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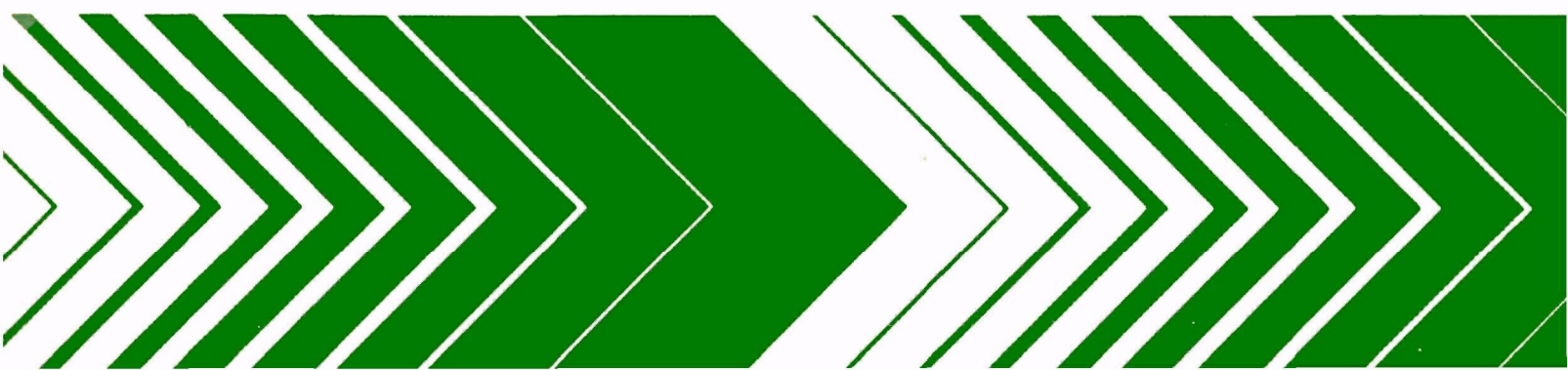
Analytical Procedures for Determining Organic Priority Pollutants in Municipal Sludges



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ANALYTICAL PROCEDURES FOR DETERMINING ORGANIC PRIORITY POLLUTANTS IN MUNICIPAL SLUDGES

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FOREWORD

The Environmental Protection Agency was created because of increasing public and government concern about the dangers of pollution to the health and welfare of the American people. Noxious air, foul water, and spoiled land are tragic testimony to the deterioration of our natural environment. The complexity of that environment and the interplay between its components require a concentrated and integrated attack on the problem.

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This report describes analytical procedures developed for the determination of semivolatile organic priority pollutants in municipal sludge at levels down to 0.01 µg/g.

ABSTRACT

An analytical procedure was developed for the determination of 54 semivolatile organic priority pollutants in sludge at levels down to 0.01 µg/g wet weight. The procedure involved extraction with methylene chloride or chloroform, cleanup of groups of compounds having common properties, and in most cases analysis of the fractions by GC-MS using high-resolution glass capillary columns and selected ion searches. The final analyses involved the analysis of three separate fractions, namely benzidines, phenols, and neutrals. The benzidines were determined by HPLC analysis using an electrochemical detector instead of by GC-MS because GC-MS sensitivity for these compounds was too low. Quantitation in the GC-MS analyses involved the internal standard method applied to selected ion responses. Relative response factors obtained from the analysis of standard solutions were used as correction factors.

The cleanup steps were considered the most critical parts of the program. Benzidines were cleaned up from neutral and acidic components by a simple acid-base extraction procedure. Phenols were cleaned up from neutral components by acid-base extraction. Fatty acids, a major interference, were separated from phenols by gel permeation chromatography using Bio-Beads S-X8. Neutrals were cleaned up from acidic components by acid-base extraction. Triglycerides, a major interference in the neutrals, were removed by gel permeation chromatography using Bio-Beads S-X8. Saturated hydrocarbons, another major interference in the neutrals, were removed by adsorption chromatography using activated silica gel. This step also removed highly polar components.

The procedure was applied to the analysis of sludge spiked with the priority pollutants of concern at a level of 0.05 µg/g, wet weight basis (1 µg/g dry weight basis for 5% solids sludge). Recoveries were generally good and in many cases were greater than 50 percent. For many of the phthalates and aromatic hydrocarbons, recoveries of several hundred percent were obtained indicative of their presence in the starting sludge. Some of the chloroethers, nitro compounds, and phenols were not recovered possibly because of retention by particulate material in the sludge, degradation during the 24-hour equilibration period, or loss in the relatively large amount of caustic wash required to remove the fatty acids. Good recoveries were obtained for benzidines even at 0.25 µg/g dry weight.

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SECTION 1

INTRODUCTION

The U.S. Environmental Protection Agency has designated 114 organic chemicals as priority pollutants and is setting limits on the discharge levels permitted for these pollutants in wastewater, sludge, landfill leachate, etc. In order to establish programs for compliance with the discharge limits satisfactory analytical methodology is needed for use by the dischargers and by EPA. The objective of the study described in this report was the development of efficient methodology for the determination of 54 of the semivolatile organic priority pollutants in municipal sewage sludge.

Those 54 chemicals were comprised of 11 acids (phenols), 2 bases (benzidines), 16 polycyclic aromatic hydrocarbons, 6 phthalates, 2 nitrosamines, 3 chloroalkyl ethers, 8 chlorinated hydrocarbons, and 6 miscellaneous neutral compounds. They are listed in Table 1. The detection limit desired was 0.3 μg/g of sludge on a dry weight basis. The sludge of interest included both primary and activated sludge prior to treatment by an anaerobic digester and the digested sludge.

TABLE 1. EPA SEMIVOLATILE ORGANIC PRIORITY POLLUTANTS

Polycyclic Aromatic Hydrocarbon	
1. Acenaphthene	9. Chrysene
2. Acenaphthylene	10. Dibenzo(a,g)anthracene
3. Anthracene	11. Fluoranthene
4. Benzo(a)anthracene	12. Fluorene
5. Benzo(b)fluoranthene	13. Indeno(1,2,3-cd)pyrene
6. Benzo(k)fluoranthene	14. Naphthalene
7. Benzo(g,h,i)perylene	15. Phenanthrene
8. Benzo(a)pyrene	16. Pyrene
Phthalates	
1. Bis(2-ethylhexyl) phthalate	4. Dimethyl phthalate
2. Butylbenzyl phthalate	5. Di-n-butyl phthalate
3. Diethyl phthalate	6. Di-n-octyl phthalate
Chlorinated Hydrocarbons	
1. 2-Chloronaphthalene	5. Hexachlorobenzene
2. 1,2-Dichlorobenzene	6. 1,2,4-Trichlorobenzene
3. 1,3-Dichlorobenzene	7. Hexachlorobutadiene
4. 1,4-Dichlorobenzene	8. Hexachlorocyclopentadiene
Chloroalkyl Ethers	
1. Bis-(2-chloroethyl) ether	3. Bis-(2-chloroisopropyl) ether
2. Bis-(2-chloroethoxy)methane	
Nitrosamines	
1. N-Nitrosodiethylamine	2. N-Nitrosodiphenylamine
Miscellaneous Neutrals	
1. 4-Bromophenyl phenyl ether	4. 2,6-Dinitrotoluene
2. 4-Chlorophenyl phenyl ether	5. Isophorone
3. 2,4-Dinitrotoluene	6. Nitrobenzene
Acids	
1. 4-Chloro-3-methylphenol	7. 2-Nitrophenol
2. 2-Chlorophenol	8. 4-Nitrophenol
3. 2,4-Dichlorophenol	9. Pentachlorophenol
4. 2,4-Dimethylphenol	10. Phenol
5. 4,6-Dinitro-2-methylphenol	11. 2,4,6-Trichlorophenol
6. 2,4-Dinitrophenol	
Bases	
1. Benzidine	2. 3,3'-Dichlorobenzidine

SECTION 2

CONCLUSIONS

The analysis scheme developed works very well in most cases for the analysis of neutrals, phenols, benzidines, pesticides, and PCBs in raw and digested sludge. The combination of extractions, gel permeation chromatography, and adsorption chromatography used for cleanup is an effective approach. The use of HPLC with an electrochemical detector is highly sensitive and selective for the determination of benzidines. The computerized GC-MS system used with glass capillary columns is highly effective as a sensitive and selective method for detecting and quantitating nearly all of the other semivolatile organic priority pollutants.

The primary remaining problems are (1) poor recoveries of nitrophenols, (2) poor recoveries of some of the more polar neutrals, namely, the nitro compounds, chloroethers, and isophorone, and (3) poor quantitative reproducibility. These problems could be caused by degradation during the 24-hour equilibration step, retention by the particulate material of the sludge, loss to the aqueous phase facilitated by soaps during caustic extraction, or alteration of separation patterns caused by large amounts of fatty acids. A systematic study should be made of each of these possible causes. Such a study could be carried out very efficiently without its being a major effort by using fatty acid and priority pollutant standards and determining recoveries by GC or HPLC.

SECTION 3

RECOMMENDATIONS

Determine the recoveries and reproducibilities of recovery of chloroether and nitrobenzenes, with and without the addition of sludge extractables, in each of the following cleanup steps: (a) caustic extraction, (b) Bio-Beads S-X8 chromatography, and (c) silica gel chromatography.

Determine the recoveries and reproducibilities of recovery of nitrophenols with and without the addition of sludge extractables, in each of the following cleanup steps: (a) Bio-Beads S-X8 chromatography, (b) caustic extraction, and (c) methylene chloride extraction of the acidified caustic extract.

Determine the effect of equilibration time on the recoveries and reproducibilities of recovery of nitrophenols and polar neutrals from spiked sludge.

Determine the effect of adding salt or a water-miscible organic solvent on the recoveries and reproducibilities of recovery of nitrophenols and polar neutrals from spiked sludge.

Use glass capillary column gas chromatography for the analyses of neutrals required in the above studies using a Hall detector for chloroethers and a thermionic detector in the nitrogen mode for the nitrobenzenes.

Use HPLC with a variable wavelength UV detector for the analyses of nitrophenols required in the above studies.

SECTION 4

STATEMENT OF THE PROBLEM

Sludge generally contains 1 to 8 percent solids on a dry weight basis and those solids in turn contain 10 to 15 percent solvent-extractable organic material. The desired detection limit of 0.3 µg/g on a dry weight basis thus corresponds to 2 to 3 µg/g of organic extractable material. The bulk of the extractable material is expected to be components such as petroleum fuels, lubricating oils, asphalt, fats, fatty acids, and detergents. The analysis problem can be equated to that of looking for a few parts per million of a lipophilic component in crankcase oil or cooking oil. In this perspective the complexity of the problem is immediately apparent. Extensive cleanup is desirable; however, the broad range of physical properties of the various priority pollutants makes it impossible to separate them as a single group. A combination of cleanup methods, detector sensitivity, and detector selectivity is needed.

If the GC-MS detection limit is 10 ng and one percent of the sample extract, e.g. 2 µl out of a total of 200 µl, is injected, at least 100 mg of total extractable material must be dealt with in the cleanup process. If the maximum concentration of total material that can be handled satisfactorily by the GC-MS system is 10 mg/ml (2 mg/200 µl) then at least a 98 percent cleanup is required. If the GC-MS detection limit is higher, an even greater degree of cleanup is required.

SECTION 5

OVERALL APPROACH

The basic approach used for determining semivolatile priority pollutants in sludge involved extraction with methylene chloride or chloroform, cleanup of groups of compounds having common properties, and analysis by GC-MS using high-resolution glass capillary columns and selected ion searches. The benzidines were an exception in that they were determined by HPLC using an electrochemical detector according to the method of Riggin and Howard*.

Several approaches to extraction were studied including freezedrying followed by Soxhlet extraction, methanol-drying followed by Soxhlet extraction, azeotropic drying and the extraction associated with it, and repetitive equilibration with solvent. The latter method, involving homogenizing to promote equilibration, centrifuging to promote phase separation, and withdrawal of the organic layer with a syringe, was chosen as an effective and convenient method. Also less decomposition would be expected using this method because of the absence of heat.

The 54 compounds of concern are comprised of neutrals, phenols and benzidines. A separate aliquot of the sludge sample was used for the determination of each of the three groups of compounds. The benzidines were cleaned up from the acidic and neutral components present in a chloroform extract by extraction into strong aqueous acid followed by neutralization and reextraction into chloroform. Because of the difficulty of protonating dichlorobenzidine, a very strong acid, 1.0N H_2SO_4, is required for this step. The use of 0.1N H_2SO_4 is entirely inadequate. All hydrocarbons, triglycerides and fatty acids are removed in this process. The use of HPLC and the selectivity of the electrochemical detector provide additional discrimination from interferences.

The phenols were cleaned up from neutral and basic components present in a methylene chloride extract by a combination of gel permeation chromatography (GPC) and acid-base extraction. The extract was first passed through a GPC column of Bio-Beads S-X8, a porous styrene-divinylbenzene copolymer, with methylene chloride as the eluting solvent. Higher molecular weight components, primarily the triglycerides, long-chain hydrocarbons, and long-chain fatty acids, elute prior to the phenols and were thus removed by this

* R. M. Riggin and C. C. Howard, Anal. Chem., 51, 210 (1979)

step. The phenols, which were contained in a lower molecular weight fraction, were cleaned up from the remaining neutral and basic components by extraction into aqueous base followed by acidification and reextraction into methylene chloride.

