C-231

~~RESEARCH ON 301H MONITORING MATTERS BEYOND THE CAPABILITIES OF THE CITY LABORATORIES~~

FINAL REPORT

TO

CITY OF LOS ANGELES

~~DEPARTMENT~~ OF SANITATION

Bureau

FROM

SOUTHERN CALIFORNIA COASTAL WATER RESEARCH PROJECT

646 W. PACIFIC COAST HIGHWAY

LONG BEACH, CALIFORNIA 90806

29 APRIL, 1988

Development of 301H Monitoring Sampling and Analysis Techniques Utilizing a Buoyed Mussel Syste

INTRODUCTION

The City of Los Angeles Department of Sanitation has applied for a 301H waiver to the US Environmental Protection Agency. If this waiver is granted, a significant increase in the City's marine monitoring program will be instituted in lieu of going to complete secondary treatment of its wastes. As part of this new marine monitoring program the Hyperion laboratory may be required to buoy clean mussels in and around the 5-mile outfall for the purpose of "biological monitoring- to integrate and thereby demonstrate exposure to pollutants over time" and subsequently to analyze them for the EPA list of "Priority Pollutants" following the protocol developed by Tetra Tech published May, 1986. To date the Hyperion laboratory personnel have had little experience in deploying and retreiving mussel buoys or using the Tetra Tech protocols to analyze for priority pollutants, except for trace metals. The purpose of this research was to assist the Hyperion scientists in preparing for satisfying these requirements.

OBJECTIVES

There were two main objectives to this project. First, as Task I, SCCWRP personnel would develop, deploy, and retreive a mussel buoy system and transfer this technology to biologists at the Hyperion laboratory. Second, as Task II, SCCWRP chemists would obtain the apparatus necessary to do head-space analysis of mussel tissues for volatile organics and they would assist the Hyperion chemists in developing the protocols necessary to analyze for base/neutral and acid extractable organics in mussel tissues. By mutual agreement, as much of the work as possible on these two tasks would be done by Hyperion scientists so that the maximum amount of experience can be gained under SCCWRP's supervision prior to initiating a 301H monitoring program.

RESULTS

TASK I- Harold Stubbs developed a sonar-release buoy system to hold bags of mussels at selected depths for extended periods of time (Appendix A) and aquired the necessary equipment. The main components were a Helle Model 5204 sonar-release system, destroyer chain for anchors, Department of Fish and Game-type nylon net mussel bags, and floats and line sufficient for the weight and depths of deployment. This system was assembled and completely tested by SCCWRP before it was ready for use.

Sources of clean mussels for transplant were investigated with Catalina, Point Dume, Point Sal, Bodega Bay and Puget Sound being considered. Catalina was selected because preliminary data indicated that trace metal and chlorinated hydrocarbon concentrations were within an acceptable range, and this site was logistically the most convenient.

Next, past mussel biomonitoring data was reviewed and it was determined that at least 5 replicates were needed for each sample to allow optimum ability to separate differences in accumulation rates from random variations. The optimum duration of exposure was determined to be bewtween 30 and 60 days to allow for maximum time to accumulate while not exposing the buoy system to unnecessary risk of loss or damage..

Finally, with the aid of Hyperion biologists, mussels were collected from the back side of Catalina Island. Subsamples were taken for T0 (Time = 0) analysis and the remainder were deployed near the 5-mile outfall on 22 August, 1985. Five bags of 20 mussels each were placed at depths of 40', 120', and 170' and were successfully retrieved on 24 October, 1985. Mussels were then immediately removed from the bags placed on dry ice and transported to the SCCWRP laboratory where they were frozen at -20C until dissection and analysis.

TASK II- Laboratory experiments at SCCWRP were performed to determine the best method for pooling samples for both trace metal and organics analysis during dissection and homogenization. The recommended technique was to dissect on Teflon sheets in a clean atmoshpere using carbon-steel bladed scalpels followed by homogenization using a Brinkmann Polytron equipped with a titanium blade. Homogenized samples could then be stored frozen at -20 C in acid-washed/kilned glass jars with Teflon liners until analysis except the volatile organics samples which were placed into clean VOA vials. These precautions reduced the potential for cross-contamination of trace metals with organics and vise versa. This method was then applied to obtain enough tissue for complete priority pollutant analysis from 5 replicates of 20 mussels from each exposure depth as well as the T0 samples.

The method proposed for volatile organics analysis was part of the priority pollutant technique reported by Tetra Tech to the EPA in May, 1986 which was a head-space analysis technique (Appendix B). Since neither SCCWRP nor Hyperion chemists had actually used this technique it involved spending a considerable amount of time on its development. The proper apparatus was constructed and tested at SCCWRP and then transported to Hyperion where personnel from both agencies worked on the development using Hyperion instrumentation. Eventually, spike-recovery tests were completed and the samples from the buoy experiment were analyzed for priority pollutant volatile organics. Results (Table 1) from these analyses indicated no significant accumulation of the volatile orgaincs. However, due to the presence of solvents getting into the samples from lab air, the detection limits for some of these compounds were quite high.

For the acid and base/neutral extractable organics, the Hyperion laboratory was already experienced through the use of similar techniques. Consequently, all that was needed from the SCCWRP chemists was to update them with the Tetra Tech protocol. The most difficult part of this technique involved the clean-up of the

samples prior to analysis using Bio-Beads. This technique was tested in the SCCWRP laboratory as well as attempted by Hyperion chemists and proved to be very difficult as well as non-reproducible, therefore it was recommended that a silica gel clean-up be used instead (Appendix C). The buoyed mussel samples for acid and base/neutral anaylsis were delivered to the Hyperion lab and are pending analysis by Hyperion chemists along with the analysis for trace metals.

CONCLUSIONS

The procedures used in "biomonitoring for pollutant exposure" using buoyed mussels were developed and the technology was transferred to the Hyperion scientists. This incorporated the mussel buoy and retrieval system, determination of a suitable collection site for clean mussels, a clean dissection and homogenization technique useful for combining trace metal and organics samples, a head-space tecnique for volatile organics, and a recommendation for using silica gel for the clean-up of acid and base/neutral samples. Using these techniques, mussels were exposed for approximately 60 days, however, at the time of this report very little chemical data was available. Therefore, it could not be determined if there was a significant bioaccumulation of any priority pollutants during this exposure.

TABLE 1. Results (ug/g) from the analysis of mussels buoyed at the Hyperion 5-mile outfall for volatile org priority pollutants using a head-space analysis technique.

COMPOUND	BLANK	T0 Rep1	T0 Rep2	T0 Rep3	40 Rep1	120 Rep1	120 Rep2	170 Rep1	170 Rep2
Bromomethane	<5	<15	96	<15	<15	<15	<15	<15	<15
Dichlorodifluorometh	<5	<15	<15	<15	<15	<15	<15	<15	<15
Chloroethane	<5	23	<10	36	<15	21	46	<15	<15
Methylene Chloride	<5	<15	74	<15	<15	20	<15	<15	<15
Trichlorofluorometh	890	<15	<15	290	<15	91	120	<15	86
1,1-Dichloroethene	<5	<15	55	11	<15	<10	<10	<15	<15
1,1-Dichloroethane	<5	<15	<15	<10	<15	<15	<15	<15	<15
1,2-Dichloroethene	<5	<15	<10	<10	<15	<15	<15	<15	<15
Chloroform	5400	5600	630	330	<15	99	200	120	120
1,2-Dichloroethane	<5	<15	<15	<15	<15	<15	<15	<15	<15
1,1,1-Trichloroethane	65	310	780	460	<15	37	40	48	23
Carbon Tetrachloride	<5	310	<15	<15	<15	<10	<15	<15	8
Bromodichloromethane	<5	<15	<15	<15	<15	<15	<15	<15	<15
1,3-Dichloropropene	<5	<15	<10	<15	<15	<15	<15	<15	<15
Trichloroethene	<5	150	33	29	<15	10	10	<10	<10
Benzene	53	<15	490	350	<15	100	170	260	250
Dibromochloromethane	<5	<15	<10	<10	<15	<15	<10	9	<15
2-Chloroethylvinyleth	<5	<15	13	<15	<15	<15	<15	<15	<15
Bromoform	<5	<15	<10	10	<15	<10	<10	<15	<10
Tetrachloroethene	21	16	270	170	<15	<10	10	39	35
Toluene	160	140	520	240	<15	160	120	110	75
Chlorobenzene	<5	<15	<10	<15	<15	<15	<15	<10	<15
Ethylbenzene	520	61	100	140	<15	<15	<15	79	70

APPENDIX A

6/14/84

SCCWRP Proposal for

MUSSEL-HOLDING BUOYS

to be used for 301h monitoring

Plan I. Sonar-release recovery method

Buoy requirements

This buoy is designed to hold bags of mussels at selected depths (10, 25, 50 m) in 60 m of water for extended periods. The buoy must remain at the point where it is installed and the mussel bags must remain within 2 vertical meters of their original depth. The mussels are to be retrieved conveniently at three month intervals. The buoy must be safely installable/retrievable by a small ship equipped as specified. No divers are to be used.

Mussel requirements

Mussels from Bodega Bay area are to be used as collecting organisms. They will be handled in accordance with methods described elsewhere. The weight of mussel soft tissue at each depth required for chemical analysis is about 500 grams. Since the wet weight of soft tissue per mussel is about 50 grams, three bags containing 25 mussels each will give three many the amount of tissue required (we note that there is little value to replication of samples composited from 10 animals).

