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SafePharm Laboratories

Dibromomethane:

ACUTE TOXICITY TO RAINBOW TROUT
(Oncorhynchus mykiss)

SPL PROJECT NUMBER: 0466/0263

OECD GUIDELINE NO. 203; US CODE OF FEDERAL REGULATIONS TITLE 40, PART 797, SECTION 1400;US EPA PESTICIDE ASSESSMENT GUIDELINES, SUB-DIVISION E, SECTION 72-1, US EPA DRAFT ECOLOGICAL EFFECTS TEST GUIDELINE, OPPTS 850.1075 AND ASTM E 729-96 STANDARD GUIDE FOR CONDUCTING ACUTE TOXICITY TESTS ON TEST MATERIALS WITH FISHES, MACROINVERTEBRATES AND AMPHIBIANS

AUTHORS: T J Goodband
D Mullee

CO SPONSORS:

Bromine Compounds Ltd.
Makleff House
P.O.B. 180
BEER-SHEVA 84101
ISRAEL

Albemarle Corporation
Health, Safety and Environment
451 Florida Street
Baton Rouge
LA 70801
UNITED STATES OF AMERICA

TEST FACILITY:

Safepharm Laboratories Limited
Shardlow Business Park
Shardlow
Derbyshire
DE72 2GD
UK

Telephone: +44 (0) 1332 792896

Facsimile: +44 (0) 1332 799018

CONTAINS NO CBI

305630

QUALITY ASSURANCE REPORT

This study type is classed as short-term. Inspection of the routine and repetitive procedures that constitute the study is carried out as a continuous process designed to encompass the major phases at or about the time this study was in progress.

This report has been audited by Safepharm Quality Assurance Unit, and is considered to be an accurate account of the data generated and of the procedures followed.

In each case, the outcome of QA evaluation is reported to the Study Director and Management on the day of evaluation. Audits of study documentation, and process inspections appropriate to the type and schedule of this study were as follows:

§	12 September 2006	Protocol Compliance Audit
	04 December 2006	Test Material Preparation
	04 December 2006	Test System Preparation
	04 December 2006	Exposure
	08 December 2006	Assessment of Response
	04, 07 December 2006	Chemical Analysis
§	21 February 2007	Draft Report Audit
§	Date of QA Signature	Final Report Audit

§ Evaluation specific to this study

..... DATE: 22 JUN 2007 .....

For Safepharm Quality Assurance Unit*

***Authorised QA Signatures:**

Head of Department:	JR Pateman CBiol MIBiol DipRQA ACQI, FRQA
Deputy Head of Department:	JM Crowther MIScT MRQA
Senior Audit Staff:	JV Johnson BSc MRQA; G Wren ONC MRQA

GLP COMPLIANCE STATEMENT

The work described was performed in compliance with UK GLP standards (Schedule 1, Good Laboratory Practice Regulations 1999 (SI 1999/3106 as amended by SI 2004/0994)). These Regulations are in accordance with GLP standards published as OECD Principles on Good Laboratory Practice (revised 1997, ENV/MC/CHEM(98)17); and are in accordance with, and implement, the requirements of Directives 2004/9/EC and 2004/10/EC.

These international standards are acceptable to the Regulatory agencies of the following countries: Australia, Austria, Belgium, Canada, the Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Israel, Italy, Japan, Republic of Korea, Luxembourg, Mexico, The Netherlands, New Zealand, Norway, Poland, Portugal, Slovenia, South Africa, Spain, Sweden, Switzerland, Turkey, the United Kingdom, and the United States of America. Specifically, these GLP standards are acceptable to the United States Environmental Protection Agency, fulfilling the requirements of 40 CFR Part 792, as amended.

This report fully and accurately reflects the procedures used and data generated.

..... Date: 22 JUN 2007

T J Goodband BSc
Study Director

The analytical data presented in this report were compiled by me or under my supervision and accurately reflect the data obtained.

..... Date: 22 JUN 2007

D Mullee CChem MRSC
Director of Analytical Services

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Dibromomethane:

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(Oncorhynchus mykiss)

SUMMARY

Introduction. A study was performed to assess the acute toxicity of the test material to Rainbow Trout (*Oncorhynchus mykiss*). The method followed that described in the OECD Guidelines for Testing of Chemicals (1992) No 203, "Fish, Acute Toxicity Test" referenced as Method C.1 of Commission Directive 92/69/EEC (which constitutes Annex V of Council Directive 67/548/EEC). US CFR Title 40 Part 797 Section 1400: US EPA Pesticide Assessment Guideline, Sub-Division E Section 72-1 and US EPA Draft Ecological Effects Test Guideline OPPTS 850.1075 and ASTM E 729-96 Standard Guide for Conducting Acute Toxicity Tests on Materials with Fishes, Macroinvertebrates and Amphibians.

Methods. Following a preliminary range-finding test, fish were exposed, in groups of ten, to an aqueous solution of the test material over a range of concentrations of 10, 18, 32, 56, and 100 mg/l for a period of 96 hours at a temperature of 13.2°C to 14.9°C. Test vessels were sealed with minimal headspace under semi-static test conditions. The number of mortalities and any sub-lethal effects of exposure in each test and control vessel were determined at 3 and 6 hours after the start of exposure and then daily throughout the test until termination after 96 hours.

Results. The 96-Hour LC_{50} based on nominal test concentrations was 45 mg/l with 95% confidence limits of 42 – 48 mg/l. The No Observed Effect Concentration was 32 mg/l.

Analysis of the fresh media at 0, 24, 48 and 72 hours showed measured test concentrations to range from 82% to 118% of nominal with the exception of the 10 mg/l test concentration replicates R_1 and R_2 at 0 hours which showed measured test concentrations of 123% and 124% respectively. As the 10 mg/l test concentration was below the No Observed Effect Concentration (NOEC) and the mean measured concentrations over the 96 hour test period of the two replicates were 114% and 111% of nominal respectively, these results were considered not to affect the outcome or validity of the test. In addition, the 32 mg/l test concentration replicate R_1 at 72 hours showed a measured concentration of 62% of nominal however, the mean measured concentration was 80% of nominal therefore this result was considered not to affect the outcome or validity of the test. As the majority of the measured concentrations were within the 80% to 120% acceptance limits it was therefore considered unnecessary to analyse the frozen duplicate samples.

Analysis of the old media at 24, 48, 72 and 96 hours showed measured test concentrations to range from 80% to 117% of nominal with the exception of the 10 mg/l replicate R_2 at 48 hours and the 32 mg/l replicate R_2 at 72 hours which showed measured test concentrations of 126% and 128% of nominal respectively. As the mean measured concentrations were 119% and 106% of nominal respectively, these results were considered not to affect the outcome or validity of the test, thus it was considered justifiable to calculate the LC_{50} values in terms of the nominal test concentrations only.

Dibromomethane:

ACUTE TOXICITY TO RAINBOW TROUT
(*Oncorhynchus mykiss*)

1. INTRODUCTION

This report contains a description of the methods used and results obtained during a study to investigate the acute toxicity of the test material to Rainbow Trout. The method followed the recommendations of the OECD Guidelines for Testing of Chemicals (1992) No 203 "Fish, Acute Toxicity Test" referenced as Method C.1 of Commission Directive 92/69/EEC (which constitutes Annex V of Council Directive 67/548/EEC), US CFR Title 40 Part 797 Section 1400: US EPA Pesticide Assessment Guideline, Sub-Division E Section 72-1 and US EPA Draft Ecological Effects Test Guideline OPPTS 850.1075 and ASTM E 729-96 Standard Guide for Conducting Acute Toxicity Tests on Materials with Fishes, Macroinvertebrates and Amphibians.

Rainbow Trout is a freshwater fish representative of a wide variety of natural habitats, and can therefore be considered as an important non-target organism in freshwater ecosystems.

The range-finding test was conducted between 16 October 2006 and 20 October 2006 and the definitive test between 18 December 2006 and 22 December 2006.

2. TEST MATERIAL AND EXPERIMENTAL PREPARATION

2.1 Description, Identification and Storage Conditions

Sponsor's identification	:	Dibromomethane
CAS No.	:	74-95-3
Description	:	Clear colourless liquid
Purity	:	99.4%
Date received	:	14 September 2006
Storage conditions	:	Room temperature in the dark

Individual samples of dibromomethane were supplied by both Co-Sponsors. These two test materials were then mixed in equal proportions and the resulting mixture analysed to determine its purity under Safepharm Laboratories Ltd Project Number 0466/0267. The Certificate of Analysis generated under Safepharm Laboratories Ltd Project Number 0466/0267, including the original sample details provided by each Co-Sponsor, the analytical parameters and the resulting test material purity is presented as Appendix 1. This mixed test material was used for all experimental testing detailed in this report.

The integrity of the supplied data relating to the identity and stability of the test material is the responsibility of the Co-Sponsors. The purity of test material has been addressed under Safepharm Laboratories Ltd Project Number 0466/0267. The Certificate of Analysis generated is presented as Appendix 1.

2.2 Experimental Preparation

For the purpose of the definitive test the test material was dissolved directly in dechlorinated tap water.

An amount of test material (5000 mg) was dissolved in dechlorinated tap water with the aid of ultrasonication for approximately 1 hour and the volume adjusted to 5 litres to give a 1000 mg/l stock solution. An aliquot (2500 ml) of the 1000 mg/l stock solution was added to a final volume of 25 litres of dechlorinated tap water, and stirred using a flat bladed mixer for approximately 1 minute, to give the 100 mg/l test concentration.

A further amount of test material (4000 mg) was dissolved in dechlorinated tap water with the aid of ultrasonication for approximately 1 hour and the volume adjusted to 2 litres to give a 2000 mg/l stock solution. An aliquot (700 ml) of the 2000 mg/l stock solution was added to a final volume of 25 litres of dechlorinated tap water, and stirred using a flat bladed mixer for approximately 1 minute, to give the 56 mg/l test concentration.

To prepare the 10, 18 and 32 mg/l test groups a further amount of test material (4000 mg) was dissolved in dechlorinated tap water with the aid of ultrasonication for approximately 1 hour and the volume adjusted to 2 litres to give a 2000 mg/l stock solution. Aliquots (125, 225 and 400 ml) of the 2000 mg/l stock solution were each separately dispersed into a final volume of 25 litres of dechlorinated tap water, and stirred using a flat bladed mixer for approximately 1 minute.

Each test concentration was prepared in duplicate to give replicates R_1 and R_2.

Each of the stock solutions was inverted several times to ensure adequate mixing and homogeneity.

The test vessels were completely filled with minimal headspace and sealed due to the suspected volatile nature of the test material. The concentration and stability of the test material in the test preparations were verified by chemical analysis at 0 (fresh media), 24, 48, 72 (old and fresh media) and 96 hours (old media) (see Appendix 2).

Chlorine analysis of the diluent water was not conducted daily as described in the OPPTS 850.1075 Guideline. However, the chlorine content of the diluent water was conducted weekly and showed measured concentrations of 0.004 to 0.005 mg/l ClO_2 for the month during which the definitive test was conducted which were considered acceptable.

3. METHODS

3.1 Test Species

The test was carried out using juvenile Rainbow Trout (*Oncorhynchus mykiss*). Fish were obtained from Brow Well Fisheries Limited, Hebdon, near Skipton, Yorkshire, UK and maintained in-house since 15 November 2006. Fish were maintained in a glass fibre tank with a "single pass" water renewal system. Fish were acclimatised to test conditions from 4 December 2006 to 18 December 2006. The lighting cycle was controlled to give a 16 hours light and 8 hours darkness cycle with 20 minute dawn and dusk transition periods.

The water temperature was controlled at 13.2°C to 13.8°C with a dissolved oxygen content of greater than or equal to 10.1 mg O_2/l. These parameters were recorded daily. Temperatures at which the stock fish were maintained were observed to be in excess of the range given in the protocol of 12 ± 1°C. This deviation was considered not to have affected the outcome or the validity of the test as zero mortality was observed in the stock fish during the acclimatisation period.

