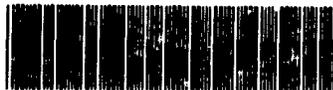




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Dr. Lynn Goldman  
Assistant Administrator  
Office of Prevention, Pesticides and Toxic Substances TS-7101  
Environmental Protection Agency  
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Washington, DC 20460

Dear Dr. Goldman:

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

OCTABROMODIPHENYL OXIDE (OBDPO): Determination of the Water Solubility.

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 M. Price*  
Courtney M. Price  
Vice President, CHEMSTAR

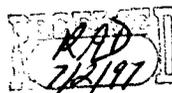
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**OCTABROMODIPHENYL OXIDE (OBDPO):  
DETERMINATION OF THE WATER SOLUBILITY**

**WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER: 439C-110**

**U.S. EPA 40 CFR Ch. 1 § 796.1860 Water Solubility (Generator Column Method)  
OECD Guideline 105 Water Solubility (Column Elution Method)**

**AUTHORS:**

Joel I. Stenzel  
Barbara J. Markley, Ph.D.

**STUDY INITIATION DATE: April 10, 1996**

**STUDY COMPLETION DATE: June 13, 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  
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**GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT**

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

**TITLE:** Octabromodiphenyl Oxide (OBDPO): Determination of the Water Solubility

**WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER:** 439C-110

**STUDY COMPLETION:** June 13, 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.

**STUDY DIRECTOR:**

Joel L. Stenzel  
Joel L. Stenzel, B.S.  
Senior Chemist

6/13/97  
DATE

**SPONSOR APPROVAL:**

Hammukh Shah  
Sponsor

6/18/97  
DATE

- 3 -

## QUALITY ASSURANCE

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

TITLE: Octabromodiphenyl Oxide (OBDFPO): Determination of the Water Solubility

WILDLIFE INTERNATIONAL LTD. PROJECT NO.: 439C-110

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 Composite Preparation	April 10, 1996	April 11, 1996	April 15, 1996
Stock Solution Preparation	August 6, 1996	August 3, 1996	August 14, 1996
Generator Column Preparation	September 25, 1996	September 26, 1996	September 26, 1996
Matrix Fortification and Sample Extraction	October 2, 1996	October 2, 1996	October 4, 1996
Draft Report and Data	March 7, 10, 11 and 12, 1997	March 12, 1997	May 12, 1997
Final Report	June 12, 1997	June 12, 1997	June 13, 1997

Susan L. Hopper  
 Susan L. Hopper  
 Senior Quality Assurance Representative

6-13-97  
 DATE

**REPORT APPROVAL**

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

TITLE: Octabromodiphenyl Oxide (OBDPO): Determination of the Water Solubility

WILDLIFE INTERNATIONAL LTD. PROJECT NO.: 439C-110

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:

Joel I. Stenzel  
Joel I. Stenzel, B.S.  
Senior Chemist

6/13/97  
DATE

MANAGEMENT:

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

6/13/97  
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-110
TEST SUBSTANCE:	Octabromodiphenyl oxide (OBDPO)
STUDY:	Octabromodiphenyl oxide (OBDPO): Determination of the Water Solubility
TEST DATES:	Experimental Start - May 3, 1996 Experimental Termination - December 12, 1996

SUMMARY:	The solubility of OBDPO in water at 25.0°C was determined to be less than 1.0 µg/L (ppb) using a column elution 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 a column elution method. Samples were eluted from a generator column and analyzed from November 13 to December 12, 1996. Raw data generated by Wildlife International Ltd. and a copy of the final report are filed under Project Number 439C-110 in archives located on the Wildlife International Ltd. site.

## OBJECTIVE

The objective of this study was to determine the solubility limit of the test substance, octabromodiphenyl oxide (OBDPO), in water at 25.0°C using a column elution method.

## EXPERIMENTAL DESIGN

A preliminary test was conducted to estimate the solubility of the test substance in reagent water at room temperature. A generator column was prepared for a preliminary test. As a result of differences in the saturation concentrations at the two flow rates, a second generator column was prepared for the definitive test. The column temperature was maintained at 25.0 °C and reagent water was pumped through it at approximately 0.5 mL per minute to elute the test substance. Samples of the eluate were collected and analyzed to determine the saturation concentration of the test substance. The flow rate was reduced to approximately half the original flow rate and the saturation concentration determined again.

**MATERIALS AND METHODS**

This study was conducted according to procedures outlined in the protocol, "Octabromodiphenyl Oxide (OBDPO): Determination of the Water Solubility" (Appendix I). The protocol was based on procedures outlined in OECD Guidelines, Method 105 (1); and EPA 40 CFR § 796.1860 (2). The column elution method was used to determine the water solubility limit of the test substance.

**Test Substance**

The test substance consisted of a composite of octabromodiphenyl oxide (OBDPO) samples received from three manufacturers. The materials 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. ID No.</u>
Albemarle Corp.	20405-1B	December 20, 1995	3517
Bromine Compounds Ltd.	951107	March 7, 1996	3601
Great Lakes Chemical Corp.	5525DI21A	March 12, 1996	3603

An equal part (500 g) of each of the manufacturer's products was placed in a 2-L, high density polyethylene (HDPE) bottle. The bottle was placed on a reciprocating shaker for two hours. The composite test substance was assigned Wildlife International Ltd. identification number 3637. Subsamples of the composite test substance were shipped to Albemarle Corporation for characterization and homogeneity analyses. The analyses were performed on May 16, 1996. The results of the analyses indicated the composite test substance was homogeneous and contained the following components:

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Hexabromodiphenyl oxide	5.5%
Heptabromodiphenyl oxide	42.3%
Octabromodiphenyl oxide	36.1%
Nonabromodiphenyl oxide	13.9%
Decabromodiphenyl oxide	2.1%

The composite test substance was stored under ambient conditions.

#### Reagent Water

The reagent water used in this study exceeded the requirements 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  $\mu\text{m}$ . 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 greater than 17.8 megohm-cm.

#### Preliminary Test

A preliminary test was performed to estimate the solubility of the test substance in reagent water. Approximately 100 mg of the composite test substance was placed in a vessel. A pre-determined volume of reagent water was added, and the vessel shaken. The solubility of the test substance was assessed by visual observations. The process was repeated twice by adding successively larger volumes of reagent water.

#### Solvents

Analytical grade ethyl acetate (Burdick and Jackson, Muskegon, MI, catalog number 100-4 or Fisher Chemical, Fairlawn, NJ, catalog number E196-4) was used as an extraction solvent for this study. Phenyl ether, with a purity of >98%, was used for preparation of calibration standards and samples. It was purchased from Fluka Chemika (Switzerland, catalog number 42730). Analytical

- 12 -

grade dimethyl formamide (Burdick and Jackson, catalog number 076-4) was used for the preparation of fortification standards.

#### Preparation of Generator Column

The inert carrier material used to charge the generator column was glass beads. The glass beads were purchased from J.T. Baker (Phillipsburg, NJ, cat. number 7467-01), and identified on the label as Ernpore, Filter Aid 400, high density glass beads. Approximately 25 mL (~69 g) of glass beads were transferred to a 1-L round-bottom flask, then rinsed with ethyl acetate to remove any potential contaminants.

A subsample of the composite test substance (350 mg) was weighed into a 500-mL Erlenmeyer flask. Ethyl acetate was added to the 300-mL mark. The flask was sonicated and swirled until the test substance appeared to be completely dissolved. The test substance solution was transferred to the round-bottom flask containing the glass beads.

A rotary evaporator was used to remove the solvent and coat the test substance onto the glass beads. The temperature of the water bath of the rotary evaporator was 43 to 44 °C. The trap was emptied after the solvent had evaporated, and the flask was replaced on the rotary evaporator for another 60 minutes, to ensure that all the solvent had been removed. The contents of the round-bottom flask then were transferred to a 150-mL beaker. Approximately 20 mL of reagent water were added, and the beaker was swirled and sonicated to remove air bubbles and homogenize the slurry.

The generator column was an Adjusta-Chrom<sup>®</sup>, jacketed, recycling column supplied by Ace Glass Inc. (Vineland, NJ, cat. number 5819). The glass column, 300 mm long with an internal diameter of 10 mm, was equipped with Teflon<sup>®</sup> plungers and glass filter discs at both ends. A small plug of silanized glass wool was placed in the bottom of the column, the plunger inserted, and end fittings fastened to the bottom. The slurry of glass beads coated with test substance was poured into the top of the column. Another small plug of silanized glass wool was placed on the top of the

column, the plunger inserted, and end fittings attached. The height of the material packed in the column was approximately 24 cm. A diagram of the generator column is presented in Figure 1.

#### Apparatus Configuration

A Cannon CT-500 Constant Temperature Bath (Cannon Instrument Company, State College, PA) was used to maintain the test temperature ( $25.0 \pm 0.1^\circ\text{C}$ ) throughout the experiment. The chiller coil of the constant temperature bath was connected to a Nessler Coolflow CFT-25 Refrigerated Recirculator (Nessler Instruments, Inc., Portsmouth, NH). Both the constant temperature bath and recirculating chiller were 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 1F680A submersible pump (Dayton Electric Mfg. Co., Chicago, IL) was placed in the constant temperature bath. The submersible pump was used to pump a continuous stream of water through the jacket surrounding the generator column to maintain a constant temperature.

A 2-liter Erlenmeyer flask was used as a reservoir for the reagent water being pumped through the generator column. The flask was filled with reagent water and submerged in the constant temperature bath so that the top of the flask remained above the surface of the water in the constant temperature bath. The inlet line of a Milton Roy miniPump<sup>®</sup> (P/N 92014901) 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 supply of reagent water in the 2-L flask was replenished intermittently during the experiment.

The eluate from the generator column was directed into a 200<sup>ml</sup> fraction collector (Isco, Inc., Lincoln, NE). The fraction collector was programmed to collect individual samples of the eluate into borosilicate glass bottles. A diagram of the apparatus configuration is presented in Figure 2.

### Sample Collection

The pump was initially set to deliver approximately 0.5 mL of reagent water per minute through the generator column. The fraction collector was programmed to collect eluate samples every 106 min. (~50 mL). The eluate was collected dropwise into 4-ounce French square bottles containing 25 mL of the extraction solvent (ethyl acetate). The sample bottles were labelled with a sequential sample number. After 94 consecutive samples were collected, the flow rate was changed to approximately half the original flow rate (~0.25 mL/min.). The fraction collector was reprogrammed to collect samples every 197 minutes (~50 mL), and sample collection was resumed. The following work day, the volume of the samples was found to be smaller than expected, and the flow rate was readjusted. The fraction collector was reprogrammed to collect samples every 200 minutes (~50 mL), and sample collection resumed with sample number 103. The sample bottles were capped and stored on a laboratory bench under ambient conditions prior to extraction and analysis.

### Analytical Method

The analytical method consisted of extracting the samples with ethyl acetate, evaporating the ethyl acetate, and reconstituting the samples in phenyl ether. A flow chart of the method is presented in Figure 3. Ethyl acetate was added to each sample bottle to adjust the volume to approximately 25 mL prior to the first extraction, to make up for any minor evaporative losses during sample collection. The sample bottle was capped and shaken for approximately 30 seconds. The ethyl acetate layer was transferred to a round-bottom flask using a disposable pipet. The sample was then extracted with another 20-mL portion of ethyl acetate. The extracts were combined in the round-bottom flask, and ~200  $\mu$ L of phenyl ether was added. The extract was placed on a rotary evaporator to remove the ethyl acetate. The remaining residue was transferred to a culture tube. The round-bottom flask was rinsed three times with ethyl acetate and the rinsates were combined in the culture tube. The ethyl acetate was evaporated from the tube under a stream of nitrogen at 47 to 50°C to less than 0.5 mL. The sample was brought to a final volume of 1.0 mL using phenyl ether and placed in a vial for analysis.

Concentrations of OBDPO in the samples were determined using a Hewlett-Packard Model 5890 Gas Chromatograph (GC). The gas chromatograph was equipped with a cyclo-splitter liner in the injection port and an electron capture detector (ECD). Chromatographic separations were achieved using an Rtx-1 capillary column (15 m  $\times$  0.53 mm, 0.1- $\mu$ m film thickness) supplied by Restek Corporation (Bellefonte, PA). Chromatographic grade helium was used as the carrier gas, and argon (95%)/methane (5%) was used as the auxiliary gas. The instrument parameters are summarized in Table 1.

