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American Chemistry Council

May 19, 2005

VIA CERTIFIED MAIL

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Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
Ariel Rios Building
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Washington, DC 20460-0001

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CONTAIN 051

8EHQ-0505-15869

RECEIVED OPPT CBIC 05 MAY 25 AM 11:04

ATTN: Toxic Substances Control Act (TSCA) Section 8(e) Coordinator

Re: Follow-up Report and Submission of a Tetrabromobisphenol A (TBPPA), CAS No.: 79-94-7, Study Report Entitled, "TBPPA: Determination of Effects on the Growth of the Common Mussel *Mytilus edulis*"

Dear TSCA 8(e) Coordinator:

The American Chemistry Council's Brominated Flame Retardants Industry Panel (BFRIP) [1] is submitting on behalf of its members the enclosed Blue (Common) mussel growth effects study, pursuant to the requirements of Section 8(e) of the Toxic Substances Control Act (TSCA). Although the information is being submitted in accordance with relevant TSCA Section 8(e) guidance of the EPA, BFRIP has made no determination as to whether a substantial risk of injury to health or the environment is actually presented by the study report. This is a follow-up to an earlier notification submitted by BFRIP on 17 December 2004 concerning preliminary information that was acquired while the study was underway. The previous submittal was assigned Document Control Number 88-050000075. The enclosed study report was undertaken, recently completed and submitted to the European Union (EU) in the context of the EU's on-going risk assessment of TBBPA. The results of the study are presented fully in the enclosed report, which reflect an overall no observed effect concentration (NOEC) of 17 µg/L based on specific growth rate of shell length and dry tissue weight between days 0 and 70.

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If you have any questions, please feel free to contact me at (703) 741-5605.

Sincerely yours,

Nancy Sandrof

Nancy Sandrof, M.P. H.
Manager, BFRIP
American Chemistry Council

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Enclosure: Study on CD-ROM

[1] The members of BFRIP are Albemarle Corporation, Ameribrom, Inc. – ICL Industrial Products, and Great Lakes Chemical Corporation.

Brixham Environmental Laboratory study number **03-0337/A**

Report Number BL8004/B

TBBPA: Determination of effects on the growth of the common mussel *Mytilus edulis*

Performing laboratories	**Brixham Environmental Laboratory** **AstraZeneca UK Limited** **Brixham** **Devon TQ5 8BA** **UK** **Wildlife International Ltd** **8598 Commerce Drive** **Easton** **Maryland 21601** **USA**
Sponsor	**ACC-BFRIP** **1300 Wilson Boulevard** **Arlington** **VA 22209** **USA**
Authors	**R J Brown** **D V Smyth** **S J Kent**
Approved by	**N Shillabeer** **April 2005**

CONFIDENTIAL (CATEGORY B)

Not to be photocopied or microfilmed. This document contains information confidential and trade secret to ACC-BFRIP. It should not be released in any form to an outside party, nor should information contained herein be used by a registration authority to support registration of any other product without the written permission of ACC-BFRIP.

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted in compliance with the United Kingdom Good Laboratory Practice Regulations. These regulations are in accordance with the OECD Principles of Good Laboratory Practice.

These international standards are acceptable to the United States Environmental Protection Agency and this study, therefore, satisfies the requirements of 40 CFR Part 160 and 40 CFR Part 792.

This study is valid for the purpose for which it was conducted and this report is a true reflection of the raw data generated.

R J Brown

R J Brown
Study Director
Brixham Environmental Laboratory

29 March 2005

Date

QUALITY ASSURANCE STATEMENT

The Quality Assurance Unit of Brixham Environmental Laboratory, AstraZeneca, has reviewed this report.

On the following dates, inspections of this study were carried out as shown below and findings were reported to the Study Director and Management.

Audit Date	**Inspection/audit**	**Findings Reported**
18 June 2004	Study Plan	18 June 2004
13 September 2004	Mussel Sampling	13 September 2004
6-8 October 2004	Stock & Feed Preparation	8 October 2004
22 November 2004	Mussel Sampling	26 November 2004
18 February 2005	Report	18 February 2005

Inspections of the analytical chemistry phase of this study are shown in a separate QA statement in Appendix 1.

Facilities and procedures associated with this type of study are periodically inspected in accordance with QA Standard Operating Procedures and the findings are reported to management.

The report is considered to accurately describe the methods and procedures used in the study and to accurately reflect the raw data of the study.

S C Lock
Quality Assurance Unit

07 April 2005
Date

AUTHENTICATION STATEMENT

I, the undersigned, hereby declare that this study was performed under my direction according to the principles of Good Laboratory Practice and that this report represents a true and accurate record of results obtained.

Study Director R J Brown — Date: 29 March 2005

The following personnel carried out work on this study:

Principal Investigator, Chemistry (Wildlife International Ltd): J MacGregor

Principal Scientist, Ecotoxicology: D V Smyth

Main report approved by Business Manager N Shillabeer — Date: 1 April 2005

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1 SUMMARY

Sponsor	ACC-BFRIP, 1300 Wilson Boulevard, Arlington, VA 22209, USA
Contact	S Kent — International telephone: (44) 1803 882882 A Leopold — International telephone: (03) 1575 573848
Location of study, raw data and final report	Brixham Environmental Laboratory, AstraZeneca UK Limited, Brixham, Devon, TQ5 8BA, UK Wildlife International Ltd, 8598 Commerce Drive, Easton, Maryland, 21601, USA
Test substance common name	Tetrabromobisphenol A (TBBPA)
Chemical abstract name	Phenol, 4,4'-(1-methylethylidene)bis[2,6-dibromo-]
Subject	TBBPA: Determination of effects on the growth of the common mussel *(Mytilus edulis)*
Test species	Common mussel *Mytilus edulis*, Batch Ref 27/04
Source of organisms	Brixham Sea Farms, Moorland View, Rocky Lane, Teignmouth, Devon, TQ14 9HF, UK
Test treatments	Dilution water control, solvent control and nominal TBBPA concentrations of 19, 38, 75, 150 and 300 $\mu g\ l^{-1}$
Length of test	Mussel exposure period 70 days flow-through, no aeration
Test dates	13 September to 22 November 2004
Nominal test temperature	15 ± 1°C
Results based on mean measured concentrations as $\mu g\ l^{-1}$ of TBBPA	Based on the means of the individual pseudo specific growth rates (SGR) for shell length and wet and dry flesh weight. Effects are based on significant differences from pooled controls

SGR (shell length) between days 0 and 14:
No observed effect (P=0.05) concentration (NOEC) = 62 $\mu g\ l^{-1}$
Lowest observed effect (P=0.05) concentration (LOEC) = 126 $\mu g\ l^{-1}$

SGR (shell length) between days 0 and 28:
No observed effect (P=0.05) concentration (NOEC) = 62 $\mu g\ l^{-1}$
Lowest observed effect (P=0.05) concentration (LOEC) = 126 $\mu g\ l^{-1}$

SGR (shell length) between days 0 and 42:
No observed effect (P=0.05) concentration (NOEC) = 32 $\mu g\ l^{-1}$
Lowest observed effect (P=0.05) concentration (LOEC) = 62 $\mu g\ l^{-1}$

SGR (shell length) between days 0 and 56:
No observed effect (P=0.05) concentration (NOEC) = 32 $\mu g\ l^{-1}$
Lowest observed effect (P=0.05) concentration (LOEC) = 62 $\mu g\ l^{-1}$

SGR (shell length) between days 0 and 70:
No observed effect (P=0.05) concentration (NOEC) = 17 $\mu g\ l^{-1}$
Lowest observed effect (P=0.05) concentration (LOEC) = 32 $\mu g\ l^{-1}$

SGR (wet tissue weight) between days 0 and 70:
No observed effect (P=0.05) concentration (NOEC) = 62 $\mu g\ l^{-1}$
Lowest observed effect (P=0.05) concentration (LOEC) = 126 $\mu g\ l^{-1}$

SGR (dry tissue weight) between days 0 and 70:
No observed effect (P=0.05) concentration (NOEC) = 17 $\mu g\ l^{-1}$
Lowest observed effect (P=0.05) concentration (LOEC) = 32 $\mu g\ l^{-1}$

Therefore the overall no observed effect concentration (P=0.05) based on SGR (shell length) and SGR (dry tissue weight) between days 0 and 70 = 17 $\mu g\ l^{-1}$

2 INTRODUCTION

At the request of ACC-BFRIP, 1300 Wilson Boulevard, Arlington, VA22209, USA, a study was undertaken to determine the effects of tetrabromobisphenol A (TBBPA) on the growth of the common mussel *Mytilus edulis*. The study was carried out under the study number 03-0337/A with exposure of mussels to TBBPA being carried out at Brixham Environmental Laboratory, AstraZeneca UK Limited, Brixham, Devon, TQ5 8BA, UK. The analytical chemistry determinations were made by Wildlife International Ltd., 8598 Commerce Drive, Easton, Maryland, 21601, USA, and are reported in Appendix 1.

The study was run between 29 July and 24 November 2004. A solubility dosing trial was conducted from 29 July to 13 August 2004 and the definitive test ran from 01 September to 24 November 2004. The mussel exposure dates were from 13 September to 22 November 2004. The original analytical chemistry data are filed in the archive at Wildlife International Ltd. All other original data, together with other relevant records are filed in the Brixham Environmental Laboratory archive.

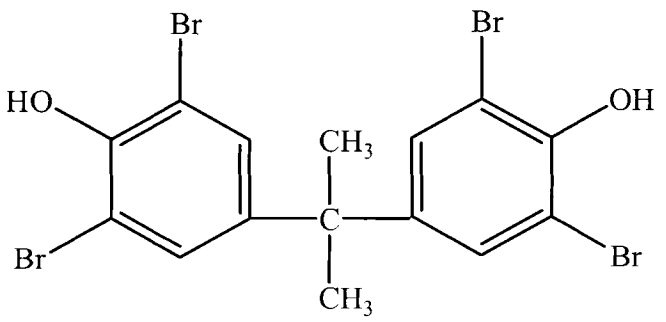
3 MATERIALS AND METHODS

3.1 Test substance

The test substance, was supplied by Wildlife International Ltd, 8598 Commerce Drive, Easton, Maryland, 21601, USA.

Chemical abstract name:	Phenol, 4,4'-(1-methylethylidene)bis[2,6-dibromo-]
CAS RN:	79-94-7

Molecular structure:



The test substance was received at Brixham Environmental Laboratory on 15 June 2004 and assigned the Brixham test substance number 03-0337. The test substance sample reference was Wildlife International Ltd. test substance 6404 and was a composite of Wildlife International Ltd. 6358, 6368, 6400, which are samples of commercial products from Albemarle Corporation, Great Lakes Chemical Corporation and the Dead Sea

Bromine Group, respectively. The test substance was supplied as a white crystalline powder. Chemical characterisation by the Albermarle Corporation, Process Development Center, Baton Rouge, LA 70821 indicates a sample purity of 99.2%.

The sample was stored at ambient temperature, in the container in which it was received, until required for testing, when an appropriate subsample was provided for the test operator.

3.2 Test organism

The test organism was the common blue mussel *Mytilus edulis*. The mussels (batch 27/04) were obtained from Brixham Sea Farms, Moorland View, Rocky Lane, Teignmouth, Devon, TQ14 9HF, UK on 01 September 2004 and held in stock tanks at Brixham Environmental Laboratory, at the nominal test temperature, in 10 μm filtered seawater until the start of the test. The sea temperature on the day the mussels were collected was 16.8°C therefore no additional acclimation to test temperature was required. No mortality or disease was observed in the batch of mussels during the holding period.

At the start of the test all mussels had a shell length between 8 and 12 mm. The mean length (± standard deviation) of the mussels (all test vessels) was 10.8 (± 0.7) mm.

3.3 Dilution water

The dilution water was natural seawater from Tor Bay, Devon, UK, filtered to 10 μm to remove particulate material. This seawater was then delivered to a temperature controlled mixing tank, in the test laboratory, where it was filtered to 1 μm before use. The seawater salinity was that of the laboratory supply, which is typically 35 ± 1‰. The water temperature was controlled to the nominal test temperature 15 ± 1°C.

The pH and salinity of the laboratory seawater supply are monitored five times per week. The seawater supply is also monitored once per month for ammonia, total organic carbon and total suspended solids and once per quarter for a range of cations and anions, trace metals, pesticides and PCBs.

4 TEST METHOD AND CONDITIONS

4.1 Apparatus

The apparatus used in this test was a dynamic, continuous flow through system (Fig 1). The test vessels, mixing chambers and stock vessels were all constructed of glass. A minimum quantity of silicone rubber and PTFE tubing was used to connect the components.

The triplicate mussel test vessels were rectangular in shape with internal dimensions of 19.0 × 15.0 × 14.6 cm (length × width × height) and a capacity of approximately 4.1 litres. The test solution volume used was approximately 3.0 litres. The test vessels had tight fitting glass covers. The test replicate tank solutions were renewed at a nominal rate of 200 ml min^{-1}, to give water removal rates of >25 litres $mussel^{-1}$ day^{-1}.

Into each test vessel a glass petri-dish (140 mm diameter) was placed for the mussels to attach.

The stock solutions were dosed to the glass mixing chambers from a syringe pump and the dilution water was supplied using a capillary flow control system. The *Tetraselmis* algal diet (Section 4.3) was introduced to the test rig dilution water inflow, via a peristaltic pump, from a stock concentrate, at the appropriate rate. Magnetic stirrers in the mixing chambers ensured thorough mixing before the test solutions passed into flow splitter cells and, via further capillary flow controls, to the exposure vessels. The dilution ratio of the stock solutions to dilution water was nominally 1:300 000 in the solvent control and all test substance concentrations.

The test was undertaken in a temperature controlled room which was set at the nominal test temperature of 15 ± 1°C. The test solutions were not aerated. The photoperiod in this study was 16 hours fluorescent light and 8 hours dark with 20 minute dawn and dusk transition periods. The light intensity was measured at least once per month.

4.2 Test procedure

The exposure phase of the test run was initiated on 13 September 2004 (exposure day 0), when test mussel populations were first placed into the test replicate vessels. The test was completed on 22 November 2004 (exposure day 70).

On day 0, batches of 10 mussels were impartially selected from a pooled population. Individual shell lengths of mussels were determined using digital callipers and the animals were then randomly placed into the petri-dishes in the replicate test tanks. Mussels were not individually identified within the tanks. The shell lengths of thirty additional mussels from the same population of animals were also determined. These mussels were then sacrificed and the individual wet flesh was removed from inside the shell and weighed. The corresponding dry flesh weights were determined after drying to a constant weight at 60°C (i.e. until the dry weights of the excess or dilution water control animals showed less than a 0.2% difference over two separate measurement occasions).

Every fourteen days the mussels were removed from the tanks and measured as described above. The mussel exposure was terminated after 10 weeks (day 70) when the dilution water control mussels had achieved a mean increase in shell length of greater than 50% of their shell length on day 0. On day 70 all mussels were carefully removed from the tanks and in addition to shell length measurements, the wet and dry mussel flesh weights were determined as described for day 0.

Approximately once per week, the test system (including tanks) were cleaned to remove faeces and detritus and to maintain effective running of the test flow-through system.

4.3 Feeding

During the holding and test periods the mussels were fed appropriate amounts of a commercially available algal diet of *Tetraselmis* sp. 'Instant algae', supplied by Reed Mariculture Ltd. Food was withheld from the mussels for at least 24 hours prior to commencement of the test.

During the test each test replicate vessel received algae at a nominal rate of 12 000 particles ml^{-1} from a concentrated stirred stock solution delivered via a peristaltic pump. The algal stock was introduced to the test rig dilution water supply (at a nominal flow rate of 1.70 ml min^{-1}), immediately before its inflow to the dilution water distribution trough. The algal stock was replaced twice per week. Dosing of the algal stock commenced on exposure day -7, after which weekly particle density determinations were made on all replicate tank inflows and outflows plus test channel flow splitter cells. The alga stock flow rates were measured twice per week.

No analyses, other than for particle densities during the study were performed on the diet.

4.4 Preparation of test solutions

Five nominal concentrations, 19, 38, 75, 150 and 300 $\mu g\ l^{-1}$ of TBBPA, together with a dilution water and solvent control were tested. The highest exposure concentration of 300 $\mu g\ l^{-1}$ TBBPA was at the maximum solubility level of the test substance within the test flow-through system. This was established during the solubility trial (Appendix 1).

Individual stock solutions were prepared by dissolving the required amount of TBBPA in Dimethylformamide (DMF). The stock solutions were ultrasonicated until the TBBPA was completely dissolved and then transferred to the syringe pump syringes. The stock solutions for the solvent control, 19, 38 and 75 $\mu g\ l^{-1}$ test solutions were clear colourless liquids. The stock solutions for the 150 and 300 $\mu g\ l^{-1}$ test solutions were clear, pale straw coloured liquids. Graduated glass syringes were filled with the appropriate stock solutions and placed onto a syringe pump. The stock solutions were delivered to the mixing chambers at a nominal flow rate of 0.002 ml min^{-1}. The stock solutions in the syringes were replenished at approximately weekly intervals.

The flow rate of water into individual test vessels was measured daily for the first four days of the exposure period and then twice per week for the rest of the test. Twice per week the flow rates for each set of stock solutions were calculated by volume displacement. The nominal dilution water flow rate to each mixing cell was 600 ml min^{-1} therefore the theoretical dilution achieved immediately before delivery to the test vessels was 300 000 times.

The solvent control and each concentration of TBBPA contained a constant nominal 3.33 $\mu l\ l^{-1}$ of DMF. The test solutions were not aerated during the test.

4.5 Analytical method

Water samples (20 ml) were taken on exposure days -10, -7, -6, 0, 4, 7, 11, 15, 22, 29, 36, 43, 50, 57 and 64 to determine the concentrations of TBBPA in the test solutions. Samples were despatched to Wildlife International, 8598 Commerce Drive, Easton, MD 21601, USA for analysis by the HPLC/MS method outlined in Appendix 1. Water samples for chemical analysis were taken from the centre of the test solutions using a graduated glass pipette.

