Biocide By-Products in Aquatic Environments

Final Report
September 10, 1976 - September 30, 1979

Prepared by R. M. Bean, C. I. Gibson, D. R. Anderson

Pacific Northwest Laboratory
Operated by
Battelle Memorial Institute

Prepared for
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Commission

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ABSTRACT

A three-year program has been conducted to study the chemistry and biological effects of products arising from the low-level chlorination of natural waters. These studies are related to environmental concerns arising from the discharge of chlorine-treated power plant cooling water. The studies have shown that addition of low levels (2 mg/l) of chlorine to natural waters produces haloforms in concentrations which are orders of magnitude lower than the LC₅₀'s measured in a number of fresh and salt water organisms. Chlorination also produces nonhaloform lipophilic organohalogen products in concentrations much lower than the haloforms, although no evidence was obtained which suggested significant biomagnification of these during chronic exposure of juvenile salmon to chlorinated fresh water. No dramatic effects were noted in organisms chronically exposed to chlorinated waters, but changes in general condition were observed.

SUMMARY

This report is intended to provide an overview of a three-year program designed to study the chemistry and biological effects of products arising from the low-level chlorination of water. The purpose of the program was to determine the potential effects of the cooling waters discharged by nuclear power plants which control biofouling through the use of chlorine treatments. Emphasis was placed on identifying the major products of chlorination, particularly those products which had a potential for long-term environmental effects, such as bioaccumulation. Biological experiments were conducted in both fresh and marine waters to determine the long-term effects of low levels of chlorination to a variety of aquatic organisms. In addition, experiments were conducted to determine the toxicity and bioaccumulation potential of chloroform to four species of fresh water fish, and of bromoform to five species of marine organisms. These latter experiments were performed because the haloforms were identified as principal products arising from the chlorination of natural waters.

The detailed methodologies, data, and interpretations leading to the observations, results and conclusions presented in this report can be found in six Topical Reports to the Nuclear Regulatory Commission. The titles and document numbers for these reports are listed in the Preface.

The principal organohalogen products formed from the low level chlorination of natural waters are haloforms; bromoform in saline waters, and chloroform in fresh waters. Concentrations of haloforms produced by addition of 2-4 mg/l chlorine are in the range of a few $\mu\text{g/l}$ (parts-per-billion). While other lipophilic organohalogen compounds are produced by the chlorination process, their concentrations appear to be considerably less than the part-per-billion level.

Chronic experiments with low levels of chlorine conducted in fresh water using juvenile trout (Salmo gairdneri) and in sea water using littleneck clams (Protothaca staminea) did not produce obvious or dramatic changes in these organisms. For the clams, there was some evidence of growth inhibition, and poor condition was indicated upon histological examination of the clam tissues.

Acute toxicity and biological uptake experiments with bromoform conducted on five marine species produced 96-hour LC_{50} 's in the range of about 7 to 40 mg/l (parts-per-million), approximately one thousand-fold higher than the bromoform concentrations produced by low-level chlorination of sea water. A similar result was observed for fresh water organisms. Ninety-six hour LC_{50} 's ranged from about 18 to 75 mg/l. Both chloroform in fresh water and bromoform in sea water were found to be rapidly absorbed into the tissues of the organisms investigated, at concentrations approximately that of the surrounding water. Upon transfer to a clean environment, haloforms were found to be rapidly depurated from the tissues of exposed organisms.

These investigations have shown that addition of low levels of chlorine to natural waters produces haloforms in concentrations orders of magnitude lower than measured 96-hr LC₅₀'s in several fresh and salt water organisms. Chlorination also produces nonhaloform lipophilic organohalogen components in concentrations much lower than the haloforms, although no evidence was obtained suggesting significant biomagnification of these components. The findings of this program are in need of verification through chemical and biological field studies conducted at actual nuclear power station locales.

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PREFACE

This is the final report for the program on Biocide By-Products in Aquatic Environments covering the period September 10, 1976 through September 30, 1979. Topical reports prepared for the program are:

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

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DEFINITION OF CHLORINE PRODUCED OXIDANT (CPO)

During the conduct of this research, all measurements of oxidant concentrations produced by the chlorination of water were performed using the amperometric procedure described in Standard Methods* for total available chlorine (free available + combined available chlorine). For many natural waters containing bromide ion, such as estuarine or marine waters, the actual oxidant species may be a combination of both free and available bromine as well as chlorine. For this reason we have used the expression "chlorine produced oxidant" (CPO) uniformly throughout this report. CPO is the concentration of total available oxidant determined by amperometric titration expressed as mg/L chlorine, regardless of the actual oxidizing species in solution.

* Standard Methods for the Examination of Water and Wastewater, 14th Ed. American Public Health Association, American Water Works Association, Water Pollution Control Federation, Washington, D.C. (1975) pp 322-325.

INTRODUCTION

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

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

This report is intended to provide an overview of a three-year program designed to study the chemistry and biological effects of products arising from the low-level chlorination of water. The purpose of the program was to determine the potential effects of the cooling waters discharged by nuclear power plants which control biofouling through the use of chlorine treatments. Emphasis was placed on major products of chlorination, particularly those products which had a potential for long term environmental effects, such as bioaccumulation. Biological experiments were conducted in both fresh and marine waters to determine the long-term effects of low levels of chlorination to a variety of aquatic organisms. In addition, experiments were conducted to determine the toxicity and bioaccumulation potential of chloroform to four species of fresh water fish, and of bromoform to five species of marine organisms. These latter experiments were performed because the haloforms were identified as principal products arising from the chlorination of natural waters.

The following three sections of this report summarize the work carried out under the three major Research Tasks of the Program: Chlorination Chemistry, Freshwater Biology, and Marine Biology. The details of

methods used, complete data sets, interpretations, and preliminary experiments performed are described in six Topical Reports to the Nuclear Regulatory Commission. The titles and document numbers for these reports are listed in the Preface to this report.

CHLORINATION CHEMISTRY

The chemistry studies pursued the following objectives:

- To apply well-documented procedures for sampling, separation, and trace analysis of organohalogen compounds formed from the low-level chlorination of fresh and marine waters.
- To use these methods to study the organic chlorination chemistry of a number of natural water bodies which are actual or potential receiving waters for chlorinated electric power plant cooling water.
- To document the extent to which addition of chlorine to these water bodies results in the formation of lipophilic organohalogen components having the potential for bioaccumulation or biomagnification.
- To determine the extent of formation of other volatile and toxic lower molecular weight halogenated organics; specifically the haloforms and phenols.

