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Investigation of Halogenated Components Formed from Chlorination of Natural Waters: Preliminary Studies

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Pacific Northwest Laboratory
Operated by
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ABSTRACT

Chlorination of power plant cooling water is extensively used as a means of controlling biofouling. This practice presents the potential for formation of halogenated organic compounds hazardous to man and his environment. Accordingly, the organic composition resulting from the chlorination of natural waters (northern Olympic Pennisula sea water and the Columbia River in Washington State) has been investigated. Nonpolar lipophilic organic halogens were extracted by passing large volumes of water over columns of XAD-2 macroreticular resins. Examination of ether extracts from the resin columns using capillary gas chromatography revealed the presence of halogenated methanes, as well as other electron-capturing components, that were not found when unchlorinated water was sampled. Examination of the chlorinated water extracts using gas chromatography/mass spectrometry revealed complex mixtures which generally were not separable into individual components, even when high efficiency WCOT capillary columns were used. The samples were separated into fractions of increasing polarity using a water-deactivated silica gel column. Fractions were thus obtained which were more amenable to GC/MS investigation. Haloforms were identified as the major halogenated product from chlorination of the waters studied. Other halogenated products were found at much lower concentrations.

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SUMMARY

This report describes the result of studies conducted to investigate appropriate sampling and analytical strategies for the detection and determination of halogenated organic compounds formed from the low level chlorination of natural waters. The work was performed as a part of a larger program to determine the synthesis, fate, and effects of chlorination products in aquatic environments. The analytical work reported here are results from the first year's efforts in a scheduled three-year study.

Emphasis was placed on those halogenated compounds that might be expected to be absorbed and biomagnified in the lipids of aquatic biota. Thus the nonpolar, presumably lipophylic organohalogens were investigated. The procedures used to isolate these compounds specifically exclude polar component types such as halogenated phenols, amines, and nitrogen heterocycles.

Samples of chlorinated natural waters were obtained from a continuous flow apparatus designed to simulated conditions of current power plant cooling water treatment practice. Organic components were concentrated by forcing chlorinated and unchlorinated water through columns of XAD-2 resin using a positive displacement pump. Ether extracts of the XAD-2 columns were analyzed for haloforms by gas chromatography. Bromoform was found to be the major consitituent in all chlorinated sea water samples. Chloroform was the major haloform produced from chlorinated Columbia River water.

The procedures used for analysis of XAD-2 extracts for components other than haloforms involved a number of preliminary separation steps prior to gas chromatographic analysis. Gel permeation chromatography was first used to determine the distribution of total halogen according to molecular weight. No halogen was detected in molecular weight fractions greater than MW 800, thus the procedure was used to remove material having molecular weight greater than 800 prior to separation according to polarity. Silica gel chromatography using hexane, then hexane-ether, was used to separate the sample into two fractions. A first fraction contained nonpolar components (e.g., hydrocarbons and halocarbons) and a second contained components of intermediate polarity.

Many of the components identified were aromatic hydrocarbons which were found in all samples studied. Procedural blanks performed by extracting freshly packed XAD-2 resin columns and subjecting the extracts to all the steps of analytical procedure did not contain these hydrocarbons. It is likely that these components were initially present in the XAD-2 polymer matrix in an unextractable form and are released during sampling, possibly because of fracturing of the resin particles as the bed is compacted. Brominated aromatic components found in samples of XAD-2 extracts of seawater are probably formed by reaction of bromine with the hydrocarbon impurities in the resin during sampling.

On the basis of these results, concentrations of nonpolar and presumably lipophilic halogenated components generated by low level chlorination of Sequim

Bay and Columbia River waters appear to be very low, in the nanogram per liter range, with the exception of haloforms, which are orders of magnitude higher.

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PREFACE.

This report includes data and analysis for the Analytical Chemistry Task of the program on Biocide By-Products in Aquatic Environments.

Reports prepared for the entire program are:

Title	Author
• Investigation of Halogenated Components Formed from Chlorination of Natural Waters: Preliminary Studies, NUREG/CR-1299	Roger M. Bean Robert G. Riley
• Acute Toxicity and Bioaccumulation of Chloroform to Four Species of Freshwater Fish Salmo gairdneri, Rainbow Trout Lepomis macrochirus, Bluegill Micropterus salmoides, Largemouth Bass Ictalurus punctatus, Channel Catfish NUREG/CR-0893	David R. Anderson E. William Lusty
• Chronic Effects of Chlorination By-Products on Rainbow Trout, Salmo gairdneri, NUREG/CR-0892	David R. Anderson Roger M. Bean Roger E. Schirmer
• Toxicity, Bioaccumulation and Depuration of Bromoform in Five Marine Species Protothaca staminea, Littleneck Clam Mercenaria mercenaria, Eastern Hard Clam, Quahog Crassostrea virginica, Eastern oyster Penaeus aztecus, Brown Shrimp Brevoortia tyrannus, Atlantic Menhaden, NUREG/CR-1297	Chalres I. Gibson Fredrick C. Tone Peter Wilkinson J. W. Blaylock Roger E. Schirmer
• Growth and Histological Effects to Protothaca staminea, (Littleneck Clam) of Long-Term Exposure to Chlorinated Sea Water, NUREG/CR-1298	Charles I. Gibson Robert E. Hillman Peter Wilkinson Dana L. Woodruff
• Analysis of Organohalogen Products from Chlorination of Natural Waters Under Simulated Biofouling Control Conditions, NUREG/CR-1301	Roger M. Bean Dale C. Mann Robert G. Riley
• Biocide By-Products in Aquatic Environments, Final Report Covering Period September 10, 1976 through September 30, 1979, NUREG/CR-1300	Roger M. Bean Charles I. Gibson David R. Anderson

ACKNOWLEDGMENTS

1.0 INTRODUCTION

Concern about the presence of halogenated organic compounds in water has been growing since Dowty et al. (1975) detected volatile organochlorine compounds in a New Orleans area municipal water treatment facility. This report was rapidly followed with evidence adduced by Rook (1974), Glaze and Henderson (1976), Jolley (1975a) and others that the presence of a wide variety of organohalogen compounds in drinking waters and wastewater effluents is a consequence of current chlorination treatment practices. Halogen-containing organic compounds have been reported to adversely affect aquatic biological species through direct toxic action (Roesijadi et al., 1976), as well as through indirect mechanisms such as interference with reproduction success (Gehrs et al., 1974), and interference with photosynthesis (Eppley et al., 1976). Further, a number of halogenated organic compounds have been found to concentrate in the tissues of aquatic organisms (Zitko and Hutzinger, 1976) which in the case of food fish, increases the potential hazard of these compounds to human health.

About 26,000 tons of chlorine are used annually in the U.S. in treatment of cooling water for electricity generating plants (Hamilton, 1978), many of which use natural riverine or estuarine waters for "once-through" cooling. The number of power plants can be expected to grow rapidly as the nation copes with increasing energy demands, with accompanying increases in chlorine admitted to the environment through cooling water treatment. Jolley et al. (1975b) provided evidence that chlorinated organics were present in samples of chlorinated cooling waters of fresh water origin, and suggested that in estuarine waters, organobromine compounds would be formed (Jolley, 1977).

The analytical studies reported here represent initial investigations on the flexibility of using macroreticular polystyrene-divinyl benzene polymer resins ("XAD-2", Rohm and Haas Company) as adsorbants for concentrating the lipophilic nonpolar haloorganic products resulting from addition of a few parts-per-million of active chlorine in the form of sodium hypochlorite. The work was conducted in support of biological studies being conducted by Battelle. The biological studies were conducted at the Marine Research Laboratory, Sequim, Washington and at the Freshwater Research Laboratories, Richland, Washington. Reports of results from the biology studies are given in the preface to this report. The chemistry studies were also intended to develop methodology for sampling chlorinated natural waters at a number of locations across the United States, and eventually for sampling chlorinated cooling water from nuclear power stations.

The principle objectives of these preliminary investigations were:

- To determine the suitability of XAD resin for concentrating lipophilic nonpolar halogenated organic material from natural waters subjected to chlorination at the few parts-per-million level.

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- To investigate capillary gas chromatography as a tool for the characterization of trace halogenated organics, using electron capture, flame ionization, and mass spectrometric detection systems.

- To develop, as required, separation and cleanup procedures for the additional characterization and simplification of complex environmental samples.

The work described in this report has been published in part (Bean et al., 1978) and was used as a basis for the selection of the sampling, separation, and analytical methods used for investigation of the chlorination of ten natural water resources (Bean et al., 1980). The procedures used for this work would not be expected to detect more polar halogenated components in water, such as the derivatives of phenols, amines, or nitrogen heterocycles. Although these more polar types have been reported in chlorinated waters (Glaze and Henderson, 1976), the procedures used herein would specifically exclude these from the analysis.

2.0 SAMPLING AND ANALYTICAL PROCEDURES

2.1 Sources of Chlorinated Fresh and Salt Water

Chlorinated and nonchlorinated sea water samples were obtained at the
Battelle-Northwest biological laboratory facilities at Sequim, Washington.
Freshwater samples were obtained at Richland, Washington. Both water systems
are relatively free from organic industrial pollutants. Chlorination of the
water samples at between one and two mg/l chlorine as NaOCl was carried out in
continuous-flow apparatus used for biological studies (Hillman et al., 1980;
Anderson and Lusty, 1980). Residence time from chlorine addition to sampling
was about two hours. Nonchlorinated and chlorinated water were sampled at the
same time, using identical sampling methods. For these studies, active chlo-
rine was not destroyed with reducing agent prior to sampling.

