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December 28, 1998

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401 M Street, S.W.
Washington, DC 20460



8EHQ-98-14304

Attention: **TSCA SECTION 8(E) COORDINATOR**

REFERENCE: 8EHQ-98-14304

Contains No CBI

Dear Sir/Madam:

As a follow-up to our previous 8(e) submission dated October 21, 1998, for a commercial material which contained ~98% Carbamothioic acid, 2-propenyl-, O-(2-methylpropyl) ester [CAS Number 86329-09-1], I am enclosing a copy of the final report entitled "AERO® 5100 Promoter - Acute Toxicity To *Daphnia magna*".

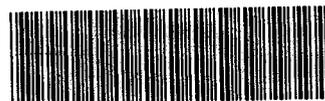
This report was received by CYTEC on December 28, 1998. This report **does not** contain confidential business information.

If you have any questions please contact me at (973) 357-3375.

Sincerely,

Patricia Ann Vernon
Product Regulatory Compliance
Manager, Asia-Pacific

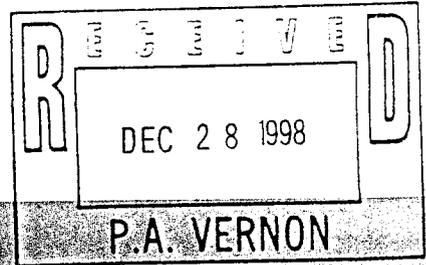
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AERO® 5100 Promoter

**ACUTE TOXICITY
TO *DAPHNIA MAGNA***

Report

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AERO® 5100 Promoter

ACUTE TOXICITY TO *DAPHNIA MAGNA*

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Sponsor

Cytec Industries Inc
Five Garret Mountain Plaza
West Paterson
NJ 07424
USA

Research Laboratory

Huntingdon Life Sciences Ltd.
P.O. Box 2,
Huntingdon,
Cambridgeshire,
ENGLAND.

Report Issued: 23 December 1998

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COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

The study described in this report was conducted in compliance with the following Good Laboratory Practice standards and I consider the data generated to be valid.

The UK Good Laboratory Practice Regulations 1997 (Statutory Instrument No 654).

EC Council Directive 87/18/EEC of 18 December 1986 (Official Journal No L 15/29).

OECD Principles of Good Laboratory Practice (as revised in 1997),
ENV/MC/CHEM(98)17.



.....
Graeme Bell,
Study Director,
Department of Ecotoxicology,
Huntingdon Life Sciences Ltd.

23 December 1998
Date

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QUALITY ASSURANCE STATEMENT

The following have been inspected or audited in relation to this study

Study Phases Inspected	Date of Inspection	Date of Reporting
Process Based Inspections		
Test media preparation	} 25-26 August 1998	27 August 1998
Experimental set-up		
Environmental measurements		
Clinical observations		
Sampling procedures		
Housing & environment		
Study data records		
Report	8 December 1998	10 December 1998

Process based inspections: At or about the time this study was in progress inspections and audits of routine and repetitive procedures employed on this type of study were carried out. These were conducted and reported to appropriate Company Management as indicated above

Report Audit: This report has been audited by the Quality Assurance Department. This audit was conducted and reported to the Study Director and Company Management as indicated above.

The methods, procedures and observations were found to be accurately described and the reported results to reflect the raw data.



 Tracy Scarfe,
 Quality Assurance Group Leader,
 Quality Assurance Department,
 Huntingdon Life Sciences Ltd.

22 DECEMBER 1998

 Date

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RESPONSIBLE PERSONNEL

STUDY MANAGEMENT

Graeme Bell, M.Sc.,
Senior Study Manager,
Department of Ecotoxicology.

CHEMICAL ANALYSIS

Ben Smith, M.Sc., M.R.S.C., C.Chem.,
Chief Chemist,
Department of Ecotoxicology

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SUMMARY

The acute toxicity of AERO® 5100 Promoter to *Daphnia magna* was assessed under static exposure conditions.

The study was conducted in accordance with EEC Methods for Determination of Ecotoxicity Annex to Directive 92/69/EEC (O.J. No. L383A, 29.12.92) Part C, Method 2 "Acute toxicity to *Daphnia*" and the OECD Guideline for Testing of Chemicals No. 202, Part I "*Daphnia* Acute Immobilisation Test".

Groups of twenty, first instar *Daphnia*, less than 24 hours old, were exposed to AERO® 5100 Promoter for 48 hours at nominal concentrations of 0.10, 0.22, 0.46, 1.0, 2.2, 4.6, 10 & 22 µg/l dispersed in Elenedt M4 medium. The numbers of immobilised daphnids were recorded for each test and control group after 24 and 48 hours and the following values were determined. All results are expressed in terms of nominal concentration.

