FINAL REPORT ACTION ITEM CHECK-OFF LIST

Chemical Name: 4,4'-(1-Methylethylidene)bis[2,6-dibromo-]phenol
Trade Name(s): BA-59P, BA-59PC, FireMaster BP-4A, CN-10-0614, CN-614, Tetrabromobisphenol A (TBBPA)
CAS No: 79-94-7
Lab Study ID No: Wildlife 439A-131
Study Title: Tetrabromobisphenol-A (TBBPA): A Prolonged Sediment Toxicity Test with *Hyalella azteca* Using Spiked Sediment

☒ Reviewed for possible:
- ☐ FIFRA 6 (a) (2) and/or
- ☒ TSCA Section 8 (e) reporting

☐ Copy of FIFRA 6 (a) (2) and/or TSCA Section 8 (e) letter to the following Agency(ies), if applicable:
- ☐ EPA-FIFRA
- ☐ EPA-TSCA
- ☐ California [FIFRA 6 (a) (2)s]
- ☐ Other States [FIFRA 6 (a) (2)s]: _____

☐ Confidentiality Statement page addressed, signed, and dated in FIFRA reports.

☐ GLP Compliance page signed and dated in FIFRA reports.

☐ Flagging Statement page addressed, signed and dated in FIFRA reports.

☒ Copy of report submitted to the Agency(ies) in conjunction and/or support of one or more of the following:
- ☐ TSCA Consent Order/Agreement
- ☐ FIFRA Registration or Re-registration
- ☐ California Registration
- ☐ EU Notification
- ☐ Japanese METI Notification
- ☐ Japanese MAFF Notification
- ☐ Canadian (DSL) Notification
- ☐ FIFRA 6 (a) (2) Submission
- ☐ TSCA 8 (e) Submission
- ☐ TSCA 8 (d) Data-Call-In
- ☐ PMN Submission
- ☒ Other: UK in regards to EURA

☐ Study Report reviewed for MSDS information.

☐ Copy of Cover & Summary report pages to Business Unit MSDS information center (domestic and international).

☐ Scan study and upload to Quick Place.

☐ All information regarding the chemical and the study report entered into the IUCLID Toxicity Data Base and uploaded to Quick Place.

☐ Send to WIL for archiving.

TETRABROMOBISPHENOL-A (TBBPA): A PROLONGED SEDIMENT TOXICITY TEST WITH *Hyalella azteca* USING SPIKED SEDIMENT

FINAL REPORT

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 439A-131

ASTM Standard E 1706-00
U. S. EPA OPPTS Number 850.1735

AUTHORS:

Henry O. Krueger, Ph.D.
Susan T. Thomas, B.S.
Timothy Z. Kendall, M.S.

STUDY INITIATION DATE: April 10, 2006

STUDY COMPLETION DATE: July 28, 2006

SUBMITTED TO:

American Chemistry Council's
Brominated Flame Retardant Industry Panel
1300 Wilson Boulevard
Arlington, Virginia 22209 USA

Wildlife International, Ltd.
8598 Commerce Drive
Easton, Maryland 21601 USA
(410) 822-8600

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

SPONSOR: American Chemistry Council's Brominated Flame Retardant Industry Panel

TITLE: Tetrabromobisphenol-A (TBBPA): A Prolonged Sediment Toxicity Test with *Hyalella azteca* Using Spiked Sediment

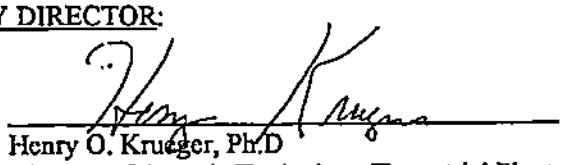
WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 439A-131

STUDY COMPLETION: July 28, 2006

This study was conducted in compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency in 40 CFR Parts 160 and 792, 17 August 1989; OECD Principles of Good Laboratory Practice (ENV/MC/CHEM (98) 17); and Japan MAFF, 11 NohSan, Notification No. 6283, Agricultural Production Bureau, 1 October 1999, with the following exception:

Periodic analyses of well water and sediment for potential contaminants were not conducted in accordance with Good Laboratory Practices; however, these analyses were performed using a certified laboratory and standard U.S. EPA analytical methods.

STUDY DIRECTOR:



Henry O. Krueger, Ph.D
Director of Aquatic Toxicology/Terrestrial Plants and Insects

28 July 06
Date

QUALITY ASSURANCE STATEMENT

This study was examined for compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency, 40 CFR Parts 160 and 792, 17 August 1989, OECD Principles of Good Laboratory Practice, (ENV/MC/CHEM(98) 17); and Japan MAFF, 11 NohSan, Notification No. 6283, Agricultural Production Bureau, 1 October 1999. The dates of all inspections and audits and the dates that any findings were reported to the Study Director and Study Director's Management were as follows:

		DATE REPORTED TO:	
ACTIVITY:	DATE CONDUCTED:	STUDY DIRECTOR	MANAGEMENT:
Protocol	April 13, 2006	April 13, 2006	May 8, 2006
Test Substance Preparation	April 13, 2006	April 13, 2006	May 8, 2006
pH Measurements	May 5, 2006	May 5, 2006	May 17, 2006
Observations	May 11, 2006	May 11, 2006	May 18, 2006
Matrix Fortifications	May 17, 2006	May 17, 2006	May 25, 2006
Analytical Data and Draft Report	June 11-13, 2006	June 13, 2006	June 23, 2006
Biological Data and Draft Report	June 12-14, 2006	June 14, 2006	June 23, 2006
Final Report	July 28, 2006	July 28, 2006	July 28, 2006

All inspections were study-based unless otherwise noted.

Linda R. Mitchell
Director of Regulatory and Ecotox Operations

28 July 2006
Date

REPORT APPROVAL

SPONSOR: American Chemistry Council's Brominated Flame Retardant Industry Panel

TITLE: Tetrabromobisphenol-A (TBBPA): A Prolonged Sediment Toxicity Test with *Hyalella azteca* Using Spiked Sediment

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 439A-131

STUDY DIRECTOR:

Henry O. Krueger, Ph. D. — Date: 28 July 06
Director of Aquatic Toxicology/Terrestrial Plants and Insects

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Supervisor

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Willard B. Nixon, Ph. D. — Date: 7/28/06
Director of Chemistry

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

TABLES

APPENDICES

TABLE OF CONTENTS (Continued)

APPENDICES

SUMMARY

SPONSORS: American Chemistry Council's Brominated Flame Retardant Industry Panel

TITLE: Tetrabromobisphenol-A (TBBPA): A Prolonged Sediment Toxicity Test with *Hyalella azteca* Using Spiked Sediment

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 439A-131

TEST DATES:		
	Study Initiation:	April 10, 2006
	Experimental Start (OECD):	April 13, 2006
	Experimental Start (EPA):	April 19, 2006
	Biological Termination:	May 17, 2006
	Experimental Termination:	May 19, 2006

LENGTH OF EXPOSURE: 28 Days

TEST ORGANISM: Amphipod (*Hyalella azteca*)

SOURCE OF TEST ORGANISMS: Environmental Consulting and Testing
Superior, WI 54880
U.S.A.

TEST CONCENTRATIONS :

Nominal
Negative Control
Solvent Control
63 mg a.i./Kg
125 mg a.i./Kg
250 mg a.i./Kg
500 mg a.i./Kg
1000 mg a.i./Kg

RESULTS: 28 Day EC50: >1000 mg a.i./Kg nominal, the highest test concentration.
NOEC: 250 mg a.i./Kg nominal, based on survival.
LOEC: 500 mg a.i./Kg nominal, based on survival.

INTRODUCTION

This study was conducted by Wildlife International, Ltd. for American Chemistry Council'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 April 19, 2006 to May 17, 2006, with dry weight data collected on May 19, 2006. Raw data generated by Wildlife International, Ltd. and a copy of the final report are filed under Project Number 439A-131 in archives located on the Wildlife International, Ltd. site.

OBJECTIVE

The objective of this study was to determine the effects of sediment-incorporated Tetrabromobisphenol A (TBBPA), on the amphipod, *Hyalella azteca*, during a 28-day exposure period under flow-through test conditions. After sediment had been added to the test compartments, a 28-day ration of food was admixed into the sediment just prior to adding the water. The test organisms were added last to the test compartments. The measured endpoints of the test were survivorship and growth as determined by dry weight measurements.

EXPERIMENTAL DESIGN

Groups of amphipods were exposed to a geometric series of five test concentrations, a negative control and a solvent control (Acetone) for 28 days under flow-through test conditions. Eight replicate test compartments were maintained in each treatment and control group, with 10 amphipods in each test compartment, for a total of 80 amphipods per group tested. Each test compartment contained a quantity of sediment and overlying water. Additional replicates were maintained in each treatment and control group for the purpose of analytical sampling of water and sediment. The "analytical" replicates sampled on Day 0 contained no amphipods, while amphipods were added at test initiation to the "analytical" replicates to be sampled on Day 7 and at test termination.

Following a range-finder test, the nominal test concentrations selected in consultation with the sponsor were 63, 125, 250, 500 and 1000 mg a.i./Kg of sediment based on the dry weight of the sediment. The results of the study are based on the nominal test concentrations. Overlying water, pore water and sediment samples were collected and analyzed from the "analytical replicates" of each treatment group and control. Samples were collected on Days 0, 7 and at the end of the test on Day 28. Results of the analyses were used to verify actual exposure over time.

Test compartments were indiscriminately and slowly positioned in a diluter unit according to treatment group approximately 48 hours prior to test initiation to condition the sediment prior to introduction of organisms. Amphipods were impartially assigned to exposure compartments at test initiation. Observations of mortality and abnormal behavior were made daily during the test. Survivorship and growth (dry weights) were determined at the end of the 28-day test period. The percent reduction in the numbers of organisms present in the treatment groups at test termination in comparison to the control group was used to determine the 28-day EC50 value. The lowest-observed-effect-concentration (LOEC) and the no-observed-effect-concentration (NOEC) were determined by the concentration-response pattern and statistical analysis of the survival and dry weight data.

MATERIALS AND METHODS

The study was conducted according to the procedures outlined in the protocol, "Tetrabromobisphenol-A (TBBPA): A Prolonged Sediment Toxicity Test with *Hyalella azteca* Using Spiked Sediment" (Appendix 1). The protocol was based on the U.S. EPA *Methods for Measuring the Toxicity and Bioaccumulation of Sediment Associated Contaminants with Freshwater Invertebrates* (1); the ASTM Standard E 1706-00: *Standard Test Methods for Measuring the Toxicity of Sediment-Associated Contaminants with Fresh Water Invertebrates* (2); and the OPPTS 850.1735: *Whole Sediment Acute Toxicity Invertebrates, Freshwater* (3).

Test Substance

The test substance used in the study consisted of a composite of TBBPA samples received from three manufacturers (Great Lakes Chemical Corporation (lot# 2008JM17B), Albemarle Corporation (lot# 25243Z-1), and Dead Sea Bromine Group (DSBG) (lot# 030036)) between June 2 and July 11, 2003. The composite sample was prepared by Wildlife International, Ltd. and assigned Wildlife International identification number 6404. Subsamples of the composite were shipped to Albemarle Corporation for characterization and purity analysis. The test substance, a white powder, was identified as: TBBPA composite of WIL 6358, 6368, 6400; CAS Number 79-94-7. The reported purity was 99.2%, with an expiration date of July 17, 2007 (Appendix 2). The test substance was stored under ambient conditions.

Test Sediment

Formulated sediment described as "Formulated Sediment A" in Kemble, et al. (6) was used as the test sediment. The formulated sediment was similar to that described in OECD Guidelines 218 (4) and 207 (5), but used alpha-cellulose as its source of organic matter instead of peat moss. Alpha-cellulose was selected by Kemble, et al. (6) as a more standardized source of organic matter than peat moss.

The sediment was composed of approximately 0.01% humic acid, 0.5% dolomite, 13% alpha-cellulose, 10% kaolin clay, and 77% industrial quartz sand (Appendix 3). The dry constituents of the sediment were mixed in a mixer for approximately 20 minutes. The percent organic carbon of the sediment was found to be 5.7. The dry sediment was stored under ambient conditions until used.

Preparation of Test Concentrations

Concentrations of the test substance were prepared on a dry weight basis, (i.e., mg test substance/Kg dry sediment). Nominal test concentrations were 63, 125, 250, 500 and 1000 mg a.i./Kg. A primary stock solution was prepared by dissolving the test substance in acetone at a nominal concentration of 100 mg a.i./mL. The primary stock was mixed by inversion and appeared clear and colorless. The primary stock was then serially diluted with acetone to prepare the four secondary stocks at nominal concentrations of 6.3, 12.5, 25.0 and 50.0 mg a.i./mL. Premixes of sediment were prepared by adding a 15-mL volume of the appropriate primary or secondary stock to 150 g of dry sediment, and mixing with a glass stir rod. The premixes were placed in a fume hood and the acetone was given an adequate amount of time to partially dissipate. The premixes were added to an additional 700 g of dry sediment in a plastic Nalgene® bottle and allowed to mix on a rotary mixer for one hour. An additional 650 g of dry sediment was added to each premix, achieving a final weight of 1500 g, and each batch was mixed for approximately 69 hours and 40 minutes on a rotary mixer. The solvent control was prepared in the same manner as the treatment groups, with the exception of not adding test material. The negative control was prepared as untreated sediment, but was placed in the rotary mixer for the same amount of time as the solvent control and treatment groups.

Eleven replicate test compartments were prepared for each treatment and control group (i.e., eight test compartments for evaluation of survival and growth, plus three compartments for analytical

purposes). For each replicate, 100 mL of sediment was placed in a 300-mL glass beaker with stainless steel mesh-covered holes on either side. Next, 28 mL of yeast, cereal grass media and trout chow (YCT), enough for 28 days, was added to each compartment that would contain organisms during the test and was mixed into the dry sediment using a glass stir rod. The sediment was then leveled to an even depth. The prepared test compartments were indiscriminately and slowly positioned in diluter tanks according to treatment group and the overlying water (approximately 150 ml) was allowed to flow through the test compartments. The sediment/water mixtures were allowed to acclimate for approximately 50 hours prior to the introduction of the test organisms. At test initiation and termination the overlying water in all test chambers appeared clear and colorless.

Test Organism

The amphipod, *Hyalella azteca*, was selected as the test species for this study. Amphipods are representative of an important group of aquatic invertebrates and were selected for use in the study based upon past history of use and ease of culturing in the laboratory. The organisms were obtained from Environmental Consulting and Testing, Superior, Wisconsin. The identity of the species was verified by the supplier. The most recent positive control test performed by Environmental Consulting and Testing on the *Hyalella azteca* culture was conducted on April 10, 2006.

The organisms were held in water from the same source as the water used during the test. During the holding period immediately preceding the test, water temperature ranged from 22.7 to 23.3°C, while the pH ranged from 8.3 to 8.7 and dissolved oxygen ranged from 7.7 to 8.3 mg/L.

During holding, the amphipods appeared normal. At test initiation, amphipods (approximately 10 days old) were collected from the culture and impartially added one and two at a time into transfer chambers until each chamber contained 10 organisms. Each group of ten organisms was impartially assigned and transferred to a test compartment. All transfers were made below the water surface using wide-bore pipettes. Ten replicate test compartments in each treatment and control group contained organisms (eight test compartments for the evaluation of effects plus two compartments for analytical purposes), while one test compartment per group was maintained without organisms, for the analyses of Day 0.

Amphipods were fed a mixture of yeast, cereal grass media and trout chow (YCT) during the holding period and during the test. A 28 mL aliquot of food (enough food for the entire study) was added to each test compartment (that contained organisms during the test) after adding the sediment, and mixed with a glass stir rod into the sediment before the overlying water was added. No additional food supplementation was made during the study.

Dilution Water

The water used for culturing and testing was freshwater obtained from a well approximately 40 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 4.

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 where the water was aerated with spray nozzles. Prior to use, the water again was filtered (0.45 µm) to remove microorganisms and particles. The results of periodic analyses performed to measure the concentrations of selected contaminants in the well water used by Wildlife International, Ltd. are presented in Appendix 5.

Test Apparatus

The test apparatus consisted of test compartments placed in stainless steel diluter tanks. The tanks were placed in a temperature-controlled water bath in a Wildlife International, Ltd. diluter unit. One tank was assigned to each treatment or control group. Standpipes were placed in each tank to maintain the water height. Each diluter tank had above it a splitting chamber. Dilution water was delivered on a timer to the splitting chamber two times per day. Each splitting chamber contained 14 syringes. Each syringe delivered dilution water to individual test compartments which were indiscriminately and carefully positioned in diluter tanks according to treatment group.

Test compartments were 300-mL glass beakers with two stainless steel mesh-covered holes on opposite sides to allow for the flow of water through the test compartment. Each beaker contained approximately 100 mL of sediment (3.4 cm in a representative test chamber) and approximately 150 mL of overlying water. The depth of overlying water in each test compartment was maintained by the water levels in the diluter tanks (e.g. 5.8 cm in a representative test compartment). The diluter was adjusted so

that approximately 793 ml of water was delivered per minute for three minutes to each splitting chamber two times per day resulting in approximately two volume additions in each test compartment per day. Test compartments were labeled with the project number, test concentration and replicate.

