PNL-2931

INTERIM REPORT

Quarterly Progress Report Covering Period October 1 Through December 31, 1978 Biocide By-Products in Aquatic Environments

March 1979

Prepared for
the U.S. Nuclear Regulatory Commission
under a Related Services Agreement with the
U.S. Department of Energy
Contract EY-76-C-06-1830

Pacific Northwest Laboratory
Operated for the U.S. Department of Energy
by Battelle Memorial Institute

Battelle

PNL-2931

PACIFIC NORTHWEST LABORATORY
operated by
BATTELLE
for the
UNITED STATES DEPARTMENT OF ENERGY
Under Contract EY-76-C-06-1830

PNL-2931

INTERIM REPORT

QUARTERLY PROGRESS REPORT COVERING PERIOD OCTOBER 1 THROUGH DECEMBER 31, 1978 BIOCIDE BY-PRODUCTS IN AQUATIC ENVIRONMENTS

D. R. Anderson
R. M. Bean
C. I. Gibson

P. R. Reed, NRC Project Manager

March 1979

Prepared for
the U. S. Nuclear Regulatory Commission
under a Related Services Agreement
with the U. S. Department of Energy
Contract EY-76-C-06-1830
FIN No. B2098-9

Pacific Northwest Laboratory
Richland, Washington 99352

SUMMARY

Work on the Biocide By-Products Program is progressing well. We are looking forward to the review of this last year's program at NRC during the Second Quarter. During the First Quarter we produced a draft of the 1978 Annual Report, which is currently undergoing internal review and clearance at Battelle. We plan to have this printed during the Second Quarter. The initiation of the NRC reporting system (NRC Manual Chapter NRC-3202) resulted in an initial delay of publication of the reports. Now that the system has been worked out, this delay can be reduced.

Analysis of water samples from several locations across the U. S. is underway. We have completed analysis using the Purge and Trap and headspace techniques on several locations. A topical report including these data will be produced following the completion of the analysis of all the samples from each sampling location. In the freshwater phase of the program we have completed fifteen chloroform acute toxicity bioassays with rainbow trout (Salmo gairdneri), bluegill (Lepomis macrochirus), and catfish (Ictalurus punctatus). Results indicate, in all cases, the LC-50 is well above that which could be expected to be discharged due to power plant chlorination. The LC-50's range from about 15 to 50 ppm chloroform depending on the species. Histological analysis is underway on clams from long-term chlorination by-product exposures. If the results of the histological analysis indicate an effect , further histological examination of samples from other concentrations will be conducted.

ANALYTICAL CHEMISTRY

Roger M. Bean, Dale C. Mann, and Barry L. Wilson

ANALYSIS OF WATER SAMPLES FOR VOLATILE ORGANICS

Chlorinated and non-chlorinated water samples from seven different bodies of water were analyzed for volatiles using two different techniques. The "Purge and Trap" method was employed (Bellar and Lichenberg, 1974) for identification and quantification of organic compounds with volatiles ranging between methylene chloride (bp=40°C) to ethyl benzene (bp=136°C). The "Headspace" technique (Bush et al., 1977) used in the analysis of water samples for chloroform, allowed us to obtain confirmation of the results obtained from the "Purge and Trap" method. Discussion under Task I will be limited this quarter to the results obtained from these analyses. Report of progress on other aspects of the chemistry task is deferred until our next reporting period.

Sampling For Volatiles Analysis

The preparation of samples of chlorinated natural waters and non-chlorinated controls is described in detail in the Annual Report for 1978 (Anderson et al., 1979). Chlorine was added to the natural water in the form of reagent grade NaOCl at the 3-4 mg/ℓ level; this procedure resulted in levels of total residual oxidant (TRO) between 0.0 and 3 mg/ℓ after a chlorine residence time of about one hour. The exact conditions prevailing at each station varied somewhat, and are given in detail in the 1978 Annual Report. "Purge and Trap" samples were taken after all TRO had been removed with a 6-fold excess of sodium sulfite and the sample had been acidified to a pH of 4.0-4.5. Samples for "Purge and Trap" analysis were taken by completely filling a 125 mg glass bottle with the sample and capping the bottle with a Hycar® septum. Headspace samples were taken by carefully removing with a syringe a 15 ml water sample directly from the chlorination apparatus (no sulfite or acid addition) and sealing it in a 125 mℓ glass bottle together with 5 g NaCl and 200 mg Na_2SO_3, using a Hycar® septum. Both "Purge and Trap" and Headspace samples were kept at wet ice temperature during shipment, and were stored in the refrigerator at 4°C until analyzed.

®Use of trademark is not an endorsement by Battelle Memorial Institute.

Purge and Trap Analysis

Procedure

The procedure used for this analysis is very similar to that discussed by Bellar and Lichtenberg (1974). A sample of water containing the volatile organics is sparged with nitrogen gas and then passed through a stainless steel column packed with porous polymer beads (in this case, Tenax®). Volatile organics, which are adsorbed on the polymer, are then introduced into the gas chromotograph (GC) or gas chromotograph/mass spectrometer (GS/MS) by heating the Tenax trap at about 200°C in a stream of helium. This process desorbs the organics from the trap and adsorbs them on to the front of the chromotographic column held at 0°C. After the volatiles have been introduced onto the column, they are separated by conventional temperature programmed gas chromotography.[a]

An important part of the procedure is the prevention of water sample exposure to an air interface prior to sparging, since the volatiles are rapidly lost to air. To avoid this loss, two hypodermic needles were inserted through the sample bottle septum, one of which was attached to a 100 ml glass syringe fitted with a stopcock valve. About 80 mℓ of water sample was then carefully drawn into the syringe and the valve closed. Thirty-five milliliters of sample was expressed from the syringe into the sparging appartus through a small-bore teflon tube, filling the apparatus from the bottom up. The remainder of the sample was stored in the syringe as a contingency sample. Just prior to sparging, one microliter of an enthanolic solution of internal standard mixture containing 0.8 mg each of bromochloromethane (Int. Std. #1), 1, 4-dichlorobutane (Int. Std. #2) and 1-cloro-2-bromopropane (Int. Std. #3)[b] was added. Nitrogen purge of the sample was at 30 ml/min for 20 minutes.

Separation of volatile components was accomplished on a 2 mm ID glass 6-ft column packed with 0.2% carbowax 1500 on carbopack, using 20 ml/min helium carrier. After an initial hold at 0°C for sample introduction (5.5 min), the chromatographic oven was brought to 45°C in 1.5 min and then programmed at 8°C to 160°C. Instrumentation was a Hewlett-Packard Model 5985 GC/MS System, including a Model 5840A Gas Chromatograph. Mass spectra were obtained by scanning in the electron

---

[a] The porous polymer traps, sparging apparatus, GC adapters, and column heater are available from Envirotest Equipment Company as part of a Trace Organic Kit .

[b] The internal standard mixture is a dilution of a mixture supplied by Supelco, Inc. Int. Std. #3 did not give spectra characteristic of the bromo compound; the spectra was consistent with 2-chloro-2-butene.

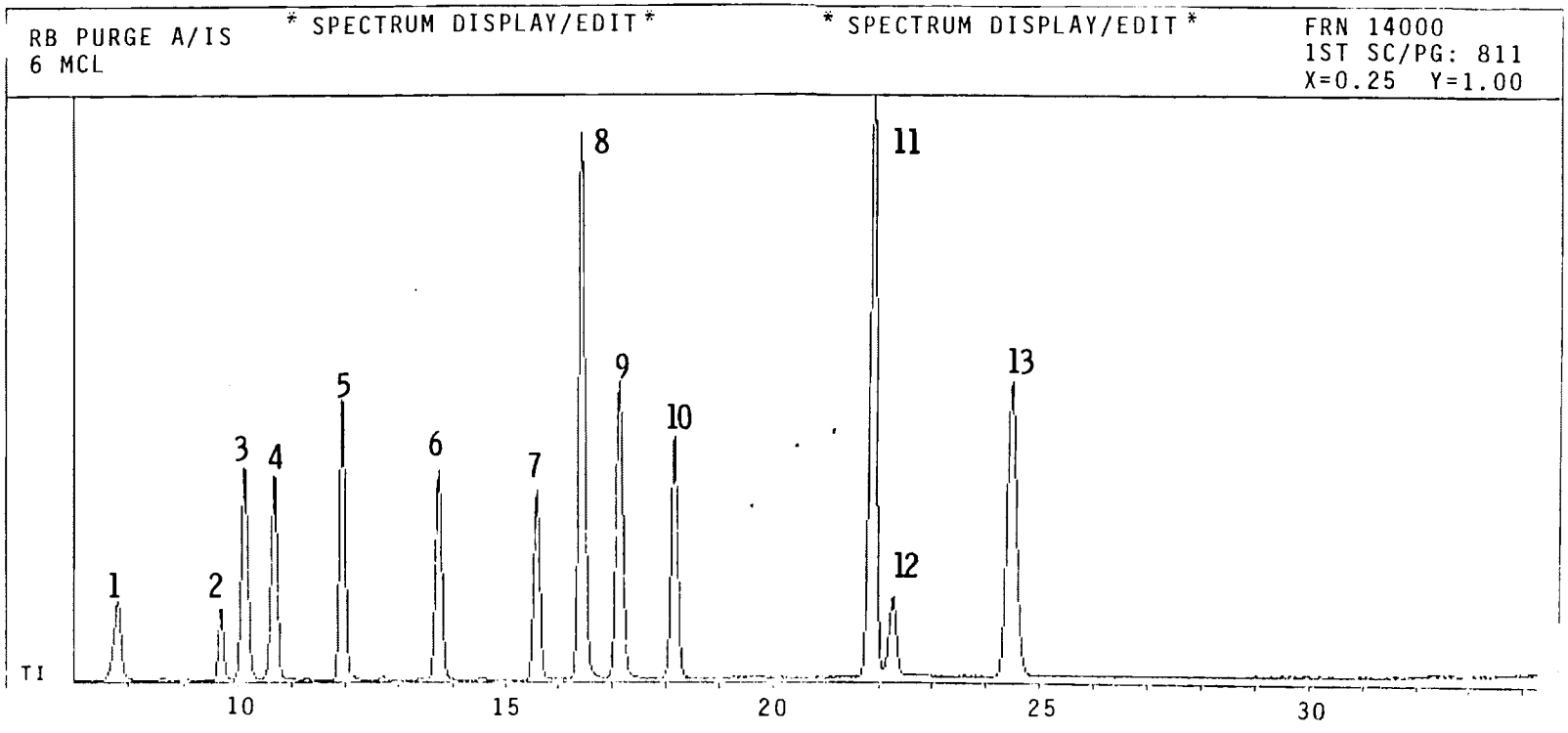
®Use of trademark is not an endorsement by Battelle Memorial Institute.

impact mode from mass 45 to 320 at 180 amu/sec at an electron multiplier voltage of 2,200.

