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BIOACCUMULATION AND DEPURATION OF BROMOFORM IN FIVE MARINE SPECIES

C. I. Gibson
F. C. Tone
R. E. Schirmer
J. W. Blaylock

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Pacific Northwest Laboratory
Richland, Washington 99352

REA

DISCLAIMER

BIOACCUMULATION AND DEPURATION OF BROMOFORM IN FIVE MARINE SPECIES

C. I. Gibson[1], F. C. Tone[2],
R. E. Schirmer[3], and J. W. Blaylock[1]

[1] Battelle Pacific Northwest Laboratory
Marine Research Laboratory
358B Washington Harbor Road
Sequim, Washington 98382

[2] Battelle Florida Marine Research Facility
Sailfish Drive-Ponce Inlet
Daytona Beach, Florida 32019

[3] Battelle Pacific Northwest Laboratory
P. O. Box 999
Richland, Washington 99352

Abstract

Bromoform has been identified as the major halogenated organic compound resulting from the chlorination of seawater. To test the potential for bioconcentration and subsequent return to man, 28-day uptake/28-day depuration studies were conducted with five commercially and recreationally important species (Protothaca staminea, Mercenaria mercenaria, Crassostrea virginica, Penaeus aztecus, Brevoortia tyrannus). The results indicate that for these species, the uptake and depuration is rapid (equilibrium is reached in 24 to 48 hours), and the concentration factors are relatively low (<1 to 10 times the water concentration).

Introduction

Bromoform has been identified as the major halogenated organic compound resulting from the chlorination of sea water[1,2]. To test the potential for bioconcentrations and subsequent return to man, 28-day uptake/28-day depuration studies were conducted with five marine species (Protothaca staminea, Mercenaria mercenaria, Crassostrea virginica, Penaeus aztecus, and Brevoortia tyrannus).

Methods and Materials

The tests with Protothaca 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. A description of collection procedures, delivery system, water analysis, and exposure system used in these tests has been described previously [3].

For the P. staminea tests, clams were exposed to target bromoform concentrations of 0 (control), 1, and 10 mg/l. Target concentrations of 0 (control) 0.1 mg/l and 1.0 mg/l were used in the tests with Crassostrea virginica, Mercenaria mercenaria, Brevoortia tyrannus and Peneaus aztecus. Subsamples were harvested on day 0, 1, 2, 4, 7, 14, 21, 28 of the uptake phase and day 1, 2, 4, 7, 14, 21, and 28 of the depuration phase and frozen in glass jars for later analysis. In some cases, mortality reduced the number available so that smaller sample groups were collected during the latter phase of the test.

Analysis of tissues for bromoform was done by homogenizing the tissue in water at 0°C and diluting with enough water to obtain a concentration of approximately one gram of tissue per 10 ml of tissue suspension. Ten to 20 ml aliquots of the aqueous tissue suspension were extracted with two 5 ml portions of hexane containing 1, 3-dibromopropane as an internal standard. The microliter samples of the hexane solution were injected into a gas chromatograph fitted with an 18" Porapak Q® column and a ^{63}Ni electron capture detector. The column was operated isothermally at 185°C. The limit of detection of this procedure was 0.5 ppb, and the coefficient of variation ranged from 1% at the 1 to 8 ppm level to 3% at levels below 0.1 ppm.

Results and Discussion

The results of the tissue analyses are presented in Table 1 through 5. The average body burdens of bromoform on each harvest date are plotted with the daily water bromoform concentrations in Figures 1 through 15.

It was difficult to hold the water bromoform concentrations within the desired target concentrations, so the average concentrations, instead of the target concentrations, for the 28-day exposure period are listed in Table 1 through 5. However, in discussions about concentration factors the body burdens are compared to the water concentration the day of harvest.

In general, the three molluscan species, P. staminea, C. virginica and M. mercenaria had tissue concentrations that were about equal to the water concentrations. There were a number of exceptions to this generalization, the most notable being the high body concentration (11.59 mg bromoform/g tissue) found on day 14 in the 1 mg bromoform/l C. virginica test. The

11.59 mg bromoform/g tissue is a concentration factor of approximately 15, a factor that is 3 times higher than any other concentration factor observed for the oysters. However, that particular oyster and 3 others appear to be exceptions to the general trend of body burdens, being approximately the same as water concentrations (Figure 1). At the 0.1 mg-bromoform/l test condition, the tissue concentrations were close to the water concentrations on the day of harvest.

Mercenaria mercenaria body burdens in the 1.0 mg-bromoform/l followed the water concentrations during the first week, but then remained lower than the water for the remainder of the exposure. At the 0.1 mg-bromoform/l the body burdens were slightly above the water concentrations but, in general, followed the water levels closely except for a single individual on day 28. P. staminea tissue concentrations also were similar to the water concentrations on day of harvest.

Menhaden and shrimp were different from the molluscs in that their body burdens were higher than the water concentrations at the 0.1 mg bromoform exposure condition, and at the 1.0 mg-bromoform/l they were higher than the water during the first week, but then fell to approximately the water concentration for the remaining 3 weeks. The control organisms also had significant levels of bromoform in their tissues during the period when bromoform was being introduced into the other test systems. In fact, even though the exposure systems were completely separate, at times there were measurable concentrations of bromoform in the control systems. However, as soon as use of bromoform in the other systems was stopped, it disappeared from the controls. It is apparent that there was sufficient bromoform vapor present in the air to allow some to enter the control exposure systems. The post exposure data, however, indicates that the water and tissue levels

of bromoform return to 0 and remain there. Therefore, the controls in these tests are actually serving as low concentration exposure systems.

The average water concentration during the 28-day exposure of menhaden in the control system was 0.03 mg bromoform/l, a concentration not different from the 0.1 mg bromoform/l target test condition average of 0.04 mg bromoform/l. The same was true for the menhaden exposures where the control average bromoform concentration was 0.03 mg/l and the 0.1 mg/l target was 0.05 mg/l.

These data indicate that bromoform is taken up by these species but that the degree of concentration is dependent on the individual, the species, and the water concentrations. For molluscs, the general trend is for the tissue concentrations to be reflections of the water concentrations. Menhaden and shrimp, however, show a tendency to concentrate bromoform by a factor of 3 to 50 above ambient water concentrations. However, there are also indications that at water concentrations above 0.1 mg/l, the body burdens decrease with time to a level of approximately 0.4 µg/g tissue, a body burden concentration similar to that found in organisms exposed to lower water concentrations.

The testing for bioaccumulation of bromoform is very difficult because of the problem associated with providing a precise concentration of bromoform in water for the 28-day exposure period. In addition, there are problems of vapor contamination of the control systems if the two are operated in the same room. However, the results of the tests presented here indicate that for the molluscs tested, the tissue concentrations will be a reflection of the ambient water concentrations, and that menhaden and shrimp will concentrate bromoform up to a point, after which there appears to be a maximum body burden that is maintained with time. Further testing is needed

to determine where within the organism the bromoform is concentrated and the factors that influence its uptake.

Acknowledgments

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Disclaimer

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

References

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3. Gibson, C. I., F. C. Tone, P. Wilkinson and J. W. Blaylock. Toxicity of effects of bromoform on five marine species. U. S. Nuclear Regulatory Commission under a Related Services Agreement with the U. S. Department of Energy, PNL-3023, (1979).

Figure Captions

1. Water and tissue concentrations of bromoform (Eastern oyster, Crassostrea virginica, 28-day uptake/28-day depuration studies. Target water bromoform concentration was 1.0 mg/l.

2. Water and tissue concentrations of bromoform (Eastern oyster, Crassostrea virginica, 28-day uptake/28-day depuration studies. Target water bromoform concentration was 0.1 mg/l.