#### Preparation of Quality Control Samples

A fortification standard of OBDPO in dimethyl formamide (DMF) was prepared at a concentration of 1.0 mg/L. This standard was used to fortify reagent water (matrix spikes) and phenyl ether (reagent spikes). A matrix blank and two matrix fortifications (1 ppb and 5 ppb) were prepared each day that samples from the generator column were extracted. A reagent blank and reagent fortification (250 ppb) were also analyzed with each sample set.

#### Calibration and Quantitation

Calibration standards of OBDPO were prepared in phenyl ether. The standards were prepared using the test substance and ranged in concentration from 25 to 250  $\mu$ g OBDPO/L. A complete set of calibration standards was analyzed before and after each set of samples, and a standard injected after a maximum of every five samples. Representative chromatograms of low and high calibration standards are shown in Figures 4 and 5, respectively. The sum of the peak areas for the major components was used to determine the instrument response. The major components used for quantitation of the test substance were heptabromodiphenyl oxide (Br7), octabromodiphenyl oxide (Br8), and nonabromodiphenyl oxide (Br9). No attempt was made to quantitate the hexabromodiphenyl oxide or decabromodiphenyl oxide components of the test substance, since peaks for these components could not be detected at the lowest calibration standard concentration (25  $\mu$ g/L). The instrument limit of detection (LOD) for this study was not experimentally determined, but was set based upon the injection volume (2  $\mu$ L) and the lowest calibration standard concentration (25  $\mu$ g/L). The LOD, thus, was set at 50 pg of OBDPO injected.

Calibration curves were calculated based on linear regression equations using the instrument responses versus the respective standard concentrations. A representative linear calibration curve is presented in Figure 6.

## RESULTS AND DISCUSSION

### Preliminary Test

The test substance at approximately 100 mg did not appear to be soluble in 10 mL, 100 mL or 1 L of reagent water. Most of the test substance was observed to settle to the bottom of the bottle. The solubility was estimated to be much less than 100 mg/L, based on visual observations.

An experiment was conducted using the column elution method prior to the definitive test. A generator column was prepared, and samples were collected at flow rates of approximately 0.5 and 0.25 mL/min. The samples were extracted and analyzed. The solubility of OBDPO was determined to be 3.9  $\mu\text{g/L}$  and  $< 0.5 \mu\text{g/L}$  at the nominal flow rates of 0.5 and 0.25 mL/min., respectively. The measured concentrations in the samples at each flow rate were not within  $\pm 30\%$  of each other; therefore, the experiment was repeated.

### Quality Control Samples

No interferences were observed at or above the limit of detection (0.5  $\mu\text{g OBDPO/L}$ ) in any of the matrix blank samples. A representative chromatogram of a matrix blank is shown in Figure 7. The instrument response for the matrix blanks (sum of peak areas) was always below the response of the lowest calibration standard. There were also no interferences observed in the reagent blank (phenyl ether) samples. A representative chromatogram of a reagent blank is shown in Figure 8.

The mean recovery from nine matrix samples fortified at 5 ppb was 102% (standard deviation 9.6), and ranged from 88% to 116%. A representative chromatogram of a 5-ppb matrix fortification is shown in Figure 9. The mean recovery from nine matrix samples fortified at 1-ppb was 113% (standard deviation 35), and ranged from 80% to 190%. A representative chromatogram

of a 1-ppb matrix fortification is shown in Figure 10. Although the low-level matrix fortifications did not always yield acceptable recoveries, none had less than 80% recovery; therefore, the 1-ppb concentration was considered the limit of quantitation (LOQ). The mean recovery from nine reagent samples fortified at 250 ppb was 99% (standard deviation 19), and ranged from 52% to 118%. A representative chromatogram of a 250-ppb reagent fortification is shown in Figure 11.

#### Column Elution

The temperature of the water bath ranged from 25.00 to 25.05°C during the experiments (Table 2). The flow rate of reagent water through the generator column was measured at the start of sample collection for each pump setting. Flow rates were also calculated based on the volume and collection time of each sample that was analyzed. The initial pump setting was 17.5 and the flow rate was measured at 0.48 mL/min. The calculated flow rates for samples collected at the initial setting averaged 0.48 mL/min. and ranged from 0.46 to 0.49 mL/min. (Table 3). After collection of sample number 94, the flow rate was reduced to approximately half the initial flow rate. The pump setting was changed to 8.0 and the flow rate was measured at 0.25 mL/min. The calculated flow rates for samples collected at this pump setting averaged 0.20 mL/min. and ranged from 0.19 to 0.21 mL/min. (Table 4).

The results from the analyses of samples eluted at a nominal flow rate of 0.5 mL/min. are presented in Table 3 and Figure 12. Most of the samples were analyzed to determine when the solubility limit was achieved. With the exception of sample sets 5 and 6, the samples were quantitated based on the sum of the peak areas of two heptabromodiphenyl oxide peaks (Br7), two octabromodiphenyl oxide peaks (Br8), and one nonabromodiphenyl oxide peak (Br9). The results from sample sets 5 and 6 were not used to determine the solubility limit of the test substance, because the samples were not considered part of the solubility plateau. The results from sample sets 5 and 6 were quantitated based on the sum of the peak areas of a single peak for each of the components (Br7, Br8 and Br9). The results from sample sets 5 and 6 were considered estimates and are presented in parentheses in the tables. The results from samples 1 to 25 closely match the pattern of results from the preliminary column. The solubility limit was considered to have been

- 18 -

achieved when the test substance concentrations of at least five consecutive samples gave similar results. The results from samples 68 to 81 met these criteria and were considered to have reached the solubility plateau. A representative chromatogram is shown in Figure 13. The mean concentration of OBDPO measured in these samples was less than 0.5 ppb, and the standard deviation could not be calculated.

The results from analyses of samples eluted at a nominal flow rate of 0.25 mL/min. are presented in Table 4 and Figure 14. The results from samples 111 to 123 were considered to have reached the solubility plateau. A representative chromatogram is shown in Figure 15. The mean concentration of OBDPO measured in these samples was 0.8 ppb with a standard deviation of 0.08.

### CONCLUSIONS

Based on the results from samples collected at both flow rates from the generator column, the solubility of OBDPO in water was determined to be less than 1.0  $\mu\text{g}$  OBDPO/L (ppb) at 25.0°C.

**REFERENCES**

1. **Organisation for Economic Cooperation and Development.** 1981. Guideline for Testing of Chemicals, Method 105, "Water Solubility" (Column Elution Method - Flask Method).
2. **U.S. Environmental Protection Agency.** 1991. 40 CFR § 796.1860, Water Solubility (generator column method). Washington, D.C.

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Table 1

## Typical Gas Chromatographic Operational Parameters

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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- $\mu$ 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 $\mu$ L
INJECTION MODE:	Splitless
CARRIER GAS:	Helium at 50 mL/minute from split vent
MAKE-UP GAS:	Argon (95%)/Methane (5%) at ~60 mL/minute from detector
OCTABROMODIPHENYL OXIDE (OBDPO) PEAK RETENTION TIMES:	14.0, 15.6, 17.2, 18.8 and 22.6 minutes

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Table 2

## Water Bath Test Temperatures

Date & Time of Observation	Temperature (°C)
11/13/96 - 3:22 pm	25.05
11/14/96 - 8:19 am	25.05
11/14/96 - 1:37 pm	25.00
11/14/96 - 4:35 pm	25.00
11/15/96 - 8:13 am	25.00
11/15/96 - 11:52 am	25.05
11/15/96 - 5:00 pm	25.05
11/16/96 - 1:51 pm	25.00
11/16/96 - 2:08 pm	25.00
11/17/96 - 5:02 pm	25.05
11/17/96 - 5:18 pm	25.05
11/18/96 - 8:03 am	25.00
11/18/96 - 2:36 pm	25.00
11/19/96 - 8:16 am	25.05
11/19/96 - 12:10 pm	25.00
11/19/96 - 4:56 pm	25.05
11/20/96 - 8:15 am	25.05
11/20/96 - 2:25 pm	25.05
11/20/96 - 5:05 pm	25.05
11/21/96 - 8:35 am	25.00
11/21/96 - 1:39 pm	25.05
11/21/96 - 4:50 pm	25.05
11/22/96 - 9:26 am	25.05
11/22/96 - 5:28 pm	25.00
11/24/96 - 12:05 pm	25.00
11/25/96 - 8:26 am	25.00

Table 3

Results for Samples Collected at a Nominal Flow Rate of 0.5 mL/min.

Sample ID (439C-110-)	Sample Volume (mL)	Collection Time (min.)	Flow Rate (mL/min.)	Measured Concentration ( $\mu\text{g}$ OBDPO/L)
1	49	106	0.463	0.9
2	49	106	0.463	[5.8] <sup>1</sup>
3	49	106	0.463	4.7
4	50	106	0.472	[5.3] <sup>1</sup>
5	50	106	0.472	4.1
6	50	106	0.472	[6.0] <sup>1</sup>
7	50	106	0.472	[6.4] <sup>1</sup>
8	50	106	0.472	4.0
9	50	106	0.472	[13.8] <sup>1</sup>
10	51	106	0.481	2.4
11	52	106	0.481	2.6
12	51	106	0.481	4.3
13	51	106	0.481	3.7
14	51	106	0.481	3.8
15	51	106	0.481	3.7
16	52	106	0.489	3.4
17	51	106	0.481	4.2
18	51	106	0.481	4.0
19	50	106	0.472	4.6
20	51	106	0.481	4.1
21	50	106	0.472	4.5
22	50	106	0.472	4.0
23	50	106	0.472	4.6
24	51	106	0.481	3.6
25	52	106	0.489	3.5
26	52	106	0.489	3.9
27	51	106	0.481	(3.2) <sup>2</sup>
28	51	106	0.481	---
29	51	106	0.481	(3.2) <sup>2</sup>
30	51	106	0.481	(3.2) <sup>2</sup>
31	51	106	0.481	(3.2) <sup>2</sup>
32	51	106	0.481	3.7
33	51	106	0.481	3.8
34	52	106	0.489	3.6
35	51	106	0.481	3.1
36	51	106	0.481	3.4
37	51	106	0.481	3.0
38	50	106	0.472	3.1
39	50	106	0.472	2.5
40	50	106	0.472	2.8
41	51	106	0.481	2.1
42	51	106	0.481	2.2

<sup>1</sup> Numbers in brackets are extrapolated values.<sup>2</sup> Numbers in parentheses are estimated values.<sup>3</sup> Sample was contaminated, no value reported.

Table 3 (Continued)

Results for Samples Collected at a Nominal Flow Rate of 0.5 mL/min.

Sample ID (439C-110-)	Sample Volume (mL)	Collection Time (min)	Flow Rate (mL/min.)	Measured Concentration ( $\mu$ g OBDF O/L)
43	51	106	0.481	2.1
44	51	106	0.481	1.9
45	51	106	0.481	1.7
46	51	106	0.481	1.6
47	50	106	0.472	1.4
48	50	106	0.472	1.2
49	50	106	0.472	1.2
50	50	106	0.472	1.1
51	50	106	0.472	1.0
52	50	106	0.472	1.0
53	50	106	0.472	0.9
54	51	106	0.481	0.8
55	51	106	0.481	0.8
56	22	46	0.472	0.7
57	51	106	0.481	0.7
58	51	106	0.481	0.8
59	51	106	0.481	0.7
60	51	106	0.481	0.7
61	50	106	0.472	0.6
62	50	106	0.472	0.6
63	50	106	0.472	0.6
64	50	106	0.472	0.6
65	51	106	0.481	0.6
66	52	106	0.489	0.5
67	52	106	0.489	0.5
68	51	106	0.481	<0.5
69	51	106	0.481	<0.5
70	51	106	0.481	<0.5
71	51	106	0.481	<0.5
72	50	106	0.472	<0.5
73	51	106	0.481	<0.5
74	51	106	0.481	<0.5
75	50	106	0.472	<0.5
76	50	106	0.472	<0.5
77	50	106	0.472	<0.5
78	50	106	0.472	<0.5
79	50	106	0.472	<0.5
80	50	106	0.472	<0.5
81	50	106	0.472	<0.5

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Table 4

Results for Samples Collected at a Nominal Flow Rate of 0.25 mL/min.

