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INTERIM REPORT

BIOCIDE BY-PRODUCTS IN AQUATIC ENVIRONMENTS

QUARTERLY REPORT

FOR THE PERIOD COVERING

APRIL 1, 1978 TO JUNE 30, 1978

by

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B30 96

Contents

SUMMARY

We are continuing to make progress in the analytical chemistry and biological effects phases of our study of chlorination by-products. As noted in earlier quarterly reports, the schedule of work for this year has been revised so that tasks originally scheduled for the fourth quarter of the year will continue into the first quarter of FY 1979. This is generally due to a delay in receipt of funds this year and delays due to the recent instigation of new DOE purchasing regulations for equipment and supplies. Additional chlorination by-products have been detected in the analytical task, and bioassays are continuing in the biological tasks.

In Task I, detailed analytical data have been obtained on the organohalogen contents of chlorinated freshwater (Columbia River) and seawater (Sequim Bay) systems. Samples have been characterized with respect to: 1) type and quantity of haloform produced from chlorination, 2) total organohalogen produced, 3) distribution of organohalogen by molecular weight, and 4) distribution of organohalogen by polarity. In addition, a number of samples have been examined for individual halogenated components. Tissues from trout obtained from the freshwater chlorination exposure system have been screened for chloroform. The pertinent information obtained from these studies is summarized below:

- Our most recent measurements of haloform concentrations generated from 2 ppm chlorine addition to Columbia River water indicate that about 35-40 μg/l (ppb) chloroform is formed, along with lesser amounts of bromodichloromethane. Similar conditions generate about 17 μg/l bromoform from Sequim Bay sea water, with lesser amounts of dibromochloromethane.
- Haloforms comprise at least 90% of the organically bound halogen formed from the fresh and seawater samples studied when chlorination is at the 2 ppm level. Higher chlorine levels may produce a higher proportion of nonhaloform organohalogen material.
- Almost all of the organically bound halogen trapped on XAD resins from the chlorinated waters studied is found in fractions having

less than 800 molecular weight. Fractions greater than 800 molecular weight contain very small or undetectable quantities of organic halogen.

- The nonhaloform organohalogen material produced appears to consist mainly of relatively polar constituents; i.e., more polar than PCB's, DDT, chloronaphthalenes, etc. There appears to be more nonpolar halogenated constituents generated from seawater chlorination than freshwater chlorination. Dichlorobenzene has been identified in a freshwater sample.
- In chlorinated Columbia River water, organochlorine is present in both phenol and acid fractions. Two dichlorophenols, a trichlorophenol and a bromochlorophenol have been identified as products of freshwater chlorination. Organohalogen is very low or not detected in seawater phenol fractions, but is present in the acid fractions.
- Measurement of chloroform levels in freshwater exposure apparatus using headspace technique has shown that chloroform levels range from 1 µg/l at the most concentrated level to 0.5 µg/l at the least concentrated level. Chloroform concentrations in the unchlorinated controls range from non-detectable to 0.5 µg/l.
- Preliminary studies indicate that there is no detectable difference in the levels of chloroform in tissues of trout, Salmo gairdneri, exposed to the highest test levels of chlorinated organics and the tissues of the controls. This observation, which needs further verification, is consistent with the small differences observed between chloroform levels in the exposure tanks.

We believe that our present procedures are capable of generating information that will be particularly applicable to the planned comparative studies of selected water resources across the United States. These comparative studies are to be initiated as soon as all of the required equipment is received and assembled. In spite of intense efforts to expedite, we have found procurement of the required components to be much slower than anticipated; however, we hope to make our first trip to the Ohio River this next quarter. It has become clear from our studies that in order

for the site-specific sampling task to be successful, a major portion of the time and effort allocated to chemistry studies must be invested in this task during the coming fiscal year. This may result in slower than planned progress in other areas.

In addition to the sampling activities, we need to extend our methods to include separation and identification of acidic components, and to find a more efficient means of isolating and chromatographing the polar, < 800 mole weight constituents which appear to comprise the majority of the nonhaloform organohalogen material.

In Task IIa, Freshwater Biology, the chronic chlorine exposures with rainbow trout, (Salmo gairdneri) are continuing and will be terminated September 27, 1978. Fish subsamples are being taken monthly for length and weight measurements and chemical analysis. Statistical analysis of the data does not reveal a trend of effect with concentration.

The testing apparatus for the acute chloroform, toxicity tests is constructed. Tests of chloroform metering systems are continuing to determine a satisfactory metering system. The chloroform toxcity tests will begin in the fourth quarter with bluegill (Lepomis macrochirus), rainbow trout (Salmo gairdneri), large mouth bass (Micropterus salmoides), and channel catfish (Ictalurus punctatus).

In Task IIb, Marine Biology, the chronic exposures of littleneck clams (Protothaca staminea) is continuing and will be terminated at the end of August, 1978. Clam samples are harvested monthly for chemical and histological examination.

The bromoform acute toxicity and bioaccumulation exposures are underway at both Daytona Beach and Sequim. Some problems have been encountered with introducing bromoform into the aquaria. This resulted in introducing bromoform vapor directly into the aquaria to achieve the concentrations necessary. From preliminary examination of the data it appears that the 96 hr LC_{50} is well above 20 mg/l bromoform for littleneck clams. Preliminary results of two acute toxicity tests with shrimp (Penaeus aztecus) show 96 hr LC_{50} of about 31 and 36 mg/l bromoform. Bromoform also produced narcosis in shrimp at concentrations above 10 mg/l.

TASK Ia, b - ANALYTICAL CHEMISTRY

Roger M. Bean, Roger E. Schirmer, Dale C. Mann

Sampling with XAD-2 columns has been conducted this quarter according to the procedure outlined in the last quarterly report. Briefly, we are destroying the chlorine residual with sodium sulfite and adjusting the pH to 5.5 prior to pumping the water at about 160 ml/min over 82 ml of XAD-2 resin. The procedure appears to work well, and is adaptable to field sampling conditions. We had hoped to be sampling in the field by this time. Delivery of fabricated components of the sampling apparatus is expected any day; we should be sampling in July or August at the latest. Several samples of chlorinated Columbia River and Sequim Bay water have been taken using our modified sampling procedure. These samples, which are described in Table 1, have been investigated intensively in our laboratories, and the results are discussed in the following sections.

EXAMINATION OF XAD-2 EXTRACTS FOR HALOFORMS AND TOTAL ORGANIC HALOGEN

At least two laboratories (Glaze, et al., 1977; Wegman and Greve, 1977) have been investigating the total organic halogen (TOX) content of waters using the microcoulometric technique. Glaze (1977) has expanded the concept to include analysis of the haloform content of waters in addition to the TOX. We hav adapted these procedures for our studies. The haloform content of our XAD-2 extracts (in ether) is determined by gas chromatography and the halogen represented by that haloform is compared with the total organic halogen. The results of these measurements are given on Table 1. The data were obtained from 3 ml sub-samples of the 200 ml ether extracts from the XAD-2 columns. The samples were at first normally stored at 4°C in teflon-capped vials to prevent evaporation and chemical change. Unfortunately we discovered during the course of the measurements that the refrigerator was a sig..ificant source of freon contamination of the samples. After this observation was made, subsequent ether sub-samples were stored at room temperature. Samples that were contaminated with freon are

TABLE 1. Comparison of haloform content in XAD-2 extracts with total organic halogen.

Sample	Description	μ mole X as $CHCl_3$	μ mole X as $CHCl_2Br$	μ mole X as $CHClBr_2$	μ mole X as $CHBr_3$	Total μ mole X as Haloform	Total μ mole X Microcoulometer	In Chlorinated Samples, % Total X as Haloform
Cl #1	Fresh Water Lab Apparatus 405.5 ℓ/2 ppm TRC[a]	18.58	2.12	—	—	20.70	28.98[b] ± 0.28	71
Cl #2	Cl #1 Control 403.9 ℓ	0.55	—	—	—	0.55	0.0[b]	—
Cl #3	Columbia River 150 ℓ/7 ppm TRC	15.52	1.44	—	—	16.96	33.41[b] ± 1.30	51
Cl #5	Columbia River Control; 310.7 ℓ	0.74	—	—	—	0.74	0.78[b] ± 0.10	—
Cl #7	Columbia River No Additives; 489.7 ℓ	0.72	—	—	—	0.72	1.25[b] ± 0.64	—
Cl #8	FW Lab - Upper Column 387.6 ℓ/0.99 ppm Cl	31.32	2.12	—	—	33.44	56.39[b] ± 2.59	59
Cl #9	FW Lab - Lower Column	27.00	1.73	—	—	28.73	41.95[b] ± 0.83	68
Cl #10	Sea Water Lab Apparatus 268.7 ℓ/1.7 ppm Cl	0.64	—	1.85	86.85	89.34	104.7 ± 6.43	85
Cl #11	Control for Cl #10; 310.5 ℓ	1.51	—	—	13.87	14.38	22.79 ± 0.88	—
Cl #12	Sea Water Lab Apparatus 335.4 ℓ/2.3 ppm	0.48	—	1.50	59.53	61.51	73.83 ± 5.19	83
Cl #13	Control for Cl #12 362.2 ℓ	—	—	—	0.43	0.43	3.08 ± 1.44	—
Cl #17	XAD-2 Extract (Blank)	—	—	—	—	—	2.80[b] ± 0.81	—
Cl #18	FW Lab Apparatus 389.6 ℓ/0.69	39.12	6.56	—	—	45.68	50.20 ± 2.97	91

[a] TRC = total residual chlorine

[b] These samples were stored in refrigerator at 4°C and were exposed to Freon (see text). Level of Freon contamination can be estimated from Cl #17.

noted on Table 1. The extent of contribution of the contaminant to the TOX measurement can be estimated by referring to the TOX results for the samples designated Cl #17, #7, #5, and #2. The TOX for these unchlorinated samples in each case corresponds to less than 3 μmole total halogen. Thus, we believe that the contribution of freon to the TOX in the refrigerated chlorinated samples is also less than 3 μmole.

It is clear from the data on Table 1 that the bulk of the organic halogen collected on the XAD resins and extracted into ether is present as haloform. In the case of the unrefrigerated samples, Cl #10, #12, and #18, there was no complicating contamination. The chlorinated seawater samples (#10 and #12) had 83 and 85 percent of the halogen as haloform, principally bromoform. The one uncontaminated freshwater sample (#18) had 91 percent of the halogen as haloform in the form of chloroform and bromodichloromethane. The appearance of the bromo compound in the freshwater samples is interesting but not surprising, since, according to Bowen (1966) bromide ion in freshwater systems is about 0.2 mg/l, one twentieth the molarity of the added chlorine.

