CN-10-0614

# The Bioaccumulation of Tetrabromobisphenol, in the Bluegill Sunfish

Project 780241

A Report
Submitted to

The Velsicol Chemical Corporation
341 East Ohio Street
Chicago, Illionois 60611

By

Stoner Laboratories, Inc.
409 Mathew Street
Santa Clara, California 95050

July 11, 1978

Donald E. Nye

## ABSTRACT

The bluegill sunfish, Lepomis macrochirus, was exposed to tetrabromobisphenol (Firemaster BP4A) in a flow-through bioassay system. All compounds were labeled with carbon-14 in the aromatic ring. Exposure was for a period of 28 days at 0.0098 ppm. This was followed by a 14 day withdrawal phase. Samples of water and both edible tissue and viscera of the fish were collected during the study for radiocarbon analysis. The tetrabromobisphenol was accumulated by the fish. Bioaccumulation in the edible tissue was 20 fold over the $^{14}C$-concentration in the water. Bioaccumulation in the viscera was 170 fold (1.69 ppm). Plateau radiocarbon levels in both the edible tissue and viscera were reached within 3-7 days of beginning the exposure phase. In the fish, radiocarbon dissipation (to less than 0.01 ppm) occurred within 3-7 days of the withdrawal phase while the half-life was less than 24 hours.

## INTRODUCTION

The object of this study was to investigate the bioaccumulation of tetrabromobisphenol in the bluegill sunfish, Lepomis macrochirus. A flow-through bioassay system developed by Stoner Laboratories was used to expose the fish to the chemical for a period of 28 days followed by a 14 day withdrawal period. This system was designed specifically to conform to the most recent draft of the Guidelines for Registering Pesticides in the United States (January 30, 1978). Published papers by Kuzeminiski et al. (1975) and Schultz (1973) were used as guides. An exposure level of 0.01 ppm was used. The rate of accumulation during the 28 day exposure phase and the bioaccumulation ratio when the fish had reach equilibrium with their environment were calculated. The half-life of the radiocarbon residue in the fish during the withdrawal phase was also determined.

## MATERIALS AND METHODS

Test Chemicals: The test chemical was supplied by the Velsicol Chemical Corporation, and was labeled with carbon-14 uniformly in the aromatic ring with a specific activity of 9.32 mCi/mmole. For the study, the compound was diluted with non-radiolabeled material to yield a final specific activity of 2.91, mCi/mmole.

Test System: The system used to deliver the chemical at a constant level to the aquaria containing the bluegill sunfish is illustrated in Figure 1. An amber glass bottle (1) was used as the chemical reservoir and contained 2950 ml of stock solution. This stock solution was delivered to the mixing chamber (3) by a peristaltic pump (2) at a rate of 0.062 ml/min. The dilution water used to make the final concentration of the chemical was tap water, aerated in a storage tank (5) and fed by gravity into the mixing chamber. Since the flow rate of the dilution water was critical for maintenance of the chemical concentration, a double needle valve system was used to control the flow rate. The flow rates for each tank at the initiation of the study, as well as other pertinent information for the chemical delivery system, are given in Table I. The chemical stock solution and the dilution water were constantly mixed using a magnetic stirrer (4) in a 2,000 ml beaker equipped with a siphon which fed directly into the test aquaria (7). For both the

test and control tank, one hundred and ten liter, glass aquaria were used to hold approximately 275,0.5-2.0 g bluegill sunfish. The aquaria were kept at the 90 liter capacity by a siphon which emptied the overflow directly into the laboratory drain.

The fish used in the study were supplied by Alex Fish Co., San Rafael, CA, and were conditioned for 14 days prior to treatment. They were exposed to the $^{14}C$-labeled chemical for 28 days using this dynamic system. At the end of the exposure period, the chemical delivery system was disconnected, allowing the fish a 14 day withdrawal phase.

