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CURRENT PRACTICE IN GC-MS ANALYSIS OF ORGANICS IN WATER

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CURRENT PRACTICE IN GC-MS ANALYSIS OF ORGANICS IN WATER

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ABSTRACT

Experiences during five years of evaluating the application of gas chromatography-mass spectrometry (GC-MS) to wastewater analysis at the Southeast Environmental Research Laboratory have resulted in the selection of recommended practices for such applications. Liquid-liquid extraction with solvents such as methylene chloride and chloroform removed greater than 50 percent of compounds found in pulp mill and petrochemical waste at concentrations of 2 µg/ℓ to 20 µg/ℓ. The Kuderna-Danish evaporator was the most effective means of concentration after extraction. Diazomethane and dimethyl sulfate proved to be the most effective of five methylation reagents studied. Packed columns were effective for gas chromatography of simple mixtures and SCOT columns provided better overall performance for complex mixtures. Computerized data reduction was essential for practical use of GC-MS for samples containing many compounds. A computerized spectra matching program proved highly effective in identifying compounds contained in the computer library. The system was shown to be effective in solving problems related to fishkills caused by pesticides, confirmation of polychlorinated biphenyl residues in water and identification of compounds discharged by over a dozen industries. Over two hundred compounds were identified in industrial effluents.

CONTENTS

FIGURES

TABLES

SECTION I

RECOMMENDATIONS

Gas chromatography-mass spectrometry should be used as the first approach to analysis of organic water pollutants when the identities of the organic constituents in the sample are in doubt. Computerized data reduction and data interpretation are recommended to improve efficiency and to eliminate the necessity for an interpretation specialist at every laboratory.

The procedures described in this report are recommended as a first step toward eventual development of a method suitable for corroborative testing and consideration as a standard method.

SECTION II

INTRODUCTION

Currently, the most effective method for identification of organic pollutants in water is gas chromatography-mass spectrometry (GC-MS).

Any compound that can be gas chromatographed without decomposition can be analyzed by GC-MS. Appendix 1, containing over two hundred compounds identified in industrial wastes, illustrates the wide applicability of GC-MS. However, some classes of compounds are better suited to other methods. Polymers, sugars, free amino acids and many other biologically derived compounds are not directly amenable to GC-MS because of their non-volatility.

One evidence of the recent popularity of GC-MS is the purchase of nineteen instruments within the last two years by EPA laboratories throughout the country. Significant time will be saved if these new users have the benefit of five years experience at the Southeast Environmental Research Laboratory in sample preparation, GC conditions, data reduction, interpretation of spectra, and all the other facets of this type of analysis. This report describes the most successful current practice in these areas and illustrates the power of GC-MS in pollution analysis.

SECTION III

SAMPLE HANDLING

Collection

Lake and river waters are not homogeneous. Industrial effluents also change with time. Some investigators prefer to take subsamples from various locations and at different times and to combine them for analysis. Many chemists avoid filtering the sample because the colloidial and suspended particulates frequently have organics absorbed on their surfaces.

Glass jars closed with Teflon-lined caps are preferred for sample containers. The jars and caps should be thoroughly cleaned with soap and water, rinsed with distilled water, and rinsed with the solvent that will later be used for extraction. After thorough drying, the jars should be tightly capped until the sample is taken. Additional jars should be prepared for distilled water blanks.

For qualitative analysis, plastic bottles can be used to collect samples that contain high concentrations of organics. For these samples, e.g., paper mill effluents and municipal sewage, the adsorption of organics on the walls of the bottle and the release of plasticizers (phthalate esters) into the sample are insignificant for short periods of storage.

The volume of sample collected is usually one or two liters. Compounds present in a one-liter sample at concentrations of 2 μg/ℓ or greater will generally give good quality spectra when processed by extraction, concentration, and GC-MS techniques. For greater sensitivity larger samples are required. For example, twenty-liter samples of municipal sewage have been processed with an apparent detection limit of 0.1 μg/ℓ based on an internal standard added to the extract before concentration.

Because samples may have to be transported or stored for considerable time before analysis, some means for stopping biological action and preventing chemical

changes is needed. Freezing is the preferred method. The addition of strong acid as a preservative is not recommended because it degrades some sensitive compounds such as geosmin and 2-methyl isoborneol (taste and odor causing compounds). Some space must be allowed in glass containers for the expansion of water as it freezes. Some workers find such a high proportion of breakage on freezing that they prefer to pack the bottles in ice for shipment and store them in a refrigerator at about 4° C. Plastic containers, particularly the popular cubitainers, do not usually rupture on freezing.

Samples are conveniently shipped by air freight. They are usually frozen, placed in styrofoam containers for insulation and protection against breakage, and further packed in dry ice in an inexpensive styrofoam chest.

Extraction

The first step in GC-MS analysis is extraction of the organic compounds from the bulk of the water. Commonly, a liter sample of water is extracted with two or three 50 to 100-ml portions of solvent. The combined extracts are dried and the solvent is evaporated to 1 ml or less for GC analysis. Recovery studies on compounds found in petrochemical refinery wastes and paper mill effluents show the effectiveness of this procedure. A liter of water, spiked with knowns, was extracted with three portions (100/50/50 ml) of chloroform (duplicates were extracted with methylene chloride), dried, and evaporated to 500 to 200 microliters for injection and quantitation by flame ionizetion GC. The recoveries were 50 to 100% at the 20 and 2 µg/ℓ levels. No significant differences were noted between methylene chloride recovery and chloroform recovery; however, methylene chloride is more likely to form emulsions. Compounds with high vapor pressures (methyl styrene, cymene, indene, methyl indene, terpinolene, camphor) gave recoveries of 50-70%, whereas low vapor pressure organics (indole, naphthalene, quinoline, biphenyl, dimethyl naphthalene, diphenyl methane, acenaphthene, bibenzyl, carbazole, benzophenone, phenanthrene, fenchone, triphenylmethane, fenchyl alcohol,

α-terpineol, and guaiacol) gave 72-100% recoveries.

Other solvents in common use are petroleum ether, hexane (also 15% diethyl ether in hexane), diethyl ether, and carbon tetrachloride. Petroleum ether and hexane are so non-polar that they do not effectively extract polar materials such as fatty acids and phenols. Ether is not recommended (except in very small quantities for diazomethane methylations) because of its flammability and inefficiency as a solvent, the danger of explosion from peroxide impurities, and the presence of additives. Various preservatives are added to ether to inhibit peroxide formation but are not usually listed on the container label. One peroxide inhibitor identified by GC-MS and later confirmed by the manufacturer was 2,6-ditertiarybutyl-p-cresol. This compound was recognized as an artifact because it also occurred in the blank. Finally, in solvent comparison studies, ether was found to be only half as effective as chloroform in extracting a paper mill effluent.

Carbon tetrachloride is sometimes preferred because it gives a narrow solvent peak on some GC columns; however, it is less efficient than chloroform for extraction of polar compounds. Carbon disulfide and various freons have the advantage of very high volatility but are difficult to purify and usually show numerous impurity peaks. Additionally, carbon disulfide is very flammable.

Extraction is affected by the pH of the sample. Organic acids are ionized salts in basic solution and will not extract into organic solvents. Therefore samples containing fatty acids (municipal sewage) or phenols (forest runoff) are best extracted at a pH <5 to insure maximum covalent character. Similarly, basic materials are best extracted at high pH. A complete, but somewhat involved, procedure for these "solubility class" separations was given by Braus, Middleton and Walton (1). We recommend extracting samples whose original pH is 5-7.5 with solvent to isolate the neutral compounds. Then the sample is acidified to pH 3 or less with hydrochloric or sulfuric acid and extracted to isolate the acids

and phenols. To isolate basic compounds, the acid solution is adjusted to pH 10 and extracted. Usually very few basic compounds are found. Many variations in procedure are possible and each problem requires an individual judgment.

Several operational details help make extraction simpler or more effective. Pesticide quality (distilled-in-glass) solvents are recommended. The separatory funnel should have a Teflon stopcock and the glass stopper should not be lubricated. If the sample contains suspended matter, a representative portion of it should be included in the sample for extraction. The first portion of extracting solvent should be used to rinse the sample container and then added to the separatory funnel. Some samples, particularly those from anaerobic sites, will form an emulsion or deposit a sludge in the solvent layer. One of the main components of this material is elemental sulfur. Some of the sulfur dissolves in the solvent and precipitates as a whitish solid when the extract is concentrated by evaporation. It usually chromatographs as a single peak during GC-MS analysis and gives a molecular ion at m/e 256 (S_8) and fragments from successive losses of 32 mass units.

To separate the sludge from the solvent or to break an emulsion, place a 2.5 cm ball of glass wool in a 2.5 cm diameter plain chromatography column--no frit--and, after wetting the wool with fresh extraction solvent, pour the emulsion through the glass wool. Ignore the debris that remains. It may be necessary to force the emulsion through with a little air pressure.

After a sample is extracted, a little water may remain dissolved in the solvent. The analyst can either dry the extract before concentration or concentrate the solvent directly. No studies have been done to show which is best. Drying is usually accomplished by filtering the extract through a column of anhydrous sodium sulfate (200 ml of solvent through a 5 cm X 2.5 cm diameter column is common). The sodium sulfate should first be heated to 500° C for 2 hours to remove organic impurities, usually phthalate esters. Another

method for drying is to pour the extract through a pre-wet glass plug as described earlier.

Some analysts simply concentrate the extract and inject it on the GC. With non-chlorinated solvents even the ultra-water-sensitive electron capture detector will accept this concentrate.

Numerous other methods have been tried to separate and concentrate organic materials. In our experience freeze concentration and steam distillation are vastly inferior to extraction. Carbon adsorption has limited use but the labor involved in processing makes it unpopular. Polyurethane foams are now being used to extract polychlorinated biphenyls directly from water. These foams, coated lightly with DC-200, also extract organochlorine pesticides. Our tests show that neither material is effective for extracting dissolved oil, phenols, or terpenes. Organic resins, in particular Rhom and Haas' XAD series, show much more promise but applications are still in the developmental stages.

Kuderna-Danish Evaporation

The best method for concentrating the organic extract is distillation with a Kuderna-Danish evaporator (Figure 1), available from Kontes. The bottom and middle flasks are filled to about half the nominal flask capacity. Boiling chips are added and the whole assembly is placed on a boiling water bath or a steam bath in a hood. The water level should be maintained just below the bottom joint and steam should bathe the rounded bottom of the middle flask. Some people let distillation become just vigorous enough to maintain liquid in the upper bubble joint; others feel that it is equally efficient, as well as quicker, to allow moderate splashing out the top. Distillation is usually continued until about one ml of liquid remains in the concentrator tube; the whole assembly is then removed and allowed to cool. As the liquid drains from the condenser it rinses the residue from the walls of the middle flask. The final volume is about five ml.

FIGURE 1. KUDERNA-DANISH EVAPORATOR

The calibrated concentrator tube is removed and placed in a beaker of warm water (<70°) and the volume reduced to any desired level, usually one ml, by directing a stream of clean dry air or other gas over the surface. A fresh glass disposable pipette is used as an air jet for each sample. The pipettes are connected to the air supply with Tygon tubing. Analysis of blanks so treated have not indicated the presence of volatile plasticizers from the tubing. If the extracting solvent is not compatible with the GC system (if it tails badly and obscures parts of the peaks), four or five volumes of a more desirable solvent are added and the volume is again reduced to the volume necessary to give adequate GC sensitivity.

Several studies (2-4) have shown that considerable loss of the extracted compounds takes place during air evaporation. For very volatile compounds, 50% loss on evaporation to about 1 ml is common; for less volatile materials such as DDT-type pesticides, losses are only a few percent. Fortunately the concentration factor increases faster than the loss factor. Therefore, enough compound for a good mass spectrum is concentrated into a solvent volume acceptable to both the GC and the MS. If the sample must be stored overnight or longer the tube should be closed with a glass stopper and placed in the refrigerator. Some prefer to store extracts in Reacti-Vials or other screw-cap vials fitted with a Teflon cap liner.

The Kuderna-Danish method is preferred to a rotary evaporator because there is less danger of contamination in the all-glass apparatus, less danger of bumping with loss of sample, and less loss due to handling since transfers do not have to be made from large round bottomed flasks to a calibrated container.

Clean-Up Techniques

When extracts of water are analyzed by GC for specific organic compounds, e.g., pesticides, the extract usually must be "cleaned-up" to remove interferences. The most widely used technique is Florisil or silica gel column chromatography, sometimes in combination with acetonitrile partitioning. Thin-layer chromatography,

preparative GC, and liquid chromatography are used less frequently. Fortunately, since the mass spectrum of a compound is much more definitive than its GC retention time, extracts for GC-MS analysis generally need not be cleaned up, particularly when solubility class separation has been used during sample extraction.

If preliminary GC runs show that clean-up of the extract is necessary, the concentrated extract is chromatographed on activated florisil. In the original technique (5), increments of solvent containing increasing proportions of ethyl ether in petroleum ether are used to elute fractions containing increasingly polar organics. We use methylene chloride rather than ethyl ether. This technique was used with a textile plant effluent being analyzed for dieldrin by GC-MS (6).

As another approach, after solubility separation of the crude extract, the neutral fraction may be further separated on a silica gel column. Elution with isooctane, benzene, 1:1 chloroform:methanol separates organics into aliphatic, aromatic, and oxygenated organic fractions, respectively (7).

Thin layer chromatography is sometimes used as a preliminary separation technique for GC-MS analysis. Samples of industrial and municipal effluents contain so many components that direct TLC of extracts usually gives only unresolved streaks. In a few case intermediate clean-up by TLC is useful, resulting in the separation of bands of compounds of the same type. This type of sample clean-up has been used in the identification of pesticide metabolites by GC-MS (8).

Preparative gas chromatography may be used as a preliminary separation technique. In many cases initial GC runs of crude sample extracts result in several overlapping peaks, even under the best of chromatographic conditions. These overlapping portions of the effluent are collected in capillary tubes, cooled if necessary by dry ice or a tissue soaked in acetone, and rechromatographed on a high resolution (usually capillary) column. With this less complex mixture, GC parameters may be varied more freely to obtain better separation. Preparative

GC was used in the separation and identification of polychlorinated biphenyl (PCB) isomers (9). In this case, as with some water samples, several sharp preparative chromatographic peaks that appeared to be one component each were shown by capillary GC to consist of two or more components.

Liquid chromatography (LC) seldom resolves sample components as well as GC, but may be considered as a preliminary separation tool in some cases. Volatile compounds collected from a LC eluate may be analyzed directly by GC-MS if the LC eluting solvent is compatible with the GC-MS system.

One advantage of LC is its amenability to chromatography of aqueous samples. Furthermore, ion-exchange LC columns provide for the separation of water soluble, non-extractable organic compounds. One established procedure (10) calls for concentration of the original water sample by direct evaporation and/or freeze drying. The concentrated sample is dissolved in a buffer solution and chromatographed on a high pressure anion-exchange column. Fractions, usually representing several overlapping peaks, are collected in their buffered eluting solution and the solution is freeze-dried. The solid residue is derivatized to make volatile components that may be analyzed directly by GC-MS.

SECTION IV

DERIVATIVE FORMATION

Many compounds are not volatile enough to be transformed to the gas phase without decomposition. Such polar compounds as carbohydrates, amino acids, sulfonic acids, and nucleic acids are not directly gas chromatographable. Some steroids, carboxylic acids, and phenols can be directly chromatographed in their free form, but require special columns and techniques not readily available. Phenols "tail" on most GC columns, steroids elute too slowly, and only recently have columns become available for the efficient separation of free long-chain carboxylic acids.

Derivatization reagents are now available for all these classes of compounds. To be useful for GC-MS analysis, a derivative must (1) be formed quantitatively from the free precursor by a rapid reaction with a readily obtainable reagent, (2) be volatile enough for vaporization in the GC inlet, (3) be thermally stable in the GC-MS system, and (4) be of a chemical class that has been studied extensively by mass spectrometry so that a spectral library is available for matching. Methyl derivatives, in the form of methyl esters of carboxylic acids or methyl ethers of phenols, and trimethylsilyl (TMS) derivatives of carbohydrates, steroids, phenols, amines, carboxylic acids, and amino acids are the most common.

Trimethylsilyl Derivatives

A variety of TMS reagents are available, some of which are tailored for specific functional groups. These reagents generally react with groups containing active hydrogen atoms (-PH, -SH, -NH, -COOH) to replace the hydrogen with a trimethylsilyl [$-Si(CH_3)_3$] moiety. Suggestions for the use of TMS reagents and information on their chromatographic and mass spectrometric properties have been collected by Pierce (11).

TMS derivatives of about fifty urinary constituents separated by high-pressure anion-exchange liquid

chromatography were prepared to increase their volatility for identification by GC and MS (12). TMS derivatives are also being used in the identification of municipal waste components separated by liquid chromatography (10).

Methyl Derivatives

Both diazomethane and dimethyl sulfate are used routinely to prepare the methyl esters of carboxylic acids and the methyl ethers of phenols.