The quantities of chloroform from the freshwater samples reported on Table 1 represent only about ten percent of the chloroform expected, based on direct chloroform measurements of the chlorinated water being sampled. If one makes the reasonable assumption that the nonhaloform organochlorine material is adsorbed on the resin more strongly than the chloroform, then it follows that practically all the organohalogen formed from the chlorination of our freshwater sample is in the form of haloform, probably well in excess of 90 percent. In the case of the seawater samples, we can say with more certainty that about 90 percent of the organohalogen is in the form of haloform, since bromoform, the principle product of seawater chlorination, is adsorbed on the XAD-2 resin with an efficiency of 80 percent.

Of special interest is the result obtained from samples Cl #3. In this sample the chlorine addition was inadvertently raised to about 7 ppm. The results indicate that the larger chlorine dose may have resulted in the formation of more nonhaloform organohalogen than with the other samples where the chlorine levels were kept to about 1-2 ppm. The observation is

clouded by freon contamination of samples and needs to be repeated. However, if this observation is true, it could have an effect on recommended chlorination practices.

HALOFORM ANALYSIS

The haloforms comprise a large percentage of the organohalogen products from chlorination of both freshwater and saltwater. We have found that a 6ft x 2mm glass column packed with Chromosorb 101 gives excellent separation of the haloforms of interest. The ether extracts are routinely analyzed on this chromatographic column at 185°C using 1,3-dibromopropane as an internal standard. Detection is by electron capture. All the haloform data presented in Table 1 were obtained by this method.

For direct analysis of chloroform in water we adapted a headspace method, since chloroform is poorly adsorbed on XAD-2 resins. The method is patterned after that reported by Bush, et al. (1977). Five grams NaCl and approximately 200 mg Na_2SO_3 are added to a 100 ml bottle capable of being sealed with a septum. A 15 ml water sample is added, the bottle is sealed and partly immersed in a water bath at 99°C for one hour. One milliliter of headspace is sampled using a sealable syringe and injected onto the Chromosorb 101 column. Detection limit is about 0.1 µg/l. Quantitation is established by comparing the area obtained to standards prepared according to the following procedure: 15 ml chloroform-free water plus salt and Na_2SO_3 are sealed in a sample bottle and 3 µl of methanol containing a known amount of chloroform is added by injection through the septum. This standard sample is then analyzed exactly as the other samples.

Biological experiment involving long-term exposure of salmonids to low-levels of chlorinated river water have also been investigated to determine concentrations of chloroform. Chloroform analyses obtained from the experiments are given in Table 2. The concentrate sample is identical to that being sampled by the XAD-2 methods discussed above. The "100%" sample is the highest level to which the trout are exposed. On 5/22 and 6/16, sampling were generally limited to one sample per exposure concentration. On 5/26, however, 5 concentrations were sampled in

TABLE 2. Chloroform Headspace Analysis of the Freshwater Low-Level Chlorination Experiment with Salmonids.

(Values reported are µg/l $CHCl_3$)

	5/22/78	5/26/78[b]	6/16/78	Average
CONCENTRATE	31.3	40.16 ± 1.39	39.09 ± 1.15	36.85 ± 4.83
"100%"	1.05 ± 0.21[a]	1.03 ± 0.11	0.93	1.00 ± 0.06
"75%"	1.00	--	0.83	0.92 ± 0.12
"50%"	0.54	0.70 ± 0.00	0.69	0.64 ± 0.08
"25%"	0.41	--	0.62	0.52 ± 0.15
"12.5%"	0.49	0.47 ± 0.05	0.6[c]	0.52 ± 0.07
"0%"	0.46	--	nd[d]	0.23
RIVER WATER	0.46	0.39 ± 0.04	--	0.43 ± 0.05

[a] Average of duplicate samples.

[b] Analyses on 5/26 were obtained from triplicate samples.

[c] Estimate, interference with peak integration.

[d] Not detected.

triplicate to determine the method reproducibility. The reproducibility of the method is quite satisfactory, and we plan to incorporate this analytical technique into our field sampling program at all freshwater locations. The chloroform data indicate that in the biological test systems, the amount of chloroform is 1 µg/l or less, and the spread between the highest and lowest concentrations of chloroform is only a factor of two. For comparison, we found the concentration of chloroform in the laboratory tap water to be 11 µg/l.

The analysis of bromoform in chlorinated seawater can be performed either by adsorption on XAD resins followed by ether extraction and gas chromatography of the ether extract, or by direct solvent extraction of the

water with hexane followed by gas chromatography. In both cases, 1,3-dibromopropane is used as internal standard. Table 3 shows the values of bromoform found in the seawater chlorination exposure apparatus (concentrate only), and compares them with the chloroform concentrations from the freshwater system. In this study it was of interest to compare the haloform concentration in the untreated chlorinated water with that found in the XAD-2 sampling apparatus after addition of acid and sodium sulfite. The results in Table 3 indicate that the manipulation of the chlorinated water by the sampling apparatus resulted in haloform losses of only about 10%. The third column in Table 3 illustrates the futility of using XAD-2 to measure chloroform concentrations in freshwater samples. Only about 10% of the chloroform is adsorbed during the large volume sampling. In contrast, XAD-2 appears to efficiently adsorb bromoform even when large volumes are sampled.

Measurement of bromoform values in the seawater exposure tanks is in progress. Solvent extraction followed by gas chromatography using electron capture detection is the method being used. Results will be presented next quarter.

TABLE 3. Comparison of Haloform Analyses

A. Freshwater Systems ($CHCl_3$)

Headspace Directly from Chlorinated Water (µg/l)	Headspace From Sampling Apparatus (µg/l)	XAD-2 column (388 liters over 82 ml Resin) (µg/l)
38.3, 39.9; $\bar{x}$=39.1	35.1, 35.3; $\bar{x}$=35.2	3.2

B. Seawater System ($CHBr_3$)

5 l over XAD-2 Directly from Chlorinated Water (µg/l)	5 l over XAD-2 from Sampling Apparatus (µg/l)	XAD-2 Column (335 liters over 82 ml Resin) (µg/l)
16.8, 17.2; $\bar{x}$=17.0	15.4	15.0

CHARACTERIZATION OF NONHALOFORM ORGANOHALOGEN COMPONENTS

Our approach to the characterization of the non-haloform halogenated organic material in our samples consists of three elements:

1) Classification of the material according to molecular weight and chemical type, using solvent extraction and chromatographic techniques.
2) Determination of the halogen content of these component classes using microcoulometric titration.
3) Investigation of suitable fractions for identity of individual components using gas chromatography/mass spectrometry (GC/MS).

We found that the identification of individual components by GC/MS is difficult because of the complexity of the samples, the very low concentrations of the individual components present, difficulty of obtaining interpretable mass spectra from the isolated components, and the general unavailability of reference standards. Our application of microcoulometric chlorine analysis to freshwater and seawater samples has shown that useful information can be obtained about the nonhaloform organohalogen material without identification of individual components. This type of information will be particularly useful during our studies comparing the chlorination behavior of a number of water bodies across the United States.

The separation scheme shown in Figure 1 summarizes the present state of our progress in the classification of chlorinated components. Briefly, the sample adsorbed on the XAD-2 resin is first extracted with Na_2CO_3 to remove acidic material, and is then extracted with ether to remove the rest of the organochlorine material. A final extraction with methanol is also performed. Phenols are removed from the rest of the acids by derivatization. The ether extract is segregated into three molecular weight fractions (<800; 800-1500; >15,000) using gel permeation chromatography (GPC). The lowest molecular weight ether fraction is further separated by silica gel chromatography into three fractions of increasing polarity. The results of analysis of the separated fractions for halogen content are

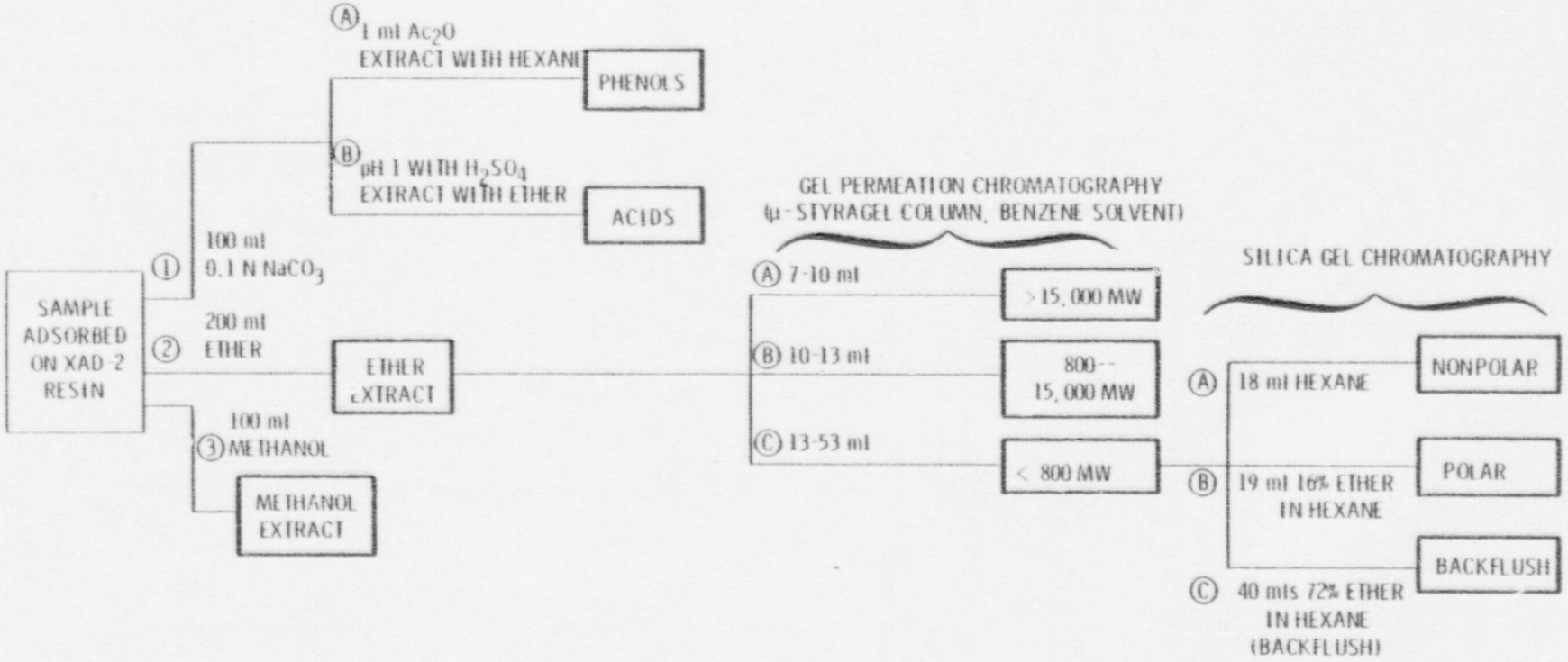

Fig. 1. Extraction and separation scheme for chlorinated water sampled by XAD-2 adsorption technique.

presented in Table 4. In order to facilitate comparison of seawater extracts with freshwater, the values on Table 4 are expressed as nanomoles halogen per liter of water extracted.

