BIOCONCENTRATION AND ELIMINATION OF ^{14}C-RESIDUES BY FATHEAD MINNOWS (*Pimephales promelas*) EXPOSED TO TETRABROMOBISPHENOL-A

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Brominated Flame Retardant Industry Panel
c/o Great Lakes Chemical Corporation
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AMENDED FINAL REPORT

STATEMENT (GLP COMPLIANCE) OF STUDY DIRECTOR

The data and report prepared for this study were produced and compiled in accordance with all pertinent EPA Good Laboratory Practice (GLP) Standards (ref. 1).

Paul H Fackler 17 Aug 89

Paul H. Fackler, Ph.D. Date
SLS Study Director

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1.0 SUMMARY

Fathead minnows (*Pimephales promelas*) were continuously exposed to a nominal concentration of 5.0 µg/L of Tetrabromobisphenol-A (TBBPA) for 24 days after which 20 fish were transferred to flowing, uncontaminated water for a depuration period of six days. The concentrations of ^{14}C-TBBPA in the test solution and exposed organisms were monitored after 0 (water only), 5 and 10 hours and on days 1, 2, 4, 9, 14, 22 and 24 of the exposure period and on days 1, 4, 5 and 6 of the depuration period. The exposure and depuration periods were experimentally defined by the uptake and elimination kinetics observed.

Radiometric analyses of the water and fish tissue revealed the following:

1. The concentration of aqueous ^{14}C-residues in the TBBPA treated exposure system remained relatively constant throughout the 24-day exposure period at a mean measured concentration (± S.D.) of 4.7 (± 0.3) µg/L. Throughout the 6 day depuration period, concentrations of ^{14}C-residues present in the water of the depuration aquarium remained below the limit of radiometric detection in water (0.29 µg/L).

2. The concentration of ^{14}C-residues in the tissue of fish exposed to ^{14}C-TBBPA reached steady state on the fourth day of the exposure period. The mean steady-state tissue concentration was 5800 µg/kg which established a bioconcentration factor (BCF) of 1200X. The 95% confidence interval of the bioconcentration factor was calculated to be 900X - 1700X. Model calculations based on the measured water and concentrations of ^{14}C-residues in the tissue of exposed fish predict a bioconcentration factor for fathead minnow of 1300X.

3. Rapid elimination of ^{14}C-residues from fathead minnows, following exposure to 4.7 µg/L ^{14}C-TBBPA, was observed during the six-day depuration period. Half-life, (50% elimination) of the ^{14}C-residues present in the tissue of fathead minnows on the last day of exposure, occurred during the first 24 hours of depuration. Elimination of 95% of

the accumulated ^{14}C residues occurred between day 1 and 4 of depuration. Following 6 days of depuration, 98% of the accumulated ^{14}C residues were eliminated from the tissues of exposed fish. Following transfer to untreated dilution water, ^{14}C-TBBPA residues did not appear to persist in fish tissue to a significant extent.

2.0 INTRODUCTION

The objective of this investigation was to define the kinetics of the uptake and elimination of ^{14}C-residues in fathead minnows (*Pimephales promelas*) continuously exposed to Tetrabromobisphenol-A (TBBPA) at a nominal concentration of 5.0 micrograms per liter (μg/L). Specifically, the major areas investigated were:

(A) The rate and extent of accumulation of ^{14}C-residues (i.e., parent compound, degradation products and metabolites calculated as TBBPA) in the tissue of fathead minnows during continuous exposure to TBBPA.

(B) The rate and extent of elimination of the accumulated ^{14}C-residues from the tissue at steady state following transfer of the exposed fish to flowing, untreated dilution water for a period of depuration.

This investigation was conducted at Springborn Life Sciences, Inc., (SLS) laboratories located in Wareham, Massachusetts. The original raw data and final report for this study are stored at the above location. The bioconcentration study was conducted, under flow-through conditions, from 23 January to 22 February 1989. A final report for this study was issued to Brominated Flame Retardant Industry Plant, c/o Great Lakes Chemical Corporation dated 16 March 1989. This amended final report, 15 August 1989, incorporates changes made as presented in Final Report Amendment.

3.0 MATERIALS AND METHODS

3.1 Test Material

The bioconcentration study was conducted according to the Springborn Life Sciences' protocol entitled "Protocol for Conducting a Dynamic Bioconcentration Study with Fathead Minnow (*Pimephales promelas*)", SLS Protocol# 020188/FM-BIOC.BFRIP. The procedures

described in this protocol generally followed those presented in EPA/OTS (Fed. Reg., 1985, 1987); Barrows *et. al.* (1980), ASTM (1984), and Spacie and Hamelink (1985) and were consistent with the requirements published in EPA's Final Test Rule 40 CFR Part 799 (Fed. Reg. Volume 52, No. 128, 6 July 1987).

Radiolabeled ^{14}C TBBPA was received from Midwest Research Institute, Kansas City, Missouri on 12 February 1988 and was stored in a freezer prior to use. The material was received in a glass vial labeled: Tetrabromobisphenol A (UL-^{14}C); Lot #MRI-77-161-19; 8.69 mCi; specific activity 9.32 mCi/mmole. Prior to use in this study, the material was purified and dissolved in 50 mL of acetone. Purification was effected by preparative TLC using Whatman - Silica Gel - 1000 μm plates. Subsequent TLC analysis indicated a radiopurity (^{14}C) of 96.0% after purification. The 50 mL solution was then radiometrically assayed to have a concentration of 8.62 mg ^{14}C-TBBPA/mL of acetone, based on the labeled specific activity.

