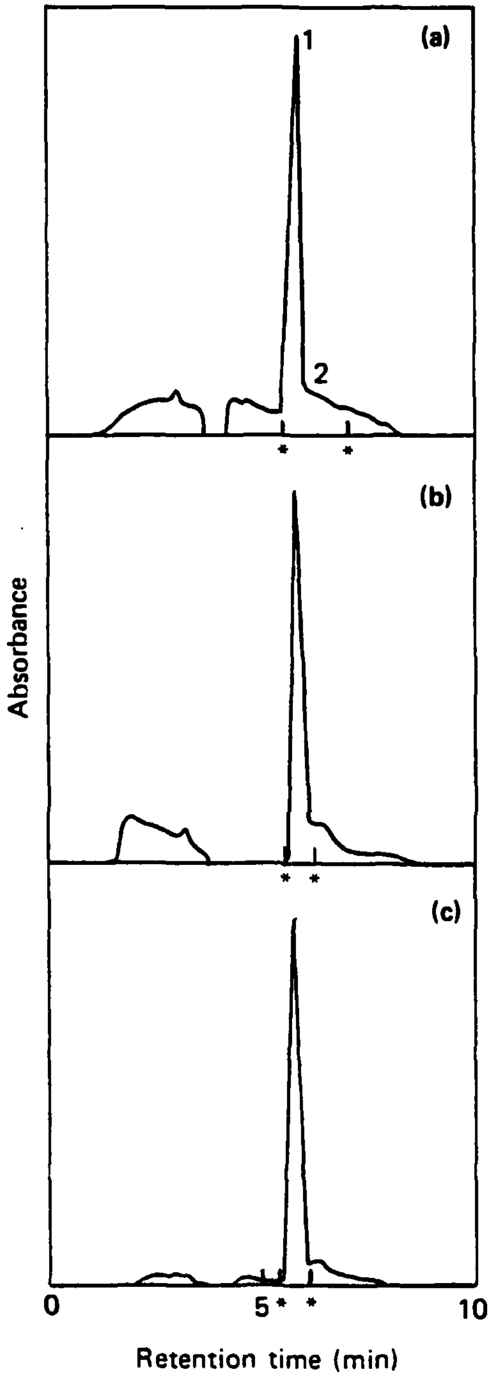

Fig. 17 HPLC chromatograms of DEGDN (28.6 mg/L) after 6(a), 24(b), and 48h (c) showing the stability of this compound in diluent water over time at 22°C. Note: The shoulder (peak 2) is due to a compound in the diluent water which absorbs at 215 nm. ** show where integrator began and ended integration of DEGDN peak.

SECTION 4

CONCLUSIONS

The methods developed in this project give accurate and precise measures of solvent yellow 33, solvent green 3, synthetic-HC smoke combustion products and DEGDN concentrations in freshwater. Detection limits range from 0.08 mg/L for the dyes to 0.286 mg/L for DEGDN by HPLC without sample concentration. Detection limits for the heavy metals (atomic absorption spectrophotometry) in synthetic-HC smoke combustion products mixture are 0.002 mg/L for aluminum, 0.0002 mg/L for cadmium, 0.002 mg/L for lead and 0.0002 mg/L for arsenic. The chlorinated organics in the synthetic-HC smoke combustion products mixture are extracted with pentane prior to injection into a gas chromatograph. This results in a 10-fold concentration and a detection limit of 0.01 mg/L using an electron capture detector. If lower detection limits for these compounds are necessary, concentration by Waters C-18 Sep-PAKS can be utilized to improve detection sensitivity. Also, the use of capillary GC would allow for greater sensitivity. The two dyes (solvent yellow 33 and solvent green 3) and DEGDN are very stable over a 48-h period at 22°C, both as standard preparations and when dissolved in water. The synthetic-HC smoke combustion products mixture is not stable over time.

SECTION 5

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