Before review of the data on Table 4, a few comments must be made. Chloroform is quite volatile (bp 61.2°C) and most of it is lost during the concentration step prior to molecular weight separation using GPC. This explains why the total amount of chlorine in the GPC fractions from the freshwater samples is very low with respect to the original ether extract. Bromoform is less volatile (bp 149.5°C) and thus may not be lost as readily during the GPC separation procedure. Generally, methanol extracts are relatively high and fairly uniform in halogen content, regardless of whether they originate from a chlorinated water sample or a control. It is likely, therefore, that the values for halogen obtained from the methanol fractions are from inorganic halogen rather than organic. Both the phenol and acid fraction procedural blanks tend to be relatively high, indicating that interfering impurities are present in our reagent. The source of these impurities is presently under investigation. The data for phenols and acids on Table 4 are reported as the value obtained for the samples minus the value obtained for the procedural blank, and are probably not very accurate.

Another observation is that the silica gel separation procedure does not yield acceptable recoveries of organohalogen material. We have evidence that this is because of irreversible adsorption of polar halogenated material. Recoveries of three standard compounds from both the GPC and the silica gel separations were studied, and the results are presented in Table 5. For these nonpolar or slightly polar compounds the recoveries were quite good. The major source of losses occurred during the final evaporation back to the original volume. Table 6 shows recoveries obtained from freshwater and seawater samples from both GPC and silica gel separation. Total halogen recovered from the GPC separation is good (74-97%), but recovery from the silica gel separation is low. It is possible therefore that the majority of the nonhaloform organohalogen material extracted from the XAD-2 columns consists of polar, relatively low molecular weight material. These results dictate that we modify our separation procedures to concentrate on the more polar materials.

TABLE 4 Organic halogen analysis of fractions obtained from freshwater and seawater samples (fractions obtained according to separation scheme on Figure 1. Halogen values are expressed as nonomoles/l).

Sample	Description	Date	Ether Extract	G.P.C. Fractions			Silica Gel Fractions			Phenols	Acids	Methanol Extract
				<800	800-15,000	>15,000	Nonpolar	Polar	Backflush			
Fresh Water Samples												
Cl # 18	Fresh Water Apparatus 0.69 TRC;[a] 390 ℓ	6/15	128.8	13.99	nd[b]	nd	4.66	1.13	2.47	0.53	3.59	39.3
Cl #8 and 9	Fresh Water Apparatus 0.99 TRC; 388 ℓ	5/10										
	Cl #8 Top Column		145.5	9.40	0.32	0.16	0.33	0.40	5.74	0.97	5.7	39.5
	Cl #9 Bottom Column		108.1	7.43	0.24	0.37	nd	0.14	1.30	0.20	3.86	29.7
Cl #5	Fresh Water Apparatus No Chlorine; 311 ℓ	4/18	2.5	0.77	nd	nd	0.06	0.20	0.15	0.61	nd	3.5
Cl #7	Columbia River Water No Additions; 490 ℓ	4/24	2.6	0.69	0.07	nd	0.06	0.05	0.16	0.62	0.11	51.0
Sea Water Samples												
Cl #10	Sea Water Apparatus[c] 1.7 TRC; 269 ℓ	5/15	389.4	107.01	0.67	0.10	18.61	8.99	11.85	nd	0.06	209.7
Cl #11	Sea Water Apparatus[c] No Chlorine; 311 ℓ	5/15	73.3	5.09	0.21	0.82	0.95	0.61	0.41	nd	0.59	80.2
Cl #12	Sea Water Apparatus 2.3 TRC; 335 ℓ	5/17	220.4	6.12	nd	nd	0.75	0.53	0.59	0.20	0.96	82.4
Cl #13	Sea Water Apparatus No Chlorine; 362 ℓ	5/17	8.5	0.57	nd	nd	0.08	nd	nd	nd	0.33	68.3
System Blank												
Cl #17	(Based on 350 ℓ sample)	6/1	7.99[d]	0.21	nd	nd	nd	nd	nd	nd	1.42	nd

[a] TRC = parts per million total residual chlorine
[b] nd = not detected
[c] Bromoform saturation experiment in progress; see text.
[d] Determined by G.C. to be exclusively due to Freon contamination

TABLE 5. Separation recovery studies.

A. Recovery of Standards from GPC Separation (values are percent of starting component)

Standard	Amount Injected	>15,000 MW	15,000-800 MW	<800 MW	Total	After Evaporation to 1.0 ml
1,4-dichlorobenzene	25.90 µg	0.0	0.0	93.9	93.9	82.2
P,P-DDT	22.06 µg	0.0	0.0	120.2	120.2	100.0
2-bromonapthalene	15.82 µg	0.0	0.0	92.4	92.4	53.9

B. Recovery of Standards from Silica Gel

Standard	Amount Injected	Nonpolar	Polar	Backflush	Total	After Evaporation to 1.0 ml
1,4-dichlorobenzene	25.90 µg	82.9	0.0	0.0	82.9	70.5
P,P-DDT	22.06 µg	96.9	11.8	0.0	108.7	74.1
2-bromonapthalene	15.82 µg	43.1	49.7	0.0	92.7	68.6

The data on Table 4 are complicated by two additional problems. Of the freshwater samples reported, only C1 #18 was not exposed to freon. Thus values for total halogen in other samples may be higher. Seawater samples were not refrigerated; however, during the sampling of one set of the seawater samples (C1 #10 and C1 #11) an experiment was being conducted in the same room which involved the saturation of water with bromoform. Apparently, this resulted in the contamination of our sampling system with bromoform, since the control had abnormally high bromoform concentrations. These two sets of samples were taken 2 days apart and were meant to be duplicates; however, the results obtained from these sets are not very comparable.

TABLE 6. Recoveries of organic chlorine from GPC and silica gel separations (values are percentages of total organic halogen determined prior to separation: GPC and silica gel experiments each assume 100% prior to separation experiment).

		GPC				Silica Gel			
		Percent Recoveries				Percent Recoveries			
Sample	Description	>15,000 MW	15,000-800 MW	<800 MW	Total	Nonpolar	Polar	Backflush	Total
Fresh Water									
Cl #18	Columbia River Water/ Fresh Water Apparatus 0.69 TRC; 390 ℓ	0.0	0.0	96.5	96.5	29.6	7.2	15.6	52.4
Cl #8 and 9	Columbia River Water Fresh Water Apparatus Tandem Columns, 0.99 TRC; 390 ℓ								
	Cl #8 Top Column	1.2	2.5	70.9	74.6	3.1	3.8	55.0	61.9
	Cl #9 Bottom Column	4.4	2.8	87.2	94.4	8.2	7.1	22.0	37.3
Sea Water									
Cl #10	Sequim Bay Sea Water 1.7 TRC; 269 ℓ	0.1	0.5	88.7	89.3	27.4	13.2	17.4	58.0
Cl #11	Sequim Bay Sea Water No Chlorine; 311 ℓ	9.9	2.5	61.3	73.7	21.7	14.1	9.4	45.2
Cl #12	Sequim Bay Sea Water 2.3 TRC; 335 ℓ	0.0	0.0	79.1	79.1	10.6	7.5	8.3	26.4
Cl #13	Sequim Bay Sea Water No Chlorine; 362 ℓ	0.0	0.0	74.8	74.8	15.6	0.0	0.0	15.6

From the data presented on Table 4 we can make the following observations about the Columbia River and Sequim Bay samples we investigated: first, the majority of the organohalogen extracted onto XAD-2 resins from the chlorinated waters is in the form of haloform; second, very little of the organohalogen extracted is in excess of 800 molecular weight; and third, most of the nonhaloform organohalogen appears to be associated with the more polar molecules.

XAD-2 resin has a relatively small pore size, and thus is not particularly suitable for extracting material of high molecular weight, such as the humic and fulvic acids. Use of larger pore size resins to collect the high molecular weight materials would be useful for determining whether incorporation of halogen into macromolecules is a significant reaction in water chlorination. This is an important environmental question since, for example, chlorinated humic and fulvic acids could be a sink for halogen, and possibly a long-term source of halogenic molecules to the environment.

GC/MS ANALYSIS OF FRESHWATER FRACTIONS

Capillary gas chromatograms are routinely obtained on the three silica gel fractions ("nonpolar", "polar", "backflush") and on the phenol fractions obtained from each XAD-2 sample. These chromatograms are used to select those fractions which have the greatest chance of giving good GC/MS results, and to supplement the GC/MS information obtained. We examined fractions obtained from the long-term freshwater chlorination exposure apparatus, mostly concentrating on the sample designated Cl #9 (see Table 1 and 4).

Our strategy for identifying the chromatographic peaks in the GC/MS which contain halogen is to run the sample in the chemical ionization (CI) mode (mild ionization mechanism), and to search the chromatogram for peaks which have spectral patterns characteristic of halogen isotope ratios. The electron capture gas chromatogram of the sample under investigation is used as a guide for determining where to look in the GC/MS chromatogram for halogen isotope ratios. When a chromatographic peak having a chlorine or bromine isotope ratio is identified, a molecular weight is assigned, if the

spectrum permits. The electron impact (EI) GC/MS mode is then applied to the sample and the spectrum of the peak of interest examined for confirmation of chlorine and for structural identification if possible.

The task is a very tedious one, since the great majority of the peaks in the chromatogram do not have isotope ratios corresponding to halogen. As our earlier studies have shown, this is particularly the case for the freshwater samples studied. Peaks which do not contain evidence of halogen in the GC/MS are not investigated further in order to reduce the time invested per sample; however with the exceptic. of the phenol fraction of Cl #9, the results obtained for the investment of technical manpower and instrument time have been disappointing. For the nonpolar fraction, only three peaks in the chromatogram were found to have isotope ratios corresponding to halogen compounds, and only one was identified (dichlorobenzene). Only one peak was found in the nonpolar fractions, and the spectra indicated no parent molecular ion. Two backflush samples only yielded three chlorine-containing peaks (unidentifiable) in the CI chromatograms, which could not be found when the corresponding EI runs were made. This last result was particularly disappointing, since our present evidence indicates that the majority of the nonhaloform organohalogen components should be in the backflush fraction (see Table 4).