Sample ID (439C-110-)	Sample Volume. (mL)	Collection Time (min.)	Flow Rate (mL/min.)	Measured Concentration ( $\mu$ g OBDPO/L)
95	38	197	0.192	(0.8) <sup>1</sup>
96	39	197	0.196	(0.8) <sup>1</sup>
97	39	197	0.196	(<0.5) <sup>1</sup>
98	39	197	0.196	0.5
99	39	197	0.196	0.8
100	39	197	0.196	0.7
101	39	197	0.196	0.7
103	41	200	0.203	(1.0) <sup>1</sup>
104	41	200	0.203	(<0.5) <sup>1</sup>
105	41	200	0.203	(1.2) <sup>1</sup>
106	41	200	0.203	(<0.5) <sup>1</sup>
107	41	200	0.203	(1.2) <sup>1</sup>
108	41	200	0.203	0.8
109	41	200	0.203	0.8
110	35	174	0.201	1.2
111	42	200	0.208	0.8
112	42	200	0.208	0.8
113	42	200	0.208	0.6
114	41	200	0.203	0.8
115	42	200	0.208	0.7
116	41	200	0.203	0.9
117	41	200	0.203	0.9
118	41	200	0.203	0.8
119	41	200	0.203	0.8
120	41	200	0.203	0.7
121	41	200	0.203	0.7
122	41	200	0.203	0.8
123	37	180	0.204	0.8

<sup>1</sup>Numbers in parentheses are estimated values.

0 0 2 6

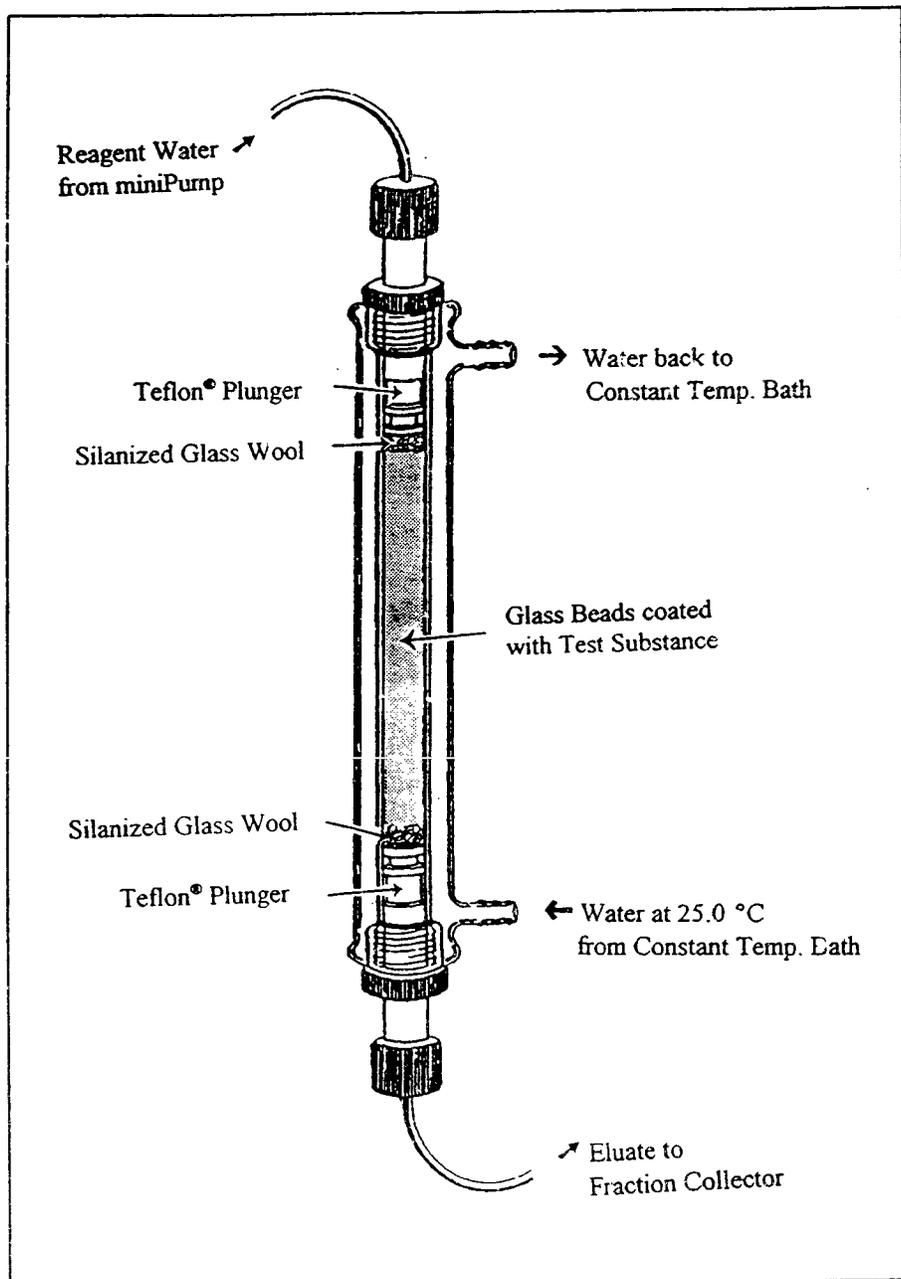


Figure 1. Diagram of generator column.

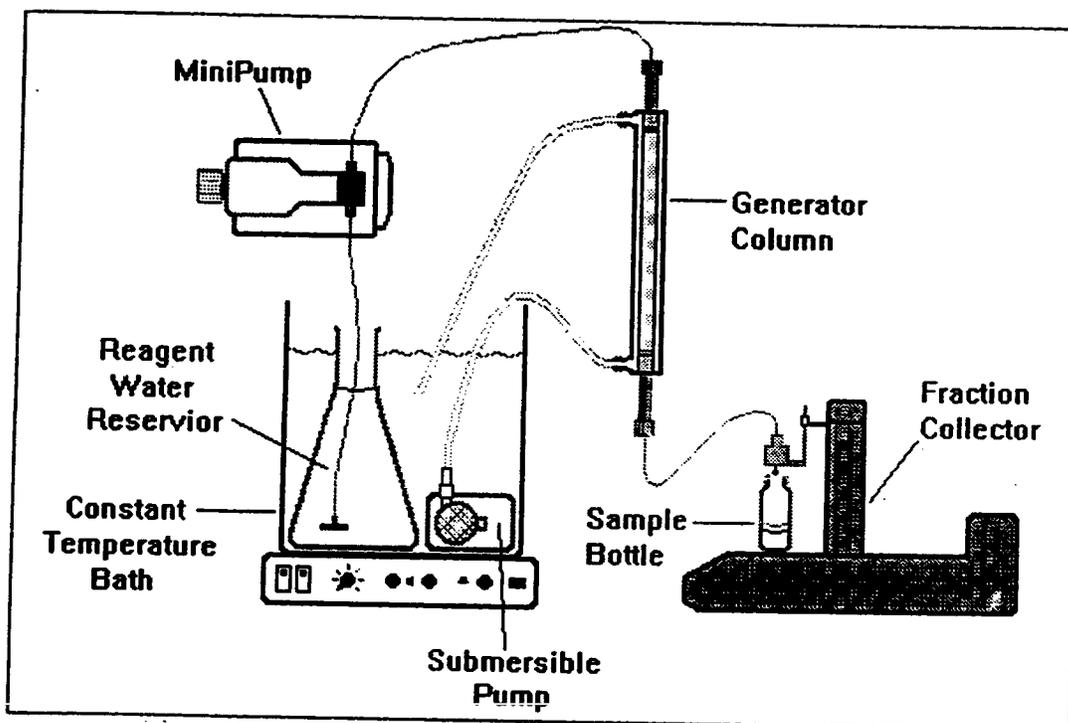


Figure 2. Diagram of apparatus configuration.

**ANALYTICAL METHOD FLOW CHART**

Add 25 mL of ethyl acetate to 4-oz. French square bottles. Label the bottles and place on fraction collector. Collect the eluate fractions from the generator column into the bottles. Cap the bottles as soon as possible after collection and store on lab bench.

↓

Prepare reagent and matrix blanks and fortifications as needed.

↓

Add ethyl acetate to the samples to adjust the volume to ~25 mL. Cap the bottles and shake for about 30 seconds. Pipet the ethyl acetate layer into a round bottom flask.

↓

Repeat the extraction with an additional 20 mL of ethyl acetate.

↓

Combine the extracts and add 200  $\mu$ L of phenyl ether (diphenyl oxide, DPO). Measure the volume of water in each sample using a graduated cylinder. Evaporate the extract on a rotovap at 30 to 50°C to less than 0.5 mL.

↓

Pipet the extract from the round bottom flask to a sample tube. Rinse the round bottom flask with 1 to 1.5 mL of ethyl acetate.

↓

Transfer the ethyl acetate to the sample tube.

↓

Repeat the rinsing steps two more times.

↓

Evaporate the sample on a nitrogen evaporator at 30 to 50°C to less than 0.5 mL. Add phenyl ether to bring the sample volume to 1.0 mL.

↓

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

Figure 3. Analytical method flow chart.

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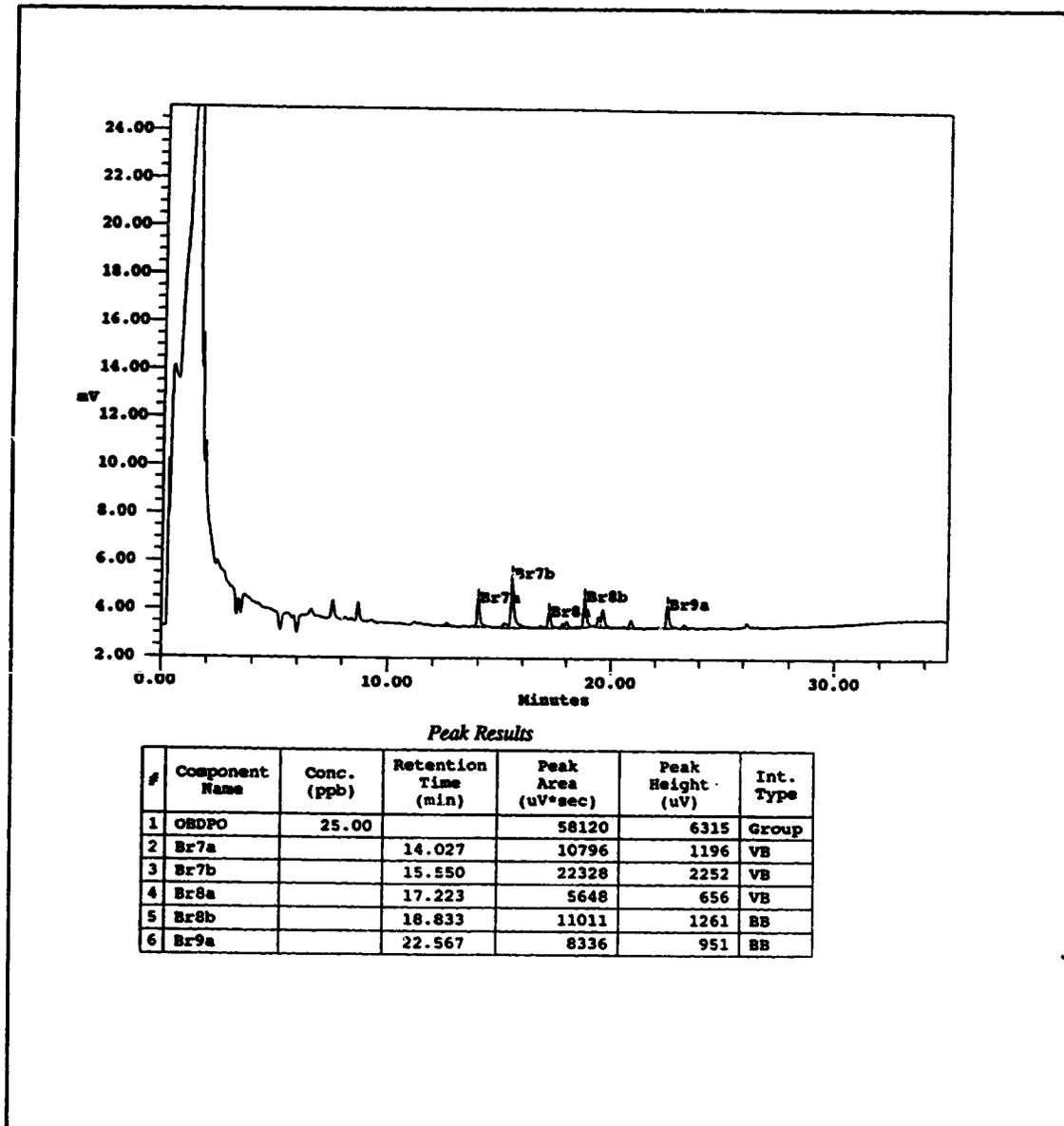


