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thesis entitled

AROMATIC COMPOUNDS IN SLUDGE: I. HPLC METHOD DEVELOPMENT AND VALIDATION. II. ANALYSIS OF SLUDGE SAMPLES FROM MICHIGAN WASTE WATER PLANT FACILITIES.

presented by

INES TORO-SUAREZ

has been accepted towards fulfillment
of the requirements for

MASTER degree in ENTOMOLOGY

Major professor

Date May 22, 1987

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By

INES TORO-SUAREZ

A THESIS

Submitted to
MICHIGAN STATE UNIVERSITY
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

DEPARTMENT OF ENTOMOLOGY

1987

ABSTRACT

AROMATIC COMPOUNDS IN SLUDGE: I. HPLC METHOD DEVELOPMENT AND VALIDATION. II. ANALYSIS OF SLUDGE SAMPLES FROM MICHIGAN WASTE WATER PLANT FACILITIES.

By

INES TORO-SUAREZ

A method which combines liquid chromatographic and spectrophotometric procedures was developed for determination of 9 aromatic compounds in sludge samples. It uses an HPLC system consisting of a C_{18} reverse phase column and UV-VIS detector at 235 nm., and has a detection range between 4 ppb for 2-mercaptobenzothiazol, and 80 ppb for Aroclor^R 1254.

With the purpose of validating the HPLC method, a secondary activated sludge was synthesized. The recovery for each step was first established with standards and then with sludge samples fortified at three different levels with the mixture of standards.

Sludge samples collected throughout the state of Michigan were also analyzed for these compounds, with exception of Fire-Master^R, using three different types of gas chromatographic detection systems. Of the 8 aromatic compounds analyzed only naphthalene, biphenyl, Hexachlorobenzene, and PCB (1248) were present at a concentration above the detection limit.

ACKNOWLEDGEMENTS

I would like to thanks my major professor Dr. Matthew Zabik for his patience, confidence and guidance. I really appreciate his efforts in coordinating everything so I could attend scientific meetings and participate in activities previously considered exclusively for men.

Without the effort of both Dr. Ruby Londono and Dr. Robert Ruppel my attendance in graduate school would be just a dream. Both of them supplied emotional support, Ruby from Colombia with her encouraging correspondences and Dr. Ruppel who offered me his family and hospitality.

Dr. Fumio Matsumura another member of my committee always had confidence in me and words encouragement for me. I am also grateful to Dr. Richard Leavitt, who was not a member of my committee but who always provided invaluable help when ever I needed it.

Thanks to Silvana Penuela who introduced me to her professor Dr. Mackenzie L. Davis from the Department of Environmental Engineering. Using their methodology and facilities, I had the opportunity to synthesize a sludge sample. I also appreciate Silvana's and Dr. Davis' personal cooperation. I am grateful to Dr. John L. Gill and Drs Ray and Judy Frankmann for helping me in the understanding and analyzing the analytical data. Also I would like to thank Dr. Victoria McGuffin for

her helpful and thought-provoking discussions about chromatography. The people in the office of the International Students and Scholars have been very cooperative. Lastly, I would like to recognize Dr. Kenneth Ebert in a very special manner.

Due to the close cooperation between our laboratory and the analytical laboratory, I had the opportunity to enjoy friendship as well as inspiring discussions with Dr. Robert Kon. I have been fortunate to meet Lester, who is always willing to offer his invaluable experience and assistance. I also want to thank Jack who along with Lester, and Bob S. helped me many times.

During this time many people have shared more than 8 hours a day in the laboratory with me. I will always remember these people for their kindness, help, discussions as well as disagreements which always ended happily. Some these people include Zwat, Nagna, Don Fontain, and Nelson. I very fortune to have friends such as Sue, Holly, and Mary Ann. I am glad to got to know Kim, Gamal and Glen.

I need to give special recognition to Dr. Robert Haack (Bob), my Spanish speaking friend. He has been the most patient, strict, and helpful editor of this thesis and many other papers. To him as well as to Sheridan, thank you very much.

From the Latin-American community Ana, Gaston, and Zusel my close close friends. I have shared work and jolliness with them all. Without their support, my time here would have been less enjoyable. Also I would like to thank my friends Marlio, Adolfo and Altair for their help.

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CHAPTER I

INTRODUCTION

With the implementation of Michigan Act No. 64, "The Hazardous Waste Management Act," the presence of toxic chemicals in concentrations greater than 1 ppm may result in some municipal sewage sludges being designated as hazardous wastes (1). The Michigan Department of Natural Resources (DNR) and the United States Environmental Protection Agency (EPA) signed a contract with researchers from Michigan State University in which the latter would 1) determine the total concentration of selected inorganic elements and organic chemicals present in 215 municipal sewage sludges from Michigan plus additional samples for the analysis of sludge process streams, and 2) would evaluate what impact, if any, these inorganic elements and organic chemicals would have for land application at the concentrations found in Michigan sewage sludges.

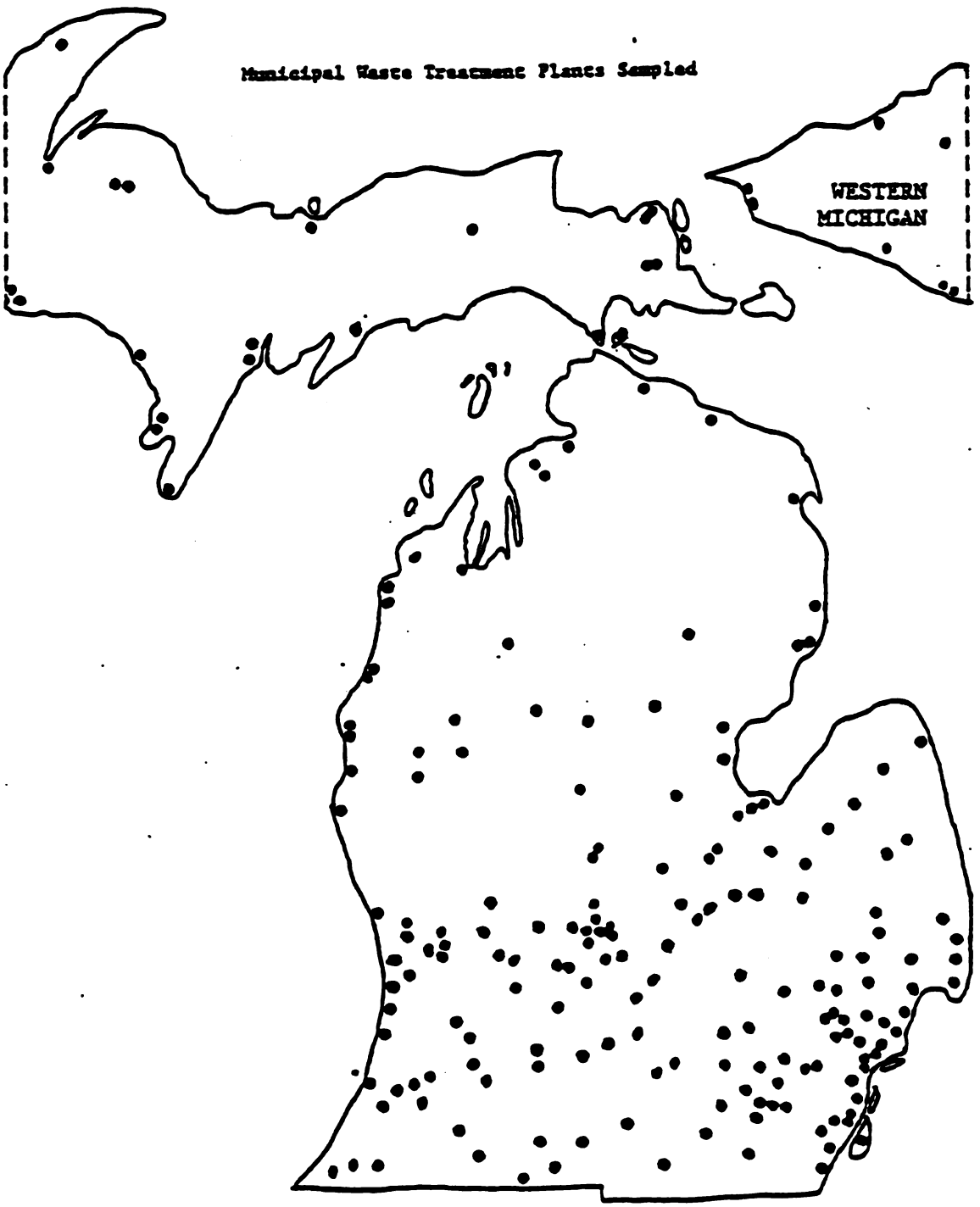
Among the organic compounds contracted for analysis was the group corresponding to aromatic hydrocarbons (Table 2.1) that are dealt with here. When conducting a literature review on analytical methodology for analysis of aromatic hydrocarbons in sludge Phyllips (2) concluded that "much attention has been given to polychlorinated biphenyls (PCB's) over the past decade, yet many other chlorinated and non-chlorinated aromatic hydrocarbons have escaped exposure." For this

reason the primary objective of this thesis has been the development of an analytical method to determine simultaneously the aromatic hydrocarbons mentioned above at a concentration over 1 ppm. Another objective has been to determine the concentration of these aromatic hydrocarbons in 236 sludge samples collected at municipal waste water plant facilities throughout Michigan (see Fig. 1). Then, based on the above findings, an evaluation of the risks involved in the land application of sludge is presented.

This thesis has five chapters, each with its own reference list. At the end of the thesis, all citations are presented in alphabetic order.

The method developed during this thesis combines the chromatographic and spectrophotometry tecniques, the process followed Taylor's (3) recommendations in which the hierarchy of methodology consists of 4 levels: technique, method, procedure, and protocol and is described in chapter 2. Chapter 3 details the steps followed in the method validation process, and starts with a description of field blank preparations (4). Chapter 4 covers the analytical results and statistical tests of the 236 sludge samples that were collected from throughout Michigan by the DNR. Chapter 5 corresponds to the overall conclusion and recommendation section.

Figure 1


Municipal Waste Treatment Plants Sampled
WESTERN
MICHIGAN

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2. Phillips, J. H. 1981. The characterization of municipal sludges for thirty-two volatile, semi-volatile and phenolic trace organic components. M.S. thesis, Michigan State University, E. Lansing.
3. Taylor, J. K. 1983. Validation of analytical methods. Anal. Chem. 55, 600A.
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PART 1

HPLC METHOD DEVELOPMENT AND VALIDATION FOR DETERMINATION OF 9 AROMATIC COMPOUNDS IN ACTIVATED SECONDARY SLUDGES.

CHAPTER II

HPLC METHOD DEVELOPMENT

Introduction

Preparation of sludge samples for the determination of organic compounds, especially the extraction procedure, is very difficult due to the formation of almost unbreakable emulsions (1,2). Additionally, when the method used for the final determination of these compounds is an electron capture detection-gas chromatography system (EC-GC), rigorous clean-up of extracts is required to eliminate compounds containing halogens, phosphorus, sulfur, nitrogen dioxide, lead, and some hydrocarbons which produce a response with an EC detector (3). The factors mentioned above contribute to inefficient recovery of organic compounds from sludge, and therefore a reduction in the number of sample-preparation steps would be useful. The objectives have been to identify the compounds listed in Fig. 1 in Michigan sludge samples and to determine their concentrations; levels over 1 ppm in sludges are considered hazardous in Michigan (2).

Aroclor^{R} 1254 has been the most widely used polychlorinated biphenyl (PCB). PCB's have been used in industry as heat transfer fluids, hydraulic fluids, solvent extenders, flame retardants, organic diluents, and dielectric fluids. The sole U.S. producer stopped their production in October 1977 (4,5). It has been a common practice to determine PCB's by EC-GLC (6,7); the Enviromental Protection Agency (EPA) recommended protocol for sludge samples uses this system (1). Lately, the characterization and quantification by open tubular column gas chromtography with electron capture detection

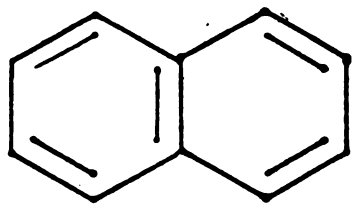
has won popularity (8,9). There are also some references using high pressure liquid chromatography (HPLC) (10,11,12).

Biphenyl and naphthalene are polynuclear aromatic hydrocarbons (PAH's), which enter the environment from motor vehicle exhaust, refuse, carbon black, creosote, soot, and residual oil. It has been generally accepted that these compounds have mutagenic and carcinogenic effects. Because PAH's are naturally fluorescent, they have been generally analyzed by reverse phase liquid chromatography (LC) using a fluorescent detector (13).

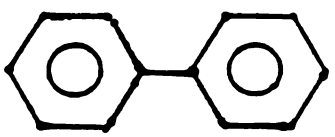
Fire Master ^{R} BP-6 belongs to the group of the polybrominated biphenyls (PBB's). Their major use is in the production of flame retardant resins of acrylonitrile, bertadiene, and styrene for business machines, electrical housing, textiles and other materials. All of these uses were discontinued in late 1974 as a result of a contamination incident in Michigan. The EC-GLC has been the most widely system for the final determination of these compounds (14,15).

Hexachlorobenzene is sold commercially as a fungicide for treatment of seed grain, as a plasticizer in polyvinyl chloride plastics, and for fire proofing textiles. It is also a by-product of the industrial production of perchloroethylene as well as an impurity in the production of several pesticides (14,16,17). The three tetrachlorobenzene isomers belong to the same group as does hexachlorobenzene and have been used as raw materials and intermediates in the manufacture of pesticides and chlorinated phenols and as process solvents (18). They have been examined by EC-GLC (14,17,18,19).

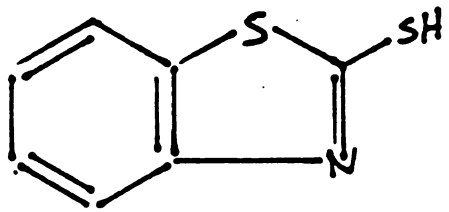
2-mercaptobenzothiazol has been used as a rubber vulcanization



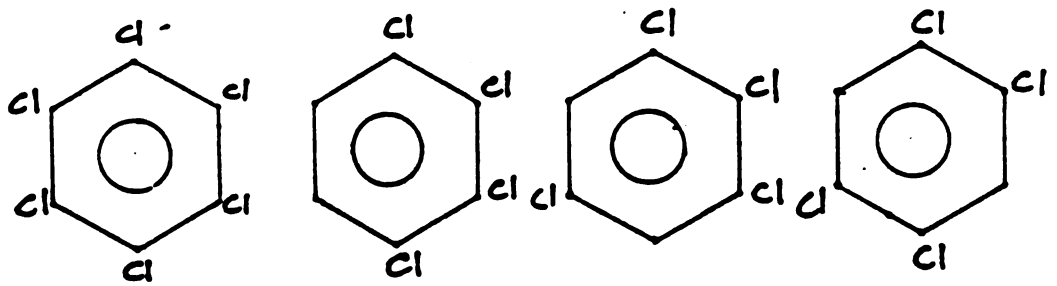
Naphthalene



Biphenyl



Mercaptobenzothiazole



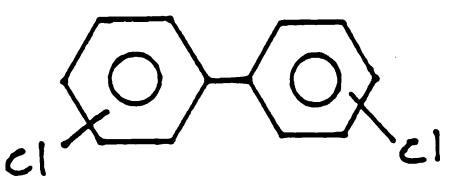
Hexachloroben-zene

1,2,3,4

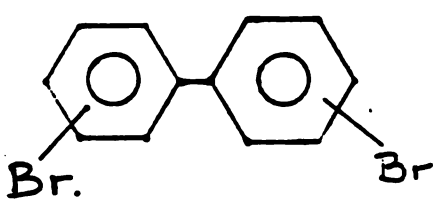
1,2,3,5

1,2,4,5

Tetrachlorobenzene



PCB



PBB

Figure 1. General chemical structure and name of nine aromatic compounds to be determined by HPLC, using a C_{18} column and an UV detector.

accelerator, whereas its salts are used as fungicides (16). No specific analytical reports were found for this compound.

Considering the above, I developed a method to determine the presence of the compounds listed in Fig. 1 by means of modern high pressure liquid chromatography in the reverse phase mode with Ultraviolet (UV) spectrophotometric detection. During the development of this method, some considerations were: 1) that because all the compounds were aromatics it should be possible to find a common determination wavelength, 2) that because the results were to be used in screening programs, the method required that sensitivity did not need to be extremely high, 3) the method should have the ability of determine all compounds simultaneously, and 4) that the method should protect the analytical column and reduce sample processing steps. In the following I present the individual steps in the development of this method and also compare its sensitivity to the traditional methods.

Methods

Apparatus:

(a) Spectrophotometer - UV-VIS microprocessor-controlled spectrophotometer, model 2600 (Gilford, Oberling, OH 44074).

(b) Liquid chromatograph - system composed of (1) solvent metering pump, model 110 (Altex Scientific Inc., Berkeley, CA 94710); (2) column compartment, model 860 (DuPont Company, Scientific and Pro-cess Instruments Division, Wilmington, DE 19898).

(c) Injector - syringe loading sample injector, model 7125 with a

20-ul loop. (Rheodyne Inc., Cotati, CA 94928).

(d) Chromatographic column - MPLC^{R} cartridge system composed of (1) analytical cartridge Speri-5-RP-18, C-18 chain, 5um, 22 cm x 4.6mm. (2) guard cartridge, Speri-5 RP-18, C18 chain, 5 um, 3 cm x 4.6 mm; saturator column, between pump and injector, silica-gel (40-70 um) 15 cm x 4.6 mm.

(e) Detector - UV-VIS variable wavelength, spectrophotometer, model 100-10 (Hitachi, Tokyo, Japan).

(f) Recorder - strip chart recoder (Linear Instruments Corp., Irvine, CA 92714).

Reagents:

(a) Solvents - acetonitrile (UV) (Burdick and Jacksons Laboratories, Inc., Muskigon, MI. 49442), methanol (HPLC grade) (J.T. Baker Chemical Comp., Phillipsburg, NJ 08865), water redistilled in Corning megapure 3 liter automatic system (Corning Waterware, Corning, NY 14830).

(b) Standards - Aroclor 1254^{R}- polychlorinated biphenyl, Lot KA628 (Monsanto Co., St. Louis, MO 63166); biphenyl, Lot 0306 MH (Aldrich Chem. Co.Inc., Milwaukee, WIS. 53233); Fire Master^{R} BP-6-hexabromobiphenyl, Lot 06151 (Michigan Chem. Corp., Chicago, ILL 60611); hexachlorobenzene, Lot 104087 (EPA analytical ref std, Research Triangle Park, NC 27711); 2-mercaptobenzothiazol, Lot 0520HK (Eastman Kodak Co.,Rochester N.Y. 14650); naphthalene- recrystallized, Lot F3I (Eastman Kodak Co., N.Y. 14650); 1,2,3,4 tetrachlorobenzene, Lot 110787 (Aldrich Chem. Co. Inc., Milwaukee, WIS. 53233); 1,2,3,5 tetrachlorobenzene, Lot 082987 (Aldrich Chem. Co. Inc., Milwaukee, WIS. 53233); 1,2,4,5 tetrachlorobenzene, Lot 1825 AJ (Aldrich Chem. Co. Inc., Milwaukee, WIS. 53233).

Standard solution preparation:

(a) Stock solutions - weigh approximately 10.0 mg of each standard compound; transfer with small portions of acetonitrile (UV) to 10-ml volumetric flask; dilute to volume with more acetonitrile; label with compound name, exact amount weighed, standard purity, solvent, date and analyst initials, wrap in aluminum foil and store in freezer when not in use.

(b) Working standard - transfer with pipet the needed volume (table 2) of each individual compound to a 10-ml volumetric flask (when necessary, prepare intermediate concentration of individual solutions); bring to volume with acetonitrile.

Chromatography:

(a) Mobile phase preparation - combine, CH_3CN and H_2O (10:1), shake well, filter through a Millipore^R system (Millipore Corp., Bedford, MA 01730) and degas thoroughly by sonication or under vacuum.

(b) System conditioning - equilibrate system with mobile phase at a flow rate of 1.2 ml/min; turn detector on and allow it to warm up. Set wave-lenght at 235 nm, calibrate the machine, and set time constant at 0.3 sec; adjust sensitivity of detector so that peak response for working standard is ca. 60% full scale recorder deflection for 20-ul injection.

(c) Sample injection - after one standard injection, inject the properly prepared sample.

Results and Discussion

The steps for the method development are discussed in cronological order.

Spectroscopic Study:

One of the most important considerations when developing a new methodology is to be able to determine the effect(s) of changing any single variable. All the compounds considered here are aromatic and thus have the well known characteristic of being able to absorb energy in the ultraviolet (UV) region of the electromagnetic spectrum (20).

UV absorption was the principal characteristic used in the development of the methodology described herein. Table 1 presents the spectroscopic characteristics of the chosen compounds (using UV acetonitrile as solvent; see methods above). As seen later, the maximum absorbance (λ_{max}) between 210-340 nm was used to determine the chromatographic characteristics of the compounds. A wavelength of 235 nm was selected because discernible detector responses were observed for all 9 compounds, even though the respective ultraviolet absorbances and absorbtivities at equimolecular concentrations varied and were not the maximum for each compound. 260 and 325 nm were the wavelenghts used to overcome the spectral interference of coextractives for selected compounds.

Column selection:

Table 1. UV spectroscopy parameters for the listed aromatic compounds.

Compound	max 200-340 nm	max	235	260	325
Aroclor 1254	217	6,105	2,544	1,018	219
Biphenyl	248	15,128	10,552	12,060	NA[1]
Firemaster	219	71,725	50,925	8,275	NA
Hexachlorobenzene	217	1246,667	306,667	20	NA
2-mercaptobenzothiazol	227	11,991	10,507	NA	19,128
Naphthalene	276	5,432	2,351	3,957	NA
1,2,3,4-Tetrachloroben[2]	204	64,931	8,212	NA	NA
1,2,3,5-Tetrachloroben.	205	58,010	6,172	NA	NA
1,2,4,5-Tetrachloroben.	205	74,368	11,520	NA	NA

[1] no absorption.

[2] Tetrachlorobenzene

Reverse phase columns have proven very useful in the LC analysis of PHA's (21,22,23). A C_{18} bounded-phase column has been commonly used with a linear solvent gradient from 60% to 100% acetonitrile. After using HPLC to characterize the behavior of a number of individual brominated biphenyls and to assess the composition of several mixtures of these compounds, De Kok et al. (15) concluded that the silicagel - dry n-hexane system was not suitable. There is a lack of information for the other compounds because traditionally they have been analyzed by gas chromatography (GC) using electron capture (EC).

Chromatographic strategy:

One of the most important goals to achieve when optimizing methods for multicompound determination is adequate resolution within an acceptable time of analysis (24). Two aditional considerations when dealing with trace analysis are sensitivity and selectivity. Each of the above point has been considered during the present method development as follow.

1. Resolution and time analysis.

The basic resolution equation (24,25), was used in the optimization process.

$$Rs = \frac{\sqrt{N}}{4} \cdot \frac{k'}{(1+k')} \cdot \frac{(\alpha - 1)}{\alpha} \qquad [1]$$

Efficiency factor | Capacity factor | Selectivity factor

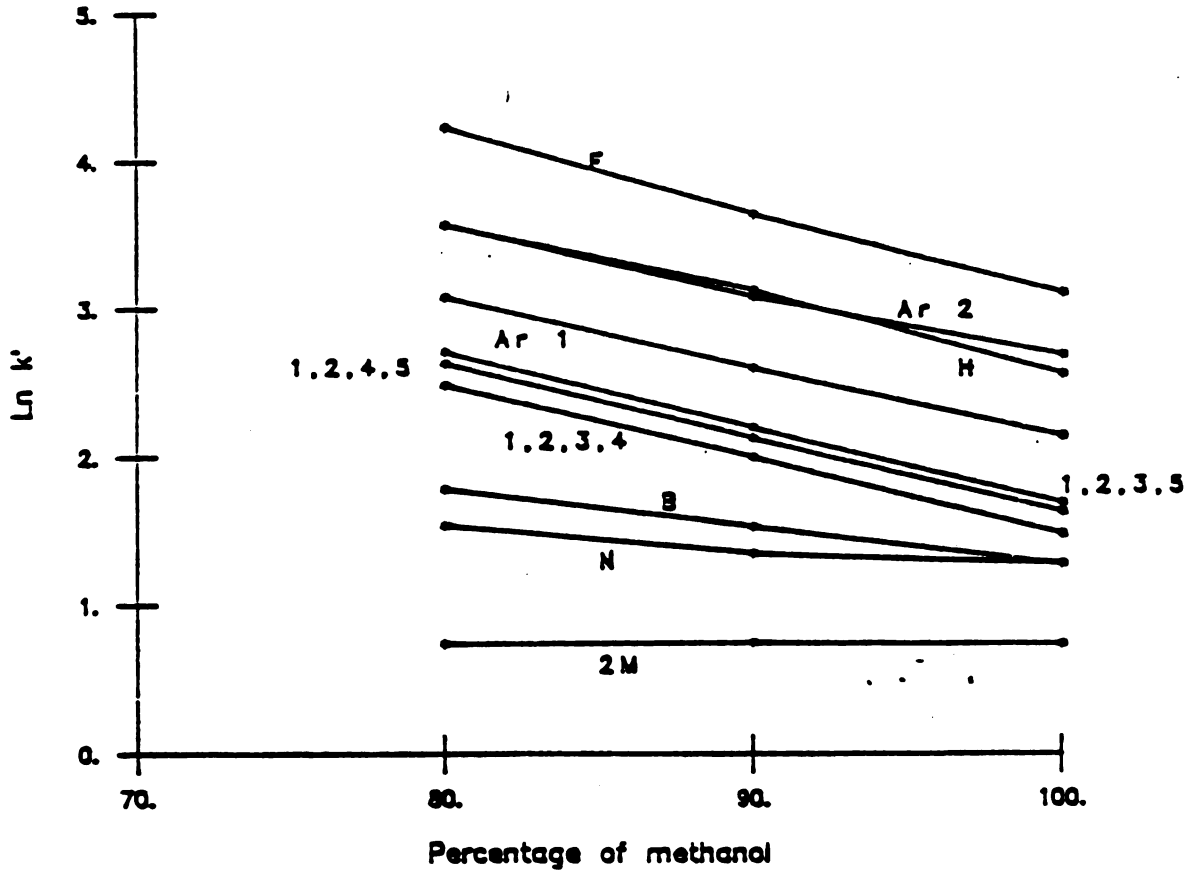


Figure 2. Capacity factor (k') of 2-mercaptobezotiazol (2M), naphthalene (N), biphenyl (B), 1,2,3,4-tetrachlorobenzene (1,2,3,4), 1,2,3,5-tetrachlorobenzene (1,2,3,5), 1,2,4,5-tetrachlorobenzene (1,2,4,5), Arochlor 1254 peak 1 (Ar 1), peak 2 (Ar 2), hexachlorobenzene (H), Fire-Master® (F), as a function of solvent strength when methanol is the organic modifier.