The stock fish were fed commercial trout pellets which were discontinued 48 hours prior to the start of the definitive test. There was zero mortality in the 7 days prior to the start of the test and the fish had a mean standard length of 4.9 cm (sd = 0.2) and a mean weight of 1.66 g (sd = 0.30) at the end of the definitive test. Based on the mean weight value this gave a loading rate of 0.67 g bodyweight/litre.

The diet and diluent water are considered not to contain any contaminant that would affect the integrity and outcome of the study.

3.2 Test Water

The test water used for both the range-finding and definitive tests was the same as that used to maintain the stock fish.

Laboratory tap water was dechlorinated by passage through an activated carbon filter (Purite Series 500) and partly softened (Elga Nimbus 1248D Duplex Water Softener) giving water with a total hardness of approximately 150 mg/l as $CaCO_3$. After dechlorination and softening the water was passed through a series of computer controlled plate heat exchangers to achieve the required temperature. Typical water quality characteristics for the tap water as supplied, prior to dechlorination and softening, are given in Appendix 3.

At the start of the study the diluent had a particulate matter concentration of 0.10 mg/l, chemical oxygen demand of 2.5, total water hardness of 124 mg/l as $CaCO_3$, alkalinity of 94 mg/l as $CaCO_3$, conductivity of 367 µS/cm, Total Organic Carbon content of less than 1 mg C/l and pH of 7.8.

3.3 Procedure

3.3.1 Range-finding test

The test concentrations to be used in the definitive test were determined by a preliminary range-finding test.

Information supplied by the Sponsor indicated that the test material may be volatile therefore the test was conducted using completely filled and sealed round glass bottomed glass vessels with no aeration.

In the range-finding test fish were exposed to a series of nominal test concentrations of 1.0, 10, and 100 mg/l. The test material was dissolved directly in dechlorinated tap water.

An amount of test material (1000 mg) was dissolved in dechlorinated tap water with the aid of ultrasonication for approximately 10 minutes and the volume adjusted to 1 litre to give a 1000 mg/l stock solution. Aliquots (20 and 200 ml) of this stock solution were each separately dispersed in a final volume of 20 litres of dechlorinated tap water, and stirred using a flat bladed mixer for approximately 1 minute, to give the test concentrations of 1.0 and 10 mg/l respectively.

A further amount of test material (2000 mg) was dissolved in dechlorinated tap water with the aid of ultrasonication for approximately 10 minutes and the volume adjusted to 1 litre to give a 2000 mg/l stock solution. The entire volume of the 2000 mg/l stock solution was dispersed in dechlorinated tap water and the volume adjusted to 20 litres, and stirred using a flat bladed mixer for approximately 1 minute, to give the test concentration of 100 mg/l.

Each of the stock solutions was inverted several times to ensure adequate mixing and homogeneity.

In the range-finding test 3 fish were added to each 20 litre test and control vessel and maintained at 13.6°C to 15.0°C in a temperature controlled room with a photoperiod of 16 hours light and 8 hours darkness with 20 minute dawn and dusk transition periods for a period of 96 hours under static test conditions.

Temperatures during the range-finding test were observed to be in excess of the range given in the protocol of 12 ± 1°C. This deviation was considered not to have affected the outcome or the validity of the test.

The control group was maintained under identical conditions but not exposed to the test material.

Data from the control group was shared with similar concurrent studies.

After 3, 6, 24, 48, 72 and 96 hours any mortalities or sub-lethal effects of exposure were determined by visual inspection of the test fish.

3.3.2 Definitive test

Based on the results of the range-finding test the following test concentrations were assigned to the definitive test: 10, 18, 32, 56 and 100 mg/l.

3.3.2.1 Preparation of the test material

For the purpose of the definitive test the required amount of test material was added to each test vessel using the method described in Section 2.2.

3.3.2.2 Exposure conditions

In the definitive test 25 litre glass exposure vessels were used for each test concentration. At the start of the test 10 fish were placed in each test vessel at random, in the test preparations. The test vessels were sealed with minimal headspace due to the suspected volatile nature of the test material and maintained at 13.2°C to 14.9°C in a temperature controlled room with a photoperiod of 16 hours light and 8 hours darkness with 20 minute dawn and dusk transition periods for a period of 96 hours. The test vessels received no auxiliary aeration. The fish were not individually identified and received no food during exposure.

The control groups were maintained under identical conditions but not exposed to the test material.

A semi-static test regime was employed in the test involving a daily renewal of the test preparations to ensure that the concentrations of the test material remained near nominal and to prevent the build up of nitrogenous waste products.

Any mortalities and sub-lethal effects of exposure were recorded at 3, 6, 24, 48, 72 and 96 hours after the start of exposure. The criteria of death were taken to be the absence of both respiratory movement and response to physical stimulation.

3.3.2.3 Physico-chemical measurements

The water temperature, pH and dissolved oxygen concentrations were recorded daily throughout the test. The measurements at 0 hours, and after each test media renewal at 24, 48 and 72 hours, represent those of the freshly prepared test preparations while the measurements taken prior to each test media renewal, and on termination of the test after 96 hours, represent those of the used or 24-Hour old test preparations. The pH and dissolved oxygen concentration were measured using a WTW pH/Oxi 340I pH and dissolved oxygen meter and the temperature was measured using a Hanna Instruments HI 93510 digital thermometer.

The water temperature was also recorded in one control vessel (replicate R_1) every hour using a Testo Data logger (Channel 2 - external) (see Figure 1).

The water hardness, particulate matter content, alkalinity, Chemical Oxygen Demand (COD), conductivity and Total Organic Carbon content of the diluent were determined at 0 hours. The water hardness and alkalinity were determined using the methods described in Field and on-site test methods for the analysis of waters (British Standard Institution, 1993) and the COD by the HACH reactor digestion method (Hach).

3.3.2.4 Verification of test concentrations

Water samples were taken from the control and all surviving test groups at 0 (fresh media), 24, 48, 72 (old and fresh media) and 96 hours (old media) for quantitative analysis.

Duplicate samples were taken and stored frozen (approximately -20°C) for further analysis if necessary.

The method of analysis, stability, recovery and test preparation analyses are described in Appendix 2.

3.3.2.5 Evaluation of data

The LC_{50} values and associated confidence limits at 3, 6, 24, 48, 72 and 96 hours were calculated by the trimmed Spearman-Karber method (Hamilton *et al* 1977) using the ToxCalc computer software package (ToxCalc 1999).

When only one partial response is shown is the trimmed Spearman-Karber method is appropriate.

4. ARCHIVES

Unless instructed otherwise by the Sponsor, all original data and the final report will be retained in the Safepharm archives for five years, after which instructions will be sought as to further retention or disposal.

5. RESULTS

5.1 Range-finding Test

Cumulative mortality data from the exposure of rainbow trout to the test material during the range-finding test are given in Table 1 and sub-lethal effects of exposure are given in Table 2.

The results showed no mortalities at the test concentrations of 1.0 mg/l. However, mortalities were observed in the 10 and 100 mg/l test concentrations.

After 96 hours exposure two mortalities were observed in the control and a single mortality was observed at the 10 mg/l test concentration. These mortalities were considered to be due to natural causes and possible low oxygen levels due to the vessels and static test conditions used, rather than a true toxic effect given that no sub-lethal effects of exposure were observed in the remaining fish and no mortalities were observed at the 1.0 mg/l test concentration. A semi-static test regime with daily monitoring of the oxygen concentration was used for the definitive test to ensure low oxygen concentrations did not affect the results.

Based on this information test concentrations of 10, 18, 32, 56 and 100 mg/l were selected for the definitive test.

5.2 Definitive Test

5.2.1 Mortality data

Cumulative mortality data from the exposure of rainbow trout to the test material during the definitive test are given in Table 3 and the relationship between percentage mortality and concentration at 96 hours is given in Figure 2.

Analysis of the mortality data by the trimmed Spearman-Karber method (Hamilton *et al* 1977) at 3, 6, 24, 48, 72 and 96 hours based on the nominal test concentrations gave the following results:

Time (h)	LC_{50} (mg/l)	95% Confidence limits (mg/l)
3	69	63 - 75
6	61	54 - 69
24	49	44 - 55
48	45	42 - 48
72	45	42 - 48
96	45	42 - 48

The results of the definitive test showed the highest test concentration resulting in 0% mortality to be 32 mg/l, the lowest test concentration resulting in 100% mortality to be 100 mg/l and the No Observed Effect Concentration (NOEC) to be 32 mg/l. The No Observed Effect Concentration is based upon zero mortalities and the absence of any sub-lethal effects of exposure at this concentration (Section 5.2.2).

The relationship between the median lethal concentration (LC_{50}) and time is presented graphically in Figure 3.

5.2.2 Sub-lethal effects

Sub-lethal effects of exposure were observed at the test concentration of 56 mg/l. This response was swimming at the surface (see Table 4).

5.2.3 Observations on test material solubility

The test preparations were observed to be clear, colourless solutions throughout the duration of the test.

5.2.4 Physico-chemical measurements

The results of the physico-chemical measurements are given in Appendix 4. Temperature was maintained at 13.2° to 14.9°C throughout the test, while there were no treatment related differences for oxygen concentration or pH.

The water temperature was also recorded in one control vessel (replicate R_1) every hour using a Testo temperature logger (see Figure 1). The temperature logger recorded temperature values between 13.2°C and 15.3°C.

Temperatures during the definitive test were observed to be in excess of the range given in the protocol of 12 ± 1°C. This deviation was considered not to have affected the outcome or the validity of the test as the control fish during testing showed no sub-lethal effects or mortality over the test period.

The oxygen concentration in the some of the test vessels for the fresh media was observed to have an air saturation value (ASV) in excess of 100%. This was considered to be due to the presence of microscopic air bubbles in the media super-saturating the diluent and was considered not to have had an impact on the outcome or integrity of the test as no adverse effects were observed.

5.2.5 Verification of test concentrations

Analysis of the fresh media at 0, 24, 48 and 72 hours (see Appendix 2) showed measured test concentrations to range from 82% to 118% of nominal with the exception of the 10 mg/l test concentration replicates R_1 and R_2 at 0 hours which showed measured test concentrations of 123% and 124% respectively. As the 10 mg/l test concentration was below the No Observed Effect Concentration (NOEC) and the mean measured concentrations over the 96 hour test period of the two replicates were 114% and 111% of nominal respectively, these results were considered not to affect the outcome or validity of the test. In addition, the 32 mg/l test concentration replicate R_1 at 72 hours showed a measured concentration of 62% of nominal however, the mean measured concentration was 80% of nominal therefore this result was considered not to affect the outcome or validity of the test. As the majority of the measured concentrations were within the 80% to 120% acceptance limits it was therefore considered unnecessary to analyse the frozen duplicate samples.

Analysis of the old media at 24, 48, 72 and 96 hours (see Appendix 2) showed measured test concentrations to range from 80% to 117% of nominal with the exception of the 10 mg/l replicate R_2 at 48 hours and the 32 mg/l replicate R_2 at 72 hours which showed measured test concentrations of 126% and 128% of nominal respectively. As the mean measured concentrations were 119% and 106% of nominal respectively, these results were considered not to affect the outcome or validity of the test, thus it was considered justifiable to calculate the LC_{50} values in terms of the nominal test concentrations only.

6. CONCLUSION

The acute toxicity of the test material to the freshwater fish rainbow trout (*Oncorhynchus mykiss*) has been investigated and gave a 96-Hour LC_{50} value of 45 mg/l with 95% confidence limits of 42 – 48 mg/l. The No Observed Effect Concentration was 32 mg/l.

7. REFERENCES

British Standard Institution (1993) Determination of Calcium and Total Hardness. Field and on-site methods for the analysis of water, (BS 1427), pp35-36.

British Standard Institution (1993) Determination of Alkalinity. Field and on-site methods for the analysis of water, (BS 1427), pp33-35.

Hamilton, M A, Russo, R C and Thurston, R V (1977) Trimmed Spearman-Karber Method for Estimating Median Lethal Concentration in Toxicity Bioassays. *Environ Sci Technol* **11**, 714-719.

ToxCalc Version 5.0.23C (1999), Tidepool Scientific Software, McKinleyville, CA 95519, USA.

