

*Chris*



FYI-1097-1303

CHEMICAL MANUFACTURERS ASSOCIATION

June 20, 1997

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

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 report is enclosed:

OCTABROMODIPHENYL OXIDE (OBDPO): A Flow-Through Life-Cycle Toxicity Test with the Cladoceran (*Daphnia magna*).

This report does not include confidential information.

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

Sincerely,

*Carol Stack*

Carol R. Stack, Ph.D.  
Acting Vice-President  
CHEMSTAR



FYI-97-001303

Enclosure



84980000005

RECEIVED  
OCT 7 11:44

RECEIVED  
OCT 14 11:34

RECEIVED  
OCT 7 11:43  
RAD  
6/25/97

**Contains No CBI**



INNOVATION, TECHNOLOGY AND RESPONSIBLE CARE® AT WORK

1300 WILSON BLVD., ARLINGTON, VA 22209 • TELEPHONE 703-741-5000 • FAX 703-741-6000



OCTABROMODIPHENYL OXIDE (OBDPO):  
A FLOW-THROUGH LIFE-CYCLE TOXICITY TEST  
WITH THE CLADOCERAN (*Daphnia magna*)

FINAL REPORT

WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER: 439A-104

Organisation for Economic Cooperation and Development  
OECD Guideline 202, Part II  
and  
TSCA Title 40 of the Federal Code of Regulation  
Part 797, Section 1330

AUTHORS:

William C. Graves  
Mark A. Mank  
James P. Swigert, Ph.D.

STUDY INITIATION DATE: June 5, 1996

STUDY COMPLETION DATE: May 8, 1997

AMENDED REPORT DATE: May 20, 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

Page 1 of 99

AMENDED

**GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT**

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

**TITLE:** Octabromodiphenyl Oxide (OBDPO): A Flow-Through Life-Cycle Toxicity Test with the Cladoceran (*Daphnia magna*)

WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER: 439A-104

STUDY COMPLETION: May 8, 1997

AMENDED REPORT DATE: May 20, 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:

Stability of the test substance under storage conditions at the test site has not been determined in accordance with Good Laboratory Practice Standards.

**STUDY DIRECTOR:**

William C. Graves  
William C. Graves  
Senior Aquatic Biologist

5-20-97  
DATE

**SPONSOR APPROVAL:**

Hammukh Shah  
Sponsor

6-2-97  
DATE

AMENDED

- 3 -

## QUALITY ASSURANCE

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	December 16, 1996	December 16, 1996	December 17, 1996
Sample Collection, Test Initiation and Water chemistry Measurements	December 18, 1996	December 18, 1996	December 18, 1996
Biological Data and Draft Report	January 28, February 5 - 7, 1997	February 7, 1997	February 11, 1997
Analytical Data and Draft Report	February 10 and 11, 1997	February 11, 1997	February 12, 1997
Final Report	May 2 and 5, 1997	May 5, 1997	May 5, 1997
Amended Final Report	May 20, 1997	May 20, 1997	May 20, 1997

Kimberly A. Hoxter  
 Kimberly A. Hoxter  
 Quality Assurance Representative

5-20-97  
 DATE

AMENDED

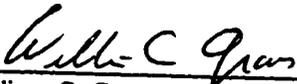
**AMENDED REPORT APPROVAL**

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

TITLE: Octabromodiphenyl Oxide (OBDPO): A Flow-Through Life-Cycle Toxicity Test with the Cladoceran (*Daphnia magna*)

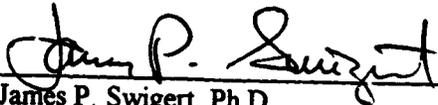
WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER: 439A-104

STUDY DIRECTOR:

  
\_\_\_\_\_  
William C. Graves  
Senior Aquatic Biologist

5-20-97  
DATE

MANAGEMENT:

  
\_\_\_\_\_  
James P. Swigert, Ph.D.  
Management, Analytical Chemistry

5/20/97  
DATE

AMENDED

## AMENDED REPORT

## TABLE OF CONTENTS

Title/Cover Page .....	1
Good Laboratory Practice Compliance Statement .....	2
Quality Assurance .....	3
Report Approval .....	4
Table of Contents .....	5
Summary .....	7
Introduction .....	8
Objective .....	3
Experimental Design .....	8
Materials and Methods .....	9
Results and Discussion .....	16
Conclusions .....	19
References .....	20

## TABLES

Table 1 - Summary of Analytical Chemistry Data .....	21
Table 2 - Temperature (°C) of Water in the Test Chambers .....	23
Table 3 - Dissolved Oxygen Content (mg/L) of Water in the Test Chambers .....	24
Table 4 - pH of Water in the Test Chambers .....	25
Table 5 - Specific Conductance, Hardness, Alkalinity and Total Organic Carbon in the Negative Control .....	26
Table 6 - Summary of Cumulative Percent Mortality and Treatment-Related Effects .....	27

**AMENDED REPORT**  
**TABLE OF CONTENTS**

- Continued -

**TABLES (Continued)**

Table 7 - Mortality/Immobility EC50 Values .....	29
Table 8 - Percent of Adults with Eggs in Brood Pouch .....	30
Table 9 - Reproduction of <i>Daphnia magna</i> During the Chronic Toxicity Test .....	31
Table 10- Clinical Observations of Neonate Daphnids .....	33
Table 11- Mean Lengths of Surviving First-Generation Daphnids .....	34
Table 12- Mean Dry Weights of Surviving First-Generation Daphnids .....	35

**APPENDICES**

Appendix I - Rangefinding Results .....	36
Appendix II - Protocol, Protocol Amendments and Deviations .....	37
Appendix III - Specific Conductance, Hardness, Alkalinity and pH of Well Water Measured During the 4-Week Period Immediately Preceding the Test ....	64
Appendix IV - Analyses of Pesticides, Organics, Metals and Other Inorganics in Wildlife International Ltd. Well Water .....	65
Appendix V - The Analysis of Octabromodiphenyl Oxide (OBDPO) in Freshwater in Support of Wildlife International Ltd. Project No.: 439A-104 .....	66
Appendix VI - Cumulative Percent Mortality and Treatment-Related Effects .....	84
Appendix VII - Neonate Production .....	91
Appendix VIII - Number of Adult Reproductive Days During the Reproduction Period ...	92
Appendix IX - Length and Dry Weight of Surviving First-Generation Daphnids .....	93
Appendix X - Personnel Involved in the Study .....	97
Appendix XI - Report Amendment .....	98

- 7 -

## SUMMARY

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

WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER:	439A-104
TEST SUBSTANCE:	Octabromodiphenyl Oxide (OBDPO)
STUDY:	Octabromodiphenyl Oxide (OBDPO): A Flow-Through Life-Cycle Toxicity Test with the Cladoceran ( <i>Daphnia magna</i> )
NOMINAL TEST CONCENTRATIONS:	Negative Control, Solvent Control, 0.13, 0.25, 0.50, 1.0 and 2.0 $\mu\text{g/L}$
TEST DATES:	Experimental Start - December 18, 1996 Biological Termination - January 9, 1997 Experimental Termination - January 28, 1997
LENGTH OF TEST:	21 Days

TEST ORGANISM:	Neonate Cladocerans ( <i>Daphnia magna</i> )
SOURCE OF TEST ORGANISMS:	Wildlife International Ltd. cultures Easton, Maryland 21601
AGE OF TEST ORGANISMS:	< 24 hours at test initiation

NO-OBSERVED-EFFECT-CONCENTRATION:	2.0 $\mu\text{g/L}$ nominal (1.7 $\mu\text{g/L}$ , measured) (Approximate limit of water solubility = < 0.5 $\mu\text{g/L}$ )
LOWEST-OBSERVED-EFFECT-CONCENTRATION:	> 2.0 $\mu\text{g/L}$ nominal (1.7 $\mu\text{g/L}$ , measured) (Approximate limit of water solubility = < 0.5 $\mu\text{g/L}$ )
MAXIMUM ACCEPTABLE TOXICANT CONCENTRATION:	> 2.0 $\mu\text{g/L}$ nominal (1.7 $\mu\text{g/L}$ , measured) (Approximate limit of water solubility = < 0.5 $\mu\text{g/L}$ )
21-DAY EC50:	> 2.0 $\mu\text{g/L}$ nominal (1.7 $\mu\text{g/L}$ , measured) (Approximate limit of water solubility = < 0.5 $\mu\text{g/L}$ )

AMENDED

- 8 -

## 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. aquatic toxicology facility in Easton, Maryland. The in-life phase of the test was conducted from December 18, 1996 to January 9, 1997. Raw data generated by Wildlife International Ltd. and a copy of the final report are filed under Project Number 439A-104 in archives located on the Wildlife International Ltd. site.

## OBJECTIVE

The objective of this study was to evaluate the acute toxicity of octabromodiphenyl oxide (OBDPO) on the survival, growth and reproduction of the cladoceran (*Daphnia magna*) during a 21-day exposure period under flow-through test conditions.

## EXPERIMENTAL DESIGN

Daphnids were exposed to a geometric series of five test concentrations, a solvent control and a negative (well water) control. Five neonate daphnids (< 24 hours old) were placed in each of two test compartments per test chamber. Test chambers were replicated so that a total of 20 daphnids were exposed to each treatment and control group. Nominal test concentrations were selected in consultation with the Sponsor, and were based upon the maximum solubility of the test substance in water (< 0.5 µg/L) and the results of an exploratory range finding toxicity test (Appendix I). Nominal test concentrations selected were 0.13, 0.25, 0.50, 1.0 and 2.0 µg/L. The 2.0 µg/L test concentration was expected to be greater than four times the maximum solubility of OBDPO in Wildlife International Ltd. well water. Mean measured test concentrations were determined from samples of test water collected from the three highest treatment groups and the control groups at test initiation, Days 7 and 14 and at test termination.

AMENDED

Delivery of the test substance was initiated approximately 45 hours prior to the introduction of the daphnids to the test water in order to achieve equilibrium of the test substance in the test chambers. Daphnids were indiscriminately assigned to exposure chambers at test initiation. Observations of survival and clinical signs of toxicity were performed daily on the first-generation daphnids. With the onset of reproduction, the number of second-generation daphnids was counted and recorded three times per week (Monday, Wednesday and Friday). The results of the test were used to calculate the EC50 (death and immobilization) and EC50 (reproduction) values, when possible, at 24 hours, 48 hours, 96 hours, 7 days, 14 days and at the end of the test (Day 21). The EC50 was defined as the concentration estimated to cause death and/or immobilization in 50% of the daphnids or reduce reproduction by 50% of the control daphnids. In addition, the results of the test were used to determine the no-observed-effect-concentration (NOEC) and the lowest-observed-effect-concentration (LOEC). The NOEC was defined as the highest concentration that produced no statistically significant ( $p > 0.05$ ) effect on survival, reproduction or growth. The LOEC was defined as the lowest concentration that produces a statistically significant ( $p \leq 0.05$ ) effect on survival, reproduction or growth. The maximum acceptable toxicant concentration (MATC) was calculated as the geometric mean of the NOEC and LOEC.

### MATERIALS AND METHODS

The study was conducted according to the procedures outlined in the protocol, Octabromodiphenyl Oxide (OBDPO): A Flow-Through Life-Cycle Toxicity Test with the Cladoceran (*Daphnia magna*) (Appendix II). The protocol was based on procedures outlined in Part II OECD Guideline for Testing Chemicals, 202: *Daphnia* sp., *Acute Immobilisation Test and Reproduction Test* (1); ASTM Standard E1193-87 *Standard Guide for Conducting Renewal Life-Cycle Toxicity Tests with Daphnia magna* (2); and Title 40 of the Code of Federal Regulations, Part 797, Section 1330, *Daphnia Chronic Toxicity Test* (3).

- 10 -

### Test Substance

The test substance was a composite sample of octabromodiphenyl oxide (OBDPO) produced by three manufacturers (Albemarle Corporation, Ameribrom LTD, and Great Lakes Chemical Corporation); composite of Wildlife International Ltd. identification numbers 3517, 3601 and 3603. The composite OBDPO sample consisted of equal parts of the three manufacturers' product and was combined at Wildlife International on April 10, 1996. The combined test substance was described as an off-white powder. The test substance was stored in a cabinet at ambient room temperature. The composite test substance was assigned Wildlife International Ltd. identification number 3637.

Prior to use in the study, equal weights of test substance from each manufacturer were placed in an appropriate plastic container and mixed on a laboratory shaker for a minimum of 24 hours to form a composite OBDPO sample for use as the test substance. Subsamples of the composite sample were collected from the left and right sides of the top, middle and bottom of the container. The subsamples were analyzed by Great Lakes Chemical Corporation to determine the homogeneity of the mixture. An additional sample of the composite was collected indiscriminately from the mixture and analyzed by Albemarle Corporation to characterize the test substance.

### Preparation of Test Concentrations

One stock solution was prepared for each of the five concentrations tested. The primary stock was prepared by dissolving octabromodiphenyl oxide (OBDPO) in dimethylformamide (DMF) at a concentration of 0.0280 mg octabromodiphenyl oxide (OBDPO)/mL. The stock solution was mixed by inversion to aid solubilization of octabromodiphenyl oxide (OBDPO). Aliquots of the primary stock solution were diluted with DMF to prepare four additional stock solutions at concentrations of 0.014, 0.0070, 0.0035 and 0.00175 mg octabromodiphenyl oxide (OBDPO)/mL. All test solutions appeared clear and colorless. Stock solutions were prepared one time during the test period. The five stocks were injected into the diluter mixing chambers where they were mixed with well water to achieve the desired test concentrations. The resultant test concentrations were not adjusted for purity of the active ingredient in the test substance. The solvent concentration in the treatment and solvent control groups was 0.07 mL/L.

### Test Organism

The cladoceran, *Daphnia magna*, was selected as the test species for this study. Daphnids are representative of an important group of aquatic invertebrates and were selected for use in the test based upon past history of use and ease of culturing in the laboratory. Daphnid neonates used in the test were less than 24-hours old and were obtained from cultures maintained by Wildlife International Ltd., Easton, Maryland.

Adult daphnids were cultured at approximately the same temperature as was used during the test. The culture water was Wildlife International Ltd. well water supplemented with calcium. Daphnids in the cultures were held for at least 20 days prior to selection of individuals for testing. The adults showed no signs of disease or stress during the holding period. During the 14-day holding period preceding the test, water temperatures ranged from 19.9 to 20.9°C. The pH of the water ranged from 8.1 to 8.7, and dissolved oxygen ranged from 8.6 to 9.2 mg/L. Instrumentation used for water measurements are described in the *Environmental Conditions* section of this report.

Neonate daphnids were obtained for testing from individual adult daphnids. The progeny from ten adults were used in the test. At test initiation, the juvenile daphnids were collected from the cultures and placed in glass beakers. The daphnids were then transferred to the test chambers using a wide-bore pipet. All transfers were conducted underneath the air/water interface. Daphnids in the cultures and those used in the test were fed a mixture of yeast, Cerophyll®, and trout chow, as well as a suspension of the freshwater green alga, *Scenedesmus capricornutum*. During the test, daphnids were fed three times daily and once on the last day of the test.

### Test Apparatus

A continuous-flow diluter was used to deliver each concentration of the test substance, a solvent control, and a negative (well water) control. Syringe pumps were used to deliver the five test substance stocks and the solvent for the solvent control into mixing chambers assigned to each treatment and control group. The stock solutions were diluted with well water in the mixing chambers in order to obtain the desired test concentrations. The flow of dilution water to the mixing

chambers was controlled by rotameters. Rotameters were calibrated prior to test initiation and at weekly intervals thereafter during the test. The flow of test water from each mixing chamber was split and allowed to flow into replicate test chambers. The proportion of test water that was split into each replicate was checked prior to the test and at weekly intervals thereafter during the test to ensure that flow rates varied by no more than  $\pm 10\%$  of the mean for the two replicates.

The diluter was adjusted so that each test chamber received approximately 6 volume additions of test water every 24 hours. The stock solution delivery pumps were calibrated before the test. The general operation of the diluter was checked visually at least two times per day during the test and once at the end of the test.

Test compartments were constructed from 500-mL glass beakers approximately 8.0 cm in diameter and 13 cm in height. Nylon mesh screen covered two holes on opposite sides of each test compartment to allow test solution to flow in and out of the test compartments. The volume of the test solution in a representative test compartment was approximately 400 mL. The test compartments were placed in 25-L stainless steel test chambers filled with approximately 22 L of test water. The depth of the test solution in a representative test compartment and test chamber was 8.0 cm and 27 cm, respectively. Test chambers were indiscriminately positioned in a temperature-controlled water bath designed to maintain a temperature of  $20 \pm 1^\circ\text{C}$ . The water bath was enclosed in a plexiglass ventilation hood in order to minimize potential for cross-contamination. The test chambers were labeled with the project number, test concentration and replicate.

#### Dilution Water

The water used for culturing and testing was freshwater obtained from a well approximately 45 meters deep located on the Wildlife International Ltd. site. The well water is characterized as moderately-hard water. The specific conductance, hardness, alkalinity, and pH of the well water during the four-week period immediately preceding the test are presented in Appendix III.

The well water was passed through a sand filter to remove particles greater than approximately 25  $\mu\text{m}$ , and pumped into a 37,800-L storage tank where the water was aerated with spray nozzles. Prior to delivery to the diluter system, the water again was filtered to remove microorganisms and particles. Prior to use, a UV sterilizer was provided as an additional method of water treatment. The results of periodic analyses performed to measure the concentrations of selected contaminants in well water used by Wildlife International Ltd are presented in Appendix IV.

#### Environmental Conditions

Lighting used to illuminate the cultures and test chambers during culturing and testing was provided by fluorescent tubes that emitted wavelengths similar to natural sunlight (Colortone® Co.). A photoperiod of 16 hours of light and 8 hours of darkness was controlled with an automatic timer. A 30-minute transition period of low light intensity was provided when lights went on and off to avoid sudden changes in lighting. Light intensity at test initiation was 347 lux at the surface of the water.

Temperature was measured in each test chamber daily during the test using a hand-held thermometer. Temperature also was measured continuously in one negative control replicate using a Fulscope ER/C Recorder. The target test temperature during the study was  $20 \pm 1^\circ\text{C}$ . Dissolved oxygen and pH were measured in alternate replicates of each treatment and control group daily during the test. Hardness, alkalinity, specific conductance and total organic carbon (TOC) were measured in alternating replicates of the negative control (dilution water) at the beginning of the test and at weekly intervals thereafter.

Measurements of pH were made using a Fisher Accumet Model 915 pH meter, and dissolved oxygen was measured using a Yellow Springs Instrument Model 51B dissolved oxygen meter. Specific conductance was measured using a Yellow Springs Instrument Model 33 Salinity-Conductivity-Temperature meter. Total organic carbon (TOC) was analyzed with a Shimadzu

- 14 -

Model 5000 TOC Analyzer. Hardness and alkalinity values were determined by titration based on procedures in *Standard Methods for the Examination of Water and Wastewater* (4).

#### Biological Observations and Measurements

Observations of each first-generation daphnid were made daily during the test. At those times, the number of dead and immobile daphnids were recorded along with the onset of reproduction and any sublethal signs of toxicity (e.g., inability to maintain position in the water column and uncoordinated swimming and cessation of feeding). The presences of eggs in the brood pouch, males, or ephippia also were recorded daily. The criteria for death included absence of heartbeat, white opaque coloration, lack of movement of appendages, and lack of response to gentle prodding. Immobilization was defined as lack of movement except for minor spontaneous random movement of the appendages.

With the onset of reproduction, the number of live and dead neonates produced by first-generation daphnids were recorded then discarded every Monday, Wednesday and Friday during the test. At those times, second-generation daphnids also were observed for abnormal development and aberrant behavior, such as inability to maintain position in the water column, uncoordinated swimming, and cessation of feeding. At the end of the test, surviving first-generation daphnids were measured for length (distance from the apex of the head to the base of the spine) and dry weight.