Dosing of the test substance to the test vessels commenced on the 01 September 2004 (exposure day -12). This was to ensure that the concentration of test substance in the tanks could be maintained in the presence of the algal food source prior to addition of the

test animals. Water samples for chemical analysis were taken on day -10 to check that the rig was dosing properly. On days -7 and -6, two water samples were taken from one tank at each test concentration for TBBPA analysis. On both occasions only one of the two water samples was centrifuged (at 40 000 g for 30 minutes) before dispatch. On day -7 (after the water samples had been taken) dosing of the algal food source commenced (see section 4.3). For all subsequent chemical analyses the water samples were not centrifuged.

4.6 Observations for mortality

Observations of mortality were made twice per week. Mussels that had moved to the water surface and were only partially submerged in test water were carefully cut free (via bysuss threads) and returned to the test vessel bottom.

4.7 Physical and chemical parameters

The temperature in one replicate control tank was determined daily, using a mercury-in-glass thermometer calibrated to 0.1°C and conforming to BS593 and also hourly using an electronic recording system. The temperatures of all test vessels were taken once per week prior to the addition of test animals and twice weekly throughout the exposure phase of the test.

Dissolved oxygen and pH in the replicate tanks was measured three times during the first week of the test exposure period and once per week at all other times. The salinity of the rig dilution water was measured once per week pre-exposure and twice per week during the exposure period.

Test dilution water parameters were regularly monitored, as detailed in Section 3.3.

5 RESULTS

5.1 Analytical data

The analytical results for the concentrations of TBBPA in the test solutions during exposure are reported in Appendix 1 and are summarised in Table 1. The highest limit of quantitation throughout the study was 2.5 $\mu g\ l^{-1}$.

Pre-exposure phase measurements (made on days -10, -7 and -6) demonstrated that the tanks were being dosed correctly and that the addition of algae on day -7 had a negligible effect on the test concentrations. A comparison of centrifuged and non-centrifuged samples taken on days -7 and -6 showed no apparent differences (Table 1). The presence of algae therefore appeared not to affect the concentration of TBBPA in solution.

The measured concentrations in the individual vessels ranged from 50 to 130% of the nominal values throughout the exposure period. The mean of the exposure phase measured concentrations ranged from 75 to 89% of nominal values. Arithmetic mean measured concentrations for this period were used in the calculation and reporting of the results.

5.2 Dosing of TBBPA

Summaries of the exposure phase dilution water and toxicant flow rates are shown in Table 2. The mean dilution ratios for the TBBPA test solutions ranged from 94 to 106% of the nominal dilution ratio. The toxicant flows were all calculated at the nominal 0.002 ml min^{-1} rate.

These data confirm that the dosing system was operating correctly and this is supported by the analytical results (Section 5.1).

5.3 Observations of mortalities

There were no observed mortalities during the period of the test, although one mussel was found to be missing from the measured 226 $\mu g\ l^{-1}$ A replicate tank on day 14. On exposure days 2 and 4, in all replicates at measured 226 $\mu g\ l^{-1}$ excessive lengths of faecal material were observed compared with the other treatments. This response was not evident after day 4.

The surfaced and partially surfaced mussels, inspected and returned to the replicate tank bottoms daily (Section 4.6), appeared to show no concentration related bias.

5.4 Mussel weights and lengths

The length of each mussel was recorded on exposure days 0, 14, 28, 42, 56 and on termination of the test, at day 70. The data obtained for each vessel and concentration, including means and standard deviations, are shown in Table 3.

The day 0 wet and dry flesh weights for excess mussels are shown in Table 4. The day 70 wet and dry flesh weight data for each vessel and test concentration are detailed in Tables 5 and 6 respectively.

5.5 Growth rates (shell length)

The specific growth rate (SGR) of mussels were analysed using procedures outlined in the OECD method 215 fish, juvenile growth test (Ref 1). For each individual mussel, the SGR length or weight per day, was calculated, based on both shell length and body weight data, using the equation:

$$r = \frac{\log_e W_2 - \overline{\log_e W_1}}{t_2 - t_1} \times 100$$

r = specific growth rate

W_1, W_2 = shell length (or tissue weight) of a particular mussel at a particular time

$\text{Log}_e\ W_2$ = logarithm of the shell length (or tissue weight) of an individual mussel at the end of the exposure period

$\overline{\text{Log}_e\ W_1}$= average of the logarithms of the shell lengths (or tissue weights) W_1 of individual mussels in the tank at the start of the exposure period. For tissue weight data

this is the average of the logarithms of the weights W_1 of excess individual mussels sacrificed on day 0.

t_1, t_2 = time (days) at start and end of exposure period

Specific growth rate (SGR) was calculated, based on shell length data (days 0-14, 0-28, 0-42, 0-56 and 0-70) and for tissue wet and dry weight data (days 0-70, Section 5.6).

For SGR based on shell lengths the data were checked for normality (Ref 2) and homogeneity of variances (Ref 3), before undergoing one-way analysis of variance (ANOVA) to establish significant differences between replicates of each treatment (P=0.05). If the data did not meet the assumptions for ANOVA, differences between replicates were detected using Wilcoxons non-parametric rank sum test (Ref 4). As there was no difference between replicates the data were pooled to consider the effect of TBBPA treatments. The dilution water control and solvent control were compared using t-tests and pooled as they were not significantly different. The data for TBBPA treatments were normal and homogenous, therefore one-way ANOVA followed by Dunnett's t-test (Ref 5) were used to identify significant differences from the solvent control and pooled controls.

Individual mussel SGRs (shell length) for the relevant time periods are detailed in Table 7. Mean and standard deviation SGRs (shell length) for the five growth periods, including statistically significant differences from the solvent control and pooled controls, are summarised in Table 8. Based on measured concentrations and significant differences from the pooled controls, the following effects were observed:

Between days 0 and 14:
No observed effect (P=0.05) concentration (NOEC) = 62 $\mu g\ l^{-1}$
Lowest observed effect (P=0.05) concentration (LOEC) = 126 $\mu g\ l^{-1}$

Between days 0 and 28:
No observed effect (P=0.05) concentration (NOEC) = 62 $\mu g\ l^{-1}$
Lowest observed effect (P=0.05) concentration (LOEC) = 126 $\mu g\ l^{-1}$

Between days 0 and 42:
No observed effect (P=0.05) concentration (NOEC) = 32 $\mu g\ l^{-1}$
Lowest observed effect (P=0.05) concentration (LOEC) = 62 $\mu g\ l^{-1}$

Between days 0 and 56:
No observed effect (P=0.05) concentration (NOEC) = 32 $\mu g\ l^{-1}$
Lowest observed effect (P=0.05) concentration (LOEC) = 62 $\mu g\ l^{-1}$

Between days 0 and 70:
No observed effect (P=0.05) concentration (NOEC) = 17 $\mu g\ l^{-1}$
Lowest observed effect (P=0.05) concentration (LOEC) = 32 $\mu g\ l^{-1}$

Therefore, based on mean measured concentrations, the lowest observed effect concentration (LOEC, P=0.05) for SGR (shell length) was 32 $\mu g\ l^{-1}$. The no observed effect concentration (NOEC, P=0.05) for SGR (shell length) was 17 $\mu g\ l^{-1}$.

5.6 Growth rates (tissue weight)

The specific growth rates (SGR) based on wet and dry tissue weight data (days 0-70) were calculated using the formula in Section 5.5.

For SGR based on wet tissue weights the data were checked for normality (Ref 2) and homogeneity of variances (Ref 3), before undergoing one-way ANOVA to establish significant differences between replicates of each treatment (P=0.05). As there was no difference between replicates the data were pooled to consider the effect of TBBPA treatments. The dilution water control and solvent control were compared using t-tests and pooled, as they were not significantly different. The data for TBBPA treatments were normal and homogenous, therefore one-way ANOVA followed by Dunnett's t-test (Ref 5) were used to identify significant differences from the solvent control and pooled controls.

Individual mussel SGRs (wet tissue weight) for the relevant time periods and significant differences from the solvent control and pooled controls are detailed in Table 9. Based on measured concentrations and significant differences from the pooled controls, the following effects were observed.

SGR (wet tissue weight):
Between days 0 and 70:

No observed effect (P=0.05) concentration (NOEC)	=	62 $\mu g\ l^{-1}$
Lowest observed effect (P=0.05) concentration (LOEC)	=	126 $\mu g\ l^{-1}$

For SGR based on dry tissue weights the data were checked for normality (Ref 2) and homogeneity of variances (Ref 3), before undergoing one-way ANOVA to establish significant differences between replicates of each treatment (P=0.05). If the data did not meet the assumptions for ANOVA, differences between replicates were detected using Wilcoxons non-parametric rank sum test (Ref 4). At the mean measured 32 $\mu g\ l^{-1}$ concentration, one test replicate (Rep A) showed significantly different SGRs (dry tissue weight) compared with the other two replicates (P=0.05). Differences in SGRs based on dry weights between treatments were therefore analysed twice, once including the data for 32 $\mu g\ l^{-1}$ replicate A and once ignoring this replicate.

The data for SGR (dry tissue weight) did not meet the assumptions for ANOVA, therefore differences between treatments were detected using Wilcoxons non-parametric rank sum test (Ref 4). The dilution water control and solvent control were compared using t-tests and as they were not significantly different, the controls were pooled.

Individual mussel SGRs (dry tissue weight) for the relevant time periods and significant differences from the solvent control and pooled controls are detailed in Table 10. There was no difference between treatments in the level of significance of the data, either including or excluding mean measured 32 $\mu g\ l^{-1}$ replicate A. The significantly different SGRs (dry tissue weight) at 32 $\mu g\ l^{-1}$ are unexpected given that there was no significant effect on the SGR (dry tissue weight) at the higher measured test concentration of 62 $\mu g\ l^{-1}$ and that there was no significant effect on SGR (wet tissue weight) at 32 $\mu g\ l^{-1}$. Measuring the wet and dry flesh weights of small mussels is a less reliable endpoint than mussel shell length, as it can be difficult to remove all the flesh from the shells.

Based on measured concentrations and significant differences from the pooled controls, the following effects were observed:

SGR (dry tissue weight)
Between days 0 and 70:

No observed effect (P=0.05) concentration (NOEC)	=	17 $\mu g\ l^{-1}$
Lowest observed effect (P=0.05) concentration (LOEC)	=	32 $\mu g\ l^{-1}$

5.7 Dosing of algal diet

Summaries of test exposure phase algal stock flow rates are shown in Table 11. The mean flows ranged between 1.62 and 1.78 ml min^{-1} (95 and 105% of the nominal flow rate, 1.70 ml min^{-1}). Weekly alga particle density determinations were made on test channel splitter cells and all replicate tank inflows plus outflows. Results are summarised in Table 12.

5.8 Physical and chemical data

During the exposure phase of the test, the pH values in the replicate tanks ranged from 7.9 to 8.1, dissolved oxygen concentrations ranged from 7.2 to 8.2 mg l^{-1} and the temperatures recorded were all within the range 15 ± 1°C. Summaries of measurements are shown in Tables 13 - 15. At no time during the course of the study was the dissolved oxygen concentration in any of the test vessels less than 60% of the air-saturation value, of 4.9 mg l^{-1} (Ref 6).

The continuous record of temperature, recorded automatically in the dilution water control, during all phases of the definitive test, remained within 15 ± 1°C. The light intensity measured during the pre-exposure phase, on day –12, was 817 lux (cosine). The light intensity was measured four times during the exposure phase of the study and ranged from a maximum of 774 lux (cosine) on day 0 to a minimum of 726 lux (cosine) at the end of the study (day 70).

Regular test dilution water salinity measurements made during the mussel exposure phase of the test, ranged from 34.5 to 35.5 ‰ (21 samples).

Full water quality parameters of the laboratory seawater supply are shown in Table 16. The water quality determinations were typical for Brixham seawater.

6 CONCLUSION

For days 0-70 mussel SGR (shell length) and SGR (dry tissue weight), the no observed effect TBBPA concentration (NOEC, P=0.05) was 17 $\mu g\ l^{-1}$ and the lowest observed effect concentration (LOEC, P=0.05) was 32 $\mu g\ l^{-1}$, based on arithmetic mean measured concentrations.

The NOEC for days 0-70 mussel SGR (wet tissue weight) was 62 $\mu g\ l^{-1}$ and the LOEC, P=0.05 was 126 $\mu g\ l^{-1}$ based on arithmetic mean measured concentrations.

7 REFERENCES

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TABLE 1

ANALYTICAL RESULTS[a]

Nominal conc of TBBPA ($\mu g\ l^{-1}$)	Exposure day measured concentrations TBBPA ($\mu g\ l^{-1}$)															Mean measured conc over the exposure duration[b] ($\mu g\ l^{-1}$)	Percentage of nominal conc
	-10	-7	-6	0	4	7	11	15	22	29	36	43	50	57	64		
Dilution water control	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	-
Solvent control	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	-
19	17.1[c]	18.1[d]	18.8[d]	19.0[c]	24.7[c]	9.53[e]	13.3[f]	12.8[g]	17.5[e]	21.1[f]	20.5[g]	15.9[e]	16.8[f]	20.1[g]	17.8[e]	17	89
38	30.7[c]	31.3[d]	29.3[d]	30.8[c]	31.6[c]	36.1[e]	24.0[f]	31.6[g]	32.8[e]	31.2[f]	35.1[g]	30.2[e]	36.8[f]	31.9[g]	32.5[e]	32	84
75	57.0[c]	70.3[d]	48.8[d]	72.8[c]	60.7[c]	68.4[e]	56.7[f]	66.8[g]	73.3[e]	73.5[f]	48.6[g]	43.5[e]	48.9[f]	75.7[g]	50.3[e]	62	83
150	137[c]	133[d]	96.3[d]	157[c]	64.4[c]	138[e]	115[f]	118[g]	139[e]	143[f]	132[g]	128[e]	110[f]	135[g]	126[e]	126	84
300	255[c]	235[d]	207[d]	228[c]	270[c]	261[e]	229[f]	232[g]	233[e]	247[f]	211[g]	221[e]	185[f]	193[g]	203[e]	226	75

a Exposure day measured concentrations are quoted to 3 significant figures, mean measured concentrations and percentages are quoted to the nearest integer
b Calculated using the arithmetic mean of the exposure day 0 to 64 results
c Mean of three (A, B and C) replicate tank samples (days -10, 0 and 4)
d Mean of duplicate samples from A replicate tanks, one sample centrifuged (40 000G, 30 minutes, 15°C) (days -7 and -6)
day –7 (no algae present): 19 $\mu g\ l^{-1}$ 16.3, 19.8 (cent): 38 $\mu g\ l^{-1}$ 31.5, 31.1 (cent): 75 $\mu g\ l^{-1}$ 65.2, 75.4 (cent): 150 $\mu g\ l^{-1}$ 135, 131 (cent): 300 $\mu g\ l^{-1}$ 233, 237 (cent)
day –6 (algae present):19 $\mu g\ l^{-1}$ 18.4, 19.2 (cent): 38 $\mu g\ l^{-1}$ 30.9, 27.7 (cent): 75 $\mu g\ l^{-1}$ 49.5, 48.1 (cent): 150 $\mu g\ l^{-1}$ 95.7, 96.8 (cent): 300 $\mu g\ l^{-1}$ 215, 199 (cent)
e Single replicate tank A sample (days 7, 22, 43, 64)
f Single replicate tank B sample (days 11, 29, 50)
g Single replicate tank C sample (days 15, 36, 57)

The highest limit of quantitation in the study was 2.5 $\mu g\ l^{-1}$

TABLE 2

SUMMARY OF EXPOSURE PHASE FLOW DATA

Nominal conc TBBPA ($\mu g\ l^{-1}$)	Mean measured concentration TBBPA ($\mu g\ l^{-1}$)	No. of readings	Replicate tank inflow rates (ml min-1)			
			Mean			Overall range of tank inflow rates
			Rep A	Rep B	Rep C	
Dilution water control	-	24	203	202	200	188 – 212
Solvent control	-	24	201	204	202	190 – 210
19	17	24	200	201	199	192 – 210
38	32	24	201	204	201	188 – 210
75	62	24	202	201	204	192 – 210
150	126	24	205	201	204	192 – 210
300	226	24	200	198	202	190 – 210

Nominal conc TBBPA ($\mu g\ l^{-1}$)	Mean measured concentration TBBPA ($\mu g\ l^{-1}$)	No. of readings	Toxicant flow rate (ml min-1)
Dilution water control	-	-	-
Solvent control	-	21	0.002 (on all occasions measured)
19	17	21	0.002 (on all occasions measured)
38	32	21	0.002 (on all occasions measured)
75	62	21	0.002 (on all occasions measured)
150	126	21	0.002 (on all occasions measured)
300	226	21	0.002 (on all occasions measured)

TABLE 3

MUSSEL LENGTHS

Exposure concentration: Dilution water control

Replicate tank	Exposure day 0	Exposure day 14	Exposure day 28	Exposure day 42	Exposure day 56	Exposure day 70
A	11.8	11.5	13.2	14.2	15.5	19.8
A	10.7	11.1	14.6	11.8	17.1	18.3
A	11.8	12.3	12.3	15.9	14.8	17.8
A	11.8	13.3	12.7	15.6	13.4	16.6
A	9.83	12.9	11.5	13.6	17.8	13.3
A	10.5	12.9	12.5	14.9	17.7	19.3
A	9.72	11.9	13.5	13.5	16.8	16.6
A	9.60	11.0	13.9	12.5	12.7	13.9
A	10.0	12.1	13.2	15.3	16.0	16.8
A	11.2	11.9	13.8	13.2	15.0	19.9
Mean	10.7	12.1	13.1	14.1	15.7	17.2
SD	0.903	0.775	0.897	1.37	1.74	2.29
B	11.0	12.7	13.7	15.1	15.9	17.2
B	11.1	12.6	14.1	16.3	15.1	17.1
B	10.1	12.2	12.8	16.9	17.7	16.0
B	11.8	12.3	13.3	15.6	15.6	18.6
B	10.5	13.2	12.6	13.9	15.1	16.2
B	10.8	13.7	15.0	14.0	14.7	20.3
B	10.1	12.3	14.8	14.7	14.6	15.7
B	10.2	12.5	12.7	14.6	15.0	18.5
B	11.4	11.8	13.5	13.7	17.2	16.0
B	11.1	12.7	14.4	13.5	18.9	16.0
Mean	10.8	12.6	13.7	14.8	16.0	17.2
SD	0.578	0.535	0.867	1.14	1.46	1.52
C	10.8	12.1	14.8	12.5	14.5	13.3
C	10.7	12.3	12.7	16.2	17.5	19.3
C	10.9	13.3	13.2	12.1	13.1	16.1
C	10.6	11.8	11.8	15.9	12.5	15.5
C	11.6	12.5	14.9	13.2	15.5	15.1
C	8.66	10.2	13.5	11.9	17.2	15.9
C	11.4	13.6	10.9	13.5	13.7	13.2
C	11.3	12.7	14.1	16.4	13.6	15.1
C	10.1	11.2	12.4	14.3	18.8	21.4
C	10.7	12.4	13.0	13.1	13.6	18.2
Mean	10.7	12.2	13.1	13.9	15.0	16.3
SD	0.832	0.985	1.27	1.71	2.15	2.60
Overall	10.7	12.3	13.3	14.3	15.6	16.9
Overall SD	0.759	0.791	1.03	1.43	1.79	2.15