Environmental Conditions

Lighting used to illuminate the culture and test chambers during holding and testing was provided by fluorescent tubes that emitted wavelengths similar to natural sunlight (Colortone® 50). A photoperiod of 16 hours of light and 8 hours of darkness was controlled with an automatic timer. A 30-minute transition period of low light intensity was provided when lights went on and off to avoid sudden changes in light intensity. Light intensity at test initiation was 207 lux at the surface of the water over one representative test chamber.

The target test temperature during the study was 23 ± 1°C. Temperature was measured in the overlying water of one alternating replicate test compartment at the beginning of the test, three times per week during the test, and at the end of the test using a hand-held liquid-in-glass thermometer. Temperature also was measured continuously in the negative control diluter tank using a Fulscope ER/C Recorder. The continuous recorder was verified with a hand-held liquid-in-glass thermometer prior to test initiation.

Dissolved oxygen and measurements of pH were made on samples of overlying water collected from one alternating replicate test compartment of each treatment and control group at the beginning of the test, three times per week during the test, and at the end of the test. Hardness, alkalinity and specific conductance were measured in a composite of the overlying water from the negative and solvent control group replicates and the highest concentration treatment group (1000 mg a.i./Kg) replicates at the beginning and end of the test. Ammonia was measured in a composite of the overlying water collected from the negative and solvent control group replicates and the highest test concentration at test initiation, weekly during the test and at the end of the test.

Light intensity was measured using a SPER Scientific Model 840006C light meter. Dissolved oxygen was measured using a Thermo Orion Model 850Aplus dissolved oxygen meter, and measurements of pH were made using a Thermo Orion Model 525Aplus pH meter. Hardness and alkalinity measurements were made by titration (7). Specific conductance was measured using a

Yellow Springs Instrument Model 33 Salinity-Conductivity-Temperature meter. Ammonia was measured using a Thermo Orion 720Aplus pH/ISE meter.

Observations

In addition to the organisms placed in the test compartments at the beginning of the test, an additional 20 organisms were impartially selected at the beginning of the test and measured for dry weight. The test compartments were observed daily to make visual assessments of abnormal behavior (e.g. leaving the sediment, climbing the walls of the test compartment). On Day 28 of the test, amphipods were removed from the sediment, and the numbers of live or dead amphipods were enumerated.

Dry Weight Measurements

At test termination, each surviving amphipod was rinsed of excess sediment, placed in a pre-weighed labeled crucible and dried for approximately 48 hours at approximately 60°C. The amphipods were weighed after allowing the crucibles to cool at room temperature for 25 to 40 minutes.

Statistical Analyses

The results of the test were based on the nominal sediment concentrations. Since the percent reduction in the number of organisms present at test termination in comparison to the negative control group was less than 50% in all treatment groups, the 28-day EC50 value was estimated to be greater than the highest sediment concentration tested.

The no-observed-effect-concentration (NOEC) and lowest-observed-effect-concentration (LOEC) were determined by visual interpretation of the dose-response pattern and statistical analyses of the survival and dry weight data. Statistical analyses were performed using TOXSTAT Version 3.5 (8, 9). The survival data was evaluated for normality using the Chi-Square Test, and for homogeneity of variance using Bartlett's Test. The dry weight (growth) data was evaluated for normality using the Chi-Square Test and for homogeneity of variance using Levene's Test.

Analyses began by first comparing the negative and solvent controls using a t-test to determine if those groups could be combined into a pooled control. In this study there were no significant differences between the negative and solvent controls; however, the mean dry weight of replicate C in the solvent control was considered an outlier since it was twice as high as the group mean value for the

solvent control. Excluding solvent control replicate C growth value reduces the variability in the solvent control group mean. The standard deviation for the solvent control changes from 0.1514 to 0.0639 after replicate C has been excluded (Table 10). Therefore, the data are presented excluding solvent control replicate C dry weight from statistical analyses and percent reduction from the pooled control (Table 10). Survival and dry weight data met the assumptions of normality and homogeneity therefore a Bonferroni t-test was used to analyze the data (8).

Analytical Sampling

Stock solutions were prepared and analyzed prior to test initiation. Analyses of sediment and water were conducted using separate test compartments collected from the control group and from each concentration treatment group on Days 0, 7 and at test termination on Day 28. Samples of overlying water, pore water and sediment were collected from each of the test concentrations at each sampling interval. The replicate used for sample collection on Day 0 did not contain amphipods, whereas at test initiation amphipods were placed in the replicates used for sample collection on Days 7 and 28. Samples of the overlying water were collected at mid-depth in the water column, and then the remaining overlying water was poured from each test chamber. The remaining sediment was collected, centrifuged, and split into separate samples of pore water and sediment. The sediment and water samples were analyzed on the day of collection.

Analytical Method

The analytical method used for the analysis of TBBPA was developed by Wildlife International, Ltd. Sediment samples (10.0 g) were weighed into French square bottles. To each sample, 100 mL of acidified methanol was added and samples were sonically disrupted for approximately 5 minutes. Following disruption, the samples were centrifuged for approximately 5 minutes. The extract was volumetrically diluted, as appropriate, using 50% methanol : 50% HPLC-grade water. An aliquot of each diluted extract was transferred to an autosampler vial for immediate HPLC analysis. A method flowchart for the analysis of sediment samples is provided in Appendix 6.1.

For freshwater analysis, the requisite volume of overlying water or pore water was transferred to a culture tube and diluted with methanol. Secondary dilutions were performed, as necessary, using 50% methanol : 50% HPLC-grade bottled water. An aliquot of each diluted sample was transferred to an

autosampler vial and submitted for analysis. A method flowchart for the analysis of freshwater samples is provided in Appendix 6.11.

Concentrations of TBBPA in the samples were determined by HPLC using an Agilent Series 1100 High Performance Liquid Chromatograph (HPLC) equipped with an Agilent Series 1100 Variable Wavelength Detector. Chromatographic separations were achieved using a YMC PACK ODS-AM column (150 mm x 4.6 mm, 3 µm particle size). Typical instrument parameters are summarized in Appendix 6.2.

An analysis was performed for the dosing solutions prior to the initiation of the study. Calibration standards, ranging in concentration from 10.0 to 100 mg a.i./L were prepared in methanol using a stock solution of TBBPA in methanol.

For the sediment and freshwater analyses, calibration standards of TBBPA ranging in concentration from 0.100 to 1.00 mg a.i./L were prepared using a stock solution of TBBPA in methanol (Appendix 6.4). Linear regression equations were generated using the peak area responses versus the respective concentrations of the calibration standards. The concentration of TBBPA in the samples was determined by substituting the peak area responses of the samples into the applicable linear regression equation. An example of the calculations for a representative sediment sample is included in Appendix 6.3.

The method limit of quantitation (LOQ) for sediment was defined as 10.0 mg a.i./Kg, calculated as the product of the concentration of the lowest calibration standard (0.100 mg a.i./L) and the dilution factor of the control samples (100). The method limit of quantitation (LOQ) for freshwater was defined as 0.200 mg a.i./L, calculated as the product of the concentration of the lowest calibration standard (0.100 mg a.i./L) and the dilution factor of the matrix blank samples (2.00).

Sediment samples were fortified at 60.0 and 994 mg a.i./Kg on Day 0, 60.0 and 1000 mg a.i./Kg Days 7, and 60.0 and 10000 mg a.i./Kg on Day 28 using the test substance or a stock solution of TBBPA test substance in methanol (Appendix 6.4), and were analyzed concurrently with the test samples. The measured concentrations for the matrix fortification samples ranged from 78.5 to 99.6% of fortified concentrations (Appendix 6.5).

Freshwater samples were fortified at 0.500 and 1.00 mg a.i./L using a stock solution of TBBPA test substance in methanol (Appendix 6.4), and were analyzed concurrently with the test samples. The measured concentrations for the matrix fortification samples ranged from 104 to 111% of fortified concentrations (Appendix 6.12).

A representative calibration curve for sediment is presented in Appendix 6.6. Representative chromatograms of low and high-level calibration standards for sediment analyses are presented in Appendices 6.7 and 6.8, respectively. A representative chromatogram of a sediment matrix fortification sample is presented in Appendix 6.9. A representative chromatogram of a sediment test sample is presented in Appendices 6.10.

A representative calibration curve for freshwater is presented in Appendix 6.13. Representative chromatograms of low and high-level calibration standards for freshwater analyses are presented in Appendices 6.14 and 6.15, respectively. A representative chromatogram of a freshwater matrix fortification sample is presented in Appendix 6.16. Representative chromatograms of an overlying water sample and a pore water sample are presented in Appendices 6.17 and 6.18, respectively.

RESULTS AND DISCUSSION

Measurement of Test Concentrations

Results of analyses to measure concentrations of TBBPA in samples collected during the definitive test are presented in Tables 1-5 and Appendix 6. Nominal TBBPA concentrations selected for use in this definitive test were 63, 125, 250, 500 and 1000 mg a.i./Kg. Samples of stock solutions collected prior to test initiation had measured concentrations that ranged from 94.5 to 99.3% of nominal concentrations (Table 1).

Samples of overlying water, pore water and sediment were collected from the negative control, solvent control, and each of the test concentrations on Days 0, 7 and 28. The purpose of these samples was to verify the concentrations in sediment at test initiation and to monitor the test substance in pore water and overlying water over the course of the study. **Results are based on the nominal concentrations in the sediment.** Negative control and solvent control samples for all three matrices on Days 0, 7 and 28 were all below the limit of quantitation (LOQ).

Measured concentrations of TBBPA in sediment from the 63, 125, 250, 500 and 1000 mg a.i./Kg nominal test concentrations on Day 0 were 34.4, 83.8, 184, 364 and 630 mg a.i./Kg, respectively (Table 2). Measured concentrations of TBBPA in sediment from the 63, 125, 250, 500 and 1000 mg a.i./Kg nominal test concentrations on Day 7 were 51.8, 117, 231, 507 and 1059 mg a.i./Kg, respectively. Measured concentrations of TBBPA in sediment from the 63, 125, 250, 500 and 1000 mg a.i./Kg nominal test concentrations on Day 28 were 65.7, 133, 218, 529 and 1183 mg a.i./Kg, respectively. All concentrations in sediment were relatively close to nominal concentrations indicating that TBBPA remained in sediment throughout the study.

Samples of overlying water in all treatment groups had concentrations below the LOQ on Days 0, 7 and 28. (Table 3). Measured concentration in pore water from the 63, 125, 250, 500 and 1000 mg a.i./Kg nominal treatment groups showed a decreasing trend with values on Day 0 of 4.88, 2.97, 2.67, 3.02 and 2.96 mg a.i./L, respectively, on Day 7 of 2.03, 0.905, 0.727, 0.849 and 0.862 mg a.i./L respectively, and on Day 28 of 0.668, 0.331, 0.315, 0.376 and 0.464 mg a.i./L respectively (Table 4).

Observations and Measurements

Measurements of temperature, dissolved oxygen and pH of the overlying water in the test chambers are presented in Tables 5, 6 and 7 respectively. Temperatures were within the 23 ± 1°C range established for the test. Dissolved oxygen concentrations were ≥ 67% (5.7 mg/L) of saturation throughout the test. Measurements of pH ranged from 7.7 to 8.2 during the test. Measurements of hardness, alkalinity and conductivity (Table 8) were typical for Wildlife International, Ltd. well water. Ammonia concentrations were always below the lowest standard of 0.17 mg/L (Table 9).

Observations of amphipods in individual replicates at each observation interval are presented in Appendix 7. All replicates appeared normal during the test, with few observations of abnormal behavior in the treatment and control groups. The mean numbers of amphipods in the negative control and solvent control groups at test termination were 8.8 and 8.4 respectively (Table 10). The mean numbers of amphipods in the 63, 125, 250, 500 and 1000 mg a.i./Kg treatment groups (nominal) were 8.9, 8.6, 8.0, 5.9 and 5.9, respectively, at test termination. The mean numbers of amphipods in the 500

and 1000 mg a.i./Kg treatment groups were significantly reduced ($p < 0.05$) from the pooled control group using a Bonferroni t-test.

At the beginning of the test, 20 organisms from the culture were impartially selected and used for the determination of dry weight. It was determined that the average individual dry weight of those twenty organisms was 0.075 mg (Appendix 8). The average individual dry weights for the surviving amphipods in each replicate at test termination are presented in Appendix 8. One outlier was identified in the solvent control (replicate C) and therefore excluded from the calculations. After excluding the outlier, the average dry weight per amphipod in the negative control and solvent control groups was 0.1997 and 0.1891 mg, respectively, when the solvent control replicate C is excluded (Table 10). The average dry weight per amphipod in the 63, 125, 250, 500 and 1000 mg a.i./Kg treatment groups was 0.2641, 0.1629, 0.1950, 0.2011 and 0.1937 mg, respectively. The dry weights of the treatment groups were not significantly reduced ($p > 0.05$) from the pooled control weights using the Bonferroni t-test, and were not concentration-dependent.

CONCLUSIONS

The 28-day EC50 value for amphipods (*Hyalella azteca*) exposed to TBBPA in sediment was greater than 1000 mg a.i./Kg, the highest nominal concentration tested. Determination of the lowest-observed-effect-concentration (LOEC) and the no-observed-effect-concentration (NOEC) was based on an evaluation of the survival and dry weight data. Based on the survival results of this study (the most sensitive endpoint), the LOEC was 500 mg a.i./Kg dry weight of sediment and the NOEC was 250 mg a.i./Kg dry weight of sediment.

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Table 1

Stock Solution Analysis for a Prolonged Sediment Toxicity Test for TBBPA with *Hyalella azteca*

Nominal Concentration (mg a.i./L)	Sample Number (439A-131-)	Measured Concentration (mg a.i./L)	Percent of Nominal[1]
6300	St-1	5956	94.5
12500	St-2	12145	97.2
25000	St-3	24681	98.7
50000	St-4	49256	98.5
100000	St-5	99313	99.3

[1] Results were generated using Excel 2000 in full precision mode. Manual calculations may differ slightly.

Table 2

Measured Concentrations of TBBPA in Sediment Samples from a Prolonged Sediment Toxicity Test with *Hyalella azteca*

Nominal Test Concentration (mg a.i./Kg)	Sample Number (439A-131-)	Sampling Interval (Day)	Measured TBBPA Concentration (mg a.i./Kg)[1,3]	Percent of Nominal[2,3]
0.0 (Negative Control)	S-1	0	< LOQ	--
	S-8	7	< LOQ	--
	S-15	28	< LOQ	--
0.0 (Solvent Control)	S-2	0	< LOQ	--
	S-9	7	< LOQ	--
	S-16	28	< LOQ	--
63	S-3	0	52.1	82.8
	S-10	7	51.8	82.2
	S-17	28	65.7	104
125	S-4	0	123	98.8
	S-11	7	117	93.3
	S-18	28	133	106
250	S-5	0	290	116
	S-12	7	231	92.5
	S-19	28	218	87.3
500	S-6	0	571	114
	S-13	7	507	101
	S-20	28	529	106
1000	S-7	0	963	96.3
	S-14	7	1059	106
	S-21	28	1183	118

[1] The limit of quantitation (LOQ) was 10.0 mg a.i./Kg, calculated as the product of the concentration of the lowest calibration standard (0.100 mg a.i./L) and the dilution factor of the control samples (100).

[2] Results were generated using Excel 2000 in the full precision mode. Manual calculations may differ slightly.

[3] Analytical results were generated using wet weights. Secondary samples were dried in an oven to determine soil content. The tabulated values are reported on a dry weight basis.

Table 3

Measured Concentrations of TBBPA in Overlying Water Samples from a Prolonged Sediment Toxicity Test with *Hyalella azteca*

Nominal Test Concentration (mg a.i./Kg)	Sample Number (439A-131-)	Sampling Interval (Day)	Measured TBBPA Concentration (mg a.i./L)[1,2]
0.0 (Negative Control)	W-1	0	< LOQ
	W-8	7	< LOQ
	W-15	28	< LOQ
0.0 (Solvent Control)	W-2	0	< LOQ
	W-9	7	< LOQ
	W-16	28	< LOQ
63	W-3	0	< LOQ
	W-10	7	< LOQ
	W-17	28	< LOQ
125	W-4	0	< LOQ
	W-11	7	< LOQ
	W-18	28	< LOQ
250	W-5	0	< LOQ
	W-12	7	< LOQ
	W-19	28	< LOQ
500	W-6	0	< LOQ
	W-13	7	< LOQ
	W-20	28	< LOQ
1000	W-7	0	< LOQ
	W-14	7	< LOQ
	W-21	28	< LOQ

[1] The limit of quantitation (LOQ) was 0.200 mg a.i./L, calculated as the product of the concentration of the lowest calibration standard (0.100 mg a.i./L) and the dilution factor of the matrix blank samples (2.00).

[2] Results were generated using Excel 2000 in the full precision mode. Manual calculations may differ slightly.

