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American
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Dr. Charlie Auer
Director
TSCA Document Control Office (7407)
EPA East Building, Room 6248
Office of Pollution Prevention and Toxics
US Environmental Protection Agency
1201 Constitution Avenue, NW
Washington DC 20460



Dear Dr. Auer:

The American Chemistry Council makes available to the public and appropriate government agencies final reports of environmental, health, and safety research that it manages. In keeping with this policy, the following reports that the American Chemistry Council's Brominated Flame Retardants Industry Panel (BFRIP) recently conducted are enclosed:

Title:

1. TBBPA: Determination of effects on the growth of the common mussel *Mytilus edulis*.
2. The *InVitro* Percutaneous Absorption of Radiolabelled Hexabromocyclododecane (HBCD) Through Human Skin

The reports do not include confidential information.

If you have any questions, please call Nancy Sandrof, Manager of BFRIP at (703) 741-5605.

Sincerely yours,

Susan A. Lewis

Enclosures

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Brixham Environmental Laboratory study number 03-0337/A

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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**

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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**

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

Brixham Environmental Laboratory Study Number: 03-0337/A

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.

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

Brixham Environmental Laboratory Study Number: 03-0337/A

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

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

Brixham Environmental Laboratory Study Number: 03-0337/A

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

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

Brixham Environmental Laboratory Study Number: 03-0337/A

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
R J Brown

29 March 2005
Date

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
N Shillabeer

1 April 2005
Date

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TBBPA: Determination of effects on the growth of the common mussel (*Mytilus edulis*)**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 $\mu\text{g l}^{-1}$

Length of test Mussel exposure period 70 days flow-through, no aeration

Test dates 13 September to 22 November 2004

Nominal test temperature $15 \pm 1^\circ\text{C}$

Results based on mean measured concentrations as $\mu\text{g l}^{-1}$ 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 $\mu\text{g l}^{-1}$
Lowest observed effect (P=0.05) concentration (LOEC) = 126 $\mu\text{g l}^{-1}$

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

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

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

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

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

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

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 $\mu\text{g l}^{-1}$

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

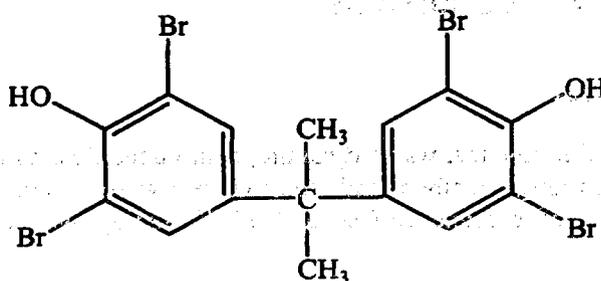
3.1 Test substance

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

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

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 (\pm standard deviation) of the mussels (all test vessels) was 10.8 (\pm 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 \pm 1‰. The water temperature was controlled to the nominal test temperature 15 \pm 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 \times 15.0 \times 14.6 cm (length \times width \times 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 $15 \pm 1^\circ\text{C}$. 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.

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

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 µg l⁻¹ of TBBPA, together with a dilution water and solvent control were tested. The highest exposure concentration of 300 µg 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 µg l⁻¹ test solutions were clear colourless liquids. The stock solutions for the 150 and 300 µg 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 µl 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 byssus 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 µg 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 µg l⁻¹ A replicate tank on day 14. On exposure days 2 and 4, in all replicates at measured 226 µg 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:

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

r = specific growth rate

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

$\log_e W_2$ = logarithm of the shell length (or tissue weight) of an individual mussel at the end of the exposure period

$\log_e W_1$ = 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

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

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 Wilcoxon's 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 $\mu\text{g l}^{-1}$

Lowest observed effect ($P=0.05$) concentration (LOEC) = 126 $\mu\text{g l}^{-1}$

Between days 0 and 28:

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

Lowest observed effect ($P=0.05$) concentration (LOEC) = 126 $\mu\text{g l}^{-1}$

Between days 0 and 42:

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

Lowest observed effect ($P=0.05$) concentration (LOEC) = 62 $\mu\text{g l}^{-1}$

Between days 0 and 56:

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

Lowest observed effect ($P=0.05$) concentration (LOEC) = 62 $\mu\text{g l}^{-1}$

Between days 0 and 70:

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

Lowest observed effect ($P=0.05$) concentration (LOEC) = 32 $\mu\text{g l}^{-1}$

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

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 $\mu\text{g l}^{-1}$
Lowest observed effect ($P=0.05$) concentration (LOEC)	= 126 $\mu\text{g l}^{-1}$

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 Wilcoxon's non-parametric rank sum test (Ref 4). At the mean measured 32 $\mu\text{g l}^{-1}$ 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 $\mu\text{g l}^{-1}$ 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 Wilcoxon's 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 $\mu\text{g l}^{-1}$ replicate A. The significantly different SGRs (dry tissue weight) at 32 $\mu\text{g l}^{-1}$ are unexpected given that there was no significant effect on the SGR (dry tissue weight) at the higher measured test concentration of 62 $\mu\text{g l}^{-1}$ and that there was no significant effect on SGR (wet tissue weight) at 32 $\mu\text{g l}^{-1}$. 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.

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

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 $\mu\text{g l}^{-1}$

Lowest observed effect (P=0.05) concentration (LOEC) = 32 $\mu\text{g l}^{-1}$

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 $15 \pm 1^\circ\text{C}$. 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 $15 \pm 1^\circ\text{C}$. 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 $\mu\text{g l}^{-1}$ and the lowest observed effect concentration (LOEC, P=0.05) was 32 $\mu\text{g l}^{-1}$, based on arithmetic mean measured concentrations.

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

7 REFERENCES

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

TABLE 1
ANALYTICAL RESULTS^a

Nominal conc of TBBPA ($\mu\text{g l}^{-1}$)	Exposure day measured concentrations TBBPA ($\mu\text{g l}^{-1}$)														Mean measured conc over the exposure duration ^b ($\mu\text{g l}^{-1}$)	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 ^e	24.7 ^e	9.53 ^e	13.3 ^f	12.8 ^g	17.5 ^g	21.1 ^f	20.5 ^g	15.9 ^f	16.8 ^f	20.1 ^g	17.8 ^e	17	89
38	30.7 ^c	31.3 ^d	29.3 ^d	30.8 ^e	31.6 ^e	36.1 ^e	24.0 ^f	31.6 ^g	32.8 ^g	31.2 ^f	35.1 ^g	30.2 ^f	36.8 ^f	31.9 ^g	32.5 ^e	32	84
75	57.0 ^c	70.3 ^d	48.8 ^d	72.8 ^e	60.7 ^e	68.4 ^e	56.7 ^f	66.8 ^g	73.3 ^g	73.5 ^f	48.6 ^g	43.5 ^f	48.9 ^f	75.7 ^g	50.3 ^e	62	83
150	137 ^c	133 ^d	96.3 ^d	157 ^e	64.4 ^e	138 ^e	115 ^f	118 ^g	139 ^g	143 ^f	132 ^g	128 ^f	110 ^f	135 ^g	126 ^e	126	84
300	255 ^c	235 ^d	207 ^d	228 ^e	270 ^e	261 ^e	229 ^f	232 ^g	233 ^g	247 ^f	211 ^g	221 ^f	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 $\mu\text{g l}^{-1}$ 16.3, 19.8 (cent): 38 $\mu\text{g l}^{-1}$ 31.5, 31.1 (cent): 75 $\mu\text{g l}^{-1}$ 65.2, 75.4 (cent): 150 $\mu\text{g l}^{-1}$ 135, 131 (cent): 300 $\mu\text{g l}^{-1}$ 233, 237 (cent)

day -6 (algae present): 19 $\mu\text{g l}^{-1}$ 18.4, 19.2 (cent): 38 $\mu\text{g l}^{-1}$ 30.9, 27.7 (cent): 75 $\mu\text{g l}^{-1}$ 49.5, 48.1 (cent): 150 $\mu\text{g l}^{-1}$ 95.7, 96.8 (cent): 300 $\mu\text{g l}^{-1}$ 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 $\mu\text{g l}^{-1}$

TABLE 2

SUMMARY OF EXPOSURE PHASE FLOW DATA

Nominal conc TBBPA ($\mu\text{g l}^{-1}$)	Mean measured concentration TBBPA ($\mu\text{g l}^{-1}$)	No. of readings	Replicate tank inflow rates (ml min ⁻¹)				Overall range of tank inflow rates
			Mean				
			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 ($\mu\text{g l}^{-1}$)	Mean measured concentration TBBPA ($\mu\text{g l}^{-1}$)	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)

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

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

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

TABLE 3 CONTD

MUSSEL LENGTHS

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

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	12.5	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) $\mu\text{g l}^{-1}$

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

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

TABLE 3 CONTD

MUSSEL LENGTHS

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

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) $\mu\text{g l}^{-1}$

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

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

TABLE 3 CONTD

MUSSEL LENGTHS

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

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 9 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

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

TABLE 5

MUSSEL WET WEIGHTS (DAY 70)

Replicate tank	Nominal (measured) concentration of TBBPA ($\mu\text{g l}^{-1}$)						
	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	113	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	39.7	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 ($\mu\text{g l}^{-1}$)						
	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

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

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

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

TABLE 7 CONTD

INDIVIDUAL PSEUDO SPECIFIC GROWTH RATES (LENGTH) OF THE MUSSELS

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

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	0.602	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) $\mu\text{g l}^{-1}$

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	0.302	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

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

TABLE 7 CONTD

INDIVIDUAL PSEUDO SPECIFIC GROWTH RATES (LENGTH) OF THE MUSSELS

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

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	0.439
B	0.656	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) $\mu\text{g l}^{-1}$

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

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

TABLE 7 CONTD

INDIVIDUAL PSEUDO SPECIFIC GROWTH RATES (LENGTH) OF THE MUSSELS

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

Replicate tank	Individual mussels pseudo specific growth rate (SGP) 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 ($\mu\text{g l}^{-1}$)	Mean measured conc TBBPA ($\mu\text{g l}^{-1}$)	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

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

TABLE 9
INDIVIDUAL PSEUDO SPECIFIC GROWTH RATES (WET WEIGHT) OF THE MUSSELS

Replicate tank	Nominal (measured) concentration of TBBPA ($\mu\text{g l}^{-1}$)						
	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	1.37	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	0.327
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	1.55	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	1.53	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	1.67	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 ($\mu\text{g l}^{-1}$)						
	Dihl 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	1.63	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	0.92	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 ($\mu\text{g l}^{-1}$)						
		Diln water control	Solvent Control	19 (12)	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 ($\mu\text{g l}^{-1}$)	Mean measured conc TBBPA ($\mu\text{g l}^{-1}$)	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 ($\mu\text{g l}^{-1}$)	Mean measured conc TBBPA ($\mu\text{g l}^{-1}$)	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 ($\mu\text{g l}^{-1}$)	Mean measured conc TBBPA ($\mu\text{g l}^{-1}$)	Number of samples	Mean ($^{\circ}\text{C}$)	Minimum ($^{\circ}\text{C}$)	Maximum ($^{\circ}\text{C}$)
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 $15 \pm 1^{\circ}\text{C}$

Continuous electronic monitoring of the control replicate B tank, with hourly recording of temperatures, throughout the whole definitive test run, showed values within $15 \pm 1^{\circ}\text{C}$

TABLE 16

WATER QUALITY PARAMETERS OF THE LABORATORY SEAWATER SUPPLY

Monthly sample

Date sampled	Test exposure day	Total ammonia as NH ₃ -N (µg l ⁻¹)	Total filterable solids (TFS) (mg l ⁻¹)	Total organic carbon (TOC) (mg l ⁻¹)
2004				
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

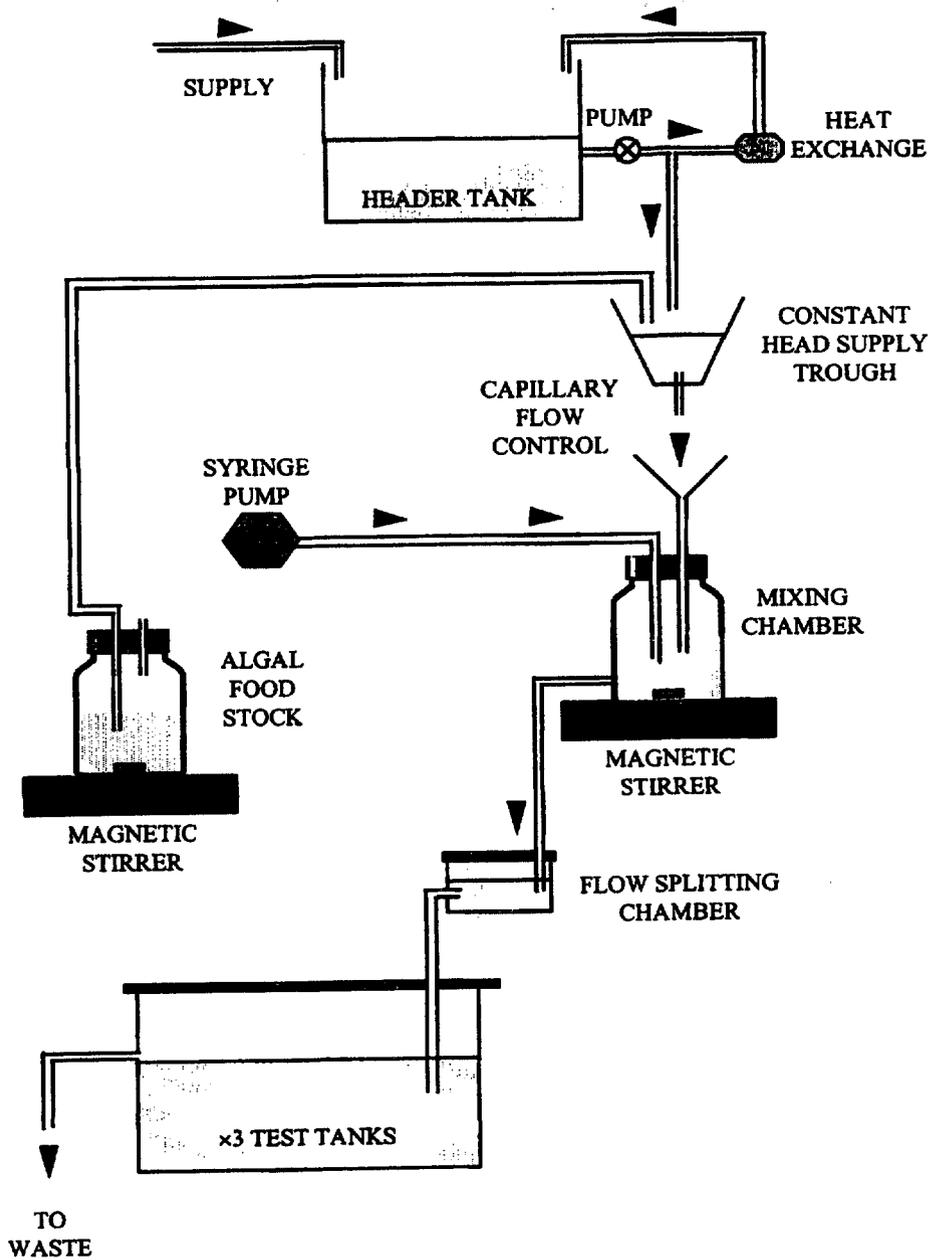
Quarterly sample (sampled 21 September 2004, exposure day 8)

Parameter	Concentration (µg 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.

