ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF ENFORCEMENT

TR-4

WORKSHOP ON

SAMPLE PREPARATION TECHNIQUES FOR ORGANIC POLLUTANT ANALYSIS

OCTOBER 2-4, 1973
DENVER, COLORADO

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Workshop on Sample Preparation Techniques for Organic Pollutant Analysis

October 2-4, 1973

Denver, Colorado

Chairman

Dr. Theodore O. Meiggs, NFIC-Denver

Discussion Leaders

Mr. James J. Lichtenberg, MDQARL

Mr. Roger C. Tindle, NFIC-Denver

Dr. Ronald G. Webb, SEWL

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Office of Enforcement
National Field Investigations Center
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Sample Preparation Techniques for
Organic Pollutant Analysis

PARTICIPANTS

Dr. Clark Allen
Enforcement & Support Branch
Region VI
1600 Patterson Street
Suite 1100
Dallas, Texas 75201

Dr. William Andrade
S & A Division
Region I
Needham Heights, Mass. 02194

Mr. James Barron
Charlottesville Laboratory
Region III
1140 River Road
Charlottesville, Virginia 22901

Mr. H. Gregory Beierl
Pesticide Laboratory
S & A Division
Region VIII
Building 45
Denver Federal Center
Denver, Colorado 80225

Mr. Thomas Bellar
Methods Development & Quality
Assurance Research Laboratory
NERC-Cincinnati
Cincinnati, Ohio 45268

Dr. Frank J. Biros
Pesticide Effects Laboratory
NERC - RTP
Research Triangle Park, N.C. 27711

Dr. Joseph Blazevich
S & A Division
Region X
14515 SE 21st Place
Bellvue, Washington 98007

Mr. Harvey W. Boyle
National Field Investigations Center
Building 53, Box 25227
Denver Federal Center
Denver, Colorado 80225

Mr. Mike Carter
Southeast Water Laboratory
College Station Road
Athens, Georgia 30601

Mr. Richard Dobbs
Water Supply Research Laboratory
NERC - Cincinnati
4676 Columbia Parkway
Cincinnati, Ohio 45226

Mr. James W. Eichelberger
Analytical Quality Control Laboratory
1014 Broadway
Cincinnati, Ohio 45202

Dr. Richard Enrione
National Field Investigations Center
5555 Ridge Avenue
Cincinnati, Ohio 45268

Mr. Mike E. Garza, Jr.
Houston Facility
S & A Division
Region VI
6608 Hornwood
Houston, Texas 77036

Dr. Gary Glass
National Water Research Laboratory
6201 Congdon Boulevard
Duluth, Minnesota 55804

Dr. Donald F. Goerlitz
U. S. Geological Survey
Water Resources Division
345 Middlefield Road
Menlo Park, California 94025

Mr. William L. Griffis
Consolidated Laboratory Services
NERC - Corvallis
200 SW 35th Street
Corvallis, Oregon 97330

Mr. Michael Gruenfeld
Edison Water Quality Research
Laboratory
Edison, New Jersey 08817

PARTICIPANTS (Cont.)

Dr. Larry Harris
Analytical Quality Control
Laboratory
1014 Broadway
Cincinnati, Ohio 45202

Mr. Lloyd Kahn
S & A Division
Region II
Edison, New Jersey 08817

Dr. Robert Kleopfer
S & A Division
Region VII
25 Funston Road
Kansas City, Kansas 66115

Mr. Fred Kopfler
Water Supply Research Laboratory
NERC - Cincinnati
4676 Columbia Parkway
Cincinnati, Ohio 45268

Mr. Thomas Leiker
National Field Investigations Center
Building 53, Box 25227
Denver Federal Center
Denver, Colorado 80225

Mr. James Lichtenberg
Method Development and Quality
Assurance Research Laboratory
NERC-Cincinnati
Cincinnati, Ohio 45268

James E. Longbottom
Method Development and Quality
Assurance Research Laboratory
NERC-Cincinnati
Cincinnati, Ohio 45268

Mr. William Loy
Laboratory Services Branch
S & A Division
Region IV
Southeast Water Laboratory
College Station Road
Athens, Georgia 30601

Dr. Theodore O. Meiggs
National Field Investigations Center
Building 53, Box 25227
Denver Federal Center
Denver, Colorado 80225

Mr. Jerry Muth
Laboratory Support Branch
S & A Division
Region IX
620 Central Avenue
Alameda, California 94501

Mr. William L. Reichel
Bureau of Sport Fisheries & Wildlife
Patuxent Wildlife Research Center
Chemistry - Pathology Building
Laurel, Maryland 20810

Dr. Peter Rogerson
National Marine Water Quality
Laboratory
South Ferry Road
Narragansett, Rhode Island 02822

Dr. Craig Shew
Robert S. Kerr Water Research Center
P. O. Box 1198
Ada, Oklahoma 74820

Dr. David L. Stalling
Bureau of Sport Fisheries & Wildlife
Fish-Pesticide Research Laboratory
PO #1
Columbia, Missouri 65201

Dr. Emilio Sturino
S & A Division
Region V
1819 West Pershing Road
Chicago, Illinois 60609

Mr. John Tilstra
S & A Division
Region VIII
Box 25345
Denver Federal Center
Denver, Colorado 80225

Mr. Roger C. Tindle
National Field Investigations Center
Building 53, Box 25227
Denver Federal Center
Denver, Colorado 80225

PARTICIPANTS (Cont.)

