ACUTE TOXICITY OF TETRABROMOBISPHENOL A TO FATHEAD MINNOW (*Pimephales promelas*) UNDER FLOW-THROUGH CONDITIONS

SUBMITTED TO:

BROMINE FLAME RETARDANT INDUSTRY PANEL

c/o GREAT LAKES CHEMICAL CORPORATION

WEST LAFAYETTE, IN 47906

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The data and report presented for this study were produced and compiled in accordance with all pertinent EPA Good Laboratory Practice regulations.

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SUMMARY

The purpose of this study was to estimate the acute toxicity (LC50) of Tetrabromobisphenol A (TBBPA) to fathead minnow (Pimephales promelas) under flow-through conditions. Twenty organisms were exposed in duplicate test aquaria to five concentrations of TBBPA, a solvent (acetone) control and a dilution water control. Procedures used during this study were consistent with the requirements published in the EPA's Tetrabromobisphenol A Final Test Rule, (Federal Register Volume 52, No. 128, 6 July 1987). During the test, nominal concentrations of 1.0, 0.65, 0.42, 0.27 and 0.18 mg active ingredient/L TBBPA were maintained by introducing approximately 9.8 aquarium volumes per day of newly prepared test solution via a continuous flow proportional diluter apparatus. The duration of the exposure was 6 days (144 hours).

Each replicate solution was sampled and analyzed for TBBPA concentration (based on radiometric analyses for ^{14}C-labeled TBBPA) at test initiation, on day 4 of the exposure period and at test termination (day 6). Based on the results of these analyses, the mean measured test concentrations were 0.63, 0.45, 0.32, 0.26 and 0.19 mg A.I./L TBBPA. The concentration of TBBPA in the highest treatment level was confirmed using high pressure liquid chromatography at test initiation and termination. Throughout the exposure period a small amount of precipitated test material was present in the diluter system's mixing chamber; however, no undissolved TBBPA (e.g., precipitate, film on the solution's

surface) was observed in any of the exposure vessels. Biological observations were made and recorded at test initiation and every 24 hours thereafter until the test was terminated.

Following 6 days (144 hours) of exposure, 100% mortality was observed in the highest mean measured concentration of TBBPA tested (0.63 mg A.I./L). The percent mortality in the remaining treatment levels ranged from 30 to 0% and followed the concentration gradient established and decreased as the concentration of test material decreased. LC50 values and corresponding confidence intervals are reported in the following table. The No Observed Effect Concentration (NOEC) through 6 days of exposure was 0.26 mg A.I./L TBBPA. Based on criteria established by U. S. EPA (1985), TBBPA is classified as highly toxic to fathead minnow (Pimephales promelas).

TEST RESULTS

LC50 (mg/A.I./L)[a]						No Observed Effect Concentration Through 96 Hours (mg A.I./L)[a]
24-Hour	48-Hour	72-Hour	96-Hour	120-Hour	144-Hour	
>0.63[b]	>0.63[b]	0.57[c] (0.53-0.62)	0.54[d] (0.45-0.63)	0.50[d] (0.45-0.63)	0.49[d] (0.45-0.63)	0.26

[a]Based on mean measured concentrations (radiometric analyses) of TBBPA as active ingredient (A.I.).

[b]LC50 value was empirically estimated as being greater than the highest concentration of TBBPA tested.

[c]LC50 value and 95% confidence interval calculated by the probit method.

[d]LC50 value estimated by nonlinear interpolation, 95% confidence interval calculated by the binomial probability method.

INTRODUCTION

The purpose of this study was to estimate the acute toxicity of Tetrabromobisphenol A (TBBPA) to fathead minnows (Pimephales promelas) under flow-through conditions. A 6-day (144-hour) toxicity test was conducted from 31 August - 6 September 1988, at the Environmental Toxicology and Chemistry Division of Springborn Life Sciences, Inc., Wareham, Massachusetts. All raw data generated and the final report are stored at the above location.

MATERIALS AND METHODS

Protocol

Procedures used in this acute toxicity test followed those described in Springborn Life Sciences study plan and test protocol entitled "Protocol for Conducting a Flow-Through Acute Toxicity Test with Tetrabromobisphenol A Fathead Minnow Following TSCA Guidelines)," Protocol #020188/FM-FA BRFIP. This protocol closely follows "Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians" (ASTM, 1980) and EPA's Tetrabromobisphenol A Final Test Rule (Federal Register Volume 52, No. 128, 6 July 1987).

Test Material

The Tetrabromobisphenol A (tested as 100% active ingredient), a white powder, was received in five aliquots from the Bromine Flame Retardant Industry Panel. Appendix I presents the description of the material received and the procedure used to formulate a composite super stock solution [labeled (^{14}C) and unlabeled] of the test article. Prior to testing, the TBBPA was stored at room temperature (approximately 22°C) in a ventilated cabinet. Test concentrations are expressed as milligrams of TBBPA per liter of solution and reported as mg A.I./L as TBBPA.

Test Organisms

The fathead minnows used during this study (SLS lot #88A41) were obtained from cultures maintained at Springborn Life Sciences, Inc., Wareham, Massachusetts. Prior to testing, these fish were held in a 500-L fiberglass tank under a photoperiod of 16 hours light and 8 hours darkness. The well water which flowed into this holding tank was characterized as having a total hardness and alkalinity range as calcium carbonate ($CaCO_3$) of 22 - 30 mg/L and 21 - 24 mg/L, respectively, and a specific conductance range of 100 - 130 micromhos per centimeter (μmhos/cm) (Weekly Gravity Feed Tank Water Quality Analysis Log Book). Other parameters monitored in the holding tank were a pH range of 6.7 - 7.1, a dissolved oxygen concentration range of 91 - 96% of saturation and a flow rate of 7.3 - 9.6 tank volume replacements/day (Weekly Record of Fish Holding

Characteristics). The temperature range in the holding tank was 21 - 22°C during the 14-day period prior to test initiation. All fish were fed a dry commercial pelleted food, ad libitum, daily except during the 48 hours prior to testing. There was no mortality in the test fish population during the two days prior to testing (Daily Record of Fish Holding Conditions). The fathead minnows used during this study were all of the same year class and had a mean (range, n = 30) wet weight and total length of 0.50 (0.20 - 0.71) grams and 36 (27 - 42) millimeters, respectively (Fish Weight and Lengths Log).

Test Dilution Water

The dilution water used to prepare the exposure solutions was from the same source as the water which flowed into the fish holding tank and was characterized as having a total hardness of 30 mg/L $CaCO_3$, a total alkalinity range of 22 - 23 mg/L, a pH of 7.1 and a specific conductance range of 100 - 110 µmhos/cm during the study period. Historical values for total organic carbon (TOC) content of the dilution water averaged 4.3 ppm TOC. The ability of several daphnid species to survive and reproduce over several generations of culture in this water source confirmed that the dilution water was of acceptable quality. In conformance with EPA-GLP, routine analyses were also conducted on representative samples from the dilution water source for the presence of pesticides and PCB's. None of the compounds have been detected in any of the water samples analyzed, in agreement with US EPA and ASTM guidelines.

Test Conditions

The definitive test was conducted using an exposure system consisting of a continuous flow serial diluter (Benoit et al 1982), a temperature-controlled water bath, and a set of 14 exposure aquaria. The test system was designed to provide five concentrations of the test material, a solvent (acetone) control and a dilution water control. The solvent control solution contained the maximum amount of acetone (CAS #67-64-1) present in any test concentration (98 µL/L). All treatment levels and the controls were maintained in duplicate. Test aquaria were labeled to identify the test sample, nominal test concentration and designated replicate. Test solutions were not aerated. A photoperiod of 16 hours light and 8 hours darkness with a light intensity of 46 - 100 footcandles at the test solution surface was maintained throughout the study period. Lighting was provided by Vita-Lite^{R} fluorescent bulbs.

Test Concentrations

Selection of TBBPA concentrations for the definitive toxicity test with fathead minnows was based on toxicity information developed at SLS through preliminary testing.

Stock Solution

A diluter stock solution of 10.2 mg/mL was prepared by mixing the appropriate volume of the labeled and nonlabeled super stock

solutions in acetone. Stock solutions were prepared on exposure days -5, -2, 1 and 4 according to the procedures described in Appendix I.

A Harvard Apparatus peristaltic pump (Model #1203) was used to deliver the stock solution of TBBPA to the diluter system where it was diluted (65% dilution factor) to provide the desired exposure concentration range. Prior to dilution, the stock solution was mixed with dilution water using a magnetic stirrer. A system similar to that used to deliver the test material was used to deliver the acetone to the solvent control aquaria.