Total ionization chromatograms of two sets of analytical standards containing 23 of the components designated as EPA priority pollutants (Environmental Protection Agency, 1977) are presented in Figures 1 and 2. All samples analyzed by the "Purge and Trap" technique were examined for spectra indicative of these components. Levels at which components could be quantitated are estimated to be 0.5 mg/ℓ. Detection levels are lower than this (>0.1 mg/ℓ) when reconstructed single ion chromatograms were examined.

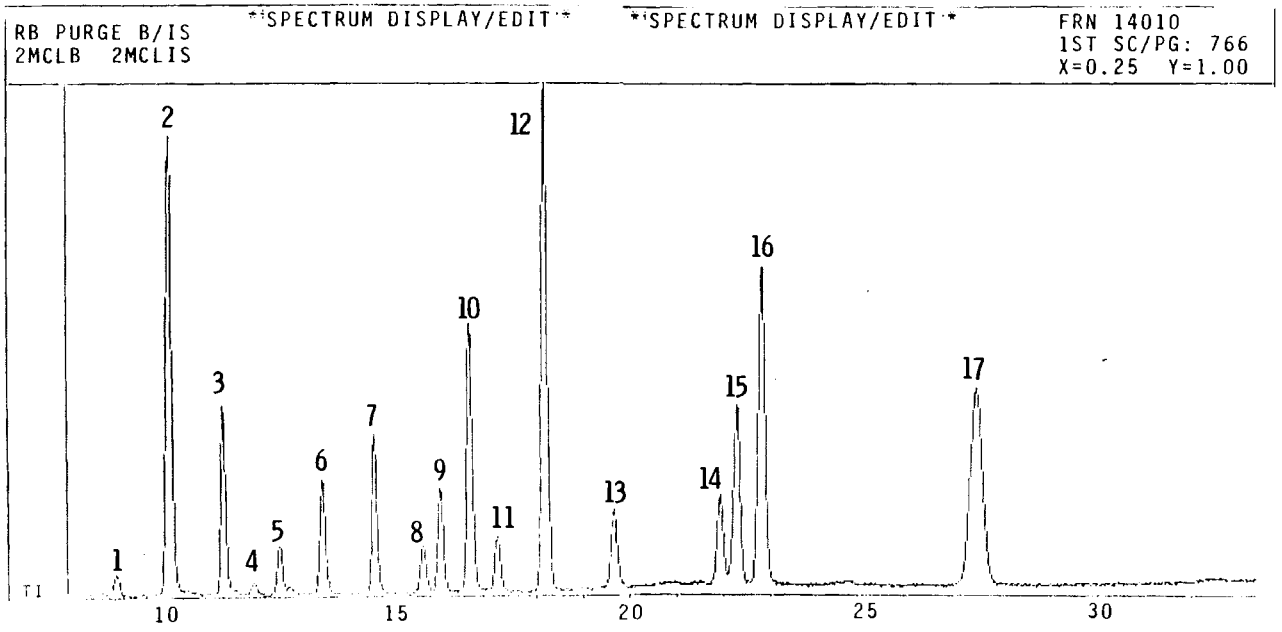
Results

At all seven stations, chlorinated samples were found to contain haloforms. These varied in type and abundance with location. In addition, most samples were found to contain carbon disulfide and methylene chloride. Chromatograms of the chlorinated water samples, standards, and procedural blank are given in Figure 3. Mixtures of the four halo forms and CS_2 and Ch_2Cl_2 were prepared at three concentration levels and analyzed in order to prepare quantitative calibration curves. These are shown in Figure 4, where the peak area divided by the internal standard area is plotted against component concentration. Under the experimental conditions used, plots of three of the four haloforms were linear over the range of concentrations examined. Bromoform data did not fit a straight line. This is presumably because bromoform's higher volatility (bp=149°C) did not permit quantitative removal from the water. On the other hand the very volatile components (CS_2 and CH_2Cl_2) also did not fit a straight line, but the curvature is opposite to that of bromoform. This suggests that at low concentrations, these highly volatile components are rapidly removed from the water, and as purging continues, are also volatilized from the trap. Hence, levels of detection are relatively high for these two components compared to the others. In all cases, accuracy suffers as concentration is increased. This was true regardless of the internal standard used for quantitation. Internal Standard #2 gave the most consistent results. Data for the highest concentrations are based on four replicate analyses, while for the two lower concentrations, data are based on three replicate analyses. The data obtained from the "Purge and Trap" samples at the seven stations examined thus far are presented in Table 1. The data are arranged so that component concentrations for chlorinated samples are in the upper left hand corner of the box corresponding to a station, and the component concentration for the non-chlorinated sample at the same station is located in the lower right hand corner. Thus, comparisons between chlorinated and non-chlorinated samples at a station can be easily made by looking diagonally across a box. At the same time, looking down vertical columns permits one to compare component concentrations at different stations for chlorinated and non-chlorinated samples. Looking across horizontal rows permits examination of different components at a station.



1. CH_2Cl_2
2. 1, 1-DICHLOROETHYLENE
3. INT. STD #1
4. 1, 1-DICHLOROETHANE
5. $CHCl_3$
6. CCl_4
7. 1, 2-DICHLOROPROPANE
8. TRICHLOROETHYLENE
9. $CHBr_2Cl$
10. INT. STD #2
11. TETRACHLOROETHYLENE
12. INT. STD #3
13. CHLOROBENZENE

Figure 1. Total ionization chromatogram of Purge and Trap Standards Mixture "A". Component concentrations are about 34µg/ℓ in water.



1. $CFCl_3$
2. INT. STD #1
3. 1,2-DICHLOROETHYLENE
4. $CHCl_3$ (IMPURITY)
5. 1,2-DICHLOROETHANE
6. 1,1,1-TRICHLOROETHANE
7. $CHBrCl_2$
8. 1,2-DICHLOROPROPANE
9. TRANS-1,2-DICHLOROPROPENE
10. BENZENE
11. C_{15}-1,2-DICHLOROPROPENE
12. INT. STD #2
13. $CHBr_3$
14. 1,1,2,2-TETRACHLOROETHANE
15. INT. STD #3
16. TOLUENE
17. ETHYLBENZENE

Figure 2. Total ionization chromatograms of Purge and Trap Standards Mixture "B". Component concentrations are about 11µg/ℓ in water.

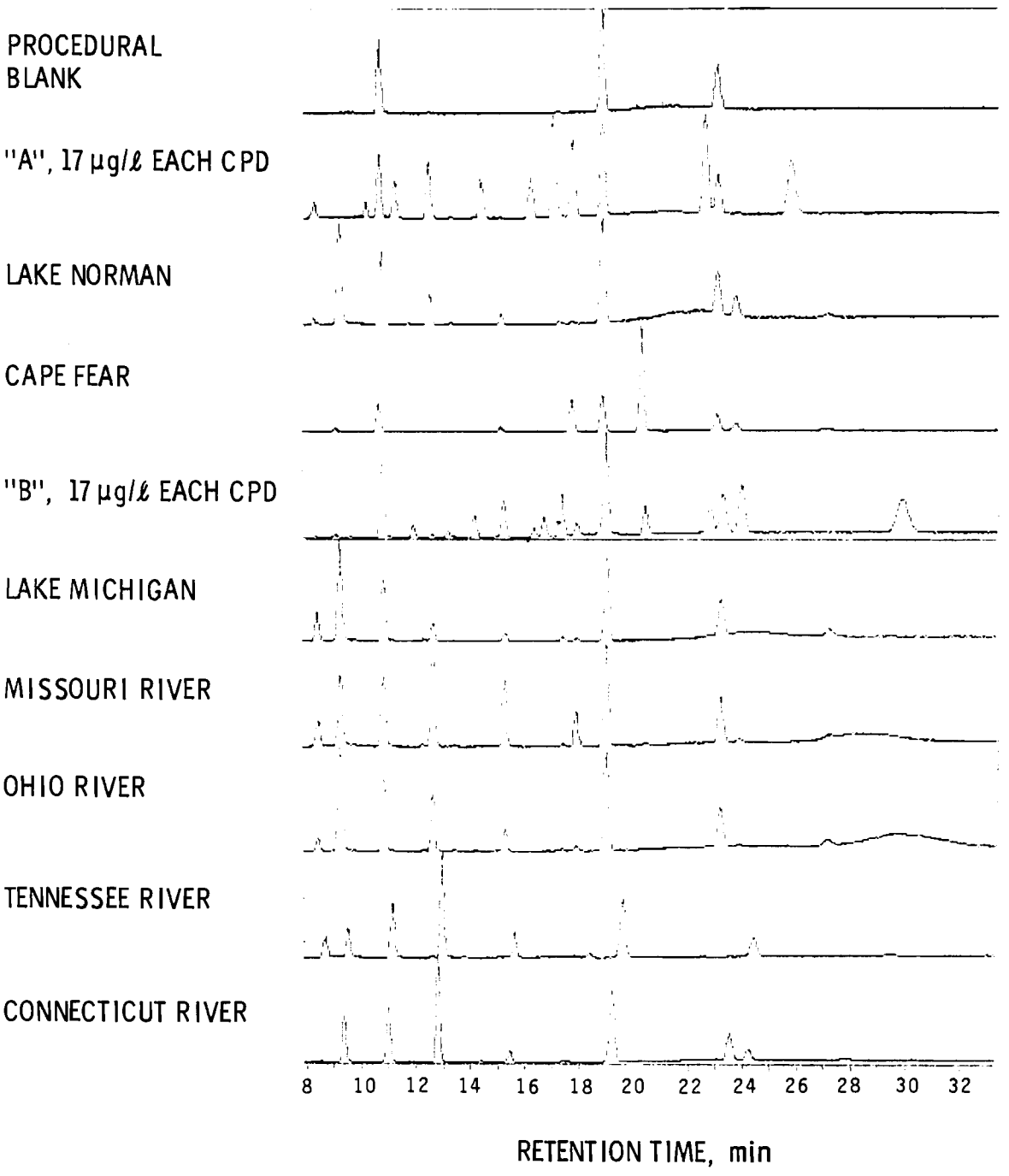


Figure 3. Total ionization chromatograms obtained from Purge and Trap Samples. The water body samples shown are all chlorinated samples.

