FINAL REPORT

Study Title

DETERMINATION OF THE CONTENT OF TETRABROMOBISPHENOL A IN THE TEST SUBSTANCE TETRABROMOBISPHENOL A (INCLUDING HOMOGENEITY)

Study Director

Ir. M.J.C. Brekelmans

Study Initiated on

02 May 2000

Study Completed on

02 May 2000

Performing Laboratory

NOTOX B.V.
Hambakenwetering 7
5231 DD 's-Hertogenbosch

Sponsor

Bromine Science & Environmental Forum (BSEF)
BSEF Secretariat co/Burson-Marsteller
118, Avenue de Cortenbergh
1000 Brussels

NOTOX Project 292815
NOTOX Substance 100422

STATEMENT OF GLP COMPLIANCE

NOTOX B.V., 's-Hertogenbosch, The Netherlands

The study described in this report has been correctly reported and was conducted in compliance with the most recent edition of:

The OECD Principles of Good Laboratory Practice

which are essentially in conformity with:

The United States Food and Drug Administration. Title 21 Code of Federal Regulations Part 58.

The United States Environmental Protection Agency (FIFRA). Title 40 Code of Federal Regulations Part 160.

The United States Environmental Protection Agency (TSCA). Title 40 Code of Federal Regulations Part 792.

Study Director

Ir. M.J.C. Brekelmans

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Date: July 07. 2000

Management

Dr. Ir. H. Willems
Head of Chemistry

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Date: July 07, 2000

QUALITY ASSURANCE STATEMENT

NOTOX B.V., 's-Hertogenbosch, The Netherlands

This report was audited by the NOTOX Quality Assurance Unit to ensure that the methods and results accurately reflect the raw data.

The dates of Quality Assurance inspections and audits are given below.
During the on-site inspections procedures applicable to this type of study were inspected.

DATES OF QAU INSPECTIONS/AUDITS	REPORTING DATES
on-site inspection (s)	
18 April 2000	18 April 2000
protocol inspection (s)	
18 April 2000	18 April 2000
report audit (s)	
18 May 2000	18 May 2000

Head of Quality Assurance
C.J. Mitchell B.Sc.

Date: 7-7-2000

SUMMARY

Determination of the % purity of the composite Tetrabromobisphenol A sample used as the test substance

A High Performance Liquid Chromatographic (HPLC) method with spectrophotometric detection was used. Measurements were performed at both 230 nm and 290 nm.

230 nm:
The content of Tetrabromobisphenol A in the test substance Tetrabromobisphenol A was determined to be 975 ± 13 g/kg (i.e. 97.5 ± 1.3%).

290 nm:
The content of Tetrabromobisphenol A in the test substance Tetrabromobisphenol A was determined to be 986 ± 35 g/kg (i.e. 98.6 ± 3.5%).

The peak areas measured at 230 nm were by a factor 4 higher than the peak areas measured at 290 nm. The measurements at 230 nm were therefore considered the most reliable. The results of the 230 nm-measurements were used for the certificate of analysis. The certificate of analysis was added to the report as an appendix.

Determination of the homogeneity of mixing of the composite Tetrabromobisphenol A sample used as test substance

The contents of Tetrabromobisphenol A in the test substance at 90%, 50% and 10% height of the test substance container were in agreement. Therefore it was concluded that the composite sample was homogeneous.

PREFACE

Sponsor	Bromine Science & Environmental Forum (BSEF) BSEF Secretariat co/Burson-Marsteller 118, Avenue de Cortenbergh 1000 Brussels
Study Monitor	Dead Sea Bromine Group Health, Safety and Environment P.O. Box 180 Beer-Sheva 84101
Testing Facility	NOTOX B.V. Hambakenwetering 7 5231 DD 's-Hertogenbosch The Netherlands
Study Director	Ir. M.J.C. Brekelmans
Study plan	Start:02 May 2000 Completed: 02 May 2000

TEST SUBSTANCE (NOTOX SUBSTANCE 100422)

Identification	Tetrabromobisphenol A
Description	White crystalline powder
Batch	Not indicated
Purity	To be determined during this NOTOX Project
Test substance storage	At room temperature in the dark
Stability under storage conditions	Stable
Expiry date	10 April 2001 (allocated by NOTOX)

The sponsor is responsible for all test substance data unless determined by NOTOX .