Test System

Each glass test aquarium measured 39 x 20 x 25 centimeters (cm) with a 14.5 cm high standpipe which maintained a constant test water volume of 11 L. The diluter was constructed to deliver 75 mL of solution per minute to each replicate test aquarium. The diluter provided approximately 9.8 solution volume replacements per aquarium every 24 hours. Test aquaria were impartially positioned in a water bath containing circulating water heated by immersion coil heaters and regulated by a mercury column thermoregulator designed to maintain the test solution temperatures at 22 ± 1°C.

Test Initiation

The test was initiated when ten fathead minnow were impartially selected and distributed to each replicate aquarium (20 per

treatment level and control). At any given time during the test, the maximum organism loading concentration was 0.046 g of biomass per liter of flowing test solution per day.

Test Monitoring

Biological observations of the fathead minnows and observations of the physical characteristics of the test solutions (e.g., precipitate, film on the solution's surface) were also made at test initiation and at each subsequent 24-hour interval. Effects during this study were based on death defined as the lack of movement by the exposed organisms (i.e., absence of gill movement and reaction to gentle prodding). Mortalities were recorded and removed from each aquarium every 24 hours during exposure. At 96 hours the LC50 was compared to the 48-hour LC50 value. If the 96-hour LC50 was less than 50% of the 48-hour LC50, the test was continued until the mean increase in mortality in any test concentration did not exceed 10% over a 24-hour period or until the test duration reached 14 days. Since during this study the 48-hour LC50 value was estimated as being greater than the highest treatment level, a comparison of the 48- and 96-hour LC50's was not possible. Therefore, the study was extended beyond 96 hours to establish an incipient LC50.

Water Quality Measurements

Dissolved oxygen concentration, temperature and pH were measured once daily in each replicate of each treatment level and the controls throughout the exposure. The temperature was continuously

monitored in one replicate (A) of the dilution water control. The pH was measured with an Instrumentation Laboratory Model #175 pH meter and combination electrode; the dissolved oxygen concentration was measured with a YSI Model #57 dissolved oxygen meter and probe and the temperature (daily measurement) was measured with a Brooklyn alcohol thermometer. Continuous monitoring of the control solution temperature was performed using a Taylor MIN-MAX thermometer. Light intensity was measured with a General Electric Model 214 light meter.

Analytical Measurements

The control and the high, middle, and low test concentrations were sampled and analyzed for TBBPA concentrations (radiometric analyses) prior to the start of the definitive exposure. Results of these pretest analyses were used to judge whether sufficient quantities of test material were being delivered and maintained in the exposure aquaria to initiate the definitive test. During the in-life phase of the definitive study, water samples were removed from both replicate test solutions of each treatment level and the controls on test days 0, 4 and 6 for analysis of TBBPA (radiometric analyses). Confirmation of the highest treatment level was performed by analyzing for TBBPA using high pressure liquid chromatography (HPLC) at test initiation and termination. Results of these analyses (HPLC) were used to verify that the measurements based on radiometric analyses represented the concentration parent TBBPA in the exposure solutions. Each exposure solution sample was collected from the approximate midpoint of the aquarium with a

volumetric pipet. In addition, quality assurance (QA) blind samples were prepared at each sampling interval and remained with the set of exposure solution samples through the analytical process. These QA samples, for both radiometric and HPLC analyses, were prepared in dilution water at TBBPA concentrations unknown to the analyst. Results of the analyses of the QA samples were used to judge the precision and quality control maintained during the analysis of exposure solution samples. All samples were filtered and analyzed within 30 minutes after removal from the exposure vessels. The analytical procedures used to analyze the exposure solution samples, radiometric and high pressure liquid chromatography, are presented in the methodology described in Appendix II. Prior to the initiation of the definitive test, a method validation recovery study was conducted at SLS for each type of analyses and established average recoveries of TBBPA from freshwater equal to 102 ± 5.03% (radiometric) and 96.1 ± 6.64% (HPLC).

Statistics

The mean measured concentrations tested (based on radiometric analyses on days 0, 4 and 6) and the corresponding mortality data derived from the toxicity test were used to estimate the median lethal concentrations (LC50) and 95% confidence intervals at each 24-hour interval of the exposure period. The LC50 is defined as the concentration of the test material in dilution water lethal to 50% of the test animal population at the stated exposure interval. If at least one test concentration caused mortality of greater than or equal to 50% of the test population, then a computer program

(Stephan, 1977, 1982) was used to calculate the LC50 values and 95% confidence intervals.

Three statistical methods were available in the computer program: moving average angle analysis, probit analysis, and nonlinear interpolation with 95% confidence intervals calculated by binomial probability. Moving average angle and probit analyses yield statistically sound results only if at least two concentrations produce a mortality of between 0 and 100% of the test organism population. The selection of reported LC50 values and 95% confidence intervals was based upon an examination of the data base and the results of the computer analysis. Selection criteria included the establishment of a concentration-effect relationship (mortality), the number of concentrations causing partial responses, and the span of responses bracketing the LC50 value. If two or more statistical methods produced acceptable results, then the method which yielded the smallest 95% confidence interval was selected. The No Observed Effect Concentration (NOEC), defined as the highest concentration tested at and below which there were no toxicant-related mortalities or physical and behavioral abnormalities (e.g., lethargy, loss of equilibrium, darkened pigmentation) was also determined.

RESULTS

Preliminary Testing

During a preliminary flow-through exposure, fathead minnows were exposed to measured concentrations of TBBPA ranging from 0.50 to 0.089 mg A.I./L. After 96 hours 40% mortality was observed at the 0.50 mg A.I./L treatment level. During the same period, no mortality was observed in the treatment level of 0.32 mg A.I./L TBBPA; however, several fish at this level did exhibit a partial loss of equilibrium. Throughout the exposure, no mortalities or adverse sublethal effects were observed in concentrations of ≤ 0.21 mg A.I./L TBBPA or among the control organisms. Based on these results, the nominal concentrations selected for the definitive test were: 1.0, 0.65, 0.42, 0.27 and 0.18 mg A.I./L TBBPA.

Definitive Test

The water quality parameters measured during the definitive study (Table 1) remained within acceptable ranges for the survival of fathead minnows and were unaffected by the concentrations of TBBPA tested.

The diluter system which prepared and delivered the test solutions to the exposure aquaria functioned properly throughout the definitive study. Review of the diluter system performance during the pretest period established that the system was preparing and delivering the appropriate concentrations of test material to the

exposure vessels. Pretest measurements of TBBPA (radiometric analyses) of the high, middle and low treatment levels resulted in measured concentrations which averaged 75% of nominal. Analyses of the solutions during the in-life portion of the definitive test established that the concentration of ^{14}C-TBBPA in the exposure solutions was generally consistent between replicate treatments and sampling intervals. Throughout the exposure period, a small amount of precipitate was present in the diluter system's mixing chamber; however, no undissolved material (e.g., precipitate, film on the solution's surface) was observed in any of the exposure vessels.

The results of the analysis (radiometric and HPLC) of exposure solutions for TBBPA during the in-life portion of the definitive exposure are presented in Table 2. Mean measured concentrations (based on day 0, 4 and 6; radiometric analyses) for this study averaged 82% of nominal and defined the exposure concentrations as 0.63, 0.45, 0.32, 0.26 and 0.19 mg A.I./L TBBPA. Coefficients of variation of the mean measured concentrations averaged 13%. Analysis of the Quality Assurance samples (Table 2) at each sampling interval (radiometric analyses) resulted in measured concentrations which were consistent with the predetermined recovery range (Appendix II) and averaged 103% of the nominal fortified levels.

Results of the analyses of the highest treatment level at test initiation and termination, using high pressure liquid chromatography, resulted in measured concentrations which were consistent with the measurements obtained during the radiometric analyses. Measured concentrations (HPLC) averaged 68% of the

nominal fortified concentrations (Table 2). Based on these data, it was established that the exposure system maintained stable concentrations of parent TBBPA and that the results of the radiometric analyses accurately define the concentration of test material in solution. Analyses of the Quality Assurance samples (Table 2) at each sampling interval (HPLC analyses) resulted in measured concentrations which were consistent with the predetermined recovery range (Appendix II) and averaged 96% of the nominal fortified levels.

The relationship between the nominal treatment levels and the mean measured concentrations established by the diluter apparatus during this study is illustrated in Figure 1. Exposures achieved were sufficient to produce a concentration-related biological response (death).