All lengths in mm
SD = Standard deviation

TABLE 3 CONTD

MUSSEL LENGTHS

Exposure concentration: Solvent control

Replicate tank	Exposure day 0	Exposure day 14	Exposure day 28	Exposure day 42	Exposure day 56	Exposure day 70
A	10.5	12.7	14.1	16.3	16.3	16.6
A	11.0	12.3	14.7	15.4	14.8	17.8
A	11.3	12.8	12.5	14.5	17.2	13.9
A	11.4	12.2	15.9	12.9	13.4	16.4
A	10.2	12.0	13.0	15.8	17.6	16.5
A	10.3	13.6	13.3	15.8	17.2	14.9
A	11.0	12.5	13.5	17.2	16.0	13.4
A	11.1	12.8	14.5	13.3	16.2	17.0
A	11.9	12.5	14.3	15.9	13.2	17.9
A	10.4	12.5	14.3	14.5	16.1	18.2
Mean	10.9	12.6	14.0	15.2	15.8	16.3
SD	0.551	0.438	0.973	1.35	1.53	1.67
B	11.2	11.7	13.8	12.3	19.8	16.4
B	10.3	12.9	15.2	15.0	16.3	16.6
B	11.9	14.3	16.7	15.3	14.7	13.4
B	10.5	11.0	11.8	14.6	16.9	14.7
B	11.3	12.5	13.8	16.1	12.9	20.8
B	10.1	11.9	13.8	18.6	15.6	15.4
B	9.86	13.4	13.6	14.0	15.6	17.8
B	11.1	13.4	13.8	14.1	14.5	17.9
B	10.3	12.4	13.5	14.6	14.7	15.6
B	11.7	12.3	14.5	14.9	16.0	17.5
Mean	10.8	12.6	14.1	15.0	15.7	16.6
SD	0.705	0.958	1.26	1.62	1.83	2.05
C	11.1	12.9	11.7	12.9	14.8	16.1
C	10.6	12.7	13.3	14.4	13.0	15.2
C	11.4	12.5	12.3	12.6	13.2	18.9
C	10.6	13.3	12.9	12.9	18.0	13.5
C	11.3	11.3	14.0	16.4	17.0	15.3
C	9.63	12.0	14.9	15.2	15.4	13.7
C	9.35	10.5	14.6	13.6	14.7	14.0
C	9.93	11.4	12.1	14.2	16.6	15.1
C	10.8	11.0	14.3	14.3	13.4	17.5
C	10.8	12.4	13.2	16.0	14.6	17.4
Mean	10.6	12.0	13.3	14.3	15.1	15.7
SD	0.698	0.913	1.10	1.31	1.69	1.80
Overall	10.8	12.4	13.8	14.8	15.5	16.2
Overall SD	0.651	0.826	1.13	1.44	1.66	1.83

All lengths in mm
SD = Standard deviation

TABLE 3 CONTD

MUSSEL LENGTHS

Nominal (mean measured) exposure concentration of TBBPA: 19 (17) $\mu g\ l^{-1}$

Replicate tank	Exposure day 0	Exposure day 14	Exposure day 28	Exposure day 42	Exposure day 56	Exposure day 70
A	11.6	12.5	14.8	16.1	16.1	14.4
A	11.1	11.4	14.3	16.0	15.3	13.8
A	11.2	13.2	14.4	14.6	17.4	16.3
A	11.5	13.2	12.9	15.5	13.8	16.4
A	11.3	12.6	14.3	14.3	17.9	12.5
A	11.0	12.7	14.6	15.1	15.5	19.4
A	9.97	10.7	11.7	15.8	17.2	16.7
A	11.5	11.7	13.8	16.1	16.2	16.1
A	9.50	13.1	12.6	13.3	12.3	18.5
A	10.5	12.6	14.3	12.1	15.7	15.9
Mean	10.9	12.4	13.8	14.9	15.7	16.0
SD	0.706	0.838	1.02	1.34	1.69	2.06
B	10.4	13.1	11.3	15.6	14.5	16.9
B	11.5	12.8	13.7	14.5	13.6	14.1
B	10.3	13.2	13.3	13.2	16.7	16.8
B	10.9	12.2	14.3	15.7	16.0	17.5
B	11.9	10.9	12.5	12.7	12.1	17.6
B	11.1	12.0	14.6	14.0	15.8	13.8
B	10.6	11.6	14.5	11.8	14.4	14.8
B	11.6	11.4	13.3	14.1	13.6	12.3
B	10.8	11.6	12.8	14.8	16.6	14.0
B	10.0	13.0	12.2	13.5	13.7	15.0
Mean	10.9	12.2	13.3	14.0	14.7	15.3
SD	0.615	0.809	1.07	1.24	1.52	1.82
C	10.4	12.0	14.6	16.5	17.1	17.3
C	11.5	11.5	12.6	14.2	14.2	14.8
C	10.3	13.1	13.0	14.6	16.7	15.0
C	11.7	11.7	15.1	12.7	15.5	14.2
C	10.2	12.2	12.4	15.5	15.6	17.2
C	10.0	11.5	15.3	14.0	13.5	17.4
C	11.7	11.4	13.2	15.6	18.1	14.5
C	11.7	13.7	13.4	16.5	16.4	16.6
C	11.8	12.5	14.2	13.2	14.6	19.1
C	9.67	13.4	12.7	13.0	13.8	18.4
Mean	10.9	12.3	13.7	14.6	15.6	16.5
SD	0.851	0.843	1.07	1.40	1.53	1.72
Overall	10.9	12.3	13.6	14.5	15.3	15.9
Overall SD	0.705	0.805	1.04	1.34	1.59	1.87

All lengths in mm
SD = Standard deviation

TABLE 3 CONTD

MUSSEL LENGTHS

Nominal (mean measured) exposure concentration of TBBPA 38 (32) $\mu g\ l^{-1}$

Replicate tank	Exposure day 0	Exposure day 14	Exposure day 28	Exposure day 42	Exposure day 56	Exposure
A	11.9	12.6	14.3	16.6	17.2	13.6
A	10.8	10.8	13.0	14.2	14.9	12.9
A	9.52	13.8	12.9	16.6	15.8	15.3
A	11.4	12.8	15.6	13.8	13.4	15.4
A	9.83	12.9	11.5	12.3	13.1	18.9
A	11.4	10.8	12.3	15.1	14.8	13.9
A	10.7	12.2	14.2	13.0	15.2	18.0
A	10.3	11.6	14.9	13.8	13.1	14.8
A	10.1	11.8	12.4	14.9	14.4	15.3
A	10.6	11.5	12.7	12.4	18.4	16.5
Mean	10.7	12.1	13.4	14.3	15.0	15.5
SD	0.752	0.964	1.30	1.54	1.74	1.89
B	11.4	12.6	12.6	13.9	16.1	14.3
B	9.78	12.3	13.1	15.0	13.4	15.3
B	10.5	12.4	13.3	13.2	15.3	16.7
B	11.5	11.3	11.5	13.9	14.9	16.3
B	10.5	11.0	13.9	13.7	12.9	16.7
B	10.5	10.9	12.8	14.9	15.1	13.8
B	11.0	12.4	12.4	12.7	16.1	14.6
B	10.7	11.8	13.7	13.5	13.7	15.0
B	9.78	11.5	13.8	14.5	14.7	16.1
B	11.7	12.6	12.6	12.1	14.2	13.9
Mean	10.7	11.9	13.0	13.7	14.6	15.3
SD	0.668	0.665	0.745	0.924	1.09	1.12
C	9.9	13.5	13.3	15.9	14.3	13.4
C	11.4	13.1	13.7	14.0	15.1	16.0
C	11.6	12.6	14.4	15.0	13.0	16.8
C	10.6	13.3	14.1	14.7	16.0	15.8
C	9.92	10.9	13.0	14.1	14.0	15.4
C	11.6	11.8	14.9	14.8	15.0	14.7
C	11.1	11.1	12.9	12.2	16.4	16.7
C	11.4	10.7	13.7	13.0	15.2	15.3
C	11.8	12.3	11.4	13.8	15.6	14.1
C	9.53	12.3	12.1	15.1	12.5	12.7
Mean	10.9	12.2	13.4	14.3	14.7	15.1
SD	0.828	1.01	1.05	1.08	1.26	1.36
Overall	10.8	12.0	13.2	14.1	14.8	15.3
Overall SD	0.733	0.870	1.04	1.20	1.35	1.45

All lengths in mm
SD = Standard deviation

TABLE 3 CONTD

MUSSEL LENGTHS

Nominal (mean measured) exposure concentration of TBBPA: 75 (62) $\mu g\ l^{-1}$

Replicate tank	Exposure day 0	Exposure day 14	Exposure day 28	Exposure day 42	Exposure day 56	Exposure
A	10.8	13.0	14.0	14.0	14.4	16.7
A	9.51	12.4	11.7	15.1	16.6	13.2
A	11.6	13.5	14.8	14.0	13.8	12.5
A	11.3	10.6	12.2	14.6	14.6	14.7
A	11.0	11.2	13.1	16.0	15.5	16.1
A	9.53	13.5	12.4	12.4	12.9	16.8
A	10.8	10.8	14.6	12.3	12.5	14.1
A	11.7	12.0	12.0	13.4	16.0	15.2
A	9.91	12.4	13.3	13.2	14.0	12.7
A	11.8	12.4	13.0	12.5	12.5	13.4
Mean	10.8	12.2	13.1	13.8	14.3	14.5
SD	0.870	1.04	1.08	1.23	1.43	1.62
B	10.9	11.9	13.7	14.2	12.9	12.7
B	9.92	12.1	13.2	14.6	13.6	15.4
B	10.8	11.5	13.9	14.1	14.2	14.5
B	10.9	12.6	13.6	14.5	15.2	14.7
B	11.2	12.3	12.3	13.1	15.0	13.3
B	10.0	13.0	13.2	13.8	13.9	16.2
B	10.1	11.3	12.6	13.3	14.6	15.0
B	10.9	12.3	13.0	14.2	14.4	14.9
B	10.6	10.8	13.1	12.7	14.0	14.7
B	11.4	11.7	11.5	11.9	12.1	14
Mean	10.7	12.0	13.0	12.5	14.0	14.5
SD	0.512	0.650	0.719	0.872	0.942	1.01
C	10.9	12.1	11.3	12.4	12.7	15.4
C	9.50	10.6	14.7	13.8	14.8	15.5
C	11.8	10.4	13.5	14.7	14.5	15.6
C	10.4	12.0	13.0	13.3	15.5	15.8
C	11.6	13.4	12.7	15.3	13.9	14.3
C	10.0	12.1	13.2	13.4	12.9	13.6
C	10.7	11.5	14.3	14.2	15.4	12.9
C	9.45	12.9	13.6	14.1	15.1	14.5
C	11.4	12.6	13.2	15.0	13.5	14.3
C	11.3	11.7	11.7	12.2	14.3	13.0
Mean	10.7	11.9	13.1	13.8	14.3	14.5
SD	0.849	0.941	1.04	1.04	0.994	1.08
Overall	10.7	12.0	13.1	13.7	14.2	14.5
Overall SD	0.737	0.868	0.928	1.02	1.11	1.22

All lengths in mm
SD = Standard deviation

TABLE 3 CONTD

MUSSEL LENGTHS

Nominal (mean measured) exposure concentration of TBBPA: 150 (126) $\mu g\ l^{-1}$

Replicate tank	Exposure day 0	Exposure day 14	Exposure day 28	Exposure day 42	Exposure day 56	Exposure day 70
A	11.4	11.7	12.3	11.8	12.8	13.2
A	11.4	10.9	12.3	12.9	13.6	14.3
A	9.37	10.7	12.2	12.4	13.0	12.9
A	11.1	11.2	11.4	13.0	11.8	14.4
A	9.96	10.6	11.8	12.6	12.4	13.6
A	10.2	11.8	13.1	12.7	12.1	13.1
A	9.78	12.6	11.7	10.5	13.5	11.9
A	11.7	11.7	12.3	13.4	13.2	10.7
A	10.8	9.88	11.2	11.4	13.7	12.1
A	9.61	11.8	10.2	11.8	10.6	14.3
Mean	10.5	11.3	11.9	12.3	12.7	13.1
SD	0.848	0.785	0.793	0.872	0.970	1.20
B	10.3	11.5	11.6	10.1	12.5	14.4
B	10.8	11.3	9.96	12.9	12.1	13.8
B	11.1	10.9	11.6	11.8	12.9	12.6
B	9.64	11.3	11.9	11.7	12.9	11.8
B	10.5	11.6	11.9	12.0	11.8	13.1
B	10.9	11.3	12.2	13.0	10.1	12.2
B	10.5	11.5	11.9	12.3	13.4	13.3
B	9.65	9.86	12.5	13.7	14.0	12.2
B	10.3	10.8	11.5	12.7	12.0	13.4
B	10.5	11.7	12.7	12.7	13.2	10.0
Mean	10.4	11.2	11.8	12.3	12.5	12.7
SD	0.481	0.544	0.748	0.981	1.08	1.23
C	9.12	12.1	11.0	12.5	14.3	13.9
C	9.62	10.9	12.9	11.6	13.5	14.6
C	9.97	11.4	12.3	12.7	11.8	15.1
C	10.8	12.1	12.9	13.3	13.6	12.7
C	11.6	10.7	12.6	13.2	11.3	11.7
C	11.3	11.9	12.2	13.5	12.8	12.1
C	11.2	9.85	13.3	12.8	14.2	14.6
C	10.7	12.1	11.8	11.4	12.1	12.3
C	9.24	10.3	11.4	12.1	12.4	12.3
C	10.7	12.2	10.6	10.9	11.5	11.7
Mean	10.4	11.4	12.1	12.4	12.8	13.1
SD	0.882	0.862	0.886	0.876	1.10	1.31
Overall	10.5	11.3	11.9	12.3	12.6	12.9
Overall SD	0.734	0.721	0.795	0.882	1.02	1.22

All lengths in mm
SD = Standard deviation

TABLE 3 CONTD

MUSSEL LENGTHS

Nominal (mean measured) exposure concentration of TBBPA: 300 (226) $\mu g\ l^{-1}$

Replicate tank	Exposure day 0	Exposure day 14	Exposure day 28	Exposure day 42	Exposure day 56	Exposure day 70
A	10.1	13.1	10.8	14.2	12.4	11.1
A	11.7	10.5	11.0	13.7	14.4	14.5
A	9.91	10.5	13.7	12.1	11.1	12.3
A	10.4	11.3	11.9	11.9	12.2	13.8
A	12.0	10.3	11.7	11.0	14.2	11.8
A	11.4	13.2	13.8	11.1	11.8	11.2
A	11.1	11.7	13.8	14.0	11.2	14.2
A	9.99	12.9	11.7	11.8	11.2	11.4
A	10.2	10.9	10.7	10.8	13.5	12.4
A	11.8	-	-	-	-	-
Mean	10.9	11.6	12.1	12.3	12.4	12.5
SD	0.825	1.18	1.30	1.34	1.29	1.32
B	11.4	11.5	13.4	12.6	12.9	14.9
B	11.1	11.9	12.1	12.9	14.8	13.0
B	9.56	12.0	12.2	11.0	14.2	14.5
B	11.9	12.1	13.9	12.2	13.1	12.3
B	10.3	12.4	13.6	14.2	11.3	11.4
B	10.9	11.6	13.1	12.3	13.8	13.8
B	10.5	12.1	10.9	13.6	14.3	14.6
B	11.5	12.7	12.5	13.7	13.0	12.3
B	11.7	10.3	13.0	12.7	12.2	13.3
B	11.3	12.8	12.1	13.9	12.3	13.0
Mean	11.0	11.9	12.7	12.9	13.2	13.3
SD	0.717	0.714	0.897	0.967	1.09	1.14
C	12.0	11.1	12.1	13.9	10.8	12.1
C	11.9	10.2	11.4	13.2	16.2	16.3
C	11.2	12.3	13.7	11.7	14.3	11.3
C	10.1	13.8	15.2	12.3	11.9	10.8
C	9.95	12.3	13.2	14.2	14.1	13.2
C	11.2	13.1	11.0	12.7	11.3	14.3
C	12.0	12.6	13.9	10.6	13.0	13.1
C	9.78	12.6	13.1	13.5	13.4	14.1
C	11.1	11.1	12.7	11.3	12.4	13.5
C	11.8	9.95	10.1	16.0	13.2	12.3
Mean	11.1	11.9	12.6	12.9	13.1	13.1
SD	0.871	1.26	1.52	1.58	1.58	1.60
Overall	11.0	11.8	12.5	12.7	12.9	13.0
Overall SD	0.785	1.05	1.24	1.30	1.33	1.36

All lengths in mm
SD = Standard deviation
- One mussel recorded missing from day 14

