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CHEMICAL MANUFACTURERS ASSOCIATION

FYI-1097-1309

September 29, 1997

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Dr. Lynn Goldman
Assistant Administrator
Office of Prevention, Pesticides and Toxic Substances TS-7101
Environmental Protection Agency
401 M Street, SW, Room 637, East Tower
Washington, DC 20460

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Dear Dr. Goldman:

The Chemical Manufacturers Association makes available to the public and appropriate government agencies final reports of environmental, health and safety research that it manages. In keeping with this policy, the following recently completed reports are enclosed:

1. PENTABROMODIPHENYL OXIDE (PeBDPO): Determination of n-Octanol Water Partition Coefficient; and
2. PENTABROMODIPHENYL OXIDE (PeBDPO): Ready Biodegradability by the Carbon Dioxide Evolution Test Method.

This report does not include confidential information.

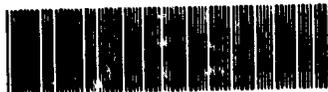
If you have any questions, please call Has Shah of my staff at 703-741-5637.

Sincerely yours,

Courtney Price

Courtney M. Price
Vice President, CHEMSTAR

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Enclosure



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**PENTABROMODIPHENYL OXIDE (PeBDPO):
DETERMINATION OF n-OCTANOL/WATER PARTITION COEFFICIENT**

WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER: 439C-108

**OPPTS 830.7560 Partition Coefficient (n-Octanol/Water),
Generator Column Method**

AUTHORS:

**Jon A. MacGregor
Willard B. Nixon, Ph.D.**

STUDY INITIATION DATE: May 9, 1997

STUDY COMPLETION DATE: September 12, 1997

Submitted to:

**Chemical Manufacturers Association's
Brominated Flame Retardant Industry Panel
1300 Wilson Boulevard
Arlington, Virginia 22209**



WILDLIFE INTERNATIONAL LTD.

**8598 Commerce Drive
Easton, Maryland 21601
(410) 822-8600**



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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

SPONSOR: Chemical Manufacturers Association's Brominated Flame Retardant Industry Panel

TITLE: Pentabromodiphenyl Oxide (PeBDPO): Determination of n-Octanol/Water Partition Coefficient

WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER: 439C-108

STUDY COMPLETION: September 12, 1997

This study was conducted in compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency in 40 CFR Part 792, 17 August 1989; OECD Principles of Good Laboratory Practices, (OECD)C(81)30 (Final)Annex 2; and Japan MHW/MITI 59 Kikyoku No. 85; EA, Kankiken No. 233: MHW, Eisei No. 38 and MITI, 63 Kikyoku No. 823 with the following exception:

Test substance characterization was not performed under Good Laboratory Practice Standards.

STUDY DIRECTOR:

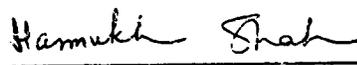


Jon A. MacGregor
Senior Chemist

9-12-97

DATE

SPONSOR APPROVAL:



Sponsor

9-15-97

DATE

- 3 -

QUALITY ASSURANCE

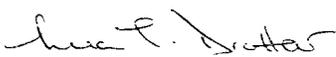
SPONSOR: Chemical Manufacturers Association's Brominated Flame Retardant Industry Panel

TITLE: Pentabromodiphenyl Oxide (PeBDPO): Determination of n-Octanol/Water Partition Coefficient

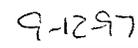
WILDLIFE INTERNATIONAL LTD. PROJECT NO.: 439C-108

This study was examined for compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency in 40 CFR Part 792, 17 August 1989; OECD Principles of Good Laboratory Practices, (OECD)C(81)30 (Final)Annex 2; and Japan MHW/MITI 59 Kikyoku No. 85; EA, Kankiken No. 233; MHW, Eisei No. 38 and MITI, 63 Kikyoku No. 823. The dates of all inspections and audits and the dates that any findings were reported to the Study Director and Laboratory Management were as follows:

| ACTIVITY: | DATE CONDUCTED: | DATE REPORTED TO: | |
|--|--------------------|--------------------|--------------------|
| | | STUDY DIRECTOR: | MANAGEMENT: |
| Test Substance Preparation and Matrix Fortifications Preparation | May 19, 1997 | May 19, 1997 | May 19, 1997 |
| Draft Report and Data | June 19, 1997 | June 19, 1997 | June 20, 1997 |
| Final Report | September 12, 1997 | September 12, 1997 | September 12, 1997 |



 Lisa T. Drott
 Quality Assurance Representative



 DATE

REPORT APPROVAL

SPONSOR: Chemical Manufacturers Association's Brominated Flame Retardant Industry Panel

TITLE: Pentabromodiphenyl Oxide (PeBDPO): Determination of n-Octanol/Water Partition Coefficient

WILDLIFE INTERNATIONAL LTD. PROJECT NO.: 439C-108

This report was reviewed by the individuals involved in the conduct and management of the study, and was found to be an accurate reflection of the methods used, data collected and results of the study.

STUDY DIRECTOR:

Jon A. MacGregor
Jon A. MacGregor
Senior Chemist

9-12-97
DATE

MANAGEMENT:

Willard B. Nixon
Willard B. Nixon, Ph.D.
Manager, Analytical Chemistry

9/12/97
DATE

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SUMMARY

| | |
|---|--|
| SPONSOR: | Chemical Manufacturers Association's Brominated Flame Retardant Industry Panel |
| SPONSOR'S REPRESENTATIVE: | Hasmukh Shah, Ph D. |
| LOCATION OF STUDY, RAW DATA AND A COPY OF THE FINAL REPORT: | Wildlife International Ltd. Easton, Maryland 21601 |

| | |
|-----------------------------|---|
| WILDLIFE INTERNATIONAL LTD. | |
| PROJECT NUMBER: | 439C-108 |
| TEST SUBSTANCE: | Pentabromodiphenyl Oxide (PeBDPO) |
| STUDY: | Pentabromodiphenyl Oxide (PeBDPO): Determination of the n-Octanol/Water Partition Coefficient |
| TEST DATES: | Experimental Start - May 19, 1997 Experimental Termination - May 27, 1997 |

| | |
|----------|---|
| SUMMARY: | The \log_{10} octanol/water partition coefficient (K_{ow}) of PeBDPO was determined to be 6.568 at $25 \pm 0.05^\circ\text{C}$ using the generator column method. |
|----------|---|

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INTRODUCTION

This study was conducted by Wildlife International Ltd. for Chemical Manufacturers Association's (CMA) Brominated Flame Retardant Industry Panel at the Wildlife International Ltd. analytical chemistry facility in Easton, Maryland. Tests were performed using the generator column method. Samples were eluted from a generator column and analyzed on May 23, 1997. Raw data generated by Wildlife International Ltd. and a copy of the final report are filed under Project Number 439C-108 in archives located on the Wildlife International Ltd. site.

OBJECTIVE

The objective of this study was to determine the n-octanol/water partition coefficient of Pentabromodiphenyl Oxide (PeBDPO) at $25 \pm 0.05^\circ\text{C}$ using the generator column method.

EXPERIMENTAL DESIGN

A single generator column was prepared for the definitive test. The column was packed with Chromosorb W HP support and loaded with an approximate 0.4% solution of the test substance in octanol. Dilutions of the test substance solution in octanol were analyzed. The column temperature was maintained at $25 \pm 0.05^\circ\text{C}$ and reagent water saturated with octanol was pumped through it at approximately 1 mL per minute to elute the test substance. Samples of the eluate were collected and analyzed to determine the concentration of the test substance in the aqueous fractions.

MATERIALS AND METHODS

This study was conducted according to procedures outlined in the protocol, "Pentabromodiphenyl Oxide (PeBDPO): Determination of n-Octanol/Water Partition Coefficient" (Appendix I). The protocol was based on procedures outlined in OPPTS 830.7560 (1). The generator column method was used to determine the partition coefficient of the test substance.

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Test Substance

The test substance consisted of a composite of pentabromodiphenyl oxide (PeBDPO) samples received from two manufacturers. The material's identity and date received from each of the manufacturers are given below:

| <u>Manufacturer</u> | <u>Lot/Batch</u> | <u>Date Received</u> | <u>Wildlife International Ltd. Identification Number</u> |
|----------------------------|------------------|----------------------|--|
| Great Lakes Chemical Corp. | 55500J18A | October 26, 1995 | 3461 |
| Akzo Nobel Chemicals, Inc. | ANCR-DFT #37 | March 20, 1996 | 3614 |

The materials were very viscous, and were warmed in an oven at approximately 60°C overnight to make it possible to pour and mix them. An equal part (450 g) of each of the manufacturer's PeBDPO material was placed in a stainless steel blender. The blender was run at the low setting for approximately 15 seconds, then at the high setting for approximately 45 seconds. The composite test substance was assigned Wildlife International Ltd. identification number 3634. Subsamples of the composite test substance were shipped to Great Lakes Chemical Corporation for characterization and homogeneity analysis. The analyses were performed on May 4, 1996. The results of the analyses indicated the test substance was homogeneous and contained the following components:

| | |
|--------------------------|-------|
| Tetrabromodiphenyl oxide | 33.7% |
| Pentabromodiphenyl oxide | 54.6% |
| Hexabromodiphenyl oxide | 11.7% |

The composite test substance was stored under ambient conditions.

Reagent Water

The reagent water used in this study met the specifications for ASTM Type II water. The water was obtained from a well located on the Wildlife International Ltd. site. The well water was pumped through a series of filters to remove microorganisms and particles greater than 0.2 μm . The water was further purified using a Culligan[®] Hi-Flo 1 Water Softener, a Culligan[®] S-Series Reverse Osmosis System, and a Barnstead NANOpure[®] ultrapure water system. The resistivity of the purified reagent water used for this study was at least 18.0 megohm-cm. The reagent water was saturated with octanol prior to use in this study.