The neutrals in a separate methylene chloride extract were separated from phenols and the large amounts of fatty acids by caustic extraction using rather large volumes of 0.1N NaOH in 10% NaCl. This left a solvent extract containing the neutral priority pollutants mixed with major amounts of triglycerides, hydrocarbons, and more polar interfering neutral components. Gel permeation chromatography, identical to that described for cleanup of phenols, was applied to the neutrals also. In this case the triglycerides eluted prior to the priority pollutants but separation of many of the hydrocarbons from the relatively large phthalate esters was not possible. Therefore, after the removal of the triglycerides by GPC, two additional GPC fractions were collected, the first of which was cleaned up further by adsorption chromatography on activated silica gel. The hydrocarbons were eluted first from the silica gel and discarded, followed by the more polar neutral components, the phthalates, chloroethers, nitroaromatics and isophorone. Any highly polar compounds were left on the silica gel column. The aromatic hydrocarbons and halocarbons were obtained in a second GPC fraction which was analyzed by GC-MS without further cleanup.

The overall analysis scheme described above, involving the use of three separate sludge samples for phenols, neutrals, and benzidines, respectively, is outlined in Figure 1. The final analyses entailed HPLC-EC analysis of a benzidine fraction and GC-MS analysis of a methylated phenol fraction and combined neutral fraction. The largest group of frequently occurring interfering compounds were the alkylbenzenes and alkylnaphthalenes present in the neutrals. Since these compounds have physical properties similar to those of dichlorobenzenes and naphthalene they cannot be removed--thus the selectivity of the GC-MS system must be relied upon for the determination of dichlorobenzenes and naphthalene in the presence of such interferences. It should also be recognized however, that the alkylbenzenes and alkylnaphthalenes may represent nearly as great an environmental hazard as some of the priority pollutants and thus their presence should be of interest.

The GC-MS system used employed high-resolution glass capillary columns and selected ion searching. Such a system provided high sensitivity, high resolution of priority pollutants from any remaining interferences, and high selectivity of detector responses. Quantitation was based upon the total area of the selected ions of a priority pollutant peak relative to the total area of the selected ions of an internal standard peak. Relative response factors obtained from the analysis of standard solutions were used as correction factors in the calculations of priority pollutant concentrations.

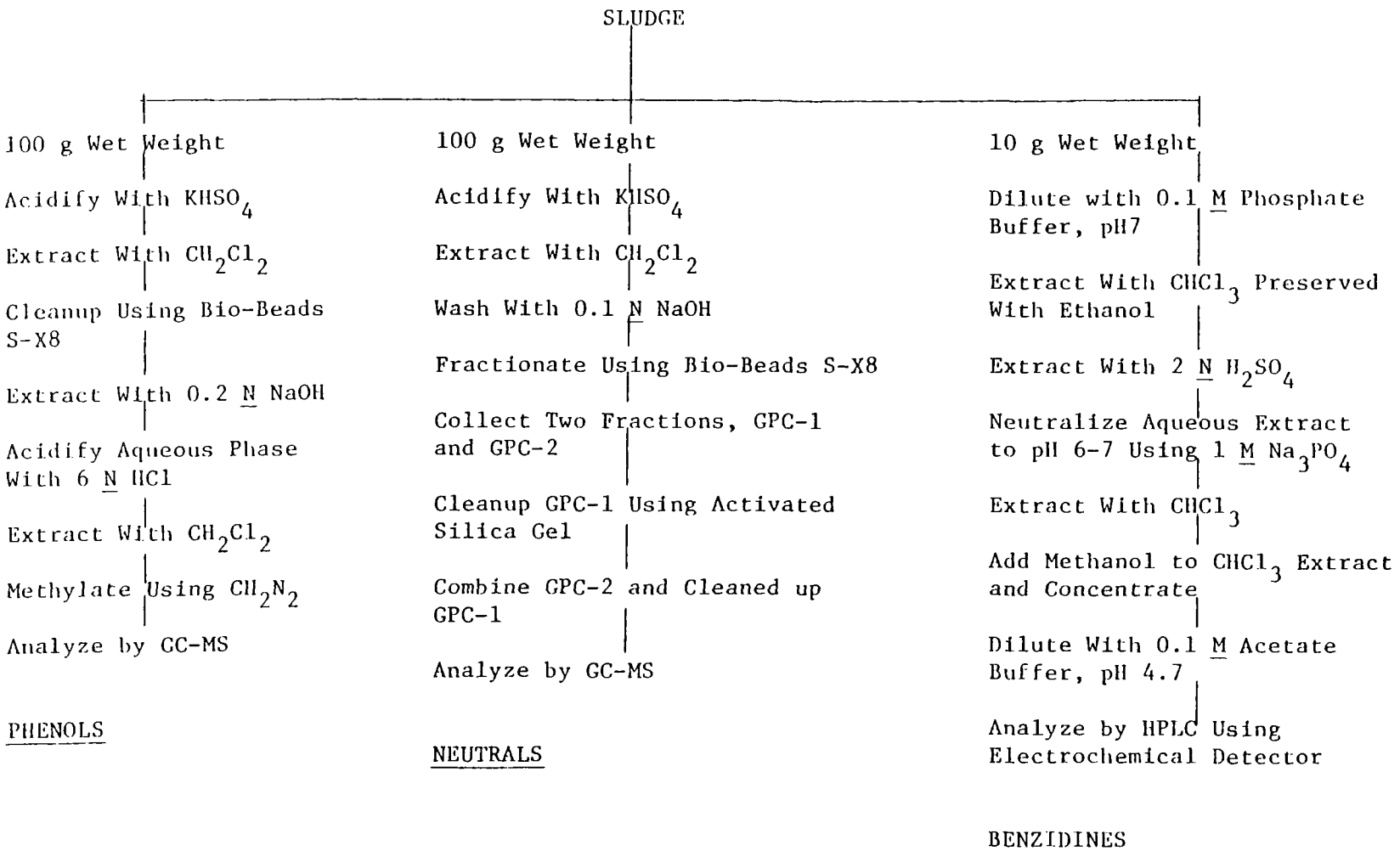

Figure 1. Scheme for determination of semivolatile priority pollutants in sludge.

SECTION 6

EXPERIMENTAL STUDIES

The experimental details of the analytical procedure developed for this program are described in Appendix A. The various studies involved in developing and evaluating the procedure are discussed below. Although there was not sufficient time for a systematic study of recoveries of all of the compounds from each step during the development of the procedure various modifications were studied briefly.

EXTRACTION

Several methods of extraction were studied including freeze drying followed by Soxhlet extraction, methanol-drying followed by Soxhlet extraction, azeotropic drying and the extraction associated with it, and repetitive equilibration with solvent. Since the extraction step was studied prior to the development of efficient cleanup methods, the effectiveness of extraction was based on the yield of total extractable material obtained.

Data comparing the effectiveness of various extraction techniques for both raw and digested sludge are shown in Table 2. The values given are in weight per cent of dry weight of sludge. The raw sludge was 4.0% solids by weight and the digested sludge was 2.8% solids by weight.

The weight per cent of extractables recovered by each method was based on the residue weight obtained by evaporating the solvent from a 100-µl aliquot of the extract.

The data suggest that extraction method 6, 7, 8, or 9 would be the best choice for the most efficient removal of the total organic material from sludge. Homogenization of the sludge with dichloromethane (Method 8) was selected for subsequent studies. The method is very rapid and simple and is carried out at room temperature. A Tekmar Tissumiser was used for the homogenization. Only Teflon and stainless steel, which are easily cleaned prior to each extraction, contact the sample. Following homogenization the sample is centirfuged and the organic layer withdrawn with a syringe. The process is repeated four times to give a total of five extracts which are combined and dried over magnesium sulfate.

It is desirable to avoid methanol in the extracting solvent because it must be removed prior to subsequent cleanup on silica gel. This additional step would require extra effort and presents a potential loss of priority

TABLE 2. YIELDS FROM VARIOUS EXTRACTIONS METHODS

Description of Extraction Method	Yield, mg per 100 mg of dry sludge	
	Raw	Digested
1. Soxhlet extract with MeOH then 1:1 benzene-MeOH	18.4	7.6
2. Freeze dry; Soxhlet extract with DCM[a]	18.7	6.9
3. Freeze dry; Soxhlet extract with 1:1 benzene-MeOH	20.2	--
4. MeOH wash; Soxhlet extract with 1:1 benzene-MeOH	15.0	9.0
5. MeOH wash; Soxhlet extract with DCM	17.4	8.3
6. MeOH wash; Soxhlet extract with MeOH then fresh 1:1 benzene-MeOH	25.6	11.3
7. Azeotropic drying with EDC[b]; centrifugation	25.6	11.6
8. Homogenization with DCM; centrifugation	22.4	12.1
9. Homogenization with 1:1 DCM-MeOH; centrifugation	26.3	12.7

a. Dichloromethane (methylene chloride)
b. Ethylene dichloride

pollutants as great as the possible increase recovery during the initial extraction.

The effectiveness of each of the five homogenization-extraction steps using dichloromethane (DCM) as the solvent was determined. For this study 100g of a wet digested sludge was extracted five times with dichloromethane. The results, shown in Table 3, indicate that the first three extractions remove 95 per cent of the total organic material obtained from the five extractions. Therefore only three extractions were used in subsequent studies.

TABLE 3. REPETITIVE HOMOGENIZATION-EXTRACTION OF SLUDGE WITH DCM

Extraction Step	Weight of Total Extract, mg	Weight Per cent of Total
1	106	50
2	79	38
3	15	7
4	4	2
5	6	3
	210	100

REMOVAL OF ACIDS FROM NEUTRALS

The approach initially tried for obtaining the neutrals involved starting with a single methylene chloride extract to obtain both a neutral fraction and an acid fraction. The acids were removed from the neutrals by extraction of the extract from 100g of wet sludge with four 50-ml portions of 0.2 N NaOH. The combined aqueous layers were acidified and back extracted with methylene chloride to give an acid fraction containing fatty acids and phenols. However, the sludge used contained very high levels of fatty acids, 1 to 2% on a wet weight basis, which formed so much soap that the caustic extraction was not efficient. Some of the netural components stayed in the soap solution and some of the soaps stayed in the methylene chloride. Emulsions were also a problem.

Because of the above problems the extraction scheme was modified by greatly increasing the amount of dilute caustic used for washing out fatty acids and by adding salt to the water to avoid emulsion formation. However, the large amount of water used made it difficult to back extract the nitrophenols into methylene chloride after acidification. Consequently a separate sludge extract was used for obtaining a cleaned up phenol fraction in which gel permeation chromatography (GPC) was used as the first cleanup step as will be discussed below.

The neutral fraction was further fractionated by GPC to give one fraction containing the phthalates and the more polar priority pollutants and a second fraction containing the aromatic hydrocarbons, halocarbons, and ethers. Only the first fraction was cleaned up further by silica gel chromatography. The two fractions were then combined in most cases to give only one final neutral extract for GC-MS analysis.

GPC STUDIES

Sephadex LH-20 Chromatography

Sephadex LH-20, a modified dextran gel was studied for use as a gel permeation fractionation step to clean up groups of compounds on the basis of molecular size. Three major conditions had to be considered. First, the solvent system had to be compatible with that of the sludge extract. Second, since Sephadex LH-20 is less dense than many common organic solvents, densities of different solvents had to be taken into consideration. Third, the solvent system chosen had to optimize separations between different sizes of molecules.

Since the concentrated sludge extract was in methylene chloride, the first choice was to use methylene chloride as the eluting solvent in the fractionation step. Since the Sephadex LH-20 was less dense than methylene chloride, this necessitated the use of an upward-flow liquid chromatographic apparatus. A 750 mm x 15 mm I.D. upward flow column was obtained for this purpose and packed with approximately 50 grams of Sephadex LH-20 swelled in methylene chloride. The apparatus was found to be an effective means of fractionating the sludge extracts but it was determined after actual application of sludge extracts that large amounts of material remained on the column after elution of the priority pollutants. This indicated the need for washing the packing with a solvent which would remove the adsorbed material or using fresh Sephadex LH-20 for each sample. Either alternative necessitated repacking the upward flow column for each sample application. Since it took considerable time to pack the upward flow apparatus, a compatible solvent system for use in a downward flow system was considered to be a time-saving alternative.

The choices of solvent systems in which the sludge was relatively soluble were somewhat limited. The sludge extract was soluble in such solvents as methylene chloride, ethylene dichloride, and benzene. The extract was moderately soluble in acetone, slightly soluble in n-butyl chloride and cyclopentane, and insoluble in methanol, ethanol, and isopropanol. Studies were also done to determine which mixtures of these and similar solvents would allow the use of a downward flow apparatus and also would be compatible with the sludge extract. Nine solvent systems were decided upon for evaluation, and are shown in Table 4. The solvent systems which provided the best conditions were 80:20 ethylene dichloride:n-butyl chloride and 70:30 methylene chloride:n-butyl chloride. The former, which contained less n-butyl chloride, was chosen for further study because it was more compatible with the sludge extract.

It was also necessary to determine the amount of material that the gel could efficiently handle. Varying amounts of sludge extract was applied to

TABLE 4. TEST SOLVENT SYSTEMS FOR SEPHADEX LH-20 FRACTIONATIONS

Solvent System	Comments
70:30 Methylene Chloride:Isopropanol	Isopropanol caused precipitation.
90:10 Ethylene Dichloride:Cyclopentane	Gave poor separation.
80:20 Ethylene Dichloride:Acetone	Gave poor separation.
80:20 Ethylene Dichloride:n-Butyl Chloride	Designated for further studies.
80:20 Ethylene Dichloride:Benzene	Benzene is restricted solvent.
70:30 Methylene Chloride:n-Butyl Chloride	Designated for further study.
70:30 Methylene Chloride:Cyclopentane	Gave poor separation.
70:30 Methylene Chloride:Benzene	Benzene is restricted solvent.
70:30 Methylene Chloride:Acetone	Gave poor separation.

the Sephadex LH-20 columns, and it was found that approximately 5 mg of extractable material could be applied for each gram of dry Sephadex LH-20 without significantly overloading the column.