#### Statistical Analyses

Statistical analyses were performed on the survival of the first-generation daphnids, the number of live young produced per adult per reproductive day, and the length and dry weight of all surviving first-generation daphnids. Survival data collected at 24 hours, 48 hours, 96 hours, 7 days, 14 days and at the end of the test (Day 21) were analyzed using contingency tables and the chi-square test to identify treatment groups that were statistically significant ( $p \leq 0.05$ ) from the control group. EC50 values for those same time periods did not warrant the use of statistical analysis and were estimated from the mortality data.

Analyses of reproduction (number of young produced per surviving adult per reproductive day) and growth (dry weights and lengths) were evaluated for normality using the Shapiro-Wilk's test and homogeneity using the Bartlett's test. Negative and solvent control data were compared using a Student's t-test and these data were pooled when no statistical differences were found. An analysis of variance test was used to determine whether or not statistically significant differences existed among the experimental groups. Those treatments statistically different from the control group were identified using the Bonferroni t-test.

The statistical analyses of survival, growth and reproduction data were used in the determination of the no-observed-effect-concentration (NOEC) and lowest-observed-effect-concentration (LOEC). The maximum acceptable toxicant concentration (MATC) was calculated as the geometric mean of the NOEC and LOEC. All statistical tests were made on a personal computer using SPSS/PC Version 2.0 (5) or TOXSTAT Version 3.2 statistical software (6).

#### Analytical Chemistry

Samples of test media solutions (test samples) were collected from both test chambers of the negative and solvent controls and the three highest treatment groups at the beginning of the test, at weekly intervals during the test, and at test termination to determine concentrations of the test substance. Due to the test method limit of quantitation (LOQ), the two lowest treatment groups were not sampled. However, the five stock samples which supplied test substance stock solution for all the treatment groups were sampled at test initiation and termination. All test samples were collected at mid-depth from each test chamber and were extracted immediately. Nominal test concentrations are reported along with mean measured test concentrations in parentheses, where appropriate. Analytical procedures used in the extraction and analyses of the samples are provided in Appendix V.

## RESULTS AND DISCUSSION

### Measurement of Test Concentrations

Results of analyses to measure concentrations of octabromodiphenyl oxide (OBDPO) in stock and water samples collected during the test are presented in Table 1 and in the analytical chemistry report (Appendix V). Nominal test concentrations selected for use in this study were 0.13, 0.25, 0.50, 1.0 and 2.0  $\mu\text{g/L}$ . Due to the test method LOQ, the two lowest treatment groups were not sampled. The mean measured test concentrations achieved in the test for the three highest treatment groups were 0.54, 0.83 and 1.7  $\mu\text{g/L}$ , which represented 108, 83 and 85% of the nominal concentrations, respectively. Stock solution samples collected at the beginning of the test ranged from 91 to 99% of the nominal values, while stock solution samples at test termination ranged from 83 to 92% of the nominal values. Based on these results, along with the test substance delivery log data and the diluter operational data, it appears that the nominal concentrations of the two lowest treatment groups, although not measured analytically, should have been close to the expected values. Mean measured test concentrations were used in determining the NOEC, LOEC, MATC and EC50 values.

### Physical and Chemical Measurements of Water

Measurements of temperature, dissolved oxygen, and pH are presented in Tables 2, 3 and 4, respectively. Water temperatures were within the  $20 \pm 1^\circ\text{C}$  range established for the test. Dissolved oxygen concentrations were  $\geq 77\%$  of saturation throughout the test. Measurements of pH ranged from 8.2 to 8.5. Measurements of specific conductance, hardness, alkalinity and total organic carbon (TOC) of the negative control are given in Table 5. No apparent differences were observed for any of these parameters throughout the exposure period.

### Survival and Biological Observations

A summary of the observations of mortality and sublethal signs of toxicity of the adult daphnids are shown in Table 6. Daily observations are provided in Appendix VI. By the end of the test, mortality percentages in the negative and solvent control groups were 5.0 and 0%, respectively.

All surviving adult daphnids in the negative and solvent control groups appeared normal and healthy throughout the test. Survival in these two groups was not statistically different ( $p > 0.05$ ) and the data from the control groups were pooled for comparison to the treatment groups. No statistically significant difference ( $p > 0.05$ ) in survival existed in the 0.13, 0.25, 0.50, 1.0 and 2.0  $\mu\text{g/L}$  treatments at 24, 48 and 96 hours, and at Days 7 and 14 or at test termination when compared to the pooled control group. Mortality in the 0.13, 0.25, 0.50, 1.0 and 2.0  $\mu\text{g/L}$  treatment groups was 10, 0, 5.0, 5.0 and 5.0%, respectively. Insufficient immobilization and/or mortality occurred during the test to calculate  $\text{EC}_{50}$  values at 24, 48 and 96 hours, and Days 7, 14 and 21. Therefore, the  $\text{EC}_{50}$  for the adult daphnids for those time periods was estimated to be greater than 2.0  $\mu\text{g/L}$  (Table 7). The  $\text{NOEC}$  for survival was 2.0  $\mu\text{g/L}$  (1.7  $\mu\text{g/L}$ , mean measured concentration), the highest concentration tested.

#### Reproduction

The percentages of adults having eggs in their brood pouch at each day of the study is shown in Table 8. Eggs were first observed in some daphnids beginning on Day 8 of the test. In general, most adults contained eggs in their brood pouch during the reproduction period of the test. No clearly defined dose-response pattern of egg production was observed in the numbers of adults holding eggs in their brood pouch.

A summary of the numbers of living and dead neonates produced per adult per reproductive day is shown in Table 9. The number of live neonates produced per reproductive day and the number of reproductive days are presented in Appendices VII and VIII, respectively. For each female, the young released per reproductive day was calculated by dividing the cumulative number of young released by the number of reproductive days. For each female, the number of reproductive days is the number of days that the female was alive from the day of first brood release of any female in the test to the end of the test. Adult daphnids in the negative and solvent control groups produced an average of 5.3 and 5.5 live young per adult per reproductive day, respectively. There was no statistical difference between these two groups, therefore, the two control groups were pooled for comparison against the treatment groups. The mean numbers of young produced per

adult per reproductive day in the 0.13, 0.25, 0.50, 1.0 and 2.0  $\mu\text{g/L}$  treatment groups were 6.7, 5.2, 5.7, 5.2 and 5.1, respectively. Neonates production in those treatment groups was not statistically different ( $p>0.05$ ) from the pooled controls.

The young produced by the adult daphnids were evaluated for mortality, immobility and abnormal behavior. A summary of the observations made on the neonates for each observation day are presented in Table 10. No dead or immobile neonates were observed in the negative and solvent control groups and all neonates produced by the adult daphnids in these groups appeared normal. Similarly, all neonates produced by the adult daphnids in the 0.13, 0.25, 0.50, 1.0 and 2.0  $\mu\text{g/L}$  treatment groups appeared normal. There were no dead or immobile neonates produced in these groups. Furthermore, there were no aborted eggs observed in any control or treatment group during the test. The NOEC for reproduction was 2.0  $\mu\text{g/L}$  (1.7  $\mu\text{g/L}$ , mean measured concentration), the highest concentration tested.

### Growth

A summary of the mean lengths and mean dry weights of the first-generation daphnids are presented in Tables 11 and 12, respectively. Individual measurements are provided in Appendix IX. The mean length and mean dry weight of the adult daphnids in the negative and solvent control groups were 4.39 mm and 0.601 mg, and 4.42 mm and 0.608 mg, respectively. When lengths and dry weights of the negative and solvent control groups were compared, no statistically significant ( $p>0.05$ ) differences were found. Therefore, those data were pooled for evaluation of treatment-related growth effects.

Mean lengths and dry weights of adult daphnids in the 0.13, 0.25, 0.50, 1.0 and 2.0  $\mu\text{g/L}$  treatment groups were 4.50, 4.50, 4.48, 4.50 and 4.44 mm, and 0.630, 0.612, 0.609, 0.637 and 0.596 mg, respectively. The mean lengths and mean dry weights of the daphnids in these groups were similar to those in the pooled control group. Any differences were slight, not dose-responsive nor statistically different ( $p>0.05$ ) from the pooled control group. The NOEC for growth was 2.0  $\mu\text{g/L}$  (1.7  $\mu\text{g/L}$ , mean measured concentration), the highest concentration tested.

- 19 -

**CONCLUSIONS**

There were no apparent treatment-related effects on survival, reproduction or growth of *Daphnia magna* exposed to octabromodiphenyl oxide (OBDPO). For this study, the 24, 48 and 96-hour, and Day 7, 14 and 21 EC50 values for survival were all greater than 2.0 µg/L (1.7 µg/L, mean measured concentration), the highest concentration tested. Similarly, the no-observed-effect-concentration (NOEC) for this study (based on survival, reproduction and growth data) was 2.0 µg/L (1.7 µg/L, mean measured concentration), the highest concentration tested, while the lowest-observed-effect-concentration (LOEC) and the maximum acceptable toxicant concentration (MATC) were estimated to be greater than 2.0 µg/L (1.7 µg/L, mean measured concentration). Nominal test concentrations were based on the maximum solubility of the test substance in water (approximately < 0.5 µg/L) and the results of an exploratory range finding toxicity test (Appendix I).

AMENDED

**REFERENCES**

- 1 **Organisation for Economic Cooperation and Development.** 1984. *Daphnia sp. Acute Immobilisation Test (24-Hour) and Reproduction Test.* OECD Guideline for Testing of Chemicals, Guideline 202. Paris.
- 2 **ASTM Standard E1193-87.** 1988. *Standard Guide for Conducting Renewal Life-Cycle Toxicity Tests with Daphnia magna.* American Society for Testing and Materials.
- 3 **Title 40 of the Code of Federal Regulations, Part 797, Section 1330.** 1994. *Daphnia Chronic Toxicity Test.*
- 4 **APHA, AWWA, WPCF.** 1985. *Standard Methods for the Examination of Water and Wastewater.* 16th Edition, American Public Health Association. American Water Works Association. Water Pollution Control Federation, New York.
- 5 **SPSS, Inc.** 1988. *SPSS/PC+ Version 2.0.* Chicago, Illinois.
- 6 **Gulley, D.D., A.M. Boelter, H.L. Bergman.** 1989. *TOXSTAT, Version 3.2.* University of Wyoming, Laramie, Wyoming.

- 21 -

Table 1  
Summary of Analytical Chemistry Data

Sponsor:		CMA's Brominated Flame Retardant Industry Panel			
Test Substance:		Octabromodiphenyl Oxide (OBDPO)			
Test Organism:		Cladoceran, <i>Daphnia magna</i>			
Dilution Water:		Well Water			
Nominal Test Concentration ( $\mu\text{g/L}$ )	Time	Replicate	Measured Concentration ( $\mu\text{g/L}$ )	Mean Measured Concentration ( $\mu\text{g/L}$ )	Mean Percent of Nominal
Negative Control	Day 0	A	< LOQ <sup>1</sup>	-	-
		B	< LOQ		
	Day 5	A	< LOQ		
		B	< LOQ		
	Day 12	A	< LOQ		
		B	< LOQ		
	Day 21	A	< LOQ		
		B	< LOQ		
Solvent Control	Day 0	A	< LOQ	-	-
		B	< LOQ		
	Day 5	A	< LOQ		
		B	< LOQ		
	Day 12	A	< LOQ		
		B	< LOQ		
	Day 21	A	< LOQ		
		B	< LOQ		
0.50	Day 0	A	0.484	0.54	103
		B	0.506		
	Day 5	A	3.81 <sup>2</sup>		
		B	0.555		
	Day 12	A	0.732		
		B	0.632		
	Day 21	A	0.451		
		B	0.413		
1.0	Day 0	A	0.891	0.83	83
		B	0.857		
	Day 5	A	0.941		
		B	0.983		
	Day 12	A	0.840		
		B	0.882		
	Day 21	A	0.577		
		B	0.681		

<sup>1</sup> The limit of quantitation (LOQ = 0.250  $\mu\text{g/L}$ ) was based upon the product of the lowest standard (25.0  $\mu\text{g/L}$ ) and the dilution factor of the control samples (0.01) analyzed concurrently with the test samples.

<sup>2</sup> Not included in the calculation of the mean.

Table 1 (Continued)  
Summary of Analytical Chemistry Data<sup>1</sup>

Sponsor: CMA's Brominated Flame Retardant Industry Panel					
Test Substance: Octabromodiphenyl Oxide (OBDPO)					
Test Organism: Cladoceran, <i>Daphnia magna</i>					
Dilution Water: Well Water					
Nominal Test Concentration ( $\mu\text{g/L}$ )	Time	Replicate	Measured Concentration ( $\mu\text{g/L}$ )	Mean Measured Concentration ( $\mu\text{g/L}$ )	Mean Percent of Nominal
2.0	Day 0	A	1.66	1.7	85
		B	1.82		
	Day 5	A	1.50		
		B	1.99		
	Day 12	A	1.87		
		B	1.90		
	Day 21	A	1.50		
		B	1.16		
Stock (mg/L) 1.2	Initiation		1.76	1.7	98
	Termination		1.55		86
3.5	Initiation		3.22	3.1	92
	Termination		3.05		87
7.0	Initiation		6.91	6.7	99
	Termination		6.43		92
14	Initiation		13.8	13	99
	Termination		11.6		83
28	Initiation		25.4	25	91
	Termination		24.0		86

<sup>1</sup> The limit of quantitation (LOQ = 0.250  $\mu\text{g/L}$ ) was based upon the product of the lowest standard (25.0  $\mu\text{g/L}$ ) and the dilution factor of the control samples (0.01) analyzed concurrently with the test samples.

Table 2

Temperature (°C) of Water in the Test Chambers

Sponsor:		OMA's Incriminated Flame Retardant Industry Panel																										
Test Substance:		Octadecylphenyl Oxide (OBDO)																										
Test Organism:		Cladocera - <i>Daphnia magna</i>																										
Exposure Water:		Well Water																										
Nominal Test Concentration (µg/L)	Test Chamber	Temperature (°C) (Day)																										
		0	1	2	3	4	5	6	7	8	9	10	11	12	12	12	14	15	16	17	18	19	20	21				
Negative Control	A	19.9	19.9	19.9	19.8	19.8	19.9	19.9	19.8	19.8	19.9	19.8	19.9	19.8	19.9	19.8	19.9	19.9	19.9	19.9	19.9	19.9	19.9	19.8	19.8			
	B	19.8	19.5	19.9	19.8	19.8	19.9	19.9	19.8	19.8	19.9	19.8	19.9	19.8	19.9	19.8	19.9	19.9	19.9	19.9	19.9	19.9	19.9	19.9	19.8	19.8		
Sewage Control	A	19.8	19.9	19.8	19.8	19.9	19.8	19.8	19.8	19.9	19.8	19.9	19.8	19.9	19.8	19.9	19.9	19.9	19.9	19.9	19.9	19.9	19.9	19.9	19.9	19.9		
	B	19.9	19.9	19.9	19.8	19.8	19.8	19.9	19.9	19.9	19.8	19.9	19.8	19.9	19.8	19.9	19.9	19.9	19.9	19.9	19.9	19.9	19.9	19.9	19.9	19.9		
0.13	A	19.8	19.9	19.8	19.8	19.8	19.8	19.8	19.9	19.9	19.8	19.9	19.8	19.9	19.8	19.9	19.9	19.9	19.9	19.9	19.9	19.9	19.9	19.9	19.8	19.8		
	B	19.8	19.9	19.8	19.8	19.8	19.8	19.9	19.9	19.8	19.8	19.9	19.8	19.9	19.8	19.9	19.9	19.9	19.9	19.9	19.9	19.9	19.9	19.9	19.9	19.8	19.8	
0.23	A	19.9	19.9	19.7	19.7	19.8	19.9	19.8	19.9	19.8	19.8	19.9	19.7	19.8	19.8	19.8	19.9	19.8	19.8	19.8	19.8	19.8	19.8	19.8	19.7	19.8		
	B	19.9	19.8	19.7	19.7	19.9	19.9	19.9	19.9	19.8	19.8	19.9	19.8	19.8	19.8	19.8	19.8	19.8	19.8	19.8	19.8	19.8	19.8	19.8	19.7	19.8		
0.50 (0.54)	A	19.7	19.8	19.8	19.8	19.9	19.9	19.8	19.7	19.9	19.8	19.8	19.8	19.8	19.8	19.8	19.8	19.8	19.8	19.8	19.8	19.8	19.8	19.7	19.9	19.9		
	B	19.9	19.8	19.8	19.8	19.8	19.8	19.8	19.8	19.8	19.8	19.8	19.8	19.8	19.8	19.8	19.8	19.8	19.8	19.8	19.8	19.8	19.8	19.8	19.8	19.8	19.8	
1.0 (0.53)	A	19.9	19.8	19.8	19.8	20.0	20.0	19.9	19.9	19.9	20.0	19.8	19.9	19.9	19.8	19.9	19.9	19.9	19.9	19.9	19.9	19.9	19.9	19.9	19.9	19.8	19.8	
	B	20.0	19.9	19.8	19.8	19.8	19.9	19.8	19.8	19.8	19.9	19.9	19.9	19.9	19.7	19.8	19.8	19.8	19.8	19.8	19.8	19.8	19.8	19.9	19.9	19.9	19.8	19.8
2.0 (1.7)	A	20.0	19.9	19.8	19.8	20.0	20.0	19.9	19.9	19.9	20.0	19.8	19.8	19.8	19.8	19.8	19.8	19.8	19.8	19.8	19.8	19.8	19.8	19.9	19.9	19.9	19.8	19.8
	B	19.9	19.8	19.8	19.8	20.0	20.0	19.9	19.9	19.9	20.0	19.8	19.8	19.8	19.8	19.8	19.8	19.8	19.8	19.8	19.8	19.8	19.8	19.9	19.9	19.9	19.9	19.8

1. Temperature was measured by a hand-held thermometer.  
 Note: Values in parentheses are mean measured test concentrations.