3. Water and tissue concentrations of bromoform (Eastern oyster, Crassostrea virginica, 28-day uptake/28-day depuration studies. Control.

4. Water and tissue concentrations of bromoform (Quahaug, Mercenaria mercenaria) 28-day uptake/28-day depuration studies. Target water bromoform concentration was 1.0 mg/l.

5. Water and tissue concentrations of bromoform (Quahaug, Mercenaria mercenaria) 28-day uptake/28-day depuration studies. Target water bromoform concentration was 0.1 mg/l.

6. Water and tissue concentrations of bromoform (Quahaug, Mercenaria mercenaria) 28-day uptake/28-day depuration studies. Control.

7. Water and tissue concentrations of bromoform (Littleneck clam, Protothaca staminea) 28-day uptake/28-day depuration studies. Target water bromoform concentration was 10 mg/l.

8. Water and tissue concentrations of bromoform (Littleneck clam, Protothaca staminea) 28-day uptake/28-day depuration studies. Target water bromoform concentration was 1.0 mg/l.

9. Water and tissue concentrations of bromoform (Littleneck clam, Protothaca staminea) 28-day uptake/28-day depuration studies. Control.

10. Water and tissue concentrations of bromoform (Menhaden, Brevoortia tyrannus) 28-day uptake/28-day depuration studies. Target water bromoform concentration was 1.0 mg/l.

11. Water and tissue concentrations of bromoform (Menhaden, Brevoortia tyrannus) 28-day uptake/28-day depuration studies. Target water bromoform concentration was 0.1 mg/l.

12. Water and tissue concentrations of bromoform (Menhaden, Brevoortia tyrannus) 28-day uptake/28-day depuration studies. Control.

13. Water and tissue concentrations of bromoform (Shrimp, Penaeus aztecus) 28-day uptake/28-day depuration studies. Target water bromoform concentration was 1.0 mg/l.

14. Water and tissue concentration of bromoform (Shrimp Penaeus aztecus) 28-day uptake/28-day depuration studies. Target water bromoform concentration was 0.1 mg/l.

15. Water and tissue concentrations of bromoform (Shrimp, Penaeus aztecus) 28-day uptake/28-day depuration studies. Control.

TABLE 1. Bromoform Concentration in each Test Organism for Eastern Oyster (Crassostrea virginica)(µg/g tissue-wet weight)

Average Bromoform Exposure Concentration (mg/l)	Tank #	1	2	4	7	14	21	28
		SAMPLE DAY - UPTAKE						
0.03	1	0.24	0.24	0.00	0.00	0.00	0.00	0.00
		0.32	0.00	0.00	0.00	0.00	0.00	0.00
		0.00	0.24	0.00	0.00	0.00	0.00	0.00
0.09	2	0.55	0.20	0.00	0.21	0.00	0.24	0.00
		0.23	0.19	0.00	0.00	0.00	0.24	0.18
		0.29	0.21	0.00	0.00	0.00	0.19	0.00
0.86	3	0.89	1.54	3.33	0.32	0.64	0.85	0.48
		1.21	0.83	1.33	0.61	11.59	1.80	0.42
		1.37	0.78	1.10	0.46	0.35	0.39	0.22

Average Bromoform Exposure Concentration (mg/l)	Tank #	1	2	4	7	14	21	28
		SAMPLE DAY - DEPURATION						
0.03	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		0.00	0.00	0.00	0.00	0.00	0.00	0.00
		0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.00	2	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		0.00	0.00	0.00	0.00	0.00	0.00	0.00
		0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.00	3	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		0.00	0.00	0.00	0.00	0.00	0.00	0.00
		0.00	0.00	0.00	0.00	0.00	0.00	0.00

TABLE 2. Bromoform Concentration in each Test Organism for Quahaug (Mercenaria mercenaria)(µg/g tissue-wet weight)

Average Bromoform Exposure Concentration (mg/l)	Tank #	1	2	4	7	14	21	28
		SAMPLE DAY - UPTAKE						
0.03	1	0.11	0.04	0.12	0.08	0.06	*	0.03
		0.11	0.07	0.00	0.07	0.07		0.00
		0.08	0.05	0.07	0.08	0.00		0.00
0.09	2	0.46	0.09	0.10	0.09	0.00	0.17	1.87
		0.42	0.15	0.09	0.03	0.07	0.15	0.25
		0.50	0.15	0.07	0.08	0.22	0.13	0.23
0.93	3	0.11	0.24	0.72	0.61	0.28	0.48	0.09
		0.06	0.14	1.01	0.33	0.31	0.70	0.21
		0.17	0.15	1.28	0.40	0.19	0.34	0.15

Average Bromoform Exposure Concentration (mg/l)	Tank #	1	2	4	7	14	21	28
		SAMPLE DAY - DEPURATION						
0.00	1	0.00	*	1.83	0.00	0.00	0.00	0.00
		0.00		105.95	0.00	0.00	0.00	0.00
		0.00		0.00	0.00	0.00	0.00	0.00
0.00	2	0.00	0.00	*	0.00	0.00	0.00	0.00
		0.00	0.13		0.00	0.00	0.00	0.00
		0.16	0.40		0.00	0.00	0.00	0.00
0.00	3	0.00	0.00	0.00	0.00	0.00	0.00	*
		0.00	0.00	0.00	0.00	0.00	0.00	
		0.17	0.00	0.00	0.00	0.00	0.00	

* No data-sample lost
* No organisms remaining due to earlier mortality

TABLE 3. Average ($\bar{x}$) Body Burden and Standard Deviation (S.D.) for Littleneck Clam (Protothaca staminea)(µg/g tissue-wet weight)

Average Bromoform Exposure Concentration	Tank #		1	2	4	7	14	21	28
			SAMPLE DAY						
0 mg/l	1	$\bar{x}$	.03	.19	.13	0	.50	.01	.01
		S.D.	±.03	±.17	±.09		±.07	±.02	±.03
2 mg/l	2	$\bar{x}$	9.94	5.59	13.65	4.08	2.22	2.05	1.08
		S.D.	±2.16	±1.38	±4.81	±.67	±1.05	±.40	±.18
19 mg/l	3	$\bar{x}$	22.14	17.63	38.05	37.29	22.05	24.85	14.25
		S.D.	±8.59	±2.30	±6.35	±7.14	±3.35	±7.90	±7.32

DEPURATION

Average ($\bar{x}$) Body Burden and Standard Deviation (S.D.) (µg/g tissue-wet weight)

Average Bromoform Exposure Concentration	Tank #		1	2	4	7	14	21	28
			SAMPLE DAY						
0 mg/l	1	$\bar{x}$	0	0	0	0	0	0	0
0 mg/l	2	$\bar{x}$	.12	.03	0	.02	0	0	0
		S.D.	±.26	±.03		±.02			
0 mg/l	3	$\bar{x}$	5.28	0.33*	0	0	0	.17	0
		S.D.	± 4.81*	0.30*	0	0	0	±.02	

* Range - 2 values only

TABLE 4. Bromoform Concentration in each Test Organism for Menhaden (Brevoortia tyrannus)(µg/g tissue-wet weight)

Average Bromoform Exposure Concentration (mg/l)	Tank #	1	2	4	7	14	21	28
		SAMPLE DAY - UPTAKE						
0.03	1 (Control)	1.24	0.00	2.10	0.43	0.18	0.43	0.00
		0.78	0.00	0.00	0.25	0.00	•	•
		1.52	0.91	0.00	0.31	0.17	•	•
0.04	2	0.33	0.51	1.16	0.28	0.20	0.39	0.15
		0.84	0.24	1.13	0.35	0.16	•	•
		0.00	1.10	1.10	0.23	0.18	•	•
0.21	3	0.79	7.61	1.09	0.29	0.13	0.30	0.67
		0.59	0.00	0.83	0.34	0.28	•	•
		1.12	0.53	0.78	0.36	0.21	•	•