Solvents

1-Octanol (certified), Fisher Chemical (Fairlawn, NJ), catalog number A 402-4, was used to prepare a stock solution of the test substance. Dimethylformamide (DMF), Burdick and Jackson (catalog number 076-1), was used to prepare stock solutions of the test substance for matrix fortifications. Diphenyl ether (DPO), purchased from Fluka Chemical (Switzerland, catalog number 42730), was used to prepare calibration standards and final sample volumes. Ethyl Acetate (EtoAc), Burdick and Jackson (catalog number 300-4), was used as an extraction solvent for this study. A solution of 5% KOH, Mallinkridt (catalog number 6984) in methanol, Burdick and Jackson (catalog number 230-4) along with a 10% phosphoric acid (85%) solution, Fisher (catalog number A242-212) and tetrahydrofuron (THF), Fisher (Catalog number T425-1) were used to rinse all glassware prior to use. All solvents used for this study were either suitable for HPLC and residue analysis or certified reagents.

Preparation of Generator Column

The generator column was supplied by At-Mar Glass Co. (Kennett Square, PA). The glass column was ~20 cm long with an internal diameter of ~6 mm, and was joined to a section with an internal diameter of ~9 mm. The entire column was enclosed in a water jacket for temperature control. A diagram of the generator column is presented in Figure 1.

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The inert support material used in the generator column was Chromosorb WHP (100 - 120 mesh), and was supplied by Supelco Inc. (Bellefonte, PA, catalog number 2-0159). A small plug of silanized glass wool was placed in the column just above the enlarged section, the column was filled with the support material, and another plug of glass wool was added to the top of the column. Gentle tapping and vibration were used to facilitate packing of the column.

A stock solution of the composite test substance was prepared by combining ~100 mg of test substance with 25 g of octanol. The nominal concentration of the stock solution was approximately 0.4% test substance by weight or approximately 2.5 mg/mL, based on the density of octanol (0.827 g/mL). The stock solution was combined and stirred at approximately 2000 rpm for approximately five minutes and then filtered through a 0.45 µm sintered disc to remove any undissolved test substance. Three subsamples of the final filtered stock solution were diluted in diphenyl ether (DPO) and analyzed to determine the concentration of test substance in the octanol. An aliquot of the stock solution (15 mL) was also used to charge the generator column. The stock solution was loaded onto the support material by applying gentle suction at the bottom of the column with the aid of a vacuum pump apparatus.

The column was then back-flushed with reagent water saturated with octanol by applying gentle suction at the top of the column to remove any entrapped air. End fittings were attached to the column after it had been flushed.

Apparatus Configuration

A Nesslab Model IC-515 Constant Temperature Water Bath (Nesslab Instruments, Inc., Portsmouth, NH) was used to maintain the test temperature ($25.0 \pm 0.05^\circ\text{C}$) throughout the experiment. The constant temperature bath was filled with water. The temperature of the water bath was monitored using an ASTM 45C kinematic viscosity thermometer (Ever Ready Thermometer Company, West Paterson, NJ).

A Teel Model 1P680A submersible pump (Dayton Electric Mfg. Co., Chicago, IL) was placed in the constant temperature bath, and was used to pump a continuous stream of water through the jacket surrounding the generator column in order to maintain a constant temperature.

A 2-L Erlenmeyer flask was used as a reservoir for the reagent water saturated with octanol that was being pumped through the generator column. The flask was submerged in the constant temperature bath. The inlet line of a Waters Model 510 solvent delivery system (Waters Associates, Milford, MA) was placed in the 2-L flask, and the top of the flask covered with aluminum foil. The pump was used to control the flow rate of the reagent water through the generator column.

The eluate from the generator column was directed to 500 mL separatory funnels containing appropriate volumes of extraction solvent. The individual samples were collected manually. A diagram of the apparatus configuration is presented in Figure 2.

Aqueous Sample Collection

Prior to the collection of aqueous samples, the pump was allowed to equilibrate for approximately one hour at a flow rate of 1.0 mL/min. Following this, the pump was set to deliver approximately 1.0 mL of reagent water saturated with octanol per minute through the generator column. The eluate was collected dropwise into 500 mL separatory funnels containing 100 mL of the extraction solvent. Three consecutive individual aqueous samples were collected at approximately four hour intervals into three separate 500 mL separatory funnels. The volume of sample collected ranged from 240 to 250 mL.

Analytical Method

The analytical method consisted of extracting the aqueous samples with ethyl acetate, evaporating the solvent, and reconstituting the sample residues in diphenyl ether (DPO). Prior to use, all glassware was base-washed using a 5% KOH in methanol solution, acid-washed using a 10% aqueous phosphoric acid solution, and then rinsed with reagent water. Acetone was then used to dry the glassware, followed by successive rinses with tetrahydrofuran and ethyl acetate solvents. Ethyl

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acetate (100 mL) was added to each separatory funnel prior to sample collection. After collection, each separatory funnel was stoppered and shaken for approximately one minute. The layers were allowed to separate and several crystals of sodium chloride were added, if necessary, to break any emulsions. The lower aqueous layer was drained into an appropriately sized Erlenmeyer flask and the ethyl acetate layer was then drained into a 250 mL round-bottom flask. The solvent volume was reduced to approximately 10 mL using rotary evaporation at 30 - 50°C. The aqueous portion was carefully poured back into the separatory funnel and 100 mL of ethyl acetate extraction solvent was added to each flask as a rinse, prior to adding it to the separatory funnels. This extraction procedure was repeated two additional times. The extracts were combined each time in the round-bottom flask. Following extraction, the volume of the aqueous sample was measured using an appropriately sized graduated cylinder. The final combined ethyl acetate extracts were reduced to approximately 2 mL using rotary evaporation at 30 - 50°C and then quantitatively transferred to individual test tubes. The extracts were then reduced to the residual octanol using a nitrogen evaporator at 30 - 50°C. The final residues were reconstituted by additions of diphenyl ether (DPO) to a final volume of 1.0 mL. The final volumes were based on visual comparison to a blank test tube containing 1.0 mL DPO (measured using a class A volumetric pipet). The final DPO extracts were transferred to labelled autosampler vials for analysis.

The preparation method used for samples of the octanol stock solution consisted of diluting the samples using DPO. Subsamples of 0.100 mL were diluted as necessary using class A volumetric glassware and mixed. Aliquots of the diluted solutions were placed in autosampler vials for analysis.

Concentrations of PeBDPO in the samples were determined using a Hewlett-Packard Model 5890 Gas Chromatograph (GC). The gas chromatograph was equipped with an electron capture detector (ECD). Chromatographic separations were achieved using an Rtx-1 capillary column (15 m X 0.53 mm, 0.1 μ m film thickness) supplied by Restek Corporation (Bellevue, PA). Chromatographic grade helium was used as the carrier gas, and argon/methane was used as the

auxiliary gas. The instrument parameters are summarized in Table 1. A method flow chart is provided in Figure 3.

Preparation of Quality Control Samples

Fortification solutions of PeBDPO were prepared in dimethylformamide (DMF) at a concentration of 1.00 mg/L. This standard was used to fortify matrix samples at 0.200 and 5.00 μg PeBDPO/L. One reagent (method) blank, two matrix blanks and two matrix fortifications (0.200 ppb and 5.00 ppb) were prepared and analyzed along with the aqueous samples collected from the generator column.

Calibration Curve and Quantitation

Calibration standards of PeBDPO were prepared in diphenyl ether (DPO). The standards were prepared using the test substance, and ranged in concentration from 50.0 to 250 μg PeBDPO/L. A set of calibration standards was analyzed before and after the set of samples, and a standard was injected a minimum of every five samples during the analytical run. The method of quantitation for the PeBDPO test substance involved summing of the Br4 and Br5 chromatographic components for each injection. A calibration curve was constructed from the linear regression equation using the respective concentration versus summed peak area responses of the calibration standards (Figure 4). Representative chromatograms of low and high calibration standards are shown in Figures 5 and 6, respectively. The concentration of PeBDPO in the samples was determined by substituting the summed peak area responses into the applicable linear regression equation generated from the calibration curve as follows:

$$\text{PeBDPO in Sample } (\mu\text{g/L}) = [(\text{Summed Peak Area} - \text{Y-Intercept/Slope}) \times \text{Dilution Factor}]$$

$$\text{Molar Concentration (M)} = \frac{\text{Measured PeBDPO Concentration (g/L)}}{\text{Molecular Weight (PeBDPO)}} \times 1$$

$$\% \text{ Recovery} = \frac{\text{Measured PeBDPO Concentration } (\mu\text{g/L})}{\text{Nominal PeBDPO Concentration } (\mu\text{g/L})}$$

The instrument limit of detection (LOD) for this study was set based upon the injection volume (2.0 μ L) and the lowest calibration standard concentration (50.0 μ g PeBDPO/L). The LOD was set at 100 pg of PeBDPO injected.

The limit of quantitation (LOQ) was 0.200 μ g PeBDPO/L calculated as the product of the lowest calibration standard (50.0 μ g PeBDPO/L) and the dilution factor of the matrix blank sample (0.0040).

RESULTS AND DISCUSSION

Quality Control Samples

No interferences were observed at or above the LOQ in the one reagent and two matrix blank samples. A chromatogram of a matrix blank is shown in Figure 7.

The percent recovery of the 0.200 and 5.00 μ g PeBDPO/L matrix fortifications were 113% and 84% of nominal concentrations. The mean recovery was calculated as 99% of nominal concentration. Representative chromatograms of the 0.200 and 5.00 μ g PeBDPO/L matrix fortification samples are shown in Figures 8 and 9, respectively.