Diazomethane. The diazomethane methylation method is simple, fast, and almost quantitative for carboxylic acids (Table 1). The procedure for diazomethane methylation is given in Appendix 2. It is the method of choice for extracts in which carboxylic acids are the most important compounds to be studied. It has been applied very successfully to the methylation of acid fractions of municipal wastes. However, diazomethane has two important disadvantages as a methylating reagent: it does not react quantitatively with all phenols, and some undesirable side reactions occur (13).

Although pentachlorophenol reacts quantitatively and other chlorinated phenols have been observed to produce methyl ethers with diazomethane, guaiacol, vanillin, and other phenols found in paper mill wastes give poor yields (Table 1).

Samples that have been extracted with chloroform, evaporated to nominal dryness and then redissolved in ether and methylated, frequently contain half a dozen or more di- and trichloroalkanes (C_2 through C_7). These are formed by the reaction of the residual chloroform and diazomethane, a reaction enhanced by exposure to uv light and excess diazomethane (14). Since methylene chloride does not react to form interfering peaks, it is now routinely used to extract samples that will be methylated with diazomethane. Another advantage of methylene chloride is that it does not have to be completely evaporated before methylation; loss of volatile phenols is thereby decreased.

Table 1

Methylation Efficiencies of Four Reagents

Compound	% Yield*				
	Diazo-methane	Dimethyl sulfate	$HCl-CH_3OH$	"Methyl-8"	"MethElute"
Guaiacol	15	90	0	0	85
Vanillin	40	70	0	0	55
Palmitic acid	80	60	90	50	70
Dehydroabietic acid	95	60	10	85	70

*Yield of methyl ether or ester, based on GC peak heights. These yields also represent losses occurring upon evaporation of solvents and other procedural errors.

Dimethyl Sulfate. In a comparison of methylation techniques using the acid fractions of kraft paper mill effluents, dimethyl sulfate gave much better yields of phenolic methyl ethers than did diazomethane (Tables 1 and 2). Dimethyl sulfate also methylates the resin acids and fatty acids found in paper mill wastes fairly well, but was not successful in methylating the acid fraction of municipal wastes (Table 2).

Dimethyl sulfate is recommended for methylation of extracts containing phenols. The procedure for dimethyl sulfate methylation is given in Appendix 3. An advantage of this method is that the aqueous sample is used directly, after extracting bases and neutrals. An important disadvantage is the time required--about four hours.

Others. Three other methylation reagents tried are compared with diazomethane and dimethyl sulfate in Tables 1 and 2. Methanolic hydrochloric acid (6N) reagent (15) (Table 2) was used with municipal waste with results comparable to those obtained with diazomethane. However, it does not methylate pure vanillin or guaiacol, and the esterification yield with dehydroabietic acid was only 10% (Table 1). The reaction is slower than diazomethane methylations, and the solvent is restricted to methanol. There are also problems with neutralization of the HCl.

"Methyl-8", dimethylformamide dimethyl acetal (16) was found to methylate palmitic acid in fair yields and dehydroabietic acid in good yields, but it did not methylate the phenols (vanillin and guaiacol). It methylated the municipal waste fraction nearly as well as diazomethane and is therefore recommended for samples containing only carboxylic acids.

The most convenient reagent tested is "MethElute", a 0.2 M solution of trimethyl anilinium hydroxide in methanol (17). The sample extract is dissolved in this reagent, and the solution is injected into the GC inlet where the methylation reaction occurs. By-products and excess starting materials appear near the solvent peak, followed by the later-eluting ethers and esters. Methylation of municipal waste and paper mill acid fractions gave results comparable to

Table 2

Qualitative Comparison of Methylation Reagents

Reagent	Phenols		Fatty Acids		Resin Acids	
	Paper mill waste	Municipal waste	Paper mill waste	Municipal waste	Paper mill waste	Municipal waste
Diazo-methane	P	F*	G	B	G	--
Dimethyl sulfate	B	P	B	P	G	--
"MethElute"	G	--	G	G**	G	--
"Methyl-8"	P	--	P	G	B	--
HCl-MeOH	--	--	--	E	--	--

B=Best, E=Excellent, G=Good, F=Fair, P=Poor

*Only one sample tested; chlorinated phenol methyl ethers were detected but yields are unknown. Cresol was not methylated.

**Two or three reagent peaks formed--they did not interfere in this analysis.

those using diazomethane. Because this reagent methylates both carboxylic acids and phenols in fairly good yields, as seen in Table 1, it has great potential but should be tested on extracts from other sources.

Ozonolysis

Ozonolysis can be used as a derivatization technique. Many unsaturated compounds can be converted to aldehydes or ketones through formation and cleavage of ozonides. The aldehyde and ketone products may be identified, leading to deduction of the original position of unsaturation. The ozone for the reaction can be generated using commonly available laboratory apparatus (18). This technique was used to differentiate the unsaturated fatty acids from their saturated analogs in municipal sewage by comparison of a sample's gas chromatogram before and after ozonolysis.

SECTION V

GAS CHROMATOGRAPHY

Most samples require some gas chromatographic experimentation to work out the optimum, or at least a useful set of chromatographic conditions prior to GC-MS analysis. Therefore, an additional gas chromatograph is useful to avoid wasting MS instrument time while gas chromatographic conditions are being determined. Glass-lined injectors should be used in both GC's to avoid decomposition of the sample by initial contact with hot metal. The detector of the auxiliary GC should be flame ionization to achieve sensitivity similar to that of the MS. The additional gas chromatograph should be of the same type as the one interfaced with the mass spectrometer because, after optimum chromatographic conditions are determined, the smallest change will affect the peak separation of a complex mixture. Often the column from the auxiliary GC is transferred to the GC-MS for the final analysis.

Columns

Packed Columns. The 1/8 inch diameter packed columns commonly used in GC laboratories are inexpensive and readily available. Most mass spectrometers will accept them. The analyst must be careful not to inject the large amounts of solvents (5-10 microliters) traditionally used because this raises the gas pressure in the MS past acceptable limits. For routine surveys in which only the more concentrated pollutants are of interest, packed columns are most often used.

Capillary Columns. Although capillary columns offer the ultimate in peak resolution they are expensive. They accept only small amounts of sample and require long separation times.

Support-coated-open-tube (SCOT) columns 0.02 inches in inside diameter, are a compromise between the traditional packed column and the true capillary column. As such, they offer some advantages of both:

- better peak resolution than packed columns with complex mixtures
- moderate size sample injections (usually 1-2 μℓ)
- chromatographic times not much longer than with packed columns (usually 20-40 minutes)
- broad choice of support-coating combinations

The technology involved in preparing them is so complex and involved that it is cheaper to buy them commercially than to develop the expertise and equipment to make them. Perkin-Elmer SCOT columns are normally terminated with a male nut that fits the PE 900 series gas chromatographs. To render these columns useful with other gas chromatographs, these male nuts need only be pushed back and replaced with 1/16-inch female slotted nuts in which the slot is made just large enough to accept the capillary tubing. Although these columns are relatively expensive, they can be returned to the factory and recoated when column deterioration becomes evident (broadened and/or tailing peaks). Some of our columns have been recoated 4 or 5 times without apparent effect on their separation efficiencies.

Column Coatings. Many liquid phases and supports are on the market. The two we have found most useful are SE-30 (non-polar) and Carbowax 20M terminated with terphthalic acid (polar). We also keep on hand DEGS, OV-17, OV-101, OV-225, DC-200, DX-300, QF-1, LB-550X, and Apiezon-L for special needs.

Flow Rate

Helium is the usual carrier gas for GC-MS systems and should also be used as the carrier gas in auxiliary gas chromatographs. The optimum carrier gas flow for GC-MS systems operating under vacuum and using a Gohlke separator is 16-18 ml/min. If about half this helium flow is used for optimizing conditions with the auxiliary GC (operating under atmospheric pressure), the other chromatographic conditions (program rate, initial temperature, initial hold) will hold when the column is

transferred to the GC-MS. The resulting chromatogram (or computer-reconstructed chromatogram) will closely approximate the chromatogram obtained on the auxiliary GC.

Solvents

Chloroform and methylene chloride are excellent solvents for extraction. They give smaller and narrower solvent peaks than hydrocarbon solvents in GC using flame ionization detection. Unfortunately in the inlet of a MS their ionization cross-sections are such that they easily trip the electronic protection circuit and cause automatic machine shutdown. As a result, no more than 1.8 µℓ of chloroform or methylene chloride solutions can be injected into our GC-MS system without taking special precautions. With solvents such as toluene, hexane, and other hydrocarbons, which do not cause this problem, up to 3 µℓ samples can be injected.

Temperature Programming

Temperature-programmed GC is nearly always used for GC-MS analysis. A medium program rate of 4° per minute is common. Frequently the program is extended to the upper limit recommended for the liquid phase. At the resulting high temperatures, considerable bleed occurs with many columns. For example, MS fragments at m/e 73, 147, and 221 resulting from dimethyl silicones appear when the usually stable silicone columns are overheated. The program should therefore be terminated short of these high temperatures.

When the initial temperature is too high, many components of interest elute with or close to the solvent. This may explain the absence of low molecular weight compounds from Appendix I. Limited experience with sub-ambient temperature programming indicates that this approach is feasible for the analysis of such compounds.

SECTION VI

MASS SPECTROMETRY

Instrumental

The first GC-MS system used at SERL in 1968 was a Perkin-Elmer/Hitachi RMU-7 double-focusing mass spectrometer connected to a Perkin-Elmer 900 gas chromatograph through a Watson-Biemann separator. The data output was to be collected and processed by computer but this portion of the system was never fully developed. Without a workable computer system, a typical twenty peak chromatogram required approximately twelve hours of manual data handling before interpretation could begin. Despite these limitations, the first system demonstrated the power of GC-MS by providing answers to problems considered too complex for other types of instrumental analysis (6, 19). Based on almost three years' experience with this system, a set of MS system criteria was prepared for use in testing and evaluating the GC-MS-computer systems available in 1971. These criteria, modified slightly (Table 3), are still valid.

Two GC-MS-computer systems (the Varian CH-7 and the Finnigan 1015) were shown to satisfy these criteria (20). Later, during 1972, an improved version of the DuPont 21-490 system was also shown to satisfy the criteria. The less expensive Finnigan system, outlined in Figure 2, was chosen and installed during 1971.

The GC, a vendor-modified Varian Model 1400 with no independent detector, serves as a specialized inlet to the mass spectrometer.

The Gohlke separator is an all-glass jet separator that separates organic samples from the helium carrier gas based on differences in the diffusion rates of the gases in a turbulent jet. Its small surface area eliminates chemisorption and catalytic degradation and thus overcomes the chief disadvantage of the Watson-Biemann fritted glass separator.

Table 3

Minimum Acceptable Criteria for
Mass Spectrometer System

Mass Spectrometer	
Scan rate	2 sec/decade or 230 amu/sec
Mass range	12-650 amu
Resolution	1/400 or unit throughout range
Minimum identifiable level of pesticide	10 ng in GC inlet
Ionizing voltage range	10-100 volts
Data System	
Field experience	Both software and hardware proven in the field
High speed storage and retrieval	Magnetic tape
Data acquisition mode	Continuous cyclic scan
Calibration method	Semi-automatic
Minimal software	Computer reconstructed chromatogram, background correction, and spectrum plotting routines

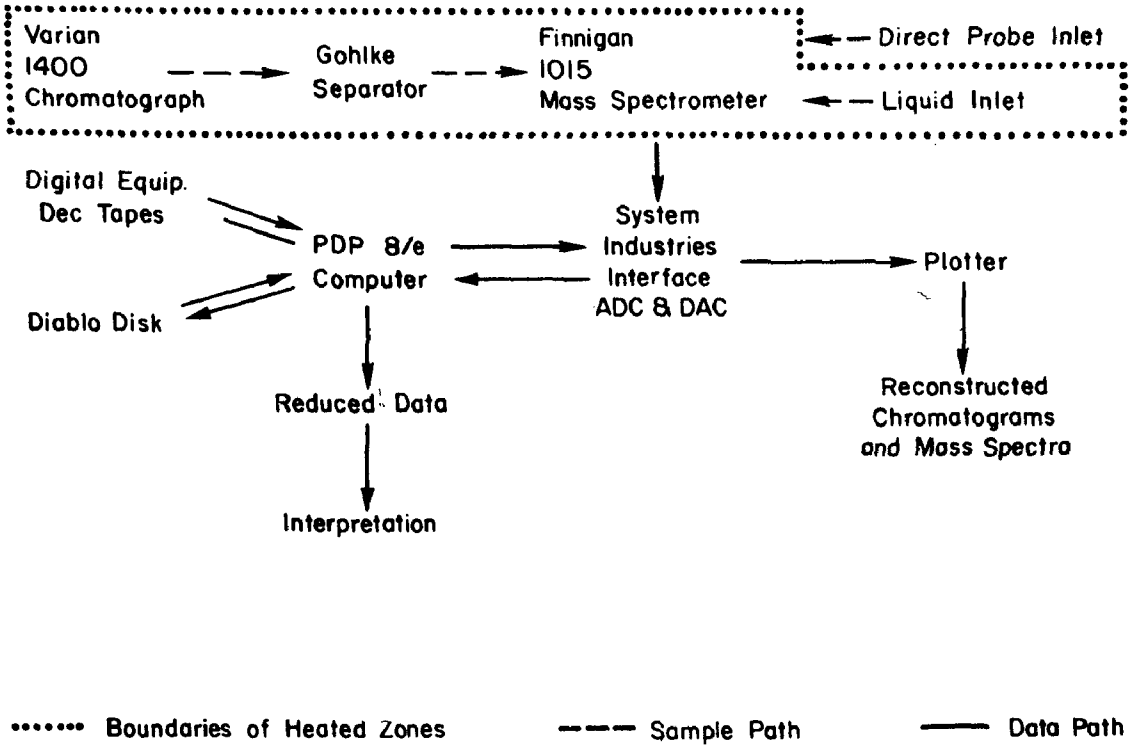

FIGURE 2. OUTLINE OF FINNIGAN GC-MS-COMPUTER SYSTEM

The Finnigan 1015 mass spectrometer is a quadrupole instrument having a range of 1-750 amu. Resolution is one mass unit throughout the range (e.g., 1/20 at mass 20, but 1/625 at mass 625). As a consequence, instrumental sensitivity at low mass is much higher than in a magnetic instrument. At a scan speed of 120 amu/sec the sensitivity is adequate to give identifiable spectra from 20 ng of material introduced into the GC inlet.

The liquid inlet is used for introduction of calibrating compounds, the direct probe for solid materials.

The System Industries interface and the analog-to-digital and digital-to-analog converters are used to permit the Digital Equipment Corporation (DEC) computer to control the mass spectrometer during calibration, data acquisition, and checking; to accept data from the mass spectrometer; and to control the Houston plotter during data reduction.

The DEC PDP 8/e computer, the heart of the data system, has a 4096 word core and an ASR33 teletypewriter. Programs, raw data, and reduced data are stored on either the two DECtape units or the Diablo disk. Output of reduced data is achieved under computer control via the plotter, the teletypewriter, or a coupling device. The coupling device connects the PDP8 via telephone lines to a large computer and permits semi-automatic spectrum identification by a matching procedure described in Section VII.

Operation

The basic steps in processing a sample by GC-MS computer are listed in Table 4 and can best be illustrated by reference to an example.

The mass spectrometer is calibrated with perfluoro-n-tributyl amine, which gives a number of characteristic fragments in the mass range 50-614. The computer uses the signals from these fragments to establish the relationship between the digital-to-analog converter values and the m/e values.

Table 4

Steps in GC-MS-Computer Data Reduction

1. Formation of calibration reference file
2. Data acquisition
3. Reconstructed gas chromatogram plot
4. Manual selection of GC peak and background spectra
5. Creation of background corrected spectra files
6. Output of plotted spectra
7. Interpretation of spectra

After the sample is injected into the GC, the mass spectrometer automatically scans its pre-set mass range every five seconds (or other pre-set interval). As it does so, the plotter draws a trace that is equivalent to a GC signal (lower trace in Figure 3). If the sample was pre-analyzed on an auxiliary GC, this trace can be used to determine when to terminate the run.

After the run is complete, the computer plots a reconstructed gas chromatogram (RGC) like that shown in the upper trace in Figure 3. The largest peak is automatically plotted at amplitude 100 and the other peaks are shown proportional to it. Each point on the spectrum number scale under the RGC represents a complete mass spectrum collected on magnetic tape. These spectra can be displayed individually and thus the analyst can see the composition of each peak.