Table 4

Measured Concentrations of TBBPA in Pore Water Samples from a Prolonged Sediment Toxicity Test with *Hyalella azteca*

Nominal Test Concentration (mg a.i./Kg)	Sample Number (439A-131-)	Sampling Interval (Day)	Measured TBBPA Concentration (mg a.i./L)[1,2]
0.0 (Negative Control)	PW-1	0	< LOQ
	PW-8	7	< LOQ
	PW-15	28	< LOQ
0.0 (Solvent Control)	PW-2	0	< LOQ
	PW-9	7	< LOQ
	PW-16	28	< LOQ
63	PW-3*	0	4.88
	PW-10	7	2.03
	PW-17	28	0.668
125	PW-4*	0	2.97
	PW-11	7	0.905
	PW-18	28	0.331
250	PW-5*	0	2.67
	PW-12	7	0.727
	PW-19	28	0.315
500	PW-6*	0	3.02
	PW-13	7	0.849
	PW-20	28	0.376
1000	PW-7*	0	2.96
	PW-14	7	0.862
	PW-21	28	0.464

[1] The limit of quantitation (LOQ) was 0.200 mg a.i./L, calculated as the product of the concentration of the lowest calibration standard (0.100 mg a.i./L) and the dilution factor of the matrix blank samples (2.00).

[2] Results were generated using Excel 2000 in the full precision mode. Manual calculations may differ slightly.

* Sample reanalyzed.

Table 5

Temperature of Overlying Water in the Test Compartments

Nominal Concentration (mg a.i./Kg)		Temperature (°C)														
		Day 0[1]	Day 1	Day 2	Day 5	Day 7	Day 9	Day 12	Day 14	Day 16	Day 19	Day 21	Day 23	Day 26	Day 27	Day 28
	Replicate:	A	B	C	D	E	F	G	H	A	B	C	D	E	F	G
Negative Control		22.5	22.7	22.4	22.7	22.5	22.9	22.4	22.5	22.7	22.5	22.5	22.6	22.6	23.2	22.7
Solvent Control		22.6	22.9	22.4	22.7	22.5	22.9	22.6	22.8	22.8	22.6	22.5	22.6	22.7	23.1	22.7
63		22.7	22.8	22.4	22.7	22.5	23.0	22.4	22.7	22.8	22.6	22.5	22.6	22.7	23.1	22.8
125		22.6	22.8	22.4	22.8	22.5	22.8	22.5	22.7	22.8	22.6	22.5	22.7	22.8	23.1	22.8
250		22.6	22.7	22.3	22.7	22.3	22.8	22.4	22.5	22.8	22.6	22.5	22.5	22.6	23.1	22.5
500		22.5	22.8	22.3	22.8	22.5	22.7	22.4	22.5	22.8	22.6	22.5	22.7	22.7	23.1	22.8
1000		22.5	22.8	22.4	22.7	22.6	22.7	22.4	22.5	22.7	22.7	22.5	22.7	22.8	23.2	22.8

[1] Temperature measured continuously in the negative control diluter tank was approximately 23.0°C during the test.

Table 6

Dissolved Oxygen in Overlying Water in the Test Compartments

Nominal Concentration (mg a.i./Kg)		Dissolved Oxygen (mg/L)														
		Day 0[1]	Day 1	Day 2	Day 5	Day 7	Day 9	Day 12	Day 14	Day 16	Day 19	Day 21	Day 23	Day 26	Day 27	Day 28
	Replicate:	A	B	C	D	E	F	G	H	A	B	C	D	E	F	G
Negative Control		6.9	7.9	7.5	7.2	7.3	7.6	7.6	6.1	7.5	6.7	7.3	6.9	7.2	7.6	6.7
Solvent Control		6.3	7.8	7.5	7.2	7.3	7.7	7.5	6.3	6.3	6.6	7.3	6.7	7.2	7.4	6.2
63		6.1	7.7	7.7	7.1	7.6	7.9	7.6	6.4	6.0	6.4	6.9	7.0	7.0	7.5	6.0
125		6.0	7.5	7.6	7.1	7.5	7.8	7.6	6.4	7.1	6.8	7.3	6.4	7.3	7.6	6.1
250		5.9	7.2	7.4	7.1	7.6	7.7	7.7	6.3	6.6	6.4	6.6	6.5	6.9	7.5	5.7
500		5.8	7.6	7.4	7.1	7.6	7.7	7.7	6.4	7.4	6.4	7.2	6.7	7.2	7.6	6.1
1000		6.5	7.7	7.4	7.4	7.5	7.6	7.3	6.7	6.8	6.6	7.2	6.5	7.1	7.7	6.1

[1] A dissolved oxygen concentration of 5.1 mg/L represents 60% saturation at 23.0°C.

Table 7

pH of Overlying Water in the Test Compartments

Nominal Concentration (mg a.i./Kg)		pH														
		Day 0[1]	Day 1	Day 2	Day 5	Day 7	Day 9	Day 12	Day 14	Day 16	Day 19	Day 21	Day 23	Day 26	Day 27	Day 28
	Replicate:	A	B	C	D	E	F	G	H	A	B	C	D	E	F	G
Negative Control		8.0	8.1	8.1	8.2	8.0	8.0	8.1	7.9	8.0	7.8	8.0	7.7	8.0	8.1	7.9
Solvent Control		8.0	8.1	8.1	8.2	8.1	8.1	8.1	7.9	7.9	7.9	8.1	7.8	8.0	8.1	7.8
63		8.0	8.1	8.1	8.2	8.2	8.2	8.1	8.1	8.0	8.0	8.1	8.0	8.0	8.1	7.8
125		8.0	8.1	8.2	8.2	8.2	8.2	8.1	8.1	8.2	8.1	8.2	8.0	8.1	8.1	7.9
250		8.0	8.1	8.2	8.2	8.2	8.2	8.2	8.1	8.1	8.0	8.1	8.0	8.1	8.2	7.9
500		8.0	8.2	8.2	8.2	8.2	8.2	8.2	8.1	8.2	8.1	8.2	8.1	8.1	8.2	8.0
1000		8.0	8.2	8.2	8.2	8.2	8.2	8.1	8.1	8.2	8.1	8.2	8.1	8.1	8.2	8.0

Table 8

Hardness, Alkalinity and Conductivity of the Overlying Water in the Test Compartments

Nominal Concentration (mg a.i./Kg)	Day 0 Hardness (mg/L as $CaCO_3$)	Day 0 Alkalinity (mg/L as $CaCO_3$)	Day 0 Conductivity (µmhos/cm)	Day 28 Hardness (mg/L as $CaCO_3$)	Day 28 Alkalinity (mg/L as $CaCO_3$)	Day 28 Conductivity (µmhos/cm)
Negative Control	116	164	300	132	176	320
Solvent Control	120	166	305	132	179	330
1000	116	166	305	124	166	320

Measurements were taken in a composite made from each of the replicates of the respective treatment groups.

Table 9

Measurements of Ammonia (mg/L) in the Overlying Water in Test Compartments

Nominal Concentration (mg a.i./Kg)	Day 0	7	14	21	28
Negative Control	<0.17[1]	<0.17	<0.17	<0.17	<0.17
Solvent Control	<0.17	<0.17	<0.17	<0.17	<0.17
1000	<0.17	<0.17	<0.17	<0.17	<0.17

Measurements were taken in a composite made from each of the replicates of the respective treatment groups.
[1]The lowest standard used for calibration was 0.17 mg/L.

Table 10

Mean Survival and Growth of Amphipods
(*Hyalella azteca*) During a Prolonged Sediment Toxicity Test

Nominal Concentration (mg a.i./Kg)	Mean Number of Surviving Amphipods (± Stdev)[1]	Percent Reduction[2] (%)	Mean Individual Dry Weight[4] (mg)	Percent Reduction[2] (%)
Negative Control	8.8 (± 1.0)	-	0.1997 (±0.0639)	-
Solvent Control	8.4 (± 1.1)	-	0.1891 (±0.0639)[5] 0.2384 (±0.1514)	-
Pooled Control	8.6 (± 1.0)	-	0.1947 (± 0.0618)[5]	-
63	8.9 (± 0.64)	-3.5	0.2641 (± 0.1336)	-36
125	8.6 (± 1.3)	0	0.1629 (± 0.0443)	16
250	8.0 (± 1.4)	7.0	0.1950 (± 0.0544)	-0.15
500	5.9 (± 2.0)[3]	31	0.2011 (± 0.0459)	-3.3
1000	5.9 (± 2.1)[3]	31	0.1937 (± 0.0478)	0.51

[1] Each replicate contained 10 Amphipods at test initiation.

[2] Percent reduction was calculated in relation to the mean of the pooled controls (excluding the solvent control replicate C value for dry weight).

[3] There were statistically significant reductions ($p<0.05$) in the mean number of surviving amphipods in comparison to the pooled control group using a Bonferroni t-test.

[4] There were no statistically significant reductions ($p>0.05$) in the mean individual dry weight of the amphipods in comparison to the pooled control group using a Bonferroni t-test.

[5] The mean individual Ash-Free Dry Weight (AFDW) for Replicate C in the solvent control was excluded since it was considered an outlier (Appendix 8). There is no difference in the conclusions from the Bonferroni t-test if Replicate C is included in or excluded from the analysis.

Appendix 1

Protocol, Amendment and Deviation

PROTOCOL

TETRABROMOBISPHENOL-A (TBBPA): A PROLONGED SEDIMENT TOXICITY TEST WITH *Hyalella azteca* USING SPIKED SEDIMENT

ASTM Standard E 1706-00

U.S. Environmental Protection Agency
Series 850 – Ecological Effects Test Guidelines
OPPTS Number 850.1735

Submitted to

American Chemistry Council's
Brominated Flame Retardant Industry Panel
1300 Wilson Boulevard
Arlington, Virginia 22209 USA

Wildlife International, Ltd.

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

February 22, 2006

Wildlife International, Ltd.

- 2 -

TETRABROMOBISPHENOL-A (TBBPA): A PROLONGED SEDIMENT TOXICITY TEST WITH *Hyalella azteca* USING SPIKED SEDIMENT

SPONSOR: American Chemistry Council's Brominated Flame Retardant Industry Panel 1300 Wilson Boulevard Arlington, Virginia 22209 USA

SPONSOR'S REPRESENTATIVE: Nancy Sandrof

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

STUDY DIRECTOR: Henry O. Krueger, Director of Aquatic Director of Aquatic Toxicology/Terrestrial Plants and Insects Wildlife International, Ltd.

LABORATORY MANAGEMENT: Willard B. Nixon, Ph. D., Director of Chemistry Wildlife International, Ltd.

FOR LABORATORY USE ONLY

Proposed Dates:

Experimental Start Date: April 12, 2006 Experimental Termination Date: May 10, 2006

Project No.: 439A-131

Test Concentrations: NC, SC, 63, 125, 250, 500, + 1000 mg/kg

Test Substance No.: 6404 Reference Substance No. (if applicable):

PROTOCOL APPROVAL

Henry Krueger — 10 Apr 06
STUDY DIRECTOR — DATE

Willard B. Nixon — 4/10/06
LABORATORY MANAGEMENT — DATE

Nancy Sandrof — 2-22-06
SPONSOR'S REPRESENTATIVE — DATE

Wildlife International, Ltd.

INTRODUCTION

Wildlife International, Ltd. will conduct a prolonged sediment toxicity test with the amphipod, *Hyalella azteca*, for the Sponsor at the Wildlife International, Ltd. aquatic toxicology facility in Easton, Maryland. The study will be based upon the U.S. EPA *Methods for Measuring the Toxicity and Bioaccumulation of Sediment Associated Contaminants with Freshwater Invertebrates*. a study design proposed by (1); the ASTM Standard E 1706-00: *Standard Test Methods for Measuring the Toxicity of Sediment-Associated Contaminants with Fresh Water Invertebrates* (2); and the U.S. EPA Series 850 - Ecological Effects Test Guidelines, OPPTS Number 850.1735: *Whole Sediment Acute Toxicity Invertebrates, Freshwater* (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.

OBJECTIVE

The objective of this study is to determine the effects of a sediment-incorporated test substance on the amphipod, *Hyalella azteca*, during a 28-day exposure period under flow-through conditions. The sediment used in the test will have an organic content of approximately 5%. Alpha-cellulose will be used as the source of organic carbon in preparation of the batch sediment used in the test. Test substance will be dry mixed into the sediment for at least overnight or over a weekend. Sediment will then be added to each test compartment, followed by the addition of a 28-day ration of food. The food is a slurry of yeast, cerophyll, and trout chow (YCT) that is mixed into the sediment in each test compartment prior to adding water. The slurry is added to each test compartment to insure that each compartment receives the same amount of food. Organisms are added to the test compartments after a 48-hour equilibration period. The measured endpoints of the test are survivorship and growth as determined by dry weight measurements.

EXPERIMENTAL DESIGN

Groups of amphipods will be exposed to a geometric series of at least five test concentrations, a negative (untreated sediment) control, and if necessary, a solvent control for approximately 28 days. Eight replicate test compartments will be maintained in each treatment and control group, with 10 amphipods in each compartment for a total of 80 individuals per test concentration. Each test compartment will contain a quantity of sediment and overlying water. Additional replicate test compartments may be included for analytical sampling of water and sediment. No amphipods will be

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placed in "analytical" replicate(s) sampled at the beginning of the test. However, the "analytical" replicate(s) sampled after the beginning of the test (e.g., on Day 7, and at the end of the test) will contain amphipods.

Test concentrations in the sediment will be prepared on a dry weight basis (i.e., mg/Kg dry soil). Nominal test concentrations will be selected in consultation with the Sponsor, and will be based upon information such as the results of exploratory range-finding toxicity data, known toxicity data, physical/chemical properties of the test substance or other relevant information. Generally, each test substance concentration used in the definitive test will be at least 60% of the next higher concentration unless information concerning the concentration-effect curve indicates that a different dilution factor would be more appropriate. Overlying water, pore water, and sediment samples from the analytical sampling test compartments will be collected from each control and treatment group at specified intervals for analysis of the test substance. Results of the analyses will be used to verify the exposure over time. The results of the study will be based on the nominal test concentrations in sediment, when appropriate.

To control bias, amphipods will be impartially assigned to exposure compartments at test initiation. No other potential sources of bias are expected to affect the results of the study. Survivorship and growth (dry weight) will be determined at the end of the test period (maximum of 28 days). Mortality in the treatment groups will be used to calculate, when possible, the EC50 value. The dose-response pattern and appropriate statistical analyses of survivorship and growth will be used to define the no-observed-effect-concentration (NOEC) and the lowest-observed-effect-concentration (LOEC).

MATERIALS AND METHODS

Test Substance

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

Wildlife International, Ltd.

Manufacturer	Lot/Batch	Date Received	Wildlife International, Ltd. Identification Number
Great Lakes Chemical Corporation	2008JM17B	June 2, 2003	6358
Albemarle Corporation	25243Z-1	July 11, 2003	6400
Bromine Compounds, Ltd.	030036	June 12, 2003	6368

The composite test substance was assigned Wildlife International, Ltd. identification number 6404 and was stored under ambient conditions. Subsamples of the composite test substance were shipped to Albemarle Corporation for characterization and purity analyses. The results of the analyses indicated the composite test substance was homogeneous and contained TBBPA with a purity of 99.20%. The test substance is stored at Wildlife International, Ltd. at room temperature.

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

Preparation of Test Concentrations

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

Stock solutions of the test substance in water, or neat test substance, will be added to dry formulated sediment to prepare batches of sediment for each treatment level. If necessary, a volatile solvent such as methanol, ethanol or acetone, that has adequate time to dissipate from the soil, will be used in one or more stock solutions to incorporate the test substance into the sediment. If an organic solvent is required, then a solvent control group will be included in the experimental design along with the negative (untreated sediment) control group. If used, the concentration of organic solvent will be minimized and will be the same in all treatment levels.

Aliquots of the stock solution will be mixed into a portion of dry sediment for each treatment level. If an organic solvent is used, the "premix" will be placed under a fume hood to allow partial evaporation of the solvent. Each premix then will be added to a larger portion of dry sediment and mixed thoroughly in a rotary mixer, or equivalent. This premix then will be mixed with additional dry sediment to prepare the final batch of treatment sediment at each concentration. The batches of

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sediment will be mixed on a motorized rotating mixer at least overnight to ensure thorough mixing prior to transfer of dry sediment to the test chambers.

After mixing, approximately 100 mL of sediment will be placed in the bottom of each test compartment (300 mL glass beaker with stainless steel mesh covered holes on opposites sides). A 28-day portion of food (YCT) will then be added to each replicate compartment that will contain organisms during the test. YCT is a slurry of yeast, cerophyll, and trout chow and in typical testing is added at a rate of 1 ml per day to overlying water. In this 28-day study approximately 28 ml of slurry will be added to the dry mixed sediment in each beaker and stirred into the sediment. The YCT is added to each beaker instead of batch mixes to be certain that each beaker receives the same amount of food. Next, approximately 150 mL of overlying water (dilution water) will be slowly added to the test compartments as they are placed in the diluter unit. After the test compartments are prepared, they will acclimate in the diluter unit for approximately 48 hours before organisms are added.

Test Organism

The amphipod, *Hyalella azteca*, has been selected as the test species for this study. Amphipods represent an important group of aquatic invertebrates, and have been selected for use in the test based upon past use history and ease of culturing in the laboratory. Amphipods to be used in the test will obtained from a commercial supplier or other reliable source, or from cultures maintained at Wildlife International, Ltd. Prior to the test, the organisms will be held in water from the same source and at approximately the same temperature as will be used in the test. Amphipods used in the test will be 7 to 14 days of age at test initiation. Enough food for 28 days (YCT) will be incorporated into the sediment. Specifications for acceptable levels of contaminants in the food for amphipods have not been established.. However, there are no levels of contaminants reasonably expected to be present in the diet that are considered to interfere with the purpose or conduct of the study

Test Sediment

Artificial sediment as described "Formulated Sediment A" in Kemble et al. (6) will be used as test sediment. This artificial sediment is similar to that described in OECD Guidelines 218 (4) and 207 (5), but uses alpha-cellulose as it's source of organic matter instead of peat moss. Alpha-cellulose was selected by Kemble, et al. (1999) as a more standardized source of organic matter than peat moss.

