FINAL REPORT

# DETERMINATION OF THE BIODEGRADABILITY OF TETRABROMOBISPHENOL A IN SOIL UNDER AEROBIC CONDITIONS

SUBMITTED TO

BROMINATED FLAME RETARDANT INDUSTRY PANEL

c/o GREAT LAKES CHEMICAL CORPORATION

WEST LAFAYETTE, INDIANA

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## Good Laboratory Practices Statement

The data and report prepared for this study were produced and compiled in accordance with all pertinent EPA Good Laboratory Practice regulations. This study was conducted based on the procedures described in the protocol of Springborn Life Sciences, Inc. (SLS) entitled "Protocol for Determining the Inherent Biodegradability in Soil under Aerobic Conditions." A copy of this protocol is maintained in SLS's project file.

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## 1.0 SUMMARY

The biodegradability of tetrabromobisphenol A (TBBPA) was tested under aerobic conditions in three different soil types. Test results showed biodegradation of TBBPA in all soil types, as determined by thin layer chromatography (TLC). However, for all soil types, 6.0% or less of the applied radioactivity was recovered in the $CO_2$ traps, suggesting only partial biodegradation to products as yet unidentified.

## 2.0 INTRODUCTION

Behavior of chemicals introduced into the environment is a function of properties and processes which control persistence, mobility and accumulation. The persistence of chemicals is often dictated by the biological processes, e.g., microbial metabolism, leading to natural degradation. Understanding the ability of microbial populations to metabolize a chemical in standard laboratory systems is an important element in the environmental safety assessment process.

The objective of this study was to determine the biodegradability of TBBPA in soil under aerobic conditions. The study was initiated on June 27, 1988 and completed on August 30, 1988. All raw data generated and a copy of the report are stored at Springborn Life Sciences, Inc. (SLS), Wareham, Massachusetts. The procedures followed are detailed in the protocol entitled "Protocol for Determining the Inherent Biodegradability in Soil under Aerobic Conditions". A copy of this protocol and protocol amendments I and II appear as Appendix I.

## 3.0 MATERIALS AND METHODS

### 3.1 Test Material

Radiolabeled $^{14}$C-TBBPA, lot no. CSL-88-164-21-10 (85 mg with a specific activity of 12.9 mCi/mmol) was received at SLS on May 3, 1988, from Chemsyn Science Laboratories, Lenexa, Kansas, and stored at -10 °C. The presumable metabolites of TBBPA, O,O' dimethyltetrabromobisphenol A (DMTBBPA) and O,O' diethyltetrabromobisphenol A (DETBBPA), were both received at SLS on May 11, 1988 from Chemsyn Science Laboratories, Lenexa, Kansas (162 mg Lot No. CSL-87-130-65-25 and 172 mg Lot No. CSL-87-130-66-20, respectively) and stored at room temperature in the dark. Approximately 1.6 kg tetrabromobisphenol A, lot no. 114-21H16B, purity 99.06%, was received at SLS on January 19, 1988 from Great Lakes Chemical Corporation and stored at room temperature in the dark.

### 3.2 Soils

The three soil types used in this study were Massachusetts Sandy Loam (MASDLM) collected at Plymouth, Massachusetts on May 17, 1988 and transported to the laboratory on May 18, 1988; a clay loam (sample No. 41-042688-CACY) collected at Fresno County, California on May 3, 1988; and a silty loam (sample No. 41-042688-ARSTLM) collected at Phillips County, Arkansas on May 11, 1988. The CACY and ARSTLM soils were supplied by W. R. Landis Associates, Valdosta, Georgia and were received at SLS on May 6 and 16, 1988, respectively.

Soil characteristics appear in Table 1. The three soil types were stored at room temperature in the dark. For the twenty six days prior to test initiation, the three soil types were kept at 40% field moisture capacity.

### 3.3 Test System

Each test system was a biometer flask, consisting of a 250 mL glass Erlenmeyer flask to which a 50 mL round bottom glass tube was fused. The Erlenmeyer part of the flask was closed with a rubber stopper through which an Ascarite filter (Ascarite II, Thomas Scientific, lot No. 0153) was inserted. The filter was provided with a stopper and a stopcock. The side tube was sealed with a stopper which was pierced by a needle. This needle, used to sample a hydroxide trapping solution, was closed on top with a piece of tubing in a tight knot. A foam plug (T1384, American Scientific Products) was placed in the side arm to trap volatile products. The Erlenmeyer, side arm and side tube were covered with aluminum foil to maintain the soil in the dark.

### 3.4 Procedure

A stock solution of $^{14}$C-TBBPA was prepared in acetone to provide for radioactivity at 1.045 $\mu$Ci/100 $\mu$L. This solution was fortified with a solution of unlabelled TBBPA in acetone at a concentration of 0.5 mg/100 $\mu$L.

Twelve biometer flasks, four replicates for each soil type, were labeled with project number, soil type, test material and replicate number and weighed. A polyurethane plug was placed in the sidearm of each test vessel. Soil, approximately 50 gram dry weight, was added to each flask. The hydroxide trapping solution, 10.0 mL of 1.0 N KOH, was carefully added to each side tube to prevent the volatile trapping plug from getting wet. Each side tube was immediately sealed with a stopper as described in Section 3.3. The soil was then dosed by distributing 100 $\mu$L of the test solution over the surface of the soil. After mixing the soil with a glass Pasteur pipette (from which the lower part was broken off and left in the flask) the flasks were sealed with the stopper holding the Ascarite filter and kept at a temperature of 21.5 ± 1.0 °C.

### 3.5 Analysis

On days 1, 2, 4, 8, 16, 32 and 64 the KOH solution was removed from the side tubes using a 10 mL syringe attached to the needle through the stopper. The recovered volume was measured by reading the graduations of the syringe. A 5.0 mL portion of 1.0 N KOH was then used to rinse the side tube, and upon removal, the recovered volume was measured in the same way. The KOH recovered from the rinse was added to the KOH originally removed from the side tube. Duplicate 1.0 mL aliquots were subsequently removed and quantitated by liquid scintillation counting (LSC).

The soil remaining in the test flasks at test termination was mixed and triplicate portions were combusted in a Packard Model 306 Tri-carb Oxidizer. The radioactive $CO_2$ formed was trapped in a mixture of Carbosorb$^R$ and Perma-Fluor V$^R$ scintillation cocktails and counted by

LSC. Recovery rates of the oxidizer were determined prior to analyzing the soil samples by combusting and quantifying the radioactivity of a standard reference material (nonactive and radioactive ($^{14}$C-) Spec-Chec ™, Packard Instrument Company, Inc.).

Prior to soil extraction, duplicate aliquots of each soil were removed for moisture determination. For each soil type the soil from two replicates was extracted with 150 mL acetone by Soxhlet extraction for at least 16 hours. Triplicate aliquots were taken of the soil after Soxhlet extraction and combusted in order to determine the amount of radioactivity remaining in the soil. The volume of recovered acetone was determined and triplicate aliquots were quantitated for radioactivity by LSC. The recovered acetone was then evaporated to dryness by rotary evaporation. The radioactive residue was dissolved in acetone (2 x 2 mL followed by a 1 x 1.0 mL rinse, brought up to a volume of 5 mL) and triplicate aliquots were removed and quantitated by LSC. An aliquot of this solution was applied to the origin of a 250 $\mu$m TLC plate. TBBPA and synthesized potential degradation products were spotted on the origin of each plate to assist in the characterization of biodegradation products formed in the soil during the 64 days of the study. Each plate was developed using a hexane:ethyl acetate (7:3) solvent system. Each plate was placed on X-ray film after drying to produce an autoradiograph. After developing the autoradiograph, each TLC plate was divided into zones, each zone was scraped into a scintillation vial, 15 mL of Monophase$^{R}$ scintillation cocktail was added, and radioactivity was quantitated by LSC.

From the replicates of which the soil had been extracted, the volatile plugs were removed and extracted with 150 mL hexane/methanol (1/4, v/v) using an overnight Soxhlet extraction procedure. The recovered volumes of hexane/methanol were measured using volumetric cylinders and triplicate samples were taken and counted by LSC.

## 4.0 CALCULATIONS

At each sampling interval the mean disintegrations per minute (DPM) per milliliter KOH recovered from the side tubes was determined along with the total amount of radioactivity recovered. The latter was also expressed as percent of the amount of radioactivity initially applied. A cumulative total of evolved $^{14}CO_2$ was calculated for each replicate and was also expressed as percent of the total amount of radioactivity initially applied to each flask.

At test termination the DPM/g soil and the total recovery of radioactivity in the soil were determined for each replicate. The latter was also expressed as the percent of radioactivity initially applied.

The radioactivity recovered in the total volume of hexane/methanol after Soxhlet extraction of the volatile plugs was determined by LSC.

A radioactivity mass balance was calculated for each soil replicate by adding the cumulative DPM recovered as $^{14}CO_2$ and the radioactivity recovered in the soil at test termination. The mean and standard deviation were calculated for each soil type.