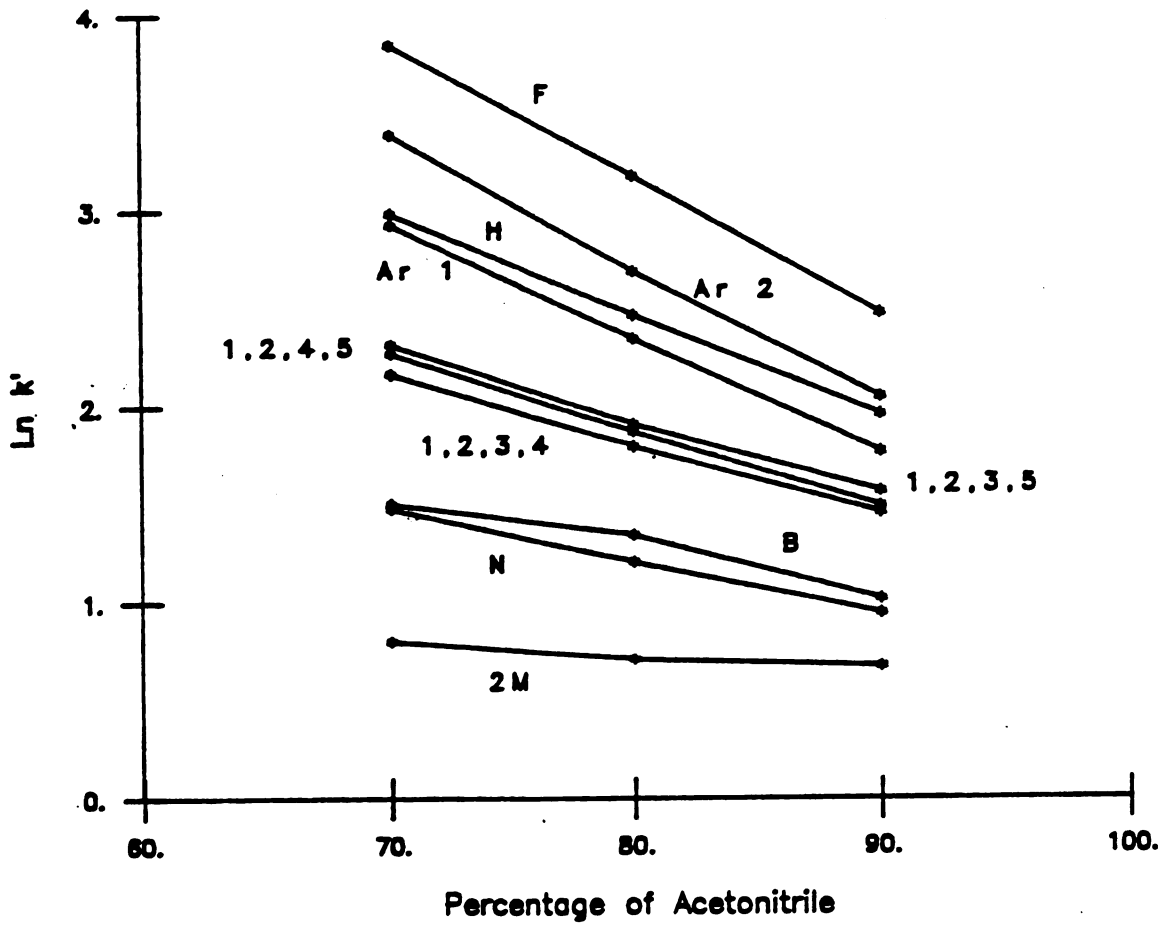


Figure 3. Capacity factor (k') of 2-mercaptobezotiazol (2M), naphthalene (N), biphenyl (B), 1,2,3,4-tetrachlorobenzene (1,2,3,4), 1,2,3,5-tetrachlorobenzene (1,2,3,5), 1,2,4,5-tetrachlorobenzene (1,2,4,5), Arochlor 1254 peak 1 (Ar 1), peak 2 (Ar 2), hexachlorobenzene (H), Fire-Master® (F), as a function of solvent strength when acetonitrile is the organic modifier.

Where N is column theoretical plates = $(t_R/\sigma)^2$, t_R is the retention time, e is the peak standard deviation. k' is capacity factor = $(t_R-t_o)/t_o$, t_o is the retention time of an unretained peak, and α is selectivity factor k'_2/k'_1. The equation factors were varied to obtain maximum resolution while minimizing overall separation time.

(a) Capacity factor optimization:

K' values are controlled by means of solvent strength. In reverse-phase chromatography, when considering water as the base solvent, the organic modifier concentration controls the solvent strength. The higher the concentration of the organic modifier the stronger the sol-vent. If at certain initial conditions the Rs must be icreased and k'is small, k' should first be increased into the optimum range $1 < k' < 10$ (25). When the time for analysis is too long and k'is out of the optimum range, the solvent strength should be increased.

K' values for the 9 compounds of interest were determined over a range of 60-100% for methanol/water (v/v) and acetonitrile /water (v/v) systems; t_0 was determined by injecting 10 ppm solution of sodium nitrate. Figures 2 and 3 show the plot of the ln k' vs % methanol and ln k' vs % acetonitrile respectively, for the 9 compounds; in this range they are linear. From the graphs, it can be observed that for the methanol/water system there are still 4 compounds over 2.3 (ln 10), when the methanol concentration is 100% but in the acetonitrile/water system only 1 compound is over 2.3 at a 90% concentration,. For this reason, acetonitrile/water at the proportion of 90% was selected.

The separation as described, to this point, was performed on an

analytical Dupont Zorbax^{R} C_{18} column. (10 um particle size).

(b) Efficiency factor optimization:

In Fig. 4 the arrow chromatogram 1, indicates a peak corresponding to hexachlorobenzene and one of the peaks of Aroclor 1254 that were not resolved by the system. The only peak on chromatogram 2 (Fig. 4) corresponds to the three positional isomers of tetrachlorobenzene; indicating that, the efficiency of the system should be increased.

The theoretical plate number (N) of the column is a useful measure of column efficiency and depends almost exclusively on the physical characteristics of the system (25), such as column length (L), mobile phase velocity (u), uniform packaging, diameter of particle packaging (d_p), etc. The plate number can be expressed in terms of plate height (H) and L as follows: $N = L/H$.

From this equation it is easy to conclude that a reduction in H will increase system efficiency. Giddings (29) introduced the concept of reduced plate height (h), which he defined as $h = H/d_p$, and demonstrated that h has a minimum of 2.4 at an optimum velocity. A large improvement in the resolution power of the system was observed after changing from a 10 um to a 5 um particle diameter, while maintaining almost all other column characteristics constant. This change allowed for the separation of the peak # 5 of Aroclor 1254 and hexachlorobenzene (Fig. 5). Good resolution, nearly base line for 1,2,3,4- and 1,2,4,5- tetrachlorobenzene, was also obtained for the three isomers of tetrachlorobenzene.

The extracolumn contributions to band broadening are added to the column contribution (Hc) to produce the apparent plate height (H) as

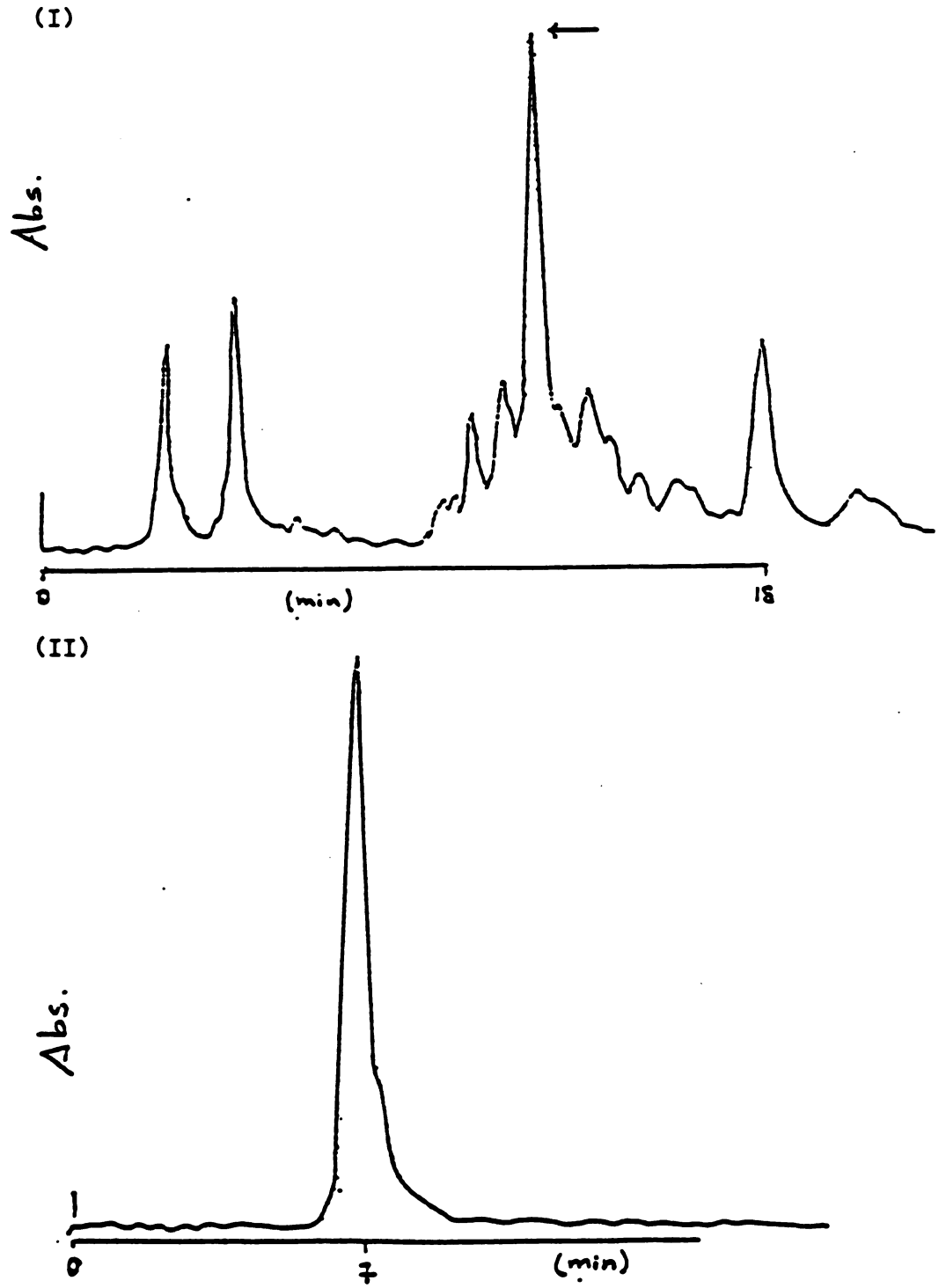


Figure 4. Coelution of (I) hexachlorobenzene and the peak # 5 of Aroclor 1254 and (II) the three positional isomers of tetrachlorobenzene in a 10 um particle size C_{18} column.

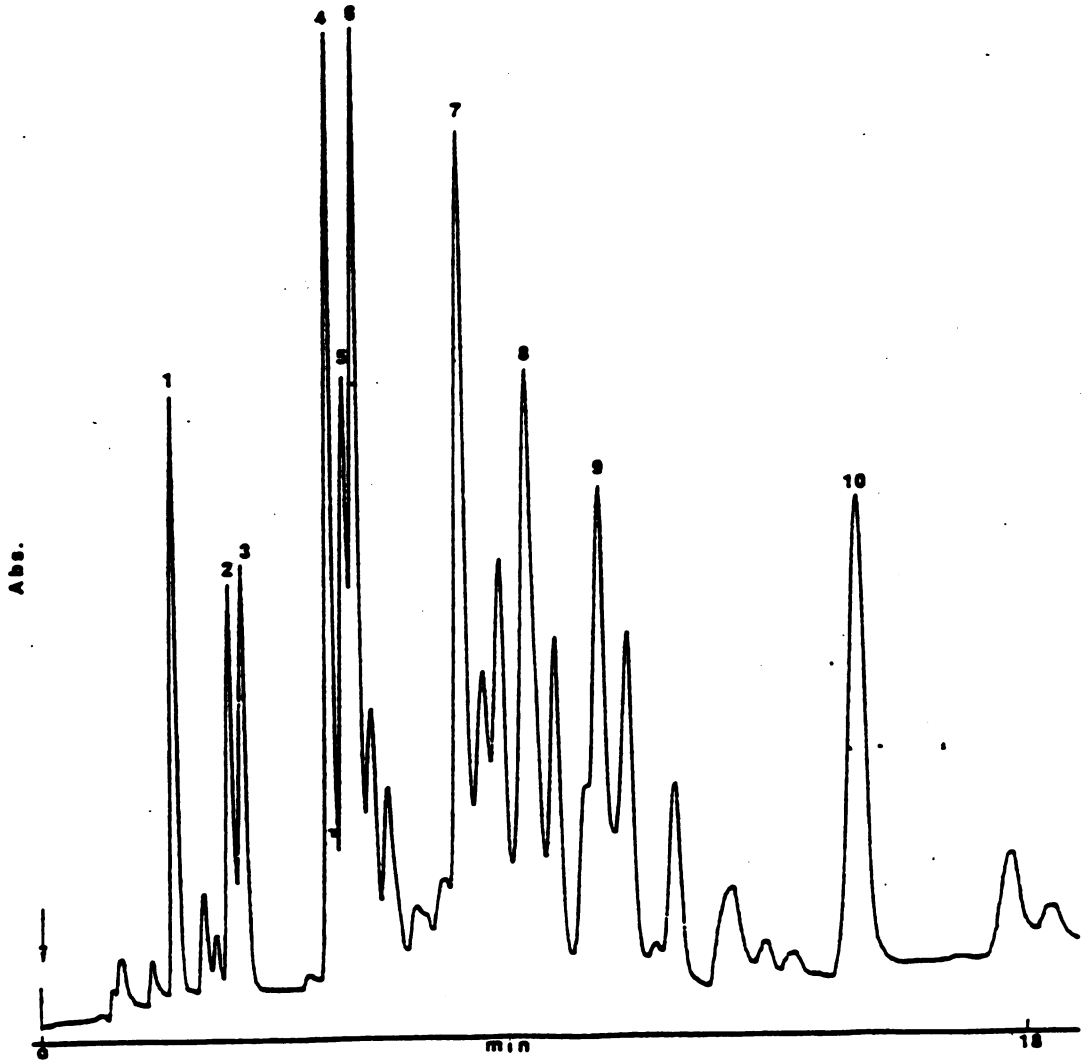


Figure 5. Separation of aromatic compounds by HPLC, on a C_{18} column, 5 um particle size. Mobil phase, acetonitrile-water (9:1). Mixture of [1] 2-mercaptobenzotiazol, [2] naphthalene, [3] biphenyl, [4] 1,2,3,4-tetrachlorobenzene, [5] 1,2,4,5-tetrachlorobenzene, [6] 1,2,3,5-tetrachlorobenzene, [7] Aroclor 1254 (first peak), [8] Hexachlorobenzene, [9] Aroclor 1254 (second peak), [10] Fire-master^{R} (only peak considered).

follows:

$$H = H_c + H_{ec} + H_t \qquad [2]$$

where H_{ec} is all extracolumn broadening factors (except H_t), and H_t is the contribution of the detector time constant t (3,26), which affects detector sensitivity to high frequency noise. The selected t was 0.3, because it provided the most efficient detector response. The minimum amount of tube connection was used between the column output and the detector in order to minimize H_{ec}.

(c) Selectivity factor optimization

The elution order of the three tetrachlorobenzene isomers, was determine to be 1,2,3,4-, 1,2,4,5-,1,2,3,5-. The possibility of increasing resolution between them was considered based on the fact that the selectivity factor could still be improved (equation 1). According to Glajch and Kirkland (24), the five most useful variables used to improve selectivity are mobile-phase composition, stationary-phase composition, temperature, primary chemical equilibria (pH or other ionic effects), and secondary chemical equilibria. During the present study the first two variables were considered when the column and solvent proportion were selected. The influence of primary chemical equilibria in this separation could not be applied due to the chemical nature of the solutes studied. Therefore, the influence of temperature on selectivity was studied in the separation of the three tetrachlorobenzene isomers.

Variation of selectivity with temperature is shown in Fig. 6 There

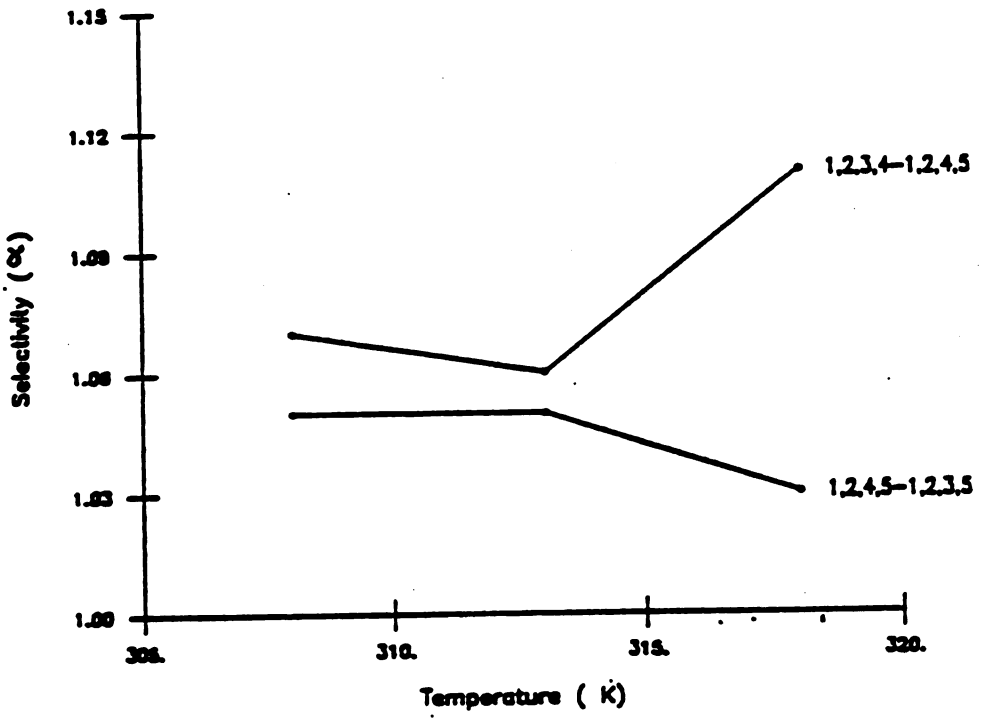


Figure 6. Selectivity (α) as a function of column temperature for the pairs 1,2,3,4- , 1,2,4,5-tetrachlorobenzene and 1,2,4,5- , -1,2,3,5 tetrachlorobenzene.

was no improvement in the system's selectivity or in the achieved resolution over the range of temperatures tested. In some cases, in fact,increasing the temperature resulted in poorer separation.

The variation of log k' with temperature [Van't Hoff plot (27)] shown in Fig. 7, indicated that the system behaved as expected. Knox and Vasvari (31) have shown that h is related to the solvent reduce velocity (v) through the equation:

$$h = B/v + Av^{0.33} + Cv \qquad [3]$$

Where A,B,C are Knox coefficients; A is related to the packing quality, B is related to the axial diffusion, and C is called the mass transfer parameter and is the only coefficient of the three that is dependent on temperature. They stated that at a given solvent flow, any improvement in the column efficiency is due to a decrease in solvent viscosity. This is of practical importance for solvent mixtures with high viscosity; this could be a reasonable explanation why no consistent results occurred when temperature was increased. That is, the high (90%) acetonitrile content, which makes the solvent viscosity very low, masked the temperature effects.

2. Sensitivity study and working standard determination.

The analyte levels that need to be measured determine the detection method selected, the amount of a sample to be taken, the degree and type of sample pretreatment to employ, and the method of analysis (32). The method described here is to be used in screening programs.

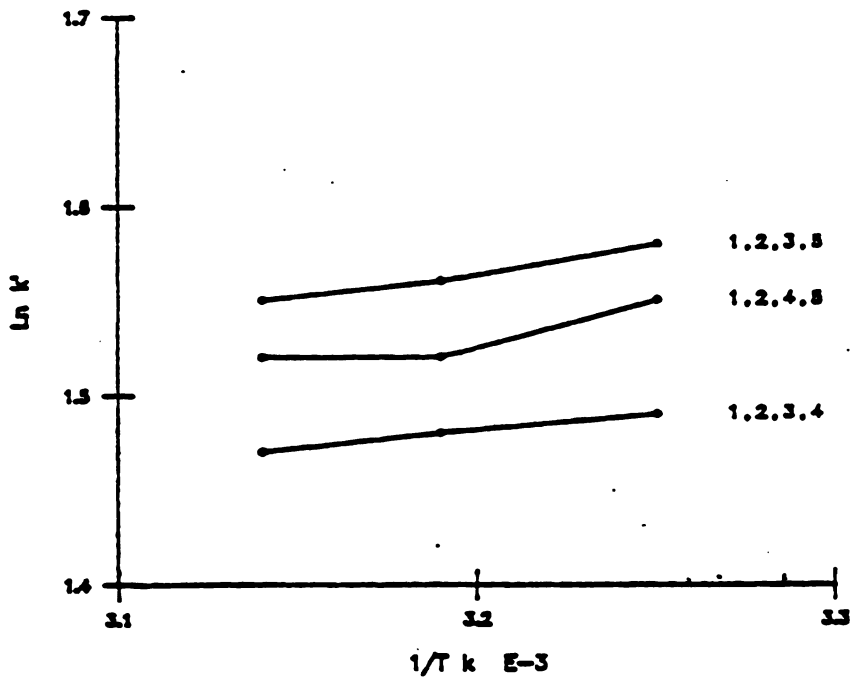


Figure 7. Van't Hoff plots for 1,2,3,4- , 1,2,4,5- and 1,2,3,5-tetrachlorobenzene.

In Michigan, for example, the presence of organic pollutants in sludge samples at a concentration over 1 ppm defines the sludge as hazardous (2). Therefore, the method used to answer this question should have a limit of detection one order of magnitude lower than 1 ppm, but not necessarily lower. Analytical methods can be unnecessarily expensive when the method sensitivity is lower than required, because in general, the lower the levels required the higher the cost of the analysis (32).

Table 2 presents the results for the calculated limit of detection, when defined as the smallest signal above 3 times the background noise (32). The concentrations recommended for the working standard will produce peak hights of approximately 60% full scale deflection, for all compounds, at the working conditions recommended here. For 25-ml sludge samples, the lowest concentration of analyte that can be detected is 4 ppb for 2 mercaptobenzothiazol, and 80 ppb for Alaclor 1254. The third column in Table 2 shows the sensitivity of the compounds in the traditional method of detection.

3. Spectroscopy selectivity.

Because of the complexity of environmental samples and the limited selectivity of most methodologies, interferences are common during analysis (32). The amount of extract purification required depends upon the selectivity of both the extraction procedure and the final determination method. The most common cleanup process features solvent partition followed by adsorption chromatography, generally using silica gel or florisil as the adsorbent Concentration of

Table 2. Detection limits (Det. L)[1] for the mixture of aromatic standards, recommended concentrations (R Con.)[2] for the preparation of the working standard when analyzing the mixture by HPLC with UV detection, minimun amount detectable in a 25 ml sludge sample MAD sl.), and minimum amount detectable as reported by others methods (MAD rep.)

Compound	Det. L ug/ml	R. con. ug/ml	MAD sl. ug/L	MAD rep.	Ref
Aroclor 1254	2.00	10.0	80	0.3[3]	7
Biphenyl	0.15	0.5	6		
Fire Master[R] BP-6	1.00	5.0	40	0.001[3]	3
Hexachlorobenzene	0.5	2.5	20	0.4[4]	19
2-mercaptobenzothiazol	0.1	0.2	4	N.F[5]	
Naphthalene	0.25	1.0	10	30.0[6]	23
1,2,3,4-tetrachlorobenzene	0.5	2.0	20	0.5[4]	19
1,2,3,5-tetrachlorobenzene	0.5	2.0	20	0.5[4]	19
1,2,4,5-tetrachlorobenzene	0.5	2.0	20	0.5[4]	19

[1] Calculated as 3 times the noise level.

[2] Concentration producing 50 to 60 % full scall deflection.

[3] mg/Kg

[4] ug/Kg

[5] N.F = non found

[6] pg/28 ul

solutions is normally required prior to, during, and after cleanup procedures (3). With sludge samples, where the sampling and extraction procedures are intrinsic sources of error, sample preparation steps should be kept to a minimum because they increase variance and mechanical loss thereby reducing method recovery (32).

Use of a guard column during HPLC will not only protect the analytical column but will allow for reduction in sample cleanup. Fig. 8 corresponds to the chromatogram of a standard fortified sludge sample after extraction, solvent partition, and evaporation. The area corresponding to 2-mercaptobenzothiazol, biphenyl, and naphthalene is covered by extraneous peaks, which could be constituents inherent in the sample or solvent contaminants or both. In solving this problem, it was decided to enhance the selectivity using the the specific adsorption wavelength that, fortunately, these compounds have.

Table 1 shows the calculated adsorptivities for the 9 compounds at 260 nm and 325 nm. These wavelenghts were used because, at 260 naphthalene and biphenyl are detected most readily, with only alaclor 1254 showing slight absorption. At 325 nm, 2-mercaptobenzothiazol is the only compound that responded visibly. Besides overcoming the interferences and increasing the sensitivity, the spectral selectivity yields the advantage of offering a way to confirm the presence of the compounds.

In summary, the method described here, could be useful for the final determination of the compounds considered in sludge samples. The next chapter covers method validation with a synthetic sludge sample.