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The soil will be composed of approximately 13% alpha-cellulose, 10% silt and clay (kaolin clay), 77% industrial quartz sand and <1% humic acid and dolomite. The dry constituents of the sediment will be mixed in a PK Twinshell or equivalent mixer. Calcium carbonate will be added as needed to adjust the pH to 7.0 ± 0.5. Organic carbon content of the final mixture of the batch of sediment used in the test should be 5% ± 0.5%. A 28-day ration of food (YCT) will be mixed into the test compartments after the sediment has been added, but immediately before adding water to the test compartments, and 48 hours before adding organisms. The amount of food added will be documented in the raw data. The percent organic carbon is based solely on alpha-cellulose as the source of organic carbon. Just as in the OECD 218 testing guideline, the food added to the test system during the test is not included in the measurement of organic carbon.

Dilution Water

Water used for the holding and testing of amphipods 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 analyses will be summarized in the final report.

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Test Apparatus

The test apparatus will consist of a Wildlife International, Ltd. diluter unit that is designed to hold up to 14 tanks in a temperature controlled water bath. Each tank can receive renewal rates that can range from static to greater than 10 tank volume additions per day. In this study there will be two renewals per day (one approximately every 12 hours).

Test compartments will consist of 300 mL glass beakers with stainless-steel mesh-covered holes on opposite sides of the beaker. The compartments will be labeled with the project number, test concentration and replicate. All replicate beakers in each treatment group will be indiscriminately positioned in one or more diluter tanks. Each beaker will contain approximately 100 ml of sediment and approximately 125 to 175 ml of overlying water. Dilution water will be delivered directly into each beaker, passively forcing water out through the holes in the sides of the beakers and exchanging the water overlying the sediment. Each replicate beaker will receive approximately two-volume additions of water per day. Proper system operation will be verified by calibration prior to initiation of the test.

Environmental Conditions

Lighting used to illuminate the cultures and test chambers during culturing and testing will be provided by fluorescent tubes that emit wavelengths similar to natural sunlight (e.g., Colortone® 50). A photoperiod of 16 hours of light and 8 hours of dark will be controlled with an automatic timer. A 30-minute transition period of low light intensity will be provided when lights go on and off to avoid sudden changes in light intensity. Light intensity will be measured at test initiation with a SPER Scientific Ltd. light meter or equivalent and should fall within the range of 100 to 1000 lux.

The target test temperature will be 23 ± 1°C. Temperature will be measured using a hand-held liquid-in-glass thermometer in the overlying water in one alternating replicate test compartment of each experimental group at the beginning and end of the test and at least three times per week during the test. Temperature also will be measured with a continuous recorder in a beaker of water placed adjacent to the test compartments or in the negative control diluter tank. Recorder measurements will be verified with a hand-held liquid-in-glass thermometer prior to test initiation.

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Dissolved oxygen will be measured in the overlying water from one alternating replicate of each experimental group daily from the beginning to the end of the test, and at least three times per week (1). Dissolved oxygen will be measured using a Thermo Orion Model 850Aplus dissolved oxygen meter, or equivalent. In the event that dissolved oxygen levels approach or fall below 60% saturation, the Sponsor will be notified, and appropriate corrective actions will be taken, if necessary, to maintain dissolved oxygen levels above 60% saturation.

Measurements of pH will be made on a sample of the overlying water from one alternating replicate of each experimental group at the beginning and end of the test and at least three times per week (1). Water pH measurements will be made using a Thermo Orion Model 525Aplus pH meter, or equivalent. If a treatment replicate reaches 100% mortality, dissolved oxygen, pH, and temperature measurements will be taken in that replicate at that time, and then discontinued.

Hardness, alkalinity, specific conductance and ammonia will be measured in a composite sample of overlying water from the control group replicates and the highest concentration treatment group replicates at the beginning and end of 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* (7). Specific conductance will be measured using a Yellow Springs Instrument Model 33 Salinity-Conductivity-Temperature meter, or equivalent. Ammonia will be measured using a Thermo Orion Model 720Aplus pH/ISE meter, or equivalent. EPA and ASTM recommend ammonia measurements at the beginning and end of the test (1, 2). Additional measurements of ammonia in overlying water will be made weekly during the study resulting in measurements on Days 0, 7, 14, 21, and at test termination (Day 28). The frequency of ammonia measurements will be evaluated during a non-GLP exploratory range-finding test to determine if additional measurements are necessary. Any change from the weekly frequency of ammonia measurements during the definitive test will be amended to the protocol.

Test Procedures and Biological Measurements

After the settling period for the water/sediment systems, one to two 7 to 14 day old amphipods will be impartially distributed to transfer containers (e.g., glass beakers) until each container holds its complement of 10 individuals. The organisms in each transfer container then will be transferred below the air/water interface to the test compartments. An additional batch of 20

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individuals will be impartially selected at the beginning of the test and will be measured for dry weight. After completing the transfer of organisms, the test compartments will be maintained in temperature-controlled diluter tanks. Test compartments will be observed daily to make visual assessments of any abnormal behavior (e.g. leaving sediment, unusual swimming).

Observations will be made at test termination to determine the number of mortalities and the number of individuals exhibiting clinical signs of toxicity or abnormal behavior. On Day 28, amphipods will be segregated from the sediment and live and dead organisms will be enumerated. When the total numbers of individuals found in each replicate at test termination are fewer than the number initially placed in each replicate, then those missing will be considered dead. Dry weights of surviving amphipods in each replicate will be measured at the end of the test to evaluate effects on growth.

Sampling for Analytical Measurements

When used, samples of the stock solutions used to spike the sediment will be collected and analyzed as soon as practical after preparation. Overlying water, pore water, and sediment samples from the analytical sampling test compartments will be collected from each concentration at the beginning of the test (no more than one hour after completing the addition of organisms to the test system), on Day 7, and at the end of the test. Water samples will be collected at mid-depth in the water column. After samples of overlying water have been taken, the remainder of the overlying water will be removed from the test compartment. The remaining sediment will be collected, centrifuged, and split into separate samples of pore water and sediment. Samples will be processed immediately for analysis when possible, or placed in an appropriate storage container (e.g., glass or polypropylene bottle) and stored under the appropriate conditions (refrigeration or ambient) until analyzed. The sampling scheme is summarized below:

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PROPOSED NUMBERS OF VERIFICATION SAMPLES

Experimental Group	Day 0			Day 7			Termination (28)		
	Wat	Sed	Pore	Wat	Sed	Pore	Wat	Sed	Pore
Stocks (if needed)	5*	-	-	-	-	-	-	-	-
Control	1	1	1	1	1	1	1	1	1
Solvent Control (if needed)	1	1	1	1	1	1	1	1	1
Level 1-Low Concentration	1	1	1	1	1	1	1	1	1
Level 2	1	1	1	1	1	1	1	1	1
Level 3	1	1	1	1	1	1	1	1	1
Level 4	1	1	1	1	1	1	1	1	1
Level 5-High Concentration	1	1	1	1	1	1	1	1	1
	12	7	7	7	7	7	7	7	7

* Stock samples are collected during pretest on the day sediments are dosed and may be analyzed before Day 0.

The above numbers of samples represent those collected from the test and do not include quality control (QC) samples such as matrix blanks and fortifications prepared and analyzed during the analytical chemistry phase of the study. At the discretion of the Study Director, samples from one or more appropriate test compartments and/or stock solutions will be collected and analyzed if an analytical error in sampling or analysis is suspected. The reason for the additional samples will be described by the Study Director and documented in the raw data and final report.

Analytical Chemistry

Chemical analysis of the samples will be performed by Wildlife International, Ltd. The analytical method used will be based upon chromatographic methodology provided by the Sponsor and/or developed at Wildlife International, Ltd. The methodology used to analyze the test samples will be documented in the raw data and summarized in the final report.

Data Analysis

When the dose-response pattern allows calculation of an EC50 value, the mortality data will be analyzed using the computer software of C.E. Stephan (8). The program was designed to calculate the EC50 value and the 95% confidence interval by probit analysis, the moving average method, or binomial probability with nonlinear interpolation (9,10,11). The EC50 value will be calculated, when possible, using mortality data collected at the end of the test.

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Growth (dry weight) data will be evaluated for normality and homogeneity of variances. If the data are deemed normal with homogeneous variances, hypothesis testing using analysis of variance (ANOVA) and multiple means tests (e.g., Dunnett's, Bonferroni, Scheffe) will be used (11,12). If the data fail the tests for normality or homogeneity, then data transformations will be tried in an attempt to correct the condition. When the data transformations fail to correct for non-normality or heterogeneity of variances, nonparametric procedures will be used to identify statistically significant differences among the experimental groups. Additional analysis of data may be conducted if deemed appropriate by the Study Director. The results of the analysis will be documented in the raw data and summarized in the final report.

RECORDS TO BE MAINTAINED

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

1. A copy of the signed protocol.
2. Identification and characterization of the test substance, if provided by the Sponsor.
3. Dates of initiation and termination of the test.
4. *Hyalella azteca* culture records.
5. Stock solution calculation and preparation, if applicable, and calculation and preparation of test concentrations.
6. Biological observations.
7. Test conditions (light intensity, photoperiod, etc.).
8. Water chemistry results (e.g., hardness and alkalinity).
9. Statistical calculations, if applicable.
10. The methods used to analyze test substance concentrations and the results of analytical measurements, if applicable.
11. Copy of final report.

FINAL REPORT

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

1. Name and address of the facility performing the study.

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2. Dates upon which the study was initiated and completed, and the definitive experimental start and termination dates.
3. A statement of compliance signed by the Study Director addressing any exceptions to Good Laboratory Practice Standards.
4. Objectives and procedures, as stated in the approved protocol, including all changes to the protocol.
5. The test substance identification including name, chemical abstract number or code number, strength, purity, composition, and other information provided by the Sponsor.
6. Stability and solubility of the test substance under the conditions of administration, if provided by the Sponsor.
7. A description of the methods used to conduct the test.
8. A description of the test organisms, including the source, scientific name, age or life stage, feed types, light intensity and photoperiod.
9. A description of the preparation of the test solutions.
10. The methods used to allocate organisms to test chambers and begin the test, the number of organisms and compartments per chamber per treatment, and the duration of the test.
11. A description of circumstances that may have affected the quality or integrity of the data.
12. The name of the Study Director and the names of other scientists, professionals, and supervisory personnel involved in the study.
13. A description of the transformations, calculations, and operations performed on the data, a summary and analysis of the biological data and analytical chemistry data, and a statement of the conclusions drawn from the analyses.
14. Statistical methods used to evaluate the data.
15. The signed and dated reports of each of the individual scientists or other professionals involved in the study, if applicable.
16. The location where raw data and final report are to be stored.

CHANGES TO PROTOCOL

Planned changes to the protocol will be in the form of written amendments signed by the Study Director and approved by 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

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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 and/or Part 792); OECD Principles of Good Laboratory Practices (ENV/MC/CHEM(98) 17); and Japan MAFF (11 NohSan, Notification No. 6283, Agricultural Production Bureau, 1 October 1999). 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.

Wildlife International, Ltd.

REFERENCES

1 **U.S. Environmental Protection Agency. 2000.** *Methods for Measuring the Toxicity and Bioaccumulation of Sediment Associated Contaminants with Freshwater Invertebrates.* EPA/600/R-99/064. 254 p.

2 **ASTM Standard E 1706-00. 2000.** *Test Method for Measuring the Toxicity of Sediment-Associated Contaminants with Fresh Water Invertebrates.* American Society for Testing and Materials.

3 **U.S. Environmental Protection Agency.** 1996. Series 850 – Ecological Effects Test Guidelines (*draft*), OPPTS Number 850.1735: *Whole Sediment Acute Toxicity Invertebrates, Freshwater.*

4 **OECD Guideline 218.** 2004. *Sediment-Water Chironomid Toxicity Test Using Spiked Sediment.* Organization for Economic Cooperation and Development. Adopted 13 April 2004.

5 **OECD Guideline 207.** 1984. *Guideline for testing of Chemicals, Earthworm, Acute Toxicity Tests.* Organization for Economic Cooperation and Development.

6 **Kemble, N.E., F.J. Dwyer, C.G. Ingersoll, T.D. Dawson, and T.J. Norberg-King.** 1999. Tolerance of freshwater Test Organisms to Formulated Sediments for use as Control Materials in Whole-Sediment Toxicity Tests, Environ. Toxicol. Chem. 18:222-230.

7 **APHA, AWWA, WPCF.** 1998. *Standard Methods for the Examination of Water and Wastewater.* 20th Edition, American Public Health Association. American Water Works Association. Water Pollution Control Federation, New York.

8 **Stephan, C.E.** 1978. U.S. EPA, Environmental Research Laboratory, Duluth, Minnesota. Personal communication.

9 **Thompson, W.R.** 1947. *Bacteriological Reviews.* Vol. II, No. 2. Pp. 115-145.

10 **Stephan, C.E.** 1977. "Methods for Calculating an LC50," *Aquatic Toxicology and Hazard Evaluations.* American Society for Testing and Materials. Publication Number STP 634, pp 65-84.

11 **Finney, D.J.** 1971. *Statistical Methods in Biological Assay.* Second edition. Griffin Press, London.

12 **West, Inc. and D.D. Gulley.** 1996. TOXSTAT® Version 3.5. Western EcoSystems Technology, Inc., Cheyenne, Wyoming.

Wildlife International, Ltd. Project Number 439A-131

AMENDMENT TO STUDY PROTOCOL

STUDY TITLE: Tetrabromobisphenol-A (TBBPA): A Prolonged Sediment Toxicity Test with *Hyalella azteca* Using Spiked Sediment

PROTOCOL NO.: 439/022206/HYA-SED/SUB439 **AMENDMENT NO.:** 1

SPONSOR: American Chemistry Council's Brominated Flame Retardant Industry Panel **PROJECT NO.:** 439A-131

EFFECTIVE DATE: April 19, 2006

AMENDMENT: Environmental Conditions, Page 9

CHANGE: Dissolved oxygen will be measured in the overlying water from one alternating replicate of each experimental group daily from the beginning to the end of the test, and at least three times per week.

TO: Dissolved oxygen will be measured in the overlying water from one alternating replicate of each experimental group at the beginning and end of the test, and at least three times per week.

REASON: Typographical error.

AMENDMENT: Final Report, Page 13

ADD: 17. A statement prepared by the Quality Assurance Unit listing the dates that study inspections and audits were made and the dates the findings were reported to the Study Director and Management.

18. If it is necessary to make corrections or additions to a final report after it has been accepted, such changes will be made in the form of an amendment issued by the Study Director. The amendment will clearly identify the part of the final report that is being amended and the reasons for the amendment, and will be signed by the Study Director.

REASON: To add final report requirements, that were inadvertently not in the original protocol.

STUDY DIRECTOR — DATE: 06 June 06

LABORATORY MANAGEMENT — DATE: 6/6/06

Wildlife International, Ltd.

Project No.: 439A-131
Page 1 of 1

DEVIATION TO STUDY PROTOCOL

STUDY TITLE: A Prolonged Sediment Toxicity Test with Hyalella azteca Using Spiked Sediment

PROTOCOL NO.: 439/022206/HYA-SED/SUB439 **DEVIATION NO.:** 1

SPONSOR: American Chemistry Council's Brominated Flame Retardant Industry Panel **PROJECT NO.:** 439A-131

DATE OF DEVIATION: April 14, 2006

DEVIATION: The formulated sediment used in the study had a percent organic carbon content of 5.7. The protocol required the organic carbon content be 5% ± 0.5%.

REASON: This slight deviation from percent organic carbon content was still considered to be within an acceptable range for this study and had no adverse impact upon the results or interpretation of the study.