2.2 Sampling Procedures

Both chlorinated and unchlorinated natural waters were sampled by pumping
through 1/2" x 9" stainless steel columns filled with 15 to 18 ml of XAD-2
resin. The XAD-2 resin (Supelco chromatographic grade) was cleaned prior to
use with the soxhlet extraction method of Junk et al. (1974). The pump used
was a variable flow, positive displacement device capable of pumping against a
pressure of 100 psi. Pumping rates ranged from 50 to 75 ml/min. Ceramic,
fluorocarbon, and 316 stainless steel were the only materials in contact with
the water. For these initial studies, residual oxidant was not destroyed by
sulfite addition, nor was there any attempt to control pH prior to column
absorption. Instrumentation is being developed for accomplishing this on a
continuous basis. The volume of water extracted using the XAD-2 adsorption
technique varied considerably but generally ranged between 200 and 500 liters.
The XAD-2 columns were kept at $4°C$ after sampling was completed.

2.3 Extraction of XAD Columns

The columns were extracted with 125 ml ether preserved with 2% ethanol (J. T.
Baker, "Resi-analyzed") following the procedure of Junk et al. (1974). All
other solvents used in these studies were Burdick and Jackson "distilled in
glass." The ether samples were treated with sodium sulfate overnight, the
final volume of ether determined, and subsampled for analysis of haloforms by
capillary gas chromatography.

2.4 Gas Chromatographic Analysis of XAD Extracts for Haloforms

Ether extracts of the XAD-2 columns were dried (sodium sulfate), sub-sampled,
and mixed with an ether solution of 1,3-dibromopropane as internal standard.
The samples were analyzed for haloforms using a Hewlett-Packard 5840A gas
chromatograph equipped with a 30 meter wall-coated open tubular capillary
column containing OV101 as liquid phase. The sample was injected at a column
as split ratio of 1:10 at a temperature of $-20°C$ and, after seven minutes, the
temperature was raised at $8°C/min$ to $65°C$. Detection was by electron
capture. Figure 1 shows a chromatogram obtained from the analysis of an XAD-2
extract from a chlorinated sea water sample.

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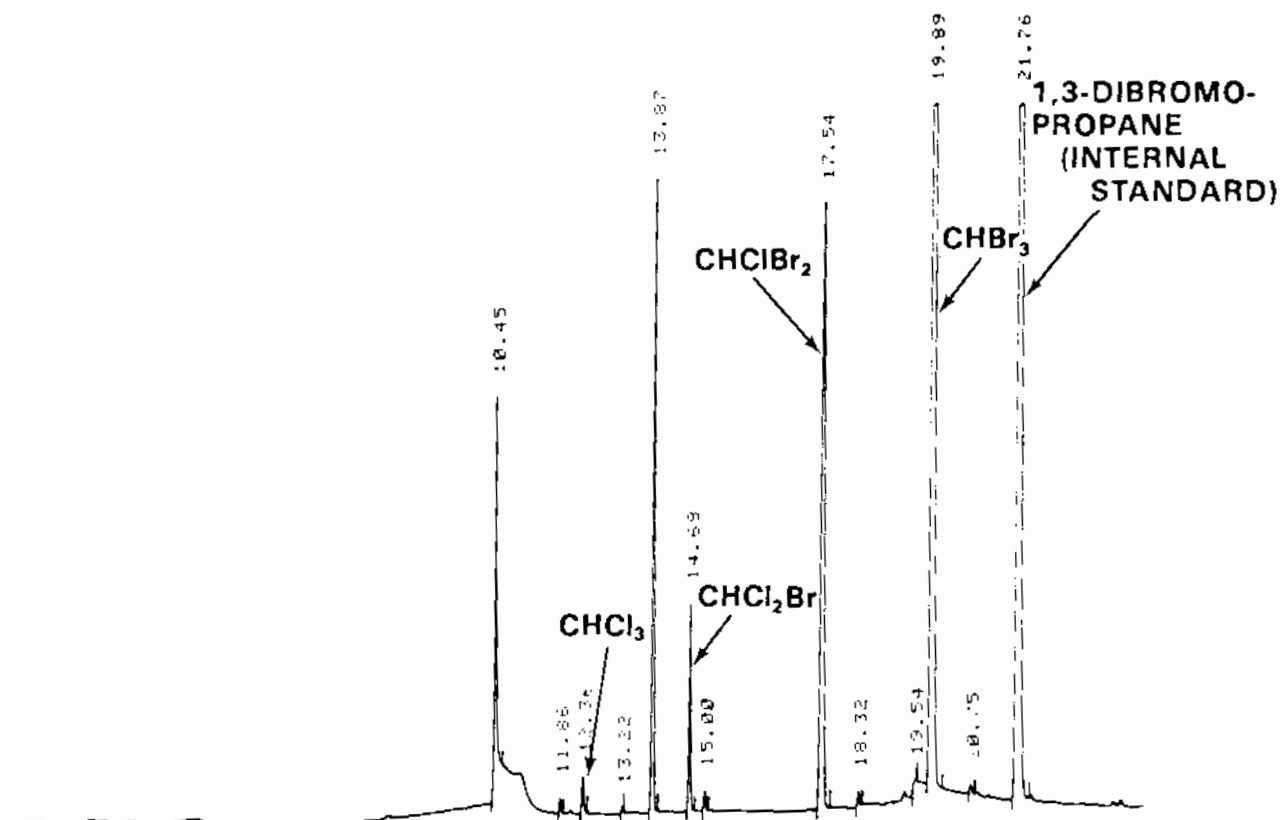
FIGURE 1. Electron capture capillary chromatogram of XAD-2 concentrate from chlorinated sea water.

$CHBr_3$ was confirmed by GC/MS, there being a strong CBr_2^+ formed at the identical retention time of an authentic sample of $CHBr_3$. The presence of $CHClBr_2$ and $CHCl_2Br$ in chlorinated sea water has been confirmed by chromatographic peak enhancement technique using authentic samples of these halomethanes.

2.5 Fractionation of XAD-2 Ether Extracts

The ether extracts from the XAD-2 columns were evaporated under a stream of dry nitrogen, and the solvent changed to benzene. Those samples which were not directly investigated by gas chromatography at this point were subjected to chromatographic fractionation. A flow diagram summarizing the separation scheme used for these studies is given in Figure 2.

The benzene solution of the ether extract was evaporated to 100 μl volume and injected into two 3/8" x 12" μ-Styragel columns (Waters Associates) connected in series. The columns were eluted with benzene at a flow of 1.0 ml/min using a Waters high pressure liquid chromatograph. The fraction eluting between 13 and 25 ml was collected for further study. Under the same conditions, polypropylene glycol (MW 800) has a retention volume of 10.8 ml and cholestane
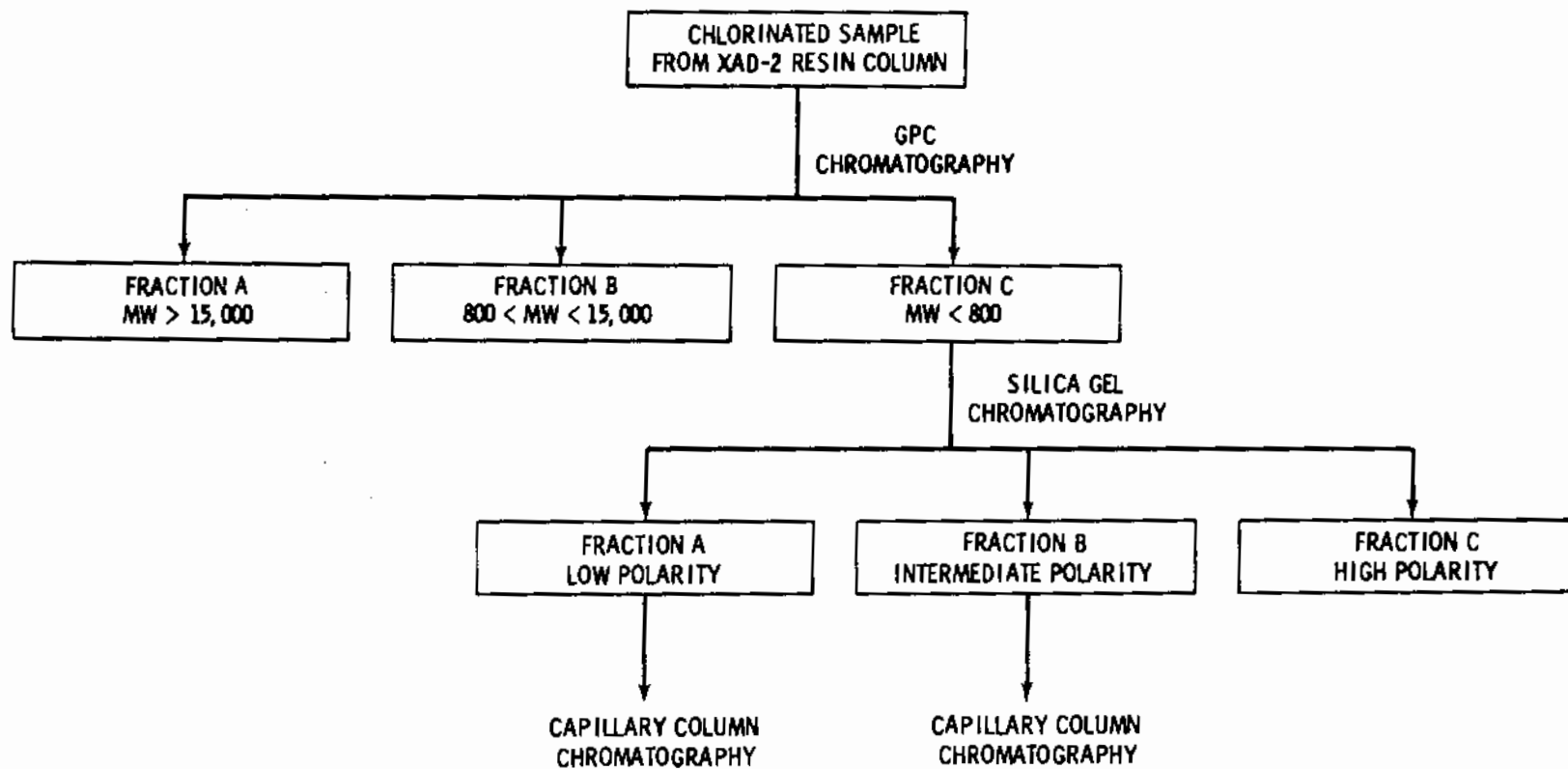
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FIGURE 2. Scheme for separation of XAD-2 samples into fractions