Note: the test substance consisted of a composite of Tetrabromobisphenol A samples received from three manufactures. The materials identities and dates received from each of the manufactures is given below:

Manufacturer	Batch	Date received	NOTOX substance
Albemarle Corp	8721-33	10 April 2000	100737
Bromine Compounds	20001096	18 April 2000	82512/B
Great Lakes Chemical Corp.	20001096	18 April 2000	82512/B

An equal quantity of each of the manufacturer's Tetrabromobisphenol A material was placed in a 500-ml glass jar with screw cap. The jar was placed on a reciprocating shaker for two hours.

ANALYTICAL STANDARD (NOTOX SUBSTANCE AS379)

Identification	Tetrabromobisphenol A (TBBA)
Description	White crystalline powder
CAS number	79-94-7
Batch number	129h-88-01 (taken from label)
Purity	99.0%
Certified	Yes
Storage conditions	At room temperature in the dark
Date of receipt	21 April 2000
Expiry date	21 April 2001 (determined at NOTOX)
Supplier	Bromine Compounds Ltd.

The sponsor is responsible for all analytical standard data unless determined by NOTOX.

PURPOSE AND PRINCIPLE

The purpose of the study was to determine the purity of the Tetrabromobisphenol A composite used as test substance. Furthermore, homogeneity of the test substance was determined.

ARCHIVING

NOTOX B.V. will archive the following data for at least 10 years:
protocol, report, test substance reference sample and raw data. Thereafter, no data will be withdrawn without the sponsor's written consent.

REAGENTS

Acetic acid	96% reinst, Merck, Darmstadt, Germany
Acetonitrile	HPLC-grade, Labscan Limited Co., Dublin, Ireland
Milli-Q water	Tap water purified by reversed osmosis and subsequently passed over activated carbon and ion-exchange cartridges; Millipore Corp., Bedford, MA, USA

ANALYTICAL METHOD

The High Performance Liquid Chromatographic method supplied by the sponsor was used. Detection was performed at two wavelengths. The conditions used are described below.

Analytical method

Column	Symmetry C 18, 150 * 4.6 (I.D.) mm; d_p=5 µm (Waters, The Netherlands)
Mobile phase	60/40/0.1 (v/v/v) acetonitrile/Milli-Q water/acetic acid
Flow	1 ml/min
Wavelength of detection	230 nm and 290 nm
Injection volume	10 µl

Calibration solutions

Six calibration solutions in mobile phase were made up from two standard solutions of the analytical standard AS379 in acetonitrile. The calibration range was 1.01 – 10.1 mg/l.

PERFORMANCE OF THE TEST

Duplicate samples were taken from the test substance at 90% height, at 50% height and at 10% height of the container. These samples, between 29 mg and 52 mg, were accurately weighed into 25 ml volumetric flasks. The flasks were filled up to the mark with mobile phase. After being sonicated for 5 minutes, these solutions were further diluted with mobile phase to obtain concentrations within the calibration range. The solutions were injected two times into the HPLC system. The chromatograms were simultaneously recorded at 230 nm and at 290 nm.

DATA HANDLING

General

1 Mean:

$$\bar{x} = \frac{1}{n}\sum_{i=1}^{n} x_i$$

where
x_i = measured value
n = number of measurements

2. Standard deviation:

$$s_{n-1} = \sqrt{\frac{\sum_{i=1}^{n}(x_i - \bar{x})^2}{(n-1)}}$$

Calibration

1. Response: R = Peak area of Tetrabromobisphenol A [units]

2. Calibration curve: The response was correlated with the concentration of Tetrabromobisphenol A using linear regression analysis (least squares method).

$$R = a * C + b$$

R = response calibration solution [units]
C = concentration of Tetrabromobisphenol A in the calibration solution [mg/l]. The concentration prepared was corrected for the purity of the analytical standard (i.e. 99.0%)
a = slope [units*l/mg]
b = intercept [units]

A calibration curve was constructed using six concentrations. For each concentration, two responses were used. The coefficient of correlation was > 0.999.