TABLE 4

MUSSEL WEIGHT DATA DAY 0 SACRIFICED MUSSELS

Mussel ID.	Shell length (mm)	Wet flesh wt (mg)	Dry flesh wt (mg)
1	10.9	28.6	4.52
2	11.0	34.5	4.20
3	9.42	23.0	1.87
4	11.8	50.7	5.39
5	11.8	41.5	4.66
6	11.2	42.7	5.07
7	10.9	32.4	3.72
8	10.1	31.8	3.67
9	11.6	44.2	5.11
10	8.90	20.7	1.14
11	10.5	28.6	2.88
12	9.70	23.4	1.38
13	10.9	34.1	3.65
14	11.0	31.6	3.07
15	9.51	24.3	2.08
16	10.5	29.5	2.57
17	11.6	40.7	4.86
18	11.5	41.3	5.02
19	10.3	27.3	2.90
20	10.1	25.9	2.83
21	11.3	39.5	5.14
22	11.9	48.3	6.25
23	12.0	42.9	4.77
24	12.0	49.0	5.75
25	10.7	32.5	2.19
26	10.9	27.4	1.63
27	11.7	45.0	4.53
28	11.8	30.6	1.95
29	10.5	27.2	2.42
30	11.1	26.1	1.94
Mean	10.9	34.2	3.57
SD	0.829	8.60	1.46

SD = Standard deviation

TABLE 5

MUSSEL WET WEIGHTS (DAY 70)

Replicate tank	Nominal (measured) concentration of TBBPA (µg l^{-1})						
	Diln water control	Solvent control	19 (17)	38 (32)	75 (62)	150 (126)	300 (226)
A	188	92.2	175	94	138	80	38.6
A	164	129	91.3	72.7	74.8	137	114
A	147	74.5	127	105	65.4	75.8	67.6
A	116	101	123	118	92.8	118	75.8
A	59.4	128	52.6	190	123	85.6	39.8
A	184	85.0	198	84.7	137	88.7	60.8
A	109	69.4	131	180	96.0	41.1	97.5
A	83.1	146	129	111	94.8	38.8	59.6
A	125	151	172	118	82.0	53.4	59.1
A	182	169	116	156	90.7	103	-
Mean	136	115	131	123	99.4	82.0	68.1
SD	44.8	34.9	42.4	39.9	25.1	31.9	24.8
B	133	122	132	115	64.8	110	115
B	146	100	86.0	94.9	129	98.4	72.7
B	101	58.9	159	147	101	72.5	106
B	163	80.2	130	119	119	60.9	77.6
B	113	241	160	136	78.5	85.5	50.6
B	213	90.3	103	78.3	130	91.4	85.3
B	117	143	92.5	91.7	113	97.1	95.1
B	136	152	48.3	88.7	142	71.8	55.9
B	76.9	96.6	80.9	94.3	118	91.6	87.2
B	107	130	121	89.7	99.3	47.2	85.8
Mean	131	121	111	105	109	82.6	83.1
SD	37.8	51.0	35.8	22.7	24.0	19.1	20.2
C	53.8	115	151	74.1	146	83.4	62.0
C	195	117	88.3	120	106	118	161
C	112	172	113	148	108	115	47.3
C	103	70.2	96.6	127	140	81.1	36.6
C	91.8	84.0	171	109	100	80.4	70.2
C	117	78.8	156	114	84.8	65.1	104
C	50.0	73.5	84.9	135	89.3	81.2	85.5
C	103	100	124	91.9	101	46.6	75.5
C	224	157	206	113	109	66.0	110
C	160	145	172	72.6	66.7	59.7	68.2
Mean	121	111	136	111	105	79.6	82.0
SD	56.6	36.6	40.9	24.7	23.7	22.7	35.9
Overall	129	116	126	113	105	81.4	78.1
Overall SD	45.8	40.2	40.0	30.0	23.8	24.3	27.7

All weights in mg
SD = Standard deviation
- One mussel recorded missing from day 14

TABLE 6

MUSSEL DRY WEIGHTS (DAY 70)

Replicate tank	Nominal (measured) concentration of TBBPA (µg l^{-1})						
	Diln water control	Solvent control	19 (17)	38 (32)	75 (62)	150 (126)	300 (226)
A	26.2	14.4	23.7	9.57	29.5	14.2	3.22
A	24.3	19.2	10.2	6.85	17.2	22.6	13.5
A	21.9	10.3	17.4	17.9	15.8	13.1	8.75
A	17.6	14.6	16.6	13.9	22.3	20.8	9.45
A	9.75	21.6	5.97	26.6	24.3	15.8	4.48
A	25.1	11.4	27.6	8.81	25.9	15.6	9.77
A	16.4	8.90	18.6	23.7	20.1	6.13	15.8
A	12.9	20.8	16.2	13.5	21.9	6.53	8.12
A	20.3	22.8	25.1	16.4	18.1	14.8	6.81
A	26.7	24.9	14.8	20.8	18.8	18.9	-
Mean	20.1	16.9	17.6	15.8	21.4	14.9	8.87
SD	5.83	5.69	6.64	6.53	4.25	5.39	3.97
B	21.2	18.6	16.1	11.9	15.5	19.7	16.3
B	23.6	14.2	9.22	10.0	25.2	15.8	10.7
B	19.8	6.75	20.4	17.0	19.3	11.4	16.3
B	24.0	10.6	17.5	12.4	23.2	11.9	10.6
B	17.1	35.2	22.9	15.5	14.1	13.8	4.77
B	31.9	12.2	13.3	0.620	24.5	15.1	11.0
B	16.7	21.2	9.42	4.48	23.4	15.7	11.0
B	19.7	22.5	3.40	4.15	26.9	10.9	5.79
B	12.6	14.4	10.7	8.88	21.9	14.8	12.4
B	16.3	19.7	18.0	8.43	17.9	11.0	12.6
Mean	20.3	17.5	14.1	9.32	21.2	14.0	11.2
SD	5.35	7.95	5.96	5.16	4.28	2.79	3.77
C	8.19	16.5	20.8	3.12	26.4	12.8	8.78
C	29.8	16.3	9.31	10.3	18.0	21.2	22.7
C	10.0	26.4	13.0	14.4	18.5	18.6	4.50
C	14.0	9.30	11.3	11.7	23.4	11.3	3.22
C	12.2	10.2	21.8	8.88	18.8	13.3	7.44
C	19.1	12.5	20.9	11.4	14.5	10.3	13.5
C	5.60	9.59	10.0	12.5	22.8	13.1	14.6
C	14.3	13.9	14.7	3.20	17.8	6.38	10.4
C	28.4	22.4	29.6	10.7	20.8	10.2	13.7
C	23.1	19.7	22.2	1.75	13.3	8.39	9.29
Mean	16.5	15.7	17.4	8.80	19.4	12.5	10.8
SD	8.37	5.76	6.65	4.47	4.03	4.46	5.63
Overall	19.0	16.7	16.4	11.3	20.7	13.8	10.3
Overall SD	6.66	6.37	6.41	6.18	4.14	4.31	4.50

All weights in mg
SD = Standard deviation
- One mussel recorded missing from day 14

TABLE 7

INDIVIDUAL PSEUDO SPECIFIC GROWTH RATES (LENGTH) OF THE MUSSELS

Exposure concentration: Dilution water control

Replicate tank	Individual mussels pseudo specific growth rate (SGR) over the period days				
	0-14	0-28	0-42	0-56	0-70
A	0.541	0.771	0.683	0.668	0.884
A	0.288	1.12	0.242	0.844	0.772
A	1.02	0.508	0.952	0.586	0.732
A	1.58	0.634	0.906	0.408	0.633
A	1.36	0.271	0.580	0.915	0.316
A	1.36	0.568	0.797	0.905	0.848
A	0.785	0.843	0.562	0.812	0.633
A	0.224	0.948	0.379	0.313	0.379
A	0.904	0.763	0.860	0.725	0.650
A	0.785	0.922	0.509	0.610	0.892
Mean	0.885	0.735	0.647	0.679	0.674
SD	0.458	0.247	0.235	0.204	0.198
B	1.16	0.851	0.799	0.691	0.665
B	1.10	0.954	0.981	0.599	0.657
B	0.873	0.608	1.07	0.883	0.562
B	0.932	0.745	0.876	0.657	0.777
B	1.44	0.552	0.602	0.599	0.580
B	1.70	1.17	0.619	0.551	0.902
B	0.932	1.127	0.735	0.539	0.535
B	1.05	0.580	0.719	0.587	0.769
B	0.635	0.798	0.567	0.832	0.562
B	1.16	1.03	0.532	1.00	0.562
Mean	1.10	0.842	0.750	0.694	0.657
SD	0.300	0.226	0.181	0.158	0.123
C	0.916	1.18	0.383	0.552	0.318
C	1.03	0.631	1.00	0.888	0.850
C	1.59	0.769	0.305	0.371	0.591
C	0.736	0.368	0.955	0.287	0.537
C	1.15	1.20	0.512	0.671	0.500
C	-0.305	0.849	0.265	0.857	0.573
C	1.75	0.0848	0.566	0.451	0.307
C	1.26	1.00	1.03	0.438	0.500
C	0.363	0.545	0.703	1.02	0.998
C	1.09	0.714	0.494	0.438	0.766
Mean	0.958	0.734	0.621	0.597	0.594
SD	0.594	0.350	0.287	0.248	0.221
Overall mean	0.981	0.770	0.673	0.656	0.642
Overall SD	0.459	0.275	0.237	0.204	0.182

SD = Standard deviation

TABLE 7 CONTD

INDIVIDUAL PSEUDO SPECIFIC GROWTH RATES (LENGTH) OF THE MUSSELS

Exposure concentration: Solvent control

Replicate tank	Individual mussels pseudo specific growth rate (SGR) over the period days				
	0-14	0-28	0-42	0-56	0-70
A	1.09	0.920	0.959	0.719	0.601
A	0.865	1.07	0.823	0.547	0.701
A	1.15	0.490	0.680	0.815	0.348
A	0.806	1.349	0.402	0.369	0.584
A	0.688	0.630	0.884	0.856	0.593
A	1.58	0.712	0.884	0.815	0.447
A	0.980	0.765	1.09	0.690	0.295
A	1.15	1.02	0.474	0.704	0.635
A	0.980	0.970	0.899	0.345	0.709
A	0.980	0.970	0.680	0.694	0.733
Mean	1.03	0.890	0.777	0.655	0.565
SD	0.245	0.247	0.216	0.180	0.152
B	0.568	0.874	0.308	1.08	0.596
B	1.27	1.22	0.781	0.734	0.613
B	2.00	1.55	0.828	0.550	0.307
B	0.127	0.314	0.717	0.799	0.440
B	1.04	0.874	0.949	0.316	0.936
B	0.689	0.874	1.29	0.656	0.506
B	1.54	0.822	0.617	0.656	0.713
B	1.54	0.874	0.634	0.525	0.721
B	0.983	0.795	0.717	0.550	0.525
B	0.925	1.05	0.765	0.701	0.689
Mean	1.07	0.925	0.761	0.657	0.605
SD	0.543	0.318	0.252	0.201	0.174
C	1.45	0.376	0.483	0.608	0.607
C	1.34	0.834	0.745	0.376	0.524
C	1.23	0.555	0.427	0.404	0.836
C	1.67	0.725	0.483	0.957	0.355
C	0.504	1.02	1.05	0.855	0.534
C	0.934	1.24	0.874	0.679	0.376
C	-0.0202	1.17	0.609	0.596	0.407
C	0.567	0.496	0.712	0.813	0.515
C	0.312	1.09	0.729	0.430	0.726
C	1.17	0.807	0.996	0.584	0.718
Mean	0.915	0.831	0.711	0.630	0.560
SD	0.550	0.296	0.217	0.198	0.161
Overall mean	1.00	0.882	0.750	0.647	0.576
Overall SD	0.457	0.281	0.223	0.187	0.159

SD = Standard deviation

TABLE 7 CONTD

INDIVIDUAL PSEUDO SPECIFIC GROWTH RATES (LENGTH) OF THE MUSSELS

Nominal (mean measured) exposure concentration of TBBPA: 19 (17) $\mu g\ l^{-1}$

Replicate tank	Individual mussels pseudo specific growth rate (SGR) over the period days				
	0-14	0-28	0-42	0-56	0-70
A	0.981	1.09	0.930	0.697	0.398
A	0.323	0.971	0.915	0.606	0.338
A	1.37	0.996	0.697	0.836	0.575
A	1.37	0.603	0.839	0.422	0.584
A	1.04	0.971	0.647	0.887	0.196
A	1.09	1.05	0.777	0.629	0.824
A	-0.129	0.254	0.885	0.815	0.610
A	0.509	0.844	0.930	0.708	0.558
A	1.32	0.519	0.475	0.217	0.756
A	1.04	0.971	0.250	0.652	0.540
Mean	0.891	0.827	0.734	0.647	0.538
SD	0.499	0.275	0.225	0.202	0.187
B	1.32	0.131	0.855	0.511	0.627
B	1.15	0.818	0.681	0.396	0.368
B	1.37	0.713	0.457	0.763	0.619
B	0.808	0.971	0.870	0.686	0.677
B	0.00364	0.491	0.365	0.187	0.685
B	0.690	1.05	0.597	0.664	0.338
B	0.448	1.02	0.190	0.498	0.438
B	0.324	0.713	0.614	0.396	0.173
B	0.448	0.576	0.729	0.752	0.358
B	1.26	0.404	0.511	0.409	0.457
Mean	0.782	0.688	0.587	0.526	0.474
SD	0.477	0.294	0.214	0.187	0.172
C	0.709	1.05	0.994	0.810	0.664
C	0.405	0.529	0.637	0.478	0.441
C	1.335	0.640	0.703	0.767	0.460
C	0.528	1.17	0.371	0.634	0.382
C	0.827	0.471	0.846	0.646	0.656
C	0.405	1.22	0.603	0.387	0.673
C	0.342	0.695	0.861	0.911	0.412
C	1.65	0.748	0.994	0.735	0.605
C	1.00	0.955	0.463	0.527	0.806
C	1.50	0.557	0.427	0.427	0.752
Mean	0.870	0.805	0.690	0.632	0.585
SD	0.483	0.276	0.229	0.175	0.150
Overall mean	0.848	0.773	0.670	0.602	0.532
Overall SD	0.472	0.279	0.224	0.190	0.171

SD = Standard deviation

TABLE 7 CONTD

INDIVIDUAL PSEUDO SPECIFIC GROWTH RATES (LENGTH) OF THE MUSSELS

Nominal (mean measured) exposure concentration of TBBPA: 38 (32) $\mu g\ l^{-1}$

Replicate tank	Individual mussels pseudo specific growth rate (SGR) over the period days				
	0-14	0-28	0-42	0-56	0-70
A	1.21	1.06	1.06	0.859	0.352
A	0.113	0.718	0.689	0.603	0.276
A	1.86	0.691	1.06	0.708	0.520
A	1.33	1.37	0.621	0.413	0.529
A	1.38	0.281	0.347	0.373	0.822
A	0.113	0.521	0.835	0.591	0.383
A	0.983	1.03	0.479	0.638	0.752
A	0.623	1.21	0.621	0.373	0.473
A	0.745	0.550	0.804	0.542	0.520
A	0.561	0.635	0.366	0.980	0.628
Mean	0.892	0.806	0.689	0.608	0.526
SD	0.568	0.344	0.255	0.201	0.172
B	1.16	0.578	0.619	0.727	0.412
B	0.984	0.717	0.800	0.399	0.509
B	1.04	0.771	0.496	0.636	0.634
B	0.378	0.252	0.619	0.588	0.599
B	0.186	0.929	0.585	0.331	0.634
B	0.121	0.634	0.785	0.612	0.361
B	1.04	0.521	0.404	0.727	0.442
B	0.688	0.877	0.550	0.438	0.480
B	0.504	0.903	0.720	0.564	0.581
B	1.16	0.578	0.289	0.502	0.371
Mean	0.726	0.676	0.587	0.553	0.502
SD	0.404	0.209	0.162	0.134	0.105
C	1.55	0.724	0.908	0.491	0.300
C	1.34	0.830	0.605	0.589	0.554
C	1.06	1.01	0.769	0.321	0.623
C	1.45	0.933	0.721	0.692	0.536
C	0.0264	0.642	0.622	0.454	0.499
C	0.593	1.13	0.737	0.577	0.433
C	0.156	0.615	0.277	0.736	0.615
C	-0.106	0.830	0.428	0.600	0.490
C	0.889	0.173	0.570	0.647	0.373
C	0.889	0.386	0.785	0.251	0.224
Mean	0.785	0.727	0.642	0.536	0.465
SD	0.600	0.288	0.185	0.157	0.132
Overall mean	0.801	0.736	0.639	0.565	0.498
Overall SD	0.517	0.281	0.202	0.164	0.137

SD = Standard deviation

TABLE 7 CONTD

INDIVIDUAL PSEUDO SPECIFIC GROWTH RATES (LENGTH) OF THE MUSSELS

Nominal (mean measured) exposure concentration of TBBPA: 75 (62) $\mu g\ l^{-1}$

Replicate tank	Individual mussels pseudo specific growth rate (SGR) over the period days				
	0-14	0-28	0-42	0-56	0-70
A	1.35	0.939	0.626	0.520	0.628
A	1.01	0.298	0.806	0.774	0.292
A	1.62	1.14	0.626	0.444	0.214
A	-0.109	0.448	0.726	0.545	0.445
A	0.285	0.702	0.944	0.651	0.575
A	1.62	0.506	0.337	0.323	0.636
A	0.0247	1.09	0.318	0.267	0.386
A	0.777	0.389	0.522	0.708	0.493
A	1.01	0.756	0.486	0.470	0.236
A	1.01	0.675	0.356	0.267	0.313
Mean	0.860	0.694	0.575	0.497	0.422
SD	0.618	0.291	0.211	0.179	0.158
B	0.787	0.896	0.683	0.341	0.250
B	0.906	0.764	0.749	0.435	0.526
B	0.543	0.948	0.666	0.512	0.440
B	1.20	0.870	0.733	0.634	0.459
B	1.02	0.511	0.491	0.610	0.316
B	1.42	0.764	0.615	0.474	0.598
B	0.417	0.598	0.527	0.562	0.488
B	1.02	0.709	0.683	0.537	0.479
B	0.0940	0.737	0.417	0.487	0.459
B	0.666	0.271	0.262	0.226	0.390
Mean	0.807	0.707	0.583	0.482	0.440
SD	0.391	0.202	0.156	0.124	0.100
C	0.896	0.204	0.357	0.310	0.524
C	-0.0498	1.14	0.612	0.584	0.533
C	-0.186	0.839	0.762	0.547	0.542
C	0.836	0.704	0.524	0.666	0.560
C	1.62	0.621	0.857	0.472	0.418
C	0.896	0.759	0.542	0.338	0.346
C	0.532	1.04	0.680	0.655	0.271
C	1.35	0.865	0.663	0.619	0.438
C	1.18	0.759	0.810	0.419	0.418
C	0.655	0.328	0.318	0.522	0.282
Mean	0.774	0.726	0.612	0.513	0.433
SD	0.571	0.289	0.181	0.126	0.107
Overall mean	0.814	0.709	0.590	0.497	0.432
Overall SD	0.518	0.255	0.178	0.141	0.121

