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August 28, 2000

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Dr. Susan Wayland
Acting 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

Contain NO CBI

[redacted]

84010000001

Dear Dr. Wayland:

The American Chemistry Council 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 report(s) that the American Chemistry Council's Brominated Flame Retardant Industry Panel recently conducted are enclosed:

1) HEXABROMOCYCLODODECANE (HBCD): A Flow-Through Bioconcentration Test with the Rainbow Trout (*Oncorhynchus mykiss*);

2) Brominated Flame Retardant End-User Survey-Phase 1, Johns Hopkins University.

These reports do not include confidential information.

If you have any questions, please call Wendy Sherman of my staff at 703-741-5639.

Sincerely yours,

Courtney M. Price

Enclosures

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HEXABROMOCYCLODODECANE (HBCD): A FLOW-THROUGH BIOCONCENTRATION TEST WITH THE RAINBOW TROUT (*Oncorhynchus mykiss*)

FINAL REPORT

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 439A-111

U.S. Environmental Protection Agency
Series 850 – Ecological Effects Test Guidelines
OPPTS Number 850.1730
and
OECD Guideline 305

AUTHORS:

Kurt R. Drottar
Henry O. Krueger, Ph.D.

STUDY INITIATION DATE: October 19, 1999

STUDY COMPLETION DATE: August 10, 2000

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

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

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

TITLE: Hexabromocyclododecane (HBCD): A Flow-Through Bioconcentration Test with the Rainbow Trout (*Oncorhynchus mykiss*)

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 439A-111

STUDY COMPLETION: August 10, 2000

This study was conducted in compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency in 40 CFR Part 160, 17 August 1989; OECD Principles of Good Laboratory Practice (ENV/MC/CHEM (98) 17); and Japan MAFF (59 NohSan, Notification No. 3850, Agricultural Production Bureau) with the following exception:

The stability of the test substance under storage conditions at the test site was not determined in accordance with Good Laboratory Practice Standards.

STUDY DIRECTOR:

Kurt R. Drottar
Senior Biologist

8/10/00
DATE

SPONSOR APPROVAL:

Wendy K. Sherman
Sponsor

8/17/00
DATE

Wendy K. Sherman
Applicant/Submitter

8/17/00
DATE

QUALITY ASSURANCE STATEMENT

This study was examined for compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency in 40 CFR Part 160, 17 August 1989; OECD Principles of Good Laboratory Practice (ENV/MC/CHEM (98) 17); and Japan MAFF (59 NohSan, Notification No. 3850, Agricultural Protection Bureau). 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	November 10, 1999	November 10, 1999	November 11, 1999
Test Substance Preparation	November 17, 1999	November 19, 1999	November 29, 1999
Test Initiation and Water Chemistry Measurements	November 23, 1999	November 23, 1999	November 24, 1999
Matrix Fortification	December 7, 1999	December 7, 1999	December 9, 1999
Analytical Data and Draft Report	March 15 – 21, 2000	March 23, 2000	March 27, 2000
Biological Data and Draft Report	April 11 – 13 and 17, 2000	April 17, 2000	April 19, 2000
Final Report	June 14, 2000	June 14, 2000	June 14, 2000

Marshall T. Hynson
Quality Assurance Program Supervisor

DATE 8/10/2000

REPORT APPROVAL

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

TITLE: Hexabromocyclododecane (HBCD): A Flow-Through Bioconcentration Test with the Rainbow Trout (*Oncorhynchus mykiss*)

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 439A-111

STUDY DIRECTOR:

Kurt R. Drottar
Senior Biologist

8/10/00
DATE

MANAGEMENT:

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

8/10/00
DATE

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SUMMARY

SPONSOR:	Chemical Manufacturers Association's Brominated Flame Retardant Industry Panel
SPONSOR'S REPRESENTATIVE:	Ms. Wendy Sherman
LOCATION OF STUDY, RAW DATA AND A COPY OF THE FINAL REPORT:	Wildlife International, Ltd. Easton, Maryland 21601

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER:	439A-111
TEST SUBSTANCE:	Hexabromocyclododecane (HBCD)
STUDY:	Hexabromocyclododecane (HBCD): A Flow-Through Bioconcentration Test with the Rainbow Trout (*Oncorhynchus mykiss*)
NOMINAL TEST CONCENTRATIONS:	Solvent Control, 0.34 and 3.4 µg HBCD/L
MEAN MEASURED TEST CONCENTRATIONS:	Solvent Control, 0.18 and 1.8 µg HBCD/L
TEST DATES:	Experimental Start – November 23, 1999 Biological Termination – February 1, 2000 Experimental Termination – February 28, 2000
LENGTH OF TEST:	70 Days (35-Day Uptake, 35-Day Depuration)

TEST ORGANISM:	Rainbow Trout (*Oncorhynchus mykiss*)
SOURCE OF TEST ORGANISMS:	Thomas Fish Company P.O. Box 851 Anderson, California 96007
AGE OF TEST ORGANISMS:	Juveniles
MEASUREMENTS OF 10 SOLVENT CONTROL FISH COLLECTED AT TEST TERMINATION:	
WEIGHT (g):	Mean = 3.46; Range = 2.33 to 5.52
STANDARD LENGTH (mm):	Mean = 72; Range = 64 to 86

RESULTS: (0.18 µg HBCD/L measured; 0.34 µg HBCD/L nominal)	Edible	Nonedible	Whole Fish
DAY 35 BCF:	6,531	20,726	13,085

Note: Steady-State was not achieved in this treatment group.

RESULTS: (1.8 µg HBCD/L measured; 3.4 µg HBCD/L nominal)	Edible	Nonedible	Whole Fish
STEADY-STATE BCF:	4,650	12,866	8,974

INTRODUCTION

A rainbow trout, *Oncorhynchus mykiss*, bioconcentration study was conducted for the Chemical Manufacturers Association's 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 November 23, 1999 to February 1, 2000. Raw data generated by Wildlife International, Ltd. and a copy of the final report are filed under Project Number 439A-111 in archives located on the Wildlife International, Ltd. site.

OBJECTIVE

The objective of this study was to determine the bioconcentration potential of hexabromocyclododecane (HBCD) in rainbow trout. The solubility of HBCD in water at 25°C is 3.4 µg HBCD/L. The 96-hour LC50 for rainbow trout is >6.8 µg HBCD/L.

EXPERIMENTAL DESIGN

The bioconcentration test consisted of a 35-day uptake phase followed by a 35-day depuration phase. During the uptake phase, the test organisms were exposed in one of three groups: 1) A solvent control (0.060 mL acetone/L); 2) A nominal concentration of 0.34 µg HBCD/L; or 3) A nominal concentration of 3.4 µg HBCD/L. The 3.4 µg HBCD/L exposure concentration was chosen by the Sponsor based on the water solubility of the test substance. At the start of the depuration phase, stock flow to the treated groups was stopped and the rainbow trout were exposed to dilution water without HBCD for the remainder of the test.

Each test chamber contained 85 rainbow trout at test initiation, and one replicate was tested for each treatment and the solvent control. Water samples were collected on Day -4 (pre-test), Day -1 (pre-test) on uptake Days 0 (0 and 4 hours), 1, 3, 7, 14, 21, 28 and 35 and on depuration Days 1, 3, 7, 10, 14, 21, 28 and 35 during the test and analyzed for HBCD using liquid chromatography-mass spectrometry (LC/MS). Tissue samples were also collected at selected water sample collection periods during the test and analyzed for HBCD by LC/MS. The results of these analyses were used to calculate the BCF values, uptake rates and depuration rates in edible tissue, nonedible tissue and whole fish.

MATERIALS AND METHODS

The study was conducted according to the procedures outlined in the protocol, "Hexabromocyclododecane (HBCD): A Flow-Through Bioconcentration Test with the Rainbow Trout (*Oncorhynchus mykiss*)" (Appendix I). The protocol was based on procedures outlined in U.S. Environmental Protection Agency Series 850 – Ecological Effects Test Guidelines OPPTS Number 850.1730 (1); *ASTM Standard E1022-84 Standard Practice for Conducting Bioconcentration Tests with Fishes and Saltwater Bivalve Molluscs* (2); and OECD Guideline for Testing of Chemicals 305, *Bioconcentration: Flow-Through Fish Test* (3).

Test Substance

The test substance consisted of a composite of HBCD samples received from three manufacturers. The material's identity and date received from each of the manufacturers is given below:

Manufacturer	Lot/Batch	Date Received	Wildlife International Ltd. Identification Number
Great Lakes Chemical Corporation	Not Given	June 19, 1998	4515
Eurobrom b.v.	971201	June 25, 1998	4520
Albemarle Corporation	33449-15X	June 29, 1998	4521

The composite test substance was assigned Wildlife International Ltd. identification number 4615 and was stored under ambient conditions. A subsample of the composite test substance was shipped to Albemarle Corporation for characterization and purity analyses (Appendix II). The results of the analyses indicated the composite test substance was homogeneous and contained the following components:

Alpha Isomer	6.4%
Beta Isomer	4.5%
Gamma Isomer	79.1%

The conclusion of the characterization was that the test article was HBCD with a purity of 90.0% HBCD isomers. The test substance was stored at room temperature.

Preparation of Test Solutions

The nominal test concentrations were 0.34 and 3.4 µg HBCD/L. A primary stock was prepared by dissolving the test substance in acetone at a concentration of 56.7 mg HBCD/L. The primary stock was inverted to mix. A secondary stock was prepared at a concentration of 5.67 mg HBCD/L by proportional dilution of the primary stock with acetone. The stock solutions were injected into the diluter mixing chambers (at a rate of 0.030 mL/minute) where they were mixed with well water (at a rate of 500 mL/minute) to achieve the desired test concentrations. The resultant test concentrations were not adjusted for the purity of the active ingredient in test substance (90.0%). The solvent control was prepared by adding acetone to the mixing chamber for the solvent control. The concentration of acetone in the solvent control and both HBCD treatment groups was 0.060 mL/L.

Test Organism

The rainbow trout, *Oncorhynchus mykiss*, was selected as the test species for this study. The rainbow trout is one of the recommended freshwater fish species for use in bioconcentration tests (1, 2, 3). Rainbow trout used in the test were obtained from Thomas Fish Company, Anderson, California. Identification of the species was verified by the supplier.

The rainbow trout were held in Wildlife International, Ltd. well water for 105 days prior to testing. The rainbow trout were fed at least once daily during holding. The fish were acclimated to test conditions for 49 hours prior to test initiation. During the holding and acclimation periods the fish showed no signs of disease or stress. During the 14-day holding period preceding the test, water temperatures ranged from 12.1 to 12.5°C. The pH of the water ranged from 8.0 to 8.3 and dissolved oxygen ranged from 9.4 to 10.0 mg/L. Instrumentation used for water measurements are described in the *Environmental Conditions* section of this report. At test initiation, the rainbow trout were collected from the acclimation tanks and indiscriminately distributed 1 to 2 at a time into the test chambers until each chamber contained 85 fish.

During the holding period preceding the test the rainbow trout were fed salmon starter food supplied by Zeigler Brothers, Inc., Gardners, Pennsylvania. Rainbow trout were not fed during the acclimation period, however, they were fed daily during the test. Feeding and sampling schedules were coordinated so that fish were sampled at least four hours after feeding.

All fish used in the test were from the same source and year class, and the standard length of the longest fish was no more than twice the length of the shortest. The length and weight of fish in the solvent control were considered to be representative of all fish used in the test. The mean total length of 10 solvent control fish measured at the end of the test was 72 mm with a range of 64 to 86 mm. The average wet weight (blotted dry) was 3.46 grams with a range of 2.33 to 5.52 grams. Loading was defined as the total wet weight of fish per liter of test water that passed through the test chamber in 24 hours, and was determined to be 0.41 g fish/L/day.

Test Apparatus

A continuous-flow diluter was used to deliver each concentration of the test substance and a solvent control. Syringe pumps (Harvard Apparatus, South Natick, MA) were used to deliver the test substance stock solutions and acetone for the solvent control into mixing chambers assigned to each treatment. The stock solutions were mixed with dilution water in the mixing chambers prior to delivery to the test chambers. The flow of dilution water to the test chambers was controlled by rotameters. The delivery of water from the rotameters was checked prior to the test and at approximately weekly intervals thereafter. Approximately 9.0 volume additions of test water were delivered to the test chambers every 24 hours. The general operation of the diluter was checked at least two times a day during the test.

Test chambers were 106-L stainless steel aquaria filled with approximately 80 L of test solution. The depth of the test water in a representative chamber was approximately 25 cm. Test chambers were indiscriminately positioned in a temperature-controlled water bath designed to maintain a constant temperature. The water bath was enclosed in a plexiglass ventilation hood in order to minimize any potential for cross-contamination. Test chambers were siphoned daily and periodically cleaned during the test to remove excess feed, fecal matter, algae and bacterial growth. Test chambers were identified by the project number, replicate and test concentration.

Dilution Water

The water used for holding 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 measurements 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 µm, and pumped into a 37,800-L storage tank and aerated with spray nozzles. Prior to use, the water again was filtered (0.45µm) to remove microorganisms and particles. 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

The target temperature range for the test was 12 ± 1°C. Temperature was recorded continuously in the solvent control with a Fulscope ER/C Recorder (1900 J Series model no. A). Temperature was also measured in all test chambers at the beginning and end of the test and at weekly intervals during the test, with a liquid-in-glass thermometer.

Dissolved oxygen was measured with a Yellow Springs Instrument Company, Inc. Model 51B dissolved oxygen meter. Measurements were made daily in each test chamber during the test. Measurements of pH were made in each test chamber at the beginning and end of the test and at weekly intervals during the test using a Fisher Accumet Model 915 pH meter.

Hardness, alkalinity, conductivity and total organic carbon (TOC) were measured in the solvent control at the beginning and end of the test, and at weekly intervals during the test. Hardness and alkalinity were measured by titration based on procedures in *Standard Methods for the Examination of Water and Wastewater* (4). Conductivity was measured using a Yellow Springs Instrument Company, Inc. Model 33 Salinity-Conductivity Temperature meter. Total organic carbon was measured using a Shimadzu model TOC-5000 total organic carbon analyzer.

Ambient room light was used to illuminate the test systems. Fluorescent tubes that emitted wavelengths similar to natural sunlight (Colortone® 50) were controlled by an automatic timer to provide a photoperiod of 16 hours of light and 8 hours of darkness. A 30-minute transition period of low light intensity was provided when lights went on and off to avoid sudden changes in light intensity.

Observations

All fish were observed once each day to evaluate the number of mortalities and the number of individuals exhibiting signs of abnormal behavior.

Procedures for Exposure of Fish to HBCD

The test chambers were conditioned by delivering HBCD to the diluter system for approximately 6 days before adding the fish. Water samples were collected twice during the pre-test period to confirm that equilibrium concentrations of test substance in the test chambers were achieved prior to adding the fish. Stock solution samples were also collected on selected days to confirm the concentration of test substance in the stock solutions.

At the end of the pre-test period, the uptake phase of the test was initiated on November 23, 1999, by placing the fish in the test chambers. Rainbow trout were impartially removed from the holding tank in groups of 1 to 2. The groups of rainbow trout were distributed among the test chambers until each test chamber contained 85 fish. The duration of the uptake phase was 35 days. At the end of the uptake phase, stock flow to the treatment groups was stopped and the rainbow trout were exposed to dilution water without HBCD for a period of 35 days.

Collection and Analysis of Water Samples

Water samples were collected on Days 0 (0 hours and 4 hours), 1, 3, 7, 14, 21, 28 and 35 of the uptake phase. Water samples were also collected on Days 1, 3, 7, 10, 14, 21, 28 and 35 of the depuration phase. At each water sampling interval, two water samples were collected from the solvent control and three samples were collected from each of the two HBCD treatment groups. One solvent control sample and two samples from each of the HBCD treatment groups were analyzed for HBCD. The remaining samples were held in reserve as backup samples. All water samples were collected from mid-depth of each test chamber using a class A volumetric pipette. The water samples were analyzed for HBCD by liquid chromatography-mass spectrometry (LC/MS). Procedures for extraction and analysis of the water samples are provided in Appendix V. All water samples were stored at approximately 4°C until analysis.