Time (hours)	EC ₅₀ (µg/l)	95% confidence limits (µg/l)
24	>22	-
48	6.8	3.2 - 15

Highest test concentration resulting in 0% immobilisation:	<0.10 µg/l*
Lowest test concentration resulting in 100% immobilisation:	>22 µg/l
'No-observed effect concentration'	0.22 µg/l

* 10% immobilisation is not considered to be biologically significant under the conditions of this study design. Consequently 0.10 µg/l can be considered to be a close approximation of the highest test concentration resulting in 0% immobilisation.

Analysis of 4.6, 10 & 22 µg/l test concentrations gave measured concentrations ranging from 144 - 107 % of nominal at 0 hours and 91 - 83 % of nominal at 48 hours.

Due to the limited sensitivity of the method of analysis (limit of quantification >4 µg/l) it was not possible to verify the aqueous test concentrations below 4.6 µg/l. However, the stock solutions used to prepare all the exposure concentrations were analysed and found to be 97 - 72 % of nominal at 0 hours, so it is reasonable to infer that near nominal concentrations were achieved at the start of the study.

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INTRODUCTION

The objective of the study was to determine the acute toxicity (48 hour median effect concentration - EC₅₀) of AERO® 5100 Promoter to *Daphnia magna*.

The study was conducted in accordance with EEC Methods for Determination of Ecotoxicity Annex to Directive 92/69/EEC (O.J. No. L383A, 29.12.92) Part C, Method 2 "Acute toxicity to *Daphnia*" and the OECD Guideline for Testing of Chemicals No. 202, Part I "*Daphnia* Acute Immobilisation Test".

The protocol was approved by Huntingdon Life Sciences management on 3 April 1998, by the Sponsor on 14 April 1998 and by the Study Director on 12 May 1998.

The experimental phase of the definitive study was conducted between 25 and 27 August 1998.

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TEST SUBSTANCE

Identity: AERO[®] 5100 Promoter

Alternative name: CT-637-97

Chemical name: Carbamothioic acid, 2-propenyl-, O-(2-methylpropyl) ester

Intended use: Flotation mineral collector

Appearance: Pale brown liquid

Storage conditions: Room temperature in the dark

Lot No: 95

Expiry date: March 1999

Purity: ~98%

Date received: 4 March 1998

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EXPERIMENTAL PROCEDURE

TEST ORGANISM

Daphnia magna (Straus) used in this study were cultured in-house and were obtained from a strain originating from the Institute National de Recherche Chimique Appliqué (IRChA), France.

Stock cultures of *Daphnia magna* were maintained in glass vessels containing approximately 800ml of Elendt M4 culture medium with a 16 hour light: 8 hour dark photoperiod at $20 \pm 2^\circ\text{C}$. Cultures were fed daily with a suspension of the unicellular green alga, *Chlorella vulgaris*. Culture conditions ensure that cultures reproduce by parthenogenesis.

The day before the start of the study, all juvenile *Daphnia* were removed from the laboratory cultures. The following morning, juveniles produced by the gravid (egg-bearing) adult *Daphnia* were removed from the culture vessels and held in a separate holding vessel; these animals, which were less than 24 hours old, were used in the test.

DILUTION WATER

The test organisms were maintained and the tests conducted in a reconstituted medium Elendt M4. The medium was prepared using analytical grade reagents and deionised water. Appendix 2 gives full details of the medium.

TEST SUBSTANCE PREPARATION

Method of preparation

An aliquot (50 mg) of test substance was dissolved in 50 ml of ethanol to give an initial stock solution of 1 mg/ml. Serial dilutions of this stock solution were prepared with ethanol. Due to the volatility of the test substance (100% volatile by weight) an "air-free" test design was employed. Bottles of a total volume of 280 ml with air-tight screw caps, were filled to capacity with Elendt M4 medium and the caps secured. Each bottle was inverted and using a syringe to puncture a Teflon coated disc, located in the centre of the bottle cap, was spiked with 28 μl of the appropriate stock solution (to give a solvent loading equivalent to 100 $\mu\text{l/l}$). Each bottle was then shaken vigorously to aid dispersion.

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Stability of test concentrations

The 22, 10 & 4.6 µg/l test concentrations were verified by chemical analysis (see THE DETERMINATION OF AERO® 5100 PROMOTER IN AQUEOUS SAMPLES). At 0 hours, additional duplicate vessels (280 ml) were prepared in an identical manner to those used in the test and their contents analysed. After 48 hours, the contents of the vessels used in the test were analysed.

EXPOSURE CONDITIONS

Experimental design

Eight test concentrations were prepared with one control and one solvent control (containing 100 µl auxiliary solvent per litre dilution water). Four replicates were prepared at each test concentration, control and solvent control. The test vessels were 280 ml capacity glass bottles with air-tight caps.

Five first instar *Daphnia* were placed without conscious bias into each bottle to give a loading of 56 ml test solution per organism. The bottle caps were tightly secured to minimise losses of test substance.