Consider the peak at spectrum number 287 (marked with an arrow in Figure 3). The spectrum stored on the tape will be that of one of the sample compounds plus various fragments from column bleed, traces of air, oil, moisture, and perhaps a small residual amount of material from the peak that eluted just prior to the one at 287. These background fragments may mask some of the peaks of

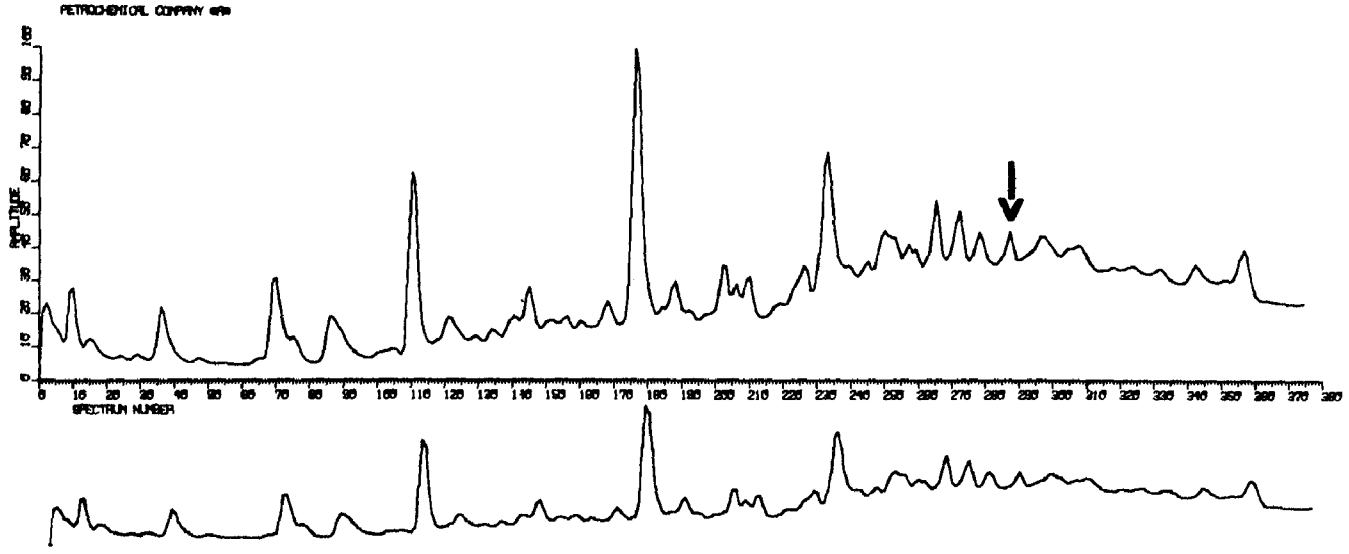

FIGURE 3. RECONSTRUCTED GAS CHROMATOGRAM (RGC) OF A PETROCHEMICAL PLANT EFFLUENT EXTRACT

the sample. To overcome this problem, the operator selects a spectrum that contains nearly the same background and none of the compound fragments and has the computer subtract the background from the sample. In this example a logical choice is the chromatographic valley at spectrum number 284. The resulting mass spectrum plotted by the computer is shown in Figure 4 (the compound structure and M^+ were added manually).

The processing of the MS data that formerly required many hours of hand calculations and graph plotting now takes less than three hours, and most of this time does not require operator attention. Only 30 minutes of operator time are required to enter into the computer the instructions to output the reduced data for a 20 peak chromatogram. Elapsed instrument time to reduce the data ranges from slightly more than one hour for the disk system to more than two-and-a-quarter hours for the tape system. The time difference is due to the time lost by the computer while searching the tapes for the appropriate data.

The overall GC-MS-computer system as outlined in Table 4 works well; however, two obvious improvements can be made in the computer programs. The first is faster data output utilizing a cathode ray tube with a hard copy device and the second, a modification to permit time-shared acquisition and processing of data. With these modifications overall data reduction time should be reduced by half.

Specialized Techniques

LMRGC. Specialized techniques of MS or data reduction can be used to detect a specific material or class of materials in a mixture. The most common technique is the generation of the limited mass reconstructed gas chromatogram (LMRGC). For example, in Figure 5, the RGC shows as peaks those spectra that contain significant numbers of any ion fragments of m/e 35 to 250. Above the RGC is the LMRGC in which the computer was instructed to respond only to those spectra that contain the m/e 149 fragment. This fragment is often

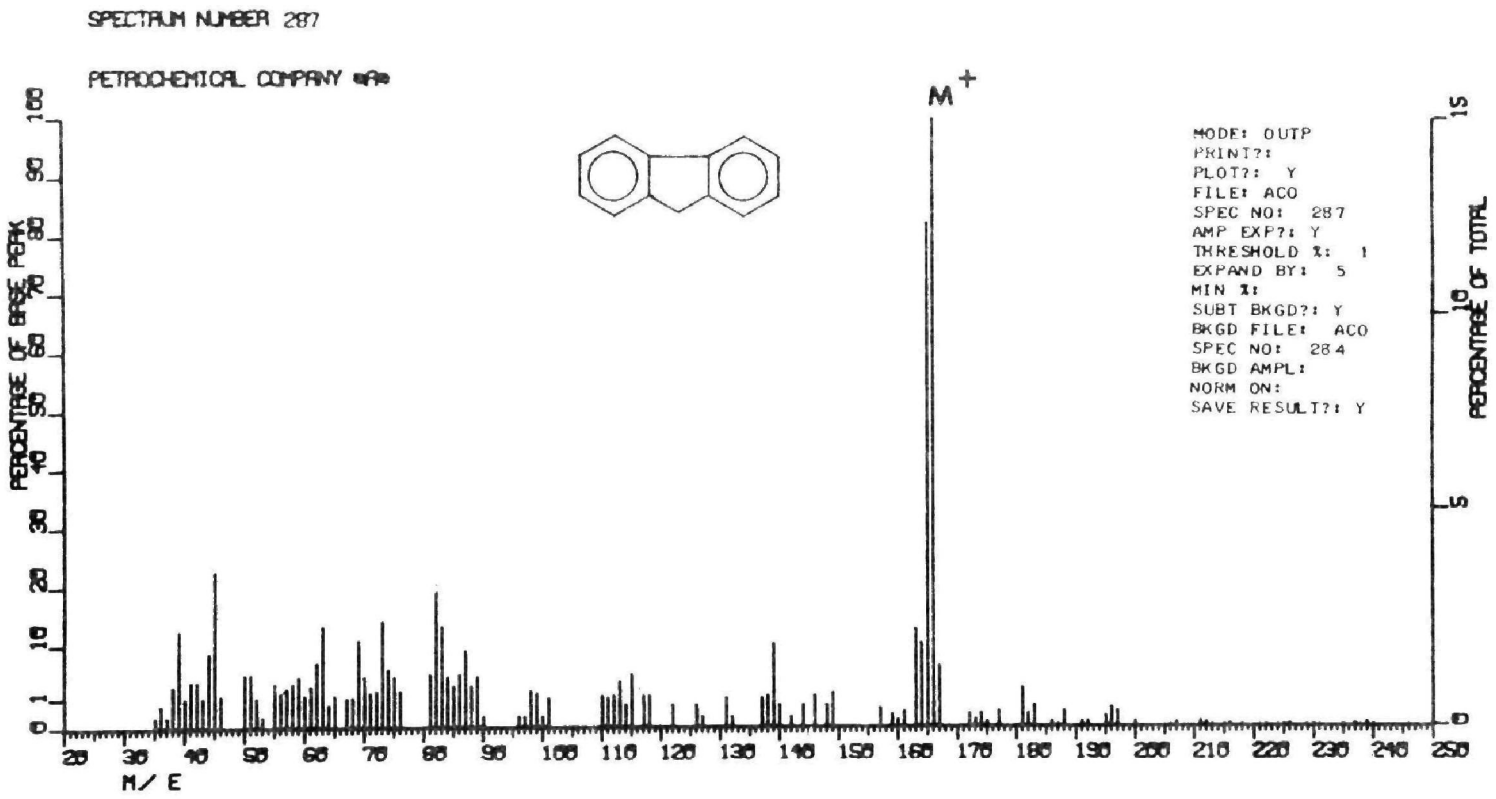

FIGURE 4. MASS SPECTRUM OF FLUORENE

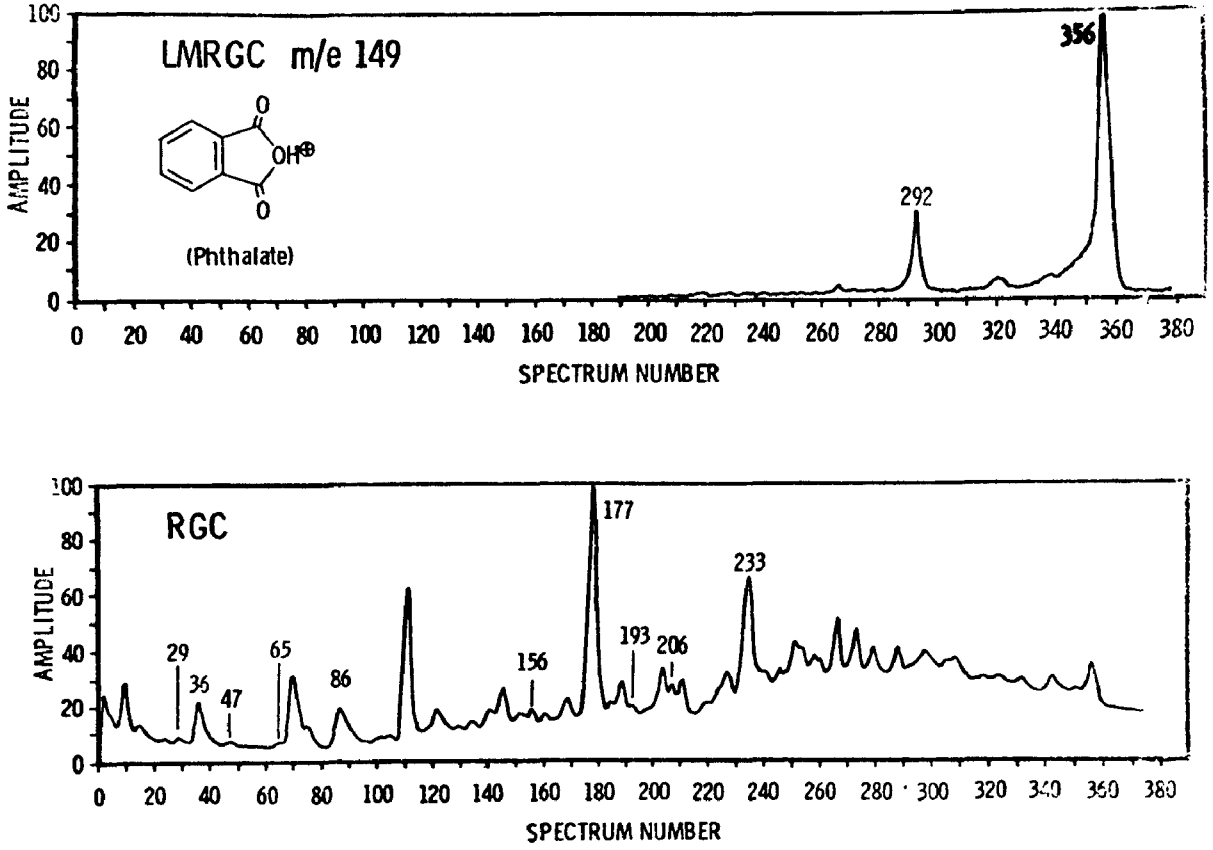

FIGURE 5. LOWER: RECONSTRUCTED GAS CHROMATOGRAM OF A PETROCHEMICAL COMPANY EFFLUENT. UPPER: LIMITED MASS RECONSTRUCTED GAS CHROMATOGRAM INDICATES ONLY SPECTRA 292 AND 356 CONTAIN SIGNIFICANT FRAGMENTS OF M/E 149, TYPICAL OF PHTHALATE ESTERS

found in the spectra of phthalate esters. The results indicate that two of the sample peaks may be phthalates. Similar characteristic fragments exist for several other classes of compounds.

Limited mass monitoring is also possible in the data acquisition step. In this mode, the mass spectrometer is stepped sequentially through a series of limited mass ranges. Longer detection times can be spent on each individual mass than in normal runs; hence, the sensitivity for a given peak can be enhanced. The technique can also be used to eliminate peaks that might otherwise interfere with the spectrum of interest. Figure 6 shows a complicated RGC from an industrial effluent. In Figure 7, the sample was rerun using the limited mass range technique. Based on the peak patterns and the particular masses monitored, the impurity was identified as a PCB mixture.

Solids Probe. Samples not amenable to GC can sometimes be introduced without decomposition into the MS through the solid probe inlet. Most GC-MS instruments have this accessary. In one example, a blue material isolated from a municipal sewage plant would not chromatograph. When introduced into the MS by the solids probe, it was identified as copper phthalocyanine. Since the uses of this compound are limited, the source was soon identified as a paint factory.