Examination of the phenol fraction of Cl #9 produced positive results, however. The procedure for isolating the phenols involves forming the acetylated derivatives, which allows them to be separated from other acidic components. The derivatization also makes GC/MS identification definitive, since a major fragmentation pathway of these molecules is to eliminate ketone. Thus, the appearance of a molecular ion plus an M-42 fragment is highly indicative of an acetyl-derivatized phenol. Figure 2 shows three single ion chromatograms corresponding to the molecular weights of four phenols identified in the phenol fraction of Cl #9. Note how clearly the peaks due to the phenolic components are enhanced with respect to the peaks in the total ionization chromatogram. We used the reconstructed single ion chromatogram capability of the GC/MS to scan the Cl #9 phenol fraction for peaks having the molecular weights of a number of phenols with different

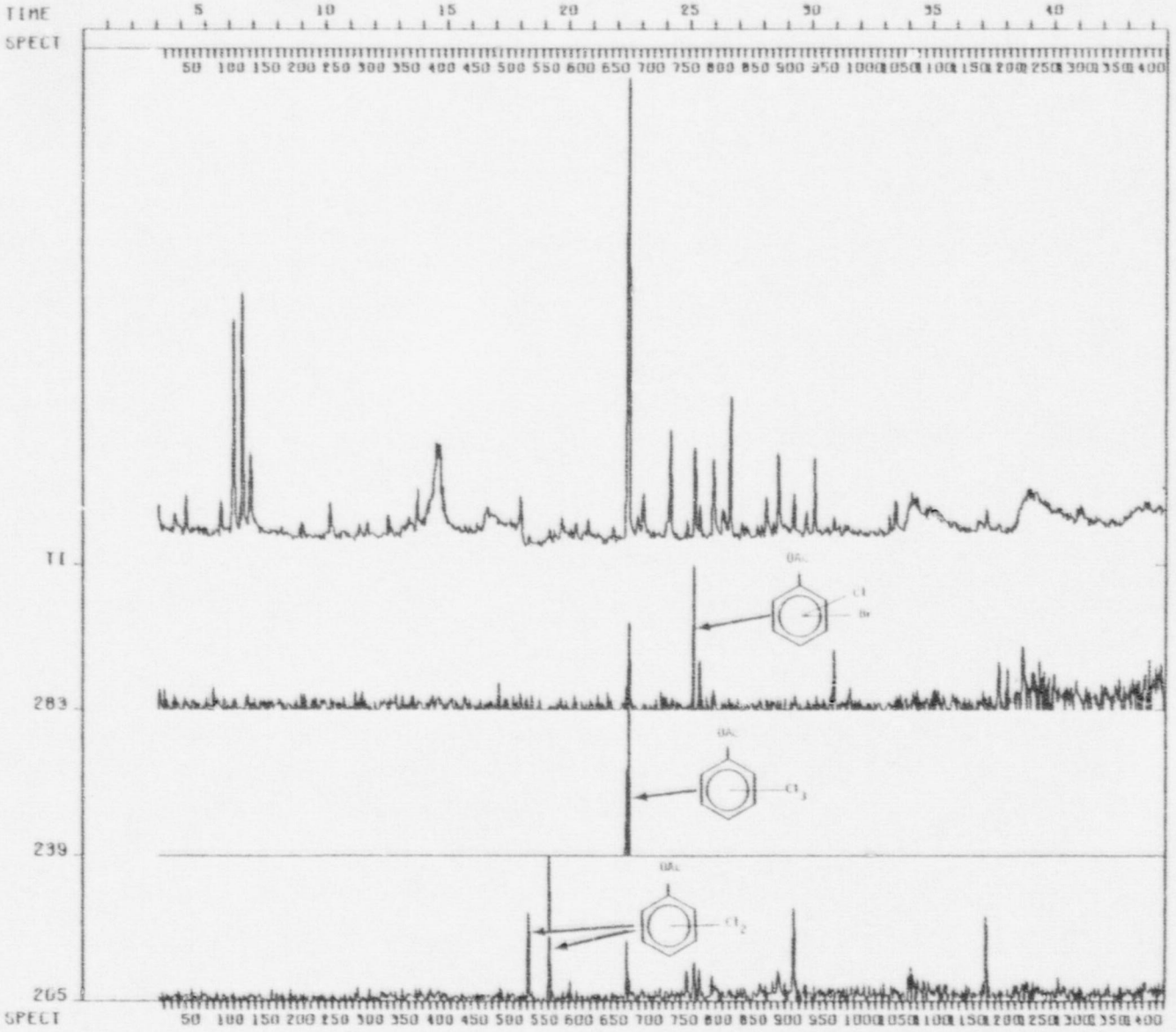

Fig. 2. Single ion chromatograms for masses 205, 239, and 283 compared to total ionization chromatogram, Chlorine #9, phenol fraction.

combinations of chlorine, bromine, and methyl groups; however, only two dichlorophenols (we presume ortho and para), trichlorophenol (probably 1, 3, 5-TCP), and a bromophenol were found. The mass spectra obtained from the electron impact scan for these compounds are given in Figs. 3, 4, and 5. Because of an unusually large mass offset in the instrument for this run, some of the mass assignments on the figures appear 1 mass lower than they should be; however, assignment of major peaks in the spectra are correct.

Although the investigation of fractions by GC/MS has been a difficult part of the present program, continued application of this methodology to our comparative sampling program is essential. As the results of the microcoulometric analyses of fractions have shown, a large part of our difficulty in finding individual components by GC/MS in the Columbia River and Sequim Bay samples is that the concentrations of these components are very low, in the ng/l range. This will probably not be the case when other less pristine waters are sampled. We must continue the GC/MS application to samples taken across the country in order to determine if there is a potential for a few major hazardous chlorinated components to be formed from the chlorination process.

ANALYSIS OF TISSUE SAMPLES FOR HALOGENATED ORGANICS

Modified Tissue Extraction Procedure

Control of the effective polarity of the extracting solvent during tissue extractions is difficult because of the high and variable quantities of water present in the sample. An attempt was made to control this problem by grinding the tissue with a larger amount of sodium sulfate than was previously used (4:1 instead of 2:1, sodium sulfate to tissue by weight). However, a significant amount of the salt dissolves when polar solvents are used for the extraction, and the salts tend to reprecipitate and plug the gel permeation column used in the subsequent fractionation step. Both the moisture and salt problems can be avoided by freeze drying the tissue prior to extraction, and this procedure has been adopted for the tissue assays. Freeze dried tissues are then soxhlet extracted using n-pentane, diethyl ether, acetone, and methanol in that order. The extractions are carried out for approximately 24 cycles with each solvent.

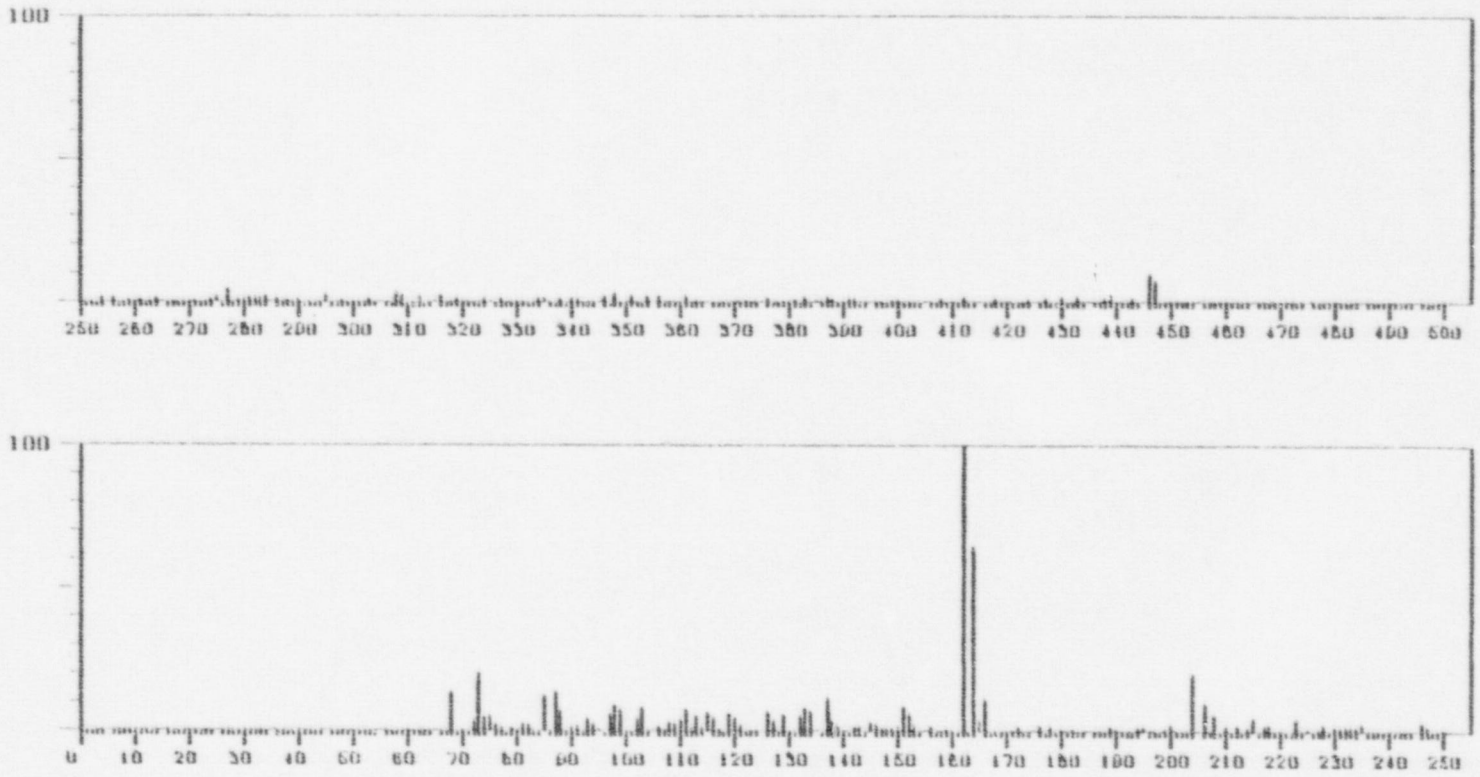

Fig. 3. Mass spectrum of peak identified as acetyl derivative of dichlorophenol.