For the soil replicates of which the soil was extracted (Soxhlet), the total amount of radio-

activity recovered in the acetone was calculated and expressed as percent of the amount of radioactivity initially applied to each flask, derived from the combustion of soil aliquots prior to extraction. After evaporating to dryness and reconstituting the residue, the radioactivity was determined and expressed as a percent of the radioactivity recovered after the Soxhlet extraction and as percent of the initial amount of radioactivity in the soil before Soxhlet extraction.

For each replicate the radioactivity recovered in the zones of the TLC plates was expressed as the percent of the amount applied on the plate, after the DPM detected in the background had been subtracted.

## 5.0 RESULTS

The recovered radioactivity in the $CO_2$ trap and soil as percent of the amount of radioactivity applied initially is presented for each soil type in Tables 2, 3 and 4. The major portion of the applied radioactivity was recovered in the soil. No radioactivity was detected in the volatile plugs after Soxhlet extraction. The maximum radioactivity recovered in the $CO_2$ trap was 5.5% (± 0.3) for CACY replicates. In ARSTLM and MASDLM 4.4% (± 0.6) and 2.4% (± 1.0) was recovered in the $CO_2$ traps, respectively. The amount of $^{14}CO_2$ evolved during the 64 days of the study is presented for each soil type in Figures 1, 2 and 3.

A complete radioactive mass balance for all MASDLM, ARSTLM and CACY replicates is provided in Tables 5, 6 and 7. The mean recoveries were approximately 80% for the MASDLM and CACY, and 60% for the ARSTLM replicates. The lower recovery for ARSTLM is probably due to inhomogeneity in the distribution of radioactivity and is evidenced by the large variability found for the concentration of material in soil. Table 6 shows the dpm per gram soil in sections C-4-a through C-4-c. Several of the combustion counts are low (below 20000 for the ARSTLM) and are most likely the cause of low recoveries.

The results of Soxhlet extraction for each soil type are presented in Table 8. Efficiency of the extraction varied for each soil type. For MASDLM and ARSTLM the recovered radioactivity was 52.4 to 86.1% and 45.5 to 48.6%, respectively. The lowest recoveries were found for the CACY replicates (10.1 to 18.6%). It should therefore be noted that the metabolite characterization of the CACY replicates on TLC plates may not represent the entire portion of the biodegradation products formed during the 64 days of the study. The high recovery of radioactivity for the MA2 replicate was probably due to incomplete mixing of the soil before combustion.

Results of TLC characterization of radioactivity extracted from soils indicated variable degradation rates of TBBPA. After 64 days of the study, the amount of TBBPA remaining in the soil was 74.3 to 81.9%, 35.9 to 40.1% and 41.1 to 43.2% for MASDLM, ARSTLM and CACY soils, respectively (Tables 9, 10 and 11). In all soil replicates two biodegradation products (Unknowns B and C) could be detected that resembled each other in mobility characteristics (Rf), but did not resemble the dimethyl or diethyl derivatives of TBBPA (Figures 4 and 5). In addition, a third biodegradation product, Unknown A could be detected in one replicate of ARSTLM soil. Figures 6, 7 and 8 depict in graphical form the TLC data.

## 6.O CONCLUSIONS

Based upon the results obtained, TBBPA is susceptible to biodegradation in soils under aerobic conditions using the procedures and methods employed in this study.

## Protocol Deviations

1. The temperature during the study varied from 20 to 25 °C (21.5 ± 1.0 °C). The protocol states that flasks are incubated at 22 ± 2 °C.

2. The protocol states that all test flasks will receive a polyurethane plug in the side arm. This was not provided for replicate 4 of Massachusetts Sandy Loam.

3. Individual and mean values for evolved $^{14}CO_2$ and $^{14}C$-volatile products have not been reported. The protocol states that these values will be reported for each sampling interval and at test termination, respectively.

It is our opinion that these deviations did not alter the interpretation of the results, or the conclusions drawn from this study.

SPRINGBORN LIFE SCIENCES, INC.

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## Quality Assurance Final Report Statement

The raw data and the 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 dates: 28 November to 2 December 1988 and 20 January 1989.

An in-life inspection was performed on 15 July 1988.

QAU inspection reports were issued to the Study Director on: 16 July 1988, 2 December 1988 and 2 January 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.

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**Table 1. Physical and Chemical Characteristics of Soils.**

| Soil Type | C.E.C.[a] | Organic Carbon[b] (%) | pH | F.M.C.[c] | Texture (%) Sand | Silty | Clay |
|---|---|---|---|---|---|---|---|
| Massachusetts Sandy Loam[d] | 10.8 | 4.4 | 7.0 | 74.8 | 83 | 13 | 4 |
| Arkansas Silty Loam[e] | 6.0 | 0.8 | 6.2 | 43.9 | 16 | 58 | 26 |
| California Clay Loam[e] | 19.6 | 1.8 | 7.6 | 75.9 | 43 | 24 | 33 |

[a] C.E.C. = Cation Exchange Capacity, meq/100 g.
[b] Percent organic carbon was calculated by dividing the percent organic matter by 1.7.
[c] F.M.C. = Field Moisture Capacity, %. Determined at Springborn Life Sciences, Inc.
[d] Characterization provided by A & L Easter Agricultural Laboratories, Inc., Richmond, VA
[e] Characterization provided by W. R. Landis Associates, Inc., Valdosta, GA

**Table 2. Distribution of Radioactivity in Massachusetts Sandy Loam Replicates as a Percent of Radioactivity Initially Applied.**

| Replicate | MA1 | MA2 | MA3 | MA4 |
|---|---|---|---|---|
| Recovery Soil | 79.0 | 60.4 | 89.1 | 75.9 |
| Recovery Volatile Traps | 1.4 | 1.7 | 2.7 | 3.6 |
| Total Recovery | 80.4 | 62.1 | 91.8 | 79.5 |
| Mean ± S.D. | | 78.5 ± 12.3 | | |

**Table 3. Distribution of Radioactivity in Arkansas Silty Loam Replicates as a Percent of Radioactivity Initially Applied.**

| Replicate | ARK1 | ARK2 | ARK3 | ARK4 |
|---|---|---|---|---|
| Recovery Soil | 62.4 | 50.0 | 54.2 | 54.1 |
| Recovery Volatile Traps | 3.7 | 4.3 | 4.6 | 5.1 |
| Total Recovery | 67.4 | 54.3 | 58.9[a] | 59.2 |
| Mean ± S.D. | | | 59.9 ± 5.5 | |

[a] Value of total recovery does not correspond with the sum of values of soil and volatile trap due to rounding.

**Table 4. Distribution of Radioactivity in California Clay Replicates as a Percent of Radioactivity Initially Applied.**

| Replicate | CAL1 | CAL2 | CAL3 | CAL4 |
|---|---|---|---|---|
| Recovery Soil | 65.6 | 69.4 | 95.3 | 72.3 |
| Recovery Volatile Traps | 5.9 | 5.4 | 5.4 | 5.3 |
| Total Recovery | 71.5 | 74.8 | 100.7 | 77.6 |
| Mean ± S.D. | | | 81.2 ± 13.3 | |

**Table 5. Radioactivity Mass Balance of Tetrabromobisphenol A in Massachusetts Sandy Loam.**

| Test Replicate | MA1 | MA2 | MA3 | MA4 |
|---|---|---|---|---|
| A. DPM initially added[a] | 2320851 | 2320851 | 2320851 | 2320851 |
| B. Total cumulative DPM of $CO_2$ and volatile traps from last sampling date | 33472 | 39837 | 61836 | 83630 |
| C. DPM in Combusted Soil | | | | |
| 1) Weight of soil in flask (g) | 49.82 | 49.86 | 49.85 | 49.82 |
| 2) a. g dry of Aliquot 1 | 0.2740 | 0.3531 | 0.2924 | 0.3934 |
| b. g dry of Aliquot 2 | 0.3244 | 0.3356 | 0.3812 | 0.3551 |
| c. g dry of Aliquot 3 | 0.3653 | 0.3164 | 0.3042 | 0.2809 |
| 3) a. DPM of Aliquot 1 | 9974.682 | 10509.57 | 10971.67 | 14096.21 |
| b. DPM of Aliquot 2 | 12565.95 | 8572.01 | 12394.90 | 12153.01 |
| c. DPM of Aliquot 3 | 12874.58 | 9203.37 | 16557.77 | 10127.69 |
| 4) DPM/g | | | | |
| a. Aliquot 1 (3a/2a) | 36403.95 | 29763.72 | 37522.81 | 35831.75 |
| b. Aliquot 2 (3b/2b) | 38735.97 | 25542.43 | 32515.48 | 34224.19 |
| c. Aliquot 3 (3c/2c) | 35243.85 | 29087.76 | 54430.54 | 36054.43 |
| 5) Mean DPM/g (4a + 4b + 4c)/3 | 36794.59 | 28131.27 | 41489.61 | 35370.12 |
| 6) Total DPM in soil (C5 X C1) | 1833106 | 1402625 | 2068257 | 1762139 |
| D. Mass Balance (as percent) $\frac{(B + C6) \times 100\%}{A}$ | 80.4 | 62.1 | 91.8 | 79.5 |
| Mean ± S.D. | | 78.5 ± 12.3 | | |

[a] This value is the mean of three independent spikes quantitated by LSC.