The mean measured concentrations (based on radiometric analyses), the corresponding mortalities and the observations made during the definitive study are presented in Table 3. After 48 hours of exposure, 45% mortality was observed in the highest mean measured test concentration of TBBPA tested (0.63 mg A.I./L). No mortalities were observed at any of the lower treatment levels (0.45 - 0.19 mg A.I./L TBBPA). Based on these data, the 48-hour LC50 for TBBPA and fathead minnows was empirically estimated as being greater than the highest test concentration (0.63 mg A.I./L). Following 96 hours of exposure, mortality of 90% and 5% was observed among fish in the two highest treatment levels, 0.63 and 0.45 mg A.I./L TBBPA, respectively. The calculated LC50 value (95% confidence interval)

at the 96-hour interval was 0.54 (0.45 - 0.63) mg A.I./L TBBPA. Since the 48-hour LC50 for this study could not be established, therefore preventing a comparison between the 48- and 96-hour LC50's, it was decided to continue the exposure beyond 96 hours until the percent mortality observed in any treatment level did not exceed 10%. The test was terminated after 6 days (144 hours) of exposure. At test termination 100% and 30% mortality was observed in two highest treatment levels. The 144-hour LC50 (95% confidence interval) for fathead minnows exposed to TBBPA was calculated by nonlinear interpolation to be 0.49 (0.45 - 0.63) mg A.I./L. The concentration-effect curves for each 24-hour interval of the definitive study are presented in Figures 2 - 7. Table 4 summarizes the LC50's and the corresponding confidence intervals established during the 6-day test. The No Observed Effect Concentration (144 hours) for fathead minnows exposed to TBBPA was determined to be 0.26 mg A.I./L. Based on EPA (1985) criteria, TBBPA would be classified as highly toxic to fathead minnows (Pimephales promelas).

Protocol Deviation

1. The protocol states that if the exposure is continued after 96 hours, the surviving fish will be fed a maintenance diet.

   Throughout this 6-day test exposed fish were not fed. Since no significant toxicant-related mortalities occurred after 96 hours and the test was terminated on day 6 (therefore only one day of feeding was omitted), this deviation did not affect the results of this study.

2. The protocol states that the test aquaria will maintain exposure solution volumes of 15 L.

   During this test the exposure solution volume maintained was equal to 11 L.

3. The protocol states that the temperature of the exposure solutions will be maintained within the range of 20-24°C.

   During this study the temperature of the exposure solutions ranged from 23-25°C.

4. The protocol states that the exposure solutions were prepared using a proportional diluter.

   During this study the exposure solutions were prepared using a serial diluter system.

Donald C Surprenant 11/16/88
Donald C. Surprenant
Study Director

QUALITY ASSURANCE UNIT STATEMENT

The raw data and final report for this study were inspected by the Springborn Life Sciences, Inc., Quality Assurance Unit (QAU) to assure compliance with the study protocol, laboratory standard operating procedures and the pertinent EPA Good Laboratory Practice Regulations on the following date(s): November 1, 2, 7, and 16, 1988. An in-life phase inspection was conducted on October 31, 1988.

QAU inspection report(s) were issued to the Study Director on September 2, November 1, 2, 7, and 16, 1988. A QAU inspection summary report is issued to the laboratory management at the end of each month.

It is the opinion of the QAU that this report accurately reflects the raw data generated during this study.

Katherine A. Grandy — 11/16/88

Katherine A. Grandy
Quality Assurance Unit

Date

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TABLES

Table 1. The water quality parameters measured during the 144-hour flow-through toxicity test exposing fathead minnows (Pimephales promelas) to TBBPA.

Nominal Concentration (mg A.I./L)	0-hour A	0-hour B	24-hour A	24-hour B	48-hour A	48-hour B	72-hour A	72-hour B	96-hour A	96-hour B	120-hour A	120-hour B	144-hour A	144-hour B
							pH							
1.0	7.1	7.2	7.2	7.2	7.1	7.2	7.0	7.1	7.2	7.2	7.2	7.2	7.2	7.2
0.65	7.2	7.2	7.2	7.2	7.1	7.1	7.1	7.1	7.2	7.2	7.2	7.2	7.2	7.2
0.42	7.3	7.3	7.2	7.3	7.1	7.2	7.0	7.1	7.2	7.1	7.3	7.3	7.2	7.2
0.27	7.3	7.3	7.2	7.3	7.1	7.2	7.1	7.1	7.2	7.2	7.3	7.3	7.2	7.2
0.18	7.3	7.3	7.3	7.3	7.2	7.2	7.2	7.1	7.2	7.2	7.2	7.3	7.2	7.2
Solvent Control	7.3	7.3	7.3	7.3	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2
Control	7.3	7.3	7.3	7.3	7.2	7.2	7.2	7.1	7.3	7.2	7.3	7.3	7.2	7.2
							Dissolved Oxygen Concentration (mg/L)							
1.0	8.0	8.2	7.1	7.6	6.6	7.5	6.9	7.3	6.8	7.3	7.4	7.5	7.3	7.9
0.65	8.1	8.0	7.1	7.5	7.5	7.0	7.3	6.8	6.8	6.8	7.5	7.4	7.5	7.5
0.42	8.1	8.1	7.3	7.4	6.8	7.5	6.8	7.3	6.9	7.0	7.5	7.5	7.7	7.9
0.27	8.0	8.2	7.2	7.7	7.2	7.7	7.0	6.9	7.3	7.6	7.5	7.8	7.4	8.0
0.18	8.0	8.3	7.5	7.7	7.0	7.6	7.2	7.2	7.5	7.4	7.9	7.9	8.1	8.1
Solvent Control	8.4	8.4	7.9	7.9	8.0	7.8	7.8	7.8	8.0	7.8	8.0	8.0	8.1	8.1
Control	8.6	8.7	8.1	8.1	8.3	8.1	8.1	8.0	8.2	8.1	8.3	8.3	8.5	8.4
							Temperature (°C)[a]							
	23		23		23		23		23		23		23	

[a] Temperature was measured in each aquarium daily. The values represent the range of the temperature measurements made in all aquaria for the specified time interval. Continuous temperature monitoring in the control (replicate A) solution established at temperature range of 23-25°C.

Table 2. Measured concentrations of TBBPA in replicate (A,B) solutions during the 144-hour flow-through exposure of fathead minnows (Pimephales promelas).

RADIOMETRIC ANALYSES

Nominal Concentration (mg A.I./L)	Measured Concentration (mg A.I./L) 0-Hour A	0-Hour B	96-Hour A	96-Hour B	144-Hour A	144-Hour B	Mean (Standard Deviation)
1.0	0.62	0.71	0.51	0.52	0.70	0.72	0.63 (0.094)
0.65	0.45	0.42	0.33	0.42	0.52	0.55	0.45 (0.077)
0.42	0.33	0.33	0.27	0.25	0.37	0.40	0.32 (0.056)
0.27	0.25	0.25	0.24	0.26	0.26	0.27	0.26 (0.012)
0.18	0.17	0.18	0.20	0.18	0.21	0.21	0.19 (0.017)
Solvent Control	<0.032	<0.032	<0.032	<0.032	<0.032	<0.032	
Control	<0.032	<0.032	<0.032	<0.032	<0.032	<0.032	
QA#1[a]	0.743 (91.5)[b]		0.421 (104)		0.869 (107)		
QA#2	0.681 (105)		0.585 (103)		0.522 (107)		
QA#3	0.488 (100)		0.873 (108)		0.256 (105)		

HPLC ANALYSES

Nominal Concentration (mg A.I./L)	Measured Concentration[c] (mg A.I./L) 0-Hour A	0-Hour B	144-Hour A	144-Hour B	Mean (Standard Deviation)
1.0	0.66	0.74	0.64	0.69	0.68 (0.045)
QA#1[a]	0.500 (100)[b]		0.462 (92.4)		
QA#2	0.990 (99.0)		0.890 (89.0)		
QA#3	1.81 (93.5)		1.71 (99.0)		

[a]QA = Quality Assurance sample.
[b]Percent of the nominal fortified concentration.
[c]Samples collected at 96 hours were analyzed at 144 hours because of an instrument malfunction. Results for the three QA samples were low and for this reason data for this interval are not reported.

Table 3. Concentrations tested and corresponding mortalities of fathead minnows (Pimephales promelas) exposed to TBBPA during a 144-hour flow-through toxicity test.

Mean Measured Concentration (mg A.I./L)	Cumulative Mortality (%)																	
	24-Hour			48-Hour			72-Hour			96-Hour			120-Hour			144-Hour		
	A	B	Mean	A	B	Mean	A	B	Mean	A	B	Mean	A	B	Mean	A	B	Mean
0.63	30	0	15[a]	70	20	45[a]	100	50	75[a]	100	80	90[a]	100	100	100	100	100	100
0.45	0	0	0[b]	0	0	0[ce]	10	0	5[cef]	10	0	5[cef]	20	20	20[afh]	30	30	30[af]
0.32	0	0	0[cg]	0	0	0[bd]	0	0	0[bg]	0	0	0[bg]	0	0	0[bgh]	0	0	0[bgh]
0.26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0[d]	0	0	0[d]
0.19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Solvent Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

[a] All fish exhibited a complete loss of equilibrium.
[b] All fish exhibited a partial loss of equilibrium.
[c] Several fish exhibited a partial loss of equilibrium.
[d] One fish exhibited darkened pigmentation.
[e] Several fish exhibited a complete loss of equilibrium.
[f] All fish exhibited darkened pigmentation.
[g] Several fish exhibited darkened pigmentation.
[h] Several fish were at the surface of the test solution.

