[redacted]

Huntingdon Life Sciences

[redacted]

ALGAL GROWTH INHIBITION ASSAY

Report

[redacted]

ALGAL GROWTH INHIBITION ASSAY

Sponsor

DuPont Speciality Chemicals,
Jackson Laboratory,
Chambers Works,
Deepwater,
NJ 08023,
USA.

Research Laboratory

Huntingdon Life Sciences Ltd.,
Eye,
Suffolk IP23 7PX,
ENGLAND.

Draft Report Issued 20 January 1999
Final Report Issued 19 March 1999

CONTENTS

CONTENTS - continued

Page

APPENDICES

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, (No. L 15/29).

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

Eileen Daly

Eileen C. Daly, Nat. Diploma, NCEA Eire,
Study Director,
Huntingdon Life Sciences Ltd.

18th March 1999

Date

QUALITY ASSURANCE STATEMENT

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

Study Phases Inspected	Date of Inspection	Date of Reporting
Protocol Audit	07 August 1998	07 August 1998
Process Based Inspections		
Counting of inoculum	10 August 1998	10 August 1998
Formulation of test medium	02 October 1998	02 October 1998
Sampling of test medium	02 October 1998	02 October 1998
Experimental set-up	30 November 1998	01 December 1998
Report Audit	05 March 1999	05 March 1999

Protocol Audit: An audit of the protocol for this study was conducted and reported to the Study Director and Company Management as indicated above.

Process based inspections: At or about the time this study was in progress inspections 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.

Helen Comb

18 March 1999

Helen Comb, B.Sc.(Hons.),
Principal Auditor,
Department of Quality Assurance,
Huntingdon Life Sciences Ltd.

Date

CONTRIBUTING SCIENTISTS

STUDY MANAGEMENT

Eileen C. Daly, Nat. Diploma, NCEA Eire
Study Director

Karen Firth, Higher National Certificate (Applied Biology)
Study Scientist

Ben Smith, B.Sc.(Hons.), M.Sc., C.Chem., M.R.S.C.
Chief Chemist

Andrew Robertson, B.Sc.(Hons.)
Senior Chemist

Richard Cubberley, B.Sc.(Hons.)
Study Analyst

Martin Nash, B.Sc.(Hons.)
Scientific Officer

SUMMARY

The effect of [redacted] on the growth of the unicellular green alga *Selenastrum capricornutum* was assessed under non-axenic conditions. Throughout the report, the exposure concentration and test results have been expressed in terms of the active ingredient (a.i.) [redacted].

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 3 "Algal Inhibition Test" and the OECD Guideline for Testing of Chemicals No. 201 "Alga, Growth Inhibition Test".

Six replicate algal cultures, with an initial cell density of 1 x 10^4/ml, were exposed to [redacted] dispersed in algal nutrient medium at a nominal concentration of 100 mg a.i./l; to aid dispersion, ultrasound treatment was employed. The cultures were incubated for 72 hours in an orbital incubator under continuous illumination at temperatures ranging from 23.2 to 23.7°C.

The measured concentration of [redacted] in unfiltered samples of medium ranged from 98.8 mg a.i./l at the start of the test to 101 mg a.i./l after 72 hours, with an overall mean measured level of 100 mg a.i./l.

Cell numbers were counted daily to monitor growth. The test results are expressed in terms of the area under the growth curve and growth rate.

Compared to the control cultures, neither the area under the growth curve nor the growth rate were reduced at a mean measured level of 100 mg a.i./l. The 72-hour median effect concentrations (E_bC_{50} and E_rC_{50}) were not identified but must be greater than 100 mg a.i./l.

The "no-observed adverse effect concentration" for inhibition of growth was considered to be 100 mg a.i./l.

Under the EC General Classification and Labelling Requirements for Dangerous Substances and Preparations, [redacted] is not considered to require classification as the 72-hour EC_{50}s are considered to be greater than the overall mean measured concentration, 100 mg a.i./l.

INTRODUCTION

This study was designed to assess the effect of [redacted] on the growth of the unicellular green alga *Selenastrum capricornutum*.

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 3 "Algal Inhibition Test" and the OECD Guideline for Testing of Chemicals No. 201 "Alga, Growth Inhibition Test".

