RESEARCH TRIANGLE INSTITUTE

PRELIMINARY STUDY ON TOXIC CHEMICALS IN ENVIRONMENTAL AND HUMAN SAMPLES

PART II: PROTOCOLS FOR ENVIRONMENTAL AND HUMAN SAMPLING AND ANALYSIS

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WORK PLAN (PHASE I)

by

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PREFACE

This volume of the work plan for the Total Exposure and Assessment Methodology (TEAM) Study contains analytical protocols that will be examined in the twelve-person Phase I Pilot Study. At the time of Volume II's draft preparation a few protocols (PNAs in particulate, metals in particulate, PCBs/Pesticides in air) had not been received from outside laboratories which had been tapped for information or participation. Upon receiving these protocols they were submitted to the TEAM Committee members for review and comment and are incorporated here.

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SECTION 1

INTRODUCTION

GENERAL PROGRAM CONCEPT

The goal of this program is to establish an ongoing human exposure monitoring capability within EPA. The ultimate output will be a field-tested methodology for measuring human exposure to toxic substances in a defined geographical area.

The basic approach is to measure individual exposures through the main exposure pathways of air, drinking water, and food. Instrumentation for individual measurements (personnel monitors) will either be evaluated in the field or, where necessary, development programs will be initiated. These monitors will be used to provide direct measurements of exposure throughout an individual's 24-hour daily routine. Monitoring in industrial and residential neighborhoods and in individual households to determine potential exposures will be carried out. To determine long-term cumulative or equilibrium dosages, body burden (concentrations in body fluids, excreta, etc.) will be determined for a range of individuals. Using individual activity pattern studies and probability sampling methods, a frequency distribution of exposures will be studied. This will permit examination of the relevence of extending these results to the entire population of the study area.

PROGRAM DESIGN

The program is divided into two phases. Phase I (the subject of this work plan) is to test methodology (sampling, survey, monitoring, chemical analysis, and statistical analysis) prior to Phase II, which involves a large number of samples. Detailed discussions of the program are in Part I of this work plan.

METHODOLOGY EVALUATION

A major objective of this program is to provide a methodology for obtaining valid estimates of multimedia exposures of individuals to any pollutant

of concern which has potential for transmission via the food, vapor, inhalable particulate, or liquid routes. Therefore, methods for collection and analysis of the pollutants must be available. To obtain valid estimates of exposure, samples must be collected using personnel monitors. This means that the most advanced techniques in this area must be tested. Furthermore, the small sample volumes collected (as opposed to a "HiVol", for example) will require the most sensitive chemical analytical methods.

Phase I will select the most likely candidate methods and test these methods. Based upon these results, the best monitoring protocols will be selected and validated for use in Phase II.

This volume describes the protocols to be investigated and the criteria for evaluation in Phase I.

SECTION 2

SUMMARY

This second volume of the work plan presents the chemical analytical protocols to be utilized in Phase I of the Total Exposure and Assessment Methodology Monitoring program. Protocols are proposed for the analysis of air (volatiles, pesticides, PCBs, PNAs, and Metals), breath (volatiles), water (volatiles, pesticides, PCBs, PNAs, and metals), blood (volatiles, pesticides, PCBs, PNAs, and metals), urine (pesticides, PCBs, PNAs, and metals), and food (volatiles, pesticides, PCBs, PNAs, and metals). In some cases, two or more protocols will be evaluated during Phase I. Based on this evaluation, the "best" protocol will be selected for use in Phase II. Selection critieria are discussed.

It is anticipated that some of the compound classes for some or all media will not provide sufficient data to warrent inclusion in Phase II. These analyses will be deleted from Phase II efforts.

This volume also discusses the status of efforts to set up a Quality Assurance Program for this study.

SECTION 3

EVALUATION OF PROTOCOLS

INTRODUCTION

As discussed below and in the Appendices, several protocols are available for a given analysis. Where possible a preferred protocol has been selected and will be used in Phase I. Presuming no major problems are encountered and the analysis is required, the protocol will be used in Phase II. In many cases, none of the available protocols is clearly superior. In this case, two or more protocols have been selected for comparative evaluation in Phase I. If one protocol proves superior in laboratory validation, it will be used for the field samples. If not, all candidate protocols will be field-tested (possibly on only part of the samples) and compared. Based on the side-by-side performance, a protocol will be selected for use in Phase II.

For some analyses, no validated protocol was found. In these cases a procedure is proposed which will be validated in the laboratory prior to field testing.

SELECTION OF CANDIDATE PROTOCOLS

Tables 3-1 and 3-2 list the toxic and hazardous chemicals which have been selected for study in Phase I of this program. Section 6 of Part I provides the rationale leading to their selection. The analytical protocols which provide for their collection and analysis in the various matrices are discussed here.

Halogenated Hydrocarbons and Benzene in Air

Criteria--

The methods must be amenable to personnel monitoring. This restricts collection devices to either active personnel monitors (pumps with a sorbent cartridge or filter) and passive monitors (permeation dosimeter). Many present serious detection limit problems due to their design for work place

Table 3-1. TOXIC CHEMICALS SELECTED FOR MONITORING IN ENVIRONMENTAL MEDIA (PHASE I)

Air					
Vapors	Particulate	Drinking water	Beverages	Food (tent.)	Household dust
Benzene	Arsenic	Benzene	Chloroform	Arsenic	Arsenic
Chloroform	Cadmium	Chloroform	1,2-Dichloroethane	Cadmium	Cadmium
1,2-Dichloroethane	Lead	1,2-Dichloroethane	1,1,1-Trichloroethane	Lead	Lead
1,1,1-Trichloroethane	----------	1,1,2-Trichloroethane	1,1,2-Trichloroethane	----------	----------
Carbon tetrachloride	Benzo(a)pyrene	Carbon tetrachloride	Vinylidene chloride	α-BHC	Benzo(a)pyrene
Vinylidene chloride	Pyrene	Vinylidene chloride	Trichloroethylene	Lindane	Fluoranthene
Trichloroethylene	Chrysene	Trichloroethylene	Tetrachloroethylene	Heptachlor	Benzo(k)fluoranthene
Tetrachloroethylene	Benzo(a)anthrene	Tetrachloroethylene	Bromodichloromethane	Heptachlor epoxide	Pyrene
Bromodichloromethane	Fluoranthene	Bromodichloromethane	Chlorobenzene	Chlordane	Chrysene
Chlorobenzene	Benzo(k)fluoranthene	Chlorobenzene	Vinyl chloride	t-Nonanchlor	Benzo(a)anthrene
1,1,2-Trichloroethane		Vinyl chloride		Oxychlordane	----------
----------		1,1,1-Trichloroethane		HCB	α-BHC
Vinyl chloride		----------		DDT/DDD/DDE	Lindane
----------		Arsenic		PCBs	Heptachlor
α-BHC		Cadmium			Chlordane
Lindane		Lead			HCB
Heptachlor		----------			DDT/DDD/DDE
Chlordane		Benzo(a)pyrene			PCBs
HCB		Fluoranthene			
DDT/DDD/DDE		Benzo(k)fluoranthene			
PCBs		Fluoranthene			
		Benzo(k)fluoranthene			
		Pyrene			
		Chrysene			
		Benzo(a)anthrene			

		α-BHC			
		Lindane			
		Heptachlor			
		Heptachlor epoxide			
		Chlordane			
		t-Nonachlor			
		Oxychlordane			
		HCB			
		DDT/DDD/DDE			
		PCBs			

Table 3-2. TOXIC CHEMICALS SELECTED FOR MEASUREMENT IN HUMAN BODY FLUIDS AND TISSUES (PHASE I)

Breath	Blood	Urine	Mother's Milk (tent.)	Hair
Benzene	Benzene	Arsenic	Benzene	Arsenic
Chloroform	--------	Cadmium	Chloroform	Cadmium
1,2-Dichloroethane	Chloroform	Lead	1,2-Dichloroethane	Lead
1,1,1-Trichloroethane	1,2-Dichloroethane	--------	1,1,1-Trichloroethane	-------
1,1,2-Trichloroethane	1,1,1-Trichloroethane	β-BHC	1,1,2-Trichloroethane	PCBs
Carbon tetrachloride	1,1,2-Trichloroethane	t-Nonachlor	Carbon tetrachloride	
Vinylidene chloride	Carbon tetrachloride	Oxychlordane	Vinylidene chloride	
Trichloroethylene	Vinylidene chloride	Heptachlor epoxide	Trichloroethylene	
Tetrachloroethylene	Trichloroethylene	PCBs	Tetrachloroethylene	
Bromodichloromethane	Tetrachloroethylene	--------	Bromodichloromethane	
Chlorobenzene	Bromodichloromethane	Benzo(a)pyrene	Chlorobenzene	
	Chlorobenzene	Fluoranthene	--------	
	--------	Benzo(k)fluoranthene	Arsenic	
	Arsenic	Pyrene	Lead	
	Cadmium	Chrysene	Cadmium	
	Lead	Benzo(a)anthrene	--------	
	--------		β-BHC	
	β-BHC		Lindane	
	Lindane		Heptachlor epoxide	
	Heptachlor expoxide		t-Nonachlor	
	t-Nonachlor		Oxychlordane	
	Oxychlordane		HCB	
	HCB		DDT/DDD/DDE	
	DDT/DDD/DDE		PCBs	
	PCBs			

	Benzo(a)pyrene			
	Fluoranthene			
	Benzo(k)fluoranthene			
	Pyrene			
	Chysene			
	Benzo(a)anthrene			

monitoring where levels are much higher than would be encountered during ambient monitoring.

Candidate Methods--

The method developed by RTI is the only known candidate method for general volatiles. A passive dosimiter for vinyl chloride appears to be sufficiently sensitive for this application and will be tested.

Pesticides and PCBs in Air

Criteria--

As with the halogenated hydrocarbons and benzene, collection must be by a personnel monitor and analysis must be both sensitive and selective.

Candidate Methods--

No validated protocols are currently available. Two candidate methods are being considered:

(1) Pesticides and PCBs are being collected by EPA/HERL-RTP using personnel monitoring pumps with a polyurethane foam (PUF) collection device. The samples can then be solvent-desorbed and analyzed by GC/ECD (personal communication, R. G. Lewis, EPA, February 1980). The method has had only limited field testing, but appears sufficiently promising to warrant inclusion as a protocol for use in this program.

(2) As an adaptation of the method for volatiles in air (Tenax) used by RTI, pesticides could be collected on a filter ahead of the Tenax cartridge and analyzed by thermal desorption/GC. This method has never been investigated, but has one distinct advantage since with thermal desorption, the whole sample is analyzed, increasing the sensitivity over solvent desorption by a factor of 100-1000.

Protocols to be Evaluated--

Both methods discussed above will be investigated further during Phase I

PNAs in Air

No validated personnel sampling methods for PNAs in air are available. Three methods will be assessed in this study:

(1) A NIOSH HPLC/Fluorescence method,

(2) Thermal Desorption/GC/MS of the filters from the volatile personnel samplers, and

(3) Analysis of the polyurethane foam extracts collected for Pesticides and PCBs by GC.

Two restrictions hamper this study:

(a) Any PNA-specific sampling would add another monitoring device to the test subject; and

(b) The concentrations of the PNAs in air are so low that sampling rates far in excess of those provided by personnel pumps would be required to obtain sufficient sample. Therefore, the analytical technique must compensate for this reduction (~thousand fold) in sample size.

Due to the lack of data on any of these techniques, all three will be tested. The first step will involve the determination of the ambient PNA levels which would be observable. If these levels are higher than generally reported ambient levels, the Project Officer will be consulted.

The methods which provide adequate sensitivity will be evaluated for selectivity, ease of use, and parity with the QA laboratory.

Metals in Air

The standard protocols for metals in air involve high volume air samplers generally weighing tens or hundreds of kg, clearly unsuitable to personnel monitoring. Three methods were considered for this program.

(1) Atomic Absorption Spectrometry (AA) of glass fiber filters from personnel samples, (RTI-in-house methodology)

(2) X-ray Fluorescence Spectrometry (XRF) of Teflon filters from personnel samples.

(3) Anodic Stripping Voltametry (SAV) of filters from personnel samples.

XRF was selected because the technique is very sensitive (50 ng/m^3 should be observable); it is non-destructive, so the filters may be retrieved for Pesticide, PCB, and/or PNA analysis; and automated analytical instrumentation available in Dr. Robert Stevens' laboratories (ERL-RTP) can perform the analyses efficiently.

Halogenated Hydrocarbons and Benzene in Water

This area has been well-researched and a large number of methods have been used in recent years. Several methods were considered:

(1) EPA/EMSL-CI (Bellar and Lichtenberg, Purge and Trap)

(2) EPA/HERL-CI (Closed Loop Stripping)

(3) RTI-Halogenated Hydrocarbon Five City Study (3-1) (Purge and Trap)

(4) RTI-Master Analytical Scheme

Evaluation Criteria--

The first method above is an "on-line" method, whereas the others generate an extract or sorbent cartridge which is stored for later analysis. This is advantageous in that samples may be extracted independent of instrumental availability.

A major option which must be considered is the GC detection system. The Bellar and Lichtenberg technique may be performed using either GC/Selective detector (HECD and PID are currently favored) or MS. The other three utilize MS. There are two distinct advantages to MS: greater compound identification confidence and archival of data (magnetic tape) for subsequent re-examination for non-target compounds. On the other hand, non-mass spectrometric detection is less expensive, since the data interpretation time is greatly reduced.

The techniques will be evaluated for reproducibility, sensitivity, selectivity, purge efficiency and parity with the QA laboratory.

Selection of Methods for Evaluation--

All methods, except the "RTI-Halogenated Hydrocarbon Five City Study" method (number 3), will be evaluated. Experiments at RTI (under the Master Analytical Scheme contract) have shown that the RTI-MAS method is superior to the other RTI method. This method compensates for the poorer sensitivity of MS by using a larger volume (200 mL) of water and increases purge efficiency through addition of sodium sulfate to the water sample to give a high ionic strength.

The Bellar and Lichtenberg technique remains the "standard for comparison" due to its wide utilization in the U.S. Its major drawback is the use of packed columns. Another disadvantage for this study is the need for PID or FID detection for benzene in addition to the HECD for the halogenated compounds

The major advantages of the closed loop stripping are the large sample concentration factor (4000 mL to .01 mL) and the use of glass capillary GC.

Pesticides and PCBs in Water

The candidate pesticides have been determined in water using a variety of methods which involve solvent extraction and GC analysis (usually GC/ECD). The principle option is in GC detector: ECD, HECD, positive ion mass spectrometry and negative ion chemical ionization mass spectrometry being the most sensitive and selective.

Evaluation Criteria--

Extraction efficiency and levels of interferences are the major evaluation criteria for the extraction/cleanup aspects of the protocols. The analytical evaluations will encompass selection of GC column and potential alternatives to ECD. This latter option will be pursued only if ECD proves inconclusive due to high background or ambiquous peak identifications.

Candidate Methods--

Two validated EPA methods have been considered:

(1) The EMSL-CI (Method 608) method, and

(2) A U. of Miami (Dr. Pfaffenberger) method.

The major difference is in choice of extraction solvent. Both methods utilize ECD as the GC detection system.

Protocols to be Evaluated--

Both the methods will be evaluated. Depending upon complexity of samples and sensitivity required, HECD, PIMS, and NICIMS may be substituted for ECD if distinct advantages are offered.

PNAs in Water

It is hoped that the same extracts used for analysis of Pesticides and PCBs in water may be analyzed for PNAs. This will require validation. Three analytical procedures were considered for evaluation:

(1) glass capillary GC/MS

(2) glass capillary GC/FID, and

(3) HPLC/Fluorescence (Method 610, EMSL-CI)

The HPLC/fluorescence method will be used in Phase I since it is already developed and can be used without a lengthy development/validation effort.

Metals in Water

Metals in water will be analyzed by graphite furnace Atomic Absorption spectrometry. This technique is routinely performed at RTI and no known alternatives present distinct advantages.

Halogenated Hydrocarbons and Benzene in Blood

Two methods have been considered:

(1) The RTI-Halogenated Hydrocarbon Five City study method and

(2) The U. of Miami method.

The former entails a headspace purge of the blood onto a Tenax cartridge for later thermal desorption/GC/MS analysis. The latter is a modification of the Bellar and Lichtenberg method for water. The foaming of the blood is controlled by a chemical antifoam. The detector usually employed is HECD. Neither method has been validated by interlaboratory testing.

Both methods will be evaluated in this study. Criteria for selection of a final protocol include sensitivity, selectivity, efficiency, and parity with QA laboratory.

Benzene in Blood

As discussed in Part I of this work plan, benzene levels in blood have been shown to be generally near the limit of detection for available analytical methodology. The protocol developed by RTI is very sensitive and has been previously validated, so it will be used to analyze for benzene in blood.

Pesticides and PCBs in Blood

Two methods are under consideration:

(1) The HERL-RTP (Thompson) method, and

(2) An RTI modification which substitutes glass capillary GC/MS analysis for GC/ECD.

The complexity of the samples (and therefore the need for MS selectivity), target compound concentration (and therefore need for ECD sensitivity) and the need for maintaining parity with the QA laboratory will determine which method is preferable.

PNAs in Blood

No standard procedures have been obtained for consideration. It is hoped that the sample extracts for Pesticides and PCB determination may be

analyzed for PNAs. This will require validation. Three analytical procedures were considered for evaluation:

(1) glass capillary GC/MS

(2) glass capillary GC/FID, and

(3) HPLC/Fluorescence

Metals in Blood

Blood will be analyzed for metals using graphite furnace Atomic Absorption spectrometry. This technique is routinely performed at RTI and no known alternatives present distinct advantages.

Halogenated Hydrocarbons and Benzene in Urine

Two methods have been considered:

(1) The RTI-Halogenated Hydrocarbon Five City Study Method, and

(2) The U. of Miami Method.

These methods are analogous to those for blood and evaluation criteria will be the same. Both methods will be evaluated.

Pesticides and PCBs in Urine

Two methods are under consideration:

(1) The HERL-RTP (Thompson) method, and

(2) An RTI modification which substitutes glass capillary GC/MS for the GC/ECD analysis.

As with blood, sample complexity, target compound concentration, and the need for maintaining parity with the QA laboratory will determine which method is preferable.

PNAs in Urine

As with blood, no standard procedures have been obtained for consideration. It is hoped that the sample extracts for Pesticide and PCB determination may be analyzed for PNAs. This will require validation. These analytical procedures were considered for evaluation:

(1) glass capillary GC/MS

(2) glass capillary GC/FID, and

(3) HPLC/Fluorescence

Metals in Urine

Metals will be analyzed by graphite furnace AA. No known methods are available which are more effective.

References

3-1 Pellizzari, E. D., M. D. Erickson and R. A. Zweidinger, "Analytical Protocols for Making a Preliminary Assessment of Halogenated Organic Compounds in Mass and Environmental Media", EPA-560/13-79-010, September 1979.

SECTION 4

QUALITY ASSURANCE

Quality assurance (QA) has been recognized as an integral part of this research project since its inception. The Quality Assurance Program consists of quality control (blanks and spiked controls to determine artifacts, losses, etc.), internal quality assurance (procedure monitoring), and external quality assurance (duplicate samples analyzed by an independent laboratory to compare results). The details of the QA program for each analysis are in each protocol (see Appendices).

Tables 4-1 and 4-2 list the number of samples and sample types to be obtained in Phase I of this study.

The general objectives of the external QA program are:

(1) The QA lab will analyze about 10% of the samples (duplicate vials, cartridges, filters, etc., where possible).

(2) Encoded (blind) blanks and spiked controls will be included with the field samples.

(3) Both qualitative and quantitative results will be compared between the principle and QA lab.

(4) The results of the QA will be reported along with the primary results. Any discrepancies will be investigated and remedied prior to Phase II.

Commitments from laboratories to perform external QA are being obtained. The current status of this effort is listed in Table 4-3.

Table 4-1. NUMBER OF SAMPLES AND SAMPLE TYPES PLANNED FOR ENVIRONMENTAL MEDIA IN PHASE I (PER TRIP)

Matrix	Chemical group	No. of field samples	No. of duplicate samples	No. of QC samples	No. of QA samples	No. of broad spectrum samples	Total samples
Air-Volatiles	Vinyl chloride	81[a]	5[a]	9[a]	9[b]	8[a],1[c]	104
	Benzene + halocarbons	81[a]	5[a]	9[a]	9[c]	-	113
	Pesticides	9[a]	1[a]	1[a]	1[d]	-	12
	PCBs	9[a]	1[a]	1[a]	1[d]	-	12
Air-Aerosols	Metals	9[e]	1[e]	1[e]	1[f]	-	12
	PNAs	9[a]	1[a]	1[a]	1[g]	-	12
Water	Benzene + halocarbons	81[a]	8[a]	9[a]	9[j]	8[a],8[j]	123
	Metals	81[a]	8[a]	9[a]	9[h]	-	107
	PNAs	9[a]	1[a]	1[a]	1[h]	-	12
Beverages & Foodstuffs	halocarbons	27[i]	3[i]	-	-	-	30
Dust	Metals	9[a]	1[a]	-	-	9	19
	PNAs	9[a]	1[a]	1[a]	-	-	11
	Pesticides	9[a]	1[a]	1[a]	1[d]	-	12
	PCBs	9[a]	1[a]	1[a]	1[d]	-	12
Food	Metals	9[i]	-	-	-	-	9
	Pesticides	9[i]	-	-	-	-	9
	PCBs	9[i]	-	-	-	-	9
						Total	618

[a] RTI.

[b] REAL.

[c] EMSL/RTP.

[d] HERL/RTP.

[e] FSU.

[f] UC/Davis.

[g] NISOH.

[h] EMSL/CI.

[i] FDA.

[j] HERL/CI.

Table 4-2. NUMBER OF ANALYSES PLANNED FOR BIOLOGICAL FLUIDS AND TISSUE IN PHASE I (PER TRIP)

Matrix	Chemical group	No. of field samples	No. of duplicate samples	No. of QC samples	No. of QA samples	No. of broad spectrum samples	Total samples
Breath	Benzene + Volatile Halogenated Compounds	21[a]	21[a]	8[a]	3[b]	2[a,c]	59
Blood	Benzene	18[a]	2[a]	8[a]	2[d]	-	30
	Volatile Halogenated Compounds	18[d]	2[d]	8[d]	2[a]	1[a]	31
	Pesticides	10[a]	2[c]	8[a]	2[e]	-	30
	PCBs	18[a]	2[a]	8[a]	2[e]	-	30
	PNAs	6[a,f]	-	8[a]	2	-	16
	Elements	18[a]	2[a]	8[a]	2[g]	-	30
Urine	Pesticides	18[a]	2[a]	8[a]	2[e]	-	30
	PCBs	18[a]	2[a]	8[a]	2[e]	-	30
	PNAs	6[a,f]	-	8[a]	2	-	16
	Elements	18[a]	2[a]	8[a]	2[g]	-	30
Hair	PCBs	3[a]	-	8[a]	1[e]	-	12
	Elements	3[a]	-	8[a]	1	-	12
						Total	352

[a]Analysis by RTI.

[b]Analysis by IITRI (requires IITRI sampling cartridges).

[c]Full interpretation of GC/MS data from selected samples. Does not require additional sampling.

[d]Analysis by U. Miami.

[e]Analysis by HERL/RTP.

[f]Selected samples analyzed. If positive results are obtained, all samples will be analyzed.

[g]HERL/CI.

Table 4-3. TEAM LEADERS IN PRIMARY AND QA LABORATORIES FOR PUBLIC HEALTH INITIATIVE CHEMICAL ANALYSIS

Medium	Class	Primary Lab (Team Leader)	QA Lab (Team Leader)
Air	Volatiles	RTI (Sparacino)	EMSL-RTP (Clements)
	Pesticides/PCBs	RTI (Sparacino)	HERL-RTP (Lewis)
	PNAs	RTI (Sparacino)	NIOSH (Larkin)
	Metals	FSU (Nelson)	U. C. at Davis (Cahill)
Breath	Volatiles	RTI (Erickson)	IITRI (Knotoszynski)
Water	Volatiles	RTI (Sparacino)	HERL-CI (Kopfler)
	Pesticides/PCBs	RTI (Sparacino)	HERL-RTP (Lewis)
	PNAs	RTI (Sparacino)	EMSL-CI (Booth)
	Metals	RTI (Handy)	EMSL-CI (Booth)
Blood	Volatiles	U. Miami (Pfaffenberger)	RTI (Erickson)
	Pesticides	RTI (Erickson)	U. Miami (Pfaffenberger)
	PNAs	RTI (Erickson)	To be determined
	Metals	RTI (Handy)	HERL-CI (Kopfler)
Urine	Volatiles	RTI (Erickson)	U. Miami (Pfaffenberger)
	Pesticides/PCBs	RTI (Erickson)	U. Miami (Pfaffenberger)
	PNAs	RTI (Erickson)	To be determined
	Metals	RTI (Handy)	HERL-CI (Kopfler)
Hair	PCBs	RTI (Erickson)	HERL-RTP (Lewis)
	Elements	RTI (Handy)	HERL-CI (Kopfler)
Food	Volatiles	FDA (Lombardo)/RTI (Pellizzari)	-
	Pesticides/PCBs	FDA	RTI
	PNAs	FDA(?)	RTI
	Metals	FDA	RTI

APPENDIX A

ANALYTICAL PROTOCOLS FOR TOXIC CHEMICALS IN ENVIRONMENTAL MEDIA

ANALYTICAL PROTOCOL: PERSONAL MONITORING OF VAPOR-PHASE ORGANIC COMPOUNDS IN AMBIENT AIR (RTI)

1.0 Principle of Method

Recovery of volatile organics from Tenax GC is accomplished by thermal desorption and purging with helium into a liquid nitrogen cooled nickel capillary trap (1-3) and then the vapors are introduced into a high resolution glass gas chromatographic column where the constitutents are separated from each other (2,4). Characterization and quantification of the constituents in the sample are accomplished by mass spectrometry either by measuring the intensity of the total ion current signal or by extracted ion current profile (2,5,6). The analysis system is shown in Figure 1.

2.0 Range and Limits of Detection

The linear range for the analysis of volatile organic compounds depends upon two principal features. The first is a function of the breakthrough volume of each specific compound which is trapped on the Tenax GC sampling cartridge and the second is related to the inherent limits of detection of the mass spectrometer for each organic (2,5-8). Thus, the range and the maximum limit of detection are a direct function of each compound which is present in the original ambient air. The linear range for quantitation using glass capillaries on a gas chromatograph/mass spectrometer/computer (GC/MS/COMP) is generally three orders of magnitude [5-5,000 ng (5-8)]. Table 1 lists the overall detection limits for some examples of volatile organics which are based on these two principles.

3.0 Interferences

For the target compounds in Table 1, no interferences have been observed. Particular attention must be paid to the preparation of clean collection devices and the use of appropriate blanks and controls to establish that the background contaminants have been removed. Otherwise, false positive detection of chloroform, toluene or benzene may occur.

Precautions must be taken for sampling caustic atmospheres which contain levels of NO_x and molecular halogens greater than 2-5 ppm and 25 ppb,

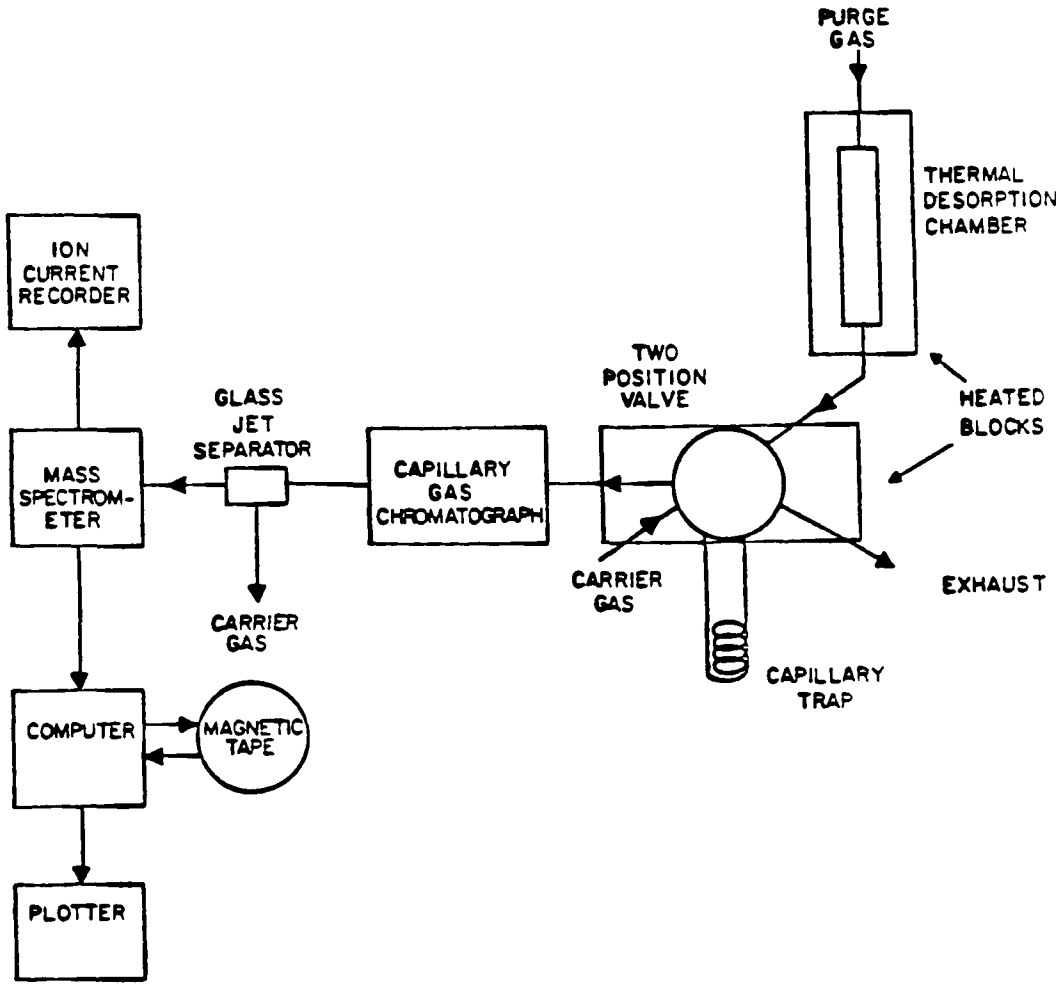

Figure 1. Analytical system for analysis of organic vapors in ambient air.

Table 1. APPROXIMATE MEASURED LIMITS OF DETECTION AND QUANTIFIABLE LIMITS FOR SELECTED VAPOR-PHASE ORGANICS IN AMBIENT AIR

Compound	m/z	LOD[a]		QL	
		µg/m^3	ppt	µg/m^3	ppt
Benzene	78	0.08	26	0.40	128
Chloroform	83/85	0.08	17	0.40	85
1,2-Dichloroethane	98/62	0.12	31	0.60	153
1,1,1-Trichloroethane	97/99	0.16	31	0.80	154
Carbon tetrachloride	117/119	0.24	39	1.20	195
Vinylidene chloride	96/98	0.12	31	0.60	156
Trichloroethylene	130/132	0.16	31	0.80	154
Tetrachloroethylene	164/166	0.24	36	1.20	179
Bromodichloromethane	127/83	0.24	37	1.20	184
Chlorobenzene	112/114	0.16	36	0.80	179
1,1,2-Trichloroethane	97/99	0.16	31	0.80	154
m-Dichlorobenzene	146/148	0.20	34	1.00	169

[a]Limit of Detection (LOD) was defined as S/N = 4 for m/z ion selected for quantification. Quantification Limit (QL) was defined as 5 x LOD or S/N = 20. Limits are based on a collection volume of 25 ℓ or breakthrough volume (70°F), which ever is smaller, for 1.5 cm x 8.0 cm Tenax GC bed volume and mass spectrometer response to that compound.

respectively (5,9,10). Quenching agents incorporated into the particulate filter should be employed in these cases (5,9,10).

4.0 Precision and Accuracy

The reproducibility of this method has been determined to range from ±10 to ±30% of the relative standard deviation for different substances when replicate sampling cartridges are examined (4-10). The inherent analytical errors are a function of several factors: [1] the ability to accurately determine the breakthrough volume and its relation to field sampling conditions for each of the organic compounds identified; [2] the accurate measurement of the ambient air volume sampled; [3] the percent recovery of the organic from the sampling cartridge after a period of storage; [4] the reproducibility of thermal desorption for a compound from the cartridge and its introduction into the analytical system; [5] the accuracy of determining the relative molar response ratios between the identified substance and the external standard used for calibrating the analytical system; [6] the reproducibility of transmitting the sample through the high resolution gas chromatographic column; and [7] the day-to-day reliability of the MS/COMP system (1-12).

The accuracy of analysis is generally ±10-30% but depends on the chemical and physical nature of the compound (2,5,6,7,12).

5.0 Apparatus and Reagents

5.1 Collection and Analysis Devices

5.1.1 Personnel Monitor Pump

A personal monitor pump (MSA Co. - Model C-200) is used for sample collection. Flow rates are adjusted to ∿0.05 ℓ/min for an 8 hr collection period. Flows are adjusted such that a total volume of ∿0.025 m^3 air is sampled for a given collection period.

5.1.2 Sampling Cartridges

The sampling tubes are prepared by packing a ten centimeter long by 1.5 cm i.d. glass tube containing 8 cm of 35/60 mesh Tenax GC with glass wool in the ends to provide support (2,11). Virgin Tenax (or material to be recycled) is extracted in a Soxhlet apparatus for a minimum of 18 hours each time with methanol and n-pentane prior to preparation of cartridge samplers (2,11). After purification of the Tenax GC sorbent and drying in a vacuum oven at

120°C for 3 to 5 hours at 28 inches of water, all the sorbent material is meshed to provide a 35/60 particle size range. Meshing and all further cartridge preparation is conducted in a "clean" room. Cartridge samplers are then prepared and conditioned at 270°C with a purified helium flow of 30 ml/min for 120 min. Prior to entering the Tenax GC cartridge the helium is purified by passing through a liquid N_2 cooled cryogenic trap. The conditioned cartridges are transferred to Kimax® (2.5 cm x 150 cm) culture tubes, immediately sealed using Teflon-lined caps and cooled. This procedure is performed in order to avoid recontamination of the sorbent bed (2,12).

5.1.3 Inlet Manifold

An inlet manifold for thermally recovering vapors trapped on Tenax sampling cartridges is used and is shown in Figure 1 (1-4).

5.1.4 Gas Chromatograph

A Varian 1700 or a Pye Unicam 102 gas chromatograph is used to house the glass capillary column and is interfaced to the inlet manifold on the Varian MAT CH-7 or LKB 2091 systems, respectively. A mass flow controller (Tylan) is used to precisely control the carrier gas. Such an analytical system was presented schematically in Figure 1.

A jet separator is employed to interface the glass capillary column to the mass spectrometer on the Varian MAT CH-7 GC/MS/COMP or LKB 2091 systems. The separator is maintained at 240°C (2,5).

5.1.5 Mass Spectrometer/Computer

A Varian MAT CH-7 or LKB 2091 mass spectrometer capable of a resolution of 1500-2,000 equipped with single ion monitoring capability is used in tandem with the Varian 1700 or Pye Unicam 102 gas chromatograph and interfaced to a Varian 620/L or PDP 11/04 computer, respectively (Figure 1).

5.2 Reagents and Materials

All reagents used are analytical reagent grade. All solvents (Burdick & Jackson) are redistilled before their use.

6.0 Procedure

6.1 Cleaning of Glassware

All glassware is washed in Isoclean/water, rinsed with deionized distilled water, acetone and air dried. Glassware is heated to 450-500°C for 2 hours to insure that all organic material has been removed prior to its use.

6.2 Collection of Volatile Organics in Ambient Air

For large sample volumes, it is important to realize that the total volume of air may cause the elution of compounds through the sampling tube if their breakthrough volume is exceeded. The breakthrough volumes of some of the volatile organics are shown in Table 2 (2,3,6,7). These breakthrough volumes have been determined and verified by previously described techniques (2,5,6). The breakthrough volume is defined as that point at which 50% of a discrete sample introduced into the cartridge is lost. Although the identity of a compound during ambient air sampling is not known (therefore, also its breakthrough volume), the compound can still be quantified after identification by GC/MS/COMP once the breakthrough volume has subsequently been established. Thus, for calculating concentration, the last portion of the sampling period which represents the volume of air sampled prior to breakthrough is selected. For cases in which the identity of a volatile organic compound is not known until after GC/MS, the breakthrough volume is subsequently determined.

Previous experiments have shown that the organic vapors collected on Tenax GC sorbent are stable and can be quantitatively recovered from the cartridge samplers up to 4 weeks after sampling when they are tightly closed in cartridge holders and placed in a second container that can be sealed, protected from light and stored at -20°C [Table 3 (1,2,6,7)].

6.2.1 Deuterated Standards

The use of deuterated compounds provides for an assessment of any premature breakthrough (and thus reduced collection efficiency) which may occur if the total vapor-phase organic load exceeds 1/10 of the cartridge capacity during field sampling. d_5-Bromoethane (B.P. 34°C), d_8-tetrahydrofuran (B.P. 65), d_6-benzene (B.P. 80), d_{10}-cyclohexene (B.P. 83) and d_5-chlorobenzene (B.P. 132) are loaded as a discrete zone onto at least 10% of all Tenax GC sampling cartridges prior to sampling. Using GC/MS/COMP the exogenous deuterated compounds are differentiated from the endogenous vapor-phase organics in ambient air.

The addition of these deuterated standards is performed by injecting 1.0 ml air/vapor of the substances onto the "front" end of the cartridge.

Table 2. TENAX GC BREAKTHROUGH VOLUMES FOR TARGET COMPOUNDS[a]

Compound	b.p. (°C)	Temperature (°F)					
		50	60	70	80	90	100
chloroform	61	56	41	32	24	17	13
carbon tetrachloride	77	45	36	28	21	17	13
1,2-dichloroethane	83	71	55	41	31	24	19
1,1,1-trichloroethane	75	31	24	20	16	12	9
tetrachloroethylene	121	481	356	261	192	141	104
trichloroethylene	87	120	89	67	51	37	28
chlorobenzene	132	1989	871	631	459	332	241

[a]For a Tenax GC bed of 1.5 x 8.0 cm.

Table 3. RECOVERY OF TARGET COMPOUNDS AFTER STORAGE

Compound	1 day	1 wk	2 wk
Benzene	100	107 ± 26	69 ± 8
Chloroform	100	83 ± 14	49 ± 4
1,2-Dichloroethane	100	100 ± 5	100 ± 7
1,1,1-Trichloroethane	100	87 ± 17	71 ± 7
Carbon tetrachloride	ND	ND	ND
Vinylidene chloride	100	92	ND
Trichloroethylene	100	87 ± 17	71 ± 7
Tetrachloroethylene	100	92 ± 1	78 ± 4
Bromodichloromethane	ND	ND	ND
Chlorobenzene	100	87 ± 3	80 ± 7
1,1,2-Trichloroethane	100	95 ± 3	92 ± 2
m-Dichlorobenzene	100	89 ± 15	85 ± 3

An air/vapor mixture stream containing 200-400 ng/ml is generated using permeation tubes of each compound with a permeation system (4,5,8,9).

The quantity of each substance on the sampling cartridges is determined by GC/MS/COMP after field sampling and the percent recovery is compared to control (unused) cartridges carried to and from the field sampling site and subjected to the same storage regime. Statistically significant differences are attributed to premature breakthrough.

6.2.2 Quantification Standards

Unique substances may be added as internal standards during sampling. Examples are the deuterated compounds listed under 6.2.1. However, the volume of air sampled is accurately known and thus external standards may be introduced into the cartridge prior to its analysis. Three standards, hexafluorobenzene, octafluorotoluene, and iodotoluene are used for the purpose of calculating RMRs and the levels in ambient air. Previous research has shown that their retention times span the chromatographic range of analysis (SE-30 coated capillary) and they do not interfere with the analysis of unknown compounds in ambient air samples.

The external standards (300-400 ng) are injected into the sampling cartridges as a 1.0 ml air/vapor mixture using a gas sampling syringe. The air/vapor mixture is synthesized using permeation tubes and a permeation system (4,5,8,9).

6.3 Analysis of Samples

The instrumental conditions for the analysis of volatile organics on the sorbent Tenax GC sampling cartridge is shown in Table 4. The thermal desorption chamber and the six port Valco valve are maintained at 270°C. The jet separator is maintained at 245°. The mass spectrometer is set to scan the mass range from approximately 20-350. The helium purge gas through the desorption chamber is adjusted to 15-20 mL/min. The nickel capillary trap on the inlet manifold is cooled with liquid nitrogen. In a typical thermal desorption cycle, a sampling cartridge is placed in the preheated desorption chamber and the helium gas is channeled through the cartridge to purge the vapors into the liquid nitrogen capillary trap [the inert activity efficiency of the trap has been shown in a previous study (4,10)]. After the desorption has been completed, the six-port valve is rotated and the

Table 4. OPERATING PARAMETERS FOR GLC-MS-COMP SYSTEM

Parameter	Setting
Inlet-manifold	
desorption chamber and valve	270°C
capillary trap - minimum	-195°C
maximum	240°C
thermal desorption time	8 min
He purge flow	15 ml/min
GLC	
85 m glass WCOT $BaCO_3$ SE-30 (0.8-1.0 μ film)	24-240°C, 4°C/min
carrier (He) flow	∿1.25 ml/min
separator/transfer line	245°C
MS	
Varian MAT CH-7	
scan range	$\underline{m}/\underline{z}$ 20 → 350
scan cycle, automatic-cyclic	1 sec/decade
filament current	300 μA
multiplier	4.0
ion source vacuum	∿4 x 10^{-6} T
LKB 2091	
scan range	$\underline{m}/\underline{z}$ 20 → 500
scan cycle, automatic	2 sec total
filament current	300 μA
multiplier	4.5
ion source vacuum	∿4 x 10^{-6} T

temperature on the capillary loop is rapidly raised (greater than 10°/min); the carrier gas then introduces the vapors onto the high resolution GC column. The glass capillary column is temperature programmed from ambient to 240°C at 4°C/min and held at the upper limit for a minimum of 10 min. After all the components have eluted the column is then cooled to ambient temperature and the next sample is processed (2).

An example of the analysis of volatile organics in ambient air is shown in Figure 2 and the background from a blank cartridge is shown in Figure 3. The high resolution glass capillary column was coated with SE-30 stationary phase which is capable of resolving a multitude of compounds to allow their subsequent identification by MS/COMP techniques; in this case over 110 compounds were identified in this chromatogram.

6.3.1 Qualitative Analysis

The mass spectral data are processed in the following manner. First, the original spectra are scanned and the reconstructed ion chromatogram (RIC) is extracted and examined. The intensity (RIC) is plotted against the spectrum number using the software package available. The information will generally indicate whether the run is suitable for further processing, since it provides some idea of the number of unknowns in the sample and the resolution obtained using the particular gc column conditions.

If mass conversion of spectral peak times to peak masses has not been performed on-the-fly during data acquisition by hardware methods than this function is next performed by software methods (magnetic systems). In either case the mass conversion is accomplished by the use of the calibration table obtained prior to sample analysis for perfluorokerosene. In general the calibration data are sufficient for an entire day's data processing; however, it is verified every eight hours.

After the spectra are obtained in mass converted form, processing proceeds either manually or by computer by comparison to a Library (13). Compound identification can involve various degrees of certainty. These levels of identification have been defined as follows:

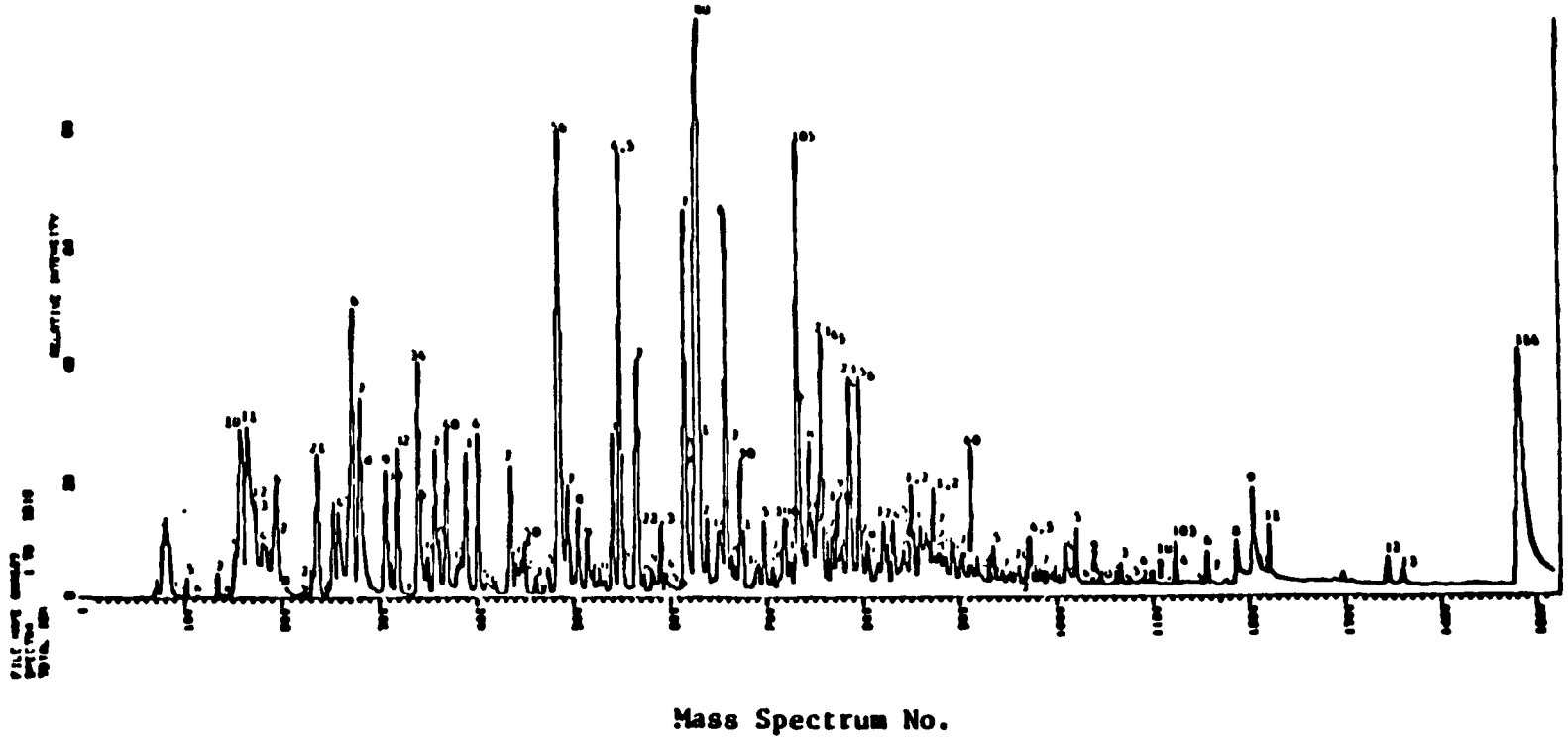

Figure 2. Profile of ambient air pollutants obtained using high resolution gas chromatography/ mass spectrometry/computer.

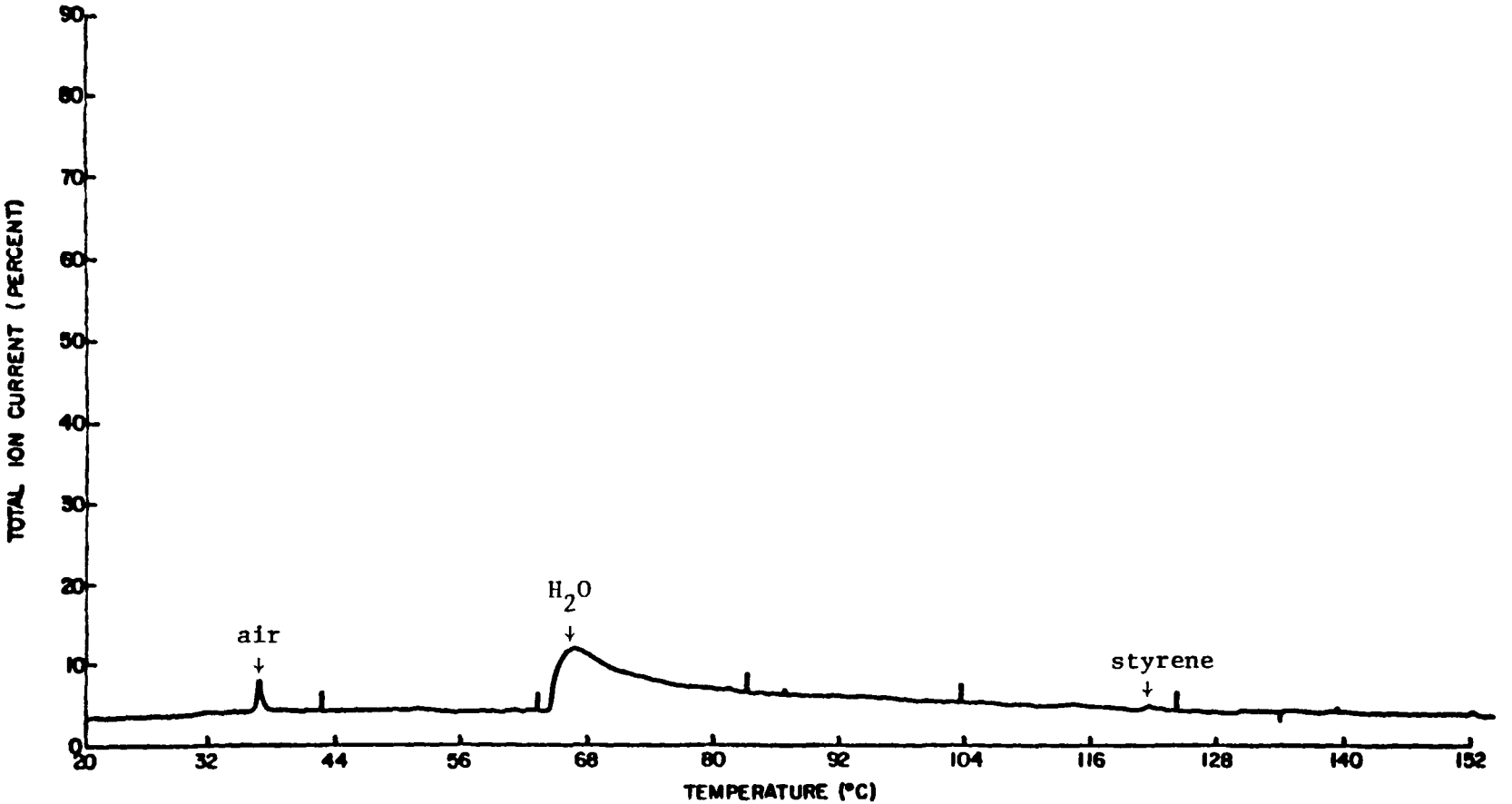

Figure 3. Background profile for Tenax GC cartridge blank.

Level I Computer Interpretation. The raw data generated from the analysis of samples are subjected to computerized deconvolution/library search and compound identification made using this approach has the lowest level of confidence. In general Level I is reserved for only those cases where compound verification is the primary intent of the qualitative analysis

Level II Manual Interpretation. The plotted mass spectra are manually interpreted by a skilled interpretor and compared to those spectra compiled in a data compendium. In general a minimum of five masses and intensities (±5% S.D.) should match between the unknown and library spectrum. This level does not utilize any further information such as retention time since many compounds the authentic compound may not be available for establishing retention times.

Level III Manual Interpretation Plus Retention Time/Boiling Point of Compound. In addition to the effort as described under Level II, the retention time of the compound is compared to the retention time which has been derived from previous chromatographic analysis. Also the boiling point of the identified compound is compared to the boiling points of other compounds in the near vicinity of the one in question when a capillary coated with a non-polar phase has been used.

Level IV Manual Interpretation Plus Retention Time of Authentic Compounds. Under this level, the authentic compound has been chromatographed on the same capillary column using identical operating conditions and the mass spectrum of the authentic compound is compared to that of the unknown.

Level V Level IV Plus Independent Confirmation Techniques. This Level utilizes other physical methods of analysis such as GC/fourier transform/IR, GC/high resolution mass spectrometry, or NMR analysis. This Level constitutes the highest degree of confidence in the identification of organic compounds.

6.3.2 Quantitation

The quantitation of constituents in ambient air samples is accomplished either by utilizing the total ion current monitor or, where necessary, from extracted ion current profiles. In order to eliminate the need to obtain complete calibration curves for each compound for which quantitative information is desired, the method of relative molar response (RMR) factors (5-10) is used. Successful use of this method requires information on the exact amount of standard added and the relationship of RMR (unknown) to the RMR (standards).

6.3.2.1 RMR Determination

The compounds to be quantified are loaded onto Tenax GC cartridges using a permeation system (4,5,8,9) or in cases where permeation tubes are not available the vaporization system shown in Figure 4 is used (5,14).

With the vaporization method helium is purified by passing it through a cryogenic trap followed by two carbon traps. The standards and substances to be quantified are prepared in methanol and a 2.0 μℓ solution is injected through the septum of the heated loading tube (250°C). The vaporized components are swept onto the Tenax GC cartridge at a rate of 200 ml/min for 6 min (total He 1.2 ℓ). Because of the low breakthrough volume for methanol (0.8 ℓ at 70°F), the majority is passed from the cartridge. This system is used to load relatively non-volatile compounds with breakthrough volumes >50 ℓ.

The method of calculating RMRs is as follows:

$$(1)\quad RMR_{unknown/standard} = \frac{A_{unk}/Moles_{unk}}{A_{std}/Moles_{std}}$$

A = system response, height or area determined by integration or triangulation.
unk = unknown
std = standard

The value of RMR is determined from at least three independent analyses during analysis of samples (5). Linearity over the dynamic range and an intercept of zero has been previously described (5,14).

$$(2)\quad RMR_{unk/std} = \frac{A_{unk}/g_{unk}/GMW_{unk}}{A_{std}/g_{std}/GMW_{std}}$$

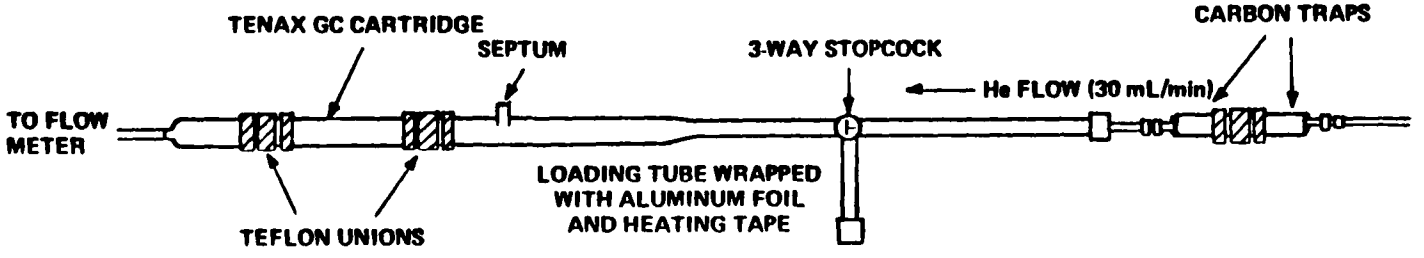

Figure 4. Schematic of vaporization unit for loading organics dissolved in methanol onto Tenax GC cartridges.

A = system response, as above
g = number of grams present
GMW = gram molecular weight

$$(3)\quad g_{unk} = \frac{A_{unk} \cdot GMW_{unk} \cdot g_{std}}{A_{std} \cdot GMW_{std} \cdot RMR_{unk/std}}$$

6.3.2.2 Calculation of Ambient Air Concentrations

Since the volume of air taken to produce a given sample is accurately known and an external standard is added to the sample, then the weight per cartridge and hence the concentration of the unknown can be determined. The approach for quantitating ambient air pollutants in this study requires that the RMR be determined for each constituent of interest during the analysis of field samples. Every sixth cartridge is a control cartridge for determining RMRs for each compound (calibration of instrument, storage and recovery). This means that when an ambient air sample is taken, the external standard is added at a known concentration prior to analysis. It is not imperative at this point to know what the RMR of each of the constituents in the sample happens to be. However, after the unknowns are identified then the RMR can subsequently be determined and the unknown concentration calculated in the original sample using the RMR. In this manner it is possible to obtain qualitative and quantitative information on the same sample with a minimum of effort.

Once the quantity of substance per cartridge has been determined, the level in ambient air is given by

$$\frac{\mu g}{m^3} = \frac{\mu g_{unk} \cdot 1000\ L}{m^3 \cdot \text{Volume Sampled (L)}}$$

7.0 Quality Assurance Program

7.1 Reagent and Glassware Control

Reagent and glassware control is required in order to minimize contamination. Sample containers, glassware, etc. are cleaned with Isoclean®, rinsed with distilled/deionized water and heat treated at 450-500°C to insure the removal of all traces of organic compounds.

7.2 Sampling Protocol and Chain of Custody

As part of the quality control procedures, sampling protocols and chain of custody forms are prepared for each sampling cartridge. Examples of these forms are given in Tables 5 and 6. The fate of each sampling cartridge is tracked from the time they are prepared until the data has been reduced to a finished form.

7.3 Blanks, Controls, Standards, and System Performance Samples

7.3.1 Blanks

Ten percent of the sampling cartridges from each batch are set aside to serve as blanks to be analyzed for background contamination. After the preparation of a set of sampling cartridges, one cartridge is checked for background prior to their committment to field sampling. Blank (unused) cartridges travel to the field site returned to the laboratory and stored along with the field samples at -20°C until ready for analysis.

7.3.2 Controls

Ten percent of the sampling cartridges are loaded with the deuterated compounds listed in 6.2.1. Sampling with control cartridges allow for an assessment of premature breakthrough if it occurs. Control cartridges are analyzed along with other samples and since deuterated compounds are employed the qualitative and quantitative analysis of these air samples proceeds unimpeded.

7.3.3 Standards for RMR Determination

The compounds listed in Table 1 are loaded onto Tenax GC cartridges from a permeation system. A minimum of three analyses is required for determining RMRs for a set of samples which are quantitatively analyzed.

7.3.4 System Performance Mixtures

The system performance standards listed in Table 7 are loaded onto Tenax GC cartridges (using the vaporization method) to determine mass calibration and intensity and chromatographic performance of the GC/MS/COMP system.

7.4 Sample Analysis

To insure the accuracy and precision of the data acquired instrument and chromatographic performance are monitored on a daily basis.

Table 5. FIELD SAMPLING PROTOCOL SHEET - A

Date: _____

Project No. (_ _ _ _)
Operator (_ _)
Trip No. (_)
Sampler (_ _)
Area _____ (_ _)
Address _____
Period (_)
Site _____(_ _ _)
Location (_)
Sample Code _____

Mutech 221 (N)

DC amps _____
Sampling rate _____ (lpm)
Vacuum _____("Hg)
End: Time _____ Ft^3 _____
Start: Time ____ Ft^3 _____
Total: (min) ___ Ft^3 _____

DuPont (D)

Sampling rate (init.) _____ (lpm)
Sampling rate (final) _____ (lpm)
End: Time _____
Start: Time _____
Total: (min) _____

MSA (M)

Sampling rate (init.) _____ (lpm)
Sampling rate (final) _____ (lpm)
End: Time _____ Count _____
Start: Time _____ Count _____
Total: (min) _____ Count _____
ml/count _____

Remarks _____

_____

Volume Air/Cartridge _____

_____

Time ___ Temp. Wet. ___ Dry _____
Rel. Humid ___% Wind Dir./Speed __/__
Cloud _____ Odor _____
Remarks _____

Time ___ Temp. Wet. ___ Dry _____
Rel. Humid ___% Wind Dir./Speed __/__
Cloud _____ Odor _____
Remarks _____

Time ___ Temp. Wet.____ Dry__ _____
Rel. Humid ___% Wind Dir./Speed __/__
Cloud _____ Odor _____
Remarks _____

Time ____ Temp. Wet ___ Dry _____
Rel. Humid ___% Wind Dir./Speed __/__
Cloud _____ Odor _____
Remarks _____

Table 6. CHAIN OF CUSTODY RECORD

Research Triangle Institute
Analytical Sciences Division
Chemistry and Life Sciences Group
Research Triangle Park, NC 27709

SAMPLE CODE:	Sample Type:	Volume Collected:
	No. of Containers:	Volume Analyzed:

Relinquished By:	Received By:	Time	Date	Operation Performed (aliquot, std. conc., remarks, etc.)

Table 7. GC/MS/COMP SYSTEM PERFORMANCE STANDARDS

Compound	Quantity (ng)
Perfluorokerosene	350
Perfluorotoluene	350
Ethylbenzene	300
p-Xylene	300
n-Octane	300
n-Decane	300
1-Octanal	300
5-Nonanone	300
Acetophenone	300
2,6-Dimethylaniline	300
2,6-Dimethylphenol	300

7.4.1 Instrument Calibration

Calibration of mass and intensity of magnetic systems employ perfluorokerosene. Table 8 lists the tolerances for each mass and intensity which the mass spectrometer must achieve.

Perfluorotoluene in the performance mixture is employed for determining instrument stability as related by mass resolution and relative ion abundance under GC conditions (5,14). The masses and intensities listed in Table 8 are compared to the results obtained on a daily basis and for each set of samples analyzed.

7.4.2 Assessment of Chromatographic Performance

The quality of the chromatography is of utmost importance since the accuracy and precision of qualitative and quantitative analysis are directly affected (5,14). Glass capillary columns are evaluated according to the following criteria:

(1) percent peak asymmetry factor (PAF)

$$\% \text{ PAF} = \frac{B}{F} \times 100$$

where B = the area of the back half of a chromatographic peak
F = area of the front half of the chromatographic peak both measured 10% above baseline

(2) effective Height Equivalent to a Theoretical Plate ($HETP_{eff}$)

$$HETP_{eff} = \frac{L}{5.54\ (X/Y)^2}$$

where X = the corrected retention distance for sweep time of the compound,
Y = chromatographic peak width at 1/2 peak height,
L = column length (mm)

(3) separation number (SN)

$$SN = \frac{D}{W_1 + W_2} - 1$$

where D = the distance between two peaks,
W_1, W_2 = widths at 1/2 height

Table 8. MASS AND INTENSITY TOLERANCES ACCEPTABLE FOR CALIBRATION OF MAGNETIC INSTRUMENTS FOR QUANTITATION

Perfluorotoluene[a]		Perfluorokerosene	
m/z	%I (C.V.)	m/z	%I (C.V.)
69	33 (5)	51	39 (10)
79	11 (10)	100	22 (8)
93	16 (8)	119	100 (0)
117	43 (8)	131	89 (5)
167	15 (7)	169	58 (4)
186	59 (5)	181	62 (6)
217	100 (0)	219	24 (5)
236	66 (4)	231	29 (8)

[a]To be achieved in the chromatography mode.

(4) resolution (R)

$$R = \frac{2\ \Delta\ W}{W_1 + W_2}$$

where ΔW = average base width,
W = peak width at base

(5) Acidity and Basicity

$$\text{Acidity} = \frac{\text{weak base (peak area or height)}}{\text{acetophenone (peak area or height)}}$$

$$\text{Basicity} = \frac{\text{weak acid (peak area or height)}}{\text{acetophenone (peak area or height)}}$$

The use of the compounds listed in Table 7 provides information as to the degree of adsorption and the type of adsorption mechanisms. 1-Octanol and 5-nonanone serves to determine the extent of deactivation of the glass surface (PAF). The acidity and basicity of the glass capillary column are assessed by the adsorption of weak bases and acids, respectively (5,14).

The resolution and separation number are determined for the compound pairs ethylbenzene:p-xylene and octane:decane, respectively. $HETP_{eff}$ is based on octane. Table 9 lists the minimum performance specifications acceptable for ambient air analysis (5). Figures 5-11 depict extracted ion current profiles used for calculating performance specifications.

7.4.3 Sequence of Sample Analysis

A strict step-sequence of analysis is followed. Upon mass and intensity calibration of the MS system, a Tenax GC cartridge loaded with the performance mixture is first analyzed. Following the performance mixture a blank and an RMR standard mixture is analyzed next, then five samples. The cycle is then repeated. At the beginning of each day the analysis cycle begins with the performance mixture, blank and RMR standards. Thus, 30% of the cartridges analyzed consist of control samples.

8.0 References

1. Pellizzari, E. D., Development of Method for Carcinogenic Vapor Analysis in Ambient Atmospheres. Publication No. EPA-650/2-74-121, Contract No. 68-02-1228, 148 pp., July, 1974.

Table 9. SPECIFICATIONS OF PERFORMANCE FOR GLASS CAPILLARY COLUMN[a]

Parameter	Test Compound(s)	Value ± S.D. (C.V.)
Resolution	Ethylbenzene:p-Xylene	1.30 ± 11 (8)
Separation No.	Octane:Decane	73 ± 6 (8)
% Peak Asymmetry Factor	1-Octanol	239 ± 141 (59)
	Nonanone	130 ± 32 (25)
	Acetophenone	260 ± 34 (13)
Acidity	2,6-Dimethylaniline:Acetophenone	1.00 ± 0.07 (9)
Basicity	2,6-Dimethylphenol:Acetophenone	0.82 ± 0.03 (5)

[a]SE-30 WCOT/$BaCO_3$, 0.48 mm i.d. x 75 m, 1 μ film thickness.

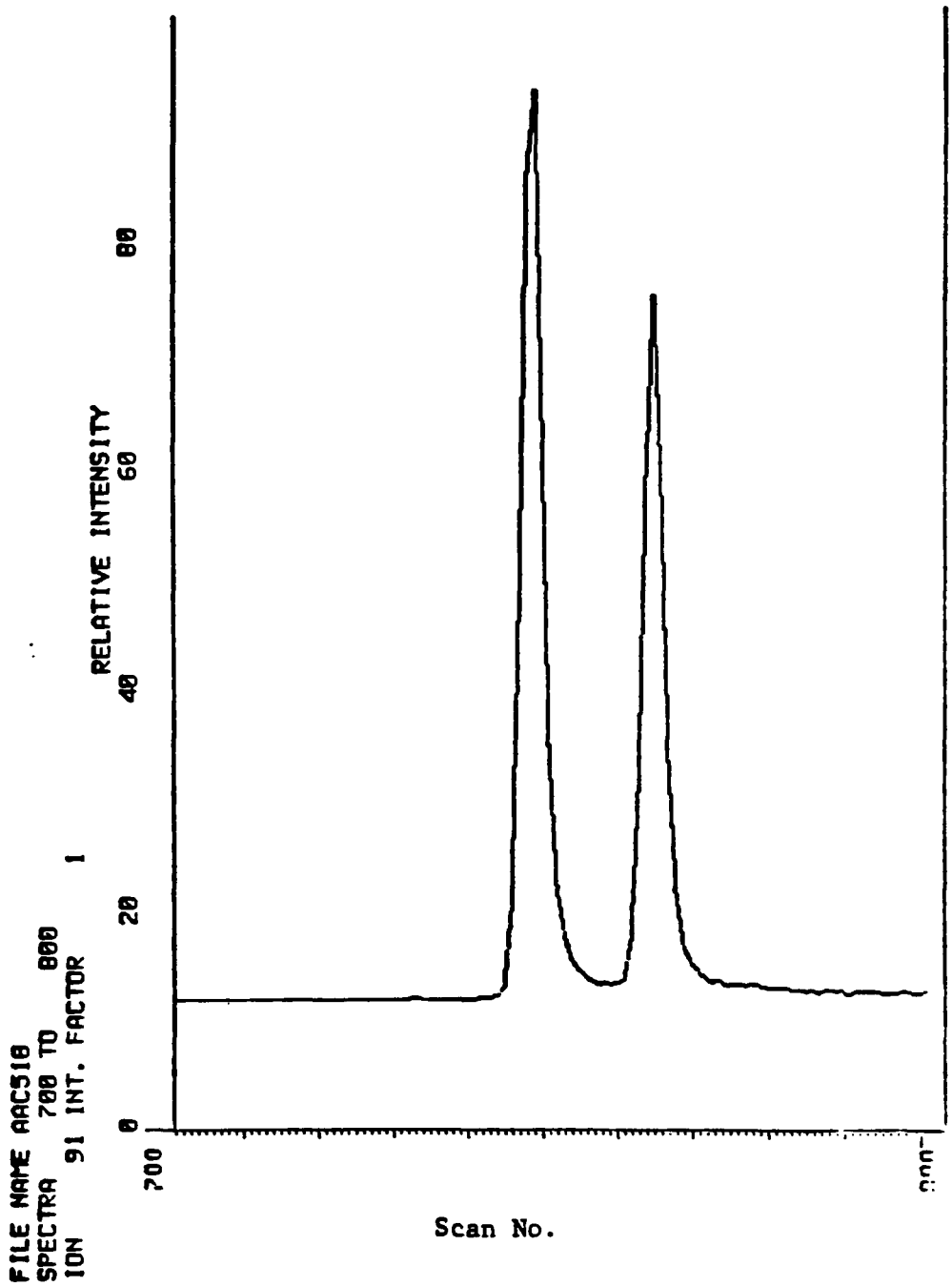

Figure 5. Extracted ion current profile of m/z 91 for p-xylene and ethylbenzene used in calculating resolution.

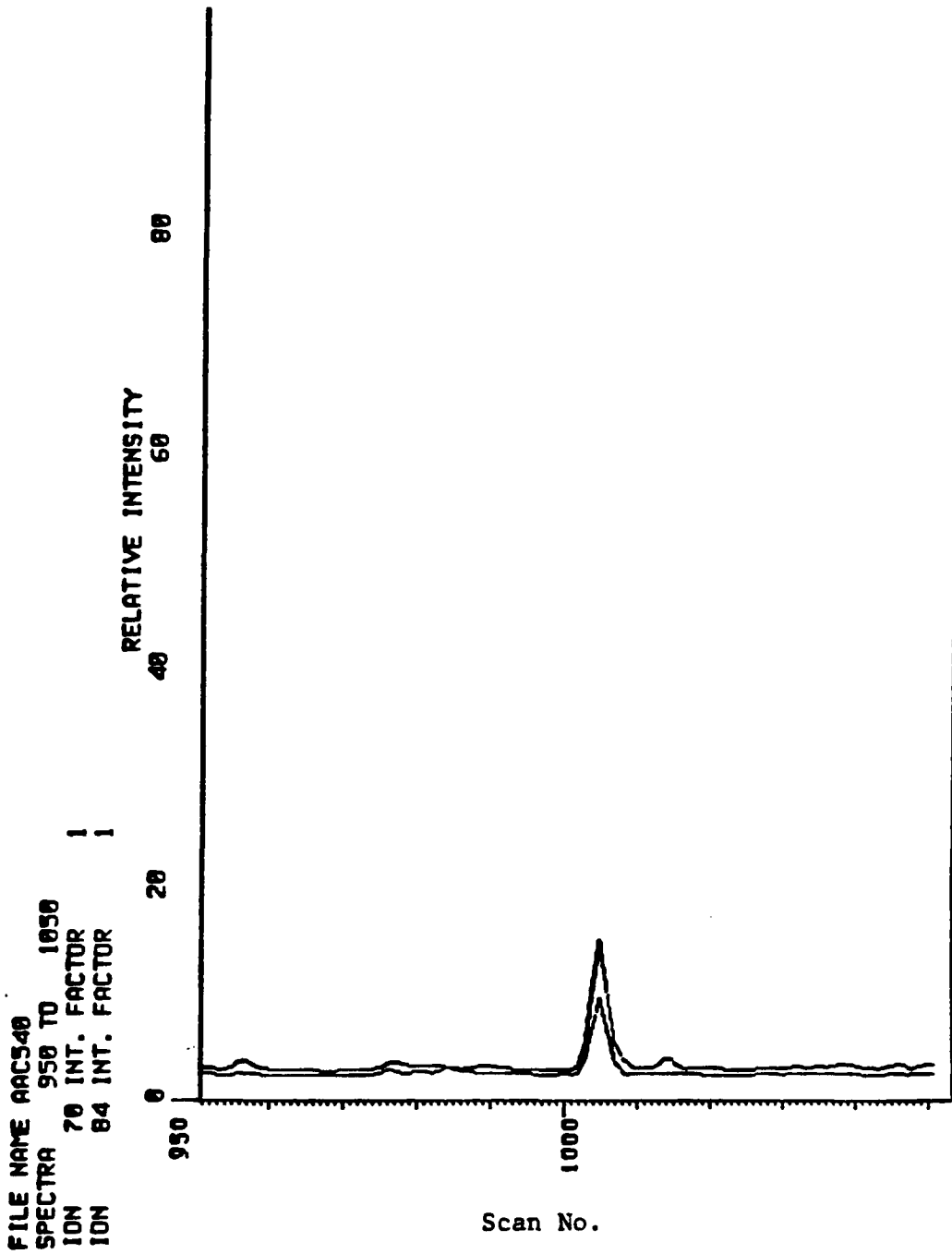

Figure 6. Extracted ion current profile of m/z 70 and 84 for 1-octanol used for calculating percent peak asymmetry factor.

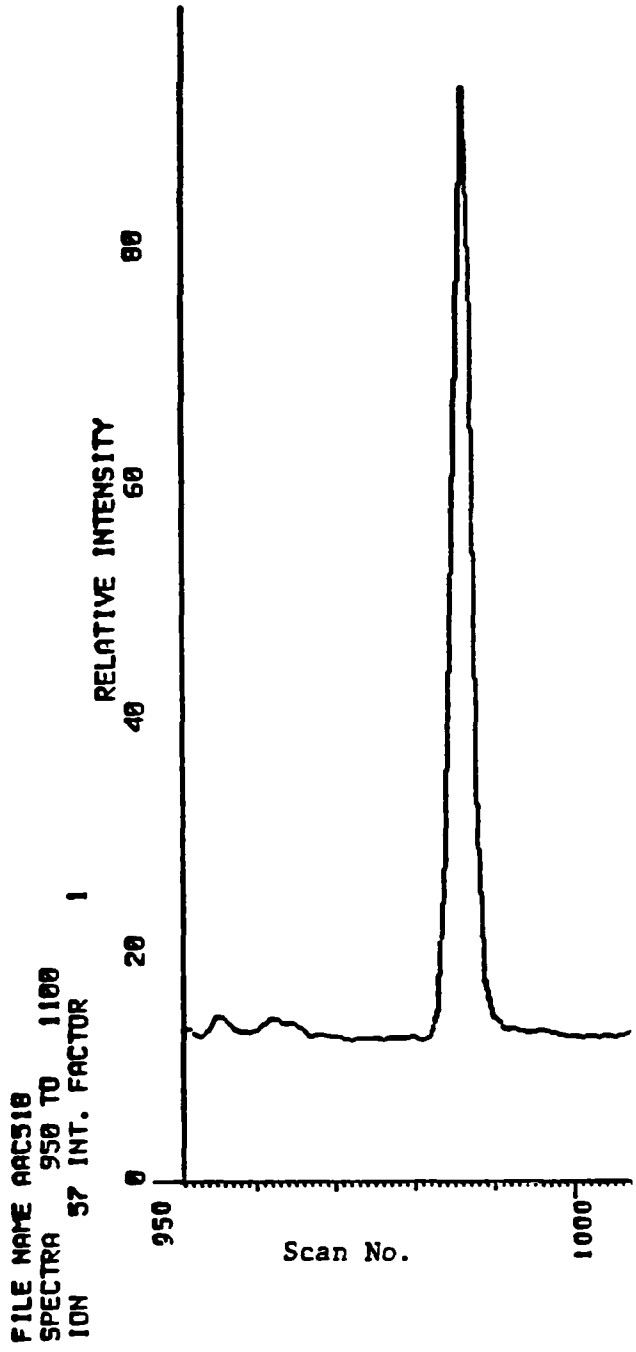

Figure 7. Extracted ion current profile of m/z 57 for nonanone used in calculating percent peak asymmetry factor.

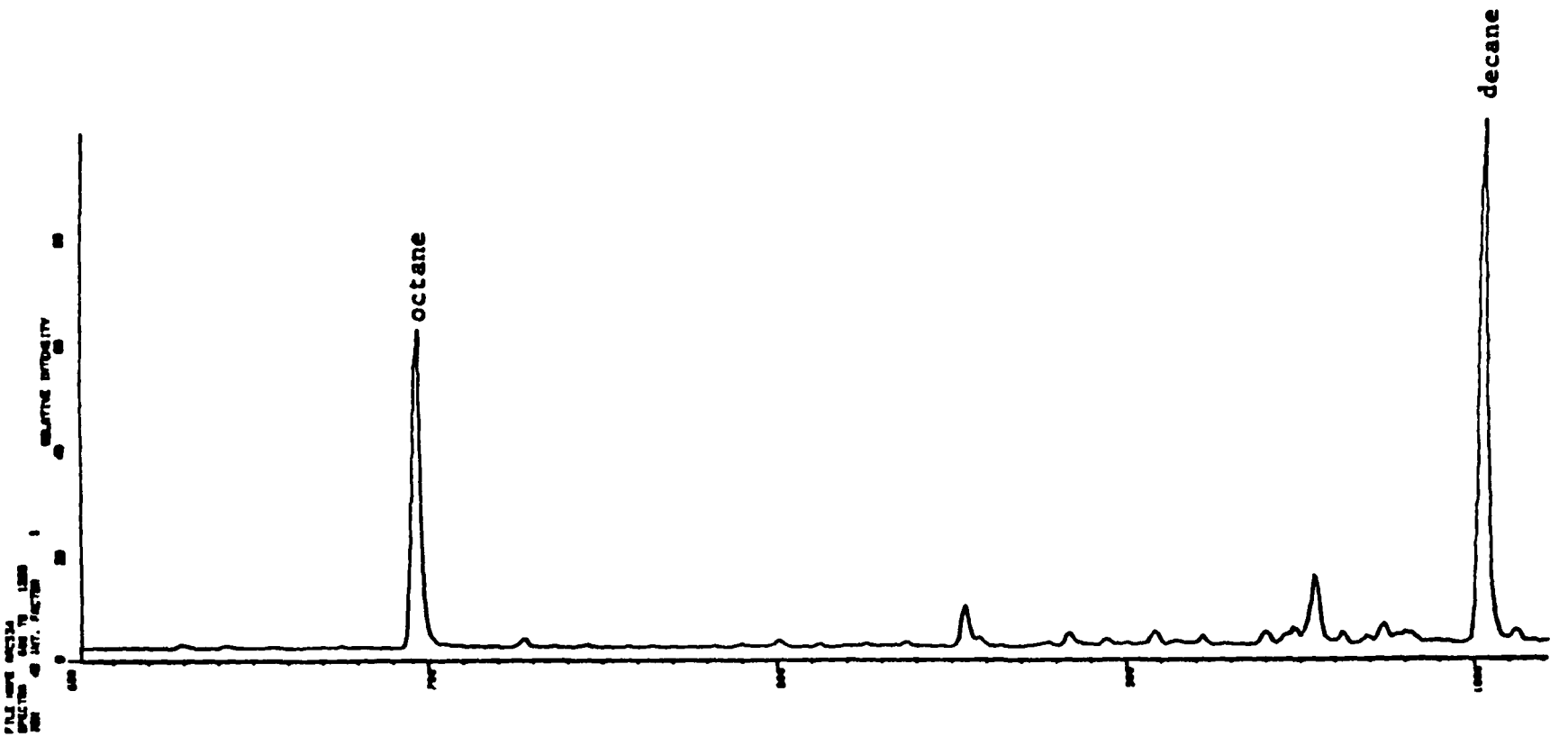

Figure 8. Extracted ion current profile of m/z 43 for octane and decane used in calculating separation number.

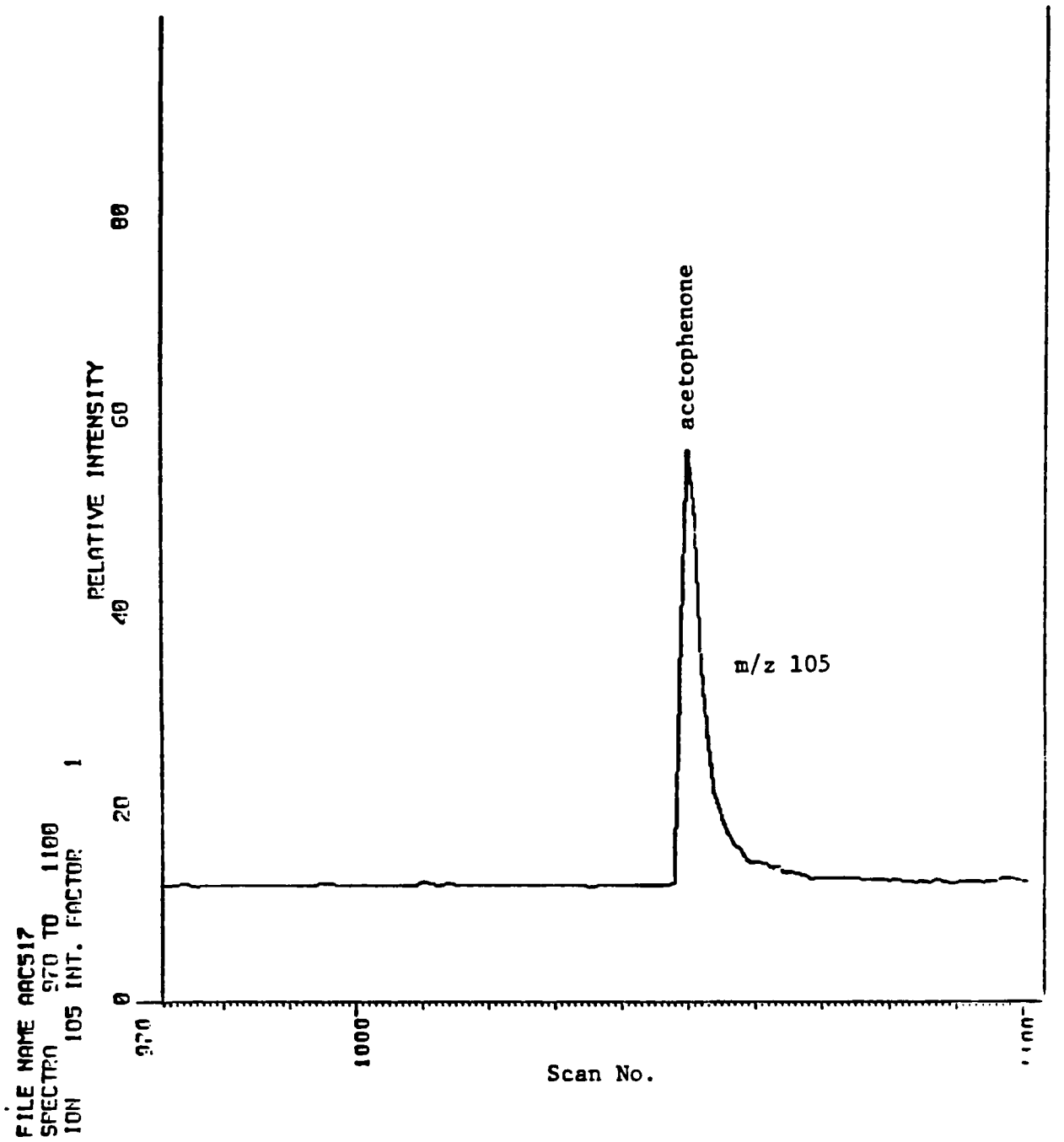

Figure 9. Extracted ion current profile of m/z 105 for acetophenone in calculating percent peak asymmetry factor.

