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DEVELOPMENT AND APPLICATION OF TEST PROCEDURES FOR SPECIFIC ORGANIC TOXIC SUBSTANCES IN WASTEWATERS

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DRAFT FINAL REPORT

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CATEGORY 1: Phthalate Esters

EPA Contract 68-03-2606
SwRI Project 01-5068-101

Prepared for

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January 1980

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SECTION 1
INTRODUCTION

This project was part of an overall program of the Environmental Protection Agency to establish test procedures for the analysis of 114 toxic substances in wastewaters. These toxic compounds have been separated into 12 categories as follows:

Category 1:	Phthalate Esters
Category 2:	Haloethers
Category 3:	Chlorinated Hydrocarbons
Category 4:	Nitrobenzenes and Isophorone
Category 5:	Nitrosamines
Category 6:	Dioxin
Category 7:	Benzidines
Category 8:	Phenols
Category 9:	Polynuclear Aromatics
Category 10:	Pesticides and PCB's
Category 11:	Purgeables
Category 12:	Acrolein, Acrylonitrile, Dichlorodifluoromethane

This is the report on Category 1: Phthalate Esters.

The specific compounds studied in this effort were:

1. Dimethyl Phthalate (DMP)
2. Diethyl Phthalate (DEP)
3. Dibutyl Phthalate (DBP)
4. Benzyl Butyl Phthalate (BBP)
5. Diethylhexyl Phthalate (DEHP)
6. Dioctyl Phthalate (DOP)

It was desirable that common sample treatment for the various categories be employed, where possible, to minimize cost of analysis of unrelated compounds in any given water sample. The efforts reported under the following performance headings were designed to provide information relative to this common purpose.

The study was conducted in two phases. In Phase I, work was conducted with clean water and was intended to provide information

which would give direction to Phase II work, conducted on actual wastewaters, and serve as a comparison base for the information developed.

The ultimate objective was to develop a method, having a maximum of applicability, which could be published in the Federal Register and carried out in most analytical laboratories. Other objectives were to develop accuracy and precision information for the various steps in the method, to develop procedure variations as required by a particular wastewater, and to maximize the use of common sample treatment steps for all of the categories of organic toxic substances listed above.

SECTION 2
CONCLUSIONS

The six phthalate esters can be gas chromatographed very well. Sensitivity quantities (producing a response 10X noise level) for the electron capture detector range from 20 to 130 picograms. Sensitivity quantities for the flame ionization detector are roughly 200-700 times greater than for the electron capture detector.

Extraction of the phthalate esters from clean water at low parts per billion (μg/L) concentrations when the pH was 2, 7, and 10 was generally 90 percent or better when using 15 percent DCM in hexane or 100 percent DCM as the extracting solvent.

At the concentrations mentioned above, some of the conditions of the preservation study produced notable effects on some of the phthalate esters. DMP and BBP extraction recoveries were reduced to 30 percent and 40 percent, respectively, after storage for 7 days in the dark at pH 10 and 24°C. Storage at 4°C under acid conditions proved satisfactory.

Recoveries from Florisil and alumina cleanup columns were excellent if the adsorbent was slightly deactivated by the addition of 3 percent water.

Five wastewaters were analyzed and none was found to be completely free of phthalate esters. The number of phthalate esters and amounts

did vary considerably with the different wastewaters.

The recoveries of the phthalate esters dosed into the wastewaters was, in general, good. Recoveries of selected phthalate esters was somewhat reduced with some wastewaters and marked reduction in recoveries from some wastewaters was noted after storage of the dosed wastewaters for 7 days at 4°C.

The principal problems encountered with the method are contamination of glassware and nonlinearity of response of the DMP and DEP with the electron capture detector.

The method is basically acceptable for use on wastewaters having a low level of interferences. It does not work well when background levels are medium to high.

SECTION 3
RECOMMENDATIONS

The gas chromatographic column used in this effort (1.5 percent SP-2250 + 1.95 percent SP-2401 on 100/120 mesh Supelcoport), although satisfactory for separation of all six phthalate esters, is only one of many GC columns that could be used. The six phthalate esters are separated primarily on the basis of boiling point and many other column packings would be satisfactory.

The electron capture detector, being much more sensitive to the phthalate esters than the flame ionization detector, is recommended for most wastewater applications. The electron capture detector would be less desirable with wastewaters contaminated with chlorinated compounds.

Either 15 percent DCM in hexane or pure DCM is satisfactory for extraction of phthalate esters from wastewaters in the pH range of 2-10. If wastewater is to be stored prior to analysis for phthalate esters, the water should be adjusted to acidic conditions and the samples should be stored in a cold (4°C) room. A preliminary evaluation of the wastewater would still be necessary to ensure that there was no degradation under these conditions.

Florisil and alumina used in column chromatography must be deactivated by addition of 3 percent water to insure recovery of all phthalate esters.

Great care should be exercised in preparing the reagents and glassware used in the analysis. Phthalate esters, due to their occurrence in many manufactured products, are a common source of sample contamination in the laboratory, and frequent reagent blanks should be run to establish levels of contamination.

Only chemists experienced with the method and with trace organic analysis in general should conduct analysis for phthalate esters.

The method is recommended primarily for wastewaters with only low levels of contamination. The collection of two fractions, instead of one, from column chromatography cleanup may be helpful in some cases and is recommended when higher levels of early eluting interferences are suspected.

SECTION 4
PHASE I

OBJECTIVES

The objectives of Phase I were limited to:

1. Review of scientific literature for key references relative to determination of the phthalate esters in water and other matrices.
2. Determination of the best gas chromatographic columns and conditions for detection of phthalate esters when using the detectors of interest.
3. Determination of extraction efficiency for phthalate esters from clean water samples.

PROGRAM DIRECTION

Before experimental work began, representatives of SwRI met in Cincinnati with the project officer and others of concern in the EPA Cincinnati laboratories to discuss and decide on certain specific aspects of the activities in Phase I. In addition to the decisions reached on GC columns, extraction solvents, and other experimental details which will be given in subsequent sections of this report, guidelines were developed as follows:

* Unusual, exotic, or overly expensive items were not desirable. Simplicity was to be preferred wherever the option existed. For example, mass spectrometry and WCOT or SCOT columns were ruled out.

* Use of chemical treatment to produce a derivative which cannot be unequivocally related to the parent substance was not acceptable.
* Quantitation by electronic integration of peak area or by peak height measurement was acceptable.

EXPERIMENTAL

Literature Search

A comprehensive literature search was made using a terminal linked to Lockheed Information Systems' DIALOG®. Chemical Abstract Condensates (CAC) were searched for the years 1972 through the present. Also, NTIS (1964 to present), Enviroline (1971 to present), and Pollution Abstracts (1970 to present) were searched. In some cases, both on-line and off-line prints were obtained, the former in order to rapidly obtain a small enriched set of references to work with and the latter to provide comprehensive coverage by retrieval of a larger set of references.

The literature search as conducted yielded over 150 references. Examination of titles and in many cases abstracts drastically reduced the number to those listed in Appendix A as being of interest to this program. The papers of G. S. Giam and his coworkers at Texas A&M are of particular interest. The paper by Giam et al. entitled "Sensitive Method for Determination of Phthalate Ester Plasticizer in Open-Ocean Biota Samples" (Anal. Chem., Vol. 47, No. 13, November 1975) was probably the most important product of the literature search. The authors report several possible laboratory sources of phthalate esters which

can/could result in contamination of samples. They also report the necessity of deactivation of Florisil to prevent loss of DEHP when column chromatography employing Florisil is used in a sample cleanup procedure.

Gas Chromatography

All six (6) phthalates have been chromatographed on two columns. The primary column (Column 1) is 1.8-m x 4-mm ID glass tubing packed with 1.5-percent SP-2250 plus 1.95-percent SP-2401 on 100/120 mesh Supelcoport. The secondary column (Column 2) has 3-percent OV-1 as its liquid phase but is otherwise the same as Column 1.

Initial investigations with Column 1 indicated that all six phthalates could be resolved at a column temperature of 200°C. However, the retention time for DOP was excessive (34 minutes) and the early eluting phthalates would be difficult to quantitate due to the proximity of the solvent peak. This would be even more critical on extracts of wastewater where the early eluters would be more likely to co-elute with interferences. Therefore, the six phthalates were divided into two groups of three for chromatography. The "low" temperature (160°C) group includes DMP, DEP, DBP, while the "high" temperature (225°C) group includes BBP, DEHP, and DOP. Table 1 gives the retention times of the six phthalate esters on Column 1. Example chromatograms of these compounds are shown as Figures 1 and 2.

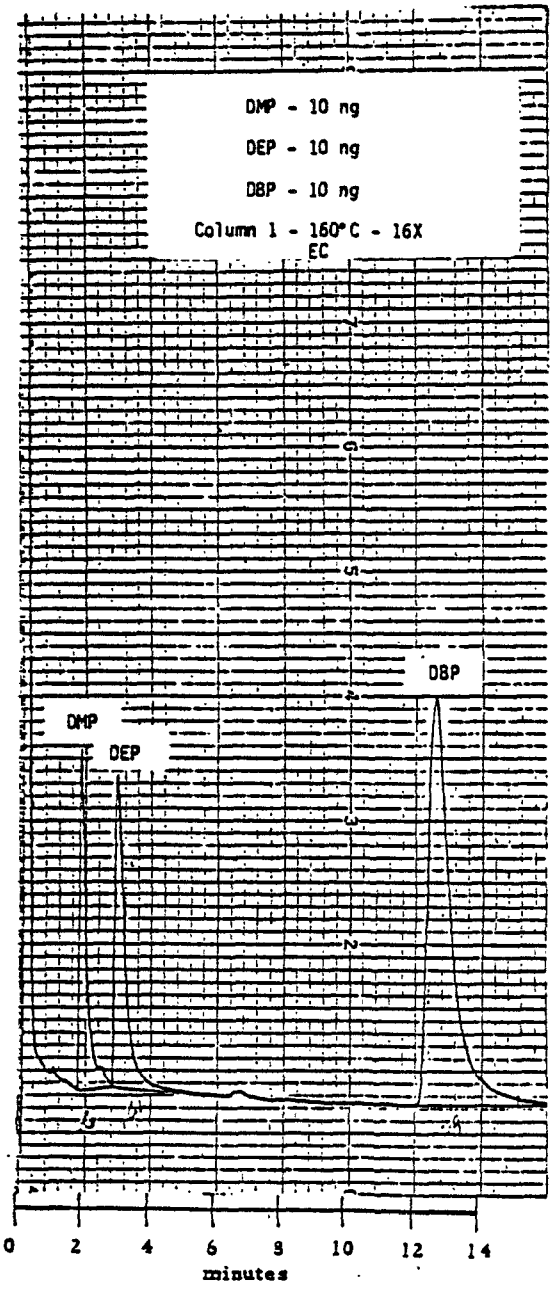

Figure 1. Standard for DMP, DEP and DBP.

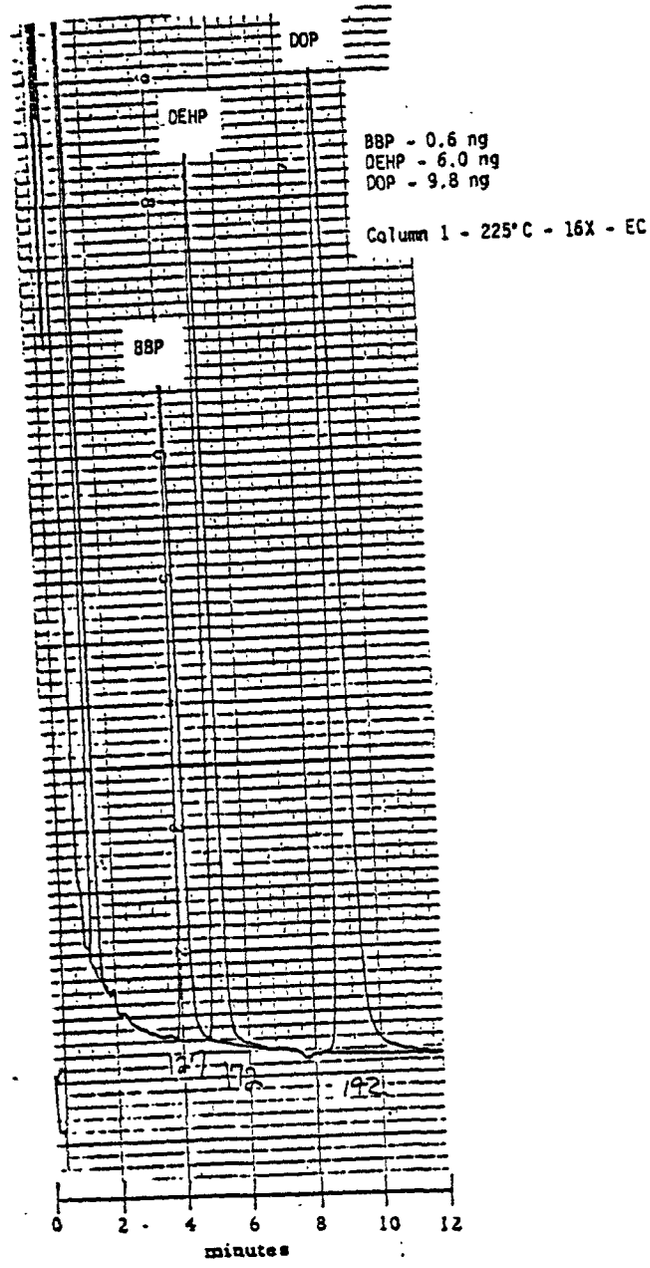

Figure 2. Standard for BBP, DEHP and DOP

TABLE 1. RETENTION TIMES
(Column 1; 60 mL/min)

Phthalate Ester		Minutes
DMP	160°C	1.9
DEP		2.9
DBP		12.6
BBP	225°C	4.1
DEHP		5.1
DOP		9.0

Investigations with Column 2 gave comparable results, leaving little basis for recommending either column over the other. Both columns perform satisfactorily, although somewhat greater sensitivities were obtained with Column 1 (see below).