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

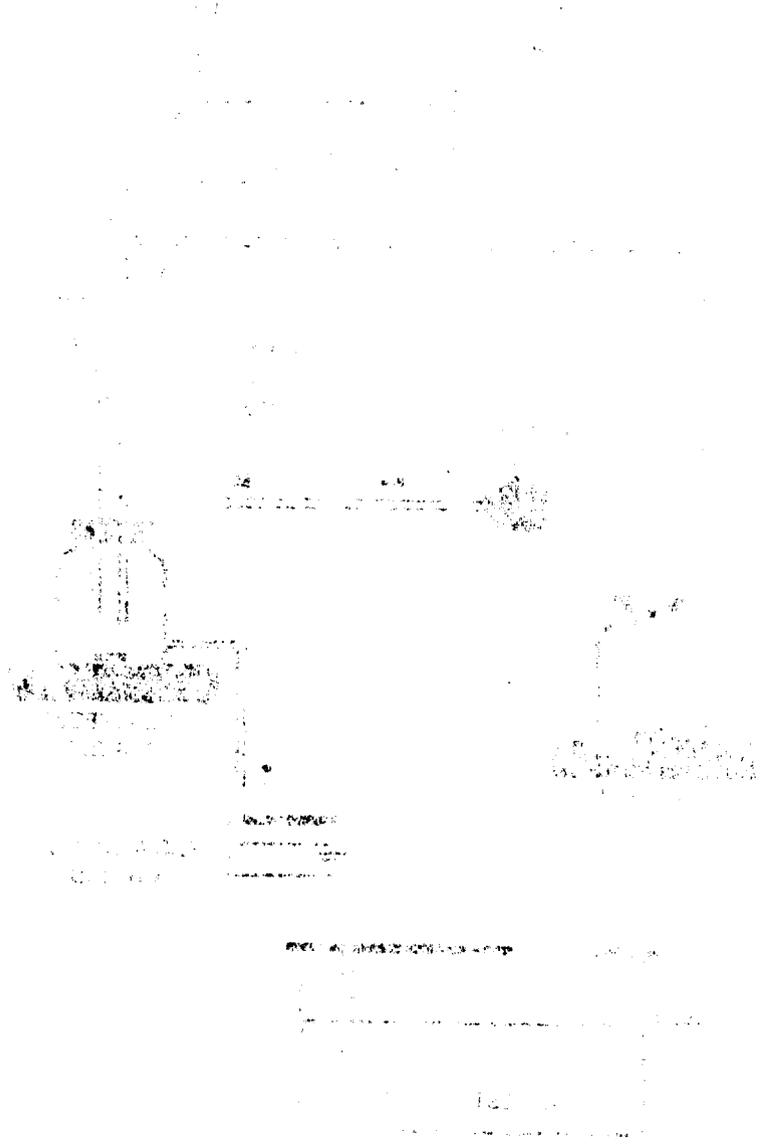
FIGURE 1
DOSING SYSTEM



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



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:

ACTIVITY:	DATE CONDUCTED:	STUDY DIRECTOR:	DATE REPORTED TO: 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

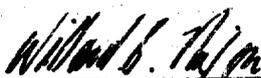
ANALYTICAL PRINCIPAL INVESTIGATOR:



Jon A. MacGregor, B.S.
Scientist

DATE 3/28/05

MANAGEMENT:



Willard B. Nixon, Ph.D.
Director, Chemistry

DATE 3/28/05

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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 (<i>Mytilus edulis</i>)
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:

<u>Manufacturer</u>	<u>Lot/Batch</u>	<u>Date Received</u>	<u>Wildlife International Ltd. ID No.</u>
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 Tetrabromobisphenyl 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 (ug 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

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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 x 2.0 mm, 5- μ m 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 μ L
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 (100 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.

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Table 5

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

Nominal Concentration ($\mu\text{g a.i./L}$)	Sample Identification	Sampling Time (Day)	Measured Concentration ($\mu\text{g a.i./L}$) 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 $\mu\text{g a.i./L}$, calculated as the product of the concentration of the lowest calibration standard (10.0 $\mu\text{g a.i./L}$) 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 ($\mu\text{g a.i./L}$)	Sample Identification	Sampling Time (Day)	Measured Concentration ($\mu\text{g a.i./L}$) 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 $\mu\text{g a.i./L}$, calculated as the product of the concentration of the lowest calibration standard (10.0 $\mu\text{g a.i./L}$) 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 ($\mu\text{g a.i./L}$)	Measured Concentration ($\mu\text{g a.i./L}$) 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 $\mu\text{g a.i./L}$, calculated as the product of the concentration of the lowest calibration standard (10.0 $\mu\text{g a.i./L}$) 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 ($\mu\text{g a.i./L}$)	Measured Concentration ($\mu\text{g a.i./L}$) 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 $\mu\text{g a.i./L}$, calculated as the product of the concentration of the lowest calibration standard (10.0 $\mu\text{g a.i./L}$) 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 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		
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		
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 ($\mu\text{g a.i./L}$)	Sample ID (03-0337/A-)	Sampling Time (Days)	Measured Concentration ($\mu\text{g a.i./L}$) ¹	Percent of Nominal ²	Mean Measured Concentration ($\mu\text{g a.i./L}$)	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 $\mu\text{g a.i./L}$, calculated as the product of the concentration of the lowest calibration standard (10.0 $\mu\text{g a.i./L}$) 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

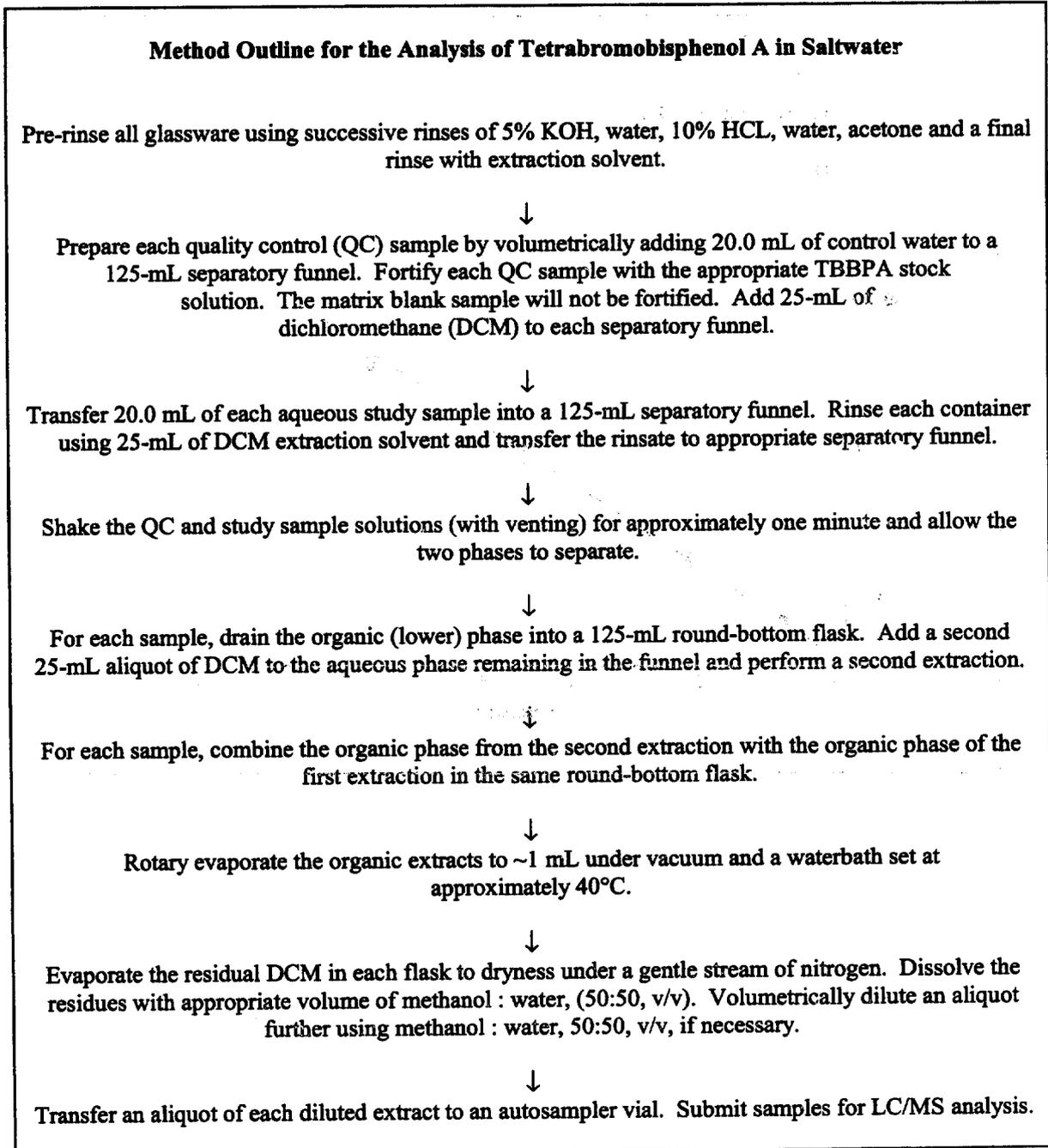


Figure 2

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

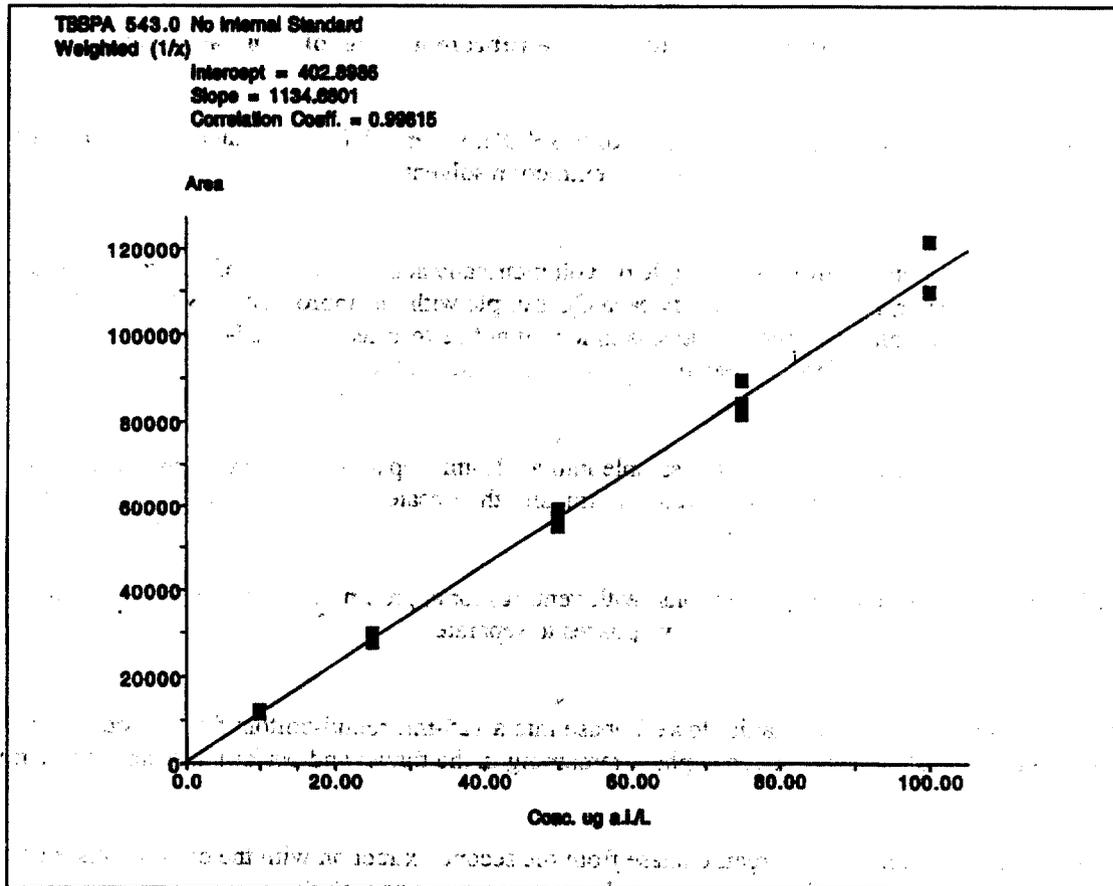
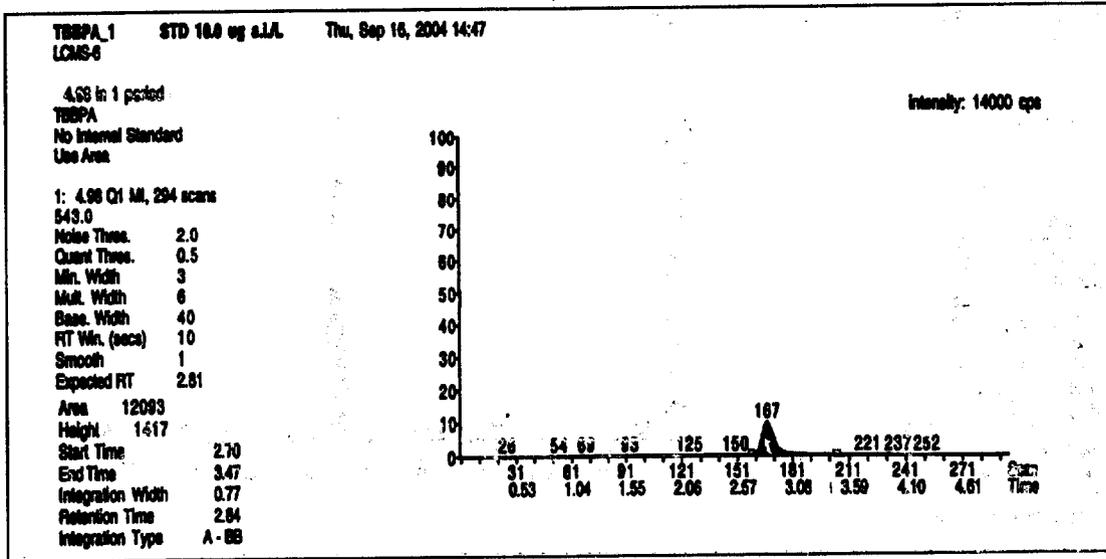