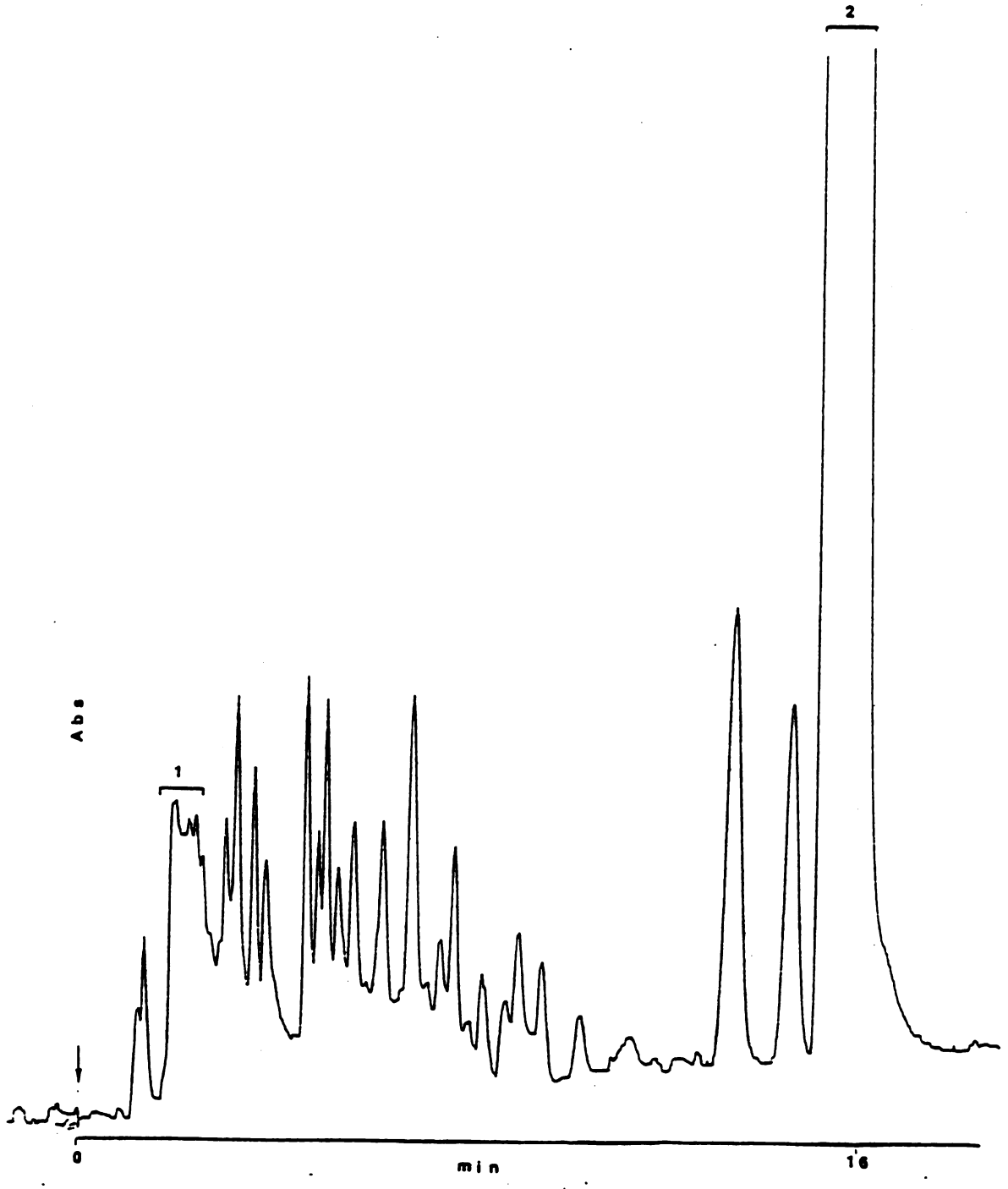


Figure 8. Sludge sample analyzed for presence of aromatic compounds, after fortification with the mixture of the standards at the ideal concentration level. (1) Coelution of interferents with 2-mercapto-benzothiazol, (2) Contamination with phthalates.

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CHAPTER III

VALIDATION OF AN HPLC METHOD WITH A SYNTHETIC SLUDGE

Introduction

Any new method or major modification of an existing method that has been developed for testinga large number of samples should be validated in the laboratory. Exceptions to this rule are standard and official methods (1). Validation is the process of determining the suitability of methodology for providing useful analytical data (2). Elements needed to obtain reliable data are dictated by the objectives, time and resources available (3). "When data requirements are ill-considered, analytical measurement can be unnecessarily expensive if the method chosen is more accurate than required, inadequate if the method chosen is more accurate than required or utterly futil if the accuracy of the method is unknown" (2).

The classical validation process consists of analyzing a sufficient number of reference samples and comparing the results to the expected or certified values. Reference samples, besides having the certified concentrations of the analyte or analytes, are similar in all respects to the test samples. When a suitable material is not available, spiked samples and surrogates may be used as reference samples (2). Matrix effects can cause wide variability in recoveries, especially with organic compounds. For this reason, in recovery studies, uncontaminated samples from control sites (field blanks) that have been spiked with analytes of interest are useful because they simulate any matrix effects. Simulated or synthetic field blanks, that best represent a sample that does not contain the analyte or analytes, may be spiked when suitable blanks from control sites are unavailable (3).

The purpose of the current method was to allow screening analysis of sludge samples. Because of the diversity of sources that contribute to sludge, field blanks for sludge were very difficult if not impossible to obtain. A method for the synthesis of secondary activated sludge was developed in Michigan State University (MSU), that uses the same basic principals for the production of activated secondary sludge. The secondary activated sludge process is one of the biological methods used to wastewater treatment. It was developed in England in 1914 by Ardern and Lockett and was so named because it involved the production of an activated mass of microorganisms that was capable of aerobically stabilizing a waste (4). The MSU method is similar in that, a bacterial culture from a wastewater facility is poured into a reaction tank (mixed liquor), where it is continuously fed oxygen. The original material is replaced daily with water. The resulting sludge is considered to be free of all possible impurities. This procedure was used for the production of the field blank used in the recovery studies conducted here.

Materials and Methods

This section describes an innovation in experimental methodology which was an outgrowth of related research literature. The method encompasses: sludge synthesis, sampling methods, determination of percent of solids, preparation of standard solutions, chromatographic standarization, concentration, selection of extraction procedure, application of the analytical method to secondary sludge fortified samples, and recovery calculations.

1. Sludge Synthesis.

The sludge sample was prepared in the laboratory using the following procedure:

a - Sixty liters of secondary sludge, collected at the Mason Waste Water Plant facility, were poured into a zinc galvanized steel tank with a capacity of approximately 100 liters (aeration tank).

b - Air was fed into the tank, at a rate of approximately 30 ml/min. during synthesis time.

c - Daily, for fifteen days:

1) Nine liters of mixed liquor were collected from the aeration tank, four liters were poured into a five liter glass beaker and the rest was discarded.

2) The four liters were continuously stirred, from which, a 50 ml sample was collected daily for a pH determination. On Mondays,

Wednesdays and Fridays a 10 ml. sample was taken to determine mixed-liquor suspended solids (MLSS), and a 1 liter sample to determine sludge volume index (SVI), defined as the volume in milliliters occupied by one gram of activated-sludge mixed-liquor solids, dry weight, after settling for 30 min in a 1000-ml graduated cylinder.

3) Nine liters of a Sodium Acetate solution (157.5 g.) were added to make-up the activated sludge that was removed.

4) A liter of the nutrient mixture (Table 1) was also added.

d - The MLSS were determined by filtering the 10 ml sample with a preweighed filter paper fixed in a Millipore Vacuum system. The filter was dried for 2 hrs in an oven at 130^{0}C. The final weight of the filter was measured after it returned to room temperature in a desicator.

e - SVI was obtained from the one liter cylinder after the sample settled for 1 hour.

2. Sampling Methods.

Without stopping air flow, which helps to mantain the homogenicity of the solids suspended in the sludge, twelve gross samples were collected from the digestion tank.

Conceptually, the tank was divided into quaters. Each sample was collected from two opposite quarters, e.g., 1 and 3, and poured into a 2 liter beaker. Then the tank was shaken manually, and portions of

Table 1. Nutrient solution composition used to feed the liquor during sludge synthesis.

Compound (saturated solution)	Volume (ml)/L
Magnesium sulphate	9.0
Iron(III) chloride	9.0
Magenesium sulphate/ sodium molibdate	9.0
Copper sulphate/zinc sulphate	9.0
Potassium dyhydrogen phosphate/ potassium hydrogen phosphate	90.0
Sodium nitrate/sodium hydrogen carbonate	90.0
Water	794.0
Sodium acetate (157.5 g.) in 9 L.	

sludge were collected from the remaining two quarters, 2 and 3. Repetition of this process filled the 2 liter beaker 3/4 full.

From each gross sample two 250 ml intermediate subsamples, and one 10 ml subsample were collected. In order to ensure homogeneity of subsamples, the beaker was placed on a magnetic stirring plate and the sludge was continuosly agitated during sampling. The containers for the 250 ml subsamples were wide-mouth glass jars which had been cleaned prior to sampling by detergent washing, distilled water and acetone rinsing, and oven drying. The caps were lined with aluminum foil to avoid contamination from the caps.

All samples were uniquely labelled and kept in a freezer until analyzed. The 10 ml subsamples from each gross sample were collected in 50 ml beakers in preparation for determination of percent of solids.

The day before analysis began, sludge samples were transferred to refrigeration at $4^{o}C$. On the day of analysis samples were brought to room temperature. Each individual jar was placed over a stirring plate and the sludge was stirred magnetically during sampling. A 50 ml sample was poured into a 250 ml beaker with a 10 ml pipette. The sample was weighed before starting the analysis.

3. Determination of Percent solids.

The 10 ml subsamples were poured quantitativaly into 50 ml glass beakers which had previously been baked at 550^{o} C and weighed when cool. These samples were then placed in an oven at $105^{o}C$ to dry to a

constant weight. After drying they were allowed to cool in a desiccator before the final weight was determined.

4. Preparation of Standard Solutions.

Concentrated stock standard solutions were prepared at a concetration of 1000 ng/ul by weighing aproximately 10.0 mg of pure standard and diluting to 10 ml with acetonitrile. The flasks were wrapped with aluminun foil.

The intermediate concentration standards were prepared by diluting 1 ml. of the stock solution in a 10 ml volumetric flask with acetonitrile. As the response of the chromatograph to the compounds was unknown a new dilution of the intermediate concentration standards was prepared (1/10) before initiating work.

A final working standard mixture was only established after the characteristic response to the individual compounds was determined, using the criterion of a 70% full scale deflection .

Stock solutions were always refrigerated.Standard solutions were also refrigerated when not in use, after marking the meniscus with tape, to allow for reconstitution to the same volume.

5. Chromatographic Standardization.

Prior to working the sludge samples, the chromatographic conditions were established with standards for compounds individually

and for the mixture of them. The steps covered in this part were: a) determining physico-chemical characteristics of the compounds, b) selection of the column and solvent system, c) retention time determination for the compounds, d) wavelength selection for the mixture, e) checking linearity of the response.

a - Determining Physico-Chemical Characteristics of the Compounds.

Literature review revealed the molecular weight, the solubility and other physico-chemical properties of the compounds.Only Ultraviolet (U.V.) spectroscopic characteristics for biphenyl, naphtalene, and hexachlorobenzene were found in the literature. Therefore, maximum absorbance wavelength and absortivity were determned for all the compounds running U.V. spectrum at different concentrations, using U.V. quality acetonitrile. A Gilford spectrophotometer 2600 was used for this purpose.

b - Selection of Column and Solvent System

A high pressure liquid chromatograph system consisting of an Altex pump a U.V.-VIS spectrophotometer (Hitachi, 100-10), a Houston recorder and an injection valve (Rhodine) with a 20 ul loop were used in the present study.

The column selected for this purpose was a C_{18} bonded phase. The initial work was performed with a 10um particles, 25 cm long column; the final study used a 5 um particle, 25 cm long column.

After preliminary use of a methanol/water solvent system, a water/acetonitrile solvent system was the final selection. The strategy followed to obtain the solvent proportion was: 1) Select the detector that gave the highest sensitivity wavelength for each compound, according to the U.V. spectrum previously taken. 2)

Equilibrate the chromatographic system with 45% acetonitrile/water mobile phase. 3) Inject each compound using the test solvent proportion at the highest sensitivity wavelenght.

6. Recovery Study.

The recovery study was divided in two parts, the first part used references compounds only, the second part used synthetic sludge samples. Figure 1 shows the steps covered by the complete analysis.

a - Recovery with Reference Compounds

1) Concentration

For all concentration assays, 1 ml of the ideal standard solution was added to 200 ml of DCM and then submitted to evaporation. When the DCM volume was ca. 5 ml, 10 ml of acetonitrile were added and the evaporation continued to ca. 0.5 ml when the sample was allowed to cool to room temperature and then transfered by washing the vessel with small portions of acetonitrile, to a sample vial of 1 ml capacity. The sample vial was covered with aluminum foil and capped tightly and refrigerated until final determination by HPLC. The recovery was calculated for each compound using the ideal standard solution as an external standard. Each assay was performed in triplicate.

Several evaporation system modifications were tried for concentrating the samples.

a) Rotavapor

A rotating vacuum type evaporator (Rotavapor) with all-glass condenser and a water bath at $40^{o}C$ was tried first for sample

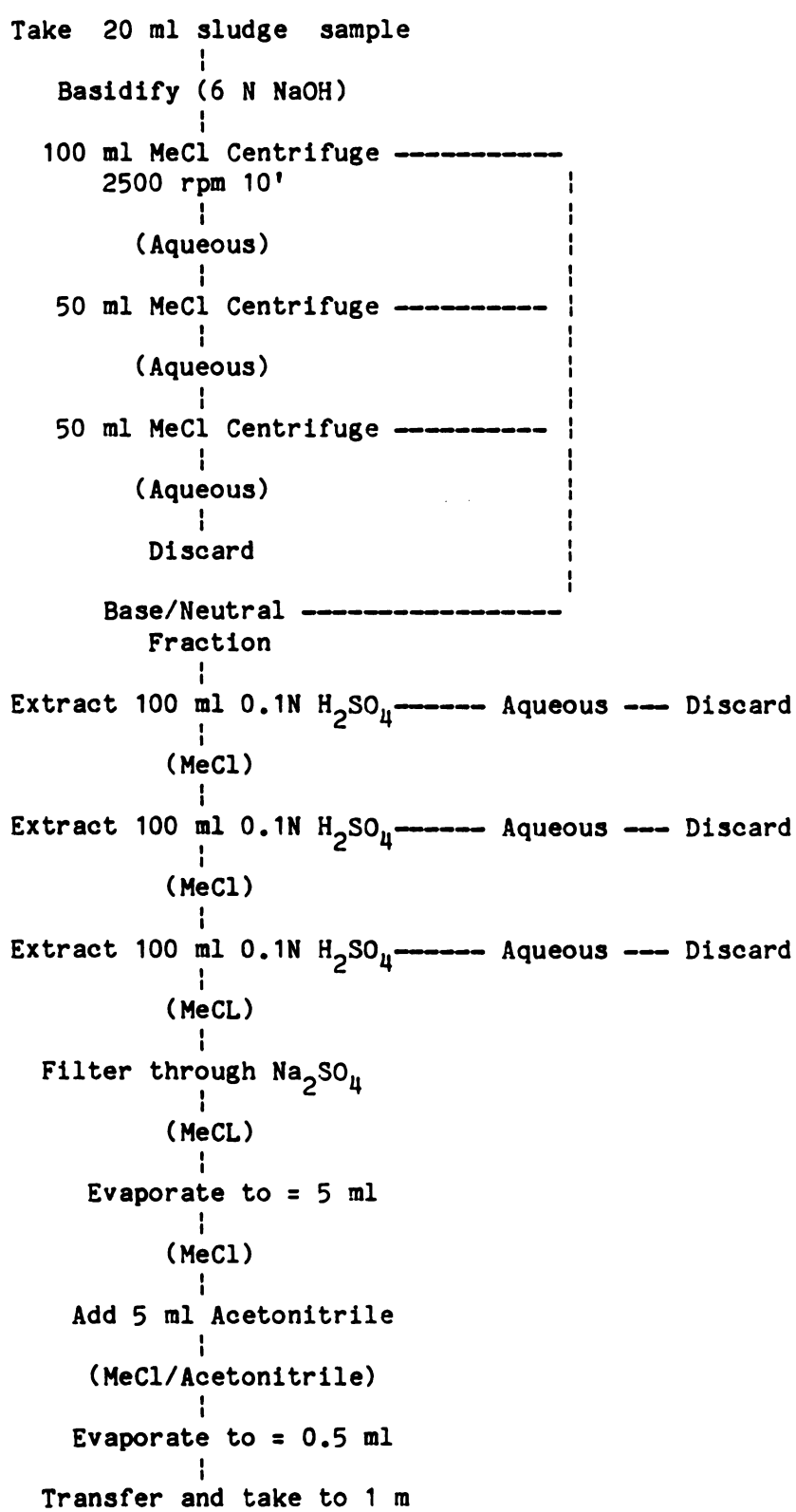

Figure 1. Sludge sample preparation scheme for analysis of aromatic compounds.

concentration. Very low recoveries for some compounds were obtained with this system. This system was modified so that the bath was used at room temperature with cold water but without success.

b) Kuderna-Danish

A Kuderna-Danish^{R} evaporative concentrator flask with a collection tube in the bottom was filled with sample, fitted to the top with a three-ball Snyder column and the tube heated in a steam bath within a hood. After the addition of acetonitrile, as described above, the tube was evaporated in a stream of dry nitrogen. Not much improvement was observed in the recovery by this method and therefore I tried another system.

c) Multi-heater

A flat-bottom boiling flask, used for collecting samples after filtration through sodium sulfate, was fitted with a three-ball Snyder column and heated over one of the hot plates of a multi-unit heater, having individual rheostat temperature controls. The column was insulated with glasswool and fixed in position with a clamp. When the DCM volume was well-reduced, the sample flask was removed, cooled, and then 10 ml of acetonitrile were added by the top of the column. The flask was returned to the heater and allowed to evaporate to near dryness. After cooling, the sample was transfered to a sample vial as described above.

2) Filtration

With the purpose of removing any residual water from the extracts, the samples were filtered through a sodium sulfate bed. To controll any possible lose of the analyte of interest, 200 ml of DCM

were fortified with 1 ml of the references compounds in a concentration equal to that determined for the ideal solution and submitted to the last two steps of the analysis. Percent recovery was calculated from the HPLC results by making appropiate comparisond with the external standard.

3) Partition into a 0.1 N sulfuric acid solution

After extraction with DCM the samples were partitioned in a dilute solution of sulfuric acid to remove any basic compound present in the extract. Again, to control the possible lose of any of the desired compounds, 200 ml of DCM fortified with 1 ml of the ideal solution were submitted to the entire procedure in triplicate. The recovery was calculated as described above.

b - Recovery Study with the Activated Secondary Sludge

1) Sampling and fortification

The use of the synthetic sludge, prepared as described above, allowed for the recovery study of the total analysis as well as the matrix effects in the extraction step of the analysis, considering that the effects of the other steps in the recovery have been individually considered with appropiate reference compounds. Seven of the original synthetic sludge samples were randomly selected for the recovery study. The samples were taken as described above under the sampling procedure after they had reached room temperature. Each sample was taken in triplicate, two of them were fortified with 1 ml of the solutions described below and allowed to equilibrate for one hour before analysis, the third sample was used as level 0. The

fortification solutions were prepared as follows:

a) Minimum Amount Detectable (MAD) solution. The minimum amount detectable for each compound was determined under chromatographic standardization, as the concentration which produced a peak height equal to three-times the signal/ noise (N/S) ratio. A solution with this concentration for each compound was prepared in acetonitrile and used to fortify the sludge samples. Fig. 2 shows the corresponding chromatogram.

b) Ideal Solution. The concentration of each compound in the ideal solution was selected in such a way that the height of the peaks produced were approximately 60% of the full scale deflection (FSD) (Fig. 2).

2) Extraction

As shown in Fig. 1, after addition of few drops of NaOH 6N, and 1 tablespoon of NaCl, each sludge sample was extracted successively with 100, 50, and 50 ml of DCM in a separatory funnel. Each time the extracts were collected in a centrifuge bottle and centrifuged at 500XG for 20 min to break the extremely heavy emulsion. After centrifugation, the DCM phase, which contained the compounds of interest, was the bottom layer. Removing it by a pipet or a hypodermic syringe was too tedious. It was found that a with little modification of a solvent dispenser the bottom phase could be easily and precisely transfered directly from the centrifuge bottle to a separatory funnel where partition in the diluted H_2SO_4 occurs (Figs 3, 4). After completion of the analysis the recoveries were calculated.

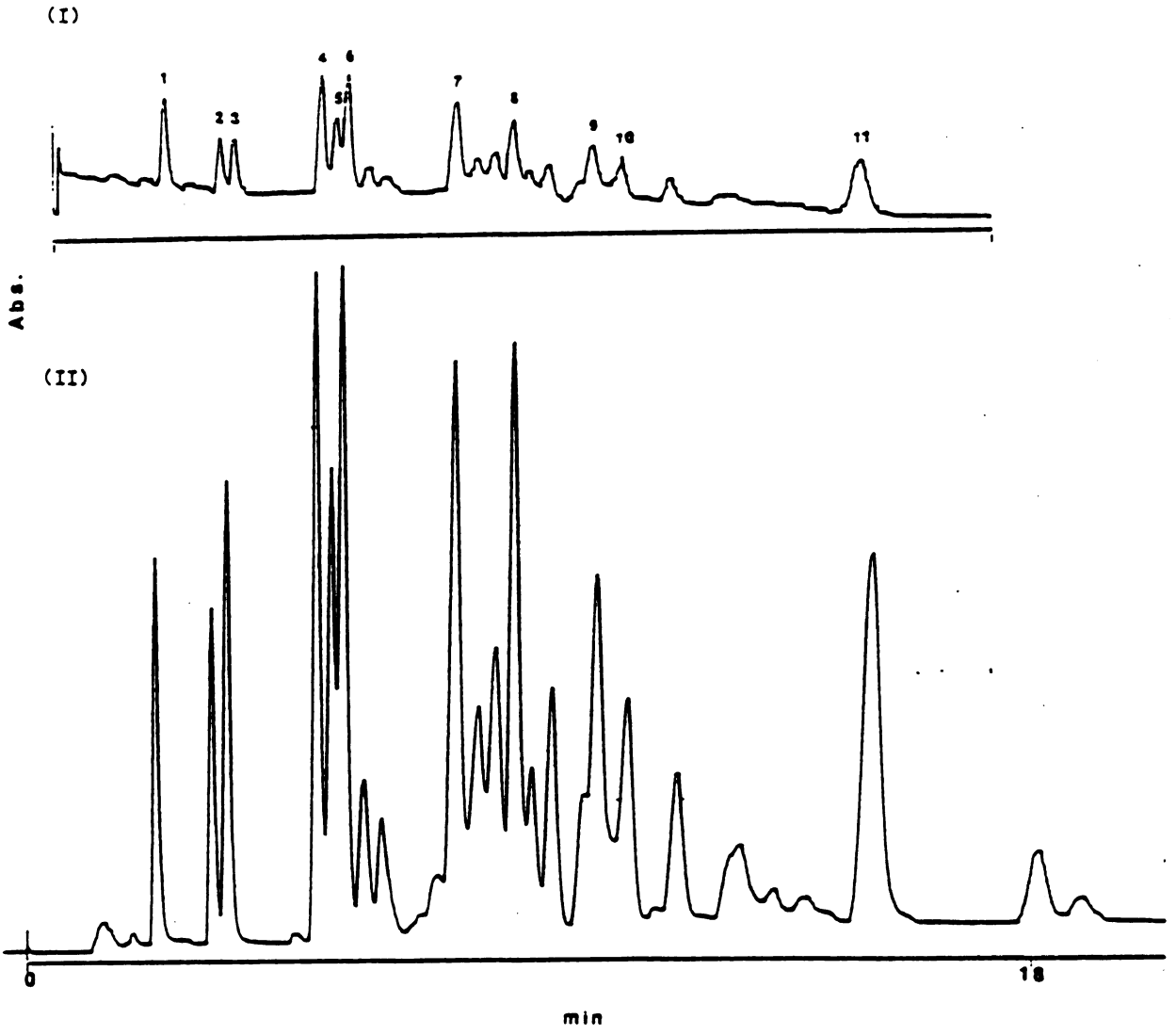

Figure 2. Chromatograms corresponding to the separation of 2-mercaptobenzothiazol (1), naphthalene (2), biphenyl (3), 1,2,3,4-tetrachlorobenzene (4), 1,2,4,5-tetrachlorobenzene (5), 1,2,3,5-tetrachlorobenzene (6), Aroclor 1254 (7,9,10), hexachlorobenzene (8), and Fire-Master (11) at two different concentrations: (I) The minimum amount detectable (MAD) defined as 3 times the noise level, (II) The ideal concentration defined as the concentration that produces an approximated scale deflection of 60 per cent.

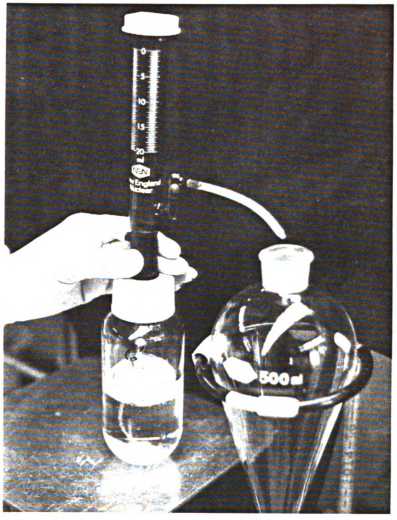

Figure 3. Commercial solvent dispenser and modified inlet tube (A) which replices normal inlet tube (B). Modification used to separate extract layers as shown in Figure 3.

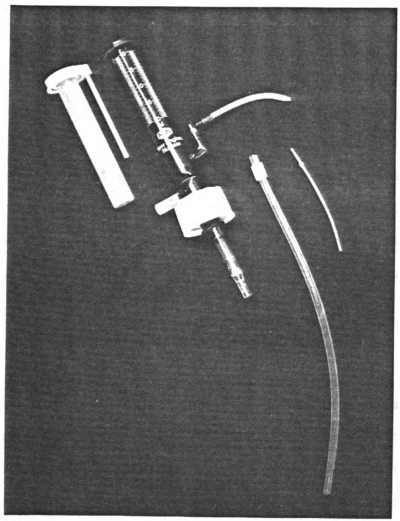

Figure 4.

Results and Discussion

1. Secondary activated sludge synthesis.

During the activated sludge synthesis period, information from Fig. 5 was used to control the concentration level of microorganisms in the reactor tank after the daily replacement of 9 liters of mixed liquor by water. As observed in this figure, the synthesis was kept between the theoretical maximum efficiency and the theoretical "Dead Curve". The abrupt change observed between day 7 and day 9 was due to the interruption of oxygen flow for 2 1/2 hours in the laboratory.

2. Control of the sampling procedure.

The sampling procedure for obtaining homogeneous sludge samples has been reported to be difficult (Philips, EPA). Considering that the activated secondary sludge has a liquid suspension condition, the use of a magnetic stirring plate was selected for agitation of the sludge during the sampling procedure. As a way of controlling the sample homogeneity the percent solids was determined for the intermediate subsample and the specific gravity calculated for the laboratory samples. The results are presented in Table 2. From the low variance of the data obtained, in both cases, I can say that agitation on a magnetic stirring plate is an appropriate way for collecting homogeneous activated secondary sludge samples.