STUDY DIRECTOR — DATE 7/13/06

LABORATORY MANAGEMENT — DATE 7/13/06

Appendix 2

Final Report on the Chemical Characterization of TBBPA

ppr. 9/24/03

CERTIFIED TRUE COPY

ALBEMARLE CORPORATION
RESEARCH AND DEVELOPMENT DEPARTMENT

FINAL REPORT ON THE CHEMICAL CHARACTERIZATION (IDENTITY, PURITY AND HOMOGENEITY) OF TETRABROMOBISPHENOL-A (TBBPA), WILDLIFE INTERNATIONAL, Ltd. TEST SUBSTANCE 6404, COMPOSITED FROM WILDLIFE INTERNATIONAL, Ltd. 6358, 6636 AND 6400

I.	Protocol Number:	TBBPA-08-01-2003
II.	Sponsor:	American Chemistry Council Brominated Flame Retardant Industry Panel 1300 Wilson Boulevard Arlington, Virginia 22209 Study Monitor: Wendy K. Sherman
III.	Analytical Testing Facilities:	Albemarle Corporation Process Development Center P. O. Box 341 Baton Rouge, LA 70821 Study Chemist: Paul F. Ranken, Ph. D.
IV.	Date of Study Initiation: Date of Study Completion:	August 12, 2003 September 24, 2003
V.	Test Article:	Tetrabromobisphenol-A (Wildlife International, Ltd. Test Substance 6404). The test article is a composite of Wildlife International, Ltd. 6358, 6368, and 6400, which are samples of commercial products from Albemarle Corporation, Great Lakes Chemical Corporation and the Dead Sea Bromine Group, respectively. The composite was prepared by Wildlife International Ltd., Easton, MD 21601.
VI.	Objective/Methodology:	This study was initiated to confirm the identity of the test article, to demonstrate the homogeneity of the test article and to demonstrate the purity of the test article. The identity of one sample of the test article, designated Characterization Sample, was confirmed by Fourier Transform Infrared

Spectroscopy using SOP No. ARS-284-R4. In this procedure, the sample infrared spectrum was compared to a standard reference spectrum of TBBPA. The homogeneity of the test article was demonstrated by determining the purity of six separate test article samples, which were taken from the top, middle and bottom of the right side of the bulk container and from the top, middle and bottom of the left side of the bulk container. The purity (area % TBBPA) of the six samples was determined by High Performance Liquid Chromatography (HPLC) using SOP No. ARS-443-R2. The homogeneity of the test article was established by demonstrating that each of the six samples had a purity (area % TBBPA) that was equal to or less than a 5% difference from the average TBBPA area % of the six samples. The six test article samples were further characterized by measuring the concentration (area%) of three potential impurities: tribromophenol, tribromobisphenol-A and o,p-tetrabromobisphenol-A. Chain of Custody and sample handling were conducted according to established standard operating procedures.

VII. Results:

Table 1 and Table 2 contain the test article analytical data from the study. The identity of the test article was confirmed by Fourier Transform Infrared Spectroscopy. The homogeneity of the test article was confirmed by HPLC analysis; all six test article samples had the same purity (<5% difference of the TBBPA area% for each sample compared to the average TBBPA area% of the six samples). Further characterization of the six test article samples was accomplished by measuring the concentration of the three expected impurities. There were no circumstances that may have affected the quality or integrity of the data.

VIII.	Regulatory Requirements:	The study conformed to the requirements of EPA TSCA (40 CFR Part 792) Good Laboratory Practice Regulations and the OECD [C(97)186/Final] Good Laboratory Practice Regulations.
IX.	Data/Record Retention:	All original data, spectra and reports will be forwarded to the Quality Assurance Unit (QAU) for a final review prior to filing in the designated Health and Environment archives at Albemarle Corporation, Health and Environment Department, 451 Florida Street, Baton Rouge, LA 70801.

Paul F. Ranken September 24, 2003

Paul F. Ranken, Ph. D. DATE
STUDY CHEMIST

Table 1. CONCLUSIONS AND TEST ARTICLE ANALYTICAL DATA.

CHEMICAL NAME: Tetrabromobisphenol-A
C.A.S. No.: 79-94-7
MOLECULAR FORMULA: $C_{15}H_{12}Br_4O_2$
PHYSICAL FORM: White Powder
CHEMICAL STRUCTURE:

ANALYSIS	RESULTS		ANALYSIS DATES	ANALYST
HPLC	Average	Difference (%) from average		
Sample	Purity (area% TBBPA)			
middle right	99.21	99.20 <5%	09/18/03	J. S. Arroyave
middle left	99.19	99.20 <5%	09/18/03	J. S. Arroyave
bottom right	99.20	99.20 <5%	09/18/03	J. S. Arroyave
top right	99.19	99.20 <5%	09/18/03	J. S. Arroyave
top left	99.19	99.20 <5%	09/18/03	J. S. Arroyave
bottom left	99.20	99.20 <5%	09/18/03	J. S. Arroyave
FTIR	The sample FT-IR spectrum matched that of the reference spectrum. All spectra are on file with the original data.		08/19/03	W. T. Cobb
CONCLUSION: Based on these analytical data, the test article identity was confirmed as tetrabromobisphenol-A. The composite sample was shown to be homogeneous with a purity of 99.20%.				

Table 2. Characterization of Test Article by HPLC (Area%)

	TBBPA	Tribromophenol	TriBPA	o,p-TBBPA
Middle Right	99.21	0.02	0.74	0.03
Middle Left	99.19	0.02	0.76	0.03
Bottom Right	99.20	0.02	0.75	0.03
Top Right	99.19	0.02	0.76	0.03
Top Left	99.19	0.02	0.76	0.03
Bottom Left	99.20	0.02	0.75	0.03
Average	99.20	0.02	0.75	0.03

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PFR 02/18/05

ALBEMARLE CORPORATION
RESEARCH AND DEVELOPMENT DEPARTMENT

FINAL REPORT ON THE CHEMICAL CHARACTERIZATION (IDENTITY, PURITY AND HOMOGENEITY) OF TETRABROMOBISPHENOL-A (TBBPA), WILDLIFE INTERNATIONAL, Ltd. TEST SUBSTANCE 6404, COMPOSITED FROM WILDLIFE INTERNATIONAL, Ltd. 6358, 6636 AND 6400

FINAL REPORT AMENDMENT

Date:	February 18, 2005
Section to be changed:	Title
Change:	Final Report On The Chemical Characterization (Identity, Purity, And Homogeneity) Of Tetrabromobisphenol-A (TBBPA), Wildlife International, Ltd. Test Substance 6404, Composited From Wildlife International, Ltd. 6358, 6368 and 6400
Reason for change:	A typographical error was made in the Final Report title. The change removes the incorrect number (6636) describing the composite composition and replaces it with the correct number (6368). The change does not alter the conclusions of the original Final Report.

Feb. 18, 2005 — Paul F. Ranken

Date — Paul F. Ranken
Study Chemist

ALBEMARLE®

June 21, 2006

Mr. Hank Kruger
Wildlife International Ltd.
8598 Commerce Drive
Easton, MD 21601

Re: TBBPA Composite 6404

Dear Mr. Kruger:

The purpose of this memo is to inform you that the expiration date for the American Chemistry Council, Brominated Flame Retardant Industry Panel TBBPA Composite (Wildlife International, Ltd. Test Substance 6404) has been extended to July 17, 2007. Please continue storing this material in a closed container at room temperature.

If you have any questions, please do not hesitate to call me at 225-359-2977.

Regards,

Paul F. Ranken
Study Chemist

cc: Marcia Hardy, HS&E

Appendix 3

Formulated Sediment Preparation[1]

Constituents	Weight (g)
Quartz Sand	30,800
Kaolin Clay	4,000
Alpha Cellulose	5,200
Dolomite	200
Humic Acid	4

[1] The constituents were mixed in PK Twinshell mixer for 20 minutes and the dry sediment was stored under ambient conditions until used. The sediment pH was 6.5.

Appendix 4

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

	Mean	Range
Specific Conductance (µmhos/cm)	301 (N = 4)	295 - 310
Hardness (mg/L as $CaCO_3$)	134 (N = 4)	128 – 136
Alkalinity (mg/L as $CaCO_3$)	182 (N = 4)	180-184
pH	8.0 (N = 4)	7..9 – 8.0

Appendix 5

Analyses of Pesticides, Organics and Metals in Wildlife International, Ltd. Well Water

Pesticides and Organics			
Component	Measured Concentration (µg/L)	Component	Measured Concentration (µg/L)
Aldrin	< 0.019	Heptachlor	< 0.0096
Alpha BHC	< 0.0096	Heptachlor Epoxide	< 0.0096
Alpha Chlordane	< 0.0096	Kepone	< 0.19
Beta BHC	< 0.038	Malathion	< 1.9
Bolstar	< 1.9	Merphos	< 1.9
Chlordane	< 0.48	Methoxychlor	< 0.096
Coumaphos	< 2.9	Methyl Parathion	< 1.9
Delta BHC	< 0.0096	Mevinphos	< 1.9
Demeton-O	< 1.9	Mirex	< 0.11
Demeton-S	< 1.9	Naled	< 2.9
Diazinon	< 1.9	o,p-DDD	< 0.019
Dichlorvos	< 1.9	o,p-DDE	< 0.019
Dieldrin	< 0.029	o,p-DDT	< 0.019
Disulfoton	< 1.9	p,p-DDD	< 0.019
Dursban (Chlorpyrifos)	< 1.9	p,p-DDE	< 0.019
Endosulfan I	< 0.0096	p,p-DDT	< 0.019
Endosulfan II	< 0.019	PCB-1016	< 0.48
Endosulfan Sulfate	< 0.019	PCB-1221	< 0.48
Endrin	< 0.019	PCB-1232	< 0.48
Endrin Aldehyde	< 0.096	PCB-1242	< 0.48
Endrin Ketone	< 0.019	PCB-1248	< 0.48
EPN	< 3.8	PCB-1254	< 0.48
Ethion	< 1.9	PCB-1260	< 0.48
Ethoprop	< 1.9	Phorate	< 1.9
Ethyl Parathion	< 1.9	Ronnel	< 1.9
Famphur	< 1.9	Stirophos	< 1.9
Fensulfothion	< 3.8	Telodrin	< 0.0096
Fenthion	< 1.9	Tokuthion	< 1.9
Gamma BHC – Lindane	< 0.0096	Toxaphene	< 0.96
Gamma Chlordane	< 0.096	Trichloronate	< 1.9
Guthion (Azinphos-methyl)	< 3.8	Trithion	< 1.9
HCB	< 0.096		

[1]Analyses performed by Lancaster Laboratories on samples collected on December 15, 2005.

Appendix 5 (Continued)

Analyses of Pesticides, Organics and Metals in Wildlife International, Ltd. Well Water[1]

Metals			
Component	Measured Concentration (mg/L)	Component	Measured Concentration (mg/L)
Aluminum	< 0.200	Magnesium	13.3
Antimony	< 0.0200	Manganese	< 0.0050
Arsenic	< 0.0200	Mercury	< 0.00020
Barium	< 0.0050	Nickel	< 0.0100
Beryllium	< 0.0050	Nitrate Nitrogen	< 0.50
Bromide	< 2.5	Nitrite Nitrogen	< 0.50
Cadmium	< 0.0050	Potassium	7.65
Calcium	33.1	Selenium	< 0.0200
Chloride	2.7	Silver	< 0.0050
Chromium	< 0.0150	Sodium	19.1
Cobalt	< 0.0050	Sulfate	< 5.0
Copper	< 0.0100	Thallium	< 0.0200
Fluoride	0.56	Vanadium	< 0.0050
Iron	< 0.200	Zinc	< 0.0200
Lead	< 0.0200		

[1]Analyses performed by Lancaster Laboratories on samples collected on December 15, 2005.

Appendix 6

The Analysis of TBBPA in Water and Sediment

Appendix 6.1

Analytical Method Flowchart for the Analysis of TBBPA in Sediment

METHOD OUTLINE FOR THE ANALYSIS OF
TBBPA IN SEDIMENT

Rinse 8 oz French square bottles with acidified methanol (0.1% phosphoric acid).
↓

Transfer the requisite volume of sediment to the French square bottles. QC samples are fortified with the appropriate Tetrabromobisphenol A stock solution or the test substance at this time. Unfortified sediment will serve for the matrix blank.
↓

Using a class A volumetric pipet, add 100 mL of acidified methanol to each sample.
↓

Sonic disrupt the samples for approximately five minutes.
↓

Transfer an aliquot of each sample into microcentrifuge tubes and centrifuge for approximately five minutes.
↓

Volumetrically dilute the centrifuged methanol extract using the requisite volume of 50% methanol : 50% water.
↓

Transfer an aliquot of each diluted extract to an autosampler vial.
Submit samples for HPLC/UV analysis.

Appendix 6.2

Typical HPLC Operational Parameters

INSTRUMENT:	Agilent Series 1100 High Performance Liquid Chromatograph (HPLC) with an Agilent Series 1100 Variable Wavelength Detector
ANALYTICAL COLUMN:	YMC PACK ODS-AM Column (150 mm x 4.6 mm, 3 µm particle size)
OVEN TEMPERATURE:	40°C
STOP TIME:	15 minutes
FLOW RATE:	1.000 mL/minute
SOLVENT A:	0.1% H_3PO_4
SOLVENT B:	CH_3CN

GRADIENT PROFILE:

Time (min)	%A	%B	Flow (mL/min)
0.01	90.0	10.0	1.000
1.00	90.0	10.0	1.000
8.00	2.0	98.0	1.000
10.00	2.0	98.0	1.000
10.10	90.0	10.0	1.000
15.00	90.0	10.0	1.000

INJECTION VOLUME:	100 µL	
TBBPA RETENTION TIME:	Sediment:	Approximately 10 minutes
	Water:	Approximately 12 minutes
PRIMARY ANALYTICAL WAVELENGTH:	286 nm	

Appendix 6.3

Example Calculations for a Representative Sediment Sample

The analytical result and percent recovery for sample number 439A-131-S-4, with a nominal concentration of 125 mg a.i./Kg, were calculated using the following equations:

$$\text{TBBPA in sample (mg a.i./Kg)} = \frac{\text{Peak Area - Y-intercept}}{\text{Slope}} \text{ X Dilution Factor} \div \text{Dry/Wet Soil Mass}$$

$$\text{Percent of nominal concentration} = \frac{\text{measured concentration of TBBPA in sample (mg a.i./Kg)}}{\text{nominal concentration of TBBPA in sample (mg a.i./Kg)}} \text{ X } 100$$

Peak Area = 30.21689
Y-intercept = -0.22939
Slope = 60.53
Dilution factor = 167
Soil = Dry Soil Mass (g)/Wet Soil Mass (g) = 7.809 g/11.50 g = 0.679

$$\text{TBBPA in sample (mg a.i./Kg)} = \frac{30.21689 + 0.22939}{60.53} \text{ X } \frac{167}{0.679}$$

Concentration of TBBPA in sample (mg a.i./Kg) = 123

$$\text{Percent of nominal concentration} = \frac{123 \text{ mg a.i./Kg}}{125 \text{ mg a.i./Kg}} \text{ X } 100$$

Percent of nominal concentration = 98.8%*

* Results were generated using Excel 2000 in the full precision mode. Manual calculations may differ slightly.

Appendix 6.4

Analytical Stocks and Standards Preparation

A stock solution of TBBPA was prepared by weighing 0.1008 g (corrected for purity) of the test substance on an analytical balance. The test substance was transferred to a 100-mL class A volumetric flask and brought to volume using methanol. This primary stock solution contained 1.00 mg a.i./mL of TBBPA. The primary stock solution was serially diluted with methanol to prepare stock solutions at concentrations of 0.100, 0.0100 and 0.00100 mg a.i./mL. For sediment analysis, matrix fortifications were prepared using the 1.00 mg a.i./mL stock solution. For overlying and pore water analyses, matrix fortifications were prepared using the 0.100 mg a.i./mL stock solution.

Calibration standards were prepared in 50% CH_3OH : 50% H_2O using the 0.100 mg a.i./mL stock solution. The following shows the dilution scheme for the set of calibration standards:

Stock Concentration (mg a.i./mL)	Aliquot (µL)	Final Volume (mL)	Standard Concentration (mg a.i./mL)
0.100	100	100	0.100
0.100	250	100	0.250
0.100	500	100	0.500
0.100	750	100	0.750
0.100	1000	100	1.00

Appendix 6.5

Matrix Fortifications Analyzed Concurrently During Sample Analyses in Sediment

Sample Number (439A-131-)	Sampling Interval (Day)	Concentrations of TBBPA		Percent Recovery[2]
		Fortified (mg a.i./Kg)	Measured[1] (mg a.i./Kg)	
SMAS-1	0	60.0	59.1	98.4
SMAS-2	0	994	780	78.5
SMAS-3	7	60.0	59.3	98.9
SMAS-4	7	1000	961	96.1
SMAS-5	28	60.0	59.7	99.4
SMAS-6	28	10000	9963[3]	99.6
			Mean =	95.2
			Standard Deviation =	8.25
			CV =	8.67%

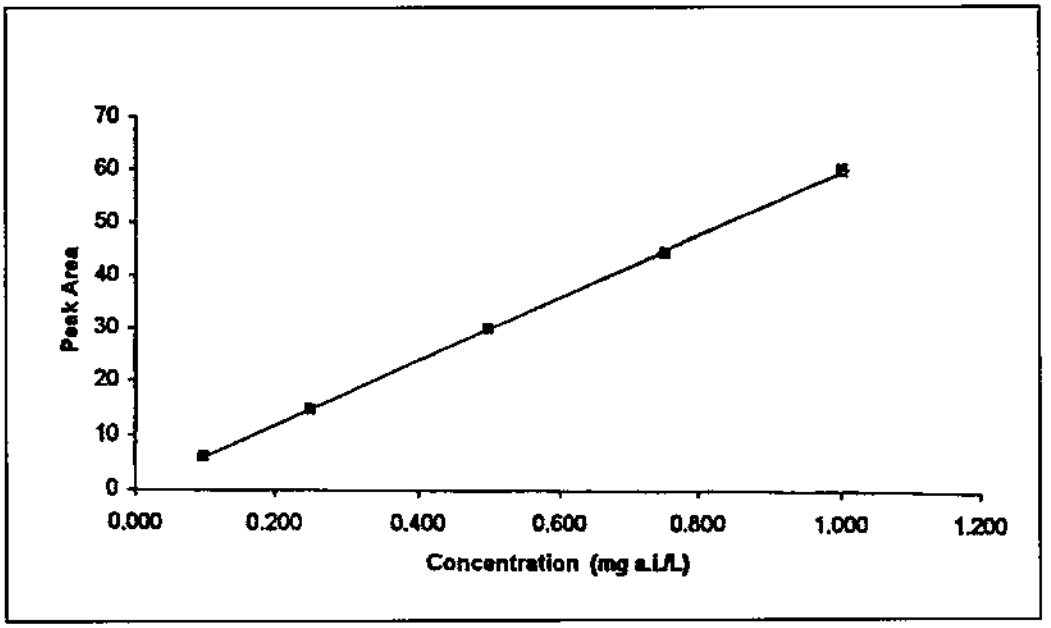
[1] The limit of quantitation (LOQ) was 10.0 mg a.i./Kg, calculated as the product of the concentration of the lowest calibration standard (0.100 mg a.i./L) and the dilution factor of the control samples (100).