Figure 4. Representative chromatogram of a low-level calibration standard.  
(3637-5C1, sample set OBROM12, vial 1)

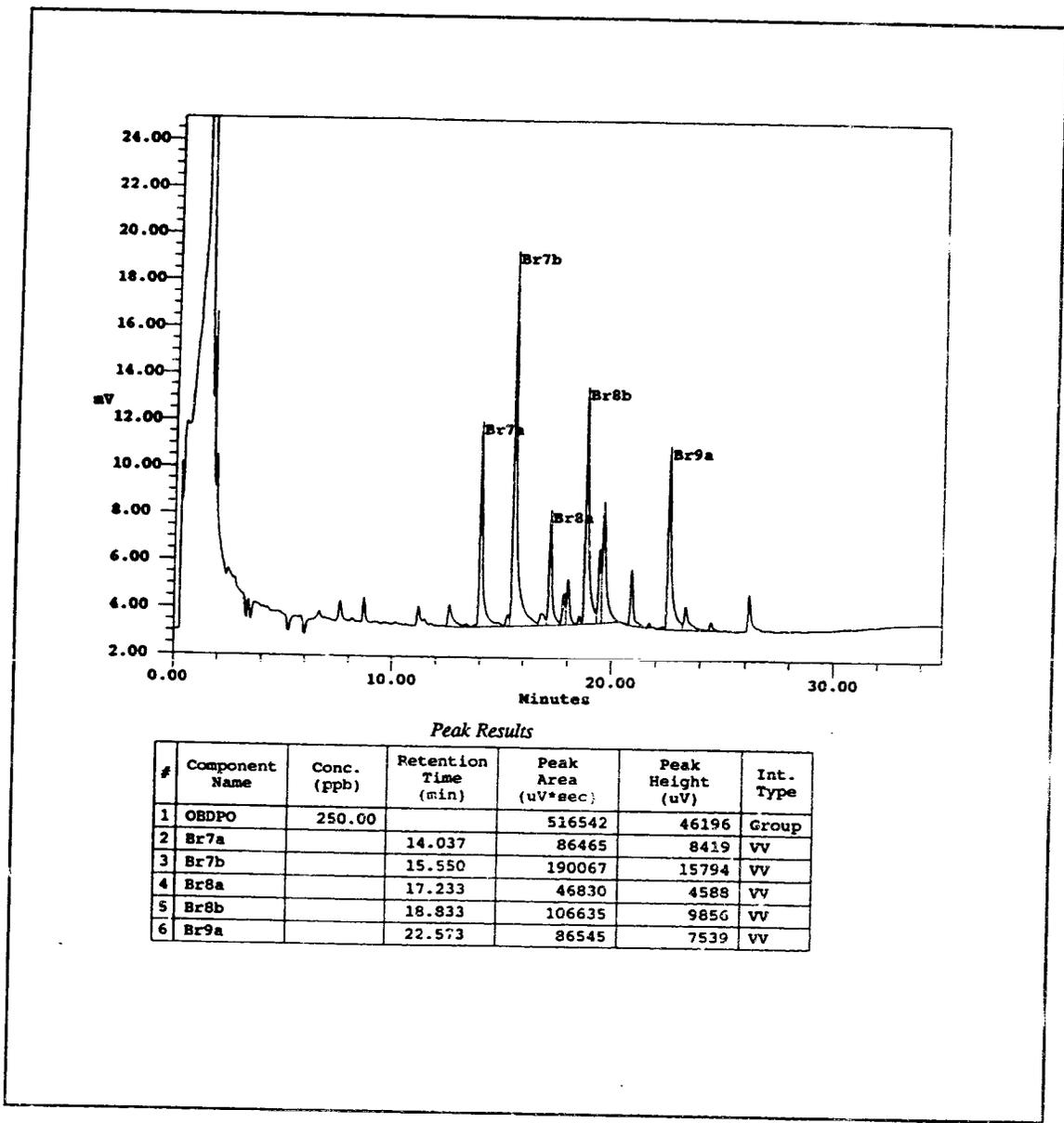


Figure 5. Representative chromatogram of a high-level calibration standard. (3637-5C5, sample set OBROM12, vial 5)

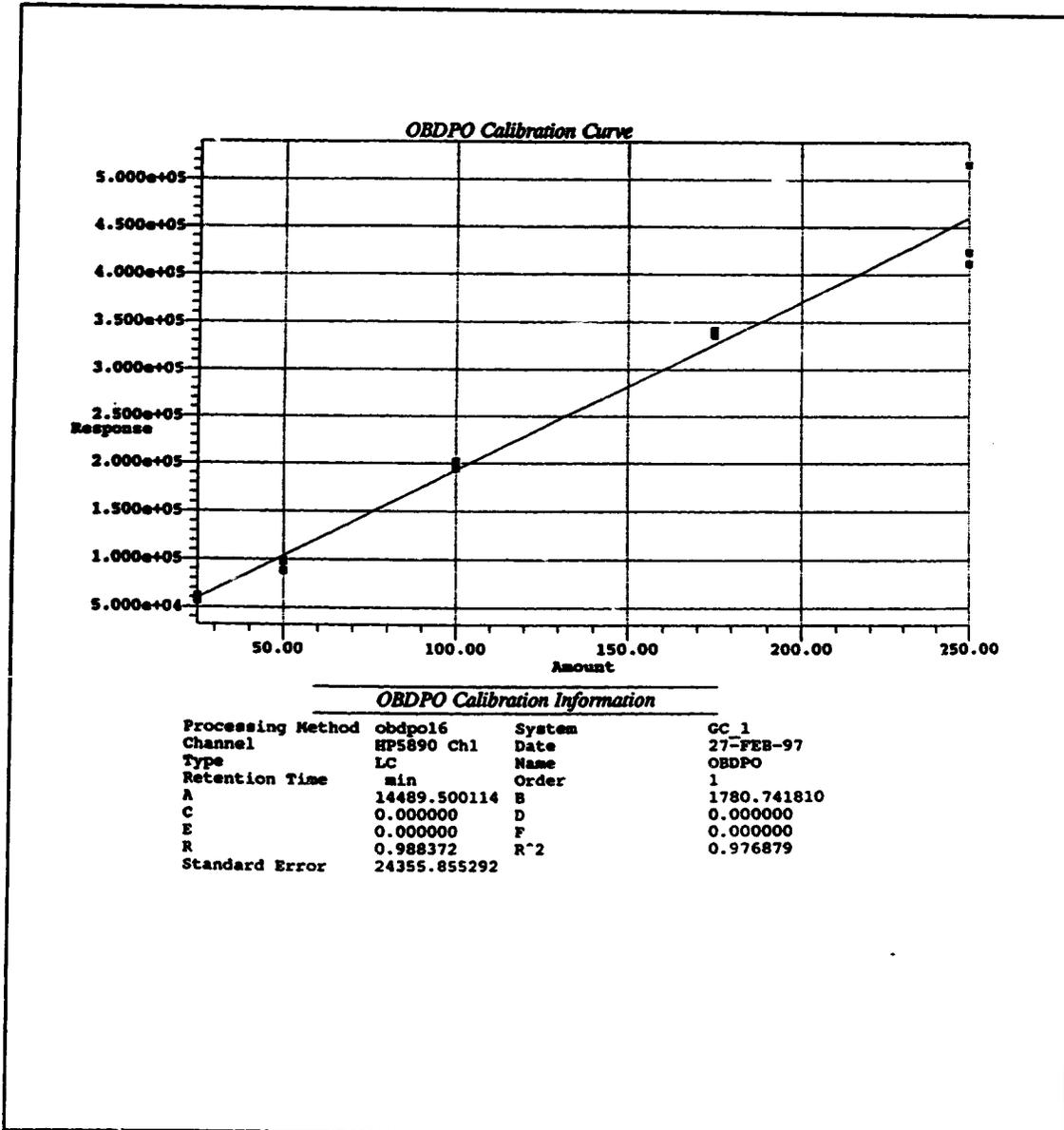
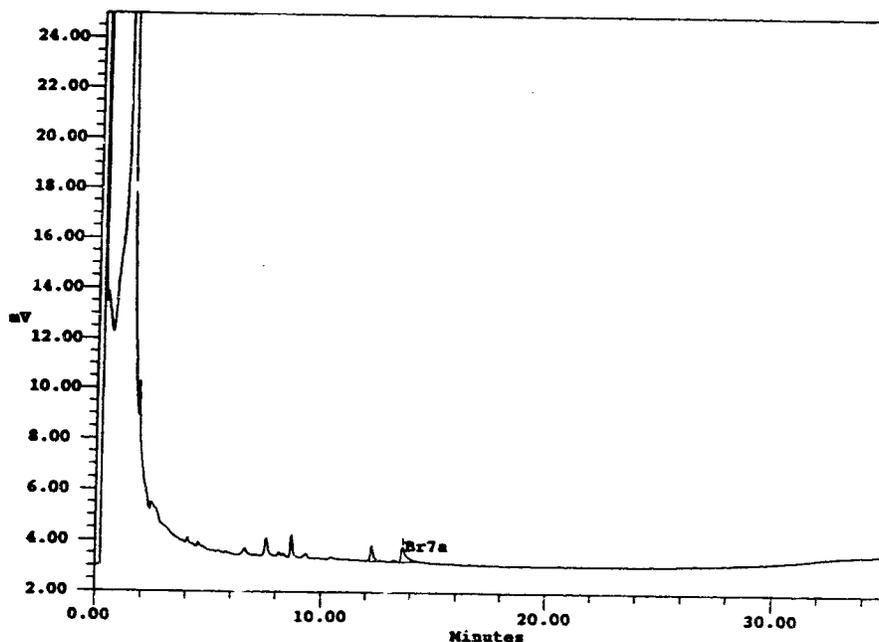


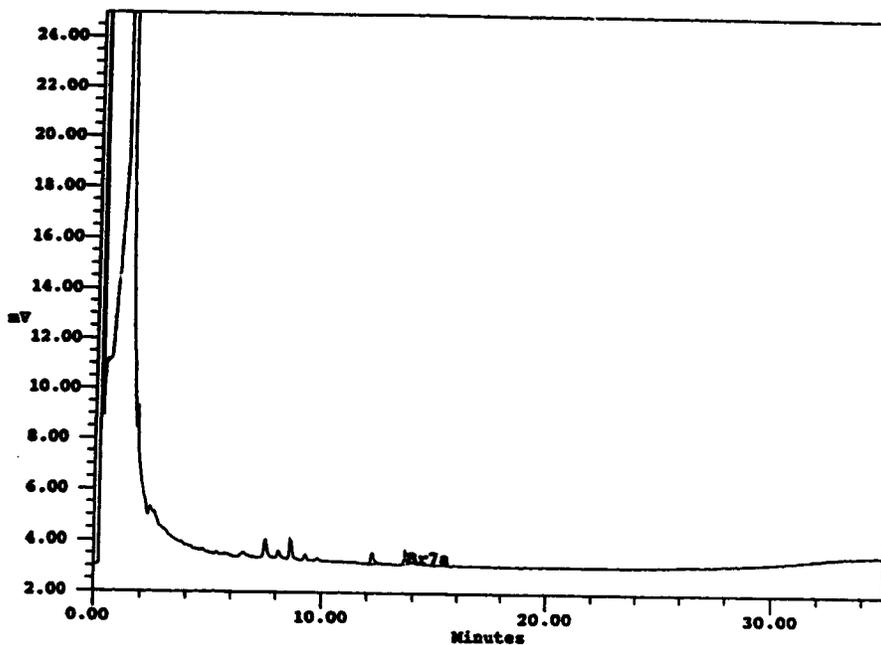
Figure 6. Representative calibration curve. (sample set OBROM12)