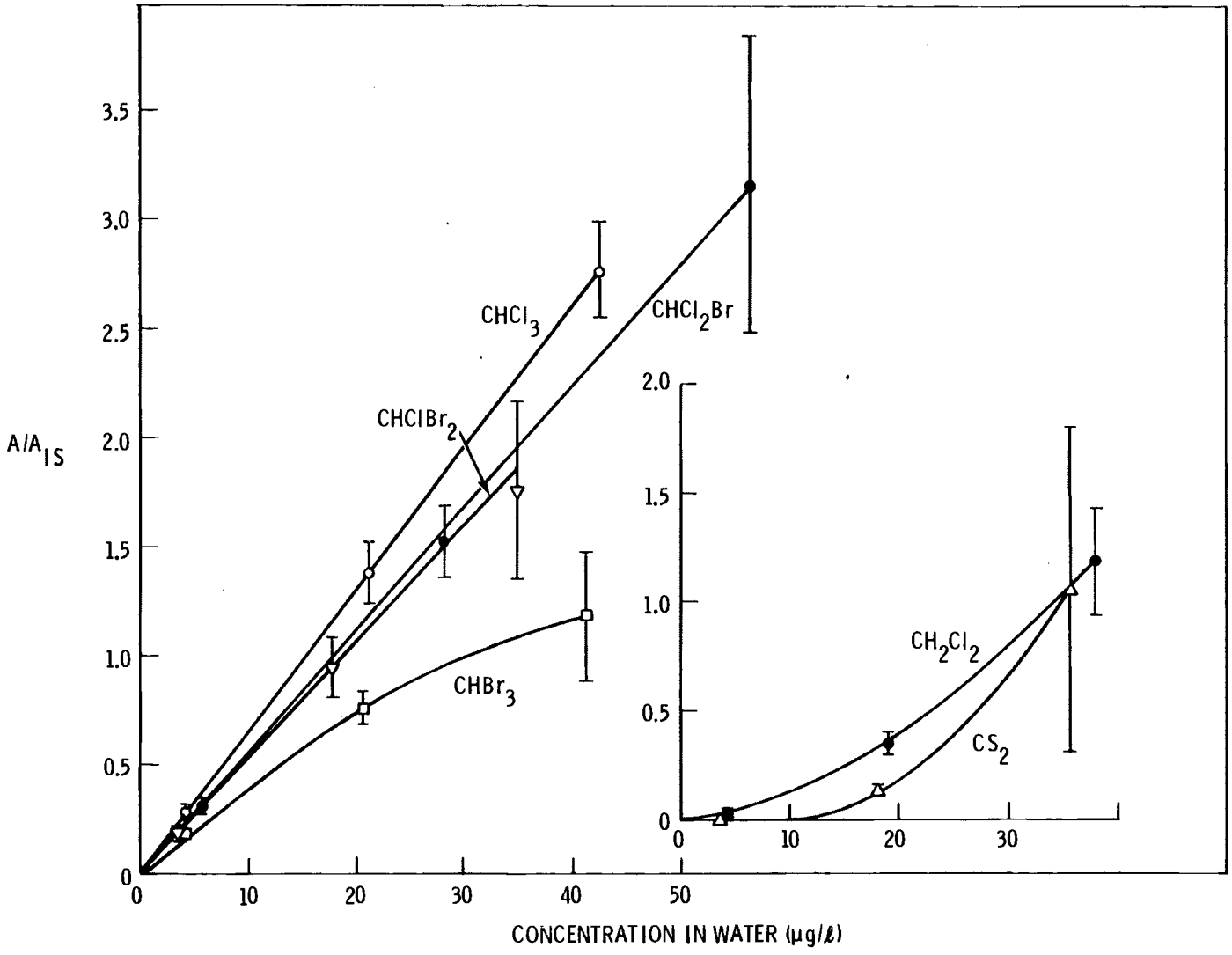


Figure 4. Calibration Curve for Purge and Trap Technique.

TABLE 1. ANALYSIS OF PURGE AND TRAP SAMPLES BY GC/MS FROM SEVEN STATIONS (concentrations are reported in micrograms per liter)

STATION		DICHLORO METHANE	CARBON DISULFIDE	$CHCl_3$	$CHCl_2Br$	$CHClBr_2$	$CHBr_3$	DICHLORO ETHYLENE	TRICHLORO ETHYLENE	TOLUENE
3. OHIO R.	Cl	9, 9	>20, >20	7.3, 5.7	3.4, 3.1	0.9, 0.9	*	*	—	tr
	N	11	>20	0.6	—	—	—	*	—	tr
4. L. MICHIGAN	Cl	17,14	>20, >20	2.7, 2.0	1.8, 1.6	0.8, 0.5	—	—	—	tr
	N	5	>20	tr	—	—	—	—	—	tr
5. MISSOURI R.	Cl	15	>20	11.5	10.3	5.8	*	—	—	tr
	N	15	>20	tr	—	—	—	—	—	tr
6. TENNESSEE R.	Cl	17, 17	>20, >20	21.2, 24.7	6.0, 7.4	1.1, 1.1	*	—	—	tr
	N	13	>20	tr	—	—	*	—	—	tr
7. CAPE FEAR	Cl	1, 3	12, 20	tr	1.9, 2.6	15.7, 17.8	‡	—	—	++
	N	3	>20	tr	—	—	*	*	—	++
8. L. NORMAN	Cl	3, 4	>20, 19	4.1, 3.1	1.7, 1.4	tr	*	*	*	++
	N	3	>20	—	—	—	—	—	—	++
9. CONNECTICUT R.	Cl	1	>20	18.7, 2.45	2.3, 3.4	tr	*	—	—	++
	N	1	>20	tr	—	—	—	*	—	++

— = not detectable
tr = trace (present in concentrations less than 0.5 $\mu g/\ell$)
* = shown to be present in single ion reconstructed chromatogram
++ = present at the several $\mu g/\ell$ level
‡ = could not be quantitated—see text

The data obtained indicate that chlorination of natural waters probably does not produce this compound in significant quantities. The only large difference between chlorinated and control concentrations of CH_2Cl_2 was at Lake Michigan site, and this does not constitute a strong case for formation of CH_2Cl_2.

The presence of relatively high concentrations of carbon disulfide in all samples was a surprise. We have not established an explanation for the presence of this molecule; however, it may be related either to our sampling equipment or to the use of sodium sulfite in excess. Experiments are planned to investigate possible origins of CS_2.

The chloroform concentrations in chlorinated samples vary widely, from traces (0.5 mg/l) in the Cape Fear samples (as might be expected in sea water, where the major product is bromoform) to over 20 mg/l in the Tennessee River. It is interesting to compare the chloroform concentrations generated in the "well-used" Ohio River with those from the rather "pristine" northern Lake Michigan. Both showed relatively low chloroform levels produced by chlorination. Perhaps the high mineral content of the Ohio inhibited chloroform formation.

The relative abundances of bromine-containing haloforms in the chlorinated samples also varied widely as did their ratios to the corresponding chloroform concentrations. The wide variation from station to station of bromine-containing haloforms indicates that this is a result of the natural bromide content of the water bodies under investigation. However, we have not ruled out as yet the possibility that there are traces of bromine in our reagent grade NaOCl, which would also contribute to the formation of bromohaloforms.

Unfortunately, the concentrations of bromoform in the chlorinated seawater sample at Cape Fear could not be estimated since the ratio of its peak area to the internal standard was 2.7, in the area of high uncertainty. It is clear that in the future, "Purge and Trap" samples to be analyzed for bromoform will have to be analyzed twice, once specifically for $CHBr_3$ using special purge conditions, and another time for the rest of the components. Quantitation of $CHBr_3$ for the Cape Fear Station will be accomplished from XAD-2 adsorption samples.

Although the results of these volatiles analyses show wide qualitative and quantitative differences from location to location, it is difficult to interpret the differences because water quality, in terms of dissolved oxygen, oxygen demand, suspended solids, dissolved organic carbon, pH, etc., can conceivably have large effects on the resulting halogen chemistry. Clearly, more laboratory and field research is required before explanations of the results of the above analyses can be given.

Our results for the seven stations are also somewhat surprising in that few samples investigated had evidence of contamination to any significant

levels by man-made volatile organics. In view of the extensive literature on organic components in drinking waters and wastewaters (e.g. Keith, 1976), we expected to find more components than we actually did. However, we are quite confident that we would have found those components searched for at least at the 0.5µg /ℓ level.

Relatively large peaks identified as toluene appeared in the total ionization chromatograms of samples from the last three stations. Since the toluene was found in both chlorinated and non-chlorinated samples, we feel that the toluene was a result of some trace contamination in our sampling equipment. Traces of benzene were also found in all samples, but benzene was also found in the blank.

HEADSPACE ANALYSIS FOR CHLOROFORM

Procedure

The procedure has been previously described in the Annual Report for 1978, and is based on that described by Bush et al. (1977). After heating the 15 ml sample described above in hot water for 1 hour, an aliquot of headspace is removed with a sealable syringe and injected into the gas chromatograph, where it is separated on a 1.83 m x 2 mm chromosorb 101 column at 150°C using an electron capture detector. Quantitation is accomplished by comparing the experimental samples to samples with known concentrations of chloroform.

Results

The results of the analyses from the headspace technique are presented in Table 2 together with the results from the "Purge and Trap" method. The agreement between values obtained at the seven stations for chloroform in chlorinated samples gives reasonable assurance that the values reported are reasonably close to actual concentration in the samples. The headspace technique is a very simple, inexpensive method of chloroform analysis, with precisions of about 10% at the low parts per billion level. Both the "Purge and Trap" analysis and the headspace technique are reasonably accurate methods. The headspace technique employs a very convenient sampling method to use at locations remote from analytical analysis.

TABLE 2. COMPARISON OF CHLOROFORM ANALYSES BY PURGE AND TRAP TECHNIQUE WITH HEADSPACE TECHNIQUE (concentrations reported at $\mu g/\ell$)[1]

	CHLORINATED		NONCLORINATED	
STATION	HEADSPACE	P+T	HEADSPACE	P+T
3. OHIO R.	7.2	6.5 ± 0.8	1.5 ± 0.3*	tr[2]
4. L. MICHIGAN	4.6 ± 0.2*	2.4 ± 0.4	1.0 ± 0.1*	tr
5. MISSOURI R.	10.4 ± 1.3	11.5	2.3 ± 0.3	tr
6. TENNESSEE R.	16.6 ± 0.4	23.0 ± 1.8	2.3 ± 0.2	tr
7. CAPE FEAR	4.1	tr	1.8	tr
8. L. NORMAN	7.7 ± 0.2	3.6 ± 0.5	1.7 ± 0.0	—
9. CONNECTICUT R.	25.9 ± 2.3	21.6 ± 2.9	1.3 ± 0.5	tr

[1]Headspace samples were sampled in triplicate and the sample variation reported as ± Standard Deviation except for those values with an asterisk, where duplicates were analysed and the variation reported as a range. Purge and trap samples were analysed in duplicate where possible, and the variation reported as a range.