Collection and Analysis of Tissue Samples

Tissue samples were collected on Days 0 (4 hours), 1, 3, 7, 14, 21, 28 and 35 of the uptake phase. Tissue samples were also collected on Days 1, 3, 7, 10, 14, 21, 28 and 35 of the depuration phase. At each tissue

sampling interval, a sufficient number of fish were collected to provide two replicate samples of solvent control fish and four replicate samples of each HBCD treatment group. Fish were impartially removed from the test chambers and euthanized by severing the spinal cord above the opercular region. The fish were blotted dry and measured for total length and wet weight within approximately 15 minutes of collection, when possible. Each fish was then rinsed with dilution water, blotted dry again and dissected into edible and nonedible tissue fractions. Dissection was accomplished by making an incision from just posterior to the base of the pectoral fin dorsally through the spinal cord. The head, fins and viscera were removed from the body and were considered to be nonedible tissue. The remaining tissue was considered the edible tissue. Tissue samples were transferred to tared scintillation vials and weighed. Procedures for extraction and analysis of the tissue samples are provided in Appendix V. All tissue samples were stored at approximately -14°C until analysis. Tissue samples collected on Day 3 and 7 of the depuration phase were not analyzed due to the pattern of depuration observed.

Tissue Lipid Content and Dry Weight

Selected fish were collected to determine lipid content and dry weight (Appendix V). The determination of lipid content and dry weight provides the potential to express BCF values in terms of lipid content and dry weight.

Data Analysis

Replicate tissue measurements were averaged to report the concentration of test substance in fish tissue. Whole fish concentrations were calculated based on the sum of the edible and nonedible parts. The steady-state bioconcentration factor (BCF) values were determined from the tissue concentrations at apparent steady-state divided by the average water concentration. Tissue concentrations were considered to be at apparent steady-state if three consecutive sets of tissue concentrations were not significantly different ($p > 0.05$) using analysis of variance. If apparent steady-state was not achieved, Day 35 BCF values were calculated using the average tissue concentration on uptake day 35.

The kinetic bioconcentration factor (BCFK), uptake rate (K_1) and depuration rate (K_2) were calculated for edible, nonedible and whole fish using BIOFAC computer software (5). BIOFAC is a nonlinear parameter estimation routine which estimates rate constants from a set of sequential time-concentration data. These rate constants are then used to calculate a BCFK (BCFK = K_1/K_2).

RESULTS AND DISCUSSION

Water Chemistry

Means and ranges of temperature, dissolved oxygen, pH of the water in the test chambers are presented in Table 1. The individual measurements are given in Appendicies VI and VII. Water temperatures in the test chambers were within the temperature range of 12± 1°C established for the test. Dissolved oxygen concentrations remained ≥8.8 mg/L (81 percent of saturation) throughout the test. Measurements of pH ranged from 7.9 to 8.3. Weekly measurements of hardness, alkalinity and conductivity remained consistent throughout the test (Table 1 and Appendix VIII).

Observations of Mortality and Clinical Signs

Observations of mortality and clinical signs are presented in Appendix IX. No mortalities were observed during the test. In addition, no treatment-related clinical signs of toxicity were observed during the test.

Concentrations of HBCD in Water

Concentrations of HBCD in the solvent control were <LOQ (<0.0250 µg HBCD/L) except on Day 28 and 35 of the depuration phase of the test (Tables 2 and 3). On Days 28 and 35, HBCD was measured. However, it is not likely that HBCD was actually present in the solvent control. It is suspected that the samples were exposed to contamination at some point during the analysis.

Measured concentrations of HBCD during the uptake phase in the 0.34 µg HBCD/L treatment group were extremely variable. The values ranged from <LOQ to 0.231 µg HBCD/L. Even with frequent cleaning of the test chamber to remove potentially adsorptive surfaces, it was difficult to maintain a constant exposure at this concentration. When concentrations measured during the uptake phase were averaged, the mean measured concentration was 0.18 µg HBCD/L which represented 53% of the nominal test concentration. Concentrations of HBCD during the depuration phase were generally <LOQ.

Measured concentrations of HBCD during the uptake phase in the 3.4 µg HBCD/L treatment group were less variable. The values ranged from 0.972 to 2.68 µg HBCD/L. When concentrations measured during the uptake phase were averaged, the mean measured concentration was 1.8 µg HBCD/L which represented 53% of

the nominal test concentration. Five of the 16 samples analyzed during the depuration phase contained measurable HBCD.

Concentrations of HBCD in Fish Tissues

Excluding two samples, there were no quantifiable HBCD concentrations in the solvent control tissues (Appendix V). The two samples which did contain HBCD appeared to have been contaminated.

The concentrations of HBCD in tissues of fish exposed to 0.18 µg HBCD/L are presented in Table 4. HBCD concentrations in edible and nonedible tissues appeared to reach steady-state at Day 21 (Figures 1-3). Uptake Day 21 and 28 tissue concentrations were not significantly different ($p > 0.05$). However on uptake Day 35, the tissue concentrations increased unexpectedly. Dunnett's test showed that the Day 35 tissue concentrations were significantly different from the Day 21 and 28 tissue concentrations ($p \leq 0.05$). Consequently, steady-state was not achieved in this treatment group. The unexpected increase in tissue concentrations on Day 35 may have been due to the variability in the measured water concentrations in this treatment group. Estimates of the BCF values for the 0.18 µg HBCD/L treatment group were based on Day 35 tissue concentrations only (Table 5). BIOFAC estimates of the time to reach 90% of steady state in edible tissue, nonedible tissue and whole fish were 118, 99 and 101 days, respectively (Table 6). During the depuration phase of the test, HBCD was eliminated slowly with estimates of time to reach 50% clearance of 35, 30 and 30 days for edible tissue, nonedible tissue and whole fish, respectively.

The concentrations of HBCD in tissues of fish exposed to 1.8 µg HBCD/L are presented in Table 7. HBCD concentrations in edible tissues appeared to reach steady-state at Day 14 (Figure 4). Analysis of variance showed that HBCD concentrations for uptake Days 14 - 35 were not significantly different ($p > 0.05$). Consequently, the BCF value for edible tissue was calculated based on the average tissue concentration for uptake Days 14 – 35 (Table 8). Similarly, HBCD concentrations in nonedible tissues reached steady-state at Day 21 (Figure 5). Analysis of variance showed that HBCD concentrations on uptake days 21, 28 and 35 were not significantly different ($p > 0.05$) and the BCF was calculated based on the average tissue concentration for days 21 - 35. Whole fish concentrations also reached steady-state on Day 21 (Figure 6) and the BCF was based on the average tissue concentrations for days 21 – 35. BIOFAC estimates of the time to reach 90% of steady state in edible tissue, nonedible tissue and whole fish were 63, 65 and 64 days, respectively (Table 9). During the

depuration phase of the test, HBCD was eliminated slowly with estimates of time to reach 50% clearance of 19, 20 and 19 days for edible tissue, nonedible tissue and whole fish, respectively.

CONCLUSIONS

Hexabromocyclododecane (HBCD) rapidly bioconcentrated in the tissues of rainbow trout (*Oncorhynchus mykiss*). After only 4 hours of exposure, measurable concentrations of HBCD were present in the fish tissues. Rainbow trout exposed to 0.18 µg HBCD/L did not reach apparent steady-state tissue concentrations during the length of the test. Rainbow trout exposed to 1.8 µg HBCD/L achieved steady-state tissue concentrations at Day 21. In this study, the BCF calculated for the 1.8 µg HBCD/L treatment group was considered to be a better estimate of the BCF because: 1) Water concentrations were maintained more consistently, and 2) Steady-state was achieved. The BCF values for edible, nonedible and whole fish were 4,650, 12,866 and 8,974, respectively. Clearance of HBCD from fish tissue during depuration was slow. The BIOFAC estimates for time to reach 50% clearance were 19, 20 and 19 days for edible, nonedible and whole fish tissue, respectively.

REFERENCES

1 **U.S. Environmental Protection Agency.** 1996. **Series 850 – Ecological Effects Test Guidelines** (*draft*), OPPTS Number 850.1730: *Fish BCF.*

2 **ASTM Standard E1022-84.** 1988. *Standard Practice for Conducting Bioconcentration Tests with Fishes and Saltwater Bivalve Molluscs.* **American Society for Testing and Materials.**

3 **OECD Guideline for Testing of Chemicals 305.** 1988. *Bioconcentration: Flow-Through Fish 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 **BIOFAC.** 1991. September 19, 1991 version. The Dow Chemical Company, Midland, Michigan.

Table 1

Means and Ranges of Water Quality Parameters

Sponsor:	Chemical Manufacturers Association's Brominated Flame Retardant Industry Panel
Test Substance:	HBCD
Test Organism:	Rainbow Trout, *Oncorhynchus mykiss*
Dilution Water:	Well Water

Uptake Phase Nominal Concentration (µg HBCD/L)	Temperature[1] (°C)	DO[2] (mg/L)	pH	Conductivity (µmhos/cm)	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)	TOC (mg C/L)
Solvent Control	12.0 11.8 – 12.3	9.6 8.8 – 10.6	8.1 7.9 - 8.3	271 270 - 280	178 172 - 184	137 132 - 140	10.4 <1 – 24.1
0.34	11.9 11.5 - 12.0	9.7 8.8 – 10.6	8.1 7.9 - 8.3				
3.4	11.8 11.4 - 12.0	9.7 8.8 – 10.6	8.1 8.0 – 8.3				

[1] Temperature measured continuously in the solvent control ranged from approximately 12.0 to 13.0°C.
[2] At a temperature of 12°C, the dissolved oxygen saturation concentration is 10.8 mg/L and 60% saturation is 6.5 mg/L.

Table 2

Concentrations of HBCD in Water Samples During the Uptake Phase

Sponsor: Chemical Manufacturers Association's Brominated Flame Retardant Industry Panel
Test Substance: HBCD
Test Organism: Rainbow Trout, *Oncorhynchus mykiss*
Dilution Water: Well Water

Nominal Concentration (µg HBCD/L)	Day of Study (µg HBCD/L)										Mean Measured Concentration	Percent of Nominal
	0 (0 Hour)	0 (4 Hours)	1	3	7	14	21	21[2]	28	35		
Solvent Control	< LOQ[1]	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	--
0.34	0.231 0.214	0.192 0.211	0.169 0.175	0.170 0.147	0.195 0.172	0.252 0.205	< LOQ < LOQ	0.117 0.126	0.155 0.157	0.163 0.178	0.18	53
3.4	2.46 2.68	2.16 2.27	2.02 2.17	2.17 1.35	2.47 2.45	2.11 2.12	1.22 0.982	0.972 1.01	1.34 1.34	1.56 1.51	1.8	53

[1]The Limit of Quantitation (LOQ) was 0.0250 µg HBCD/L.
[2]Additional samples were collected on Day 21 due to low recoveries.

Table 3

Concentrations of HBCD in Water During the Depuration Phase

Sponsor:	Chemical Manufacturers Association's Brominated Flame Retardant Industry Panel
Test Substance:	HBCD
Test Organism:	Rainbow Trout, *Oncorhynchus mykiss*
Dilution Water:	Well Water

Uptake Phase Mean Measured Concentration (µg HBCD/L)	Day of Study (µg HBCD/L)							
	1	3	7	10	14	21	28	35
Solvent Control	<LOQ[1]	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.0461[2]	0.0496[2]
0.18	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.0306	0.0542
	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
1.8	<LOQ	0.300	<LOQ	<LOQ	<LOQ	<LOQ	0.0412	<LOQ
	0.142	<LOQ	<LOQ	0.0288	<LOQ	<LOQ	<LOQ	0.0648

[1]The Limit of Quantitation (LOQ) was 0.0250 µg HBCD/L.
[2]Samples presumed to be contaminated.

Table 4

HBCD Concentrations in Edible, Nonedible and Whole Fish Tissues of Rainbow Trout Exposed to 0.18 µg HBCD/L

Sponsor: Chemical Manufacturers Association's Brominated Flame Retardant Industry Panel
Test Substance: HBCD
Test Organism: Rainbow Trout, *Oncorhynchus mykiss*
Dilution Water: Well Water

Sample ID	Uptake Day	Edible Tissue Concentration (µg HBCD/Kg)	Edible Tissue Weight (g)	Nonedible Tissue Concentration (µg HBCD/Kg)	Nonedible Tissue Weight (g)	Whole Fish Concentration[1] (µg HBCD/Kg)
E4/N4	0 (4 hours)	13.9	1.5641	32.8	1.3507	22.7
E5/N5	0 (4 hours)	13.9	1.4558	24.8	1.2641	19.0
E6/N6	0 (4 hours)	10.0	1.3705	24.4	1.2802	17.0
E7/N7	0 (4 hours)	8.38	2.4433	27.0	2.0041	16.8
E17/N17	1	79.9	2.3969	123	1.9973	99.5
E18/N18	1	67.0	2.2607	91.8	1.7977	78.0
E19/N19	1	90.6	2.1009	165	1.7498	124
E20/N20	1	85.4	2.3081	97.4	1.8944	90.8
E30/N30	3	229	1.3582	382	1.0778	297
E31/N31	3	232	1.2416	310	1.0750	268
E32/N32	3	213	1.7536	366	1.0463	270
E33/N33	3	225	1.2130	456	0.8662	321
E43/N43	7	318	1.2910	733	1.1209	511
E44/N44	7	361	1.4897	1129	1.2845	717
E45/N45	7	436	1.4840	1199	1.2958	792
E46/N46	7	415	2.3696	583	1.9002	490
E56/N56	14	462	2.1449	937	1.7711	677
E57/N57	14	517	1.9038	983	1.8699	748
E58/N58	14	592	1.9857	861	1.9349	725
E59/N59	14	524	1.5568	1130	1.6737	838

[1] Whole Fish Concentration = $\frac{\text{(edible wt. X edible conc.) + (nonedible wt. X nonedible conc.)}}{\text{(edible wt. + nonedible wt.)}}$

Table 4 (Continued)

HBCD Concentrations in Edible, Nonedible and Whole Fish Tissues of Rainbow Trout Exposed to 0.18 µg HBCD/L

Sponsor: Chemical Manufacturers Association's Brominated Flame Retardant Industry Panel
Test Substance: HBCD
Test Organism: Rainbow Trout, *Oncorhynchus mykiss*
Dilution Water: Well Water

Sample ID	Uptake Day	Edible Tissue Concentration (µg HBCD/Kg)	Edible Tissue Weight (g)	Nonedible Tissue Concentration (µg HBCD/Kg)	Nonedible Tissue Weight (g)	Whole Fish Concentration[1] (µg HBCD/Kg)
E69/N69	21	665	3.3308	1744	3.0509	1181
E70/N70	21	767	2.0901	1765	1.7854	1227
E71/N71	21	671	1.8612	2636	1.6679	1600
E72/N72	21	801	1.7825	1621	1.5895	1188
E82/N82	28	751	1.6704	1532	1.8072	1157
E83/N83	28	881	1.6154	2386	1.8349	1681
E84/N84	28	727	1.4706	2238	1.7193	1541
E85/N85	28	740	1.4309	2426	1.5337	1612
E95/N95	35	1617	2.5726	3396	2.0240	2400
E96/N96	35	1155	1.6082	3890	1.4210	2438
E97/N97	35	888	1.9934	3051	1.5687	1841
E98/N98	35	1042	1.2609	4586	1.1621	2742

[1]Whole Fish Concentration = $\frac{(\text{edible wt. X edible conc.}) + (\text{nonedible wt. X nonedible conc.})}{(\text{edible wt.} + \text{nonedible wt.})}$