After this process of optimizing the column run conditions, several standards containing various classes of priority pollutants were run on the column. It became evident that very polar compounds such as phenols did not elute from the column.

BioBeads Chromatography

BioBeads are porous styrene-divinylbenzene copolymers which do not have severe adsorption problems. The process of optimizing solvent systems was repeated, mainly concentrating on those solvent systems which were successful when used with Sephadex LH-20. Toluene was suggested by the manufacturer as a possible solvent for this application and was studied along with acetone, ethylene dichloride, methylene chloride, and 50:50 methylene chloride:acetone. Also studied were the different types of BioBeads differentiated by increasing pore sizes and hence increasing size exclusion limits. The grades studied were BioBeads S-X12, S-X8, S-X4 and S-X3, in order of increasing size exclusion limits.

Toluene was found to be unsatisfactory as a solvent since it did not display good separating abilities. All of the other solvent systems displayed good separation abilities. Acetone was rejected because it did not dissolve the sludge extractables as well as the other solvents.

As the size exclusion limit of the BioBeads gel was increased its bed volume and separation ability increased. This indicated a tradeoff between good separation ability and short fractionation times. BioBeads S-X8 provided good fractionation capabilities and an acceptable analysis time and was chosen for further study. Elution profiles of whole sludge extracts on BioBeads S-X12, S-X8, and S-X4 are shown in Figure 2.

The column flow parameter was found to be an important factor in optimization of this technique. The flow rate was optimized at approximately 4 to 8 ml/hour/cm^2. Therefore, a 15 mm I.D. column, which has a cross-sectional area of 1.8 cm^2, would have an optimum flow rate of 7 to 14 ml/hour.

It was anticipated that certain classes of compounds, specifically fatty acids, triglycerides, and saturated hydrocarbons, would be present in large amounts in the sludge extracts and would subsequently cause problems in detecting low levels of the priority pollutants. It was therefore necessary to ascertain the efficiency of the BioBeads S-X8 system in separating these interfering compounds from the priority pollutants. Figure 3 represents three samples run on a 40 mm x 9 mm I.D. column packed with 10 grams of BioBeads S-X8 in 50:50 methylene chloride:acetone. The first sample (Figure 3(a)) was whole sludge extract, the second sample (Figure 3(b)) was composed of fatty acids, and the third sample (Figure 3(c)) contained triglycerides. It was ascertained by subsequent gas chromatographic analysis that the later eluting material, as indicated in Figure 3(a), was composed

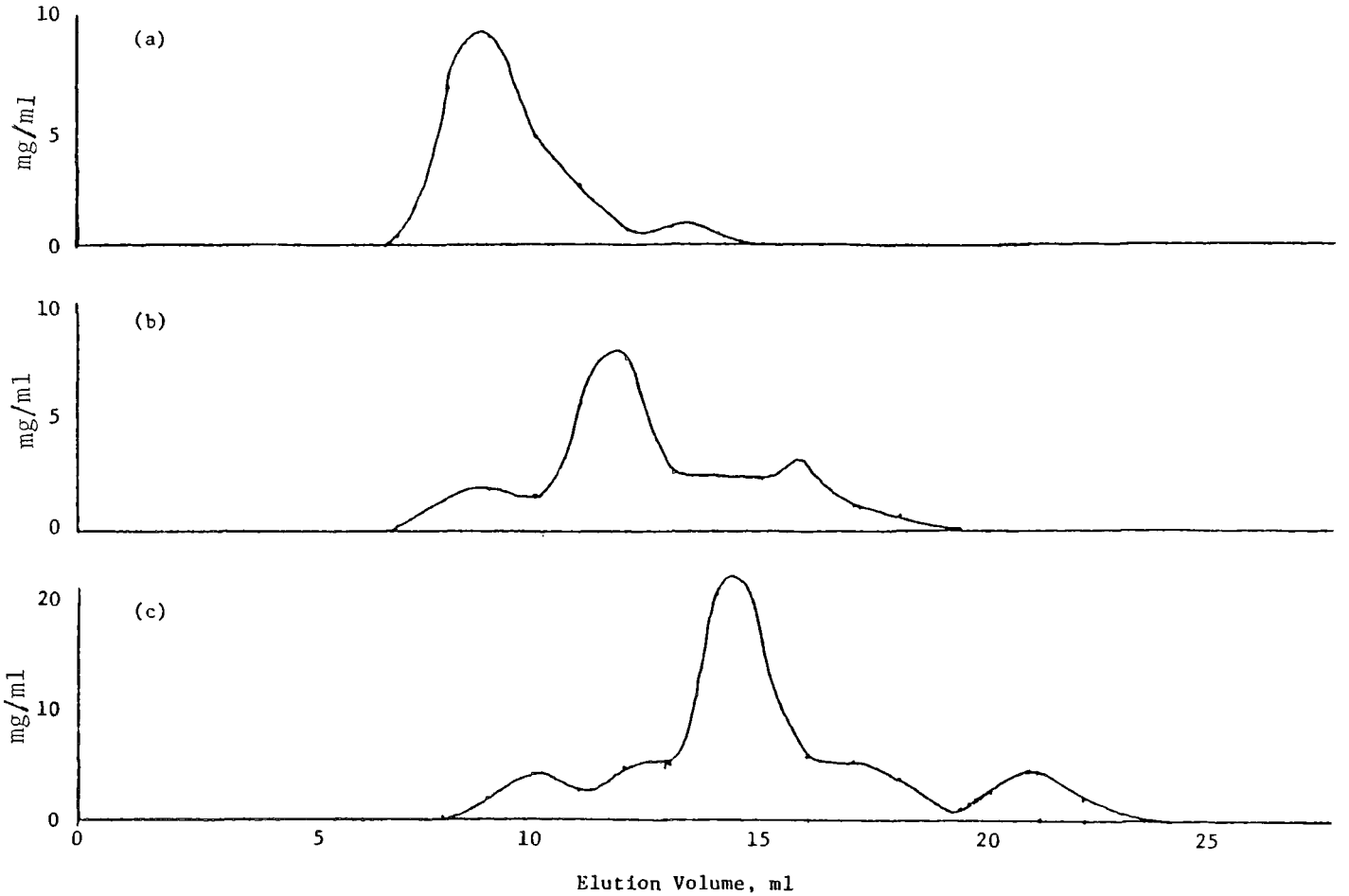

Figure 2. Elution profiles of whole sludge extract on (a) BioBeads S-X12, (b) BioBeads S-X8, and (c) BioBeads S-X4

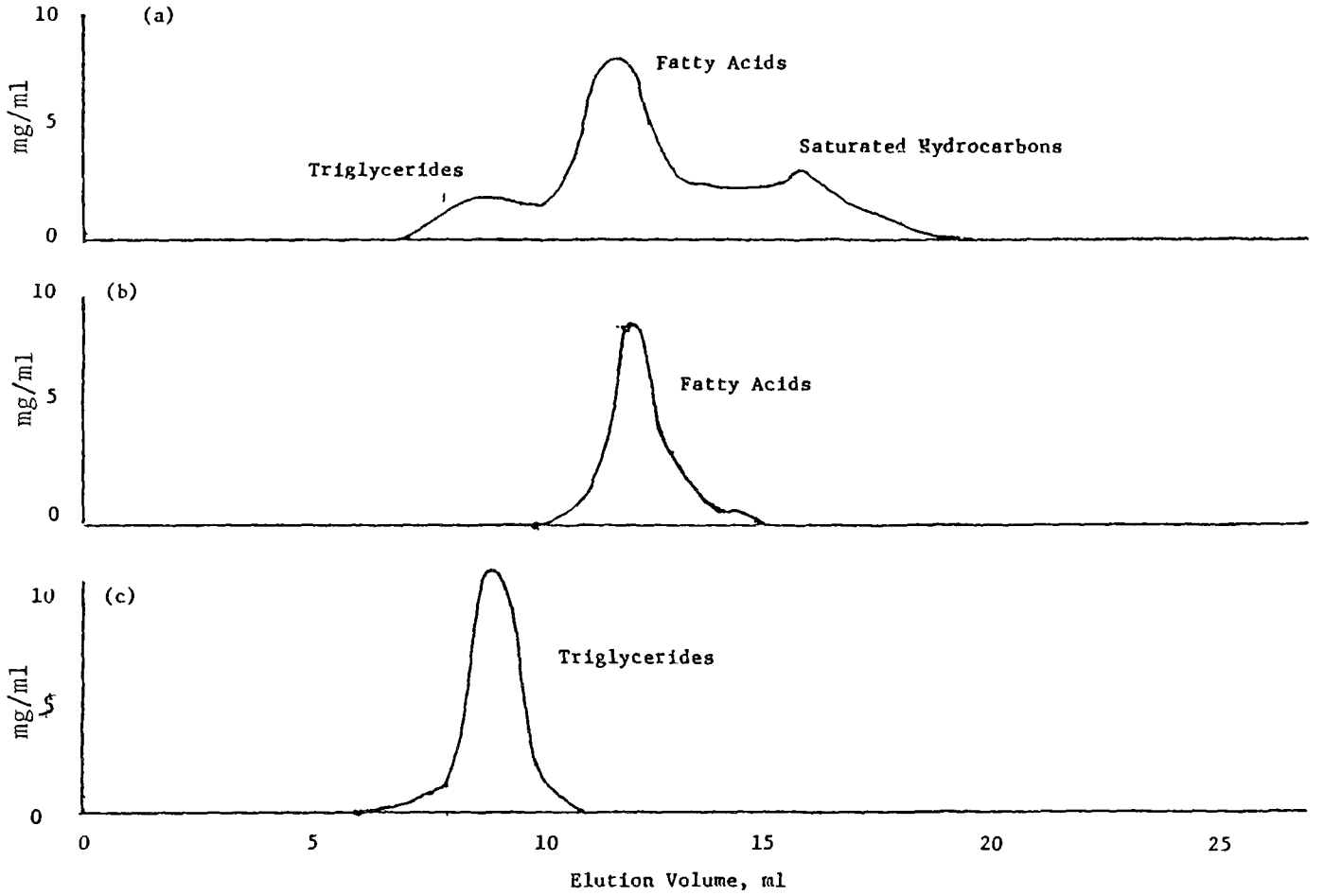

Figure 3. Elution profiles of (a) whole sludge extract, (b) fatty acids, and (c) triglycerides on BioBeads S-X8

of saturated hydrocarbons.

Capacity tests were also performed using BioBeads S-X8, and it was estimated that a maximum of 5 milligrams of sludge extractables per gram of BioBeads S-X8 could be applied without significantly overloading the column.

The use of BioBeads S-X8 in the place of Sephadex LH-20 eliminated the adsorption problems encountered in the latter. When whole sludge was run through a BioBeads S-X8 column, virtually all of the material was recovered from the gel. This was also evident when phenol standards were run through the BioBeads S-X8 and no adsorbance problems were observed. Since material did not seem to adsorb to this column it was possible to reuse this column for more than one sample, thus saving the time necessary to pack new BioBeads S-X8 columns and also eliminating the necessity of purchasing large amounts of the gel. This also made the use of BioBeads S-X8 upward flow column packed in methylene chloride more desirable since the need for repeated repacking of the column was eliminated. The size of the column was increased to increase its capacity to handle up to 1 gram of extractable material.

The performance of the column in terms of efficiency and retention times of reference compounds was studied. Approximately 2500 theoretical plates were achieved for the elution of di-n-octyl phthalate. The capacity factors, K', for a number of reference compounds are given in Table 5.

TABLE 5. CAPACITY FACTORS OF REFERENCE COMPOUNDS ELUTED WITH METHYLENE CHLORIDE FROM BIO-BEADS S-X8

Compound	Capacity Factor K'
Di-n-octyl phthalate	0.40
Dimethyl phthalate	0.67
Phenylacetic acid	0.70
2,4-Dinitrophenol	0.76
Hexaethylbenzene	0.83
2-Nitrophenol	0.92
4-Nitrophenol	0.99
2,4-Dimethylphenol	1.01
Phenol	1.08
2-Chlorophenol	1.09
2,4-Dichlorophenol	1.11
Benzene	1.19
Dibenz(a,h)anthracene	1.19
Pyrene	1.31
Sulfur	1.80

It is of particular interest to note that the K' for sulfur is considerably higher than that of the other compounds. Because of this the GPC

cleanup works very well for the removal of sulfur which would otherwise interfere with the GC-MS analysis.

SILICA GEL CLEANUP

It was necessary to include relatively large molecules in the fraction taken from the BioBeads cleanup of the neutral fraction in order to analyze for the larger phthalates. This necessitated a cleanup step to remove the relatively large amounts of long-chain hydrocarbons that were contained in that fraction. Silica gel chromatography was evaluated as a method to fractionate this extract on the basis of compound polarity. Standards containing a wide variety of compounds were run on silica gel, and the type of solvent system needed to elute each compound was noted. Nonpolar compound such as saturated hydrocarbons eluted with nonpolar solvent such as petroleum ether. Addition of increasing percentages of methylene chloride in petroleum ether eluted more polar molecules such as subsituted benzenes, naphthalenes and polynuclear aromatic hydrocarbons. It was necessary to go to 5% acetone in methylene chloride to elute polar neutral compounds such as isophorone, nitro compounds, and the phthalates. Very polar molecules remained on the silica gel.