Figure 3

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

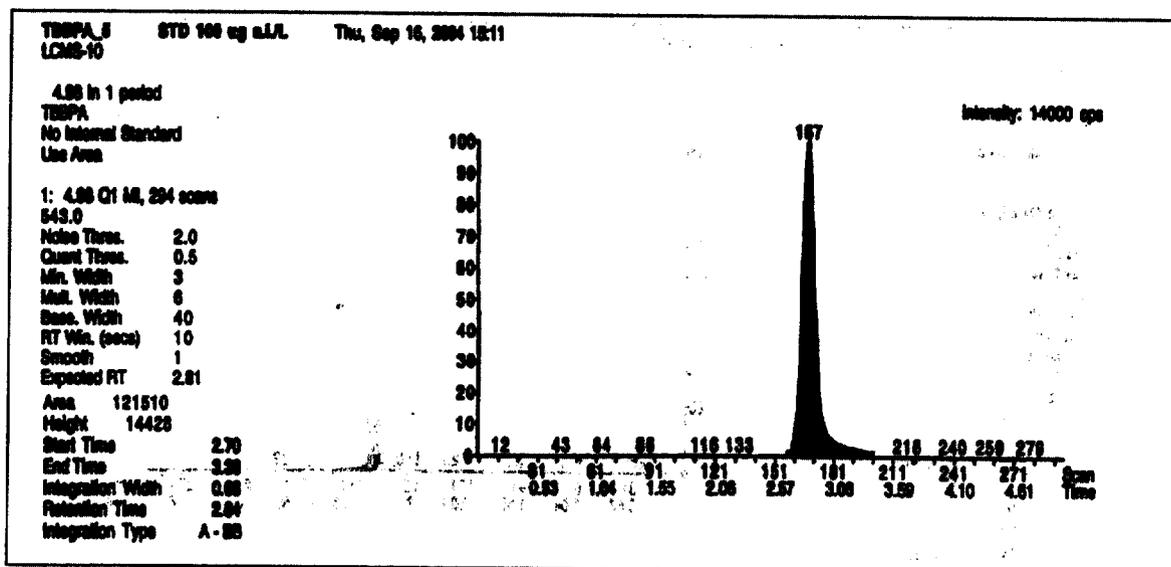


Nominal concentration: 10.0 µg a.i./L

- 29 -

Figure 4

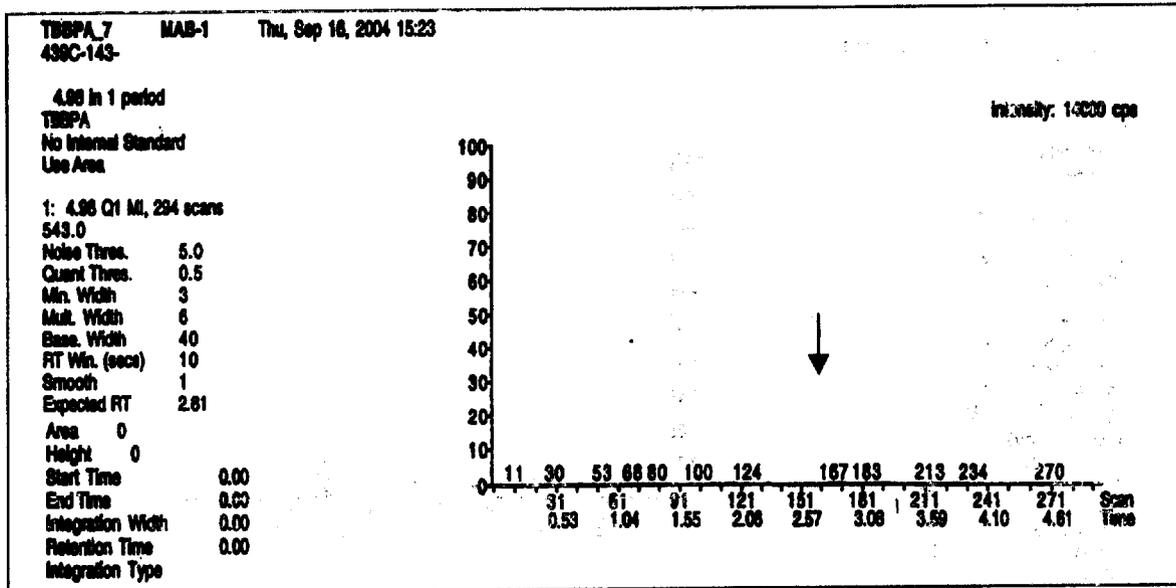
Representative Chromatogram of a High-level Saltwater Tetrabromobisphenol A Calibration Standard Analyzed by HPLC/MS



Nominal concentration: 100 µg a.i./L

Figure 5

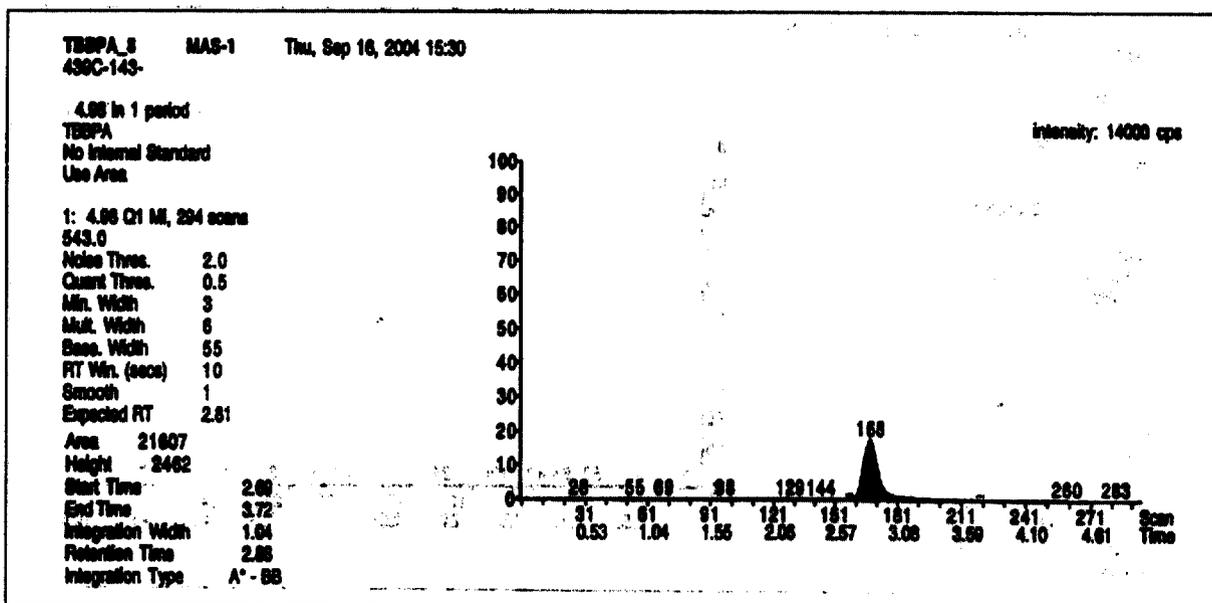
Representative Chromatogram of a Saltwater Matrix Blank Sample Analyzed by HPLC/MS



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

Figure 6

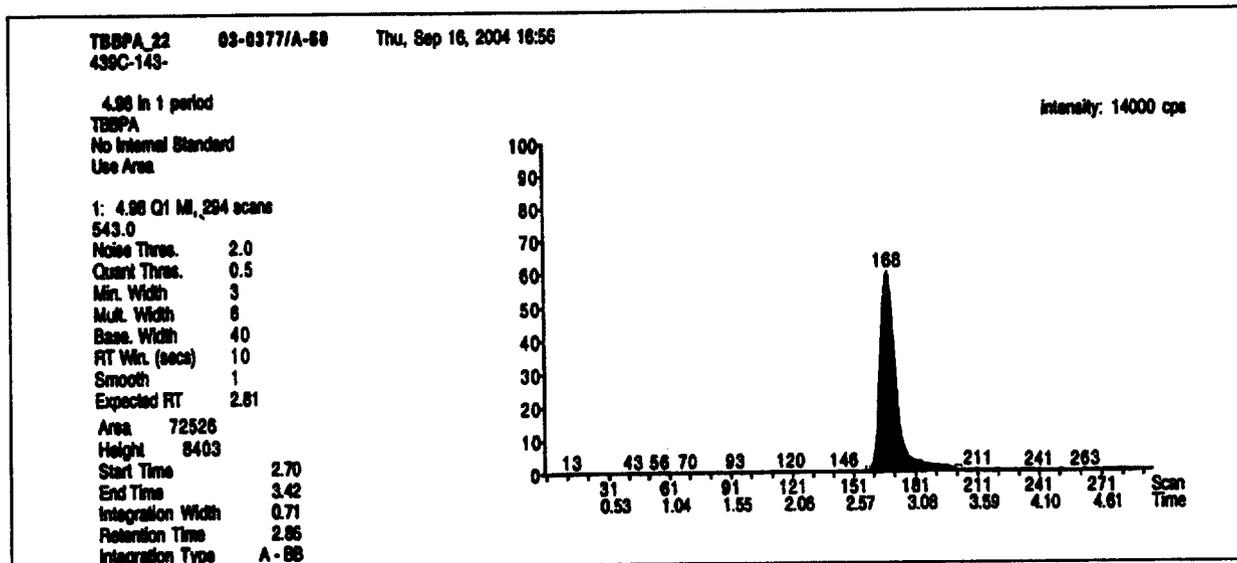
Representative Chromatogram of a Saltwater Matrix Fortification Sample Analyzed by HPLC/MS



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**

CERTIFIED TRUE COPY
per Taylor

ALBEMARLE CORPORATION
RESEARCH AND DEVELOPMENT DEPARTMENT

FINAL REPORT ON THE CHEMICAL CHARACTERIZATION (IDENTITY, PURITY AND
HOMOGENEITY) OF TETRABROMOBIPHENOL-A (TBBPA), WILDLIFE
INTERNATIONAL, LTD. TEST SUBSTANCE 6404, COMPOSITED FROM WILDLIFE
INTERNATIONAL, LTD. 6358, 6368 AND 6400

- I. Protocol Number: TBBPA-02-01-2003
- II. Sponsor:
 Great Lakes Chemistry Council
 Great Lakes Flame Retardant Industry Panel
 1500 Wilson Boulevard
 Arlington, Virginia 22209
 Study Monitor: Wendy K. Sherman
- III. Analytical Testing Facilities:
 Albemarle Corporation
 Process Development Center
 P. O. Box 341
 Bozart Rouge, LA 70821
 Study Chemist: Paul F. Ranken, Ph. D.
- IV. Date of Study Initiation: August 12, 2003
 Date of Study Completion: September 24, 2003
- V. Test Article:
 Tetrabromobiphenol-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
 Chemical 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
 determine 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 (umol/L) of three potential impurities: tribromophenol, tribromobiphenyl-A and o,p-tetrabromobiphenyl-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 suspected impurities. There were no chromatograms that may have affected the quality or integrity of the data.

F1

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
Paul F. Ranken, Ph. D.
STUDY CHEMIST

September 24, 2003
DATE

Table 1. CONCLUSIONS AND TEST ARTICLE ANALYTICAL DATA.

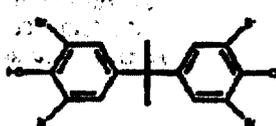
<p>CHEMICAL NAME: Tebutrombiphenyl-A C.A.S. No.: 79-94-7 MOLECULAR FORMULA: C₁₈H₁₉BrO₂ PHYSICAL FORM: White Powder CHEMICAL STRUCTURE</p>					
					
ANALYSIS	RESULTS			ANALYSIS DATES	ANALYST
RFIC	Purity (mean ± STMPA)	Average	Difference (%) from average		
Sample					
middle right	99.21	99.20	<1%	08/18/03	J. S. Ameyere
middle left	99.18	99.20	<1%	08/18/03	J. S. Ameyere
bottom right	99.20	99.20	<1%	08/18/03	J. S. Ameyere
top right	99.19	99.20	<1%	08/18/03	J. S. Ameyere
top left	99.19	99.20	<1%	08/18/03	J. S. Ameyere
bottom left	99.20	99.20	<1%	08/18/03	J. S. Ameyere
FTIR	The sample FT-IR spectrum matched that of the reference spectrum. All spectra are on file with the original data.			08/18/03	W. T. CGM
<p>CONCLUSION: Based on these analytical data, the test article identity was confirmed as tebutrombiphenyl-A. The sample was shown to be homogeneous with a purity of 99.20%.</p>					

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

	TBBPA	Trbromophenol	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

CONTAIN NO CR!