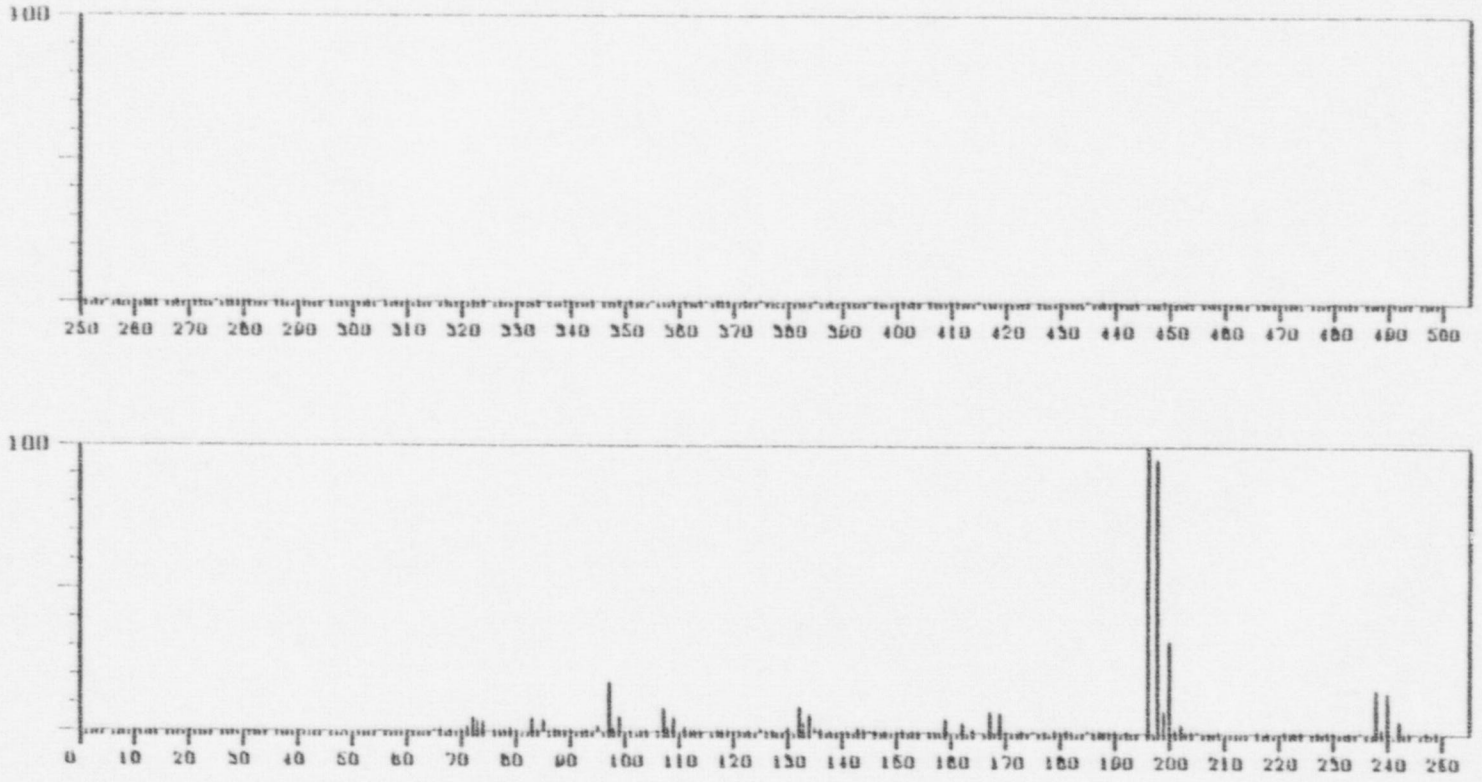

Fig. 4. Mass spectrum of peak identified as acetyl derivative of trichlorophenol.

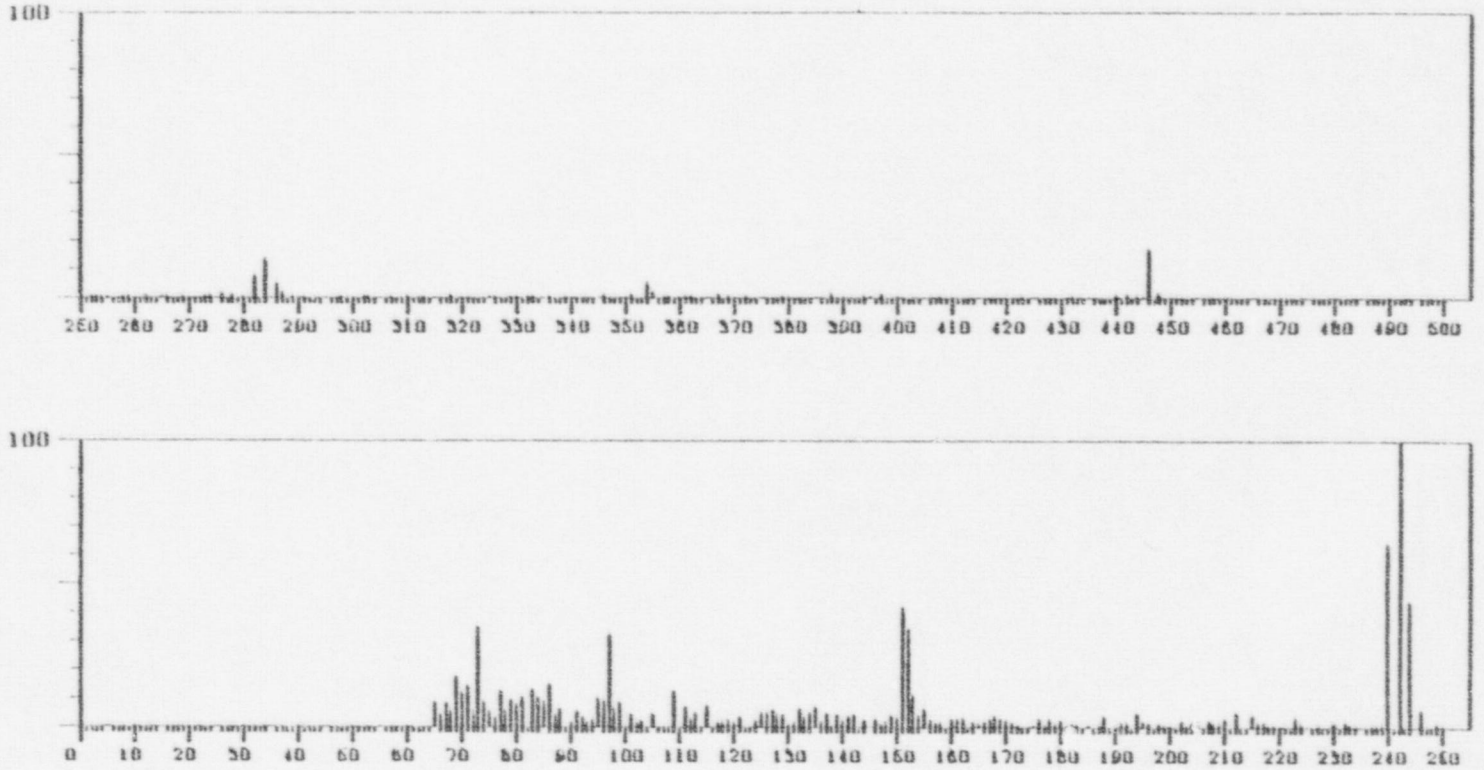

Fig. 5. Mass spectrum of peak identified as acetyl derivative of bromochlorophenol.

A sample of 6.19 g of freeze dried control rainbow trout (Group 2B, equivalent to 26.02 g fresh fish) was sequentially soxhlet extracted using 100 ml portions of the four solvents. Since the methanol extract was deep yellow, an extraction with a second 100 ml portion of methanol was also made to check for completeness of the first extraction; 7.4 g of freeze dried exposed rainbow trout (Group 3A, equivalent to 23.66 g fresh fish) were extracted in the same manner as the controls. The extraction of littleneck clams is in progress using this procedure.

Characterization of Rainbow Trout Tissue Extracts

The extracts of the Rainbow Trout tissues have been examined by gas chromatography using a 60 M SE54 glass capillary column and both flame (FID) and election capture (EC) detectors. The column temperature was held at 80°C for 5 minutes after injection and then programmed to 240°C at 4°C/min. Comparison of the FID and EC traces suggest the presence of several halogen containing compounds, but there were no qualitative differences between the chromotogram from control and exposed fish. These samples will be concentrated, fractionated and examined for lower levels of halogenated compounds during the next quarter.

Examination of Rainbow Trout Tissues for Chloroform Residues

Chloroform is known to be the major by-product of chlorination of fresh water, but it would probably be lost during freeze drying of the tissues and would elute too rapidly from the gas chromatograph to be detected under the conditions used for characterizing the tissue extracts. Therefore, a separate analysis for chloroform in rainbow trout tissue was made by adapting a procedure reported by Bush et al. (1977) for chloroform in water samples.

A single fish was weighed and sliced into several strips while still frozen. The tissues were placed into a 12 ml septum cap bottle containing 15 ml of high purity water which had been boiled to remove chloroform residues; 5 g of sodium chloride was added to the vial, the vial was capped, heated at 100°C for 1 hour, and the headspace analyzed for chloroform by gas

chromatography on a Chromosorb 101 column operated at 150°C using an EC detector. The peak in the chromatogram with the same retention time as chloroform is calculated as chloroform, although the possibility of a second substance with the same retention characteristics being present in the samples cannot be ruled out at this time. The results are summarized in Table 7. A high level of chloroform was found in the tissues, but there is no difference between exposed and control fish. The absence of a difference in levels between the two groups is not surprising in view of the fact that the chloroform level in the water in the control aquarium was approximately one quarter the level found in the treated water, as shown in Table 2.

Table 7. Estimated Chloroform in Rainbow Trout Tissue.

Exposed (3A)		Control (2B)	
Fish Weight, grams	$CHCl_3$ ng/g tissue	Fish Weight, grams	$CHCl_3$, ng/g tissue
3.88	187	3.08	239
5.70	103	6.43	85
3.17	252	5.35	177
6.57	85	6.22	78
5.26	60		
	$\bar{x}$ = 137		$\bar{x}$ = 145
	σ = 80		σ = 77

TASK IIa - FRESHWATER BIOLOGY

David R. Anderson

CHRONIC CHLORINE EXPOSURES

We have completed three subsamplings of rainbow trout, Salmo gairdneri, at the time of this report. The chronic chlorine study will be completed September 28, 1978. There are five test concentrations and a control (0, 12.5, 25, 50, 75, 100%). Details of the experimental design of the chronic study are in the 2nd Quarterly Report of 1978.

The mortality rate thoughout the test (Table 8) is low, less than one fish per week. The overall trend in mortality appears to be one of decreasing mortality with increasing concentrations. This may be due to a prophylactic effect of the chlorination by-products. A statistical analysis of this trend will be made at the end of the study to see if it continues during the disease-prone warm months of the year.

Using a projected growth rate from the first chronic bioassay, the subsampling scheme (Table 9), is designed to maintain the fish biomass in each aquarium less than the recommended maximum level of 2 liters of water per gram of fish per day (Sprague, 1973). Results of morphological measurements of the fish from each subsample are in Tables 10-12. A plot of the means and range of fork length, weight, and condition factors ($k = \frac{W}{\ell^3}$) for each test group in each subsample are in Figures 6, 7 and 8. Visual comparison between test groups for each subsample reveals little difference in mean and range for fork length, weight and condition factor at each subsample. There is a general trend of growth in both length and weight from subsamples 1-3 as would be expected. Also, there is an expected decrease in condition factor from subsamples 1-3 due to changes in body proportions of juvenile fish as they mature. Juvenile salmonids characteristically have a higher condition factor, larger head/body ratio and length to weight ratio. As they mature these ratios change. Under normal conditions one would expect allometric growth to become evident after the first year. Thus, a generally decreasing trend in condition factor

could be expected with time, up to one year. Comparisons of condition factors as an indication of an effect of test concentration are not valid for fish of different ages. Condition factor is only useful to indicate an effect on juvenile fish of the same age.