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Table 1 Cumulative Mortality Data in the Range-finding Test

Nominal Concentration (mg/l)	Cumulative Mortality (Initial Population = 3)					
	3 Hours	6 Hours	24 Hours	48 Hours	72 Hours	96 Hours
Control	0	0	0	0	0	2*
1.0	0	0	0	0	0	0
10	0	0	0	0	0	1*
100	0	2	3	3	3	3

* Mortalities considered to be due to natural causes and possible low oxygen levels due to the vessels used, rather than a true toxic effect given that no sub-lethal effects of exposure were observed in the remaining fish and no mortalities were observed at the 1.0 mg/l test concentration.

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Table 2 Sub-lethal Effects of Exposure in the Range-finding Test

Nominal Concentration (mg/l)	Sub-lethal Effects	Time (Hours)					
		3	6	24	48	72	96
Control	No abnormalities detected						
1.0	No abnormalities detected						
10	No abnormalities detected						
100	Swimming at surface	3/3	1/1	A/D			

A/D = All fish dead

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Table 3 Cumulative Mortality Data in the Definitive Test

Nominal Concentration (mg/l)		Cumulative Mortality (Initial Population =10)						% Mortality
		3 Hours	6 Hours	24 Hours	48 Hours	72 Hours	96 Hours	96 Hours
Control	R_1	0	0	0	0	0	0	0
	R_2	0	0	0	0	0	0	0
10	R_1	0	0	0	0	0	0	0
	R_2	0	0	0	0	0	0	0
18	R_1	0	0	0	0	0	0	0
	R_2	0	0	0	0	0	0	0
32	R_1	0	0	0	0	0	0	0
	R_2	0	0	0	0	0	0	0
56	R_1	2	4	8	9	9	9	90
	R_2	1	3	7	9	9	9	90
100	R_1	10	10	10	10	10	10	100
	R_2	10	10	10	10	10	10	100

R_1 and R_2 = Replicates 1 and 2

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Table 4 Sub-lethal Effects of Exposure in the Definitive Test

Nominal Concentration (mg/l)		Sub-lethal Effects	Time (Hours)					
			3	6	24	48	72	96
Control	R_1	No abnormalities detected						
	R_2	No abnormalities detected						
10	R_1	No abnormalities detected						
	R_2	No abnormalities detected						
18	R_1	No abnormalities detected						
	R_2	No abnormalities detected						
32	R_1	No abnormalities detected						
	R_2	No abnormalities detected						
56	R_1	Swimming at surface	8/8	6/6	2/2	1/1	1/1	1/1
	R_2	Swimming at surface	9/9	7/7	3/3	1/1	1/1	1/1
100	R_1	No abnormalities detected	A/D					
	R_2	No abnormalities detected	A/D					

R_1 and R_2 = Replicates 1 and 2
A/D = All fish dead

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Figure 1 **Temperature Measurements Recorded Over the 96-Hour Test Period Using a Testo Temperature Logger**

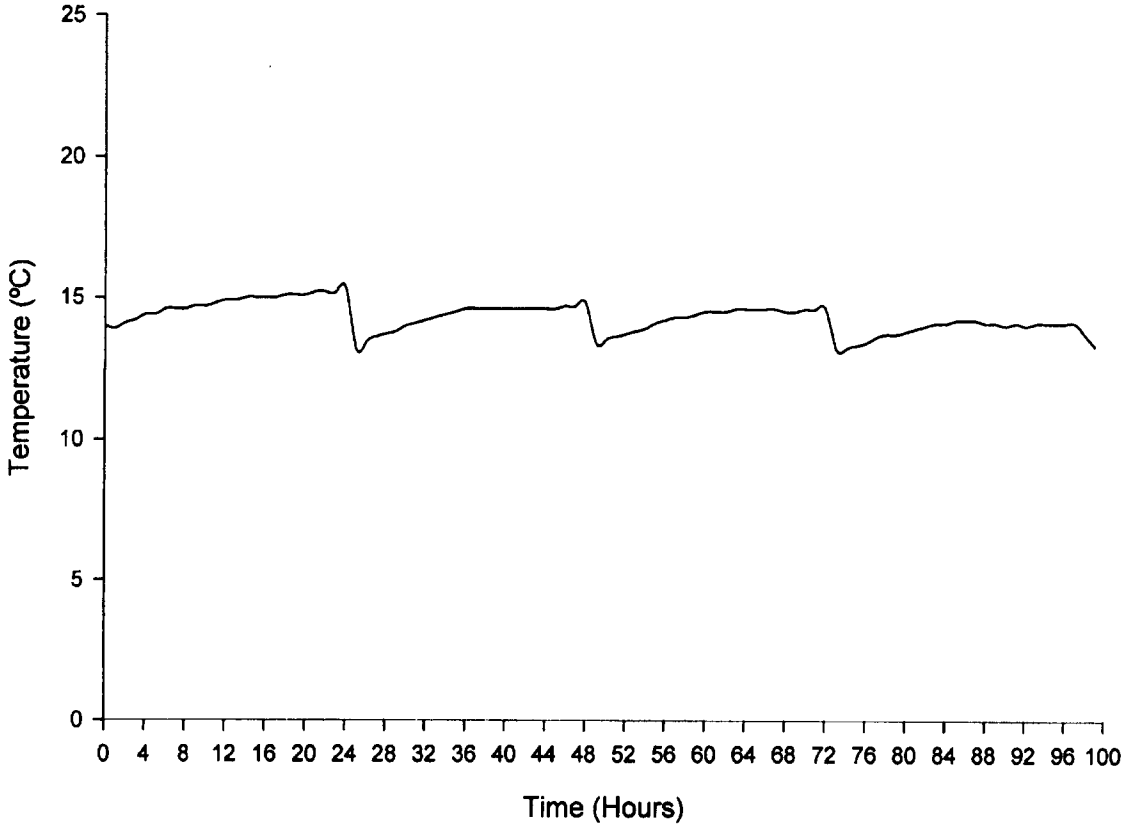

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Figure 2 Concentration-Mortality Curve

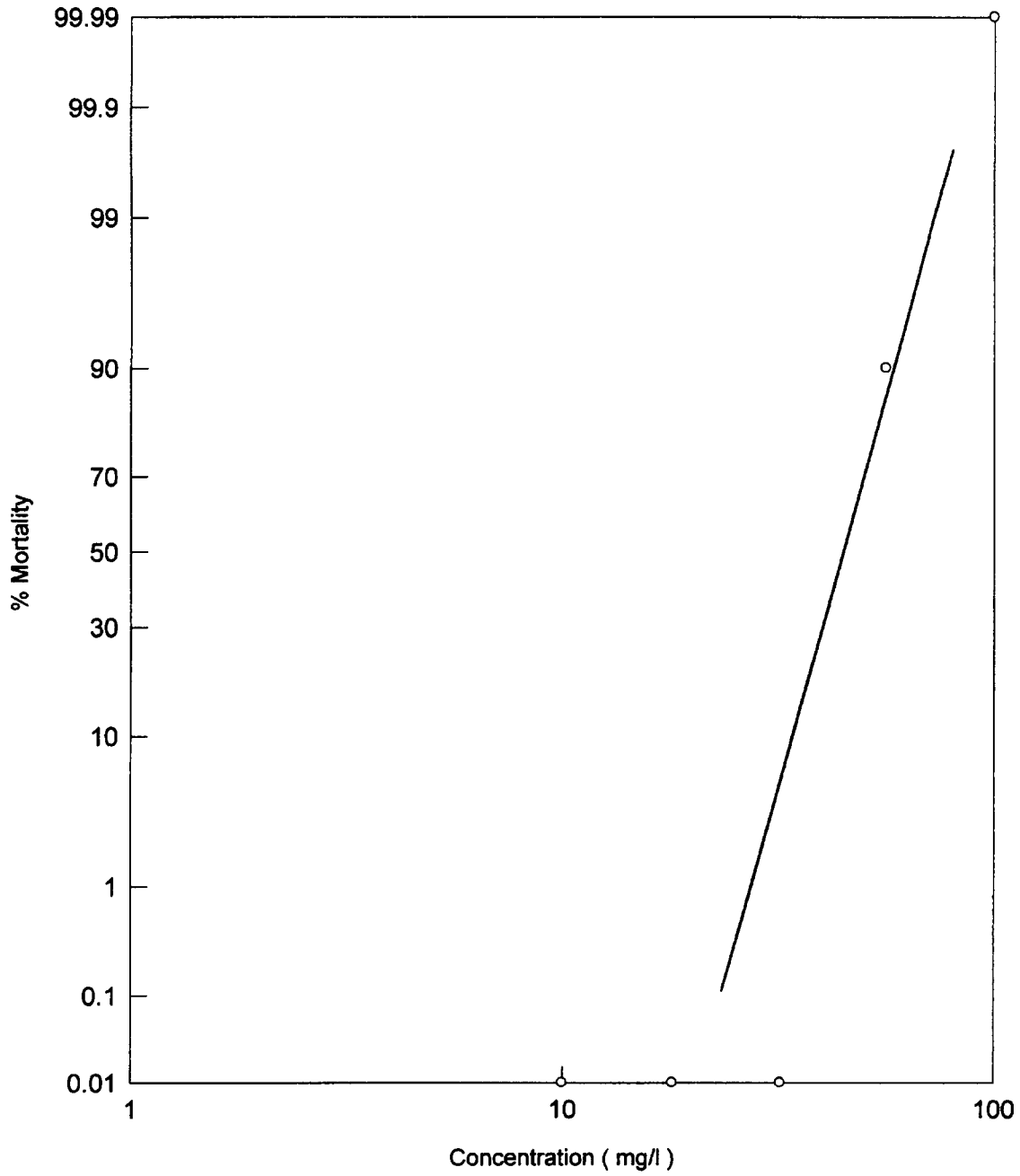

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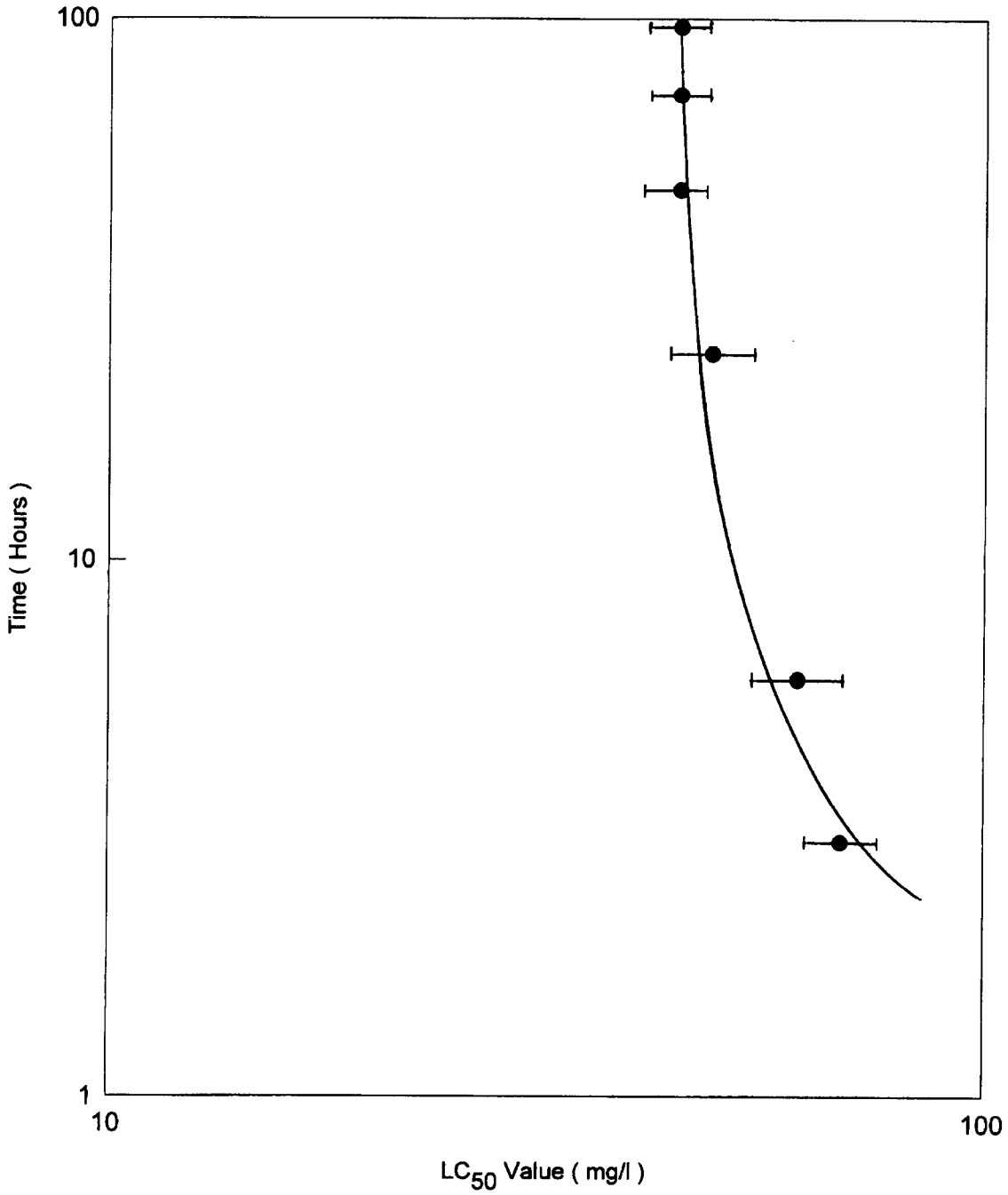
Figure 3 Toxicity Curve