Selection of test concentrations

Preliminary range finding tests were conducted using test concentrations of 0.01 µg/l to 100 µg/l, under similar exposure conditions to those subsequently used in the definitive test. The results of these preliminary range finding tests indicated that the 'No-observed effect concentration' (NOEC) was approximately 0.1 µg/l and the lowest test concentration resulting in 100% immobilisation was approximately 100 µg/l.

Test concentrations

The following test concentrations were prepared based on the results of the earlier range finding study.

Nominal test concentrations:	22, 10, 4.6, 2.2, 1.0, 0.46, 0.22 & 0.10 µg/l
Mean measured test concentrations:	22, 9.5, 5.1 µg/l*

* Limit of quantification > 4 µg/l

Nominal exposure concentrations quoted in this report refer to the test material as received; no allowance has been made for a purity of less than 100%.

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Medium renewal

Daphnia were exposed to the test or control conditions for a period of 48 hours without renewal of test media.

Environmental conditions

Cultures were maintained at $20\pm 2^{\circ}\text{C}$, with a photoperiod of 16 hours light: 8 hours dark and without supplementary aeration or feeding during the 48 hour exposure period.

The temperature, pH and dissolved oxygen levels in each vessel were recorded at the start and at the end of the study (see Table 2 and Appendix 1).

Criterion of effect

Daphnia were considered to be immobile if they were unable to swim within approximately 15 seconds following gentle agitation of the test vessel.

EVALUATION OF DATA

EC_{50} values and 95% confidence limits were calculated using the Thompson and Weil model (Thompson and Weil, 1952).

References

Thompson, W.R. & Weil, C.S., 1952, *Biometrics* 8: 51 -54.

ARCHIVES

All specimens, raw data and study related documents generated during the course of the study at Huntingdon Life Sciences, together with a copy of the final report will be lodged in the Huntingdon Life Sciences Archive.

Such specimens and records will be retained for a minimum period of five years from the date of issue of the final report. At the end of the five year retention period the Sponsor will be contacted and advice sought on the future requirements. Under no circumstances will any item be discarded without the Sponsor's knowledge.

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RESULTS

Cumulative immobilisation data are given in Table 1 and the relationship between percentage immobilisation and concentration at 48 hours is given in Figure 1. All results are expressed in terms of nominal concentration (see Table 3, Appendix 3 and THE DETERMINATION OF AERO® 5100 PROMOTER IN AQUEOUS SAMPLES).

Analysis of 4.6, 10 & 22 µg/l test concentrations gave measured concentrations ranging from 144 - 107 % of nominal at 0 hours and 91 - 83 % of nominal at 48 hours.

Due to the limited sensitivity of the method of analysis (limit of quantification >4 µg/l) it was not possible to verify the aqueous test concentrations below 4.6 µg/l. However, the stock solutions used to prepare all the exposure concentrations were analysed and found to be 97 - 72 % of nominal at 0 hours, so it is reasonable to infer that near nominal concentrations were achieved at the start of the study.

Analysis of the immobility data gave the following results:

Time (hours)	EC ₅₀ (µg/l)	95% confidence limits (µg/l)
24	>22	-
48	6.8	3.2 - 15

Highest test concentration resulting in 0% immobilisation:	<0.10 µg/l*
Lowest test concentration resulting in 100% immobilisation:	>22 µg/l
'No-observed effect concentration'	0.22 µg/l

* 10% immobilisation is not considered to be biologically significant under the conditions of this study design. Consequently 0.10 µg/l can be considered to be a close approximation of the highest test concentration resulting in 0% immobilisation.

Individual pH, temperature and dissolved oxygen values remained within acceptable limits during the study (see Appendix 1). A summary table giving mean values for each control and test group is given in Table 2.