Average Bromoform Exposure Concentration (mg/l)	Tank #	1	2	4	7	14	21	28
		SAMPLE DAY - DEPURATION						
0.00	1	0.00	0.00	0.00	0.00	•	•	•
		•	•	•	•			
		•	•	•	•			
0.00	2	0.00	0.00	0.00	0.00	•	•	•
		•	•	•	•			
		•	•	•	•			
0.00	3	0.00	0.00	0.00	•	•	•	•
		•	•	•				
		•	•	•				

• No data
• No organisms remaining due to earlier mortality

TABLE 5. Bromoform Concentration in each Test Organism for Shrimp (Penaeus aztecus)(µg/g tissue-wet weight)

Average Bromoform Exposure Concentration (mg/l)	Tank #	1	2	4	7	14	21	28
		SAMPLE DAY - UPTAKE						
0.02	1	0.49 0.55 0.49	0.64 0.79 0.57	0.33 0.31 0.31	0.41 0.43 0.38	0.33 0.23 0.32	0.42 • •	0.26 • •
0.05	2	0.51 0.50 0.44	0.67 0.50 0.81	0.32 0.39 0.60	0.39 0.24 0.34	0.33 0.30 0.35	0.38 • •	0.00 • •
0.29	3	•	0.83 0.63 0.95	0	0	0.31 0.35 0.37	0.70 • •	0.37 • •
Average Bromoform Exposure Concentration (mg/l)	Tank #	1	2	4	7	14	21	28
		SAMPLE DAY - DEPURATION						
0.00	1	0.00 • •	0.00 • •	0.00 • •	0.00 0.00 •	0.15 0.00 •	0.00 0.00 0.00	• • •
0.00	2	0.00	0.00	0.00	0.00 0.00	0.00 0.00 0.00	0.00 0.00 0.00	0.00 0.00
0.00	3	0.00	0.00	0.00	0.00	0.00 0.00 •	0.00 0.00 •	• • •

0 Sample not processed
• Sample Lost
• No organism remaining due to earlier mortality

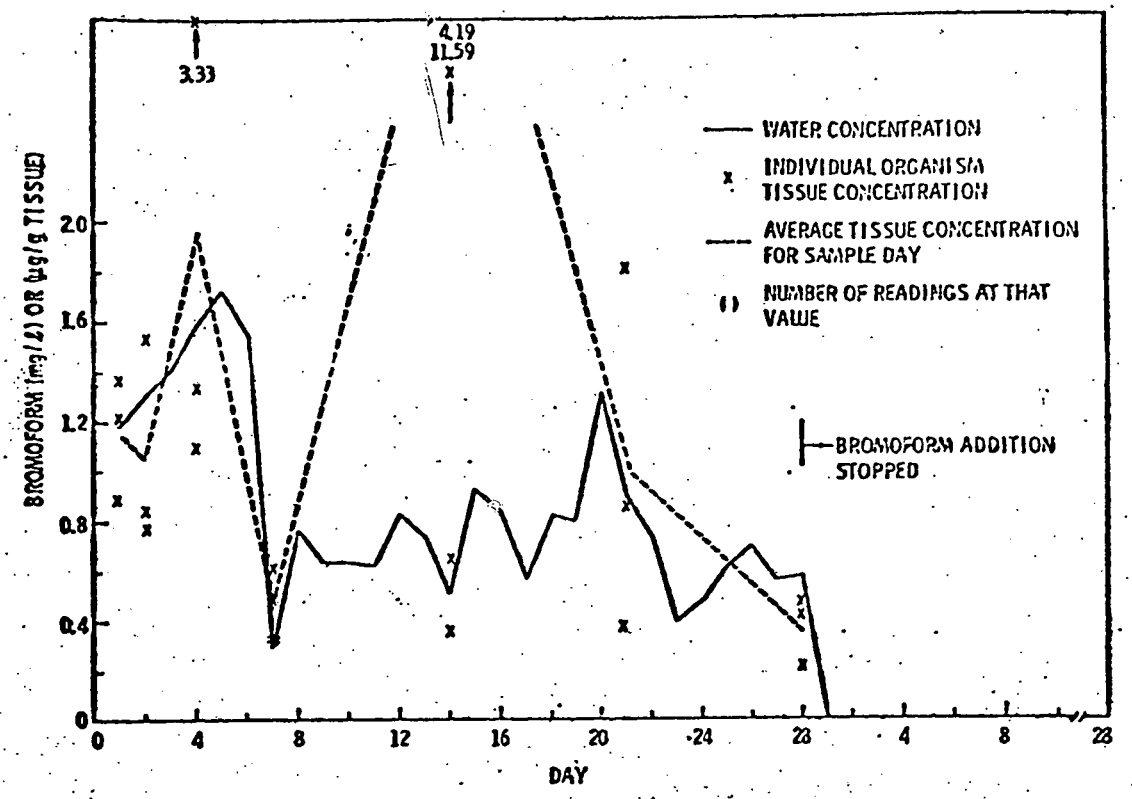

FIGURE 1. Water and tissue concentrations of bromoform (Eastern oyster, Crassostrea virginica), 28-day uptake/28-day depuration studies. Target water bromoform concentration was 1.0 mg/ℓ.

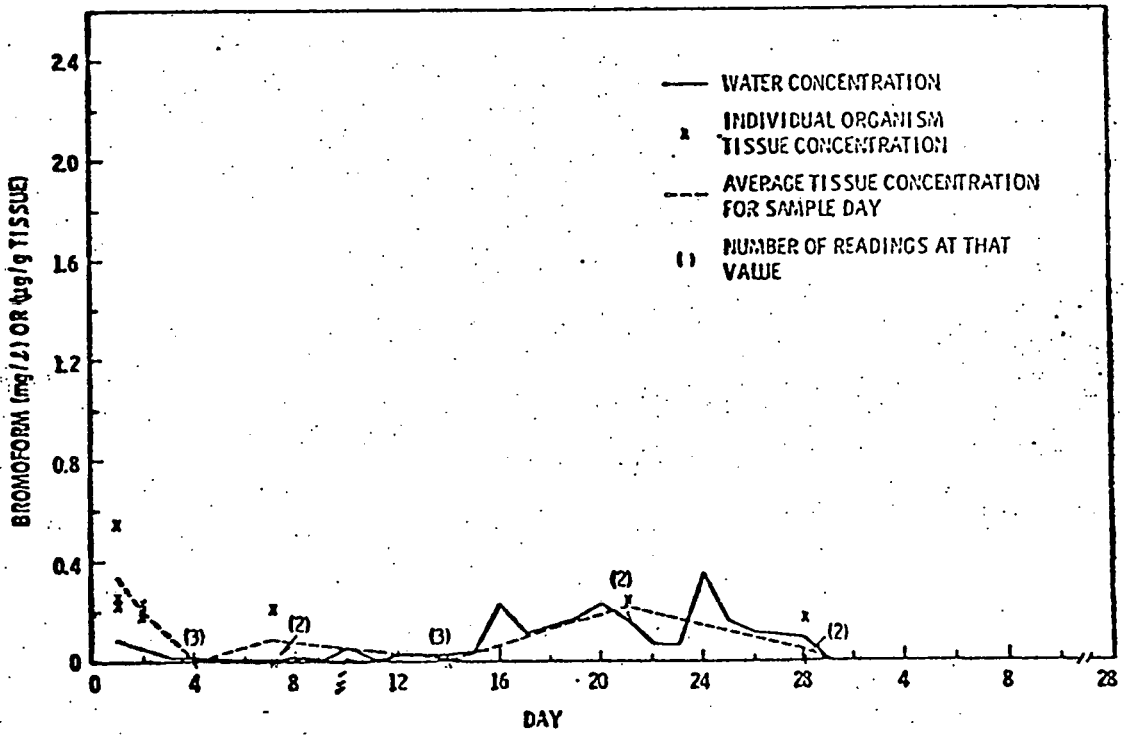

FIGURE 2. Water and tissue concentrations of bromoform (Eastern oyster, Crassostrea virginica), 28-day uptake/28-day depuration studies. Target water bromoform concentration was 0.1 mg/ℓ.