PACKED COLUMN GC-MS ANALYSIS

In an effort to simplify the GC-MS analysis somewhat and utilize previously developed EPA methods, the use of packed GC columns instead of a capillary column was studied very briefly. It was found that the sensitivities achieved using packed columns, in terms of GC-MS peak areas, were generally poorer, by a factor of 5 to 10, than those achieved using capillary columns. Resolution, of course, was much lower with the packed columns. The results of a single comparison of the two techniques applied to the determination of components in spiked sludge are given in Table 6. The amounts found using a capillary column were somewhat higher than those found with packed columns. In some cases where components were found by the capillary technique none were found using packed columns. Additional studies are necessary for making a more valid comparison. However, since the confirmation of the identity of each component detected by a selected ion search is based upon its full mass range spectrum, the superior resolution of capillary column systems should give significantly fewer false negatives in a matrix as complex as sludge extracts.

ANALYSIS OF PHENOLS

Methylation of phenols by diazomethane is used to improve their gas chromatographic properties. Although the more acidic phenols, e.g. nitrophenols and trichlorophenol, methylate rather completely, the less acidic phenols, e.g. dimethylphenol, chlorophenol, or phenol itself, are only partially derivatized if at all.

One approach that was studied in an effort to achieve an improvement in the analysis of phenols, was the use of a packed GC column, 1% SP-1240DA, for the analysis of free phenols. The column gave good separation and good peak shape for all of the phenols however the sensitivity was lower than that

obtained with a capillary column. The packed column data for phenols in Table 6 was obtained using 1% SP-1240DA. Despite the disadvantage of poorer sensitivity the packed column approach offers the advantage of eliminating the methylation step and therefore solves the problem of incomplete methylation of the less acidic phenols.

Another approach to the derivatization problem is the use of a different derivatizing agent that might be suitable for all phenols. With this in mind we investigated briefly the preparation of pentafluorobenzyl derivatives by reaction with pentafluorobenzyl bromide (PFBB) in the presence of potassium carbonate and a crown ether. The method worked very well for the less acidic phenols but did not work for nitrophenols at low levels. Since the less acidic phenols can be determined satisfactorily as free phenols using a capillary column and the more acidic phenols are readily methylated by diazomethane this method was retained as the method of choice.

DETERMINATION OF PESTICIDES

The pesticides and PCBs included in the list of priority pollutants are similar to the semivolatile neutrals in terms of solubility, polarity, and size. Accordingly they would be expected to be found in the same fraction as the neutrals in the analysis scheme developed on this program. This means that the pesticides and PCBs could be determined at the same time that the neutrals are being determined with no additional effort other than the additional selected ion searches and calibration studies to establish retention times and response factors.

In an effort to validate this concept a sample of digested sludge was spiked with aldrin, endrin, heptachlor, p,p'-DDT, p,p'-DDE, p,p'-DDD, γ-BHC, and Arochlor 1254 at a level of 10 µg/100 g wet weight and analyzed using the procedure in Appendix A. All of the spiked components were found in the GPC-2 neutral fraction. The recoveries were greater than 50% for each component. The results indicate that separate analysis schemes for pesticides and PCBs are not necessary.

ANALYSIS OF SPIKED AND NONSPIKED SAMPLES

Samples of distilled water, raw municipal sludge, and digested municipal sludge were spiked with all 54 of the priority pollutants of concern and analyzed in triplicate. Similar analyses were performed for nonspiked samples in triplicate. The spiking level varied from 2.5 to 6 µg/100g depending upon the concentrations in standard solutions obtained from Supelco, Inc. The analytical procedures used was that described in detail in Appendix A. The recoveries obtained are given in Tables 7, 8, and 9.

The recoveries from the water samples were very good for nearly all of the components except for dibenzo(a,h)anthracene and some of the phenols. This indicates that with a few exceptions the components are not being lost by the cleanup procedure in the absence of interferences. Most of the components are also recovered from raw or digested sludge, however, in many cases, a meaningful value for percent recovery could not be calculated because of the very significant but highly variable amounts found in the

TABLE 6. COMPARISON OF PACKED COLUMN AND CAPILLARY COLUMN GC ANALYSIS FOR DETERMINING THE RECOVERY OF PRIORITY POLLUTANTS FROM DIGESTED MUNICIPAL SLUDGE[a]

Compound[b]	Amount Added, µg/100 ml	Amount Recovered, µg/100 ml, Using Given GC Method	
		Packed Column[c]	Capillary Column[d]
Bis-(2-chloroethyl) ether	5.0	NA[e]	NA
1,3-Dichlorobenzene	5.0		2.1
1,4-Dichlorobenzene	5.0	4.6[f]	2.1
1,2-Dichlorobenzene	5.0		2.1
Bis-(2-chloroisopropyl) ether	5.0	NA	NA
N-Nitrosodipropylamine	5.0	NA	NA
Nitrobenzene	5.0	NA	NA
Bis-(2-chloroethoxy)methane	5.0	NA	NA
1,2,4-Trichlorobenzene	5.0	1.9	6.7
Naphthalene	5.0	5.7	12
Hexachlorobutadiene	5.0	ND[g]	1.1
2-Chloronaphthalene	5.0	ND	3.4
2,6-Dinitrotoluene	5.0	NA	NA
Dimethyl phthalate	5.0	NA	NA
Acenaphthylene	5.0	ND	ND
Acenaphthene	5.0	ND	1.8
2,4-Dinitrotoluene	5.0	NA	NA
Diethyl phthalate	5.0	NA	NA
Fluorene	5.0	ND	7.7
4-Chlorophenyl phenyl ether	5.0	ND	ND

TABLE 6. (Continued)

Compound[b]	Amount Added, µg/100 ml	Amount Recovered, µg/100 ml, Using Given GC Method	
		Packed Column[c]	Capillary Column[d]
N-Nitrosodiphenylamine	5.0	NA	NA
4-Bromophenyl phenyl ether	5.0	0.4	1.9
Hexachlorobenzene	5.0	0.5	2.2
Phenanthrene	5.0		15
Anthracene	5.0	9.0[h]	15
Di-n-butyl phthalate	5.0	NA	NA
Fluoranthene	2.5	1.1	6.0
Pyrene	2.5	1.5	7.7
Butylbenzyl phthalate	5.0	NA	NA
Chrysene	2.5	ND	6.1
Benzo(a)anthracene	2.5	ND	6.1
Bis(2-ethylhexyl)phthalate	5.0	NA	NA
Di-n-octyl phthalate	5.0	NA	NA
Benzo(b)fluoranthene	2.5	ND	3.7
Benzo(k)fluoranthene	2.5	ND	3.7
Benzo(a)pyrene	2.5	ND	4.0
Benzo(g,h,i)perylene	2.5	ND	2.1
Indeno(1,2,3-cd)pyrene	2.5	ND	2.3
Dibenzo(a,h)anthracene	2.5	ND	ND
Acids			
2-Chlorophenol	5.0	1.3	1.2

TABLE 6. (Continued)

Compound[b]	Amount Added, µg/100 ml	Amount Recovered, µg/100 ml, Using Given GC Method: Packed Column[c]	Capillary Column[d]
Phenol	6.0	7.0	11.0
2,4-Dimethylphenol	5.0	2.0	1.6
2,4-Dichlorophenol	5.0	3.0	6.1
2,4,6-Trichlorophenol	5.0	3.0	5.1
2-Nitrophenol	5.0	ND	ND
4-Chloromethyphenol	5.0	3.0	10
4-Nitrophenol	5.0	ND	ND
4,6-Dinitro-o-cresol	5.0	ND	ND
Pentachlorophenol	5.0	3.0	3.3
2,4-Dinitrophenol	5.0	ND	ND

a. The digested sludge used had a dry solids content of 2.5 g/100 ml and a total lipids content of 0.5 g/100 ml.

b. The priority pollutant standards used were purchased from Supelco, Inc.

c. A 2 m x 2 mm I.D. glass column packed with 3% SP-2250-DB on 100-120 mesh Supelcoport was used for neutrals and a 2 m x 2 mm I.D. glass column packed with 1% SP-1240-DA on 100-200 Supelcoport was used for free phenols.

d. A 30 m x 0.2 mm I.D. glass capillary column coated with SE-30 was used for neutrals and methylated phenols.

e. Not appropriate; only fraction GPC-2 and the acid fraction were analyzed using the packed column; this component does not appear in these fractions.

f. Total for all three isomers.

g. Not detected.

h. Total for phenanthrene and anthracene.

TABLE 7. RECOVERY OF PRIORITY POLLUTANTS FROM WATER

Compound[a]	Amount Added, µg/100 ml	Amount Recovered, µg/100 ml, in Given Sample								Average Recovery,[b] %
		Unspiked				Spiked				
		1	2	3	Avg.	1	2	3	Avg.	
Neutrals										
Bis-(2-chloroethyl) ether	5.0	ND[e]	ND	ND	--	3.3	1.3	(f)	2.3	46
1,3-Dichlorobenzene	5.0	ND	ND	ND	--	3.9	2.8	(f)	3.4	67
1,4-Dichlorobenzene	5.0	ND	ND	ND	--	3.9	2.8	(f)	3.4	67
1,2-Dichlorobenzene	5.0	ND	ND	ND	--	6.0	2.9	(f)	4.5	89
Bis-(2-chloroisopropyl) ether	5.0	ND	ND	ND	--	5.4	1.1	(f)	3.3	65
N-Nitrosodipropylamine	5.0	ND	ND	ND	--	6.8	1.1	(f)	4.0	79
Nitrobenzene	5.0	ND	ND	ND	--	4.8	4.3	(f)	4.6	91
Bis-(2-chloroethoxy) methane	5.0	ND	ND	ND	--	3.9	2.6	(f)	3.3	65
1,2,4-Trichlorobenzene	5.0	ND	ND	ND	--	5.0	3.5	(f)	4.3	85
Naphthalene	5.0	0.4	ND	0.3	0.2	6.7	4.0	(f)	5.4	104
Hexachlorobutadiene	5.0	ND	ND	ND	--	2.8	4.7	(f)	3.8	75
2-Chloronaphthalene	5.0	ND	ND	ND	--	6.6	3.2	(f)	4.9	98
2,6-Dinitrotoluene	5.0	ND	ND	ND	--	5.6	ND	(f)	2.8	56
Dimethyl phthalate	5.0	0.2	ND	ND	0.1	9.0	5.6	(f)	7.3	144
Acenaphthylene	5.0	ND	ND	ND	--	7.3	5.5	(f)	6.4	128
Acenaphthene	5.0	ND	ND	ND	--	7.4	4.7	(f)	6.1	121
2,4-Dinitrotoluene	5.0	ND	ND	ND	--	5.3	ND	(f)	2.7	54
Diethyl phthalate	5.0	0.8	0.2	0.6	0.5	7.3	5.8	(f)	6.6	121

TABLE 7. (Continued)

Compound[a]	Amount Added, µg/100 ml	Amount Recovered, µg/100 ml, in Given Sample: Unspiked 1	Unspiked 2	Unspiked 3	Unspiked Avg.	Spiked 1	Spiked 2	Spiked 3	Spiked Avg.	Average Recovery,[b] %
Acids										
2-Chlorophenol[c]	5.0	ND	ND	ND	--	0.7	0.9	ND	0.5	10
Phenol[c]	6.0	0.9	ND	ND	0.3	2.7	ND	ND	0.9	10
2,4-Dimethylphenol[c]	5.0	ND	ND	ND	--	ND	ND	ND	--	--
2,4-Dichlorophenol[c]	5.0	ND	ND	ND	--	4.0	0.6	2.2	2.3	46
2,4,6-Trichlorophenol[d]	5.0	ND	ND	ND	--	4.8	ND	4.5	3.1	62
2-Nitrophenol[d]	5.0	ND	ND	ND	--	ND	ND	ND	--	--
4-Chloromethylphenol[c]	5.0	ND	ND	ND	--	6.4	ND	8.6	5.0	100
4-Nitrophenol[d]	5.0	ND	ND	ND	--	ND	ND	ND	--	--
4,6-Dinitro-o-cresol[d]	5.0	ND	ND	ND	--	5.5	ND	ND	1.9	38
Pentachlorophenol[d]	5.0	ND	ND	ND	--	4.7	0.1	3.1	2.6	52
2,4-Dinitrophenol[d]	5.0	ND	ND	ND	--	ND	ND	ND	--	--
Bases										
Benzidine	0.6	ND	ND	ND	--	0.4	0.4	0.4	0.4	67
3,3'-Dichlorobenzidine	0.6	ND	ND	ND	--	0.4	0.6	0.6	0.5	83

a. The priority pollutant standards used were purchased from Supelco, Inc.

b. $\frac{\text{(Avg. Recovered from Spiked Sample) - (Avg. Recovered from Unspiked Sample)}}{\text{Amount Added}} \times 100$

c. Determined as the free phenol

d. Determined as the methyl ester

e. Not detected

f. Neutral fraction lost

TABLE 7. (Continued)