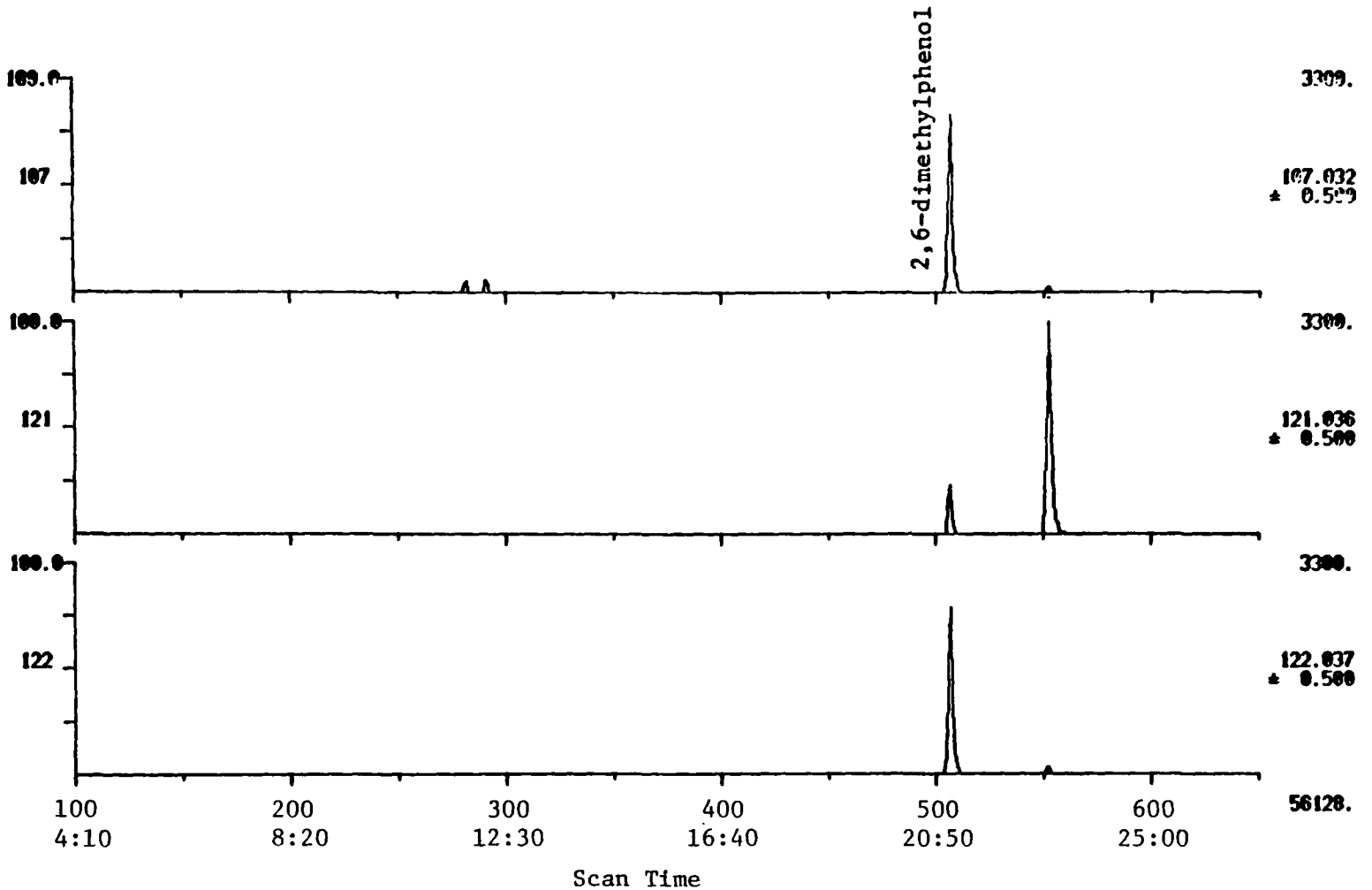

Figure 10. Extracted ion current profile of m/z 107, 121 and 122 of 2,6-dimethylphenol in mixture.

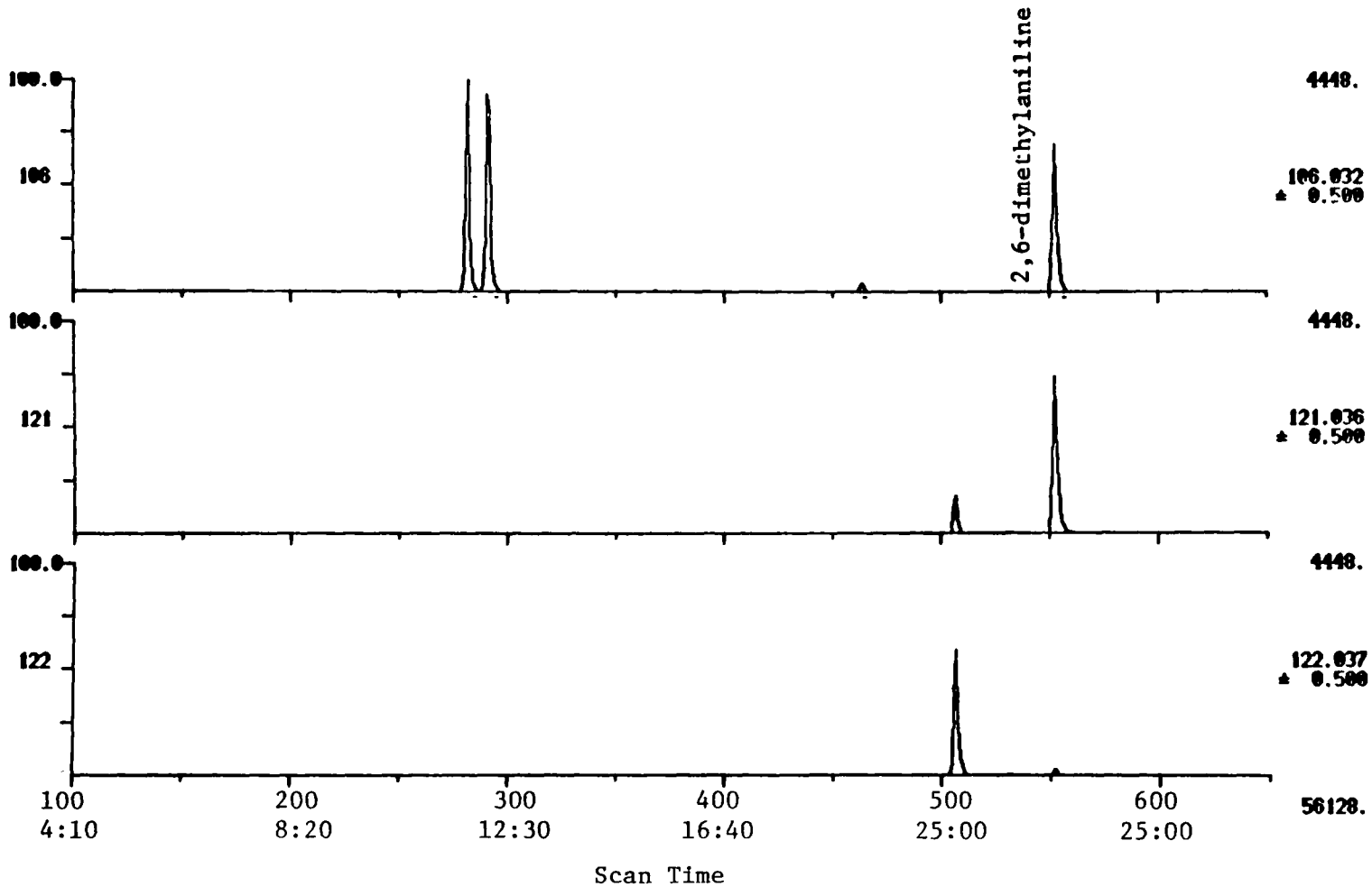

Figure 11. Extracted ion current profile of m/z 106, 121, and 122 for 2,6-dimethylaniline in performance mixture.

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11. Pellizzari, E. D., J. E. Bunch, R. E. Berkley and J. McRae, Anal. Chem., 48, 803 (1976).
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13. "Eight Peak Index of Mass Spectra", Vol. I, (Tables 1 and 2) and II (Table 3), Mass Spectrometry Data Centre, AWRE, Aldermaston, Reading, RF74PR, UF, 1970.
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Written analytical protocol prepared 5/6/80.

ANALYTICAL PROTOCOL: POLYNUCLEAR AROMATIC HYDROCARBONS IN DRINKING WATER

1.0 Principle of the Method

This method is applicable for the determination, from drinking water, of the following polynuclear aromatic hydrocarbons (PAH):

Acenaphthene
Acenaphthylene
Anthracene
Benzo(a)anthracene
Benzo(b)fluoranthene
Benzo(a)pyrene
Benzo(b)fluoranthene
Benzo(ghi)perylene
Benzo(k)fluoranthene
Chrysene
Dibenzo(a,h)anthracene
Fluoranthene
Fluorene
Indeno(1,2,3-cd)pyrene
Naphthalene
Phenanthrene
Pyrene

This method contains both liquid and gas chromatographic approaches, depending upon the needs of the analyst. The gas chromatographic procedure cannot adequately resolve the following four pairs of compounds: Anthracene and phenanthrene; chrysene and benzo(a)anthracene; benzo(b)fluoranthene and benzo(k)fluoranthene; and dibenzo(a,h)anthracene and indeno(1,2,3-cd)pyrene. Unless the purposes of the analysis can be served by reporting a sum for an unresolved pair, the liquid chromatographic or capillary gas chromatographic approaches must be used for these compounds. The liquid chromatographic method will resolve all of the 16 compounds listed above.

This method is recommended for use only by experienced residue analysts or under the close supervision of such qualified persons.

2.0 Range and Detection Limit

The sensitivity of this method is usually dependent upon the level of interferences rather than instrumental limitations. The limits of detection listed in Table 1 for the liquid chromatographic approach represent sensitivities than can be achieved in wastewaters in the absence of interferences. Gas chromatography (packed column) can produce detection limits at least as low as the HPLC approach, while capillary gas chromatography will produce significantly lower limits.

3.0 Interferences

Solvents, reagents, glassware, and other sample processing hardware may yield discrete artifacts and/or elevated baselines causing misinterpretation of the chromatograms. All of these materials must be demonstrated to be free from interferences under the conditions of the analysis by running method blanks. Specific selection of reagents and purification of solvents by distillation in all-glass systems may be required.

Interferences coextracted from the samples will vary considerably from source to source, depending upon the diversity of the industrial complex or municipality being sampled. While a general clean-up technique is provided as part of this method, unique samples may require additional clean-up approaches to achieve the sensitivities stated in Table 1.

The extent of interferences that may be encountered using liquid chromatographic techniques has not been fully assessed. Although the chromatographic conditions described allow for a unique resolution of the specific PAH compounds covered by this method, other PAH compounds may interfere.

Capillary gas chromatographic methods, with inherently greater resolution than either HPLC or packed column GC, will minimize the extent of interferences.

4.0 Precision and Accuracy

The U. S. EPA Environmental Monitoring and Support Laboratory in Cincinnati is in the process of conducting an interlaboratory method study to determine the accuracy and precision of this test procedure.

Table 1. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY OF PAHs

Compound[b]	Retention time (min)	Detection limit (μg/L)[a]	
		UV	Fluorescence
Naphthalene	16.17	2.5	20.0
Acenaphthylene	18.10	5.0	100.0
Acenaphthene	20.14	3.0	4.0
Fluorene	20.89	0.5	2.0
Phenanthrene	22.32	0.25	1.2
Anthracene	23.78	0.10	1.5
Fluoranthene	25.00	0.50	0.05
Pyrene	25.94	0.10	0.05
Benzo(a)anthracene	29.26	0.20	0.04
Chrysene	30.14	0.20	0.5
Benzo(b)fluoranthene	32.44	1.0	0.04
Benzo(k)fluoranthene	33.91	0.30	0.04
Benzo(a)pyrene	34.95	0.25	0.04
Dibenzo(a,h)anthracene	37.06	1.0	0.08
Benzo(ghi)perylene	37.82	0.75	0.2
Indeno(1,2,3-cd)pyrene	39.21	0.30	0.1

[a]Detection limit is calculated from the minimum detectable HPLC response being equal to five times the background noise, assuming an equivalent of a 2 ml final volume of the 1 liter sample extract, and assuming an HPLC injection of 2 microliters.

[b]HPLC conditions: Reverse phase HC-ODS Sil-X 2.6 x 250 mm Perkin-Elmer column; isocratic elution for 5 min using 40% acetonitrile/60% water, then linear gradient elution to 100% acetonitrile over 25 minutes, flow rate is 0.5 mL/min.

5.0 Apparatus and Reagents

5.1 Sampling equipment, for discrete or composite sampling

5.1.1 Grab sample bottle-amber glass, 1-liter or 1-quart volume. French or Boston Round design is recommended. The container must be washed and solvent rinsed before use to minimize interferences.

5.1.2 Bottle caps-threaded to screw on to the sample bottles. Caps must be lined with Teflon. Foil may be substituted if sample is not corrosive.

5.2 Compositing equipment-automatic or manual compositing system

Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated during sampling. No Tygon or rubber tubing may be used in the system.

5.3 Separatory funnel-2000 mL, with Teflon stopcock

5.4 Drying column-20 mm ID pyrex chromatographic column with coarse frit

5.5 Kuderna-Danish (K-D) apparatus

5.5.1 Concentrator tube-10 mL, graduated (Kontex K-570050-1025 or equivalent). Calibration must be checked. Ground glass stopper (size 19/22 joint) is used to prevent evaporation of extracts.

5.5.2 Evaporative flask-500 mL (Kontes K-57001-0500 or equivalent). Attach to concentrator tube with springs (Kontes K-66270-0012).

5.5.3 Snyder column-three-ball macro (Kontes K503000-0121 or equivalent).

5.5.4 Snyder column-two-ball micro (Kontes K-569001-0219 or equivalent).

5.5.5 Boiling chips-solvent extracted, approximately 10/40 mesh.

5.6 Water bath-heated, with concentric ring cover, capable of temperature control (±2°C). The bath should be used in a hood.

5.7 HPLC Apparatus:

5.7.1 Gradient pumping system, constant flow.

5.7.2 Reverse phase column, 5 micron HC-ODS Sil-X, 250 mm x 2.6 mm ID (Perkin Elmer No. 809-0716 or equivalent).

5.7.3 Fluorescence detector, for excitation at 280 nm and emission at 389 nm.

5.7.4 UV detector, 254 nm, coupled to fluorescence detector.

5.7.5 Strip chart recorder compatible with detectors, (a data system for measuring peak areas is recommended).

5.8 Gas chromatograph-Analytical system complete with gas chromatograph suited for on-column injection and all required accessories including dual flame ionization detectors, column supplies, recorder, gases, syringes. A data system for measuring peak areas is recommended.

5.9 Chromatographic column-250 mm long x 10 mm ID with coarse fritted disc at bottom and Teflon stopcock.

5.2 Reagents

5.2.1 Preservatives

5.2.1.1 Sodium hyroxide-(ACS) 10N in distilled water.

5.2.1.2 Sulfuric acid-(ACS) Mix equal volumes of conc. H_2SO_4 with distilled water.

5.2.1.3 Sodium thiosulfate-(ACS) Granular.

5.2.2 Methylene chloride, Pentane, Cyclohexane, High Purity Water-HPLC quality, distilled in glass.

5.2.3 Sodium sulfate-(ACS) Granular, anhydrous (purified by heating at 400°C for 4 hrs in a shallow tray).

5.2.4 Stock standards-Prepare stock standard solutions at a concentration of 1.00 µg/µL by dissolving 0.100 grams of assayed reference material in pesticide quality isooctane or other appropriate solvent and diluting to volume in a 100 mL ground glass stoppered volumetric flask. The stock solution is transferred to ground glass stoppered reagent bottles, stored in a refrigerator, and checked frequently for signs of degradation or evaporation, expecially just prior to preparing working standards from them.

5.2.5 Acetonitrile-Spectral quality.

5.2.6 Silica gel-100/120 mesh desiccant (Davison Chemical grade 923 or equivalent). Before use, activate for at least 16 hours at 130°C in a foil covered glass container.

6.0 Procedure

6.1 Collection of Sample

Grab samples must be collected in glass containers. Conventional sampling practices should be followed, except that the bottle must not be

prewashed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be free of Tygon and other potential sources of contamination.

The samples must be iced or refrigerated from the time of collection until extraction. Chemical preservatives should not be used in the field unless more than 24 hours will elapse before delivery to the laboratory. If the samples will not be extracted within 48 hours of collection, adjust the sample to a pH range of 6.0-8.0 with sodium hydroxide or sulfuric acid and add 35 mg sodium thiosulfate per part per million of free chlorine per liter.

All samples must be extracted within 7 days and completely analyzed within 30 days of collection.

6.2 Sample Extraction

Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a two-liter separatory funnel. Check the pH of the sample with wide-range pH paper and adjust to within the range of 5-9 with sodium hydroxide or sulfuric acid.

Add 60 mL methylene chloride to the sample bottle, seal, and shake 30 seconds to rinse the inner walls. Transfer the solvent into the separatory funnel, and extract the sample by shaking the funnel for two minutes with periodic venting to release vapor pressure. Allow the organic layer to separate from the water phase for a minimum of ten minutes. If the emulsion interface between layers is more than one-third the size of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, or centrifugation. Collect the methylene chloride extract in a 250-mL Erlenmeyer flask.

Add a second 60-mL volume of methylene chloride to the sample bottle and complete the extraction procedure a second time, combining the extracts in the Erlenmeyer flask.

Perform a third extraction in the same manner. Pour the combined extract through a drying column containing 3-4 inches of anhydrous sodium sulfate, and collect it in a 500-mL Kuderna-Danish (K-D) flask equipped with a 10 mL concentrator tube. Rinse the Erlenmeyer flask and column with 20-30 mL methylene chloride to complete the quantitative transfer.

Add 1-2 clean boiling chips to the flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL methylene chloride to the top. Place the K-D apparatus on a hot water bath (60-65°C) so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed in vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15-20 minutes. At the proper rate of distillation the balls of the column will actively chatter but the chambers will not flood. When the apparatus volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain for at least 10 minutes while cooling. Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1-2 mL of methylene chloride. A 5 mL syringe is recommended for this operation. Stopper the concentrator tube and store refrigerated if further processing will not be performed immediately.

Determine the original sample volume by refilling the sample bottle to the mark and transferring the liquid to a 1000 mL graduated cylinder. Record the sample volume to the nearest 5 mL.

If the sample requires cleanup before chromatographic analysis, proceed to Section 6.3. If the sample does not require cleanup, or if the need for cleanup is unknown, analyze an aliquot of the extract according to Section 6.5.

6.3 Cleanup and Separation

Before the silica gel cleanup technique can be utilized, the extract solvent must be exchanged to cyclohexane. Add a 1-10 mL aliquot of sample extract (in methylene chloride) and a boiling chip to a clean K-D concentrator tube. Add 4 mL cyclohexane and attach a micro-Snyder column. Prewet the micro-Snyder column by adding 0.5 mL methylene chloride to the top. Place the micro K-D apparatus on a boiling (100°C) water bath so that the concentrator tube is partially immersed in the hot water. Adjust the vertical position of the apparatus and the water temperature as required to complete concentration in 5-10 minutes. At the proper rate of distillation the balls of the column will actively chatter but the chambers will not flood. When the apparent volume of the liquid reaches 0.5 mL, remove K-D apparatus and allow it to drain for at least 10 minutes while cooling.

Remove the micro-Snyder column and rinse its lower joint into the concentrator tube with a minimum of cyclohexane. Adjust the extract volume to about 2 mL.

6.3.2 Silica Gel Column Cleanup for PAHs

Prepare a slurry of 10 g activated silica gel in methylene chloride and place this in a 10 mm ID chromatography column. Gently tap the column to settle the silica gel and elute the methylene chloride. Add 1-2 cm of anhydrous sodium sulfate to the top of the silica gel.

Preelute the column with 40 mL pentane. Discard the eluate and just prior to exposure of the sodium sulfate layer to the air, transfer the 2 mL cyclohexane sample extract onto the column, using an additional 2 mL of cyclohexane to complete the transfer. Just prior to exposure of the sodium sulfate layer to the air, add 25 mL pentane and continue elution of the column. Discard the pentane eluate.

Elute the column with 25 mL of 40% methylene chloride/60% pentane and collect the eluate in a 500 mL K-D flask equipped with a 10 mL concentrator tube. Elution of the column should be at a rate of about 2 mL/min. Concentrate the collected fraction to less than 10 mL by K-D techniques as in 6.2, using pentane to rinse the walls of the glassware. Proceed with HPLC or gas chromatographic analysis.

6.4 Calibration

Prepare calibration standards that contain the compounds of interest, either singly or mixed together. The standards should be prepared at concentrations covering two or more orders of magnitude that will completely bracket the working range of the chromatographic system. If the sensitivity of the detection system can be calculated from Table 1 as 100 µg/L in the final extract, for example, prepare standards at 10 µg/L, 50 µg/L, 100 µg/L, 500 µg/L, etc. so that injections of 1-5 µL of each calibration standard will define the linearity of the detector in the working range.

Assemble the necessary HPLC or gas chromatographic apparatus and establish operating parameters equivalent to those indicated in Table 1 or 2. By injecting calibration standards, establish the sensitivity limit of the detectors and the linear range of the analytical systems for each compound.

Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interferences from the reagents.

6.5 Analysis

6.5.1 HPLC

To the extract in the concentrator tube, add 4 mL acetonitrile and a new boiling chip, then attach a micro-Snyder column. Increase the temperature of the hot water bath to 95-100°C. Concentrate the solvent as above. After cooling, remove the micro-Snyder column and rinse its lower joint into the concentrator tube with about 0.2 mL acetonitrile. Adjust the extract volume to 1.0 mL.

Table 1 summarizes the recommended HPLC column materials and operating conditions for the instrument. Included in this table are estimated retention times and sensitivities that should be achieved by this method. An example of the separation achieved by this column is shown in Figure 1. Calibrate the system daily with a minimum of three injection of calibration standards.

Inject 2-5 µL of the sample extract with a high pressure syringe or sample injection loop. Record the volume injected to the nearest 0.05 µL, and the resulting peak size, in area units. If the peak area exceeds the linear range of the system, dilute the extract and reanalyze. If the peak area measurement is prevented by the presence of interference, further cleanup is required. The UV detector is recommended for the determination of naphthalene and acenaphthylene and the fluorescence detector is recommended for the remaining PAHs.

6.5.2 Gas Chromatography

The gas chromatographic procedure will not resolve certain isomeric pairs as indicated in Table 2. The liquid chromatographic procedure (6.5.1) must be used for these materials.

To achieve maximum sensitivity with this method, the extract must be concentrated to 1.0 mL. Add a clean boiling chip to the methylene chloride extract in the concentrator tube. Attach a two-ball micro-Snyder column. Preset the micro-Snyder column by adding about 0.5 ml of methylene chloride to the top. Place this micro K-D apparatus on a hot water bath (60-65°C) so

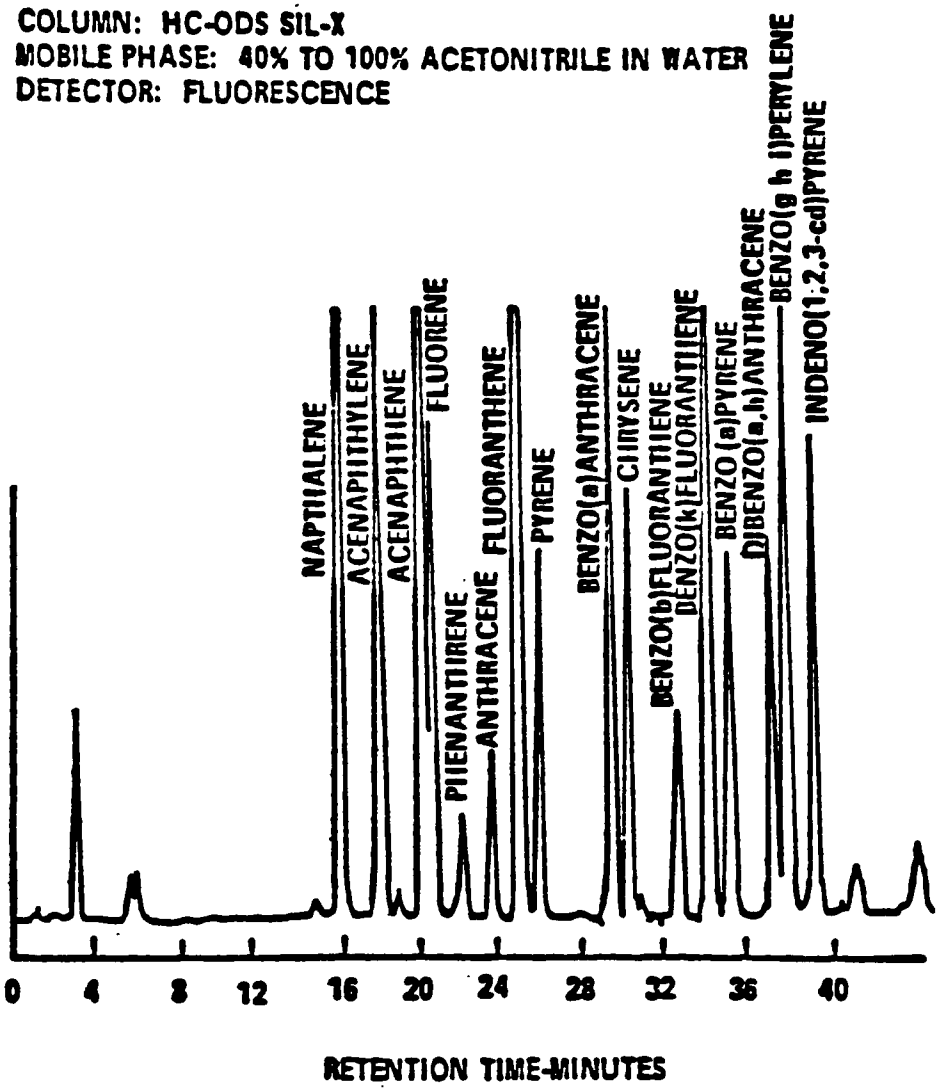


Figure 1. Liquid chromatogram of polynuclear aromatics.

Table 2. GAS CHROMATOGRAPHY OF PAHs

Compound[a]	Retention time (min)
Naphthalene	4.5
Acenaphthylene	10.4
Acenaphthene	10.8
Fluorene	12.6
Phenanthrene	15.9
Anthracene	15.9
Fluoranthene	19.6
Pyrene	20.6
Benzo(a)anthracene	20.6
Chrysene	24.7
Benzo(b)fluoranthene	28.0
Benzo(k)fluoranthene	28.0
Benzo(a)pyrene	29.4
Dibenzo(a,h)anthracene	36.2
Indeno(1,2,3-cd)pyrene	36.2
Benzo(ghi)perylene	38.6

[a]GC conditions: Chromosorb W-AW-DMDCS 100/120 mesh coated with 3% OV-17, packed in a 6' x 2 mm ID glass column, with nitrogen carrier gas at 40 mL/min flow rate. Column temperature was held at 100°C for 4 minutes, then programmed at 8°/minute to a final hold at 280°C.

that the concentrator tube is partially immersed in the hot water. Adjust the vertical position of the apparatus and water temperature as required to complete the concentration in 5 to 10 minutes. At the proper rate of distillation the balls will actively chatter but the chambers will not flood. When the apparent volume of liquid reaches 0.5 mL, remove the K-D apparatus and allow it to drain for at least 10 minutes while cooling. Remove the micro-Snyder column and rinse its lower joint into the concentrator tube with a small volume of methylene chloride. Adjust the final volume to 1.0 mL and stopper the concentrator tube.

Table 2 describes the recommended gas chromatographic column material and operating conditions for the instrument. Included in this table are estimated retention times that should be achieved by this method. Calibrate the gas chromatographic system daily with a minimum of three injections of calibration standards.

Inject 2-5 μL of the sample extract using the solvent flush technique. Smaller (1.0 μL) volumes can be injected if automatic devices are employed. Record the volume injected to the nearest 0.05 μL, and the resulting peak size, in area units. If the peak area exceeds the linear range of the system, dilute the extract and reanalyze. If the peak area measurement is prevented by the presence of interferences, further cleanup is required.

6.6 Calculations

Determine the concentration of individual compounds according to the formula:

$$\text{Concentration, } \mu g/L = \frac{(A)(B)(V_t)}{(V_i)(V_s)}$$

where:

A = calibration factor for chromatographic system, in nanograms material per area unit
B = peak size in injection of sample extract, in area units
V_i = volume of extract injected (μL)
V_t = volume of total extract (μL)
V_s = volume of water extracted (mL)

Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, all data obtained should be reported.

7.0 Quality Assurance Program

7.1 Quality Control

Before processing any samples, the analyst should demonstrate through the analysis of a distilled water method blank, that all glassware and reagents are interference-free. Each time a set of samples is extracted or there is a change in reagents, a method blank should be processed as a safeguard against laboratory contamination.

Standard quality assurance practices should be used with this method. Field replicates should be collected to validate the precision of the sampling technique. Laboratory replicates should be analyzed to validate the precision of the sampling technique. Laboratory replicates should be analyzed to validate the precision of the analysis. Fortified samples should be analyzed to validate the accuracy of the analysis. Where doubt exists over the identification of a peak on the chromatogram, confirmatory techniques such as fraction collection and GC-mass spectroscopy should be used.

8.0 References

8.1 "Development and Application of Test Procedures for Specific Organic Toxic Substances in Wastewaters, Category 9-PAHs", Report for EPA Contract 68-03-2624 (in preparation).

ANALYTICAL PROTOCOL: VINYL CHLORIDE FROM PERSONAL MONITORING DEVICE

1.0 Principal of the Method

The method for measuring the exposure of personnel to vinyl chloride utilizes the permeation technique for sampling. The vinyl chloride that permeates the membrane is trapped on activated charcoal which is removed for subsequent determination by gas chromatography. The monitor is about the size of a standard film badge, weighs less than 35 g, and requires no source of power. The method is insensitive to temperature and humidity. It is ideally suited to personal monitoring programs because the analytical data represent a time-weighted-average exposure and require no further data reduction step.

2.0 Range and Detection Limit

The method has been validated for a concentration range (8 hr air sample) of 5 ppb to 50 ppm. The lower value represents the dilution limit when analysis is accomplished via thermal desorption techniques. When interferences preclude the use of this approach, solvent desorption is used. The limit of detection is then 20 ppb.

3.0 Interferences

Studies have shown that sulfur dioxide, nitrogen dioxide, ozone and chlorine do not interfere with the analysis of vinyl chloride using the personal monitor device. The only known interferent is ethylene chloride which is converted to vinyl chloride by heat treatment of the trapping medium during assay. The solvent desorption technique, described below, precludes this artifactual formation of vinyl chloride. Ambient humidity does not affect the operation and assay of the permeation device.

4.0 Precision and Accuracy

The level of precision at the detection limit (5 ppb) is within $\pm$ 50%. At concentrations greater than 100 ppb the precision is 5% for the thermal desorption mode of analysis; the solvent desorption procedure yields a precision of ~5% at 500 ppb. At this concentration (500 ppb) the accuracy for replicate determinations was $\pm$ 2.5%.

5.0 Apparatus and Reagents

5.1 Sampling Device

The sampling device is a badge having dimensions of 41 mm by 48 mm and a thickness of 7 mm (Figure 1). An internal cavity is covered by a permeable membrane through which vinyl chloride passes at a rate proportional to the external concentration. The vinyl chloride that permeates through the membrane is adsorbed on activated charcoal which is later removed from the device after completion of the exposure, and the amount adsorbed is then determined by gas chromatography.

5.2 Adsorber

The adsorber used is Darco Activated Charcoal, 20-40 mesh, supplied by Matheson, Coleman and Bell. It is pretreated to remove moisture and any organics present by heating to 350^{o}C under a flow of inert gas.

5.3 Reagents

All gases used in the preparation of permeation tubes are chemically pure grade. All liquids used are reagent grade. Purity of each organic reagent is checked by gas chromatography.

5.4 Instrumentation

5.4.1 Calibration Device

The permeation device used in this work is the same as that described previously (1), except the absorbing solution is replaced by a known quantity of activated charcoal which serves as an adsorber for the vinyl chloride that permeates the membrane.

The membrane material used for the device is a disk of single-backed dimethylsilicone rubber (General Electric Co., One River Road, Schnectady, N. Y. 12305). The standard permeation tube described by O'Keeffe and Ortman (2) and Scaringelli et al. (3) is used. These tubes are unsuitable for studies at very low levels; therefore, for concentrations below 0.05 ppm it is necessary to use a low-level permeation tube which is similar to the reservoir device of Saltman et al (4).

A reservoir of 3 mL is used with an active tube length of as little as 1 mm. This device, which has been used successfully for other gases, has a very short active area and is capable of accurately dispensing levels as low as 20 ng/min of vinyl chloride. Permeation tubes containing vinyl chloride

have a tendency to form a solid material, presumably a polymeric species, despite precautions taken to entrap inhibitor within the tube. This is not a serious problem as long as there is liquid visible in the tube.

5.4.2 Analytical Instrumentation

A Varian Model 1200 gas chromatograph along with a Honeywell-Brown recorder equipped with a Disc integrator, or equivalent instrumentation, is used in all investigations. The gas chromatograph is equipped with a flame ionization detector and is modified to permit the use of an external sampling system (Figure 2) consisting of an interchangeable sampling loop in a thermally shielded compartment which can be rapidly heated to 300°C. The sample loop consists of a 15 cm x 6.35 mm o.d. stainless steel tube. The tube is placed inside a brass tee and is heated by the exhaust from a heat gun, (Master Appliance Corp., Model No. HG-501) being directed through the side of the tee. A four-way valve provides a bypass for the helium carrier gas to permit changing of the sample loop without interrupting the determination.

6.0 Procedure

6.1 Calibration

6.1.1 Permeation Device

The membrane material used in the device is not entirely of uniform thickness; hence, each permeation device under study is individually calibrated using the apparatus illustrated in Figure 3. Calibration is accomplished by cleaning and dehumidifying air from the laboratory by pumping it though columns of activated charcoal and silica gel. Finally, the clean, dry air is passed at a predetermined flow rate over a permeation tube, of the design of O'Keeffe and Ortman (2). The permeation tube emits vinyl chloride at a known constant rate, thereby providing a primary standard for the calibration procedure. The standard concentration of vinyl chloride in air is passed through the exposure chamber where the permeation devices are exposed for calibration.

The determination of the amount of vinyl chloride adsorbed on the charcoal from the device allows the calculation of a permeation constant for each device. This constant is calculated from the following equation:

$$k = \frac{Ct}{w}$$

where k = constant (about 0.5); C = concentration of vinyl chloride, ppm; t=

time of exposure, and w = amount of vinyl chloride adsorbed, μg.

6.1.2 Gas Chromatograph

The response factor of the gas chromatograph is determined by preparing a standard containing a known amount of vinyl chloride adsorbed on 1 g of activated charcoal. This is accomplished by collecting the vinyl chloride from a stream of known concentration by passing the standard through a tube containing the charcoal sample. A backup tube is used to assure that no breakthrough of vinyl chloride occurs. These are then analyzed along with the unknown samples. This provides an accurate measure of response of the instrument to the levels of vinyl chloride being determined. It also compensates for the fact than vinyl chloride may not be completely desorbed from the charcoal. No breakthrough of vinyl chloride is observed for any standard studied over the range of 0.2 to 200 ng.

6.2 Sample Collection

The method utlizes a permeable membrane to collect the air sample at a rate proprotional to concentration. The device is worn by individuals as a badge in a manner that permits free access of ambient air to the device.

6.3 Storage of Samples

The monitor may be stored in an air-tight container for at least 8 h without loss of vinyl chloride. However, for longer storage, the charcoal should be transferred to a sealed vial. Samples stored in vials for periods up to 6 months have shown no significant losses. It is not advisable to store samples collected in a complex environment for this length of time. It should also be noted that with monitors that are exposed to uncontaminated air for 8 h, no detectable losses are observed indicating that desorption through the membrane is very slow.

6.4 Analytical Method

The chromatographic determination of vinyl chloride used for these studies primarily employs the thermal desorption process. The charcoal sample is placed in a sample loop and the loop mounted in the desorption oven. Standards are first transferred to a vial, then to the sample loop. The column is cooled to 25°C and the sample loop purged with the helium carrier gas. Then, with the carrier gas still flowing through the sample loop, the loop is heated by the hot air from the heat gun. The conditions necessary for optimum desorption were found to be 5 min desorption at 300°C.

After the completion of the thermal desorption process, the vinyl chloride from the sample is trapped on the head of the analytical column. This column is a 2-m by 2.4 mm i.d. stainless steel tube packed with Chromosorb 102 (Johns-Manville). The carrier gas flow is shunted around the sample loop by the bypass valve, and the column oven heated to 90°C. Under these conditions, vinyl chloride exhibits a rentention time of ∿ 4 min. After the emergence of the vinyl chloride, the column is heated to 200°C to remove other substances and ready the column for the next determination. Carbon disulfide extraction and analysis procedures are also applicable for use with the permeation sampling technique. The procedure involves extraction of the charcoal with 5 mL of CS_2 in a vial sealed with a Teflon-backed rubber septum. The mixture is allowed to develop for 30 min with periodic agitation. Both the sample and solvent are chilled to dry ice temperatures before extraction, and sample vials are sealed with Teflon-backed rubber septa.

The permeation constant, which with care can be determined to a precision of 3%, is used for the calculation of the average concentration of vinyl chloride in an unknown atmosphere by using the equation:

$$C = \frac{wk}{t}$$

where C = time-weighted-average vinyl chloride concentration, ppm.

7.0 Quality Control Program

The exercise of control of the quality of the data generated during the sampling and analysis of vinyl chloride using the permeation device is achieved through the use of sampling blanks, and lab controls. The analysis of blanks that have experienced field environments, without exposure, determines background levels. Control samples allow for updating gas chromatographic response factors. Both types of blanks will signal interferences, either from the atmosphere or from the instrumentation (septa, fittings, etc).

The permeation rate for a given personal monitor should be checked periodically using the calibration apparatus described above (6.1.1). The analysis of each sample extract should be conducted in duplicate. For all sample handling, extraction, and storage, only cleaned materials (glass, Teflon, stainless steel) should be used.

8.0 References

8-1 Reiszner, K. D. West, P. W., Environ. Sci. Technol., 7, 526 (1973).

8-2 O'Keeffe, A. E., Ortman, G. C., Anal. Chem., 38, 760 (1966).

8-3 Scaringelli, F. P., Frey, S. A., Saltzman, B. E., Anal. Chem., 42, 871 (1970).

8-4 Saltzman, B. E., Burgh, W. R., Romaswamy, G. K., Environ. Sci. Technol., 5, 1121 (1971).

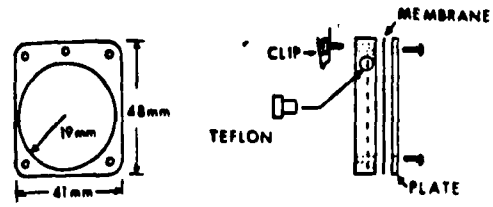


Figure 1. Personal monitoring device

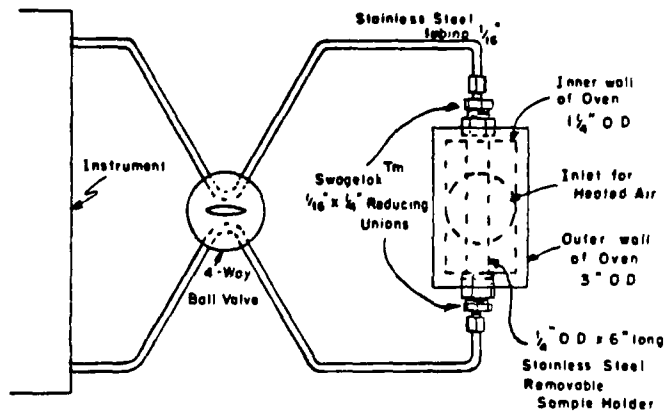


Figure 2. Apparatus for thermal desorption of vinyl chloride

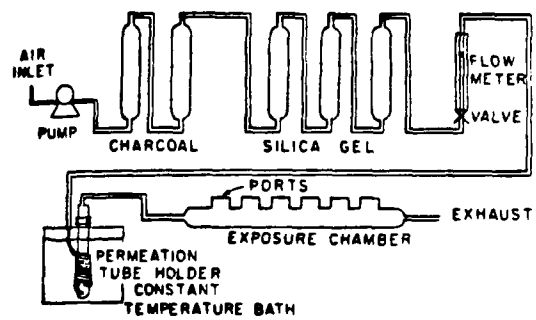


Figure 3. Calibration apparatus

Adapted from "Personal Vinyl Chloride Monitoring Device with Permeation Technique for Sampling", L. H. Nelms, K. D. Reiszner and P. W. West, Anal. Chem., 49, 994 (1977).

ANALYTICAL PROTOCOL: ARSENIC, CADMIUM AND LEAD IN AMBIENT AIR PARTICULATE

The analyses for the target metals will be accomplished by proton induced X-ray emission (PIXE) analysis under the direction of Dr. J. W. Nelson at Florida State University. The technique has been validated for a large number of metals from air aerosol from a number of different sources. Sensitivites are sufficient for the analysis of some 20 metals from aerosol obtained from 120 L of air. Complete specifications and methodology are provided in the following references:

1. Hudson, G. M., H. C. Kaufmann, J. W. Nelson and M. A. Bonacci, Nuclear Instruments and Methods, 168, 259 (1980).
2. Johansson, T. B., R. E. VanGrieken, J. W. Nelson and J. W. Winchester, Anal. Chem., 47, 855 (1975).
3. Jensen, B. and J. W. Nelson, "Nuclear Methods in Environmental Research", CONF-740701 (Oak Ridge, Tennessee:USERDA Technical Information Center, 1974), p. 366.

ANALYTICAL PROTOCOL: ORGANOCHLORINE PESTICIDES AND PCBS IN AIR

1.0 Principle of the Method

Air is sampled using a personal monitoring pump (2-4 L/min), and a cartridge of polyurethane foam (PUF) for trapping the target compounds. The PUF is Soxhlet extracted with hexane-diethyl ether (95:5 v/v). Chlorinated pesticides and PCBs are measured by EC-GC after column chromatographic clean-up on alumina. PCBs are separated from technical chlordane and other pesticides by column chromatography on silicic acid deactivatived with 3% distilled water.

2.0 Range and Detection Limit

The limits of detection for selected organochlorine pesticides and PCBs as extracted from PUF plugs according to this protocol are given in Table 1. Levels vary from several hundred picograms up to ~10 ng for the pesticides, and from ~25-35 ng for various Arochor mixtures. Also shown are the limits of measurement which consist of the low detectable quantities as determined by injection of standards onto the GC system. The range of detection is unspecified in the EPA protocol.

3.0 Interferences

Unspecified in EPA protocol.

4.0 Precision and Accuracy

The precision and accuracy of the recovery and collection efficiency are shown in Table 2 for selected pesticides and 3 Arochlors. Recoveries were determined by spiking PUF plugs via syringe with solutions of known concentrations of target compounds. The collection efficiencies were measured by vaporizing known quantities of analytes into the PUF, and are corrected by the recovery values.

5.0 Apparatus and Reagents

5.1 Sampling

5.1.1 Pumps

DuPont Constant Flow Sampling Pump, Model P4000A (includes charger), Catalog No. 66-241. DuPont, Applied Technology Division, Wilmington, DE 19898 or MSA Monitatire™ Sampler, Model S, Catalog No. 458475 and charger No. 456059. Mine Safety Applicances Company, 600 Penn Center Boulevard,

Table 1. LIMITS OF DETECTION (EC) AND AMOUNTS COMMONLY FOUND ON FOAM

Compound	Measurement limit[a] pg	Amount on control foam ng
γ-BHC	3	0.3
aldrin	5	0.6
p,p'-DDE	10	1.5
p,p'-DDT	30	10
mirex	10	2
tech chlordane	55	5
Aroclor 1242	90	30
Aroclor 1254	150	25
Aroclor 1260	520	34

[a]This is defined as the amount necessary to give a peak 10% of full scale deflection at attenuation X2. For most compounds this is 10-15 times noise (or 5-7 times the detection limit).

Table 2. EFFICIENCY OF FOAM AS A COLLECTOR OF PESTICIDES

	Collection efficiency (collected for recovery			Recovery efficiency			Retention efficiency		
	$\bar{x}$	% RSD	n	$\bar{x}$	% RSD	n	$\bar{x}$	% RSD	n
aldrin	59	12	12	91	2	4	67	13	4
p,p'-DDE	102	11	12	96	2	4	100	6	4
p,p'-DDT	98	21	12	101	2	4	97	8	4
mirex	86	22	7	89	1	4	94	5	4
tech chlordane	-	-	-	103	7	5	-	-	-
Aroclor 1242	96	15	6	100	3	5	97	14	7
Aroclor 1254	95	7	6	99	5	5	101	11	7
Aroclor 1260	109	5	11	98	7	5			

Pittsburgh, PA 15235. Both of these small, battery operated pumps are capable of pumping air through an 18 mm diameter x 50 mm cylindrical PUF plus at 2.5 to 4 liters/minute for at least 8 hours with a fully charged battery pack. The DuPont pump has the advantage that it will automatically adjust its pumping rate to compensate for changes in flow resistance (e.g., due to accumulation of particulate matter at the intake of the collection module). It also operates more quietly than the MSA and can be programmed to stop sampling after a prescribed period.

5.1.2 Collection Device

A glass tube, 10 cm x 1.5 cm I.D. is used which contains ~15 ml of PUF (8 x 1.5 cm). The foam plug is cut slightly oversized for a compression fit. An all Teflon filter housing allows for collection of particulate matter at the intake end of the cartridge. The cartridge is attached via a compression fitting and short length of flexible tubing to the pumping device.

5.2 Analysis

5.2.1 Gas chromatograph, Tracor 222 or 560, equipped with linearized ^{63}Ni FPD, and electrolytic conductivity detectors, or equivalent.

5.2.2 Glassware. Centrifuge tubes, 15 mL, graduated; separatory funnel, 500 ml; Buchner filtration device.

5.2.3 Extractors, Soxhlet, 1000, 500 and 250 mL.

5.2.4 Clean-up microcolumn, 10 cm x 5 mm i.d. disposable pipet or Chromaflex column, size 22, 20 cm x 7 mm, Kontes, Vineland, NJ, K 420100-0022.

5.2.5 Chromatoflo chromatography column, 25 cm x 9 mm i.d., Pierce #29020, equipped with a Teflon mesh support membrane, Pierce #29268, lower end plate, adapter, and 500 mL solvent reservoir (Ace #5824-10).

5.2.6 Rotary vacuum evaporator, e.g., Buchi, with 250, 500, and 1000 mL round bottom flasks.

5.2.7 Solvents, glass distilled, pesticide quality, or equivalent. Diethyl ether, analytical reagent grade, Mallinckrodt #0850, containing 2% ethanol.

5.2.8 Pesticide standards and commercial PCB mixtures, 98-100% pure, obtainable from the Pesticide Repository, U. S. EPA, EDT, HERL, Research

Triangle Park, NC (MD-69). Individual PCBs, obtainable from RFR Corp., Hope, RI.

5.2.9 Column chromatography, stationary phase.

5.2.9.1 Alumina, basic, 60 mesh, Alfa Products. Adjust to Brockmann activity IV by adding 6% (w/v) distilled water to the adsorbent in a flask, stoppering, and shaking well; allow to equilibrate for at least 15 hours before use. Discard after two weeks.

5.2.9.2 Silicic acid, Mallinckrodt AR, 100 mesh; heat at 130°C for at least 7 hours and cool to room temperature in a desiccator; to deactivate, weigh into a bottle, add 3% (w/w) distilled water, seal tightly, shake well, and place in a desiccator for at least 15 hours. Discard any adsorbent not used within one week.

6.0 Procedure

6.1 Extraction of PUF Plug

Place the foam plug in a Soxhlet extractor, handling with forceps rather than hands.

NOTE: After sampling, the foam plugs should have been wrapped in aluminum foil until analysis. Use plugs carried to the field along with those employed for sampling as controls.

Extract with an appropriate volume of n-hexane-acetone-diethyl ether (47:47:6 v/v) for 8-12 hours at 8 cycles per hour with the smaller Soxhlet. Remove the boiling flask to a rotary evaporator and reduce the solvent volume to approximately 5 mL. Transfer the concentrate to a 15 mL graduated centrifuge tube with rinsing.

6.2 Analysis

6.2.1 Sample Preparation

Reduce the volume in the 15 mL tube to below 1 mL by careful evaporation under a gentle stream of nitrogen at room temperature. Carry out alumina cleanup as follows:

Place a small plug of pre-extracted glass wool in the Chromaflex column and wash with 10 mL of hexane. Pack the column with 10 cm of activity grade IV alumina. Transfer the sample from the centrifuge tube to the top of the column; rinse the tube three times with 1 ml portions of n-hexane, adding each rinse to the column. Elute the column at a rate of ca. 0.5 mL per

minute with 10 ml of n-hexane, collecting the eluate in a 15 mL centrifuge tube. Adjust the final volume of the eluate to 10 mL for gas chromatographic analysis.

When necessary, separate PCBs from technical chlordane by silicic acid chromatography as follows:

Place 3 grams of deactived silicic acid in a Chromatoflo column assembly. Wash the column with hexane. Place the sample, concentrated to less than 1 mL, on the column and add 130 mL of hexane to the reservoir. Apply nitrogen pressure to the column to increase the flow rate to ca. 1 mL/minute. Collect the eluate in three fractions: Fraction I (0-30 ml) contains all the HCB and Aroclor 1254 and most of the Aroclor 1242; Fraction II (31-50 mL) contains the remainder of Aroclor 1242, p,p'-DDE, some of the o,p'-DDT and toxaphene, and the early eluting peaks of technical chlordane; Fraction III (51-130 mL) contains the remainder of the technical chlordane, including all of the cis- and trans-chlordane, p,p'-DDT, and 30% of the toxaphene. Elute dieldrin, p,p'-DDD, 6% of the toxaphene, and the remaining pesticides with 15 mL of dichloromethane. Adjust the fraction volumes and analyze by GC. Blank values of unused plugs determined by extraction and alumina clean-up of the extract should be equivalent to <1 pg/m^3.

6.2.2 Gas Chromatography

Determine target pesticides on a 183 cm x 4 mm i.d. glass column packed with 1.5% OV-17/1.95 % OV-210 and/or 4% SE-30/6% OV-210 on 80-100 mesh Gas Chrom Q; column, 200°C; injection port, 215°C; nitrogen carrier gas, 60-85 mL/minute; electron capture detector.

Determine PCBs by EC-GC under the above conditions on a similar column packed with 3% OV-1 on Gas Chrom Q at 180°C. Alternatively, use columns containing 3% OV-225 on Supelcoport, 80-100 mesh or 4% SE-30/6% OV-210 on Gas Chrom Q, 100-200 mesh at 200°C.

Quantitate peaks in the usual way, i.e., by measuring peak heights to the nearest mm when the base width is <1 cm or via peak areas by integration or triangulation for broader peaks. Confirm results as required by combined GC/MS or some other appropriate procedure (EPA Pesticide Analytical Quality Control Manual, Chapter 8).

Commercial PCB mixtures are quantitated by comparisons of the total heights or areas of GC peaks with the corresponding peaks in the standard used. The absolute retention times on the 3% OV-1 column for the peaks used were as follows:

Aroclor 1242 - 2.39, 2.65, 3.11, 3.33, 3.94, 4.37, 4.67, 5.59, and 6.25 minutes

Aroclor 1254 - 3.81, 4.28, 4.61, 5.55, 6.68, 7.76, 8.23, 9.83, 11.47, and 13.67 minutes.

With the SE-30/OV-210 column, the total peak heights of the peaks shown in Figure 1 can be used for quantitation. (Make Aroclor standards by dissolving the Aroclor in isooctane, and prepare dilutions in hexane. Store stock solutions in brown bottles at -10°C. Remake working standards periodically from these and store in a refrigerator when not in use).

7.0 Quality Assurance Program

7.1 Quality Control

In-house control of all instruments, solvents, and procedures will follow the detailed guidelines specified in "Manual for Analytical Quality Control for Pesticides and Related Compounds in Human and Environmental Samples", EPA Report No. EPA-600/1-79-008.

Blank PUF plugs and plugs loaded with known amounts of standards are prepared for each sampling trip. A portion of the samples are designated "lab blanks/controls" and remain in the laboratory; a futher portion are designated "field blanks/controls", and are carried to the field in the same containers as the sample plugs. This procedure not only provides a check on possible contamination during transport and storage, but also sllows calculation of overall recoveries during the storage and analysis phases.

7.2 Quality Assurance

A specified number of duplicate samples (Table 6-7, Part I of this Work Plan) will be provided to a separate laboratory (HERL/RTP) for analysis. The results will be compared with those obtained at the primary lab (RTI).

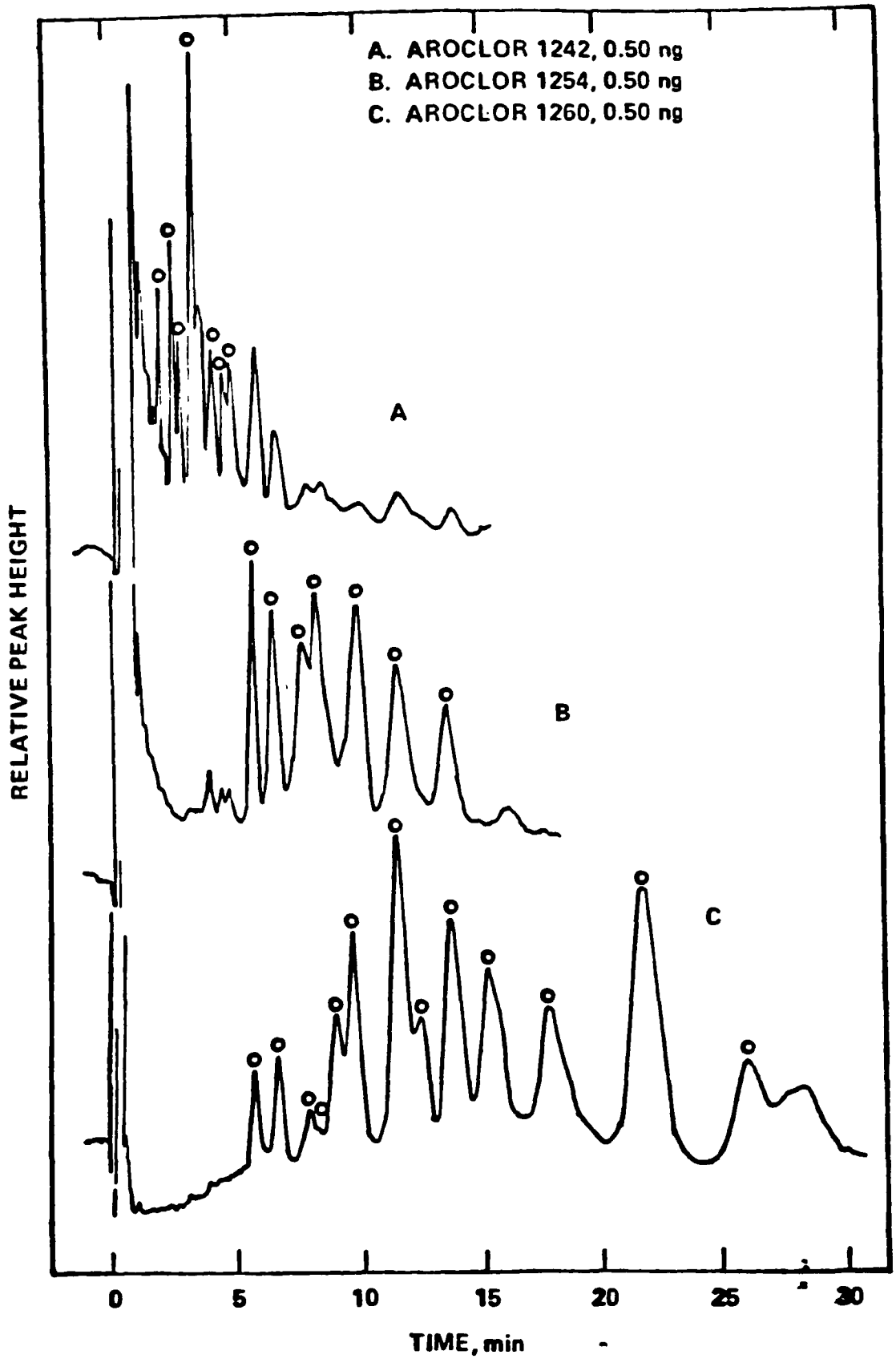


Figure 1. Chromatograms showing peaks used in quantifying PCB, SE-30/OV-210.

ANALYTICAL PROTOCOL: VOLATILE ORGANOCHLORIDES AND BENZENE BY THE PURGE AND TRAP METHOD

1.0 Principle of the Method

This method is applicable in the determination of the halogenated compounds listed in Table 1 contained in carbon filtered drinking water or raw source water. It is also applicable for the determination of benzene.

Organohalides are extracted by an inert gas which is bubbled through the aqueous sample. The organohalides, noted in Table 1 along with other organic constituents which exhibit low water solubility and boil less than 200°C, are efficiently transferred from the aqueous phase to the gaseous phase. These compounds are swept from the purging device and are trapped in a short column containing a carefully selected sorbent combination. After a predetermined period of time, the trapped components are thermally desorbed and backflushed onto the head of a gas chromatographic column and separated under programmed conditions. The effluent is split and measurement is accomplished with both a halogen specific detector, which eliminates interference problems commonly encountered with universal or semispecific detectors, and a flame ionization or photoionization detector. The extraction/concentration technique enhances the quantities of organohalides injected into the gas chromatograph by a factor of 1000 over direct injection gas chromatography.

This method is recommended for use only by analysts experienced in the measurement of purgeable organics at the µg/L level or by experienced technicians under the close supervision of a qualified analyst.

2.0 Range and Detection Limit

The actual detection limits are highly dependent upon the ability of the analyst to properly maintain the entire analytical system. Using carefully optimized equipment the method has been proven to be useful for the detection and measurement of multicomponent mixtures spiked into carbon-filtered finished water and raw source water at concentrations between 0.2 and 0.4 µg/L. The method as described is capable of accurately measuring those compounds mentioned in Table 1 over a concentration range of 0.10 to 5.0 µg/L. Additionally, it is possible to measure

Table 1. ORGANOHALIDES TESTED USING PURGE AND TRAP METHOD

Compound	Retention Time (SEC) Column I	Column II	Estimated [A] lower Limit of detection ug/l
chloromethane	90	317	0.009
bromomethane	130	423	0.03
dichlorodifloromethane	157	?	0.03
vinyl chloride	160	317	0.01
chloroethane	200	521	0.01
dichloromethane	315	607	0.01
fluorotrichloromethane	431	?	0.01
allylchloride	475	?	0.02
1,1-dichloroethylene	476	463	0.006
bromochloromethane	509	760	0.02
1,1-dichloroethane	558	754	0.004
cistrans 1,2-dichloro-ethylene	605	563	0.006
cis-1,2-dichloro-ethylene	605	726	0.006
chloroform	641	725	0.006
1,2-dichloroethane	684	921	0.006
dibromomethane	698	895	0.03
1,1,1-trichloroethane	756	786	0.005
carbon tetrachloride	781	664	0.007
bromodichloromethane	819	877	0.006
2,3-dichloropropene	891	?	0.007
1,2-dichloropropane	895	997	0.004
1,1-dichloropropene	904	?	0.01
trans-1,3 dichloropropene	913	997	0.006
1,1,2-TriChloroEthylene	948	787	0.005
1,3-dichloropropane	973	?	0.01
chlorodibromomethane	989	997	0.01
1,1,2-trichloroethane	991	1084	0.006
cis-1,3-dichloropropene	992	1078	0.008
1,2-dibromoethane	1046	1131	0.01
2-chloroethylethyl ether	1056	?	0.03
2-chloroethylvinyl ether	1080	?	0.06
bromoform	1154	1150	0.02
1,1,1,2-tetrachloroethane	1163	1302	0.003
1,2,3-trichloropropane	1279	?	0.007
chlorocyclohexane	1283	?	0.01
1,1,2,2-tetrachloroethane	1297	?	0.006
1,1,2,2-tetrachloroethylene	1300	898	0.007
pentachloroethane	1300	?	0.01
1-chlorocyclohexene-1	1345	1186	0.03
chlorobenzene	1451	1130	0.03

(continued)

Table 1 (cont'd.)

1-chlorohexane	1499	1229	0.05
bis-2-chloroethylether	1500	?	0.2
bromobenzene	1626	?	0.07
o-chlorotoluene	1927	1320	0.07
bis-2-chloroisopropyl ether	1931	?	0.04
m-dichlorobenzene	2042	1346	0.04
o-dichlorobenzene	2094	1411	0.04
p-dichlorobenzene	2127	1340	0.04

A = See 10.3

individual organohalides up to 1500 µg/L. However, the ability to measure complex mixtures containing co-eluting or partially resolved organohalides with concentration differences larger than a factor of 10 is hampered.

3.0 Interferences

Impurities contained in the purge gas and organic compounds out-gasing from the plumbing ahead of the trap usually account for the majority of contamination problems. The presence of such interferences is easily monitored using the quality control program described herein. Sample blanks are normally run between each set of samples. When a positive organohalide reponse is noted in the sample blank, the analyst should analyze a method blank in order to identify the source of contamination. Method blanks are run by charging the purging device with organic-free water and analyzing it in the normal manner.

Whenever organohalides are noted in the method blank, the analyst should change the purge gas source and regenerate the molecular sieve purge gas filter. Subtracting blank values from sample results is not recommended. The use of non-TFE plastic tubing, non-TFE thread sealants, or flow controllers with rubber components in the purging device should be avoided since such materials out-gas organic compounds which will be concentrated in the trap during the purge operation. Such out-gasing problems are common whenever new equipment is put into service. With use, minor out-gasing problems generally cure themselves.

Several instances of accidental sample contamination have been noted and attributed to diffusion of volatile organics through the septum seal and into the sample during shipment and storage. The sample blank is used as a monitor for this problem.

For compounds that are not efficiently purged, such as bromoform, small variations in sample volume, purge time, purge flow rate, purging device geometry, or purge temperature can effect the analytical result. Therefore, samples and standards must be analyzed under identical conditions.

Cross contamination can occur whenever high level and low level samples are sequentially analyzed. To reduce the liklihood of this, the

purging device and sample syringe should be rinsed out twice between samples with organic-free water. Whenever an unusually concentrated sample is encountered, it is necessary that it be followed by a sample blank analysis to check for sample cross contamination. For samples containing large amounts of water soluble materials, suspended solids, high boiling compounds or high organohalide levels it may be necessary to wash out the purging device with a soap solution, rinse with distilled water, and then dry in a 105°C oven between analyses.

Qualitative misidentifications are a problem in gas chromatographic analysis. Whenever samples whose qualitative nature is unknown are analyzed, the following precautionary measures should be incorporated into the analysis.

1. Perform duplicate analyses using the two recommended columns which provide different retention order and retention times for many organohalides.
2. Whenever possible use GC/MS techniques which provide unequivocal qualitative identifications.

4.0 Precision and Accuracy

4.1 Control Samples

Both Ohio River water (ORW) and carbon-filtered tap (CFT) water were spiked with known amounts of organohalides. The spiked solutions were then sealed in septum-seal vials both with and without sodium thiosulfate (thio) and sodium sulfite (sulfite) then stored on the bench top for up to four weeks. Samples were randomly analyzed on four occasions. When matrix effects were noted or suspected then that data was not included in the following single laboratory precision and accuracy statement. Table 2 shows the accuracy and precision data obtained from this study.

4.2 Blank Samples

Organic-free water was dosed with mixtures of organohalides. The dosed water was used to fill septum seal vials which were stored under ambient conditions. The dosed samples were randomly analyzed over a 2-week period of time. The data listed in Table 3 reflect the errors due to the analytical procedure and storage.

Table 2. SINGLE LAB ACCURACY AND PRECISION FOR PURGE AND TRAP METHOD
HALL 700A ELECTROLYTIC CONDUCTIVITY DETECTOR

Compound	Spike Level µg/ℓ	C.F.T.	O.R.W.	Preserved Sample Thio. Sulfide	Mean % Recovery	Number of Samples	Std. Deviation	Rel. Std. Deviation	Maximum Holding Time (Days)
chloromethane	0.40	yes[C]	yes[C]	yes[A,B,C]	93	16	.034	8.5	21
bromomethane	0.40	yes[C]	yes[C]	yes[C]	85	8	.025	6.3	2
vinyl chloride	0.20	no	yes	no	110	12	.029	15	6
dichlorodifluoromethane	0.40	yes	yes	yes	103	12	.081	20	27
chloroethane	0.40	yes	yes	yes	93	20	.071	18	21
dichloromethane	0.20	no	yes	no	85	17	.024	12	27
fluorotrichloromethane	0.40	yes	yes	yes[A]	90	21	.037	9.3	27
1,1-dichloroethylene	0.40	yes[C]	yes[C]	yes[C]	88	18	.037	9.3	27
allylchloride	0.40	yes[C]	yes[C]	yes[C]	85	8	.046	12	2
bromochloromethane	0.40	yes	yes	yes	90	19	.038	9.5	21
1,1-dichloroethane	0.20	no	yes	no	95	17	.012	6.0	27
cis+trans-1,2-dichloroethylene	0.20	no	yes	no	95	17	.011	5.5	27
cis-1,2-dichloroethylene	0.40	yes	yes	yes	88	20	.028	7.0	21
1,2-dichloroethane	0.20	no	yes	no	110	17	.014	7.0	27
dibromomethane	0.40	yes	yes	yes	100	5	.032	8.0	21
1,1,1-trichloroethane	0.40	yes	yes	yes	93	20	.032	8.0	21
carbon tetrachloride	0.20	no	yes	no	90	17	.014	7.0	27
bromodichloromethane	0.20	no	yes	no	100	17	.013	6.5	27
2,3-dichloropropene	0.20	no	yesC	no	95	14	.012	6.0	6
1,2-dichloropropane	0.40	yes	yes	yes[A]	95	20	.014	3.5	21
1,1-dichloropropene	0.40	yes	yes	yes[C]	88	18	.037	9.3	27
trans-1,3 dichloropropene	0.40	yes[C]	yes[C]	yes[C]	88	4	.000	000	1
cis-1,3-dichloropropene	0.40	yes[C]	yes[C]	yes[C]	90	4	.050	12.5	1
1,1,2-trichloroethane	0.40	yes	yes	yes	95	15	.024	6.0	27
1,3-dichloropropane	0.40	yes	yes	yes	98	21	.026	6.5	27

(continued)

Table 2 (cont'd.)

Compound	Spike Level µg/ℓ	C.F.T.	O.R.W.	Preserved Sample Thio. Sulfide	Mean % Recovery	Number of Samples	Std. Deviation	Rel. Std. Deviation	Maximum Holding Time (Days)
chlorodibromomethane	0.20	no	yes	no	95	17	.014	7.0	27
1,1,2-trichloroethylene	0.20	no	yes	no[B]	94	17	.012	6.0	27
1,2-dibromoethane	0.40	yes	yes	yes[A]	93	18	.050	12.5	21
2-chloroethylether ether	0.40	yes	yes	yes	95	18	.030	7.5	27
2-chloroethylvinyl ether	0.40	yes	yes	yes	100	21	.031	7.8	27
bromoform	0.20	no	yes	no	95	17	.030	15.0	27
1,1,1,2-tetrachloro-ethane	0.40	yes	yes	yes	93	20	.032	8.0	21
1,2,3-trichloropropane	0.40	yes	yes	yes	100	20	.038	9.5	21
chlorocyclohexane	0.40	yes	yes	yes	93	21	.033	8.3	27
1,1,2,2-tetrachloro-ethane	0.40	yes	yes	yes[A]	95	18	.036	9.0	21
1,1,2,2-tetrachloro-ethylene	0.20	no	yes	no	90	17	.019	9.5	27
pentachloroethane	0.40	yes	yes	yes	98	21	.039	9.8	27
1-chlorocyclohexene-1	0.40	yes	yes	yes	93	21	.051	12.8	27
chlorobenzene	0.40	yes[C]	yes[C]	yes[C]	88	18	.037	9.3	21
1-chlorohexane	0.40	yes[C]	yes[C]	yes[C]	83	4	.022	5.5	1
bis-2-chloroethylether	0.40	yes	yes	yes	100	16	.065	16.	9
bromobenzene	0.40	yes	yes	yes	93	20	.047	12	21
o-chlorotoluene	0.40	yes	yes	yes	85	20	.037	9.3	21
bis-2-chloroisopropyl ether	0.40	yes	yes	yes	125	21	.11	28.	27
m-dichlorobenzene	0.40	yes	yes	yes	95	21	.033	8.3	27
o-dichlorobenzene	0.40	yes	yes	yes	95	21	.053	13.	27
p-dichlorobenzene	0.40	yes	yes	yes	90	20	.051	13.	21

A - matrix effect noted due to the presence of sodium sulfite
B - matrix effect noted due to the presence of sodium thiosulfate
C - matrix effect noted due to the sample storage (recommended storage time noted in maximum age column)

Table 3. SINGLE LABORATORY ACCURACY AND PRECISION FOR TRIHALOMETHANES HALL 700 ELECTROLYTIC CONDUCTIVITY DETECTOR

Dose (ug/l)	Number samples	Mean (ug/l)	Standard deviation
Chloroform			
1.19	12	1.21	0.14
11.9	8	11.3	0.16
119	11	105	7.9
Bromodichloromethane			
1.60	12	1.52	0.05
16.0	8	15.1	0.39
160	11	145	10.2
Chlorodibromomethane			
1.96	12	1.91	0.09
19.6	8	19.1	0.70
196	11	185	10.6
Bromoform			
2.31	12	2.33	0.16
23.1	8	22.5	1.38
231	11	223	16.3

5.0 Apparatus and Reagents

5.1 Purge and Trap Device

The purge and trap equipment consists of three separate pieces of apparatus: the purging device, trap, and desorber. Construction details for a purging device and an easily automated trap-desorber hybrid which has proven to be exceptionally efficient and reproducible are shown in Figures 1 through 4.

5.1.1 Purging Device

Construction details are given in Figure 1 for an all-glass 5 mL purging device. The glass frit installed at the base of the sample chamber allows finely divided gas bubbles to pass through the sample while the sample is restrained above the frit. Gaseous volumes above the sample are kept to a minimum to eliminate dead volume effects, yet allowing sufficient space for most foams to disperse. The inlet and exit ports are constructed from heavy walled 1/4 inch glass tubing so that leak-free removable connections can be made using "finger-tight" compression fittings containing Teflon ferrules. The removable foam trap is used to control samples that foam. Purging device design has been found to be critical for low level analyses. For this reason variations from Figure 1 will require method revalidation.

5.1.2 Trapping Device

The trap (Figure 2) is a short gas chromatographic column which at 22°C retards the flow of the compounds of interest while venting the purge gas. The trap is constructed with a low thermal mass so that it can be rapidly heated for efficient desorption, then rapidly cooled to room temperature for recycling. Variations in the trap ID, wall thickness, sorbents, sorbent packing order, and sorbent mass adversely affect the trapping and desorption efficiencies for certain compounds shown in Table 1. For this reason, any changes in the trap design will require method revalidation. Pack the trap according to Figure 2. In order to function properly the trap must be packed in the following order: Place the glass wool plug in the inlet end of the trap, follow with the OV-1, Tenax, silica gel, charcoal, and finally, the second glass wool plug.

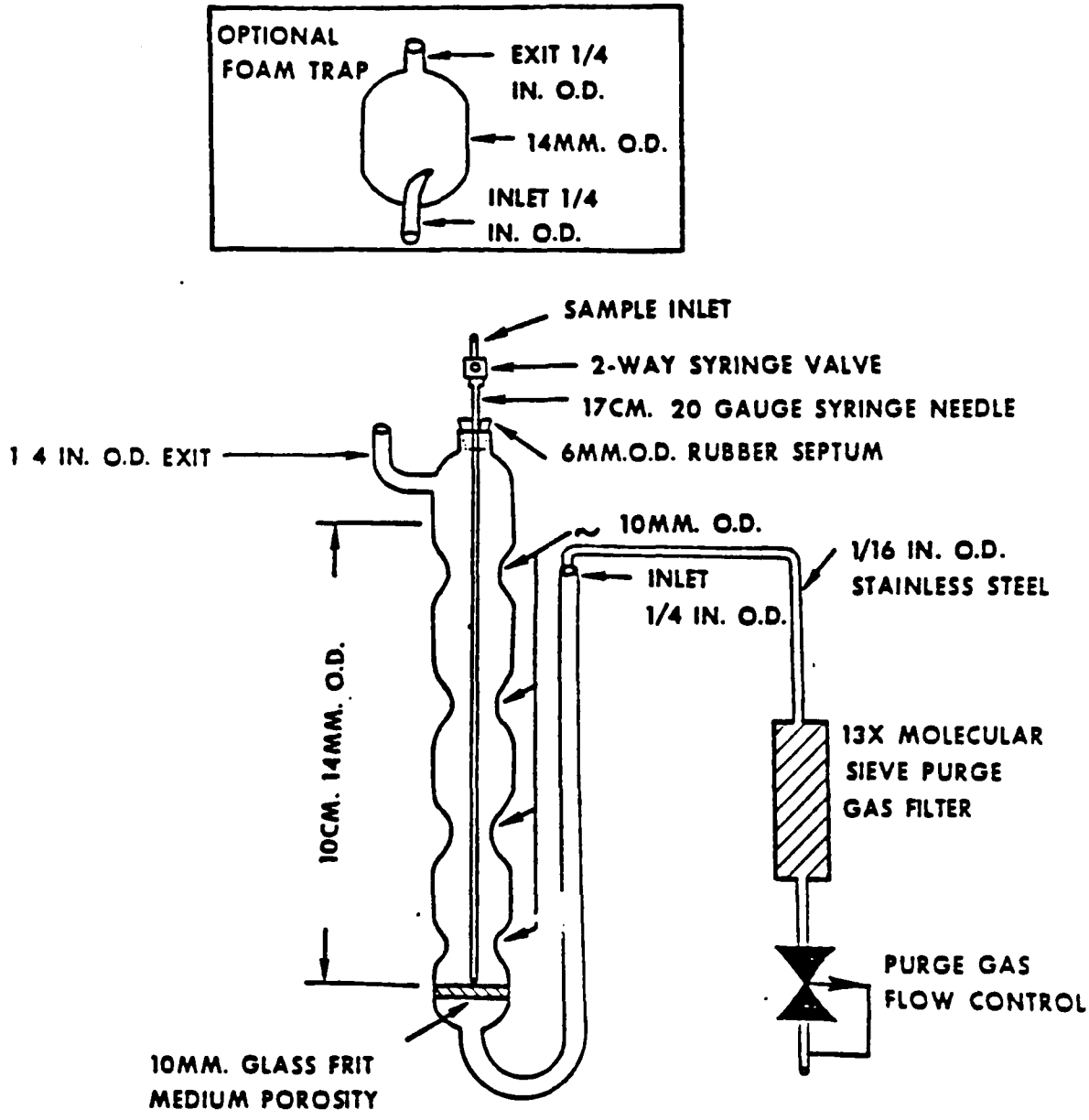


Figure 1. Purging device.

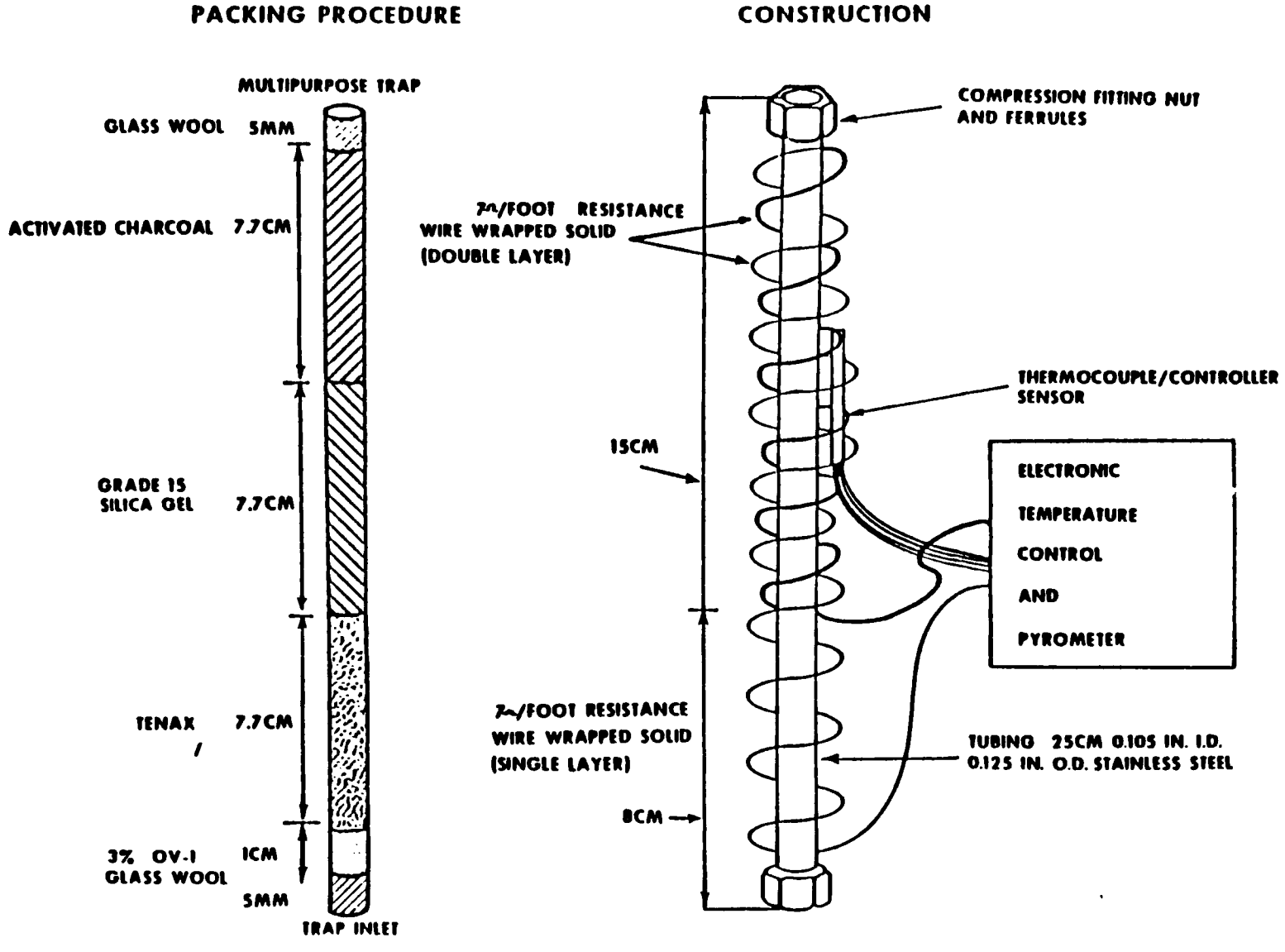


Figure 2. Trap.

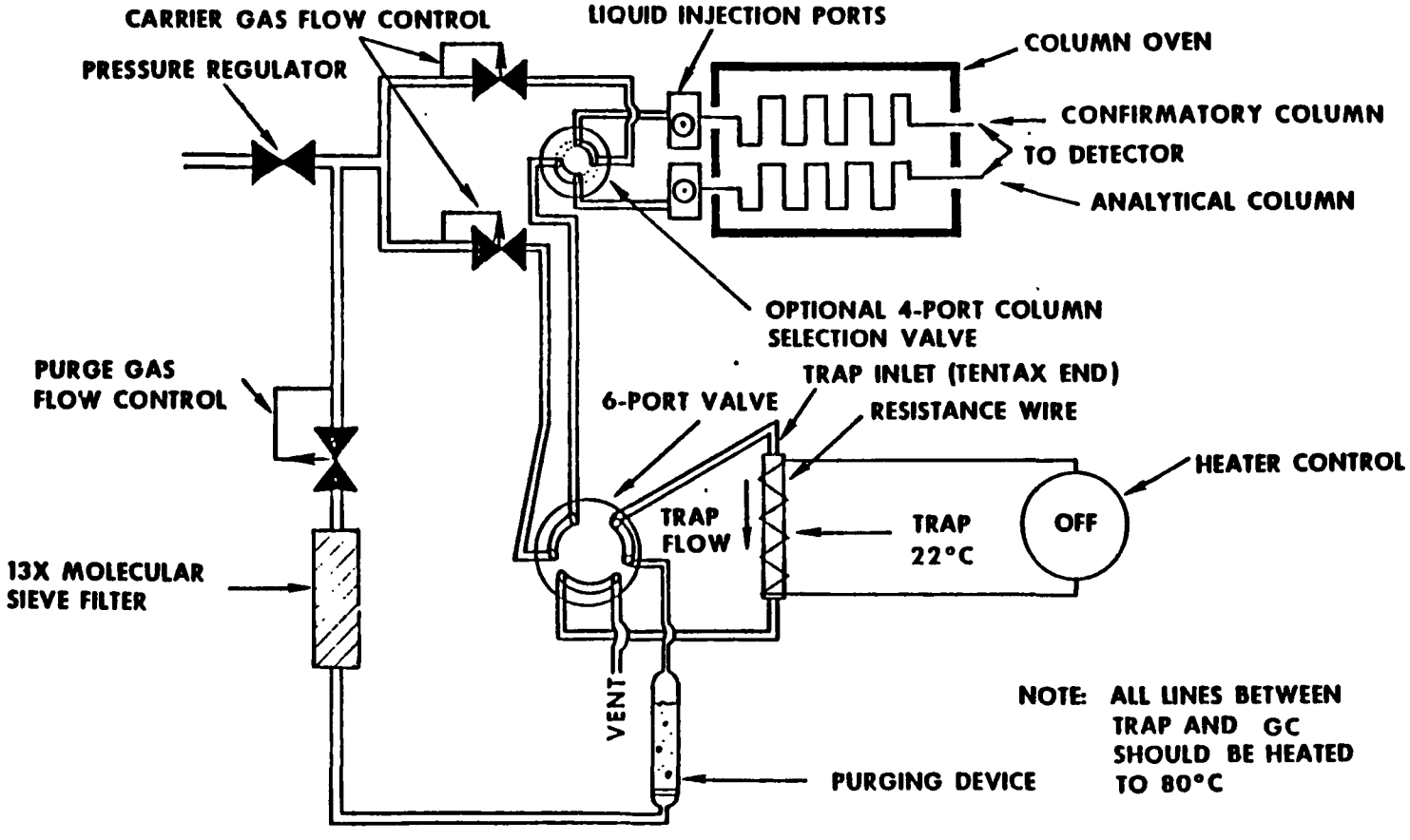


Figure 3. Purge-trap system (purge-sorb mode).

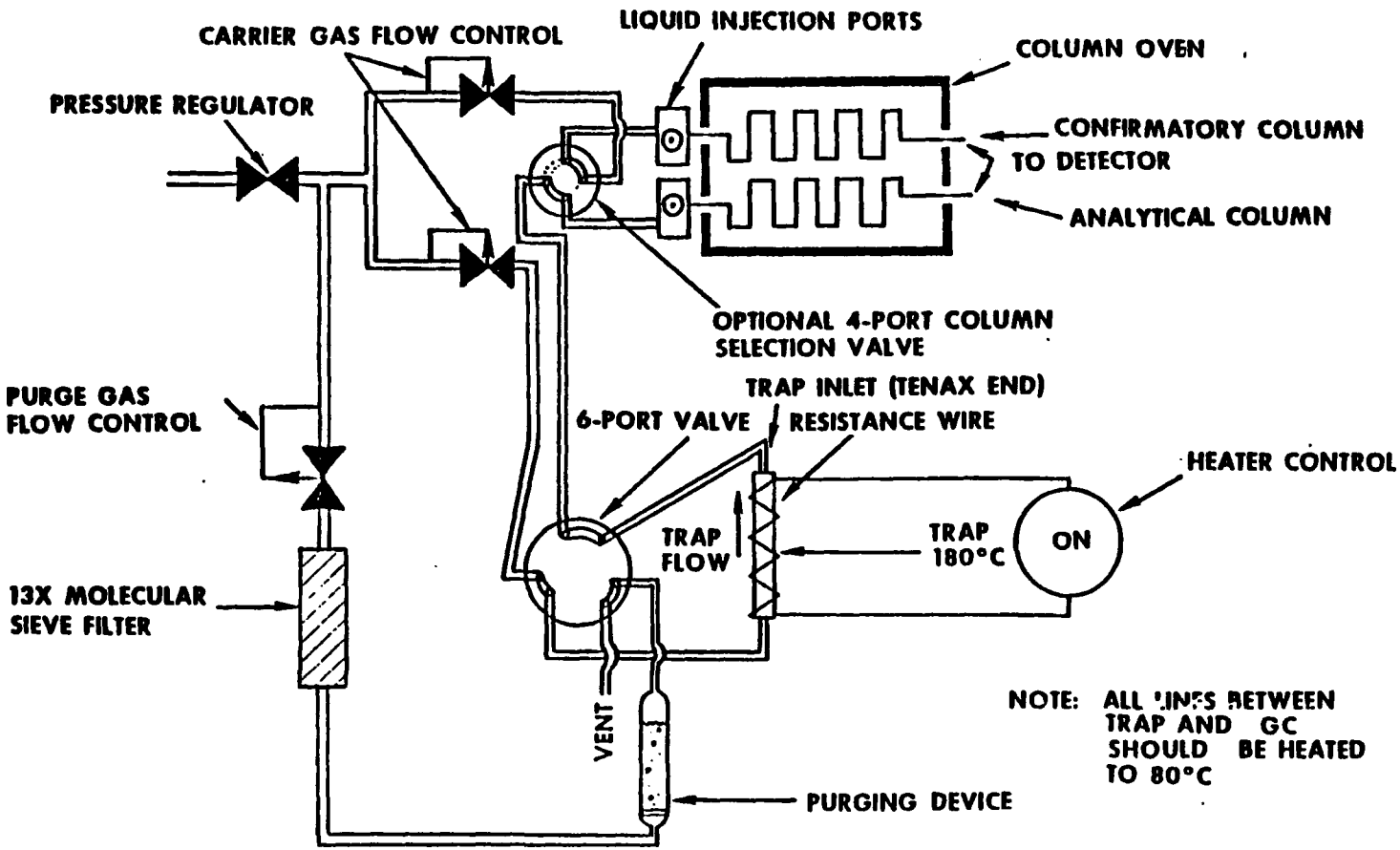


Figure 4. Purge-trap system (desorb mode).