~~CONFIDENTIAL~~

The *In Vitro* Percutaneous Absorption of Radiolabelled Hexabromocyclododecane (HBCD) Through Human Skin

Inveresk Study Number 774740

Inveresk Report Number 25031

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L8

CONFIDENTIAL

Inveresk Report Number 25031

***The In Vitro Percutaneous Absorption of Radiolabelled
Hexabromocyclododecane (HBCD) Through Human Skin***

Author:

C S Roper

Sponsor:

American Chemistry Council
Brominated Flame Retardant Industry Panel
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USA

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Tranent
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Scotland
UK

Final Page of Report: 41

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Authentication

"I, the undersigned, hereby declare that this study was performed under my direction and in accordance with the OECD Principles of Good Laboratory Practice as set forth by the United Kingdom Department of Health. The study was conducted according to the procedures herein described and this report represents a true and accurate record of the results obtained."



Clive S Roper BSc PhD
Study Director

26 April 2005

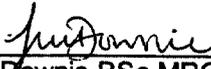
Date

Quality Assurance Statement

Inveresk Quality Assurance Unit conducted the following QA functions on this study.

<u>Date of QA Activity</u>	<u>Phase</u>	<u>Date of Report to Management and Study Director</u>
05 January 2005	Protocol Review	05 January 2005
06 January 2005	Dose Preparation Review/ Dosing/ Protocol Compliance	12 January 2005

This report is considered to describe accurately the procedures used in the study and the results obtained.



Janet M Downie BSc MRQA
Quality Assurance

25 April 2005

Date

Personnel Involved

Study Director:

Clive S Roper BSc PhD

Staff Involved:

Leanne Stupart BSc
Lucy F Crow BSc
Jill Runciman
David Fallis BSc
Frank Toner BSc

Quality Assurance:

Margaret Tiffney BSc
Ellen Carnegie BSc MSc
Jane P Dunsire CBiol MIBiol
Janet M Downie BSc MRQA

1 Summary

Hexabromocyclododecane (CAS No. 25637-99-4), also known as HBCD, is a brominated flame retardant that is managed under the American Chemistry Council – Brominated Flame Retardant Industry Panel (ACC-BFRIP) consortium.

HBCD is a homogeneous mixture of three diastereomers (alpha, beta and gamma). The gamma is present at the highest percentage level of the three when manufactured. HBCD has very low solubility in most common solvents.

As part of the safety evaluation of HBCD, a study was conducted to assess the rate and extent of absorption of HBCD following topical application of HBCD to human skin.

The split-thickness skin membranes were mounted into flow-through diffusion cells. Receptor fluid (tissue culture medium containing bovine serum albumin, glucose, streptomycin and penicillin G) was pumped underneath the skin at a flow rate of ca 1.5 mL/h and a tritiated water barrier integrity test performed. The skin surface temperature was maintained at ca 32°C throughout. All skin samples with a tritiated water permeability coefficient (k_p) less than 2.5×10^{-3} cm/h were accepted for use.

[14 C]-HBCD was applied in an acetone vehicle at ca 47 $\mu\text{L}/\text{cm}^2$ in 5 applications of 6.0 μL over a ca 15 min period to human split-thickness skin membranes mounted in flow-through diffusion cells *in vitro*. The [14 C]-HBCD could not be applied as the powder as the mass to be applied (ca 640 μg , 1 mg/cm^2) could not be accurately dispensed. Therefore, [14 C]-HBCD was applied as a solution using acetone as the vehicle. The test preparation ([14 C]-HBCD in acetone) could not be applied in a single volume application because the solubility of HBCD in acetone was too low. The acetone evaporated rapidly from the skin surface leaving behind the [14 C]-HBCD.

Absorption was assessed by collecting receptor fluid in hourly fractions from 0-8 h post dose and then in 2-hourly fractions from 8-24 h post dose. At 24 h post dose, the exposure was terminated by washing and drying the skin. The stratum corneum was then removed by successive tape stripping. All samples were analysed by liquid scintillation counting.

A summary of the results is provided in the table below.

	Mean	SD
Target HBCD Application Rate ($\mu\text{g}/\text{cm}^2$)	1000	-
Actual HBCD Application Rate ($\mu\text{g}/\text{cm}^2$)	950	-
Dislodgeable Dose ($\mu\text{g equiv.}/\text{cm}^2$)	600.90	114.17
Unabsorbed Dose ($\mu\text{g equiv.}/\text{cm}^2$)	903.76	42.07
Absorbed Dose ($\mu\text{g equiv.}/\text{cm}^2$)	0.06	0.04
Dermal Delivery ($\mu\text{g equiv.}/\text{cm}^2$)	12.82	4.65
Mass Balance ($\mu\text{g equiv.}/\text{cm}^2$)	916.58	40.36
Dislodgeable Dose (% Applied Dose)	63.37	12.04
Unabsorbed Dose (% Applied Dose)	95.31	4.43
Absorbed Dose (% Applied Dose)	0.01	0.00
Dermal Delivery (% Applied Dose)	1.35	0.49
Mass Balance (% Applied Dose)	96.67	4.25

In conclusion, following topical application of [¹⁴C]-HBCD to human split-thickness skin *in vitro*, the absorbed dose and dermal delivery were 0.01% (0.06 µg.equiv./cm²) and 1.35% (12.82 µg.equiv./cm²) of the applied dose, respectively. At 8 h post dose, 34.62% of the applied dose was removed from the skin by washing and drying. At 24 h a further 28.76% was recovered in the 24 h skin drying and cell wash. Therefore, the dislodgeable dose was 63.37% of the applied dose. The stratum corneum contained a further 31.49% of the applied dose. The bulk of this (25.70%) was recovered in the first 5 tape strips. Since the bulk of the stratum corneum associated material was found in the first 5 tapes strips, this indicated that the [¹⁴C]-HBCD was on the surface of the skin and that the stratum corneum was an efficient barrier to [¹⁴C]-HBCD penetration.

2 Introduction

Hexabromocyclododecane (CAS No. 25637-99-4), also known as HBCD, is a brominated flame retardant that is managed under the American Chemistry Council – Brominated Flame Retardant Industry Panel (ACC-BFRIP) consortium.

HBCD is a homogeneous mixture of three diastereomers (alpha, beta and gamma). The gamma is present at the highest percentage level of the three when manufactured. HBCD has very low solubility in most common solvents.

As part of the safety evaluation of HBCD, a study was conducted to assess the rate and extent of absorption of HBCD following topical application of HBCD to human skin.

The study was conducted at:

Inveresk
Tranent
EH33 2NE
Scotland
UK

Key dates in the conduct of the study were as follows:

Study Initiation:	22 December 2004
Experimental Start Date:	05 January 2005
Experimental Completion Date:	19 January 2005
Study Completion Date:	See Authentication page for date of Study Director's Signature

All data generated and recorded during this study, including a copy of the final report, will be stored in the Scientific Archives of Inveresk for 5 years after issue of the final report. At the end of the 5 year period the Sponsor will be consulted regarding the transfer, disposal or continued storage of raw data.

This study was performed in accordance with the following documents:

OECD Guideline for Testing of Chemicals, Guideline 428: Skin Absorption: *In Vitro* Method (2004).

OECD Environmental Health and Safety Publications Series on Testing and Assessment No. 28. Guidance Document for the Conduct of Skin Absorption Studies (2004).

3 Experimental Procedure

3.1 Materials

3.1.1 Radiolabelled Test Item

[¹⁴C]-Hexabromocyclododecane (HBCD) in acetone at a radioactive concentration of 1.0 mCi/mL, lot no. EPPS-03-021-57-08, with a supplied specific activity of 24.5 mCi/mmol, was supplied by EaglePicher Pharmaceutical Services, LLC, Lenexa, Kansas, USA. The test item was stored at ca -20°C in the dark. A copy of the Certificate of Analysis is provided in Appendix 1.

3.1.2 Non-Radiolabelled Test Item

HBCD Composite, batch no. TS6541, expiry date 13 December 2005, was supplied by Wildlife International, Ltd, Ecotoxicology & Analytical Testing Services, Easton, MD, USA and was stored at ambient temperature in the dark. A copy of the chemical characterization report is provided in Appendix 2.

3.1.3 Other Materials

Aquasafe 500 liquid scintillation fluid was obtained from Zinsser Analytic, Maidenhead, UK.

Carbo-Sorb[®] CO₂ absorbing fluid, Permafluor[®]E⁺ scintillation fluid and Spec-Chec[™]-¹⁴C were supplied by Canberra Packard Limited, Pangbourne, UK.

All other materials were obtained by Inveresk.

3.2 Confirmation of Radiochemical Purity of [¹⁴C]-HBCD

The radiochemical purity of [¹⁴C]-HBCD was determined prior to dose preparation with analysis by HPLC using the following equipment and conditions:

Equipment

HP 1050 Series HPLC
Gilson 401 Diluter
Gilson 231 Sample injector
HP 1050 Series u.v. Detector

Canberra Packard Radiomatic[™] Flo-one[®] Beta, Flow Scintillation Analyser (Model 150TR)

Conditions

Column: Nova-pak C18 (150 mm x 3.9 mm i.d., 5 µm)
Mobile Phase: Solvent A = Water
Solvent B = Acetonitrile

Solvent System (Gradient)	Time (mins)	Solvent A (%)	Solvent B (%)
	0	25	75
	12	25	75
	17	0	100
	25	0	100

Flow Rate: 0.8 mL/min
U.V. Detection: 220 nm
Scintillant: Ultima-Flo™
Data Collection: ATLAS 2002 (Thermo LabSystems) Product Version 6.18

The chemical authenticity of the [¹⁴C]-HBCD was confirmed by co-chromatography with authentic non-radiolabelled HBCD. The radiochemical purity of [¹⁴C]-HBCD was determined to be 97.0%. A representative HPLC chromatogram is presented in Appendix 3. The three main peaks were associated with the three diastereomers.

3.3 Human Skin Samples

Seven samples of full-thickness breast human skin were obtained from patients (aged 19 to 68 years old), who gave informed consent for their skin to be taken for scientific research purposes, prior to undergoing routine surgery at the Plastic Surgery Unit, St Johns Hospital, West Lothian NHS Trust, Livingston, UK. The skin was transferred to Inveresk stored on ice and cleaned of subcutaneous fat and connective tissue using a scalpel blade. The skins were washed in cold running water and dried using "blue roll" tissue paper. The sample was then cut into smaller pieces (where appropriate), wrapped in aluminium foil, put into self sealing plastic bags and stored at ca -20°C until required. The age and sex of the donor and site from which the skin was taken were recorded. The sample details are as shown in Appendix 4.

3.4 Preparation of Split-Thickness Skin Membranes

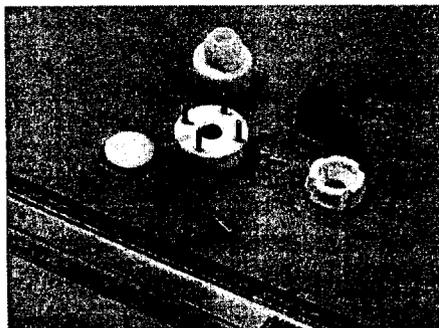
When required, the human skin samples were removed from storage and allowed to thaw at ambient temperature. The thickness of the uncut skin membranes was measured using a micrometer. Split-thickness membranes were prepared by pinning the full-thickness skin, stratum corneum uppermost, onto a raised cork board and cutting at a setting equivalent to 200-400 µm depth using a Zimmer electric dermatome. The membranes were then laid out onto aluminium foil and the thickness of the membranes measured using a micrometer. The thickness of the full-thickness and split-thickness membranes is provided in Appendix 5.

3.5 Flow-Through Diffusion Cell Apparatus

An automated flow-through diffusion cell apparatus (Scott/Dick, University of Newcastle-upon-Tyne, UK) was used (see photograph overleaf). The flow-through cells were placed in a steel manifold heated *via* a circulating water bath to maintain the skin surface temperature at ca 32°C (Appendix 6). The cells were connected to multi-channel peristaltic pumps from their afferent ports, with the receptor fluid effluent dropping *via* fine bore tubing into scintillation vials on a fraction collector.

The surface area of exposed skin within the cells was 0.64 cm². The receptor chamber volume was 0.25 mL. The peristaltic pumps were adjusted to maintain a flow-rate of ca 1.5 mL/h (Appendix 6).

A Photograph of the Flow-Through Diffusion Cell



3.6 Receptor Fluid

A tissue culture medium containing bovine serum albumin (ca 5%, w/v), glucose (ca 1%, w/v), streptomycin (ca 0.1 mg/mL) and penicillin G (ca 100 units/mL) was used as the receptor fluid for the tritiated water barrier integrity and test item permeability assessments.

3.7 Solubility of Test Item in Receptor Fluid

HBCD has a solubility in water of 65.2 ppb, a toluene solubility of >5% (w/w) and an acetone solubility of >1% (w/w). Therefore, any normally used receptor fluid (OECD Guideline 428) will not have sufficient HBCD solubility. Hence, a physiological receptor fluid was chosen. If absorption was high, then HBCD would be found in the skin layers and would therefore be included in the dermal delivery figure.

3.8 Flow-Through Diffusion Cell Preparation

Sections of split-thickness skin membrane, ca 1.5 x 1.5 cm, were cut out, positioned on the receptor chamber of the diffusion cell, containing a magnetic flea, and the donor chamber was tightened into place with screws. The cells were then placed in the heated manifold and connected to the peristaltic pump. The Variomag magnetic stirrer was switched on to mix the contents of the receptor chamber. An equilibration period of ca 15 min was allowed while receptor fluid was pumped through the receptor chambers at ca 1.5 mL/h. The effluent was then collected for ca 30 min and retained as blank samples for use in the tritiated water barrier integrity assessment.

3.9 Barrier Integrity Assessment

Tritiated water (250 µL, radioactivity ca 100,000 d.p.m.) was applied to the surface of each skin sample and the donor chamber occluded. Penetration of tritiated water was assessed by collecting hourly fractions for 2 hours and analysing the fractions by liquid

scintillation counting. Permeability coefficients (k_p) were calculated for each skin sample. Any human skin sample exhibiting a k_p greater than 2.5×10^{-3} cm/h was excluded from subsequent absorption measurements. A cross reference of skin sample number and donor and its corresponding tritiated water permeability coefficient (k_p) is presented in Appendix 7. At the end of the 2 h period, residual tritiated water was removed from the skin surface by rinsing with water (ca 2 mL) and the skin was dried with tissue paper. An equilibration period was allowed prior to collection of the pre-dose sample which was collected for ca 30 min.

