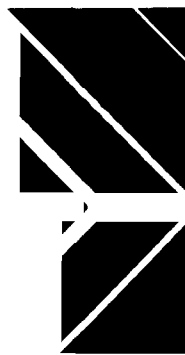
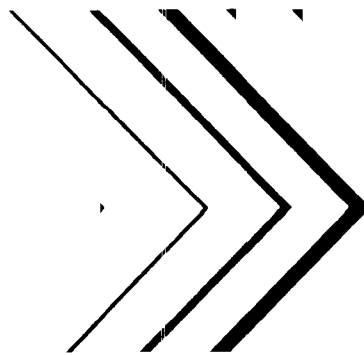
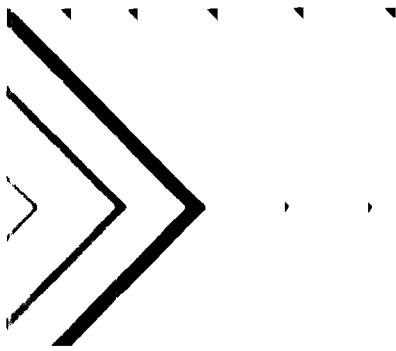




Research and Development

Evaluation of Protocols for Pesticides and PCB's in Raw Wastewater



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EPA-600/2-79-166
November 1979

EVALUATION OF PROTOCOLS FOR PESTICIDES AND PCB'S IN RAW WASTEWATER

by

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FOREWORD

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

Research and development is that necessary first step in problem solution and it involves defining the problem, measuring its impact, and searching for solutions. The Municipal Environmental Research Laboratory develops new and improved technology and systems for the prevention, treatment, and management of wastewater and solid and hazardous waste pollutant discharges from municipal and community sources, for the preservation and treatment of public drinking water supplies, and to minimize the adverse economic, social, health, and aesthetic effects of pollution. This publication is one of the products of that research; a most vital communications link between the researcher and the user community.

The research and the results reported in this document confirm the applicability of the standard EPA protocol to the evaluation of the priority pesticides and PCB's in raw waste waters. The confirmation of this procedure insures that high quality data for pesticides and PCB will be obtained from the planned national surveys of priority pollutants in publicly owned treatment plants.

Francis T. Mayo
Director
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ABSTRACT

The general EPA protocol for screening industrial effluents for priority pollutants (Federal Register 38, No. 75, Part II), has been tested for its applicability to the analysis of the priority pollutant pesticides and PCB's in raw waste water. For this study, samples of raw waste water (RWW) before and after the grit chamber were obtained from the municipal sewage treatment plant in Brockton, Massachusetts and the analysis was performed on the samples as collected and after dosing with 1 - 30 ppb of the priority pollutant pesticides and PCB's.

In testing and validating the protocol as adapted for raw waste water samples, the experimental design was planned to allow the assessment of losses associated with each step of the total procedure. The overall procedure evaluated consists of the following steps: extraction with 15% methylene chloride/hexane with centrifugation to break up the emulsion, concentration by Kuderna-Danish evaporation, removal of interferences by acetonitrile partition, chromatography on Florisil and Sephadex LH-20, and sulfur removal by treatment with mercury. Samples were assayed by gas chromatography using an electron capture detector. Compound confirmation was done with GC/MS using GC conditions identical to those used for GC/ECD analysis.

Recovery data have been obtained for alpha-, beta-, gamma- and delta-BHC, aldrin, dieldrin, endrin, endrin aldehyde, heptachlor, heptachlor epoxide, p,p'-DDD, p,p'-DDE, p,p'-DDT, endosulfan I, endosulfan II, endosulfan sulfate, chlordane, Arochlors 1016, 1254 and 1260 when added to RWW at levels of 1 to 30 ppb. The data obtained show that the Kuderna-Danish evaporation step could be a significant source of sample loss unless the evaporation process is carried out at a fast rate. Treatment with mercury effectively cleans up the extracts with no significant loss of pesticides. Sample clean up on Sephadex LH-20 is recommended as an alternative to the Florisil column clean up procedure, in as much as it offers several advantages and performs essentially the same clean up function as Florisil.

The method tested works well for part per billion determinations, however, there is a need to assess its practical application to raw waste water in as much as the pesticide levels normally found in most raw waste water samples appear to be at parts per trillion level.

This report was submitted in fulfillment of Contract No. 68-01-3857, Task 5, by Arthur D. Little, Inc. under the sponsorship of the U.S. Environmental Protection Agency. This report covers the period June 5, 1978, to October 1978, and work was completed as of November 1978.

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SECTION 1

INTRODUCTION

Publicly owned treatment works are regarded as one of the most significant sources of environmental exposure to priority pollutants. However, insufficient exposure data exists because current methodology does not provide reliable and valid information due to analytical interferences and lack of adequate methods. Thus, the Municipal Environmental Research Laboratory, Environmental Protection Agency sponsored an overall program on the development of protocols for the determination of priority pollutants in raw waste water and sludges derived from municipal sewerage treatment plants.

The experimental program described in this report deal specifically with the evaluation of test protocols for only the priority pollutant pesticides and PCB's in raw waste water. The main thrust of the study was directed at evaluating and/or modifying the standard EPA protocol for priority pollutant pesticides and PCB's. This standard protocol is described in "Sampling and Analysis Procedures for Screening of Industrial Effluents for Priority Pollutants," U.S. EPA Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268, March 1977, revised April 1977 (Federal Register 38, No. 75, Part II).

SECTION 2

CONCLUSIONS AND RECOMMENDATIONS

The overall method for the priority pollutant pesticides and PCB's as tested within the scope of this program, works well at the ppb levels. The clean up methods tested show that the mercury treatment is an effective sulfur removal procedure with minimal sample loss. The Sephadex LH-20 and Florisil column chromatography procedure also works well. Of these two methods, however, the Sephadex LH-20 offers the advantages of providing a cheaper and a less tedious procedure with better sample recoveries, as shown in Table 1, without sacrificing the clean up action of the Florisil. Thus, the preferable clean up sequence is sulfur removal followed by Sephadex LH-20 chromatography.

The issue which needs further resolution relates to the fact that it appears that the actual amounts of the pesticides present in raw waste water are in the ppt level. This information is based on the data for the Brockton samples and other POTW samples obtained from Cincinnati facilities in a separate ADL program also for EPA.* Thus, reliability of the method for ppt analysis needs further investigation if a hazard is presented by ppt levels of pesticides in raw waste water.

*EPA Contract No. 68-01-3857, Task 6, Publicly Owned Treatment Water Works Study, 1978.

TABLE 1. COMPARISON OF ANALYTICAL RECOVERY: SEPHADEX LH-20 VS. FLORISIL

	Sample M6-1*				Sample M6-2*				Sample M6-5**				Sample M6-6**			
	MC/ Hexane	Sephadex	Florisil	MC/ Hexane	Sephadex	Florisil	MC/ Hexane	Sephadex	Florisil	MC/ Hexane	Sephadex	Florisil	MC/ Hexane	Sephadex	Florisil	MC/ Hexane
α -BHC	93	90	65	87	86	67	87	86	67	87	85	65	88	84	68	
λ -BHC	95	91	70	89	87	68	92	90	68	92	90	68	92	97	71	
Heptachlor	76	49	62	70	48	63	62	46	61	63	46	61	63	55	61	
Δ -BHC	94	73	67	89	80	71	108	72	64	101	84	76	101	84	76	
Heptachlor Epoxide	82	89	73	72	89	69	100	83	69	96	81	71	96	81	71	
Endosulfan I	64	85	79	58	75	74	75	87	73	72	94	81	72	94	81	
DDE	71	60	70	53	54	62	79	63	63	75	73	63	75	73	63	
Endrin	86	89	95	79	84	66	83	83	65	82	94	76	82	94	76	
DDD	94	94	72	93	100	70	92	81	64	94	99	57	94	99	57	
DDT	73	55	79	60	61	74	70	50	60	62	65	50	62	65	50	
Endrin aldehyde	37	32	13	40	28	13	13	4	5	13	3	4	13	3	4	
Endosulfan sulfate	99	100	21*	91	90	17*	86	102	25*	87	105	21*	87	105	21*	
Arochlor 1260	114	57	65	114	88	72	141	98	64	124	82	68	124	82	68	

* These samples were stored 0 hours before extraction

** These samples were stored 48 hours at 40°F before extraction

Δ This value includes the sum found in the 6%, 15% and 50% ether/petroleum ether fractions

SECTION 3

APPROACH AND GENERAL EXPERIMENTAL CONDITIONS

PROCEDURE AND APPROACH

The basic procedure which has been evaluated and verified as applicable to raw waste water (RWW) samples consists of the following steps: extraction of the pH7 RWW with 15% methylene chloride/hexane and centrifugation to break up the emulsion, sample concentration by Kuderna-Danish evaporation, and analysis by gas chromatography with an electron capture detector. Removal of interferences by four techniques, i.e., acetonitrile partition, chromatography on Florisil and on Sephadex LH-20, sulfur removal by mercury treatment, was also investigated.

The experiments were designed such that GC analysis was carried out at each appropriate interim stage in order to identify the various steps in the procedure where sample loss could occur and the magnitude of such losses. This interim monitoring was possible for samples spiked with 30 µg/liter of the chlorinated priority pesticides (except chlordane and toxaphene) because of their high sensitivity. The basic methodology and approach adapted is illustrated in Figure 1.

ANALYTICAL CONDITIONS AND GROUPING OF PESTICIDES

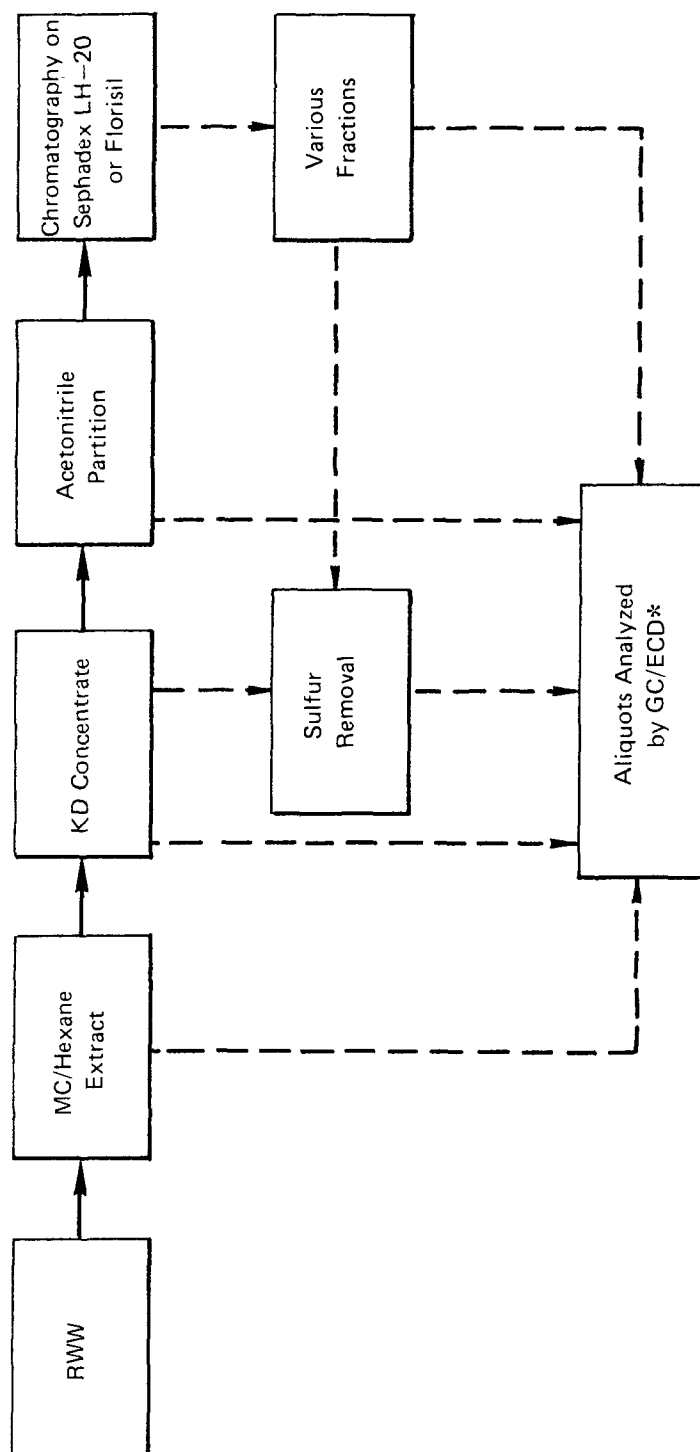
The primary analytical instrument used for these studies was a Perkin Elmer Model 900 gas chromatograph with a Ni-63 electron capture detector. A Spectra Physics System 1 computing integrator was generally used for quantitation except for cases where the interferences cause erroneous integration, in which case peak height or peak area measurement by planimetry was used as necessary. The other operating conditions are as follows:

Column 1: Glass 180 cm x 2-mm ID, packed with 1.5% SP 2250/1.95%
SP 2401 on 100/120 mesh Supelcoport

Carrier gas: 95% Argon/5% methane at a column flow rate of 45 ml
per minute plus auxiliary flow rate of 25 ml per
minute through detector.

Column Temperature: 195°C

Detector Temperature: 310°C



*Selected samples analyzed by GC/MS for confirmation.

Figure 1. Diagrammatic representation of general approach and methodology evaluated.

Injection Port Temperature: 295°C

Sample Aliquot Injected: 2 microliters

Unless otherwise specified, this instrumental setup was used for the various analyses. At the operating conditions described above, the sixteen pesticides, which are pure compounds (99%), give a chromatographic resolution pattern indicated by the relative retention times (RRT) shown in Table 2. Note that heptachlor epoxide was used as the reference compound instead of the commonly used aldrin because heptachlor epoxide appeared to be more stable than aldrin under the conditions used for these studies. As the RRT values indicate, complete resolution of the sixteen pure pesticides is not achieved with the 180 cm x 2-mm ID column used. Compound confirmation was done with GC/MS using GC conditions identical to those used for GC/ECD analysis.

A Hewlett Packard Model 5840A equipped with an electron capture detector and a glass column, 180 cm x 4 mm ID, packed with 1.5% SP 2250/1.95% SP 2401 on 100/120 mesh Supelcoport (designated as Column 2) was also used in a few experiments in this program. The RRT values of the compounds of interest in this column are also shown in Table 2; only two of the 16 pesticides are not resolved by this column.

Two pesticide groupings designated as Mix 2 (M2) and Mix 3 (M3) were used for most of this study. The composition of these two mixtures is indicated in Table 2; each mixture consists of chromatographically resolved peaks as shown in Figures 2 and 3. The other pesticides, chlordane and toxaphene, and the PCB's, which are by themselves complex mixtures, were each studied as single compounds. As a reference point, representative chromatograms of chlordane, toxaphene, Arochlors 1016, 1242, 1254 and 1260 are shown respectively in Figures 4 - 8.

The chemical formula of the priority pollutant pesticides are illustrated in Appendix A.

As a final permutation, two other mixtures designated as Mix 5 (M5) and Mix 6 (M6) were also used. M5 contains all the sixteen pure pesticides while M6 contains 12 pesticides and Arochlor 1260 in approximately equal amounts. The GC/ECD chromatogram of M6, shown in Figure 9, illustrates that when the Arochlor is present in the sample at concentrations equal to that of the pesticides, the typical Arochlor 1260 chromatographic pattern (see Figure 8) is hidden by the pesticide peaks. Only the major Arochlor 1260 peak No. 10 (retention time = 9 minutes) is discernible in the M6 reference standard solution; this peak corresponds to the peak No. 13 in Figure 9 and was used to estimate the Arochlor 1260 content of the various M6 samples.

The Mix 5 (M5) samples which contain the sixteen pesticides were analyzed using two different instruments (i.e., the Perkin Elmer Model 900 and a Hewlett Packard Model 5840A gas chromatographs) and glass columns of the same length but different internal dimensions

TABLE 2. LIST OF PRIORITY POLLUTANT PESTICIDES AND PCB'S

Pesticides and PCB's	RRT [*]	Composition of mixtures used		Column Δ RRT
		2	3	
α -BHC	0.38	●	x	0.37
λ -BHC (lindane)	0.47	●		0.46
β -BHC	0.54		x	0.52
Heptachlor	0.56	●		0.56
Δ -BHC	0.62	●		0.60
Aldrin	0.68		x	0.67
Heptachlor epoxide **	1.00	●	x	1.00
Endosulfan I	1.25	●	x	1.25
p,p' DDE	1.45	●		1.43
Dieldrin	1.52		x	1.53
Endrin	1.85	●		1.85
p,p' DDD	2.22	●		2.21
Endosulfan II	2.22		x	2.21
p,p' DDT	2.66	●		2.62
Endrin aldehyde	2.94	●		2.92
Endosulfan sulfate	3.60	●	x	3.55
Chlordane				
Toxaphene				
Arochlors 1016				
1221				
1232				
1242				
1248				
1254				
1260				

*The column was glass, 180 cm x 2 mm ID packed with 1.5% SP 2250/1.95% SP 2401 on 100/120 mesh Supelcoport.

**RRT reference point

Δ - Glass, 180 cm x 4 mm ID packed with same column packing as above.

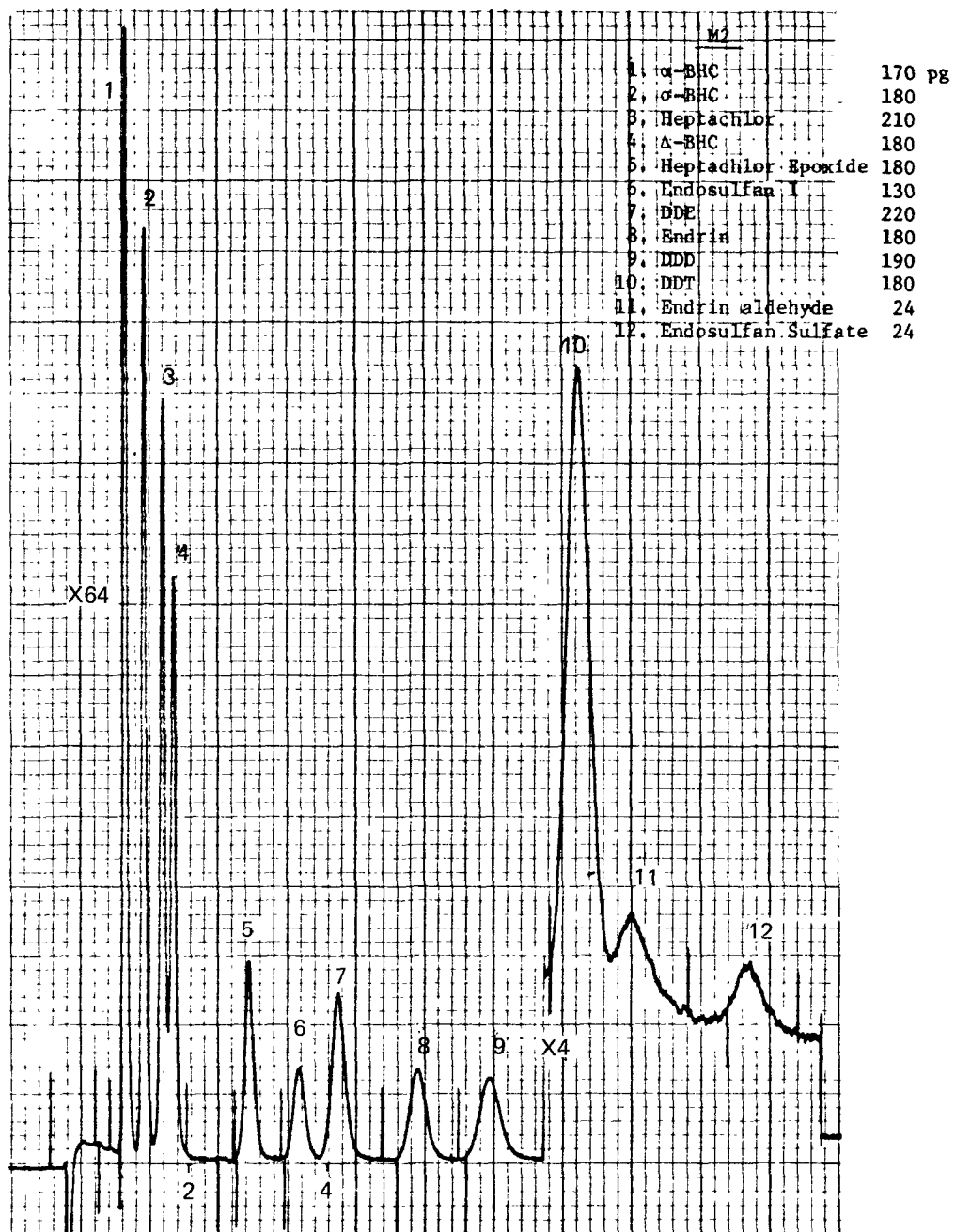


Figure 2. Chromatogram of mix 2 (M2).

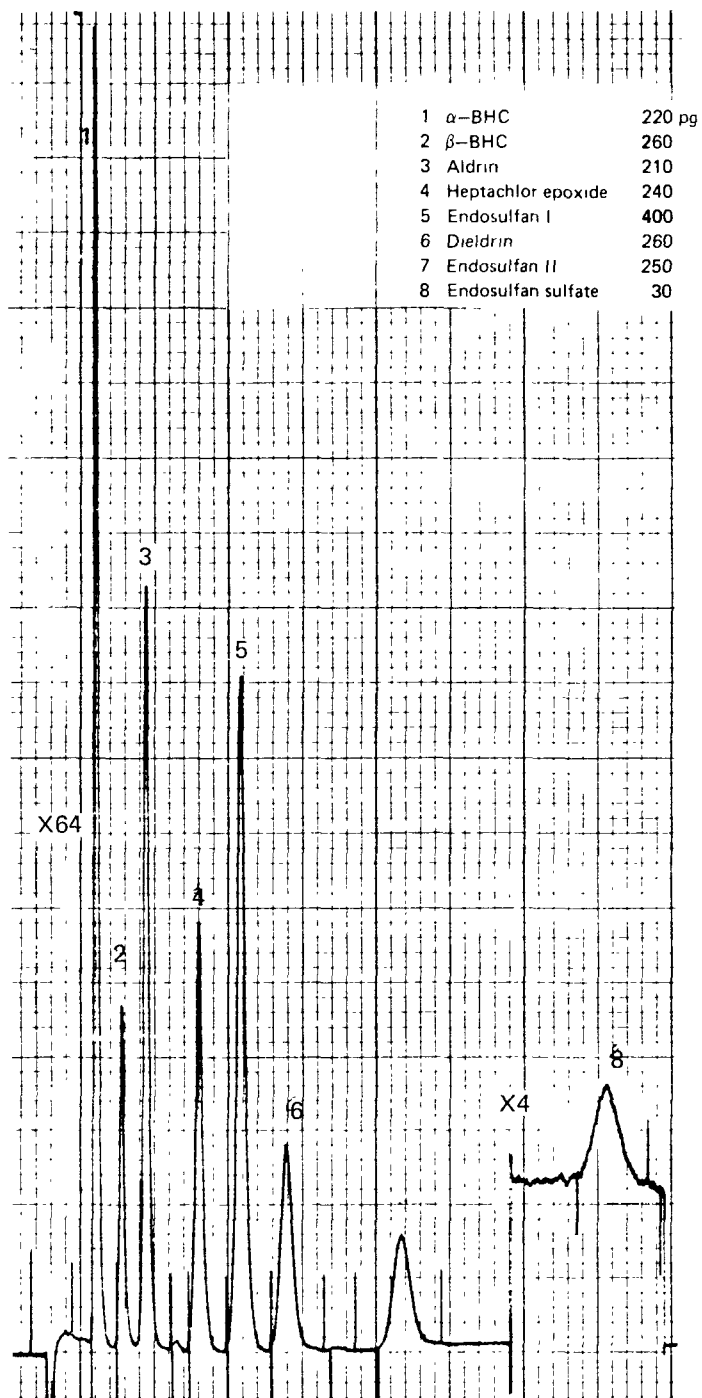


Figure 3. Chromatogram of mix 3 (M3).