Dibromomethane: ACUTE TOXICITY TO RAINBOW TROUT

Appendix 1 Certificate of Analysis

SafePharm Laboratories

SafePharm Laboratories Ltd
Shardlow Business Park
London Road, Shardlow
Derbyshire DE72 2GD
Tel: 01332 792896 Fax: 01332 799018
Website: www.safepharm.co.uk

MIXING AND ANALYSIS OF TEST MATERIAL SAMPLES

CERTIFICATE OF ANALYSIS

TEST MATERIAL	:	Dibromomethane	
PROJECT NUMBER	:	0466/0267	
CO-SPONSORS	:	Bromine Compounds Ltd. Makleff House P.O.B. 180 BEER-SHEVA 84101 ISRAEL	Albemarle Corporation Health, Safety and Environment 451 Florida Street Baton Rouge LA 70801 UNITED STATES OF AMERICA
TEST DATES	:	14 September 2006 to 28 September 2006	

Test Material Details:

Supplier	:	Bromine Compounds Ltd	Albemarle Corporation
Name	:	Dibromomethane	Dibromomethane
Description	:	clear, colourless liquid	clear, colourless liquid
Lot number	:	20050298	DBM082506
Date Received	:	12 September 2006	01 September 2006
Storage Conditions	:	room temperature, in the dark	room temperature, in the dark

Testing Details:

The above two test materials were mixed in equal proportions and the purity of the resulting mixture determined by gas chromatography (GC) area normalisation. The purity was determined to be 99.4%.

Analytical Conditions:

Column	:	ZB-624 (30 m x 0.25 mm id, 1.40 µm film)
Oven temperature program	:	initial temperature : 40°C rate: 5°C/minute final temperature: 260°C for 5 minutes
Injector temperature	:	250°C
Flame ionisation detector temperature	:	300°C
Injection volume	:	1 µl
Retention time	:	approximately 9.5 minutes for Dibromomethane

Registered in England No. 3009027

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Appendix 1 (continued) Certificate of Analysis

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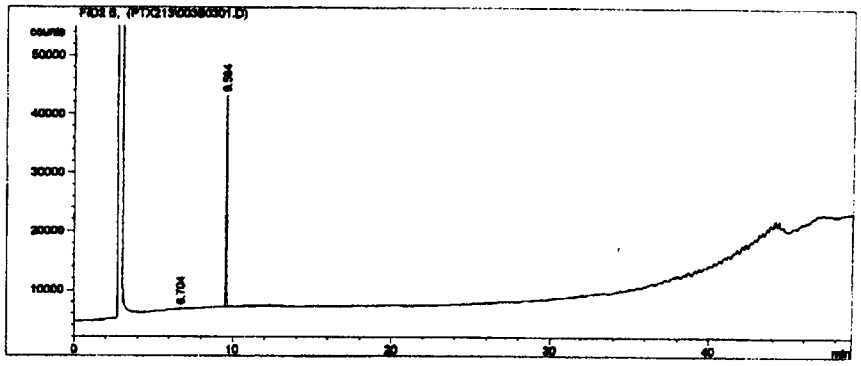
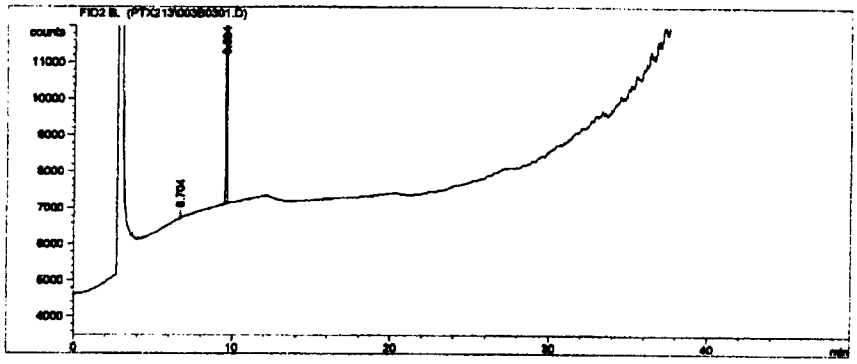
Typical Chromatography

Sample Solution, 6.14 x 10^3 mg/l in Methanol



Sample Solution (Expanded Scale)



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I the undersigned, hereby declare that this certificate of analysis accurately reflects the original data generated in SPL Project Number 0466/0267.

This study was performed in compliance with the UK GLP Regulations 1999 (as amended in 2004) and this certificate accurately reflects the results obtained. The original data and the Certificate of Analysis will be kept for five years in the archives of Safepharm Laboratories Ltd.

Signed: ..... Date: 02 NOV 2006
B J O'Connor BSc (Hons) MRSC
Study Director

Studies of this type are monitored and reported by Safepharm Laboratories Quality Assurance Unit as part of a routine sampling programme: 14, 26 September 2006.

The certificate was audited on 02 November 2006 by Safepharm Laboratories Quality Assurance Unit. It is considered to be an accurate account of the data generated and of the results produced.

Signed: ..... Date: 02 NOV 2006
For Safepharm Quality Assurance Unit

Registered in England No. 3009027

Appendix 2 Verification of Test Concentrations

1. METHOD OF ANALYSIS

1.1 Introduction

The test material concentration in the test samples was determined by gas chromatography (GC) using an external standard. The test material gave a chromatographic profile consisting of a single peak.

The method was developed by the Department of Analytical Services, Safepharm Laboratories Limited.

1.2 Sample Preparation

A volume of test sample was diluted with acetone to give a final theoretical concentration of 5.0 to 50 mg/l.

1.3 Standards

Standard solutions of test material were prepared in acetone: water* (50:50 v/v) at a nominal concentration of 20 mg/l.

1.4 Procedure

The standards and samples were analysed by GC using the following conditions:

GC System	:	Agilent Technologies 5890 incorporating autosampler and workstation
Column	:	ZB-5 (30 m x 0.53 mm id, 5 µm film)
Oven temperature program	:	initial 40°C for 2 minutes rate 1 3°C/minute to 70°C rate 2 20°C/minute final 130°C for 2 minutes

* Prepared by ELGA Purelab Option R-15 BP water purification

Appendix 2 (continued) Verification of Test Concentrations

Injector temperature	:	300°C
Detector temperature	:	300°C
Carrier gas and pressure	:	nitrogen at 5 psi
Injection volume	:	2 µl
Injection mode	:	splitless purge on at 1 minute
Detector	:	flame ionisation detector (FID)
Retention time	:	approximately 6 minutes

2. VALIDATION

2.1 Linearity

A range of standard solutions covering 2.0 to 610 mg/l (40% of the lowest working sample concentration to 1220% of the highest working sample concentration) was analysed.

Linearity was confirmed (R^2 = 0.9994) in the range 0 to 610 mg/l.

The results are presented graphically on page 29.

2.2 Recoveries

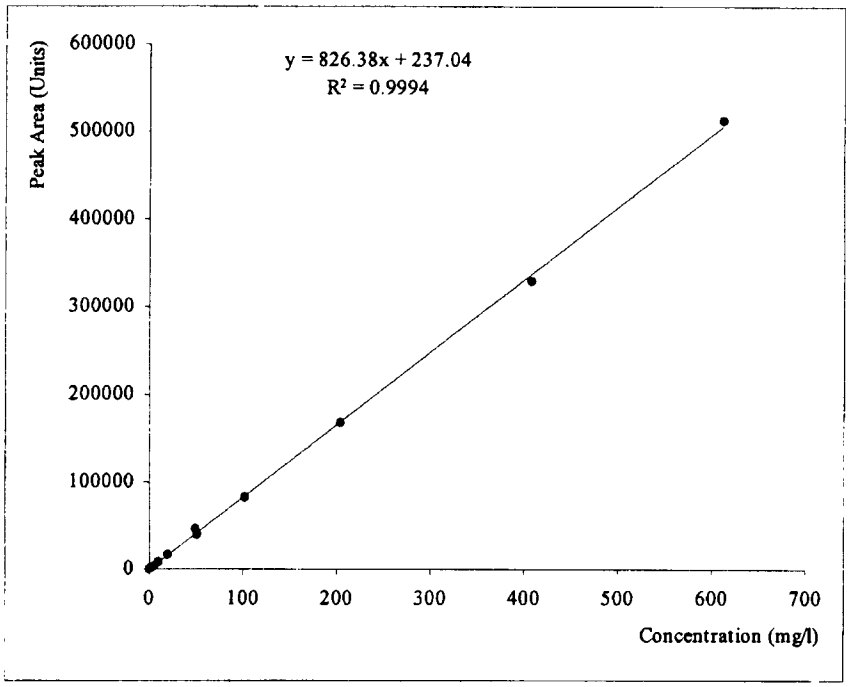
A range of preliminary test samples, accurately fortified at known concentrations of test material, was prepared and analysed.

The recovery samples were prepared by direct addition of the test material to a sample of test medium.

Dibromomethane: ACUTE TOXICITY TO RAINBOW TROUT

Appendix 2 (continued) Verification of Test Concentrations

Linearity of Detector Response



Appendix 2 (continued) Verification of Test Concentrations

Fortification (mg/l)	Recoveries		
	(mg/l)	(%)	Mean %
10.9	11.6	107	108
10.9	11.8	108	
34.8	30.9	89	92
34.8	32.8	94	
109	94.5	87	88
109	96.8	89	

The method has been considered to be sufficiently accurate for the purposes of this test. The test sample results have not been corrected for recovery.

2.3 Limit of Quantitation

The limit of quantitation has been assessed down to 4.1 mg/l.

3. STABILITY

The stability samples were carried out for The Acute Toxicity to *Daphnia magna* study (Safepharm Laboratories Project Number: 0466/0264).

A range of preliminary test samples was prepared, analysed initially and then after storage in sealed glass vessels at ambient temperature in light and dark conditions for approximately 48 hours (greater than the period of media renewal). In addition test samples were tested for stability without prior mixing (sonication) the test sample bottles to assess for losses due to adsorption and/or insolubility. Test samples were also tested for stability in open vessels to assess for losses due to volatility.

Dibromomethane: ACUTE TOXICITY TO RAINBOW TROUT

Appendix 2 (continued) Verification of Test Concentrations

Nominal concentration (mg/l)	10	32	100
Concentration found initially (mg/l)	11.7	31.9	95.6
Concentration found after storage in light conditions (mg/l)	11.9	29.5	95.6
Expressed as a percent of the initial concentration	102	92	100
Concentration found after storage in dark conditions (mg/l)	13.2	26.5	86.4
Expressed as a percent of the initial concentration	113	83	90
Concentration found after storage in dark conditions (mg/l) – unsonicated sample	10.1	34.5	79.8
Expressed as a percent of the initial concentration	86	108	83
Concentration found after storage in dark conditions (mg/l) – open vessel	3.05	6.49	19.6
Expressed as a percent of the initial concentration	26	20	21

The data showed the test material to be stable over a 48-Hour period; therefore it was considered that the test material would be stable over the 24-Hour period of media renewal and hence it was considered unnecessary to generate this data.