3.10 Formulation of Test Preparation

HBCD Composite (0.03744 g) was weighed into a 2 mL volumetric flask. [^{14}C]-HBCD in acetone stock solution (200 μL) was transferred into the volumetric flask. The volumetric flask was made up to the 2 mL line with acetone and mixed by inversion until the test item had dissolved. Seven 6.4 μL aliquots were taken into scintillation vials, mixed with 10 mL scintillant and analysed by liquid scintillation counting: The aliquots were homogeneous with a coefficient of variation of 3.47%. The mean mass of [^{14}C]-HBCD in each 6.4 μL aliquot was 136.24 μg . Therefore, it was calculated that 30.1 μL would contain 640 μg . To ensure optimal delivery, it was decided to apply the test preparation to the skin in five 6.0 μL aliquots within ca 15 min. This test preparation was accepted for dosing.

3.11 Application of Test Preparation

The test preparation was applied over the stratum corneum surface of the exposed skin using an M25 Gilson Microman positive displacement pipette set to deliver 6.0 μL , once the acetone had evaporated from the skin surface this was repeated 4 more times until a total of 30 μL (47 $\mu\text{L}/\text{cm}^2$) had been applied to the skin. The dosing procedure took a total of ca 15 min to complete. The donor chambers were left open to the atmosphere. To accurately quantify the radioactivity applied to the skin samples, 7 aliquots (30 μL) of the test preparation were collected in Combustococones[®] in the same manner at the time of dosing. These mock dose samples were used to calculate mass balance.

3.12 Analysis of Mock Dose Samples

The mock dose samples (Section 3.11) were analysed by combustion/ liquid scintillation counting. The total mass of [^{14}C]-HBCD applied to the skin was determined to be 711.92 μg (1.1 mg/cm^2), which was 111.24% of the target application of 640 μg (1 mg/cm^2).

As this was much higher than anticipated, it was believed that the mock dosing samples may not have been representative of the dosing to the skin. The Combustococones[®] contain absorptive pads and the skin is non absorptive to solvents. To demonstrate this, 6.0 μL of the dosing solution was applied to six Combustococones[®] and this was repeated to each Combustococone[®] 4 times (total volume 30 μL). At the same time, 6.0 μL of the dosing solution was transferred to 20 scintillation vials. Acetone (1 mL) and then scintillant (10 mL) was then added. The Combustococones[®] were then analysed by combustion/ liquid scintillation counting and the scintillation vial samples were analysed by liquid scintillation counting. The counts for each group of 5

vials were added together. The results showed that the scintillation vials contained only 85.24% of the radioactivity of the combustion samples. A conversion factor of 85.24% was, therefore, applied to the mock dose samples. The corrected total mass of [¹⁴C]-HBCD applied to the skin was calculated to be 606.84 µg (0.95 mg/cm²), which was 94.82% of the target application of 640 µg (1 mg/cm²).

3.13 Sampling Information

3.13.1 Receptor Fluid

Receptor fluid was collected in hourly fractions from 0-8 h post dose and then in 2 hourly fractions from 8-24 h post dose. All receptor fluid samples were mixed with ca 10 mL scintillation fluid and analysed by liquid scintillation counting.

3.14 Terminal Procedures – 8 h Post Dose

At 8 h post dose, the exposed skin surface was washed (skin wash 8 h) with ca 10 mL of a ca 2% (v/v) soap solution (Radox Supersoap). The skin wash was aspirated with a pipette and collected into a pre-weighed vial and mixed with ca 10 mL acetone. The mass of skin wash was weighed and duplicate weighed, ca 1 mL, aliquots taken for analysis by liquid scintillation counting. The pipette tip was cut up and placed into a scintillation vial, mixed with scintillant and analysed directly by liquid scintillation counting. The cell and skin surface was dried with tissue paper swabs (skin swab 8 h). These swabs were pooled and placed in a Combustocone[®] for subsequent analysis by combustion/ liquid scintillation counting.

Initial analysis of the duplicate aliquots for the skin wash 8 h samples indicated that the samples were not homogeneous. The remainder of each sample (ca 18 mL) was divided between 8 vials (7 new vials and the bulk sample vial) and these samples were then analysed directly by liquid scintillation counting. The pipette tip was analysed as described previously. The counts for each portion were added to the counts previously obtained from the duplicate analysis to constitute a total recovery value.

3.15 Terminal Procedures – 24 h Post Dose

At 24 h post dose (*i.e.* following a 16 h monitoring period), each diffusion cell was disconnected from the receptor fluid pump lines. The underside of the skin was washed (receptor rinse) with receptor fluid (1-2 mL), which was mixed with scintillation fluid (10 mL) and analysed by liquid scintillation counting. The receptor rinse represented the absorbed test item, which was in the receptor chamber but had not been collected in the 22-24 h receptor fluid fraction.

The cell and skin surface were dried with a tissue paper swab (skin swab 24 h) and the cell dismantled. The skin was removed from the cell and dried with a further tissue paper swab. These swabs were pooled and placed in a Combustocone[®] for subsequent analysis by combustion/ liquid scintillation counting.

The donor and receptor chambers were transferred to pre-weighed pots (cell wash) containing a known weight (ca 40 mL) of acetone. The pots containing the cells were

left to extract the test item for ca 30 mins and then sonicated for ca 10 mins and then duplicate weighed aliquots (1 mL) were taken for analysis by liquid scintillation counting. The donor and receptor chambers were then removed from the cell wash pots.

The stratum corneum was removed with 20 successive tape strips (Guilbert, Niceday). The first 5 tapes were pooled together in a Combustocone[®]. This was repeated for tapes 6-10, 11-15 and 16-20. The skin under the cell (unexposed skin) was cut away from the exposed skin with scissors. These samples were placed into Combustocones[®] for subsequent analysis by combustion/ liquid scintillation counting.

3.16 Storage of Samples

All bulk samples not immediately analysed were stored at ca -20°C. After analysis, samples were returned to storage at ca -20°C.

3.17 Combustion Analysis

Mock dose, skin, tape strip and tissue swab samples were combusted using a Model 307 Tri-Carb Automatic Sample Oxidiser (Canberra Packard Limited). The resultant ¹⁴CO₂ generated was absorbed in Carbo-Sorb[®] (8 mL) and mixed with Permaflour[®]E⁺ scintillation fluid (10 mL). Combustion efficiency and carry over were checked at the start of each run of 30 samples by combusting quality control standards containing Spec-Chec^{TM-14}C. Combustion efficiency was within the range of 97-103%.

3.18 Quantification of Total Radioactivity

All samples, except for the tritiated water samples, were counted for 5 min together with representative blanks using a liquid scintillation analyser (Packard 2100-TR) with automatic quench correction by external standard. Representative blank sample values were subtracted from sample count rates to give net d.p.m. per sample. Prior to analysis, samples were allowed to stabilise with regard to light and temperature. The tritiated water samples were treated as above, except that they were subject to liquid scintillation counting for 1 min only.

3.19 Limit of Reliable Measurement

A limit of reliable measurement of 30 d.p.m. above background has been instituted in these laboratories. Any occasions where results arose from data below the limit of reliable measurement have been noted in the results section of the report.

4 Calculations

The following calculations were performed:

4.1 Permeability Coefficient (k_p) of Water

Cumulative absorption of tritiated water was calculated for each skin sample by summing the net d.p.m. for each hourly fraction from 0 to 2 h. The slope of the absorption *versus* time curve from 0-2 h (i.e. 3 data points) was calculated by linear regression to give an absorption rate (d.p.m./cm²/h).

$$\text{Absorption rate (d.p.m./cm}^2\text{/h)} = \frac{\text{slope (d.p.m./h)}}{\text{exposed area (cm}^2\text{)}}$$

This was then converted to the permeability coefficient (k_p) from the dose application rate of tritiated water as follows:

$$K_p = \frac{\text{absorption rate (d.p.m./cm}^2\text{/h)}}{\text{application rate (d.p.m./cm}^3\text{)}}$$

4.2 Absorption of Radiolabelled Test Item (Flux and Percentage Absorbed)

The absorbed dose was calculated from each individual sample (receptor fluid samples were given as cumulative absorbed dose) radioactivity (d.p.m.), specific activity (SA) and dose area as follows:

$$\text{Absorbed dose (}\mu\text{g equiv./cm}^2\text{)} = \frac{\text{sample radioactivity (d.p.m.)}}{\text{SA (d.p.m./}\mu\text{g equiv.)} \times \text{exposure area (cm}^2\text{)}}$$

In addition, the percentage absorbed dose was also calculated for each sample as follows:

$$\text{Absorbed dose (\%)} = \frac{\text{sample radioactivity (d.p.m.)} \times 100\%}{\text{applied dose (d.p.m.)}}$$

4.3 Data Presentation

Data presented in results, tables, figures and appendices are computer generated and rounded appropriately for inclusion in the report. As a consequence, calculation of values from data presented will, in some instances, yield minor variations.

5 Definitions

The definitions used in this report were taken directly from the OECD guidance document as follows:

Absorbed Dose (*in vitro*)

The mass of test item reaching the receptor fluid or systemic circulation within a specified period of time.

Absorbable Dose

Represents that present on or in the skin following washing.

Absorption (Dermal, Percutaneous and Skin Absorption)

Diffusion of chemicals from the outer surface of the skin to the receptor fluid or systemic circulation.

Absorption Profile

A graphical representation of cumulative absorption as a function of time.

Absorption Rate

Mass of test item passing through a unit area of skin into the receptor fluid or systemic circulation, per unit time (in $\mu\text{g}/\text{cm}^2/\text{h}$).

Adsorption

Reversible binding or adherence of the test item to any component of the test system.

Applied Dose

The mass of test preparation containing a specified mass of test item applied per cm^2 of skin.

Dermal Delivery

The sum of the applied dose found in the treated skin and the absorbed dose at the end of the experiment.

Dislodgeable Dose

The mass of test item that is removable from the application site.

Exposure Period

The time from application of test preparation to removal at skin washing.

Finite Dose

The amount of test preparation applied to the skin where a maximum absorption rate of the test item may be achieved for a certain time interval but is not maintained.

Flux

The mass of the test item passing through a unit area of skin per unit of time under steady-state conditions (in $\mu\text{g}/\text{cm}^2/\text{h}$).

In-Use Preparation

The preparation of test item which relates directly to potential human exposure (e.g. cosmetic or agrochemical formulations and dilutions thereof, a mixture of industrial chemicals in a solvent, etc).

Infinite Dose

The amount of test preparation applied to the skin where a maximum absorption rate of the test item is achieved and maintained.

Lag Time

Derived from a graph of cumulative absorbed dose and time. Intercept of the tangent of the linear part of the absorption profile with the x-axis.

Penetration Enhancer

An adjuvant, which facilitates penetration of the test item through skin.

Percentage Absorption

The mass of test item absorbed (over a given time period) divided by the mass of test item applied multiplied by 100.

Permeability Coefficient (K_p)

A value, in units of cm/h , that represents the rate at which a chemical penetrates the skin. This is calculated from the flux divided by the applied concentration.

Steady-State

The part of the absorption profile where the absorption rate remains constant.

Test Substance (Test Item)

A single chemical entity whose penetration characteristics are under investigation.

Test Preparation

The actual material which is applied to the skin. Usually the test preparation will be the "in-use" preparation that reflects actual use conditions; alternatively it may be a mixture of the test item in a carrier or solvent to facilitate application to the skin.

Unabsorbed Dose

Represents that washed from the skin surface after exposure and any present on the non-occlusive cover, including any dose shown to volatilise from the skin during exposure.

This is also defined as the sum of the mass of test item in the dislodgeable dose and stratum corneum.

6 Results and Discussion

A total of 9 samples of human skin, obtained from 6 different donors, were dosed topically with [^{14}C]-HBCD in an acetone vehicle. Cell 2 was rejected from the mean \pm SD as it had a poor mass balance (less than 90%). Cell 9 was rejected as it had just failed the tritiated water barrier integrity assessment (2.6×10^{-3} cm/h, rejection criterion $> 2.5 \times 10^{-3}$ cm/h) and it had a high value in the exposed skin (7.57%, which was outside the mean \pm 2SD of the other samples of exposed skin). Therefore, the following results are based on 7 samples of skin obtained from 5 different donors.

The distribution of radioactivity at 24 h post dose is shown in Table 1. At 8 h post dose, 34.62% of the applied dose was washed off (6.98%, 0.19% and 27.45% were recovered in the skin wash 8 h, tip and tissue swab 8 h, respectively). At 24 h post dose, the mean mass balance was 96.67% of the applied dose. The tissue swab 24 h and cell wash contained 5.70% and 23.06% of the applied dose, respectively. Therefore, at 24 h post dose, the dislodgeable dose was 63.37% of the applied dose. The mean total unabsorbed dose was 95.31% of the applied dose. This consisted of the dislodgeable dose, unexposed skin (0.45%) and the radioactivity associated with the stratum corneum (31.49%). The stratum corneum acted as a good barrier to the test item as the bulk of the radioactivity (25.70%) was recovered in the outermost 5 tape strips (tape strips 1-5). Considerably less radioactivity was recovered with each of the subsequent 3 groups of tape strips (3.12%, 1.54% and 1.13% in tape strips 6-10, 11-15 and 16-20, respectively). This indicated that the HBCD was on the skin surface and would be anticipated to be in the stratum corneum which would be sloughed off of the skin. The absorbed dose (0.01%) was the sum of the receptor fluid (0.01%) and the receptor rinse ($<0.01\%$). Dermal delivery (1.35%) was the sum of the absorbed dose and the exposed skin (1.34%). The absorption profile is provided in Table 2 and graphically in Figure 1.

