

REPORT

SKELETONEMA COSTATUM

MARINE ALGAL GROWTH INHIBITION TEST

WITH

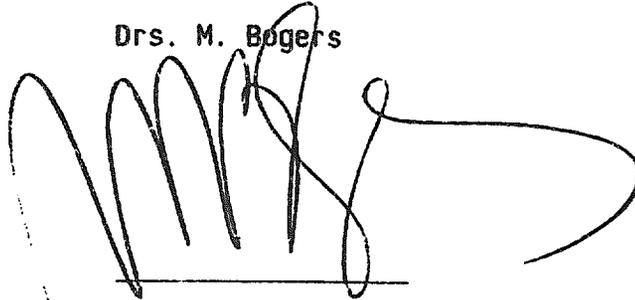
SURFACTANT F058

RCC NOTOX Project 087751
RCC NOTOX Substance 27378

REPORT APPROVAL

STUDY DIRECTOR:

Drs. M. Bogers

A large, stylized handwritten signature in black ink, consisting of several loops and a long horizontal stroke at the end.

date: 16 February, 1993

MANAGEMENT:

Ing. E.J. van de Waart
(Section Head, Genetic &
Eco-Toxicology)

A handwritten signature in black ink, appearing to be 'E.J. van de Waart', written over a horizontal line.

date: 19/02/1993

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SUMMARY

Skeletonema costatum, marine algal growth inhibition test with SURFACTANT F058.

Exponentially growing cultures of Skeletonema costatum were exposed in a static test to different concentrations of SURFACTANT F058 in ISO-medium for 72 hours. The initial cell density in the final test was 1×10^4 cells/ml. The nominal concentrations tested in the final study were 0.10, 0.32, 1.0, 3.2 and 10 mg/l with a blank control (0 mg/l).

Under the conditions of the present study with Skeletonema costatum, SURFACTANT F058 inhibited cell growth significantly at concentrations higher than 0.10 mg/l ($P=0.05$, Williams' test). Statistically significant reduction of growth rate (0-72h) was recorded at 1.0 mg/l and higher concentrations ($P=0.05$, Williams' test). Significant recovery of S.costatum growth (24-72h) was observed at SURFACTANT F058 concentrations up to and including 1.0 mg/l (nominal). Concentrations of 3.2 mg/l and higher induced total inhibition of cell growth.

The nominal 72-hour EC50 for cell growth inhibition ($E_gC50:0-72h$) was 0.30 mg/l (95% fiducial limits: 0.010 - 1.07 mg/l), whereas the EC50 for growth rate reduction ($E_R C50:0-72h$) was between 1.0 and 3.2 mg/l.

The No Observed Effect Concentration (NOEC) for cell growth inhibition was 0.10 mg/l (0-72h), whereas it was 0.32 mg/l for growth rate reduction (0-72h).

PREFACE

GENERAL

Title Skeletonema costatum, marine algal growth inhibition test with SURFACTANT F058.

Sponsor Dowell Schlumberger Inc.
P.O. Box 2710
TULSA, Oklahoma 74101
USA

Study Monitor Mr. D. DeBolt

Testing Facility RCC NOTOX B.V.
Hambakenwetering 3
5231 DD 's-Hertogenbosch
The Netherlands

RCC NOTOX Project 087751

Test substance SURFACTANT F058

Test system Algae (Skeletonema costatum)

PROJECT STAFF

Study Director Drs. M. Bogers (RCC NOTOX B.V.)

Technical Coordinator Ing. J.J.C. van der Pol (RCC NOTOX B.V.)

SCHEDULE

Start of the study 2 November, 1992

Completion of the study 26 November, 1992

QUALITY ASSURANCE STATEMENT

RCC NOTOX B.V.
5231 DD 's-Hertogenbosch / The Netherlands

RCC NOTOX Project 087751
Test Substance SURFACTANT F058
Study Director Drs. M. Bogers
Title Skeletonema costatum, marine algal growth
 inhibition test with SURFACTANT F058.

Study procedures were subjected to periodic inspections.

This report was audited by the Quality Assurance Unit and, as far as can be reasonably established, the methods and results accurately reflect the raw data.

Dates of QAU Inspections / Audits	Reporting Date
16-10-1992	16-10-1992
12-11-1992	12-11-1992
01-02-1993	01-02-1993

General non study specific processes are also inspected at least once every 3 months and results reported to management.