(MW 386) has a retention volume of 13.6. The fraction collected from 13 to 25 ml is thus designated as the <800 MW fraction. The <800 MW fraction was evaporated to 100 µl, and was further separated on a 3/8" x 12" deactivated silica gel column (MCB SX 144-7, 10 water deactivated) using 20 ml hexane at 2 ml/min to elute a nonpolar fraction (Fraction A), followed by 20 ml of a mixture of 16 ethanol-free ether in hexane, also at 2 ml/min, to elute a more polar fraction (Fraction B). The uneluted material was back-flushed from the column using a 40/60 ether/hexane mixture. Fractions A and B were evaporated to a volume of ca. 50 µl.

2.6 Capillary Gas Chromatography of XAD-2 Extracts

Gas chromatography of ether extracts and subfractions was carried out using a Hewlett-Packard Model 5840 equipped with cryogenics, flame ionization detector (FID) and electron capture detector (ECD). The chromatographic column and splitter assembly were purchased from J&W Scientific, Inc. The 30 m column was found to have 101,000 effective theoretical plates at $k = 17.03$ and was one-half of a 60 meter OV101 capillary. The other half of the column was used to perform the GC/MS analyses.

Chromatographic conditions were: Helium carrier gas flow, 1.30 ml/min at 16 psig; split ratio, 6.7 to 1; program, 4 min at 65°C followed by 4°C/min to 250°C and 20 min hold; injector temperature, 310°C. The above conditions were constant regardless of the detector used. When the column was connected to the electron capture detector (ECD), the following conditions obtained: detector temperature, 350°C; argon/methane flow to detector, 30 ml/min; detector attenuation, 8" slope sensitivity, 28. Samples were normally evaporated to a suitable volume such that the detector attenuation for the ECD was 8. Subsequent evaporation of the sample to somewhat less than half that volume permitted analysis of the sample using the following FID conditions: detector temperature, 310°C; nitrogen make-up to detector, 20 ml/min; detector attenuation, 2; slope sensitivity, 0.2.

Changing the column from ECD to FID takes less than 10 minutes. The oven is cooled to 30°C and the column, securing nut, and graphite ferrule moved to the other detector and locked in place being careful not to touch with fingers the portion of the column inserted into the detector base. This procedure is followed by changing the signal, attenuation, and slope sensitivity appropriate for the detector used.

2.7 Capillary Gas Chromatography/Mass Spectrometry of XAD-2 Extracts

The GC/MS instrument used in this work was a Hewlett-Packard 5982A system which includes a HP 5710 gas chromatograph. The GC injection port was fitted with a splitter identical to that used with the EC/FID instrument and was operated as nearly as possible under identical flow and temperature conditions. The back end of the column was connected directly to the standard transfer line intended for chemical ionization GC/MS operation, so that effluent passed directly into the ion source. In CI operation, preheated

reagent gas, usually methane, was added at the column exit, in a manner similar to the addition of make-up gas in conventional capillary chromatography. The 30 m OV101 WCOT column used in this work was matched with the column used in the EC/FID instrument as described above. GC resolution obtained with this system was very nearly the same as on the other instrument, with most differences attributable to the different injection port heaters, geometries, and pressure differences. Chromatographic conditions were identical to those used for the EC/FID instrument, using a split ratio of about 6 to 1. Mass spectra were recorded every 0.9 sec, scanning over a mass range of 40-400 (EI) and 100-460 (CI). The data were stored on disks for off-line data reduction.

3.0 ANALYSIS OF XAD-2 EXTRACTS FOR HALOFORMS

Ether extracts from XAD-2 columns used to sample both chlorinated and non-chlorinated sea water were analyzed for haloforms by capillary gas chromatography. The primary objective of these analyses was to determine the feasibility of using XAD resins for analysis of chlorinated waters in field operations. The results of these studies are presented in Table 1. The first three experiments in Table 1 give results from the analyses of large volume samples using different quantities of XAD resin. Since the quantity of bromoform adsorbed appeared to be a function of the quantity of resin used, it was apparent that the large sample volumes were overloading the resin with bromoform. Table 1 shows the results obtained from a fourth experiment in which two XAD-2 columns were connected in series and nine liters of chlorinated sea water sampled. The results indicate that about 30 μg/l $CHBr_3$ were formed in the Sequim Bay sea water under the chlorination conditions used. Seventy-nine percent of the bromoform was trapped on the upper column. This would indicate an adsorption efficiency somewhat lower than that recently reported by Glaze et al. (87%, 1977), probably because we employed a higher sample-to-resin ratio.

In addition to the sea water samples, we have investigated the presence of chloroform in chlorinated samples of Columbia River water. Through use of columns connected in series, we found that XAD-2 resin is not a suitable absorbent for chloroform at the chlorination concentrations under study. Chlorinated river water (12.5 liters) was pumped through two 3/8" XAD-2 columns. The top column was found to contain 3.3 μg $CHCl_3$ per ml resin, the bottom column contained 4.3 μg/ml. Other haloforms were not detected. Thus, while $CHCl_3$ was found in chlorinated fresh water, it was not possible to measure the quantities formed by this technique at this time.

The origin of chloroform in chlorinated drinking waters and wastewaters has been discussed by Rook (1977), who presented evidence for the formation of this compound from reaction of chlorine with fulvic acids. An EPA survey (Symons, 1975) has shown that chloroform is present at the parts-per-billion level in all water issuing from treatment plants using chlorine, and that the chloroform concentration positively correlates with the organic content of the untreated water.

TABLE 1. Analysis of sea water-pumped XAD-2 resin for halomethanes (values reported as µg/liter of sea water pumped through resin[a])

Trial No.	Water Chlorinated	Volume of Pumped (1)	Volume of Resin (ml)	$CHCl_2Br$ µg/l	$CHClBr_2$ µg/l	$CHBr_3$ µg/l
1.	Yes	238	8.0	<0.01	0.13	2.11
	No	231	9.0	nd[c]	nd	0.04
2.	Yes	425	15.5	nd	0.44	8.33
	No	510	15.6	nd	nd	0.01
3.	Yes	634	6.3	<0.01	0.13	2.37
	No	604	7.6	nd	nd	0.01
4.	Yes	9.0	8.0 (top)[b]	nd	0.14	23.39
	No	9.0	10.8 (bottom)	nd	nd	6.33
					TOTAL	29.72

[a] Only traces of $CHCl_3$ (0.01 to 0.03 µg/l) were found in the samples
[b] Two columns were connected in series and then separately analyzed. See text.
[c] nd — not detected

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4.0 INITIAL CHARACTERIZATION OF CHLORINATED AND NONCHLORINATED WATER EXTRACTS BY CAPILLARY CHROMATOGRAPHY

A first goal of the chromatographic studies was to compare the response of the electron capture detector to components from the XAD extracts of both chlorinated and nonchlorinated water. Capillary chromatography of the XAD-2 ether extracts in benzene produced an immediate analytical result: A series of electron-capturing peaks was observed in the ether extracts of nonchlorinated Columbia River water (Figure 3). This pattern was found to be identical in relative peak intensities and relative retention times to that of Aroclor 1254, a well-known polychlorinated biphenyl (PCB) mixture (Figure 4). The concentration of Aroclor found in the Columbia River sample was in the ng/l range. In the corresponding chlorinated Columbia River sample (Figure 5) the Arcolor pattern remains identifiable, even in the presence of many other electron-capturing components, and indicates that while chlorination may have produced additional chlorine-containing components, significant additional PCB chlorination did not occur. The power of capillary chromatography is well illustrated here, in that this observation could not have been made with these samples using conventional chromatography.

Figures 3 and 5 also serve to illustrate some of the difficulties in working with the unrefined ether extracts of natural waters. In general, attempts to analyze the crude ether extracts directly on the gas chromatograph were not successful. It was evident from the dark greenish-brown appearance of the extracts that significant quantities of high molecular weight material were present. Thus, there was the likelihood that artifacts would be produced from pyrolytic reactions occurring in the injection port of the chromatograph. From inspection of the chromatograms obtained, we have formed the qualitative judgment that these types of reactions were occurring; however, this was not investigated in detail, since only a few of the crude extract samples could be run before the chromatographic column became plugged at the injection port end. This problem could be temporarily rectified by breaking off as much as one meter of column length. Inspection of the sealed capillary under magnification showed that, in addition to dark material within, the liquid phase had pulled away from the capillary wall. The glass liner of the injection port accumulated a black residue of carbonaceous material.

Another problem associated with the crude ether extracts was the complexity of the samples. The samples were sufficiently complex that most components would not be resolved on the capillary column. Thus, flame ionization chromatograms of the extracts were principally unresolved envelopes. The largest significant peaks were phthalate esters. Electron capturing components, being fewer in number, were better resolved from each other; however, there was little observable correspondence between peaks in electron capture chromatograms and those in the flame ionization chromatograms. Investigation of the samples by GC/MS was also unfruitful, since clean spectra could not be obtained. Single mass chromatograms generated from the spectral data were almost as complex as the total ion chromatograms for all masses investigated.

