ANNUAL REPORT

**The Synthesis, Pathways, Effects, and
Fate of Chlorination By-Products in
Freshwater, Estuarine and
Marine Environments**

Period Covered: September 10, 1976 -
September 30, 1977

David R. Anderson
Roger M. Bean
Charles I. Gibson
Thomas O. Thatcher

June 1978

Pacific Northwest Laboratory
Richland, Washington 99352
Operated for the
U.S. Department of Energy
by

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PACIFIC NORTHWEST LABORATORY
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Summary

The program has maintained a productive output throughout the year. The major goals of each task have been accomplished in accordance with the time schedules set forth in the Work Plan, November 10, 1976, Figure 1. The program has produced four reports that have been presented at technical conferences. The reports are included in the Appendix. This is the first year of funding of this program.

The study is composed of analytical chemistry and biological divisions with freshwater and marine biological subdivisions. The objective of the analytical phase is to identify those chemical compounds, other than the "free and combined available halogen", which result from the addition of chlorine to fresh or saltwater. The objectives of the biological studies are to investigate the immediate and relatively long-term toxicity of several chlorination by-products to selected aquatic biota; to follow their pathways of action; and to analyze for bioaccumulation or biomagnification.

Initial analytical experimentation has been directed toward isolating and identifying nonpolar, lipophylic organohalogens that might be expected to be absorbed and biomagnified in the lipids of aquatic biota. Chlorinated natural fresh and marine water samples were obtained from Task IIa and b. 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 constituent in all chlorinated sea water samples. In contrast, chloroform was the only haloform produced from chlorinated freshwater.

Many components identified in the marine samples are aromatic hydrocarbons which were found in all samples studied. Our hypothesis is that they are present in the XAD-2 polymer matrix in an unextractable form and are released during sampling.

With the exception of the haloforms, the level of nonpolar lipophylic halogenated compounds present in the chlorinated samples is in the nanogram per liter range. The haloforms are several orders of magnitude higher.

The Freshwater Biology 6-month chronic bioassay on rainbow trout has produced several results. The mortality rate, although low, indicated an interesting trend in response to increasing concentration of chlorination by-products. In addition to observed mortality, concentration was inversely related to other fish losses which are hypothesized to result from decreased aggressiveness. Fish length and weight at six months was also significantly reduced with increasing concentrations of chlorination by-products. An additional chronic bioassay is scheduled for FY 1978 to corroborate these results.

A chronic bioassay of chlorination by-products was conducted with little-neck clams in the marine phase of the program. Subsamples were taken

periodically for histological studies and for chlorination by-product analysis. Results of mortality and growth differences due to chlorination by-products are inconclusive. A second chronic bioassay is planned in FY 1978 with modifications to respond to questions posed during the first bioassay.

Plans for FY 1978

A detailed description of the program work plan for FY 1978 will be contained in the first quarterly report, January, 1978. A summary of ~~these~~ plans for each task follows.

In Task I, Analytical Chemistry, sampling of chlorinated waters using XAD-2 resins will continue with modifications for pH adjustment and quenching of active oxidant prior to adsorption. Other methodologies will be used for the examination of halogenated phenols in water samples and for analyses of total organic halogen in organic water fractions and tissues. Total organo-halogen determinations will be particularly important when applied to higher molecular weight materials which cannot be studied by GC/MS techniques. Tissue analysis will be conducted for those components which bioaccumulate in tissue, because they can have environmental impact far in excess of that initially predicted from aquatic concentrations.

There are three major efforts in the Task IIa Freshwater Biology to be conducted in FY 1978: 1) A second six month chronic chlorination by-products bioassay with rainbow trout will be conducted with periodic sampling for bioaccumulation and morphological measurements. 2) Acute toxicity tests will be conducted with chemical compounds (chloroform) selected from the results of the analytical task and with the consent of NRC technical staff, on four species of freshwater fish: rainbow trout (Salmo gairdneri), bluegill (Lepomis macrochirus),

largemouth bass (Micropterus salmoides), and channel catfish (Ictalurus punctatus).

3) Chlorine isotope studies will be used to trace the path of chlorine and/or chlorination by-products in rainbow trout during short-term (1-2 day) exposures.

In Task IIb Marine Biology efforts in FY 1978 to be conducted follow. A second six month chronic chlorination by-product bioassay with littleneck clams will be conducted. Halogenation by-products identified during the first year of the program (bromoform) will be used in relatively short term exposures to determine their effects on: littleneck clams (Protothaca staminea), quahaug (Mercinaria mercenaria), the eastcoast oyster (Crassostrea virginica), white shrimp (Litopenaeus setiferus), and the Atlantic menhaden (Brevoortia tyrannus). Although we are aware of the difficulties in working with adult menhaden alive in aquaria, our best effort will be applied to accomplish the stated objectives.

TABLE 1. Work Plan Schedule, November 10, 1976

	FY 1977				FY 1978				FY 1979			
	1	2	3	4	1	2	3	4	1	2	3	4
Task Ia and Ib.												
Literature Search & Planning												
Organohalogen Detection in Water												
Routine Analytical Methods Development												
GC/MS Identification of Compounds												
Organohalogen Detection in Tissue												
Identification of Metabolic Products and Mechanisms of Formation of Halogenated Components												
Final Report Write-up and Submission												
Task IIa												
Literature Search & Planning												
Chronic Chlorine Exposures												
Acute Cl By-Product Exposures												
Biological Half-Life Cl By-Products												
Toxic Mode of Action												
Final Report Write-up and Submission												
Task IIb.												
Literature Search & Planning												
Chronic Exposures of Clams to Cl By-Products												
Bioaccumulation Analyses												
Acute Cl By-Product Exposures												
Chronic Exposures with Individual Halogenated Organics												
Final Report Write-up & Submission												

Note: "*" = a definite sampling point; "___" = continuous; "....." = indefinite initiation or termination.

Task Ia, b - ANALYTICAL CHEMISTRY OF FRESH AND MARINE WATERS

- R. M. Bean

Activities this year have concentrated on reviewing the literature, acquiring instrumentation, and techniques necessary to conduct the program. Stress has been placed on developing water sampling methods which are relatively easy to perform at locations remote from laboratory facilities. This is consistent with our long-range plans to investigate power plant cooling effluents for halogenated components. An electron capture, capillary gas chromatograph has been acquired, made operational, and has been a principle investigative analytical tool. Much effort has been expended to insure that results obtained on this instrument are consistent with those obtained on the capillary gas chromatograph/mass spectrophotometer (GC/MS), the other major analytical instrument used in the program. Another major activity has been the investigation of methodology for selective removal of high molecular weight polar material from water samples which interfered with the analytical studies. The extreme complexity of the samples and the very low concentrations of individual halogenated components in the samples have been the major problems encountered in the initial analytical studies thus far. Identification of these components is therefore proving to be quite difficult.

Results of our initial analytical investigations have been summarized in two symposia presentations. The first, given at the Symposium on High Resolution Gas Chromatography at the American Chemical Society Meeting in Chicago, August 28, 1977, is entitled "Investigation of Trace Organic Constituents in Chlorinated Natural Waters Using Glass WCOT Columns". The second was presented at the Conference on Water Chlorination in Gatlinburg, TN on November 1, and

is entitled "Investigation of Halogenated Components Formed from Chlorination of Marine Water". Both these papers will be published as chapters in the proceedings of the conferences early in 1978. They are appended to this report.

SUMMARY OF PROGRESS, FY 1977

Initial experimentation has been directed toward isolating and identifying halogenated components formed in natural waters under conditions similar to those in cooling water treatment. Emphasis has been on those halogenated compounds that might be expected to be absorbed and biomagnified in the lipids of aquatic biota. Thus, for these studies, we investigated the nonpolar, presumably lipophylic organohalogens formed. The procedures used to isolate these compounds (Figure 1) specifically exclude more polar component types such as halogenated phenols, amines, and nitrogen heterocycles that are under investigation using other methodologies.

Samples of chlorinated natural waters, from Tasks II a and b, were obtained from a continuous flow apparatus designed to simulate 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 constituent in all chlorinated sea water samples. In contrast, chloroform was the only haloform produced from chlorinated freshwater.

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

remove material having molecular weight greater than 800. 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 (e.g., ketones and esters) Figure 1.

Many of the components identified are 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 the analytical procedure did not contain these hydrocarbons. Our present hypothesis is that these components are 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. The brominated aromatic components in silica gel Fraction A are probably formed by reaction of bromine with the hydrocarbon impurities in the resin during sampling.