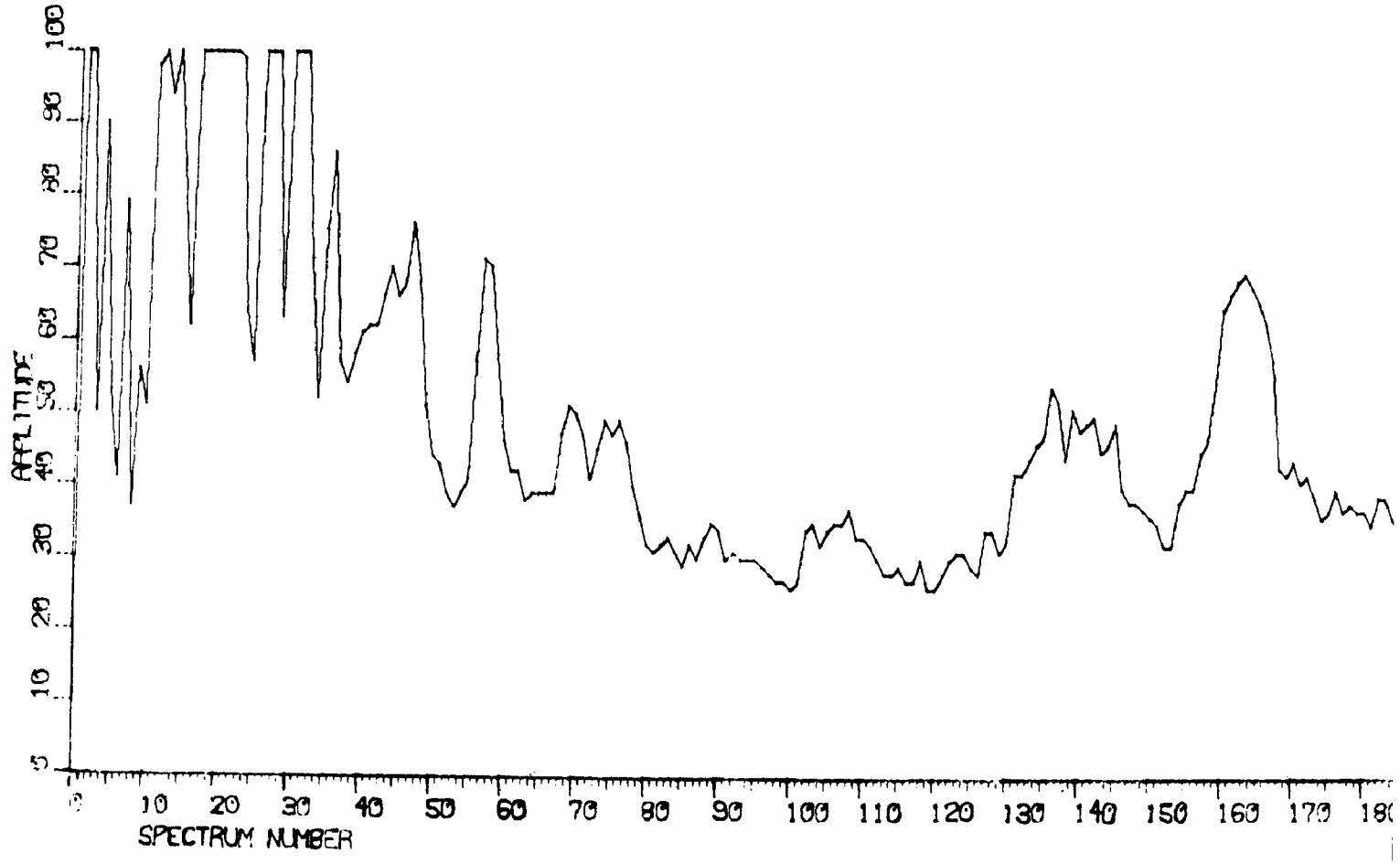

FIGURE 6. COMPLICATED RGC FROM AN INDUSTRIAL EFFLUENT EXTRACT

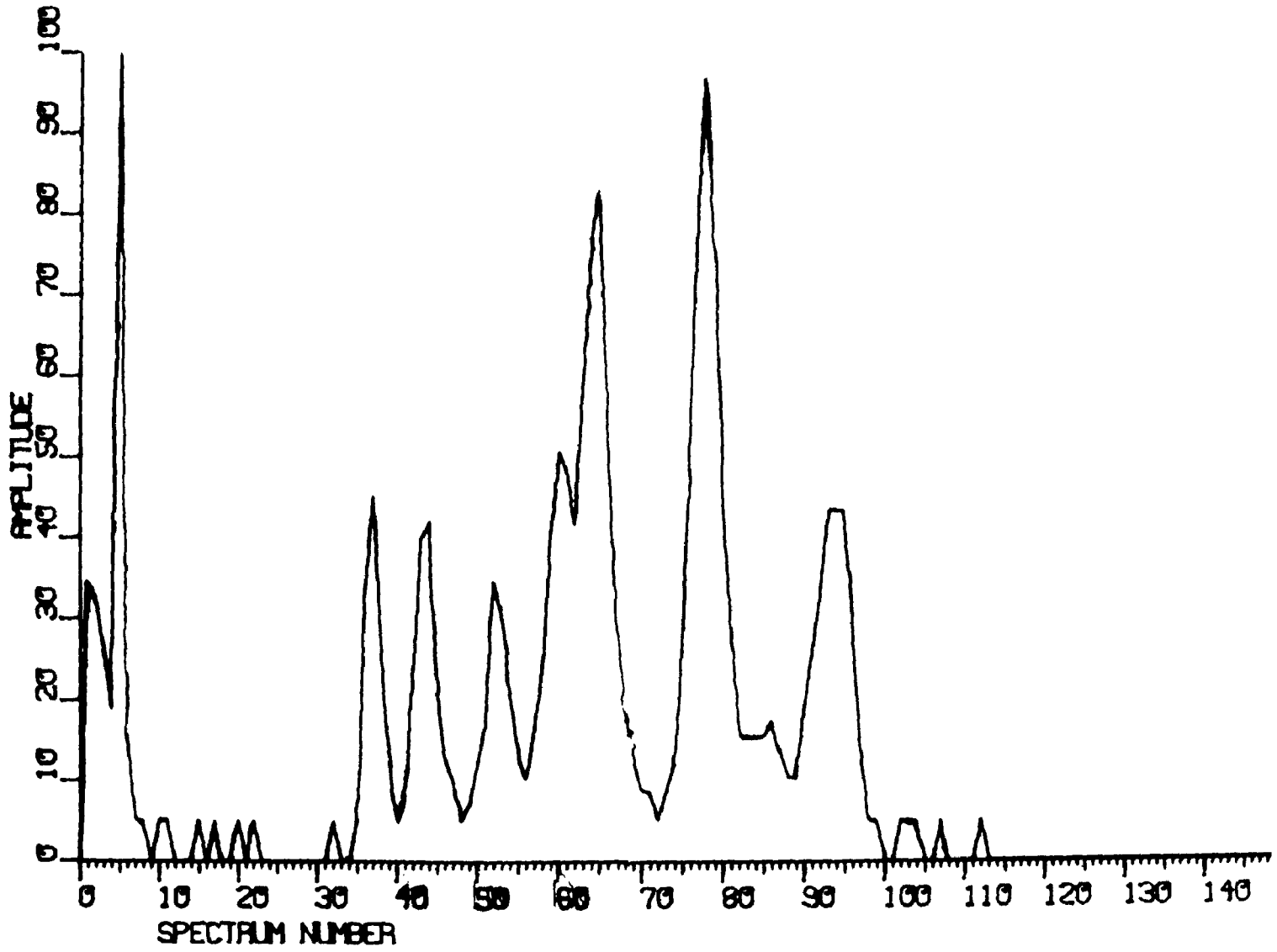

FIGURE 7. RGC OF EFFLUENT EXTRACT FROM FIGURE 6 USING LIMITED MASS TECHNIQUE TO ENHANCE SENSITIVITY AND REDUCE INTERFERENCES

SECTION VII

INTERPRETATION AND COMPUTER MATCHING

The mass spectrum provides a chemical fingerprint that is characteristic of a material and can be interpreted to give the structure. The majority of organic spectra can be interpreted through a combination of experience and a knowledge of chemistry and mass spectral fragmentation theory. Depending on the complexity and uniqueness of the spectrum and the analyst's familiarity with the compound or class of compounds, interpretation can be made in a matter of seconds or it can require hours or even days. Tables that list the eight or ten most intense peaks (21-23) enable the analyst to search for compounds at a rate of about 10 minutes per search. This becomes tedious if more than one or two spectra are analyzed. Computerized empirical spectra matching program can identify spectra at a much faster rate.

Many matching schemes using computers have been described in the literature. After a thorough survey of the literature and of EPA's needs, a research grant was awarded to Battelle Columbus Laboratories to modify the spectra matching system of Hertz, Hites, and Biemann (24). The modifications included increasing the speed of matching and constructing a data base suitable for pollutant identification.

Matching, taking advantage of the information redundancy of mass spectra, is based only on the two most intense peaks in every 14 amu slot. There are four main steps in the matching process:

1) Screening based on molecular weight range

2) Screening based on most intense peak of the unknown spectrum

3) Pre-searching based on spectrum family

4) Calculation of the similarity index and ordering of best matches based on peak-by-peak comparison of unknown to those reference spectra that passed the pre-search.

This modified program, SEWL3P, has been evaluated and improved during the past year. Matching the spectrum of an unknown against the present data base of 11,000 spectra (10,600 general spectra from the Aldermaston collection and 400 pollutant spectra from Southeast Environmental Research Laboratory and Battelle) requires approximately 45 seconds. The similarity index gives the user an immediate indication of whether the "best hit" is a poor match (<0.2 if no closely related compound is in data base), one of several fair matches (0.2-0.35 if the correct compound is not in data base), or a good match (>0.35 where the second best hit is significantly lower).

In cases where the correct answer is not included in the data base, related compounds are usually found as the best hits. This can lead to a rapid identification by other means. In one study made at the Southeast Environmental Research Laboratory, 50% of the unknowns present in an environmental sample were found correctly as the best hit; 8%, as the second best; and 2% as the third best.

Addition of new pollutant spectra to the library takes place continuously. As the data base grows, the success of the system is expected to improve.

To reduce the operator time involved in utilizing this system, PDP8 utility programs were developed with Battelle that enable a laboratory to transfer all of the necessary data from the laboratory computer through a telephone coupling device to the central matching computer. These programs also eliminate human errors and prejudices in selection and transmission of data.

At the time the National Water Contaminants Characterization Research Program and Battelle were developing this system, a somewhat less sophisticated matching program was developed at the National Institutes of Health (25) and a simple one at Finnigan Corporation and System Industries (26). A comparison of these programs is given in Table 5. Each has certain strengths that adapt it to different phases of pollutant identification.

Table 5

Comparison of Three Spectra Matching Programs

Parameter	System		
	EPA/Battelle	NIH	Finnigan/ System Industries
Basis for match	Masses and intensities of the two most intense peaks in all 14-amu slots	Masses and intensities selected from the two most intense peaks in 14-amu slots	Masses of the most intense peak in all 14-amu slots
Average number of input peaks used	~25	~4	~15
Data selection and entry	Semi-automatic or manual	Manual	Manual (semi-automatic version has been written)
Average match time for best fit (based on present library size)	45 seconds	Screens only. Does not choose a "best fit" Screening time (including dialog) approximately 2 minutes for 4 mass peaks.	<15 seconds for System 250 disk <3 minutes for System 150 DECtape
Output ordered and ranked by	Similarity index (high→low)	File number (low→high)	Number of mismatched peaks (low→high)
Effect of an extraneous peak	Slight reduction in similarity index	Eliminates the correct match	Increases the mismatch index by 1
Number of spectra in data base	11,000	8,800	300
Expansion of data base	EPA program manager	User	User
Present availability to Agency users	Nationwide on Battelle time-shared computer	Nationwide on NIH time-shared computer	System Industries PDP8 routine--also available on GE time-shared computer
Minimum hardware required	Acousticoupler & TTY	Acousticoupler & TTY	TTY
Preferred hardware*	Coupler, KL8E/c, and CRT	Coupler & CRT	CRT
Estimated cost per match (excluding library storage costs)	$1.50	$1.00	Free

*Comparisons of match time and costs made on this basis.

The EPA/Battelle program is the most useful because it quickly provides ordered listings of the best matches with minimal operator handling. These listings are based on detailed comparison of both masses and intensities for all peaks in the reduced unknown spectrum with those of the 11,000 reduced spectra of the library. When the correct compound is not in the data base, the best matches have low similarity indices but generally are related to the correct compound.

The NIH system provides sequential screenings of the data base through comparison of the intensities of user-selected masses from the unknown with those of the corresponding masses in reference spectra. This system has been useful in suggesting possible structural fragments associated with the selected masses.

The Finnigan/System Industries program appears well suited for low-cost, rapid, in-house matching of spectra against small data bases. The library furnished with the system was developed for drug enforcement use (26) and is not applicable to environmental pollutants. Work is underway to develop a similar library of the 240 industrial organic pollutants listed in Appendix 1. When this is completed, a better assessment of the Finnigan/System Industries program can be made.

SECTION VIII

CONFIRMATORY TECHNIQUES

Although the mass spectrum of a compound can be described as a fingerprint, it is not always completely unique. The spectra of different members of homologous series (hydrocarbons and fatty acids) and of isomers (polychlorobiphenyls and alkanes) are usually indistinguishable. In the case of m- and p-cresol, not only are the spectra indistinguishable, but on many GC columns the retention times are the same.

A broad range of confidence levels exist for an identification by GC-MS. The lowest level is identification based entirely on a chemist's interpretation of the spectrum without any reference to a standard or known spectrum. Next is a poor match with a standard spectrum or a low similarity index (<0.2) from a machine search. Computer matches with similarity indices above 0.4 or close resemblance with spectra from the literature are termed tentative identifications. An even higher level of confidence is placed in a good computer or literature MS match combined with a GC retention time match with a known measured in the laboratory. This level is accepted as a confirmed match in our laboratory, particularly when the compound has been run here earlier and its spectrum placed in our files and in the computer library. The best identification based solely on GC-MS is a match of both the GC and MS data with a known run under the same conditions. The ultimate in identification is agreement between GC-MS and other independent methods, e.g., NMR or IR. Unfortunately, the amounts of sample required usually preclude the use of conventional NMR and IR in water analysis.

SECTION IX

CASE HISTORIES

Black Warrior River and Locust Fork Branch Fish Kills

Fish kills in the Black Warrior River occurred at the Locust Fork Branch, near Birmingport, Alabama, in October, 1969, and again near Demopolis, Alabama, in September, 1970. The 1969 kill involved 750 thousand fish and the 1970 incident killed 8 thousand fish. Both kills were suspected to have been caused by the spraying of malathion in conjunction with a U. S. Corps of Engineers mosquito control program in the area. The presence of malathion was confirmed by GC-MS in extracts of both the Locust Fork Branch (Figure 8-A) and the Black Warrior River area near Demopolis (Figure 8-B).

The molecular ion (m/e 330) was not observed in spectra of either of the samples or of the standard when it was introduced from the gas chromatographic column (Figure 8-C). Instead, the fragment (m/e 173) resulting from cleavage of $(CH_3O)_2PS_2^{\cdot}$ was the largest significant ion. A small parent ion was found in a sample introduced by the direct probe (Figure 8-D). The fragmentation pattern of 8-D is significantly different from 8-C, primarily because of different sample pressures in the ion source, illustrating the necessity of comparing mass spectra of samples with standards under identical conditions. The major fragmentations of malathion are indicated in Figure 8-D.

The results of these analyses were used in a brief prepared by the Alabama Department of Conservation and submitted to the Alabama State Attorney.

Polychlorinated Biphenyls (PCB's)

Aroclors, manufactured by the chlorination of biphenyl, are distillation fractions containing 20 or more PCB isomers. They are used widely in industry and have become ubiquitous pollutants of the aquatic environment. We have used mass spectrometry several times to confirm the presence of PCB's, identified tentatively by

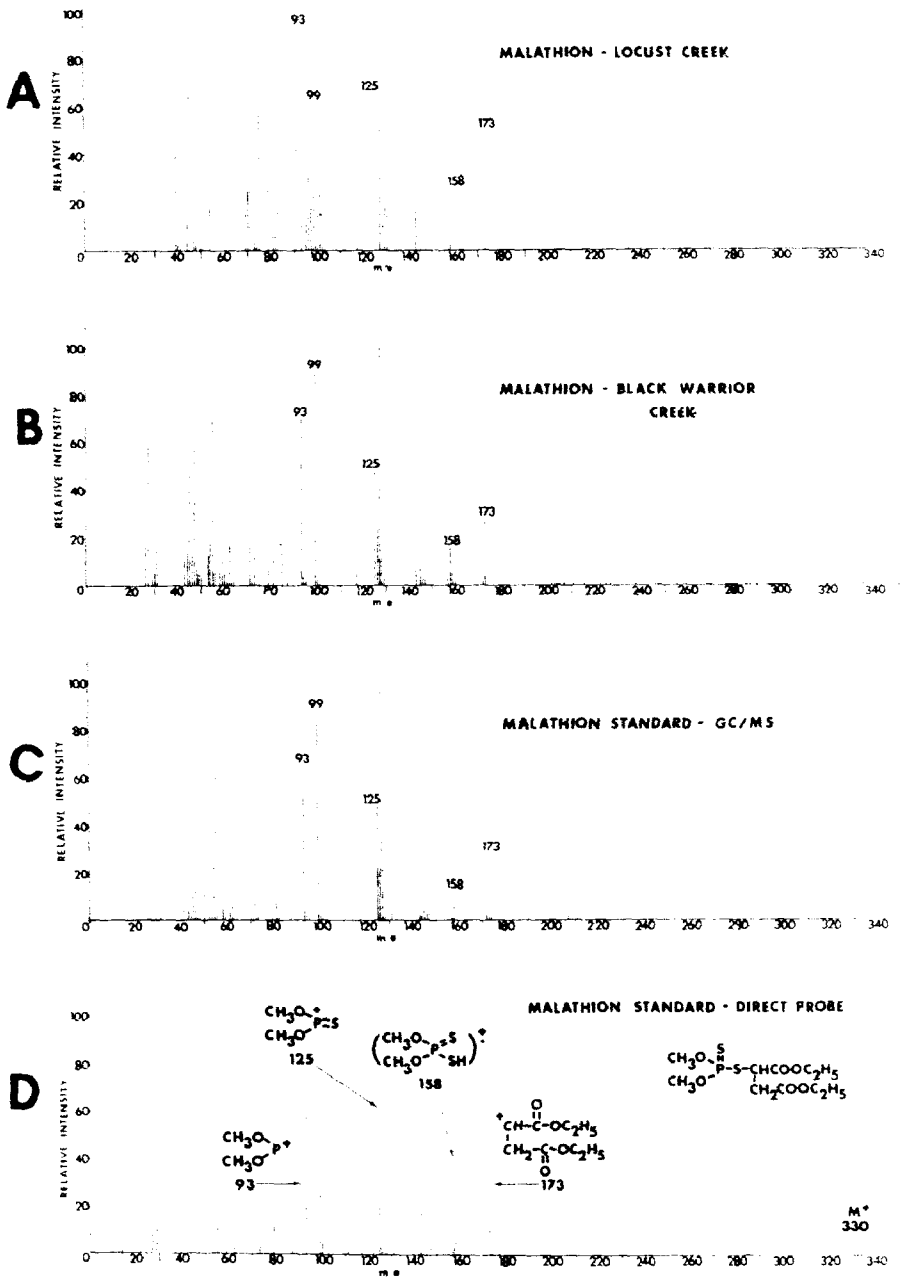

FIGURE 8. MASS SPECTRA OF EXTRACTS AND STANDARDS CONTAINING MALATHION

electron capture GC, in water and mud samples. The FID chromatograms of the extracts are invariably complicated, but the specificity of the mass spectrometer allows unequivocable identifications of submicrogram amounts of PCB's.

In one case, a Florida Bay sediment extract, cleaned on a florisil column, was analyzed for Aroclor 1254. The GC effluent was split 1:1 between the flame detector and the MS. The GC flame detector pattern is shown in Figure 9. Eleven of these peaks were shown by MS to be PCB's. Their retention times and chlorine numbers (number of chlorine atoms per PCB molecule, as determined by MS) correspond with those of an Aroclor 1254 standard, also shown in Figure 9.

Mass spectra showing several chlorine isotope clusters for some of the PCB's from the sediment are compared with corresponding spectra from an Aroclor 1254 standard in Figure 10. The parent ion was observed in all the PCB's. The major fragmentation path is loss of successive chlorine atoms from the parent molecule.

Industrial Effluent Characterization

In April, 1971, EPA Division of Field Investigations, Denver, Colorado, requested chemical characterization of the wastewaters from seven different companies in an industrial area of western Louisiana.

One liter of the effluent from a petrochemical company was extracted with chloroform; the organic layer was dried over anhydrous sodium sulfate and concentrated in a Kuderna-Danish apparatus to about 1 ml. Further concentration (to 0.25 ml) was effected by carefully blowing dry nitrogen over the sample, which was warmed only by body heat from the fingers. The concentrated sample was chromatographed on a 50 ft SCOT column coated with carbowax 20M-TPA.