In order to accomplish these objectives it was necessary to construct a portable apparatus for the continuous low-level chlorination of natural waters, and to sample and analyze the chlorinated water in a manner consistent with the objectives. Although humic acids from natural waters have been shown to react with chlorine (Oliver, 1978), these materials were not considered in our sampling and analytical scheme because of their relatively low potential for immediate aquatic toxicity or bioaccumulation. The method selected for sampling and concentrating lipophilic halogenated organics is the very well documented XAD resin adsorption technique. The method has been exhaustively studied (Junk et al., 1974, 1976) from the points of view of technique, resin purity, and efficiency of recovery of a wide variety of compound types. The technique has been applied extensively to studies of haloorganics in treated wastewater (Glaze, et al., 1975, 1976, 1977). An extensive review of the technique has been published by Dressler (1979).

The use of these columns precluded the concentration of organics with relatively reactive chlorine-nitrogen bonds, such as the chloramines, since prior to column adsorption, destruction of active chlorine with sodium sulfite was necessary to avoid additional chlorination reaction with the material trapped on the column (Bean, 1978; Glaze, 1977).

The analysis of lipophilic halogen compounds adsorbed on the XAD-2 resin using microcoulometry to determine the total organic chlorine in the adsorbed material is based on the work of Glaze et al. (1977), who suggested that nonhaloform organic halogen trapped on XAD-2 resin was an important water quality parameter. Although we adapted the general

strategy of determining organic halogen, we also subjected the material to further separation steps in order to classify the type of material adsorbed according to molecular weight and polarity.

The analysis of volatile organics in the chlorinated and control waters was accomplished using both "headspace" (Bush et al., 1977) and "purge-and-trap" methods (Bellar and Lichtenberg, 1974). Methods of this general type have been reviewed recently by Drozd and Novak (1979).

Halogenated phenols are not necessarily considered lipophiles since they do not adsorb well on XAD-2 at neutral or basic pH (Junk et al., 1974). However, we chose to add phenols to our analytical scheme because they are a chemical class which is likely to be formed from the chlorination of natural waters.

Substantial effort was made during the course of this program to document the methods used with respect to reproducibility, reliability, and efficiency of recovery. A complete discussion can be found in Bean et al. (1980).

Sampling Chlorinated Natural Waters at Ten U.S. Locations

Ten sites were chosen for sampling (Figure 1). Portable equipment was constructed which continuously pumps water from the natural environment and treats it with from 2 to 5 parts-per-million chlorine as NaOCl (Figure 2). A schematic of the apparatus is shown in Figure 3. A baffled chlorine contact chamber allows a residence time of about an hour before the chlorinated water is treated with Na₂SO₃ and brought to a pH of ~4.5 with reagent grade H₂SO₄ by an automatic pH controller. The treated water is then pumped through the XAD-2 column using a positive displacement pump. Two identical devices are used, one for the chlorinated samples and one to simultaneously sample unchlorinated water as a control (Figure 4). To minimize contamination of water samples by the sampling apparatus, the only materials in contact with the sample are polypropylene, teflon, ceramic, and stainless steel.

Other types of water samples were taken from both chlorinated and unchlorinated samples for analyses by methods for which the high volume samples are not appropriate.

- Purge-and-Trap Samples--These samples were analyzed for volatile components using the purge-and-trap technique of Bellar and Lichtenberg (1974).
- Headspace Samples--These samples were analyzed for haloform according to the method of Bush et al. (1977).
- Bulk Samples--Approximately 5 liters of untreated water were sampled in bulk as a contingency sample.

FIGURE 1. Locations of Sampling Stations

FIGURE 2. Portable Apparatus Constructed to Pump Natural Water and Add Chlorine

PUMP A - TUBING PUMP (FLOW RATE 0.06 - 2280 ml/min)
 PUMP B } PERISTALTIC
 PUMP C } (FLOW RATE 0.7-70 ml/hour)
 PUMP D } POSITIVE DISPLACEMENT PUMP
 PUMP E } (FLOW RATE 0-450 ml/min - pressures to 100 psig)

FIGURE 3. Schematic of Apparatus for field Sampling of Chlorinated Natural Waters

FIGURE 4. Duplicate Apparatus on Beach at Station 10, San Onofre (left) and Station 4, Lake Michigan

Results

Data on volatile components was generated primarily using the Purge-and-Trap (P&T) technique, since the method is suitable for the identification and quantification of many important volatile toxic organic chemicals, including twenty-three compounds listed by the Environmental Protection Agency as priority pollutants.

The results of the P&T analysis for nine stations are presented in Table 1. The data are arranged such that the results for the chlorinated water samples are presented at the upper left-hand corner of each box, and the results for the corresponding unchlorinated control are presented in the lower right-hand corner of the same box.

As reported by many workers, haloforms are found to be the principle volatile products arising from the chlorination of natural waters. The data presented in the Table are in complete agreement with these findings. The waters containing significant quantities of sea water (Cape Fear, 50% sea water; San Onofre, 100%) produced primarily bromoform, consistent with previous observations (Carpenter, 1978; Helz, 1979). The concentrations of haloforms varied widely among locations, however. Chloroform production at fresh water locations varied from 2 $\mu\text{g}/\text{l}$ (L. Michigan) to 25 $\mu\text{g}/\text{l}$ (Tennessee R.). Traces of chloroform were found in seven of nine control waters.

A major observation to be made about the waters sampled and analysed by P&T is that they were remarkably free from contamination by volatile nonhaloform organic compounds. Although each GC/MS run was searched using single ion reconstruction technique for all of the 23 priority pollutants, Table 1 lists all of the compounds actually found in the samples which were not found in procedural blanks. Concentrations of haloforms found in our samples were on the same order of magnitude as those found in many drinking waters.

The samples taken by pumping large volumes of water over XAD resins were extracted with ether and the extracts analyzed for chlorinated organic material which was not haloform. The nonhaloform organohalogen material was further separated into three fractions of increasing polarity using silica gel chromatography. The ether extracts and the corresponding fractions were analyzed for total organic halogen by microcoulometry. This data was combined with the Purge-and-Trap data to compare the amount of halogen added as chlorine with the amount of halogen showing up as either haloform or nonhaloform organic chlorine in the chlorinated water. These results, shown in Table 2, show that on the order of one percent of the added chlorine was used to form haloforms, while the amount of chlorine used to form nonhaloform organohalogen material was much less. Thus, most of the added chlorine, probably greater than 95%, does not form lipophilic organohalogen material.