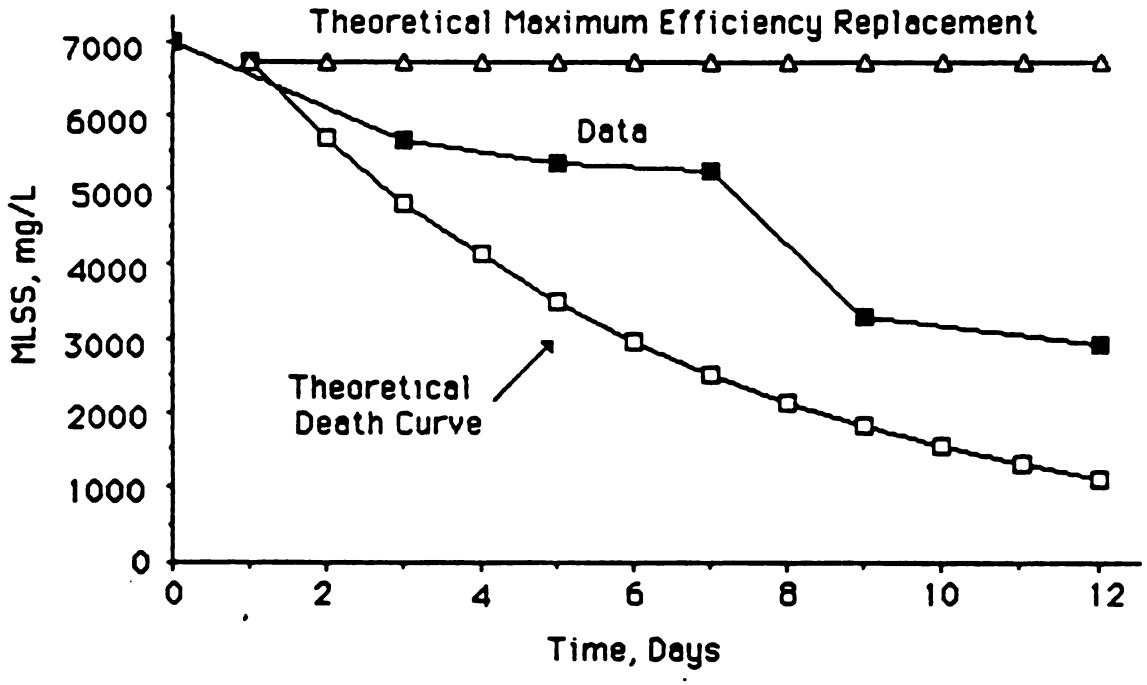

Figure 5. Synthetic Sludge Efficiency Control

Table 2. Statistical results of using agitation of sludge on a magnetic stirring plate during the sampling procedure.

Sampling step	Control method	Mean	Std. dev.	Variance
Interm. subsample	Percent solids	20.03 [1)]	0.11	0.01
Laboratory sample	Specific gravity	1.01 [2)]	0.01	0.0001

1) Mean of 12 samples.
2) Mean of 7 samples.

3. Recovery study.

In an attempt to improve the overall reliability, the recovery of every step in the procedure was evaluated. The general procedure covers sampling, extraction, partition into water, filtration and evaporation. It is important to recall at this point that no column cleanup procedure was used with the sludge samples. The results will be reported starting with the evaporation and finishing with the extraction considering that the last involves all the steps of the procedure.

a. Concentration of sample solutions

For injection in the liquid chromatograph the samples should be in the same solvent as the mobile phase besides this, the sample volume should be reduced to the point that the analyte concentration is sufficient for detection. For evaporation of DCM from the extracts, in the preliminary work with reference compounds a rotatory vacuum evaporator with a water bath at 40^0C was used. After observing systematic lose of biphenyl, naphthalene, and the isomers of tetrachlorobenzene, alternative methods of evaporation were tested.

The first attempt was a Kuderna-Danish evaporative concentrator flask fitted on top with the Snyder reflux column. After several columns blew up due to sudden changes in pressure of the steam bath, as well as considering the inconveniene of processing the extracts individually and not obtaining much improvement in recovery, it was decided to use the set-up described as evaporation with a multi-heater. A comparasion of percent recovery for biphenyl, naphthalene,

and 1,2,3,4-tetrachlorobenzene DCM solutions when evaporated using a rotatory vacuum evaporator and the system introduced here is presented in Tables 3. A general improvement in the recovery of approximately 100% was observed for biphenyl and naphthalene when using the method 2. The recovery of 1,2,3,4-tetrachlorobenzene also increased by 50% when using method 2. Table 4. is a summary of the recovery of the mixture of the standard compounds at the ideal concentration level when evaporated as described in materials and methods under multi-heater. With exception of 2-Mercaptobenzotiazol, the recovery obtained was in general good and the reproducibility (precision) in an acceptable range, as can be observed from the coefficient of variation (C.V.) range (2.89% - 26.46%).

b. Filtration

Control of the filtration step was obtained by analyzing in triplicate in the usual manner. Table 5 shows the results of the recovery study including a statistical analysis. The comparison of these data with those in Table 4 suggests that there is not appreciable lose of compounds during filtration; with the slight differences normal in experimental results. A lower range in the C.V. (10.16% - 19.22%) indicated more precision in the process.

c. Partition

Alkalized, fortified DCM solutions in triplicate, were partitioned three times in slightly acidified water (0.1 N H_2SO_4) and analyzed in a manner identical to the preceding work. The recovery and the usual statistical data are summarized in Table 6. It is important

Table 3. Percent recovery of reference compounds subjected to two different concentration procedures.

Replic.	Biphenyl		Naphthalene		1,2,3,4 tetrachl[3].	
#	Method 1[1]	Method 2[2]	Method 1	Method 2	Method 1	Method 2
1	52.88	100.00	32.81	89.60	56.63	98.83
2	46.91	91.00	35.85	87.65	68.33	90.99
3	43.73	97.09	38.99	81.16	62.21	88.26
Mean	47.84	96.03	35.89	86.14	62.39	92.69
St.Dev.	4.65	4.59	3.09	4.42	5.85	5.49

[1] Method 1 = rotavapor
[2] Method 2 = multi-heater
[3] tetrachlorobenzene

Table 4. Percent recovery, mean, standard deviation (St. Dev.) and coefficient of variation (C.V.) for the mixture of reference compounds when submitted to the multi-heater concentration method.

Compound	Replication 1	2	3	Mean	St. Dev.	C.V.
Mercaptobenzot.[1]	-[2]	-	-	-	-	-
Naphthalene	104.17	86.79	75.00	88.65	14.67	16.55
Biphenyl	121.07	80.94	84.95	95.63	22.07	23.08
1,2,3,4-tetrac.[3]	116.67	68.52	88.89	91.36	24.17	26.46
1,2,4,5-tetrac.	73.29	69.86	63.01	68.72	5.23	7.61
1,2,3,5-tetrac.	75.76	72.73	67.17	74.24	2.14	2.89
Araclor 1254	94.49	70.34	72.03	78.95	13.49	17.07
	82.61	73.91	73.91	78.26	6.15	7.86
Hexachlorobenz[4].	89.26	79.34	74.38	80.99	7.58	9.35
Fire-Master[R]	86.67	73.89	75.56	78.70	6.95	8.83

[1] Mercaptobenzotiazol.
[2] not present or concentration below detection limited.
[3] Tetrachlorobenzene.
[4] Hexachlorobenzene.

Table 5. Percent recovery, mean, standard deviation (St. Dev.) and coefficient of variation (C.V.) for reference compounds from filtration through a sodium sulfate bed.

Compound	Replication 1	2	3	Mean	St. Dev.	C.V.
Mercaptobenzotiazol	-[1]	-	-	-	-	-
Naphthalene	86.79	62.50	75.00	74.76	12.15	16.25
Biphenyl	98.03	80.94	84.95	87.97	8.94	10.16
1,2,3,4-tetrac.[2]	72.13	68.52	88.89	76.51	10.87	14.21
1,2,4,5-tetrac.	73.29	60.29	75.00	69.53	8.04	11.57
1,2,3,5-tetrac.	75.76	63.64	77.27	72.22	7.47	10.35
Araclor 1254	94.07	67.80	89.00	83.62	13.94	16.67
	94.37	64.79	87.32	82.16	15.45	18.80
Hexachlorobenzene	89.26	62.90	90.32	80.83	15.53	19.22
Fire-Master^{R}	86.67	67.82	96.55	83.68	14.60	17.44

[1] Not present or concentration below detection limit.
[2] Tetrachlorbenzene.

Table 6. Percent recovery, mean, standard deviation (St.Dev.) and coefficient of variation (C.V.) for reference compounds from partition in H_2SO_4 solution.

Compound	Replication 1	2	3	Mean	St. Dev.	C.V.
Mercaptobenzotiazol	2.31	3.7	7.41	4.47	2.64	59.06
Naphthalene	56.06	76.55	77.36	69.99	12.07	17.25
Biphenyl	24.59	64.97	65.00	51.52	23.32	45.26
1,2,3,4-tetrac.[1)]	60.0	72.73	76.36	69.70	8.59	12.32
1,2,4,5-tetrac.	55.63	69.01	73.24	65.96	9.19	13.93
1,2,3,5-tetrac.	53.55	66.35	72.99	64.30	9.88	15.37
Araclor 1254	49.00	68.27	71.49	62.92	12.16	19.33
	49.34	66.45	69.08	61.62	10.72	17.40
Hexachlorobenzene	39.55	48.47	51.81	46.61	6.34	13.60
Fire-Master^R	55.80	67.40	96.13	72.84	21.10	28.97

1) Tetrachlorobenzene.

to consider that the results of adding this step to the recovery study correspond to the total process recovery with standard compounds, equivalent to 100% extraction efficiency. With the exception of biphenyl and Fire-Master^{R} the C.V. were all between 10 to 20 %, indicating reasonable precision. However, comparison of recovery results with those in Table 5 indicates that there was some lose of certain compounds in the partition process. The most obvious were hexachlorobenzene and biphenyl with an approximate loses of 30% each. The lose of Araclor 1254 was also relatively high.

d. Fortified sample recovery

Up to this point, each experimental variable has been evaluated as a possible source of error. Sludge is known as one of the most complex materials to be analyzed, mainly due to matrix effects. Among the different sludge types, the secondary activated sludge has the added complexity of having a very high microbial activity. When selecting the sampling design, 7 random samples were selected among the 12 original samples, with all samples then analyzed for the study compounds at three levels of fortification (treatments) as described under materials and methods. The first level (zero), was used as a control. With this design the error component associated with the matrix effect should be smaller than when random samples are used for each treatment. There is a reduction in the degrees of freedom which is compensated for the decreased matrix effect.

Recovery was calculated in the usual way, comparing peak heights for individuals with external standards of the same concentration of the fortified solutions. The percent recoveries for the individual

Table 7. Concentration of the listed compounds found in the original sample when compared to the MAD[1] standard.

Compound	Sludge Sample Number 1	2	3	4	5	6	7
Mercatobenzotiazol	18.87	-[2]	4.65	12.10	-	43.1	7.98
Naphthalene	-	-	14.42	11.96	9.03	10.68	8.01
Biphenyl	-	-	7.61	7.61	-	93.08	20.00
1,2,3,4-tetrac.[3]	7.79	-	-	-	-	3.19	16.20
1,2,4,5-tetrac.	10.92	-	-	-	-	3.65	1.49
1,2,3,5-tetrac.	7.41	-	-	-	-	20.83	2.36
Araclor 1254	-	-	-	-	-	68.42	-
	-	-	-	-	-	-	-
	-	-	-	-	-	-	-
Hexachlorobenzene	-	-	-	-	-	-	-
Fire-Master®	90.77	9.09	51.20	53.85	100.7	86.39	78.79

1 Minimum Amount Detectable.
2 Not present or concentration below detection limit.
3 Tetrachlorobenzene.

compounds at the three different fortification levels were reported in tables 7, 8, and 9. Table 10 is a summary of the means, standard deviations and coefficients of variation of these recoveries. By simple observation high variability was found among samples. An analysis of variance was applied to the data with the purpose of finding if the observed differences in the treatment means were significant at the 0.05 confidence level. As is shown in table 11, all the treatment means were significant at the designed confident level.

The Dunnett test is used when pairwise comparisons consist of differences between one treatment condition (usually a control or baseline condition) and several others (usually experimental conditions) (7). Due to the fact that I used a zero level or baseline condition, I was interested in comparing this level with the other two fortification levels. Additionally, I wanted to evaluate the significance of the differences observed between these two groups. With these purposes in mind the Dunnett test was applied and the results obtained were summarized in table 12.

Considering the comparisons between the zero level and the fortification treatments, the results were unexpected for 1,2,3,5 - tetrachlorobenzene. This was due to the lose of this compound in the procedure at the minimum detection level. At the ideal concentration level, the results were not significant for biphenyl probably due to the high concentration of biphenyl found in one of the control sludge samples (table 7) which affect the mean of the group.

There is a problem regarding Fire-Master^{R}, when comparing the significance levels between the two treatments. The problem resulted from the fact that the water used during the analysis was contaminated

Table 8. Percent recovery of aromatic compounds from sludge samples spiked at a level equal to the Minimum Amount Detectable (MAD) for the listed compounds.

Compound	Sludge Sample Number						
	1	2	3	4	5	6	7
Mercaptobenzotiazol	22.58	24.19	25.81	22.58	22.58	29.03	29.03
Naphthalene	40.00	45.33	32.00	40.00	57.33	78.05	39.02
Biphenyl	68.18	77.27	36.36	22.73	68.18	350.00	116.67
1,2,3,4-tetrac.[1]	71.70	67.92	50.94	65.45	65.45	52.73	87.27
1,2,4,5-tetrac.	-[2]	-	-	24.24	-	-	39.39
1,2,3,5-tetrac.	119.23	67.31	38.46	44.64	89.29	16.07	35.71
Araclor 1254	270.27	264.86	94.59	125.64	228.21	112.82	89.74
	54.55	40.91	40.91	48.01	28.00	48.00	60.00
	33.33	40.00	80.00	276.47	41.18	111.76	58.82
Hexachlorobenzene	85.71	53.57	42.86	103.57	60.71	189.29	142.86
Fire-Master®	310.00	350.00	346.67	659.26	466.67	455.56	411.11

[1] Tetrachlorobenzene.
[2] Not present or concentration below detection limit.

Table 9. Percent recovery of aromatic compounds from sludge samples spiked at a level equal to ideal solution concentration.[1]

Compound	Sludge Sample Number						
	1	2	3	4	5	6	7
Mercaptobenzotiazol	22.01	-[2]	-	-	-	55.85	28.19
Naphthalene	67.02	40.13	-	69.33	46.40	46.41	38.81
Biphenyl	81.77	80.49	-	77.17	69.61	131.54	91.54
1,2,3,4-tetrac.[3]	62.77	47.66	48.13	55.56	47.67	64.22	68.06
1,2,4,5-tetrac.	50.40	25.34	32.09	44.82	35.15	41.38	44.64
1,2,3,5-tetrac.	52.27	29.52	36.19	44.53	39.78	42.95	42.92
Araclor 1254	43.84	61.90	55.95	59.50	63.64	99.12	140.79
	39.47	61.17	44.66	45.83	45.14	35.21	54.17
	32.61	61.97	56.34	53.00	48.00	40.43	61.76
Hexachlorobenzene	38.03	50.31	33.74	37.97	35.44	63.54	76.38
Fire-Master^{R}	96.30	101.40	71.29	83.00	71.60	108.84	211.11

[1] Concentration producing 60% full scale deflection
[2] Not present or concentration below detection limit.
[3] Tetrachlorobenzene

Table 10. Comparison of the mean percent recovery, standard deviation (S.D.), and Coefficient of Variation (C.V.) of sludge samples, spiked at three different concentration levels.[1]

Compound	Spike level								
	0			MAD			Ideal Std.		
	Mean	S.D.	C.V.	Mean	S.D.	C.V.	Mean	S.D.	C.V.
Mercaptobenzotiazol	17.34	15.35	88.52	25.11	2.92	11.63	15.15	21.57	142.38
Naphthalene	10.82	2.52	23.29	51.26	14.89	29.05	44.01	22.95	52.15
Biphenyl	32.07	41.10	128.16	126.08	114.76	91.02	76.02	39.15	51.50
1,2,3,4-tetrac.	9.06	6.60	72.85	65.92	12.19	18.49	56.30	8.75	15.54
1,2,4,5-tetrac.	5.35	4.94	92.34	9.09	16.13	33.66	39.12	8.66	22.14
1,2,3,5-tetrac.	10.20	9.55	93.63	58.67	35.65	60.76	41.17	7.11	17.27
Araclor 1254	-	-	-	169.45	81.45	48.07	74.96	33.63	44.86
	-	-	-	96.94	5.31	5.48	47.17	16.40	34.77
	-	-	-	45.77	19.16	41.86	46.52	8.72	18.74
Hexachlorobenzene	-	-	-	91.65	86.02	93.86	50.59	11.00	21.74
Fire-Master®	57.26	31.60	46.98	428.47	112.76	26.32	106.22	48.46	45.62

[1] level 0 = not fortified, MAD = fortification standard concentration at the minimum amount detectable, Ideal Std.= fortification standard concentration producing 60% full scale deflection.

Table 11. F-test values and significance levels (critical table values: 3.89 for p=0.05, 6.93 for p=0.01, and 13.0 for p=0.001) for the analysis of variance of the recovery in sludge samples of the listed compounds.

Compound	Value	P<
Naphthalene	9.18	.01
Biphenyl	7.46	.01
1,2,3,4-tetrac.[1)]	209.47	.001
1,2,4,5-tetrac.	28.44	.001
1,2,3,5-tetrac.	11.80	.01
Alaclor 1254	13.02	.001
	80.15	.001
	5.94	.05
Hexachlorobenzene	19.69	.001
Fire-Master^{R}	42.53	.001

1) Tetrachlorobenzene.

Table 12. Significant levels after comparing the fortification levels using the Dunnett test (critical values: 2.5 for p=0.05, 3.39 for p=0.01).

Compound	Significant levels		
	Zero[1] vs MAD	Zero vs Ideal	MAD vs Ideal[1]
Naphthalene	.01	.05	n.s.[2]
Biphenyl	.01	n.s.	n.s.
1,2,3,4-tetrac.	.01	.01	.05
1,2,4,5-tetrac.	n.s.	.01	.01
1,2,3,5-tetrac.	.01	.05	n.s.
Alaclor 1254	.01	n.s.	.05
	.01	.01	n.s.
	.01	n.s.	n.s.
Hexachlorobenzene	.01	.05	.05
Fire-Master[R]	.01	n.s.	.01

[1] Zero = not fortified samples, MAD = fortification standard concentration equal to minimum amount detectable, ideal concentration = fortification standard concentration, 60% of full scale deflection.

[2] Not significant.

with phathalates. These compounds can be considered as a confounding variable with Fire-Master^{R} because one of them has the same retention time as the main peak of Fire-Master^{R} making the recovery of this compound until 6 times bigger than expected. Similar effect was found for 1,2,3,5 tetrachlorobenzene due to the lose of the compound at the low level, as mention before.

The values observed for 2-mercaptobenzothiazol could be due to contamination with another compound with same retention time and UV response or contamination with the same compound which made the total concentration higher that the ideal concentration. At this level, with the evaporation process used, I could not detect that compound.

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PART II

CHAPTER IV

AROMATIC COMPOUNDS IN SLUDGE SAMPLES FROM MICHIGAN WASTE WATER PLANT FACILITIES

INTRODUCTION

Sludge is the liquid, semiliquid or solid residue obtained from the processing of waste water. Waste water flows consist of four major sources: domestic and industrial input, frequently considered together, infiltration/inflow input and storm water input. Besides nutrients, these waters can bring potentially toxic heavy metals and trace elements, bacteria, fungi and microbes, and toxic organic compounds all of them contributing to potential sludge contamination.

One of the goals of the Federal Water Pollution Control Act of 1972 (PL 92-500), was to obtain zero contaminants by 1985 in waste water going to rivers or oceans. As a result, the number of waste water treatment plant facilities has increased as well as the amount of sludge, so much in fact that the disposal of sludge has become a problem.

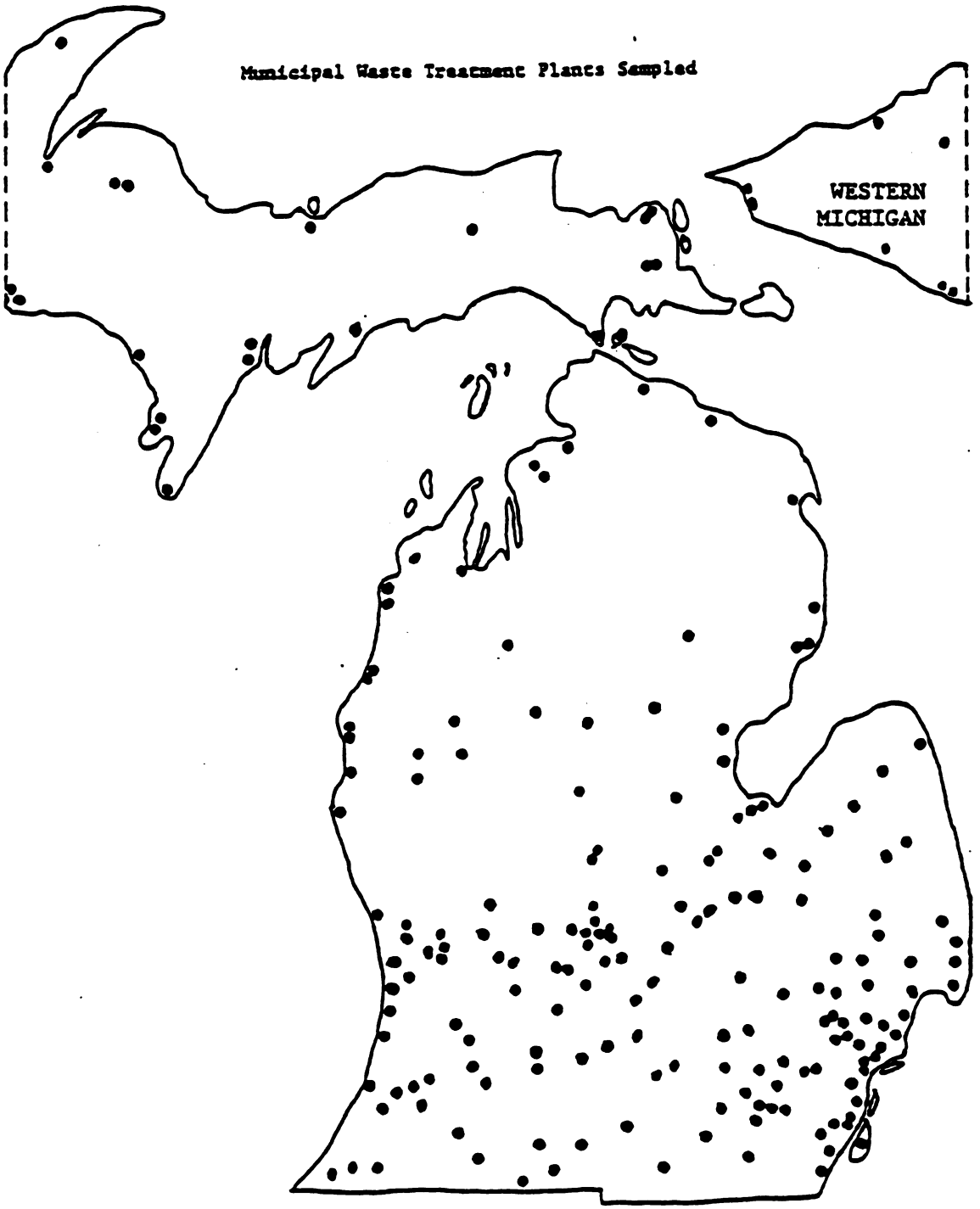
Among the sludge disposal methods, land application is receiving considerable amounts. This is being done as a means of disposal, as well as a means of reclaiming marginal land and as a means of utilizing the nutrient content in sludge (1). But with the incresed use of sludge in land application the potential for distributing toxic or hazardous substances into the environment which could result in the uptake of chemical into the food chain is now been considered. As a consequence, federal, state and local agencies are requiring a closer look at the usage and disposal methods for toxic and hazardous compounds. Under Michigan's Act 64, the "Hazardous Waste Management Act", sludge that contain hazardous organic materials present at concentrations from 1 to 1000 ppm are designated "hazardous waste". This designation tags the

sludge as a potential environmental pollutant and requires that it be disposed of properly and monitored continually by the waste-water treatment facility affected. Ultimately, the Michigan Department of Natural Resources (DNR) must enforce and regulate the management of such wastes (2).

In order to help evaluate whether some sewage sludge in Michigan might be classified as hazardous, the Michigan DNR and the United States Environmental Protection Agency (EPA) contracted with researchers at Michigan State University. The sludge samples arrived from throughout the state (Fig. 1) and were analyzed for organic and inorganic toxic substances or potential xenobiotics. The organic compounds were classified into the following groups: phenols, purgeables, aromatic hydrocarbons, bases phthalates, nitrobenzenes, and triaryl phosphate esters (3).

In this study, the group corresponding to the aromatic hydrocarbons (Appendix) were analyzed. With the results, the correlation between simultaneous presence of the compounds has been calculated and will be considered. The influence, if any, of the percent of industrial input, the facility flow and the percent of solids present in the samples has been calculated and will be compared with some bar graphs drawn with the same data.

Figure 1


Municipal Waste Treatment Plants Sampled
WESTERN
MICHIGAN

Materials and Methods

At the onset of the project (3) of which this is the part corresponding to the aromatic hydrocarbons group, no analytical techniques for analysis of organics in sludge were available expect "Interim Method" proposed by the Environmental Monitoring and Support Laboratory in Cincinnati, Ohio and these methods had not been well tested on large numbers and types of samples. Several techniques were tried and tested in order to determine the most cost-effective, accurate and reproducible analysis scheme possible. Municipal sludge contains 15-20 percent solvent extractable materials, all of which contribute to interferences upon analysis. Therefore, an extensive extraction and cleanup scheme was necessary in order to separate the interfering components from those which were to be analyzed.

1. Overall scheme

The following pages present a flow diagram for the general analysis scheme used to separate, characterize and quantify all organic components of interest (Figs. 2 and 3).

All sample containers and teflon seals were detergent washed, rinsed three times each with tap water, distilled water, acetone and finally hexane. The glassware was allowed to dry in a 105^{o}C oven for one hour and cooled in an area known to be free of organics. Caution was taken not to heat teflon seals for more than one hour at 105^{o}C preventing degradation of the silicone layer.