Table 4. The LC50 values (95% confidence interval) and No Observed Effect Concentration observed during the 144-hour flow-through toxicity test exposing fathead minnows (Pimephales promelas) to TBBPA.

LC50 (mg A.I./L)[a]						No Observed Effect Concentration Through 144 Hours (mg A.I./L)[a]
24-Hour	48-Hour	72-Hour	96-Hour	120-Hour	144-Hour	
>0.63[b]	>0.63[b]	0.57[c] (0.53-0.62)	0.54[d] (0.45-0.63)	0.50[d] (0.45-0.63)	0.49[d] (0.45-0.63)	0.26

[a] Based on mean measured concentrations (radiometric analyses) of TBBPA as active ingredient (A.I.).

[b] LC50 value was empirically estimated as being greater than the highest mean measured tested.

[c] LC50 value and 95% confidence interval calculated by probit analyses.

[d] LC50 value estimated by nonlinear interpolation, 95% confidence interval calculated by the binomial probability method.

FIGURES

Figure 1. Graphical illustration of the relationship between mean measured concentrations and the nominal test concentrations during the acute toxicity exposure of fathead minnows (Pimephales promelas).

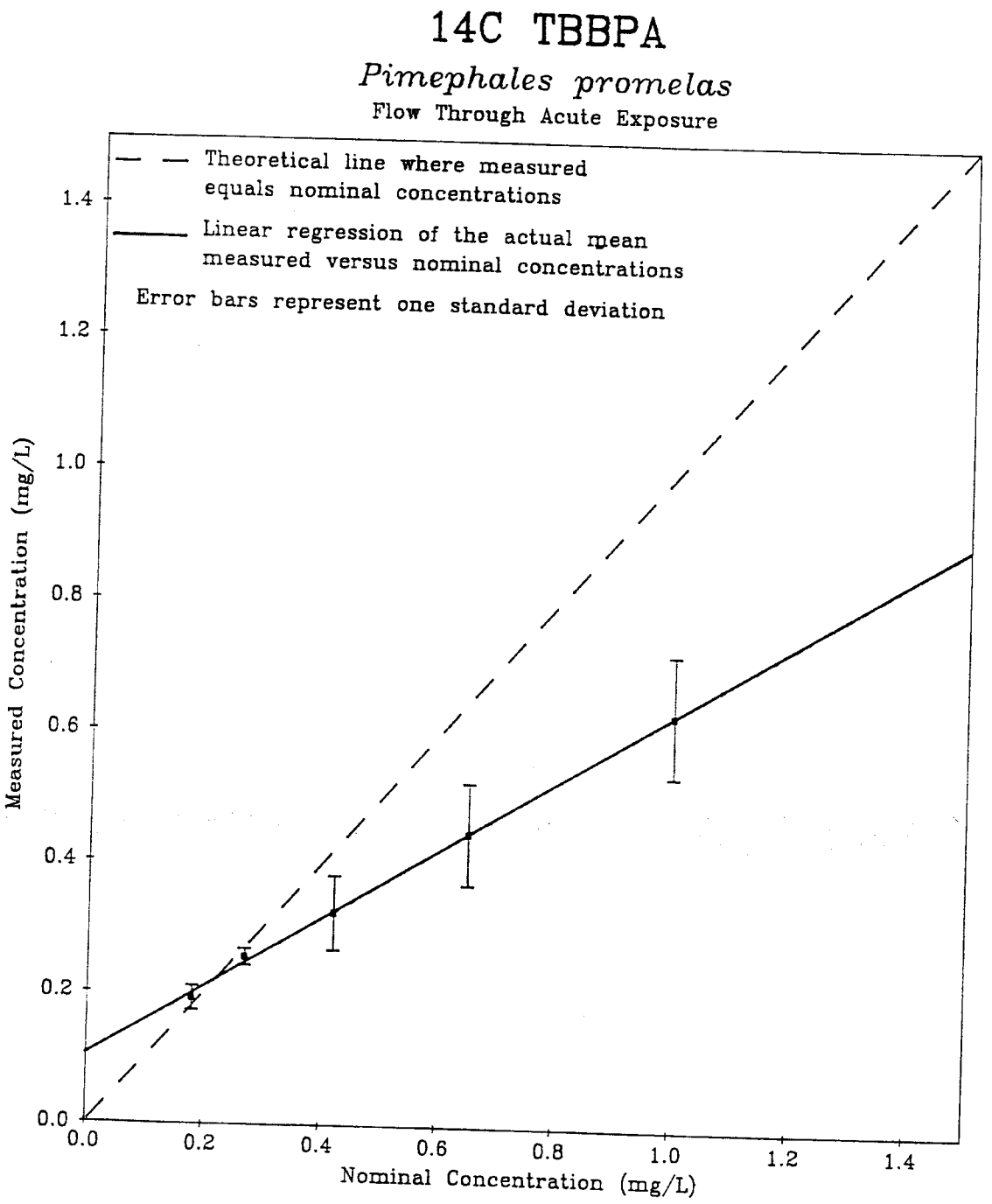


Figure 2. The concentration response (mortality) curve for fathead minnows (Pimephales promelas) after 24 hours exposure to TBBPA.

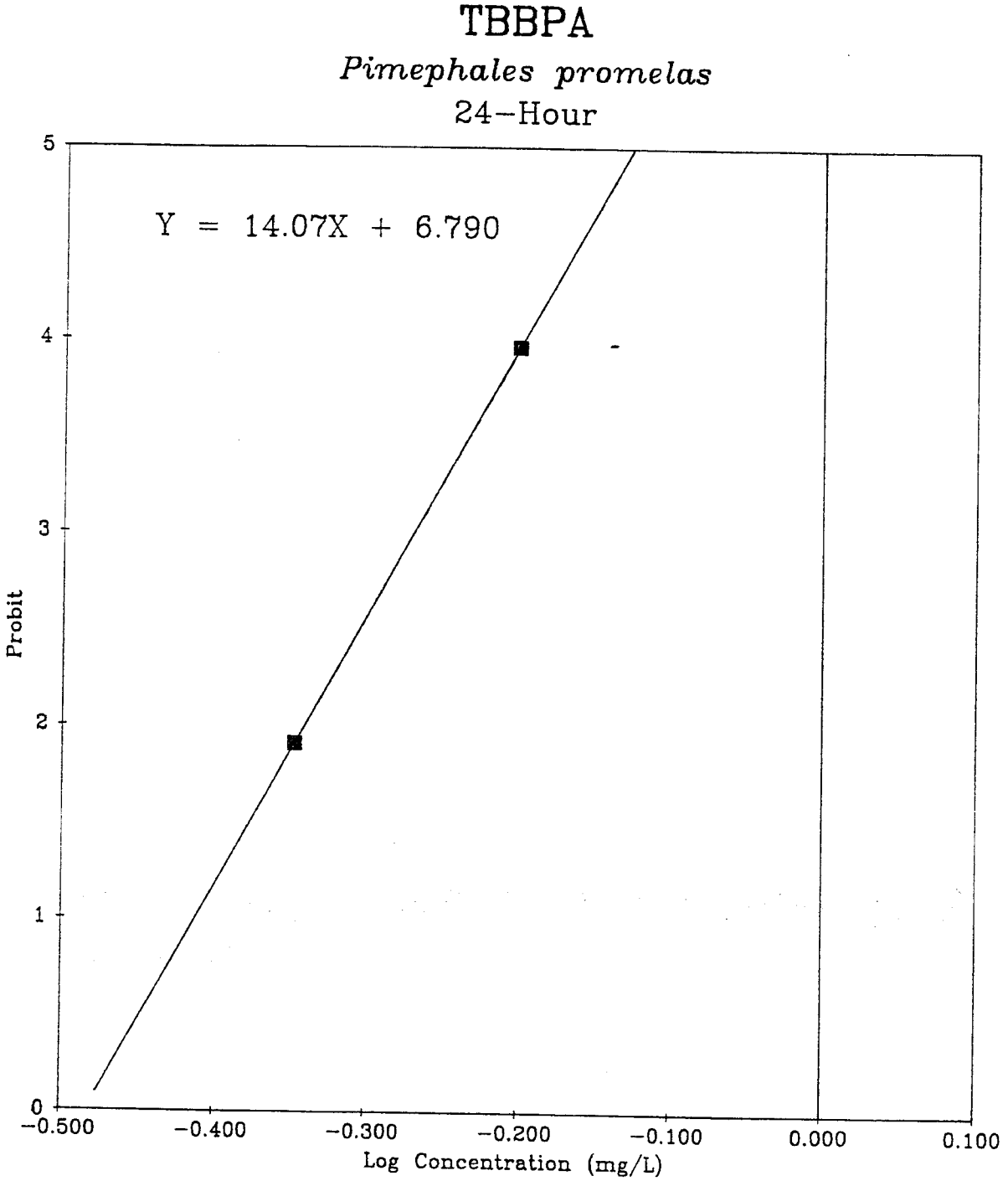


Figure 3. The concentration response (mortality) curve for fathead minnows (Pimephales promelas) after 48 hours exposure to TBBPA.