Column Elution

The temperature of the water bath remained constant at $25.0 \pm 0.05^\circ\text{C}$ throughout the experiment (Table 2).

The nominal flow rate of reagent water through the generator column was measured prior to the start of sample collection. Flow rates were also calculated based on the volume and collection time of each sample that was analyzed. The pump setting was 1.0 mL/min. and the flow rate was measured at 1.0 mL/min. The calculated flow rates for samples averaged 1.02 mL/min. and ranged from 1.00 to 1.04 mL/min. (Table 3).

The results from the analyses of aqueous samples eluted from the generator column are presented in Table 3. A representative chromatogram is shown in Figure 10. The mean concentration of PeBDPO measured in these samples was 0.6714 μg PeBDPO/L, or 1.24×10^{-9} M (molecular weight of PeBDPO is 540.7 g/mole).

The results from the analyses of the octanol stock solution are presented in Table 4. A representative chromatogram is shown in Figure 11. The mean concentration of PeBDPO measured in these samples was 2.48 g PeBDPO/L, or 4.59×10^{-3} M (molecular weight of PeBDPO is 540.7 g/mole).

CONCLUSIONS

The octanol/water partition (K_{ow}) coefficient was calculated from the following equation:

$$K_{ow} = \frac{\text{Measured Concentration in Octanol (M)}}{\text{Measured Concentration in Aqueous Samples (M)}}$$

Based on the results from octanol samples collected from the stock solution and aqueous samples collected from the generator column, the mean octanol/water partition coefficient (K_{ow}) for PeBDPO was determined to be 3.70×10^6 ($\log_{10}K_{ow}$ is 6.568).

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REFERENCES

1. **U.S. Environmental Protection Agency.** 1996. Product Properties Test Guidelines, OPPTS 830.7560, Partition Coefficient (n-Octanol/Water), Generator Column Method. Washington, D C

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Table 1

Typical Gas Chromatographic Operational Parameters

| | |
|--|---|
| INSTRUMENT: | Hewlett-Packard Model 5890 Gas chromatograph (GC) Equipped with a Model G1030A Chemstation |
| DETECTOR: | Hewlett-Packard Electron-Capture Detector (ECD) |
| ANALYTICAL COLUMN: | Restek-Rtx-1 Column (15 m x 0.53 mm, 0.1 μ m film thickness) |
| INJECTOR TEMPERATURE: | 300 °C |
| OVEN: | Initial temperature: 150 °C Initial hold time: 2 minutes Ramp: 5 °C/minute Final temperature: 300 °C Final hold time: 3 minutes |
| DETECTOR TEMPERATURE: | 320 °C |
| INJECTION VOLUME: | 2 μ L, splitless |
| CARRIER GAS: | Helium, ~4-6 mL/minute (~4 p.s.i., CHP) |
| MAKE-UP GAS: | Argon/Methane, ~45 mL/minute |
| PENTABROMODIPHENYL OXIDE (PeBDPO) COMPONENT PEAK RETENTION TIMES: | Br4 = 6.5 minutes Br5 = 9.8 minutes |

Table 2

Water Bath Test Temperatures

| Date & Time of Observation | Water Bath Temperature (°C) |
|-------------------------------|--------------------------------|
| 5/19/97 11:30 | 25.00 |
| 5/19/97 15:30 | 25.00 |
| 5/19/97 19:30 | 25.05 |
| 5/19/97 23:30 | 25.05 |

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Table 3

Results for Aqueous Samples Collected from the Generator Column

| Sample ID (439C-108-) | Summed Peak Area (uV*sec) | Sample Volume (mL) | Final Volume (mL) | Collection Time (min.) | Calculated Flow Rate (mL/min.) | Measured Concentration (μ g PeBDPO/L) | Measured Concentration (M) |
|--------------------------|------------------------------------|--------------------------|-------------------------|------------------------------|--------------------------------------|--|----------------------------------|
| 1 | 211,722 | 245 | 1.0 | 240 | 1.02 | 0.6065 | 1.12×10^{-9} |
| 2 | 238,596 | 250 | 1.0 | 240 | 1.04 | 0.6708 | 1.24×10^{-9} |
| 3 | 249,129 | 240 | 1.0 | 240 | 1.00 | 0.7370 | 1.36×10^{-9} |

$\bar{x} = 0.6714$ $\bar{x} = 1.24 \times 10^{-9}$
SD = 0.0653 SD = 1.20×10^{-10}

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Table 4

Results for Octanol Stock Solution Samples

| Sample ID (439C-108-) | Summed Peak Area (uV*sec) | Sample Volume (mL) | Final Volume (mL) | Dilution Factor | Measured Concentration ($\mu\text{g PeBDPO/L}$) | Measured Concentration (M) |
|--------------------------|------------------------------------|--------------------------|-------------------------|--------------------|---|----------------------------------|
| 0-1 | 133,559 | 0.100 | 100 | 25.0 | 2,259,367 | 4.17×10^{-3} |
| 0-2 | 150,031 | 0.100 | 100 | 25.0 | 2,562,561 | 4.74×10^{-3} |
| 0-3 | 153,512 | 0.100 | 100 | 25.0 | 2,626,635 | 4.86×10^{-3} |
| | | | | | $\bar{x} = 2,482,854$ | $\bar{x} = 4.59 \times 10^{-3}$ |
| | | | | | SD = 196,179 | SD = 3.69×10^{-4} |

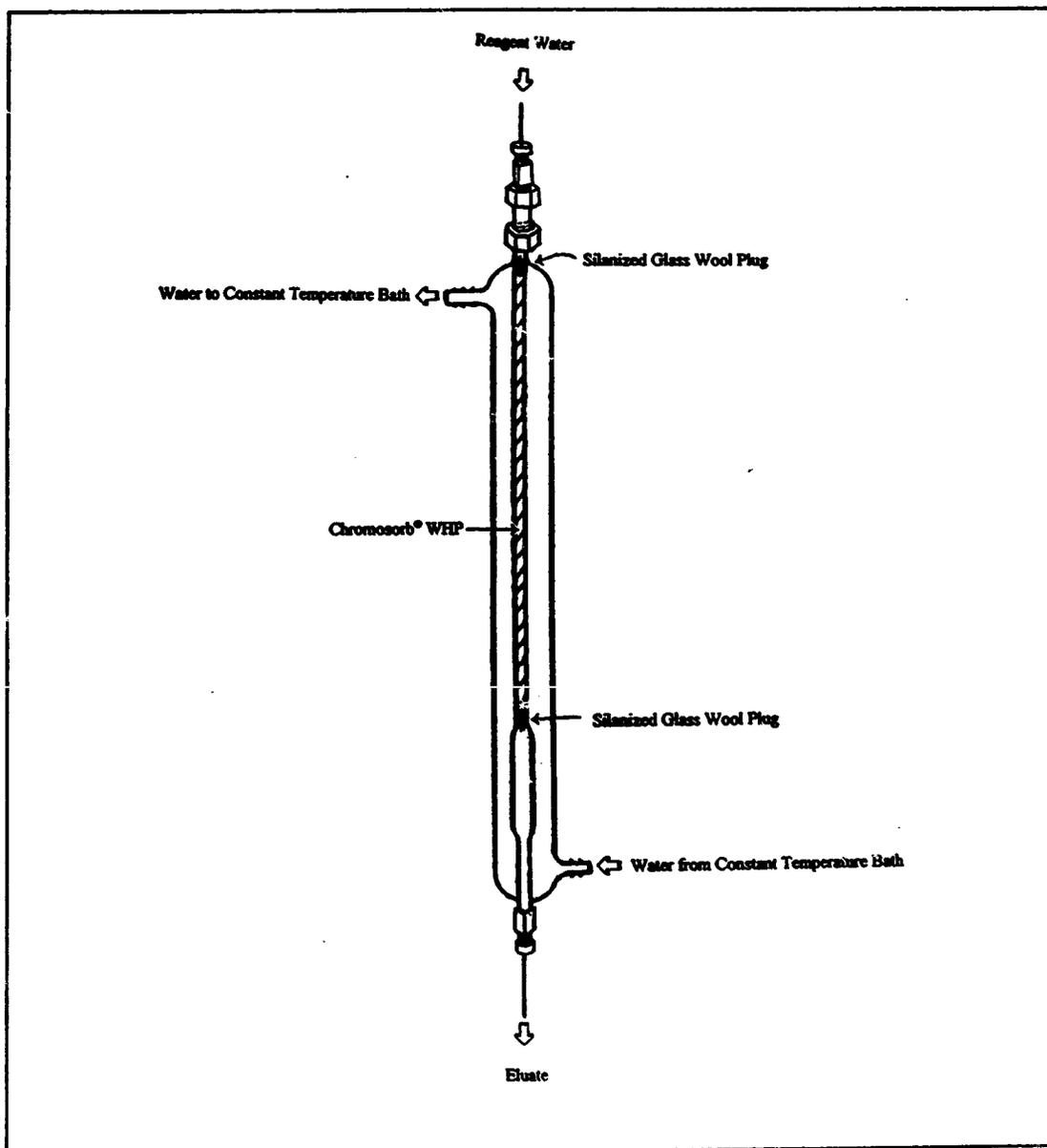


Figure 1. Diagram of generator column.