3.2 Test Organisms

The fathead minnows (SLS Lot #88A73) used during this study were obtained from a population reared at SLS and held under a photoperiod of 16 hours of light and 8 hours of darkness. Fish were fed a dry pelleted food, ad libitum, daily except during the 24 hours prior to testing. No mortality occurred in the test population during the 48 hours prior to test initiation. The mean (standard deviation) total length and weight of the fish used during this study were 39 (± 4) mm and 0.57 (± 0.20) grams, respectively. All fish used were reproductively immature based on the size of test organisms. The culture water used to rear these organisms was drawn from a 125-meter deep bedrock well into a concrete reservoir where it was aerated and supplemented with well water from the town of Wareham, Massachusetts. The well water which flowed into the holding tank was characterized as having a total hardness and alkalinity (as calcium carbonate - $CaCO_3$) of 28 mg/L and 20 - 26 mg/L, respectively, and a specific conductance of 100 μmhos/cm (Weekly Gravity Feed Tank Water Quality Analysis Log Book). Other parameters measured in the holding tank were a pH range of 6.8 - 6.9, a dissolved oxygen concentration range of 79 - 81% of saturation, a flow

rate of 7.3 - 7.9 tank volume replacements per day, and a temperature range of 21 - 23° C (Weekly Record of Fish Holding Characteristics). The test fish were held under these conditions for a minimum of 14 days prior to testing.

3.3 Test Dilution Water

The dilution water was obtained from the same source as the water which flowed into the fish holding tank and was characterized as having a total hardness range of 26 - 30 mg/L $CaCO_3$, a total alkalinity range of 21 - 24 mg/L $CaCO_3$, a pH range of 6.8 - 7.3 and a specific conductance range of 100 - 130 μmhos/cm. The ability of several daphnid species to survive and reproduce in this water source over several generations of culture 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, metals 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. Results of analyses performed during the six months prior to testing are provided in Appendix I.

3.4 Test System

The exposure solutions were prepared during this study using a modified diluter system, similar to that described by Mount and Brungs (1967). The system was calibrated to deliver 2.0 L of water to each test aquarium per diluter cycle. The system cycled an average of 257 times per day. The glass exposure aquaria measured 76 x 40 x 30 centimeters (cm) with a 24.7 centimeter high sidedrain to maintain a test solution volume of approximately 75 liters. The flow rate of solution delivered to each aquaria provided 6.9 volume turnovers per day. This turnover rate is equivalent to a 90% aquaria volume replacement time of 8 hours (Sprague, 1969). Illumination of the test area was provided by Vitalite fluorescent bulbs for 16 hours daily at an intensity of 34 - 36 footcandles at the test solution surface.

3.5 Test Concentrations

Selection of the exposure concentration for the bioconcentration study was based on a flow-through acute exposure of fathead minnows previously conducted at SLS. Results of this acute test established a 96-hour LC50 for TBBPA and fathead minnows of 0.50 mg/L (SLS report #88-10-2834). The concentration selected for the bioconcentration study was 1/100 of this LC50 value, or 5.0 μg/L TBBPA. This concentration was selected in order to minimize any chronic effects of the test material on fathead minnows.

3.6 Stock Solutions

A stock solution, at a concentration of 1.11 mg/mL ^{14}C TBBPA was prepared by diluting 9.7 mL of the radiolabeled stock solution (8.62 mg TBBPA /mL acetone) to a total volume of 75 mL with acetone (CAS# 67-64-1). Syringe delivery mechanisms and 50-mL Glenco gas-tight syringes located above the treatment and solvent control aquaria were calibrated to deliver 0.009 mL of the appropriate stock solution of test material (treatment) or acetone (solvent control) to 2.0 L of dilution water with each diluter cycle. The solvent control solution contained 4.5 μL/L acetone, which was equivalent to the concentration of solvent present in the treatment level aquarium.

3.7 Test Initiation and Monitoring

The exposure system was in operation approximately 3 weeks prior to initiation of the bioconcentration study. The exposure was initiated on 23 January 1989 by placing 91 fathead minnows into each test aquarium (treatment level and solvent control). During the study, fish were fed once daily with a commercial pelleted fish food at a rate of 2% of the biomass present in the exposure aquaria. Excess food was siphoned from the aquaria one-half hour after each feeding, which were regulated to be no less than 24 hours prior to tissue sample collection. The temperature and dissolved oxygen concentration were measured in each aquarium daily and the pH three times weekly. Dissolved oxygen concentration was measured with a YSI Model #57 dissolved oxygen meter and probe; pH was measured with a LaMotte

Model HA pH meter; specific conductance was measured with a YSI Model #33 S-C-T meter and temperature was measured with a Brooklyn alcohol thermometer. The temperature was continuously monitored in one test aquarium with a Brooklyn Min-Max thermometer. Light intensity was measured using a General Electric Model 214 light meter.