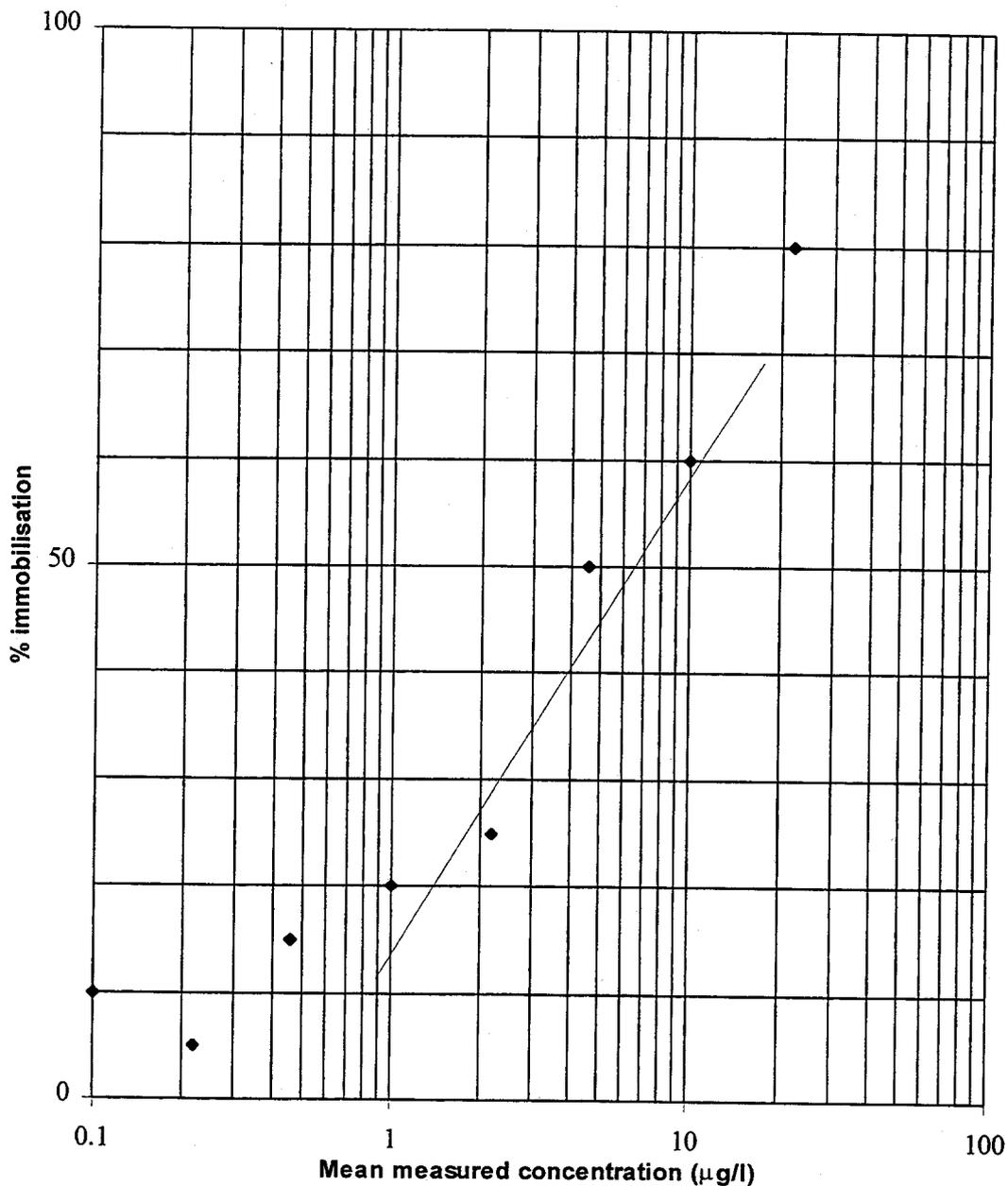
CONCLUSION

The 48 hour EC₅₀ (immobilisation) value for AERO® 5100 Promoter with *Daphnia magna* was determined to be 6.8 µg/l. The 'No-observed effect concentration' (NOEC) was 0.22 µg/l.

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FIGURE 1

Concentration-response curve for
Daphnia magna exposed for 48 hours to AERO® 5100 Promoter



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TABLE 1

**Cumulative immobilisation data for *Daphnia magna*
exposed for 48 hours to AERO® 5100 Promoter**

Nominal concentration $\mu\text{g/l}$	Geometric mean measured concentration $\mu\text{g/l}$	Cumulative immobilised <i>Daphnia magna</i> (initial population: 5 per replicate)												
		24 hours						48 hours						
		R ₁	R ₂	R ₃	R ₄	Total	%	R ₁	R ₂	R ₃	R ₄	Total	%	
Control	-	0	0	0	0	0	0	0	0	0	0	1	1	5
Solvent Control	ND	0	0	0	0	0	0	0	0	0	0	0	0	0
0.10	*	0	0	0	0	0	0	1	1	0	0	2	2	10
0.22	*	0	0	0	0	0	0	0	0	0	1	1	1	5
0.46	*	0	0	0	1	1	5	0	1	0	2	3	3	15
1.0	*	0	0	0	2	2	10	1	0	0	3	4	4	20
2.2	*	0	0	1	0	1	5	1	0	2	2	5	5	25
4.6	5.1	1	1	1	0	3	15	3	3	2	2	10	10	50
10	9.5	1	1	3	2	7	35	5	2	3	2	12	12	60
22	22	2	1	2	3	8	40	4	2	5	5	16	16	80

R₁ - R₄ Replicate No.