Table 3

Dissolved Oxygen Content (mg/L) of Water in the Test Chambers

Sponsor: CMA's Brominated Flame Retardant Industry Panel  
 Test Substance: Octabromodiphenyl Oxide (OBDDPO)  
 Test Organism: Cladoceran, *Daphnia magna*  
 Dilution Water: Well Water

Nominal Test Concentration ( $\mu\text{g/L}$ )	Test Chamber Replicate	Dissolved Oxygen (mg/L) (Day)																					
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Negative Control	A	8.8	8.8	8.8	8.8	8.8	8.6	8.2	8.3	8.4	8.4	8.2	8.4	8.4	8.4	8.2	8.5	8.5	8.2	8.6	8.4	8.4	8.4
	B	9.0	9.0	9.0	9.0	8.7	8.7	8.2	8.2	8.2	8.2	8.1	8.1	8.4	8.4	8.5	8.5	8.5	8.2	8.6	8.4	8.4	8.4
Solvent Control	A	8.9	8.8	8.8	8.8	8.8	8.4	8.2	8.1	8.1	8.2	8.0	8.0	8.2	8.2	7.9	7.9	7.9	7.9	7.0	7.0	7.0	7.1
	B	9.0	8.8	8.8	8.7	8.7	8.4	8.2	8.1	8.1	8.0	8.0	7.8	8.2	8.2	7.9	7.7	7.7	7.4	7.9	7.2	7.2	7.4
0.13	A	8.9	8.8	8.8	8.8	8.8	8.5	8.2	8.1	8.1	8.0	8.0	7.8	7.7	7.7	7.7	7.7	7.7	7.7	7.0	7.2	7.2	7.0
	B	9.0	9.0	9.0	9.0	8.8	8.8	8.2	8.2	8.2	8.0	8.0	7.7	7.7	7.7	7.4	7.4	7.4	7.4	7.4	7.4	7.4	7.0
0.50 (0.54)	A	8.9	8.7	8.7	8.7	8.4	8.4	8.1	8.1	8.1	8.0	8.0	8.0	8.2	8.2	8.3	8.3	8.3	8.2	8.6	7.3	7.3	7.6
	B	9.0	8.8	8.8	8.8	8.8	8.8	8.1	8.1	8.0	8.0	8.0	8.0	8.2	8.2	8.3	8.3	8.3	8.2	8.6	7.6	7.6	7.6
1.0 (0.83)	A	8.9	8.7	8.7	8.8	8.4	8.4	8.0	8.0	8.0	8.0	8.1	8.1	8.1	8.2	8.3	8.2	8.2	8.2	8.0	7.4	7.4	7.3
	B	9.0	8.8	8.8	8.8	8.8	8.8	7.7	7.7	7.7	8.0	8.1	8.1	8.2	8.2	8.2	8.2	8.2	8.0	8.0	7.5	7.5	7.3
2.0 (1.7)	A	8.9	8.8	8.8	8.8	8.4	8.4	8.1	8.1	8.1	8.2	8.1	8.1	8.1	8.2	8.2	8.2	8.2	8.2	8.1	7.4	7.4	7.3
	B	9.0	8.8	8.8	8.8	8.8	8.8	8.1	8.1	8.0	8.0	8.0	8.1	8.1	8.1	8.1	8.1	8.1	8.2	8.1	7.4	7.4	7.3

<sup>1</sup> A dissolved oxygen concentration of approximately 7.0 mg/L represents 77% of saturation,  $-1.6^{\circ}\text{C}$  in freshwater.  
 Note: Values in parentheses are mean measured test concentrations.

Table 4  
pH of Water in the Test Chambers

Sponsor:		CMA's Brominated Flame Retardant Industry Panel																					
Test Substance:		Octabromodiphenyl Oxide (OBDDPO)																					
Test Organism:		Cladoceran, <i>Daphnia magna</i>																					
Dilution Water:		Well Water																					
Nominal Test Concentration (µg/L)	Test Chamber	pH (Day)																					
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Negative Control	A	8.4	8.4	8.4	8.4	8.4	8.3	8.3	8.3	8.3	8.5	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.3	8.3
	B	8.4	8.4	8.4	8.4	8.4	8.3	8.3	8.3	8.3	8.3	8.3	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.3	8.3
Solvent Control	A	8.4	8.4	8.4	8.4	8.4	8.3	8.3	8.3	8.3	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.3	8.3
	B	8.4	8.4	8.4	8.4	8.4	8.3	8.3	8.3	8.3	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.3	8.3
0.13	A	8.4	8.4	8.5	8.5	8.5	8.3	8.3	8.3	8.3	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.3	8.3	8.3
	B	8.4	8.4	8.5	8.5	8.5	8.3	8.3	8.3	8.3	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.3	8.3	8.3
0.25	A	8.4	8.4	8.5	8.5	8.5	8.3	8.3	8.3	8.3	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.3	8.3	8.3
	B	8.4	8.4	8.5	8.5	8.5	8.3	8.3	8.3	8.3	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.3	8.3	8.3
0.50 (0.54)	A	8.4	8.4	8.5	8.5	8.5	8.3	8.3	8.3	8.3	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.3	8.3	8.3
	B	8.4	8.4	8.5	8.5	8.5	8.3	8.3	8.3	8.3	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.3	8.3	8.3
1.0 (0.83)	A	8.4	8.4	8.5	8.5	8.5	8.3	8.3	8.3	8.3	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.3	8.3	8.3
	B	8.4	8.4	8.5	8.5	8.5	8.3	8.3	8.3	8.3	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.3	8.3	8.3
2.7 (1.7)	A	8.4	8.4	8.5	8.5	8.5	8.3	8.3	8.3	8.3	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.3	8.3	8.3
	B	8.4	8.4	8.5	8.5	8.5	8.3	8.3	8.3	8.3	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.3	8.3	8.3

Note: Values in parentheses are mean measured test concentrations.

- 26 -

Table 5

**Specific Conductance, Hardness, Alkalinity and Total Organic Carbon  
in the Negative Control**

Negative Control				
Parameter	Time			
	Day 0, Rep A	Day 7, Rep B	Day 14, Rep A	Day 21, Rep A
Sponsor:	CMA's Brominated Flame Retardant Industry Panel			
Test Substance:	Octabromodiphenyl Oxide (OBDPO)			
Test Organism:	Cladoceran, <i>Daphnia magna</i>			
Dilution Water:	Well Water			
Specific Conductance ( $\mu$ mhos/cm)	310	305	310	320
Hardness (mg/L as CaCO <sub>3</sub> )	132	136	132	136
Alkalinity (mg/L as CaCO <sub>3</sub> )	180	184	178	182
Total Organic Carbon (mgC/L)	< 1.0	< 1.0	< 1.0 (Rep B)	< 1.0 (Rep A)

Table 6

Summary of Cumulative Percent Mortality and Treatment-Related Effects<sup>1</sup>

Nominal Test Concentration ( $\mu\text{g/L}$ )	Day 1			Day 2			Day 4		
	% Mortality	% Immobile	Effects	% Mortality	% Immobile	Effects	% Mortality	% Immobile	Effects
Negative Control	0	0	20 AN	0	0	20 AN	0	0	20 AN
Solvent Control	0	0	20 AN	0	0	20 AN	0	0	20 AN
0.13	0	0	20 AN	0	0	20 AN	0	0	20 AN
0.25	0	0	20 AN	0	0	20 AN	0	0	20 AN
0.50 (0.54)	0	0	20 AN	0	0	20 AN	0	0	20 AN
1.0 (0.83)	0	0	20 AN	0	0	20 AN	0	0	20 AN
2.0 (1.7)	0	0	20 AN	0	0	20 AN	0	0	20 AN

<sup>1</sup> Observed Effects: AN = All organisms appeared normal and no unusual behavior observed; C = Lethargy.

Note: Mortality values are cumulative while observations are specific to the day of observation. Values in parentheses are mean measured test concentrations.

Table 6 (Continued)  
 Summary of Cumulative Percent Mortality and Treatment-Related Effects<sup>1</sup>

Sponsor: CMA's Brominated Flame Retardant Industry Panel									
Test Substance: Octabromodiphenyl Oxide (OBDFPO)									
Test Organism: Cladoceran, <i>Daphnia magna</i>									
Dilution Water: Well Water									
Nominal Test Concentration (µg/L)	Day 7			Day 14			Day 21		
	% Mortality	% Immobile	Effects	% Mortality	% Immobile	Effects	% Mortality	% Immobile	Effects
Negative Control	0	0	20 AN	0	0	20 AN	5.0	0	19 AN
Solvent Control	0	0	20 AN	0	0	20 AN	0	0	20 AN
0.13	0	0	20 AN	5.0	0	19 AN	10	0	18 AN
0.25	0	0	20 AN	0	0	20 AN	0	0	20 AN
0.50 (0.54)	0	0	20 AN	0	0	20 AN	5.0	0	19 AN
1.0 (0.83)	0	0	20 AN	C	0	20 AN	5.0	0	18AN;1C
2.0 (1.7)	0	0	20 AN	5.0	0	19 AN	5.0	0	18 AN;1C

<sup>1</sup> Observed Effects: AN = All organisms appeared normal and no unusual behavior observed; C = Lethargy.

Note: Mortality values are cumulative while observations are specific to the day of observation. Values in parentheses are mean measured concentrations.

- 29 -

Table 7  
Mortality/Immobility EC50 Values

Sponsor: CMA's Brominated Flame Retardant Industry Panel				
Test Substance: Octabromodiphenyl Oxide (OBDPO)				
Test Organism: Cladoceran, <i>Daphnia magna</i>				
Dilution Water: Well Water				
Time	EC50 ( $\mu\text{g/L}$ )	Lower 95% Confidence Limits <sup>1</sup>	Upper 95% Confidence Limits <sup>1</sup>	Statistical Method
24 Hours	> 2.0	--	--	NA
48 Hours	> 2.0	--	--	NA
96 Hours	> 2.0	--	--	NA
Day 7	> 2.0	--	--	NA
Day 14	> 2.0	--	--	NA
Day 21	> 2.0	--	--	NA

<sup>1</sup> Confidence limits could not be calculated with the data obtained.

NA - Statistical method not used. Estimation made by visual interpretation of the mortality/immobility data.

Table 8  
Percent of Adults with Eggs in Brood Pouch

Day	CMA's Brominated Flame Retardant Industry Panel						
	Negative Control	Solvent Control	0.13 µg/L	0.25 µg/L	0.50 µg/L (0.54 µg/L)	1.0 µg/L (0.83 µg/L)	2.0 µg/L (1.7 µg/L)
5	25.0	15.0	30.0	15.0	20.0	30.0	10.0
6	45.0	35.0	40.0	35.0	45.0	50.0	40.0
7	100	95.0	100	100	100	100	100
8	100 <sup>1</sup>	95.0 <sup>1</sup>	100 <sup>1</sup>	100 <sup>1</sup>	100 <sup>1</sup>	100 <sup>1</sup>	100 <sup>1</sup>
9	100	95.0	100	100	100	100	100
10	100	95.0	90.0	95.0	100	90.0	100
11	100	100	100	95.0	100	100	100
12	100	100	100	100	100	100	100
13	95.0	95.0	94.7	100	100	100	78.9
14	100	95.0	94.7	100	100	100	100
15	90.0	80.0	89.5	95.0	90.0	95.0	94.7
16	85.0	75.0	89.5	90.0	95.0	85.0	94.7
17	100	95.0	88.9	85.0	100	85.0	89.5
18	100	100	94.4	85.0	90.0	90.0	94.7
19	100	100	100	85.0	100	80.0	100
20	94.7	90.0	100	100	90.0	94.7	84.2
21	94.7	100	88.9	95.0	89.5	89.5	94.7

<sup>1</sup> Neonates present by Day 8

Note: Values in parentheses are mean measured test concentrations.

Table 9

Reproduction of *Daphnia magna* During the Chronic Toxicity Test

Nominal Test Concentration ( $\mu\text{g/L}$ )	Replicate	Beaker	No. Repro- Days	No. Dead/Imm. Young Produced	No. Live Young Produced	No. Dead/Imm. Young/Adult/Repro-Day	No. Live Young/Adult/Repro-Day	Replicate Mean		Treatment Mean	
								No. Live Young/Adult/Repro-Day	No. Dead/Imm. Young/Adult/Repro-Day	Treatment Mean Live Young/Adult/Repro-Day	Treatment Mean Dead/Imm. Young/Adult/Repro-Day
Negative Control	A	1	70	0	388	0	5.5	5.5	5.3	0	
	B	2	67	0	363	0	5.4	5.0			
	A	1	70	0	340	0	4.9				
	B	2	70	0	358	0	5.1				
	A	1	70	0	351	0	5.0	5.1	5.5	0	
	B	2	70	0	369	0	5.3	5.9			
0.13	A	1	62	0	497	0	8.0	6.6	6.7	0	
	B	2	66	0	347	0	5.3	6.8			
	A	1	70	0	474	0	6.8				
	B	2	70	0	484	0	6.9				
	A	1	70	0	312	0	4.5	5.3	5.2	0	
	B	2	70	0	435	0	6.2	5.0			
0.50 (0.54)	A	1	70	0	341	0	4.9	5.0	5.7	0	
	B	2	70	0	362	0	5.2	6.5			
	A	1	70	0	319	0	4.6	5.0	5.7	0	
	B	2	70	0	377	0	5.4	6.5			
	A	1	70	0	434	0	6.2	6.8			
	B	2	70	0	476	0	6.8				

Note: The mean number of neonates produced per adult per reproductive day in the pooled control group was 5.4. Values in parentheses are mean measured test concentrations.

Table 9 (Continued)  
 Reproduction of *Daphnia magna* During the Chronic Toxicity Test

Sponsor: CMA's Brominated Flame Retardant Industry Panel													
Test Substance: Octabromodiphenyl Oxide (OBDFO)													
Test Organism: Cladoceran, <i>Daphnia magna</i>													
Dilution Water: Well Water													
Nominal Test Concentration ( $\mu\text{g/L}$ )	Replicate	Beaker	No. Repro- Days	No. Dead/Imm. Young Produced	No. Live Young Produced	No. Dead/Imm. Young/Adult/Repro-Day	No. Live Young/Adult/Repro-Day	Replicate Mean		Treatment		Treatment Mean	
								No. Live Young/Adult/Repro-Day	No. Live Young/Adult/Repro-Day	Mean Live Young/Adult/Repro-Day	Mean Live Young/Adult/Repro-Day	Dead/Imm. Young/Adult/Repro-Day	Dead/Imm. Young/Adult/Repro-Day
1.0 (0.83)	A	1	70	0	319	0	4.6	5.6	5.2	0			
		2	68	0	460	6	6.8						
	B	1	70	0	321	0	4.6	4.8					
		2	70	0	351	0	5.0						
2.0 (1.7)	A	1	61	0	289	0	4.7	5.4	5.1	0			
		2	70	0	415	0	5.9						
	B	1	70	0	360	0	5.1	4.9					
		2	70	0	320	0	4.6						

Note: The mean number of neonates produced per adult per reproductive day in the pooled control group was 5.4. Values in parentheses are mean measured test concentrations.

Table 10  
Clinical Observations<sup>1</sup> of Neonate Daphnids

Sponsor:		CMA's Brominated Flame Retardant Industry Panel				
Test Substance:		Octabromodiphenyl Oxide (OBDPO)				
Test Organism:		Cladoceran, <i>Daphnia magna</i>				
Dilution Water:		Well Water				
Nominal Test Concentration ( $\mu\text{g/L}$ )	Day of Study					
	9	12	14	16	19	21
Negative Control	115 AN	374 AN	139 AN	295 AN	161 AN	365 AN
Solvent Control	68 AN	432 AN	426 AN	198 AN	342 AN	80 AN
0.13	171 AN	495 AN	508 AN	134 AN	370 AN	124 AN
0.25	133 AN	521 AN	88 AN	323 AN	250 AN	135 AN
0.50 (0.54)	96 AN	399 AN	110 AN	233 AN	439 AN	329 AN
1.0 (0.83)	75 AN	507 AN	304 AN	200 AN	256 AN	109 AN
2.0 (1.7)	72 AN	315 AN	62 AN	319 AN	401 AN	215 AN

<sup>1</sup> Observations: AN = Appeared Normal  
Note: Values in parentheses are mean measured test concentrations.

Table 11

## Mean Lengths of Surviving First-Generation Daphnids

Nominal Test Concentration ( $\mu\text{g/L}$ )	Replicate	No. per Replicate	Replicate Mean Length (mm)	Treatment Mean Length (mm) ( $\pm$ SD)
Negative Control	A	9	4.33	4.39 ( $\pm$ 0.078)
	B	10	4.44	
Solvent Control	A	10	4.45	4.42 ( $\pm$ 0.042)
	B	10	4.39	
0.13	A	8	4.46	4.50 ( $\pm$ 0.057)
	B	10	4.54	
0.25	A	10	4.47	4.50 ( $\pm$ 0.042)
	B	10	4.53	
0.50 (0.54)	A	9	4.44	4.48 ( $\pm$ 0.057)
	B	10	4.52	
1.0 (0.83)	A	9	4.45	4.50 ( $\pm$ 0.071)
	B	10	4.55	
2.0 (1.7)	A	9	4.41	4.44 ( $\pm$ 0.035)
	B	10	4.46	

Note: The mean length in the pooled control group was 4.40 ( $\pm$  0.055) mm.  
Values in parentheses are mean measured test concentrations.

- 35 -

Table 12

## Mean Dry Weights of Surviving First-Generation Daphnids

Sponsor: CMA's Brominated Flame Retardant Industry Panel				
Test Substance: Octabromodiphenyl Oxide (OBDPO)				
Test Organism: Cladoceran, <i>Daphnia magna</i>				
Dilution Water: Well Water				
Nominal Test Concentration ( $\mu\text{g/L}$ )	Replicate	No. per Replicate	Replicate Mean Dry Weight (mg)	Treatment Mean Dry Weight (mg) ( $\pm$ SD)
Negative Control	A	9	0.577	0.601 ( $\pm$ 0.034)
	B	10	0.625	
Solvent Control	A	10	0.522	0.608 ( $\pm$ 0.021)
	B	10	0.593	
0.13	A	8	0.599	0.630 ( $\pm$ 0.043)
	B	10	0.660	
0.25	A	10	0.597	0.612 ( $\pm$ 0.021)
	B	10	0.627	
0.50 (0.54)	A	9	0.600	0.609 ( $\pm$ 0.012)
	B	10	0.617	
1.0 (0.83)	A	9	0.607	0.637 ( $\pm$ 0.042)
	B	10	0.666	
2.0 (1.7)	A	9	0.587	0.596 ( $\pm$ 0.012)
	B	10	0.604	

Note: The mean dry weight in the pooled control group was 0.604 ( $\pm$  0.023) mg.  
Values in parentheses are mean measured test concentrations.

APPENDIX I

Rangefinding Results

Sponsor: CMA's Brominated Flame Retardant Industry Panel  
 Test Substance: Octabromodiphenyl Oxide (OBDPO)  
 Test Organism: Cladoceran, *Daphnia magna*  
 Dilution Water: Well Water

Test Concentration (mg/L)	Number Exposed	Observations										Total # Neonates Produced			
		Day 0 (1.25 h.)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9		Percent Survival		
Negative Control	10	10 AN	10 AN	10 AN	10 AN	10 AN	10 AN	10 AN	10 AN	10 AN	10 AN	10 AN	10 AN	100	32
Solvent Control	10	10 AN	10 AN	10 AN	10 AN	10 AN	10 AN	10 AN	10 AN	10 AN	10 AN	10 AN	10 AN	100	21
0.0081	10	10 AN	10 AN	10 AN	10 AN	10 AN	10 AN	10 AN	10 AN	10 AN	10 AN	10 AN	10 AN	100	0
0.027	10	10 AN	10 AN	10 AN	10 AN	10 AN	10 AN	10 AN	10 AN	10 AN	10 AN	10 AN	10 AN	100	19
0.09	10	10 AN	10 AN	10 AN	10 AN	10 AN	10 AN	10 AN	10 AN	10 AN	10 AN	10 AN	10 AN	100	27
0.3	10	10 AN	10 AN	10 AN	10 AN	10 AN	10 AN	10 AN	10 AN	10 AN	10 AN	10 AN	10 AN	100	0
1.0	10	10 AN	10 C	3 AN; 3 I; 4 Dead	3 AN; 3 I; 3 Dead	3 AN	3 AN	30	0						

Observation Codes: AN - Appears Normal; C - Lethargy; I - Immobile.

APPENDIX II

Protocol, Protocol Amendments and Deviations

APPENDIX II

WILDLIFE INTERNATIONAL LTD.

Project No.: 439A-104  
Page 1 of 3

AMENDMENT TO STUDY PROTOCOL

**STUDY TITLE:** OCTABROMODIPHENYL OXIDE (OBDPO): A FLOW-THROUGH LIFE-CYCLE TOXICITY TEST WITH THE LADOCERAN (*Daphnia magna*)

**PROTOCOL NO.:** 439041296/DP-LC2/SUB439

**AMENDMENT NO.:** 1

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

**PROJECT NO.:** 439A-104

**EFFECTIVE DATE:** December 18, 1996

**AMENDMENT:** Page 2:

**Add: PROPOSED DATES:**

Experimental Start Date: December 18, 1996

Experimental Termination Date: January 8, 1997

**STUDY ROOM:** Duster #4

**TEST CONCENTRATIONS:** 0.13, 0.25, 0.50, 1.0 and 2.6 µg/L and Negative and Solvent Controls

**TEST SUBSTANCE NO.:** 3637

Receipt Date: April 11, 1996

**REASON:** The above information was not available at the time the protocol was signed and is needed to complete the protocol

**AMENDMENT:** Page 3, Experimental Design Section, Add the following sentence at the end of the 2nd paragraph:

Stock samples will also be collected and analyzed.