The exposure of fathead minnows to TBBPA, at a nominal concentration of 5.0 µg/L, was continuous until a steady state tissue residue concentration was established. Steady state, defined as three consecutive sampling intervals for which the measured ^{14}C-tissue concentrations were not significantly different, was determined by analysis of variance. Steady state represents the equilibrium concentration of accumulated ^{14}C-residues in the tissue of fathead minnow. Once equilibrium was achieved twenty fish from the treatment level aquarium were transferred to a clean aquarium into which untreated dilution water was introduced at a rate equal to the flow rate during the exposure period. The remaining fish were dissected into 3 tissue portions (muscle, viscera and carcass), then frozen and retained for possible future metabolite identification. Results of these analyses appear in Appendix I.

The depuration period was maintained in order to estimate the extent and rate of elimination of the accumulated ^{14}C-TBBPA residues from the exposed fish. Depuration was terminated when more than 95% of the residues, present in the fish tissue at the termination of exposure, were eliminated.

3.8 Bioconcentration Factor Computation

The bioconcentration factor (BCF) was calculated for TBBPA and fathead minnows, on a whole body fish tissue basis by dividing the mean measured equilibrium ^{14}C-tissue concentration by the mean measured water concentration of TBBPA in the test solution during the same period. For comparison, an additional method of calculating the bioconcentration factor was performed wherein the bioconcentration factor is the ratio of the uptake constant (K_u) to the depuration constant (K_d) (Spacie and Hamelink, 1985). The uptake and depura-

tion constants (K_u) and (K_d) were obtained using the following equations:

for the uptake phase

$$C = (K_u/K_d) \times C_w \times [1 - e^{(-K_d t)}] \quad (1)$$

for the depuration phase

$$C = (K_u/K_d) \times C_w \times e^{(-K_d t)} \quad (2)$$

Where:
- K_d = depuration constant (day^{-1})
- K_u = uptake constant (day^{-1})
- t = time in days
- C = tissue concentration at time t ($\mu g/kg$)
- C_w = steady state water concentration ($\mu g/L$)

$BCF = K_u/K_d$

Confidence intervals were determined using the following equations:

97.5% Confidence Interval (tissue, water) = Mean ± t x S.E.

where: t = Student's t-Statistic at P = 0.025 and (n - 1) degrees of freedom

S.E. = Standard Error (Standard deviation/$\sqrt{n}$)

95% confidence interval = $L_T/U_W - U_T/L_W$

where: U_T and L_T = upper and lower 97.5% confidence interval for tissue, respectively

U_W and L_W = upper and lower 97.5% confidence intervals for water, respectively

$L_T/U_W \leq$ 95% Confidence Interval BCF $\leq U_T/L_W$

3.9 Analytical Measurements

System calibration measurements were performed by collecting and analyzing triplicate 5 mL water samples 4 and 3 days prior to the introduction of fish to the treatment aquaria. During the study, the concentration of the ^{14}C-residues in the water of the TBBPA treatment and depuration aquaria were monitored by the removal of samples from the treatment level solution and the control at 0, 5 and 10 hours and on days 1, 2, 4, 9, 14, 22 and 24, of exposure and days 1, 4, 5 and 6 of depuration. Each 5 mL sample was removed using a glass volumetric pipet and transferred to individual glass scintillation vials containing 15 mL of Monophase^{R}. The ^{14}C-activity of the water samples was measured by placing the scintillation vials containing the solution sample and scintillation fluid in a liquid scintillation spectrometer (Beckman Model LS 1801) and recording the number of disintegrations per minute. Analytical results were combined and reported as the mean measured concentration in μg ^{14}C-TBBPA/L of test solution (N=3) for each sampling interval and system, i.e., treated aquarium, solvent control aquarium.

In order to quantify the accumulation and elimination of ^{14}C-residues in the whole body tissue of fathead minnows, four fish were collected and analyzed from the treatment aquarium at 5 and 10 hours and on days 1, 2, 4, 9, 14, 22 and 24 exposure and on days 1, 4, 5, and 6 of depuration. Four solvent control fish were removed on each of the sampling days but only analyzed on days 0 (5 hour), 9, 14, 22 and 24 of exposure and day 6 of depuration in order to quantify background ^{14}C-residues as ^{14}C-TBBPA in fish tissue. Each tissue sample was placed in a pre-tared Combusto-Cone^{R} (a pressed cellulose-based material, Packard Instrument Company) and weighed. To aid combustion, approximately 0.2 g of cellulose powder (Whatman CC4L) was added to each cone containing the sample. Each sample was air-dried for at least 24 hours at ambient temperature and then placed in a Packard Model 306 Sample Oxidizer and combusted. The resulting $^{14}CO_2$ was trapped as a carbonate salt in a mixture of Carbosorb^{R} (a basic amine) and scintillation fluid (4 g 98% PPO, a primary scintillator + 2% bis/MSB, a secondary scintillator per liter of toluene) in a glass scintillation

vial. Each vial was then placed in a liquid scintillation spectrometer and the disintegrations per minute determined. Analytical results were combined and are reported on a whole body basis as μg ^{14}C-TBBPA/kg of fish tissue (N=4).

Recovery rates of the oxidizer were determined prior to analyzing each set of samples by combusting and counting the activity of a standard reference material (Spec Chec ^{14}C-standard) and comparing the calculated value to the measurement of the spiked standard. Recovery rates were determined to be 97 - 104% Experimental data were not adjusted for percentage recovery.