Manager, Quality Assurance Unit

C.J. Mitchell B. Sc.
i.a.



Date: 23 February, 1993

STATEMENT OF GLP COMPLIANCE

RCC NOTOX Project 087751
Test Substance SURFACTANT F058
Study Director Drs. M. Bogers
Title Skeletonema costatum, marine algal growth
 inhibition test with SURFACTANT F058.

To the best of my knowledge and belief, the study described in this report was conducted in compliance with the most recent edition of:

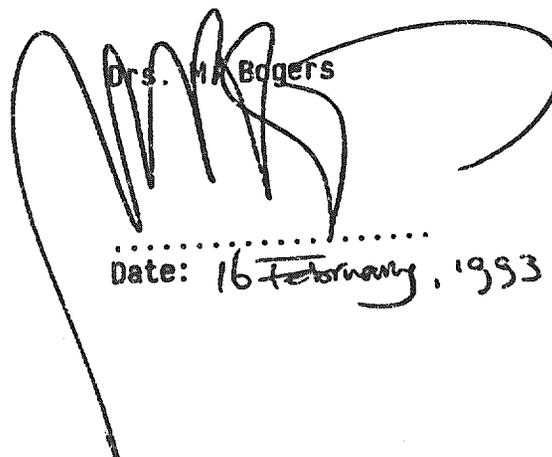
OECD Principles of Good Laboratory Practice, Paris, France.

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

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

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

Study Director


Drs. M. Bogers
Date: 16 February, 1993

GUIDELINES

The study procedure described in this report is based on the following guidelines for fresh water algae:

The Organisation for Economic Co-operation and Development (OECD), OECD guideline for Testing of Chemicals no. 201: "Algae, Growth Inhibition Test" adopted June 7, 1984, with some modifications.

European Economic Community (EEC), EEC Directive 67/548 amended November 18, 1987 (87/302), DJEC L133 V31, Part C: Methods for the determination of ecotoxicity, "Algal Inhibition Test".

In addition the study procedure is based on the Parcom Ring test protocol: "Technical support document for the ISO DP 10253 Standard Method".

ARCHIVING

RCC NOTOX B.V. will archive the following data for at least 10 years: protocol, report, test substance reference sample, all specimens and raw data.

OBJECTIVE

PURPOSE

The purpose of the study was to evaluate the test substance for its ability to inhibit the growth of the marine algal species Skeletonema costatum in a short-term experiment.

DEFINITIONS

Cell density is the number of cells per millilitre.

Growth is the increase in cell density over the test period.

Growth rate is the increase in cell density per unit time.

EC50 is the concentration of test substance which results in a 50% reduction in either growth or growth rate relative to the control.

No Observed Effect Concentration (NOEC) is the highest tested concentration at which the measured parameter(s) show(s) no significant inhibition of growth relative to control values.

MATERIALS AND METHODS

TEST SYSTEM

Species	<u>Skeletonema costatum</u> , Strain: NIVA BAC1
Reason for selection	This system is an unicellular algal species sensitive to toxic substances in the marine ecosystem and has been selected as an internationally accepted species.
Control of sensitivity	A reference test with potassium dichromate (Merck, Art. 4864) is carried out approximately every 3 months. The results of the most recent test are appended to this report.

TEST SUBSTANCE

Identification	SURFACTANT F058
Description	Clear liquid
Batch	IB 92 302
Purity	100%
Instructions for test substance storage	At room temperature in the dark
Stability under storage conditions	Stable
Expiry date	April 1, 1993
Stable for at least 96 hours in vehicle	Water: yes

RANGE-FINDING TEST

A range-finding test preceded the final test to provide information about the range of concentrations to be used in the final test. The range to which algae were exposed was 0.1 to 1000 mg/l, increasing by a factor 10.