Reversing the packing order (placing the charcoal in the trap first) will cause the silica gel and Tenax layers to become contaminated with charcoal dust causing poor desorption efficiencies. Be sure to install the trap so that the effluent from the purging device enters the Tenax end of the Trap.

5.1.3 Desorber Assembly

Details for the desorber are shown in Figures 3 and 4. With the 6-port valve in the Purge-Sorb position (See Figure 3), the effluent from the purging device passes through the trap where the flow rate of the organics is retarded. The GC carrier gas also passes through the 6-port valve and is returned to the GC. With the 6-port valve in the Purge-Sorb position, the operation of the GC is in no way impaired; therefore, routine liquid injection analyses can be performed using the gas chromatograph in this mode. After the sample has been purged, the 6-port valve is turned to the desorb position (See Figure 4). In this configuration, the trap is coupled in series with the gas chromatographic column allowing the carrier gas to back-flush the trapped materials into the analytical column. Just as the valve is actuated the power is turned on to the resistance wire wrapped around the trap. The power is supplied by an electronic temperature controller. Using this device, the silica gel/charcoal area of the trap is rapidly heated to 180°C with minimal temperature overshoot and then maintained at 180°C. The trapped compounds are released as a "plug" to the gas chromatograph by this heat and backflush step. Normally, packed columns with theoretical efficiencies near 500 plates/foot under programmed temperature conditions can accept such desorb injections without altering peak geometry. Substituting a non-controlled power supply, such as a manually-operated variable transformer, will provide non-reproducible retention times and poor quantitative data unless Injection Procedure is used.

A commercial device manufactured by Tekmar has been tested and shown to be equivalent: Tekmar, P. O. Box 37202, Cincinnati, Ohio 45202. This device or its equivalent may be used as long as the proper trap and purging device are installed on the instrument.

5.2 Gas Chromatograph

The chromatograph must be temperature programmable and equipped with a halide specific detector.

5.2.1 Column I is a highly efficient column which provides outstanding separations for a wide variety of organic compounds. Because of its ability to resolve complex mixtures of organochlorine compounds, Column I should be used as the primary analytical column (See Figure 5).

5.2.1.1 Column I parameters: Dimensions - eight feet long x 0.1 inch ID stainless steel or glass tubing. Packing - 1% SP-1000 on Carbopack-B (60/80) mesh. Carrier Gas - helium at 40 mL/minute. Temperature program sequence: 45°C isothermal for 3 minutes, program at 8°C/minute to 220°C then hold for 15 minutes or until all compounds have eluted. NOTE: It has been found that during handling, packing, and programming, active sites are exposed on the Carbopack-B packing. This results in tailing peak geometry and poor resolution of many constituents. To correct this, pack the first 5 cm of the column with 3% SP-1000 on Chromosorb-W 60/80 followed by the Carbopack-B Packing. Condition the precolumn and the Carbopack columns with carrier gas flow at 220°C overnight. Pneumatic shocks and rough treatment of packed columns will cause excessive fracturing of the Carbopack. If pressure in excess of 60 psi is required to obtain 40 mL/minute carrier flow then the column should be repacked.

5.2.2 Column II provides unique organohalide-separations when compared to those obtained from Column I (see Figure 6). However, since the resolution between various compounds is generally not as good as those with Column I, it is recommended that column II be used as a qualitative confirmatory column for unknown samples when GC/MS confirmation is not possible.

5.2.2.1 Column II parameters: Dimensions: six feet long x 0.1 inch ID stainless steel or glass. Packing: n-octane on Porasil-C (100/120 mesh). Carrier Gas: helium at 40 mL/minute. Temperature program sequence: 50°C isothermal for 3 minutes, program at 6°/minutes to 170°C, then hold for 4 minutes or until all compounds have eluted.

5.2.3 Detector - A halogen specific detector must be used in order to eliminate misidentifications due to non-organohalides which are coextracted during the purge step.

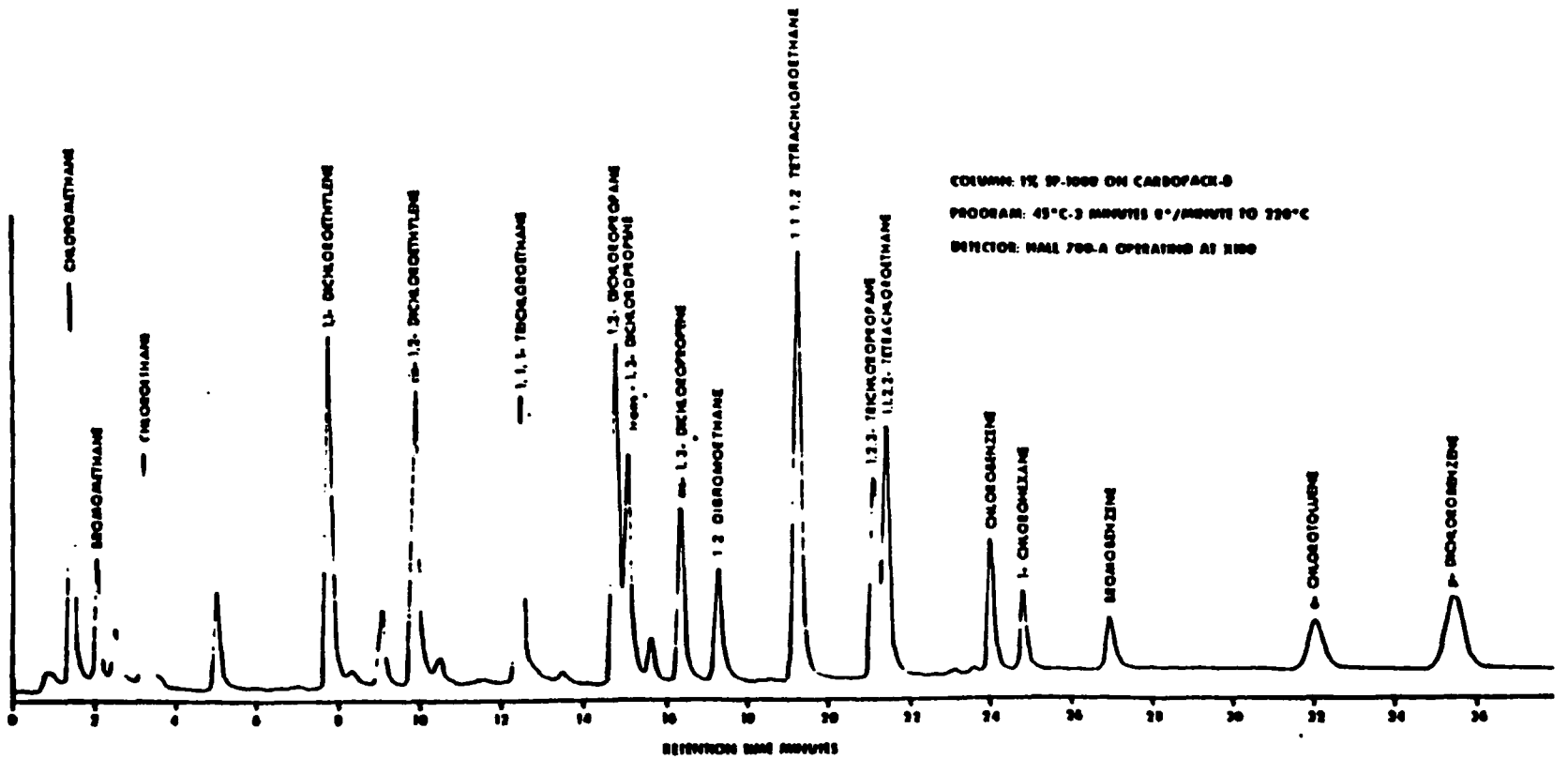


Figure 5. Chromatogram of 0.4 µg/L standard.

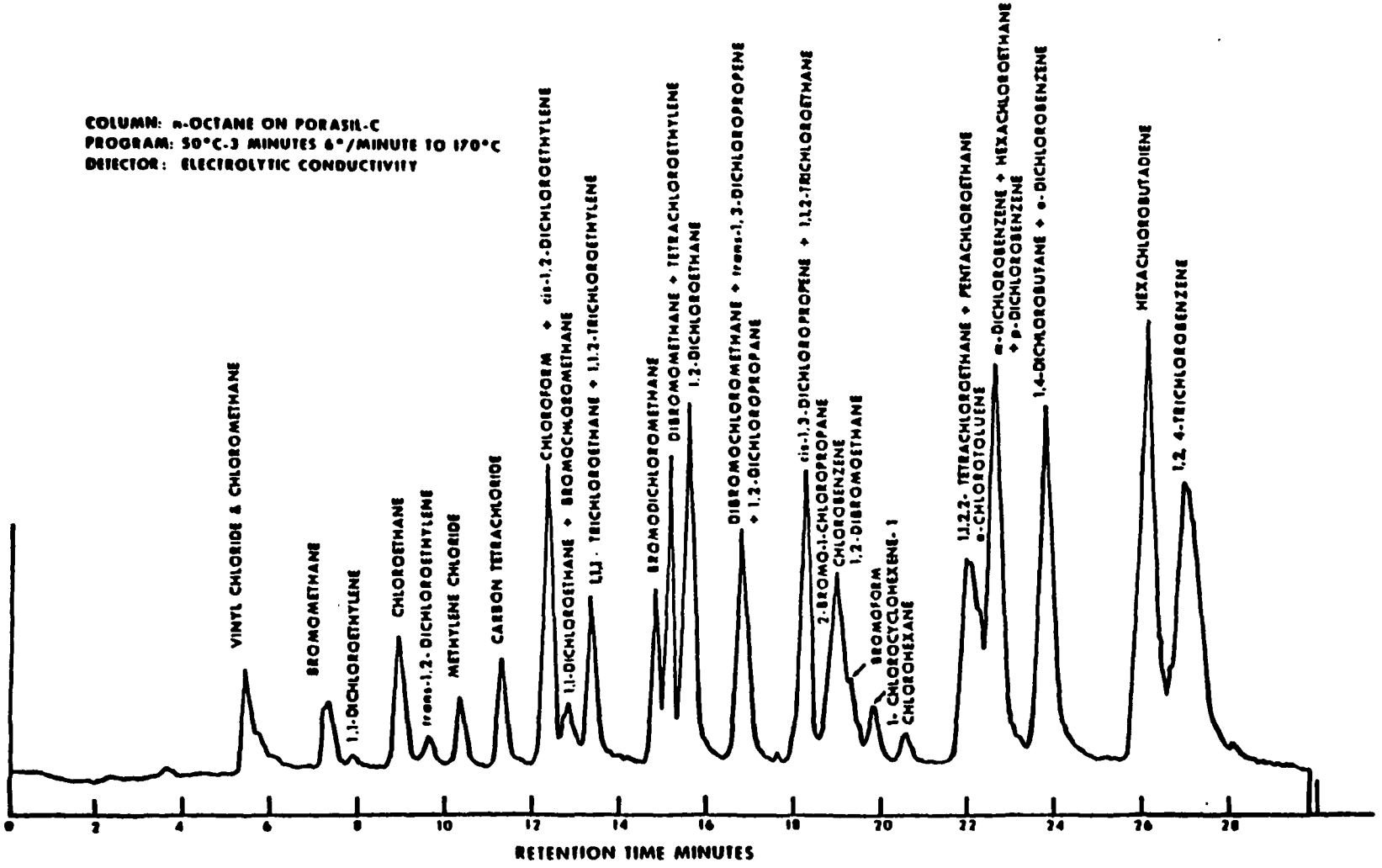


Figure 6. Chromatogram of organohalides.

5.2.3.1 A Hall model 700-A available from Tracor has been tested and found to provide the sensitivity needed to produce meaningful analyses down to 0.10 µg/L for most organohalides with a relative standard deviation of less than 10%.

Operating conditions for Hall 700-A Detector:

Reactor tube: nickel 1/16" O.D.

Reactor temperature: 810°C

Reactor base temperature: 225°C

Electrolyte: 100% n-propyl alcohol

Electrolyte flow rate: 0.8 mL/minute

Reaction gas: hydrogen at 40 mL/minute

Carrier gas: helium at 40 mL/minute

5.2.3.2 Other halogen specific detectors including electrolytic conductivity and microcoulometric titration can be used. However, the stability and sensitivity of these detectors limit the method to measurements down to 1.0 µg/L with a relative standard deviation near 10%.

5.3 Sample Containers - 40 mL screw cap vials sealed with Teflon faced silicone septa.

Vials and caps - Pierce #13075 or equivalent

Septa - Pierce #12722 or equilvalent

5.4 Syringes - 5 mL hypodermic with luerlok tip (2 each).

5.5 Micro syringes- 10, 100 µL.

5.6 Micro syringe - 25 µL with a 2" by 0.006 inch I.D. needle (Hamilton #702N or equilvalent).

5.7 2-way syringe valve with Luer ends (3 each).

5.8 Modified 500 and 1000 mL volumetric flasks. See Figure 7.

5.9 Syringe - 5 mL gas-tight with shut-off valve.

5.10 Trap Materials

5.10.1 Porous polymer packing 60/80 mesh chromatographic grade Tenax GC (2,6-diphenylene oxide).

5.10.2 OV-1 (3%) on Chromosorb-W 60/80 mesh.

5.10.3 Silica gel-(35/60 mesh) - Davison, grade-15 or equivalent.

5.10.4 Coconut charcoal (26 mesh) Barnaby Chaney, CA-580-26 lot #M-2649 or equilvalent.

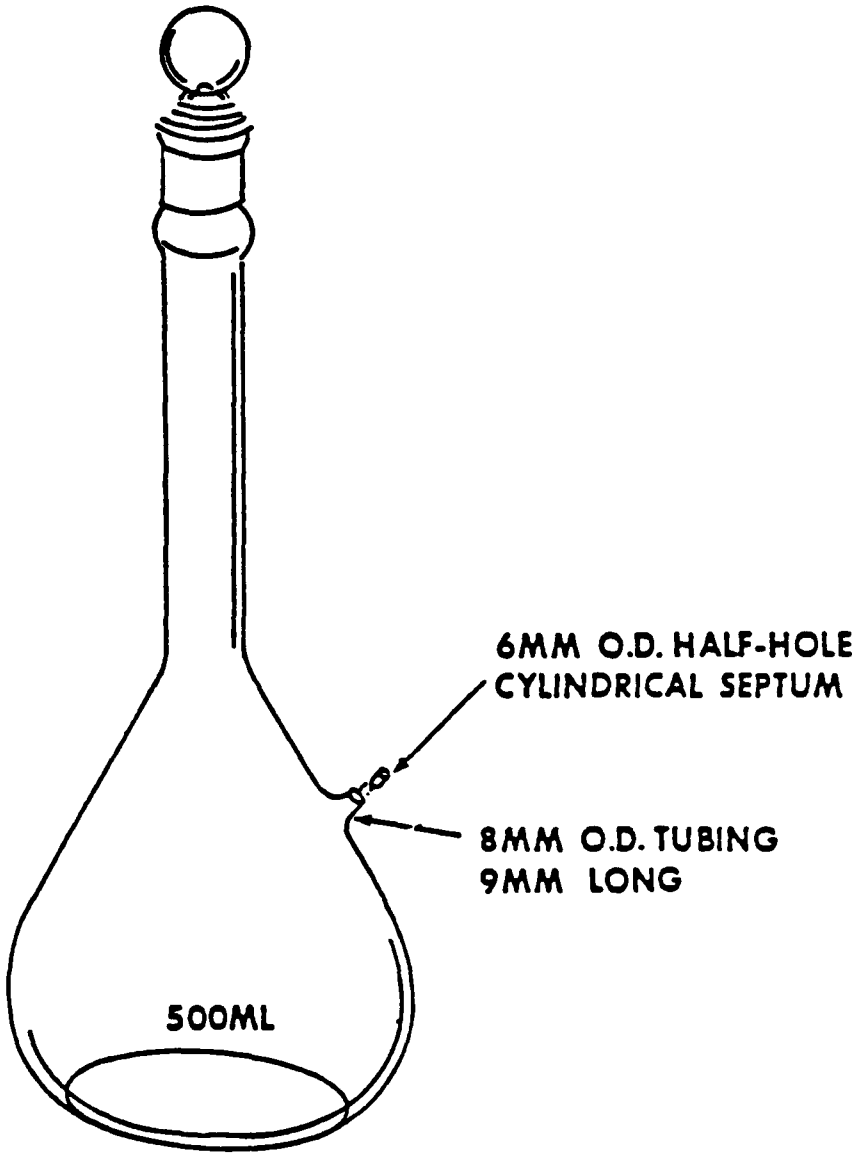


Figure 7. Modified volumetric flask.

5.11 SP-1000 (1%) on Carbopack-B 60/80 mesh available from Supelco.

5.12 n-Octane on Porasil-C (100/120 mesh) avilable from Waters Associates.

5.13 SP-1000 (3%) on Chromosorb-W (60/80 mesh).

5.14 Dechlorinating compound-crystalline sodium thiosulfate, A.C.S. Reagent Grade.

5.15 Activated carbon (for preparation of organic-free water) - Filtra sorb-200, available from Calgon Corp., Pittsburgh, PA, or equivalent.

5.16 Organic-free water

5.16.1 Organic-free water is defined as water free of interference when employed in the purge and trap procedure described herein. It is generated by passing tap water through a carbon filter bed containing about 1 lb. of activated carbon.

5.16.2 A Millipore Super-Q Water System or its equivalent may be used to generate organic-free deionized water.

5.16.3 Organic-free water may also be prepared by boiling water for 15 minutes. Subsequently, while maintaining the temperature at 90°C bubble a contaminant free inert gas through the water for one hour. While still hot, transfer the water to a narrow mouth screw cap bottle with a Teflon seal. NOTE: Test organic free water daily by analyzing according to paragraph 8.

5.17 Standards

5.17.1 Obtain 97% pure reagent grade reference standards.

5.18 Standard Stock Solutions (compounds boiling above room temperature).

NOTE: Because of the toxicity of organohalides, it is necessary to prepare primary dilutions in a hood. It is further recommended that a NIOSH/MESA approved toxic gas respirator be used when the analyst handles high concentrations of such materials.

5.18.1 Place about 9.8 mL of methyl alcohol into a 10 mL a ground glass stoppered volumetric flask.

5.18.2 Allow the flask to stand, unstoppered, for about 10 minutes or until all alcohol wetted surfaces have dried.

5.18.3 Weigh the flask to the nearest 0.1 mg.

5.18.4 Using a 100 µL syringe, immediately add 2 drops of the reference standard to flask, then reweigh. Be sure that the 2 drops fall directly into the alcohol without contacting the neck of the flask.

5.18.5 Dilute to volume, stopper, then mix by inverting the flask several times.

5.18.6 Calculate the concentration in micrograms per microliter from the net gain in weight.

5.18.7 Transfer the standard solution to a 10 mL screw-cap bottle with a Teflon cap liner.

5.18.8 Store the solution at 4°C.

NOTE: With the exception of 2-chloroethylvinyl ether standard solutions prepared in methyl alcohol are stable up to 4 weeks when stored under these conditions. They should be discarded after that time has elapsed.

5.19 Standard Stock Solutions (Gaseous Compounds)

5.19.1 Place about 9.8 mL of methyl alcohol into a 10.0 mL ground glass stoppered volumetric flask.

5.19.2 Allow the flask to stand unstoppered about 10 minutes or until all alcohol wetted surfaces have dried.

5.19.3 Weigh to the nearest 0.1 mg.

5.19.4 Fill a 5 mL valved gas-tight syringe with the reference standard to the 5.0 mL mark.

5.19.5 Lower the needle to 5 mm above the methyl alcohol menicus.

5.19.6 Slowly inject the reference standard into the neck of flask (the gas will rapidly dissolve into the methyl alcohol).

5.19.7 Immediately reweigh the flask to the nearest 0.1 mg.

5.19.8 Dilute to volume, stopper, then mix by inverting the flask several times.

5.19.9 Transfer the standard solution to a 10 mL screw - Cap bottle with a Teflon cap-liner.

5.19.10 Store stock solutions at 0°C.

5.19.11 Stock solutions prepared from gaseous compounds are generally not stable for periods exceeding 1 week. They should be discarded after that time.

5.19.13 Calculate the concentration in micrograms per microliter from the net gain in weight.

5.20 Calibration Standards

5.20.1 In order to prepare accurate aqueous standard solutions the following precautions must be observed.

a. Do not inject more than 20 µL of alcoholic standards into 100 mL of organic-free water.

b. Use a 25 µLl Hamilton 702N microsyringe or equivalent. (Variations in needle geometry will adversely effect the ability to deliver reproducible volumes of methanolic standards into water).

c. Rapidly inject the alcoholic standard into the expanded area of the filled volumetric flask. Figure 7. Remove the needle as fast as possible after injection.

d. Mix aqueou standards by inverting the flask three times only.

e. Discard the contents contained in the neck of the flask. Fill the sample syringe from the standard solution contained in the expanded area of the flask as directed in paragraph 8.5.

f. Never use pipets to dilute or transfer samples or aqueous standards.

g. Aqueous standards are not stable and should be

h. Aqueous standards are not stable and should be discarded after one hour unless stored and sealed according to 6.4.

5.20.2 Prepare, from the standard stock solutions, secondary dilution mixtures in methyl alcohol so that a 20 µL injection into 100, 500, or 1000 mL of organic-free water will generate a calibration standard which produces a response close (± 10%) to that of the unknowns.

5.20.3 Purge and analyze the aqueous calibration standards in the same manner as the unknowns.

5.21 Quality Check Standard (0.40 µg/L)

5.21.1 From the standard stock solutions, prepare a secondary dilution in methyl alcohol containing 10 ng/µL of each compound normally monitored. NOTE: It may be necessary to prepare two or more quality check standards so that all of the compounds in each mixture are adequately resolved for quantitative measure ment.

5.21.2 Daily, inject 20.0 µL of this mixture into 500 mL of organic-free water and analyze according to the Procedure Section 6.

6.0 Procedure

6.1 Sample Collection and Handling

6.1.1 The sample containers should have a total volume in excess of 40 mL. Narrow mouth screw cap bottles with the TFE fluorocarbon faced silicone septa cap liners are strongly recommended. Crimp-seal serum vials with TFE fluorocarbon faced septa are acceptable if the seal is properly made and maintained during shipment.

6.1.2 Sample Bottle Preparation

Wash all sample bottles and TFE seals in detergent. Rinse with tap water and finally with distilled water. Allow the bottles and seals to air dry at room temperature, then place in a 105°C oven for one hour, then allow to cool in an area known to be free of organics.

NOTE: Do not heat the TFE seals for extended periods of time (i.e., more than 1 hour) because the silicone layer slowly degrades at 105°C. When cool, seal the bottles with the TFE seals that will be used for sealing the samples.

6.2 Sample Preservation - Sodium thiosulfate, a chemical dechlorinating agent, is added to samples containing free chlorine in order to arrest the formation of trihalomethanes after sample collection (1). If chemical preservation is employed, the preservative is also added to

the blanks. The chemical preservative (2.5 to 5 mg/40 mL) is added to the empty sample bottles just prior to shipping to the sampling site. Do not add sodium thiosulfate to samples when data on maximum trihalomethane formation is desired. See Table II in order to determine the stability of various organohalides in the presence of sodium thiosulfate

6.3 Sample Collection

Collect a miminum of two replicates from each sample source. Fill the sample bottles in such a manner that no air bubbles pass through the sample as the bottle is being filled. Seal the bottles so that no air bubbles are entrapped in it. Maintain the hermetic seal on the sample bottle until time of analysts.

6.3.1 Sample from a water tap.

Turn on water and allow the system to flush. When the temperature of the water has stabilized, adjust the flow to about 500 mL/minute and collect duplicate samples from the flowing stream.

6.3.2 Sampling from an open body of water.

Fill a 1-quart wide-mouth bottle or 1-liter beaker with sample from a representative area. Carefully fill a minimum of two sample bottles from the sampling container as noted above. If preservative has been added to the sample bottles, then fill with sample just to overflowing, seal the bottle, and shake vigorously for 1 minute.

6.3.3 Sealing practice for septum seal screw cap bottles.

Open top bottle and fill to overflowing, place on a level surface, position the TFE side of the spetum seal upon the convex sample meniscus and seal the bottle by screwing the cap on tightly. Invert the sample and lightly tap the cap on a solid surface. The absence of entrapped air indicates a successful seal. If bubbles are present, open the bottle, add a few additional drops of sample and reseal bottle as above. NOTE: If the septum seals are inverted (i.e., the silicone side against the sample) then significant organohalide losses will occur in shipment and storage.

6.4 Preparation of Blanks

Sample blanks must be prepared and accompany the samples wherever the samples are shipped or stored. If the samples are immediately analyzed at the sampling site then blanks are not required.

Prepare blanks in replicate at the laboratory by filling and sealing a minimum of two sample bottles with pre-tested organic-free water just prior to shipping the sample bottles to the sampling site. If the sample is to be preserved, add an identical amount of preservative to the blanks. Ship the blanks to and from the sampling site along with the sample bottles. Store the blanks and the samples collected from a given source (sample set), together. A sample set is defined as all the samples collected from a given source (i.e., at a water treatment plant, the replicate raw source waters, the replicate finished waters and the replicate blank samples comprise the sample set). Store the sample set in an area known to be free of organic vapors. See Table II for maximum storage time.

6.5 Conditioning Traps

6.5.1 Condition newly packed traps overnight at 200°C by backflushing with an inert gas flow of at least 20 mL/min. Vent the trap effluent to the room, not to the analytical column. Prior to daily use, condition traps 10 minutes while backflushing at 180°C. The trap may be vented to the analytical column; however, after conditioning the column must be programmed prior to use.

6.6 Extraction and Analysis

Adjust the purge gas (nitrogen or helium) flow rate to 40 mL/min. Attach the trap inlet to the purging device. Turn the valve to the purge-sorb position (Figure 3). Open the syringe valve located on the purging device sample introduction needle. Remove the plungers from two 5 mL syringes and attach a closed syringe valve to each. Open the sample bottle (or standard) and carefully pour the sample into one of the syringe barrels until it overflows. Replace the syringe plunger and compress the sample. Open the syringe valve and vent any residual air while adjusting the sample volume to 5.0 mL. Close the valve. Fill the second syringe in an identical manner from the same sample bottle. This second syringe is reserved for a replicate analysis, if necessary.

Attach the syringe-valve assembly to the syringe valve on the purging device. Open the syringe valve and inject the sample into the purging chamber. Close both valves. Purge the sample for 11.0 $\pm$ .05 minutes. After the 11 minute purge time, attach the trap to the chromatograph (turn

the valve to the desorb position) and introduce the trapped materials to the GC column by rapidly heating the trap to 180°C while backflushing the trap with an inert gas between 20 and 60 mL/min for 4 minutes. If the trap can be rapidly heated to 180°C and maintained at this temperature, the GC analysis can begin as the sample is desorbed, i.e., the column is at the initial 45°C operating temperature. The equipment described in Figure 4 will perform accordingly.

With other types of equipment where the trap is not rapidly heated or is not heated in a reproducible manner, it is necessary to transfer the contents of the trap into the analytical column at 30°C where it is once again trapped. Once the transfer is complete (4 minutes), the column is rapidly heated to the initial operating temperature for analysis. Note: In some cases it may be necessary to cool the column down to 0°C. If injection procedure 8.9.1 is used and the early eluting peaks in the resulting chromatogram have poor geometry or variable retention times, then method 8.9.2 should be used.

While the extracted sample is being introduced into the gas chromatograph, empty the purging device using the sample introduction syringe, follow by two 5 mL flushes of organic-free water. After the purging device has been emptied, leave the syringe valve open to allow the purge gas to vent through the sample introduction needle. After desorbing the sample for approximately four minutes recondition the trap by returning the valve to the sorb position, wait 15 seconds then close the syringe valve on the purging device. Maintain the trap temperature at 180°C. After approximately seven minutes turn off the trap power and open the syringe valve. NOTE: If the operations described in 8.11 are omitted then large amounts of water will be injected into the column which will cause large narrow peaks to appear in the early elution area of the chromatogram from the following analysis.

Analyze each sample and sample blank from the sample set in an identical manner (see 6.4.9.5) on the same day. Prepare calibration standards from the standard stock solutions (5.20) in organic-free water that are close to the unknown in composition and concentration. The concentrations should be such that no more than 20 µL of the secondary dilution need be added to 100

to 1000 mL of organic-free water to produce a standard at the same level as the unknown.

6.7 Calculations

Quantify the unknowns by comparing the peak height of the unknowns to the standard peak height (obtained from calibration standards). Round off the data to the nearest .01 μg/L.

$$\mu g/L = \frac{\text{peak height sample}}{\text{peak height standard}} \times (\text{concn. std., } \mu g/L)$$

Report the results obtained from the EMSL Quality Control Sample and the lower limit of detection estimates along with the data for the unknown samples. Calculate the limit of detection (LOD) for each organohalide not detected using the following criteria:

$$\text{LOD } (\mu g/L) = 0.4 \frac{(A \times ATT)}{(B \times ATT)}$$

where: A = 5 times the noise level in (mm) at the exact retention time of the organohalide or the baseline displacement in (mm) from the theoretical zero at the exact retention time of the organohalide. B = peak height (mm) of 0.4 μg/L quality check standard ATT = Attenuation factor.

7.0 Quality Assurance Program

7.1 Analytical Quality Control

Analyze the 0.40 μg/L quality check sample daily before any samples are analyzed. Instrument status checks and olower limit of detection estimators based upon response factor calculations at five times the noise level are obtained from these data. In addition, respone factor data obtained from the 0.40 μg/L quality check standard can be used to estimate the concentration of the unknowns. From this information the appropriate standard dilutions can be determined.

Analyze the EMSL-Cincinnati volatile organics quality control samples or their equivalent on a quarterly basis. Analyze the sample blank or a method blank to monitor for potential interferences as described in Section 3.0. Perform the following instrument status checks using the data gathered from blanks duplicate analyses and the quality check sample.

7.1.1 Peak Geometry Check

7.1.1.1 All of the peaks contained in the quality check chromatogram must appear to be sharp and symetrical. Peak tailing in excess of that shown in the method chromatogram Figure 5 must be corrected.

Tailing problems are generally traceable to:

A. Active sites on column - repack
B. Reactor temperature too low.
C. Reactor base temperature too low.
D. Contaminated reactor tube - recondition/replace.
E. Contaminated reactor transfer line - replace
F. Detector flow too low.
G. Spent ion exchange column - replace.

7.1.1.2 If only the compounds eluting before chloroform give random responses, unusually wide peak widths, are poorly resolved, or are missing, the problem is usually traceable to the trap/desorber.

7.1.1.3 If only brominated compounds show poor peak geometry or do not properly respond at low concentrations, repack the trap.

7.1.1.4 If negative peaks appear in the chromatogram replace the ion exchange column and replace electrolyte.

7.1.1.5 Retention times for the organohalides should remain constant throughout the day (less than 10% variance).

7.1.2 Replicate analyses

A properly operating gas chromatograph should perform with an average relative standard deviation of less than 6% over a concentration range of 0.1 to 100 µg/L. Poor precision is generally traceable to pneumatic leaks especially around the detector reactor inlet and exit.

The method blank analysis should represent less than a 0.1 µg/L response or less than a 10%. Interference for those compounds that occur routinely.

Any instrument not performing according to 7.1 specifications should be considered "out of control". The instrument must be "in control" before acceptable data can be generated.

7.1.3 Confirmatory Analyses

Confirmatory analyses are performed using dissimilar columns. If sufficient material is present then confirmatory analyses are performed by gas chromatography-mass spectrometry.

Aqueous standards and unknowns are always extracted and analyzed under identical conditions in order to compensate for extraction losses.

8.0 References

1. Identification and Analysis of Organic Pollutants in Water, Keith, L. H., Ann Arbor Science, p. 87 (1976).

ANALYTICAL PROTOCOL: DETERMINATION OF ORGANIC CONTAMINANTS BY GROB CLOSED-LOOP-STRIPPING ANALYSIS (CLSA)

1.0 Principle of the Method

In 1973 in Zurich, Switzerland, Grob (1) reported on CLSA for the measurement of semivolatile, intermediate molecular weight organics in drinking water at the part-per-trillion (nanogram-per-liter) level. Grob CLSA is accomplished by making the sample vessel, containing a headspace, part of a closed system in which the entrapped air is continually circulated through the sample and an activated carbon filter. As the air passes through the sample, organic compounds are purged from the water into the headspace air. The carbon filter then adsorbs these compounds from the air. The carbon filters are extracted with a small amount of solvent and the extracts are analyzed by capillary GC/MS.

2.0 Range and Detection Limit

The CLS technique is capable of analyzing water samples containing purgeable organic contaminants at the low ng/L (ppt) level. Reported analyses (2,3) indicate an acceptable analytical concentration range of ∿1-100 ng/L.

3.0 Interferences

Impurities in the purge gas and plumbing ahead of the trap can lead to contamination problems. The analytical system can be demonstrated to be free from contamination under the conditions of the analysis by running method blanks. Method blanks are run by charging the purging device with organic-free water and analyzing it in a normal manner. The use of non-TFE plastic tubing, non-TFE thread sealants, or flow controllers with rubber components in the purging device is avoided.

Samples can be contaminated by diffusion of volatile organics (particularly freons and methylene chloride) during shipment and storage. A sample blank prepared from organic-free water and carried through the sampling and handling protocol can serve as a check on such contamination.

Cross contamination can occur whenever high level and low level samples are sequentially analyzed. To reduce the likelihood of this, the purging device is rinsed between samples with organic-free water. Whenever an

unusually concentrated sample is encountered, it is followed by an analysis of organic-free water to check for cross contamination.

The use of a high resolution separation technique (capillary GC) coupled with a highly specific detection device (MS) reduces the likelihood of interference problems. Published reports (2,3,4) have shown that the method is not seriously impaired by interferences when environmental samples were analyzed.

4.0 Precision and Accuracy

Statistical analyses (SA) to determine standard deviations have been performed on RRTs, GC peak areas, response factors, amounts, and recovery efficiencies. SA for both total ions and single ion quantitation have been compared. Greater qualitative and quantitative accuracy was achieved by single ion quantitation for complex environmental samples due to co-eluting GC peaks. Table 1 shows the SA results of the repetitive direct injections with quantitation results based on a single ion. This table shows that peak areas vary an average of 13% whereas the amounts vary an average of 9%. Since the sensitivity of the mass spectrometer fluctuates daily, these area variations are expected. However, most importantly, the internal standard method of quantitation compensates for these changes when the amounts are calculated by the computer.

5.0 Apparatus and Reagents

The original closed-loop stripping apparatus of Grob (1) has been modified for increased durability and ease of sample handling. With the exception of the sample container, all glass parts have been eliminated and the apparatus constructed of type 304 stainless steel and Teflon® as seen in Figures 1 and 2; tubing connections are made with Swagelok® fittings containing Teflon ferrules.

The sample container is a one-gallon jug on which the neck opening has been ground flat to ensure a leak-proof seal during the vapor phase stripping. With this system, a sample can be collected and analyzed in the same container. Contamination is minimized, spillage and errors introduced by sample transfer are eliminated, sample changeover is simplified, and the time required is greatly reduced. Figure 3 shows a schematic diagram of the closed-loop stripping apparatus.

Table 1. STATISTICAL ANALYSIS OF REPETITIVE DIRECT INJECTION STUDY WITH QUANTITATION BASED ON A SINGLE ION

Compound	Quantitation Mass (m/e)	Relative Retention Time (RRT)*	Area	Amount* (ng)
Bis-(2-chloroethyl)ether	93	0.525 ± .001	125,339 ± 20,978	61.0 ± 3.1
1,4-Dichlorobenzene	146	0.557 ± .001	174,043 ± 21,770	50.0 ± 4.6
2-Ethyl-1,4-dimethylbenzene	119	0.635 ± .001	231,704 ± 30,954	42.8 ± 3.4
1,2,4-Trichlorobenzene	180	0.728 ± .001	183,366 ± 22,306	72.6 ± 6.5
Hexachloro-1,3-butadiene	225	0.776 ± .000	79,039 ± 8,047	84.0 ± 9.6
1-Chlorododecane (I.S.)	91	1.000 ± .000	37,512 ± 7,667	52.0 ± 0.0
2,2',4,5,5'-Pentachlorobiphenyl	254	1.447 ± .002	17,564 ± 1,505	50.0 ± 7.1

*Average value ± standard deviation/based on 10 injections.

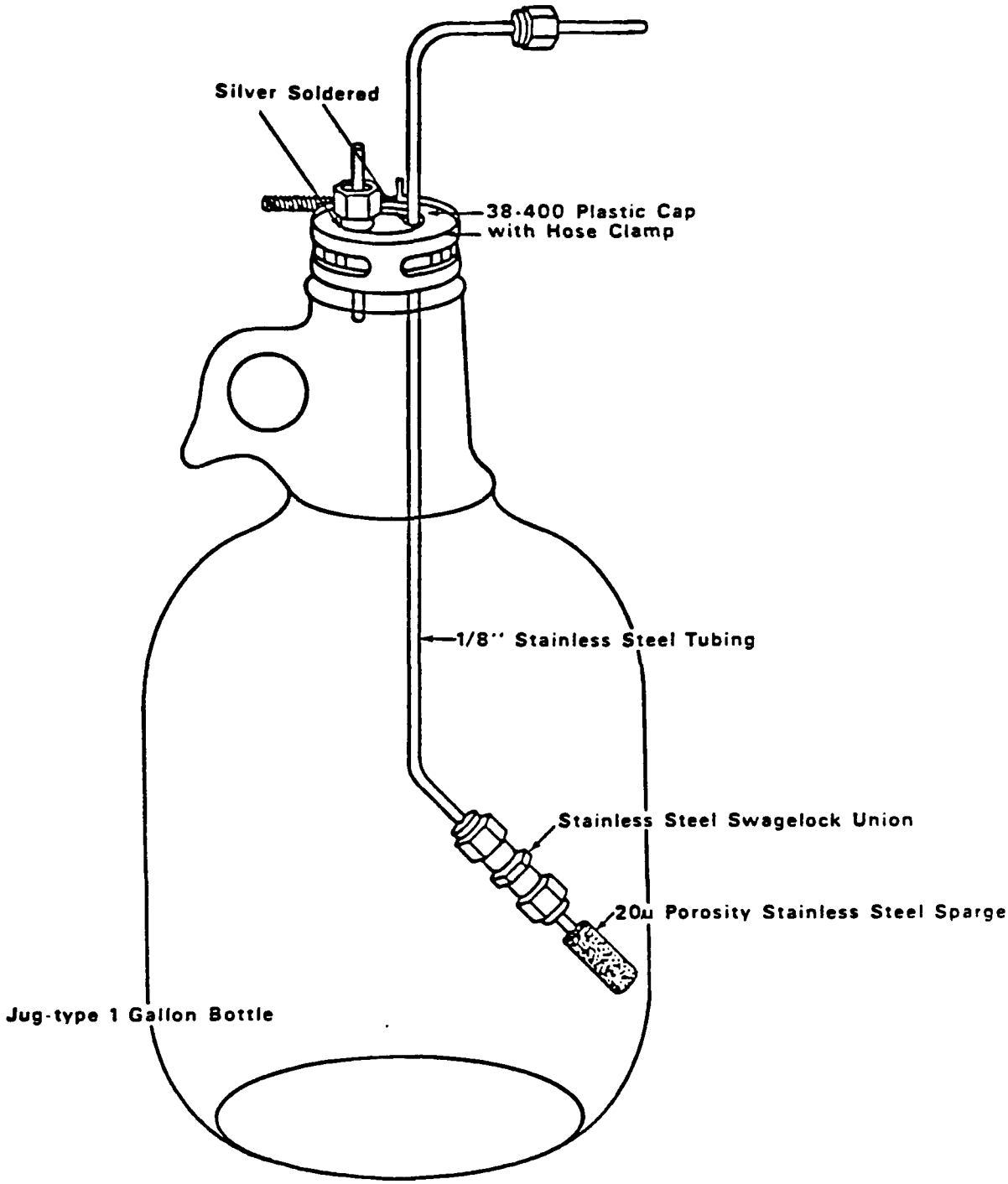


Figure 1. Sample container for CLSA.

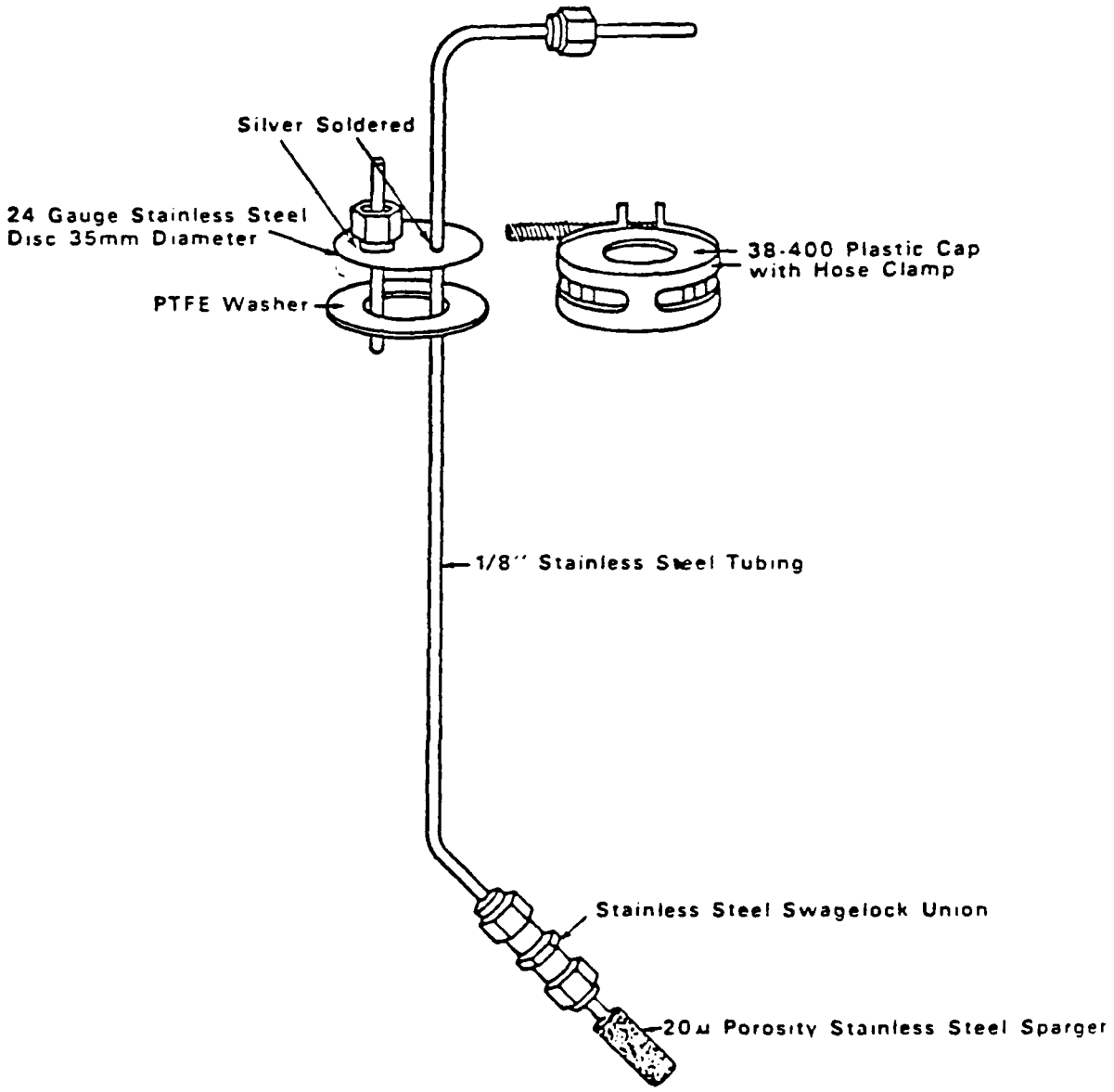


Figure 2. CLSA sample container plumbing and fittings.

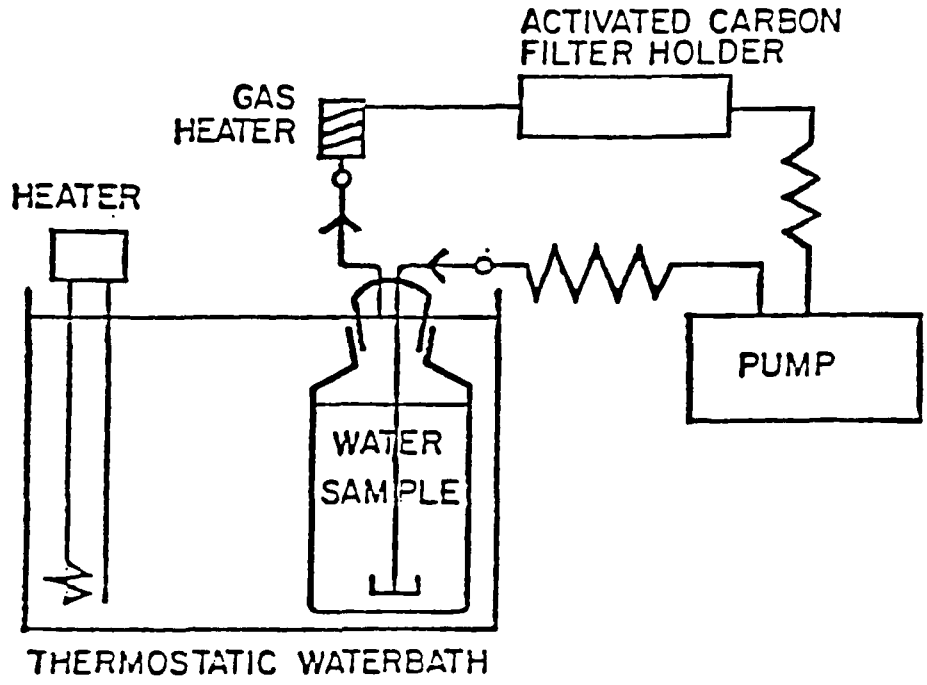


Figure 3. Schematic diagram of closed loop stripping apparatus.

6.0 Procedure

6.1 Sample Collection

Water samples are collected directly in muffled (400°C) bottles containing 100 mg of sodium sulfite to quench residual chlorine and 40 mg of mercury chloride ($HgCl_2$) to retard bacterial growth. The bottle is filled completely without any headspace and the Teflon-lined caps are applied securely. Samples are stored at 5°C until analyzed.

6.2 Sample Stripping

The HERL closed-loop stripping apparatus is shown schematically in Figure 3. The water bath temperature is thermostatically controlled at 30°C; the preheater temperature is maintained at 80°C to prevent condensation of vapors on the carbon filter; the filter holder is insulated from the surrounding atmosphere and its temperature is maintained about 40°C.

CLSA is initiated by decanting a small amount of the water sample down to the "one-gallon" mark, adding 0.6 μL of internal standard solution (1-chloroalkanes in acetone) and assembling the purging apparatus. The 0.6 μL of internal standard solution when added to the sample gives a concentration of 52 ng/L each of 1-chlorohexane, 1-chlorooctane, 1-chlorododecane and 1-chlorohexadecane and 260 ng/L of 1-chlorooctadecane. The latter amount is added due to poorer recovery efficiency of 1-chlorooctadecane. With a clean, dry, carbon filter in place, the sample is purged by recirculating the headspace for 2 hours.

6.3 Carbon Filter Extraction

The carbon filter extraction procedure using carbon disulfide is very similar to that used by Grob and others (1,2,3,5). The carbon filter is removed and assembled into the extraction apparatus (5). The filter is butted up tightly to the receiver tube and secured with a Teflon sleeve. The first 6 μL aliquot of CS_2 is added directly to the top of the carbon and is refluxed back and forth through the carbon 3 times by alternately cooling the filter with ice to pull the solvent down just below the carbon, then warming with the hand to push the solvent back through. When the solvent is pulled through a fourth time, the solvent is transferred to the receiver by swinging the tube in a long downward arc. As most of the solvent aliquot will be required to wet the carbon, only about 2-3 μL will be recovered. The

process is repeated with three additional aliquots of 2 μL each which results in a total sample volume of about 8 μL. Two microliters of this final volume are used for $(GC)^2$ or $(GC)^2$/MS analysis. The entire extraction procedure takes about 10 minutes to complete.

6.4 Analysis

For this protocol all $(GC)^2$/MS analyses are performed on a Finnigan Model 3300 mass spectrometer equipped with an Incos Model 2300 data system computer. A Finnigan Model 9500 gas chromatograph equipped with a Grob designed splitless injector (6) and a 60m x 0.25 mm i.d. WCOT glass capillary SP2100 column was interfaced to the mass spectrometer with a glass-lined stainless steel dual transfer line held at 230°C.

Two microliters (2 μL) of sample in CS_2 are injected with the split closed. The split is opened (20:1 ratio) after 30 sec. Grob's hot needle technique (7) is used for injection of all samples and standards. The helium carrier gas flow rate is about 3 mL/min at 25°C; the linear velocity is 25 cm/sec with a helium head pressure of 20 psi (no flow controller). The injector is maintained at 260°C. The column is temperature programmed: 20°C isothermal (cooled with liquid nitrogen) for approximately 8 min, then 2°C/min to 250°C. Mass spectra are acquired at the rate of one per 2 sec from 14-450 amu at 70 eV and 90°C source temperature. Source pressure is approximately 5×10^{-6} torr. For a typical $(GC)^2$/MS analysis of this type, 3900 scans are recorded (130 minutes from injection to termination). Confirmation of identifications are made by comparison of GC and GC/MS properties with those of authentic standards or library spectra.

6.5 Quantitation

Automatic computerized quantitation procedures based on the internal standard method of quantitation are used in this method. The quantitation formula used by the computer is:

$$\text{Amount} = \frac{\text{Area x Internal Standard Amount}}{\text{Internal Standard Area x Response Factor}}$$

where

$$\text{Response factor} = \frac{\text{Area x Internal Standard Amount}}{\text{Amount x Internal Standard Area}}$$

7.0 Quality Assurance

Since many organics in water can be measured at 5 ng/L or less with CLSA, it is extremely necessary to incorporate procedural blanks into the analytical scheme. Prior to analyzing actual samples of drinking water, filter blanks and CLSA blanks of low organic water (Milli-Q® Water) containing the same internal standard mix are performed. When spiking water with low levels of organic chemicals for recovery studies one must consider the background level of these chemicals in the spiking medium for correct quantitation results.

Blank water samples are run between highly contaminated environmental samples. If a series of samples is to be analyzed, the least contaminated samples are run first.

Standard quality assurance practices should be used with this method. Field replicates should be collected to validate the precision of the sampling technique. Laboratory replicates should be analyzed to validate the precision of the analysis. Fortified samples should be analyzed to validate the accuracy of the analysis.

8.0 References

8-1 Grob, K., Organic Substances in Potable Water and in its Precursor, Part I. Methods for Their Determination by Gas-Liquid Chromatography, J. Chromatogr., 84, 255 (1973).

8-2 Stieglitz, L., Roth, W., Kuhn, W., and Leger, W., The Behavior of Organohalides in the Treatment of Drinking Water, Vom Wasser, 47, 347 (1976).

8-3 Schwarzenbach, R. P., Molnar-Kubica, E., Giger, W., and Wakeham, S. G., Distribution, Residence Time, and Fluxes of Tetrachloroethylene and 1,4-Dichlorobenzene in Lake Zurich, Zwitzerland, Envir. Sci. and Tech., 13(11), 1367 (Nov. 1979).

8-4 Zurcher, F. and Giger, W., The Study of Volatile Organic Compounds in the Glatt River, Vom Wasser, 47, 37 (1976).

8-5 Grob, K. and Zurcher, F., Stripping of Trace Organic Substances from Water Equipment and Procedure, J. Chromatogr., 117, 285 (1976).

8-6 Grob, K. and Grob, K., Jr., Splitless Injection and the Solvent Effect, HRC & CC, 1(1), 57 (July 1978).

8-7 Grob, K. and Grob, G., Practical Capillary Gas Chromatography - a Systematic Approach, HRC & CC, 2(3), 109 (Mar. 1979).

ANALYTICAL PROTOCOL: ANALYSIS OF PURGEABLE ORGANIC COMPOUNDS IN WATER (MASTER ANALYTICAL SCHEME)

1.0 Principle of the Method

This method describes the determination of purgeable organic compounds in drinking water, surface waters and treated municipal and industrial wastewater effluents. The procedure utilizes gas stripping analysis to effectively partition semi-soluble and insoluble volatile organic compounds between gaseous and aqueous phases followed by trapping on a Tenax GC cartridge Exposed cartridges are thermally desorbed and the compounds released are analyzed by GC/MS/COMP using capillary columns. Summarized recovery data relating to the procedure below is presented in Figure 1 and Table 1.

2.0 Range and Detection Limits

Detection limits are:

0.1 ppb in drinking water
1.0 ppb in surface water
10 ppb in industrial wastewater effluent

3.0 Interferences

Background from the Tenax cartridge can present problems with identification and quantitation. Phenolics and amines can mask other compounds of interest (because of chromatographic tailing). If the pH of sample is adjusted to seven the phenols and amines are in an ionic state and will not purge.

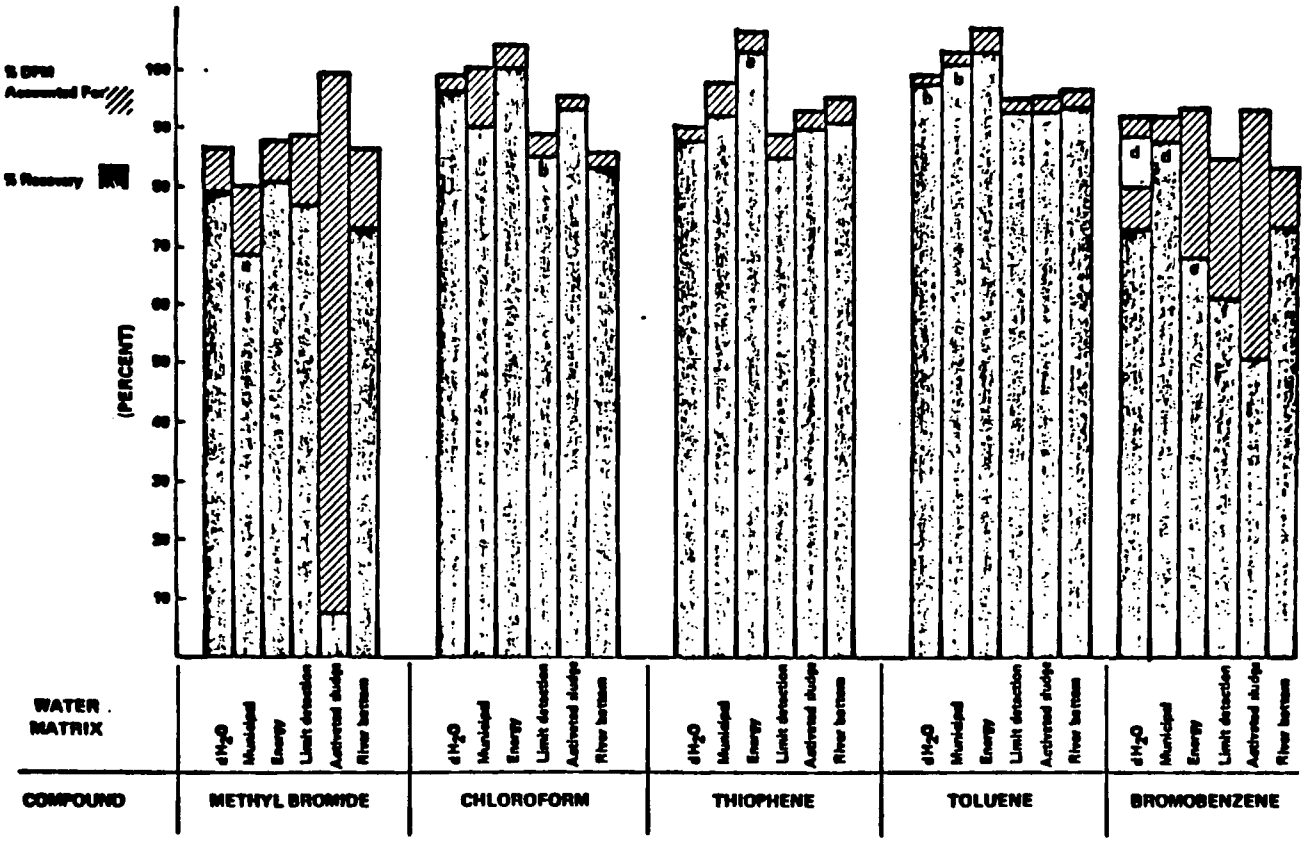
4.0 Precision and Accuracy

Table 1 presents mean % recovery and confidence limits for five compounds in six different water matrices. Minor variations in recovery data are due to the fact that the experimental procedure was modified slightly during the study.

5.0 Apparatus and Reagents

For Purging --

1. Purge flask, 200 mL capacity (Figure 2).
2. Tenax GC sorbent cartridges.
3. Flowmeter, (10-100 mL/min).



[a] Two cartridges stored overnight give low recovery.
[b] Duplicate data only.
[c] Experimental procedure altered:semi-closed system.
[d] Experiments performed with old stock of bromobenzene.
[e] Mean of 2 triplicate experiments.
[f] Single determination only.

Figure 1. Recoveries for selected compounds in various water matrices and particulate types.

Table 1. SUMMARY OF RECOVERY DATA FOR SELECTED COMPOUNDS IN VARIOUS WATER MATRICES[a,b]

Compound	Mean % recovery ± standard deviation (C.V.)	
methyl bromide[c]	76 ± 5 (7)	81 (30)
chloroform	91 ± 6 (7)	91 (12)
thiophene	92 ± 6 (7)	92 (11)
toluene	97 ± 4 (5)	97 (14)
bromobenzene	66 ± 9 (14)	71 (8)

[a]Experiments were performed at the 10 ppb level with the exception of those at the limit of detection (0.2 to 2 ppb).

[b]Water matrices examined were: distilled water, 10% municipal wastewater effluent, 10% energy effluent, distilled water (limit of detection), 500 ppm activated sludge, and 500 ppm river bottom particulates.

[c]Does not include activated sludge results.

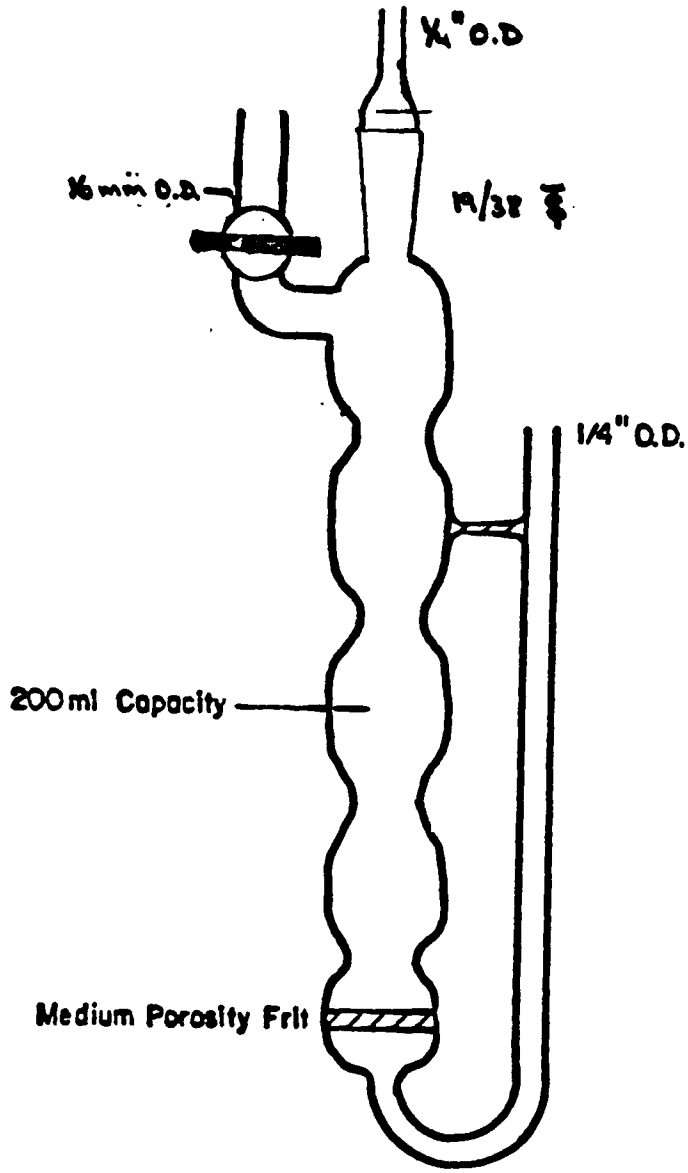


Figure 2. Purge Flask, 200 mL capacity.

4. Reagents
    a. Sodium sulfate, anhydrous powder (ACS grade).
    b. Phospate buffer, 2.0M, pH7.
    c. High purity water, purged.
    d. Helium gas, scrubbed to remove volatiles.
5. Liquid-nitrogen trap (3' of 1/4" O.D. copper tubing, coiled and immersed in liquid nitrogen).
6. Pipets, volumetric, 1 mL and 20 mL.
7. Beckmann fittings, #416.
8. Quick-connect fittings, 1/4" tube, double end shut-off.
9. Water bath, 30°C.

6.0 Procedure

6.1 Sample Collection, Preservation and Handling

6.1.1 Sample Collection

Grab samples must be collected in glass containers having a total volume in excess of 40 mL. Fill the sample bottles in such a manner that no air bubbles pass through the sample as the bottle is being filled. Seal the bottle so that no air bubbles are entrapped in it. Maintain the hermetic seal on the sample bottle until time of analysis.

6.1.2 Sample Preservation

The samples must be iced or refrigerated from the time of collection until extraction. If the sample contains free or combined chlorine, add sodium thiosulfate preservative (10 mg/40 mL will suffice for up to 5 ppm Cl_2) to the empty sample bottles just prior to shipping to the sampling site, fill with sample just to overflowing, seal the bottle, and shake vigorously for 1 minute.

6.1.2 Sample Handling

All samples should be analyzed within 14 days of collection.

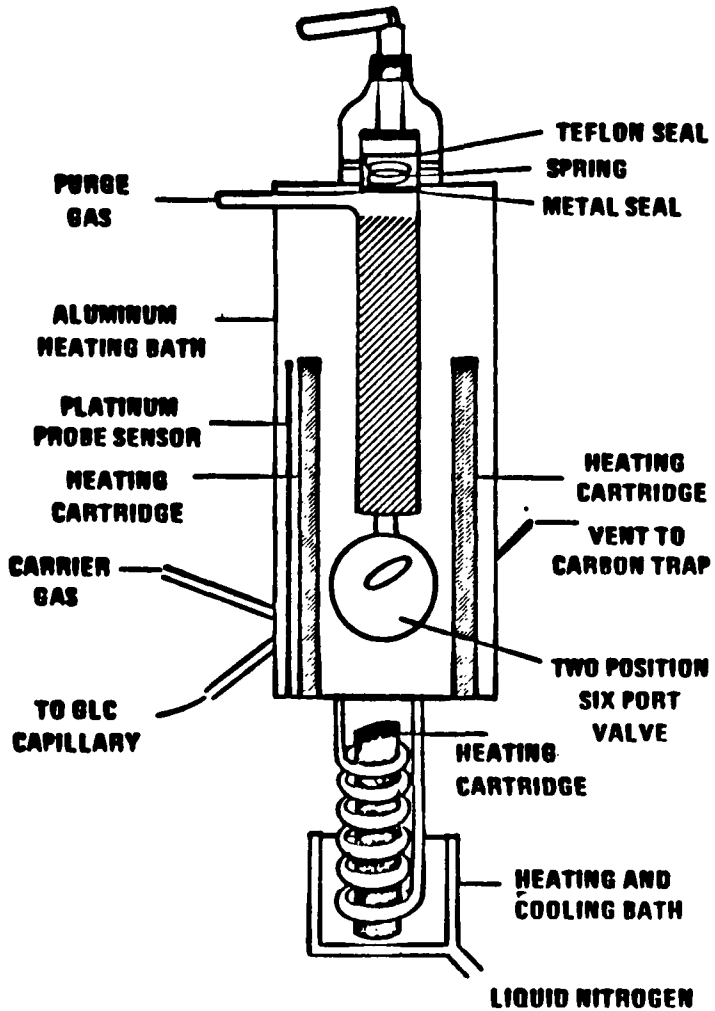
6.2 Extraction/Purge/Etc

6.2.1 Tenax GC Cartridge Preparation

Tenax GC is prepared by extraction with redistilled, pesticide analysis grade methanol in a Soxhlet apparatus for 48 hours with a cycle time of approximately fifteen minutes followed by a similar extraction with redistilled, pesticide analysis grade pentane. After air drying, the Tenax is

placed in a vacuum oven at 100°C for at least 24 hours. Cartridges (1.3 x 6.0 cm; 35/60 mesh) are prepared in 1.6 x 10 cm Pyrex tubes with 1 cm glass wool (silanized) plugs at each end and thermally desorbed at 260°C for a minimum of 2 hours under a helium flow of 30 mL/min. Following thermal desorption the cartridges are transferred, hot, to Pyrex culture tubes (25 x 150 mm) with Teflon®-lined screw caps and cooled to room temperature. The Teflon cap-liners are a major source of contamination and must be carefully solvent rinsed and vacuum-dried overnight. All gases which come in contact with the Tenax GC cartridges, as well as water samples must be passed through liquid-nitrogen traps to remove volatile components. Cartridge preparation and thermal desorption is done in a lab free of solvent vapors.

Tenax GC background is checked in a manner identical to the procedure for analyzing loaded cartridges. Injection of adsorbed materials from Tenax GC onto a gas chromatographic column is accomplished using the thermal desorption system illustrated in Figure 3 and 4. This system consist of four main components: a desorption chamber, a six-port, two-position, high-temperature, low-volume, valve (Valco Instruments, Inc.), a Ni capillary cyrogenic trap, and a temperature controller. The stainless steel thermal desorption chamber and six-port valve are encased in a common aluminum sandwich which serves as a heating block. The chamber itself has an overall length of 12 cm and accommodates a Pyrex sampling cartridge of dimensions 13 mm i.d. x 10 mm o.d. and 10 cm length. Two, 150 W, 115V heating cartridges are used to heat the aluminum sandwich and the temperature is controlled and monitored with iron-constantan thermocouples and output on a pyrometer (Omega Engineering, Inc.). The desorption chamber is connected to the valve with a short section of Ni capillary tubing (0.50 mm i.d., 1.47 mm o.d.). Similar capillary tubing is used for the cryogenic trap (Figure 3). This trap, constructed of aluminum, is cooled to -195°C by passing cryogenically cooled nitrogen gas through stainless steel tubing (18" x 1/6" o.d. x 0.04" i.d.) prior to entry into the body of the trap. This allows collection and concentration of vapors desorbed from the sorbent cartridge. The vapors are injected onto the GC column by rapid heating of the capillary trap to 270°C provided by a 150 W cartridge heater located inside the aluminum cylinder. The desorption chamber is interfaced to the capillary GC column with a



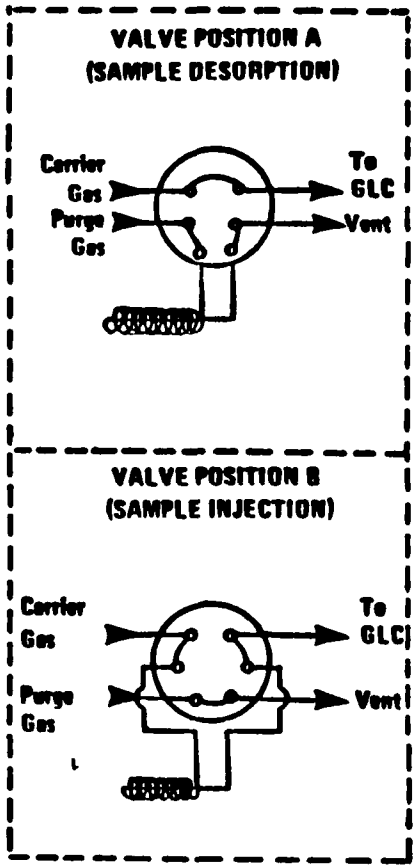


Figure 3. Thermal desorption inlet-manifold for Tenax GC cartridges.

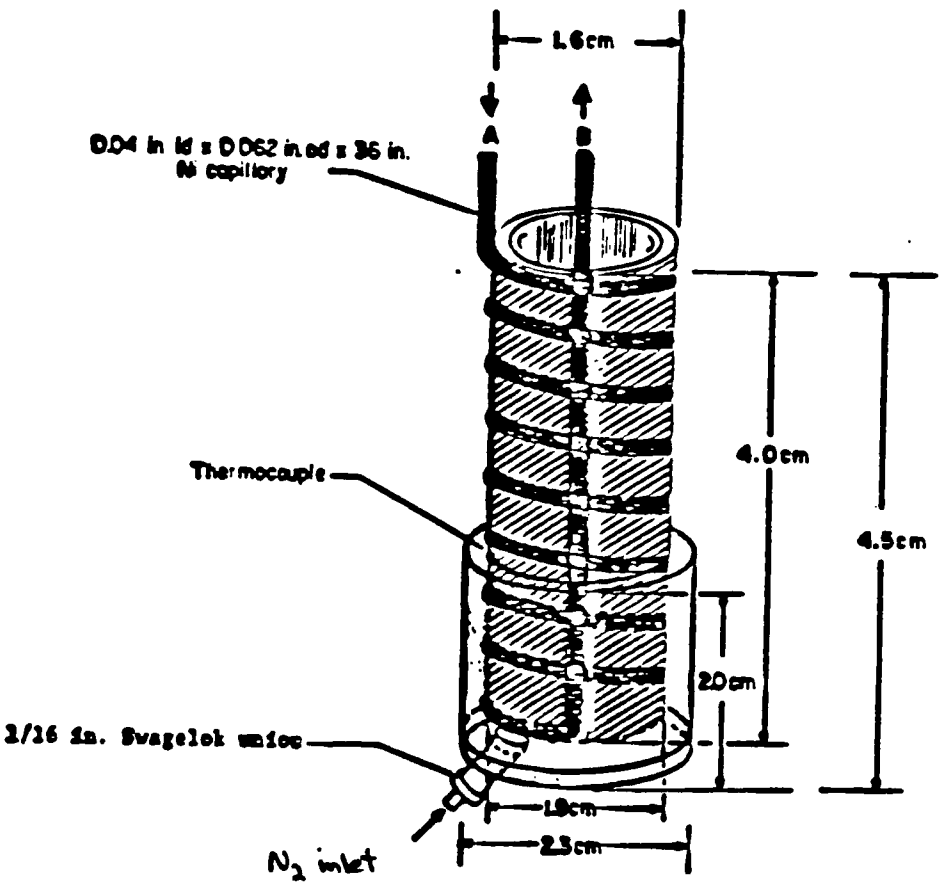


Figure 4. Cryo-heater module for inlet-manifold.

minimal length of gold-plated Ni capillary tubing (0.40 mm i.d., 1.57 mm o.d.), deactivated with OV-17. Connection between the Ni transfer line and glass capillary is made with 1/16" stainless steel "zero dead volume" union (Swagelok®, Crawford Fitting Co.) using stainless steel and Vespel® Ferrules.

In a typical thermal desorption/injection cycle, an exposed cartridge is placed in the preheated (240°C) chamber with a flow of He gas (15 mL/min) through the cartridge to purge the desorbed vapors into the cryogenic trap; this constitutes valve position A (Figure 3). After 8 min of thermal desorption, the six-port valve is rotated to position B, the temperature on the capillary trap is rapidly raised (>100°/min), and carrier gas sweeps the vapors onto the gas chromatographic column. Upon reaching the maximum trap temperature, the trap heater is turned off; however, the valve is retained in position B with the cartridge in the desorption chamber. After approximately 30 min the cartridge is removed and the valve is returned to Position A. The removal of the cartridge and the switching of the valve should be done simultaneously or as closely together as possible.

The criteria for Tenax GC cartridge acceptability are, for the most part, empirical, and are based on the following chromatographic evidence obtained at 128 x 10^{-12} amps full scale on a Varian 3700 gas chromatograph using a 50 m x 0.25 mm SE-30 WCOT column temperature programmed from 40 to 220°C at 4°/min:

1. No peaks with the exception of pentane and methanol, over 10% full scale. Pentane and methanol are generally 30% full scale.
2. No more than 15 peaks over 3% full scale.

6.2.2 Distilled/Deionized Water Purification

Interfering volatile substances in distilled and/or deionized water are removed by purging with purified helium at 90°C and 50 mL/min for at least two hours. Water purified in this manner should be used immediately.

6.2.3 Sample Extraction

1. Assemble the purge flask, except for the Tenax GC cartridge, and place in a 30°C bath.

2. Weigh out 60 g of anhydrous Na_2SO_4 and transfer to the purge flask. Purge with helium* at 20 mL/min for approximately 15 minutes.
3. With the Tenax GC cartridge in place, the flask and contents at 30°C, the helium flow stopped and the gas inlet tube stoppered, transfer exactly 200 mL of sample to the flask as quickly as possible. Immediately, close the stopcock just ahead of the cartridge, remove the flask from the batch and shake vigorously to dissolve the salt.
4. Return the flask to the water bath, reconnect the gas line, open the stopcock and purge the sample with 500 mL helium using a flow rate between 10 and 100 mL/min.
5. Remove the exposed cartridge to its screw-capped culture tube and store, if necessary, at -10°C in a sealed metal container.
6. Analyze cartridges by capillary GC/MS/COMP.

6.3 Analysis

Analyses of the catridges as done on a glass capillary column - 50 m x 0.5 mm i.d., coated with SE-30 (tentatively) by a GC/MS/COMP system.

6.4 Qualitative Identification

Qualitative analysis is to be performed either by computer searches or manual interpretation of information acquired by utilizing a full scan mode.

6.5 Quantitation

A software program will provide for calculating the quantity of each component in each of the sample extracts using relative molar response factors (ratioed to the internal deuterated standards). Also it will correct for the recovery of each organic compound from the water matrix based on the recovery data which was generated under this program or by the user. Finally, it will calculate the concentration of each organic in the original water sample.

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*The cryogenic trap must be used at all times to prevent contamination of the sample with volatile components in the purge gas.

7.0 Quality Assurance Program

An on-going quality assurance program is required to assure the data quality. Quality control (QC) procedures determine artifacts, losses, etc. through a system of blanks and controls. Quality assurance (QA) procedures monitor the execution of the procedure and check data interpretations and calculations.

7.1 Quality Control

Prior to a field sampling trip, ehough blanks and controls are prepared to equal 10%, each (2 minimum) of the anticipated number of field samples. Blanks consist of 40 mL of purged distilled water in the same type of sampling container as is used in the field. Controls consist of the 40 mL of purged distilled water and are spiked with known compounds. These blanks and controls are carried to the field and receive the same handling as the field samples. Workup and analyses of field blanks and controls is interpreted with field samples on a regular basis. This method allows assessment of sample storage stability.

Table 2 presents a typical set of blanks and controls for quality control on a field trip were 50 tap water samples are to be collected.

7.1.2 Procedural Blanks and Controls

7.1.2.1 Extraction Blanks

With each set of samples, a procedural blank is run. This consists of 10 mL of prepurged distilled water which is extracted under the same conditions as the samples. These blanks are designed to detect artifacts from dirty glassware, laboratory atmosphere instrusion, and other sources

7.1.2.2 GC/MS Procedural Control

At the start of each working day, a mixture of 2,6-dimethylphenol, 2,6-dimethylaniline, and acetophenone (PA mixture) is analyzed to monitor the capillary GC column performance. This also serves to check the mass spectrometer tuning.

Field samples, field controls, field blanks, and procedural blanks are queued up for GC/MS analysis such that at least one QC sample is run each working day. In addition, a standard solution is analyzed each day to serve as a procedural control and also to update the RMR value.

Table 2. QC SAMPLES FOR A TYPICAL SAMPLING TRIP

Sample type	Number	Comment
Field Blank	5	Freeze after preparation vary to field, store with field samples
Field Control	5	Store with field blanks
Lab Blank	5	Freeze after preparation, store in same freezer as field samples will be stored
Lab Control	5	Store with lab blanks

Thus, in a typical working day, 4 field samples, 1 blank or control, and 1 RMR standard are run.

7.2 Quality Assurance

Both internal and external quality assurance procedures are to be followed. Internal quality assurance procedures assure the continuity and consistency of the data. External quality assurance procedures (interlaboratory checks) verify or dispute the accuracy of the data.

7.2.1 Internal Quality Assurance

7.2.1.1 Supervision and Monitoring of Activities

There are three levels of quality assurance (QA). The primary quality assurance is the person conducting the sampling and/or analysis. This person must be aware of their actions, observe events which may effect the data, and maintain appropriate records. At the second level, the chemist's supervisor monitors their daily activities, reviews the notebooks, checks data and calculations, and assists in "troubleshooting" problems. At the tertiary level, a QA coordinator interviews all personnel on the project. The interviews cover the operations they perform (precisely), the data they obtain, a spot-check of their calculations, and any problems they have had.

7.2.1.2 Documentation

Chain of Custody

From the initial preparation of a sample container through reporting of the analytical results, each sample is accompanied by a chain of custody sheet. Each person signs in the time of receipt, operations performed, and transmittal of the sample. This record is important for tracing a contaminant, bad standard, or some other problem.

Sampling Protocol Sheets

When a sample is collected, a sampling protocol sheet is filled in which contains a discrete sample code which identifies project number, area, site, locations, trip number, sampling period, and sample type. Also included are sample times, volumes, addresses, meteorology, and other pertinent information. Where appropriate, a map is made to precisely identify the location.

Sample Log

Upon return from a sampling trip, each sample code is entered into a sample log book. This log is updated as samples proceed through workup and analysis. Thus, at a glance, project personnel can tell the status of each sample and find out how many are at different stages in the analytical protocol.

GC/MS Log

Each sample run by GC/MS is logged into a notebook, detailing analysis conditions, where the data are archived, and what hardcopy data has been produced.

7.2.2 External Quality Assurance

Samples will be analyzed by a designated quality assurance laboratory Samples, controls and blanks will be shipped directly from the field to the laboratory for analysis. They will report the result to the primary laboratory for correlation with primary data.

7.2.2.1 Selection of Samples for Quality Assurance

Approximately 10% of the field samples (2 minimum) will be collected in duplicate for shipment to the quality assurance laboratory. The selection process will be random with the following restrictions: If any stratefication of sites is known, purposive, selection of quality assurance sites may be used to get representative samples (up river vs down river sites).

7.2.2.2 QC Samples

Along with the QA duplicate samples, at least one QC control and one QC blank must be included with the QA samples. An example is shown in Table 3 for a trip collecting 50 samples.

7.2.2.3 Sample Codes

All samples shipped to the QA laboratory should be encoded so that the laboratory cannot discern between samples and controls. This "blind" protocol will add validity to the results.

7.2.2.4 Shipping

Samples should be shipped on dry ice directly to the QA laboratory by an appropriate air carrier (e.g., Federal Express, Eastern Sprint) in well insulated and packed cartons.

Table 3. SAMPLES TO BE COLLECTED AND SHIPPED TO QA LABORATORY FOR A TYPICAL SAMPLING TRIP

Sample	Number	Comment
Duplicate Sample	5	Random selection unless prior information stratifies sites
Field Blank	1	Ship with samples
Field Control	1	Ship with samples

ANALYTICAL PROTOCOL: POLYNUCLEAR AROMATIC HYDROCARBONS IN AIR

1.0 Principle of the Method

Air is drawn through a sampling train consisting of a filter followed by a polyurethane foam (PUF) plug. Only particulate material trapped by the filter is analyzed for PNAs. The filter is extracted with cyclohexane. The resulting solution is fractionated by chromatography on a short silica gel bed, followed by thin layer chromatography on cellulose. The solution resulting from this clean-up is analyzed by liquid chromatography with fluorescence detection; if sufficient sensitivity and/or resolution is not attained, the solution will be analyzed by capillary gas chromatography with flame ionization detection (GC-FID). This methodology is based on procedures developed at NIOSH (Cincinnati), Division of Physical Sciences and Engineering, Measurements Research Branch, Organic Methods Development Section.

2.0 Range and Detection Limit

For this procedure the lower limit of measurement for all target PNAs is approximately 100 ng per sample. The concentration range embraced by the procedure was not specified in the NIOSH protocol.

3.0 Interferences

Unspecified in NIOSH protocol.

4.0 Precision and Accuracy

Unspecified in NIOSH protocol.

5.0 Apparatus and Reagents

5.1 Sampling

5.1.1 Filters - Glass filter, 25 mm diameter (Gelman Type A/E); Teflon, 25 mm diameter (Gelman Type TF).

5.1.2 Filter holder - Gelman, open, Delrin; Beckman union reducer, Cat. No. 830517.

5.1.3 Personal sampling pumps capable of sampling at 2 L/min with the sampling train in line (MSA Model G or equivalent). The pumps must be calibrated with a representative sampling train in line.

5.2 Analysis

5.2.1 High pressure liquid chromatograph, automated (Two Waters M6000A pumps, a Waters 660 solvent programmer, and a Waters WISP 710 auto sampler).

5.2.2 LC column, 25 cm x 6.4 mm inside diamter, packed with Vydac 201TP reverse phase, 10 μm particle size, or equivalent.

5.2.3 Constant temperature water jacket for LC column.

5.2.4 Fluorescence detector, monitoring emission at 425 nm with excitation at 340 nm, 300 μL flow cell (Farrand Mark I spectrophotofluorometer), or equivalent.

5.2.5 Laboratory data system (Hewlett-Packard 3354B), or equivalent.

6.2.6 Scintillation vials, 20 mL, with polyethylene-lined screw caps.

5.2.7 Ultrasonic water bath (Cole-Palmer Model 8845-60), or equivalent.

5.2.8 TLC plates, 20 cm x 30 cm, precoated with MN 300 cellulose normal, layer thickness 250 μm (Analtech).

5.2.9 TLC tank, 27 cm x 21.5 cm x 7.5 cm, with glass-plate cover and filter paper lining one large side.

5.2.10 Polyperfluoroethylene filter, 25 mm diameter, unlaminated, 0.5 μm pore size (Millipore fluoro pore #FHUP 025-00).

5.2.11 Holder, 25 mm filter, glass (Millipore #XX-10-025-00).

5.2.12 Cartridges, silica gel, 2 cm x 1 cm diameter (Waters SEP-PAK).

5.2.13 Syringe, 10 mL, glass.

5.2.14 Vials, 5 mL, conical-cavitied, with screw caps and Teflon-lined septa.

5.2.15 Vial heater, block type (Supelco Blok Heater), or equivalent.

5.2.16 Sample concentrator, 6-port, positive-gas-flow type.

5.2.17 Pipets, Eppindorf, 10- and 25-μL.

5.2.18 UV light box.

5.2.19 TLC plate zone collectors, made in-house as follows: The tip of a Pasteur pipet is removed to give a glass tube 8 cm long x 7 mm in diameter. A quarter of a 25 mm polyperfluoroethylene filter (Item I.A.10) is wrapped over and around the smooth end of the tube, which is then gently inserted into flexible tubing connected to house vacuum.

5.2.20 Pipets, 1-, 2-, 5-, 9- and 10 mL.

5.2.21 Flasks, volumetric, 10- and 100 mL, low-actinic, tinted red.

5.2.22 Culture tubes, 13 mm x 100 mm, round-bottomed, with screw caps.

5.2.23 Centrifuge.

5.2.24 Centrifuge tubes, tapered-bottom, 15 mL, graduated with 0.1 mL subdivisions.

5.2.25 Solvents (Burdick and Jackson) - acetonitrile, methanol, cyclohexane, methylene chloride, 2-propanol, acetone, water (deionized, distilled)

5.2.26 Mobile phases.

Solvent A: 48.4:43.8:7.8 Acetonitrile-water-methanol by volume

Solvent B: 61.5:38.5 methanol-acetonitrile by volume

Solvent C: 2:2:1 water-2-propanol-acetone by volume

Solvent D: 9:1 hexane-methylene chloride by volume

5.2.27 Reference standards - fluoranthene, pyrene, benz(a)anthracene, chrysene, benzo(a)pyrene, benzo(e)pyrene.

6.0 Procedure

6.1 Sampling

Immediately before sampling, connect the sampling train to a piece of flexible tubing connected to a personal sampling pump. Position the sampling device vertically with the face of the filter cassette down. This will prevent channeling of the sorbent beds. Upon completion of the sampling, transfer the particulate filter to a clean glass container, covered with foil and sealed with Teflon-lined screw-cap vials. Note sampling flow, length of sampling period, and ambient temperature and pressure. Store the samples in a refrigerator and protect from light until they are analyzed.