Dr. Gilman Veith
National Water Quality Laboratory
6201 Congdon Boulevard
Duluth, Minnesota 55804

Mr. Larry Wapensky
S & A Division
Region VIII
490 Orchard Street
Golden, Colorado 80401

Mr. Virgil L. Warren
National Field Investigations Center
Building 53, Box 25227
Denver Federal Center
Denver, Colorado 80225

Dr. James Watson
National Field Investigations Center
Building 53, Box 25227
Denver Federal Center
Denver, Colorado 80225

Dr. Ronald G. Webb
Southeast Water Laboratory
College Station Road
Athens, Georgia 30601

Mr. Robert E. White
Bureau of Sport Fisheries & Wildlife
Wildlife Research Center
Building 16
Denver Federal Center
Denver, Colorado 80225

Dr. A. J. Wilson
Gulf Breeze Laboratory
Sabine Island
Gulf Breeze, Florida 32561

INTRODUCTION

Throughout our Country, large quantities of industrial organic chemicals are being discharged daily to our rivers and lakes. Historically, the primary concern for these pollutants has been the oxygen demand they exert upon the receiving waters. However, it has become increasingly apparent that many of these organic chemicals can produce other adverse affects. Many of these compounds are highly toxic to aquatic life, some are carcinogenic, mutagenic, or teratogenic, while others undergo bio-concentration within the food chain. As a result, the discharge of these materials into the aqueous environment can pose a grave threat to the receiving water biota and in some cases even to the ultimate consumer - man.

Before much progress can be made to reduce this form of pollution analytical techniques must be developed to identify and quantitate individual chemical pollutants. However, the complex mixtures of organic compounds, and the low concentrations that are normally encountered, have made the analytical task formidable. Recent advance in analytical techniques and instrumentation have allowed some progress to be made in this difficult task. Consequently, a number of water laboratories have begun to apply these new techniques to the analysis of industrial-waste discharges and the receiving-water systems.

The purpose and goal of this workshop was to bring together the chemists who are responsible for organic-pollutant analysis, and to serve as a forum to exchange the varied experiences and accomplishments that have occurred in this rapidly developing field. The emphasis of

the workshop was placed upon the problems of sample collection, extraction, and fractionation prior to detection of the pollutants of interest by the appropriate detection techniques. Wherever possible, methods or procedures were stressed that were applicable to the analysis for general classes of organic compounds as opposed to procedures for individual compound identifications.

What follows is a summation of the techniques discussed at the workshop. Many of these are currently being used by water laboratories to analyze industrial effluents, natural waters, bottom sediments, and aquatic biota for industrial and agricultural organic-chemical pollutants. In addition, some discussion is provided regarding analytical quality control in the organic laboratory as well as a summation of miscellaneous, general comments that were expressed at the meeting.

It is felt that the summary of this workshop will serve as a guide to current practices in the analysis for organic-chemical pollutants, and draw attention to those areas where more information is needed.

I. SAMPLE COLLECTION AND PRESERVATION

Sample collection and preservation is an area where considerable research and development is needed. There was general agreement among the workshop participants that there are, at present, no definitive guides to sample collection and preservation for organic-pollutant analysis. In addition, less than ten percent of the laboratories represented indicated that they routinely participate in the actual collection of samples for organic analysis. This would indicate that very few chemists have direct input into the planning and conducting of sampling programs. In this respect, there was unanimous agreement that the analyst should participate in the design of the sample collection process, particularly with respect to the preparation of sample containers, check on purity of solvents and reagents, etc. Also, there was agreement that the chemists should be allowed to train sampling crews to avoid potential sources of sample contamination.

In light of the expressed attitudes, it seems reasonable that some group within EPA should be assigned the task of preparing a sampling manual for use in training and guiding sampling crews who are to collect samples for organic analysis.

By far, the largest number of samples analyzed by the various laboratories are grab-water samples. Despite this fact, there seemed to be little real knowledge of the effects of collection method, container materials, handling procedures, etc. on the quality of the resulting sample. However, it should be emphasized that discussions did not include the relatively well-developed area of pesticide residue

analysis since the planners of the workshop felt that pesticide residue methodology is probably not sufficiently applicable to the problem of sampling for the wide variety of industrial organic compounds that may be encountered.

There was considerable discussion on the choice of sample container to be used in grab sampling. Most attendees agreed that glass bottles or jars were preferred, but one chemist suggested the possibility of sample contamination due to leaching of materials from soft glass. The mechanism of contamination from this source is not known, but the use of borosilicate glass (Pyrex, Kimax, etc.) seems to avoid the problem. This phenomenon needs to be studied further.

Most attendees felt that teflon-cap liners should be used to avoid sample contamination and losses, but a limited amount of evidence suggests that some solutes, notably PCBs, may be lost to, or through teflon liners. In these cases, aluminum-foil liners proved superior. Again, research is needed in this area.

Some attendees pointed out that serious losses of solutes from water samples may occur due to volatilization from the water surface. This effect has been particularly noticed with petroleum samples. A recent paper by Mackay and Wolkoff [*Env. Sci. Technol.*, 7, 611 (1973)] attempted to mathematically define the losses that occur due to vaporization of various compounds, including Aroclor 1260. The authors made some assumptions regarding vapor densities, etc., and produced some rather interesting conclusions. For example, if we assume that the sample bottle commonly used by EPA laboratories contains 850 ml of liquid and 50 ml of air space, then at 4°C about 20 percent of the

Aroclor 1260 from an initially saturated solution will be found in the air space. Also, since this is the equilibrium situation, shaking the sample should not redissolve the vaporized material. At higher temperatures, these losses would be even greater due to the greater evaporation of water into the air space. While some of the assumptions used for this estimate are probably not entirely true, the order of magnitude is probably correct. Thus, vaporization from a water sample into the air space in a partially filled jar may represent a major source of error in the analysis of grab samples for PCBs, aromatics, alkanes, and other organic materials. Obviously, the smaller the air space above the sample, the smaller the losses that may occur due to vaporization. However, as pointed out by some of the Workshop participants, if there is sufficient petroleum, or other organic material, to form a microscopic layer over the surface of the water, then losses due to capillarity (creeping of the organics out of the minute space between the jar lip and the cap liner) may become significant when the jar is nearly full. Again, we have a problem that deserves considerable attention.

Contamination of grab samples was a major concern of most Workshop participants. Contamination can occur in many ways; one of the most common results from inadequate pre-cleaning of sample containers. To minimize this, the chemists should provide properly prepared (prewashed) containers to the sampling crews.

Other sources of contamination are the caps used to seal sample bottles (metal caps must be freed of lacquer prior to use, while plastic caps may contain plasticizers), sealing tape used to assure that the caps remain tightly closed, glassware or other sampling gear used to prepare the sample for transfer to the sample bottle, reagents and solvents used for preservation, and possible other sources. The best answers to the contamination problem seem to be careful preparation of the sample containers before use, training of field crews so that they will avoid possible contamination sources, and careful pre-screening (and if necessary, pre-cleaning) of reagents and solvents for field use. Among the most common contaminants are phthalate esters, but many other types of compounds may be encountered.