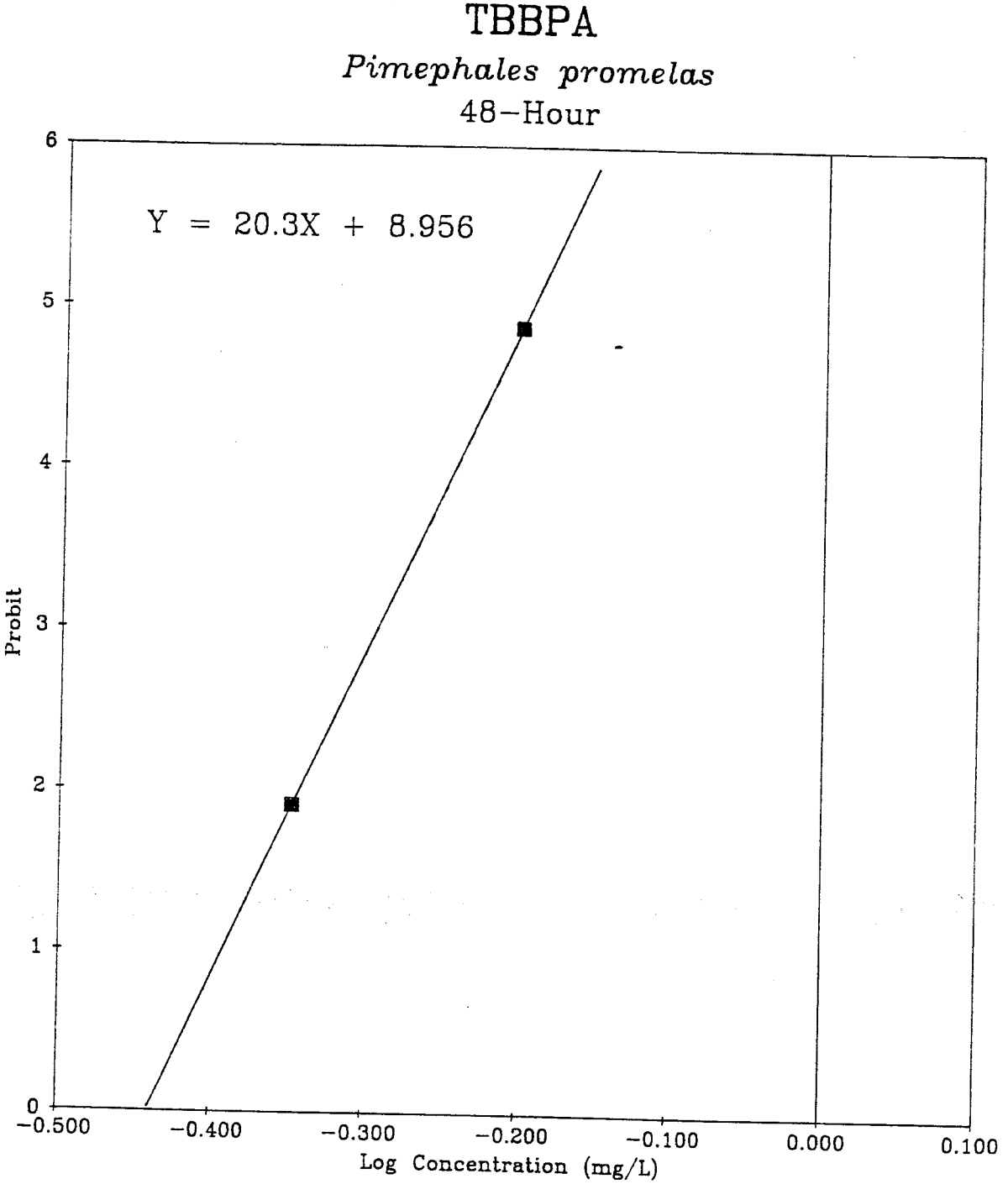


Figure 4. The concentration response (mortality) curve for fathead minnows (Pimephales promelas) after 72 hours exposure to TBBPA.

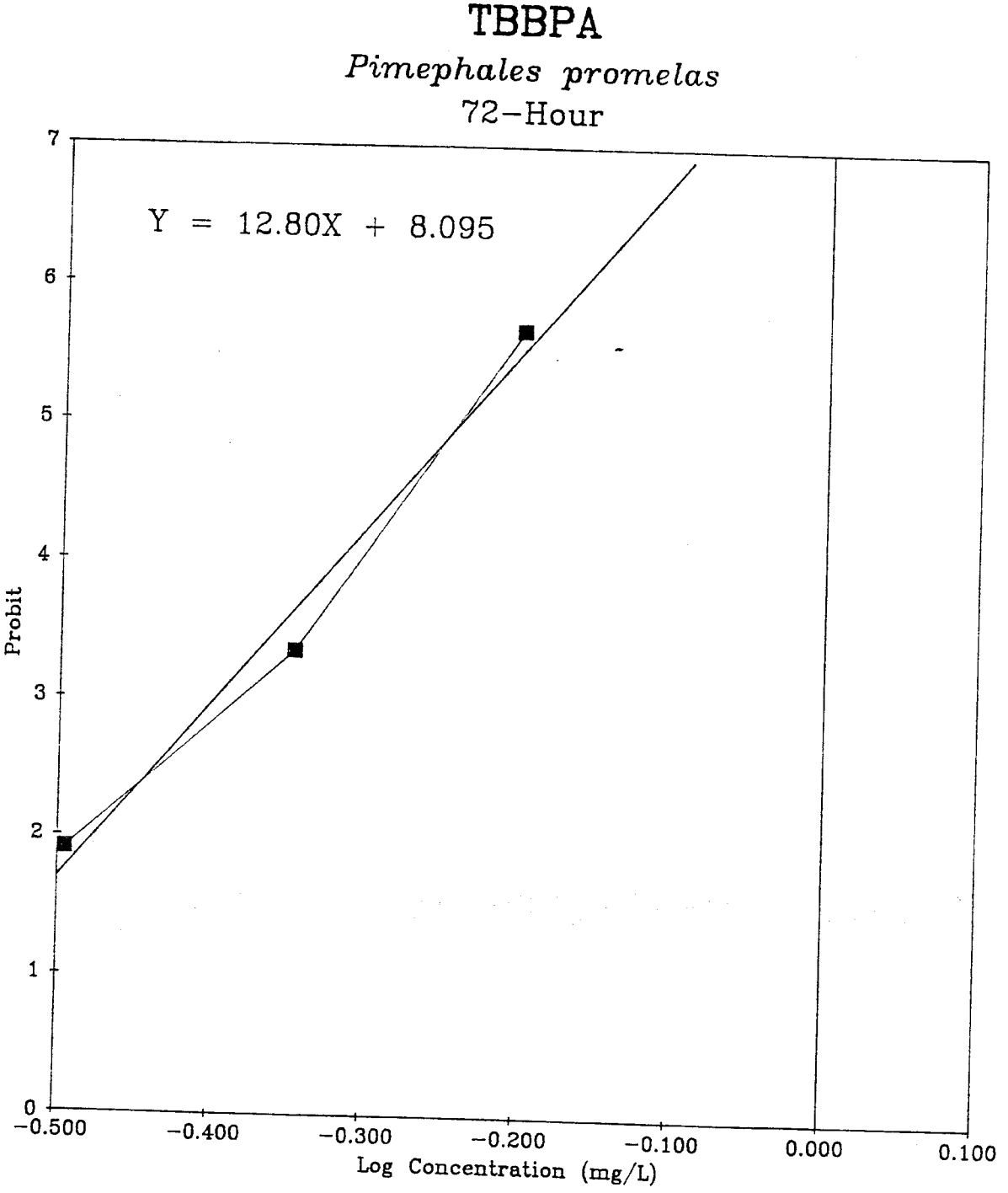


Figure 5. The concentration response (mortality) curve for fathead minnows (Pimephales promelas) after 96 hours exposure to TBBPA.