Samples

The content of Tetrabromobisphenol A in the test substance was expressed in g/kg:.

$$C = \frac{(R-b) * V * d}{a * w} \quad [g/kg]$$

R = response sample [units]
V = volume volumetric flask [ml]
d = dilution factor
a = slope [units*l/mg]
b = intercept [units]
w = weight sample [mg]

Relative to prepared:

$$\frac{\text{Concentration analysed}}{\text{Concentration prepared}} * 100 \, [\%]$$

Relative to mean concentration:

$$\frac{\text{Concentration analysed}}{\text{Mean of concentrations analysed of 6 samples}} * 100 \, [\%]$$

RESULTS

HPLC chromatograms (recorded at 230 nm) of an analytical standard solution, a test substance solution and a blank solution (mobile phase) are shown in Figures 1 to 3. The chromatograms of the blank showed no peak with the same retention as Tetrabromobisphenol A. The chromatograms recorded at 290 nm were comparable with the chromatograms recorded at 230 nm.

The results of the analytical measurements at 230 nm and 290 nm are summarised in Table 1 and Table 2, respectively.

The calculations were performed using not-rounded concentrations. Therefore some differences might be observed when re-calculating the mean values and standard deviations as mentioned in the tables below. The concentrations analysed were given for duplicate samples. The mean of duplicate analysis was given for each sample. The maximum deviation between the responses (n=2) was <10% for each sample.

Table 1 Analytical measurements at 230 nm

Wavelength of detection	Sample position	Concentration analysed [mg/g]	Relative to mean concentration [%]
230	90% height	960	99
		958	98
	50% height	979	100
		975	100
	10% height	986	101
		990	102

Table 2 Analytical measurements at 290 nm

Wavelength of detection	Sample position	Concentration analysed [mg/g]	Relative to mean concentration [%]
290	90% height	954	97
		966	98
	50% height	1019	103
		947	96
	10% height	1001	101
		1030	104

Tables 1 and 2 show that the contents of Tetrabromobisphenol A in the test substance at 90%, 50% and 10% height of the test substance container were in agreement. Therefore it was concluded that the test substance Tetrabromobisphenol A is homogeneous.

From the concentrations analysed, the mean and the standard deviation were calculated. The results are summarised in Table 3.

Table 3 Content of Tetrabromobisphenol A in the test substance.

Wavelength of detection [nm]	Mean of the concentrations analysed [g/kg]	Standard deviation [g/kg]	Content of Tetrabromobisphenol A in the test substance [%]
230	975	13	97.5 ± 1.3
290	986	35	98.6 ± 3.5

The peak areas measured at 230 nm were by a factor 4 higher than the peak areas measured at 290 nm. The measurements at 230 nm were therefore considered the most reliable. The results of the 230 nm-measurements were used for the certificate of analysis. The certificate of analysis was added to the report as an appendix.