TEST PROCEDURES AND CONDITIONS

Test type	Static																																							
Test vessels	100 ml, all-glass																																							
Medium	<p>ISO-medium formulated according to the International Standards "Water quality - Marine algal growth inhibition test" with <u>Skeletonema costatum</u> and <u>Phaeodactylum tricornutum</u> October, 1988 (formulated using natural seawater, in such a way that precipitation did not occur). The ISO-medium has the following composition</p> <table border="0"> <tr> <td>FeCl₃.6H₂O</td> <td>140</td> <td>µg/l (Fe)</td> </tr> <tr> <td>MnCl₂.4H₂O</td> <td>605</td> <td>µg/l (Mn)</td> </tr> <tr> <td>ZnSO₄.7H₂O</td> <td>150</td> <td>µg/l (Zn)</td> </tr> <tr> <td>CuSO₄.5H₂O</td> <td>0.6</td> <td>µg/l (Cu)</td> </tr> <tr> <td>CoCl₂.6H₂O</td> <td>1.5</td> <td>µg/l (Co)</td> </tr> <tr> <td>H₃BO₃</td> <td>17.1</td> <td>mg/l</td> </tr> <tr> <td>Na₂EDTA</td> <td>15.0</td> <td>mg/l</td> </tr> <tr> <td>Thiamin hydrochloride</td> <td>25</td> <td>µg/l</td> </tr> <tr> <td>Biotin</td> <td>0.005</td> <td>µg/l</td> </tr> <tr> <td>B₁₂</td> <td>0.05</td> <td>µg/l</td> </tr> <tr> <td>K₃PO₄</td> <td>3.0</td> <td>µg/l</td> </tr> <tr> <td>NaNO₃</td> <td>50</td> <td>µg/l</td> </tr> <tr> <td>Na₂SiO₃.5H₂O</td> <td>14.9</td> <td>µg/l</td> </tr> </table>	FeCl ₃ .6H ₂ O	140	µg/l (Fe)	MnCl ₂ .4H ₂ O	605	µg/l (Mn)	ZnSO ₄ .7H ₂ O	150	µg/l (Zn)	CuSO ₄ .5H ₂ O	0.6	µg/l (Cu)	CoCl ₂ .6H ₂ O	1.5	µg/l (Co)	H ₃ BO ₃	17.1	mg/l	Na ₂ EDTA	15.0	mg/l	Thiamin hydrochloride	25	µg/l	Biotin	0.005	µg/l	B ₁₂	0.05	µg/l	K ₃ PO ₄	3.0	µg/l	NaNO ₃	50	µg/l	Na ₂ SiO ₃ .5H ₂ O	14.9	µg/l
FeCl ₃ .6H ₂ O	140	µg/l (Fe)																																						
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Na ₂ SiO ₃ .5H ₂ O	14.9	µg/l																																						
Cell density	<p>Using a 3 days old preculture with a cell density of 133.4×10^4 cells/ml, cell suspensions of 2×10^4 cells/ml were prepared. At each concentration, samples of these suspensions were mixed with test media containing test substance at a ratio of 1:1 resulting in an initial cell density of 1×10^4 cells/ml.</p>																																							
Test duration	72 hours																																							
Illumination	Continuously using TLD-lamps of 18 Watt (Philips, Spain), yielding 6000-8000 lux.																																							
Temperature of the medium	Optimal range: $20 \pm 2^\circ\text{C}$																																							
Incubation	During incubation the algal cells were kept in suspension by continuous shaking.																																							

Test concentrations	0, 0.10, 0.32, 1.0, 3.2, 10 mg/l.
Control	Test medium without test substance or other additives (0 mg/l).
Replicas	3 replicas of each test concentration. 6 replicas of 0 mg/l.

PREPARATION OF TEST MEDIA

Initially, a pre-stock solution in ISO-medium was prepared with a nominal concentration of 2000 mg/l. A volume of 10 ml of this stock solution was diluted up to 200 ml of ISO-medium to provide a concentration of 100 mg/l. Exact aliquots of this stock solution were diluted up to 100 ml of ISO-medium. Subsequently, these solutions were mixed with ISO-medium containing 2×10^4 algal cells/ml at a ratio of 1:1. Each vessel contained a final volume of 50 ml. At the start of the test all test solutions appeared clear and colourless.

MEASUREMENTS AND RECORDINGS

pH	At the beginning and at the end of the test.
Temperature of the medium	Every day in one of the control vessels
Cell densities	
Frequency	Daily, beginning at the start of the test.
Method	At the beginning of the test cells were counted by microscope using a counting chamber. Thereafter cell densities were determined by spectrophotometric measurement of samples at 720 nm using a Lambda 1 Spectrophotometer (Perkin Elmer, Illinois, USA), with a cuvette of 5 cm path-length. Algal medium was used as blank.

ACCEPTABILITY OF THE TEST

The cell density in the control cultures must have increased by a factor of at least 16 within three days.