At the beginning of this work, the electron capture detector was considered to be the primary detector to be used in the analysis of phthalates, and the flame ionization detector was designated the alternate detector. Experimentation confirmed that the electron capture detector is preferred over the flame ionization detector on the bases of the greater sensitivity and selectivity. Table 2 gives comparative sensitivity values for the two detectors.

TABLE 2. SENSITIVITY VALUES
(Amount in nanograms required to produce response 10X noise level)

	Detector		
	Electron Capture		Flame Ionization
Phthalate Ester	Column 1	Column 2	Column 1
DMP	0.11	1.3	18.8
DEP	0.13	1.9	31.3
DBP	0.02	0.3	13.9
BBP	0.02	0.5	15.3
DEHP	0.04	0.7	20.3
DOP	0.11	0.9	31.3

The gas chromatographs used in this work were a Hewlett-Packard 5710A with a Ni-63 electron capture detector and a Hewlett-Packard 5710A with a flame ionization detector. Operating conditions of these instruments were:

	ECD	FID
Oven temperature	variable	variable
Detector temperature	300°C	250°C
Carrier gas	5% methane in argon	helium
Carrier gas flow rate	60 mL/min	60 mL/min
Flame gases	---	H_2 + air

Extraction Study

The extraction study was initiated to determine the recoveries of the six phthalates of interest from clean water at pH 2, 7, and 10 using 15 percent dichloromethane (DCM) in hexane and 100 percent DCM as the extracting solvents.

The water used in the extraction study is a naturally buffered well water obtained from the SwRI supply line prior to chlorination. Samples of this water were collected and transported to the laboratory in empty solvent bottles which had previously contained Burdick and Jackson solvents. The pH of the untreated water is close to 8. Adjustment to the required pH's was accomplished by adding strong acid or base as follows:

pH 2 - 2.5 mL 18N H_2SO_4

pH 7 - 0.1 mL 18N H_2SO_4

pH 10 - 0.2 mL 10N NaOH

The water was found to be very low in electron capture sensitive materials as determined through comparison of an extract of the water with a glassware blank. Chromatograms in these instances were very similar, strongly indicating that the water could not be the source of any

large interferences.

The water was dosed with acetone solutions of the six phthalate esters, each solution containing 100 times the sensitivity amount per microliter, as determined earlier for each phthalate. By adding 100 µL of the appropriate acetone solution to a liter of water, dosed water samples for extraction were prepared.

Poor reproducibility, contamination problems and loss of sensitivity after replacement of a defective EC detector prompted an increase in dosing levels after two attempts failed to get usable data from the extraction study. Only the data from the third effort are reported, as shown in Table 3.

TABLE 3. QUANTITIES OF SPIKE FOR EXTRACTION STUDY

Phthalate	Dosing Solution µg/mL	Conc. in 1 Liter ng/mL
DMP	100	10
DEP	100	10
DBP	100	10
BBP	6	0.6
DEHP	60	6.0
DOP	97.5	9.8

The one-liter samples of water were dosed while in one-liter Erlenmeyer flasks, then poured into two-liter separatory funnels for extraction by either 15 percent DCM in hexane or 100 percent DCM in accordance with the procedure found in the Federal Register, Volume 38, No. 125, Friday, June 29, 1973, page 17320. Three dosed samples were extracted at pH 2 and 10 with each solvent. At pH 7, four dosed samples were extracted with each solvent. Thus, 20 samples were extracted for each group. The final volume of the extract concentrate was always

10 mL, and 10 μL of the concentrate was always injected into the GC. At the time dosing of the samples occurred, 100 μL of the dosing solution was added to 10 mL of hexane which then served as the standard against which all recoveries were measured.

The Federal Register procedure calls for the separated extracts to be drained through three to four inches of anhydrous sodium sulfate into a Kuderna-Danish flask. Frequent plugging of the tube containing the sodium sulfate was encountered. Usually unplugging could be achieved by applying a slight pressure to the top of the tube by means of a 2-ounce rubber bulb. It was discovered that the column of sodium sulfate could be decreased in height to 3 to 4 centimeters without any obvious effect on the analytical results and with a more rapid delivery of extract into the Kuderna-Danish flask. In the course of unplugging the sodium sulfate tube, a small amount of water was sometimes forced into the Kuderna-Danish flask. This situation produces no harmful effects unless the quantity is so great as to cause an intolerable volume error in determining the final 10-mL volume or unless the water phase should be accidentally injected into the GC.

When the extracting solvent is 100 percent DCM, it is necessary to remove essentially all of the DCM prior to analysis. This was done by taking the extract to a volume of 10 to 15 mL, adding 75 to 100 mL of hexane, and then reconcentrating to the final volume. The DCM extracts usually produced a wider "solvent" peak than those produced by 15 percent DCM in hexane. This peak broadening was not reduced when the amount of hexane added before reconcentration was increased

in amounts up to 200 mL. The peak broadening incurred is not a serious problem, however.

Occasionally, an emulsion resulted during the extraction process. This occurred usually with the 15 percent DCM in hexane solvent and at pH 2 and 7. After draining off most of the water phase, the emulsion could be broken almost always by shaking or swirling the separatory funnel. When emulsification occurred, the solvent phase was usually not as well separated from the water phase as when emulsification did not occur, resulting in a higher rate of plugging of the sodium sulfate tube.

The most serious problem encountered was the inability to obtain chromatograms of blanks for water, glassware and sodium sulfate which were devoid of interfering peaks.

The most consistent interfering peaks were observed at the same retention times as BBP and DEHP. Other interfering peaks with retention times matching the other four phthalates were present at a lesser frequency. Figure 3 is a chromatogram (at 225°C) of an unspiked water extract using DCM. This chromatogram illustrates the magnitude of the contamination problem.

The interfering peak from Figure 3 was concentrated and analyzed by GC/MS to determine the nature of the contaminant. This interference was identified as an adipate, possibly the ethylhexyl.

Elimination of contamination was never completely accomplished and periodically caused serious problems with the phthalate determinations. Whether glassware was cleaned and heated to 275°C overnight before

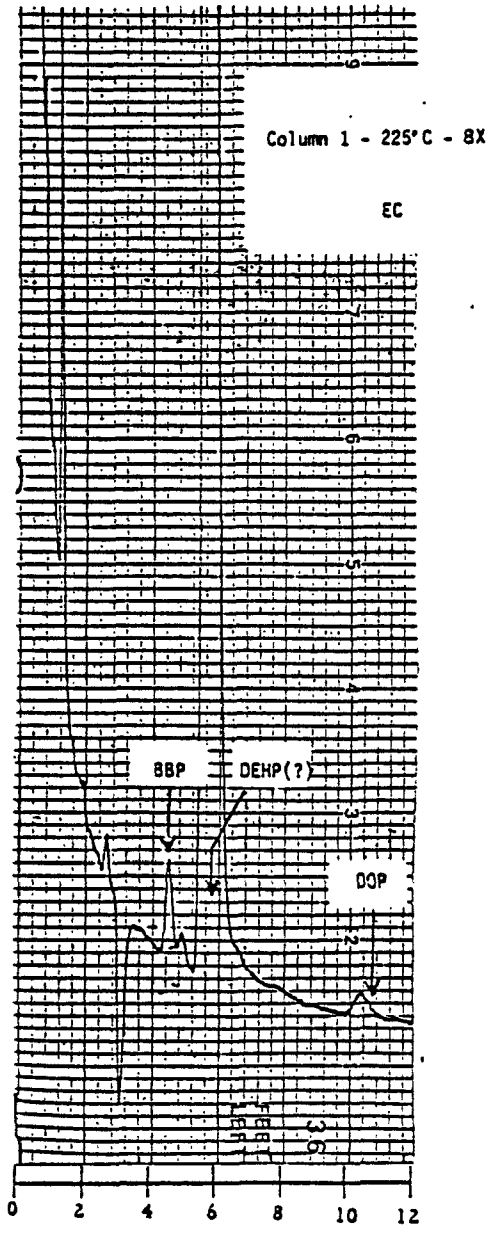

Figure 3. Unspiked water extract (100% DCM)

use or was heated for several hours at 450°C before use seemed to have little effect. Leaving the glassware in a chromic acid solution (40 mL saturated $K_2Cr_2O_7$ - H_2SO_4 solution in 1 liter of water) for 12 to 18 hours and rinsing several times with deionized water, acetone and petroleum ether prior to use seemed to give the best results. Early in the study, the sodium sulfate was heated overnight at 450-500°C before use. Some improvement resulted when this practice was supplanted by Soxhlet extraction of the sodium sulfate with DCM for two days.[1]

The sodium sulfate, the holding tube and the glass wool plug were all rinsed with DCM and hexane immediately before use.

After increasing the phthalate dosing levels, the problem with contamination became manageable, and the data acquired during the third extraction effort allowed reliable conclusions to be made with respect to the extraction efficiencies of the solvents tested.

Percent recovery data were obtained by both computer integrated peak areas and by peak height measurement. Only data based on peak height measurement are reported here.

The data acquired in the extraction study are presented in Tables 4 and 5. The data have not been corrected for blank extractions.

Data Analysis--

The results of the extraction study were submitted to statistical analysis to determine which of the factors affected the recovery efficiency of the method. The statistical model for these data was a two-way analysis of variance (ANOVA) defined by:

TABLE 4. EXTRACTION STUDY RESULTS - 100% DCM % RECOVERY

pH	Extract	DMP	DEP	DBP	BBP	DEHP	DOP
2	1	96	89	63	88	102	94
	2	125	98	81	88	92	90
	3	67	71	40	90	101	96
	Mean	96	86	61	89	98	93
7	1	135	103	97	118	104	99
	2	143	106	95	110	99	97
	3	144	103	95	109	98	96
	4	115	99	94	98	98	94
	Mean	134	103	95	109	100	97
10	1	108	100	96	91	100	90
	2	119	115	100	91	99	95
	3	113	111	88	90	112	94
	Mean	113	109	95	91	104	93

TABLE 5. EXTRACTION STUDY RESULTS - 15% DCM IN HEXANE % RECOVERY

pH	Extract	DMP	DEP	DBP	BBP	DEHP	DOP
2	1	107	104	100	93	99	95
	2	105	101	87	92	96	95
	3	100	99	87	84	127	91
	Mean	104	101	91	90	107	94
7	1	116	101	104	-	-	91
	2	111	101	-	90	101	92
	3	109	103	104	85	104	91
	4	104	101	97	94	98	93
	Mean	110	102	102	90	101	92
10	1	110	104	95	98	105	97
	2	110	103	93	94	102	96
	3	113	104	95	98	112	99
	Mean	111	104	94	97	110	97

- data not available - contamination (?)

$$Y_{ijk} = \mu + \tau_i + \rho_j + \tau\rho_{ij} + \varepsilon_{ijk}$$

where Y_{ijk} - observed recovery for solvent system i and pH j on replicate k, i=1,2, j=1,2,3, k=1,2,3 or 1,...,4

μ - overall mean

τ_i - effect due to solvent system i

ρ_j - effect due to pH j

$\tau\rho_{ij}$ - interactive effect between solvent i and pH j

and ε_{ijk} - random error associated with Y_{ijk}

Both solvent and pH are fixed effects, and all F-ratios were calculated using the mean square for error as the denominator. The 5-percent significance level was used on all tests.

The interaction between solvent and pH, if significant, implies that the combination of a particular pH and a particular solvent performed better (worse) than could be predicted by using the results from the two factors taken separately. For example, if a difference of 7 percent is observed between solvents and 6 percent between pH's, then using the best combination would result in a predicted difference of 13 percent, the sum of the two differences. If the observed difference were to be 20 percent, then the interaction term in the ANOVA would be significant. This merely implies that the best estimate of the expected recovery should be obtained from the results on that particular combination, not from the pH or solvent means themselves. Interaction is used in terms of a data analytical rather than physical approach.

A factor or interaction is considered to be significant if an F-ratio calculated as part of the ANOVA procedure exceeds the corresponding tabled value at the 5-percent significance level. This implies that

there was more variability among the levels of a factor, e.g., among the three pH's, than there was among replicate analyses under identical conditions. In some cases, however, the precision of the individual analyses allows differences to be termed significant that are too small to be of any practical importance. This should be considered in evaluating the results contained in this section.

Separate ANOVA's were conducted for each of the compounds in the group, and the results were then synthesized to give the recommended extraction conditions. There are several values in the data set which appear unusual, either high or low, but none of these were eliminated or replaced prior to conducting the ANOVA. The rationale behind this was that there was insufficient information concerning the method to warrant the use of an outlier test, which may eliminate values likely to occur in actual use of the procedure. It was noted where a particular significant result occurred due to one or more of these values and a qualifying statement was added, where appropriate.

For those factors determined to be significant, a means separation procedure was used, Fisher's LSD statistic, to determine where the differences lay. The LSD is calculated according to the formula:

$$LSD_{.05} = t_{.05}(\nu)\sqrt{\left(\frac{1}{n_1} + \frac{1}{n_2}\right) MS_E}$$

where $LSD_{.05}$ - test statistic at 5-percent significance level

$t_{.05}(\nu)$ - student's value with ν degrees of freedom at the 5-percent level

n_1, n_2 - number of observations used to obtain the mean values

and MS_E - mean square for error from the ANOVA table.

Factor means are presented in the statistical summary tables in Appendix B, along with the LSD values. The results obtained for each of the phthalate esters and the overall conclusions are summarized below.