* Below limit of quantification 4 $\mu\text{g/l}$

ND None detected

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TABLE 2

AERO® 5100 Promoter: Environmental parameters
Mean values for pH, temperature and dissolved oxygen

Nominal concentration µg/l	Geometric mean concentration µg/l	Mean values		
		pH	mgO ₂ /l	T°C
Control	-	7.4	8.0	19
Solvent Control	ND	7.5	8.2	19
0.10	*	7.5	7.5	19
0.22	*	7.5	7.8	19
0.46	*	7.4	8.1	19
1.0	*	7.5	8.1	19
2.2	*	7.6	7.9	19
4.6	5.1	7.5	7.9	19
10	9.5	7.5	7.8	19
22	22	7.6	7.5	19

* Below limit of quantification 4 µg/l

ND None detected

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TABLE 3

**AERO® 5100 Promoter: Measured concentrations
Mean values and percentages of nominal**

Nominal concentration µg/l	Number of samples analysed	Mean measured concentration µg/l	% Nominal
Control	-	-	-
Solvent Control	2	ND	-
0.10	-	*	-
0.22	-	*	-
0.46	-	*	-
1.0	-	*	-
2.2	-	*	-
4.6	2	5.1	111
10	2	9.5	95
22	2	22	100

* Below limit of quantification 4 µg/l

ND None detected

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APPENDIX 1

AERO® 5100 Promoter: Environmental measurements
Individual records of pH, temperature and dissolved oxygen

Concentration (µg/l)		0 hours			48 hours		
		pH	mgO ₂ /l	T°C	pH	mgO ₂ /l	T°C
Control	R ₁	7.5	8.6	19	7.3	7.4	19
	R ₂	7.5	8.6	19	7.3	7.5	19
	R ₃	7.5	8.6	19	7.3	7.4	19
	R ₄	7.5	8.6	19	7.3	7.6	19
Solvent control	R ₁	7.6	8.6	19	7.3	7.8	19
	R ₂	7.6	8.6	19	7.3	8.0	19
	R ₃	7.6	8.6	19	7.3	7.9	19
	R ₄	7.6	8.6	19	7.3	7.4	19
0.1	R ₁	7.7	7.4	19	7.3	7.5	19
	R ₂	7.7	7.4	19	7.3	7.6	19
	R ₃	7.7	7.4	19	7.3	7.5	19
	R ₄	7.7	7.4	19	7.3	7.5	19
0.22	R ₁	8.3	8.4	19	6.8	6.9	19
	R ₂	8.3	8.4	19	6.7	7.3	19
	R ₃	8.3	8.4	19	6.8	7.2	19
	R ₄	8.3	8.4	19	6.8	7.0	19
0.46	R ₁	8.0	8.8	19	6.9	7.4	19
	R ₂	8.0	8.8	19	6.9	7.3	19
	R ₃	8.0	8.8	19	6.9	7.5	19
	R ₄	8.0	8.8	19	6.8	7.3	19
1.0	R ₁	7.8	8.6	19	7.2	7.9	19
	R ₂	7.8	8.6	19	7.2	7.8	19
	R ₃	7.8	8.6	19	7.1	7.4	19
	R ₄	7.8	8.6	19	7.2	7.3	19
2.2	R ₁	7.8	8.8	19	7.3	7.2	19
	R ₂	7.8	8.8	19	7.3	7.1	19
	R ₃	7.8	8.8	19	7.3	7.0	19
	R ₄	7.8	8.8	19	7.3	6.8	19
4.6	R ₁	7.7	9.0	19	7.3	7.1	19
	R ₂	7.7	9.0	19	7.3	6.8	19
	R ₃	7.7	9.0	19	7.3	6.8	19
	R ₄	7.7	9.0	19	7.3	6.8	19
10	R ₁	7.7	9.0	19	7.3	6.7	19
	R ₂	7.7	9.0	19	7.3	6.7	19
	R ₃	7.7	9.0	19	7.3	6.5	19
	R ₄	7.7	9.0	19	7.3	6.6	19
22	R ₁	7.9	8.8	19	7.3	6.1	19
	R ₂	7.9	8.8	19	7.3	6.3	19
	R ₃	7.9	8.8	19	7.3	6.2	19
	R ₄	7.9	8.8	19	7.3	6.2	19

R₁ - R₄ Replicate No.

pH meter: Sentron 1001

Dissolved oxygen meter: YSI model 57

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APPENDIX 2

Elendt M4 medium

1.	Trace elements	mg/l
	H ₃ BO ₃	2.86
	MnCl ₂ .4H ₂ O	0.36
	LiCl	0.31
	RbCl	0.071
	SrCl ₂ .6H ₂ O	0.152
	NaBr	0.016
	Na ₂ MoO ₄ .2H ₂ O	0.063
	CuCl ₂ .2H ₂ O	0.017
	ZnCl ₂	0.013
	CoCl ₂ .6H ₂ O	0.010
	KI	0.0033
	Na ₂ .SeO ₃	0.0022
	NH ₄ VO ₃	0.00058
	Fe-EDTA solution	3.50
2.	Macro nutrients	mg/l
	CaCl ₂ .2H ₂ O	294
	MgSO ₄ .7H ₂ O	123
	KCL	5.80
	NaHCO ₃	64.8
	Na ₂ .SiO ₃ .9H ₂ O	10.0
	NaNO ₃	0.274
	KH ₂ PO ₄	0.143
	K ₂ HPO ₄	0.184
3.	Vitamins	mg/l
	Thiamine hydrochloride	0.075
	Cyanocobalamine (Vitamin B12)	0.0010
	Biotin (Vitamin H)	0.00075

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The above analytical grade reagents are dissolved in deionised water.