Table 4 (Continued)

HBCD Concentrations in Edible, Nonedible and Whole Fish
Tissues of Rainbow Trout Exposed to 0.18 µg HBCD/L

Sponsor: Chemical Manufacturers Association's Brominated Flame Retardant Industry Panel
Test Substance: HBCD
Test Organism: Rainbow Trout, *Oncorhynchus mykiss*
Dilution Water: Well Water

Sample ID	Depuration Day	Edible Tissue Concentration (µg HBCD/Kg)	Edible Tissue Weight (g)	Nonedible Tissue Concentration (µg HBCD/Kg)	Nonedible Tissue Weight (g)	Whole Fish Concentration[1] (µg HBCD/Kg)
E106/N106	1	1100	2.4333	1769	2.3973	1432
E107/N107	1	1074	1.3764	2161	1.5885	1656
E108/N108	1	1047	1.4125	2334	1.7169	1753
E109/N109	1	1493	1.0815	2021	1.3982	1791
E139/N139	10	738	1.1499	1749	1.3073	1276
E140/N140	10	874	0.9618	4473	1.1118	2804
E141/N141	10	1002	1.0696	3185	1.2114	2161
E142/N142	10	1131	0.9121	4059	1.1301	2751
E150/N150	14	815	1.3703	2292	1.4152	1565
E151/N151	14	946	1.7360	2842	1.6515	1870
E152/N152	14	1018	1.2102	3662	1.2372	2355
E153/N153	14	700	1.7052	3167	1.57[illegible]5	1884

[1]Whole Fish Concentration = $\frac{\text{(edible wt. X edible conc.) + (nonedible wt. X nonedible conc.)}}{\text{(edible wt. + nonedible wt.)}}$

Table 4 (Continued)

HBCD Concentrations in Edible, Nonedible and Whole Fish Tissues of Rainbow Trout Exposed to 0.18 µg HBCD/L

Sponsor: Chemical Manufacturers Association's Brominated Flame Retardant Industry Panel
Test Substance: HBCD
Test Organism: Rainbow Trout, *Oncorhynchus mykiss*
Dilution Water: Well Water

Sample ID	Depuration Day	Edible Tissue Concentration (µg HBCD/Kg)	Edible Tissue Weight (g)	Nonedible Tissue Concentration (µg HBCD/Kg)	Nonedible Tissue Weight (g)	Whole Fish Concentration[1] (µg HBCD/Kg)
E161/N161	21	1872	1.7683	1977	1.7717	1925
E162/N162	21	--[2]	1.0609	1348	1.1569	--[3]
E163/N163	21	1235	2.2179	2497	1.8875	1815
E164/N164	21	835	1.6039	1090	1.5778	961
E172/N172	28	745	1.0772	1362	1.1195	1059
E173/N173	28	610	1.6895	614	1.5630	612
E174/N174	28	246	2.4913	1362	2.2456	775
E175/N175	28	468	2.7381	1476	2.4948	949
E183/N183	35	894	1.4476	1597	1.5441	1257
E184/N184	35	126	2.2725	330	1.9845	221
E185/N185	35	707	2.2240	1049	1.9948	869
E186/N186	35	741	1.0413	--[2]	1.0473	--[3]

[1]Whole Fish Concentration = $\frac{\text{(edible wt. X edible conc.) + (nonedible wt. X nonedible conc.)}}{\text{(edible wt. + nonedible wt.)}}$

[2]Sample contaminated
[3]Whole fish concentration could not be calculated.

Table 5

Day 35 BCF Estimates for Rainbow Trout Exposed to 0.18 µg HBCD/L

Sponsor:	Chemical Manufacturers Association's Brominated Flame Retardant Industry Panel
Test Substance:	HBCD
Test Organism:	Rainbow Trout, *Oncorhynchus mykiss*
Dilution Water:	Well Water

Tissue Type	Mean Measured Test Concentration (µg HBCD/L)	Mean Measured Day 35 Tissue Concentration (µg HBCD/Kg)	Day 35 BCF
Edible	0.18	1,175.5	6,531
Nonedible	0.18	3,730.75	20,726
Whole Fish	0.18	2,355.25	13,085

Table 6

BIOFAC Model Estimates for Rainbow Trout Exposed to 0.18 µg HBCD/L

Sponsor:	Chemical Manufacturers Association's Brominated Flame Retardant Industry Panel
Test Substance:	HBCD
Test Organism:	Rainbow Trout, *Oncorhynchus mykiss*
Dilution Water:	Well Water

Tissue Type	Kinetic Bioconcentration Factor (BCFK)	Uptake Rate Constant (k_1, $L \cdot Kg^{-1} \cdot Day^{-1}$)	Depuration Rate Constant (k_2, Day^{-1})	Estimate Time to Reach 90% Steady State (Days)	Estimated Time to Reach 50% Clearance (Days)
Edible	14,039	275	0.0196	118	35.4
Nonedible	30,242	701	0.0232	99.3	29.9
Whole Fish	21,940	500	0.0228	101	30.4

Table 7

HBCD Concentrations in Edible, Nonedible and Whole Fish Tissues of Rainbow Trout Exposed to 1.8 µg HBCD/L

Sponsor: Chemical Manufacturers Association's Brominated Flame Retardant Industry Panel
Test Substance: HBCD
Test Organism: Rainbow Trout, *Oncorhynchus mykiss*
Dilution Water: Well Water

Sample ID	Uptake Day	Edible Tissue Concentration (µg HBCD/Kg)	Edible Tissue Weight (g)	Nonedible Tissue Concentration (µg HBCD/Kg)	Nonedible Tissue Weight (g)	Whole Fish Concentration[1] (µg HBCD/Kg)
E9/N9	0 (4 hours)	132	1.3508	175	1.2955	153
E10/N10	0 (4 hours)	110	2.5779	269	2.2808	185
E11/N11	0 (4 hours)	119	1.9132	216	1.6847	164
E12/N12	0 (4 hours)	75.9	1.6168	401	1.5500	235
E22/N22	1	657	1.9264	991	1.6815	813
E23/N23	1	740	1.8537	1450	1.7584	1086
E24/N24	1	895	2.8838	1203	2.5892	1041
E25/N25	1	529	2.2368	1157	2.0366	828
E35/N35	3	2848	1.2694	4455	0.9832	3549
E36/N36	3	3200	1.5575	5170	1.0289	3984
E37/N37	3	3086	1.0597	3797	0.8309	3398
E38/N38	3	1202	1.9177	4902	1.3430	2726
E48/N48	7	4542	2.3545	11043	1.9485	7486
E49/N49	7	6300	1.2714	12053	1.3356	9247
E50/N50	7	5798	1.8194	11329	1.7754	8530
E51/N51	7	3938	1.4523	13440	1.3052	8436
E61/N61	14	5727	2.0403	14530	1.9756	10058
E62/N62	14	5734	2.2546	11688	2.1097	8612
E63/N63	14	7764	1.9041	19332	1.7054	13230
E64/N64	14	7985	1.6724	15593	1.5604	11657

[1]Whole Fish Concentration = $\frac{\text{(edible wt. X edible conc.) + (nonedible wt. X nonedible conc.)}}{\text{(edible wt. + nonedible wt.)}}$

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Table 7 (Continued)

HBCD Concentrations in Edible, Nonedible and Whole Fish Tissues of Rainbow Trout Exposed to 1.8 µg HBCD/L

Sponsor: Chemical Manufacturers Association's Brominated Flame Retardant Industry Panel
Test Substance: HBCD
Test Organism: Rainbow Trout, *Oncorhynchus mykiss*
Dilution Water: Well Water

Sample ID	Uptake Day	Edible Tissue Concentration (µg HBCD/Kg)	Edible Tissue Weight (g)	Nonedible Tissue Concentration (µg HBCD/Kg)	Nonedible Tissue Weight (g)	Whole Fish Concentration[1] (µg HBCD/Kg)
E74/N74	21	8814	2.6499	24197	2.4278	16169
E75/N75	21	9801	1.6744	29737	1.5764	19469
E76/N76	21	8101	1.6934	28478	1.6278	18088
E77/N77	21	12607	1.5397	19567	1.4856	16025
E87/N87	28	4205	2.1502	21523	2.1049	12772
E88/N88	28	8905	2.0656	21455	2.3595	15597
E89/N89	28	10061	1.9036	16465	2.1876	13485
E90/N90	28	9759	1.6929	26294	1.7189	18090
E99/N99	35	6550	2.3350	15931	2.2233	11126
E100/N100	35	8035	1.8894	29848	2.1399	19620
E101/N101	35	10692	1.6189	29841	1.9766	21219
E102/N102	35	9174	1.3193	14563	1.6753	12189

[1]Whole Fish Concentration = $\frac{\text{(edible wt. X edible conc.) + (nonedible wt. X nonedible conc.)}}{\text{(edible wt. + nonedible wt.)}}$

Table 7 (Continued)

HBCD Concentrations in Edible, Nonedible and Whole Fish
Tissues of Rainbow Trout Exposed to 1.8 µg HBCD/L

Sponsor: Chemical Manufacturers Association's Brominated Flame Retardant Industry Panel
Test Substance: HBCD
Test Organism: Rainbow Trout, *Oncorhynchus mykiss*
Dilution Water: Well Water

Sample ID	Depuration Day	Edible Tissue Concentration (µg HBCD/Kg)	Edible Tissue Weight (g)	Nonedible Tissue Concentration (µg HBCD/Kg)	Nonedible Tissue Weight (g)	Whole Fish Concentration[1] (µg HBCD/Kg)
E110/N110	1	9477	1.3927	15609	1.7595	12900
E111/N111	1	11443	1.6451	22868	1.8841	17542
E112/N112	1	15998	1.1291	29797	1.3117	23414
E113/N113	1	2646	1.3459	26946	1.4684	15325
E143/N143	10	7626	1.2653	29146	1.3037	18547
E144/N144	10	10999	1.2525	49662	1.2570	30365
E145/N145	10	7991	1.9346	28026	2.1985	18648
E146/N146	10	5354	0.9461	28059	1.1445	17784
E154/N154	14	10419	1.5073	12683	1.6794	11612
E155/N155	14	9916	3.2649	17418	2.4738	13150
E156/N156	14	7877	1.5836	16952	1.5890	12422
E157/N157	14	6575	2.3430	22385	2.1846	14203

[1]Whole Fish Concentration = $\frac{\text{(edible wt. X edible conc.) + (nonedible wt. X nonedible conc.)}}{\text{(edible wt. + nonedible wt.)}}$

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Table 7 (Continued)

HBCD Concentrations in Edible, Nonedible and Whole Fish Tissues of Rainbow Trout Exposed to 1.8 µg HBCD/L

Sponsor: Chemical Manufacturers Association's Brominated Flame Retardant Industry Panel
Test Substance: HBCD
Test Organism: Rainbow Trout, *Oncorhynchus mykiss*
Dilution Water: Well Water

Sample ID	Depuration Day	Edible Tissue Concentration (µg HBCD/Kg)	Edible Tissue Weight (g)	Nonedible Tissue Concentration (µg HBCD/Kg)	Nonedible Tissue Weight (g)	Whole Fish Concentration[1] (µg HBCD/Kg)
E165/N165	21	7195	1.6321	13482	1.5362	10243
E166/N166	21	7358	2.9487	14236	2.4623	10488
E167/N167	21	8609	2.3526	18354	2.4214	13552
E168/N168	21	11730	1.4194	10613	1.4749	11161
E176/N176	28	1022	2.2929	14190	2.1302	7364
E177/N177	28	1308	1.3544	11546	1.5451	6764
E178/N178	28	3047	1.2849	1164	1.4847	2038
E179/N179	28	1543	0.8676	2717	0.9912	2144
E187/N187	35	3072	1.6754	7999	1.8852	5681
E188/N188	35	5803	1.9002	9499	1.9272	7664
E189/N189	35	3100	1.4604	9054	1.4891	6106
E190/N190	35	4077	0.9763	6745	1.0245	5443

[1] Whole Fish Concentration = $\frac{\text{(edible wt. X edible conc.) + (nonedible wt. X nonedible conc.)}}{\text{(edible wt. + nonedible wt.)}}$

Table 8

Steady-State BCF Values for Rainbow Trout Exposed to 1.8 µg HBCD/L

Sponsor:	Chemical Manufacturers Association's Brominated Flame Retardant Industry Panel
Test Substance:	HBCD
Test Organism:	Rainbow Trout, *Oncorhynchus mykiss*
Dilution Water:	Well Water

Tissue Type	Mean Measured Test Concentration (µg HBCD/L)	Uptake Days at Steady-State	Mean Measured Steady-State Tissue Concentration (µg HBCD/Kg)	Steady-State BCF
Edible	1.8	14, 21, 28, 35	8,370	4650
Nonedible	1.8	21, 28, 35	23,158	12866
Whole Fish	1.8	21, 28, 35	16,154	8974

Table 9

BIOFAC Model Estimates for Rainbow Trout Exposed to 1.8 µg HBCD/L

Sponsor:	Chemical Manufacturers Association's Brominated Flame Retardant Industry Panel
Test Substance:	HBCD
Test Organism:	Rainbow Trout, *Oncorhynchus mykiss*
Dilution Water:	Well Water

Tissue Type	Kinetic Bioconcentration Factor (BCFK)	Uptake Rate Constant (k_1, $L \cdot Kg^{-1} \cdot Day^{-1}$)	Depuration Rate Constant (k_2, Day^{-1})	Estimate Time to Reach 90% Steady State (Days)	Estimated Time to Reach 50% Clearance (Days)
Edible	9,826	362	0.0369	62.5	18.8
Nonedible	23,303	821	0.0352	65.4	19.7
Whole Fish	16,450	591	0.0359	64.1	19.3

Figure 1. Concentrations of HBCD in Edible Fish Tissues of Rainbow Trout Exposed to 0.18 µg HBCD/L.

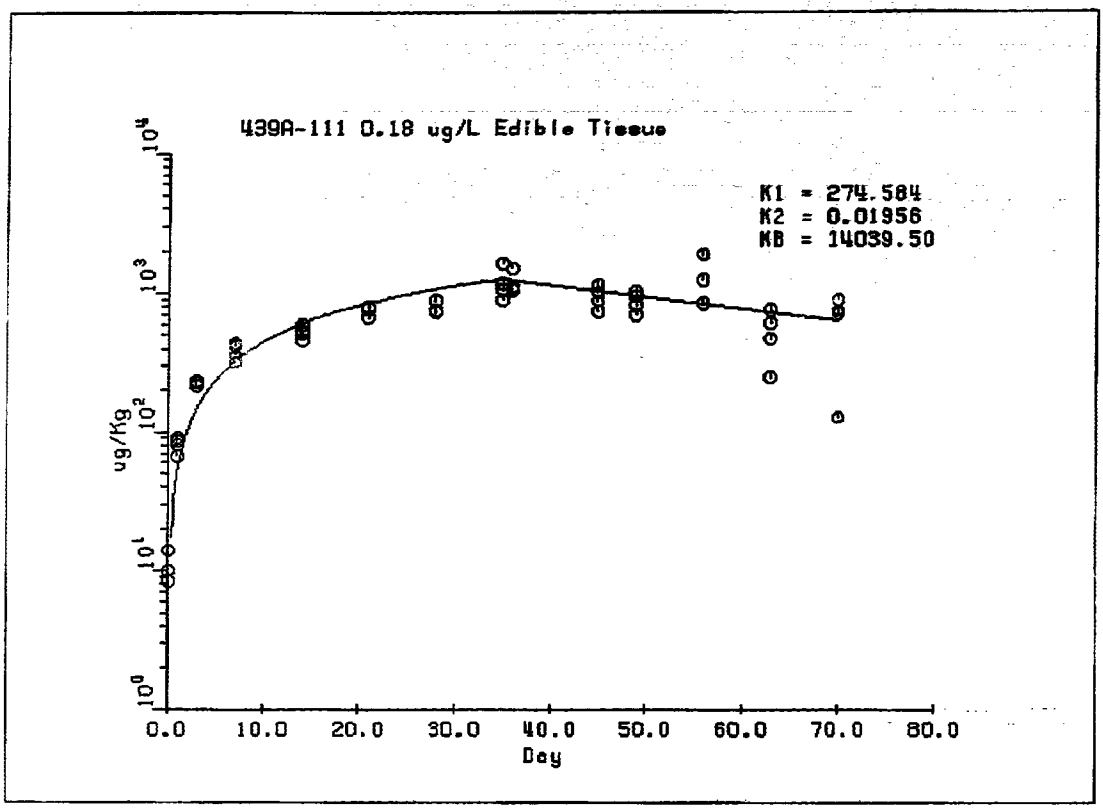


Figure 2. Concentrations of HBCD in Nonedible Fish Tissues of Rainbow Trout Exposed to 0.18 µg HBCD/L.