Compound[a]	Amount Added, µg/100 ml	Amount Recovered, µg/100 ml, in Given Sample								Average Recovery,[b] %
		Unspiked				Spiked				
		1	2	3	Avg.	1	2	3	Avg.	
Neutrals										
Fluorene	5.0	ND	ND	ND	--	7.4	5.1	(f)	6.3	125
4-Chlorophenyl phenyl ether	5.0	ND	ND	ND	--	4.5	4.1	(f)	4.3	86
N-Nitrosodiphenylamine	5.0	ND	ND	ND	--	2.0	5.7	(f)	3.9	77
4-Bromophenyl phenyl ether	5.0	ND	ND	ND	--	5.1	6.2	(f)	5.7	113
Hexachlorobenzene	5.0	ND	ND	ND	--	5.7	6.2	(f)	6.0	119
Phenanthrene	5.0	0.6	ND	ND	0.2	5.6	6.3	(f)	6.0	116
Anthracene	5.0	ND	ND	ND	--	7.6	7.8	(f)	7.7	154
Di-n-butyl phthalate	5.0	3.7	0.6	0.9	1.7	10.9	6.6	(f)	8.8	142
Fluoranthene	2.5	0.3	ND	ND	0.1	2.1	3.4	(f)	2.8	108
Pyrene	2.5	0.4	ND	ND	0.1	2.5	4.6	(f)	3.6	140
Butylbenzyl phthalate	5.0	27	0.3	1.7	0.7	7.4	2.6	(f)	5.0	86
Chrysene	2.5	ND	ND	ND	--	2.5	3.2	(f)	2.9	114
Benzo(a)anthracene	2.5	ND	ND	ND	--	2.5	3.2	(f)	2.9	114
Bis(2-ethylhexyl)phthalate	5.0	0.9	0.1	0.9	0.6	11.2	0.3	(f)	5.8	104
Di-n-octyl phthalate	5.0	0.4	ND	0.3	0.2	8.3	0.4	(f)	4.4	84
Benzo(b)fluoranthene	2.5	ND	ND	ND	--	2.5	2.2	(f)	2.4	96
Benzo(k)fluoranthene	2.5	ND	ND	ND	--	2.5	2.2	(f)	2.4	96
Benzo(a)pyrene	2.5	ND	ND	ND	--	2.2	1.6	(f)	1.9	76
Benzo(g,h,i)perylene	2.5	ND	ND	ND	--	0.6	0.9	(f)	0.8	30
Indeno(1,2,3-cd)pyrene	2.5	ND	ND	ND	--	2.0	0.3	(f)	1.2	48
Dibenzo(a,h)anthracene	2.5	ND	ND	ND	--	ND	ND	(f)	--	--

TABLE 8. RECOVERY OF PRIORITY POLLUTANTS FROM DIGESTED MUNICIPAL SLUDGE[b]

Compound[a]	Amount Added, µg/100 ml	Amount Recovered, µg/100 ml, in Given Sample								Average Recovery,[c] %
		Unspiked				Spiked				
		1	2	3	Avg.	1	2	3	Avg.	
Neutrals										
Bis-(2-chloroethyl) ether	5.0	ND[f]	ND	ND	---	ND	ND	ND	--	--
1,3-Dichlorobenzene	5.0	ND	9.3	1.8	3.7	0.1	10	2.1	4.1	NM[g]
1,4-Dichlorobenzene	5.0	ND	9.3	1.8	3.7	0.1	10	2.1	4.1	NM
1,2-Dichlorobenzene	5.0	ND	9.3	1.8	3.7	0.1	10	2.1	4.1	NM
Bis-(2-chloroisopropyl) ether	5.0	ND	ND	ND	---	ND	ND	ND	--	--
N-Nitrosodipropylamine	5.0	ND	ND	ND	---	ND	1.5	5.4	2.3	46
Nitrobenzene	5.0	ND	ND	ND	---	ND	ND	ND	--	--
Bis-(2-chloroethoxy)methane	5.0	ND	ND	ND	---	ND	ND	ND	--	--
1,2,4-Trichlorobenzene	5.0	0.3	2.8	0.4	1.2	1.0	13	6.7	6.9	NM
Naphthalene	5.0	5.1	32	5.8	14	3.7	38	12	18	NM
Hexachlorobutadiene	5.0	ND	ND	ND	---	0.5	4.9	1.1	2.2	44
2-Chloronaphthalene	5.0	ND	ND	ND	---	0.7	10.1	3.4	4.7	94
2,6-Dinitrotoluene	5.0	ND	ND	ND	---	ND	ND	ND	--	--
Dimethyl phthalate	5.0	ND	ND	1.0	0.3	0.7	5.2	14	6.7	128
Acenaphthylene	5.0	ND	ND	ND	---	ND	ND	ND	--	--
Acenaphthene	5.0	1.4	6.2	1.7	2.4	0.6	6.4	1.8	2.9	NM
2,4-Dinitrotoluene	5.0	ND	ND	ND	---	ND	ND	ND	--	--
Diethyl phthalate	5.0	37	0.4	ND	12	1.5	5.0	16	7.5	NM

TABLE 8. (Continued)

Compound	Amount Added, µg/100 ml	Amount Recovered, µg/100 ml, in Given Sample								Average Recovery, %
		Unspiked				Spiked				
		1	2	3	Avg.	1	2	3	Avg.	
Neutrals										
Fluorene	5.0	1.9	6.6	ND	2.8	1.0	16.0	7.7	8.5	NM
4-Chlorophenyl phenyl ether	5.0	ND	ND	ND	--	4.0	ND	ND	1.3	26
N-Nitrosodiphenylamine	5.0	ND	ND	ND	--	ND	ND	ND	--	--
4-Bromophenyl phenyl ether	5.0	ND	ND	ND	--	0.7	4.3	1.9	2.3	46
Hexachlorobenzene	5.0	ND	ND	ND	--	ND	3.8	2.2	2.0	40
Phenanthrene	5.0	8.1	35	6.3	16	7.7	39	15	21	NM
Anthracene	5.0	8.1	35	6.3	16	7.7	39	15	21	NM
Di-n-butyl phthalate	5.0	7.4	4.0	2.5	27	13.1	9.7	32	18	NM
Fluoranthene	2.5	3.8	16	3.9	7.9	3.4	18	6.0	9.1	NM
Pyrene	2.5	4.8	24	5.1	12	4.1	24	7.7	12	NM
Butylbenzyl phthalate	5.0	150	33	60	81	47	64	400	170	NM
Chrysene	2.5	3.2	15.0	4.2	7.5	3.6	14	6.1	7.9	NM
Benzo(a)anthracene	2.5	3.2	15.0	4.2	7.5	3.6	14	6.1	7.9	NM
Bis(2-ethylhexyl)phthalate	5.0	690	13	12	240	150	21	100	90	NM
Di-n-octyl phthalate	5.0	190	9.7	8.1	93	64	9	66	46	NM
Benzo(b)fluoranthene	2.5	0.7	4.9	1.9	2.5	1.0	4.3	3.7	3.0	NM
Benzo(k)fluoranthene	2.5	0.7	4.9	1.9	2.5	1.0	4.3	3.7	3.0	NM
Benzo(a)pyrene	2.5	ND	6.4	2.4	2.9	1.0	7.2	4.0	4.1	NM
Benzo(g,h,i)perylene	2.5	ND	3.2	0.8	1.3	1.7	0.8	2.1	1.5	NM
Indeno(1,2,3-cd)pyrene	2.5	ND	3.9	1.1	1.7	2.2	1.6	2.3	2.0	NM
Dibenzo(a,h)anthracene	2.5	ND	ND	ND	--	ND	ND	ND	--	--

TABLE 8. (Continued)

Compound	Amount Added, µg/100 ml	Amount Recovered, µg/100 ml, in Given Sample: Unspiked 1	Unspiked 2	Unspiked 3	Unspiked Avg.	Spiked 1	Spiked 2	Spiked 3	Spiked Avg.	Average Recovery, %
Acids										
2-Chlorophenol[d]	5.0	ND	ND	ND	--	1.2	4.5	3.5	3.1	6.2
Phenol[d]	6.0	ND	15	0.4	5.1	11.0	14	0.8	8.6	NM
2,4-Dimethylphenol[d]	5.0	ND	ND	ND	--	1.6	ND	1.5	1.0	20
2,4-Dichlorophenol[d]	5.0	ND	ND	ND	--	6.1	5.1	5.6	5.6	112
2,4,6-Trichlorophenol[e]	5.0	ND	ND	ND	--	5.1	3.2	4.9	4.4	88
2-Nitrophenol[e]	5.0	ND	ND	ND	--	ND	ND	ND	--	--
4-Chloromethyphenol[d]	5.0	ND	ND	ND	--	10	ND	2.9	4.3	86
4-Nitrophenol[e]	5.0	ND	ND	ND	--	ND	ND	ND	--	--
4,6-Dinitro-o-cresol[e]	5.0	ND	ND	ND	--	ND	ND	ND	--	--
Pentachlorophenol[e]	5.0	ND	ND	ND	--	3.3	1.6	2.6	2.5	50
2,4-Dinitrophenol[e]	5.0	ND	ND	ND	--	ND	ND	ND	--	--
Bases										
Benzidine	0.6	ND	ND	ND	--	0.4	0.4	1.2	0.7	117
3,3'-Dichlorobenzidine	0.6	ND	ND	ND	--	0.3	0.2	0.6	0.4	67

a. The priority pollutant standards used were purchased from Supelco, Inc.

b. The digested sludge used had a dry solids content of 2.5 g/100 ml and a total lipid content of 0.5 g/100 ml.

c. $\frac{\text{(Avg. Recovered from Spiked Sample) - (Avg. Recovered from Unspiked Sample)}}{\text{Amount Added}} \times 100$

d. Determined as the free phenol

e. Determined as the methyl ether

f. Not detected

g. Not meaningful because of large amounts and/or wide variations in amounts found in unspiked samples as well as in spiked samples.

TABLE 9. RECOVERY OF PRIORITY POLLUTANTS FROM RAW SLUDGE[b]

Compound[a]	Amount Added, µg/100 ml	Amount Recovered, µg/100 ml, in Given Sample								Average Recovery[c], %
		Unspiked				Spiked				
		1	2	3	Avg.	1	2	3	Avg.	
Neutrals										
Bis-(2-chloroethyl)ether	5.0	ND[f]	ND	ND	--	6.1	6.1	0.2	4.1	82
1,3-Dichlorobenzene	5.0	ND	55	15	33	0.2	25	20	15	NM[g]
1,4-Dichlorobenzene	5.0	ND	55	15	33	0.2	25	20	15	NM
1,2-Dichlorobenzene	5.0	ND	55	15	33	0.2	25	20	15	NM
Bis-(2-chloroisopropyl) ether	5.0	ND	ND	ND	--	ND	ND	ND	--	NM
N-Nitrosodipropylamine	5.0	ND	ND	ND	--	2.3	6.7	6.2	5.1	102
Nitrobenzene	5.0	ND	ND	ND	--	ND	ND	ND	--	--
Bis-(2-chloroethoxy)methane	5.0	ND	ND	ND	--	7.2	7.3	6.3	6.9	138
1,2,4-Trichlorobenzene	5.0	ND	ND	0.4	0.1	2.0	7.5	8.6	6.0	118
Naphthalene	5.0	ND	65	30	32	7.5	35	46	29	NM
Hexachlorobutadiene	5.0	ND	ND	ND	--	1.0	1.8	2.6	1.8	36
2-Chloronaphthalene	5.0	ND	ND	ND	--	ND	ND	1.0	0.3	6
2,6-Dinitrotoluene	5.0	ND	ND	ND	--	ND	ND	ND	--	--
Dimethyl phthalate	5.0	ND	0.6	0.2	0.3	6.1	5.9	6.0	6.0	114
Acenaphthylene	5.0	ND	ND	ND	--	ND	ND	ND	--	--
Acenaphthene	5.0	ND	21	5.9	9.0	4.0	26	9.1	13	NM
2,4-Dinitrotoluene	5.0	ND	ND	ND	--	ND	ND	ND	--	--
Diethyl phthalate	5.0	0.7	1.1	4.5	2.1	3.5	6.9	7.2	5.9	76
Fluorene	5.0	5.0	30	22	17	8.0	19	27	18	NM

TABLE 9. (Continued)

Compound	Amount Added, g/100 ml	Amount Recovered, g/100 ml, in Given Sample								Average Recovery, %
		Unspiked				Spiked				
		1	2	3	Avg.	1	2	3	Avg.	
Neutrals										
4-Chlorophenyl phenyl ether	5.0	ND	ND	ND	--	1.2	3.3	3.2	2.6	54
N-Nitrosodiphenylamine	5.0	ND	ND	ND	--	2.4	5.0	3.3	3.6	72
4-Bromophenyl phenyl ether	5.0	ND	ND	ND	--	3.1	2.4	2.4	2.6	52
Hexachlorobenzene	5.0	ND	ND	ND	--	2.5	1.4	2.4	2.1	42
Phenanthrene	5.0	39	27	50	39	34	38	68	47	NM
Anthracene	5.0	39	27	50	39	34	38	68	47	NM
Di-n-butyl phthalate	5.0	13	53	22	29	64	55	14	44	NM
Fluoranthene	2.5	25	34	8.9	23	11	26	14	17	NM
Pyrene	2.5	19	35	8.9	21	12	26	13	17	NM
Butylbenzyl phthalate	5.0	280	180	40	170	450	180	45	225	NM
Chrysene	2.5	7.6	22	7.5	12	8.6	25	9.1	14	NM
Benzo(a)anthracene	2.5	7.6	22	7.5	12	8.6	25	9.1	14	NM
Bis(2-ethylhexyl)phthalate	5.0	140	44	29	71	100	45	44	63	NM
Di-n-octyl phthalate	5.0	78	48	26	51	19	34	30	28	NM
Benzo(b)fluoranthene	2.5	3.3	5.7	2.3	3.8	3.5	12	4.5	6.7	116
Benzo(k)fluoranthene	2.5	3.3	5.7	2.3	3.8	3.5	12	4.5	6.7	116
Benzo(a)pyrene	2.5	4.3	5.9	2.4	4.2	5.2	7.6	5.9	6.2	80
Benzo(g,h,i)perylene	2.5	1.3	4.8	ND	2.0	ND	2.2	2.5	1.6	NM
Indeno(1,2,3-cd)pyrene	2.5	2.7	5.5	ND	2.7	ND	3.3	3.3	2.2	NM
Dibenzo(a,h)anthracene	2.5	ND	ND	ND	--	ND	ND	ND	--	--