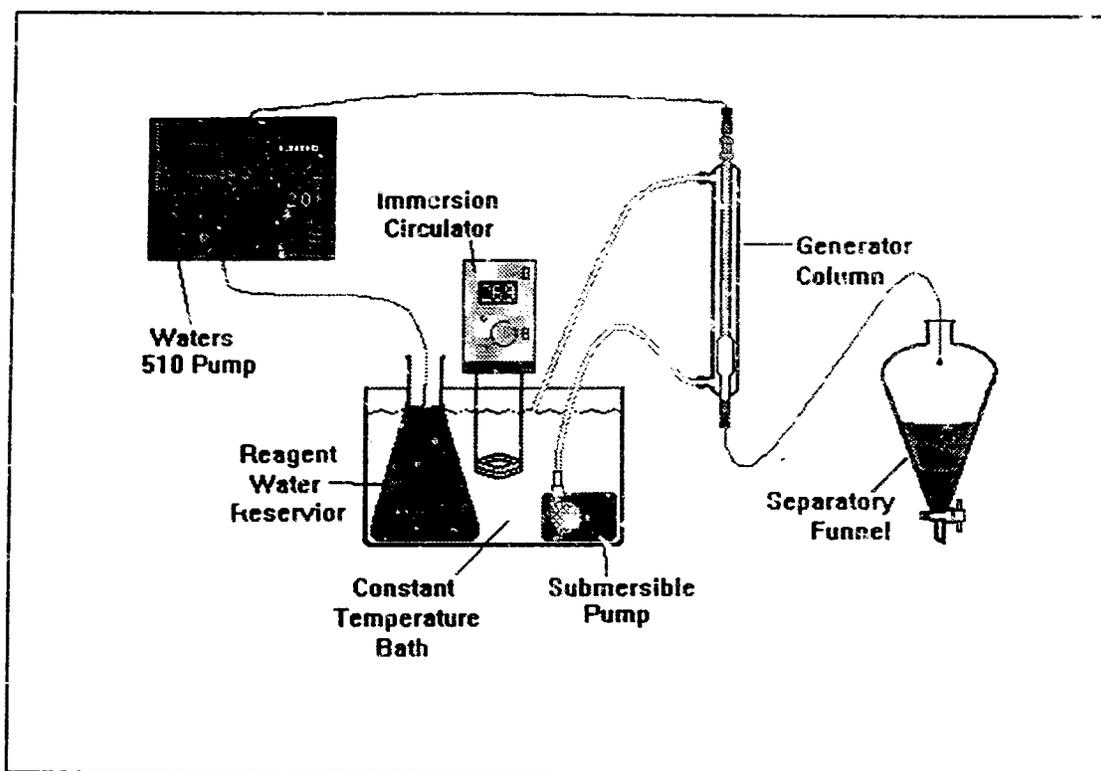


Figure 2. Diagram of apparatus configuration.

ANALYTICAL METHOD FLOW CHART

Measure 250 mL samples of octanol saturated NANOpure® water or column eluate into 500 mL separatory funnels using 250 mL graduated cylinders, or equivalent

Fortify with the appropriate standard solution; swirl to mix. Add 100 mL of ethyl acetate to each bottle.

Shake each sample for approximately one minute. Allow layers to separate. Add several crystals of NaCl to break emulsions, if necessary. Drain aqueous layer into appropriately sized flask. Drain ethyl acetate into 250 mL round-bottom flasks. Reduce volume to approximately 10 mL by rotary evaporation.

Pour aqueous sample back into separatory funnel. Add 100 mL of ethyl acetate extraction solvent to aqueous flask to rinse. Transfer to separatory funnel.

Repeat the extraction procedure above two additional times.

Combine the extracts into the same flask after reducing solvent volume each time.

Measure the volume of water in each aqueous generator column sample using an appropriately sized graduated cylinder.

Evaporate the extract to approximately 2 mL on a rotovap at 30 to 50°C. Quantitatively transfer to test tubes.

Reduce to residual octanol on nitrogen evaporator at 30 to 50°C.

Reconstitute the extracts using additions of diphenyl ether (DPO) to bring the final sample volume to 1.0 mL. Volume based on visual comparisons to a blank tube containing 1.0 mL of diphenyl ether measured using a class A volumetric pipet.

Transfer the phenyl ether extract into a vial for analysis using GC/ECD.

Figure 3. An analytical method flow chart.

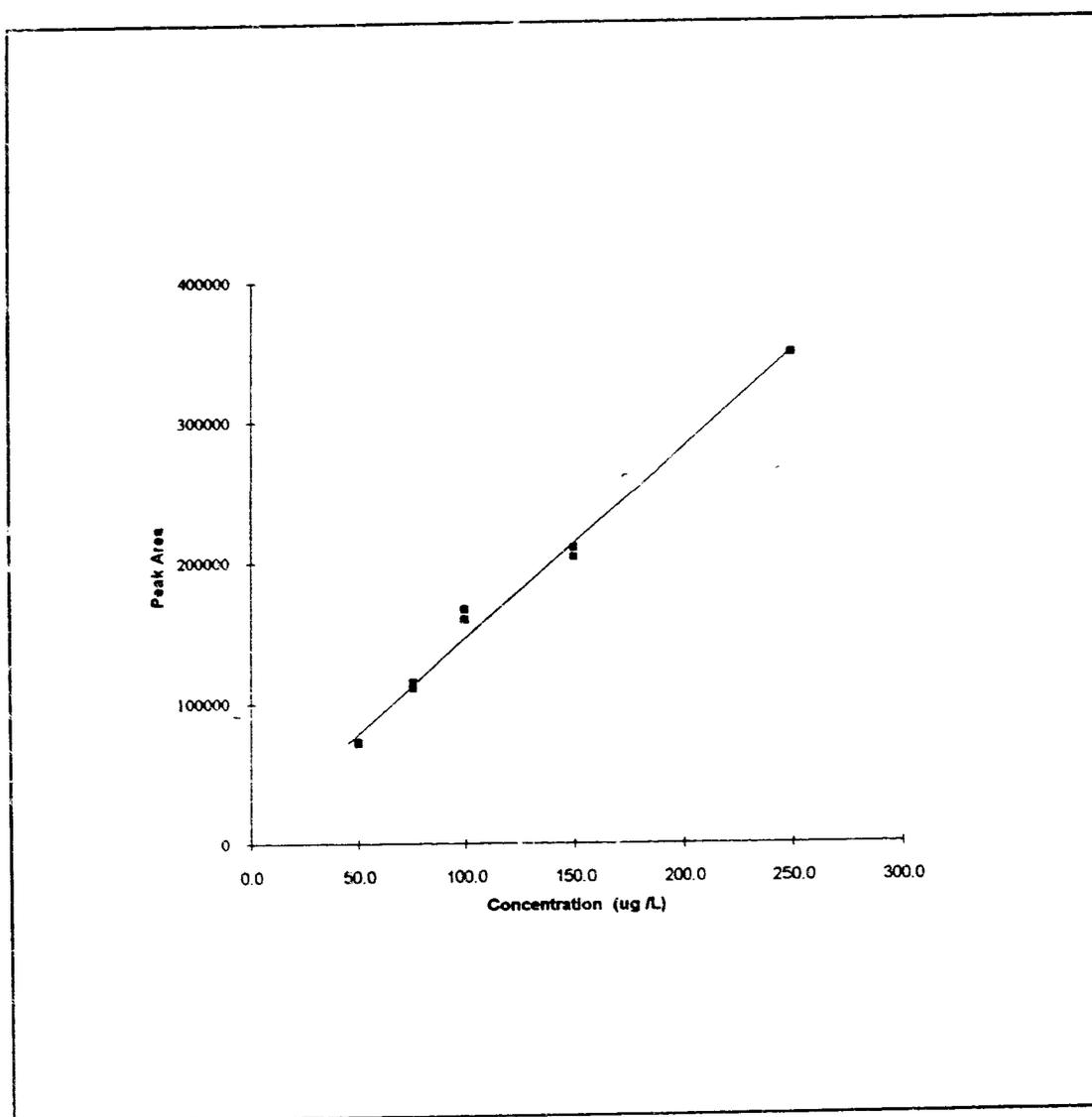


Figure 4. Representative calibration curve. Intercept = 10811.6389; Slope = 1358.2053;
 $r^2 = 0.9880$.