DATA HANDLING

Calibration curve:

At the end of the final test, a calibration curve was made using dilutions of one of the negative control cultures. Cell density was plotted versus extinction using spectrophotometric measurements of 10 dilutions with different cell densities. The calibration curve was composed using linear regression. The equation of this curve was then used to calculate the cell densities of the various test media at different points in time during the test period.

Determination of the NOEC and calculation of the EC50:

For determination of the NOEC and the EC50 the approaches recommended in the OECD guideline (201, adopted 7 June 1984) were used. An effect was considered to be significant if statistical analysis of the data obtained for the test concentrations compared with those obtained in the negative control revealed significant reduction of growth or inhibition of growth rate (Williams' test, TOXSTAT Release 3.0, September 1989, D.D. Gulley, A.M. Boelter, H.L. Bergman)

Comparison of areas under the growth curves:

The area below the growth curve was calculated using the formula:

$$A = \frac{N_1 - N_0 \times t_1}{2} + \frac{N_1 + N_2 - 2N_0 \times (t_2 - t_1)}{2} + \frac{N_{n-1} + N_n - 2N_0 \times (t_n - t_{n-1})}{2}$$

Where: A = area

N_0 = nominal number of cells/ml at the start of the test

N_1 = measured number of cells/ml at t_1

N_n = measured number of cells/ml at t_n

t_1 = time of first measurements after beginning of the test

t_n = time of n^{th} measurement after beginning of the test

The percentage inhibition of cell growth at each test concentration (I_T) was calculated using the following formula:

$$I_T = \frac{A_C - A_T}{A_C} \times 100$$

Where: A_C = area below the growth curve obtained in the control

A_T = area below the growth curve at each test substance concentration

Growth inhibition was calculated for the total period of 72h.

Comparison of growth rates:

The average specific growth rate (μ) for exponentially growing cultures was calculated as:

$$\mu = \frac{\ln N_n - \ln N_1}{t_n - t_1}$$

The average growth rate at each test substance concentration was then compared to the control value and the percentage reduction in growth rate was calculated.

EC50

The percentages of cell growth and growth rate reduction were determined at the different test concentrations relative to the mean values for the control (0 mg/l). These data were used to estimate the EC50 value for growth inhibition (E_{gC50} (0-72h)) and growth rate reduction (E_{rC50} (0-72h)).

RESULTS

RANGE-FINDING TEST

In the range-finding test, inhibition of algal growth was observed at nominal concentrations of 10 mg/l and higher.

FINAL TEST: MEAN CELL DENSITIES

Table 1 shows the mean cell densities measured at 24-hour intervals at the different concentrations of SURFACTANT F058. The respective growth curves are shown in Figure 1 (See the appendix I for the worksheet data of the calibration curve, individual extinctions, cell densities, cell growth and growth rate).

From 24 hours following the start of the exposure onwards, exponential increase in cell growth was recorded at 0 mg/l, whereas the slopes of the different growth curves decreased with increasing test concentration (see Figure 1).

FINAL TEST: INHIBITION OF CELL GROWTH AND REDUCTION OF GROWTH RATE

Table 2 shows the calculation of the percentage of inhibition of cell growth and the percentage of growth rate reduction. Statistical analysis of the data for areas under the growth curves (cell growth) and the growth rates are shown in Appendix II.

Statistically significant inhibition of cell growth was observed from 0.32 mg/l onwards ($P=0.05$, Williams' test). However, statistically significant reduction of growth rate (0-72h) was recorded at 1.0 mg/l and higher concentrations ($P=0.05$, Williams' test). Recovery of cell growth rate was recorded at both 0.32 and 1.0 mg/l owing to a delayed exponential cell growth during the last part of the exposure period.

FINAL TEST: EXPERIMENTAL CONDITIONS

Table 3 shows the values for pH recorded at the start and the end of the test.

The pH was 8.1 at the beginning of the test and ranged from 8.3 to 9.0 at the end of the test.

The temperature of the test medium varied from 20.5 to 21°C during the test period.

FINAL TEST: ACCEPTABILITY OF THE TEST

In the controls, the cell density increased by an average factor of 75 within 3 days. Further all test conditions remained within the ranges prescribed by the protocol.

