A 01

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Date Produced	Date Received	TSCA Section
05/29/96	12/23/98	8E
Submitting Organization		
CIBA SPECIALTY CHEMS USA		
Contractor		
TERRA MILJOLABORATORIUM A/S		
Document Title		
INITIAL SUBMISSION: LTR FR CIBA SPECIALTY CHEMS USA TO USEPA RE (4) AQUATIC TOXICITY STUDIES OF PERCOL LT 31, PERCOL LT 35, ANTIFOAM H2 & ALCOMER 280L, W/ATTCHMTS & DATED 12/23/98		
Chemical Category		
DIMETHYLAMINE-ETHYLENEDIAMINE-EPICHLOROHYDRIN COPOLYMER; *		

A 03

Ciba Specialty Chemicals
USA

Water
Treatments

8EHQ-1098-14342
14357

Ciba


8EHQ-98-14342

December 23, 1998

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Office of Polilution Prevention and Toxics
U.S. Environmental Protection Agency
401 M Street, S.W.
Washington, DC 20460

CONTAINS NO CBI

99 JAN 27 PM 12:02
OPPT NCIC

Re: TSCA Section 8(e) Notice

Dear Sir or Madam:

Ciba Specialty Chemicals Water Treatments, Inc. is submitting the four aquatic toxicity studies discussed below pursuant to Section 8(e) of the Toxic Substances Control Act ("TSCA"). These studies calculated LC_{50} values near or less than 1.0 mg/l for certain products. These are commercial products, and, as such, there is an inference that they will be released to the environment. We believe that the aquatic toxicity of these classes of products was well-known to the Agency by the time these data became known to our predecessor, Allied Colloids, Inc. However, because it is difficult to reconstruct the state of the Agency's past knowledge, these studies are being submitted in an abundance of caution.

96-Hour Multiple Concentration Static Bioassay, Percol LT 31: Percol LT 31 is a dimethylamine-ethylenediamine-epichlorohydrin copolymer, CAS No. 42751-79-1, in aqueous solution. It is used as a coagulant in treating potable water. This product was determined to have an aquatic LC_{50} of 0.35 ppm in rainbow trout.

96-Hour Multiple Concentration Static Bioassay, Percol LT 31 and LT 35: Percol LT 35 is a homopolymer of diallyldimethylammonium chloride, CAS No. 26062-79-3, in aqueous solution that is also used as a coagulant in treating potable water. Percol LT 31 was determined to have a LC_{50} of 1.18 ppm in rainbow trout, while Percol LT 35 was determined to have a LC_{50} of 0.77 ppm.

Growth Inhibition in the Marine Alga *Skeletonema costatum*, Antifoam H2: Antifoam H2 contains a blend of fatty acid PEG esters. It is sold as an antifoam agent primarily

Allied Colloids
Ciba Specialty Chemicals Water Treatments, Inc.
2301 Wilroy Rd.
P.O. Box 820
Suffolk, Virginia 23439-0820
Tel. 757 538 3700
Fax 757 538 3989

in the textile industry. A 72-hour EC_{50} of 0.47 mg/l was determined based upon algal growth inhibition.

Growth Inhibition in the Marine Alga *Skeletonema costatum*, Alcomer 280L: Alcomer 280L contains a cationic copolymer of acrylamide and a cationic quaternary amino ester suspended in a mineral oil. This product is used as a wastewater sludge dewatering aide and as a viscosifier for acidizing in the oil drilling industry. A 72-hour EC_{50} of 0.83 mg/l was determined based upon algal growth inhibition.