6.2 Analysis

Prior to use for sample fractionation the TLC plates must be conditioned by exposure to solvent C. Develop a plate with solvent C. In about 3 hours the solvent front will be within 1 cm of the top of the plate.

Transfer the filter to a 20 mL scintillation vial. Add 5 mL of cyclohexane. After insuring that the filter is covered by the solvent, seal the vial and agitate it in an ultrasonic water bath for 1 hour. Filter the sample solution through a 0.5 μm pore size polyperfluoroethylene filter using positive nitrogen pressure. Collect the filtrate in a clean 20 mL scintillation vial. About 4 mL of the solution is recovered.

Transfer a 3 mL aliquot of the sample solution to the barrel of a 10 mL syringe fitted with a silica gel cartridge. Insert the plunger and force

the solution through the silica gel bed over a 10-s period. Discard the eluate. In order to prevent disturbance of the silica gel bed, before removing the plunger for the addition of solvent D, remove the silica gel bed. Add 3 mL of solvent D to the syringe, and force it through the silica gel bed, collecting the eluate in a 5 mL vial. Add an additional 2 mL of solvent D to the syringe and force it through the silica gel bed, combining the eluate with the previous 3 mL.

With the water bath set around 60°C, concentrate the sample solution to about 2 mL under a gentle stream of nitrogen. Add 1 mL of acetonitrile and continue the concentration until the volume has been reduced to about 0.1 mL. In order to minimize sample loss, DO NOT ALLOW RESIDUE TO FORM ON THE SIDES OF THE VIAL OR ALLOW THE SAMPLE TO GO TO DRYNESS. Remove the developed blank TLC plate from the developing chamber and allow it to dry approximately 20 min. Spot the sample in 10 μL or smaller aliquots at the origin, centered about 4 cm from the bottom of the plate. Rinse the vial two times with 25 μL portions of acetonitrile, each time spotting the washing with the sample. Five samples can be run on a plate. Include in these samples one blank and one control sample of sufficient concentration that the analyte will be visible when viewed under short-wave UV light.

At 30 minutes past the time the blank TLC plate was removed from the developing chamber, return the spotted plate to the chamber and develop the chromatogram with solvent C. If the TLC plate becomes too dry prior to development of the chromatogram, the polycyclic aromatics will move with the solvent front. After removing the developed TLC plate from the tank, visualize the spots of the chromatograms by exposing the plate to short-wave UV light. There is no need to dry the TLC plate. Using the control sample as a reference, mark spots or areas corresponding to the analytes for each of the five samples. The polycyclic aromatics usually are located between R_f 0.62 and R_f 0.81.

Using the zone collector described in Section 5.2.19, remove the cellulose containing the polynuclear aromatic fraction of the samples from the TLC plate. Transfer the cellulose and the filter and glass tube of the spot collector to a culture tube. Add 2 mL of acetonitrile. After insuring that

all of the cellulose is covered with acetonitrile, agitate the test tube in an ultrasonic bath for 1 hour.

Centrifuge with sample. Transfer a 200 μL aliquot of the supernatant solution to an auto sampler vial for LC analysis.

Set up the LC system for the following conditions:

Mobile phase flow	1.0 L/min
Mobile phase	solvents A and B
Mobile phase program	
injection to 37 min	linear increase in solvent B from 30% to 100%
37 min to 55 min	100% solvent B
55 min to 75 min	30% solvent B
Column temperature	28°C

Under these conditions the retention times were:

fluoranthene	12.6 min
pyrene	14.0 min
benz(a)anthracene	20.1 min
chrysene	22.1 min
benzo(e)pyrene	25.5 min
benzo(a)pyrene	32.2 min

Inject a 10 μL aliquot of the sample into the LC system and begin the solvent program. Use of an auto sampler to run a series of samples and standards at regular intervals maximizes the reproducibility of the chromatography. Determine the area of the analyte chromatographic peaks using the laboratory data system. Use the detector monitoring emission $\geq$370 nm for the chrysene measurement; use the other detector for obtaining measurements of the other five analytes. Check to see that the level of each analyte falls within the range of the standard curve.

If the levels of one or more components is above the range of the standard curve, dilute an aliquot to an appropriate concentration and rerun the sample as above. If the levels of one or more components is below the range of the standard curve, continue with the procedure below. Recombine the remainder of the 200 μL aliquot in the auto sampler vial with the bulk of the sample in the culture tube. Filter the sample under positive nitrogen

pressure through a polyperfluoroethylene filter, collecting the filtrate in a graduated centrifuge tube. Rinse the residue with four 2 mL portions of acetonitrile, adding the rinsings to the first filtrate. Using the vial heater set around 120°C, reduce the volume of the solution to below 0.5 mL under a gentle stream of nitrogen. Cool the sample to room temperature. Adjust the sample volume to 0.5 mL with acetonitrile. Inject a 125 µL aliquot into the LC system and begin the solvent program. Analyze the samples under the same chromatographic conditions as described above.

6.3 Calibration and Standards

Prepare stock solutions of each polycyclic aromatic in acetonitrile at the following concentrations:

fluoroanthrene	1 mg/mL
pyrene	1 mg/mL
benz(a)anthracene	1 mg/mL
chrysene	0.5 mg/mL
benz(e)pyrene	1 mg/mL
benzo(a)pyrene	0.5 mg/mL

Prepare a combined stock solution at the concentration 0.1 mg/mL by transferring 2 mL aliquots of the chrysene and benzo(a)pyrene stock solutions and 1 mL aliquots of the others to a 10 mL volumetric flask and diluting to the mark with acetonitrile. This solution may be stored in a refrigerator if transferred to a 20 mL scintillation vial, which is capped and wrapped in aluminum foil. Using the combined stock solution, prepare five or six standards covering the range 1-25 µg/mL. Use these standards for the analysis of the dilute samples. With the same combined stock solution, prepare five or six standards covering the range 0.1-5 µg/mL. Use these standards for the analysis of the concentrated samples.

Analyze the standards with the samples. Construct calibration curves of peak area plotted against concentration for each analyte. Prepare separate curves for the analysis of dilute and concentrated samples.

6.4 Calculations

Read the concentration c (µg/mL) of the analyte in the solution from the appropriate calibration curve. Calculate the amount w (µg) of analyte in the sample by the equation:

$$w = \frac{c \times v \times F}{0.6}$$

where

v = volume of solution containing the sample workup (either 2 mL or 0.5 mL)
F = dilution factor, if sample diluted in step III-C.14
0.6 = fraction of total sample submitted to workup and analysis

Subtract from w contributions of the blank to give W.

Calculate the air concentration C (mg/m^3) of the analyte as follows:

$$C = \frac{W}{V}$$

where

V = volume (L) of air sampled

7.0 Quality Assurance Program

7.1 Quality Control

Laboratory and field blanks are prepared and analyzed as a check on background contamination. Two of each will be analyzed. The filters for the blanks will be obtained from the same lot as the filters used for field samples. One duplicate sample will be taken and analyzed as a means of checking method reproducibility.

7.2 Quality Assurance

Of the eleven samples to be collected and analyzed, two will be shipped to the QA laboratory (NIOSH, Cincinnati) for analysis, and comparison of results with those obtained at the primary lab.

ANALYTICAL PROTOCOL: ORGANOCHLORINE PESTICIDES AND PCBs IN DRINKING WATER

1.0 Principal of Method

This method covers the determination of certain organochlorine pesticides and polychlorinated biphenyls (PCBs) found in drinking water. The following compounds may be determined by this method:

Aldrin	Endrin
a-BHC	Endrin Aldehyde
b-BHC	Heptachlor
d-BHC	Heptachlor Epoxide
g-BHC	Toxaphene
Chlordane	PCB-1016
4,4'-DDD	PCB-1221
4,4'-DDE	PCB-1232
4,4'-DDT	PCB-1242
Dieldrin	PCB-1248
Endosulfan I	PCB-1254
Endosulfan II	PCB-1260
Endosulfan Sulfate	

A 1-liter sample of wastewater is extracted with methylene chloride using separatory funnel techniques. The extract is dried and concentrated to a volume of 10 mL or less. Chromatographic conditions are described which allow for the accurate measurement of the compounds in the extract. If interferences are encountered, the method provides selected general purpose cleanup procedures to aid the analyst in their elimination.

This method is recommended for use only by experienced residue analysts or under the close supervision of such qualified persons.

2.0 Range and Detection Limit

The sensitivity of this method is usually dependent upon the level of interferences rather than instrumental limitations. The limits of detection listed in Table 1 represent sensitivities that can be achieved in wastewaters.

3.0 Interferences

Solvents, reagents, glassware, and other sample processing hardware may yield discrete artifacts and/or elevated baselines causing misinterpretation

Table 1. GAS CHROMATOGRAPHY OF PESTICIDES AND PCB's

Parameter	Retention time (min) Column 1	Column 2	Detection limit (µg/L)**
Aldrin	2.40	4.10	0.003
a-BHC	1.35	1.82	0.002
b-BHC	1.90	1.97	0.004
d-BHC	2.15	2.20	0.004
g-BHC	1.70	2.13	0.002
Chlordane	*	*	0.04
4,4'-DDD	7.83	9.08	0.012
4,4'-DDE	5.13	7.15	0.006
4,4'-DDT	9.40	11.75	0.016
Dieldrin	5.45	7.23	0.006
Endosulfan I	4.50	6.20	0.005
Endosulfan II	8.00	8.28	0.01
Endosulfan sulfate	14.22	10.70	0.03
Endrin	6.55	8.10	0.009
Endrin aldehyde	11.82	9.30	0.023
Heptachlor	2.00	3.35	0.002
Heptachlor epoxide	3.50	5.00	0.004
Toxaphene	*	*	0.40
PCB-1016	*	*	0.04
PCB-1221	*	*	0.10
PCB-1232	*	*	0.10
PCB-1242	*	*	0.05
PCB-1248	*	*	0.08
PCB-1254	*	*	0.08
PCB-1260	*	*	0.15

* Multiple peak response. See Figures 2-10.

** Detection limit is calculated from the minimum detectable GC response being equal to five times the GC background noise, assuming a 10 ml final volume of the 1 liter sample extract, and assuming a GC injection of 5 microliters.

Column 1 conditions: Supelcoport 100/120 mesh coated with 1.5% SP-2250/1.95% SP-2401 packed in a 180 cm long x 4 mm ID glass column with 5% Methane/95% Argon carrier gas at 60 ml/min flow rate. Column temperature is 200°C.

Column 2 conditions: Supelcoport 100/120 mesh coated with 3% OV-1 in a 180 cm long x 4 mm ID glass column with 5% Methane/95% Argon carrier gas at 60 ml/min flow rate. Column temperature is 200°C.

of gas chromatograms. All of these materials must be demonstrated to be free blanks. Specific selection of reagents and purification of solvents by distillation in all-glass systems may be required. Interferences coextracted from the samples will vary considerably from source to source, depending upon the source of the water being sampled. While general cleanup techniques are provided as part of this method, unique samples may require additional cleanup approaches to achieve the sensitivities stated in Table 1.

Glassware must be scrupulously clean. Clean as soon as possible after use by rinsing with the last solvent used. This should be followed by detergent washing in hot water. Rinse with tap water, distilled water, acetone and finally pesticide quality hexane. Heavily contaminated glassware may require treatment in a muffle furnace at 400°C for 15 to 30 minutes. Some high boiling materials, such as PCBs, may not be eliminated by this treatment. Volumetric ware should not be heated in a muffle furnace. Glassware should be stored immediately after drying or cooling to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.

Interferences by phthalate esters can pose a problem in the 15% and 50% fractions from the Florisil fractionation. These interferences can be minimized by avoiding contact with any plastic materials. The presence of phthalate esters is indicated in samples that respond to electron capture detectors but not to microcoulometric or electrolytic conductivity (halogen mode) detectors.

4.0 Precision and Accuracy

The results of studies delineating the precision and accuracy of this method have not yet been determined. The sensitivity of this method with the EPA (HERL-RTP) protocol for response in river water, which is similar to the protocol outlined here, indicates acceptable levels of precision for recovery of the chlorinated pesticides and PCBs would be obtained.

5.0 Apparatus and Reagents

5.1 Sampling Equipment (for discrete or composite sampling)

5.1.1 Grab sample bottle - amber glass, liter or quart volume. French or Boston Round design is recommended. The container must be washed and solvent rinsed before use to minimize interferences.

5.1.2 Bottle Caps - Threaded to screw on sample bottles. Caps must be lined with Teflon. Foil may be substituted if sample is not corrosive.

5.1.3 Compositing equipment - Automatic or manual compositing system. Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated during sampling. No Tygon or rubber tubing or fittings may be used in the system.

5.2 Separatory funnel - 2000 mL, with Teflon stopcock.

5.3 Drying column - A 20 mm ID pyrex chromatographic column with coarse frit.

5.4 Kuderna-Danish (K-D) Apparatus

5.4.1 Concentrator tube - 10 mL, graduated (Kontes K-570050-1025 or equivalent). Calibrations must be checked at 1.0 and 10.0 mL level. Ground glass stopper (size 19/22 joint) is used to prevent evaporation of extracts.

5.4.2 Evaporative flask - 500 mL (Kontes K-57001-0500 or equivalent). Attach to concentrator tube with springs. (Kontes K-662750-012)

5.4.3 Snyder column - three-ball macro (Kontes K503000-0121 or equivalent).

5.4.4 Boiling chips-extracted, approximately 10/40 mesh.

5.5 Water Bath - Heated, with concentric ring cover, capable of temperature control (± 2°C). The bath should be used in a hood.

5.6 Gas chromatograph - Analytical system complete with gas chromatograph suitable for on-solumn injection and all required accessories including electron capture or halogen-specific detector, column supplies, recorder, gases, syringes. A data system for measuring peak areas is recommended.

5.7 Chromatographic column - Pyrex, 400 mm x 25 mm OD, with coarse fritted plate and Teflon stopcock (Kontes K-42054-213 or equivalent).

5.8 Preservatives:

5.8.1 Sodium hydroxide - (ACS) 10 N in distilled water.

5.8.2 Sulfuric acid - (ACS) Mix equal volumes of conc. H_2SO_4 with distilled water.

5.9 Methylene chloride - Pesticides quality or equivalent.

5.10 Sodium Sulfate - (ACS) Granular, anhydrous (purified by heating at 400°C for 4 hrs.) especially just prior to preparing working standards from them.

5.11 Boiling chips - Hengar granules (Hengar Co.; Fisher Co.) or equivalent.

5.12 Mercury - triple distilled.

5.13 Aluminum oxide - basic or neutral, active.

5.14 Hexane - pesticide residue analysis grade.

5.15 Isooctane (2,2,4-trimethyl pentane) - pesticide residue analysis grade.

5.16 Acetone - pesticide residue analysis grade.

5.17 Diethyl ether - preserved with 2% ethanol.

5.17.1 Must be free of peroxides as indicated by EM Quant test strips (EM Laboratories, Inc., 500 Executive Blvd., Elmsford, N.Y. 10523).

5.17.2 If test indicates, remove peroxides by eluting over basic or neutral grade aluminum oxide. Retest before using.

5.18 Florisil - PR grade (60/100 mesh); purchase activated at 1250°F and store in glass containers with glass stoppers or foil-lined screw caps. Before use activate each batch at least 16 hours at 130°C in a foil covered glass container.

5.19 Stock standards - Prepare stock standard solutions at a concentration of 1.00 µg/µL by dissolving 0.100 grams of assayed reference material in pesticide quality isooctane or other appropriate solvent and diluting to volume in a 100 mL ground glass stoppered volumetric flask. The stock solution is transferred to ground glass stoppered reagent bottles, stored in a refrigerator, and checked frequently for signs of degradation or evaporation.

6.0 Procedure

6.1 Sample Collection

Grap samples must be collected in glass containers. Conventional sampling pratcies should be followed, except that the bottle must not be prewashed with sample before collection. Composite samples should be

collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be free of Tygon and other potential sources of contamination. The samples must be iced or refrigerated from the time of collection until extraction. Chemical preservatives should not be used in the field unless more than 24 hours will elapse before delivery to the laboratory. If the samples will not be extracted within 48 hours of collection, the sample should be adjusted to a pH range of 6.0-8.0 with sodium hydroxide or sulfuric acid. All samples must be extracted within 7 days and completely analyzed within 30 days of collection.

6.2 Sample Extraction

Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a two-liter separatory funnel. Check the pH with wide-range paper and adjust to within the range of 5-9 with sodium hydroxide or sulfuric acid. Add 60 mL methylene chloride to the sample bottle and shake 30 seconds to rinse the walls. Transfer the solvent into the separatory funnel, and extract the sample by shaking the funnel for two minutes with periodic venting to release vapor pressure. Allow the organic layer to separate from the water phase for a minimum of ten minutes. If the emulsion interface between layers is more than one-third the size of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, or centrifugation. Collect the methylene chloride extract in a 250-mL Ehrlenmeyer flask.

Add a second 60-mL volume of methylene chloride to the sample bottle and complete the extraction procedure a second time, combining the extracts in the Ehrlenmeyer flask.

Perform a third extraction in the same manner. Pour the combined extract through a drying column containing 3-4 inches of anhydrous sodium sulfate, and collect it in a 500-mL Kuderna-Danish (K-D) flask equipped with a 10 mL concentrator tube. Rinse the Ehrlenmeyer flask and column with 20-30 mL methylene chloride to complete the quantitative transfer.

Add 1-2 clean boiling chips to the flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL methylene chloride to the top. Place the K-D apparatus on a steaming hot (60-65°C) water bath so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed in steam. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15-20 minutes. At the proper rate of distillation the balls of the column will actively chatter but the chambers will not flood. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain for at least 10 minutes while cooling.

Increase the temperature of the hot water bath to about 80°C. Momentarily remove the Snyder column, add 50 ml of hexane and a new boiling chip and reattach the Snyder column. Pour about 1 mL of hexane into the top of the Snyder column and concentrate the solvent extract as before. Elapsed time of concentration should be 5 to 10 minutes. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain at least 10 minutes while cooling. Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1-2 mL of hexane, and adjust the volume to 10 mL. A 5-mL syringe is recommended for this operation. Stopper the concentrator tube and store refrigerated if further processing will not be performed immediately. If the sample extract requires no further cleanup, proceed with gas chromatographic analysis. If the sample requires cleanup, proceed to 6.3

Determine the original sample volume by refilling the sample bottle to the mark and transferring the liquid to a 1000 mL graduated cylinder. Record the sample volume to the nearest 5 mL.

6.3 Cleanup and Separation

Cleanup procedures are used to extend the sensitivity of a method by minimizing or eliminating interferences that mask or otherwise disfigure the gas chromatographic response to the pesticides and PCBs. The Florisil column allows for a select fracionation of the compounds

and will eliminate polar materials. Elemental sulfur interferes with the electron capture gas chromatography of certain pesticides and can be removed by the techniques described below.

6.3.1 Florisil Column Cleanup

Add a weight of Florisil, nominally 21 g, but predetermined by calibration, to a chromatographic column. Settle the Florisil by tapping the column. Add sodium sulfate to the top of the Florisil to form a layer 1-2 cm deep. Add 60 mL of hexane to wet and rinse the sodium sulfate and Florisil. Just prior to exposure of the sodium sulfate to air, stop the draining of the hexane by closing the stopcock on the chromatography column. Discard the eluate.

Add the sample extract from the K-D concentrator tube to the Florisil column. Rinse the tube twice with 1-2 mL hexane, adding each rinse to the column. Place a 500 mL K-D flask and clean concentrator tube under the chromatography column. Drain the column into the flask until the sodium sulfate layer is nearly exposed. Elute the column with 200 mL of 6% ethyl ether in hexane (Fraction 1) using a drip rate of about 5 mL/min. Remove the K-D flask and set aside for later concentration. Elute the column again, using 200 mL of 15% ethyl ether in hexane (Fraction 2), into a second K-D flask. Perform the third elution using 200 mL of 50% ethyl ether in hexane (Fraction 3). The elution patterns for the pesticides and PCBs are shown in Table 2.

Concentrate the eluates by standard K-D techniques (6.2), substituting hexane for methylene chloride and using the water bath at about 85°C. Adjust final volume to 10 mL with hexane. Analyze by gas chromatography.

Elemental sulfur will usually elute entirely in Fraction 1. To remove sulfur interference from this fraction or the original extract, pipet 1.00 mL of the concentrated extract into a clean concentrator tube or Teflon-sealed vial. Add 1-3 drops of mercury and seal. Agitate the contents of the vial for 15-30 seconds. Place the vial in an upright position on a reciprocal laboratory shaker and shake for 2 hours. Analyze by gas chromatography.

Table 2. DISTRIBUTION AND RECOVERY OF CHLORINATED PESTICIDES AND PCBs USING FLOROSIL COLUMN CHROMATOGRAPHY

Parameter	Recovery (%) by fraction* 1(6%)	2(15%)	3(50%)
Aldrin	100		
a-BHC	100		
b-BHC	97		
d-BHC	98		
g-BHC	100		
Chlordane	100		
4,4'-DDD	99		
4,4'-DDE	98		
4,4'-DDT	100		
Dieldrin	0	100	
Endosulfan I	37	64	
Endosulfan II	0	7	91
Endosulfan sulfate	0	0	106
Endrin	4	96	
Endrin aldehyde	0	68	26
Heptachlor	100		
Heptachlor epoxide	100		
Toxaphene	96		
PCB-1016	97		
PCB-1221	97		
PCB-1232	95	4	
PCB-1242	97		
PCB-1248	103		
PCB-1254	90		
PCB-1260	95		

*From: "Development and Application of Text Procedures for Specific Organic Toxic Substances in Wastewaters. Category 10-Pesticides and PCB's. Report for EPA Contract 68-03-2606.

6.4 Analysis

6.4.1 Gas Chromatography

Table 1 summarizes some recommended gas chromatographic column materials and operating conditions for the instrument. Included in this table are estimated retention times and sensitivites that should be achieved by this method. Examples of the separations achieved by these columns are shown in Figures 1 through 10. Calibrate the system daily with a minimum of three injections of calibration standards.

Inject 2-5 µL of the sample extract using the solvent-flush technique. Smaller (1.0 µL) volumes can be injected if automatic devices are employed. Record the volume injected to the nearest 0.05 µL, and the resulting peak size, in area units. If the peak area exceeds the linear range of the system, dilute the extract and reanalyze. If the peak area measurement is prevented by the presence of interferences, further cleanup is required.

6.4.2 Calibration

Prepare calibration standards that contain the compounds of interest, either singly or mixed together. The standards should be prepared at concentrations covering two or more orders of magnitude that will completely bracket the working range of the chromatographic system. If the sensitivity of the detection system can be calculated from Table 1, as 100 µg/L in the final extract for example, prepare standards at 10 µg/L, 50 µg/L, 100 µg/L, 500 µg/L, etc., so that injections of 1-5 µL of each calibration standard will define the linearity of the detector in the working range.

Assemble the necessary gas chromatographic apparatus and establish operating parameters equivalent to those indicated in Table 1. By injecting calibration standards, establish the sensitivity limit of the detector and the linear range of the analytical system for each compound.

Before using any cleanup procedure, the analyst must process a series of calibration standards through the system to validate elution patterns and the absence of interferences from the reagents.

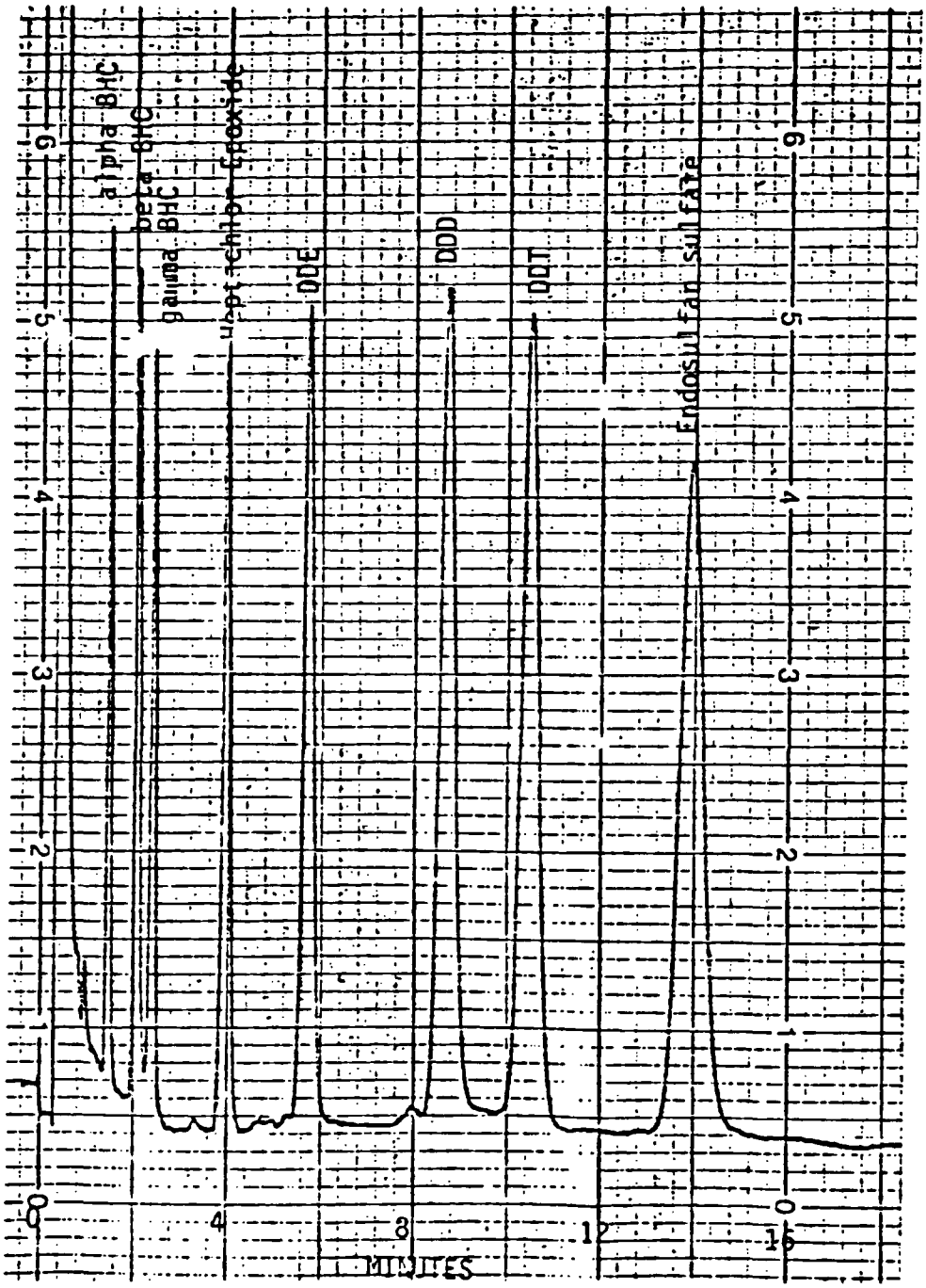


Figure 1. EC Gas Chromatography of Organochlorine Pesticides on Column 1. For Conditions, See Table I.

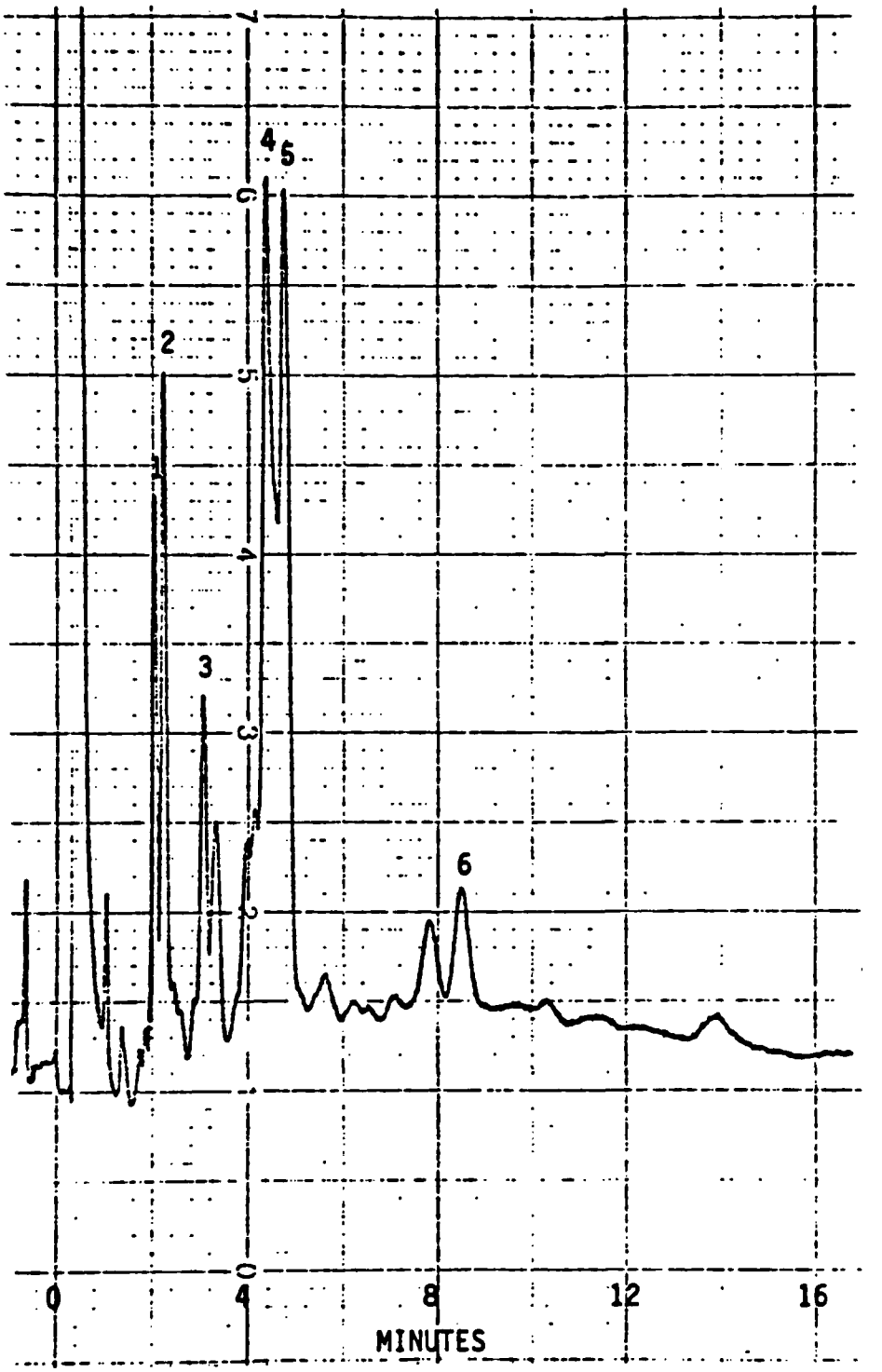


Figure 2. EC Gas Chromatography of Chlordane on Column 1. For Conditions, See Table I.

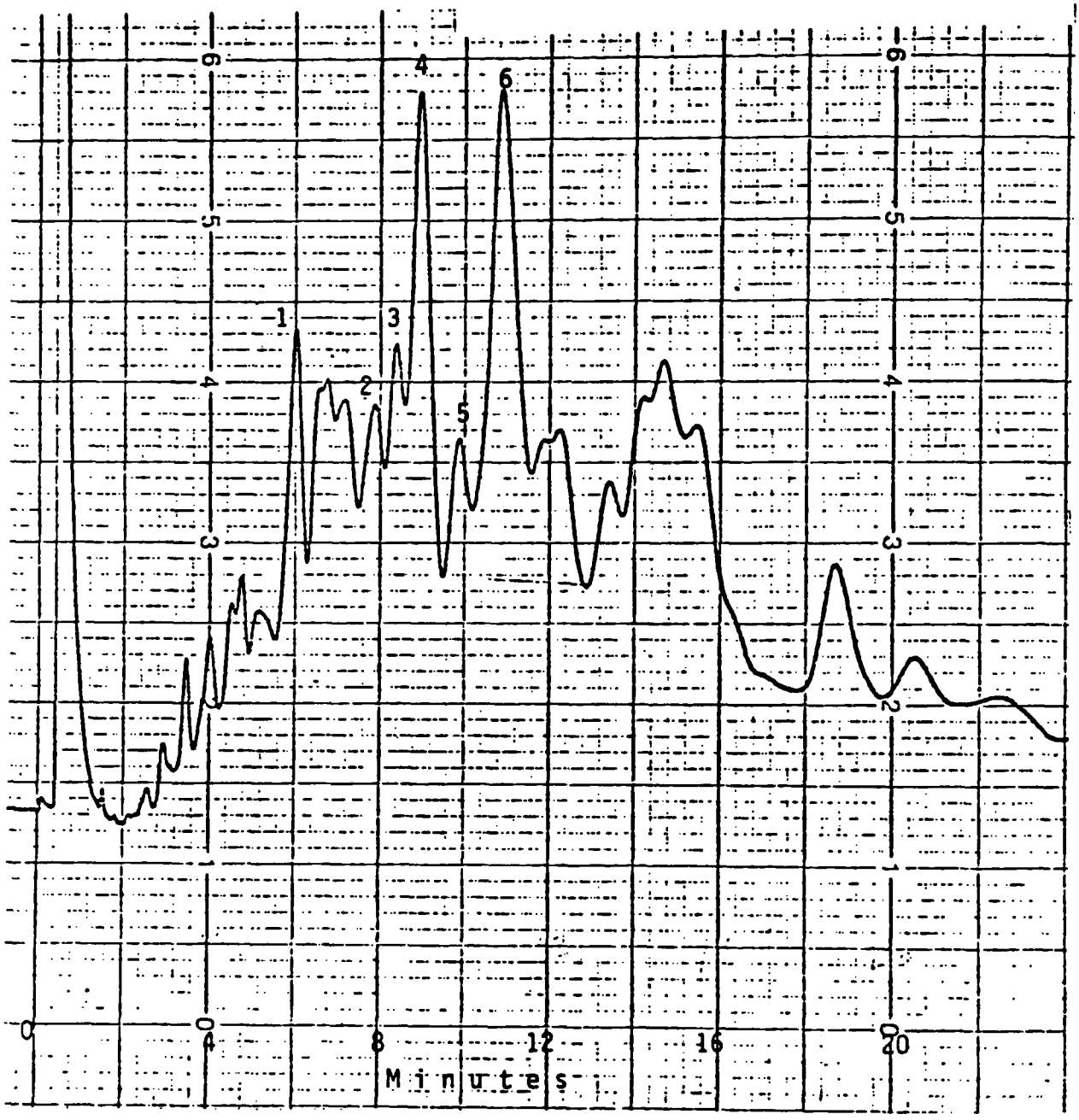


Figure 3. EC Gas Chromatography of Toxaphene on Column 1.
cFor Conditions, See Table I.

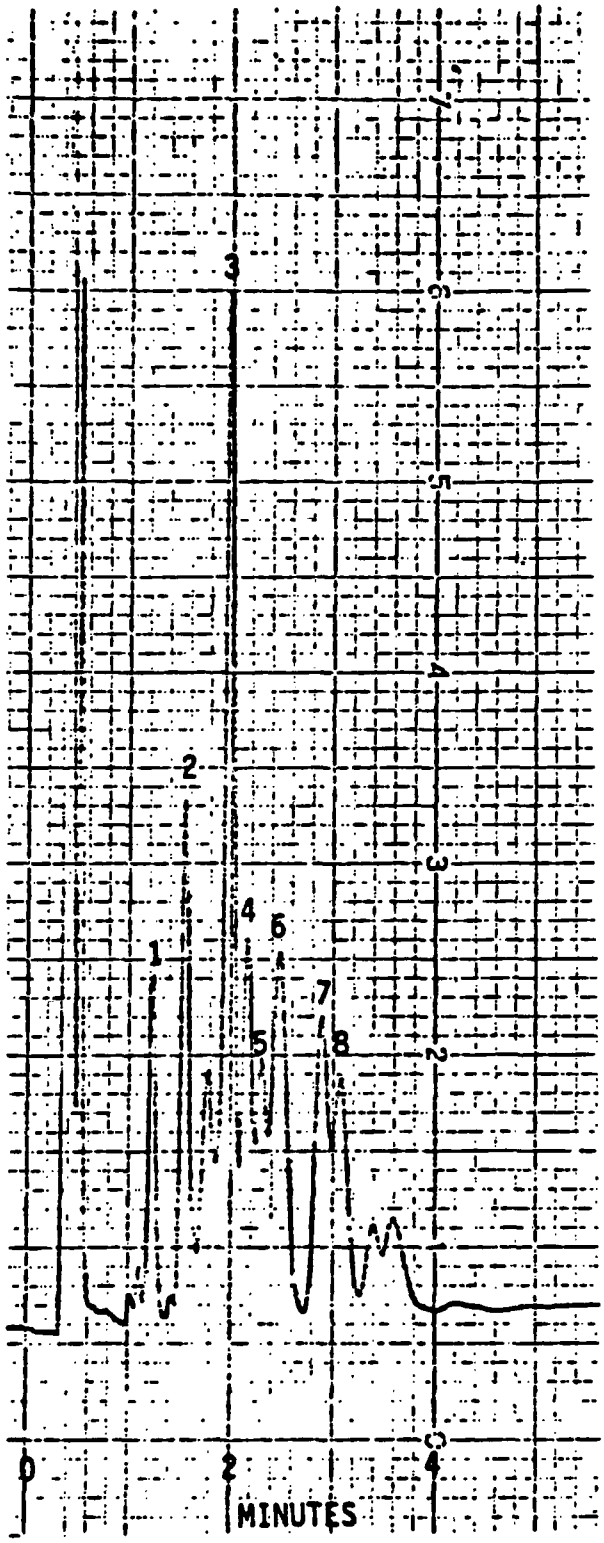


Figure 4. EC Gas Chromatography of PCB 1016 on Column 1. For conditions, see Table 1.

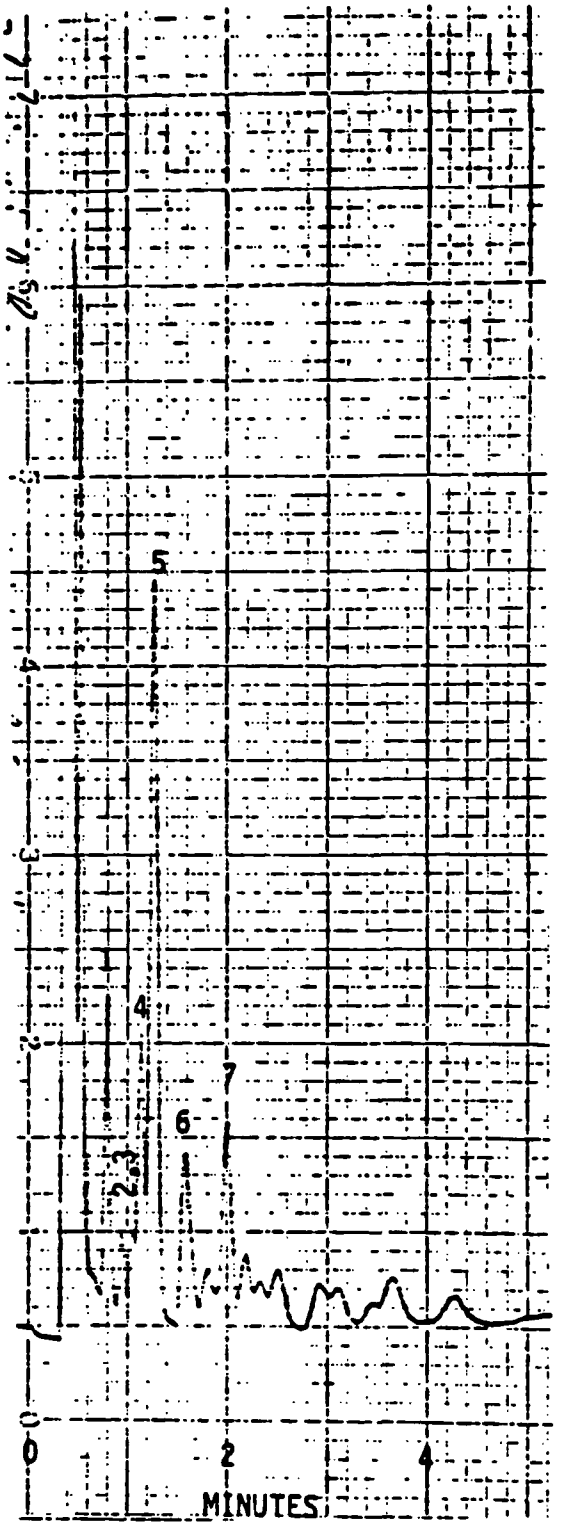


Figure 5. EC Gas Chromatography of PCB 1221 on Column 1. For conditions, see Table 1.

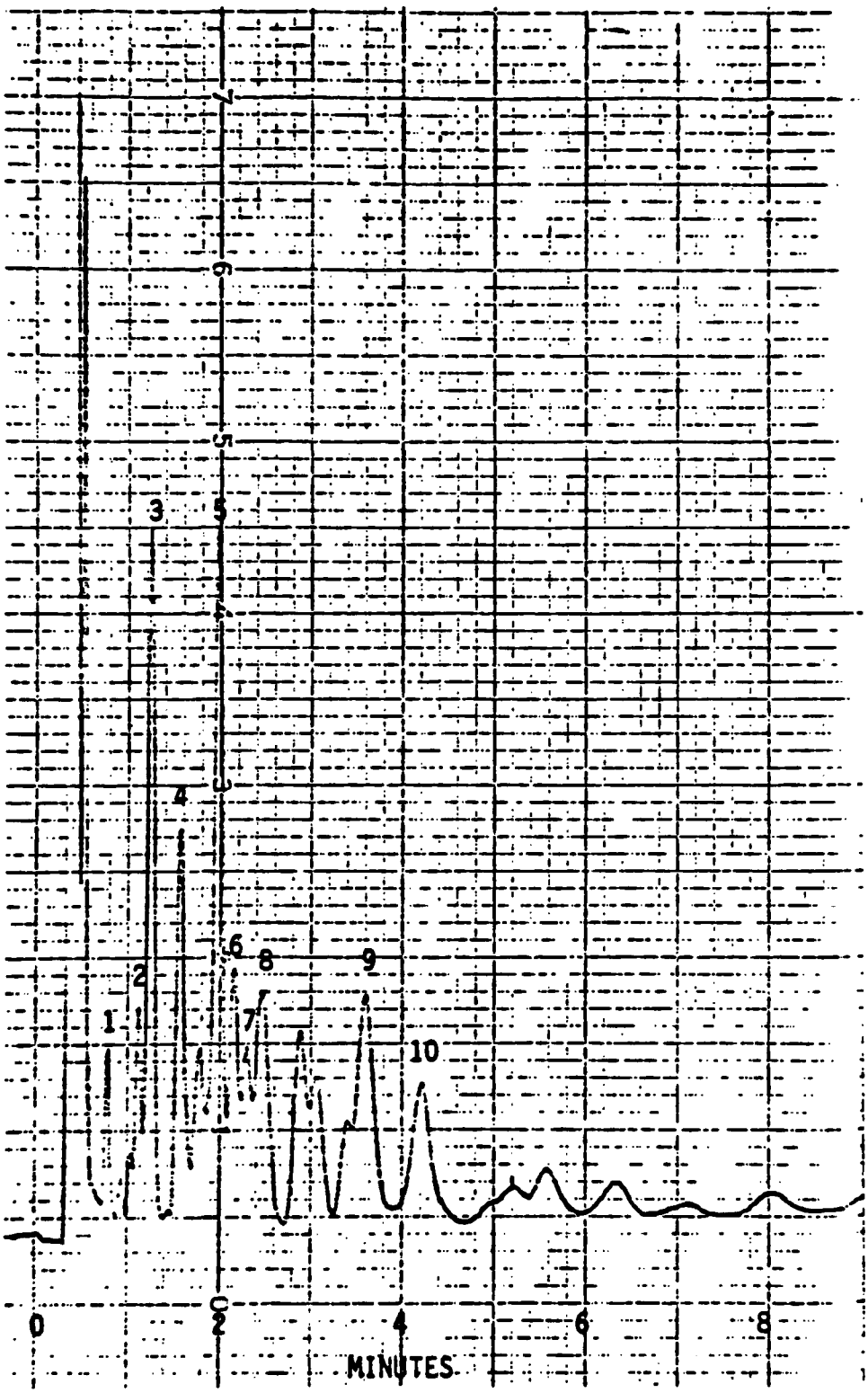


Figure 6. EC Gas Chromatography of PCB 1232 on Column 1. For conditions, see Table 1.

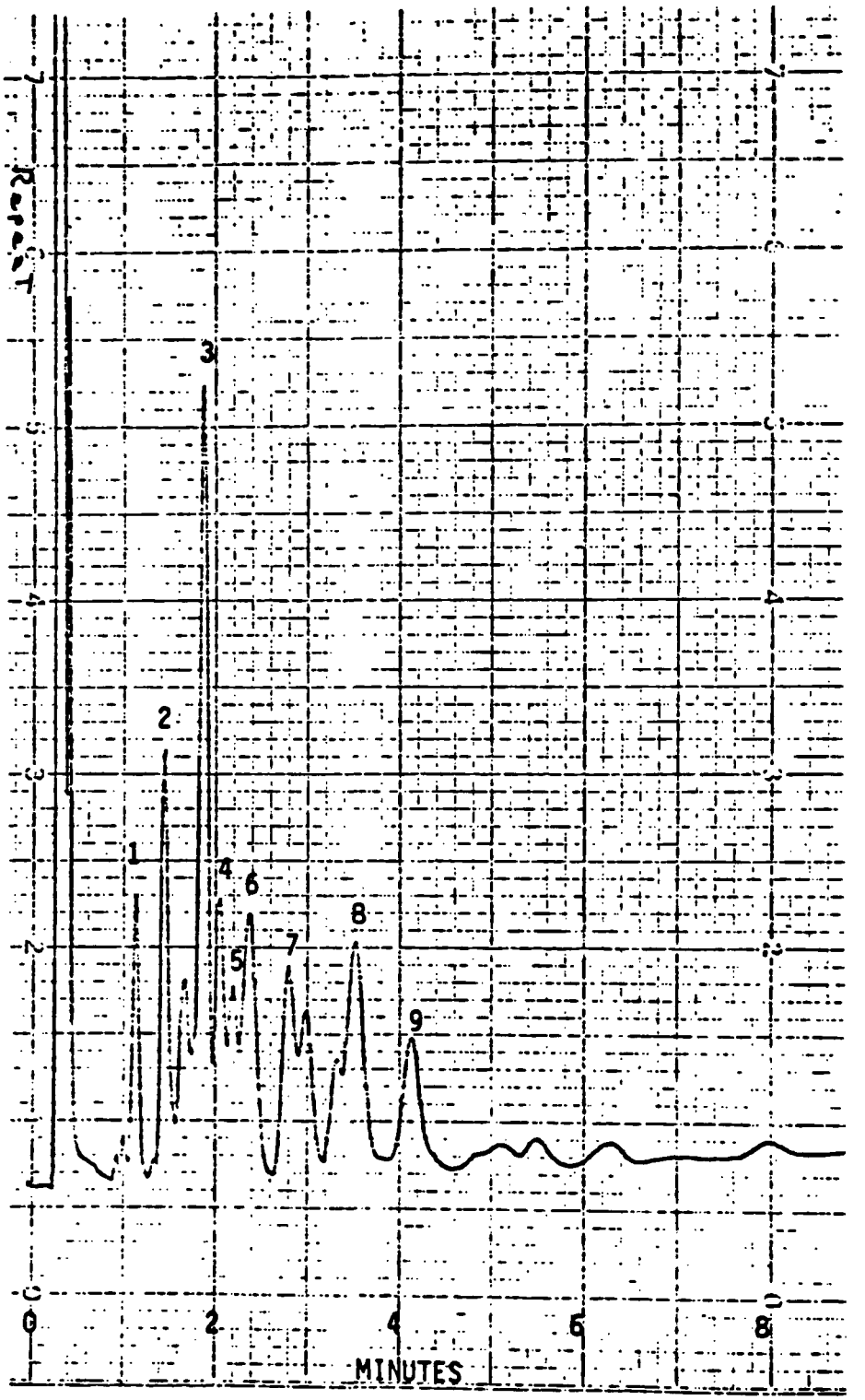


Figure 7. EC Gas Chromatography of PCB -1242 on Column 1. For Conditions, See Table I.

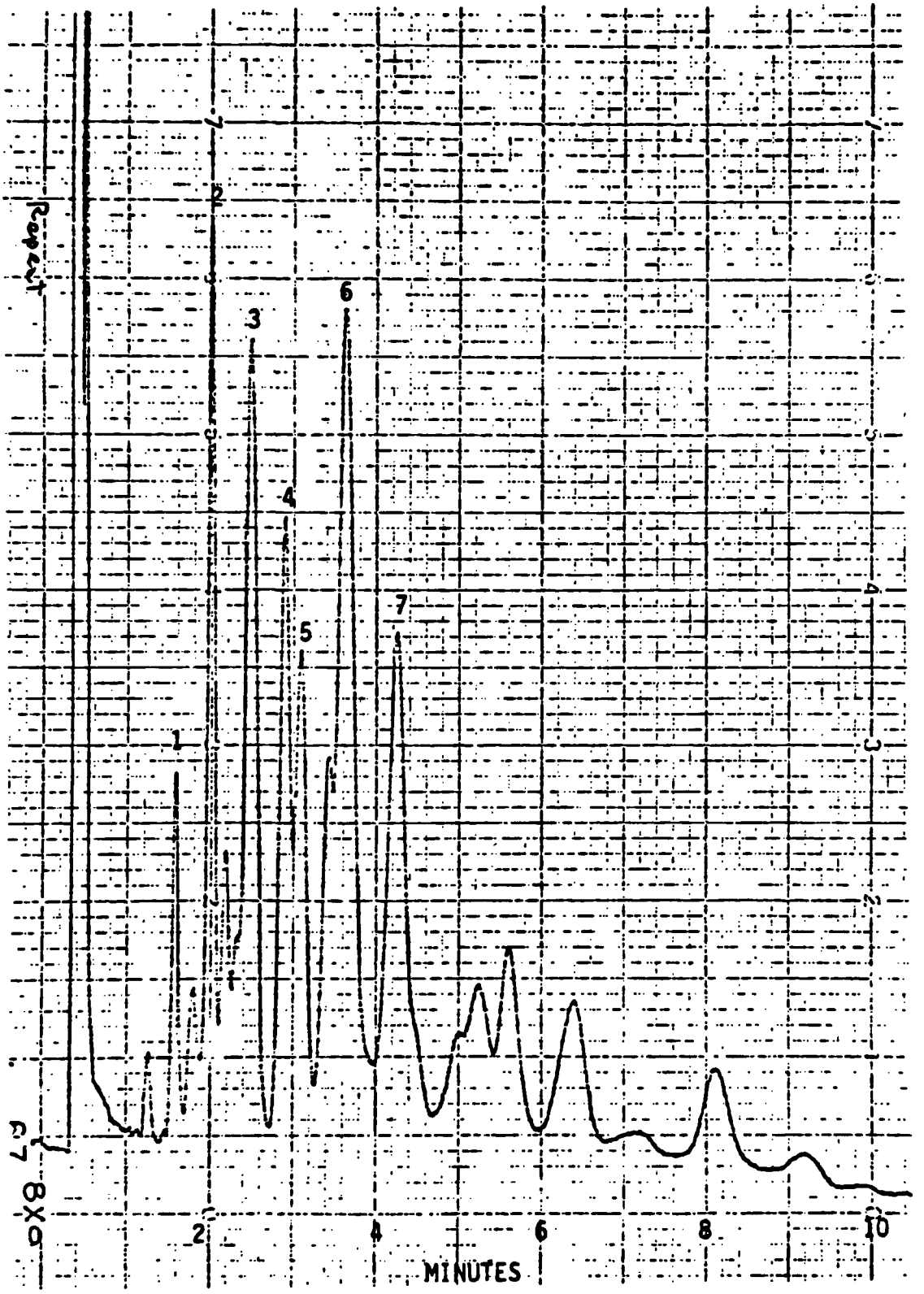


Figure 8. EC Gas Chromatography of PCB 1248 on Column 1. For conditions, see Table 1.

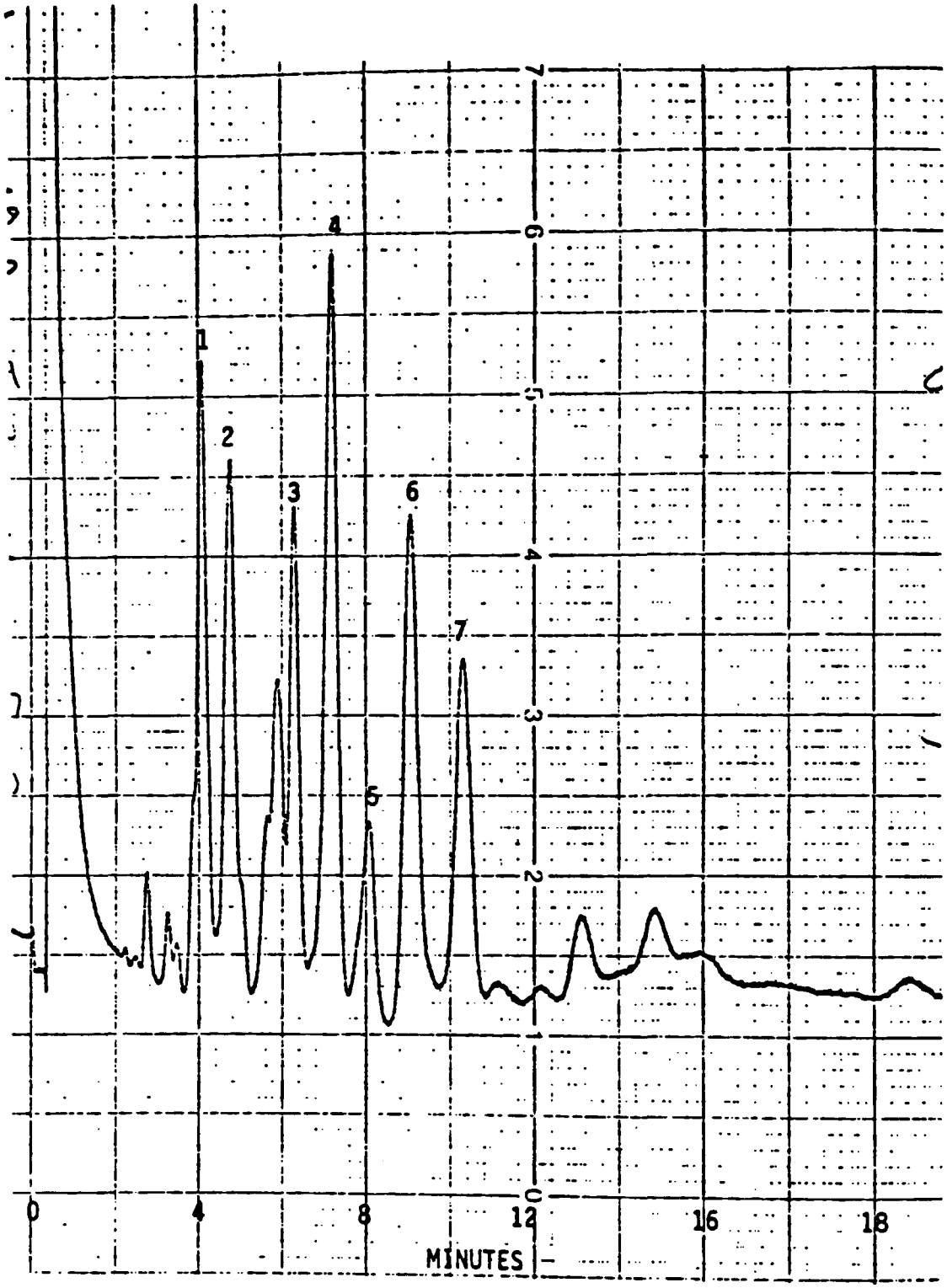


Figure 9. EC Gas Chromatography of PCB - 1254 on Column 1. For Conditions, See Table I.

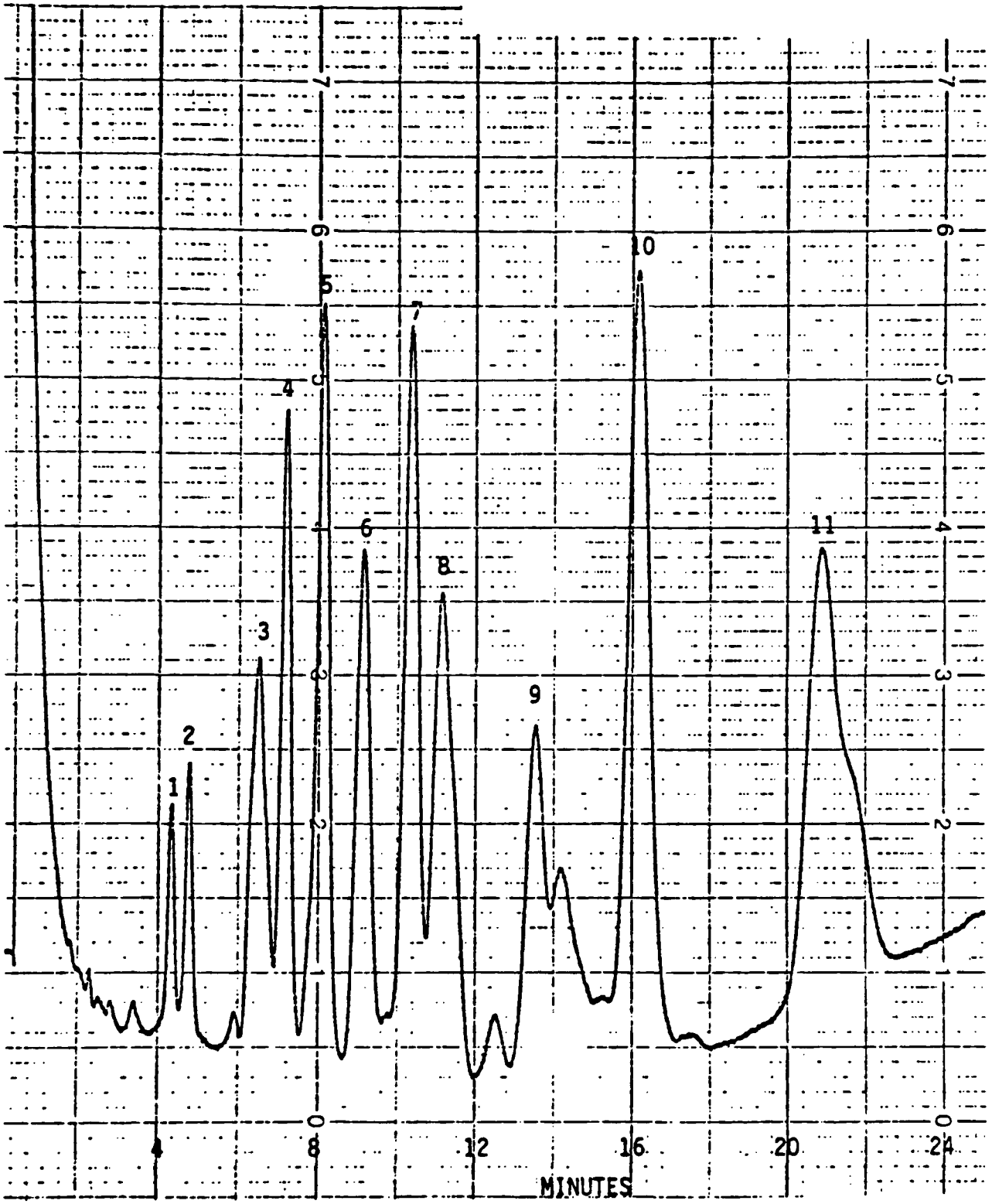


Figure 10. EC Gas Chromatography of PCB - 1260 on Column 1. For Conditions, See Table I.

6.4.3 Calculations

Determine the concentration of the individual compounds according to the formula:

$$\text{Concentration, } \mu g/L = \frac{(A)\ (B)\ (Vt)}{(V_i)\ \ (V_s)}$$

where A = Calibration factor for chromatographic system, in nanograms material per area unit.

B = Peak size in injection of sample extract, in area units

V_i = Volume of extract injected (μL)

V_t = Volume of total extract (μL)

V_s = Volume of water extracted (mL)

Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, all data obtained should be reported.

7.0 Quality Control

Before processing any samples, the analyst should demonstrate through the analysis of a distilled water method blank, that all glassware and reagents are interference-free. Each time a set of samples is extracted or there is a change in reagents, a method blank should be processed as a safeguard against chronic laboratory contamination.

Standard quality assurance practices should be used with this method. Field replicates should be collected to validate the accuracy of the analysis Where doubt exists over the identification of a peak on the chromatogram, confirmatory techniques such as mass spectroscopy should be used.

8.0 References

8-1 "Development and Application of Test Procedures for Specific Organic Toxic Substances in Wastewaters. Category 10-Pesticides and PCBs." Report for EPA Contract 68-03-2606.

ANALYTICAL PROTOCOL: DETERMINATION OF ORGANOCHLORIDE PESTICIDES AND METABOLITES IN DRINKING WATER (U. OF MIAMI)

1.0 Principle of the Method

Drinking water is extracted with methylene chloride and the extract is concentrated. The concentrate is eluted through deactivated silica gel first with hexane as the solvent and then with benzene/hexane, 3:2. The two fractions are analyzed by GC/ECD for twelve pesticides and metabolites: hexachlorobenzene, β-hexachlorocyclohexane (β-BNC), heptachlor, oxychlordane, heptachlor epoxide, trans-nonachlor, p,p'-DDE, dieldrin, o,p-DDT, p,p'-DDT, α-chlordane and δ-chlordane.

2.0 Range and Detection Limit

Although the linear dynamic range of an ECD is not very large the quantitative range of detection can be greatly expanded by successive dilution of the extracts. Theoretically this can make the range virtually infinite. The detection limits for the pesticides sought in this study are listed in Table 1.

3.0 Interferences (1)

Interferences in sample analysis and quantification using GC/ECD are manifested in the electron capturing ability of the given contaminant. The relative purity of the water matrices (i.e., residential drinking water) minimizes large interferences.

4.0 Precision and Accuracy

No data applicable to precision measurement was not presented in this study. Recovery studies performed in duplicate on the pesticides of interest is presented in Table 2. The blank control for this study showed none of the compounds of interest in concentrations exceeding the limit of detection.

5.0 Apparatus and Readgents

5.1 Apparatus

1. A Tracor Model 220 gas chromatograph equipped with a ^{3}H electron capture detector.
2. Column: 6 ft x 6.35 mm I.D. glass U-tube packed with 1.5% OV-17 + 1.95% QF-1 on 100/120 Chromosorb W.

Table 1. PERCENT RECOVERY, DETECTOR SENSITIVITY AND LIMITS OF DETECTABILITY OF PESTICIDE AND METABOLITES IN WATER

Compound	Detector Sensitivity (pg)	Limit of Detectability (pptr)
β-HCH	6	25
Heptachlor	3	10
Oxychlordane	3	13
Heptachlor epoxide	5	18
trans-Nonachlor	4	15
p,p'-DDE	4	17
Dieldrin	8	30
o,p'-DDT	11	44
p,p'-DDT	14	56
HCB	2	6
γ-Chlordane	6	23
α-Chlordane	7	28

Table 2. PERCENT RECOVERY OF OC'S AND OC METABOLITES IN WATER

Compound	Fraction	Recovery (%)
β-HCH	11	88
Haptachlor	1	81
Oxychlordane	1	89
Heptachlor epoxide	11	86
trans-Nonachlor	1	93
p,p'-DDE	1	97
Dieldrin	11	93
o,p'-DDT	1	96
p,p'-DDT	1	96
HCB	1	79
γ-Chlordane	1	86
α-Chlordane	1	94
Mean		90

3. 2.2 cm x 30 cm Pyrex® filtering column
4. 125 mL separatory funnel
5. 15 mL centrifuge tubes
6. 7 mm i.d. ChromaFlex® column

5.2 Reagents

1. Methylene Chloride, Nanograde® from Mallinckrodt Chemicals
2. Hexane, Nanograde® from Mallinckrodt Chemicals
3. Deionized water washed twice with benzene
4. Benzene, Nanograde® from Mallinckrodt Chemicals
5. Na_2SO_4 aunydrous
6. "Keeper" solution of 1% USP paraffin oil/hexane
7. Silica Gel, Woelm, activity grade I from Waters Associates, Inc., deactivated with 20% water.

6.0 Procedure

6.1 Collection of Samples

Drinking water samples are collected from kitchen water faucets. Each 1 L sample is contained in a glass bottle with a ground glass stopper and stored, removed from any source of organo-chlorine pesticide, at 4°C until analyzed.

6.2 Extraction

1. Add 5 mL of methylene chloride to 50 mL of water in a 125 mL sep funnel and shake vigorously for 2 min.
2. Drain the organic (lower) phase through a 2.2 cm x 30 cm filter column with a 2 cm layer of Na_2SO_4 into a 15 mL conical centrifuge tube.
3. repeat 1 & 2
4. Rinse column with an additional 4 mL of solvent and combine all elutes.
5. Add 5 drops of "Keeper" (see Reagents) and concentrate to 0.5 mL under a stream of dry nitrogen at 40°C.
6. Prepare a silica gel column by adding to a 7 mm i.d. column plugged with a small pad of glass wool, 1 g of deactivated (20% water) silica gel. Settle the silica gel with firm tapping and top with 2.5 cm $NaSO_4$.

7. Wash column with 10 mL of hexane and discard. As the hexane reaches the Na_2SO_4 place a 15 mL centrifuge tube under the column.
8. Transfer sample to column using a disposable pipet, rinse concentrating tube with 1 mL of hexane and transfer wash to the column using same disposable pipet.
9. repeat 8 wash step 2 more times
10. Add 6.5 mL of hexane to the column and combine effluents.
11. Add 5 drops of "keeper", concentrate to 0.2 ml and redilute to 1 mL. This is fraction I to be analysed by GC/ECD. (see Table 2 for compounds eluted in this fraction
12. As soon as fraction I has eluted change to another centrifuge tube and add 15 mL of 3:2 benzene:hexane (V/V) to the column and elute. This is fraction II
13. Add 5 drops of keeper and concentrate to 0.2 ml, and redilute with hexane to 1 mL. This solution is then ready for analysis by GC/ECD.

6.3 Analysis

The GC operating conditions for the analysis of pesticides is as follows:

1. N_2 carrier flow rate of 60 mL/min
2. Injection port temperature: 215°C
3. Column temperature: 200°C (isothermal)
4. Detector temperature: 210°

These parameters were chosen to maximize column efficiency and minimize analysis time, the latest eluting pesticide, p,p'-DDT eluting in 18 min.

The usual injection was 10-μL and convenient electrometer settings were 10 x 8 and 10 x 16.

6.4 Qualitative Identification

Qualitative identification is made based on relative retention times and is subject to the limitations of this methodology (2).

6.5 Quantitation

Quantitation is based on peak heights for the early eluting sharp peaked compounds and on peak areas for the later eluting broader peaks (e.g., p,p'-DDT) (2).

7.0 Quality Assurance Program

An on-going quality assurance program is required to assure the data quality. Quality control (QC) procedures determine artifacts, losses, etc. through a system of blanks and controls. Quality assurance (QA) procedures monitor the execution of the procedure and check data interpretations and calculations.

7.1 Quality Control

7.1.1 Field Blanks and Controls

Prior to a field sampling trip, enough blanks and controls are prepared to equal 10% each (2 minimum) of the anticipated number of field samples. Blanks consist of 50 mL of water in the same type of sampling container as is used in the field. Controls consist of 50 mL of water spiked at 50-202 ng with the compounds listed in Table 3. These blanks and controls are carried to the field and receive the same handling as the field samples. Workup and analysis of field blanks and controls is interspersed with the field samples on a regular basis. This method allows assessment of sample storage stability.

Table 4 presents a typical set of blanks and controls for QC on a field trip where 50 water samples are to be collected.

7.1.2 Procedural Blanks and Controls

7.1.2.1 Extraction Blanks

With each set of samples, a procedural blank is run. This consists of 50 mL of prepurged distilled water which is extracted under the same conditions as the samples. These blanks are designed to detect artifacts from dirty glassware, laboratory atmosphere intrusion, and other sources.

7.1.2.2 GC/ECD Procedural Control

Field samples, field controls, field blanks, and procedural blanks are queued up for GC/ECD analysis such that at least one QC sample is run each working day. In addition, a standard solution is analyzed each day to serve as a procedural control. Thus, in a typical working day, 4 field samples and 1 blank or control are run.

In addition standard solutions are run at regular intervals at least early, middle and later in a day) to monitor detector response and update the analytical curve.

Table 3. PESTICIDES USED AS CONTROLS IN THIS STUDY

	Pesticide	Amount
Reference I	3-Hexachlorocyclohexane	100 ng
	Heptachlor	60 ng
	Aldrin	51 ng
	Oxychlordane	95 ng
	Heptachlor epoxide	59 ng
	trans-Nonachlor	103 ng
	pp'-DDE	100 ng
	Dieldrin	100 ng
	o-p'-DDT	202 ng
	pp'-DDT	200 ng
Reference II	Hexachlorobenzene	19 ng
	α-Chlordane	100 ng
	γ-Chlordane	101 ng

Table 4. WATER QC SAMPLES FOR A TYPICAL SAMPLING TRIP

Sample Type	Number	Comments
Field Blank	5	refrigerate (40) after preparation, carry to field, store with field samples
Field Control	5	Store with field blanks
Lab Blank	5	Refrigerate after preparation store in same freezer as field samples will be stored
Lab control	5	Store with Lab Blanks

7.2 Quality Assurance

Both internal and external QA procedures are to be followed. Internal QA procedures assure the continuity and consistancy of the data. External QA procedures (interlaboratory checks) verify or dispute the accuracy of the data being generated in the primary laboratory.

7.2.1 Internal Quality Assurance

7.2.1.1 Supervision and Monitoring of Activities

There are three levels of quality assurance (QA). The primary quality assurance is the person conducting the sampling and/or analysis. This person must be aware of their actions, observe events which may effect the data, and maintain appropriate records. At the second level, the chemist's supervisor monitors their daily activities, reviews the notebook, checks data and calculations, and assists in "troubleshooting" problems. At the tertiary level, a QA coordinator interviews all personnel on the project. The interviews cover the operations they perform (precisely), the data they obtain, a spot-check of their calculations, and any problems they have had.

7.2.1.2 Documentation

Chain of Custody

From the initial preparation of a sample container through reporting of the analytical results, each sample is accompanied by a chain of custody sheet. Each person signs in the time of receipt, operations performed, and transmittal of the sample. This record is important for tracing a contaminant, bad standard, or some other problem.

Sampling Protocol Sheets

When a sample is collected, a sampling protocol sheet is filled in which contains a discrete sample code which identifies project number, area, site, locations, trip number, sampling period, and sample type. Also included are sample times, volumes, addresses, meteorology, and other pertinent information. Where appropriate, a map is made to precisely identify the location.

Sample Log

Upon return from a sampling trip, each sample code is entered into a sample log book. This log is updated as samples proceed through workup and analysis. Thus, at a glance, project personnel can tell the status of each sample and find out how many are at different stages in the analytical protocol

7.2.2 External Quality Assurance

Samples will be analyzed by a designated QA laboratory. Samples, controls, and blanks will be shipped directly from the field to the QA laboratory for analysis. They will report the results to the primary laboratory for correlation with the primary data.

7.2.2.1 Selection of Samples for QA

Approximately 10% of the field samples (2 minimum) will be collected in duplicate (i.e., 2 50 mL water samples) for shipment to the QA laboratory. This selection process will be random with the following restrictions:

(1) The subject must consent to the additional water collection

(2) If any stratification of subjects is known, purposive selection of QA subjects may be used to get representative samples (e.g., occupationally exposed vs. "normal" individuals)

7.2.2.2 QC Samples

Along with the QA duplicate samples, at least one QC control and one QC blank must be included with the QA samples. An example is shown in Table 5 for a trip collecting 50 samples.

7.2.2.3 Sample Codes

All samples shipped to the QA laboratory should be encoded so that the laboratory cannot discern between samples and controls. This "blind" protocol will add validity to the results.

7.2.2.4 Shipping

Samples should be shipped on ice directly to the QA laboratory by an appropriate air carrier (e.g., Federal Express, Eastern Sprint) in well insulated and packed cartons.

8.0 References

(1) Pellizzari, E.D., M. D. Erickson and R.A. Zweidinger, "Analytical Protocols for Making a Preliminary Assessment of Halogenated Organic Compounds in Man and Environmental Media", Appendix G, Pg 165. Revised April 79.

Table 5. SAMPLES TO BE COLLECTED AND SHIPPED TO QA LABORATORY FOR A TYPICAL SAMPLING TRIP

Sample	Number	Comment
Duplicate Sample	5	Random selection unless prior information stratifies subjects
Field Blank	1	Ship with samples
Field Control	1	Ship with samples

ANALYTICAL PROTOCOL: SAMPLING AND ANALYSIS OF ARSENIC, CADMIUM, AND LEAD IN DRINKING WATER (RTI)

1.0 Principle of Method

The analysis of arsenic, cadmium, and lead in drinking water is carried out using atomic absorption spectrophotometry. Increased sensitivity is achieved by atomizing the metal in a graphite furnace with continuous deuterium background correction. Arsenic determinations are performed on solutions containing 1000 ppm nickel.

2.0 Range and Detection Limit

The minimum detection limit (MDL) and range for the metal assays in drinking water are shown below.

Metal	MDL (µg/L)	Max. Conc. (µg/L)
Arsenic	3.50	200.0
Cadmium	0.04	50.0
Lead	0.35	50.0

Samples containing higher metal concentrations may be analyzed by suitable dilution with 0.5 of nitric acid. Dilution for arsenic determinations is made with 1.0% nitric acid containing 1000 ppm nickel.

3.0 Interferences

No known chemical or spectral interferences exist in the analysis of arsenic, cadmium, or lead in drinking water.

4.0 Precision and Accuracy

The precision and accuracy associated with these analyses is a function of sample metal concentration at the detection limit, the total measurement error is ± 100%. Based on the results of a previous study (1), the metal analyses are performed with the following precision (relative standard deviation) and accuracy (relative error). The total analysis error is also given (2).

Metal	Range (µg/L)	Precision (% RSD)	Accuracy (% RE)	Total Error (%)
Arsenic	10-30	10	10	30
Cadmium	0.5-1.0	10	10	30
Lead	10-30	5	10	20

5.0 Apparatus and Reagents

A commercially available stock solution containing 1000 ppm metal is used for the preparation of the calibration standards. The concentrated nitric acid is reagent grade quality and the deionized water used in this study is prefiltered and subjected to the action of an activated carbon cartridge and two sequential ion exchange units.

The glassware used for the preparation of the calibration solutions must be subjected to a nitric acid cleaning protocol.

All volumetric flasks should be soaked overnight in 20% nitric acid, rinsed with deionized water, soaking for an additional 15-18 hours in a 5% nitric acid bath, followed by a deionized water rinse. The flasks are completely filled with 0.5% nitric acid and stored in this manner. Prior to use, each flask is emptied and rinsed well with deionized water. Pipets are soaked in 5% nitric acid, rinsed well with deionized water, rinsed well with deionized water, air-dried, and stored in a clean, dust-free environment.

Sample cups for the graphite furnace autosampler may be made of polystyrene or Teflon. The former type requires overnight soaking in 1% nitric acid and followed by rinsing with deionized water. The latter type may be soaked overnight in 20% nitric acid, rinsed, and dried in a 105°C oven.

Nickel chloride hexahydrate is used for adjusting the nickel concentration in all samples and standards to 1000 ppm.

6.0 Procedure

6.1 Collection of Samples

Drinking water samples are collected in 4-ounce wide mouth polyethylene bottles with a Polyseal cap and spiked with 0.5 ml concentrated nitric acid/100 ml sample. The sample is labeled and its location and other pertinent data recorded on a protocol sheet.

6.2 Extraction, Cleanup, and Concentration

None

6.3 Instrumental

A Perkin-Elmer Model 403 Spectrophotometer, equipped with a HGA-2000 furnace attachment with deuterium background correction is used for this analysis. An electrodeless discharge lamp is used as the light source and the furnace atmomization response traced on a Perkin-Elmer Model 056 recorder

An AS-1 Autosampler is used to increase throughput and/or to improve pack reproducibility and sensitivity.

Arsenic: Wavelength - 193.7 nm
Gas Interrupt (N_2) - Auto Furnace Cycle Conditions -
Dry: 200°C for 20 sec.
Char: 1200°C for 30 sec.
Atomize: 2500°C for 8 sec.
Injection Volume - 20 µl

Cadmium: Wavelength - 228.8 nm
Gas Interrupt (N_2) - Auto
Furnace Cycle Conditions -
Dry: 200°C for 20 sec.
Char: 400°C for 20 sec.
Atomize: 2000°C for 8 sec.
Injection Volume - 20 µl

Lead: Wavelength - 217.0 nm
Gas Interrupt (N_2) - Auto
Furnace Cycle Conditions -
Dry: 200°C for 20 sec.
Char: 550°C for 20 sec.
Atomize: 2000°C for 8 sec.
Injection Volume - 20 µl

6.4 Qualitative and Quantitative Analysis

6.4.1 Qualitative Analysis

N/A

6.4.2 Quantitative Analysis

The instrument is calibrated with four aqueous standards and a reagent blank.

Calibration Range:

Arsenic -	0.0 to 4.0 ng/20 µl
Cadium -	0.0 to 1.0 ng/20 µl
Lead -	0.0 to 1.0 ng/20 µl

An exponential of the form $y = Ae^{bx}-M$ provides the best representation of the analytical curve. The values of the x,y calibration pairs are entered into a Monroe Calculator Model 1880 programmed to regress the data to the exponential and to provide values for the constants A, b, and M.

Sample peak heights are measured manually and expressed in units of millivolts. The calibration constants, A, b, and M are entered into the storage banks of a Texas Instrument Calculator Model 57 and the metal concentration results obtained by keying in peak height data. Sample peak measurements and concentration results are recorded on a calculation worksheet

$y = Ae^{bx}-M$, ng/20 µl

Units Conversion: ng/20 µ → µg/liter

$$\frac{ng}{20\ \mu l}\left(\frac{\mu g}{1000\ ng}\right)\left(\frac{10^6\ \mu l}{liter}\right) = 50\ \frac{\mu g}{liter}$$

$y = 50\ (Ae^{bx}-M)$, µg/liter

$y = 50D\ (Ae^{bx}-M)$

y = metal concentration in sample, µg/liter,

x = sample peak height, mv

D = dilution factor

7.0 Quality Assurance Program

An on-going quality assurance program is required to assure the data quality. Quality control (QC) procedures determine artifacts, losses, etc., through a system of blanks and controls. Quality assurance (QA) procedures monitor the execution of the procedure and check data interpretation and calculations.

7.1 Quality Control

7.1.1 Field Controls

Prior to field sampling, several blanks and spiked water samples (10% of anticipated number of field samples, are prepared. These field controls are placed in a 4-ounce polyethylene bottle (Polyseal cap), adjusted to 0.5% nitric acid and carried to the sampling site. They are subjected to the same handling and storage conditions as field samples. The analysis of these samples is a part of each water analytical run. Within the precision

of the assay, the calculated metal concentration of these controls is a measure of the contamination/loss during field storage and transit to RTI.

7.1.2 Internal Quality Control

7.1.2.1 Calibration Standards and Blanks

The instrument is calibrated before aech analytical run with four standard solutions and a reagent blank. Evidence of contamination or instrument malfunction is evident at this time. Such problems are resolved before initiating sample analysis.

7.1.2.2 Conditioning of Graphite Tube

The instrument is calibrated before each analytical run with four standard solutions and a reagent blank. Evidence of contamination or instrument malfunction is evident at this time. Such problems are resolved before initiating samples analysis.

7.1.2.2 Conditioning of Graphite Tube

Before each analytical run, the graphite tube is conditioned by injecting 10 to 20 20 µl aliquots of one of the calibration standards. This operation insures acceptable precision during sample analysis.

7.1.2.3 Duplicate Injections

Reproducibility of peak response is continuously monitored during sample analysis. All standard and sample solutions receive two successive injections into the graphite furnace. Signal agreement between the duplicate injections is evaluated according to the following criterion:

First Signal % of Full Scale	% Maximum Permissible Variation (% MPV)	Permissible Range of Second Signal, % of Full Scale
90	± 4%	86-94
80	± 5%	76-84
70	± 6%	66-74
60	± 7%	56-64
50	± 8%	46-54
40	± 10%	36-44
30	± 30%	26-34
20	± 20%	16-24
10	± 30%	7-13
5	± 60%	2-8
2	±100%	0-4

If the second injection gives a signal which falls outside the permissible range, a third injection is performed. The peak measurement not in agreement with the maching pair is discarded.

All calibration and sample calculations are based on the mean of the duplicate determinations.

7.1.2.4 Standard Checks

Instrument performance is monitored during each analytical run. After the analysis of every 12-16 samples, one of the calibration standards is reinjected into the furnace. The standard which most closely matches the sample peak heights is selected as the check solution. A metal concentration is calculated for the check standard based on its peak height during the calibration run. Similar calculations are carried out for each check response and the observed changes in metal concentration expressed in terms of standard deviation units (SDU).

$$SDU = \frac{(\text{Calibration Value} - \text{Check Value})\ 100}{(\text{Calibration Value})\ (\%\ \text{of RSD})}$$

The analysis is under control when the SDU < 2.0. Standard checks which indicate a variation in peak response greater than 2.0 SDU are unacceptable. In this event, the graphite tube is changed, conditioned, and the system recalibrated. Quality control charts are graphed to show this change in instrument performance with time.

7.2 Quality Assurance

Both internal and external QA procedures are to be followed. Internal QA procedure assume the continuity and consistancy of the data. External QA procedures (interlaboratory checks) verify or dispute the accuracy of the data being generated in the primary laboratory.

7.2.1 Interal Quality Assurance

7.2.1.1 Supervision and Monitoring Activities

There are three levels of quality assurance (QA). The primary quality assurance is the person conducting the sampling and/or analysis. This person must be aware of their actions, observe events which may effect the data, and maintain appropriate records. At the second level, the chemist's supervisor monitors their daily activities, reviews the notebook, checks data and calculations, and assists in "troubleshooting" problems. At the

tertiary level, a QA coordinator interviews all personnel on the project. The interviews cover the operations they perform (precisely), the data they obtain, a spot-check of their calculations, and any problems they have had.

7.1.2.2 Documentation

Chain of Custody

From the initial preparation of a sample container through reporting of the analytical results, each sample is accompanied by a chain of custody sheet. Each person signs in the time of receipt, operations performed, and transmittal of the sample. This record is important for tracing a contaminant, bad standard, or some other problems.

Sampling Protocol Sheets

When a sample is collected, a sampling protocol sheet is filled in which contains a discrete sample code which identifies project number, area, site, locations, trip number, sampling period, and sample type. Also included are sample times, volumes, addresses, meteorology, and other pertinent information. Where appropriate, a map is made to precisely identify the location.

Sample Log

Upon return from a sampling trip, each sample code is entered into a sample log book. This log is updated as samples proceed through workup and analysis. Thus, at a glance, project personnel can tell the status of each sample and find out how many are at different stages in the analytical protocol.

Instrument Log

Each sample analysis is logged into a notebook, detailing analysis conditions.

7.2.2 External Quality Assurance

Samples will be analyzed by a designated QA laboratory. Samples, controls, and blanks will be shipped directly from the field to the QA laboratory for analysis. They will report the results to the primary laboratory for correlation with the primary data.

7.2.2.1 Selection of Samples for QA

Approximately 10% of the field samples (2 minimum) will be collected in duplicate for shipment to the QA laboratory. This selection process will be random with the following restrictions:

(1) The donor must consent to the additional collection.

(2) If any stratification of donors is known, purposive selection of QA donors may be used to get representative samples (e.g., occupationally exposed vs "normal" individuals or upwind vs downwind residents).

7.2.2.2 QC Samples

Along with the QA duplicate samples, at least one QC control and one QC blank must be included with the QC samples. An example is shown in Table 7 for a trip collecting 50 samples.

7.2.2.3 Sample Codes

All samples shipped to the QA laboratory should be encoded so that the laboratory cannot discern between samples and controls. This "blind" protocol will add validity to the results.

7.2.2.4 Shipping

Samples should be shipped on dry ice directly to the QA laboratory by an appropriate air carrier (e.g., Federal Express, Eastern Sprint) in well insulated and packed cartons.

8.0 References

8-1 "Epidemiologic Study Conducted in Populations Living Around Non-Ferrous Smelters", Final Report for Contract No. 68-02-2442 (in preparation).