Figure 3 shows the ion current summation (ICS) and, plotted above it, the computer-reconstructed gas chromatogram (RGC). Although many of the RGC peaks exhibited a variation in height or area relative to the FID chromatogram obtained prior to the GC-MS run,

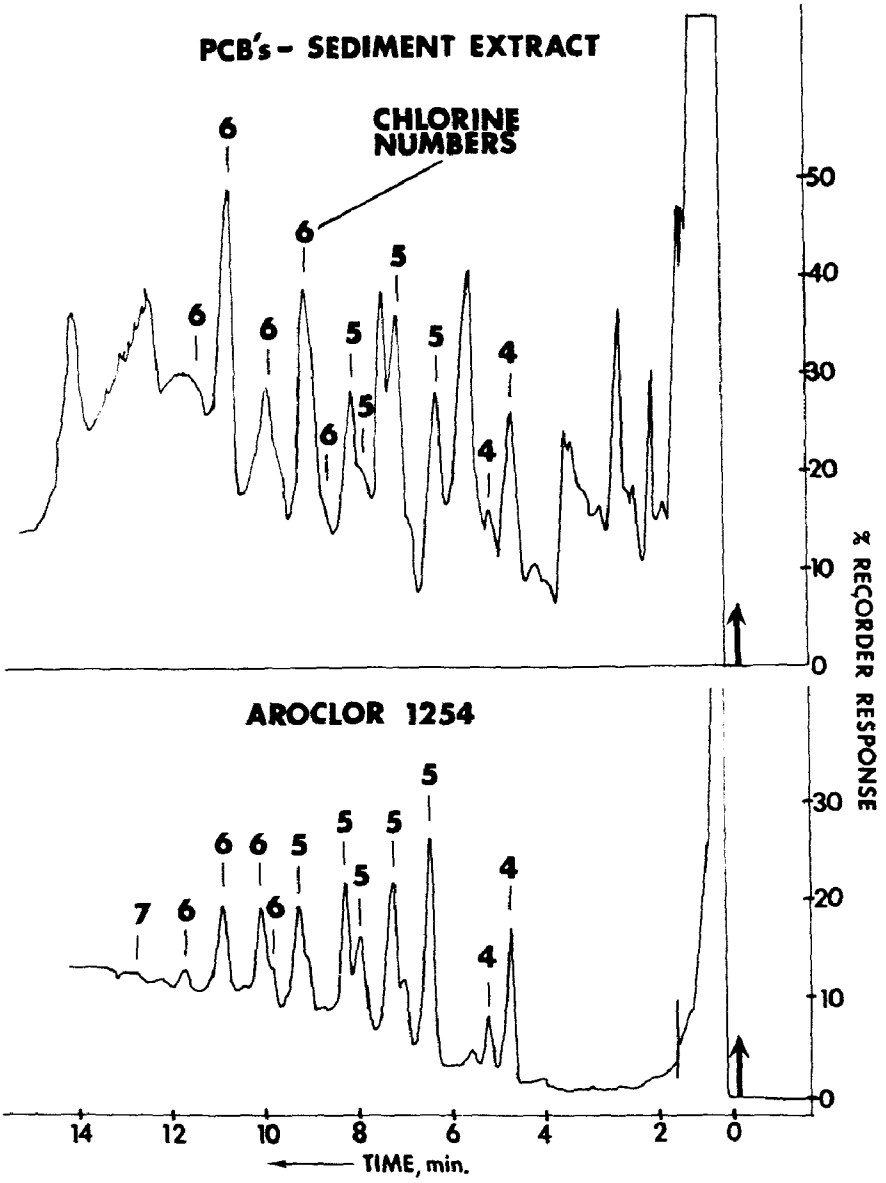

FIGURE 9. FLAME IONIZATION DETECTOR GAS CHROMATOGRAMS OF PCB'S FROM THE ENVIRONMENT AND IN AN AROCLOR STANDARD. THE NUMBERS ARE CHLORINE ATOMS PER PCB MOLECULE DETERMINED BY MS

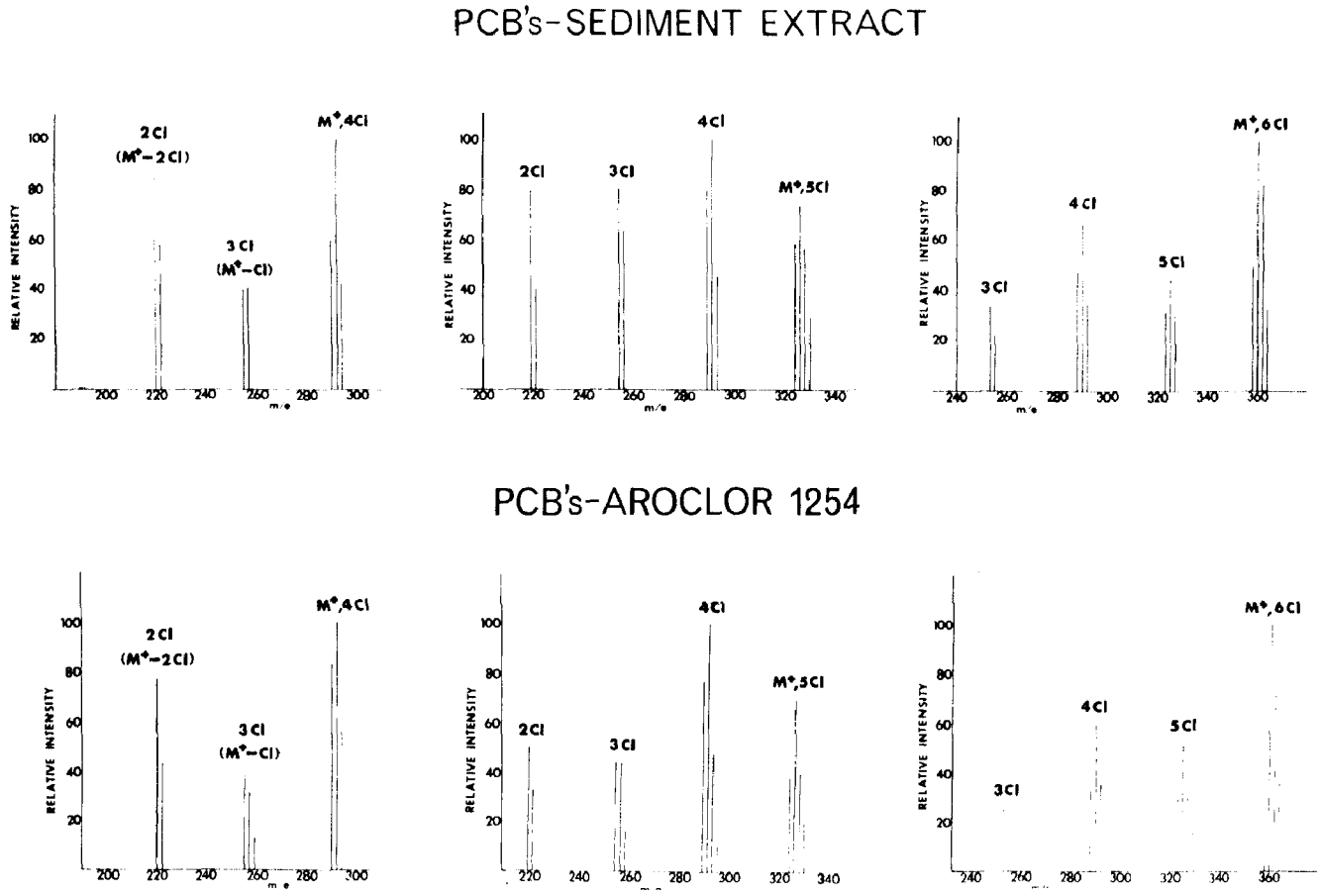

FIGURE 10. PARTIAL MASS SPECTRA OF SOME OF THE PCB'S SHOWN IN FIGURE 9

the resemblance was sufficient to relate the FID GC peaks to their RGC counterparts.

After the RGC and desired LMRGC's were plotted, requests for spectra, with background or interfering peak subtraction, were logged into the computer. When the spectra of the individual peaks were produced by the computer, they were identified by the spectra matching program through the central data bank located at the Battelle Research Institute, Columbus, Ohio. Table 6 shows the list of compounds identified in the plant wastewater.

Correlation of Compounds in Natural Waters with Industrial Wastewater Discharges

In the summer of 1972 the Organic Analysis Unit of the Lower Mississippi River Field Facility requested our assistance in determining whether paper mills in that region contributed measurably to organic pollution of the Mississippi River. Only one mill will be discussed as an example.

The mill wastewater (1.8 ℓ) was extracted with two 500 ml portions of chloroform. Combined extracts were dried over sodium sulfate and concentrated to 1 ml in a Kuderna-Danish apparatus. The extract was further concentrated to 0.3 ml by blowing dry nitrogen over the sample.

Six liters of Mississippi River water sampled 0.25 mi above the mill (control sample) and 6 ℓ sampled 0.25 mi below the mill were each extracted with two 1 ℓ portions of chloroform. Combined extracts of each sample were dried over sodium sulfate, concentrated to 1 ml in a Kuderna-Danish apparatus, and further concentrated to 0.25 ml with dry nitrogen. In addition to the upriver control sample, a solvent blank was necessary because of the very large concentration factors (8,000X) required. Two liters of the same lot of chloroform were therefore treated similarly.

Table 6

Compounds Identified in Wastewater
of Petrochemical Company

RGC Spectrum # (from Figure 3)	Compound Name
2	m-xylene*
4	p-xylene*
10	1,5-cyclooctadiene
16	o-xylene*
29	isopropylbenzene (cumene)
36	styrene*
47	o-ethyltoluene
65	o-methylstyrene*
70	diacetone alcohol
75	indan*
86	2-butoxyethanol
89	β-methylstyrene
109	indene*
121	dimethylfuran isomer
129	n-pentadecane
140	1-methylindene*
145	3-methylindene
156	acetophenone
160	n-hexadecane
168	α-terpineol
177	naphthalene*
193	α-methylbenzyl alcohol
202	2-methylnaphthalene*
206	benzyl alcohol
210	1-methylnaphthalene*
221	ethylnaphthalene isomer
233	2,6-dimethylnaphthalene*
233	phenol*
244	methyl ethyl naphthalene isomer
249	cresol isomer
256	acenaphthene
265	acenaphthalene
278	methylbiphenyl isomer
287	fluorene
292	phthalate diester
296	3,3-diphenylpropanol
356	phthalate diester

*Identification was confirmed with a standard.

The results of the GC-MS analysis are summarized in Figures 11-14. The solvent contained the impurities shown in Figure 11. Figure 12 shows that the upriver control sample contains only one compound (guaiacol) that is commonly found in paper mill effluents. The paper mill effluent contained eleven compounds (Figure 13). Five of these compounds were confirmed in the downriver sample (camphor, fenchyl alcohol, terpinene-4-ol, alpha-terpineol and anethole isomer "C") as shown in Figure 14.

Waste Treatment

The cases already discussed are examples of typical short-term problems that confront most service laboratories. They usually require a few man-days to several man-months effort. In addition to these, several extensive projects based on GC-MS analysis are in progress at SERL. GC-MS is invaluable in studying the effects of waste treatment practices in areas such as municipal sewage, paper mills, textile mills, and refineries. In these studies, GC-MS determines

- the chemicals present in the raw wastewaters,
- which chemicals are reduced or removed by the treatment,
- which chemicals resist the treatment and are discharged,
- which new chemicals are produced by the treatment, and
- which treatment is effective with each compound.

Each of these studies is a major effort that requires development of specific techniques for sampling, extraction, cleanup, concentration, and GC-MS. In addition to providing answers to specific pollution problems, these studies are also demonstrating that GC-MS is an indispensable tool in water pollution studies.