APPENDIX 3

Summary of Analytical Chemistry Results

Test solutions

Occasion	Nominal concentration (µg/l)	Measured concentration (µg/l)	Expressed as a percentage of nominal
0 hours	Control	ND	-
	4.6	6.634	144
	10	10.94	109
	22	23.59	107
48 hours	Control	ND	-
	4.6	3.924	85.3
	10	8.328	83.3
	22	20.04	91.1

Stock solutions

Nominal concentration (mg/ml)	Measured concentration (mg/ml)	Expressed as a percentage of nominal
1.0	0.7207	72.1
2.2	1.669	75.9
4.6	4.193	91.2
10	9.246	92.5
22	20.66	93.9
46	43.69	95.0
100	97.43	97.4
220	200.1	91.0
1000	954.8	95.5

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Summary of Analytical Chemistry Results (continued)**Procedural recoveries (test levels)**

Occasion	Fortified concentration (µg/l)	Recovery as a percentage of fortified
0 hours	Control	ND
	10.88	92.9
	10.88	93.9
48 hours	Control	ND
	10.88	91.9
	10.88	94.8
Mean (RSD)		93.3 (1.3)

Procedural recoveries (stock levels)

Fortified concentration (mg/ml)	Recovery as a percentage of fortified
21.76	101
21.76	97.7
Mean	99.1

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THE DETERMINATION OF AERO® 5100 PROMOTER IN AQUEOUS SAMPLES

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SAMPLE ANALYSIS**Method 1 (22 µg/l and lower)**

Aqueous samples (500 ml) were extracted by liquid-liquid partitioning into chloroform (3×50 ml); the organic extracts were evaporated to dryness in the presence of a keeper (10 µl dodecane) and the residues dissolved in sufficient chloroform to bring the expected AERO® 5100 promoter concentration within the calibration range.

The processed samples were analysed for AERO® 5100 promoter by gas chromatography using a flame ionisation detector.

Method 2 (stock solutions)

Stock solutions in ethanol were diluted with ethanol where necessary to bring the expected AERO® 5100 promoter concentration within the calibration range.

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CHROMATOGRAPHY INSTRUMENTATION AND CONDITIONS

A gas chromatography system comprising autosampler, injector, oven, detector (flame ionization), and data collection system was used.

Column		
	Material:	Fused silica capillary
	Type:	5% phenylmethyl silicone
	Dimensions (l × id):	30 m × 0.53 mm
Carrier gas:		Nitrogen
Flow:		1.5 ml/min
Oven temperature settings		
	Initial	50°C held for 1 min
	Rate	25°C/min
	Final	200 °C held for 5 mins
Injector:		
	Type:	Splitless
	Injection volume:	1 µl (5µl for Daphnia, version 5)
	Temperature:	200°C
Septum purge		
	Gas type:	Nitrogen
	Flow:	26 ml/min
Detector		
	Type:	Flame ionisation
	Temperature	250°C
	Makeup gas	Nitrogen
	Makeup flow	28.5 ml/min
	Hydrogen	35 ml/min
	Air	400ml/min

Under the above conditions AERO® 5100 promoter chromatographed as a single peak see Figures 2 and 4.

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CALIBRATION SOLUTIONS

Calibration solutions were prepared with the same batch (L95) of AERO® 5100 promoter used in the biological phase of the study. Results are consequently expressed in terms of the test material as supplied.

Working calibration solutions in the nominal range 12.5 to 1 mg/l were prepared by volumetric dilution with chloroform (test solutions) or ethanol (stock solutions) of a primary standard prepared in ethanol.

CALCULATIONS

AERO® 5100 promoter concentrations were determined using external standards.

Peak height responses for AERO® 5100 promoter in the calibration standards chromatography showed a quadratic relationship with calibration standard concentrations.

$$C_A \text{ (mg/l)} = \frac{-b \pm \sqrt{(b^2 - 4a(c - y))}}{2a}$$

y = Peak height response for sample
a, b, c = coefficients for the quadratic equation below

$$y = ax^2 + bx + c$$

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VALIDATION OF THE ANALYTICAL PROCEDURE

The analytical procedure was validated by determining the linearity of response of the analytical system, specificity of chromatographic analysis, the limits of detection and quantification and the method's accuracy and precision (Table 1).

During the course of the study the performance of the method was monitored by the analysis of quality control samples.