TABLE 1. Concentrations of Volatile Organics in Natural Waters as Analyzed by the "Purge and Trap" Method (concentrations are reported in micrograms per liter)

STATION (DATE) (CI ADDED mg/l)	DICHLORO METHANE	CARBON DISULFIDE	CHCl ₃	CHCl ₂ Br	CHClBr ₂	CHBr ₃	DICHLORO ETHYLENE	TRICHLORO ETHYLENE	TOLUENE
1. COLUMBIA R. (WA) (5/79) (2.7-3.0)	CI 1 N 1	720 1	12.0,13.5 tr	tr —	— —	— —	— —	* *	++ ++
3. OHIO R (WV) (8/78) (4.0-5.3)	CI 9.9 N 11	>20,>20 >20	7.3,5.7 0.6	3.4,3.1 —	0.9,0.9 —	* —	* *	— —	tr tr
4. L. MICHIGAN (MI) (8/78) (3.0-3.8)	CI 17,14 N 5	>20,>20 >20	2.7,2.0 tr	1.8,1.6 —	0.8,0.5 —	— —	— —	— —	tr tr
5. MISSOURI R. (MO) (9/78) (3.6-4.8)	CI 15 N 15	>20 >20	11.5 tr	10.3 —	5.8 —	* —	— —	— —	tr tr
6. TENNESSEE R. (KY) (9/78) (4.3-4.7)	CI 17,17 N 13	>20,>20 >20	21.2,24.7 tr	6.0,7.4 —	1.1,1.1 —	* *	— —	— —	tr tr
7. CAPE FEAR (NC) (10/78) (4.8-5.7)	CI 1.3 N 3	12,10 >20	tr tr	1.9,2.6 —	15.7,17.8 —	54.5† *	— *	— —	++ ++
8. L. NORMAN (NC) (10/78) (4.1)	CI 3.4 N 3	>20,19 >20	4.1,3.1 —	1.7,1.4 —	tr —	* —	* —	* —	++ ++
9. CONNECTICUT R. (CT) (10/78) (4.3-5.0)	CI 1 N 1	>20 >20	18.7,24.5 tr	2.3,3.4 —	tr —	* —	— *	— —	++ ++
10. SAN ONOFRE (CA) (2/79) (2.9-3.2)	CI 6 N 1	— —	— —	— —	tr —	13.0,17.0 —	— —	* *	++ ++

— = not detected

tr = trace (present in concentrations less than 0.5 µg/l)

* = shown to be present in single ion reconstructed chromatogram

++ = present at the several µg/l level

† = analyzed using XAD-2 method

TABLE 2. Halogen Recovery in Organic Fractions Expressed as $\mu\text{g}/\ell$ Chlorine

STATION (DATE) (Cl ADDED mg/l)		CHLORINE TO HALOFORMS (PURGE & TRAP)	CHLORINE TO NONHALOFORMS (<800 MW)	CHLORINE TO SILICA GEL FRACTIONS		
				HEX/ETHER	ETHER	MeOH
1. COLUMBIA R. (WA) (5/79) (2.7-3.0)	‡Cl	11.9	.37	.19	.07	.06
	‡N	tr	.06	.01	.04	.01
2. SEQUIM BAY (WA) (8/78) (1.8)	Cl	8.7*	1.48	.37	.23	.04
	N	nd	.09	.01	.01	.02
3. OHIO R. (WV) (8/78) (4.0-5.3)	Cl	8.4	.49,.62	.07,.13	.07,.07	.02,.09
	N	0.5	.18	.05	.06	.01
4. L. MICHIGAN (MI) (8/78) (3.0-3.8)	Cl	3.5	.39,.40	.10,.11	.06,.04	.01,.01
	N	tr	.08	.02	.02	.01
5. MISSOURI R. (MO) (9/78) (3.6-4.8)	Cl	19.9	.84,.99	.20,.22	.32,.18	.07,.07
	N	tr	.16	.04	.07	.03
6. TENNESSEE R. (KY) (9/78) (4.3-4.7)	Cl	25.4	1.26,1.28	.35,.21	.40,.39	.21,.14
	N	tr	.12	.06	.03	.06
7. CAPE FEAR (NC) (10/78) (4.8-5.7)	Cl	32.9*	3.14	.94	.32	nd
	N	tr	.29	.03	.22	.01
8. L. NORMAN (NC) (10/78) (4.1)	Cl	4.2	1.04,1.03	.21,.23	.42,.37	.16,.08
	N	nd	.05	.02	.02	.02
9. CONNECTICUT R. (CT) (10/78) (4.3-5.0)	Cl	21.1	.57	.15	.30	.07
	N	tr	.15	.05	.06	.02
10. SAN ONOFRE (CA) (2/79) (2.9-3.2)	Cl	6.3	1.43	.58	.43	.13
	N	nd	.06	.01	.03	.01

‡Cl = Chlorinated

N = Nonchlorinated

*Bromoform analysis performed using XAD-2 adsorption method

TABLE 3. Phenols Identified in Chlorinated Water Samples.^(a) (Retention times for phenols relative to trichlorophenol)^(b)

Phenolic Compound	#1 Columbia River	#3 Ohio River	#5 Missouri River	#6 Tennessee River	#7 Cape Fear	#8 Lake Norman	#9 Connecticut River
Chlorophenol			0.637			0.680	0.693(?)
Methylchlorophenol		0.802	0.807				0.830
Dimethylchlorophenol				1.036			1.021
Dichlorophenol	0.854 0.885	0.815(?) 0.850	0.821	0.821 0.860		0.844(?)	0.855
Bromophenol			1.086(?)				
Methyldichlorophenol	1.055(?)		1.081				0.989 1.064(?)
Dimethyldichlorophenol							1.131
Trichlorophenol	1.000	1.000(?)	1.000	1.000	1.000	1.000	1.000
Bromochlorophenol			0.946	0.955			0.961
Bromodichlorophenol			1.130 1.139(?)	1.135 1.149(?)	1.144 1.135		
Dimethyldibromophenol					1.423		
Dibromochlorophenol			1.265 1.269		1.265 1.274		
Tribromophenol					1.395		

^a A question mark indicates that spectral quality left doubt as to identity

^b Two retention times indicates two isomers found

FRESHWATER BIOLOGY

The study on effects of chlorination by-products in freshwater systems was conducted in two phases: first, chronic exposure of freshwater fish to chlorination by-products was undertaken to determine long range effects on mortality, growth, and condition; second, the effects of chloroform on mortality and bioaccumulation were investigated on four freshwater fish species.