Integrated sampling comprises the various techniques whereby samples are taken over an extended period of time, and in which the rate of sampling is related in some manner to time or the rate of flow of the sampled body. The commonly practiced technique of manually compositing grab samples over a period of time is an example of this type of sampling. Compositing was not discussed to any extent during the workshop, however, as usually practiced, solute losses due to vaporization probably represent a major problem when this method is used.

The Workshop participants showed considerable interest in the use of macroreticular resins for integrated sampling. Four of the represented laboratories, including three from EPA, had previously worked with macroreticular resin columns. Those that had used resins agreed that this approach to sampling seems to hold a great deal of promise.

In a recent example [R. Tindle, *AQC Newletter*, #19, October, 1973], a mixed bed of Amberlite XAD-2 and XAD-7 (50:50 mixture) had been used in a sampling column, which also contained polyurethane-foam plugs both before and after the resin bed, to sample for several pesticide and industrial-organic compounds ranging from hydrocarbons to phenols. This system exhibited good, trapping efficiencies (>90 percent) for most tested compounds, and upon elution gave overall recoveries generally of >90 percent.

Some Workshop attendees pointed out that recoveries may be flow-rate dependent and some gel-filtration effects (exclusion of large molecules) may be noted.

The greatest potential for resin-column use seems to lie in the area of long-term (24-96 hours) sampling for low levels of organics in lakes, streams, etc. Much work needs to be done to define the usefulness and limitations of this method. However, the potential usefulness certainly justifies a concerted initial evaluation.

Some of the characteristics of the resin-column samplers that need to be defined are:

(a) quantitative aspects - "What compounds are quantitatively trapped and over what concentration range?"

(b) preparation of resins - "How can resins best be prepared for use?"

(c) column capacity - "What is the capacity of a particular size of column? Are there interactions between solutes?"

(d) preservation of columns - "How can columns be preserved before extraction?"

(e) particulates on columns - "How should particulates be handled? What are the effects of partial plugging?"

(f) elution procedures - "What is best method of eluting columns? Are separation based on pH or solubility feasible?"

There was some discussion of carbon adsorption. Very few, if any, of the represented laboratories now use this method, although three Regional Offices appear to be considering use of this method for monitoring purposes.

There was almost no discussion of methods of collecting tissue and sediment samples. What discussion occurred centered around the idea that EPA needs a written set of guidelines regarding the collection of these types of samples.

Discussions on the methods of preserving samples quickly revealed an almost total lack of knowledge regarding the effects of various alternative methods of preservation. In general, most laboratories simply place bottled-grab samples on ice for shipment as a means of preservation. However, it was clear that no one really knew whether this approach is effective in preserving samples containing a variety of industrial-organic compounds.

Some attendees suggested the use of solvent in sample bottles as a means of preservation. There was, however, little agreement as to which solvent should be used. For low-boiling pollutants, the use of hexadecane as a keeper solvent seems to have some utility. Various solvents, including methylene chloride, Freon TF, hexane, isooctane, etc., were

suggested for use as keepers for higher-boiling pollutants. Some form of keeper in the bottle would seem desirable to reduce possible loss by volatilization as discussed previously.

Petroleum-containing samples are preserved by the addition of sulfuric acid [M. Gruenfeld, *Env. Sci. Technol.*, 7, 636 (1973)], while formalin was suggested for PCB-containing samples [T. A. Bellar and J. J. Lichtenberg, "Some Factors Affecting the Recovery of PCB's From Water and Bottom Samples," CIC-CCIW Symposium on Water Quality Parameters, Burlington, Ontario, November, 1973]. Copper sulfate and phosphoric acid are often used to preserve samples for phenols analysis.

Tissue- and bottom-sediment samples are preserved by freezing by almost all represented labs. A recent paper [Butler, *Pest. Monitor. J.*, 6, 238 (1963)] reported the use of a mixture of 90 percent anhydrous sodium sulfate and 10 percent Quso G30 (micro-fine precipitated silica) to preserve field-blended-tissue samples. This method allowed the storage of desiccated-tissue samples for at least 14 days without loss or degradation of pesticide residues.

This area of preservation of samples is another highly important area requiring extensive research. Hopefully, the various EPA research groups will expend the needed effort to define suitable preservation techniques for both water and other samples.

II. EXTRACTION PROCEDURES

Extraction procedures cover a wide variety of techniques whereby the organic pollutant(s) of interest are transferred from the inorganic or biological matrix (i.e., water, sediment, or tissue) and usually concentrated prior to chemical characterization. During the Workshop, extraction techniques were discussed for separating organic pollutants from water and wastewater samples, tissue, bottom sediment, and sludge samples.

The simplest situation occurs when no extraction is required. In this case, the chemist may apply such techniques as direct aqueous-injection gas chromatography, head-space analysis, trapping of volatile components by either gas purging or cold trapping and finally, steam distillation.

The technique of direct aqueous-injection gas chromatography (GC) was familiar to most workshop participants. Those who had applied it had found it most useful for the analysis of volatile organics in effluents where the detection limit of approximately 1 mg/l is adequate. The use of pre-columns to prevent salts and other non-volatiles from damaging the GC column was recommended by several participants. Quartz inserts or lengths of column tubing at the head of the GC column, either empty or packed with quartz wool, can be employed for this purpose. These inserts can be changed or cleaned with a minimum of effort. Direct aqueous injection is currently being recommended by the EPA Methods Development and Quality Assurance Research Laboratory (MDQARL) in the analysis for chlorinated and aliphatic solvents. For these analyses,

the halogen specific micro-coulometric or the non-specific flame ionization detectors are employed. Direct aqueous injection is recommended by ASTM for the analysis of phenols in their "Standard Method of Test for Phenols in Water by Gas Liquid Chromatography" (D2580) and for the analysis of volatile organic matter in water in their Method (D2908). Additional methods of direct aqueous injection for organic acids, nitrites, and aliphatic hydrocarbons are presently being prepared by ASTM and others [D. Brown, *AQC Newsletter*; (19), 5 (1973)].