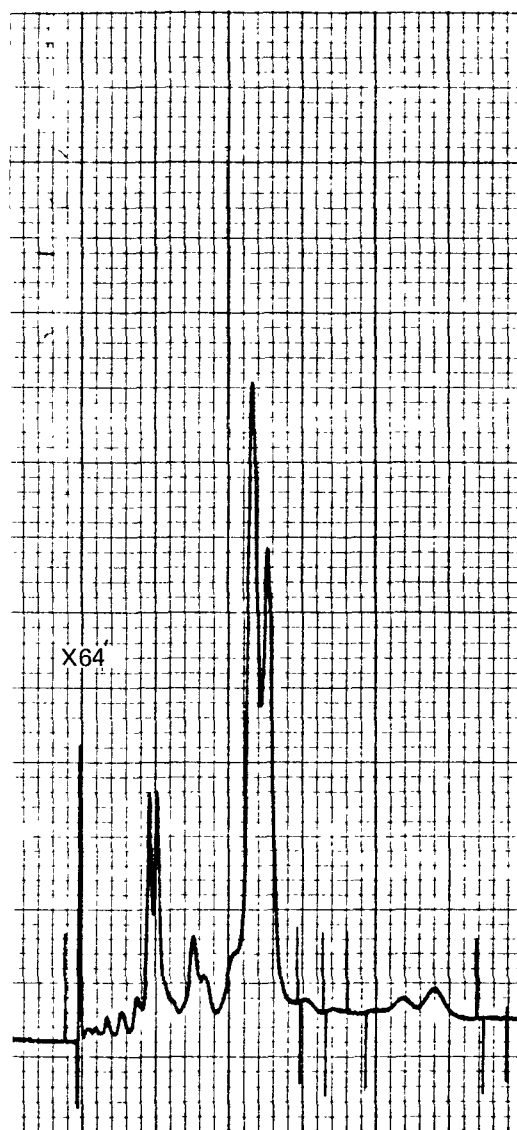


Figure 4. Chromatogram of Chlordane (2.4 ng).

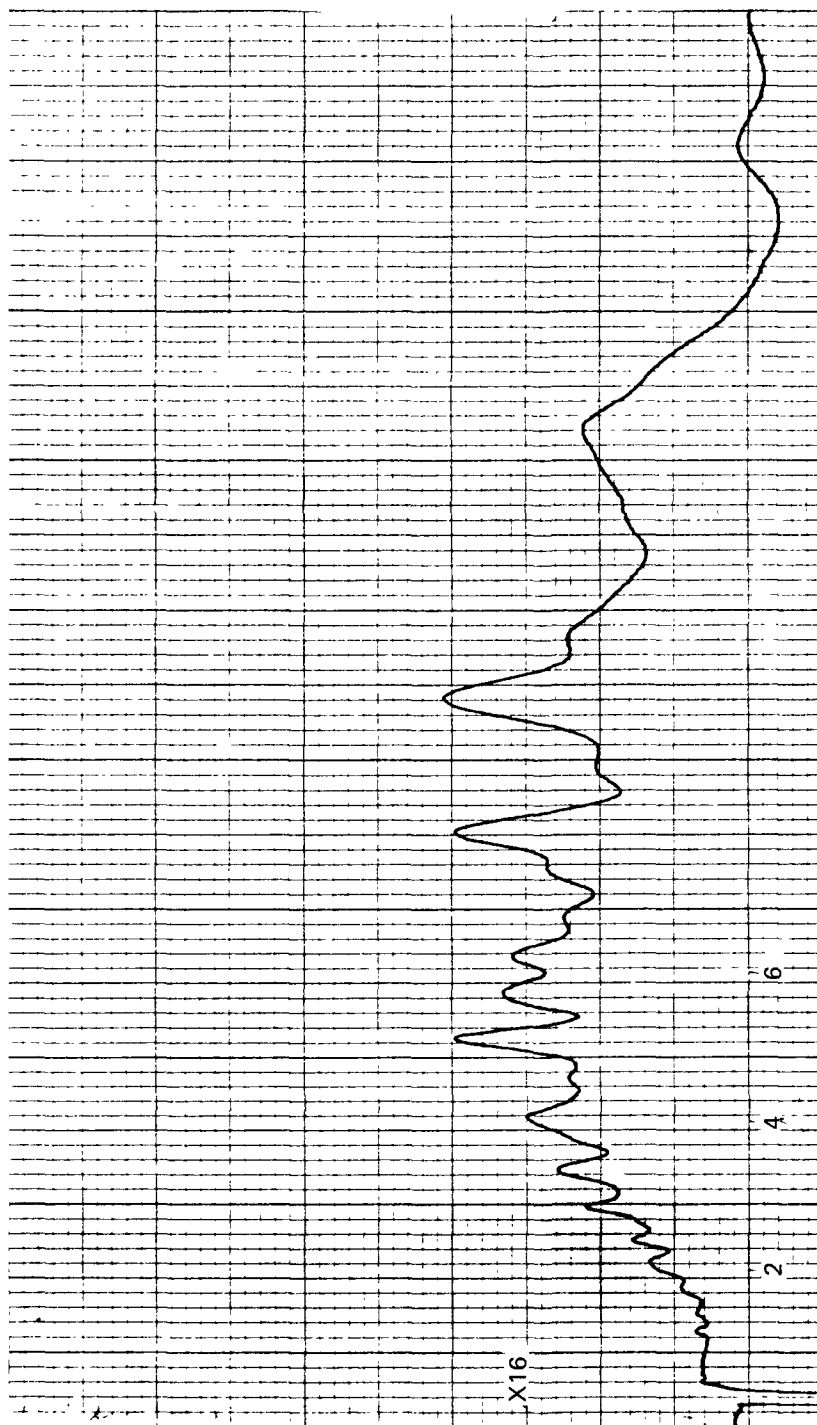


Figure 5. Chromatogram of toxaphene (7.5 ng).

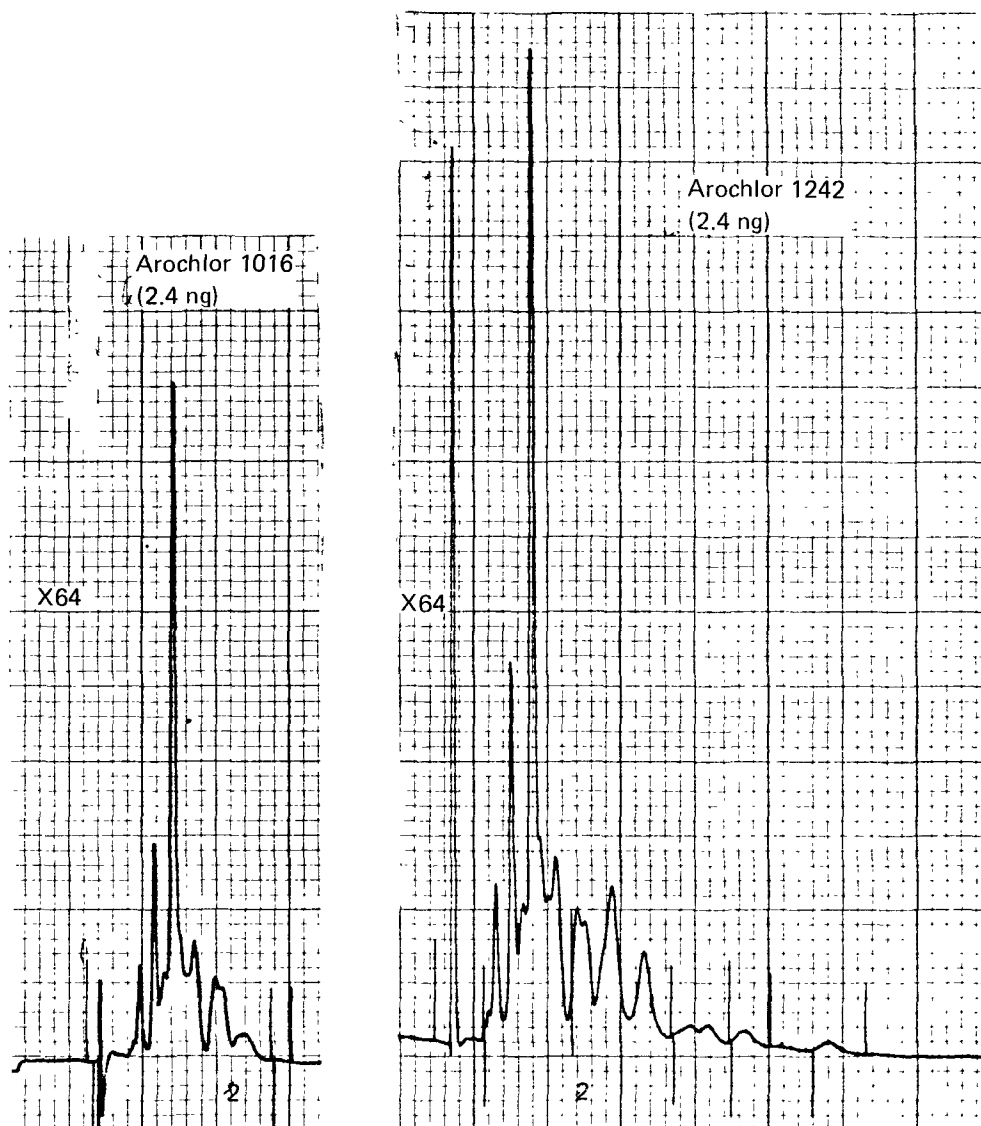


Figure 6. Chromatograms of Arochlors 1016 and 1242.

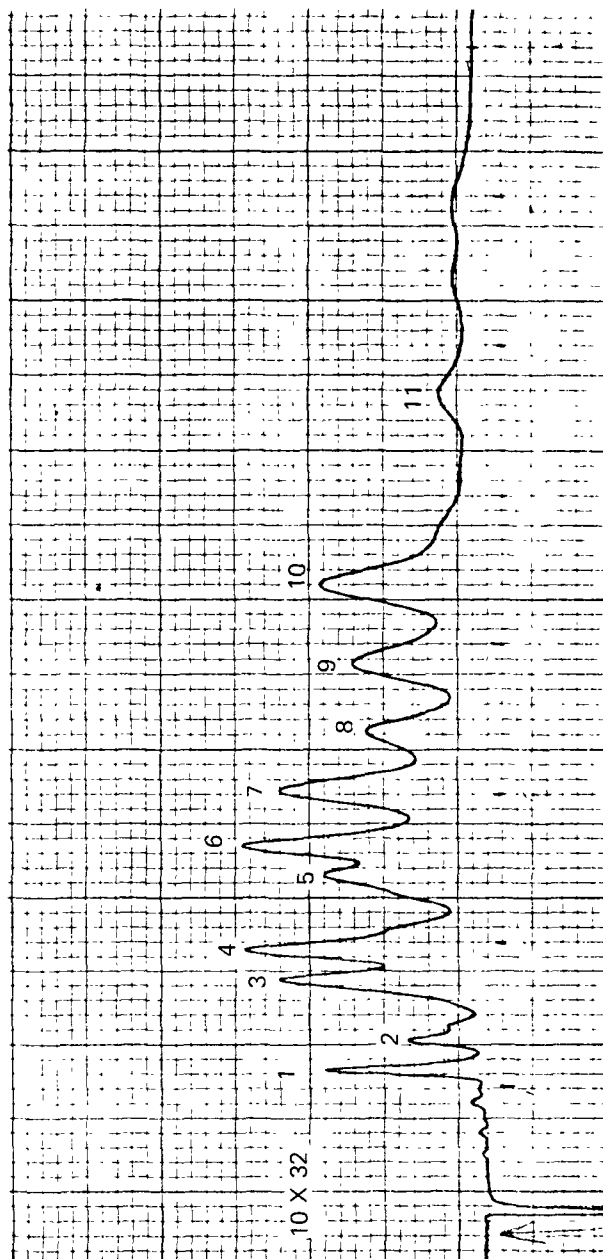


Figure 7. Chromatogram of Arochlor 1254 (1.2 ng).

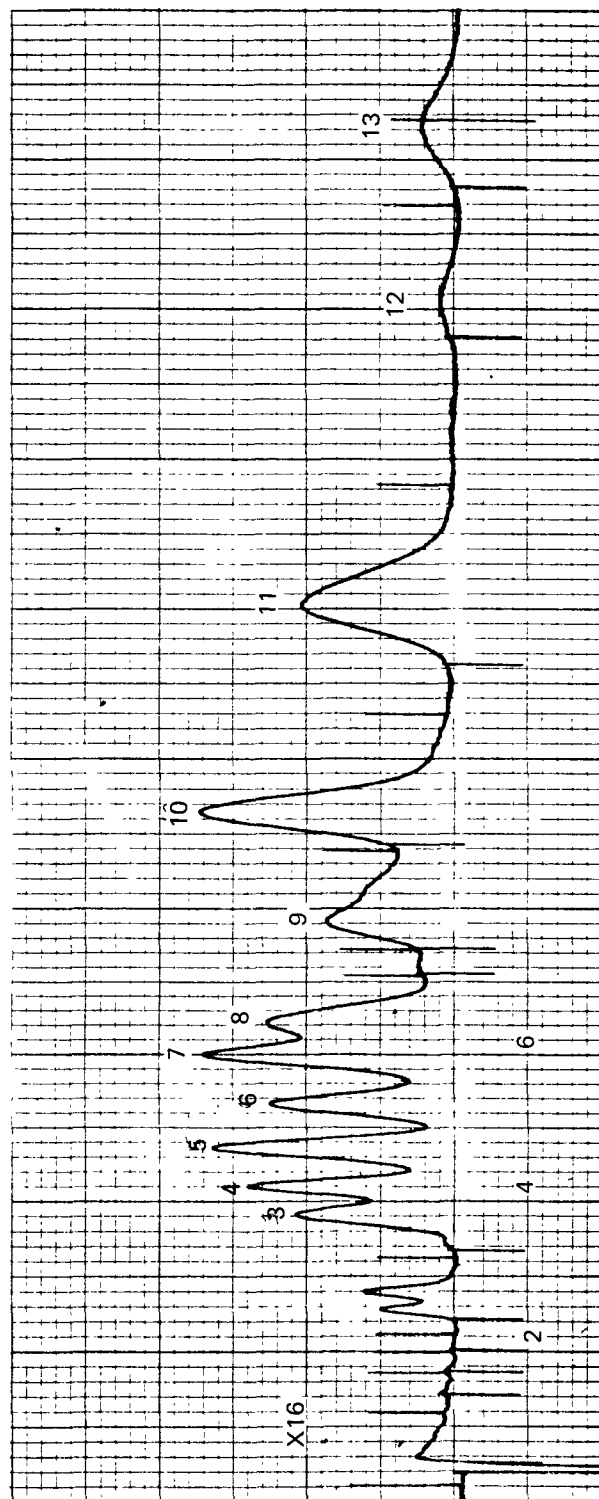


Figure 8. Chromatogram of Arochlor 1260 (1.8 ng).

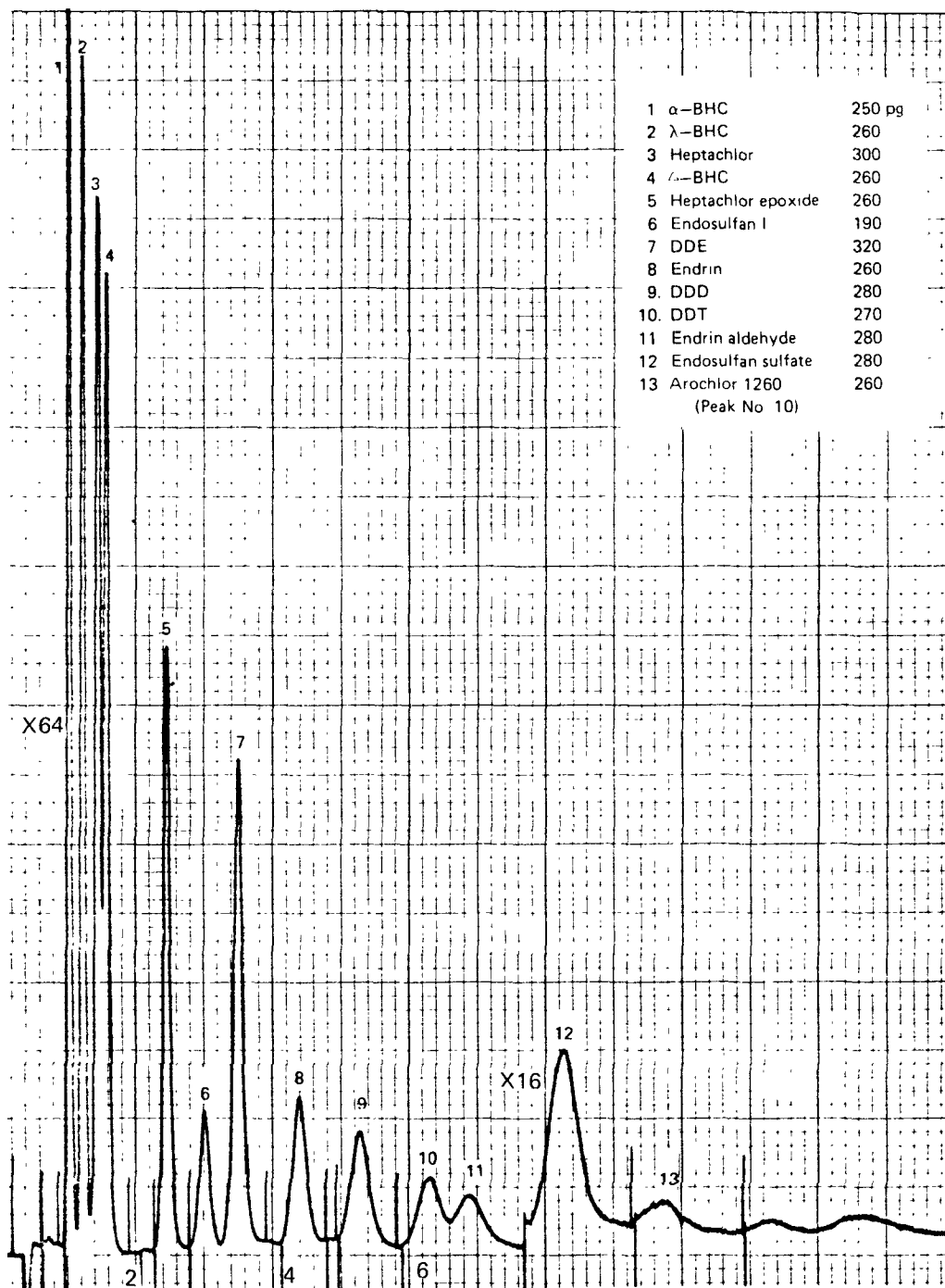


Figure 9. Chromatogram of Mix 6 (M6).

(2-mm ID vs 4-mm ID) packed with the same column packing described earlier. Representative chromatograms obtained for these two conditions are shown in Figures 10 and 11. In the 2-mm ID column (Figure 10), only thirteen peaks are observed because of three pairs of unresolved pesticides, while the 4-mm ID column gives complete resolution of the sixteen pesticides with the exception of DDD and endosulfan II which elutes together.

Various samples of raw waste water were spiked with these pesticide mixtures or with single entities of the complex compounds at test levels of 10 - 30 $\mu\text{g/liter}$ and 1 - 5 $\mu\text{g/liter}$.

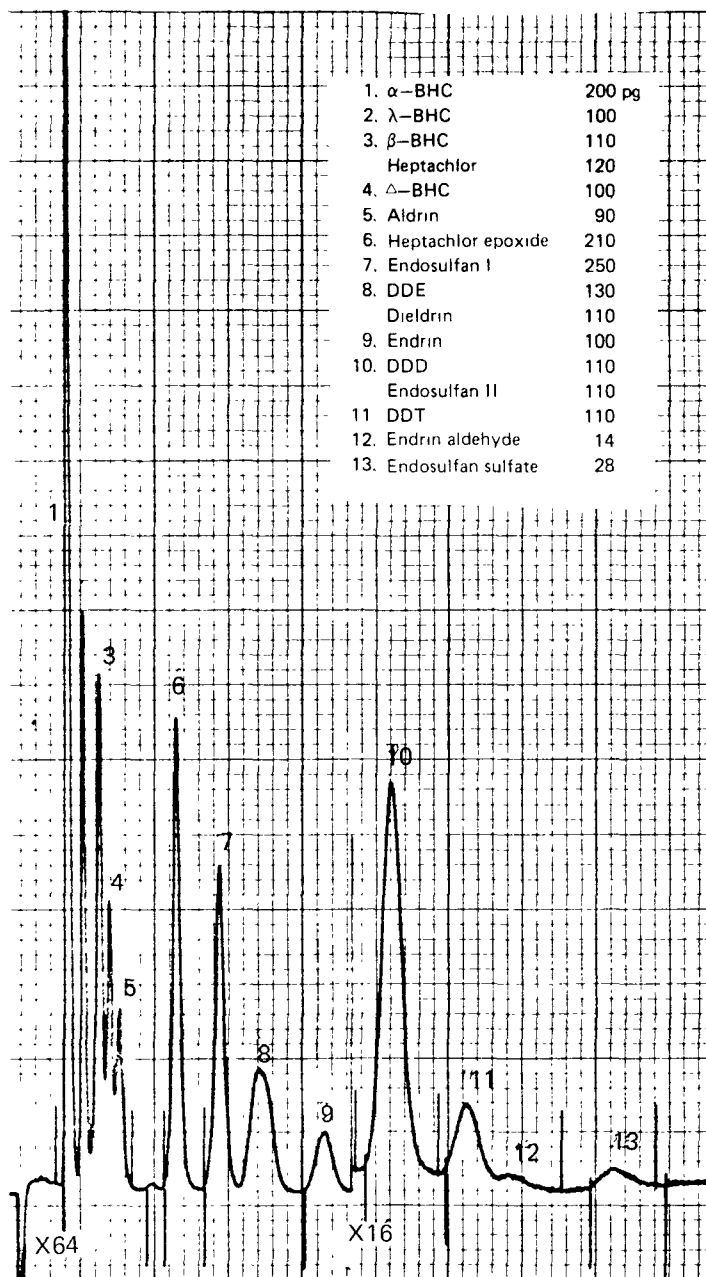


Figure 10. Chromatogram of Mix 5 (M5) using Perkin Elmer 900, 180 cm x 2 mm ID column.

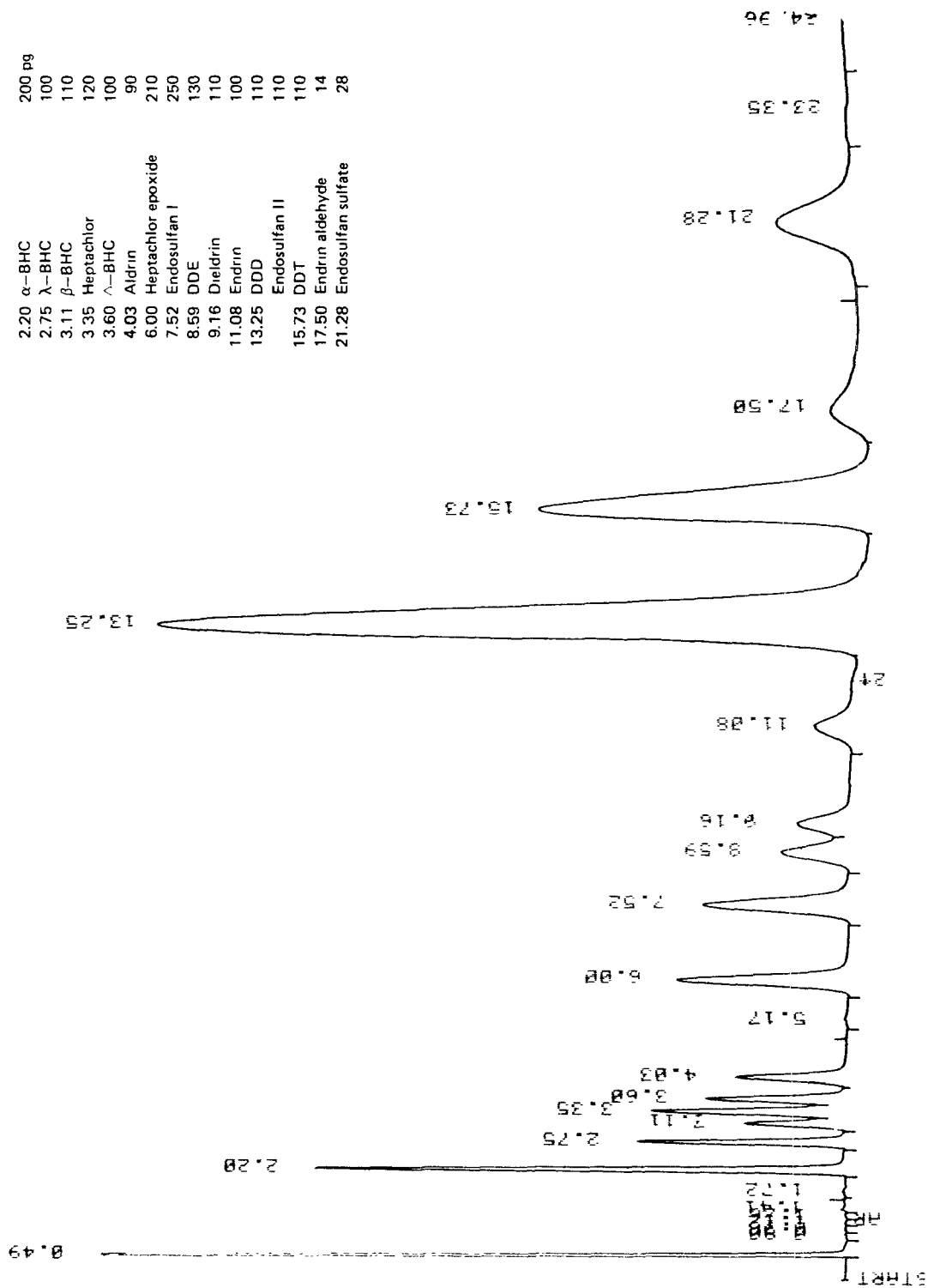


Figure 11. Chromatogram of Mix 5 (M5) using Hewlett Packard Model 5840 A, 180 cm x 4 mm ID column.