Chronic Effects of Chlorination By-Products

The chronic chlorination by-products study of rainbow trout was made on an exposure system that was designed to maximize production of chlorination by-products at chlorination levels of 1-2 ppm under a temperature regime which could be expected to occur under normal power plant operations. Chlorinated and unchlorinated river water were proportionally mixed to produce the maximum test concentration of approximately 0.02 ppm chlorine produced oxidant (CPO) which is considered the 100% test group. The four other test groups were based upon dilutions of 75%, 50%, 25%, and 12% of the 100% test concentration and a control.

Initially, two hundred fish were randomly selected and placed in each duplicate aquarium for each test concentration (Figure 5). The sequence of test concentrations was selected from a random numbers table.

Test mortality was low, approximately one fish per aquarium per week. There was no apparent effect on mortality of chronic exposure to low level CPO at any concentration tested. However there was a statistically significant difference between the 50% group and controls indicating the controls were in better condition than the 50% group. This was corroborated also by the length and weight differences between control and the 50% group. The observed difference in the 50% test group was not judged to be a result of the exposure to CPO or chlorination by-products. Results of morphological measurements of fish from each subsample in the test reveal little difference in mean and range for fork length, weight and condition factor at each subsample. An increase in both length and weight with time was found, as expected.

The results have indicated that there was no effect on growth or mortality resulting from chlorination by-product toxicity. Nevertheless, an effect on the health of the test fish as indicated by condition factor was found.

Chloroform was found in fish tissues, but no difference was noted between exposed and control fish. The absence of a difference in levels between the two groups is not surprising considering the chloroform level in the control aquarium water was approximately one quarter the level found in the treated water. The identity and concentrations of chloroform in the tissues of exposed fishes was confirmed by GC/MS.

FIGURE 5. Exposure System for Chlorination By-Products

Acute Toxicity and Bioaccumulation of Chloroform

Objectives of this phase of the study were to determine the acute toxicity and bioaccumulation of chloroform, a major fresh water chlorination by-product, to four species of fresh water fishes. Choice of chloroform as a toxicant was based on results of the analytical phase of the Chlorination By-Products Program. Studies conducted to determine the biologically available chlorinated organics produced from chlorination of fresh water across the United States indicated that chloroform was the major chlorination by-product produced in fresh water. Therefore we conducted acute (96-hr) toxicity studies with rainbow trout (Salmo gairdneri), bluegill (Lepomis macrochirus), channel catfish (Ictalurus punctatus) and largemouth bass (Micropterus salmoides). The species were selected because of their economic and ecological importance in aquatic ecosystems near power plants using fresh water as a secondary coolant. A major objective of the acute toxicity tests was to provide data for the evaluation of potential toxicity resulting from chloroform produced during power plant chlorination. Acute 96-hr LC₅₀'s were determined and compared to expected levels of chloroform produced during power plant chlorination.

Chloroform saturated water cannot be effectively prepared by simply stirring the two together due to the slow rate of solution, so a flow-through toxicant delivery system was constructed (Figure 6) that produced a continuous supply of stock solution saturated with chloroform at 8000 ppm. A complete description of the apparatus can be found in Anderson and Lusty (1980). Toxicity studies were conducted on the four species for 96 hr.

In order to minimize effects of fish growth on the test results, the complete series of toxicity tests for each species except for bluegill were completed before testing another species. Testing was suspended for bluegill during an outbreak of columnaris infection. Apparently the stress of handling fish prior to the outset of the bioassay was sufficient to cause an outbreak of the disease. Chloroform concentrations greater than 14.9 ppm were sufficient to inhibit or reduce the columnaris infection.

The experimental design of the toxicity tests is in accordance with that recommended by Sprague (1973). Chloroform concentration sequence in the test system is random. The sequence of loading fish into the aquaria for each test is performed following a random numbers table. This randomization minimizes the possibility of the testing system arrangement and fish loading selectivity on the outcome of the toxicity test.

FIGURE 6. Delivery System Used for Acute Chloroform Tests of Fresh Water Species

Statistical analyses of the toxicity test results were analyzed on a PDP 1170 computer. Stephan's (1977) program was used to compute the LC₅₀'s and 95% confidence intervals. The program computes LC₅₀'s by probit, moving average and binomial computational procedures.

Test fish can be placed in two groups by sensitivity to chloroform. The first group, trout and bluegill, with 96-hr LC₅₀'s less than 25 ppm chloroform and the second group, largemouth bass and catfish, with LC₅₀'s greater than 25 ppm (Tables 4-7).

Distinct behavioral differences were noted between largemouth bass exposed to toxic levels of chloroform and controls. Exposed fish exhibited color changes, and "head down" position while resting. Occasionally the fish in the "head down" posture would swim energetically and erratically colliding with the top and sides of the aquarium. The energetic swimming was intermittent and occasionally concomitant with a sudden noise in the room. The behavioral response may be described as an excessive startle response.

Preliminary studies were conducted with trout tissues in order to determine the number of methanol extractions required to remove chloroform from fish tissue. These preliminary studies indicated that 97.5% of the chloroform was removed during the first two chloroform extractions of fish tissue. Only in very few instances was any chloroform found in the third extraction. The samples were analyzed in a random order to minimize analytical bias.

Rainbow trout exhibited the highest bioaccumulation factor of any of the species tested. Within 1 hr of exposure, the tissue level of chloroform in trout was 4 µg of chloroform per gram of fish tissue. This is the approximate tissue level achieved in other test species at 4 hr of exposure. Chloroform level in trout tissue then nearly doubled to 7 µg per gram of tissue at 4 hr. At the 24 hr sampling, the mean chloroform concentration of 5.5 µg CHCl₃/g tissue, was lower than at 4 hr. Preliminary tests were conducted with a much higher level of chloroform than the one per million level finally used to determine the period of exposure and depuration. They showed that chloroform levels did not increase from 4 to 8 hr. The 8-hr sampling time was eliminated and replaced by a 24-hr sampling to provide time for an equilibrium between fish and water concentrations to occur.