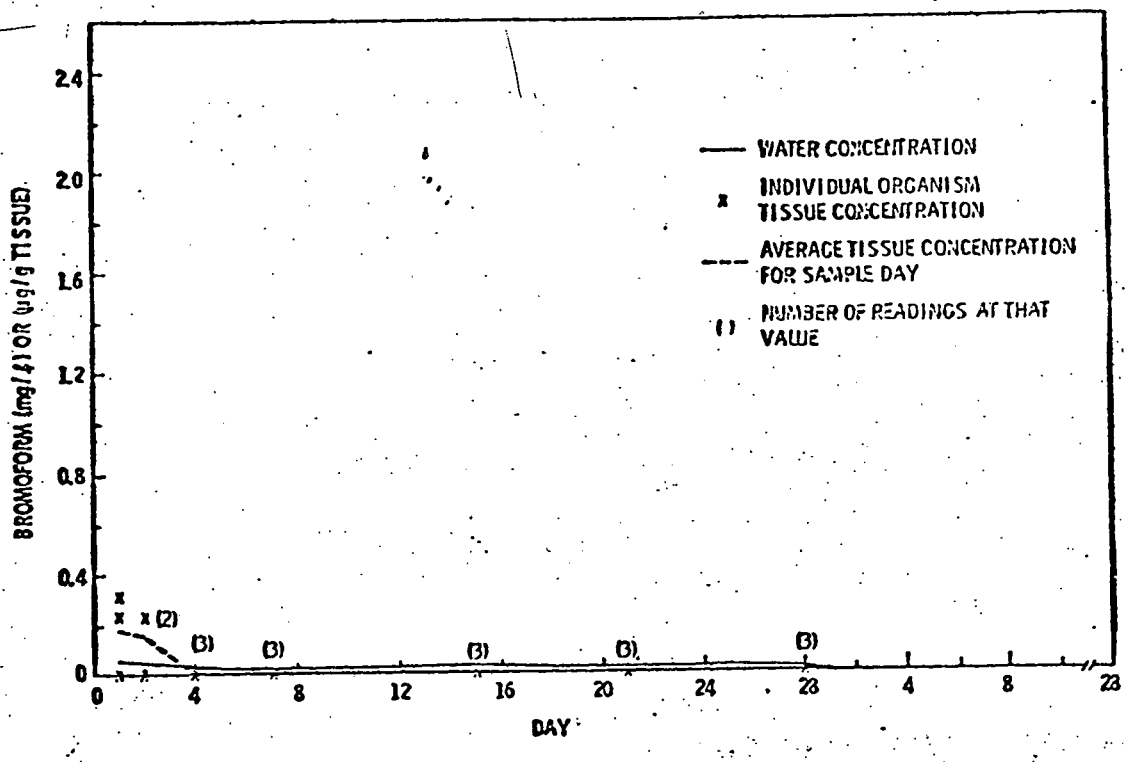

FIGURE 3. Water and tissue concentrations of bromoform (Eastern oyster, Crassostrea virginica), 28-day uptake/28-day depuration studies. Control.

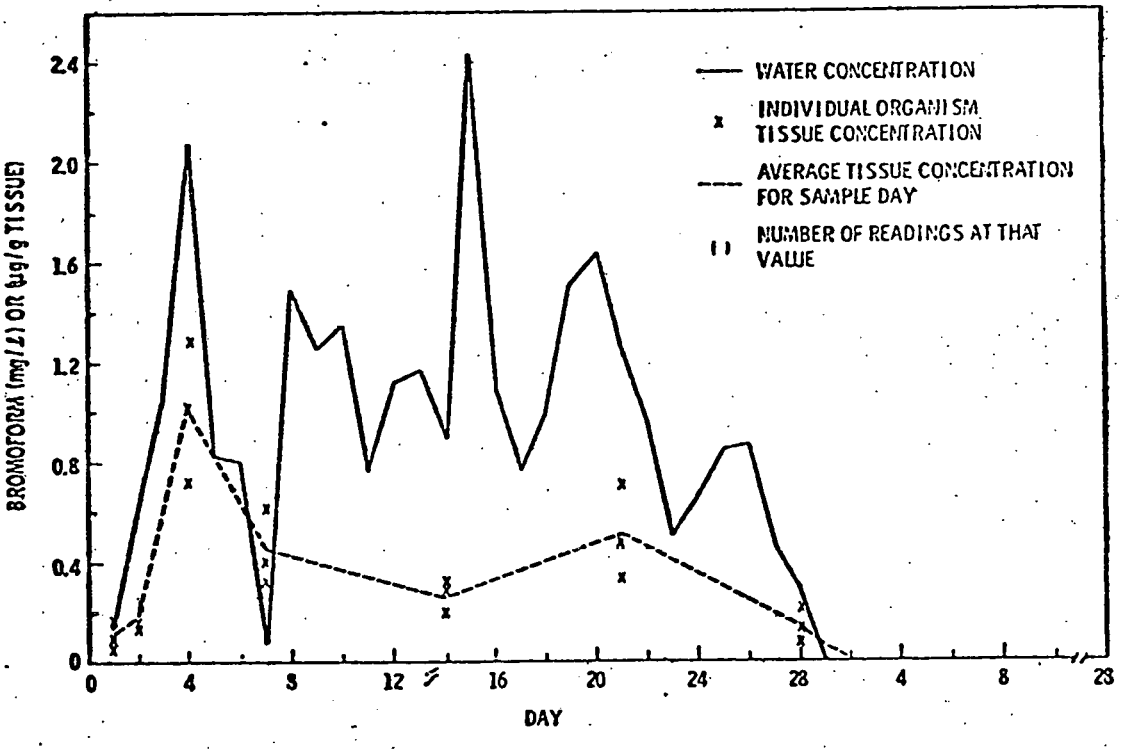

FIGURE 4. Water and tissue concentrations of bromoform (Quahaug, Mercenaria mercenaria) 28-day uptake/28-day depuration studies. Target water bromoform concentration was 1.0 mg/ℓ.