8-2 McFarren, E. F., Lishka, R. J. and Parker, J. H., Criterion for Judging Acceptability of Analytical Methods, Anal. Chem., 42(3), 358 (1970).

ANALYTICAL PROTOCOL: SAMPLING AND ANALYSIS OF ARSENIC, CADMIUM AND LEAD IN WATER (EMSL-CI)

1.0 Principle of Method

Drinking water is analysed for arsenic, cadmium and lead by atomic absorption spectroscopy (A.A.). Metals in solution are readily determined by A.A. The procedure used here is furnace atomic absorption because of greater sensitivity and less interference from chemicals and sample matrices than with a flame.

2.0 Range and Detection Limit

Arsenic and Lead - Optimal Concentration Range 5-100 µg/L
Detection Limit - 1 µg/L

Cadmium - Optimal Concentration Range .5-10 µg/L
Detection Limit - 0.1 µg/L

With quantitative dilution the upper limit of range can be increased indefinitely.

3.0 Interferences

This technique is subject to chemical and matrix interference, the presence or absence of which should be verified by a standard additions method on a spiked and unspiked sample diluted to a least half of its original concentration.

There are several other potential interferences associated with furnace AA such as presence of organics in the matrix, absorptive gas generation, spectral interference from other elements present, carbide formation, and external contamination of the sample. The analysis therefore should be done by an analyst experienced with the potential sources of error associated with the method, and the techniques for correcting them.

4.0 Precision and Accuracy

In a single laboratory (EMSL), using Cincinnati, Ohio tap water spiked at concentrations of 20, 50 and 100 µg As/1, the standard deviations were ±0.7, ±1.1 and ±1.6 respectively. Recoveries at these levels were 105%, 106% and 101%, respectively, Cincinnati, Ohio tap water spiked at concentrations of 2.5, 5.0 and 10.0 µg Cd/1, gave standard deviations of ±0.10, ±0.16 and ±0.33, respectively. Recoveries at these levels were 96%, 99% and 98%,

respectively, and Cincinnati, Ohio tap water spiked at concentrations of 25, 50, and 100 µg Pb/l, the standard deviations were ±1.3, ±1.6, and ±3.7, respectively. Recoveries at these levels were 88%, 92%, and 95% respectively.

5.0 Apparatus and Reagents

1. Atomic absorption spectrophotometer: Single or dual channel, single-or double-beam instrument having a grating monochromator, photomultiplier detector, adjustable slits, a wavelength range of 190 to 800 nm, and provisions for interfacing with a strip chart recorder.
2. Hollow cathode lamps: Single element lamps are to be preferred but multi-element lamps may be used. Electrodeless discharge lamps may also be used when available.
3. Graphite furnace: Any furnace device capable of reaching the specified temperatures is satisfactory.
4. Strip chart recorder: A recorder is strongly recommended for furnace work so that there will be a permanent record and any problems with the analysis such as drift, incomplete atomization, losses during charring, changes in sensitivity, etc., can be easily recognized.
5. Pipets: Microliter with disposable tips. Sizes can range from 5 to 100 microliters as required. NOTE: Pipet tips which are white in color, do not contain CdS, and have been found suitable for research work are available from Ulster Scientific, Inc. 53 Main St. Highland, NY 12528 (914)691-7500.
7. Separatory flasks: 250 ml, or larger, for extraction with organic solvents. NOTE: Glassware; all glassware, linear polyethylene, polyproplyene or Teflon containers, including sample bottles, should be washed with detergent, rinsed with tap water, 1:1 nitric acid, tap water, 1:1 hydrochloric acid, tap water and deionized distilled water in that order.
8. Borosilicate glass distillation apparatus.

5.2 Reagents

1. Deionized distilled water: Prepare by passing distilled water through a mixed bed of cation and anion exchange resins.
2. Use deionized distilled water for the preparation of all reagents, calibration standards, and as dilution water.
3. Nitric acid (conc.): If metal impurities are found to be present, distill reagent grade nitric acid in a borosilicate glass distillation apparatus or use a spectrograde acid. Caution: Distillation should be performed in hood with protective sash in place.
4. Nitric Acid (1:1): Prepare a 1:1 dilution with deionized, distilled water by adding the conc. acid to an equal volume of water.
5. Argon and Nitrogen: Used as furnace purge gas.
6. Arsenic Analyses

   Arsenic Trioxide, As_2O_3, analytical reagent grade

   Nickel Nitrate, $Ni(NO_3)_2 \cdot 6H_2O$, analytical reagent grade
7. Cadmium Analysis

   Cadmium Sulfate ($3CdSO_4 \cdot 8H_2O$); analytical reagent grade

   Ammonium phosphate $(NH_4)_2HPO_4$ analytical reagent grade.
8. Lead Analysis

   Lead Nitrate ($Pb(NO_3)_2$) analytical reagent grade

   Lanthanum Oxide (La_2O_3) analytical reagent grade

6.0 Procedure

6.1 Sample Handling and Preservation

For the determination of trace metals, contamination and loss are of prime concern. Dust in the laboratory environment, impurities in reagents and impurities on laboratory apparatus which the sample contacts are all sources of potential contamination. For liquid samples, containers can introduce either positive or negative errors in the measurement of trace metals by (a) contributing contaminants through leaching or surface desorption and (b) by depleting concentrations through adsorption. Thus the collection and treatment of the sample prior to analysis requires particular attention. The sample bottle whether borosilicate glass, linear polyethylene, polypropylene or Teflon should be thoroughly washed with detergent and tap water; rinsed with 1:1 nitric acid, tap water, 1:1 hydrochloric acid, tap water and

finally deionized distilled water in that order. Before collection of the sample a decision must be made as to the type of data desired, i.e., dissolved, suspended, total or total recoverable. For container preference, maximum holding time and sample preservation at time of collection see Table 1.

To determine total recoverable metals, acidify the entire sample at the time of collection with conc. redistilled HNO_3, 5 ml/l. At the time of analysis a 100 ml aliquot of well mixed sample may be transferred to a beaker or flask and heated on a steam bath or hot plate until the volume has been reduced to 15-20 ml, making certain the samples do not boil. After this treatment the sample is filtered to remove silicates and other insoluble material and the volume adjusted to 100 ml. The sample is then ready for analysis. Concentrations so determined shall be reported as "total".

6.2.1 Preparation of Standard Arsenic Solution

1. Stock solution: Dissolve 1.320 g of arsenic trioxide, As_2O_3 (analytical reagent grade) in 100 ml of deionized distilled water containing 4 g NaOH. Acidify the solution with 20 ml conc. HNO_3 and dilute to 1 liter. 1 ml = 1 mg As (1000 mg/l).
2. Nickel Nitrate Solution, 5%: Dissolve 24.780 g of ACS reagent grade $Ni(NO_3)_2 \cdot 6H_2O$ in deionized distilled water and make up to 100 ml.
3. Nickel Nitrate Solution, 1%: Dilute 20 ml of the 5% nickel nitrate to 100 ml with deionized distilled water.
4. Working Arsenic Solution: Prepare dilutions of the stock solution to be used as calibration standards at the time of analysis. Withdraw appropriate aliquots of the stock solution, add 1 ml of conc. HNO_3, 2 ml of 30% H_2O_2, and 2 ml of the 5% nickel nitrate solution. Dilute to 100 ml with deionized distilled water.

6.2.2 Arsenic Sample Preparation

1. Transfer 100 ml of well-mixed sample to a 250 ml Griffin beaker, add 2 ml of 30% H_2O_2 and sufficient conc. HNO_3 to result in an acid concentration of 1% (v/v). Heat for 1 hour at 95°C or until the volume is slightly less than 50 ml.
2. Cool and bring back to 50 ml with deionized distilled water.

Table 1. RECOMMENDATION FOR SAMPLING AND PRESERVATION OF SAMPLES ACCORDING TO MEASUREMENTS

Metals	Volume Required(mℓ)	Container	Preservative	Maximum Holding Time
Dissolved	200	polyethylene with propylene cap no liner	Filter onsite HNO_3 to pH < 2	6 mos
Suspended	200	"	Filter onsite	6 mos
Total	100		HNO_3 to pH < 2	6 mos

3. Pipet 5 ml of this digested solution into a 10-ml volumetric flask, add 1 ml of the 1% nickel nitrate solution and dilute to 10 ml with deionized distilled water. The sample is now ready for injection into the furnace.

6.2.3 Preparation of Standard Cadmium Solution

1. Stock Solution: Carefully weigh 2.282 g of cadmium sulfate ($3CdSO_4 \cdot 8H_2O$, analytical reagent grade) and dissolve in deionized distilled water. 1 ml = 1 mg Cd(1000 mg/l).
2. Ammonium Phosphate solution (40%): Dissolve 40 grams of ammonium phosphate, $(NH_4)_2HPO_4$ (analytical reagent grade) in deionized distilled water and dilute to 100 ml.
3. Prepare dilutions of the stock cadmium solution to be used as calibration standards at the time of analysis. To each 100 ml of standard and sample alike add 2.0 ml of the ammonium phosphate solution. The calibration standards should be prepared to contain 0.5% (v/v) HNO_3.

6.2.4 Cadmium Sample Preparation

Prepare as described under Sample Handling and Preservation (above). Sample and solutions for analyses should contain 0.5% (v/v) HNO_3.

6.2.5 Preparation of Standard Lead Solution

1. Stock Solution: Carefully weigh 1.599 g of lead nitrate, $Pb(NO_3)_2$ (analytical reagent grade), and dissolve in deionized distilled water. When solution is complete, acidify with 10 ml redistilled HNO_3 and dilute to 1 liter with deionized distilled water. 1 ml = 1 mg Pb (1000 mg/l).
2. Lanthanum Nitrate Solution: Dissolve 58.64 g of ACS reagent grade La_2O_3 in 100 ml conc. HNO_3 and dilute to 1000 ml with deionized distilled water. 1 ml = 50 mg La.
3. Working Lead Solution: Prepare dilutions of the stock lead solution to be used as calibration standards at the time of analysis. Each calibration standard should contain 0.5% (v/v) HNO_3. To each 100 ml of diluted standard add 10 ml of the lanthanum nitrate solution.

6.2.6 Lead Sample Preparation

1. Prepare as described under Sample Handling and Preservation (above). Sample solutions for analyses should contain 0.5% (v/v) HNO_3.
2. To each 100 ml of prepared sample solution add 10 ml of the lanthanum nitrate solution.

6.2.7 Calibration Standards and Curves

Calibration standards are prepared by diluting the stock metal solutions at the time of analysis. For best results, calibration standards should be prepared fresh each time an analysis is to be made and discarded after use. Prepare a blank and at least four calibration standards in graduated amounts in the appropriate range. Beginning with the blank and working toward the highest standard, aspirate the solutions and record the readings. Repeat the operation with both the calibration standards and the samples a sufficient number of times to secure a reliable average reading for each solution.

6.3 Analysis

6.3.1 Furnace Procedure

Furnace devices (flameless atomization) are a most useful means of extending detection limits. Because of differences between various makes and models of satisfactory instruments, no detailed operating instructions can be given for each instrument. Instead, the analyst should follow the instructions provided by the manufacturer of his particular instrument and use as a guide the temperature settings and other instrument conditions listed for each metal. The listing for each metal are the recommended settings working on a Perker-Elmer HGA-2100.

6.3.2 Arsenic Analysis

Instrument Parameters (General)

1. Drying Time and Temp: 30 sec-125°C.
2. Ashing Time and Temp: 30 sec-1100°C.
3. Atomizing Time and Temp: 10 sec-2700°C.
4. Purge Gas Atmosphere: Argon
5. Wavelength: 193.7 nm
6. Other operating parameters should be set as specified by the particular instrument manufacturer.

6.3.3 Cadmium Analysis

Instrument Parameters (General)

1. Drying Time and Temp: 30 sec-125°C.
2. Ashing Time and Temp: 30 sec-500°C.
3. Atomizing Time and Temp: 10 sec-1900°C.
4. Purge Gas Atmosphere: Argon
5. Wavelength: 228.8 nm
6. Other operating parameters should be set as specified by the particular instrument manufacturer.

6.3.4 Lead Analysis

Instrument Parameters(General)

1. Drying Time and Temp: 30 sec-125°C.
2. Ashing Time and Temp: 30 sec-500°C.
3. Atomizing Time and Temp: 10 sec-2700°C.
4. Purge Gas Atmosphere: Argon
5. Wavelength: 283.3 nm
6. Other operating parameters should be set as specified by the particular instrument manufacturer.

6.3.5 General Notes

The following points may be helpful in the analyses of samples. With flameless atomization, background correction becomes of high importance especially below 350 nm. This is because certain samples, when atomized, may absorb or scatter light from the hollow cathode lamp. It can be caused by the presence of gaseous molecular species, salt particules, or smoke in the sample beam. If no correction is made, sample absorbance will be greater than it should be, and the analytical result will be erroneously high. If during atomization all the analyte is not volatilized and removed from the furnace, memory effects will occur. This condition is dependent on several factors such as the volatility of the element and its chemical form, whether pyrolytic graphite is used, the rate of atomization and furnace design. If this situation is detected through blank burns, the tube should be cleaned by operating the furnace at full power for the required time period as needed at regular intervals in the analytical scheme. Some of the smaller size furnace devices, or newer furnaces equipped with feedback temperature control

(Instrumentation Laboratories MODEL 555, Perkin-Elmer MODELS HGA 2200 and HGA 76B, and Varian MODEL CRA-90) employing faster rates of atomization, can be operated using lower atomization temperatures for shorter time periods than those listed in this manual. Although prior digestion of the sample in many cases is not required, providing a representative aliquot of sample can be pepeted into the furnace, it will provide for a more uniform matrix and possibly lessen matrix effects. Inject a measured microliter aliquot of sample into the furnace and atomize. If the concentration found is greater than the highest standard, the sample should be diluted in the same acid matrix and reanalyzed. The use of multiple injections can improve accuracy and help detect furnace pipetting errors. To verify the absence of interference, follow the procedure as given in part 5.2.1. A check standard should be run approximately after every 10 sample injections. Standards are run in part to monitor the life and performance of the graphite tube. Lack of reproducibility or significant change in the signal for the standard indicates that the tube should be replaced. Even though tube life depends on sample matrix and atomization temperature, a conservative estimate would be that a tube will last at least 50 firings. A pyrolytic-coating would extend that estimate by a factor of 3.

6.4 Qualitative Identification

Not applicable. (determined by wavelength being monitored).

6.5 Quantitation

For determination of metal concentration by the furnace: Read the metal value in µg/l from the calibration curve or directly from the readout system of the instrument.

If different size furnace injection volumes are used for samples than for standards:

µg/l of metal in sample = Z(S/U)

where:

Z = µg/l of metal read from calibration curve or readout system

S = ul volume standard injected into furnace for calibration curve

U = ul volume of sample injected for analysis

If dilution of sample was required but sample injection volume same as for standard:

µg/l of metal in sample = Z[(C+B)/C]

where:

Z = µg/l metal in diluted aliquot from calibration curve

B = ml of deionized distilled water used for dilution

C = ml of sample aliquot

7.0 Quality Assurance Program

An on-going quality assurance program is required to assure the data quality. Quality control (QC) procedures determine artifacts, losses, etc. through a system of blanks and controls. Quality assurance (QA) procedures monitor the execution of the procedure and check data interpretations and calculations.

7.1 Quality Control

7.1.1 Procedural Quality Control

Minimum Requirements

All quality control data should be maintained and available for easy reference or inspection. An unknown performance sample (when available) must be analyzed once per year for the metals measured. Results must be within the control limit established by EPA. If problems arise, they should be corrected, and a follow-up performance sample should be analyzed.

Minimum Daily Control

After a calibration curve composed of a minimum of a reagent blank and three standards has been prepared, subsequent calibration curves must be verified by use of at least a reagent blank and one standard at or near the MCL. Daily checks must be within ±10 percent of original curve. If 20 or more samples per day are analyzed, the working standard curve msut be verified by running an additional standard at or near the MCL every 20 samples. Checks must be within ±10 percent of original curve.

Optional Requirements

A current service contract should be in effect on balances and the atomic absorption spectrophotometer. Class S weights should be available to make periodic checks on balances. Chemicals should be dated upon receipt of shipment and replaced as needed or before shelf life has been exceeded. A known reference sample (when available) should be analyzed once per quarter for the metals measured. The measured value should be within the control

limits established by EPA. At least one duplicate sample should be run every 10 samples, or with each set of samples to verify precision of the method. Checks should be within the control limit established by EPA. Standard deviaiton should be obtained and documented for all measurements being conducted. Quality Control charts or a tabulation of mean and standard deviation should be used to document validity of data on a daily basis.

7.1.2 Field Blanks and Controls

Prior to a field sampling trip, enough blanks and controls are prepared to equal 10% each (2 minimum) of the anticipated number of field samples. Blanks consist of 100 ml of water in the same type of sampling container as is used in the field. Controls consist of 100 ml of water spiked at 1 μg arsenic and lead and 0.1 μ cadmium. These blanks and controls are carried to the field and receive the same handling as the field samples. Workup and analysis of field blanks and controls is interspersed with the field samples on a regular basis. This method allows assessment of sample storage stability. Table 2 presents a typical set of blanks and controls for QC on a field sampling trip when 50 water samples are to be collected.

7.2 Quality Assurance

Both internal and external QA procedures are to followed. Internal QA procedure assure the continuity and consistency of the data. External QA procedures (interlaboratory checks) verify or dispute the accuracy of the data being generated in the primary laboratory.

7.2.1 Internal Quality Assurance

7.2.1.1 Supervision and Monitoring of Activities

There are three levels of quality assurance (QA). The primary quality assurance is the person conducting the sampling and/or analysis. This person must be aware of their actions, observe events which may effect the data, and maintain appropriate records. At the second level, the chemist's supervisor monitors their daily activities, reviews the notebook, checks data and calculations, and assists in "troubleshooting" problems. At the tertiary level, a QA coordinator interviews all personnel on the project. The interviews cover the operations they perform (precisely), the data they obtain, a spot-check of their calculations, and any problems they have had.

Table 2. WATER QC SAMPLES FOR METALS ANALYSIS--
TYPICAL SAMPLING TRIP (50 SAMPLES)

Type	Number	Comments[a]
Lab Blank	5	Store at 4°C in refrigerator to be used for field samples
Lab Control	5	Store with Lab Blanks
Field Blank	5	Accompanies field samples from lab to field & return
Field Control	5	Same as field blanks.

[a] All preserved at pH< 2 with HNO3

Documentation

Chain of Custody

From the initial preparation of a sample container through reporting of the analytical results, each sample is accompanied by a chain of custody sheet. Each person signs in the time of receipt, operations performed, and transmittal of the sample. This record is important for tracing a contaminant, bad standard, or some other problem.

Sampling Protocol Sheets

When a sample is collected, a sampling protocol sheet is filled in which contains a discrete sample code which identifies project number, area, site, locations, trip number, sampling period, and sample type. Also included are sample times, volumes, addresses, meteorology, and other pertinent information. Where appropriate, a map is made to precisely identify the location.

Sample Log

Upon return from a sampling trip, each sample code is entered into a sample log book. This log is updated as samples proceed through workup and analysis. Thus, at a glance, project personnel can tell the status of each sample and find out how many are at different stages in the analytical protocol.

7.2.2 External Quality Assurance

Samples will be analyzed by a designated QA laboratory. Samples, controls, and blanks will be shipped directly from the field to the QA laboratory for analysis. They will report the results to the primary laboratory for correlation with the primary data.

7.2.2.1 Selection of Samples for QA

Approximately 10% of the field samples (2 minimum) will be collected randomly in duplicate for shipment to QA laboratory.

7.2.2.2 QC Samples

Along with the QA duplicate samples, at least one QC control and one QC blank must be included with the QA samples. An example is shown in Table 3 for a trip collecting 50 samples.

7.2.2.3 Sample Codes

All samples shipped to the QA laboratory should be encoded so that the laboratory cannot discern between samples and controls. This "blind" protocol will add validity to the results.

Table 3. SAMPLES TO BE COLLECTED AND SHIPPED TO QA LABORATORY FOR A TYPICAL SAMPLING TRIP

Sample	Number	Comments
Duplicate Sample	5	Random selection unless prior information stratifies subjects
Field Blank	1	Ship with samples
Field Control	1	Ship with samples

7.2.2.4 Shipping

Samples should be shipped directly to the QA laboratory by an appropriate air carrier (e.g. Federal Express, Eastern Spint) in well insulated and packed cartons.

Adapted from Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, OH, "Methods for Chemical Analysis of Water and Wastes", EPA publication No.-EPA-600/4-79-020, March, 1979.

* Indicates section not in original protocol. Added here for application to this research project.

ANALYTICAL PROTOCOL: VOLATILE HALOGENATED HYDROCARBONS IN BEVERAGES AND FOODSTUFFS (FDA)

The target compounds will be analyzed from beverages and foods as obtained from the food sources used by the study volunteers. Specific items to be obtained will be determined from the individual diaries maintained for each respondent during the sampling period. The collections and compositing of food and beverage samples will be carried out in accordance with the FDA protocols included herein (Attachment A). The collection of samples will be the responsibility of RTI; compositing will be carried out by Howard University personnel under separate contract. All samples will be analyzed by FDA/Washington as indicated in the following letter of intent (Attachment B). The addition of an analytical chemist on loan from RTI to FDA (6 months) will allow for the implementation of a full analytical program for the Phase I samples.

ATTACHMENT A

ATTACHMENT A

INSTRUCTIONS FOR COLLECTION OF FOOD PRODUCTS

Food items are to be purchased as listed in Composites I, II, X and XII of the Shopping/Compositing Guide for the Northeast region.

Attempt to collect each market basket on a one stop basis. Where certain food items are not available at one store, visit as many stores as necessary to collect all of the items required under the Shopping/Compositing Guide. List each store visited.

Use local chains or large independent grocery stores as sample sources. National chains may be used if local stores are not available, or to supplement collection of items not found at local stores.

Give preference to the sampling of locally produced food items, whenever possible.

The quantities to be collected for the individual products in the Shopping/Compositing Guide are sufficient for most analyses to be performed. If these products are not available in the unit size shown, collect the next larger size or sufficient smaller units to meet the requirement. Never collect less than the amount stated.

Where more than one form of a single food item is listed on the Shopping/Compositing Guide and one form is preferred over the other, it has been underlined. Collect the preferred form if available, e.g., Adult Sub Number "63 Broccoli fresh or frozen" indicates that fresh broccoli will be collected if it is available. If fresh broccoli is not available, collect frozen broccoli.

Collect units with legible coding if the food product bears a code. Make sure all units bear the same code if more than one unit is collected to obtain the amount required by the Shopping Compositing Guide.

List the store name and address, date collected, sample number and brand names for each item on the attached Shopping/Compositing Guide sheets for each composite.

The food items should then be packaged for shipping as outlined in the attached section on packaging food products. This should be done as quickly as possible, maintaining refrigerated or frozen products in that state until they can be further packaged.

A set of heat sealable polymer pouches will be necessary to protect certain food items during shipment. A heat sealing device is also necessary. KAPAK/SCOTCHPAK BRAND sealable pouches are recommended.

Coolers will be necessary to ship frozen or chilled items. Ice and dry ice will also be necessary. The coolers and mailable packages should be

shipped no later than the day following the collection by Federal Express or similar delivery service to the address listed below. Please call Mr. Entz at 202-245-1380 immediately upon shipping samples and provide the shipper and invoice number for future tracking.

Richard Entz
Division of Chemical Technology (HFF-424)
Food and Drug Administration
Room 4818
200 C Street, S.W.
Washington, DC 20204
202-245-1380

Send completed copies of the Shopping/Compositing Guide for each composite and samples of unused packaging materials to Mr. Entz.

PACKAGING OF FOOD PRODUCTS FOR TOTAL DIET TYPE COMPOSITES FOR VOLATILES ANALYSIS (PACKAGING PRIOR TO SHIPMENT TO DCT)

Composite I

Subsample #	Food	Remarks
1	Milk	Original container sealed in plastic[a] pouch shipped in cooler on Dry Ice
4	Ice Cream	
5	Cottage Cheese	Original container sealed
6	Processed Cheese	in plastic pouch
7	Natural cheese	Shipped in cooler with
8	Butter	dry ice (CO_2)
9	Skim milk	
2	Evaporated milk (canned)	Ship in original
3	Non fat Dry Milk	container. No refrigeration necessary

[a]Heatsealable Plastic Pouch: KAPAK/SCOTCHPAK BRAND or equivalent to be used Bags should be heat sealed, use the extra heavy weight bags.

Composite II

Subsample #	Food	Instructions
12,13,14,15,16,17 19,20,21,23,24 25,26,27	Fish & meats, frozen and fresh	Wrap original package in prewashed[a] aluminum foil, seal in plastic pouch[b] ship in cooler with dry ice.
18	Canned tuna or salmon	Ship as is, no refrigeration necessary.
22	Eggs	Seal package in plastic pouch,[b] wrap with packaging material to avoid breakage, ship in cooler on ice.

[a]Prewashed aluminum foil - wash foil with acetone and air dry.

[b]Heatsealable Plastic Pouch: KAPAK/SCOTCHPAK BRAND or equivalent to be used. Bags should be heat sealed, use the extra heavy weight bags.

Composite X

Subsample #	Food	Remarks
108	Salad dressing (mayonnaise)	Ship in original container (glass) with adequate protection from breakage.
109	Salad dressing - French	
110	Salad dressing - other	
111	Shortening	
112	Peanut butter	Place in plastic bag. No refrigeration necessary
113	Margarine	Seal original container in plastic pouch,[a] ship frozen on dry ice (CO_2) in cooler.

[a]Heatsealable Plastic Pouch: KAPAK/SCOTCHPAK BRAND or equivalent to be used. Bags should be heat sealed, use the extra heavy weight bags.

Composite XII

Subsample #	Food	Remarks
123	Tea	Ship in original container. No refrigeration necessary.
123	Coffee, ground	
128	Coffee, instant	
125	Cocoa	
126	Cola soft drink	Purchase in glass bottles.
127	Non-cola soft drink	Ship in original container. No refrigeration necessary.
129	Water	Let 2 gallons run from tap before collecting 1 gallon sample in prewashed bottle with Teflon seal. Fill bottle to overflowing and seal with Teflon faced cap. Ship in cooler with ice and packaging material to prevent breakage.

Shipping Containers Summary

Cooler - (H_2O) ice:

Subsamples 22, 129

Cooler (CO_2) Dry Ice:

Subsamples 1,9,4,5,6,7,8,12,13,14,15,16,17,19,20,21,23,24,25,26,27,113

Containers - Packaged to eliminate breaking:

Subsamples 2,3,18,108,109,110,111,112,123,124,125,126,127,128

Canned items need not be specially packaged.

Ship items to:

Richard Entz
Division of Chemical Technology, HFF-424
Food and Drug Administration
200 C St., S.W., Room 4818
Washington, DC 20204

PROTOCOL FOR FOOD PREPARATION AND COMPOSITING FOR JOINT FDA/EPA STUDY ON VOLATILE HALOCARBONS IN FOODS

This draft protocol provides instructions for preparation, compositing, and packaging of selected foods prior to analysis for volatile halocarbons. The major goals of this work are to provide an uncontaminated, stable, homogeneous food composite for analysis. These instructions are based on the Food and Drug Administration's (FDA) Total Diet Study.

The attached Total Diet Studies Shopping/Composite Guide for composites I, II, X, and XII will be used as instructions for collection and the amount of each food item to add to the final composites. Two sets of composites I, II, X, and XII are to be collected and prepared by personnel from Howard University in the Washington, DC area during the first or second week of June and the first week of July 1980. Instructions for sample collection and storage are attached. These same food items will be collected from Northern New Jersey in the middle of June, July, and August and be shipped to the Division of Chemical Technology (DCT). DCT will arrange for transportation and participate in overseeing of the initial food preparation and compositing at Howard University.

Specific food preparation and packaging instructions are included with each of the attached composite sheets. These instructions are intended to provide guidance for obtaining homogeneous composites with minimum loss of volatile halocarbons. Written records of specific chopping/homogenization equipment, homogenization times, food mixing and equipment cleaning procedures, and changes from this protocol should be maintained and copies provided to DCT.

INSTRUCTIONS FOR SAMPLE PREPARATION, COMPOSITING, AND STORAGE FOR FOOD ITEMS FROM TOTAL DIET STUDIES - ADULT SHOPPING/COMPOSITING GUIDE

Composite I

- Frozen items, (milk, skim milk, ice cream and butter subsamples) are allowed to soften (ice cream and butter) or thaw (milk, skim milk) in the original packages.
- The cheese should be grated/ground while still hard (cold) and then let come to near room temperature (cottage cheese need not be ground).
- Individual subsamples (food components of the composite) are weighed in amounts listed in the compositing guide.
- These subsamples are then added to the blender and mixed until a homogeneous mixture is attained.
- It may be necessary to mix the weighed portions of butter and cheese in a polytron head blender until homogeneous before addition to remainder of composite.
- The final composite mixture is placed in the provided 1 quart mason jar, (prewashed) with aluminum foil liner and two 40 mL screw cap vials with Teflon lined septa. These composite mixtures should be labeled and frozen.
- Portions of the subsamples are to be stored frozen, in the manner listed as follows:

Subsample Number	Food Item(s)	Storage
1	Milk	Retain 2 x 1 Quart bottle (Frozen)
2	Evaporated	Retain 2 x 40 mL vials (Frozen)
9	Skim Milk	Retain 1 x 250 mL (pint) bottle (frozen)
4-8	Ice Cream, Cheeses, Butter	Retain ca. 100 g in 4 oz bottle (frozen)
3	Nonfat Dry Milk	Retain ca. 100 g in 4 oz bottle

Comments

- Recommend blender sufficient to hold 2 gallons to composite these food items.
- Specific type of blender (Hobart or Waring, etc.) not important except that it be able to develop a homogeneous mixture (no settling out or separation for at least 15 minutes).
- All composites/subsamples to be frozen.
- Bottles are to have Teflon seal/cap cover or (pre-cleaned) aluminum foil where necessary (4 oz bottle).
- All bottles are to be prewashed (by DCT).
- Individual items can be weighed onto aluminum foil (prewashed with isopropanol) or beakers (prewashed).
- Use of a top loading balance accure to 0.1 g is necessary.

INSTRUCTIONS FOR SAMPLE PREPARATION, COMPOSITING, AND STORAGE FOR FOOD ITEMS FROM TOTAL DIET STUDIES - ADULT SHOPPING/COMPOSITING GUIDE

Composite II

Some items (subsamples 12,13,14,15,16,17,21,23,24,25,26,27) will be cooked before compositing. See instructions for food preparation (Attachment A). Those items needing cooking are listed in the Shopping/Compositing Guide under "Diet" as processor. (Note that bacon drippings need not be retained).

- After cooking, individual food items should be ground/homogenized after bones are removed. Other food items: canned fish (18), luncheon meat (19), frankfurters (20) and eggs (22) should be homogenized. Meats may be ground in a Hobart or similar meat chopper.

- Individual items should be weighed prior to addition to a blender for compositing (Waring commercial blender recommended).

- Blend for minimum time necessary to obtain a homogenous mixture.

- Composite to be stored frozen in 1 quart jar with prewashed aluminum cap liner and in 2 x 40 mL screw cap vials.

- After homogenization and removal of a portion for the composites, individual subsample components (ca. 100 g each) are to be stored in 4 oz bottles with aluminum foil liners or in heat sealed retort pouches with appropriate labels. These should then be frozen. Compositing will follow the cooking/homogenization of individual components as quickly as possible (no intermediate storage needed). Indvidual samples can then be retained in 100 g quantities and the remainder discarded.

Composite X

Frozen items, after thawing sufficiently to be handled, should be weighed individually according to the compositing guide. Approximately 100 grams should be saved for storage in 4 oz wide mouth jars with washed aluminum foil liners for lid or 125 mL bottles. The composite can be blended using a (Hobart or similar) chopper/blender and placed in a prewashed 1 quart jar and 2 x 40 mL Teflon sealed vials. All composites and subsamples should be frozen after packaging.

NOTE:

Chunky style peanut butter (#112) may require blending to chop the peanuts.

INSTRUCTIONS FOR SAMPLE PREPARATION, COMPOSITING, AND STORAGE FOR FOOD ITEMS FROM TOTAL DIET STUDIES - ADULT SHOPPING/COMPOSITING GUIDE

Composite XII

- Tea and coffee are to be prepared with the supplied drinking water according to the instructions at the bottom of the compositing guide. Other components are weighed and added in a blender, homogenized and the resulting composite stored in 2 x 1 quart bottle and 2 x 40 mL vials and refrigerated.

- Individual components should be stored as follows: Tea leaves (123), coffee (124), cocoa (125), coffee instant (128), in 4 oz bottles (refrigeration not necessary).

- The drinking water and cola soft-drinks should be stored in the provided Teflon sealed 1 quart and 125 mL bottles respectively and refrigerated.

ATTACHMENT B

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
WASHINGTON, D.C. 20204

February 25, 1980

Lance Wallace, Ph.D.
EPA RD-680
401 M Street, SW
Washington, DC 20460

Dear Dr. Wallace:

As suggested in our recent meeting with Dr. Jelinek and Mr. Burke, we are providing you with our thoughts on how the food of Northern New Jersey residents might be collected, prepared and analyzed for certain organic contaminants.

As previously pointed out, the problems associated with the collection and transportation of the actual table-ready diets of 9 families over a period of several months appear to be overwhelming. Additionally, this matter was discussed with our Epidemiology Unit, which concluded that the only practical and statistically valid approach would be to sample one general diet for the area, such as that used by FDA in its Total Diet studies. A listing of the foods in the total diet for the Northeast is enclosed.

If this approach is selected, you might consider the analysis of Composites I, II, X and XII. As can be observed, these for the most part comprise the fatty food and beverage portions.

Another very important reason for recommending a general (vs. individual) diet is that it is the most practical in an analytical sense. A changing matrix would result in an analytical nightmare, as recovery/validation studies would be required for most samples. On the other hand, the selection of a general diet would necessitate but a single investigation for each diet composite.

You also requested an estimate of cost, time frames, expected accomplishments, etc., if FDA were to carry out the food portion of the pilot study.

For the group of compounds including chloroform, carbon tetrachloride, tri- and tetrachloroethylene, methyl chloroform, 1,2-dichloroethane, ethylene dibromide and bromodichloromethane, approximately 4 person months would be needed to adapt/validate our methodology for the 4 food composites. If 10 diet samples were collected (a total of 40 analytical samples), analysis would

require about 3 person months. If the chlorobenzenes, vinyl chloride (VC) and vinylidene chloride (VCl_2) were included, the required time and resources would roughly double. Analysis for the chlorobenzenes necessitates a different procedure, while additional methodology would have to be developed for VC and VCl_2. The same would be true for benzene.

We are prepared to absorb part of the cost of the study, as the investigation would provide us useful information. In addition, we would need to add a chemist to our staff to carry out the bulk of the work. If limited to the first group of chemicals, it is estimated that the pilot project could be completed in about 6 months after start-up. A total of a year or more would be needed if chlorobenzenes, VC, VCl_2 and benzene were included.

Items such as sample collection, preparation and shipping are not included in the time/resource estimates. It is assumed that these activities would be carried out or arranged for by RTI, your contractor for the other phases of the project.

We will be happy to discuss these matters further at your convenience.

Sincerely yours,

Pat Lombardo

P. Lombardo
Chemical Industry Practices Branch
Division of Chemical Technology
Bureau of Foods

ANALYTICAL PROTOCOL: POLYNUCLEAR AROMATIC HYDROCARBONS FROM HOUSEHOLD DUST

The determination of the target PNAs from household dust will involve application of the EMSL (Cincinnati) analysis protocol for PNAs from water. Dust samples will be collected on Teflon filters, (25 mm diameter) using the modified vacuum cleaner described for metal analysis. Each Teflon filter will be halved, one half to be used for organochlorine pesticides and PCB determination, the other half for PNAs analysis. The assay specifications (range, sensitivity, etc.) are as delineated in the EMSL procedures.

ANALYTICAL PROTOCOL: SAMPLING AND ANALYSIS OF ARSENIC, CADMIUM, AND LEAD IN HOUSE DUST (RTI)

1.0 Principle of Method

The analysis of arsenic, cadmium, and lead in house dust is carried out using atomic absorption spectrophotometry. Increased sensitivity is achieved by atomizing the metal in a graphite furnace with continuous background correction. Arsenic determinations are performed on solutions containing 1000 ppm nickel.

2.0 Range and Detection Limit

The minimum detection limit (MDL) and range for the metal assays in house dust are shown below (assume 25 mg sample weight).

Metal	MDL	Max. Conc.
Arsenic	0.50 µg/g	100.0 µg/g
Cadmium	0.50	100.0
Lead	0.50	400.0

Samples containing higher metal concentrations may be analyzed by suitable dilution with 1.0% nitric acid. Dilution for arsenic determinations is made with 1.0% nitric acid containing 1000 ppm.

3.0 Interferences

No known chemical or spectral interferences exist in the analysis of arsenic, cadmium, or lead in house dust.

4.0 Precision and Accuracy

The precision and accuracy associated with these analyses is a function of sample metal concentration. At the detection limit, the total measurement error is ± 100%. Based on the results of a previous study (1), the metal analyses are performed with the following precision (relative standard deviation) and the total analysis error (estimated) is also given (2).

Metal	Range	Precision (%RSD)	Accuracy (%RE)	Estimated Total Error (%)
Arsenic	5-30 µg/g	10	10-20	30-40
Cadmium	10-50	10	10-20	30-40
Lead	100-500	10	10-20	30-40

5.0 Apparatus and Reagents

A commercially available stock solution containing 1000 ppm metal is used for the preparation of the calibration standards. The concentrated nitric acid is reagent grade quality and the deionized water used in this study will be prefiltered and subjected to the action of an activated carbon cartridge and two sequential ion exchange units.

The glassware used for sample workup and the preparation of the calibration solutions must be subjected to a nitric acid protocol.

All volumetric flasks and beakers should be soaked overnight in 20% nitric acid, rinsed with deionized water, soaking for an additional 15-18 hours in a 5% nitric acid bath, followed by a copious deionized water rinse. The flasks are completely filled with 0.5% nitric acid and stored in this manner. Prior to use, each flask is emptied and rinsed well with deionized water. Pipets are soaked in 5% nitric acid, rinsed well with deionized water, air-dried, and stored in a clean, dust-free environment.

Sample cups for the graphite furnace auto sampler may be made of polystyrene or Teflon. The former type requires overnight soaking in 1% nitric acid and followed by rinsing with deionized water. The latter type may be soaked overnight in 20% nitric acid, rinsed, and dried in a 105°C oven.

Nickel chloride hexahydrate is used for adjusting the nickel concentration to 1000 ppm in solutions slated for arsenic analysis.

6.0 Procedure (3)

6.1 Collection of Samples

House dust samples are collected on a filter medium (glass fiber, Teflon, etc.) with a modified, portable vacuum cleaner. The sample plus filter is placed in a Zip-Loc bag, labelled, and all pertinent information recorded on a protocol sheet. The collection device is cleaned after every sampling with a canister of pressurized Freon (Dust-Off).

6.2 Extraction, Cleanup, and Concentration

The Zip-Loc bag containing the filter plus collected dust is placed in a dessicator (less than 50% relative humidity), equilibrated overnight and a weight recorded for the filter plus sample. This is accomplished by cutting the creases of the bag and scrapping the dust material onto a sheet of tared powder paper.

The net sample weight is determined by subtracting the tare filter weight. Each collection (filter plus dust) is placed in a 3-dram vial with a Teflon- lined cap and allowed to stand overnight under an accurately delivered 10.0 ml volume of 1% HNO_3. The mixture is heated at 50°-65°C for 45 minutes and the digestion finished by treatment with 0.2 ml of 30% H_2O_2 at 50-65°C for 15 minutes. The vial is capped during this operation. A blank filter is carried through the same workup. Each digest is clarified by passing the supernatant through a plug of acid-washed glass wool and stored in a 2-dram vial with a Teflon- lined cap until analyzed.

6.3 Instrumental

A Perkin-Elmer Model 403 Spectrophotometer, equipped with a HGA-2000 furnace attachment with deuterium background correction is used for this analysis. An electrodeless discharge lamp is used as the light source and the furnace atomization response traced on a Perkin-Elmer Model 056 recorder AS-1 Autosampler may be used to increase throughput and/or to improve peak reproducibility and sensitivity.

Arsenic: Wavelength - 193.7 nm
Gas Interrupt (N_2) - Auto
Furnace Cycle Conditions -
Dry: 200°C for 20 sec.
Char: 1200°C for 30 sec.
Atomize: 2500°C for 8 sec.
Injection Volume - 20 µl

Cadmium: Wavelength - 228.8 nm
Gas Interrupt (N_2) - Manual
Furnace Cycle Conditions -
Dry: 200°C for 20 sec.
Char: 400°C for 30 sec.
Atomize: 2000°C for 8 sec.
Injection Volume - 20 µl

Lead: Wavelength - 217.0 nm
Gas Interrupt (N_2) - Auto

Furnace Cycle Conditions -

Dry: 200°C for 20 sec.

Char: 500°C for 20 sec.

Atomize: 2000°C for 8 sec.

Injection Volume - 20 µl

6.4 Qualitative and Quantitative Analysis

6.4.1 Qualitative Analysis

N/A

6.4.2 Quantitative Analysis

The instrument is calibrated with a four aqueous standards in 1.0% nitric acid, a reagent blank, and a flask filter subjected to the same workup as samples.

Calibration Range -

Arsenic - 0.0 to 5.0 ng/20 µl

Cadmium - 0.0 to 5.0 ng/20 µl

Lead - 0.0 to 20.0 ng/20 µl

An exponential of the form $y = Ae^{bx}-M$ provides the best representation of the analytical curve. The values of the x, y calibration pairs are entered into a Monroe Calculator Model 1880 programmed to regress the data to the exponential and to provide values for the constants A, b, and M.

Sample peak heights are measured manually and expressed in units of millivolts. The calibration constants A, b, and M are entered into the storage banks of a Texas Instrument Calculator Model 57 and the metal concentration results obtained by keying in peak height data. Sample peak measurements and concentration results are recorded on a calculation worksheet.

$y = Ae^{bx} - M$, ng/20 µl

$y = y_s$ (uncorr. metal conc. in sample) - y_m (metal conc. in matrix)

$y_s = Ae^{bx_s} - M$

$y_m = Ae^{bx_m} - M$

$$y = (Ae^{bx_s} - M) - (Ae^{bx_m} - M)$$

$$y = A(e^{bx_s} - e^{bx_m})$$

Units Conversion: ng/20 µl → µg/gm

$$\frac{ng}{20\ \mu l}\left(\frac{1000\ \mu l}{ml}\right)\left(\frac{\mu g}{1000\ ng}\right)\left(\frac{V\ ml}{W\ gm}\right) = \frac{V}{20\ W}\ \frac{\mu g}{gm}$$

V = volume of dust digest, ml

W = weight of dust sample, gm

$$y = (V/20W)\ A(e^{bx_s} - e^{bx_m}),\ \mu g/gm$$

y = metal concentration in sample, µg/gm,

x_s = sample peak height, mv,

y_s = uncorrected metal concentration in sample,

x_m = matrix peak height, mv,

y_m = matrix blank (metal concentration in dust digest due to impurities in filter medium),

7.0 Quality Assurance Program

An on-going quality assurance program is required to assure the data quality. Quality control (QC) procedures determine artifacts, losses, etc. through a system of blanks and controls. Quality assurance (QA) procedures monitor the execution of the procedure and check data interpretations and calculations.

7.1 Quality Control

7.1.1 Field Controls

Prior to field sampling, several control dust collections (10% of anticipated number of field samples) are obtained. Each dust sample is mixed well and divided into two portions. One aliquot is placed in a container identical to that used for field samples, sent to the site, and subjected to the same handling and storage conditions as field samples. The other aliquot is stored at RTI in a dust-free environment. On receipt of samples at RTI, both portions of the control dust collections are worked up and analyzed as a part

of each dust analytical run. Within the precision of the assay, the difference in calculated metal concentrations of the two control dust aliquots is a measure of the contamination/loss during field storage and transit to RTI.

7.1.2 Internal Quality Control

7.1.2.1 Calibration Standards and Blanks

The instrument is calibrated before each analytical run with four standard solutions and a reagent blank. Evidence of contamination or instrument malfunction is evident at this time. Such problems are resolved before initiating sample analysis.

7.1.2.2 Conditioning of Graphite Tube

Before each analytical run, the graphite tube is conditioned by injecting 10 to 20 20 µl aliquots of one of the calibration standards. This operation insures acceptable precision during sample analysis.

7.1.2.3 Duplicate Injections

Reproducibility of peak response is continuously monitored during sample analysis. All standard and sample solutions receive two successive injections into the graphite furnace. Signal agreement between the duplicate injections is evaluated according to the following criterion:

First Signal, % of Full Scale	% Maximum Permissible Variation (% MPV)	Permissible Range of Second Signal, % of Full Scale
90	± 4%	86-94
80	± 5	76-84
70	± 6	66-74
60	± 7	56-64
50	± 8	46-54
40	± 10	36-44
30	± 13	26-34
20	± 20	16-24
10	± 30	7-13
5	± 60	2-8
2	±100	0-4

If the second injection gives a signal which falls outside the permissable range, a third injection is performed. The peak measurement not in agreement with the matching pair is discarded.

All calibration and sample calculations are based on the mean of the duplicate determinations.

7.1.2.4 Standard Checks

Instrument performance is monitored during each analytical run. After the analysis of every 12-16 samples one of the calibration standards is reinjected into the furnace. The standard which most closely matches the sample peak heights is selected as the check solution. A metal concentration is calculated for the check standard, based on its peak height during the calibration run. Similar calculations are carried out for each check response and the observed changes in metal concentration expressed in terms of standard deviation units (SDU),

$$SDU = \frac{(\text{Calibration Value}-\text{Check Value})100}{(\text{Calibration Value})(\%\ \text{RSD})}$$

The analysis is under control when the SDU <2.0. Standard checks which indicate a variation in peak response greater than 2.0 SDU are unacceptable. In this event, the graphite tube is changed, conditioned, and the system recalibrated. Quality control charts are graphed to show this change in instrument performance with time.

7.2 Quality Assurance

Both internal and external QA procedures are to be followed. Internal QA procedures assume the continuity and consistency of the data. External QA procedures (interlaboratory checks) verify or dispute the accuracy of the data being generated in the primary laboratory.

7.2.1 Internal Quality Assurance

7.2.1.1 Supervision and Monitoring of Activities

There are three levels of quality assurance (QA). The primary quality assurance is the person conducting the sampling and/or analysis. This person must be aware of their actions, observe events which may effect the data, and maintain appropriate records. At the second level, the chemist's supervisor monitors their daily activities, reviews the notebook, checks data and calculations, and assists in "troubleshooting" problems. At the tertiary level, a QA coordinator interviews all personnel on the project. The interviews

cover the operations they perform (precisely), the data they obtain, a spot-check of their calculations, and any problems they have had.

7.2.1.2 Documentation

Chain of Custody

From the initial preparation of a sample container through reporting of the analytical results, each sample is accompanied by a chain of custody sheet. Each person signs in the time of receipt, operations performed, and transmittal of the sample. This record is important for tracing a contiminant, bad standard, or some other problem.

Sampling Protocol Sheets

When a sample is collected, a sampling protocol sheet is filled in which contains a discrete sample code which identifies project number, area, site, locations, trip number, sampling period, and sample type. Also included are sample times, volumes, addresses, meteorology, and other pertinent information. Where appropriate, a map is made to precisely identify the location.

Sample Log

Upon return from a sampling trip, each sample code is entered into a sample log book. This log is updated as samples proceed through workup and analysis. Thus, at a glance, project personnel can tell the status of each sample and find out how many are at different stages in the analytical protocol.

Instrument Log

Each sample analysis is logged into a notebook, detailing analysis conditions.

7.2.2 External Quality Assurance

Samples will be analyzed by a designated QA laboratory. Samples, controls, and blanks will be shipped directly from the field to the QA laboratory for analysis. They will report the results to the primary laboratory for correlation with the primary data.

7.2.2.1 Selection of Samples for QA

Approximately 10% of the field samples (2 minimum) will be collected in duplicate for shipment to the QA laboratory. This selection process will be random with the following restrictions:

(1) The donor must consent to the additional collection

(2) If any stratification of donors is known, purposive selection of QA donors may be used to get representative samples (e.g., occupationally exposes vs. "normal" individuals or upwind vs. downwind residents).

7.2.2.2 QC Samples

Along with the QA duplicate samples, at least one QC control and one QC blank must be included with the QA samples. An example is shown in Table 1 for a trip collecting 50 samples.

7.2.2.3 Sample Codes

All samples shipped to the QA laboratory should be encoded so that the laboratory cannot discern between samples and controls. This "blind" protocol will add validity to the results.

7.2.2.4 Shipping

Samples should be shipped on dry ice directly to the QA laboratory by an appropriate air carrier (e.g., Federal Express, Eastern Spint) in well insulated and packed cartons.

8.0 References

8-1 "Epidemiologic Study Conducted in Populations Living Around Non-Ferrous Smelters", Final Report for Contract No. 68-02-2442 (in preparation).

8-2 McFarren, E. F., Lishka, R. J., and Parker, J. H., Criterion for Judging Acceptability of Analytical Methods, Anal. Chem., 42(3), 358 (1970).

8-3 Handy, R. W., et al., "Analysis of Housedust for Trace Metal Consent by Atomic Absorption Spectrophotometry", Paper No. 33 presented at the 31st ACS Southeastern Regional Meeting at Roanoake, VA, Oct. 24-26, 1979.

Table 1. SAMPLES TO BE COLLECTED AND SHIPPED TO QA LABORATORY FOR A TYPICAL SAMPLING TRIP

Sample	Number	Comment
Duplicate sample	5	Random selection unless prior information stratifies subjects
Field blank	1	Ship with samples
Field control	1	Ship with samples

ANALYTICAL PROTOCOL: ORGANOCHLORINE PESTICIDES AND PCBs IN HOUSEHOLD DUST

This protocol is identical to "Organochlorine Pesticides and PCBs in Drinking Water (Method 608)" except for sampling and sample extraction. Dust samples will be collected using the modified vacuum cleaner with filter described in the protocol for metals in dust. Each filter is halved (clean surgical scissors) and the filter half designated for pesticides/PCBs analysis is immersed in ∿ 5 ml toluene (glass distilled) and sonicated (ultrasonic bath) for 5 minutes. The toluene is decanted, and a fresh portion is added and the sonification process is repeated. A further repetition provides ∿ 15 ml toluene extract. The combined extracts are concentrated to ∿ 1 ml via the K-D apparatus (Method 608), and further concentrated to dryness via a gentle stream of nitrogen (using a 3-ball Snyder column - balls removed - to minimize sample losses). The residue is dissolved, with sonication, in ∿ 1 ml hexane and treated according to the procedures outlined in Method 608.

APPENDIX B

ANALYTICAL PROTOCOLS FOR TOXIC CHEMICALS IN BODY BURDEN SAMPLES

ANALYTICAL PROTOCOL: SAMPLING AND ANALYSIS PROCEDURE FOR HUMAN BREATH SAMPLES (RTI)

1.0 Principle of Method

The breath sample is collected on a Tenax GC cartridge using a specially designed spirometer and low hydrocarbon air ("ultrapure") or equivalent (1). The Tenax cartridge is then dried over $CaSO_4$ and analyzed by thermal desorption into a gas chromatograph (WCOT glass column)-mass spectrometer [(GC/MS), Figure 1].

2.0 Range and Limits of Detection

The linear range for the analysis of volatile organic compounds depends upon two principal features. The first is a function of the breakthrough volume (Table 1) of each specific compound which is trapped on the Tenax GC sampling cartridge and the second is related to the inherent limits of detection of the mass spectrometer for each organic (3,6-9). Thus, the range and the maximum limit of detection are a direct function of each compound which is present in the original breath samples. The linear range for quantitation using glass capillaries on a gas chromatograph/mass spectrometer/computer (GC/MS/COMP) is generally three orders of magnitude [5-5,000 ng (5-8)]. Table 2 lists the overall detection limits for some examples of volatile organics which are based on these two principles (1,8).

3.0 Interferences

For the target compounds in Table 2, no interferences have been observed. Particular attention must be paid to the preparation of clean collection devices and the use of appropriate blanks and controls to establish that the background contaminants have been removed. Otherwise, false positive detection of chloroform may occur.

4.0 Precision and Accuracy

The reproducibility of this method has been determined to range from ±10 to ±30% of the relative standard deviation for different substances when replicate sampling cartridges are examined (5-11). The inherent analytical errors are a function of several factors: [1] the ability to accurately determine the breakthrough volume and its relation to field sampling conditions for each of the organic compounds identified; [2] the accurate

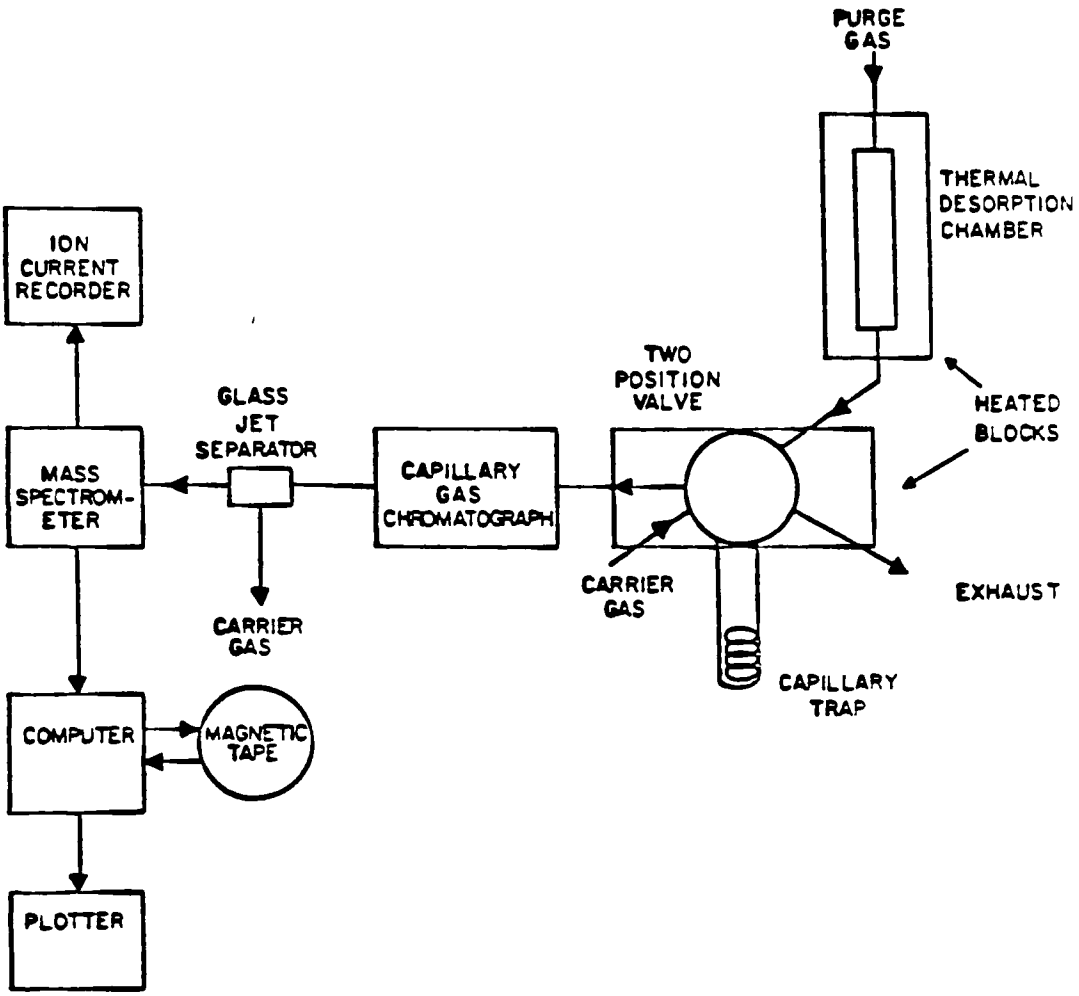


Figure 1. Analytical system for analysis of organic vapors in ambient air.

Table 1. TENAX GC BREAKTHROUGH VOLUMES FOR TARGET COMPOUNDS[a]

Compound	b.p. (°C)	Temperature (°F)					
		50	60	70	80	90	100
chloroform	61	56	41	32	24	17	13
carbon tetrachloride	77	45	36	28	21	17	13
1,2-dichloroethane	83	71	55	41	31	24	19
1,1,1-trichloroethane	75	31	24	20	16	12	9
tetrachloroethylene	121	481	356	261	192	141	104
trichloroethylene	87	120	89	67	51	37	28
chlorobenzene	132	1989	871	631	459	332	241

[a]For a Tenax GC bed of 1.5 x 8.0 cm.

Table 2. APPROXIMATE MEASURED LIMITS OF DETECTION AND QUANTIFIABLE LIMITS FOR SELECTED VAPOR-PHASE ORGANICS IN BREATH

Compound	m/z	LOD[a]		QL	
		$\mu g/m^3$	ppt	$\mu g/m^3$	ppt
Benzene	78	0.11	35	0.55	176
Chloroform	83/85	0.11	23	0.55	116
1,2-Dichloroethane	98/62	0.16	41	0.82	209
1,1,1-Trichloroethane	97/99	0.22	42	1.10	208
Carbon tetrachloride	117/119	ND	ND	ND	ND
Vinylidene chloride	96/98	0.16	42	0.82	214
Trichloroethylene	130/132	0.22	42	1.10	211
Tetrachloroethylene	164/166	0.33	49	1.65	245
Bromodichloromethane	127/83	0.33	51	1.65	253
Chlorobenzene	112/114	0.22	49	1.10	245
1,1,2-Trichloroethane	97/99	0.22	42	1.10	208
m-Dichlorobenzene	146/148	0.27	46	1.37	231

[a]Limits of detection (LOD) was defined as S/N = 4 for ion selected for quantification. Quantification Limit (QL) was defined as 5 x LOD or S/N = 20. Limits are based on a collection volume of 20 ℓ or breakthrough volume (70°F), whichever is smaller, for 1.5 xm x 8.0 cm Tenax GC bed volume and mass spectrometer response to that compound.

measurement of the ambient air volume sampled; [3] the percent recovery of the organic from the sampling cartridge after a period of storage; [4] the reproducibility of thermal desorption for a compound from the cartridge and its introduction into the analytical system; [5] the accuracy of determining the relative molar response ratios between the identified substance and the external standard used for calibrating the analytical system; [6] the reproducibility of transmitting the sample through the high resolution gas chromatographic column; and [7] the day-to-day reliability of the MS/COMP system (2-13).

The accuracy of analysis is generally ±10-30% but depends on the chemical and physical nature of the compound (3,6,7,8,13).

5.0 Apparatus and Reagents

5.1 Collection and Analysis Devices

5.1.1 Spirometer

The spirometer is diagrammed in Figure 2. The valves in the Douglas mouth piece must be replaced with Tedlar or similar material. A bubbler filled with distilled deionized water is placed in-line with the air tank to humidify the air for subject comfort (1).

5.1.2 Sampling Cartridges

The sampling tubes are prepared by packing a ten centimeter long by 1.5 cm i.d. glass tube containing 8 cm of 35/60 mesh Tenax GC with glass wool in the ends to provide support (3,12). Virgin Tenax (or material to be recycled) is extracted in a Soxhlet apparatus for a minimum of 18 hours each time with methanol and n-pentane prior to preparation of cartridge samplers (3,12). After purification of the Tenax GC sorbent and drying in a vacuum oven at 120°C for 3 to 5 hours at 28 inches of water, all the sorbent material is meshed to provide a 35/60 particle size range. Meshing and all further cartridge preparation is conducted in a "clean" room. Cartridge samplers are then prepared and conditioned at 270°C with a purified helium flow of 30 ml/min for 120 min. Prior to entering the Tenax GC cartridge the helium is purified by passing through a liquid N_2 cooled cryogenic trap. The conditioned cartridges are transferred to Kimax® (2.5 cm x 150 cm) culture tubes, immediately sealed using Teflon-lined caps and

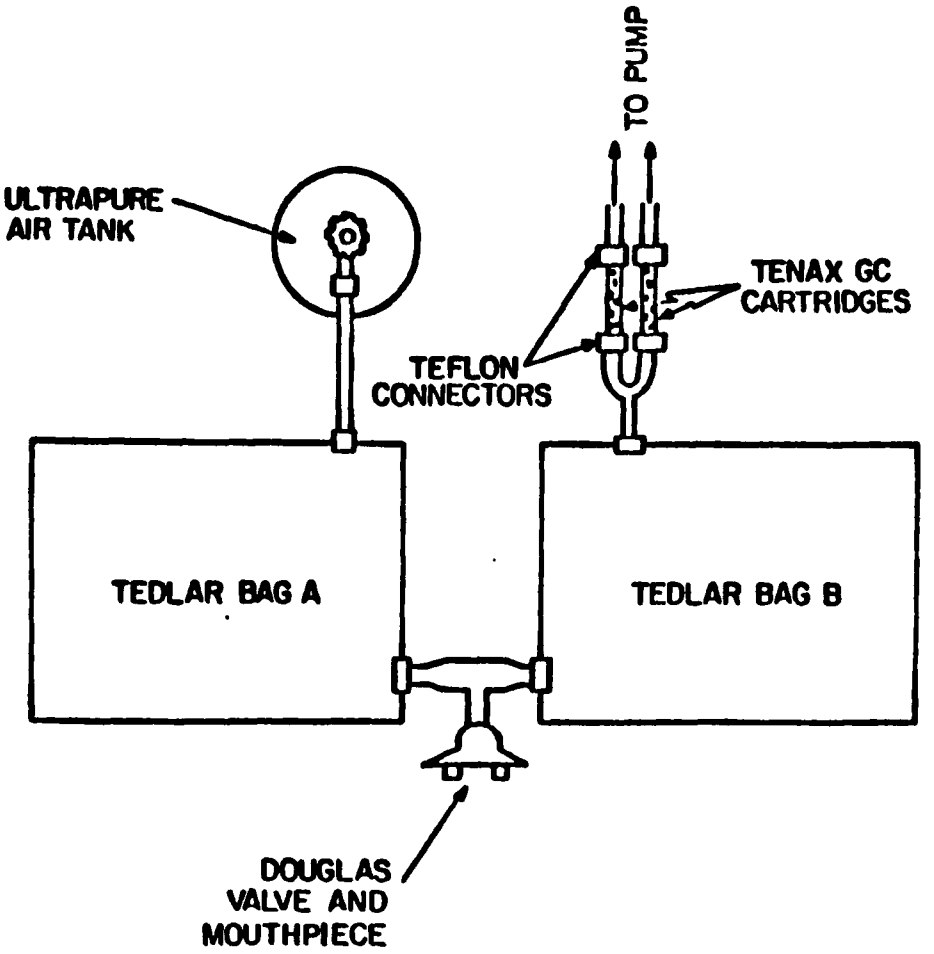


Figure 2. Schematic of spirometer for collection of breath samples.

cooled. This procedure is performed in order to avoid recontamination of the sorbent bed (3,13).

Kimax® culture tubes are cleaned as described under Section 6.1. Teflon liners are washed in Isoclean/water, rinsed with deionized distilled water, acetone and air dried. Subsequently the liners are soaked in methanol for 2-3 hr, then air dried and placed into the culture tube caps (which are already aluminum foil lined).

Calcium sulfate is cleaned by heating in a muffle furnace at 400-500°C. Approximately 2 g is placed in the Kimax® culture tube, followed by glass wool and the thermally conditioned Tenax GC cartridges.

5.1.3 Inlet Manifold

An inlet manifold for thermally recovering vapors trapped on Tenax sampling cartridges is used and is shown in Figure 1 (2-5).

5.1.4 Gas Chromatograph

A Varian 1700 or a Pye Unicam 102 gas chromatograph is used to house the glass capillary column and is interfaced to the inlet manifold on the Varian MAT CH-7 or LKB 2091 systems, respectively. A mass flow controller (Tylan) is used to precisely control the carrier gas. Such an analytical system was presented schematically in Figure 1.

A jet separator is employed to interface the glass capillary column to the mass spectrometer on the Varian MAT CH-7 GC/MS/COMP or LKB 2091 systems. The separator is maintained at 240°C (3,6).

5.1.5 Mass Spectrometer/Computer

A Varian MAT CH-7 or LKB 2091 mass spectrometer capable of a resolution of 1500-2,000 equipped with single ion monitoring capability is used in tandem with the Varian 1700 or Pye Unicam 102 gas chromatograph and interfaced to a Varian 620/L or PDP 11/04 computer, respectively (Figure 1).

5.2 Reagents and Materials

All reagents used are analytical reagent grade. All solvents (Burdick & Jackson) are redistilled before their use.

6.0 Procedure

6.1 Cleaning of Glassware

All glassware is washed in Isoclean/water, rinsed with deionized distilled water, acetone and air dried. Glassware is heated to 450-500°C

for 2 hours to insure that all organic material has been removed prior to its use.

6.2 Collection of Samples

The subject is seated in a comfortable chair and the mouthpiece height adjusted to a convenient level. A long spring clamp is used to seal the air flow from Bag A to the mouthpiece (Fig. 2). A plug is placed in the mouthpiece opening until the test begins to prevent room air contamination. Air flow from the air tank is started and when the 50 L Bag A is about half full, the mouthpiece is attached, the clamp and plug removed and the subject may begin to breathe on the apparatus. The nose clips must also be in place at this time. After a minute or two, the Nutech Model 221 sampler pump (Nutech, Durham, NC) is started with the flow at approximately 7 L/min. The flow may be adjusted to match the individual subject's respiration rate. It is useful to furl Bag B using spring clips to avoid using this bag as an exponential dilutor but retaining the safety factor of the 25 L bag capacity. After a predetermined volume (20 L) of breath has been sampled, the test is terminated. The subject is removed from the apparatus and the nose clips removed from the subject. The Tenax cartridges are removed and stored in culture tubes. The entire apparatus is then flushed with pure air to decontaminate it for the next use. This is best done by successively filling and evacuating the bags. The mouthpiece is sterilized by placing in alcohol after each use. The Tenax cartridges are desiccated over $CaSO_4$ before analysis by placing ~2 g in the bottom of a culture tube and covering with glass wool. The cartidge is then sealed in the culture tube for 2-4 hr.

Previous experiments have shown that the organic vapors collected on Tenax GC sorbent are stable and can be quantitatively recovered from the cartridge samplers up to 4 weeks after sampling when they are tightly closed in cartridge holders and placed in a second container that can be sealed, protected from light and stored at -20°C [Table 3 (2,3,7,8)].

6.2.1 Deuterated Standards

The use of deuterated compounds provides for an assessment of any premature breakthrough (and thus reduced collection efficiency) which may occur if the total vapor-phase organic load exceeds 1/10 of the cartridge

Table 3. RECOVERY OF TARGET COMPOUNDS AFTER STORAGE

Compound	1 day	1 wk	2 wk
Benzene	100	107 ± 26	69 ± 8
Chloroform	100	83 ± 14	49 ± 4
1,2-Dichloroethane	100	100 ± 5	100 ± 7
1,1,1-Trichloroethane	100	87 ± 17	71 ± 7
Carbon tetrachloride	ND	ND	ND
Vinylidene chloride	100	92	ND
Trichloroethylene	100	87 ± 17	71 ± 7
Tetrachloroethylene	100	92 ± 1	78 ± 4
Bromodichloromethane	ND	ND	ND
Chlorobenzene	100	87 ± 3	80 ± 7
1,1,2-Trichloroethane	100	95 ± 3	92 ± 2
m-Dichlorobenzene	100	89 ± 15	85 ± 3

capacity during sampling of breath. d_5-Bromoethane (B.P. 34°C), d_8-tetrahydrofuran (B.P. 65), d_6-benzene (B.P. 80), d_{10}-cyclohexene (B.P. 83) and d_5-chlorobenzene (B.P. 132) are loaded as a discrete zone onto at least 10% of all Tenax GC sampling cartridges prior to sampling. Using GC/MS/COMP the exogenous deuterated compounds are differentiated from the endogenous vapor-phase organics in breath samples.

The addition of these deuterated standards is performed by injecting 1.0 mL air/vapor of the substances onto the "front" end of the cartridge. An air/vapor mixture stream containing 200-400 ng/mL is generated using permeation tubes of each compound with a permeation system (5,6,9,10).

The quantity of each substance on the sampling cartridges is determined by GC/MS/COMP after breath sampling and the percent recovery is compared to control (unused) cartridges carried to and from the field sampling site and subjected to the same storage regime. Statistically significant differences are attributed to premature breakthrough.

6.2.2 Quantification Standards

Unique substances may be added as internal standards during sampling. Examples are the deuterated compounds listed under 6.2.1. However, the volume of air sampled is accurately known and thus external standards may be introduced into the cartridge prior to its analysis. Three standards, hexafluorobenzene, octafluorotoluene, and iodotoluene are used for the purpose of calculating RMRs and the levels in human breath. Previous research has shown that their retention times span the chromatographic range of analysis (SE-30 coated capillary) and they do not interfere with the analysis of unknown compounds in human breath samples.

The external standards (300-400 ng) are injected into the sampling cartridges as a 1.0 mL air/vapor mixture using a gas sampling syringe. The air/vapor mixture is synthesized using permeation tubes and a permeation system (5,6,9,10).

6.3 Analysis of Samples

The instrumental conditions for the analysis of volatile organics on the sorbent Tenax GC sampling cartridge is shown in Table 4. The thermal desorption chamber and the six port Valco valve are maintained at 270°C. The jet separator is maintained at 245°. The mass spectrometer is set to

Table 4. OPERATING PARAMETERS FOR GLC-MS-COMP SYSTEM

Parameter	Setting
Inlet-manifold	
desorption chamber and valve	270°C
capillary trap - minimum	-195°C
maximum	240°C
thermal desorption time	8 min
He purge flow	15 ml/min
GLC	
85 m glass WCOT $BaCO_3$ SE-30 (0.8-1.0 μ film)	24-240°C, 4°C/min
carrier (He) flow	∿1.25 ml/min
separator/transfer line	245°C
MS	
Varian MAT CH-7	
scan range	$\underline{m}/\underline{z}$ 20 → 350
scan cycle, automatic-cyclic	1 sec/decade
filament current	300 μA
multiplier	4.0
ion source vacuum	∿4 x 10^{-6} T
LKB 2091	
scan range	$\underline{m}/\underline{z}$ 20 → 500
scan cycle, automatic	2 sec total
filament current	300 μA
multiplier	4.5
ion source vacuum	∿4 x 10^{-6} T

scan the mass range from approximately 20-350. The helium purge gas through the desorption chamber is adjusted to 15-20 mL/min. The nickel capillary trap on the inlet manifold is cooled with liquid nitrogen. In a typical thermal desorption cycle, a sampling cartridge is placed in the preheated desorption chamber and the helium gas is channeled through the cartridge to purge the vapors into the liquid nitrogen capillary trap [the inert activity efficiency of the trap has been shown in a previous study (5,11)]. After the desorption has been completed, the six-port valve is rotated and the temperature on the capillary loop is rapidly raised (greater than 10°/min); the carrier gas then introduces the vapors onto the high resolution GC column. The glass capillary column is temperature programmed from ambient to 240°C at 4°C/min and held at the upper limit for a minimum of 10 min. After all the components have eluted the column is then cooled to ambient temperature and the next sample is processed (3).

6.3.1 Qualitative Analysis

The mass spectral data are processed in the following manner. First, the original spectra are scanned and the reconstructed ion chromatogram (RIC) is extracted and examined. The intensity (RIC) is plotted against the spectrum number using the software package available. The information will generally indicate whether the run is suitable for further processing, since it provides some idea of the number of unknowns in the sample and the resolution obtained using the particular gc column conditions.

If mass conversion of spectral peak times to peak masses has not been performed on-the-fly during data acquisition by hardware methods than this function is next performed by software methods (magnetic systems). In either case the mass conversion is accomplished by the use of the calibration table obtained prior to sample analysis for perfluorokerosene. In general the calibration data are sufficient for an entire day's data processing; however, it is verified every eight hours.

After the spectra are obtained in mass converted form, processing proceeds either manually or by computer by comparison to a Library (14). Compound identification can involve various degrees of certainty. These levels of identification have been defined as follows:

Level I Computer Interpretation. The raw data generated from the analysis of samples are subjected to computerized deconvolution/library search and compound identification made using this approach has the lowest level of confidence. In general Level I is reserved for only those cases where compound verification is the primary intent of the qualitative analysis

Level II Manual Interpretation. The plotted mass spectra are manually interpreted by a skilled interpretor and compared to those spectra compiled in a data compendium. In general a minimum of five masses and intensities (±5% S.D.) should match between the unknown and library spectrum. This level does not utilize any further information such as retention time since many compounds the authentic compound may not be available for establishing retention times.

Level III Manual Interpretation Plus Retention Time/Boiling Point of Compound. In addition to the effort as described under Level II, the retention time of the compound is compared to the retention time which has been derived from previous chromatographic analysis. Also the boiling point of the identified compound is compared to the boiling points of other compounds in the near vicinity of the one in question when a capillary coated with a non-polar phase has been used.

Level IV Manual Interpretation Plus Retention Time of Authentic Compounds. Under this level, the authentic compound has been chromatographed on the same capillary column using identical operating conditions and the mass spectrum of the authentic compound is compared to that of the unknown.

Level V Level IV Plus Independent Confirmation Techniques. This Level utilizes other physical methods of analysis such as GC/fourier transform/IR, GC/high resolution mass spectrometry, or NMR analysis. This Level constitutes the highest degree of confidence in the identification of organic compounds.

6.3.2 Quantitation

The quantitation of constituents in breath samples is accomplished either by utilizing the total ion current monitor or, where necessary, from extracted ion current profiles. In order to eliminate the need to obtain complete calibration curves for each compound for which quantitative information is desired, the method of relative molar response (RMR) factors (6-11) is used. Successful use of this method requires information on the exact amount of standard added and the relationship of RMR (unknown) to the RMR (standards).

6.3.2.1 RMR Determination

The compounds to be quantified are loaded onto Tenax GC cartridges using a permeation system (5,6,9,10) or in cases where permeation tubes are not available the vaporization system shown in Figure 3 is used (6,15).

With the vaporization method helium is purified by passing it through a cryogenic trap followed by two carbon traps. The standards and substances to be quantified are prepared in methanol and a 2.0 μℓ solution is injected through the septum of the heated loading tube (250°C). The vaporized components are swept onto the Tenax GC cartridge at a rate of 200 ml/min for 6 min (total He 1.2 ℓ). Because of the low breakthrough volume for methanol (0.8 ℓ at 70°F), the majority is passed from the cartridge. This system is used to load relatively non-volatile compounds with breakthrough volumes >50 ℓ.

The method of calculating RMRs is as follows:

$$(1)\quad RMR_{unknown/standard} = \frac{A_{unk}/Moles_{unk}}{A_{std}/Moles_{std}}$$

A = system response, height or area determined by integration or triangulation.
unk = unknown
std = standard

The value of RMR is determined from at least three independent analyses during analysis of samples (6). Linearity over the dynamic range and an intercept of zero has been previously described (6,15).

$$(2)\quad RMR_{unk/std} = \frac{A_{unk}/g_{unk}/GMW_{unk}}{A_{std}/g_{std}/GMW_{std}}$$

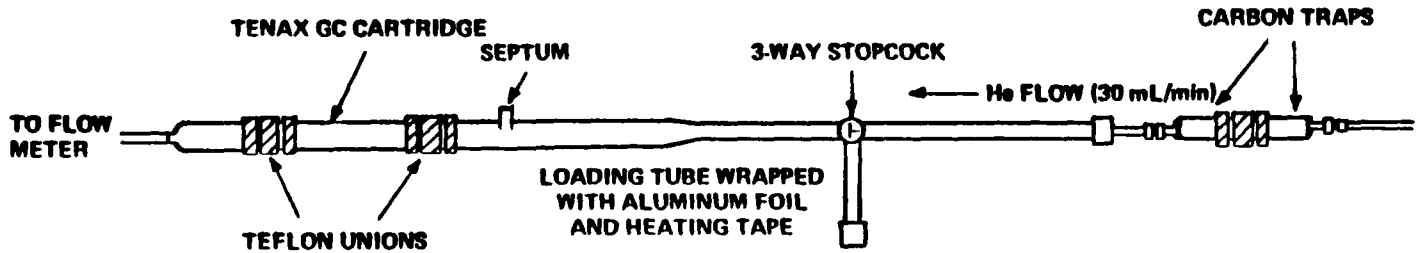


Figure 3. Schematic of vaporization unit for loading organics dissolved in methanol onto Tenax GC cartridges.

A = system response, as above
g = number of grams present
GMW = gram molecular weight

$$(3) \quad g_{unk} = \frac{A_{unk} \cdot GMW_{unk} \cdot g_{std}}{A_{std} \cdot GMW_{std} \cdot RMR_{unk/std}}$$

6.3.2.2 Calculation of Organic Vapor Concentrations in Breath

Since the volume of breath taken to produce a given sample is accurately known and an external standard is added to the sample, then the weight per cartridge and hence the concentration of the unknown can be determined. The approach for quantitating ambient air pollutants in this study requires that the RMR be determined for each constituent of interest during the analysis of field samples. Every sixth cartridge is a control cartridge for determining RMRs for each compound (calibration of instrument, storage and recovery). This means that when a breath sample is taken, the external standard is added at a known concentration prior to analysis. It is not imperative at this point to know what the RMR of each of the constituents in the sample happens to be. However, after the unknowns are identified then the RMR can subsequently be determined and the unknown concentration calculated in the original sample using the RMR. In this manner it is possible to obtain qualitative and quantitative information on the same sample with a minimum of effort.

Once the quantity of substance per cartridge has been determined, the level in breath is given by

$$\frac{\mu g}{m^3} = \frac{\mu g_{unk} \cdot 1000 \text{ L}}{m^3 \cdot \text{Volume Sampled (L)}}$$

7.0 Quality Assurance Program

Both internal and external QA procedures are to be followed. Internal QA procedures assure the continuity and consistency of the data. External QA procedures (interlaboratory checks) verify or dispute the accuracy of the data being generated in the primary laboratory.

7.1 Internal Quality Assurance

7.1.1 Supervision and Monitoring of Activities

There are three levels of quality assurance (QA). The primary quality assurance is the person conducting the sampling and/or analysis. This person must be aware of his actions, observe events which may effect the data, and maintain appropriate records. At the second level, the chemist's supervisor monitors his daily activities, reviews the notebook, checks data and calculations, and assists in "troubleshooting" problems. At the tertiary level, a QA coordinator interviews all personnel on the project. The interviews cover the operations they perform (precisely), the data they obtain, a spot-check of their calculations, and any problems they have had.

7.1.2 Reagent and Glassware Control

Reagent and glassware control is required in order to minimize contamination. Sample containers, glassware, etc. are cleaned with Isoclean®, rinsed with distilled/deionized water and heat treated at 450-500°C to insure the removal of all traces of organic compounds.

7.1.3 Sampling Protocol and Chain of Custody

As part of the quality control procedures, sampling protocols and chain of custody forms are prepared for each sampling cartridge. Examples of these forms are given in Tables 5 and 6. The fate of each sampling cartridge is tracked from the time they are prepared until the data has been reduced to a finished form.

7.4 Blanks, Controls, Standards, and System Performance Samples

7.1.4.1 Blanks

Ten percent of the sampling cartridges from each batch are set aside to serve as blanks to be analyzed for background contamination. After the preparation of a set of sampling cartridges, one cartridge is checked for background prior to their committment to field sampling. Blank (unused) cartridges travel to the field site returned to the laboratory and stored along with the field samples at -20°C until ready for analysis.

7.1.4.2 Controls

Ten percent of the sampling cartridges are loaded with the deuterated compounds listed in 6.2.1. Sampling with control cartridges allow for an assessment of premature breakthrough if it occurs. Control cartridges are

Table 5. FIELD SAMPLING PROTOCOL SHEET - A

Date: _____

Project No. (_ _ _ _)
Operator (_ _)
Trip No. (_)
Sampler (_ _)
Area _____ (_ _)
Address _____
Peribd (_)
Site _____ (_ _ _)
Location (_)
Sample Code _____

Mutech 221 (M)

DC amps _____

Sampling rate _____ (lpm)

Vacuum _____ ("Hg)

End: Time _____ Ft^3 _____

Start: Time _____ Ft^3 _____

Total: (min) _____ Ft^3 _____

DuPont (D)

Sampling rate (init.) _____ (lpm)

Sampling rate (final) _____ (lpm)

End: Time _____

Start: Time _____

Total: (min) _____

MSA (M)

Sampling rate (init.) _____ (lpm)

Sampling rate (final) _____ (lpm)

End: Time _____ Count _____

Start: Time _____ Count _____

Total: (min) _____ Count _____

ml/count _____

Remarks _____

_____

Volume Air/Cartridge _____

_____

Time ___ Temp. Wet. ___ Dry _____
Rel. Humid ___% Wind Dir./Speed __/__
Cloud _____ Odor _____
Remarks _____

Time ___ Temp. Wet. ___ Dry _____
Rel. Humid ___% Wind Dir./Speed __/__
Cloud _____ Odor _____
Remarks _____

Time ___ Temp. Wet. ___ Dry _____
Rel. Humid ___% Wind Dir./Speed __/__
Cloud _____ Odor _____
Remarks _____

Time ___ Temp. Wet ___ Dry _____
Rel. Humid ___% Wind Dir./Speed __/__
Cloud _____ Odor _____
Remarks _____

Table 6. CHAIN OF CUSTODY RECORD

Research Triangle Institute
Analytical Sciences Division
Chemistry and Life Sciences Group
Research Triangle Park, NC 27709

SAMPLE CODE:	Sample Type:	Volume Collected:
	No. of Containers:	Volume Analyzed:

Relinquished By:	Received By:	Time	Date	Operation Performed (aliquot, std. conc remarks, etc.)

analyzed along with other samples and since deuterated compounds are employed the qualitative and quantitative analysis of these air samples proceeds unimpeded.

7.1.4.3 Standards for RMR Determination

The compounds listed in Table 2 are loaded (250-450 ng each) onto Tenax GC cartridges from a permeation system. A minimum of three analyses is required for determining RMRs for a set of samples which are quantitatively analyzed.

7.1.4.4 System Performance Mixtures

The system performance standards listed in Table 7 are loaded onto Tenax GC cartridges (using the vaporization method) to determine mass calibration and intensity and chromatographic performance of the GC/MS/COMP system.

7.1.2 Sample Analysis

To insure the accuracy and precision of the data acquired instrument and chromatographic performance are monitored on a daily basis.

7.1.2.1 Instrument Calibration

Calibration of mass and intensity of magnetic systems employs perfluorokerosene. Table 8 lists the tolerances for each mass and intensity which the mass spectrometer must achieve.

Perfluorotoluene in the performance mixture is employed for determining instrument stability as related by mass resolution and relative ion abundance under GC conditions (6,15). The masses and intensities listed in Table 8 are compared to the results obtained on a daily basis and for each set of samples analyzed.

7.1.2.2 Assessment of Chromatographic Performance

The quality of the chromatography is of utmost importance since the accuracy and precision of qualitative and quantitative analysis are directly affected (6,15). Glass capillary columns are evaluated according to the following criteria:

(1) percent peak asymmetry factor (PAF)

$$\% \text{ PAF} = \frac{B}{F} \times 100$$

Table 7. GC/MS/COMP SYSTEM PERFORMANCE STANDARDS

Compound	Quantity (ng)
Perfluorokerosene	350
Perfluorotoluene	350
Ethylbenzene	300
p-Xylene	300
n-Octane	300
n-Decane	300
1-Octanal	300
5-Nonanone	300
Acetophenone	300
2,6-Dimethylaniline	300
2,6-Dimethylphenol	300

Table 8. MASS AND INTENSITY TOLERANCES ACCEPTABLE FOR CALIBRATION OF MAGNETIC INSTRUMENTS FOR QUANTITATION

Perfluorotoluene[a]		Perfluorokerosene	
m/z	%I (C.V.)	m/z	%I (C.V.)
69	33 (5)	51	39 (10)
79	11 (10)	100	22 (8)
93	16 (8)	119	100 (0)
117	43 (8)	131	89 (5)
167	15 (7)	169	58 (4)
186	59 (5)	181	62 (6)
217	100 (0)	219	24 (5)
236	66 (4)	231	29 (8)

[a]To be achieved in the chromatography mode.

where B = the area of the back half of a chromatographic peak
F = area of the front half of the chromatographic peak both measured 10% above baseline

(2) effective Height Equivalent to a Theoretical Plate ($HETP_{eff}$)

$$HETP_{eff} = \frac{L}{5.54\ (X/Y)^2}$$

where X = the corrected retention distance for sweep time of the compound,
Y = chromatographic peak width at 1/2 peak height,
L = column length (mm)

(3) separation number (SN)

$$SN = \frac{D}{W_1 + W_2} - 1$$

where D = the distance between two peaks,
W_1, W_2 = widths at 1/2 height

(4) resolution (R)

$$R = \frac{2\ \Delta\ W}{W_1 + W_2}$$

where ΔW = average base width,
W = peak width at base

(5) Acidity and Basicity

$$\text{Acidity} = \frac{\text{weak base (peak area or height)}}{\text{acetophenone (peak area or height)}}$$

$$\text{Basicity} = \frac{\text{weak acid (peak area or height)}}{\text{acetophenone (peak area or height)}}$$

The use of the compounds listed in Table 7 provides information as to the degree of adsorption and the type of adsorption mechanisms. 1-Octanol and 5-nonanone serves to determine the extent of deactivation of the glass surface (PAF). The acidity and basicity of the glass capillary column are assessed by the adsorption of weak bases and acids, respectively (6,15).