The results of the unsonicated stability vessel showed no evidence of insolubility or adherence to glass.

The results of the open vessel stability showed significant evidence of volatility of the test material.

Dibromomethane: ACUTE TOXICITY TO RAINBOW TROUT

Appendix 2 (continued) Verification of Test Concentrations

4. RESULTS

Sample	Nominal Concentration (mg/l)	Concentration Found (mg/l)	Expressed as a Percent of the Nominal Concentration (%)
0 Hours	Control R_1	<LOQ	-
	Control R_2	<LOQ	-
	10 R_1	12.3	123
	10 R_2	12.4	124
	18 R_1	17.9	99
	18 R_2	17.9	99
	32 R_1	31.2	97
	32 R_2	31.0	97
	56 R_1	52.0	93
	56 R_2	52.7	94
	100 R_1	90.5	91
	100 R_2	95.5	95
24 Hours Old Media	Control R_1	<LOQ	-
	Control R_2	<LOQ	-
	10 R_1	10.3	103
	10 R_2	9.80	98
	18 R_1	17.0	94
	18 R_2	17.7	98
	32 R_1	29.4	92
	32 R_2	28.8	90
	56 R_1	51.5	92
	56 R_2	49.7	89
	100 R_1	89.4	89
	100 R_2	92.9	93

LOQ = Limit of quantitation
R_1 and R_2 = Replicates 1 and 2

Dibromomethane: ACUTE TOXICITY TO RAINBOW TROUT

Appendix 2 (continued) Verification of Test Concentrations

Sample	Nominal Concentration (mg/l)	Concentration Found (mg/l)	Expressed as a Percent of the Nominal Concentration (%)
24 Hours Fresh Media	Control R_1	<LOQ	-
	Control R_2	<LOQ	-
	10 R_1	11.1	111
	10 R_2	11.0	110
	18 R_1	19.2	107
	18 R_2	17.6	98
	32 R_1	30.2	94
	32 R_2	30.3	95
	56 R_1	53.3	95
	56 R_2	53.3	95
48 Hours Old Media	Control R_1	<LOQ	-
	Control R_2	<LOQ	-
	10 R_1	11.2	112
	10 R_2	12.6	126
	18 R_1	18.3	101
	18 R_2	18.7	104
	32 R_1	31.6	99
	32 R_2	30.8	96
	56 R_1	53.3	95
	56 R_2	54.1	97
48 Hours Fresh Media	Control R_1	<LOQ	-
	Control R_2	<LOQ	-
	10 R_1	11.8	118
	10 R_2	11.7	117
	18 R_1	18.4	102
	18 R_2	18.9	105
	32 R_1	30.5	95
	32 R_2	34.9	109
	56 R_1	48.9	87
	56 R_2	51.5	92

LOQ = Limit of quantitation
R_1 and R_2 = Replicates 1 and 2

Dibromomethane: ACUTE TOXICITY TO RAINBOW TROUT

Appendix 2 (continued) Verification of Test Concentrations

Sample	Nominal Concentration (mg/l)	Concentration Found (mg/l)	Expressed as a Percent of the Nominal Concentration (%)
72 Hours Old Media	Control R_1	<LOQ	-
	Control R_2	<LOQ	-
	10 R_1	10.7	107
	10 R_2	10.1	101
	18 R_1	16.6	92
	18 R_2	15.3	85
	32 R_1	26.9	84
	32 R_2	41.1	128
	56 R_1	45.0	80
	56 R_2	48.1	86
72 Hours Fresh Media	Control R_1	<LOQ	-
	Control R_2	<LOQ	-
	10 R_1	11.8	118
	10 R_2	10.3	103
	18 R_1	14.7	82
	18 R_2	18.1	101
	32 R_1	19.7	62
	32 R_2	31.6	99
	56 R_1	61.2	109
	56 R_2	51.7	92
96 Hours Old Media	Control R_1	<LOQ	-
	Control R_2	<LOQ	-
	10 R_1	11.7	117
	10 R_2	11.0	110
	18 R_1	18.4	102
	18 R_2	16.9	94
	32 R_1	30.8	96
	32 R_2	31.5	98
	56 R_1	49.5	88
	56 R_2	50.3	90

LOQ = Limit of quantitation
R_1 and R_2 = Replicates 1 and 2

Appendix 2 (continued) Verification of Test Concentrations

5. DISCUSSION

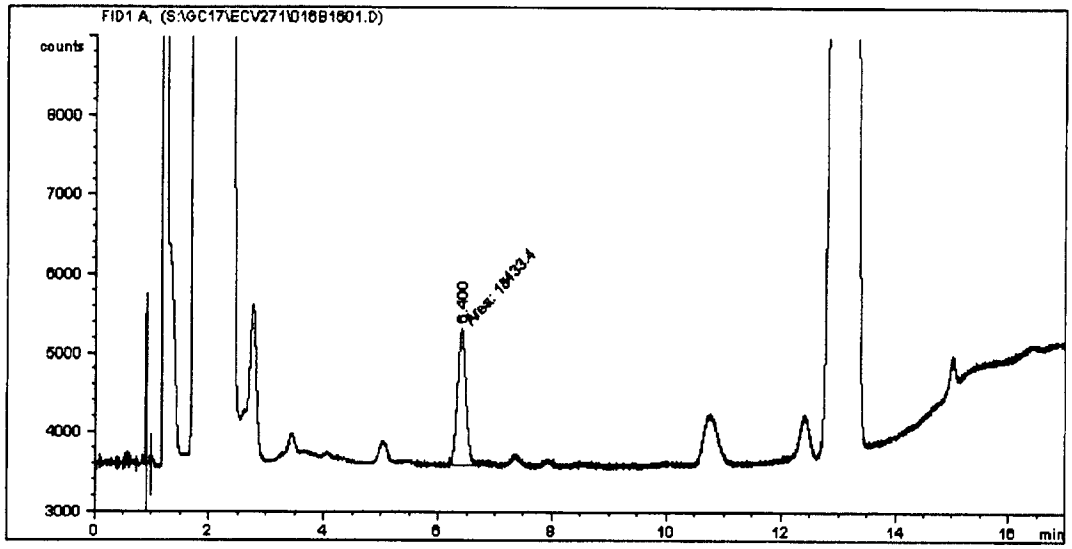
The detection system was found to have acceptable linearity. The analytical procedure had acceptable recoveries of test material in test medium (80% to 120% of nominal value). A method of analysis was validated and proven to be suitable for use.

The test material was stable in the test medium for the duration of the period of media renewal.

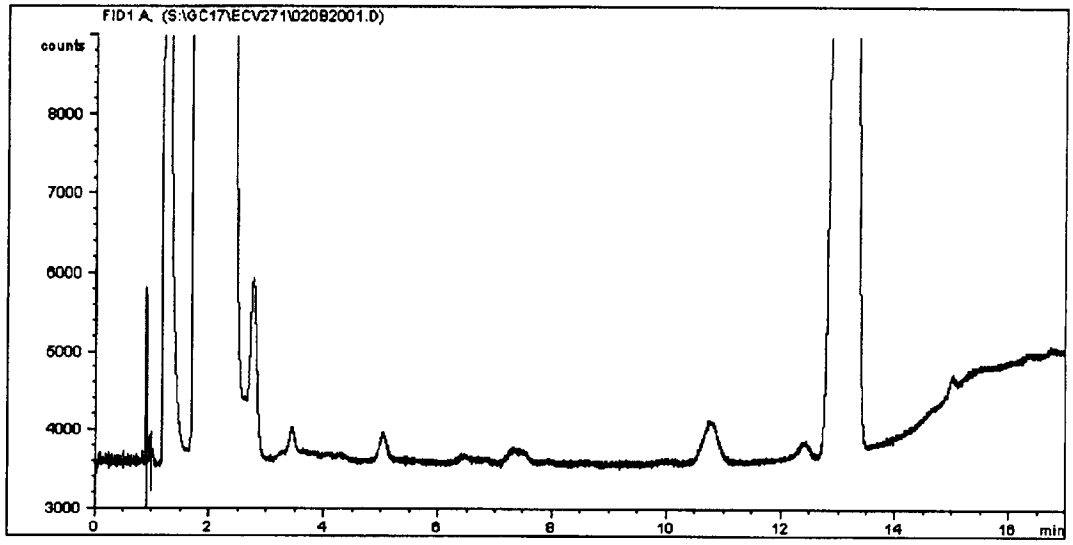
Dibromomethane: ACUTE TOXICITY TO RAINBOW TROUT