The distribution, by mass, of [^{14}C]-HBCD at 24 h post dose is shown in Table 3. The mass balance, dislodgeable, unabsorbed, dermal delivery and absorbed doses were 916.58, 600.90, 903.76, 12.82 and 0.06 $\mu\text{g.equiv./cm}^2$, respectively. The absorption profile is provided in Table 4 and graphically in Figure 2. There were two steady state fluxes observed in this study. The first was attained from 0-6 h post dose. The mean steady state flux rate over this period was calculated to be 1.36 ng equiv./ cm^2/h . The second was attained from 6 h to 24 h post dose. The mean steady state flux rate over this period was calculated to be 2.64 ng equiv./ cm^2/h .

The individual flux rates are provided in Table 5 and graphically in Figure 3.

7 Conclusion

In conclusion, following topical application of [¹⁴C]-HBCD to human split-thickness skin *in vitro*, the absorbed dose and dermal delivery were 0.01% (0.06 µg.equiv./cm²) and 1.35% (12.82 µg.equiv./cm²) of the applied dose, respectively. At 8 h post dose, 34.62% of the applied dose was removed from the skin by washing and drying. At 24 h a further 28.76% was recovered in the 24 h skin drying and cell wash. Therefore, the dislodgeable dose was 63.37% of the applied dose. The stratum corneum contained a further 31.49% of the applied dose. The bulk of this (25.70%) was recovered in the first 5 tape strips. Since the bulk of the stratum corneum associated material was found in the first 5 tapes strips, this indicated that the [¹⁴C]-HBCD was on the surface of the skin and that the stratum corneum was an efficient barrier to [¹⁴C]-HBCD penetration.

8 Tables

Table 1 Distribution of Radioactivity (% Applied Dose) at 24 h Post Dose Following Topical Application of HBCD to Human Split-Thickness Skin

	Cell Number and Donor Number														Mean	SD
	Cell 2 0105	Cell 4 0082	Cell 5 0067	Cell 8 0086	Cell 9 0105	Cell 11 0082	Cell 12 0067	Cell 13 0070	Cell 14 0109							
Skin Wash 8 h	13.96	4.50	8.17	9.33	6.08	10.67	3.41	8.15	4.66	6.98	2.77					
Tissue Swab 8 h	16.36	48.17	13.24	27.86	22.28	25.49	16.07	40.84	20.48	27.45	12.86					
Pipette Tips 8 h	0.21	0.01	0.24	0.14	0.14	0.26	0.08	0.20	0.37	0.19	0.12					
Tissue Swab 24 h	1.17	2.05	3.68	2.99	1.95	7.85	9.51	7.88	5.93	5.70	2.85					
Cell Wash	26.39	10.82	38.18	31.96	26.17	31.31	12.06	14.12	22.95	23.06	11.00					
Dislodgeable Dose	58.09	65.55	63.51	72.28	56.62	75.57	41.13	71.19	54.39	63.37	12.04					
Stratum corneum 1-5	18.09	33.40	23.48	20.83	25.67	15.07	42.06	18.58	26.49	25.70	9.31					
Stratum corneum 6-10	2.65	1.60	1.71	3.04	2.04	1.77	5.43	3.54	4.74	3.12	1.54					
Stratum corneum 11-15	1.53	1.05	0.91	1.38	4.56	1.21	1.16	1.16	3.90	1.54	1.05					
Stratum corneum 16-20	0.97	0.59	0.83	1.59	1.47	0.72	1.13	1.09	1.94	1.13	0.49					
Stratum Corneum	23.25	36.65	26.92	26.85	33.74	18.78	49.78	24.38	37.08	31.49	10.39					
Unexposed Skin	0.14	0.10	0.51	0.64	0.57	0.73	0.28	0.59	0.31	0.45	0.23					
Total Unabsorbed	81.47	102.29	90.94	99.77	90.92	95.08	91.19	96.16	91.77	95.31	4.43					
Exposed Skin	4.11	0.38	1.49	1.54	7.57	1.53	1.37	1.96	1.14	1.34	0.49					
Receptor Fluid	0.01	*0.00	*0.00	0.01	0.01	0.00	0.01	0.01	0.01	*0.01	0.00					
Receptor Rinse	*0.00	*0.00	*0.00	0.00	*0.00	0.00	*0.00	0.00	0.00	*0.00	0.00					
Total Absorbed	0.01	0.00	0.00	0.01	0.01	0.00	0.01	0.01	0.01	0.01	0.00					
Dermal Delivery	4.12	0.38	1.50	1.55	7.58	1.54	1.37	1.96	1.16	1.35	0.49					
Mass Balance	85.59	102.67	92.43	101.32	98.50	96.62	92.56	98.12	92.93	96.67	4.25					

* Results calculated from data less than 30 d.p.m. above background
 ° Mean includes results calculated from data less than 30 d.p.m. above background

Cell 2 was rejected from Mean and SD due to mass balance being less than 90%
 Cell 9 was rejected as it had high tritiated water barrier integrity value and a high exposed skin value

Table 2 Cumulative Absorption (% Applied Dose) of [¹⁴C]-HBCD into Receptor Fluid Following Topical Application of HBCD to Human Split-Thickness Skin

Time (h)	Cell Number and Donor Number														Mean	SD	
	Cell 2 0105	Cell 4 0082	Cell 5 0067	Cell 8 0086	Cell 9 0105	Cell 11 0082	Cell 12 0067	Cell 13 0070	Cell 14 0109								
0	*0.000	*0.000	*0.000	*0.000	*0.000	*0.000	*0.000	*0.000	*0.000	*0.000	*0.000	*0.000	*0.000	*0.000	*0.000	*0.000	0.000
1	*0.000	*0.000	*0.000	*0.000	*0.000	*0.000	*0.000	*0.000	*0.000	*0.000	*0.000	*0.000	*0.000	*0.000	*0.000	*0.000	0.000
2	*0.000	*0.000	*0.000	*0.000	*0.001	*0.000	*0.000	*0.000	*0.000	*0.000	*0.000	*0.000	*0.000	*0.001	*0.000	*0.000	0.000
3	*0.000	*0.000	*0.000	*0.000	*0.001	*0.000	*0.000	*0.000	*0.000	*0.000	*0.000	*0.000	*0.000	*0.001	*0.000	*0.000	0.000
4	*0.001	*0.000	*0.000	*0.000	*0.001	*0.000	*0.000	*0.000	*0.000	*0.000	*0.000	*0.000	*0.000	*0.001	*0.000	*0.000	0.000
5	*0.001	*0.001	*0.000	*0.001	*0.001	*0.000	*0.001	*0.001	*0.001	*0.000	*0.001	*0.001	*0.001	*0.001	*0.001	*0.000	0.000
6	*0.001	*0.001	*0.001	*0.001	*0.001	*0.001	*0.001	*0.001	*0.001	*0.001	*0.001	*0.001	*0.001	*0.001	*0.001	*0.000	0.000
7	*0.001	*0.001	*0.001	*0.001	*0.001	*0.001	*0.001	*0.001	*0.001	*0.001	*0.001	*0.001	*0.001	*0.001	*0.001	*0.000	0.000
8	*0.002	*0.001	*0.001	*0.001	*0.001	*0.001	*0.001	*0.001	*0.001	*0.001	*0.001	*0.001	*0.001	*0.001	*0.001	*0.001	0.001
10	*0.002	*0.001	*0.002	*0.002	*0.002	*0.002	*0.002	*0.002	*0.002	*0.001	*0.001	*0.002	*0.002	*0.002	*0.002	*0.001	0.001
12	*0.002	*0.002	*0.002	*0.003	*0.003	*0.003	*0.003	*0.003	*0.003	*0.001	*0.001	*0.002	*0.003	*0.004	*0.002	*0.001	0.001
14	0.003	*0.002	*0.002	*0.002	0.004	*0.002	*0.006	0.005	0.004	*0.001	*0.001	*0.002	0.003	0.004	*0.003	*0.001	0.001
16	*0.003	*0.002	*0.002	*0.002	*0.002	*0.002	*0.006	0.005	0.005	*0.002	*0.002	0.003	0.004	0.005	*0.003	*0.002	0.002
18	0.004	*0.002	*0.002	*0.002	*0.005	*0.002	0.007	*0.005	0.006	*0.002	*0.002	0.003	*0.005	0.006	*0.004	*0.002	0.002
20	0.004	*0.002	0.003	0.003	0.006	0.003	0.009	0.006	0.006	*0.002	*0.002	*0.004	*0.005	0.007	*0.005	*0.003	0.003
22	0.005	*0.003	0.004	0.011	0.006	0.006	0.011	0.006	0.006	*0.002	*0.002	0.005	*0.006	0.008	*0.005	*0.003	0.003
24	0.006	*0.003	*0.004	0.012	0.007	0.003	0.012	0.007	0.007	0.003	0.003	0.005	0.006	0.008	*0.006	*0.004	0.004

* Results calculated from data less than 30 d.p.m. above background
 ° Mean includes results calculated from data less than 30 d.p.m. above background

Cell 2 was rejected from Mean and SD due to mass balance being less than 90%
 Cell 9 was rejected as it had high tritiated water barrier integrity value and a high exposed skin value

Table 3 *Distribution of [¹⁴C]-HBCD (µg equiv./cm²) at 24 h Post Dose Following Topical Application of HBCD to Human Split-Thickness Skin*

	Cell Number and Donor Number														Mean	SD
	Cell 2 0105	Cell 4 0082	Cell 5 0067	Cell 8 0086	Cell 9 0105	Cell 11 0082	Cell 12 0067	Cell 13 0070	Cell 14 0109							
Dislodgeable Dose	550.81	621.56	602.19	685.42	536.82	716.53	389.94	675.01	515.66	600.90	114.17					
Stratum Corneum	220.42	347.47	255.22	254.56	319.87	178.04	472.05	231.14	351.56	298.58	98.47					
Total Unabsorbed	772.52	969.93	862.27	946.06	862.09	901.52	864.67	911.74	870.13	903.76	42.07					
Total Absorbed	0.06	0.03	0.04	0.12	0.07	0.03	0.05	0.06	0.12	0.06	0.04					
Dermal Delivery	39.02	3.64	14.20	14.70	71.87	14.57	13.01	18.62	10.98	12.82	4.65					
Total Recovery	811.54	973.57	876.46	960.77	933.96	916.09	877.68	930.37	881.11	916.58	40.36					

Cell 2 was rejected from Mean and SD due to mass balance being less than 90%
 Cell 9 was rejected as it had high tritiated water barrier integrity value and a high exposed skin value

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Table 4 Cumulative Absorption (ng equiv./cm²) of [¹⁴C]-HBCD into Receptor Fluid Following Topical Application of HBCD to Human Split-Thickness Skin

Time (h)	Cell Number and Donor Number														Mean	SD
	Cell 2 0105	Cell 4 0082	Cell 5 0067	Cell 8 0086	Cell 9 0105	Cell 11 0082	Cell 12 0067	Cell 13 0070	Cell 14 0109							
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	1.12	1.15	1.83	1.41	2.31	1.14	1.41	2.31	1.14	0.69	1.57	1.89	1.38	0.43		
2	2.71	2.26	3.07	2.24	5.11	2.28	2.24	5.11	2.28	3.02	3.69	5.00	3.08	1.01		
3	3.22	3.28	3.07	3.58	7.47	3.27	3.58	7.47	3.27	4.02	4.68	8.02	4.27	1.74		
4	5.74	4.50	3.38	4.61	10.00	3.72	4.61	10.00	3.72	4.52	6.39	10.59	5.39	2.48		
5	7.17	5.01	4.42	6.47	11.35	4.21	6.47	11.35	4.21	5.42	8.14	12.48	6.59	2.93		
6	8.62	5.97	6.16	8.08	12.62	5.44	8.08	12.62	5.44	6.45	10.30	14.63	8.15	3.31		
7	11.02	7.37	8.07	10.80	14.88	6.44	10.80	14.88	6.44	8.06	12.28	18.42	10.20	4.15		
8	14.37	8.42	9.21	13.14	16.98	8.09	13.14	16.98	8.09	9.31	17.68	21.02	12.41	5.11		
10	18.57	11.13	14.93	22.88	20.78	8.99	22.88	20.78	8.99	15.15	22.08	30.85	18.00	7.65		
12	21.05	15.04	19.16	31.48	24.51	9.55	31.48	24.51	9.55	18.73	27.02	37.28	22.61	9.73		
14	26.29	17.85	20.93	41.24	37.37	11.86	41.24	37.37	11.86	22.62	31.76	42.27	26.94	11.74		
16	30.65	19.34	21.88	55.52	44.03	14.75	55.52	44.03	14.75	28.43	42.58	47.76	32.89	15.71		
18	36.20	20.84	23.05	70.93	47.62	15.78	70.93	47.62	15.78	33.17	45.72	61.04	38.65	21.24		
20	41.56	22.42	29.50	88.14	52.59	17.87	88.14	52.59	17.87	36.26	47.98	66.33	44.07	25.43		
22	48.50	24.40	35.77	103.17	58.99	19.30	103.17	58.99	19.30	43.11	52.40	73.15	50.18	29.49		
24	53.96	26.94	36.78	117.68	64.58	24.11	117.68	64.58	24.11	48.18	57.16	79.02	55.70	33.24		

Cell 2 was rejected from Mean and SD due to mass balance being less than 90%
Cell 9 was rejected as it had high tritiated water barrier integrity value and a high exposed skin value