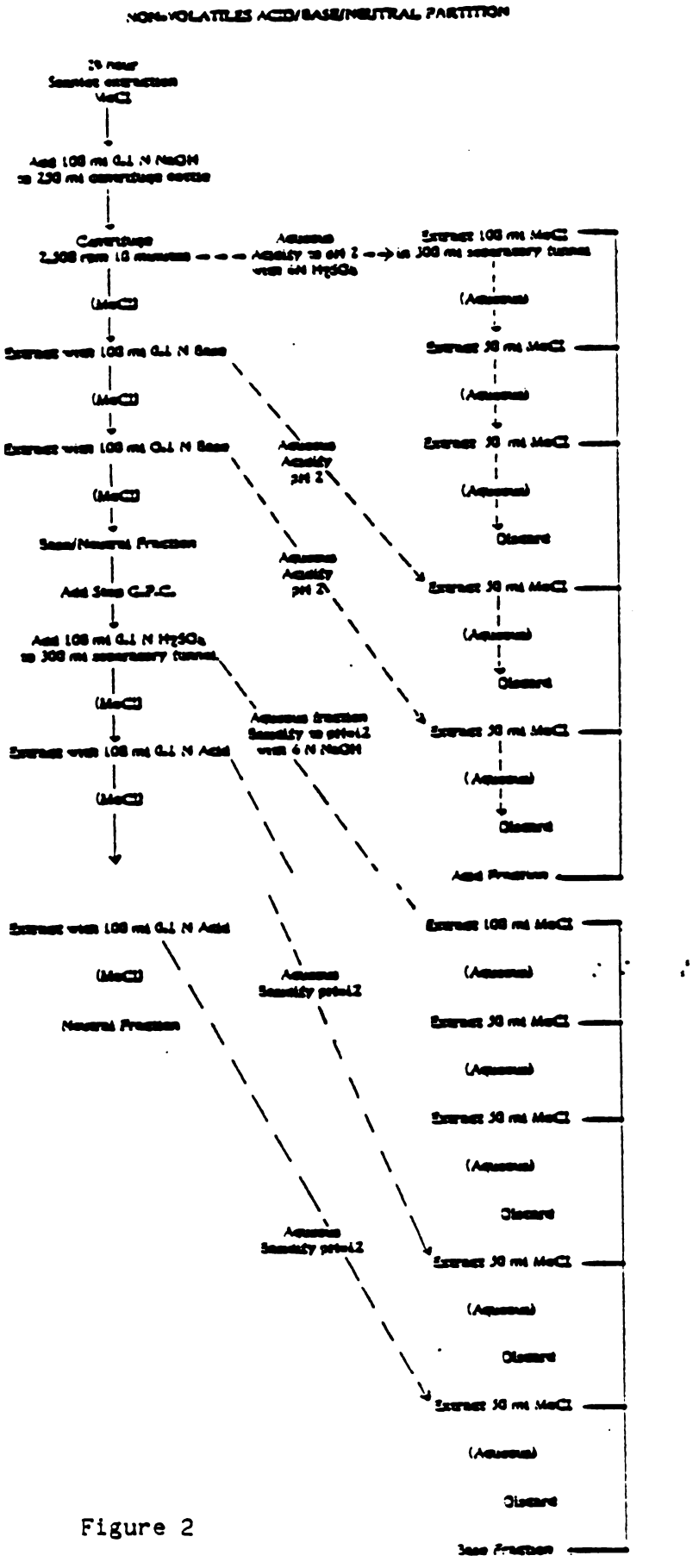

NON-VOLATILES ACID/BASE/NEUTRAL PARTITION
Add 100 ml 0.1 N NaOH
Centrifuge
Extract with 100 ml 0.1 N Base
Extract with 100 ml 0.1 N Base
Base/Neutral Fraction
Add Base G.P.C.
Add 100 ml 0.1 N H2SO4
Extract with 100 ml 0.1 N Acid
Extract with 100 ml 0.1 N Acid
Neutral Fraction
Aqueous Acidify pH 2
Aqueous Acidify pH 2
Aqueous fraction Basify to pH=12 with 6 N NaOH
Aqueous Basify pH=12
Aqueous Basify pH=12
Extract 100 ml MeCl2
Extract 50 ml MeCl2
Extract 50 ml MeCl2
Discard
Extract 50 ml MeCl2
Discard
Extract 50 ml MeCl2
Discard
Acid Fraction
Extract 100 ml MeCl2
Extract 50 ml MeCl2
Extract 50 ml MeCl2
Discard
Extract 50 ml MeCl2
Discard
Extract 50 ml MeCl2
Discard
Base Fraction

Figure 2

LIQUID SAMPLES PHENOL ACID/BASE/NEUTRAL PARTITION

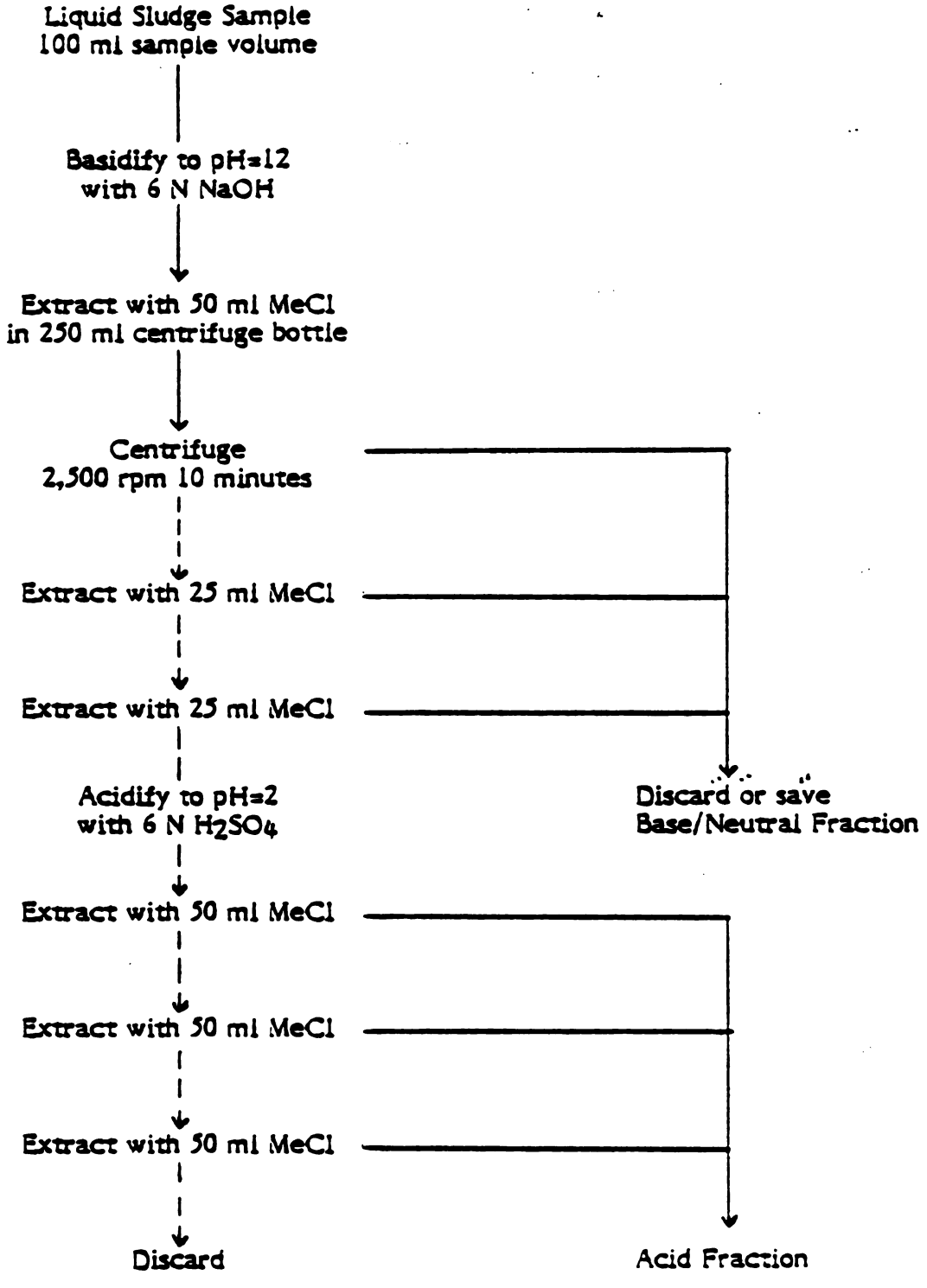


Figure 3

2. Sampling procedure

Non-volatile samples were collected in 2,000 ml wide mouth, amber screw cap jars. Foil lined caps were used for each sample. After labeling each sample bottle with pencil or indelible ink they were refrigerated at $4^{o}C$ and transported to the laboratory. Sample preservation was strongly recommended, however, this was rejected by the Michigan Department of Natural Resources who were responsible for sample collection, sample site, name of individual which collected the sample, as well as any other pertinent information.

For data handling, manipulation and storage, a program was written for a Digital PDP-11 computer. This program had the following purposes:

a. To store information:
    1) All sample sites
    2) All cities, counties or townships
    3) The popolation served by each treatment facility
    4) The percent industrial input into each treatment system
    5) All municipal sewage plant treatment parameters
    6) All solids parameters
    7) All organic compound levels
b. To perform various mathematical and statistical functions on the data.
c. To list specific information requested from all sites in a variety of formats.

3. Extraction and cleanup

With the exception of volatiles, semi-volatiles and phenols in liquid samples a continuous soxhlet extraction was employed. All non-volatile samples were routinely dried at 50°C and then placed in cellulous thimbles for 24-hour soxhlet extraction process with 200 ml of methylene chloride. The methylene chloride sludge extract was then put through an extensive acid/base/neutral extraction process including a gel permeation chromatography step for the combined base/ neutral group (Fig. 3).

The neutral fraction was roto-evaporated down to 1 ml prior to cleanup via an activated florisil column. Ten grams of florisil activated overnight at 550°C was dry packed in a 10 ml diameter column plugged with oven burned silnized glass wool. A vibrator was used to achieve a consistent homogeneous packing. After topping of the florisil with approximately 4 cm of anhydrous sodium sulfate, the column was pre-rinsed with 100 mls of glass distilled hexane and kept wetted while the sample was applied on the top of the column. At this point several solvent solutions ranging from nonpolar to polar were used to elute the column; certain fractions retained and others discarded as described in figure 4.

The first fraction contains nonchlorinated aromatic such as biphenyl and naphthalene as well as a mixture of chlorinated hydrocarbons. Since certain chlorinated pesticides (DDE, DDT) and chlorinated aromatic hydrocarbons (PCBs) co-chromatogram, it was necessary to separate them prior to analysis by gas chromatography. A method has been described for the separation of polychlorinated biphenyls from DDT metabolites (Armour, 1970). This method uses silica gel deactivated with 3% H_2O by weight and separates the DDT metabolites from PCBs via

Elutriate	Fraction	Contents
50 ml Hexane	1	Nonchlorinated aromatics
50 ml Hexane	1	Chlorinated hydrocarbons
50 ml 6% E.E. in Hexane	1	Chlorinated pesticides
50 ml 6% E.E. in Hexane	waste	
50 ml 15% E.E. in Hexane	2	Triaryl phosphate esters
50 ml 15% E.E. in Hexane	2	Triaryl phosphate esters
50 ml 50% E.E. in Hexane	2	Triaryl phosphate esters
50 ml 50% E.E. in Hexane	waste	
50 ml 10% MeCl in Hexane	waste	
50 ml 10% MeCl in Hexane	waste	
50 ml 10% Acetone in MeCl	3	Phathalates
50 ml 10% Acetone in MeCl	3	Phathalates
50 ml 50% Acetonitrile in Methanol	3	Phathalates
50 ml of Acetonitrile	waste	

Figure 4. Solvent elution system used with the florisil column cleanup and type of compounds collected in each fraction.

to elution mixtures as shown in figure 5.

4. Chromatographic analysis

After combining fractions A and B with the Phathalates (fraction 3) the volume was reduced to 1 ml. the eight compounds of interest were determined simultaneously with other compounds in four different chromatographic system which allowed maximum sensitivities (Fig. 6).

Elutriate	Fraction	Contents
250 mls n-hexane	A	PCB's and similar compounds
220 mls Acetone:Hexane:Methylene chloride 1:19:80	waste	DDT metabolites
250 mls Acetonitrile:Methylene Chloride 20:80	B	Nitrobenzenes and similar compounds

Figure 5. Solvent system used in the separation of non-volatiles compounds by a silica gel column.

Flame Ionization Detector

Capillary SE-30, 15 meters

Column Flow = 1.5 ml/min H_2

Makeup = 28 ml/min H_2

Air = 300 ml/min

Oven temperature:

Initial = 200°C for 3 min.

Program rate = 10 per min.

Final = 240°C for 15 min.

Compounds of interest in order of retention time:

Naphthalene

Biphenyl

Dimethylphathalate

Diethylphthalate

Dibuthylphthalate

Buthylbenzylphthalate

Dioctylphthalate

Di-n-octylphthalate

Electron Capture Detector

Capillary SE-30, 15 meters

Column Flow = 1.5 ml/min H_2

Makeup = 28 ml/min N_2

Injector = 220°C

Detector = 280°C

Oven temperature = 200°C

Compounds of interest in order of retention time:

1-Chloro-4-nitrobenzene

1-Chloro-2-nitrobenzene

1,2,4,5-tetrachlorobenzene

1,2,3,5-tetrachlorobenzene

1,2,3,4-tetrachlorobenzene

1-chloro-2,6-dinitrobenzene

1-chloro-3,4-dinitrobenzene

1-chloro-2,4-dinitrobenzene

Hexachlorobezene

Pentachloronitrobenzene

Polychlorinated biphenyls

N-P Flame Ionization Detector	Flame Photometric Detector
	Sulfur Mode
Capillary SE-30, 15 meters	3% OV-101 on Qf-1, 60-80 mesh
Hydrogen purge	Hydrogen = 28 ml/min.
Hydrogen = 28 ml/min.	Air = 280 ml/min.
Column Flow = He at 28 ml/min.	Column Flow = He at 30 ml/min.
Oven = 180°C	Oven = 250°C
Injector = 220°C	Injector = 260°C
Compounds of interest in order of retention time:	Compounds of interest in order of retention time:
Nitrobenzene	**3-Mercaptobenzothiazol**
1-Chloro-4-nitrobenzene	
1-Chloro-2-nitrobenzene	
2,6-dinitrotoluene	
2,4-dinitrotoluene	
1-Chloro-2,6-dinitrobenzene	
1-Chloro-3,4-dinitrobenzene	
1-Chloro-2,4-dinitrobenzene	
Pentachloronitrobenzene	
3-mercaptobenzothiazole	

Figure 6. Description of the four chromatographic systems used to determine the neutral organic compounds in sludge samples (The compounds corresponding to the aromatic hydrocarbon group are in bold letters).

Results and Discussion.

The results obtained from the sample analyses for the various compounds of interest are shown in the appendix. (The footnote explains the notation used in the listing).

Of the eight compounds analyzed for only four gave concentrations above the detection limit. These compounds were naphthalene, biphenyl, hexachlorobenzene, and among the PCB's, only Arachlor 1248. From now on, Araclor 1248 will be called PCB (1248).

As a general exploration of the data, certain values were calculated for compounds with concentrations above the detection limit. The results of calculations are summarized in tables 1 and 2.

Table 1 shows the percent of values below the detection limit, the central tendency of the data (mean and median) and the range limits as measure of dispertion. It is observed that: (a) a very high proportion of zeros are present (see footnote Table 2); (b) the central tendency values differ in one or two orders of magnitude; and (c) the dispersion of data is very wide, from two to three orders of magnitude. In such cases, where data do not follow the normal distribution or a known distribution, a non-parametric statistic can be used to examine the data (4).

It was observed that in many cases two or more compounds were present in the same site (Table 2). The degree of association between pairs of compounds was tested by rank correlation.

Table 1. Summary* of sludge samples containing measurable residues in mg/kg.

Compound name	% values below detection limit	Mean	Median	Range High	Range Low
Naphthalene	49.58	1.54E 02	4.25E 01	6.61E 03	0.00E 00
Biphenyl	66.95	2.96E 01	0.00E 00	1.73E 03	0.00E 00
Hexachlorob.[1)]	56.96	2.01E 02	0.00E 00	2.62E 04	0.00E 00
PCB(1248)	89.74	8.23E 00	0.00E 00	6.20E 02	0.00E 00

* Each concentration obtained below detection limit was considered zero (0) for the calculations.

Table 2. Number of sites containing detectable residue combinations (0-4) of Naphthalene (N), Biphenyl (B), Hexachlorobenzene (H),and PCB(1248) (P).

# of compounds present	Combinations						Total sites
none							64
one(1)	N	B	H	P			
	34	11	28	2			75
two(2)	NB	NH	NP	BH	BP	HP	
	18	23	2	14	0	2	59
three(3)	NBH	NBP	NHP	BHP			
	21	7	6	1			35
four(4)	NBHP						
	4						4

The influence that various characteristics of wastewater treatment facilities have on the results was examined using Levy's test.The concentration of samples will be grouped according to ranges for the different variables with the purpose of applying Levy's test which will be explain in detail. Bar graphs will be drawn with the mean and median values for each group in the y axis and the range number in the x axis.

1. Rank Correlation.

The degree of association among two or more variables with data that are not distrbuted as bivariate normal can be determined by the rank correlation (4). The coefficient of rank correlation or Sperman's coefficient may be computed from

$$(\text{rho})\ \rho = 1 - \frac{6\,\Sigma d^2}{n(n^2 - 1)} \qquad [1]$$

where:

n = sample size

d^2= squares of the difference in the ranks.

The results summarized in table 3 show that every compound has a level of association with the others, but in some pairs it is very slight (e.g., Hexachlorobenzene and PCB). Naphthalene and

Table 3. Spearman Correlation Coefficients of residue concentrations including non detectable values for selected pairs of compounds*.

Compound Pair	ρ [rho]	Num.of sites N	Significance
Naph. and Biphen.[1)]	.31	235	.001
Naph. and Hexach.	.14	236	.015
Naph. and PCB(1248)	.25	233	.001
Biph. and Hexach.	.15	236	.010
Biph. and PCB(1248)	.14	233	.014
Hexa. and PCB(1248)	.11	234	.044

*The Spearman Correlation Coefficients were also calculated among all sites when values below the detection limit were excluded. The rho values thus obtained were much lower but had the same tendency i.e., the highest association was found between Naphthalene and Biphenyl followed by Naphthalene and PCB(1248).

1) Naph. = Naphthalene, Biphen. = Biphenyl, Hexach. = Hexachlorobenzene.

biphenyl have the highest degree of association (ρ = .31, significance .001). Naphthalene and PCB (1248) have a degree of association of ρ=.25

The relationship between naphthalene and biphenyl is logical considering that they often have a common source. The same is true for naphthalene and PCB (1248).

2. Levy's Test and Bar Graphs.

Levy's test (5) may be applied when one has data from experiments in which some of the expected results are not observed, when the data have excessive number of zeros or when the data have severe skewness. The results obtained in this analysis fall in the last two categories (Table 1.).

In the test procedure, the data are ordered from the smallest to the highest (ignoring groups) and the "grand median" (m_n) is obtained. This value is compared with the data in the groups.

The number of observations in each group exceeding m_n is determined and is known as a_i. Based on the hypothesis, in this specific case, that if a variable is influencing the concentration obtained, the number of values over the grand median in each group must be different, in other words, $a_i - a_{i'}$ when i=i' is the test statistic. To find if the difference between two groups is significant, this is compared with a critical value (C.V.) calculated with the next formula:

$$C.V. = (q_{\alpha,t,\infty}) [rh(n-h)/n(n-1)]^{1/2} \qquad [2]$$

where:

t = number of groups

r = observations by group

n = (t)(r)

h = n/2 or (n-1)/2

$q_{\alpha,t,\infty}$ = is the percentage points of studentized range tables (5).

Two groups are significantly different if the test statistic exceeds the appropiate critical value.

The variables that are hypothesized to influence the concentration of those aromatic compounds that have values over the detection limit are (a) percent industrial input, (b) size of treatment facility, and (c) percent of solids in the sample.

The definition and values used for percent industrial input are the same as those established by Phillips (7). The numbers represent the total volume of waste containing critical materials divided by the flow of the treatment facility. I belief that this could be one of the major origins of the contaminant products and the validity of this is what should be observed with the statistical test.

The size of the treatment facility can be defined by the flow in millions of gallons per day (M.G.D.). The question can be asked, "Will larger plants create more contaminated sludge than smaller plants?. What should be tested, when the samples are grouped according to flow classes, is the influence of clean effluent flow

rate in the levels of contaminant found in the sludge. This test would be made because the contaminants in question have been removed from the effluent and should be present in the sludge.

The last variable that should be correlated with the results is the percent of solids in the samples. This value was obtained for every sample and has been used for the final calculation of aromatic compound concentrations. It is one of the variables that is expected to have a very high influence on the results, because the compounds may be expected to bind to solid materials.

To prove the validity of the hypothesis, Levy's test is applied to the data after grouping according to the increased value of the different variables for each compound.

a. Effect of percent indusrial input on aromatic compounds levels.

The data have been organized in order of percent industrial input from smallest to largest and then divided into 4 groups with concentrations of individual compounds for 58 sites. In all, 7 sites were not considered, 5 because values were not available and 2 in order to mantain equal sample sizes among the four groups (Table 5).

Common Computations:

t= 4

r= 58

n=232

h=116

Table 4. Selected range values of Percent Industrial Input used to create groups used inthis study.

Group	Range Percent Ind. Input
1	0
2	0 - 0.35
3	0.36 - 12.99
4	13 -

Table 5. Critical values (C.V.) for different levels of probability (α). The 3.816 value for sample concentrations, grouped by ranges of percent Industrial input.

α	$q_{\alpha,4,\infty}$*	C.V.=3.816$q_{\alpha,4,\infty}$
0.2	2.784	10.624
0.1	3.240	12.364
0.05	3.633	13.863
0.01	4.403	16.802

*Percent points of studentized ranges tables.

Table 6. Grand median (m_n) without considering groups; mean and median for each group of residue concentrations when the samples were grouped by percent of Industrial Input and a_i^*. Data were caculated for Levy's test and Bar graphs.

	RANGE											
	1			2			3			4		
	Mean	Median	a_i	Mean	median	a_i	Mean	Median	a_i	Mean	Median	a_i
Naphthal. m_n=0.966	143.	2.11	32	83.82	0	25	145.	4.54	34	257.	0	25
Biphenyl m_n=0	11.5	0	15	23.90	0	18	25.1	0	19	60	0	26
Hexachlo. m_n=0	71.5	0.119	29	548.29	0	23	31.6	0	24	169	0	25
PCB(1248) m_n=0	12.9	0	8	9.54	0	6	7.5	0	7	3.8	0	7

*Number of observations in each group exceeding grand median.

Table 7. Results for Levy's statistical test $|a_i - a_{i'}|$ [1] for i=i'. Sample concentrations grouped by to percent Industrial Input.

i':	2	$\underset{3}{\underset{1}{\|a_1-a_{i'}\|}}_4$		$\underset{3}{\underset{2}{\|a_2-a_{i'}\|}}_4$		$\underset{4}{\underset{3}{\|a_3-a_{i'}\|}}$
Naphthalene	7	2	7	9	0	9
Biphenyl	3	4	11*	8	8	7
Hexachlorob.	6	5	4	2	1	1
PCB(1248)	2	1	1	1	1	0

1) a_i or $a_{i'}$ is the number of observations in each group exceeding grand median.

* $P<0.2$

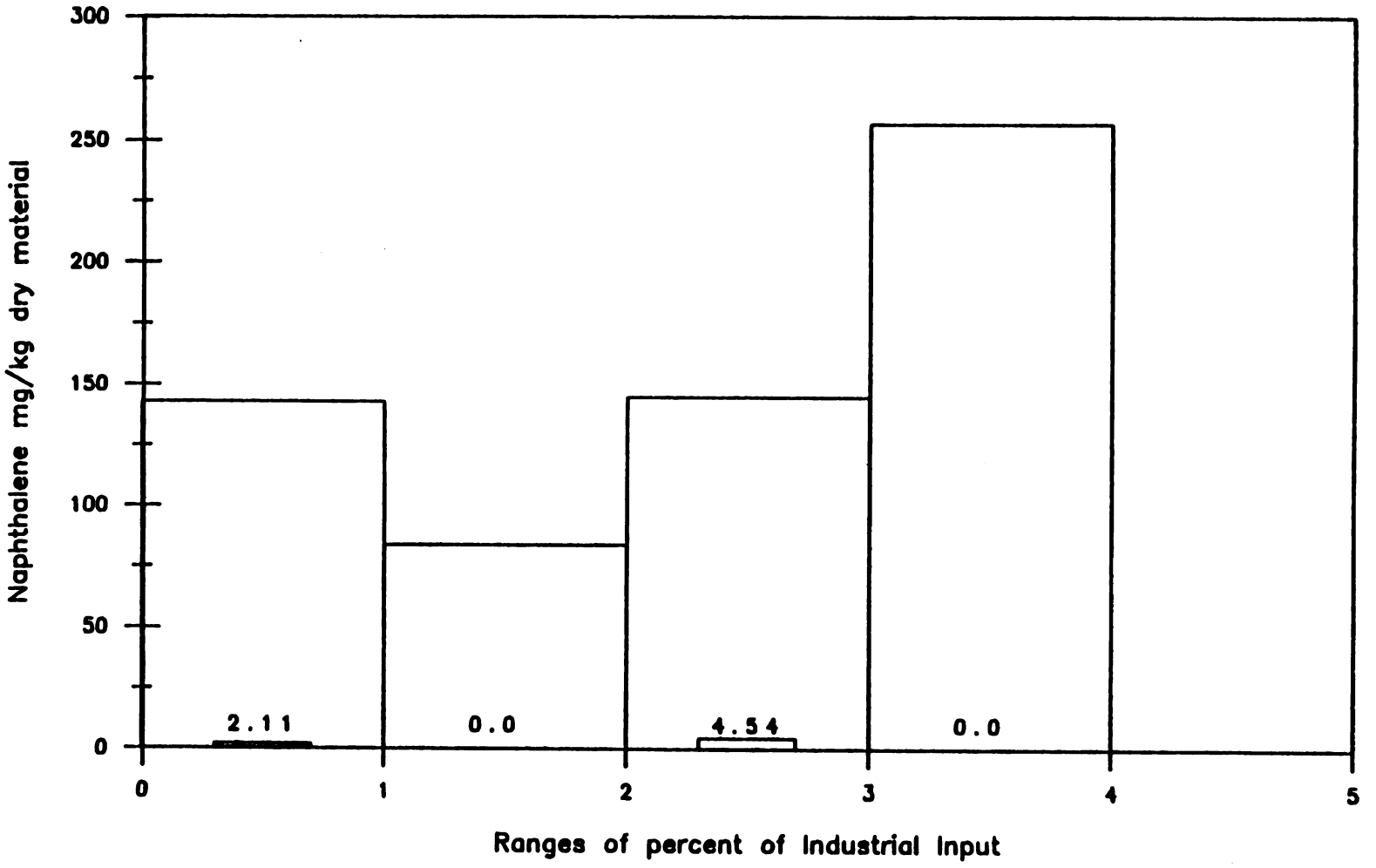


Figure 7. Means (big bar top line) and medians (small bar) of Naphthalene concentrations for different ranges of industrial inputs.