[2] Results were generated using Excel 2000 in the full precision mode. Manual calculations may differ slightly.

[3] The analytical result is correctly reported. The QC sample used to fortify sediment on Day 28 was inadvertently fortified with the incorrect stock (10,000 instead of 1000 mg a.i./Kg). When noted, the extract was diluted into the range of the standard curve and reanalyzed.

Appendix 6.6

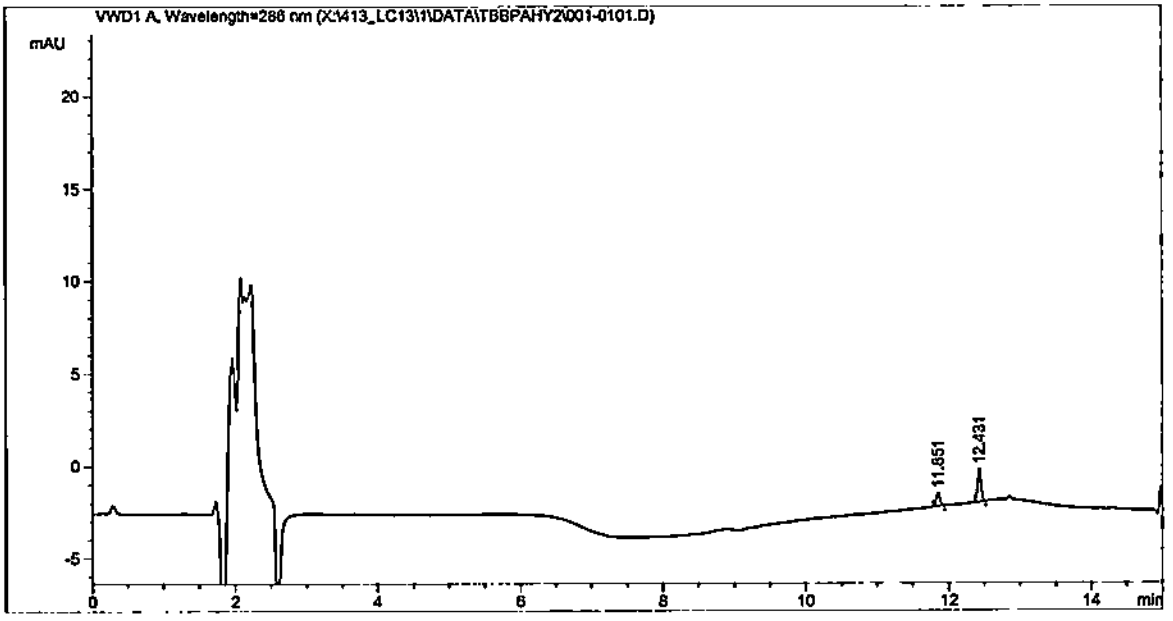
Representative Calibration Curve for TBBPA in Sediment



Slope = 60.53; Y-Intercept = -0.22939; $R^2 = 0.9998$

Appendix 6.7

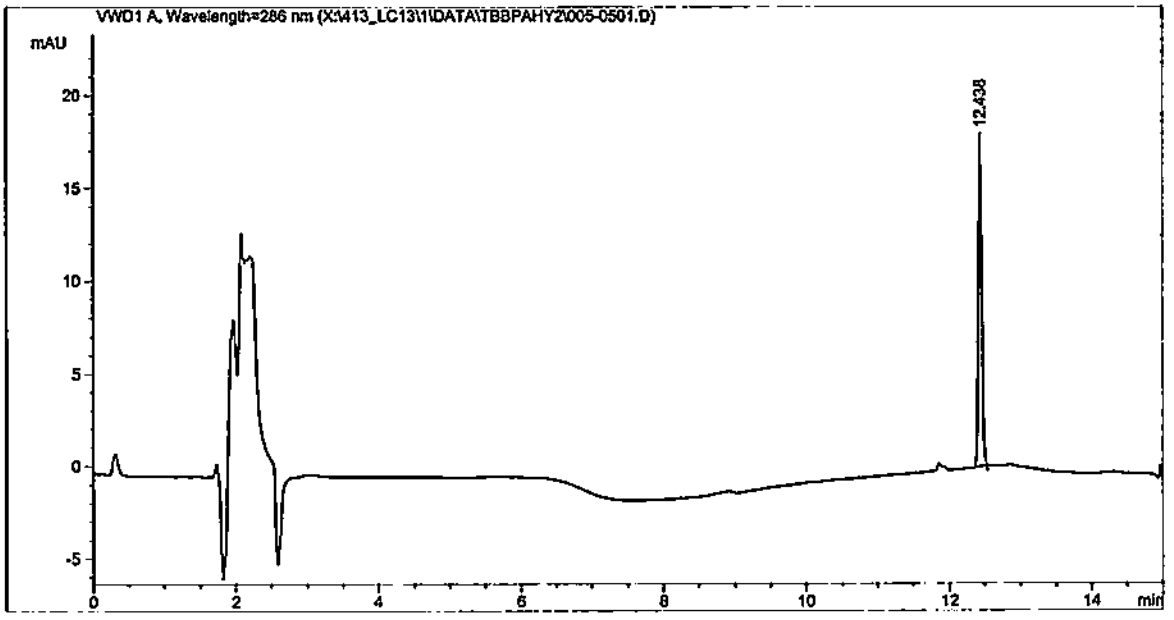
Chromatogram of a Low-level TBBPA Calibration Standard for Sediment Analyses



Nominal concentration: 0.100 mg a.i./L

Appendix 6.8

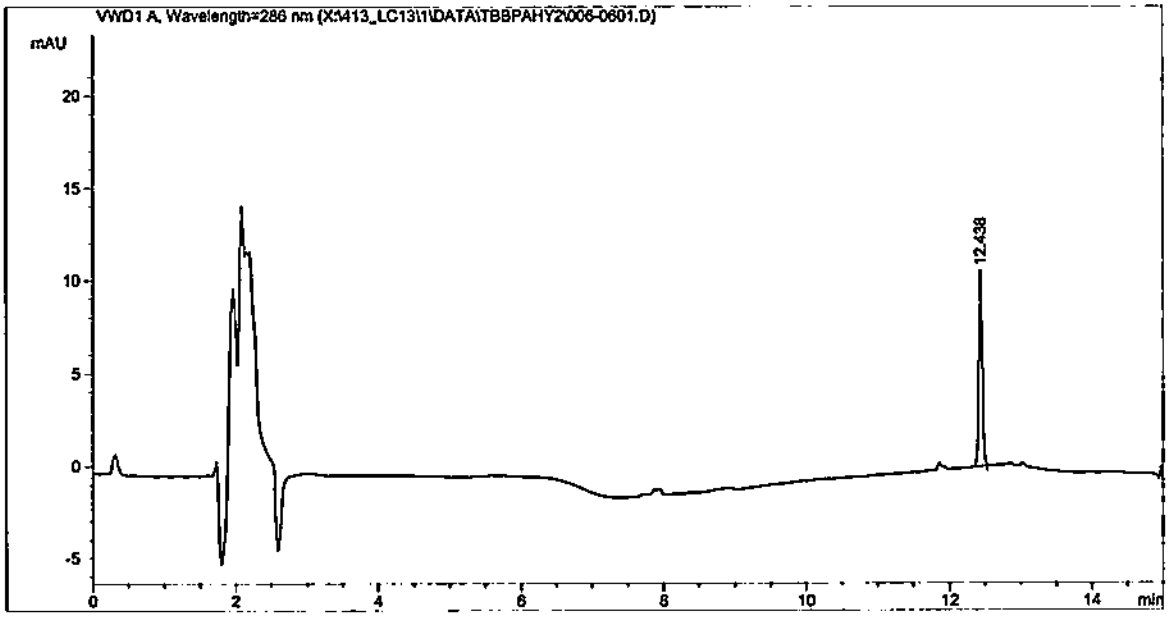
Chromatogram of a High-level TBBPA Calibration Standard for Sediment Analyses



Nominal concentration: 1.00 mg a.i./L

Appendix 6.9

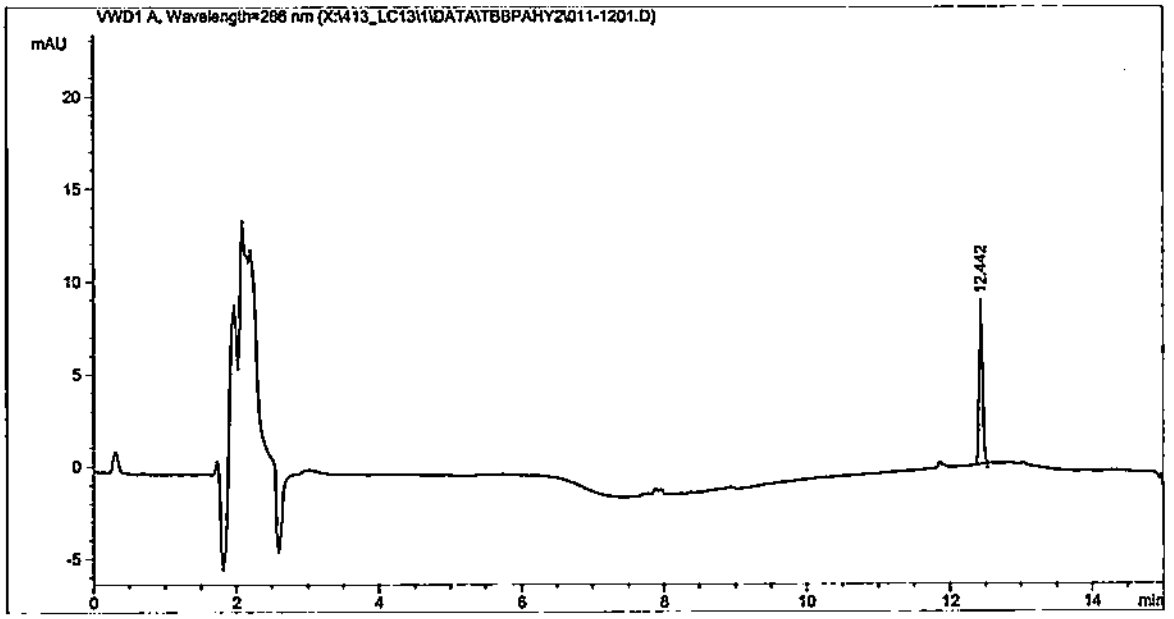
Chromatogram of a Matrix Fortification in Sediment



Sample number 439A-131-SMAS-1; 60.0 mg a.i./Kg, nominal concentration.

Appendix 6.10

Chromatogram of a Day 0 Sediment Sample



Sample number 439A-131-S-4; 125 mg a.i./Kg nominal concentration

Appendix 6.11

Analytical Method Flowchart for the Analysis of TBBPA in Freshwater

METHOD OUTLINE FOR THE ANALYSIS OF TBBPA IN FRESHWATER

Dilute samples in equal volume of methanol, secondary dilutions in 50% methanol : 50% HPLC-grade bottled water using gas-tight syringes and culture tubes.

↓

Ampulate and submit samples for analysis.

Appendix 6.12

Matrix Fortifications Analyzed Concurrently During Sample Analyses in Freshwater

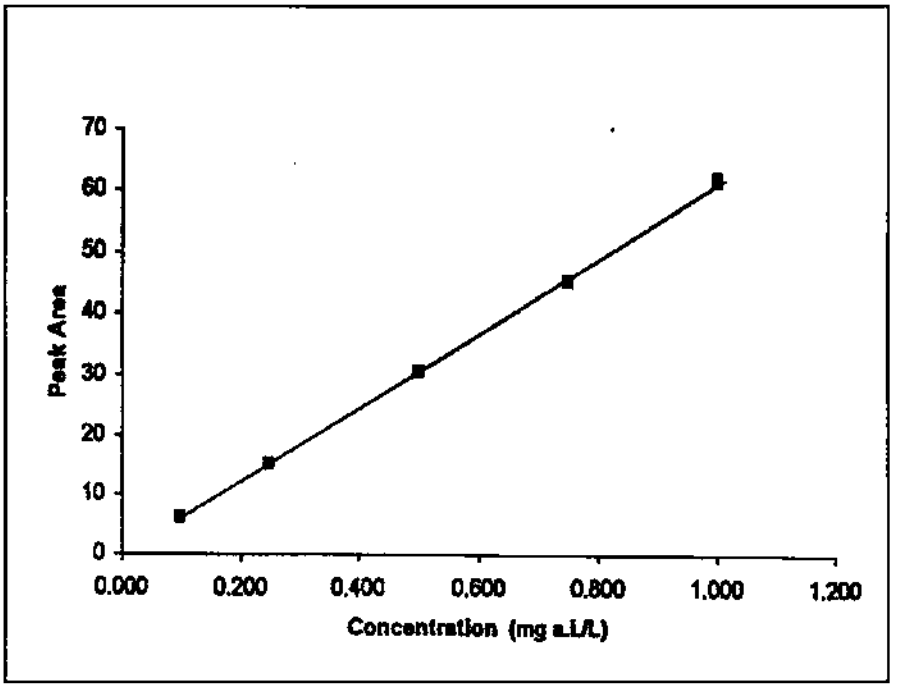
Sample Number (439A-131-)	Sampling Interval (Day)	Concentrations of TBBPA		Percent Recovery[2]
		Fortified (mg a.i./L)	Measured[1] (mg a.i./L)	
WMAS-1	0	0.500	0.527	105
WMAS-2	0	1.00	1.04	104
WMAS-3	7	0.500	0.537	107
WMAS-4	7	1.00	1.11	111
WMAS-5	28	0.500	0.534	107
WMAS-6	28	1.00	1.05	105
			Mean =	107
			Standard Deviation =	2.51
			CV =	2.35%

[1] The limit of quantitation (LOQ) was 0.200 mg a.i./L, calculated as the product of the concentration of the lowest calibration standard (0.100 mg a.i./L) and the dilution factor of the matrix blank samples (2.00).

[2] Results were generated using Excel 2000 in the full precision mode. Manual calculations may differ slightly.

Appendix 6.13

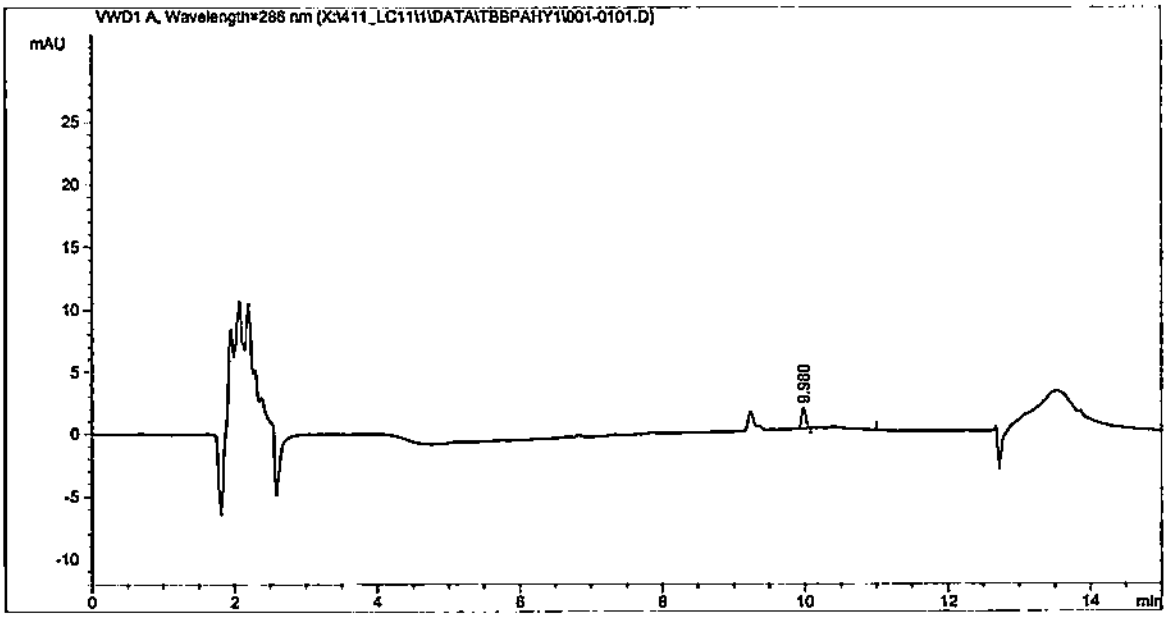
Representative Calibration Curve for TBBPA in Freshwater