DMP--The dimethyl phthalate results are summarized in Table B.1 of Appendix B. The only significant term in the ANOVA was pH, with pH 2 giving 100 percent recovery with statistically equivalent results at pH 10, 112.1 percent recovery. These results are considered somewhat spurious, however, in light of complicating factors. There was considerable variability in the data, with an overall standard deviation of 13.1 percent recovery, due largely to the 100 percent DCM data. The DCM-pH 2 results ranged from 67 to 125 percent recovery, and the average result of 96 percent recovery seems fortuitous. For the 15 percent DCM in hexane data, the replicate values and the pH-to-pH values were fairly consistent, with no real tendency observable. In view of the data, it is felt that little weight should be given to this result.

Neither the solvent system nor the interaction term was significant, and no preference for a solvent or solvent-pH combination was indicated for these data. The conclusion then for the DMP is that no clear tendency for better recovery was observed for any of the factors in the experiment.

DEP--The statistical analyses for diethyl phthalate are summarized in Table B.2 of Appendix B. For these data, both the pH and the pH-solvent interaction terms were significant. The pH means indicate that pH's 7 and 10 were equivalent with sample means of 102.1 and 106.2 percent recovery, respectively, and the pH 2 results were significantly

lower, with an average of 93.7 percent recovery. The pH 2 data were affected by one low value with 100 percent DCM but would have indicated that a different true mean could be expectd even if this value had been excluded. Similarly, the solvent-pH interaction term indicates that the low average value for 100 percent DCM-pH 2 was the principal difference among the means, but this would remain true if the average of two results, excluding the one low one, was used. The interaction term revealed that the low pH 2 results are a function of the low 100 percent DCM-pH 2 data and not a true pH effect. The indication is then that if 100 percent DCM is used as the extraction solvent, the wastewater should first be adjusted to a neutral-to-basic condition. If 15 percent DCM in hexane is used, no pH adjustment would be necessary.

DBP--The summary of the statistical analyses on the dibutyl phthalate data is given in Table B.3 of Appendix B. Both solvent and pH terms were significant, as well as the solvent-pH interaction. Higher recoveries were obtained using the 15 percent DCM-hexane system with 95.8 percent recovery compared to 84.9 percent for the 100 percent DCM. The mean recoveries for the varying pH's indicate equivalent recoveries at pH's 7 and 10, 98.0 and 94.5 percent, respectively, with lower overall recovery at pH 2 of 76.3 percent.

The interaction means indicate, however, that in both of these cases low recovery with 100 percent DCM-pH 2 was the reason for these differences. For the remaining solvent-pH combinations, the average recovery ranged from 91.3 to 101.7 percent, all acceptable results. As in the previous data, the conclusion is that if 100 percent DCM

is used, an adjustment to a neutral-to-basic condition would be required, but no adjustment of pH need be made if 15 percent DCM in hexane is used as the extraction solvent.

BBP--The summary of the ANOVA on the benzyl-butyl phthalate data is shown in Table B.4 of Appendix B. The significant terms were the solvent, the pH, and the solvent-pH interaction. The preferred solvent was 100 percent DCM with a recovery of 97.3 percent compared to 93.7 percent for 15 percent DCM in hexane. Better results were obtained at pH 7 than at pH 2 or 10, 100.6 percent recovery versus 89.2 and 93.7, respectively. The separation of the interaction means, however, revealed that these differences were influenced by a high average result for 100 percent DCM-pH 7 and are not truly pH and solvent effects. All of the solvent-pH combinations were equivalent with the exception of the 100 percent DCM-pH 7, which had an average recovery of 108.8 percent. The remaining combinations showed recoveries of 88.7 to 96.7 percent and were all acceptable recoveries. The conclusion for BBP then is that either solvent may be used, but if 100 percent DCM is used, the pH of the wastewater should be adjusted to a nonneutral condition.

DEHP--The summary of the statistical analyses on the diethylhexyl phthalate data is contained in Table B.5 of Appendix B. For these data there were no significant terms in the ANOVA, and the conclusion is that neither solvent nor pH affected the recovery of this compound. The overall mean recovery was 103.1 percent.

DOP--The statistical analyses on the dioctyl phthalate data are summarized in Table B.6 of Appendix B. These data gave one significant

term in the ANOVA, the solvent-pH interaction term. The best results were obtained with 100 percent DCM-pH 7 and 15 percent DCM-hexane-pH 10 with 96.5 and 97.3 percent recovery, respectively. The other four combinations were equivalent to each other ranging from 91.8 to 93.3 percent recovery so all combinations produced good recoveries. The preference for those two sets of conditions is slight and may not be of practical significance.

Summary--

The results of the analyses on the six compounds are summarized in Table 6. From this it can be seen that no clear tendency can be detected for one solvent system to produce superior recoveries or for the recoveries to be influenced by the pH of the water. The principal differences occurred when 100 percent DCM was used and a particular pH gave unacceptable results, especially in the cases of DEP and DBP. When 15 percent DCM in hexane was used as the extraction solvent, no differences were detected among the pH's. Another factor in the evaluation is that the 15 pecent DCM-hexane solvent system produced more consistent results with fewer of the apparent contaminations and none of the low recoveries. The conclusion then is that 15 percent DCM-hexane should be used for extracting the wastewater and no adjustment of the pH need be made. The mean recoveries obtained with 15 percent DCM-hexane at the three pH's are shown at the bottom of the summary table to indicate the recovery obtained.

Preservation Study

The preservation study was conducted to determine the effects of a 7-day storage period at various conditions on the recovery of

TABLE 6. SUMMARY OF EXTRACTION STUDY ANALYSES

Compound	FACTOR		
	Solvent	pH	Solvent x pH
DMP	--*	U†	---
DEP	--	7,10	not 100% DCM-pH 2
DBP	15% DCM-hexane	7,10	not 100% DCM-pH 2
BBP	100% DCM	7	not 100% DCM-pH 7
DEHP	--	--	---
DOP	--	--	100% DCM-pH 7 15% DCM-hexane-pH 10

Means (15% DCM in Hexane)

pH	COMPOUND					
	DMP	DEP	DBP	BBP	DEHP	DOP
2	104.0	101.3	91.3	89.7	107.3‡	93.7
7	110.0	101.5	101.7	89.7	101.3	91.8
10	111.0	103.7	94.3	96.7	109.7‡	97.3

* Line indicates no significant difference.

† U indicates data do not lend themselves to ready interpretation.

‡ Influenced by single high value.

the six phthalate esters of interest from dosed water samples. Each sample consisted of one quart of water dosed with six phthalates, as in the extraction study (see Table 3). Two replicates for each of twelve conditions of pH, temperature, and residual chlorine were prepared for each group as shown in the following model:

	4°C		24°C	
	0 ppm Cl	2 ppm Cl	0 ppm Cl	2 ppm Cl
pH 2	2	2	2	2
pH 7	2	2	2	2
pH 10	2	2	2	2

The 2 ppm residual chlorine level was obtained, where required, by adding 160 microliters of Mallinckrodt sodium hydrochlorite analytical reagent (5 percent minimum available Cl). This quantity of hypochlorite solution was determined by preparing a standard chlorine solution which was diluted to five different concentrations that were used to prepare an absorbance versus concentration curve.[2] Storage containers were one-quart, flint-glass, round, narrow-mouth bottles with aluminum foil-lined caps. A sample was prepared by filling the bottle about two-thirds full with pH-adjusted water, adding 160 microliters of sodium hypochlorite (when required), swirling vigorously, adding the 100 microliters of dosing solution into the vortex, and adding the remainder of the pH-adjusted water while that already in the bottle was still swirling. The bottle was capped immediately and stored in a closed cardboard box at 4°C or 24°C. Care was taken not to slosh the bottle contents onto the aluminum lining of the cap after closure.

After storage, samples were extracted and analyzed as in the extraction study.

The third extraction study had not been completed when the preservation study was started. This made it necessary to select an extraction solvent and extraction pH without the benefit of prior data. The solvent selected was 15 percent DCM-hexane. Considerations in selecting this solvent were its proven extraction efficiency with other priority pollutants (pesticides and PCB's), its relative general use, and the ideal situation of conforming as many priority pollutant methodologies to a standard procedure as possible. The preservation study samples were extracted at their given pH without any prior adjustment.

Data obtained in the preservation study was given in Table 7. The data presented are uncorrected for values obtained for blanks at each of the 12 test conditions because of the variation of these values.

Data Analysis--

The results of the preservation study were examined by submitting the percent recoveries to an analysis of variance (ANOVA) procedure to determine which of the factors affected the recovery upon storage.

The ANOVA for these data is based on a 3x2x2 factorial experiment with two replicates of each combination. The model is presented by:

$$Y_{ijkl} = \mu + \rho_i + \tau_j + \gamma_k + \rho\tau_{ij} + \rho\gamma_{ik} + \tau\gamma_{jk} + \rho\tau\gamma_{ijk} + \varepsilon_{ijkl}$$

where

Y_{ijkl} - observed recovery for pH i, temperature j, chlorine level k and repetition l

μ - overall mean

ρ_i - effect of pH i, i=1,2,3

TABLE 7. RESULTS OF PRESERVATION STUDY - DMP, DEP, DBP, BBP, DEHP, DOP
(PERCENT RECOVERY - 7 - DAY STORAGE PERIOD)

pH	Temperature °C	Chlorine ppm	Replicate	DMP	DEP	DBP	BBP	DEHP	DOP
2	4	0	1	*	*	*	105	108	107
			2	103	97	79	88	91	90
		2	1	104	95	89	98	96	99
			2	104	94	89	93	96	95
	24	0	1	110	103	86	88	101	93
			2	109	99	90	91	118	91
		2	1	116	100	91	85	88	92
			2	115	103	89	103	104	95
7	4	0	1	93	98	85	78	89	94
			2	92	97	84	67	86	90
		2	1	97	100	91	77	88	92
			2	94	100	88	77	84	90
	24	0	1	112	100	92	73	99	69
			2	*	*	*	*	*	*
		2	1	100	98	89	70	85	95
			2	100	100	105	68	104	91
10	4	0	1	94	100	90	79	86	83
			2	90	97	94	87	92	88
		2	1	89	97	95	84	84	83
			2	87	103	94	79	75	75
	24	0	1	30	85	74	40	89	81
			2	32	85	81	41	94	89
		2	1	26	80	68	37	93	88
			2	25	78	71	35	94	87

* data not available - contamination (?)

τ_j - effect of temperature j, j=1,2

γ_k - effect of chlorine level k, k=1,2

$\rho\tau_{ij}$, $\rho\gamma_{ik}$, $\tau\gamma_{jk}$ - two-factor interactions

$\rho\tau\gamma_{ijk}$ - three-factor interaction

and ε_{ijkl} - random error associated with Y_{ijkl}

All terms (pH, temperature, chlorine) are considered fixed effects, and all F-ratios calculated against the error mean square. Each compound is investigated separately and the results synthesized to obtain a recommended set of conditions for storage. A separate ANOVA table is presented for each phthalate ester in Appendix C to this report. For those factors which were significant, the means for each level or each combination are broken out and a least significant difference (LSD) statistic used to determine where the differences lie. The LSD is calculated according to the formula:

$$LSD_{.05} = \sqrt{2}\ t_{.05}(\nu)\sqrt{\hat{\sigma}^2/n}$$

where

$t_{.05}(\nu)$ - Student's t value with ν degrees of freedom at the 5-percent significance level

$\hat{\sigma}^2$ - mean square for replicates from the ANOVA

and n - number of values used to obtain each sample mean.

The means and LSD's are also shown in the statistical summary table for each compound in Appendix C.

As in the extraction study, the interaction factors are to be interpreted as an instance where the recovery obtained was better (worse) than could be expected by observation of the average results

for pH, temperature, and/or chlorine taken individually. The conclusion is that the means of the combinations should be used to provide the estimates of percent recovery, and no physical interpretation is implied. The precision of the analyses allowed, in some cases, relatively small differences to be termed significant, while there may be no practical value to the difference between average recoveries in these cases.

DMP--The results for dimethyl phthalate showed that all main effects and interactions had a significant effect on the recoveries obtained. The most prominent effects, as determined by the magnitude of the mean square for each factor, were pH, temperature, and the pH-temperature interaction. The LSD for the pH factor showed that all three sample means could be considered to be estimating different true (population) means. At pH 7, the average recovery was 100.0 percent, while 108.0 percent was obtained for pH 2 and 59.1 percent for pH 10. The best conditions for this phthalate then was storage at neutral conditions with basic conditions clearly unacceptable. The better temperature for storage was 4°C with an average recovery of 95.8 percent versus 82.2 percent at room temperature.

The pH-temperature combination means gave more information. At pH's 2 and 7, averages above 100 percent were obtained at room temperature storage (112.5, 106), while the pH 2-4°C mean was 103.5 and the pH 7-4°C mean was 94.0. The mean recovery for pH 10-4°C was 90.0 percent, an acceptable result, but the recovery for pH 10-24°C was only 28.2 percent. The average recovery shown above for pH 10 thus was greatly affected by the results from room temperature storage.

Clearly then the data indicate that cold storage is recommended for preserving samples for DMP, and some preference is noted for neutral conditions. None of the pH's is unacceptable under storage at 4°C, but the results at pH 2 were closest to 100 percent, although slightly above. There was no effect on the results at pH 2 or 7 resulting from the presence or absence of residual chlorine in the water.

DEP--There were three significant terms in the ANOVA of the diethyl phthalate data, pH, temperature, and the pH-temperature interaction. The pH results indicated that better recovery was obtained at pH's of 2 and 7 (98.5 and 99.1 percent recovery, respectively) than at pH 10 (90.6 percent recovery). Storage at 4°C produced a significantly higher average result, but the difference was slight, with 97.9 percent observed for 4°C and 94.2 percent recovery for 24°C.

The pH-temperature interaction means revealed a similar pattern to the DMP results. The principal difference among the means was the low average recovery (82.0 percent) for the samples stored at pH 10 and at room temperature. Neither the chlorine term nor any of the interactions involving chlorine were significant, and the conclusion is that residual chlorine in the water will not affect the results obtained for DEP upon storage of the samples.