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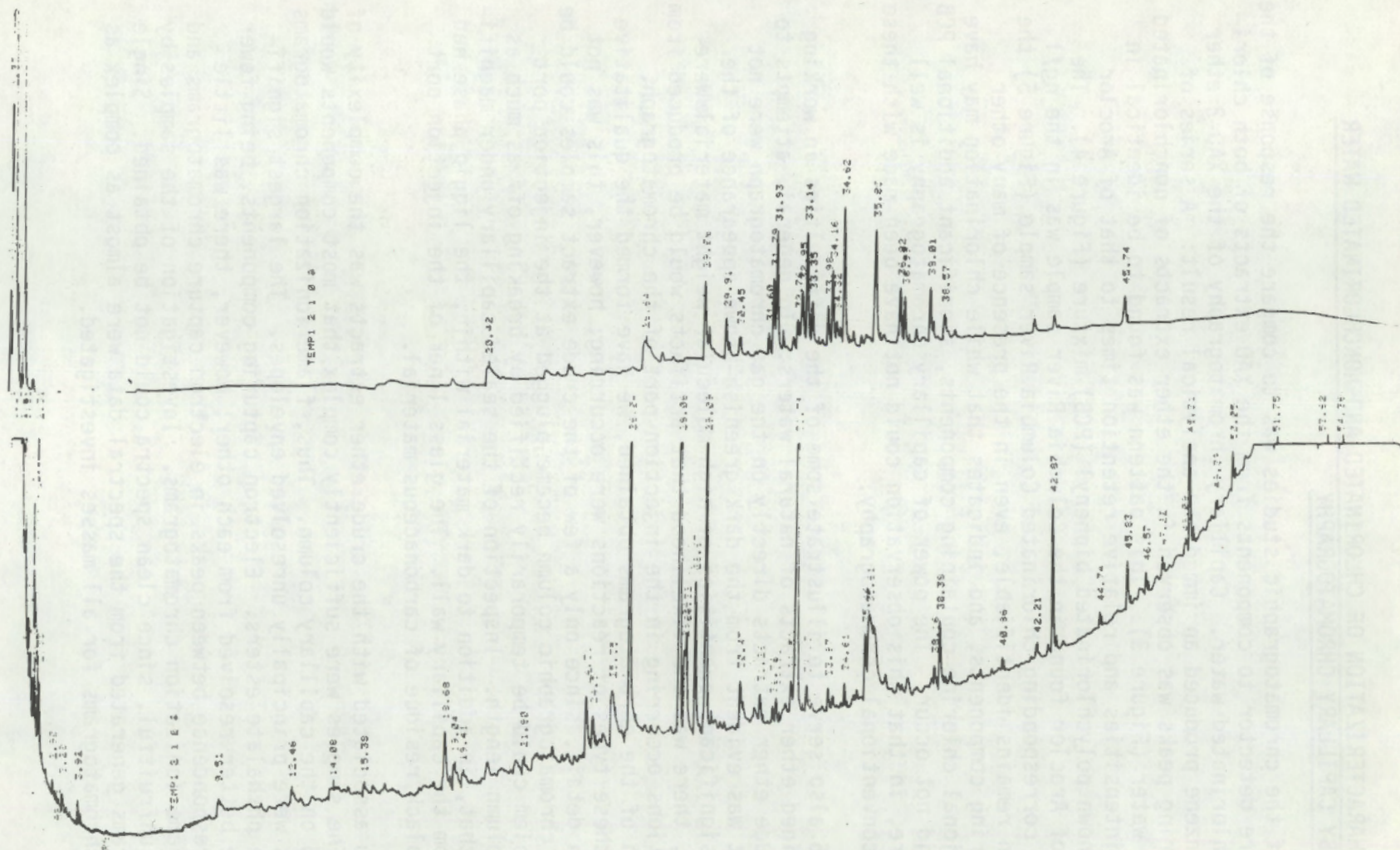
FIGURE 3. Above, electron capture chromatogram of the extract from several hundred liters of nonchlorinated Columbia River water. Below, corresponding flame ionization chromatogram.

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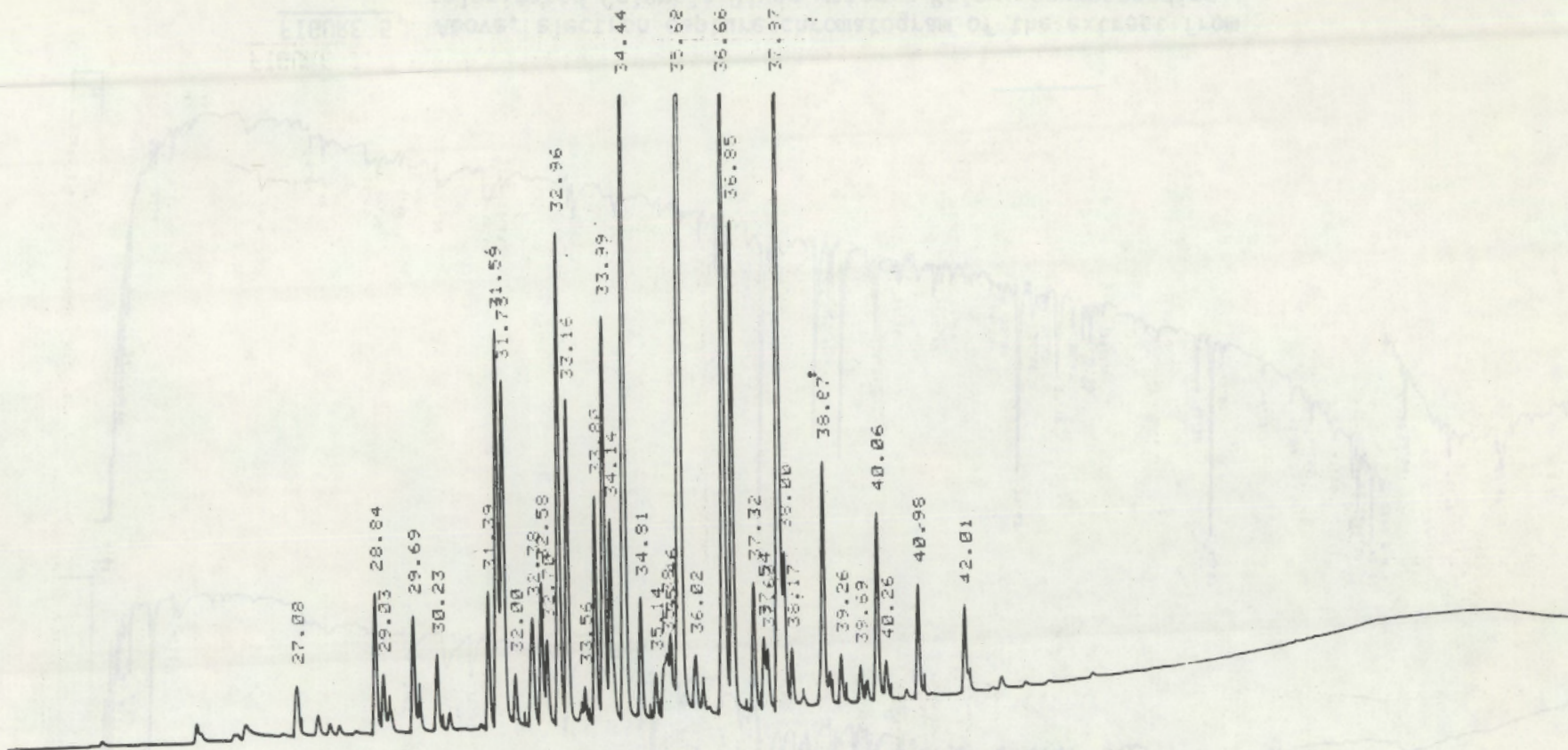
FIGURE 4. Electron capture chromatogram of Aroclor 1254, showing a similarity
of relative peak heights and retention times to Figure 3. Absolute
retention times differ because of slightly different chromato-
graphic conditions.
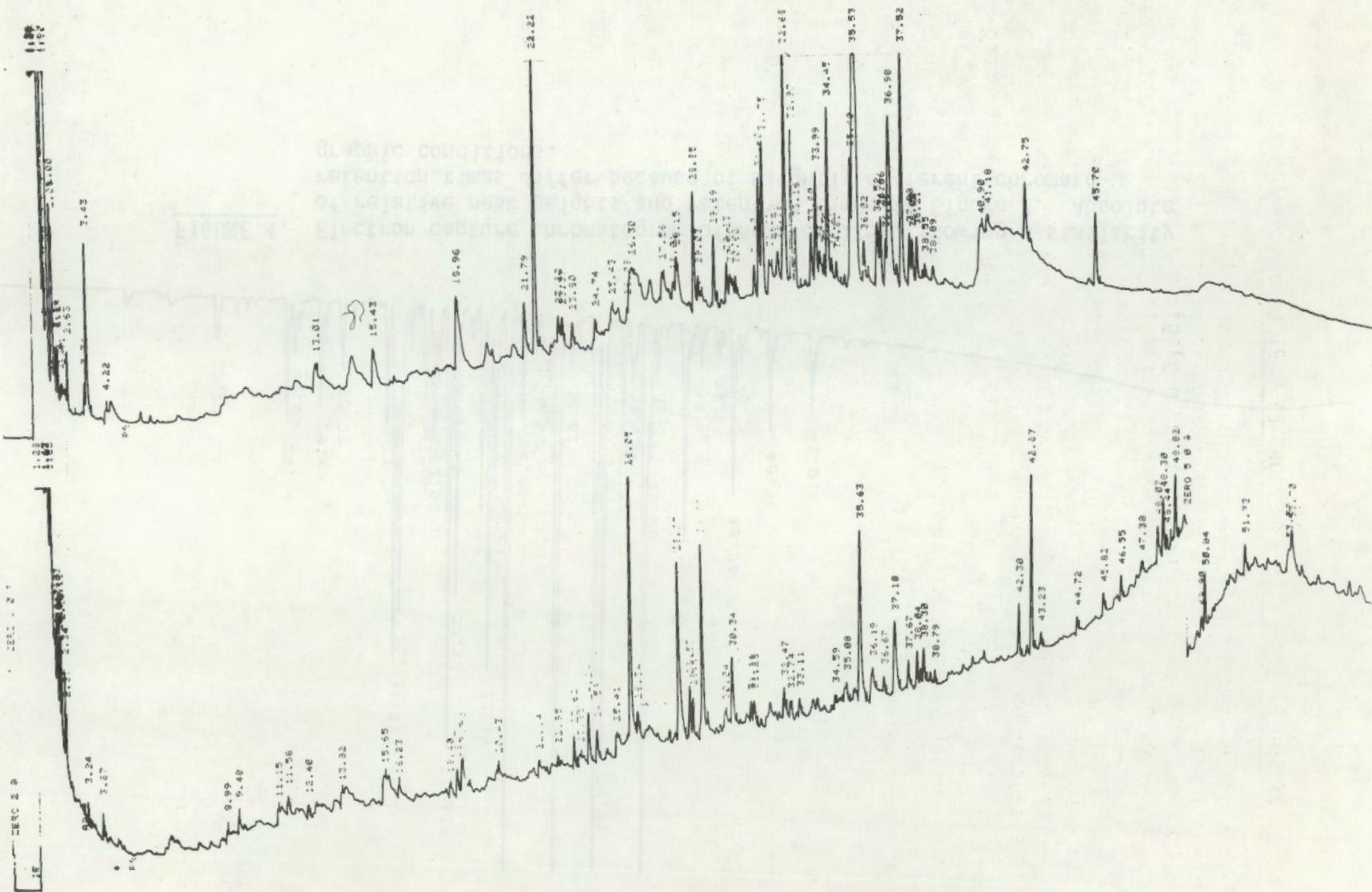
FIGURE 5. Above, electron capture chromatogram of the extract from chlorinated Columbia River water. Below, corresponding flame ionization chromatogram. The chlorinated sample was obtained simultaneously with the nonchlorinated sample (Figure 3).