TABLE 9. (Continued)

Compound	Amount Added µg/100 ml	Amount Recovered, µg/100 ml, in Given Sample: Unspiked 1	Unspiked 2	Unspiked 3	Unspiked Avg.	Spiked 1	Spiked 2	Spiked 3	Spiked Avg.	Average Recovery, %
Acids										
2-Chlorophenol[d]	5.0	ND	ND	ND	--	ND	ND	ND	--	--
Phenol[d]	6.0	14	ND	ND	5	16	ND	14	10	NM
2,4-Dimethylphenol[d]	5.0	ND	ND	ND	--	ND	ND	ND	--	--
2,4-Dichlorophenol[d]	5.0	ND	ND	ND	--	1.4	ND	3.9	1.8	36
2,4,6-Trichlorophenol[e]	5.0	ND	ND	ND	--	0.2	ND	2.0	0.7	14
2-Nitrophenol[e]	5.0	ND	ND	ND	--	ND	ND	ND	--	--
4-Chloromethylphenol[d]	5.0	ND	ND	ND	--	2.9	ND	ND	1.0	20
4-Nitrophenol[e]	5.0	ND	ND	ND	--	ND	ND	ND	--	--
4,6-Dinitro-o-cresol[e]	5.0	ND	ND	ND	--	ND	ND	ND	--	--
Pentachlorophenol[e]	5.0	ND	ND	ND	--	2.6	ND	6.7	3.1	62
2,4-Dinitrophenol[e]	5.0	ND	ND	ND	--	ND	ND	ND	--	--
Bases										
Benzidine	1.2	ND	ND	ND	--	0.8	0.8	0.8	0.8	100
3,3-Dichlorobenzidine	1.2	ND	ND	ND	--	1.2	1.2	1.6	1.3	108

a. The priority pollutant standards used were purchased from Supelco, Inc.

b. The raw sludge used had a dry solids content of 4.4 µg/100 ml and a total lipid content of 1.4 µg/100 ml.

c. $\frac{\text{(Avg. Recovered from Spiked Sample) - (Avg. Recovered from Unspiked Sample)}}{\text{Amount Added}} \times 100$

d. Determined as the free phenol

e. Determined as the methyl ether

f. Not detected

g. Not meaningful because of large amounts and/or wide variations in amounts found in unspiked samples as well as in spiked samples.

unspiked samples. There was a noticeable lack of success in recovering some of the more polar neutrals and the nitrophenols. The poor recoveries could have been caused by various factors including degradation during the 24-hour period of equilibration with sludge, poor extraction from the sludge matrix, loss to the aqueous phase during caustic extraction steps, and alteration of separation patterns caused by large amounts of fatty acids. These are discussed under Recommendations.

APPENDIX

S100 METHOD FOR SEMIVOLATILE ORGANIC COMPONENTS

110 Scope and Application

111 This method covers the determination of 54 semi-volatile organic priority pollutants. A complete list of these compounds is given in Table 111.

112 The method is applicable to the measurement of these compounds in raw and digested municipal sludge.

113 The method is capable in most cases of detecting 2 µg of a priority pollutant per 100 ml of wet sludge (0.5 µg/g dry weight basis for sludge containing 4% solids).

120 Summary

121 This method offers separate procedures for the analysis of the acidic, neutral, and basic components. The procedure involves the use of repetitive solvent extraction to efficiently recover components from the sludge matrix. Gel permeation chromatography, silica-gel chromatography, and acid-base extractions are used as cleanup procedures to eliminate interferences. The acidic (phenolic) and neutral components are determined by GC-MS analysis using high-resolution glass capillary columns and selected ion searches. The bases, benzidine and 3,3-dichlorobenzidine, are determined by HPLC analysis using an electrochemical detector. The overall analysis scheme is shown in Figure 121.

122 This method is recommended for use only by analysts experienced in liquid chromatography, glass capillary column GC-MS analysis, and trace organic analysis of environmental samples, or under the close supervision of such qualified persons.

130 Apparatus and Reagents

131 For sample extraction, Section 150:

131.1 Tekmar tissuemizer
131.2 Solvents, distilled-in-glass grade:
a. Methylene chloride
b. Chloroform preserved with ethanol

TABLE 111. EPA SEMIVOLATILE ORGANIC PRIORITY POLLUTANTS

Polycyclic Aromatic Hydrocarbon

1. Acenaphthene
2. Acenaphthylene
3. Anthracene
4. Benzo(a)anthracene
5. Benzo(b)fluoranthene
6. Benzo(k)fluoranthene
7. Benzo(g,h,i)perylene
8. Benzo(a)pyrene
9. Chrysene
10. Dibenzo(a,g)anthracene
11. Fluoranthene
12. Fluorene
13. Indeno(1,2,3-cd)pyrene
14. Naphthalene
15. Phenanthrene
16. Pyrene

Phthalates

1. Bis(2-ethylhexyl) phthalate
2. Butylbenzyl phthalate
3. Diethyl phthalate
4. Dimethyl phthalate
5. Di-n-butyl phthalate
6. Di-n-octyl phthalate

Chlorinated Hydrocarbons

1. 2-Chloronaphthalene
2. 1,2-Dichlorobenzene
3. 1,3-Dichlorobenzene
4. 1,4-Dichlorobenzene
5. Hexachlorobenzene
6. 1,2,4-Trichlorobenzene
7. Hexachlorobutadiene
8. Hexachlorocyclopentadiene

Chloroalkyl Ethers

1. Bis-(2-chloroethyl) ether
2. Bis-(2-chloroethoxy) methane
3. Bis-(2-chloroisopropyl) ether

Nitrosamines

1. N-Nitrosodiethylamine
2. N-Nitrosodiphenylamine

Miscellaneous Neutrals

1. 4-Bromophenyl phenyl ether
2. 4-Chlorophenyl phenyl ether
3. 2,4-Dinitrotoluene
4. 2,6-Dinitrotoluene
5. Isophorone
6. Nitrobenzene

Acids

1. 4-Chloro-3-methylphenol
2. 2-Chlorophenol
3. 2,4-Dichlorophenol
4. 2,4-Dimethylphenol
5. 4,6-Dinitro-2-methylphenol
6. 2,4-Dinitrophenol
7. 2-Nitrophenol
8. 4-Nitrophenol
9. Pentachlorophenol
10. Phenol
11. 2,4,6-Trichlorophenol

Bases

1. Benzidine
2. 3,3'-Dichlorobenzidine

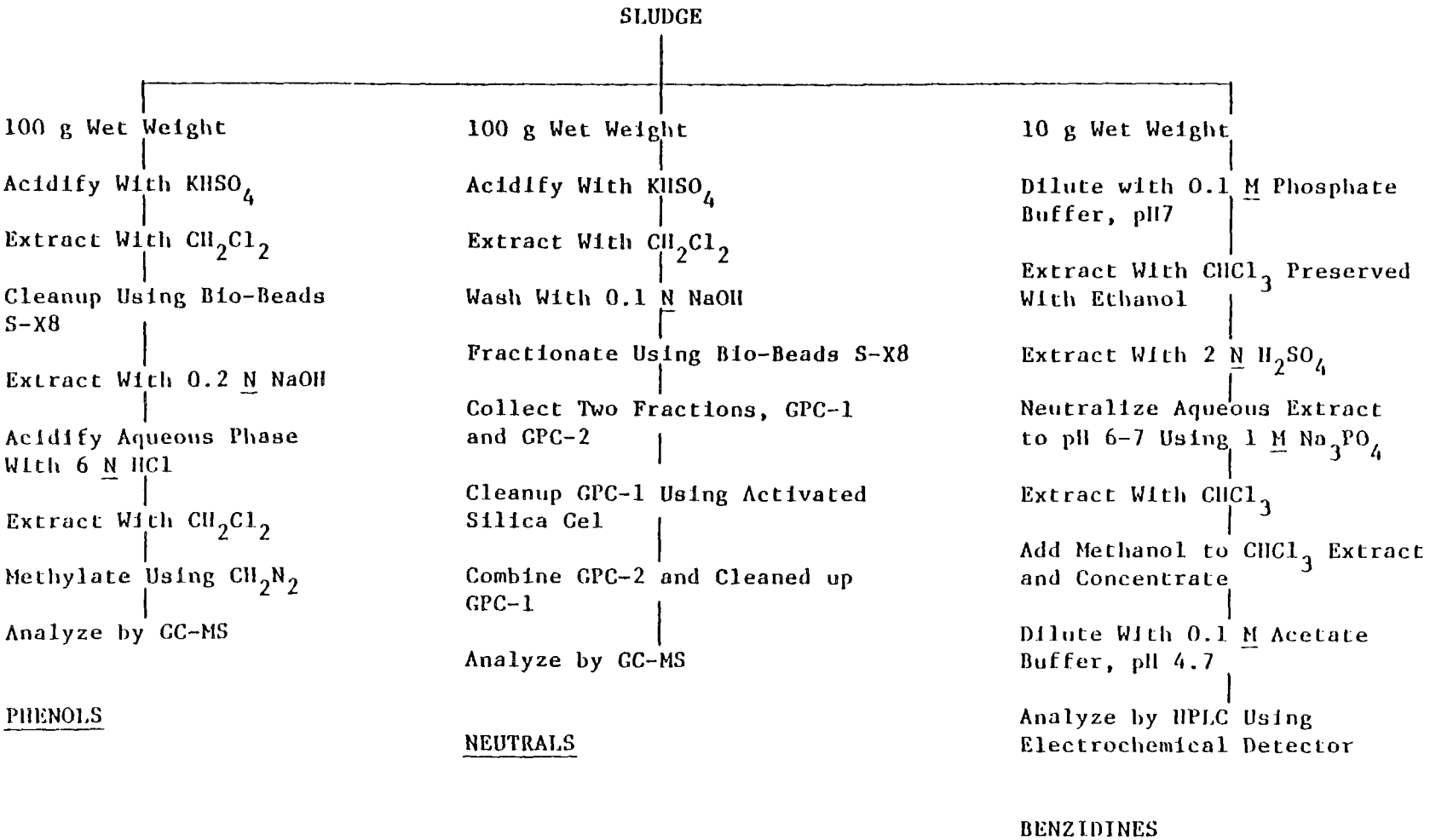

Figure 121. Scheme for determination of semivolatile priority pollutants in sludge.

c. Methanol

131.3 Glassware
- a. Centrifuge tubes - 50 ml and 200 ml, with Teflon-lined screw caps
- b. Round-bottom flasks - 500 ml and 100 ml, with 24/40 joints
- c. Vortex evaporator tubes - 15 ml
- d. Separatory funnels - 125 ml with Teflon stopcocks

131.4 Rotating evaporator

131.5 Vortex evaporator

131.6 Reagents
- a. Magnesium sulfate, anhydrous - conditioned at 450°C
- b. Potassium bisulfate, anhydrous - conditioned at 450°C
- c. Phosphate buffer - 0.1 M, pH 7

131.7 Syringe - 50 ml with 8-inch 15-gauge square-tipped needle

131.8 Microbalance

131.9 Aluminum foil pans - 25 mm

132 For removal of interferences, Section 160.

132.1 Reagents
- a. Magnesium sulfate, anhydrous - conditioned at 450°C
- b. Sodium hydroxide - 0.1 N in 10% NaCl
- c. Sodium hydroxide - 20%
- d. Hydrochloric acid - 6 N
- e. Trisodium phosphate - 0.4 M
- f. Sulfuric acid - 2 N
- g. Diazald
- h. Acetate buffer - 0.1 M, pH 4.7

132.2 Glassware
- a. Separatory funnels - 60 ml, 125 ml, and 500 ml with Teflon stopcocks
- b. Micro diazomethane generating apparatus - obtained from Paxton Woods Glass Shop, 7500 Brill Road, Cincinnati, Ohio
- c. Round-bottom flasks - 100 ml and 500 ml
- d. Vortex evaporator tubes - 15 ml graduated, screw cap, conical centrifuge tubes
- e. Centrifuge tubes - 50 ml and 200 ml, screw cap
- f. Chromatography column - 400 mm x 9 mm I.D. Lab-Crest column with 100-ml reservoir, scintered glass frit, Teflon stopcock, and Solv-Seal joints

132.3 Solvents, distilled-in-glass grade
- a. Methylene chloride
- b. Petroleum ether, b.p. 30-60°C
- c. Acetone
- d. Hexane
- e. Ethylene dichloride
- f. Chloroform preserved with ethanol

132.4 Silica gel - 100-200 mesh Davison grade 923, activated for 16 hours at 150°C

132.5 Standard solutions
- a. GPC calibration solution for neutrals - methylene chloride containing 1 mg each of di-n-tridecyl phthalate, di-n-octyl phthalate, 4-chlorophenyl phenyl ether, dimethyl phthalate, pyrene, and sulfur per ml
- b. GPC calibraton solution for acids - methylene chloride containing 1 mg each of 4-phenylbutyric acid, 2,4-dinitrophenol, 2,4-dichlorophenol, and sulfur per ml
- c. DDA internal standard solution - 10 mg of deca-deuteroanthracene in 100 ml of heptane

132.6 Rotating evaporator

132.7 Vortex evaporator

132.8 Gel-permeation chromatography system*
- a. Chromatographic column - 1200 mm x 25 mm I.D. glass
- b. Bio-Beads S-X8 - 200 g per column
- c. Pump - capable of constant flow of 0.1 to 5 ml/min at up to 100 psi
- d. Injector - with 5 ml loop
- e. Ultra-violet detector - 254 nm
- f. Strip-chart recorder

132.9 Centrifuge capable of handling 50-ml tubes

133 For quantitation, Section 160:

133.1 GC-MS system
- a. Capable of scanning from 50 to 450 a.m.u. every 2 seconds
- b. Capable of producing a recognizable mass spectrum at unit resolution from 10 ng of methyl stearate when the sample is introduced through the GC inlet
- c. Interfaced with a gas chromatograph equipped with an injector system designed for splitless injection glass capillary column work. All sections of the transfer lines must be glass or glass-lined and deactivated with Carbowax 20M
- d. Interfaced with a computer data system having a selected ion search program, i.e., a program capable of searching a full mass range total ion chromatogram for selected ions after the run is completed**
- e. Glass capillary column - 30 m x 0.2 mm I.D. coated with SE-30

133.2 HPLC System
- a. Pump - capable of constant flow of 0.1 to 5 ml/min at up to 5000 psi

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*For the processing of large numbers of samples a GPC Auto Prep 1001, available from Analytical Biochemistry Laboratories, Inc., or equivalent may be used with an operations procedure that will give performance equal to that described herein.