TABLE 8. Rainbow trout mortalities during the 2nd chronic chlorination by-product exposure.

Test Group	Aquaria	Subsample 1	2	3	Total	Mortality Rate (fish/week)
0%	2A	0	0	0	0	0.00
	2B	1	4	0	5	0.42
	Σ	1	4	0	5	0.21
12.5%	1A	0	1	2	3	0.25
	1B	0	3	0	3	0.25
	Σ	0	4	2	6	0.25
25%	6A	0	1	0	1	0.08
	6B	1	0	2	3	0.25
	Σ	1	1	2	4	0.17
50%	4A	0	0	2	2	0.17
	4B	0	0	2	2	0.17
	Σ	0	0	4	4	0.17
75%	5A	0	0	1	1	0.08
	5B	0	0	0	0	0.00
	Σ	0	0	1	1	0.04
100%	3A	0	1	1	2	0.17
	3B	0	0	0	0	0.00
	Σ	0	1	1	2	0.09

A statistical analysis of the data from each subsample has shown no significant effect of test concentration ($\alpha = 0.05$) with the following exceptions: adjusted mean lengths of subsample 3 between control and 75% (Aq 16); and adjusted mean weight of subsamples 1 and 3 of control and 12.5%. It is not clear at this time whether these differences indicate an effect due to exposure alone. Effects of crowding, feeding, and an effects due to the sequence of aquaria may contribute to the differences. Further information from later subsamples will provide more information to evaluate morphological differences and to provide a definitive indication of the cause of these differences.

TABLE 9. Fish subsampling scheme for the second chronic chlorination by-product bioassay.

Subsampling schedule (weeks)	Subsample size	Fish remaining for next period	Total fish per aquaria
4	25	175	200
8	25	150	200
12	25	125	200
16	35	90	200
20	30	60	200
24	30	30	200
28	30	0	200

TABLE 10. Length, weight and condition factor for rainbow trout from subsample 1, March 15 - April 12, 1978.

Test Group	Aquarium Number	n	Length (cm)* $\bar{x}$	Length (cm)* s	Weight (g) $\bar{x}$	Weight (g) s	Condition Factor $\bar{x}$	Condition Factor s
0 %	2A	23	5.7	0.5	2.44	0.79	1.26	0.08
	2B	32	5.5	0.5	2.20	0.66	1.26	0.09
	Σ	55	5.6	0.5	2.30	0.72	1.26	0.86
12.5%	1A	26	5.8	0.5	2.20	0.76	1.10	0.13
	1B	23	5.9	0.4	2.57	0.68	1.30	0.10
	Σ	49	5.8	0.5	2.38	0.74	1.19	0.15
25 %	6A	24	5.7	0.4	2.42	0.67	1.25	0.11
	6B	18	5.4	0.5	1.92	0.55	1.19	0.10
	Σ	52	5.6	0.5	2.20	0.66	1.22	0.11
50 %	4A	24	5.6	0.4	2.19	0.57	1.25	0.13
	4B	15	5.9	0.4	2.56	0.58	1.23	0.07
	Σ	39	5.7	0.4	2.34	0.60	1.24	0.11
75 %	5A	22	5.5	0.5	2.06	0.84	1.19	0.12
	5B	19	5.6	0.3	2.12	0.36	1.21	0.12
	Σ	41	5.5	0.5	2.09	0.66	1.20	0.12
100 %	3A	23	5.6	0.5	2.79	2.01	1.70	1.88
	3B	22	5.8	0.5	2.43	0.70	1.22	0.12
	Σ	45	5.7	0.5	2.61	1.53	1.46	1.35
Initial Size		200	5.6	3.8	1.67	0.40	1.08	0.09

n = number of fish sampled

$\bar{x}$ = mean

s = standard deviation

* = fork length

TABLE 11. Length, weight and condition factor for rainbow trout from subsample 2, March 15 - May 11, 1978.

Test Group	Aquarium Number	n	Length (cm)* $\bar{x}$	Length (cm)* s	Weight (g) $\bar{x}$	Weight (g) s	Condition Factor $\bar{x}$	Condition Factor s
0 %	2A	33	5.9	0.6	2.53	0.87	1.20	0.14
	2B	19	5.9	0.6	2.45	1.04	1.16	0.12
	Σ	52	5.9	0.6	2.50	0.93	1.18	0.13
12.5%	1A	20	5.8	0.7	2.43	1.18	1.13	0.14
	1B	22	5.9	0.5	2.39	0.67	1.17	0.11
	Σ	42	5.8	0.6	2.41	0.94	1.15	0.12
25 %	6A	24	5.8	0.4	2.48	0.67	1.22	0.09
	6B	24	6.1	0.6	2.86	1.08	1.23	0.10
	Σ	48	5.9	0.5	2.67	0.90	1.23	0.09
50 %	4A	26	5.8	0.6	2.47	0.79	1.20	0.07
	4B	28	6.0	0.7	2.95	1.53	1.26	0.27
	Σ	54	5.9	0.6	2.72	1.24	1.23	0.10
75 %	5A	24	5.8	0.6	2.42	0.80	1.19	0.10
	5B	24	5.9	0.7	2.74	1.20	1.25	0.20
	Σ	48	5.9	0.6	2.58	1.02	1.22	0.16
100 %	3A	23	6.3	0.8	3.13	1.27	1.19	0.13
	3B	25	6.0	0.7	2.70	1.13	1.22	0.11
	Σ	48	6.1	0.7	2.90	1.22	1.21	0.12
Inital Size		200	5.6	3.8	1.67	0.40	1.08	0.09

n = number of fish sampled

$\bar{x}$ = mean

s = standard deviation

* = fork length

TABLE 12. Length, weight and condition factor for rainbow trout from subsample 3, March 15 - June 7, 1978.

Test Group	Aquarium Number	n	Length (cm)* $\bar{x}$	Length (cm)* s	Weight (g) $\bar{x}$	Weight (g) s	Condition Factor $\bar{x}$	Condition Factor s
0 %	2A	22	6.4	0.8	3.18	1.19	1.18	0.06
	2B	25	6.7	0.9	3.49	1.69	1.10	0.09
	Σ	47	6.5	0.9	3.34	1.47	1.14	0.09
12.5%	1A	14		0.9	3.25	1.68	1.13	0.06
	1B	24	6.2	0.8	2.99	1.29	1.21	0.10
	Σ	38	6.3	0.8	3.08	1.43	1.18	0.09
25 %	6A	22	6.4	0.9	3.21	1.35	1.13	0.10
	6B	23	7.0	1.0	4.28	2.00	1.17	0.07
	Σ	45	6.7	1.0	3.76	1.78	1.15	0.08
50 %	4A	21	6.9	1.2	4.22	2.83	1.17	0.09
	4B	21	6.8	0.8	3.89	1.49	1.17	0.05
	Σ	42	6.8	1.0	4.06	2.24	1.17	0.07
75 %	5A	23	6.8	0.8	3.79	1.44	1.16	0.09
	5B	21	6.7	0.8	3.62	1.40	1.17	0.08
	Σ	44	6.7	0.8	3.71	1.41	1.16	0.09
100 %	3A	25	6.7	1.0	3.90	1.95	1.18	0.08
	3B	23	6.9	1.2	4.34	2.72	1.18	0.09
	Σ	48	6.8	1.1	4.11	2.33	1.18	0.08
Initial Size		200	5.6	3.8	1.67	0.40	1.08	0.09

n = number of fish sampled

$\bar{x}$ = mean

s = standard deviation

* = fork length

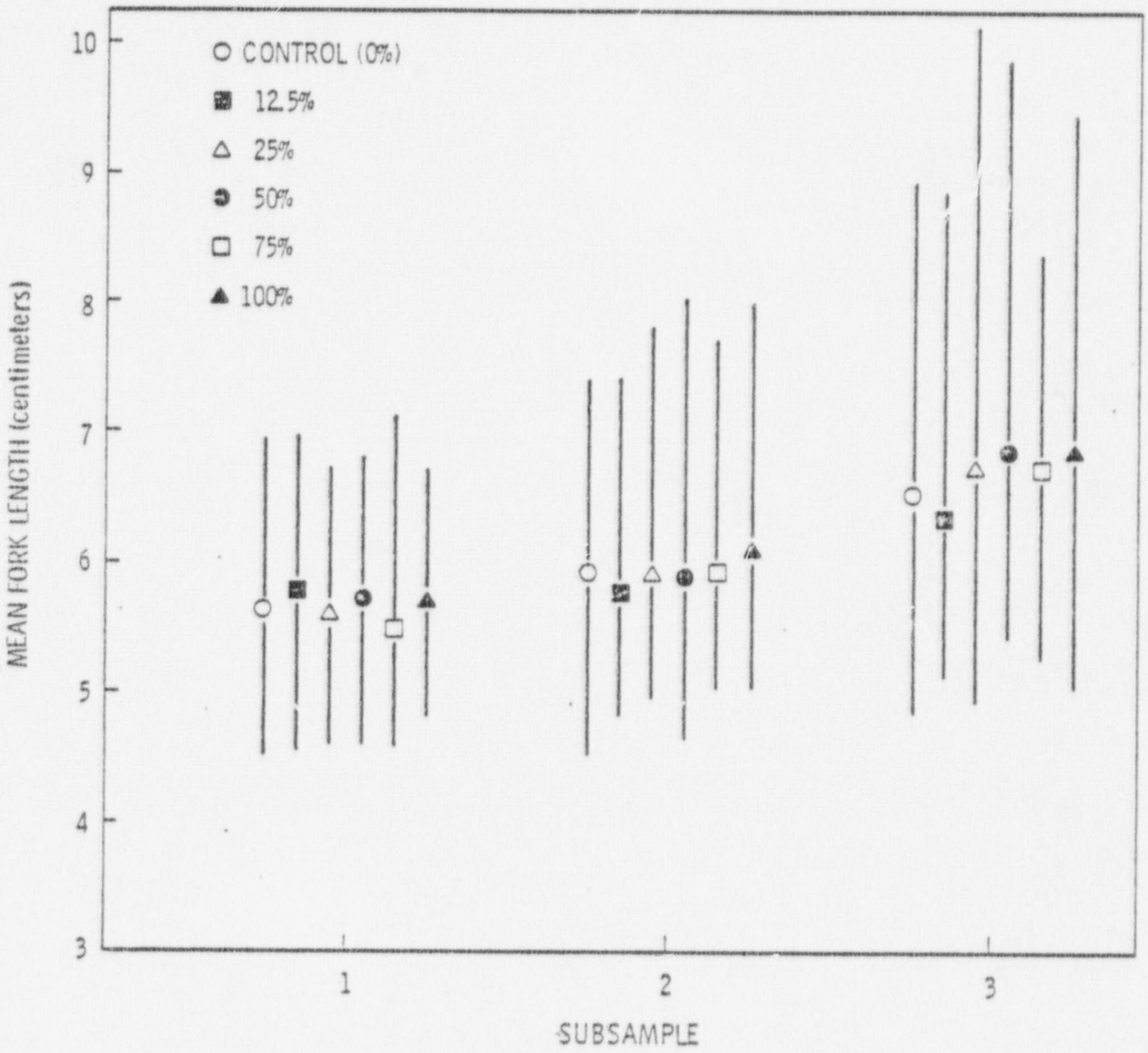

FIGURE 6. Mean fork length and range of rainbow trout for each test concentration at the first three monthly subsamplings.