SECTION 4

RESULTS AND DISCUSSION

RAW WASTE WATER SAMPLES

Samples were collected at the Municipal Sewerage Treatment Plant in Brockton, Massachusetts. The treatment facilities are of the activated sludge type, and include units for grit removal, comminution and screening, primary settling using longitudinal tanks, aeration using mechanical aerators, final settling tanks of the circular type with provisions for rapid removal of returned sludge, chlorination and sludge treatment including thickening of the waste activated sludge by flotation process, digesters, counter-current elutriation and vacuum filtration. Sludge gas is used for heating the digesters, the digester filter building and the operating building.

Brockton has small industries and many of the industries, which include a tannery and a large laundry business, now empty into the sewerage system. Of the current total population of 85,000, only 77,000 are connected to the sewer system. The average flow is 12 million gallons per day (MGD) with a maximum flow capacity of 30 MGD.

Samples of raw waste water (RWW), before and after the grit chambers, were collected on 6/8/78, 7/11/78 and 7/25/78; these samples were used in the various spiking and recovery experiments. Of these three samples, the "cleanest" was the 6/8/78 samples which were collected soon after a rainfall of 0.35 inches; the other two sampling dates were planned such that there was no rainfall for at least five days before the sampling date.

Various aliquots of the RWW samples were extracted to serve as blanks and controls for the respective spiked samples. Typically, 0.5 liter of RWW was extracted with 15% methylene chloride/hexane, concentrated to 10.0 ml and a 2.0 microliter aliquot was analyzed by GC/ECD. Representative chromatograms of the "before" and "after" grit chamber RWW samples collected during these three dates are shown in Figures 12 and 13 (6/8/78), 14 and 15 (7/11/78), and 16 and 17 (7/25/78). There appears to be no significant difference between the "before" and "after" the grit chamber samples. The chromatograms also illustrate that the blank levels became progressively worse as the sampling period moved deeper into the summer.

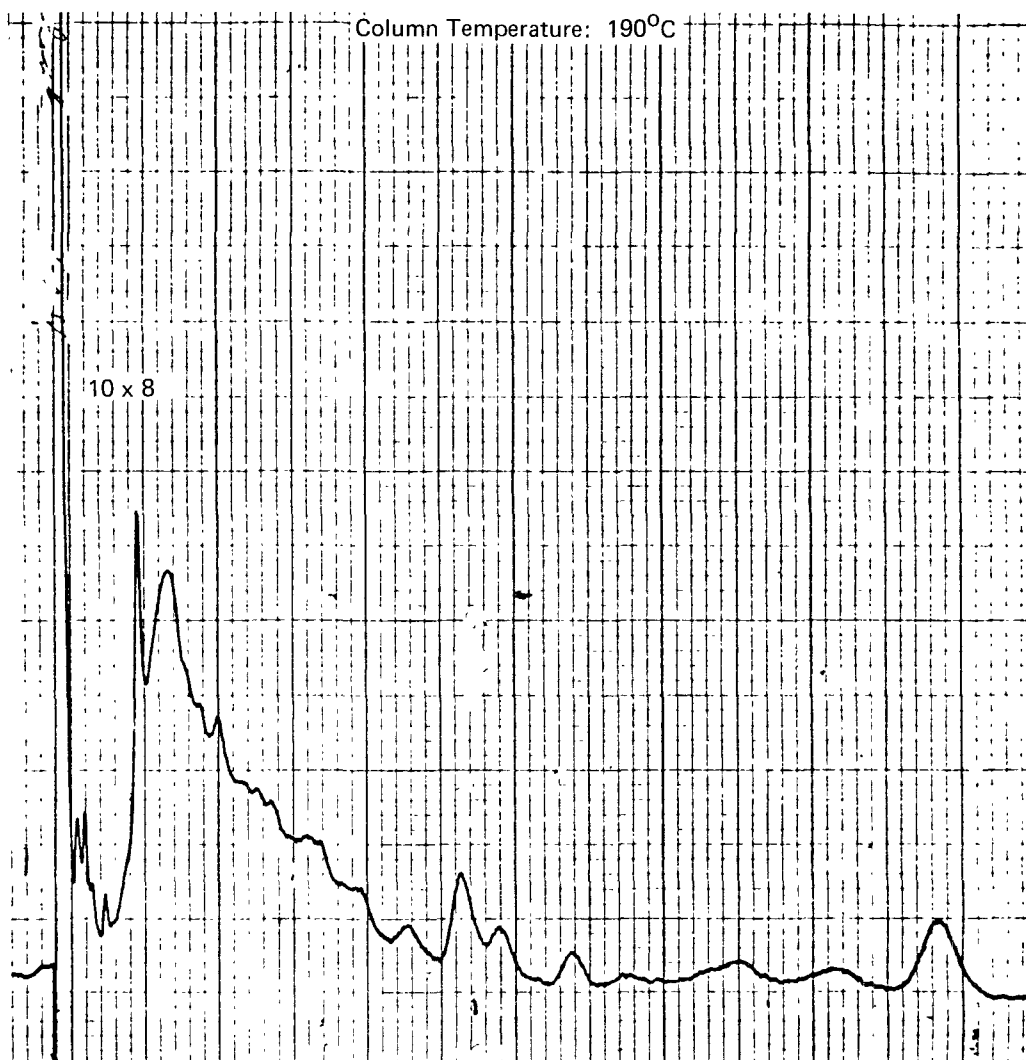


Figure 12. Chromatogram of extract of RWW before grit chamber (sample 6/8/78).

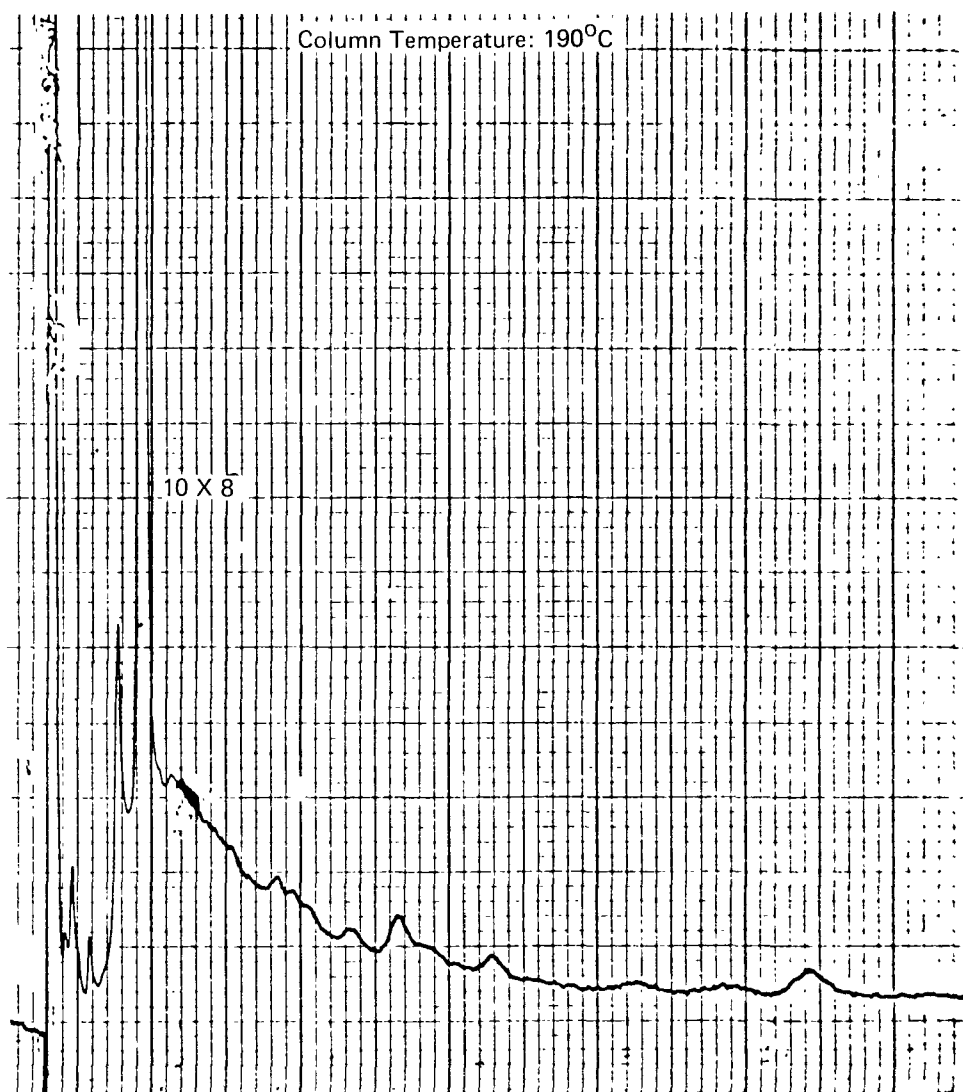


Figure 13. Chromatogram of extract of RWW after grit chamber (sample 6/8/78).

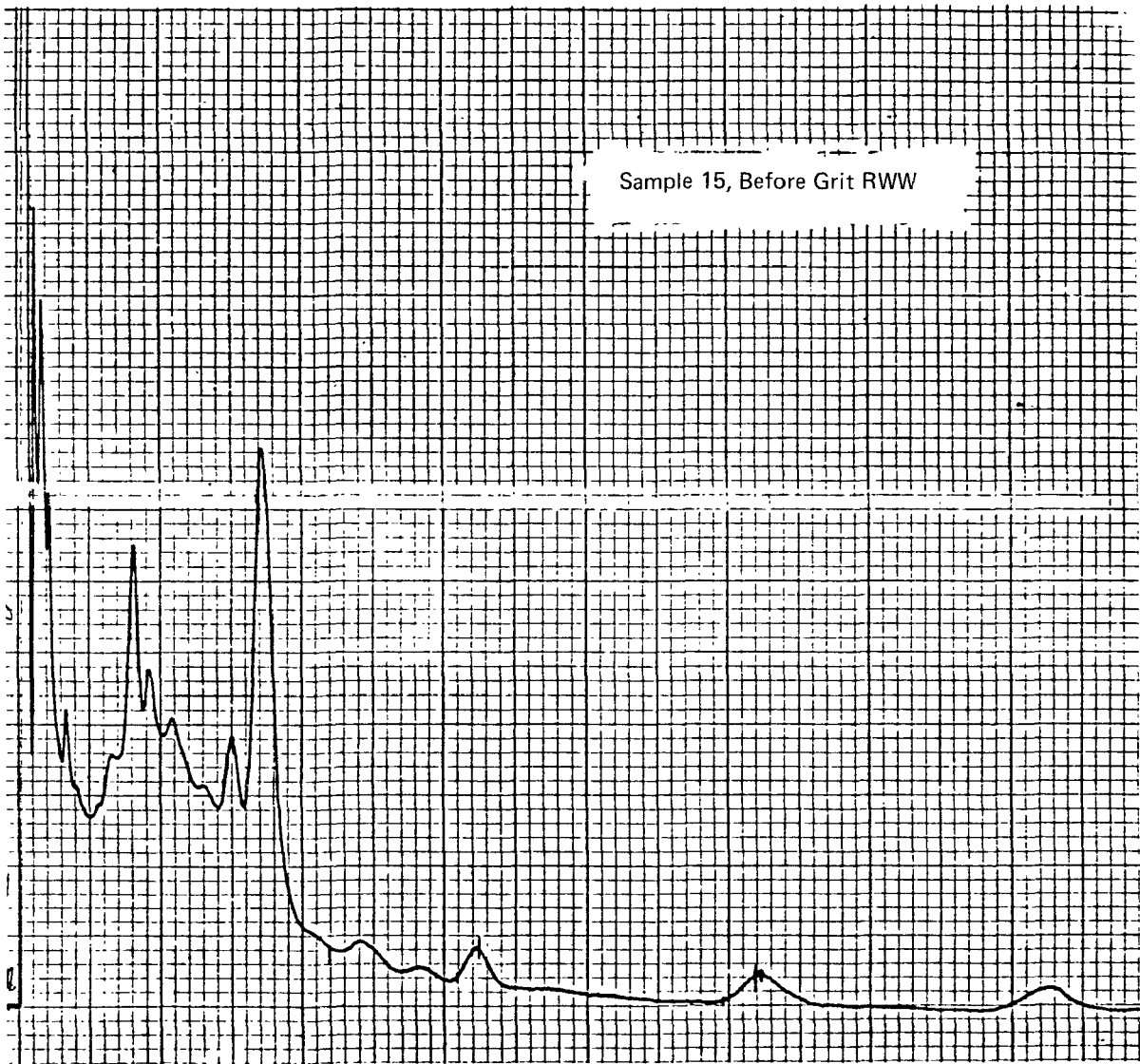


Figure 14. Chromatogram of extract of RWW before grit chamber (sample 7/11/78).

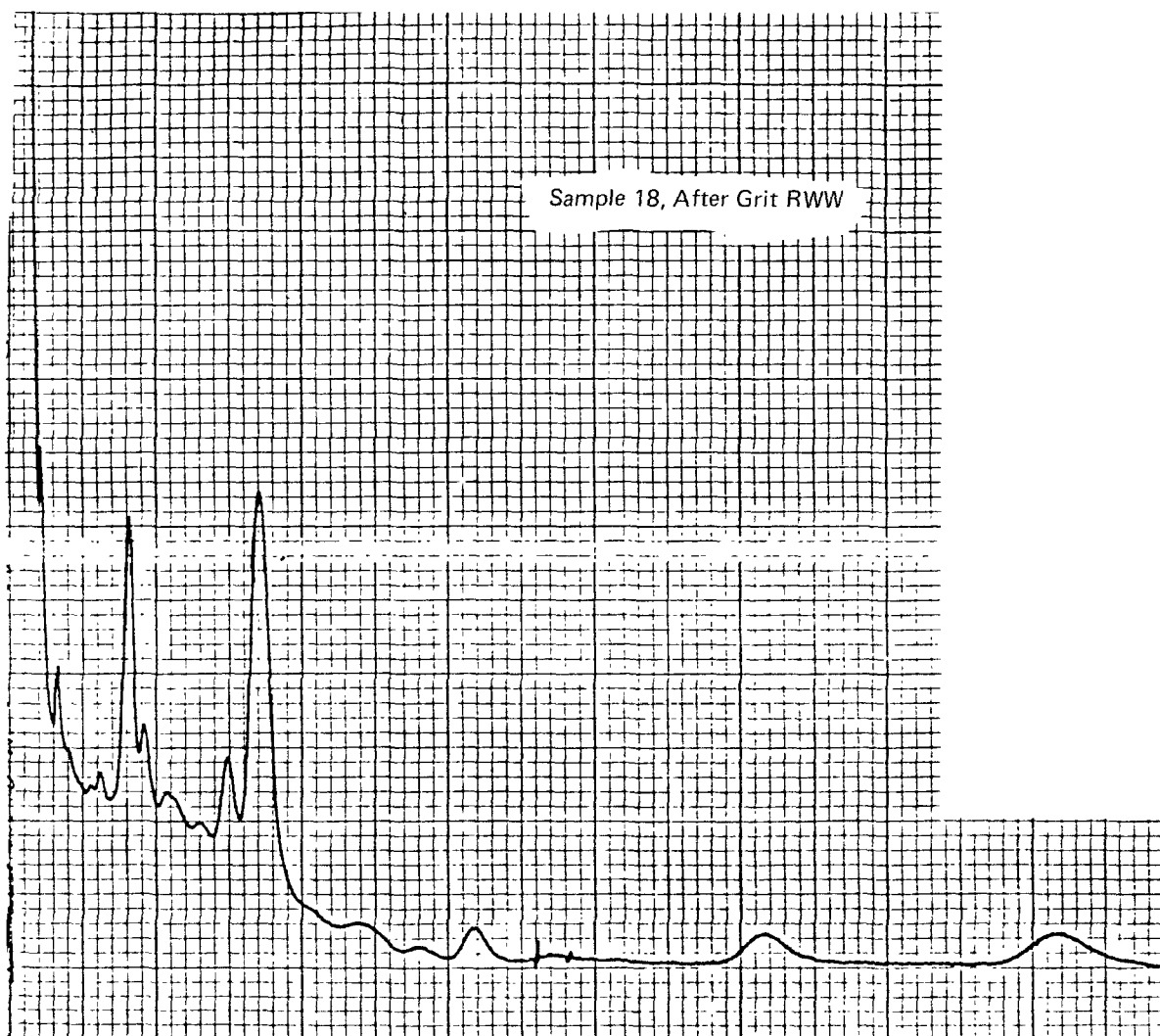


Figure 15. Chromatogram of extract of RWW after grit chamber (sample 7/11/78).

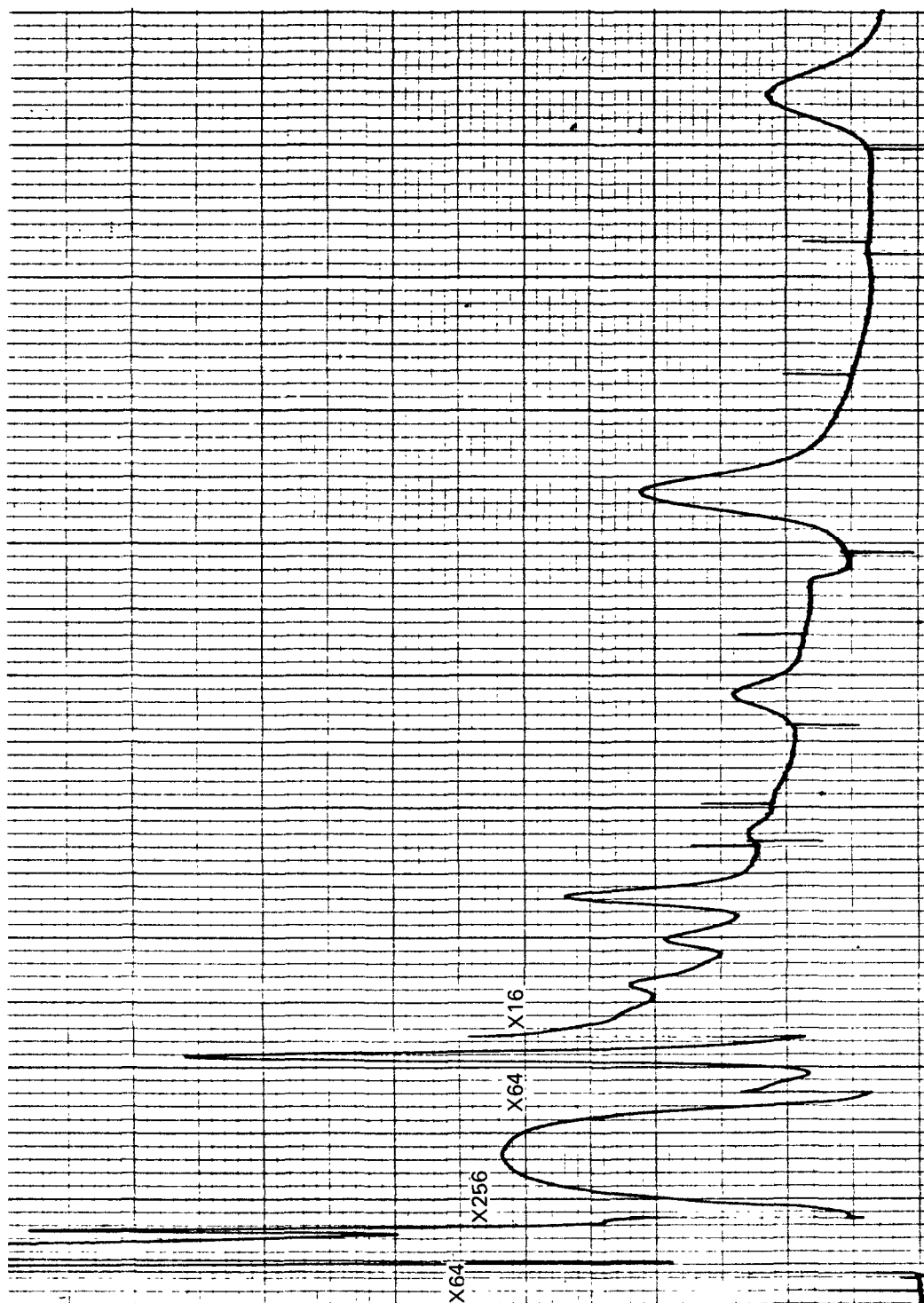


Figure 16. Chromatogram of extract of RWW before grit chamber (sample 7/25/78).

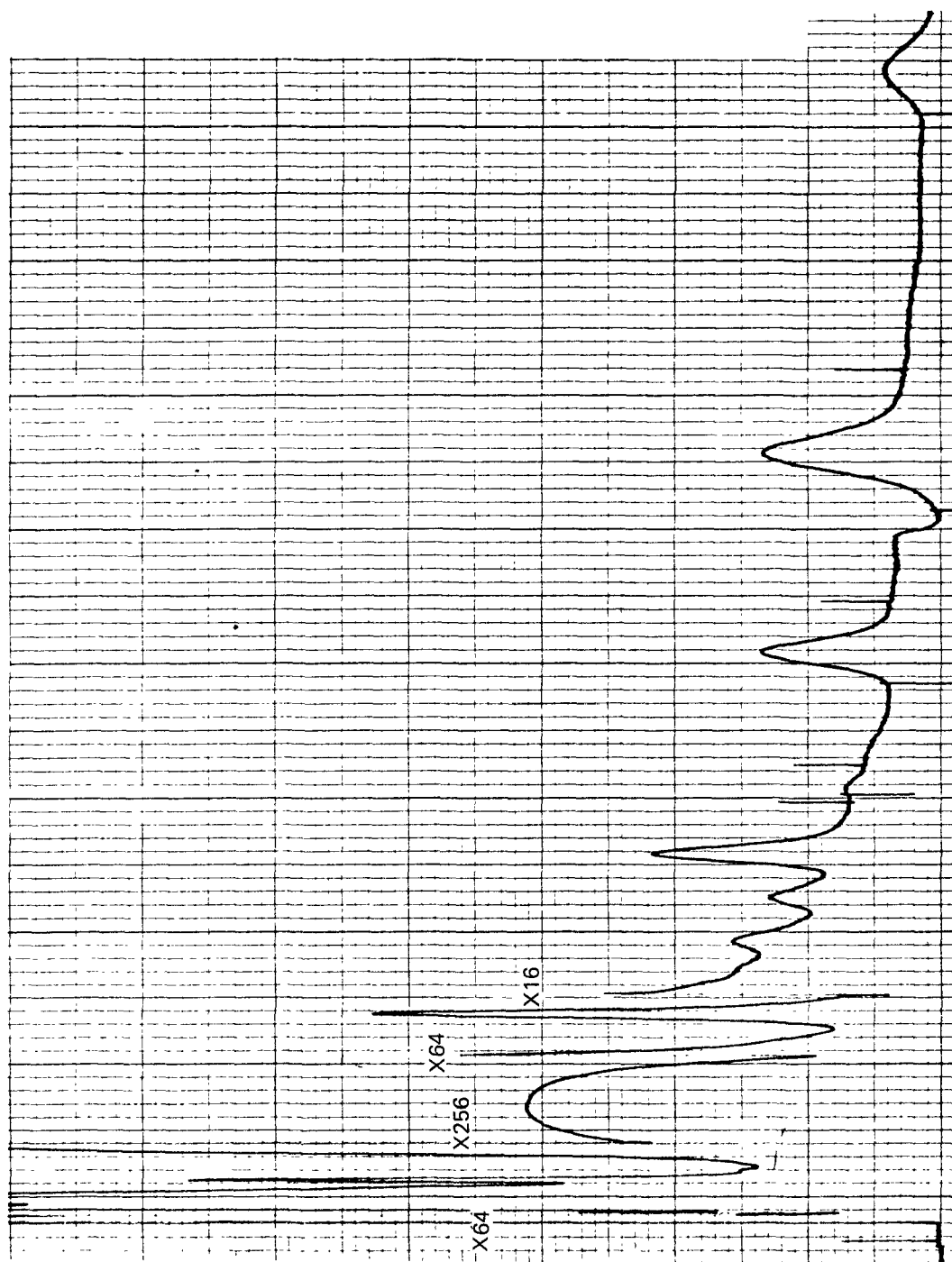


Figure 17. Chromatogram of extract of RW after grit chamber (sample 7/25/78).