Appendix 2 (continued) Verification of Test Concentrations

6. TYPICAL CHROMATOGRAPHY



Standard 20 mg/l

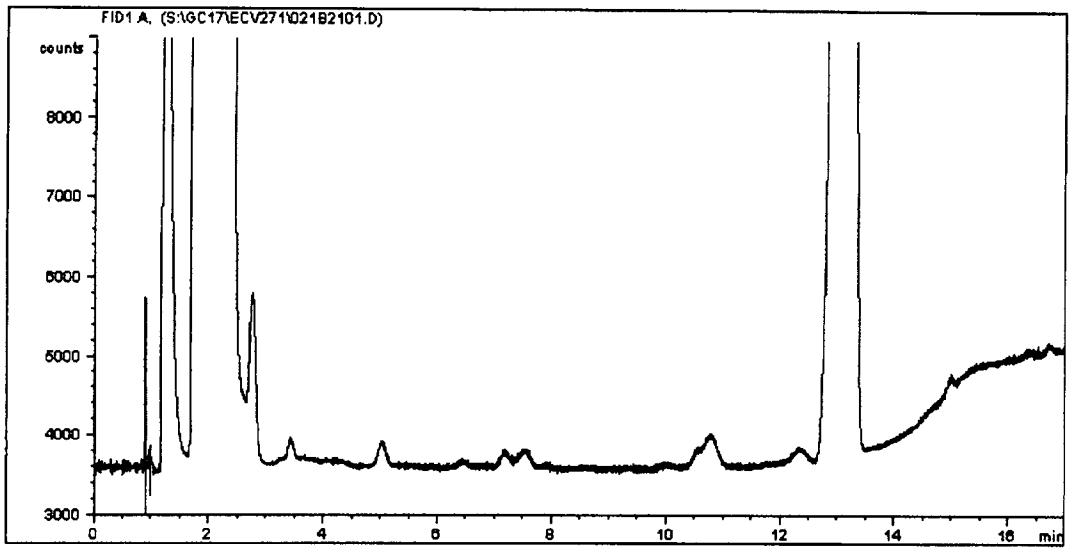


Control Sample R_1

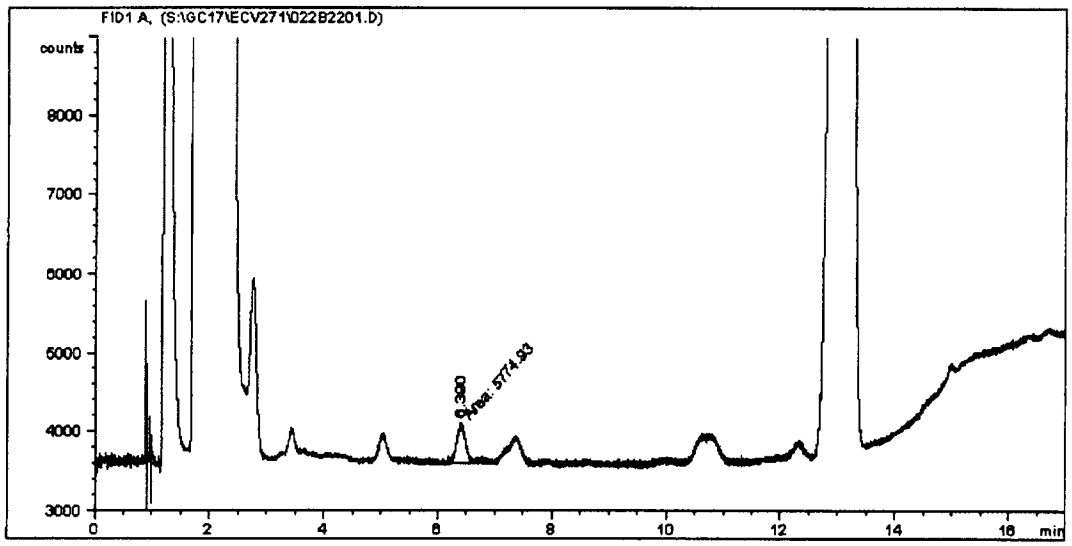
R_1 = Replicate 1

Dibromomethane: ACUTE TOXICITY TO RAINBOW TROUT

Appendix 2 (continued) Verification of Test Concentrations



Control Sample R_2

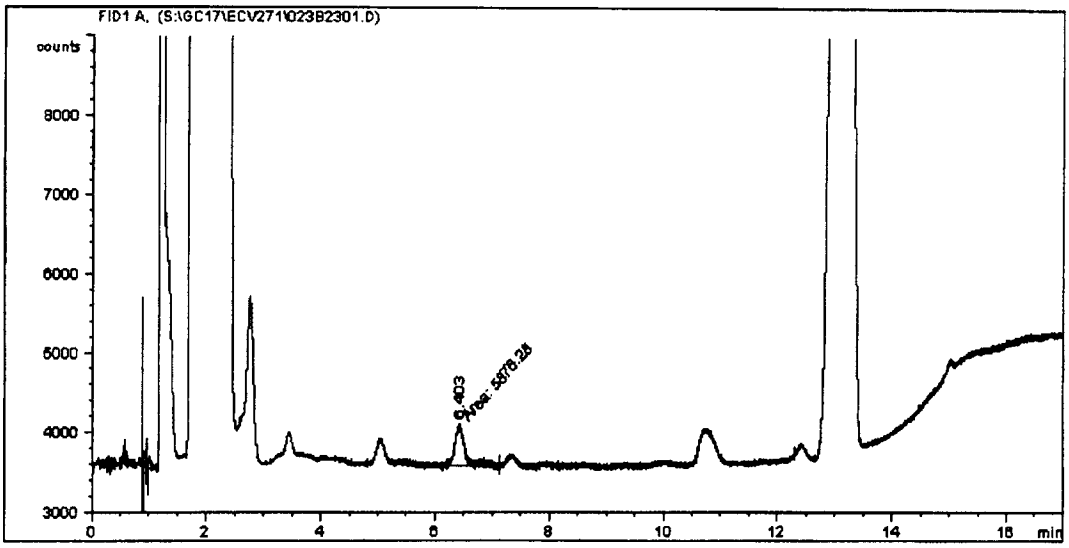


Test Sample 10 mg/l R_1

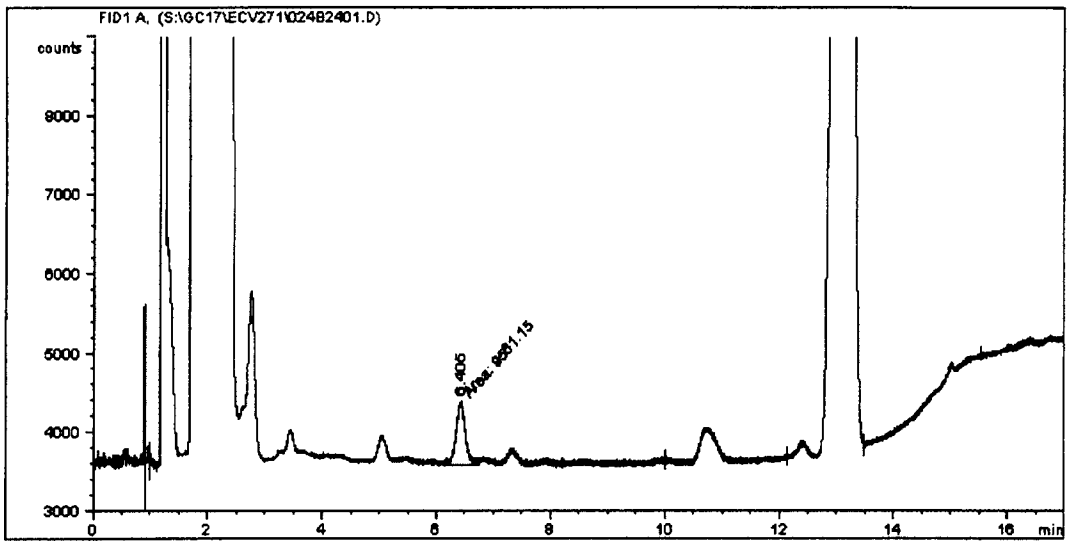
R_1 and R_2 = Replicates 1 and 2

Dibromomethane: ACUTE TOXICITY TO RAINBOW TROUT

Appendix 2 (continued) Verification of Test Concentrations



Test Sample 10 mg/l R_2

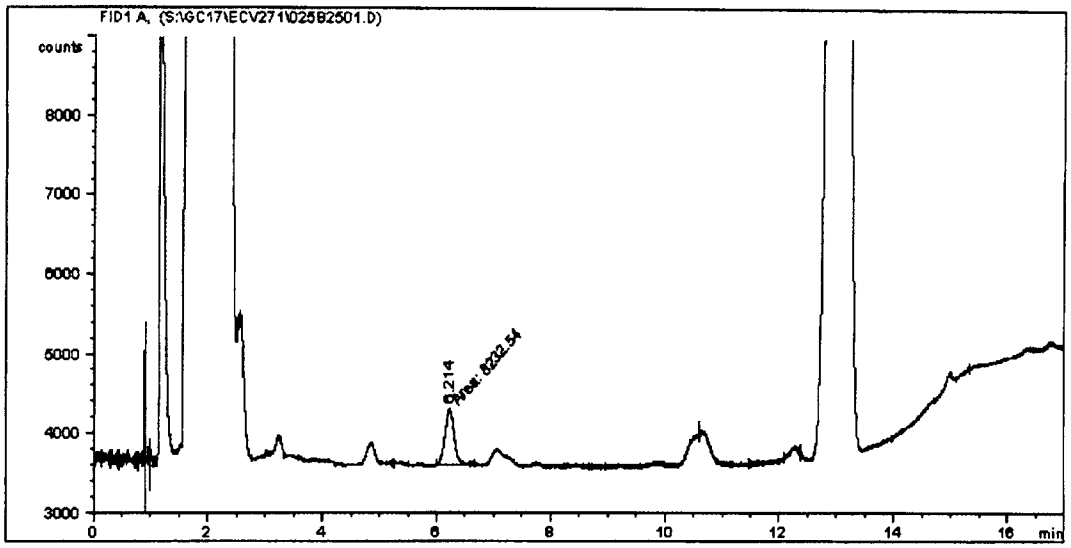


Test Sample 18 mg/l R_1

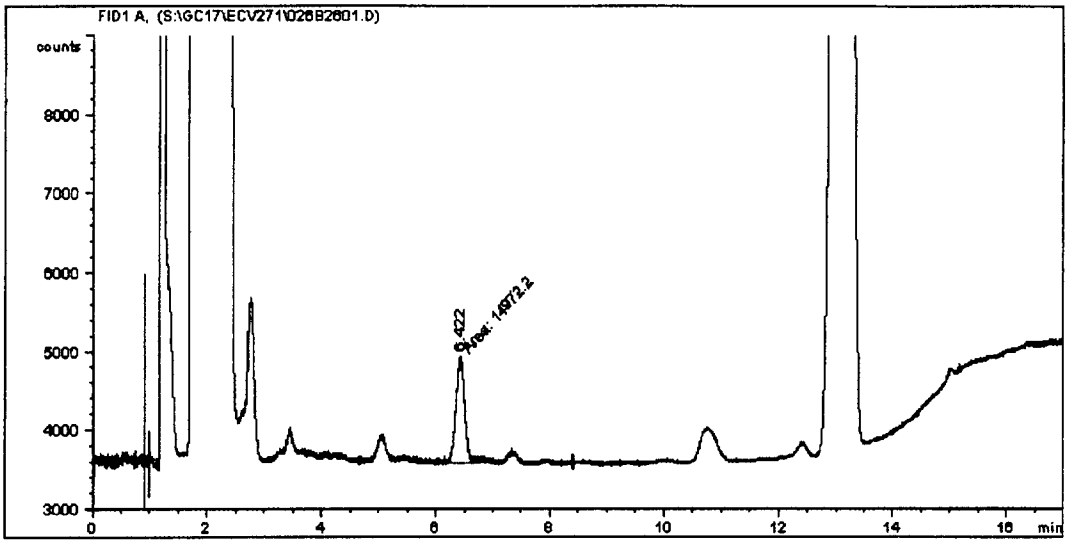
R_1 and R_2 = Replicates 1 and 2

Dibromomethane: ACUTE TOXICITY TO RAINBOW TROUT

Appendix 2 (continued) Verification of Test Concentrations



Test Sample 18 mg/l R_2

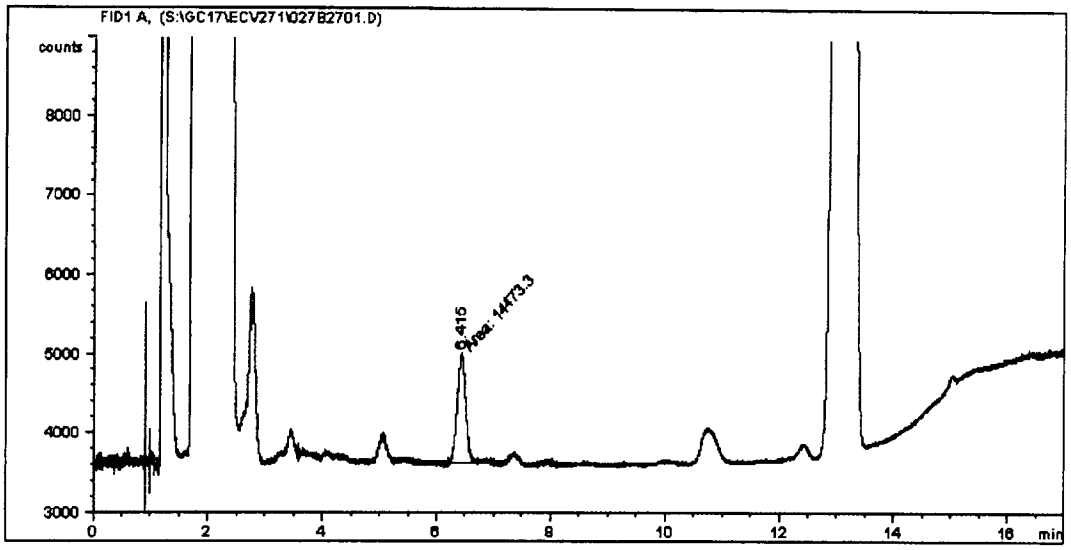


Test Sample 32 mg/l R_1

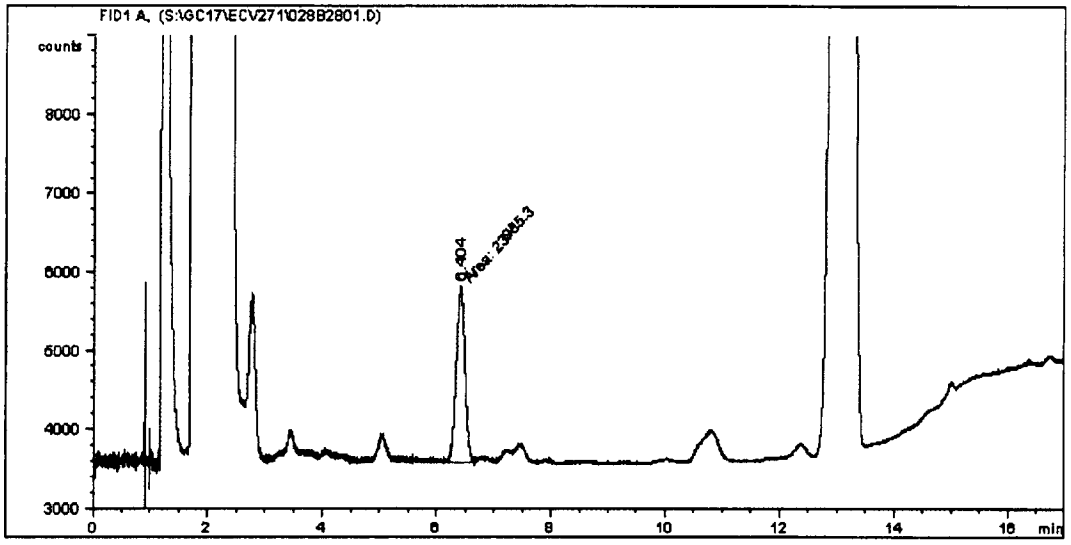
R_1 and R_2 = Replicates 1 and 2

Dibromomethane: ACUTE TOXICITY TO RAINBOW TROUT

Appendix 2 (continued) Verification of Test Concentrations



Test Sample 32 mg/l R_2

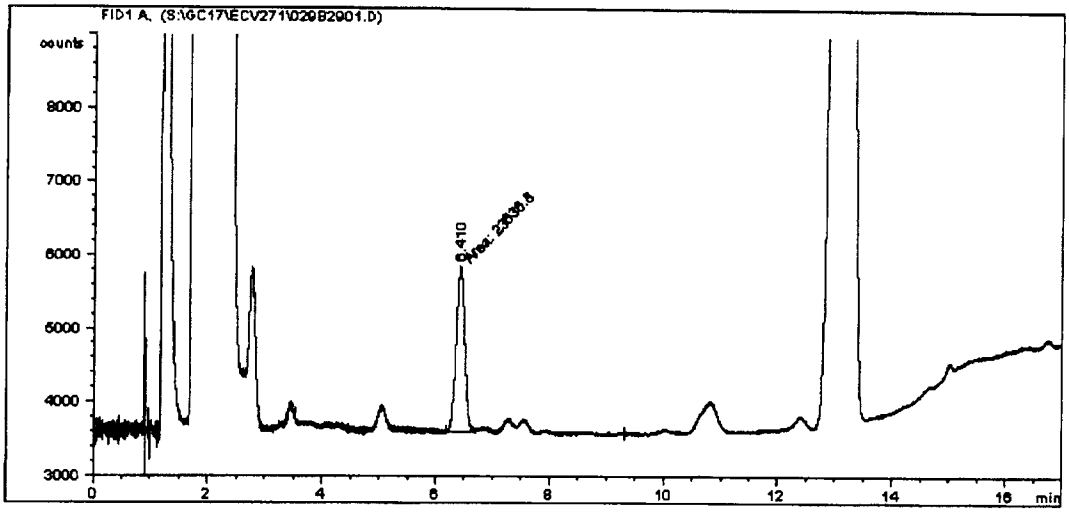


Test Sample 56 mg/l R_1

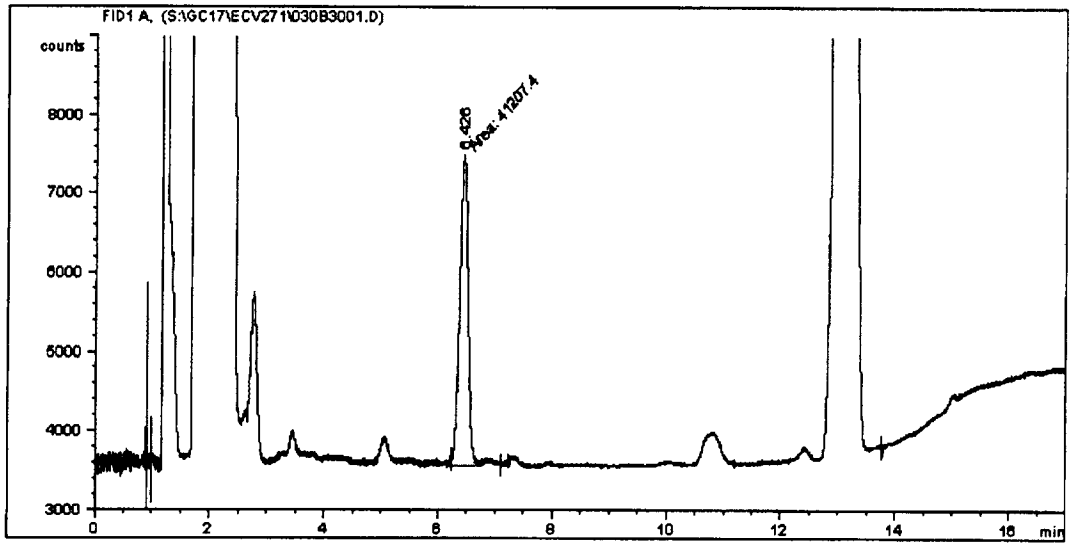
R_1 and R_2 = Replicates 1 and 2

Dibromomethane: ACUTE TOXICITY TO RAINBOW TROUT

Appendix 2 (continued) Verification of Test Concentrations



Test Sample 56 mg/l R_2

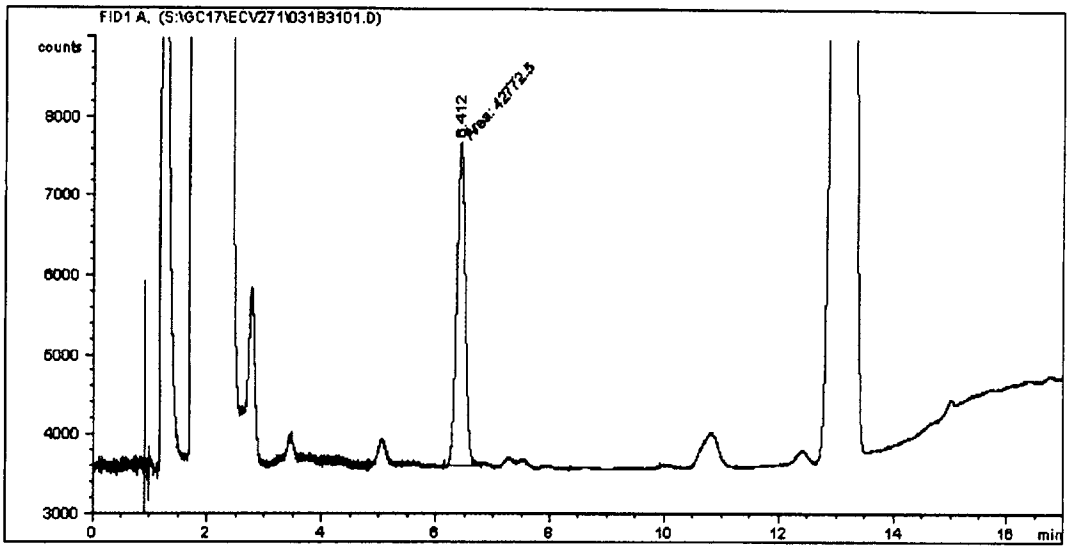


Test Sample 100 mg/l R_1

R_1 and R_2 = Replicates 1 and 2

Dibromomethane: ACUTE TOXICITY TO RAINBOW TROUT

Appendix 2 (continued) Verification of Test Concentrations



Test Sample 100 mg/l R_2

R_2 = Replicate 2

Dibromomethane: ACUTE TOXICITY TO RAINBOW TROUT

Appendix 3 Typical Water Quality Characteristics

WATER SUPPLY ZONE ZDB13 ALVASTON

REPORTING PERIOD: 01/01/2005 - 31/12/2005

PARAMETER	ANALYSIS UNITS	NO. SAMPLES TAKEN	CONCENTRATION OR VALUE (ALL SAMPLES)			PCV	SAMPLES CONTRAVENING PCV	
			MINIMUM	AVERAGE	MAXIMUM		NUMBER	PERCENT
1,2 Dichloroethane	µg/l	8	<0.080	<0.090	0.120	3	0	0.00
2, 3, 6 - TBA	µg/l	8	<0.002	<0.002	0.002	0.1	0	0.00
2, 4 - DB	µg/l	8	<0.003	<0.003	0.003	0.1	0	0.00
2, 4, 5, - TCPA	µg/l	8	<0.003	<0.003	0.003	0.1	0	0.00
2,4-D	µg/l	8	<0.003	<0.012	0.031	0.1	0	0.00
Aldrin	µg/l	8	<0.002	<0.002	0.002	0.03	0	0.00
Alpha-HCH	µg/l	8	<0.001	<0.001	0.001	0.1	0	0.00
Aluminium	µg/l	52	<5.000	<14.135	47.000	200	0	0.00
Antimony	µg/l	8	<0.240	<0.556	1.200	5	0	0.00