[2]tr = trace (present in concentrations less than .5 $\mu g/\ell$)

FRESHWATER BIOLOGY

David R. Anderson and E. W. (Bill) Lusty

CHLOROFORM TOXICITY TESTS

Fifteen acute 96 hr toxicity bioassays of chloroform were completed with rainbow trout, bluegill and channel catfish. To conduct these tests we constructed a toxicant delivery system that produced chloroform saturated water, ∿8000 ppm chloroform, as a stock solution. Saturating water with chloroform is not readily accomplished simply by mixing. Thus, we used a 0.61 m glass saturation column filled with 3 mm glass beads, with bands of glass wool at 15 mm intervals. Chloroform was pumped into the top of the glass saturation column using a manostat variable speed cassette pump at a flow rate of ∿1.0 mℓ/min. Deionized water was added at ∿90 mℓ/min to maintain a constant head in the column. The solution flowed into a magnetically stirred four-liter amber-glass carboy. A second four-liter amber-glass carboy which was not stirred followed. This permitted settling of fine chloroform droplets prior to pumping the saturated solution to the head tank or delivery manifold.

A stainless steel, positive displacement pump with a ceramic head was used for pumping the chloroform saturated water. The saturated solution was metered into funnels with dilution water from the head tank or delivery manifold. During each test the dilution water flow rates to each aquaria were equal. Dilution water flow was adjusted to a level ranging from 1 to 1.5 ℓ/min during a test, depending on the fish oxygen demands and the ASTM recommended flow rate (Sprague, 1973). Aerated Columbia River Water was maintained at a constant temperature within 0.1°C. The diurnal light cycle was automatically controlled to a 12 hr light/dark cycle. Fish were held in this temperature and light regime at least 2 weeks prior to testing. Fish were not fed during the test or two days prior to the test. With the exception of largemouth bass which were fed live rainbow trout, all fish were fed silvercup®fish food. In previous toxicity tests at Battelle, this diet has been shown to provide adequate nutritional requirements. Tests by Mehrle et al. (1977), corroborate our results.

Chloroform alters the behavior of rainbow trout and largemouth bass prior to loss of equilibrium and death. This response is similar to a pharmacological response in humans to chloroform in which they become very energetic prior to unconsciousness. With rainbow trout there is a definite change in social structuring or behavior in test aquaria at chloroform concentrations > 10 ppm. Fish congregate near

®Use of trademark is not an endorsement by Battelle Memorial Institute.

the surface of the test aquaria, whereas they are evenly distributed in control aquaria. There is an apparent increased sensitivity to light or noise disturbances in fish exposed to chloroform compared with controls. Sudden illumination of the room at night sends the fish, rainbow trout, bluegill and largemouth bass, into frenzied activity.

Mortality resulting from chloroform exposure shows a sharp threshold effect, resulting in a high incidence of 100% mortality or 100% survival among experimental organisms over a narrow range. Partial test group mortality rarely occurred. These bioassay results are difficult to interpret with probit analysis, which requires several partial kills to maximize the accuracy of the LC-50 and minimize the confidence intervals. Thus the binomial method and the moving average method are also used to compute LC-50 (Stephan, 1977).

Tables 3, 4 & 5 contain mortality information at 12 hr, 24 hr, 48 hr and 96 hr census times throughout each bioassay 6 & 7. These results were used to compute LC-50's (Tables 6 & 7). Results of chloroform toxicitv tests with channel catfish are inconclusive with respect to a chloroform LC-50. In accordance with the NRC scope of work, adult catfish were tested. Adult channel catfish exhibit aggressive behavior in fish densities (5-10 fish per aquarium) normally used in toxicity tests. Mortality occurred in all aquaria as a result of aggression with the dominant fish surviving. In tests with one fish per aquarium, no mortalities occurred to a maximum chloroform level of 68 ppm. The possibility of additional tests with juvenile channel catfish will be discussed with NRC staff.

Fish mortality rates from chloroform exposure differ between species. With rainbow trout and largemouth bass mortality occurs primarily within the first two days of the test. With bluegill, mortality occurs during the latter half of the 96 hr toxicity tests.

The LC-50's for each species are several orders of magnitude above the chloroform levels found in the analytical chemistry studies of chlorinated water across the U. S., Table 1. Although chloroform is toxic in the tens of parts per million, this is well above that which could be expected to be produced under normal power plant chlorination conditions, which the chemistry studies simulate. Also, the behavioral effects noted occur well above projected levels from power plants. Using acute toxicity to adult fish as the only criterion, it does not appear that chloroform is a potential problem in power plant discharges. Other criteria such as reproductive effects, carcinogenicity, tetratogenicity and mutagenicity of chloroform exposure may alter this perspective of environmental problems resulting from power plant generation of chloroform.

Table 3. Rainbow Trout Mortalities During 96 Hour Acute Toxicity Tests with Chloroform.

Bioassay #4 10 fish per aquarium

Census Times	12 Hr	24 Hr	48 Hr	96 Hr
$CHCl_3$ (ppm)				
16.4	0	0	0	0
12.1	0	0	0	0
10.1	0	0	0	0
12.4	0	0	0	0
0.0	0	0	0	0
16.9	0	0	0	0

Bioassay #5 10 fish per aquarium

Census Times	12 Hr	24 Hr	48 Hr	96 Hr
$CHCl_3$ (ppm)				
20.0	0	0	1	7
11.3	0	0	0	0
12.0	0	0	0	0
13.2	0	0	0	0
0.0	0	0	0	0
22.9	0	1	9	10

Bioassay #6 10 fish per aquarium

19.5	0	0	5	7
15.4	0	8	8	8
15.0	0	0	0	0
16.6	0	0	0	0
0.0	0	0	0	0
25.5	0	1	1	7

Bioassay #7 20 fish per aquarium

27.0	3	13	19	19
19.6	0	0	0	4
15.1	0	0	0	0
10.6	0	0	0	0
0.0	0	0	0	0
27.8	1	11	16	20

Bioassay #13 20 fish per aquarium

18.6	0	0	10	17
8.4	0	0	0	0
3.3	0	0	0	0
12.3	0	0	3	6
0.0	0	0	0	0
12.1	0	0	0	0

Bioassay #15 20 fish per aquarium

26.6	15	21	21	21
9.8	0	0	0	0
15.3	0	0	0	0
18.6	0	0	3	19
0.0	0	0	0	0
20.6	3	16	20	20

Table 4. Bluegill Mortalities During 96 Hour Acute Toxicity Tests With Chloroform.

Bioassay #1 10 fish per aquarium

Census Times	12 Hr	24 Hr	48 Hr	96 Hr
$CHCl_3$ (ppm)				
1.2	0	0	0	0
0.1	0	0	0	0
1.4	0	0	0	0
10.5	0	0	0	0
0.0	0	0	0	0
11.1	0	0	0	0

Bioassay #2 10 fish per aquarium

Census Times	12 Hr	24 hr	48 Hr	96 Hr
$CHCl_3$ (ppm)				
2.4	0	0	0	0
0.4	0	0	0	0
3.0	0	0	0	0
13.7	1	1	1	1
0.0	0	0	0	0
21.4	9	9	10	10

Bioassay #3 10 fish per aquarium

18.7	0	0	-	-
14.1	1	1	-	-
16.3	1	1	-	-
14.5	0	1	-	-
0.0	0	0	-	-
23.9	6	8	-	-

Bioassay #8 5 fish per aquarium

25.3	3	3	4	5
18.7	0	0	0	0
12.7	0	0	0	0
14.7	0	0	0	0
0.0	0	0	0	0
21.1	1	1	1	1

Bioassay #9 5 fish per aquarium

28.8	5	5	5	5
22.1	3	3	4	5
4.9	0	0	0	0
21.4	3	3	4	4
0.0	0	0	0	0
15.2	3	3	3	3

Bioassay #10 5 fish per aquarium

26.6	3	4	4	4
15.4	2	2	2	3
15.4	0	0	0	0
20.3	1	2	3	3
0.0	0	0	0	0
32.2	5	5	5	5

Table 4. (Continued) Bluegill Mortalities During 96 Hour Acute Toxicity Tests With Chloroform.

Bioassay #12 5 fish per aquarium

Census Times	12 Hr	24 Hr	48 Hr	96 Hr
$CHCl_3$ (ppm)				
29.3	5	5	5	5
21.6	1	1	2	2
5.7	0	0	0	0
20.2	2	2	2	3
0.0	0	0	0	0
25.2	3	3	3	4

Table 5. Catfish Mortalities During 96 Hour Acute Toxicity Test With Chloroform.

Bioassay #11 3 fish per aquarium

Census Times	12 Hr	24 Hr	48 Hr	96 Hr
$CHCl_3$ (ppm)				
25.3	0	0	0	0
19.7	0	0	0	0
0.0	0	0	0	0
7.4	0	0	0	0
0.0	0	0	0	0
4.7	0	0	0	0

Table 6. Acute Chloroform LC-50's With Rainbow Trout at 8°C in Columbia River Water.

Census Times	12 Hr.	24 Hr.	48 Hr.	96 Hr.
Bioassay No.				
4	a	a	a	a
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

Table 7. Acute Chloroform LC-50's with Bluegill at 25°C in Columbia River Water.

Census Times	12 Hr.	24 Hr.	48 Hr.	96 Hr.
Bioassay No.				
1	a	a	a	a
2	17.1	17.1	16.3	16.3
3	23.9	21.8	b	b
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 - No mortalities occurred in all test groups.

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

CHLORINE ISOTOPE STUDIES

A series of paired three day ^{36}Cl exposures were conducted. One exposure of twelve fish was with sodium hypochlorite ($NaO^{36}Cl$) at about 40 ppb total residual chlorine (TRCl) and one exposure was with an equivalent level of chlorine isotope as its sodium salt ($Na^{36}Cl$). Analysis of the results is underway. Fish were sampled at selected intervals, dissected and the following tissues were digested and counted for ^{36}Cl:

- Blood
- Brain
- Eye
- Gill
- Bile and Gall Bladder
- Large Intestine (evacuated)
- Upper Gastrointestinal tract including pyloric cacae
- Spleen
- Kidney
- Liver
- White Muscle
- Red Muscle
- Opercular Bone

MARINE AND ESTUARINE BIOLOGY

Charles I. Gibson

BROMOFORM STUDIES

Acute Effects of Bromoform on Selected Marine Species

Acute tests with Protothaca staminea, Mercenaria mercenaria, Crassostrea virginica, Brevoortia tyrannus, and Penaeus aztecus were completed in FY78. A paper discussing the results of these tests was presented at the Second Workshop on Marine and Freshwater Application of Ozone, October 31-November 5, 1978, in Orlando, Florida. The paper, appended to this report, is now in review for consideration for publication in the meeting proceedings. No further acute testing is anticipated.

Bioaccumulation and Depuration

The tissue analyses for bromoform were completed during this quarter. At this time, the data are still being analyzed. We anticipate having a full discussion of these data completed by the end of the third quarter. We anticipate that a topical report on the bioaccumulation studies will be completed by the end of the fiscal year.