Peak Results

#	Component Name	Retention Time (min)	Peak Area (uV*sec)	Peak Height (uV)	Int. Type	Sample Volume	Measured Conc. (ppb)
1	OBDDPO		8471	601	Group	50	
2	Br7a	13.687	8471	601	BB	50	
3	Br7b	15.700			Missing	50	
4	Br8a	17.700			Missing	50	
5	Br8b	19.700			Missing	50	
6	Br9a	21.700			Missing	50	

Figure 7. Representative chromatogram of a matrix blank.  
(439C-110-MAB-5, sample set OBROM12, vial 9)



Peak Results

Component Name	Retention Time (min)	Peak Area (uV*sec)	Peak Height (uV)	Int. Type	Sample Volume	Measured Conc. (ppb)
1 OBDPO		2278	204	Group	1	
2 Br7a	15.647	2278	204	BB	1	
3 Br7b	15.700			Missing	1	
4 Br8a	17.000			Missing	1	
5 Br8b	19.000			Missing	1	
6 Br9a	22.700			Missing	1	

Figure 8. Representative chromatogram of a reagent blank.  
(439C-110-RGB-5, sample set OBROM12, vial 6)

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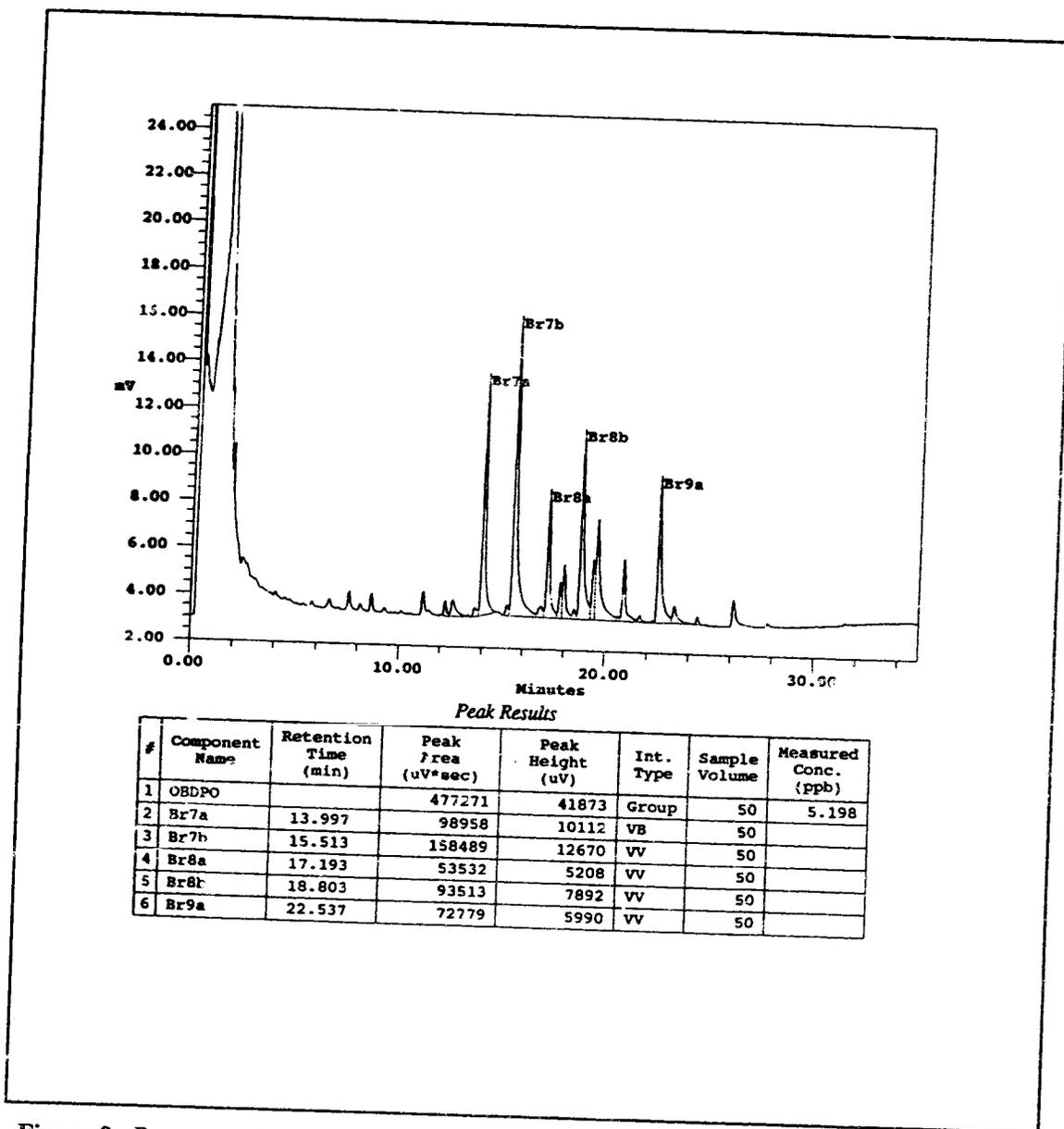
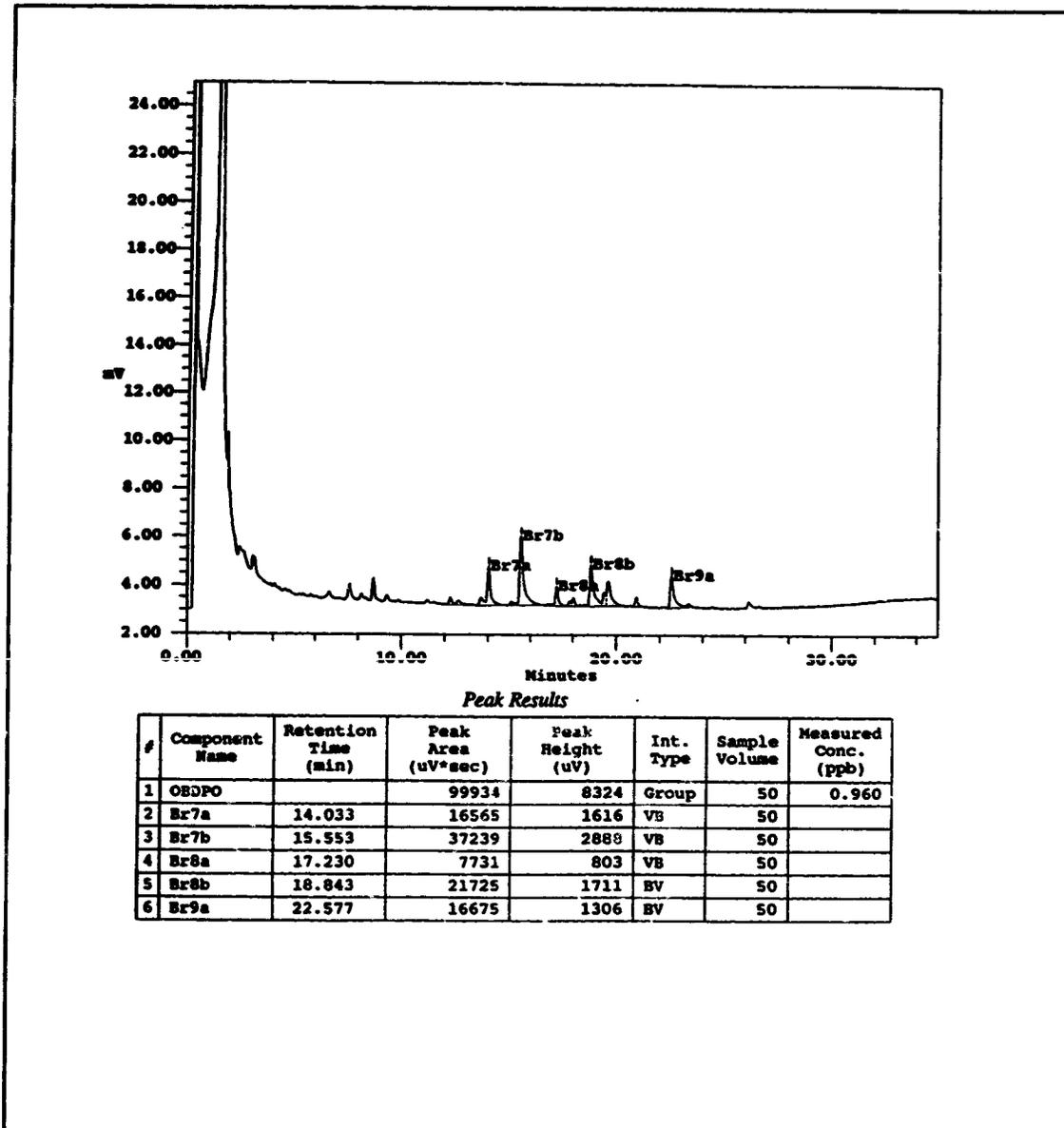


Figure 9. Representative chromatogram of a 5-ppb matrix fortification. (439C-110-MAS-10, sample set OBROM12, vial 11)

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**Figure 10.** Representative chromatogram of a 1-ppb matrix fortification.  
(439C-110-MAS-9, sample set OBROM12, vial 10)

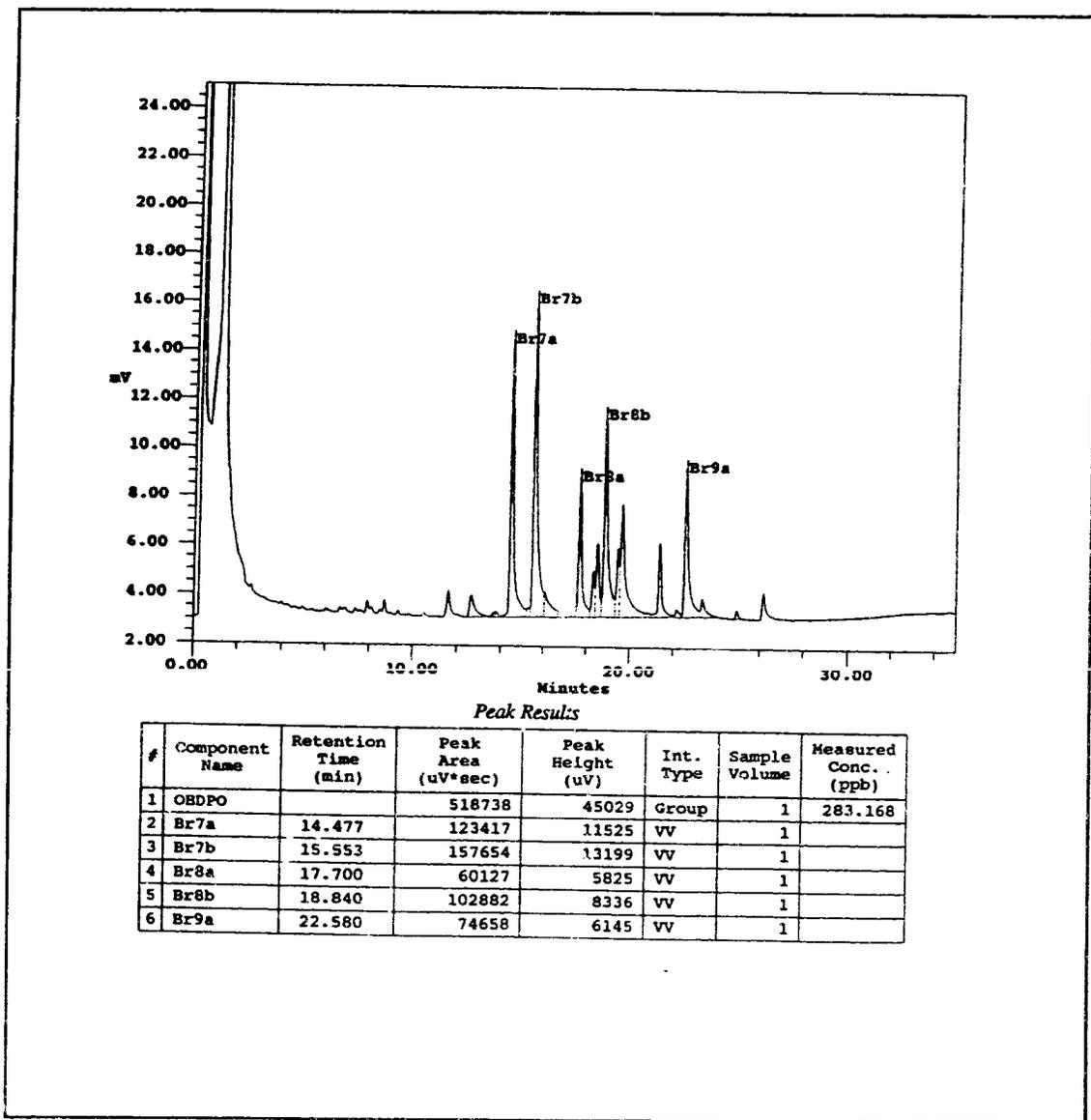


Figure 11. Representative chromatogram of a reagent fortification. (439C-110 RGS-5, sample set OBROM12, vial 7)