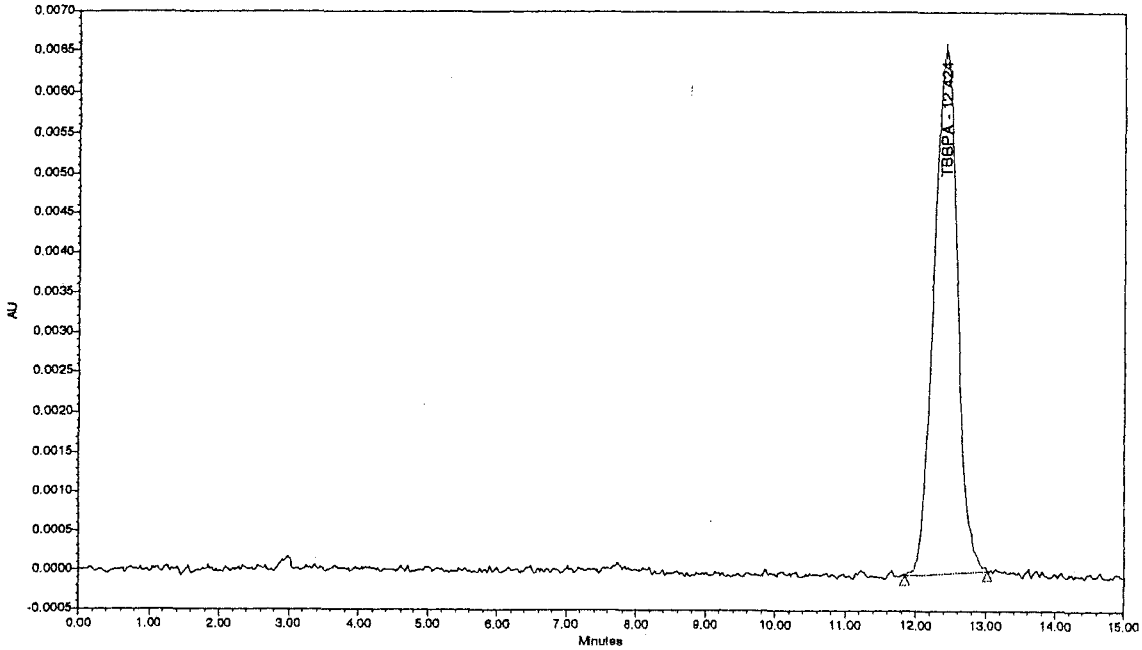

Figure 1 HPLC chromatogram of an analytical standard solution (6.06 mg/l) [res.id. 1211].

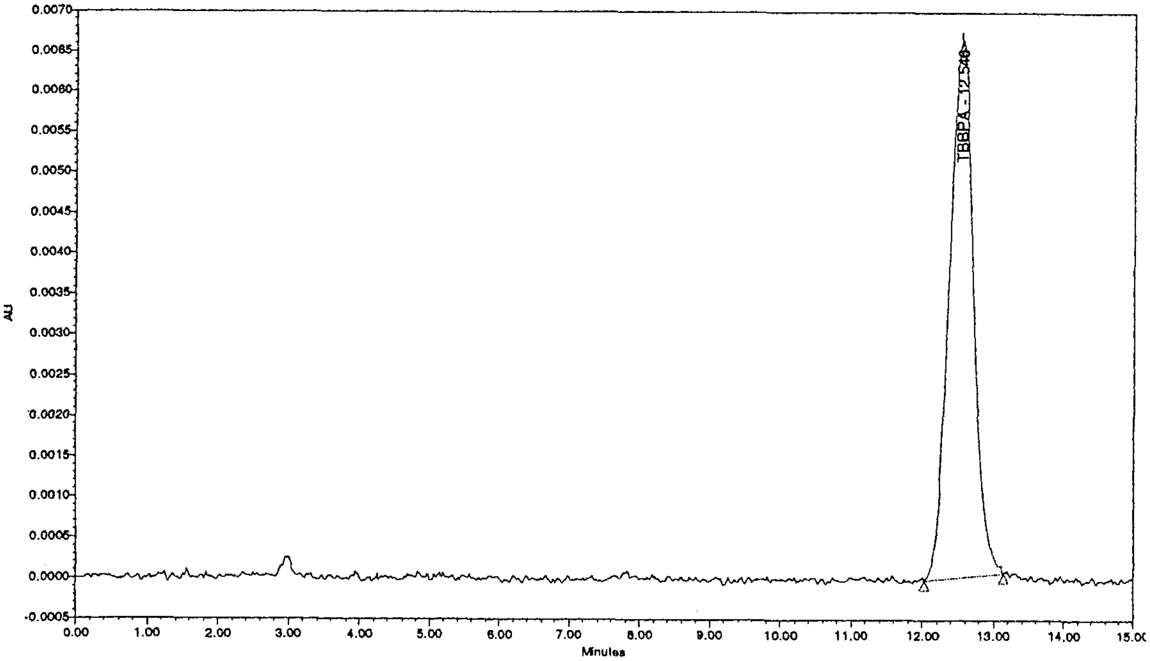

Figure 2 HPLC chromatogram of a test substance solution [res.id. 1257]. The test substance sample was taken from the middle of the container.