The resolution and separation number are determined for the compound pairs ethylbenzene:p-xylene and octane:decane, respectively. $HETP_{eff}$ is based on octane. Table 9 lists the minimum performance specifications acceptable for breath analysis (6). Figures 4-10 depict extracted ion current profiles used for calculating performance specifications.

Table 9. SPECIFICATIONS OF PERFORMANCE FOR GLASS CAPILLARY COLUMN[a]

Parameter	Test Compound(s)	Value ± S.D. (C.V.)
Resolution	Ethylbenzene:p-Xylene	1.30 ± 11 (8)
Separation No.	Octane:Decane	73 ± 6 (8)
% Peak Asymmetry Factor	1-Octanol	239 ± 141 (59)
	Nonanone	130 ± 32 (25)
	Acetophenone	260 ± 34 (13)
Acidity	2,6-Dimethylaniline:Acetophenone	1.00 ± 0.07 (9)
Basicity	2,6-Dimethylphenol:Acetophenone	0.82 ± 0.03 (5)

[a]SE-30 WCOT/$BaCO_3$, 0.48 mm i.d. x 75 m, 1 μ film thickness.

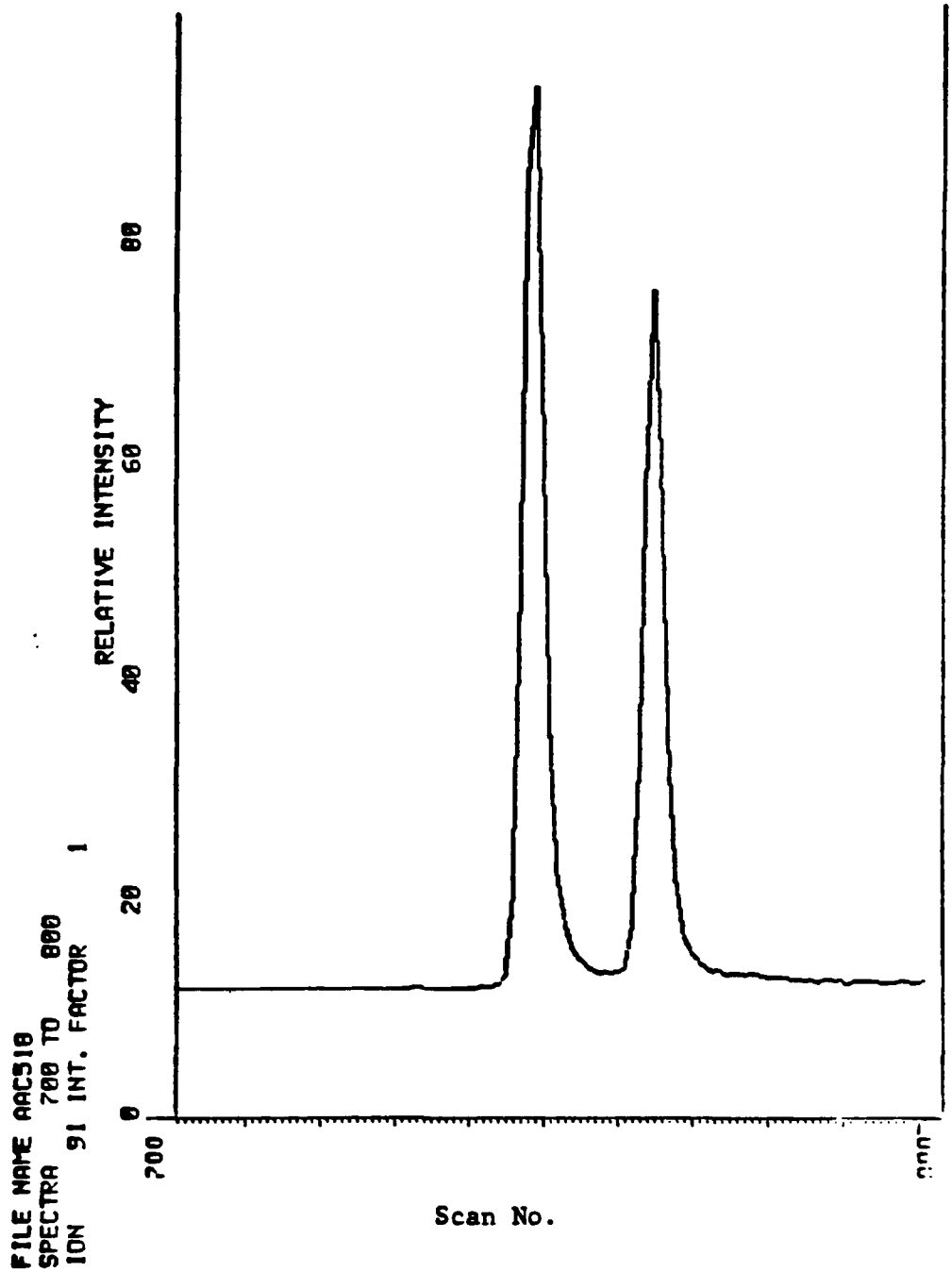


Figure 4. Extracted ion current profile of m/z 91 for p-xylene and ethylbenzene used in calculating resolution.

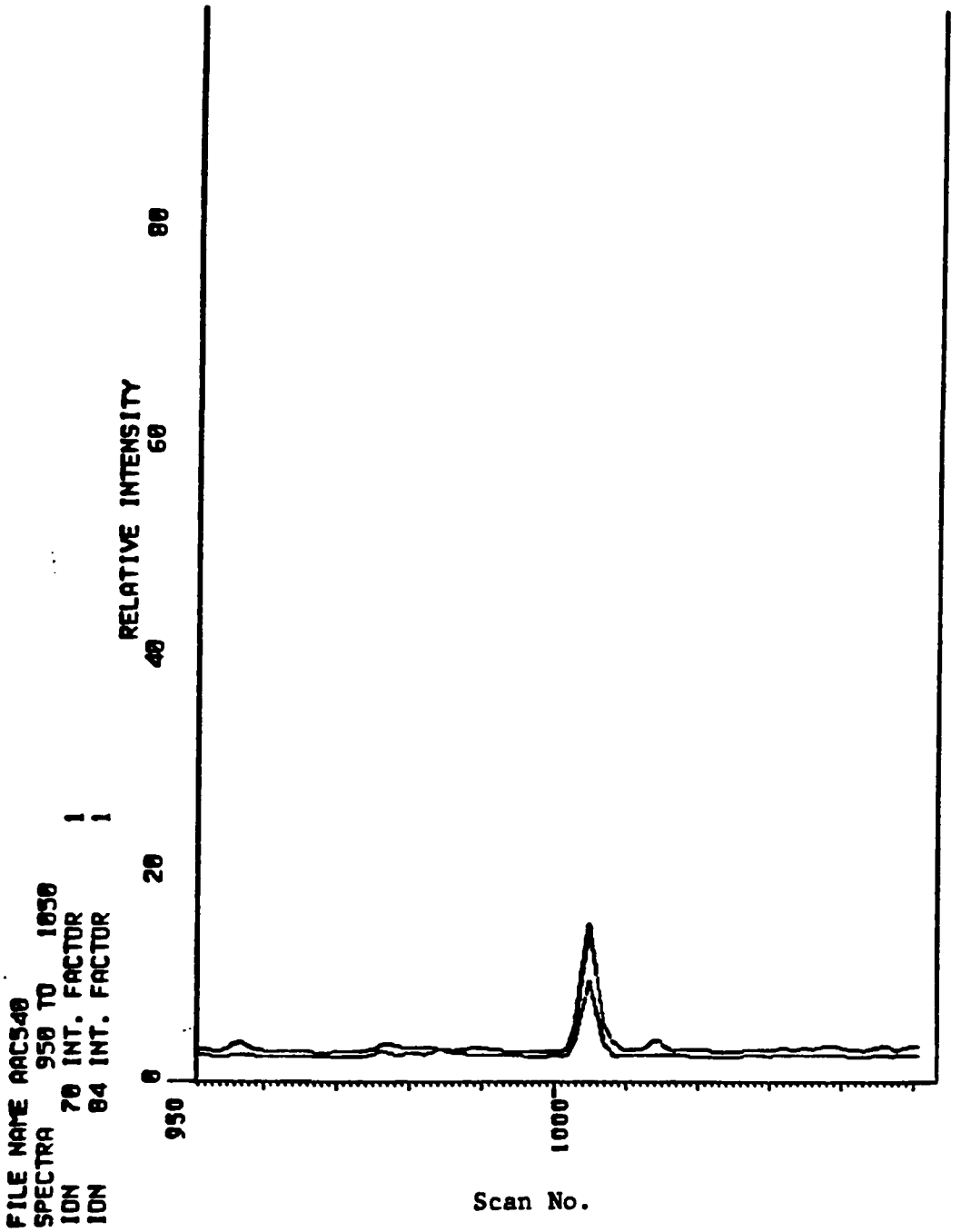


Figure 5. Extracted ion current profile of m/z 70 and 84 for 1-octanol used for calculating percent peak asymmetry factor.

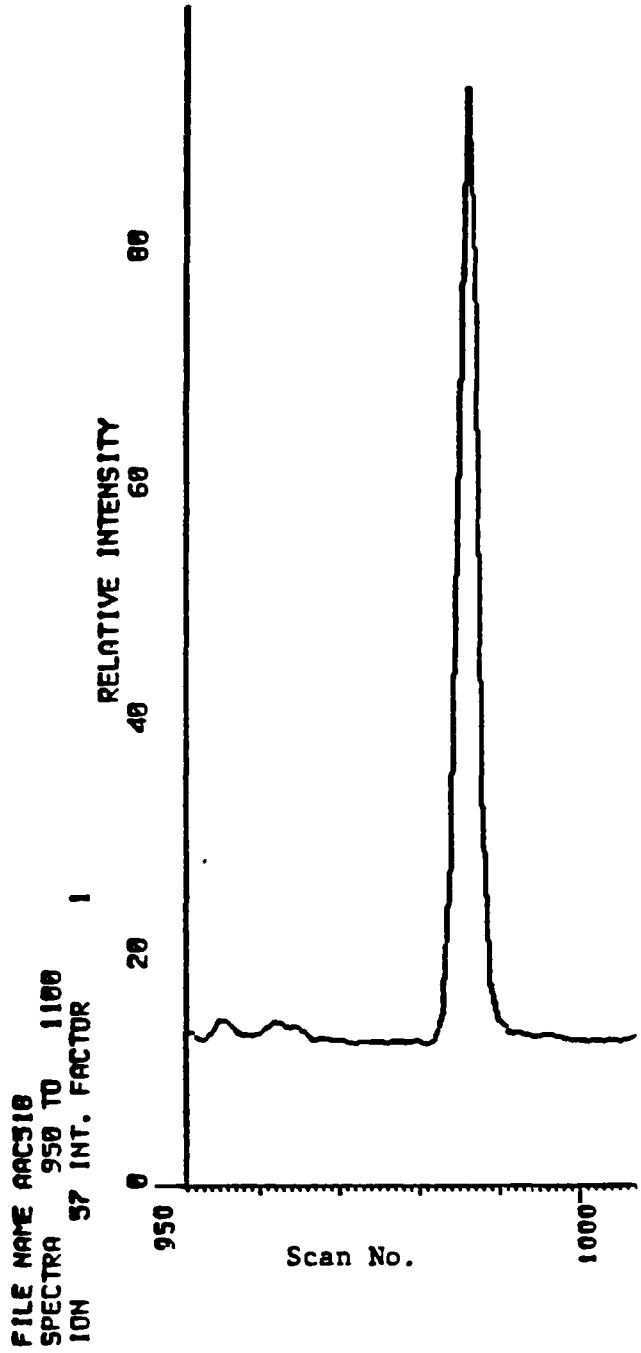


Figure 6. Extracted ion current profile of m/z 57 for nonanone used in calculating percent peak asymmetry factor.

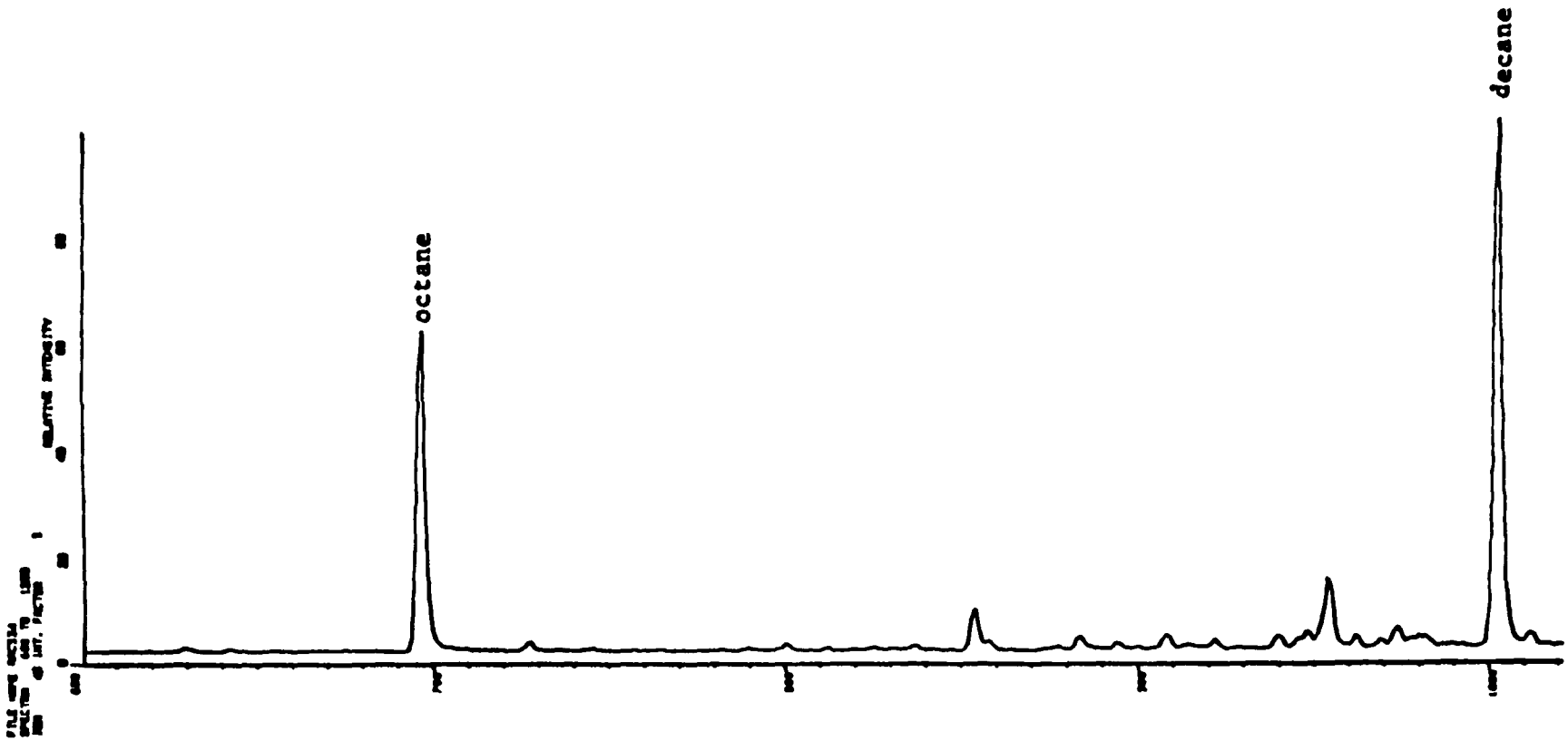


Figure 7. Extracted ion current profile of m/z 43 for octane and decane used in calculating separation number.

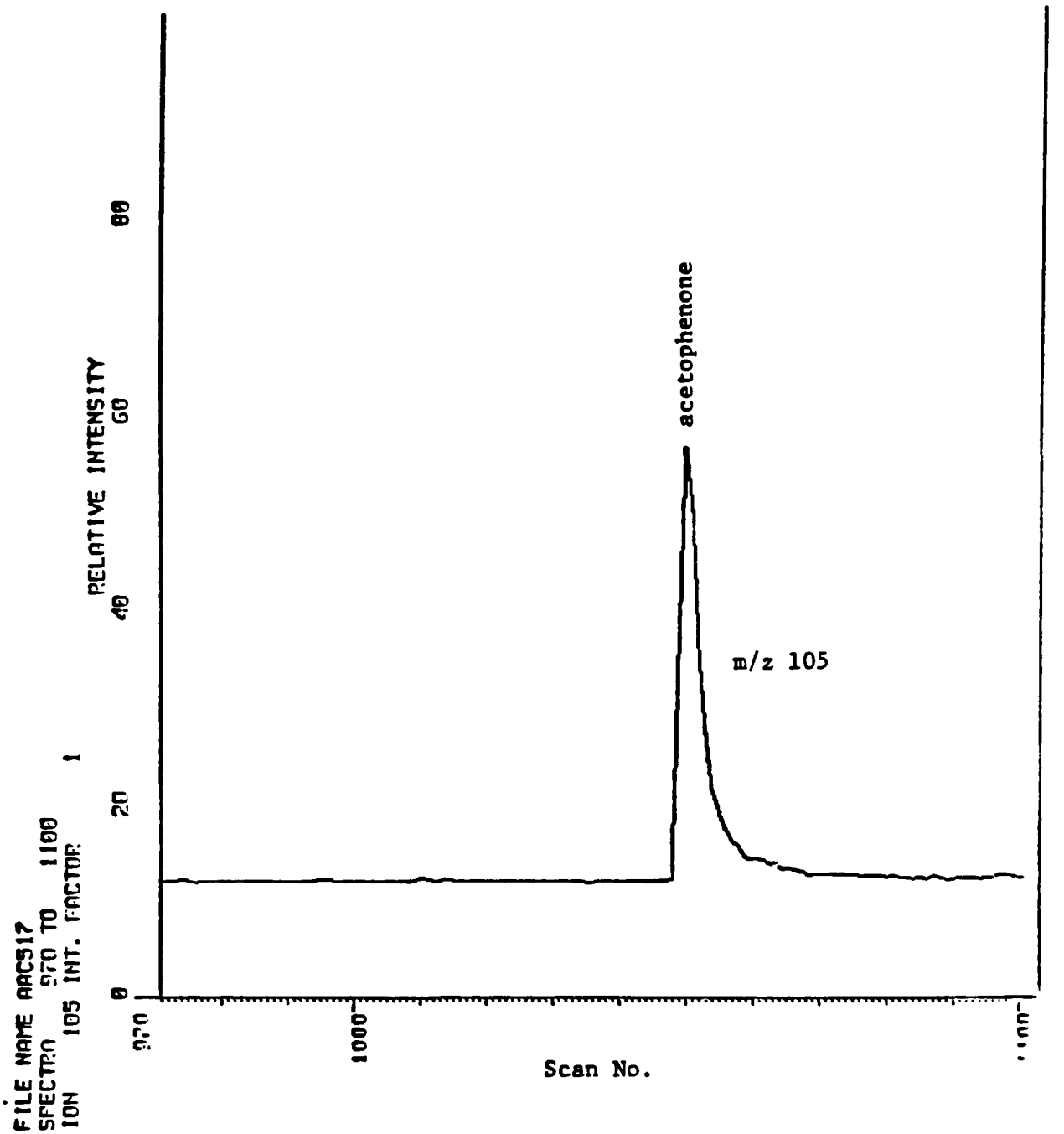


Figure 8. Extracted ion current profile of m/z 105 for acetophenone in calculating percent peak asymmetry factor.

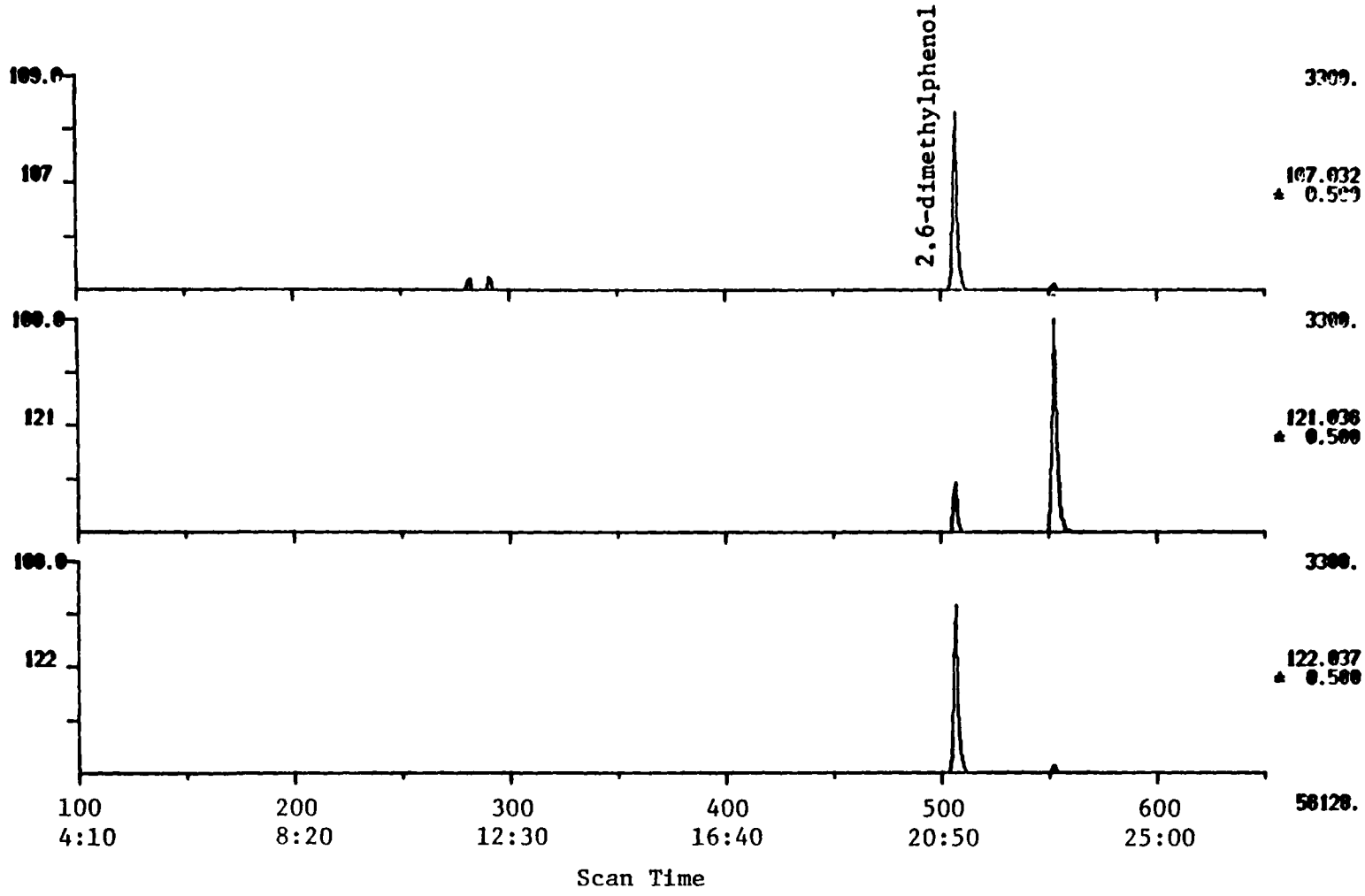


Figure 9. Extracted ion current profile of m/z 107, 121 and 122 of 2,6-dimethylphenol in mixture.

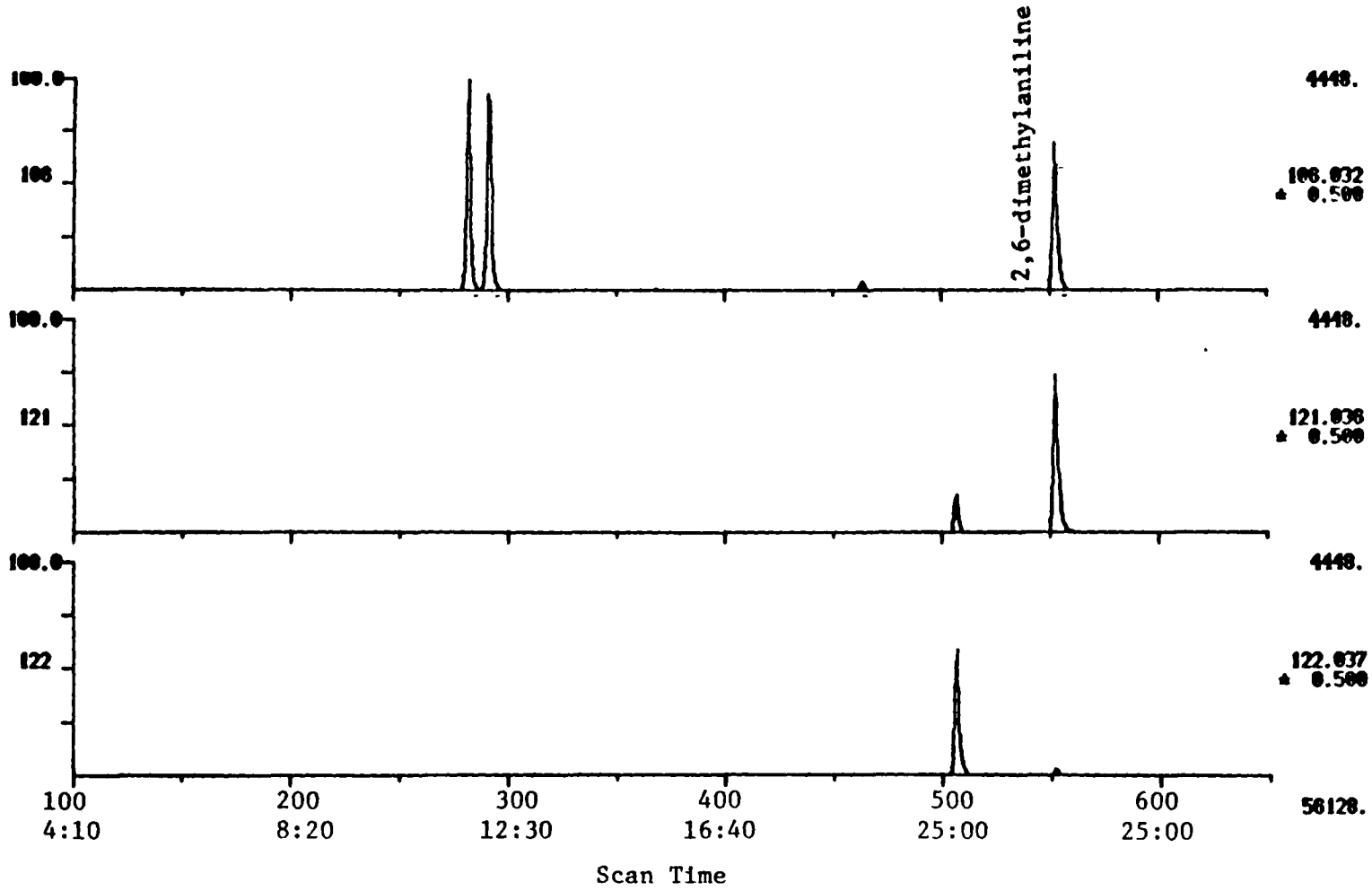


Figure 10. Extracted ion current profile of m/z 106, 121, and 122 for 2,6-dimethylaniline in performance mixture.

7.1.2.3 Sequence of Sample Analysis

A strict step-sequence of analysis is followed. Upon mass and intensity calibration of the MS system, a Tenax GC cartridge loaded with the performance mixture is first analyzed. Following the performance mixture a blank and an RMR standard mixture is analyzed next, then five samples. The cycle is then repeated. At the beginning of each day the analysis cycle begins with the performance mixture, blank and RMR standards. Thus, 30% of the cartridges analyzed consist of control samples.

7.2 External Quality Assurance

Samples will be analyzed by a designated QA laboratory. Samples, controls, and blanks will be shipped directly from the field to the QA laboratory for analysis. They will report the results to the primary laboratories for correlation with the primary data.

7.2.1 Selection of Samples for QA

All of the field samples will be collected in duplicate for replicate analyses. Approximately 10% of the duplicates will be shipped to the QA laboratory. This selection process will be random, unless any stratification of donors is known. If so, purposive selection of QA donors may be used to get representative samples (e.g., occupationally exposed vs. "normal" individuals or upwind vs. downwind residents).

7.2.2 QC Samples

Along with the QA duplicate samples, at least one QC control and one QC blank must be included with the QA samples. An example is shown in Table 10 for a trip collecting 50 samples.

7.2.3 Sample Codes

All samples shipped to the QA laboratory should be encoded so that the laboratory cannot discern between samples and controls. This "blind" protocol will add validity to the results.

7.2.4 Shipping

Samples should be shipped on dry ice directly to the QA laboratory by an appropriate air carrier (e.g., Federal Express, Eastern Sprint) in well insulated and packed cartons.

8.0 References

1. Zweidinger, R., et al., Final Draft Report on Benzene, EPA Contract No. 68-01-3849, 1980, in preparation.
2. Pellizzari, E. D., "Development of Method for Carcinogenic Vapor Analysis in Ambient Atmospheres", Publication No. EPA-650/2-74-121, Contract No. 68-02-1228, 148 pp., July, 1974.
3. Pellizzari, E. D., "Development of Analytical Techniques for Measuring Ambient Atmospheric Carcinogenic Vapors", Publication No. EPA-600/2-75-075, Contract No. 68-02-1228, 187 pp., November, 1975.
4. Pellizzari, E. D., J. E. Bunch, B. H. Carpenter and E. Sawicki, Environ. Sci. Technol., 9, 552 (1975).
5. Pellizzari, E. D., "The Measurement of Carcinogenic Vapors in Ambient Atmospheres", Publication No. EPA-600-7-77-055, Contract No. 68-02-1228, 288 p., June, 1977.
6. Pellizzari, E. D., "Evaluation of the Basic GC/MS Computer Analysis Technique for Pollutant Analysis", Final Report, EPA Contract No. 68-02-2998.
7. Pellizzari, E. D. and L. W. Little, "Collection and Analysis of Purgeable Organics Emitted from Treatment Plants", Final Report, EPA Contract No. 68-03-2681, 216 pp.
8. Pellizzari, E. D., unpublished results.
9. Pellizzari, E. D., "Analysis of Organic Air Pollutants by Gas Chromatography and Mass Spectroscopy", EPA-600/2-77-100, June 1977, 114 pg.
10. Pellizzari, E. D., "Analysis of Organic Air Pollutants by Gas Chromatography and Mass Spectroscopy", EPA-600/2-79-057, March 1979, 243 pg.
11. Pellizzari, E. D., "Ambient Air Carcinogenic Vapors Improved Sampling and Analytical Techniques and Field Studies", EPA-600/2-79-081, May 1979, 340 pg.
12. Pellizzari, E. D., J. E. Bunch, R. E. Berkley and J. McRae, Anal. Chem., 48, 803 (1976).
13. Pellizzari, E. D., J. E. Bunch, R. E. Berkley and J. McRae, Anal. Lett., 9, 45 (1976).

14. "Eight Peak Index of Mass Spectra", Vol. I, (Tables 1 and 2) and II (Table 3), Mass Spectrometry Data Centre, AWRE, Aldermaston, Reading, RF74PR, UF, 1970.

15. Pellizzari, E. D., et al., "Master Scheme for the Analysis of Organic Compounds in Water Part III: Experimental Development and Results", EPA Contract No. 68-03-2704, March 1980.

Written analytical protocol prepared 5/6/80.

ANALYTICAL PROTOCOL: SAMPLING AND ANALYSIS FOR BENZENE IN BLOOD

1.0 Principle of Method

A blood sample is equilibrated at 37°C with an air space of determined volume until equilibrium is attained. The entire headspace is then purged into a cryogenic trap which can be placed in line with a gc as a sample loop and heated. In this manner, the recovery is determined by the partition between fluid and air and avoids the many artifacts and other problems introduced by purging (i.e., foaming, precipitation occulsion, and sorbent background).

2.0 Detection Limit

The range is limited by the limit of detection on one extreme and by the chromatographic capacity of the capillary on the other or $\sim 10^4$. Minimum detectable concentration for the method is estimated to be 1.6 µg/l (95% confidence level).

3.0 Interferences

No interferences have been observed, however high levels of other hydrocarbons in the sample could cause the benzene peak to be obscured.

4.0 Precision and Accuracy

Precision at 500 µg/l is 8% relative standard deviation increasing to 33% at 1.8 µg/l. Recovery of control samples spiked at 5 µg/l was 98%.

5.0 Apparatus and Materials

1. A thermostated two-position, six-port valve with nickel capillary trap as indicated in Figure 1.
2. A gas chromatograph with flame ionization detector.
3. Thermostated oven (37°C).
4. Glass capillary gas chromatography column with SE30 liquid phase.
5. Glass hypodermic syringes (10 ml) and needles.
6. Silicone rubber septum material.
7. Liquid nitrogen.
8. Ultrapure air (<0.1 ppm total hydrocarbon).
9. Vacuum blood sampling tubes (Venoject KT200SKA, Kimble).

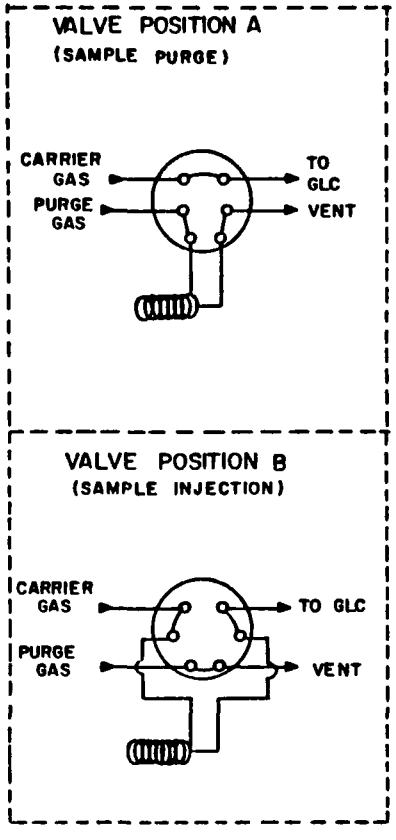


Figure 1. Six-port, 2 position valve for the introduction of headspace samples.

6.0 Procedure

6.1 Collection of Blood Sample

Blood samples are collected from selected participants from a brachial vein by venipuncture using a 10 ml Venoject tube. These blood samples are collected by experienced medical personnel using accepted medical procedures.

6.2 Analysis of Samples

Pre-equilibrate a 10 ml glass syringe at 37°C (>30 min), remove the needle from the syringe and inject 1.0 ml of blood (sample, standard or blank) into the syringe which has been sealed around the plunger with saturated lithium chloride. Adjust the volume to 10 ml by filling the syringe with "ultra pure" air, replace the needle on the syringe and seal it by inserting into a piece of silicone septum material. Incubate the entire syringe assembly at 37°C for 20 min. After the incubation, the needle is removed and the syringe connected to the cryogenic trap via an 18 gauge needle. The total air space in the syringe is purged through the trap. An additional 1 ml of air is purged through the trap from another syringe. The latter step is to prevent sample holdup in the transfer lines to the trap.

At this point, the coolant (liquid nitrogen) is removed from the trap, the valve rotated and the trap rapidly heated to 175°C. The GC operating parameters are given in Table 1.

Calibration of the GC is obtained by analyzing blood spiked with known amounts of benzene and blood blanks under identical conditions to the sample.

6.3 Quantitation

Peak areas of benzene in unknown samples are compared to calibration curves generated with known amounts of added benzene. This results in the following relationship:

$$\text{Concentration of benzene } (\mu g/l) = \frac{A_{unk}/g_{std}}{A_{std}}$$

where

A_{unk} is the peak area of the sample,
g_{std} is the amount of benzene added to the standard
A_{std} is the peak area of the standard.

Table 1. OPERATING PARAMETERS FOR GC/FID ANALYSIS OF BENZENE IN BLOOD AND URINE

Parameter	Value
Column	
47 M glass WCOT SE-30, $BaCO_3$	50°C initial for 3 min then 4°/min to 200°C
Helium carrier gas flow rate	2.1 ml/min

7.0 Quality Assurance Program

An on-going quality assurance program is required to assure the data quality. Quality control (QC) procedures determine artifacts, losses, etc. through a system of blanks and controls. Quality assurance (QA) procedures monitor the execution of the procedure and check data interpretations and calculations.

7.1 Quality Control

7.1.1 Field Blanks and Controls

Prior to a field sampling trip, enough blanks and controls are prepared to equal 10% each (2 minimum) of the anticipated number of field samples. Blanks consist of 10 ml of water in the same type of sampling container as is used in the field. Controls consists of 10 ml of plasma spiked at 50 ng with benzene. These blanks and controls are carried to the field and receive the same handling as the field samples. Workup and analysis of field blanks and controls is interspersed with the field samples on a regular basis. This method allows assessment of sample storage stability.

Table 2 presents a typical set of blanks and controls for QC on a field trip where 50 blood samples are to be collected.

7.1.2 Procedural Blanks and Controls

7.1.2.1 Extraction Blanks

With each set of samples, a procedural blank is run. This consists of 10 ml of prepurged distilled water which is extracted under the same conditions as the samples. These blanks are designed to detect artifacts from dirty glassware, laboratory atmosphere intrusion, and other sources.

7.1.2.2 GC Procedural Control

At the start of each working day, a standard test mixture is analyzed to monitor the capillary GC column performance.

Field samples, field controls, field blanks, and procedural blanks are queued up for analysis such that at least one QC sample is run each working day. In addition, a standard solution is analyzed each day to serve as a procedural control and also to update the calibration value. Thus, in a typical working day, 4 field samples, 1 blank or control, and 1 calibration standard are run.

Table 2. BLOOD QC SAMPLES FOR A TYPICAL SAMPLING TRIP

Sample Type	Number	Comments
Field Blank	5	Freeze after preparation, carry to field, store with field samples.
Field Control	5	Store with field blanks.
Lab Blank	5	Freeze after preparation, store in same freezer as field samples will be stored.
Lab Control	5	Store with Lab Blanks

7.2 Quality Assurance

Both internal and external QA procedures are to be followed. Internal QA procedures assure the continuity and consistency of the data. External QA procedures (interlaboratory checks) verify or dispute the accuracy of the data being generated in the primary laboratory.

7.2.1 Internal Quality Assurance

7.2.1.1 Supervision and Monitoring of Activities

There are three levels of quality assurance (QA). The primary quality assurance is the person conducting the sampling and/or analysis. This person must be aware of their actions, observe events which may affect the data, and maintain appropriate records. At the second level, the chemist's supervisor monitors their daily activities, reviews the notebook, checks data and calculations, and assists in "troubleshooting" problems. At the tertiary level, a QA coordinator interviews all personnel on the project. The interviews cover the operations they perform (precisely), the data they obtain, a spot-check of their calculations, and any problems they have had.

7.2.1.2 Documentation

Chain of Custody

From the initial preparation of a sample container through reporting of the analytical results, each sample is accompanied by a chain of custody sheet. Each person signs in the time of receipt, operations performed, and transmittal of the sample. This record is important for tracing a contaminant, bad standard, or some other problem.

Sampling Protocol Sheets

When a sample is collected, a sampling protocol sheet is filled in which contains a discrete sample code which identifies project number, area, site, locations, trip number, sampling period, and sample type. Also included are sample times, volumes, address, and other pertinent information. Where appropriate, a map is made to precisely identify the location.

Sample Log

Upon return from a sampling trip, each sample code is entered into a sample log book. This log is updated as samples proceed through workup and analysis. Thus, at a glance, project personnel can tell the status of each

sample and find out how many are at different stages in the analytical protocol.

GC Log

Each sample run by GC is logged into a notebook, detailing analysis conditions.

7.2.2 External Quality Assurance

Samples will be analyzed by a designated QA laboratory. Samples, controls, and blanks will be shipped directly from the field to the QA laboratory for analysis. They will report the results to the primary laboratory for correlation with the primary data.

7.2.2.1 Selection of Samples for QA

Approximately 10% of the field samples (2 minimum) will be collected in duplicate (i.e., two 10 ml blood samples) for shipment to the QA laboratory. This selection process will be random with the following restrictions:

(1) The donor must consent to the additional hair collection.

(2) If any stratification of donors is known, purposive selection of QA donors may be used to get representative samples (e.g. occupationally exposed vs. "normal" individuals or upwind vs. downwind residents).

7.2.2.2 QC Samples

Along with the QA duplicate samples, at least one QC control and one QC blank must be included with the QA samples. An example is shown in Table 3 for a trip collecting 50 samples.

7.2.2.3 Sample Codes

All samples shipped to the QA laboratory should be encoded so that the laboratory cannot discern between samples and controls. This "blind" protocol will add validity to the results.

7.2.2.4 Shipping

Samples should be shipped on dry ice directly to the QA laboratory by an appropriate air carrier (e.g. Federal Express, Eastern Sprint) in well insulated and packed cartons.

Table 3. SAMPLES TO BE COLLECTED AND SHIPPED TO QA LABORATORY FOR A TYPICAL SAMPLING TRIP

Sample	Number	Comments
Duplicate Sample	5	Random selection unless prior information stratifies subject
Field Blank	1	Ship with samples
Field Control	1	Ship with samples

8.0 References

1. Zweidinger, R. A., S. D. Cooper, B. S. H. Harris, III, T. D. Hartwell, R. E. Folsom, Jr., E. D. Pellizzari, A. W. Sherdon, T. K. Wong and H. S. Zelon, "Measurement of Benzene Body-Burden for Populations Potentially Environmentally Exposed to Benzene", EPA Contract No. 68-01-3849, Final Report (in preparation).

Revised April, 1980

ANALYTICAL PROTOCOL: SAMPLING AND ANALYSIS OF VOLATILE PURGEABLE HALOGENATED HYDROCARBONS IN HUMAN BLOOD SERUM AND URINE (U. MIAMI)

1.0 Principle of the Method

Human blood serum or urine is analysed for volatile purgeable halogenated hydrocarbons (VPHH's) by a purge/trap/desorb procedure based on that of Bellar and Lichtenberg. No extraction or clean-up step is required and the cost per analysis is reasonable. Each analysis is completed in about 30 minutes.

Successfully analyzing blood and urine is dependent on circumventing foaming problems. To circumvent or greatly reduce foaming problems, 0.5 mL of a 1% solution of Dow Corning Antifoam Emulsion B is purged 20 min with pure inert gas prior to the addition of the blood serum.

A small condenser, attached to the effluent arm of the purging device also aids in keeping foaming serum out of the Tekmar instrument. The serum, urine/antifoam mixture is purged for 30 min at room temperature and then at 115°. The elevated temperature presumably denatures the proteins, one of the causes of foaming; it is also necessary to counteract the inherent binding capacity of serum for halogenated organic compounds. In vitro spiked compounds can be recovered completely at room temperature or 115°. Samples obtained from dosed animals (in vivo work) require 115° to effect complete removal of volatile purgeable halocarbons.

2.0 Range and Detection Limits

Detection limits are:

chloroform - 0.05 µg/L
carbon tetrachloride - 0.05 µg/L
bromodichloromethane - 0.1 µg/L
trichloroethylene - 5 µg/L

3.0 Interferences

Co-eluting halogenated compounds present problems with identification and quantitation. Co-elution can be circumvented by the use of different columns such as Carbopack C or Tenax GC. There is some concern that chloroform is being generated in situ from trichloroacetic acid. This is currently under investigation.

4.0 Precision and Accuracy

One serum sample was analyzed ten times over a two-day period. The chloroform concentration ranged from 23 to 36 µg/l with a mean value of 27 µg/l and a standard deviation of 4. Accuracy has not been determined.

5.0 Apparatus and Reagents

5.1 Apparatus

1. A Tekmar Model LSC-1 liquid sample concentrator
2. Tracor Model 222 gas-liquid chromatograph (GLC)
3. Hall electrolytic conductivity detector which is operated in the halide specific mode.
4. Chromatographic column, 2-m x 6.4-mm I.D. glass U-tube containing n-octane on 100-120 mesh Porasil C packing.
5. A Finnigan Model 4000 gas -liquid chromatograph/mass spectrometer (GLC/MS) analytical system interfaced to a Tekmar liquid sample concentrator
6. Both the GLC and GLC/MS systems utilized a hot plate stirrer and a glyceral bath to heat the sample in the Tekmar purging device.

5.2 Solvents and Reagents

1. Chloroform, pesticide grade, Fisher Scientific Co.
2. Carbon tetrachloride, pesticide grade.
3. Hexane, pesticide grade, Fisher Scientific Co.
4. Trichloroethylene, Aldrich Chemical Co.
5. 1,2-dichloroethane, Aldrich Chemical Co.
6. Bromoform, Aldrich Chemical Co.
7. Bromodichloromethane, Columbia Organic Chemical Co.
8. Dibromochloromethane, Columbia Organic Chemical Co.
9. Dow Corning Antifoam Emulsion B, Fisher Schientific Co.
10. n-octane on 100-120 mesh Porasil C chromatographic packing, Supelco Inc.

6.0 Procedure

6.1 Collection and Handling of Samples

Blood is collected in vacuum tubes suitable for use in gc applications, viz., Venoject® L428 (Kimble) tubes (see page 208). Blood plasma is separated from the cells and stored at 4° prior to analysis.

First morning urine samples are collected in wide-mouthed bottles, sealed and stored at 4° until analysis.

6.2 Preparation of Standards and Samplers

6.2.1 Preparation of Standards

6.2.1.1 Two mL each of carbon tetrachloride, dibromochloromethane and bromoform and 1 mL each of chloroform, trichloroethylene, bromodichloromethane and 1,2 dichloroethane are diluted with hexane to final volume of 100 mL (Solution 1). Appropriate amounts of 1,1,1-trichloroethane, 1,1,2-trichloroethane, vinylidene chloride, and chlorobenzene can also be diluted to prepare standards.

6.2.1.2 One ml of solution 1 is quantitatively diluted to 100 ml with hexane (solution 2), and a convenient working standard prepared by diluting 0.1 ml of solution 2 to 25 ml with hexane. Use of 5 µl of this solution leads to acceptable peak heights when the Hall detector attenuation is 10 x 8.

6.2.1.3 A standard curve is obtained by using three hexane dilutions of solution 2: the working standard, 0.1 ml diluted to 50 ml (for 10 x 4 attenuation) and 0.2 ml diluted to 25 ml (for 10 x 16 attenuation). Although solutions 1 and 2 are stable at room temperature, fresh working standards must be made daily.

6.2.2 Procedure for Blood Serum and Urine

6.2.2.1 One ml of 1% aqueous antifoam is added to the 5 ml purging device and the sample concentrator is operated in the Trap Bake Mode for 20 min while the trap temperature was 200°. The trap is then cooled to the ambient temperature.

6.2.2.2 By means of a gas tight syring, 0.5 ml of serum or 2 ml of urine is introduced into the purging device and a purge flow-rate of 10 ml/min was started. During analyses the stripping gas volume is 0.3 L.

6.2.2.3 The lower portion of the purging device is immersed in a 115° stirred glycerol bath for 30 min. To prevent steam contamination of the Tenax/silica gel trap, a small glass vapor trap or interceptor is placed between the purging device and the adsorbent trap.

6.2.2.4 After the purge/trap period is complete, the adsorbed compounds are desorbed and transferred to the analytical column (60°) by heating the trap at 150° for 6 min.

6.3 Analysis

6.3.1 The GLC operating conditions include: a nitrogen carrier gas flow-rate of 30 ml/min, an inlet temperature of 140°, and a transfer line temperature of 210°. The GLC column is temperature programmed 7°/min to 140°.

6.3.2 The Hall detector furnace is maintained at 900° with a hydrogen flow-rate of 40 ml/min and a solvent (1:1 n-propanol:distilled water) flow of 0.4 ml/min.

6.4 Qualitative Identification

Identifying of compounds for LSC/GLC/HECD system is done by retention times. This is confirmed by LSC/GLC/MS methodology. The confirmations are based on relative retention data and mass fragmentation data.

6.5 Quantitation

LSC/GLC/HECD chromatograms are quantified by using the external standard method described in section 6.2.1.3.

*7.0 Quality Assurance Program

An on-going quality assurance program is required to assure the data quality. Quality control (QC) procedures determine artifacts, lossed, etc. through a system of blanks and controls. Quality assurance (QA) procedures monitor the execution of the procedure and check data interpretations and calculations.

7.1 Quality Control

7.1.1 Field Blanks and Controls

Prior to a field sampling trip, enough blanks and controls are prepared to equal 10% each (2 minimum) of the anticipated number of field samples. Blanks consist of 10 ml of water in the same type of sampling container as is used in the field. Controls consist of 10 ml of plasma or urine spiked at 10-15 ng with chloroform, 1,2-dichloroethane, 1,1,1-trichloroethane, 1,1,2-trichloroethane, carbon tetrachloride, vinylidene chloride, trichloroethylene, tetrachloroethylene, bromodichloromethane, chlorobenzene and m-dichlorobenzene. These blanks and controls are carried to the field and

receive the same handling as the field samples. Workup and analysis of field blanks and controls is interspersed with the field samples on a regular basis. This method allows assessment of sample storage stability.

Table 1 presents a typical set of blanks and controls for QC on a field trip where 50 blood or urine samples are to be collected.

7.1.2 Procedural Blanks and Controls

With each set of samples, a procedural blank is run. This consists of 2 ml of prepurged distilled water which is analyzed under the same conditions as the samples. These blanks are designed to detect artifacts from dirty glassware, laboratory atmosphere intrusion, and other sources.

7.1.2.2 GC/MS Procedural Control

Field samples, field controls, field blanks, and procedural blanks are queued up for GC/MS analysis such that at least one QC sample is run each working day. In addition, a standard solution is analyzed each day to serve as a procedural control and also to update the RMR value. Thus, in a typical working day, 4 field samples, 1 blank or control, and 1 RMR standard are run.

The Finnigan GC/MS is a quadrupole mass spectrometer which requires frequent tuning. Daily tuning is achieved using FC-43 and decafluorotriphenylphosphine (DFTPP).

7.2 Quality Assurance

Both internal and external QA procedures are to be followed. Internal QA procedure assure the continuity and consistancy of the data. External QA procedures (interlaboratory checks) verify or dispute the accuracy of the data being generated in the primary laboratory.

7.2.1 Internal Quality Assurance

7.2.1.1 Supervision and Monitoring of Activities

There are three levels of quality assurance (QA). The primary quality assurance is the person conducting the sampling and/or analysis. This person must be aware of their actions, observe events which may affect the data, and maintain appropriate records. At the second level, the chemist's supervisor monitors their daily activities, reviews the notebook, checks data and calculations, and assists in "troubleshooting" problems. At the tertiary level, a QA coordinator interviews all personnel on the project.

Table 1. BLOOD QC SAMPLES FOR A TYPICAL SAMPLING TRIP

Sample Type	Number	Comments
Field Blank	5	Freeze after preparation, carry to field, store with field samples.
Field Control	5	Store with field blanks.
Lab Blank	5	Freeze after preparation, store in same freezer as field samples will be stored.
Lab Control	5	Store with Lab Blanks

The interviews cover the operations they perform (precisely), the data they obtain, a spot-check of their calculations, and any problems they have had.

7.2.1.2 Documentation

Chain of Custody

From the initial preparation of a sample container through reporting of the analytical results, each sample is accompanied by a chain of custody sheet. Each person signs in the time of receipt, operations performed, and transmittal of the sample. This record is important for tracing a contaminant bad standard, or some other problem.

Sampling Protocol Sheets

When a sample is collected, a sampling protocol sheet is filled in which contains a discrete sample code which identifies project number, area, site, locations, trip number, sampling period, and sample type. Also included are sample times, volumes, address and other pertinent information. Where appropriate, a map is made to precisely identify the location.

Sample Log

Upon return from a sampling trip, each sample code is entered into a sample log book. This log is updated as samples proceed through workup and analysis. Thus, at a glance, project personnel can tell the status of each sample and find out how many are at different stages in the analytical protocol.

GC/MS Log

Each sample run by GC/MS is logged into a notebook, detailing analysis conditions, where the data are archived, and what hardcopy data has been produced.

7.2.2 External Quality Assurance

Samples will be analyzed by a designated QA laboratory. Samples, controls, and blanks will be shipped directly from the field to the QA laboratory for analysis. They will report the results to the primary laboratory for correlation with the primary data.

7.2.2.1 Selection of Samples for QA

Approximately 10% of the field samples (2 minimum) will be collected in duplicate (i.e., two 10 ml blood or two 120 mL urine samples) for shipment

to the QA laboratory. This selection process will be random with the following restrictions:

(1) The donor must consent to the additional blood and urine collection.

(2) If any stratification of donors is known, purposive selection of QA donors may be used to get representative samples (e.g., occupationally exposed vs. "normal" individuals or upwind vs. downwind residents).

7.2.2.2 QC Samples

Along with the QA duplicate samples, at least one QC control and one QC blank must be included with the QA samples. An example is shown in Table 2 for a trip collecting 50 samples.

7.2.2.3 Sample Codes

Sll samples shipped to the QA laboratory should be encoded so that the laboratory cannot discern between samples and controls. This "blind" protocol will add validity to the results.

7.2.2.4 Shipping

Samples should be shipped on dry ice directly to the QA laboratory by an appropriate air carrier (e.g., Federal Express, Eastern Sprint) in well insulated and packed cartons.

8.0 References

1. Sherma, J., "Manual of Analytical Quality Control for Pesticides in Human and Environmental Media" EPA -600/1-76-017, 2K, 33 (1979).
2. ibid, Section 4, A, (6), p 2.
3. ibid, Section 4, A, (4), p 4.

Adapated from: "Determination of Volatile Purgeable Halogenated Hydrocarbons in Human Adipose Tissue and Blood Serum: Peoples, A. J., Pfaffenberger, C. D., Shafik, T. M., Enos, H. F., University of Miami School of Medicine, Department of Epidemiology and Public Health, Chemical Epidemiology Division, Miami Florida.

* Indicates sections not in original protocol, added here for application to this research project.

Table 2. SAMPLES TO BE COLLECTED AND SHIPPED TO QA LABORATORY FOR A TYPICAL SAMPLING TRIP

Sample	Number	Comments
Duplicate Sample	5	Random selection unless prior information stratifies subjects
Field Blank	1	Ship with samples
Field Control	1	Ship with samples

ANALYTICAL PROTOCOL: SAMPLING AND ANALYSIS OF PURGEABLE HALOGENATED HYDROCARBONS IN BLOOD (RTI)

1.0 Principle of the Method

Volatile compounds are recovered from a blood sample by warming the sample and purging an inert gas over the warm sample. The vapors are then trapped on a Tenax cartridge which is analyzed by thermal desorption interfaced to GC/MS.

2.0 Range and Sensitivity

For a typical organic compound approximately 30 ng are required for a mass spectral identification using high resolution glass capillary GC/MS analysis. Based on a 10 ml blood sample, the limit of detection is about 3 ng/ml or 3 ppb. The dynamic range for a purged sample is $\sim 10^4$, however, smaller samples may be purged and the range increased commensurately.

3.0 Interferences

Two possible types of interference must be considered: (1) material present in the sample which physically prevents the effective purge of the sample, and (2) a material which interferes with the analysis of the purged sample. In the former case, several techniques have been developed to handle such problems (e.g., foaming) by diluting and stirring the sample, or the use of chemical antifoaming agents. The second case is minimized by the use of GC/MS for the analysis since unique combinations of m/z and retention times can be selected for most compounds. This permits the analysis of compounds even though chromatographic resolution is not obtained.

4.0 Precision and Accuracy

The purge and trap technique was validated using four ^{14}C-labeled model compounds and six "cold" model compounds with an average recovery of 98.3% ±14.2%. Based on these data, expected recoveries of purgeable halogenated organics from blood are about 80% or better. Within the precision requirements of this study, these recovery values indicate that the method is essentially quantitative.

5.0 Apparatus and Reagents

5.1 Sampling Apparatus

Vacutainers "suitable for GC", Venoject L 428 (Kimble, or other high purity vacutainers; ice bath; disposable Pasteur pipettes and bulbs, and cleaned and oven-treated shell vials with Teflon-lined screw caps.

5.2 Purge Apparatus

The apparatus required is shown in Figure 1.

5.3 Tenax Cartridges

Tenax cartridges are prepared and the background checked as described in Section 6.1.1 of Protocol A-8 (Analysis of Purgeable Organic Compounds in Water [Master Analytical Scheme]).

5.4 GC/MS/COMP

The volatile halogenated hydrocarbons purged from water are analyzed on either an LKB 2091 GC/MS with an LKB 2031 data system or a Varian MAT CH-7 GC/MS with a Varian 620/I data system. The sample, concentrated on a Tenax GC cartridge is thermally desorbed using an inlet manifold system (2-6). The operating conditions for the thermal desorption unit and the analysis Tenax GC cartridges are given in Table 1.

5.5 Reagents and Solvents

1. Pentane, Burdick and Jackson distilled in glass, redistilled prior to use,
2. Methanol, Burdick and Jackson distilled in glass, redistilled prior to use.

6.0 Procedure

6.1 Collection of Samples

Blood samples are collected in replicate 10 ml vacutainer tubes containing an anticoagulant. Using a qualified phlebotomist, the samples are collected by brachial venipuncture. Glass syringes represent the optimal collection device, since no polymeric material which may contaiminate the sample comes in contact with the blood. However, sterilization of large numbers of glass syringes in the field is not practical, so vacutainers will be used.

Possible contamination by permeation through the rubber septum caps of the vacutainers is a cause for concern. Teflon-lined vacutainers are not

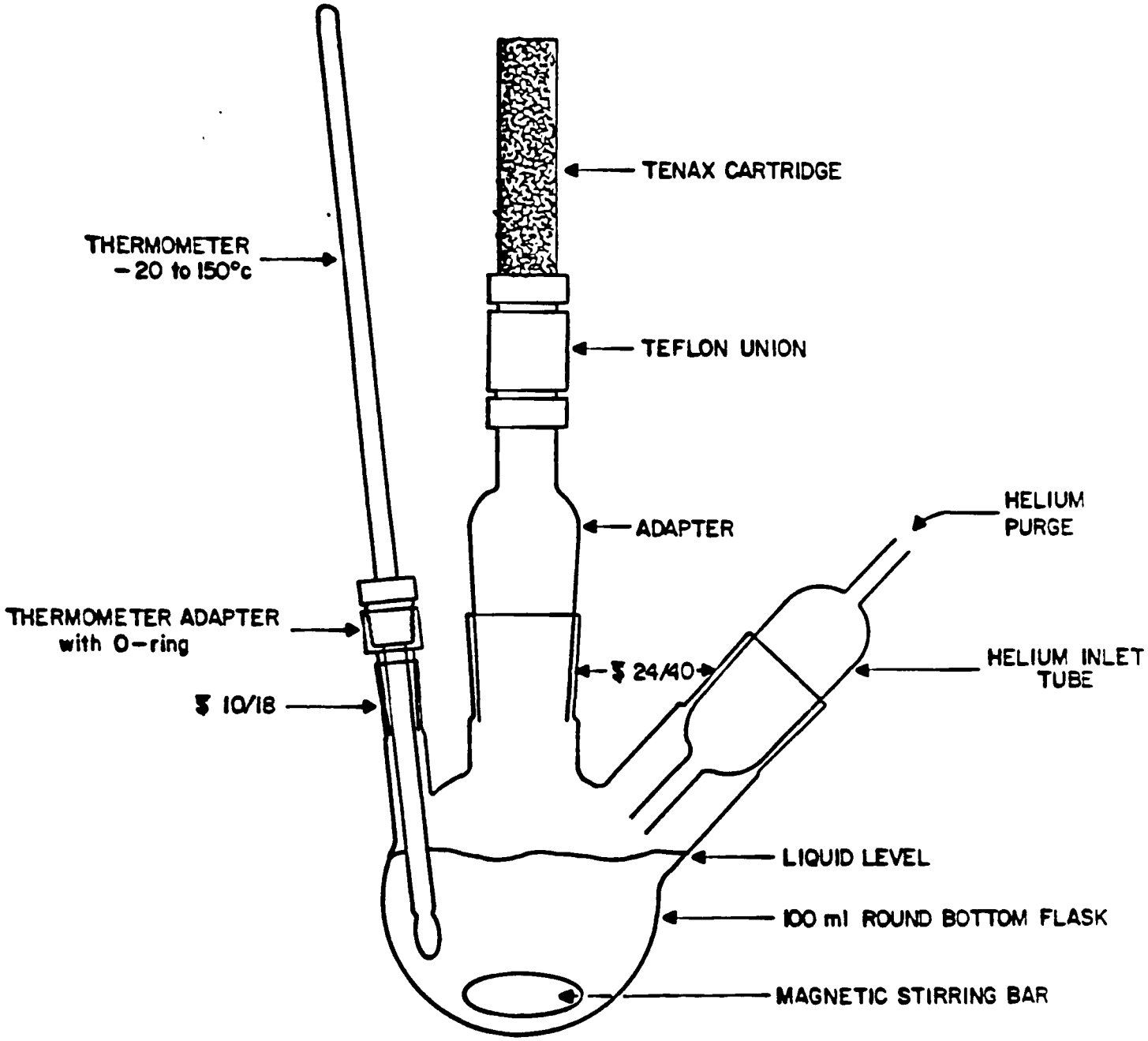


Figure 1. Headspace using apparatus for blood, urine and tissue samples.

Table 1. INSTRUMENTAL OPERATING CONDITIONS

	LKB 2091	Varian MAT CH-7
Desorption chamber temperature	270	265
Desorption chamber He flow	15 ml/min	10 ml/min
Desoprtion time	8.0 min	8.0 min
Capillary Trap Temperature during desorption	-196°C	-196°C
Temperature of capillary trap during injection onto column	-196°C to 250°C - then held at 190°C	
Time of He flow through capillary trap	12 3/4 min	12 3/4 min
He flow through column [sweep time]	9.5 min	4 min
Carrier flow	2.0 ml/min	1.0 ml/min
Capillary column	100 m SE-30 SCOT	20 m SE-30 WCOT
Column temperature	30°C for 2 min, then 4°/min to 240°	20 → 240° at 4°/min
Scan range	5-490 dalton	20 → 500 dalton
Scan rate	2 sec full scale	1 sec/decade
Scan cycle time	2.4 sec	4.5 sec
Scan mode	parabolic	exponential
Trap current	4A	
Filament current	50μA	300μA
Accelerating volatage	3.5 kV	2kV

available but manufacturers recommended special vacutainers "suitable for GC" (Venoject L 428, Kimble). Validation experiments for benzene under a separate contract (EPA No. 68-01-3849, Task I) found the background of these tubes to be acceptable.

Following collection, shipping and storage procedures must assure that the purgeable halogenated organics remain intact in the blood sample. Leakage of the vacutainer caps has been observed and permeation through the cap material is suspected. Accordingly, these containers are not suitable for storage. In the procedure used for this project, the blood sample is chilled and transferred to a clean shell vial with a Teflon-lined screw cap. The cap is then taped shut. This procedure has been validated and field tested on EPA Contract No. 68-01-3849, Task I.

6.2 Purge of Volatile Organics

1. Measure a 10 ml aliquot of whole blood (if available), previously chilled to 4°C, into the purge flask (Fig. 1).
2. Dilute sample to 50 ml with purged distilled water and add stir bar.
3. Assemble apparatus, start stirring and raise the temperature to 50°C.
4. Adjust helium flow to 25 ml/min and purge for 90 min.
5. After 90 min disassemble apparatus and transfer Tenax cartridge to a Kimax culture tube with 2 g calcium sulfate dessicant for 4 hours of drying.
6. Transfer Tenax cartridge to an identical Kimax culture tube without calcium sulfate, seal in a paint can and store in freezer until analysis.

6.3 Analysis of Sample Purged on Cartridge

The insturmental conditions for the analysis of halogenated hydrocarbons of the sorbent Tenax GC sampling cartridge is shown in Table 1. The thermal desorption chamber and six-port valve are maintained at 270° and 200°C, respectively. The helium purge gas through the desorption chamber is adjusted to 15-20 ml/min. The nickel capillary trap at the inlet manifold is cooled with liquid nitrogen. In a typical thermal desorption cycle a sampling cartridge is placed in the preheated desorption chamber and helium gas is

channeled through the cartridge to purge the vapors into the liquid nitrogen cooled nickel capillary trap. After desorption the six-port valve is rotated and the temperature on the capillary loop is rapidly raised; the carrier gas then introduces the vapors onto the high resolution GLC column. The glass capillary column is temperature programmed from 20° to 240°C at 4°/min and held at the upper limit for a minimum of 10 min. After all of the components have eluted from the capillary column the analytical column is then cooled to ambient temperature and the next sample is processed.

6.4 Quantitation

All data are acquired in the full scan mode. Quantitation of the halogenated compounds of interest is accomplished by utilizing selected ion plots, SIPs, which are plots of the intensity of specific ions (obtained from full scan data) vs. time. Using SIPs of ions characteristic of a given compound in conjunction with retention times permits quantitation of components of overlapping peaks. Two external standards, perfluorobenzene and perfluorotoluene, were added to each Tenax GC cartridge in known quantities just prior to analysis. In order to eliminate the need to construct complete calibration curves for each compound quantitated, the method of relative molar response (RMR) is used. In this method the relationship of the RMR of the unknown to the RMR of the standard is determined as follows:

$$RMR_{unknown} = \frac{A_{unk}/Moles_{unk}}{A_{std}/Moles_{std}}$$

$$RMR_{unk/std} = \frac{A_{unk}/g_{unk}/GMW_{unk}}{A_{std}/g_{std}/GMW_{std}}$$

where A = peak response of a selected ion,
g = number of grams present, and
GMW = gram molecular weight.

Thus, in the sample being analyzed:

$$g_{unknown} = \frac{(A_{unk})(GMW_{unk})(g_{unk})}{(A_{std})(GMW_{std})(RMR_{unk/std})}$$

The value of an RMR is determined from at least three independent analyses of standards of accurately known concentration prepared using a gas permeation system (5). The precision of this method has been determined to be generally ±10 percent when replicate sampling cartridges are examined.

7.0 Quality Assurance Program

An on-going quality assurance program is required to assure the data quality. Quality control (QC) procedures determine artifacts, lossed, etc. through a system of blanks and controls. Quality assurance (QA) procedures monitor the execution of the procedure and check data interpretations and calculations.

7.1 Quality Control

7.1.1 Field Blanks and Controls

Prior to a field sampling trip, enough blanks and controls are prepared to equal 10% each (2 minimum) of the anticipated number of field samples. Blanks consist of 10 ml of water in the same type of sampling container as is used in the field. Controls consist of 10 ml of plasma spiked at 100-450 ng with chloroform, 1,2-dichloroethane, 1,1,1-trichloroethane, 1,1,2-trichloroethane, carbon tetrachloride, vinylidene chloride, trichloroethylene, tetrachloroethylene, bromodichloromethane, chlorobenzene, m-dichlorobenzene, and vinyl chloride. These blanks and controls are carried to the field and receive the same handling as the field samples. Workup and analysis of field blanks and controls is interspersed with the field samples on a regular basis. This method allows assessment of sample storage stability.

Table 2 presents a typical set of blanks and controls for QC on a field trip where 50 blood samples are to be collected.

7.1.2 Procedural Blanks and Controls

7.1.2.1 Extraction Blanks

With each set of samples, a procedural blank is run. This consists of 10 ml of prepurged distilled water which is extracted under the same conditions as the samples. These blanks are designed to detect artifacts from dirty glassware, laboratory atmosphere intrusion, and other sources.

7.1.2.2 GC/MS Procedural Control

At the start of each working day, a mixture of 2,6-dimethylphenol, 2,6-dimethylaniline, and acetophenone (PA mixture) is analyzed to monitor

Table 2. BLOOD QC SAMPLES FOR A TYPICAL SAMPLING TRIP

Sample Type	Number	Comments
Field Blank	5	Freeze after preparation, carry to field, store with field samples.
Field Control	5	Store with field blanks.
Lab Blank	5	Freeze after preparation, store in same freezer as field samples will be stored.
Lab Control	5	Store with Lab Blanks

the capillary GC column performance. This also serves to check the mass spectrometer tuning.

Field samples, field controls, field blanks, and procedural blanks are queued up for GC/MS analysis such that at least one QC sample is run each working day. In addition, a standard solution is analyzed each day to serve as a procedural control and also to update the RMR value. Thus, in a typical working day, 4 field samples, 1 blank or control, and 1 RMR standard are run.

The Finnigan GC/MS is a quadrupole mass spectrometer which requires frequent tuning. Daily tuning is achieved using FC-43 and decafluorotriphenylphosphine (DFTPP).

7.2 Quality Assurance

Both internal and external QA procedures are to be followed. Internal QA procedure assure the continuity and consistancy of the data. External QA procedures (interlaboratory checks) verify or dispute the accuracy of the data being generated in the primary laboratory.

7.2.1 Internal Quality Assurance

7.2.1.1 Supervision and Monitoring of Activities

There are three levels of quality assurance (QA). The primary quality assurance is the person conducting the sampling and/or analysis. This person must be aware of their actions, observe events which may affect the data, and maintain appropriate records. At the second level, the chemist's supervisor monitors their daily activities, reviews the notebook, checks data and calculations, and assists in "troubleshooting" problems. At the tertiary level, a QA coordinator interviews all personnel on the project. The interviews cover the operations they perform (precisely), the data they obtain, a spot-check of their calculations, and any problems they have had.

7.2.1.2 Documentation

Chain of Custody

From the initial preparation of a sample container through reporting of the analytical results, each sample is accompanied by a chain of custody sheet. Each person signs in the time of receipt, operations performed, and transmittal of the sample. This record is important for tracing a contaminant, bad standard, or some other problem.

Sampling Protocol Sheets

When a sample is collected, a sampling protocol sheet is filled in which contains a discrete sample code which identifies project number, area, site, locations, trip number, sampling period, and sample type. Also included are sample times, volumes, address and other pertinent information. Where appropriate, a map is made to precisely identify the location.

Sample Log

Upon return from a sampling trip, each sample code is entered into a sample log book. This log is updated as samples proceed through workup and analysis. Thus, at a glance, project personnel can tell the status of each sample and find out how many are at different stages in the analytical protocol.

GC/MS Log

Each sample run by GC/MS is logged into a notebook, detailing analysis conditions, where the data are archived, and what hardcopy data has been produced.

7.2.2 External Quality Assurance

Samples will be analyzed by a designated QA laboratory. Samples, controls, and blanks will be shipped directly from the field to the QA laboratory for analysis. They will report the results to the primary laboratory for correlation with the primary data.

7.2.2.1 Selection of Samples for QA

Approximately 10% of the field samples (2 minimum) will be collected in duplicate (i.e., two 10 ml blood samples) for shipment to the QA laboratory. This selection process will be random with the following restrictions:

(1) The donor must consent to the additional blood collection.

(2) If any stratification of donors is known, purposive selection of QA donors may be used to get representative samples (e.g., occupationally exposed vs. "normal" individuals or upwind vs. downwind residents).

7.2.2.2 QC Samples

Along with the QA duplicate samples, at least one QC control and one QC blank must be included with the QA samples. An example is shown in Table 3 for a trip collecting 50 samples.

Table 3. SAMPLES TO BE COLLECTED AND SHIPPED TO QA LABORATORY FOR A TYPICAL SAMPLING TRIP

Sample	Number	Comments
Duplicate Sample	5	Random selection unless prior information stratifies subjects
Field Blank	1	Ship with samples
Field Control	1	Ship with samples

7.2.2.3 Sample Codes

Sll samples shipped to the QA laboratory should be encoded so that the laboratory cannot discern between samples and controls. This "blind" protocol will add validity to the results.

7.2.2.4 Shipping

Samples should be shipped on dry ice directly to the QA laboratory by an appropriate air carrier (e.g., Federal Express, Eastern Sprint) in well insulated and packed cartons.

8.0 References

1. Pellizzari, E.D., M.D. Erickson and R.A. Zweidinger, "Analytical Protocols for Making a Preliminary Assessment of Halogenated Organic Compounds in Man and Environmental Media", Appendix C, Pg 116-117, Revised April 79.
2. Pellizzari, E.D., Development of Method for Carcinogenic Vapor Analysis in Ambient Atmospheres. Publication No. EPA-650/2-74-121, Contract No. 68-02-1228, 148 pp., July, 1974.
3. Pellizzari, E.D., Development of Analytical Techniques for Measuring Ambient Atmospheric Carcinogenic Vapors. Publication No. EPA-600/2-76-076, Contract No. 68-02-1228, 185 pp., November, 1975.
4. Pellizzari, E.D., J.E. Bunch, B.H. Carpenter and E. Sawicki, Environ. Sci. Tech., 9, 552 (1975).
5. Pellizzari, E.D., B.H. Carpenter, J.E. Bunch and E. Sawicki, Environ. Sci. Tech., 9, 556, (1975).
6. Pellizzari, E.D., Development of Analytical Techniques for Measuring Ambient Atmospheric Carcinogenic Vapors, Publication No. EPA-600/7-77-055, 288 pp., June 1977.

Revised April 1980

ANALYTICAL PROTOCOL: SAMPLING AND ANALYSIS OF ARSENIC, CADMIUM, AND LEAD IN WHOLE BLOOD (RTI)

1.0 Principle of Method

The analysis of arsenic, cadmium, and lead in urine is carried out using atomic absorption spectrophotometry. Increased sensitivity is achieved by atomizing the metal in a graphite furnace with continuous deuterium background correction. Sample workup for arsenic analysis includes an extraction from the blood matrix and furnace atomization of solutions containing 1000 ppm nickel.

2.0 Range and Detection Limit

The minimum detection limit (MDL) and range for the metal assays in urine are shown below.

Metal	MDL	Max. Conc.
Arsenic	not established	
Cadmium	0.05	10.0
Lead	1.00	100.0

Samples containing higher metal concentrations may be analyzed by suitable dilution with 0.5% nitric acid. Dilution for arsenic determinations is made with 0.005 M dichromate solution containing 1000 ppm nickel in 1.0% nitric acid.

3.0 Interferences

No known chemical or spectral interferences exist in the analysis of arsenic, cadmium or lead in whole blood. Severe matrix interferences with arsenic analysis are minimized by incorporating the toluene extraction step into the workup procedure.

4.0 Precision and Accuracy

The precision and accuracy associated with these analyses is a function of sample metal concentration. At the detection limit, the total measurement error is $\pm$ 100%. Based on the results of a previous study (1), the metal analyses are performed with the following precision (relative standard deviation) and accuracy (relative error). The total analysis error is also given (2).

Metal	Range	Precision (% RSD)	Accuracy (% RE)	Total Error (%)
Cadmium	0.3-5.0	15	10	40
Lead	5.0-100.0	10	5	25

The precision and accuracy for the arsenic analysis has not been determined.

5.0 Apparatus and Reagents

A commercially available stock solution containing 1000 ppm metal is used for the preparation of the calibration standards. The concentrated nitric acid is reagent grade quality and the deionized water used in this study is prefiltered and subjected to the action of an activated carbon cartridge and two sequential ion exchange units.

The glassware used for sample workup and the preparation of the calibration solutions must be subjected to a nitric acid cleaning protocol.

All volumetric flasks should be soaked overnight in 20% nitric acid, rinsed with deionized water, soaking for an additional 15-18 hours in a 5% nitric acid bath, followed by a copious deionized water rinse. The flasks are completely filled with 0.5% nitric acid and stored in this manner. Prior to use, each flask is emptied and rinsed well with deionized water. Pipets are soaked in 5% nitric acid, rinsed well with deionized water, air-dried and stored in a clean, dust-free environment.

All beakers used for blood operations require additional pretreatment. Clean beakers (soaked in 20% and 5% nitric acid) are "predigested" by heating 10-25 ml of conc. nitric to reflux (with watchglass), cooling, and discarding the acid. The beakers are rinsed thoroughly with deionized water and used for a sample digestion within 30 minutes. The beakers are never allowed to go dry.

Sample cup for the graphite furnace autosampler may be made of polystyrene or Teflon. The former type requires overnight soaking in 1% nitric acid and followed by rinsing with deionized water. The latter type may be soaked overnight in 20% nitric acid, rinsed, and dried in a 105°C oven.

Nickel chloride hexahydrate is used for adjusting the nickel concentration to 1000 ppm in all solutions slated for arsenic analysis.

6.0 Procedure

6.1 Collection of Samples

Whole blood samples are collected by veinpuncture from a brachial (arm) vein. The blood is drawn into a Vacutainer tube (B.D. No. 4727, low trace metal content) containing an EDTA anticoagulant. The tube is labeled and all pertinent information recorded on a protocol sheet.

6.2 Extraction, Cleanup, and Extraction

6.2.1 Cadmium and Lead Analysis

One ml of whole blood is treated with 10.0 ml of conc. HNO_3 at 85-90°C in a predigested beaker. The heating is continued for 2 hours 45 minutes with a watchglass placed on top of the beaker to minimize losses. The beaker is cooled and a total of 1.0 ml of 30% H_2O_2 is added in 0.2 ml portions The contents of the beaker is heated at 85-90°C for an additional 15 minutes. The watchglass is removed and the heating continued to reduce the digest volume to 1-2 ml. The residue is transferred to a 10 ml volumetric flask and diluted to the mark with 0.5% HNO_3. The sample solution is stored in 1 oz. polyethylene bottle (with screw cap) at ambient temperatures until analyzed.

6.2.2 Arsenic Analysis

One ml of whole blood is treated with 0.1 ml of a 10^4 ppm nickel solution and digested with 10.0 ml of conc. HNO_3 as described in Section 6.2.1. After treatment with 30% H_2O_2 the digestate is cautiously evaporated to dryness. The residue is transferred to a clean 4 oz glass bottle with 25 ml of 4:1 conc. HCl and the mixture allowed to stand at room temperature for 3-7 days. At the end of this period, 10 ml of 0.5 M $SnCl_2$ and 5 ml of 30% KI is added to the sample digest and the total allowed to stand at room temperature and for 30 minutes. Forty ml of conc. HCl and 10.0 ml of toluene is added to the mixture and the arsenic bodies extracted into the organic phase. Half of the toluene layer (5.0 ml) is withdrawn and mixed with 2.0 ml of a 0.005 M dichromate solution containing 1000 ppm nickel in 1.0% HNO_3. The arsenic compounds are back-extracted into the aqueous phase and stored in polypropylene bottles until ready for analysis.

6.3 Instrumental

A Perkin-Elmer Model 403 Spectrophotometer, equipped with a HGA-2000 furnace attachment with deuterium background correction is used for this analysis. An electrodeless discharge lamp is used as the light source and the furnace atomization response traced on a Perkin-Elmer Model 056 recorder An AS-1 Autosampler may be used to increase throughput and/or to improve peak reproducibility and sensitivity.

Arsenic: Wavelength - 193.7 nm
Gas Interrupt (N_2) Auto
Furnace Cycle Conditions -
Dry: 200°C for 30 sec.
Char: 15000°C for 35 sec.
Atomize: 2500°C for 6 sec.
Injection Volume - 20 µl

Cadmium: Wavelength - 228.8 nm
Gas Interrupt (N_2) - Manual
Furnace Cycle Conditions -
Dry: 200°C for 20 sec.
Char: 400°C for 30 sec.
Atomize: 2000°C for 8 sec.
Injection Volume - 20 µl

Lead: Wavelength - 217.0 nm
Gas Interrupt (N_2) - Auto
Furnace Cycle Conditions -
Dry: 200°C for 20 sec.
Char: 500°C for 30 sec.
Atomize: 2000°C for 8 sec.
Injection Volume - 20 µl

6.4 Qualitative and Quantitative Analysis

6.4.1 Qualitative Analysis

N/A

6.4.2 Quantitative Analysis

The instrument is calibrated with a digested control blood spiked at four different concentration, an unspiked control urine, and a reagent blank.

Calibration Range (spike concentration):

Arsenic - 0.0 to 10.0 μg/100 ml.

Cadmium - 0.0 to 10.0

Lead - 0.0 to 100.0

An exponential of the form $y = Ae^{bx}-M$ provides the best representation of the analytical curve. The values of the x,y calibration pairs are entered into a Monroe Calculator Model 1880 programmed to regress the data to the exponential and to provide values for the constants A, b, and M.

Sample peak heights are measured manually and expressed in units of millivolts. The standard additions calibration constants A, b, and M are entered into the storage banks of a Texas Instrument Calculator Model 57 and the metal concentration results obtained by keying in peak height data. Sample peak measurements and concentration results are recorded on a calculation worksheet.

$y = Ae^{bx}-M$, μg/100 ml

$y = y_s$ (metal conc. in sample relative to control blood) + y_c (metal conc. in control blood)

$y_s = Ae^{bx_s}-M$

$y_c = -y_o - -(Ae^{bx_o}-M)$

$y = A(e^{bx_s}-e^{bx_o})$

y = metal concentration in sample, μg/100 ml,

y = reagent blank peak height, mv

y_o = standard additions metal concentration corresponding to reagent blank signal ($y_o \leq 0$),

$y_c = -y_o$ = metal concentration in control blood, μg/100 ml,

x_s = sample peak height, mv

y_s = concentration differential between sample and control blood. This blood may be either negative ($y \leq y_c$) or positive ($y_c < y$)

7.0 Quality Assurance Program

An on-going quality assurance program is required to assure the data quality. Quality control (QC) procedures determine artifacts, losses, etc., through a system of blanks and controls. Quality assurance (QA) procedures monitor the execution of the procedure and check data interpretations and calculations.

7.1 Quality Control

7.1.1 Field Control

Prior to field sampling, several control whole blood collections (10% of anticipated number of field samples) are obtained. Each blood sample is collected in duplicate. One tube from each collection is sent to the site and subjected to the same handling and storage conditions as field samples. The other tube is stored at RTI in a dust-free environment. On receipt of samples at RTI, both tubes of the control blood collections are worked up and analyzed as a part of each blood analytical run. Within the precision of the assay, the difference in calculated metal concentrations of the two control blood tubes is a measure of the contamination/loss during field storage, and transit to RTI.

7.1.2 Internal Quality Control

7.1.2.1 Calibration Standards and Blanks

The instrument is calibrated before each analytical run with four standard solutions and a reagent blank. Evidence of contamination or instrument malfunction is evident at this time. Such problems are resolved before initiating sample analysis.

7.1.2.2 Conditioning of Graphite Tube

Before each analytical run, the graphite tube is conditioned by injecting 10 to 20-20 µl aliquots of one of the calibration standards. This operation insures acceptable precision during sample analysis.

7.1.2.3 Duplicate Injections

Reproducibility of peak response is continuously monitored during sample analysis. All standard and sample solutions receive two successive injections into the graphite furnace. Signal agreement between the duplicate injections is evaluated according to the following criterion:

First Signal % of Full Signal	% Maximum Permissible Variation (% MPV)	Permissible Range of Second Signal, % of Full Scale
90	± 4%	86-94
80	± 5%	76-84
70	± 6%	66-74
60	± 7%	56-64
50	± 8%	46-54
40	± 10%	36-44
30	± 13%	26-34
20	± 20%	16-24
10	± 30%	7-13
5	± 60%	2-8
2	±100%	0-4

If the second injection gives a signal which falls outside the permissible range, a third injection is performed. The peak measurement not in agreement with the matching pair is discarded.

All calibration and sample calculations are based on the mean of the duplicate determinations.

7.1.2.4 Standard Checks

Instrument performance is monitored during each analytical run. After the analysis of every 12-16 samples one of the calibration standards is reinjected into the furnace. The standard which most closely matches the sample peak heights is selected as the check solution. A metal concentration is calculated for the check standard based on its peak height during the calculation run. Similar calculations are carried out for each chech response and the observed changes in metal concentration expressed in terms of standard deviation units (SDU).

$$SDU = \frac{(\text{Calibration value - check Value})\ 100}{(\text{Calibration Value})(\%\ \text{of RSD})}$$

The analysis is under control when the SDU < 2.0. Standard checks which indicate a variation in peak response greater than 2.0 SDU are unacceptable. In this event, the graphite tube is changed, conditioned, and the system recalibrated. Quality control charts are graphed to show this change in instrument performance with time.

7.2 Quality Assurance

Both internal and external QA procedures are to be followed. Internal QA procedures assume the continuity and consistency of the data. External QA procedures (interlaboratory checks) verify or dispute the accuracy of the data being generated in the primary laboratory.