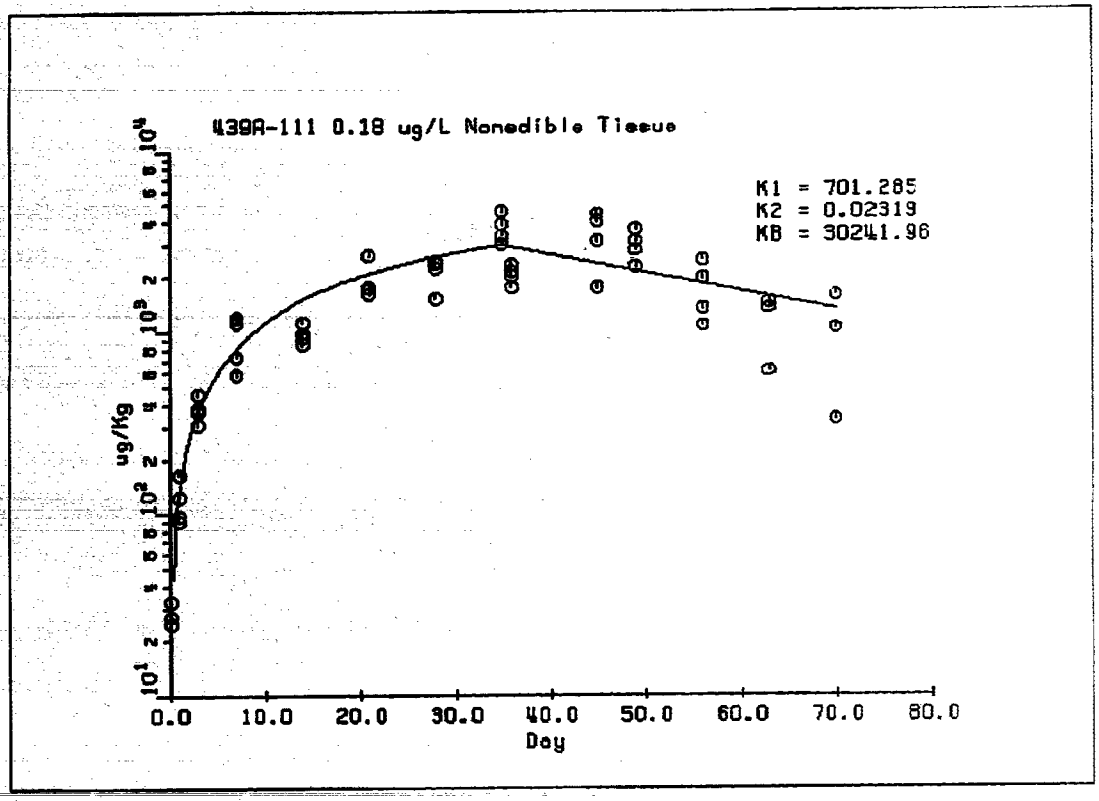


Figure 3. Concentrations of HBCD in Whole Fish Tissues of Rainbow Trout Exposed to 0.18 µg HBCD/L.

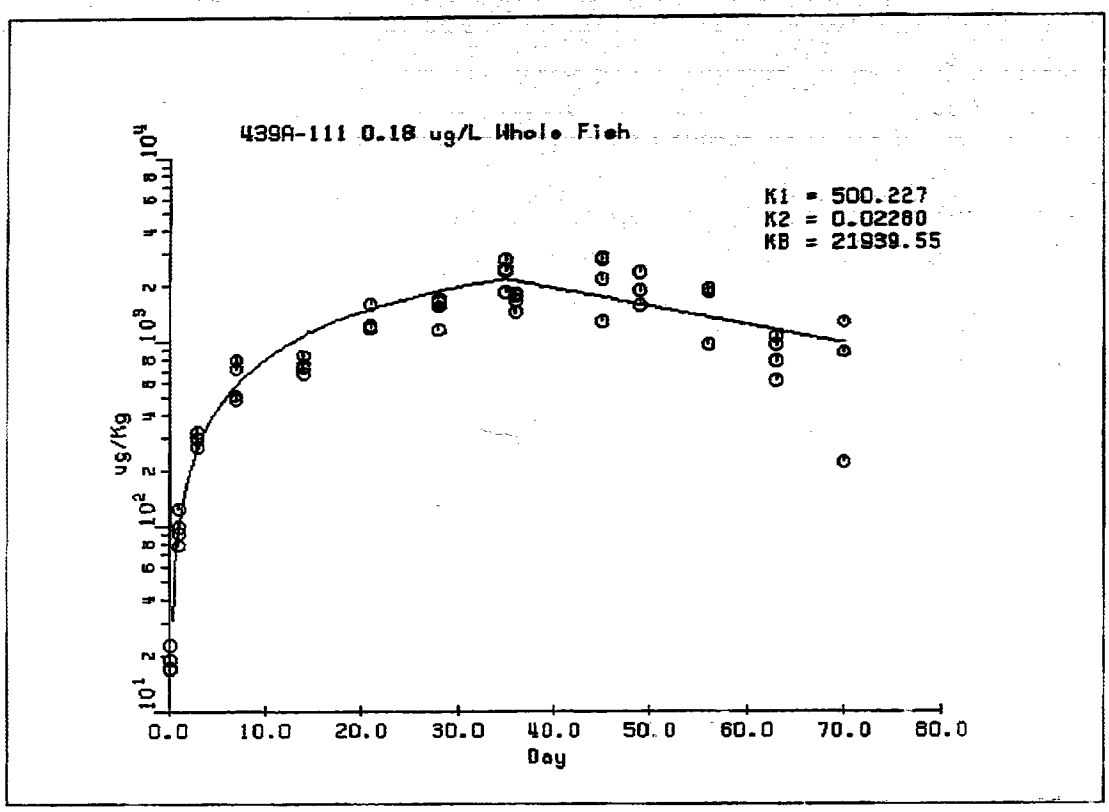


Figure 4. Concentrations of HBCD in Edible Fish Tissues of Rainbow Trout Exposed to 1.8 µg HBCD/L.

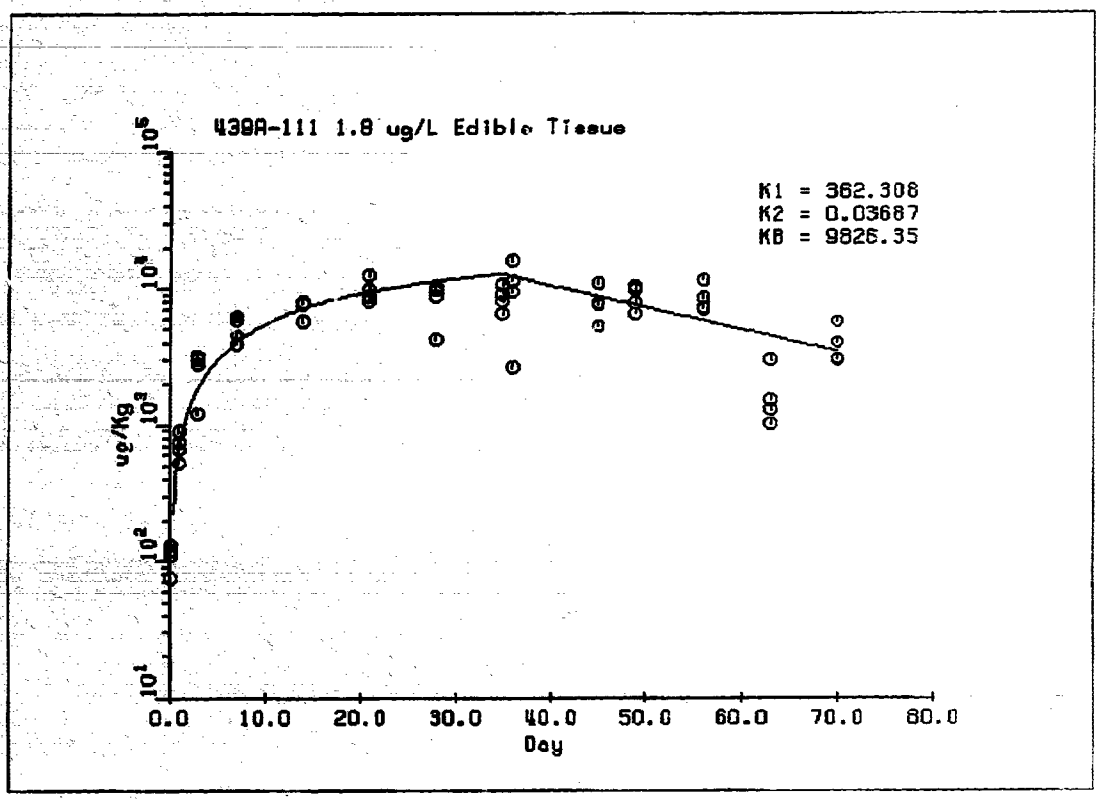


Figure 5. Concentrations of HBCD in Nonedible Fish Tissues of Rainbow Trout Exposed to 1.8 µg HBCD/L.

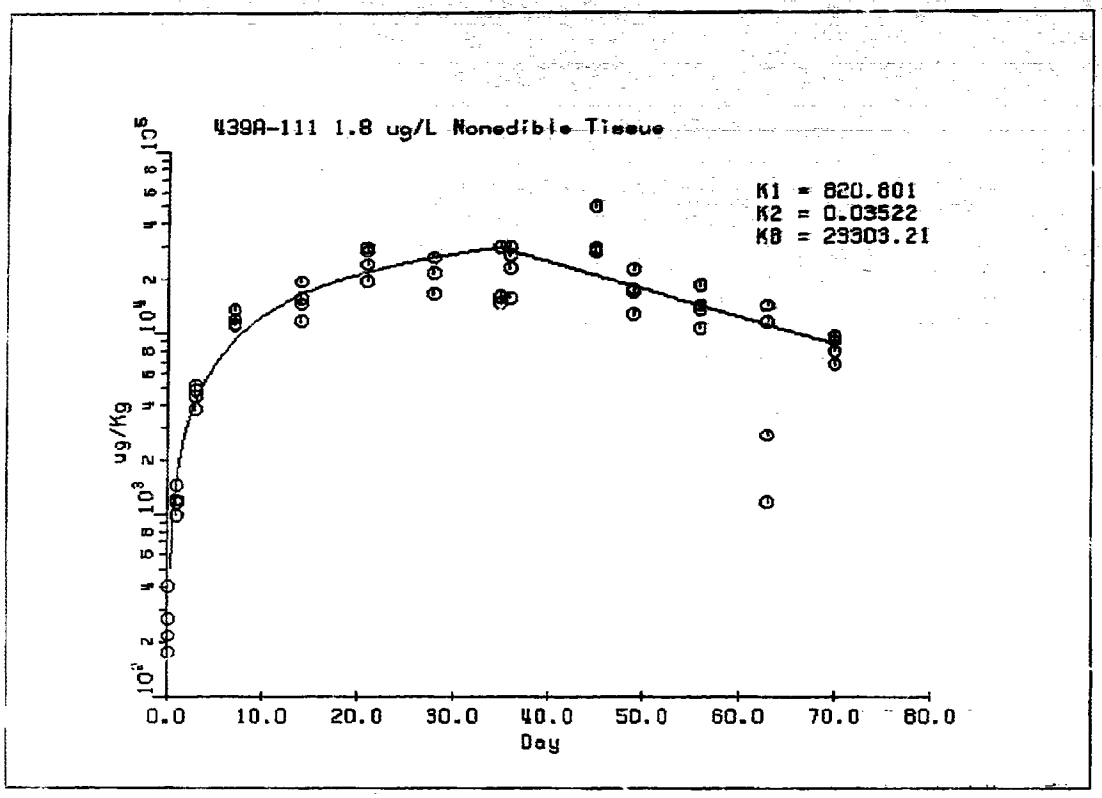
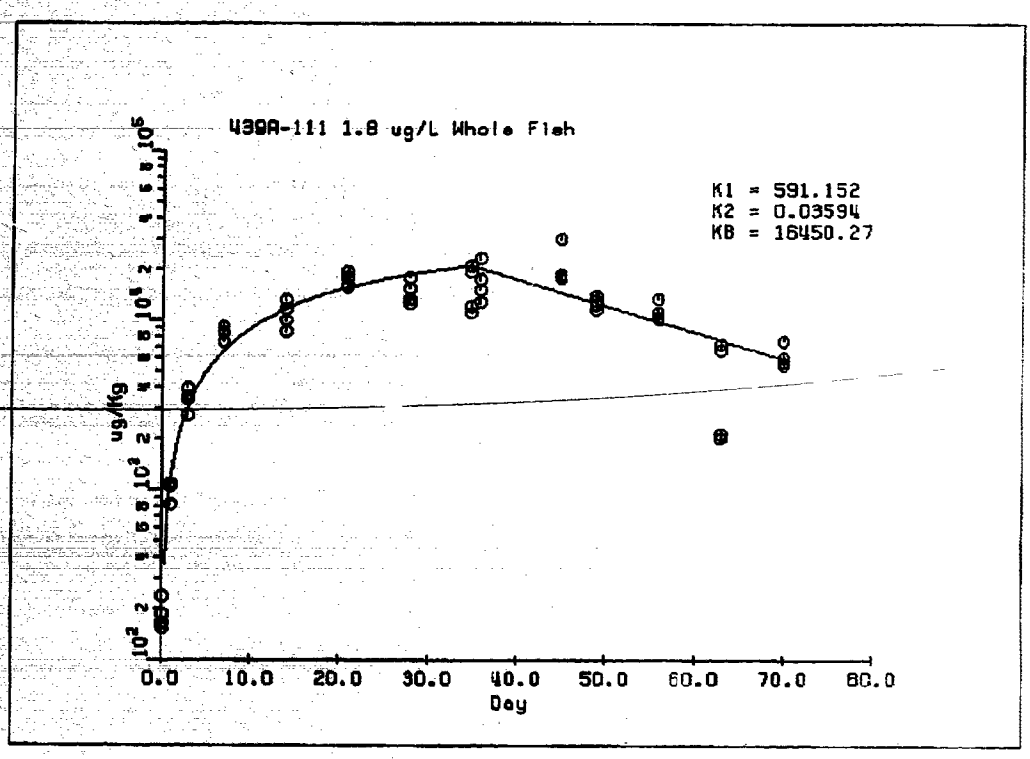


Figure 6. Concentrations of HBCD in Whole Fish Tissues of Rainbow Trout Exposed to 1.8 µg HBCD/L.