CHRONIC CHLORINATION BY-PRODUCTS

The long-term exposure of P. staminea was terminated on November 8, 1978. During this eight month experiment, littleneck clams were exposed to 0, 6, 12, 25, 50 and 100 ppb total residual oxidant (TRO). Subsamples of clams from each exposure condition were harvested monthly, measured, weighed and preserved for later histological examination and analysis for halogenated organic uptake. Specimens for histological examination were shipped to Dr. Robert Hillmann, Battelle, W. F. Clapp Laboratory, Duxbury, Massachusetts. We anticipate having the initial results from his investigation by the end of the second quarter and a detailed report by the end of the third quarter. No chemical analysis of the preserved (frozen) specimens is scheduled at this time. We anticipate that a topical report on the growth and histological studies will be completed by the end of the fiscal year.

REFERENCES

Anderson, D.R., R. M. Bean, and C. I. Gibson. 1979. Biocide By-Products in Aquatic Environments Annual Report FY 1978. Pacific Northwest Laboratory, Richland, WA. PNL-2806. NUREG/CR-0504.

Bellar, T. A., and J. J. Lichtenberg. 1974. Determining volatile organics at microgram-per-liter levels by gas chromatography. Journal AWWA, 66:739-744.

Bush, B., R. S. Narang, and S. Syrotynski. 1977. Screening for halo-organics in New York State drinking water. Bull. Env. Cont. & Toxicol. 18:436-440.

Environmental Protection Agency. 1977. Sampling and Analysis Procedures for Survey of Industrial Effluents for Priority Pollutants. IFB No.WA-77-B133. (Available from US EPA Effluent Guidelines Division, Washington, D.C. 20460). Also, see Supelco, Inc., Bulletin 775, Water Pollution Analysis and Standards, Bellefont, Pa., 1978.

Keith, L. H. 1976. Identification and Analysis of Organic Pollutants in Water . Ann Arbor Science Publishers, Inc., Ann Arbor, Michigan.

Mehrle, P. M., Mayer, F. L., and Johnson, W. W., Diet Quality in Fish Toxicology: Effects on Acute and Chronic Toxicity, Aquatic Toxicology and Hazard Evaluation, ASTM STP 634, F. L. Mayer, and J. L. Hamelink, Eds., American Society for Testing and Materials, pp. 269-280.

Sprague, J. B., 1973. The ABC's of Pollutant Bioassay Using Fish, Biological Methods for the Assessment of Water Quality, ASTM STP 528, American Society for Testing and Materials, pp.6-30.

Stephan, C. E., 1977. Methods for Calculating an LC_{50}, Aquatic Toxicology and Hazard Evaluation, ASTM STP 634, F. L. Mayer and J. L. Hamelink, Eds., American Society for Testing and Materials, pp.65-84.

APPENDIX

TOXICITY AND EFFECTS OF BROMOFORM ON FIVE MARINE SPECIES

by

Charles I. Gibson, Frederick C. Tone,
Peter Wilkinson and J.W. Blaylock

BATTELLE
Marine Research Laboratory
Route 5, Box 1000
Sequim, Washington 98382
USA

Toxicity and Effects of Bromoform on Five Marine Species

Charles I. Gibson[1], Frederick C. Tone[2], Peter Wilkinson[1], and J. W. Blaylock[1]

BATTELLE[1]
Pacific Northwest Laboratory
Marine Research Laboratory
Route 5, Box 1000
Sequim, Washington 98382

BATTELLE[2]
Florida Marine Research Facility
Sailfish Drive Ponce Inlet
Datona Beach, Florida 32019

ABSTRACT

Bromoform has been identified as the single most abundant halogenated organic compound produced by the chlorination of marine waters. To determine the potential biological effects of its release into marine waters, short-term toxicity bioassays and 28-day uptake/28-day depuration studies were conducted with five marine species: Protothaca staminea, Mercenaria mercenaria, Crassostrea virginica, Penaeus aztecus and Brevoortia tyrannus. The bioassay studies indicate that 96-hr LC50's ranged from approximately 7 ppm for B. tyrannus to greater than 40 ppm for P. staminea. The behavior of P. aztecus and B. tyrannus was significantly altered by exposure to bromoform.

This paper is based on work prepared under Nuclear Regulatory Commission Contract #60193001-B2098.

INTRODUCTION

Bromoform has been identified as the major organohalogen compound formed by the chlorination of sea water (Bean et al., 1978; Carpenter and Smith, 1978). Coastal-sited steam electric stations chlorinate cooling water to prevent condensor fouling and slime buildup and, thus, may have the potential to release considerable amounts of bromoform into the marine ecosystem. To test the relative toxicity of bromoform and the probability of bioaccumulation, a series of bioassays with bromoform were conducted using five marine species. The purpose of the bioassays was to obtain an estimate of the potential toxicity and bioaccumulation of bromoform by organisms that may be exposed to cooling water discharges. This report discusses the toxicity of bromoform to five species of marine organisms: littleneck clam (Protothaca staminea), Eastern oyster (Crassostrea virginica), quahaug (Mercenaria mercenaria), shrimp (Penaeus aztecus), and menhaden (Brevoortia tyrannus).

METHODS AND MATERIALS

The bioassays with P. staminea were conducted at the Battelle Marine Research Laboratory, Sequim, Washington, and those with M. mercenaria, C. virginica, P. aztecus and B. tyrannus were conducted at the Battelle Florida Marine Research Facility, Daytona Beach, Florida.

Collection and Exposure - Sequim

Specimens of P. staminea were collected from Sequim Bay, Washington, and held in unfiltered, ambient running sea water for four days prior to testing. There was less than 1% mortality during the holding period. The exposure of P. staminea was conducted by bubbling air saturated with bromoform directly into the exposure tanks (Figure 1). The tanks were 30-liter glass aquaria

layered with approximately 5 cm of coarse sand. Seventy-five clams were randomly selected and placed in each tank. Concentration of bromoform and mortality was monitored five days a week. Bromoform/air flows were adjusted to maintain nominal concentrations of 0, 1, 5, 10, and 20 mg/l.

Collection and Exposure - Daytona

With the exception of shrimp (P. aztecus) which were purchased from a local bait shrimp dealer, clams (M. mercenaria), oysters (C. virginica) and juvenile menhaden (B. tyrannus), were collected by Battelle staff members in the Halifax River within one mile of the Florida Marine Research facility. Shrimp and menhaden were held in 11,350-liter, outdoor holding tanks with a continually circulating supply of Halifax River sea water, filtered through sand and activated carbon. Purina Trout Chow® was fed daily at a rate of 5% body weight. Clams and oysters, held in 265-liter water tables, were supplied with unfiltered water as a food source. Clams were placed in 5 cm of sand. All organisms were held for at least one week prior to exposure with less than 1% mortality. LC50 values were calculated by the method of Litchfield and Wilcoxon (1948).

Bromoform exposures were conducted with a modified Chadwick® diluter (Figure 2). Exposure chambers were 40-liter glass aquaria. Sea water used for the experiments was pumped through activated carbon filters before being returned to the Halifax River.

Bromoform was introduced into the sea water by bubbling air through liquid bromoform and then into sea water in a mixing chamber via silicone tubing and a glass airstone. Air flow was regulated with glass air valves and rates were measured with Gilmont® flow meters. The concentration of bromoform in the

sea water was controlled by regulating the air and seawater flow rate to the mixing chamber.

Sampling and Monitoring Procedures

During all exposures at Daytona, daily water quality measurements were taken of incoming diluter water. Water quality parameters had the following ranges: salinity, 25 to 35 ppt; temperature, 25 to 30°C; ph, 8.0 to 8.5; D.O., 5.5 to 7.5 mg/l. Water samples were taken for bromoform analysis from selected exposure tanks at each concentration, and were collected daily in amber glass bottles and immediately refrigerated for shipment to Sequim. Water and airflow rates were monitored daily.

At Sequim, water quality was not monitored. Previous studies have shown the water quality to be stable with only slight seasonal variation. Salinity ranges from 29 to 31 ppt and temperature from 7 to 13°C. Oxygen is normally 100% saturation (9.4 mg/l) expect in late August and September when concentrations down to 80% saturation have been observed. Water samples for bromoform analysis were collected and stored under refrigeration.

Bromoform Analysis

Water samples collected from the bioassay tanks were stored in tightly capped and completely filled 60 ml bottles at 4°C prior to analysis. Subsamples (5 to 10 ml) were removed from the bottles and transferred to 25 ml screw-cap vials containing 10 ml hexane. The vials were hand-shaken for 90 seconds and allowed to stand until phase separation occurred. One ml of the hexane phase was transferred to a 2 ml Hewlett Packard® autosampler vial with septum cap. An internal standard was then added to the vial by syringe (3 µl of 152 µg/ml, 3-dibromopropane). The samples were then analyzed

by electron capture gas chromatography, utilizing a Hewlett Packard model 5840® with autosampler.

The analysis conditions were as follows: Column - 30 meter SP2100 glass capillary with 15 to 1 split ratio; carrier gas - helium; oven temperature - 85°C; detector - $^{63}N_1$ electron capture. Calculation of sample concentration was conducted by the internal standard calibration method.

RESULTS

Protothaca staminea

Preliminary testing indicated that obtaining a 96-hr LC50 would be difficult because the clams closed up and were not pumping water when exposed to high levels of bromoform. At concentrations in the 300 to 400 mg/l range, P. staminea retract their siphons and close. They remain closed, and in one test where the concentration was approximately 800 mg/l, the clams died in the closed position. Therefore, to obtain an estimate of the toxicity and potential for bioaccumulations, an exposure for 28 days at 1, 5, 10 and 20 mg/l was conducted. Considerable difficulty was experienced in maintaining the desired bromoform concentrations. At the highest level (nominal 20 mg/l) the average of 20 values for the 28-day exposure was 27 mg/l with a range from 9 mg/l to 76 mg/l. A plot of the concentrations measured are given in Figure 3. The other exposure tanks had similar variation (Figures 4, 5, & 6) and averaged 2 mg/l, 7 mg/l, and 19 mg/l for the target concentrations of 1, 5, and 10 mg/l, respectively.

Mortality was observed only in the two higher concentrations. At the highest concentrations (average 27 mg/l), 21 of 60 clams were found dead on Day-7 of the exposure, and then 10 of 24 clams were found dead on Day-25. In the 10 mg/l

exposure (average concentration of 19 mg/l), 9 of 45 clams were found dead on Day-25. During the depuration cycle of the test, two more mortalities occurred at the highest concentrations; one recorded on Day-4, and one recorded on Day-7. The exact day of death for these individuals could not be determined. Of interest is the fact that no mortalities occurred at the lower two test levels through the 28-day uptake or 28-day depuration period.

Penaeus aztecus

The calculated 96-hr LC50 for P. aztecus was 26 mg/l with a 95% confidence interval between 33 mg/l and 20 mg/l. Of interest was the behavior exhibited by the shrimp at two levels of concentration. At bromoform/airflow rates delivering 19 mg/l and above, an avoidance response to the bromoform source occurred within 60 seconds of exposure. At flow rates delivering 31 mg/l and above, a narcotic-like effect, where the shrimp were observed lying on their sides on the bottom of the tank with their abdominal appendages undulating, occurred within 120 minutes and continued throughout the experiment or until death.