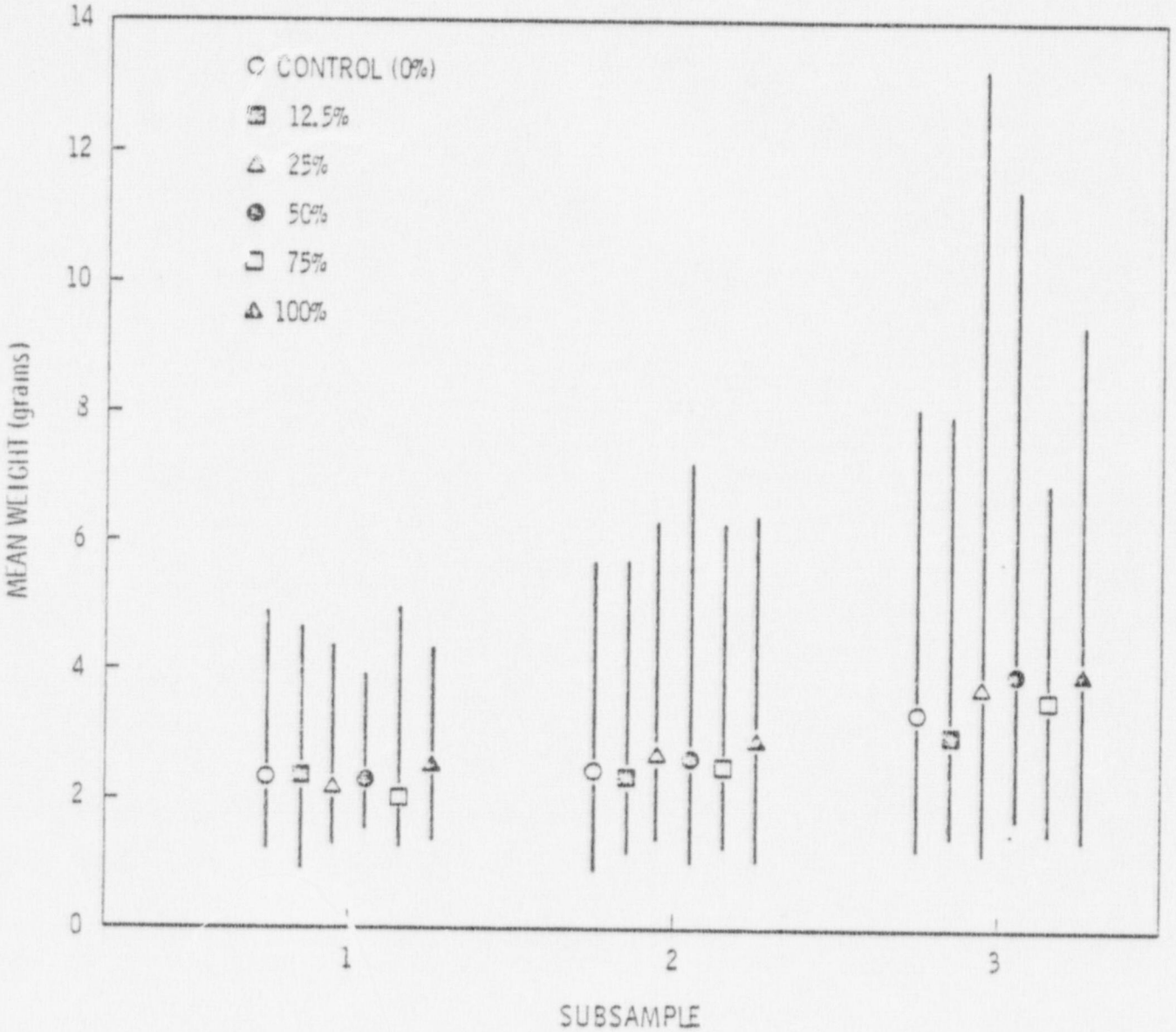

FIGURE 7. Mean weight and range of rainbow trout test concentration at the first three monthly subsamplings.

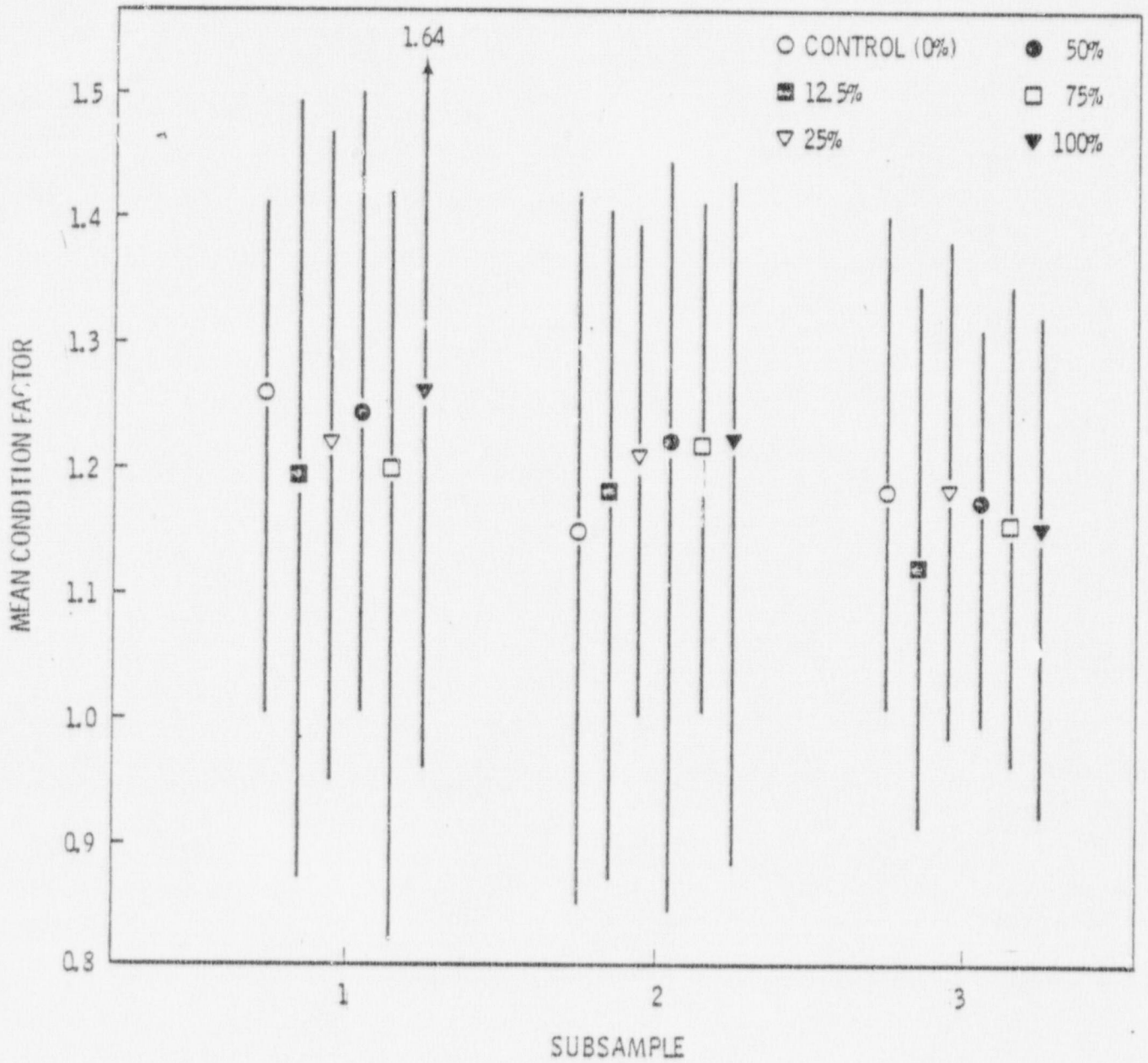

FIGURE 8. Mean condition factor and range for rainbow trout for each test concentration at the first three monthly subsamplings.

ACUTE CHLOROFORM STUDIES

The Toxicant Delivery System for the acute chloroform studies has been constructed (Figure 9). Due to the OSHA standards of chloroform in air, we modified of the system to include a fume hood above the toxicant metering apparatus to remove chloroform vapors.

Chloroform has proven to be very difficult to work with. Aside from its health hazard, several problems have arisen due to its chemical nature. We were unable to use a Marriot Bottle in the toxicant metering apparatus. A Marriot Bottle did not meter volumes the viscosity of the chloroform consistently with the varying bottle volumes because of the viscosity of the chloroform. In switching to a pump and silicone tubing we found that the chloroform dissolved the walls of the tubing. Chloroform is proving to be difficult to handle.

It is projected that most of the acute toxicity tests will be completed this fiscal year. We plan to test bluegill (Lepomis macrochirus), largemouth bass (Micropterus salmoides), rainbow trout (Salmo gairdneri), and channel catfish (Ictalurus punctatus), in that order. The number of tests completed depends upon resolution of the toxicant metering problems. Thomas O. Thatcher is consulting with us on the chloroform acute toxicity tests and will continue to assist in interpreting the results of these tests.