APPENDIX I

Protocol, Protocol Amendment and Protocol Deviation

PROTOCOL

HEXABROMOCYCLODODECANE (HBCD): A FLOW-THROUGH BIOCONCENTRATION TEST WITH THE RAINBOW TROUT (*Oncorhynchus mykiss*)

U.S. Environmental Protection Agency
Series 850 - Ecological Effects Test Guidelines
OPPTS Number 850.1730
and
OECD Guideline 305

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

October 6, 1999

HEXABROMOCYCLODODECANE (HBCD): A FLOW-THROUGH BIOCONCENTRATION TEST WITH THE RAINBOW TROUT (*Oncorhynchus mykiss*)

SPONSOR:	Chemical Manufacturers Association's Brominated Flame Retardant Industry Panel 1300 Wilson Boulevard Arlington, Virginia 22209
SPONSOR'S REPRESENTATIVE:	Ms. Wendy Sherman
TESTING FACILITY:	Wildlife International, Ltd. 8598 Commerce Drive Easton, Maryland 21601
STUDY DIRECTOR:	Kurt Drottar, Senior Aquatic Biologist
LABORATORY MANAGEMENT:	Henry O. Krueger, Ph.D. Director of Aquatic Toxicology & Non-Target Plants

FOR LABORATORY USE ONLY

Proposed Dates:

Experimental Start Date: _____ Experimental Termination Date: _____

Project No.: 439A-111

Test Concentrations: 0.34 and 3.4 µg a.i./L

Test Substance No.: 3577 Reference Substance No. (if applicable): _____

PROTOCOL APPROVAL

STUDY DIRECTOR	DATE 10/19/99
LABORATORY MANAGEMENT	DATE 10/19/99
SPONSOR'S REPRESENTATIVE	DATE 10/13/99

PROTOCOL NO. 439/100699/RBT-BIO/SUB439

INTRODUCTION

Wildlife International, Ltd. will conduct a bioconcentration test with the rainbow trout (*Oncorhynchus mykiss*) for the Sponsor at the Wildlife International, Ltd. aquatic toxicology facility in Easton, Maryland. The study will be performed based on procedures in U.S. Environmental Protection Agency Series 850 - Ecological Effects Test Guidelines OPPTS Number 850.1730 (1); *ASTM Standard E1022-84 Standard Practice for Conducting Bioconcentration Tests with Fishes and Saltwater Bivalve Molluscs* (2); and OECD Guideline for Testing of Chemicals 305, *Bioaccumulation: Flow-Through Fish 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 obtain laboratory data characterizing the bioconcentration potential of the test substance in the rainbow trout, *Oncorhynchus mykiss*. The solubility of HBCD in water at 25°C is 3.4 µg/L (ppb). The acute 96-hour LC50 for rainbow trout is >6.8 µg/L.

EXPERIMENTAL DESIGN

Rainbow trout will be exposed to two test concentrations (x and 10x) and a solvent (dimethylformamide, DMF) control. Each group will consist of one test chamber. The test will be divided into two phases: uptake and depuration. During the uptake phase, fish in the treatment groups will be exposed to sublethal concentrations of HBCD, while fish in the control group will be exposed to dilution water containing the same amount of solvent as that used for the treatment groups. The duration of the uptake phase (2 hours to 28 days) and the depuration phase (6 hours to 60 days) may vary according to the time required to reach steady-state. The time to steady-state will be estimated prior to test initiation. During both phases of the test, test organisms and water samples are collected and analyzed for HBCD content. These values will be used to determine the steady-state concentration, the uptake rate constant (k_1), the depuration rate constant (k_2), the kinetic bioconcentration factor (BCFK) and the steady state bioconcentration factor (BCF) for whole body tissues, edible tissue (skin on) and nonedible tissue.

MATERIALS AND METHODS

Test Substance

The test substance consists of a composite of hexabromocyclododecane (HBCD) samples received from three manufacturers. The materials' identity and date received from each of the manufacturers is give n below:

Manufacturer	Lot/Batch	Date Received	Wildlife International Ltd. ID No.
Great Lakes Chemical Corp.	635297G-1	October 26, 1995	3462
Albemarle Corp.	33449-15X	December 20, 1995	3519
Bromine Compounds Ltd.	950303	February 5, 1996	3551

An equal part (300 g) of each of the manufacturer's HBCD material was placed in a 2-L, high density polyethylene (HDPE) bottle. The bottle was placed on a reciprocating shaker for two hours. The composite test substance was assigned Wildlife International, Ltd. identification number 3577 and is being stored under ambient conditions. Initial analyses of subsamples of the composite were performed on March 20, 1996. The results of the analyses indicated the composite test substance was homogeneous and contained the following components:

HBCD - beta isomer	8.5%
HBCD - alpha isomer	6.0%
HBCD - gamma isomer	79.1%
Total HBCD	93.6%

Another subsample of the composite test substance will be shipped to Albemarle Corp. for characterization and homogeneity analyses to demonstrate stability and confirm its activity for the purpose of the study.

Preparation of Test Concentrations

A primary stock solution of HBCD will be prepared by transferring the HBCD to a volumetric flask and diluting to an appropriate volume with DMF. The solvent concentration in the solvent control and the HBCD treatment groups will not exceed 0.1 mL/L.

Test Organism

The test organism used in this study will be the rainbow trout, *Oncorhynchus mykiss*. Rainbow trout represent an important group of aquatic vertebrates, and have been selected for use in the test based upon past use history in the laboratory. Fish will be obtained from a commercial supplier or hatchery, and the identity of the species will be verified by the supplier or by Wildlife International, Ltd. personnel using appropriate taxonomic keys, such as Eddy (3). Fish will be gradually acclimated to Wildlife International, Ltd. well water, and held for a period of at least 14 days prior to the test. If the fish are cultured at water temperatures that differ from the test temperature, they will be brought to test temperature at a rate not exceeding 3°C per 72 hours during holding. If mortality exceeds 3% during the 48-hour period immediately preceding the test, the entire batch of fish will be rejected or held for an additional 14-day period to ensure that they are healthy.

Fish will be handled as little as possible, but when handling is necessary, it will be done carefully, gently, and quickly. To control bias, fish will be removed from holding tanks with nets and impartially distributed to the test chambers. No other forms of bias are expected to affect the results of the study.

The minimum acceptable size of individual test organisms will be determined by the quantity of tissue required for measurement of residues. Loading (the total wet weight of fish per liter of test solution) will not exceed 1.0 gram of fish per liter of solution that passes through a test chamber in 24 hours.

During the holding period the test fish will be fed at least once daily. The diet will consist of live or frozen brine shrimp nauplii (*Artemia sp.*), and/or commercial food during holding and testing. Specifications for acceptable levels of contaminants in fish 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.

Dilution Water

Water used for the holding and testing of rainbow trout will be obtained from a well approximately 40 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 0.45 μm in order to remove fine particles. Water used for holding and testing is characterized as moderately hard. Typical values for hardness, alkalinity, pH and specific conductance are approximately:

Hardness, mg/L as $CaCO_3$	145
Alkalinity, mg/L as $CaCO_3$	190
pH	8.1
Specific Conductance, μmhos/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 well water and results of the most recent GLP analyses will be summarized in the final report.

Diluter System and Test Conditions

A continuous-flow diluter will be used to provide two concentrations of the test substance (0.34 and 3.4 μg/L) and a control. The flow of dilution water to control and treatment test chambers will be regulated by rotameters. A syringe or peristaltic pump will be used to inject the test substance working stock solution into a mixing chamber where dilution to the test concentration occurs. Solvent without the test substance will be introduced to the control test chamber in an identical manner.

Flow rates will be adjusted so that each test chamber receives at least six volume additions of water every 24 hours. Delivery of the test substance working stock solution will be initiated at least 48 hours prior to the test. Duplicate water samples from each test chamber will be collected at least twice to verify the establishment of equilibrium concentrations of HBCD in the test chambers before introduction of the test organisms. The general operation of the diluter will be checked visually at least two times per day during the test.

Test chambers will be 52-L Teflon®-lined stainless steel test chambers containing approximately 45 L of test solution. Test chambers will be placed in a water bath to maintain a test temperature of 12±1°C. The test chambers and water bath will be enclosed in a plexiglass hood in

order to minimize potential cross-contamination. Ambient light during testing will be provided by fluorescent tubes that emit wavelengths similar to natural sunlight (e.g. Colortone® 50 or equivalent). A photoperiod of 16 hours of light and 8 hours of darkness will be controlled with an automatic timer. A 30 minute transition period of low light intensity will be provided when lights go on or off to avoid sudden changes in light intensity. Test chambers will be identified by the project number, replicate and test concentration.

PROCEDURES

The test will be divided into two phases: uptake and depuration. Organisms in the solvent control group will be exposed to well water in the uptake and depuration phases. Organisms in the treatment groups will be exposed to the test substance in the uptake phase and well water in the depuration phase.

Selection of Test Concentrations

The test concentrations selected will not stress, irritate, or otherwise adversely affect the organisms. The test concentrations were selected in conjunction with the Sponsor based on acute or chronic toxicity data. The two exposure concentrations of 0.34 and 3.4 µg/L differ by a factor of 10.

Chemical/Physical Measurements

Temperature will be monitored and recorded continuously during the entire test in the solvent control test chamber using a Fulscope ER/C Recorder (1900 J Series Model A), or equivalent. Temperature also will be measured in every test chamber at the beginning and end of the test and at weekly intervals during the test using a liquid-in-glass thermometer.

Dissolved oxygen will be measured daily in every test chamber during the test using a Yellow Spring Instruments Model 51B dissolved oxygen meter, or equivalent. In the event that dissolved oxygen levels fall below 60% saturation, appropriate actions will be taken after consultation with the Sponsor.

Measurements of pH will be made in every test chamber at the beginning and end of the test and at weekly intervals during the test using a Fisher Accumet Model 915 pH meter, or equivalent. Hardness, alkalinity, conductivity and total organic carbon will be measured in the control treatment

at the beginning and end of the test and at weekly intervals during the test. Hardness and alkalinity measurements will be made by titration using procedures based on methods in *Standard Methods for the Examination of Water and Wastewater* (3). Conductivity will be measured using a Yellow Springs Instrument Model 33 Salinity-Conductivity-Temperature meter, or equivalent. Total organic carbon will be measured using a Shimadzu model TOC-5000 total organic carbon analyzer, or equivalent.

Biological Measurements

Observations of behavior and mortality will be made daily. For a test to be considered valid, mortality and abnormal behavior should not be apparent in more than 10% of the fish in the treatment or control groups.

Duration of Uptake Phase

The statistically optimum duration of the uptake phase (u) is near $u = 1.6/k_2$, but not more than $3.0/k_2$, which is equivalent to 95 percent of steady state. A pre-test estimate of k_2 will be obtained from a test with a similar material, water solubility data, or the n-octanol/water partition coefficient. The uptake phase will continue until the concentration of HBCD residues in body tissues reaches steady-state, but not for more than 30 days. The criterion for attainment of steady-state is that three consecutive HBCD residue concentrations in fish are not significantly different from each other.

Duration of Depuration Phase

Two times u is usually a sufficient time for about 95 percent removal of the body burden ($t_{1/2} = 0.69/k_2$), however, the depuration phase will continue until the concentration of HBCD residue in the organisms attains any one of the following three conditions:

1. Residues reach less than 10% of steady-state; or
2. Residues fall below the LOQ; or
3. Sixty (60) days of depuration have elapsed.

Sample Intervals for Water and Fish Collection

Fish and water will be sampled no less than five times during the uptake phase and four times during the depuration phase of the study at the same intervals. In addition, control and treated water

will be sampled no less than twice prior to addition of fish to the chambers to confirm the concentrations desired. Control fish for the preparation of analytical quality control samples will be drawn from the same batch of fish used for the study, if possible. Additional control samples of fish and water may be collected and prepared as stability samples at least one week prior to test initiation. In the absence of information on the bioconcentration potential of the test substance and/or reliable physico-chemical data from which bioconcentration potential may be estimated, the recommended sampling schedule is as follows.

Phase	Sample Interval	Water[1]	Fish[2]	Action
Pre-Uptake[3]	$-n_1$	X	-	Start
	$-n_2$	X	-	
	0	X	-	Add fish
Uptake	~4 hours	X	X	
	1 day	X	X	
	2 day	X	X	
	7	X	X	
	14	X	X	
	21	X	X	
	28	X	X	Transfer
Depuration	1	X	X	
	3	X	X	
	7	X	X	
	10	X	X	
	14[4]	X	X	

[1] At least five replicates of water samples will be collected at each interval listed for the analysis of HBCD. Additional samples will be drawn as necessary for the determination of pH, hardness, alkalinity, specific conductance, and total organic carbon (TOC).

[2] Sufficient fish will be collected to allow the preparation of four replicate samples from each treatment group and duplicate samples from the control group for the analysis of HBCD. Additional samples will be drawn as necessary for the analysis of dry weight and lipid content.

[3] Pre-Uptake water sampling ($-n_1$, $-n_2$) for confirmation of the treatment levels prior to addition of fish.

[4] Depuration phase sampling will continue until the concentration of HBCD residue in the organisms attains any one of the conditions outlined in the duration of depuration phase on page 8.

The number and frequency of sample intervals may be changed based on physico-chemical properties of the test substance from which predictions of bioconcentration rate can be derived and/or at the direction of the Sponsor.

Water Sampling

At least five replicates of water samples will be collected at each interval listed for the analysis of HBCD. The HBCD concentration will be determined for a minimum of one replicate of a control and two replicates at each treatment level for each interval in which samples are analyzed (as specified by the Study Director). The remaining replicates of samples drawn for HBCD analysis will be held in reserve and may be analyzed at the direction of the Study Director or Chemistry Principal Investigator. The concentration of HBCD in the water at each sample interval will be presented as the average of the replicate measurements, as applicable.

Additional samples will be drawn as necessary for the determination of pH, hardness, alkalinity, specific conductance, and total organic carbon (TOC).

Fish Sampling

To assess the HBCD content in fish tissues, sufficient fish will be collected to allow the preparation of four replicate samples from each treatment group and duplicate samples from the control group. The HBCD concentration will be determined for a minimum of one replicate of a control and four replicates at each treatment level for each interval in which samples are analyzed (as specified by the Study Director). The remaining replicates of samples drawn for HBCD analysis will be held in reserve and may be analyzed at the direction of the Study Director or Chemistry Principal Investigator. The concentration of HBCD in fish at each sample interval will be presented as the average of the replicate measurements.

Fish sampled for determination of HBCD content will be impartially removed from the test chambers, rinsed with dilution water, blotted dry, and sacrificed. The weight (wet weight, blotted dry) and total length of each fish will be determined within approximately 15 minutes of collection, if possible. Each fish will be separated into edible and nonedible tissue. Fish will be stored frozen if not analyzed immediately.

Additional samples will be drawn as necessary for the analysis of dry weight and lipid content..

Analytical Method

Water and tissue samples will be analyzed for HBCD using liquid chromatography-mass spectrometry. The analytical methodology used will be described in the final report.

Tissue Lipid Content

At a minimum, fish will be sampled from the control aquarium at Day 0 (upon transfer of fish to aquaria), at an interval where the HBCD concentration in fish has reached steady state (or following 28 days of exposure), and at the end of the depuration period. Fish will be sampled to generate four samples from the control and two treated aquaria at each of the intervals noted. The lipid content of the tissue homogenates will be determined by chloroform/methanol extraction. The tissue percent solids will also be determined to allow quantitation on a dry-weight basis.

Data Analysis

Results of tissue analyses will be presented on a wet weight basis. The steady-state bioconcentration factor for the test substance (BCF), kinetic bioconcentration factor (BCFK) uptake rate (k_1), and depuration rate (k_2) will be expressed for edible tissue, nonedible tissue and whole fish.

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 test.
4. Test organism holding and acclimation records.
5. Methods used to prepare stock solutions and dilutions of the test substance.
6. Daily observations.
7. Water chemistry calculations (e.g., hardness and alkalinity).
8. Organism weight and length measurements, if applicable.
9. The methods used to analyze test substance concentrations and the results of analytical measurements.
10. Statistical calculations.