**Table 6. Radioactivity Mass Balance of Tetrabromobisphenol A in Arkansas Silty Loam.**

| Test Replicate | ARK1 | ARK2 | ARK3 | ARK4 |
|---|---|---|---|---|
| A. DPM initially added[a] | 2320851 | 2320851 | 2320851 | 2320851 |
| B. Total cumulative DPM of $CO_2$ and volatile traps from last sampling date | 85833 | 99525 | 107837 | 118074 |
| C. DPM in Combusted Soil | | | | |
| 1) Weight of soil in flask (g) | 46.71 | 51.06 | 51.06 | 44.09 |
| 2) a. g dry of Aliquot 1 | 0.2765 | 0.3746 | 0.3161 | 0.2746 |
| b. g dry of Aliquot 2 | 0.2598 | 0.3653 | 0.3014 | 0.3073 |
| c. g dry of Aliquot 3 | 0.3426 | 0.3069 | 0.3823 | 0.3494 |
| 3) a. DPM of Aliquot 1 | 12693.74 | 11569.73 | 5165.367 | 6808.085 |
| b. DPM of Aliquot 2 | 8160.129 | 6412.543 | 11386.83 | 9929.754 |
| c. DPM of Aliquot 3 | 6031.371 | 6037.830 | 7583.331 | 9912.987 |
| 4) DPM/g | | | | |
| a. Aliquot 1 (3a/2a) | 45908.64 | 30885.56 | 16340.927 | 24792.735 |
| b. Aliquot 2 (3b/2b) | 31409.27 | 17554.182 | 37779.79 | 32312.899 |
| c. Aliquot 3 (3c/2c) | 17604.702 | 19673.607 | 19836.074 | 28371.457 |
| 5) Mean DPM/g (4a + 4b + 4c)/3 | 31640.87 | 22704.45 | 24652.26 | 28492.36 |
| 6) Total DPM in soil (C5 X C1) | 1477945 | 1159289 | 1258744 | 1256228 |
| D. Mass Balance (as percent) $\frac{(B + C6) \times 100\%}{A}$ | 67.4 | 54.2 | 58.9 | 59.2 |
| Mean ± S.D. | | 59.9 ± 5.5 | | |

[a] This value is the mean of three independent spikes quantitated by LSC.

**Table 7. Radioactivity Mass Balance of Tetrabromobisphenol A in California Clay Loam.**

| Test Replicate | CAL1 | CAL2 | CAL3 | CAL4 |
|---|---|---|---|---|
| A. DPM initially added[a] | 2320851 | 2320851 | 2320851 | 2320851 |
| B. Total cumulative DPM of $CO_2$ and volatile traps from last sampling date | 137279 | 125637 | 124774 | 122013 |
| C. DPM in Combusted Soil | | | | |
| 1) Weight of soil in flask (g) | 51.47 | 51.47 | 51.32 | 51.50 |
| 2) a. g dry of Aliquot 1 | 0.3077 | 0.3093 | 0.3988 | 0.3569 |
| b. g dry of Aliquot 2 | 0.3542 | 0.4068 | 0.4185 | 0.4827 |
| c. g dry of Aliquot 3 | 0.4706 | 0.3774 | 0.4280 | 0.4179 |
| 3) a. DPM of Aliquot 1 | 8468.148 | 12659.61 | 9213.058 | 10986.28 |
| b. DPM of Aliquot 2 | 8156.165 | 10047.18 | 26481.63 | 11288.35 |
| c. DPM of Aliquot 3 | 17979.62 | 10660.05 | 18403.38 | 18219.43 |
| 4) DPM/g | | | | |
| a. Aliquot 1 (3a/2a) | 27520.793 | 40929.87 | 23101.951 | 30782.52 |
| b. Aliquot 2 (3b/2b) | 23027.005 | 24698.08 | 63277.49 | 23385.85 |
| c. Aliquot 3 (3c/2c) | 38205.74 | 28246.03 | 42998.55 | 43597.58 |
| 5) Mean DPM/g (4a + 4b + 4c)/3 | 29584.51 | 31291.33 | 43125.99 | 32588.65 |
| 6) Total DPM in soil (C5 X C1) | 1522715 | 1610565 | 2213226 | 1678315 |
| D. Mass Balance (as percent) $\frac{(B + C6) \times 100\%}{A}$ | 71.5 | 74.8 | 100.7 | 77.6 |
| Mean ± S.D. | | 81.2 ± 13.3 | | |

[a] This value is the mean of three independent spikes quantitated by LSC.

**Table 8. Mass Balance of Soxhlet Extractions.**

| Replicate | MA2 | MA3 | ARK2 | ARK3 | CAL2 | CAL3 |
|---|---|---|---|---|---|---|
| A. Pre-Soxhlet | | | | | | |
| Dry g soil | 15.6059 | 15.9527 | 17.5256 | 18.7658 | 14.5409 | 16.9936 |
| Mean DPM/g | 28131.27 | 41489.61 | 22704.45 | 24652.26 | 31291.33 | 43125.99 |
| Total DPM | 439014 | 661871 | 397909 | 462619 | 455004 | 732866 |
| B. Post-Soxhlet Recoveries: | | | | | | |
| I. Acetone | | | | | | |
| Volume (mL) | 144 | 150 | 164 | 150 | 172 | 198 |
| Mean DPM/mL | 2828.365 | 2452.597 | 1178.986 | 1602.022 | 522.206 | 401.563 |
| Total DPM | 407285 | 367890 | 193354 | 240303 | 89819 | 79509 |
| Percent[b] | 92.8 | 55.6 | 48.6 | 51.9 | 19.7 | 10.8 |
| Mean % | 74.2 | | 50.3 | | 15.3 | |
| II. Soil | | | | | | |
| Mean DPM/g | 26478 | 22198 | 17910 | 14755 | 21908 | 22354 |
| Total DPM | 413213 | 354118 | 313883 | 276889 | 318562 | 379875 |
| Percent[b] | 94.1 | 53.5 | 78.9 | 59.8 | 70.0 | 51.8 |
| C. Post-Rotorvap | | | | | | |
| DPM/5 mL | 377843 | 346757 | 179390 | 223653 | 84370 | 73797 |
| Total DPM [a] | 378071 | 346952 | 181091 | 224788 | 84586 | 73931 |
| Percent[c] | 92.8 | 94.3 | 93.7 | 93.5 | 94.2 | 93.0 |
| Percent[b] | 86.1 | 52.4 | 45.5 | 48.6 | 18.6 | 10.1 |
| Mean | 69.3 | | 47.1 | | 14.4 | |

[a] Total DPM = DPM/5 mL + DPM remaining in roundbottom flask.
[b] Percent of Total DPM Pre-Soxhlet
[c] Percent of Total DPM from Acetone Post-Soxhlet

**Table 9. Percentages[a] of radioactivity recovered from the TLC plates after Soxhlet extraction of Massachusetts Sandy Loam soil.**

| Replicate | MA-2 | MA-3 |
|---|---|---|
| Origin | 12.3 | 3.5 |
| Low Rf[b] | 3.0 | 1.8 |
| Unknown A | ND[f] | ND[f] |
| Unknown B | 4.2 | 6.5 |
| TBBPA | 74.3 | 81.9 |
| Unknown C | 6.2 | 5.7 |
| Derivatives[c] | 0.0 | 0.5 |
| High Rf[d] | 0.0 | 0.2 |
| DPM Rec.[e] | 6593 | 6287 |
| DPM Appl. | 7543 | 6935 |
| % Rec. | 87 | 91 |

[a]DPM per zone(s) / Total DPM recovered X 100%.
[b]Low Rf-refers to zone(s) between origin and Unknown A.
[c]Derivatives refer to dimethyl and diethyl TBBPA.
[d]High Rf-refers to zone(s) above TBBPA dimethyl and diethyl derivatives.
[e]DPM Recovered
[f]Not Detected, Radioactivity attributed to low Rf.

**Table 10. Percentages[a] of radioactivity recovered from the TLC plates after Soxhlet extraction of Arkansas Silty Loam soil.**

| Replicate | ARK-2 | ARK-3 |
|---|---|---|
| Origin | 28.3 | 27.4 |
| Low Rf[b] | 22.0 | 19.2 |
| Unknown A | ND[f] | 7.0 |
| Unknown B | 8.2 | 7.4 |
| TBBPA | 40.1 | 35.9 |
| Unknown C | 1.5 | 3.1 |
| Derivatives[c] | 0.0 | 0.0 |
| High Rf[d] | 0.0 | 0.0 |
| DPM Rec.[e] | 2948 | 3667 |
| DPM Appl. | 3588 | 4473 |
| % Rec. | 82 | 82 |

[a]DPM per zone(s) / Total DPM recovered X 100%.
[b]Low Rf-refers to zone(s) between origin and Unknown A.
[c]Derivatives refer to dimethyl and diethyl TBBPA.
[d]High Rf-refers to zone(s) above TBBPA dimethyl and diethyl derivatives.
[e]DPM Recovered
[f]Not Detected, Radioactivity attributed to low Rf.