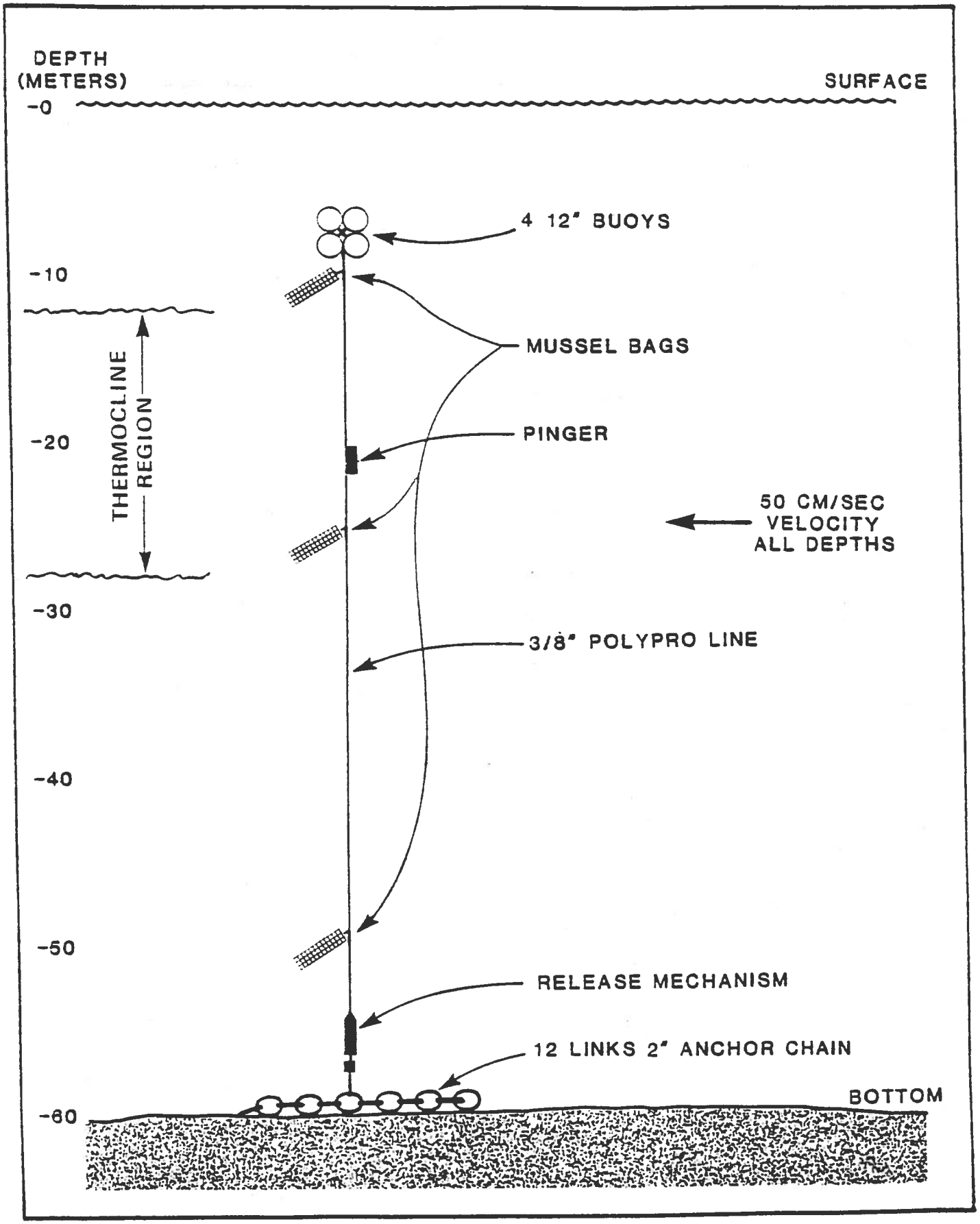
Ship requirements

- Large enough to do the job
- Loran C
- Echo sounder
- Winch capable of handling 400 lbs in a seaway

Design requirements

Based on our experience with many types of buoys for various purposes we propose a single line taut-moored buoy. The characteristics for which we have designed this system are as follows:

1. 60 meter depth
2. Highest likely velocity in Santa Monica Bay 50 cm/second thruout water column
3. Upper mussel bags not to go over 2 m below installed height
4. Anchor to remain in place with 100 pounds (37 kilos) horizontal drag
5. Drag increase of 25% because of marine growth on buoy line.
6. Means of confirming that buoy is in place without raising it. Also to be used for finding the array if release fails


DEPTH
(METERS)
SURFACE
-0
4 12" BUOYS
-10
MUSSEL BAGS
THERMOCLINE
REGION
-20
PINGER
50 CM/SEC
VELOCITY
ALL DEPTHS
-30
3/8" POLYPRO LINE
-40
-50
RELEASE MECHANISM
12 LINKS 2" ANCHOR CHAIN
BOTTOM
-60

Component characteristics and approximate costs

		cost per buo
Buoys (4)		
Lane Inst. Co. 12" heavy duty oceanographic floats #1011-H	$30. each or	$ 120.00
Lowering line		
1/2" poly pro yellow, twisted 600' coil		300.00*
Buoy line		
3/8' poly propelyene line, yellow, twisted 600' coils for $200		100.00
Mussel bags		
nylon mesh bags 3" x 30" Nylon Net Co. 9 bags per buoy @ $2.00		18.00
Anchor		
12 links of 2" anchor chain, 25lbs/link 300lbs @ 0.25/lb		75.00
Release		
Helle Engineering Co.		
Command Module 5148	$1,850*	
Release Module 5204	2,870	
10 mech release links	1,000	
	$5,720	
Pinger		
Helle Engineering Co.		
10 Battery 2250-1116 (6 mo. life)	$1,000*	
1 directional antenna 6501	2,950	
1 pinger/receiver	6,100	
	5,200	
Attachments to line		
electrical ties (nylon of bags to main lines thimbles, electrical tape		
	TOTAL	$11,533.00
		-5,100.00

*only one per group of buoys

Therefore, second and subsequent costs per system are $ 6,433.00

Method of installation

Connect buoys to each other and to pre-measured and marked buoy line. Attach mussel bags with nylon ties. Bring ship to site using loran. Launch buoy first and stream the anchor line. Run 1/2" line thru anchor and secure the bitter end on deck. Launch anchor and lower by slipping the standing end around a cleat. When anchor is on bottom, release bitter end and retrieve the slip line.

Retrieval

Push sonar release button, wait for surfacing (about 2 minutes). Come alongside buoy, grasp with boathook, attach snap-hook on end of BT winch wire. Hoist buoy aboard (use steadying lines if needed), Pull in line by hand. Remove mussel bags. Clean release mechanism, recock it, attach to new line and clean buoy. Launch the replacement buoy as above.

For the past year SCCWRP has been using Helle Engineering Release Module for our current meter program, to date we have 25 out of 25 releases.

We have used many methods of moorings in the ocean both deep and shallow and at best achieved 75% recovery--this is not acceptable, all information is lost as well as expensive instruments. Surface markers are an invitation to steal, subsurface markers on a single moor are hard to locate, trimoors take up too much room and are easily snagged by fishermen, anchors or gillnets.

We would propose the following.

1. Weights could be made of concrete in sizes that can be handled by one person and be joined together at sea to obtain desired anchor weight as this will be left on bottom when released.

2. Taut-line of 3/8" PolyPro will be used to secure mussel bags.

3. Two choices of float can be used 12" or 8". Perhaps 12" present too much of a drag on mussel line.

Our procedure for deployment is to first deploy floats and mussel line behind the boat then run 3/8" line through the anchor and secure bitter end on deck, lowering anchor to bottom by slipping standing end of line from cleat release bitter end and retrieve slip line; carefully recording Loran numbers and depth along with any visual landmark where possible.

Many areas selected for deployment are known fishing areas (hook and line, crab and lobster, gillnets) in shipping lanes. This should be avoided when possible.

Plan II. Recovery using line to marker buoy.

In this plan the mussel buoy is the same as that previously described except that the anchor is lighter. It is recovered by picking up a light line that connects the top of the mussel buoy to a Petersen yacht racing buoy. The arrangement shown in Figure 2 is the same as given in our original paper on mussel buoys (Dr. David Young in Marine Pollution Bulletin, August 1976) and used in EPA publication 600/4-83-000 REF 3941C, C. Weber, ed., 1984. Details of the Peterson Company's buoy is given in Figure 3 (their drawing).

The Peterson Company, 8271 Flight Avenue, Midway City, Californi 92655, 213/436-7941 - boat, 714/894-2523 - home, does not sell these buoys but they will install and remove them for $1800 and pick up, clean, return them once a year for $1320.

If the mussel buoy and the Petersen buoy are connected as shown, it is a simple matter to pick up the connecting line with a grapnel to begin recovery. The cost of the two recovery systems are similar; the choice depends on whether it is preferable to have a surface buoy that can be run down by ships (and used by fishermen as an mooring) to the entirely underwater system.

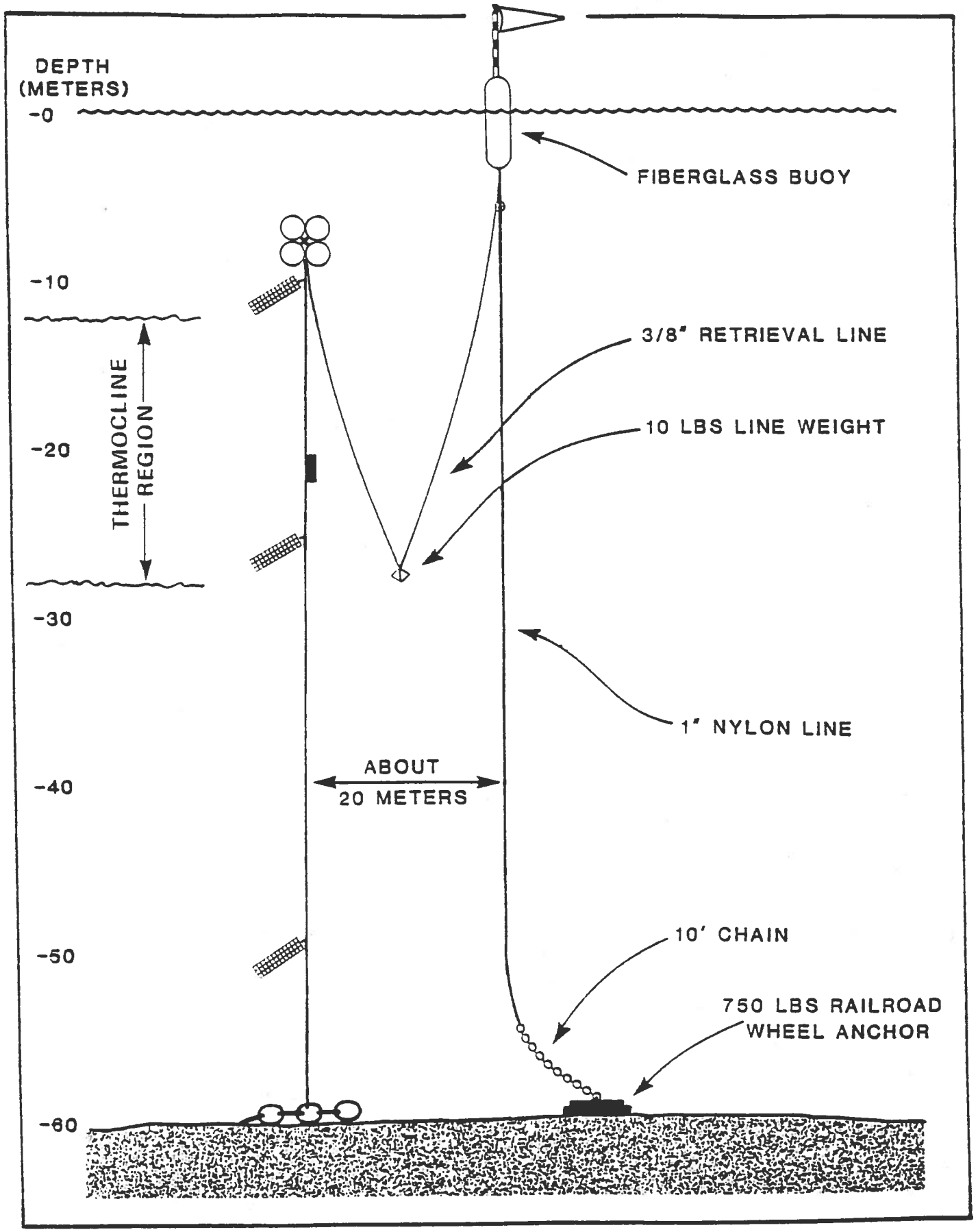


Figure 2. Alternate mussel monitoring buoy. Same as plan 1, but is retrieved using line from Petersen yacht racing buoy.