Arsenic	µg/l	8	<0.500	<0.638	1.600	10	0	0.00
Benazolin	µg/l	8	<0.002	<0.002	0.002	0.1	0	0.00
Bentazone	µg/l	8	<0.005	<0.005	0.005	0.1	0	0.00
Benzene	µg/l	8	<0.060	<0.083	0.150	1	0	0.00
Benzo 3,4 Pyrene	µg/l	8	<0.001	<0.001	0.001	0.01	0	0.00
Beta - HCH	µg/l	8	<0.002	<0.002	0.002	0.1	0	0.00
Boron	mg/l	8	0.010	0.032	0.064	1	0	0.00
Bromate	µg/l	8	<0.600	<0.675	0.800	10	0	0.00
Bromoxynil	µg/l	8	<0.001	<0.001	0.002	0.1	0	0.00
Cadmium	µg/l	8	<0.230	<0.246	0.320	5	0	0.00
Chlordane A	µg/l	8	<0.002	<0.002	0.002	0.1	0	0.00
Chlorothalonil	µg/l	8	<0.001	<0.001	0.001	0.1	0	0.00
Chromium	µg/l	8	<0.340	<0.420	0.520	50	0	0.00
Cis - Permethrin	µg/l	8	<0.001	<0.001	0.001	0.1	0	0.00
Clopyralid	µg/l	8	<0.005	<0.010	0.034	0.1	0	0.00
Colour	mg/l	26	<0.400	<0.738	1.200	20	0	0.00
Copper	mg/l	8	<0.001	<0.004	0.007	2	0	0.00
Cyanide	µg/l	8	<0.220	<0.295	0.590	50	0	0.00
Cyfluthrin	µg/l	8	<0.040	<0.040	0.040	0.1	0	0.00
Cypermethrin	µg/l	8	<0.040	<0.040	0.040	0.1	0	0.00
D.D.E - Para Para	µg/l	8	<0.002	<0.002	0.002	0.1	0	0.00
D.D.E - Ortho Para	µg/l	8	<0.002	<0.002	0.002	0.1	0	0.00
D.D.T - Ortho Para	µg/l	8	<0.002	<0.002	0.002	0.1	0	0.00
D.D.T - Para Para	µg/l	8	<0.002	<0.002	0.002	0.1	0	0.00
Delta - HCH	µg/l	8	<0.002	<0.002	0.002	0.1	0	0.00
Deltamethrin	µg/l	8	<0.020	<0.020	0.020	0.1	0	0.00
Dicamba	µg/l	8	<0.001	<0.001	0.001	0.1	0	0.00
Dichlobenil	µg/l	8	<0.001	<0.001	0.001	0.1	0	0.00
Dichloprop	µg/l	8	<0.002	<0.002	0.002	0.1	0	0.00
Dieldrin	µg/l	8	<0.002	<0.002	0.002	0.03	0	0.00
E. coli	No. / 100 ml	180	0.000	0.000	0.000	0	0	0.00
Endosulfan A	µg/l	8	<0.001	<0.001	0.001	0.1	0	0.00
Endosulfan B	µg/l	8	<0.004	<0.004	0.004	0.1	0	0.00
Endrin	µg/l	8	<0.002	<0.002	0.002	0.1	0	0.00
Enterococci	No. / 100 ml	8	0.000	<0.000	0.000	0	0	0.00
Fenoprop	µg/l	8	<0.003	<0.003	0.003	0.1	0	0.00
Fenvalerate	µg/l	8	<0.020	<0.020	0.020	0.1	0	0.00
Fluoride	mg/l	8	0.240	0.326	0.430	1.5	0	0.00
Fluroxypyr	µg/l	8	<0.003	<0.003	0.003	0.1	0	0.00
Gamma - HCH	µg/l	8	<0.002	<0.002	0.002	0.1	0	0.00
Heptachlor	µg/l	8	<0.002	<0.002	0.002	0.03	0	0.00
Heptachlor epoxide	µg/l	8	<0.002	<0.002	0.002	0.03	0	0.00
Hexachlorobenzene	µg/l	8	<0.001	<0.001	0.001	0.1	0	0.00
Hexachlorobutadiene	µg/l	8	<0.002	<0.002	0.002	0.1	0	0.00
Ioxynil	µg/l	8	<0.002	<0.002	0.002	0.1	0	0.00
Iron	µg/l	52	<7.000	<30.442	76.000	200	0	0.00
Isodrin	µg/l	8	<0.002	<0.002	0.002	0.1	0	0.00
Lead	µg/l	8	<1.000	<1.913	5.000	25	0	0.00
Manganese	µg/l	52	<1.500	<1.690	3.400	50	0	0.00
MCPA	µg/l	8	<0.002	<0.008	0.023	0.1	0	0.00
MCPB	µg/l	8	<0.006	<0.006	0.006	0.1	0	0.00