On the basis of our results thus far, concentrations of nonpolar and presumably lipophylic 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. Although destruction of active chlorine prior to XAD adsorption should eliminate brominated artifacts, continued problems with contaminants can be expected since many substances, phthalates for example, are ubiquitous in the environment at these levels.

PLANS FOR FY 1978

Sampling of chlorinated waters using XAD-2 resins will continue this fiscal year. Sampling equipment modifications will include pH adjustment and quenching of active oxidant with sodium sulfite prior to XAD adsorption. In addition,

other methodology for the identification of specific halogenated phenols in water samples will be explored, since identification of this class of compounds is potentially an important one. Instrumentation will be acquired for the analysis of water samples and separated organic fractions for total organic halogen. Such procedures will improve the chemical definition of the bioassay water as well as identifying those organic fractions which are relatively high in halo-organics, and thus meriting detailed chemical investigations. Total organohalogen determinations will be particularly important when applied to higher molecular weight materials which are not volatile, and thus cannot be studied by GC/MS techniques.

Also planned for this year is the examination of biological tissues for halogenated components. This phase of the program has assumed additional importance in view of the low levels of halo-organics being found in chlorinated waters. Components which bioaccumulate in living tissue can have environmental impact far in excess of that initially predicted from aquatic concentrations. Since some halogenated trace components have been shown to biomagnify by factors as high as 10^6 (chlorinated hydrocarbons for example), tissue analysis may be the best methodology for identifying components as biohazards.

FIGURE 1. Sample Separation Procedures

TASK IIa - FRESHWATER BIOLOGY

David R. Anderson

The Freshwater Biology task is on schedule with the final work plan of November 11, 1976. Primary objectives of the first year are to conduct a chronic chlorination by-products bioassay with rainbow trout (Salmo gairdneri) to examine effects on growth, mortality, bioaccumulation of chlorination by-products, and to provide samples of chlorinated water for chemical analysis under Task I, Analytical Chemistry. These objectives have been accomplished. A second chronic bioassay with rainbow trout (RBT), to corroborate results obtained during the first bioassay is scheduled to begin during the second year of the program. Modifications in the toxicant delivery system to reduce the formation of chlorination by-product artifacts will be made prior to initiation of the second chronic bioassay.

The design of the toxicant delivery system used in the first chronic bioassay is shown in Figure 1. Chlorinated and unchlorinated Columbia River water are proportionally mixed to produce the maximum test concentration of approximately 0.02 ppm total residual chlorine (TRCl) which is considered the 100% test group. There are five other test groups (Table 1) based upon dilutions from the 100% test group concentration, 75%, 50%, 25%, 12% and a control. Total residual chlorine (TRCl) was measured by amperometric titration. Concentrations of TRCl throughout the chronic bioassay were maintained by altering the amount of sodium hypochlorite (NaOCl) added to compensate for changes in chlorine demand of the test water.

To begin the bioassay, two hundred RBT were placed in each aquaria following the sequence from a random numbers table to eliminate bias in fish selection. Concentration sequence of TRCl was also selected using a random numbers table to eliminate bias in the sequence of concentrations.

The level of fish biomass was initially less than the level recommended by Sprague (1973), 2-3 grams of fish/liter/day. Fish were subsampled at three and six months to reduce the biomass and thus maintain it at a level less than 2 grams of fish/liter/day.

The fish subsampled are weighed, measured, and frozen for use in the development of analytical techniques for chlorination by-products in fish tissues (Task I, Analytical Chemistry). Shortly after the six month subsampling, a pump failure resulted in mortality of the controls and fish at several other test levels. Thus, the results presented are as of the six month subsampling.

Mortality rate throughout the experiment was low, generally less than one fish per aquarium per week. The total number of mortalities from each test concentration indicates an interesting trend of mortality and TRCl test concentration or resultant chlorination by-product concentration (Table 2). Test concentration appears related to number of fish unaccounted for (Table 2). At this time, these losses are attributed to two causes: 1) cannibalism within each aquaria, and 2) fish leaving the aquaria by jumping into the one inch diameter standpipe drain. Cannibalism has been reported in chronic bioassays by Sprague (1973). We suspect it may have occurred but have no confirming observations. We have observed the second behavior and will modify the test system by screening the tops of the standpipes for the second chronic bioassay. These results raise questions as to what effect chlorinated natural waters may have on basic behavioral patterns necessary for survival such as foraging behavior or aggressiveness.

Results of the length and weight measurements taken at the three and six month subsampling periods are shown in Tables 3 through 6. No significant

differences were found in length or weight between controls and other test levels at the three month subsampling (Tables 3 and 4). In order to obtain a constant coefficient of variation, length and weight data were logarithmically transformed prior to analysis to normalize the data. Analysis of variance indicates a significant difference ($P = 0.10$) at the six month subsampling between control and 50% and 75% groups (Table 6) in both length and weight. The 100% group was not significantly different from controls in either length or weight.

There appears to be a sublethal effect of TRCI or chlorination by-products which results in a decrease in growth in rainbow trout. It is not clear why the 100% test group did not show this effect. One hypothesis is that the effect is related to fish biomass in the aquaria. Since there was greater biomass in the 100% aquaria, due to mortalities in the 50% and 75% aquaria, the concentration of chlorinated organics per gram of fish was lower in the 100% aquaria. The second chronic bioassay scheduled for FY 1978 will provide further information to verify or refute this hypothesis.

Fulton's coefficient of condition ($\frac{W}{L^3} = K$) or condition factor was computed at the three and six month subsamplings (Table 5). This length (L) weight (W) relationship can also be expressed in a general formula of $W = \alpha L^\beta$, or as a regression $\ln W = \ln \alpha + \beta \ln L$ where β is the slope and $\ln \alpha$ is the length-weight intercept (Table 6). Analysis of covariance of length-weight relationship indicated no significant difference in slope (β) between control and test groups. Analysis of the adjusted means adjusted to the common slope ($\beta = 3.02$) indicates a significant difference between the control and the 50% test group. When compared to the control, none of the other test groups differed significantly using the procedures by Dunnett (1964). Condition

factor is an estimate of the overall health of fish of the same age and species. The statistically significant difference between the 50% group and controls indicates the controls are healthier or in better condition than the 50% group. This is corroborated by the length and weight differences between control and the 50% group also. Whether this is an effect of chlorinated organics will not be clear until results are available from the second chronic bioassay.

PLANS FOR FY 1978

During the upcoming year, a chronic bioassay with RBT will be initiated with some modifications in the pretreatment of the chlorinated water before it enters the test aquaria. The water will be heated 10°C above ambient and the chlorine added as sodium hypochlorite. The water will then be cooled to approximately ambient temperature after an hour contact time. Then it will enter the dilution system and associated test aquaria. Both control and chlorinated water will be treated similarly. Pretreatment is designed to serve two major functions: 1) enhance chlorination by-product formation and 2) simulate the thermal regime through a power plant and transit time through a discharge canal or pipe prior to dilution.

Total halogenated organic measurements will be made in the chronic bioassay using a Dohrmann Microcoulometric Device. This may also be used on tissue samples, but it will be dependent upon the development of tissue analytical techniques.

Studies of acute effects of chloroform will be conducted during the next year. These will include bioassays on rainbow trout, Salmo gairdneri, channel catfish, Ictalurus punctatus, bluegill, Lepomis macrochirus, and smallmouth bass, Micropterus dolomieu.

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Figure 1.
TOXICANT DELIVERY SYSTEM

TABLE 1. Mean total residual chlorine (TRCl) in parts per million measured by Amperometric Titration during the six month bioassay.