**REASON:** At the request of the Sponsor, stock samples will be collected and analyzed.

**AMENDMENT:** Page 7, Test Apparatus section, 7th sentence.

**Change:** Daphnids will be held in test compartments suspended in Teflon<sup>®</sup>-lined 8-L polyethylene test chambers or non-lined stainless steel chambers. Test chambers will hold approximately 6.5 liter of water.

**To:** Daphnids will be held in test compartments suspended in 25-L stainless steel chambers. Test chambers will hold approximately 22 liters of water.

**REASON:** Clarification of the size and volume of test solutions to be used in the study.

cypa

*Reviewed  
KH 12-18-96*

## APPENDIX II

WILDLIFE INTERNATIONAL LTD.

Project No.: 439A-104

Page 2 of 3

## AMENDMENT: Page 9, Sampling for Analytical Measurements

Change: Water samples will be collected from alternate test chambers, at the beginning of the test, at approximately weekly intervals during the test, and at the end of the test to determine concentrations of the test substance. In the event that 100% mortality occurs in any treatment, then sampling of that treatment will terminate following the next sampling interval. Samples will be collected at mid-depth from each test chamber and analyzed immediately, or placed in an appropriate storage container, (e.g., glass or polyethylene bottle) and stored under refrigeration until analyzed. The sample scheme is summarized below.

## PROPOSED NUMBERS OF SAMPLES

Experimental Group	Day 0	Day 7	Day 14	Day 21
Control	2	2	2	2
Solvent Control (if needed)	2	2	2	2
Level 1-Low Concentration	2	2	2	2
Level 2	2	2	2	2
Level 3	2	2	2	2
Level 4	2	2	2	2
Level 5 - High Concentration	2	2	2	2
<b>TOTALS</b>	<b>14</b>	<b>14</b>	<b>14</b>	<b>14</b>

Total Number of Samples = 56

Note: Water samples will be collected from both test chambers of the negative and solvent controls and 0.5, 1.0 and 2.0  $\mu\text{g/L}$  treatment groups at the beginning of the test, at approximately weekly intervals during the test, and at the end of the test to determine concentrations of the test substance. Stock samples will also be collected initiation of the test and termination. In the event that 100% mortality occurs in any treatment, then sampling of that treatment will terminate following the next sampling interval. Samples will be collected at mid-depth from each test chamber and analyzed immediately, extracted, or placed in an appropriate storage container, (e.g., glass or polyethylene bottle) and stored under refrigeration until analyzed. The sample scheme is summarized below.

APPENDIX II

WILDLIFE INTERNATIONAL LTD.

Project No.: 439A-104  
Page 3 of 3

PROPOSED NUMBERS OF WATER VERIFICATION SAMPLES

Experimental Group	Day 0	Week 1	Week 2	Week 3
Control	2	2	2	2
Solvent Control	2	2	2	2
0.5	2	2	2	2
1.0	2	2	2	2
2.0	2	2	2	2
Water Verification	10	10	10	10
Stock Samples	5	-	-	5

Total Number of Samples = 50

REASON:

Change made at the request of the Sponsor.

W. C. Ginn  
STUDY DIRECTOR

12-18-96  
DATE

James Stewart  
LABORATORY MANAGEMENT

12/18/96  
DATE

Ramell Shah  
SPONSOR PRINCIPAL CONTACT

12/26/96  
DATE

APPENDIX II

WILDLIFE INTERNATIONAL LTD.

Project No.: 439A-104  
Page 1 of 2

AMENDMENT TO STUDY PROTOCOL

STUDY TITLE: OCTABROMODIPHENYL OXIDE (OBDPO): A FLOW-THROUGH LIFE-CYCLE TOXICITY TEST WITH THE CLADOCERAN (*Daphnia magna*)

PROTOCOL NO.: 439/041296/DAP-LC2/SUB439

AMENDMENT NO.: 2

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

PROJECT NO.: 439A-104

EFFECTIVE DATE: December 18, 1996

AMENDMENT: Add the following page as Appendix II.

REASON: To complete Appendix II (Analytical Method).

W. K. C. Ginn  
STUDY DIRECTOR

12-18-96  
DATE

James Stewart  
LABORATORY MANAGEMENT

12/18/96  
DATE

Ramukh Shah  
SPONSOR PRINCIPAL CONTACT

12/26/96  
DATE

*GA review  
12-18-96*

## APPENDIX II

WILDLIFE INTERNATIONAL LTD.

Project No.: 439A-104

Page 2 of 2

Method Outline for the Processing of  
OBDPO in Well Water

Rinse separatory funnels, roundbottom flasks and centrifuge tubes with ethyl acetate.

Prepare quality control samples by directly fortifying well water contained in separatory funnels with an appropriate stock solution.

Volumetrically sample 100 mL of each test solution directly into its respective separatory funnel.

Using a 50 mL graduated cylinder, add 50 mL of ethyl acetate to each sample. Stopper and shake each sample (with venting) for approximately one minute. Allow the organic and aqueous layers to separate. Drain the aqueous (lower) layer into a 250-mL beaker. Drain the ethyl acetate (upper) layer into a 250-mL roundbottom flask.

Return the aqueous layer to the separatory funnel. Add 50 mL of ethyl acetate to the beaker and swirl the beaker. Pour the ethyl acetate rinse into the separatory funnel containing the aqueous fraction. Shake and partition as described in the step above and combine each extract in its respective roundbottom flask; the total volume should be approximately 100 mL.

Rotary evaporate each sample to approximately 2-3 mL using a waterbath; maintain at 40-50°C. Do not evaporate to dryness.

Add approximately 2-3 mL of ethyl acetate to each flask. Swirl to dissolve residues. Transfer the extract to a labeled 15-mL centrifuge tube. Add an additional 2-4 mL of ethyl acetate to the roundbottom flask; swirl and combine the rinse and the previous rinse in the centrifuge tube.

Place the samples in a waterbath maintained at 40-50°C and evaporate the samples to dryness under a gentle stream of nitrogen.

Add the requisite volume of diphenylether to each centrifuge tube and vortex for 10-20 seconds.

Transfer the diluted samples to autosampler vials and submit for GC/ECD analysis.

For stock solutions, spike the requisite volume of each diluter stock into a 10-mL volumetric flask containing 4-6 mL of diphenylether. Adjust to volume with diphenylether.

- 43 -

APPENDIX II

PROTOCOL

OCTABROMODIPHENYL OXIDE (OBDPO): A FLOW-THROUGH LIFE-CYCLE  
TOXICITY TEST WITH THE CLADOCERAN (*Daphnia magna*)

Organisation for Economic Cooperation and Development  
OECD Guideline 202, Part II

and

TSCA Title 40 of the Federal Code of Regulation  
Part 797, Section 1330

Submitted to

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



WILDLIFE INTERNATIONAL LTD.



2598 Commerce Drive  
Easton, Maryland 21601  
(410) 822-8600

April 12, 1996

PROTOCOL NO.: 439/04 1296/DAP-I,C2/SUB439

APPENDIX II

WILDLIFE INTERNATIONAL LTD.

OCTABROMODIPHENYL OXIDE (OBDPO): A FLOW-THROUGH LIFE-CYCLE TOXICITY TEST WITH THE CLADOCERAN (*Daphnia magna*)

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

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

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

**STUDY DIRECTOR:** William C. Graves, Senior Aquatic Biologist

**LABORATORY MANAGEMENT:** James P. Swigert, Ph.D.  
Manager of Aquatic Toxicology

FOR LABORATORY USE ONLY

Proposed Dates:	
Experimental Start Date: _____	Experimental Termination Date: _____
Project No.: <u>439A-104</u>	Study Room: _____
Test Concentration: _____	Int/Date: _____
Test Substance No.: _____	Receipt Date: _____

PROTOCOL APPROVAL

William C. Graves  
STUDY DIRECTOR

6-5-96  
DATE

James P. Swigert  
LABORATORY MANAGEMENT

6/5/96  
DATE

Hamukh Shah  
SPONSOR'S REPRESENTATIVE

May 28, 1996  
DATE

- 45 -

## APPENDIX II

WILDLIFE INTERNATIONAL LTD.

- 3 -

**INTRODUCTION**

Wildlife International Ltd. will conduct a flow-through life cycle toxicity test with the cladoceran, *Daphnia magna*, for the Sponsor at the Wildlife International Ltd. aquatic toxicology facility in Easton, Maryland. The study will be performed based on procedures in Part II OECD Guideline for Testing Chemicals, 202: *Daphnia sp., Acute Immobilisation Test and Reproduction Test* (1); ASTM Standard E1193-87 *Standard Guide for Conducting Renewal Life-Cycle Toxicity Tests with Daphnia magna* (2); and Title 40 of the Code of Federal Regulations, Part 797, Section 1330, *Daphnia Chronic Toxicity Test* (3). 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 effects of a test substance on the survival, growth and reproduction of the cladoceran, *Daphnia magna*, under flow-through test conditions for a period of 21 days.

**EXPERIMENTAL DESIGN**

Daphnids will be exposed to a geometric series of at least five test concentrations, a negative (dilution water) control, and if necessary, a solvent control for 21 days. Five neonate daphnids (< 24 hours old) will be placed in each of two test compartments per test chamber. Test chambers will be replicated so that a total of 20 daphnids are exposed to each treatment and control group.

Nominal test concentrations will be selected in consultation with the Sponsor, and will be based upon information such as the results of exploratory range-finding toxicity data, known toxicity data, physical/chemical properties of the test substance or other relevant information. Target concentrations will not exceed 1000 mg/L or the solubility limit of the test substance in water (whichever is lower). Generally, each test substance concentration will be at least 50% of the next higher one unless information concerning the concentration-effect curve indicates that a different dilution factor would be more appropriate. Water samples from appropriate test chambers will be collected at specified intervals for analysis of the test substance. Results of the analyses will be used to calculate mean measured test concentrations.

To control bias, neonate daphnids will be impartially assigned to transfer chambers at test initiation. No other potential sources of bias are expected to affect the results of the study. Observations of survival and clinical signs of toxicity will be performed daily on the first-generation

PROTOCOL NO.: 439/041296/DAP-LC2/SUB439

- 46 -

## APPENDIX II

WILDLIFE INTERNATIONAL LTD.

- 1 -

daphnids. With the onset of reproduction, the number of second generation daphnids will be counted and recorded three times per week (Monday, Wednesday and Friday). The results of the test will be used to calculate the ECS0 (death and immobilization) and ECS0 (reproduction) values when possible at 24 hours, 48 hours, 96 hours, 7 days, 14 days and at the end of the test (Day 21). The ECS0 is defined as the concentration estimated to cause death and/or immobilization in 50% of the daphnids or reduce reproduction by 50% of the control daphnids. In addition, the results of the test will be used to calculate the no observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC). The NOEC is defined as the highest concentration that produces no statistically significant effect on survival, reproduction or growth. The LOEC is defined as the lowest concentration that produces a statistically significant effect on survival, reproduction or growth. The maximum acceptable toxicant concentration (MATC) will be calculated as the geometric mean of the NOEC and LOEC.

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

The test substance will be a composite sample of octabromodiphenyl oxide (OBDO) produced by three manufacturers (Albemarle Corporation, Ameribrom LTD, and Great Lakes Chemical Corporation). The composite OBDO 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 OBDO sample for use as the test substance. 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 OBDO 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 OBDO 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

PROTOCOL NO.: 439/041296/DAP-LC2/SUB439

0 0 4 8

- 47 -

## APPENDIX II

WILDLIFE INTERNATIONAL LTD.

- 5 -

SUBSTANCE BY SPONGOR (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.

Preparation of Test Concentrations

The test substance will be administered to the test organism in water. This route of administration was selected because it represents the most likely route of exposure to aquatic organisms.

The test substance will be mixed directly into the dilution water or may be first mixed with a solvent. If a solvent is used, the test substance will be dissolved in the solvent to form a stock solution that will subsequently be added to the dilution water. Reverse osmosis water will be the solvent of choice, although dimethyl formamide, triethylene glycol, methanol, ethanol, or acetone also may be used. If an organic solvent is required, then a solvent control group will be included in the experimental design along with a negative (dilution water) control group. The concentration of the organic solvent will not exceed 0.1 mL/L, when possible. The solvent concentration in the solvent control group will be equal to the highest solvent concentration in test chambers containing the test substance.

Test Organism

The cladoceran, *Daphnia magna*, has been selected as the test species for this study. Daphnids are representative of an important group of aquatic invertebrates, and have been selected for use in the test based upon past use history and ease of culturing in the laboratory. Daphnid neonates to be used in the test will be less than 24 hours old and will be obtained from cultures maintained at Wildlife International Ltd., Easton, Maryland. The identity of the species will be verified by the supplier of the original culture or by Wildlife International Ltd. personnel using appropriate taxonomic keys such as Pennak (4).

Daphnids will be cultured in water from the same source and at approximately the same temperature as will be used during the test, except that the culture water is supplemented with selenium. Daphnids in the cultures producing neonates for the test will be held for at least 10 days prior to collection of the neonates for testing. Neonates from daphnids that show signs of disease or

PROTOCOL NO.: 439/041296/DAP-LC2/SUB439

- 48 -

## APPENDIX II

WILDLIFE INTERNATIONAL LTD.

- 6 -

stress will not be used as test organisms. Daphnids in holding that produce ephippia also will not be used to supply neonates for testing.

Daphnids in the cultures will be fed once daily. The diet will be a mixture of yeast, Cerophyll®<sup>®</sup>, and trout chow (YCT), supplemented with a suspension of the freshwater green alga *Solenastrum capricornutum*. Food (YCT) provided to daphnids will be analyzed at least once annually to ensure that there are no contaminants at levels known to be capable of interfering with the study. Specifications for acceptable levels of contaminants in daphnid diets have not been established. However, there are no known levels of contaminants reasonably expected to be present in the diet that are considered to interfere with the purpose or conduct of the test.

Neonates will be obtained for testing from at least three individual adults. Prior to test initiation, the neonates will be collected from cultures and transferred to glass beakers or directly to the test compartments. The daphnids will be released into the test compartments below the water surface using a wide-bore pipette. Daphnids will be fed daily during the test using the same diet as used in the cultures. The frequency of feeding will be documented in the raw data and the final report.

Dilution Water

Water used for the culturing and testing of daphnids will be obtained from a well approximately 45 meters deep located on the Wildlife International Ltd. site. The water will be passed through a sand filter and pumped into a 37,800-L storage tank where the water will be aerated with spray nozzles. Prior to use the water will be filtered to 0.2  $\mu$ m and passed through an ultraviolet sterilizer in order to remove microorganisms and fine particles. Water used for culturing and testing is characterized as moderately hard. Typical values for hardness, alkalinity, pH and specific conductance are approximately:

Hardness, mg/L as CaCO <sub>3</sub>	145
Alkalinity, mg/L as CaCO <sub>3</sub>	190
pH	8.1
Specific Conductance, $\mu$ mhos/cm	330

Hardness, alkalinity, pH and specific conductance will be measured weekly to monitor the consistency of the well water. Means and ranges of the measured parameters for the four-week period preceding the test will be provided in the final report. Analyses will be performed at least once annually to determine the concentrations of selected organic and inorganic constituents of the

PROTOCOL NO.: 439/041296/DAP-LC2/SUB439

## APPENDIX II

WILDLIFE INTERNATIONAL LTD.

well water. A list of the parameters routinely measured along with detection limits is presented in Table 1.

**Test Apparatus**

A continuous-flow diluter will be used to provide each concentration of the test substance, a negative (dilution water) control, and a solvent control, when necessary. A syringe pump, peristaltic pump, or a similar device will be used to deliver the test substance to mixing chambers where the test substance will be mixed with dilution water. The flow of dilution water into each mixing chamber will be controlled using rotameters. The rotameters will be calibrated prior to the test and verified and/or calibrated at least once a week during the test. After mixing, test solutions will be split to each replicate chamber. The proportion of water split to each replicate will be checked prior to the study and approximately weekly thereafter to ensure that these flow rates vary by no more than  $\pm 1\%$  of the mean flow rate of the two replicates. Daphnids will be held in test compartments suspended in Teflon®-lined, 8-L polyethylene test chambers or non-lined stainless steel chambers. Test chambers will hold approximately 6.5 liters of water. Test compartments will be constructed from 500-mL glass beakers, approximately 8.0 cm in diameter and 13 cm in height. Nylon mesh screen will cover two holes on opposite sides of each compartment to permit test solution to flow into and out of the compartment. Two compartments will be suspended in each replicate test chamber. Test chambers will be indiscriminately positioned in a temperature-controlled water bath to maintain a temperature of  $20 \pm 1^\circ\text{C}$ . The water bath will be enclosed in a plexiglass ventilation hood in order to minimize potential cross contamination between test systems. Test chambers will be labelled with the project number, test concentration and replicate.

The diluter will be adjusted so that each test chamber receives at least 5 volume additions of test solution every 24 hours. Peristaltic pumps will be calibrated and/or verified before the test and at least once a week during the test. Syringe pumps, if used, will be calibrated before the test. The delivery of test substance to test chambers will begin at least 4 hours prior to the test in order to establish equilibrium concentrations of the test substance. The general operation of the diluter will be checked visually at least two times per day during the test and at least once at the beginning and end of the test.

- 50 -

## APPENDIX II

WILDLIFE INTERNATIONAL LTD.

- 8 -

Environmental Conditions

Lighting used to illuminate the cultures and test chambers during culturing and testing will be provided by fluorescent tubes that emit wavelengths similar to natural sunlight (e.g., Colortone® 50). A photoperiod of 16 hours of light and 8 hours of dark will be controlled with an automatic timer. A 30-minute transition of low light intensity will be provided when lights go on and off to avoid sudden changes in light intensity. Light intensity will be measured at test initiation with a SPER Scientific Ltd. light meter or equivalent.

The target test temperature will be  $20 \pm 1^\circ\text{C}$ . Temperature will be measured in each test chamber at the beginning of the test and at daily intervals during the test using a hand-held thermometer. Temperature also will be measured with a continuous recorder in one negative control replicate. Recorder measurements will be verified with a hand-held thermometer prior to test initiation and at least weekly thereafter.

Dissolved oxygen will be measured in alternate replicates of the treatment and control group(s) at test initiation and daily thereafter using a Yellow Springs Instrument Model 51B dissolved oxygen meter, or equivalent. In the event that dissolved oxygen levels fall below 60% saturation, dissolved oxygen measurements will be made in every test chamber and appropriate actions will be taken after consultation with the Sponsor. Measurements of pH will be made in alternate replicates of each treatment and control group at test initiation and daily thereafter using a Fisher Accumet Model 915 pH meter, or equivalent. If a treatment group reaches 100% mortality, dissolved oxygen, pH and temperature measurements will be taken at that time, then discontinued.

Hardness, alkalinity, specific conductance and total organic carbon (TOC) will be measured in alternate replicates of the negative (dilation water) control at test initiation and at weekly intervals until test termination. Hardness and alkalinity measurements will be made by titration using procedures based on methods in *Standard Methods for the Examination of Water and Wastewater* (5). Specific conductance will be measured using a Yellow Springs Instrument Model 33 Salinity-Conductivity-Temperature meter, or equivalent. Total organic carbon will be analyzed with a Shimadzu Model 5000 TOC analyzer.