The blank levels associated with the 7/25/78 RWW samples presented serious interference problems for studies at the 1 - 5 ppb for the early eluting pesticides such as the BHC's and even at 30 ppb levels for chlordane and Arochlor 1016.

RECOVERY AFTER EXTRACTION AND KD CONCENTRATION

The initial experiments on extraction with 15% methylene chloride/hexane and Kuderna-Danish concentration showed that the KD evaporation step can be a significant source of sample loss unless proper measures are adapted. Some data which indicate this loss are shown in Tables 3 and 4. The data in Table 3 also indicate the importance of the solvent used to dissolve the pesticides and PCB's for spiking, i.e., a water miscible solvent like methanol gave higher recoveries than hexane.

With respect to the KD concentration step, it has been determined that a rapid evaporation rate must be maintained to avoid sample loss. The rapid rate means that concentrating 150 ml of 15% methylene chloride/hexane to 10 ml, including two rinse cycles with 20 - 30 ml hexane per cycle, should take no more than 60 minutes. Good recoveries were obtained when the necessary precautions were followed.

The data in Tables 5 to 9 show that extraction with 15% methylene chloride/hexane gives adequate and good recoveries of the pesticides and PCB's (Arochlor 1254 and 1260) from raw waste water at test levels of 30 $\mu\text{g/liter}$. Tables 5 and 6 also show the comparative data when the methylene chloride/hexane extract was analyzed before and after KD concentration to 10.0 ml. The concentrated samples had to be rediluted 1 to 10 in order to be within the narrow linear working range* of the GC/ECD used. It should be noted that for these early tests where the 6/8/78 RWW samples were spiked with the pesticides at test levels of 30 $\mu\text{g/liter}$, the necessary redilution step dilutes the waste water interferences to insignificant levels and allows for the assay of these samples without the need for sample clean up.

The various data show no significant loss associated with the KD concentration process when a rapid evaporation rate was used. The data also shows that there is no statistically significant difference between the recoveries obtained from the "before" and "after" the grit chamber RWW samples. Thus, the four samples for each experiment set were treated as replicate samples.

The data in Table 10 summarizes the results for the pesticides at test levels of 30 $\mu\text{g/liter}$ and show good recoveries of at least 75% for all the pesticides cited except for heptachlor, aldrin and endrin aldehyde, with respective recoveries of 66, 74 and 47%. The recoveries of endrin aldehyde have been consistently low throughout these studies.

* Note that appropriate calibration curves were used for the quantitative measurements.

TABLE 3. SAMPLE LOSS ON KD: RECOVERY OF ALDRIN, HEPTACHLOR EPOXIDE, DIELDRIN

Sample: 1 liter distilled water spiked with pesticides at 30-50 µg/liter

<u>Sample Code</u>	<u>Aldrin</u>		<u>Heptachlor epoxide</u>		<u>Dieldrin</u>	
	<u>MC/Hex</u>	<u>KD</u>	<u>MC/Hex</u>	<u>KD</u>	<u>MC/Hex</u>	<u>KD</u>
1 (a)	49	10	82	13	66	8
2 (a)	48	32	81	50	77	52
3 (b)	97	NA	101	NA	101	NA
4 (b)	91	NA	99	NA	95	NA

(a) A solution of the pesticides in hexane was used for spiking.

(b) A solution of the pesticides in methanol was used for spiking.

NA Not analyzed.

TABLE 4. SAMPLE LOSS ON KD: RECOVERY OF AROCHLOR 1254

Sample: 1 liter distilled water spiked with 30 µg Arochlor 1254

<u>Quantitative Data</u>	Sample 5		Sample 6	
	<u>MC/Hex</u>	<u>KD</u>	<u>MC/Hex</u>	<u>KD</u>
Peak 1	61	26	82	51
Peak 4	62	27	85	51
Peak 7	56	27	82	53
Peak 10	56	29	80	54

TABLE 5. % RECOVERY OF PESTICIDES FROM RAW WASTE WATER*

Pesticide	Added µg/liter	(CH ₂ Cl ₂ /Hexane Extract, Kuderna-Danish Evaporation)				-----After grit RW-----			
		-----Before grit RW-----		-----After grit RW-----		-----Before grit RW-----		-----After grit RW-----	
		Sample 11 MC/Hex	KD	Sample 12 MC/Hex	KD	Sample 13 MC/Hex	KD	Sample 14 MC/Hex	KD
α-BHC	28	114	89	99	83	78	88	81	101
λ-BHC	30	100	89	96	82	75	87	80	101
Heptachlor	34	55	70	70	62	80	66	76	81
Heptachlor epoxide	29	82	87	87	78	90	78	92	90
Endosulfan 1	22	67	84	82	75	82	75	69	85
p,p' DDE	37	73	72	82	64	80	73	82	81
p,p' DDD	32	84	90	84	83	84	83	84	96
p,p' DDT	30	79	51	51	72	83	79	80	94

* 0.5 liter of raw waste water was used for these experiments.

TABLE 6. % RECOVERY OF PESTICIDES FROM RAW WASTE WATER

Pesticide	Added $\mu\text{g/liter}$	(Sample Size: 0.5 liter)				-----After grit RW-----			
		-----Before grit RW-----		Sample M3-2		Sample M3-3		Sample M3-4	
		Sample M3-1	KD	MC/Hex	KD	Sample M3-3	KD	MC/Hex	KD
α -BHC	28	71	81	70	79	70	79	73	79
β -BHC	32	76	81	74	78	71	75	75	79
Aldrin	26	65	78	67	73	61	71	66	72
Heptachlor epoxide	29	80	88	83	85	79	83	83	85
Endosulfan I	50	82	96	86	92	80	86	86	90
Dieldrin	33	80	80	82	79	82	80	82	80
Endosulfan II	31	92	92	96	94	95	88	90	99
Endosulfan sulfate*	4	106	117	84	83	82	111	112	103

*The values reported have been corrected for a component which behaves like endosulfan sulfate present at ca. 1 $\mu\text{g/liter}$ in the raw waste water.

TABLE 7. % RECOVERY OF VARIOUS PESTICIDES FROM RWW

(Samples analyzed after KD concentration of 15% MC/hexane extract)

Pesticide	Added $\mu\text{g/liter}$	Before grit RWW		After grit RWW	
		M2-5	M2-6	M2-7	M2-8
α -BHC	28	94	90	94	87
λ -BHC	30	88	88	97	87
Heptachlor	34	63	59	80	62
Δ -BHC	30	77	91	101	98
Heptachlor epoxide	29	100	88	100	81
Endosulfan I	22	103	92	104	97
DDE	36	87	73	90	84
Endrin	30	110	104	106	101
DDD	32	92	98	91	87
DDT	30	98	78	109	105
Endrin aldehyde	4	40	35	53	59
Endosulfan sulfate*	4	56	70	75	84

*The reported values have been corrected for a component which behaves like endosulfan sulfate present in the raw waste water at ca. 1 $\mu\text{g/liter}$.

TABLE 8. RECOVERY OF AROCHLOR 1254 FROM RWW

(Sample size: 0.5 liter of RWW spiked at 30 µg/liter)

<u>Sample code</u>	<u>Peak selected for quantitation</u>		
	<u>6</u>	<u>7</u>	<u>10</u>
1254-1*	69	91	89
1254-2*	65	91	85
1254-3**	69	87	82
1254-4**	71	91	81
Average	69	90	84
σ	2.6	2.0	3.6

* RWW, before grit chamber.

** RWW, after grit chamber.

TABLE 9. RECOVERY OF AROCHLOR 1260 FROM RWW

(Sample size: 0.5 liter of RWW spiked at 30 µg/liter)

<u>Sample code</u>	<u>Peak selected for quantitation</u>		
	<u>7</u>	<u>8</u>	<u>10</u>
1260-1*	87	83	62
1260-2*	80	80	87
1260-3**	67	64	61
1260-4**	83	75	73
Average	79	75	71
σ	8.7	8.2	12.1

* RWW, before grit chamber.

** RWW, after grit chamber.

TABLE 10. RECOVERY OF PESTICIDES AND PCB's AT TEST LEVEL OF 30 µg/LITER

(Data based on analysis after KD concentration of Mc/hexane extract)

<u>Pesticide & PCB</u>	<u>Average recovery*</u>	<u>σ</u>
α-BHC	85	6.7
λ-BHC	90	4.7
β-BHC	78	2.5
Heptachlor	66	9.5
Δ-BHC	92	10.7
Aldrin	74	3.1
Heptachlor epoxide	89	7.3
Endosulfan I	95	6.2
DDE	84	7.4
Dieldrin	80	0.5
Endrin	105	3.8
DDD	92	4.5
Endosulfan II	93	4.3
DDT	98	13.8
Endrin aldehyde**	47	11.1
Endosulfan sulfate**	87Δ	21.0Δ
Arochlor 1254	90	2.0
Arochlor 1260	79	8.7

* Each value is based on the average of 4 samples except for α-BHC, heptachlor epoxide, endosulfan I and endosulfan sulfate which are based on 8 samples.

** The samples were spiked at a test level of 4 µg/liter.

Δ The raw waste water samples contain a component with a retention time equal to endosulfan sulfate. The average value was estimated to be 1 µg/liter with a standard deviation of 0.7 or an RSD of 70%; the value for each sample was corrected for this blank value.

SEPARATION PROCEDURES FOR CLEAN UP

The various clean up procedures evaluated are discussed below. The silica gel column clean up procedure suggested in the standard EPA method for separating PCB's from pesticides was not tried because of time and budget limitations and the fact that the data reported indicate that the procedure does not effectively separate the chlorinated pesticides from the PCB's for all practical purposes.

Acetonitrile Partition

A few experiments were carried out to determine the recovery of some pesticides when the clean up procedure described in Appendix B is used. The data in Tables 11 and 12 indicate that there is no significant loss of any of the twelve pesticides tested. The Table 12 data should be compared with individual sample data in Table 7. Observe that the recovery for endrin aldehyde has been consistently low.

Chromatography on Florisil

Sample clean up using the standard Florisil column procedure does not effectively eliminate the major interference from raw waste water which elutes within the first three minutes; this so called "hump" is not pronounced in the raw waste water samples collected on 7/25/78. (See Figures 16 and 17). The chromatograms shown in Figures 18 and 19 illustrate the components which elute in the 6% and the 15% ether/petroleum ether fractions, respectively. The "hump" which elutes in the 6% ether fraction can mask the early eluting pesticides (the BHC's, aldrin, heptachlor and heptachlor epoxide) and PCB's (Arochlors 1016, 1221, 1232, 1242) which would all elute in the 6% fraction. In a later experiment discussed in the following section, it was observed that the "hump" can be eliminated by treatment with mercury to remove sulfur.

Several extracts of raw waste water spiked with various pesticides at levels of 3, 10 and 30 $\mu\text{g/liter}$ together with Arochlor 1260, at 10 $\mu\text{g/liter}$ were chromatographed on Florisil. The 6%, 15%, and 50% ether/petroleum ether fractions were collected, concentrated by KD and analyzed by gas chromatography. The recovery data for these samples are shown in Tables 13 to 15. For the M6 samples reported in Table 15, the sample size chromatographed is only one-half of the total extract (0.5 liter RWW in 10 ml) spiked with 10 $\mu\text{g/liter}$. Thus, a total of 2 to 3 μg of each pesticide and Arochlor 1260 was applied to the column. In addition, the 6% fraction was treated with mercury prior to GC analysis. The data obtained show that 30 - 40% losses of the pesticides and Arochlor 1260 are observed. Endrin aldehyde and endosulfan sulfate (refer to Table 15) show very low values.

The general elution pattern from Florisil is shown in Table 16. Most of the pesticides elute in the 6% fraction. Others, such as endosulfan I, endosulfan II and dieldrin are split in two fractions.

TABLE 11. % RECOVERY OF PESTICIDES: ACETONITRILE PARTITION EXPERIMENTS

Pesticide	Added [*] µg/liter	Trial		
		I	II	III
α-BHC	28	103	106	106
λ- BHC	30	108	103	100
Heptachlor	34	99	96	94
Δ-BHC	30	105	100	97
Heptachlor epoxide	29	101	95	96
Endosulfan I	22	104	97	98
p,p' DDE	37	100	93	94
Endrin	30	106	110	110

*These samples were not carried through the CH₂Cl₂/Hexane extraction nor Kuderna-Danish concentration, but the amount of pesticide added to 10 ml hexane solution used for the subsequent acetonitrile partition is equivalent to that present in 0.5 liter of raw waste water at specified concentrations.

TABLE 12. EFFECT OF ACETONITRILE PARTITION ON PESTICIDE RECOVERY*

<u>Pesticide</u>	<u>Before grit RWW</u>		<u>After grit RWW</u>	
	<u>M2-5</u>	<u>M2-6</u>	<u>M2-7</u>	<u>M2-8</u>
α -BHC	90	87	90	90
λ -BHC	93	90	96	96
Heptachlor	101	83	95	97
Δ -BHC	107	89	107	101
Heptachlor epoxide	98	92	98	98
Endosulfan I	91	89	97	94
DDE	80	73	82	82
Endrin	94	92	98	97
DDD	83	84	90	82
DDT	95	74	93	97
Endrin aldehyde	30	35	35	35
Endosulfan sulfate	95	90	115	110

* These data were obtained on spiked RWW samples which have gone through methylene chloride/hexane extraction, KD concentration, acetonitrile partition and a second KD concentration. Compare with Table 7 data.

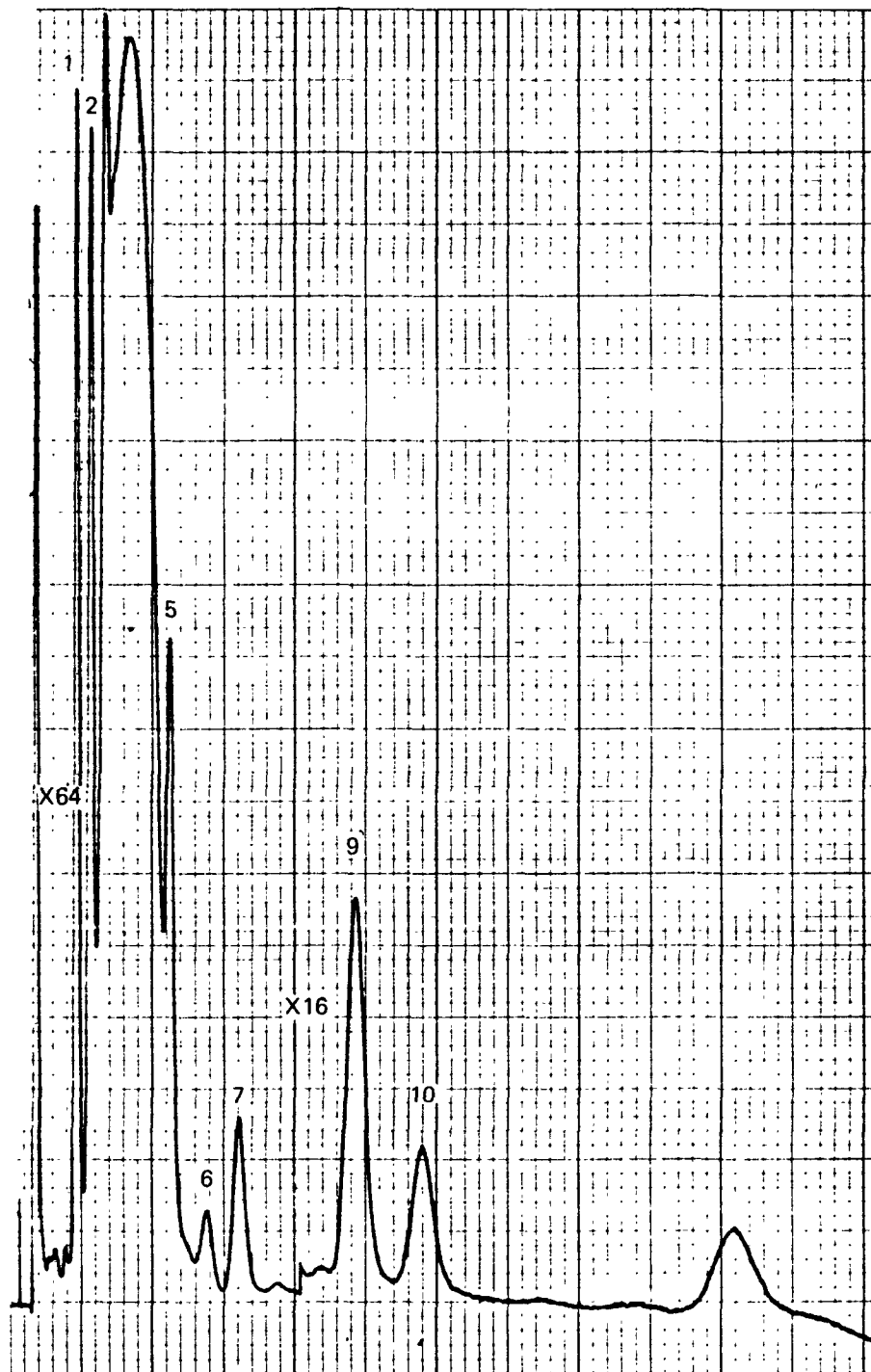


Figure 18. Chromatogram of 6% ether/petroleum ether fraction (sample M2-9, 3 µg/liter).

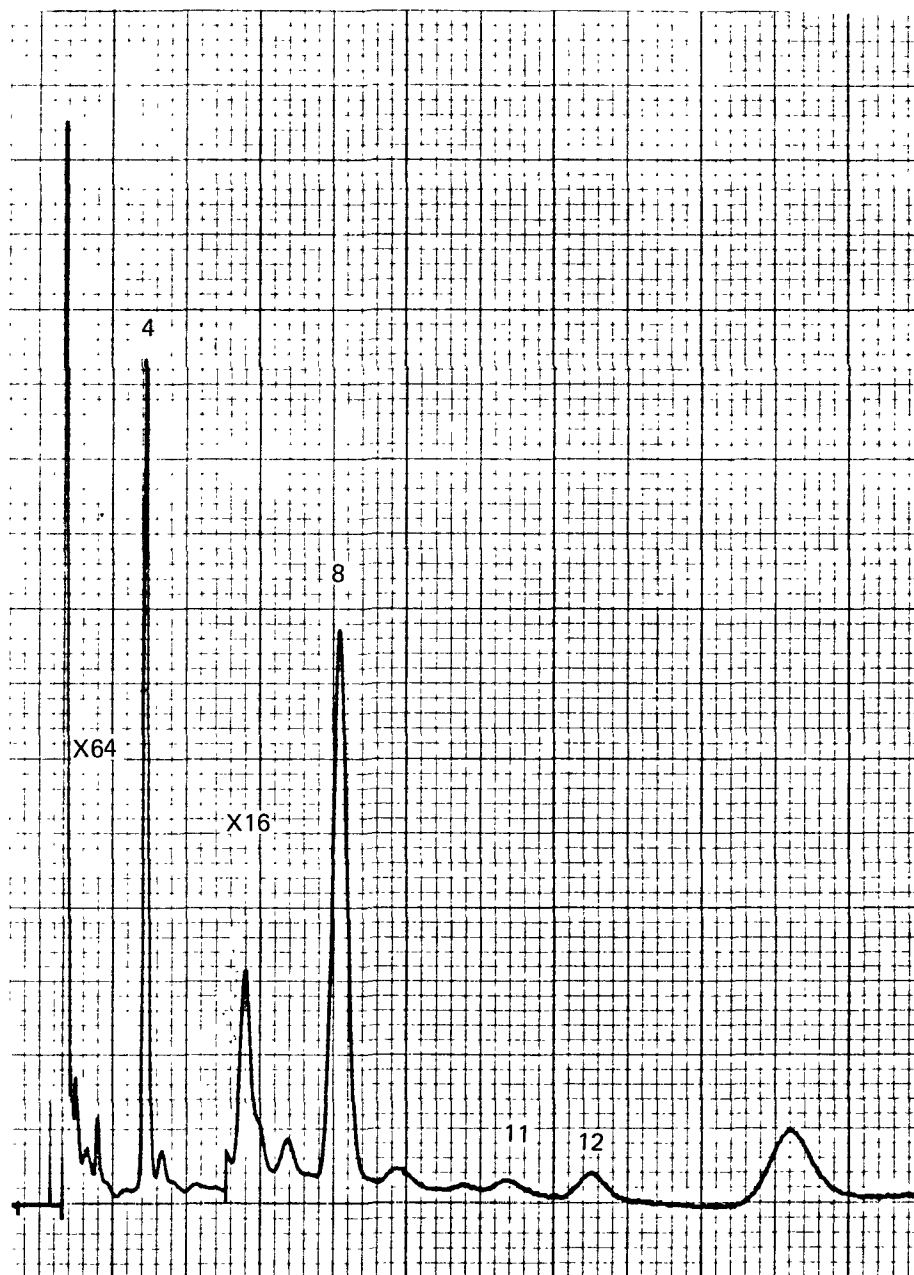


Figure 19. Chromatogram of 15% ether/petroleum ether fraction (sample M2-9, 3 µg/liter).

TABLE 13. ANALYTICAL DATA FOR FLORISIL FRACTIONS EXPRESSED AS % RECOVERY*

Pesticide	M2-9, 3 ppb		M2-5, 30 ppb	
	6% Ether	15% Ether	6% Ether	15% Ether
α -BHC	74	--	91	--
λ -BHC	72	--	86	--
Heptachlor	Masked	--	103	--
Δ -BHC	--	112	--	98
Heptachlor epoxide	60	1	73	0.6
Endosulfan I	50	--	41	39
DDE	52	--	52	--
Endrin	--	88	--	84
DDD	82	--	81	--
DDT	60	--	77	--
Endrin aldehyde	--	25	--	39
Endosulfan sulfate	--	91**	--	79**

* % Recovery = (μg Found \div μg Added to RWW) \times 100

** This value has not been corrected for blanks.

TABLE 14. ANALYTICAL DATA FOR FLORISIL FRACTIONS EXPRESSED AS % RECOVERY*

<u>Pesticide</u>	M3-5, 3 ppb		M3-6, 3 ppb	
	<u>6% Ether</u>	<u>15% Ether</u>	<u>6% Ether</u>	<u>15% Ether</u>
α-BHC	68	--	80	--
β-BHC	59	--	111	5
Aldrin	Masked	--	Masked	--
Heptachlor epoxide	43	--	57	--
Endosulfan I	32	5	58	5
Dieldrin	15	36	27	63
Endosulfan II	--	16	6	70
Endosulfan sulfate	--	--	--	85**

* % Recovery = (μg Found \div μg Added to RWW) x 100

** This value has not been corrected for blanks.

TABLE 15. FLORISIL FRACTIONS*, **: ANALYTICAL RECOVERY OF VARIOUS PESTICIDES AND AROCHLOR 1260

	M6-1, 10 ppb		M6-2, 10 ppb		M6-5, 10 ppb		M6-6, 10 ppb	
	6% Fr.	15% Fr.	6% Fr.	15% Fr.	6% Fr.	15% Fr.	6% Fr.	15% Fr.
α -BHC	65	--	67	--	65	--	68	--
λ -BHC	70	--	68	--	68	--	71	--
Heptachlor	62	--	63	--	61	--	61	--
Δ -BHC	--	67	--	71	--	63	--	76
Heptachlor epoxide	73	--	69	--	69	--	71	--
Endosulfan I	55	24	46	28	47	26	52	29
DDE	70	--	62	--	63	--	63	--
Endrin	--	95	--	66	--	65	--	76
DDD	72	--	70	--	64	--	57	--
DDT	79	--	74	--	60	--	50	--
Endrin aldehyde	--	13	--	13	--	5	--	4
Endosulfan sulfate**	5	4	4	3	6	4	7	--
Arochlor 1260	65	--	72	--	64	--	68	--

* The 6% fractions were treated with mercury to eliminate the sulfur interference which manifests itself as a "hump" in the early region of the chromatogram.

** The 50% fractions were analyzed and only endosulfan sulfate was found at levels of 10 - 15% in these fractions.

TABLE 16. FLORISIL CHROMATOGRAPHY: OBSERVED ELUTION PATTERN
OF PRIORITY POLLUTANT PESTICIDES AND PCB's

<u>Compound</u>	<u>Major proportion found in</u>		
	<u>6% fraction</u>	<u>15% fraction</u>	<u>50% fraction</u>
α -BHC	X		
λ -BHC	X		
β -BHC	X		
Heptachlor	X		
Δ -BHC		X	
Aldrin	X		
Heptachlor epoxide	X		
Endosulfan I	X	X	
DDE	X		
Dieldrin	X	X	
Endrin		X	
DDD	X		
Endosulfan II		X	X
DDT	X		
Endrin aldehyde		X	
Endosulfan sulfate	?	?	X
PCB's	X		

The results obtained show that the 6%, 15% and 50% fractions should all be analyzed since some of the pesticides elute in these fractions.