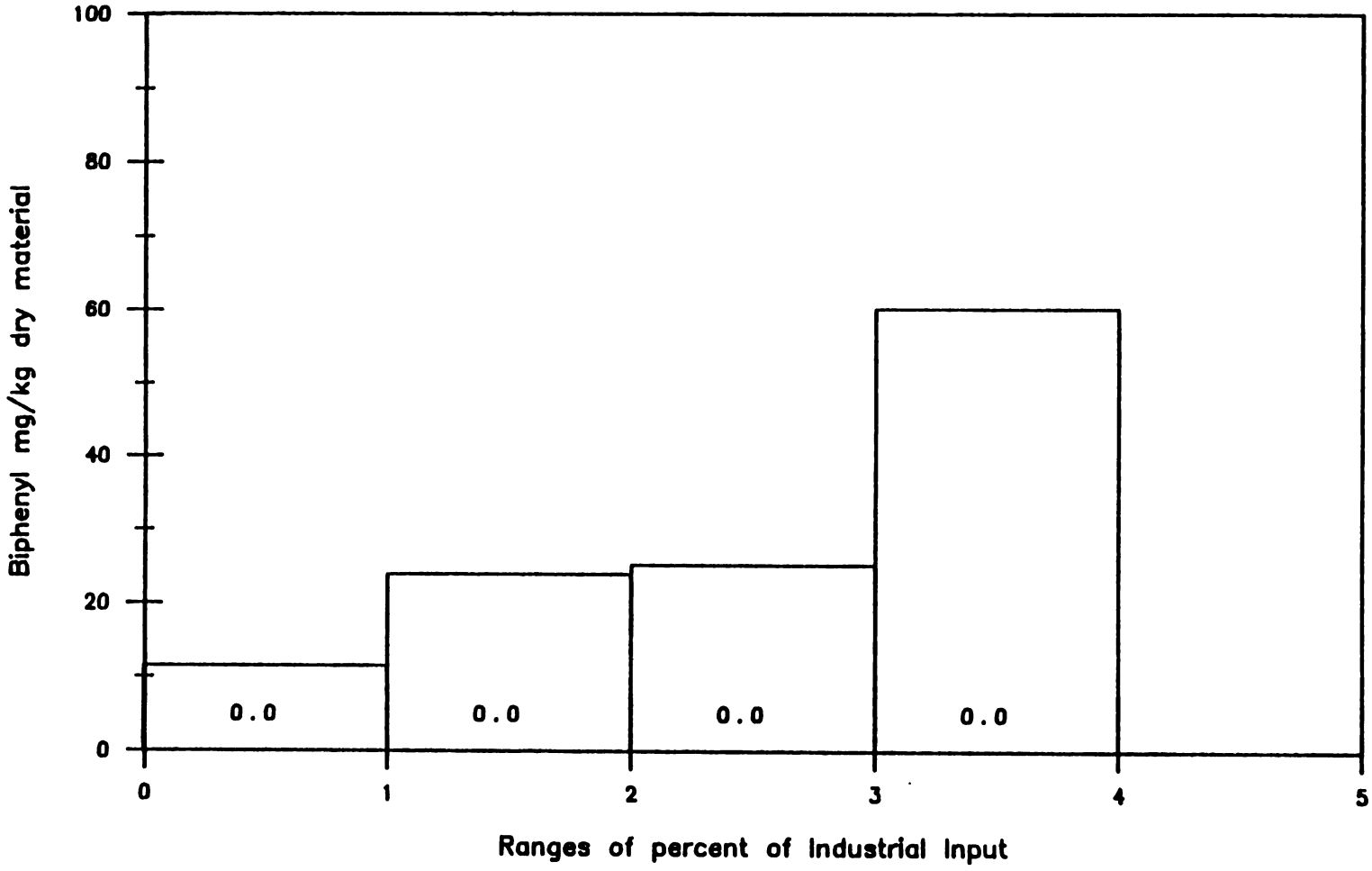


Figure 8. Means (big bar top line) and medians (small bar) of Biphenyl concentrations for different ranges of industrial inputs.

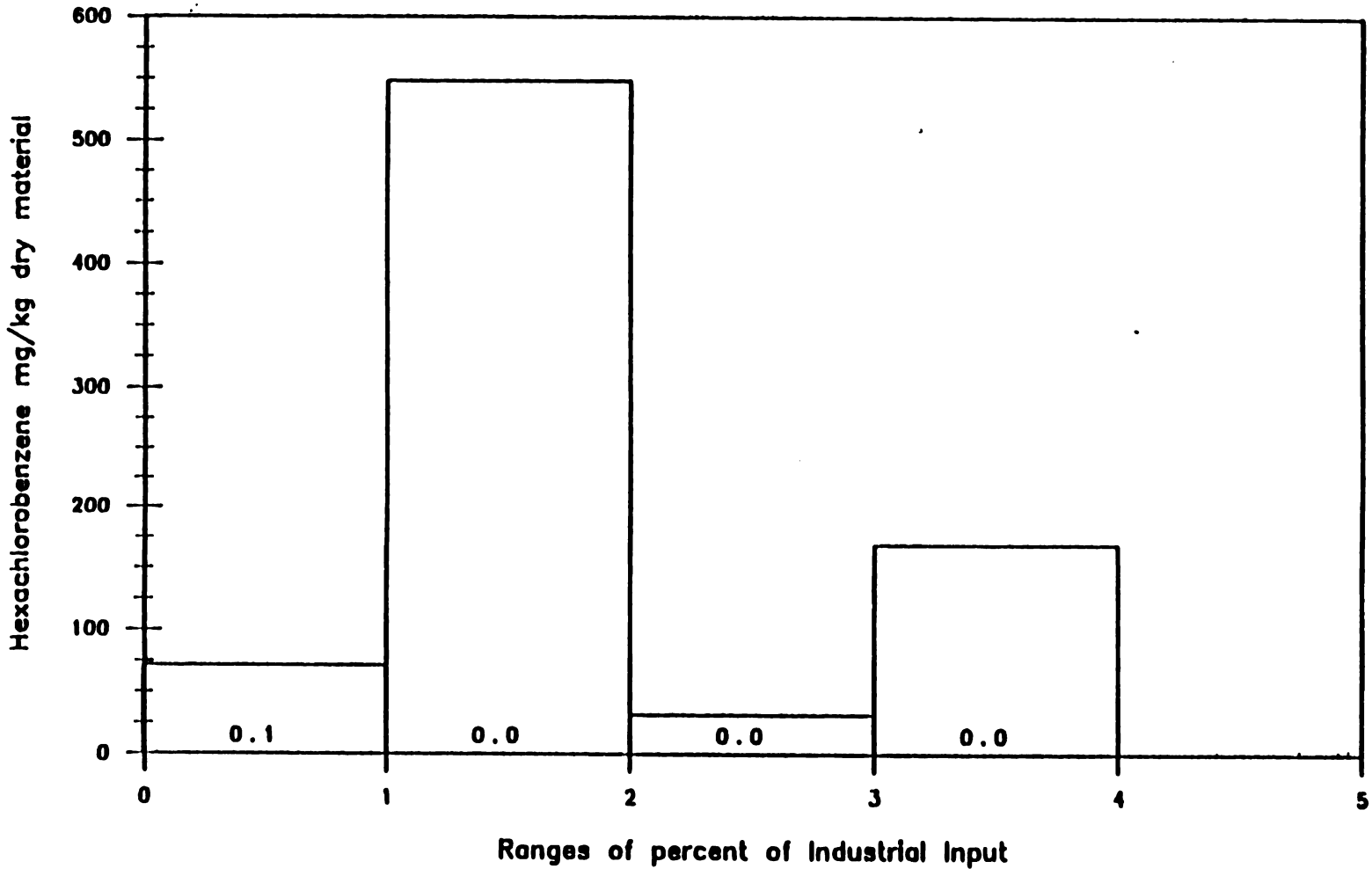


Figure 9. Means (big bar top line) and medians (small bar) of Hexachlorobenzene concentrations for different ranges of industrial inputs.

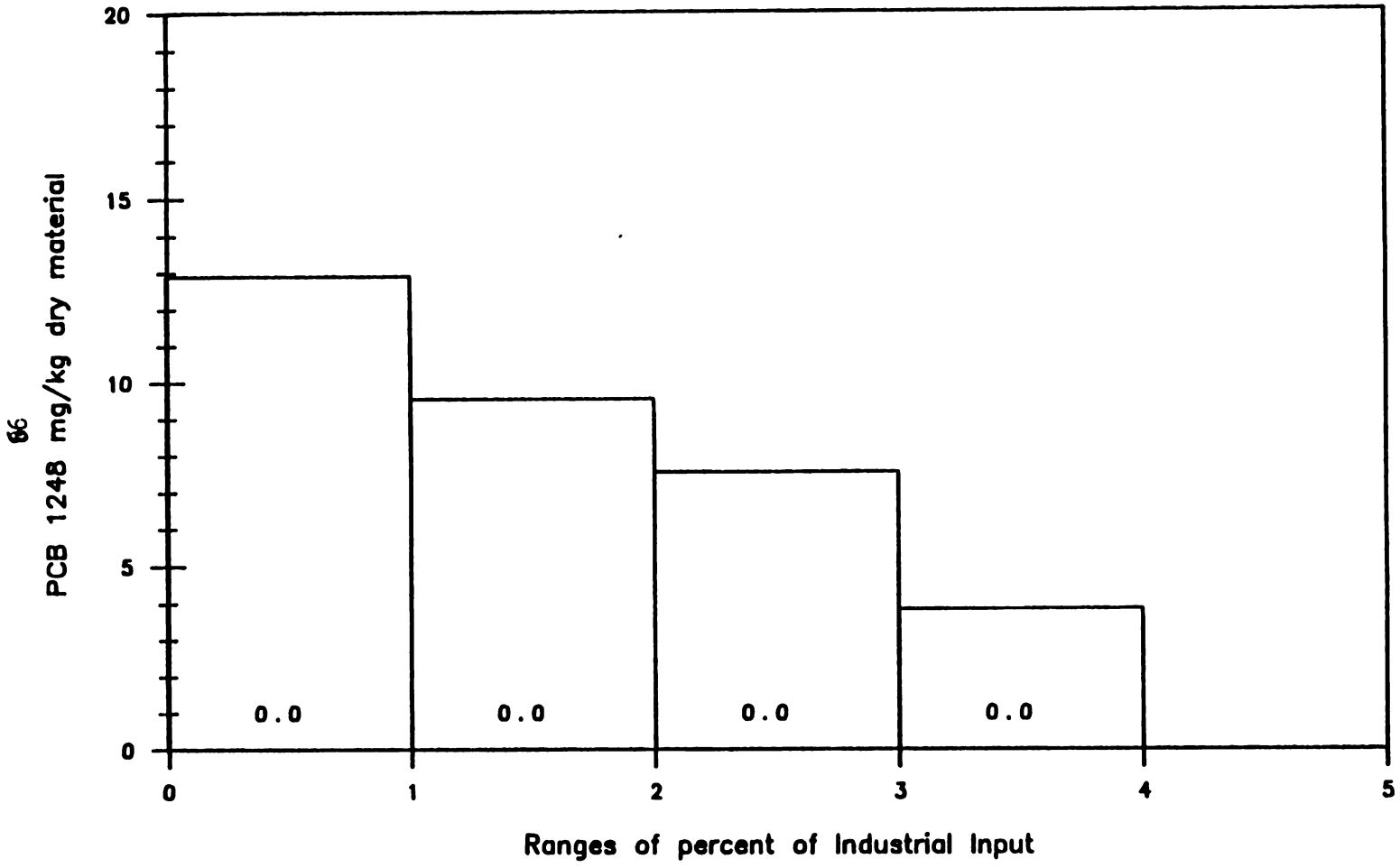


Figure 10. Means (big bar top line) and medians (small bar) of PCB 1248 concentrations for different ranges of industrial inputs.

$$[rh(n-h)/n(n-1)]^{1/2} = [58x116(232-116)/232(232-1)]^{1/2} = 3.816$$

Test Statistic (for each pair of groups): $|a_i - a_{i'}|$, $i \neq i'$

Tables 5,6, and 7 summarized the results .

None of the differences observed in Table 7 was strongly significant. In fact, only one difference was significant at the 20% level of type I statistical error. It is entirely possible that this one happened by chance alone. In the bar graphs (Figs. 7 to 10) only naphthalene and hexachlorobenzene shown some groups with medians diferent from zero (two for naphthalene, one for hexachloro- benzene) but there was not observed any consistent tendency in the data.

b. Effect of size of treatment facility (Flow in M.G.D) on the presence of aromatic compounds.

To establish the groups, the sites were ranked according to the flow of each facility from smallest to largest and then divided into 5 groups, every one with 45 sites. Of the 238 sites, 13 were not taken into account, 5 because there were no samples available, 6 because the flow was not known, and 2 more to mantain equal sample sizes among groups (Table 8).

Table 8. Selected value of flow(MGD) in facilities used to create the groups used in this study.

Group	Range Flow(MGD)
1	0.0 - 0.29
2	0.30 - 0.59
3	0.60 - 1.24
4	1.25 - 3.8
5	4.00 - 1,200.0

Common Computations

t= 5

r= 45

n=225

h=112

$[rh(n-h)/n(n-1)]^{1/2} = [45x112(225-112)/225(224)]^{1/2} = 3.361$

Test Statistic (for each pair of groups): $|a_1-a_1'|$, i=i'.

Results are summarized in tables 9,10, and 11.

As seen in Table 11 and graphs 11,12,13, the biphenyl concentration is possibly affected by flow. The other compounds are apparently not affected by flow.

c. Effect of percent solids on the amount of aromatic compounds found in municipal sludge.

After ranking the 234 sites (no samples were available for 4 sites) according to percent solids, they were organized into 6 groups of 39 units each (Table 13).

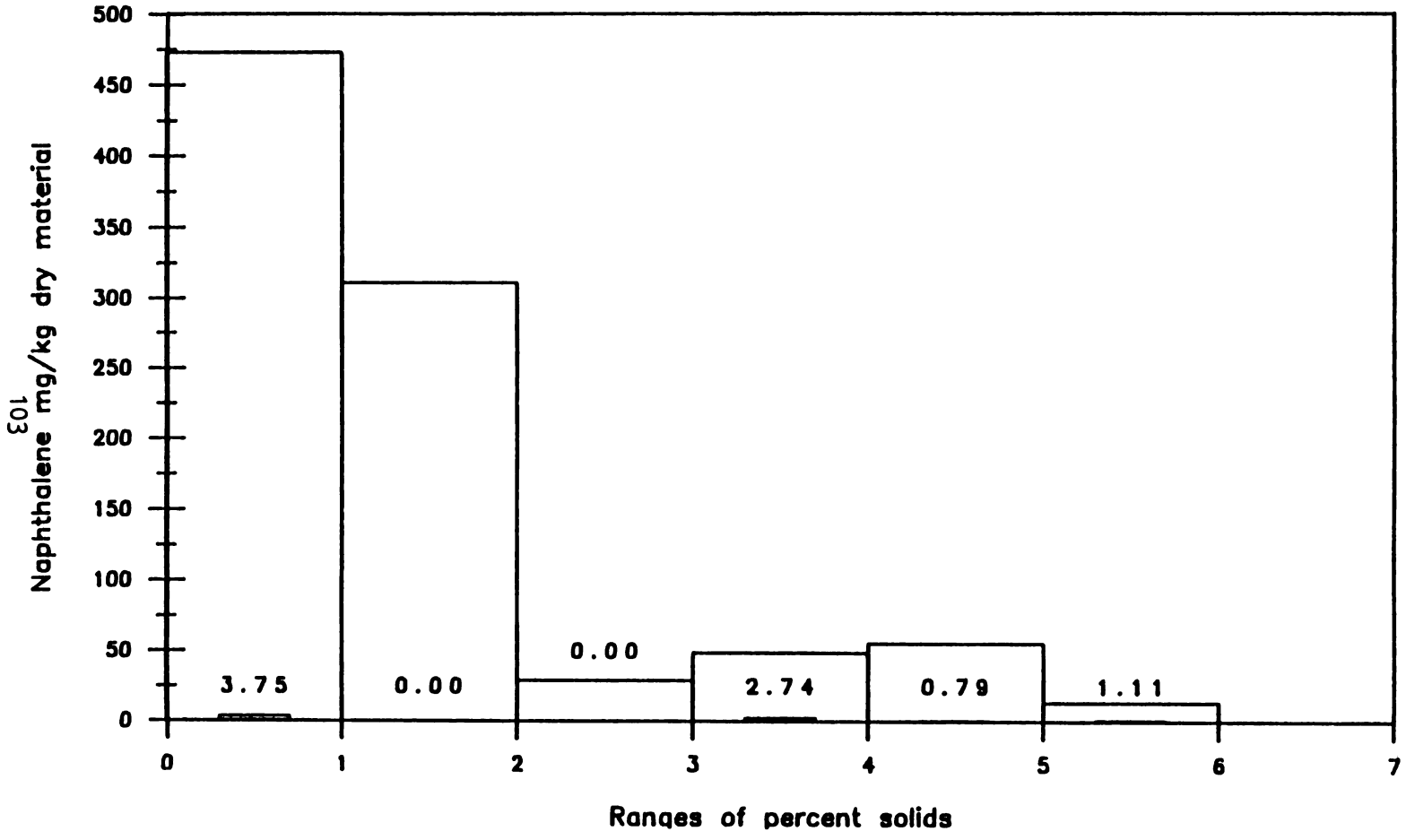


Figure 11. Means (big bar top line) and medians (small bar) of Naphthalene concentrations for different ranges of treatment plant flow.

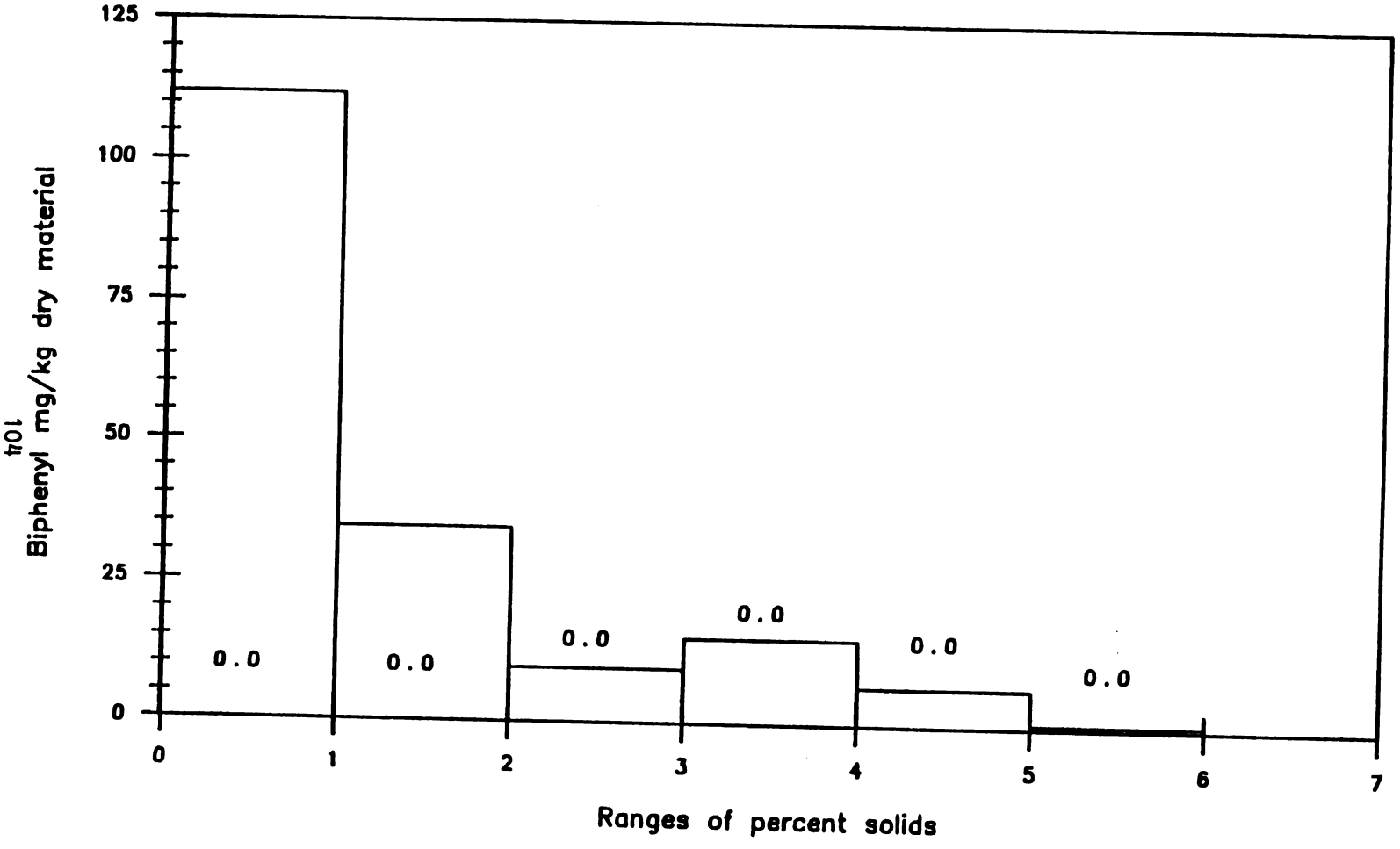


Figure 12. Means (big bar top line) and medians (small bar) of Biphenyl concentrations for different ranges of treatment plant flow.

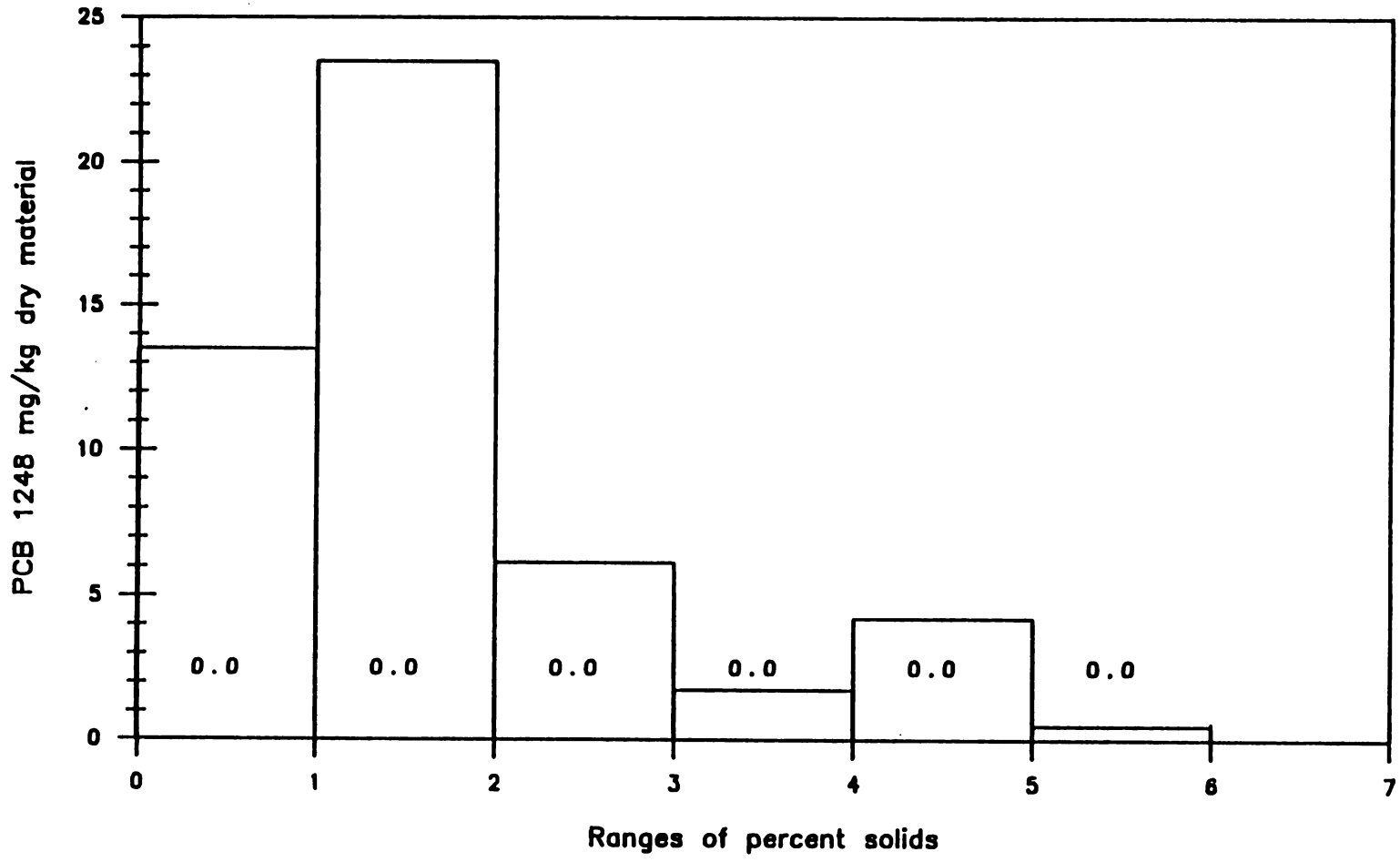


Figure 13. Means (big bar top line) and medians (small bar) of PCB 1248 concentrations for different ranges of treatment plant flow.

Table 9. Critical values (C.V.) for different levels of probability (α). The 3.361 value for samples concentrations, grouped by to ranges of flow facility.

α	$q_{\alpha,5,\infty}$*	C.V.=3.361$q_{\alpha,5,\infty}$
0.2	3.037	10.207
0.1	3.478	11.689
0.05	3.858	12.967
0.01	4.603	15.471

*Percent points of studentized range tables.

Table 10. Grand median (m_n) without considering groups; mean and median for each group of residue concentrations when the samples were grouped according to treatment plant flow and a_2*. Data used in Levy's test and Bar graphs.

	RANGE														
	1			2			3			4			5		
	Mean	Median	a_1	Mean	Median	a_2	Mean	Median	a_3	Mean	Median	a_4	Mean	Median	a_5
Naphthalene m_n=.7947	290	1.11	23	54.6	0	21	173	0	21	127.77	0	26	168	0	21
Biphenyl m_n=0	19.1	0	12	21.6	0	15	32.2	0	23	35.062	0	26	49.88	0	19
Hexachlorob m_n=0	116	1.77	23	38	0	20	670	0	20	44.977	0	18	192.177	0	19
PCB (1248) m_n=0	23.1	0	7	1.31	0	2	6.99	0	3	5.806	0	7	7.493	0	6

*Number of observations in each group exceeding grand median.