Brevoortia tyrannus

The calculated 96-hr LC50 was 12 mg/l with a 95% confidence interval between 15 mg/l and 9 mg/l. As the menhaden approached death they began to lose equilibrium and lie on their side at the bottom of the tank. Opercular movement gradually decreased until all movement stopped.

Crassostrea virginica and Mercenaria mercenaria

A 96-hr exposure period appears to be inadequate to generate meaningful LC50 data on clams and oysters. At concentrations above 10 mg/l, filtering ceases and the bivalves close and remain closed for much of the exposure period.

At the end of 96 hours there were no mortalities with either M. mercenaria or C. virginica. However, mortalities occurred during the 3-day period immediately following the 96 hours of exposure to bromoform. Based on this latent mortality data, the 50% mortality concentration for C. virginica and M. mercenaria was in the range of 40 mg/l and 140 mg/l, respectively.

DISCUSSION

The results of the bioassays indicate that bromoform does not cause acute effects to the species tested at concentrations below 1 mg/l. Menhaden were the most sensitive, with a 96-hr LC50 of 10 mg/l. Shrimp were next in sensivity with a 96-hr LC50 of 26 mg/l. The bivalves tested had 96-hr LC50's that were apparently above 40 mg/l. These bromoform concentrations are well above those one would expect in a power plant discharge, based on the findings of Carpenter and Smith (1978) and Bean et al. (1978). They reported bromoform concentrations of 30 to 350 ppb in sea water that had been chlorinated at a rate of 1 to 4 ppm. This is a conversion rate of about .02 to .08 parts bromoform for each part chlorine added. At this conversion rate, chlorine would have to be added at a rate of 500 mg/l to form sufficient bromoform to cause acute effects. At this rate of chlorination (unless there is an extremely heavy chlorine demand), the residual oxidant will have a much more pronounced effect than bromoform. The literature reports that total residual oxidant causes acute effects to the tested species in the 1.5 mg/l to .005 mg/l range (Roberts et al., 1975; Thatcher, 1978; Scott et al., 1978).

The mortalities noted in the P. staminea 27 mg/l uptake/depuration exposure are curious in that they appear to happen at two single points in time. Both occurrences were four days after peak exposure concentrations were experienced (Figure 3). The first mortality occurred after a peak of 56 mg/l bromoform,

and the second occurred after a peak of 76 mg/l bromoform. Thus, in this exposure tank it appears that there may be a threshold concentration above which mortality begins.

The mortality that occurred in the 19 mg/l exposure did not follow the pattern found at the higher level. The concentration in this system was not as variable as in the 27 mg/l exposure, and the mortality did not occur until ten days after a peak concentration occurred.

The delayed mortalities noted in the oyster tests and the above clam mortalities indicate that the action of the bromoform at high concentrations can cause severe enough damage to prevent recovery. This action can result from short-term exposure to high concentrations (probably greater than 50 mg/l) or longer term exposure to high concentrations in the 20 to 30 mg/l range. In regard to concern about bromoform being released from steam electric stations, the exact concentrations required for either is academic, since these levels are approximately 1000 times those expected to be found.

At sublethal concentrations, the menhaden and shrimp exhibited some qualitative behavorial changes. After 48 hours, juvenile (under 7 cm T.L.) menhaden exposed to 6 mg/l and 9 mg/l bromoform exhibited extreme excitation to external stimuli such as loud noises, quick movements or sudden light changes. These stimuli would cause the fish to swim rapidly in random directions, and frequently hit the tank walls. In control tanks and at the higher concentrations, this response did not occur. This excitability continued for up to 20 days after the exposure to bromoform had been terminated.

The shrimp responded similarly at bromoform concentrations between .4 mg/l and 6 mg/l. However, at concentrations below 3 mg/l the response was no longer evident within one hour after bromoform addition was stopped. At concentrations between 3 mg/l and 6 mg/l, the response continued for at least one day.

These observations are qualitative but completely opposite of the response noted for those organisms that died. At the higher levels the bromoform appeared to act as a narcotic. Both shrimp and menhaden would gradually slow down, lose orientation and eventually stop pleopod or opercular movement. This condition was reversible for the shrimp, and the animal would recover within a few hours if the bromoform input was stopped before pleopod motion ceased.

Based on the 96-hr LC50 studies and mortality data from the uptake and depuration studies, the potential for acute environmental effects (to the studied species) from bromoform created through chlorination of steam electric station cooling waters is minimal. The behaviorial responses noted should be considered subjective observations that may or may not be related to bromoform exposures. To determine if the behaviorial responses noted are, in fact, real changes and caused by bromoform, further research will be necessary.

Bioaccumulation data, which was not available at this writing, will provide insights into the potential for long-term effects on food web transfer of bromoform via those species that spend significant time in discharge streams.

Acknowledgment

This paper is based on work prepared under Contract #60193301-B2098 with the Nuclear Regulatory Commission.

Disclaimer: *The registered trademarks are referenced for reader convenience in replicating experiments and do not represent endorsement by Battelle, Pacific Northwest Laboratories.*

REFERENCES

Bean, R. M., R. G. Riley, and P. W. Ryan. Investigation of halogenated components formed from chlorination of marine water. In: Water Chlorination - Environmental Impact and Health Effects, Vol. 2, pp. 223-234. (R.L. Jolley, H. Gorchev, D.H. Hamilton, Jr., eds.) Ann Arbor Science, Ann Arbor, Mich. 1978.

Carpenter, J. H. and C. A. Smith. Reactions in chlorinated sea water. In: Water Chlorination - Environmental Impact and Health Effects, Vol. 2., pp. 195-208. (R.L. Jolley, H. Gorchev, D.H. Hamilton, Jr., eds). Ann Arbor Science, Ann Arbor, Mich. 1978.

Litchfield, J. T. Jr., and F. W. Wilcoxon. A simplified method of evaluating dose-effect experiments. J. Pharmacal. Exp. Theraput. 96:99-113, 1949.

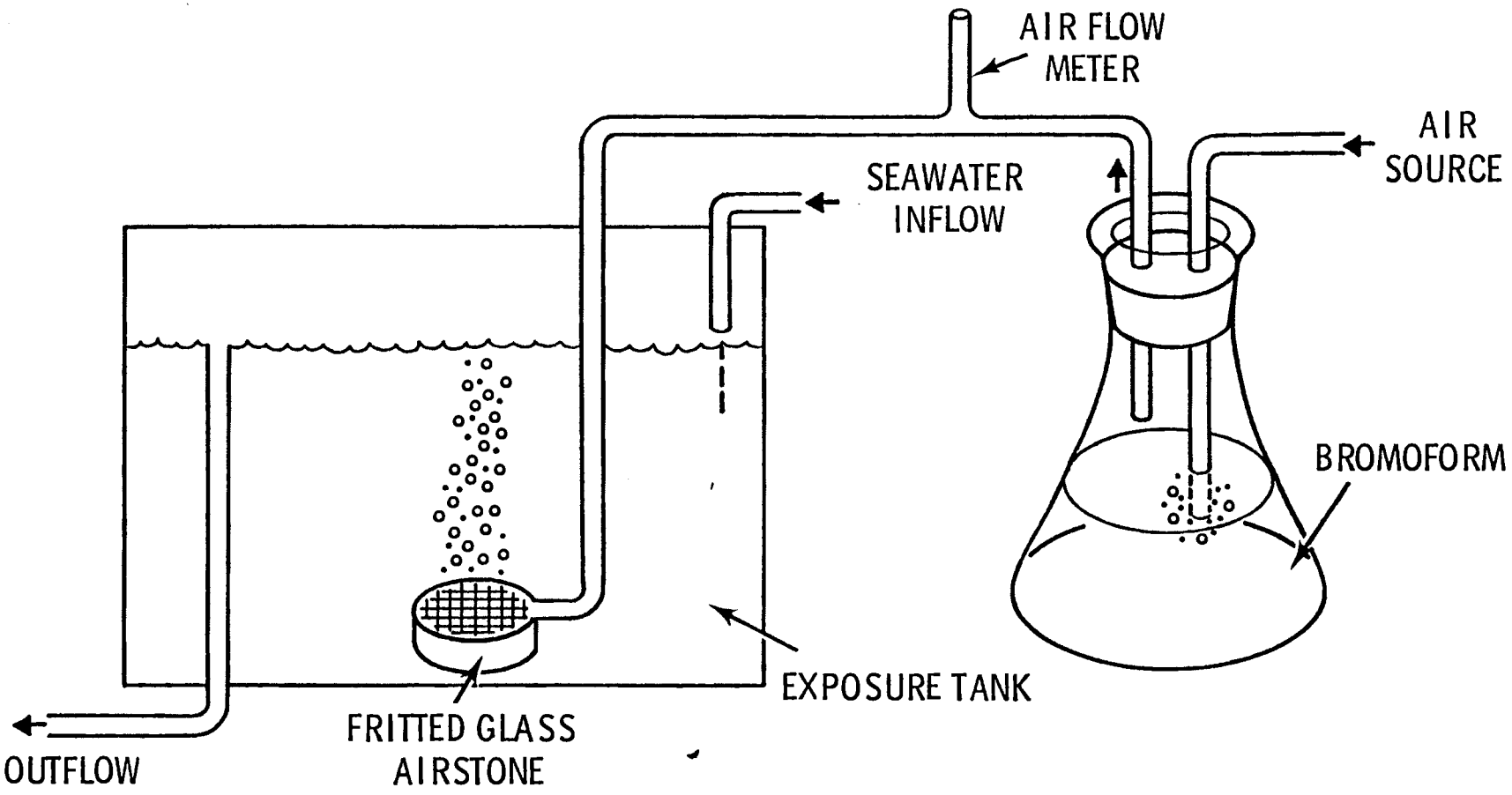
Roberts, M. H. Jr., and R. A. Gleeson. Acute toxicity of bromochlorinated seawater to selected estuarine species with a comparison to chlorinated seawater toxicity. Mar. Environm. Res. 1:19-30. 1978.

Scott, G. I. and Middaugh, D. P. Seasonal chronic toxicity of chlorination to the American oyster, Crassostrea virginica(G). In: Water Chlorination Environmental Impact and Health Effects, Vol. 2, pp. 311-328. (R.L. Jolley, H. Gorchev, D.H. Hamilton, Jr., eds). Ann Arbor Science, Ann Arbor, Mich. 1978.