Fish sensitivity to chloroform as indicated by 96-hr LC₅₀'s ranges from 18 ppm for rainbow trout and bluegill to 51 and 75 ppm for largemouth bass and channel catfish, respectively. Although the 96-hr LC₅₀'s provide an indication of the sensitivity of fish to chloroform, examination of the mortality rates during each toxicity test provides additional information. Mortality rates for bluegill and largemouth bass were high during the first day of exposure with little further mortality occurring later in the toxicity test. Trout and catfish, which had the

TABLE 4. Acute Chloroform LC₅₀'s (ppm) With Largemouth Bass at 19°C in Columbia River Water

Census Times	12 hr	24 hr	48 hr	96 hr
Toxicity Test No.				
16	a	a	a	a
17	56.2	56.2	b	b
18	50.4	50.4	50.4	c
19	55.8	55.8	55.8	55.8
20	52.5	52.5	52.5	52.5
21	45.4	45.4	45.4	45.4

a No mortalities occurred in all test groups.

b Only one mortality occurred, insufficient data for LC₅₀ calculation.

c Test terminated prior to the 48-hr census time.

TABLE 5. Acute Chloroform LC₅₀'s (ppm) With Channel Catfish at 19°C in Columbia River Water

Census Times	12 hr	24 hr	48 hr	96 hr
Toxicity Test No.				
11	a	a	b	b
22	a	a	a	a
25	a	a	a	a
26	135 ^c	135 ^c	b	b
27	126 ^d	126 ^d	101 ^e	b
28	a	a	a	75 ^d

a Insufficient mortality to compute an LC₅₀.

b Test terminated.

c Binomial method.

d Moving average method.

e Probit method.

TABLE 6. Acute Chloroform LC₅₀'s (ppm) With Bluegill at 25°C in Columbia River Water

<u>Census Times</u>	<u>12 hr</u>	<u>24 hr</u>	<u>48 hr</u>	<u>96 hr</u>
<u>Toxicity</u> <u>Test No.</u>				
2	17.1	17.1	16.3	16.2
3	23.9	21.8	a	a
8	24.2	24.2	23.1	22.3
9	16.2	16.2	14.6	13.3
10	24.4	20.2	19.4	18.3
12	24.1	24.1	22.3	20.8

a Test terminated prior to 48-hr census time due to disease mortality in the control aquarium.

TABLE 7. Acute Chloroform LC₅₀'s (ppm) With Rainbow Trout at 13°C in Columbia River Water

<u>Census Times</u>	<u>12 hr</u>	<u>24 hr</u>	<u>48 hr</u>	<u>96 hr</u>
<u>Toxicity</u> <u>Text No.</u>				
5	-	-	21.4	18.2
6	-	-	-	18.4
7	37.1	26.1	23.6	22.1
13	-	-	18.6	15.1
15	24.5	20.0	19.3	17.1

lowest and highest 96-hr LC₅₀'s respectively, demonstrated similar mortality patterns at test concentrations. They tended to exhibit an initial tolerance and low mortality with increasing mortality later in the toxicity test. Although with relatively high chloroform concentrations in regard to the LC₅₀, mortality was high during the initial portion of the test.

Toxicity Test No.		Toxicity Test No.	
1	17.1	8	24.1
2	23.9	9	24.1
3	24.2	10	24.1
4	24.2	11	24.1
5	24.2	12	24.1
6	24.2	13	24.1
7	24.2	14	24.1
8	24.2	15	24.1
9	24.2	16	24.1
10	24.2	17	24.1
11	24.2	18	24.1
12	24.2	19	24.1
13	24.2	20	24.1
14	24.2	21	24.1
15	24.2	22	24.1
16	24.2	23	24.1
17	24.2	24	24.1
18	24.2	25	24.1
19	24.2	26	24.1
20	24.2	27	24.1
21	24.2	28	24.1
22	24.2	29	24.1
23	24.2	30	24.1
24	24.2	31	24.1
25	24.2	32	24.1
26	24.2	33	24.1
27	24.2	34	24.1
28	24.2	35	24.1
29	24.2	36	24.1
30	24.2	37	24.1
31	24.2	38	24.1
32	24.2	39	24.1
33	24.2	40	24.1
34	24.2	41	24.1
35	24.2	42	24.1
36	24.2	43	24.1
37	24.2	44	24.1
38	24.2	45	24.1
39	24.2	46	24.1
40	24.2	47	24.1
41	24.2	48	24.1
42	24.2	49	24.1
43	24.2	50	24.1
44	24.2	51	24.1
45	24.2	52	24.1
46	24.2	53	24.1
47	24.2	54	24.1
48	24.2	55	24.1
49	24.2	56	24.1
50	24.2	57	24.1
51	24.2	58	24.1
52	24.2	59	24.1
53	24.2	60	24.1
54	24.2	61	24.1
55	24.2	62	24.1
56	24.2	63	24.1
57	24.2	64	24.1
58	24.2	65	24.1
59	24.2	66	24.1
60	24.2	67	24.1
61	24.2	68	24.1
62	24.2	69	24.1
63	24.2	70	24.1
64	24.2	71	24.1
65	24.2	72	24.1
66	24.2	73	24.1
67	24.2	74	24.1
68	24.2	75	24.1
69	24.2	76	24.1
70	24.2	77	24.1
71	24.2	78	24.1
72	24.2	79	24.1
73	24.2	80	24.1
74	24.2	81	24.1
75	24.2	82	24.1
76	24.2	83	24.1
77	24.2	84	24.1
78	24.2	85	24.1
79	24.2	86	24.1
80	24.2	87	24.1
81	24.2	88	24.1
82	24.2	89	24.1
83	24.2	90	24.1
84	24.2	91	24.1
85	24.2	92	24.1
86	24.2	93	24.1
87	24.2	94	24.1
88	24.2	95	24.1
89	24.2	96	24.1
90	24.2	97	24.1
91	24.2	98	24.1
92	24.2	99	24.1
93	24.2	100	24.1

a Test terminated prior to 48-hr census time due to disease mortality in the control aquarium.

TABLE 2. Acute Chloroform LC₅₀'s (ppm) with Rainbow Trout at 12°C in Columbia River Water.