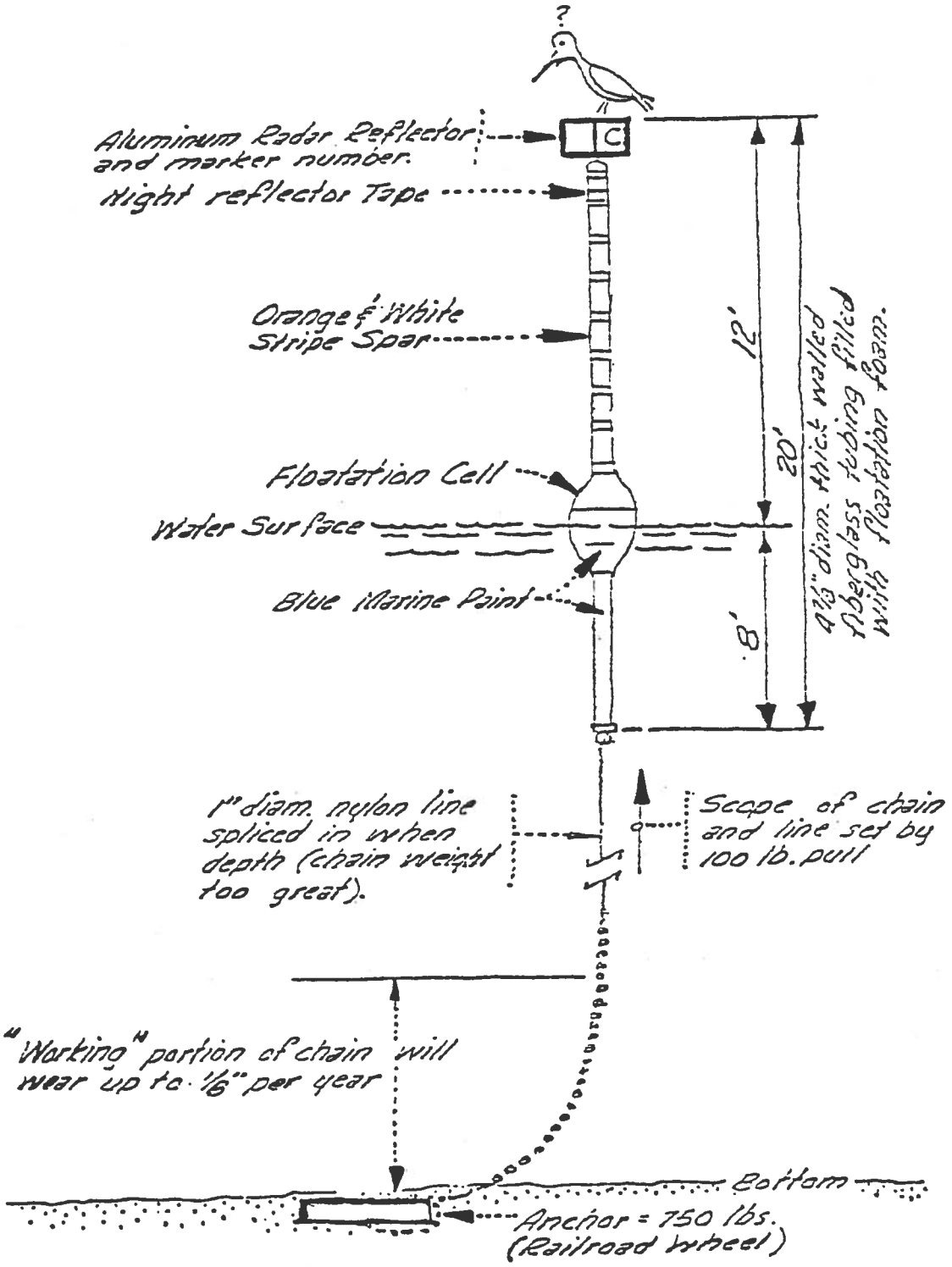


Figure 3. Petersen Yacht racing buoy.

APPENDIX B

EPA Contract No. 68-01-6938
TC 3953-03

Final Report

BIOACCUMULATION MONITORING GUIDANCE:

4. ANALYTICAL METHODS FOR U.S. EPA PRIORITY POLLUTANTS AND 301(h) PESTICIDES IN TISSUES FROM ESTUARINE AND MARINE ORGANISMS

for

Marine Operations Division
Office of Marine and Estuarine Protection
U.S. Environmental Protection Agency
401 M Street SW (WH-556M)
Washington, DC 20460

May, 1986

by

Tetra Tech, Inc.
11820 Northup Way, Suite 100
Bellevue, Washington 98005

SECTION II

ANALYSIS OF VOLATILE ORGANIC COMPOUNDS IN ESTUARINE AND MARINE TISSUES

CONTENTS

ANALYSIS OF VOLATILE ORGANIC COMPOUNDS IN ESTUARINE AND MARINE TISSUES

1.0 SCOPE AND APPLICATION

1.1 This method is designed to determine the volatile toxic organic pollutants associated with Clean Water Act Section 301(h) regulation [40 CFR 125.58(k) and (v)] and additional compounds amenable to purge and trap gas chromatography-mass spectrometry (GC/MS) (Table II-1).

1.2 The chemical compounds listed in Table II-1 can be determined in biological tissue samples collected from estuarine and marine environments by this method.

1.3 The detection limit of this method is usually dependent on the level of interferences rather than instrumental limitations.

Lower limits of detection (LLD) are established by analysts based on their experience with the instrumentation and with interferences in the sample matrix being analyzed. LLD are greater than instrumental detection limits because they take into account sample interferences. To estimate LLD, the noise level should be determined in the retention window for the quantitation mass of representative analytes. These determinations should be made for at least three field samples in the sample set under analysis. The signal required to exceed the average noise level by at least a factor of two should then be estimated. This signal is the minimum response required to identify a potential signal for quantification. The LLD is the concentration corresponding to the level of this signal based on calibrated response factors. Based on best professional judgment, this LLD would then be applied to samples in the set with comparable or lower interference. Samples with much higher interferences (e.g., at least a factor of two higher) should be assigned LLD at a multiple of the original LLD.

These LLD values may be less than the rigorously defined method detection limits specified in the revised "Guidelines Establishing Test Procedures for the Analysis of Pollutants" (40 CFR Part 136, 10/26/84). This latter procedure requires the analysis of seven replicate samples and a statistical determination of th emethod detection limit with 99 percent confidence. Data quantified between the LLD and the rigorous method detection limit are valid and useful in environmental investigations of low-level contamination, but have a lower statistical confidence associated with them than data quantified above the method detection limit.

The LLD are roughly 5-10 ppb (wet weight) with the exception of acrolein and acrylonitrile, which have not been thoroughly tested on tissue matrices with this method.

2.0 SUMMARY OF METHOD

2.1 Volatile organic compounds are vacuum extracted from a macerated, 5-g (wet wt) tissue sample and concentrated in a cryogenic trap cooled with liquid nitrogen (references 1 and 2). The cryogenic trap is then transferred to a conventional purge-and-trap device. The extract is diluted to a 5 mL volume with water and treated as an aqueous sample. In the purge-and-trap device, the volatile organic compounds are purged from the aqueous phase into a gaseous phase with an inert carrier gas. The volatile compounds are passed into a sorbent column and trapped. After purging is completed, the trap is backflushed and heated rapidly to desorb the compounds into a gas chromatograph (GC). The compounds are separated by GC and detected with a mass spectrometer (MS).

Analysis is carried out by GC/MS either according to the isotope dilution technique (U.S. EPA Method 1624 Revision B; reference 3) or U.S. EPA Method 624 (reference 4). Both of these methods were developed for water wastewater sample matrices. The isotope dilution technique, which requires spiking the sample with a mixture of stable isotope labeled analogs of the analytes, is the technique of choice because it provides reliable recovery data for

each analyte. Method 624 requires spiking samples with only three surrogate compounds and does not allow for recovery corrections. If uniformly high recoveries can be attained with Method 624, then addition of numerous labeled compounds (Method 1624B) and recovery corrections are unnecessary. However, until such performance can be demonstrated, Method 1624B provides a detailed and valuable assessment of analytical performance.

Hiatt (reference 5) proposed another vacuum distillation procedure that did not include a purge-and-trap device. In this technique, volatile organic compounds are transferred directly from a cryogenically cooled trap to a fused-silica capillary column for GC/MS analysis. This capillary column technique allows for optimum resolution and rapid conditioning between samples. However, the performance of the technique has not been thoroughly tested (reference 6). A potential problem is that water can enter the capillary column and cause chromatographic problems or freeze, effectively plugging the column. Thus, Hiatt's original procedure (references 1 and 2), which has been tested more thoroughly, is recommended here.

Vacuum distillation is recommended rather than direct purge-and-trap extraction (i.e., without vacuum distillation) because the former technique has been demonstrated to allow for better recoveries of spiked compounds than the latter technique (reference 1; comparisons based on similar spiking levels).

2.2 Laboratories may use alternative analytical procedures provided that evidence of performance comparable to the recommended procedure is provided.

3.0 INTERFERENCES

3.1 Impurities in the purge gas, organic compounds out-gassing from the plumbing upstream of the trap, and solvent vapors in the laboratory account for the majority of contamination problems. The analytical system is demonstrated to be free from interferences under conditions of the analysis by analyzing blanks initially and with each sample lot (samples analyzed

on the same 8 h shift), as described in Sect. 9.3. Common laboratory solvents (e.g., methylene chloride) are often contaminants in volatiles analyses.

3.1.1 Newly packed traps should be conditioned overnight at 170°-180°C by backflushing with an inert gas at a flow rate of 20-30 mL/min. Traps must be conditioned daily for a minimum of 10 min before use.

3.2 There is potential for ambient contamination of samples and extracts when using vacuum and cryogenic concentration techniques. Care must be taken to eliminate any leaks in the vacuum extraction and concentration device. A critical source of potential contamination is pump oil vapor and exhaust from the vacuum pump; this should not be a problem if the system is properly sealed. A cold trap is placed between the vacuum pump and concentration trap to prevent condensation of pump oil vapors in the concentration trap (Figure II-1). All materials in the vacuum extraction and concentration device that contact the sample and its vapors must be made of stainless steel and/or borosilicate glass. All connections and seals must be free of elastomers or grease that either outgas or allow penetration of ambient contaminant vapors.

3.3 Samples can be contaminated by diffusion of volatile organic compounds (particularly methylene chloride) through the bottle seal during shipment and storage. A field blank prepared from reagent water and carried through the sampling and handling protocol serves as a check on such contamination.

3.4 Contamination by carry-over can occur when high level and low level samples are analyzed sequentially. When an unusually contaminated sample is analyzed, it should be followed by analysis of a reagent water blank to check for carry-over. Because the transfer lines, trap, and other parts of the system can retain contaminants and interferences, frequent bakeout and purging of the entire system may be required.

4.0 SAFETY

4.1 The toxicity or carcinogenicity of each compound or reagent used in this method has not been precisely determined; however, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of data handling sheets should also be made available to all personnel involved in these analyses. Additional information on laboratory safety can be found in references 7-9.

4.2 The following compounds covered by this method have been tentatively classified as known or suspected human or mammalian carcinogens: benzene, carbon tetrachloride, chloroform, and vinyl chloride. Primary standards of these toxic compounds should be prepared in a hood, and a NIOSH/MESA-approved toxic gas respirator should be worn when high concentrations are handled.

4.3 The following safety measures must be employed when handling cryogenic and vacuum systems:

4.3.1 Liquid nitrogen (LN_2) must not be allowed to contact flesh since it will cause extreme frostbite and deaden (kill) tissues.

4.3.2 The concentrator and cold traps must never be closed off or sealed after allowing any concentration of liquid air. The liquid air will vaporize, resulting in tremendous pressure build up and explosive damage to the vacuum system. Always vent any vessel <u>immediately</u> after removing the cryogenic or LN_2 bath. Wear safety goggles when working with cryogenic and vacuum systems.