DBP--The dibutyl phthalate results were analyzed according to the ANOVA model above and gave two significant terms, pH and the pH-temperature interaction. The best results were obtained under storage at pH 7 with 90.8 percent recovery on average. The mean for pH 2 (86.5 percent recovery) was indistinguishable from the pH 7 mean using

the LSD statistic, but storage at pH 10 produced a significantly lower result (83.4 percent recovery). As in the previous data, however, the interaction term revealed that the principal reason for this difference was the low result for pH 10-24°C. No effect was noted due to the presence or absence of residual chlorine in the water, and this does not appear to be a concern in analyzing for this compound.

BBP--The recoveries for benzyl-butyl phthalate gave three significant terms in the ANOVA, pH, temperature, and the pH-temperature interaction. The three pH's produced distinct means for this compound, with storage at pH 2 giving the best results, 93.8 percent recovery, followed by pH 7 and pH 10 (72.8 and 60.3 percent recovery, respectively). Higher results were obtained on average when storage was at 4°C as opposed to room temperature, with mean recoveries of 84.3 percent and 67.0 percent, respectively. From the pH-temperature interaction, however, no significant difference was observed between the 4°C and 24°C results at pH 2 (96.0 and 91.8 percent recovery, respectively). As a result, for this compound an adjustment to pH 2 prior to storage would remove the necessity for cold storage. As in the previous analyses, the significance of the pH-temperature interaction is largely due to the loss of BBP at pH 10-24°C, and no chlorine effect, either alone or in combination, was observed in the results.

DEHP--The diethylhexyl phthalate data gave one significant factor in the ANOVA, the pH of the water. The best results were obtained when the samples were stored at pH 2, with an average of 100.8 percent recovery. The average for pH 7, 91.8 percent recovery, was indistinguishable

from the pH 2 mean using the LSD statistic, but only marginally. No other factors or interactions were significant, and the results were not affected by either the presence or absence of chlorine.

DOP--The dioctyl phthalate results gave one significant main effect, pH, and three significant interaction terms, pH-temperature, pH-chlorine, and temperature-chlorine. The pH results were consistent with the other compounds, showing best recovery at pH 2 (95.2 percent) with pH's 7 and 10 lower and equivalent to each other (86.2 and 84.2 percent, respectively). The pH-temperature interaction term revealed that equivalent results were obtained at pH 2 for either 4° or 24°C, and at pH 7-4°C. The other combinations were equivalent to each other but lower.

The two interaction terms involving chlorine provided no information that would indicate a problem for these analyses. For the pH 2 data, the same average result (95.2 percent recovery) was obtained for both 0 and 2 ppm residual chlorine in the water, which was the highest average obtained. For the temperature-chlorine interaction term, the data indicated that storage at 24°C-0 ppm chlorine gave a lower average result than the other combinations. However, previous results indicate a preference for cold storage so the presence or absence of chlorine, assuming storage at 4°C, is not a problem for this compound.

Summary--

The results of the analyses on the six compounds in this category are summarized in Table 8. On the basis of the trends shown, the recommended conditions for storage would specify an adjustment of

TABLE 8. SUMMARY OF RESULTS OF PRESERVATION STUDY

Compound	FACTOR						
	pH	Temp	Cl	pH x Temp	pH x Cl	Temp x Cl	pH x Temp x Cl
DMP	7	4	0	not (10,24)	U*	eq. at 4°	U
DEP	2,7	4	-†	not (10,24)	-	-	-
DBP	2,7	-	-	(2,24), (7,24) (10,4)	-	-	-
BBP	2	4	-	(2,4), (2,24)	-	-	-
DEHP	2,7	-	-	--	-	-	-
DOP	2	-	-	(2,4), (2,24) (7,4)	eq. at pH 2	not (24,0)	-

Mean Recovery (pH 2-4°C)

DMP	DEP	DBP	BBP	DEHP	DOP
103-112‡	95.8	84.0	96.0	100.2	97.8

*U - results do not lend themselves to ready interpretation.
† - line indicates no significant difference.
‡ - depending on presence/absence of residual chlorine.

the water to acidic conditions, and storage at a temperature of 4°C. Under these conditions, no interference can be expected from residual chlorine up to the 2 ppm level.

Adjustment of wastewater samples to pH 2 may not be practical under field conditions and may be avoided with minimal effect on the determination of phthalate esters, provided the samples are stored at 4°C.

SECTION 5
PHASE II

OBJECTIVES

The objectives of Phase II were as follows:

1. Investigation of two liquid-solid chromatographic cleanup procedures, including the determination of the separation patterns and recoveries for all of the substances of interest.
2. Application of the method to five wastewater samples from industrial discharges. This aspect of the work included modifications of the method as required to analyze the wastewaters. These modifications would be based on experience gained earlier in the program and on new developments reported in scientific literature.
3. Generation of accuracy and precision data for all substances of interest by dosing them into the five wastewaters. Determinations would be made immediately after dosing and after one week of storage at the best conditions observed in Phase I.

PROGRAM DIRECTION

Representatives of SwRI met with the project officer in Cincinnati before Phase II work commenced. Discussion with respect to Phase II was primarily on the cleanup columns and procedures to be used. Florisil and alumina were selected as the adsorptive solids. Fractionation of compounds was limited to that which can be obtained on the above-

named solids. Fractionation on secondary columns such as silica gel or charcoal was considered to be beyond the scope of this program.

DCM was chosen as the extracting solvent to be used with the wastewater samples.

LIQUID-SOLID COLUMN CHROMATOGRAPHY

The first column chromatography method to be investigated for cleanup of phthalate esters was based on the work reported by C. S. Giam et al.[3] It was found by Giam et al. that incomplete recoveries of phthalate esters were obtained from Florisil unless the Florisil was deactivated with 3 percent water. Two procedures were developed: one employing 35 grams of Florisil for samples with a high lipid content and the second procedure using only 10 grams of Florisil for samples with a low lipid content.

Initial studies using the 10 grams of deactivated Florisil (3 percent H_2O) and the reported solvent system indicated that modification of the procedure was required. The dimethyl and diethyl phthalates are more polar than the other phthalates used by Giam and were not eluted from the column when following the reported procedure.

The modified procedure, used in this study, is as follows:

Prepare Florisil (PR) by heating to 400°C overnight. Then add 3 percent H_2O (w/w) and mix thoroughly. Let stand for at least 2 hours. Use 10-mm ID column chromatography tube with glass frit and Teflon stopcock (Kontes Catalog K420540-0213).

1. Add 10 g of Florisil (3 percent H_2O) to the 1-cm ID chromatography tube and tap to settle.

2. Add ≈1 cm Na_2SO_4 to top of column.

3. Add hexane to tube and pre-elute 40 mL. Discard.

4. Drain until solvent level is even with top of Na_2SO_4.

5. Add sample in ≈2 mL hexane to top of column.

6. Drain sample onto column (to top of Na_2SO_4).

7. Wash sides of tube with ≈2-mL hexane and drain into top of Na_2SO_4.

8. Add hexane to top of column and elute 40 mL of hexane. Discard this fraction.

9. Drain hexane to top of Na_2SO_4 and add ≈2 mL 20 percent ether/hexane.

10. Drain new solvent to top of Na_2SO_4. Add more 20 percent ether/hexane.

11. Place a 500-mL Kuderna-Danish evaporator below the column.

12. Collect 100 mL of the 20 percent ether/hexane eluate in the K-D evaporator.

13. Evaporate the above eluate to <10 mL on a steam bath.

14. Transfer to a solvent-rinsed 4-dram vial with Teflon cap liner.

15. Adjust final volume of concentrate to 10 mL. Evaporate under nitrogen if necessary.

The second column procedure developed for this program, using alumina as the adsorbent, is as follows:

Prepare Alumina (Woelm N) by heating to 400°C overnight. Then add 3 percent H_2O (w/w) and mix thoroughly. Let stand overnight in sealed jar. Use 10-mm ID column chromatography tube with glass frit and Teflon stopcock (Kontes Catalog K420540-0213).

1. Add 10 g of Alumina (3 percent H_2O) to the 1-cm ID chromatography tube and tap lightly to settle.

2. Add ≈1 cm Na_2SO_4 to top of column.

3. Add hexane to tube and pre-elute 40 mL. Discard.

4. Drain until solvent level is even with top of Na_2SO_4.

5. Add sample in ≈2 mL hexane to top of column.

6. Drain sample onto column (to top of Na_2SO_4).

7. Wash sides of tube with ≈2 mL hexane and drain into top of Na_2SO_4.

8. Add hexane to top of column and elute 35 mL of hexane. Discard this fraction.

9. Place a 500 mL Kuderna-Danish evaporator below the column.

10. Drain hexane to top of Na_2SO_4 and add ≈2-mL 20 percent ether/hexane.

11. Drain new solvent to top of Na_2SO_4. Add more 20 percent ether/hexane.

12. Collect 140 mL of the 20 percent ether/hexane eluate in the K-D evaporator.

13. Evaporate the above eluate to ≈10 mL on a steam bath.

14. Transfer to a solvent-rinsed 4-dram vial with Teflon cap liner. Rinse twice with hexane and add rinses to vial.

15. Adjust final volume of concentrate to 10 mL. Evaporate under nitrogen if necessary.

A gel permeation column using Sephadex was considered for use in this work, but when a computer search of the literature failed to give any references relative to the use of this substance other than for removal of lipids, no further use was contemplated. Lipids are not a problem contaminant with most nondomestic wastewaters.

The cleanup procedures using Florisil and alumina were evaluated as to the recoveries that could be obtained when doses of the six phthalates were applied to columns of these adsorbents. Table 9 shows the recoveries obtained from five replicate samples when using the Florisil cleanup column. Table 10 shows the recoveries obtained from

TABLE 9. RECOVERY* (%) FROM FLORISIL PR COLUMN

Sample	DMP	DEP	DBP	BBP	DEHP	DOP
1	114	114	99	93	98	97
2	115	115	97	92	96	94
3	114	114	97	92	97	96
4	115	117	98	93	96	94
5	117	118	99	94	97	95
Avg	115	116	98	93	97	95

TABLE 10. RECOVERY* (%) FROM ALUMINA(N) COLUMN

Sample	DMP	DFP	DBP	BBP	DEHP	DOP
1	114	108	97	100	87	95
2	112	107	97	102	85	95
3	114	109	98	99	97	98
4	115	111	99	99	99	99
5	115	111	97	98	84	93
Avg	114	109	98	100	91	96

* Amount added to column in 2 mL hexane.

DMP-15μg, DEP-15μg, DBP-8μg, BBP-1μg, DEHP-8μg, DOP-20μg

five replicate s mples using the Alumina N cleanup column. The recoveries for all six test phthalates from both materials appear to be very good, averaging 90 percent or better. The high recoveries for DMP and DEP were unexplained since blank tests showed only small (2-3 percent) interferences or contamination for these two compounds. A lack of a linear detector response could have been responsible.

WASTEWATER APPLICATION

With the assistance and approval of the project officer, five wastewaters were procured and analyzed. All samples were put in clean, one-gallon bottles and shipped unrefrigerated via air to Southwest Research Institute laboratories. Upon receipt at SwRI, all the wastewater from a particular source (except Wastewater 2) were pooled, adjusted to pH 5-7 if necessary, returned to the bottles in which they were shipped, and stored at 4°C (in the dark) until used. Due to the high acid content of Wastewater 2 (approximately 48 g/L NaOH required to neutralize), it was stored at 4°C as received.

In order to develop method improvements and to provide base data for dosing and recovery experiments and for the accuracy and precision evaluations to follow, each wastewater was analyzed in triplicate for each substance of interest in this program.

One liter of wastewaters was extracted three times using 60-mL DCM for each extraction. The combined extract was dried with Na_2SO_4 and placed in a Kuderna-Danish evaporator. The DCM extract was concentrated to 5-10 mL, 90 mL of hexane was added, and the extract was concentrated to slightly less than 10 mL in the K-D. The sample was then transferred

to small vial and concentrated to 2 mL. This 2-mL concentrated extract was then subjected to the Florisil cleanup procedure as previously described. One procedural change, used only with Wastewaters 3, 4, and 5, was attempted and with some success. In the Florisil cleanup procedure, instead of collecting a single fraction of 100 mL of eluting solvent containing all the phthalate esters of interest, a two-fraction collection was made. Fraction 1 (first 60 mL) contained nearly all of the DOP and DEHP, most of the DBP and BBP and, in some instances, much of the early GC-eluting material. Fraction 2 (next 40 mL) contained all of the DMP, most of the DEP, some DEP and BBP and, in some instances, reduced amounts of early GC-eluting material(s) which otherwise interfere with detection of the DMP and DEP. This method of collecting two fractions instead of one may be of some help in the analysis for DMP and DEP. For the analysis of the other phthalate esters, it is best to combine fractions (or collect only the single fraction) before GC analysis and thus avoid the complications of partial separation of some of the phthalate esters.

Wastewaters 1 and 2 were analyzed by collection and analysis of only one fraction. Wastewaters 3, 4, and 5 were examined by collection of the two fractions. Chromatograms of Fraction 2, analyzed for DMP and DEP only and of combined Fractions 1 and 2, analyzed for the remaining phthalate esters, are shown in Figures D.1 and D.2 in Appendix D for dosed Wastewater 3.

Chromatograms of a standard mixture and Wastewater 1 through 5 (single fraction collection) are shown in Appendix E.

The gas chromatography for the wastewater analysis was performed using Column 1. Samples were analyzed by holding the column at 180°C for 16 minutes for determination of DMP, DEP, and DBP. The temperature was then raised rapidly to 220°C to decrease elution time of the BBP, DEHP, and DOP. All analytical data were obtained using peak height measurements. All extracts were diluted so compounds of interest would not go off scale at 8X, thus assuring linearity of the EC detector for DBP, BBP, DEHP, and DOP. No linear range for DMP and DEP was found with the EC detector, and analytical data for these two compounds were obtained from calibration curves.