SD = Standard deviation

TABLE 7 CONTD

INDIVIDUAL PSEUDO SPECIFIC GROWTH RATES (LENGTH) OF THE MUSSELS

Nominal (mean measured) exposure concentration of TBBPA: 150 (126) $\mu g\ l^{-1}$

Replicate tank	Individual mussels pseudo specific growth rate (SGR) over the period days				
	0-14	0-28	0-42	0-56	0-70
A	0.772	0.565	0.278	0.353	0.327
A	0.266	0.565	0.490	0.462	0.441
A	0.134	0.536	0.396	0.381	0.294
A	0.460	0.293	0.508	0.208	0.451
A	0.0669	0.416	0.434	0.297	0.369
A	0.833	0.790	0.453	0.253	0.316
A	1.30	0.386	-0.00028	0.449	0.179
A	0.772	0.565	0.580	0.408	0.0268
A	-0.436	0.230	0.196	0.475	0.202
A	0.833	-0.104	0.278	0.0167	0.441
Mean	0.500	0.424	0.361	0.330	0.305
SD	0.502	0.245	0.174	0.142	0.136
B	0.712	0.387	-0.0717	0.327	0.464
B	0.587	-0.157	0.511	0.269	0.403
B	0.329	0.387	0.299	0.383	0.273
B	0.587	0.478	0.278	0.383	0.179
B	0.774	0.478	0.339	0.224	0.329
B	0.587	0.567	0.529	-0.0538	0.227
B	0.712	0.478	0.397	0.451	0.350
B	-0.387	0.654	0.654	0.529	0.227
B	0.264	0.356	0.474	0.254	0.361
B	0.835	0.711	0.474	0.424	-0.0572
Mean	0.500	0.434	0.388	0.319	0.275
SD	0.361	0.238	0.198	0.162	0.146
C	1.09	0.203	0.440	0.570	0.416
C	0.342	0.773	0.262	0.467	0.486
C	0.662	0.602	0.478	0.227	0.534
C	1.09	0.773	0.588	0.481	0.287
C	0.209	0.688	0.570	0.150	0.170
C	0.969	0.573	0.623	0.372	0.218
C	-0.382	0.882	0.496	0.558	0.486
C	1.09	0.454	0.221	0.272	0.241
C	-0.063	0.331	0.363	0.316	0.241
C	1.15	0.0712	0.114	0.181	0.170
Mean	0.615	0.535	0.415	0.359	0.325
SD	0.555	0.266	0.171	0.154	0.141
Overall mean	0.538	0.464	0.388	0.336	0.302
Overall SD	0.466	0.246	0.177	0.149	0.138

SD = Standard deviation

TABLE 7 CONTD

INDIVIDUAL PSEUDO SPECIFIC GROWTH RATES (LENGTH) OF THE MUSSELS

Nominal (mean measured) exposure concentration of TBBPA: 300 (226) $\mu g\ l^{-1}$

Replicate tank	Individual mussels pseudo specific growth rate (SGR) over the period days				
	0-14	0-28	0-42	0-56	0-70
A	1.36	-0.0116	0.645	0.241	0.0349
A	-0.222	0.0550	0.559	0.508	0.417
A	-0.222	0.839	0.264	0.0436	0.182
A	0.302	0.336	0.224	0.212	0.346
A	-0.360	0.275	0.0367	0.483	0.122
A	1.41	0.865	0.0582	0.153	0.0477
A	0.551	0.865	0.611	0.0597	0.387
A	1.25	0.275	0.204	0.0597	0.0730
A	0.0447	-0.0438	-0.00704	0.393	0.193
A	-	-	-	-	-
Mean	0.457	0.384	0.288	0.239	0.200
SD	0.720	0.378	0.255	0.183	0.148
B	0.321	0.707	0.325	0.285	0.434
B	0.565	0.342	0.381	0.531	0.239
B	0.625	0.372	0.00125	0.457	0.395
B	0.685	0.838	0.248	0.313	0.160
B	0.859	0.760	0.609	0.0490	0.052
B	0.383	0.626	0.267	0.406	0.325
B	0.685	-0.0307	0.506	0.469	0.405
B	1.03	0.458	0.524	0.299	0.160
B	-0.466	0.598	0.343	0.186	0.272
B	1.09	0.342	0.558	0.200	0.239
Mean	0.577	0.501	0.376	0.320	0.268
SD	0.442	0.258	0.182	0.149	0.124
C	0.0184	0.317	0.542	-0.0443	0.127
C	-0.586	0.104	0.419	0.680	0.553
C	0.752	0.761	0.131	0.457	0.0292
C	1.57	1.13	0.251	0.129	-0.0355
C	0.752	0.628	0.593	0.432	0.251
C	1.20	-0.0231	0.327	0.0365	0.366
C	0.924	0.813	-0.104	0.287	0.240
C	0.924	0.601	0.472	0.341	0.345
C	0.0184	0.490	0.0487	0.202	0.283
C	-0.763	-0.328	0.877	0.314	0.150
Mean	0.481	0.449	0.356	0.283	0.231
SD	0.772	0.437	0.288	0.214	0.172
Overall mean	0.507	0.447	0.342	0.282	0.234
Overall SD	0.637	0.355	0.235	0.180	0.147

SD = Standard deviation
- One mussel recorded missing from day 14

TABLE 8

MEAN PSEUDO SPECIFIC GROWTH RATE (SHELL LENGTH) INDICATING SIGNIFICANT EFFECTS

Nominal conc TBBPA ($\mu g\ l^{-1}$)	Mean measured conc TBBPA ($\mu g\ l^{-1}$)	Mean pseudo specific growth rate (shell length) and (standard deviations) during period days				
		0-14	0-28	0-42	0-56	0-70
Dilution water control	-	0.981 (0.459)	0.770 (0.275)	0.673 (0.237)	0.656 (0.204)	0.642 (0.182)
Solvent control	-	1.00 (0.457)	0.882 (0.281)	0.750 (0.223)	0.647 (0.187)	0.576 (0.159)
19	17	0.848 (0.472)	0.773 (0.279)	0.670 (0.224)	0.602 (0.190)	0.532 (0.171)
38	32	0.801 (0.517)	0.736 (0.281)	0.639 (0.202)	0.565 (0.164)	0.498* (0.137)
75	62	0.814 (0.518)	0.709 # (0.255)	0.590* # (0.178)	0.497* # (0.141)	0.432* # (0.121)
150	126	0.538* # (0.466)	0.464* # (0.246)	0.388* # (0.177)	0.336* # (0.148)	0.302* # (0.138)
300	226	0.507* # (0.637)	0.447* # (0.355)	0.342* # (0.239)	0.282* # (0.180)	0.234* # (0.147)

* Indicates a significant difference (P=0.05) in mean specific growth rate between treatment and the pooled control

\# Indicates a significant difference (P=0.05) in mean specific growth rate between treatment and the solvent control

TABLE 9

INDIVIDUAL PSEUDO SPECIFIC GROWTH RATES (WET WEIGHT) OF THE MUSSELS

Replicate tank	Nominal (measured) concentration of TBBPA (µg l^{-1})						
	Diln water	Solvent control	19 (17)	38 (32)	75 (62)	150 (126)	300 (226)
A	2.48	1.46	2.37	1.48	2.04	1.25	0.216
A	2.29	1.95	1.45	1.12	1.16	2.02	1.76
A	2.13	1.16	1.91	1.64	0.970	1.18	1.02
A	1.79	1.59	1.87	1.81	1.47	1.81	1.18
A	0.833	1.93	0.658	2.50	1.87	1.36	0.260
A	2.45	1.34	2.56	1.34	2.02	1.41	0.867
A	1.69	1.06	1.96	2.42	1.52	0.305	1.54
A	1.31	2.12	1.94	1.73	1.50	0.225	0.838
A	1.89	2.17	2.35	1.81	1.29	0.681	0.827
A	2.43	2.33	1.79	2.22	1.44	1.61	-
Mean	1.93	1.71	1.89	1.81	1.53	1.19	0.946
SD	0.543	0.448	0.540	0.453	0.355	0.606	0.515
B	1.99	1.86	1.97	1.78	0.957	1.71	1.78
B	2.12	1.57	1.36	1.50	1.94	1.55	1.12
B	1.59	0.821	2.24	2.13	1.60	1.12	1.66
B	2.28	1.26	1.95	1.83	1.82	0.868	1.22
B	1.75	2.83	2.25	2.02	1.23	1.35	0.604
B	2.66	1.43	1.62	1.23	1.95	1.45	1.35
B	1.80	2.09	1.47	1.45	1.75	1.53	1.50
B	2.02	2.18	0.537	1.41	2.08	1.10	0.747
B	1.20	1.53	1.27	1.49	1.82	1.45	1.38
B	1.68	1.96	1.85	1.42	1.57	0.505	1.36
Mean	1.91	1.75	1.65	1.63	1.67	1.26	1.27
SD	0.401	0.559	0.521	0.294	0.348	0.367	0.370
C	0.692	1.78	2.16	1.15	2.12	1.32	0.894
C	2.53	1.81	1.40	1.84	1.66	1.81	2.26
C	1.74	2.35	1.75	2.14	1.69	1.78	0.508
C	1.62	1.07	1.53	1.92	2.05	1.28	0.143
C	1.46	1.33	2.34	1.71	1.58	1.27	1.07
C	1.81	1.24	2.21	1.77	1.34	0.965	1.64
C	0.587	1.14	1.34	2.00	1.42	1.28	1.35
C	1.61	1.58	1.89	1.46	1.59	0.487	1.18
C	2.73	2.22	2.61	1.75	1.70	0.984	1.71
C	2.25	2.10	2.35	1.12	1.00	0.840	1.03
Mean	1.70	1.66	1.96	1.69	1.61	1.20	1.18
SD	0.699	0.465	0.441	0.343	0.326	0.406	0.608
Overall	1.85	1.71	1.83	1.71	1.61	1.22*	1.14*
Overall SD	0.551	0.477	0.503	0.364	0.336	0.456	0.508

SD = Standard deviation
- One mussel recorded missing from day 14
* Indicates a significant difference (p=0.05) in mean specific growth rate (wet weight) from the solvent control and the pooled controls

TABLE 10

INDIVIDUAL PSEUDO SPECIFIC GROWTH RATES (DRY WEIGHT) OF THE MUSSELS

Replicate tank	Nominal (measured) concentration of TBBPA (µg l^{-1})						
	Diln water control	Solvent control	19 (17)	38 (32)	75 (62)	150 (126)	300 (226)
A	2.98	2.13	2.84	1.54	3.15	2.11	-0.0125
A	2.87	2.54	163	1.07	2.38	2.77	2.03
A	2.72	1.65	2.40	2.44	2.26	2.00	1.42
A	2.42	2.15	2.33	2.07	2.75	2.65	1.53
A	1.57	2.71	0.87	3.00	2.87	2.26	0.459
A	2.92	1.79	3.06	1.43	2.96	2.24	1.57
A	2.32	1.44	2.49	2.84	2.60	0.907	2.26
A	1.97	2.65	2.29	2.04	2.72	1.00	1.31
A	2.62	2.78	2.92	2.31	2.45	2.16	1.06
A	3.01	2.91	2.16	2.65	2.51	2.51	-
Mean	2.54	2.28	2.30	2.14	2.67	2.06	1.29
SD	0.477	0.519	0.651	0.636	0.279	0.634	0.714
B	2.68	2.49	2.29	1.85	2.23	2.57	2.31
B	2.83	2.11	1.49	1.60	2.93	2.26	1.71
B	2.58	1.04	2.62	2.37	2.54	1.80	2.31
B	2.85	1.69	2.40	1.91	2.81	1.86	1.68
B	2.37	3.40	2.79	2.23	2.10	2.06	0.549
B	3.26	1.89	2.01	-2.37	2.89	2.20	1.74
B	2.34	2.68	1.52	0.459	2.82	2.25	1.75
B	2.57	2.76	0.0652	0.350	3.02	1.73	0.826
B	1.94	2.13	1.71	1.44	2.72	2.16	1.92
B	2.31	2.58	2.45	1.36	2.44	1.74	1.94
Mean	2.57	2.28	1.94	1.12	2.65	2.06	1.67
SD	0.366	0.656	0.798	1.395	0.309	0.277	0.570
C	1.32	2.32	2.65	-0.0575	2.99	1.96	1.42
C	3.17	2.30	1.50	1.65	2.45	2.68	2.77
C	1.61	3.00	1.98	2.13	2.49	2.49	0.466
C	2.08	1.50	1.78	1.83	2.82	1.77	-0.0125
C	1.89	1.63	2.72	1.44	2.51	2.01	1.18
C	2.53	1.92	2.66	1.79	2.14	1.65	2.04
C	0.778	1.55	1.61	1.93	2.79	1.99	2.15
C	2.12	2.08	2.16	-0.02	2.43	0.964	1.66
C	3.10	2.76	3.16	1.70	2.65	1.63	2.05
C	2.80	2.57	2.75	-0.884	2.01	1.36	1.50
Mean	2.14	2.16	2.30	1.15	2.53	1.85	1.52
SD	0.777	0.519	0.564	1.06	0.302	0.503	0.825
Overall	2.42	2.24	2.18	1.47* #	2.61	1.99*	1.50* #
Overall SD	0.583	0.551	0.677	1.14	0.293	0.487	0.703

SD = Standard deviation
- One mussel recorded missing from day 14
* Indicates a significant difference (p=0.05) in mean specific growth rate (dry weight) from the pooled controls
# Indicates a significant difference (p=0.05) in mean specific growth rate (dry weight) from the solvent control

TABLE 11

EXPOSURE PHASE MEAN ALGAL STOCK FLOW RATES

Algal stock number	Test exposure (days)	Stock volume pumped (ml)	Pumping duration (mins)	Mean stock flow rate (ml min^{-1})
3	0 - 4	9680	5815	1.66
4	4 - 8	9230	5515	1.67
5	8 - 11	7500	4505	1.66
6	11 - 15	9350	5640	1.66
7	15 - 18	7300	4455	1.64
8	18 - 22	9370	5498	1.70
9	22 - 25	7520	4542	1.66
10	25 - 29	9400	5535	1.70
11	29 - 32	8110	4615	1.76
12	32 - 36	8980	5495	1.63
13	36 - 39	7420	4590	1.62
14	39 - 43	9580	5735	1.67
15	43 - 46	7450	4320	1.72
16	46 - 50	9540	5665	1.68
17	50 - 53	7420	4425	1.68
18	53 - 57	9190	5495	1.67
19	57 - 60	7590	4520	1.68
20	60 - 64	9500	5740	1.66
21	64 - 67	7150	4315	1.66
22	67 - 70	7700	4335	1.78

TABLE 12

SUMMARY OF EXPOSURE PHASE TEST CHANNEL ALGA PARTICLE DENSITY DETERMINATIONS

Test 'rig' position		Nominal (mean measured) conc TBBPA ($\mu g l^{-1}$)						
		Diln water control	Solvent Control	19 (17)	38 (32)	75 (62)	150 (126)	300 (226)
Splitter Cells	Mean	1.23	1.33	1.26	1.26	1.28	1.28	1.21
	Minimum	1.01	1.04	0.932	0.989	0.985	0.992	0.959
	Maximum	1.45	1.55	1.54	1.47	1.51	1.59	1.45
	n	10	10	10	10	10	10	10
Tank in flows	Mean	1.09	1.27	1.19	1.21	1.18	1.16	1.19
	Minimum	0.880	0.956	0.913	0.850	0.742	0.891	0.901
	Maximum	1.38	1.68	1.43	1.44	1.53	1.38	1.51
	n	30	30	30	30	30	30	30
Tank out flows	Mean	0.992	1.10	1.02	1.08	1.07	1.10	1.09
	Minimum	0.750	0.816	0.721	0.806	0.743	0.770	0.834
	Maximum	1.33	1.51	1.35	1.37	1.37	1.32	1.48
	n	30	30	30	30	30	30	30

Algal particle densities expressed as $\times 10^4$ particles ml^{-1} and quoted to 3 sig figs
n indicates the number of samples

TABLE 13

SUMMARY OF EXPOSURE PHASE pH MEASUREMENTS IN THE TEST VESSELS

Nominal conc TBBPA ($\mu g\ l^{-1}$)	Mean measured conc TBBPA ($\mu g\ l^{-1}$)	Number of samples	Minimum	Maximum
Dilution water control	-	39	7.9	8.0
Solvent control	-	39	7.9	8.0
19	17	39	7.9	8.1
38	32	39	7.9	8.1
75	62	39	7.9	8.1
150	126	39	7.9	8.1
300	226	39	7.9	8.1

TABLE 14

SUMMARY OF EXPOSURE PHASE DISSOLVED OXYGEN CONCENTRATIONS IN THE TEST VESSELS

Nominal conc TBBPA ($\mu g\ l^{-1}$)	Mean measured conc TBBPA ($\mu g\ l^{-1}$)	Number of samples	Mean ($mg\ l^{-1}$)	Minimum ($mg\ l^{-1}$)	Maximum ($mg\ l^{-1}$)
Dilution water control	-	39	7.8	7.4	8.2
Solvent control	-	39	7.6	7.2	8.0
19	17	39	7.7	7.2	8.0
38	32	39	7.7	7.2	8.0
75	62	39	7.7	7.4	8.0
150	126	39	7.7	7.2	8.0
300	226	39	7.7	7.2	8.2