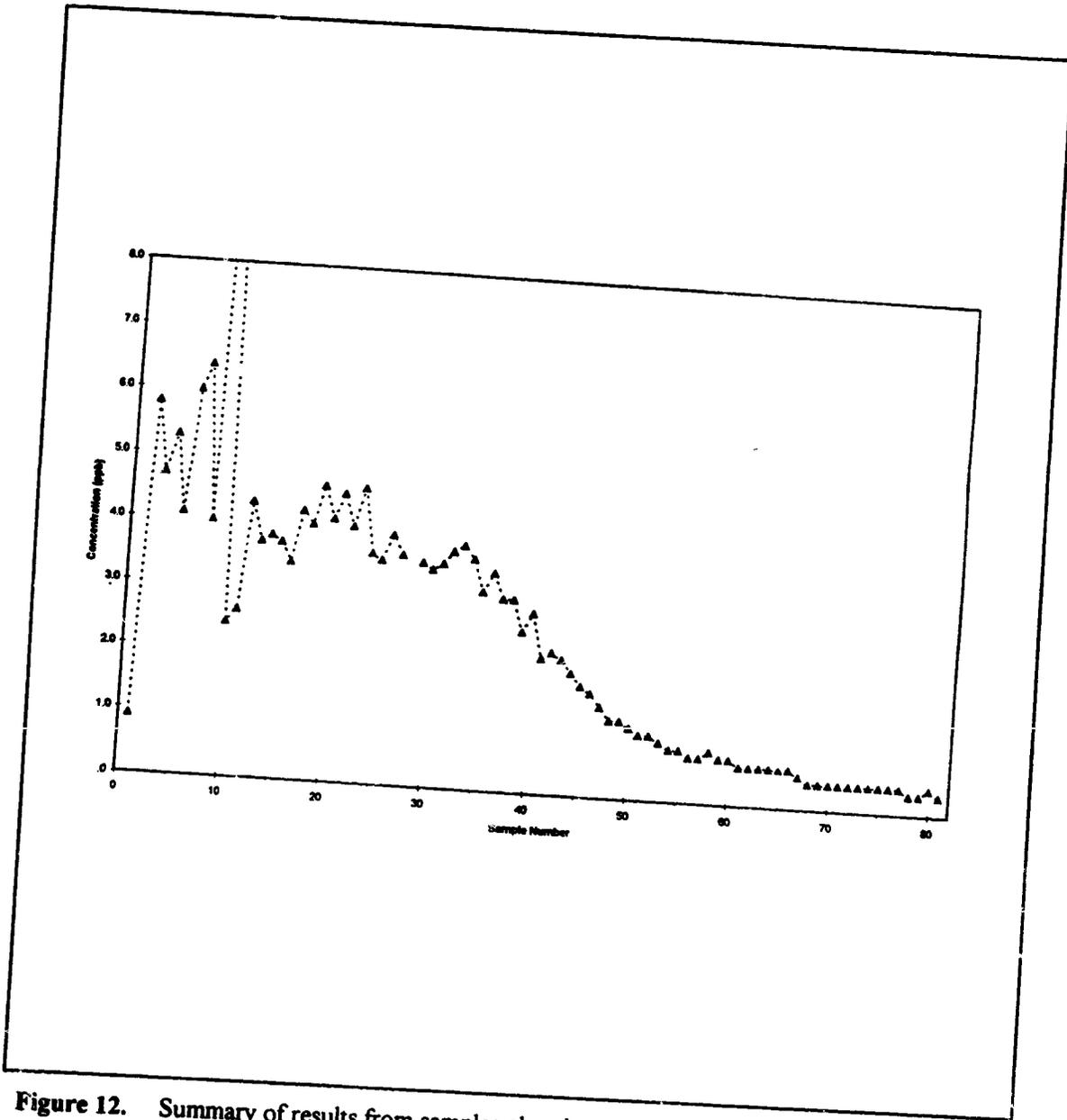
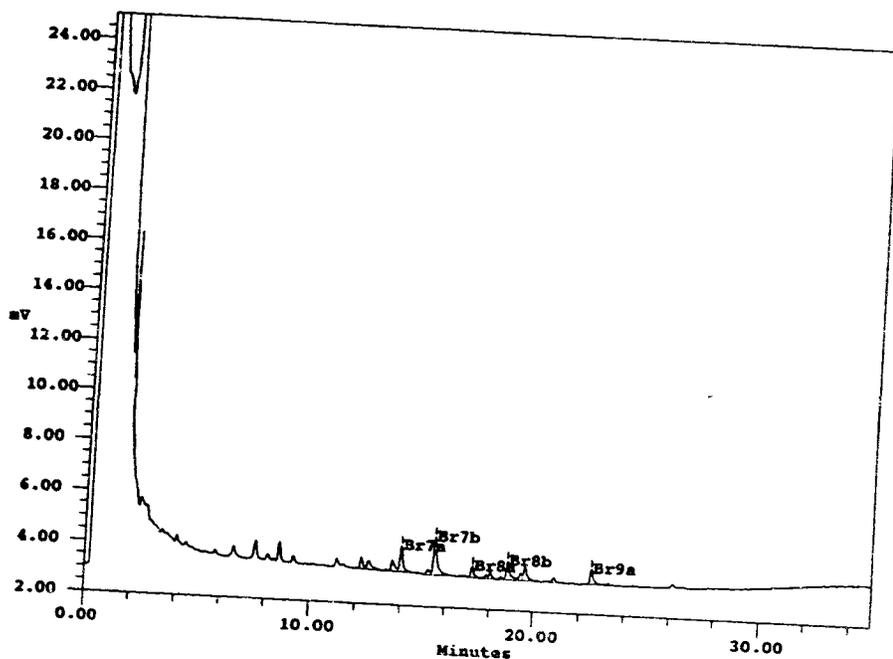


Figure 12. Summary of results from samples eluted at a nominal flow rate of 0.5 mL/min.



Peak Results

#	Component Name	Retention Time (min)	Peak Area (uV*sec)	Peak Height (uV)	Int. Type	Sample Volume	Measured Conc. (ppb)
1	OBDPO		45194	4371	Group	50	0.345
2	Br7a	14.020	9696	1043	VB	50	
3	Br7b	15.520	17172	1517	VB	50	
4	Br8a	17.217	4330	452	VB	50	
5	Br8b	18.810	8148	751	VV	50	
6	Br9a	22.547	5848	607	BB	50	

13. Representative chromatogram of a sample eluted at a nominal flow rate of 0.5 mL/min. (439C-110-#81, sample set OBROM12, vial 29)

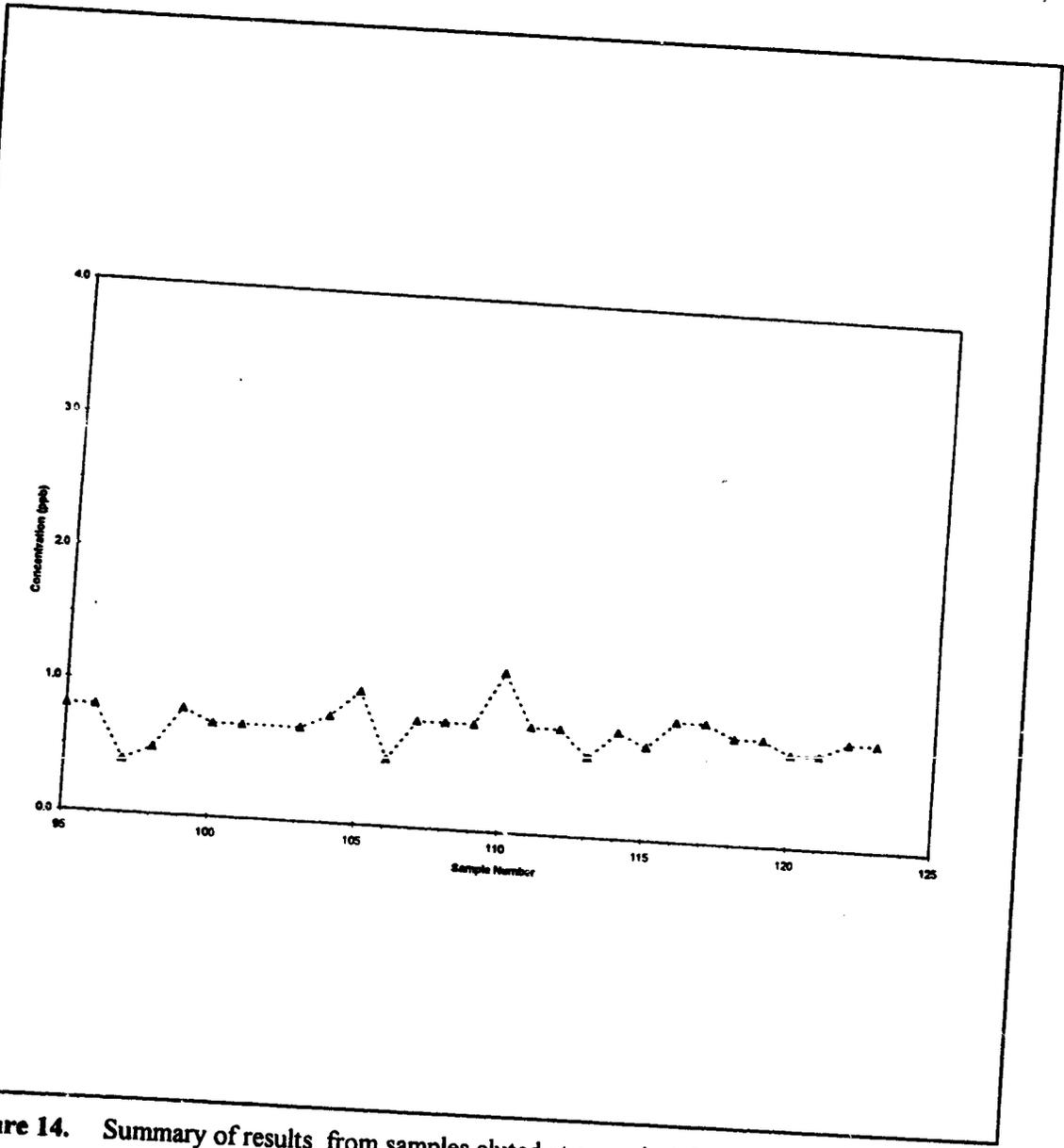
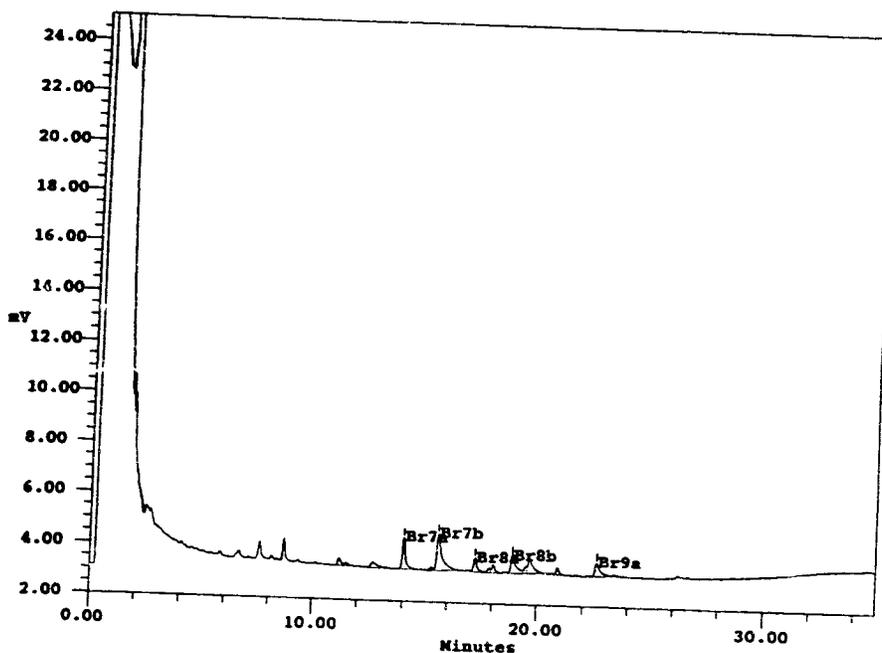


Figure 14. Summary of results from samples eluted at a nominal flow rate of 0.25 mL/min.