Counting efficiencies of all experimental samples were determined using an external standard and a factory prepared calibration curve (Beckman Instruments). All test samples were counted for a maximum of 100 minutes or until a 2 sigma error of 5% was attained. Using the criterion and the calculations described in Standard Methods for the Examination of Water and Wastewater, it was determined that a minimum net counts per minute (cpm) for all samples of 48 - 67 (dependent upon background) had an associated counting 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.

Background levels of radiation for liquid and combusted samples were determined monthly. The background levels during this study ranged from 39.35 to 42.32 cpm for liquid samples and was 41.83 to 55.50 cpm for combusted samples.

The minimum detectable ^{14}C-residue concentration was dependent on the counting efficiency, sample size (milliliters or grams) and the acceptable minimum net cpm (see above).

Quality Assurance (QA) samples were prepared and analyzed with both water and oxidized tissue samples at each interval. The QA samples analyzed with the water samples

were prepared by injecting a known amount of ^{14}C-TBBPA into 5 mL of dilution water. The QA samples analyzed with oxidized tissue were prepared by injecting a known amount of ^{14}C-TBBPA into a pre-tared Combusto-Cone^{R} containing approximately 0.30 g cellulose powder and combusting in the same manner as the experimental samples. Analyses of the QA samples were used to determine the accuracy and quality control maintained during the analytical process. A recovery study performed prior to test initiation, resulted in a mean recovery of 95.8 ± 8.93% for analysis of water, and 98.0 ± 9.30% for combusted samples. Ordinary rejection criteria for data sets utilized at Springborn Life Sciences consist of, among other things, rejection of data intervals where more than one Quality Assurance (QA) sample recovery percentage is more than three standard deviations from the mean demonstrated during the method validation. Based on these criteria, Quality Assurance samples for day 14 of the exposure period for tissue analysis were rejected. Therefore, analytical measurements of the concentration of ^{14}C-TBBPA in the tissue during this sampling interval were not included in any calculations. The calculations used to determine the concentration of ^{14}C-residues in each sample were the following:

Total ^{14}C-residues calculated as ^{14}C-TBBPA (μg/kg or μg/L) =

$$\frac{\text{Net dpm}}{\text{Specific Activity of } ^{14}\text{C-TBBPA} \quad X \quad \text{Sample Size kilograms or liter}}$$

Where: Net dpm = disintegrations per minute calculated by the instrument after background subtraction.

Specific activity of ^{14}C-TBBPA = 38040.978 dpm/μg

4.0 RESULTS

4.1 Evaluation of Bioconcentration

The bioconcentration study exposing fathead minnows to a nominal concentration of 5.0 µg/L TBBPA and a solvent control solution was terminated after 24 days of exposure and 6 days of depuration (30 days in-life). During both the exposure and depuration periods, no mortalities were observed, all fish exhibited normal behavior and appeared to be in good physical condition.

4.2 Water Quality Measurements

During this study, the measured water quality parameters varied minimally between test aquaria and remained within acceptable ranges for the survival and normal behavior of fathead minnows. Dissolved oxygen levels in the treatment and solvent control aquaria ranged between 8.6 and 9.6 mg/L and were comparable during the exposure and depuration periods. Test temperature, 19 - 21° C, and pH, 7.0 - 7.6, were similarly comparable and indicated consistent control of the test system conditions. Results of water quality measurements made during this study are summarized in Table 1.

4.3 Measurements of ^{14}C-TBBPA in Water

Analyses of the test solution samples removed 4 and 3 days before the exposure was initiated resulted in measured concentrations which were representative of the nominal treatment level and were generally consistent between the two sampling intervals. The average concentration of TBBPA measured in the pretest samples (N =6) was 4.7 (± 0.34) µg/L. Based on these results, fathead minnows were introduced into the treatment and solvent control aquaria and the exposure was initiated.

The ^{14}C residue concentrations, reported as TBBPA, measured in the treatment aquarium solution during the exposure period of the study are presented in Table 2. The

mean measured ^{14}C TBBPA concentration during the exposure period was 4.7 ± 0.3 µg/L. The mean concentration was 94% of the nominal concentration of 5.0 µg/L and varied minimally (coefficient of variation was 6.4%). During the depuration period, no detectable levels of ^{14}C residues were present at the limit of detection (0.29 µg/L). Analyses of the solvent control solution (Table 4) established that no detectable ^{14}C residues were present in the control aquaria water during the entire study.

4.4 Measurements of ^{14}C-TBBPA in Fish

The ^{14}C residue concentrations measured in the tissue of fathead minnows during the exposure period are also presented in Table 2 and illustrated in Figure 1. Statistical comparison (analyses of variance) of the ^{14}C residue concentrations measured in the tissue of fathead minnows exposed to TBBPA established that no significant difference existed between the residue levels measured on days 4, 9 and 22. Based on these data, it was determined that steady state for the ^{14}C residue concentration in fish tissue was reached on day 4 of the exposure period. During steady state, the mean measured equilibrium concentration of ^{14}C-residues in fathead minnow whole body tissue was 5800 (± 1300) µg/kg and ranged from 5000 to 6600 µg/kg for exposure days 4, 9, 22 and 24. Based on the mean equilibrium tissue concentration 5800 µg/kg and a mean water concentration during this period of 4.7 µg/L, the bioconcentration factor for TBBPA in fathead minnows was 1200X with a 95% confidence interval of 900X - 1700X.