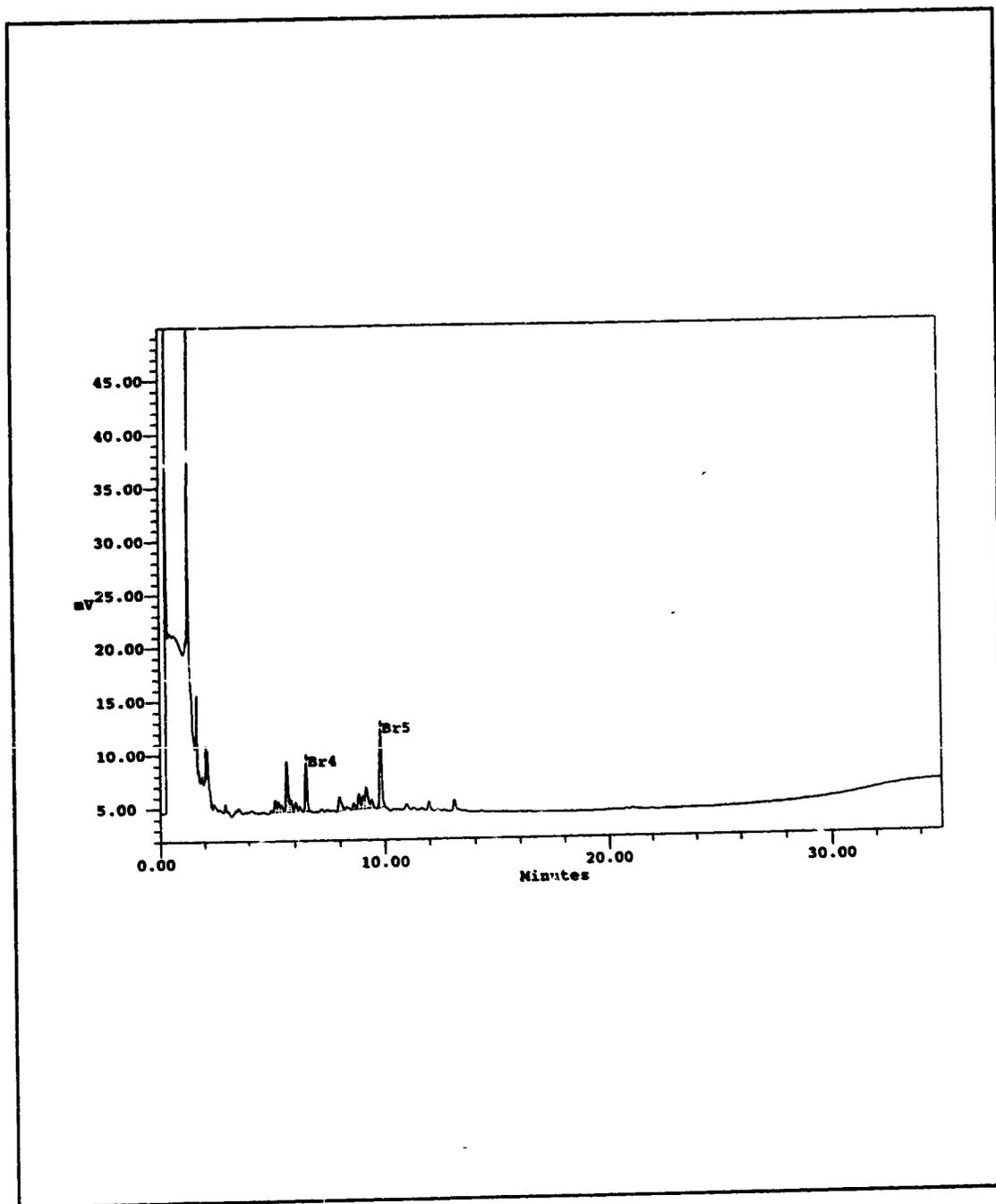


Figure 5. Representative chromatogram of a low-level calibration standard (50.0 µg PeBDPO/L).

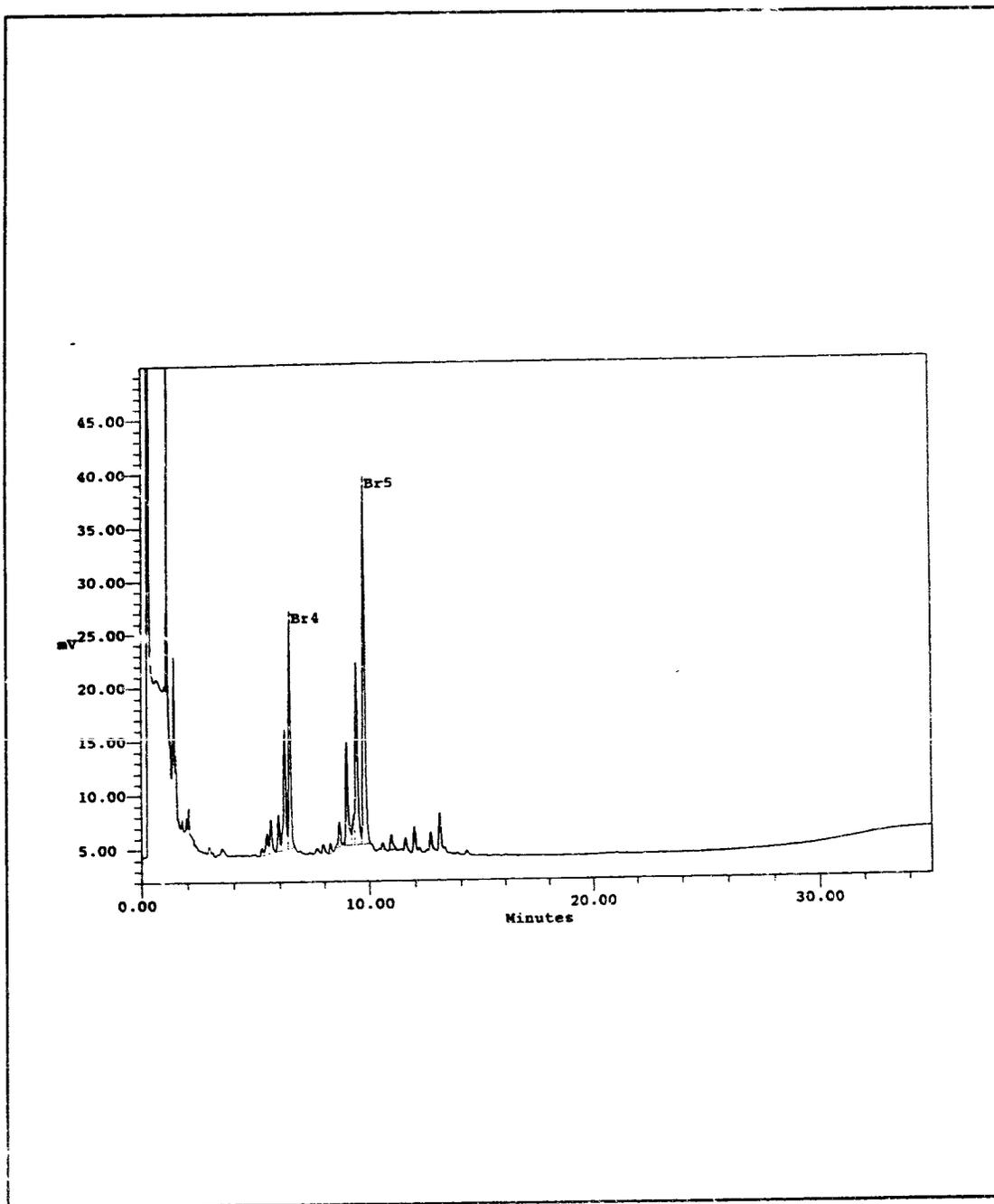


Figure 6. Representative chromatogram of a high-level calibration standard (250 μ g PeBDPO/L).

**PENTABROMODIPHENYL OXIDE (PeBDPO): READY BIODEGRADABILITY
BY THE CARBON DIOXIDE EVOLUTION TEST METHOD**

WILDLIFE INTERNATIONAL LTD. PROJECT NO.: 439E-103A

**AUTHORS:
Edward C. Schaefer
Doug Haberlein**

STUDY INITIATION DATE: August 27, 1996

STUDY COMPLETION DATE: August 25, 1997

Submitted to:

**Chemical Manufacturers Association's
Brominated Flame Retardant Industry Panel
1300 Wilson Boulevard
Arlington, Virginia 22209**



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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

SPONSOR: Chemical Manufacturers Association's Brominated Flame Retardant Industry Panel

TITLE: Pentabromodiphenyl Oxide (PeBDPO): Ready Biodegradability by the Carbon Dioxide Evolution Test Method

WILDLIFE INTERNATIONAL LTD. PROJECT NO.: 439E-103A

STUDY COMPLETION: August 25, 1997

This study was conducted in compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency in 40 CFR Part 792 17 August 1989; OECD Principles of Good Laboratory Practices, (OECD) C(81)30(Final) Annex 2; and Japan MHW/MITI 59 Kikyoku No. 85; EA, Kankiken No. 233; MHW, Fisei No. 33 and METI, 63 Kikyoku No. 823, with the following exceptions:

Characterization and stability of the test substance was not conducted in compliance with Good Laboratory Practice Standards.

Characterization and stability of the reference substance, obtained from J.T. Baker (Phillipsburg, NJ), was not conducted in compliance with Good Laboratory Practice Standards.

STUDY DIRECTOR:


Edward C. Schaefer, B.S.
Manager, Biodegradation

8-25-97
DATE

SPONSOR APPROVAL:


Sponsor

8-29-97
DATE

- 3 -

**QUALITY ASSURANCE
PENTABROMODIPHENYL OXIDE (PeBDPO): READY BIODEGRADABILITY
BY THE CARBON DIOXIDE EVOLUTION TEST METHOD
PROJECT NO.: 439E-103A**

This study was examined for compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency, Office of Pesticide Programs in 40 CFR Part 792, 17 August 1989; OECD Principles of Good Laboratory Practices, (OECD)C(81)30 (Final)Annex 2; and Japan MHW/MITI 59 Kikyoku No. 85; EA, Kankiken No. 233: MHW, Eisei No. 38 and MITI, 63 Kikyoku No. 823. The dates of all inspections and audits and the dates that any findings were reported to the Study Director and Laboratory Management were as follows:

| ACTIVITY: | DATE CONDUCTED: | DATE REPORTED TO: | |
|--|--------------------|--------------------|--------------------|
| | | STUDY DIRECTOR: | MANAGEMENT: |
| 439E-103 | | | |
| Test Chamber Preparation | September 4, 1996 | September 5, 1996 | September 9, 1996 |
| Pour Plate Preparation | September 4, 1996 | September 4, 1996 | September 6, 1996 |
| Test Substance Preparation and Test Initiation | September 5, 1996 | September 12, 1996 | September 26, 1996 |
| Sample Collection | September 19, 1996 | September 19, 1996 | September 26, 1996 |
| 439E-103A | | | |
| Test Chamber Preparation | October 1, 1996 | October 1, 1996 | October 29, 1996 |
| Raw Data and Draft Report | January 22, 1997 | January 22, 1997 | January 23, 1997 |
| Final Report | August 21, 1997 | August 21, 1997 | August 25, 1997 |