**The system should have capabilities which at least meet the requirements of the EMSL guidelines contianed in "Sampling and Analysis Procedures for Screening of Industrial Effluents for Priority Pollutants", April, 1977.

b. High-pressure injector - with 50 µl loop
c. Chromatographic column - 4.6 mm I.D. x 25 cm stainless steel, packed with Lichrosorb RP-2, 5 micron particle diameter
d. Electrochemical detector - equipped with a thin layer glassy carbon electrode
e. Strip-chart recorder - 1 to 10 volts full scale
f. Mobile phase - 50:50 acetonitrile: 0.1 M acetate buffer, pH 4.7

133.3 Syringes - 10 µl and 100 µl

133.4 Standard solutions - 0.2 mg/ml of neutrals, 0.5 mg/ml of phenols, and 0.1 mg/ml of benzidines in methanol.

140 Sampling and Preservation

141 Samples should be collected in 2000-ml wide-mouth glass containers with clean Teflon-lined or foil-lined caps. The containers should be heated in an oven at 450-500°C overnight to remove any traces or organic contamination before use. The containers should be filled no more than two-thirds full with sample to minimize breakage during freezing.

142 Samples should be refrigerated at 4°C immediately after collection and extracted within 24 hours. If extraction within 24 hours is not possible the samples should be frozen. Samples may be stored for up to 30 days at -20°C or indefinitely at -75°C. In order to prevent breakage during storage it is essential that the container not be permitted to be slightly warmed and recooled.

150 Sample Extraction

151 Three separate samples are extracted, one each for neutral, acidic, and basic fractions.

152 Neutral fraction

152.1 Place 100 g of homogeneous sludge into a 200-ml centrifuge tube and acidify with 5 g of $KHSO_4$.

152.2 Add 100 ml methylene chloride to the centrifuge tube and homogenize for one minute with a tissuemizer. As a safety precaution in case of breakage place the glass tubes in the metal holders of the centrifuge prior to homogenization.

152.3 Cap the centrifuge tubes tightly with Teflon-lined screw caps and centrifuge to achieve good phase separation. Remove the methylene chloride layer with a 50-ml syringe and transfer to a 500-ml round-bottom flask.

152.4 Repeat extraction procedure two more times and combine the methylene chloride layers.

152.5 Concentrate to 60-80 ml on a rotating evaporator at 35°C.

153 Acid fraction

153.1 Extract 100 g of homogenized sludge following the procedure of 152.1 to 152.4 and dry the combined extracts by shaking with 2 g of $MgSO_4$.

153.2 Decant the extract into a 500-ml round-bottom flask and concentrate to 50 ml on a rotating evaporator at 35°C. Transfer concentrate to a 100-ml round-bottom flask and concentrate to 8-10 ml. Transfer to a 15-ml vortex evaporator tube and make up to 10 ml with methylene chloride. Mix thoroughly. Obtain a residue weight of 100 µl of the 10-ml concentrate and calculate the total amount of material in the sample. The residue weight is determined by placing the 100 µl on a tared aluminum foil pan, allowing the solvent to evaporate, and reweighing the pan using a microbalance. Concentrate the extract further as necessary on a vortex evaporator at 25°C to adjust the final total volume to 1.0 ml for every 200 mg of material in the sample. Centrifuge the concentrate to remove traces of particulate material.

154 Basic fraction

154.1 Place 10 g of homogenized sludge in a 50-ml centrifuge tube and dilute with 20 ml 0.1 M phosphate buffer (pH 7). Add 10 ml of chloroform and homogenize the mixture for one minute using a tissuemizer. Centrifuge to achieve a good phase separation and remove the chloroform layer using a 50-ml syringe.

154.2 Repeat the above extraction two more times using 10 ml of chloroform each time and combine the chloroform layers.

160 Removal of Interferences

161 The cleanup procedures described in this section are designed to remove the major interfering classes of compounds found in sludge extracts, namely triglycerides, fatty acids, and long-chain hydrocarbons.

162 Neutral fraction - Base extraction is used to remove phenols and fatty acids. Gel permeation chromatography is used to remove triglycerides and to obtain an intermediate-sized molecule fraction containing the higher alkyl phthalates and a small-molecule fraction containing the remaining neutral compounds of interest. Silica gel chromatography is used to remove saturated hydrocarbons from the higher alkyl phthalates.

162.1 Transfer the extract from Section 152.5 to a 1000-ml separatory funnel and add 200 ml of petroleum ether. Extract three times with 400-ml portions of 0.1 N NaOH in 10% NaCl followed by two washes with 200-ml portions of 10% NaCl and discard the aqueous layers. If an emulsion forms

at the solvent interface, collect the emulsion in a centrifuge tube and centrifuge to separate the layers.

162.2 Dry the final organic layer by shaking with 2 g of $MgSO_4$.

162.3 Concentrate to 200 mg/ml as described in Section 153.2. Centrifuge the concentrate to remove any traces of particulate material.

162.4 Prepare a 1200 mm x 25 mm I.D. gel permeation chromatography (GPC) column by slurry packing using 200 g of Bio Beads S-X8 that have been swelled in methylene chloride for at least 4 hours. Prior to initial use, rinse the column with methylene chloride at 1 ml/min for 16 hours to remove any traces of contaminants. Calibrate the system by injecting 5 ml of the GPC calibration solution for neutrals (132.5-a), eluting with methylene chloride at 2 ml/min for at least 3 hours and observing the resultant UV detector trace. The column may be used indefinitely as long as no darkening or pressure increases occur and a column efficiency of at least 1200 theoretical plates is acheived. The pressure should not be permitted to exceed 50 psi. Recalibrate the system daily.

162.5 Inject up to 5 ml of the neutral concentrate (from 162.3) onto the GPC column and elute with methylene chloride at 2 ml/min for at least 3 hours. Discard the first fraction that elutes up to a retention time represented by the minimum between the di-n-tridecyl phthalate peak and the di-n-octyl phthalate peak in the calibration run. Collect as Fraction GPC-1 the next fraction eluting up to a retention time represented by the minimum between the dimethyl phthalate peak and the 4-chlorophenyl phenyl ether peak in the calibration run. Collect as Fraction GPC-2 the remaining eluate that elutes up to a retention time represented by the minimum between the pyrene peak and the sulfur peak in the calibration run.

162.6 Apply the above GPC separation to any remaining neutral concentrate using only up to 5 ml at a time. Combine the fractions to give one "Fraction GPC-1" and one "Fraction GPC-2".

162.7 Prepare a 400 mm x 9 mm I.D. silica gel chromatography column by slurry packing using 20 g of activated silica gel suspended in 25% acetone in methylene chloride. Wash the column, using gravity flow, with 50 ml of 25% acetone in methylene chloride to remove any traces of impurities and then wash it with 50 ml of petroleum ether to remove the polar solvent. Care should be taken to avoid any bubbles of air or solvent vapor in the column. Solvent flow should be continued only until the solvent level is within 1 mm of the top of the silica gel.

162.8 Concentrate Fraction GPC-1 to 2 ml using the procedures described in Section 153.2 and add 2 ml of petroleum ether.

162.9 Apply the Fraction GPC-1 concentrate to the freshly prepared silica gel column and open the stopcock to permit flow until the liquid level is within 1 mm of the top of the

silica gel. In a similar fashion rinse the sample onto the column completely with two 1-ml portions of 50% methylene chloride in petroleum ether.

162.10 Elute the column with 50 ml of 50% methylene chloride in petroleum ether to remove interfering nonpolar and slightly polar components. Discard this eluate.

162.11 Elute the column with 50 ml of 25% acetone in methylene chloride and collect the eluate as the phthalate fraction. The highly polar components remain on the silica gel which is then discarded.

162.12 Combine the phthalate fraction with Fraction GPC-2 and concentrate to 10 ml using the procedures described in Section 153.2. Add 0.1 ml of the DDA internal standard solution and 1.0 ml of ethylene chloride and concentrate to 0.2 ml using a vortex evaporator. This is the final neutral fraction used for GC-MS analysis.

163 Acid fraction - Gel permeation chromatography is used to remove triglycerides and fatty acids. Acid-base extraction is used to remove the remaining neutral and basic components.

163.1 Calibrate the GPC system described in Section 162.4 by injecting 5 ml of the GPC calibration solution for acids (132.5-b), eluting with methylene chloride at 2 ml/min for at least 3 hours and observing the resultant UV detector trace. Inject up to 5 ml of the acid concentrate (from 153.2) onto the GPC column. Elute with methylene chloride at 2 ml/min for at least 3 hours. Discard the first fraction that elutes up to a retention time represented by the minimum between the 4-phenylbutyric acid peak and the 2,4-dinitrophenol peak in the calibration run. Collect as the phenolic fraction the remaining eluate that elutes up to a retention time represented by the minimum between the 2,4-dichlorophenol peak and the sulfur peak in the calibration run.

163.2 Apply the above GPC separation to any remaining acid concentrate using only up to 5 ml at a time. Combine the phenolic fractions.

163.3 Concentrate the combined phenolic fractions to 2 ml using the procedures described in Section 153.2. Transfer the concentrate to a 50-ml centrifuge tube and add 20 ml of hexane. Extract two times with 20 ml of 0.1 N NaOH in 10% NaCl. Centrifuge if necessary to facilitate phase separation. Combine the aqueous layers in a 200-ml centrifuge tube and acidify with 1 ml of 6 N HCl.

163.4 Extract the acidified aqueous phase two times with 20-ml portions of methylene chloride. Centrifuge, if necessary, to facilitate phase separation. Combine the organic layers and dry over $MgSO_4$.

163.5 Add 0.1 ml of the DDA internal standard solution and 10 ml of ethylene dichloride and concentrate to 3 ml using the procedures described in Section 153.2.

163.6 Methylate the phenols by bubbling diazomethane, generated from Diazald, into the solution until it turns yellow. Cap the sample and keep it at room temperature for 30 minutes. If the yellow color dissipates during this time add more diazomethane.

163.7 Concentrate the sample to 0.2 ml in a vortex evaporator at 30°C. This is the acid fraction for GC-MS analysis.

164 Basic fraction

164.1 Transfer the chloroform extract from 154.2 to a 50-ml centrifuge tube and extract twice with 10 ml 2 N H_2SO_4 using a 50 ml syringe to withdraw the aqueous layer from the bottom.

164.2 Combine aqueous layers in a 50-ml beaker containing a magnetic stirring bar and neutralize by the dropwise addition with stirring of 1 ml of 0.4 M Na_3PO_4 followed by the dropwise addition over at least a two-minute period of 20% NaOH to pH 6-7 (approximately 7 ml will be required). Do not allow the sample pH to ever exceed pH 8.

164.3 Transfer the neutralized aqueous extract to a 60-ml separatory funnel and extract twice with 10 ml portions of chloroform. Wash the combined chloroform extracts with 5 ml of distilled water. Add 5 ml of methanol to the chloroform extract and concentrate to 0.2 ml using a vortex evaporator at 25°C.

164.4 Dilute to 1 ml with 0.1 M acetate buffer (pH 4.7). This is the basic fraction used for the analysis of benzidines by HPLC.

170 Quantitation

171 The neutral and acidic (phenolic) components are quantitated by GC-MS analysis using an SE-30 glass capillary column. The basic components (benzidines) are quantitated by HPLC analysis using an electrochemical detector.

172 Neutral and acidic fractions

172.1 Inject 2 µl of sample into the GC-MS using the splitless mode with the injector at 270°C, column at 60°C, transfer line at 280°C, and helium carrier gas flow at approximately 2 ml/min. Hold the column temperature at 60°C for 5 minutes, then temperature program at 4 degrees per minute to 270°C and hold at 270°C for 15 minutes. Scan m/e values of 40-450 at approximately 30 scans per minute. Start data acquisition four minutes after injection.