During the exposure, low levels of ^{14}C-residues were measured in the solvent control fish. Since no ^{14}C-residues were detected in the water samples removed from the solvent control aquarium, the detectable levels of residues measured in the fish tissue from this aquaria were believed due to sample contamination during the sampling or combustion processes. The detectable concentration of ^{14}C-residues measured in the control fish was < 0.5% of the mean ^{14}C-residue concentrations measured in fish exposed to TBBPA.

4.5 Quality Control Measurements

Results of the analysis of Quality Assurance samples analyzed concurrently with water samples established a mean (standard deviation) recovery of 96.6 ± 10.5% (Table 5). Analyses of the Quality Assurance samples analyzed concurrently with the tissue samples resulted in a mean (standard deviation) recovery of 105 ± 17.8% (Table 6). Laboratory performance indicated that satisfactory control was exercised over the precision and accuracy of the analysis of water and fish tissue samples collected during the reported bioconcentration study.

4.6 Bioconcentration Factor

A bioconcentration factor for tissue was also calculated by using the equations on page 10 to determine the uptake constant (K_u) and depuration constant (K_d) for tissue, where K_u/K_d equals the predicted bioconcentration factor (BCF).

Measured Exposure Level	(K_u/K_d)	Predicted BCF	Measured BCF
4.7 µg/L	2400/1.8	1300X	1200X

A comparison of the bioconcentration factors derived by these two methods is presented in Figure 1. The values used for the observed data points are presented in Table 2. Model predictions are in good agreement with experimentally derived results.

4.7 Depuration Phase

Results of the tissue analyses for fathead minnows transferred to flowing, uncontaminated water after 24 days of exposure to TBBPA are presented in Table 3. Rapid elimination of the accumulated ^{14}C residues from the tissue was observed. Half-life, or the time when 50% of the accumulated ^{14}C residue was eliminated, was achieved within the first 24 hours of the depuration period. Elimination of 95% of the ^{14}C residues was achieved

between days 1 and 4 of depuration. The study was terminated after 6 days of depuration. By this time, 98% of the accumulated ^{14}C-residues had been eliminated from the exposed fish. The rapid elimination of ^{14}C-residues from the tissue of fathead minnows is illustrated in Figure 1.

4.8 Conclusion

Results of this study indicate that continuously exposed fathead minnows readily accumulate ^{14}C-TBBPA reaching steady-state equilibrium conditions within 4 days. Extending the period of continuous exposure, up to 24 days, did not increase the accumulation or equilibrium concentration in ^{14}C-residues observed. In depuration, the transferred fathead minnows rapidly and nearly completely eliminated the accumulated ^{14}C-residue. By day 6 of the elimination period in untreated dilution water only 2% of the accumulated ^{14}C-residue remained in the exposed fish. The bioconcentration factor (BCF) for TBBPA was greater than 1000X but residues are not expected to persist in fathead minnows once removed from conditions of continuous exposure.

DEVIATIONS FROM PROTOCOL

1. The protocol states that the exposure solution temperature will be maintained at 22 ± 2° C. On one occasion, (exposure day 12) the temperature in the system reached 19° C.

2. The protocol contains a procedure for calculating the 95% confidence interval of the BCFs which was corrected and appears on page 11 of this report. The protocol states that the standard error in S.D./n. The standard error is S.D./$\sqrt{n}$.

3. Quality Assurance samples were analyzed with each sampling interval and were prepared in dilution water. The protocol states that they will be analyzed with every other interval and will be prepared in distilled water.

4. The radiolabeled stock was prepared as described on page 7 of this report. The protocol states that the entire contents of the vial will be diluted with acetone and was not applicable to this study.

5. A different method for calculating K_u and K_d was used employing a computer program to select the best fit for the equation found on page 12 of this report with the experimental data. The protocol states that these calculations will be done by hand.

It is our opinion that these deviations did not effect the results of this study.

Emily Dionne 8-17-89

Emily Dionne Date
Principal Investigator

Paul H Fackler 17 Aug 89

Paul H. Fackler, Ph.D. Date
SLS 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): 8, 9, 13, 14, 15 and 16 March 1989. The Appendix was audited on 16 August 1989.

QAU inspection report(s) were issued to the Study Director on 8, 14 and 16 March and 16 August 1989. 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.

Patricia D. Royal 8/17/89

Patricia D. Royal Date
Manager, Regulatory Affairs and
Quality Assurance

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TABLES

Table 1. Water quality analysis of test solutions during the 30 day (24 day exposure, 6 day depuration) bioconcentration study with ^{14}C-TBBPA and fathead minnows (*Pimephales promelas*).

	Mean (Standard Deviation) Range		
Aquarium	Dissolved Oxygen (mg/L)	Temperature (°C)	pH
Treatment	9.1 (0.3)[a] 8.6-9.5	20 (0.6) 19-21	—[c] 7.0-7.6
Solvent Control	9.2 (0.3)[b] 8.7-9.6	20 (0.6) 19-21	—[c] 7.0-7.4

[a] 100% of saturation at 20° C.
[b] 101% of saturation at 20° C.
[c] Not applicable.