Sephadex LH-20

This is the main clean up procedure evaluated in this study because it offered the following advantages:

- a) the method eliminated the interference due to the "hump" derived from raw waste water;
- b) it separated the "mass" of the samples from the pesticides;
- c) the solvent volumes of each fraction are small and avoid the additional concentration step required for the Florisil column procedure.

The development of the Sephadex LH-20 fractionation scheme followed a course whereby column bed volumes (1.9 x 7 cm, 1.9 x 14 cm, 1.9 x 19 cm) and single solvent or solvent combinations compatible with an electron capture detector were tried. Based on a few quick tests and initial observations, the column dimension of 1.9 x 19 cm and the solvent system methanol/toluene (1:1) was chosen for evaluation.

The gel was allowed to swell in methanol for at least overnight, and packed as a slurry in methanol into a glass column plugged with a small wad of precleaned glass wool. The column was tapped while packing and more gel was added as needed to fill the column to a height of 19 cm (i.e., column bed volume = 54 ml). The packed column was then washed first with 150 ml methanol followed by at least 150 ml of methanol/toluene (1:1). This treatment cleans the Sephadex and prepares it for sample application. During the course of these studies, four such columns were packed and reused repetitively, employing the precaution that after the completion of every sample elution, the column was rewashed with at least 150 ml of methanol/toluene (1:1) before a new sample was applied.

Basically two fractionation schemes, henceforth referred to as Fractionation Schemes 1 and 2, were tested. The main difference was that Scheme 1 consisted of collecting 5 ml fractions vs. 10 ml fractions for Scheme 2. In addition, for Scheme 1, the samples were concentrated in a nitrogen stream to 1 - 2 ml before loading to the column. In Scheme 2, 5.0 ml aliquots of the RWW extracts which have been concentrated to 10 ml were loaded to the column. It is not clear at this point whether or not the 2 vs. 5 ml of sample exerts a real difference in the fractionation scheme, but it is duly noted in as much as the sample solution is in hexane which causes some shrinkage of the gel. The advantage of using a 5 ml sample aliquot (vs. 2 ml) is that it minimizes the possibility of sample loss during further concentration of the sample extract.

The elution pattern for Fractionation Scheme 1 is described in Table 17. The nature of the clean up which can be achieved by this technique is shown by the chromatograms in Figures 20 to 25. Figure 20 is the chromatogram of a concentrated RWW extract (Sample M2-12) containing 3 ppb of a dozen pesticides (Mix 2). Note that the region from 0 to 3 minutes is masked by the "hump" characteristic of the RWW extract. When this sample was subjected to a Sephadex clean up procedure, five fractions (5 to 9) were observed to contain the pesticides and the main early eluting "hump" was eliminated. The chromatograms of these five fractions are shown, respectively, in Figures 21 to 25. Quantitative analysis of these fractions reveal a recovery and pesticide distribution pattern in the various fractions as typified by the data shown in Table 18. Although most of the pesticides elute in Fractions 6 and 7, some separation of certain pesticides do occur such as Δ -BHC which elutes mainly in Fraction 8.

By using this fractionation scheme, eight samples of raw waste water extracts were precleaned and the pertinent fractions were analyzed. The recoveries obtained for sixteen pesticides are summarized in Table 19. The data reflects the overall recovery from the extraction through the entire clean up process. It is probable that most of the sample loss occurred during the auxiliary sample concentration from 10 to 2 ml, under a nitrogen stream, just prior to sample loading on the Sephadex.

In subsequent experimental runs, two modifications were introduced; i.e., the auxiliary sample concentration step was eliminated and 10 ml fractions were collected in order to minimize the number of fractions which must be analyzed. This revised fractionation scheme is shown in Table 20.

Eight samples (M6 series) were fractionated in this fashion and analyzed. The samples subjected to the Sephadex clean up process were extracts of raw waste water spiked with 10 $\mu\text{g/liter}$ of each of twelve pesticides plus Arochlor 1260. The extracts were initially analyzed after KD concentration and then half of each sample was subjected to the Sephadex clean up procedure. (The other half of four samples were chromatographed on Florisil; see Table 15 data). The individual data for these eight samples are shown in Tables 21 to 28.

Two other samples (M5 series), which are extracts of raw waste water spiked with sixteen pesticides at test levels ranging from 1 to 10 $\mu\text{g/liter}$, were also fractionated on Sephadex LH-20. Fractions 4 and 5 were desulfurized by mercury treatment and analyzed by gas chromatography. The GC instrument used for this analysis was the Hewlett Packard Model 5840A. The data for these two samples are shown in Tables 29 and 30.

After GC analysis, the Sephadex LH-20 fractions were transferred to a tared aluminum pan, the solvent was allowed to evaporate by air drying, and the pans were weighed. Table 31 shows the data for the seven fractions of four samples and indicates that most of the sample mass

TABLE 17. CHROMATOGRAPHIC SEPARATION ON SEPHADEX LH-20

Fractionation scheme 1

Sample volume: 1 - 2 ml in hexane

Elluent: methanol/toluene (1:1)

<u>Fraction no.</u>	<u>Sequential effluent volume cut-off, ml</u>	<u>Color of fraction</u>	<u>Observations</u>
1	0-10	Colorless	At the end of this fraction, three zones are observed--a broad yellow zone at the front followed by a translucent and an opaque zone.
2	10-15	Colorless	
3	15-20	Yellow	Yellow zone begins to elute.
4	20-25	Dk. Yellow	Translucent band also begins to elute.
5	25-30	Lt. Yellow	Opaque zone elutes. Pesticides and PCB's elute in these fractions.
6	30-35	Colorless	
7	35-40	Colorless	
8	40-45	Colorless	
9	45-50	Colorless	
10	50-55	Colorless	
11	55-60	Colorless	
12	60-65	Colorless	

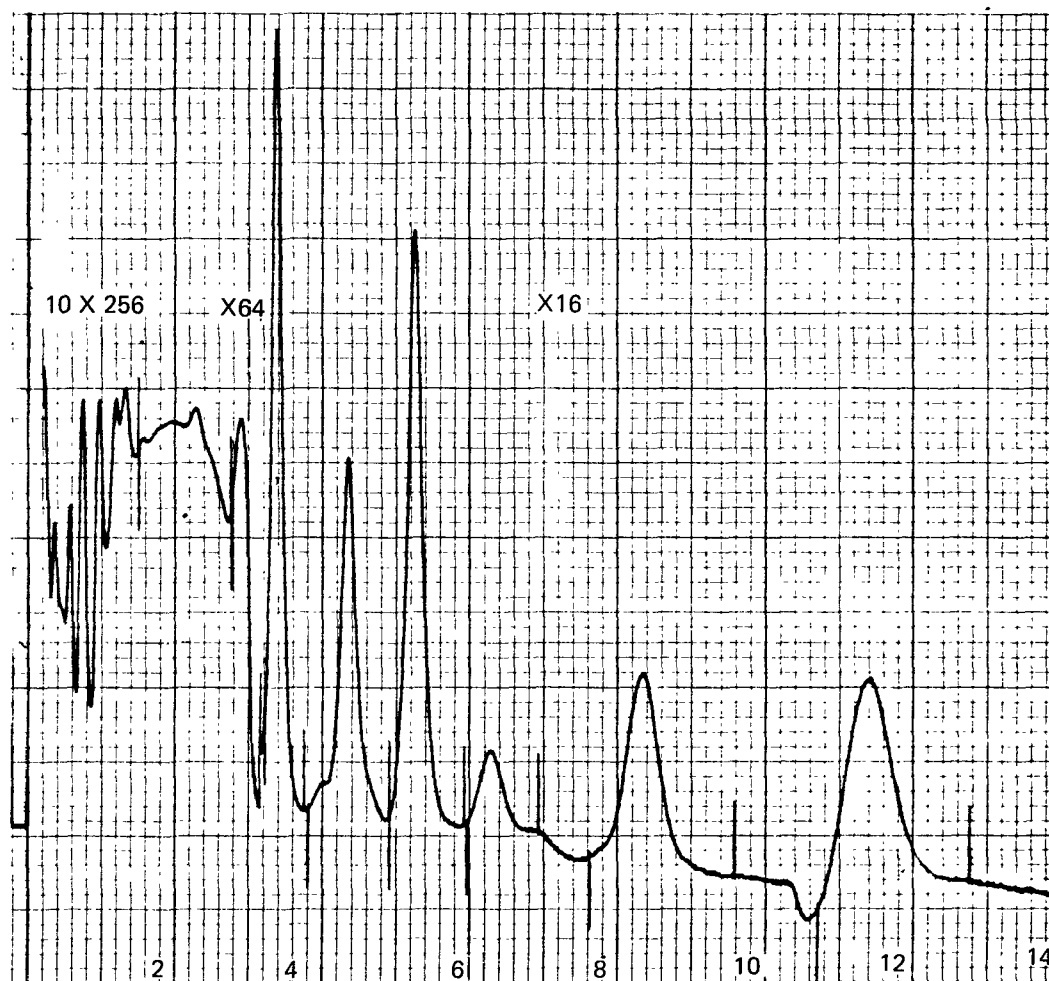


Figure 20. Chromatogram of extract of RWW spiked with Mix 2 at 3 µg/liter (sample M2-12).



Figure 21. Chromatogram of Sephadex LH-20 Fraction 5 (sample M2-12, scheme 1).



Figure 22. Chromatogram of Sephadex LH-20 Fraction 6 (sample M2-12, scheme 1).

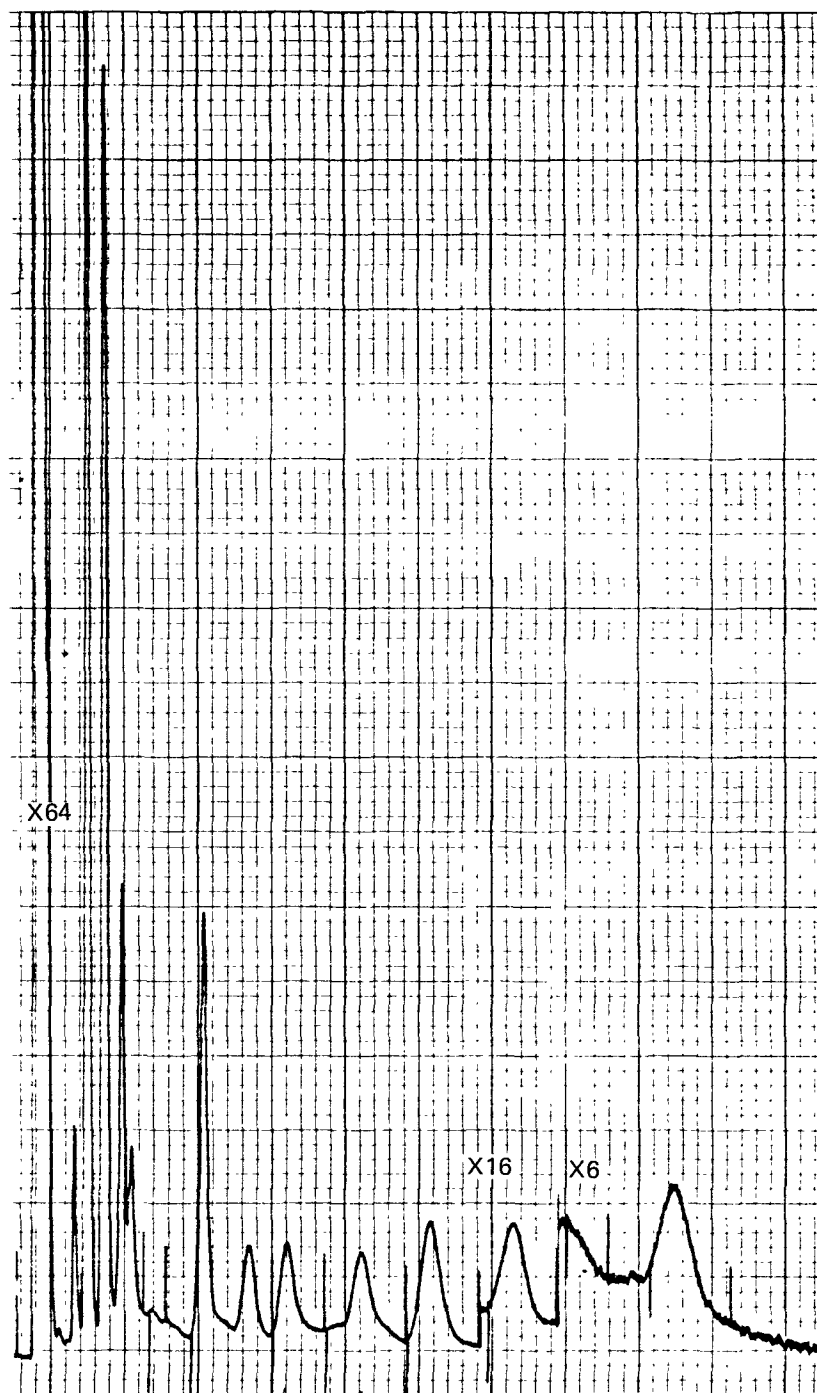


Figure 23. Chromatogram of Sephadex LH-20 Fraction 7 (sample M2-12, scheme 1).

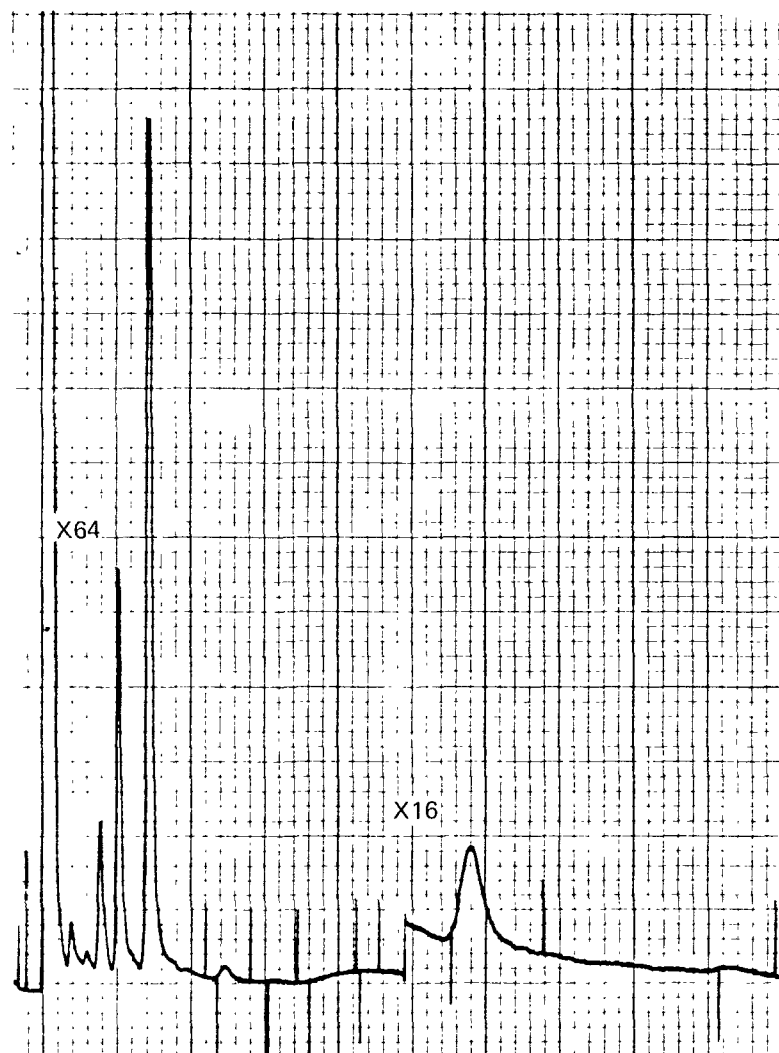


Figure 24. Chromatogram of Sephadex LH-20 Fraction 8 (sample M2-12, scheme 1).

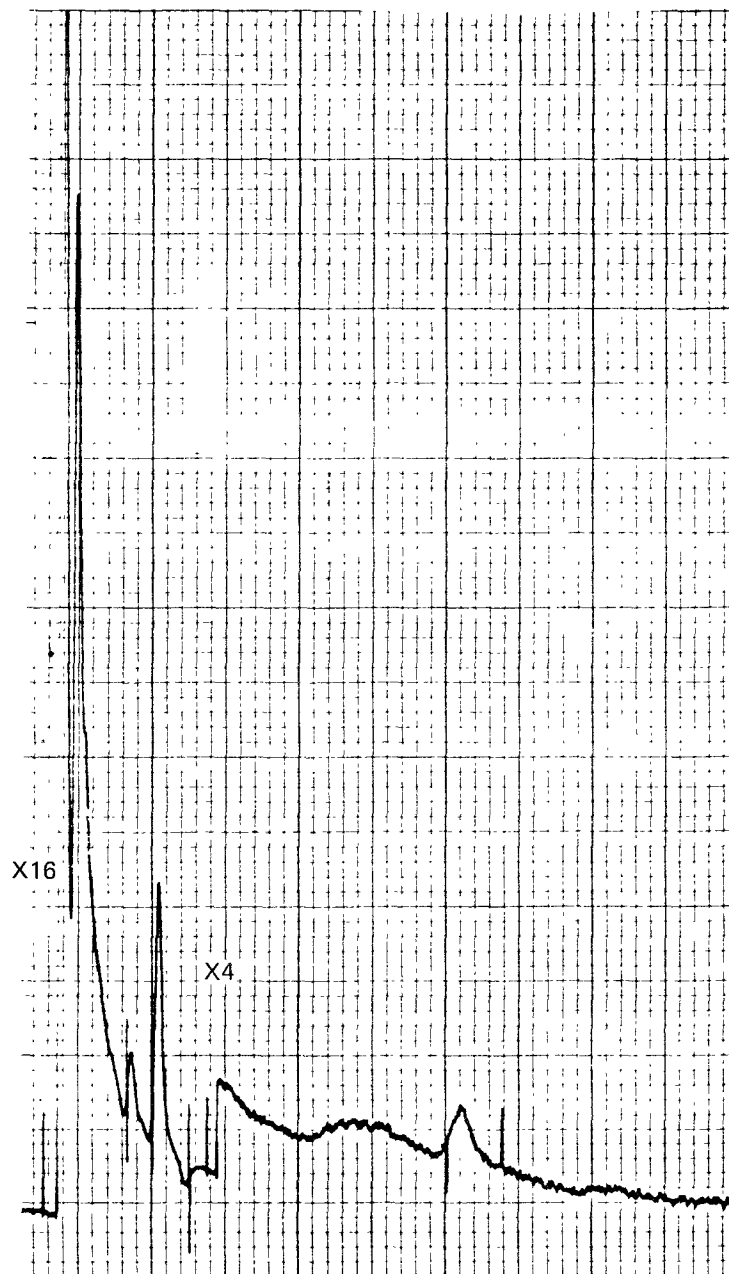


Figure 25. Chromatogram of Sephadex LH-20 Fraction 9 (sample M2-12, scheme 1).

TABLE 18. ANALYTICAL DATA FOR SEPHADEX LH-20 FRACTIONS: SAMPLE M2-12, 3 ppb

Pesticide	Added to RWW µg/liter	-----% Found *-----						Total
		Fraction 5	Fraction 6	Fraction 7	Fraction 8	Fraction 9		
α-BHC	2.8	0.4	2.2	40.0	4.1	--	47	
λ-BHC	3.0	--	0.4	40.7	12.7	0.7	54	
Heptachlor	3.4	5.9	38.8	14.1	--	--	59	
Δ-BHC	3.0	--	--	4.7	55.3	6.0	66	
Heptachlor epoxide	2.9	--	23.4	38.6	1.4	--	63	
Endosulfan I	2.2	--	30.9	30.0	0.5	--	61	
DDE	3.6	7.2	22.2	12.8	--	--	42	
Endrin	3.0	0.7	34.7	26.0	--	--	61	
DDD	3.2	0.4	26.7	40.6	9.4	1.2	78	
DDT	3.0	2.0	29.3	17.3	--	--	49	
Endrin aldehyde	0.4	ND	ND	ND	ND	ND		
Endosulfan sulfate	0.4	Dominated by blank from raw waste water estimated to be 1 µg/liter						

Weight of residue **, mg..... 6.1 5.2 1.6 0.4 0.7

* Values are expressed as percent of the original amount added to RWW found in each fraction.

** The mass found in fractions 1, 2, 3, and 4, respectively, are: 0.7, 1.3, 7.3 and 10.5 mg

TABLE 19. RECOVERY OF VARIOUS PESTICIDES FROM RWW AFTER CLEAN UP ON SEPHADEX LH-20

(Fractionation scheme 1)		
<u>Pesticide</u>	-----Average % recovery-----*	
	<u>30 ppb</u>	<u>3 ppb</u>
α -BHC	64**	52**
λ -BHC	56	54
β -BHC	96	85
Heptachlor	73	62
Δ -BHC	72	64
Aldrin	46	44
Heptachlor epoxide	80**	62
Endosulfan I	64**	59**
DDE	62	42
Dieldrin	76	60
Endrin	66	58
DDD	76	72
Endosulfan II	82	64
DDT	38	46
Endrin aldehyde	--	41
Endosulfan sulfate	--	79**

*Each value is based on the average of two independent samples.

**Average based on four independent samples.

TABLE 20. CHROMATOGRAPHIC SEPARATION ON SEPHADEX LH-20

Fractionation scheme 2			
Sample volume: 5 ml in hexane			
Eluent: methanol/toluene (1:1)			
<u>Fraction no.</u>	<u>Sequential effluent volume cut off, ml</u>	<u>Color of fraction</u>	<u>Observations</u>
1	0-10	Colorless	Three zones migrate down the column--a broad yellow zone followed by distinct translucent and opaque zones.
2	10-20	Colorless	
3	20-30	Dk. Yellow	Yellow band elutes here.
4	30-40	Yellow	Translucent and opaque zones elute. Pesticides and PCB's elute in these fractions.
5	40-50	Colorless	
6	50-60	Colorless	
7	60-70	Colorless	

TABLE 21. ANALYTICAL DATA* FOR SEPHADEX LH-20 FRACTIONS: SAMPLE M6-1

<u>Pesticide</u>	<u>Fraction 4</u>	<u>Fraction 5</u>	<u>Fraction 6</u>	<u>Total</u>
α -BHC	0.3	89.4	--	90
λ -BHC	--	87.3	3.8	91
Heptachlor	20.7	28.0	--	49
Δ -BHC	--	63.4	9.2	73
Heptachlor epoxide	0.8	88.4	--	89
Endosulfan I	5.8	79.5	--	85
DDE	30.3	29.9	--	60
Endrin	7.9	80.7	--	89
DDD	--	93.0	1.4	94
DDT	11.2	43.7	--	55
Endrin aldehyde	8.0	23.9	--	32
Endosulfan sulfate	3.3	96.9	--	100
Arochlor 1260				

*Values are expressed as percent of the original amount added to RWW found in each fraction.

TABLE 22. ANALYTICAL DATA* FOR SEPHADEX LH-20 FRACTIONS: SAMPLE M6-2

<u>Pesticide</u>	<u>Fraction 4</u>	<u>Fraction 5</u>	<u>Fraction 6</u>	<u>Total</u>
α - BHC	0.3	83.7	2.0	86
λ - BHC	--	81.4	6.1	87
Heptachlor	20.7	27.7	--	48
Δ -BHC	--	59.6	20.8	80
Heptachlor epoxide	2.6	86.0	--	89
Endosulfan I	6.3	68.9	--	75
DDE	23.8	22.8	7.4	54
Endrin	12.5	71.6	--	84
DDD	4.6	92.9	2.1	100
DDT	15.7	45.5	--	61
Endrin aldehyde	--	27.9	--	28
Endosulfan sulfate	4.4	82.9	2.5	90
Arochlor 1260				

*Values are expressed as percent of the original amount added to RWV found in each fraction.

TABLE 23. ANALYTICAL DATA* FOR SEPHADEX LH-20 FRACTIONS: SAMPLE M6-3

<u>Pesticide</u>	<u>Fraction 4</u>	<u>Fraction 5</u>	<u>Fraction 6</u>	<u>Total</u>
α -BHC	24.4	65.4	--	90
λ -BHC	6.9	78.5	2.4	88
Heptachlor	51.7	8.0	--	60
Δ -BHC	--	90.0	--	90
Heptachlor epoxide	50.0	21.3	--	71
Endosulfan I	62.6	26.8	--	89
DDE	66.0	19.1	--	85
Endrin	61.4	29.2	--	91
DDD	46.4	44.3	--	91
DDT	43.7	18.9	--	63
Endrin aldehyde	13.8	21.4	--	35
Endosulfan sulfate	32.6	69.5	--	108
Arochlor 1260				

*Values are expressed as percent of the original amount added to RWW found in each fraction.