Head-space analysis can also be applied for the determination of volatile-organic materials although few of the Workshop participants had actually employed it in practice. The use of infrared spectroscopy has been reported for the quantitative identification of head-space gases in oil samples.

Other techniques for the analysis of volatile organics use gas purging to remove volatiles from the samples. Tom Bellar reported the use of nitrogen gas to purge volatile-organic components from water samples. The evolved organics are then collected on an adsorbent column (Chromosorb 103). The collection column is then inserted into the injection port of a gas chromatograph and the trapped components analyzed under temperature-program conditions. The technique has been applied to a variety of chlorinated and non-chlorinated aromatic and aliphatic solvents. Under ambient conditions, the recovery of relatively insoluble organic compounds has been found more efficient than the recovery of highly water-soluble compounds. Instead of collecting the purged volatile-organics on a column, the materials may be collected in a cold trap. This technique has been used by several of the

laboratories in attendance and is a relatively common technique in the analysis of atmospheric samples.

Steam distillation can be used to reduce the sample volume and to concentrate organic components that are volatile under such conditions. Samples thus concentrated can be analyzed by direct-aqueous injection or by solvent extraction prior to GC or other types of detection. However, possible hydrolysis of sample components must be carefully considered when ever this technique is employed.

Liquid-liquid extraction is by far the most common type of extraction technique in dealing with water and wastewater samples. The Workshop participants were queried as to the most common types of solvents used for this purpose and it was found that the solvents most commonly used were methylene chloride or chloroform, followed by ethyl ether, hexane, methylene chloride-hexane, and finally by ethyl ether-hexane mixture. Other solvents that were mentioned but not widely used, were carbon tetrachloride for oils, freon for oils, benzene-hexane, hexane-acetone and hexadecane. A consensus of the Workshop participants was that, whenever possible, non-flammable solvents such as methylene chloride or freon should be used. The potential explosive hazard of diethyl ether and other flammable solvents should not preclude there use when needed. However, the chemist must be aware of the hazard and take measures to minimize the possibility of accident. Obviously, the solvent of choice will depend upon the types of pollutants to be analyzed or to be characterized.

In the case where a wide variety of organic types are to be determined, liquid-liquid extraction can be used as a fractionation tool as

well as a separation technique. A procedure for the separation of neutrals, acids, and bases was described by William Loy of the Southeast Water Laboratory. In this procedure, conditions for the initial extraction of the sample are determined by the pH of the sample as received. The sample is shaken to provide homogeneity and is divided into two equal portions for replicate analysis. If the pH of the sample is between 5 and 14, the sample is initially extracted with hexane to recover the "neutral organics" which are then analyzed by gas chromatography. After the neutrals have been removed, the sample is then acidified to pH 2 and extracted with methylene chloride. The methylene chloride extract is then concentrated and divided equally. One aliquot is analyzed directly by gas chromatography, the second is esterified, using diazomethane prior to analysis by gas chromatography. If the original sample has a pH less than 5, the sample is acidified to pH 2 and extracted only with methylene chloride. The methylene chloride extract is then divided and analyzed or esterified as above. In some cases, organic bases may be recovered by adjusting the pH of the sample to greater than 10 and extracting with methylene chloride or other appropriate solvent. Extraction of the samples may be carried out using separatory funnels or a magnetic stirring device. The magentic stirring approach is satisfactory when extracting with a lighter-than-water solvent; it is not very efficient when using a solvent that is heavier than water.

A good discussion of liquid-liquid extraction can be found in the ASTM Manual, Part 23, Method D-2778 "Solvent Extraction of Organic Matter from Water". This method describes a general approach that will

separate a wide variety of organic components and allows the analyst to select from a variety of solvents as required to meet his needs.

Workshop participants reported that recoveries from industrial waste samples were variable and often poor. Salting out was suggested as a method for improving recovery. The use of sub-ultrasonic (polytron) or ultrasonic treatment to break up the suspended solids in a sample was also suggested as a means of improving the extraction efficiency. It should be noted that heavier-than-water solvents can cause problems during phase separation when the samples contain fibers for other solid materials that tend to settle to the bottom.

A procedure for breaking emulsions by pouring the sample through glass wool was presented to the Workshop by William Loy. The procedure calls for passing the organic layer through a column of 2-3 inches of Pyrex glass wool (prerinsed with methylene chloride) and collecting it in a beaker. If necessary, the solvent is forced through the glass wool by applying mild air pressure. If a layer of water is present after passing through the glass wool once, it is passed through a second column for final drying. Some unanswered questions regarding this technique are the following: Are organics that may be occluded in emulsified material lost as this material is removed by the glass wool? Does the glass wool do an adequate job of removing the water from the solvent extract? Mr. Loy is working to answer these and other questions.

Sulfur is often extracted from environmental samples into the organic layer. In order to avoid sulfur interference, the sulfur may be removed with mercury or copper powder (*Bull. Environ. Cont. and Toxic.*, 6, 9(1971).

An alternate approach for recovery of organic pollutants from water and wastewater is adsorption on organic resins or activated carbon followed by solvent extraction of the resin or carbon to desorb the organic pollutants of interest. A number of Workshop participants have used the Rohm and Haas XAD resins for the recovery of a variety of organic compounds. Though this relatively new technique is not yet fully developed, it has shown considerable promise for some applications. A preliminary literature review of work with this technique has been prepared by Roger Tindle, NFIC-Denver.

Investigations of procedures for extracting organic materials from the XAD resins are in progress. A number of solvents have already been applied singly, in series, or as mixtures. Examples are acetone, methanol, or ethanol used singly or acetone followed by methylene chloride followed by acetone again in series, or a single elution using a mixture of acetone in chloroform. Some degree of class separation based upon pH can be achieved using the resins, however, more work needs to be done in this area. Some of the problems surrounding the use of the resins are the same as those encountered for the carbon adsorption technique. These include, variable particle size, background interference, unknown efficiency, plugging by suspended matter, etc.; however, there are a number of decided advantages of the resin over the carbon such as lack of active sites which minimize chemical changes on the resin surface.