Biological Observations

The first generation daphnids will be observed daily during the test for survival, the onset of reproduction, and clinical signs of toxicity. The criteria for death include absence of heartbeat, white opaque coloration, lack of movement of appendages and lack of response to gentle prodding. Immobilization is defined as a lack of movement except for minor spontaneous random movement of the appendages. Examples of clinical signs of toxicity include inability to maintain position in the

PROTOCOL NO.: 439/041296/DAP-LC2/SUB439

APPENDIX

WILDLIFE INTERNATIONAL LTD.

water column, uncoordinated swimming and cessation of feeding. The presence of eggs in the brood pouch, aborted eggs, males, or ephippia does not constitute injury.

The number of second-generation daphnids will be counted and recorded three times weekly (Monday, Wednesday, Friday). At each observation, the percentage of mortality will be recorded, and the second-generation daphnids will be measured for length (distance from the apex of the rostrum to the base of the pedicel) and dry weight.

Sampling for Analytical Measurements

Water samples will be collected from chambers and chambers of the topography of the test, at approximately weekly intervals during the test, and at the end of the test to determine concentrations of the test substance. In the event that 100% mortality occurs in any treatment, the sampling of the treatment will terminate following the next sampling interval. Samples will be collected and kept from each test chamber and analyzed immediately or placed in a polypropylene storage container (e.g., glass or polypropylene bottle) and stored under refrigeration until analyzed. The sample data is summarized below:

Experimental Group	PROPOSED NUMBER OF SAMPLES			
	Day 1	Day 2	Day 4	Day 7
Control	1	1	1	1
Solvent Control (if needed)	1	1	1	1
Level 1-Low Concentration	1	1	1	1
Level 2	1	1	1	1
Level 3	1	1	1	1
Level 4	1	1	1	1
Level 5-High Concentration	1	1	1	1
TOTALS	10	10	10	10

Total Number of Samples = 40

The above numbers of samples represent those collected from the test and do not include quality control (QC) samples such as reagents, standards and field samples prepared and analyzed during the analytical chemistry phase of the study. At the discretion of the Study Director, more samples also will be collected from at least one appropriate chamber whenever a malfunction is observed in any part of the test substance delivery system.

- 52 -

## APPENDIX II

WILDLIFE INTERNATIONAL LTD.

- 10 -

**Analytical Chemistry**

Chemical analysis of the samples will be performed by Wildlife International Ltd. The analytical method used will be based upon methodology provided by the Sponsor (Appendix II). The methodology used to analyze the test samples will be documented in the raw data and summarized in the final report.

**Data Analysis**

Statistical analyses will be performed on the survival of first-generation daphnids, the number of live young produced per adult per reproductive day, and the length and dry weight of all surviving first-generation daphnids.

EC50 values with 95% confidence intervals will be calculated when possible by probit analysis, the moving average-angle method, or binomial probability (6, 7, 8, 9) using mortality/immobilization and reproduction data collected at 24 hours, 48 hours, 96 hours, 7 days, 14 days, and at the end of the test (Day 21). Survival data also will be evaluated at those same times to identify those treatments statistically different from the control group using 2 X 2 contingency tables or a similar statistical comparison test. If a solvent control group is used in addition to a negative control group, these two groups will be compared by a Student's t-test. If no statistical differences are found, then the data of the two control groups may be pooled. If statistical differences are found, then either the negative or solvent control groups will be used to evaluate the treatment-related effects.

Analyses of reproduction (number of young produced per surviving adult per reproductive day) and growth (weight and length) data will be evaluated for normality and homogeneity of variances. If data are deemed normal with homogeneous variances, hypothesis testing using analysis of variance (ANOVA) and multiple means tests (e.g., Dunnett's, Bonferroni, Scheffe) will be used. If data fail the tests for normality or homogeneity, then transformations will be tried in an attempt to correct the condition. When the data transformations fail to correct for non-normality or heterogeneity of variances, nonparametric procedures will be used to identify statistically significant differences among the experimental groups. If a solvent control group is used in addition to a negative control group, then two groups will be compared by a Student's test-test. If no statistical differences are found, then the two groups may be pooled. If statistical differences are found, then either the negative or solvent control group will be used to evaluate the treatment-related effects.

The statistical analyses of survival, growth and reproduction data will aid but may not be exclusively used in the determination of the no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC). The maximum acceptable toxicant concentration (MATC)

PROTOCOL NO.: 439/041296/DAP-LC2/SUB439

0.059

## APPENDIX II

WILDLIFE INTERNATIONAL LTD.

will be calculated as the geometric mean of the NOEC and LOEC. All statistical evaluations will be done using commercial available computer software such as TOXSTAT (10) or SPSS/PC+ (11).

**RECORDS TO BE MAINTAINED**

Records to be maintained for data generated at 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 termination of the study.
4. Length and dry weight measurements.
5. Reproduction parameters.
6. Test organism culture records.
7. Results of range-finding tests, when applicable.
8. Stock solution calculation and preparation.
9. Daily observations.
10. Water chemistry calculations (e.g., hardness and alkalinity).
11. If applicable, the methods used to analyze test substance concentrations and the results of analytical measurements.
12. Statistical calculations.
13. Test conditions and physical/chemical measurements.
14. Calculation and preparation of test concentrations.
15. Copy of 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. Dates upon which the study was initiated and completed. It is the responsibility of the Sponsor to provide the final date that data are recorded for chemistry pathology and/or supporting evaluations that may be generated at other laboratories.
3. A statement of compliance signed by the Study Director addressing any exceptions to Good Laboratory Practice Standards.

0-055

## APPENDIX II

WILDLIFE INTERNATIONAL LTD.

4. Objectives and procedures, as stated in the approved protocol, including changes in the original protocol.
5. The test substance identification, including name, chemical abstract number or code number, strength, purity, composition, and other information provided by the Sponsor.
6. Stability and solubility of the test substance under the conditions of administration, if provided by the Sponsor or contracted to Wildlife International Ltd.
7. A description of the methods used to conduct the test.
8. A description of the test organisms, including the source, scientific name, age, life stage, food types, light intensity, and photoperiod.
9. A description of the preparation of the test solutions, the methods used to allocate organisms to test chambers and begin the test, the number of organisms and chambers per treatment, and the duration of the test.
10. A description of circumstances that may have affected the quality or integrity of the data.
11. The name of the Study Director and the names of other scientists, professionals, and supervisory personnel involved in the study.
12. A description of the transformations, calculations, and operations performed on the data, a summary and analysis of the biological data and analytical chemistry data, and a statement of the conclusions drawn from the analyses.
13. Statistical methods used to evaluate the data.
14. The signed and dated reports of each of the individual scientists or other professionals involved in the study.
15. The location where raw data and final report are to be stored.
16. A statement prepared by the Quality Assurance Unit listing the dates that study inspections were made and findings reported to the Study Director/Management.
17. If it is necessary to make corrections or additions to a final report after it has been accepted, such changes will be made in the form of an amendment issued by the Study Director. The amendment will clearly identify the part of the final report that is being amended and the reasons for the amendment, and will be signed and dated by the Study Director.

- 55 -

APPENDIX II

WILDLIFE INTERNATIONAL LTD.

- 13 -

**CHANGING OF PROTOCOL**

Planned changes to the protocol will be in the form of written amendments signed by the Study Director and the Sponsor's Representative. 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 in accordance with Good Laboratory Practice Standards for EPA (40 CFR Part 792) and OECD (ISBN 92-84-12367-9). 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 (e.g., residue analyses or pathology). 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.

PROTOCOL NO.: 439/041296/DAP-LC2/SUB439

## APPENDIX II

WILDLIFE INTERNATIONAL LTD.REFERENCES

- 1 OECD. 1984. Guideline 202, Part II: *Daphnia sp.*. Acute Immobilisation Test and Reproduction Test.
- 2 ASTM Standard E1193-87. 1988. Standard Guide for Conducting Renewal Life-Cycle Toxicity Tests with *Daphnia magna*. American Society for Testing and Materials.
- 3 Title 40 of the Code of Federal Regulations, Part 797, Section 1330. 1994. *Daphnia Chronic Toxicity Test*.
- 4 Pennak, R.W. 1978. *Freshwater Invertebrates of the United States*. 2nd Ed. 365 p.
- 5 APHA, AWWA, WPCF. 1985. *Standard Methods for the Examination of Water and Wastewater*. 16th Edition, American Public Health Association. American Water Works Association. Water Pollution Control Federation, New York.
- 6 Finney, D.J. 1971. *Statistical Methods in Biological Assay*. Second Edition. Griffin Press, London.
- 7 Thompson, W.R. *Bacteriological Reviews*. Vol. II, No. 2, pp. 115-143.
- 8 Stephan, C.E. 1977. "Methods for Calculating and LC50", *Aquatic Toxicology and Hazard Evaluation*. American Society for Testing and Materials. Publication Number STP 634, pp. 65-84.
- 9 Stephan, C.E. 1978. U.S. Environmental Protection Agency, Environmental Research Laboratory, Duluth, Minnesota. Personal communication.
- 10 Galley, D.D., A.M. Bechter, H.L. Bergman. 1989. TOXSTAT, Version 3.2. University of Wyoming, Laramie, Wyoming.
- 11 SPSS, Inc. 1988. SPSS/PC+ Version 2.0. Chicago, Illinois.

## APPENDIX II

WILDLIFE INTERNATIONAL LTD.

TABLE I

PESTICIDES, ORGANICS, METALS AND OTHER INORGANICS ANALYZED  
IN WILDLIFE INTERNATIONAL LTD. WELL WATER

ANALYSIS	TARGET LIMIT OF DETECTION
<b>Miscellaneous Measurements</b>	
Total Dissolved Solids	4 mg/L
Ammonia Nitrogen	0.02 mg/L
Total Organic Carbon	1 mg/L
Total Cyanide	0.001 mg/L
<b>Organochlorines and PCBs</b>	
Aldrin	≤ 0.01 µg/L
Alpha BHC	≤ 0.01 µg/L
Beta BHC	≤ 0.01 µg/L
Delta BHC	≤ 0.01 µg/L
Gamma BHC (Lindane)	≤ 0.01 µg/L
Chlordane	≤ 0.03 µg/L
DDD, pp'	≤ 0.01 µg/L
DDE, pp'	≤ 0.01 µg/L
DDT, pp'	≤ 0.01 µg/L
Dieldrin	≤ 0.01 µg/L
Endosulfan, A	≤ 0.01 µg/L
Endosulfan, B	≤ 0.01 µg/L
Endosulfan Sulfate	≤ 0.01 µg/L
Endrin	≤ 0.01 µg/L
Endrin Aldehyde	≤ 0.01 µg/L
Heptachlor	≤ 0.01 µg/L
<del>Methoxychlor</del>	≤ 0.01 µg/L
Heptachlor Epoxide	≤ 0.01 µg/L
Toxaphene	≤ 0.06 µg/L
PCB-1016	≤ 0.3 µg/L
PCB-1221	≤ 0.3 µg/L
PCB-1232	≤ 0.3 µg/L
PCB-1242	≤ 0.3 µg/L
PCB-1248	≤ 0.3 µg/L
PCB-1254	≤ 0.3 µg/L
PCB-1260	≤ 0.3 µg/L

Actual analysis is based on available methodologies at the testing facility. Results from each annual analysis with the limit of detection for each chemical is retained in the Wildlife International Ltd. archives.

- 58 -

## APPENDIX II

WILDLIFE INTERNATIONAL LTD.

- 16 -

TABLE I (Continued)

PESTICIDES, ORGANICS, METALS AND OTHER INORGANICS ANALYZED  
IN WILDLIFE INTERNATIONAL LTD. WELL WATER

ANALYSIS	TARGET LIMIT OF DETECTION
<b>Organophosphorus &amp; Organonitrogen Pesticides</b>	
Azinphos Methyl	<3 µg/L
Chlorpyrifos	<0.3 µg/L
Coumaphos	<3 µg/L
Demeton	<0.3 µg/L
Diazinon	<0.3 µg/L
Dichlorvos	<0.3 µg/L
Dimethoate	<0.3 µg/L
Disulfoton	<2 µg/L
EPN	<0.5 µg/L
Ethoprop	<0.5 µg/L
Fenthion	<0.5 µg/L
Fenitrothion	<3 µg/L
Malathion	<0.5 µg/L
Mephos	<0.5 µg/L
Mevinphos	<0.5 µg/L
Monochrotophos	<3 µg/L
Naled	<2 µg/L
Methyparathion	<0.3 µg/L
Parathion	<0.3 µg/L
Phorate	<0.3 µg/L
Romel	<2 µg/L
Stirofos	<0.6 µg/L
<del>Sulfotone</del>	<0.3 µg/L
Sulprofos	<0.3 µg/L
Tepp	<0.3 µg/L
Toxathion	<0.3 µg/L
Trichloronate	<0.3 µg/L

Actual analysis is based on available methodologies at the testing facility. Results from each annual analysis with the limit of detection for each chemical is retained in the Wildlife International Ltd. archives.

## APPENDIX II

WILDLIFE INTERNATIONAL LTD.

TABLE I (Continued)

PESTICIDES, ORGANICS, METALS AND OTHER INORGANICS ANALYZED  
IN WILDLIFE INTERNATIONAL LTD. WELL WATER

ANALYSIS	TARGET LIMIT OF DETECTION
<b>Chlorophenoxy Acid Herbicides</b>	
2,4-D, Total	< 0.02 µg/L
2,4-DB	< 0.02 µg/L
2,4,5-T Water	< 0.02 µg/L
2,4,5-TP/Silvex	< 0.02 µg/L
Dalapon	< 0.02 µg/L
Dicamba (Banvel)	< 0.02 µg/L
Dichloroprop	< 0.02 µg/L
Dinoseb	< 0.02 µg/L
MCPA	< 0.4 µg/L
MCPP	< 0.4 µg/L
<b>Metals and Other Inorganics</b>	
Aluminum	< 40 µg/L
Arsenic	< 3 µg/L
Beryllium	< 5 µg/L
Cadmium	< 5 µg/L
Calcium	< 500 µg/L
Chromium	< 5 µg/L
Copper	< 5 µg/L
Iron	< 45 µg/L
Lead	< 3 µg/L
Magnesium	< 3 µg/L
Manganese	< 3 µg/L
Mercury	< 3 µg/L
Nickel	< 10 µg/L
Potassium	< 500 µg/L
Selenium	< 3 µg/L
Silver	< 5 µg/L
Sodium	< 500 µg/L
Zinc	< 15 µg/L
Molybdenum	< 10 µg/L
Actual analysis is based on available methodologies at the testing facility. Results from each annual analysis with the limit of detection for each chemical is retained in the Wildlife International Ltd. archives.	

APPENDIX II

WILDLIFE INTERNATIONAL LTD.

APPENDIX I  
IDENTIFICATION OF TEST SUBSTANCE BY SPONSOR

To be Completed by Sponsor

I. Test Substance Identity (name to be used in the report): \_\_\_\_\_  
Reference Standard (if applicable): Analytical Standard: \_\_\_\_\_  
Internal Standard: \_\_\_\_\_

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

Test Substance 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 and reference standard been determined prior to its use in 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: \_\_\_\_\_

- 61 -

APPENDIX II

WILDLIFE INTERNATIONAL LTD.

---

- 19 -

APPENDIX II

Analytical Method to be Provided by Sponsor

APPENDIX II

WILDLIFE INTERNATIONAL LTD.

PROJECT NO.: 439A-104  
Page 1 of 1

DEVIATION TO STUDY PROTOCOL

STUDY TITLE: OCTABROMODIPHENYL <sup>Ⓢ 2-11-97</sup>OXIDE (OBDPO): A FLOW-THROUGH LIFE-  
CYCLE TOXICITY TEST WITH THE CLADOCERAN (*Daphnia magna*)

PROTOCOL NO.: 439041296/DAP-LC2/SUB43 <sup>Ⓢ 2-11-97</sup> DEVIATION NO.: 1

SPONSOR: Chemical Manufacturers Association PROJECT NO.: 439A-104

DATE OF DE FACTO DEVIATION: January 1, 1997

DEVIATION:

On Day 14 of the test, total organic carbon sample was collected from replicate B of the negative control group rather than replicate A (th : alternative replicate).

REASON:

Technician oversight. This should not adversely affect the results of the study.

Walter C. Gross  
STUDY DIRECTOR

1-21-97  
DATE

James Stewart  
LABORATORY MANAGEMENT

1/22/97  
DATE

APPENDIX II

WILDLIFE INTERNATIONAL LTD.

PROJECT NO.: 439A-104  
Page 1 of 1

DEVIATION TO STUDY PROTOCOL

STUDY TITLE: OCTABROMODIPHENYL OXIDE (OBDPO): A FLOW-THROUGH LIFE-CYCLE TOXICITY TEST WITH THE CLADOCERAN (*Daphnia magna*)

PROTOCOL NO.: 439/041296/DAP-LC2/SUB439

DEVIATION NO.: 2

SPONSOR: Chemical Manufacturers Association

PROJECT NO.: 439A-104

DATE OF DE FACTO DEVIATION: May 20, 1996

DEVIATION:

In addition to sending samples of the composited test material to Albemarle Corporation for analysis, samples were sent to Great Lakes Chemical Corporation for analysis.

REASON:

Sponsor request. This should not adversely affect the results of the study.

Walt-C Qu  
STUDY DIRECTOR

2-11-97  
DATE

James Stewart  
LABORATORY MANAGEMENT

2/11/97  
DATE

## APPENDIX III

Specific Conductance, Hardness, Alkalinity and pH of Well Water Measured  
During the 4-Week Period Immediately Preceding the Test

Sponsor:	CMA's Brominated Flame Retardant Industry Panel	
Test Substance:	Octabromodiphenyl Oxide (OBDPO)	
Test Organism:	Cladoceran, <i>Daphnia magna</i>	
Dilution Water:	Well Water	
	Mean	Range
Specific Conductance ( $\mu$ mhos/cm)	305 (N = 4)	300 - 310
Hardness (mg/L as CaCO <sub>3</sub> )	127 (N = 4)	124 - 128
Alkalinity (mg/L as CaCO <sub>3</sub> )	175 (N = 4)	174 - 176
pH	8.3 (N = 4)	8.3 - 8.4

APPENDIX IV  
Analyses of Pesticides, Organics, Metals and Other Inorganics  
in Wildlife International Ltd. Well Water<sup>1</sup>

Sponsor:	CMA's Brominated Flame Retardant Industry Panel		
Test Substance:	Octabromodiphenyl Oxide (OBDPO)		
Test Organism:	Cladoceran, <i>Daphnia magna</i>		
Dilution Water:	Well Water		
ANALYSIS		MEASURED CONCENTRATION	
<b>Miscellaneous Measurements</b>			
Total Dissolved Solids		261	mg/L
Ammonia Nitrogen	<	0.050	mg/L
Total Organic Carbon <sup>2</sup>	<	1.0	mg/L
Total Cyanide	<	5.0	µg/L
<b>Organochlorines and PCBs</b>			
Aldrin	<	0.005	µg/L
Alpha BHC	<	0.005	µg/L
Beta BHC	<	0.005	µg/L
Delta BHC	<	0.005	µg/L
Gamma BHC (Lindane)	<	0.005	µg/L
Chlordane	<	0.025	µg/L
DDD, pp'	<	0.005	µg/L
DDE, pp'	<	0.005	µg/L
DDT, pp'	<	0.005	µg/L
Dieldrin	<	0.005	µg/L
Endosulfan, A	<	0.005	µg/L
Endosulfan, B	<	0.005	µg/L
Endosulfan Sulfate	<	0.005	µg/L
Endrin	<	0.005	µg/L
Endrin Aldehyde	<	0.005	µg/L
Heptachlor	<	0.005	µg/L
Methoxychlor	<	0.005	µg/L
Heptachlor Epoxide	<	0.005	µg/L
Toxaphene	<	0.500	µg/L
PCB-1016	<	0.250	µg/L
PCB-1221	<	0.250	µg/L
PCB-1232	<	0.250	µg/L
PCB-1242	<	0.250	µg/L
PCB-1248	<	0.250	µg/L
PCB-1254	<	0.250	µg/L
PCB-1260	<	0.250	µg/L
<b>Metals and Other Inorganics</b>			
Aluminum		69.9	µg/L
Arsenic	<	2.5	µg/L
Beryllium	<	4.0	µg/L
Boron		142	µg/L
Cadmium	<	5.0	µg/L
Calcium		34.3	mg/L
Chromium	<	10.0	µg/L
Cobalt	<	20.0	µg/L
Copper		40.4	µg/L
Iron	<	45.0	µg/L
Lead	<	2.0	µg/L
Magnesium		13.5	mg/L
Manganese	<	5.0	µg/L
Mercury	<	0.20	µg/L
Molybdenum	<	10.0	µg/L
Nickel	<	15.0	µg/L
Potassium		6.16	mg/L
Selenium	<	2.5	µg/L
Silver	<	5.0	µg/L
Sodium		21.6	mg/L
Zinc	<	30.0	µg/L

<sup>1</sup> Analyses performed by Environmental Science & Engineering, Inc., Gainesville, Florida for samples collected on August 21, 1996.