TABLE 15

SUMMARY OF EXPOSURE PHASE TEMPERATURE MEASUREMENTS IN THE TEST VESSELS

Nominal conc TBBPA ($\mu g\ l^{-1}$)	Mean measured conc TBBPA ($\mu g\ l^{-1}$)	Number of samples	Mean (°C)	Minimum (°C)	Maximum (°C)
Dilution water control	-	66	15.2	14.9	15.4
Solvent control	-	66	15.2	14.8	15.3
19	17	66	15.2	14.9	15.4
38	32	66	15.2	14.8	15.4
75	62	66	15.2	14.9	15.4
150	126	66	15.2	14.8	15.4
300	226	66	15.2	14.9	15.4

Dilution water control replicate B tank measurements, made daily throughout the exposure period were within 15 ± 1 °C

Continuous electronic monitoring of the control replicate B tank, with hourly recording of temperatures, throughout the whole definitive test run, showed values within 15 ± 1°C

TABLE 16

WATER QUALITY PARAMETERS OF THE LABORATORY SEAWATER SUPPLY

Monthly sample

Date sampled 2004	Test exposure day	Total ammonia as NH_3-N ($\mu g\ l^{-1}$)	Total filterable solids (TFS) ($mg\ l^{-1}$)	Total organic carbon (TOC) ($mg\ l^{-1}$)
21 September	8	<5.00	10.00	1.61
19 October	36	<5.00	2.00	1.71
11 November	59	<5.00	12.00	1.45

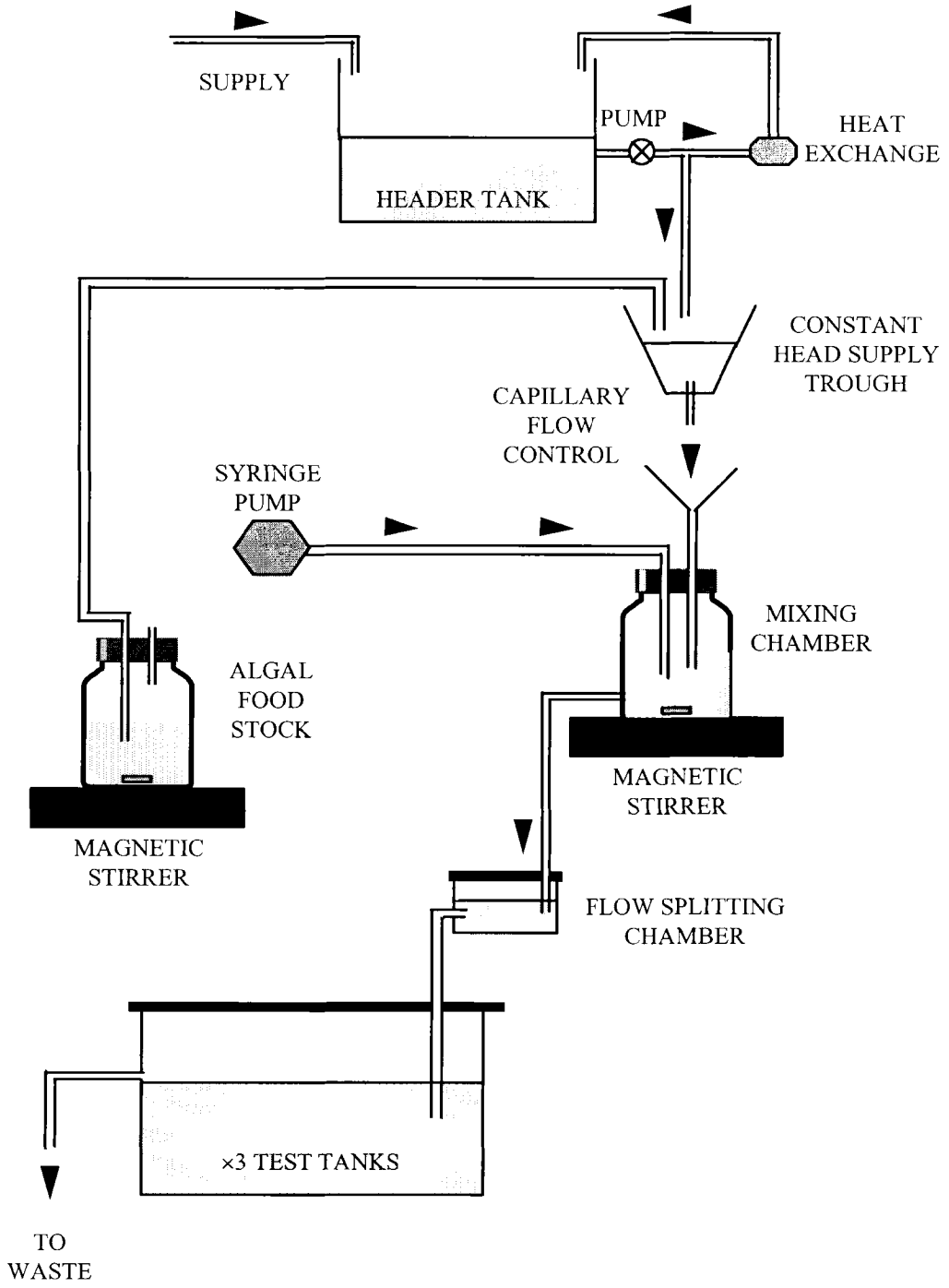
Quarterly sample (sampled 21 September 2004, exposure day 8)

Parameter	Concentration ($\mu g\ l^{-1}$)
Cadmium	<0.0400
Mercury	<0.008
Silver	2.880
Aluminium	<40.000
Arsenic	1.610
Chromium	0.181
Cobalt	2.100
Copper	1.340
Iron	19.500
Lead	0.040
Manganese	<2.000
Nickel	0.750
Zinc	3.020
Boron	4010.000
Highest OC Pesticide	<0.2000
Highest OP pesticide	<0.0100
Highest PCB result	<0.0100

All analyses conducted at Environment Agency, Llanelli Laboratory, Penyfai House, 19 Penyfai Lane, Furnace, Llanelli, SA15 4EL. This is a non GLP compliant facility. All reports archived at Brixham Environmental Laboratory

FIGURE 1

DOSING SYSTEM



APPENDIX 1

CHEMISTRY REPORT

This Appendix, which follows page 46 (circulation list), details the chemistry phase of this study, conducted by Wildlife International Ltd., 8598 Commerce Drive, Easton, Maryland, 21601, USA.

CIRCULATION

Copy number		
1 (single sided bound)	Nancy Sandrof	ACC-BFRIP, 1300 Wilson Boulevard, Arlington, VA 22209, USA
2 (PDF)	Nancy Sandrof	Nancy_Sandrof@americanchemistry.com
3 (PDF)	A Leopold	aleopold@wildlifeinternational.com
4 - 7 (double sided bound)	Reports Centre	Brixham Environmental Laboratory, AstraZeneca UK Limited

TETRABROMOBISPHENOL A: DETERMINATION OF THE EFFECT ON THE GROWTH OF THE COMMON MUSSEL (*Mytilus edulis*)

FINAL REPORT

ASTRAZENECA STUDY NO.: 03-0337/A
WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 439C-143

AUTHORS:

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

SPONSOR:

American Chemistry Council – BFRIP
1300 Wilson Blvd.
Arlington, VA 22209

ANALYTICAL PHASE PERFORMED BY:

Wildlife International, Ltd.

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

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

SPONSOR: American Chemistry Council-BFRIP

TITLE: Tetrabromobisphenol A: Determination of the Effect on the Growth of the Common Mussel (*Mytilus edulis*)

ASTRAZENECA STUDY NO.: 03-0337/A

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 439C-143

ANALYTICAL PHASE COMPLETION: March 28, 2005

The analytical phase of the study was conducted in compliance with OECD Principles of Good Laboratory Practice (ENV/MC/CHEM (98) 17).

ANALYTICAL PRINCIPAL INVESTIGATOR:

Jon A. MacGregor, B.S.
Scientist

3/28/05
DATE

QUALITY ASSURANCE STATEMENT

The analytical phase of the study was examined for compliance with Good Laboratory Practice Standards as published by OECD Principles of Good Laboratory Practice (ENV/MC/CHEM (98) 17). The dates of all inspections and audits and the dates that any findings were reported to the Study Director and Laboratory Management were as follows:

		DATE REPORTED TO:	
ACTIVITY:	DATE CONDUCTED:	STUDY DIRECTOR:	MANAGEMENT:
Matrix Fortification	September 15, 2004	September 22, 2004	September 22, 2004
Sample Preparation	October 28, 2004	November 5, 2004	November 5, 2004
Analytical Phase Data and Draft Report	January 27 and 28, 2005	February 8, 2005	February 8, 2005
Analytical Phase Data and Draft Report	February 16, 2005	February 18, 2005	February 18, 2005
Analytical Phase Final Report	March 28, 2005	March 28, 2005	March 28, 2005

Marshall T. Hynson
Quality Assurance Program Supervisor

3/28/2005
DATE

REPORT APPROVAL

SPONSOR: American Chemistry Council-BFRIP

TITLE: Tetrabromobisphenol A: Determination of the Effect on the Growth of the Common Mussel (*Mytilus edulis*)

ASTRAZENECA STUDY NO.: 03-0337/A

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 439C-143

ANALYTICAL PRINCIPAL INVESTIGATOR:

Jon A. MacGregor, B.S. DATE 3/28/05
Scientist

MANAGEMENT:

Willard B. Nixon, Ph.D. DATE 3/28/05
Director, Chemistry

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SUMMARY

SPONSOR:	American Chemistry Council-BFRIP
SPONSOR'S REPRESENTATIVE:	Christopher Cleet, Manager of BFRIP Panel
LOCATION OF ANALYTICAL RAW DATA AND A COPY OF THE ANALYTICAL REPORT:	Wildlife International, Ltd. 8598 Commerce Drive Easton, Maryland 21601

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER:	439C-143
ASTRAZENECA STUDY NUMBER:	03-0337/A
TEST SUBSTANCE:	Tetrabromobisphenol A (TBBPA)
REPORT:	Tetrabromobisphenol A: Determination of the Effect on the Growth of the Common Mussel (*Mytilus edulis*)
TEST DATES:	Analytical Experimental Start – September 7, 2004 Analytical Experimental Termination – November 19, 2004

Saltwater samples collected from a growth study of the common mussel (*Mytilus edulis*) were analyzed for Tetrabromobisphenol A concentrations using high performance liquid chromatography (HPLC) with mass selective detection (MS). Prior to the initiation of the definitive study, a solubility trial was performed to determine the functional water solubility of the TBBPA test substance in the test delivery system and to insure testing at the maximum solubility level. The high concentration evaluated was 1000 µg a.i./L, which demonstrated measured results ranging from 243 to 636 µg a.i./L throughout Days 3, 6, and 10 of the investigation. Based on these results, the high concentration selected for the definitive study was 300 µg a.i./L. Saltwater verification samples collected at pre-test #1 and analyzed by HPLC/MS yielded results ranging from 62.1-95.8% of the nominal concentrations. Saltwater samples were also collected at two additional pre-test intervals. Pre-test #2 samples were collected and analyzed to show any potential effects of centrifugation on measured sample results, without algae cells present. Pre-test #3 samples were collected and analyzed to show any potential effects of centrifugation on measured sample results, with algae cells present. Pre-test #2 and Pre-test #3 verification samples yielded results ranging from 77.5 - 104% and 63.8 - 101% of the nominal concentrations, respectively. Comparison of the measured results of the three intervals demonstrated that centrifugation of samples had no significant effect on the resulting measured concentrations of the samples, and would not be necessary during the definitive study. Verification samples collected on day 0 of the exposure ranged from 74.7-113% of the nominal concentrations. Verification samples collected on days 4,7, 11, 15, 22, 29, 36, 43, 50, 57 and 64 (termination) yielded measured results ranging from 41.1-130%, 50.2 - 95.0%, 63.1- 76.6%, 67.4 - 89.1%, 77.8 - 97.7%, 82.1 - 111%, 64.8 - 108%, 58.0 - 85.6%, 61.6 - 96.8%, 64.2 - 106%, and 67.1 - 93.6% of the nominal concentrations, respectively. Quality control fortification samples analyzed concurrently with the study samples yielded recoveries ranging from 91.8-104% of the nominal concentrations. All control samples were devoid of Tetrabromobisphenol A.

APPENDIX 1

INTRODUCTION

Saltwater samples were collected during growth study designed to determine the effect of Tetrabromobisphenol A on the common mussel (*Mytilus edulis*). The concentrations of Tetrabromobisphenol A were measured in saltwater samples collected from the control and test substance treatments at three separate pre-test intervals (centrifugation trials), Day 0, biweekly during first two weeks of the study, and weekly throughout the remainder of the study until termination on day 64.

This analytical phase of the study was conducted by Wildlife International, Ltd. and identified as Project Number 439C-143. Water samples were received from Brixham Environmental Laboratories of AstraZeneca and analyzed by Wildlife International, Ltd. between September 7 and November 19, 2004 using high performance liquid chromatography (HPLC) with mass spectrometric detection (MS). Raw data generated by Wildlife International, Ltd. and the original analytical final report are kept on file in Wildlife International, Ltd. archives.

MATERIALS AND METHODS

Test Substance

The test substance for the study consisted of a composite of tetrabromobisphenol A (TBBPA) samples received from three manufacturers. The material's identity and date received from each of the manufacturers is given below:

Manufacturer	Lot/Batch	Date Received	Wildlife International Ltd. ID No.
Great Lakes Chemical Corp.	2008JM17B	June 2, 2003	6358
Albemarle Corp.	25243Z-1	July 11, 2003	6400
DSBG	030036	June 12, 2003	6368

An equal part (4374 g) of each of the manufacturer's TBBPA material was placed in a container and mixed on a shaker table for thirty minutes. The composite test substance, a white powder, was assigned the Wildlife International, Ltd. identification number 6404. Subsamples of the composite test substance were shipped to Albemarle Corporation for characterization and homogeneity analyses (Appendix 3). The analyses were

performed on September 18, 2003. The results of the analyses indicated the composite test substance was homogeneous and contained the following components:

Tetrabromobisphenol-A	99.2%
o,p′- Tetrabromobisphenol-A	0.03%
2,4,6-Tribromophenol	0.02%
Tribromobisphenol-A	0.75%

The composite test substance was stored under ambient conditions.

Reagents and Solvents

All solvents used in this study were HPLC grade or equivalent. All reagents were ACS reagent grade or equivalent. NANOpure® water (equivalent to ASTM Type II Designation D1193-91) was used (1).

REFERENCES

1 **American Society for Testing and Materials.** 1991. Standard Specification for Reagent Water. D1193-91, ASTM Section II Water and Environmental Technology, Vol. 11.01: 45-47.

APPENDIX 2

Analytical Method for the Analysis of Tetrabromobisphenol A in Saltwater Samples
by HPLC/MS

Analytical Method for the Analysis of Tetrobromobisphenyl in Saltwater Matrix

The method used for the analysis of saltwater samples was developed and validated by Wildlife International, Ltd. under project number 439C-142. Twenty mL aliquots of saltwater matrix were measured and transferred to 125-mL separatory funnels. QC samples were fortified with the appropriate Tetrabromobisphenol A stock solution. Unfortified saltwater served as the matrix blank. Twenty-five mLs of dichloromethane (DCM) was added to each separatory funnel. For the study samples, the entire sample volume measured at Brixham Laboratory was transferred to the separatory funnel and the sample container was rinsed with the first 25-mL volume of DCM to remove any potentially adsorbed TBBPA residues. Samples were extracted by shaking for approximately one minute with venting. The lower organic phase layer was drained into a 125-mL round bottom flask. The extraction was repeated using a second 25-mL aliquot of DCM. The DCM extracts were combined in the roundbottom flask from previous extraction. The extracts were initially reduced to approximately 1 mL using rotary evaporation at approximately 40°C and then to complete dryness using a gentle stream of nitrogen. The residues were reconstituted using volumetric additions of the requisite volume of methanol:water (50:50, v/v) solution. Further dilutions were made using the same solution, as necessary to dilute the samples into the calibration range. Aliquots from each final diluted extract were transferred to autosampler vials and submitted for HPLC/MS analysis.

Concentrations of Tetrabromobisphenol A in the saltwater sample extracts were determined using a Hewlett-Packard Model 1100 High Performance Liquid Chromatograph coupled with a Perkin-Elmer SCIEX API 100 LC Mass Spectrometer and Perkin-Elmer SCIEX Heated Nebulizer ion source (HPLC/MS) operated in the negative, selective ion monitoring (SIM) mode. Chromatographic separations were achieved using a Keystone Betasil C-18 column (50 mm X 0.2 mm, 5 µm particle size). The instrument parameters are summarized in Table 1. A method flow chart for the analysis of saltwater samples is provided in Figure 1.

Stock Preparation

A stock solution of TBBPA was prepared by accurately 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. From the 1.00 mg a.i./mL stock solution, 0.100, 0.0100, 0.00100 and 0.000100 mg a.i./mL stock solutions were prepared in methanol. The 0.0100 and 0.00100 stock solutions were used to fortify the method verification samples.