0-040



Peak Results

#	Component Name	Retention Time (min)	Peak Area (uV*sec)	Peak Height (uV)	Int. Type	Sample Volume	Measured Conc. (ppb)
1	OBDFC		59898	4494	Group	37	0.811
2	Br7a	14.040	12499	1268	VB	37	
3	Br7b	15.583	23068	1460	VB	37	
4	Br8a	17.237	5657	555	VV	37	
5	Br8b	18.880	10527	676	VV	37	
6	Br9a	22.617	8147	535	BV	37	

Figure 15. Representative chromatogram of a sample eluted at a nominal flow rate of 0.25 mL/min. (439C-110-#123, sample set OBROM10, vial 31)

APPENDIX I

Protocol, Amendment and Deviations

## APPENDIX I

WILDLIFE INTERNATIONAL LTD.

Project Number: 439C-110  
Page 1 of 2

## AMENDMENT TO STUDY PROTOCOL

STUDY TITLE: OCTABROMODIPHENYL OXIDE (OBDPO): DETERMINATION OF THE WATER SOLUBILITY

PROTOCOL NO.: 439C308/6105-01/SU13439

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

EFFECTIVE DATE: September 25, 1996

AMENDMENT NO.: 1

PROJECT NO.: 439C-110

AMENDMENT: Page 5, Test Procedure

Change: The entire test procedure section as follows.

Approximately 25 ml. of carrier material (glass beads) will be rinsed with an appropriate solvent and transferred to a round bottom flask. A stock solution of the test substance will be prepared in ethyl acetate and added to the carrier material so that approximately 300 mg of test substance are added. The flask will be placed on a rotary evaporator to evaporate the solvent and deposit the test substance on the carrier material. After the solvent has been thoroughly evaporated, a slurry of the loaded carrier material will be prepared in water. The slurry will be sonicated briefly to remove air bubbles and to dissolve some of the test substance. A generator column will be prepared as follows: a plug of silanized glass wool will be inserted into one end of the glass column. The slurry of loaded carrier material in water will be added to the column, followed by another plug of silanized glass wool. The generator column will be labeled with the project number, test substance number, and a unique column ID code.

The generator column will be held at a fixed temperature of  $25.0 \pm 0.5$  °C, and water will be pumped through the generator column at a rate of approximately 0.5 ml/min. The flow rate will be calibrated prior to and after sample collection. The eluate from the generator column will be directed to collection vessels containing the extraction solvent (ethyl acetate). The collection times for the water samples will be calculated based on the calibrated pump flow rates and desired sample volumes. Samples will be identified with the project number, test substance number, column ID code, and unique sample ID code.

Samples will be analyzed using the following procedure: the volume of the extraction solvent will be adjusted to approximately half the volume of the water sample. The vessel will be capped with a Teflon<sup>®</sup>-coated lid, and the sample will be shaken for ~30 seconds. The extraction solvent layer will be transferred to a round bottom flask. The extraction process will be repeated a second time, and both extracts will be combined in the round bottom flask. A small amount of diphenyl ether will be added and the extract will be evaporated to near dryness (<0.5 mL) on a rotary evaporator at 30-50 °C. The test substance will be quantitatively transferred from the round bottom flask to a sample tube using three 1 ml. to 1.5 ml. portions of extraction solvent. The tube will be placed under a stream of nitrogen until evaporated to <0.5 mL. Diphenyl ether will be added to each tube to bring the final sample volume to 1.0 mL. The tube will be vortexed briefly and the sample will be placed in a vial for analysis using GC/ECD.

*QA review  
KH 9-25-96*

APPENDIX I

The apparatus will be allowed to run until at least five consecutive samples differ by less than 30% in a random fashion. The flow rate will then be reduced to approximately half the previous flow rate. The test will continue in this fashion until at least five consecutive samples from two consecutive flow rates differ by less than 30% in a random fashion.

REASON:

The exact test procedures and analytical methods to be used for this study were not available at the time the protocol was signed. The procedures have been added to complete the protocol.

Gul el. Hameed  
STUDY DIRECTOR

9/25/96  
DATE

B. J. Mandley  
LABORATORY MANAGEMENT

9/26/96  
DATE

Harmukh Shah  
SPONSOR'S REPRESENTATIVE

11-25-96  
DATE

APPENDIX I

Project Number: 439C-110  
Page 1 of 1

DEVIATION TO STUDY PROTOCOL

STUDY TITLE: OCTABROMODIPHENYL OXIDE (OBDPO): DETERMINATION OF THE WATER SOLUBILITY

PROTOCOL NO.: 439/030896/105-OT/SUB439

DEVIATION NO.: 1

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

PROJECT NO.: 439C-110

DATE OF DEVIATION: November 24, 1996

DEVIATION: The protocol states the flow rate will be reduced to approximately half the previous flow rate and the test will continue until results from two consecutive flow rates differ by less than 30%. At the end of the test, the flow rate was increased to greater than the original flow rate.

REASON: At the conclusion of the experimental work for this study, the flow of reagent water through the generator column was not stopped right away, but was increased to a rate of approximately 0.8 ml/min. More samples were collected, extracted and analyzed. This work was performed at the request of the Sponsor's Technical Representative, but was never amended to the protocol. The mean measured concentration from samples collected at the higher flow rate was less than 0.5 µg OBDPO/L. The deviation had no effect on the results generated during the study.

Gerald Atteny  
STUDY DIRECTOR

5/12/97  
DATE

A. Munkley  
LABORATORY MANAGEMENT

5/12/97  
DATE

APPENDIX I

Project No.: 439C-110  
Page 1 of 1

DEVIATION TO STUDY PROTOCOL

STUDY TITLE: OCTABROMODIPHENYL OXIDE (OBDPO): DETERMINATION OF  
THE WATER SOLUBILITY

PROTOCOL NO.: 439/030896/105-OT/SUB439

DEVIATION NO.: 2

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

PROJECT NO.: 439C-110

DATE OF DEVIATION: May 8, 1997

DEVIATION: The protocol states the concentrations will be expressed in milligrams per liter (mg/L). Concentrations were calculated and reported in micrograms per liter ( $\mu\text{g/L}$ ) or parts per billion (ppb).

REASON: The solubility of the test substance was less than 1 mg/L; therefore, it was more appropriate to express results in  $\mu\text{g/L}$ . The test guidelines no longer specify the units to be used for reporting results. An amendment was never written to change the concentration units specified in the protocol. The deviation had no impact on the results of this study.

Paul L. Atwood  
STUDY DIRECTOR

5/12/97  
DATE

A. Markley  
LABORATORY MANAGEMENT

5/12/97  
DATE

APPENDIX I

Project Number. 439C-110  
Page 1 of 1

DEVIATION TO STUDY PROTOCOL

STUDY TITLE: OCTABROMODIPHENYL OXIDE (OBDPO): DETERMINATION OF THE WATER SOLUBILITY

PROTOCOL NO.: 439/030896/105-OT/SUB439

DEVIATION NO.: 3

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

PROJECT NO.: 439C-110

DATE OF DEVIATION: October 4, 1996

DEVIATION: The protocol states the temperature of the generator column will be maintained at  $25 \pm 0.5^\circ\text{C}$ . The temperature of the water bath was found to be approximately  $22.5^\circ\text{C}$  on the morning of 10/4/96, during the preliminary test with the first column.

REASON: The water level in the constant temperature bath dropped overnight to a level below the sensor, because of evaporative losses. The heater coil of the water bath automatically shut off, and the water cooled to room temperature. The problem was corrected within twenty minutes on the morning of 10/4/96. The deviation happened during the preliminary test with the first column, and did not effect the results of the study

Gal. L. Stuyvel  
STUDY DIRECTOR

5/12/97  
DATE

B. J. Mackley  
LABORATORY MANAGEMENT

5/12/97  
DATE

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**APPENDIX I**

**PROTOCOL**

**OCTABROMODIPHENYL OXIDE (OBDPO):  
DETERMINATION OF THE WATER SOLUBILITY**

**Organisation for Economic Cooperation and Development  
OECD Guideline 105**

and

**TSCA Title 40 of the Federal Code of Regulations  
Part 796, Section 1840**

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**

April 10, 1996

**PROTOCOL NO.: 439/030896/105-OT/SUB439**

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APPENDIX I

WILDLIFE INTERNATIONAL LTD.

OCTABROMODIPHENYL OXIDE (OBDPO):  
DETERMINATION OF THE WATER SOLUBILITY

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

**SPONSOR'S REPRESENTATIVE:** Dr. Hasmukh Shah

**TESTING FACILITY:** Wildlife International Ltd.  
8598 Commerce Drive  
Easton, Maryland 21601

**STUDY DIRECTOR:** Joel Stenzel  
Senior Scientist

**LABORATORY MANAGEMENT:** Barbara J. Markley, Ph.D.  
Manager of Analytical Chemistry

FOR LABORATORY USE ONLY

Proposed Dates:	
Experimental Start Date: <u>4/10/96</u>	Experimental Termination Date: <u>7/1/96</u>
Project No.: <u>439C-110</u>	Int/Date: <u>GED 4/10/96</u>
Test Substance No.: <u>3517, 3401, 3403</u>	Receipt Date: <u>12/24/95, 3/7/96, 3/12/96</u>

PROTOCOL APPROVAL

<u>Joel Stenzel</u> STUDY DIRECTOR	<u>4/10/96</u> DATE
<u>Barbara J. Markley</u> LABORATORY MANAGEMENT	<u>4/10/96</u> DATE
<u>Hasmukh Shah</u> SPONSOR'S REPRESENTATIVE	<u>4 - 9 - 96</u> DATE

PROTOCOL NO.: 439/030896/105-OT/SUB439

## APPENDIX I

WILDLIFE INTERNATIONAL LTD.

- 3 -

**INTRODUCTION**

Wildlife International Ltd. will determine the water solubility of the test substance. The study will be conducted at the Wildlife International Ltd. analytical chemistry facility in Easton, Maryland. The study will be performed based on procedures in OECD Guideline for Testing Chemicals, 105: *Water Solubility* (1) and TSCA Title 40 of the Federal Code of Regulations, Part 796, Section 1840: *Water Solubility* (2). The method of analysis for quantification provided by the Sponsor will be verified at Wildlife International Ltd. Raw data for all work performed at Wildlife International Ltd. and a copy of the final report will be filed by project number in archives located on the Wildlife International Ltd. site or at an alternative location to be specified in the final report.

**PURPOSE**

The purpose of this study is to determine the water solubility of the test substance.