Susan L. Hopper
Susan L. Hopper, B.A.
Senior Quality Assurance Representative

8-25-97
DATE

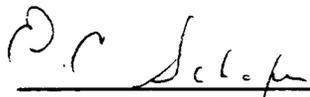
REPORT APPROVAL

SPONSOR: Chemical Manufacturers Association's Brominated Flame Retardant Industry Panel

TITLE: Pentabromodiphenyl Oxide (PeBDPO): Ready Biodegradability by the Carbon Dioxide Evolution Test Method

WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER: 439E-103A

STUDY DIRECTOR:

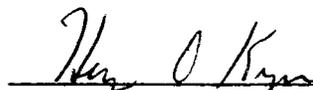


Edward C. Schaefer
Manager, Biodegradation

8-25-97

DATE

MANAGEMENT:



Henry O. Krueger, Ph.D.
Director of Aquatic Toxicology and Non-Target Plants

8/25/97

DATE



Linda R. Mitchell
Manager, Quality Assurance

25 August 1997

DATE

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SUMMARY

The ready biodegradability of pentabromodiphenyl oxide (PeBDPO) was determined by the Carbon Dioxide Evolution Test Method (OECD Guideline 301B). Tests of ready biodegradability are stringent tests that provide limited opportunity for acclimation and biodegradation to occur. In the CO₂ test, inoculated mineral medium is dosed with a known amount of test substance as the nominal sole source of organic carbon and aerated with CO₂-free air. The CO₂ produced from the mineralization of organic carbon within the test chambers is displaced by the flow of CO₂-free air and trapped as K₂CO₃ in a KOH trapping solution. The amount of CO₂ produced by the test substance (corrected for that evolved by the blank inoculum) is expressed as a percentage of the theoretical amount of CO₂ (TCO₂) that could have been produced if complete biodegradation of the test substance occurred.

Minimal degradation of the test substance was observed over a 93-day test period. The standard 28-day test period (1,2) was extended so that sufficient opportunity for biodegradation would be provided. Pentabromodiphenyl oxide (PeBDPO) evolved an average of approximately 0.0 and 2.4% TCO₂ on days 29 and 93, respectively.

STUDY IDENTIFICATION

| | |
|---|--|
| SPONSOR: | Chemical Manufacturers Association's Brominated Flame Retardant Industry Panel |
| SPONSOR'S REPRESENTATIVE: | Hasmukh Shah Ph.D. |
| LOCATION OF STUDY, RAW DATA AND THE FINAL REPORT: | Wildlife International Ltd. Easton, MD 21601 |

| | |
|---|---|
| WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER: | 439E-103A |
| TEST SUBSTANCE: | Pentabromodiphenyl Oxide (PeBDPO) |
| STUDY: | Pentabromodiphenyl Oxide (PeBDPC): Ready Biodegradability by the Carbon Dioxide Evolution Test Method |
| TEST CONCENTRATION: | 10 mg C/L |
| TEST DATES: | Experimental Start - October 2, 1996 Experimental Termination - January 3, 1997 |
| LENGTH OF BIOLOGICAL PHASE: | 93 Days |

INTRODUCTION

Tests of ready biodegradability, by definition, provide limited opportunity for acclimation and biodegradation to occur. A positive result in a test of ready biodegradability is an indication that the test substance will undergo rapid and ultimate biodegradation in the environment. A negative result in a test of ready biodegradability does not necessarily mean that the test substance will not be biodegraded under relevant environmental conditions but that additional testing may be needed.

Original raw data generated by Wildlife International Ltd. and the original final report are filed under Project Number 439E-103A in the archives located on the Wildlife International Ltd. site.

OBJECTIVE

The objective of the study was to measure the amount of carbon dioxide (CO₂) produced from the biodegradation of the test substance. This value is expressed as a percentage of the theoretical amount of CO₂ (TCO₂) that could have been produced if complete biodegradation of the test substance occurred. Biodegradation involves the breakdown of an organic chemical to CO₂ and other inorganic constituents. Comparing the amount of CO₂ evolved in a test system containing the test substance as the sole carbon source to the TCO₂ provides an accurate assessment of the biodegradability of the test substance under the conditions tested.

EXPERIMENTAL DESIGN

The test contained a blank control group, a reference group, and a treatment group. Each group contained two replicate test chambers. The blank control was used to measure the background CO₂ production of the inoculum and was not dosed with a carbon source. The reference chambers were used to check the viability of the inoculum and were dosed with sodium benzoate, a substance

known to be biodegradable, at a concentration of 10 mg Carbon/L. The treatment group was used to evaluate the test substance at 10 mg C/L. The CO₂ produced from the degradation of organic carbon sources within the test chambers was trapped as K₂CO₃ in KOH trapping solution. The traps were removed at intervals during the test and an aliquot of each trapping solution was analyzed for inorganic carbon.

MATERIALS AND METHODS

This study was conducted according to the procedures outlined in the protocol, "Pentabromodiphenyl Oxide (PeBDPO): Ready Biodegradability by the Carbon Dioxide Evolution Test Method" (Appendix I). The protocol was based on the procedures specified in the OECD Guideline for Testing of Chemicals, Method 301B (1); and EEC Method C.4-C (2).

Test Substance

The test substance used in this study was a composite of the following two samples of pentabromodiphenyl oxide (PeBDPO):

Manufacturer: Great Lakes Chemical
Sample ID: Pentabromodiphenyl oxide
Lot. No.: 55500J18A
Expiration Date: Not Given
Date Received: October 26, 1995
Wildlife ID: 3461

Manufacturer: Akzo Nobel Chemicals Inc.
Sample ID: Pentabromodiphenyl oxide

NB Ref. No.: ANCR-DF T#37
Expiration Date: Not Given
Date Received: March 20, 1996
Wildlife ID: 3614

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The composite PeBDPO sample was prepared on April 9, 1996 and was assigned Wildlife International Ltd. identification number 3634. The composite was prepared by heating the products to approximately 60°C, combining equal parts of the products, and blending for approximately one minute. Six subsamples of the composite sample were collected from the container. The subsamples were shipped to Great Lakes Chemical Corporation for analysis to verify the homogeneity of the mixture.

The theoretical carbon content of the test substance mixture was 26.2%.

Stock Solution Preparation

A stock solution of the reference substance, sodium benzoate, was prepared at a nominal concentration of 400 mg C/L in Nanopure water (ASTM Type I Reagent Grade Water). The total organic carbon (TOC) concentration of the reference substance stock solution was determined prior to dosing to confirm the nominal concentration. The dosing volume of the reference solution was calculated based on the measured TOC concentrations. The reference substance stock solution was stored in a refrigerator for approximately 2 days.

Test Medium

The test medium was a modified biochemical oxygen demand (BOD) test dilution water and was prepared using high quality water as described in the Protocol (Appendix I).

Test Apparatus and Conditions

The test chambers were amber 4-liter gas washing bottles. The air entering the chambers was passed through Drierite to remove ambient moisture and then through Ascarite to produce CO₂-free air. The air exiting the test chambers was passed through a series of three gas washing bottles each containing approximately 100 mL of 0.5 N KOH to trap the CO₂ that had evolved within the chamber. An additional set of gas washing bottles that were not connected to a chamber were maintained concurrently with the traps connected to the chambers. These bottles contained approximately 100 mL of 0.5 N KOH and the amount of CO₂ detected in the KOH solution was

subtracted from the CO₂ in the blank control traps to determine the amount of CO₂ produced by the inoculum in the blank control. The test was conducted at 20±3°C. Test chambers were identified by project number, test substance ID, test concentration, and vessel number. Magnetic stir bars and stir plates were used to mix the contents of the test chambers. Stir plate motors were cycled on and off approximately every 15 minutes to prevent the transfer of heat from the stirrer motors to the test chambers.

Test Inoculum

Activated sludge was collected from the Prospect Bay Wastewater Treatment Facility on October 1, 1996. The sludge was sieved using a 2 mm screen and then aerated for approximately four hours. After the aeration period, the sludge was homogenized in a blender at medium speed for approximately 2 minutes. The sludge then was allowed to settle for approximately 30 minutes. The supernatant was used as the inoculum for this study and was used the same day that it was prepared. A total suspended solids measurement and standard plate count were performed on the inoculum. The plates were incubated at 20±3°C for approximately 48 hours and then the number of colony forming units was enumerated.

Preparation of Test Chambers

The following was added to each chamber:

- 1) 2470 mL of Nanopure water
- 2) 3 mL of ammonium sulfate solution (4.0%)
3 mL calcium chloride solution (2.75%)
12 mL of ferric chloride solution (0.025%)
3 mL of magnesium sulfate solution (2.25%)
6 mL of phosphate buffer (pH 7.2)
- 3) 30 mL of the activated sludge inoculum

All chambers were aerated with CO₂ free air for approximately 24 hours at a rate of 50 to 100 mL per minute to purge the systems of CO₂. After the aeration period, the flow of CO₂ free air

was stopped, and three CO₂ traps each containing approximately 100 mL of 0.5 N KOH were connected to the exit air lines of each chamber. An amount of test substance necessary to deliver 10 mg C/L was added to the treatment group test chambers by direct weight addition. A volume of reference substance stock solution necessary to deliver 10 mg C/L was added to the reference group chambers. The volume within all chambers was brought up to 3000 mL by the addition of Nanopure water.

Biodegradation Test Initiation

The biodegradation test was started by bubbling CO₂ free air through each of the test chambers at a rate of 50 to 100 mL per minute. The CO₂ produced from the degradation of organic carbon sources within the test chamber was trapped as K₂CO₃ in the KOH solution and the amount of inorganic carbon in the trapping solution was measured at various intervals during the study, using a Shimadzu TOC-5000 carbon analyzer.