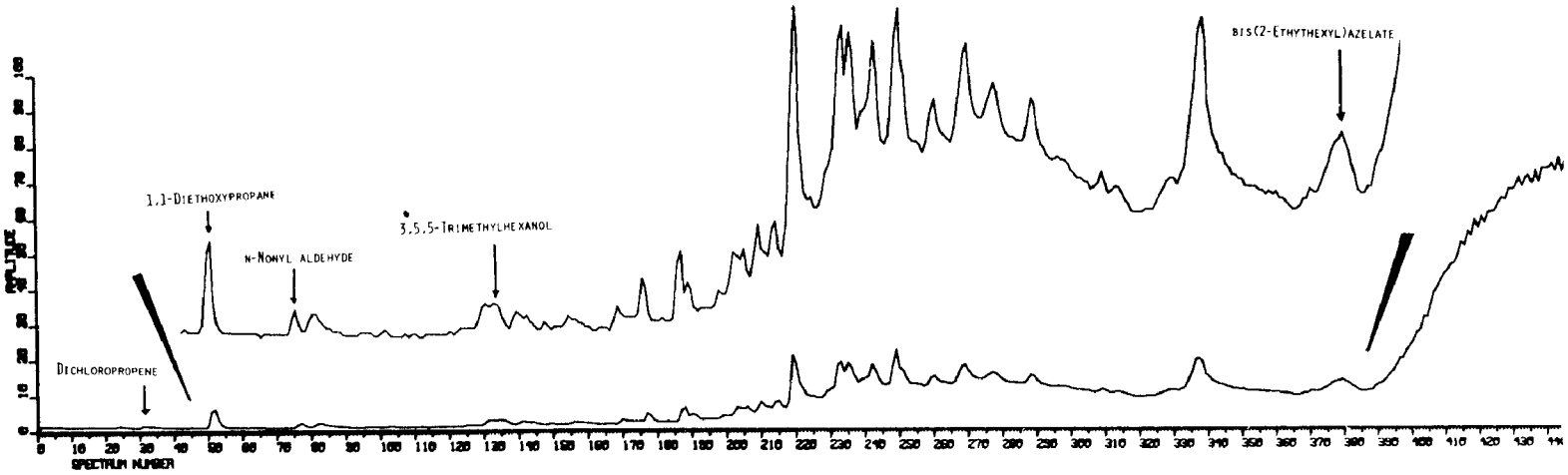

FIGURE 11. COMPUTER RECONSTRUCTED CHROMATOGRAM OF CHLOROFORM BLANK

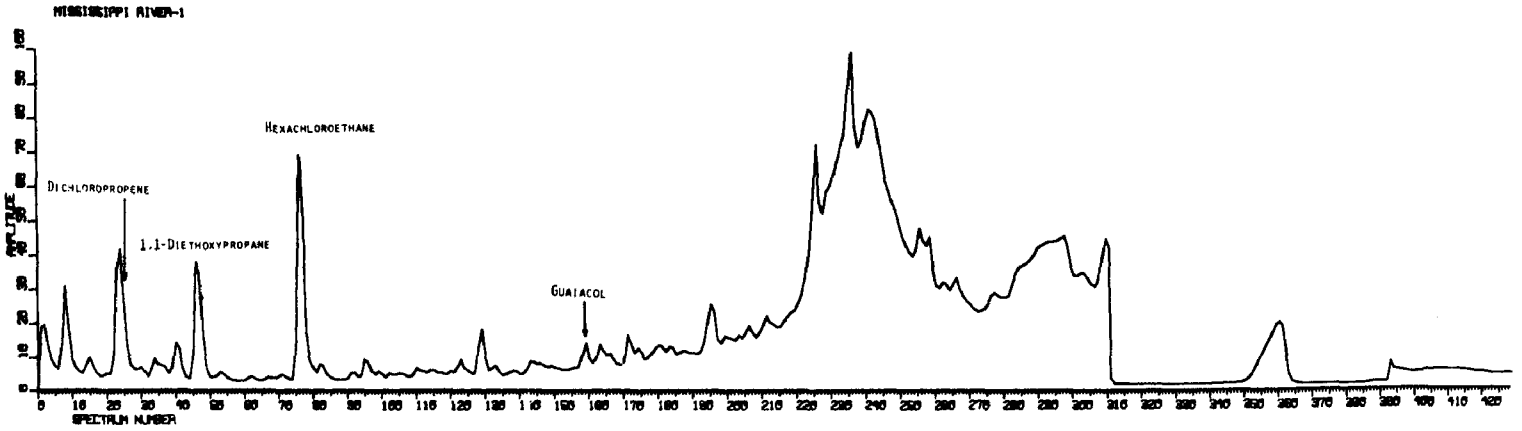

FIGURE 12. RGC OF UPRIVER CONTROL SAMPLE EXTRACT

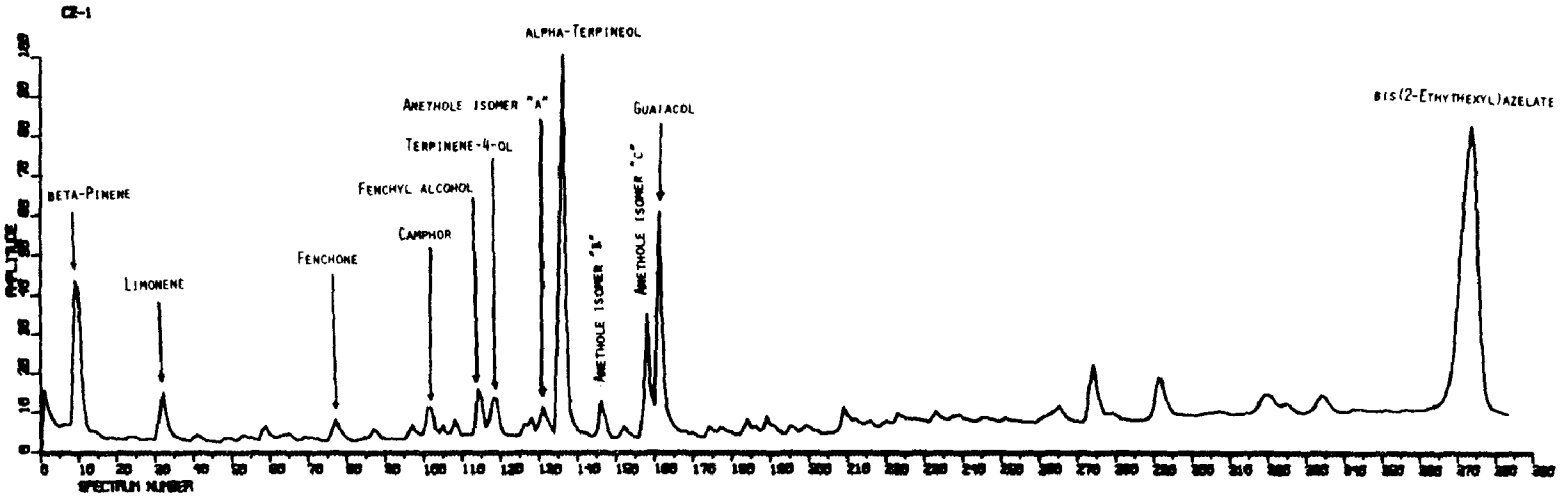

FIGURE 13. RGC OF PAPER MILL EFFLUENT EXTRACT

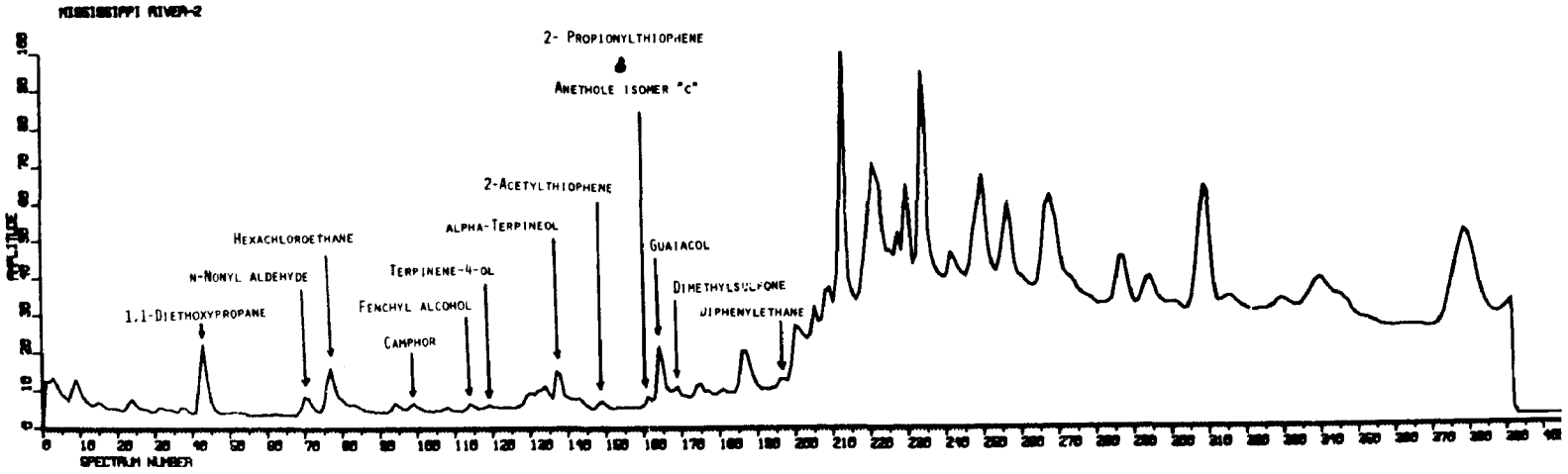

FIGURE 14. RGC OF DOWNSTREAM SAMPLE EXTRACT

SECTION X

ACKNOWLEDGEMENTS

Mary Walker, Ann Alford and Mike Carter, mass spectrometrists at the Southeast Environmental Research Laboratory, have made substantial contributions to this report and to the application of GC-MS to water pollution problems.

The data on extraction efficiency were contributed by A. D. Thruston, Jr.

William Loy, Surveillance and Analysis Division, Region IV, has made extensive application of GC-MS and his contribution to Appendix One is appreciated.

SECTION XI

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SECTION XII

APPENDICES

APPENDIX ONE

ORGANIC COMPOUNDS IDENTIFIED IN WATER BY
GAS CHROMATOGRAPHY-MASS SPECTROMETRY

Compound (1)	Sample source	Concentrations, toxicities (4), comments, analyst
6,8,11,13-Abietatetraen-18-oic acid (2,C) (3)	Paper mill's raw waste and trickling filter effluent	Toxic to salmon at 2-5 mg/1 (10). Keith.
13-Abieten-18-oic acid (3)	Paper mill's raw waste and trickling filter effluent	Toxic to salmon at 2-5 mg/1 (10). Keith.
Abietic acid (C) (3)	Paper mill's raw waste and lagoon	Toxic to salmon at 5 mg/1 (10). Keith, Loy.
Acenaphthalene	Petrochemical plant's five-day lagoon effluent	Keith.
Acenaphthene	Petrochemical plant's five-day lagoon effluent	Caused skin tumors in mice (9). Keith.
" (C)	Wood preserving plant's lagoon effluent	Loy.
" (C)	Wood preserving plant's settling pond	0.2 mg/1. Loy.
"	Pesticide plant's raw effluent	McGuire.
Acetophenone (C)	Chlorinated paraffin plant's lagoon	0.29 mg/1; LD50 in rats is 3g/kg (7). Loy.
"	Petrochemical plant's five-day lagoon effluent	Keith.

Acetosyringone (C)	Gulf coast paper mill's settling pond	0.14 mg/1. Loy.
Acetovanillone (C)	Gulf coast paper mill's settling pond	Estimated at 0.025 mg/1. Loy.
" (C)	Paper mill's raw waste and lagoon	Keith, Loy.
2-Acetylthiophene	Paper mill's raw waste	Keith.
Acrylonitrile (C)	Acrylic fiber plant's settling pond	100 mg/1; 24 hr. TLm to bluegills is 22.5 mg/1 and 30 mg/1 caused 100% mortality to pin perch (8). Garrison.
Adipic acid (C)	Nylon plant's raw waste	3.7 mg/1; 24 hr. TLm for bluegills is 330 mg/1 (11). Loy.
Adiponitrile (C)	Nylon plant's raw waste	320 mg/1; 24 hr. TLm for bluegills is 815 mg/1 (8). Loy.
Aldrin	Pesticide plant's raw effluent	96 hr. TLm at 25° C is 0.01 mg/1 for bluegill (8). McGuire.
m-Anethole	Paper mill's raw waste	Keith.
o-Anethole	Paper mill's raw waste	Keith.
p-Anethole	Paper mill's raw waste	Keith.
Anthraquinone (C)	Wood preserving plant's settling pond	0.05 mg/1. Loy.
Anteisomargaric acid (C) (3)	Paper mill's raw waste and five-day lagoon	Keith.

Anteisopentadecanoic acid (C) (3)	Paper mill's five-day lagoon	Keith
Arachidic acid (3)	Paper mill's raw waste	MLD for fish is 5 mg/l of the sodium salt (8). Garrison.
Arachidonic acid (C) (3)	Paper mill's five-day lagoon	Keith.
Behenic acid (C) (3)	Paper mill's raw effluent and five-day lagoon	Keith.
Benzaldehyde (C)	Paper mill's raw waste	Keith.
Benzyl alcohol	Petrochemical plant's five-day lagoon effluent	Keith.
2-Benzothiazole (C)	Latex accelerators and thickeners plant's holding pond	0.16 mg/1; LD50 for mice is 100 mg/kg (7). Loy.
" (C)	Synthetic rubber plant's aerated lagoon	Very disagreeable odor. Loy.
Biphenyl	River below textile finishing plant	LD50 in rats is 2.2 g/kg (7). Loy.
Borneol	Paper mill's raw waste and trickling filter effluent	Toxic--probable human lethal dose is 50-500 mg (5). Keith, Loy.
1-Butanol (C)	Petrochemical (alcohols) plant's raw effluent	16.0 mg/1; 90 lb/day discharge. Keith.
2-Butoxyethanol	Petrochemical plant's five-day lagoon effluent	Keith.
n-Butylisothiocyanate (C)	Latex accelerators and thickeners plant's holding pond	Raw effluent 0.5 mg/1 and holding pond 0.1 mg/1. Loy.

Camphor (C)	Paper mill's raw waste and trickling filter effluent	Minimum detectable taste is 2 mg/1 (8). Keith, Loy.
" (C)	Gulf coast paper mill's settling pond	0.031 mg/1. Loy.
Caproic acid (C)	Nylon plant's raw waste	220 mg/1; 24 hr. TLm for bluegills is 200 mg/1 (11). Loy.
Carbazole (C)	Wood preserving plant's settling pond	0.27 mg/1; intraperitoneal LD50 for rats is 200 mg/kg (9). Loy.
Chlordane	Pesticide plant's raw effluent	96 hr. TLM at 25° C is 0.02 mg/1 for bluegill (8). McGuire.
Chlordene	Pesticide plant's raw waste	McGuire.
o-Chlorobenzoic acid (C) (3)	Chlorinated paraffin plant's lagoon	0.24 mg/1. Loy.
bis-(2-Chloroethoxy) methane (C)	Synthetic rubber plant's treated waste	140 mg/1. Loy.
bis-2-Chloroethyl ether (C)	Synthetic rubber plant's treated waste	0.16 mg/1. Loy
bis-2-Chloroisopropyl ether (C)	Glycol plant's thickening and sedimentation pond	Loy.
trans-Communic acid (3)	Paper mill's raw waste and trickling filter effluent	Toxic to salmon at 2-5 mg/1 (10). Keith.
o-Cresol (C)	Wood preserving plant's settling pond	1.4 mg/1; 48 hr. TLm for fathead minnows is 24 mg/1; odor threshold is 0.07 mg/1 at 30° C (8). Loy.

o-Cresol (C)	Petrorefinery's eight-hour lagoon effluent	0.120 mg/l; 300 lb/day discharge. Keith.
m-Cresol (C)	Wood preserving plant's settling pond	2.5 mg/l; 24 hr. TLm for carp is 24 mg/l; odor threshold is 0.33 mg/l at 30° C (8). Loy.
p-Cresol (C)	Paper mill's raw waste and lagoon	More toxic than phenol (5); odor threshold 0.05 mg/l (8). Loy.
Cumene (isopropylbenzene)	Petrochemical plant's five-day lagoon effluent	Keith.
Cyclohexanol (C)	Nylon plant's raw waste	LD50 to rats is 1-10 g/kg (12). Loy.
1,5-Cyclooctadiene	Petrochemical plant's five-day lagoon effluent	Keith.
p-Cymene (C)	Paper mill's raw waste and trickling filter effluent	Keith.
"	Pesticide plant's raw waste	McGuire.
Decane (C)	Polyolefin plant's lagoon	0.03 mg/l. Keith.
1-Decanol (C)	Petrochemical (alcohols) plant's raw effluent	2.5 mg/l; 15 lb/day discharge. Keith.
Dehydroabietic acid (C) (3)	Wood preserving plant's settling pond	0.02 mg/l; the sodium salt is toxic to salmon at 5 mg/l (10); LD50 in rats is 1g/kg (7). Loy.
" (C) (3)	Paper mill's raw waste and trickling filter effluent	Keith.

Dehydroabietic acid (C) (3)	Gulf coast paper mill's settling pond	0.47 mg/1. Loy.
" (C) (3)	Tall oil refinery's settling pond	Loy.
Diacetone alcohol	Petrochemical plant's five-day lagoon effluent	Causes liver damage and anemia in animals (5). Keith.
4,4'-Diamino-dicyclohexyl methane	Nylon and polyester plant's effluent after neutralization and sedimentation	0.4 mg/1. Loy.
Dibenzofuran (C)	Wood preserving plant's settling pond	0.12 mg/1. Loy.
" (C)	Wood preserving plant's lagoon effluent	Loy.
" (C)	Nylon plant's settling pond	Garrison.
2,3-Dibromo-1-propanol (C)	Acrylic fibers plant's settling pond	0.5 mg/1. Loy.
Dibromopropene isomer	Acrylic fibers plant's settling pond	Garrison.
Dibutylamine (C)	Latex accelerators and thickeners plant's raw effluent	Less than 1 mg/1; LD50 in rats is 550 mg/kg (7). Loy.
Dieldrin (C)	Anaerobic lagoon of yarn finishing mill	48 hr. TLm for bluegill is 3.4 µg/1 and 0.3 µg/1 for marine shrimp; lethal to rainbow trout after three months' exposure to 1 µg/1 (5). Garrison.

Dieldrin	Pesticide plant's raw effluent	96 hr. TLm values for several fish species are 0.005-0.05 mg/1 (8). McGuire.
N,N-Diethylformamide (C)	Latex accelerators and thickeners plant's raw effluent	Less than 1 mg/1. Loy.
Diethyl phthalate (C)	Synthetic rubber plant's settling pond	Loy.
3,4-Dihydroxyacetophenone (pungenin) (3)	Paper mill's trickling filter effluent	Probably low T&O threshold. Keith.
3,5-Dimethoxy-4-hydroxy-acetophenone (C) (3)	Paper mill's raw effluent and five-day lagoon	Keith.
2,4-Dimethyldiphenylsulfone	Nylon plant's settling pond	Garrison.
"	Acrylic fibers plant's settling pond	Garrison.
Dimethyl furan isomer	Petrochemical plant's five-day lagoon effluent	Keith.
2,6-Dimethyl naphthalene (C)	Petrochemical plant's five-day lagoon effluent	0.015 mg/1. Keith
Dimethyl naphthalene isomer	Pesticide plant's raw effluent	McGuire.
Dimethyl phthalate (C)	Plastic (PVA) plant's settling pond	LD50 to rats is 8400 mg/kg (7). Loy.
" (C)	Synthetic rubber plant's settling pond	Loy.

Dimethyl pyridine isomer	Wood preserving plant's settling pond	0.1-0.2 mg/1. Loy.
Dimethyl quinoline isomers	Wood preserving plant's settling pond	0.1 mg/1. Loy.
Dimethyl sulfone (C)	Paper mill's raw waste and trickling filter effluent	Keith.
Dimethyl sulfoxide (C)	Paper mill's raw waste and trickling filter effluent	Keith.
10,12-Dimethyl tridecanoic acid (3)	Paper mill's five-day lagoon	Keith.
4,6-Dinitro-o-cresol (C) (2-methyl-4,6-dinitrophenol)	Specialty chemical plant's effluent	18 mg/1; minimum lethal dose at 6 hr. was 3-4 mg/1 for minnows (8). Loy.
2,4-Dinitrotoluene (C)	Explosives (DNT) plant's raw waste and settling pond effluent	190 mg/1 in raw waste. Loy.
2,6-Dinitrotoluene (C)	Explosives (DNT) plant's raw waste and settling pond effluent	150 mg/1 in raw waste and 0.02 mg/1 in pond effluent. Loy.
" (C)	TNT plant's raw effluent	0.68 mg/1. Loy.
3,4-Dinitrotoluene (C)	Explosives (DNT) plant's raw waste and settling pond effluent	40 mg/1 in raw waste. Loy, Garrison.
Diphenylene sulfide (C)	Wood preserving plant's settling pond	0.1 mg/1. Loy.

Diphenyl ether	Pesticide plant's raw effluent	Threshold odor and taste level is 0.013 mg/1 (8). McGuire.
3,3-Diphenylpropanol	Petrochemical plant's five-day lagoon effluent	Keith.
2,6-Di-t-butyl-p-benzo-quinone (C)	Surface drainage from closed waste treatment system of particle board plant	Estimated at 0.01 mg/1. Loy.
p-Dithiane (C)	Synthetic rubber plant's treated waste	0.12 mg/1; strong offensive odor. Loy.
Dodecane (C)	Petrorefinery's lagoon effluent after activated sludge treatment	0.22 mg/1; 0.4 lb/day discharge. Keith.
" (C)	Petrorefinery's eight-hour lagoon effluent	0.031 mg/1; 79 lb/day discharge. Keith.
"	Paper mill's raw effluent	Kerosene based defoamer. Keith.
Eicosane (C_{20}) (C)	Petrorefinery's lagoon effluent after activated sludge treatment	0.30 mg/1; 2.9 lb/day discharge. Keith.
Endrin	Pesticide plant's raw effluent	TLm values are less than 0.005 mg/1 for six fish species (8). McGuire.
Ethyl carbamate (C)	Paper mill's trickling filter and aerated lagoon	Keith.
2-Ethyl-1-hexanol (C)	Gulf coast paper mill's settling pond	0.019 mg/1. LD50 to rats is 3200 mg/kg (7). Loy.