7.2.1.1 Supervision and Monitoring of Activities

There are three levels of quality assurance (QA). The primary quality assurance is the person conducting the sampling and/or analysis. This person must be aware of their actions, observe events which may effect the data, and maintain appropriate records. At the second level, the chemist's supervisor monitors their daily activities, reviews the notebook, checks data and calculations, and assists in "troubleshooting" problems. At the tertiary levels, a QA coordinator interviews all personnel on the project. The interviews cover the operations they perform (precisely), the data they obtain, a spot-check of their calculations, and any problems they have had.

7.2.1.2 Documentation

Chain of Custody

From the initial preparation of a sample container through reporting of the analytical results, each sample is accompanied by a chain of custody sheet. Each person signs in the time of receipt, operations performed, and transmittal of the sample. This record is important for tracing a contaminant, bad standard, or some other problem.

Sampling Protocol Sheets

When a sample is collected, a sampling protocol sheet is filled in which contains a discrete sample code which identifies project number, area, site, locations, trip number, sampling period, and sample type. Also

included are sample times, volumes, addresses, meteorology, and other pertinent information. Where appropriate, a map is made to precisely identify the location.

Sample Log

Upon return from a sampling trip, each sample code is entered into a sample log book. This log is updated as samples proceed through workup and analysis. Thus, at a glance, project personnel can tell the status of each sample and find out how many are at different stages in the analytical protocol.

Instrument Log

Each sample analysis is logged into a notebook, detailing analysis conditions.

7.2.2 External Quality Assurance

Samples will be analyzed by a designated QA laboratory. Samples, controls, and blanks will be shipped directly from the field to the QA laboratory for analysis. They will report the results to the primary laboratory for correlation with the primary data.

7.2.2.1 Selection of Samples for QA

Approximately 10% of the field samples (2 minimum) will be collected in duplicate shipment to the QA laboratory. This selection process will be random with the following restrictions:

(1) The donor must consent to the additional collection.

(2) If any stratification of donors is known, purposive selection of QA donors may be used to get representative samples (e.g. occupationally exposed vs "normal" individuals or upwind vs downwind residents).

7.2.2.2 QC Samples

Along with the QA duplicate samples, at least one QC control and one QC blank must be included with the QA samples. An example is shown in Table 7 for a trip collecting 50 samples.

7.2.2.3 Sample Codes

All samples shipped to the QA laboratory should be encoded so that the laboratory cannot discern between samples and controls. This "blind" protocol will add validity to the results.

7.2.2.4 Shipping

Samples should be shipped on dry ice directly to the QA laboratory by an appropriate air carrier (e.g., Federal Express, Eastern Sprint) in well insulated and packed cartons.

8.0 References

8-1 "Epidemiologic Study Conducted in Populations Living Around Non-Ferrous Smelters", Final Report for Contract No. 68-02-2442 (in preparation).

8-2 McFarren, E. F., Lishka, R. J. and Parker, J. H., Criterion for Judging Acceptability of Analytical Methods, Anal. Chem., 42(3), 358 (1970).

ANALYTICAL PROTOCOL: ANALYSIS OF HUMAN SERUM AND URINE FOR EXTRACTABLES (HERL-RTP)

*1.0 Principle of the Method

Semi-volatile hydrocarbons are extracted from blood plasma and urine with organic solvents, dried, and concentrated to an appropriate volume for analysis using gas chromatography/electron capture detection (GC/ECD).

*2.0 Range and Detection Limit

The lower limit of detectability as established by the EPA Analytical Chemistry Committee for chlorinated pesticides in serum is as follows:

β-BHC, lindane, aldrin, heptachlor, heptachlor epoxide, o,p'-DDE, p,p'-DDE, dieldrin---------- 1 ppb.

Endrin, o,p'-DDT, p,p'-DDD, p,p'-DDT---------- 2 ppb.

Although the linear dynamic range of an electron capture detector is not very large the range of this method can be greatly enhanced by dilution of the sample.

*3.0 Interferences

Interferences in sample analysis and quantification using GC/ECD are manifested in the electron capture ability of the given contaminant. Blood and urine extracts which have not been cleaned up contain repidly eluting components which exhibit high electron capturing properties; these interferences can be largely removed by a fractionation process with an activated Florisil column as discussed below.

*4.0 Precision and Accuracy

Recoveries from spiked serum samples in a three year study gave average recoveries of 96% ± 13% (1). The four compounds used were not specified.

5.0 Apparatus and Reagents

1. A rotary mixer so designed as to accomodate the 16 mm culture tubes and which may be operated at a rotary speed of 50 rpm. Fisher Scientific Company, Roto-Rack™, Cat. No. 14-456.
2. Gas chromatograph fitted with electron capture detector. Recommended GLC columns and operating parameters are given in reference (2).

3. Tubes, Culture, 16 x 125 mm, fitted with screw caps, size 15-415 with Teflon-faced rubber liners, Corning No. 9826.
4. Micro-Snyder column modified, with 10/22 ℸ joint, Kontes No. K-569251.
5. Concentrator tube, 10 ml, grad. 0 to 0.1 and 2 to 10 x 1, 19/22 ℸ joint, size 1025, Kontes No. K-570050.
6. Syringe, 100 µl, Hamilton No. 710 or equivalent.
7. Vortex Genie mixer.
8. Pipet, Mohr type, 1 ml grad. in 0.01 ml increments. Corning No. 7063 or equivalent.
9. Pipets, transfer, 2-, 5-, and 6-ml Corning No. 7100 or the equivalent.
10. Beads, solid, glass, 3 mm, Corning No. 7268 or the equivalent.
11. Six-place tube carrier, stainless steel. May be fabricated at local tin shop (see original reference).
12. Water bath capable of holding temp. of 95 to 100°C.
13. Centrifuge with head to accommodate the Corning No. 9826 tube, capable of speed of 2,000 rpm.
14. Hexane, distilled in glass, pesticide grade.

If Florisil column cleanup is necessary the additional following apparatus and reagents are necessary:

1. Funnels, glass, ca 60 mm diameter.
2. Separatory funnels - 125 ml and 1 liter, Kimble 29048-F, or equiv.
3. Chromatographic columns -25 mm o.d. x 300 mm long, with Teflon stopcocks, without fritted glass plates, Kontes 420530, Size 241.
4. Erlenmeyer flasks -500 ml capacity.
5. Kuderna-Danish concentrator fitted with grad. evaporative concentrator tube. Available from the Kontes Glass Company, each component bearing the following stock numbers:
   a. Flask, 500 ml, stock #K-570001
   b. Snyder Column, 3-ball, stock #K-503000

c. Steel springs, 1/2", stock #K-662750

d. Concentrator tubes, 10 ml, size 1025, stock #K-570050

6. Glass wool - Corning #3950 or equivalent.

5.2 Reagents

1. Petroleum ether - Pesticide Quality, redistilled in glass, b.p. 30°C - 60°C.

NOTE: If this method is used for the detection and quantitation of organophosphorous compounds, some special factors must be considered. The presence of any peroxides in the ethyl ether and/or impurities in the pet. ether can result in extremely low recoveries. Recovery efficiency should be predetermined on standard mixtures containing the specific compounds of interest. If low recoveries are obtained, it may be necessary to try an alternate manufacturer's pet. ether.

2. Diethyl ether - AR grade, peroxide free, Mallinckrodt #0850 or the equivalent. The ether must contain 2% (v/v) absolute ethanol. Some of the AR grade ethers contain 2% ethanol, added as a stabilizer, and it is therefore unnecessary to add ethanol unless peroxides are found and removed.

NOTE: To determine the absence of peroxides in the ether, add 1 ml of freshly prepared 10% KCl solution to 10 ml of ether in a clean 25-ml cylinder previously rinsed with the ether. Shake and let stand 1 minute. A yellow color in the ether layer indicates the presence of peroxides which must be removed before using. See Misc. Note 4 at end of procedure. The peroxide test should be repeated at weekly intervals on any single bottle or can as it is possible for peroxides to form from repeated opening of the container.

3. Eluting mixture, 6% (6+94) - purified diethyl ether (60 ml) is diluted to 1000 ml with redistilled petroleum ether and anhydrous sodium sulfate (10-25 g) is added to remove moisture.

4. Eluting mixture, 15% (15+85) - purified diethyl ether (150 ml) is diluted to 1000 ml with redistilled petroleum ether and dried as described above.

NOTE: Neither of the eluting mixtures should be held longer than 24 hours after mixing.

5. Florisil, 60/100 mesh, PR grade, to be stored at 130°C until used.

NOTE: (1) In a high humidity room, the column may pick up enough moisture during packing to influence the elution pattern. To insure uniformity of the Florisil fractionation, it is recommended to those laboratories with sufficiently large drying ovens that the columns be packed ahead of time and held (at least overnight) at 130°C until used. (2) Florisil furnished to the contract laboratories by the RTP, NC laboratory on order, has been activated by the manufacturer, and elution pattern data is included with each shipment. However, each laboratory should determine their own pesticide recovery and elution pattern on each new lot received, as environmental conditions in the various laboratories may differ somewhat from that in RTP, NC. Each new batch should be tested by the procedure described previously for assurance that the operator can obtain recoveries and compound elution patterns comparable to the data given on the accompanying table.

6. Acetonitrile, pesticide grade, saturated with pet. ether.

NOTE: Occasional lots of CH_3CN are impure and require redistillation. Generally, vapors from inpure acetonitrile will turn litmus paper blue when the moistened paper is held over the mouth of the bottle.

7. Anhydrous sodium sulfate, reagent grade granular, Mallinkrodt stock #8024 or the equivalent.

NOTE: When each new bottle is opened, it should be tested for contaminants that will produce peaks by Electron Capture Gas Liquid Chromatography. This may be done by transferring ca 10 grams to a 125 ml Erlenmeyer flask, adding 50 ml pet. ether, stoppering and shaking vigorously for 1 minute. Decant extract into a 100 ml beaker and evaporate down to ca 5 ml. Inject 5 µl into the Gas Liquid Chromatograph and observe chromatogram for contaminants. When impurities are found, it is necessary to remove them by extraction. This may be done by using hexane in a continuously cycling Soxhlet extraction apparatus or by several successive rinses with hexane in a beaker. The material is then dried in an oven and kept in a glass-stoppered container.

NOTE: See Note for sodium sulfate, Step 7, above.

8. MgO-Celite mixture (1:1) weigh equal parts of reagent grade MgO and Celite 545 and mix thoroughly. (optional)

6.0 Procedure

*6.1 Collection of Samples

6.1.1 Blood

Blood samples are obtained in 10 ml vacutainer tubes "suitable for GC" (Venoject L428, Kimble) containing 15 mg tripotassium salt of EDTA and 20 μg potassium sorbate.

6.1.2 Urine

Place whole blood sample in the refrigerator for about 30 minutes for a settling period and then centrifuge for a sufficient time for the separation of at least 3 ml of clear serum - generally 10 minutes at 2,500 r.p.m. Whether or not the analysis is to be conducted immediately, it is desirable at this point to transfer the 2 ml sample aliquot to the 16 x 125 mm culture tube used for extraction. If analysis cannot be run immediately, place in refrigerator at 2-5°C for periods of up to 24 hours before analysis. If time interval to analysis exceeds 24 hours, the tube should be stored in a deep freeze at -15 to -25°C. Stored in this manner, analysis may be delayed for periods up to a month without undue effects on the chlorinated pesticides present.

First morning urines are collected in a 120 mL wide-mouthed glass bottles. The sample is stored at 4° awaiting analysis.

6.2 Extraction, Cleanup, and Concentration

6.2.1 Serum Samples

1. Mix blood serum sample thoroughly and, with a volumetric pipet, transfer 2 ml to a 15 ml round bottom culture tube.

NOTE: In case of the presence of any flocculent or sedimentary material, it is strongly recommended that the sample be centrifuged ca 5 minutes @ 2,000 r.p.m. before pipetting the 2 ml aliquot. Failure to observe this point may result in poor reproducibility of replicated analyses of the same sample.

2. Add 6 ml hexane from a volumetric pepet. Tightly stopper the culture tube with a Teflon-lined screw cap. Place tube on rotator.

3. Set rotator speed at 50 r.p.m. and rotate for 2 hours.

NOTE: (1) This speed may vary from 50 to 44 r.p.m. but should be confined to this range. (2) Unless the sample is extremely old, emulsion

formation should present no problem. In case it occurs, centrifuge at 2,000 r.p.m. 4 to 5 minutes, or longer if necessary, to effect sufficient separation to permit withdrawal of the 5 ml aliquot of clear extract.

4. With a volumetric pipet, transfer 5 ml of the hexane extract to a 10 ml grad. concentrator tube, add one 3 mm glass bead, and attach a modified micro-Snyder column. Evaporate the extract in a steam or hot water bath at 100°C to a volume slightly less than that which is estimated as appropriate to accommodate (1) the current level of electron capture detector sensitivity, and (2) the expected residue range in the particular sample. When working with general population blood of low pesticide levels, it may be necessary to evaporate to ca 0.5 ml.

NOTE: (1) With some experience the operator can complete the evaporation step in less than 5 minutes. The tube must be withdrawn from the water when boiling agitation becomes too vigorous. Immersion and withdrawal are alternated based on observation of boil agitation. (2) Up to six tubes of extract may be evaporated simultaneously by using the special rack shown in Fig. 2. Time and motion studies have shown that the time required for the evaporation period is equal to that required for a single tube. (3) When working with blood from high exposure donors, the 5 ml aliquot may require dilution rather than concentration. This can be determined by a preliminary analysis of the 5-ml aliquot. (4) With lower concentrations, use higher degree of concentration of samples.

5. Allow the tube to cool (3 to 5 minutes), remove the micro-Snyder column and rinse down the sides of the tube and the column joint with hexane. The volume used will depend on the desired dilution.

NOTE: (1) When a minimal dilution is required after evaporation, a 100 µl syringe is useful in performing the hexane rinse. (2) To obtain a suitable extract concentration for p,p'-DDE, it is generally necessary to adjust the extract volume to a level in excess of 1 ml. In this case, add hexane until the meniscus is exactly at the 1-ml mark on the concentrator tube. Then use a 1-ml Mohr pipet for total volumes up to 3 ml. For larger volumes, use a 5 ml Mohr pipet, carefully measuring the volume of hexane delivered. Above

the 1 ml graduation mark, the concentrator tube calibrations are not sufficiently accurate for use in this analysis. It is also good practice to check the graduation marks up to 1 ml for all concentrator tubes used in this analysis.

6. Stopper the concentrator tube and hold on the Vortex mixer, set for high speed for ca. 30 seconds for volumes of 6 ml or less. It is safer practice to mix a full minute for larger volumes.

6.2.2 Urine Samples

The urine is treated exactly as the blood serum except that 5 mL are analysed rather than 2 mL.

6.2.3 Optional Florisil Cleanup

If interferences from unwanted electron capturing materials hinder analysis and quantitation of desired peaks, a Florisil column cleanup can be incorporated into the extraction scheme. The procedure for pooled serum is as follows:

6.2.3.1 Extraction

1. Measure 50 ml of serum into a 1 L sep. funnel containing 190 ml of CH_3CN, 200 ml of aqueous 2% Na_2SO_4 and 50 ml of hexane.
2. Stopper, shake funnel vigorously 2 minutes, and allow the layers to separate.
3. Draw off the aqueous (lower) layer into a second 1-L sep. funnel and percolate the hexane layer through a 2-inch column of anhydrous Na_2SO_4 into a 500 ml Kuderna-Danish flask fitted with a 10-ml grad., evap. concentrator tube containing one 3-mm glass bead.
4. Add another 50-ml portion of hexane to the aqueous solution in the second 1-L separator; stopper and shake vigorously another two minutes. When layers have separated, draw aqueous layer back into the first 1 L separator and percolate the hexane layer through the Na_2SO_4 into the K-D flask. Repeat the extraction twice more resulting in a total hexane extract of 200 ml.

5. Assemble K-D evaporator and concentrate extract to ca 3 ml. Disassemble evaporator rinsing tube joint with a small volume of hexane and dilute extract to exactly 5 ml. Stopper and shake on Vortex mixer 2 minutes.

6.2.3.2 Florisil Fractionation

1. Prepare a chromatographic column containing 4 inches (after setting) of activated Florisil topped with 1/2 inch of anhydrous, granular Na_2SO_4. A small wad of glass wool, preextracted with pet. ether, is placed at the bottom of the column to retain the Florisil.

NOTE: (1) Florisil is activated by heating for at least 5 hr at 130°. (2) If the oven is of sufficient size, the columns may be prepacked and stored in the oven, withdrawing columns a few minutes before use. (3) The amount of Florisil needed for proper elution should be determined for each lot of Florisil.

2. Place a 500-ml Erlenmeyer flask under the column and prewet the packing with pet. ether (40-50 ml, or a sufficient volume to completely cover the Na_2SO_4 layer).

NOTE: From this point and through the elution process, the solvent level should never be allowed to go below the top of the Na_2SO_4 layer. If air is introduced, channeling may occur, making for an inefficient column.

3. Using a 5-ml Mohr or a long disposable pipet, immediately transfer the tissue extract (ca 5 ml) from the evaporator tube onto the column and permit it to percolate through.
4. Rinse tube with two successive 5-ml portions of pet. ether, carefully transferring each portion to the column with the pipet.

NOTE: Use of the Mohr or disposable pipet to deliver the extract directly onto the column precludes the need to rinse down the sides of the column.

5. Prepare two Kuderna-Danish evaporative assemblies complete with 10 ml graduated evaporative concentrator tubes. Place one glass bead in each concentrator tube.

6. Replace the 500-ml Erlenmeyer flask under each column with a 500 ml Kuderna-Danish assembly and commence elution with 200 ml of 6% diethyl ether in pet. ether (Fraction I). The elution rate should be 5 ml per minute. When the last of the eluting solvent reaches the top of the Na_2SO_4 layer, place a second 500 ml Kuderna-Danish assembly under the column and continue elution with 200 ml of 15% diethyl ether in pet. ether (Fraction II).
7. To the second fraction only, add 1.0 ml of hexane containing 200 nanograms of aldrin, place both Kuderna-Danish evaporator assemblies in a water bath and concentrate extract until ca 5 ml remain in the tube.
8. Remove assemblies from bath and cool to ambient temperature.
9. Disconnect collection tube from Kuderna-Danish flask and carefully rinse joint with a little hexane.
10. Attach modified micro-Snyder column to collection tubes, place tubes back in water bath and concentrate extracts to 1 ml. If preferred, this may be done at room temperature under a stream of nitrogen.
11. Remove from bath, and cool to ambient temperature. Disconnect tubes and rinse joints with a little hexane.

NOTE: The extent of dilution or concentration of the extract at this point is dependent on the pesticide concentration in the substrate being analyzed and the sensitivity and linear range of the Electron Capture Detector being used in the analysis.

12. Should it prove necessary to conduct further cleanup on the 15% fraction, transfer 10 grams MgO-Celite mixture to a chromatographic column using vacuum to pack. Prewash with ca 40 ml pet. ether, discard prewash and place a Kuderna-Danish receiver under column. Transfer concentrated Florisil eluate to column using small portions to pet. ether. Force sample and washings into the MgO-Celite mixture by slight air pressure and elute column with 100 ml pet. ether. Concentrate to a suitable volume and proceed with Gas Liquid Chromatography.

NOTE: Standard Recoveries should be made through column to ensure quantitative recoveries.

6.3 Analysis

Detection and quantification of semi-volatile halogenated hydrocarbons is made using a model MT-220 gas chromatograph manufactured by Tracor, Inc. Austin, TX, equipped with a tritium foil electron capture detector. Separation is achieved using a 6 foot x 5/32" i.d. silanized glass column packed with 1.5% OV-17/1.95% OV-210 on a 80/100 mesh silanized support. Confirmatory separation may be effected using an identical column packed with 5% OV-210 on a 100/120 mesh silanized support. A flow rate of 50-70 ml/min is recommended for the first column at an operating temperature of 200° (isothermal) while 45-60 ml/min is recommend for the second column at an operating temperature of 180° (isothermal). Both columns should be operated with a detector temperature of 205°C.

NOTE: Laboratories may substitute GC/ECD equipment from other manufactures and use 2 mm i.d. glass columns with a carrier gas flow rate of 20-30 mL/min.

6.4 Qualitative Identification

The peaks obtained are qualitatively identified by relative retention times which are listed in Tables 1 & 2 for the primary and the confirmatory columns. If the relative retention times are correct for both columns a nearly positive identification has been made. Identification can be improved by such techniques as TLC and/or electrolytic conductivity detection (3).

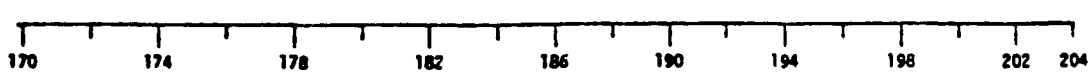
6.5 Quantitation

Quantitation is accomplished using either peak height if the peaks are tall and narrow or peak ht. x width at half height if the peaks are symmetric and rather broad (e.g., late eluting peaks). These peak measurements are compared to peak measurements of standard solutions of known concentration. It must be remembered that the peak heights of the sample and standard should be within 25% of one another to assure precise quantitation due to the small linear dynamic range of an ECD. It is also recommended that a sample injection of at least 5 µl be employed to minimize injection error.

Table 1. RELATIVE RETENTION TIMES ON 1.5% OV-17/1.95% OC-210

Column Temperature, °C.

170		174		178		182		186		190		194		198		202	204	Compound
0.25	0.25	0.26	0.26	0.26	0.27	0.27	0.27	0.28	0.28	0.28	0.[illegible]	0.29	0.29	0.30	0.30	0.30	0.31	Dimethyl Phthalate
0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.33	0.[illegible]	0.33	0.33	0.33	0.33	0.33	0.33	Mevinphos
0.34	0.34	0.35	0.35	0.36	0.36	0.36	0.37	0.37	0.38	0.38	0.38	0.39	0.39	0.40	0.40	0.40	0.41	Tecnazene
0.38	0.38	0.38	0.38	0.39	0.39	0.39	0.39	0.39	0.40	0.40	0.4[illegible]	0.40	0.41	0.41	0.41	0.41	0.41	Diethyl Phthalate
0.44	0.45	0.45	0.45	0.45	0.45	0.45	0.46	0.46	0.46	0.46	0.46	0.46	0.47	0.47	0.47	0.47	0.47	2,4-D(ME)
0.42	0.42	0.43	0.43	0.44	0.44	0.44	0.45	0.45	0.45	0.46	0.46	0.47	0.47	0.48	0.48	0.48	0.49	Hexachlorobenzene
0.48	0.48	0.49	0.49	0.50	0.50	0.50	0.51	0.51	0.52	0.52	0.5[illegible]	0.53	0.53	0.54	0.54	0.54	0.55	a-BHC
0.54	0.54	0.54	0.54	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.56	0.56	0.56	0.56	0.56	0.56	CDEC
0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	2,4-D(IPE)
0.54	0.54	0.55	0.55	0.56	0.56	0.56	0.57	0.57	0.58	0.58	0.58	0.59	0.59	0.60	0.60	0.60	0.61	Chlordene
0.67	0.67	0.66	0.66	0.66	0.66	0.66	0.65	0.65	0.65	0.65	0.65	0.65	0.64	0.64	0.64	0.64	0.64	Diazinon
0.65	0.65	0.65	0.65	0.66	0.66	0.66	0.66	0.66	0.67	0.67	0.67	0.67	0.67	0.68	0.68	0.68	0.68	PCNB
0.66	0.67	0.67	0.67	0.67	0.67	0.67	0.68	0.68	0.68	0.68	0.68	0.68	0.69	0.69	0.69	0.69	0.69	Lindane
0.76	0.76	0.76	0.76	0.75	0.75	0.75	0.75	0.75	0.74	0.74	0.74	0.74	0.73	0.73	0.73	0.73	0.73	2,4,5-T(ME)
0.82	0.82	0.82	0.82	0.81	0.81	0.81	0.81	0.81	0.81	0.81	0.80	0.80	0.80	0.80	0.80	0.80	0.80	g-BHC
0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	Heptachlo
0.94	0.94	0.93	0.92	0.92	0.91	0.90	0.90	0.89	0.88	0.88	0.87	0.87	0.86	0.85	0.85	0.84	0.83	2,4,5-T(IPE)
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	Aldrin (REFERENCE)
1.17	1.16	1.15	1.14	1.13	1.11	1.10	1.09	1.08	1.07	1.06	1.05	1.03	1.02	1.01	1.00	0.99	0.98	Dimethoate
1.17	1.16	1.16	1.15	1.14	1.14	1.13	1.12	1.12	1.11	1.10	1.09	1.09	1.08	1.07	1.07	1.06	1.05	Ronnel
1.49	1.48	1.45	1.43	1.41	1.40	1.38	1.36	1.34	1.32	1.31	1.29	1.27	1.25	1.23	1.22	1.20	1.18	Dibutyl Phthalate
1.41	1.40	1.39	1.38	1.36	1.35	1.34	1.33	1.32	1.31	1.30	1.29	1.28	1.27	1.26	1.25	1.24	1.23	1-Hydroxychlordene
1.49	1.49	1.47	1.47	1.46	1.45	1.44	1.44	1.43	1.42	1.42	1.41	1.40	1.39	1.39	1.38	1.37	1.36	Oxychlordane
1.71	1.69	1.67	1.66	1.64	1.62	1.60	1.59	1.57	1.55	1.53	1.52	1.50	1.48	1.47	1.45	1.43	1.41	M. Parathion
1.70	1.69	1.68	1.67	1.66	1.65	1.64	1.63	1.62	1.61	1.59	1.58	1.57	1.56	1.55	1.54	1.53	1.52	Heptachlor Epoxide
1.82	1.80	1.78	1.76	1.74	1.72	1.70	1.68	1.66	1.64	1.62	1.60	1.58	1.56	1.54	1.52	1.50	1.48	DCPA
2.07	2.04	2.01	1.98	1.95	1.92	1.89	1.87	1.84	1.81	1.78	1.75	1.72	1.69	1.66	1.63	1.60	1.57	Malathion
1.92	1.91	1.89	1.88	1.86	1.85	1.83	1.81	1.80	1.78	1.77	1.75	1.74	1.72	1.71	1.69	1.68	1.66	Chlordane, Gamma
2.02	2.00	1.99	1.97	1.95	1.93	1.92	1.90	1.88	1.86	1.85	1.83	1.82	1.79	1.78	1.76	1.74	1.73	Trans-Nonachlor
2.14	2.12	2.09	2.07	2.05	2.03	2.01	1.98	1.96	1.94	1.92	1.90	1.88	1.86	1.84	1.82	1.79	1.77	o,p'-DDE
2.32	2.28	2.25	2.22	2.19	2.16	2.13	2.09	2.06	2.03	2.00	1.97	1.93	1.90	1.87	1.84	1.81	1.78	E. Parathion
2.15	2.13	2.11	2.09	2.07	2.05	2.03	2.01	1.99	1.97	1.96	1.94	1.92	1.90	1.88	1.86	1.84	1.82	Chlordane, Alpha
2.20	2.18	2.16	2.15	2.13	2.11	2.10	2.08	2.06	2.05	2.03	2.01	2.00	1.98	1.97	1.95	1.93	1.91	Endosulfan I
2.75	2.72	2.68	2.64	2.61	2.58	2.54	2.51	2.47	2.43	2.40	2.37	2.33	2.30	2.27	2.23	2.20	2.17	p,p'-DDE
2.97	2.93	2.88	2.84	2.79	2.75	2.71	2.66	2.62	2.57	2.53	2.49	2.44	2.40	2.35	2.31	2.27	2.22	DDA(ME)
2.80	2.77	2.75	2.72	2.69	2.67	2.64	2.61	2.59	2.56	2.53	2.51	2.48	2.45	2.43	2.40	2.37	2.35	Dieldrin
3.34	3.29	3.25	3.20	3.15	3.11	3.06	3.01	2.97	2.92	2.88	2.83	2.77	2.74	2.69	2.65	2.60	2.56	o,p'-DDD
3.26	3.23	3.19	3.16	3.13	3.09	3.06	3.03	3.00	2.96	2.93	2.90	2.87	2.83	2.80	2.77	2.74	2.70	Chlordecone
3.47	3.43	3.40	3.36	3.33	3.29	3.26	3.22	3.18	3.15	3.12	3.08	3.04	3.01	2.97	2.93	2.90	2.87	Endrin
3.98	3.94	3.88	3.83	3.77	3.71	3.66	3.60	3.54	3.48	3.43	3.38	3.32	3.27	3.21	3.16	3.10	3.04	o,p'-DDT
4.65	4.57	4.49	4.41	4.33	4.26	4.18	4.10	4.02	3.94	3.87	3.79	3.71	3.64	3.61	3.48	3.40	3.32	p,p'-DDD
4.45	4.39	4.34	4.28	4.23	4.17	4.11	4.05	3.99	3.94	3.88	3.82	3.76	3.71	3.65	3.59	3.54	3.48	Endosulfan II
5.57	5.48	5.39	5.29	5.20	5.11	5.01	4.92	4.83	4.74	4.64	4.55	4.46	4.36	4.27	4.18	4.09	4.00	p,p'-DDT
6.1	5.97	5.85	5.73	5.61	5.49	5.36	5.24	5.12	5.00	4.88	4.76	4.64	4.52	4.40	4.28	4.16	4.04	Ethion
6.4	6.2	6.1	5.99	5.88	5.76	5.64	5.52	5.40	5.28	5.16	5.04	4.92	4.80	4.68	4.56	4.44	4.32	Carbophenothion
7.7	7.6	7.5	7.3	7.3	7.1	7.0	6.9	6.8	6.7	6.6	6.5	6.4	6.3	6.2	6.1	6.0	5.85	Mirex
10.7	10.5	10.3	10.1	9.9	9.7	9.5	9.3	9.1	8.9	8.7	8.5	8.3	8.1	7.9	7.7	7.5	7.3	Endrin Ketone "153"
13.1	12.7	12.4	12.0	11.6	11.2	10.8	10.4	10.0	9.7	9.3	8.9	8.5	8.1	7.7	7.3	7.0	6.6	Dioctyl Phthalate
12.4	12.1	11.8	11.6	11.3	11.0	10.7	10.4	10.1	9.8	9.5	9.3	9.0	8.7	8.4	8.1	7.8	7.5	Methoxychlor
16.9	16.5	16.1	15.7	15.3	14.9	14.5	14.1	13.7	13.3	12.9	12.5	12.1	11.7	11.3	10.9	10.5	10.2	Tetradifon
22.1	21.5	20.9	20.3	19.6	19.0	18.4	17.7	17.1	16.5	15.8	15.2	14.6	14.0	13.4	12.7	12.1	11.5	Diphenyl Phthalate

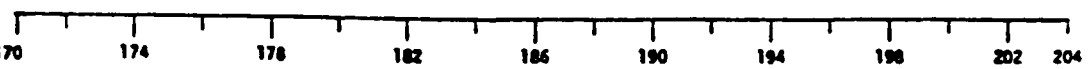


Retention ratios, relative to aldrin, of 49 compounds at temperatures from 170 to 204°C; support of Gas Chrom Q, 100/120 mesh; electron capture detector; tritium source, parallel plate; all absolute retentions measured from injection point. Arrow indicates optimum column operating temperature with carrier flow at 60 ml per minute.

Table 2. RELATIVE RETENTION TIMES ON 5% OV-210

Column Temperature, °C.

170		174		178		182		186		190		194		198		202	204	Compound
0.43	0.43	0.44	0.45	0.45	0.46	0.46	0.47	0.48	0.48	0.49	0.49	0.50	0.51	0.51	0.52	0.52	0.53	Hexachlorobenzene
0.51	0.51	0.51	0.51	0.52	0.52	0.52	0.53	0.53	0.53	0.53	0.54	0.54	0.54	0.54	0.55	0.55	0.55	Dimethyl Phthalate
0.52	0.53	0.53	0.54	0.54	0.55	0.55	0.55	0.56	0.56	0.57	0.57	0.58	0.58	0.59	0.59	0.60	0.60	Tecnazene
0.58	0.59	0.59	0.60	0.60	0.61	0.61	0.62	0.62	0.63	0.63	0.64	0.64	0.65	0.65	0.66	0.67	0.67	Chlordene
0.58	0.58	0.59	0.60	0.61	0.62	0.62	0.63	0.64	0.65	0.66	0.66	0.67	0.68	0.69	0.70	0.70	0.71	a-BHC
0.65	0.66	0.66	0.66	0.67	0.67	0.67	0.67	0.68	0.68	0.68	0.69	0.69	0.69	0.70	0.70	0.70	0.70	CDEC
0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.68	0.68	0.68	0.68	0.68	0.68	0.68	0.68	0.68	Mevinphos
0.73	0.73	0.72	0.72	0.72	0.72	0.72	0.71	0.71	0.71	0.71	0.71	0.70	0.70	0.70	0.70	0.70	0.69	Diethyl Phthalate
0.75	0.75	0.75	0.74	0.74	0.74	0.74	0.73	0.73	0.73	0.72	0.72	0.72	0.71	0.71	0.71	0.71	0.70	Diazinon
0.78	0.79	0.79	0.79	0.79	0.79	0.80	0.80	0.80	0.80	0.80	0.81	0.81	0.81	0.81	0.81	0.82	0.82	Lindane
0.85	0.85	0.85	0.84	0.84	0.84	0.84	0.84	0.83	0.83	0.83	0.83	0.83	0.82	0.82	0.82	0.82	0.82	2,4-D(IPE)
0.83	0.83	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.85	0.85	0.85	0.85	0.85	0.85	PCNB
0.86	0.86	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.88	0.88	0.88	0.88	0.88	0.88	Heptachlor
0.93	0.93	0.92	0.92	0.92	0.92	0.92	0.92	0.92	0.92	0.92	0.92	0.91	0.91	0.91	0.91	0.91	0.91	g-BHC
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	Aldrin (REFERENCE)
1.41	1.39	1.38	1.37	1.35	1.34	1.33	1.32	1.30	1.29	1.28	1.26	1.25	1.24	1.22	1.21	1.20	1.19	Ronnel
1.44	1.43	1.42	1.41	1.40	1.39	1.38	1.37	1.36	1.36	1.35	1.34	1.33	1.32	1.31	1.30	1.29	1.28	1-Hydroxychlordene
1.59	1.58	1.57	1.56	1.55	1.54	1.53	1.52	1.51	1.50	1.49	1.48	1.47	1.45	1.44	1.43	1.42	1.41	Oxychlordane
1.66	1.64	1.63	1.61	1.60	1.58	1.56	1.55	1.53	1.52	1.50	1.48	1.47	1.45	1.44	1.42	1.40	1.39	o,p'-DDE
1.88	1.87	1.85	1.83	1.81	1.80	1.78	1.76	1.74	1.73	1.71	1.69	1.68	1.66	1.64	1.62	1.61	1.59	Trans-Nonachlor
1.88	1.87	1.85	1.83	1.82	1.80	1.78	1.77	1.75	1.73	1.72	1.70	1.68	1.67	1.65	1.63	1.62	1.60	Chlordane, Gamma
1.96	1.94	1.92	1.91	1.89	1.87	1.85	1.84	1.83	1.80	1.79	1.77	1.75	1.74	1.72	1.70	1.68	1.67	Heptachlor Epoxide
2.05	2.03	2.02	2.00	1.98	1.96	1.94	1.92	1.90	1.89	1.87	1.85	1.83	1.81	1.79	1.77	1.75	1.74	Chlordane, Alpha
2.25	2.21	2.17	2.13	2.10	2.06	2.02	1.99	1.95	1.91	1.87	1.84	1.80	1.76	1.72	1.69	1.65	1.61	Dibutyl Phthalate
2.21	2.18	2.16	2.13	2.10	2.09	2.04	2.01	1.98	1.95	1.92	1.89	1.87	1.84	1.81	1.78	1.75	1.72	Dimethoate
2.21	2.19	2.16	2.13	2.11	2.09	2.05	2.02	2.00	1.97	1.94	1.92	1.89	1.85	1.84	1.81	1.78	1.75	p,p'-DDE
2.54	2.52	2.49	2.46	2.44	2.41	2.38	2.35	2.33	2.30	2.27	2.25	2.22	2.19	2.17	2.14	2.11	2.08	Endosulfan I
2.60	2.58	2.55	2.53	2.50	2.48	2.46	2.43	2.41	2.38	2.36	2.34	2.31	2.29	2.26	2.24	2.22	2.19	o,p'-DDD
2.69	2.65	2.61	2.57	2.55	2.49	2.45	2.41	2.37	2.33	2.29	2.25	2.21	2.17	2.13	2.09	2.05	2.01	DCPA
2.83	2.79	2.76	2.72	2.69	2.66	2.62	2.59	2.55	2.52	2.49	2.45	2.42	2.38	2.35	2.32	2.28	2.25	Chlordecone
2.97	2.92	2.86	2.81	2.75	2.70	2.65	2.59	2.54	2.48	2.43	2.38	2.32	2.27	2.21	2.16	2.11	2.05	o,p'-DDT
3.00	2.95	2.89	2.84	2.79	2.73	2.68	2.63	2.58	2.52	2.47	2.42	2.36	2.31	2.26	2.21	2.15	2.10	Malathion
2.95	2.91	2.87	2.82	2.78	2.74	2.70	2.66	2.61	2.57	2.53	2.49	2.45	2.40	2.36	2.32	2.28	2.24	M. Parathion
3.08	3.05	3.01	2.98	2.94	2.91	2.87	2.84	2.80	2.77	2.73	2.70	2.66	2.63	2.59	2.56	2.52	2.49	Dieldrin
3.71	3.66	3.61	3.56	3.51	3.46	3.41	3.36	3.31	3.26	3.21	3.12	3.11	3.06	3.01	2.96	2.91	2.86	Endrin
4.01	3.94	3.88	3.81	3.74	3.67	3.60	3.53	3.46	3.39	3.32	3.25	3.19	3.12	3.05	2.98	2.91	2.84	p,p'-DDD
4.45	4.31	4.17	4.04	3.90	3.76	3.62	3.49	3.35	3.21	3.08	2.94	2.80	2.67	2.53	2.39	2.25	2.12	E. Parathion
4.15	4.09	4.03	3.98	3.92	3.86	3.80	3.74	3.69	3.63	3.57	3.51	3.45	3.40	3.34	3.28	3.22	3.16	Mirex
4.38	4.31	4.23	4.16	4.08	4.01	3.93	3.85	3.78	3.70	3.63	3.55	3.47	3.40	3.32	3.25	3.17	3.09	p,p'-DDT
4.78	4.70	4.63	4.55	4.48	4.40	4.33	4.25	4.18	4.11	4.03	3.96	3.88	3.81	3.73	3.66	3.58	3.50	Endosulfan II
5.28	5.17	5.06	4.95	4.84	4.73	4.62	4.51	4.40	4.28	4.17	4.06	3.95	3.84	3.73	3.62	3.51	3.40	Carboophenothion
5.90	5.77	5.63	5.50	5.36	5.23	5.09	4.96	4.82	4.69	4.55	4.42	4.28	4.15	4.01	3.89	3.74	3.61	Ethion
7.3	7.1	6.9	6.7	6.55	6.4	6.2	6.0	5.84	5.66	5.49	5.31	5.13	4.95	4.77	4.60	4.42	4.24	Methoxychlor
13.6	13.1	12.7	12.3	11.9	11.4	11.0	10.6	10.2	9.7	9.3	8.9	8.5	8.0	7.6	7.2	6.8	6.4	Dioctyl Phthalate
12.9	12.5	12.3	12.0	11.8	11.5	11.2	10.9	10.6	10.3	10.1	9.8	9.5	9.2	8.9	8.6	8.4	8.1	Endrin Ketone "153"
20.0	19.4	18.9	18.3	17.8	17.3	16.7	16.2	15.6	15.1	14.6	14.0	13.5	13.0	12.4	11.9	11.3	10.8	Tetradifon
21.0	20.4	19.7	19.1	18.5	17.8	17.2	16.5	15.9	15.2	14.6	13.9	13.3	12.6	12.0	11.4	10.7	10.1	Diphenyl Phthalate



Retention ratios, relative to aldrin, of 47 compounds at temperatures from 170 to 204°C; support of gas Chrom Q, 80/100 mesh; electron capture detector; ^{63}Ni source; all absolute retentions measured from injection point. Arrow indicated optimum column operating temperature with carrier flow at 50 ml per min.

*7.0 Quality Assurance Program

An on-going quality assurance program is required to assure the data quality. Quality control (QC) procedures determine artifacts, losses, etc. through a system of blanks and controls. Quality assurance (QA) procedures monitor the execution of the procedure and check data interpretations and calculations.

7.1 Quality Control

7.1.1 Field Blanks and Controls

Prior to a field sampling trip, enough blanks and controls are prepared to equal 10% each (2 minimum) of the anticipated number of field samples. Blanks consist of 10 ml of water in the same type of sampling container as is used in the field. Controls consist of 10 ml of plasma spiked at 10-15 ng/ml with the compounds listed in Table 3. For urine 10 mL of water serves as a blank and 10 mL of water spiked with 10-15 ng/ml of the compounds in Table 3 serves as a control sample. These blanks and controls are carried to the field and receive the same handling as the field samples. Workup and analysis of field blanks and controls is interspersed with the field samples on a regular basis. This method allows assessment of sample storage stability

Table 4 presents a typical set of blanks and controls for QC on a field trip where 50 blood samples are to be collected.

7.1.2 Procedural Blanks and Controls

With each set of samples, a procedural blank is run. This consists of 10 ml of prepurged distilled water which is extracted under the same conditions as the samples. These blanks are designed to detect artifacts from dirty glassware, laboratory atmosphere intrusion, and other sources.

7.2 Quality Assurance

Both internal and external QA procedures are to be followed. Internal QA procedure assure the continuity and consistancy of the data. External QA procedures (interlaboratory checks) verify or dispute the accuracy of the data being generated in the primary laboratory.

7.2.1 Internal Quality Assurance

7.2.1.1 Supervision and Monitoring of Activities

There are three levels of quality assurance (QA). The primary quality assurance is the person conducting the sampling and/or analysis. This

Table 3. SEMI-VOLATILE HALOGENATED HYDROCARBONS IN METHANOL SPIKING SOLUTION

Compound	Compound
4-Chlorobiphenyl	Heptachlor epoxide
α-BHC	Dieldrin
β-BHC	p,p'-DDE
γ-BHC	p,p'-DDT
4,4'-Dichlorobiphenyl	2,2',3,3',6,6'-Hexachlorobiphenyl
2,4,5-Trichlorobiphenyl	trans-Nonachlor
Heptachlor	Oxychlordane
Aldrin	HCB

Table 4. BLOOD QC SAMPLES FOR A TYPICAL SAMPLING TRIP

Sample Type	Number	Comments
Field Blank	5	Freeze after preparation, carry to field, store with field samples.
Field Control	5	Store with field blanks.
Lab Blank	5	Freeze after preparation, store in same freezer as field samples will be stored.
Lab Control	5	Store with Lab Blanks

person must be aware of their actions, observe events which may affect the data, and maintain appropriate records. At the second level, the chemist's supervisor monitors their daily activities, reviews the notebook, checks data and calculations, and assists in "troubleshooting" problems. At the tertiary level, a QA coordinator interviews all personnel on the project. The interviews cover the operations they perform (precisely), the data they obtain, a spot-check of their calculations, and any problems they have had.

7.2.1.2 Documentation

Chain of Custody

From the initial preparation of a sample container through reporting of the analytical results, each sample is accompanied by a chain of custody sheet. Each person signs in the time of receipt, operations performed, and transmittal of the sample. This record is important for tracing a contaminant bad standard, or some other problem.

Sampling Protocol Sheets

When a sample is collected, a sampling protocol sheet is filled in which contains a discrete sample code which identifies project number, area, site, locations, trip number, sampling period, and sample type. Also included are sample times, volumes, address and other pertinent information. Where appropriate, a map is made to precisely identify the location.

Sample Log

Upon return from a sampling trip, each sample code is entered into a sample log book. This log is updated as samples proceed through workup and analysis. Thus, at a glance, project personnel can tell the status of each sample and find out how many are at different stages in the analytical protocol.

GC/MS Log

Each sample run by GC/MS is logged into a notebook, detailing analysis conditions, where the data are archived, and what hardcopy data has been produced.

7.2.2 External Quality Assurance

Samples will be analyzed by a designated QA laboratory. Samples, controls, and blanks will be shipped directly from the field to the QA

laboratory for analysis. They will report the results to the primary laboratory for correlation with the primary data.

7.2.2.1 Selection of Samples for QA

Approximately 10% of the field samples (2 minimum) will be collected in duplicate (i.e., two 10 ml blood or two 120 mL urine samples) for shipment to the QA laboratory. This selection process will be random with the following restrictions:

(1) The donor must consent to the additional blood collection.

(2) If any stratification of donors is known, purposive selection of QA donors may be used to get representative samples (e.g., occupationally exposed vs. "normal" individuals or upwind vs. downwind residents).

7.2.2.2 QC Samples

Along with the QA duplicate samples, at least one QC control and one QC blank must be included with the QA samples. An example is shown in Table 5 for a trip collecting 50 samples.

7.2.2.3 Sample Codes

Sll samples shipped to the QA laboratory should be encoded so that the laboratory cannot discern between samples and controls. This "blind" protocol will add validity to the results.

7.2.2.4 Shipping

Samples should be shipped on dry ice directly to the QA laboratory by an appropriate air carrier (e.g., Federal Express, Eastern Sprint) in well insulated and packed cartons.

8.0 References

1. Sherma, J., "Manual of Analytical Quality Control for Pesticides in Human and Environmental Media" EPA -600/1-76-017, 2K, 33 (1979).
2. ibid, Section 4, A, (6), p 2.
3. ibid, Section 4, A, (4), p 4.

Table 5. SAMPLES TO BE COLLECTED AND SHIPPED TO QA LABORATORY FOR A TYPICAL SAMPLING TRIP

Sample	Number	Comments
Duplicate Sample	5	Random selection unless prior information stratifies subjects
Field Blank	1	Ship with samples
Field Control	1	Ship with samples

Adapted from:

Thompson, J. F., Analysis of Pesticide Residues in Human and Environmental Samples, A Compilation of Methods Selected for Use in Pesticide Monitoring Programs, Environ. Toxicol. Div., Health Effects Research Lab., USEPA, RTP, NC, December, 1977. Sections 4A, 5A(1), and 5A(3)(a).

* Indicates a section not in original protocol, added here for application to this research project.

ANALYTICAL PROTOCOL: SAMPLING AND ANALYSIS OF EXTRACTABLE HALOGENATED ORGANICS IN BLOOD AND URINE (RTI)

1.0 Principle of Method

Semi-volatile halogenated hydrocarbons are extracted from blood plasma or urine with organic solvents, dried, and concentrated to an appropriate volume for quantification using a gas chromatograph/electron capture detector (GC/ECD). Identifications are confirmed by GC/ECD using a second column and, when sufficiently concentrated, by GC/MS/COMP. Blood and urine samples are optionally subjected to liquid chromatographic cleanup on Florisil if severe interferences are encountered. This procedure was adapted from that of Thompson (1).

2.0 Range and Limit of Detection

The sensitivity of response to GC/ECD is a function of the instrument, the compound, and the matrix from which it is extracted. Acceptable recoveries from human plasma at approximately 5 ppb (parts per billion) have been achieved.

3.0 Interferences

Interferences in sample analysis and quantification using GC/ECD are manifested in the electron capturing ability of the given contaminant. Blood extracts which have not been cleaned up contain rapidly eluting components which exhibit high electron capturing properties; these interferences can largely be removed by gradient liquid-solid chromatography on 2% aqueous deactivated Florisil, as discussed below.

4.0 Precision and Accuracy

Recovery studies were initiated with a wide variety of model halogenated hydrocarbons (e.g., trifluralin, α-BHC, β-BHC, γ-BHC, 2,4,5-trichlorobiphenyl, heptachlor, aldrin, heptachlor epoxide, endosulfan, p,p'-DDE and dieldrin). For 2.0 ml aliquots of human plasma and 3.0 ml aliquots of urine spiked with 14 ng of the above halogenated hydrocarbons and equilibrated for 19 hr at 4°C, a mean recovery of 53.1 ± 12.6% was obtained for blood plasma (2).

5.0 Apparatus and Reagents

5.1 Sampling Apparatus

Blood samples are obtained in 10 ml vacutainer tubes "suitable for GC" (Venoject L428, Kimble) containing 15 mg tripotassium salt of EDTA and 20 μg potassium sorbate.

5.2 Extraction Apparatus

1. Glass culture tubes (16 x 125 mm) and caps equipped with Teflon liners, reciprocal shaker (ca. 40 oscillations/minute);
2. 500 ml Kuderna-Danish evaporators, receiving tubes and three ball Snyder columns;
3. glass bottles and caps equipped with Teflon liners;
4. reactivials®;
5. centifuge
6. 22 mm i.d. chromatography columns.

5.3 Solvents and Reagents

1. Hexane (Burdick and Jackson) distilled in glass, redistilled prior to use.
2. Methylene chloride (Burdick and Jackson) distilled in glass, redistilled prior to use.
3. Anhydrous sodium sulfate, (extracted with pentane in Soxhlet for 24 hr and stored in oven at 140°C).
4. Florisil, 60/100 mesh (activated by heating for at least 5 hr at 130°C).

   NOTE: For a more complete treatment on the handling and characteristics of Florisil batches refer to the "EPA Manual of Analytical Methods for the Analysis of Pesticide Residues in Human and Environmental Samples", Section 3, D.

6.0 Procedure

6.1 Collection of Samples

Blood is obtained from volunteers by a qualified phlebotomist using vacutainers. Samples are inverted to ensure dispersal of the anticoagulant and are then centrifuged for 45 min at 9000 rpm. Plasma is then pipetted off, stored in a shell vial with teflon-lined screw caps and frozen as soon as possible.

First morning urines are collected by the volunteers in 120 ml wide-mouthed bottles. The samples are then picked up by a member of the sampling team, and stored in a refrigerator or on ice until ready for analysis.

6.2 Extraction, Cleanup, and Concentration

6.2.1 Plasma

1. Transfer 2.0 ml of plasma to a 12 x 125 mm culture tube.
2. Pipet in 7.0 ml of hexane and cap.
3. Shake on a shaker bath at 40 oscillations/minute for 24 hr.
4. Remove sample from bath, centrifuge for v5 minutes to improve separation of layers, transfer 5.0 ml of organic extract to glass bottle with Teflon liner.
5. Pipet 5.0 ml additional hexane into serum and repeat (3).
6. Repeat (4), combine extracts and dry for at least 30 min over 0.5 g anhydrous Na_2SO_4.
7. Transfer to a 500 ml K-D flask (micro K-D optional), concentrate to 2-4 ml and cool to ambient temperature.
8. Rinse sides of K-D with 1.5 ml hexane and blow under dry nitrogen to about 1 ml.
9. Transfer to a reactivial® previously calibrated to a specific volume and further concentrate.

6.2.2 Urine

1. Transfer 5.0 ml of urine to a 12 x 125 mm culture tube.
2. Follow steps 2 through 9 of the procedure for the extraction of blood serum for organochloride pesticides.

6.3 Instrumental

The detection and quantification of semi-volatile halogenated hydrocarbons is made using a Series 4400 Fisher/Victoreen Gas Chromatograph equipped with a tritium foil electron capture detector. Separation is effected on a 40 m, 0.38 mm i.d., glass SCOT capillary column coated with 1% SE-30 on 0.32% Tullanox (3,4). Maximum efficiency is obtained with a flow rate of 2.5 ml/min of nitrogen gas with makeup nitrogen gas adjusted to a total flow of 25.0 ml/min, column 220°C (isothermal), and detector 285°C.

As a confirmatory column a 190 cm x 0.2 cm i.d. 1.5% OV-17/1.95% QF-1 on 80/100 Chromosorb W-HP packing is employed. Efficient responses are

obtained for flow rates of 18 ml/min at identical column and detector temperatures.

Final confirmation of the identity of the components of sufficiently concentrated extracts (generally greater than 10 ng/µl) can be made using gas chromatography/mass spectrometry/computer (GC/MS/COMP) (Finnigan 3300).

The GC/MS/COMP systems used are a Finnigan 3300 GC/MS/COMP and an LKB 2091 GC/MS equipped with an LKB 2031 data system. Chromatographic conditions for the Finnigan 3300 are 20 m x 0.38 mm i.d., 1% SE-30 SCOT capillary operated isothermally at 235°C and a flow rate of 2.0 ml/min helium. Splitless injection (0.2-0.3 µl) is used, with standard electron impact (70 eV) ionization conditions.

The LKB 2091 is operated using a 18 m 1% SE-30/$BaCO_3$ WCOT capillary column at 240°, isothermal for PCBs and a 40 m x 0.38 mm i.d. 1% SE-30 SCOT capillary column at 230° isothermal for the pesticides. In both cases, the column flow rate is 2 ml/min with 20 ml/min split off at the injector. The mass spectrometer is operated under standard electron impact conditions.

NOTE: Similar GC columns may be substituted for those prescribed. GC conditions may change accordingly.

6.4 Qualitative and Quantitative Analysis

6.4.1 Qualitative Analysis

Alternate single injection of extracts and standard solutions is the routine procedure for processing samples. If the retention time of a given component of an extract suggests the presence of a standard compound, a repetitive injection is then made. Tentative identification is made if the deviation between the two respective means is no greater than three percent. A similar criterion is then applied to the retention times of both extract and standard component upon a second confirmatory column. Qualitative identification of a component is made if both criteria are satisfied.

6.4.2 Quantitative Analysis

A mean linear response range of 5-160 pg/µl has been established for the compounds trifluralin and γ-BHC on a 1% SE-30/0.32% Tullanox 40 m, 0.38 mm i.d. SCOT capillary column installed in a Series 4400 Fisher/Victoreen Gas Chromatograph. Quantification of a given component is made by a comparison of the means of recorder trace areas of two extract and two standard

solutions within this linear response range. The precision of the concentration of a given component is normally less than ten percent of the mean concentrations and is obtained by propagation of the standard deviations of the responses of both the extract and standard solutions. The effective concentration multiplied by the volume of extract results in the total amount of extracted material.

The areas employed in the quantification of blood extracts must be normalized to compensate for the volume of extract that is not analyzed. The removal of the first 5.0 ml aliquot from the 7.0 ml extract leaves 2/7 of the extracted material behind. Since the partition coefficients for semi-volatiles in human plasma and urine are large, one approximates that the second extraction contributes more as a dilution gradient than as an extracting solvent. Under this approximation, 5/7 of the original 2/7 remaining in the organic extract would be obtained from the second aliquot. One would then have $5/7 + (2/7)\cdot(5/7) = 45/49$ of the total initial extract to analyze, and the area responses for the extracts would necessarily require normalization by $49/45 = 1.089$ prior to comparison to standard solutions. This methodology was initiated due to the formation of complex emulsions between the plasma and organic solvent which prevented the easy removal of all of the organic extract. This procedure assumes an infinite partition coefficient and that the second extraction does not influence the total recovery.

If the extracts are colored, the presence of lipids may interfere with either analysis or concentration of the extracts due to precipitation. In this case, the sample should be transferred to a 22 mm i.d. column containing 1.6 g of 2% aqueous-deactivated Florisil and eluted with 10 ml each of hexane, 5% $MeCl_2$/hex, 10% $MeCl_2$/hex, 15% $MeCl_2$/hex, 20% $MeCl_2$/hex, 30% $MeCl_2$/hex, 50% $MeCl_2$/hex, and $MeCl_2$. The extracts are concentrated and analyzed. Most semi-volatile halogenated hydrocarbons are expected to appear in the first five fractions. This estimate is based upon elution data of pesticides on Florisil (5) and has not been subjected to full experimental verification.

6.4.3 GC/MS/COMP Confirmation

The chromatography conditions are similar to those used for GC/ECD. The samples for this study are to be screened by GC/ECD and confirmed (if sufficiently concentrated) by GC/MS/COMP. Therefore, the retention times of the two techniques must be similar. GC/ECD must operate isothermally, so the GC/MS/COMP conditions reflect this restriction.

The Finnigan 3300 and the LKB 2091 systems may be operated in both the full scan and selected ion monitoring (SIM) modes. In the full scan mode, full spectra are collected. Spectra or mass fragmentograms (single ion plots) may be plotted for interpretation. In the SIM mode, only a small number (up to 9 for the Finnigan 3300 and up to 16 for the LKB 2091) of ions are monitored. Full spectra are not collected. The advantage of this method is that the detector spends more time "looking" at the selected ion and therefore better (generally 10-50 times) sensitivity is obtained.

To determine the limits of detection, standard solutions of selected pesticides and PCB isomers have been analyzed on the Finnigan 3300 and LKB 2091. In the full scan mode, the limit of detection was the amount of compound required for an interpretable spectrum. In the SIM mode, the limit of detection was the amount of compound required to yield a peak 2-4 times the noise level.

The estimated limits of detection for the Finnigan 3300 and LKB 2091 are presented in Table 1.

Quantitation using GC/MS/COMP is achieved by comparing the computer-calculated integrated area of the unknown with the integrated response for a known amount of standard. To compensate for differences in ionization cross-section, the relative molar response of authentic compounds is obtained

The calculation of the relative molar response (RMR) factor allows the estimation of the levels of sample components without establishing a calibration curve. The RMR is calculated as the integrated peak area of a known amount of the compound, A°_{unk}, with respect to the integrated peak area of a known standard, A°_{std} (in this case d_{10}-pyrene), according to the equation

Table 1. ESTIMATED LIMITS OF DETECTION FOR EXTRACTABLE HALOGENATED ORGANICS ANALYSIS[a]

Compound	LKB 2091[b]			Finnigan 3300[a]		
	Full scan ng/µl	SIM m/z	SIM ng/µl	Full scan ng/µl	SIM m/z	SIM ng/µl
γ-BHC (lindane)	>12<20	181	0.10-0.4	5-10	181	1
heptachlor	12	272	0.10-0.4	10-20	272	1-1.5
chlordane	∿30[c]	375	5	25-50	375	5-10
p,p'-DDE	12	246	>0.3	5-10	246	0.5-1
2-chlorobiphenyl	∿1	188	0.004	∿2.5	188	∿0.025
hexachlorobiphenyl	<1	360	∿0.016	25-50	360	∿0.15
decachlorobiphenyl	12	498	0.42	150	498	∿0.3

[a]See text for conditions.

[b]15:1 split injection, only 1/15 of injection is on column.

[c]0.2 µl injected with no split.

$$R = \frac{A^{\circ}_{unk}/moles_{unk}}{A^{\circ}_{std}/moles_{std}} = \frac{(A^{\circ}_{unk})\ (mw_{unk})\ (g_{std})}{(A^{\circ}_{unk})\ (mw_{std})\ (g_{unk})} \qquad \text{(Eq. 1)}$$

From this calculated value, the concentration of an identified compound in a sample is calculated by rearranging Equation 1 to give

$$g_{unk} = \frac{(A_{unk})\ (mw_{unk})\ (g_{std})}{(A_{std})\ (mw_{std})\ (RMR)} \qquad \text{(Eq. 2)}$$

The use of RMR for quantitation by GC/MS has been successful in repeated applications to similar research problems.

The RMRs for the compounds were calculated from the numerical integrations of peaks observed in the appropriate MID channel. Typical RMRs listed in Table 2 and 3 are mean values of three injections of each of three replicate standard mixtures.

The RMRs given here are to be regarded as typical values. Not only must they be determined for each instrument, but day-to-day variations are sometimes large enough to require daily calibration.

7.0 Quality Assurance Program

An on-going quality assurance program is required to assure the data quality. Quality control (QC) procedures determine artifacts, losses, etc. through a system of blanks and controls. Quality assurance (QA) procedures monitor the execution of the procedure and check data interpretations and calculations.

7.1 Quality Control

7.1.1 Field Blanks and Controls

Prior to a field sampling trip, enough blanks and controls are prepared to equal 10% each (2 minimum) of the anticipated number of field samples. Blanks consist of 10 ml of water in the same type of sampling container as is used in the field. Controls consist of 10 ml of plasma or water (urine control) spiked at 10-15 ng/ml with the compounds listed in Table 4. These blanks and controls are carried to the field and receive the same handling as the field samples. Workup and analysis of field blanks and controls is interspersed with the field samples on a regular basis. This method allows assessment of sample storage stability.

Table 2. RMRs FOR PCBs AND PESTICIDES OF INTEREST TO THIS PROGRAM[a]

Compound	Concentration	Ion	RMR
2-chlorobiphenyl	104 ng/µl	188	elutes with solvent and was two scans wide·
	3.8 ng/µl		not determinable
hexachlorobiphenyl	570 ng/µl	360	.38 ± 3%
	10.4 ng/µl		.35 ± 10%
decachlorobiphenyl	1156 ng/µl	498	.14 ± 7%
	8.4 ng/µl		not determinable
lindane	100 ng/µl	181	.74 ± 9%
		183	.62 ± 12%
heptachlor	100 ng/µl	272	.74 ± 6%
p,p'-DDE	100 ng/µl	246	.45 ± 6%
chlordane (peak 1)	100 ng/µl	373	.71 ± 5%
		375	.65 ± 5%
chlordane (peak 2)	100 ng/µl	373	.051 ± 6%
		375	.045 ± 13%

[a]Standard is d_{10}-pyrene (m/z= 212).

Table 3. RMR FACTORS FOR STANDARD PCB SOLUTIONS, SELECTED ION MONITORING MODE

	Standard	RMR m/z 188 2-Chlorobiphenyl		RMR m/z 358 Hexachlorobiphenyl		RMR m/z 498 Decachlorobiphenyl	
I	PCB-STD-20	0.60		0.257		0.341	
II	PCB-STD-2	0.620 0.811 0.466 0.643	0.640 ± .171	0.291 0.334 0.319 0.321	0.325 ± .009	0.430 0.474 0.462 0.431	0.456 ± .018
III	PCB-STD-0.2	0.566 0.840 0.637 0.597 0.705	0.699 +.171	0.366 0.293 0.301 0.239 0.273	0.294 ± .072	0.372 0.361 0.373 0.303 0.394	0.361 ± .033
IV	PCB-STD-0.04	1.020 0.692 0.576	0.763 ± .257	0.320 0.528 0.528	0.459 ± .072	0.287 0.543 0.372	0.401 ± .142

[a]Standard is d_{10}-pyrene (m/z = 212).

Table 4. SEMI-VOLATILE HALOGENATED HYDROCARBONS IN METHANOL SPIKING SOLUTION

Compound	Compound
α-BHC	Heptachlor epoxide
β-BHC	Dieldrin
γ-BHC	p,p'-DDE
4,4'-Dichlorobiphenyl	p,p'-DDT
2,4,5-Trichlorobipenyl	2,2',3,3',6,6'-Hexachlorobiphenyl
Heptachlor	trans-Nonachlor
Aldrin	Oxychlordane
	HCB

Table 5 presents a typical set of blanks and controls for QC on a field trip where 50 blood samples are to be collected.

7.1.2 Procedural Blanks and Controls

7.1.2.1 Extraction Blanks

With each set of samples, a procedural blank is run. This consists of 10 ml of prepurged distilled water which is extracted under the same conditions as the samples. These blanks are designed to detect artifacts from dirty glassware, laboratory atmosphere intrusion, and other sources.

7.1.2.2 GC/MS Procedural Control

At the start of each working day, a mixture of 2,6-dimethylphenol, 2,6-dimethylaniline, and acetophenone (PA mixture) is analyzed to monitor the capillary GC column performance. This also serves to check the mass spectrometer tuning.

Field samples, field controls, field blanks, and procedural blanks are queued up for GC/MS analysis such that at least one QC sample is run each working day. In addition, a standard solution is analyzed each day to serve as a procedural control and also to update the RMR value. Thus, in a typical working day, 4 field samples, 1 blank or control, and 1 RMR standard are run.

The Finnigan GC/MS is a quadrupole mass spectrometer which requires frequent tuning. Daily tuning is achieved using FC-43 and decafluorotriphenyl phosphine (DFTPP).

7.2 Quality Assurance

Both internal and external QA procedures are to be followed. Internal QA procedures assure the continuity and consistency of the data. External QA procedures (interlaboratory checks) verify or dispute the accuracy of the data being generated in the primary laboratory.

7.2.1 Internal Quality Assurance

7.2.1.1 Supervision and Monitoring of Activities

There are three levels of quality assurance (QA). The primary quality assurance is the person conducting the sampling and/or analysis. This person must be aware of their actions, observe events which may affect the data, and maintain appropriate records. At the second level, the chemist's supervisor monitors their daily activities, reviews the notebook, checks

Table 5. BLOOD QC SAMPLES FOR A TYPICAL SAMPLING TRIP

Sample type	Number	Comments
Field Blank	5	Freeze after preparation, carry to field, store with field samples.
Field Control	5	Store with field blanks
Lab Blank	5	Freeze after preparation, store in same freezer as field samples will be stored
Lab Control	5	Store with lab blanks

data and calculations, and assists in "troubleshooting" problems. At the tertiary level, a QA coordinator interviews all personnel on the project. The interviews cover the operations they perform (precisely), the data they obtain, a spot-check of their calculations, and any problems they have had.

7.2.1.2 Documentation

From the initial preparation of a sample container through reporting of the analytical results, each sample is accompanied by a chain of custody sheet. Each person signs in the time of receipt, operations performed, and transmittal of the sample. This record is important for tracing a contaminant, bad standard, or some other problem.

Sampling Protocol Sheets--When a sample is collected, a sampling protocol sheet is filled in which contains a discrete sample code which identifies project number, area, site, locations, trip number, sampling period, and sample type. Also included are sample times, volumes, addresses, and other pertinent information. Where appropriate, a map is made to precisely identify the location.

Sample Log--Upon return from a sampling trip, each sample code is entered into a sample log book. This log is updated as samples proceed through workup and analysis. Thus, at a glance, project personnel can tell the status of each sample and find out how many are at different stages in the analytical protocol.

GC/MS Log--Each sample run by GC/MS is logged into a notebook, detailing analysis conditions, where the data are archived, and what hardcopy data has been produced.

7.2.2 External Quality Assurance

Samples will be analyzed by a designated QA laboratory. Samples, controls, and blanks will be shipped directly from the field to the QA laboratory for analysis. They will report the results to the primary laboratories for correlation with the primary data.

7.2.2.1 Selection of Samples for QA

Approximately 10% of the field samples (2 minimum) will be collected in duplicate (i.e. two 10 ml blood samples and two 25 ml urine samples) for shipment to the QA laboratory. This selection process will be random with the following restrictions:

(1) The donor must consent ot the additional blood and urine collection.

(2) If any stratification of donors is known, purposive selection of QA donors may be used to get representative samples (e.g. occupationally exposed vs. "normal" individuals or upwind vs. downwind residents).

7.2.2.2 QC Samples

Along with the QA duplicate samples, at least one QC control and one QC blank must be included with the QA samples. An example is shown in Table 6 for a trip collecting 50 samples.

7.2.2.3 Sample Codes

All samples shipped to the QA laboratory should be encoded so that the laboratory cannot discern between samples and controls. This "blind" protocol will add validity to the results.

7.2.2.4 Shipping

Samples should be shipped on dry ice directly to the QA laboratory by an appropriate air carrier (e.g. Federal Express, Eastern Sprint) in well insulated and packed cartons.

8.0 References

1. Pellizzari, E. D., M. D. Erickson, and Zweidinger, R. A., "Analytical Protocols for Making a Preliminary Assessment of Halogenated Organic Compounds in Man and Environmental Media", EPA-560/13-79-010, September 1979, Appendix F, p. 151.

2. Thompson, J. F., "Analysis of Pesticide Residues in Human and Environmental Samples, A Compilation of Metods Selected for Use in Pesticide Monitoring Programs", Environ. Toxicol. Div., Health Effects Research Lab., USEPA, RTP, NC, December (1974).

3. Hines, J. R., Shaprio, R., Pellizzari, E. and Schwartz, A., HRC and CC, submitted for publication (1978).

4. Hines, J. R., Shapiro, R., Schwartz, A. and Pellizzari, E., HRC and CC, submitted for publication (1978).

5. Sherma, J., "Manual of Analytical Quality Control for Pesticides and Related Compounds", EPA-600/1-76-017, Table 7-1.

Revised, April 1980

Table 6. SAMPLES TO BE COLLECTED AND SHIPPED TO QA LABORATORY FOR A TYPICAL SAMPLING TRIP

Sample	Number	Comment
Duplicate sample	5	Random selection unless prior information stratified subjects
Field blank	1	Ship with samples
Field Control	1	Ship with samples

ANALYTICAL PROTOCOL: POLYNUCLEAR AROMATIC HYDROCARBONS IN BLOOD AND URINE (RTI)

1.0 Principle of Method

Polynuclear aromatic hydrocarbons (PAHs) are extracted from blood plasma or urine with organic solvents, dried and concentrated to an appropriate volume for quantification using gas chromatographic/flame ionization detection (GC/FID). An alternate method for analysis if high performance liquid chromatography (HPLC). Blood or urine samples are optionally subjected to liquid chromatographic cleanup if severe interferences are encountered.

2.0 Range and Limit of Detection

The sensitivity of response to GC/FID is a function of the instrument, the compound and the sample matrix from which it is extracted. The limit of detection for this method is dependent on the level of interferences from sample matrix rather than instrumental limitations. With no interferences the detection limits of the PAHs of interest are about 1 ng with packed column gas chromatography. Detection limits for HPLC analysis are given in Table 1.

3.0 Interferences

Solvents, reagents, glassware, and other sample processing hardware may yield discrete artifacts and/or elevated baselines causing misinterpretation of the chromatograms. All of these materials must be demonstrated to be free from interferences under the conditions of the analysis by running method blanks. Specific selection of reagents and purification of solvents by distillation in all-glass systems may be required.

Interferences can be coextracted from the samples. Thus samples may need a clean-up step to approach the 1 ng detection limit.

Capillary gas chromatographic methods, with inherently greater resolution than packed column GC, minimizes the extent of interferences.

4.0 Precision and Accuracy

The method has not been validated.

Table 1. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY OF PAHs[a]

Compound[c]	Retention time (min)	Detection limit (μg/L)[b]	
		UV	Fluorescence
Naphthalene	16.17	2.5	20.0
Acenaphthylene	18.10	5.0	100.0
Acenaphthene	20.14	3.0	4.0
Fluorene	20.89	0.5	2.0
Phenanthrene	22.32	0.25	1.2
Anthracene	23.78	0.10	1.5
Fluoranthene	25.00	0.50	0.05
Pyrene	25.94	0.10	0.05
Benzo(a)anthracene	29.26	0.20	0.04
Chrysene	30.14	0.20	0.5
Benzo(b)fluoranthene	32.44	1.0	0.04
Benzo(k)fluoranthene	33.91	0.30	0.04
Benzo(a)pyrene	34.95	0.25	0.04
Dibenzo(a,h)anthracene	37.06	1.0	0.08
Benzo(ghi)perylene	37.82	0.75	0.2
Indeno(1,2,3-cd)pyrene	39.21	0.30	0.1

[a]Source: "Development and Application of Test Procedures for Specific Organic Toxic Substances in Wastewaters, Category 9-PAHs", Report for EPA Contract 68-03-2624 (in preparation).

[b]Detection limit is calculated from the minimum detectable HPLC response being equal to five times the background noise, assuming an equivalent of a 2 ml final volume of the 1 liter sample extract, and assuming an HPLC injection of 2 microliters.

[c]HPLC conditions: Reverse phase HC-ODS Sil-X 2.6 x 250 mm Perkin-Elmer column; isocratic elution for 5 min using 40% acetonitrile/60% water, then linear gradient elution to 100% acetonitrile over 25 minutes, flow rate is 0.5 ml/min.

5.0 Apparatus and Reagents

5.1 Sampling Apparatus

5.1.1 Blood

Blood samples are obtained in 10 ml vacutainer tubes made of borosilicate glass with silicone stoppers (Venoject-KT-200-SKA) containing heparin as an anticoagulant.

5.1.2 Urine

Urine samples are obtained in 120 ml wide-mouthed bottles washed, solvent rinsed once oven dried before use. Screw caps with Teflon liners must be used on sample bottles.

5.2 Extraction Apparatus

1. Glass culture tubes (16 x 125 mm) and caps equipped with Teflon liners, reciprocal shaker (ca. 40 oscillations/minute);
2. 500 ml Kuderna-Danish evaporators, receiving tubes and three ball Snyder columns;
3. glass bottles and caps equipped with Teflon liners;
4. reactivials®;
5. centrifuge;
6. 22 mm i.d. chromatography columns.

5.3 Solvents and Reagents

1. Hexane (Burdick and Jackson) distilled in glass, redistilled prior to use.
2. Methylene chloride (Burdick and Jackson) distilled in glass, redistilled prior to use.
3. Anhydrous sodium sulfate, (extracted with pentane in Soxhlet for 24 hr and stored in oven at 140°C).
4. Florisil, 60/100 mesh (activated by heating for at least 5 hr at 130°C).

NOTE: For a more complete treatment on the handling and characteristics of Florisil batches refer to the "EPA Manual of Analytical Methods for the Analysis of Pesticide Residues in Human and Environmental Samples", Section 3, D.

6.0 Procedure

6.1 Collection of Samples

6.1.1 Blood

Blood is obtained from volunteers by a qualified phlebotomist using vacutainers. Samples are inverted to ensure dispersal of the anticoagulant and are then centrifuged for 45 min at 2500 rpm. Plasma is then pipetted off, stored in a shell vial with teflon-lined screw caps and frozen as soon as possible.

6.1.2 Urine

First morning urines are collected by the volunteers in 120 ml wide-mouthed bottles. The samples are then picked up by a member of the sampling team, and stored in a refrigerator or on ice until ready for analysis.

6.2 Extraction, Cleanup, and Concentration

6.2.1 Plasma

1. Transfer 2.0 ml of plasma to a 12 x 125 mm culture tube.
2. Pipet in 7.0 ml of hexane and cap.
3. Shake on a shaker bath at 40 oscillations/minute for 3 hr.
4. Remove sample from bath, centrifuge for ~5 minutes to improve separation of layers, transfer 5.0 ml of organic extract to glass bottle with Teflon liner.
5. Pipet into serum 5.0 ml additional hexane and repeat (3).
6. Repeat (4), combine extracts and dry for at least 30 min over 0.5 g anhydrous Na_2SO_4.
7. Transfer to a 500 ml K-D flask (micro K-D optional), concentrate to 2-4 ml and cool to ambient temperature.
8. Rinse sides of K-D with 1.5 ml hexane and blow under dry nitrogen to about 1 ml.
9. Transfer to a reactivial® previously calibrated to a specific volume and further concentrate.

6.2.2 Urine

1. Transfer 5.0 ml of urine to a 12 x 125 mm culture tube.
2. Follows steps 2 through 9 of the procedure for the extraction of blood plasma for organochlorine pesticides.

6.2.3 Optional Florisil Cleanup

If interferences hinder analysis and quantitation of desired peaks, a Florisil column clean-up can be incorporated into the extraction scheme.

6.2.3.1 Florosil Fractionation

1. Prepare a chromatographic column containing 4 inches (after settling) of activated Florisil topped with 1/2 inch of anhydrous, granular Na_2SO_4. A small wad of glass wool, preextracted with hexane, is placed at the bottom of the column to retain the Florisil.

NOTE: (1) Florisil is activated by heating for at least 5 hr at 130°. (2) If the oven is of sufficient size, the columns may be prepacked and stored in the oven, withdrawing columns a few minutes before use. (3) The amount of Florisil needed for proper elution should be determined for each lot of Florisil.

2. Place a 500 ml Erlenmeyer flask under the column and prewet the packing with hexane (40-50 ml, or a sufficient volume to completely cover the Na_2SO_4 layer).

NOTE: From this point and through the elution process, the solvent level should never be allowed to go below the top of the Na_2SO_4 layer. If air is introduced, channeling may occur, making for an inefficient column.

3. Using a 5 ml Mohr or a long disposable pipet, immediately transfer the tissue extract (ca. 5 ml) from the evaporator tube onto the column and permit it to percolate through.
4. Rinse tube with two successive 5 ml portions of hexane, carefully transferring each portion to the column with the pipet.

NOTE: Use of the Mohr or disposable pipet to deliver the extract directly onto the column precludes the need to rinse down the sides of the column.

5. Prepare two Kuderna-Danish evaporative assemblies complete with 10 ml graduated evaporative concentrator tubes. Place one glass bead in each concentrator tube.
6. Replace the 500 ml Erlenmeyer flask under each column with a 500 ml Kuderna-Danish assembly and commence elution with 200 ml of 6% diethyl ether in hexane (Fraction I). The elution rate should be 5 ml per minute. When the last of the eluting solvent reaches the

top of the Na_2SO_4 layer, place a second 500 ml Kuderna-Danish assembly under the column and continue elution with 200 ml of 15% diethyl ether in hexane (Fraction II).

7. To the second fraction only, add 1.0 ml of hexane containing 200 nanograms of aldrin, place both Kuderna-Danish evaporator assemblies in a water bath and concentrate extract until ca. 5 ml remain in the tube.
8. Remove assemblies from bath and cool to ambient temperature.
9. Disconnect collection tube from Kuderna-Danish flask and carefully rinse joint with a little hexane.
10. Attach modified micro-Snyder column to collection tubes, place tubes back in water bath and concentrate extracts to 1 ml. If preferred, this may be done at room temperature under a stream of nitrogen.
11. Remove from bath, and cool to ambient temperature. Disconnect tubes and rinse joints with a little hexane.

NOTE: The extent of dilution or concentration of the extract at this point is dependent on the pesticide concentration in the substrate being analyzed and the sensitivity of the flame ionization detector being used in the analysis.

12. Should it prove necessary to conduct further cleanup on the 15% fraction, transfer 10 grams MgO-Celite mixture to a chromatographic column using vacuum to pack. Prewash with ca. 40 ml hexane, discard prewash and place a Kuderna-Danish receiver under column. Transfer concentrated Florisil eluate to column using small portions of hexane. Force sample and washings into the MgO-Celite mixture by slight air pressure and elute column with 100 ml hexane. Concentrate to a suitable volume and proceed with gas liquid chromatography.

NOTE: Standard recoveries should be made through column to ensure quantitative recoveries.

6.3 Analysis

Table 2 describes the recommended gas chromatographic column material and operating conditions for the instrument. Included in this table are

Table 2. GAS CHROMATOGRAPHY OF PAHs

Compound[a]	Retention time (min)
Naphthalene	4.5
Acenaphthylene	10.4
Acenaphthene	10.8
Fluorene	12.6
Phenanthrene	15.9
Anthracene	15.9
Fluoranthene	19.6
Pyrene	20.6
Benzo(a)anthracene	20.6
Chrysene	24.7
Benzo(b)fluoranthene	28.0
Benzo(k)fluoranthene	28.0
Benzo(a)pyrene	29.4
Dibenzo(a,h)anthracene	36.2
Indeno(1,2,3-cd)pyrene	36.2
Benzo(ghi)perylene	38.6

[a]GC conditions: Chromosorb W-AW-DMDCS 100/120 mesh coated with 3% OV-17, packed in a 6' x 2 mm ID glass column, with nitrogen carrier gas at 40 mL/min flow rate. Column temperature was held at 100°C for 4 minutes, then programmed at 8°/minute to a final hold at 280°C.

estimated retention times that should be achieved by this method. Calibrate the gas chromatographic system daily with a minimum of three injections of calibration standards.

Prepare calibration standards that contain the compounds of interest, either singly or mixed together. The standards should be prepared at concentrations covering two or more orders of magnitude that will completely bracket the working range of the chromatographic system.

Inject 2-5 µl of the sample extract using the solvent flush technique. Smaller (1.0 µl) volumes can be injected if automatic devices are employed. Record the volume injected to the nearest 0.05 µl, and the resulting peak size, in area units. If the peak area exceeds the linear range of the system, dilute the extract and reanalyze. If the peak area measurement is prevented by the presence of interferences, further cleanup is required.

Table 1 describes the recommended HPLC column material and operating conditions for the instrument.

6.4 Calculations

Determine the concentration of individual compounds according to the formula:

$$\text{Concentration, } \mu g/l = \frac{(A)\ (B)\ (V_t)}{(V_i)\ (V_s)}$$

where:

A = calibration factor for chromatographic system, in nanograms material per area unit
B = peak size in injection of sample extract, in area units
V_i = volume of extract injected (µl)
V_t = volume of total extract (µl)
V_s = volume of water extracted (ml)

Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, all data obtained should be reported.

7.0 Quality Assurance Program

An on-going quality assurance program is required to assure the data quality. Quality control (QC) procedures determine artifacts, losses, etc. through a system of blanks and controls. Quality assurance (QA) procedures

monitor the execution of the procedure and check data interpretations and calculations.

7.1 Quality Control

7.1.1 Blanks and Controls

Prior to a field sampling trip, enough blanks and controls are prepared to equal 10% each (2 minimum) of the anticipated number of field samples. Lab blanks and controls are stored in the laboratory and constitute "storage control". Field blanks and controls are carried to the field and receive the same handling as the field samples and constitute "transportation control" Workup and analysis of blanks and controls is interspersed with the field samples on a regular basis. This method allows assessment of sample storage stability.

Table 3 presents a typical set of blanks and controls for QC on a field trip where 50 blood or urine samples are to be collected.

7.1.1.1 Blood

Blood blanks consist of 10 ml of water in a vacutainer tube. Controls consist of 10 ml of water spiked with 10-15 ng of the compounds listed in Table 4.

7.1.1.2 Urine

Urine blanks consist of 120 ml of distilled water. Controls consist of 120 ml of distilled water spiked with 10-15 ng of the compounds listed in Table 4.

7.1.2 Procedural Blanks and Controls

7.1.2.1 Extraction Blanks

With each set of samples, a procedural blank is run. This consists of 10 ml of prepurged distilled water which is extracted under the same conditions as the samples. These blanks are designed to detect artifacts from dirty glassware, laboratory atmosphere intrusion, and other sources.

7.2 Quality Assurance

Both internal and external QA procedures are to be followed. Internal QA procedures assure the continuity and consistency of the data. External QA procedures (interlaboratory checks) verify or dispute the accuracy of the data being generated in the primary laboratory.

Table 3. BLOOD QC SAMPLES FOR A TYPICAL SAMPLING TRIP

Sample type	Number	Comments
Field Blank	5	Freeze after preparation, carry to field, store with field samples.
Field Control	5	Store with field blanks
Lab Blank	5	Freeze after preparation, store in same freezer as field samples will be stored
Lab Control	5	Store with lab blanks

Table 4. POLYNUCLEAR AROMATIC HYDROCARBONS IN METHANOL SPIKING SOLUTION

Compound	Compound
Fluoranthene	Chrysene
Benzo(a)pyrene	Benzo(a)anthracene
Pyrene	

7.2.1 Internal Quality Assurance

7.2.1.1 Supervision and Monitoring of Activities

There are three levels of quality assurance (QA). The primary quality assurance is the person conducting the sampling and/or analysis. This person must be aware of their actions, observe events which may affect the data, and maintain appropriate records. At the second level, the chemist's supervisor monitors their daily activities, reviews the notebook, checks data and calculations, and assists in "troubleshooting" problems. At the tertiary level, a QA coordinator interviews all personnel on the project. The interviews cover the operations they perform (precisely), the data they obtain, a spot-check of their calculations, and any problems they have had.

7.2.1.2 Documentation

From the initial preparation of a sample container through reporting of the analytical results, each sample is accompanied by a chain of custody sheet. Each person signs in the time of receipt, operations performed, and transmittal of the sample. This record is important for tracing a contaminant, bad standard, or some other problem.

Sampling Protocol Sheets--When a sample is collected, a sampling protocol sheet is filled in which contains a discrete sample code which identifies project number, area, site, locations, trip number, sampling period, and sample type. Also included are sample times, volumes, addresses, meteorology, and other pertinent information. Where appropriate, a map is made to precisely identify the location.

Sample Log--Upon return from a sampling trip, each sample code is entered into a sample log book. This log is updated as samples proceed through workup and analysis. Thus, at a glance, project personnel can tell the status of each sample and find out how many are at different stages in the analytical protocol.

7.2.2 External Quality Assurance

Samples will be analyzed by a designated QA laboratory. Samples, controls, and blanks will be shipped directly from the field to the QA laboratory for analysis. They will report the results to the primary laboratories for correlation with the primary data.

7.2.2.1 Selection of Samples for QA

Approximately 10% of the field samples (2 minimum) will be collected in duplicate (i.e. two 10 ml blood samples) and two 25 ml urine samples for shipment to the QA laboratory. This selection process will be random with the following restrictions:

(1) The donor must consent to the additional hair collection.

(2) If any stratification of donors is known, purposive selection of QA donors may be used to get representative samples (e.g. occupationally exposed vs. "normal" individuals or upwind vs. downwind residents).

7.2.2.2 QC Samples

Along with the QA duplicate samples, at least one QC control and one QC blank must be included with the QA samples. An example is shown in Table 5 for a trip collecting 50 samples.