DETERMINATION OF EC50 VALUES AND NOEC

The nominal 72-hour EC50 for cell growth inhibition ($E_{gC50:0-72h}$) was 0.30 mg/l with 95% fiducial limits of 0.010 and 1.07 mg/l (Table 4, regression line: $\log_{10}(\text{conc.}) = 2.62 + (\text{probit} - 5.24) / 1.53$).

The nominal 72-hour EC50 for growth rate reduction ($E_{rC50:0-72h}$) was between 1.0 and 3.2 mg/l with no significant reduction at 0.32 mg/l and total (ca. 100%) reduction at 3.2 mg/l.

The NOEC for cell growth inhibition was 0.10 mg/l (0-72h), whereas it was 0.32 mg/l for growth rate reduction (0-72h).

CONCLUSIONS

Under the conditions of the present study with Skeletonema costatum, SURFACTANT F058 inhibited cell growth significantly at concentrations higher than 0.10 mg/l (nominal NOE_{gC}) and reduced growth rate (0-72h) significantly at concentrations higher than 0.32 mg/l (nominal NOE_{rC}). Significant recovery of S. costatum growth was observed at SURFACTANT F058 concentrations up to and including 1.0 mg/l (nominal). Concentrations of 3.2 mg/l and higher induced total inhibition of cell growth.

The nominal 72-hour EC50 for cell growth inhibition ($E_{gC50:0-72h}$) was 0.30 mg/l (95% fiducial limits: 0.010 - 1.07 mg/l), whereas the EC50 for growth rate reduction ($E_{rC50:0-72h}$) was between 1.0 and 3.2 mg/l.

Table 1: Calculated mean cell densities¹:

Nominal concentration (mg/l)	Mean cell densities during exposure			
	0h	24h	48h	72h
0.00	1.00	3.43	29.46	74.55
0.10	1.00	2.81	17.38	59.63
0.32	1.00	1.66	10.64	50.93
1.00	1.00	1.28	4.82	28.28
3.20	1.00	1.15	1.00	1.00
10.00	1.00	1.94	1.03	1.22

¹ Number of cells inoculated at t=0: 1.0×10^4 cells/ml. A variation of ± 0.01 is acceptable owing to rounding off by the program used for calculations.

Table 2: Percentage inhibition of cell growth and percentage reduction of growth rate¹.

Nominal concentration (mg/l)	Cell growth (0-72h):		Growth rate (cells/ml/h ²):			
	Area (A)	Inhibition (%)	24-72h $\mu^2=$	reduction (%)	0-72h $\mu^2=$	reduction (%)
0.00	1623.94		0.06481		0.05986	
0.10	1139.97	29.80	0.06446	0.53	0.05521	7.77
0.32	846.34	47.88*	0.06408	1.12	0.04908	18.02
1.0	425.65	73.79*	0.06444	0.56	0.04618	22.86*
3.2	3.71	99.77**	-0.00265	104.09**	0.00000	100.00**
10.0	25.92	98.40**	-0.00891	113.75**	0.00234	96.10**

¹ A variation of ± 0.01 is acceptable owing to rounding off by the program used for calculations.

$$^2 \mu = \frac{\ln N_n - \ln N_1}{t_n - t_1}$$

* Significantly different from control (Williams' test, P=0.05, see Appendix II)

** Total inhibition of growth or reduction of growth rate.

Table 3: pH values recorded during the final study.

Nominal concentration (mg/l)	vessel	pH-values	
		0h	72h
0	A	8.1	8.8
0.10	A	8.1	8.8
0.32	A	8.1	9.0
1.0	A	8.1	8.4
3.2	A	8.1	8.3
10	A	8.1	8.3