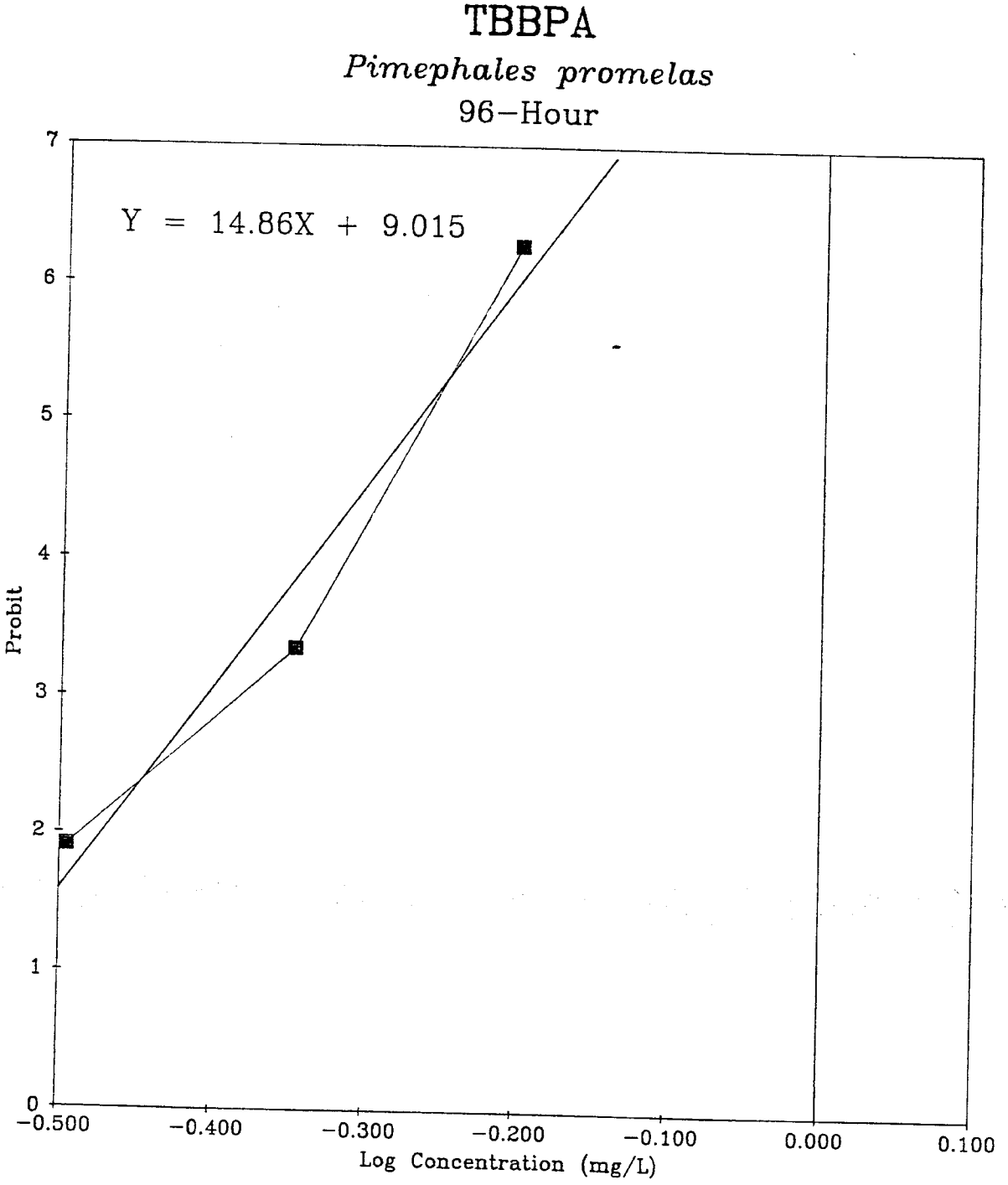


Figure 6. The concentration response (mortality) curve for fathead minnows (Pimephales promelas) after 120 hours exposure to TBBPA.

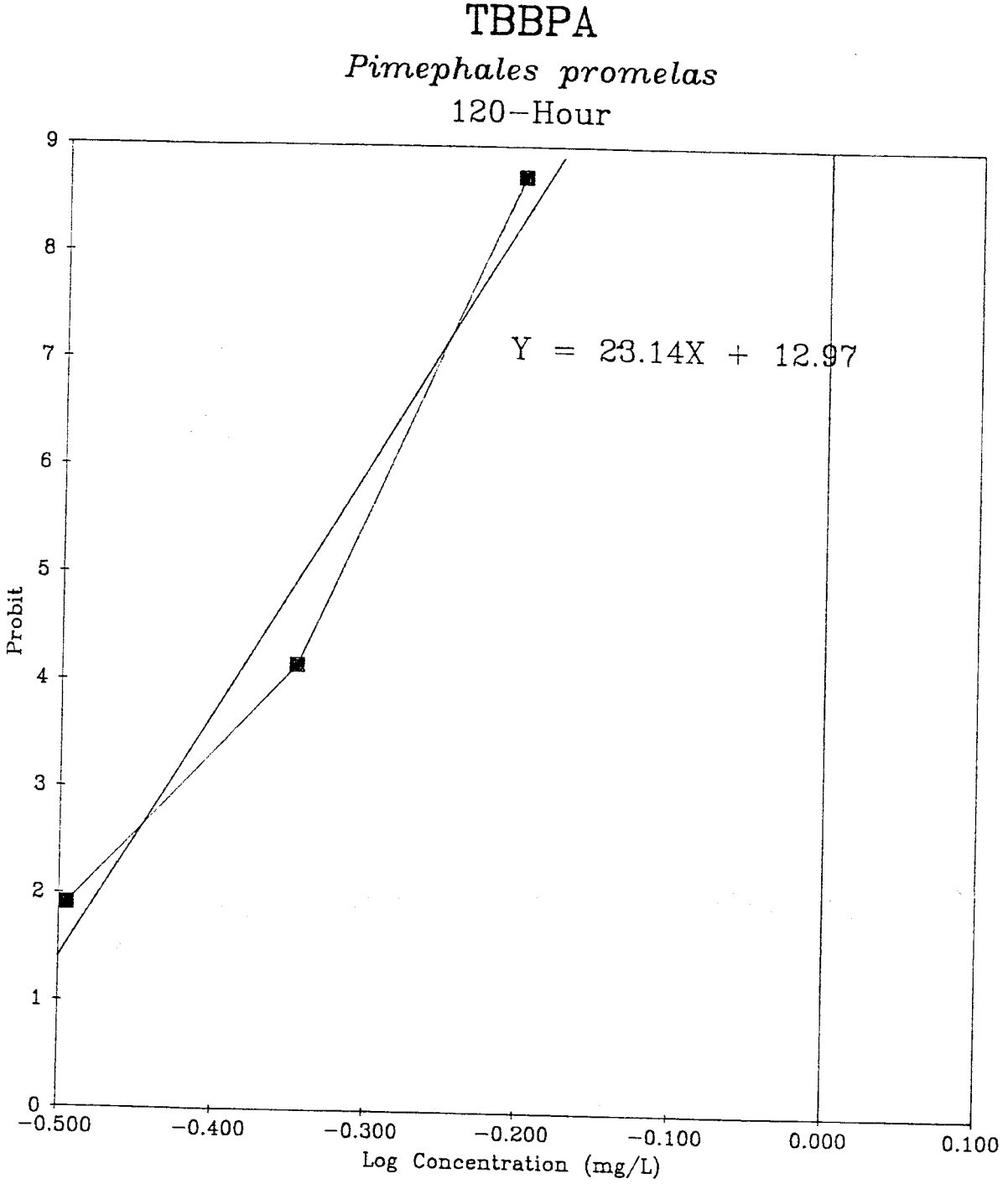
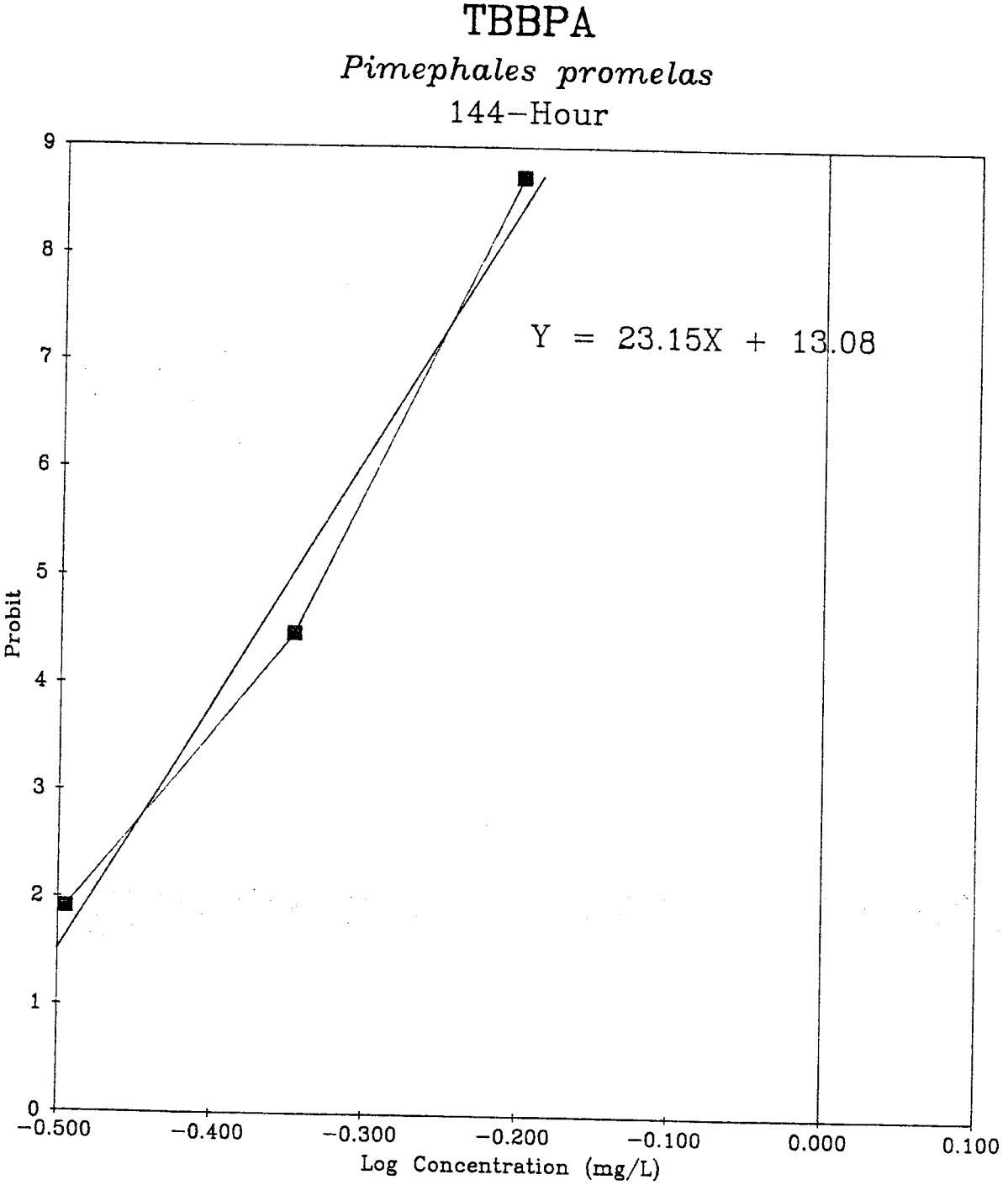