PCV = Prescribed concentration or value for mandatory parameters and specified limit or value for non-mandatory parameters (indicators and disinfectants)

Dibromomethane: ACUTE TOXICITY TO RAINBOW TROUT

Appendix 3 (continued) Typical Water Quality Characteristics

WATER SUPPLY ZONE ZDB13 ALVASTON

REPORTING PERIOD: 01/01/2005 - 31/12/2005

PARAMETER	ANALYSIS UNITS	NO. SAMPLES TAKEN	CONCENTRATION OR VALUE (ALL SAMPLES)			PCV	SAMPLES CONTRAVENING PCV	
			MINIMUM	AVERAGE	MAXIMUM		NUMBER	PERCENT
Mecoprop	µg/l	8	<0.001	<0.006	0.014	0.1	0	0.00
Mercury	µg/l	8	<0.015	<0.021	0.061	1	0	0.00
Methoxychlor	µg/l	8	<0.002	<0.002	0.002	0.1	0	0.00
Nickel	µg/l	8	<1.400	<1.925	2.400	20	0	0.00
Nitrate	mg/l	8	10.400	12.500	15.100	50	0	0.00
Nitrite	mg/l	8	<0.002	<0.002	0.002	0.5	0	0.00
Nitrite/Nitrate Calculated	mg/l	8	<0.260	<0.270	0.300	<1***	0	0.00
Odour	Dilution Number	26	0.000	0.000	0.000	3 at 25°C	0	0.00
PAH	µg/l	8	0.000	0.000	0.000	0.1	0	0.00
PCB – Arochlor 1254	µg/l	8	<0.003	<0.003	0.003	0.1	0	0.00
pH	pH value	52	7.100	7.564	7.800	Max 9.5, Min 6.5	0	0.00
Selenium	µg/l	8	0.480	0.671	0.990	10	0	0.00
Sodium	µg/l	8	19.000	25.625	44.000	200	0	0.00
T.D.E – Ortho Para	µg/l	8	<0.002	<0.002	0.002	0.1	0	0.00
T.D.E – Para Para	µg/l	8	<0.002	<0.002	0.002	0.1	0	0.00
Taste	Dilution Number	26	0.000	0.000	0.000	3 at 25°C	0	0.00
Tetrachloroethane and Trichloroethane	µg/l	8	0.000	0.048	0.170	10	0	0.00
Tetrachloromethane	µg/l	8	<0.020	<0.033	0.040	3	0	0.00
Total Pesticides	µg/l	8	0.000	0.028	0.066	0.5	0	0.00
Total Trihalomethanes	µg/l	8	23.400	39.525	53.700	100	0	0.00
Trans - Permethrin	µg/l	8	<0.002	<0.002	0.002	0.1	0	0.00
Trichlorobenzene	µg/l	8	<0.001	<0.001	0.001	0.1	0	0.00
Triclopyr	µg/l	8	<0.004	<0.005	0.010	0.1	0	0.00
Turbidity	NTU	52	<0.050	<0.109	0.250	4	0	0.00
Free Chlorine	mg/l	180	0.010	0.211	0.480	-**	-	-
Total Chlorine	mg/l	180	0.080	0.287	0.550	-**	-	-
Ammonium	mg/l	52	<0.021	<0.022	0.032	0.5	0	0.00
Chloride	mg/l	8	21.800	26.513	33.900	250	0	0.00
Clostridium perfringens	No. / 100 ml	26	0.000	0.000	0.000	0	0	0.00
Coliform Bacteria	No. / 100 ml	180	0.000	0.000	0.000	0	0	0.00
Colony Count after 72 hours at 22°C	No. / 100 ml	52	0.000	2.712	84.000	-**	-	-
Colony Counts after 48 hours at 37°C	No. / 100 ml	52	0.000	1.885	31.000	-**	-	-
Coductivity	µS/cm at 20°C	26	331.000	410.769	539.000	2500	0	0.00
Gross Alpha Activity	Bq/l	8	<0.019	<0.033	0.047	0.1***	-	-
Gross Beta Activity	Bq/l	8	0.040	0.079	0.122	1***	-	-
Sulphate	mg/l	8	58.800	72.838	105.000	250	0	0.00
Total Organic Carbon	mg/l	8	1.380	1.573	2.040	-**	-	-

PCV = Prescribed concentration or value for mandatory parameters and specified limit or value for non-mandatory parameters (indicators and disinfectants)

Dibromomethane: ACUTE TOXICITY TO RAINBOW TROUT

Appendix 4 Physico-Chemical Measurements

Nominal Concentration (mg/l)	Time (Hours)											
	0 Hours (Fresh Media)				24 Hours (Old Media)				24 Hours (Fresh Media)			
	pH	mg O_2/l	%ASV*	T°C	pH	mg O_2/l	%ASV*	T°C	pH	mg O_2/l	%ASV*	T°C
Control R_1	7.8	10.7	104	13.8	7.3	6.7	66	14.8	7.6	10.9	106	13.4
Control R_2	7.7	10.7	104	13.6	7.3	6.8	67	14.7	7.6	11.0	107	13.2
10 R_1	7.7	10.7	104	13.8	7.2	7.0	69	14.7	7.6	10.6	103	13.7
10 R_2	7.7	10.6	103	13.9	7.3	7.0	69	14.6	7.6	10.6	103	13.3
18 R_1	7.7	10.7	104	13.8	7.3	7.0	69	14.7	7.7	10.7	104	13.8
18 R_2	7.7	10.7	104	13.8	7.3	6.8	67	14.8	7.6	10.7	104	13.8
32 R_1	7.7	10.6	103	13.7	7.3	7.0	69	14.7	7.6	10.6	103	13.7
32 R_2	7.7	10.7	104	13.7	7.3	7.0	69	14.6	7.6	10.7	104	13.9
56 R_1	7.8	10.6	103	13.8	7.4	8.6	85	14.7	7.5	10.5	102	13.9
56 R_2	7.7	10.7	104	14.2	7.4	8.2	81	14.7	7.6	10.5	102	13.9
100 R_1	7.7	10.5	102	14.7	7.6	10.1	100	14.6	-	-	A/D	-
100 R_2	7.7	10.5	102	14.9	7.6	10.2	100	14.6	-	-	A/D	-

Nominal Concentration (mg/l)	Time (Hours)											
	48 Hours (Old Media)				48 Hours (Fresh Media)				72 Hours (Old Media)			
	pH	mg O_2/l	%ASV*	T°C	pH	mg O_2/l	%ASV*	T°C	pH	mg O_2/l	%ASV*	T°C
Control R_1	7.5	7.5	74	14.7	7.7	10.4	101	13.7	7.5	7.9	78	14.5
Control R_2	7.4	7.5	74	14.6	7.7	10.4	101	13.6	7.4	7.6	74	14.4
10 R_1	7.3	7.3	72	14.6	7.7	10.4	101	13.6	7.4	8.2	80	14.1
10 R_2	7.3	7.3	72	14.7	7.7	10.3	100	13.7	7.4	7.6	74	14.2
18 R_1	7.3	7.4	73	14.6	7.7	10.3	100	13.7	7.4	8.1	79	14.2
18 R_2	7.3	7.5	74	14.6	7.7	10.4	101	13.7	7.3	7.5	73	14.1
32 R_1	7.3	7.3	72	14.7	7.7	10.4	101	13.5	7.4	8.2	81	14.2
32 R_2	7.3	7.2	71	14.6	7.7	10.4	101	13.6	7.3	7.8	77	14.2
56 R_1	7.5	9.7	96	14.5	7.7	10.4	101	13.6	7.6	10.2	99	14.2
56 R_2	7.5	9.2	91	14.6	7.7	10.4	101	13.6	7.6	10.2	101	14.5
100 R_1	-	-	A/D	-	-	-	-	-	-	-	-	-
100 R_2	-	-	A/D	-	-	-	-	-	-	-	-	-

*ASV = Dissolved oxygen concentration expressed as a percentage of Air Saturation Value
R_1 and R_2 = Replicates 1 and 2
A/D = All fish dead

Dibromomethane: ACUTE TOXICITY TO RAINBOW TROUT

Appendix 4 (continued) Physico-Chemical Measurements

Nominal Concentration (mg/l)	Time (Hours)							
	72 Hours (Fresh Media)				96 Hours (Old Media)			
	pH	mg O_2/l	%ASV*	T°C	pH	mg O_2/l	%ASV*	T°C
Control R_1	7.6	10.6	101	13.3	7.4	8.0	78	14.1
Control R_2	7.6	10.5	100	13.5	7.5	8.3	81	14.0
10 R_1	7.6	10.7	104	13.9	7.4	8.3	81	13.6
10 R_2	7.6	10.7	104	14.0	7.4	8.0	78	13.7
18 R_1	7.6	10.7	104	13.9	7.4	8.3	81	13.7
18 R_2	7.6	10.6	103	14.0	7.4	8.0	78	13.7
32 R_1	7.6	10.7	104	14.0	7.4	8.1	79	13.8
32 R_2	7.6	10.6	103	14.0	7.4	7.9	77	13.9
56 R_1	7.6	10.7	104	14.0	7.4	10.3	100	13.8
56 R_2	7.6	10.7	104	13.9	7.7	10.4	101	14.0
100 R_1	-	-	A/D	-	-	-	-	-
100 R_2	-	-	A/D	-	-	-	-	-

* ASV = Dissolved oxygen concentration expressed as a percentage of Air Saturation Value
R_1 and R_2 = Replicates 1 and 2
A/D = All fish dead

Dibromomethane: ACUTE TOXICITY TO RAINBOW TROUT

Appendix 5 Statement of GLP Compliance in Accordance with Directive 2004/9/EC

Department of Health

THE DEPARTMENT OF HEALTH OF THE GOVERNMENT OF THE UNITED KINGDOM

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE
IN ACCORDANCE WITH DIRECTIVE 2004/9/EC

LABORATORY	TEST TYPE
SafePharm Laboratories Ltd. **Shardlow Business Park** **London Road** **Shardlow** **Derby** **DE72 2GD**	**Analytical Chemistry** **Environmental Fate** **Environmental Toxicity** **Mutagenicity** **Phys/Chem Testing** **Toxicology**

DATE OF INSPECTION

30th August 2005

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above laboratory as part of the UK GLP Compliance Programme.

At the time of inspection no deviations were found of sufficient magnitude to affect the validity of non-clinical studies performed at these facilities.

Bryan J Wright 21/11/05.

Mr. Bryan J. Wright
Head, UK GLP Monitoring Authority