Test Group	Entering Aquaria		In the Aquaria	
	$\bar{x}$	s	$\bar{x}$	s
0 %	<.001	-	<.001	-
12.5%	~.001	.001	<.001	-
25 %	.004	.003	<.001	-
50 %	.012	.005	.006	.005
75 %	.026	.008	.015	.008
100 %	.034	.011	.021	.010
Contact Chamber	.037	.013		

TABLE 2. An accounting of rainbow trout mortalities during the 6 month bioassay

Test Group	Aquarium Number	Total Number of Fish	Subsample #1	Subsample #2	Live Fish After Second Subsample	Mortality	Disease	Fish Unaccounted For **
0 %	2A	200	104	30	22	10	2	32
	2B	200	110	33	22	1	2	32
	Σ	400	214	63	44	11	4	64
12.5%	1A	200	106	27	20	11	0	36
	1B	200	119	11	22	15	8	25
	Σ	400	225	38	42	26	8	61
25 %	6A	200	128	4	12	3	37*	16
	6B	200	104	32	22	2	2	38
	Σ	400	232	36	34	5	39	54
50 %	4A	200	103	49	22	3	1	22
	4B	200	114	5	22	4	33*	22
	Σ	400	217	54	44	7	34	44
75 %	5A	200	123	15	22	21	1	18
	5B	200	105	21	22	14	7	31
	Σ	400	228	36	44	35	8	49
100 % ***	3A	200	116	35	22	8	0	19
	3B	200	114	34	22	11	4	15
	Σ	400	230	69	44	19	4	34
Totals		2400	1346	296	252	103	97	306

*Mortalities occurred due to low D. O., plugged incoming water line.

**See text for a further explanation

***The mean TRC1 concentration entering the 100% group is 0.034 mg/l.

TABLE 3. Lengths in centimeters of RBT subsamples periodically during the 6 month chronic bioassay.

Test Group	Aquarium Number	3 Months			6 Months		
		<u>n</u>	<u>$\bar{x}$</u>	<u>s</u>	<u>n</u>	<u>$\bar{x}$</u>	<u>s</u>
0 %	2A	104	7.0	1.1	30	8.9	1.5
	2B	110	7.3	0.8	33	9.7	1.5
	Σ	214	7.2	1.0	63	9.4	1.6
12.5%	1A	106	7.2	1.0	27	9.2	1.2
	1B	118	7.0	0.8	11	8.8	1.4
	Σ	224	7.1	0.9	38	9.1	1.3
25 %	6A	127	7.0	1.1	4	9.3	1.5
	6B	103	7.2	1.0	32	8.8	1.4
	Σ	232	7.1	1.1	36	8.9	1.4
50 %	4A	103	7.1	1.2	49	8.2	1.0
	4B	114	7.0	1.0	5	8.3	0.7
	Σ	217	7.0	1.1	54	8.2	1.0
75 %	5A	123	7.0	1.0	15	9.0	1.1
	5B	105	7.1	1.2	21	8.0	1.0
	Σ	228	7.0	1.1	36	8.4	1.1
100 %	3A	115	7.0	1.0	35	8.8	1.1
	3B	114	7.0	1.0	34	8.8	1.2
	Σ	229	7.0	1.0	69	8.8	1.3
Beginning of Bioassay		20	4.4	.37			

n = Number of fish per sample

$\bar{x}$ = Mean

s = Standard Deviation

Table 4. Weights in grams of RBT subsampled periodically during the 6 month chronic bioassay.

Test Group	Aquarium Number	3 Months			6 Months		
		<u>n</u>	<u>$\bar{x}$</u>	<u>s</u>	<u>n</u>	<u>$\bar{x}$</u>	<u>s</u>
0 %	2A	104	4.61	2.29	30	9.98	6.00
	2B	110	4.95	1.74	33	11.61	6.05
	Σ	214	4.87	1.81	63	11.90	6.18
12.5%	1A	106	4.72	1.87	27	10.44	4.66
	1B	118	4.48	1.71	11	9.51	4.75
	Σ	224	4.59	1.79	38	10.17	4.64
25 %	6A	128	4.50	2.15	4	12.18	6.58
	6B	104	4.78	2.09	32	9.70	4.57
	Σ	232	4.62	2.12	36	9.98	4.78
50 %	4A	103	4.74	2.70	49	7.84	3.03
	4B	114	4.54	2.07	5	8.43	2.50
	Σ	217	4.63	2.38	54	7.89	2.97
75 %	5A	123	4.51	2.02	15	10.28	4.07
	5B	105	4.53	2.31	21	7.01	2.87
	Σ	228	4.52	2.15	36	8.37	3.74
100 %	3A	115	4.42	2.17	35	9.82	4.63
	3B	114	4.43	1.86	34	9.61	4.16
	Σ	229	4.43	2.02	69	9.72	4.37
Beginning of Bioassay		20	1.35	0.38			

n = Number of fish per subsample

$\bar{x}$ = Mean

s = Standard Deviation

TABLE 5. Condition factor of RBT for chronic bioassay at 3 and 6 month subsamples.

Test Group	Aquarium Number	3 Months		3 Months	
		n	$\bar{x}$	n	$\bar{x}$
0 %	2A	104	1.227	30	1.274
	2B	110	1.201	33	1.316
	Σ	214	1.214	63	1.296
12.5%	1A	106	1.212	27	1.270
	1B	118	1.233	11	1.318
	Σ	224	1.223	38	1.284
25 %	6A	127	1.231	4	1.407
	6B	103	1.221	32	1.343
	Σ	232	1.216	36	1.350
50 %	4A	103	1.231	49	1.352
	4B	114	1.327	5	1.431
	Σ	217	1.235	54	1.360
75 %	5A	123	1.220	15	1.362
	5B	105	1.181	21	1.274
	Σ	228	1.202	36	1.311
100 %	3A	115	1.232	35	1.343
	3B	114	1.216	34	1.354
	Σ	229	1.224	69	1.349

n = Number of fish per subsample

$\bar{x}$ = Mean

TABLE 6. Data summary at 6 months.

<u>Test Group</u>	<u>Number of fish</u>	<u>Length mean</u>	<u>S.E.⁴</u>	<u>Weight Mean</u>	<u>S.E.⁴</u>	<u>Length-Weight Intercept²</u>	<u>Regression Slope¹</u>	<u>Adjusted Mean Length³</u>
0 %	59	9.3	0.20	11.2	0.44	-4.40	3.11	2.14
12.5%	38	9.1	0.21	10.2	0.75	-4.41	2.90	2.12
25 %	36	8.9	0.23	10.0	0.80	-4.36	2.84	2.18
50 %	54	8.2*	0.13	7.9*	0.40	-4.35	2.99	2.19
75 %	36	8.4*	0.19	8.4*	0.62	-4.39	3.29	2.15
100 %	69	8.8	0.16	9.7	0.53	-4.36	3.00	2.18

* = Differs from control $\alpha = .10$

1 = Regression equation $\ln w = \ln \alpha + \beta \ln l, w = \alpha l^\beta$

2 = Intercept based on common slope, $\beta = 3.02$

3 = Adjusted means using a common β

4 = Standard error of the mean

Task IIB - ESTUARINE AND MARINE BIOLOGY - T. O. Thatcher

Objectives of the estuarine and marine biology segment of the program are to expose littleneck clams, Protothaca staminea, to chlorinated sea water and study bioaccumulation of halogenated by-product compounds, histological damage, effects on growth, and to provide samples of chlorinated sea water for chemical analysis described under another section Task I. Two separate systems were maintained to accomplish these biological and chemical objectives.

To examine uptake of halogenated organic compounds as well as effects of such compounds, clams were exposed to various concentrations of chlorinated sea water for six months. Initial sea water chlorination produced five total residual oxidant (TRO) levels ranging from 0.008 to 0.133 mg/l, plus a control. The system is designed with two series of chambers, an initial chlorine contact chamber followed by the exposure chamber which contains clams. Resultant exposure chamber concentrations ranged from undetectable to 0.016 mg/l TRO. This expected reduction is due in part to chlorine demand of the sea water (which includes the formation of halogenated organic by-products). Samples of exposed and control clams were taken during the six month exposure period and preserved. Specimens to be analyzed later for halogenated compounds were frozen, and others were preserved in an appropriate fixative for histological examination. Growth of exposed clams, measured as increase in length of shell, was also examined during the six month exposure.

Data for the six month growth period is summarized in Table I. These data indicated no significant increase in shell length in either the control or exposed organisms. Either no growth occurred or shell length measurements did not detect any growth which may have occurred. Samples for histological examination have been sent to Dr. Robert Hillman at our east coast marine laboratory.

Initial examinations are expected to be completed in the first quarter of CY 1978. Frozen samples will be analyzed for bioaccumulation of halogenated organic by-products when appropriate procedures have been developed.

Recently, a new exposure system was completed that supplies both high concentrations (1-2 mg/l TR0) necessary for chemical characterization of chlorinated sea water, the low concentrations needed for clam exposures and incorporates a 10°C temperature increase during the initial chlorination period to simulate power plant conditions. Initial chlorination is at a level of approximately 1.5 mg/l TR0.