Census Times		Toxicity Test No.	
15 hr	24 hr	48 hr	96 hr
1	17.1	24.2	24.2
2	23.9	24.2	24.2
3	24.2	24.2	24.2
4	24.2	24.2	24.2
5	24.2	24.2	24.2
6	24.2	24.2	24.2
7	24.2	24.2	24.2
8	24.2	24.2	24.2
9	24.2	24.2	24.2
10	24.2	24.2	24.2
11	24.2	24.2	24.2
12	24.2	24.2	24.2
13	24.2	24.2	24.2
14	24.2	24.2	24.2
15	24.2	24.2	24.2
16	24.2	24.2	24.2
17	24.2	24.2	24.2
18	24.2	24.2	24.2
19	24.2	24.2	24.2
20	24.2	24.2	24.2
21	24.2	24.2	24.2
22	24.2	24.2	24.2
23	24.2	24.2	24.2
24	24.2	24.2	24.2
25	24.2	24.2	24.2
26	24.2	24.2	24.2
27	24.2	24.2	24.2
28	24.2	24.2	24.2
29	24.2	24.2	24.2
30	24.2	24.2	24.2
31	24.2	24.2	24.2
32	24.2	24.2	24.2
33	24.2	24.2	24.2
34	24.2	24.2	24.2
35	24.2	24.2	24.2
36	24.2	24.2	24.2
37	24.2	24.2	24.2
38	24.2	24.2	24.2
39	24.2	24.2	24.2
40	24.2	24.2	24.2
41	24.2	24.2	24.2
42	24.2	24.2	24.2
43	24.2	24.2	24.2
44	24.2	24.2	24.2
45	24.2	24.2	24.2
46	24.2	24.2	24.2
47	24.2	24.2	24.2
48	24.2	24.2	24.2
49	24.2	24.2	24.2
50	24.2	24.2	24.2
51	24.2	24.2	24.2
52	24.2	24.2	24.2
53	24.2	24.2	24.2
54	24.2	24.2	24.2
55	24.2	24.2	24.2
56	24.2	24.2	24.2
57	24.2	24.2	24.2
58	24.2	24.2	24.2
59	24.2	24.2	24.2
60	24.2	24.2	24.2
61	24.2	24.2	24.2
62	24.2	24.2	24.2
63	24.2	24.2	24.2
64	24.2	24.2	24.2
65	24.2	24.2	24.2
66	24.2	24.2	24.2
67	24.2	24.2	24.2
68	24.2	24.2	24.2
69	24.2	24.2	24.2
70	24.2	24.2	24.2
71	24.2	24.2	24.2
72	24.2	24.2	24.2
73	24.2	24.2	24.2
74	24.2	24.2	24.2
75	24.2	24.2	24.2
76	24.2	24.2	24.2
77	24.2	24.2	24.2
78	24.2	24.2	24.2
79	24.2	24.2	24.2
80	24.2	24.2	24.2
81	24.2	24.2	24.2
82	24.2	24.2	24.2
83	24.2	24.2	24.2
84	24.2	24.2	24.2
85	24.2	24.2	24.2
86	24.2	24.2	24.2
87	24.2	24.2	24.2
88	24.2	24.2	24.2
89	24.2	24.2	24.2
90	24.2	24.2	24.2
91	24.2	24.2	24.2
92	24.2	24.2	24.2
93	24.2	24.2	24.2
94	24.2	24.2	24.2
95	24.2	24.2	24.2
96	24.2	24.2	24.2
97	24.2	24.2	24.2
98	24.2	24.2	24.2
99	24.2	24.2	24.2
100	24.2	24.2	24.2

MARINE BIOLOGY

The Marine and Estuarine Biology Task was designed and performed as a supportive effort to the main objective of the program. The primary objective of this task was to provide a laboratory system capable of producing chlorinated sea water that would be representative of cooling system discharge water from a steam electric station, using unpolluted sea water as cooling system circulating water. In addition to this primary objective, data on the biological effects of chlorinated sea water and its by-products were to be obtained as time and resources permitted. Where halogenated by-products of sea water chlorination were identified, preliminary investigation of their toxicity and potential for bioaccumulation were to be tested.

Using these guidelines the following program outline was formulated:

- (1) A flow-through sea water system that was chlorinated at a rate of approximately 1.5 ppm Cl_2 would be constructed and operated at the Sequim Marine Research Laboratory. Sequim Bay, the source of sea water for the Marine Research Laboratory, is a relatively pristine body of water that has no local industrial contamination and only minimal potential domestic contamination. Therefore, it was felt that the likelihood of background halogenated organics would be minimal, and the problems of background interference in attempting to identify those compounds created by chlorination would be minimized.

- (2) In conjunction with the operation of this system, littleneck clams, Protothaca staminea, would be exposed to selected dilutions of the chlorinated sea water to look for acute and chronic effects and bioaccumulation of halogenated organics. The littleneck clam was selected because of its economic and recreational importance, the fact that it is nonmobile and, therefore, likely to be subjected to a consistent exposure regime in the natural environment, and the demonstrated bioaccumulation ability of molluscs for halogenated organics.

- (3) When halogenated organic products were identified, their toxicity and bioaccumulation would be tested with representative marine species.

The flowing sea water system, providing a continuous source of chlorinated sea water, began operation in October of 1977. Initially, a system using the Mount-Brungs diluter concept was used. However, because of difficulty in maintaining this type of system with sea water, it was decided to switch to a manifold-type system. The manifold-type system that was developed and put into use in December 1977 is shown in Figure 7. It proved to be a very reliable design and it functioned without problems from December 1977 through November 1978, when long-term testing was terminated. The results of the chemical analysis of water from this system for halogenated organics are discussed in the Chemistry Section of this report.

FIGURE 7. Toxicant Delivery System for Sea Water/Bromoform Bioassays Conducted at Sequim

The littleneck clams exposed to chlorinated sea water were examined for growth effects, histological effects, and bromoform accumulation. Groups of clams were exposed to chlorinated sea water/unchlorinated sea water mixtures that had in-tank Chlorine Produced Oxidant (CPO) concentrations of 0, 6, 12, 25, 50, and 100 $\mu\text{g}/\ell$. For histological purposes, clams were harvested from the exposure tanks after 1, 2, 3, 4, 5, and 6 months of exposure. Clams for growth measurements and bromoform analyses were harvested after 1, 2, 3, 4, 5, 6, and 8 months.

The histological examination of the clams indicated that they were in relatively poor condition after one month in the system. However, after the first month there was a general improvement at all test conditions.

Beyond the second month there was general improvement or maintenance of "status quo" for the organisms in the control, 6, 12, and 25 $\mu\text{g-CPO}/\ell$ test conditions and a general decline at the 50 and 100 $\mu\text{g-CPO}/\ell$ conditions. Figures 8 and 9 compared digestive glands of the clam taken from the control tank after six months to digestive gland of clam exposed to 100 ppb CPO for six months. Figures 10 and 11 compare gills and Figures 12 and 13 compare intestinal tissues after similar treatments.

The growth data showed that the clams did not begin adding new shell in the 25, 50, and 100 $\mu\text{g-CPO}/\ell$ test conditions, but did show positive signs of growth after 5 months in the two lower concentrations and control conditions. A more detailed discussion of the results are provided in Gibson et al. (1980b).