**Table 11. Percentages[a] of radioactivity recovered from the TLC plates after Soxhlet extraction of California Clay soil.**

| Replicate | CAL-2 | CAL-3 |
|---|---|---|
| Origin | 18.7 | 18.0 |
| Low Rf[b] | 15.1 | 13.3 |
| Unknown A | ND[f] | ND[f] |
| Unknown B | 11.0 | 11.0 |
| TBBPA | 43.2 | 41.1 |
| Unknown C | 8.8 | 16.2 |
| Derivatives[c] | 1.3 | 0.4 |
| High Rf[d] | 1.9 | 0.0 |
| DPM Rec.[e] | 1243 | 1248 |
| DPM Appl. | 1476 | 1687 |
| % Rec. | 84 | 74 |

[a] DPM per zone(s) / Total DPM recovered X 100%.
[b] Low Rf-refers to zone(s) between origin and Unknown A.
[c] Derivatives refer to dimethyl and diethyl TBBPA.
[d] High Rf-refers to zone(s) above TBBPA dimethyl and diethyl derivatives.
[e] DPM Recovered
[f] Not Detected, Radioactivity attributed to low Rf.

Figure 1. The amount of $^{14}CO_2$ produced in the Massachusetts Sandy Loam as a function of time based on the total applied DPM per flask.

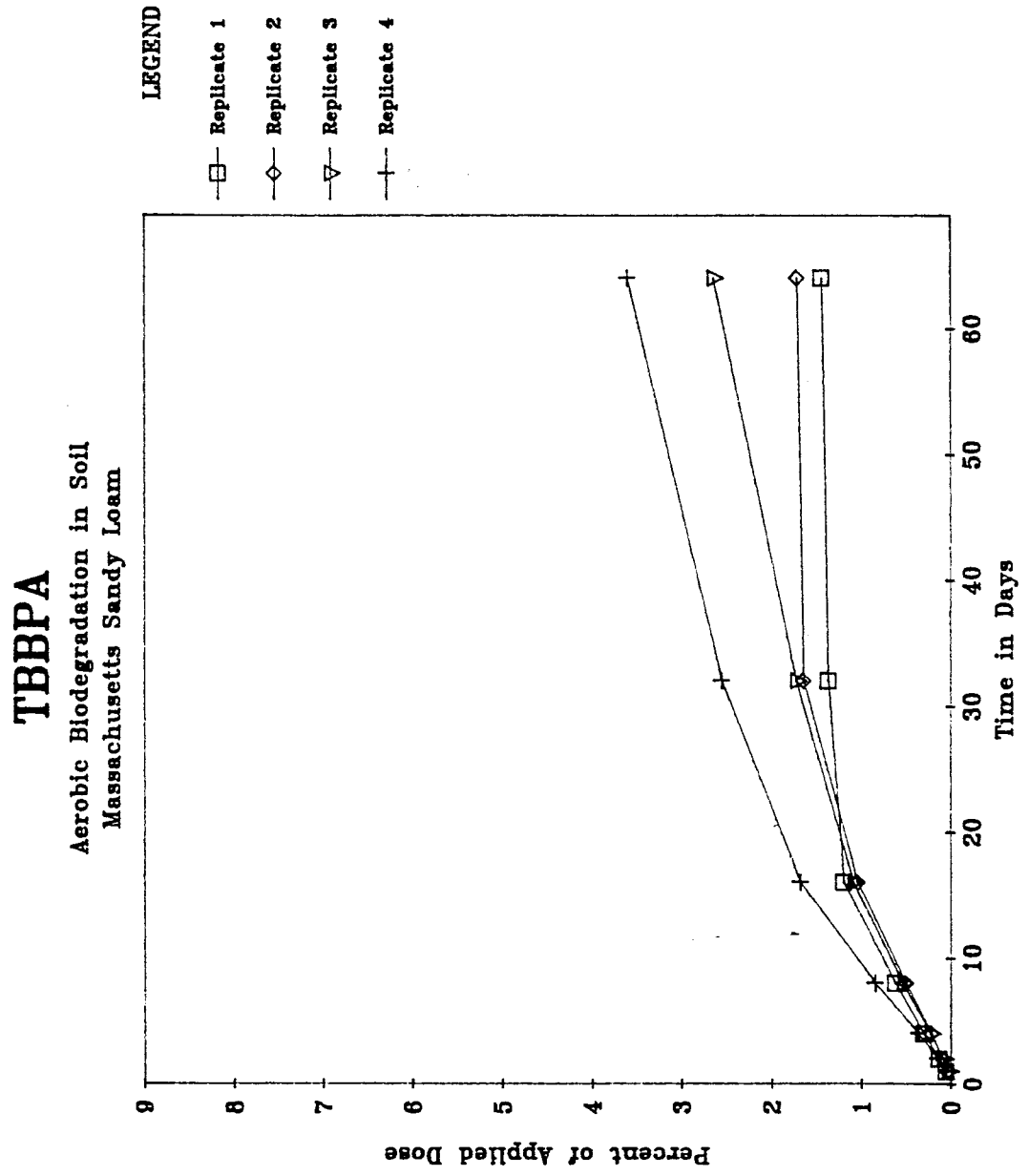


Figure 2. The amount of $^{14}CO_2$ produced in the Arkansas Silty Loam as a function of time based on the total applied DPM per flask.

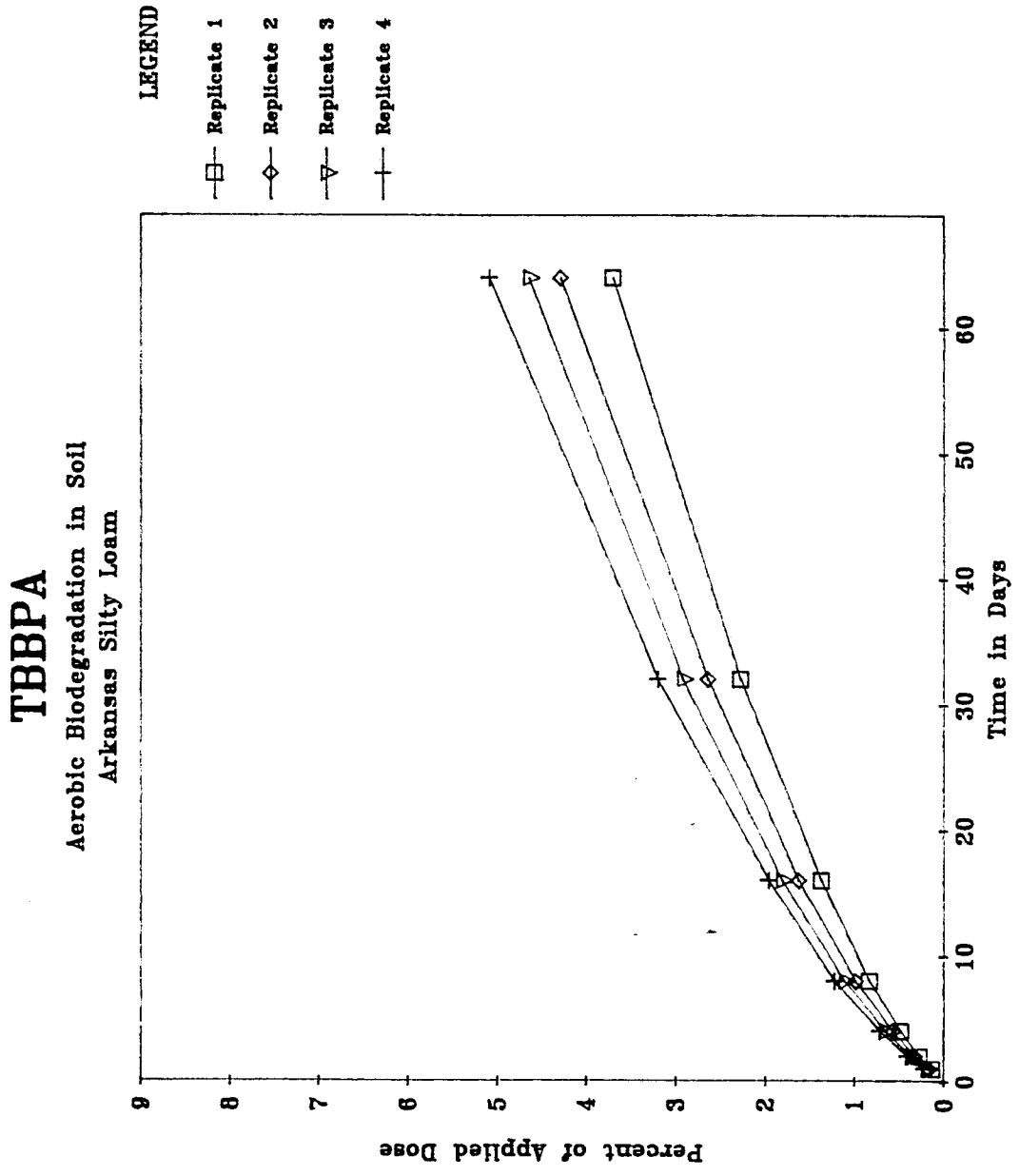


Figure 3. The amount of $^{14}CO_2$ produced in the California Clay as a function of time based on the total applied DPM per flask.