PLANS FOR FY 1978

In the upcoming year, the program will continue examining the effects of long-term exposures on growth, histopathology, and bioaccumulation. Growth studies will use the biological parameters, weight, volume, and condition in addition to the length measurements. Acute toxicity tests with bromoform will be conducted.

TABLE I. Growth data for Littleneck clams, Protothaca staminea,^(a)
exposed to chlorinated seawater.^(e)

Test Group	$\bar{x}$ ^(b) Initial TR0 (mg/l)	$\bar{x}$ Exposure TR0 (mg/l)	Clam Size, mm		Shell Growth, mm	
			$\bar{x}$	s	$\bar{x}$ (Range)	s ^(f)
1	0.00	≤ 0.00	45.2	5.8	-.13 (-.30 to +.03)	.08
2	.008	$\leq .01$	46.2	7.1	-.10 (-.23 to .00)	.05
3	.016	$\leq .01$	45.0	7.9	-.10 (-.20 to +.10)	.05
4	.032	$\leq .01$	45.0	8.4	-.10 (-.23 to +.25)	.08
5	.065	$\leq .01$	46.0	6.4	-.10 (-.28 to +.13)	.05
6	.133 ^(c)	$\leq .016$ ^(d)	45.0	6.4	-.08 (-.18 to +.05)	.08

(a) The growth data represent 60 clams per exposure chamber.

(b) TR0 = Total Residual Oxidant.

(c) 0.133 is the mean over the 6 months, the range was .08 - .17, Standard Deviation was .025, the other concentrations were calculated from successive dilutions of 50%.

(d) 0.016 is the mean over the 6 months, the range was .01 - .03, Standard Deviation was .007, the other test group concentrations were consistently found to be less than 0.01 mg/l.

(e) Other water quality parameters:	Mean	Range	Std. Dev.
Dissolved Oxygen (mg/l)	8.11	7.45- 8.40	0.23
pH	7.93	7.67- 8.13	0.09
Temperature (°C)	15.01	14.2 -15.8	0.25
Salinity (‰)	30.43	29.8 -31.1	0.33

(f) s = Standard Deviation

APPENDIX

INVESTIGATION OF TRACE ORGANIC COMPONENTS IN
CHLORINATED NATURAL WATERS USING GLASS WCOT COLUMNS

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INVESTIGATION OF TRACE ORGANIC COMPONENTS IN
CHLORINATED NATURAL WATERS USING GLASS WCOT COLUMNS

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Glass wall-coated open tubular column chromatography has been used for the separation of organic components in fresh and saline waters after treatment with chlorine at concentrations approximating those in power plant cooling waters. Examination of the organic constituents isolated from water samples using an XAD-2 resin column has revealed that a complex mixture of electron-capturing components are produced by chlorination. The analytical scheme for the study of halogenated components consists of clean up steps performed by high speed liquid chromatography followed by gas-liquid chromatography on glass WCOT columns using flame ionization and electron capture GC detectors. Capillary GC/MS was also employed, using electron impact and chemical ionization techniques. Significant problems arose with respect to retaining the identity of hundreds of component peaks as they emerged from different chromatographic columns in different instruments using the different detection systems. Therefore, it was necessary to use procedures for insuring the reproducibility of retention times in different GC detection modes, and where this was not possible, to develop intercalibration techniques. Concentrations of nonpolar and presumably lipophylic, halogenated components formed by the chlorination of relatively uncontaminated natural waters appear to be very low (in the ng/l range), with the exception of the haloforms.

INTRODUCTION

Concern about the presence of halogenated organic compounds in water has been growing since Dowty, et al⁽¹⁾ detected volatile organochlorine compounds in a New Orleans area municipal water treatment facility. This report was rapidly followed with evidence adduced by Rook⁽²⁾, Glaze and Henderson⁽³⁾, Jolley⁽⁴⁾, 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⁽⁵⁾, as well as through indirect mechanisms, such as interference with reproductive success⁽⁶⁾, and interference with photosynthesis⁽⁷⁾. Further, a number of halogenated organic compounds have been found to concentrate in the tissues of aquatic organisms^(8,9), which in the case of food fish, increases the hazard of these compounds to human health.

In 1975, about 100,000 tons of chlorine were used in the treatment of cooling water for electricity generating plants⁽¹⁰⁾, 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. The studies reported here are part of an integrated research program to characterize the halogen-containing products which may arise from cooling water treatment, and to determine their toxicity and biological availability to aquatic life.

Initial analytical development has concentrated on the extraction, concentration, separation, and identification of volatile, lipophylic organohalogens formed from chlorination of both fresh and marine waters. Extraction and concentration of

nonpolar organics from water for the analysis of municipal wastewater were adapted from a procedure used by Glaze and Henderson⁽³⁾. In this procedure, the water sample is forced through a column of XAD-2 resin, the resin extracted with ether, and the concentrated ether extract analyzed by gas chromatographic and GC/MS techniques. Departures from the procedures of Glaze and Henderson were necessary because of the low concentrations of organic components found in these natural waters, and because of the exceedingly complex nature of the samples examined. The procedures used for this work would not be expected to detect more polar components in water, such as the halogenated derivatives of phenols, amines, or nitrogen heterocycles. Although these more polar types have been reported in chlorinated waters^(3,4), the procedures used herein would specifically exclude these from the analysis.

EXPERIMENTAL

Chlorinated water samples were obtained using the Battelle-Northwest biological laboratory facilities at Sequim, Washington (seawater from Sequim Bay) and at Richland, Washington (fresh water from the Columbia River). 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 a continuous-flow apparatus. Residence time from chlorine addition to sampling was about two hours.

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.⁽¹¹⁾. 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.

The columns were extracted with 125 ml ether preserved with 2% ethanol (J. T. Baker, "Resi-analyzed") following the procedure of Junk et al.⁽¹¹⁾. All other solvents used in these procedures were Burdick and Jackson "distilled in glass." The ether extracts were evaporated under a stream of dry nitrogen, the solvent changed to benzene. Those samples which were not directly investigated by gas chromatography at this point were subjected to the separation scheme described below.

The benzene solution of the ether extract was evaporated to 100 μ l volume and injected into two 3/8" x 12" μ -Styragel^R 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 (MW 386) has a retention volume of 13.6. The fraction collected from 13-25 ml is thus designated as the <800 MW fraction. The <800 MW fraction was evaporated to 100 μ l, 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 μ l and stored in tapered-bottom 0.3 ml vials.

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 used for this work were purchased from J&W Scientific, Inc. The 30 m column used for this work was found to have 101,000 effective theoretical plates at $k = 17.03$ and was one-half of a 60 meter OV-101 capillary. The other half of the column was used to perform the GC/MS analyses.

Chromatographic conditions were as follows: 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 is a simple procedure that 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.

The computerized 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 OV-101 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 disk for off-line data reduction.

DISCUSSION

Preliminary studies of concentrated ether extracts from the XAD-2 resins indicated that large samples would be required in order to obtain sufficient material for characterization. At present, water samples of between 200 and 400 liters are being processed. Preliminary chromatography of these large volume samples produced an immediate analytical result: A series of electron-capturing peaks was observed in the ether extracts of unchlorinated Columbia River water (Figure 1). 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 2). The concentration of

FIGURE 2. Electron-capture chromatogram of Aroclor 1254, showing similarity of relative peak heights and retention times to Figure 1. Absolute retention times differ because of slightly different chromatographic conditions.

Aroclor found in the Columbia River sample was in the ng/l range. In the corresponding chlorinated Columbia River sample (Figure 3) the Aroclor 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 1 and 3 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 could not be resolved on the capillary column. Thus, flame ionization chromatograms of the

FIGURE 3. Above, electron capture chromatogram of the extract from chlorinated Columbia River water. The chlorinated sample was obtained simultaneously with the unchlorinated sample (Figure 1). Below, corresponding flame ionization chromatogram.

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.

The above experiences serve to emphasize the importance of adopting sample cleanup techniques in the analysis of environmental samples for trace constituents in order to avoid artifacts and to reduce sample complexity. The separation of the sample by gel permeation chromatography permitted the rejection of all material in excess of 800 molecular weight. A chromatogram obtained from a seawater extract after the molecular weight separation step is shown in Figure 4. 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 μ l. A comparison of electron capture chromatograms of Fraction A obtained from chlorinated and unchlorinated seawater samples (Figure 5) 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 6.