Figure 1: Growth curves at different concentrations of SURFACTANT F058

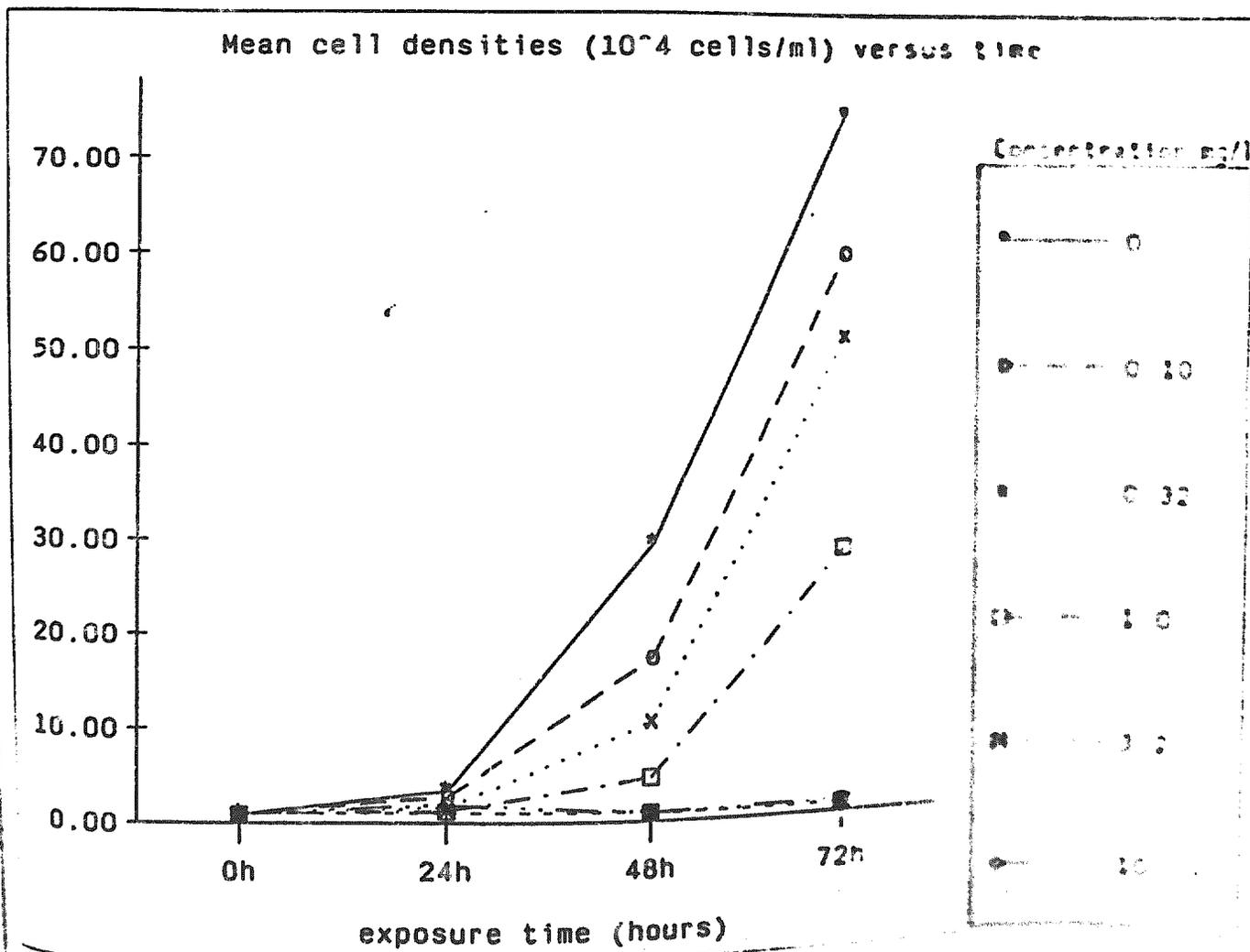


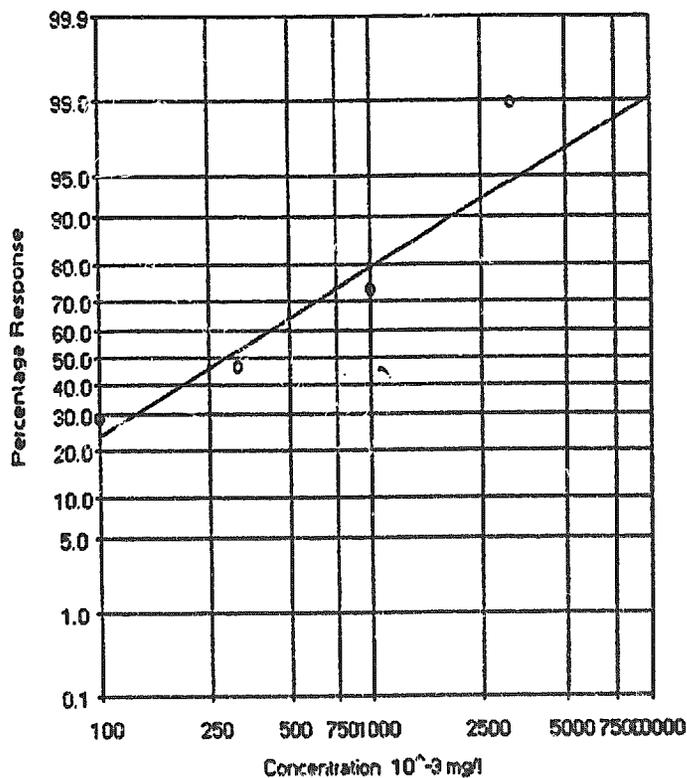
Table 4: Calculation of the EC50 for inhibition of cell growth:

$EC_{50}(0-72h) = 295 \cdot 10^{-3} \text{ mg/l}$
 95% fiducial limits: $9.53 \cdot 10^{-3} - 1065 \cdot 10^{-3} \text{ mg/l}$

heterogeneous data, $h=4.52$
 index of regression significance: $g=0.75$
 $\chi^2 = 9.03$, with 2 degrees of freedom
 regression line: $\log_{10}(\text{conc.}) = 2.62 + (\text{probit} - 5.24) / 1.53$

concentration mg/l	group $\mu\text{g/l}$	response size	corrected fraction	expected fraction	χ^2
0.10	100	100	0.29	0.24	1.58
0.32	320	100	0.47	0.52	1.09
1.0	1000	100	0.73	0.79	2.30
3.2	3200	100	0.99	0.94	4.06
					9.03

Figure 2: Percentage inhibition of cell growth as function of the log concentration of SURFACTANT F058.



REFERENCE TEST

RCC NOTOX PCSK-2

Skeletonema costatum, marine algal growth inhibition test with potassium dichromate.

September, 1992.

This reference test was carried out to check the sensitivity of the test system used by RCC NOTOX to potassium dichromate (Merck, Art. 4864, Batch 2750398).

Concentrations : 0, 0.56, 1.0, 1.8, 3.2 and 5.6 mg/l.

Results: The nominal 72-hour EC50 for growth inhibition ($E_{p}C_{50:0-72h}$) was 1.96 mg/l (95% confidence interval of 1.45 - 3.45 mg/l). The nominal 72-hour EC50 for growth rate reduction ($E_{r}C_{50:0-72h}$) could not be calculated and was between 3.2 and 5.6 mg/l.

The raw data and report of this study are kept in the RCC NOTOX archives. The test was performed under GLP conditions with a QA-check.

APPENDIX I WORKSHEET DATA

Table I1: Calculation of the calibration curve:

x	y	Calculated y
0.51	16.50	28.08
1.06	23.50	33.79
2.12	38.50	44.84
4.28	58.50	67.32
8.55	115.50	111.91
17.10	206.00	201.09
34.20	410.50	379.45
51.30	578	557.81
68.40	748	736.18
85.50	880	914.54

x = cell density ($\times 10^{-4}$ cells/ml)

y = extinction (/1000)

intercept = 22.7272

r = 0.9984

slope = 10.4305

n = 10.00

Extinction at 1.00×10^{-4} cells 33.16

Table I2: Individual values for extinction

concentration mg/l	vessel nr.	hours of exposure		
		24h	48h	72h
0.00	1			
	2	66	305	743
	3	50	277	782
	4	44	211	799
	5	66	413	783
	6	62	369	821
0.10	1	63	405	874
	2	54	251	810
	3	34	57	292
0.32	1	68	304	832
	2	42	145	708
	3	30	36	103
1.0	1	45	220	851
	2	33	74	312
	3	37	84	387
3.2	1	38	61	254
	2	33	19	18
	3	28	10	7
10.0	1	38	21	21
	2	34	25	23
	3	44	30	33
		51	34	40

APPENDIX I (continued)

Table I3: Cell densities calculated from the individual extinction values

concentration mg/l	vessel nr.	Cell densities (10^4 cells /ml) per vessel at			
		0h	24h	48h	72h
0.00	1	1.00	4.15	27.06	69.61
	2	1.00	2.61	24.38	72.73
	3	1.00	2.04	18.05	74.62
	4	1.00	4.15	37.42	72.69
	5	1.00	3.77	33.20	74.99
	6	1.00	3.86	36.65	81.41
0.10	1	1.00	3.00	21.89	79.40
	2	1.00	1.08	3.29	29.82
	3	1.00	4.34	26.97	77.99
0.32	1	1.00	1.85	11.72	65.76
	2	1.00	1.00	1.27	7.76
	3	1.00	2.14	18.91	79.41
1.0	1	1.00	1.00	4.92	27.73
	2	1.00	1.37	5.87	34.93
	3	1.00	1.46	3.67	22.17
3.2	1	1.00	1.00	1.00	1.00
	2	1.00	1.00	1.00	1.00
	3	1.00	1.46	1.00	1.00
10.0	1	1.00	1.08	1.00	1.00
	2	1.00	2.04	1.00	1.00
	3	1.00	2.71	1.06	1.66