Ciba Specialty Chemicals Water Treatments, Inc., formerly known as Allied Colloids, Inc. ("Allied Colloids"), is a subsidiary of Ciba Specialty Chemicals Corporation ("Ciba"). Ciba has a worldwide policy for environmental and safety audits and due diligence that is designed to meet the standards of EPA's policy on "Incentives for Self-Policing: Discovery, Disclosure, Correction and Prevention of Violations," 60 Fed. Reg. 66706 (Dec. 22, 1995). Ciba undertook a program of TSCA management systems audits in 1997, and these audits are continuing. Earlier this year, Ciba acquired the business of Allied Colloids and has been incorporating it into Ciba's audit and due diligence program. As a part of this program, a targeted toxicology self-audit of the former Allied Colloids facility in Bradford, England, was recently completed to ensure that any studies potentially reportable under Section 8(e) would be submitted to EPA. During the course of this review of toxicological files in England, the studies discussed above were identified. To the best of our knowledge, these studies were not previously known to U.S. employees of Allied Colloids, Ciba, or their subsidiaries. We are also participating in EPA's "Compliance Incentive Program" for the Industrial Organic Chemical sector (SIC Code 2869), and will submit to EPA by January 31, 1999, a final report identifying all potential areas of noncompliance uncovered pursuant to this program.

Please call me at (757) 538-3700 if you have any questions regarding this matter.

Sincerely yours,

Paul Whitwell, ES

Paul Whitwell
Technical Manager
Ciba Specialty Chemicals Water Treatments, Inc.

Enclosures

cc: Aquanetta Dickens, U.S. EPA, Region III

A 05

CHEMICAL & GEOLOGICAL LABORATORIES LTD.
14203-129 AVENUE, EDMONTON, ALBERTA T5L 4N9 TELEPHONE (403) 454-1504

96 HOUR MULTIPLE CONCENTRATION STATIC BIOASSAY

LABORATORY REPORT NUMBER: EB-83-5596-3

1. GENERAL INFORMATION

Organization or Company: ALLIED COLLOIDS (CANADA) INC.

Land location of sample:

Source of test water: A PRODUCT CALLED "PERCOL LT-31"

Sample point:

Name and qualification of supervising biologist: MR. B. SALAHUB, B.Sc. (ZOOLOGY)

Name and qualification of individual conducting the test: MR. B. SALAHUB, B.Sc. (ZOOLOGY)

Test species: *Salmo gairdneri* Richardson (Rainbow Trout)

Source of test species: SUN VALLEY TROUT FARMS LTD.

Mean length and weight of fish ± 1 S.D.: 3 cm 1 g

Mortality rate of test species in the 4 days prior to test: NIL

Source of holding water: Dechlorinated City of Edmonton tap water @ 15°C

2. SAMPLING INFORMATION

Date sampled: Time sampled:

Date received in lab.: MARCH 11, 1983 Time received in lab.:

Method of sampling: (a) grab (b) Composite (24 hrs.?)