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

1.0

FIGURE 4. Above, electron capture chromatogram of an extract from Sequim Bay seawater after removal of material in excess of 800 molecular weight by gel permeation chromatography. Below, corresponding flame ionization chromatogram.

FIGURE 5. Above, electron capture chromatogram from Fraction A of a chlorinated seawater sample. Below, electron capture chromatogram from unchlorinated sample.

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 1 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 FID and ECD, respectively.

TABLE I. Correspondence Between Retention Times Obtained Using Flame Ionization and Electron Capture Detectors

Component	FID	ECD	FID-ECD
	Retention Time Min $\pm$ s.d.	Retention Time Min $\pm$ s.d.	Difference $\pm$ s.d.
1-Cl C ₈	12.78 $\pm$ 0.054	12.66 $\pm$ 0.087	0.12 $\pm$ 0.10
1-Cl C ₉	16.43 $\pm$ 0.063	16.38 $\pm$ 0.090	0.05 $\pm$ 0.11
1-Cl C ₁₀	20.08 $\pm$ 0.058	20.07 $\pm$ 0.077	0.01 $\pm$ 0.10
1-Cl C ₁₆	39.23 $\pm$ 0.059	39.26 $\pm$ 0.074	-0.03 $\pm$ 0.09
1-Cl C ₁₈	44.44 $\pm$ 0.061	44.48 $\pm$ 0.075	-0.04 $\pm$ 0.10
1-Cl C ₂₀	49.23 $\pm$ 0.060	49.25 $\pm$ 0.078	-0.02 $\pm$ 0.10

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 μ l prior to injection.

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. It is physically located several miles from the laboratory in which the samples are generated and investigated using FID and ECD gas chromatography. 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 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.

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 II and Figure 7 give results obtained for the non-polar fraction (Fraction A) derived from several hundred liters of seawater from Sequim Bay. The darkened peaks in the EC/FID chromatograms in Figure 7 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 II lists bromine-containing components identified using the dual-detection criteria discussed

TABLE II. Comparison of Retention Times Obtained for Bromo-organic and Aromatic Hydrocarbon Components on GC/MS and FID/ECD Instruments, Nonpolar Fraction from Chlorinated Seawater 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
CHBr ₃	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	--
Bromotrimethyl- benzene	18.55	21.13	21.11	21.08
169	19.55	22.14	22.14	22.13
Biphenyl	21.88	24.53	24.47	--
Bromomethyl- naphthalene	28.60	31.42	31.55	31.59
Bromomethyl- naphthalene	29.08	31.91	31.93	31.95
Bromomethyl- naphthalene	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
Bromodimethyl- naphthalene	32.21	35.19	35.38	35.31
235	32.64	35.65	35.82	35.80
Bromotrimethyl- naphthalene	35.66	38.76	38.86	38.70
Pyrene	41.10	44.41	44.58	--

FIGURE 7. Above, flame ionization chromatogram of Fraction A from Sequim Bay seawater. Below, corresponding electron capture chromatogram. Darkened areas indicate those peaks arising from brominated organic compounds.

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 good 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 have 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 of CI and its tendency to concentrate the entire ion current into a small number of mass peaks are additional advantages. Following the ECD and CI analysis to locate compounds of interest, the remainder of the sample was used to obtain EI spectra which contain structural information complementary to the CI data.

Sample complexity and the generally low concentrations of the components of interest force us to work with spectra of uncertain quality. The results have been mixed. Experience has shown that available libraries contain very few spectra which are similar to those which we attribute to possible halogenation products, and the combination of EI 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 at present left unassigned.

A number of halogenated components have been identified in the samples thus far investigated. The principle halogenated products are the haloforms. Under the conditions employed, about 30 $\mu\text{g/l}$ of bromoform is formed in seawater. It is quite likely, however, that many of the compounds are formed as a

result of secondary reactions of excess halogen with sample impurities. Although the XAD-2 resin is soxhlet extracted scrupulously with three different solvents, aromatic hydrocarbons have appeared in all samples investigated thus far. 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. These components can then be halogenated during sampling, and indeed, brominated derivatives of these hydrocarbons are found in the seawater samples. Interestingly, few chlorinated aromatic hydrocarbons are found in fresh water samples even though the corresponding unchlorinated hydrocarbons were present.

It is apparent from the results 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 $\mu\text{g/ml}$). This corresponds to individual component concentrations in the parts per trillion range. At these concentrations, problems with experimental artifacts are major obstacles, since a number of substances are practically ubiquitous in the environment at these levels⁽¹²⁾.

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names are given to assist in replication of the analysis and use does not constitute endorsement by Battelle Memorial Institute.

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INVESTIGATION OF HALOGENATED COMPONENTS FORMED
FROM CHLORINATION OF ESTUARINE WATER

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FROM CHLORINATION OF ESTUARINE WATER

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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 relatively pristine estuarine waters of the northern Olympic Peninsula is currently under investigation. Organic matter is extracted by passing large volumes of seawater over columns of XAD-2 macroreticular resins. Examination of ether extracts from the resin columns using capillary gas chromatography has revealed the presence of halogenated methanes, as well as other electron-capturing components, that are not found when unchlorinated seawater is sampled. Examination of the unrefined chlorinated seawater extracts using gas chromatography/mass spectrometry has revealed a complex mixture which generally was not separable into individual components, even when high efficiency WCOT capillary columns were used. As an aid to the GC/MS investigations, the concentrated XAD-2 column ether extracts were further fractionated using high speed liquid chromatographic techniques. The samples were first separated into molecular weight fractions using a microstyragel column, and then further separated into fractions of increasing polarity using a water-deactivated silica gel column. Fractions were thus obtained which give baseline separation of components and which are more amenable to GC/MS investigation.

INTRODUCTION

Halogenated organic compounds in drinking water¹ and in treated waste waters^{2,3} have been studied over the past several years. However, the potential for formation of organohalogen products in chlorine-treated power plant cooling waters has received attention only recently. About 100,000 tons of chlorine were used to treat cooling waters from electricity generating plants in 1975⁴ and this use of chlorine will probably increase as additional conventional and nuclear power plants are brought into service. Jolley et al.⁵ recently 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.⁶

Our studies are part of an interdisciplinary program designed to investigate the formation of halogenated organic compounds in both fresh and saline cooling waters, and to determine their toxicity and biological availability to aquatic organisms. Much of our initial experimentation was directed toward isolating and identifying halogenated components formed in seawater under conditions similar to those used in cooling water treatment, particularly those halogenated compounds that might be expected to be absorbed and biomagnified in the lipids of aquatic biota. Thus, for these studies, we investigated the nonpolar, presumably lipophilic organohalogens formed in seawater. The procedures used to identify these compounds would specifically exclude more polar component types such as halogenated phenols, amines, and nitrogen heterocycles that are being investigated using other methodologies.

EXPERIMENTAL

Samples of chlorinated seawater were obtained from a continuous flow apparatus located at the Battelle-Northwest Marine Research Laboratory at Sequim, Washington. The apparatus was designed to simulate conditions of current power plant cooling water treatment practice. The waters of Sequim Bay are relatively pristine, containing about one mg/liter organic carbon. Chlorination as NaOCl was carried out at between one and two mg/liter chlorine and the seawater was sampled approximately two hours after treatment.

Organic components were concentrated by forcing chlorinated and unchlorinated seawater through columns of XAD-2 resin using a positive displacement pump. The procedures used were recently reported⁷ and are similar to those discussed by Glaze et al.⁸ Samples taken for study were usually several hundred liters in volume. Because of the large volumes involved, we did not destroy the active chlorine prior to resin adsorption in these experiments, although procedures for continuous additions of reductant are being implemented.

Ether extracts of the XAD-2 columns were dried over sodium sulfate, subsampled 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 1:10 split ratio 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.