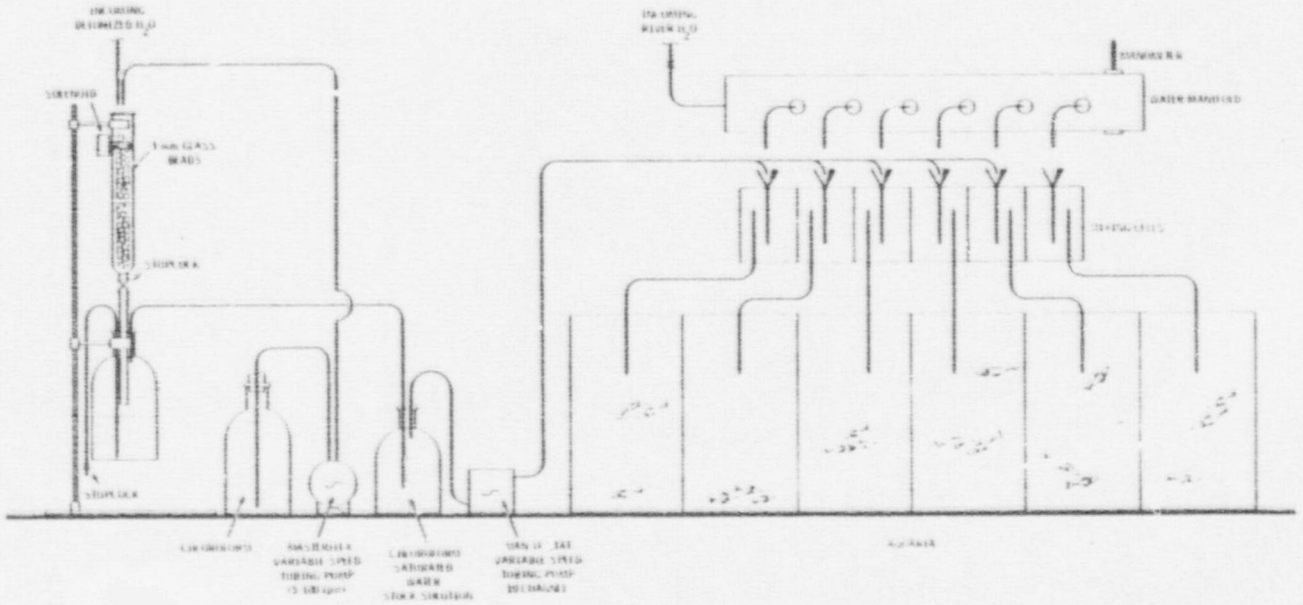

FIGURE 9. Chloroform Toxicant Delivery System.

TASK IIb - MARINE BIOLOGY

Charles I. Gibson

CHRONIC CHLORINE EXPOSURES

The exposure of littleneck clams, Protothaca staminea, to sublethal concentrations of chlorinated seawater were continued during the third quarter. Samples were harvested monthly from each of the test concentrations (control, 100, 50, 25, 12 and 6 ppb TRO) for chemical analysis and histopathological examination. The six-month exposure is scheduled to be terminated at the end of August, 1978.

BROMOFORM TOXICITY AND BIOACCUMULATION EXPOSURE

The introduction of bromoform into seawater in a soluble form still remains a major problem. After extensive experimentation, it has been concluded that bubbling bromoform saturated air in each aquaria is the best method. A schematic of the procedure is presented in Figure 10. The concentration of bromoform in the exposure tank is controlled by the rate of air flow and water exchange.

Clam sensitivity to bromoform was tested by observing the clams while the bromoform concentration was gradually increased. When the first sign of reaction (withdrawal of the siphons) was observed, a sample of the water was taken and analyzed. It was found that P. staminea withdrew their siphons at about 400 mg/l bromoform. When the bromoform was stopped and the exposure tank allowed to purge, the clams resumed siphoning after approximately 60 minutes. Because of this reaction and our experience of clams dying in the closed position (reported in the last Quarterly Report), we decided to run a 28-day uptake/28-day depuration exposure at several concentrations and monitor mortality. By doing this, we felt we would be able to obtain data on the toxicity of bromoform at concentrations that are higher than those likely to occur in the natural environment while also obtaining bioaccumulation data.

The test was initiated on May 30, 1978. Groups of 75 clams were placed in five tanks with nominal concentrations of bromoform of 0, 1, 5, 10, 20 mg/l. Samples were harvested on day 0, 1, 2 ,4, 7, 14, 21 and 28 and

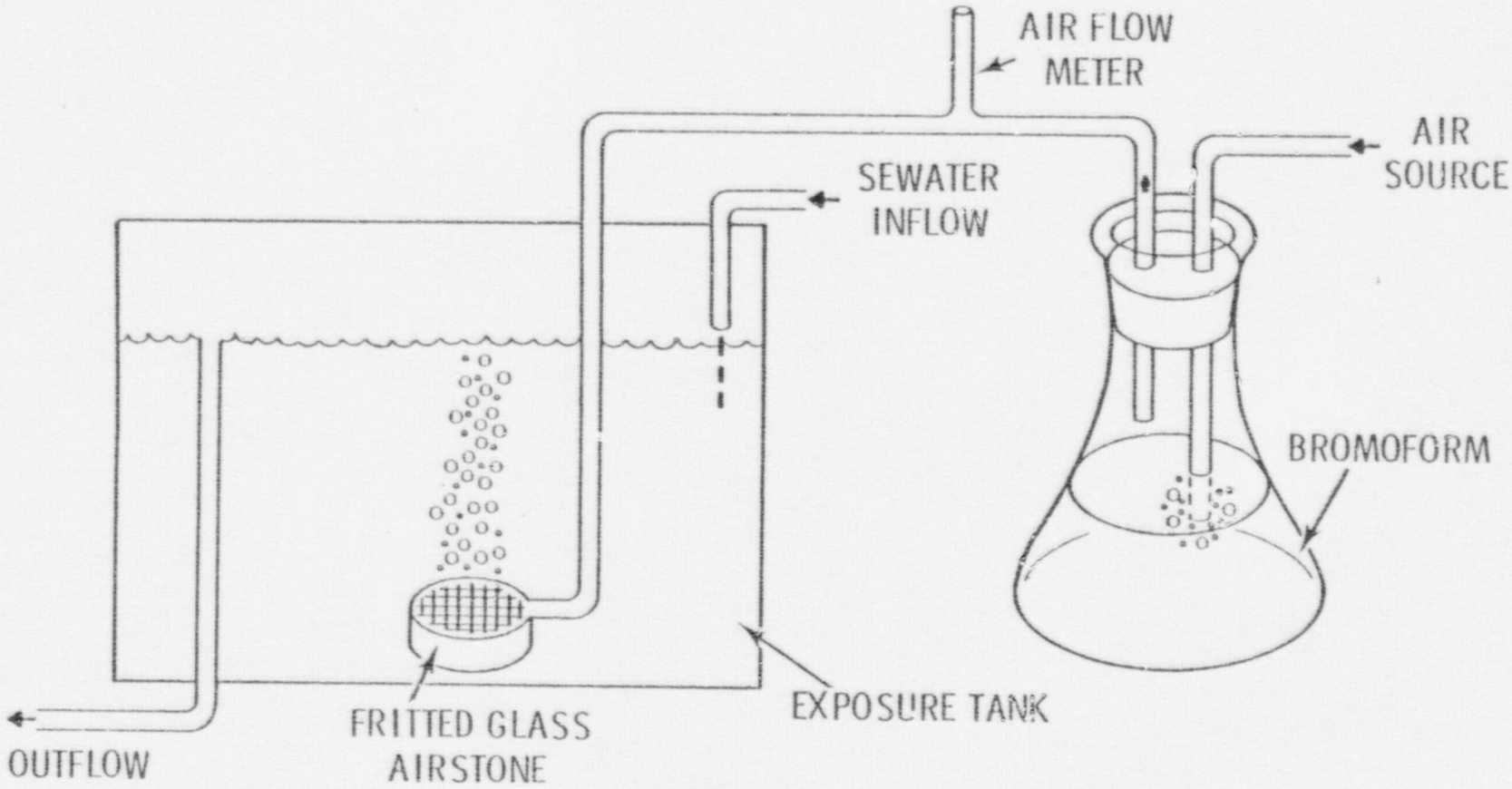

FIGURE 10. Toxicant delivery system for seawater/bromoform bioassays.

frozen for later chemical analysis. Clams are also being harvested for chemical analysis on days 1, 2, 4, 7, 14, 21, and 28 of the depuration cycle. Mortality occurred in the 20 and 10 mg/l concentrations but did not occur in the control or 1 and 5 mg/l tanks. There was considerable variation in the concentrations of bromoform at each of the predicted levels. These variations were generally upwards, so that the average exposure was higher than initially planned. The average values were 0. 1.6, 6.8, 19.5, and 26.8 mg/l. No mortalities were observed until the sixth day when 21 of the remaining 60 clams died at the 20 mg/l test level. On the 24th day, 10 of the remaining 24 died at the 20 mg/l concentration, and nine of the remaining 45 died at the 10 mg/l nominal test concentration. No further mortal- ities occurred during the uptake phase of the study.

Based on our experience to date, we hypothesize that the 96-hr LC_{50} concentration is well above 20 mg/l. It is unlikely organisms will experience lethal levels under ordinary circumstances when one considers that our analytical studies have found bromoform to be formed in concentrations of approximately 30 μg/l when seawater is chlorinated at a rate of 1.5 mg/l.

The work at the Daytona laboratory has been hampered by the same bromoform delivery problems experienced at Sequim, plus some unexpected analytical difficulties. The laboratory at which we had planned to have our bromoform water analysis conducted was found to be inadequate. We modified our plans and are now shipping the samples to Sequim for bromoform determinations. This creates some delays in results, but gives the advantage of having the same analytical quality control throughout all bromoform toxicity studies.

The samples are shipped air freight from Daytona at 7:30 A.M. EDT and arrive in Sequim the same day at 6:30 P.M. PDT, giving a transit time of approximately 14 hours. To assure that the delay caused by holding the samples and then shipping did not affect the concentration, we stored replicate samples at Sequim for an eight-day period. The initial sample concentration was 21.3 mg/l (average of three samples S.D. $\pm$ 3.4 mg/l), and the concentration after eight days was 21.3 mg/l (average of six samples S.D. $\pm$ 4.0 mg/l) indicating that no significant change has occurred during the storage period.

Two acute 96-hr studies have been completed with the shrimp, Penaeus aztecus. Computer analysis of the data for a LC_{50} as determined by the probit method has not been completed; however, a plot of the data on log/probability paper gave a 96-hr LC_{50} of 36 mg/l for the first run and 31 mg/l for the second run. These values are in close agreement and at levels that are well beyond those expected to occur in the environment. Therefore, at this time, a third acute test is not planned.

Of interest is the behavior of the shrimp when exposed to bromoform. As with the bivalves, the shrimp can detect bromoform. When they detect it they avoid the source. During the second acute test, the following observations were made:

a) At all concentrations (nominal, 50, 40, 30, and 20 mg/l) except 10 mg/l, the shrimp attempted to leave the tank or avoid the air stone through which the bromoform was entering within 60 seconds of the addition of bromoform.

b) A narcotic effect occurred. Shrimp lost locomotive ability and equilibrium eventually ending up on the sides with appendages undulating within 30 minutes at 50 mg/l concentration; 60 minutes at 40 mg/l; 90 minutes at 30 mg/l; and 120 minutes at 20 mg/l. Narcosis did not occur at the 10 mg/l concentration or in the control.

c) At the termination of the test, a number of individuals fully recovered their equilibrium within one hour after the flow of bromoform was discontinued.

Based on our experience with the littleneck clam, acute bioassays with oysters (Crassostrea virginica) and hardshell clams (Mercenaria mercenaria) are not planned at this time. Instead, 28-day uptake/28-day depuration studies will be run at 20, 10, 5, and 1 mg/l and monitored for mortality. Shrimp uptake and depuration studies will be run at 20, 10, 5, and 1 mg/l and monitored for mortality. Shrimp uptake and depuration studies will be run at 1 ppm.

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