Handling method: (a) Temperature maintained during transport: AMBIENT

(b) Temperature maintained during storage: ROOM

(c) Length of storage @ 4°C: N/A

(d) Pretreatment of sample: N/A

3. TEST INFORMATION

Date test started: MARCH 13, 1983

Size of test container: 25 litre

Material of test container: Disposable plastic bags inside plastic container

Volume of test solution per container: 20 litres

Number of fish per container: 10

Test temperature ± variance: 15 ± 1°C

Method of aeration: bubbler

Concentrations of test material: 0.1, 0.2, 0.3, 0.4, 0.5 ppm by Volume

Source of dilution water: holding water

A 06

BIOASSAY RESULTS

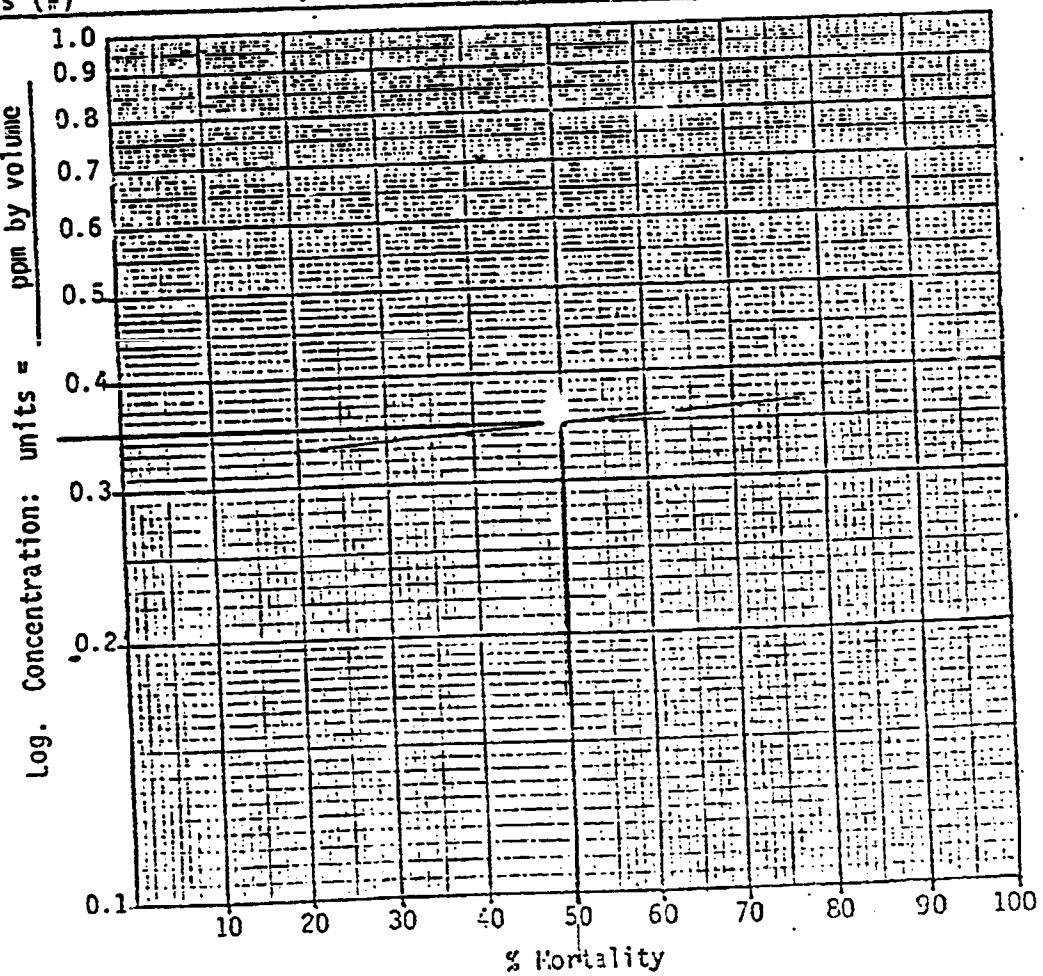
LABORATORY REPORT NUMBER: EP-83-5596-3

ncentrations ppm by volume	0.1	0.2	0.3	0.4	0.5				Control
TART Dissolved Oxygen (mg/l)	>8	>8	>8	>8	>8				>8
pH	8.2	8.2	8.2	8.2	8.2				8.2
Temperature (°C)	15	15	15	15	15				15
Other									
HR. Number Surviving	10	10	10	10	10				10
¼ Stress (#)									
HR. Number Surviving	10	10	10	10	10				10
½. Stress (#)									
HR. Number Surviving	10	10	10	10	10				10
1 Stress (#)									
HRS. Number Surviving	10	10	10	10	10				10
2 Stress (#)									
HRS. Number Surviving	10	10	10	10	10				10
4 Stress (#)									
HRS. Number Surviving	10	10	10	10	10				10
5 Stress (#)									
RS. Number Surviving	10	10	10	10	10				10
24 Dissolved Oxygen (mg/l)	>8	>8	>8	>8	>8				>8
pH	8.2	8.2	8.2	8.2	8.2				8.2
Temperature (°C)	15	15	15	15	15				15
Stress (#)									
RS. Number Surviving	10	10	10	9	1				10
48 Dissolved Oxygen (mg/l)	>8	>8	>8	>8	>8				>8
pH	8.2	8.2	8.2	8.2	8.2				8.2
Temperature (°C)	15	15	15	15	15				15
Stress (#)									