The procedures used for the analysis of seawater 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 remove material having molecular weight greater than 800. Silica gel chromatography using hexane, then hexane-ether, was used to separate the sample into two fractions. Fraction A contained nonpolar components (e.g., hydrocarbons and halocarbons) and Fraction B contained components of intermediate polarity (e.g. ketones and esters). Both fractions were investigated by capillary gas chromatography. Temperature programming was from 65°C to 250°C at 4°C/min. Four detection systems were used: flame ionization, electron capture, electron impact mass spectroscopy, and chemical ionization mass spectroscopy. The separation and gas chromatographic techniques used to study the nonhaloform halogenated organics were described at a recent symposium on capillary chromatography.⁷

RESULTS AND DISCUSSION

Haloform Formation

Our initial analyses for haloforms were performed on XAD-2 extracts obtained from several hundred liters of seawater. An electron capture gas chromatogram obtained from one of these samples is given in Fig. 1. The principle component

FIGURE I. Electron capture capillary chromatogram of KAD-2 concentrate from chlorinated seawater.

found in all seawater samples was bromoform. Smaller quantities of dibromochloromethane and traces of dichlorobromomethane were found. Interestingly, chloroform was not present in significant concentrations. The first two experiments on Table I give results from the analyses of large volume samples using different quantities of KAD 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 I shows the results obtained from a third experiment in which two KAD-2 columns were connected in series and nine liters of chlorinated seawater sampled. The results indicate that about 30 $\mu\text{g/l}$ CHBr_3 were formed in the Sequim Bay seawater 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.⁸ (87%), probably because we employed a higher sample-to-resin ratio. It is of some interest that bromoform was found in all unchlorinated seawater samples analyzed and probably represents environmental concentrations.

TABLE I. Analysis of XAD-2 extracts of seawater for halomethanes

EXP #	Chlorine Addition	Sample Vol. (l)	Volume of Resin (ml)	CHCl ₂ Br (ug/l)	CHClBr ₂ (ug/l)	CHBr ₃ (ug/l)
1	Yes	238	8.0	<0.01	0.13	2.11
	No	231	9.0	nd*	nd	0.04
2	Yes	425	15.5	nd	0.44	8.33
	No	510	15.6	nd	nd	0.01
3	Yes	9.0	8.0 (Top)	nd	0.14	23.39
	Yes	9.0	10.8 (Bottom)	nd	nd	6.33

*not detected

Formation of Other Halogenated Components of Low Polarity

The liquid chromatographic separations applied to the XAD-2 extracts were critical to the overall analytical procedure. Fractionation of the organic material using gel permeation chromatography indicated the presence of material in excess of 800 molecular weight. Direct injection of the extracts into the gas chromatograph resulted in pyrolysis of the sample in the injection port. Chromatograms obtained on unrefined samples indicated poor resolution of individual components, even on chromatographic columns having as high as 100,000 effective theoretical plates. The poor resolution was reflected in preliminary runs made with capillary GC/MS in which all but the most prominent chromatographic peaks gave uninterpretable spectra indicative of multicomponent composition. The cleanup steps applied to the extracts gave samples which yielded baseline separations of most component peaks.

Capillary chromatograms obtained from Fraction A (nonpolar components) and B (components of intermediate polarity) using the flame ionization detector are shown in Fig. 2. These two fractions were derived from the XAD-2 extract of over 600 liters of chlorinated seawater. Final volume of the fractions was adjusted to less than 50 μ l prior to GC analysis. The chromatographic peaks denoted by asterisks or numbers represent components that met two criteria: 1) exhibited response from the electron capture detector, and 2) gave chemical ionization mass spectra having peaks characteristic of bromine isotope ratios. A component was required to meet both of these criteria to positively designate it as bromine-containing. The identities of numbered peaks in Fig. 2 are listed on Table II.

Fig. 2. Above, gas chromatogram of Fraction A from XAD-2 extract of seawater 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 1.

TABLE II. Components identified by GC/MS in Fractions A and B obtained from XAD-2 extract of seawater*

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

*Hydrocarbons were identified by comparison of spectra with authentic samples; identities of brominated components were assigned solely on the basis of spectral interpretation.

Many of the components identified in Fraction A are aromatic hydrocarbons. At first these components were thought to arise from petroleum contamination of the seawater samples since oil pollution studies are a part of ongoing research at the Sequim Laboratory. However, these same hydrocarbons were found in all samples studied, including those derived from fresh water sources. Procedural blanks performed by extracting freshly packed XAD-2 resin columns and subjecting the extracts to all the steps of the analytical procedure did not contain these hydrocarbons. Our present belief is that these components are initially present in the XAD-2 polymer matrix in an unextractable form and are released during the sampling operation, possibly because of fracturing of the resin particles as the bed is compacted. 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, do appear from their spectra to arise from the chlorination of seawater.

On the basis of our results thus far obtained, the concentrations of non-polar and presumably lipophylic halogenated components generated by the low level chlorination of relatively pristine seawater appear to be very low, in the nanogram per liter range, with the exception of the haloforms. Identifica-

tion of halogenated components at these concentrations is proving to be a difficult task, particularly since brominated standards are lacking and many of the components under investigation give singularly uninformative spectra under electron impact conditions. Although destruction of active chlorine prior to XAD adsorption should eliminate brominated artifacts, continued problems with contaminants can be expected since many substances are ubiquitous in the environment at these levels.

Acknowledgments

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CONTRASTS BETWEEN CHLORINATION EFFECTS IN
FRESHWATER AND SEAWATER ENVIRONMENTS

Presented at the American Fisheries Society, Western
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CONTRASTS BETWEEN CHLORINATION EFFECTS IN FRESHWATER AND SEAWATER ENVIRONMENTS

by

David Anderson and Thomas Thatcher

The earliest recorded practice of chlorination on a large scale was in 1854 in London, when the Royal Sewage Commission used chlorine to treat the city's sewage. Since that time, chlorine has been used in many ways for treating potable waters, waste waters and cooling waters. All of these uses, plus industrial processes such as the bleaching of wood pulp, used in the manufacturing of light colored papers, have the potential to introduce chlorine or its by-products into surface waters.

The chemistry of chlorine reactions in water is very complex, especially in saline waters, as shown in Figure 1. It makes little difference whether the chlorine is introduced as gas (Cl_2), as sodium hypochlorite (NaOCl) or calcium hypochlorite ($\text{Ca}(\text{OCl})_2$). The chlorine gas rapidly reacts with water to form hypochlorous acid (HOCl) which is in equilibrium with the hypochlorite ion (OCl^-). In the presence of ammonia or amino groups, chloramines are formed. Typically, monochloramine or dichloramine is formed, but in rare instances trichloramine may also be formed. Further reactions may occur in the presence of suitable substrates or precursors leading to by-products chlorinated organic.

In sea water, additional complexity is involved. In sea water the reaction is more complex involving oxidation of bromide ion by chlorine or hypochlorite to bromate which reacts as shown in Figure 1. Bromide ion is present in full strength sea water at about 65 mg/l.

The general result of the reaction is the formation of hypobromous acid (HOBr) and hypobromite ion (OBr^-) rather than the HOCl and the OCl^- which are formed in fresh water. Similar compounds involving iodine may also be formed. The addition of chlorine to sea water may lead to the formation of bromamines if the appropriate amino groups or ammonia are present, although some chloramines may be found also. The bromamines in sea water are typically di- and tribromamine, unlike the mono- and dichloramines commonly found in fresh water. Further reactions in the presence of the appropriate precursors or substrates may lead to the formation of bromo-organic by-products.

Some examples of chlorinated organics that have been formed in fresh water are shown in Figure 2. Approximately 100 chloro-organic compounds have been identified so far. Freshwater investigators have observed that up to 3% of the initially introduced chlorine may be found in these chlorinated by-products. We anticipate that the percentage found in sea water may be higher due to the presence of a greater variety of potential chemical precursors.

As biologists our interests with aqueous chlorine stems from the potentially toxic effects of the chlorine in its acutely toxic forms; commonly referred to as Total Residual Chlorine (TRCl), or more accurately as Total Residual Oxidant (TRO). The TRCl and TRO would include all those components shown in Figure 1 except the chloro- and bromo-organics. Chronically toxic forms could include chlor- or bromamines which, in some cases, may persist for days, and more complex by-products which may persist for weeks or months.

In recent years, there have been a number of good literature reviews published describing the acute toxicity of the TRCl or TRO components.

Among these are the following: Becker and Thatcher, 1973, who discussed both freshwater and sea water used as a cooling medium in electrical generating plants; Brooks and Seegart, 1976, who discussed freshwater problems with some sea water references also; Davis and Middaugh, 1976, who discussed sea water chlorination; Mattice and Zittel, 1976, who discussed both freshwater and sea water; and Page, 1975, who discussed, principally, freshwater problems. The data presented in these reviews includes that published through part of 1975. It suggests that the toxicity of chlorinated freshwater is approximately one order of magnitude greater than that of chlorinated sea water. Mattice and Zittel presented this concept in graphic fashion, similar to Figure 3. For exposures up to approximately 2 days in length, chlorinated sea water is more toxic than freshwater, but in longer exposures, freshwater is cited as being more toxic.