The protocol was approved by Huntingdon Life Sciences Management on 7 July 1998, by the Sponsor on 17 July 1998, and by the Study Director on 3 August 1998.

The experimental phase of the study was conducted between 4 September and 2 October 1998 and the results of chemical analysis were issued by 12 October 1998.

Information provided by the Sponsor indicated that the solubility of [redacted] in water was [redacted] by weight at 35 - 40°C and that its purity was [redacted] Throughout this report, the exposure concentration and the test results have been expressed in terms of the active ingredient. Also, the Sponsor indicated that at room temperature the test substance was a suspension in water and upon standing it would separate out into its component phases; accordingly, at the recommendation of the Sponsor, the suspension was warmed to 35 - 40°C (in a water bath) with gentle stirring to obtain an homogenous composition before use.

The results of the most recent laboratory reference test using potassium dichromate indicated that its 72-hour E_bC_{50} to *Selenastrum capricornutum* was 0.62 mg/l; this was within the range typically obtained in this laboratory (0.3 to 1 mg/l).

TEST SUBSTANCE

Identity:	[redacted]
Chemical name:	[redacted]
Appearance:	[redacted]
Storage conditions:	Room temperature
Lot number:	[redacted]
Expiry date:	2 years from date of receipt
Purity:	[redacted]
Sample received:	23 June 1998

EXPERIMENTAL PROCEDURE

TEST SPECIES

Name

Selenastrum capricornutum, Strain No. CCAP 278/4.

Source

Axenic, uni-algal, agar slope cultures were obtained from the Culture Collection of Algae and Protozoa, Institute of Freshwater Ecology, Cumbria, UK and arrived on 26 August 1998.

Pre-culture

The agar slope cultures were stored in an illuminated refrigerator. Sterile algal nutrient medium (Appendix 1) was inoculated with cells aseptically removed from the slope culture; these primary liquid cultures (50 ml) were incubated for approximately three days in an orbital incubator under continuous illumination at nominal temperatures in the range 21 to 25°C and typically had a cell density of 1.2×10^6 cells/ml. An aliquot of a primary culture was diluted using sterile algal nutrient medium to give a test inoculum with a cell density of 2.5×10^4 cells/ml before use.

CULTURE MEDIUM

Sterile algal nutrient medium as recommended in Official Journal No. L383A Part C.3 and OECD Procedure 201 (see Appendix 1).

TEST SUBSTANCE PREPARATION

Method of preparation

Based on information provided by the Sponsor, the test substance was warmed (to *c*.38°C) in a water bath and gently swirled to provide an homogeneous mixture before weighing.

A concentrated stock was prepared by adding the test substance (800 mg, as received) directly to sterile algal nutrient medium (approximately 180 ml). This was treated with ultrasound for twenty minutes and its pH adjusted from 2.8 to 7.9 with 0.5 ml sodium hydroxide (1M). After adjustment to a volume of 200 ml, an aliquot (10 ml) of the stock was added to each test flask.

Stability of test concentrations

The test concentration of [redacted] was measured using an HPLC method of chemical analysis (Appendix 2). Its stability in dilution medium under refrigerated storage conditions was determined before the start of the study.

At the start of the definitive test, four samples (100 ml) were taken from the freshly-prepared control and test media; after 72 hours, the contents of the replicate flasks for each group were pooled and further samples taken for analysis. Additional samples were also taken from flasks containing [redacted] at 100 mg/l but with no algal cells, in order to obtain information on the extent of adsorption/absorption of the test substance by the algal cells. The samples were stored in a refrigerator before duplicates were transferred to the Huntingdon Research Centre, Cambridgeshire, for analysis; the other samples from each occasion of analysis remained in storage in case further analysis was required.

EXPOSURE CONDITIONS

Experimental design

A preliminary rangefinding test was followed by a definitive (limit) test with one test concentration plus an algal nutrient medium control group. Ten flasks were established for each control and test group. Six flasks from each group were incubated and the others were used for water quality measurements and chemical analysis at the start.