5.0 APPARATUS AND EQUIPMENT

5.1 Sample Handling Equipment

5.1.1 Stainless steel spatula, rinsed with methanol and oven-dried at 150° C.

5.1.2 Sample vessel - Pyrex flask with 15 mm O-ring connector, washed with detergent and rinsed with distilled water and oven-dried at 450° C.

5.1.3 O-ring, Buna N, sonicated with 50 percent methanol/water then dried by vacuum at 60° C.

5.1.4 Tissue homogenizer (e.g., Tekmar Tissuemizer, Tekmar Co., Cincinnati, OH) - must be free of volatiles and solvents before use.

5.2 Apparatus for Vacuum Distillation and Cryogenic Concentration (Figure II-1).

5.2.1 Vacuum pump, capable of achieving 10^{-3} Torr and 25 L/min.

5.2.2 Vaccum/pressure gauge - with a range of subatmospheric pressure to 10 psi.

5.2.3 Concentrator trap or purge flask, 25 mL capacity (Tekmar Part No. 14-0957-024 or equivalent) modified with 9 mm O-ring connectors.

5.2.4 Cold trap - glass trap (easily produced by glassblowing, Figure II-1) with O-ring fittings (e.g., Kontes 671750-009).

5.2.5 Transfer line, 1/4 in o.d. glass-lined stainless steel tubing. Lines should be kept as short as possible to minimize sample carryover.

5.2.6 Vacuum valves, Nupro B-4BKT or equivalent.

5.2.7 Dewar flasks, 665 mL or 1,000 mL, for liquid nitrogen bath.

5.2.8 Assorted compression fittings and graphite ferrules (Figure II-1).

5.2.9 Ultrasonic bath, Branisonic 12 or equivalent.

5.2.10 Heater tape - to wrap around stainless steel lines and valve bodies to maintain a temperature of 60° C.

5.2.11 Pinch clamps, Thomas - to secure O-ring connections.

5.3 Purge-and-Trap Device - capable of meeting specifications listed in U.S. EPA Method 1624 B (see below). Complete devices consisting of a purging device (the concentrator trap, Sect. 5.2.3), a Tenax/silica trap, and a desorber are commercially available (e.g., Tekmar Model LSC-2, Tekmar Co., Cincinnati, OH).

5.3.1 Trap - 25 to 30 cm x 2.5 mm i.d. minimum, containing the following:

5.3.1.1 Methyl silicone packing - one ±0.2 cm, 3 percent OV-1 on 60/80 mesh Chromosorb W, or equivalent.

5.3.1.2 Porous polymer - 15 ±1.0 cm, Tenax GC (2,6-diphenylene oxide polymer), 60/80 mesh, chromatographic grade, or equivalent.

5.3.1.3 Silica gel - 8 ±1.0 cm, Davison Chemical, 35/60 mesh, grade 15, or equivalent.

5.3.2 Desorber - should heat the trap to 175 ±5° C in 45 sec or less. The polymer section of the trap should not exceed 180° C, and the remaining sections should not exceed 220° C.

5.3.3 Commercial purge and trap devices are easily coupled to GC systems.

5.4 GC/MS (Gas Chromatograph-Mass Spectrometer) System.

5.4.1 GC - should be linearly temperature programmable with initial and final temperature holds.

5.4.2 GC column - 6 ft long x 0.1 in i.d. (stainless steel or glass) packed with 1 percent SP-1000 on Carbopak B, 60/80 mesh or equivalent.

5.4.3 MS - 70 eV electron impact ionization; capable of repeatedly scanning from 20 to 250 amu every 2 to 3 sec.

5.4.4 GC/MS interface - GC to MS interfaces constructed of all-glass or glass-lined materials are recommended. Glass can be deactivated by silanizing with dichloro-dimethyl silane.

5.5 Data System - should collect and record MS data, store mass intensity data in spectral libraries, process GC/MS data and generate reports, and should calculate and record response factors.

5.5.1 Data acquisition - mass spectra should be collected continuously throughout the analysis and stored on a mass storage device.

5.5.2 Mass spectral libraries - user created libraries containing mass spectra obtained from analysis of authentic standards should be employed to reverse search GC/MS runs for the compounds of interest.

5.5.3 Data processing - the data system should be used to search, locate, identify, and quantify the compounds of interest in each GC/MS analysis. Software routines should be employed to compute retention times and extracted ion current profile (EICP) areas. Displays of spectra, mass chromatograms, and library comparisons are required to verify results.

5.5.4 Response factors and multipoint calibrations -- the data system should be used to record and maintain lists of response factors (response ratios for isotope dilution) and generate multi-point calibration curves.

Computations of relative standard deviation (coefficient of variation) are useful for testing calibration linearity.

5.6 Other Materials

5.6.1 Syringe, 10 uL ± 1 percent of volume.

5.6.2 Syringe, 50 uL ± 1 percent of volume.

5.6.3 Syringe, 5 mL ± 1 percent of volume, gas-tight with shut-off.

5.6.4 Bubble flowmeter.

6.0 REAGENTS AND CONSUMABLE MATERIALS

6.1 Reagent Water

6.1.1 Reagent water is defined as water free of interferences (i.e., interferents are not observed at the detection limits of the compounds of interest).

6.1.2 Prepare water by boiling 1 L of freshly distilled water down to 900 mL and transferring the water to a 1-L volumetric flask that has been modified by replacing the ground glass joint with a 15-mm i.d., Buna-N O-ring connector.

6.1.3 Connect the flask to the distillation apparatus at the sample chamber site and evacuate for 15 min while continuously agitating the flask in an ultrasonic cleaner.

6.1.4 After evacuation, release an inert gas (N_2 or He can be used) into the flask until equilibrium is obtained, then seal with a cap made from a Buna-N O-ring connector.

6.2 Methanol - pesticide quality or equivalent.

6.3 Standard Solutions - purchased as solutions or mixtures with certification as to their purity, concentration, and authenticity, or prepared from materials of known purity and composition. If compound purity is 96 percent or greater, the weight may be used without correction to calculate the concentration of the standard.

6.4 Preparation of Stock Solutions - prepare in methanol using liquid or gaseous standards per the steps below. Observe the safety precautions given in Sect. 4.

6.4.1 Place approximately 9.8 mL of methanol in a 10 mL ground glass stoppered volumetric flask. Allow the flask to stand unstoppered for approximately 10 min or until all methanol-wetted surfaces have dried. In each case, weigh the flask, immediately add the compound, then immediately reweigh to prevent evaporation losses from affecting the measurement.

6.4.1.1 Liquids - using a 100 uL syringe, permit two drops of liquid to fall into the methanol without contacting the neck of the flask. Alternatively, inject a known volume of the compound into the methanol in the flask using a micro-syringe. With the exception of 2-chloroethylvinyl ether, stock standards of compounds that boil above room temperature are generally stable for at least 4 wk when stored at 40 C.

6.4.1.2 Gases (chloromethane, bromomethane, chloroethane, vinyl chloride) - fill a valved 5 mL gas-tight syringe with the compound. Lower the needle to approximately 5 mm above the methanol meniscus. Slowly introduce the compound above the surface of the meniscus. The gas will dissolve rapidly in the methanol.

6.4.2 Fill the flask to volume, stopper, then mix by inverting several times. Calculate the concentration in mg mL (ug uL) from the weight gain (or density if a known volume was injected).

6.4.3 Transfer the stock solution to a Teflon sealed screw-cap bottle. Store, with minimal headspace, in the dark at -10 to -20° C.

6.4.4 Prepare fresh standards weekly for the gases and 2-chloroethylvinyl ether. All other standards are replaced after 1 mo, or sooner if comparison with check standards indicates a change in concentration of over 10 percent. Quality control check standards that can be used to determine the accuracy of calibration standards are available from the U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio.

6.5 Labeled Compound Spiking Solution - from stock standard solutions prepared as above, or from mixtures, prepare the spiking solution to contain a concentration such that a 5-10 uL spike into each 5 mL sample "extract", blank, or aqueous standard analyzed will result in a concentration of 10 ng/mL of each labeled compound. For the gases and for the water soluble compounds (acrolein, acrylonitrile), a concentration of 50 ng/mL may be used. Include the internal standards (Sect. 8.1.2) in this solution so that a concentration of 10 ng/mL in each sample, blank, or aqueous standard will be produced.

6.6 Secondary Standards - using stock solutions, prepare a secondary standard in methanol to contain each pollutant at a concentration of 250 ug/mL. For the gases and water soluble compounds (Sect. 6.5), a concentration of 1.25 mg/mL may be used.

6.7 Aqueous Calibration Standards - the concentrations of calibration solutions suggested in this section are intended to bracket concentrations that will be encountered during sample analysis that will not overload the analytical system. Use sufficient amounts of the secondary standard (Sect. 6.6) and reagent water to produce concentrations of 5, 10, 20, 50, and 100 ug/L in the aqueous calibration standards. The concentrations of gases and water soluble compounds will be higher (i.e., 25, 50, 100, 250, and 500 ug/L). Analysts may use a wider range of standard concentrations if linearity can be demonstrated.

6.8 Aqueous Performance Standard - an aqueous standard containing all pollutants, internal standards, labeled compounds, and BFB (4-bromofluorobenzene) is prepared daily, and analyzed each shift to demonstrate performance (Sect. 11). This standard should contain either 10 or 50 ug/L of the labeled and pollutant gases and water soluble compounds, 5 ug/L of BFB, and 10 ug/L of all other pollutants, labeled compounds, and internal standards. It may be the nominal 10 ug/L aqueous calibration standard (Sect. 6.7).

6.9 A methanolic standard containing all pollutants and internal standards is prepared to demonstrate recovery of these compounds when syringe injection and purge-and-trap analyses are compared. This standard should contain either 10 ug/mL or 50 ug/mL of the gases and water soluble compounds, and 10 ug/mL of the remaining pollutants and internal standards (consistent with the amounts in the aqueous performance standard in Sect. 6.8).

6.10 Other standards that may be needed are those for testing of BFB performance (Sect. 8.2.1) and for collecting mass spectra for storage in spectral libraries (Sect. 8.1.4).

6.11 High Purity Helium - 99.999 percent.

6.12 Liquid Nitrogen (LN_2).

7.0 SAMPLE COLLECTION, PREPARATION, AND STORAGE

7.1 In the field, sources of contamination include sampling gear, grease from ship winches or cables, ship engine exhaust, dust, and ice used for cooling. Efforts should be made to minimize handling and to avoid sources of contamination. This will usually require that resection (i.e., surgical removal) of tissue be performed in a controlled environment (e.g., a laboratory). For example to avoid contamination from ice, the samples should be wrapped in aluminum foil, placed in watertight plastic bags and immediately iced in a covered ice chest. Aluminum foil should be cleaned by heating at over 105° C before use. Solvent cleaning is unacceptable unless heating is performed afterward. Organisms should not be frozen prior to resection

if analyses will only be conducted on selected tissues, because freezing may cause internal organs to rupture and contaminate other tissue (e.g., muscle). If organisms are eviscerated on board the survey vessel, the remaining tissue may be wrapped as described above and frozen.

7.2 To avoid cross-contamination, all equipment used in sample handling should be thoroughly cleaned before each sample is processed. All instruments must be of a material that can be easily cleaned (e.g., stainless steel, anodized aluminum, or borosilicate glass). Before the next sample is processed, instruments should be washed with a detergent solution, rinsed with tap water, soaked in high-purity acetone and methylene chloride, and finally rinsed with reagent water.