Sample Collection and Analysis

The CO₂ traps were removed for analysis on days 1, 5, 9, 12, 15, 19, 22, 26, 29, 35, 40, 47, 54, 61, 68, 76, 82 and 89. The CO₂ trap nearest the chamber was removed and analyzed for inorganic carbon. The two remaining traps were placed one position closer to the test chamber and a new trap was placed on the end of the series.

Test Termination

On the 92nd day of the test, an aliquot of the contents of each test chamber was removed and the pH determined. The contents of each chamber then were acidified by the addition of 1 mL of concentrated hydrochloric acid to drive off inorganic carbonate. The chambers were aerated overnight after which the trapping solutions closest to the test chambers were analyzed for inorganic carbon.

Calculations

The results of the inorganic carbon analyses of the CO₂ traps were converted to mg CO₂ produced using the following equation:

$$\text{mg CO}_2 = \text{result (mgC/L)} \times \text{vol. of KOH (L)} \times 3.67 \text{ mg CO}_2/\text{mg C}$$

The percentage of theoretical carbon dioxide (%TCO₂) evolved was calculated as follows:

$$\% \text{TCO}_2 = \frac{\text{mg CO}_2 \text{ produced}}{(\text{mg of carbon in test}) (3.67 \text{ mg CO}_2/\text{mg Carbon})} \times 100$$

The cumulative %TCO₂ was plotted against time and is presented in Figure 1.

RESULTS AND DISCUSSION

Stock Solution Analysis

The measured total organic carbon (TOC) concentration of the reference substance stock solution used to dose the reference group test chambers was 398.4 mg C/L. The volume of reference substance stock solution used to dose the chambers was adjusted based on the measured TOC value so that the desired amount of carbon was delivered.

Observations and Measurements

The temperature range recorded during the test was 18.3 to 20.6°C and was within the protocol specified range throughout the test. The average measured total suspended solids (TSS) concentration of the inoculum was 219 mg/L. The solids concentration of the test solution was approximately 2.2 mg/L and was within the acceptable limit specified in the test guideline (1). The result of the standard plate count performed on the inoculum was 2.16 x 10⁶ CFU/mL. The standard plate count result indicated that the inoculum used in this study contained an acceptable number of viable microbes according to the referenced guideline (2).

The measured pH of the test chamber contents on Day 92 are presented in Table 1. The pH values ranged from 5.68 to 6.90. The measured concentrations of carbon in the carbon dioxide trapping solutions are presented in Table 2. The cumulative milligrams of carbon dioxide produced over the test period are presented in Table 3. The average total amount of CO₂ evolved by the control chambers was approximately 61.5 milligrams of CO₂. When this value was corrected for the amount of CO₂ in the trapping solution (potassium hydroxide solution, even when freshly prepared, contains carbonates) the average amount of CO₂ actually evolved by the control was 20.4 milligrams. The amount of CO₂ evolved by the control chambers did not exceed the 40 mg/L (120 mg/3 liters) value considered the acceptable limit for CO₂ evolution tests (1).

The cumulative percentages of theoretical carbon dioxide evolved from the test and control flasks are presented in Table 4. A plot of the cumulative percent of theoretical CO₂ (%TCO₂) produced over time is presented in Figure 1. The viability of the inoculum and validity of the test were supported by the results of the reference substance, sodium benzoate, from which 97.8% of theoretical CO₂ was evolved. An average percent biodegradation of greater than 60% was achieved within 5 days, thereby fulfilling the criteria for a valid test by reaching the pass level by day 14 (1). The average cumulative percent of theoretical carbon dioxide produced by the test substance was 0.0 and 2.4% on days 29 and 93, respectively. The standard 28-day test period (1,2) was extended so that sufficient opportunity for biodegradation would be provided.

CONCLUSIONS

Minimal degradation of the test substance was observed over a 93-day test period. The standard 28-day test period (1,2) was extended so that sufficient opportunity for biodegradation would be provided. Pentabromodiphenyl oxide (PeBDPO) evolved an average of approximately 0.0 and 2.4% TCO₂ on days 29 and 93, respectively. Evidence of ready biodegradability in a Carbon Dioxide Evolution Test is 60% TCO₂ within the 28-day test period (1,2). In addition, the pass value must be reached within 10 days of achieving 10% TCO₂ (1,2).

REFERENCES

1. **Organisation for Economic Cooperation and Development.** 1992. *Ready Biodegradability: CO₂ Evolution (Modified Sturm Test)*. OECD Guideline 301B.
2. **Official Journal of the European Communities.** 1967. *Determination of Ready Biodegradability: CO₂ Evolution Test, Method C.4-C.*

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Table 1

Measured pH of Test and Control Solutions at Test Termination

| Test Substance | pH |
|------------------------------------|------|
| Control Rep. A | 6.83 |
| Control Rep. B | 6.90 |
| Sodium Benzoate Rep. A (10 mg C/L) | 6.02 |
| Sodium Benzoate Rep. B (10 mg C/L) | 5.68 |
| PeBDPO Rep. A (10 mg C/L) | 6.56 |
| PeBDPO Rep. B (10 mg C/L) | 6.47 |

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Table 2

Inorganic Carbon Present in the Trapping Solution at
Various Intervals During the Study (mg C/L)

| Date | Day | Control Rep. A | Control Rep. B | Benzoate Rep. A | Benzoate Rep. B | FeBDPO Rep. A | FeBDPO Rep. B | Blank |
|--------|-----|-------------------|-------------------|--------------------|--------------------|------------------|------------------|------------------|
| Oct-03 | 1 | 9.9 | 9.4 | 12.1 | 10.0 | 9.5 | 9.5 | 4.9 ² |
| Oct-07 | 5 | 13.0 | 12.5 | 208.8 | 216.0 | 13.9 | 15.5 | 3.0 |
| Oct-11 | 9 | 9.5 | 9.1 | 52.0 ¹ | 54.5 | 11.3 | 10.9 | 5.0 |
| Oct-14 | 12 | 7.0 | 7.5 | 22.4 | 21.5 | 6.9 | 6.7 | 5.6 |
| Oct-17 | 15 | 12.8 | 6.9 | 18.5 | 18.3 | 5.8 | 5.6 | 5.6 |
| Oct-21 | 19 | 7.1 | 6.7 | 17.7 | 14.0 | 5.8 | 10.8 | 4.9 ² |
| Oct-24 | 22 | 6.3 | 5.5 | 11.5 | 9.2 | 7.0 | 8.8 | 6.7 |
| Oct-28 | 26 | 8.6 | 6.1 | 10.8 | 11.1 | 6.9 | 7.5 | 5.7 |
| Oct-31 | 29 | 16.7 | 8.2 | 7.3 | 7.5 | 4.7 ² | 5.9 | 6.5 |
| Nov-06 | 35 | 14.2 | 10.8 | 9.2 | 9.8 | 10.9 | 8.6 | 5.1 |
| Nov-11 | 40 | 10.6 | 12.0 | 12.7 | 12.3 | 10.2 | 10.4 | 8.5 |
| Nov-18 | 47 | 8.4 | 7.3 | 8.9 | 11.7 | 8.8 | 9.1 | 5.4 |
| Nov-25 | 54 | 8.1 | 9.9 | 7.0 | 28.1 | 22.1 | 8.6 | 5.9 |
| Dec-02 | 61 | 8.8 | 7.1 | 6.2 | 10.3 | 10.5 | 8.2 | 8.0 |
| Dec-09 | 68 | 6.7 | 6.9 | 5.7 | 8.2 | 8.2 | 7.2 | 10.0 |
| Dec-17 | 76 | 9.6 | 8.6 | 6.3 | 9.3 | 10.5 | 9.5 | 5.7 |
| Dec-23 | 82 | 6.1 | 10.8 | 11.8 | 8.3 | 9.0 | 9.4 | 5.6 |
| Dec-30 | 89 | 5.1 | 6.2 | 8.2 | 7.5 | 8.7 | 8.1 | 4.9 ² |
| Jan-03 | 93 | 6.7 | 8.2 | 8.8 | 8.4 | 9.2 | 9.4 | 4.9 ² |

¹ Result was above the highest calibration standard.

² Result was below the lowest calibration standard.