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5.0 CHARACTERIZATION OF SEPARATED FRACTIONS BY CAPILLARY CHROMATOGRAPHY

The separation of the sample by gel permeation chromatography permitted the rejection of all material in excess of 800 molecular weight after it had been determined that the >800 MW fraction contained negligible halogen content. A chromatogram obtained from a sea water extract after the molecular weight separation step is shown in Figure 6. Fractionation of the sample over deactivated silica gel permitted the isolation of the relatively nonpolar, more lipophylic compounds of interest. Gas chromatography of both Fraction A (hexane elution from silica gel) and Fraction B (16 ether in hexane) could be accomplished with baseline separation of most components even after the samples had been evaporated to as little as 10 μl. A comparison of electron capture chromatograms of Fraction A obtained from chlorinated and nonchlorinated seawater samples (Figure 7) clearly shows the enhancement of electron capturing activity introduced by chlorination. The chromatographic results obtained from Fraction B of the same water sample are shown in Figure 8.
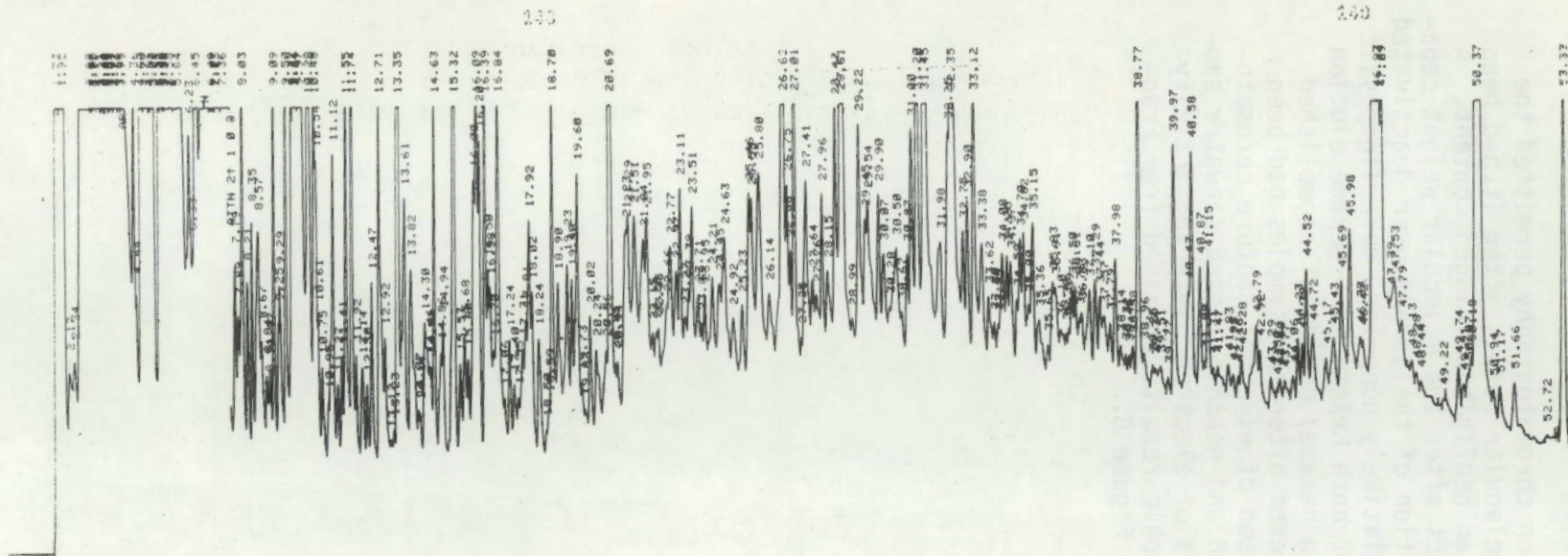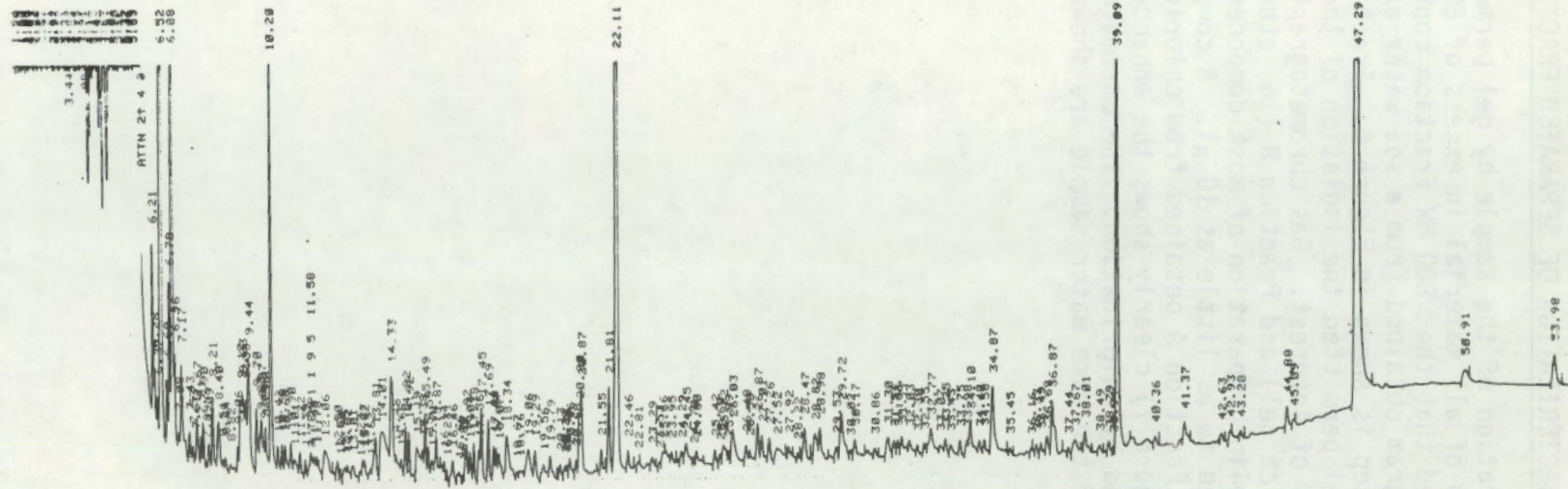
FIGURE 6. Above, electron capture chromatogram of an extract from Sequim Bay sea water after removal of material in excess of 800 molecular weight by gel permeation chromatography. Below, corresponding flame ionization chromatogram.
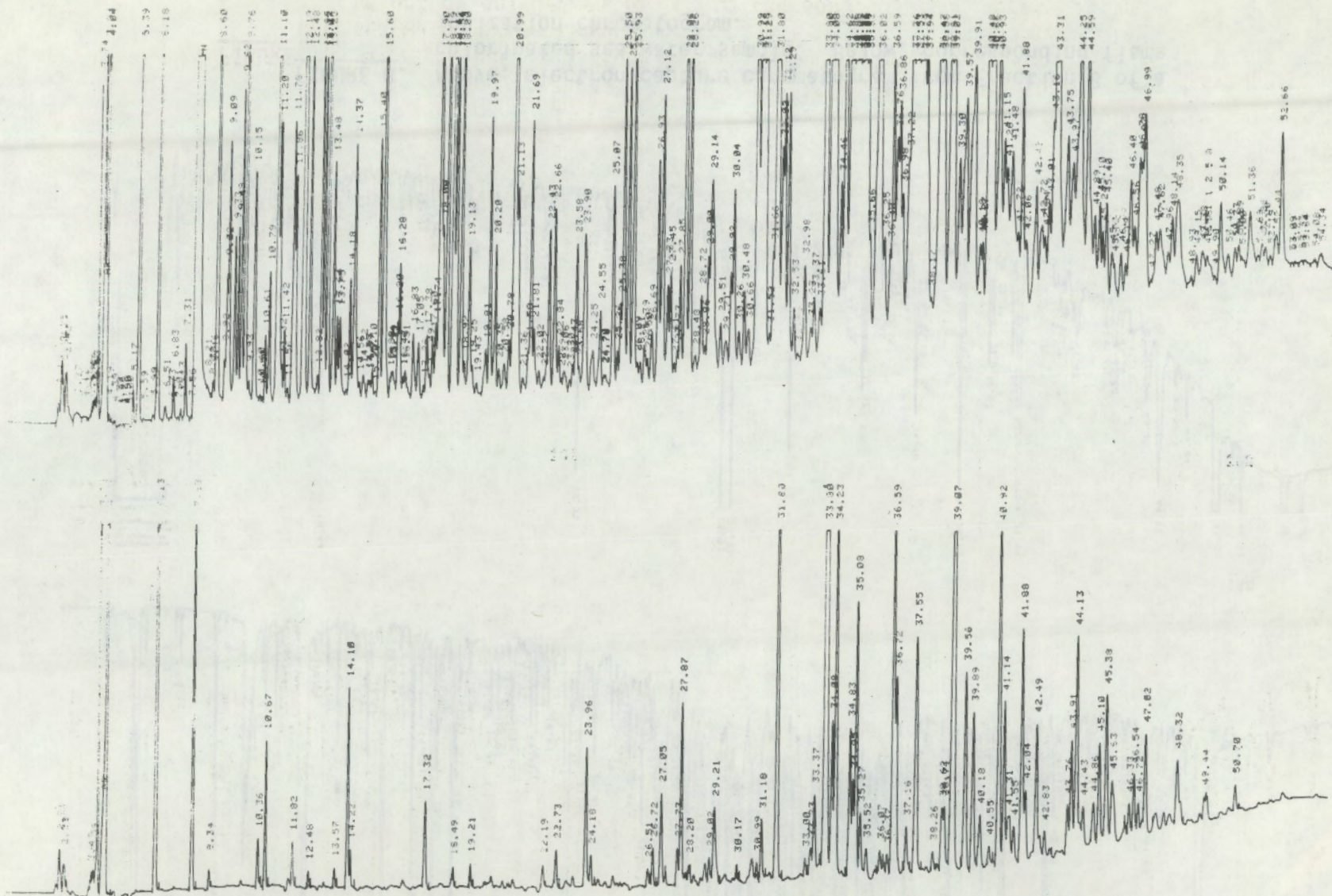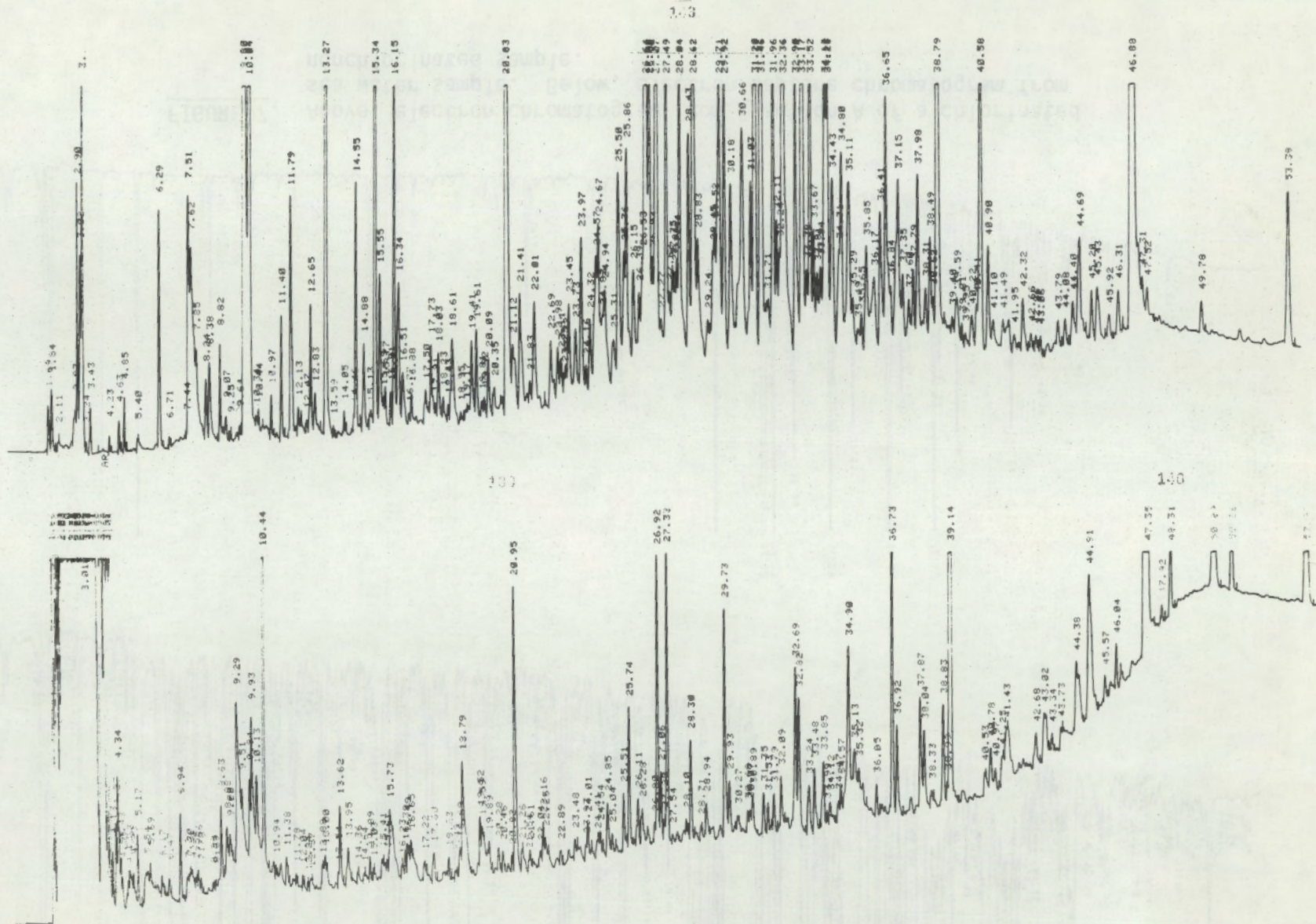
FIGURE 7. Above, electron chromatogram from Fraction A of a chlorinated sea water sample. Below, electron capture chromatogram from nonchlorinated sample.