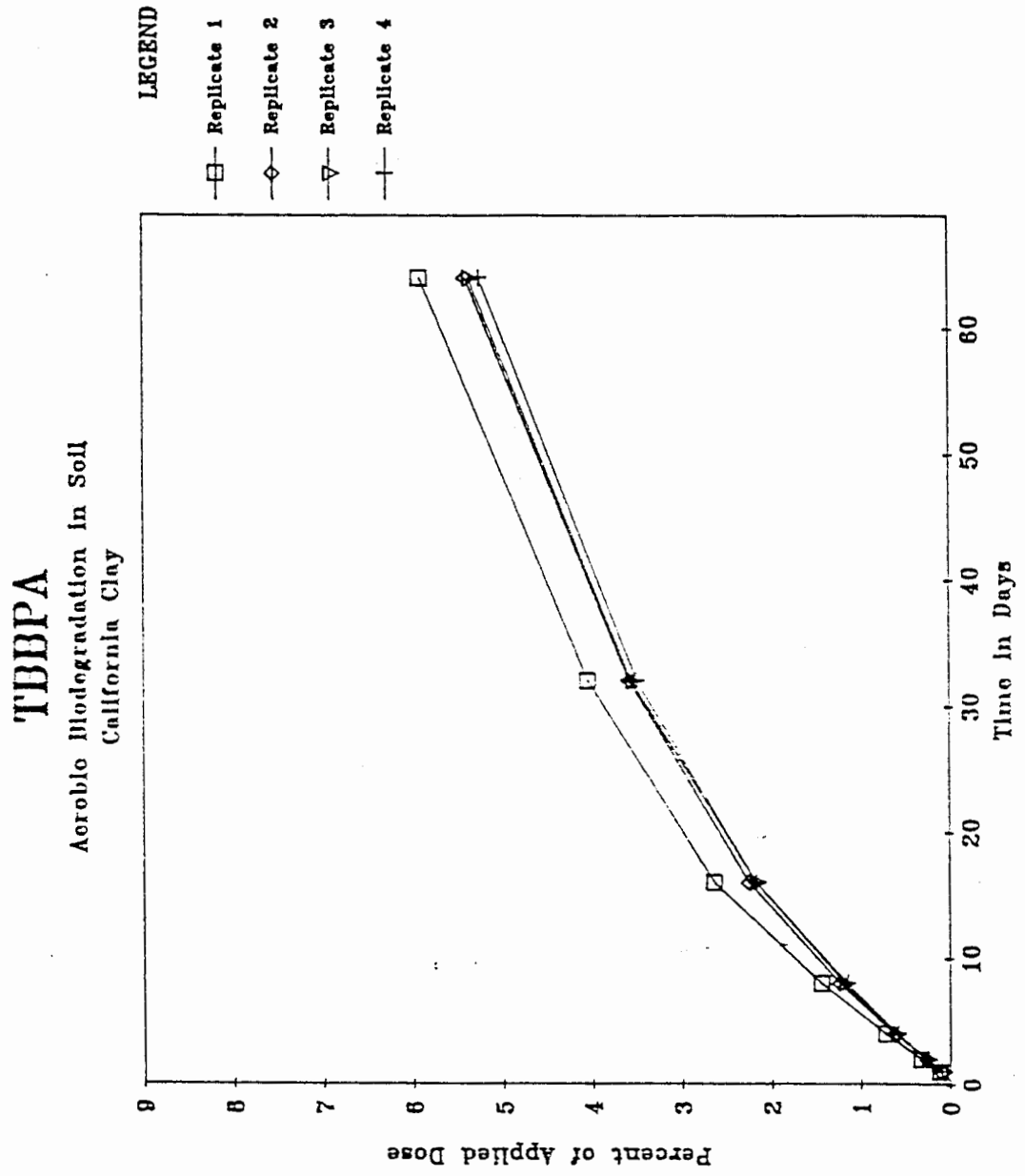


**Figure 4.** **Copy of Autoradiograph produced from the soil extracts of MASDLM and ARSTLM.**

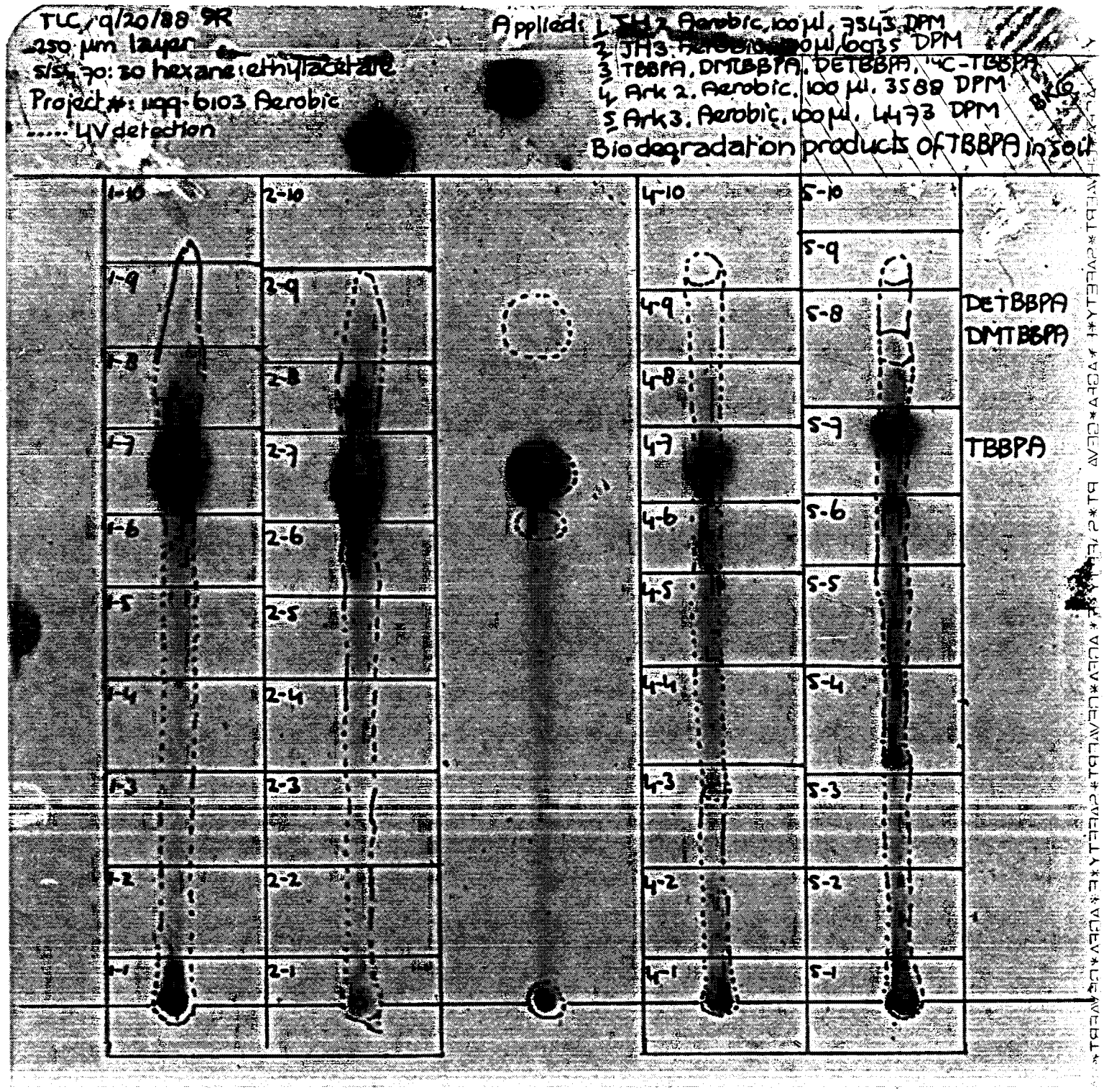


**Figure 5. Copy of Autoradiograph produced from the soil extracts of CACY.**

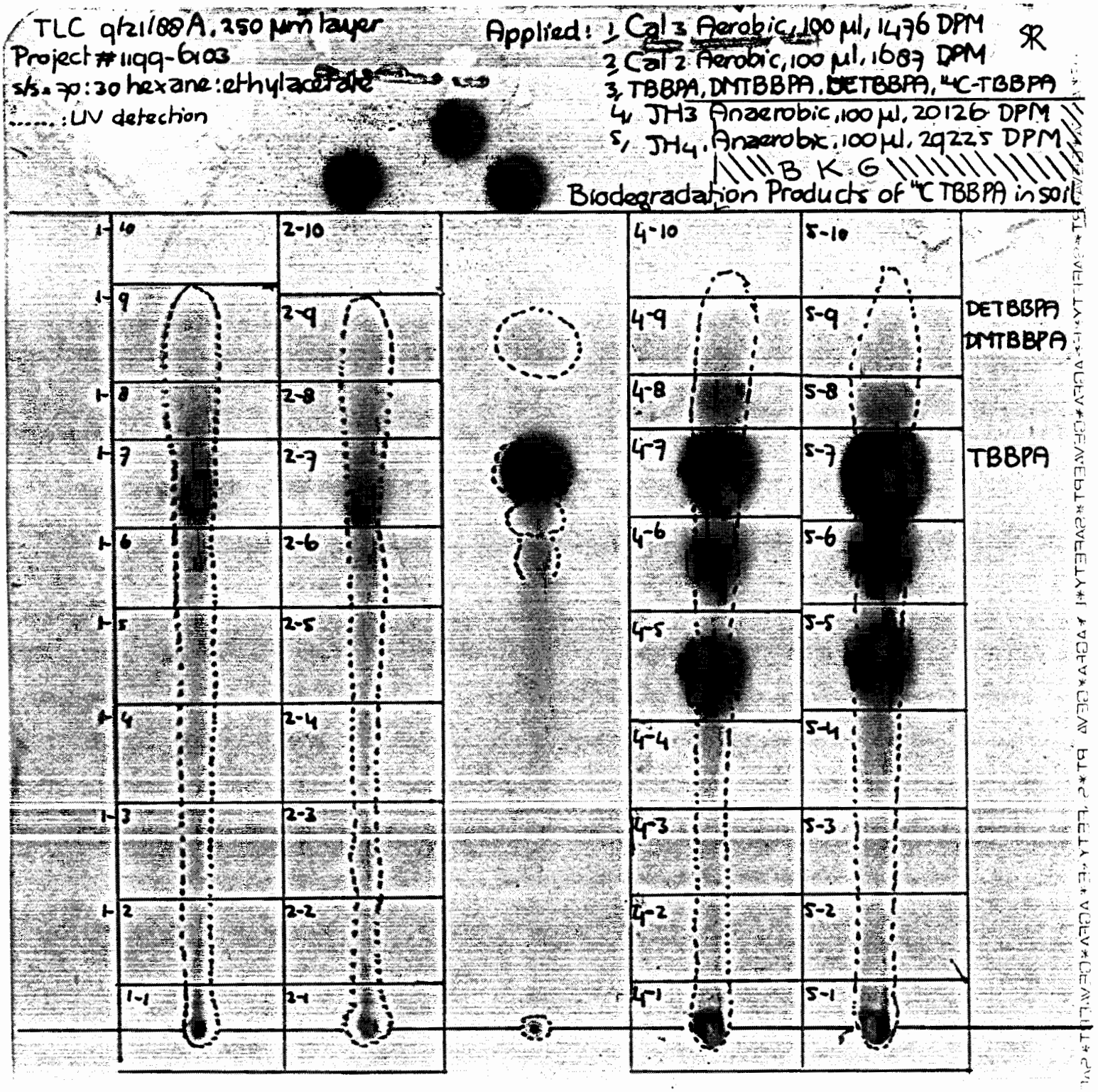