<sup>2</sup> Analyses performed by Wildlife International Ltd. for the sample collected on August 14, 1996.

- 66 -

APPENDIX V

THE ANALYSIS OF OCTABROMODIPHENYL OXIDE (OBDPO) IN FRESHWATER

IN SUPPORT OF

WILDLIFE INTERNATIONAL LTD. PROJECT NO.: 439A-104

- 67 -

APPENDIX V

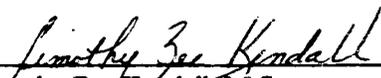
REPORT APPROVAL

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

TITLE: Octabromodiphenyl Oxide (OBDDPO): A Flow-Through Life-Cycle Toxicity Test with the Cladoceran (*Daphnia magna*)

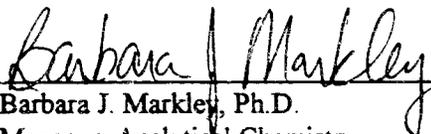
WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER: 439A-104

PRINCIPAL INVESTIGATOR:

  
\_\_\_\_\_  
Timothy Zee Kendall, M.S.  
Laboratory Supervisor, Analytical Chemistry

5/8/97  
\_\_\_\_\_  
DATE

MANAGEMENT:

  
\_\_\_\_\_  
Barbara J. Markley, Ph.D.  
Manager, Analytical Chemistry

5/8/97  
\_\_\_\_\_  
DATE

## APPENDIX V

Introduction

Samples of test media solutions (test samples) were collected from a flow-through life-cycle aquatic toxicity study designed to determine the effects of octabromodiphenyl oxide (OBDPO) to the cladoceran (*Daphnia magna*). This study was conducted by Wildlife International Ltd. and identified as Project Number 439A-104. The analyses of these test samples were performed at Wildlife International Ltd. by gas chromatography (GC) with electron-capture detection (ECD). Test samples were received for analysis and extracted between December 18, 1996 and January 8, 1997 and analyzed between January 14, 1997 and January 28, 1997.

Test Substances

The composite test substance (OBDPO) used for the analytical portion of this study was prepared by Wildlife International Ltd. and assigned identification number 3637 (Refer to page 10 regarding composite test substance preparation). The test substance was used to prepare calibration standards and fortification samples.

Analytical Method

The method used for the analysis of the test samples was based upon methodology provided by the Sponsors.

The analytical method consisted of volumetrically pipetting a 100-mL portion of test solution directly into a separatory funnel. Added to the sample was 50 mL of ethyl acetate. The solution was shaken (with venting) for approximately one minute after which the two liquid phases were allowed to separate. The lower aqueous phase was drained into a beaker and the remaining ethyl acetate extract was drained into a 250-mL roundbottom flask. The aqueous phase was returned to the separatory funnel and an additional 50 mL of ethyl acetate was added to the beaker. The ethyl acetate was gently swirled within the beaker before being transferred to the separatory funnel. The extraction/separation process was repeated and the organic extract was combined in the roundbottom

## APPENDIX V

flask with the first extract. Using a waterbath maintained at a temperature between 40-50°C, the extract was rotary evaporated to a volume of approximately 2-3 mL. This solution was transferred, with rinsing, to a graduated 15-mL centrifuge tube and evaporated to dryness under a gentle stream of nitrogen. The extract was dissolved using either 1 or 2 mL diphenylether and vortexed 10-20 seconds. The diluted extract was transferred to autosampler vials and submitted for analysis by gas chromatography.

Concentrations of octabromodiphenyl oxide (OBDPO) in the test samples were determined by gas chromatography (GC) equipped with an electron-capture detector (ECD). Chromatographic separations were achieved using a Restek Rtx-1 column (15 m x 0.53 mm ID, 0.10  $\mu\text{m}$  film thickness). The instrumental parameters are summarized in Table 1 and a method flow chart is provided in Figure 1.

#### Calibration Curve, Limit of Detection and Limit of Quantitation

Calibration standards for octabromodiphenyl oxide (OBDPO), ranging in concentration from 25.0 to 250  $\mu\text{g/L}$ , were analyzed with each series of test samples. Linear regression equations were generated using the sum of peak area responses of the components of octabromodiphenyl oxide (OBDPO) versus the respective concentrations of the calibration standards. A representative calibration curve is presented in Figure 2. The concentration of octabromodiphenyl oxide (OBDPO) in the test samples was determined by substituting the sum of peak area responses into the applicable linear regression equation. Representative chromatograms of low and high calibration standards are shown in Figures 3 and 4, respectively.

The instrument limit of detection (LOD) for this study was set based upon the injection volume (2  $\mu\text{L}$ ) and the lowest standard concentration (25.0  $\mu\text{g/L}$ ). The LOD was set at 50 pg injected on-column. The method limit of quantitation (LOQ) for these analyses was set at

- 70 -

## APPENDIX V

0.250  $\mu\text{g/L}$  based upon the product of the lowest standard and the dilution factor of the control samples analyzed concurrently with the test samples.

Matrix Blank and Fortification Samples

Along with the actual test sample analyses, four matrix blanks were analyzed to determine possible interference. No interferences were observed at or above the LOQ during the test sample analyses (Table 2). A representative chromatogram of a matrix blank is presented in Figure 5.

Well water samples were fortified at 0.400, 1.00 and 3.00  $\mu\text{g/L}$  and analyzed concurrently with the test samples to determine the mean procedural recovery (Table 3). A representative chromatogram of a matrix fortification is presented in Figure 6. Sample concentrations were adjusted for a mean procedural recovery of 119%.

RESULTSSample Analysis

Test samples were collected from the flow-through life-cycle toxicity study with cladoceran (*Daphnia magna*) at test initiation (Day 0), at Day 5, at Day 12, and at test termination (Day 21). The measured concentrations of octabromodiphenyl oxide (OBDPO) in the test samples collected at study initiation (Day 0) ranged from 83 to 101% of the nominal concentrations (Table 5). Test samples collected at Days 5, 12 and 21 (test termination) had measured concentration ranges of 75 to 111%, 84 to 146% and 58 to 90% of nominal concentrations, respectively. Stock solutions analyzed at test initiation and test termination yielded mean percent recoveries of 94%, 89%, 96%, 93% and 89% for the 1.8, 3.5, 7.0, 14 and 28 mg/L stock solutions, respectively (Table ). A representative chromatogram of a test sample taken at Day 21 is shown in Figure 7.

- 71 -

## APPENDIX V

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 $\mu$ m film thickness)	
INJECTOR TEMPERATURE:	300°C	
OVEN:	Initial temperature:	150°C
	Initial hold time:	2.00 minute
	Ramp:	5.0°C/minute
	Final temperature:	300°C
	Final hold time:	3.00 minutes
DETECTOR TEMPERATURE:	300°C	
INJECTION VOLUME (splitless):	2 $\mu$ L	
CARRIER GAS (Helium):	5 p.s.i. (head pressure)	
MAKE-UP GAS (Argon/Methane):	65 mL/minute	
OC TABROMODIPHENYL OXIDE (OBDPO) PEAK RETENTION TIMES AND RANGE:	14-23 minutes	

---

- 72 -

## APPENDIX V

Table 2

## Matrix Blanks Analyzed Concurrently During Test Sample Analysis

Sample		Measured Concentration of Octabromodiphenyl Oxide (OBDPO) ( $\mu\text{g/L}$ ) <sup>1</sup>
Number (439A-104-)	Type	
MAB-1	Matrix Blank	< LOQ
MAB-2	Matrix Blank	< LOQ
MAB-3	Matrix Blank	< LOQ
MAB-4	Matrix Blank	< LOQ

<sup>1</sup> The limit of quantitation (LOQ = 0.250  $\mu\text{g/L}$ ) was based upon the product of the lowest standard (25.0  $\mu\text{g/L}$ ) and the dilution factor of the control samples (0.01) analyzed concurrently with the test samples.

- 73 -

## APPENDIX V

Table 3

## Matrix Fortifications Analyzed Concurrently During Test Sample Analysis

Sample Number (439A-104-)	Concentration of Octabromodiphenyl Oxide (OBDPO) ( $\mu\text{g/L}$ )		Percent Recovery
	Fortified	Measured	
MAS-1	0.400	0.389	97.2
MAS-2	1.00	1.25	125
MAS-3	3.00	3.32	111
MAS-4	0.400	0.476	119
MAS-5	1.00	1.13	113
MAS-6	3.00	3.43	114
MAS-7	0.400	0.575	144
MAS-8	1.00	1.32	132
MAS-9	3.00	3.54	118
MAS-10	0.400	0.529	132
MAS-11	1.00	1.16	116
MAS-12	3.00	3.37	112
		Mean =	119%
		Standard Deviation =	12.3
		n =	12

Results were generated using Excel 4.0 in the full precision mode. Calculated values may differ slightly.

## APPENDIX V

Table 4

Measured Concentrations of Octabromodiphenyl Oxide (OBDDPO) in Stock Solutions from a Daphnia Flow-Through Life-Cycle Toxicity Test

Nominal Stock Concentration (mg/L)	Sample Number (439A-104-)	Sampling Time (Days)	Octabromodiphenyl Oxide (OBDDPO) Concentration (mg/L)		Mean (mg/L)	Mean Percent of Nominal
			Measured	Corrected <sup>1</sup>		
1.8	S-1	0	2.09	1.76	1.7	94
	S-6	21	1.84	1.55		
3.5	S-2	0	3.83	3.22	3.1	89
	S-7	21	3.63	3.05		
7.0	S-3	0	8.22	6.91	6.7	96
	S-8	21	7.65	6.43		
14	S-4	0	16.4	13.8	13	93
	S-9	21	13.8	11.6		
28	S-5	0	30.2	25.4	25	89
	S-10	21	28.6	24.0		

<sup>1</sup> Values were corrected for a mean procedural recovery of 119%.

## APPENDIX V

Table 5

Measured Concentrations of Octabromodiphenyl Oxide (OBDDPO) in Freshwater Test Samples from a  
Daphnia Flow-Through Life-Cycle Toxicity Test

Nominal Test Concentration ( $\mu\text{g/L}$ )	Sample Number (439A-104-)	Sampling Time (Days)	Octabromodiphenyl Oxide (OBDDPO) Concentration ( $\mu\text{g/L}$ )		Mean ( $\mu\text{g/L}$ )	Mean Percent of Nominal
			Measured <sup>1</sup>	Corrected <sup>2</sup>		
0.0 (Negative Control)	1	0	<LOQ	--	--	--
	2	0	<LOQ	--	--	--
	11	5	<LOQ	--	--	--
	12	5	<LOQ	--	--	--
	21	12	<LOQ	--	--	--
	22	12	<LOQ	--	--	--
	31	21	<LOQ	--	--	--
	32	21	<LOQ	--	--	--
0.0 (Solvent Control)	3	0	<LOQ	--	--	--
	4	0	<LOQ	--	--	--
	13	5	<LOQ	--	--	--
	14	5	<LOQ	--	--	--
	23	12	<LOQ	--	--	--
	24	12	<LOQ	--	--	--
	33	21	<LOQ	--	--	--
	34	21	<LOQ	--	--	--
0.50	5	0	0.576	0.484	0.54	108
	6	0	0.602	0.506		
	15	5	4.53*	--		
	16	5	0.661	0.555		
	25	12	0.871	0.732		
	26	12	0.752	0.632		
	35	21	0.537	0.451		
	36	21	0.491	0.413		

<sup>1</sup> The limit of quantitation (LOQ = 0.250  $\mu\text{g/L}$ ) was based upon the product of the lowest standard (25.0  $\mu\text{g/L}$ ) and the dilution factor of the control samples (0.01) analyzed concurrently with the test samples.

<sup>2</sup> Values were corrected for a mean procedural recovery of 119%.

\* Not included in the calculation of the mean.

## APPENDIX V

Table 5 (Continued)  
 Measured Concentrations of Octabromodiphenyl Oxide (OBDDPO) in Freshwater Test Samples  
 from a Daphnia Flow-Through Life-Cycle Toxicity Test

Nominal Test Concentration ( $\mu\text{g/L}$ )	Sample Number (439A-104-)	Sampling Time (Days)	Octabromodiphenyl Oxide (OBDDPO) Concentration ( $\mu\text{g/L}$ )		Mean ( $\mu\text{g/L}$ )	Mean Percent of Nominal			
			Measured <sup>1</sup>	Corrected <sup>2</sup>					
1.0	7	0	1.06	0.891	0.83	83			
	8	0	1.02	0.857					
	17	5	1.12	0.941					
	18	5	1.17	0.983					
	27	12	1.00	0.840					
	28	12	1.05	0.882					
	37	21	0.687	0.577					
	38	21	0.810	0.681					
	2.0	9	0	1.98			1.66	1.7	85
		10	0	2.16			1.82		
19		5	1.79	1.50					
20		5	2.37	1.99					
29		12	2.23	1.87					
30		12	2.26	1.90					
39		21	1.79	1.50					
40		21	1.38	1.16					

<sup>1</sup> The limit of quantitation (LOQ = 0.250  $\mu\text{g/L}$ ) was based upon the product of the lowest standard (25.0  $\mu\text{g/L}$ ) and the dilution factor of the control samples (0.01) analyzed concurrently with the test samples.

<sup>2</sup> Values were corrected for a mean procedural recovery of 119%.

## APPENDIX V

**METHOD OUTLINE FOR THE PROCESSING OF OCTABROMODIPHENYL OXIDE (OBDPO) IN WELL WATER**

Rinse separatory funnels, roundbottom flasks and centrifuge tubes with ethyl acetate.

↓

Prepare quality control samples by directly fortifying well water contained in separatory funnels with an appropriate stock solution.

↓

Volumetrically sample 100 mL of each test solution directly into its respective separatory funnel.

↓

Using a 50 mL graduated cylinder, add 50 mL of ethyl acetate to each sample. Stopper and shake each sample (with venting) for approximately one minute. Allow the organic and aqueous layers to separate. Drain the aqueous (lower) layer into a 250-mL beaker. Drain the ethyl acetate (upper) layer into a 250 mL roundbottom flask.

↓

Return the aqueous layer to the separatory funnel. Add 50 mL of ethyl acetate to the beaker and swirl the beaker. Pour the ethyl acetate rinse into the separatory funnel containing the aqueous fraction. Shake and partition as described above and combine each extract in its respective roundbottom flask; the total volume should be approximately 100 mL.

↓

Rotary evaporate each sample to approximately 2-3 mL using a waterbath maintained at 40-50°C. Do not evaporate to dryness.

↓

Add approximately 2-3 mL of ethyl acetate to each flask. Swirl to dissolve residues. Transfer the extract to a labelled 15-mL centrifuge tube. Add an additional 2-4 mL of ethyl acetate to the roundbottom flask; swirl and combine the rinse with the previous rinse in the centrifuge tube.

↓

Place samples in a waterbath maintained at 40-50°C and evaporate the samples to dryness under a gentle stream of nitrogen.

↓

Add the requisite volume of diphenylether to each centrifuge tube and vortex for 10-20 seconds.

↓

Transfer the diluted samples to autosampler vials and submit for GC/ECD analysis.

↓

For stock solutions, spike the requisite volume of each diluter stock into a 10-mL volumetric flask containing 4-6 mL of diphenylether. Adjust to volume with diphenylether.

Figure 1. Analytical method flow chart for the analysis of octabromodiphenyl oxide (OBDPO) in freshwater.

## APPENDIX V

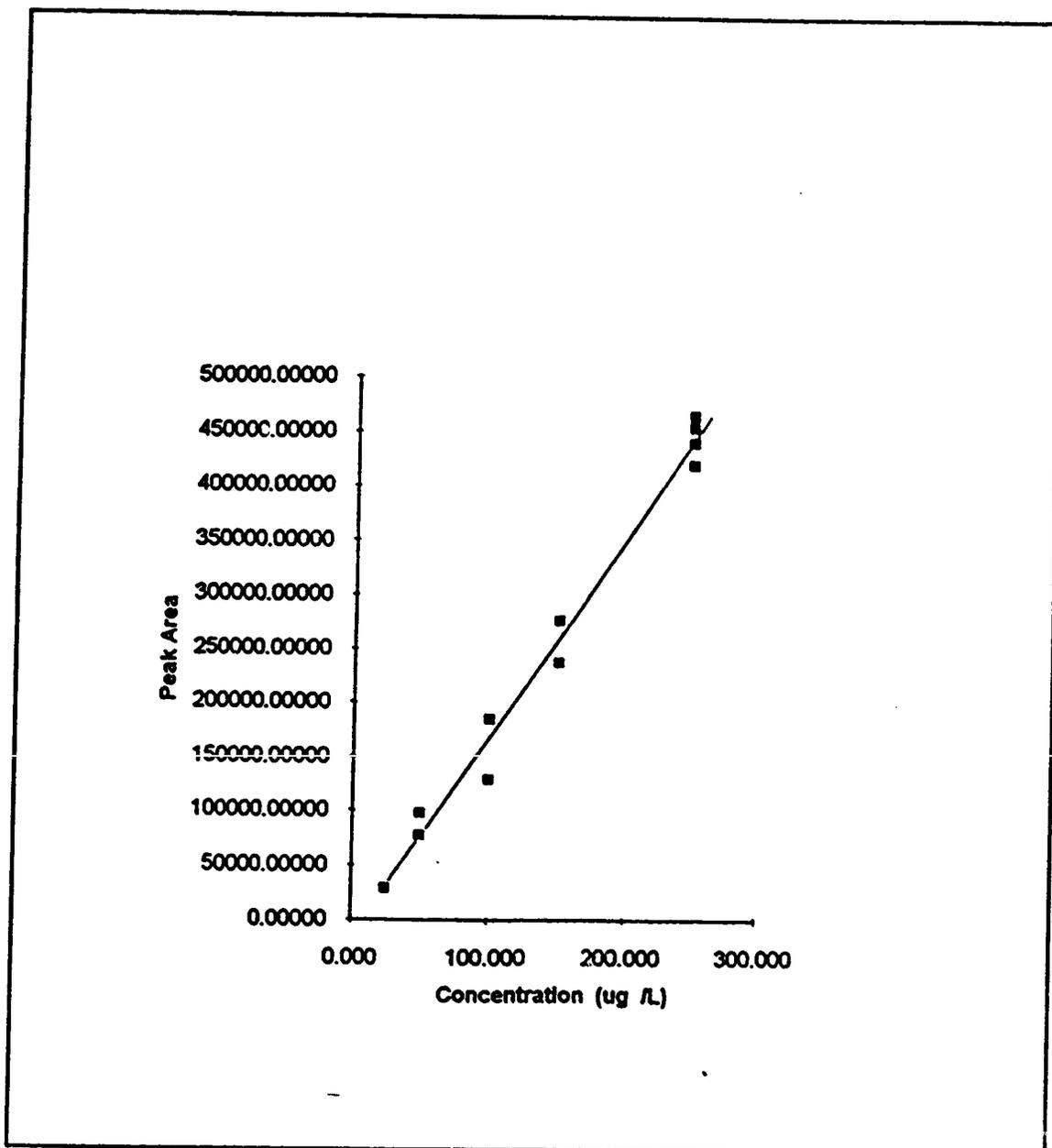


Figure 2. A representative calibration curve for octabromodiphenyl oxide (OBDPO).  
Slope = 1831.70; Intercept = -15639.9.