Table 11. Results for Levy's statitical test $|a_i - a_{i'}|$[1] for i=i'. Sample concentrations grouped by to facility flow.

i':	2	$\|a_1 - a_{i'}\|$ 3	4	5	$\|a_2 - a_{i'}\|$ 3	4	5	$\|a_3 - a_{i'}\|$ 4	5	$\|a_4 - a_{i'}\|$ 5
Naphthalene	2	2	3	2	0	5	0	5	0	5
Biphenyl	3	11*	14**	7	8	11	4	3	4	7
Hexachlorob.	3	3	5	4	0	2	1	2	1	1
PCB(1248)	5	4	0	1	1	5	4	4	3	1

[1]) a_i or $a_{i'}$ is the number of observations in each group exceeding grand median (m_n).

* P<0.2

** P<0.5

Table 12. Selected range values of percent solids present in samples used to create the groups used in this study.

Group	Range Flow(MGD)
1	0.0 - 2.6
2	2.6 - 4.4
3	4.5 - 6.7
4	6.75 - 13.2
5	13.3 - 29.9
6	30.0 - 91.0

Common Computations:

t=6

r=39

n=234

h=117

$$[rh(n-h)/n(n-1)]^{1/2} = [39x117(234-117)/234(233)]^{1/2} = 3.129$$

Test Statistic (for each pair of groups): $|a_i-a_i'|, i \neq i'$.

Results are summarized in Tables 13, 14 and 15.

The values presented in Table 15 indicate that there were no clear statistical differences among samples with respect to percent solids. Although in the bar graphs (Figs. 14 to 16) consistently the group corresponding to the lowest solid content had the highest mean, it is necessary to keep in mind that the difference in value between the mean and the median is skewed upward because of very high concentration values.

Table 13. Critical values (C.V.) for different levels of probability (α). The 3.129 value for sample concentrations, grouped by to ranges of percent solids.

α	$q_{\alpha,6,\infty}$*	C.V.=$3.361q_{\alpha,6,\infty}$
0.2	3.037	10.207
0.1	3.478	11.689
0.05	3.858	12.967
0.01	4.603	15.471

*Percent points of studentized range tables.

Table 14. Grand median (m_n) without considering groups; mean and median for each group of residue concentration when the samples were grouped by percent of solids in the samples and a_i*. Data used for levy's test and bar graphs.

	RANGE																	
	1			2			3			4			5			6		
	Mean	Median	a_i	Mean	Median	a_i	Mean	Median	a_i	Mean	Median	a_i	Mean	Median	a_i	Mean	Median	a_i
Naphthalene m_n=.806	473	3.75	20	311	0	17	29	0	15	48.8	2.74	25	55.6	.795	19	13.5	1.11	20
Biphenyl m_n=0	112	0	12	34.4	0	15	9.7	0	9	15.2	0	13	6.82	0	16	.814	0	13
Hexachlorob m_n=0	883	0	16	239	0	17	76.1	0	15	26.2	.238	20	15.2	0	14	5.86	0	19
PCB (1248) m_n=0	13.5	0	4	23.5	0	4	6.15	0	5	1.75	0	3	4.22	0	5	.509	0	5

*Number of observation in each group exceeding grand median

Table 15. Results for Levy's statistical test $|a_i - a_{i'}|^*$ for $i \neq i'$. Sample concentrations grouped by to % of solids.

	$\|a_1-a_{i'}\|$					$\|a_2-a_{i'}\|$				$\|a_3-a_{i'}\|$			$\|a_4-a_{i'}\|$		$\|a_5-a_{i'}\|$
i':	2	3	4	5	6	3	4	5	6	4	5	6	5	6	6
Naphthalene	3	5	5	1	0	2	8	2	3	10	4	5	6	5	1
Biphenyl	3	3	1	4	1	6	2	1	2	4	7	4	3	0	1
Hexachlorob.	1	1	4	2	3	2	3	3	2	5	1	4	6	1	5
PCB(1248)	0	1	1	1	1	1	1	1	1	2	0	0	2	2	0

*a_i or $a_{i'}$ is the number of observations in each group exceeding grand median (m_n).

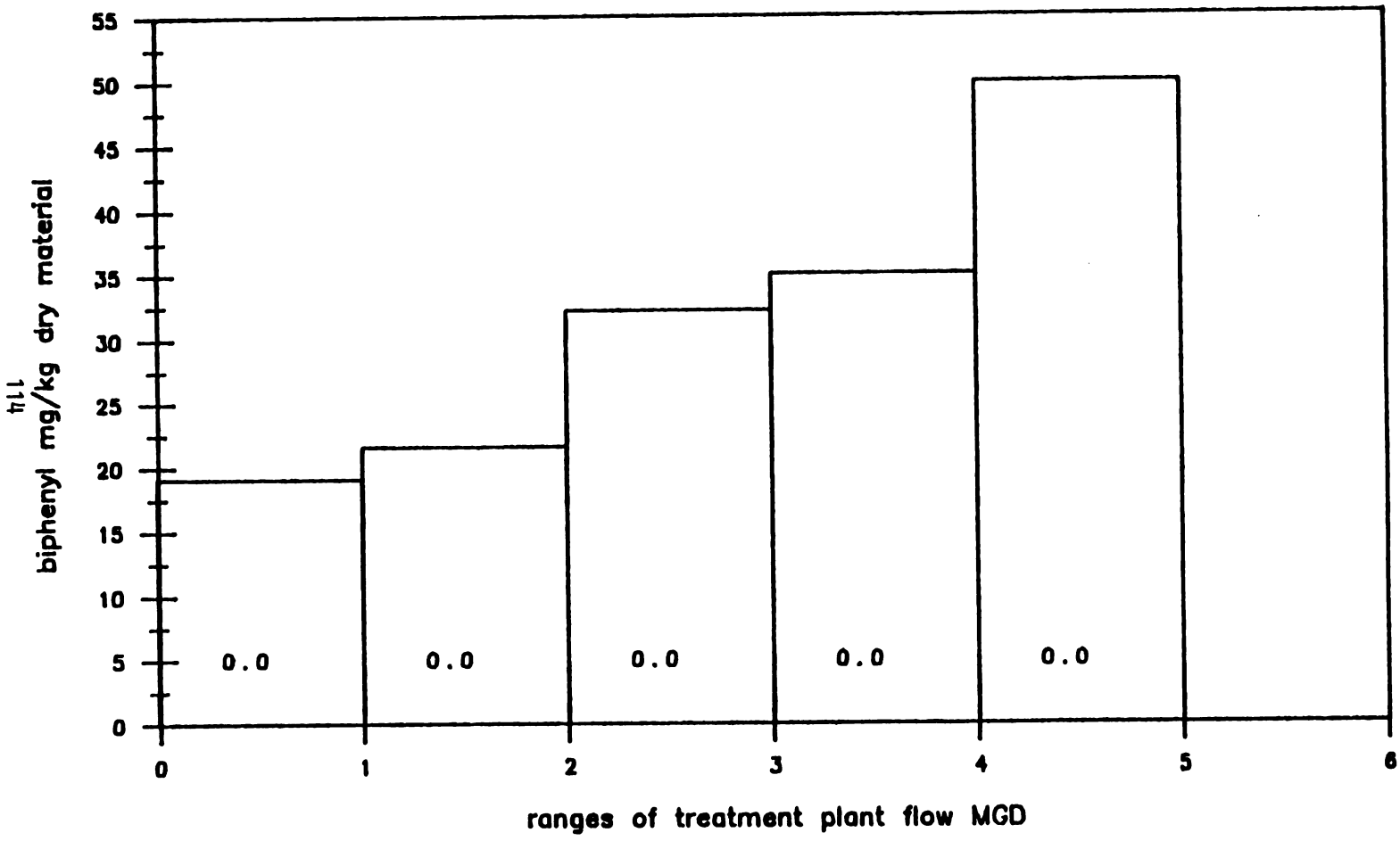


Figure 14. Means (big bar top line) and medians (small bar) of Biphenyl concentrations for different ranges of percent solids.

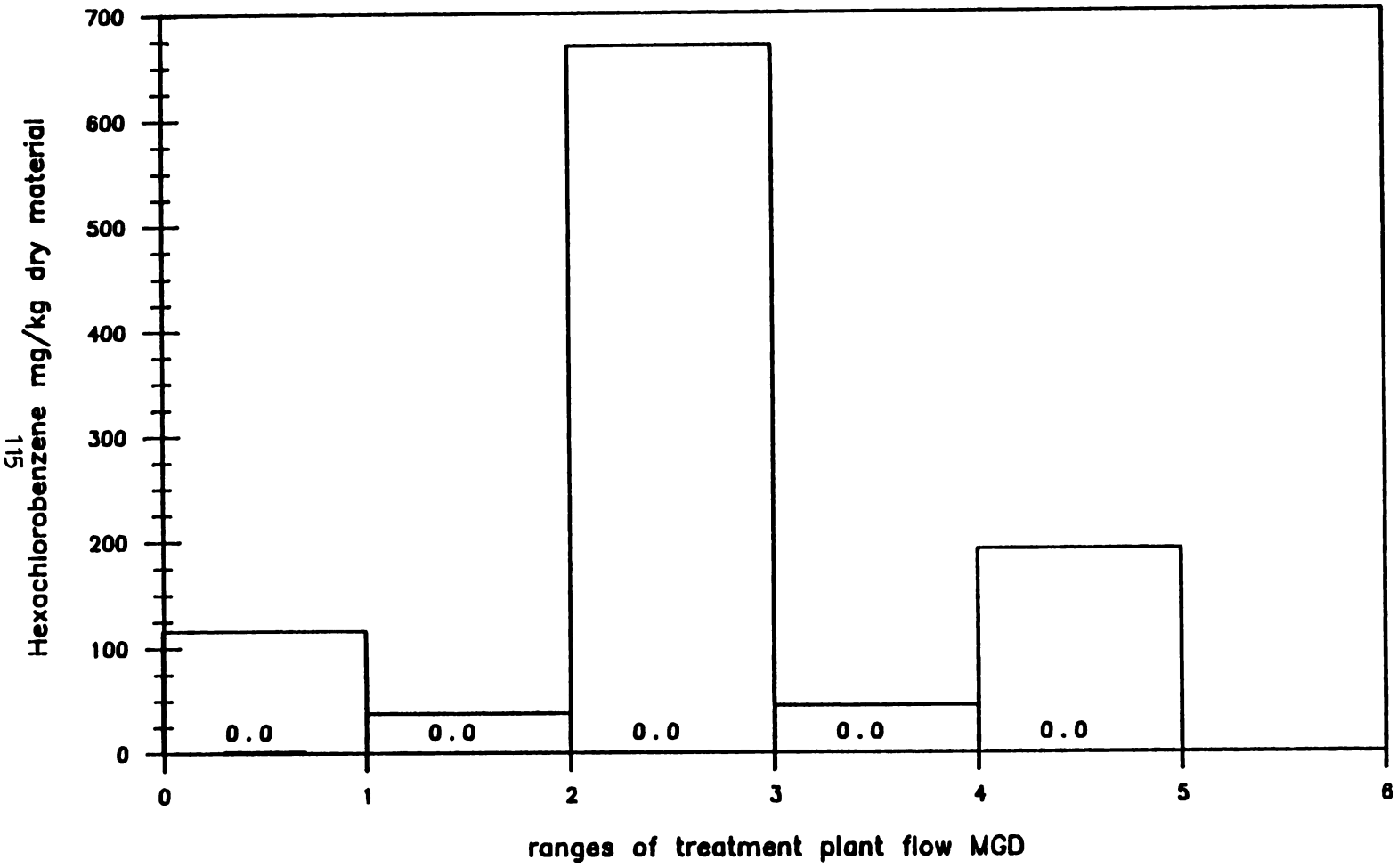


Figure 15. Means (big bar top line) and medians (small bar) of Hexachlorobenzene for different ranges of percent solids.

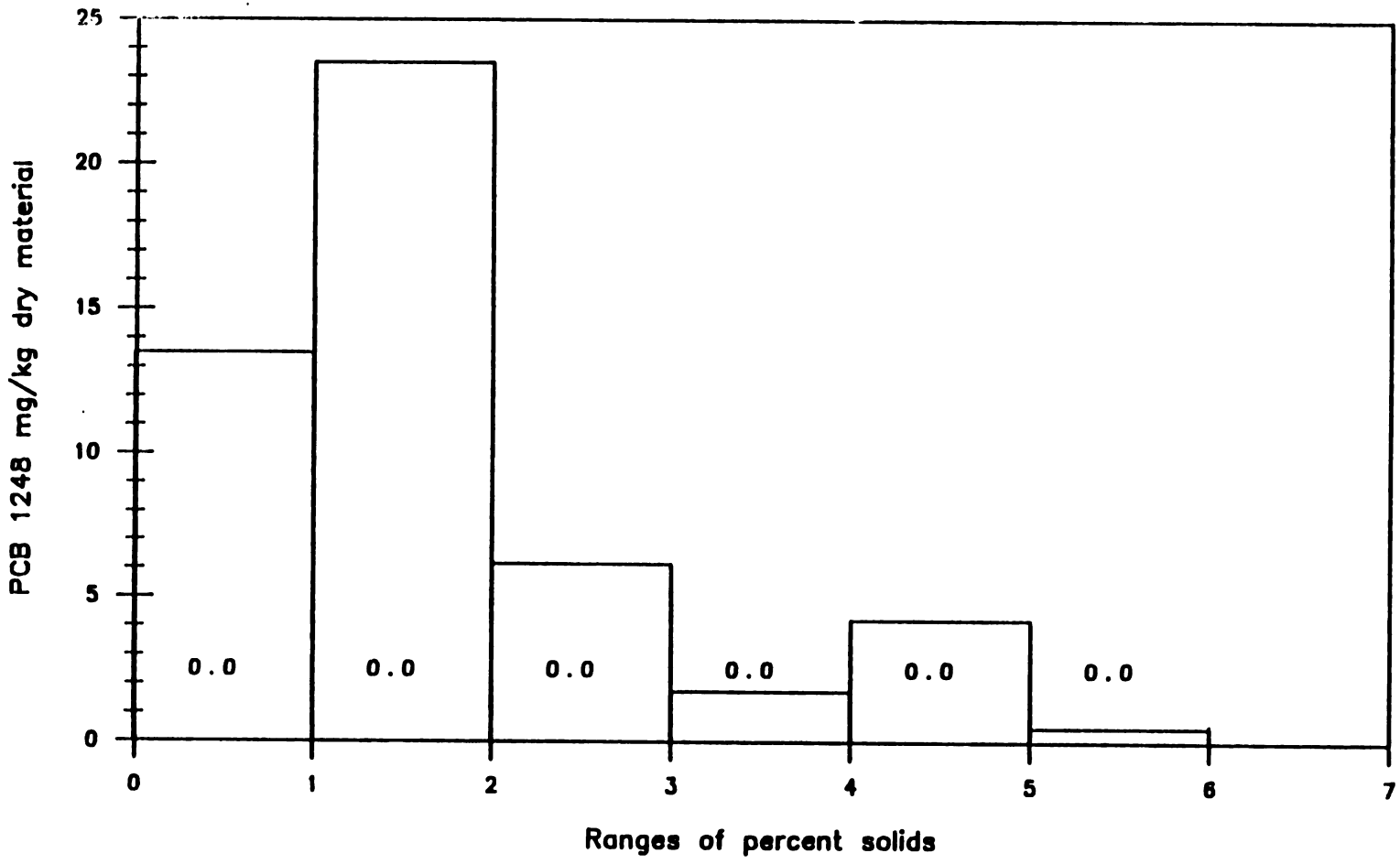


Figure 16. Means (big bar top line) and medians (small bar) of PCB 1248 concentrations for different ranges of percent solids.

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CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

Comparing the chromatographic systems used to determine the eight aromatic compounds allowed me to conclude that the High Pressure Liquid Chromatographic (HPLC) system developed and presented here has many advantages over gas chromatographic systems used for the Michigan state samples analysis. They are:

1) It allows for the simultaneous determination of the eight compounds of interest; gas chromatography with electron capture detector only determined five of the compounds simultaneously.

2) With the HPLC system no cleanup or separation was required during the sample preparation, even when using a material as difficult as secondary activated sludge. With gas chromatography, an exhaustive cleanup was required especially when using the electron capture detector.

3) The HPLC system can be used for confirmation or to overcome interference from compounds that coelute with 2-mercaptobenzothiazol, naphthalene, or/and biphenyl. At 260 nm, naphthalene and biphenyl are detected selectively and at 320 nm, 2-mercaptobenzothiazol is the only member of the group absorbing.

4) Besides the compounds contracted to be determined in the Michigan sludge samples, this method can determine polybrominated biphenyls such as Fire-master^{R}.

In addition, water samples can be analyzed following basically the same procedure. The presence of phthalates as contaminants in sludge and water samples shown also the possibility of determining these

compounds with the same chromatographic system.

I suggest that the resolution power of the system can be increased by adding a third modifier at the milimole level. I also recommend that a multi-heater, a flat bottom flask, and a snyder column be combained to evaporate extracts with difficult compounds such as naphthalene.

Of the eight compounds determined in the Michigan sludge samples, naphthalene, byphenol, hexachlorobenzene, and Aloclor 1248 were found at concentrations above the detection limit. The concentration of these compounds in some samples was very high; however, it is difficult to conclude anything because only a sample and not even a sample composited over time was taken from each location.

APPENDIX

APPENDIX A

Naphthalene, biphenyl, hexachlorobenzene, octachlorostyrene, PCB aroclor 1254, 1,2,3,4-tetrachlorobenzene, 1,2,3,5-tetrachlorobenzene, and 1,2,4,5-tetrachlorobenzene measured in 239 sludge samples (mg/kg, dry weight basis).

	Naphthalene	Biphenyl	Hexachlorobenzene	Octachlorostyrene	PCB's	1,2,3,4-tetrachlorobenzene	1,2,3,5-tetrachlorobenzene	1,2,4,5-tetrachlorobenzene
1	2.1E 01	57.4E 01	37.4E 01	4.1E 01	00.0E 01	2.1E 01	2.1E 01	2.1E 01
2	2.4E 02	31.7E 03	15.1E 03	4.0E 02	00.0E 01	2.4E 02	2.4E 02	2.4E 02
3	2.1E 02	2.1E 03	21.1E 02	4.2E 02	00.0E 01	2.1E 02	2.1E 02	2.1E 02
4	1.5E 02	1.5E 02	47.3E 02	3.0E 02	1.5E 02	1.5E 02	1.5E 02	1.5E 02
5	1.1E 01	42.1E 03	10.5E 01	2.1E 01	1.1E 01	1.1E 01	1.1E 01	1.1E 01
6	2.2E 02	43.7E 03	33.3E 01	4.4E 02	2.2E 02	2.2E 02	2.2E 02	2.2E 02
7	3.0E 02	25.5E 01	37.7E 01	7.5E 02	3.0E 02	3.0E 02	3.0E 02	3.0E 02
8	4.2E 02	70.0E 01	4.2E 02	8.3E 02	4.2E 02	4.2E 02	4.2E 02	4.2E 02
9	2.2E 02	2.2E 02	2.1E 02	4.3E 02	2.2E 02	2.2E 02	2.2E 02	2.2E 02
10	2.1E 02	2.1E 02	24.1E 00	4.3E 02	2.1E 02	2.1E 02	2.1E 02	2.1E 02
11	7.0E 02	25.1E 01	15.2E 01	1.4E 01	90.4E 00	7.0E 02	7.0E 02	7.0E 02
12	4.2E 02	4.2E 02	2.9E 01	0.3E 02	14.7E 01	4.2E 02	4.2E 02	4.2E 02
13	2.9E 01	2.9E 01	-2.9E 01	5.7E 01	-2.9E 01	2.9E 01	2.9E 01	2.9E 01
14	2.2E 01	2.2E 01	-2.2E 01	4.4E 01	-2.2E 01	2.2E 01	2.2E 01	2.2E 01
15	10.3E 00	11.3E 01	-4.9E 02	-1.4E 01	-4.9E 02	6.9E 02	6.9E 02	6.9E 02
16	52.4E 01	-2.4E 01	-2.4E 01	-4.8E 01	-2.4E 01	-2.4E 01	-2.4E 01	-2.4E 01
17	24.0E 01	-1.4E 01	-1.4E 01	-2.8E 01	-1.4E 01	-1.4E 01	-1.4E 01	-1.4E 01
18	94.5E 01	-2.4E 00	-2.4E 00	-4.7E 00	-2.4E 00	-2.4E 00	-2.4E 00	-2.4E 00
19	1.5E 01	-1.5E 01	30.3E 00	-3.1E 01	-1.5E 01	-1.5E 01	-1.5E 01	-1.5E 01
20	2.1E 01	-2.1E 01	21.0E-01	-4.2E 01	-2.1E-01	-2.1E-01	-2.1E-01	-2.1E 01
21	2.4E 01	-2.4E 01	18.0E 00	-5.1E 01	-2.4E 01	-2.4E 01	-2.4E 01	-5.7E 02
22	17.0E 00	17.0E 00	59.9E 00	-5.3E 01	-2.7E 01	-2.7E 01	-2.7E 01	-3.3E 00
23	2.7E 01	24.9E 01	40.1E-01	-5.3E 01	-2.7E-01	-2.7E-01	-2.7E 01	-4.3E 02
24	2.0E 01	-2.0E 01	-2.0E 01	-4.0E 01	-2.0E 01	-2.0E 01	-2.0E 01	-7.1E 01
25	1.9E 01	-1.9E 01	-1.9E-01	-3.8E 01	-1.9E 01	-1.9E 01	-1.9E 01	-2.0E 02
26	4.1E 02	-4.1E 02	-4.1E-02	-8.3E-02	-4.1E-02	-4.1E-02	-4.1E 02	-1.0E 02
27	4.9E 02	-4.9E 02	-4.9E-02	-9.8E-02	-4.9E-02	-4.9E-02	-4.9E 02	-1.4E 02
28	1.5E 01	-1.5E 01	-1.5E-01	-3.0E-01	-1.5E-01	-1.5E-01	-1.5E 01	-1.5E 02
29	5.1E 02	24.7E 00	20.5E 01	-1.0E-01	41.0E-01	-5.1E-02	-5.1E-02	-1.3E 02
30	30.7E 02	45.4E 00	23.0E 02	-7.1E-01	-3.4E-01	-3.4E-01	-3.4E 01	-3.4E 02
31	2.1E 01	90.0E-01	34.8E 00	-4.2E-01	-2.1E-01	-2.1E-01	-2.1E-01	-5.9E 02
32	4.0E 02	27.4E 02	17.5E 00	-1.4E-01	-6.8E-02	-6.8E-02	-6.8E-02	-3.0E 01
33	59.2E 00	12.1E 01	10.3E-01	-9.3E-02	-4.7E-02	-4.7E-02	-4.7E-02	-4.5E 02
34	24.4E 00	11.0E-01	85.3E-01	-1.3E 01	-4.5E-02	-4.5E-02	-4.5E-02	-1.4E 01
35	11.3E 00	-7.0E 02	84.7E 00	-1.4E-01	-7.0E-02	-7.0E-02	-7.0E-02	-6.0E 02
36	24.4E 00	-9.1E 02	-9.1E-02	-1.8E-01	-9.1E-02	-9.1E-02	-9.1E-02	-2.1E 01
37	2.1E 02	-2.1E 02	-2.1E-02	-4.2E-02	-2.1E-02	-2.1E-02	-2.1E-02	-3.7E 01
38	5.0E 02	-5.0E 02	-5.0E-02	-1.0E-01	-5.0E-02	-5.0E-02	-5.0E-02	-2.2E 00
39	1.4E 01	-1.4E 01	-1.4E-01	-3.2E-01	-1.4E-01	-1.4E-01	-1.4E-01	-1.0E 00
40	2.1E	-2.1E 01	-2.1E-01	-4.1E	-2.1E-01	-2.1E-01	-2.1E-01	-2.0E 01
41	21.0E 00	31.3E 01	-5.7E-02	-1.1E 01	-5.7E 02	-5.7E-02	-5.7E-02	-5.7E 02
42	3.3E 00	-3.3E 00	-3.3E 00	-6.7E 00	-3.3E 00	-3.3E 00	-3.3E 00	-3.3E 00
43	4.3E 02	-4.3E 02	-4.3E-02	-8.5E-02	-4.3E-02	-4.3E-02	-4.3E 02	-4.3E 02
44	7.1E 00	-7.1E 01	-7.1E-01	-1.4E 00	-7.1E-01	-7.1E-01	-7.1E 01	-7.1E 01
45	2.0E 02	-2.0E 02	-2.0E-02	-5.5E-02	-2.0E-02	-2.0E-02	-2.0E 02	-2.0E 02
46	29.0E 01	42.4E 01	-3.4E-02	-3.4E-02	82.0E-01	-1.4E-02	-1.4E 02	-1.0E 02
47	27.0E 01	48.7E 00	-3.2E 02	-3.2E 02	41.1E 01	-1.5E-02	-1.5E 02	-1.4E 02
48	1.5E 02	71.7E-01	-2.9E-02	-2.9E-02	-1.5E 02	-1.3E-02	-1.3E 02	-1.3E 02
49	74.3E 01	-1.3E 02	-2.8E-02	-2.8E-02	-1.4E-02	-1.3E-02	-1.3E 02	-3.4E 02
50	1.4E 02	-3.4E 02	-6.8E-02	-6.8E-02	-3.4E-02	-3.4E-02	-3.4E 02	-5.9E 02
51	5.9E 02	-5.9E 02	-1.2E 01	-1.2E 01	-5.9E 02	-5.9E-02	-5.9E 02	-3.0E 01
52	3.0E 01	-3.0E 01	-5.9E 01	-5.9E 01	-3.0E 01	-3.0E-01	-3.0E 01	-4.5E 02
53	4.5E 02	-4.5E 02	-9.0E-02	-9.0E-02	-4.5E 02	-4.5E-02	-4.5E 02	-1.4E 01
54	14.0E 01	71.8E 00	15.2E 00	-2.7E-01	-1.4E 01	-1.4E-01	-1.4E 01	-6.0E 02
55	84.7E 01	-4.0E-02	70.3E-01	-1.4E 01	-6.8E-02	-6.8E-02	-6.8E-02	-2.1E 01
56	10.4E 00	44.7E 01	-2.1E-01	-4.2E-01	-2.1E-01	-2.1E-01	-2.1E 01	-3.7E 01
57	12.3E 02	15.7E 00	-3.7E-01	-7.3E 01	-3.7E 01	-3.7E-01	-3.7E 01	-2.2E 00
58	5.1E 01	20.1E 01	-2.2E 00	-4.4E 00	-2.2E 00	-2.2E 00	-2.2E 00	-1.0E 00
59	2.3E 01	53.1E 01	55.0E 01	-2.1E 00	-1.0E 00	-1.0E 00	-1.0E 00	-2.0E 01
60		-2.0E 01	-2.0E 01	-4.0E-01	2.0E 01	-2.0E-01	-2.0E 01	-2.0E 01