Figure 7. The concentration response (mortality) curve for fathead minnows (Pimephales promelas) after 144 hours exposure to TBBPA.



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APPENDIX I

Description of the test material (Tetrabromobisphenol A) and procedures used to prepare the super stock solutions of the test article.

Description of the five sample lots of TBBPA (nonlabeled) received at Springborn Life Sciences, Inc., from the Bromine Flame Retardant Industry Panel.

Manufacturer	Lot Number	Date Received
Bromine Compounds Ltd.	7137	12/22/87
Great Lakes Chemical Corp.	114-21H-16B	1/19/88
Ethyl Corporation	25739-36	2/5/88
Bromine Compounds Ltd.	7137	3/17/88
Ethyl Corporation	25813-96	4/29/88

The following table lists the quantity of each sample used to formulate the TBBPA (nonlabeled) super stock solution used during the 144-hour exposure of fathead minnows (Pimephales promelas).

Manufacturer	Lot Number	Quantity Used
Bromine Compounds Ltd.	7137	20.0257 grams
Great Lakes Chemical Corp.	114-21H-16B	40.0146 grams
Ethyl Corporation	25739-36	20.0052 grams
Bromine Compounds Ltd.	7137	20.0070 grams
Ethyl Corporation	25813-96	20.0135 grams

The above quantity of each sample was mixed in a 1000-mL glass volumetric flask, then diluted to volume with acetone to produce a stock solution of 120 mg A.I./mL TBBPA. Stock solution concentration is based on 100% active ingredient.

The following is a description of the procedure used to prepare the super stock solution of ^{14}C-labeled TBBPA for the 144-hour exposure of fathead minnows (Pimephales promelas) to TBBPA.

Manufacturer	Specific Activity	Date Received
Chemsyn Science Laboratories Lot #CSL-88-164-21-10 (tested as 100% A.I.)	12.9 mCi/mmole	5/3/88

81.2 mg of ^{14}C-labeled TBBPA was diluted with acetone to volume in a 50-mL glass volumetric flask to produce a stock solution of 1.624 mg ^{14}C-TBBPA/mL -

The following is a description of the procedure used to prepare the diluter stock solutions for the 144-hour exposure of fathead minnows (Pimephales promelas).

Nominal[a] Concentration	Date Prepared	mL of Super Stock[b] (nonlabeled)	mL of Super Stock (Labeled)	Total Diluter[d] Stock Solution Volume
10.2 mg A.I./mL	8/29/88[e]			
10.2 mg A.I./mL	9/1/88	8.44	4.1	100
10.2 mg A.I./mL	9/4/88	8.44	4.1	100

[a] Nominal concentration represents the total amount of labeled and nonlabeled material.

[b] Super stock concentration (nonlabeled) = 120 mg A.I./mL.

[c] Super stock concentration (labeled) = 1.624 mg A.I./mL.

[d] Diluted to total volume with acetone.

[e] Diluter stock solution prepared on 8/29/88 was formulated by adding 12.5 mL of a nonlabeled super stock solution (120 mg/mL) and 0.44 mL labeled super stock solution (1.624 mg A.I./mL) to 270 mL of an existing diluter stock solution containing 5.1 mg TBBPA/mL acetone (prepared 8/26/88). The existing diluter stock solution contained 1.28% labeled TBBPA.

APPENDIX II

Analytical Methodology for the Measurement of TBBPA in Freshwater

PART I

Radioassay Methodology

1.0 OBJECTIVE:

This study was designed to validate a procedure for quantitative analysis of ^{14}C-Tetrabromo[ring-u-^{14}C]bisphenol A (TBBPA) in freshwater.

2.0 SUMMARY:

Water samples were filtered prior to analysis by liquid scintillation counting subsequent to the addition of scintillation cocktail.

3.0 EQUIPMENT AND REAGENTS:

3.1 Equipment

1. Scintillation vials: 22 mL, Wheaton.
2. Syringes: Hamilton, assorted volumes and micro mate 10 mL.
3. Liquid scintillation counter: Beckman LS 1801.
4. Pipets: volumetric, class A, 5 mL.
5. Wheaton vials: 100 mL with crimp caps and Teflon^R septa.
6. Volumetric flasks: assorted sizes
7. Acrodisc filters: Gelman Acrodisc-CR, 0.45 micron, Teflon

3.2 Reagents

1. Monophase S^R - Packard Instrument Company.
2. Radiolabeled (^{14}C) Tetrabromo[ring[-u-$^{1}4C$]bisphenol A, 85 mg, S.A. = 12.9 mCi/mmole Lot # CSL-88-164-21-10 supplied by Chemsyn Science Laboratories, Lenexa, KS; as 100% active ingredient.

4.0 PROCEDURE

4.1 Superstock Preparation

A 1.624 mg/mL superstock was prepared by quantitative transfer of the radiolabeled material (81.2 mg) into a total volume of 50.0 mL acetone.

4.2 Determination of Specific activity

Specific activity was determined by spiking 10 µL of the 1.624 mg/mL stock into a scintillation vial containing 15 mL of Monophase S^R (prepared in triplicate). The ^{14}C-activity of each sample was measured by placing the vial in a Beckman LS 1801 scintillation counter and recording disintegrations per minute (dpm). The dpm for each sample were divided by the total µg spiked to yield dpm/µg. This measured specific activity, used for calculation of all test and quality assurance samples, was a mean of the above triplicate analyses. The mean measured specific activity was determined to be 52742 dpms/µg (100% of theoretical).

4.3 Sample Preparation and Analysis

Samples (approximately 7.0 mL) were taken with a 10-mL syringe. An Acrodisc filter was then placed on the end of the syringe and filtered into a beaker. Five mL was then volumetrically transferred into a scintillation vial.