11. A copy of the final report.

FINAL REPORT

A 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:

1. Name and address of the facility performing the study.
2. Dates on 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.
4. Objectives and procedures stated in the approved protocol, including any changes in the original protocol.
5. Statistical methods employed for analyzing the data.
6. The test, control and reference substances identified by name, chemical abstracts number or code number, strength, purity, and composition or other appropriate characteristics, if provided by the Sponsor.
7. Stability and, when relevant to the conduct of the study, the solubility of the test, control and reference substances under the conditions of administration, if provided by the Sponsor or contracted to Wildlife International Ltd.
8. A description of the methods used.
9. A description of the test system used. Where applicable, the final report shall include the number of animals used, body weight range, source of supply, species, age, and procedure used for identification.
10. A description of the dosage, dosage regimen, route of administration, and duration.
11. A description of all circumstances that may have affected the quality or integrity of the data.
12. The name of the Study Director, the names of other scientists or professionals, and the names of all supervisory personnel, involved in the study.
13. A description of the transformations, calculations, or operations performed on the data, a summary and analysis of the data, and a statement of the conclusions drawn from the analysis.
14. The signed and dated reports of each of the individual scientists or other professionals involved in the study, if applicable.

15. The location where all specimens, raw data, and the final report are to be stored.
16. A statement prepared by the Quality Assurance Unit listing the dates that study inspections and audits were made and the dates of any findings reported to the Study Director and Management.
17. If it is necessary to make corrections or additions to a final report after it has been accepted, such changes shall be in the form of amendment by the Study Director. The amendment should clearly identify the part of the final report that is being added to or corrected and the reasons for the correction or addition. Amendments shall be signed and dated by the Study Director.

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 signed by the Study Director and 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 160); OECD Principles of Good Laboratory Practice (ENV/MC/CHEM (98) 17); and Japan MAFF (59 NohSan, Notification No. 3850, Agricultural Production Bureau). 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.

REFERENCES

1 U.S. Environmental Protection Agency. 1996. Series 850- Ecological Effects Test Guidelines (*draft*), OPPTS Number 850.1730: *Flow-Through Fish Test.*

2 ASTM Standard E1022-81. 1988. *Standard Practice for Conducting Bioconcentration Tests with Fishes and Saltwater Bivalve Molluscs.* American Society for Testing and Materials.

3 OECD Guideline for Testing of Chemicals 305. 1996. *Bioconcentration: Flow-Through Fish Test.*

4 APHA, AWWA, WPCF. 1995. *Standard Methods for the Examination of Water and Wastewater.* 17th Edition, American Public Health Association. American Water Works Association. Water Pollution Control Federation, New York.

APPENDIX I

IDENTIFICATION OF TEST SUBSTANCE BY SPONSOR

To be Completed by Sponsor

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

Test Substance Sample Code or Batch Number: Wildlife International Ltd. Identification No. 3577

Test Substance Purity (% Active Ingredient): 93.67 - HBCD 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? X Yes ____ No

III. Test Substance Storage Conditions

Please indicate the recommended storage conditions at Wildlife International, Ltd.

Ambient

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.

x 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: _____

AMENDMENT TO STUDY PROTOCOL

STUDY TITLE: HEXABROMOCYCLODODECANE (HBCD): A FLOW-THROUGH BIOCONCENTRATION TEST WITH THE RAINBOW TROUT (*Oncorhynchus mykiss*)

PROTOCOL NO.: 439/100699/RBT-BIO/SUB439 **AMENDMENT NO.:** 1

SPONSOR: Chemical Manufacturers Association's Brominated Flame Retardant Industry Panel **PROJECT NO.:** 439A-111

EFFECTIVE DATE: November 10, 1999

AMENDMENT: Page 2

Add: Experimental Start Date: 11/17/99
Experimental Termination Date: 12/29/99

REASON: The above information was not known when the protocol was signed by the Study Director.

AMENDMENT: Page 2

Change: Test Substance No.: 3577

To: Test Substance No.: 4615

REASON: The Sponsor requested that Wildlife use the most recent composite sample.

AMENDMENT: Page 2

Change: Test Concentrations: 0.34 and 3.4 µg a.i./L.

To: Test Concentrations: 0.34 and 3.4 µg HBCD/L

REASON: The test will be performed on an AS IS basis.

AMENDMENT: Test Substance, Page 4

Delete: Entire Section

REASON: The section pertains to test substance No. 3577 which will not be used.

KRD:\439A111\pro1

Reviewed by QA
QAC 11-10-99

AMENDMENT: Test Organism, Page 5

Change: During the holding period the test fish will be fed at least once daily.

To: During the holding and testing periods, the fish will be fed at least once daily. Uneaten food and feces will be siphoned from the test chambers shortly after feeding (30 minutes to 1 hour).

REASON: To clarify feeding during the test and to allow for siphoning.

AMENDMENT: Diluter System and Test Conditions, Page 6

Change: Test chambers will be 52-L Teflon®-lined stainless steel test chambers containing approximately 45 L of test solution.

To: Test chambers will be 106-L stainless steel test chambers containing approximately 80 L of test solution.

REASON: To correct the size of the test chambers and the volume of test solution.

AMENDMENT: Water Sampling, Page 10

Change: At least five replicates of water samples will be collected at each interval listed for the analysis of HBCD.

To: At each sampling interval, two water samples will be collected from the control and three water samples will be collected from each of the two HBCD treatment groups.

REASON: To clarify the number of water samples which will be collected.

AMENDMENT: Fish Sampling, Page 10

Change: Each fish will be separated into edible and nonedible tissue.

To: Each fish will be separated into edible and nonedible tissue and the wet weight of each tissue fraction (i.e., edible and nonedible) will be recorded.

REASON: Edible and nonedible tissue weights are needed calculate whole body concentrations.

AMENDMENT: Fish Sampling, Page 11

Delete: Additional samples will be drawn as necessary for the analysis of dry weight and lipid content.

REASON: Dry weight determinations will not be made.

STUDY DIRECTOR	DATE 11/10/99
LABORATORY MANAGEMENT	DATE 11/10/99
SPONSOR'S REPRESENTATIVE	DATE 11/16/99

KRD:\439A111\pro1

DEVIATION TO STUDY PROTOCOL

STUDY TITLE: HEXABROMOCYCLODODECANE (HBCD): A FLOW-THROUGH BIOCONCENTRATION TEST WITH THE RAINBOW TROUT (*Oncorhynchus mykiss*)

PROTOCOL NO.: 439/100699/RBT-BIO/SUB439 **DEVIATION NO.:** 1

SPONSOR: Chemical Manufacturers Association's Brominated Flame Retardant Industry Panel **PROJECT NO.:** 439A-111

DATE OF DEFACTO DEVIATION: February 25, 2000

DEVIATION: Fish dry weights were determined. Dry weight measurements were amended out of the protocol in Amendment No. 1.

REASON: The chemist did not realize that dry weights had been amended out of the protocol. It is the best judgement of the Study Director that this deviation did not adversely affect the results of the study.

STUDY DIRECTOR — DATE 4/5/00

LABORATORY MANAGEMENT — DATE 4/5/00

KRD:\439A111\prd1

APPENDIX II

Test Substance Characterization

Brominated Flame Retardant End-User Survey – Phase 1

Final Report

Submitted To:

Wendy K. Sherman
Chemical Manufacturers Association
Brominated Flame Retardant Industry Panel
1300 Wilson Blvd.
Arlington, VA 22209

Submitted By:

Patrick N. Breysse, Ph.D., C.I.H.
Julie Kacergis, M.H.S.
Johns Hopkins University
School of Hygiene and Public Health
Department of Environmental Health Sciences
615 N. Wolfe Street, Room W6010
Baltimore, MD 21205

May 19, 2000

Abstract

The first phase of a comprehensive assessment of occupational exposures to selected brominated flame retardants (BFRs) has been completed. The BFRs included in this assessment are decabromodiphenyl oxide (DBDPO), octabromodiphenyl oxide (OBDPO), pentabromo-diphenyl oxide (PeBDPO), hexabromocyclododecane (HBCD), and tetrabromobisphenol A (TBBPA). Phase I consisted of an evaluation of the extent to which existing data can be used to accurately characterize end-user exposures. An exposure questionnaire was designed and mailed to 276 customers of BFRIP member companies. A short one-page questionnaire was developed to be used, with follow-up phone calls of customers who did not respond to the mailed questionnaire. Eighteen customers that reported having exposure data were contacted and asked to share the data. Of these companies exposure data were received from nine.

Most companies do not routinely collect exposure data directly relevant to this study. Total dust samples are the most common type of exposure data collected. The mean and geometric mean dust concentration based on 52 personal samples provided by seven companies are 1.67 and 0.63 mg/m^3, respectively. Interpretation of these results is limited, however, due to the lack of information on sample duration and purpose of sampling. Based on a limited amount of data provided primarily by a single company, antimony exposures may exceed the exposure standards and guidelines with mean and geometric means equal to 0.86 and 0.07 mg/m^3 based on 41 samples, respectively. Three companies reported hydrogen bromide concentration; all were non-detectable.

No direct airborne BFR exposures were reported. One company provided information termed "% brominated product." These data were subsequently used to estimate airborne BFR levels in the absence of definitive data on the compounds of interest. The estimates indicate most (85%) airborne exposures are less than 1 mg/m^3 and range from less than 0.001 mg/m^3 to greater than 50 mg/m^3.

The results of this survey indicate that the exposure data provided by end-users are of limited utility for risk-assessment purposes and suggest that a more detailed end-user exposure assessment is needed.

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I. Introduction

The purpose of this project is to evaluate occupational exposures to selected brominated flame retardants (BFRs) for the various industries that incorporate these substances into a polymer matrix (end-users). The BFRs included in this study are decabromodiphenyl oxide (DBDPO), octabromodiphenyl oxide (OBDPO), pentabromodiphenyl oxide (PeBDPO), hexabromocyclo-dodecane (HBCD), and tetrabromobisphenol A (TBBPA). This research was supported by the Brominated Flame Retardant Industry Panel (BFRIP) of the Chemical Manufacturers Association.

Exposures to the five selected BFRs are being evaluated in a phased approach. Phase I consisted of an evaluation of the extent to which existing data can be used to accurately characterize end-user exposures. Subsequent phases may encompass a variety of activities including developing sampling and analytical methods, conducting a detailed exposure assessment, evaluating exposure surrogates, and developing biomarkers of BFR exposure. This report summarizes the results of the Phase I survey of existing end-user BFR exposure data. This survey was conducted from October 1999 to April 2000.

II. Background

Flame retardant chemicals are used in a wide range of commercial product applications including construction materials such as paints and adhesives, as well as plastics, textiles, fabrics, and other home furnishings.[1] In particular, BFRs are suitable for use in high performance thermoplastic resins used in electrical and high temperature applications.[2] There is limited BFR exposure information in the published literature, particularly for end-users.[3] One recent study reported quantitative exposure estimates of polybrominated diphenyl ethers in an electronics dismantling plant ranging from 0.08 to 200 ng/m^3.[4]

Currently, the only occupational exposure standards or guidelines for BFRs other than DBDPO is the total nuisance dust Permissible Exposure Limit (PEL) set by the U.S. Occupational Safety and Health Administration (OSHA) at 15 mg/m^3 and the Threshold Limit Value (TLV) recommended by the American Conference of Governmental Industrial Hygienists (ACGIH) at 10 mg/m^3. The American Industrial Hygiene Association (AIHA) Worker Environmental Exposure Level (WEEL) for DBDPO is 5 mg/m^3.[5] A few exposure standards might indirectly affect people using BFRs such as the hydrogen bromide (HBr) and antimony standards. Hydrogen bromide gas is a byproduct of the combustion of BFRs, and some companies that use heat to process BFRs will test for HBr to ensure that their systems are not causing HBr off-gassing. The OSHA PEL for hydrogen bromide is 10 mg/m^3, and the ACGIH Ceiling is 9.9 mg/m^3. When added to polymers along with BFRs, antimony trioxide induces synergistic effects on flame retardancy. Companies using antimony trioxide may have dust samples analyzed for antimony content. The OSHA PEL and ACGIH TLV for antimony are both equal to 0.5 mg/m^3.

III. Methods

Phase 1 included the following steps:

- A preliminary list of end-user companies, their addresses, and points of contact was obtained from BFR manufacturing companies;
- A detailed exposure questionnaire (referred to as the long questionnaire) was developed and mailed to end-user companies in order to catalogue the extent to which exposure data are available (Appendix A);

- Companies that did not respond to the long questionnaire were called and administered a short questionnaire over the telephone (Appendix B);
- Companies that reported having exposure data were asked to share the data in order to develop an exposure information profile for the industry; and
- The exposure data were statistically summarized.

The long questionnaire that was mailed to all potential BFR end-users had five parts: 1) company identification; 2) company description, including the types of BFRs used and the manner in which they are handled; 3) workforce demographics; 4) personal protective equipment used while working with BFRs; and 5) exposure information including the amount and types of dust samples taken and whether they were analyzed for BFR content or for BFR additives (e.g., antimony trioxide). This questionnaire was mailed with a cover letter from BFRIP explaining the purpose of the study and encouraging companies to participate in the survey. To facilitate return, a return-address envelope was included in each mailing. Standard follow-up procedures, e.g., phone call reminders, emails, and faxes, were used to achieve as high a response rate as possible in the time allotted.

Due to time constraints and concern about the amount of effort needed to complete the long questionnaire, a short questionnaire that could be administered over the telephone during follow-up calls was developed. The short questionnaire asked what types of BFRs were used and how they were used; what products were being made with the BFRs, whether exposure samples were taken and, if so, how many; and whether the company would be willing to share the exposure data with us. The long questionnaire did not ask for the exposure data, but any company that reported having exposure data was contacted and asked if they would be willing to send the data. Appendix C contains a flow chart of the methods used to survey end-user companies.

Lists of companies in North America who purchase BFRs were provided by three BFR manufacturers: Great Lakes Chemical Corporation, Albemarle Corporation, and Ameribrom, Inc. The three lists were compiled, and companies with duplicate addresses were removed. Detailed questionnaires were mailed to all of the contact addresses, of which fifteen were Canadian, one was Mexican and the rest had U.S. addresses. Over the course of the survey a few companies were uncontactable due to incorrect addresses; they were removed from the database (note that correct addresses were sought through internet and phonebook searches) giving a final end-user database that included 276 companies. The final list of companies has been kept confidential and has not been shared with any BFRIP member companies.

IV. Results

Table 1 summarizes the results of survey response information. Twenty-seven and 68 companies responded to the long and short questionnaires, respectively. Of these 95 companies (34% response rate), 18 reported having exposure data and nine provided exposure data for analysis. Twenty-nine of the responding companies did not provide information either because they did not have any exposure data, or for various other reasons including confidentiality issues, not using BFRs, and not enough time or staff available to complete the questionnaires. Many company addresses in the original database did not include a phone number. Attempts were made to locate telephone numbers using phone directory and internet searches. All companies for which a phone number or email address was available were contacted at least once and, in some cases, several times. Of the remaining companies 140 were contacted at least once (many were contacted multiple times) and 41 companies could not be reached.

Nine companies supplied exposure data including total dust, antimony trioxide, hydrogen bromide, and (in one case) "brominated product." A summary of company characteristics for these nine companies is presented in Table 2. Detailed company characteristics are available only for those companies that completed the long questionnaire.

Airborne total dust samples represent the most common exposure data collected, with seven of the nine companies reporting results. Table 3 summarizes total dust samples by company number and whether they were personal or area samples. These results are presented without regard to sample time. Detailed information on each sample is contained in Appendix D, which indicates that the sample times are highly variable. Some of the results were based on full-shift samples, some were for a relatively short duration, and some results were provided with no sample time information. A number of the results provided without sample times were referred to as "TWA" samples without any other descriptor. Table 3 contains the results of 16 and 52 area and personal total dust samples, respectively. The personal total dust samples averaged 1.67 mg/m^3 and ranged from less than 0.12 to 15.4 mg/m^3. The area total dust samples averaged 1.81 mg/m^3 and ranged from less than 0.18 to 4.09 mg/m^3. The highest personal exposure estimate, 15.4 mg/m^3, was collected by Company 4 without reference to sample time. The next highest sample, 8.9 mg/m^3, was collected on an operator at a hopper feed tank and is described by Company 6 as having been collected over a short (unspecified) sampling time. Table 4 summarizes total dust sampling results by BFR type. Most of the air sampling was conducted during DBDPO handling.