Figure 6. Distribution of radioactive constituents in the soil extracts from Massachusetts Sandy Loam.

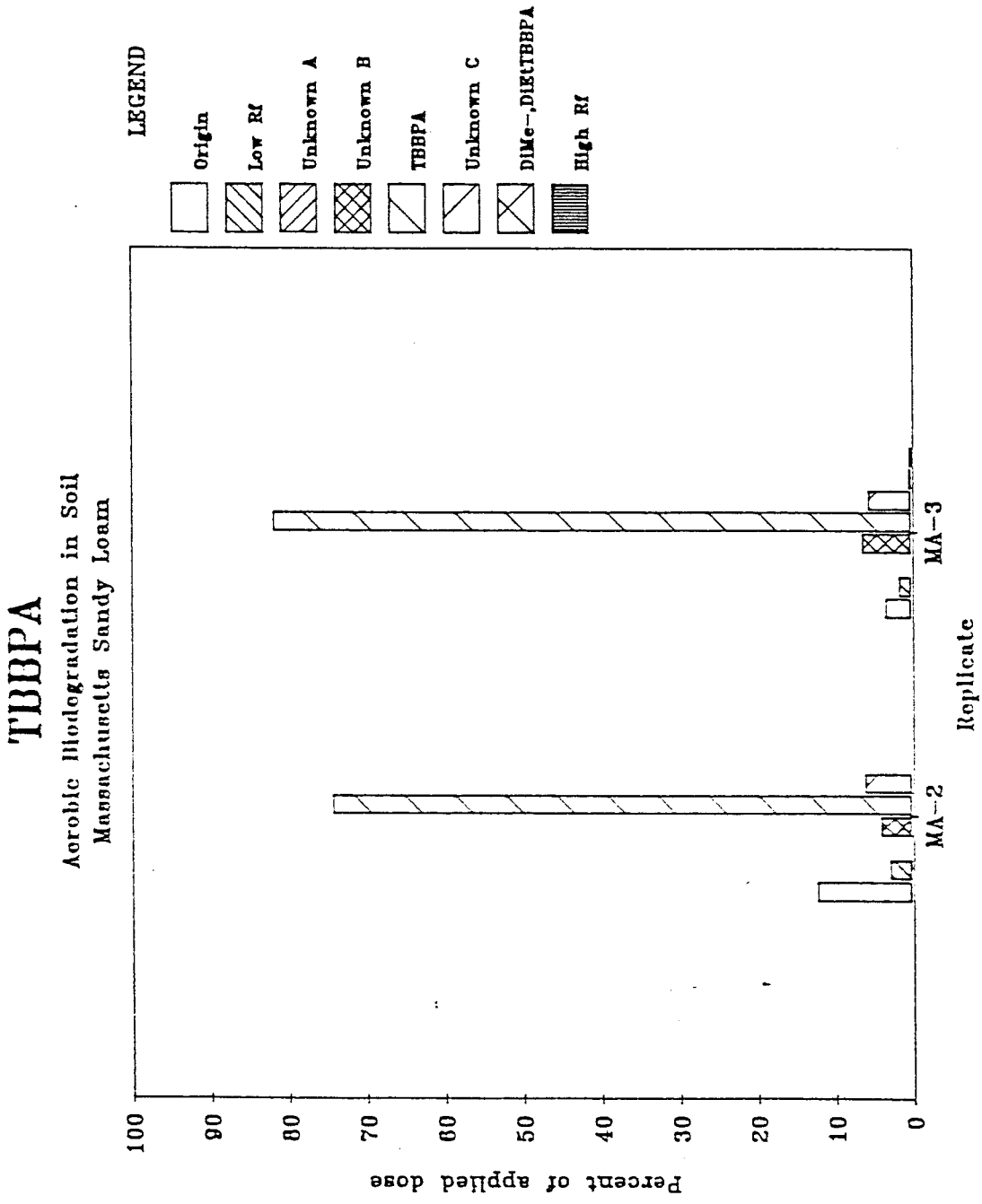


Figure 7. Distribution of radioactive constituents in the soil extracts from Arkansas Silty Loam.