FIGURE 8. Above, electron capture chromatogram from Fraction B of a
chlorinated sea water sample. Below, corresponding flame
ionization chromatogram.

6.0 USE OF COMBINED DETECTORS FOR IDENTIFICATION OF CAPILLARY CHROMATOGRAPHIC PEAKS

6.1 Comparison of Chromatographic Retention Times Using Electron Capture and Flame Ionization Detectors

The plan of attack for identification of halogenated components by GC/MS was to obtain FID and ECD chromatograms prior to the GC/MS analysis. Correspondence in retention times of peaks in both detection systems would be evidence that the eluting component contained halogen, and was present in sufficient quantity for GC/MS analysis. Identification of halogen compounds prior to GC/MS analysis could considerably reduce the time required on the mass spectrometer. As the initial results revealed almost no correspondence between ECD and FID peaks, a series of 1-chloroalkanes was separated on the column using both detection systems. Initial results revealed that, under the conditions used, the emergence times of these components were as much as 0.30 min longer when the flame detector was used than for the ECD. The lengthening of retention times was a result of back-pressure generated in the detector at high make-up nitrogen flow rates. Raising the carrier gas pressure and lowering the nitrogen make-up pressure and flow resulted in satisfactory correspondence between peaks obtained in FID and ECD modes of operation. Table 2 shows the data obtained from six FID and six ECD chromatograms obtained over a period of four days.

In other experiments conducted over a period of one day, the reproducibility of retention times was somewhat better, averaging ± 0.04 min and ± 0.05 min for FIC and ECD, respectively.

The general lack of correspondence initially obtained between ECD and FID peaks was primarily due to sensitivity differences between the two detectors, rather than retention time differences. The flame ionization detector was not sensitive enough to detect halogenated components giving large peaks from the ECD until samples were concentrated to about 20 μl prior to injection.

6.2 Comparison of Chromatographic Retention Times Between Gas Chromatography and GC/MS

The GC/MS instrument available for this work could not be exclusively dedicated to this program, since it is a general service instrument shared by a wide variety of research programs. Although the use of matched columns insured that comparable resolution and similar chromatograms were obtained for both instruments, retention times obtained on the two instruments varied by as much as three minutes. It thus became necessary to calibrate both GC/MS and EC/FID instruments for retention time in order to retain the identity of separated components.