A 07

LABORATORY REPORT NUMBER: EB-83-5596-3

ntrations	ppm by volume	0.1	0.2	0.3	0.4	0.5				Control
RS.	Number Surviving	10	10	10	2	0				10
	Dissolved Oxygen (mg/l)	>8	>8	>8	>8	>8				>8
72.	pH	8.2	8.2	8.2	8.2	8.2				8.2
	Temperature (°C)	15	15	15	15	15				15
	Stress (#)									
RS.	Number Surviving	10	10	10	0	0				10
	% Survival	100	100	100	0	0				100
96	Dissolved Oxygen (mg/l)	>8	>8	>8	-	-				>8
	pH	8.2	8.2	8.2	-	-				8.2
	Temperature (°C)	15	15	15	-	-				15
	Stress (#)									



LC50: 0.35 ppm by volume

-CSAGS C Month AUGUST Year 1984 Licence No. RF 006

PART 4 - FISH TOXICITY TEST

1. GENERAL INFORMATION

Source of test water: GRAHAM R.M. JONES

Organization or Company: ALLIED COLLOIDS

Land location of sample:

Description of sample source: e.g. west tailings pond

Testing agency: Chemex Labs (Alberta) Ltd.

Location of laboratory: 2021 - 41 Ave. N.E., Calgary

Name and qualifications of supervising biologist:

Name and qualifications of individual conducting the test:

ROHAN KERNER LAB TECH

2. SAMPLING METHOD

Date sampled:

Time sampled:

Date received in Laboratory:

Time received in Laboratory:

Method of sampling: (a) Grab:

(b) Composite (24 hours?):

Handling method: (a) Temperature maintained during transport: Ambient

(b) Temperature maintained during storage: Ambient

(c) Length of storage at 4°C: NIL

(d) Pre-treatment of sample: None

PRE-TEST

Test species: Salmo gairdneri (Rainbow trout)

Source of test species: SUN VALLEY TROUT FARMS INC. MISSION, B.C.

Mean length of test species ± 1 S.D. : 2.3 ± 0.3 cm

Mean weight of test species ± 1 S.D. : 0.5 ± 0.1 gm

Acclimation procedure (dilution water & temperature): City of Calgary tap water

L. TEST

Date of test: AUGUST 17, 1984

Type of test: e.g., 24-hour static, 96-hour continuous: LC50

Size of test container: 2 LITER

Material of test container: e.g., glass, plastic GLASS

Volume of test solution per container: 2 LITER

Number of test animals per vessel: 10

Test temperature ± variance: 15°C

Method of aeration, if any: AQUARIUM PUMP

Method of solution renewal, if any: If applicable, flow rate, turnover volume, volume of renewal solution, etc. NONE

Diluter flow rates: Table 3

Concentrations of test material: *

Source of dilution water, if any: e.g. from holding tank, etc. DISTILLED WATER

*	LT 31	0.7, 1.4, 2.5 ppm. - LC50	1.18 ppm*
	LT 35	0.7, 1.0, 2.5 ppm - LC50	0.77 ppm*
	SP1	0.75, 1.25, 2.5 ppm - LC50	0.98 ppm*

* all concentrations are calculated on a volume to volume ratio.

Signature [signature]