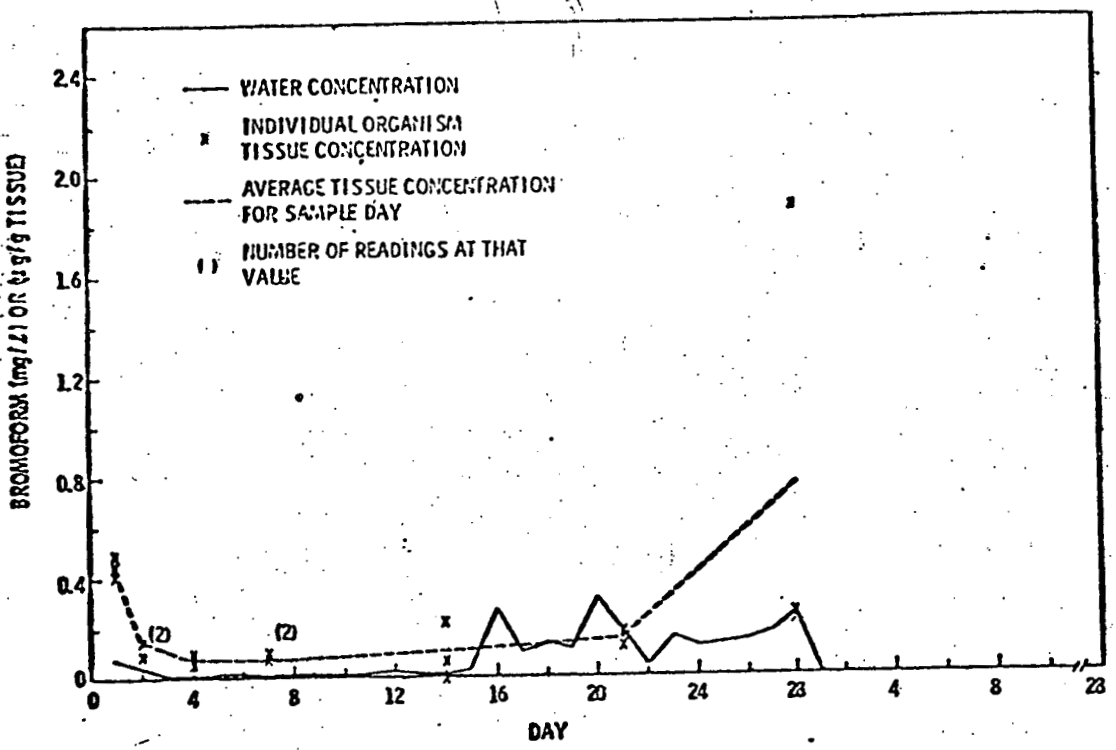

FIGURE 5. Water and tissue concentrations of bromoform (Quahaug, Mercenaria mercenaria) 28-day uptake/28-day depuration studies. Target water bromoform concentration was 0.1 mg/ℓ.

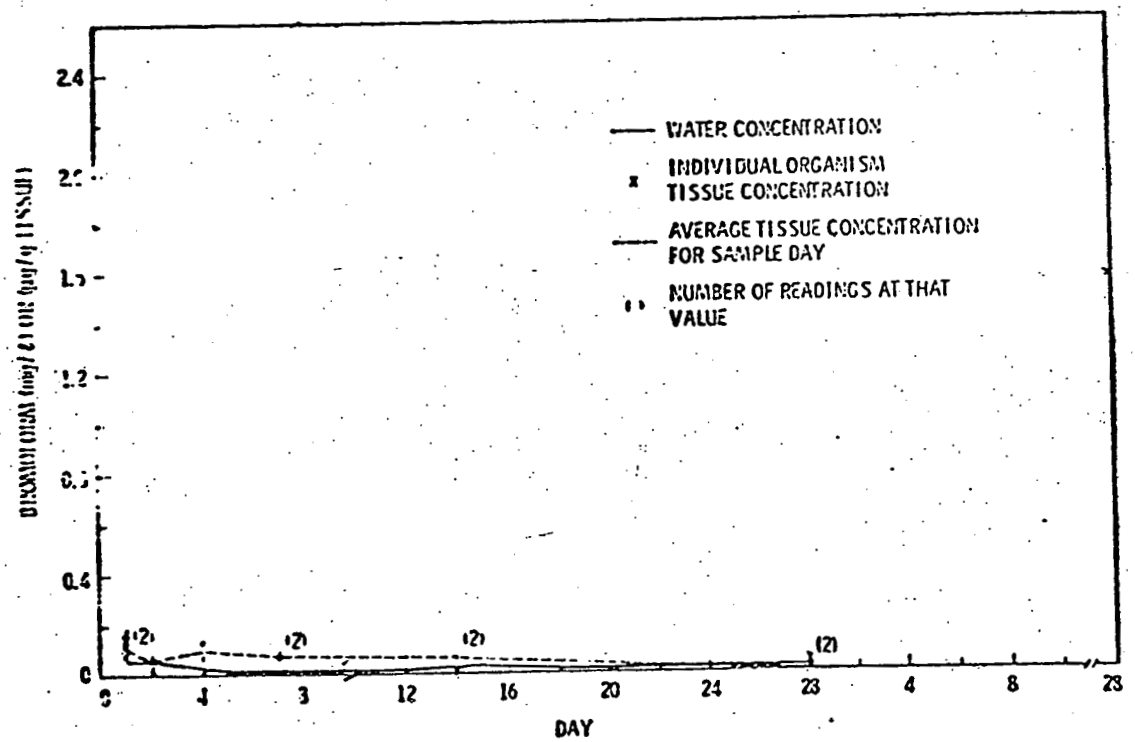

FIGURE 6. Water and tissue concentrations of bromoform (Quahaug, Mercenaria mercenaria) 28-day uptake/28-day depuration studies. Control.

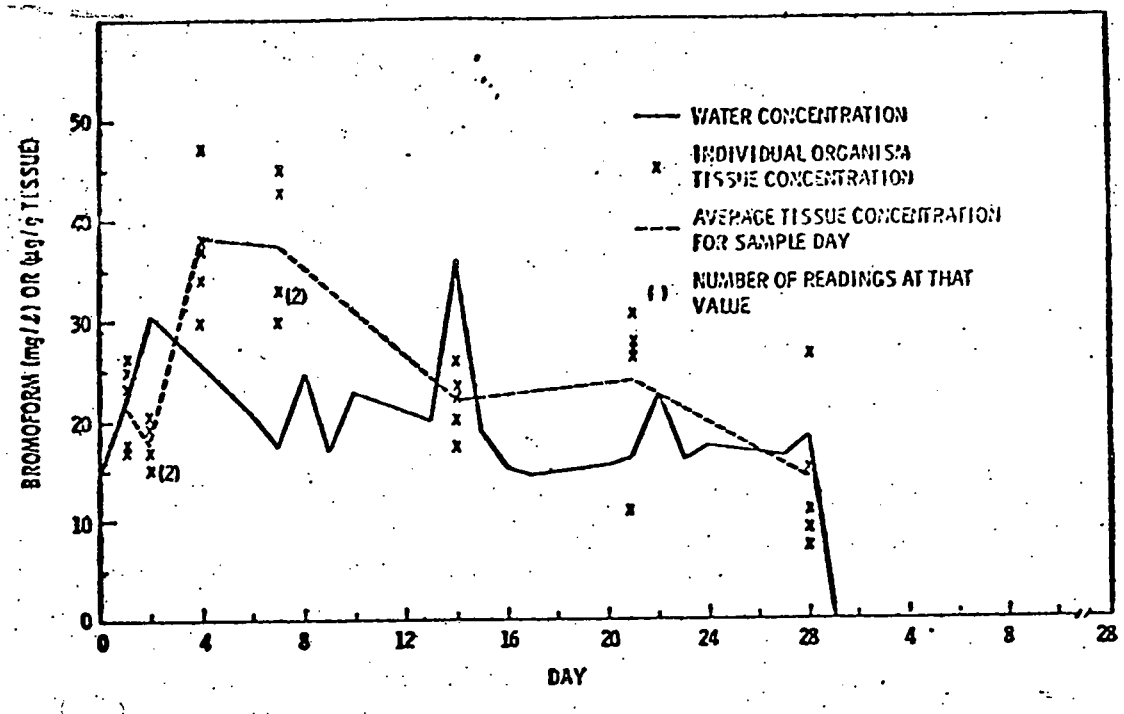

FIGURE 7. Water and tissue concentrations of bromoform (Littleneck clam, Protothaca staminea) 28-day uptake/28-day depuration studies. Target water bromoform concentration was 10 mg/ℓ.