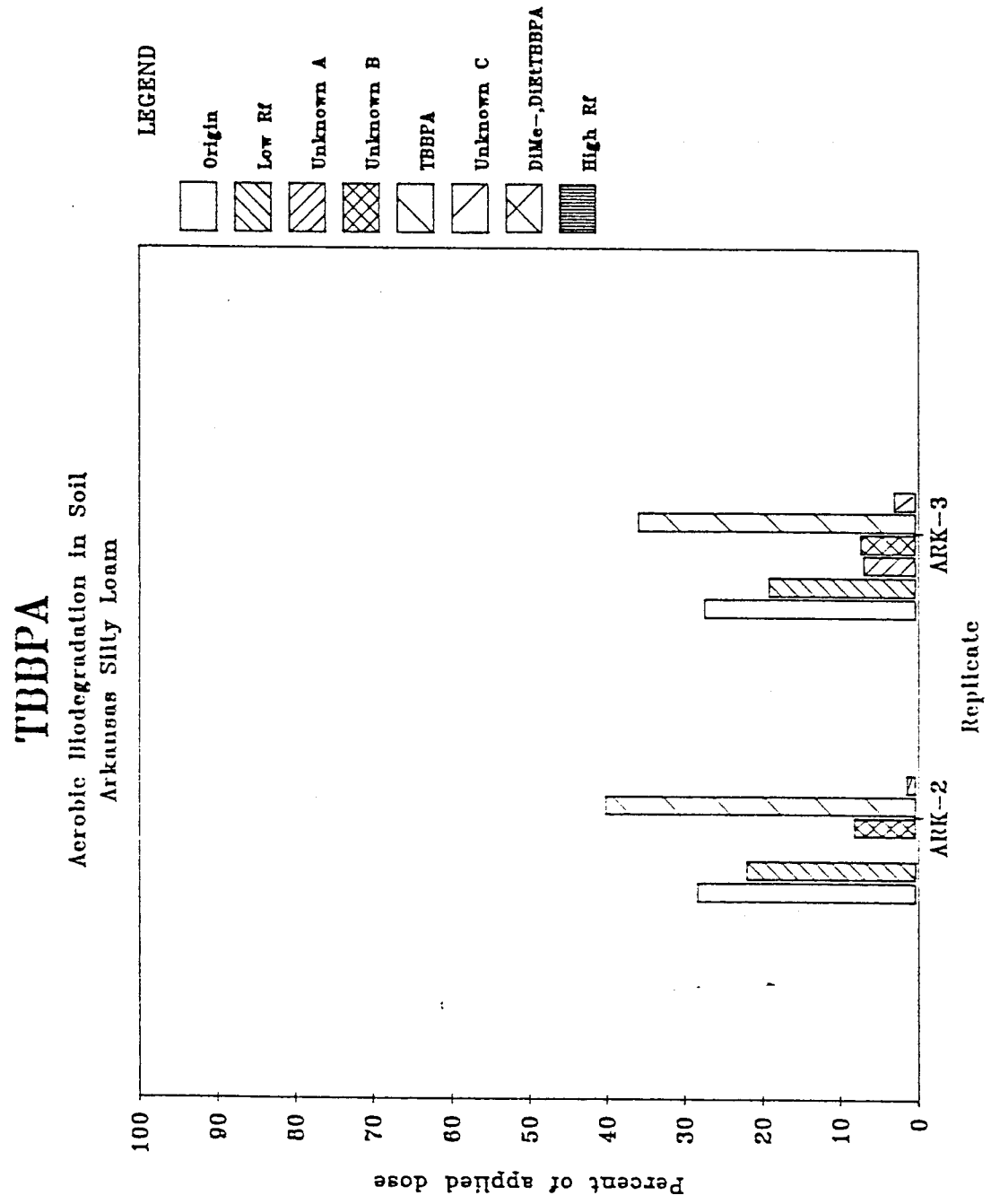
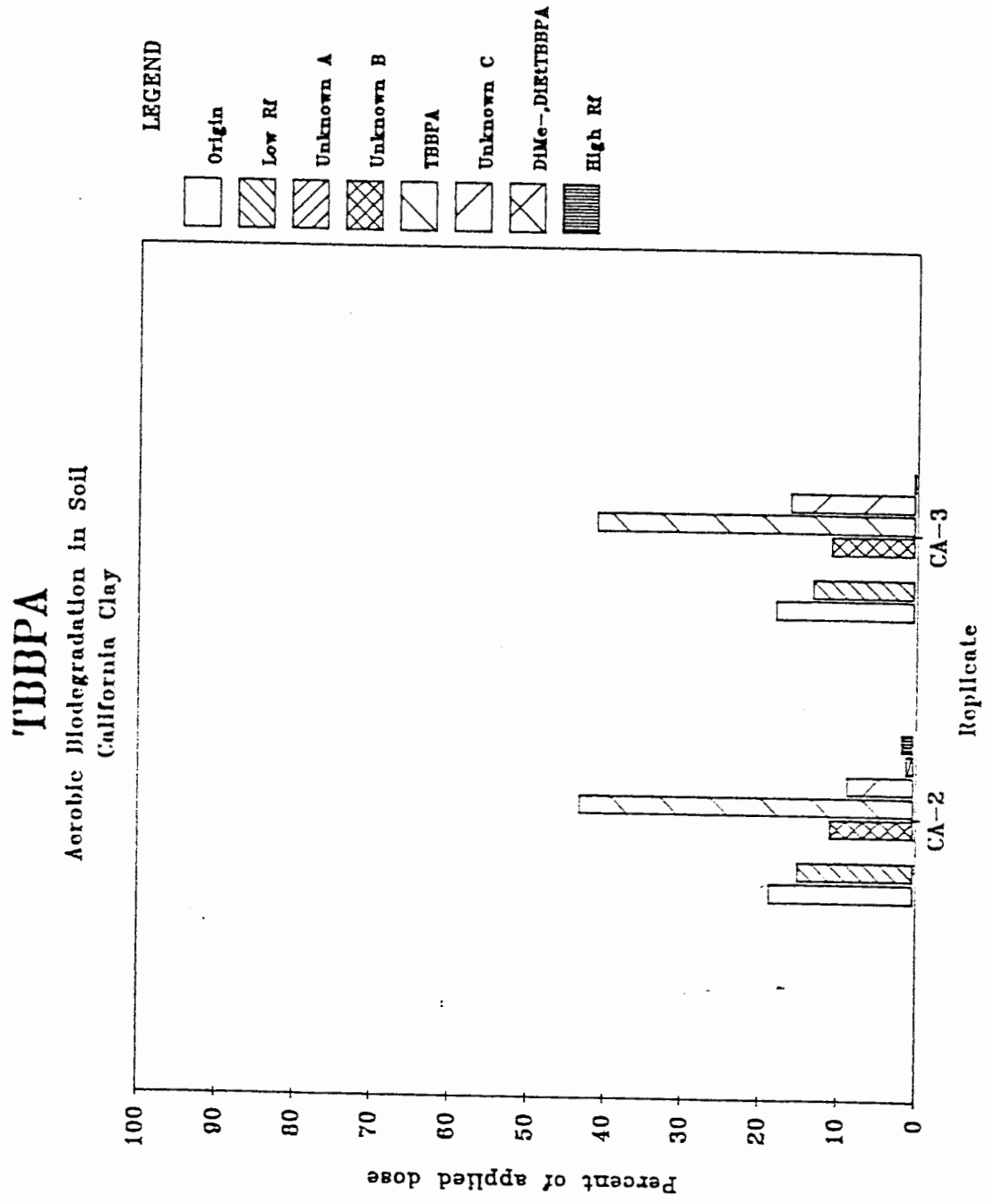


Figure 8. Distribution of radioactive constituents in the soil extracts from California Clay.



# APPENDIX I

(Study Protocol)

**Springborn Life Sciences, Inc.**
**Environmental Toxicology & Chemistry Division**
790 Main Street • Wareham, Massachusetts 02571 • (617) 295-2550 • Telex 4436041 • Facsimile (203) 749-7533

## STUDY PLAN AND TEST PROTOCOL

**PROTOCOL TITLE: Protocol for Determining the Inherent Biodegradability in Soil under Aerobic Conditions.**

**TO BE COMPLETED BY THE STUDY SPONSOR:**

Study Sponsor: Brominated Flame Retardant Industry Panel
Represented By: Great Lakes Chemical Corporation, Highway 52, N.W.
West Lafayette, IN 47906 Telephone: 317+463-2511
Sponsor Representative: Dennis L. McFadden, Ph.D.
Test Substance: Tetrabromobisphenol A
Purity: _____ CAS# or LOT#: _____
Additional Comments and/or Modifications: _____

Dennis L. McFadden, 6/15/88
Sponsor Approval Date

**TO BE COMPLETED BY SLS BEFORE TEST INITIATION:**

Testing Facility: Springborn Life Sciences, Inc.
Study Director: John P. Martinson
SLS Project No.: 1199-6103
Test Concentrations: 0.50mg/50g
Solvent: _____ CAS# or LOT#: _____
Proposed Start Date of Study: _____
Proposed Completion Date of Study: _____
Additional Comments and/or Modifications: _____

John P. Martinson, 6-27-88
Study Director Date

# DETERMINATION OF INHERENT BIODEGRADABILITY IN SOIL UNDER AEROBIC CONDITIONS

## SUMMARY

The $^{14}C$-radiolabeled test material is incorporated into 50 g of soil and incubated aerobically in a biometer flask. Release of $^{14}CO_2$ is trapped by alkali absorption and periodically measured by liquid scintillation counting. Volatile parent or degradation products are quantitated at the point of 50 % degradation or at test termination by extraction of a volatile trapping plug of polyurethane. At the point of 50 % degradation or at test termination the soil is extracted to determine the percentage of bound test material or metabolic products. The amount of $^{14}CO_2$ recovered, as a percentage of the $^{14}C$-test material initially applied to the soil, is plotted versus time.

## MATERIALS AND METHODS

A. Soil- Three soil types are used:

(a) Alfisol: pH 5.5-6.5, organic carbon content (OC) 1-1.5%, clay content (CC) 10-20%, cation exchange capacity (CEC) 10-15 mval.

(b) Spodosol: pH 4.0-5.0, OC 1.5-3.5%, CC ≤ 10%, CEC < 10mval.;

(c) Entisol: pH 6.6-8.0, OC 1-4%, CC 11-25%, CEC >10 mval.

Prior to testing, each of three types of soils are characterized to establish soil type, organic carbon content, pH, cation exchange capacity, field moisture capacity and texture (% sand, % silt and % clay). Soil is sieved through a 2 mm mesh screen to assure uniformity, and maintained at 40 % of field moisture capacity for 2 weeks at 22 ± 2 °C prior to test initiation.

B. Test Vessels- The test vessel is a biometer flask consisting of a 250 mL Erlenmeyer flask to which a 50 mL round bottom glass tube is fused (Figure 1). The biometer flask is charged by injecting 10 mL of 0.1 N KOH from a calibrated syringe into the glass tube. If requested, a polyurethane plug is placed into the side arm of the biometer flask to trap volatile products.