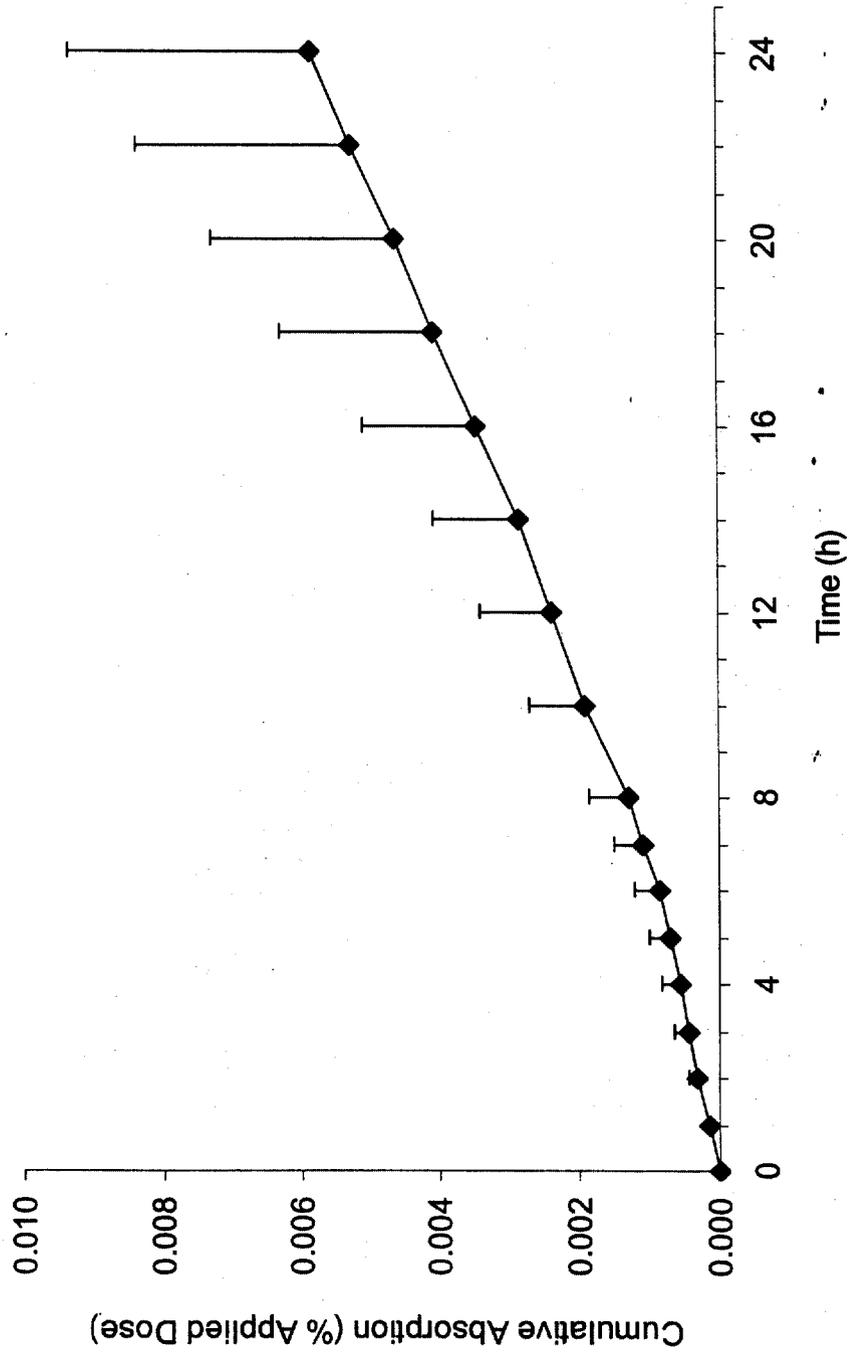
Table 5 Individual Flux Rates (ng equiv./cm²/h) of [¹⁴C]-HBCD into Receptor Fluid Following Topical Application of HBCD to Human Split-Thickness Skin

Time (h)	Cell Number and Donor Number														Mean	SD
	Cell 2 0105	Cell 4 0082	Cell 5 0067	Cell 8 0086	Cell 9 0105	Cell 11 0082	Cell 12 0067	Cell 13 0070	Cell 14 0109							
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	1.12	1.15	1.83	1.41	2.31	1.14	1.14	0.69	1.57	1.89	1.38	1.38	1.89	1.38	0.43	0.43
2	1.59	1.10	1.24	0.83	2.81	1.14	1.14	2.33	2.12	3.11	1.70	1.70	3.11	1.70	0.83	0.83
3	0.51	1.02	0.00	1.34	2.35	0.99	0.99	1.00	0.99	3.03	1.19	1.19	3.03	1.19	0.91	0.91
4	2.52	1.22	0.31	1.03	2.53	0.45	0.45	0.50	1.71	2.57	1.11	1.11	2.57	1.11	0.81	0.81
5	1.44	0.51	1.04	1.86	1.34	0.49	0.49	0.90	1.75	1.89	1.21	1.21	1.89	1.21	0.62	0.62
6	1.44	0.96	1.74	1.61	1.27	1.24	1.24	1.03	2.16	2.16	1.56	1.56	2.16	1.56	0.50	0.50
7	2.40	1.40	1.91	2.72	2.26	0.99	0.99	1.61	1.98	3.78	2.06	2.06	3.78	2.06	0.93	0.93
8	3.35	1.05	1.14	2.33	2.10	1.65	1.65	1.25	5.40	2.60	2.20	2.20	2.60	2.20	1.53	1.53
10	2.10	1.36	2.86	4.87	1.90	0.45	0.45	2.92	2.20	4.91	2.80	2.80	4.91	2.80	1.67	1.67
12	1.24	1.95	2.11	4.30	1.87	0.28	0.28	1.79	2.47	3.21	2.30	2.30	3.21	2.30	1.25	1.25
14	2.62	1.41	0.89	4.88	6.43	1.16	1.16	1.95	2.37	2.50	2.16	2.16	2.50	2.16	1.34	1.34
16	2.18	0.74	0.47	7.14	3.33	1.44	1.44	2.90	5.41	2.74	2.98	2.98	2.74	2.98	2.48	2.48
18	2.77	0.75	0.59	7.71	1.80	0.51	0.51	2.37	1.57	6.64	2.88	2.88	6.64	2.88	3.02	3.02
20	2.68	0.79	3.22	8.60	2.48	1.04	1.04	1.54	1.13	2.64	2.71	2.71	2.64	2.71	2.75	2.75
22	3.47	0.99	3.14	7.52	3.20	0.71	0.71	3.42	2.21	3.41	3.06	3.06	3.41	3.06	2.26	2.26
24	2.73	1.27	0.50	7.26	2.79	2.41	2.41	2.54	2.38	2.94	2.76	2.76	2.94	2.76	2.16	2.16

Cell 2 was rejected from Mean and SD due to mass balance being less than 90%
Cell 9 was rejected as it had high tritiated water barrier integrity value and a high exposed skin value

9 **Figures**

Figure 1 Absorption Profile of [¹⁴C]-HBCD (% Applied Dose) in Receptor Fluid Following Topical Application of HBCD to Human Split-Thickness Skin (Mean + SD, n = 7)



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Figure 2 Absorption Profile of [¹⁴C]-HBCD (ng equiv./cm²) in Receptor Fluid Following Topical Application of HBCD to Human Split-Thickness Skin (Mean ± SD, n = 7)

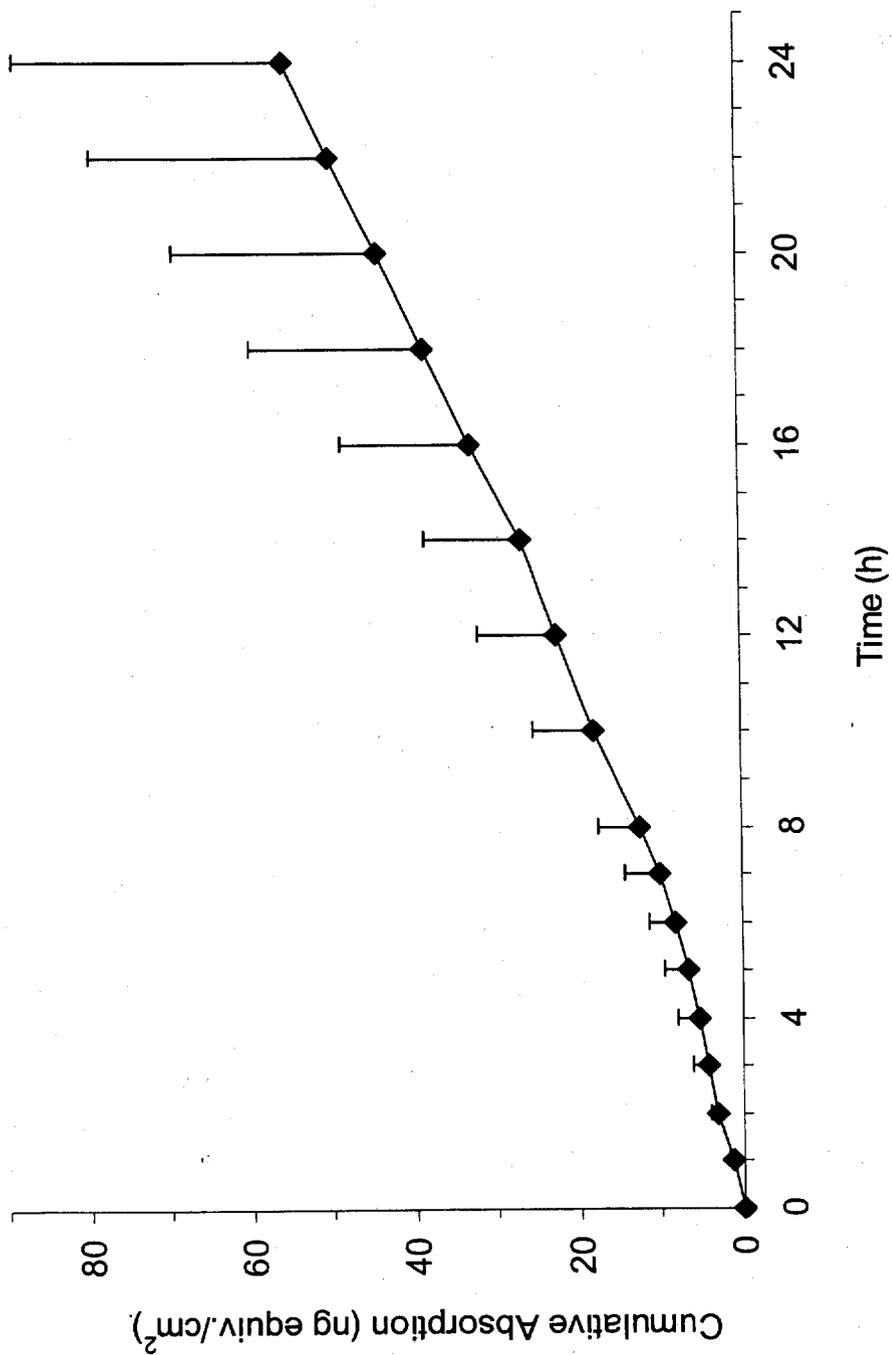
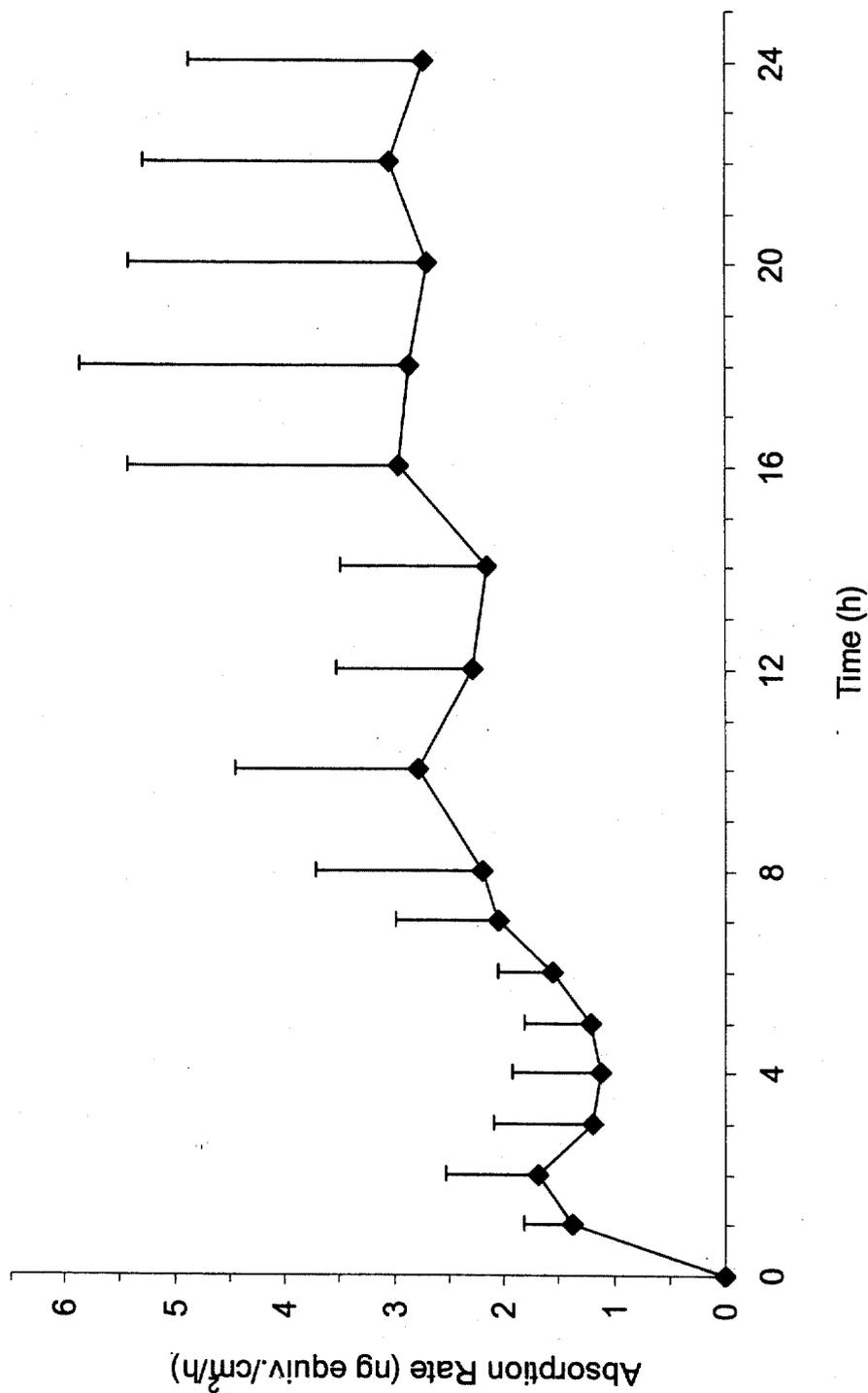


Figure 3 Flux Rate Profile of [¹⁴C]-HBCD (ng equiv./cm²/h) in Receptor Fluid Following Topical Application of HBCD to Human Split-Thickness Skin (Mean ± SD, n = 7)



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10 Appendices

Appendix 1 Certificate of Analysis for [¹⁴C]-Hexabromocyclododecane

EaglePicher

13605 W. 96th Terrace
Lenexa, Kansas 66215-1297
Phone: (800) 233-6643
(913) 541-0525
Fax: (913) 888-3582

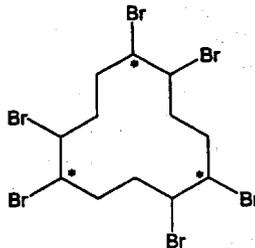
ANALYTICAL DATA SUMMARY PROJECT NO. 6622.00

COMPOUND: [¹⁴C]Hexabromocyclododecane

MOLECULAR FORMULA: C₁₂H₁₈Br₆

MOLECULAR WEIGHT: 641.73

STRUCTURE:



LOT NUMBER: EPPS-03-021-57-08

DATE OF ANALYSIS: January 14, 2004

RADIOCHEMICAL PURITY: 7.74% Alpha by HPLC
7.84% Beta by HPLC
81.5% Gamma by HPLC

SPECIFIC ACTIVITY: 24.5 mCi/mmol (906.5 MBq/mmol) by Gravimetric determination

CONCENTRATION: 1.0 mCi/mL (37.0 MBq/mL) in acetone

PACKAGING: 1 x 1 mCi (1 x 37.0 MBq) in flame-sealed ampule

PHYSICAL DESCRIPTION: Clear solution

RECOMMENDED STORAGE AND HANDLING CONDITIONS: Store under inert atmosphere at -20 °C protected from light.