Table 2. **Measured ^{14}C-residue concentrations, calculated as TBBPA in the water and tissue of fathead minnows (*Pimephales promelas*) during 24 days of continuous aqueous exposure to TBBPA at a mean measured water concentration of 4.7 ± 0.3 µg/L.**

Day	Exposure Concentration (µg/L)[a]	Range (µg/L)	^{14}C-Tissue Residue Concentration (whole body) (µg/kg)[b]	Range (µg/kg)
		Exposure Phase		
0-Hour	4.8 (0.1)	4.8-4.9	——	
5-Hour	4.5 (0.1)	4.5-4.6	2400 (290)	2000-2700
10-Hour	4.5 (0.1)	4.4-4.6	2800 (510)	2400-3500
1	5.0 (0.1)	4.9-5.1	5000 (560)	4400-5700
2	4.5 (0.2)	4.4-4.7	7500 (520)	6800-8000
4	4.4 (0.1)	4.2-4.4	6600 (1800)	4500-8800
9	4.8 (0.2)	4.6-5.0	5200 (630)	4600-6000
14	5.3 (0.2)	5.1-5.5	4300 (1700)[c]	2200-6000
22	4.8 (0.2)	4.6-4.9	5000 (770)	4300-6100
24	4.3 (0.1)	4.1-4.4	6500 (1400)	4900-7800

[a] Mean and standard deviation (S.D.) based on radiometric analysis of triplicate samples.
[b] Mean (S.D.) based on analysis of the tissue of 4 fish.
[c] Results of sample analyses were not used as results of analyses of Quality Assurance Samples for this interval were outside the acceptable range established by this laboratory (i.e. three standard deviations from the average recovery determined during the method validation study).

Table 3. **Measured ^{14}C-residue concentrations, calculated as TBBPA in the water and tissue of Fathead minnows (*Pimephales promelas*) during 6 days of depuration in flowing untreated dilution water.**

Day	Exposure Concentration (µg/L)	^{14}C-Tissue Residue Concentration (whole body) (µg/kg)[a]	Range (µg/kg)
1	< 0.29[b]	1200 (740)	240-2000
4	< 0.29	170 (58)	140-260
5	< 0.29	120 (26)	96-160
6	< 0.29	120 (24)	95-150

[a] Mean (S.D.) based on analysis of tissue portions of 4 fish.
[b] Water concentrations listed for the depuration phase are the minimum detection limit.

Table 4. Measured ^{14}C-residue concentrations, calculated as TBBPA, in the solvent control water and solvent control tissue of fathead minnows (*Pimephales promelas*) during the 30 day study.

Day	Measured Water Concentration (µg/L)[a]	^{14}C-Tissue Residue Concentration Whole Body (µg/kg)[b]
	Exposure Phase	
0	< 0.28	——
5 hour	< 0.28	9.7
9	< 0.29	2.8[d]
14	< 0.29	< 2.7[c,e]
22	< 0.29	< 3.7[f]
24	< 0.29	< 3.6[g]
	Depuration Phase	
6	< 0.29	5.1

[a] Water concentrations listed are the minimum detection limit.
[b] Samples were apparently contaminated with low levels of ^{14}C during the sample preparation or combustion procedure, as water concentrations were consistently below detection.
[c] Results of analyses of Quality Assurance samples for this interval were outside the acceptable established by this laboratory.
[d] Mean, N=3. One sample was below limit of detection at < 1.71 µg/kg.
[e] One of 4 samples contained measurable levels of ^{14}C-residues at 2.6 µg/kg.
[f] Two of 4 samples contained measurable levels of ^{14}C-residues at 9.7 and 13 µg/kg.
[g] Two of 4 samples contained measurable levels of ^{14}C-residues at 4.8 and 3.7 µg/kg.

Table 5. **Analytical results for Quality Assurance samples analyzed concurrently with water samples during the 30 day (24 day exposure, 6 day depuration) bioconcentration study with TBBPA and fathead minnows (*Pimephales promelas*).**

Sampling Interval	Theoretical Concentration (µg/L)	Measured Concentration (µg/L)	% Recovery
		Exposure Period	
0 Hour	5.16	3.27	63.4
	5.85	4.54	77.6
	6.54	5.47	83.6
5 Hour	5.33	4.17	78.2
	6.19	5.23	84.5
	6.88	5.93	86.2
10 Hour	5.68	4.90	86.3
	6.19	5.45	88.0
	5.16	4.59	89.0
Day 1	5.16	3.73	72.3
	5.50	5.25	95.5
	6.19	5.96	96.3
Day 2	5.16	4.96	96.1
	6.02	5.91	98.2
	6.88	7.06	103
Day 4	5.16	5.03	97.5
	5.85	5.61	95.9
	6.88	7.19	105
Day 9[a]	5.16	5.15	99.8
	5.68	5.86	103
	6.54	6.84	105

Table 5. (Continued).