Before the start of the test, the required number of test vessels (250 ml conical flasks), each containing algal medium (50 ml), were loosely stoppered with non-absorbent cotton wool, covered with aluminium foil which was secured by autoclave tape and sterilised by autoclaving (121°C for 15 minutes). Following the addition of algal inoculum (40 ml) and the test substance (as a 10 ml aliquot of aqueous stock), the cell density in each flask was approximately 1 x 10^4 cells/ml. Each flask was then loosely plugged with non-absorbent cotton wool.

The control cultures were prepared by adding aliquots (10 ml) of algal nutrient medium and test inoculum (40 ml) to the flasks containing sterile algal nutrient medium (50 ml).

Test concentrations

The rangefinding study used test concentrations of 1, 10 and 100 mg a.i./l. The definitive (limit) test employed a nominal concentration of 100 mg a.i./l.

Environmental conditions

Conical flasks each containing control or test culture (100 ml) were placed in an illuminated orbital incubator according to a random number sequence. The cultures were incubated, without renewal of medium, for 72 hours under continuous illumination of approximately 7488 lux provided by 6 x 30 W "cool white" 1 metre fluorescent tubes. The temperature was maintained at 23 ± 2°C.

The temperature and pH of control and test flasks at the start and end of the test were recorded. Gaseous exchange and suspension of the algal cells were ensured by the action of the orbital shaker, oscillating at a nominal 150 cycles per minute. The minimum, maximum and ambient temperature and light intensity in the test area were determined each day.

MEASUREMENT OF GROWTH

Samples were taken from control and test flasks at 24, 48 and 72 hours and the cell densities measured using a haemacytometer (Improved Neubauer). The estimate of cell numbers in each sample was based on the mean of four or eight consecutive counts.

EVALUATION OF DATA

The area under each growth curve (cell density *v* time) is taken to be an index of growth and is calculated using the equation:

$$A = \frac{N_1 - N_o}{2} \times t_1 + \frac{N_1 + N_2 - 2N_o}{2} \times [t_2 - t_1] + \ldots + \frac{N_{n-1} + N_n - 2N_o}{2} \times [t_n - t_{n-1}]$$

where A = area
N_o = nominal cell densities at t_o
N_1, N_2 = measured cell densities at t_1, t_2
N_n = measured cell densities at t_n
t_1, t_2 = time of first or second measurement (hours from start)
t_n = time of n_{th} measurement (hours from start)
n = number of measurements taken after the beginning of the test

Percentage inhibition of growth at the test concentration (I_A) is calculated by comparing the area under the test curve (A_t) with that under the control curve (A_c) as appropriate using the equation:

$$I_A = \frac{A_c - A_t}{A_c} \times 100$$

The E_bC_{50} ("x" h) is the median effect concentration for inhibition of growth based on a comparison of areas under the growth curves after "x" hours. The E_bC_{50} was not calculated because growth was not inhibited.

The average specific growth rate (μ) for each exponentially growing culture is also calculated from the appropriate section of the growth curve by the equation:

$$\mu = \frac{\ln N_n - \ln N_o}{t_n - t_o}$$

Where t_o is the time at the beginning of the test.

The E_rC_{50} ("x" - "y" h) is the median effect concentration for inhibition of growth based on a comparison of growth rates from "x" to "y" hours. The E_rC_{50} was not calculated because growth was not inhibited.

The "no-observed effect concentration" could not be identified statistically using Dunnett's multicomparison test to compare the percentage inhibition in the test group with that for the control cultures (Dunnett, C.W. 1955, 1964) because the mean cell density for the exposure concentration was greater than that for the control group.

PROTOCOL DEVIATIONS

One test flask was not inoculated with algal cells at the start of the test, in error. However, this is not considered to have affected the integrity of the study.

MAINTENANCE OF RECORDS

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.

RESULTS

Chemical analysis

The results of chemical analysis are given in Table 1 and an example chromatogram is illustrated in Figure 1.

The mean measured concentration of [redacted] ranged from 98.8 mg a.i. /l at the start of the test to 101 mg a.i./l after 72 hours, with an overall mean measured level of 100 mg a.i./l.