The investigation of the environmental consequences of sea water chlorination is just beginning. Very little has been done since an early study by Galtsof in 1946 with oysters. Recently environmental impact statements for nuclear electrical generating stations have required information on potential effects of cooling water chlorination. Current marine and estuarine studies of chlorine toxicity will define the range of toxicity to a number of marine organisms as similar studies have done in freshwater. Studies by both Roberts of VIMS on the East Coast, and our West Coast Battelle Marine Laboratory, have reported toxic effects equal to and less than 0.005 mg/l (5 ppb) for sea water organisms.

There are reasons why the toxicity of chlorinated sea water may differ from chlorinated freshwater. First, a rapid exchange between chlorine and

bromine as the available oxidant takes place when chlorine is added to sea water. Also, if there is a greater potential for the formation of by-products as has been suggested in estuarine and marine waters, then acute toxicity problems in sea water would likely be short-lived, or in close proximity to the point of effluent discharge. Although there would be additional chlorination by-products which may be of concern, there are still many unanswered questions.

Many studies have been performed on acute effects, but for several reasons we feel that long-term exposures will be the major problem posed by chlorine on aquatic life. Basically, these reasons are: (1) rapid degradation of the acutely toxic forms, with the resultant by-product formation; (2) dilution of the acutely toxic forms by receiving waters, (3) state and federal regulations and recent information on the required dosage levels, which tend to decrease the levels of acutely toxic forms in the aquatic environments. Thus, the basic concern will be aquatic exposures to either continuous low levels of TRC1 or TR0, or chlorination by-products.

The goals of our freshwater and sea water chlorination study program are shown in Figure 4. The freshwater portion of the program is being conducted by D. R. Anderson in Richland, Washington, which is approximately 200 miles east of Seattle. The sea water portion of the program is being conducted by Thatcher at the Sequim, Marine Research Laboratory, which is located about 50 miles northwest of Seattle. The analytical chemistry for both freshwater and marine programs is being conducted by Roger Bean, who is at the Battelle Richland Laboratories. Our first goal is to identify chlorination by-products formed in both fresh water and sea water. Roger

is using several gas chromatography (GC) and high pressure liquid chromatography (HPLC) plus final identification with a gas chromatograph/mass spectrophotometer (GC/MS). Samples for chemical analysis are supplied from both the freshwater and sea water portions of the program. These water samples are prepared by pumping from 300 to 600 liters of water over an XAD-2 resin column. The columns are then extracted with ether then methanol which may then be analyzed directly by GC or may be separated by HPLC into fractions containing similar molecular weight compounds which are then analyzed by GC then GC/MS. Details of the analytical technique may be obtained directly from Dr. Bean. The next goal will be to determine chronic toxicity of the chlorination by-products. This will include effects on growth and mortality. In the freshwater phase of the program we are using rainbow trout (Salmo gairdneri). In the sea water phase, we are using the native littleneck clam (Protothaca staminea). Both of these species are important in commercial and recreational fisheries. Another goal will be to study the potential bioaccumulation of any of the chlorination by-products. These studies also will be conducted with the rainbow trout and the littleneck clam. Finally, a goal of the program will then be field verification of the chlorination by-products formed with those formed at operating electrical generating stations.

The program has been in operation for almost 9 months now, and we have some preliminary results. The results are outlined in Figure 5. We are progressing well in the development of specific chlorination by-product analysis techniques in fresh and sea water. So far, we have identified

chloroform, bromoform, and other halomethanes. Also there are many other unidentified peaks on the GC scans whose identities are being pursued. The analytical technique development for chlorination by-products in tissue samples will begin soon.

In the freshwater phase of the program, some trout mortalities have shown up after 4 months of exposure to very low levels of TRCl in the freshwater studies. The highest TRCl concentration in these chambers is about 0.04 mg/l, and noteworthy mortalities have occurred down to concentrations of 50% of the high concentrations. Tissue samples are taken periodically from the trout, and are being frozen until the techniques are developed for the analysis of potential bioaccumulation of the chlorination by-products. In the sea water biology phase of the program, there have been no mortalities to the clams which have been exposed for 6 months. Growth data for these adult clams from the first 6 months was inconclusive. This may be due to the indicator of growth possibly because we were using shell measurements. We are now beginning a new series of exposures, in which we are going to use the bio-energetic approach to the study of growth in addition to tissue weight and shell length measurements. Periodically, we are subsampling and freezing clam tissues to be analyzed for potential bioaccumulation of chlorination by-products later.

We would like to acknowledge the sponsor of this research, the Nuclear Regulatory Commission. We appreciate their support.

This presentation was made at the annual meeting of the Western Region-American Fisheries Society, July 11-13, 1977, at Tucson, Arizona.

Slide 1

CHLORINATED (SALT) WATER

FIGURE 2

E X A M P L E S O F C H L O R O - O R G A N I C F O R M E D (F W)

Chloroform

Chlorosalicyclic Acid

Chlorobenzoic Acid

Chlorophenol

Chlororesorcinol

Chlorouracil

Chlorouridine

Chlorocaffeine

Chloro Amino Purine

Plus 75 or More Others

FIGURE 3

FIGURE 4

G O A L S

Identify Chlorination By-Product Formed in FW and SW

Determine Chronic Toxicity of By-Products in Growth, Mortality, Etc.

Study Bioaccumulation of By-Product in Trout and Clams

Field Verification

FIGURE 5

R E S U L T S

By-Product Analysis Techniques

- Water and Tissue
- Chloroform, bromoform, halo methanes and many peaks by GC not yet identified

Freshwater

- Trout Mortalities
- Tissues Waiting

Sea Water

- Growth Data
- Tissues Waiting

RECENT RESEARCH ON CHLORINATION BY-PRODUCTS
IN FRESH AND MARINE WATERS

Presented at the American Fisheries Society International Meeting in November, 1977, Vancouver, B.C.

RECENT RESEARCH ON CHLORINATION BY-PRODUCTS IN FRESH AND MARINE WATERS

Thomas O. Thatcher and David R. Anderson

American Fisheries Society Meeting

November, 1977

Vancouver, British Columbia

This is a light review of current research of chlorination by-products in fresh and marine waters. This work is part of a program studying chlorination by-products sponsored by the Nuclear Regulatory Commission.

Chlorine, either in solid, liquid, or gaseous form, has been used for about a century for a variety of purposes which lead to its eventual discharge into surface waters. Included among these uses are (a) the treatment of potable waters, recreational waters, various waste waters, cooling waters, and (b) applications in numerous industrial processes. Although the water and waste treatment uses are more universally recognized, industrial applications constitute a significant portion of the total used. For example, in the early 1970s, approximately 2 million tons of chlorine were used in pulp and paper manufacturing annually, compared to about 1/10-1/2 million tons for various sanitation purposes. In other words, approximately 10 times more is used in the pulp and paper industry than for sanitation.

Aqueous chlorine species usually form HOCl (hypochlorous acid), OCl^- (hypochlorite ion), and chloramines or bromamines. These are very reactive chemically and biologically which is why they are in common use as disinfectants or biocides. A simplified characterization of this reaction process, which occurs when chlorine is added to water, is shown in Slide 1. As you can see, depending on the quality of the receiving water, the reaction series can be quite complex. This is true in fresh water alone, but when sea water is

involved, the possibilities are multiplied. Considering the entire series of possible reactions, a wide variety of halogenated by-products may be formed: inorganic halides which are relatively nontoxic and are not shown here; chloramines or bromamines, which may be inorganic or organic; and, halogenated organics. The intent of this presentation is to focus on the bottom line compounds shown in this diagram--the organic compounds formed which contain halogens as a result of a chlorination process. For this overview, I have relied on two principal sources. First was the available literature, including reports presented at several 1975 and 1976 conferences involving effects of chlorination practices, and, second--for the most up-to-date information--was through personal communication with researchers actively engaged in chlorine studies.

The reasons for concern about chlorinated or halogenated organic materials are based primarily on two factors:

1. their typical attraction for lipids or fats and the consequential potential for accumulating in living organisms and being transferred through food chains, and
2. their potential for toxic effects on aquatic organisms resulting from exposure to halogenated water or from consuming food organisms containing accumulations of them.