C. Soil Treatment- Soil, 50 g dry weight, is placed into the Erlenmeyer flask. The radiolabeled test material (100 µL) is delivered from a calibrated syringe over the surface of the soil. The soil is then mixed with a glass Pasteur pipette (from which the lower part is removed and left in the flask).

D. Aerobic Incubation- Immediately after soil replicates (4) are treated with the test material the biometer flasks are sealed and placed in an environmental chamber. Flasks are incubated in the dark at 22 ± 2 °C.

E. Sampling and Analysis- Sampling and analysis of the $^{14}CO_2$ trapping solution is performed on Days 1, 2, 4, 8, 16, 32 and, if necessary, 64. At each sampling interval the trapping solution is removed via syringe and the side tube is rinsed with 5.0 mL of 0.1 N KOH. Duplicate 1.0 mL aliquots of the trapping solution, with rinse, are quantitated via liquid scintillation counting (LSC) to determine total $^{14}CO_2$ evolved. Prior to recharging the side tube with fresh trapping solution, three 25 mL volumes of air are drawn through the test vessel to maintain the soil in an aerobic state.

Analysis of $^{14}C$-volatile material absorbed onto the polyurethane foam plug is performed at the point of 50 % degradation or at test termination (64 days maximum). The polyurethane plug is removed and extracted with a n-hexane:methanol solution (1:4) using a Soxhlet apparatus. Duplicate 1.0 mL aliquots of the extract are quantitated by LSC.

If requested, at the point of 50 % degradation or at test termination, the soil from duplicate biometer flasks may be extracted by appropriate methods (reflux, soxhlet or shaker method) with 100 mL acetone (or other suitable solvent recommended by the Sponsor). The extractable and non-extractable proportions of the test material are determined by direct LSC and combustion radioassay, respectively. Soil is oxidized in a Packard Model 306 Tri-Carb Oxidizer and the $^{14}CO_2$ trapped in a mixture of Carbosorb and scintillation cocktail.

All quantitation of $^{14}C$-radioactivity is performed utilizing a Beckman LS-1801 Liquid Scintillation Counter calibrated with factory standards.

## CALCULATIONS

The percent biodegradation is calculated at each sampling interval by dividing the total $^{14}CO_2$ recovered in the trapping solution by the total $^{14}C$-radioactivity added and multiplying by 100. A complete material balance is provided at test termination and expressed as a percentage of the applied radioactivity.

## RESULTS

Individual and mean values for evolved $^{14}CO_2$ are reported for each sampling interval along with cumulative values. At the 50 % degradation point or at test termination, individual and mean values for trapped $^{14}C$-volatile products and extractable and non-extractable residue values are reported along with a complete material balance.

## REPORTING

The report will be a typed document, submitted in triplicate, describing the results of the study, and will be signed by the Study Director and Quality Assurance Unit. It will include, but not be limited to, the following:

1) Dates on which the study began and ended.

2) Name and address of the testing laboratory.

3) Location where the test was performed.

4) Name(s) of principle investigator(s).

5) Signatures of the senior scientific personnel responsible for the study.

6) A full description of the experimental design and procedures followed and a description of the test equipment used.

7) Identification of the test substance including chemical name and percentage of active ingredient, molecular structure, location of the radiolabel and qualitative and quantitative descriptions of the chemical composition (Sponsor supplied).

8) Manufacturer and lot and sample numbers of the test substance.

9) Properties of the test substance including physical state, pH, and stability (Sponsor supplied).

10) The principle mathematical equations used in generating and analyzing the data as well as calculations using these equations. Tabular and graphical representations of the data.

11) Soil characteristics.

12) Data evaluation and conclusions.

13) Dates of Quality Assurance audits, data inspections, and certification of report approval.

14) Location of raw data and report.

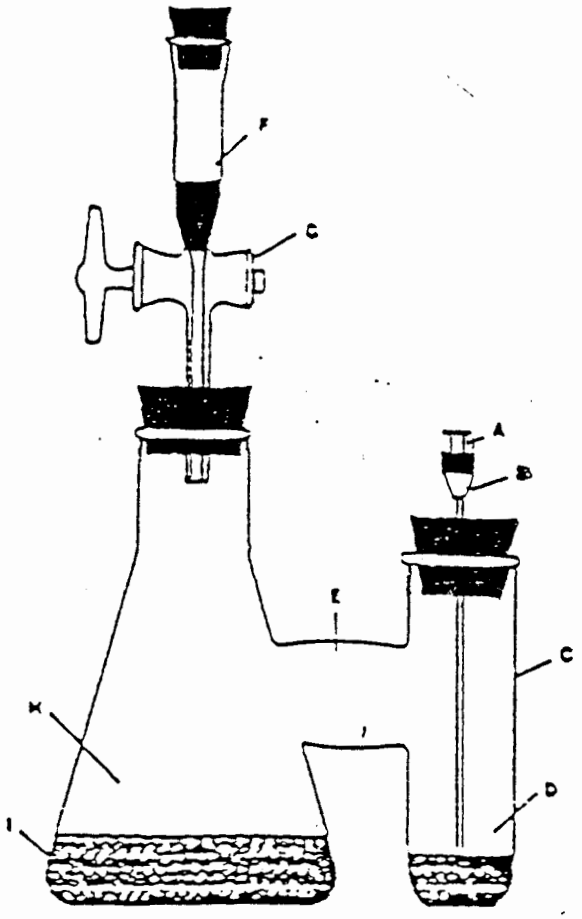
15) A complete description of any protocol deviations and the impact expected.

## SPECIAL PROVISIONS

GOOD LABORATORY PRACTICES (GLP): All test procedures, documentation, records, and reports will comply with the U. S.Environmental Protection Agency's Good Laboratory Practices as promulgated under the Toxic Substances Control Act (FEDERAL REGISTER, Part III, 29 November 1983).

TEST MATERIAL DISPOSAL: After 60 days of the issuance of the final report, the test material will be returned to the Sponsor's project officer, at Sponsor expense, unless different arrangements are made.

FIGURE 1. SCHEMATIC PRESENTATION OF BIOMETER FLASK




A. Small Stopper
B. Needle
C. Side Tube
D. Alkali Trap (0.1 N KOH)
E. Side Arm
F. Ascarite Filter
G. Stopcock
H. 250-mL Erlenmeyer Flask
I. Soil

AMENDMENT TO
STUDY PLAN AND TEST PROTOCOL

PROTOCOL TITLE AND NUMBER: Protocol for Determining the Inherent Biodegradability in Soil under Aerobic Conditions.

No 012988/304A.BFRIP

SLS PROJECT NUMBER: 1199-6103 AMENDMENT NUMBER: 1

SPONSOR: Brominated Flame Retardant Industry Panel

TEST MATERIAL: Tetrabromobisphenol A

PROTOCOL MODIFICATIONS AND/OR ADDITIONS

1. Protocol Modification: The protocol states that the following soil types with the specified characteristics will be used for the study:

Alfisol: pH 5.5-6.5, organic carbon content (OC) 1-1.5 %, clay content (CC) 10-20 %, cation exchange capacity (CEC) 10-15 mval.

Spodosol: pH 4.0-5.0, OC 1.5-3.5 %, CC $\leq$ 10 %, CEC < 10 mval.

Entisol: pH 6.6-8.0, OC 1-4 %, CC 11-25 %, CEC > 10 mval.

The following soils with the specified characteristics were actually selected for use:

Loamy Sand: pH 7.0, OC 4.4 %, CC 4 %, CEC 10.8 meq/100 g.

Silt Loam: pH 6.2, OC 0.8 %, CC 26.0 %, CEC 6.0 meq/100 g.

Clay Loam: pH 7.6, OC 1.8 %, CC 33.0 % CEC 19.6 meq/100 g.

Reason for change: Soils were selected from the available inventories. Selection was based upon using soils with a wide range of the above characteristics.

APPROVAL SIGNATURES:

Sponsor Representative Dennis L. McFadden Date 8/30/88

SLS Study Director John L. Martinson Date 8-29-88

**Springborn Life Sciences, Inc.**
Environmental Toxicology & Chemistry Division
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AMENDMENT TO
STUDY PLAN AND TEST PROTOCOL

PROTOCOL TITLE & NUMBER: **Protocol for Determining the Inherent Biodegradability in Soil under Aerobic Conditions. No. 012988/304A-AN.BFRIP**

SLS PROJECT NUMBER: 1199-1287-6103-760 AMENDMENT NUMBER: 2

SPONSOR: Brominated Flame Retardant Industry Panel

TEST MATERIAL: Tetrabromobisphenol A (TBBPA)

PROTOCOL MODIFICATIONS AND/OR ADDITIONS:

1. Study Director:

Change from John P. Martinson to Paul H. Fackler, Ph.D.

APPROVAL SIGNATURES:

Sponsor Representative Dennis McFadden Date 12/15/88

SLS Study Director Paul H Fackler Date 1 Dec 88