Polyurethane foams have also been employed with the extraction of certain organics from water. In general, the foams have been found to work well for the extraction of non-polar compounds, e.g., PCB's; however, they are not very effective for the extraction of more polar

compounds. A combination of the XAD resins and the polyurethane foams have been applied by NFIC-Denver with good success.

Historically, carbon adsorption has been widely used for the separation of organic components from water. However, due to its non-quantitative nature, expense of sampling, etc. it has fallen into disuse. The normal procedure for removing organics from carbon has been air drying of the carbon, followed by extraction first with chloroform followed by extraction with alcohol. Dr. Clark Allen reported at the Workshop that a considerable increase in extraction efficiency can be obtained if the carbon is dried by freeze drying instead of air drying. Apparently, much greater removal of water is obtained this way and more thorough contact is achieved between the carbon and the extraction solvent. Although the Methods Development and Quality Assurance Research Laboratory has now terminated surveillance operations using the carbon adsorption technique, there is still some interest among the Regions in the use of this technique for separating and identifying organic compounds from water.

A number of the EPA laboratories have found the need, on occasion, to analyze tissue samples for organic chemical pollutants. It is anticipated that the need for this type of activity will be increased since, especially from an enforcement standpoint, there is a legal necessity to demonstrate the effects of pollutants on the environment. Measuring the uptake of chemical pollutants by aquatic life is one way to demonstrate this.

The extraction of tissue samples for organic pollutants is considerably different from the extraction of water and wastewater samples.

Most work in this area has centered around the analysis for pesticides and/or petroleum products. Certain types of organic pollutants, such as oils, can be extracted by adding an organic solvent while the tissue sample is being masserated in a blender. This technique has been found to be especially useful when using sub-ultrasonic mixers, such as the Polytron (Brinkman instruments) and the Tissu-Mizer (Tek-Mar Company). Several Workshop participants have investigated this technique and feel that it shows promise for certain applications [*J. Agr. Food Chem.* 20. 48, (1972)].

Blenders can also be used to prepare samples for column extraction. For this technique, the sample is ground in the presence of dry ice and sodium sulfate [*J. Agr. Food Chem.*, 18, 948, (1970)]. Following grinding, the dry ice is allowed to sublime from the sample leaving a fine powdery material. Once the tissue has been dried in this fashion, it can be packed in a chromatographic column and extracted by elution with a solvent such as acetone, methanol, or acetone-haxane (1:1) [(*Bull. Envir. Contam. Toxi.*, 7, 1151, (1972) and Southeast Water Laboratory, EPA, Athens, Georgia, Method No. SP-8/71]. An alternate technique is freeze drying the tissue samples. Dr. A. Wilson of EPA's Gulf Breeze Laboratory has used this method for preparation of phytoplankton samples.

Tissue samples can also be extracted by use of a soxhlet extractor. This seems to be about as commonly applied as the column extraction technique. Some of the solvents employed for soxhlet extraction are petroleum ether, methylene chloride, acetone-hexane, methylene chloride-hexane, acetonitrile, ethyl acetate, and acetone-benzene. The use of

phosphoric acid acetone (1:2) has been used for the extraction of pentachlorophenols.

Bottom sediment and sludge samples can be extracted by techniques that are similar to those applied to tissue samples, namely, column extraction, soxhlet extraction and blender extraction. Sample pre-treatment, however, varies depending upon whether the extraction is to be done under wet or "dry" conditions. Workshop participants discussed five different approaches to pre-treatment, namely: 1) air drying at ambient conditions, followed by grinding with a mortar and pestle and addition of 10 percent water followed by soxhlet extraction; 2) partial air drying (30-40 percent moisture) at ambient conditions and blending with sodium sulfate followed by column extraction; 3) decanting excess water and blending the wet sample with sodium sulfate followed by column extraction; 4) decanting excess water and extracting the wet sample by shaking with solvent using no dessicant; and 5) blending of the wet sample directly with solvent.

Once a sample has been "dried" it may then be extracted either by column elution or soxhlet extraction. Solvents normally employed are acetone-hexane, acetonitrile-hexane, methylene chloride, acetonitrile, ethyl acetate, and acetone-benzene. Soxhlet extraction of sediment samples has been described many times ("The Identification and Measurement of Chlorinated Hydrocarbon Pesticides and Surface Waters", U. S. Department of the Interior, 1014 Broadway, Cincinnati, Ohio, Publication WP-22, 1966.) This technique is found to give good recovery of compounds that are stable and do not volatilize under the conditions of treatment.

Organic pollutants can be extracted from sediments by mixing directly with the solvent of choice. Although no laboratories are presently using Waring Blenders for this type of extraction, both mechanical shaking ("Methods for the Analysis of Organic Substances in Water", Book 5, Chapter 83, Techniques of Water Resources Investigations, the U.S.G.S., 1972.) and sub-ultrasonic mixing are being employed with varying degrees of success. The latter technique looks especially promising; however, further work is required to access its full utility.

In general, it was concluded at the Workshop that air drying of the sample is not a good practice when a broad spectrum of organic compounds is to be determined. Significant amounts of very volatile organic compounds can be lost if air drying is employed, e.g., BHC has been found to volitalize readily under these conditions. On the other hand, extraction of some compounds from environmentally contaminated sediments has been significantly more efficient when carried out on an air-dried sample. Thomas Bellar of the MDQARL reported that both PCBs and dieldrin are more efficiently extracted from natural samples that have been dried in this fashion.

It was recognized by the Workshop participants that a great deal of work needs to be done to determine which extraction procedure, if any, is superior. However, an even greater area of concern is the wide variations that occur during sample collection. It was the general concession that for tissues and sediments, the sampling variations and biological variations are much larger than analytical variations and often account for the wide discrepancies in replicate analysis.