After 72 hours, analysis of medium containing [redacted] which had been incubated without algal cells gave similar results to test medium incubated in the presence of algal cells (99.7 mg a.i./l compared to 101 mg a.i./l); this indicates that the presence of algal cells had not affected the stability of [redacted]

Algal growth

Individual cell densities for each culture and the mean values are given in Table 2. The calculated area under the growth curve and average specific growth rate values are given in Table 3 and are expressed in terms of percentage inhibition by comparing the test group value with that of the control curve.

Compared to the control cultures the area under the growth curve and the growth rate at a mean measured level of 100 mg a.i./l were significantly higher (Dunnett's test). The 72-hour median effect concentrations (E_bC_{50} and E_rC_{50}) were not identified but must be greater than 100 mg a.i./l.

The no-observed-effect concentration (NOEC) could not be identified statistically because the mean cell density for the exposure concentration was greater than that for the control group. Therefore, the "no-observed adverse effect concentration" was considered to be 100 mg a.i./l.

Under the EC General Classification and Labelling Requirements for Dangerous Substances and Preparations, [redacted] is not considered to require classification as the 72-hour EC_{50}s are considered to be greater than the overall mean measured concentration, 100 mg a.i./l.

Observations

No microscopic abnormalities of the cells were detected.

Environmental parameters

The measurements of water quality (temperature and pH) in control and test flasks are summarised in Table 4; they remained within acceptable limits throughout the study.

The temperature of the incubator ranged between 23.2 and 23.7°C.

The test medium was a colourless dispersion with undissolved material visible at its surface and on the bases of the flasks.

CONCLUSIONS

[redacted] was not found to be inhibitory to *Selenastrum capricornutum* when dissolved in algal nutrient medium at a mean measured level of 100 mg a.i./l.

The 72-hour median effect concentrations (E_bC_{50} and E_rC_{50}) for inhibition of growth were not identified but must be greater than 100 mg a.i./l.

The no-observed adverse effect concentration (NOEC) for inhibition of growth was considered to be 100 mg a.i./l.

REFERENCES

DUNNETT, C.W. (1955) "A multiple comparison procedure for comparing several treatments with a control". Journal of the American Statistical Association, 50, 1096-1121.

DUNNETT, C.W. (1964) "New tables for multiple comparisons with a control". Biometrics, 20, 482-491.

Official Journal of the European Communities Commission Directive (1 March 1991). Annex VI General classification and labelling requirements for dangerous substances and preparations. Part II "*Classification on the Basis of Environmental Effect*" p.62 – 64.

FIGURE 1

Typical sample chromatography - 400 mg/l (100 mg a.i./l) taken on Day 0 of the test

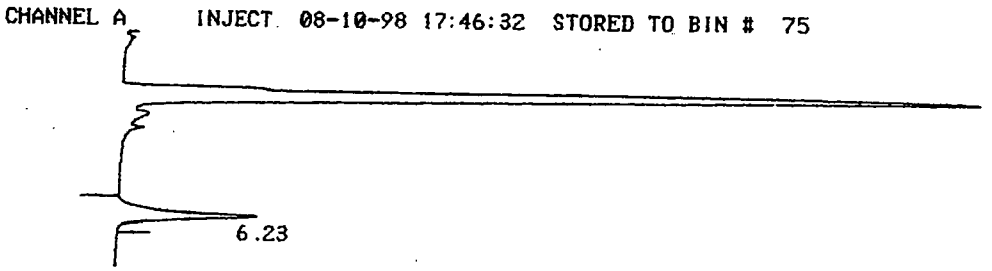


TABLE 1

Measured concentrations [redacted]

Nominal conc$, (mg/l)	Measured [redacted] concentrations (mg/l) 0 hours	% N	72 hours	%N	%ti	Overall mean
control	nd nd	-	nd nd	-	-	-
100	98.9 98.6	99	103 99.5	101	103	100
100 (no algae)	-	-	99.8 99.5	100	-	99.7

$: in terms of the active ingredient [redacted]
nd : none detected (<2.5 mg active ingredient/l).
%N : mean measured concentration expressed as a percentage of the nominal concentration.
%ti : mean measured concentration after 72 hours expressed as a percentage of the mean starting concentration.