7.3 Resection should be carried out by or under the supervision of a competent biologist. Each organism should be handled with clean stainless steel or quartz instruments (except for external surfaces). The specimens should come into contact with precleaned glass surfaces only. Polypropylene and polyethylene surfaces are a potential source of contaminatin and should not be used. To control contamination when resecting tissue, separate sets of utensil should be used for removing outer tissue and for dissecting tissue for analysis. For fish samples, special care must be taken to avoid contaminating target tissues (especially muscle) with slime and/or adhering sediment from the fish exterior (skin) during resection. The incision "troughs" are subject to such contamination; thus, they should not be included in the sample. In the case of muscle, a "core" of tissue is taken from within the area boarded by the incision troughs, without contacting them. Unless specifically sought as a sample, the dark muscle tissue that may exist in the vicinity of the lateral line should not be mixed with the light muscle tissue that constitutes the rest of the muscle tissue mass.

7.4 The resected tissue sample should be placed in a clean glass or TFE container which has been washed with detergent, rinsed twice with tap water, rinsed once with distilled water, and heated at 105° C for several hours. Jars should be heated at 105° C and allowed to cool immediately before use.

7.5 The U.S. EPA and other federal agencies (e.g., National Bureau of Standards) have not yet provided specific guidance regarding holding times and temperatures for tissue samples to be analyzed for volatile organic compounds. Until U.S. EPA develops definitive guidance, the following holding conditions should be observed. Resected tissue samples should be maintained at -20° C and analyzed as soon as possible, but within 10 days of sample receipt. The 10 day holding time is based on the Contract Laboratory Program regulations for sediments to be analyzed for volatiles (reference 10).

8.0 CALIBRATION AND STANDARDIZATION

8.1 Initial Calibration

8.1.1 Calibration by the isotope dilution technique -- the isotope dilution technique is used for the purgeable organic compounds when appropriate labeled compounds are available and when interferences do not preclude the analysis. If labeled compounds are not available or interferences are present, the internal standard technique (Sect. 8.1.2) is used. A calibration curve encompassing the concentration range of interest is prepared for each compound determined. The relative response (RR) vs. concentration (ug/L) is plotted or computed using a linear regression. An example of a calibration curve for a pollutant and its labeled analog is given in Figure II-2. Also shown are the ±10 percent error limits (dotted lines). Relative response is determined according to the procedures described below. A minimum of five data points is required for calibration (Sect. 6.7).

8.1.1.1 The relative response (RR) of pollutant to labeled compound is determined from isotope ratio values calculated from acquired data. Three isotope ratios are used in this process:

R_x = the isotope ratio measured in the pure pollutant (Figure II-3A)
R_y = the isotope ratio of pure labeled compound (Figure II-3B)
R_m = the isotope ratio measured in the analytical mixture of the pollutant and labeled compounds (Figure II-3C).

The correct way to calculate RR is:

$$RR = (R_y - R_m)(R_x + 1)/(R_m - R_x)(R_y + 1).$$

If R_m is not between $2R_y$ and $0.5R_x$, the method does not apply and the sample is analyzed by the internal standard technique (Sect. 8.1.2).

8.1.1.2 In most cases, the retention times of the pollutant and labeled compound are the same and isotope ratios (R's) can be calculated from the EICP areas, where:

$$R = (\text{area at } m_1/z)/(\text{area at } m_2/z)$$

If either of the areas is zero, it is assigned a value of one in the calculations; that is, if: area of m_1/z=50,721, and area of m_2/z=0, then R=50721/1= 50720. The m/z's are always selected such that $R_x > R_y$. When there is a difference in retention times (RT) between the pollutant and labeled compounds, special precautions are required to determine the isotope ratios.

R_x, R_y, and R_m are defined as follows:

R_x = [area m_1/z (at RT_2)]/1
R_y = 1/[area m_2/z (at RT_1)]
R_m = [area m_1/z (at RT_2)]/[area m_2/z (at RT_1)].

8.1.1.3 An example of the above calculations can be taken from the data plotted in Figure II-3 for a pollutant and its labeled analog. For these data, R_x=168920/1= 168920, R_y=1/60960=0.00001640, and R_m=96868/82508 =1.174. The RR for the above data is then calculated using the equation given in Sect. 8.1.1.1. For the example, RR=1.174. Note: Not all labeled compounds elute before their pollutant analogs.

8.1.1.4 To calibrate the analytical system by isotope dilution, analyze a 5 mL aliquot of each of the aqueous calibration standards (Sect. 6.7)

spiked with an appropriate constant amount of the labeled compound spiking solution (Sect. 6.5), using the purge and trap procedure in Sect. 10. Compute the RR at each concentration.

8.1.1.5 Linearity - if the ratio of relative response to concentration for any compound is constant (less than 20 percent coefficient of variation) over the five point calibration range, an averaged relative response/concentration ratio may be used for that compound; otherwise, the complete calibration curve for that compound should be used over the 5 point calibration range.

8.1.2 Calibration by internal standard - used when criteria for isotope dilution (Sect. 8.1.1) cannot be met. The method is applied to pollutants having no labeled analog and to the labeled compounds themselves. The internal standards used for volatiles analyses are bromochloromethane, 2-bromo-1-chloropropane, and 1,4-dichlorobutane. Concentrations of the labeled compounds and pollutants without labeled analogs are computed relative to the nearest eluted internal standard.

8.1.2.1 Response factors - calibration requires the determination of response factors (RF), which are defined by the following equation:

$$RF = (A_s \times C_{is})/(A_{is} \times C_s)$$

where:

A_s = the EICP area at the characteristic m/z for the compound in the daily standard

A_{is} = the EICP area at the characteristic m/z for the internal standard

C_{is} = the concentration (ug/L) of the internal standard

C_s = the concentration of the pollutant in the daily standard.

8.1.2.2 The response factor is determined at 5, 10, 20, 50, and 100 ug/L for the pollutants (optionally at five times these concentrations for gases and water soluble pollutants - see Sect. 6.6 and 6.7), in a way

analogous to that for calibration by isotope dilution (Sect. 8.1.1.4). The RF is plotted against concentration for each compound in the standard (C_s) to produce a calibration curve.

8.1.2.3 Linearity - if the response factor (RF) for any compound is constant (less than 35 percent coefficient of variation) over the five point calibration range, an averaged response factor may be used for that compound; otherwise, the complete calibration curve for that compound should be used over the five point range.

8.1.3 Combined calibration - by adding the isotopically labeled compounds and internal standards (Sect. 6.5) to the aqueous calibration standards (Sect. 5.7), a single set of analyses can be used to produce calibration curves for the isotope dilution and internal standard methods.

8.1.4 Mass spectral libraries - detection and identification of the compound of interest during calibration and sample analysis are dependent upon the spectra stored in user created libraries.

8.1.4.1 Obtain a mass spectrum of each pollutant and labeled compound and each internal standard by analyzing an authentic standard either singly or as part of a mixture in which there is no interference between closely eluted components. That only a single compound is present is determined by examination of the spectrum. Fragments not attributable to the compound under study indicate the presence of an interfering compound. Adjust the analytical conditions and scan rate (for this test only) to produce an undistorted spectrum at the GC peak maximum. An undistorted spectrum will usually be obtained if five complete spectra are collected across the upper half of the GC peak. Software algorithms designed to "enhance" the spectrum may eliminate distortion, but may also eliminate authentic ions or introduce other distortion.

8.1.4.2 Obtain the authentic reference spectrum under BFB tuning conditions (Table II-2) to normalize it to spectra from other instruments.

8.1.4.3 The spectrum is edited by saving the five most intense mass spectral peaks and all other mass spectral peaks greater than 10 percent of the base peak. This spectrum is stored for reverse search and for compound confirmation.

8.2 Ongoing Calibration

8.2.1 The BFB standard must be analyzed at the beginning of each 8-hr shift. The tuning criteria in Table II-2 must be met before blanks and samples may be analyzed.

8.2.2 At the beginning and end of each 8-hr shift, system calibration should be verified by purging the aqueous performance standard (Sect. 6.8).

8.2.2.1 Calibration is tested by computing the concentration of unlabeled compounds by the isotope dilution technique (Sect. 8.1.1) for compounds with labeled analogs. Concentrations of unlabeled compounds without labeled analogs are calculated according to the internal standard technique (Sect. 8.1.2).

A complete (five-point) recalibration should be performed when results vary from predicted concentrations by more than ±25 percent. The last sample analyzed before failing criteria should then be reanalyzed. If the results differ by more than ±20 percent (i.e., twice the median reproducibility for replicate analysis of tissue samples, Table II-3), then it is to be assumed that the instrument was out of control during the original analysis and the earlier data should be rejected. Reanalysis of samples should progress in reverse order until it is determined that there is ≤20 percent difference between initial and reanalysis results.

9.0 QUALITY CONTROL [For further guidance, see Quality Assurance/Quality Control (QA/QC) for 301(h) Monitoring Programs: Guidance on Field and Laboratory Methods (Tetra Tech 1986).]

9.1 Each laboratory that uses this method is required to operate a formal quality assurance program. The minimum requirements of this program consist

of an initial demonstration of laboratory capability, analysis of samples spiked with labeled compounds to evaluate and document data quality, and analysis of standards and blanks as tests of continued performance.

9.2 Initial Demonstration of Laboratory Capability

9.2.1 Analyze the aqueous performance standard (Sect. 6.8) according to the purge-and-trap procedure in Sect. 10. Compute the area at the primary m/z (Table II-1) for each compound. Compare these areas to those obtained by injecting one uL of the methanolic standard (Sect. 6.9) to determine compound recovery. The recovery should be greater than 20 percent for the water soluble compounds (acrolein and acrylonitrile), and 60-110 percent for all other compounds. This recovery should be demonstrated initially for each purge-and-trap GC/MS system. The test should be repeated only if the purge and trap or GC/MS systems are modified in any way that might result in a change in recovery.

9.3 Blanks

9.3.1 Reagent water blanks must be analyzed to demonstrate freedom from carry-over (Sect. 3) and contamination.

9.3.1.1 The level at which the purge-and-trap system will carry greater than 5 ug/L of a pollutant of interest into a succeeding blank should be determined by analyzing successively larger concentrations of these compounds. When a sample contains this concentration or more, a blank should be analyzed immediately following this sample to demonstrate no carry-over at the 5 ug/L level.

9.3.1.2 With each sample lot (samples analyzed on the same 8-h shift), a blank should be analyzed immediately after analysis of the aqueous performance standard (Sect. 8.2.2) to demonstrate freedom from contamination. If any of the compounds of interest, except common laboratory contaminants (e.g., methylene chloride and toluene), or any potentially interfering compound is found in a blank at greater than 10 ug/L (assuming a response

factor of 1 relative to the nearest eluted internal standard for compounds not listed in Table II-1), analysis of samples is halted until the source of contamination is eliminated and a blank shows no evidence of contamination at this level. This control action also applies if methylene chloride or toluene is detected in a blank at greater than 50 ug/L.

9.4 Sample Spiking

9.4.1 The laboratory should spike all samples with labeled compounds to assess method performance on the sample matrix.

9.4.2 Spike and analyze each sample according to the method beginning in Sect. 10.

9.4.3 Compute the percent recovery (P) of the labeled compounds using the internal standard technique (Sect. 8.1.2).

9.5 Replicates

9.5.1 Replicate analyses (i.e., analyses of two subsamples from the same tissue homogenate) must be performed to monitor laboratory precision.