## APPENDIX V

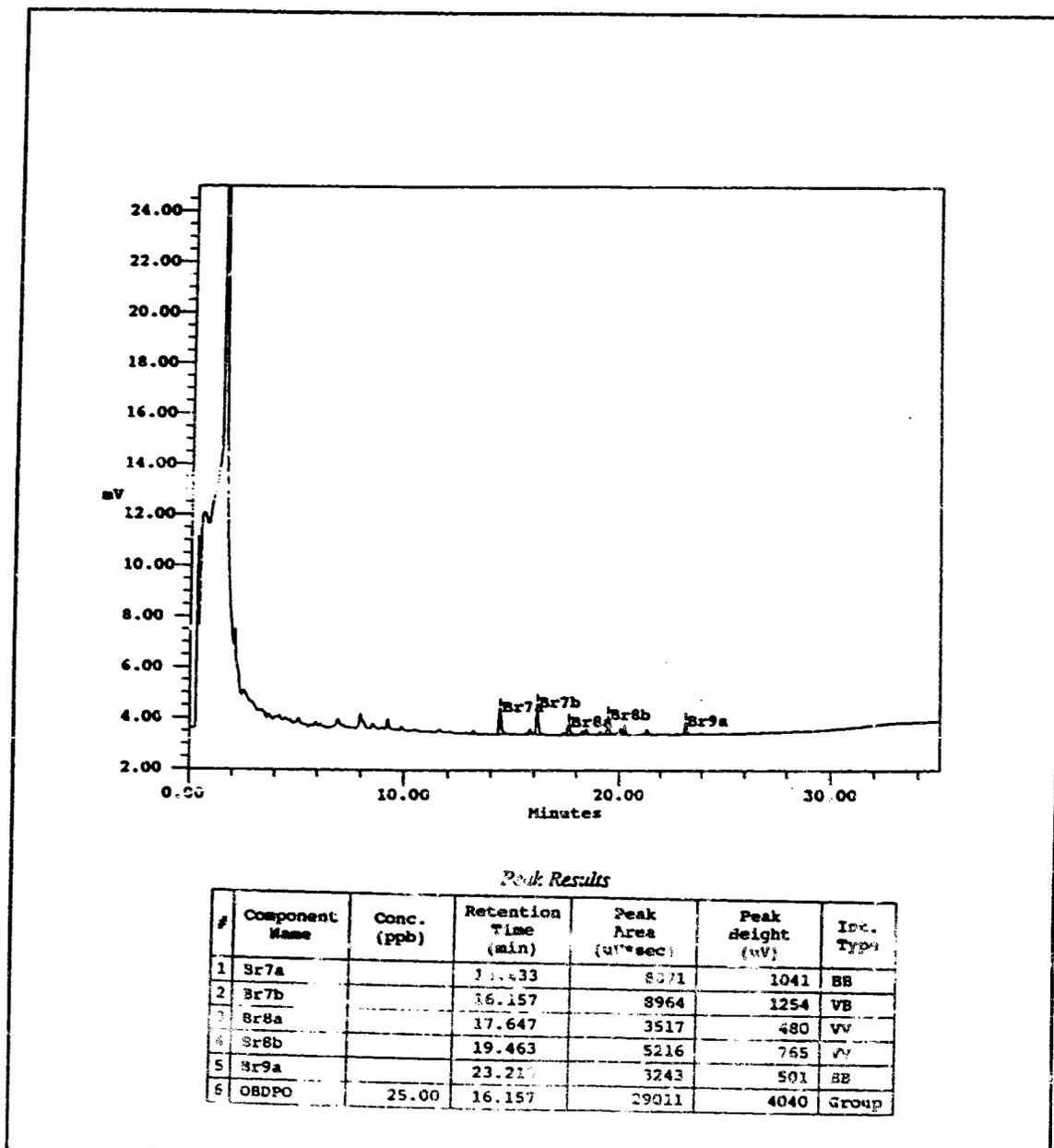


Figure 3. A representative chromatogram of a 25.0 µg/L octabromodiphenyl oxide (OBDDPO) standard (0.05 ng on-column).

## APPENDIX V

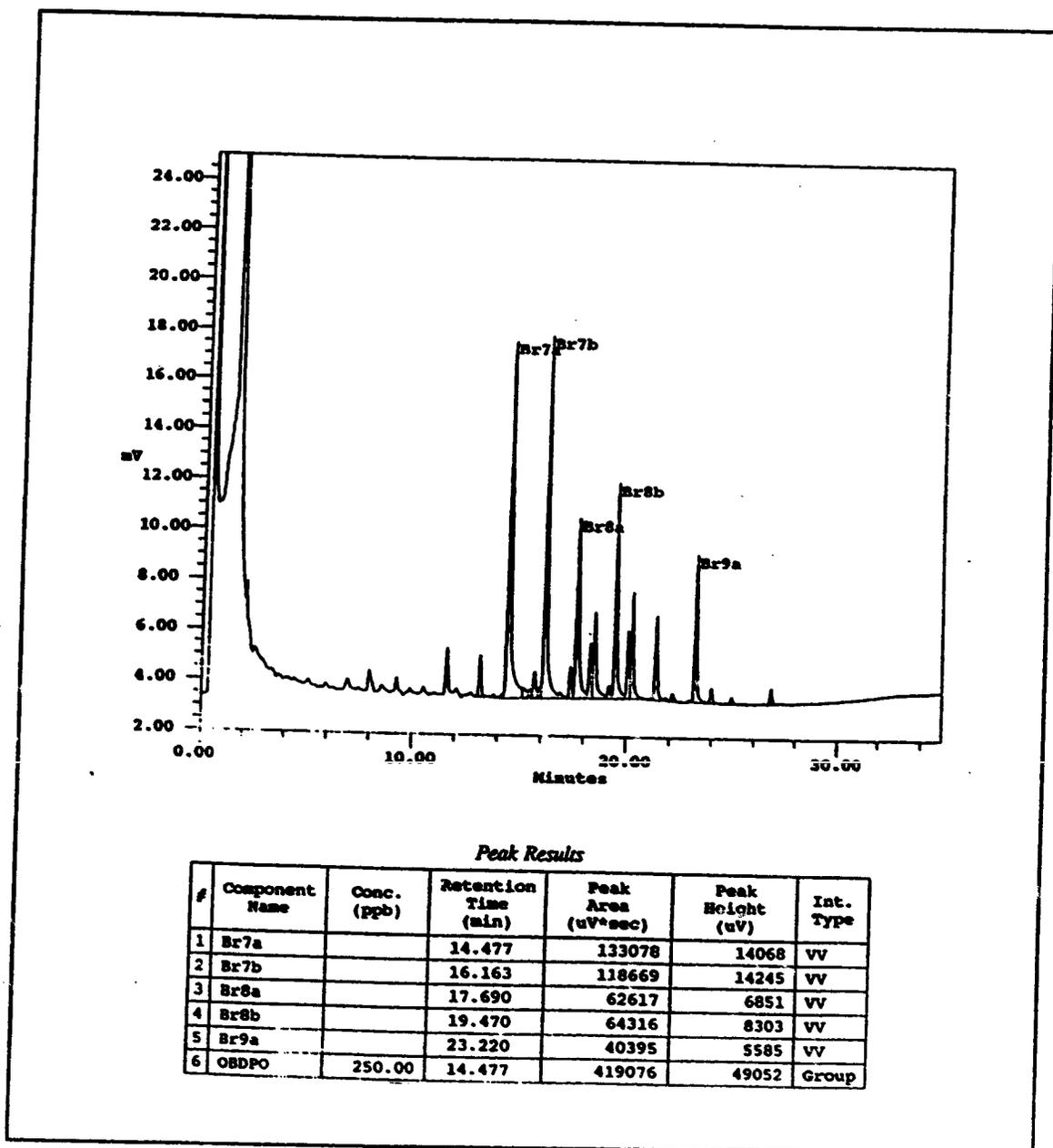
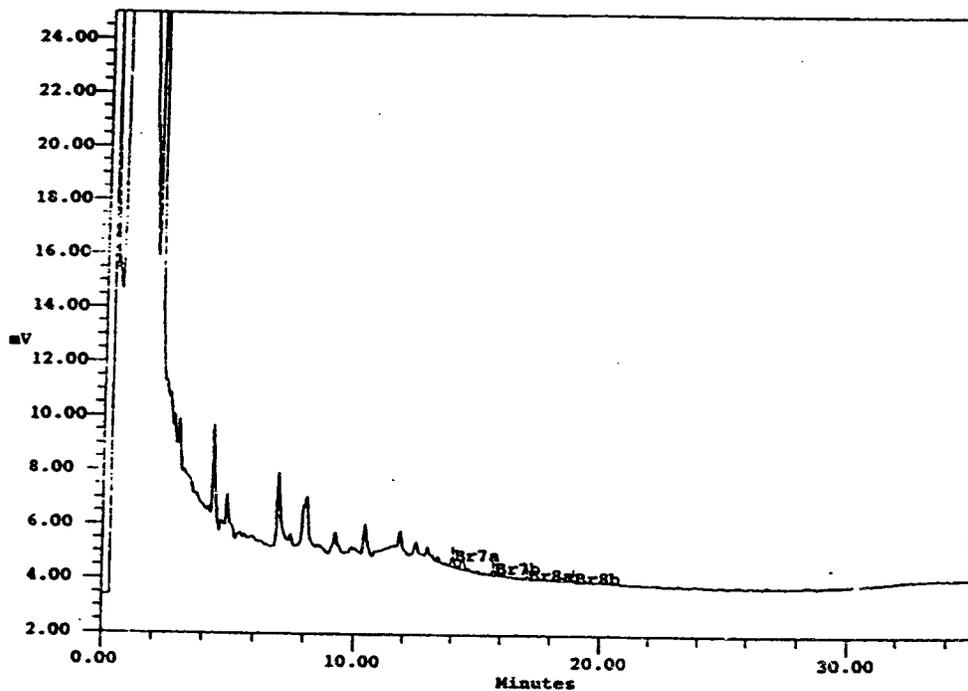


Figure 4. A representative chromatogram of a 250  $\mu\text{g/L}$  octabromodiphenyl oxide (OBDDPO) standard (0.50 ng on-column).

APPENDIX V

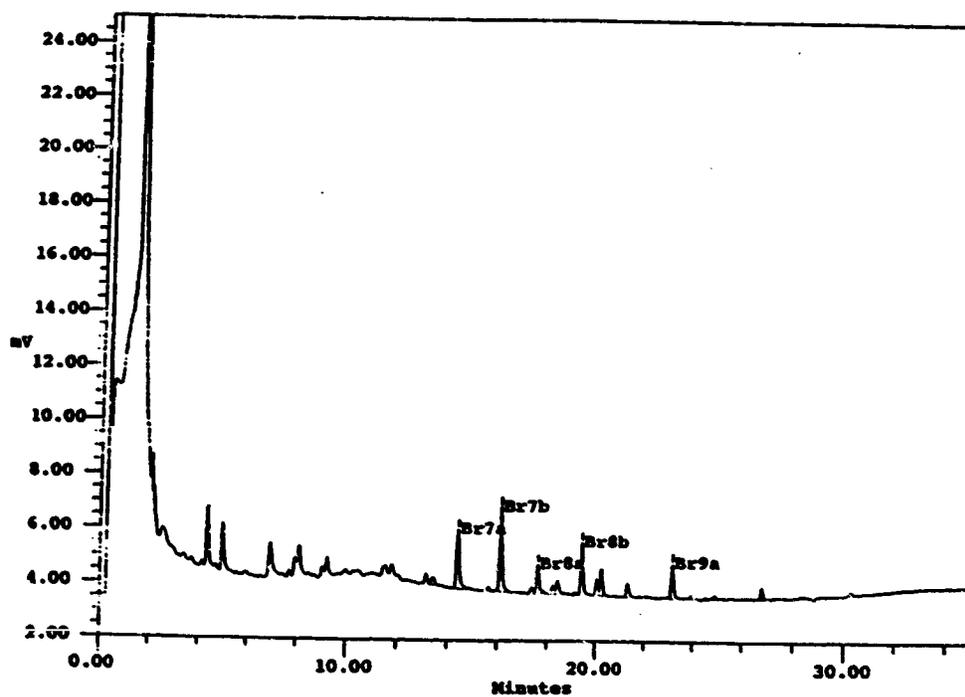


Peak Results

#	Component Name	Retention Time (min)	Peak Area (uV*sec)	Peak Height (uV)	Int. Type	Dilution Factor	Measured Conc. (ppb)
1	Br7a	14.080	3493	389	BV	1.000	
2	Br7b	15.727	1911	215	BB	1.000	
3	Br8a	17.067	1082	115	BB	1.000	
4	Br8b	18.953	1091	110	VV	1.000	
5	Br9a	23.000			Missing	1.000	
6	OBDPO	14.080	7577	828	Group	1.000	12.675

Figure 5. A representative chromatogram of a matrix blank, 439A-104-MAB-2.

## APPENDIX V

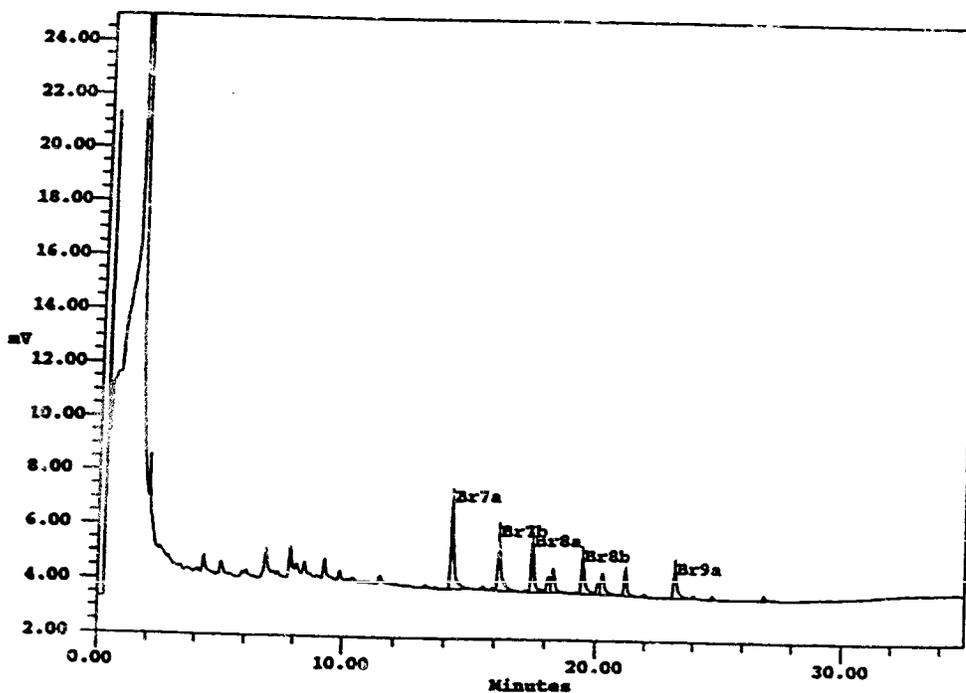


## Peak Results

#	Component Name	Retention Time (min)	Peak Area (uV*sec)	Peak Height (uV)	Int. Type	Dilution Factor	Measured Conc. (ppb)
1	Br7a	14.423	18163	2227	VB	1.000	
2	Br7b	16.137	23300	3162	VB	1.000	
3	Br8a	17.637	8181	1094	VV	1.000	
4	Br8b	19.443	13101	1950	BB	1.000	
5	Br9a	23.197	8745	1283	VB	1.000	
6	OBDDPO	16.137	71491	9716	Group	1.000	47.569

Figure 6. A representative chromatogram of a matrix fortification, 439A-104-MAS-4 (0.400  $\mu\text{g/L}$ ).

APPENDIX V



Peak Results

#	Component Name	Retention Time (min)	Peak Area (uV*sec)	Peak Height (uV)	Int. Type	Dilution Factor	Measured Conc. (ppb)
2	Br7a	14.303	31362	3409	VB	1.000	
2	Br7b	16.163	22264	2167	VB	1.000	
3	Br8a	17.510	16320	1865	VV	1.000	
4	Br8b	19.473	14215	1376	VV	1.000	
5	Br9a	23.230	11476	1066	BV	1.000	
5	OBDFPO	14.303	95638	9884	Group	1.000	68.709

Figure 7. A representative chromatogram of a test sample on Day 21, 439A-104-37 (1.00 µg/L nominal concentration).

## APPENDIX VI

Cumulative Percent Mortality and Treatment-Related Effects<sup>1</sup>

Sponsor: CMA's Brominated Flame Retardant Industry Panel  
 Test Substance: Octabromodiphenyl Oxide (OBDDPO)  
 Test Organism: Cladoceran, *Daphnia magna*  
 Dilution Water: Well Water

Nominal Test Concentration ( $\mu\text{g/L}$ )	Initial Number Exposed	Day 1		Day 2		Day 3	
		Number Immobile	Cumulative Dead	Number Immobile	Cumulative Dead	Number Immobile	Cumulative Dead
Negative Control	20	0	0	0	0	0	0
Scientific Control	20	0	0	0	0	0	0
0.13	20	0	0	0	0	0	0
0.25	20	0	0	0	0	0	0
0.50 (0.54)	20	0	0	0	0	0	0
1.0 (0.83)	20	0	0	0	0	0	0
2.0 (1.7)	20	0	0	0	0	0	0

<sup>1</sup> Observed Effects: AN = Appeared Normal; C = Lethargy.

Note: Immobility data are not cumulative. The numbers of daphnids described as immobile were made on the surviving daphnids in each compartment. Values in parentheses are mean measured test concentrations.

## APPENDIX VI (Continued)

Cumulative Percent Mortality and Treatment-Related Effects<sup>1</sup>

Sponsor: CMA's Brominated Flame Retardant Industry Panel  
 Test Substance: Octabromodiphenyl Oxide (OBDPO)  
 Test Organism: Cladoceran, *Daphnia magna*  
 Dilution Water: Well Water

Nominal Test Concentration ( $\mu\text{g/l.}$ )	Initial Number Exposed	Day 4			Day 5			Day 6		
		Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects
Negative Control	20	0	0	20 AN	0	0	20 AN	0	0	20 AN
Solvent Control	20	0	0	20 AN	0	0	20 AN	0	0	20 AN
0.13	20	0	0	20 AN	0	0	20 AN	0	0	20 AN
0.25	20	0	0	20 AN	0	0	20 AN	0	0	20 AN
0.50 (0.54)	20	0	0	20 AN	0	0	20 AN	0	0	20 AN
1.0 (0.83)	20	0	0	20 AN	0	0	20 AN	0	0	20 AN
2.0 (1.7)	20	0	0	20 AN	0	0	20 AN	0	0	20 AN

<sup>1</sup> Observed Effects: AN = Appeared Normal; C = Lethargy.

Note: Immobility data are not cumulative. The numbers of daphnids described as immobile were made on the surviving daphnids in each compartment. Values in parentheses are mean measured test concentrations.