The concentration of bromoform found in the tissues of the clams harvested from the exposure tanks was relatively low (0 to 352 $\mu\text{g}/\text{g}$ tissue) and did not show a pattern of bioconcentration (Table 8). The higher concentrations found in the clams in the first two months may be attributed to better conditions (more lipids) and/or possible contamination of the water supply by bromoform vapor resulting from toxicity tests being conducted in the same room at the same time. Use of bromoform in the room was discontinued on June 17, therefore, the presence of bromoform in tissues after that date would have to be by uptake from the chlorinated sea water or retained from earlier uptake. Data from the uptake and depuration studies indicate that bromoform is depurated from clam tissues in 24 to 48 hours. However, the appearance of bromoform in the controls after June may indicate that very low concentrations may remain. Studies by Bean and Riley (1980) indicate that a 4% conversion of Cl_2 to bromoform expected in the test tank receiving 100% chlorinated sea water would be approximately 60 $\mu\text{g}/\ell$.

CEREBRO-
VISCERAL
CONNECTIVE
NERVE

DIGESTIVE
TUBULES

CONNECTIVE
TISSUE

FIGURE 8. Section Through Digestive Gland of Clam Exposed in Control Tank for 6 Months. Masson's Stain, 10 X Objective.

CEREBRO-
VISCERAL
CONNECTIVE
NERVE

DIGESTIVE
TUBULES

FIGURE 9. Section Through Digestive Gland of Clam Exposed to 100 ppb CPO for 6 Months. Note Necrotic Nerve and Digestive Tubule, Tubule Epithelium, As Well As Loss of Connective Tissue. Sections Tend to Stain Less Well. Masson's Stain, 10 X Objective.

FIGURE 10. Section Through Gill of Clam Exposed in Control Tank for 6 Months. Ordinary Filaments Are Distinct and the Tufts of Laterofrontal and Lateral Cilia Are Easily Seen. Masson's Stain, 10 X Objective.

ORDINARY
FILAMENTS

PIECES OF
SLOUGHED
EPITHELIUM

FIGURE 11. Section Through Gill of Clam Exposed to 100 ppb CPD for 6 Months. Note Necrotic Appearance of Filaments. Tips of Filaments are Indistinct and No Cilia Apparent. Sloughed Epithelium Can be Seen in Section. Masson's Stain, 10 X Objective.

FIGURE 12. Section Through Intestine of Clam Exposed in Control Tank for 6 Months. Note Distinct Cilia Extending Into Lumen of Intestine. Columnar Epithelial Cells Are Healthy Where Attached to Basal Membrane. Masson's Stain, 10 X Objective.

FIGURE 13. Section Through Intestine of Clam Exposed to 100 ppb CPO for 6 Months. Note Increased Vacuolization and Necrotic Zone Near Basal Membrane. Masson's Stain, 10 X Objective.

TABLE 8. Bromoform Concentrations in Clams ($\mu\text{g/g}$ wet wt) Exposed to Chlorinated Sea Water Containing Sublethal Concentrations of Chlorine Produced Oxidant (CPO).

CPO $\mu\text{g/l}$	-----Date of Harvest-----							
	3/1	4/3	5/2	5/30	6/29	8/1	9/5	11/8
Control	0*	0 226 107	0 6 0	12 5 0	N.D.	N.D.	0 10 0	1 9 0 ^a
6		97 166 0	20 15 0	0 0 0	0 0 0	2 0 0	0 0 0	0 ^b
12		33 183 238 296	56 9 0	2 169 0 9	0 0 0	0 10 14 18	1 9 3 20 0	2 40 0 ^c
25		24 123 74 42	72- 13 80 39	348 26 20 9	17 0 0 35	18 14 14 17	0 0 208 0	3 2 2 0 ^d
50		107 34 97 352	21 16 6 82	7 11 1 8	13 44 25 41	4 0 0 14	0 0 0 0	2 0 ^e
100		72 95 103 89	150 153 64 60	14 43 46 32	N.D.	0 22 26 8	6 2 0 2	18 28 22 0 ^f

* Represents 13 individuals

a Represents 9 individuals

b Represents 11 individuals

c Represents 4 individuals

d Represents 7 individuals

e Represents 6 individuals

f Represents 4 individuals

The finding of bromoform in the control system raises questions about the possible compounding effect this could have had on the organisms in the growth and histological studies. An examination of the data does not reveal any such effect. The phenomena noted (growth inhibition and general cellular degradation) maintain a trend that was consistent through the whole exposure period and directly related to CPO concentrations. The bromoform was present in the room only through the first 4 months of the study and would have been in equal concentrations in all systems. Although these observations do not rule out combined effects, it appears that the phenomena observed were due to the chlorinated sea water and related to the concentration of CPO present.

Acute Toxicity and Bioaccumulation of Bromoform

Bromoform was identified as the major halogenated organic created by the chlorination of sea water and, therefore, a series of bioassays were undertaken to determine its toxicity to commercially and recreationally important marine species. The species selected by NRC to be tested were the Eastern oyster (Crassostrea virginica), Eastern hardclam (Mercenaria mercenaria), brown shrimp (Penaeus aztecus), Menhaden (Brevoortia tyrannus), and littleneck clam (Protothaca staminea). Toxicity testing was done in flow-through systems that had bromoform injected via spragging the sea water with air saturated with bromoform. The testing of bromoform for toxicity was difficult because of its volatility, the difficulty in getting it into solution, and the narcotic effect on organisms. However, the 96-hr LC₅₀'s (Table 9) show that it requires concentrations 2 to 3 orders of magnitude above those expected to be produced via normal chlorination practices to produce acute effects. Details of the testing methods and results are presented in Gibson et al. (1979).

Bioaccumulation and depuration studies with bromoform were conducted with the same five marine species. Again, the establishment of constant concentration of bromoform in seawater presented a major problem. However, the results indicate that for the mollusks (oysters, eastern clams, and littleneck clams) the body burdens were similar to water concentrations, whereas, for the brown shrimp and menhaden there were indications of biomagnification at lower water concentrations but that a maximum body burden was attained. In all species, the bromoform was depurated within 24 to 48 hours after exposure was terminated. The details of these tests are provided in Gibson et al. (1980a).

TABLE 9. Mortality and Average Exposure Concentrations for the 96-hr Exposure of Shrimp, Clams, Menhaden and Oysters to Bromoform.