TABLE 2

Cell densities - control and test cultures

Mean measured [redacted] concentration$ (mg/l)		Cell densities (cells x 10^4/ml)		
		24 hours	48 hours	72 hours
Control	R_1	4.50	19.6	47.0
	R_2	4.13	20.0	62.0
	R_3	*	*	*
	R_4	3.75	18.3	54.6
	R_5	4.38	20.3	50.1
	R_6	3.25	19.5	57.0
	Mean	4.00	19.5	54.1
100	R_1	4.13	23.6	75.1
	R_2	5.13	30.3	75.3
	R_3	3.50	28.0	67.1
	R_4	2.88	34.0	70.4
	R_5	4.63	31.8	58.5
	R_6	4.75	31.3	60.8
	Mean	4.17	29.8	67.9

R_1 - R_6 Replicates 1 – 6
* Flask not inoculated with algae in error.
$ In terms of the active ingredient [redacted]

TABLE 3

Inhibition of growth

Mean measured [redacted] concentration$ (mg/l)		Area under curve# @ 72 h	Mean (% Inhibition)	Growth rate§ (0-72 h)	Mean (% Inhibition)
Control	R_1	1082	1155	5.347	5.537
	R_2	1263		5.732	
	R_3	*		*	
	R_4	1124		5.556	
	R_5	1134		5.436	
	R_6	1170		5.615	
100	R_1	1507	1571	5.998	5.851
	R_2	1694	(0)	6.002	(0)
	R_3	1501		5.842	
	R_4	1670		5.909	
	R_5	1516		5.651	
	R_6	1535		5.705	

R_1 - R_6 Replicates 1 – 6
* Flask not inoculated with algae in error.
$ In terms of the active ingredient [redacted]
\# x 10^4
§ x 10^{-2}

TABLE 4

Environmental parameters - temperature and pH

Mean measured [redacted] conc.,$ (mg/l)	Temperature °C		pH	
	0 h	72 h	0 h	72 h
Control	24.3	23.5 - 24.0	7.9	7.6 - 7.7
100	24.4	23.6 - 24.1	7.9	7.5 - 7.7

$ In terms of the active ingredient [redacted]

APPENDIX 1

Algal nutrient medium (OECD)

Four stock solutions were prepared according to the following table, using filtered, dechlorinated tap water which had been softened and treated by reverse osmosis, before microfiltration and purification (resistivity of 18 Megohm/cm). Stock solutions were sterilised by autoclaving (solutions 1-3) or by membrane filtration (solution 4) before being stored at 4°C in the dark.

Aliquots of stock solutions 1-4 were further diluted with the same diluent and autoclaved again to produce the working strength nutrient medium. The pH of the medium after equilibration with air is approximately 8.

Nutrient	Concentration in stock solution (g/l)	Volume of stock solution per litre of final medium (ml)	Final concentration in test solution (mg/l)
Stock solution 1: macro-nutrients			
NH_4Cl	1.5	10	15
$MgCl_2.6H_2O$	1.2		12
$CaCl_2.2H_2O$	1.8		18
$MgSO_4.7H_2O$	1.5		15
KH_2PO_4	0.16		1.6
Stock solution 2: Fe-EDTA			
$FeCl_3.6H_2O$	0.08	1	0.08
$Na_2EDTA.2H_2O$	0.1		0.1
Stock solution 3: trace elements			
H_3BO_3	0.185	1	0.185
$MnCl_2.4H_2O$	0.415		0.415
$ZnCl_2$	3×10^{-3}		3×10^{-3}
$CoCl_2.6H_2O$	1.5×10^{-3}		1.5×10^{-3}
$CuCl_2.2H_2O$	10^{-5}		10^{-5}
$Na_2MoO_4.2H_2O$	7×10^{-3}		7×10^{-3}
Stock solution 4: $NaHCO_3$			
$NaHCO_3$	50	1	50

APPENDIX 2

The determination of [redacted] in aqueous media

SAMPLE ANALYSIS

The aqueous samples were diluted with sodium hydroxide and acetonitrile to bring the expected [redacted] concentrations within the calibration range. Determination of [redacted] was by high performance liquid chromatography (HPLC) using a conductivity detector[1].

CHROMATOGRAPHY INSTRUMENTATION AND CONDITIONS

A high performance liquid chromatography system comprising autosampler, pump, conductivity detector, anion suppressor and data collection system was used.