9.5.2 At least one laboratory duplicate should be run for cases of up to 20 samples. For cases of over 20 samples, one blind triplicate and additional duplicates must be run for a minimum of 5 percent replication overall.

10.0 PROCEDURE

10.1 Sample Processing

10.1.1 Mince tissue sample with a scalpel and homogenize the sample to a uniform consistency with a micro-grinder. Care must be taken to ensure that the micro-grinder is thoroughly cleaned after each use. This usually entails disassembly of the unit. Devices with large surface areas (e.g., blenders, meat grinders) should not be used, as they are difficult to clean

and a small sample is difficult to remove after grinding. Liquid associated with the sample should be retained throughout the procedure.

10.1.2 Dry weight determination - If sample size permits and dry-weight concentrations are required, dry weight determinations may be performed as follows: transfer an aliquot of approximately 3 g (weighed to the nearest 0.1 g) to a preweighed dish. Allow the sample to dry in an oven at 105° C overnight and determine the solid residue weight to the nearest 0.1 g. The percent total solids is calculated as:

$$T_S = [\text{dry residue wt (g)}]/[\text{wet sample wt (g)}]$$

Dry weight determinations should not be made at the cost of having insufficient sample for volatiles analysis. Significant decreases in the size of samples used for extraction will decrease attainable detection limits.

10.1.3 Immediately after homogenization, use a stainless steel spatula to transfer a 5-g aliquot to a preweighed sample vessel (Sect. 5.1.2). Weigh the transferred portion to the nearest 0.1 g.

10.1.4 Spike 50 nanograms of each labeled compound (or 250 nanograms of gaseous and water soluble compounds) into 2 mL of reagent water and add to the sample matrix. Seal the sample vessel with an O-ring connector and clamp and sonicate for 10 min. After sonication, store the sample contained in the sample vessel overnight in a refrigerator/freezer and analyze the next day.

10.2 Vaccum Distillation and Concentration Reference 2)

10.2.1 The vacuum extractor must be airtight and free of moisture before an extraction can be started.

10.2.2 A clean 100 mL pyrex flask is connected to the vacuum distillation apparatus at the sample vessel site (see Figure II-1), the vacuum pump started, and V_2-V_4 opened to evacuate the apparatus. Line condensation

is prevented by warming the transfer lines while evacuating the system. Heating tape is effective in creating even transfer line temperatures and can be used continuously during the procedure.

10.2.3 The vacuum apparatus is pressurized with helium by closing V_3 and opening V_1. The apparatus is tested for leaks with a helium leak detector or Snoop®, and appropriate adjustments are made as necessary. When the apparatus has been found to be airtight, close V_1, open V_3 and then heat the transfer lines and concentrator trap to 100° C for 5 min to eliminate any residual contamination.

10.2.4 The flask containing the sample should be immersed in liquid nitrogen, before the flask is uncapped. To begin the distillation, close V_2 (with V_3 and V_4 remaining open), cool the concentrator trap with a liquid nitrogen bath, and replace the empty sample vessel with the cooled sample flask. Disconnect the vacuum source by closing V_3. Open V_2 to permit vapors from the sample vessel to reach the concentrator trap. Immerse the sample vessel in a 50° C water bath and sonicate for 5 min.

10.2.5 Connect the vacuum source to the sample vessel by opening V_3. The lower pressure hastens the transfer of volatile compounds from the sample to the cooled concentrator trap. After 15 min of vacuum, close V_3 and open V_1 to fill the system with helium to atmospheric pressure. Close V_1 and V_2 to isolate the condensate. The distillation is now completed and the condensate is ready for transfer to a purge-and-trap device. The condensate can be held in the liquid nitrogen bath for up to 1 h prior to analysis. Care should be taken to ensure that moisture does not freeze in the narrow glass tubing in the concentrator trap. Careful drying of the system prior to analysis and maintenance of an airtight system will preclude this problem.

10.2.6 Disconnect the sample concentrator trap from the vacuum apparatus and connect it to the purge-and-trap device. Some outgassing is observed when the sample condensate is melted; therefore, the condensate should be kept frozen until the concentrator trap is attached to the purge-and-

trap device. After attachment, warm the concentrator trap walls to loosen the condensate and allow the ring of ice formed during condensation to drop to the bottom of the trap. To this partially melted extract add 3 mL of reagent water containing 50 ng of each of the internal standards (bromochloromethane, 2-bromo-1-chloropropane, and 1,4-dichlorobutane). The internal standards are added after vacuum extraction to allow the analyst the assess analytical losses of labeled compounds during the extraction/ concentration procedure.

10.3 Purge-and-Trap Procedure

10.3.1 Because commercial purge flasks must be slightly modified (with O-ring fittings) to be attached to the vacuum distillation apparatus, a simple O-ring adapter is necessary to connect the purge flask to the commercial device for which it was designed. The modified purge flask (Sect. 5.2.3) used in this procedure can be fitted to a commercial purge-and-trap device (e.g., a Tekmar ALS interfaced with a Tekmar LSC-2) with 9 mm O-ring fittings fused to short sections of glass tubing. Commercial purge-and-trap devices are almost entirely automated and are easy to operate with manufacturer's instructions.

10.3.2 Purge the extract solution with the concentrator trap immersed in an ice-water bath for 5 min followed by immersion in a 55° C-water bath for an additional 7 min. This provides conditions for reproducibly melting the frozen extracts in order to obtain reproducible purging efficiencies.

10.3.3 The GC conditions for analysis are as follows:

Injector zone temp.	225° C
Initial GC oven temp.	60° C
Final GC temp.	175° C
Initial hold time	3 min
Ramp rate	8° C/min
Final hold time	24 min
Jet separator oven temp.	225° C

10.4 Qualitative Determination - accomplished by comparison of data from analysis of a sample or blank with data from analysis of the shift standard (Sect. 8.2.2). Identification is confirmed when spectra and retention times agree according to the criteria below.

10.4.1 Labeled compounds and pollutants having no labeled analog:

10.4.1.1 The signals for all characteristic masses stored in the spectral library (Sect. 3.1.4.3) should be present and should maximize within the same two consecutive scans.

10.4.1.2 Either 1) the background corrected EICP areas or 2) the corrected relative intensities of the mass spectral peaks at the GC peak maximum should agree within a factor of two (0.5 to 2 times) for all masses stored in the library.

10.4.1.3 The retention time relative to the nearest eluted internal standard should be within ± 7 scans or ± 20 sec, whichever is greater, of this difference in the shift standard.

10.4.2 Pollutants having a labeled analog:

10.4.2.1 The signals for all characteristic masses stored in the spectral library should be present and should maximize within the same two consecutive scans.

10.4.2.2 Either 1) the background corrected EICP areas or 2) the corrected relative intensities of the mass spectral peaks at the GC peak maximum should agree within a factor of two for all masses stored in the spectral library.

10.4.2.3 The retention time difference between the pollutant and its labeled analog should agree within ± 2 scans or ± 6 sec, whichever is greater, of this difference in the shift standard.

10.4.2.4 If the experimental mass spectrum contains masses that are not present in the reference spectrum, an experienced spectrometrist is to determine the presence or absence of the compound.

10.5 Tentatively Identified Compounds (GC/MS Analysis) - The ten non-target peaks of greatest area in the RIC (reconstructed ion chromatogram) should be identified and quantified, if possible.

10.5.1 Guidelines for making tentative identification (reference 10):

(1) Tentative identifications should be based on a forward search of the EPA/NIH mass spectral library. Sample spectra should be visually compared with the most similar library match.

(2) Relative intensities of major ions in the reference spectrum (ions greater than 10 percent of the most abundant ion) should be present in the sample spectrum.

(3) The relative intensities of the major ions should agree within ±20 percent. (Example: For an ion with an abundance of 50 percent in the standard spectra, the corresponding sample ion abundance must be between 30 and 70 percent.)

(4) Molecular ions present in reference spectrum should be present in sample spectrum.

(5) Ions present in the reference spectrum but not in the sample spectrum should be reviewed for possible subtraction from the sample spectrum because of background contamination or co-eluting compounds. Data system library reduction programs can sometimes create these discrepancies.

10.5.1.1 If, in the opinion of the mass spectral specialist, no valid tentative identification can be made, the compound should be reported

as unknown. The mass spectral specialist should give additional classification of the unknown compound if possible (e.g., unknown hydrocarbon, unknown aromatic compound, unknown chlorinated compound). If probable molecular weights can be distinguished, include them.

10.5.2 Tentative quantification - quantification of TICs will be based on the internal standard technique and an assumed response factor of one (in the absence of data from authentic standards). The uncertain nature of this quantification should be clearly noted in the data report.

11.0 QUANTITATIVE DETERMINATION (CALCULATIONS)

11.1 Isotope Dilution - by adding a known amount of a labeled compound to every sample prior to vacuum distillation, correction for recovery of the pollutant can be made because the pollutant and its labeled analog exhibit similar behavior during purging, desorption, and gas chromatography. Note that pollutants and their labeled analogs are not always retained identically by complex matrices, so their behavior during the extraction step may differ. Use of this technique is to enable correction for analytical losses after extraction, not for matrix recovery.

11.1.1 Relative response (RR) values for sample mixtures are used in conjunction with calibration curves described in Sect. 8.1.1 to determine concentrations directly, so long as labeled compound spiking levels are constant.

11.1.2 For the isotope dilution technique, concentration is calculated as follows:

$$C\ (\mu g/kg, \text{ wet wt tissue}) = \frac{C_A\ (\mu g/kg) \times RR \times n}{\sum_{i=1}^{n} \frac{RR_i \times Z_{Ai}}{Z_i}}$$

where

C_A = the concentration of the stable isotope labeled compound as spiked into the sample

RR = relative response of unlabeled pollutant to isotope labeled surrogate in the sample

RR_i = relative response at i^{th} point in calibration

Z_i = absolute amount of unlabeled compound at i^{th} point of calibration

Z_{Ai} = absolute amount of labeled compound at i^{th} point in calibration

n = number of calibration points.

11.2 Internal Standard - calculate the concentration using the response factor determined from calibration data (Sect. 8.1.2) and the following equation:

Concentration = $(A_s \times C_{is})/(A_{is} \times RF)$ where the terms are as defined in Sect. 8.1.2.1, except that C_{is} is in ug/kg (wet tissue) and A_s is the EICP area at the characteristic m/z for the analyte in the sample.

11.3 If the EICP area at the quantitation mass for any compound exceeds the calibration range of the system, a smaller sample aliquot should be analyzed if possible. However, sample sizes of less than 0.5 g are not recommended because such small samples may not be representative.

11.4 Report results for all pollutants and labeled compounds found in samples, in ug/kg (wet weight, unless dry weight is required) to three significant figures. Pollutants and labeled compounds in blanks should be reported in ng/sample.

12.0 PRECISION AND ACCURACY

12.1 Recoveries from replicate spiked water and tissue analyses are presented in Table II-3 (references 1 and 2). These analyses were not performed with the isotope dilution technique and recovery results are uncorrected.

13.0 REFERENCES

1. Hiatt, M.H., "Analysis of Fish and Sediment for Volatile Priority Pollutants," Anal. Chem. Vol. 53, 1981, pp. 1541-1543.

2. Hiatt, M.H., and T.L. Jones. Isolation of Purgeable Organics from Solid Matrices by Vacuum Distillation. U.S. Environmental Protection Agency, Region IX, Las Vegas Laboratory, 1984.