Tables 11 through 15 show the results of the analysis of raw Wastewaters 1 through 5.

Tables 16 through 19 show the results of the analysis of Wastewaters 1, 3, 4, and 5 dosed with the six phthalate esters under study.

The data presented in the tables referred to below were used to calculate a "Method Recovery", i.e., the percent recovered of the amount added at zero time, and a "Preservation Recovery", i.e., the percent remaining after seven days of the total amount in the dosed wastewater at zero time. This was performed as follows:

let k = background (amount found in the undosed wastewater)

let y = quantity added to wastewater

let x_0 = quantity found in dosed wastewater at zero time for replicates 1, 2, or 3.

let $x_{0\ avg}$ = average quantity found in dosed wastewater at zero time

TABLE 11. ANALYSIS OF WASTEWATER 1 FOR PHTHALATES, µg/L

Replicate	DMP	DEP	DBP	BBP	DEHP	DOP
1	I*	I	157	8.0	11.6	0
2	I	I	151	12.8	13.9	0
3	I	I	157	6.2	13.9	0
"k" µg/L Average	I	I	155	9.0	13.1	0

*I - Interfering Peak(s)

TABLE 12. ANALYSIS OF WASTEWATER 2 FOR PHTHALATES, µg/L

Replicate	DMP	DEP	DBP	BBP	DEHP	DOP
1	I*	1430	I	232	I	3260
2	I	1500	I	304	I	3820
3	I	1580	I	304	I	4020
"k" µg/L Average	I	1500	I	280	I	3700

*I - Interfering Peak(s)

TABLE 13. ANALYSIS OF WASTEWATER 3 FOR PHTHALATES, µg/L

Replicate	DMP	DEP	DBP	BBP	DEHP	DOP
1	0	0	2.4	0.8	6.6	0
2	0	0	1.8	0.6	6.5	0
3	0	0	2.9	1.0	6.6	0
"k" µg/L Average	0	0	2.3	0.8	6.6	0

TABLE 14. ANALYSIS OF WASTEWATER 4 FOR PHTHALATES, µg/L

Replicate	DMP	DEP	DBP	BBP	DEHP	DOP
1	0	0	0	0.7	1.0	0
2	0	0	0	0.4	1.0	0
3	0	0	0	0.9	1.0	0
"k" µg/L Average	0	0	0	0.7	1.0	0

TABLE 15. ANALYSIS OF WASTEWATER 5 FOR PHTHALATES, μg/L

Replicate	DMP	DEP	DBP	BBP	DEHP	DOP
1	0	5.8	1.9	3.2	255	0
2	0	5.8	3.7	2.5	269	0
3	0	3.8	3.7	2.1	285	0
"k" μg/L Average	0	5.1	3.1	2.6	270	0

TABLE 16. ANALYSIS OF WASTEWATER 1 DOSED WITH PHTHALATES

Replicate	Dose, µg/L					
	0 DMP	0 DEP	1500 DBP‡	100 BBP‡	150 DEHP‡	150 DOP‡
Zero time						
1			1380	86	122	119
Method recovery,%			82	77	69	79
2			1360	86	116	116
Method recovery,%			80	77	69	77
3			1360	86	117	116
Method recovery,%			80	77	73	77
X_0 Avg.			1370	86	118	117
k	I*	I*	155	9.0	13.1	0
Method recovery,% Avg.			81	77	70	78
7 days at 4°C						
1			1390	85	111	112
Preservation recovery,%			101	99	94	96
2			1370	80	109	121
Preservation recovery,%			100	93	92	103
3			1610	77	116	116
Preservation recovery,%			118	90	98	99
Preservation Recovery, % Average	I*	I*	106	94	95	99

* Huge interferences made spiking unfeasible at µg/L levels.

† Extract diluted 500x to see DBP in linear range of EC detector.

‡ Extract diluted 50x to see BBP, DEHP, & DOP in linear range of EC detector.

TABLE 17. ANALYSIS OF WASTEWATER 3 DOSED WITH PHTHALATES

Replicate	Dose, µg/L					
	45 DMP*	45 DEP*	24 DBP†	3 BBP†	24 DEHP†	60 DOP†
Zero Time						
1	45	45	25.6	3.8	24.1	46.4
Method Recovery,%	100	100	97	100	73	77
2	45	45	23.2	3.6	20.2	34.4
Method Recovery,%	100	100	87	93	57	57
3	45	45	23.8	4.4	22.2	37.6
Method Recovery,%	100	100	90	120	65	63
X_0 Avg.	45	45	24.2	4.0	22.2	39.5
k	0	0	2.3	0.8	6.6	0
Method Recovery,%Avg.	100	100	91	124	65	66
7 Days at 4°C						
1	36.0	39.3	18.7	3.1	23.4	36.5
Preservation Recovery,%	80	87	77	79	105	92
2	29.7	38.1	16.4	3.4	24.5	34.4
Preservation Recovery,%	66	85	68	85	110	87
3	32.1	39.3	14.5	3.0	24.6	32.2
Preservation Recovery,%	71	87	60	76	111	82
Preservation Recovery % Avg.	72	86	68	80	109	87

*DMP & DEP determined from Fraction 2 of Florisil eluate diluted 15x to come within EC detector calibration range.

†DBP, BBP, DEHP, & DOP determined from total Florisil eluate (Fracs. 1&2 recombined) diluted 3x to come within linear range of EC detector.

TABLE 18. ANALYSIS OF WASTEWATER 4 DOSED WITH PHTHALATES

Replicate	Dose, µg/L					
	15 DMP*	15 DEP	20 DBP	5 BBP	40 DEHP	40 DOP
Zero Time						
1	12.9	12.2	17.6	4.2	19.4	18.3
Method Recovery,%	86	81	88	70	47	46
2	13.2	12.3	18.2	4.6	19.4	19.4
Method Recovery,%	88	82	91	78	47	49
3	13.3	12.6	18.9	4.7	23.0	21.7
Method Recovery,%	89	84	95	80	56	54
X_0 Avg.	13.1	12.4	18.2	4.5	20.6	19.8
k	0	0	0	0.7	1.0	0
Method Recovery,% Avg.	88	82	91	76	50	50
7 Days at 4°C						
1	13.7	12.9	18.2	4.3	27.9	26.5
Preservation Recovery,%	105	104	100	96	134	134
2	14.3	13.2	18.6	4.3	29.5	28.7
Preservation Recovery,%	109	106	102	96	143	145
3	14.1	13.1	18.1	4.3	29.3	27.6
Preservation Recovery,%	108	106	99	96	142	139
Preservation Recovery % Avg.	107	106	100	96	140	139

* All components determined from total extract diluted 1:5 to come in linear or calibration range of EC detector.

TABLE 19. ANALYSIS OF WASTEWATER 5 DOSED WITH PHTHALATES

Replicate	Dose, µg/L					
	50 DMP*	50 DEP*	30 DBP‡	30 BBP‡	1000 DEHP‡	150 DOP‡
Zero Time						
1	50	52	18	23	980	140
Method Recovery,%	100	94	49	68	71	93
2	49	51	15	22	980	140
Method Recovery,%	98	92	40	65	71	93
3	47	50	13	19	980	140
Method Recovery,%	94	90	33	55	71	93
X_0 Avg.	49	51	15.3	21.3	980	140
k	0	5.1	3.1	2.6	270	0
Method Recovery,% Avg.	97	92	41	63	71	93
7 Days at 4°C						
1	50	49	9	13	740	110
Preservation Recovery,%	102	96	59	61	76	79
2	49	49	9	15	770	110
Preservation Recovery,%	100	96	59	70	79	79
3	48	48	10	14	780	110
Preservation Recovery,%	98	94	65	65	80	79
Preservation Recovery % Avg.	100	95	61	65	78	79

* DMP & DEP determined from Fraction 2 of Florisil eluate diluted 50x to come within EC detector calibration range.

‡ DBP, BBP, DEHP, & DOP determined from total Florisil eluate (Fracs. 1&2 recombined) diluted 25x to come within linear range of EC detector.

let $x_{7\ avg}$ = average quantity found in dosed wastewater after seven days for replicates 1, 2, and 3.

Then

"Method Recovery" = $(x_0-k)100/y$ % (calculated for each replicate)

and

"Preservation Recovery" = $(x_7)(100)/x_{0\ avg}$ % (calculated for each replicate)

No dosed recovery or preservation data were obtained on Wastewater 2 due to obvious deterioration of the sample.

ACCURACY AND PRECISION

Approach

The accuracy and precision assessment for the method was of a limited nature due to the number and types of analytical results obtained. According to the design of the program three replicates of each of five wastewaters were to be dosed for the compounds of interest and analyzed, both at a zero time and after seven days storage under the appropriate conditions. This provided 15 determinations of recovery of the dose for statistical analyses. However, the five wastewaters could not be considered equivalent, either in their nature or in the behavior of the method when applied, so combining the results to form an overall evaluation of the method was inappropriate. Instead, the results of each wastewater were treated individually, and no general statement about the method was attempted.

The accuracy of a method refers to its ability to obtain on average the true value, within the limits of the variability inherent in the

method. For this study, the accuracy standard used was the percent recovery of the amount of the particular phthalate ester dosed into the wastewater, with 100 percent representing an accurate method. Precision relates to the variability among measurements arising from various sources and is considered separately from the accuracy evaluation. It is possible for a method to be precise, meaning having little variability among measurements, without being considered accurate. For this study, the precision measurement used was the range or the difference between the highest and lowest recoveries. While the range is sensitive to single extreme values, it was deemed more appropriate for these data than other measures, given the number of observations available.

The recoveries obtained after seven days storage were examined separately to evaluate the effects of storage on the observed levels of these compounds. These recoveries are based on the total amount present at time zero rather than the dosing level, since it can be assumed that any degradation would affect both the initial amount and the dosed amount in equal proportions.

Results

Wastewater 1--

Neither the DMP nor the DEP was dosed into Wastewater 1, either at time zero or after storage due to the interferences. Consistent results were obtained for the other four compounds studied, however, with recoveries from 70 percent for DEHP to 81 percent for DBP. In each of these cases, the precision of the analyses was good, with ranges of 2, 0, 4, and 2 percent recovery for DBP, BBP, DEHP, and DOP, respectively.

The preservation recoveries were generally good for the four higher-boiling phthalates, but more variable than the zero-day analyses. The recoveries were from a low of 94 percent to a high of 106, with ranges of 6, 7, 9, and 18. The 106 average for DBP was influenced by a single value of 118, and the indication is that recovery for these compounds was not affected by the storage conditions in this wastewater.

Wastewater 3--

The analyses on this wastewater produced results which ranged from low to very good, depending upon the particular compound. Results for both DMP and DEP were very good, with triplicate analyses indicating 100 percent recovery of the spike. The recovery of DBP was 91 percent, on average, but with a range of 10 percent recovery. The results for the remaining phthalates were not good in this wastewater, however. The average recovery of BBP was 104 percent, but the individual recoveries had a range of 27 percent (93-120). DEHP and DOP were recovered at 65 and 66 percent, respectively, of the dose level with ranges of 16 and 20 percent recovery.

The preservation data were also inconsistent from one compound to the next. For the six compounds studied, the average recovery relative to the zero-day analyses went from 68 percent for DBP to 109 percent for DEHP. These analyses were more variable than the zero-day analyses in the cases of DMP and DBP, equivalent for DEP, and considerably less variable for the remainder. The ranges of the triplicate analyses at zero-time were 27, 16, and 20 for DBP, DEHP,

and DOP, respectively, at the initial analyses compared to 9, 6, and 10 for these compounds after seven-days storage.

Wastewater 4--

The analyses on Wastewater 4 for DMP and DEP produced good recovery, 88 and 82 percent, respectively, with ranges of 3 and 2 percent. Recovery of 91 percent on average was noted for DBP but more variability with a range of 10 percent recovery. Lower recoveries were obtained for the remaining three phthalates, with average percent recoveries of 76, 50, and 50 for BBP, DEHP, and DOP, respectively, with ranges of 10, 9, and 8.

The preservation data were good for the first four compounds, going from 96 to 107 percent with ranges from 0 to 4 percent recovery. For the DEHP and DOP analyses, however, the average recoveries were 140 and 139 percent with ranges of 9 and 11, respectively. These recoveries are comparable to 70 and 69 percent, respectively, of the original dosed amount and likely indicate a problem with the initial analyses.

Wastewater 5--

The results for both DMP and DEP were good in this wastewater, with average recoveries of 97 and 92 percent, respectively, and ranges of 6 and 4 percent recovery. The DBP and BBP results were low and variable, with average recoveries of 41 and 63 and ranges of 16 and 13 percent recovery, respectively. The DEHP and DOP values were consistent but only 71 percent of the dose was recovered on the DEHP, while 93 percent was recovered on the DOP. For both of these compounds there was a zero range, with all three analyses showing the same recovery.

The preservation recoveries were fairly consistent for all of the compounds, with ranges of recovery of 0 to 9 percent. However, the level of recovery could be broken down into three groups: DMP-DEP, DBP-BBP, DEHP-DOP. The recovery after storage was 95 to 100 for the first pair, 61 to 65 for the second and 78 to 79 for the third. The 61 percent recovery represents only 25 percent of the initial dosed amount remaining after seven days and indicates that storage in this wastewater would not be recommended for these analyses.

Summary

The accuracy and precision evaluations on phthalate esters in wastewater lead to the following conclusions. There are no assurances that acceptable data will be obtained when analyzing for phthalate esters using this method, as in the case of Wastewater 2 where no usable results were obtainable, and in Wastewater 1, where none were obtainable for DMP or DEP. The recoveries that can be expected for the compounds studied ranged from 40 to 100 percent, depending upon which compound and which wastewater. In general, the precision of the analyses was acceptable to good, with ranges of less than 10 percent recovery common.