	Naphthalene	Biphenyl	Acenaphthylene	Acenaphthene	PCB's	1,2,3,4-Tetrachlorobenzene	1,2,3,5-Tetrachlorobenzene	1,2,4,5-Tetrachlorobenzene
41	1.5E 01	1.5E 01	1.5E 01	7.1E 01	1.5E 01	1.5E 01	1.5E 01	1.5E 01
42	10.0E 00	2.0E 01	2.0E 01	4.0E 01	2.0E 01	2.0E 01	2.0E 01	2.0E 01
43	10.3E 01	31.1E 02	47.0E 01	2.2E 02	1.1E 02	1.1E 02	1.1E 02	1.1E 02
44	17.2E 00	26.3E 01	17.7E 00	2.0E 02	1.4E 02	1.4E 02	1.4E 02	1.4E 02
45	10.7E 01	25.0E 01	25.7E 01	4.1E 01	24.3E 01	3.0E 01	3.0E 01	3.0E 01
46	1.0E 01	1.0E 01	50.0E 00	2.1E 01	1.0E 01	1.0E 01	1.0E 01	1.0E 01
47	1.4E 02	1.4E 02	44.2E 01	2.7E 02	1.4E 02	1.4E 02	1.4E 02	1.4E 02
48	1.7E 01	14.9E 01	1.7E 01	7.4E 01	1.7E 01	1.7E 01	1.7E 01	1.7E 01
49	22.4E 00	2.4E 02	23.1E 01	5.1E 02	2.4E 02	2.4E 02	2.4E 02	2.4E 02
50	21.0E 01	2.7E 02	2.7E 02	5.5E 02	2.7E 02	2.7E 02	2.7E 02	2.7E 02
51	3.4E 02	3.4E 02	3.4E 02	6.8E 02	3.4E 02	3.4E 02	3.4E 02	3.4E 02
52	20.3E 00	41.1E 00	4.7E 02	9.5E 02	4.7E 02	4.7E 02	4.7E 02	4.7E 02
53	12.0E 01	21.3E 01	2.1E 01	4.2E 01	2.1E 01	2.1E 01	2.1E 01	2.1E 01
54	20.9E 01	21.2E 01	9.9E 02	2.0E 01	9.9E 02	9.9E 02	9.9E 02	9.9E 02
55	2.5E 01	2.5E 01	15.5E 01	4.9E 01	2.5E 01	2.5E 01	2.5E 01	2.5E 01
56	52.4E 00	1.9E 02	11.4E 00	3.6E 02	1.9E 02	1.9E 02	1.9E 02	1.9E 02
57	1.3E 02	43.3E 01	58.4E 01	2.6E 02	1.3E 02	1.3E 02	1.3E 02	1.3E 02
58	2.2E 01	2.2E 01	14.0E 00	4.3E 01	2.2E 01	2.2E 01	2.2E 01	2.2E 01
59	2.3E 02	2.3E 02	10.0E 02	4.7E 02	2.3E 02	2.3E 02	2.3E 02	2.3E 02
60	1.1E 02	1.1E 02	1.1E 02	2.3E 02	1.1E 02	1.1E 02	1.1E 02	1.1E 02
61	1.6E 01	1.6E 01	1.6E 01	3.1E 01	1.6E 01	1.6E 01	1.6E 01	1.6E 01
62	49.0E 01	5.1E 02	5.1E 02	1.0E 01	5.1E 02	5.1E 02	5.1E 02	5.1E 02
63	99.3E 01	2.1E 02	2.1E 02	4.2E 02	2.1E 02	2.1E 02	2.1E 02	2.1E 02
64	37.0E 00	7.4E 02	7.4E 02	1.5E 01	7.4E 02	7.4E 02	7.4E 02	7.4E 02
65	3.0E 01	3.0E 01	3.0E 01	7.7E 01	3.0E 01	3.0E 01	3.0E 01	3.0E 01
66	40.4E 00	1.0E 02	13.0E 00	3.7E 02	1.0E 02	1.0E 02	1.0E 02	1.0E 02
67	23.2E 00	44.7E 01	25.6E 01	5.1E 01	2.4E 01	2.4E 01	2.4E 01	2.4E 01
68	6.7E 01	6.7E 01	15.1E 01	1.3E 00	6.7E 01	6.7E 01	6.7E 01	6.7E 01
69	14.7E 02	44.2E 01	3.5E 02	7.0E 02	81.2E 00	3.5E 02	3.5E 02	3.5E 02
70	6.3E 01	6.3E 01	17.9E 01	1.3E 00	6.3E 01	6.3E 01	6.3E 01	6.3E 01
71	3.0E 01	3.0E 01	3.0E 01	7.4E 01	3.0E 01	3.0E 01	3.0E 01	3.0E 01
72	1.3E 01	1.3E 01	1.3E 01	4.7E 01	1.3E 01	1.3E 01	1.3E 01	1.3E 01
73	49.5E 00	2.3E 01	2.3E 01	4.7E 01	2.3E 01	2.3E 01	2.3E 01	2.3E 01
74	14.3E 01	7.9E 02	23.0E 02	1.4E 01	7.9E 02	7.9E 02	7.9E 02	7.9E 02
75	41.7E 01	4.2E 01	20.0E 01	8.3E 01	4.2E 01	4.2E 01	4.2E 01	4.2E 01
76	10.2E 01	1.2E 01	75.3E 00	2.4E 01	1.2E 01	1.2E 01	1.2E 01	1.2E 01
77	8.7E 02	8.7E 02	14.3E 00	1.7E 01	8.7E 02	8.7E 02	8.7E 02	8.7E 02
78	2.5E 01	22.1E 00	30.0E 00	5.0E 01	2.5E 01	2.5E 01	2.5E 01	2.5E 01
79	2.9E 01	2.9E 01	17.1E 00	5.7E 01	2.9E 01	2.9E 01	2.9E 01	2.9E 01
80	11.1E 01	2.5E 02	55.2E 00	5.0E 02	2.5E 02	2.5E 02	2.5E 02	2.5E 02
81	8.3E 01	8.3E 01	8.3E 01	1.7E 00	8.3E 01	8.3E 01	8.3E 01	8.3E 01
82	4.4E 02	4.4E 02	8.8E 02	4.4E 02	4.4E 02	4.4E 02	4.4E 02	4.4E 02
83	6.0E 02	6.0E 02	21.2E 01	6.0E 02	6.0E 02	6.0E 02	6.0E 02	6.0E 02
84	2.7E 01	2.7E 01	53.3E 02	5.3E 01	2.7E 01	2.7E 01	2.7E 01	2.7E 01
85	13.3E 01	13.3E 01	3.3E 02	6.6E 02	3.3E 02	3.3E 02	3.3E 02	3.3E 02
86	5.5E 02	5.5E 02	5.5E 02	1.1E 01	5.5E 02	5.5E 02	5.5E 02	5.5E 02
87	12.0E 01	92.1E 01	24.1E 01	5.2E 02	2.4E 02	2.4E 02	2.4E 02	2.4E 02
88	1.3E 01	1.3E 01	19.1E 00	2.6E 01	1.3E 01	1.3E 01	1.3E 01	1.3E 01
89	4.1E 02	4.1E 02	4.1E 02	8.2E 02	4.1E 02	4.1E 02	4.1E 02	4.1E 02
90	6.5E 02	6.5E 02	6.5E 02	1.3E 01	6.5E 02	6.5E 02	6.5E 02	6.5E 02
91	1.3E 02	1.3E 02	1.3E 02	2.7E 02	13.1E 01	1.3E 02	1.3E 02	1.3E 02
92	2.1E 02	2.1E 02	24.4E 01	4.3E 02	2.1E 02	2.1E 02	2.1E 02	2.1E 02
93	1.1E 02	1.1E 02	24.3E 00	2.2E 02	26.4E 01	1.1E 02	1.1E 02	1.1E 02
94	3.3E 01	3.3E 01	99.0E 00	6.7E 01	43.0E 01	3.3E 01	3.3E 01	3.3E 01
95	2.4E 01	23.1E 01	21.1E 01	5.1E 01	2.4E 01	2.4E 01	2.4E 01	2.4E 01
96	1.1E 01	1.1E 01	37.6E 01	2.2E 01	1.1E 01	1.1E 01	1.1E 01	1.1E 01
97	11.3E 01	15.0E 01	4.3E 01	8.7E 01	4.3E 01	4.3E 01	4.3E 01	4.3E 01
98	54.1E 01	1.3E 00	1.3E 00	2.5E 00	1.3E 00	1.3E 00	1.3E 00	1.3E 00
99	11.7E 00	1.5E 02	1.5E 02	3.0E 02	1.5E 02	1.5E 02	1.5E 02	1.5E 02
100	5.0E 00	5.0E 00	5.0E 00	1.0E 01	5.0E 00	5.0E 00	5.0E 00	5.0E 00

continued.

	Hydrolase	Phenyl	Hexachlorobenzene	Mercaptobenzothiazole	PCB's	1,2,3,4-tetrachlorobenzene	1,2,3,5-tetrachlorobenzene	1,2,4,5-tetrachlorobenzene
121	13.7E 02	5.4E 00	5.4E 00	1.1E 01	5.4E 00	5.4E 00	5.4E 00	5.4E 00
122	33.4E 01	1.0E 01	1.0E 01	3.6E 01	1.0E 01	1.0E 01	1.0E 01	1.0E 01
123	11.0E 00	1.2E 02	43.9E 01	2.4E 02	1.2E 02	1.2E 02	1.2E 02	1.2E 02
124	20.4E 00	40.6E 02	23.0E 00	6.3E 02	3.1E 02	3.1E 02	3.1E 02	3.1E 02
125	35.0E 01	4.3E 01	23.7E 01	8.7E 01	4.3E 01	4.3E 01	4.3E 01	4.3E 01
126	N.G.A.	N.G.A.	N.G.A.	N.G.A.	N.G.A.	N.G.A.	N.G.A.	N.G.A.
127	1.4E 02	1.4E 02	1.4E 02	3.3E 02	1.4E 02	1.4E 02	1.4E 02	1.4E 02
128	74.4E 00	49.7E 01	47.6E 00	1.0E 01	9.0E 02	9.0E 02	9.0E 02	9.0E 02
129	1.3E 01	58.3E 01	39.7E 01	2.6E 01	1.3E 01	1.3E 01	1.3E 01	1.3E 01
130	1.0E 01	1.0E 01	85.9E 00	3.7E 01	1.0E 01	1.0E 01	1.0E 01	1.0E 01
131	66.5E 00	53.0E 00	6.7E 02	1.3E 01	17.3E 00	6.7E 02	6.7E 02	6.7E 02
132	30.6E 01	19.4E 01	8.7E 01	1.7E 00	8.7E 01	8.7E 01	8.7E 01	8.7E 01
133	60.1E 01	21.4E 01	63.0E 01	1.4E 00	6.9E 01	6.9E 01	6.9E 01	6.9E 01
134	22.3E 00	3.3E 01	10.0E 00	6.7E 01	3.3E 01	3.3E 01	3.3E 01	3.3E 01
135	10.3E 00	2.1E 01	14.0E 00	4.3E 01	2.1E 01	2.1E 01	2.1E 01	2.1E 01
136	11.0E 00	2.5E 01	2.5E 01	5.0E 01	2.5E 01	2.5E 01	2.5E 01	2.5E 01
137	5.3E 02	5.3E 02	5.3E 02	1.1E 01	5.3E 02	5.3E 02	5.3E 02	5.3E 02
138	6.3E 01	6.3E 01	6.3E 01	1.3E 00	6.3E 01	6.3E 01	6.3E 01	6.3E 01
139	81.4E 01	15.0E 01	5.0E 01	1.0E 00	5.0E 01	5.0E 01	5.0E 01	5.0E 01
140	47.2E 01	2.7E 02	2.7E 02	5.4E 02	2.7E 02	2.7E 02	2.7E 02	2.7E 02
141	20.1E 01	1.3E 01	1.3E 01	2.7E 01	1.3E 01	1.3E 01	1.3E 01	1.3E 01
142	59.4E 01	2.1E 02	2.1E 02	4.3E 02	2.1E 02	2.1E 02	2.1E 02	2.1E 02
143	29.9E 01	1.6E 01	68.0E 00	3.1E 01	1.6E 01	1.6E 01	1.6E 01	1.6E 01
144	47.1E 00	2.1E 01	10.6E 00	4.2E 01	2.1E 01	2.1E 01	2.1E 01	2.1E 01
145	11.1E 00	26.0E 02	21.4E 01	2.7E 01	1.3E 01	1.3E 01	1.3E 01	1.3E 01
146	9.1E 01	54.5E 01	90.9E 01	1.0E 00	9.1E 01	9.1E 01	9.1E 01	9.1E 01
147	2.4E 01	31.7E 01	2.4E 01	4.9E 01	2.4E 01	2.4E 01	2.4E 01	2.4E 01
148	3.9E 00	47.1E 00	3.9E 00	7.8E 00	3.9E 00	3.9E 00	3.9E 00	3.9E 00
149	36.7E 00	8.3E 01	8.3E 01	1.7E 00	8.3E 01	8.3E 01	8.3E 01	8.3E 01
150	83.0E 00	2.1E 01	12.0E 00	4.2E 01	25.3E 00	2.1E 01	2.1E 01	2.1E 01
151	3.4E 01	3.4E 01	31.2E 00	6.8E 01	3.4E 01	3.4E 01	3.4E 01	3.4E 01
152	1.1E 00	1.1E 00	24.0E 00	2.2E 00	1.1E 00	1.1E 00	1.1E 00	1.1E 00
153	2.0E 01	2.0E 01	2.0E 01	4.1E 01	2.0E 01	2.0E 01	2.0E 01	2.0E 01
154	3.3E 01	3.3E 01	3.3E 01	6.7E 01	3.3E 01	3.3E 01	3.3E 01	3.3E 01
155	33.6E 00	53.3E 00	1.4E 01	2.7E 01	1.4E 01	1.4E 01	1.4E 01	1.4E 01
156	35.6E 00	2.5E 01	2.5E 01	4.9E 01	2.5E 01	2.5E 01	2.5E 01	2.5E 01
157	14.0E 00	1.3E 01	1.3E 01	2.6E 01	1.3E 01	1.3E 01	1.3E 01	1.3E 01
158	70.3E 00	2.4E 01	2.4E 01	4.7E 01	2.4E 01	2.4E 01	2.4E 01	2.4E 01
159	1.0E 01	1.0E 01	1.0E 01	3.5E 01	1.0E 01	1.0E 01	1.0E 01	1.0E 01
160	5.1E 00	5.1E 00	5.1E 00	1.0E 01	5.1E 00	5.1E 00	5.1E 00	5.1E 00
161	22.4E 00	1.1E 01	53.0E 01	2.2E 01	1.1E 01	1.1E 01	1.1E 01	1.1E 01
162	45.4E 00	1.7E 01	64.1E 01	3.4E 01	1.7E 01	1.7E 01	1.7E 01	1.7E 01
163	70.6E 00	9.9E 02	17.7E 01	2.0E 01	12.0E 00	9.9E 02	9.9E 02	9.9E 02
164	1.4E 00	1.4E 00	1.4E 00	2.7E 00	82.2E 00	1.4E 00	1.4E 00	1.4E 00
165	2.2E 02	2.2E 02	2.2E 02	4.4E 02	2.2E 02	2.2E 02	2.2E 02	2.2E 02
166	17.7E 01	3.0E 00	32.7E 01	7.7E 00	3.0E 00	3.0E 00	3.0E 00	3.0E 00
167	27.5E 01	4.7E 02	23.7E 01	9.5E 02	4.7E 02	4.7E 02	4.7E 02	4.7E 02
168	59.0E 00	1.2E 01	1.2E 01	2.5E 01	1.2E 01	1.2E 01	1.2E 01	1.2E 01
169	1.3E 01	11.3E 00	34.1E 01	4.7E 01	3.3E 01	3.3E 01	3.3E 01	3.3E 01
170	2.0E 01	2.0E 01	2.0E 01	3.9E 01	2.0E 01	2.0E 01	2.0E 01	2.0E 01
171	1.7E 00	1.7E 00	1.7E 00	3.3E 00	1.7E 00	1.7E 00	1.7E 00	1.7E 00
172	64.5E 01	83.9E 02	3.2E 02	6.5E 02	64.5E 02	3.2E 02	3.2E 02	3.2E 02
173	86.9E 01	62.9E 00	7.1E 01	1.4E 00	7.1E 01	7.1E 01	7.1E 01	7.1E 01
174	66.1E 02	42.3E 01	11.0E 01	6.7E 00	3.3E 00	3.3E 00	3.3E 00	3.3E 00
175	7.7E 01	7.7E 01	7.7E 01	1.5E 00	7.7E 01	7.7E 01	7.7E 01	7.7E 01
176	2.2E 01	2.2E 01	2.2E 01	4.4E 01	2.2E 01	2.2E 01	2.2E 01	2.2E 01
177	11.7E 00	7.7E 02	7.7E 02	1.5E 01	7.7E 02	7.7E 02	7.7E 02	7.7E 02
178	22.3E 01	2.2E 01	10.4E 02	4.3E 01	2.2E 01	2.2E 01	2.2E 01	2.2E 01
179	1.1E 01	60.3E 00	54.5E 00	2.2E 01	1.1E 01	1.1E 01	1.1E 01	1.1E 01
180	1.2E 01	1.2E 01	1.2E 01	2.4E 01	1.2E 01	1.2E 01	1.2E 01	1.2E 01

101	1.3E 01	1.3E 01	1.3E 01	2.7E 01	1.3E 01	1.3E 01	1.3E 01	1.3E 01
102	9.7E 02	9.7E 02	9.7E 02	1.9E 01	9.7E 02	9.7E 02	9.7E 02	9.7E 02
103	81.0E 02	9.1E 02	9.1E 02	1.0E 01	9.1E 02	9.1E 02	9.1E 02	9.1E 02
104	63.5E 00	1.0E 01	1.0E 01	2.0E 01	1.0E 01	1.0E 01	1.0E 01	1.0E 01
105	15.0E 01	44.6E 01	3.2E 02	6.3E 02	1.2E 02	1.2E 02	1.2E 02	1.2E 02
106	5.0E 01	5.0E 01	26.2E 01	1.0E 00	5.0E 01	5.0E 01	5.0E 01	5.0E 01
107	3.0E 01	3.0E 01	3.0E 01	7.7E 01	3.0E 01	3.0E 01	3.0E 01	3.0E 01
108	4.0E 02	38.5E 01	4.0E 02	8.1E 02	4.0E 02	4.0E 02	4.0E 02	4.0E 02
109	3.4E 01	17.9E 00	3.4E 01	7.1E 01	3.4E 01	3.4E 01	3.4E 01	3.4E 01
110	1.2E 01	1.2E 01	1.2E 01	2.4E 01	1.2E 01	1.2E 01	1.2E 01	1.2E 01
111	20.4E 00	15.5E 00	11.9E 00	1.7E 01	0.3E 02	0.3E 02	0.3E 02	0.3E 02
112	16.5E 01	77.7E 00	54.3E 00	1.5E 01	8.0E 02	8.0E 02	8.0E 02	8.0E 02
113	39.9E 01	78.0E 00	1.0E 01	2.1E 01	54.3E 00	1.0E 01	1.0E 01	1.0E 01
114	1.3E 01	1.3E 01	1.3E 01	2.6E 01	1.3E 01	1.3E 01	1.3E 01	1.3E 01
115	3.0E 01	3.0E 01	3.0E 01	7.7E 01	3.0E 01	3.0E 01	3.0E 01	3.0E 01
116	5.2E 02	15.6E 01	29.6E 01	1.0E 01	5.2E 02	5.2E 02	5.2E 02	5.2E 02
117	1.0E 01	82.3E 01	52.1E 01	2.1E 01	1.0E 01	1.0E 01	1.0E 01	1.0E 01
118	1.5E 01	96.9E 01	1.5E 01	3.1E 01	1.5E 01	1.5E 01	1.5E 01	1.5E 01
119	1.9E 01	1.9E 01	14.0E 00	3.0E 01	1.9E 01	1.9E 01	1.9E 01	1.9E 01
120	2.5E 01	2.5E 01	28.1E 01	5.0E 01	2.5E 01	2.5E 01	2.5E 01	2.5E 01
121	11.1E 02	1.3E 00	27.0E 02	-2.6E 00	1.3E 00	-1.3E 00	-1.3E 00	1.3E 00
122	26.7E 00	1.5E 01	40.0E 00	3.0E 01	41.0E 00	-1.5E 01	1.5E 01	1.5E 01
123	0.3E 01	0.3E 01	59.8E 01	-1.7E 00	16.7E 00	-0.3E-01	-0.3E-01	0.3E 01
124	-1.0E 01	-1.0E 01	47.9E 01	-2.1E 01	-1.0E 01	-1.0E 01	-1.0E 01	1.0E 01
125	79.0E 00	39.0E 01	78.7E 01	-1.9E 01	9.3E 02	-9.3E-02	-9.3E-02	9.3E 02
126	33.3E 01	54.2E 01	-2.1E-01	-4.2E-01	-2.1E 01	-2.1E 01	-2.1E 01	2.1E 01
127	77.5E 00	0.3E 01	-0.3E-01	-1.7E 00	38.3E 01	-0.3E-01	-0.3E 01	0.3E 01
128	10.0E 01	2.2E 01	-2.2E-01	-4.3E 01	24.1E 00	-2.2E 01	-2.2E 01	2.2E 01
129	3.3E 01	3.3E 01	-3.3E-01	-6.7E-01	-3.3E 01	-3.3E-01	-3.3E 01	3.3E 01
130	1.9E 01	1.9E-01	-1.9E-01	-3.8E-01	-1.9E 01	-1.9E-01	-1.9E 01	1.9E 01
131	71.6E 00	6.9E-02	-6.9E-02	-1.4E-01	-6.9E-02	-6.9E-02	-6.9E-02	6.9E 02
132	25.2E 01	15.7E 01	-2.6E-01	-5.1E-01	-2.6E-01	-2.6E-01	-2.6E 01	2.6E-01
133	N.S.A.	N.S.A.	N.S.A.	N.S.A.	N.S.A.	N.S.A.	N.S.A.	N.S.A.
134	2.4E 02	-2.4E 02	-2.4E-02	-4.9E 02	-2.4E-02	-2.4E-02	-2.4E 02	-2.4E-02
135	79.5E 02	79.5E 02	-6.6E-02	-1.3E-01	-6.6E-02	-6.6E-02	-6.6E-02	-6.6E-02
136	13.9E 01	19.4E 01	-3.2E 01	-6.5E-01	-3.2E 01	-3.2E 01	-3.2E 01	3.2E 01
137	37.5E 01	1.3E 00	-1.3E 00	-2.5E 00	-1.3E 00	-1.3E 00	-1.3E 00	1.3E 00
138	3.4E 01	-3.4E 01	-3.4E 01	-6.9E-01	-3.4E 01	-3.4E 01	-3.4E 01	3.4E 01
139	2.1E 01	-2.1E 01	-2.1E 01	-4.2E-01	-2.1E 01	-2.1E 01	-2.1E 01	2.1E 01
140	32.0E 02	17.3E 02	18.1E 02	-2.9E 00	-1.4E 00	-1.4E 00	1.4E 00	1.4E 00
141	15.3E 00	73.2E 00	89.9E 01	-4.3E 01	38.9E 00	-2.1E 01	-2.1E 01	2.1E 01
142	1.9E 02	17.4E 02	-1.9E-02	-3.9E-02	-1.9E-02	-1.9E-02	-1.9E 02	1.9E 02
143	91.7E 01	-2.1E 01	-2.1E 01	4.2E 01	-2.1E 01	-2.1E 01	-2.1E 01	2.1E 01
144	N.S.A.	N.S.A.	N.S.A.	N.S.A.	N.S.A.	N.S.A.	N.S.A.	N.S.A.
145	29.6E 02	2.3E 01	55.0E 02	-4.6E 01	-2.3E-01	-2.3E-01	2.3E 01	2.3E 01
146	91.5E 00	30.7E 01	-2.9E-01	-5.9E 01	44.7E 00	2.9E 01	2.9E 01	2.9E 01
147	2.2E 01	30.2E 00	-2.2E-01	4.4E 01	-2.2E 01	2.2E 01	2.2E 01	2.2E 01
148	4.0E 01	4.0E 01	-4.0E 01	-9.5E 01	-4.0E 01	-4.0E 01	4.0E 01	4.0E 01
149	30.6E 01	-5.2E 02	41.5E 01	-1.0E-01	5.2E 02	-5.2E 02	5.2E 02	5.2E 02
150	5.3E 01	-5.3E 01	37.9E 00	-1.1E 00	-5.3E 01	-5.3E 01	5.3E 01	5.3E 01
151	63.0E 01	36.6E 01	62.9E 00	-2.9E 00	-1.4E 00	-1.4E 00	1.4E 00	1.4E 00
152	15.4E 01	25.5E 01	-6.7E-02	-1.3E 01	-6.7E-02	-6.7E-02	6.7E 02	-6.7E 02
153	2.1E 01	29.0E 00	-2.1E-01	-4.2E 01	2.1E 01	-2.1E-01	2.1E 01	2.1E 01
154	1.9E 02	26.4E 02	-1.9E-02	-3.8E 02	-1.9E 02	-1.9E 02	1.9E 02	1.9E 02
155	N.S.A.	N.S.A.	N.S.A.	N.S.A.	N.S.A.	N.S.A.	N.S.A.	N.S.A.
156	N.S.A.	N.S.A.	N.S.A.	N.S.A.	N.S.A.	N.S.A.	N.S.A.	N.S.A.
157	64.1E 01	7.4E 02	20.1E 01	-1.5E 01	7.4E 02	-7.4E 02	7.4E 02	7.4E 02
158	7.4E 02	7.4E 02	95.3E 00	-1.5E 01	7.4E 02	7.4E 02	7.4E 02	7.4E 02
159	7.9E 02	7.9E 02	22.2E 01	-1.6E 01	7.9E 02	-7.9E 02	7.9E 02	7.9E 02

(1) ON THE DESIGNATIONS ARE USED IN CONCENTRATION LISTINGS: (I) 00.0E 01 MEANS THE SAMPLE WAS NOT ANALYZED FOR THE ORGANIC; (II) N.S.A. MEANS NO SAMPLE WAS AVAILABLE FOR ANALYSIS; (III) A NEGATIVE NUMBER, E.G., -4.2E 00, MEANS THAT THE CONCENTRATION WAS BELOW THE DETECTION LIMITS AND (IV) A POSITIVE NUMBER, E.G., 58.2E 01, IS THE MEASURED CONCENTRATION IN UG/L OR UG/KG THE DECIMAL IS ADJUSTED BY THE EXPONENT GIVEN, I.E., 5.0E 01 MEANS 50.