The extremely small sample sizes mitigated against the use of an internal standard for retention time calibration. The procedure adopted was to chromatograph a series of normal alkanes with carbon numbers ranging from C_8 to C_{28} prior to analysis of the sample in either GC or GC/MS instruments. Using the retention times obtained for the standards on each instrument, the

TABLE 2. Correspondence between retention times obtained using flame ionization and electron capture detectors

Component	FID Retention Time Min + s.d.	ECD Retention Time Min + s.d.	FIO-ECO Difference + s.d.
1-Cl C_8	12.78 + 0.054	12.66 + 0.087	0.12 + 0.10
1-Cl C_9	16.43 + 0.063	16.38 + 0.090	0.05 + 0.11
1-Cl C_{10}	20.08 + 0.058	20.07 + 0.077	0.01 + 0.10
1-Cl C_{16}	39.23 + 0.059	39.26 + 0.074	-0.03 + 0.09
1-Cl C_{18}	44.44 + 0.061	44.48 + 0.075	-0.04 + 0.10
1-Cl C_{20}	49.23 + 0.060	49.25 + 0.078	-0.02 + 0.10

retention time of an environmental sample component peak on one instrument was calculated by linear interpolation. Thus:

$$P_B + C_{XB} + \frac{P_A - C_{XA}}{C_{(X+1)A} - C_{XA}} \left[C_{(X+1)B} - C_{XB} \right] ;$$

where C_X refers to the retention time of n-alkane of carbon number X, C_{X+1} refers to the retention time of the next highest n-alkane, and P refers to the peak of interest occurring at a retention time intermediate to C_X and C_{X+1}. A and B refer to instrument A and instrument B.

6.3 GC/MS - Electron Capture Identification of Components in XAD-2 Extracts

In principle, one would like to use the ECD chromatogram to locate possible halogenated components, find the corresponding peak in the MS chromatogram and identify the electron capturing compound on the basis of this mass spectrum. In practice, the MS is so much less sensitive and less selective than the ECD that many ECD peaks cannot be detected in the GC/MS runs and the mass spectrum at the appropriate retention time may be due exclusively, partially, or not at all to the electron-capturing material. The most successful approach was a somewhat less direct one. The GC/MS data was first scrutinized for indications of halogenated species, the ECD chromatogram inspected for confirmation and for major omissions, then corresponding mass spectra examined for identification if possible.

The presence of Br and Cl is most clearly indicated in the mass spectrum by clusters of mass peaks with relative intensities characteristic of the halogen isotope ratios. But unresolved interferences, complex fragmentations and vagaries of the computer peak detection algorithm at low intensities can either distort real clusters or create misleading patterns. Hence, the ECD activity was required for identification of halogenated species.

Table 3 and Figure 9 give results obtained for the non-polar fraction (Fraction A) derived from several hundred liters of sea water from Sequim Bay. The darkened peaks in the EC/FID chromatograms in Figure 9 correspond to compounds which both chemical ionization GC/MS and electron capture GC indicate are halogen containing. Electron capturing components which did not give mass spectra having isotope characteristics of chlorine or bromine were rejected. Table 3 lists bromine-containing components identified using the dual-detection criteria discussed above, plus some aromatic hydrocarbons found to be present. The observed retention times for the components in both EC and FID detection modes in general are in agreement with the retention times calculated by interpolation from the GC/MS analysis.

The fragmentation of halogenated species under electron impact conditions frequently involves a primary loss of X or HX, so that the isotope cluster information is lost from the mass spectrum. For this reason, we relied heavily on chemical ionization mass spectrometry, which produces much less fragmentation and increases the probability that halogen-containing ions will contribute their characteristic clusters to the spectra. The high ionization efficiency

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TABLE 3. Comparison of retention times obtained for bromo-organic and aromatic hydrocarbon components on GC/MS and FID/ECD instruments, nonpolar fraction from chlorinated sea water extract.

Component (or mass of characteristic Br-containing ion)	GC/MS Retention Time, Min	Calculated Retention Time on FID/ECD, Min	Actual FID Retention Time, Min	Actual ECD Retention Time, Min
CHBr₃	4.80	7.41	7.48	7.52
149	5.12	7.80	7.80	7.83
149	6.25	8.97	8.95	8.98
149	7.62	10.27	10.36	10.39
Trimethylbenzene	8.28	10.90	10.92	--
149	8.85	11.45	11.56	11.64
163	9.92	12.51	12.56	12.64
Naphthalene	15.00	17.56	17.63	--
Bromotrimethylbenzene	18.55	21.13	21.11	21.08
169	19.55	22.14	22.14	22.13
Biphenyl	21.88	24.53	24.47	--
Bromomethylnaphthalene	28.60	31.42	31.55	31.59
Bromomethylnaphthalene	29.08	31.91	31.93	31.95
Bromomethylnaphthalene	29.18	32.02	32.06	32.16
249	30.70	33.61	33.60	33.72
235	31.01	33.93	34.01	34.02
219	31.32	34.26	34.35	34.32
249	31.82	34.78	34.88	34.85
251	31.98	34.95	35.16	35.09
Bromodimethylnaphthalene	32.21	35.19	35.38	35.31
235	32.64	35.65	35.82	35.80
Bromotrimethylnaphthalene	35.66	38.76	38.86	38.70
Pyrene	41.10	44.41	44.58	--

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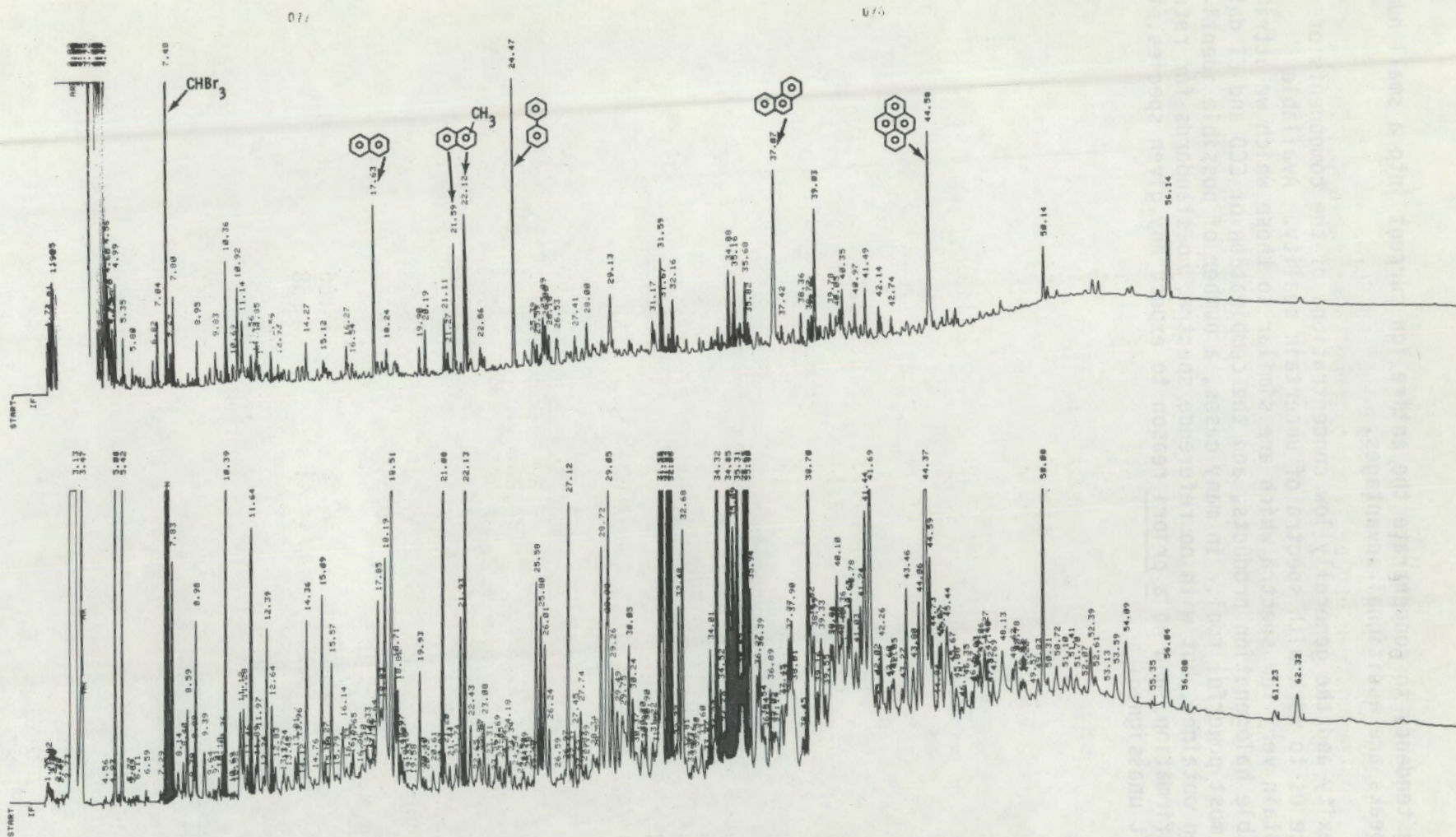
FIGURE 9. Above, flame ionization chromatogram of Fraction A from Sequim Bay sea water. Below, corresponding electron capture chromatogram. Darkened areas indicate those peaks arising from brominated organic compounds.

of CI and its tendency to concentrate the entire ion current into a small number of mass peaks are additional advantages.

Sample complexity and the generally low concentrations of the components of interest force us to work with spectra of uncertain quality. Available libraries contain very few spectra which are similar to those which we attribute to possible halogenation products, and the combination of ECD and CI data has been our most powerful tool. In many cases, a number of possible identities have been obtained, but with no reference spectra, no standards for retention time confirmation, and no a priori reason to expect any given species, the peaks are left unassigned.