As you know, a major class of insecticides contains chlorinated organics. These are principally, if not entirely, composed of molecules containing numerous chlorine atoms. Katz and Cohn, in a 1976 paper, observed that in considering these polychlorinated insecticides, the toxicity is generally related to the number of chlorines added to the parent compound. Some of these toxic compounds or similar compounds may be formed spontaneously at ambient

temperature in the presence of "excess" chlorine without catalysts other than sunlight. As examples, they list the formation of several familiar insecticides.

As far as I have been able to determine, none of these insecticides have been reported to be formed by real-world chlorination processes that I am considering here. However, the fact that the nearly universal presence of some of these chlorinated materials has not been definitely attributed to everyday chlorination practices, does not mean that these reactions do not occur. It may reflect the lack of research funding allotted to investigating these potential sources.

The widespread realization of the presence of a potential problem due to the chlorination of effluents has stemmed primarily from the work of several freshwater investigations in the early 1970s. These studies with sewage effluents and surface waters, in particular the work of Jolley and his associates at Oak Ridge National Lab in Tennessee, Glaze and his co-workers in Texas, and Murphy and others at McMaster University in Ontario, have identified approximately 100 chlorinated by-products in chlorinated sources which were either absent or at lower concentrations before chlorination. The next transparency illustrates examples of some of these compounds. As you can see, depending on the organic precursors present in chlorinated water, a large variety of materials can be generated.

Much of the concern of the earlier studies has been, and continues to be, the effects of these chlorination by-products in human drinking waters. As a result, research into the chemical reactions in sea water, and into the effect of these chlorinated compounds upon fresh and sea water organisms has lagged behind. However, work in both fields is underway now, and results are becoming available.

Freshwater work is going on at a number of labs. I have already mentioned Jolley's work at the Oak Ridge Lab. Other researchers there, including Gehrs and his associates, are looking at potential effects of some of the chlorination by-products which have been identified in sewage effluents. In one study with two such compounds, 4-chlororesorcinol and 5-chlorouracil, reductions in the hatchability of carp eggs were identified at very low concentrations. It is also noteworthy to point out that in another study with 5-chlorouracil, this group of investigators observed spinal deformities in newly hatched carp. This observation causes one to wonder if morphological deformities reported in 3 or 4 other instances of chronic chlorine exposure may, in fact, be due to persistent halogenated organics formed during the experiments. In yet another series of experiments with these same by-products, it was determined that reproductive success of Daphnia magna was reduced at 10 ppb. Elsewhere, the EPA reported that four of the by-products identified by Jolley can cause fish flesh tainting in concentrations from 0.1 to 35 ppb.

The EPA Duluth Lab work by Dough Kuehl, or EPA contract work by researchers in Minnesota and Michigan, have identified a number of chlorinated compounds in tissues of freshwater fish exposed to chlorinated sewage effluent. In a 1976 report, they list di and trichlorophenols, di and trichlorobenzenes and trichloroanisoles as having been found in all fish (fathead minnows) raised in the treated sewage. These compounds were absent or at lower concentrations in fish from untreated effluents. More recently, they have identified some 20 brominated compounds in tissues of fathead minnow exposed to sewage effluents treated by bromine chloride. These by-products included brominated compounds including phenols, anisoles, and indoles, and suggest compounds to evaluate when the potential environmental problems of sea water chlorination are investigated.

Two other research reports could be mentioned here. Carlson and his co-workers in 1975, and Gaffney in 1977, predict that PCBs can be formed during water and waste treatment processes, and the higher the level of chlorination, the greater the potential.

At our Battelle-Northwest lab in Richland, Washington, we are currently working on a new program sponsored by the Nuclear Regulatory Commission which has the objective of identifying chlorinated organic by-products resulting from the chlorination of fresh water which may be used for cooling purposes at an electrical generating plant. Another objective of equal importance is the determination of the biological significance of these by-products. David R. Anderson is conducting the freshwater biological investigations on rainbow trout Salmo gairdneri. Roger Bean and Bob Riley are the chemists who are developing the analytical techniques for detecting and quantifying the chlorination by-products in the water and fish. They are employing various chromatographic techniques for product separation and Gas Chromatographic-Mass Spectrophotometric analyses for final identification. Chlorinating relatively high quality Columbia River water produces chloroform and volatile electron capturing compounds other than haloforms, but the volatile organic fraction is exceedingly complex. The identification of individual components in these freshwater samples is still continuing. Tissues of rainbow trout which have been exposed to this same chlorinated water have been frozen for analysis after the appropriate techniques are developed or modified as required. In future work, other chlorinated natural freshwaters in the vicinity of operating power plants will be investigated for chlorinated by-products.

Next, I want to talk about research in progress with chlorinated sea water. As I mentioned previously, this work has begun in earnest only in the last few years. Several labs are now involved and I want to describe the work on going at two: The EPA Bears Bluff Lab in South Carolina, and the other being our Battelle Marine Research Lab, located at Sequim, Washington. The research at Sequim is the sea water portion of the same Nuclear Regulatory Commission program underway at our freshwater facility in Richland, which I described earlier.

During sea water chlorination, the natural presence of up to 65 ppm of bromide results in the incorporation of bromine into the by-products, as you will recall from an earlier diagram I presented. As a result, bromoform is the haloform produced in sea water, and it has been the predominating halogenated by-product observed by Bean and Riley in chlorinated Sequim Bay water samples. When chlorination was maintained at approximately 1.5 mg/l measured as is common as Total Residual Oxidant (TRO), up to 30 ppb of bromoform was measured in the sea water. Several other trihalomethanes (such as bromodichloromethane or chlorodibromomethane) were also identified but their concentrations were less than 1 ppb. Many other peaks are present in the chromatograms and identifications are being sought. This work has recently been described in more detail in a paper by Bean, Ryan and Riley entitled "Investigations of Trace Organic Compounds in Chlorinated Natural Waters Using Glass W.C.O.T. Columns, which was presented at the American Chemical Society meeting in Chicago earlier this month.

In our sea water studies, we are using the native littleneck clam as the experimental animal. They were selected because when living in effluent

areas they cannot avoid the halogenated effluents. Clam tissues from chronic exposures to chlorinated water are being kept frozen for analysis. During this next year, we will also be conducting some short-term toxicity tests with bromoform. Additionally, we will be evaluating clam response to chronic exposures by measuring a bioenergetic parameter, the scope for growth. The scope for growth is simply the calories of energy remaining and available for growth after all the other energy requiring needs of the animal have been satisfied. Field verifications of chlorination by-products produced in these experiments will also be sought at operating power plants.

Now a few words about current EPA research. Their chlorine program is principally directed by Will Davis at the Bear's Bluff Lab and includes work by Middaugh and co-workers at that location, and by contractors at the University of Maryland and the University of Miami. The principal results from their research, as well as ours, and those of other research labs across the country will be presented at a Chlorination Conference to be conducted at Gatlinburg, Tennessee, from October 31 through November 4 of this year. A preliminary report of the EPA findings includes hydrogen cyanide in addition to bromoform and other brominated by-products. The hydrogen cyanide apparently resulted from the chlorination of natural humic acids in the sea water. They also report that exposure of chlorinated sea water to sunlight increases formation of halogenated organics--in the case of bromoform, a 10-fold increase was detected. These investigators also are evaluating the bioaccumulation potential for these by-products of chlorination.

It seems appropriate at this point to mention a method that appears to be a very useful tool for biologists and chemists alike in this area of research. Glaze and his associates at North Texas State University have described the

determination of "carbon bound halogen $\mu\text{g/l}$ (ppb) level in water samples with a commercially available instrument and readily obtainable materials" (to use their words). This article appears in the July, 1977, issue of Environmental Science and Technology.

In closing, I feel we are just now really getting into the "meat" of the research in our investigation of the potential environmental impact of these halogenated by-products resulting from various chlorination procedures. Now that water and tissue analytical techniques are being fine tuned, the upcoming research should bring into focus in the near future, the true magnitude of the problem (or the non-problem) in real-world conditions.

Slide 1

CHLORINATED (SALT) WATER

Slide II

E X A M P L E S O F C H L O R O - O R G A N I C S F O R M E D (F W)

Chloroform

Chlorosalicylic Acid

Chlorobenzoic Acid

Chlorophenol

Chlorouracil

Chlorouridine

Chlorocaffeine

Chloroaminopurine

Plus 75 or More Others

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