2-Ethyl-1-hexanol (C)	Laboratory sewage	Webb.
" (C)	Plastic (PVA) plant's settling pond	Loy.
"	River below textile finishing plant	Loy.
Ethylidenecyclopentane	Paper mill's raw waste	Keith.
Ethyl isothiocyanate (C)	Latex accelerators & thickeners plant's raw effluent	Less than 1.5 mg/l; used as a military poison gas (7). Loy.
Ethyl naphthalene isomer	Petrochemical plant's five-day lagoon effluent	Keith.
Ethyl naphthalene isomer	Pesticide plant's raw effluent	McGuire.
m-Ethyl phenol (C)	Paper mill's raw waste and lagoon	Loy.
Ethyl phenylacetate (C)	Resin plant's lime treated holding pond effluent	Loy.
o-Ethyl toluene	Petrochemical plant's five-day lagoon effluent	Keith.
Eugenol	Paper mill's raw waste and lagoon	LD50 in rats is 2 g/kg (7). Keith.
Fenchyl alcohol (C)	Paper mill's raw waste and trickling filter effluent	Taste threshold 2 mg/1 (5). Keith, Loy.
Fenchone (C)	Paper mill's raw waste and trickling filter effluent	Keith.
Fluoranthene (C)	Wood preserving plant's settling pond	0.6 mg/1; oral LD50 for rats is 2000 mg/kg (9). Loy.

Fluorene (C)	Wood preserving plant's settling pond	0.17 mg/1. Loy.
"	Petrochemical plant's five-day lagoon effluent	Keith.
2-Formylthiophene	Paper mill's raw waste	Keith.
Furfural (3)	Paper mill's raw waste	Ingestion of 0.06g produces persistent headache (7). Keith.
" (C)	Synthetic rubber plant's settling pond	0.002 mg/1. Keith.
Guaiacol (C)	Gulf coast paper mill's settling pond	0.43 mg/1; toxic to perch at 70-80 mg/1; odor threshold is 0.002 mg/1 (8). Loy.
" (C) (3)	Paper mill's raw waste and trickling filter effluent	Keith, Loy.
Heneicosane (C_{21}) (C)	Petrorefinery's lagoon effluent after activated sludge treatment	0.19 mg/1; 1.8 lb/day discharge. Keith.
Heptachlor	Pesticide plant's raw waste	McGuire.
Heptachloronorbornene isomers	Pesticide plant's raw effluent	McGuire.
Heptadecane	Nylon plant's settling pond	Garrison.
" (C)	Petrorefinery's eight-hour lagoon effluent	0.022 mg/1; 53 lb/day discharge. Keith.

Heptadecane (C)	Petrorefinery's lagoon effluent after activated sludge treatment	0.34 mg/1; 3.3 lb/day discharge. Keith.
Hexachlor epoxide	Pesticide plant's raw waste	McGuire.
Hexachlorobenzene (C)	Chlorinated solvents plant's raw effluent	Loy.
Hexachlorobutadiene	Pesticide plant's raw effluent	McGuire.
Hexachlorocyclopentadiene	Pesticide plant's raw waste	McGuire.
Hexachloronorbornadiene isomers	Pesticide plant's raw effluent	McGuire.
Hexadecane (C)	Nylon plant's settling pond	Garrison.
" (C)	Petrorefinery's eight-hour lagoon effluent	0.026 mg/1; 66 lb/day discharge. Keith.
" (C)	Petrorefinery's lagoon effluent after activated sludge treatment	0.42 mg/1; 4.0 lb/day discharge. Keith.
"	Paper mill's raw waste	Kerosene based defoamer. Keith.
"	Petrochemical plant's five-day lagoon effluent	Keith.
Hexadieneal	Pesticide plant's raw effluent	McGuire.
1-Hexanol (C)	Petrochemical (alcohols) plant's raw effluent	65.0 mg/1; 375 lb/day discharge. Keith.

Homovanillic acid (3)	Paper mill's raw waste and five-day lagoon	Keith.
p-Hydroxyacetophenone (C)	Paper mill's raw waste and lagoon	Keith.
p-Hydroxybenzaldehyde (C) (3)	Paper mill's raw waste and lagoon	Keith.
o-Hydroxybenzoic acid (3)	Paper mill's raw waste	Keith.
Hydroxybiphenyl isomer	Pesticide plant's raw effluent	McGuire.
4-Hydroxy-3 methoxypropiophenone (C) (3)	Paper mill's raw effluent	Keith.
p-Hydroxythiophenol (3)	Paper mill's raw waste	Keith.
Indan (C)	Petrochemical plant's five-day lagoon effluent	0.007 mg/1. Keith.
Indene (C)	Petrochemical plant's five-day lagoon effluent	0.026 mg/1. Keith.
Isodrin	Pesticide plant's raw effluent	TLm for bluegill is 0.006 mg/1. (8). McGuire.
Isoeugenol	Paper mill's raw waste and lagoon	Keith.
Isopalmitic acid (C) (3)	Paper mill's five-day lagoon	Keith.
Isopentyl alcohol (C)	Laboratory sewage	0.17 mg/1. Webb.
Isooctyl phthalate (C)	Nylon plant's raw waste	Loy.
Isopimaric acid (C) (3)	Paper mill's raw waste and trickling filter effluent	Toxic to salmon at 2-5 mg/1 (10). Keith.

Jasmone	Pesticide plant's raw effluent	McGuire.
Lignoceric acid (3)	Paper mill's raw waste	MLD for fish is 5 mg/l of the sodium salt (8). Garrison
Limonene (C)	Paper mill's raw waste and trickling filter effluent	Keith.
Linoleic acid (C) (3)	Paper mill's raw waste and lagoon	MLD for fish is 10 mg/l of the sodium salt (8). Keith.
Mandelic acid (3)	Paper mill's raw waste	Keith.
Margaric acid (C) (3)	Paper mill's raw waste	MLD for fish is 5 mg/l of the sodium salt (8). Keith.
2-Mercaptobenzothiazole (C)	Synthetic rubber plant's aerated lagoon	Very disagreeable odor. Loy.
" (C)	Paper mill's raw waste and lagoon	Probably highly toxic. Keith.
alpha-Methylbenzyl alcohol	Petrochemical plant's five-day lagoon effluent	Keith.
Methyl biphenyl isomer	Petrochemical plant's five-day lagoon effluent	Keith.
Methyl 3,4-Dimethoxybenzyl ether (3)	Paper mill's raw waste	Keith.
2-Methyl-4-ethyl dioxolane (C)	Fiberglass plant's effluent	Distinct odor of black walnuts. Loy.
Methyl ethyl naphthalene isomer	Petrochemical plant's five-day lagoon effluent	Keith.

1-Methyl indene (C)	Petrochemical plant's five-day lagoon effluent	0.002 mg/1. Keith.
3-Methyl indene	Petrochemical plant's five-day lagoon effluent	0.003 mg/1. Keith.
1-Methyl naphthalene	River below textile finishing plant	Probable lethal human dose 500-5000 mg/kg (5). Loy.
" (C)	Petrorefinery's eight-hour lagoon effluent	0.005 mg/1; 12 lb/day discharge. Keith.
" (C)	Petrochemical plant's five-day lagoon effluent	0.02 mg/1. Keith.
" (C)	Synthetic rubber plant's settling pond	0.002 mg/1. Keith.
2-Methyl naphthalene (C)	Petrorefinery's eight-hour lagoon effluent	0.013 mg/1; 33 lb/day discharge. Keith.
" (C)	Petrochemical plant's five-day lagoon effluent	0.03 mg/1. Keith.
Methyl naphthalene isomer	Wood preserving plant's lagoon effluent	Loy.
Methyl naphthalene isomers	Pesticide plant's raw effluent	McGuire.
13-Methyl pentadecanoic acid (3)	Paper mill's five-day lagoon	Keith.
Methyl phenanthrene	Wood preserving plant's lagoon effluent	Loy.

Methyl quinoline isomers	Wood preserving plant's settling pond	0.5 mg/1. Loy.
o-Methylstyrene (C)	Petrochemical plant's five-day effluent	0.001 mg/1. Keith.
beta-Methylstyrene	Petrochemical plant's five-day lagoon effluent	Keith.
Methyl trisulfide	Paper mill's raw waste	Keith.
Myristic acid (C) (3)	Paper mill's raw waste	MLD for fish is 5 mg/1 of the sodium salt (8). Keith.
Naphthalene (C)	Nylon plant's settling pond	MLD to minnows for 6 hrs. is 15 mg/1 (8). Garrison.
" (C)	Surface drainage from closed treatment of system of particle board plant	Less than 0.01 mg/1. Loy.
" (C)	Petrochemical plant's five-day lagoon effluent	0.05 mg/1. Keith.
"	Pesticide plant's raw waste	McGuire.
2-Naphthoic acid (C)	Wood preserving plant's settling pond	0.16 mg/1. Loy.
Neoabietic acid (C) (3)	Paper mill's raw waste	Toxic to salmon at 2-5 mg/1 (10). Loy, Keith.
Nitrobenzene (C)	Chemical company's lagoon after steam stripping	0.11 mg/1; minimum lethal dose at 6 hr. was 90-100 mg/1 for minnows (8); approximate conc. in water causing faint odor is 0.03 mg/1 (8). Loy.

2-Nitro-p-cresol (C)	Chemical company's lagoon after steam stripping	9.3 mg/1. Loy.
o-Nitrophenol (C)	Chemical company's lagoon after steam stripping	1.4 mg/1; minimum lethal dose at 6 hr. was 125-130 mg/1 for minnows (8). Loy.
o-Nitrotoluene (C)	Paper mill's five-day lagoon	Keith.
" (C)	TNT plant's raw effluent	0.15 mg/1. Loy.
" (C)	DNT plant's raw effluent	7.8 mg/1 in raw waste and 0.012 in pond effluent. Loy.
m-Nitrotoluene	DNT plant's raw effluent	Garrison.
p-Nitrotoluene (C)	Chemical company's lagoon after steam stripping	0.04 mg/1; minimum lethal dose at 6 hr. was 45-50 mg/1 for minnows (8). Loy.
" (C)	DNT plant's raw effluent	8.8 mg/1 in raw waste. Loy, Garrison.
Nonachlor	Pesticide plant's raw effluent	McGuire
Nonadecane (C)	Petrorefinery's lagoon effluent after activated sludge treatment	0.31 mg/1; 3.0 lb/day discharge. Keith.
" (C)	Petrorefinery's eight-hour lagoon effluent	0.013 mg/1; 33 lb/day discharge. Keith.
Nonylphenol (C)	Anaerobic lagoon of yarn finishing mill	LD50 for rats (orally) is 400-1400 mg/kg (6); estimated human lethal dose 500-5000 mg/kg (5). Garrison.

Nonylphenol	River below textile finishing plant	Loy.
Norcamphor	Paper mill's raw waste	Keith.
beta-Ocimene (C)	Paper mill's raw waste	Loy.
1-Octanol (C)	Petrochemical (alcohols) plant's raw effluent	19.0 mg/1; 110 lb/day discharge. Keith.
Octachlorocyclopentene	Pesticide plant's raw effluent	McGuire.
Octadecane (C)	Petrorefinery's eight-hour lagoon effluent	0.017 mg/1; 43 lb/day discharge. Keith.
" (C)	Nylon plant's settling pond	Garrison.
Oleic acid (C)	Tall oil refinery's settling pond	MLD for fish is 5 mg/1 of the sodium salt (8). Loy.
" (C) (3)	Paper mill's raw waste and trickling filter effluent	Keith, Loy.
Octylphenol	River below textile finishing plant	LD50 to mice is 25-50 mg/kg (6). Loy.
Palmitic acid (3)	Textile chemical plant's raw effluent	MLD for fish is 5 mg/1 of the sodium salt (8). Garrison.
" (C)	Tall oil refinery's settling pond	Loy.
" (C) (3)	Paper mill's raw waste and trickling filter effluent	Keith, Loy.
" (C) (3)	Gulf coast paper mill's settling pond	0.013 mg/1. Loy.

Palmitoleic acid (C) (3)	Paper mill's five-day lagoon	Keith.
Pentachlorocyclopentadiene isomers	Pesticide plant's raw effluent	McGuire.
Pentachloronorbornadiene isomer	Pesticide plant's raw effluent	McGuire.
Pentachloronorbornene isomer	Pesticide plant's raw effluent	McGuire.
Pentachloronorbornene isomer	Pesticide plant's raw waste	McGuire.
Pentachloronorbornadiene epoxide isomer	Pesticide plant's raw waste	McGuire.
Pentachlorophenol (C)	Latex accelerators and thickeners plant's holding pond	0.4 mg/1; 0.2 to 0.6 mg/1 toxic to 19 varieties of fish; odor threshold of 0.86 mg/1 at 30° C (8). Loy.
" (C) (3)	Wood preserving plant's raw effluent	Garrison.
" (C)	Resin plant's lime treated holding pond effluent	Loy.
" (C)	Synthetic rubber plant's aerated lagoon	Loy.
" (C)	Wood preserving plant's lagoon effluent	Loy.
Pentadecane (C)	Petrorefinery's eight-hour lagoon effluent	0.030 mg/1; 76 lb/day discharge. Keith.

Pentadecane (C)	Petrorefinery's lagoon effluent after activated sludge treatment	0.49 mg/1; 4.8 lb/day discharge. Keith.
"	Paper mill's raw waste	Kerosene based defoamer. Keith.
"	Petrochemical plant's five-day lagoon effluent	Keith.
Pentadecanoic acid (C) (3)	Paper mill's lagoon	MLD for fish is 5 mg/1 of the sodium salt (8). Keith.
Phenanthrene (C)	Wood preserving plant's lagoon effluent	5 mg/1 killed rainbow trout and bluegills in 24 hrs. (8). Loy.
" (C)	Wood preserving plant's settling pond	1.4 mg/1. Loy.
Phenol (C)	Laboratory sewage	96 hr. TLm for bluegills is 13.5 mg/1 and odor threshold is 0.02-0.03 mg/1 (8). Webb.
" (C)	Petrorefinery's eight-hour lagoon effluent	0.2 mg/1; 510 lb/day discharge. Keith.
" (C)	Wood preserving plant's settling pond	0.66 mg/1. Loy.
" (C)	Petrochemical plant's five-day lagoon effluent	0.06 mg/1. Keith.
" (C) (3)	Paper mill's raw waste	Keith.
Phenyl ether (C)	Nylon plant's settling pond	0.05 mg/1; odor threshold is 0.013 mg/1 (8). Garrison.

o-Phenylphenol	River below textile finishing plant	Probable lethal human dose 500-5000 mg/kg (5). Loy.
Pimaric acid (C) (3)	Paper mill's raw waste and trickling filter effluent	Toxic to salmon at 2-5 mg/1 (10). Keith, Loy.
" (C) (3)	Gulf coast paper mill's settling pond	0.12 mg/1. Loy.
beta-Pinene (C)	Paper mill's raw waste	Loy.
Pinene isomer	Gulf coast paper mill's settling pond	0.008 mg/1. Loy.
Polychlorinated biphenyls (Arochlor 1254) (C)	Nylon plant's raw waste	0.2 µg/1. Loy.
2-Propionylthiophene	Paper mill's raw waste	Keith.
4-n-Propylphenol (C)	Paper mill's raw waste and lagoon	Loy.
Pyrene (C)	Wood preserving plant's settling pond	0.33 mg/1. Loy.
Quinoline (C)	Wood preserving plant's settling pond	1.5 mg/1; oral LD50 for rats is 460 mg/kg (9); 5 mg/1 was lethal to bluegills in 4 hrs. at 13° C (8). Loy.
Sandaracopimeric acid (C) (3)	Paper mill's raw waste and lagoon	Toxic to salmon at 2-5 mg/1 (10). Keith.
Stearic acid (3)	Textile chemical plant's raw effluent	MLD for fish is 5 mg/1 of the sodium salt (8). Garrison.