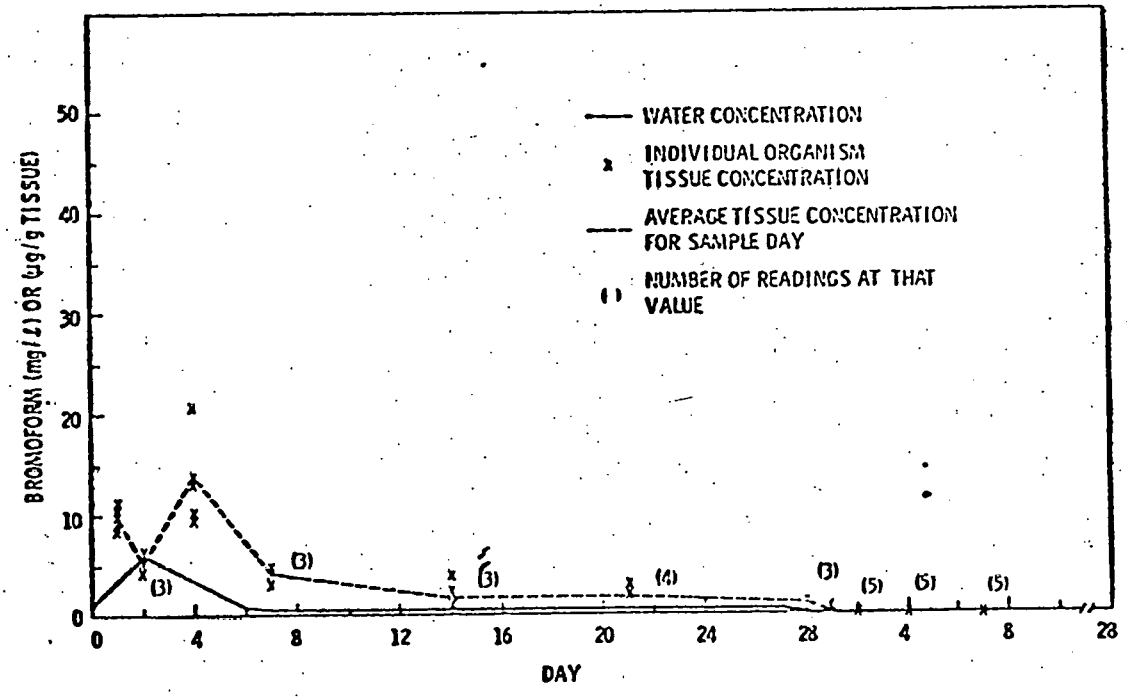

FIGURE 8. Water and tissue concentrations of bromoform (Littleneck clam, Protothaca staminea) 28-day uptake/28 day depuration studies. Target water bromoform concentration was 1.0 mg/ℓ.

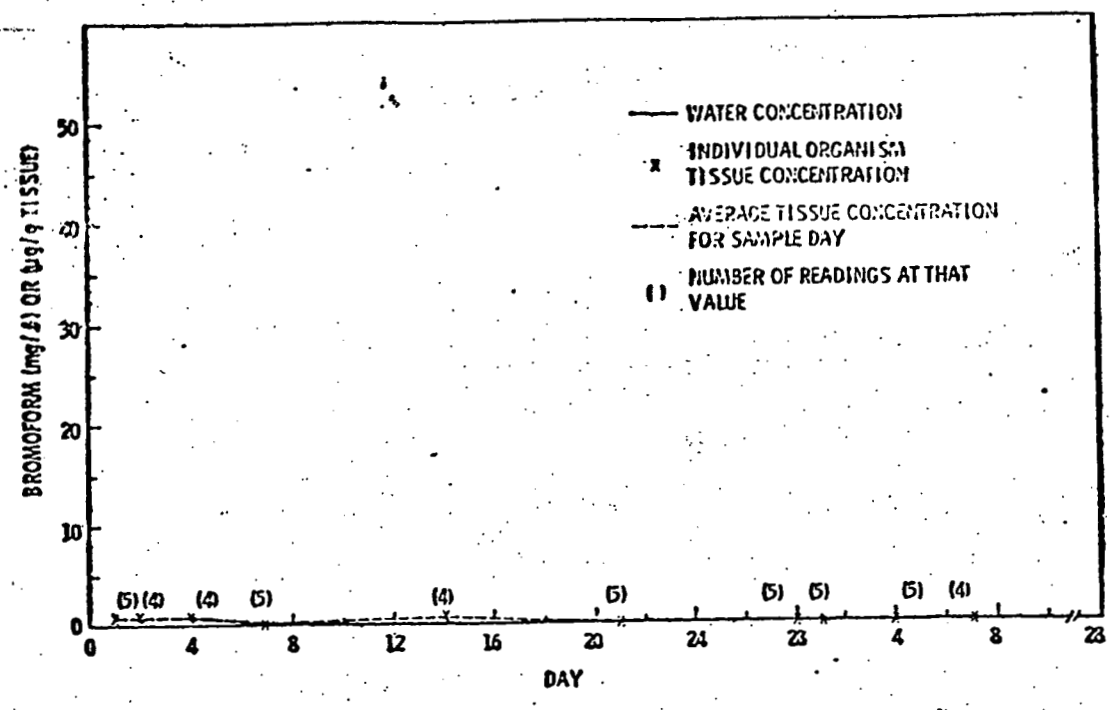

FIGURE 9. Water and tissue concentrations of bromoform (Littleneck clam, Protothaca staminea) 28-day uptake/28-day depuration studies. Control.

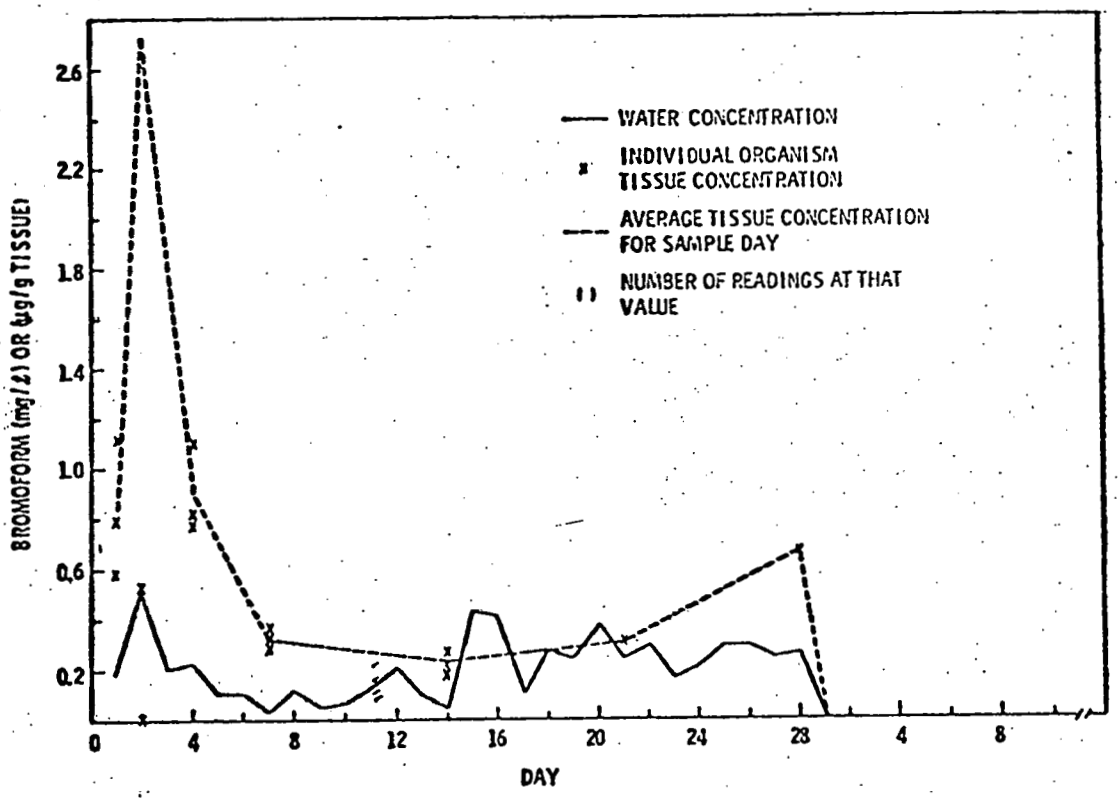

FIGURE 10. Water and tissue concentrations of bromoform (Menhaden, Brevoortia tyrannus) 28-day uptake/28-day depuration studies. Target water bromoform concentration was 1.0 mg/ℓ.