III. FRACTIONATION AND DERIVATIZATION PROCEDURES

Samples that are too complex to be separated under normal GC conditions are usually subjected to some form of fractionation during, or after, extraction. Six workshop attendees reported that they frequently use some form of acid-base separation for water samples, particularly industrial effluents. They preferred to use a simple two- or three-step scheme although they were aware of Braus, Middleton and Walton's more complex scheme that separates neutral compounds, strong acids, phenols, bases and amphoteric compounds [*Anal. Chem.* 23, 1160 (1951)]. Typically, they extract the sample, as received, to isolate neutral compounds. Then they acidify to about pH 2 and extract to isolate acids and phenols. Phosphoric, sulfuric, or hydrochloric acids are used for this step. The aqueous layer is then adjusted to pH greater than 8 with ammonia or dilute sodium hydroxide to form free bases and the sample is extracted a third time.

Some acids and phenols from the acid fraction can be analyzed without further treatment. Acetic through hexanoic acids can be chromatographed directly on Chromosorb 101 in an all-glass system. Carbowax 20M and FFAP have been used to analyze simple phenols, cresols and similar materials in paper-mill effluents. The longer acids and more complex phenols are usually converted to methyl esters and ethers. The most commonly used methylating agent is diazomethane. Phenols react more completely with this reagent when a little boron trifluoride in methanol is added as a catalyst. The extract must not contain any chloroform because it reacts with diazomethane to form di- and

trichlorinated alkanes up to seven carbons long that complicate the GC analysis. Methylene chloride does not cause this problem. A discussion of methylation procedures, including several on-column reagents is given in the report from SERL on "Current Practice in GC-MS Analysis of Organics in Water" (EPA-R2-73-277).

Other groups feel that trimethylsilyl (TMS) derivatives give more definitive mass spectra than methyl derivatives. They recommend BSTFA (N,O-bis-trimethylsilyl trifluoroacetamide) as the reagent of choice. One trade name is Regisil. Another derivatization mentioned, but apparently rarely used, is to form pentafluorobenzyl ethers, thioethers or esters from phenols, mercaptans and acids. [Kawahara, *Anal. Chem.* 40, 1009 and 2073 (1968)].

In contrast to neutral compounds and acids, very few bases have been identified in the environment. The Workshop participants agreed that judging from manufacturing data, industrial usage and the size of the basic fractions in past CCE studies, these compounds must be in the environment but we are not seeing them. This is a major weakness in our present analytical techniques.

Among the bases that have been found are picolines (methyl pyridine isomers) in river water, dibutyl amine from a latex-additives plant and quinoline and di- and trimethyl pyridines from a wood-preserving plant. Several dichloroaniline isomers and other nitrogen containing compounds in industrial effluents were identified by NFIC-Denver after conversion to TMS derivatives. Several aromatic amines from biological sources were reported as analyzed in good

yield by conversion to the amides with pentafluoropropionic anhydride and analysis by EC-GC.

Some methods-development work on amines has been done. Many simple amines can be gas chromatographed by direct aqueous injection on Tenax columns. One report was that Chromosorb 101 can also be used for this purpose although the 103 material is recommended. Another worker found that methyl and dimethyl amines can be sampled by the headspace-gas technique and then analyzed by GC on OV-101. Low-molecular weight amines were also reported as separated from other impurities by adsorption on weak acid cation exchange resins.

A wide variety of post-extraction chromatographic cleanup techniques were discussed. Oils are frequently chromatographed on Florisil or silica gel in a manner similar to pesticides. Some use a column containing silica gel on top of alumina. This column, deactivated with 4 percent water, was reported to separate oils from sewage when eluted with carbon tetrachloride. Another observation was that a useful second dimension of proof in oil fingerprinting by GC was to separate the oil into aliphatic, aromatic and oxygenated fractions by eluting from silica gel with isooctane, benzene and 1:1 chloroform-methanol by the method of Rosen and Middleton [*Anal. Chem.*, 27, 790 (1955)]. Phenols can be isolated from carbon chloroform extracts (CCE's) by extracting the chloroform with base, extracting the acidified-aqueous layer with ether, and then chromatography on Florisil with ether as the eluting solvent.

Thin-layer chromatography (TLC) would seem to be a very powerful technique in view of its low cost, and the visual impact it can have in a courtroom. In practice however, it is used little in pollution analysis because of its lack of discrimination and sensitivity. Polynuclear aromatic hydrocarbons (i.e., benzopyrene) have been separated and detected; phenols from CCE's have been detected down to one microgram per spot, and some amines can be analyzed by TLC. Identification of sources of oil spills by TLC has been extensively studied, partially through EPA grants to Esso and Phillips Petroleum, but there are still problems with the method. Two areas for future research on TLC were suggested -- reversed phase TLC for polar compounds, and detection of specific classes of compounds in industrial effluents by specific spray reagents. Nobody seemed to be planning any immediate activity in these areas.

Another method mentioned for detecting specific compound groups was the use of GC subtraction columns. These are short lengths of tubing containing a chemical that removes specific compounds. They are placed between the GC injector and the column. Boric acid subtracts alcohols, o-dianisidine subtracts aldehydes and ketones, phosphoric acid subtracts epoxides [Beroza, *JAOAC*, 54, 251 (1971); see also *Chem. Abst.*, 74, 134709z (1971)]. No one reported first-hand experience with this procedure.

Liquid chromatography as a cleanup method has not been extensively applied. It was not an improvement over column techniques for cleanup of oils for fluorescence analysis. There was one report of permaphase

columns bleeding enough to show up later in GC-MS analysis of the individual fractions.

Of at least ten LC's in EPA labs, four have been bought within the last year and are too new to be properly evaluated. It was generally concluded that a detector breakthrough will have to be made before LC finds extensive application in pollution analysis.

Probably the most promising cleanup technique is some form of automated gel-permeation chromatography. Gel permeation is not universally applicable, but it is useful for eliminating interference from compounds of molecular weights greater than about 700. One example cited was in analysis of an oily-fish extract containing PCB's toxaphene and chlordane. Gel permeation allowed isolation of the combined pesticide mixture. Further cleanup on silica gel and alumina was required before the separate materials could be analyzed. NFIC-Denver plans to evaluate the application of gel permeation to industrial effluents.