3. Fed. Register, Volume 49, No. 209, October 26, 1984, pp. 43407-43415.

4. Fed. Register, Volume 49, No. 209, October 26, 1984, pp. 43373-43384.

5. Hiatt, M.H., "Determination of Volatile Organic Compounds in Fish Samples by Vacuum Distillation and Fused Silica Capillary Gas Chromatography/Mass Spectrometry," Anal. Chem. Vol. 55, 1983, pp. 506-516.

6. Hiatt, M.H. 4 November 1985. Personal Communication (phone by Mr. Harry Beller). Analytical Technologies, Incorporated, National City, CA.

7. "Working with Carcinogens," DHEW, PHS, NIOSH. Publication 77-206 (1977).

8. "OSHA Safety and Health Standards, General Industry," 29 CFR 1910, OSHA 2206, (1976).

9. "Safety in Academic Chemistry Laboratories," American Chemical Society Publication, Committee on Chemical Safety (1979).

10. U.S. Environmental Protection Agency. 1984 (revised January, 1985). U.S. EPA Contract Laboratory Program - statement of work for organics analysis, multi-media, multi-concentration. IFB WA 85-J176, J177, J178.

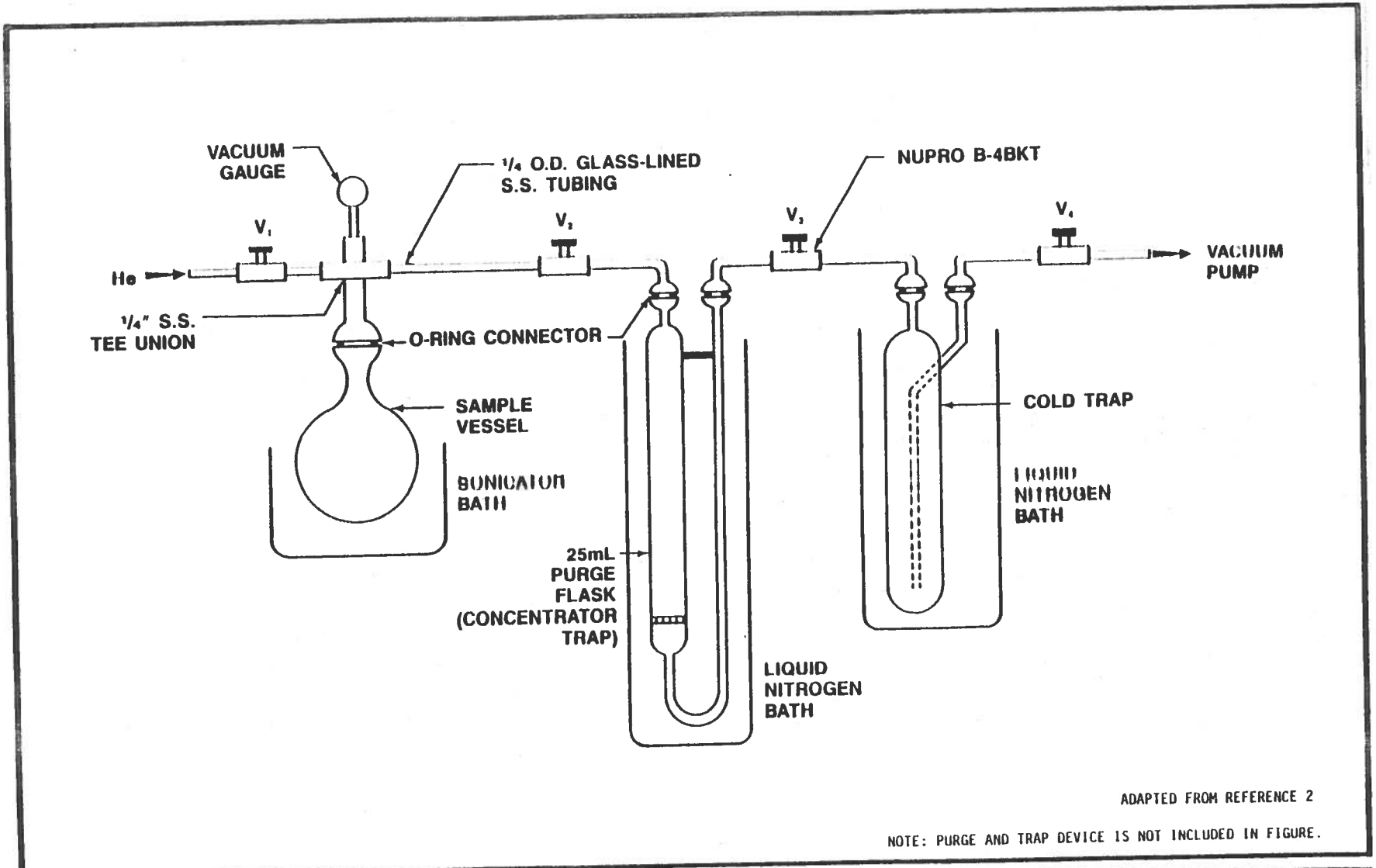
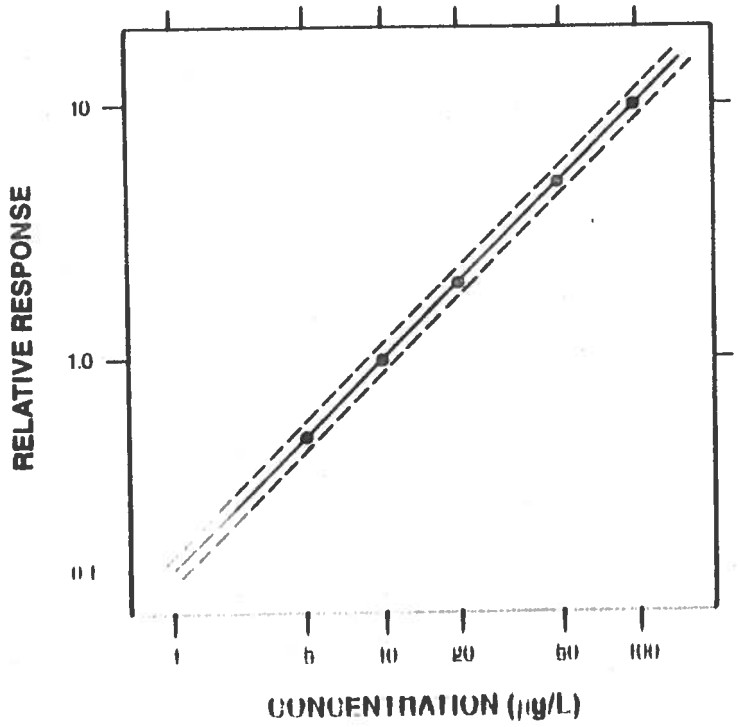
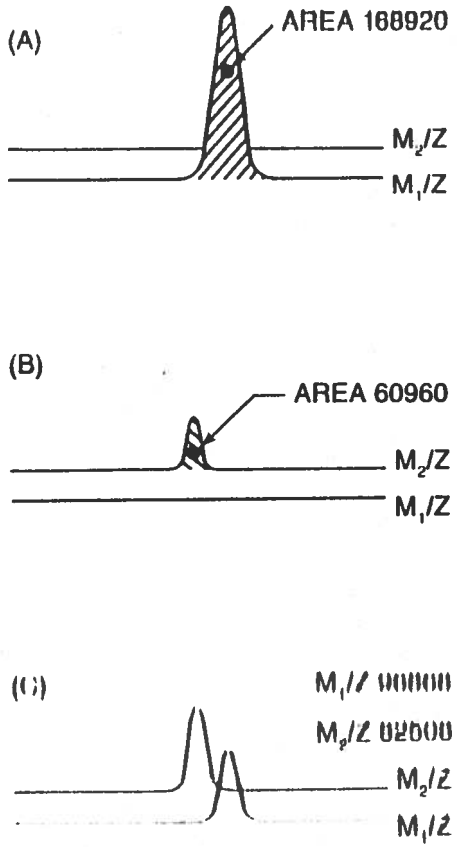


Figure II-1. Apparatus for vacuum distillation and cryogenic concentration.



ADAPTED FROM REFERENCE 3

2.



ADAPTED FROM REFERENCE 3

3.

Figure II-2. Relative response calibration curve.
Figure II-3. Extracted ion current profiles for (A) the unlabeled pollutant, (B) the labeled analog, and (C) a mixture of the labeled and the unlabeled compounds.

TABLE II-1. VOLATILE ORGANIC ANALYTES

Analyte	CASRN	ODES	Quantitation Ion (m/z)	Secondary Ion(s)
Acrolein	107-02-8	ACROLEIN	56	55
Acrylonitrile	107-13-1	ACRYLNTRLE	53	51, 52
Benzene	71-43-2	BENZENE	78	--
Bromodichloromethane	75-27-4	2CLBRMETHA	83	85, 129
Bromoform	75-25-2	BROMOFORM	173	171, 175
Bromomethane	74-83-9	METHYL BR	94	96
Carbon tetrachloride	56-23-5	CARBON TET	117	119, 121
Chlorobenzene	108-90-7	CLBNZ	112	114
Chloroethane	75-00-3	ETHYL CL	64	66
2-chloroethylvinyl ether	110-75-8	2-CLEVE	63	65, 106
Chloroform	67-66-3	CHLOROFORM	83	85
Chloromethane	74-87-3	METHYL CL	50	52
Dibromochloromethane	124-48-1	2BRCLMETH	129	206, 208, 127
1,1-dichloroethane	75-34-3	11-2CLETH	63	65, 83
1,2-dichloroethane	107-06-2	12-2CLETH	62	64, 98
1,1-dichloroethene	75-35-4	11-2CLETHE	96	61, 98
trans-1,2-dichloroethene	156-60-5	12-2CLETHE	96	61, 98
1,2-dichloropropane	78-87-5	12-2CLPRP	63	65, 114
cis-1,3-dichloropropene	10061-01-5	CIS-2CLPRP	75	77
trans-1,3-dichloropropene	10061-02-6	TRS-2CLPRP	75	77
Ethylbenzene	100-41-4	ETHYLBENZ	106	91
Methylene chloride	75-09-2	METHYLE CL	84	86
1,1,2,2-tetrachloroethane	79-34-5	4CLETHAN	83	85, 168
Tetrachloroethene	127-18-4	4CLETHE	164	129, 131, 166
Toluene	108-88-3	TOLUENE	92	91
1,1,1-trichloroethane	71-55-6	111-3CLETH	97	99, 117, 119
1,1,2-trichloroethane	79-00-5	112-3CLETH	97	83, 85, 99
Trichloroethene	79-01-6	3CLETHE	130	95, 97, 132
Vinyl chloride	75-01-4	VINYL CL	62	64

TABLE II-2. BFB MASS-INTENSITY SPECIFICATION

Mass	Intensity Required
50	15-40% of mass 95
75	30-60% of mass 95
95	Base peak, 100% relative abundance
96	5-9% of mass 95
173	<2% of mass 174
174	>50% of mass 95
175	5-9% of mass 174
176	>95% but <101% of mass 174
177	5-9% of mass 176.