Column	
Type:	PLRP-S supplied by Polymer Laboratories
Dimensions (l × id):	250 × 4.6 mm
Temperature:	Ambient
Mobile phase	
Composition:	Acetonitrile : aqueous buffer solution (25 : 75% v/v)
Flow rate:	1.0 ml/min
Suppresser type:	
Regenerant composition:	50 mN sulphuric acid
Flow rate:	2.5 ml/min
Injection volume:	100 µl

Aqueous buffer solution: 2mM ammonium hydroxide/1mM sodium carbonate (made up in ultra high purity water) and filtered through 0.2 micron cellulose nitrate filter paper.

Under the above conditions [redacted] chromatographed as a single peak (Figure 3).

[1] A method contained in a fax dated 23 July 1998 from Kavsy D Dastur, Dupont Specialty Chemicals was modified to comply with Huntingdon Life Sciences standard operating procedures and instrumentation.

CALIBRATION SOLUTIONS

Calibration solutions were prepared with the same batch of [redacted] used in the preparation of the toxicity test solutions.

Trout and Algae Studies

Working calibration solutions in the nominal range 40 to 500 mg/l (equal to 10 to 125 mg a.i./l) were prepared by volumetric dilution with acetonitrile: 100 mM sodium hydroxide (25:75% v/v) of a primary standard prepared in ultra high purity water.

Daphnia Study

Working calibration solutions in the nominal range 4.0 to 50.0 mg/l (equal to 1.0 to 12.5 mg a.i./l) were prepared by volumetric dilution with acetonitrile : 100 mM sodium hydroxide (25: 75% v/v) of a primary standard prepared in ultra high purity water.

CALCULATIONS

[redacted] concentrations were determined using mean bracketing standards.

The mean peak height responses were calculated for [redacted] in bracketing standard chromatograms. [redacted] concentration in each sample was then calculated using the following equation:

$$\text{Concentration [redacted] (mg/l)} = \frac{Y \times A}{Z} \times F$$

Where
- Y = Detector response to [redacted]
- Z = Mean detector response to bracketing standard.
- A = Concentration of bracketing standard (mg/l).
- F = Factor to take into account sample processing.

The purity (active ingredient) of the test substance is given in the Test Substance Data Sheet as [redacted] Nominal and fortified concentrations are reported as [redacted] as supplied and in terms of the active ingredient.

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 limit of detection, and the method's accuracy and precision.
During the course of the study the performance of the method was monitored by the analysis of quality control samples.

TABLE 1

Validation recoveries of [redacted] from fortified samples of dilution media

Medium	Fortification level (mg/l)		Recovery as a % of fortification level
	As supplied	As active ingredient	
Dechlorinated tap water	Control	Control	ND
	387.9	96.98	95.8
	387.9	96.98	104
Algal	Control	Control	ND
	535.5	133.9	92.0
	530.0	132.5	101
	214.2	53.55	92.6
	212.0	53.00	95.5
Elendt M4	Control	Control	ND
	9.93	2.483	98.5
	9.93	2.483	95.9
	496.5	124.1	103
	496.5	124.1	103
Overall mean (RSD)			98.1 (4.2)

Active ingredient content of [redacted]
RSD: relative standard deviation.
ND: none detected; less than the limit of detection (trout and algal studies : 2.5 mg a.i./l; *Daphnia* study: 0.5 mg a.i./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.