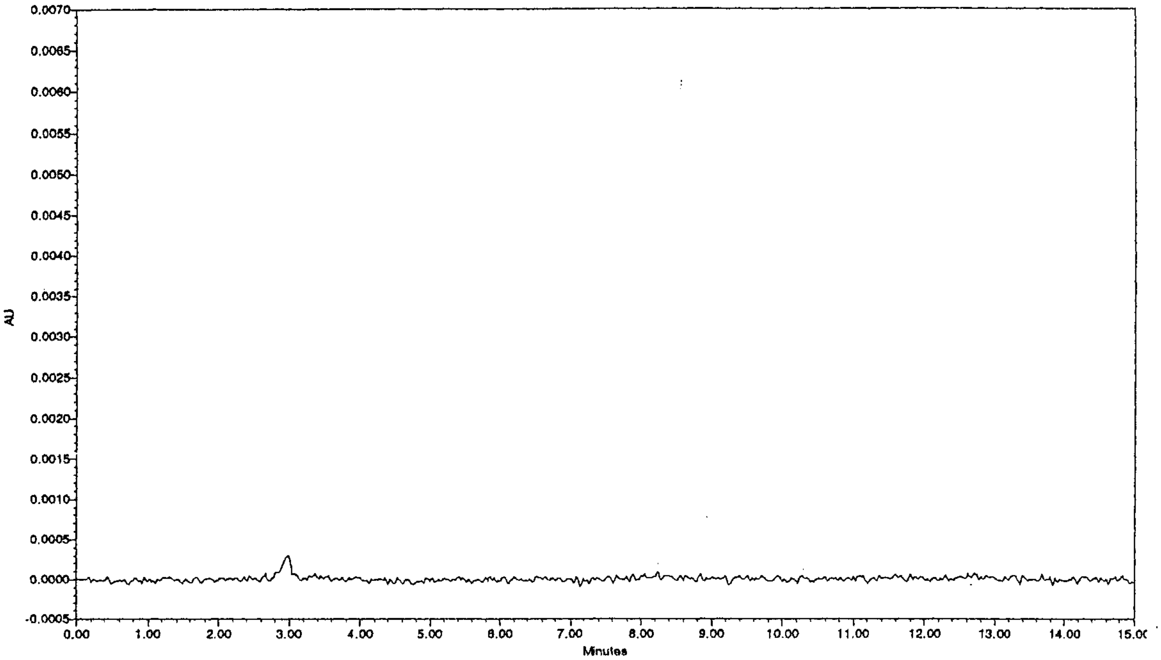

Figure 3 HPLC chromatogram of a blank solution (mobile phase) [res.id. 1251].

APPENDIX

CERTIFICATE OF ANALYSIS OF TETRABROMOBISPHENOL A

This certificate of analysis is an appendix of NOTOX Project 292815, "Determination of the content of Tetrabromobisphenol A in the test substance Tetrabromobisphenol A (including homogeneity)".

SPONSOR

Company name	Bromine Science & Environmental Forum (BSEF) BSEF Secretariat co/Burson-Marsteller 118, Avenue de Cortenbergh 1000 Brussels

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CHEMICAL ANALYSIS

Date of analysis: 02 May 2000

Content of Tetrabromobisphenol A in the test substance Tetrabromobisphenol A:

97.5 ± 1.3 % (w/w) (mean ± s_{n-1}; n=6); HPLC at 230 nm

APPROVAL

Ir. M.J.C. Brekelmans
(Study Director)

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Date: July 07. 2000