Antimony sample results are summarized in Table 5, with 42 samples from four companies (one area, the rest personal) presented. Personal exposures to antimony ranged from 0.003 to 22 mg/m^3. Company 8 reported the results of 34 personal samples, averaging 0.97 mg/m^3. No descriptive information was presented with any of the samples reported by Company 8. Company 8 sample results, which dominate the results presented in Table 4 (see Appendix D), were reported in terms of antimony concentration along with the percent antimony in each sample. The percent antimony in these samples was highly variable and ranged from 1 to 37%.

Three companies submitted hydrogen bromide exposure data. These companies were using heat in one or more processes and were concerned about possible thermal degradation of BFRs. None of the 20 sample results submitted were above the detection limits (<0.002 to <0.2 ppm).

One company submitted 34 dust samples that had been analyzed for "brominated product" without reference to an analytical method. These results were expressed in terms of percent "brominated product" along with antimony concentration data (see discussion above for antimony results for Company 8). The percentages of "brominated product" in these 34 air samples ranged from 0 to 37.7%. By using the ratio of "% brominated compound" to the % antimony, the concentration of "brominated product" was estimated from the antimony concentration data (see Appendix D). Using this approach, the "brominated compound" concentrations for these 34 samples were highly variable and ranged from 0.7 µg/m^3 to 79.2 mg/m^3. It was not possible to compute descriptive statistics for these 34 samples, because no information on the limit of detection was given for samples with no (0 %) brominated compound detected. Roughly one-third of these 34 samples had non-detectable brominated compound and 29 of the 34 results (85%) suggest BFR exposures were less than 1 mg/m^3. Another important limitation of these data is the fact that no descriptive information was provided along with these results. We have no knowledge, for example, of the sample time.

V. Conclusions

The following can be concluded from this survey of BFR end-user companies:

- Most companies do not routinely collect exposure data directly relevant to BFR use.
- Total dust samples are the most common type of exposure data collected. Based on the limited total dust exposure data provided by seven companies, it can be preliminarily concluded that total dust exposures were typically well below the OSHA PEL and the ACGIH TLV, with a mean and geometric mean of 52 personal samples equal to 1.67 and 0.63 mg/m^3, respectively. Interpretation of these results is limited, however, due to the lack of information on sample duration.
- Based on a limited amount of data provided primarily by a single company, the antimony exposures during BFR use can exceed the PEL and the TLV, with mean and geometric means equal to 0.86 and 0.07 mg/m^3, respectively.
- Three companies reported hydrogen bromide exposures that were less than the limit of detection.
- No direct airborne BFR exposures were reported. One company provided information termed "% brominated product." This data was subsequently used to estimate airborne BFR levels in the absence of definitive data on the compounds of interest. The estimates indicate most (85%) airborne exposures are less than 1 mg/m^3 and range from less than 0.001 mg/m^3 to greater than 50 mg/m^3.
- These results, by themselves, are of limited utility for risk-assessment purposes and suggest that a more detailed end-user exposure assessment is needed.

VI. References

1. Ulsamer AG, Osterberg RE, and McLaughlin JR: Flame-retardant chemicals in textiles. *Clinical Toxicology*, 17(1):101-131 (1980).

2. Norris JM, Kociba RJ, Schwertz BA, Rose JQ, Humiston CG, Jewett GL, Gehring PJ, and Mailhes JB: Toxicology of octabromobiphenyl and decabromodiphenyl oxide. *Environmental Health Perspectives*, 11:153-161 (1975).

3. International Agency for Research on Cancer: *IARC Monograph - Some Flame Retardants and Textile Chemicals, and Exposures in the Textile Manufacturing Industry*, 48:73-85 (1990).

4. Sjödin A, Hagmar L, Klasson-Wehler, Kronholm-Diab K, Jakobsson E and Bergman A: Flame retardant exposure: Polybrominated diphenyl ethers in blood from Swedish workers. *Environmental Health Perspectives*, 107(8):643-647 (1999).

5. American Industrial Hygiene Association Workplace Environmental Exposure Level Guide. Decabromodiphenyl Oxide. *Am. Ind. Hyg. Assoc. J.*, 42:A-76-77 (1981).

[illegible]	
Total number of end-user companies in database	276
Number of long questionnaires mailed	276
Number of companies responding to long questionnaire	27
Number of companies responding to short questionnaire	68
Total number of companies responding to either long and short questionnaire	95
Number of contacts with exposure data (reported on long or short questionnaire)	18
Number of contacts who have sent exposure data	9
Total number companies that have not responded or been contacted	181
Number of follow - up calls for 181 contacts that have not responded	140

[illegible]						
Company Number	Location	Products	Processes	Number of Production Employees	Years in Business	Years using BFRs
1	Midwest	Compounded Engineering Products	Dry blending and extrusion	70	40	17
2	MidAtlantic	Fiberglass reinforced plastics	Pultrusion, batch mixing	300	70	30
3	Southwest	Polyester and vinyl ester resins	Reacting and blending	21	26	20
4	Southwest	Polypropylene resin compounding	Dry blending and extrusion	Not given	Not given	Not given
5	Southeast	Compounding	Dry blending and extrusion	200	10	5
6	Midwest	Plastics	Dry blending and extrusion	Not given	Not given	Not given
7	Southeast	Water-based dispersions	Wet blending	6	12	12
8	Midwest	Thermoplastic compounding	Dry blending	96	40	15
9	Northeast	Wire cable jacketing	Dry blending and extrusion	Not given	Not given	Not given

Company Number	Type of Sample[1]	N[2]	Range (mg/m³)	Mean (mg/m³)	Std. Dev.[3] (mg/m³)	GM[4] (mg/m³)	GSD[5]
1	A	5	<0.18 - <1.5	N/A**	N/A	N/A	N/A
1	P	9	<0.12 - 1.00	0.39	0.29	0.30	2.26
2	P	3	1.20 - 1.70	1.43	0.25	1.42	1.19
3	P	11	<0.13 - 1.77	0.66	0.61	0.42	2.81
4	P	14	0.18 - 15.40	3.09	4.98	1.04	4.35
5	A	1	4.09	N/A	N/A	N/A	N/A
5	P	10	<0.32 - 7.60	1.43	2.24	0.74	2.98
6	A	6	<5.0 - <5.0	N/A	N/A	N/A	N/A
6	P	1	8.90	N/A	N/A	N/A	N/A
7	A	4	0.03 - 0.46	0.30	0.19	0.21	3.63
7	P	4	0.08 - 4.80	1.30	2.33	0.31	6.46
Overall	A	16	<0.18 - 4.09	1.81	1.64	0.86	4.59
Overall	P	52	<0.12 - 15.4	1.67	3.12	0.63	3.69

[1] A= area sample, P= personal sample,
[2] N = number of samples,
[3] Std. Dev. = standard deviation,
[4] GM= geometric mean,
[5] GSD = geometric standard deviation, N/A = not applicable
Note: For samples reported as less than the limit of detection (LOD), the LOD/√2 was substituted for all statistical calculations.

Type of BFR[1]	Type of Sample[2]	N[3]	Range (mg/m³)	Mean (mg/m³)	Std. Dev.[4] (mg/m³)	GM[5] (mg/m³)	GSD[6]
DBDPO	A	9	<0.18 – 0.46	0.43	0.38	0.29	2.99
DBDPO	P	30	<0.12 - 15.4	1.88	3.63	0.63	4.03
HBCD	A	6	<5.0 - <5.0	N/A	N/A	N/A	N/A
HBCD	P	1	8.9	N/A	N/A	N/A	N/A
TBBPA	P	11	<0.13 - 1.77	0.66	0.61	0.42	2.81
Unspecified	A	1	4.09	N/A	N/A	N/A	N/A
Unspecified	P	10	<0.32 - 7.6	1.43	2.24	0.74	2.98

[1] DBDPO = decabromodiphenyl oxide, HBCD = hexabromocyclododecane, TBBPA = tetrabromobisphenol A,
[2] A= area sample, P= personal sample,
[3] N = number of samples,
[4] Std. Dev. = standard deviation,
[5] GM= geometric mean,
[6] GSD = geometric standard deviation, N/A = not applicable
Note: For samples reported as less than the limit of detection (LOD), the LOD/√2 was substituted for all statistical calculations.

Table 5: Summary of [illegible]

Company Number	Type of Sample[1]	N[2]	Range (mg/m³)	Mean (mg/m³)	Std. Dev.[3] (mg/m³)	GM[4] (mg/m³)	GSD[5]
2	P	3	0.052 - 0.100	0.07	0.03	0.07	1.4
4	P	3	0.58 - 0.99	0.73	0.22	0.71	1.33
8	P	34	0.003 - 22.0	0.97	3.82	0.06	9.59
9	A	1	<0.011	N/A	N/A	N/A	N/A
9	P	1	<0.011	N/A	N/A	N/A	N/A
Overall	A	1	<0.011	N/A	N/A	N/A	N/A
Overall	P	41	0.003 - 22.0	0.86	3.48	0.07	8.88

[1] A= area sample, P= personal sample,
[2] N = number of samples,
[3] Std. Dev. = standard deviation,
[4] GM= geometric mean,
[5] GSD = geometric standard deviation, N/A = not applicable

E II

Appendix A
Long Questionnaire

Brominated Flame Retardant

End-User Exposure Questionnaire

Johns Hopkins University
School of Hygiene and Public Health
615 N. Wolfe Street
Room W6010
Baltimore, MD 21205

For More Information Contact:

Patrick N. Breysse, PhD, CIH
Johns Hopkins University

pbreysse@jhsph.edu
410-955-3608

or

Wendy Sherman
Chemical Manufacturers Association
Brominated Flame Retardant Industrial Panel
Wendy_Sherman@cmhaq.com
703-741-5639

A. COMPANY IDENTIFICATION

Company Name: _____

Division: _____

Address: _____

City, State, Zip or Postal Code:

_____

Person Completing Questionnaire:

Name: _____

Title: _____

Address (if different from above):

_____

_____

_____

Phone: _____ Fax: _____

Please provide the name and phone number of a person at your facility who is responsible for or familiar with industrial hygiene and safety in your company. This person will be contacted if additional information is required.

Name: _____

Title: _____

Phone: _____ Fax: _____

B. COMPANY DESCRIPTION

Please describe the production process(es) and products manufactured at your plant that involve the use of brominated flame retardants (include attachment if necessary):

_____

_____

_____

Is work conducted: (check all that apply) Indoors _____ Outdoors _____ Underground _____

Individual brominated flame retardants (BFR) that might be found at your plant: (check all that apply)

_____ Decabromodiphenyl oxide (DBDPO) -or- Saytex 102E, Saytex 102, DE-83, DE-83R, FR-1210

_____ Octabromodiphenyl oxide (OBDPO) -or- DE-79

_____ Hexabromocyclododecane (HBCD) -or- Saytex HBCD LM, Saytex HBCD HM, Saytex HBCD SF, CD 75, SP 75, SP 75C, FR-1206

_____ Tetrabromobisphenol A (TBBPA or TBBA) -or- Saytex RB100, BA-59P, FR-1524

_____ Pentabromodiphenyloxide (PeBDPO) -or- DE-71, DE-60 F

Others:(please list) _____

How many years has your facility been operating _____, and how many years has it been using brominated flame retardants _____?

Please describe specifically how flame retardants are used and handled in your facility (include an attachment if needed):

_____

_____

_____

_____

_____

_____

C. WORKFORCE DEMOGRAPHICS

How many production workers are currently employed in your plant? _____

How many non-production workers are currently employed in your plant? _____

*What is the age range of your workforce (i.e. youngest to oldest)?_____

*How many of your current workers have been employed at your facility for

less than 5 years? _____

5 - 10 years? _____

10 - 15 years? _____

more than 15 years? _____

*Of the total number of workers, roughly how many worked with flame retardant operations directly in the years

1990 - present? _____

1980 - 1989? _____

1970 - 1979? _____

D. PERSONAL PROTECTIVE EQUIPMENT USED WHILE WORKING WITH

Please check all that apply:

Processes Involving BFRs	Gloves	Dust Respirator	Supplied Air Respirator	Protective Clothing
Bulk Transport				
Milling/Grinding				
Mixing				
Maintenance Activities				

Company:_____

E. EXPOSURE INFORMATION (This information applies to general dust sampling.)

Date	Sampling Method (check all methods used at your facility)	How often is sampling conducted for most of the workers who are monitored? (circle one)	How many total samples have you collected? Avg/yr	Total/period
1990 to pres.	Personal air samples _____	Daily Weekly Monthly Annually Other	_____	_____
	Total dust _____	Daily Weekly Monthly Annually Other	_____	_____
	Respirable dust _____	Daily Weekly Monthly Annually Other	_____	_____
	Inhalable dust _____	Daily Weekly Monthly Annually Other	_____	_____
	Area air samples _____	Daily Weekly Monthly Annually Other	_____	_____
	Total dust _____	Daily Weekly Monthly Annually Other	_____	_____
	Respirable dust _____	Daily Weekly Monthly Annually Other	_____	_____
	Inhalable dust _____	Daily Weekly Monthly Annually Other	_____	_____
	Other (please describe):	Daily Weekly Monthly Annually Other	_____	_____
1980 to 1989	Personal air samples _____	Daily Weekly Monthly Annually Other	_____	_____
	Total dust _____	Daily Weekly Monthly Annually Other	_____	_____
	Respirable dust _____	Daily Weekly Monthly Annually Other	_____	_____
	Inhalable dust _____	Daily Weekly Monthly Annually Other	_____	_____
	Area air samples _____	Daily Weekly Monthly Annually Other	_____	_____
	Total dust _____	Daily Weekly Monthly Annually Other	_____	_____
	Respirable dust _____	Daily Weekly Monthly Annually Other	_____	_____
	Inhalable dust _____	Daily Weekly Monthly Annually Other	_____	_____
	Other (please describe):	Daily Weekly Monthly Annually Other	_____	_____
1970 to 1979	Personal air samples _____	Daily Weekly Monthly Annually Other	_____	_____
	Total dust _____	Daily Weekly Monthly Annually Other	_____	_____
	Respirable dust _____	Daily Weekly Monthly Annually Other	_____	_____
	Inhalable dust _____	Daily Weekly Monthly Annually Other	_____	_____
	Area air samples _____	Daily Weekly Monthly Annually Other	_____	_____
	Total dust _____	Daily Weekly Monthly Annually Other	_____	_____
	Respirable dust _____	Daily Weekly Monthly Annually Other	_____	_____
	Inhalable dust _____	Daily Weekly Monthly Annually Other	_____	_____
	Other (please describe):	Daily Weekly Monthly Annually Other	_____	_____

Company:_____

Have you ever analyzed your dust samples for BFR content? Yes_____ No_____

If yes, what method was used: _____

How many samples were analyzed for BFR content:

From 1990-present? _____ Avg Annual Total? _____ Total for entire period? _____
From 1980-1989? _____ Avg Annual Total? _____ Total for entire period? _____
From 1970-1979? _____ Avg Annual Total? _____ Total for entire period? _____

Have you ever sampled and analyzed for antimony trioxide? Yes_____ No_____

If yes, when was the sampling done and how many samples were collected:

From 1990-present? Yes_____ No_____ Avg Annual Total? _____ Total for entire period? _____
From 1980-1989? Yes_____ No_____ Avg Annual Total? _____ Total for entire period? _____
From 1970-1979? Yes_____ No_____ Avg Annual Total? _____ Total for entire period? _____

Have you sampled for BFR additives other than antimony trioxide? Yes_____ No_____
If yes, please list these additives: _____