TABLE 2

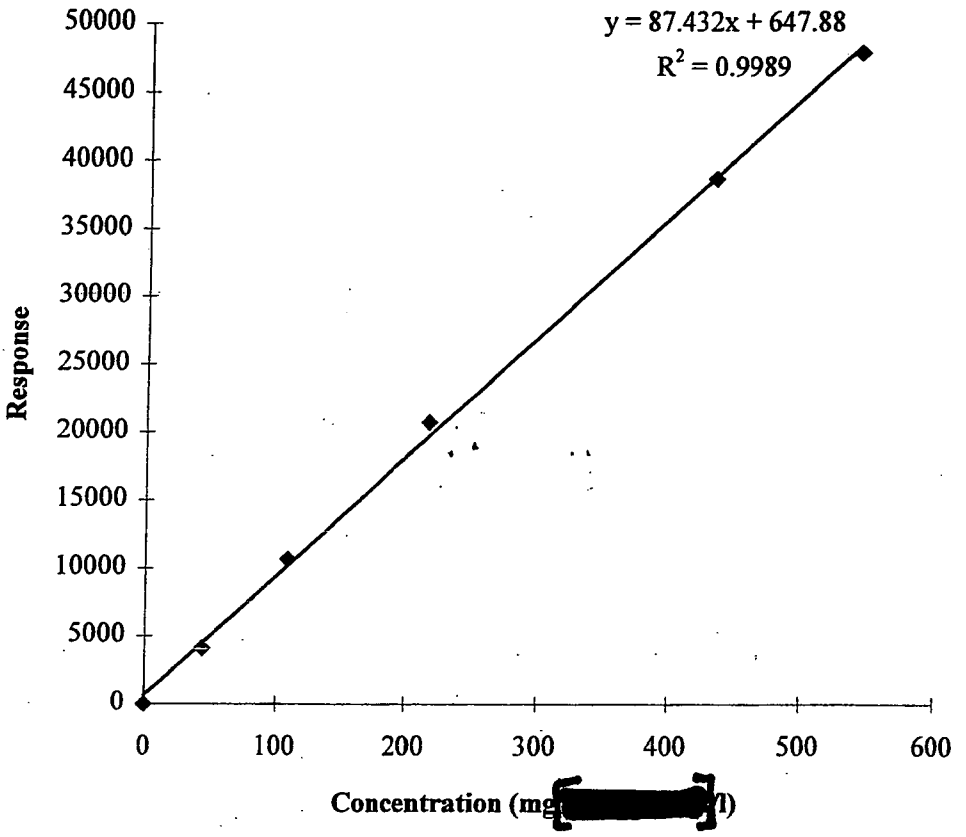
Stability of [redacted] in dilution medium

Storage conditions	Time-point	
	0 hours	20 hours
Procedural recovery [1]	110	-
Procedural recovery [1]	112	-
Light, sealed, room temperature [1]	-	103
Light, sealed, room temperature [1]	-	106
Dark, sealed, 4°C [1]	-	109
Dark, sealed, 4°C [1]	-	93.1
Procedural recovery [2]	-	109
Procedural recovery [2]	-	98.9

[1] Fortification level: 11.20 mg/l as [redacted]
[2] Fortification level: 11.65 mg/l as [redacted]
Results are given as percentage recoveries of [redacted] after storage for the indicated time.

FIGURE 1

Standard Calibration for [redacted]
(*ca* 40 - 500 mg/l)