Sampling Interval	Theoretical Concentration (µg/L)	Measured Concentration (µg/L)	% Recovery
		Exposure Period (Continued)	
Day 14	5.16	4.77	92.4
	6.02	5.80	96.3
	6.88	7.13	104
Day 22	5.16	5.43	105
	6.02	6.17	102
	6.88	7.39	107
Day 24	5.16	5.56	108
	6.02	6.32	105
	6.88	7.45	108
		Depuration Period	
Day 1	5.16	4.63	89.7
	5.85	5.88	101
	6.88	7.22	105
Day 4	5.16	5.40	105
	5.85	5.96	102
	6.88	6.99	102
Day 5	5.16	5.22	101
	6.88	7.09	103
	6.54	6.99	107
Day 6	5.16	5.20	101
	6.02	6.27	104
	6.88	7.22	105

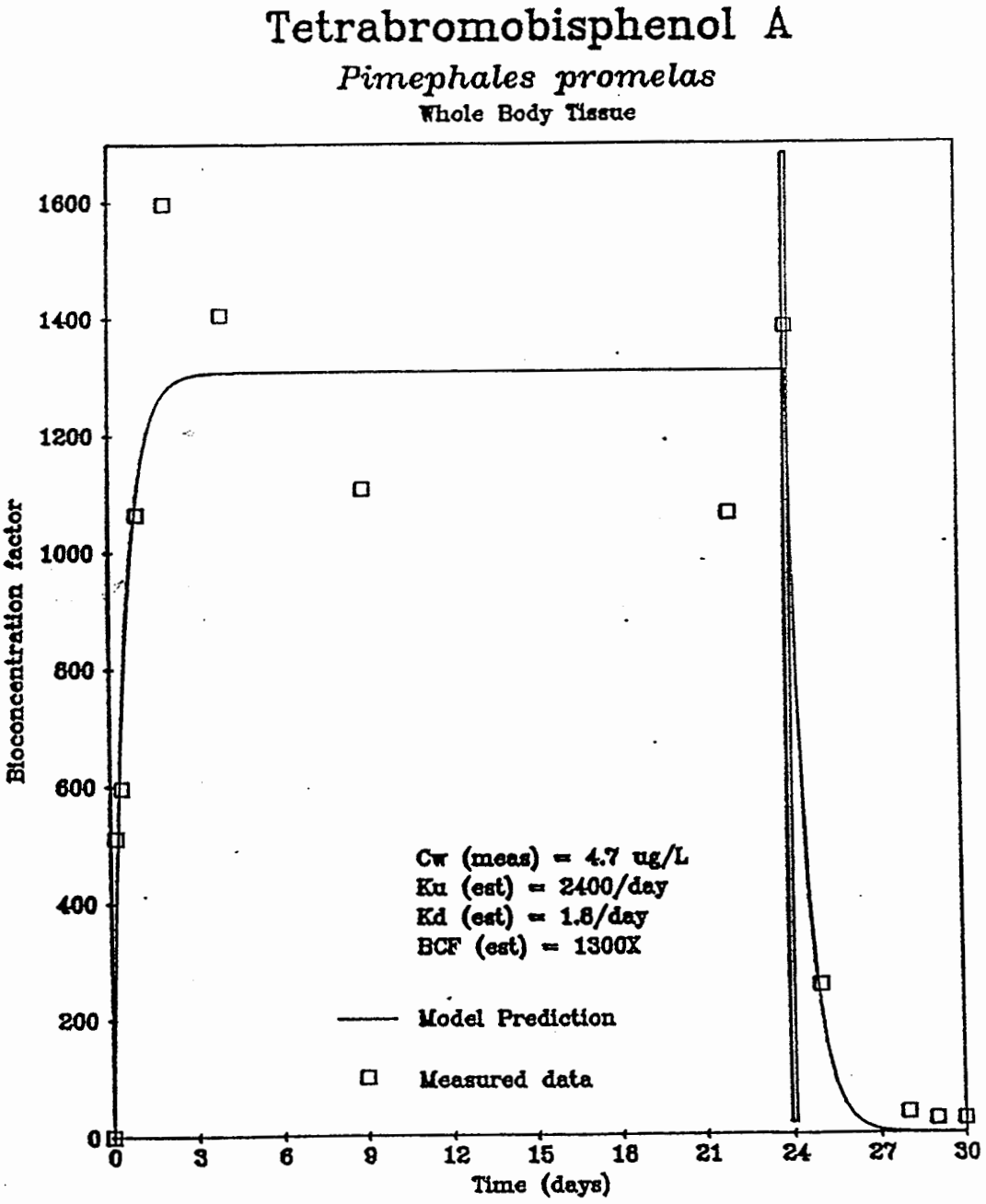
Mean recovery = 96.6 ± 10.5 (N = 42)

Table 6. Analytical results for Quality Assurance samples analyzed concurrently with tissue samples during the 30 day (24 day exposure, 6 day depuration) bioconcentration study with ^{14}C-TBBPA and fathead minnows (*Pimephales promelas*).

Sampling Interval	Theoretical Concentration (µg/kg)	Measured Concentration (µg/kg)	% Recovery
		Exposure Period	
0 Hour	3110	3290	106
	2590	2740	106
	4190	4390	105
5 Hour	207	163	78.7
	164	138	84.1
	146	116	79.5
10 Hour	57.0	55.9	98.1
	93.0	92.9	99.9
	92.9	94.6	102
Day 1	161	142	88.2
	119	121	102
	149	143	96.0
Day 2	54.1	52.8	97.6
	97.0	87.3	90.0
	214	180	84.1
Day 4	69.0	76.1	110
	126	115	91.3
	107	132	123
Day 9	201	176	87.6
	120	104	86.7
	3030	2830	93.4
Day 14[a]	35.4	53.6	151
	73.2	109	149
	230	311	135

FIGURE

Figure 1. **A comparison of measured tissue concentrations of ^{14}C-TBBPA versus those predicted by the model for tissue in Fathead minnows (*Pimephales promelas*).**