**EXPERIMENTAL DESIGN**

The study will be conducted using a generator column elution method which is applicable to any liquid or solid organic test substance that is stable in water, and has a solubility less than 1000 ppm and greater than 10 ppb. Preliminary tests may be performed to estimate the water solubility of the test substance and verify the suitability of the analytical method. For the definitive test, at least two runs will be performed at different flow rates, and at least five samples will be collected from each run for analysis of test substance concentrations. Runs will be repeated using successively lower flow rates until the results from two consecutive runs give the same solubility (less than 30% difference). Samples will be collected and/or analyzed until the results from five consecutive samples do not differ by more than 30% in a random fashion.

**MATERIALS AND METHODS****Test Substance**

The test substance will be a composite sample of octabromodiphenyl oxide (OBDPO) produced by three manufacturers (Aldemarle Corporation, Americbrom LTD, and Great Lakes Chemical Corporation). The composite OBDPO sample will be composed of equal parts of each of the three manufacturers' product. Prior to use in the study, equal weights of test substance from each manufacturer will be placed in an appropriate plastic container and mixed on a laboratory shaker for a minimum of two hours to form a composite OBDPO sample for use as the test substance.

PROTOCOL NO.: 439/030896/105-OT/SUB439

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## APPENDIX I

WILDLIFE INTERNATIONAL LTD.

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Subsamples of the composite sample will be collected from the left and right sides of the top, middle and bottom of the container. The subsamples will be analyzed by Albemarle Corporation to determine the homogeneity of the mixture. An additional sample of the composite will be collected indiscriminately from the mixture and analyzed by Albemarle Corporation to characterize the test substance. If additional mixing or other procedures are necessary based on the results of the analysis, details of the procedure will be amended to the protocol. The mean OBDPO percent of the homogeneity samples will be used for the study.

Information on the characterization of test, control or reference substances is required by Good Laboratory Practice Standards (GLP), 40 CFR Part 792.31. The Sponsor is responsible for providing Wildlife International Ltd. written verification that the OBDPO composite sample has been characterized according to GLP's prior to its use in the study. If written verification of GLP test substance characterization is not provided to Wildlife International Ltd., it will be noted in the compliance statement of the final report. The attached form IDENTIFICATION OF TEST SUBSTANCE BY SPONSOR (Appendix I) will be used to provide information necessary for GLP compliance.

The Sponsor is responsible for all information related to the test substance and agrees to accept any unused test substance and/or test substance containers remaining at the end of the study.

Reagents

Water that meets ASTM Type II standards (ASTM D 1193-77) will be used. Other solvents may be needed for the analytical method or preparation of stock solutions and analytical standards. All solvents will be ACS reagent grade or better. The carrier material for the column elution method will be either glass beads or silica.

Preliminary Test Procedure

A preliminary test may be run to estimate the solubility of the test substance in water at room temperature. A small amount (e.g. 10 mg) of the test substance will be placed in a vessel. Water will be added in successively greater volumes to test solubility at approximately 10, 1, 0.1 and 0.01 grams per liter, as needed. After each addition of water, the vessel will be shaken vigorously for 10 minutes, allowed to stand at room temperature, then visually examined for any undissolved parts of

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the test substance. As the volume increases, the contents of the vessel may be transferred to larger vessels or the test may be restarted with a smaller amount of test substance. The test will end when the test substance appears to be completely dissolved or when solubility is estimated to be less than 0.01 g/L. If solubility appears to be greater than 0.1 g/L, the Sponsor will be consulted to select a more appropriate method for determining solubility. If the temperature dependence of solubility is anticipated to be greater than 3% per °C, the Sponsor will be consulted to select appropriate additional test temperatures.

Test Procedure

Approximately 600 mg of carrier material will be weighed and transferred to a 50 mL round bottom flask. If the test substance is a liquid, a weighed amount will be added directly to the carrier material. If the test substance is a solid, a stock solution containing approximately 1% of the test substance in a volatile solvent will be added to the carrier material. The flask will be placed on a rotary evaporator to evaporate the solvent and/or evenly coat the carrier material with the test substance. The loaded carrier material will be allowed to soak for approximately 2 hours in approximately 5 mL of water. A generator column will then be prepared as follows: a plug of silanized glass wool will be inserted into one end of the glass column. The slurry of loaded carrier material in water will be added to the column, followed by another plug of silanized glass wool to retain the carrier material. Each generator column will be labelled with the project number, test substance number, and a unique column ID code.

Water will be pumped through the generator column at a fixed temperature of  $25.0^{\circ}\text{C} \pm 0.1^{\circ}\text{C}$  at a rate of approximately 1 mL/hour. The eluate from the generator column will be directed to either a rotary switching valve connected to an HPLC system or a collection vessel. The collection vessel will be used when HPLC analysis is not suitable. The apparatus will be allowed to run until five consecutive samples differ by less than 30% in a random fashion. The test will then be repeated at successively lower flow rates until two consecutive runs give the same solubility.

If samples will be analyzed by HPLC, the rotary valve will be placed in the inject position for approximately 5 minutes, and the eluate will be directed to a waste container. At the same time, water will be pumped through the HPLC system to flush out any solvent remaining from previous runs. The valve will then be switched to the load position, and the eluate directed through an

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extractor column. The extractor column will be packed with a substrate (e.g. C<sub>18</sub>/Corasil) that will adsorb the test substance. At the same time the valve is switched, a preweighed bottle will be placed at the waste position. An appropriate amount of water will be collected. The amount of water will depend on the amount of test substance needed for analysis. As the sample is loaded onto the extractor column, the mobile phase of the HPLC system will be changed to a solvent/water ratio that will elute the test substance from the extractor column. The valve will be returned to the inject position. The size of the sample loaded onto the extractor column will be determined from the amount of water collected in the bottle. The eluate from the extractor column will be directed through an analytical column and detector on the HPLC system, as specified in the method supplied by the Sponsor. The concentration of test substance in the water will be calculated based on the weight of the water sample collected and the amount of test substance determined from the HPLC analysis.

If HPLC analysis is not suitable, the eluate will be collected in a waste container for approximately 15 minutes. A collection vessel containing a preweighed amount of extracting solvent will be used to collect each water sample. Samples will be identified with the project number, test substance number, column ID code, and unique sample ID code. The water samples will be introduced below the surface of the solvent, and the weights will be determined. The water sample will be analyzed using the method supplied by the Sponsor.

Method

The analytical method to be used will be based upon procedures provided by the Sponsor. The method will be appended to this protocol in Appendix II and referenced in the report. The method may be verified as part of the preliminary tests, and may be modified to measure the expected test substance concentrations in water. Major modifications to the method will be approved by the Sponsor and Study Director, and documented in the data.

Calculations

The concentration of the test substance in each sample will be expressed in milligrams per liter. The average solubility and standard deviation will be calculated for at least five samples from each run.

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Sample Handling & Safety

The Sponsor will identify any special handling or safety precautions to be used with the above-referenced test substance. All normal precautions with respect to handling and storage will be taken.

Sample and Test Substance Retention

Test substance and test substance containers remaining at the end of the study will be returned to the sponsor. After finalization of the report, Wildlife International Ltd. will return samples generated from this test to the Sponsor or will dispose of samples with written authorization by the Sponsor.

RECORDS TO BE MAINTAINED

Records to be maintained for data generated by Wildlife International Ltd. will include, but not be limited to:

1. A copy of the signed protocol.
2. Identification and characterization of the test substance, if provided by the Sponsor.
3. Dates of initiation and completion of the test.
4. ~~Dates of experimental start and termination.~~
5. Storage conditions of the test substance.
6. Test substance use log.
7. Concentration calculations and records of solution preparation.
8. Instrument operating conditions and chromatograms.
9. Statistical calculations.
10. Test conditions.
11. A copy of the final report.

FINAL REPORT

A final report of the results of the study will be prepared by Wildlife International Ltd. The report will include, but not be limited to the following, when applicable:

1. Name and address of the facility performing the study.
2. Name and address of the Sponsor.
3. Dates upon which the study was initiated and completed.
4. A statement of compliance signed by the Study Director addressing any exceptions to Good Laboratory Practice Standards.

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5. Purpose and procedure, as stated in the approve protocol, including a copy of the protocol, and all amendments and deviations to the protocol.
6. The test substance identification, including name, chemical abstract number or code number, purity, composition, empirical formula, molecular formula, manufacturer's lot/batch number, dissociation in water, method of analysis, and any other information provided by the Sponsor
7. Description of the test method or reference to the method used along with any modifications made.
8. The individual concentrations, flow rates, and pHs of each sample.
9. The means and standard deviations from at least five samples from the saturation plateau of each run.
10. The average of the two successive, acceptable runs.
11. Description of any problems experienced and how they were resolved.
12. A statement prepared by the Quality Assurance Unit listing the dates that study inspections and audits were made and findings reported to the Study Director and Management.

CHANGING OF PROTOCOL

Planned changes to the protocol will be in the form of written amendments signed by the Study Director and the Sponsor. Amendments will be considered as part of the protocol and will be attached to the final protocol. Any other changes will be in the form of written deviations filed with the raw data. All changes to the protocol will be indicated in the final report.

GOOD LABORATORY PRACTICES

This study will be conducted according to the Good Laboratory Practices described in OECD (ISBN 92-84-12367-9) and EPA (40 CFR Part 792). Each study conducted by Wildlife International Ltd. is routinely examined by the Wildlife International Ltd. Quality Assurance Unit for compliance with Good Laboratory Practices, Standard Operating Procedures and the specified protocol. A statement of compliance with Good Laboratory Practices will be prepared for all portions of the study conducted by Wildlife International Ltd. The Sponsor will be responsible for compliance with Good Laboratory Practices for procedures performed by other laboratories. Raw data for all work performed at Wildlife International Ltd. and a copy of the final report will be filed by project number in archives located on the Wildlife International Ltd. site or at an alternative location to be specified in the final report.

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REFERENCES

- 1 Organisation for Economic Cooperation and Development. 1984. *Guideline for Testing of Chemicals, 105: Water Solubility.*
- 2 TSCA Title 40 of the Federal Code of Regulations. 1994. Part 796, Section 1840: *Water Solubility.*

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APPENDIX I  
IDENTIFICATION OF TEST SUBSTANCE BY SPONSOR

To be Completed by Sponsor

I. Test Substance Identity (name to be used in the report): \_\_\_\_\_

Sample Code or Batch Number: \_\_\_\_\_

Purity (% Active Ingredient): \_\_\_\_\_ Expiration Date: \_\_\_\_\_

II. Test Substance Characterization

Have the identity, strength, purity and composition or other characteristics which appropriately define the test substance been determined prior to the start of this study in accordance with GLP Standards? Yes \_\_\_ No \_\_\_

III. Test Substance Storage Conditions

Please indicate the recommended storage conditions at Wildlife International Ltd.

Has the stability of the test substance under these storage conditions been determined in accordance with GLP Standards? Yes \_\_\_ No \_\_\_

Other pertinent stability information: \_\_\_\_\_

IV. Test Concentrations:

Adjust test concentration to 100% a.i. based upon the purity (%) given above.

Do not adjust test concentration to 100% a.i. Test the material AS IS.

V. Toxicity Information:

Mammalian: Rat LD50 \_\_\_\_\_ Mouse LD50 \_\_\_\_\_

Aquatic: Invertebrate Toxicity (EC/LC50) Fish Toxicity (LC50)

Other Toxicity Information (including findings of chronic and subchronic tests): \_\_\_\_\_

VI. Classification of the Compound:

\_\_\_\_\_ Insecticide \_\_\_\_\_ Herbicide \_\_\_\_\_ Fungicide  
\_\_\_\_\_ Microbial Agent \_\_\_\_\_ Economic Poison

Other: \_\_\_\_\_

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APPENDIX II

Analytical Method to be Provided by Sponsor

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APPENDIX II

Personnel Involved in the Study

The following key Wildlife International Ltd. personnel were involved in the conduct or management of this study:

1. Willard B. Nixon, Ph.D., Manager, Analytical Chemistry
2. Barbara J. Markley, Ph.D., Manager, Analytical Chemistry
3. Joel I. Stenzel, Senior Chemist