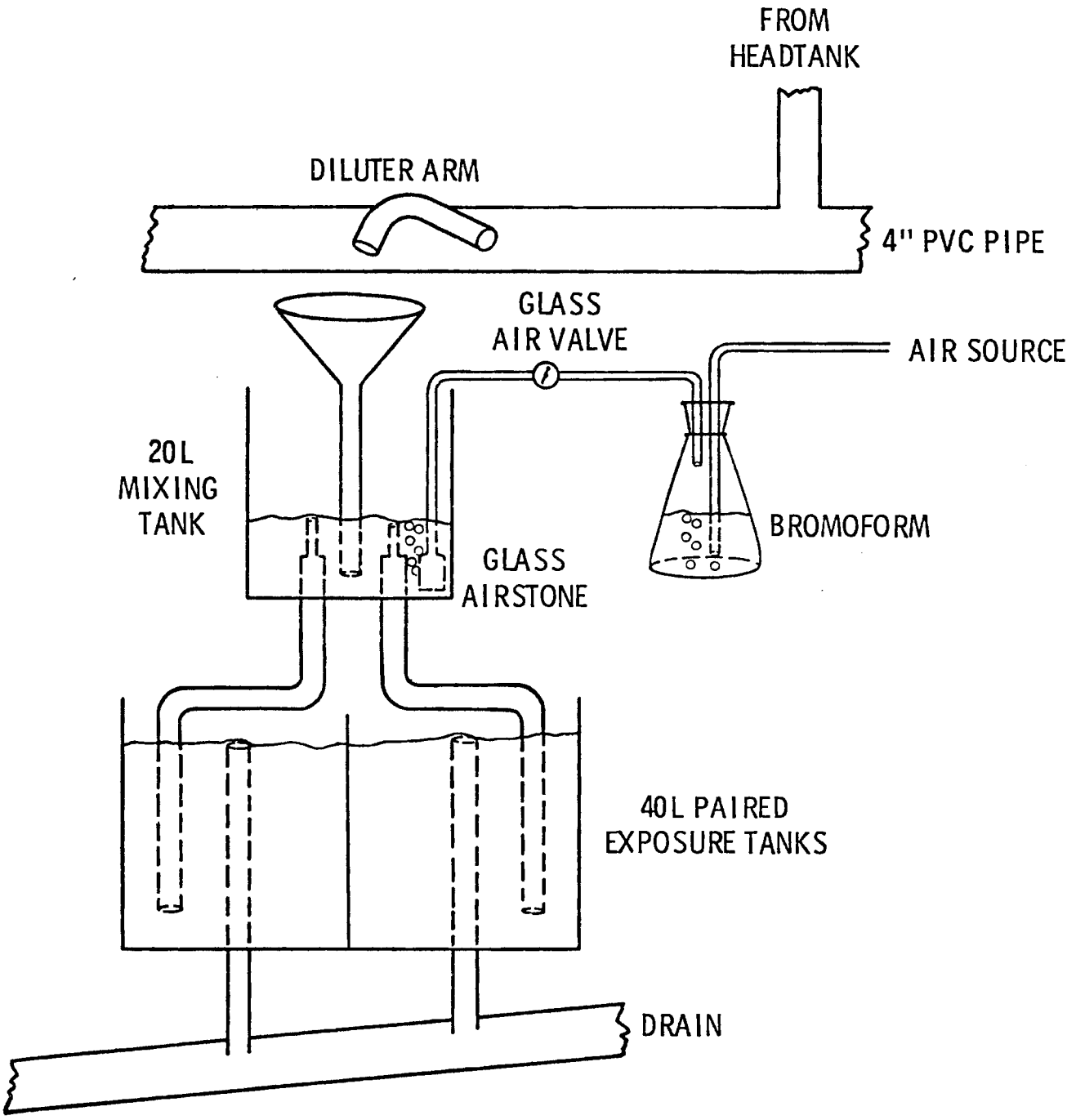
Thatcher, T. O. The relative sensitivity of Pacific Northwest fishes and invertebrates to chlorinated sea water. In: Water Chlorination - Environmental Impact and Health Effects. Vol 2. pp. 341-350. (R. L. Jolley, H. Gorchev, D.H Hamilton, Jr., eds). Ann Arbor Science, Ann Arbor, Mich. 1978.

FIGURE CAPTION

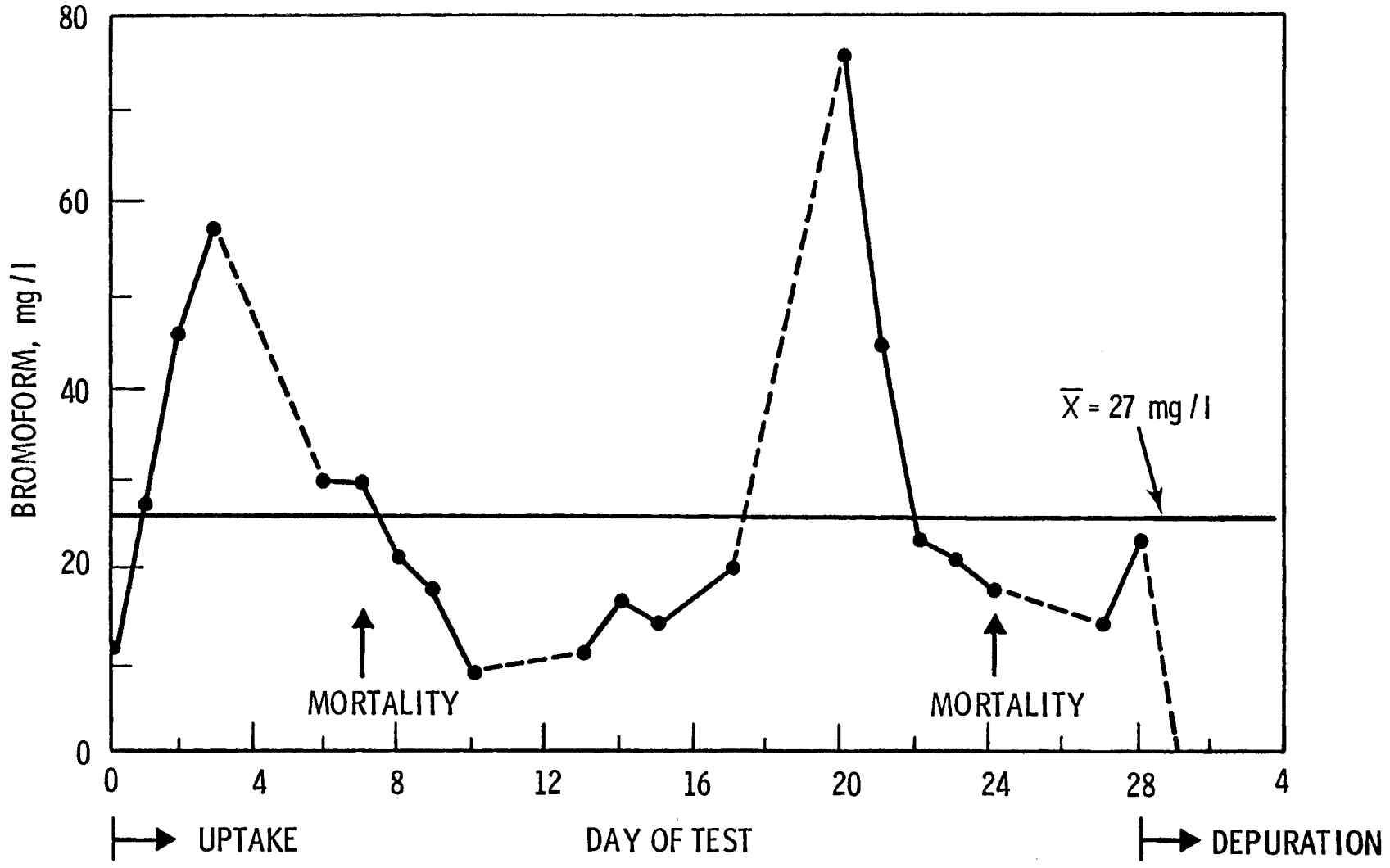
Fig. 1	Toxicant delivery system for seawater/bromoform bioassays conducted at Sequim
Fig. 2	A section of the bromoform exposure system used at the Daytona Beach laboratory
Fig. 3	Measured Concentrations in 20 mg/l exposure tank
Fig. 4	Measured Concentrations in 10 mg/l exposure tank
Fig. 5	Measured Concentrations in 5 mg/l exposure tank
Fig. 6	Measured Concentrations in 1 mg/l exposure tank