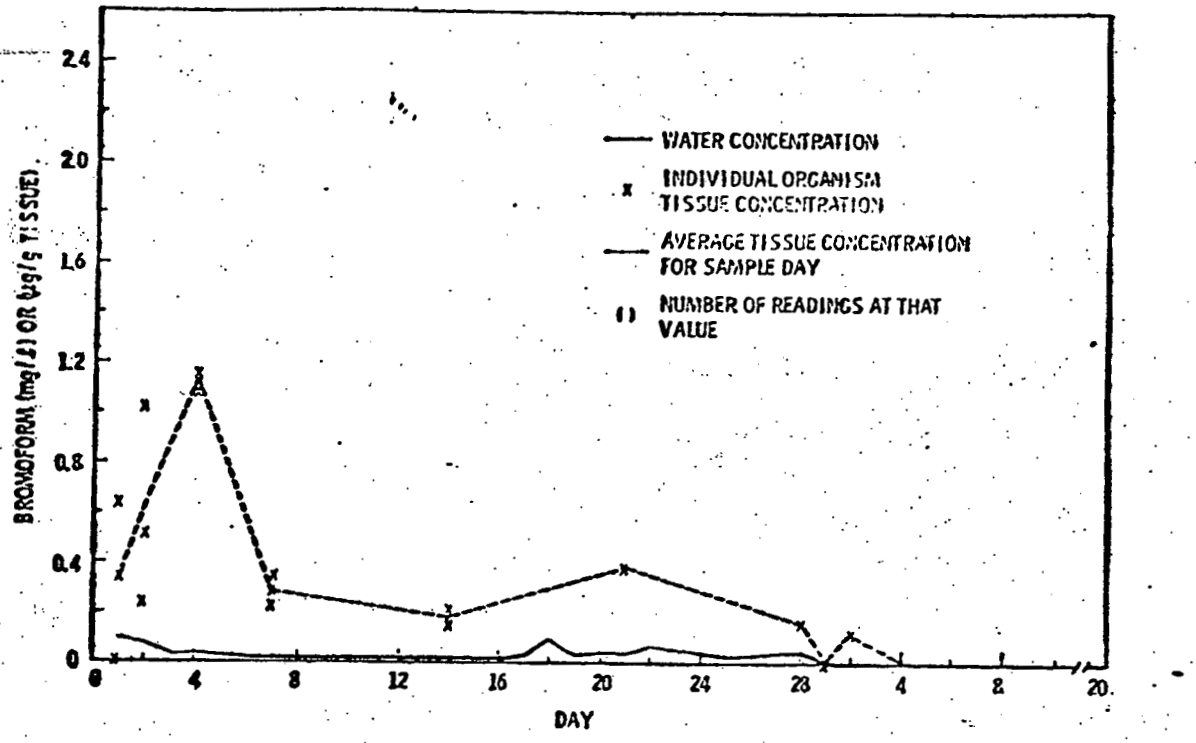

FIGURE 11. Water and tissue concentrations of bromoform (Menhaden, Brevoortia tyrannus) 28-day uptake/28-day depuration studies. Target water bromoform concentration was 0.1ℓ.

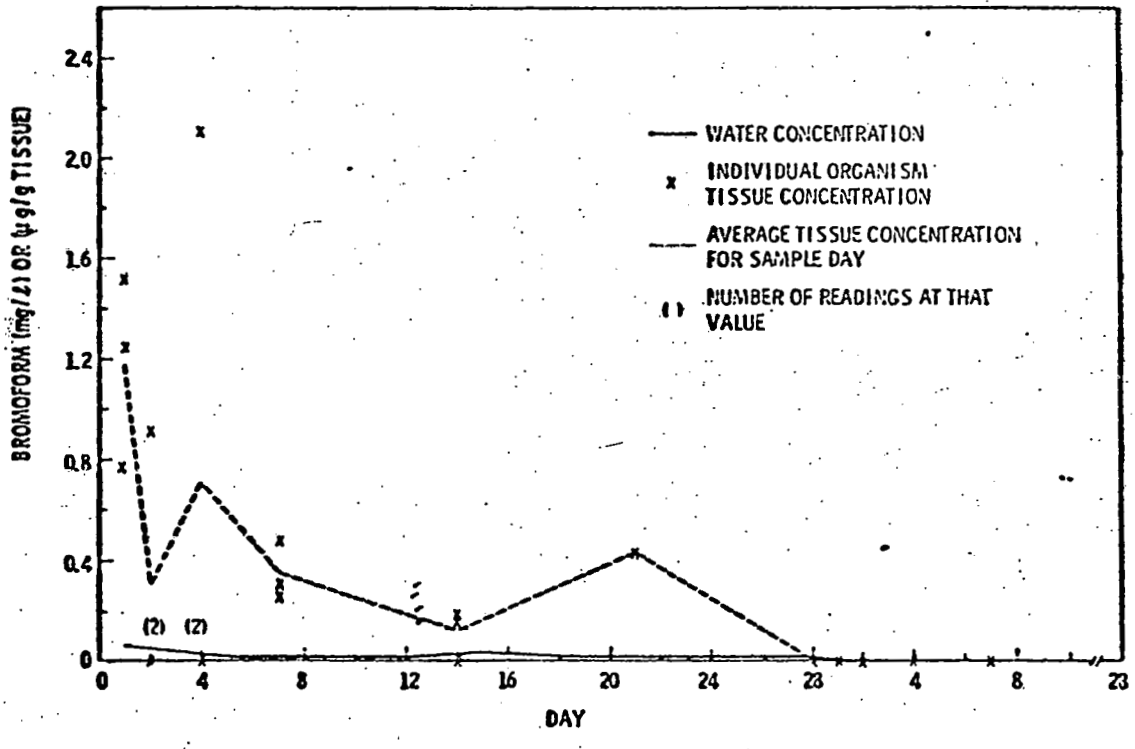

FIGURE 12. Water and tissue concentrations of bromoform (Menhaden, Brevoortia tyrannus) 28-day uptake/28-day depuration studies. Control.