Appendix 1 (Continued)

Analytical Data Summary
Project No.: 6622.00
Date: 1/15/2004
Page: 2

CHROMATOGRAPHIC DATA:

High Performance Liquid Chromatography: (Figures 1-4)

Instrument: Varian Prostar LC-6L

Injector: Prostar 410 autosampler, 20 µl injections

Sample Solvent: 100% Acetonitrile

Column: Waters NovaPak, 3.9 x 150 mm

Mobile Phase: Solvent A: Water

Solvent B: Acetonitrile

<u>Time</u>	<u>A</u>	<u>B</u>
0	25	75
12	25	75
17	0	100
25	0	100

Flow Rate: 0.8 mL/min

UV Detector: Prostar 310

Wavelength: 220 nm

Integrator: Varian Star Workstation v. 5.51

Radiodetector: Packard Flow Scintillation Analyzer 500TR

Splitter: 50%

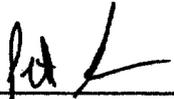
Cocktail: Fisher Scintisafe Plus 50%

Total Flow Rate: 2.0 mL/min

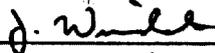
Flow Cell Volume: 100 µl

Background: 42 cpm

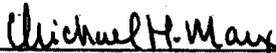
APPROVAL:

 1/15/04

Analyst

 01-15-04

Approved

 1-15-04

Approved

**Appendix 2 Chemical Characterization Report for
Hexabromocyclododecane (HBCD)**

**ALBEMARLE CORPORATION
RESEARCH AND DEVELOPMENT DEPARTMENT**

**FINAL REPORT ON THE CHEMICAL CHARACTERIZATION (IDENTITY AND
HOMOGENEITY) OF HEXABROMOCYCLODODECANE (HBCD), WIL TEST
SUBSTANCE #6541-1103**

- I. Reference Protocol Number: HBCD-11-18-2003
- II. Sponsor: American Chemistry Council
Brominated Flame Retardant Industry Panel
1300 Wilson Boulevard
Arlington, Virginia 22209
Study Monitor: Francis Maher
- III. Analytical Testing Facilities: Albemarle Corporation
Process Development Center
Gulf States Road
Baton Rouge, LA 70805
Study Chemist: Paul F. Ranken, Ph. D.
- IV. Dates of Performance: Study Initiation Date: November 25, 2003
Study Completion Date: February 3, 2004
- V. Test Article: Hexabromocyclododecane (WIL Test
Substance #6541-1103). The test article was a
composite of commercial products from
Albemarle Corporation, Great Lakes
Chemical Corporation and Eurobrom B.V.
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 and to demonstrate
the homogeneity of the test article. The
identity of one sample of the test article, taken
from the middle center of the bulk container
and designated "Characterization Sample
#MC1-6541-1103", was confirmed by Fourier

CERTIFIED TRUE COPY

Paul F. Ranken
Feb. 3, 2004

Appendix 2 (Continued)

Transform Infrared Spectroscopy using SOP No. ARS-284-R4. In this procedure, the sample infrared spectrum was compared to a standard reference spectrum of HBCD. The HBCD infrared spectrum published in the Aldrich Library of FT-IR Spectra, Volume 1, page 107A, was used as the reference spectrum. The homogeneity of the test article was demonstrated by determining the composition (area % of the three HBCD diastereomers) of six separate test article samples which were taken from the top, middle and bottom right side and from the top, middle and bottom left side of the bulk container. The composition of the six samples was determined by High Performance Liquid Chromatography (HPLC) using SOP No. ARS-432-R1. Each of the six samples was analyzed in triplicate and the composition of each sample expressed as the average area % of each of the three diastereomers. Chain of Custody and sample handling were conducted according to established standard operating procedures.

VIII. Results:

The attached Conclusions and test article analytical data contain all of the test results 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 composition (<5 % difference of the HBCD diastereomers area % for each sample compared to the average HBCD diastereomer area % of the six samples). Results of the eighteen analyses are given in Table 1.

IX. Deviations:

Two deviations occurred from SOP No. ARS-432-R1. The SOP required that each sample be analyzed in duplicate. Each sample was analyzed in triplicate, as specified in the analytical protocol. In addition, the SOP

Appendix 2 (Continued)

required that a representative sample be labeled as a "lab/reference standard" and be analyzed. Pass or failure of subsequent samples in the study was to be determined by comparison to this original sample. The representative sample was not designated nor analyzed and no comparisons to subsequent samples were made. The pass/fail criterion for the test article in this study was outlined in the analytical protocol and was not based on a "lab/reference standard". The pass/fail criterion in SOP No. ARS-432-R1 was not applicable to this study.

The sponsor study monitor (Wendy K. Sherman), listed on protocol, was replaced by Francis Maher, prior to completion of the study, on or about December 1, 2003. The protocol was not amended at the time of the change.

These deviations did not affect the quality or integrity of the data generated.

X. Regulatory Requirements:

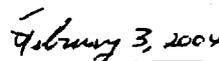
The study conformed to the requirements of EPA TSCA (40 CFR Part 792) Good Laboratory Practice Regulations and the OECD [C (97) 186/Final] Good Laboratory Practice Regulations.

XI. 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, Ph. D.
STUDY CHEMIST



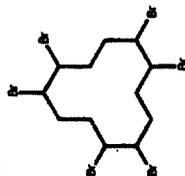
DATE

Appendix 2 (Continued)

CONCLUSIONS AND TEST ARTICLE ANALYTICAL DATA (INITIAL ANALYSES)

CHEMICAL NAME: Hexabromocyclodecane
TEST ARTICLE: HBCD Composite, WIL Test Substance #6541-1103
C.A.S. No.: 3194-55-6

MOLECULAR FORMULA: C₁₂H₁₈Br₆
PHYSICAL FORM: White Powder
CHEMICAL STRUCTURE:



ANALYSIS	RESULTS				ANALYSIS DATES	ANALYST
	Alpha Average	% Difference from Mean	Beta Average	% Difference from Mean		
FT-IR	The sample FT-IR spectrum matched that of the Aldrich reference spectrum. All spectra are on file with the original data.				12/10/03	W. T. Cobb
HPLC	Area %				12/15/03	J. S. Arroyave
				Gamma Average	% Difference from Mean	
Top Right	10.46	1.75	8.98	80.56	0.55	
Middle Right	10.58	2.92	8.89	80.53	0.59	
Bottom Right	10.74	4.47	8.95	80.31	0.86	
Top Left	9.81	4.57	8.41	81.78	0.95	
Middle Left	9.99	2.82	8.42	81.59	0.72	
Bottom Left	10.08	1.95	8.65	81.27	0.32	
Mean	10.28		8.72	81.01		

CONCLUSION: Based on these analytical data, the test article was identified as HBCD. The test article was homogeneous.

Appendix 2 (Continued)

Table 1. Test Article HPLC Analytical Data

Sample	Alpha Area %	Beta Area %	Gamma Area %
Top Right	10.4	8.88	80.72
Top Right	10.64	9.13	80.23
Top Right	10.34	8.93	80.73
Average	10.46	8.98	80.56
Middle Right	10.61	9.00	80.39
Middle Right	10.65	8.91	80.44
Middle Right	10.49	8.76	80.75
Average	10.58	8.89	80.53
Bottom Right	10.97	9.18	79.85
Bottom Right	10.50	8.74	80.75
Bottom Right	10.74	8.92	80.34
Average	10.74	8.95	80.31
Top Left	9.85	8.45	81.70
Top Left	9.70	8.33	81.97
Top Left	9.87	8.45	81.68
Average	9.81	8.41	81.78
Middle Left	10.00	8.43	81.56
Middle Left	9.90	8.25	81.85
Middle Left	10.08	8.57	81.35
Average	9.99	8.42	81.59
Bottom Left	10.02	8.66	81.32
Bottom Left	9.97	8.50	81.53
Bottom Left	10.24	8.80	80.96
Average	10.08	8.65	81.27
Mean (n = 18)	10.28	8.72	81.01

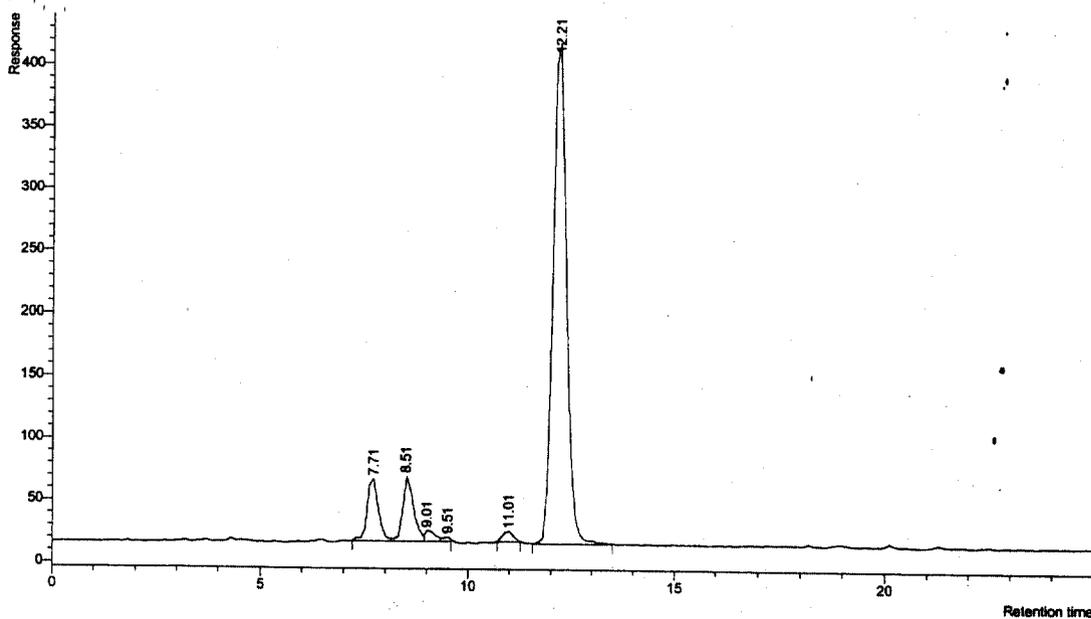
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Appendix 3 Radiochemical Purity of [¹⁴C]-HBCD

Hot/Cold mix (6,1)
Acquired 05 January 2005 16:01:43

774740,instrument152,74005Jan051146,6,1



74005Jan051146, 6.1, radiotrace 152, hot/cold mix

Peak	Retention Time (min)	Peak Height (mV)	Peak Area (mV)	Peak Name	Area (%)
1	7.712	50.3794	945.294	HBCD (alpha)	8.24
2	8.512	52.2109	933.047	HBCD (beta)	8.14
3	9.013	8.96558	143.438		1.25
4	9.509	3.56897	45.0287		0.39
5	11.011	8.35618	153.309		1.34
6	12.213	402.574	9246.45	HBCD (gamma)	80.6

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Appendix 4 Human Skin Donor Details

Inveresk Donor No.	Sex/Age	Site
0067	F/68Y	Breast
0109	F/51Y	Breast
0110	F/38Y	Breast
0070	F/32Y	Breast
0105	F/19Y	Breast
0082	F/46Y	Breast
0086	F/33Y	Breast

Appendix 5 Thickness of Full and Split-Thickness Skin Membranes

Inveresk Donor No.	Membrane Thickness (μm)	
	Full-Thickness Skin	Split -Thickness Skin
0067	1790	380
0109	1440	390
0110	1270	400
0070	1400	390
0105	1160	380
0082	1000	390
0086	1240	350



Appendix 6 **Flow Rate and Temperature Calibration**

	Cell Numbers	Mean	SD	CV (%)
Flow Rate (mL/h)	1-14	1.55	0.02	1.52
Temperature (°C)	1-14	32.4	0.2	0.60

Appendix 7 Cross Reference of Skin Sample Number with Skin Donor and Tritiated Water Permeability Coefficient

Cell No	Inveresk Donor No.	K_p ($\times 10^{-3}$ cm/h)	Use/Reject
1	0086	3.3	Reject
2	0105	2.1	Use
3	0110	5.0	Reject
4	0082	1.4	Use
5	0067	1.7	Use
6	0070	16.7	Reject
7	0109	8.0	Reject
8	0086	2.1	Use
9*	0105	2.6*	Use*
10	0110	3.4	Reject
11	0082	1.6	Use
12	0067	1.4	Use
13	0070	1.7	Use
14	0109	2.4	Use

Rejection criterion, reject if $k_p > 2.5 \times 10^{-3}$ cm/h

* Cell 9 was used as this was on the borderline. There were enough samples for the study without this sample (protocol requirement minimum 8 skin samples), however, this was dosed as a precaution

CONTAIN NO CBI