TABLE 24. ANALYTICAL DATA* FOR SEPHADEX LH-20 FRACTIONS: SAMPLE M6-4

<u>Pesticide</u>	<u>Fraction 4</u>	<u>Fraction 5</u>	<u>Fraction 6</u>	<u>Total</u>
α -BHC	16.7	71.1	--	88
λ -BHC	5.8	75.4	3.1	84
Heptachlor	22.7	50.0	--	73
Δ -BHC	--	77.7	2.3	80
Heptachlor epoxide	15.5	58.1	--	74
Endosulfan I	26.8	59.0	--	86
DDE	37.0	37.7	--	75
Endrin	26.9	56.3	--	83
DDD	25.5	62.4	0.4	88
DDT	17.5	42.0	--	60
Endrin aldehyde	11.6	21.7	--	33
Endosulfan sulfate	19.9	72.1	--	102
Arochlor 1260				

*Values are expressed as percent of the original amount added to RWW found in each fraction.

TABLE 25. ANALYTICAL DATA* FOR SEPHADEX LH-20 FRACTIONS: SAMPLE M6-5

<u>Pesticide</u>	<u>Fraction 4</u>	<u>Fraction 5</u>	<u>Fraction 6</u>	<u>Total</u>
α -BHC	--	85.0	--	85
λ -BHC	--	87.7	2.5	90
Heptachlor	6.3	39.3	--	46
Δ -BHC	--	66.5	5.8	72
Heptachlor epoxide	0.4	81.8	0.9	83
Endosulfan I	5.3	81.0	0.5	87
DDE	13.3	48.5	1.0	63
Endrin	2.6	80.3	--	83
DDD	1.4	83.0	--	84
DDT	6.3	43.3	--	50
Endrin aldehyde	4.0	ND	--	
Endosulfan sulfate	2.2	99.9	--	102
Arochlor 1260				

*Values are expressed as percent of the original amount added to RWW found in each fraction.

TABLE 26. ANALYTICAL DATA* FOR SEPHADEX LH-20 FRACTIONS: SAMPLE M6-6

<u>Pesticide</u>	<u>Fraction 4</u>	<u>Fraction 5</u>	<u>Fraction 6</u>	<u>Total</u>
α -BHC	2.4	82.1	--	84
λ -BHC	1.4	89.6	6.5	97
Heptachlor	24.0	31.3	--	55
Δ -BHC	--	65.8	17.7	84
Heptachlor epoxide	8.1	73.3	--	81
Endosulfan I	23.7	70.0	--	94
DDE	30.9	40.1	1.7	73
Endrin	20.8	73.5	--	94
DDD	15.6	83.3	--	99
DDT	19.4	45.2	--	65
Endrin aldehyde	--	2.5	--	
Endosulfan sulfate	10.1	95.0	--	105
Arochlor 1260				

*Values are expressed as percent of the original amount added to RWW found in each fraction.

TABLE 27. ANALYTICAL DATA* FOR SEPHADEX LH-20 FRACTIONS: SAMPLE M6-7

<u>Pesticide</u>	<u>Fraction 4</u>	<u>Fraction 5</u>	<u>Fraction 6</u>	<u>Total</u>
α -BHC	--	87.7	--	88
λ -BHC	--	87.7	--	88
Heptachlor	11.1	36.9	--	48
Δ -BHC	--	68.6	7.0	76
Heptachlor epoxide	7.0	84.9	--	92
Endosulfan I	11.6	84.0	--	96
DDE	21.4	46.7	--	68
Endrin	9.8	85.2	--	95
DDD	6.8	81.8	--	89
DDT	11.5	43.6	--	55
Endrin aldehyde	5.6	8.7	--	14
Endosulfan sulfate	5.6	85.2	--	91
Arochlor 1260				

*Values are expressed as percent of the original amount added to RWW found in each fraction.

TABLE 28. ANALYTICAL DATA* FOR SEPHADEX LH-20 FRACTIONS: SAMPLE M6-8

<u>Pesticide</u>	<u>Fraction 4</u>	<u>Fraction 5</u>	<u>Fraction 6</u>	<u>Total</u>
α -BHC	28.5	44.0	None found	73
λ -BHC	22.5	49.8	in this	72
Heptachlor	26.3	10.0	fraction	46
Δ -BHC	11.4	63.9		75
Heptachlor epoxide	37.8	31.8		70
Endosulfan I	42.2	36.2		79
DDE	45.2	21.0		66
Endrin	43.5	37.9		81
DDD	32.9	42.6		76
DDT	22.7	23.2		46
Endrin aldehyde	0	3.7		4
Endosulfan sulfate	44.6	46.0		91
Arochlor 1260				

*Values are expressed as percent of the original amount added to RWW found in each fraction.

TABLE 29. ANALYTICAL DATA* FOR SEPHADEX LH-20 FRACTIONS: SAMPLE M5-1

<u>Pesticide</u>	<u>Fraction 4</u>	<u>Fraction 5</u>	<u>Total</u>
α -BHC	65.3	22.4	88
λ -BHC	50.6	44.6	95
B-BHC	12.5	75.9	88
Heptachlor	94.0	2.6	97
Δ -BHC	10.8	83.8	95
Aldrin	76.2	1.9	78
Heptachlor epoxide	86.8	8.2	95
Endosulfan I	91.5	4.0	95
DDE	78.8	--	79
Dieldrin	95.7	--	96
Endrin	88.5	--	86
DDD + Endosulfan II	49.7	23.3	73
DDT	40.6	6.0	47
Endrin aldehyde	37	*	37
Endosulfan sulfate	90	*	90

*These peaks were masked by artifacts.

TABLE 30. ANALYTICAL DATA* FOR SEPHADEX LH-20 FRACTIONS: SAMPLE M5-3

<u>Pesticide</u>	<u>Fraction 4</u>	<u>Fraction 5</u>	<u>Total</u>
α -BHC	60.7	31.6	92
λ -BHC	41.2	52.6	94
B-BHC	14.2	76.0	90
Heptachlor	71.5	14.3	86
Δ -BHC	10.3	83.9	94
Aldrin	74.0	11.5	87
Heptachlor expoxide	70.2	18.8	89
Endosulfan I	74.4	17.0	91
DDE	73.2	--	73
Dieldrin	72.4	27.2	100
Endrin	66.2	11.7	78
DDD + Endosulfan II	61.4	24.5	86
DDT	49.4	16.5	66
Endrin aldehyde	28	Masked*	28
Endosulfan sulfate	68.9	*	69

*These peaks were masked by artifacts.

TABLE 31. DISTRIBUTION OF SAMPLE MASS IN VARIOUS SEPHADEX LH-20 FRACTIONS

Fraction no.	-----mg Found*-----			
	<u>M6-1</u>	<u>M6-2</u>	<u>M6-7</u>	<u>M6-8</u>
1	2.8	0.4	0.5	1.0
2	1.9	1.0	0.6	1.2
3	4.5	5.5	2.7	2.5
4	13.5	14.3	9.1	10.5
5	1.1	1.4	0.9	0.5
6	0.4	0.6	0.9	0.9
7	0.7	0.9	0.7	0.8

*These values represent the weight of the total residue in each fraction which were obtained by drying each fraction in a tared, aluminum weighing pan.

elutes in Fraction 4. Test for sulfur in a continuous series of seven fractions reveal the presence of sulfur in Fractions 3, 4 and 5, with the most amount found in Fraction 4.

The general trend of the data (in Tables 21 to 30) indicates that the pesticides elute primarily in Fractions 4 and 5, although there is some "bounce" in the relative distribution between these two fractions. Fraction 3 does not contain pesticides, but Fraction 6 has been found to contain a relatively small amount. The data are most encouraging in that the pesticides reproducibly elute in the relative retention volume (V_R , defined as the ratio of the elution volume to the total volume of the gel) range of 0.56 to 0.93 which corresponds to Fractions 4 and 5.

The elution pattern of phthalate esters which are common interferences in pesticide assay was also determined. Fifty micrograms of diethyl-, dibutyl-, and dioctyl phthalate was added to 5.0 ml of hexane and the sample was chromatographed on Sephadex LH-20. The six fractions of three replicate samples were analyzed by gas chromatography. Diethyl phthalate was hidden in the solvent response, but the data for dibutyl and dioctyl phthalates, summarized in Table 32, illustrates that the phthalates elute ahead of the pesticides and PCB's; i.e., Fractions 2 and 3 contain the phthalate esters.

Experiments were also conducted to determine the effect of concentrating combined fractions by KD. For this study, a few pesticides and Arochlor 1016 was added to 30 ml of methanol/toluene (1:1) at levels shown in Table 33. Six replicate samples were concentrated by KD to 5 - 10 ml, and the samples were analyzed. The data in Table 33 show that a loss of 20 to 30% generally occur; in a few cases, less than 50% recovery are indicated.

The procedure recommended and described in Appendix B calls for collecting together Fractions 1 to 3 (30 ml), Fractions 4 and 5 (20 ml), and Fraction 6 (10 ml). The first 30 ml fraction is discarded, the second 20 ml fraction is analyzed, preferably avoiding KD concentration, and the third 10 ml fraction is collected separately as an insurance, but avoids further dilution of the major pesticide fractions.

The Sephadex LH-20 clean up method achieves the same purpose as the Florisil clean up and provides the following relative advantages:

- a) the method is simple and fast,
- b) avoids cumbersome distillation of solvents and sorbent clean up associated with the Florisil method, and gives better recovery because it eliminates one other concentration step.

TABLE 32. ELUTION PATTERN OF PHTHALATE ESTERS ON SEPHADEX LH-20

Fractionation scheme 2		
Average % found in fraction*		
<u>Fraction no.</u>	<u>Dibutyl phthalate</u>	<u>Diethyl phthalate</u>
1	--	--
2	10	36
3	50	51
4	18	--
5	--	--
6	--	--
Total	78%	87%

*Averages of three independent determinations

TABLE 33. RECOVERY OF PESTICIDES & PCB AFTER KD CONCENTRATION OF METHANOL/
TOLUENE (50:50) SOLUTION

<u>Pesticide and PCB</u>	<u>Total μg added</u>	<u>-----% Recovery-----</u>					
		<u>#1</u>	<u>#2</u>	<u>#3</u>	<u>#4</u>	<u>#5</u>	<u>#6</u>
α-BHC	1.4	79	49	69	46	53	51
Δ-BHC	3.0	80	*	66	*	27	77
Heptachlor Epoxide	2.9	88	80	80	80	82	69
Endosulfan 1	3.1	81	64	*	*	*	68
DDE	2.8	84	91	79	82	88	72
Endrin	3.0	99	91	91	89	96	83
DDD	3.2	84	82	77	77	88	80
DDT	3.0	89	70	72	72	94	84
Arochlor 1016	15.0	84	88	75	84	83	57

*These values were significantly lower than the calibration curve range
and are estimated to correspond to less than 10% recovery.

Sulfur Removal

Metallic mercury was used to remove sulfur interferences. The method was applied directly to extracts of raw waste water, to the 6% ether/petroleum ether fraction, and to the Sephadex LH-20 fractions.

Initial experiments were performed on a 6% ether/petroleum ether fraction which showed a "hump" in the chromatogram and had a sulfur like odor. A 2 ml aliquot of this fraction was treated with two to three drops of metallic mercury. A black precipitate formed and the mercury was immediately separated from the solvent layer by centrifugation and decantation and an aliquot of the sample analyzed. The comparative chromatograms of the fractions before and after treatment with mercury are shown in Figures 26 and 27 respectively, which clearly show that the "hump" is sulfur related.

In view of these observations, subsequent experiments were conducted to determine the effect of this mercury treatment on the recovery of the pesticides. The data shown in Tables 34 and 35 for various fractions from Florisil and Sephadex LH-20 before and after treatment with mercury, show virtually no loss for sixteen pesticides.

Experiments were also conducted to test the effect of the sulfur removal procedure directly on the concentrated raw waste water extracts. As noted earlier, the 7/25/78 raw waste water samples showed significant levels of the early eluting "hump" which masked chlordane and Arochlor 1016, even at test levels of 30 µg/liter (see Figure 28 for sample chromatogram). These samples were again treated with mercury and analyzed. The results obtained for four replicate samples for chlordane and Arochlor 1016 are shown respectively in Tables 35 and 36. The average recoveries were 104% for chlordane and 74% for Arochlor 1016.

EFFECT OF STORAGE ON SAMPLE STABILITY

Three liter aliquots of the "before" the grit chamber and "after" the grit chamber raw waste water samples were spiked with Mix 6 at a test level of 10 µg/liter. Each sample was mixed thoroughly and homogenized in a blender. Immediately after the sample preparation, duplicate 0.5 liter aliquots of the two samples were extracted and concentrated to 10 ml. (Samples M6-1, M6-2, M6-3, M6-4). The remaining portion of the spiked samples were stored at 40°F for 48 hours and again duplicate 0.5 liter aliquots of both samples were processed similarly (Samples M6-5, M6-6, M6-7 and M6-8).

The data summarized in Table 38 illustrates that the pesticides and Arochlor 1260 are stable under these storage conditions. Of the twelve pesticides, only endrin aldehyde and possibly heptachlor appear to be significantly lower in the stored samples.

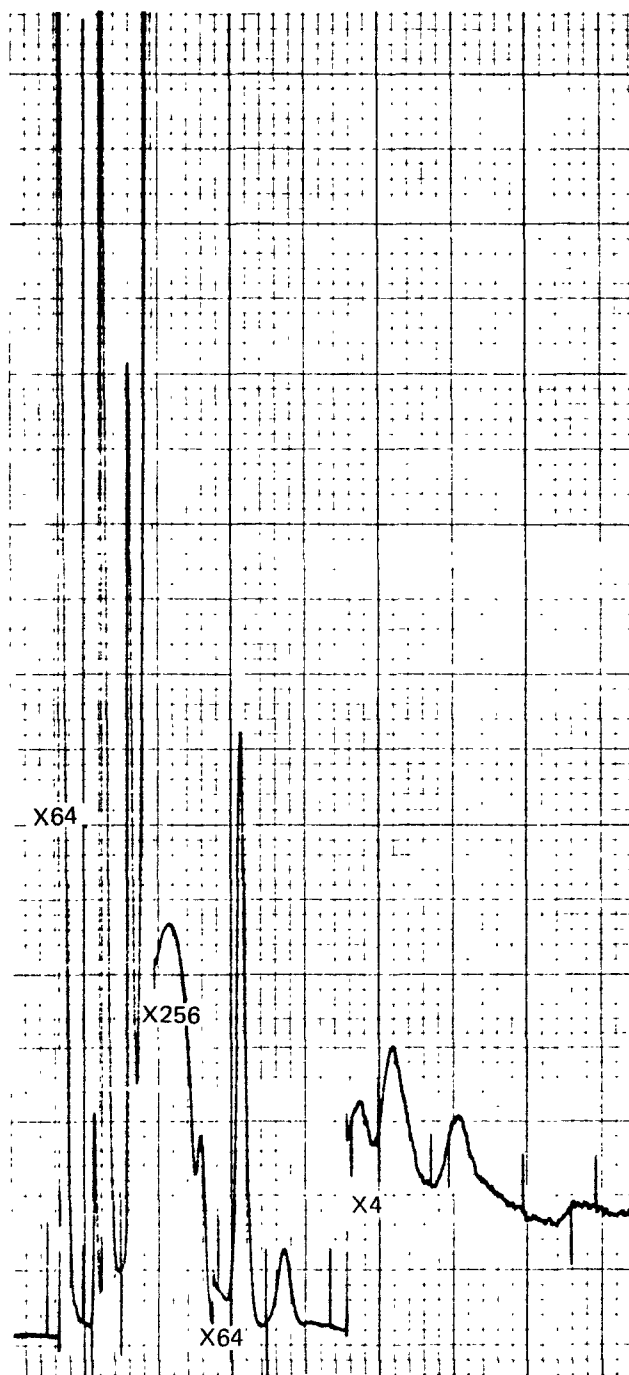


Figure 26. Chromatogram of Florisil Fraction in 6% ether/petroleum ether before treatment with mercury (sample M3-6).

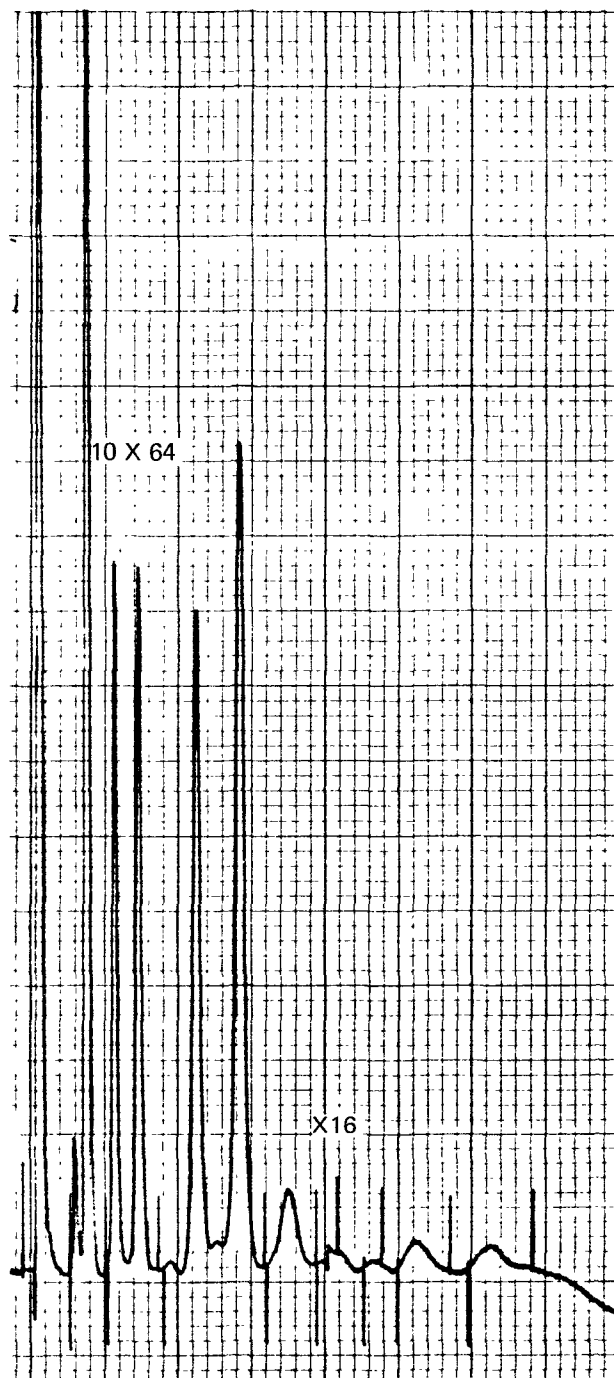


Figure 27. Chromatogram of Florisil Fraction in 6% ether/petroleum ether after treatment with mercury (sample M3-6).

TABLE 34. EFFECT OF Hg TREATMENT FOR SULFUR REMOVAL ON PESTICIDE RECOVERY

Pesticide	-----ng Found-----			
	M3-5, 6% ether fraction		M3-6, 6% ether fraction	
	Untreated	Hg treated	Untreated	Hg treated
α -BHC	950	800	1100	1000
β -BHC	960 (?)	490	1800	1750
Aldrin	Masked	350	Masked	780
Heptachlor epoxide	630	440	1200	1050
Endosulfan I	800	950	1400	1200
Dieldrin	250	270	440	530
Endosulfan II	--	--	90	110

TABLE 35. EFFECT OF Hg TREATMENT FOR SULFUR REMOVAL ON PESTICIDE RECOVERY

Pesticide	-----nanograms found-----									
	M6-3, LH fraction 5		M6-4, LH fraction 5		M6-5, LH fraction 5		M6-5, LH fraction 5		M6-5, LH fraction 5	
	Untreated	Hg treated	Untreated	Hg treated	Untreated	Hg treated	Untreated	Hg treated	Untreated	Hg treated
α -BHC	1610	1670	1750	1730	1880	1760	1830	1820		
λ -BHC	2040	2160	1960	1910	1910	1890	2040	2010		
Heptachlor	240	240	1500	1750	1180	1190	940	950		
Δ -BHC	2340	2450	2020	2030	1730	1660	1710	1670		
Heptachlor epoxide	530	560	1480	1520	2140	2030	1900	1860		
Endosulfan I	490	520	1125	1120	1570	1500	1350	1300		
DDE	635	600	1230	1190	1630	1580	1270	1260		
Endrin	730	810	1430	1540	2150	2050	1950	1920		
DDD	1210	1290	1760	1760	2370	2290	2410	2220		
DDT	470	530	1090	1160	990	1330	1200	1230		
Endrin aldehyde	530	650	620	580	ND	ND	ND	ND		
Endosulfan sulfate	1930	2210	2130	2180	2280	2210	2240	2360		

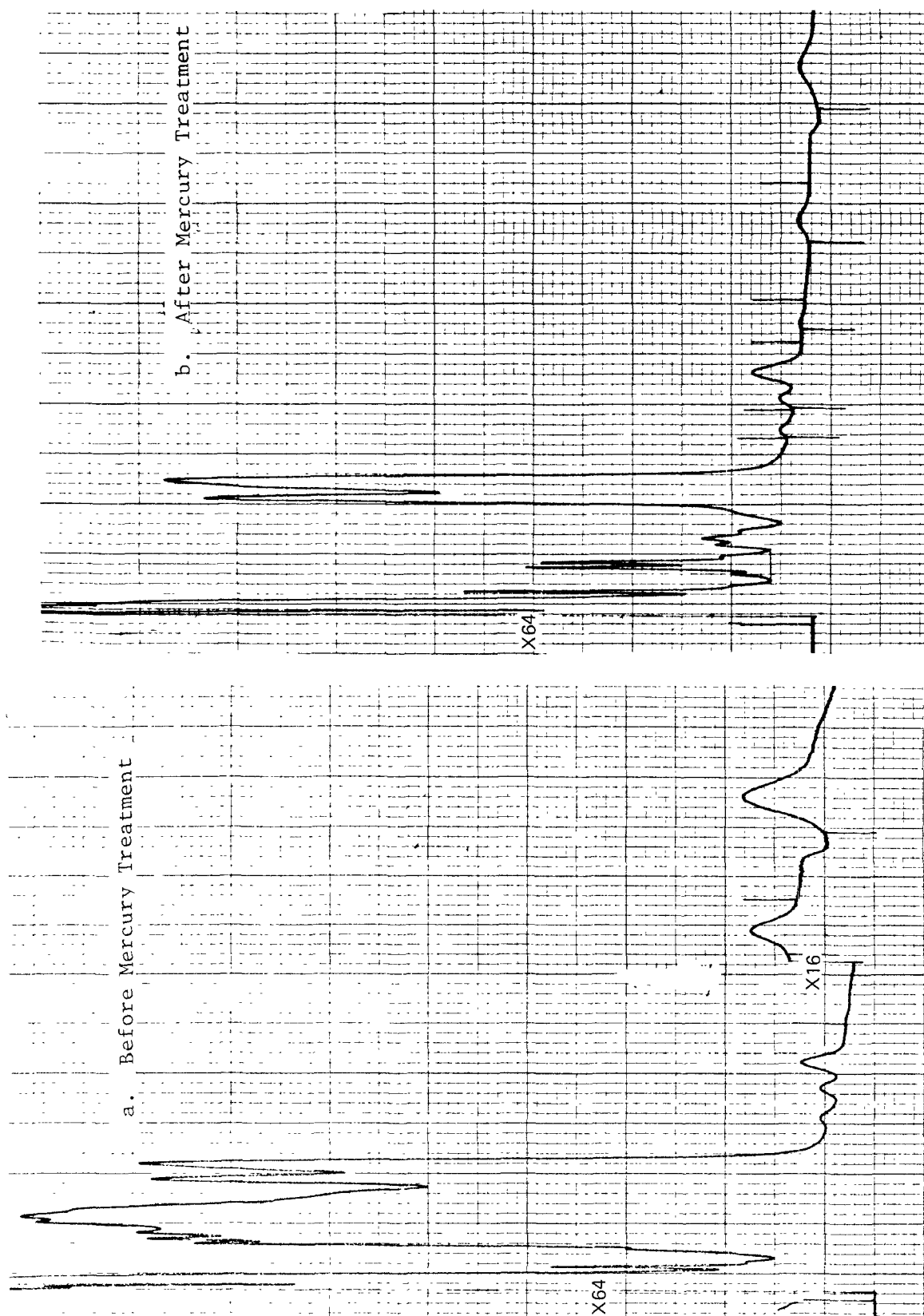


Figure 28. Chlordane in RRW extract before and after mercury treatment (test level = 30 $\mu\text{g/liter}$)

TABLE 36. RECOVERY OF CHLORDANE FROM RWW*

<u>Single code</u>	<u>RWW source</u>	<u>µg/liter</u>		<u>Percent recovery</u>
		<u>Added</u>	<u>Found</u>	
C -1	Before Grit	29.6	30.6	103
C -2	Before Grit	29.6	32.6	110
C -3	After Grit	29.6	31.2	105
C -4	After Grit	29.6	29.4	99
Average.....104				
σ4.6				

*Quantitative analysis of these samples was possible only after sulfur removal.