TABLE II-3. PERCENT SPIKE RECOVERIES FOR VOLATILE PRIORITY POLLUTANTS USING VACUUM DISTILLATION[a]

Spiking Compound	Average Percent Recovery (Water)[b]	Average Percent Recovery (Tissue)[c]
Chloromethane	105 ± 12	85 ± 22
Bromomethane	110 ± 13	126 ± 75
Vinyl chloride	83 ± 12	64 ± 11
Chloroethane	103 ± 15	69 ± 22
Methylene chloride	125 ± 22	LOD[d]
1,1-dichloroethene	98 ± 5	74 ± 8
1,1-dichloroethane	96 ± 5	90 ± 6
trans-1,2-dichloroethene	98 ± 5	86 ± 9
Chloroform	98 ± 5	107 ± 31
1,2-dichloroethane	98 ± 10	92 ± 5
1,1,1-trichloroethane	104 ± 9	92 ± 8
Carbon tetrachloride	102 ± 11	91 ± 9
Acrylonitrile	85 ± 13	NA[e]
Bromodichloromethane	108 ± 12	64 ± 11
1,2-dichloropropane	104 ± 7	54 ± 7
trans-1,3-dichloropropene	109 ± 9	52 ± 9
Trichloroethene	105 ± 9	65 ± 11
Benzene	106 ± 7	57 ± 10
Dibromochloromethane	102 ± 11	58 ± 9
1,1,2-trichloroethane	98 ± 8	68 ± 7
cis-1,3-dichloropropene	109 ± 9	54 ± 9
Bromoform	104 ± 14	ND[f]
Tetrachloroethene	106 ± 9	ND
1,1,2,2-tetrachloroethane	90 ± 9	61 ± 10
Toluene	106 ± 7	ND
Chlorobenzene	101 ± 7	64 ± 15
Ethylbenzene	103 ± 8	ND
2-chloroethyl vinyl ether	94 ± 51	--
Acrolein	113 ± 78	NA
Average compound recovery	102 ± 8	78 ± 20

[a] From references 1 and 2.
[b] Reagent water was spiked with 25 µg/L of each compound except acrolein and acrylonitrile, which were added at 100 µg/L. The recoveries are averaged from 9 analyses and were calculated by comparing vacuum extracted determinations to determinations for which spikes were added directly to a purge-and-trap device.
[c] Ten-gram fish samples were spiked at 25 ppb. The recoveries were averaged from 12 analyses.
[d] Laboratory contamination of fish prevented the generation of valid data.
[e] Compound was not added to this matrix.
[f] Not determined.

APPENDIX C

SOUTHERN CALIFORNIA COASTAL WATER RESEARCH PROJECT
STANDARD OPERATING PROCEDURE
FOR

CLEAN-UP OF SOLVENT EXTRACTS PRIOR TO ANALYSIS FOR POLYCYCLIC AROMATIC HYDROCARBONS AND ALIPHATIC HYDROCARBONS

1.0 OBJECTIVE

The objective of this SOP is to describe a standard method for the clean-up of chloroform or methylene chloride extracts prior to the analysis of these extracts for aliphatic hydrocarbons and polycyclic aromatic hydrocarbons by either GC-FID or GC-MS. The method is applicable to extracts derived from biological tissues, marine sediments, and aqueous samples including effluent, seawater, microlayer, and river run-off.

2.0 PREPARATION

2.1 Supplies and Equipment

2.1.1. 25 ml pear-shaped boiling flask with 14/20 ground glass joint.

2.1.2. Glass adaptor, 24/40 joint (female) to 14/20 joint (male).

2.1.3. Roto-evaporator.

2.1.4. Glass chromatography column with Teflon stopcock, 10 mm I.D. by 30 cm long.

2.1.5. Glass wool, silanized (Supelco #2-0410).

2.1.6. Miscellaneous beakers, pipettes, funnels, etc.

2.1.7. Sample vial, amber and clear, 1.5 ml, with Teflon lined screw cap (e.g. Varian Autosampler vials).

2.1.8. Ring stand with clamps.

2.1.9. Glass syringe (2 to 3 cc).

2.2 Reagents

2.2.1. Pesticide quality n-hexane (Burdick & Jackson #217 Non-Spectro hexane).

2.2.2. Pesticide quality methylene chloride (Burdick &

Jackson #300 methylene chloride with cyclohexene preservative).

2.2.3. Silica gel, 40-140 mesh (J.T. Baker #3404), activated.

2.3 Activation of Silica Gel

2.3.1. Fill a porcelain crucible of appropriate size with silica gel and cover with a lid.

2.3.2. Activate silica gel by heating at ~~400~~°C for approximately ~~12~~ 15-16 hours. ✓

2.3.3. Allow silica gel to cool to room temperature, transfer to a glass storage container, and store under hexane.

2.4 Glassware Cleaning

2.4.1. All glassware is cleaned by washing with soap and water, rinsing with tap water, rinsing with deionized water, and kilning at 1050°F (566°C) for four hours prior to use.

2.4.2. New 1.5 ml autosampler vials will not require washing or kilning prior to use.

2.4.3. See Attachment 8.3 for proper operation of the electric kiln.

2.5 Other Cleaning

2.5.1. Teflon stopcocks, glass syringe bodies, syringe plungers, and syringe needles are cleaned by rinsing with acetone, then sonicating in acetone for 5 minutes.

3.0 PROCEDURE

3.1 Extract Clean-Up

3.1.1. Using a glass syringe, transfer the chloroform or methylene chloride extract (20 ml total) into a clean 25 ml pear-shaped boiling flask.

3.1.2. Reduce the volume of the extract to less than 1 ml by roto-evaporation (see Attachment 8.4 for proper operation of the Buchi Roto-Evaporator). Do not

allow the extract to dry out.

3.1.3. Add approximately 20 ml of n-hexane to the reduced extract and reduce the volume once again to less than 1 ml by roto-evaporation. Do not allow the extract to dry out.

3.1.4. Calibrate a clean, glass chromatography column (10 mm I.D. by 30 cm long, with Teflon stopcock) by marking it at four locations with a marking pen. Make the first calibration line 17 cm above the bottom of the glass column. Using the 17 cm line as a reference, mark 3 additional lines ~~cm~~ above the 17mm mark corresponding to volumes of 5 ml, 8 ml, and 10 ml. These lines will be used to measure the gel column and to measure the volume of eluent added to and drained from the column.

3.1.5. Install the Teflon stopcock into the glass column. Push a small plug of silanized glass wool into the column and seat it against the bottom of the column.

3.1.6. Attach the glass column to a ring stand with one or two clamps. Position a beaker below the column to collect waste solvent.

3.1.7. Using a Teflon wash bottle containing a mixture of hexane/methylene chloride (3:2), rinse the glass column and the glass wool. Collect the rinsate into a waste beaker.

3.1.8. Rinse the column and glass wool with n-hexane only, collecting the rinsate into a waste beaker. Close the stopcock, and fill the column with several inches of n-hexane.

3.1.9. Open the stopcock, then with a large bore glass pipette, dispense activated silica gel into the column to a settled height of approximately one inch above the 17 cm mark. Leave the stopcock open during packing to facilitate setting of the gel. Keep the hexane level above the gel level.

3.1.10. Gently tap the column to promote packing of the gel. Continue tapping until the gel stops settling. If the top of the silica gel column is below the 17 cm mark, add a little more gel to the

column. Continue this process until the packed gel column is 17 cm high.

3.1.11. Rinse the sides of the glass column with approximately 5 ml of n-hexane, allowing the n-hexane to drain into a waste container through the open stopcock. Close the stopcock when the n-hexane is only a few mm above the top of the gel.

3.1.12. In the remaining steps, you will be directed to apply sample and solvents to the gel column. When doing so, take care not to unduly disturb the top of the gel column. When draining the column, do not (unless otherwise directed) allow the solvent level to recede below the gel level. Close the stopcock just as the solvent level reaches the top of the gel column.

3.1.13. Using a clean glass 3 cc syringe, remove the concentrated sample extract from the 25 ml pear-shaped boiling flask.

3.1.14. Open the stopcock and drain the hexane off the gel column until it is just below the top of the gel. Close the stopcock. Position the tip of the syringe just above the gel column and gently dispense the sample extract onto the gel column. Open the stopcock and allow the sample to recede to just below the top of the gel. Close the stopcock.

3.1.15. Rinse the pear-shaped boiling flask with a small quantity of n-hexane, and gently add the rinsate to the gel column with the 3 cc glass syringe. As before, open the stopcock and allow rinsate to recede to just below the top of the gel. Repeat this step once more, but open the stopcock only until rinse solution just covers top of gel column.

3.1.16. Using the calibrated marking on the glass chromatography column, gently add 5 ml of n-hexane to the gel column (being careful not to disturb the top of the gel column), open the stopcock and drain 5 ml of eluant into a waste beaker. The remaining n-hexane should just cover the top of the gel column.

3.1.17. In a like manner, gently add 10 ml of n-hexane to the gel column, open the stopcock, and collect 10 ml of eluate into a clean, labeled 25 ml

pear-shaped boiling flask. Repeat once more, adding 5 ml of eluate. Total eluate collected will be 15 ml. This combined eluate is termed the F1 fraction and contains the aliphatic hydrocarbons.

3.1.18. Using the calibrated marking on the glass column as a guide, gently add 8 ml of a n-hexane/ methylene chloride (3:2) mixture to the gel column, open the stopcock, and drain 8 ml of eluant into a waste beaker.

3.1.19. Gently add 10 ml of the n-hexane/methylene chloride (3:2) solution to the gel column, open the stopcock, and collect 10 ml of eluate into a clean, labeled 25 ml pear-shaped boiling flask. Repeat this step twice more, each time collecting 10 ml of eluate, for a total of 30 ml. The combined eluate is termed the F2 fraction and contains the polycyclic aromatic hydrocarbons.

3.1.20. Dispose of the used silica gel into a waste container. Set the glass column aside for cleaning and kilning.

3.1.21. Roto-evaporate both the F1 and F2 fractions to a volume of less than 1 ml. Rinse the roto-evaporator trap and adapter between fractions and between samples with hexane (after roto-evaporating the F1 fraction) or hexane/methylene chloride (after roto-evaporating the F2 fraction).

3.2.22. Using a clean glass syringe, transfer the F1 fraction to a clear, labeled 1.5 ml sample vial. Cap with a Teflon-lined screw top. Label the vial with the sample code and the designation F1.

3.2.23. Transfer the F2 fraction to an amber, labeled 1.5 ml sample vial. Cap with a Teflon-lined screw top. Label the vial with sample code and the designation F2.

4.0 DATA CALCULATION AND REPORTING

Not applicable

5.0 QUALITY CONTROL

5.1 Blank

5.1.1. The extraction blank is run through the clean-up procedure along with the actual samples.

6.0 TRAINING

6.1 Persons performing this procedure must read this SOP and must receive a verbal and visual demonstration. No proficiency test is required.

7.0 SAFETY

7.1 The clean-up procedure must be performed in an explosion proof fume hood.

7.2 This procedure utilizes two potentially harmful solvents.

7.2.1. Hexane is flammable and is a skin irritant. Avoid breathing and contact.

7.2.2. Methylene chloride is a suspected carcinogen and is a skin irritant. Avoid breathing and contact.

7.2.3. For more specific safety information regarding these two solvents, see Attachments 8.1 and 8.2.

7.3 Dispose of waste solvent into the appropriate waste container.

7.4 Observe safe laboratory practices.

8.0 ATTACHMENTS

8.1 MSDS - hexane
8.2 MSDS - methylene chloride
8.3 Operation of Electric Kiln
8.4 Operation of Buchi Roto Evaporator