Have you collected any BFR surface contamination samples? Yes_____ No_____
If yes, please describe: _____

Have you determined size distribution of airborne dust? Yes_____ No_____
If yes, please describe your methods: _____

Please describe the format of <u>most</u> of your exposure data: (check one)

Paper records stored on site: _____ Paper records stored elsewhere: _____
Mainframe data sets: _____ PC-based data sets: _____
MAC-based data sets: _____ Other: (please describe) _____

Comments: _____

Please list any other substances that are routinely monitored in your facility (for example, asbestos, silica, acid fumes, and organic compounds) and describe the sampling and analytical methods used for these substances:

_____

_____

_____

_____

E 05

Appendix B
Short Questionnaire

Brominated Flame Retardant End-Use Exposure Questionnaire

Date: _____

Company Name: _____

Address: _____

City, State, Zip or Postal Code: _____

Name & Title of person completing questionnaire: _____

Phone: _____

Fax: _____

1. What types of BFRs are used at your plant?

_____ Decabromodiphenyl oxide (DBDPO) -or- Saytex 102E, Saytex 102, DE-83, DE-83R, FR-1210,
_____ Octabromodiphenyl oxide (OBDPO) -or- DE-79,
_____ Hexabromocyclododecane (HBCD) -or- Saytex HBCD LM, Saytex HBCD HM, Saytex HBCD SF, CD 75, SP 75, SP 75C, FR-1206,
_____ Tetrabromobisphenol A (TBBPA or TBBA) -or- Saytex RB100, BA-59P, FR-1524,
_____ Pentabromodiphenyloxide (PeBDPO) -or- DE-71, DE-60 F
_____ Other type _____

2. Are BFRs used in _____ dry or _____ liquid form in your facility?

3. Are BFRs _____ mixed, _____ milled , or _____ ground with other products?

4. What types of products are BFRs used in? _____ Wire coatings? _____Furniture?
_____ Auto interiors? _____ Insulation? _____ Other, describe _____

5. Have you ever done personal or area air sampling for _____ total, _____ respirable, or _____ inhalable BFR dust? If so, roughly how many samples have you taken:

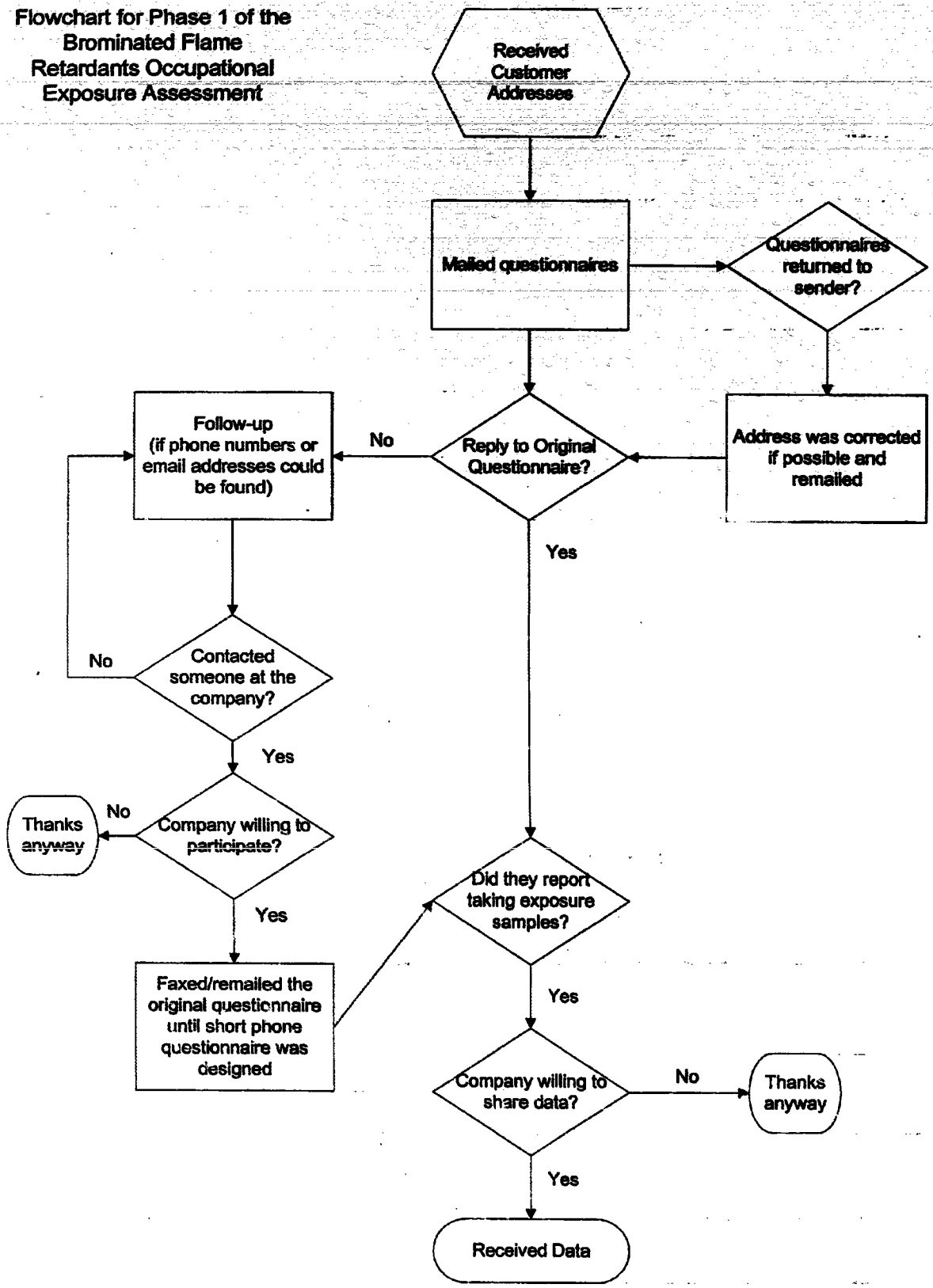
_____ 1-50,
_____ 50-100,
_____ 100-500,
_____ 500-1000,
_____ More than 1000?

6. Would you be willing to share the data with us? _____ Yes _____ No
Personal identifiers would be hidden.

F 07

Appendix C
Flowchart of Survey Methods

Flowchart for Phase 1 of the Brominated Flame Retardants Occupational Exposure Assessment



F 09

Appendix D
Detailed Dust and Antimony Data

Appendix D –Detailed Dust and Antimony Data

Description of Dust Data

Compartment Number	Sample Number	Type of Data	Sampling Time (min)	Sample Volume (liters)	Less than (<)	Total Dust Conc (mg/m3)	Type of BFR	Comments
1	1	A	Not given	Not given	<	0.18	DBDPO	
1	2	A	Not given	Not given	<	0.3	DBDPO	
1	3	A	Not given	Not given	<	0.33	DBDPO	
1	4	A	Not given	Not given	<	1.5	DBDPO	
1	5	A	Not given	Not given	<	1.5	DBDPO	
1	6	P	Not given	Not given		0.14	DBDPO	TWA
1	7	P	Not given	Not given		0.26	DBDPO	TWA
1	8	P	Not given	Not given		0.4	DBDPO	TWA
1	9	P	Not given	Not given		0.5	DBDPO	TWA
1	10	P	Not given	Not given		0.51	DBDPO	
1	11	P	Not given	Not given		0.52	DBDPO	TWA
1	12	P	Not given	Not given		1	DBDPO	
1	13	P	Not given	Not given	<	0.12	DBDPO	TWA
1	14	P	Not given	Not given	<	0.2	DBDPO	TWA
2	1	P	161	322		1.2	DBDPO	Operator at catalyst mixer
2	2	P	138	276		1.4	DBDPO	Operator at resin mixer
2	3	P	157	314		1.7	DBDPO	Operator at resin mixer
3	1	P	225	450		0.15	TBBPA	Operator at hock deck
3	2	P	185	370		0.17	TBBPA	Operator at hock deck
3	3	P	229	458		0.19	TBBPA	Operator in gel hock area
3	4	P	263	Not given		0.28	TBBPA	Operator in resin area
3	5	P	207	414		0.32	TBBPA	Operator at hock deck
3	6	P	280	560		0.71	TBBPA	Operator at hock deck
3	7	P	275	Not given		0.8	TBBPA	Operator at hock deck
3	8	P	216	432		1.1	TBBPA	Operator in gellcoat area
3	9	P	248	496		1.63	TBBPA	Operator in gellcoat area
3	10	P	163	326		1.77	TBBPA	Operator in gel hock area
3	11	P	266	Not given	<	0.13	TBBPA	Operator in gellcoat area
4	1	P	Not given	Not given		0.18	DBDPO	
4	2	P	Not given	Not given		0.26	DBDPO	
4	3	P	Not given	Not given		0.29	DBDPO	

4	4	P	Not given	Not given		0.37	DBDPO	
4	5	P	Not given	Not given		0.48	DBDPO	
4	6	P	Not given	Not given		0.49	DBDPO	
4	7	P	Not given	Not given		0.5	DBDPO	
4	8	P	Not given	Not given		0.56	DBDPO	
4	9	P	Not given	Not given		1	DBDPO	
4	10	P	Not given	Not given		1.2	DBDPO	
4	11	P	Not given	Not given		4.7	DBDPO	
4	12	P	Not given	Not given		4.9	DBDPO	
4	13	P	Not given	Not given		13	DBDPO	
4	14	P	Not given	Not given		15.4	DBDPO	
5	1	A	97	Not given		4.09	Other	Chrystalline poly area
5	2	P	190	Not given		0.26	Other	Intern in R&D
5	3	P	116	Not given		0.86	Other	Lab tech in polypropylene area
5	4	P	175	Not given		1.74	Other	Lab tech in prod devel area
5	5	P	125	Not given		7.6	Other	Lab tech in polypropylene area
5	6	P	154	Not given	<	0.32	Other	Lab tech in elec mat R&D
5	7	P	128	Not given	<	0.39	Other	Lab tech in elec mat R&D
5	8	P	75	Not given	<	0.66	Other	Lab tech in chrystalline poly area
5	9	P	66	Not given	<	0.76	Other	Lab tech in prod devel area
5	10	P	62	Not given	<	0.81	Other	Lab tech in chrystalline poly area
5	11	P	20	Not given	<	2.5	Other	Lab tech in chrystalline poly area
6	1	A	Not given	Not given	<	5	HBCD	TWA-Extruder mix panel -short sampling time
6	2	A	Not given	Not given	<	5	HBCD	TWA-Color concentrate gaylord-short sampling time
6	3	A	Not given	Not given	<	5	HBCD	TWA-Surger hopper tower-short sampling time
6	4	A	Not given	Not given	<	5	HBCD	TWA-Weight augur-short sampling time
6	5	A	Not given	Not given	<	5	HBCD	TWA-Control switches-short sampling time
6	6	A	Not given	Not given	<	5	HBCD	TWA-Color concentrate hopper-short sampling time
6	7	P	Not given	Not given		8.9	HBCD	TWA-Operator at hopper feed tank-short sampling
7	1	A	Not given	Not given		0.03	DBDPO	TWA-Mix tank
7	2	A	Not given	Not given		0.32	DBDPO	TWA-Drum off
7	3	A	Not given	Not given		0.4	DBDPO	TWA-Drum off
7	4	A	Not given	Not given		0.46	DBDPO	TWA-Mix tank
7	5	P	Not given	Not given		0.08	DBDPO	TWA-Operator
7	6	P	Not given	Not given		0.12	DBDPO	TWA-Operator
7	7	P	Not given	Not given		0.2	DBDPO	TWA-Operator
7	8	P	Not given	Not given		4.8	DBDPO	TWA-Operator

Description of Antimony Data

Company Number	Sample Number	Type	Sample Time (mins)	Sample Volume (liters)	Less than(<)	Antimony(mg/m3)	Type of BFR	Comments
2	1	P	161	322		0.052	DBDPO	Operator at the catalyst mixer
2	2	P	138	276		0.063	DBDPO	Operator at the resin mixer
2	3	P	157	314		0.1	DBDPO	Operator at the resin mixer
4	1	P	Not given	Not given		0.584	DBDPO	
4	2	P	Not given	Not given		0.618	DBDPO	TWA
4	3	P	Not given	Not given		0.985	DBDPO	
8	1	P	Not given	Not given		0.003	DBDPO	
8	2	P	Not given	Not given		0.003	DBDPO	
8	3	P	Not given	Not given		0.003	DBDPO	
8	4	P	Not given	Not given		0.003	DBDPO	
8	5	P	Not given	Not given		0.003	DBDPO	
8	6	P	Not given	Not given		0.004	DBDPO	
8	7	P	Not given	Not given		0.006	DBDPO	
8	8	P	Not given	Not given		0.007	DBDPO	
8	9	P	Not given	Not given		0.008	DBDPO	
8	10	P	Not given	Not given		0.009	DBDPO	
8	11	P	Not given	Not given		0.014	DBDPO	
8	12	P	Not given	Not given		0.018	DBDPO	
8	13	P	Not given	Not given		0.023	DBDPO	
8	14	P	Not given	Not given		0.042	DBDPO	
8	15	P	Not given	Not given		0.042	DBDPO	
8	16	P	Not given	Not given		0.056	DBDPO	
8	17	P	Not given	Not given		0.062	DBDPO	
8	18	P	Not given	Not given		0.062	DBDPO	
8	19	P	Not given	Not given		0.068	DBDPO	
8	20	P	Not given	Not given		0.076	DBDPO	
8	21	P	Not given	Not given		0.085	DBDPO	
8	22	P	Not given	Not given		0.11	DBDPO	
8	23	P	Not given	Not given		0.15	DBDPO	
8	24	P	Not given	Not given		0.19	DBDPO	
8	25	P	Not given	Not given		0.21	DBDPO	
8	26	P	Not given	Not given		0.21	DBDPO	

8	27	P	Not given	Not given		0.21	DBDPO	
8	28	P	Not given	Not given		0.29	DBDPO	
8	29	P	Not given	Not given		0.34	DBDPO	
8	30	P	Not given	Not given		0.75	DBDPO	
8	31	P	Not given	Not given		1.4	DBDPO	
8	32	P	Not given	Not given		1.6	DBDPO	
8	33	P	Not given	Not given		4.8	DBDPO	
8	34	P	Not given	Not given		22	DBDPO	
9	1	A	450	Not given	<	0.011	DBDPO	Area sample at extruder
9	2	P	450	Not given	<	0.011	DBDPO	Machine operator

FIG 13

F 14

Bromin. .ed Product Data from Company 8

Antimony (%)	Brominated Product (%)	Antimony (mg/m3)	Brominated Product (estimated mg/m3)
1.5	3.85	0.15	0.385
1.5	3.85	0.75	1.92
1.25	3.75	0.042	0.126
1	20	1.6	32
1.25	3.75	0.21	0.63
3.07	0.72	0.0029	0.0007
3.07	0.72	0.34	0.08
3.07	0.72	0.062	0.015
3.07	0.72	0.003	0.0007
3.07	0.72	0.0078	0.0018
28.04	0	0.018	0
3.18	0	0.062	0
3.18	0	0.0067	0
1.5	3.85	0.042	0.11
3.18	0	0.0057	0
1	3.25	0.056	0.18
2	6	0.023	0.069
2	6	0.0089	0.0267
2.36	5.64	0.0029	0.0069
1.25	4.5	0.19	0.0684
1.25	4.5	22	79.2
37.74	0	0.29	0
3.18	0	0.003	0
37.74	0	0.068	0
4.79	20	1.4	5.845
3.18	0	0.003	0
3.18	0	0.076	0
1.08	1.08	0.21	0.21
1.5	3.9	0.085	0.22
1.3	5	4.8	18.46
1.08	1.08	0.11	0.11
3.18	0	0.21	0
28.04	0	0.014	0
27.7	37.7	0.004	0.0054