Date: AUGUST 21, 1984

SAMPLE 21 51

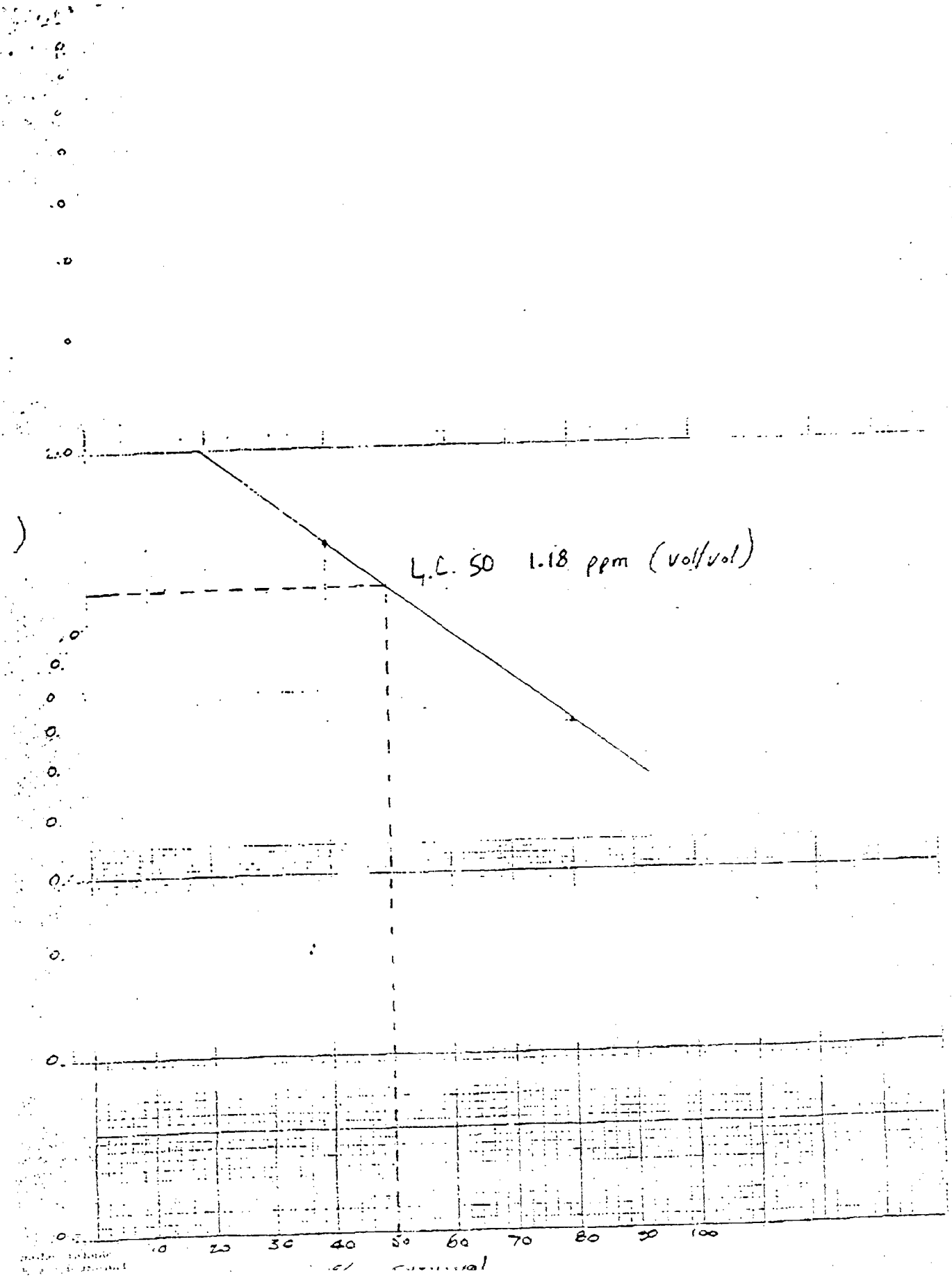


TABLE II

BIOASSAY - NUMERICAL RESULTS

START	LT31 CONCENTRATIONS	0.7	1.4	2.5						CONTROL
	D.O.	7.48	7.40	7.38						7.50
	PH	6.97	6.99	7.00						6.98
	TEMPERATURE	15	15							15
	OTHER									
1 HOUR	NO. SURVIVING	10	10	10						10
	% SURVIVAL	100	100	100						100
	D.O.	7.48	7.40	7.38						7.50
	PH	7.01	7.00	7.02						6.99
	TEMPERATURE	15	15	15						15
	OTHER									
2 HOURS	NO. SURVIVING	10	8	0						10
	% SURVIVAL	100	80	0						100
	D.O.	7.51	7.45	7.44						7.47
	PH	7.08	7.05	7.10						7.03
	TEMPERATURE	15	15	15						15
	OTHER									
4 HOURS	NO. SURVIVING	10	7							10
	% SURVIVAL	100	70							100
	D.O.	7.45	7.40							7.45
	PH	7.11	7.10							7.06
	TEMPERATURE	15	15							15
	OTHER									
8 HOURS	NO. SURVIVING	10	6							10
	% SURVIVAL	100	60							100
	D.O.	7.48	7.50							7.50
	PH	7.18	7.14							7.10
	TEMPERATURE	15	15							15
	OTHER									