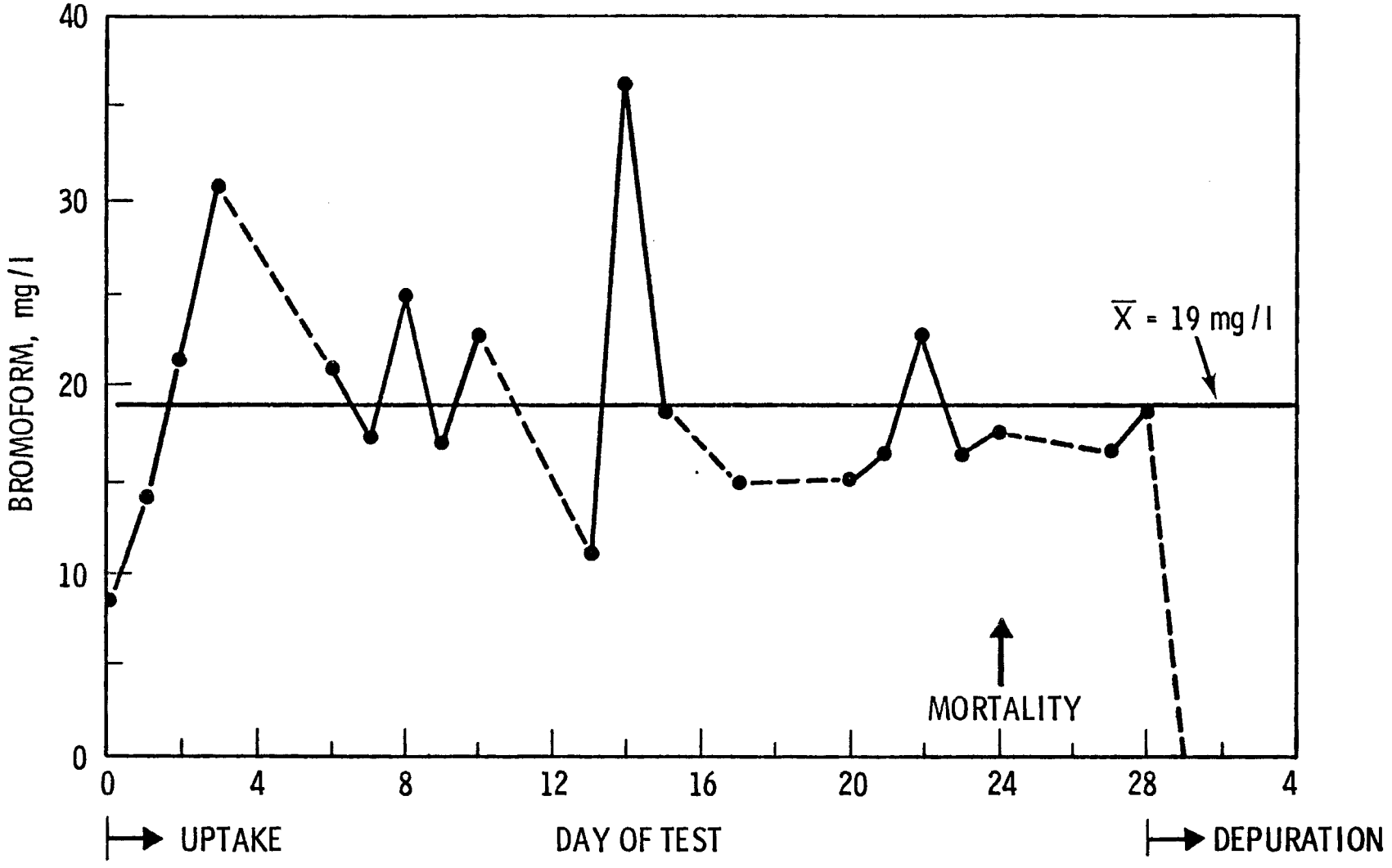


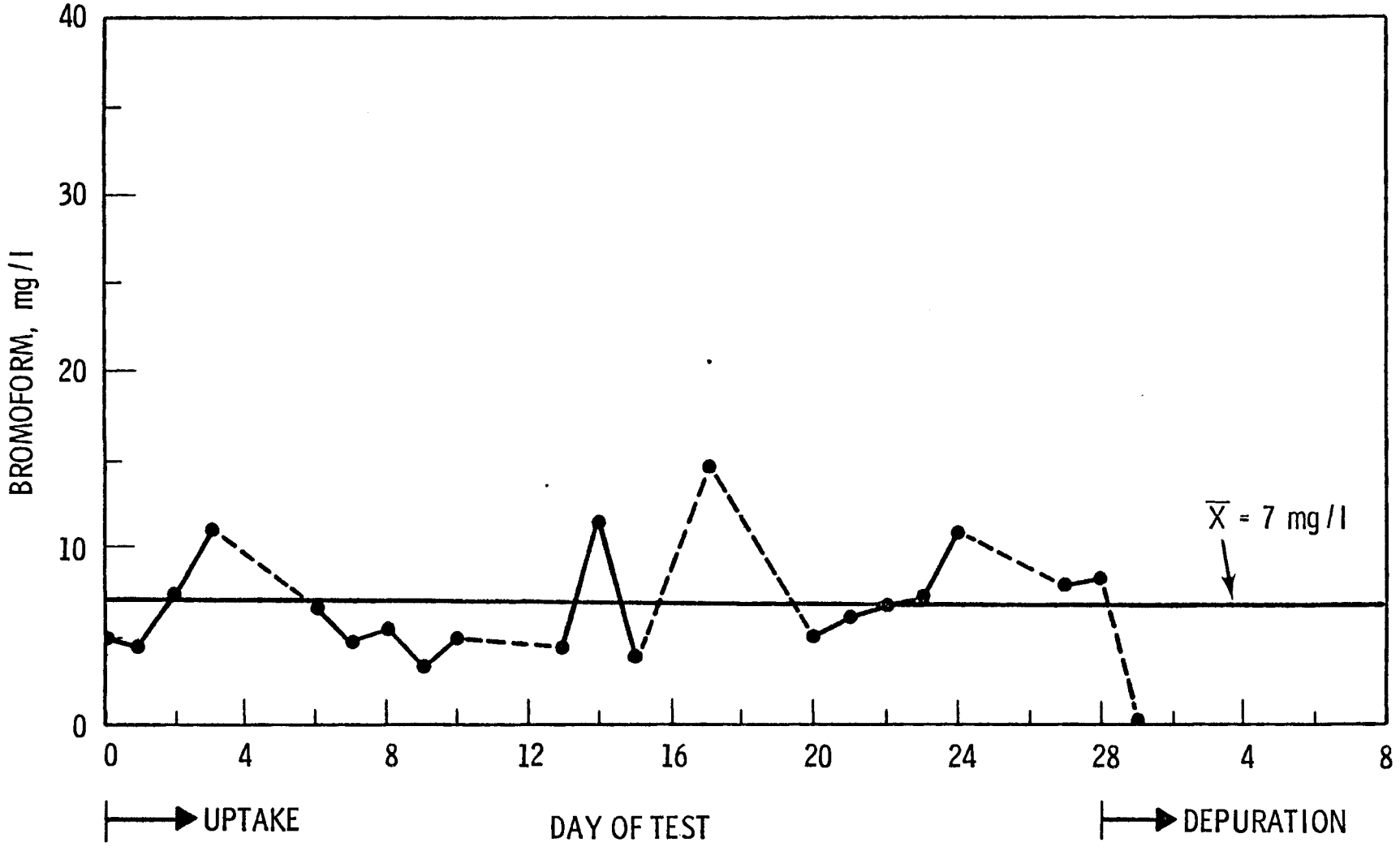
TOXICANT DELIVERY SYSTEM FOR SEAWATER / BROMOFORM BIOASSAYS CONDUCTED AT SEQUIM

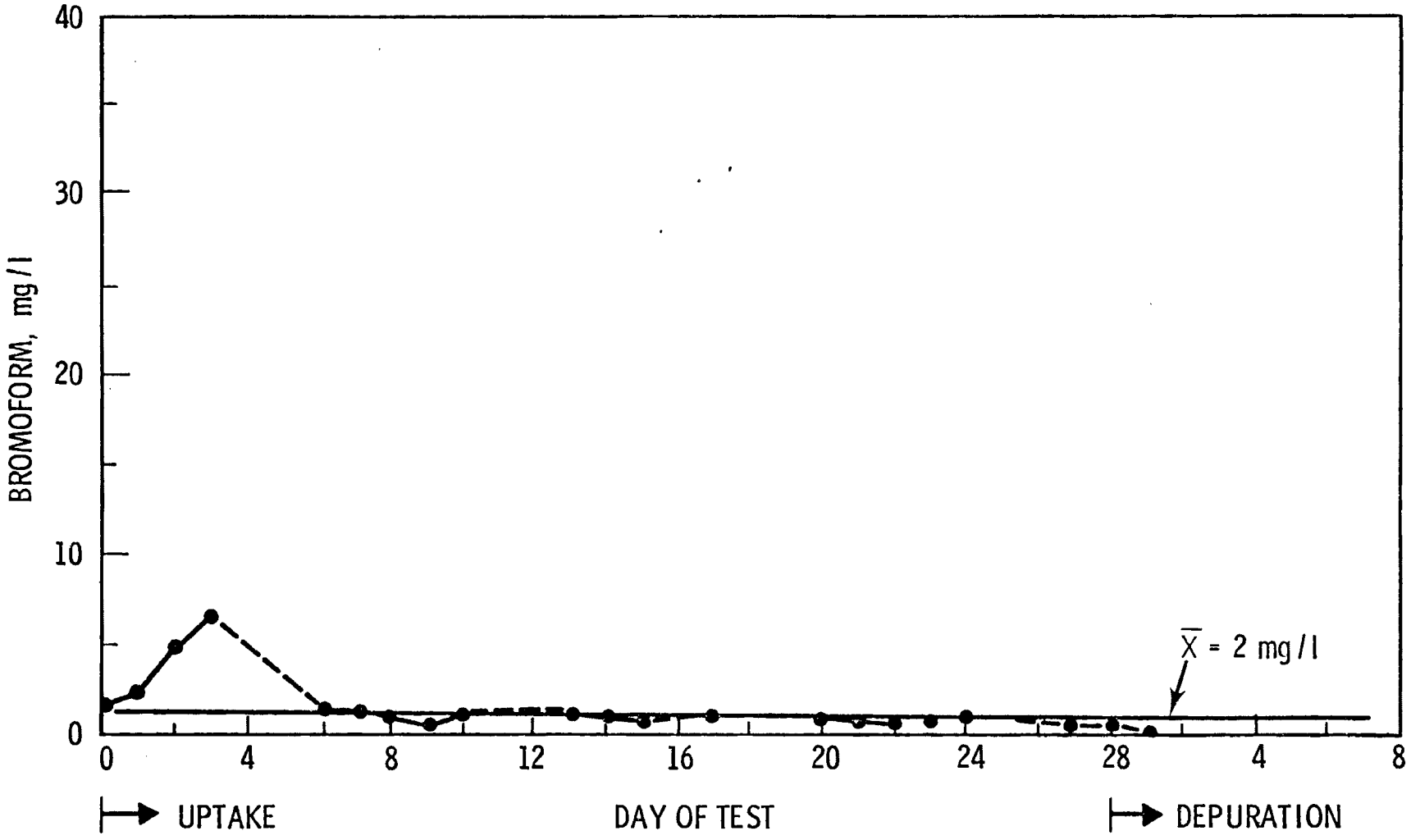


A SECTION OF THE BROMOFORM EXPOSURE SYSTEM USED AT THE DAYTONA BEACH LABORATORY


80
60
40
20
0
BROMOFORM, mg/l
$\overline{X}$ = 27 mg/l
MORTALITY
MORTALITY
0
4
8
12
16
20
24
28
4
UPTAKE
DAY OF TEST
DEPURATION


40
30
20
10
0
BROMOFORM, mg/l
$\overline{X}$ = 19 mg/l
MORTALITY
0
4
8
12
16
20
24
28
4
UPTAKE
DAY OF TEST
DEPURATION


40
30
20
10
0
BROMOFORM, mg/l
$\overline{X}$ = 7 mg/l
0
4
8
12
16
20
24
28
4
8
UPTAKE
DAY OF TEST
DEPURATION


40
30
20
10
0
BROMOFORM, mg/l
$\bar{X}$ = 2 mg/l
0
4
8
12
16
20
24
28
4
8
UPTAKE
DAY OF TEST
DEPURATION

No. of Copies

OFFSITE

19

A. A. Churm
DOE Patent Division
9800 S. Cass Avenue
Argonne, IL 60439

Phillip R. Reed (10)
Bob Samworth
John C. Lehr
NRC
7915 Eastern Avenue
Silver Springs, MD 20910

J. Donald Johnson
Department of Environmental Science and Engineers
School of Public Health
University of North Carolina
Chapel Hill, N. C. 27514

Milton H. Lietzke
P.O. Box X
Oak Ridge National Laboratory
Oak Ridge, Tennessee 37830

Quentin J. Stober
FRI WH-10
University of Washington
Seattle, WA 98195

NRC Division of Technical (2)
Information and Document Control
Washington, DC 20555

DOE TIC (2)

No. of Copies

ONSITE

64

Pacific Northwest Laboratory

Publishing Coordination (2)
Technical Information (5)

DR Anderson (20)	WL Templeton
RM Bean (10)	TO Thatcher
RF Foster	FC Tone
CI Gibson (10)	BE Vaughan
NR Legato	DG Watson
DC Mann	RE Wildung
TL Page	File
RG Riley	LB
RE Schirmer	