Sampling and Radioassay: Water samples (3 ml) were collected on a daily basis from the test tank and control tank. They were assayed by direct liquid scintillation counting in 15 ml of Packard Insta-gel liquid scintillation cocktail using a Nuclear Chicago Isocap-300 liquid scintillation spectrometer. Fish samples (approximately 10 g) were collected on days 1, 3, 7, 10, 14, 21, and 28 of the treatment phase and days 1, 3, 7, 10, and 14 of the withdrawal phase. Radioassay of the fish was performed after separation of edible and visceral tissue. The edible sample was taken by removing tissue (including skin and the fin) from the dorsal half of the fish above the gut cavity and by segmenting the fish vertically posterior to the anal vent. These two portions were combined into one edible tissue sample. The remainder of the fish was used for the visceral sample. Both the edible and visceral samples were homogenized by grinding in a mortar and pestle with dry ice. The dry ice was allowed to sublime, and a 0.1-0.5 subsample was analyzed by combustion using the Harvey Biological Materials Oxidizer and trapping of the liberated $^{14}CO_2$. All radioassays were performed in triplicate, and the results are expressed as equivalent ppm based on the specific activity of the radiolabeled chemicals. The limit of detection was 0.002 ppm in the water and 0.01 ppm in the fish (2 x background).

## RESULTS AND DISCUSSION

A number of minor difficulties arose at the initiation of the study due to both the nature of the chemicals and the design of the chemical delivery system. The most serious of these occurred three to five days after the intiation of the treatment phase. The aqueous ethanol solution used in the chemical delivery system was an ideal carbon source for slime bacteria of the genus Sphaerotilus, which occur in the Santa Clara water system. A bacterial layer covered the internal surfaces of the systems, including the control: the mixing chambers, the siphon tubes leading to the aquaria, the aquaria, and the siphon tubes leading from the tanks. The bacteria proliferated very rapidly and nearly shut down the system. A filter was installed on each tank to remove the bulk of the bacteria. Because it was thought that the bacteria might adsorb the chemical and thereby act as a source of biomagnification, they were analyzed by combustion. The bacteria did not contain radiocarbon levels above the concentration of the water and were thus determined not to be a source of biomagnification for the test chemicals.

Fish mortality data for the treatment and control aquaria is given in Table II. Fish mortality occurred early in the study due to the range in size of the fish and competition for food between the fish. Mortality was only observed with fish in the 0.5-1.0 g size range.

The results of the analyses of the fish and water samples are found in Table III and are graphically illustrated in Figure 2. The mean concentration of the test chemical in the water during the treatment phase of the study was 0.0098 + 0.0014. This concentration should have been attained within 2 days of initiation of the treatment phase due to the 150 liter/day water exchange; however, no radiocarbon could be found 2 days after beginning the study. This was principally due to the low water solubility of the compound and the affinity for the plastic tubing of the chemical delivery system. Technicon type autoanalyzer tubing was used to deliver the chemical from the stock containers through the peristaltic pump to the mixing chamber. Upon initiation of the treatment phase, this section of tubing (1.5 m) quantitatively absorbed the tribromobisphenol. Two days elapsed before corrective measures were taken. Teflon tubing was substituted up to and away from the peristaltic pump (#2, Figure 1), decreasing the residence time of the chemical in the Technicon type

tubing and the surface area available for absorption. This change of tubing was considered the beginning of the treatment phase.

Once delivery of the chemical to the aquaria had begun, fish samples were collected and analyzed. Tetrabromobisphenol was accumulated by the fish. Figure 2 indicates that radiocarbon levels in both the edible and visceral tissue of the fish reached plateau within 3 days of initiation of the treatment phase. The average radiocarbon level in the edible tissue was 0.196 ppm which represents a 20 fold accumulation over the concentration in the water. The radiocarbon levels in the viscera were more variable than those of the edible tissue but were generally 9 times higher than in the edible tissue (at 1.69 ppm). This value represents a 170 fold accumulation over the radiocarbon concentration in the water.