172.2 Locate the priority pollutant compounds in the GC-MS runs by selected ion searches (see 133.1-d) (SIS). In this method, the computer is instructed to search the full mass range mass spectra for several specified m/e values which are characteristic of the compound of interest. These

TABLE 172.2. GC-MS DATA USED FOR DETERMINING SEMIVOLATILE PRIORITY POLLUTANTS

Compound	Approximate Retention Time, min.[a]	M.W.	Ions Used For Quantitation, m/e (intensity)
	Neutrals		
Bis-(2-chloroethyl) ether	10.5	142	93(100), 63(99), 95(31)
1,3-Dichlorobenzene	10.6	146	146(100), 148(65), 111(35)
1,4-Dichlorobenzene	10.6	146	146(100), 148(65), 111(35)
1,2-Dichlorobenzene	11.2	146	146(100), 148(65), 111(35)
Bis-(2-chloroisopropyl) ether	11.7	170	45(100), 77(19), 79(12)
N-Nitrosodipropylamine	12.6	130	70(100), 130(30)
Nitrobenzene	13.4	123	77(100), 123(50)
Isophorone	14.6	138	82(100), 138(15)
Bis-(2-chloroethoxy)methane	15.5	180	93(100), 95(32), 123(21)
1,2,4-Trichlorobenzene	16.8	180	180(100), 182(97)
Naphthalene	17.1	128	128(100), 127(15)
Hexachlorobutadiene	18.8	258	225(100), 227(60)
Hexachlorocyclopentadiene	22.7	270	237(100), 235(63), 272(12)
2-Chloronaphthalene	24.0	162	162(100), 127(40)
2,6-Dinitrotoluene	25.5	182	165(100), 89(80)
Dimethyl phthalate	26.3	194	163(100), 77(21)
Acenaphthalene	27.0	152	152(100), 151(23)
Acenaphthene	27.2	154	153(100), 154(90)
2,4-Dinitrotoluene	27.9	182	165(100), 63(60)
Diethyl phthalate	30.4	222	149(100), 178(25)
Fluorene	30.5	166	166(100), 165(90)
4-Chlorophenyl phenyl ether	30.6	204	204(100), 141(75)
N-Nitrosodiphenylamine[b]	31.4	198	169(100), 168(72)
4-Bromophenyl phenyl ether	33.0	248	248(100), 250(95)
Hexachlorobenzene	33.4	282	282(100), 284(77)
Phenanthrene	35.6	178	178(100), 176(17)
Anthracene	35.9	178	178(100), 176(17)
Di-n-butyl phthalate	40.1	278	149(100), 104(10)
Fluoranthene	42.5	202	202(100), 101(25)
Pyrene	43.7	202	202(100), 101(25)
Butylbenzyl phthalate	48.5	298	149(100), 91(62)
Chrysene	50.9	228	228(100), 226(27)
Benzo(a)anthracene	51.2	228	228(100)
Bis(2-ethylhexyl) phthalate	52.9	390	149(100), 167(38)
Di-n-octyl phthalate	55.9	390	149(100), 167(34)
Benzo(b)fluoranthene	56.4	252	252(100), 253(23)
Benzo(k)fluoranthene	56.9	252	252(100), 253(23)
Benzo(a)pyrene	62.8	252	252(100), 253(23)
Benzo(g,h,i)perylene	66.8	276	276(100)
Indeno(1,2,3-cd)pyrene	68.3	276	276(100)
Dibenzo(a,h)anthracene	72.5	278	278(100)

TABLE 172.2. (Continued)

Compound	Approximate Retention Time, min.[a]	M.W.	Ions Used for Quantitation, m/e (intensity)
	Phenols		
2-Chlorophenol[c]	11.0	128	128(100), 130(33)
Phenol[c]	11.7	94	94(100), 66(60), 65(35)
2,4-Dimethylphenol[c]	16.9	122	107(100), 121(97), 122(85)
2,4-Dichlorophenol[c]	18.1	162	162(100), 164(66)
2,4,6-Trichlorophenol(Me)[d]	22.6	210	195(100), 197(95), 167(75)
2-Nitrophenol(Me)[d]	22.9	153	77(100), 106(80), 92(65)
4-Chloro-3-methylphenol[c]	23.3	142	107(100), 142(69), 77(62)
4-Nitrophenol(Me)[d]	25.8	153	107(100), 77(82), 153(12)
4,6-Dinitro-o-cresol(Me)[d]	33.8	212	89(100), 165(61), 182(58)
Pentachlorophenol(Me)[d]	34.9	278	237(100), 265(91), 280(70)
2,4-Dinitrophenol(Me)[d]	35.5	198	76(100), 151(69), 168(62)
	Internal Standard		
Decadeuteroanthracene	35.9	188	188(100)

a. GC conditions: 30 m x 0.2 mm I.D. glass capillary column coated with SE-30; hold the column temperature at 60°C for 5 minutes then program at 4 degree per minute to 270°C and hold at 270°C for 15 minutes.

b. Decomposes upon injection in the GC to diphenylamine; therefore, it is detected as diphenylamine.

c. Determined as the free phenol.

d. Determined as the methyl ether.

selected ions are given in Table 172.2. Whenever a peak is tentatively identified by SIS methods as a priority pollutant, its full mass spectrum and retention time should be studied manually for positive confirmation. The area counts for the selected ions of each identified priority pollutant and the DDA internal standard are then obtained by the computer.

172.3 Quantitate the identified priority pollutants using the following equation:

$$X = \frac{PP}{DDA} \times \frac{10}{RF} \times \frac{1}{W}$$

where X = concentration of the priority pollutant in the sludge in µg/g dry weight

PP = area counts obtained for the priority pollutant

DDA = area counts found for decadeuteroanthracene

RF = response factor of the priority pollutant relative to that of DDA as determined by GC-MS analysis of standard solutions

W = dry weight, g, of 100 g of wet sludge

Response factors for the particular GC-MS system used should be determined at least weekly.

173 Basic fraction

173.1 Inject 50 µl of the final base extract into the sample loop of the HPLC system described in Section 133.2. Use a flow rate of 0.8 ml/min and operate the detector at 0.8 volts versus a standard calomel electrode.

173.2 Quantitate the benzidine and 3,3'-dichlorobenzidine on the basis of peak heights using the following equation:

$$X = \frac{H_{sample}}{H_{std}} \times \frac{5}{W}$$

where X = concentration of the benzidine in the sludge in µg/g dry weight

H_{sample} = peak height of the benzidine found in the sample

H_{std} = peak height of the benzidine obtained from 50 µl of a standard solution containing 0.5 µg/ml of each of the two benzidines

W = dry weight, g, of 100 g of wet sludge

180 Quality Assurance

181 Sample processing

181.1 Process blanks - In order to assess any contamination sources during sample extraction and cleanup, at least one process blank (distilled water) should be run concurrently with each set of 10 sludge samples. The resulting neutral and acidic fractions may be analyzed by GC alone instead of by GC-MS.

181.2 Spiked process blanks - In order to assess recovery efficiencies of the extraction and cleanup procedures used, in the absence of matrix effects, at least one spiked blank should be run concurrently with each set of 10 sludge samples. The spike should contain 10 µg of each of the priority pollutants of concern. The resulting neutral and acidic fractions may be analyzed by GC alone instead of by GC-MS. Recoveries of at least 25% for each component should be achieved. Occasionally in order to assess the lower sensitivity of the method a spike containing only 2 µg per 100 ml should be used. At the 2 µg level recoveries of at least 25% should not be expected for all compounds.

181.3 Spiked sludge - In order to assess overall recovery efficiencies, including matrix effects, and also assess the reproducibility of the method, triplicate samples of a representative sludge should be run with every 10 sludge samples. The sludge should be spiked with each of the priority pollutants of concern at a level of 10 µg per 100 ml. The spiked sludges should be equilbriated at 4°C for 1 hour prior to extraction. Recoveries of at least 10 percent for each component should be achieved. Occasionally in order to assess the lower sensitivity of the method a spike level of only 2 µg per 100 ml should be used. At the 2 µg level recoveries of at least 10% should not be expected for all compounds.

181.4 Replicate samples - In order to assess the reproducibility of the method, one of every 10 sludge samples should be run in triplicate.

182 GPC fractionation - The GPC system should be calibrated daily or after every 10 runs to determine retention times and column efficiency by injecting 5 ml of the GPC calibration solutions. The column efficiency or number of theoretical plates (N) is calculated from the retention time, t, and the peak width at half height, $W_{1/2}$, obtained for the di-n-octyl phthalate peak on the UV trace using the equation

$$N = 5.5\left(\frac{t}{W_{1/2}}\right)^2$$

The column should not be used unless at least 1200 theoretical plates can be achieved.

183 GC-MS analysis - Strict performance standards need to be maintained to ensure that the GC-MS system is providing adequate sensitivity and high quality chromatograms and mass spectra.

183.1 Capillary column performance - Prior to installing the glass capillary column in the GC-MS, evaluate the column in a GC system designed for all-glass capillary column work. With the helium carrier gas flow adjusted to 30-40 cm/sec, the split ratio adjusted to 10:1 and, column oven temperature set at 100°C, inject 2 µl of a column performance test mixture containing 25 ng/µl each of 2,6-dimethylphenol, 2,4-dimethylaniline, n-decyl alcohol, n-decyl aldehyde, n-tridecane, and n-tetradecane.

a. The acidity of the column is measured as the ratio of the peak height of dimethylphenol to that of dimethylaniline. A value of 0.5 to 2.0 is considered acceptable.

b. The polarity of the column is determined from the degree of tailing of the n-decyl alcohol peak. This is evaluated by drawing a perpendicular from the apex of the peak to the baseline and measuring, at one-tenth peak height, the width from the front of the peak to the perpendicular line and from the back of the peak to the perpendicular line. If the width at the back of the peak is greater than four times the width of the front of the peak, the column is too polar for sample analyses. Low polarity is particularly important for achieving satisfactory sensitivity in the analyses of polycyclic aromatic hydrocarbons and more polar compounds.

c. The number of effective plates (N_{eff}) is determined from the n-tetradecane peak using the equation:

$$N_{eff} = 5.5\left(\frac{t_c}{W_{1/2}}\right)^2$$

where t_c = the corrected retention time of n-tetradecane, and
$W_{1/2}$ = the peak width at half height.
The number of effective plates of acceptable columns must be at least 50,000. Only columns which meet <u>all</u> of the above criteria are to be used for the GC-MS analyses.

183.2 Mass spectrometer tune up - Calibrate and tune the mass spectrometer each day before samples are analyzed. After tuning and calibration has been completed, admit decafluorotriphenylphosphine (DFTPP) into the mass spectrometer via the solids probe. Its spectrum should be comparable to that described by Eichelberger, et al. [Anal. Chem., 46, 995 (1975)]. Retune and recalibrate the instrument if necessary.

183.3 Capillary column GC-MS performance - Evaluate the performance of the complete glass capillary column GC-MS system each day prior to its use for the analysis of neutral fractions. Using splitless injection techniques and the standard sample analysis conditions inject 2.0 µl of a heptane solution containing 20 ng each of 1-naphthylamine, 2-naphthol, 1-pentadecanol, DFTPP, 3-methylnonadecane, tridecylcyclohexane, n-eicosane, pyrene, n-heneicosane, and methyl stearate. The total ion chromatogram is used to assess the system performance.

a. The acidity of the system is measured as the ratio of the peak areas of the naphthylamine and naphthol. It must be between 0.5 and 2.0 to be acceptable.
b. The polarity of the system is measured by the peak height of 1-pentadecanol, pyrene, or methyl stearate relative to that of n-eicosane. In each case a value of at least 0.2 must be obtained.
c. The sensitivity of the system is measured by the signal to noise ratio obtained for pyrene. It must be at least 20.
d. The resolution of the system is assessed by the resolution achieved between tridecylcyclohexane and 3-methylnonadecane. The resolution should be sufficient to give a valley between the two peaks which is no more than 20% of the peak heights.
e. The high/low mass spectral balance of the system is determined from the mass spectrum of DFTPP. The ratio of the intensities of the 442 ion and the 198 ion should be 0.4 to 0.7. This is a check on the earlier MS tuneup.

183.4 Response factors and retention times - Once each week or each time a substantal change in GC-MS performance is observed, the response factors and retention times for the priority pollutants should be determined. This is accomplished by analyzing 2 µl of standard solutions containing 50 ng/µl of decadeuteroanthracene and each of the neutral and acidic priority pollutants.

184 HPLC analysis - With each set of samples analyzed, analyze a standard containing 0.5 µg/ml of each of the two benzidines. The retention times and peak heights are used for the detection and quantitation of the benzidines in the samples.

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16. ABSTRACT

An analytical procedure was developed for the determination of 54 semi-volatile organic priority pollutants in sludge at levels down to 0.01 μg/g wet weight. The procedure involved extraction with methylene chloride or chloroform, cleanup of groups of compounds having common properties, and in most cases analysis of the fractions by GC-MS using high-resolution glass capillary columns and selected ion searches. The final analyses involved the analysis of three separate fractions, namely benzidines, phenols, and neutrals. The benzidines were determined by HPLC analysis using an electrochemical detector instead of by GC-MS because GC-MS sensitivity for these compounds was too low. Quantitation in the GC-MS analyses involved the internal standard method applied to selected ion responses. Relative response factors obtained from the analysis of standard solutions were used as correction factors.

17. KEY WORDS AND DOCUMENT ANALYSIS

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