## APPENDIX VI (Continued)

Cumulative Percent Mortality and Treatment-Related Effects<sup>1</sup>

Sponsor: CMA's Brominated Flame Retardant Industry Panel  
 Test Substance: Octabromodiphenyl Oxide (OBDPO)  
 Test Organism: Cladoceran, *Daphnia magna*  
 Dilution Water: Well Water

Nominal Test Concentration ( $\mu\text{g/L}$ )	Initial Number Exposed	Day 7			Day 8			Day 9		
		Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects
Negative Control	20	0	0	20 AN	0	0	20 AN	0	0	20 AN
Solvent Control	20	0	0	20 AN	0	0	20 AN	0	0	20 AN
0.13	20	0	0	20 AN	0	0	20 AN	0	0	20 AN
0.25	20	0	0	20 AN	0	0	20 AN	0	0	20 AN
0.50 (0.54)	20	0	0	20 AN	0	0	20 AN	0	0	20 AN
1.0 (0.83)	20	0	0	20 AN	0	0	20 AN	0	0	20 AN
2.0 (1.7)	20	0	0	20 AN	0	0	20 AN	0	1	19 AN

<sup>1</sup> Observed Effects: AN = Appeared Normal; C = Lethargy.

Note: Immobility data are not cumulative. The numbers of daphnids described as immobile were made on the surviving daphnids in each compartment. Values in parentheses are mean measured test concentrations.

APPENDIX VI (Continued)

Cumulative Percent Mortality and Treatment-Related Effects<sup>1</sup>

Sponsor: CMA's Brominated Flame Retardant Industry Panel  
 Test Substance: Octabromodiphenyl Oxide (OBDPO)  
 Test Organism: *Cadocera, Daphnia magna*  
 Dilution Water: Well Water

Nominal Test Concentration (µg/L)	Initial Number Exposed	Day 10			Day 11			Day 12		
		Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects
Negative Control	20	0	0	20 AN	0	0	20 AN	0	0	20 AN
Solvent Control	20	0	0	20 AN	0	0	20 AN	0	0	20 AN
0.13	20	0	0	20 AN	0	0	20 AN	0	0	20 AN
0.25	20	0	0	20 AN	0	0	20 AN	0	0	20 AN
0.50 (0.54)	20	0	0	20 AN	0	0	20 AN	0	0	20 AN
1.0 (0.63)	20	0	0	20 AN	0	0	20 AN	0	0	20 AN
2.0 (1.7)	20	0	1	19 AN	0	1	19 AN	0	1	19 AN

<sup>1</sup> Observed Effects: AN = Appeared Normal; C = Lethargy.

Note: Immobility data are not cumulative. The numbers of daphnids described as immobile were made on the surviving daphnids in each compartment. Values in parentheses are mean measured test concentrations.

## APPENDIX VI (Continued)

Cumulative Percent Mortality and Treatment-Related Effects<sup>1</sup>

Sponsor: CMA's Brominated Flame Retardant Industry Panel  
 Test Substance: Octabromodiphenyl Oxide (OBDPO)  
 Test Organism: Cladoceran, *Daphnia magna*  
 Dilution Water: Well Water

Nominal Test Concentration ( $\mu\text{g/L}$ )	Initial Number Exposed	Day 13			Day 14			Day 15		
		Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects
Negative Control	20	0	0	20 AN	0	0	20 AN	0	0	20 AN
Solvent Control	20	0	0	20 AN	0	0	20 AN	0	0	20 AN
0.13	20	0	1	19 AN	0	1	19 AN	0	1	19 AN
0.25	20	0	0	20 AN	0	0	20 AN	0	0	20 AN
0.50 (0.54)	20	0	0	20 AN	0	0	20 AN	0	0	20 AN
1.0 (0.83)	20	0	0	20 AN	0	0	20 AN	0	0	20 AN
2.0 (1.7)	20	0	1	19 AN	0	1	19 AN	0	1	19 AN

<sup>1</sup> Observed Effects: AN = Appeared Normal; C = Lethargy.

Note: Immobility data are not cumulative. The numbers of daphnids described as immobile were made on the surviving daphnids in each compartment. Values in parentheses are mean measured test concentrations.

## APPENDIX VI (Continued)

Cumulative Percent Mortality and Treatment-Related Effects<sup>1</sup>

Sponsor: CMA's Brominated Flame Retardant Industry Panel  
 Test Substance: Octadecylphenyl Oxide (OBDPO)  
 Test Organism: Cladoceran, *Daphnia magna*  
 Dilution Water: Well Water

Nominal Test Concentration (µg/L)	Initial Number Exposed	Day 16			Day 17			Day 18		
		Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects
Negative Control	20	0	0	20 AN	0	0	20 AN	0	1	19 AN
Solvent Control	20	0	0	20 AN	0	0	20 AN	0	0	20 AN
0.13	20	0	1	19 AN	0	2	18 AN	0	2	18 AN
0.25	20	0	0	20 AN	0	0	20 AN	0	0	20 AN
0.50 (0.54)	20	0	0	20 AN	0	0	20 AN	0	0	20 AN
1.0 (0.83)	20	0	0	20 AN	0	0	20 AN	0	0	20 AN
2.0 (1.7)	20	0	1	19 AN	0	1	19 AN	0	1	19 AN

<sup>1</sup> Observed Effects: AN = Appetite, Normal; C = Letargy.

Note: Immobility data are not qualitative. The numbers of daphnids described as immobile were made on the surviving daphnids in each compartment. Values in parentheses are mean measured test concentrations.

## APPENDIX VI (Continued)

Cumulative Percent Mortality and Treatment-Related Effects<sup>1</sup>

Sponsor: CMA's Brominated Flame Retardant Industry Panel

Test Substance: Octabromodiphenyl Oxide (OBDDPO)

Test Organism: Cladoceran, *Daphnia magna*

Dilution Water: Well Water

Nominal Test Concentration ( $\mu\text{g/L}$ )	Initial Number Exposed	Day 19			Day 20			Day 21		
		Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects	Number Immobile	Cumulative Dead	Effects
Negative Control	20	0	1	19 AN	0	1	19 AN	0	1	19 AN
Solvent Control	20	0	0	20 AN	0	0	20 AN	0	0	20 AN
0.13	20	0	2	18 AN	0	2	18 AN	0	2	18 AN
0.25	20	0	0	20 AN	0	0	20 AN	0	0	20 AN
0.50 (0.54)	20	0	0	20 AN	0	0	19AN;1C	0	1	19 AN
1.0 (0.83)	20	0	1	18AN;1C	0	1	18AN;1C	0	1	18AN;1C
2.0 (1.7)	20	0	1	19AN	0	1	19AN	0	1	18AN;1C

<sup>1</sup> Observed Effects: AN = Appeared Normal; C = Lethargy.

Note: Immobility data are not cumulative. The numbers of daphnids described as immobile were made on the surviving daphnids in each compartment. Values in parentheses are mean measured test concentrations.

## APPENDIX VII

Neonate Production<sup>1</sup>

Sponsor:		CMA's Brominated Flame Retardant Industry Panel						
Test Substance:		Octa-bromodiphenyl Oxide (OBDPO)						
Test Organism:		Cladoceran, <i>Daphnia magna</i>						
Dilution Water:		Well Water						
Nominal Test Concentration ( $\mu\text{g/L}$ )	Replicate	Day						Total
		9	12	14	16	19	21	
Negative Control	A	21	124	76	47	41	79	1449
	B	32	73	29	74	56	99	
	C	28	103	12	88	26	83	
	D	34	74	22	86	38	104	
Solvent Control	A	20	89	88	43	99	12	1546
	B	18	114	67	84	72	14	
	C	8	102	162	26	79	35	
	D	22	127	109	45	92	19	
0.13	A	62	165	137	38	56	39	1802
	B	34	132	29	43	85	24	
	C	32	89	193	21	110	29	
	D	43	109	149	32	119	32	
0.25	A	39	67	10	48	106	42	1450
	B	24	178	41	87	71	34	
	C	34	149	10	97	29	22	
	D	36	127	27	91	44	37	
0.50 (0.54)	A	34	123	16	73	32	41	1606
	B	15	92	25	51	89	105	
	C	33	102	6	79	135	79	
	D	14	82	63	30	183	104	
1.0 (0.83)	A	14	139	54	42	48	22	1451
	B	32	159	53	126	61	29	
	C	20	99	111	27	45	19	
	D	9	110	86	5	102	39	
2.0 (1.7)	A	27	69	10	56	79	48	1384
	B	9	103	9	77	123	94	
	C	22	64	30	76	119	49	
	D	14	79	13	110	80	24	

<sup>1</sup> All neonates produced were alive and appeared normal.

Note: Values in parentheses are mean measured test concentrations.

## APPENDIX VIII

## Number of Adult Reproductive Days During the Reproduction Period

		Day														Totals	
Treatment	Replicate	8	9	10	11	12	13	14	15	16	17	18	19	20	21		
Negative Control	A	5	5	5	5	5	5	5	5	5	5	5	5	5	5	70	
	B	5	5	5	5	5	5	5	5	5	5	5	5	4	4	4	67
	C	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	70
	D	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	70
Solvent Control	A	5	5	5	5	5	5	5	5	5	5	5	5	5	5	70	
	B	5	5	5	5	5	5	5	5	5	5	5	5	5	5	70	
	C	5	5	5	5	5	5	5	5	5	5	5	5	5	5	70	
	D	5	5	5	5	5	5	5	5	5	5	5	5	5	5	70	
0.13	A	5	5	5	5	5	5	4	4	4	4	4	4	4	4	62	
	B	5	5	5	5	5	5	5	5	5	5	4	4	4	4	66	
	C	5	5	5	5	5	5	5	5	5	5	5	5	5	5	70	
	D	5	5	5	5	5	5	5	5	5	5	5	5	5	5	70	
0.25	A	5	5	5	5	5	5	5	5	5	5	5	5	5	5	70	
	B	5	5	5	5	5	5	5	5	5	5	5	5	5	5	70	
	C	5	5	5	5	5	5	5	5	5	5	5	5	5	5	70	
	D	5	5	5	5	5	5	5	5	5	5	5	5	5	5	70	
0.50 (0.54)	A	5	5	5	5	5	5	5	5	5	5	5	5	5	5	70	
	B	5	5	5	5	5	5	5	5	5	5	5	5	5	5	70	
	C	5	5	5	5	5	5	5	5	5	5	5	5	5	5	70	
	D	5	5	5	5	5	5	5	5	5	5	5	5	5	5	70	
1.0 (0.83)	A	5	5	5	5	5	5	5	5	5	5	5	5	5	5	70	
	B	5	5	5	5	5	5	5	5	5	5	5	5	4	4	68	
	C	5	5	5	5	5	5	5	5	5	5	5	5	5	5	70	
	D	5	5	5	5	5	5	5	5	5	5	5	5	5	5	70	
2.0 (1.7)	A	5	5	5	5	5	4	4	4	4	4	4	4	4	4	61	
	B	5	5	5	5	5	5	5	5	5	5	5	5	5	5	70	
	C	5	5	5	5	5	5	5	5	5	5	5	5	5	5	70	
	D	5	5	5	5	5	5	5	5	5	5	5	5	5	5	70	

Note: Values in parentheses are mean measured test concentrations.

## APPENDIX IX

## Length and Dry Weight of Surviving First-Generation Daphnids

Sponsor: CMA's Brominated Flame Retardant Industry Panel					
Test Substance: Octabromodiphenyl Oxide (OBDO)					
Test Organism: Cladoceran, <i>Daphnia magna</i>					
Dilution Water: Well Water					
Nominal Test Concentration ( $\mu\text{g/L}$ )	Replicate	Individual Length (mm)	Replicate Mean Length (mm)	Individual Dry Weights (mg)	Replicate Mean Dry Weights (mg)
Negative Control	A	4.50	4.33	0.60	0.577
		4.30		0.54	
		4.15		0.49	
		4.25		0.52	
		4.30		0.56	
		4.10		0.51	
		4.05		0.50	
		4.70		0.78	
		4.60		0.69	
	B	4.20	4.44	0.54	0.625
		4.45		0.59	
		4.10		0.53	
		4.60		0.71	
		4.40		0.59	
		4.70		0.76	
		4.20		0.53	
		4.40		0.58	
		4.45		0.55	
Solvent Control	A	4.35	4.45	0.53	0.622
		4.50		0.64	
		4.85		0.85	
		4.25		0.54	
		4.20		0.55	
		4.40		0.58	
		4.35		0.57	
		4.60		0.68	
		4.40		0.59	
	4.60	0.69			
	B	4.30	4.39	0.58	0.593
		4.70		0.81	
		4.15		0.54	
		4.30		0.56	
		4.25		0.54	
		4.50		0.63	
		4.40		0.55	
		4.40		0.51	
4.45		0.58			
4.45	0.63				

- 94 -

## APPENDIX IX (Continued)

## Length and Dry Weight of Surviving First-Generation Daphnids

Sponsor:		CMA's Brominated Flame Retardant Industry Panel			
Test Substance:		Octabromodiphenyl Oxide (OBDPO)			
Test Organism:		Mudocera, <i>Daphnia magna</i>			
Dilution Water:		Well Water			
Nominal Test Concentration ( $\mu\text{g/l}$ )	Replicate	Individual Length (mm)	Replicate Mean Length (mm)	Individual Dry Weights (mg)	Replicate Mean Dry Weights (mg)
0.13	A	4.45	4.46	0.57	0.599
		4.30		0.53	
		4.50		0.59	
		4.65		0.79	
		4.45		0.56	
		4.35		0.56	
		4.45		0.57	
		4.55		0.62	
		4.65		0.70	
	B	4.75	4.54	0.79	0.660
		4.35		0.58	
		4.40		0.56	
		4.35		0.53	
		4.75		0.69	
		4.40		0.58	
		4.80		0.90	
		4.55		0.67	
		4.40		0.60	
0.25	A	4.70	4.47	0.61	0.597
		4.40		0.58	
		4.45		0.57	
		4.50		0.59	
		4.35		0.53	
		4.40		0.59	
		4.65		0.63	
		4.25		0.53	
		4.70		0.79	
	4.25	0.55			
	B	4.65	4.53	0.66	0.627
		4.45		0.58	
		4.35		0.54	
		4.40		0.58	
		4.40		0.54	
		4.45		0.57	
		4.80		0.79	
		4.70		0.72	
4.45		0.58			
4.65	0.7				

## APPENDIX IX (Continued)

## Length and Dry Weight of Surviving First-Generation Daphnids

Sponsor:		CMA's Brominated Flame Retardant Industry Panel			
Test Substance:		Octabromodiphenyl Oxide (OBDDPO)			
Test Organism:		Cladoceran, <i>Daphnia magna</i>			
Dilution Water:		Well Water			
Nominal Test Concentration ( $\mu\text{g/L}$ )	Replicate	Individual Length (mm)	Replicate Mean Length (mm)	Individual Dry Weights (mg)	Replicate Mean Dry Weights (mg)
0.50 (0.54)	A	4.80	4.44	0.83	0.600
		4.15		0.53	
		4.40		0.57	
		4.35		0.57	
		4.45		0.55	
		4.50		0.59	
		4.45		0.59	
		4.40		0.58	
		4.45		0.59	
	B	4.65	4.52	0.61	0.617
		4.60		0.62	
		4.55		0.58	
		4.60		0.64	
		4.75		0.73	
		4.55		0.69	
		4.45		0.56	
		4.30		0.57	
		4.60		0.65	
1.0 (0.83)	A	4.45	4.45	0.59	0.607
		4.35		0.59	
		4.55		0.64	
		4.65		0.71	
		4.35		0.61	
		4.45		0.60	
		4.50		0.59	
		4.35		0.55	
		4.40		0.58	
	B	4.35	4.55	0.59	0.666
		4.45		0.60	
		4.65		0.64	
		4.65		0.68	
		4.70		0.81	
		4.50		0.61	
		4.30		0.54	
		4.70		0.90	
		4.65		0.62	
4.55	0.67				

Note: Values in parentheses are mean measured test concentrations.

- 96 -

## APPENDIX IX (Continued)

## Length and Dry Weight of Surviving First-Generation Daphnids

Nominal Test Concentration ( $\mu\text{g/L}$ )		Replicate	Individual Length (mm)	Replicate Mean Length (mm)	Individual Dry Weights (mg)	Replicate Mean Dry Weights (mg)
2.0 (1.7)		A	4.35	4.41	0.56	0.587
			4.40		0.55	
			4.15		0.50	
			4.45		0.59	
			4.55		0.69	
			4.60		0.72	
			4.35		0.57	
			4.40		0.52	
			4.45		0.58	
		B	4.40	4.46	0.57	0.604
			4.45		0.59	
			4.60		0.67	
			4.35		0.52	
			4.60		0.69	
			4.40		0.53	
			4.35		0.55	
			4.30		0.50	
			4.55		0.69	
			4.60		0.73	

Note: Values in parentheses are mean measured test concentrations.

APPENDIX X

Personnel Involved in the Study

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

1. James P. Swigert, Ph.D., Manager, Aquatic Toxicology
2. Barbara J. Markley, Ph.D., Manager, Analytical Chemistry
3. Timothy Z. Kendall, Laboratory Supervisor, Analytical Chemistry
4. William C. Graves, Senior Aquatic Biologist
5. Mark A. Mank, Aquatic Laboratory Supervisor

- 98 -

APPENDIX XI  
REPORT AMENDMENT  
OCTABROMODIPHENYL OXIDE (OBDPO)  
A FLOW-THROUGH LIFE-CYCLE TOXICITY TEST  
WITH THE CLADOCERAN (*Daphnia magna*)  
WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER: 439A-104

Rationale for Amendment:

After finalization of the report, it became apparent that the solubility of the test substance in water was not 2.0 µg/L, but < 0.5 µg/L and therefore had to be changed in the report.

1. Original Report: Title Page  
Amended Report: The amended report date was added. The total number of pages was changed from 97 to 99.  
Reason: To indicate that the report was an amended version.
2. Original Report: Page 2  
Amended Report: New signatures and dates were added.  
Reason: To provide new signature and date for the amended final report.
3. Original Report: Page 3  
Amended Report: New signature and dates were added  
Reason: To indicate the date(s) the amended final report was audited and reported to the Study Director/Management.
4. Original Report: Page 4  
Amended Report:
  - a. New signatures and dates were added.
  - b. Change: Mark Jaber, Wildlife International Toxicologist  
To: James P. Swigert, Ph.D.Reason:
  - a. To indicate the dates of the approval of the amended final report
  - b. To indicate that James P. Swigert, Ph.D. will approve the amended final report for laboratory management.

- 99 -

5. Original Report: Pages 5 and 6  
Amended Report: "Amended Report" was added to the Table of Contents.  
Appendix XI- Report Amendment was added to the Table of Contents.  
Reason: To revise the Table of Contents to include new Appendix XI.
6. Original Report: Page 7  
Amended Report: Approximate limit of water solubility was changed from 2  $\mu\text{g/L}$  to < 0.5  $\mu\text{g/L}$ .  
Reason: To provide the correct water solubility limit of the test substance.
7. Original Report: Page 8, Experimental Design section, 4th and 5th sentences  
Amended Report: (< 0.5  $\mu\text{g/L}$ ) was added in the 4th sentence, and the 6th sentence was reworded based on the test substance water solubility.  
Reason: To provide the correct water solubility limit of the test substance and the basis for selection of the maximum nominal test concentration.
8. Original Report: Page 19, last sentence.  
Amended Report: (approximately 2  $\mu\text{g/L}$ ) was changed to (approximately < 0.5  $\mu\text{g/L}$ )  
Reason: To provide the correct water solubility limit of the test substance.

## AMENDMENT SIGNATURES:

Will C. Gray 5-20-97  
Study Director DATE

Jerry P. Deeryant 5/20/97  
Management DATE

## REVIEWED BY:

Kimberly A. Hoxter 5-20-97  
Kimberly A. Hoxter DATE  
Quality Assurance Representative