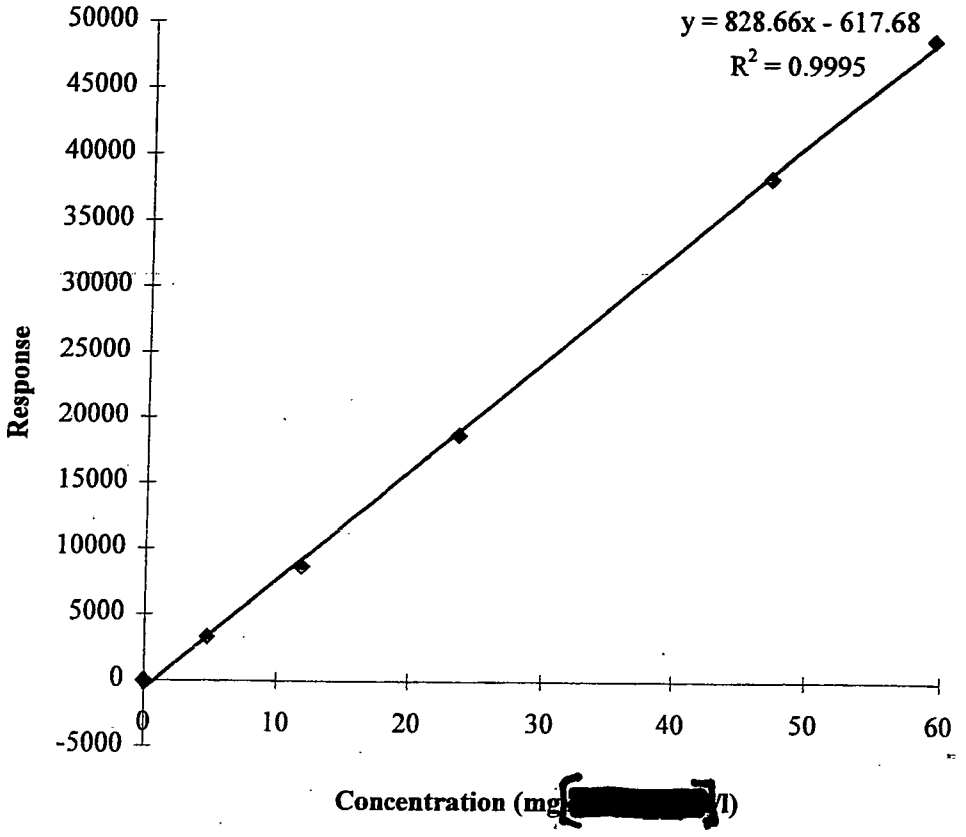
Run no.:DPT/439/013

Standard concentration (mg/l)	Peak height
0.0	NOP
43.57	4075
108.9	10668
217.9	20734
435.7	38644
544.7	47864

NOP: no observable peak.

FIGURE 2

Standard Calibration for [redacted]
(*ca* 4 to 50 mg/l)



Run no.:DPT/440/002

Standard concentration (mg/l)	Peak height
0.0	NOP
4.720	3295
11.80	8657
23.60	18669
47.20	38224
59.00	48699

NOP: no observable peak.

FIGURE 3

Typical calibration chromatography - 217.9 mg/l (54.47 mg/l as a.i.)

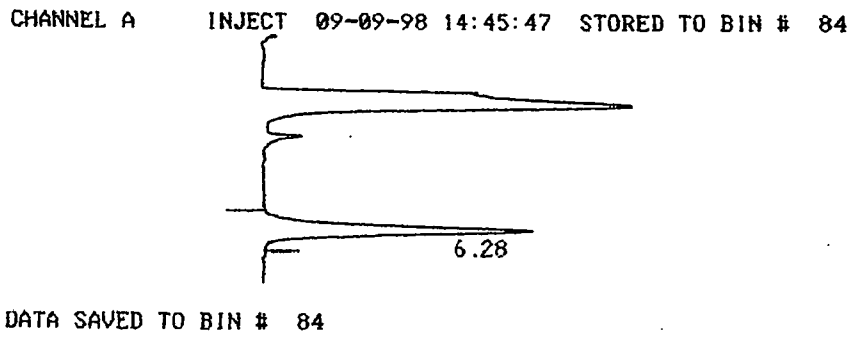


FIGURE 4

Typical chromatography - Unfortified algal medium

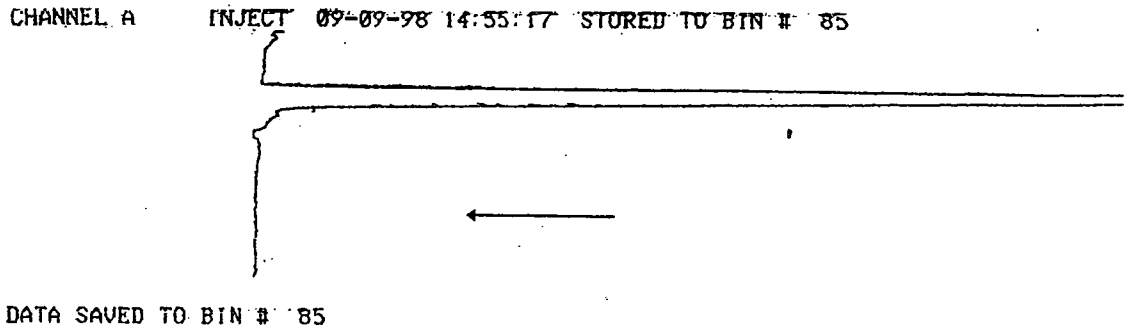


FIGURE 5

Typical sample chromatography - algal medium fortified at 535.5 mg/l (133.9 mg/l as a.i.)