Table I4: Calculation of growth and growth rate per vessel

Concentration	vessel	Area (A) Growth rate cells/h		
		0-72h	24-72h	0-72h
0.00	1	1517.71	0.05859	0.05882
	2	1461.34	0.06930	0.05955
	3	1315.23	0.07494	0.05986
	4	1812.23	0.05971	0.05957
	5	1745.30	0.06275	0.06025
	6	1891.51	0.06356	0.06114
0.10	1	1442.93	0.06720	0.06005
	2	354.59	0.06611	0.04515
	3	1622.40	0.06007	0.06044
0.32	1	1054.07	0.07440	0.05813
	2	86.89	0.04251	0.02834
	3	1398.06	0.07533	0.06076
1.00	1	414.78	0.06922	0.04615
	2	532.91	0.06749	0.04935
	3	329.28	0.05662	0.04304
3.20	1	0.00	0.00000	0.00000
	2	0.00	0.00000	0.00000
	3	11.14	-0.00794	0.00000
10.00	1	1.94	-0.00162	0.00000
	2	24.95	-0.01485	0.00000
	3	50.86	-0.01027	0.00701

APPENDIX II STATISTICS

STATISTICAL ANALYSIS OF THE AREAS UNDER THE GROWTH CURVES (0.10)

Table II1: Shapiro Wilks test for normality

D = 2141468.889
W = 0.923

Critical W (P = 0.05) (n = 15) = 0.881
Critical W (P = 0.01) (n = 15) = 0.835

Data PASS normality test at P=0.01 level. Continue analysis.

Table II2: Bartlett's test for homogeneity of variance

Calculated B statistic = 8.34
Table Chi-square value = 11.34 (alpha = 0.01)
Table Chi-square value = 7.81 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 2.75
Used for Chi-square table value ==> df (#groups-1) = 3

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

Table II3A: Williams' test (Isotonic regression model) Table 1 of 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	0.00	6	1623.937	1623.937	1623.937
2	0.10	3	1139.973	1139.973	1139.973
3	0.32	3	846.340	846.340	846.340
4	1.00	3	425.657	425.657	425.657

Table II3B: Williams' test (Isotonic regression model) Table 2 of 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
0.00	1623.937				
0.10	1139.973	1.551		1.80	k= 1, v=11
0.32	846.340	2.492	*	1.89	k= 2, v=11
1.00	425.657	3.841	*	1.92	k= 3, v=11

s = 393.406

Note: df used for table values are approximate when v > 20.

APPENDIX II STATISTICS (CONTINUED)

STATISTICAL ANALYSIS OF GROWTH RATES (0-72):

Table II4: Shapiro Wilks test for normality

D = 0.001
W = 0.875

Critical W (P = 0.05) (n = 15) = 0.881
Critical W (P = 0.01) (n = 15) = 0.835

Data PASS normality test at P=0.01 level. Continue analysis.

Table II5: Bartlett's test for homogeneity of variance

Calculated B statistic = 15.79
Table Chi-square value = 11.34 (alpha = 0.01)
Table Chi-square value = 7.61 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 2.75
Used for Chi-square table value ==> df (#groups-1) = 3

Data FAIL homogeneity test at 0.01 level at every transformation.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

Table II5A: Williams' test (Isotonic regression model) Table 1 of 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	0.00	6	0.060	0.060	0.060
2	0.10	3	0.055	0.055	0.055
3	0.32	3	0.049	0.049	0.049
4	1.00	3	0.046	0.046	0.046

Table II5B: Williams' test (Isotonic regression model) Table 2 of 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
0.00	0.060				
0.10	0.055	0.786		1.80	k= 1, v=11
0.32	0.049	1.824		1.89	k= 2, v=11
1.00	0.046	2.313	*	1.92	k= 3, v=11

* = 0.008

Note: df used for table values are approximate when v > 20.

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