The overriding conclusion is that the recovery and the ability to store the water for later analysis are a function of the wastewater. Storage frequently resulted in significant losses of the study materials and in less precise determinations and cannot be recommended as a general rule.

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3. Giam, C. S., Chan, H. S., and Neff, G. S., "Sensitive Method for Determination of Phthalate Ester Plasticizers in Open-Ocean Biota Samples," Analytical Chemistry, 47:2225, November 1975.

APPENDIX A

KEY REFERENCES FROM LITERATURE SEARCH

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APPENDIX B

STATISTICAL ANALYSES
PHASE I EXTRACTION STUDY

TABLE B-1. STATISTICAL SUMMARY FOR DMP - EXTRACTION STUDY

Anova Table

Source	df	SS	MS	F
Solvents	1	320.00	320.00	2.87
pH's	2	1679.29	839.65	4.92*
Interaction	2	960.29	480.15	2.81
Error	14	2391.42	170.82	
Total	19	5351.00		

Mean = 112.5

Cell Means

Solvent

1	2
116.5	108.5

pH (LSD = 16.2)

7	7	10
100.0	122.1	112.1

Interaction

(1,2)	(1,7)	(1,10)	(2,2)	(2,7)	(2,10)
96.0	134.2	113.3	104.0	110.0	111.0

* Significant at 0.05 level.

TABLE B-2. STATISTICAL SUMMARY FOR DEP - EXTRACTION STUDY

Anova Table

Source	df	SS	MS	F
Solvents	1	33.80	33.80	<1
pH's	2	492.16	246.08	6.38*
Interaction	2	359.49	179.75	4.66*
Error	14	539.75	38.55	
Total	19	1425.20		

Mean = 100.8

Cell Means

Solvent

1	2
99.5	102.1

pH (LSD = 4.7)

2	7	10
93.7	102.1	106.2

Interaction (LSD= 6.7)

(1,2)	(1,7)	(1,10)	(2,2)	(2,7)	(2,10)
86.0	102.8	108.7	101.3	101.5	103.7

* Significant at 0.05 level.

TABLE B-3. STATISTICAL SUMMARY FOR DBP - EXTRACTION STUDY

Anova Table

Source	df	SS	MS	F
Solvents	1	560.49	560.49	6.80*
pH's	2	1690.11	845.06	10.25*
Interaction	2	860.27	430.14	5.22*
Error	13	1072.08	82.47	
Total	18	4182.55		

Mean= 90.0

Cell Means

Solvent

1	2
84.9	95.8

pH (LSD =10.6)

2	7	10
76.3	98.0	94.5

Interaction (LSD= 14.9)

(1,2)	(1,7)	(1,10)	(2,2)	(2,7)	(2,10)
61.3	95.2	94.7	91.3	101.7	94.3

* Significant at 0.05 level.

TABLE B-4. STATISTICAL SUMMARY FOR BBP - EXTRACTION STUDY

Anova Table

Source	df	SS	MS	F
Solvents	1	133.06	133.06	5.65*
pH's	2	431.28	215.64	9.16*
Interaction	2	546.74	273.37	11.61
Error	13	306.08	23.54	
Total	18	1417.16		

Mean = 94.8

Cell Means

Solvent

1	2
97.3	92.0

pH

2	7	10
89.2	100.6	93.7

Interaction

(1,2)	(1,7)	(1,10)	(2,2)	(2,7)	(2,10)
88.7	108.8	90.7	89.7	89.7	96.7

* Significant at 0.05 level.

TABLE B-5. STATISTICAL SUMMARY FOR DEHP - EXTRACTION STUDY

Anova Table

Source	df	SS	MS	F
Solvents	1	143.29	143.29	1.82
pH's	2	132.19	66.10	<1
Interaction	2	34.89	17.45	<1
Error	13	1025.42	78.88	
Total	18	1335.79		

Mean = 103.1

Cell Means

Solvent

1	2
100.5	106.0

pH

2	7	10
102.8	100.3	106.7

Interaction

(1,2)	(1,7)	(1,10)	(2,2)	(2,7)	(2,10)
98.3	99.8	103.7	107.3	101.3	109.7

* Significant at 0.05 level.

TABLE B-6. STATISTICAL SUMMARY FOR DDP - EXTRACTION STUDY

Anova Table

Source	df	SS	MS	F
Solvents	1	1.25	1.25	<1
pH's	2	8.54	4.27	<1
Interaction	2	72.21	36.11	7.94*
Error	14	63.75	4.55	
Total	19	145.75		

Mean = 94.2

Cell Means

Solvent

1	2
94.5	94.0

pH

2	7	10
93.5	94.1	95.2

Interaction (LSD = 3.5)

(1,2)	(1,7)	(1,10)	(2,2)	(2,7)	(2,10)
93.3	96.5	93.0	93.7	91.8	97.3

* Significant at 0.05 level.

APPENDIX C

ANOVA TABLES
PHASE I PRESERVATION STUDY

TABLE C-T. STATISTICAL SUMMARY FOR DMP - PRESERVATION STUDY

ANOVA Table

Source	df	SS	MS	F
pH	2	10,996.08	5,498.04	2,972*
Temperature	1	1,107.04	1,107.04	598*
Chlorine	1	22.04	22.04	11.91*
pH x T	2	6,969.08	3,484.54	1,884*
pH x C	2	88.08	44.04	23.82*
T x C	1	22.04	22.04	11.91*
pH x T x C	2	104.08	52.04	28*
Residual	10	18.50	1.85	
TOTAL	21	19,326.94		

Mean = 89.0

Cell Means

pH (LSD=1.4)

2	7	10
108.0	100.0	59.1

Temperature, °C

4	24
95.8	82.2

pH-Temperature

(2,4)	(2,24)	(7,4)	(7,24)	(10,4)	(10,24)
103.5	112.5	94.0	106.0	90.0	28.2

* Significant at 0.05 level.

TABLE C-2. STATISTICAL SUMMARY FOR DEP - PRESERVATION STUDY

ANOVA Table

Source	df	SS	MS	F
pH	2	359.08	179.54	45*
Temperature	1	80.67	80.67	20.17*
Chlorine	1	4.17	4.17	1.04
pH x T	2	576.08	288.04	72.01*
pH x C	2	9.08	4.54	1.14
T x C	1	10.67	10.67	2.67
pH x T x C	2	28.08	14.04	3.51
Residual	10	40.00	4.00	
TOTAL	21	1,107.83		

Mean = 96.1

Cell Means

pH (LSD=2.1)

2	7	10
98.5	99.1	90.6

Temperature, °C

4	24
97.9	94.2

pH-Temperature (LSD-3.0)

(2.4)	(2,24)	(7,4)	(7,24)	(10,4)	(10,24)
95.8	101.2	98.8	99.5	99.2	82.0

* Significant at 0.05 level.

TABLE C-3. STATISTICAL SUMMARY FOR DBP - PRESERVATION STUDY

ANOVA Table

Source	df	SS	MS	F
pH	2	219.25	109.62	6.07*
Temperature	1	35.04	35.04	1.94
Chlorine	1	45.38	45.38	2.51
pH x T	2	907.58	453.79	25.14*
pH x C	2	91.75	45.88	2.54
T x C	1	57.04	57.04	3.16
pH x T x C	2	30.08	15.04	<1
Residual	10	108.50	18.05	
TOTAL	21	1,566.62		

Mean = 86.9

Cell Means

pH (LSD=4.6)

2	7	10
86.5	90.8	83.4

pH-Temperature (LSD=6.3)

(2,4)	(2,24)	(7,4)	(7,24)	(10,4)	(10,24)
84.0	89.0	87.0	94.5	93.3	73.5

* Significant at 0.05 level.

TABLE C-4. STATISTICAL SUMMARY FOR BBP - PRESERVATION STUDY

ANOVA Table

Source	df	SS	MS	F
pH	2	4,616.08	2,308.04	58*
Temperature	1	1,802.67	1,802.67	45*
Chlorine	1	0.67	0.67	<1
pH x T	2	2,133.58	1,066.79	27*
pH x C	2	23.58	11.79	<1
T x C	1	6.00	6.00	<1
pH x T x C	2	49.75	24.88	<1
Residual	11	433.00	39.68	
TOTAL	22	9,065.33		

Mean = 75.6

Cell Means

pH (LSD=6.9)

2	7	10
93.8	72.8	60.3

Temperature, °C

4	24
84.3	67.0

pH-Temperature

(2,4)	(2,24)	(7,4)	(7,24)	(10,4)	(10,24)
96.0	91.8	74.8	71.0	82.3	38.3

* Significant at 0.05 level.

TABLE C-5. STATISTICAL SUMMARY FOR DEHP - PRESERVATION STUDY

ANOVA Table

Source	df	SS	MS	F
pH	2	709.08	354.54	5.72
Temperature	1	287.04	287.04	4.63
Chlorine	1	108.38	108.38	1.75
pH x T	2	29.08	14.54	<1
pH x C	2	57.25	28.62	<1
T x C	1	2.04	2.04	<1
pH x T x C	2	188.53	94.29	1.52
Residual	11	681.50	61.95	
TOTAL	22	2,062.90		

Mean = 93.0

Cell Means

pH (LSD=8.6)		
2	7	10
100.2	91.8	87.1

* Significant at 0.05 level.

TABLE C-6. STATISTICAL SUMMARY FOR DOP - PRESERVATION STUDY

ANOVA Table

Source	df	SS	MS	F
pH	2	549.33	274.67	11.90*
Temperature	1	88.17	88.17	3.82
Chlorine	1	60.17	60.17	2.61
pH x T	2	214.33	107.17	4.64*
pH x C	2	212.33	106.17	4.60*
T x C	1	228.17	228.17	9.88*
pH x T x C	2	129.33	64.67	2.80
Residual	11	254.00	23.09	
TOTAL	22	1,735.83		

Mean = 88.5

Cell Means

pH (LSD=5.3)

2	7	10
95.2	86.2	84.2

pH-Temperature (LSD=7.5)

(2,4)	(2,24)	(7,4)	(7,24)	(10,4)	(10,24)
97.8	92.8	91.5	81.0	82.2	86.2

pH-Chlorine (LSD=7.5)

(2,0)	(2,2)	(7,0)	(7,2)	(10,0)	(10,2)
95.2	95.2	80.5	92.0	85.2	83.2

Temperature-Chlorine (LSD=6.1)

(4,0)	(4,2)	(24,0)	(24,2)
92.0	89.0	82.0	91.3

* Significant at 0.05 level.