TABLE 37. RECOVERY OF AROCHLOR 1016 FROM RWW*

<u>Single code</u>	<u>RWW source</u>	<u>µg/liter</u>		<u>Percent recovery</u>
		<u>Added</u>	<u>Found</u>	
1016-1	Before Grit	30	24.0	80
1016-2	Before Grit	30	23.8	79
1016-3	After Grit	30	18.0	60
1016-4	After Grit	30	23.2	77
Average..... 74				
σ 9.4				

*Quantitative analysis of these samples was possible only after sulfur removal.

TABLE 38. EFFECT OF SAMPLE STORAGE TIME ON PESTICIDE RECOVERY

Pesticide	Added µg/liter	-----Samples Stored 0 Hours-----						-----Samples Stored 48 Hours, 40°F-----					
		Before Grit M6-1	Before Grit RWV M6-2	After Grit M6-3	After Grit RWV M6-4	Avg.	σ	Before Grit M6-5	Before Grit RWV M6-6	After Grit M6-7	After Grit RWV M6-8	Avg.	σ
α-BHC	10	93	87	88	89	89	2.6	87	88	81	83	85	3.3
λ-BHC	11	95	89	90	91	91	2.6	92	92	82	85	88	5.1
Heptachlor	13	76	70	83	82	78	6.0	62	63	67	71	66	4.1
Δ-BHC	11	94	89	92	94	92	2.4	108	101	86	92	97	9.7
Heptachlor epoxide	11	82	72	83	84	80	5.6	100	96	84	90	92	7.0
Endosulfan I	8	64	58	66	69	64	4.6	75	72	64	70	70	4.6
DDE	13	71	53	68	60	63	8.1	79	75	62	64	70	8.3
Endrin	11	86	79	87	87	85	3.9	83	82	79	86	82	2.9
DDD	12	94	93	102	99	97	4.2	92	94	94	101	95	3.9
DDT	11	73	60	60	63	64	6.2	70	62	55	53	60	7.7
Endrin aldehyde	11	37	40	46	45	42	4.2	13	13	15	16	14	1.5
Endosulfan sulfate	11	99	91	107	103	100	6.8	86	87	95	103	93	7.9
Arochlor	10	114	114	NA	NA	141		141	124	NA	NA		

NA - Not available; the data for these samples were not processed.

COMPARISON OF DATA DERIVED FROM TWO GC UNITS

As another estimate of the degree of variability or agreement which can be expected, four samples of raw waste water extracts were analyzed using two different GC units. The response of the Perkin Elmer 900 equipped with a 180 cm by 2-mm ID glass column (Column 1) used for most of these studies was compared with that of the Hewlett Packard 5840A equipped with a 180 cm by 4-mm ID glass column (Column 2). Two advantages are provided by the latter set up in that the Hewlett Packard 5840A has a higher sensitivity than the Perkin Elmer 900 and the 4-mm ID column gives better resolution of the sixteen pesticides; i.e., only DDD and endosulfan II overlap vs. three pairs of unresolved components with the 2-mm ID column.

The four samples were spiked with a mixture of sixteen pesticides at individual concentrations ranging from 0.6 to 10 µg/liter. The samples were extracted, concentrated, treated with mercury and analyzed; the samples were rediluted by a factor of 4 before analysis. Four calibration standards were used to calibrate the two analytical instruments.

The results obtained are shown in Tables 39 and 40. In the PE-900/Column 1 (Table 39), average recoveries less than 75% were obtained for combined β-BHC and heptachlor, combined DDE and dieldrin, and aldrin; the endrin aldehyde appeared to be present at trace levels. The HP-5840A/Column 2 data (Table 40), show average recoveries greater than 80% for all pesticides except endrin aldehyde with an average recovery of 35%. The RWW interference peak, which behaves like endosulfan sulfate in Column 1, shows up to be a different component partially resolved from endosulfan sulfate (RT = 21 minutes) in Column 2. Chromatograms on Column 2 of a spiked RWW extract and a corresponding blank are shown in Figures 29 and 30 respectively.

DETECTION LIMITS AND GC/MS ANALYSIS

The concentration range evaluated in this program was 1 to 30 µg/liter and the data obtained for the spiked samples show that the method is applicable to ppb analysis. However, the level of what appears to be some pesticides found in the raw waste water samples are in the parts per trillion (ppt) level.

The data obtained from extracts of the 7/25/78 raw waste water are shown in Table 41. These extracts, in duplicate, were treated with mercury to eliminate the major sample interference and analyzed by the PE-900 and the HP-5840A. The sample peaks which appear to conform to the indicated pesticides are shown in Table 41. The data based on the two columns with identical substrates but different dimensions indicate the presence of λ-BHC, DDD and/or endosulfan II. GC/MS analysis of a similar sample indicated only a BHC, most likely the λ-BHC. The estimated concentration of the λ-BHC by the GC/ECD analysis is 0.02 to 0.3 µg/liter. The uncertainties associated with these values would negate any assumption that the GC/MS analysis can be used for confirmation at the ppt level.

TABLE 39. PESTICIDE RECOVERY FROM RWV: ANALYTICAL DATA FROM PE-900/COLUMN 1

Pesticide	Added µg/liter	Before grit RWV		After grit RWV		Average	σ
		M5-1	M5-2	M5-3	M5-4		
α-BHC	10	83	83	85	83	84	1.0
λ-BHC	5	82	83	98	92	89	7.6
β-BHC	6	69*	65*	78*	66*	70*	5.9*
Heptachlor	6						
Δ-BHC	5	96	95	110	106	102	7.4
Aldrin	5	46	48	53	44	48	3.9
Heptachlor epoxide	10	78	74	91	81	81	7.2
Endosulfan I	13	76	76	93	79	81	8.1
DDE	6	67*	61*	71*	61*	65*	4.9*
Dieldrin	6						
Endrin	5	71	71	82	76	75	5.2
DDD	6	89*	89*	97*	91*	92*	3.8*
Endosulfan II	5						
DDT	5	84	71	76	75	77	5.4
Endrin aldehyde	0.6	Not measureable					
Endosulfan sulfate	1**	85	64	89	84	80	11.0

*Combined value for the two unresolved pesticides

**The values have been corrected for a component with the same retention time as endosulfan sulfate.

TABLE 40. PESTICIDE RECOVERY FROM RW: ANALYTICAL DATA FROM HP-5840A/COLUMN 2

Pesticide	Added µg/liter	Before grit RW		After grit RW		Average	σ
		M5-1	M5-2	M5-3	M5-4		
α-BHC	10	94	99	88	82	91	7.4
λ-BHC	5	92	103	92	82	92	8.6
β-BHC	6	89	98	88	84	90	5.9
Heptachlor	6	87	93	83	79	85	6.0
Δ-BHC	5	92	103	89	83	92	8.4
Aldrin	5	86	89	78	76	82	6.2
Heptachlor epoxide	10	92	96	85	83	89	6.1
Endosulfan I	13	91	94	86	84	89	4.6
DDE	6	87	88	81	74	83	6.4
Dieldrin	6	98	100	91	90	95	5.0
Endrin	5	88	88	85	76	84	5.7
DDD	6	99*	103*	102*	103*	102*	
Endosulfan II	5						1.9
DDT	5	96	98	86	84	91	7.0
Endrin aldehyde	0.6	40	23	40	35	35	8.0
Endosulfan sulfate	1**	117	118	107	113	114	5.0

* Combined value for the two unresolved pesticides.

** The values have been corrected for a component with the same retention time as endosulfan sulfate.

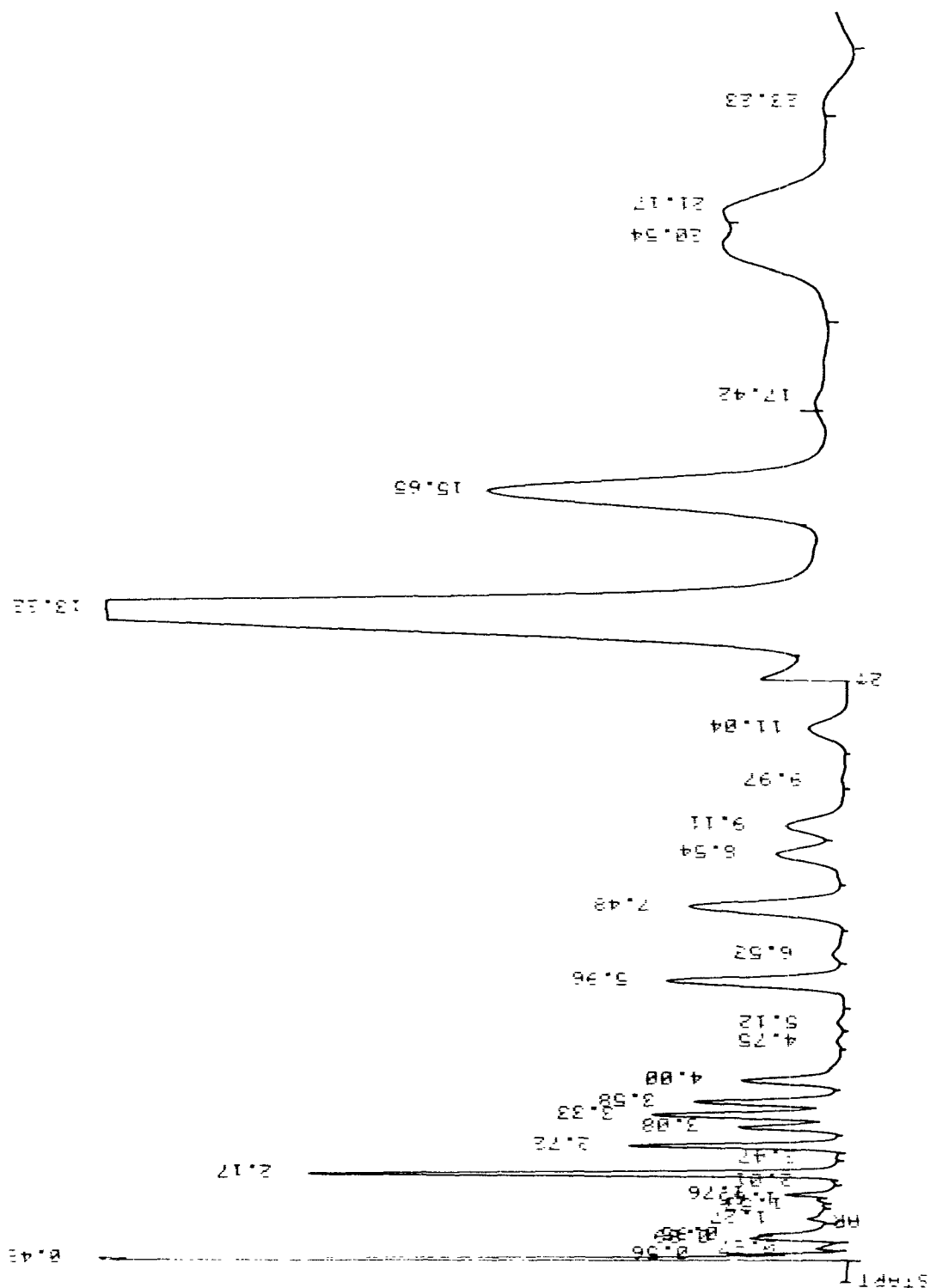


Figure 29. Chromatogram of sample M5-1 on HP-5840A/column 2.

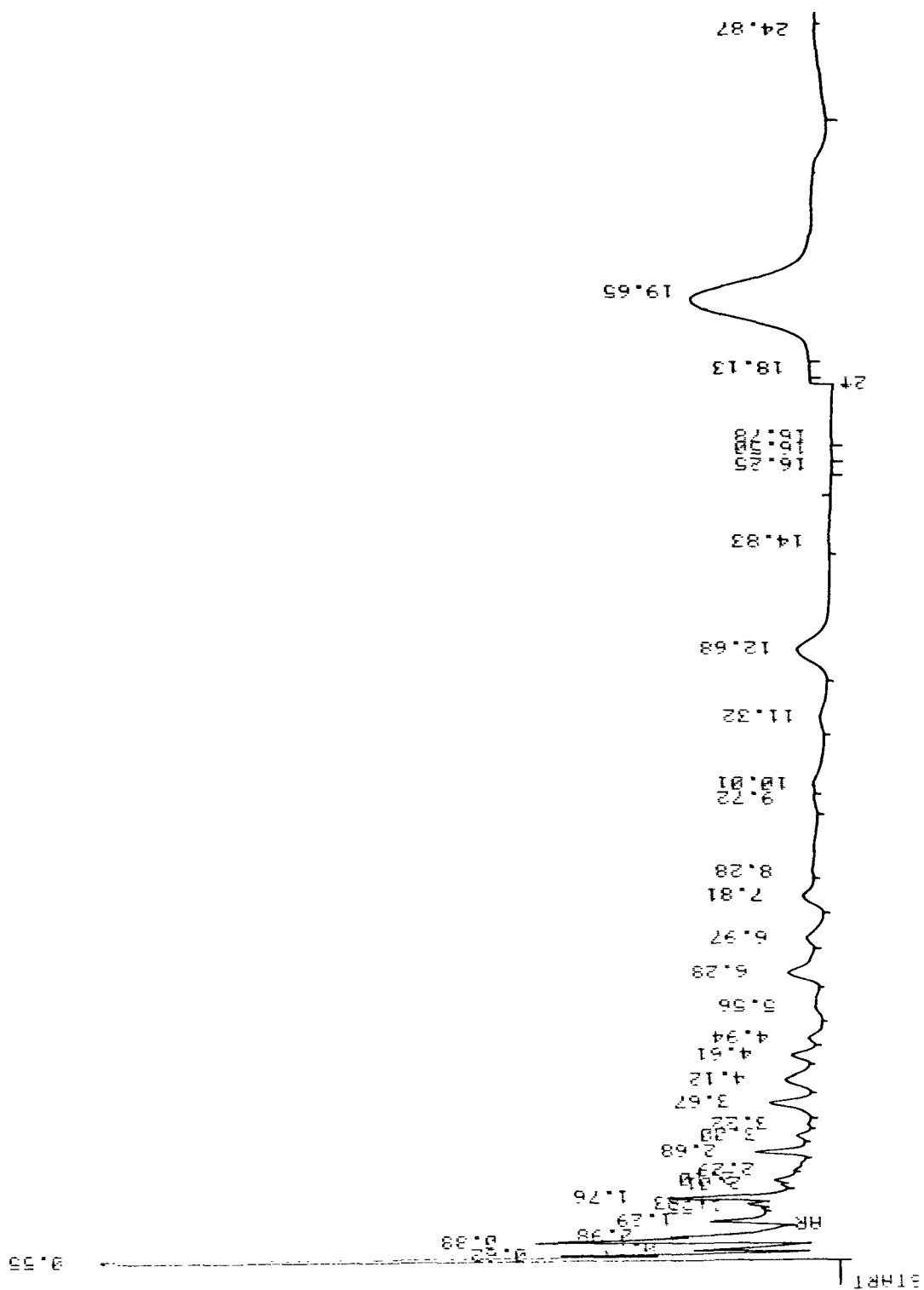


Figure 30. Chromatogram of RW blank on HP-5840A/column 2.

TABLE 41. ANALYTICAL DATA ON RAW WASTE WATER SAMPLES

<u>Pesticide</u>	Estimated Concentration, $\mu\text{g/liter}^*$	
	<u>PE-900/Column 1</u>	<u>HP 5840 A/Column 2</u>
λ -BHC	0.02	0.30
β -BHC	---	0.18
Δ -BHC	0.03	---
Aldrin	0.01	---
DDD	0.90*	0.80**
Endosulfan II		
Endosulfan sulfate	1.0	---

* Values reported are an average of two samples.

** DDD and endosulfan II are not resolved.

There is no doubt that the sensitivity of the electron capture detector is more than adequate for doing pesticide analysis on "clean" samples at the ppt level. However, there are practical and economic issues involved in doing ppt analysis in raw waste water by GC/ECD because of the interference problems. The standard protocol requires identification based on RRT on two columns and GC/MS confirmation. Since the GC/MS cannot be used at ppt levels, one alternative would be to use a third column. On the basis of using three columns for identification, and depending on the extent of the sample clean up necessary, a rough estimate of the cost per sample to do the ppt analysis is shown below.

One stage (sulfur removal)	\$125/sample
Two stages (sulfur removal and Sephadex)	250
Two stages (sulfur removal and Florisil)	325
Three stages (sulfur removal and Florisil, and Silica gel)	750

It is noted that for this task, the GC/ECD procedure was used for quantitative and qualitative analysis of the pesticides and PCB's in the raw waste water extracts subjected to the concentration and clean-up procedures discussed in the preceding sections. Compound confirmation was done with GC/MS using GC conditions identical to those used for GC/ECD analysis; the column was 1.5% SP-2250/1.95% SP-2401 on 100/120 mesh Supelcoport. GC/MS calibration was done by the use of standard solutions of pesticides and PCB's. It has been established that 10 ng of each pesticide, except endrin and DDT, which requires 30 ng, must be injected into the system for proper GC/MS confirmation; a sample injection of 2- μ L is used. Thus, each RWW sample extract was further concentrated to a smaller volume based on the GC/ECD quantitative results prior to GC/MS analysis. Using this approach, it was found that the RWW samples collected at the Brockton Sewerage Treatment Plant contained BHC, most likely λ -BHC. It is further noted that in a separate task, also for EPA, where approximately 200 RWW water samples from various sites in the U.S. have been collected, 21 samples showed pesticides tentatively identified by the GC/ECD method; of these 21 samples, only 5 samples have been confirmed to contain pesticides by GC/MS analysis. The pesticides found in these samples at 1 to 3 μ g/liter include heptachlor epoxide, heptachlor, and aldrin.

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APPENDIX A

CHEMICAL FORMULA OF PRIORITY POLLUTANT PESTICIDES

VARIOUS NAMES FOR PESTICIDES

FORMULA

BHC's

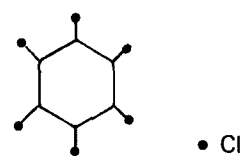
1,2,3,4,5,6—Hexachlorocyclohexane,
Benzene hexachloride,
P:99%
MW = 290

α — isomer — Alpha BHC

β — isomer — Beta BHC

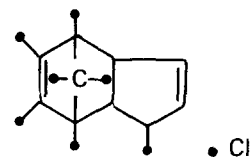
δ — isomer — Delta BHC

γ — isomer — Lindane, Gamma-BHC, Streunex, Tri-6,
Aparasin®, Aphtiria, Ben-Hex®, HCH®,
Lorexane®, Streunex®, HGI®
LD50 = 125 mg/kg



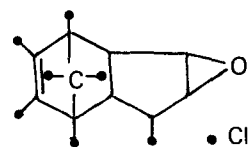
Heptachlor

1,4,5,6,7,8,8—Heptachloro-3a,4,7,7a-tetra-
hydro-4,7-methanoindene
E3314®, Velsicol 104®, Drinox®
P:99% → LD50 = 195 mg/kg
MW = 373



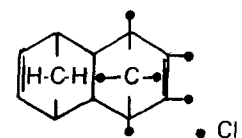
Heptachlor Epoxide

1,4,5,6,7,8,8—Heptachloro-2,3-epoxy-3a,4,7,
7a-tetrahydro-4,7-methanoindan
P:99%
MW = 389



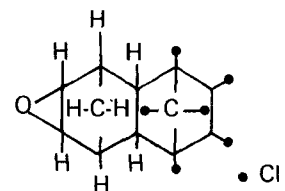
Aldrin

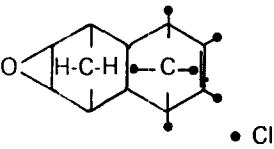
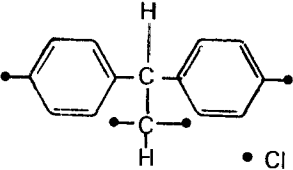
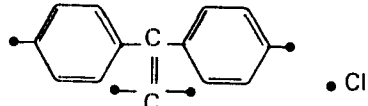
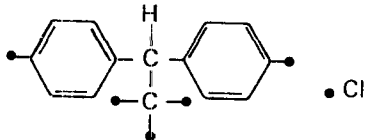
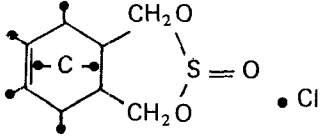
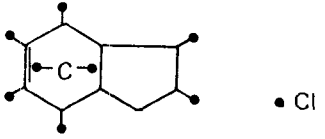
1,2,3,4,10,10-Hexachloro-1,4,4a,5,8,8a-
hexadhydro-endo-exo-1,4:5,8-dimethano-
naphthalene, Aldrin®
1,2,3,4,10,10-Hexachloro-1,4,4a,5,8,8a-
hexahydro-1,4, endo-exo-5,8-dimethylnaphthalene
P:99% LD50 = 55 mg/kg
MW = 365



Dieldrin

1,2,3,4,10,10-Hexachloro-6,7-epoxy-1,4,
4a,5,6,7,8,8a-octahydro-endo-exo-1,4:5,
8-dimethanonaphthalene
Compound 497®, HEOD, Dieldrin, Octalox®
P:98.5% LD50 = 10-102 mg/kg
MW = 383



Endrin	1,2,3,4,10,10-Hexachloro-6,7-epoxy-1,4,4a-5,6,7,8,8a-octahydro-1,4-endo-endo-5,8-dimethanonaphthalene Endrin, Experimental insecticide 269® P:98% LD50 = 7.5-17 mg/kg MW = 383	
p, p'—DDD	1,1-Bis[p-chlorophenyl] 2,2-dichloroethane TDE, Rothane®, Dichlorodiphenyl dichloroethane, 2,2-bis[p-chlorophenyl] - 1,1-dichloroethane P:96% LD50 = 3400 mg/kg MW = 320	
p,p' — DDE	1,1 Dichloro 2,2, bis [p-chlorophenyl] ethylene 0.1 g/1.0g P:99% MW = 318	
p,p' — DDT	1,1,1-Trichloro-2,2-bis [p-chlorophenyl] ethane 1g/10g P:99% LD50 = 250 mg/kg MW = 354	
Endosulfans	1,4,5,6,7,7-Hexachloro-5-norbornene-2,3- 1g/10g dimethanol cyclic sulfite Chlorthiepin®, Malix®, Hoe 2671®, Thiodan®, Cycloclan®, Beosit®, Thimul®, Thifor®, 6,7,8,9,10,10-Hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3 benzo-dioxathiepin 3-oxide P:98.9% LD50 = 80-110 mg/kg MW = 407	
Chlordane	1,2,4,5,6,7,8,8-Octachloro-3a,4,7,7a- 1g/10g tetrahydro-4,7-methanoindene CD-68®, Toxichlor®, Velsicol® 1068, Octachlor®, Octa-Klor® 1,2,4,5,6,7,8,8-Octachloro-4,7-methane-3a,4,7,7a-tetrahydroindane P:95% LD50 = 452-590 mg/kg MW = 409.75	
Toxaphene	Polychloro bicyclic terpenes Campheclor, Chlorinated camphene®, Geniphen®, Penphen®, Phenacide®, Phenetox®, Synthetic 3956®, Toxakil®, Toxaphene®, Hercules 3956®, Alltex® P:65% Cl LD50 = 90 mg/kg	$C_{10}H_{16-x}Cl_x$

APPENDIX B

PROCEDURE FOR THE DETERMINATION OF PRIORITY POLLUTANT PESTICIDES AND PCB's IN RAW WASTE WATER

SCOPE AND APPLICATION

1. This method covers the determination of the various priority pollutant pesticides and PCB's in raw waste water.
2. The method is based on the standard EPA method for these priority pollutants in industrial effluents and has been tested for the determination of the following pollutants: alpha-, beta-, delta-, gamma-BHC, aldrin, dieldrin, endrin, endrin aldehyde, heptachlor, heptachlor epoxide, p,p'-DDD, p,p'-DDE, p,p'-DDT, endosulfan I, endosulfan II, endosulfan sulfate, chlordane, and the arochlors.
3. For a sample size of 1 liter, the working limit of the method is estimated to be 1 µg/l liter for all the pesticides except for chlordane whose working limit is estimated to be 5 µg/liter, the PCB's also have a working limit of 5 µg/liter.
4. For samples containing high levels of the PCB's relative to the chlorinated pesticides (i.e., for example, a 20 to 1 ratio) differentiation of the pesticides from the PCB's is not possible. In this case, confirmation by GC/MS is necessary.

SUMMARY AND PRINCIPLE OF THE METHOD

1. The priority pollutant pesticides and PCB's are extracted from pH7 raw waste water with 15% methylene chloride/hexane; centrifugation is used to break up the emulsion. The extract is concentrated to 5 to 10 ml in a Kuderna-Danish evaporator. Sample clean-up and removal of interferences is carried out by a combination of one or more of the following methods: acetonitrile partition to remove fatty acids, treatment with mercury to remove sulfur and chromatographic clean-up techniques using either Florisil or Sephadex LH-20. Detection and measurement is accomplished using an electron capture detector (or alternatively a microcoulometric or electrolytic conductivity detector). Identification is made from relative retention times and gas chromatographic patterns obtained through the use of two or more unlike columns. Results are reported in micrograms per liter.