Slope = 61.84; Y-Intercept = -0.29470; $R^2 = 0.9995$

Appendix 6.14

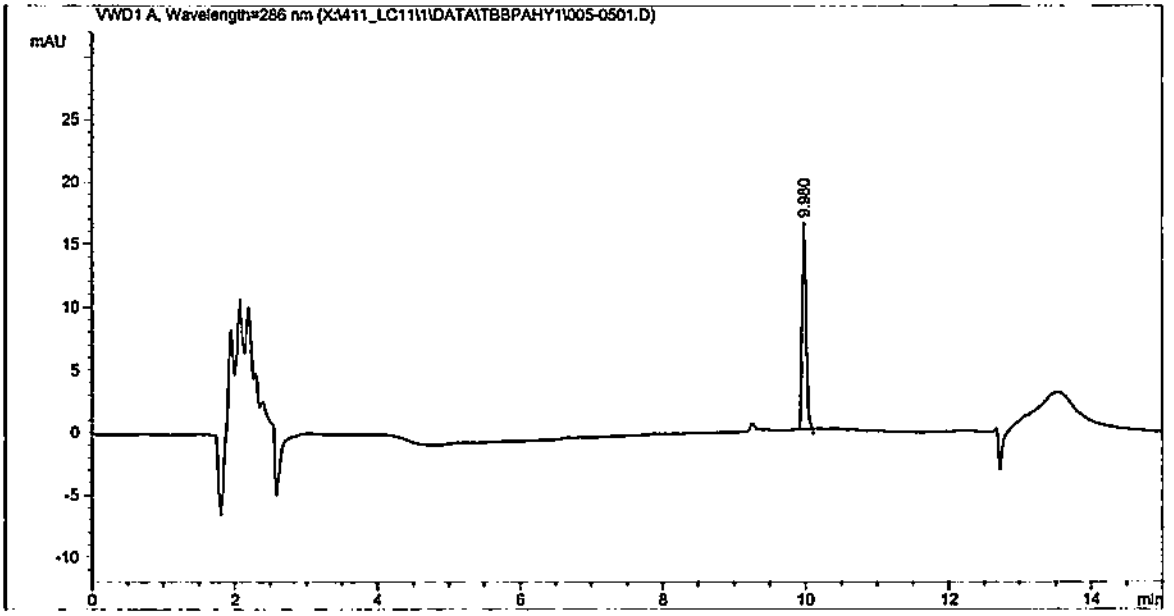
Chromatogram of a Low-level TBBPA Calibration Standard for Freshwater Analyses



Nominal concentration: 0.100 mg a.i./L

Appendix 6.15

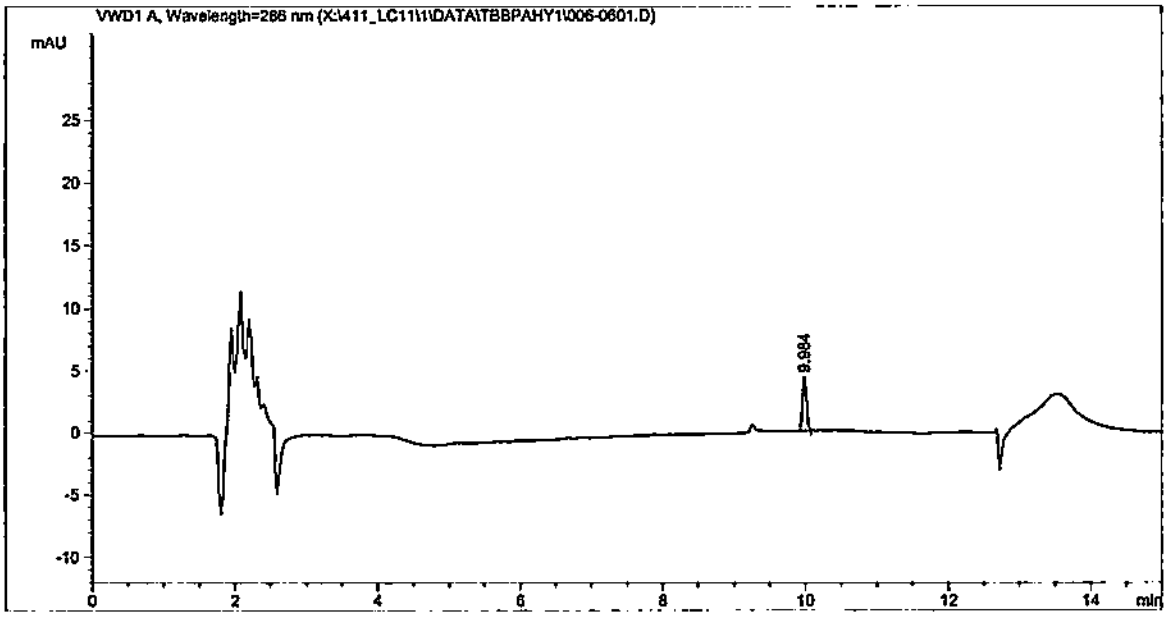
Chromatogram of a High-level TBBPA Calibration Standard for Freshwater Analyses



Nominal concentration: 1.00 mg a.i./L

Appendix 6.16

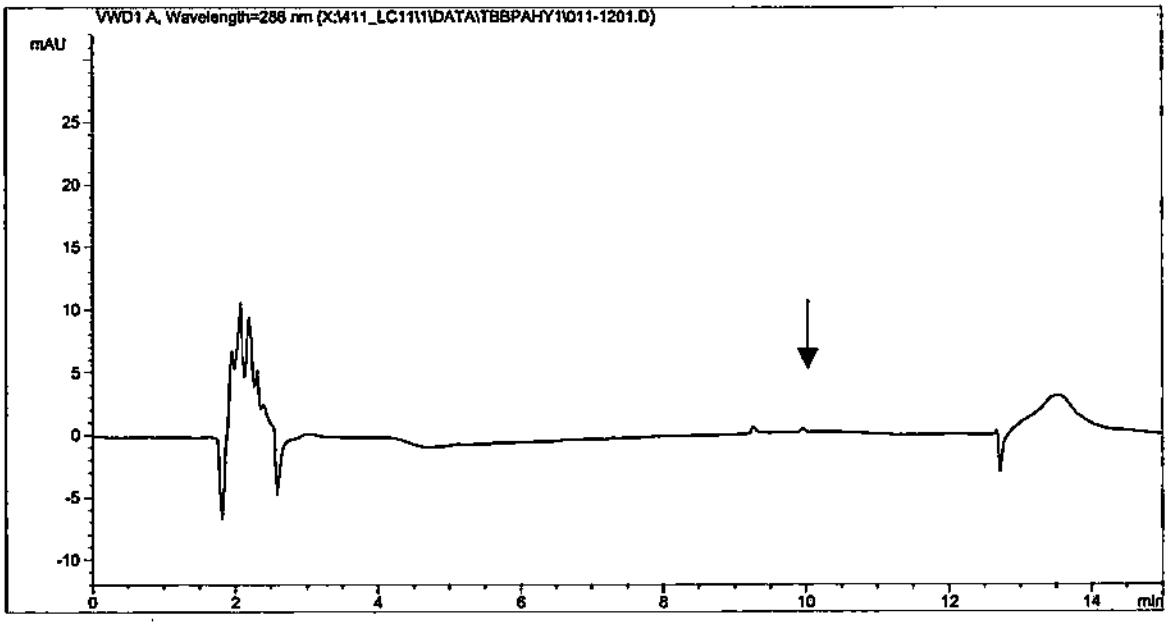
Chromatogram of a Matrix Fortification in Freshwater



Sample number 439A-131-WMAS-1; 0.500 mg a.i./L nominal concentration.

Appendix 6.17

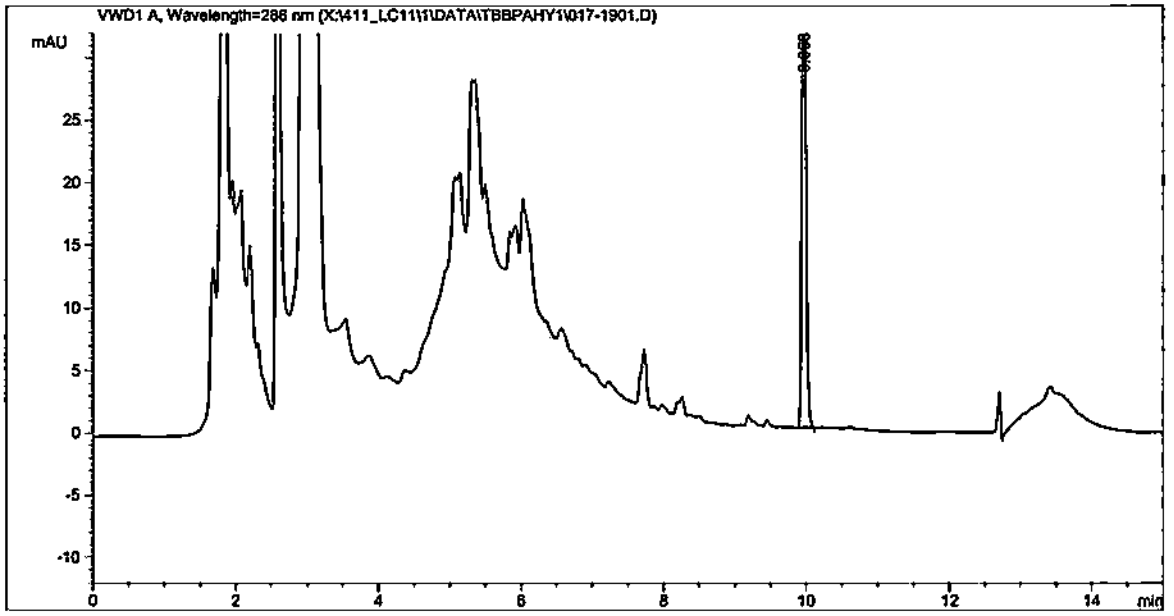
Chromatogram of a Day 0 Overlying Water Sample



Sample number 439A-131-W-4; 125 mg a.i./Kg nominal concentration. The arrow indicates the approximate retention time of TBBPA.

Appendix 6.18

Chromatogram of a Day 0 Pore Water Sample



Sample number 439A-131-PW-3; 63 mg a.i./Kg nominal concentration.

Appendix 7

Observations[1] of Effects by Replicate in a Prolonged Sediment Toxicity Test with the Amphipod (*Hyalella azteca*)

Nominal Concentration (mg a.i./Kg)	Replicate	Number Exposed	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Negative Control	A	10	AN	AN	AN	AN	AN	AN	AN
	B	10	AN	AN	AN	AN	AN	AN	AN
	C	10	AN	AN	AN	AN	AN	AN	AN
	D	10	AN	AN	AN	AN	AN	AN	AN
	E	10	AN	AN	AN	AN	AN	AN	AN
	F	10	AN	AN	AN	AN	AN	AN	AN
	G	10	AN	AN	AN	AN	AN	AN	AN
	H	10	AN	AN	AN	AN	AN	AN	AN
Solvent Control	A	10	AN	AN	AN	AN	AN	AN	AN
	B	10	AN	AN	AN	AN	AN	AN	AN
	C	10	AN	AN	AN	AN	AN	AN	AN
	D	10	AN	AN	AN	AN	AN	AN	AN
	E	10	AN	AN	AN	AN	AN	AN	AN
	F	10	AN	AN	AN	AN	AN	AN	AN
	G	10	AN	AN	AN	AN	AN	AN	AN
	H	10	AN	AN	AN	AN	AN	AN	AN

[1]Observations: AN = appear normal.

Appendix 7 (Continued)

Observations[1] of Effects by Replicate in a Prolonged Sediment Toxicity Test with the Amphipod (*Hyalella azteca*)

Nominal Concentration (mg a.i./Kg)	Replicate	Number Exposed	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
63	A	10	AN	AN	AN	AN	AN	AN	AN
	B	10	AN	AN	AN	AN	AN	AN	AN
	C	10	AN	AN	AN	AN	AN	AN	AN
	D	10	AN	AN	AN	AN	AN	AN	AN
	E	10	AN	AN	AN	AN	AN	AN	AN
	F	10	AN	AN	AN	AN	AN	AN	AN
	G	10	AN	AN	AN	AN	AN	AN	AN
	H	10	AN	AN	AN	AN	AN	AN	AN
125	A	10	AN	AN	AN	AN	AN	AN	AN
	B	10	AN	AN	AN	AN	AN	AN	AN
	C	10	AN	AN	AN	AN	AN	AN	AN
	D	10	AN	AN	AN	AN	AN	AN	AN
	E	10	AN	AN	AN	AN	AN	AN	AN
	F	10	AN	AN	AN	AN	AN	AN	AN
	G	10	AN	AN	AN	AN	AN	AN	AN
	H	10	AN	AN	AN	AN	AN	AN	AN

[1]Observations: AN = appear normal.

Appendix 7 (Continued)

Observations[1] of Effects by Replicate in a Prolonged Sediment Toxicity Test with the Amphipod (*Hyalella azteca*)

Nominal Concentration (mg a.i./Kg)	Replicate	Number Exposed	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
250	A	10	AN	AN	AN	AN	AN	AN	AN
	B	10	AN	AN	AN	AN	AN	AN	AN
	C	10	AN	AN	AN	AN	AN	AN	AN
	D	10	AN	AN	AN	AN	AN	AN	AN
	E	10	AN	AN	AN	AN	AN	AN	AN
	F	10	AN	AN	AN	AN	AN	AN	AN
	G	10	AN	AN	AN	AN	AN	AN	AN
	H	10	AN	AN	AN	AN	AN	AN	AN
500	A	10	AN	AN	AN	AN	AN	AN	AN
	B	10	AN	AN	AN	AN	AN	AN	AN
	C	10	AN	AN	AN	AN	AN	AN	AN
	D	10	AN	AN	AN	AN	AN	AN	AN
	E	10	AN	AN	AN	AN	AN	AN	AN
	F	10	AN	AN	AN	AN	AN	AN	AN
	G	10	AN	AN	AN	AN	AN	AN	AN
	H	10	AN	AN	AN	AN	AN	AN	AN

[1]Observations: AN = appear normal.

Appendix 7 (Continued)

Observations[1] of Effects by Replicate in a Prolonged Sediment Toxicity Test with the Amphipod (*Hyalella azteca*)

Nominal Concentration (mg a.i./Kg)	Replicate	Number Exposed	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
1000	A	10	AN	AN	AN	AN	AN	AN	AN
	B	10	AN	AN	AN	AN	AN	AN	AN
	C	10	AN	AN	AN	AN	AN	AN	AN
	D	10	AN	AN	AN	AN	AN	AN	AN
	E	10	AN	AN	AN	AN	AN	AN	AN
	F	10	AN	AN	AN	AN	AN	AN	AN
	G	10	AN	AN	AN	AN	AN	AN	AN
	H	10	AN	AN	AN	AN	AN	AN	AN

[1]Observations: AN = appear normal.

Appendix 7 (Continued)

Observations[1] of Effects by Replicate in a Prolonged Sediment Toxicity Test with the Amphipod (*Hyalella azteca*)

Nominal Concentration (mg a.i./Kg)	Replicate	Number Exposed	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13
Negative Control	A	10	AN	AN	AN	AN	AN	AN	AN
	B	10	AN	AN	AN	AN	AN	AN	AN
	C	10	AN	AN	AN	AN	AN	AN	AN
	D	10	AN	AN	AN	AN	AN	AN	AN
	E	10	AN	AN	AN	AN	AN	AN	AN
	F	10	AN	AN	AN	AN	AN	AN	AN
	G	10	AN	AN	AN	AN	AN	AN	AN
	H	10	AN	AN	AN	AN	AN	AN	AN
Solvent Control	A	10	AN	AN	AN	AN	AN	AN	AN
	B	10	AN	AN	AN	AN	AN	AN	AN
	C	10	AN	AN	AN	AN	AN	AN	AN
	D	10	AN	AN	AN	AN	AN	AN	AN
	E	10	AN	AN	AN	AN	AN	AN	AN
	F	10	AN	AN	AN	AN	AN	AN	AN
	G	10	AN	AN	AN	AN	AN	AN	AN
	H	10	AN	AN	AN	AN	AN	AN	AN

[1]Observations: AN = appear normal.

Appendix 7 (Continued)

Observations[1] of Effects by Replicate in a Prolonged Sediment Toxicity Test with the Amphipod (*Hyalella azteca*)

Nominal Concentration (mg a.i./Kg)	Replicate	Number Exposed	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13
63	A	10	AN	AN	AN	AN	AN	AN	AN
	B	10	AN	AN	AN	AN	AN	AN	AN
	C	10	AN	AN	AN	AN	AN	AN	AN
	D	10	AN	AN	AN	AN	AN	AN	AN
	E	10	AN	AN	AN	AN	AN	AN	AN
	F	10	AN	AN	AN	AN	AN	AN	AN
	G	10	AN	AN	AN	AN	AN	AN	AN
	H	10	AN	AN	AN	AN	AN	AN	AN
125	A	10	AN	AN	AN	1U; AN	AN	AN	AN
	B	10	AN	AN	AN	AN	AN	AN	AN
	C	10	AN	AN	AN	AN	AN	AN	AN
	D	10	AN	AN	AN	AN	AN	AN	AN
	E	10	AN	AN	AN	AN	AN	AN	AN
	F	10	AN	AN	AN	AN	AN	AN	AN
	G	10	AN	AN	AN	AN	AN	AN	AN
	H	10	AN	AN	AN	AN	AN	AN	AN

[1]Observations: AN = appear normal; U = organism observed swimming in the water column.