IV. QUALITY CONTROL IN THE ORGANIC LABORATORY

There are two major categories to be considered when discussing analytical quality control in the organic laboratory. The first is the qualitative aspects of the analysis, that is, the degree of certainty that the unknown constituent has been correctly identified. The second category involves the quantitative aspects of the analysis, that is, the acceptability of the precision and the accuracy of the results obtained. A discussion of this subject is presented in the "Handbook for Analytical Quality Control in Water and Wastewater Laboratories", Analytical Quality Control Laboratory, Cincinnati, Ohio, 1972.

In regard to qualitative control, it was recognized by the Workshop participants that the first step requires checking and eliminating interfering background components from all reagents, solvents, glassware and other equipment employed in the analysis. Once the analyst has assured himself that interferences are not present, he must then recognize that selective extraction of particular compounds may occur depending upon the pH of the sample, the solvents used, and other factors. Consequently, various separation or "cleanup operations" may be required to provide additional support for the qualitative identification of specific compounds. Final qualitative identifications can be achieved by a variety of techniques. The current best methods are GC-MS, infrared spectroscopy, and multiple column gas chromatography. The latter technique is enhanced when a semispecific detector such as the FPD or Coulsen micro-coulometric can be used. Obviously in all cases of instrumental analysis, close control must be maintained of the instrumental parameters.

In regard to the quantitative aspects of quality control, both replicate and spiked sample analyses must be performed periodically to assure the precision and accuracy of the test; however, due to the complexition of organic analysis, time constraints are often the controlling factor in limiting the number of replicates or spiked sample analyses that can be performed. As a guide to the types of techniques that can be employed, several of the Workshop participants described the quality control procedures that they presently employ in their laboratories. These are summarized below.

William Loy, Chemical Services Branch, Southeast Water Laboratory -- At this laboratory all water samples are analyzed in duplicate. When samples are to be analyzed for a broad spectrum of industrial chemicals, selected samples are spiked with a mixture of six known organic compounds. These known compounds cover the range of volatile, basic, acidic, and neutral compounds at a concentration of 100 µg/l in acetone. Problems with recovery have been encountered only when large amounts of particulates are present in the sample.

James Lichtenberg, Methods Development Quality Assurance Research Laboratory -- In this laboratory one set of duplicate samples is run with each series that is analyzed, usually one duplicate for every nine samples. Simultaneously, one sample is dosed with a mixture of known compounds of the same class as those to be determined and analyzed along with the other samples.

Robert White, Wildlife Research Laboratory -- In this laboratory, which deals primarily with tissue analysis, every ninth sample is

repeated though not at the same time as the first analysis. The second analysis is randomly performed either by the same or different analyst. When running the repeat analysis, the chemist goes back to his primary reference standard-stock solution to insure that the standard used in the initial analysis was accurate. The results are independently reviewed by a second analyst before being reported. Control charts are maintained for several concentration levels using a computer program devised by this laboratory. From time to time, collaborative studies are conducted with other laboratories.

Dr. David Stalling, Fish Pesticide Research Laboratory -- This laboratory uses many of the techniques described above; however, they also use alternate test procedures to check upon the reliability of the reported results. Primarily, they use carbon-14 tagged materials to check each step in the analytical procedures. With this system, each analyst is required to withdraw 10 percent of the sample extract obtained from each step of the analysis, e.g., extraction, concentration, eluted fractions from cleanup steps, etc. These aliquots are then analyzed by liquid scintillation and the recovery in each step is determined. These results are then compared with the results obtained by routinely applied techniques, such as gas chromatography. So far, the technique has been applied primarily for quality control during tissue analysis and for such analysis, no GC interference is noted at the dosing levels required. However, in the analysis of the low levels of organics found in natural waters, such an interference may be a problem.

This approach to quality control appears to be quite intriguing, especially because once it is set up it is very easy to operate. Liquid scintillation counting requires a minimal amount of time and effort. Consequently, much more quality control information can be gathered than by conventional techniques. One of the main problems with this technique is the cost of the carbon-14 labeled compounds and the accessibility of a liquid scintillation counter. It is not inconceivable, however, that one central location could provide this service to many of the EPA laboratories.

A variety of other techniques were discussed by the Workshop participants which should help in the quality control program. For example, several of the participants use internal standards for both qualitative and quantitive purposes. In one laboratory, a known reference standard equivalent to the tentatively identified unknown is added to the sample and the gas chromatographic response compared to that produced by the reference standard alone. In another case, the response factor of a selected internal standard (not the same as the compound identified) relative to the compound to be identified is determined. This factor is then used for future calculations of the quantitative results.

In the quantitation of gas chromatographic peaks, it was generally observed by the Workshop participants that peak area is more accurate for later eluting peaks. Peak height, however, is best for very early eluting peaks. It should be noted that the volume of an injection affects the peak width and therefore the injected volume should be

close to the same for both the sample and reference standard. In all cases, the detector must be operated within its linear range.

Sample injection technique is critical during gas chromatographic analysis. A number of laboratories use the solvent-flush technique in which a small volume of pure solvent is pulled up into the barrel of the syringe before the sample. Upon injection, this pure solvent flushes all the sample from the needle and complete transfer of the sample is assured. When they are available, automatic sample injectors have been found to give very reproducible results and their use should be encouraged whenever possible. In all cases, the analyst is encouraged to use the technique best suited to him.

V. GENERAL COMMENTS

A variety of items not covered in the preceding chapters were brought up during the general discussion period of the Workshop. Some of these items are summarized below in varying detail.

A number of Workshop participants were greatly interested in the proposed list of Toxic Substances [*Federal Register*, Vol. 38, No. 173, Sept. 7, 1973)]. Much of this interest was in the form of concern for the brevity of the proposed list and questions as to why the materials listed were the ones chosen. As no answers to these questions were forthcoming, the discussion shifted to analytical procedures for measuring these toxic substances.

Most participants agreed that suitable procedures were presently available for measuring polychlorinated biphenyls and the chlorinated hydrocarbon pesticides, aldrin, dieldrin, toxaphene, etc. However, little information was available regarding the analysis for benzidine (4,4-diaminodiphenyl) and its salts.