Counting efficiencies of all experimental samples were determined using an external standard and factory prepared calibration curve. All test samples were counted for a maximum of 100 minutes or until a 2 sigma error of 5% was attained. Using this criterion and the calculations described in Standard Methods for the Examination of Water and Wastewater (APHA et al.), it was determined at the 95% confidence level that a minimum net counts per minute (cpm) for all samples of 53 cpm (background 43.48 cpm) had an associated error of less than 10%. This percentage was the maximum acceptable error and was associated with the minimum net cpm of that sample; the counting error decreased as the sample activity increased.

The minimum detectable ^{14}C-residue concentration was dependent on counting efficiency, volume of each sample and the minimum net cpm acceptable. Three quality assurance (QA) samples were prepared and analyzed at each sampling interval. Analysis of QA samples was used to determine the accuracy of the analytical procedure used.

The calculation used in determining the concentration of ^{14}C-residues in the test sample was:

$$\frac{\text{Net dpm}}{(\text{Specific Activity of } ^{14}C\text{-TBBPA}) \quad (\text{Sample size, mL}) \quad (\%\ ^{14}C\text{-activity of the stock})}$$

where:

Net dpm = disintegrations calculated by instrument after background calculation

Specific Activity of ^{14}C-TBBPA = 52742 dpm/μg

Sample size = Initial volume of sample (mL)

% ^{14}C-activity of the stock = 100%

5.0 RESULTS

Analytical results for the recovery of ^{14}C-TBBPA from freshwater as presented in Table 1A.

Table 1A. Analytical results for the recovery of ^{14}C-TBBPA from freshwater.

Nominal Fortified Concentration (mg/L)		Sample Volume (mL)	Concentration Recovered (mg/L)	% Recovered
10.6	A	10.0	11.6	109
	B	10.0	11.5	108
	C	10.0	10.6	100
5.2	A	10.0	5.47	105
	B	10.0	5.41	104
	C	10.0	4.88	93.8
0.309	A	10.0	0.331	107
	B	10.0	0.325	105
	C	10.0	0.295	95.5
0.162	A	10.0	0.156	96.3
	B	10.0	0.165	102
	C	10.0	0.164	101
Control	A	10.0	<0.000219	NA
	B	10.0	<0.000219	NA
	C	10.0	<0.000219	NA

Average recovery = 102% ± 5.03.

Part II

High Pressure Liquid Chromatography Methodology

1.0 OBJECTIVE:

This study was designed to validate a procedure for quantitative analysis of Tetrabromobisphenol A (TBBPA) in freshwater.

2.0 SUMMARY:

All samples were analyzed by HPLC by direct aqueous injection, following 0.45 micron (Teflon) filtration.

3.0 EQUIPMENT AND REAGENTS

3.1 Equipment

1. Balance:S/P 182, four-place analytical balance
2. Beakers: Pyrex, assorted sizes
3. Flasks: Volumetric, assorted sizes
4. Instrument: Waters Model 510 liquid chromatograph solvent pump equipped with Waters Intelligent Sample Processor Model 710B, Kratos Model 757 variable wavelength detector and Hewlett-Packard Model 3388 A Integrator
5. Pipets: Volumetric (Class A), assorted sizes
6. Serum bottles: Wheaton, assorted sizes, with teflon-lined lids and metal crimp tops
7. Syringes: Hamilton, assorted sizes
8. Filters: Gelman Acrodisc-CR 0.45 micron

3.2 Reagents

1. Acetonitrile, Burdick and Jackson, HPLC grade, UV cutoff @188 nm
2. TBBPA, Lot No. 7173, Bromine Compounds, Ltd.; as 100% active ingredient
3. Water, Burdick and Jackson, HPLC grade

4.0 PROCEDURE

4.1 Preparation of Stock

Approximately (ca.) 0.1 gram (g) of TBBPA was weighed on a balance in a 100-milliliter (mL) volumetric flask and solubilized in acetonitrile. The TBBPA stock solution (ca. 1 µg A.I./µL) was stored refrigerated (4 - 10°C) in a 100-mL amber serum vial with a teflon-lined lid. This stock was then used, with appropriate dilution, for quantitation and fortification.

4.2 Sample Fortification

Method validation/recovery samples were prepared using freshwater. The aqueous samples were fortified with dilutions of the TBBPA stock solution. The fortification levels produced were 0.500, 1.00, and 3.00 µg A.I./mL (three replicates at each level). An additional three freshwater samples (100 mL) were left unfortified to be utilized as blank control samples.

4.3 High Pressure Liquid Chromatography

High Pressure Liquid Chromatographic (HPLC) analysis was conducted utilizing the following instrumental conditions:

Instrument: Waters Model 510 liquid chromatograph solvent pump equipped with Waters Intelligent Sample Processor Model 710B, Kratos Model 757 Variable Wavelength Detector and Hewlett-Packard Model 3388A Integrator

Column: Phenomenex Ultremex C_{18} (5μm) 250 mm (length) x 4.6 mm ID

Mobile Phase: 80% Acetonitrile: 20% HPLC grade water

Flow: 1.50 mL/minute

Pressure: 1000 p.s.i.

Chart Speed: 0.3 cm/minute

Wavelength: 230 nm

Injection Volume: 25 μL

Instrument Sensitivity: 0.10 AUFS

Peak Width: 0.1 seconds

Attenuation: 2^1

Threshold: 1

4.4 Analysis

The TBBPA stock was diluted with 50/50 acetonitrile-HPLC grade water to prepare appropriate HPLC calibration standards of 200, 350, 500, 1000, and 1500 μg A.I./L for the method validation. Analyses of the samples and standards were performed by programmed injection. A standard curve was constructed by plotting the peak height observed versus the concentration (μg A.I./L) of the standard injected.

Linear regression analysis was used to determine the concentration of TBBPA found in the sample.

4.5 Calculations

The following equation was utilized in calculating analytical results:

Analytical Result (μg A.I./L) = A x D.F.

where:

Analytical Result = concentration of TBBPA

A = concentration (μg A.I./L) of sample from the regression analysis

D.F. = dilution factor, ratio of final volume (mL) of the sample to volume (mL) of sample used

5.0 RESULTS AND DISCUSSION:

Analytical results for the recovery of TBBPA from freshwater are presented in Table 2A.

A representative chromatogram showing the recovery of TBBPA from freshwater is presented in Figure 1A.

The linear regression analysis for the standards used in the recovery study is shown in Figure 2A.

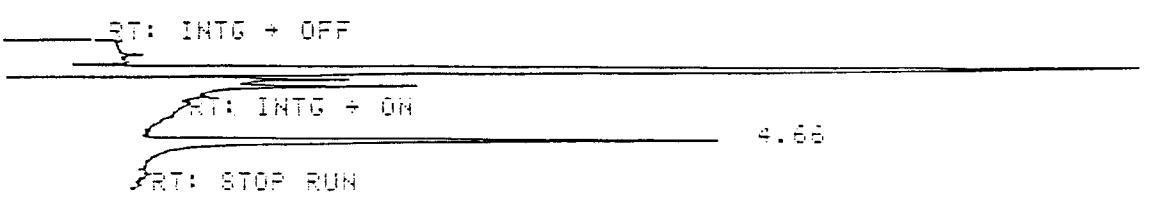
Table 2A. Analytical results for the recovery of TBBPA from freshwater.

Sample ID (µg A.I./mL)		Volume (mL)	Concentration Recovered (µg A.I./mL)	% Recovered
3.00	A	100	2.67	89.0
	B	100	2.70	90.0
	C	100	2.54	84.7
1.00	A	100	0.989	98.9
	B	100	0.994	99.4
	C	100	0.971	97.1
0.500	A	100	0.518	104
	B	100	0.515	103
	C	100	0.492	98.4
Control	A	100	<2.22	N/A
	B	100	<2.22	N/A
	C	100	<2.22	N/A

Average recovery: 96.1% (±6.64).

Theoretical minimum detectable concentration is <2.22 µg A.I./mL.

Figure 1A. A representative chromatogram showing the recovery of TBBPA from freshwater.



[hp] 3388A MANUAL INJECTION @ 19:00 JUL 28, 1988
AREA %

RT	AREA	TYPE	WIDTH	HEIGHT	BASELINE	AREA %
0.00			BASELINE @ START RUN = 0.52			
0.00			THRESHOLD @ START RUN = 1			
0.00			PEAK WIDTH @ START RUN = 0.04			
0.01			RT: INTG + OFF			
3.50			RT: INTG + ON			
4.66	174.76	BB	------#	16.17	1.37	100.000

1.00 ± µg/mL

TOTAL AREA = 174.76
MULTIPLIER = 1

Figure 2A. The linear regression analysis for the standards used in the recovery study.