Calibration standards were prepared in methanol : water (50:50, v/v) using the 0.0100 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 (mL)	Final Volume (mL)	Standard Concentration (µg a.i./L)
0.0100	0.100	100	10.0
0.0100	0.250	100	25.0
0.0100	0.500	100	50.0
0.0100	0.750	100	75.0
0.0100	1.00	100	100

Table 1

Typical HPLC/MS Operational Parameters for the Analysis of Tetrabromobisphenol A in Saltwater Samples

INSTRUMENT:	Hewlett-Packard Model 1100 High Performance Liquid Chromatograph (HPLC) with a Perkin-Elmer API 100LC Mass Spectrometer (MS)
ION SOURCE:	Perkin-Elmer SCIEX Heated Nebulizer Operated in Selective Ion Monitoring (SIM) Mode
ANALYTICAL COLUMN:	Keystone Betasil C-18 Column (50 mm × 2.0 mm, 5-µm particle size) with a Keystone Javelin Guard Column (20 x 2.0 mm)
OVEN TEMPERATURE:	40 °C
STOP TIME:	5.00 min
FLOW RATE:	0.250 mL/min
MOBILE PHASE:	
SOLVENT A:	20% - Formic Acid (0.1%)
SOLVENT B:	80% - Methanol
INJECTION VOLUME:	10.0 µL
GAS 1:	Air – 60 psi
GAS 2:	Nitrogen 60 psi; <1 L/min.
TETRABROMOBISPHENOL A PEAK RETENTION TIME:	Approximately 2.8 minutes
TETRABROMOBISPHENOL A MONITORED MASS:	542.7 amu

Table 2

Results of Solubility Trial of Tetrabromobisphenol A in Saltwater

Sample Identification	Nominal Concentration (µg a.i./L)	Analytical Result (µg a.i./L)
SOL CON A – Day 3	0	< LOQ
SOL CON B – Day 3	0	< LOQ
SOL CON C – Day 3	0	< LOQ
SOL CON A – Day 6	0	< LOQ
SOL CON A – Day 10	0	< LOQ
SOL CON B – Day 10	0	< LOQ
100 A – Day 3	100	105
100 B – Day 3	100	105
100 C – Day 3	100	108
100 A – Day 6	100	93.5
100 A – Day 10	100	85.3
100 B – Day 10	100	86.4
250 A – Day 3	250	230
250 B – Day 3	250	244
250 C – Day 3	250	272
250 A – Day 6	250	225
250 A – Day 10	250	184
250 B – Day 10	250	190
1000 A – Day 3	1000	253
1000 B – Day 3	1000	261
1000 C – Day 3	1000	343
1000 A – Day 6	1000	636
1000 A – Day 10	1000	259
1000 B – Day 10	1000	243

[1] The limit of quantitation (LOQ) was 0.25 µg a.i./L, calculated as the product of the concentration of the lowest calibration standard (1.00 µg a.i./L) and the dilution factor of the matrix blanks (0.250).

[2] Results were generated using MacQuan Version 1.6. Manual calculations may differ slightly.

Table 3

Results of Matrix Blank and Fortification Sample Analyses for the Solubility Trial

Nominal Concentration (µg a.i./L)	Sample Identification	Sampling Interval (Day)	Measured Concentration (µg a.i./L)[1]	Percent of Nominal[2]
0.0	SOLMAB-1	3	< LOQ	--
0.0	SOLMAB-2	6	< LOQ	--
0.0	SOLMAB-3	10	< LOQ	--
0.500	SOLMAS-1	3	0.471	94.2
50.0	SOLMAS-2	3	51.6	103
1500	SOLMAS-3	3	1603	107
0.500	SOLMAS-4	6	0.451	90.2
50.0	SOLMAS-5	6	49.9	99.8
1500	SOLMAS-6	6	1546	103
0.500	SOLMAS-7	10	0.480	96.1
50.0	SOLMAS-8	10	51.2	102
1500	SOLMAS-9	10	1544	103

[1] The limit of quantitation (LOQ) was 0.25 µg a.i./L, calculated as the product of the concentration of the lowest calibration standard (1.00 µg a.i./L) and the dilution factor of the matrix blanks (0.250).

[2] Results were generated using MacQuan Version 1.6. Manual calculations may differ slightly.

Table 4

Results of Pre-Test #1 Saltwater Samples Analyses by HPLC/MS

Nominal Concentration (µg a.i./L)	Sample Identification	Sampling Time (Day)	Measured Concentration (µg a.i./L)[1]	Percent of Nominal[2]
0.0	PTMAB-1	-10	< LOQ	--
5.00	PTMAS-1	-10	5.02	100
350	PTMAS-2	-10	360	103
Negative Control	03-0337/A-1	-10	< LOQ	--
0.0	03-0337/A-2	-10	< LOQ	--
	03-0337/A-3	-10	< LOQ	--
Solvent Control	03-0337/A-4	-10	< LOQ	--
0.0	03-0337/A-5	-10	< LOQ	--
	03-0337/A-6	-10	< LOQ	--
19	03-0337/A-7	-10	18.2	95.8
	03-0337/A-8	-10	16.9	88.7
	03-0337/A-9	-10	16.1	84.6
38	03-0337/A-10	-10	29.7	78.0
	03-0337/A-11	-10	29.2	77.0
	03-0337/A-12	-10	33.3	87.7
75	03-0337/A-13	-10	65.6	87.5
	03-0337/A-14	-10	46.6	62.1
	03-0337/A-15	-10	58.8	78.4
150	03-0337/A-16	-10	134	89.5
	03-0337/A-17	-10	141	93.8
	03-0337/A-18	-10	136	90.8
300	03-0337/A-19	-10	251	83.6
	03-0337/A-20	-10	252	83.9
	03-0337/A-21	-10	263	87.7

[1] The limit of quantitation (LOQ) was 2.5 µg a.i./L, calculated as the product of the concentration of the lowest calibration standard (10.0 µg a.i./L) and the dilution factor of the matrix blanks (0.250).

[2] Results were generated using MacQuan Version 1.6. Manual calculations may differ slightly.

Table 5

Results of Pre-Test #2 Saltwater Samples Analyses by HPLC/MS
(centrifugation without algae)

Nominal Concentration (µg a.i./L)	Sample Identification	Sampling Time (Day)	Measured Concentration (µg a.i./L)[1]	Percent of Nominal[2]
0.0	PTMAB-2	-7	< LOQ	--
5.00	PTMAS-3	-7	4.89	97.9
350	PTMAS-4	-7	354	101
Negative Control	03-0337/A-22	-7	< LOQ	--
0.0	03-0337/A-23*	-7	< LOQ	--
Solvent Control	03-0337/A-24	-7	< LOQ	--
0.0	03-0337/A-25*	-7	< LOQ	--
19	03-0337/A-26	-7	16.3	85.9
	03-0337/A-27*	-7	19.8	104
38	03-0337/A-28	-7	31.5	83.0
	03-0337/A-29*	-7	31.1	81.8
75	03-0337/A-30	-7	65.2	86.9
	03-0337/A-31*	-7	75.4	100
150	03-0337/A-32	-7	135	89.9
	03-0337/A-33*	-7	131	87.6
300	03-0337/A-34	-7	233	77.5
	03-0337/A-35*	-7	237	78.9

[1] The limit of quantitation (LOQ) was 2.5 µg a.i./L, calculated as the product of the concentration of the lowest calibration standard (10.0 µg a.i./L) and the dilution factor of the matrix blanks (0.250).

[2] Results were generated using MacQuan Version 1.6. Manual calculations may differ slightly.

* Samples centrifuged.

Table 6

Results of Pre-Test #3 Saltwater Samples Analyses by HPLC/MS
(centrifugation with algae)

Nominal Concentration (µg a.i./L)	Sample Identification	Sampling Time (Day)	Measured Concentration (µg a.i./L)[1]	Percent of Nominal[2]
0.0	PTMAB-3	-6	< LOQ	--
5.00	PTMAS-5	-6	4.97	99.5
350	PTMAS-6	-6	352	100
Negative Control	03-0337/A-36	-6	< LOQ	--
0.0	03-0337/A-37*	-6	< LOQ	--
Solvent Control	03-0337/A-38	-6	< LOQ	--
0.0	03-0337/A-39*	-6	< LOQ	--
19	03-0337/A-40	-6	18.4	97.1
	03-0337/A-41*	-6	19.2	101
38	03-0337/A-42	-6	30.9	81.3
	03-0337/A-43*	-6	27.7	73.0
75	03-0337/A-44	-6	49.5	66.1
	03-0337/A-45*	-6	48.1	64.1
150	03-0337/A-46	-6	95.7	63.8
	03-0337/A-47*	-6	96.8	64.6
300	03-0337/A-48	-6	215	71.8
	03-0337/A-49*	-6	199	66.4

[1] The limit of quantitation (LOQ) was 2.5 µg a.i./L, calculated as the product of the concentration of the lowest calibration standard (10.0 µg a.i./L) and the dilution factor of the matrix blanks (0.250).

[2] Results were generated using MacQuan Version 1.6. Manual calculations may differ slightly.

* Samples centrifuged.

Table 7

Results of Matrix Blank and Fortification Sample Analyses by HPLC/MS

Sample Number (439C-143)	Sample Type	Fortified Concentration (µg a.i./L)	Measured Concentration (µg a.i./L) [1]	Percent Recovery[2]
MAB-1	Matrix Blank	0.0	< LOQ	--
MAB-2	Matrix Blank	0.0	< LOQ	--
MAB-3	Matrix Blank	0.0	< LOQ	--
MAB-4	Matrix Blank	0.0	< LOQ	--
MAB-5	Matrix Blank	0.0	< LOQ	--
MAB-6	Matrix Blank	0.0	< LOQ	--
MAB-7	Matrix Blank	0.0	< LOQ	--
MAB-8	Matrix Blank	0.0	< LOQ	--
MAB-9	Matrix Blank	0.0	< LOQ	--
MAB-10	Matrix Blank	0.0	< LOQ	--
MAB-11	Matrix Blank	0.0	< LOQ	--
MAB-12	Matrix Blank	0.0	< LOQ	--
MAS-1	Matrix Fortification	5.00	4.67	93.4
MAS-2	Matrix Fortification	350	365	104
MAS-3	Matrix Fortification	5.00	4.85	97.0
MAS-4	Matrix Fortification	350	359	103
MAS-5	Matrix Fortification	5.00	4.81	96.1
MAS-6	Matrix Fortification	350	353	101
MAS-7	Matrix Fortification	5.00	4.88	97.6
MAS-8	Matrix Fortification	350	357	102
MAS-9	Matrix Fortification	5.00	4.87	97.5
MAS-10	Matrix Fortification	350	364	104
MAS-11	Matrix Fortification	5.00	5.14	103
MAS-12	Matrix Fortification	350	354	101

[1] The limit of quantitation (LOQ) was 2.5 µg a.i./L, calculated as the product of the concentration of the lowest calibration standard (10.0 µg a.i./L) and the dilution factor of the matrix blanks (0.250).

[2] Results were generated using MacQuan Version 1.6. Manual calculations may differ slightly.

Table 7 (Continued)

Results of Matrix Blank and Fortification Sample Analyses by HPLC/MS

Sample Number (439C-143)	Sample Type	Fortified Concentration (µg a.i./L)	Measured Concentration (µg a.i./L) [1]	Percent Recovery[2]
MAS-13	Matrix Fortification	5.00	4.59	91.8
MAS-14	Matrix Fortification	350	359	102
MAS-15	Matrix Fortification	5.00	4.83	96.7
MAS-16	Matrix Fortification	350	357	102
MAS-17	Matrix Fortification	5.00	4.95	99.0
MAS-18	Matrix Fortification	350	364	104
MAS-19	Matrix Fortification	5.00	5.21	104
MAS-20	Matrix Fortification	350	355	101
MAS-21	Matrix Fortification	5.00	4.88	97.6
MAS-22	Matrix Fortification	350	360	103
MAS-23	Matrix Fortification	5.00	4.80	95.9
MAS-24	Matrix Fortification	350	351	100
			Mean =	99.9
			Standard Deviation =	3.53
			CV =	3.54%

[1] The limit of quantitation (LOQ) was 2.5 µg a.i./L, calculated as the product of the concentration of the lowest calibration standard (10.0 µg a.i./L) and the dilution factor of the matrix blanks (0.250).

[2] Results were generated using MacQuan Version 1.6. Manual calculations may differ slightly.

Table 8

Results of Definitive Test Saltwater Verification Sample Analyses by HPLC/MS

Nominal Test Concentration (µg a.i./L)	Sample ID (03-0337/A-)	Sampling Time (Days)	Measured Concentration (µg a.i./L)[1]	Percent of Nominal[2]	Mean Measured Concentration (µg a.i./L)	Mean Measured Percent of Nominal
Negative Control 0.0	50	0	< LOQ	--	--	--
	51	0	< LOQ	--		
	52	0	< LOQ	--		
	71	4	< LOQ	--		
	72	4	< LOQ	--		
	73	4	< LOQ	--		
	92	7	< LOQ	--		
	99	11	< LOQ	--		
	106	15	< LOQ	--		
	113	22	< LOQ	--		
	120	29	< LOQ	--		
	127	36	< LOQ	--		
	134	43	< LOQ	--		
	141	50	< LOQ	--		
	148	57	< LOQ	--		
	155	64	< LOQ	--		
Solvent Control 0.0	53	0	< LOQ	--	--	--
	54	0	< LOQ	--		
	55	0	< LOQ	--		
	74	4	< LOQ	--		
	75	4	< LOQ	--		
	76	4	< LOQ	--		
	93	7	< LOQ	--		
	100	11	< LOQ	--		
	107	15	< LOQ	--		
	114	22	< LOQ	--		
	121	29	< LOQ	--		
	128	36	< LOQ	--		
	135	43	< LOQ	--		
	142	50	< LOQ	--		
	149	57	< LOQ	--		
	156	64	< LOQ	--		

[1] The limit of quantitation (LOQ) was 2.5 µg a.i./L, calculated as the product of the concentration of the lowest calibration standard (10.0 µg a.i./L) and the dilution factor of the matrix blanks (0.250).

[2] Results were generated using MacQuan Version 1.6. Manual calculations may differ slightly.

Table 8 (Continued)

Results of Definitive Test Saltwater Verification Sample Analyses by HPLC/MS

Nominal Test Concentration (µg a.i./L)	Sample ID (03-0337/A-)	Sampling Time (Days)	Measured Concentration (µg a.i./L)[1]	Percent of Nominal[2]	Mean Measured Concentration (µg a.i./L)	Mean Measured Percent of Nominal
19	56,57,58*	0	19.0	100	17	89
	77,78,79*	4	24.7	130		
	94	7	9.53	50.2		
	101	11	13.3	70.1		
	108	15	12.8	67.4		
	115	22	17.5	91.9		
	122	29	21.1	111		
	129	36	20.5	108		
	136	43	15.9	83.5		
	143	50	16.8	88.4		
	150	57	20.1	106		
	157	64	17.8	93.6		
38	59,60,61*	0	30.8	81.1	32	84
	80,81,82*	4	31.6	83.2		
	95	7	36.1	95.0		
	102	11	24.0	63.1		
	109	15	31.6	83.2		
	116	22	32.8	86.3		
	123	29	31.2	82.1		
	130	36	35.1	92.4		
	137	43	30.2	79.5		
	144	50	36.8	96.8		
	151	57	31.9	83.9		
	158	64	32.5	85.5		

[1] The limit of quantitation (LOQ) was 2.5 µg a.i./L, calculated as the product of the concentration of the lowest calibration standard (10.0 µg a.i./L) and the dilution factor of the matrix blanks (0.250).
[2] Results were generated using MacQuan Version 1.6. Manual calculations may differ slightly.
* Mean value of triplicate of analysis.

Table 8 (Continued)

Results of Definitive Test Saltwater Verification Sample Analyses by HPLC/MS

Nominal Test Concentration (µg a.i./L)	Sample ID (03-0337/A-)	Sampling Time (Days)	Measured Concentration (µg a.i./L)[1]	Percent of Nominal[2]	Mean Measured Concentration (µg a.i./L)	Mean Measured Percent of Nominal
75	62,63,64*	0	72.8	97.0	62	83
	83,84,85*	4	60.7	80.9		
	96	7	68.4	91.2		
	103	11	56.7	75.6		
	110	15	66.8	89.1		
	117	22	73.3	97.7		
	124	29	73.5	97.9		
	131	36	48.6	64.8		
	138	43	43.5	58.0		
	145	50	48.9	65.2		
	152	57	75.7	101		
	159	64	50.3	67.1		
150	65,66,67*	0	157	105	126	84
	86,87,88*	4	64.4	84.8		
	97	7	138	92.3		
	104	11	115	76.6		
	111	15	118	78.5		
	118	22	139	92.9		
	125	29	143	95.5		
	132	36	132	87.8		
	139	43	128	85.6		
	146	50	110	73.3		
	153	57	135	90.3		
	160	64	130	86.6		

[1] The limit of quantitation (LOQ) was 2.5 µg a.i./L, calculated as the product of the concentration of the lowest calibration standard (10.0 µg a.i./L) and the dilution factor of the matrix blanks (0.250).
[2] Results were generated using MacQuan Version 1.6. Manual calculations may differ slightly.
* Mean value of triplicate analysis.

Table 8 (Continued)

Results of Definitive Test Saltwater Verification Sample Analyses by HPLC/MS

Nominal Test Concentration (µg a.i./L)	Sample ID (03-0337/A-)	Sampling Time (Days)	Measured Concentration (µg a.i./L)[1]	Percent of Nominal[2]	Mean Measured Concentration (µg a.i./L)	Mean Measured Percent of Nominal
300	68,69,70*	0	228	75.9	226	75
	89,90,91*	4	270	89.9		
	98	7	261	87.1		
	105	11	229	76.3		
	112	15	232	77.4		
	119	22	233	77.8		
	126	29	247	82.5		
	133	36	211	70.2		
	140	43	221	73.8		
	(300B)**	50	185	61.6		
	154	57	193	64.2		
	161	64	203	67.8		

[1] The limit of quantitation (LOQ) was 2.5 µg a.i./L, calculated as the product of the concentration of the lowest calibration standard (10.0 µg a.i./L) and the dilution factor of the matrix blanks (0.250).
[2] Results were generated using MacQuan Version 1.6. Manual calculations may differ slightly.
* Mean value of triplicate analysis.
** Sample reanalyzed from back-up samples collected on Day 50. Original sample broken during shipment.

Figure 1

Flowchart for the Analysis of Tetrabromobisphenol A in Saltwater Analyzed by HPLC/MS

Method Outline for the Analysis of Tetrabromobisphenol A in Saltwater

Pre-rinse all glassware using successive rinses of 5% KOH, water, 10% HCL, water, acetone and a final rinse with extraction solvent.

↓

Prepare each quality control (QC) sample by volumetrically adding 20.0 mL of control water to a 125-mL separatory funnel. Fortify each QC sample with the appropriate TBBPA stock solution. The matrix blank sample will not be fortified. Add 25-mL of dichloromethane (DCM) to each separatory funnel.