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APPENDIX I

Characterization of ^{14}C-Residues Remaining in Fathead Minnow Tissue After 24 Days of Exposure to ^{14}C-TBBPA

Extraction Validation

In order to demonstrate that a viable extraction procedure existed prior to extraction of the ^{14}C-residues remaining in tissue from the bioconcentration studies (oyster and fathead minnow), a portion of fathead minnow tissue was fortified with ^{14}C-TBBPA and extracted. The extraction was conducted according to the following procedure. A Soxhlet extraction thimble was assembled using a 250 mL round bottom flask over which was attached a 50 mL thimble (Whatman, double thickness, 25 x 80 mm). Approximately 150 mL acetone and 8 boiling stones were added to the round bottom flask and the system connected to cooling water to condense the acetone. The apparatus was allowed to reflux overnight. After the heating was disconnected and the acetone allowed to cool, the apparatus was disassembled and all interior surfaces rinsed with more acetone in order to collect all ^{14}C-residues in the round bottom flask. Triplicate 1 mL aliquots of the combined acetone volume were taken for liquid scintillation counting (LSC). The acetone was then rotary evaporated to a volume suitable for further use, usually a small volume (1 - 5 mL) for spotting on TLC plates.

On 15 March 1989, a 2.88 gram portion of fathead minnow tissue was fortified with approximately 22.2 μg ^{14}C-TBBPA, producing a tissue concentration of 7700 μg/kg. This was similar to the maximum tissue concentration established for fathead minnow during the 24 day exposure to TBBPA, and was slightly higher than the steady state concentration of 5800 μg/kg established during the bioconcentration study. The fortified tissue portion contained approximately 857000 dpm of ^{14}C-TBBPA. After 17 hours of heating, the apparatus was rinsed with acetone and the recovered volume of solvent determined to be 126 mL. Triplicate 1 mL aliquots were assayed by LSC and determined to contain 6970 dpm/mL. This corresponds to a total of 878000 dpm for 126 mL acetone, or a 102% recovery. A similar procedure was developed and validated for extraction of TBBPA from soil and is detailed in separate reports,

"Determination of the Biodegradability of Tetrabromobisphenol A in Soil Under Aerobic Conditions", Springborn Life Sciences, Inc. Report #88-11-2848, and "Determination of the Biodegradability of Tetrabromobisphenol A in Soil Under Anaerobic Conditions", Springborn Life Sciences, Inc. Report #88-11-2849.

Extraction of Fathead Minnow Tissue

On 16 March 1989, a 0.9166 gram portion of fathead minnow carcass and a 0.4981 gram portion of fathead minnow viscera from day 24 of exposure, archived under freezer conditions, were extracted with acetone for 16 hours using the procedure described above. Resulting dpm recovered for each sample were 93900 for the carcass and 375000 for the viscera. These extracts were rotary evaporated to approximately 1 to 2 mL in preparation for thin layer chromatography (TLC). The carcass extract was spotted on a 250 μm silica plate along with unlabeled TBBPA in the following manner. One spot consisted of 10 μL of an acetone stock of unlabeled TBBPA (120 mg/mL), a second spot consisted of 10 μL of an acetone stock of ^{14}C-TBBPA (8.62 mg/mL) and a third spot consisted of a portion of the carcass extract co-spotted with 10 μL of the unlabeled TBBPA stock. The third spot containing the extract was visibly larger than the other two largely due to the water that was carried through the extraction procedure. The viscera extract was spotted in a similar manner. Each plate was developed using an hexane:ethyl (7:3, v/v) acetate solvent system, dried, visualized under UV light and placed on X-ray film to produce an autoradiograph. After developing the autoradiograph, each TLC plate was divided into zones and each zone scraped into separate scintillation vials. Radioactivity in each zone was subsequently determined by LSC.

Results

Results of the radioassay of the TLC plates are presented below.

FATHEAD MINNOW CARCASS TISSUE

Zone	DPMS in Zone	DPMS minus Background	Percent of DPMS Recovered
Origin - 1	45003	44987	75.6
2	326	310	0.5
3	4843	4827	8.1
TBBPA - 4	9075	9059	15.2
5	146	130	0.2
6	106	90	0.2
7	95	79	0.1
Background	16	0	0
Total Recovered		59482	100.0

FATHEAD MINNOW VISCERA TISSUE

Zone	DPMS in Zone	DPMS minus Background	Percent of DPMS Recovered
Origin - 1	174963	174925	77.3
2	2178	2140	0.9
3	1179	1141	0.5
4	24844	24806	11.0
TBBPA - 5	22062	22024	9.7
6	708	670	0.3
7	518	480	0.2
8	221	183	0.1
Background	38	0	0
Total Recovered		226369	100.0

Results of these analyses demonstrate that a significant portion of the extracted ^{14}C-residues remain at the origin of the TLC plate. This would suggest that some of the metabolites formed have become more polar than TBBPA and bind more strongly to silica than TBBPA. Similarly, very little of the ^{14}C-residues applied to the plate have a larger Rf than TBBPA implying that more nonpolar metabolites are not formed. The plates developed a

relatively large spot at the origin, due to the water that was co-extracted with the ^{14}C-residues. Spotting of this aqueous extract produced, through capillary action, a relatively large spot and made identification of the TBBPA zone difficult.