Once the chemical delivery system was disconnected (day 28), the radiocarbon level in the water decreased to 0.002 ppm on day 29 and was below the limit of detection after day 31. The radiocarbon levels in the sunfish, both in the edible tissue and the viscera, decreased nearly as rapidly and were below the limit of detection (0.01 ppm) 3 to 7 days after beginning the withdrawal phase. The half-life for the radiocarbon in edible and visceral tissue was less than 24 hours (Table III).

Tetrabromobisphenol (Firemaster BP4A) did not show a potential for bioaccumulation in this aquatic system. Although the bluegill sunfish did absorb the compound during the treatment phase, once withdrawal of the chamical had begun, the fish completely eliminated the radiocarbon residues within 7 days.

## REFERENCES

Krezeminiski, S. F., Brackett, C. K., and Fisher, J. D. 1975. Fate of microbial 3-isothiazolone compounds in the environment: Modes and rates of dissipation. J. Agr. Food Chem. 23:1060.

Schultz, D. P. 1973. Dynamics of a salt of (2,4-dichlorophenory)-acetic acid in fish, water, and hydrosoil. J. Agr. Food Chem. 21:186.

TABLE I

| Chemical | Flow Rate | Chemical Stock Concentration | Solvent Carrier | Aquaria Final Concentration |
|---|---|---|---|---|
| Control | 105 ml/min | -- | 10% Acetone-water | -- |
| tetrabromobisphenol | 106 ml/min | 16.75 ppm | 40% Ethanol-water | 0.01 ppm |

TABLE II

Individual Mortality Data for the
Bluegill Sunfish Bioaccumulation Study

| | Test Compound/Mortality [a] | |
|---|---|---|
| Day | Control | Tetrabromobisphenol |
| 1 | - | - |
| 2 | 1 | - |
| 3 | 1 | 2 |
| 4 | 2 | - |
| 5 | - | - |
| 6 | 2 | - |
| 7 | 2 | 1 |
| 8 | 4 | 1 |
| 9 | - | - |
| 10 | 1 | 2 |
| 11 | 2 | - |
| 12 | 3 | - |
| 13 | 1 | - |
| 14 | - | - |
| 15 | 2 | - |
| 16 | - | - |
| 17 | - | - |
| 18 | 1 | 1 |
| 19 | 1 | - |
| 21 | 2 | - |
| 22-42 | | |
| Total | 25 | 7 |

[a] Number of individuals found dead at the morning feeding

TABLE III

Bioaccumulation of tetrabromobisphenol in the Bluegill Sunfish

| Interval (Day) | Concentration (Parts per Million) tetrabromobisphenol | | |
|---|---|---|---|
| | Water | Bluegill Sunfish | |
| Treatment Phase | | Edible | Viscera |
| 1 | 0.005 | 0.043 | 1.252 |
| 3 | 0.007 | 0.157 | 1.718 |
| 7 | 0.009 | 0.160 | 0.808 |
| 10 | 0.010 | 0.168 | 1.480 |
| 14 | 0.011 | 0.260 | 2.840 |
| 21 | 0.010 | 0.260 | 1.870 |
| 28 | 0.010 | 0.170 | 1.421 |
| Withdrawal Phase | | | |
| 1 | 0.002 | 0.040 | 0.99 |
| 3 | ND | 0.035 | 0.091 |
| 7 | ND | ND | 0.010 |
| 10 | ND | ND | ND |
| 14 | ND | ND | ND |

FIGURE 1 The chemical delivery system used in the bluegill sunfish bioaccumulation study.