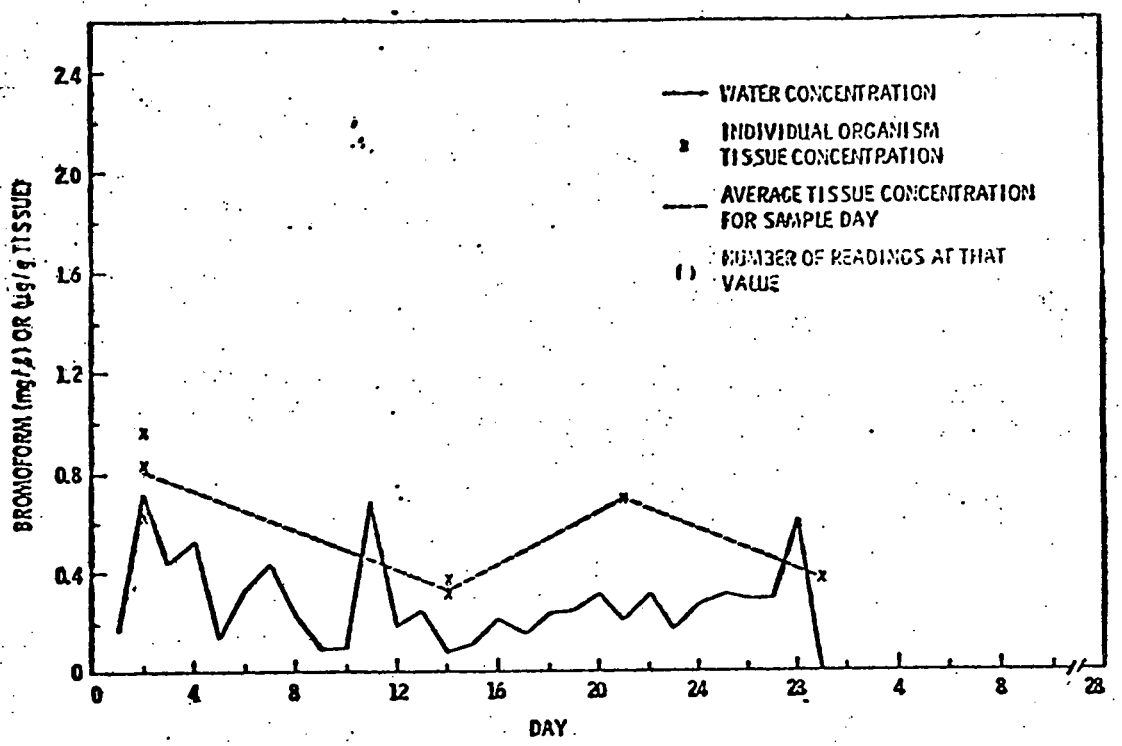

FIGURE 13. Water and tissue concentrations of bromoform (Shrimp, Penaeus aztecus) 28-day uptake/28-day depuration studies. Target water bromoform concentration was 1.0 mg/ℓ.

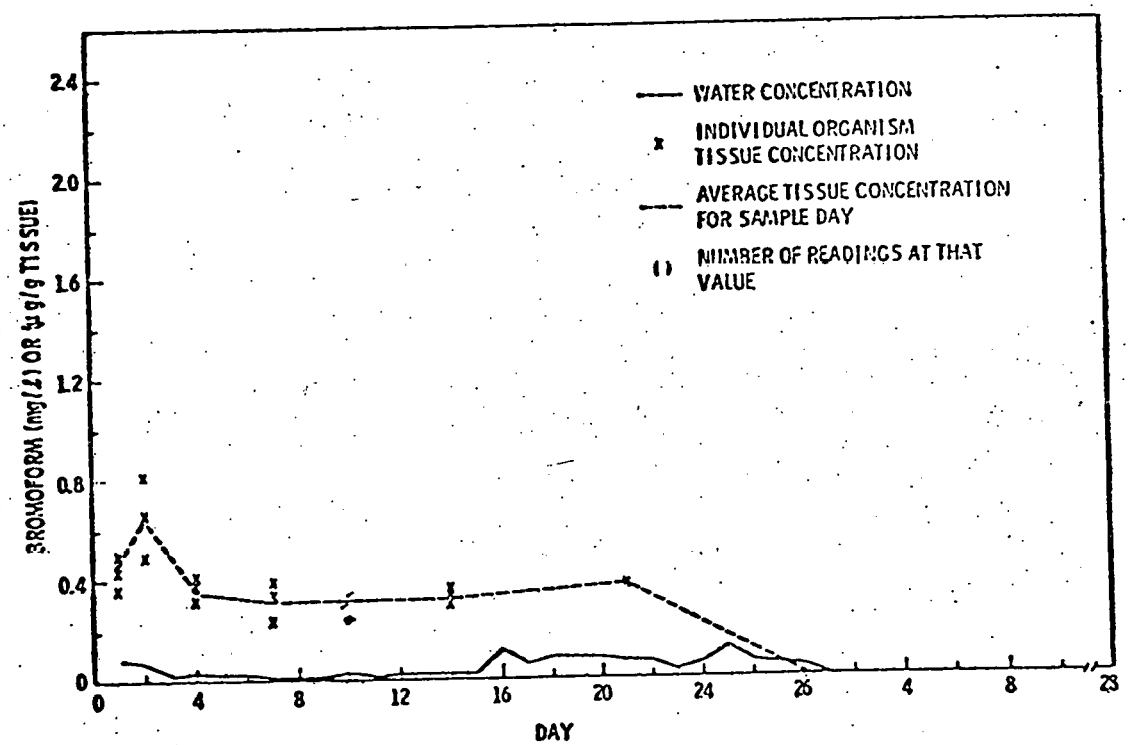

FIGURE 14. Water and tissue concentrations of bromoform (Shrimp, Penaeus aztecus) 28-day uptake/28-day depuration studies. Target water bromoform concentration was 0.1 mg/ℓ.

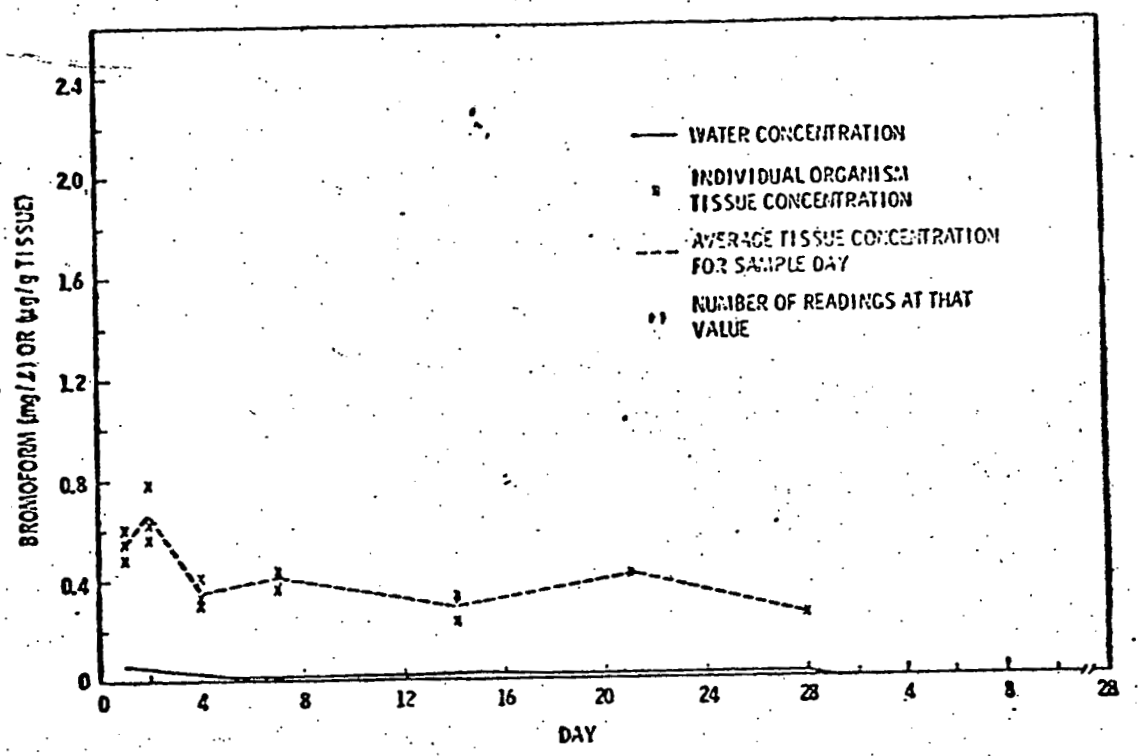

FIGURE 15. Water and tissue concentrations of bromoform (Shrimp, Penaeus aztecus) 28-day uptake/28-day depuration studies. Control.