INTERFERENCES

1. Solvents, reagents, glassware, and other hardware used may yield discrete artifacts and/or elevated baselines which can cause misinterpretation of gas chromatograms. All of these materials must be demonstrated to be free from interferences under the conditions of the analysis. Specific selection of reagents and purification of solvents by distillation in all glass systems may be required.
2. The interferences in raw waste water are high and varied and often pose great difficulty in the accurate and precise measurement of chlorinated pesticides and the PCB's. Separation and clean-up procedures are generally required to eliminate these interferences; however, such techniques may result in some loss of the organo-chlorine compounds. For this reason, great care should be exercised in the selection and use of sample clean-up methods. While it is not possible to describe procedures for overcoming all of the interferences that may be encountered in municipal raw waste water, four clean-up procedures which have been evaluated for raw waste water are described in the section.
3. The PCB's, when present in high concentrations (ie., 10 µg/liter or greater), will act as severe interferences for the priority pesticides. And conversely, the priority pollutant pesticides will interfere with the PCB's.
4. Phthalate esters, certain organophosphorus pesticides, and elemental sulfur will interfere when using an electron capture detector. These materials do not interfere when the microcoulometric or electrolytic conductivity detectors are used in the halogen mode.

APPARATUS AND MATERIALS

1. Gas chromatograph - equipped with glass lined injection port.
2. Detector options:
 - Electron capture - radioactive (tritium or nickel 63)
 - Microcoulometric titration
 - Electrolytic conductivity.
3. Recorder - potentiometric strip chart (10 in.) compatible with the detector.
4. An electronic integrator or some other suitable method for measuring peak areas.
5. Gas chromatographic column, glass (180 cm long x 4-mm ID) packed with one of the following substrates:
 - 1.5% SP2250/1.95% SP2401 on 100/120 mesh Supelcoport,
 - 3% OV-1 on 100/120 Supelcoport or Gas Chrom Q.
 - 5% OV-210 on 100/120 mesh Supelcoport or Gas Chrom Q.

6% QF1/4% SE-30 on 100/120 mesh Supelcoport or Gas Chrom Q.

6. Kuderna-Danish (KD) glassware (Kontes)
 - Snyder column - three ball (macro) and two ball (micro)
 - Evaporative flasks - 100 ml
 - Receiver ampuls - 10 ml, graduated
 - Ampul stoppers
7. Chromatographic column - Chromaflex (400 mm long x 19 mm ID) with coarse fritted plate on bottom and Teflon stopcock; 250 ml reservoir bulb at top of column with flared out funnel shape at top of bulb - a special order (Kontes K-420540-9011).
8. Chromatographic column - pyrex (approximately 300 mm long x 19 mm ID) with Teflon stopcock.
9. Micro syringes - 10, 25, 50 and 100 μ l.
10. Separatory funnels - 125 ml, 1000 ml and 2000 ml with Teflon stopcock.
11. Blender - high speed, glass or stainless steel cup.
12. Test tubes - 8 ml with Teflon lined screw caps.
13. Graduated cylinders - 100, 250, and 1000 ml.
14. Assorted glassware.
15. Florisil - PR grade (60 - 100 mesh); purchase activated at 1250°F and store in the dark in glass containers with glass stoppers or foil-lined screw caps. Before use, pre-clean and activate each batch overnight at 130°C in foil-covered glass container. Determine lauric-acid value (see section of Determination of Lauric-acid value). The following apparatus are needed for this determination:
 - Buret - 25 ml with 1/10 ml graduations,
 - Erlenmeyer flasks - 125 ml narrow mouth and 25 ml, glass stoppered,
 - Pipet - 10 and 20 ml transfer,
 - Volumetric flask - 500 ml.
16. Sephadex LH-20 (Pharmacia Fine Chemicals). The dry bead diameter is 25 - 100 microns.
17. Boiling chips, Teflon.

REAGENTS, SOLVENTS, AND STANDARDS

1. Ferrous Sulfate - (ACS) 30% solution in distilled water,
2. Potassium Iodide - (ACS) 10% solution in distilled water.

3. Sodium Chloride - (ACS) saturated solution in distilled water (pre-rinse NaCl with hexane).
4. Sodium Hydroxide - (ACS) 10 N in distilled water.
5. Sodium Sulfate - (ACS) granular, anhydrous (pre-cleaned and conditioned at 400°C for 4 hours).
6. Mercury - (ACS).
7. Sulfuric Acid - (ACS) mix equal volumes of concentrated H_2SO_4 with distilled water.
8. Methylene Chloride - nanograde, redistill in glass if necessary.
9. Hexane - nanograde, redistill in glass if necessary.
10. Acetonitrile - nanograde, redistill in glass if necessary.
11. Methanol - nanograde, redistill in glass if necessary.
12. Toluene - nanograde, redistill in glass if necessary.
13. Petroleum Ether (boiling range 30° - 60°C) - nanograde, redistill in glass if necessary.
14. Diethyl Ether - nanograde, redistilled in glass if necessary.

Must contain 2% alcohol and be free of peroxides by following test: to 10 ml of ether in glass-stoppered cylinder previously rinsed with ether, add one ml of freshly prepared 10% KI solution. Shake and let stand one minute. No yellow color should be observed in either layer.

Decompose ether peroxide by adding 40 g of 30% ferrous sulfate solution to each liter of solvent. CAUTION: reaction may be vigorous if the solvent contains a high concentration of peroxides.

Distill deperoxidized ether in glass and add 2% ethanol.

15. Reagents and solvents for determination of Lauric Acid Value.

Alcohol, ethyl - USP or absolute, neutralized to phenolphthalein.

Hexane - distilled from all glass apparatus.

Lauric acid - purified, CP.

Lauric acid solution - transfer 10.000 g lauric acid to 500 ml volumetric flask, dissolve in hexane, and dilute to 500 ml (1 ml = 20 mg).

Phenolphthalein indicator - dissolve 1 g in alcohol and dilute to 100 ml.

Sodium hydroxide - dissolve 20 g NaOH (pellets, reagent grade) in water and dilute to 500 ml (1N). Dilute 25 ml 1N NaOH to 100 ml with water (0.05N). Standardize as follows: weigh 100 - 200 mg lauric acid into 125 ml Erlenmeyer flask. Add 50 ml neutralized ethyl alcohol and 3 drops phenolphthalein indicator; titrate to permanent end point. Calculate mg lauric acid/ml 0.05N NaOH (about 10 mg/ml).

16. Pesticides and PCB standards - Reference Grade Analytical Standards including the following:

Alpha-BHC

Beta-BHC

Delta-BHC

Gamma-BHC

Aldrin

Dieldrin

Endrin

Endrin aldehyde

Heptachlor

Heptachlor epoxide

p,p'-DDD

p,p'-DDE

p,p'-DDT

Endosulfan I

Endosulfan II

Endosulfan sulfate

Chlordane

Arochlors 1016, 1221, 1232, 1242, 1248, 1254, 1260

CALIBRATION

1. Gas chromatographic operating conditions are considered acceptable if the response to dicapthon is at least 50% of full scale when ≤ 0.06 ng is injected for electron capture detection and ≤ 100 ng is injected for

microcoulometric or electrolytic conductivity detection; or alternatively, for an electron capture detector, the response of 0.05 ng of aldrin is at least 50% of full scale. For all quantitative measurements, the detector noise level should be less than 2% of full scale.

2. Standards are injected frequently as a check on the stability of operating conditions.
3. The elution order and retention ratios of various organochlorine pesticides are provided in Table 1-B as a guide.

QUALITY CONTROL

1. Duplicate and spiked sample analyses are recommended as quality control checks. When the routine occurrence of a pesticide is being observed, the use of quality control charts is recommended.

SAMPLE PREPARATION

1. Blend the sample if suspended matter is present and adjust pH to near neutral (pH 6.5 - 7.5) with 50% sulfuric acid or 10 N sodium hydroxide.
2. Quantitatively transfer a 1 liter aliquot of homogenized sample into a 2 liter separatory funnel.

EXTRACTION

1. Add 60 ml of 15% methylene chloride in hexane (v:v) to the sample in the separatory funnel and shake vigorously for two minutes.
2. Allow sample to stand for at least 15 minutes and draw the bottom aqueous layer into a one liter Erlenmeyer flask, retaining any emulsion layer with the top organic solvent layer. Transfer the top organic solvent layer into a 250 ml centrifuge bottle.
3. Centrifuge the organic solvent layer at 1000 rpm for 5 - 30 minutes to break up emulsion. With the aid of a disposable pipet, draw the clear top **organic layer**, pass it through a column containing 3 - 4 inches of anhydrous sodium sulfate, and collect it in a 500 ml KD flask equipped with a 10 ml ampul.
4. Return both the aqueous layers in the Erlenmeyer flask and in the centrifuge bottle back into the separatory funnel. Rinse both Erlenmeyer flask and centrifuge bottle with 60 ml of 15% methylene chloride/hexane, transfer these solvent rinsings into the separatory funnel and repeat the extraction procedure a second time including centrifugation step and combine the clear organic extract with the first extract in the K-D assembly.
5. Repeat the extraction process in the manner a third time.

TABLE 1-B. RETENTION TIME OF PRIORITY POLLUTANT PESTICIDES RELATIVE TO
HEPTACHLOR EPOXIDE

Pesticide	1.5% SP2550*	1.5% OV-17**	5% OV-210 ^Δ	3% OV-1 ^Δ
	+ 1.95% SP2401	+ 1.95% QF-1		
α-BHC	0.37	0.35	0.33	0.27
λ-BHC (lindane)	0.46	0.45	0.42	0.34
β-BHC	0.52	--	--	--
Heptachlor	0.56	0.53	0.45	0.61
Δ-BHC	0.60	--	--	--
Aldrin	0.67	0.65	0.52	0.78
Heptachlor epoxide	1.00	1.00	1.00	1.00
Endosulfan I	1.25	1.27	1.28	1.27
p,p' DDE	1.43	1.45	1.09	1.56
Dieldrin	1.53	1.56	1.55	1.51
Endrin	1.85	1.90	1.84	1.70
p,p' DDD	2.21	2.26	1.94	2.04
Endosulfan II	2.21	2.33	2.38	1.76
p,p' DDT	2.62	2.71	2.11	2.73
Endrin aldehyde	2.92	--	--	--
Endosulfan sulfate	3.55	--	--	--

* Glass column 180 cm x 4 mm ID, solid support 100/120 mesh Supelcoport, column temperature 200°C, Argon/Methane Carrier flow rate at 60 ml/minute.

** Same conditions as (1) except solid support is 100/120 mesh Gas-Chrom Q.

Δ Glass column 180 x 4 mm ID, solid support 100/120 Gas-Chrom Q, column temperature 200°C, Argon/Methane Carrier flow rate at 70 ml/minute.

6. Add a piece of Teflon boiling chip and concentrate the extract in the KD evaporator on a hot water bath. This evaporation step should be conducted at a fast rate. It should take no more than 60 minutes to concentrate the combined extracts, including two sequential rinsings of the KD assembly with 25 ml of hexane and evaporation to 5.0 ml. (Tests have shown that a slow evaporation rate yields poor recoveries.)
7. Analyze by gas chromatography. Perform any sample dilution or clean-up necessary as indicated by this initial GC analysis.

CLEAN-UP AND SEPARATION PROCEDURES

1. Interferences in the form of distinct peaks and/or high background in the initial gas chromatographic analysis, as well as the physical characteristics of the extract (color, cloudiness, viscosity) and background knowledge of the sample will indicate whether clean-up is required. When these interfere with measurement of the pesticides, or affect column life or detector sensitivity, proceed as directed below.
2. Sulfur removal - the procedure described below eliminates a common interference found in raw waste water. Tests have shown that the method can be applied directly to the concentrated extract or to the respective column fractions with quantitative recovery of the priority pollutant pesticide and PCB's. However, the analyst must demonstrate quantitative recovery of specific pesticides and PCB's at least once.

Take a 2 ml aliquot of the sample extract or column fraction and transfer to a centrifuge tube with ground-glass stopper.

With the aid of a disposable pipet, add 1 drop of metallic mercury to the centrifuge tube and shake gently. Observe for the formation of a black precipitate which indicates the presence of sulfur. Let stand for 2 minutes only, centrifuge quickly and immediately transfer this solution into a second tube, avoiding transfer of any mercury or black precipitate. Repeat this process two to three times but do not allow the sample solution to stay in contact with the mercury for longer than 5 minutes.

Analyze by gas chromatography.

3. Acetonitrile partition - this procedure is used to isolate fats and oils from the sample extracts. Tests have shown that the priority pollutant pesticides and PCB's are quantitatively recovered by this procedure; however, the analyst must demonstrate the efficiency of the partitioning for specific pesticides and PCB's at least once.

Quantitatively transfer the previously concentrated extract to a 125 ml separatory funnel with enough hexane to bring the final volume to 15 ml. Extract the sample four times by shaking vigorously for one minute with 30 ml portions of hexane-saturated acetonitrile.

Combine and transfer the acetonitrile phases to a 1 liter separatory funnel and add 650 ml of distilled water and 40 ml of saturated sodium chloride solution. Mix thoroughly for 30-45 seconds. Extract with two 100 ml portions of hexane by vigorously shaking about 15 seconds.

Combine the hexane extracts in a 1 liter separatory funnel and wash with two 100 ml portions of distilled water. Discard the water layer and pour the hexane layer through a 3-4 inch anhydrous sodium sulfate column into a 500 ml KD flask equipped with a 10-ml ampul. Rinse the separatory funnel and column with three 10-ml portions of hexane.

Concentrate the extracts to 5-10 ml in the KD evaporator in a hot water bath.

Analyze by gas chromatography unless a need for further clean-up is indicated.

4. Florisil column adsorption chromatography

Clean-up of Florisil and column preparation:

Clean-up the Florisil before use by washing the Florisil with boiling water (distilled, or preferably filtered through a Milli Q unit or equivalent); 200 g of Florisil requires three washes with 1 liter of boiling water each time. Allow most of the water to drain and dry the Florisil at 130°C for 4 hours. Then calcine the Florisil by heating in a muffle furnace at 675°C for 25 hours. Store in a dessicator and keep protected from light.

Determine lauric acid value as described below.

Just before use, weigh the amount needed based on the lauric acid value in a 125 ml beaker, cover with foil and heat again for 1 hour at 130°C. Immediately after removing from the oven, pack the Florisil into the Chromaflex column with gentle tapping to let the Florisil settle and proceed as directed under column chromatography procedure.

Determination of lauric acid value - a rapid method for determining adsorptive capacity of Florisil is based on adsorption of lauric acid from hexane solution (6)(8). An excess of lauric acid is used and amount not adsorbed is measured by alkali titration. Weight of lauric acid adsorbed is used to calculate, by simple proportion, equivalent quantities of Florisil for batches having different adsorptive capacities. The procedure is as follows:

Transfer 2.000 g Florisil to 25 ml glass stoppered Erlenmeyer flasks. Cover loosely with aluminum foil and heat overnight at 130°C. Stopper, cool to room temperature, add 20.0 ml

lauric acid solution (400 mg), stopper, and shake occasionally for 15 minutes. Let adsorbent settle and pipet 10.0 ml of supernatant into 125 ml Erlenmeyer flask. Avoid inclusion of any Florisil.

Add 50 ml neutral alcohol and 3 drops indicator solution; titrate with 0.05N to a permanent end point.

Calculate amount of lauric acid adsorbed on Florisil as follows:

$$\text{Lauric acid value} = \frac{\text{mg lauric acid}}{\text{g Florisil}} = \frac{200 - (\text{ml required for titration} \times \text{mg lauric acid/ml } 0.05\text{N NaOH})}{\text{g Florisil}}$$

To obtain an equivalent quantity of any batch and multiply by 20 g. The pattern of elution of pesticides are indicated below and must be independently verified by the analyst.

Column chromatography procedure

Adjust the sample extract volume to 10 ml with hexane.

After settling the Florisil by tapping the column, add about one-half inch layer of anhydrous granular sodium sulfate to the top. Allow Florisil to cool.

Pre-elute the column, after cooling, with 50-60 ml of petroleum ether. Discard the eluate and just prior to exposure of the sulfate layer to air, quantitatively transfer the sample extract into the column by decantation and subsequent petroleum ether washings. Adjust the elution rate to about 5 ml per minute and, separately, collect up to three eluates in 500 ml KD flasks equipped with 10 ml ampuls. (See eluate composition below). Perform the first elution with 200 ml of 6% ethyl ether, and the second elution with 200 ml of 15% ethyl ether in petroleum ether. Perform the third elution with 200 ml of 50% ethyl ether - petroleum ether and the fourth elution with 200 ml of 100% ethyl ether.

Concentration the eluates to 6-10 ml in the KD evaporator in a hot water bath.

Analyze by gas chromatography.

Eluate composition by using an equivalent quantity of any batch of Florisil as determined by its lauric acid value, the pesticides will be separated into the eluates indicated on the next page.

6% Eluate

Aldrin	DDT	Pentachloronitrobenzene
BHC	Heptachlor	Strobane
Chlordane	Heptachlor epoxide	Toxaphene
DDD	Lindane	Trifluralin
DDE	Methoxychlor	PCB's
	mirex	

15% Eluate

Endosulfan I
Endrin
Dieldrin
Dichloran
Phthalate esters

50% Eluate

Endosulfan II
Captan

Certain thiophosphate pesticides will occur in each of the above fractions as well as the 100% fraction. For additional information regarding eluate composition, refer to the FDA Pesticide Analytical Manual (6).

5. Chromatography on Sephadex LH-20

This is suggested as an alternative to the Florisil column clean-up procedure for the removal of phthalate interferences and other high molecular weight interferences. Limited tests have shown that the phthalates elute earlier than the priority pollutant pesticides and PCB's. Tests have also shown quantitative recovery of these chlorinated compounds using this chromatographic clean-up procedure. However, the analyst must demonstrate the qualitative recovery of the priority pollutant pesticides and PCB's with the procedure described.

Preparation of chromatographic column

Allow the Sephadex LH-20 beads to swell in methanol for at least one over night period. Approximately 13-14 g of dry beads are needed to pack one column.

Plug the effluent end of a glass column (300 mm x 19 mm ID) with a small wad of pre-cleaned glass wool. Mark the glass column at a point 19 cm above the glass wool plug. Fill the column half-way with methanol, gently swirl the swollen gel to make a fairly thick slurry in methanol, open column stop-cock and pack the column with enough gel to fill the column exactly to the 19 cm mark. To prepare a well packed column, it is advisable to add the gel slurry uninterrupted and employ gentle tapping as the gel settles. A well packed column should be free of cracks and air bubbles. (The total bed volume is 54 ml.)

To clean the gel, wash the column bed with at least 200 ml of methanol and collect a 20 ml effluent. Analyze effluent by gas chromatography. If the analysis shows presence of interferences, wash column with more methanol until the column effluent is free of interferences; if the effluent is "clean," proceed to the next step.

Wash the column gel bed with 100 ml of methanol/toluene (1:1). After this conditioning cycle, adjust column flow-rate to 1.0 ml per minute and collect 20 ml of effluent. Analyze effluent by gas chromatography. This effluent should be "free" of interferences.

Prepare column for sample application by allowing the solvent to drain until the solvent level reaches the top level of the gel bed. Close column stopcock.

Chromatographic separation

A sample size of 5 ml in hexane is carefully added to the column minimizing agitation of the gel by carefully pouring solution either down the wall of the column or down a glass rod.

Position a 50 ml graduated tube beneath the column and open stopcock. When the sample level reaches the top of the gel bed, rinse the glass rod and/or wall of the column with 2-3 ml of methanol/toluene (1:1). Let the rinse solvent reach the level of the gel bed again before gradually adding 50 ml of methanol to toluene to the column. Maintain column flow rate at 1 ml per minute and collect a total of 30 ml of effluent. (Fraction 1.) This fraction contains the phthalates and other interferences with molecular weights greater than the priority pollutant pesticides and PCB's/

In another graduated tube, collect the next 20 ml of effluent (Fraction 2.) This fraction contains priority pollutant pesticides and PCB's.

Collect the next 10 ml of effluent. (Fraction 3.) This fraction serves as assurance that the pesticide and PCB's are completely eluted but avoids dilution of the preceding fraction.

Analyze fractions 2 and 3 by gas chromatography.

Addendum notes on Sephadex LH-20

The elution volume cut off for the three fractions were based on studies which showed that for a column gel bed of the given dimensions, at least 80% of the total pesticides and PCB's

elute in the relative retention volume (V_r) range of .055-0.93, where V_r is defined as:

$$V_r = \frac{\text{Elution Volume}}{\text{Total bed volume}}$$

These V_r range correspond to the elution volume sequence of 30-50 ml (Fraction 2).

Limited experiments here also show that Fraction 3 (V_r range = 0.93-1.1) may contain a small amount, a 10-20% of the pesticide sample. However, collecting fractions 2 and 3 together is not recommended because of the dilution effect and the observed losses associated with KD concentration. Tests have shown that when 1 μ g of chlorinated pesticide and 15 μ g of PCB were added to 30 ml of methanol/toluene (1:1) and then concentrated to 10 ml by KD evaporation, losses of as much as 30-50% of these chlorinated components can occur.

CALIBRATION OF STANDARDS

1. Prepare a calibration curve for the specific pesticides and PCB's of interest to cover a linear response range. Use at least three different concentrations.
2. Calculate the calibration constant (k) by the nanograms of components.

CALCULATION AND REPORTING RESULTS

1. Determine the pesticide concentration as described below expressing results in micrograms per liter which is numerically equal to nanograms per ml.

$$\text{micrograms/liter} = \frac{A \times V_t}{k \times V_i \times V_w}$$

where:

A = Peak area of sample
k = Calibration constant, area per nanogram
 V_i = Value of extract injected (μ l)
 V_t = Value of total extract (μ l)
 V_w = Value of water extracted (ml)

TECHNICAL REPORT DATA <i>(Please read Instructions on the reverse before completing)</i>		
1. REPORT NO EPA-600/2-79-166	2.	3. RECIPIENT'S ACCESSION NO.
4. TITLE AND SUBTITLE EVALUATION OF PROTOCOLS FOR PESTICIDES AND PCB'S IN RAW WASTEWATER	5. REPORT DATE November 1979 (Issuing Date)	6. PERFORMING ORGANIZATION CODE
	8. PERFORMING ORGANIZATION REPORT NO.	
7. AUTHOR(S) Alegria B. Caragay Philip L. Levins	10. PROGRAM ELEMENT NO. 1BC611, SOS #5, Task AI-04	
9. PERFORMING ORGANIZATION NAME AND ADDRESS Arthur D. Little, Inc. 20 Acorn Park Cambridge, Massachusetts 02140	11. CONTRACT/GRANT NO. 68-01-3857	
	13. TYPE OF REPORT AND PERIOD COVERED FINAL REPORT 6/78-10/78	
12. SPONSORING AGENCY NAME AND ADDRESS Municipal Environmental Research Laboratory-Cin., OH Office of Research and Development U.S. Environmental Protection Agency Cincinnati, Ohio 45268	14. SPONSORING AGENCY CODE EPA/600/14	
	15. SUPPLEMENTARY NOTES Project Officer: Robert T. Williams (513) 684-7626	
16. ABSTRACT <p>The general EPA protocol for screening industrial effluents for priority pollutants (Federal Register <u>38</u>, No. 75, Part II), has been tested for its applicability to the analysis of the priority pollutant pesticides and PCB's in raw wastewater. Raw wastewater from the municipal sewage treatment plant in Brockton, Massachusetts was dosed with 1-30 ppb of the priority pollutant pesticides and PCB's.</p> <p>The overall procedure evaluated consists of the following steps: extraction with 15% methylene chloride/hexane with centrifugation to break up the emulsion, concentration by Kuderna-Danish evaporation, removal of interferences by acetonitrile partition, chromatography on Florisil and Sephadex LH-20, and sulfur removal by treatment with mercury. Samples were assayed by gas chromatography using an electron capture detector.</p> <p>The data obtained show that the Kuderna-Danish evaporation step could be a significant source of sample loss unless the evaporation process is carried out at a fast rate. Treatment with mercury effectively cleans up the extracts with no significant loss of pesticides. Sample clean-up on a Sephadex LH-20 is recommended as an alternative to the Florisil column clean-up procedure. The method tested works well for parts per billion determination.</p>		
17. KEY WORDS AND DOCUMENT ANALYSIS		
d. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Pesticides Extraction Chemical Analysis Centrifuging DDT Dieldrin	Arochlors Polychlorinated Biphenyls Priority Pollutants	07C
18. DISTRIBUTION STATEMENT RELEASE TO PUBLIC	19. SECURITY CLASS (This Report) UNCLASSIFIED	21. NO. OF PAGES 110
	20. SECURITY CLASS (This page) UNCLASSIFIED	22. PRICE