(1) stock chemical reservoir
(2) peristaltic pump
(3) mixing chamber
(4) magnetic stirrer
(5) dilution water reservoir
(6) double needle valve regulator for dilution water
(7) test tank

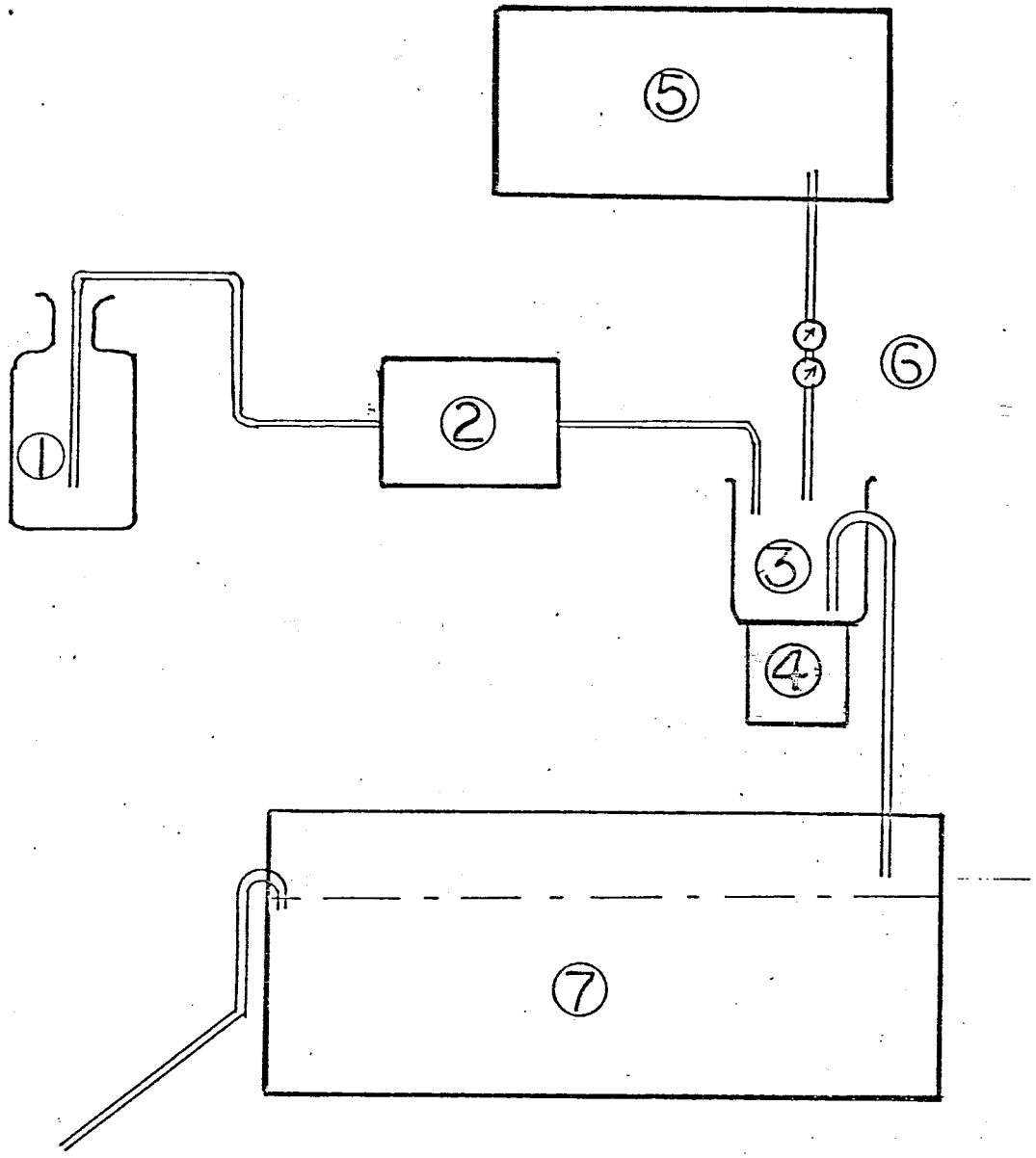
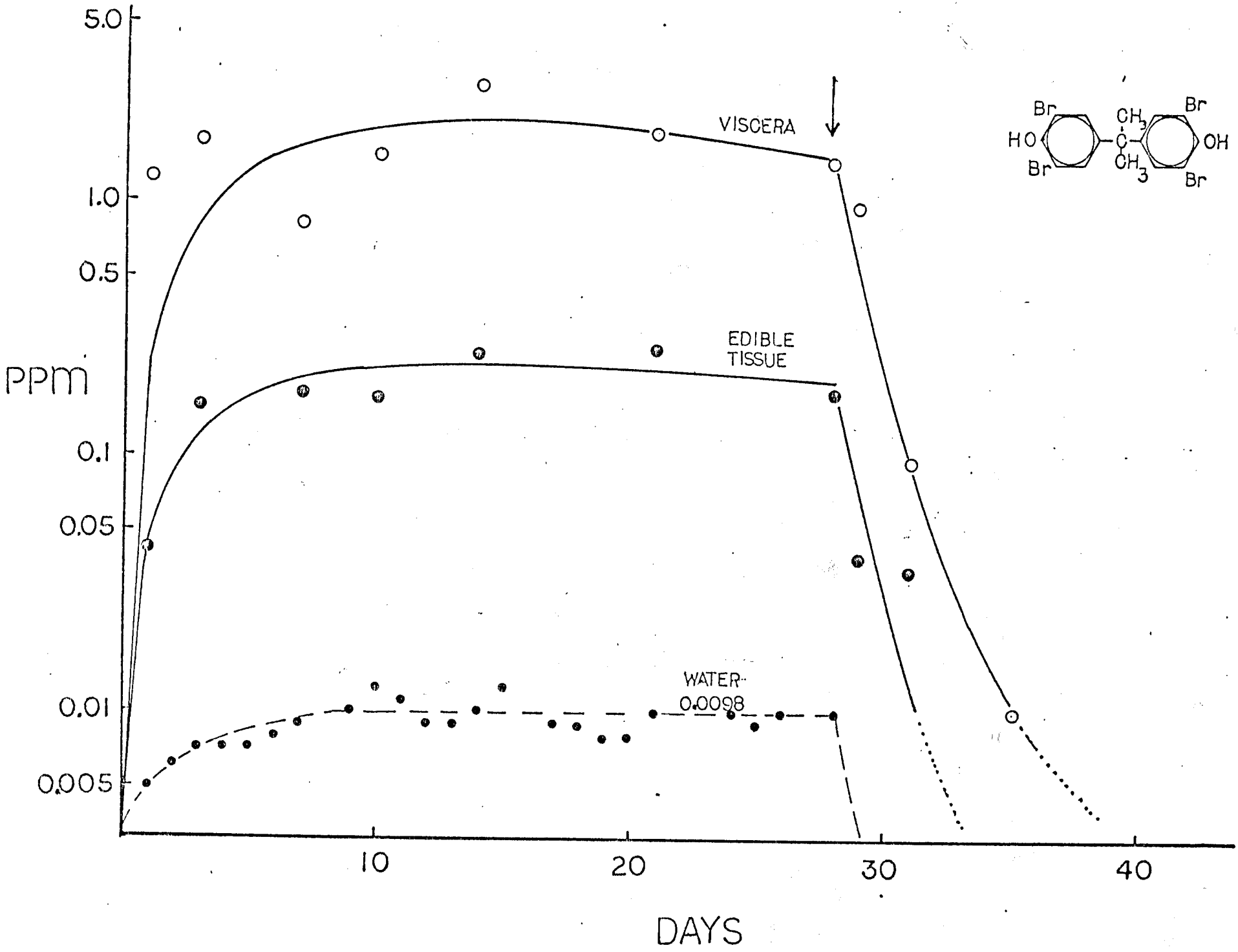

⑤
⑥
①
②
③
④
⑦

FIGURE 2 Bioaccumulation of tetrabromobisphenol in the edible tissue and viscera of the bluegill sunfish, Lepomis macrochirus. The arrow indicates beginning of the withdrawal phase. The concentration is given in tetrabromobisphenol equivalents based on the starting specific activity.


5.0
1.0
0.5
0.1
0.05
0.01
0.005
PPM
VISCERA
EDIBLE TISSUE
WATER 0.0098
10
20
30
40
DAYS
Br
HO
Br
CH3
C
CH3
Br
OH
Br