7.0 ORIGIN OF COMPONENTS ISOLATED FROM XAD EXTRACTS

Figure 10 and Table 4 serve to summarize the components investigated in Fractions A and B from an XAD-2 extract of 200 liters of chlorinated sea water. Chlorinated compounds corresponding to the brominated ones listed on Table 4 were not found in similar fresh water samples. It is quite likely that many of the compounds we have identified are the products of secondary reactions of excess halogen with sample impurities. Although the XAD-2 resin is soxhlet extracted scrupulously with three different solvents, aromatic hydrocarbons appeared in all samples investigated. These hydrocarbons (naphthalenes, phenanthrenes, pyrene) are present as impurities in the uncleaned resin, but are not found when the cleaned column is extracted and the extract treated as a procedural blank. It is likely that these components are released from the XAD polymer matrix during the sampling of the water when the resin fractures under the fairly high back pressures produced. This belief is reinforced by the observation that the resin contains aromatic hydrocarbons as impurities when received from the supplier. These impurities must be removed by exhaustive soxhlet extraction. The above observations also led us to conclude that the brominated aromatic components in Fraction A are formed by reaction of bromine with the hydrocarbon impurities during sampling. While the concentrations of these experimental artifacts represent only a few nanograms per gram of XAD-2 resin, they appear in concentrations comparable to other halogenated components in Fractions A and B which, although unidentified, may truely arise from the chlorination of sea water. Interestingly, few chlorinated aromatic hydrocarbons are found in fresh water samples even though the corresponding nonchlorinated hydrocarbons were present.

It is apparent from the result obtained that with the exception of the haloforms, the quantity of nonpolar halogenated organics formed during the chlorination of natural waters is exceedingly low in the relatively pristine natural waters thus far investigated. Sample concentration factors are on the order of 10^6, and we still are approaching the limits of detection by GC/MS (on the order of one $\mu g/ml$). This corresponds to individual component concentrations in the parts per trillion range. Although destruction of active chlorine prior to XAO adsorption should eliminate brominated artifacts, continued problems with contaminants can be expected since many substances are ubiquitous in the environment at these levels.
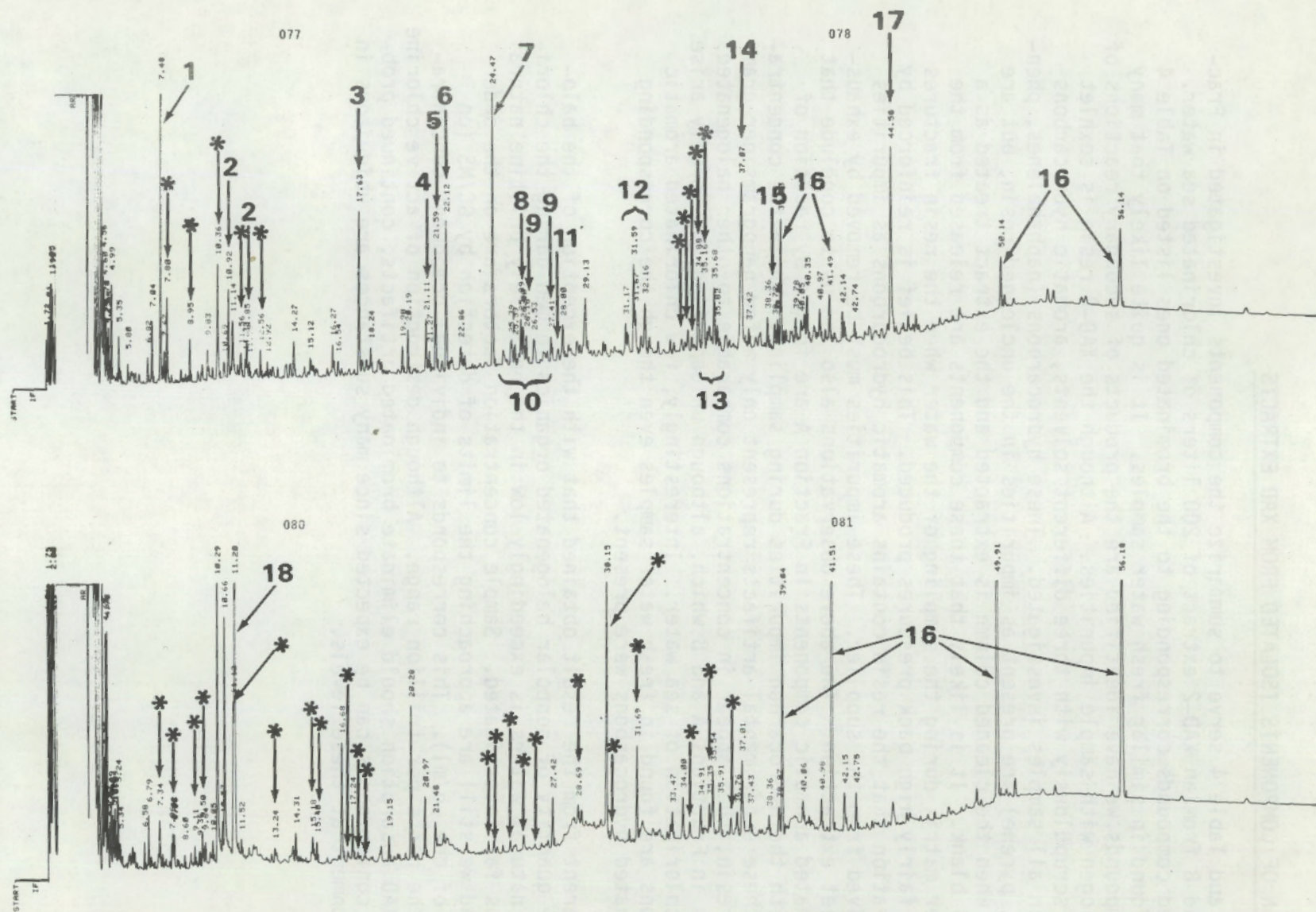
FIGURE 10. Above, gas chromatogram of Fraction A from XAD-2 extract of sea water using flame ionization detector. Below, chromatogram of Fraction B. Chromatographic peaks denoted by an asterisk represent unidentified bromine-containing components (confirmed by GC/MS and electron capture). Peaks denoted by numbers have been identified and are listed by number on Table 4.

TABLE 4. Components identified by GC/MS in Fractions A and B obtained from XAD-2 extract of sea water [a]

1. Bromoform
2. Trimethylbenzene
3. Naphthalene
4. Bromotrimethylbenzene
5. 2-Methylnaphthalene
6. 1-Methylnaphthalene
7. Biphenyl
8. Bromotetralin
9. Bromotetramethylbenzene
10. Dimethylnaphthalenes
11. Bromonaphathalene
12. Bromomethylnaphthalenes
13. Bromobiphenyls and Bromodimethylnaphthalenes
14. Phenanthrene
15. Bromotrimethylnaphthalene
16. Phthalate Esters
17. Pyrene
18. Bromacetal

[a] Hydrocarbons were identified by comparison of spectra and authentic samples; identities of brominated components were assigned solely on the basis of spectral interpretation.

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8.0 CONCLUSIONS AND RECOMMENDATIONS

- It is apparent from the results of this study that when natural waters are chlorinated with sodium hypochlorite at chlorine concentrations between 1 and 2 mg/l that the major halogenated products are halomethane compounds, principally $CHBr_3$ in sea water and chloroform in fresh water.

- While electron capture chromatograms have indicated the presence of other higher molecular weight components, our studies to-date suggest that volatile halogenated components readily adsorbed by XAD-2 resin (and by inference, presumably readily absorbed by animal tissues) are formed in concentrations which approximate already existing levels of nonpolar halogenated components in the environment; e.g., DDT, PCB, etc.

- Our development studies on relatively pure fresh and saline water systems have been of great advantage during the methodological development stages of these studies. The low levels of halogenated organics produced have made us acutely aware of the possible sources of contamination and the levels at which they may be introduced. At the same time, we have used the samples obtained in the development of the necessary microanalytical and sample cleanup techniques required for the next stages of the chemical characterization phase of the program.

- Sampling large volumes of water through XAD-2 resin columns can produce chlorinated artifacts in the samples if the active chlorine is not destroyed prior to sampling. While the addition of a reducing agent to the water to be sampled may alter the chemistry of the sample, it is probably preferable not to add it. Efforts should be made to reduce the back pressure on the column resin to avoid fracturing the resin, as this appears to be a source of the artifacts.

- Chlorination of estuarine systems in the heavily industralized Northeast, or riverine systems such as the Ohio and Mississippi Rivers could result in the generation of relatively high concentrations of halogenated organics presenting considerable hazard to people as well as their environment. Investigation of the potential of the more polluted bodies of water to form halogenated organic compounds is a logical next step in the program plan. Should already existing pollutant molecules in water bodies prove to be major precursors of halogenated organics upon chlorination, then we might expect to find a different list of components at each individual sampling location.

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16. ABSTRACT Chlorination of power plant cooling water is extensively used as a means of controlling biofouling. This practice presents the potential for formation of halogenated organic compounds hazardous to man and his environment. Accordingly, the organic composition resulting from the chlorination of natural waters (northern Olympic Pennisula sea water and the Columbia River in Washington State) has been investigated. Nonpolar lipophilic organic halogens were extracted by passing large volumes of water over columns of XAD-2 macroreticular resins. Examination of ether extracts from the resin columns using capillary gas chromatography revealed the presence of halogenated methanes, as well as other electron-capturing components, that were not found when unchlorinated water was sampled. Examination of the chlorinated water extracts using gas chromatography/mass spectrometry revealed complex mixtures which generally were not separable into individual components, even when high efficiency WCOT capillary columns were used. The samples were separated into fractions of increasing polarity using a water-deactivated silica gel column. Fractions were thus obtained which were more amenable to GC/MS investigation. Haloforms were identified as the major halogenated product from chlorination of the waters studied. Other halogenated products were found at much lower

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