Water Concentrations mg/l	% Mortality after 96 hours	% Mortality after 3 days
Shrimp:		
57	100	
47	100	
43	100	
39	85	
31	40	
26.5	40	
19	15	
16	15	
9.4	5	
3.3	0	
* Calculated LC ₅₀ - 26 mg/l - 95% Confidence Interval 33 mg/l to 20 mg/l		
Menhaden:		
39.5	100	
19.75	100	
13.8	100	
8.55	30	
6.0	10	
* Calculated LC ₅₀ - 12 mg/l - 95% Confidence Interval 15 mg/l to 9 mg/l		
Oysters:		
39.5	10	50
19.75	0	20
13.8	0	10
8.55	0	20
6.0	0	20
Control	0	10
Insufficient mortality to calculate LC ₅₀		
Clams:		
62.7	20	
40.5	10	
21.0	0	
9.2	0	
0.5	0	
0.32	0	
Insufficient mortality to calculate LC ₅₀		

* LC₅₀ calculated by the method of Litchfield and Wilcoxon (1949)

CONCLUSIONS

- Chlorination of natural waters in concentrations of a few milligrams per liter results in an increase in the quantity of chlorinated organic material which can be extracted using XAD-2 resins.
- Haloforms are the predominant stable lipophilic products resulting from the low level chlorination of natural waters. Concentrations of haloforms found in these studies ranged from 2 to 55 $\mu\text{g}/\ell$. Haloforms account for most of the organically bound chlorine found in XAD-2 extracts of the water.
- Concentrations of nonhaloform stable lipophilic halogenated compounds produced by low level water chlorination appear to be very low, on the order of nanograms per liter. Less stable products, such as halogenated amines may be found in higher concentrations, but would not be detected with the procedures used.
- Trace amounts of bromide in fresh water bodies have a profound influence on the distribution of haloform types produced by chlorination. Bromide concentrations in excess of ten micrograms per liter produced significant quantities of mono- and dibrominated haloforms.
- Halogenated phenols have been found to be chlorination products in seven of the ten locations. They were not found in chlorinated seawater, but were found at the Cape Fear estuary, which contained fifty percent sea water.
- Given the time and exposure conditions of a 6-mo exposure, there was no apparent long-term effect of low-level chlorination on rainbow trout mortality or growth.
- Rainbow trout tissues from the first chronic chlorination by-products exposure at 6-mo were analyzed for chlorinated organics. No significant differences in chlorinated organics were found between exposed and control fish.
- Measurement of chloroform in the fresh water exposure apparatus using headspace technique shows that chloroform levels range from 1 $\mu\text{g}/\ell$ to 0.5 $\mu\text{g}/\ell$. Chloroform concentrations in the unchlorinated controls range from non-detectable to 0.5 $\mu\text{g}/\ell$.
- Under the test conditions used, CPD concentrations of 50 and 100 $\mu\text{g}/\ell$ had an adverse effect on the growth of littleneck clams. However, the control group and groups exposed to target CPD concentrations of 6, 12, and 25 $\mu\text{g}/\ell$ had positive growth.

- Histological examination of the clams showed stress conditions at the beginning of the exposure, but the clams in the control and lower CPO concentrations (6, 12 and 25 $\mu\text{g}/\text{l}$) recovered while those at the higher concentrations (50 and 100 $\mu\text{g}/\text{l}$) had significant tissue damage at the end of the test period (6 months).
- The ultimate consequences of the lack of growth and tissue damage on the ability of the clams to survive and reproduce was not determined. However, the data indicates that clam populations that are continually exposed to CPO concentrations of 50 $\mu\text{g}/\text{l}$ or higher will be under greater stress than those exposed to concentrations of 25 $\mu\text{g}/\text{l}$ or less.
- The 96-hr LC_{50} 's were 18.2 ppm for rainbow trout and bluegill, 51.2 ppm for largemouth bass and 75 ppm for channel catfish.
- Although chloroform was observed to be lethal to these fish, lethal levels are several orders of magnitude above that expected to be produced under normal power plant operating conditions. Observed changes in fish behavior also occurred at chloroform threshold levels above that expected to be discharged from power plants.
- Chloroform accumulated in fish tissues at three to seven times the water concentration during exposure of up to 4 hr, but rapid depuration of tissue chloroform levels was observed in most species.
- Although most power plants sited on fresh water normally chlorinate intermittently, accumulation of chloroform in fish tissues can be expected during these short term exposures.
- Toxicity tests on marine organisms with bromoform have indicated that for brown shrimp the calculated 96-hr LC_{50} is 26 mg/l. The 96-hr LC_{50} concentration for menhaden was calculated to be 12 mg/l. Behavioral changes were observed in both menhaden and shrimp exposed to sublethal concentrations of bromoform. The extent and consequences of the behavioral changes noted on the survival of the shrimp and menhaden are not known.
- Standard 96-hr LC_{50} values for bromoform were not calculated for littleneck clams, quahogs or Eastern oysters. Based on latent mortalities and mortalities in the 28-day uptake exposures, it is estimated that the 96-hr LC_{50} value would be greater than 30 to 40 mg/l.
- All species tested rapidly took and depurated bromoform. The mollusk species had tissue concentrations that were above water concentrations during the first week of exposure, but the tissue concentrations decreased during the last three weeks of exposure and were reflective of the ambient water concentrations.

- In shrimp and menhaden the tissue concentrations were also highest during the first week of exposure. After that they fell to a concentration of approximately 0.4 $\mu\text{g/g}$ and remained there for the remaining three weeks of exposure. The tissue concentrations of 0.4 $\mu\text{g/g}$ appear to be maintained independent of the water concentration.

RECOMMENDATIONS

- Although haloforms have been identified to be the major products of low-level chlorination of natural waters, with other lipophilic products formed in much lower amounts, a sampling program should be undertaken at nuclear power stations to verify these findings, and to determine the effects of plant operations on the quantities and distributions of halogenated organic products.
- Future research should examine the long term effects of repeated accumulation and depuration of chloroform that fish may experience as a result of power plant chlorination.
- Given the lipophilic nature of chloroform and the high lipid content of fish eggs relatively high chloroform concentrations may occur in the developing gonads of female fish. The implications of this to fish reproduction are unknown.
- Studies of the combined effects of chlorine and chlorination by-products should be conducted since these will occur simultaneously in power plant discharges and the demonstrated effects of chlorine on membrane permeability may exacerbate the effects of chlorination by-products.
- Studies are also recommended in the long-term effects of chloroform on reproduction, carcinogenicity, teratogenicity and mutagenicity in fresh water biota.
- Field sampling of mollusk populations exposed to CPO should be undertaken to verify the existence of similar tissue damage in the natural environment.

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