Appendix 7 (Continued)

Observations[1] of Effects by Replicate in a Prolonged Sediment Toxicity Test with the Amphipod (*Hyalella azteca*)

Nominal Concentration (mg a.i./Kg)	Replicate	Number Exposed	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13
250	A	10	AN	AN	AN	AN	AN	AN	AN
	B	10	AN	AN	AN	AN	AN	AN	AN
	C	10	AN	AN	AN	AN	AN	AN	AN
	D	10	AN	AN	AN	AN	AN	AN	AN
	E	10	AN	AN	AN	AN	AN	AN	AN
	F	10	AN	AN	AN	AN	AN	AN	AN
	G	10	AN	AN	AN	AN	AN	AN	AN
	H	10	AN	AN	AN	AN	AN	AN	AN
500	A	10	AN	AN	AN	AN	AN	AN	AN
	B	10	AN	AN	AN	AN	AN	AN	AN
	C	10	AN	AN	AN	AN	AN	AN	AN
	D	10	AN	AN	AN	AN	AN	AN	AN
	E	10	AN	AN	AN	AN	AN	AN	AN
	F	10	AN	AN	AN	AN	AN	AN	AN
	G	10	AN	AN	AN	AN	AN	AN	AN
	H	10	AN	AN	AN	AN	AN	AN	AN

[1]Observations: AN = appear normal.

Appendix 7 (Continued)

Observations[1] of Effects by Replicate in a Prolonged Sediment Toxicity Test with the Amphipod (*Hyalella azteca*)

Nominal Concentration (mg a.i./Kg)	Replicate	Number Exposed	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13
1000	A	10	AN	AN	AN	AN	AN	AN	AN
	B	10	AN	AN	AN	AN	AN	AN	AN
	C	10	AN	AN	AN	AN	AN	AN	AN
	D	10	AN	AN	AN	AN	AN	AN	AN
	E	10	AN	AN	AN	AN	AN	AN	AN
	F	10	AN	AN	AN	AN	AN	AN	AN
	G	10	AN	AN	AN	AN	AN	AN	AN
	H	10	AN	AN	AN	AN	AN	AN	AN

[1]Observations: AN = appear normal.

Appendix 7 (Continued)

Observations[1] of Effects by Replicate in a Prolonged Sediment Toxicity Test with the Amphipod (*Hyalella azteca*)

Nominal Concentration (mg a.i./Kg)	Replicate	Number Exposed	Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20
Negative Control	A	10	AN	AN	AN	AN	AN	AN	AN
	B	10	AN	AN	AN	AN	AN	AN	AN
	C	10	AN	AN	AN	AN	AN	AN	AN
	D	10	AN	AN	AN	AN	AN	AN	AN
	E	10	AN	AN	AN	AN	AN	AN	AN
	F	10	AN	AN	AN	AN	AN	AN	AN
	G	10	AN	AN	AN	AN	AN	AN	AN
	H	10	AN	AN	AN	AN	AN	AN	AN
Solvent Control	A	10	AN	AN	AN	AN	AN	AN	AN
	B	10	AN	AN	AN	AN	AN	AN	AN
	C	10	AN	AN	AN	AN	AN	AN	AN
	D	10	AN	AN	AN	AN	AN	AN	AN
	E	10	AN	AN	AN	AN	AN	AN	AN
	F	10	AN	AN	AN	AN	AN	AN	AN
	G	10	AN	AN	AN	AN	AN	AN	AN
	H	10	AN	AN	AN	AN	AN	AN	AN

[1]Observations: AN = appear normal.

Appendix 7 (Continued)

Observations[1] of Effects by Replicate in a Prolonged Sediment Toxicity Test with the Amphipod (*Hyalella azteca*)

Nominal Concentration (mg a.i./Kg)	Replicate	Number Exposed	Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20
63	A	10	AN	AN	AN	AN	AN	AN	AN
	B	10	AN	AN	AN	AN	AN	AN	AN
	C	10	AN	AN	AN	AN	AN	AN	AN
	D	10	AN	AN	AN	AN	AN	AN	AN
	E	10	AN	AN	AN	AN	AN	AN	AN
	F	10	AN	AN	AN	AN	AN	AN	AN
	G	10	AN	AN	AN	AN	AN	AN	AN
	H	10	AN	AN	AN	AN	AN	AN	AN
125	A	10	AN	AN	AN	AN	AN	AN	AN
	B	10	AN	AN	AN	AN	AN	AN	AN
	C	10	AN	AN	AN	AN	AN	AN	AN
	D	10	AN	AN	AN	AN	AN	AN	AN
	E	10	AN	AN	AN	AN	AN	AN	AN
	F	10	AN	AN	AN	AN	AN	AN	AN
	G	10	AN	AN	AN	AN	AN	AN	AN
	H	10	AN	AN	AN	AN	AN	AN	AN

[1]Observations: AN = appear normal.

Appendix 7 (Continued)

Observations[1] of Effects by Replicate in a Prolonged Sediment Toxicity Test with the Amphipod (*Hyalella azteca*)

Nominal Concentration (mg a.i./Kg)	Replicate	Number Exposed	Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20
250	A	0	AN	AN	AN	AN	AN	AN	AN
	B	0	AN	AN	AN	AN	AN	AN	AN
	C	0	AN	AN	AN	AN	AN	AN	AN
	D	0	AN	AN	AN	AN	AN	AN	AN
	E	0	AN	AN	AN	AN	AN	AN	AN
	F	0	AN	AN	AN	AN	AN	AN	AN
	G	0	AN	AN	AN	AN	AN	AN	AN
	H	0	AN	AN	AN	AN	AN	AN	AN
500	A	0	AN	AN	AN	AN	AN	AN	AN
	B	0	AN	AN	AN	AN	AN	AN	AN
	C	0	AN	AN	AN	AN	AN	AN	AN
	D	0	AN	AN	AN	AN	AN	AN	AN
	E	0	AN	AN	AN	AN	AN	AN	AN
	F	0	AN	AN	AN	AN	AN	AN	AN
	G	0	AN	AN	AN	AN	AN	AN	AN
	H	0	AN	AN	AN	AN	AN	AN	AN

[1]Observations: AN = appear normal.

Appendix 7 (Continued)

Observations[1] of Effects by Replicate in a Prolonged Sediment Toxicity Test with the Amphipod (*Hyalella azteca*)

Nominal Concentration (mg a.i./Kg)	Replicate	Number Exposed	Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20
1000	A	10	AN	AN	AN	AN	AN	AN	AN
	B	10	AN	AN	AN	AN	AN	AN	AN
	C	10	AN	AN	AN	AN	AN	AN	AN
	D	10	AN	AN	AN	AN	AN	AN	AN
	E	10	AN	AN	AN	AN	AN	AN	AN
	F	10	AN	AN	AN	AN	AN	AN	AN
	G	10	AN	AN	AN	AN	AN	AN	AN
	H	10	AN	AN	AN	AN	AN	AN	AN

[1]Observations: AN = appear normal.

Appendix 7 (Continued)

Observations[1] of Effects by Replicate in a Prolonged Sediment Toxicity Test with the Amphipod (*Hyalella azteca*)

Nominal Concentration (mg a.i./Kg)	Replicate	Number Exposed	Day 21	Day 22	Day 23	Day 24	Day 25	Day 26	Day 27
Negative Control	A	10	AN	AN	AN	AN	AN	AN	AN
	B	10	AN	AN	AN	AN	AN	AN	AN
	C	10	AN	AN	AN	AN	AN	AN	AN
	D	10	AN	AN	AN	AN	AN	AN	AN
	E	10	AN	AN	AN	AN	AN	AN	AN
	F	10	AN	AN	AN	AN	AN	AN	AN
	G	10	AN	AN	AN	AN	AN	AN	AN
	H	10	AN	AN	AN	AN	1G; AN	AN	AN
Solvent Control	A	10	AN	AN	AN	AN	AN	AN	AN
	B	10	AN	AN	AN	AN	AN	AN	AN
	C	10	AN	AN	AN	AN	AN	AN	AN
	D	10	AN	AN	AN	AN	AN	AN	AN
	E	10	AN	AN	AN	AN	AN	AN	AN
	F	10	AN	AN	AN	AN	AN	AN	AN
	G	10	AN	AN	AN	AN	AN	AN	AN
	H	10	AN	AN	AN	AN	AN	AN	AN

[1]Observations: AN = appear normal; G = leaving the sediment or on the surface of the sediment.

Appendix 7 (Continued)

Observations[1] of Effects by Replicate in a Prolonged Sediment Toxicity Test with the Amphipod (*Hyalella azteca*)

Nominal Concentration (mg a.i./Kg)	Replicate	Number Exposed	Day 21	Day 22	Day 23	Day 24	Day 25	Day 26	Day 27
63	A	10	AN	AN	AN	AN	AN	AN	AN
	B	10	AN	AN	AN	AN	AN	AN	AN
	C	10	AN	AN	AN	AN	AN	AN	AN
	D	10	AN	AN	AN	AN	AN	AN	AN
	E	10	AN	AN	AN	AN	AN	AN	AN
	F	10	AN	AN	AN	AN	AN	AN	AN
	G	10	AN	AN	AN	AN	AN	AN	AN
	H	10	AN	AN	AN	AN	AN	AN	AN
125	A	10	AN	AN	AN	AN	AN	AN	AN
	B	10	AN	AN	AN	AN	AN	AN	AN
	C	10	AN	AN	AN	AN	AN	AN	AN
	D	10	AN	AN	AN	AN	AN	AN	AN
	E	10	AN	AN	AN	AN	AN	AN	AN
	F	10	AN	AN	AN	AN	AN	AN	AN
	G	10	AN	AN	AN	AN	AN	AN	AN
	H	10	AN	AN	AN	AN	AN	AN	AN

[1]Observations: AN = appear normal.

Appendix 7 (Continued)

Observations[1] of Mortality and Effects by Replicate in a Prolonged Sediment Toxicity Test with the Amphipod (*Hyalella azteca*)

Nominal Concentration (mg a.i./Kg)	Replicate	Number Exposed	Day 21	Day 22	Day 23	Day 24	Day 25	Day 26	Day 27
250	A	10	AN	AN	AN	AN	AN	AN	AN
	B	10	AN	AN	1G; AN	AN	AN	AN	AN
	C	10	AN	AN	AN	AN	AN	AN	AN
	D	10	AN	AN	AN	AN	AN	AN	AN
	E	10	AN	AN	AN	AN	AN	AN	AN
	F	10	AN	AN	AN	AN	AN	AN	AN
	G	10	AN	AN	AN	AN	AN	AN	AN
	H	10	AN	AN	AN	AN	AN	AN	AN
500	A	10	AN	AN	AN	AN	AN	AN	AN
	B	10	AN	AN	AN	AN	AN	AN	AN
	C	10	AN	AN	AN	AN	AN	AN	AN
	D	10	AN	AN	AN	AN	AN	AN	AN
	E	10	AN	AN	AN	AN	AN	AN	AN
	F	10	AN	AN	AN	AN	AN	AN	AN
	G	10	AN	AN	AN	AN	AN	AN	AN
	H	10	AN	AN	AN	AN	AN	AN	AN

[1]Observations: AN = appear normal; G = leaving the sediment or on the surface of the sediment.

Appendix 7 (Continued)

Observations[1] of Effects by Replicate in a Prolonged Sediment Toxicity Test with the Amphipod (*Hyalella azteca*)

Nominal Concentration (mg a.i./Kg)	Replicate	Number Exposed	Day 21	Day 22	Day 23	Day 24	Day 25	Day 26	Day 27
1000	A	10	AN	AN	AN	AN	AN	AN	AN
	B	10	AN	AN	AN	AN	AN	AN	AN
	C	10	AN	AN	AN	AN	AN	AN	AN
	D	10	AN	AN	AN	AN	AN	AN	AN
	E	10	AN	AN	AN	AN	AN	AN	AN
	F	10	AN	AN	AN	AN	AN	AN	AN
	G	10	AN	AN	AN	AN	AN	AN	AN
	H	10	AN	AN	AN	AN	AN	AN	AN

[1]Observations: AN = appear normal.

Appendix 7 (Continued)

Observations[1] of Effects by Replicate in a Prolonged Sediment Toxicity Test with the Amphipod (*Hyalella azteca*)

Nominal Concentration (mg a.i./Kg)	Replicate	Day 28		
		No. Dead	Obs.	No. Survivors
Negative Control	A	0	10 AN	10
	B	0	10 AN	10
	C	1	9 AN	9
	D	2	8 AN	8
	E	3	7 AN	7
	F	2	8 AN	8
	G	1	9 AN	9
	H	1	9 AN	9
			Mean (± Stdev)	8.8 (± 1.0)
Solvent Control	A	1	9 AN	9
	B	1	9 AN	9
	C	4	6 AN	6
	D	1	9 AN	9
	E	1	9 AN	9
	F	1	9 AN	9
	G	2	8 AN	8
	H	2	8 AN	8
			Mean (± Stdev)	8.4 (± 1.1)

[1]Observations: AN = appear normal.

Appendix 7 (Continued)

Observations[1] of Effects by Replicate in a Prolonged Sediment Toxicity Test with the Amphipod (*Hyalella azteca*)

Nominal Concentration (mg a.i./Kg)	Replicate	Day 28		
		No. Dead	Obs.	No. Survivors
63	A	0	10 AN	10
	B	1	9 AN	9
	C	1	9 AN	9
	D	1	9 AN	9
	E	1	9 AN	9
	F	2	8 AN	8
	G	2	8 AN	8
	H	1	9 AN	9
			Mean (± Stdev)	8.9 (± 0.64)
125	A	1	9 AN	9
	B	4	6 AN	6
	C	0	10 AN	10
	D	0	10 AN	10
	E	2	8 AN	8
	F	1	9 AN	9
	G	2	8 AN	8
	H	1	9 AN	9
			Mean (± Stdev)	8.6 (± 1.3)

[1]Observations: AN = appear normal.

Appendix 7 (Continued)

Observations[1] of Effects by Replicate in a Prolonged Sediment Toxicity Test with the Amphipod (*Hyalella azteca*)

Nominal Concentration (mg a.i./Kg)	Replicate	Day 28		
		No. Dead	Obs.	No. Survivors
250	A	0	10 AN	10
	B	1	9 AN	9
	C	3	7 AN	7
	D	3	7 AN	7
	E	4	6 AN	6
	F	1	9 AN	9
	G	1	9 AN	9
	H	3	7 AN	7
			Mean (± Stdev)	8.0 (± 1.4)
500	A	2	8 AN	8
	B	1	9 AN	9
	C	6	4 AN	4
	D	7	3 AN	3
	E	4	6 AN	6
	F	4	6 AN	6
	G	5	5 AN	5
	H	4	6 AN	6
			Mean (± Stdev)	5.9 (± 2.0)

[1]Observations: AN = appear normal.

Appendix 7 (Continued)

Observations[1] of Effects by Replicate in a Prolonged Sediment Toxicity Test with the Amphipod (*Hyalella azteca*)

Nominal Concentration (mg a.i./Kg)	Replicate	Day 28		
		No. Dead	Obs.	No. Survivors
1000	A	8	2 AN	2
	B	2	8 AN	8
	C	3	7 AN	7
	D	3	7 AN	7
	E	5	5 AN	5
	F	2	8 AN	8
	G	6	4 AN	4
	H	4	6 AN	6
			Mean (± Stdev)	5.9 (± 2.1)

[1]Observations: AN = appear normal.

Appendix 8

Dry Weights by Replicate in a Prolonged Sediment Toxicity Test with the Amphipod (*Hyalella azteca*)

	Average Individual Dry Weight (mg) by Nominal Concentration						
Replicate	Negative Control	Solvent Control	63 mg a.i./Kg	125 mg a.i./Kg	250 mg a.i./Kg	500 mg a.i./Kg	1000 mg a.i./Kg
A	0.1600	0.2778	0.4000	0.1556	0.1700	0.1625	0.2000
B	0.1500	0.2444	0.5111	0.1333	0.1111	0.2444	0.2000
C	0.2000	0.5833	0.1556	0.2400	0.2000	0.2250	0.1286
D	0.1750	0.1222	0.2889	0.1900	0.1714	0.1333	0.1429
E	0.1429	0.2000	0.1667	0.1875	0.2833	0.2667	0.2200
F	0.3250	0.1667	0.2750	0.1111	0.1667	0.2167	0.1750
G	0.2667	0.1000	0.1375	0.1750	0.2000	0.1600	0.2000
H	0.1778	0.2125	0.1778	0.1111	0.2571	0.2000	0.2833
Mean ± Std. Dev.	0.1997 ± 0.0639	0.2384 ± 0.151	0.2641 ± 0.134	0.1629 ± 0.0443	0.1950 ± 0.0544	0.2011 ± 0.0459	0.1937 ± 0.0478

On Day 0, 20 organisms were impartially removed from the culture and used to determine dry weight. The average individual dry weight was determined to be 0.075 mg.

Appendix 9

Personnel Involved in the Study

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

1. Henry O. Krueger, Ph.D., Director of Aquatic Toxicology/Terrestrial Plants and Insects
2. Willard B. Nixon, Ph.D., Director of Chemistry
3. Timothy Z. Kendall, Supervisor
4. Amy S. Blankinship, Laboratory Supervisor
5. Susan T. Thomas, Aquatic Biologist