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TABLE 1

Validation recoveries of AERO® 5100 promoter from fortified samples of dilution media

Medium	Method	Fortification level (mg/l)	Recovery as a % of fortification level
Dechlorinated tap water	1	Control	ND
	1	2.044	97.0, 96.6
Algal	1	Control	ND
	1	0.2555	83.8, 88.1
	1	1.022	97.5, 97.7
Elendt M4	1	15.33	100, 97.9
	2 ¹	Control	(0.0007 mg/l)
	2 ¹	0.0003475	59.0, 49.3
	2 ¹	0.001022	76.3, 73.3
	2 ¹	0.005110	85.7, 93.8
	2	Control	ND
	2	0.004088	95.3, 90.1
	2	0.01022	107, 105
	2	0.04088	103, 102
	2	0.1513	93.9, 97.7
Mean (RSD)			97.0 (6.2)

RSD: Relative standard deviation

ND: none detected; less than the limit of detection (Method 1: 0.06 mg/l, Method 2: 0.001 mg/l),

The limit of detection is defined as the analyte concentration in a processed sample which would give a peak equal to 3 × local base-line noise.

¹ Recoveries analysed using an altered method (calibration range 1.3 to 0.08 mg/l, 5µl injection volume), method was not successful and therefore not used for analysis of samples. The control value has been subtracted from the results. Results not included in calculation of the mean

The LOQ for the method was 0.004 mg/l and was defined as the lowest successful validated sample level (recovery 80 - 120 % with an RSD ≤ 20 %).

TABLE 2

Validation recoveries of AERO® 5100 promoter from stock solutions in ethanol

Nominal Concentration (mg/l)	Recovery expressed as a percentage of nominal
1.088	94.0 95.5
2.397	94.3 95.6
1303	104 104
Mean (RSD)	98.0 (5.1)

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TABLE 3**Stability of AERO® 5100 promoter in dilution medium**

Storage conditions	Time-point	Time-point	
	0 hours	24 hours	
Light, unsealed, room temperature, 24 hours		94.9,	95.8
Light, sealed, room temperature, 24 hours		95.2	
Dark, sealed, room temperature, 24 hours		97.2,	97.5
Dark, sealed, 4°C, 24 hours		97.1,	98.9
Procedural recoveries	102,	99.6	

Fortification level: 2.0442 mg/l.

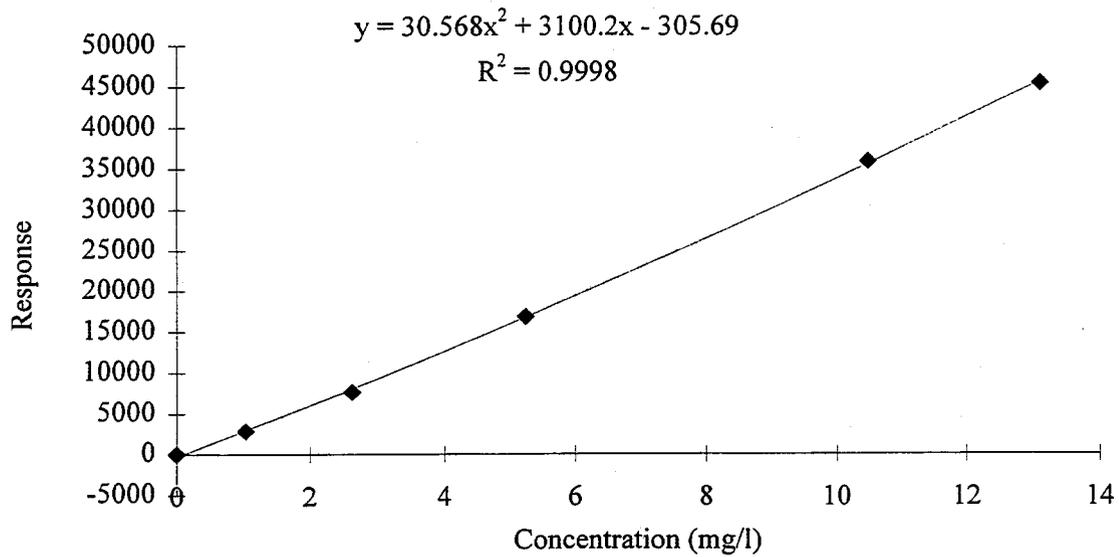
Results are given percentage recoveries of AERO 5100 Promoter after storage for the indicated time.

Duplicate sample for light, sealed, room temperature, 24 hours lost during analysis.