Stearic acid (C) (3)	Gulf coast paper mill's settling pond	0.02 mg/l. Loy.
Styrene (C)	Petrochemical plant's five-day lagoon effluent	0.03 mg/l; LD50 in rats is 5 g/kg (5). Keith.
" (C)	Synthetic rubber plant's settling pond	0.003 mg/l. Keith.
Syringaldehyde (C)	Gulf coast paper mill's settling pond	Estimated at 0.01 mg/l. Loy.
" (C) (3)	Paper mill's lagoon	Keith, Loy.
Terpinene-4-ol	Paper mill's raw waste	Keith.
alpha-Terpineol (C)	Nylon plant's settling pond	Garrison.
" (C)	Paper mill's raw waste and trickling filter effluent	Keith, Loy.
"	Petrochemical plant's five-day lagoon effluent	Keith.
Terpineol isomer	Gulf coast paper mill's settling pond	0.200 mg/l. Loy.
Terpinolene	Paper mill's raw waste	Kerosene based defoamer. Keith.
1,1,2,2-Tetrachloroethane (C)	Chlorinated solvents plant's raw effluent	2.2 mg/l; LD50 intravenous in rabbits is 80 mg/kg (7). Keith.
Tetrachlorophenol isomer (3)	Wood preserving plant's raw effluent	Odor threshold of 0.9 mg/l at 30° C (8); probable lethal human dose is 50-500 mg/kg (5). Garrison.

Tetradecane (C)	Petrorefinery's lagoon effluent after activated sludge treatment	0.58 mg/1; 5.6 lb/day discharge. Keith.
" (C)	Petrorefinery's eight-hour lagoon effluent	0.039 mg/1; 99 lb/day discharge. Keith.
Tetramethylbenzene isomer	Pesticide plant's raw waste	McGuire.
2,2'-Thiodiethanol (C) (Thiodiglycol)	Synthetic rubber plant's treated waste	Estimated at 2 mg/1. Loy.
Toluic acid (C) (3)	Chlorinated paraffin plant's lagoon	0.24 mg/1. Loy.
Trichlorobenzene isomer	River below textile finishing plant	May cause liver damage; estimated human lethal dose is 50-500 mg/kg (5). Loy.
Trichlorobenzene isomer	Textile chemical plant's raw effluent	Estimated lethal human dose is 50-500 mg/kg (5). Garrison.
Trichlorocyclopentene isomers	Pesticide plant's raw effluent	McGuire.
1,1,2-Trichloroethane (C)	Chlorinated solvents plant's raw effluent	5.4 mg/1; TLm for marine pinperch is 150-175 mg/1 (5). Loy.
Trichloroguaiacol (C) (3)	Paper mill's raw waste	Present in sample toxic to salmon (10). Keith.
n-Tridecane (C)	Petrorefinery's eight-hour lagoon effluent	0.042 mg/1; 107 lb/day discharge. Keith.
" (C)	Petrorefinery's lagoon effluent after activated sludge treatment.	0.39 mg/1; 3.8 lb/day discharge. Keith.

n-Tridecane (C)	Paper mill's raw waste	Kerosene based defoamer. Keith.
Triethylurea (C)	Latex accelerators & thickeners plant's raw effluent	6.4 mg/1. Loy.
3,4,5-Trimethoxyaceto-phenone (C) (3)	Paper mill's raw waste and trick-ling filter effluent	Keith.
2,4,6-Trimethylpyridine (C)	Wood preserving plant's settling pond	0.3 mg/1. Loy.
2,4,6-Trinitrotoluene (C)	TNT plant's raw effluent	0.7 mg/1; MLD for minnows over 6 hrs. is 4 mg/1 (8). Loy.
n-Undecane	Paper mill's raw waste	Keith.
" (C)	Petrorefinery's eight-hour lagoon effluent	0.027 mg/1; 69 1b/day discharge. Keith.
" (C)	Polyolefin plant's lagoon	0.02 mg/1. Keith.
" (C)	Petrorefinery's lagoon effluent after activated sludge treat-ment	0.05 mg/1; 0.4 1b/day discharge. Keith.
Valeric acid (C)	Nylon plant's raw waste	500 mg/1; 48 hr. TLm for daphnia magna is 4-5 mg/1 (11). Loy.
Vanillin (C)	Paper mill's raw waste and trick-ling filter effluent	Odor threshold 0.15 mg/1 (8). Keith, Loy.
" (C)	Gulf coast paper mill's settling pond	Estimated at 0.02 mg/1. Loy.
Veratraldehyde (C)	Paper mill's raw waste & lagoon	Keith.

o-Xylene (C)	Synthetic resin plant's settling pond	Loy.
" (C)	Petrochemical plant's five-day lagoon effluent	0.006 mg/1. Keith.
m-Xylene (C)	Petrochemical plant's five-day lagoon effluent	0.008 mg/1; MLD is 10-90 mg/1; taste and odor threshold 0.3-1.0 mg/1 (8). Keith.
p-Xylene (C)	Petrochemical plant's five-day lagoon effluent	0.002 mg/1. Keith.
2,5-Xylenol (C)	Wood preserving plant's settling pond	0.82 mg/1; behavior similar to 3,4-xylenol (8). Loy.
3,4-Xylenol (C)	Wood preserving plant's settling pond	0.5 mg/1; 24 hr. TLm for carp is 30 mg/1; odor threshold similar to phenol (8). Loy.
3,5-Xylenol (C)	Wood preserving plant's settling pond	1.5 mg/1; 24 hr. TLm for carp is 53 mg/1; odor threshold similar to phenol (8). Loy.

(1) Arranged alphabetically. Prefixes showing position (e.g. : p-, alpha-, bis-, trans-) are not considered part of the name for this purpose.

(2) Confirmed identifications are marked (C). All others are to be regarded as tentative. Using only GC-MS data, identifications can be confirmed by concurrent examination of the sample and a known compound for duplication of GC retention time and MS fragmentation pattern. As a substitute for the concurrent mass spectrum, the MS comparison can be with data from the literature, from computer files, or from other reference collections.

(3) Converted to the methyl ester or ether (usually by diazomethane or dimethylsulfate) before analysis.

(4) Brief discussions of how toxicity data are obtained for water pollutants are found in references (8) and (11). LD50 (lethal dose, 50%) is the amount of compound administered by direct feeding or injection that kills 50 percent of the test animals. The term TLm (tolerance limit, median) designates the exposure concentration required to kill 50 percent of the test organisms within a specified time period, e.g. 96 hrs. MLD (minimum lethal dose) is the minimum concentration required to kill one or more of the test species.

(5) Gleason, M. N., Gassel, R. E., Hodge, H. C., and Smith, R. R., Chemical Toxicology of Commercial Products, The Williams & Wilkens Co., Baltimore, Md., 3rd Edition (1969).

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(7) The Merck Index of Chemicals and Drugs, Merck & Co., Inc., Rahway, N.J., 7th Ed (1960).

(8) Water Quality Criteria, Edited by McKee, J. E. and Wolf, H. W., California State Water Resources Control Board, 2nd Edition (1963).

(9) Survey of Compounds Which Have Been Tested for Carcinogenic Activity, Supplement 2, USDHEW, Public Health Service, Bethesda, MD. (1969).

(10) Rogers, I. H., "Isolation and Chemical Identification of Toxic Components of Kraft Mill Wastes," presented at the joint annual meeting of the Pacific Coast and Western Technical Sections of the Canadian Pulp and Paper Association, Jasper, Alberta, May 25-27, 1972.

(11) Water Quality Criteria Data Book, Volume 3, "Effects of Chemicals on Aquatic Life," by Battelle's Columbus Laboratories for the Environmental Protection Agency, Project No. 18050 GWV (1971).

(12) Handbook of Analytical Toxicology, The Chemical Rubber Co., Cleveland, Ohio (1969).

APPENDIX TWO

PROCEDURE FOR DIAZOMETHANE METHYLATION

The apparatus is shown in Figure 15.

1. Evaporate the sample extract just to dryness with a stream of nitrogen in a centrifuge tube, the bottom tube of a Kuderna-Danish apparatus, or the sample storage vial. A small amount of methylene chloride may be retained, but the presence of chloroform may produce artifacts. Dissolve the extract in one-half to one milliliter of distilled-in-glass ethyl ether.

2. Add about 5 ml of distilled-in-glass ether to the first tube of the apparatus to saturate the nitrogen carrier gas with ether. Add 0.7 ml of ether, 0.7 ml of carbitol, 2-(2-ethoxyethoxy)ethanol, 1.0 ml of 37% aqueous KOH (not over 2 days old), and 0.1-0.2 g of N-methyl-N-nitroso-p-toluenesulfonamide ("Diazald," Aldrich Chemical Co.) to the second tube. The base immediately begins to release diazomethane from the sulfonamide.

3. Immediately position the second test tube and adjust the nitrogen flow to about 10 ml per minute. Caution! Diazomethane is an extremely toxic and explosive gas. A good fume hood and safety glasses are mandatory. No chipped glassware should be used, as rough glass surfaces catalyze decomposition of diazomethane.

4. Position the third tube (a safety trap to prevent reagent carry-over) and the sample tube to bubble the nitrogen and diazomethane gas mixture through the sample. Continue the reaction until the slight yellow color of diazomethane persists in the sample solution (from a few seconds to 30 minutes, depending upon the sample concentration). In the case of dark colored extracts in which the diazomethane is not visible, a reaction time of 30 minutes is recommended.

5. Allow the esterified sample to stand unstoppered in the hood for 15 to 30 minutes to allow excess diazomethane to escape from the ether solution. Discard all waste from the reaction with care and rinse the apparatus with acetone. Evaporate the sample to the volume necessary for gas chromatography.

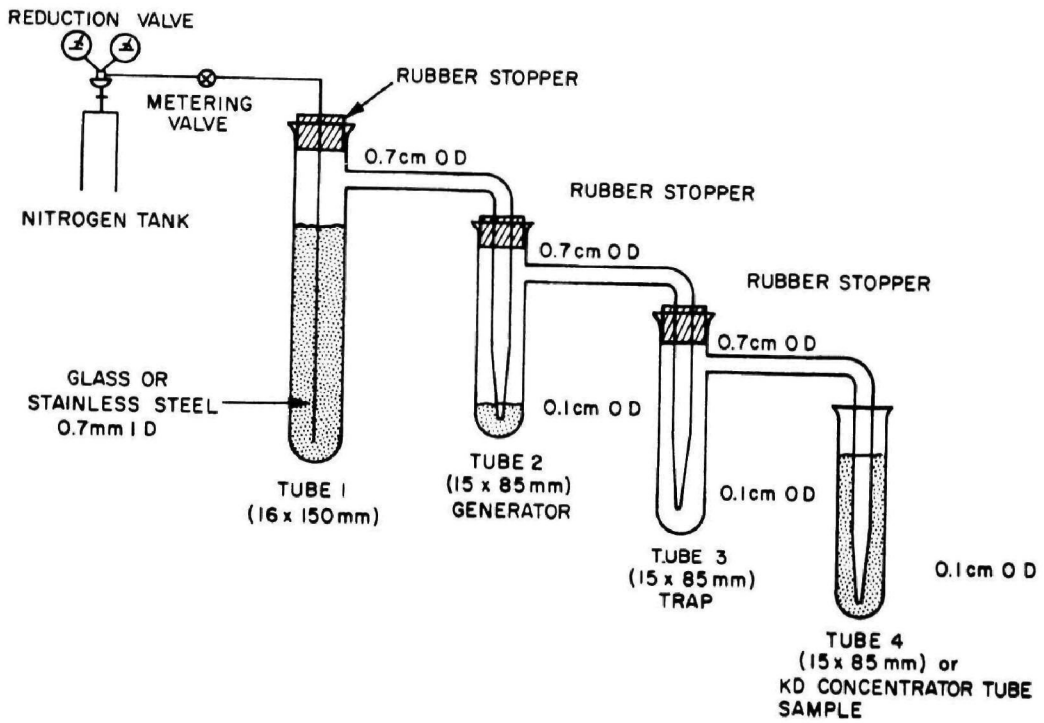

FIGURE 15. APPARATUS FOR DIAZOMETHANE METHYLATION

APPENDIX THREE

PROCEDURE FOR DIMETHYL SULFATE METHYLATION

The apparatus is shown in Figure 16.

1. Bring the original sample (300 ml) to pH 11 and extract with chloroform to remove neutral and basic compounds.

2. A 500-ml 3-neck (standard taper 24/40) round bottom flask, equipped with a fourth neck for a thermometer, is fitted with two pressure-equalizing addition funnels, the probe of a single-probe pH meter, and a magnetic stirrer.

3. Nitrogen is introduced into the top of the first addition funnel and exits from the top of the second one. Place forty ml of Eastman reagent grade dimethyl sulfate into the first addition funnel and a 50% solution of sodium hydroxide (80 ml) into the second.

4. Pour the sample into the flask and flush the system with nitrogen.

5. After raising the temperature to 85° C, begin dropwise addition of both the dimethylsulfate and the sodium hydroxide solution. Maintain temperature between 80-90° (Caution--exothermic reaction. Have ice available to add to water bath.) and the pH between 10.5-11. Since dimethylsulfate is not readily soluble in water vigorous stirring must be used. The addition time is about 1 hour.

6. After all the dimethylsulfate is added, maintain the reaction vessel at 85-90° for an additional 15-20 minutes and then cool to room temperature.

7. Add 5 ml concentrated ammonium hydroxide to destroy excess dimethylsulfate and re-extract the reaction mixture with chloroform to remove the methyl esters of acids and the methyl ethers of phenols.

8. Dry the chloroform extract and evaporate to the appropriate volume for GC analysis.

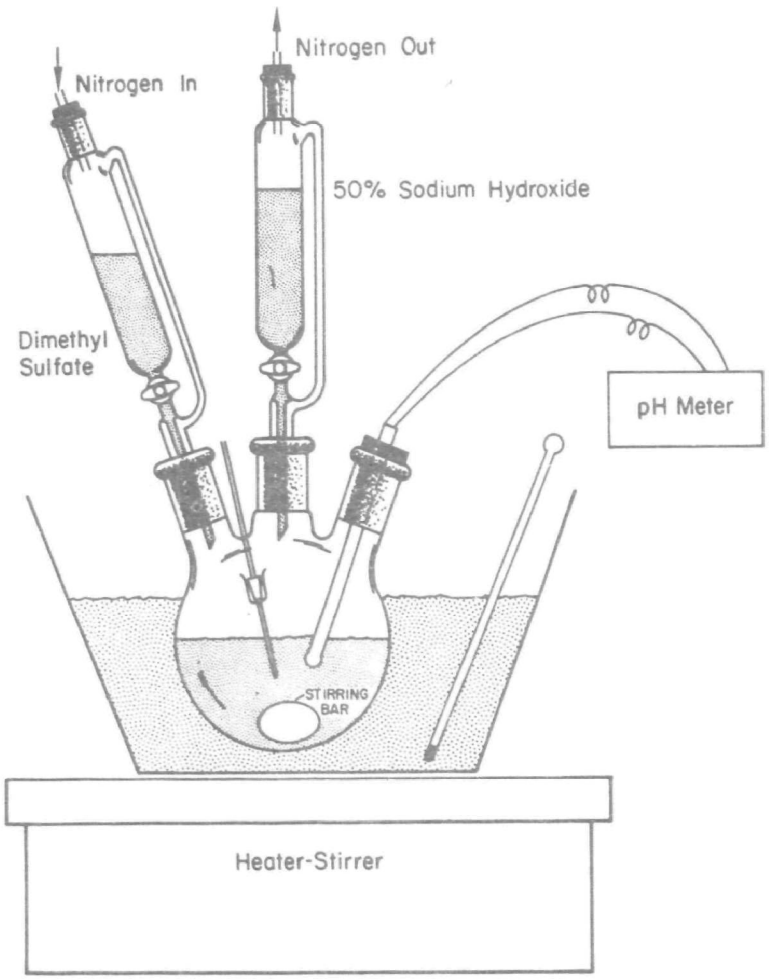

FIGURE 16. APPARATUS FOR DIMETHYL SULFATE METHYLATION

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16. Abstract Experiences during five years of evaluating the application of gas chromatography-mass spectrometry (GC-MS) to wastewater analysis at the Southeast Environmental Research Laboratory have resulted in the selection of recommended practices for such applications. Liquid-liquid extraction with solvents such as methylene chloride and chloroform removed greater than 50 percent of compounds found in pulp mill and petrochemical waste at concentrations of 2 µg/ℓ to 20 µg/ℓ. The Kuderna-Danish evaporator was the most effective means of concentration after extraction. Diazomethane and dimethyl sulfate proved to be the most effective of five methylation reagents studied. Packed columns were effective for gas chromatography of simple mixtures and SCOT columns provided better overall performance for complex mixtures. Computerized data reduction was essential for practical use of GC-MS for samples containing many compounds. A computerized spectra matching program proved highly effective in identifying compounds contained in the computer library. The system was shown to be effective in solving problems related to fishkills caused by pesticides, confirmation of polychlorinated biphenyl residues in water and identification of compounds discharged by over a dozen industries. Over two hundred compounds were identified in industrial effluents.

17a. Descriptors *Pollutant Identification, *Water Sampling, *Solvent Extractions, *Gas Chromatography, *Mass Spectrometry, Industrial Wastes, Organic Wastes, Data Storage and Retrieval, Water Pollution Sources, Water Chemistry

17b. Identifiers *GC-MS, GC/MS, *Computer-aided Organic Compound Identification, Derivative Formation, Clean-up, Case Histories, Kuderna-Danish Evaporator

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