7.2.2.3 Sample Codes

All samples shipped to the QA laboratory should be encoded so that the laboratory cannot discern between samples and controls. This "blind" protocol will add validity to the results.

7.2.2.4 Shipping

Samples should be shipped on dry ice directly to the QA laboratory by an appropriate air carrier (e.g. Federal Express, Eastern Sprint) in well insulated and packed cartons.

8.0 References

1. Thompson, J. F., "Analysis of Pesticide Residues in Human and Environmental Samples, A Compilation of Methods Selected for Use in Pesticide Monitoring Programs", Environ. Toxicol. Div., Health Effects Research Lab., USEPA, RTP, NC, December (1974).

2. Pellizzari, E. D., M. D. Erickson, and Zweidinger, R. A., "Analytical Protocols for Making a Preliminary Assessment of Halogenated Organic Compounds in Man and Environmental Media", EPA-560/13-79-010, September 1979, Appendix F, p. 151.

3. "Development and Application of Test Procedures for Specific Organic Toxic Substances in Wastewaters, Category 9-PAHs", Report for EPA Contract 68-03-2624 (in preparation).

Revised, May, 1980

Table 5. SAMPLES TO BE COLLECTED AND SHIPPED TO QA LABORATORY FOR A TYPICAL SAMPLING TRIP

Sample	Number	Comment
Duplicate sample	5	Random selection unless prior information stratified subjects
Field blank	1	Ship with samples
Field Control	1	Ship with samples

ANALYTICAL PROTOCOL: SAMPLING AND ANALYSIS OF PURGEABLE HALOGENATED ORGANICS IN URINE (RTI)

1.0 Principle of the Method

Volatile compounds are recovered from a urine sample by warming the sample and purging an inert gas over the warm sample. The vapors are then trapped on a Tenax cartridge and subsequently can be analyzed by thermal desorption interfaced to GC/MS.

2.0 Range and Sensitivity

For a typical organic compound approximately 30 ng are required for mass spectral identification using high resolution glass capillary GC/MS analysis. Based on a 25 ml urine sample, a limit of detection of about 1.2 μg/l (1.2 ppb) may be expected. The dynamic range for a purged sample is $\sim 10^4$; however, smaller samples may be purged and the range increased commensurately.

3.0 Interferences

Two possible types of interference must be considered: (1) material present in the sample which physically prevents the effective purge of the sample, and (2) a material which interferes with the analysis of the purged sample. In the former case, several techniques have been developed to handle such problems (e.g., foaming) by diluting and stirring the sample. The second case is minimized by the use of GC/MS for the analysis since unique combinations of m/z and retention time can be selected for most compounds. This permits the analysis of compounds even though chromatographic resolution is not obtained.

4.0 Precision and Accuracy

The purge and trap technique was validated using four ^{14}C-labeled model compounds and six "cold" model compounds yielding an average recovery of 82.9% ± 20.8% (1).

Based on these data, expected recoveries of purgeable halogenated organics from urine are about 80% or better. Within the precision requirements of this study, these recovery values indicate that the method is essentially quantitative.

5.0 Apparatus and Reagents

5.1 Sampling

Urine samples are collected in 120 ml cleaned and oven-treated glass bottles and sealed with Teflon-lined caps.

5.2 Purge Apparatus

The apparatus required is shown in Figure 1.

5.3 Tenax Cartridges

Tenax cartridges are prepared and the background checked as described in Section 6.1.1 of Protocol A-8 (Analysis of Purgeable Organic Compounds in Water [Master Analytical Scheme]).

5.4 GC/MS/COMP

The volatile halogenated hyrocarbons purged from water are analyzed on either an LKB 2091 GC/MS with an LKB 2031 data system or a Varian MAT CH-7 GC/MS with a Varian 620/i data system. The sample, concentrated on a Tenax GC cartridge, is thermally desorbed using an inlet manifold system.

The operating conditions for the thermal desorption unit and the analysis of Tenax GC cartridges are given in Table 7.

5.5 Reagents and Solvents

1. Pentane, Burdick and Jackson distilled in glass, redistilled.
2. Methanol, Burdick and Jackson distilled in glass, redistilled prior to use.

6.0 Procedure

6.1 Collection of Samples

Each participant is provided with a clean 120 ml (4 oz.) bottle and asked to collect the first urine sample in the morning. In addition, spot urine samples are collected from selected participants when tissue, breath and blood samples are collected. These samples serve both as backups and to determine individual variability.

6.2 Purge of Volatile Organics

1. Measure a 25 ml aliquot of urine, previously chilled to 4°C, into the purge flask (Fig. 1).
2. Dilute sample to 50 ml with purged distilled water and add stir bar.

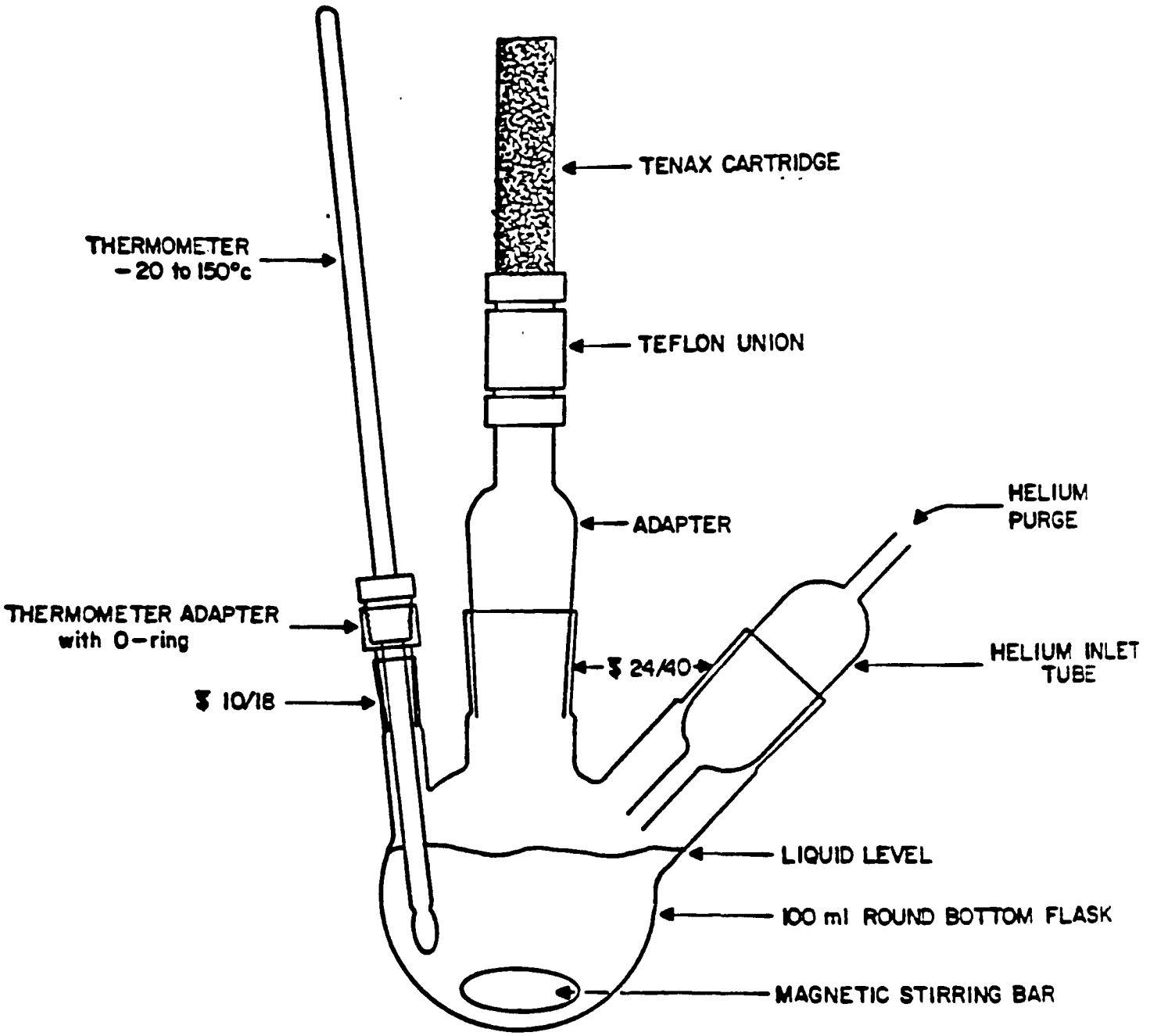


Figure 1. Headspace using apparatus for blood, urine, and tissue samples.

3. Assemble apparatus, start stirring and raise the temperature to 50°C.
4. Adjust helium flow to 25 ml/min and purge for 90 min.
5. After 90 min disassemble apparatus and transfer Tenax cartridge to a Kimax culture tube with 2 g calcium sulfate dessicant for 4 hours of drying.
6. Transfer Tenax cartridge to an identical Kimax culture tube without calcium sulfate, seal in a paint can and store in freezer until analysis.

6.3 Analysis of Sample Purged on Cartridge

The instrumental conditions for the analysis of halogenated hydrocarbons of the sorbent Tenax GC sampling cartridge is shown in Table 1. The thermal desorption chamber and six-port valve are maintained at 270° and 200°C, respectively. The helium purge gas through the desorption chamber is adjusted to 15-20 ml/min. The nickel capillary trap at the inlet manifold is cooled with liquid nitrogen. In a typical thermal desorption cycle a sampling cartridge is placed in the preheated desorption chamber and helium gas is channeled through the cartridge to purge the vapors into the liquid nitrogen cooled nickel capillary trap. After desorption the six-port valve is rotated and the temperature on the capillary loop is rapidly raised; the carrier gas then introduces the vapors onto the high resolution GLC column. The glass capillary column is temperature programmed from 20° to 240°C at 4°/min and held at the upper limit for a minimum of 10 min. After all of the components have eluted from the capillary column the analytical column is then cooled to ambient temperature and the next sample is processed.

6.4 Quantitation

All data are acquired in the full scan mode. Quantitation of the halogenated compounds of interest is accomplished by utilizing selected ion plots, SIPs, which are plots of the intensity of specific ions (obtained from full scan data) vs time. Using SIPs of ions characteristic of a given compound in conjunction with retention times permits quantitation of components of overlapping peaks. Two external standards, perfluorobenzene and perfluorotoluene, were added to each Tenax GC cartridge in known quantities just prior to analysis. In order to eliminate the need to construct complete

Table 1. INSTRUMENTAL OPERATING CONDITIONS

	LKB 2091	Varian MAT CH-7
Desorption chamber temperature	270	265
Desorption chamber He flow	15 ml/min	10 ml/min
Desorption time	8.0 min	8.0 min
Capillary trap temperature during desorption	-196°C	-196°C
Temperature of capillary trap during injection onto column	-196°C to 250°C - then held at 190°C	
Time of He flow through capillary trap	12 3/4 min	12 3/4 min
He flow through column [sweep time]	9.5 min	4 min
Carrier flow	2.0 ml/min	1.0 ml/min
Capillary column	100 m SE-30 SCOT	70 m SE-30 WCOT
Column temperature	30°C for 2 min then 4°/min to 240°	20 → 240° at 4°min
Scan range	5-490 dalton	20 → 500 dalton
Scan rate	2 sec full scale	1 sec/decade
Scan cycle time	2.4 sec	4.5 sec
Scan mode	parabolic	exponential
Trap current	4A	
Filament Current	50 μA	300 μA
Accelerating voltage	3.5 kV	2 kV

calibration curves for each compound quantitated, the method of relative molar response (RMR) is used. In this method the relationship of the RMR as the unknown to the RMR of the standard is determined as follows:

$$RMR_{unknown/standard} = \frac{A_{unk}/Moles_{unk}}{A_{std}/Moles_{std}}$$

$$RMR_{unk/std} = \frac{A_{unk}/g_{unk}/GMW_{unk}}{A_{std}/g_{std}/GMW_{std}}$$

where A = peak response of a selected ion,
g = number of grams present, and
GMW = gram molecular weight

Thus, in the sample analyzed:

$$g_{unknown} = \frac{(A_{unk})(GMW_{unk})(g_{std})}{(A_{std})(GMW_{std})(RMR_{unk/std})}$$

The value of an RMR is determined at least three independent analyses of standards of accurately known concentration purged using a gas permeation system (D5). The precision of this method has been determined to be generally ± percent when replicate sampling cartridges are examined.

7.0 Quality Assurance Program

An on-going quality assurance program is required to assure the data quality. Quality control (QC) procedures determine artifacts, losses, etc., through a system of blanks and controls. Quality assurance (QA) procedures monitor the execution of the procedure and check data interpretations and calculations.

7.1 Quality Control

7.1.1 Field and Lab Blanks and Controls

Prior to a field sampling trip, enough blanks and controls are prepared to equal 10% each (2 minimum) of the anticipated number of field samples. Blanks consist of 100 ml of water in the same type of sampling container as is used in the field. Controls consist of 100 ml of urine spiked at

100-150 ng with chloroform, 1,2-Dichloroethane, 1,1,1-Trichloroethane, Carbon tetrachloride, Trichloroethylene, Tetrachloroethylene, Chlorobenzene, m-dichlorobenzene, Bromodichloromethane, and vinyl chloride. Field blanks and controls are carried to the field and receive the same handling as the field samples. Lab blanks and controls are prepared at the same time as the field blanks and controls and are stored at -4°C. Workup and analysis of field blanks and controls is interspersed with the field samples on a regular basis. This method allows assessment of sample storage stability.

Table 2 presents a typical set of blanks and controls for QC on a field trip where 50 urine samples are to be collected.

7.1.2 Procedural Blanks and Controls

7.1.2.1 Extraction Blanks

With each set of samples, a procedural blank is run. This consists of 10 ml of purged, distilled water which is extracted under the same conditions as the samples. These blanks are designed to detect artifacts from dirty glassware, laboratory atmoshpere intrusion, and other sources.
In addition Tenax cartridge blanks are analyzed to determine cartridge background.

7.1.2.2 GC/MS Procedural Control

At the start of each working day, a mixture of 2,6-dimethylphenol, 2,6-dimethylaniline, and acetophenone (PA mixture) is analyzed to monitor the capillary GC column performance. This also serves to check the mass spectrometer tuning.

Field samples, field controls, field blanks, and procedural blanks are queued up for GC/MS analysis such that at least one QC sample is run each working day. In addition, a standard solution is analyzed each day to serve as a procedural control and also to update the RMR value. Thus, in a typical working day, 4 field samples, 1 blank or control, and 1 RMR standard are run.

The Finnigan GC/MS is a quadrupole mass spectrometer which requires frequent tuning. Daily tuning is achieved using FC-43 and decafluorotriphenylphosphine (DFTPP).

Table 2. URINE QC SAMPLES FOR A TYPICAL SAMPLING TRIP

Sample Type	Number	Comments
Field Blank	5	Freeze after preparation, carry to field, store with field samples
Field Control	5	Store with field blanks
Lab Blank	5	Freeze after preparation, store in same freezer as field samples will be stored
Lab Control	5	Store with lab blanks

7.2 Quality Assurance

Both internal and external QA procedures are to be followed. Internal QA procedures assume the continuity and consistency of the data. External QA procedure (interlaboratory checks) verify or dispute the accuracy of the data being generated in the primary labortory.

7.2.1 Internal Quality Assurance

7.2.1.1 Supervision and Monitoring Activities

There are three levels of quality assurance (QA). The primary quality assurance is the person conducting the sampling and/or analysis. This person must be aware of their actions, observe events which may affect the data, and maintain appropriate records. At the second level, the chemist's supervisor monitors their daily activities, and reviews the notebook, checks data and calculations, and assists in "troubleshooting" problems. At the tertiary level, a QA coordinator interviews all personnel on the project. The interviews cover the operations they perform (precisely), the data they obtain, a spot-check of their calculations, and any problems they have had.

7.2.1.2 Documentaion

Chain of Custody

From the initial preparation of a sample transfer through reporting of the analytical results, each sample is accompanied by a chain of custody sheet. Each person signs in the time of receipt, operations performed, and transmittal of the sample. This record is important for tracing a contaminant, bad standard, or some other problem.

Sampling Protocol Sheets

When a sample is collected, a sampling protocol sheet is filled in which contains a discrete sample code which identifies project number, area, site, locations, trip number, sampling period, and sample type. Also included are sample times, volumes, addresses, and other pertinent information. Where appropriate, a map is made to precisely identify the location.

Sample Log

Upon return from a sampling trip, each sample code is entered into a sample log book. This log is updated as samples proceed through workup and analysis. Thus, at a glance, project personnel can tell the status of each

sample and find out how many are at different stages in the analytical protocol.

GC/MS Log

Each sample run by GC/MS is logged into a notebook, detailing analysis conditions, where the data are archived, and what hardcopy data has been produced.

7.2.2 External Quality Assurance

Samples will be analyzed by a designated QA laboratory. Samples, controls, and blanks will be shipped directly from the field to the QA laboratory for analysis. They will report the results to the primary laboratory for correlation with the primary data.

7.2.2.1 Selection of Samples for QA

Approximately 10% of the field samples (2 minimum) will be collected in duplicate (i.e., two 100 ml min samples) for shipment to the QA laboratory. This selection process will be random with the following restrictions:

1. The donor must consent to the additional urine collection.
2. If any stratification of donors is known, purposive selection of QA donors may be used to get representative samples (e.g., occupationally exposed vs "normal" individuals or upwind vs downwind residents).

7.2.2.2 QC Samples

Along with the QA duplicate samples, at least one QC control and one QC blank must be included with the QA samples. An example is shown in Table 3 for a trip collecting 50 samples.

7.2.2.3 Sample Codes

All samples shipped to the QA laboratory should be encoded so that the laboratory cannot discern between samples and controls. This "blind" protocol will add validity to the results.

7.2.2.4 Shipping

Samples should be shipped on dry ice directly to the QA laboratory by an appropriate air carrier (e.g., Federal Express, Eastern Sprint) in well insulated and packed cartons.

Table 3. SAMPLES TO BE COLLECTED AND SHIPPED TO QA LABORATORY FOR A TYPICAL SAMPLING TRIP

Sample	Number	Comments
Duplicate sample	5	Random selection unless prior information stratifies subjects
Field Blank	1	Ship with samples
Field Control	1	Ship with samples

8.0 References

1. Pellizzari, E. D., Erickson, M. D., and Zweidinger, R. A. "Analytical Protocols for Making a Preliminary Assessment of Halogenated Organic Compounds in Man and Environmental Media". Appendix pg. Revised April 1979.
2. Pellizzari, E. D., Development of Method for Carcinogenic Vapor Analysis in Ambient Atmospheres, Publication No. EPA-650/2-74-121, Contract No. 68-02-1228, 148 pp., July, 1974.
3. Pellizzari, E. D., Development of Analytical Techniques for Measuring Ambient Atmospheric Carcinogenic Vapors. Publication No. EPA-600/2-76-076, Contract No. 68-02-1228, 185 pp., November, 1975.
4. Pellizzari, E. D., J. E. Bunch, B. H. Carpenter and E. Sawicki, Environ. Sci. Tech., 9, 556 (1975).
5. Pellizzari, E. D., B. H. Carpenter, J. E. Bunch and E. Sawicki, Environ. Sci. Tech., 9, 556 (1975).
6. Pellizzari, E. D., Development of Analytical Techniques for Measuring Ambient Atmospheric Carcinogenic Vapors. Publication No. EPA-600/7-77-055, 288 pp., June, 1977.

Revised April 1980

ANALYTICAL PROTOCOL: SAMPLING AND ANALYSIS OF ARSENIC, CADMIUM, AND LEAD IN URINE (RTI)

1.0 Principle of Method

The analysis of arsenic, cadmium, and lead in urine is carried out using atomic absorption spectrophotometry. Increased sensitivity is achieved by atomizing the metal in a graphite furnace with continuous deuterium background correction. Sample workup for arsenic analysis includes an extraction from the urine matrix and furnace atomization of solutions containing 1000 ppm nickel.

2.0 Range and Detection Limit

The minimum detection limit (MDL) and range for the metal assays in urine are shown below.

Metal	MDL	Max. Conc.
Arsenic	0.10 µg/100 ml	6.0 µg/100 ml
Cadmium	0.01	0.50
Lead	0.20	10.0

Samples containing higher metal concentrations may be analyzed by suitable dilution with 0.5% nitric acid. Dilution for arsenic determinations is made with 0.005 M dichromate solution containing 1000 ppm nickel in 1.0% nitric acid.

3.0 Interferences

No known chemical or spectral interferences exist in the analysis of arsenic, cadmium or lead in urine. Severe matrix interferences in the arsenic analysis are minimized by incorporating the toluene extraction step into the workup procedure.

4.0 Precision and Accuracy

The precision and accuracy associated with these analyses is a function of sample metal concentration. At the detection limit, the total measurement error is $\pm$ 100%. Based on the results of a previous study (1), the metal analyses are performed with the following precision (relative standard deviation). The total analysis error (estimated) is also given (2).

Metal	Range	Precision (% RSD)	Accuracy (% RE)	Estimated Total Error (%)
Arsenic	0.5-2.0 µg/100 ml	15	5-10	35-40
Cadmium	0.03-0.07	20	5-10	45-50
Lead	0.5-1.5	15	5-10	35-40

5.0 Apparatus and Reagents

A commercially available stock solution containing 1000 ppm metal is used for the preparation of the calibration standards. The concentrated nitric acid is reagent grade quality and the deionized water used in this study will be prefiltered and subjected to the action of an activated carbon cartridge and two sequential ion exchange units.

The glassware used for sample workup and the preparation of calibration solutions must be subjected to a nitric acid cleaning protocol.

All volumetric flasks, beakers, and digestion bottles should be soaked overnight in 20% nitric acid, rinsed with deionized water, soaking for an additional 15-18 hours in a 5% nitric acid bath, followed by a copious deionized water rinse. The flasks are completely filled with 0.5% nitric acid and stored in this manner. Prior to use, each flask is emptied and rinsed well with deionized water. Pipets are soaked in 5% nitric acid, rinsed well with deionized water, air-dried, and stored in a clean, dust-free environment.

All beakers used for urine digestions require additional pretreatment. Clean beakers (soaked in 20% and 5% nitric acid) are "predigested" by heating 10-25 ml of conc. nitric to reflux (with watchglass), cooling, and discarding the acid. The beakers are rinsed thoroughly with deionized water and used for a sample digestion within 30 minutes. The beakers are never allowed to go dry.

Sample cups for the graphite furnace autosampler may be made of polyptyrene or Teflon. The former type requires overnight soaking in 1% nitric acid and followed by rinsing with deionized water. The latter type may be soaked overnight in 20% nitric acid, rinsed, and dried in a 105°C oven.

Nickel chloride hexahydrate is used for adjusting the nickel concentrations to 1000 ppm in all solution slated for arsenic analysis.

6.0 Procedure

6.1 Collection of Samples

Urine samples are collected in a 4-ounce polyethylene bottle with a Polyseal cap. To insure sample stability and to improve handling during sample workup, the specimen is spiked with concentrated nitric acid shortly after collection (1.0 ml/100 ml urine). The bottle is labeled and all pertinent information recorded on a protocol sheet.

6.2 Extraction, Cleanup, and Concentration

6.2.1 Cadmium and Lead Analysis

Two ml of urine, acidified at the site, is added to a 50 ml predigested beaker and treated with 10.0 ml of conc. HNO_3 at 85-90°C for one hour. The watchglass is removed from the beaker and the digest volume reduced to approximately 1.0 ml. The residue is cooled and transferred to a 10 ml volumetric flask and diluted to the mark with 0.5% HNO_3. The sample solution is stored in a 1 oz. polypropylene bottle (with screw cap) at ambient temperatures until ready for analysis.

6.2.2 Arsenic Analysis (3)

A 5.0 ml aliquot of urine is added to 20 ml of low arsenic conc. HCl in a clean 4 oz. glass bottle and the mixture allowed to stand at room temperature for 3-7 days. At the end of this period, 10 ml of 0.5 M $SnCl_2$ and 5 ml of 30% KI is added to the sample digest and the total allowed to stand at room temperature and for 30 minutes. Forty ml of conc. HCl and 10.0 ml of toluene is added to the mixture and the arsenic bodies extracted into the organic phase. Half of the toluene layer (5.0 ml) is withdrawn and mixed with 2.0 ml of a 0.005 M dichromate solution containing 1000 ppm nickel in 1.0% HNO_3. The arsenic compounds are back-extracted into the aqueous phase and stored in polypropylene bottles until ready for analysis.

6.3 Instrumental

A Perkin-Elmer Model 403 Spectrophotometer, equipped with a HGA-2000 furnace attachment with deuterium background correction is used for this analysis. An electrodeless discharge lamp is used as the light source and the furnace atomization response traced on a Perkin-Elmer Model 056 recorder. An AS-1 Autosampler may be used to increase throughput and/or to improve peak reproducibility and sensitivity.

Arsenic: Wavelength - 193.7 nm

Gas Interrupt (N_2) - Auto

Furnace Cycle Conditions -

Dry: 200°C for 20 sec.

Char: 1200°C for 30 sec.

Atomize: 2500°C for 8 sec.

Injection Volume - 20 µl

Cadmium: Wavelength - 228.8 nm

Gas Interrupt (N_2) - Auto

Furnace Cycle Conditions -

Dry: 200°C for 20 sec.

Char: 400°C for 30 sec.

Atomize: 1500°C for 8 sec.

Injection Volume - 20 µl

Lead: Wavelength- 217.0 nm

Gas Interrupt (N_2) - Auto

Furnace Cycle Conditions -

Dry: 200°C for 20 sec.

Char: 500°C for 30 sec.

Atomize: 2000°C for 8 sec.

Injection Volume - 20 µl

6.4 Qualitative and Quantitative Analysis

6.4.1 Qualitative Analysis

N/A

6.4.2 Quantitative Analysis

The instrument is calibrated with a digested control urine spiked at four different concentrations, an unspiked control urine, and a reagent blank.

Calibration Range (spike concentration):

Arsenic - 0.0 to 6.0 µg/100 ml

Cadmium - 0.0 to 0.5

Lead - 0.0 to 10.0

An exponential of the form $y = Ae^{bx}-M$ provides the best representation of the analytical curve. The values of the x,y calibration pairs are

entered into a Monroe Calculator Model 1880 programmed to regress the data to the exponential and to provide values for the constants, A, b, and M.

Sample peak heights are measured manually and expressed in units of millivolts. The standard additions calibration constants A, b, and M are entered into the storage banks of a Texas Instrument Calculator Model 57 and the metal concentration results obtained by keying in peak height data. Sample peak measurements and concentration result are recorded in a calculation worksheet.

$$y = Ae^{bx}-M, \ \mu g/100 \ ml$$

$y = y_s$ (metal conc. in sample relative to control urine + y_c (metal conc. in control urine)

$$y_s = (Ae^{bx_s}-M)$$

$$y_c = -y_o = -(Ae^{bx_o}-M)$$

$$y = (Ae^{bx_s}-M) - (Ae^{bx_o}-M)$$

$$y = A(e^{bx_s}-e^{bx_o})$$

x_o = reagent blank peak height, mv

y_o = standard additions metal concentration corresponding to reagent blank signal ($y \leq 0$),

$y_c = -y_o$ = metal concentration in control urine, µg/100 ml,

x_s = sample peak height, mv

y_s = concentration differential between sample and control urine. This value may be either negative ($y \leq y$) or positive ($y \leq y$)

7.0 Quality Assurance Program

An on-going quality assurance program is required to assure the data quality. Quality control (QC) procedures determine artifacts, losses, etc., through a system of blanks and controls. Quality assurance (QA) procedures monitor the execution of the procedure and check data interpretations and calculations.

7.1 Quality Control

7.1.1 Field Controls

Prior to field sampling, several control urine collections (10% of anticipated number of field samples) are obtained. Each urine sample is divided into two portions. One aliquot is placed in a container identical to that used for field sample, sent to the site, and subjected to the same handling and storage conditions as field samples. The other aliquot is stored at RTI in a dust-free environment. On receipt of samples at RTI, both portions of the control urine collection is worked up and analyzed as a part of each urine analytical run. Within the precision of the assay, the difference in calculated metal concentrations of the two control urine aliquots is a measure of the contamination/loss during field storage, and transit to RTI.

7.1.2 Internal Quality Control

7.1.2.1 Calibration Standards and Blanks

The instrument is calibrated before each analytical run with four standard solutions and a reagent blank. Evidence of contamination or instrument malfunction is evident at this time. Such problems are resolved before initiating sample analysis.

7.1.2.2 Conditioning of Graphite Tube

Before each analytical run, the graphite tube is conditioned by injecting 10 to 20 20 µl aliquots of one of the calibration standards. This operation insure acceptable precision during sample analysis.

7.1.2.3 Duplicate Injections

Reproducibility of peak response is contiuously monitored during sample analysis. All standard and sample solutions receive two successive injections into the graphite furnace. Signal agreement between the duplicate injections is evaluated according to the following criterion:

First Signal % of Full Scale	% Maximum Permissible Variation (% MPV)	Permissible Range of Second Signal, % of Full Scale
90	± 4%	86-94
80	± 5%	76-84
70	± 6%	66-74
60	± 7%	56-64

First Signal % of Full Scale	% Maximum Permissible Variation (% MPV)	Permissible Range of Second Signal, % of Full Scale
50	± 8%	46-54
40	± 10%	36-44
30	± 13%	26-34
20	± 20%	16-24
10	± 30%	7-13
5	± 60%	2-8
2	±100%	0-4

If the second injection gives a signal which falls outside the permissible range, a third injection is performed. The peak measurement not in agreement with the matching pair is discarded.

All calibration and sample calculations are based on the mean of the duplicate determinations.

7.1.2.4 Standard Checks

Instrument performance is monitored during each analytical run. After the analysis of every 12-16 samples one of the calibration standards is reinjected into the furnace. The standard which most closely matches the sample peak heights is selected as the check solution. A metal concentration is calculated for the check standrad based on its peak height during the calibration run. Similar calculations are carried out for each check response and the observed changes in metal concentration expressed in terms of standard deviation units (SDU).

$$SDU = \frac{(\text{Calibration Value} - \text{Check Value})100}{(\text{Calibration Value})\ (\%\ \text{RSD})}$$

The analysis is under control when the SDU < 2.0. Standard checks which indicate a variation in peak response greater than 2.0 SDU are unacceptable. In this event, the graphite tube is changed, conditioned, and the system recalibrated. Quality control charts are graphed to show this change in instrument performance with time.

7.2 Quality Assurance

Both internal and external QA procedures are to be followed. Internal QA procedures assume the continuity and consistency of the data. External

QA procedures (interlaboratory checks) verify or dispute the accuracy of the data being generated in the primary laboratory.

7.2.1 Internal Quality Assurance

7.2.1.1 Supervision and Monitoring of Activities

There are three levels of quality assurance (QA). The primary quality assurance is the person conducting the sampling and/or analysis. This person must be aware of their actions, observe events which may effect the data, and maintain appropriate records. At the second level, the chemist's supervisor monitors their daily activities, reviews the notebook, checks data and calculations, and assists in "troubleshooting" problems. At the tertiary level, a QA coordinator interviews all personnel on the project. The interviews cover the operations they perform (precisely), the data they obtain, a spot-check of their calculations, and any problems they have had.

7.2.1.2 Documentation

Chain of Custody

From the initial preparation of a sample container through reporting of the analytical results, each sample is accompanied by a chain of custody sheet. Each person signs in the time of receipt, operations performed, and transmittal of the sample. This record is important for tracing a contaminant, bad standard, or some other problem.

Sampling Protocol Sheets

When a sample is collected, a sampling protocol sheet is filled in which contains a discrete sample code which identifies project number, area, site, locations, trip number, sampling period, and sample type. Also included are sample times, volumes, addresses, meteorology, and other pertinent information. Where appropriate, a map is made to precisely identify the location.

Sample Log

Upon return from a sampling trip, each sample code is entered into a sample log book. This log is updated as samples proceed through workup and analysis. Thus, at a glance, project personnel can tell the status of each sample and find out how many are at different stages in the analytical protocol.

Instrument Log

Each sample analysis is logged into a notebook, detailing analysis conditions.

7.2.2 External Quality Assurance

Samples will be analyzed by a designated QA laboratory. Samples, controls, and blanks will be shipped directly from the field to the QA laboratory for analysis. They will report the results to the primary laboratory for correlation with the primary data.

7.2.2.1 Selection of Samples for QA

Approximately 10% of the field samples (2 minimum) will be collected in duplicate shipment to the QA laboratory. This selection process will be random with the following restrictions:

(1) The donor must consent to the additional collection.

(2) If any stratification of donors is known, purposive selection of QA donors may be used to get representative samples (e.g., occupationally exposed vs "normal" individuals or upwind vs downwind residents).

7.2.2.2 QC Samples

Along with the QA duplicate samples, at least one QC control and one QC blank must be included with the QA samples. An example is shown in Table 7 for a trip collecting 50 samples.

7.2.2.3 Sample Codes

All samples shipped to the QA laboratory should be encoded so that the laboratory cannot discern between samples and controls. This "blind" protocol will add validity to the results.

7.2.2.4 Shipping

Samples should be shipped on dry ice directly to the QA laboratory by an appropriate air carrier (e.g., Federal Express, Eastern Spring) in well insulated and packed cartons.

8.0 References

8-1 "Epidemiologic Study Conducted in Populations Living Around Non-Ferrous Smelters", Final Report for Contract No. 68-02-2442 (in preparation).

8-2 McFarren, E. F., Lishka, R. J., and Parker, J. H., Criterion for Judging Acceptability of Analytical Methods, Anal. Chem., 42(3), 358 (1970).

8-3 Handy, R. W. and Natschke, D. F., "Analysis of Arsenic in Whole Blood (Urine)", Paper No. 43 presented at the 30th ACS Southeastern Regional Meeting at Savannah, GA, Nov. 8-10, 1978.

ANALYTICAL PROTOCOL: SAMPLING AND ANALYSIS OF EXTRACTABLE HALOGENATED ORGANICS IN HAIR (RTI)

1.0 Principle of Method

Semi-volatile halogenated hydrocarbons are extracted from hair with organic solvents, dried, and concentrated to an appropriate volume for quantification using a gas chromatograph/electron capture detector (GC/ECD). Identifications are confirmed by GC/ECD using a second column and, when sufficiently concentrated, by GC/MS/COMP. Hair samples are optionally subjected to liquid chromatographic cleanup on Florisil if severe interferences are encountered.

2.0 Range and Limit of Detection

The sensitivity of response to GC/ECD is a function of the instrument, the compound, and the matrix from which it is extracted. If an instrumental sensitivity of 1.0 pg/µl is achieved and 15 g of hair can be extracted, a detection limit of 7 ppb may be realized. This is probably a lower limit.

3.0 Interferences

Interferences in sample analysis and quantification using GC/ECD are manifested in the electron capturing ability of the given contaminant. Additional specificity may be obtained by the use of GC/MS (especially the negative ion chemical ionization mode), which is very sensitive and selective toward halogenated organics.

4.0 Precision and Accuracy

This method has not been validated.

5.0 Apparatus and Reagents

5.1 Sampling Apparatus

1. Scissors (cleaned with a solvent such as toluene or alcohol).
2. Bottles (120 ml, wide-mouth), foil-lined caps and teflon-liners.

5.2 Extraction Apparatus

1. Soxhlet extractor (Fisher Cat. No. 9-556B this includes condenser, Soxhlet tube and flask);
2. Variac and heating mantles;
3. Extraction thimbles;

4. 500 mL Kuderna-Danish evaporators, receiving tubes and three ball Snyder columns;
5. glass bottles and caps equipped with Teflon liners;
6. reactivials®;
7. 10 mm i.d. chromatography columns;
8. 125 ml Erlenmeyer flasks.

5.3 Solvents and Reagents

1. Hexane (Burdick and Jackson) distilled in glass, redistilled prior to use.
2. Toluene (Burdick and Jackson) distilled in glass, redistilled prior to use.
3. Anhydrous granular sodium sulfate, (extracted with pentane in Soxhlet for 24 hr and stored in oven at 140°C).
4. Florisil, 60/100 mesh (activated by heating for at least 5 hr at 130°C).

   NOTE: For a more complete treatment on the handling and characteristics of Florisil batches refer to the "EPA Manual of Analytical Methods for the Analysis of Pesticide Residues in Human and Environmental Samples", Section 3, D (1).

6.0 Procedure

6.1 Collection of Samples

Hair samples are collected using solvent-cleaned (toluene or alcohol) scissors and transferred directly to a precleaned glass bottle (120 ml). It is prefereable to collect long shrands clipped close to the scalp to integrate over the hair growth period. Second preference is clippings from short-haired regions such as the nape of the neck to get the most recent growth. As a final resort, any clippings are useable.

As much hair as possible is desired up to that which will conveniently fill the sample bottle. A minimum would be about 5 g.

6.2 Extraction, Cleanup, and Concentration

6.2.1 Extraction

1. The hair sample is weighed and up to 15 g placed in a precleaned Soxhlet extraction thimble. The sample is extracted with toluene for ~16 hr (overnight).

2. The extract is concentrated in a Kuderna-Danish evaporator. At this point, an aliquot is gently taken to dryness with a stream nitrogen and weighed to determine "oil content". The aliquot is returned to the main sample.
3. Cool the KD to ambient temperature, rinse the sides with 1.5 ml hexane, and concentrate by micro KD to 1 ml.
4. Transfer extract to a reactivial®, previously calibrated to a desirable volume and make to volume.
5. As an option, proceed with Florisil cleanup.

6.2.2 Optional Florisil Cleanup (semi-micro)

If interferences from unwanted electron capturing materials hinder analysis and quantitation of desired peaks, a Florisil column cleanup can be incorporated into the extraction scheme.

1. Prepare a chromatographic column containing 10 cm (after settling) of activated Florisil topped with 1 cm of anhydrous, granular Na_2SO_4. A small wad of glass wool, preextracted with hexane, is placed at the bottom of the column to retain the Florisil.

NOTE: (1) Florisil is activated by heating for at least 5 hr at 130°. (2) If the oven is of sufficient size, the columns may be prepacked and stored in the oven, withdrawing columns a few minutes before use. (3) The amount of Florisil needed for proper elution should be determined for each lot of Florisil.

2. Place a 125 ml Erlenmeyer flask under the column and prewet the packing with hexane (40-50 ml, or a sufficient volume to completely cover the Na_2SO_4 layer).

NOTE: From this point and through the elution process, the solvent level should never be allowed to go below the top of the Na_2SO_4 layer. If air is introduced, channeling may occur, making for an inefficient column.

3. Using a long disposable pipet, immediately transfer the hair extract (ca. 1 ml) from the evaporator tube onto the column and permit it to percolate through.
4. Rinse tube with two successive 1 ml portions of hexane, carefully transferring each portion to the column with the pipet.

NOTE: Use of the disposable pipet to deliver the extract directly onto the column precludes the need to rinse down the sides of the column.

5. Prepare two Kuderna-Danish evaporative assemblies complete with 10 ml graduated evaporative concentrator tubes. Place 2 or 3 boiling chips in each concentrator tube.
6. Replace the 125 ml Erlenmeyer flask under each column with a 500 ml Kuderna-Danish assembly and commence elution with 50 ml of 6% diethyl ether in hexane (Fraction I). The elution rate should be 5 ml per minute. When the last of the eluting solvent reaches the top of the Na_2SO_4 layer, place a second 500 ml Kuderna-Danish assembly under the column and continue elution with 50 ml of 15% diethyl ether in hexane (Fraction II).
7. To the second fraction only, add 1.0 ml of hexane containing 200 nanograms of aldrin, place both Kuderna-Danish evaporator assemblies in a water bath and concentrate extract until ca. 5 ml remain in the tube.
8. Remove assemblies from bath and cool to ambient temperature.
9. Disconnect collection tube from Kuderna-Danish flask and carefully rinse joint with a little hexane.
10. Attach modified micro-Snyder column to collection tubes, place tubes back in water bath and concentrate extracts to 1 ml. If preferred, this may be done at room temperature under a stream of nitrogen.
11. Remove from bath, and cool to ambient temperature. Disconnect tubes and rinse joints with a little hexane.

NOTE: The extent of dilution or concentration of the extract at this point is dependent on the pesticide concentration in the substrate being analyzed and the sensitivity and linear range of the Electron Capture Detector being used in the analysis.

12. Should it prove necessary to conduct further cleanup on the 15% fraction, transfer 12 grams MgO-Celite mixture to a chromatographic column using vacuum to pack. Prewash with ca. 10 ml hexane, discard prewash and place a Kuderna-Danish receiver under column. Transfer concentrated Florisil eluate to column using small portions

of hexane. Force sample and washings into the MgO-Celite mixture by slight air pressure and elute column with 25 ml hexane. Concentrate to a suitable volume and proceed with gas liquid chromatography.

NOTE: Standard recoveries should be made through column to ensure quantitative recoveries.

6.3 Instrumental

The detection and quantification of semi-volatile halogenated hydrocarbons is made using a Series 4400 Fisher/Victoreen Gas Chromatograph equipped with a tritium foil electron capture detector. Separation is effected on a 40 m, 0.38 mm i.d., glass SCOT capillary column coated with 1% SE-30 on 0.32% Tullanox (3,4). Maximum efficiency is obtained with a flow rate of 2.5 ml/min of nitrogen gas with makeup nitrogen gas adjusted to a total flow of 25.0 ml/min, column 220°C (isothermal), and detector 285°C.

As a confirmatory column a 190 cm x 0.2 cm i.d. 1.5% OV-17/1.95% QF-1 on 80/100 Chromosorb W-HP packing is employed. Efficient responses are obtained for flow rates of 18 ml/min at identical column and detector temperatures.

Final confirmation of the identity of the components of sufficiently concentrated extracts (generally greater than 10 ng/µl) can be made using gas chromatography/mass spectrometry/computer (GC/MS/COMP) (Finnigan 3300).

The GC/MS/COMP systems used are a Finnigan 3300 GC/MS/COMP and an LKB 2091 GC/MS equipped with an LKB 2031 data system. Chromatographic conditions for the Finnigan 3300 are 20 m x 0.38 mm i.d., 1% SE-30 SCOT capillary operated isothermally at 235°C and a flow rate of 2.0 ml/min helium. Splitless injection (0.2-0.3 µl) is used, with standard electron impact (70 eV) ionization conditions.

The LKB 2091 is operated using a 18 m 1% SE-30/$BaCO_3$ WCOT capillary column at 240°, isothermal for PCBs and a 40 m x 0.38 mm i.d. 1% SE-30 SCOT capillary column at 230° isothermal for the pesticides. In both cases, the column flow rate is 2 ml/min with 20 ml/min split off at the injector. The mass spectrometer is operated under standard electron impact conditions.

NOTE: Similar GC columns may be substituted for those prescribed. GC conditions may change accordingly.

6.4 Qualitative and Quantitative Analysis

6.4.1 Qualitative Analysis

Alternate single injections of extracts and standard solutions is the routine procedure for processing samples. If the retention time of a given component of an extract suggests the presence of a standard compound, a repetitive injection is then made. Tentative identification is made if the deviation between the two respective means is no greater than three percent. A similar criterion is then applied to the retention times of both extract and standard component upon a second confirmatory column. Qualitative identification of a component is made if both criteria are satisfied.

6.4.2 Quantitative Analysis

A mean linear response range of 5-160 pg/μl has been established for the compounds trifluralin and γ-BHC on a 1% SE-30/0.32% Tullanox 40 m, 0.38 mm i.d. SCOT capillary column installed in a Series 4400 Fisher/Victoreen Gas Chromatograph. Quantification of a given component is made by a comparison of the means of recorder trace areas of two extract and two standard solutions within this linear response range. The precision of the concentration of a given component is normally less than ten percent of the mean concentrations and is obtained by propagation of the standard deviations of the responses of both the extract and standard solutions. The effective concentration multiplied by the volume of extract results in the total amount of extracted material.

6.4.3 GC/MS/COMP Confirmation

The chromatography conditions are similar to those used for GC/ECD. The samples for this study are to be screened by GC/ECD and confirmed (if sufficiently concentrated) by GC/MS/COMP. Therefore, the retention times of the two techniques must be similar. GC/ECD must operate isothermally, so the GC/MS/COMP conditions reflect this restriction.

The Finnigan 3300 and the LKB 2091 systems may be operated in both the full scan and selected ion monitoring (SIM) modes. In the full scan mode, full spectra are collected. Spectra or mass fragmentograms (single ion plots) may be plotted for interpretation. In the SIM mode, only a small number (up to 9 for the Finnigan 3300 and up to 16 for the LKB 2091) of ions are monitored. Full spectra are not collected. The advantage of this

method is that the detector spends more time "looking" at the selected ion and therefore better (generally 10-50 times) sensitivity is obtained. As an option, GC/Negation Ion Chemical ionization mass spectrometry may be employed. This technique is still under validation, and must be employed with caution.

To determine the limits of detection, standard solutions of selected pesticides and PCB isomers have been analyzed on the Finnigan 3300 and LKB 2091. In the full scan mode, the limit of detection was the amount of compound required for an interpretable spectrum. In the SIM mode, the limit of detection was the amount of compound required to yield a peak 2-4 times the noise level.

The estimated limits of detection for the Finnigan 3300 and LKB 2091 are presented in Table 1.

Quantitation using GC/MS/COMP is achieved by comparing the computer-calculated integrated area of the unknown with the integrated response for a known amount of standard. To compensate for differences in ionization cross-section, the relative molar response of authentic compounds is obtained.

The calculation of the relative molar response (RMR) factor allows the estimation of the levels of sample components without establishing a calibration curve. The RMR is calculated as the integrated peak area of a known amount of the compound, A°_{unk}, with respect to the integrated peak area of a known standard, A°_{std} (in this case d_{10}-pyrene), according to the equation

$$R = \frac{A^{\circ}_{unk}/moles_{unk}}{A^{\circ}_{std}/moles_{std}} = \frac{(A^{\circ}_{unk})\ (mw_{unk})\ (g_{std})}{(A^{\circ}_{unk})\ (mw_{std})\ (g_{unk})} \qquad \text{(Eq. 1)}$$

From this calculated value, the concentration of an identified compound in a sample is calculated by rearranging Equation 1 to give

$$g_{unk} = \frac{(A_{unk})\ (mw_{unk})\ (g_{std})}{(A_{std})\ (mw_{std})\ (RMR)} \qquad \text{(Eq. 2)}$$

The use of RMR for quantitation by GC/MS has been successful in repeated applications to similar research problems.

Table 1. ESTIMATED LIMITS OF DETECTION FOR EXTRACTABLE HALOGENATED ORGANIC ANALYSIS[a]

	LKB 2091[b]			Finnigan 3300[a]		
		SIM			SIM	
Compound	Full scan ng/μl	m/z	ng/μl	Full scan ng/μl	m/z	ng/μl
γ-BHC (lindane)	>12<20	181	0.10-0.4	5-10	181	1
heptachlor	12	272	0.10-0.4	10-20	272	1-1.5
chlordane	∿30[c]	375	5	25-50	375	5-10
p,p'-DDE	12	246	>0.3	5-10	246	0.5-1
2-chlorobiphenyl	∿1	188	0.004	∿2.5	188	∿0.025
hexachlorobiphenyl	<1	360	∿0.016	25-50	360	∿0.15
decachlorobiphenyl	12	498	0.42	150	498	∿0.3

[a]See text for conditions.

[b]15:1 split injection, only 1/15 of injection is on column.

[c]0.2 μl injected with no split.

The RMRs for the compounds were calculated from the numerical integrations of peaks observed in the appropriate MID channel. Typical RMRs listed in Table 2 and 3 are mean values of three injections of each of three replicate standard mixtures.

The RMRs given here are to be regarded as typical values. Not only must they be determined for each instrument, but day-to-day variations are sometimes large enough to require daily calibration.

7.0 Quality Assurance Program

An on-going quality assurance program is required to assure the data quality. Quality control (QC) procedures determine artifacts, losses, etc. through a system of blanks and controls. Quality assurance (QA) procedures monitor the execution of the procedure and check data interpretations and calculations.

7.1 Quality Control

7.1.1 Field Blanks and Controls

Prior to a field sampling trip, enough blanks and controls are prepared to equal 10% each (2 minimum) of the anticipated number of field samples. Blanks consist of an empty sampling container. Controls consist of 15 g of hair spiked at 100-150 ng with the compounds listed in Table 4. These blanks and controls are carried to the field and receive the same handling as the field samples. Workup and analysis of field blanks and controls is interspersed with the field samples on a regular basis. This method allows assessment of sample storage stability.

Table 5 presents a typical set of blanks and controls for QC on a field trip where 9 hair samples are to be collected.

7.1.2 Procedural Blanks and Controls

7.1.2.1 Extraction Blanks

With each set of samples, a procedural blank is run. This consists of a Soxhlet apparatus and thimble which is extracted under the same conditions as the samples. These blanks are designed to detect artifacts from dirty glassware, laboratory atmosphere intrusion, and other sources.

7.1.2.2 GC/MS Procedural Control

At the start of each working day, a mixture of 2,6-dimethylphenol, 2,6-dimethylaniline, and acetophenone (PA mixture) is analyzed to monitor

Table 2. RMRs FOR PCBs AND PESTICIDES OF INTEREST TO THIS PROGRAM[a]

Compound	Concentration	Ion	RMR
2-chlorobiphenyl	104 ng/µl	188	elutes with solvent and was two scans wide- not determinable
	3.8 ng/µl		
hexachlorobiphenyl	570 ng/µl	360	0.38 ± 3%
	10.4 ng/µl		0.35 ± 10%
decachlorobiphenyl	1156 ng/µl	498	0.14 ± 7%
	8.4 ng/µl		not determinable
lindane	100 ng/µl	181	0.74 ± 9%
		183	0.62 ± 12%
heptachlor	100 ng/µl	272	0.74 ± 6%
p,p'-DDE	100 ng/µl	246	0.45 ± 6%
chlordane (peak 1)	100 ng/µl	373	0.71 ± 5%
		375	0.65 ± 5%
chlordane (peak 2)	100 ng/µl	373	0.051 ± 6%
		375	0.045 ± 13%

[a]Standard is d_{10}-pyrene (m/z = 212).

Table 3. RMR FACTORS FOR STANDARD PCB SOLUTIONS, SELECTED ION MONITORING MODE

Standard	RMR m/z 188 2-Chlorobiphenyl	RMR m/z 358 Hexachlorobiphenyl	RMR m/z 498 Decachlorobiphenyl
I PCB-STD-20	0.60	0.257	0.341
II PCB-STD-2	0.620 0.811 0.466 0.643 } 0.640 ± .171	0.291 0.334 0.319 0.321 } 0.325 ± .009	0.430 0.474 0.462 0.431 } 0.456 ± .018
III PCB-STD-0.2	0.566 0.840 0.637 0.597 0.705 } 0.699 ± .171	0.366 0.293 0.301 0.239 0.273 } 0.294 ± .072	0.372 0.361 0.373 0.303 0.394 } 0.361 ± .033
IV PCB-STD-0.04	1.020 0.692 0.576 } 0.763 ± .257	0.320 0.528 0.528 } 0.459 ± .072	0.287 0.543 0.372 } 0.401 ± .142

[a]Standard is d_{10}-pyrene (m/z = 212).

Table 4. SEMI-VOLATILE HALOGENATED HYDROCARBONS IN METHANOL SPIKING SOLUTION

Compound	Compound
α-BHC	Heptachlor epoxide
β-BHC	Dieldrin
γ-BHC	p,p'-DDE
4,4'-Dichlorobiphenyl	p,p'-DDT
2,4,5-Trichlorobiphenyl	2,2',3,3',6,6'-Hexachlorobiphenyl
Heptachlor	trans-Nonachlor
Aldrin	Oxychlordane
	HCB

Table 5. HAIR QC SAMPLES FOR A TYPICAL SAMPLING TRIP

Sample type	Number	Comments
Field Blank	2	Freeze after preparation, carry to field, store with field samples
Field Control	2	Store with field blanks
Lab Blank	2	Freeze after preparation, store in same freezer as field samples will be stored
Lab Control	2	Store with lab blanks

the capillary GC column performance. This also serves to check the mass spectrometer tuning.

Field samples, field controls, field blanks, and procedural blanks are queued up for GC/MS analysis such that at least one QC sample is run each working day. In addition, a standard solution is analyzed each day to serve as a procedural control and also to update the RMR value. Thus, in a typical working day, 4 field samples, 1 blank or control, and 1 RMR standard are run.

The Finnigan GC/MS is a quadrupole mass spectrometer which requires frequent tuning. Daily tuning is achieved using FC-43 and decafluorotriphenylphosphine (DFTPP).

7.2 Quality Assurance

Both internal and external QA procedures are to be followed. Internal QA procedures assure the continuity and consistency of the data. External QA procedures (interlaboratory checks) verify or dispute the accuracy of the data being generated in the primary laboratory.

7.2.1 Internal Quality Assurance

7.2.1.1 Supervision and Monitoring of Activities

There are three levels of quality assurance (QA). The primary quality assurance is the person conducting the sampling and/or analysis. This person must be aware of their actions, observe events which may affect the data, and maintain appropriate records. At the second level, the chemist's supervisor monitors their daily activities, reviews the notebook, checks data and calculations, and assists in "troubleshooting" problems. At the tertiary level, a QA coordinator interviews all personnel on the project. The interviews cover the operations they perform (precisely), the data they obtain, a spot-check of their calculations, and any problems they have had.

7.2.1.2 Documentation

From the initial preparation of a sample container through reporting of the analytical results, each sample is accompanied by a chain of custody sheet. Each person signs in the time of receipt, operations performed, and transmittal of the sample. This record is important for tracing a contaminant, bad standard, or some other problem.

Sampling Protocol Sheets--When a sample is collected, a sampling protocol sheet is filled in which contains a discrete sample code which identifies project number, area, site, locations, trip number, sampling period, and sample type. Also included are sample times, volumes, address, and other pertinent information. Where appropriate, a map is made to precisely identify the location.

Sample Log--Upon return from a sampling trip, each sample code is entered into a sample log book. This log is updated as samples proceed through workup and analysis. Thus, at a glance, project personnel can tell the status of each sample and find out how many are at different stages in the analytical protocol.

GC/MS Log--Each sample run by GC/MS is logged into a notebook, detailing analysis conditions, where the data are archived, and what hardcopy data has been produced.

7.2.2 External Quality Assurance

Samples will be analyzed by a designated QA laboratory. Samples, controls, and blanks will be shipped directly from the field to the QA laboratory for analysis. They will report the results to the primary laboratories for correlation with the primary data.

7.2.2.1 Selection of Samples for QA

Approximately 10% of the field samples (2 minimum) will be collected in duplicate for shipment to the QA laboratory. This selection process will be random with the following restrictions:

(1) The donor must consent to the additional hair collection.

(2) If any stratification of donors is known, purposive selection of QA donors may be used to get representative samples (e.g. occupationally exposed vs. "normal" individuals or upwind vs. downwind residents).

7.2.2.2 QC Samples

Along with the QA duplicate samples, at least one QC control and one QC blank must be included with the QA samples. An example is shown in Table 6 for a trip collecting 50 samples.

Table 6. SAMPLES TO BE COLLECTED AND SHIPPED TO QA LABORATORY FOR A TYPICAL SAMPLING TRIP

Sample	Number	Comment
Duplicate sample	5	Random selection unless prior information stratified subjects
Field blank	1	Ship with samples
Field Control	1	Ship with samples

7.2.2.3 Sample Codes

All samples shipped to the QA laboratory should be encoded so that the laboratory cannot discern between samples and controls. This "blind" protocol will add validity to the results.

7.2.2.4 Shipping

Samples should be shipped on dry ice directly to the QA laboratory by an appropriate air carrier (e.g. Federal Express, Eastern Sprint) in well insulated and packed cartons.

8.0 References

1. Thompson, J. F., "Analysis of Pesticide Residues in Human and Environmental Samples, A Compilation of Methods Selected for Use in Pesticide Monitoring Programs", Environ. Toxicol. Div., Health Effects Research Lab., USEPA, RTP, NC, June (1977).

Revised, April, 1980

ANALYTICAL PROTOCOL: SAMPLING AND ANALYSIS OF POLYNUCLEAR AROMATIC HYDROCARBONS IN HAIR (RTI)

1.0 Principle of Method

Polynuclear aromatic hydrocarbons (PAHs) are extracted from hair with an organic solvent, dried, and concentrated to an appropriate volume for quantification by gas chromatography/flame ionization detector (GC/FID). Analysis could also be done by high performance liquid chromatography (HPLC). Hair samples are optionally subjected to liquid chromatographic clean-up on silica gel if severe interferences are encountered. This method determines PAHs in "total hair" (including oil, dust, etc.), not "washed hair".

2.0 Range and Limit of Detection

In the absence of interferences from the sample matrix, detection limits of about 1 ng/µl are anticipated with GC/FID. A detection limit of 7 ppm may be realized if 15 g of hair is extracted. Detection limits for HPLC analysis are given in Table 1.

3.0 Interferences

Solvents, reagents, glassware, and other sample processing apparatus may yield discrete artifacts and/or elevated baselines causing misinterpretation of the chromatograms. All of these materials must be demonstrated to be free from interferences under the conditions of the analysis by running method blanks. Specific selection of reagents and purification of solvents by distillation in all-glass systems may be required.

Capillary gas chromatographic methods, with inherently greater resolution than packed column GC, minimize the extent of interferences.

4.0 Precision and Accuracy

This method has not been validated.

5.0 Apparatus and Reagents

5.1 Sampling Apparatus

1. Scissors (cleaned with a solvent such as toluene or alcohol).
2. Bottles (120 ml, wide-mouth), foil-lined caps and teflon-liners.

5.2 Extraction Apparatus

1. Soxhlet extractor (Fisher Cat. No. 9-556B this includes condenser, Soxhlet tube and flask);

Table 1. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY OF PAHs[a]

Compound[c]	Retention time (min)	Detection limit (μg/L)[b]	
		UV	Fluorescence
Naphthalene	16.17	2.5	20.0
Acenaphthylene	18.10	5.0	100.0
Acenaphthene	20.14	3.0	4.0
Fluorene	20.89	0.5	2.0
Phenanthrene	22.32	0.25	1.2
Anthracene	23.78	0.10	1.5
Fluoranthene	25.00	0.50	0.05
Pyrene	25.94	0.10	0.05
Benzo(a)anthracene	29.26	0.20	0.04
Chrysene	30.14	0.20	0.5
Benzo(b)fluoranthene	32.44	1.0	0.04
Benzo(k)fluoranthene	33.91	0.30	0.04
Benzo(a)pyrene	34.95	0.25	0.04
Dibenzo(a,h)anthracene	37.06	1.0	0.08
Benzo(ghi)perylene	37.82	0.75	0.2
Indeno(1,2,3-cd)pyrene	39.21	0.30	0.1

[a]Source: "Development and Application of Test Procedures for Specific Organic Toxic Substances in Wastewaters, Category 9-PAHs", Report for EPA Contract 68-03-2624 (in preparation).

[b]Detection limit is calculated from the minimum detectable HPLC response being equal to five times the background noise, assuming an equivalent of a 2 ml final volume of the 1 liter sample extract, and assuming an HPLC injection of 2 microliters.

[c]HPLC conditions: Reverse phase HC-ODS Sil-X 2.6 x 250 mm Perkin-Elmer column; isocratic elution for 5 min using 40% acetonitrile/60% water, then linear gradient elution to 100% acetonitrile over 25 minutes, flow rate is 0.5 ml/min.

2. Variac and heating mantles;
3. Extraction thimbles;
4. 500 mL Kuderna-Danish evaporators, receiving tubes and three ball Snyder columns;
5. Glass bottles and caps equipped with Teflon liners;
6. Reactivials®;
7. 10 mm i.d. chromatography columns;

5.3 Solvents and Reagents

1. Toluene (Burdick and Jackson) distilled in glass.
2. Cyclohexane (Burdick and Jackson) distilled in glass.
3. Pentane (Burdick and Jackson) distilled in glass.
4. Methylene chloride (Burdick and Jackson) distilled in glass.
5. Anhydrous, granular sodium sulfate (purified by heating at 400°C for 4 hrs in a shallow tray).
6. Silica gel-100/120 mesh desiccant (Davison Chemical grade 923 or equivalent). Before use, activate for at least 16 hours at 130°C in a foil covered glass container.

6.0 Procedure

6.1 Collection of Samples

Hair samples are collected using solvent-cleaned (toluene or alcohol) scissors and transferred directly to a precleaned glass bottle (120 ml). It is preferable to collect long strands clipped close to the scalp to integrate over the hair growth period. Second preference is clippings from short-haired regions such as the nape of the neck to get the most recent growth. As a final resort, any clippings are useable.

As much hair as possible is desired up to that which will conveniently fill the sample bottle. A minimum would be about 5 g.

6.2 Extraction, Cleanup, and Concentration

6.2.1 Extraction

1. The hair sample is weighed and up to 15 g placed in a precleaned Soxhlet extraction thimble. The sample is extracted with toluene for ~16 hr (overnight).

2. The extract is concentrated in a Kuderna-Danish evaporator. At this point, an aliquot is gently taken to dryness with a stream nitrogen and weighed to determine "oil content". The aliquot is returned to the main sample.
3. Cool the KD to ambient temperature, rinse the sides with 1.5 ml hexane, and concentrate by micro KD to 1 ml.
4. Transfer extract to a reactivial®, previously calibrated to a desirable volume and make to volume.
5. As an option, proceed with silica gel.

6.2.2 Optional Silica Gel Cleanup

Before the silica gel cleanup technique can be utilized, the extract solvent must be exchanged to cyclohexane. Add a 1-10 ml aliquot of sample extract (in toluene) and a boiling chip to a clean K-D concentrator tube. Prewet the micro-Snyder column by adding 0.5 ml toluene to the top. Place the micro K-D apparatus on a boiling (100°C) water bath so that the concentrator tube is partially immersed in the hot water. Adjust the vertical position of the apparatus and the water temperature as required to complete concentration in 5-10 minutes. At the proper rate of distillation the balls of the column will actively chatter but the chambers will not flood. When the apparent volume of the liquid reaches 0.1 ml, remove K-D apparatus and allow it to drain for at least 10 minutes while cooling. Remove the micro-Snyder column and rinse its lower joint into the concentrator tube with a minimum of cyclohexane. Adjust the extract volume to about 2 ml.

Prepare a slurry of 10 g activated silica gel in methylene chloride and place this in a 10 mm ID chromatography column. Gently tap the column to settle the silica gel and elute the methylene chloride. Add 1-2 cm of anhydrous sodium sulfate to the top of the silica gel.

Preelute the column with 40 ml pentane. Discard the eluate and just prior to exposure of the sodium sulfate layer to the air, transfer the 2 ml cyclohexane sample extract onto the column, using an additional 2 ml of cyclohexane to complete the transfer. Just prior to exposure of the sodium sulfate layer to the air, add 25 ml pentane and continue elution of the column. Discard the pentane eluate.

Elute the column with 25 ml of 40% methylene chloride/60% pentane and collect the eluate in a 500-ml K-D flask equipped with a 10 ml concentrator tube. Elution of the column should be at a rate of about 2 ml/min. Concentrate the collected fraction to less than 10 ml by K-D techniques as in 6.2.1, using pentane to rinse the walls of the glassware. Proceed with HPLC or gas chromatographic analysis.

6.3 Calibration

Prepare calibration standards that contain the compounds of interest, either singly or mixed together. The standards should be prepared at concentrations covering two or more orders of magnitude that will completely bracket the working range of the chromatographic system. If the sensitivity of the detection system can be calculated as 100 µg/l in the final extract, for example, prepare standards at 10 µg/l, 50 µg/l, 100 µg/l, 500 µg/l, etc. so that injections of 1-5 µl of each calibration standard will define the linearity of the detector in the working range.

Assemble the necessary gas chromatographic apparatus and establish operating parameters equivalent to those indicated in Table 1 or 2. By injecting calibration standards, establish the sensitivity limit of the detectors and the linear range of the analytical systems for each compound.

6.4 Analysis

To achieve maximum sensitivity with this GC, the extract must be concentrated to 1.0 ml. Add a clean boiling chip to the methylene chloride extract in the concentrator tube. Attach a two-ball micro-Snyder column. Preset the micro-Snyder column by adding about 0.5 ml of methylene chloride to the top. Place this micro K-D apparatus on a hot water bath (60-65°C) so that the concentrator tube is partially immersed in the hot water. Adjust the vertical position of the apparatus and water temperature as required to complete the concentration in 5 to 10 minutes. At the proper rate of distillation the balls will actively chatter but the chambers will not flood. When the apparent volume of liquid reaches 0.5 ml, remove the K-D apparatus and allow it to drain for at least 10 minutes while cooling. Remove the micro-Snyder column and rinse its lower joint into the concentrator tube with a small volume of methylene chloride. Adjust the final volume to 1.0 mL and stopper the concentrator tube.

Table 2 describes the recommended gas chromatographic column material and operating conditions for the instrument. Included in this table are estimated retention times that should be achieved by this method. Calibrate the gas chromatographic system daily with a minimum of three injections of calibration standards.

Inject 2-5 µl of the sample extract using the solvent flush technique. Smaller (1.0 µl) volumes can be injected if automatic devices are employed. Record the volume injected to the nearest 0.05 µl, and the resulting peak size, in area units. If the peak area exceeds the linear range of the system, dilute the extract and reanalyze. If the peak area measurement is prevented by the presence of interferences, further cleanup is required.

Table 1 describes the recommended HPLC column material and operating conditions for the instrument.

6.5 Calculations

Determine the concentration of individual compounds according to the formula:

$$\text{Concentration, } \mu g/l = \frac{(A)(B)(V_t)}{(V_i)(V_s)}$$

where:

A = calibration factor for chromatographic system, in nanograms material per area unit
B = peak size in injection of sample extract, in area units
V_i = volume of extract injected (µl)
V_t = volume of total extract (µl)
V_s = volume of water extracted (ml)

Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, all data obtained should be reported.

7.0 Quality Assurance Program

An on-going quality assurance program is required to assure the data quality. Quality control (QC) procedures determine artifacts, losses, etc. through a system of blanks and controls. Quality assurance (QA) procedures monitor the execution of the procedure and check data interpretations and calculations.

Table 2. GAS CHROMATOGRAPHY OF PAHs[a]

Compound[b]	Retention time (min)
Naphthalene	4.5
Acenaphthylene	10.4
Acenaphthene	10.8
Fluorene	12.6
Phenanthrene	15.9
Anthracene	15.9
Fluoranthene	19.6
Pyrene	20.6
Benzo(a)anthracene	20.6
Chrysene	24.7
Benzo(b)fluoranthene	28.0
Benzo(k)fluoranthene	28.0
Benzo(a)pyrene	29.4
Dibenzo(a,h)anthracene	36.2
Indeno(1,2,3-cd)pyrene	36.2
Benzo(ghi)perylene	38.6

[a]Source: "Development and Application of Test Procedures for Specific Organic Toxic Substances in Wastewaters, Category 9-PAHs", Report for EPA Contract 68-03-2624 (in preparation).

[b]GC conditions: Chromosorb W-AW-DMDCS 100/120 mesh coated with 3% OV-17, packed in a 6' x 2 mm ID glass column, with nitrogen carrier gas at 40 ml/min flow rate. Column temperature was held at 100°C for 4 minutes, then programmed at 8°/minute to a final hold at 280°C.

7.1 Quality Control

7.1.1 Field Blanks and Controls

Prior to a field sampling trip, enough blanks and controls are prepared to equal 10% each (2 minimum) of the anticipated number of field samples. Blanks consist of an empty sampling container. Controls consist of 15 g of washed hair spiked at 100-150 ng with the compounds listed in Table 3. These blanks and controls are carried to the field and receive the same handling as the field samples. Workup and analysis of field blanks and controls is interspersed with the field samples on a regular basis. This method allows assessment of sample storage stability.

Table 4 presents a typical set of blanks and controls for QC on a field trip where 9 hair samples are to be collected.

7.1.2 Procedural Blanks and Controls

With each set of samples, a procedural blank is run. This consists of a Soxhlet apparatus and thimble which is extracted under the same conditions as the samples. These blanks are designed to detect artifacts from dirty glassware, laboratory atmosphere intrusion, and other sources.

7.2 Quality Assurance

Both internal and external QA procedures are to be followed. Internal QA procedures assure the continuity and consistency of the data. External QA procedures (interlaboratory checks) verify or dispute the accuracy of the data being generated in the primary laboratory.

7.2.1 Internal Quality Assurance

7.2.1.1 Supervision and Monitoring of Activities

There are three levels of quality assurance (QA). The primary quality assurance is the person conducting the sampling and/or analysis. This person must be aware of their actions, observe events which may affect the data, and maintain appropriate records. At the second level, the chemist's supervisor monitors their daily activities, reviews the notebook, checks data and calculations, and assists in "troubleshooting" problems. At the tertiary level, a QA coordinator interviews all personnel on the project. The interviews cover the operations they perform (precisely), the data they obtain, a spot-check of their calculations, and any problems they have had.

Table 3. POLYNUCLEAR AROMATIC HYDROCARBONS IN METHANOL SPIKING SOLUTION

Compound	Compound
Fluoranthene	Chrysene
Benzo(a)pyrene	Benzo(a)anthracene
Pyrene	

Table 4. HAIR QC SAMPLES FOR A TYPICAL SAMPLING TRIP

Sample type	Number	Comments
Field Blank	2	Freeze after preparation, carry to field, store with field samples.
Field Control	2	Store with field blanks
Lab Blank	2	Freeze after preparation, store in same freezer as field samples will be stored
Lab Control	2	Store with lab blanks

7.2.1.2 Documentation

From the initial preparation of a sample container through reporting of the analytical results, each sample is accompanied by a chain of custody sheet. Each person signs in the time of receipt, operations performed, and transmittal of the sample. This record is important for tracing a contaminant, bad standard, or some other problem.

Sampling Protocol Sheets--When a sample is collected, a sampling protocol sheet is filled in which contains a discrete sample code which identifies project number, area, site, locations, trip number, sampling period, and sample type. Also included are sample times, volumes, address, and other pertinent information. Where appropriate, a map is made to precisely identify the location.

Sample Log--Upon return from a sampling trip, each sample code is entered into a sample log book. This log is updated as samples proceed through workup and analysis. Thus, at a glance, project personnel can tell the status of each sample and find out how many are at different stages in the analytical protocol.

GC/MS Log--Each sample run by GC/MS is logged into a notebook, detailing analysis conditions, where the data are archived, and what hardcopy data has been produced.

7.2.2 External Quality Assurance

Samples will be analyzed by a designated QA laboratory. Samples, controls, and blanks will be shipped directly from the field to the QA laboratory for analysis. They will report the results to the primary laboratories for correlation with the primary data.

7.2.2.1 Selection of Samples for QA

Approximately 10% of the field samples (2 minimum) will be collected in duplicate for shipment to the QA laboratory. This selection process will be random with the following restrictions:

(1) The donor must consent to the additional hair collection.

(2) If any stratification of donors is known, purposive selection of QA donors may be used to get representative samples (e.g. occupationally exposed vs. "normal" individuals or upwind vs. downwind residents).

ANALYTICAL PROTOCOL: SAMPLING AND ANALYSIS OF ARSENIC, CADMIUM, AND LEAD IN SCALP HAIR (RTI)

1.0 Principle of Method

The analysis of arsenic, cadmium, and lead in scalp hair is carried out using atomic absorption spectrophotometry. Increased sensitivity is achieved by atomizing the metal in a graphite furnace with continuous deuterium background correction. Arsenic determinations are performed on solutions containing 1000 ppm nickel.

2.0 Range and Detection Limit

The minimum detection limit (MDL) and range for the metal assays in scalp hair are shown below.

Metal	MDL (μg/g)	Max. Conc. (μg/g)
Arsenic	0.08	3.0
Cadmium	0.06	3.0
Lead	0.25	30.0

Samples containing higher metal concentration may be analyzed by suitable dilution with 0.5% nitric acid. Dilution for arsenic determinations is made 0.5% nitric acid containing 1000 ppm.

3.0 Interferences

No known chemical or spectral interferences exist in the analysis of arsenic, cadmium, or lead in scalp hair.

4.0 Precision and Accuracy

The precision and accuracy associated with these analyses is a function of sample metal concentration. At the detection limit, the total measurement error is ± 100%. Based on the results of a previous study (1), the metal analyses are performed with the following precision (relative standard deviation). The total analysis error (estimated) is also given (2).

Metal	Range (μg/g)	Precision (% RSD)	Accuracy (% RE)	Estimated Total Error (%)
Arsenic	0.1-0.8	15	5-10	35-40
Cadmium	1-3	15	5-10	35-40
Lead	10-30	10	5-10	25-30

Table 5. SAMPLES TO BE COLLECTED AND SHIPPED TO QA LABORATORY FOR A TYPICAL SAMPLING TRIP

Sample	Number	Comment
Duplicate sample	5	Random selection unless prior information stratified subjects
Field blank	1	Ship with samples
Field Control	1	Ship with samples

6.2 Evaporation, Cleanup, and Concentration (3)

6.2.1 Washing of Hair Samples

The total hair sample is placed on a clean sheet of paper, cut into four approximately equal sections with a pair of stainless steel scissors and then placed in an acid-washed 100 ml beaker.

The hair sections are covered with 50 ml of a 1:1 methanol/ether mixture and held 30 minutes at room temperature with occasional stirring. This operation is successful in removing fat-soluble surface contaminants.

The supernatant liquid is decanted and the hair cleansed with approximately 50 ml of a 10% Prell solution. This is carried out by mixing the contents of the beaker in an ultrasonic bath for 30 minutes. A square section of panty hose, secured with a rubber bank, is placed over the beaker and the detergent mixture decanted through the hose. The hair is rinsed repeatedly with deionized water, until the rinse water is suds-free.

To insure complete removal of all surface contaminants, the Prell washing and the deionized water rinsing operations are repeated. The sample is then dried overnight in a 100°C oven. At this point, the hair material is further sectioned into a 1 to 2 cm pieces and a 250 mg portion is weighed for digestion and subsequent analysis.

6.2.1 Digestion of Hair Samples

The weighed hair sample is added to a predigested beaker and heated in 10 ml of conc. HNO_3. A watchglass is placed on each beaker and the total heated at 85-90°C for 3 hours.

The watchglass is removed and the solution evaporated to 1-2 ml. The residue is transferred to a 10 ml volumetric flask and diluted to the mark with 0.5% HNO_3.

Instrumental

A Perkin-Elmer Model 403 Spectrophotometer, equipped with a HGA-2000 furnace attachment with deuterium background correction is used for this analysis. An arsenic electrodeless discharge lamp is used as the light source and the furnace atomization response traced on a Perkin-Elmer Model

5.0 Apparatus and Reagents

A commercially available stock solution containing 1000 ppm metal is used for the preparation of the calibration standards. The concentrated nitric acid is reagent grade quality and the deionized water used in this study is prefiltered and subjected to the action of an activated carbon cartridge and two sequential ion exchange units.

The glassware used for sample workup and the preparation of the calibration solutions must be subjected to a nitric acid cleaning protocol.

All volumetric flasks should be soaked overnight in 20% nitric acid, rinsed with deionized water, soaking for an additional 15-18 hours in a 5% nitric acid bath, followed by a copious deionized water rinse. The flasks are completely filled with 0.5% nitric acid and stored in this manner. Prior to use, each flask is emptied and rinsed well with deionized water. Pipets are soaked in 5% nitric acid, rinsed well with deionized water, air-dried, and stored in a clean, dust-free environment.

All beakers used for hair digestions require additional pretreatment. Clean beakers (soaked in 20% and 5% nitric acid) are "predigested" by heating 10-25 ml of conc. nitric to reflux (with watchglass), cooling, and discarding the acid. The beakers are rinsed thoroughly with deionized water and used for a sample digestion within 30 minutes. The beakers are never allowed to go dry.

Sample cups for the graphite furnace autosampler may be made of polystyrene or Teflon. The former type requires overnight soaking in 1% nitric acid and followed by rinsing with deionized water. The latter type may be soaked overnight in 20% nitric acid, rinsed, and dried in a 105°C oven.

Nickel chloride hexahydrate is used for adjusting the nickel concentration in all samples and standards to 1000 ppm.

6.0 Procedures

6.1 Collection of Samples

Full strand hair samples are collected with a comb and a pair of thinning shears. The collection is placed in a zip-loc bag, labeled, and all pertinent information recorded on a protocol sheet.

Calibration Range (spike concentration):

Arsenic - 0.0 to 3.0 μg/g

Cadmium - 0.0 to 3.0 μg/g

Lead - 0.0 to 30.0 μg/g

An exponential of the form $y = Ae^{bx}-M$ provides the best representation of the analytical curve. The values of the x,y calibration pairs are entered into a Monroe Calculator Model 1800 programmed to regress the data to the exponential and to provide value for the constants, A, b, and M.

Sample peak heights are measured manually and expressed in units of millivolts. The standard additions calibration constants, A, b, and M are entered into the storage banks of a Texas Instrument Calculator Model 57 and the metal concentration results obtained by keying in peak height data. Sample peak measurements and concentration results are recorded on a calculation worksheet.

$y = Ae^{bx}-M$, μg/g

$y = y_s$ (metal conc. in sample relative to control hair) +
y_c (metal conc. in control hair)

$y_s = Ae^{bx_s}-M$

$y_c = -y_o = -(Ae^{bx_o}-M)$

$y = (Ae^{bx_s}-M) - (Ae^{bx_o}-M)$

$y = A(e^{bx_s}-e^{bx_o})$

y = metal concentration in sample μg/g

x_o = reagent blank peak height, mv

y_o = standard additions metal concentration corresponding to reagent blank signal ($y_o \leq 0$),

$y_c = -y_o$ = metal concentration in control hair, μg/g

x_s = sample peak height, mv,

y_s = concentration differential between sample and control hair. This value may be either negative ($y < y_c$) or positive ($y_c < y$)

056 recorder. An AS-1 Autosampler may be used to increase throughput and/or to improve peak reproducibility and sensitivity.

Arsenic: Wavelength - 193.7 nm
Gas Interrupt (N_2) Auto
Furnace Cycle Conditions -
Dry: 200°C for 30 sec.
Char: 1500°C for 35 sec.
Atomize: 2500°C for 6 sec.
Injection Volume - 20 µl

Cadmium: Wavelength - 228.8 nm
Gas Interrupt (N_2) - Manual
Furnace Cycle Conditions -
Dry: 200°C for 20 sec.
Char: 400°C for 20 sec.
Atomize: 1900°C for 6 sec.
Injection Volume - 20 µl

Lead: Wavelength - 217.0 nm
Gas Interrupt (N_2) - Auto
Furnace Cycle Conditions -
Dry: 200°C for 20 sec.
Char: 500°C for 20 sec.
Atomize: 2000°C for 20 sec.
Injection Volume - 20 µl

6.4 Qualitative and Quantitative Analysis

6.4.1 Qualitative Analyses

N/A

6.4.2 Quantitative Analysis

The instrument is calibrated with a digested control of hair material spiked at four different concentrations, an unspiked control hair, and a reagent blank.

7.1.2.3 Duplicate Injections

Reproducibility of peak response is continuously monitored during sample analysis. All standard and sample solutions receive two successive injections into the graphite furnace. Signal agreement between the duplicate injections is evaluated according to the following criterion:

First Signal % of Full Signal	% Maximum Permissible Variation (% MPV)	Permissible Range of Second Signal, % of Full Scale
90	± 4%	86-94
80	± 5%	76-84
70	± 6%	66-74
60	± 7%	56-64
50	± 8%	46-54
40	± 10%	36-44
30	± 13%	26-34
20	± 20%	16-24
10	± 30%	7-13
5	± 60%	2-8
2	±100%	0-4

If the second injection gives a signal which falls outside the permissible range, a third injection is performed. The peak measurement not in agreement with the matching pair is discarded.

All calibration and sample calculations are based on the mean of the duplicate determinations.

7.1.2.4 Standard Checks

Instrument performance is monitored during each analytical run. After the analysis of every 12-16 samples one of the calibration standards is reinjected into the furnace. The standard which most closely matches the sample peak heights is selected as the check solution. A metal concentration is calculated for the check standard based on its peak height during the calculation run. Similar calculations are carried out for each check response and the observed changes in metal concentration expressed in terms of standard deviation units (SDU).

$$SDU = \frac{(\text{Calibration value} - \text{Check Value})\ 100}{(\text{Calibration Value})(\%\ \text{RSD})}$$

Arsenic calculations may be based on a linear regression of the calibration data.

7.0 Quality Assurance Program

An on-going quality assurance program is required to assure the data quality. Quality control (QC) procedures determine artifacts, losses, etc., through a system of blanks and controls. Quality assurance (QA) procedures monitor the execution of the procedure and check data interpretations and calculations.

7.1 Quality Control

7.1.1 Field Controls

Prior to field sampling, several control hair collections (10% of anticipated number of field samples) are obtained. Each hair sample is cut into small pieces, mixed well, and divided into two portions. One aliquot is placed in a container identical to that used for field samples, sent to the site, and subjected to the same handling and storage conditions as field samples. The other aliquot is stored at RTI in a dust-free environment. On receipt of samples at RTI, both portions of the control hair collection are worked up and analyzed as a part of each hair analytical run. Within the precision of the assay, the difference in calculated metal concentrations of the two control hair aliquots is a measure of the contamination/loss during field storage, and transit to RTI.

7.1.2 Internal Quality Control

7.1.2.1 Calibration Standards and Blanks

The instrument is calibrated before each analytical run with four standard solutions and a reagent blank. Evidence of contamination or instrument malfunction is evident at this time. Such problems are resolved before initiating sample analysis.

7.1.2.2 Conditioning of Graphite Tube

Before each analytical run, the graphite tube is conditioned by injecting 10 to 20 20 µl aliquots of one of the calibration standards. This operation insures acceptable precision during sample analysis.

Sample Log

Upon return from a sampling trip, each sample code is entered into a sample log book. This log is updated as samples proceed through workup and analysis. Thus, at a glance, project personnel can tell the status of each sample and find out how many are at different stages in the analytical protocol.

Instrument Log

Each sample analysis is logged into a notebook, detailing analysis conditions.

7.2.2 External Quality Assurance

Samples will be analyzed by a designated QA laboratory. Samples, controls, and blanks will be shipped directly from the field to the QA laboratory for analysis. They will report the results to the primary laboratory for correlation with the primary data.

7.2.2.1 Selection of Samples for QA

Approximately 10% of the field samples (2 minimum) will be collected in duplicate shipment to the QA laboratory. This selection process will be random with the following restrictions:

(1) The donor must consent to the additional collection.

(2) If any stratification of donors is known, purposive selection of QA donors may be used to get representative samples (e.g. occupationally exposed vs "normal" individuals or upwind vs downwind residents).

7.2.2.2 QC Samples

Along with the QA duplicate samples, at least one QC control and one QC blank must be included with the QA samples. An example is shown in Table 1 for a trip collecting 50 samples.

7.2.2.3 Sample Codes

All samples shipped to the QA laboratory should be encoded so that the laboratory cannot discern between samples and controls. This "blind" protocol will add validity to the results.

The analysis is under control when the SDU < 2.0. Standard checks which indicate a variation in peak response greater than 2.0 SDU are unacceptable. In this event, the graphite tube is changed, conditioned, and the system recalibrated. Quality control charts are graphed to show this change in instrument performance with time.

7.2 Quality Assurance

Both internal and external QA procedures are to be followed. Internal QA procedures assume the continuity and consistency of the data. External QA procedures (interlaboratory checks) verify or dispute the accuracy of the data being generated in the primary laboratory.

7.2.1.1 Supervision and Monitoring of Activities

There are three levels of quality assurance (QA). The primary quality assurance is the person conducting the sampling and/or analysis. This person must be aware of their actions, observe events which may effect the data, and maintain appropriate records. At the second level, the chemist's supervisor monitors their daily activities, reviews the notebook, checks data and calculations, and assists in "troubleshooting" problems. At the tertiary levels, a QA coordinator interviews all personnel on the project. The interviews cover the operations they perform (precisely), the data they obtain, a spot-check of their calculations, and any problems they have had.

7.2.1.2 Documentation

Chain of Custody

From the initial preparation of a sample container through reporting of the analytical results, each sample is accompanied by a chain of custody sheet. Each person signs in the time of receipt, operations performed, and transmittal of the sample. This record is important for tracing a contaminant, bad standard, or some other problem.

Sampling Protocol Sheets

When a sample is collected, a sampling protocol sheet is filled in which contains a discrete sample code which identifies project number, area, site, locations, trip number, sampling period, and sample type. Also included are sample times, volumes, addresses, meteorology, and other pertinent information. Where appropriate, a map is made to precisely identify the location.

Table 1. SAMPLES TO BE COLLECTED AND SHIPPED TO QA LABORATORY FOR A TYPICAL SAMPLING TRIP

Sample	Number	Comment
Duplicate sample	5	Random selection unless prior information stratifies subjects
Field blank	1	Ship with samples
Field control	1	Ship with samples