APPENDIX D

CHROMATOGRAMS OF WASTEWATER FRACTIONS
PHASE II

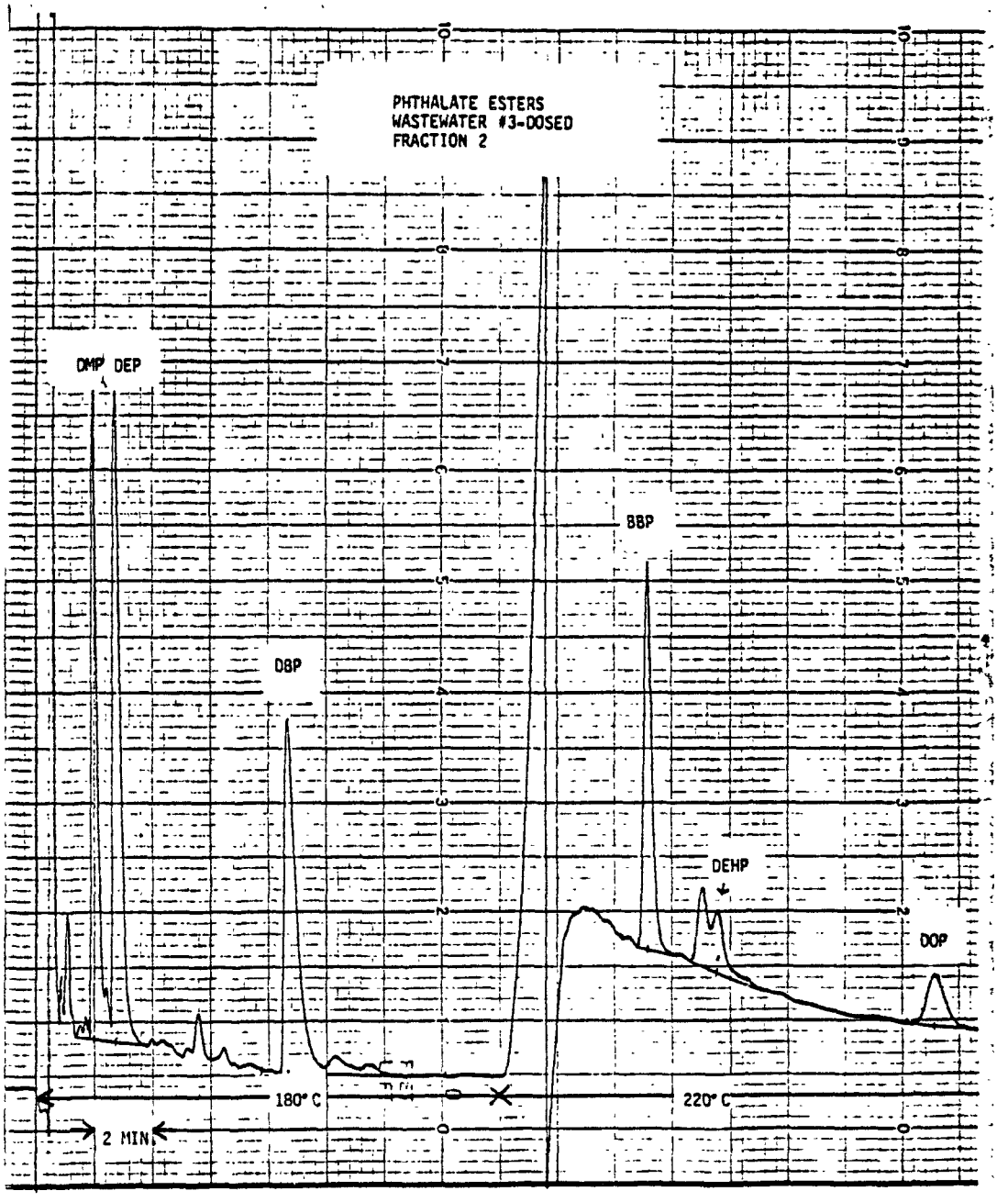

Figure D.1. Chromatogram Wastewater 3 - Fraction 2

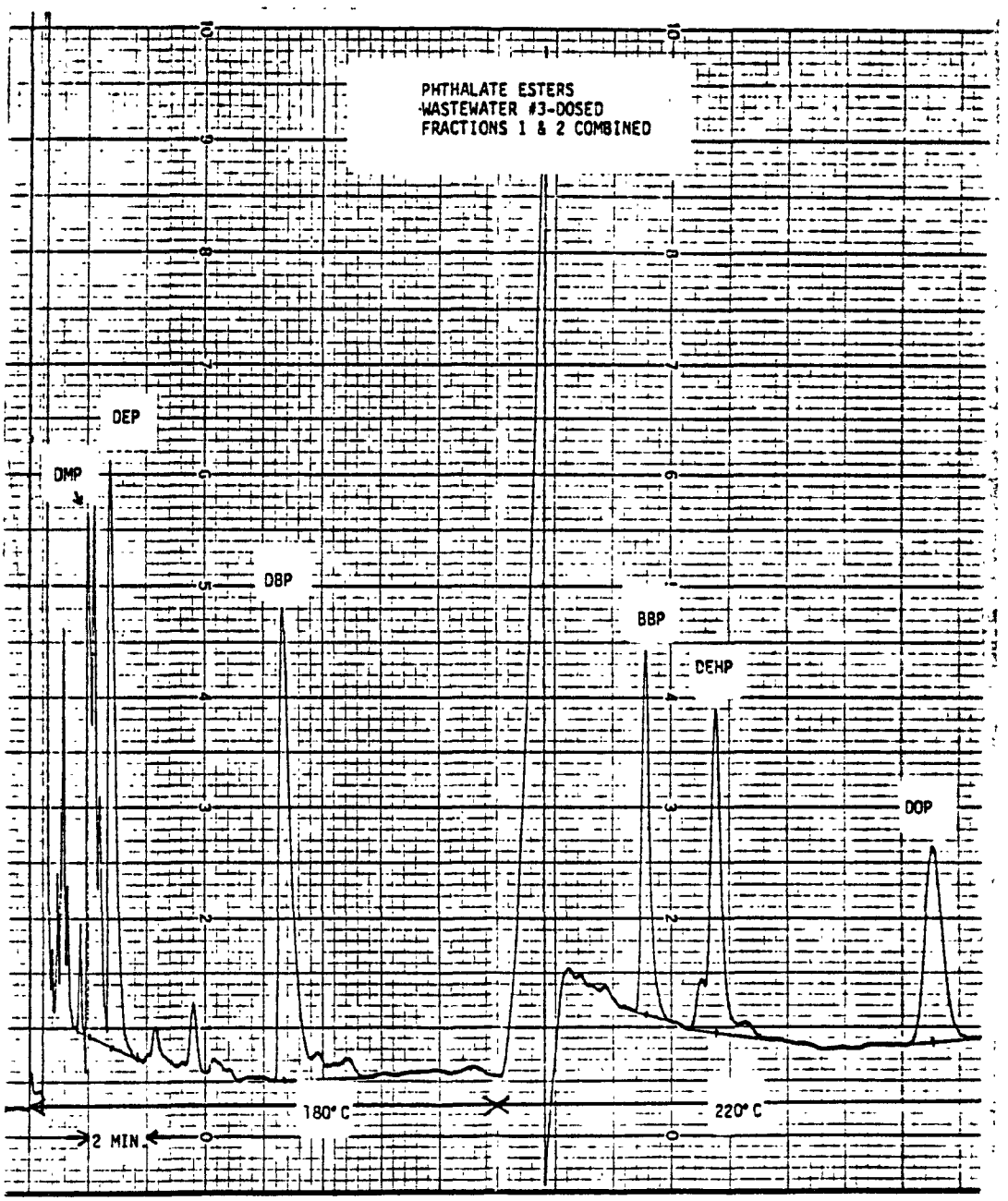

Figure D.2. Chromatogram Wastewater 3 - Fractions 1 & 2 Combined

APPENDIX E

CHROMATOGRAMS OF WASTEWATERS 1 THROUGH 5
SINGLE FRACTION COLLECTION - PHASE II

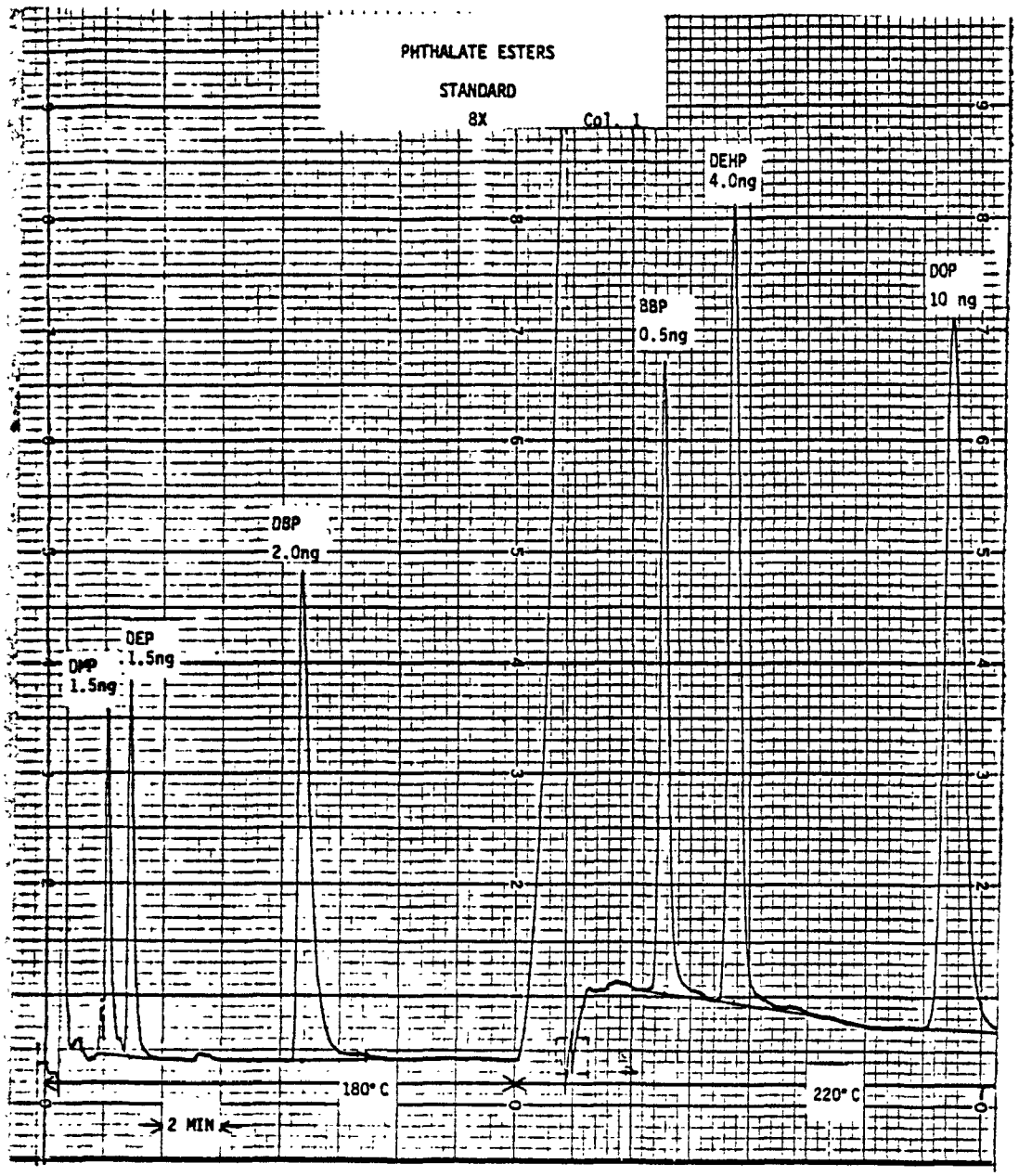