The MDQARL is presently working on methods for benzidine. A colorimetric method is currently recommended [M. A. El-Dib, *JAOAC*, 54, (6), 1383 (1971)]; however, it is not specific for benzidine. Work is presently underway on a thin-layer modification of this method and a GC procedure, both of which would be more specific. It should be noted that the free base form of benzidine can be chromatographed on SE-30 columns and also on Tenax columns although some partial adsorption is observed with the latter.

Benzidine can be removed from water by carbon adsorption of the HCl salt; however, the salt apparently cannot be recovered from the carbon by chloroform or alcohol extraction. A search was made of the CCE extracts on file from the Surveillance Network of the FWPCA, and no benzidine was found.

The MDQARL has found that the free base can be quantitatively extracted from water at pH 10 with chloroform. Water samples of benzidine do not appear to be stable. Benzidine was found to react rapidly with Cincinnati tap water (presumably the chlorine) to form a precipitate. Even standards made up with distilled water turned cloudy within one week.

An alternate approach was suggested by Dr. David L. Stalling. Trifluoroacetic anhydride is a good derivatizing agent for amines, and Dr. Stalling suggests that this reagent may form derivatives with benzidine that will be easily chromatographed. This, of course, will need to be checked. With no other comments concerning the Toxic Substance List, the discussion turned to the Ocean Dumping Criteria.

These Criteria were recently promulgated by EPA. The Proposed Criteria appeared in the *Federal Register*, Vol. 38, No. 94, May 16, 1973 and the Final Criteria were published in the October 15, 1973 *Federal Register*, Vol. 38, No. 198, Part II. The Criteria lists a number of potential organic pollutants that require "special consideration" prior to issuance of a dumping permit. Consequently, many Workshop participants felt they may be required at sometime in the future, to analyze wastes for these materials and, as a result, they were quite interested in any information as to how to perform such tests.

First on the list were organosilicon compounds. No one at the Workshop was aware of any pollution problems associated with organosilicon compounds and consequently it was unclear just what compounds would be of most concern.

In regard to other organometallic pollutants, it was evident that little work had been done in this area. The National Water Quality Laboratory at Duluth has apparently looked very briefly at organocadmium and organocopper compounds. The Edison Laboratory has had some experience with organolead materials in oil wastes.

The Ocean Dumping Criteria also listed aliphatic solvents as waste components that require "special consideration." A variety of methods appear to be available for this analysis, namely, direct aqueous injection, head space analysis, GC analysis of volatile components trapped on Chromasorb 101 or other material following purge by inert gas, and finally, extraction with a high-boiling solvent such as hexadecane followed by GC analysis. The method of choice would depend upon the needs of the particular laboratory although all the cited procedures seem workable.

The Workshop participants felt that they could test for phenols either by the steam distillation - 4-aminoantipyrine - procedure in *Standard Methods* or by the gas chromatographic procedure in the *ASTM Manual, Part 23.*

Plastics, plastic intermediates and byproducts seemed to be an unknown quantity to the Workshop participants. Undoubtedly, many compounds in this category could be identified by gas chromatography/mass spectrometry following work up procedures previously discussed,

i.e., phthalate plasticisers [D. L. Stalling, *et. al., Environmental Health Prospectives*, 159 (1973)]. However, before additional tests can be considered, we will need more information concerning just what compounds in this category actually represent a pollution hazard.

Analytical procedures for amines were discussed previously.

Polynuclear aromatic hydrocarbons can be identified by a variety of procedures. Several participants felt that the easiest procedures to apply were colormetric, as recommended by the World Health Organization, and thin-layer chromatography [E. Sawicki, *et. al., Health Lab. Sci. 7* (1)68 (1970)] even though the specificity of these procedures is unknown. In addition, many of the aromatic hydrocarbons can be separated and identified by gas chromatography. Participants recommended columns of OV-1, Dexil, and Apiezon L. Undoubtedly, others are available. Liquid chromatography has also been used and appears to hold considerable promise [N. F. Ives and L. Giuffrida, *JAOAC*, 55, (4), 757 (1972)]. In tissue samples, polynuclear aromatic hydrocarbons can be identified by fluorescence following a rigorous clean-up procedure [J. W. Howard, *et. al., JAOAC*, 51, 122 (1968); *AOAC Methods, 11th Ed. 21.001*, pg 361 (1971]. From the above discussion, it was evident that a number of potentially suitable procedures are available, however, at present, none of the participants had applied any of these tests to industrial waste-waters, sludges, or dredge spoil.

Little work has been done on detergents other than extraction of ABS or LAS by the MBAS tests described in *Standard Methods*. The MDQARL has used TLC procedures to identify polyoxyethylene-type detergents in CCE extracts from carbon filters [*ASTM Special Tech. Pub.* No. 448, p 78

(1969)]. No other analytical procedures were mentioned by the Workshop participants. This may be a potential-problem area since there are published references to the extreme toxicity of some surfactants to aquatic life [D. J. Wildish, and W. G. Carson, *Fisheries Research Board of Canada Report*, Series No. 1212, October (1972); D. J. Wildish, *Water Research*, 6, 759 (1972)].

A brief discussion took place at the Workshop regarding the limiting-permissible concentrations of pollutants listed in the Criteria. The final revision of the Criteria uses the bioassay tests as the basis for limiting the concentrations of pollutants. It was generally agreed by the Workshop participants that neither bioassay nor concentration limits would be satisfactory by themselves. Hopefully, sometime in the future, limitations will be based upon some suitable combination of pollutant concentrations and bioassay information.

In regard to bioassay and toxicity studies, it was pointed out that a number of computer-based information systems are presently available that store toxicological information. Should any of the EPA offices need such information, NFIC-Denver is tied into a number of these library systems, notably, TOXICON and others, and will be glad to assist in gathering the necessary data.

At the conclusion of the Workshop, it was brought out that a real need exists for some simplified, screening methods of analysis. Quite obviously, the complexities and time requirements of detailed organic analysis of industrial wastes preclude the ability to monitor a large

number of waste streams. Hopefully, procedures can and will be devised whereby a large number of samples can be quickly screened and only those that test above a certain level will need to be set aside for detailed analysis. Certainly that is a worthwhile goal and we would encourage any thoughts on the matter.