↓

Transfer 20.0 mL of each aqueous study sample into a 125-mL separatory funnel. Rinse each container using 25-mL of DCM extraction solvent and transfer the rinsate to appropriate separatory funnel.

↓

Shake the QC and study sample solutions (with venting) for approximately one minute and allow the two phases to separate.

↓

For each sample, drain the organic (lower) phase into a 125-mL round-bottom flask. Add a second 25-mL aliquot of DCM to the aqueous phase remaining in the funnel and perform a second extraction.

↓

For each sample, combine the organic phase from the second extraction with the organic phase of the first extraction in the same round-bottom flask.

↓

Rotary evaporate the organic extracts to ~1 mL under vacuum and a waterbath set at approximately 40°C.

↓

Evaporate the residual DCM in each flask to dryness under a gentle stream of nitrogen. Dissolve the residues with appropriate volume of methanol : water, (50:50, v/v). Volumetrically dilute an aliquot further using methanol : water, 50:50, v/v, if necessary.

↓

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

Figure 2

Representative Calibration Curve for Tetrabromobisphenol A in Saltwater Analyzed by HPLC/MS

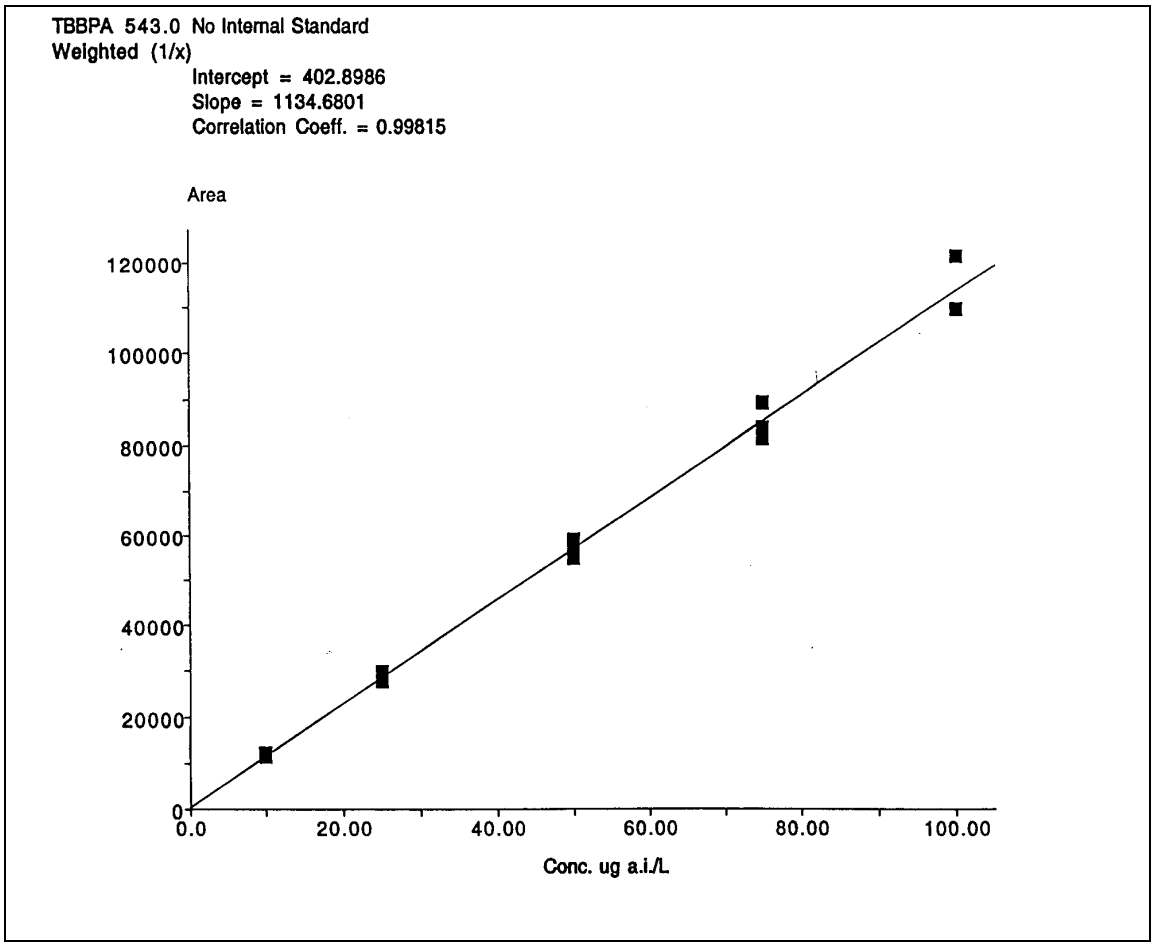
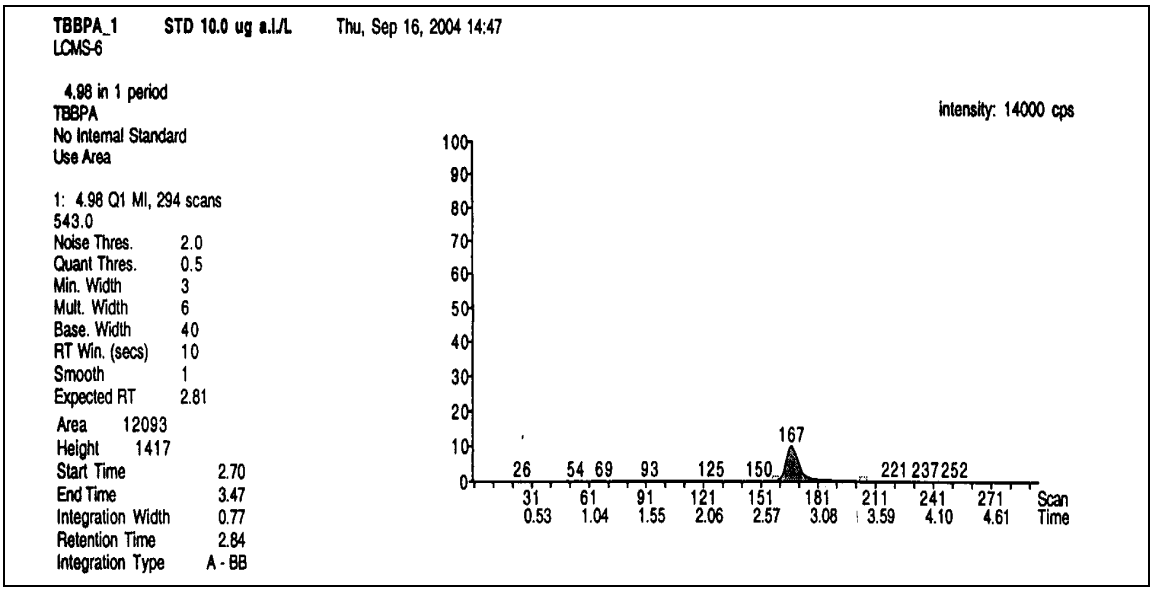


Figure 3

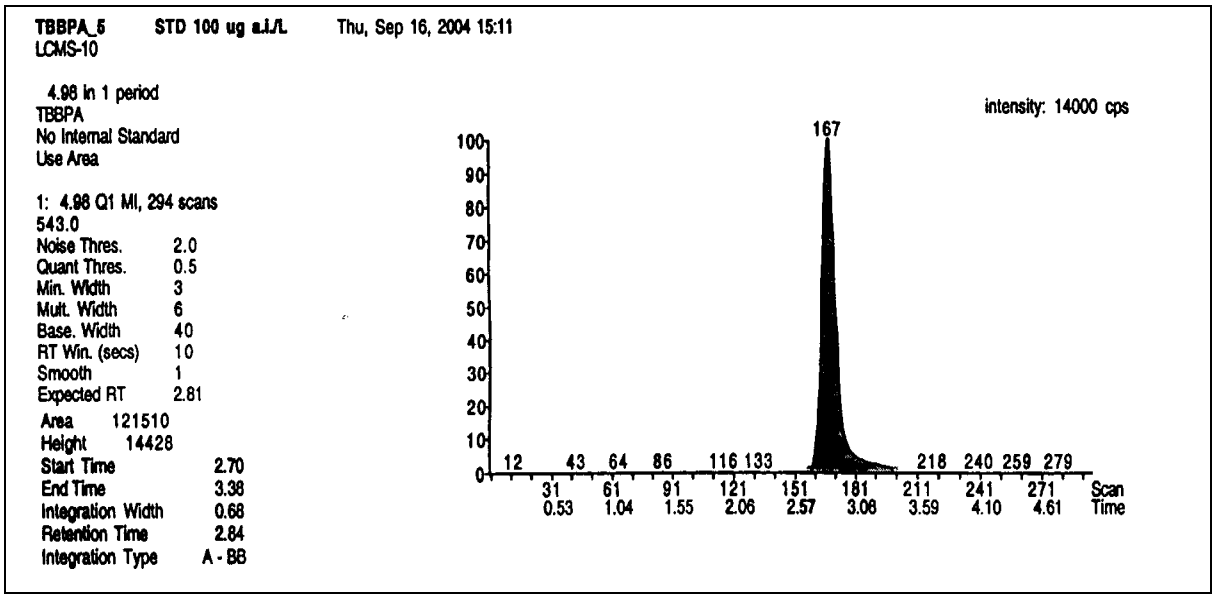
Representative Chromatogram of a Low-level Saltwater Tetrabromobisphenol A Calibration Standard Analyzed by HPLC/MS



Nominal concentration: 10.0 µg a.i./L

Figure 4

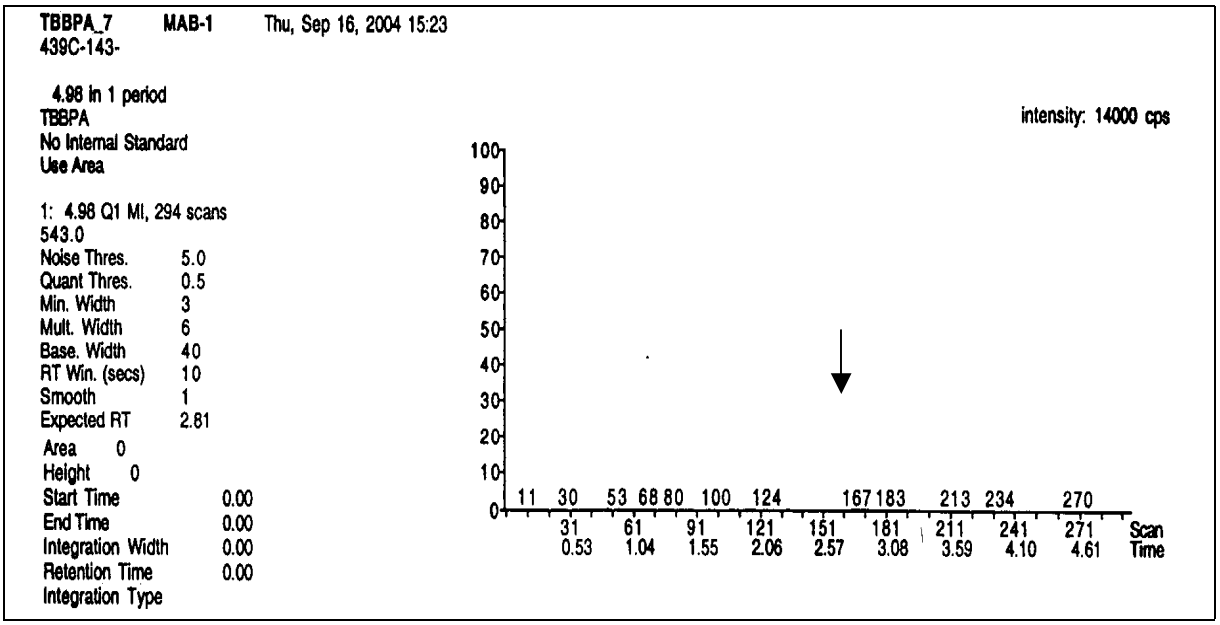
Representative Chromatogram of a High-level Saltwater Tetrabromobisphenol A Calibration Standard Analyzed by HPLC/MS



Nominal concentration: 100 µg a.i./L

Figure 5

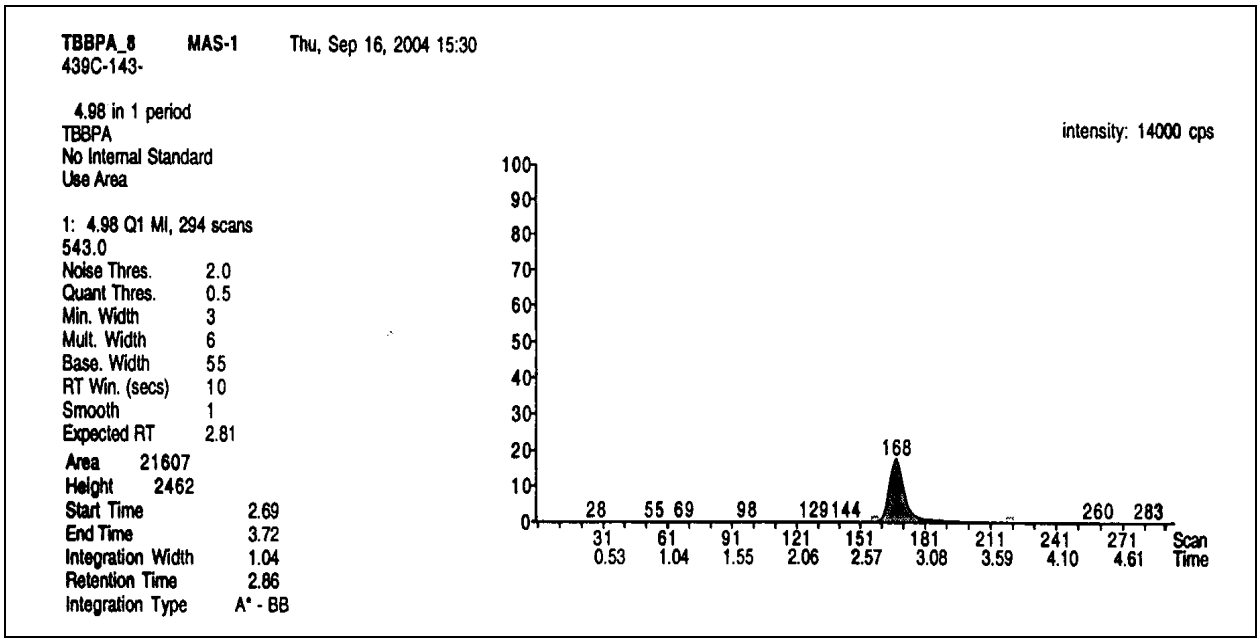
Representative Chromatogram of a Saltwater Matrix Blank Sample Analyzed by HPLC/MS



Sample number 439C-143-MAB-1. The arrow indicates the approximate retention time of Tetrabromobisphenol A

Figure 6

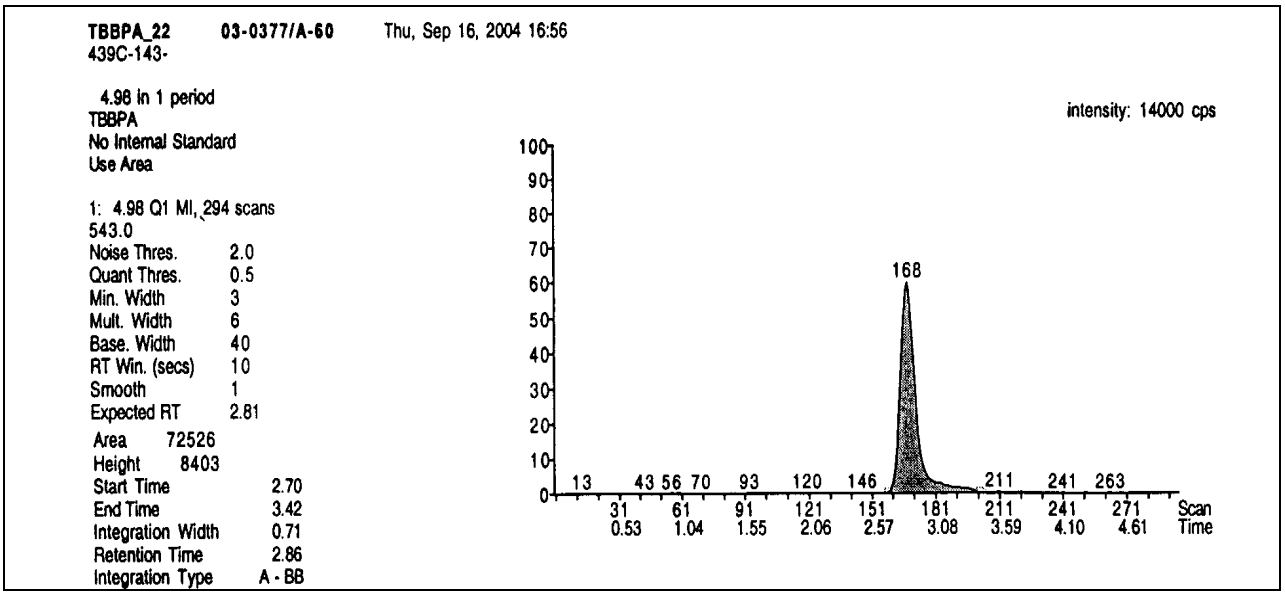
Representative Chromatogram of a Saltwater Matrix Fortification Sample Analyzed by HPLC/MS



Sample number 439C-143-MAS-1; 5.00 µg a.i./L nominal concentration

Figure 7

Representative Chromatogram of a Saltwater Test Sample Analyzed by HPLC/MS



Sample number 03-0377/A-60, Day 0; 38 µg a.i./L nominal concentration

Appendix 3

Characterization and Purity Analysis for the
Composite Test Substance

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PR 8/20/03

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.

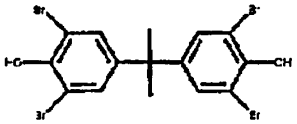
Paul F. Ranken September 24, 2003

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					
Sample	Purity (area% TBBPA)	Average	Difference (%) from average		
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

Appendix 4

Personnel Involved in the Study

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

1. Willard B. Nixon, Ph.D., Director, Chemistry
2. Jon A. MacGregor, B.S., Scientist