Figure E.1. Standard

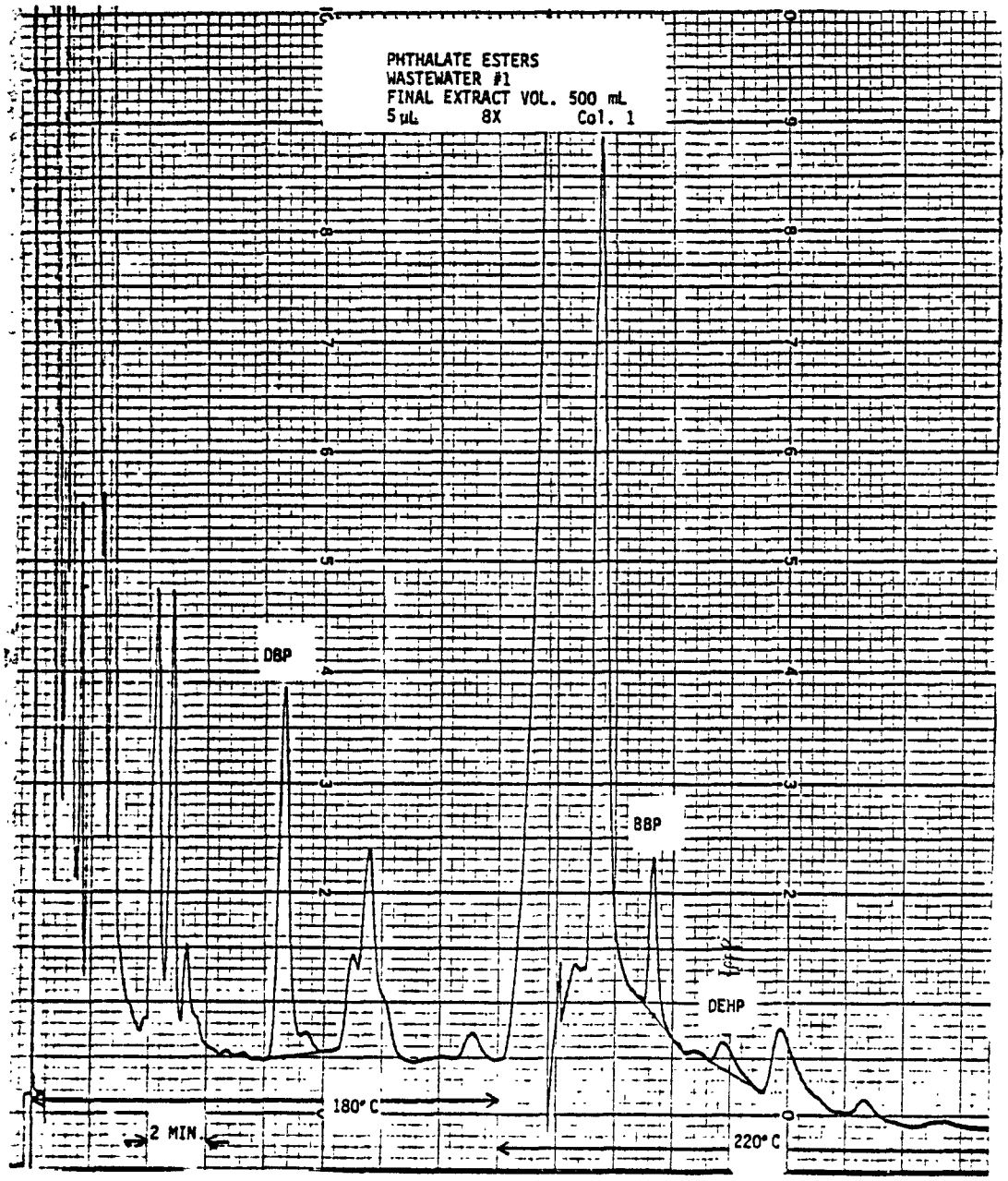

Figure E.2. Wastewater 1

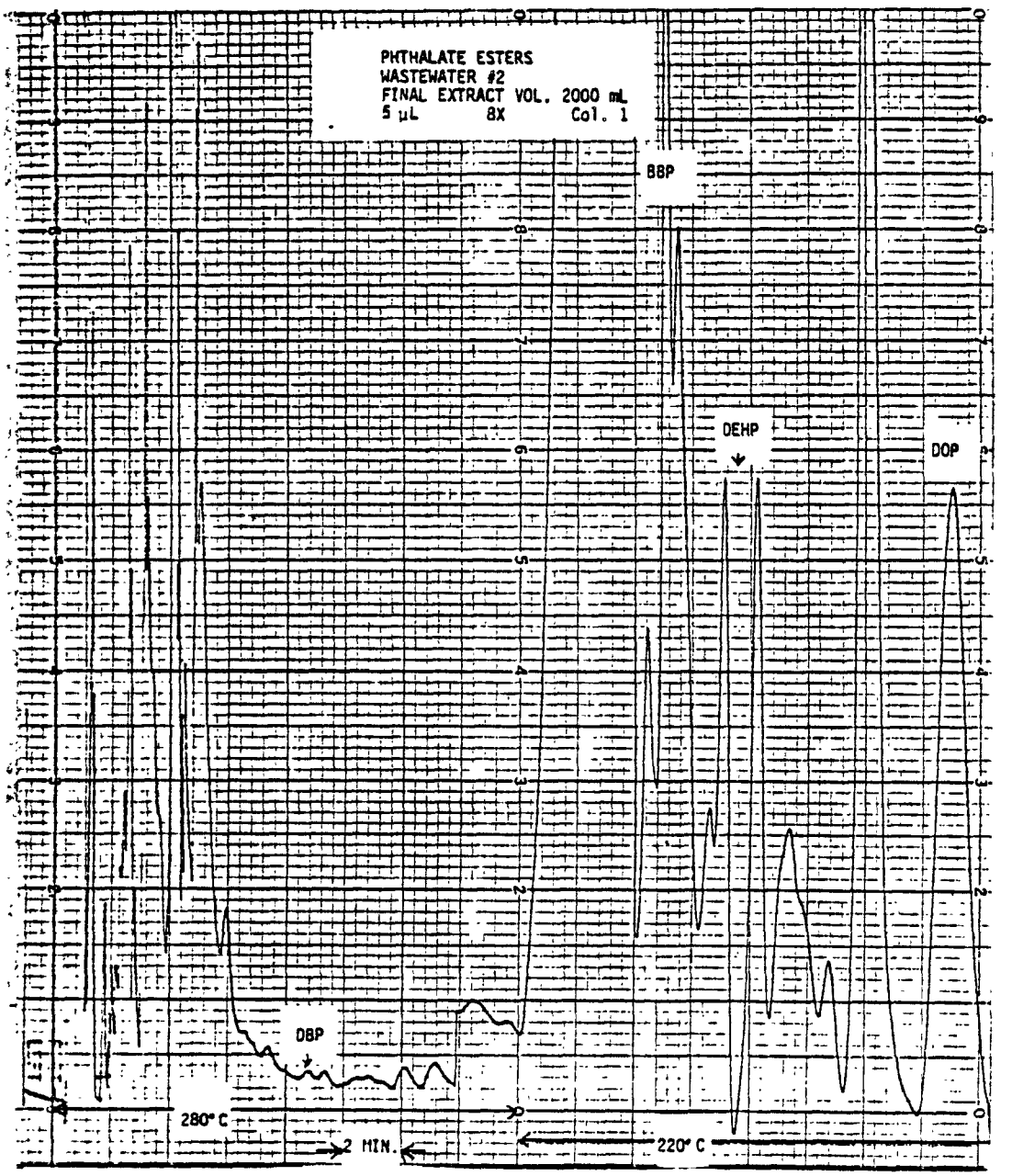

Figure E-3. Wastewater 2

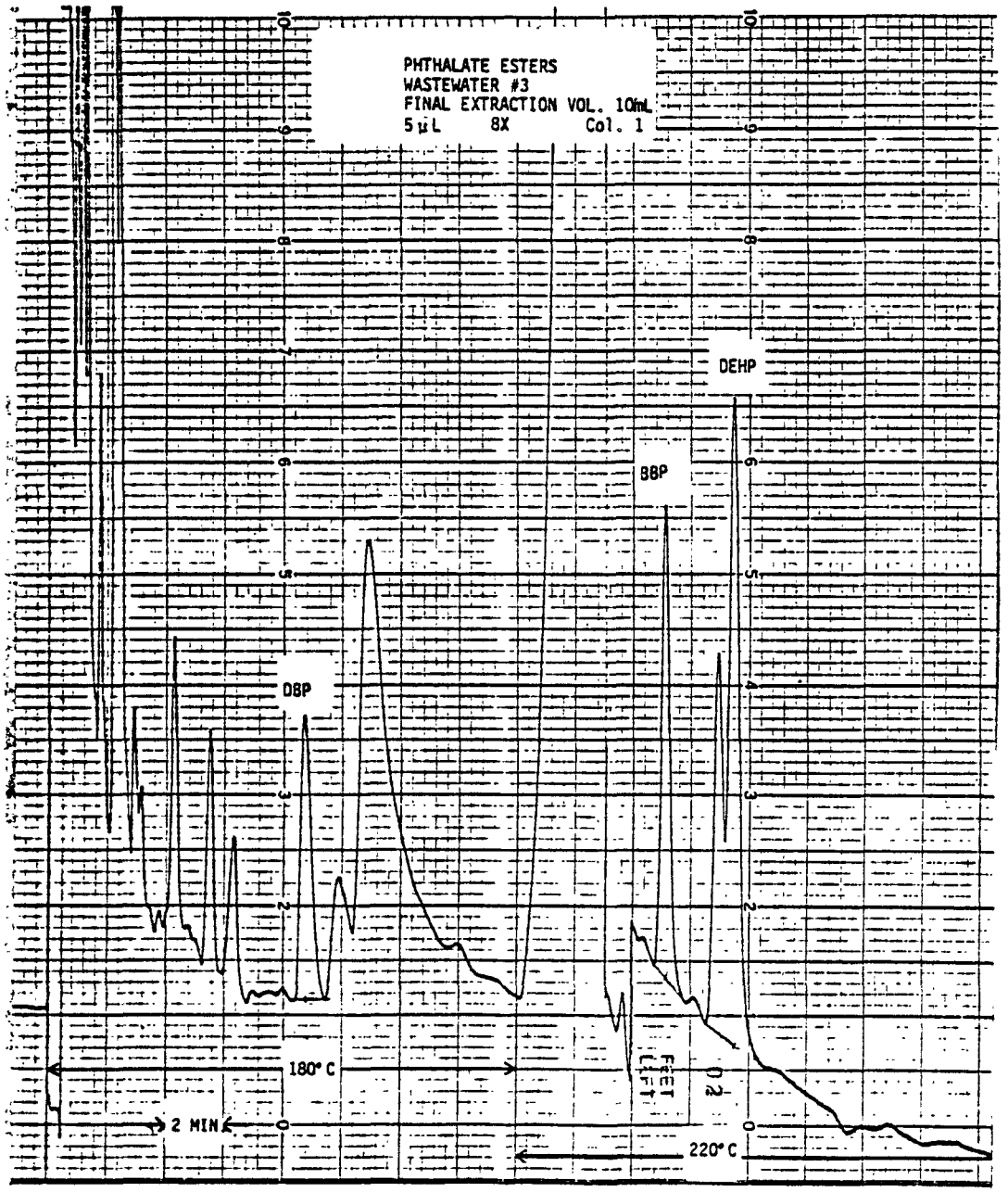

Figure E.4. Wastewater 3

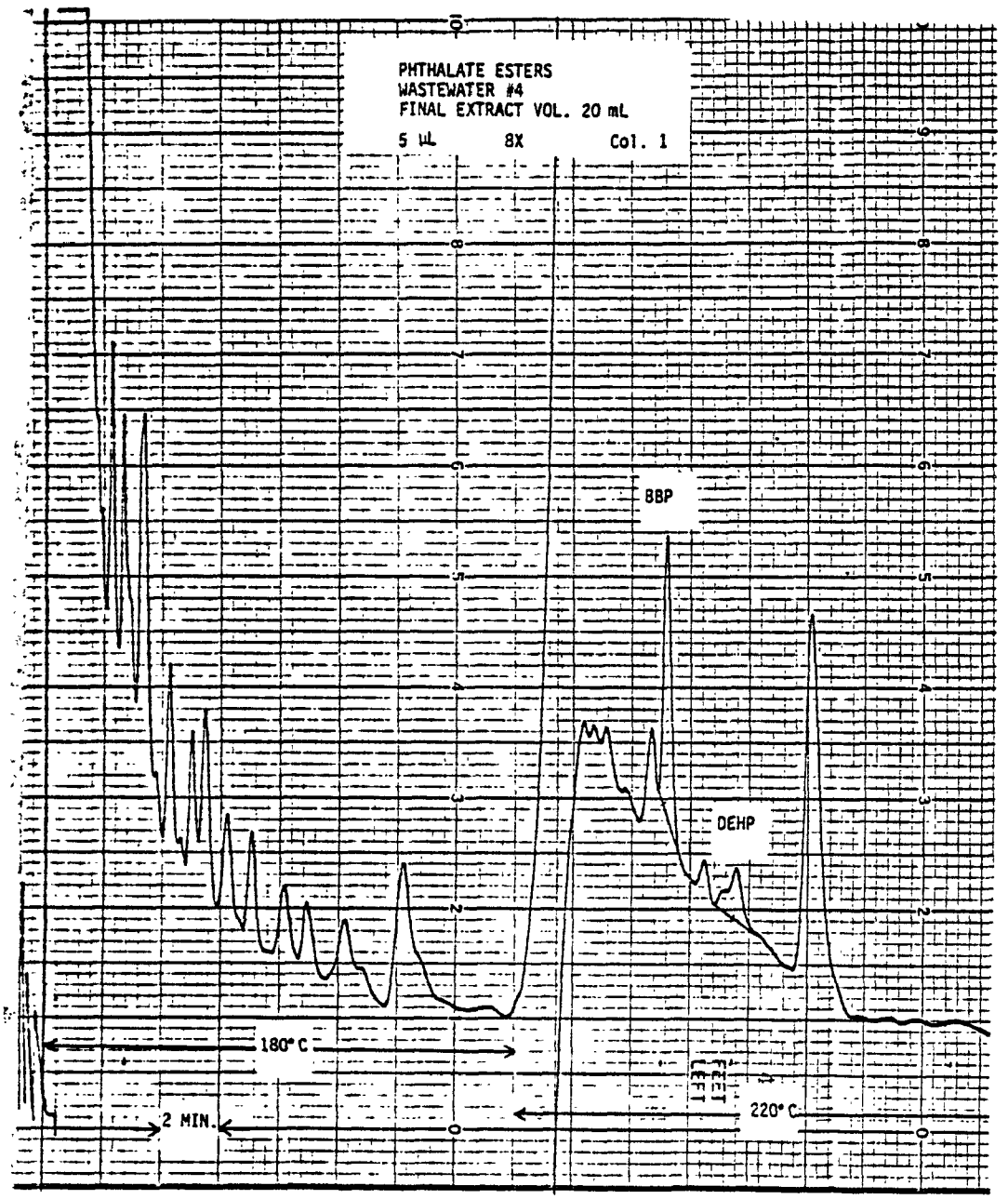

Figure E.5. Wastewater 4

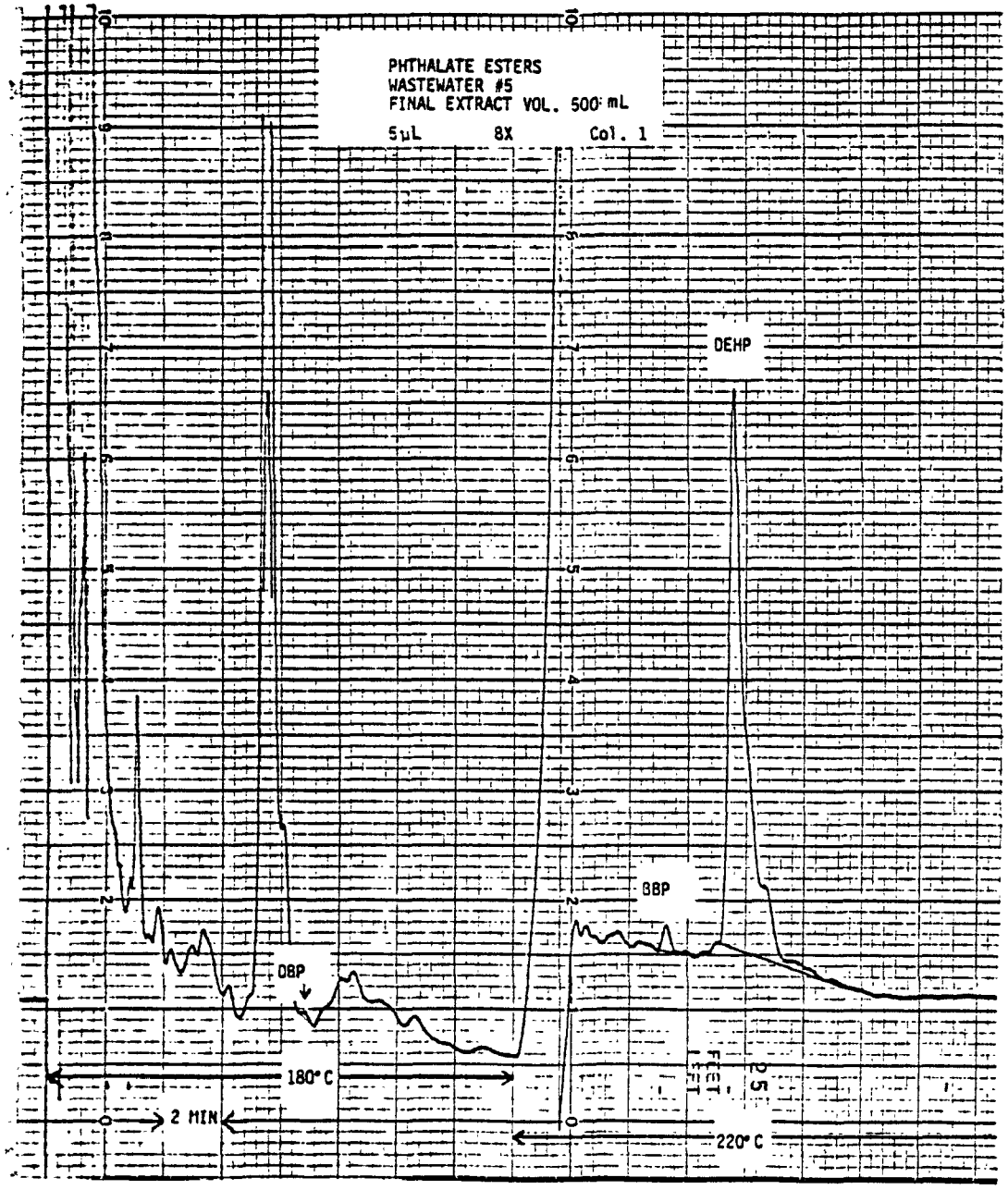

Figure E.6. Wastewater 5