Table 3

Cumulative Milligrams of Carbon Dioxide Evolved¹

| Date | Day | Control Rep. A | Control Rep. B | Benzoate ² Rep. A | Benzoate ² Rep. B | PeBDPO ² Rep. A | PeBDPO ² Rep. B | Control ³ Rep. A | Control ³ Rep. B | Blank |
|--------|-----|-------------------|-------------------|---------------------------------|---------------------------------|-------------------------------|-------------------------------|--------------------------------|--------------------------------|-------|
| Oct-03 | 1 | 3.6 | 3.4 | 0.9 | 0.2 | 0.0 | 0.0 | 1.8 | 1.6 | 1.8 |
| Oct-07 | 5 | 8.4 | 8.0 | 72.9 | 74.7 | 0.4 | 1.0 | 5.5 | 5.1 | 2.9 |
| Oct-11 | 9 | 11.9 | 11.4 | 88.5 | 91.2 | 1.0 | 1.5 | 7.2 | 6.7 | 4.7 |
| Oct-14 | 12 | 14.5 | 14.1 | 94.1 | 96.5 | 1.0 | 1.3 | 7.7 | 7.3 | 6.8 |
| Oct-17 | 15 | 19.2 | 16.7 | 97.2 | 99.6 | 0.0 | 0.0 | 10.4 | 7.9 | 8.8 |
| Oct-21 | 19 | 21.8 | 19.1 | 101.2 | 102.2 | 0.0 | 1.2 | 11.2 | 8.5 | 10.6 |
| Oct-24 | 22 | 24.1 | 21.1 | 103.3 | 103.5 | 0.0 | 2.3 | 11.0 | 8.0 | 13.1 |
| Oct-28 | 26 | 27.2 | 23.4 | 104.5 | 104.8 | 0.0 | 2.3 | 12.0 | 8.2 | 15.2 |
| Oct-31 | 29 | 33.4 | 26.4 | 102.6 | 103.0 | 0.0 | 0.0 | 15.8 | 8.8 | 17.6 |
| Nov-06 | 35 | 38.6 | 30.4 | 101.4 | 102.0 | 0.0 | 0.0 | 19.1 | 10.9 | 19.5 |
| Nov-11 | 40 | 42.5 | 34.8 | 101.9 | 102.3 | 0.0 | 0.0 | 19.9 | 12.2 | 22.6 |
| Nov-18 | 47 | 45.5 | 37.4 | 102.3 | 103.8 | 0.0 | 0.0 | 20.9 | 12.8 | 24.6 |
| Nov-25 | 54 | 48.5 | 41.1 | 101.6 | 110.8 | 0.6 | 0.0 | 21.8 | 14.4 | 26.7 |
| Dec-02 | 61 | 51.7 | 43.7 | 101.0 | 111.7 | 1.6 | 0.0 | 22.0 | 14.0 | 29.7 |
| Dec-09 | 68 | 54.2 | 46.2 | 100.6 | 112.2 | 2.1 | 0.0 | 20.9 | 12.9 | 33.3 |
| Dec-17 | 76 | 57.7 | 49.4 | 99.5 | 112.2 | 2.6 | 0.0 | 22.3 | 14.0 | 35.4 |
| Dec-23 | 82 | 60.0 | 53.3 | 100.7 | 112.2 | 2.8 | 0.0 | 22.5 | 15.8 | 37.5 |
| Dec-30 | 89 | 61.8 | 55.6 | 101.7 | 112.9 | 3.9 | 0.1 | 22.5 | 16.3 | 39.3 |
| Jan-03 | 93 | 64.3 | 58.6 | 102.1 | 113.2 | 4.5 | 0.8 | 23.2 | 17.5 | 41.1 |
| Mean | | 61.5 | | 107.7 | | 2.7 | | 20.4 | | NA |

¹ The results of the inorganic carbon analyses of the CO₂ traps were converted to mg CO₂ produced using the following equation:

$$\text{mg CO}_2 = \text{result (mgC/L)} \times \text{vol. of KOH (L)} \times 3.67 \text{ mg CO}_2/\text{mg C}$$

² Corrected for the average amount of CO₂ evolved by control to correct for the amount of CO₂ attributed to the inoculum and the KOH.

³ Value has been corrected for the amount of CO₂ in the trapping solution since potassium hydroxide solution, even when freshly prepared, contains carbonates.

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Table 4
Cumulative Percent of Theoretical Carbon Dioxide Evolved¹

| Date | Day | Control Rep. A. | Control Rep. B | Benzoate Rep. A | Benzoate Rep. B | PeBDPO Rep. A | PeBDPO Rep. B |
|--|-----|--------------------|-------------------|--------------------|--------------------|------------------|------------------|
| Oct-03 | 1 | NA | NA | 0.8 | 0.2 | 0.0 | 0.0 |
| Oct-07 | 5 | NA | NA | 66.2 | 67.8 | 0.4 | 0.9 |
| Oct-11 | 9 | NA | NA | 80.4 | 82.8 | 0.9 | 1.4 |
| Oct-14 | 12 | NA | NA | 85.5 | 87.6 | 0.9 | 1.2 |
| Oct-17 | 15 | NA | NA | 88.3 | 90.5 | 0.0 | 0.0 |
| Oct-21 | 19 | NA | NA | 91.9 | 92.8 | 0.0 | 1.1 |
| Oct-24 | 22 | NA | NA | 93.8 | 94.0 | 0.0 | 2.1 |
| Oct-28 | 26 | NA | NA | 94.9 | 95.2 | 0.0 | 2.1 |
| Oct-31 | 29 | NA | NA | 93.2 | 93.6 | 0.0 | 0.0 |
| Nov-06 | 35 | NA | NA | 92.1 | 92.6 | 0.0 | 0.0 |
| Nov-11 | 40 | NA | NA | 92.6 | 92.9 | 0.0 | 0.0 |
| Nov-18 | 47 | NA | NA | 92.9 | 94.3 | 0.0 | 0.0 |
| Nov-25 | 54 | NA | NA | 92.3 | 100.6 | 0.5 | 0.0 |
| Dec-02 | 61 | NA | NA | 91.7 | 101.5 | 1.5 | 0.0 |
| Dec-09 | 68 | NA | NA | 91.4 | 101.9 | 1.9 | 0.0 |
| Dec-17 | 76 | NA | NA | 90.4 | 101.9 | 2.4 | 0.0 |
| Dec-23 | 82 | NA | NA | 91.5 | 101.9 | 2.5 | 0.0 |
| Dec-30 | 89 | NA | NA | 92.4 | 102.5 | 3.5 | 0.1 |
| Jan-03 | 93 | NA | NA | 92.7 | 102.8 | 4.1 | 0.7 |
| Mean Cumulative Percent TCO ₂ | | | | 97.8 | | 2.4 | |

¹ The percentage of TCO₂ was calculated using the following equation:

$$\% \text{TCO}_2 = \frac{\text{mg CO}_2 \text{ produced}}{(\text{mg of carbon in test}) (3.67 \text{ mg CO}_2 / \text{mg Carbon})} \times 100$$

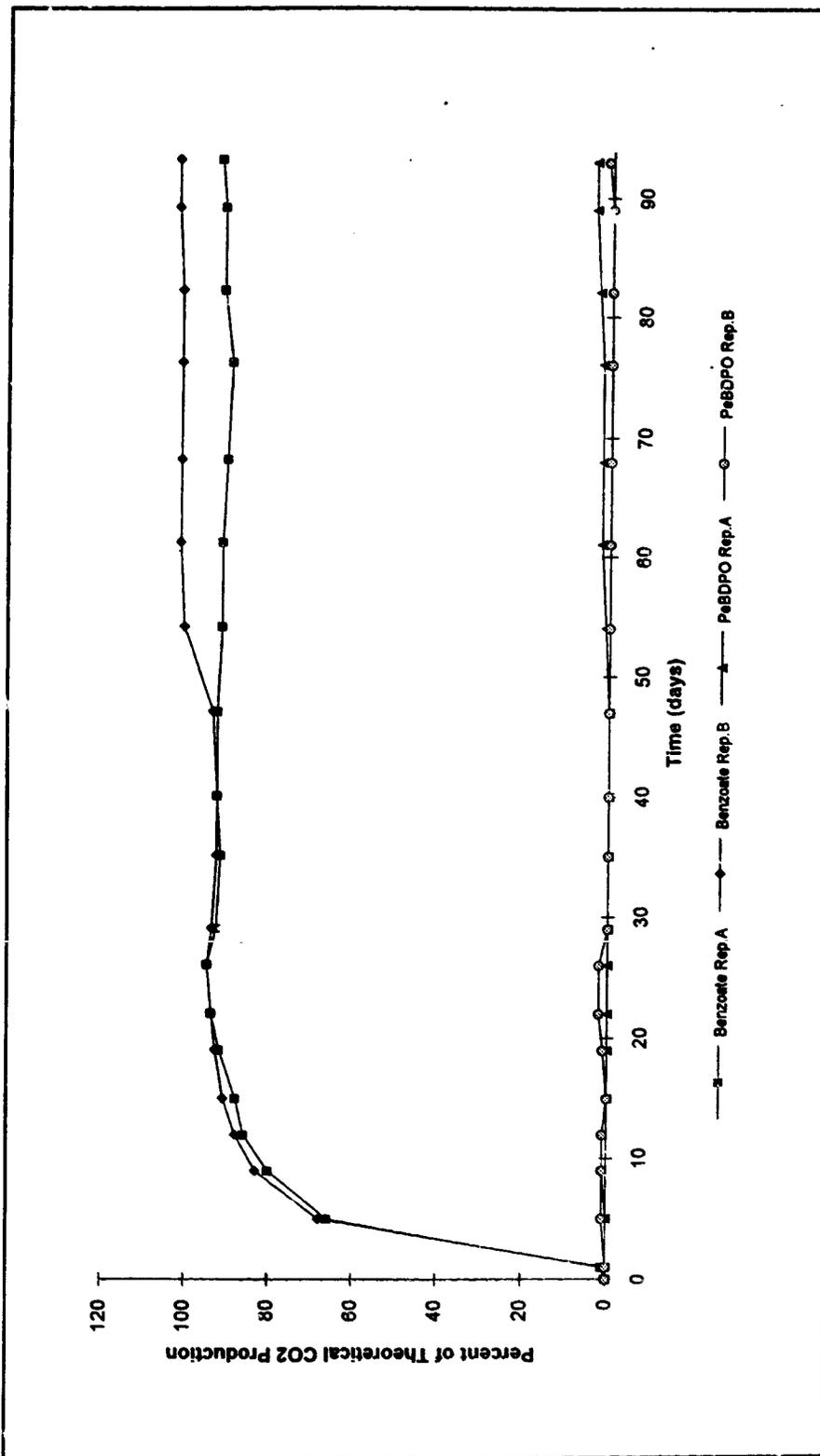


Figure 1. Cumulative Percentage of Theoretical Carbon Dioxide Production.

APPENDIX A

Protocol, Amendments and Deviation