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FIGURE 1

Chemical Calibration Graph for AERO® 5100 promoter



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Run number : CTI 65/019

Standard concentration (mg/l)	Peak height
13.10	45347
10.48	35845
5.239	16861
2.620	7643
1.048	2853
0	0

FIGURE 2

Typical calibration chromatography - 13.10 mg/l

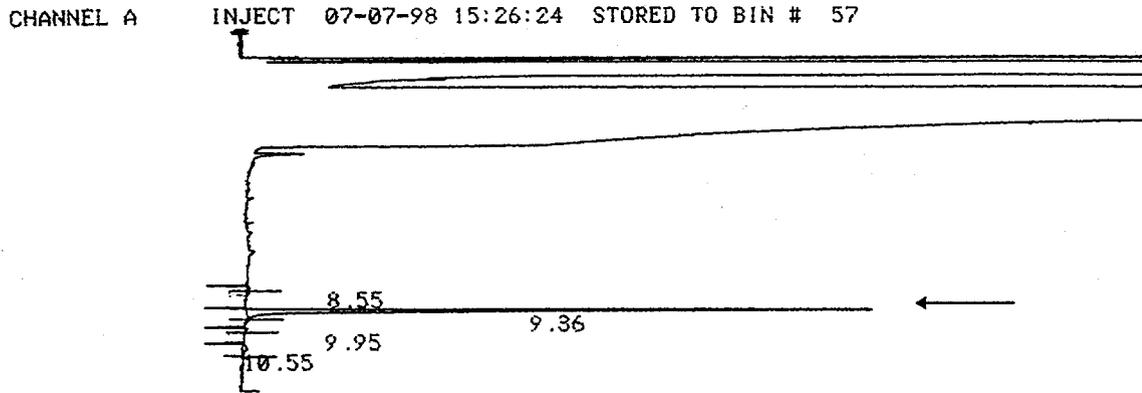
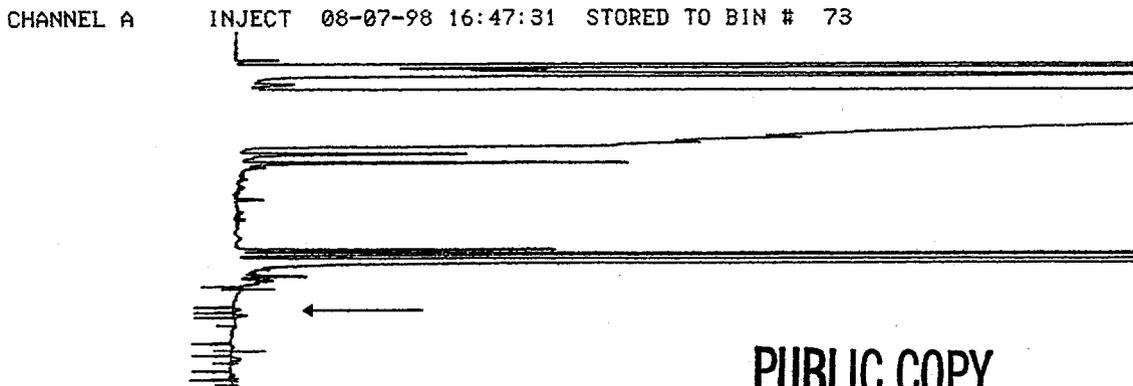


FIGURE 3

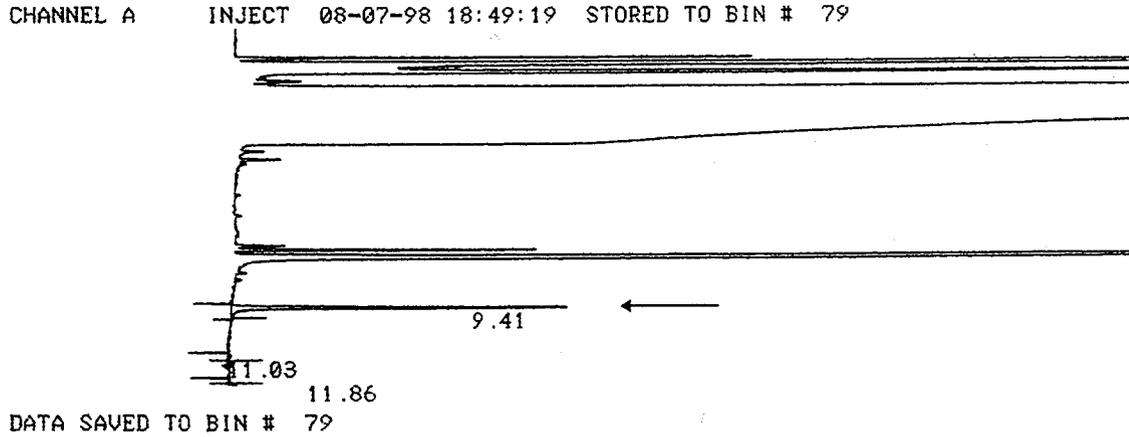
Typical chromatography - Elendt M4 - recovery control



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FIGURE 4

Typical chromatography - sample of Elendt medium, fortified at 0.1513 mg/l



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