LT31

TABLE II (CONTINUED) - 2 -

24 HOURS	CONCENTRATION	0.7	1.4	2.5							CONTROL
	NO. SURVIVING	10	6								10
	% SURVIVAL	100	60								100
	D.O.	7.40	7.50								7.45
	PH	7.28	7.24								7.20
	TEMPERATURE	15	15								15
	OTHER										
48 HOURS	NO. SURVIVING	8	4								10
	% SURVIVAL	80	40								100
	D.O.	7.38	7.46								7.47
	PH	7.33	7.30								7.24
	TEMPERATURE	15	15								15
	OTHER										
72 HOURS	NO. SURVIVING	8	4								10
	% SURVIVAL	80	40								100
	D.O.	7.43	7.46								7.48
	PH	7.36	7.35								7.29
	TEMPERATURE	15	15								15
	OTHER										
96 HOURS	NO. SURVIVING	8	4								10
	% SURVIVAL	80	40								100
	D.O.	7.47	7.47								7.42
	PH	7.41	7.44								7.21
	TEMPERATURE	15	15								15
	OTHER										

TABLE II

BIOASSAY - NUMERICAL RESULTS

SP1

START	CONCENTRATIONS	0.75	1.25	2.5						CONTROL
	D.O.	7.40	7.38	7.51						7.50
	PH	7.00	7.01	7.01						6.98
	TEMPERATURE	15	15	15						15
	OTHER									
1 HOUR	NO. SURVIVING	10	10	10						10
	% SURVIVAL	100	100	100						100
	D.O.	7.40	7.40	7.51						7.50
	PH	7.01	7.01	7.04						6.99
	TEMPERATURE	15	15	15						15
	OTHER									
2 HOURS	NO. SURVIVING	10	9	2						10
	% SURVIVAL	100	90	20						100
	D.O.	7.45	7.40	7.50						7.47
	PH	7.05	7.05	7.10						7.03
	TEMPERATURE	15	15	15						15
	OTHER									
4 HOURS	NO. SURVIVING	10	8	0						10
	% SURVIVAL	100	80	0						100
	D.O.	7.43	7.50	7.42						7.45
	PH	7.05	7.10	7.19						7.06
	TEMPERATURE	15	15	15						15
	OTHER									
8 HOURS	NO. SURVIVING	10	5							10
	% SURVIVAL	100	50							100
	D.O.	7.50	7.50							7.50
	PH	7.19	7.18							7.10
	TEMPERATURE	15	15							15
	OTHER									

SP1

TABLE 11 (CONTINUED)

24 HOURS	CONCENTRATION	0.75	1.25	2.5								C
	NO. SURVIVING	9	5									10
	% SURVIVAL	90	50									100
	D.O.	7.53	7.44									7.4
	PH	7.25	7.20									7.2
	TEMPERATURE	15	15									15
	OTHER											
48 HOURS	NO. SURVIVING	9	4									10
	% SURVIVAL	90	40									100
	D.O.	7.47	7.41									7 47
	PH	7.28	7.23									7.24
	TEMPERATURE	15	15									15
	OTHER											
72 HOURS	NO. SURVIVING	7	3									10
	% SURVIVAL	70	30									100
	D.O.	7.46	7.48									7.48
	PH	7.31	7.29									7.29
	TEMPERATURE	15	15									15
	OTHER											
96 HOURS	NO. SURVIVING	7	3									10
	% SURVIVAL	70	30									100
	D.O.	7.42	7.39									7.42
	PH	7.36	7.32									7.31
	TEMPERATURE	15	15									15
	OTHER											

TABLE II

LT35 BIOASSAY - NUMERICAL RESULTS

START	CONCENTRATIONS	0.7	1.0	2.5						CONTROL
	D.O.	7.41	7.45	7.43						7.50
	PH	6.98	6.95	6.99						6.98
	TEMPERATURE	15	15	15						15
	OTHER									
1 HOUR	NO. SURVIVING	10	10	10						10
	% SURVIVAL	100	100	100						100
	D.O.	7.40	7.45	7.43						7.50
	PH	7.01	7.00	7.02						6.99
	TEMPERATURE	15	15	15						15
	OTHER									
2 HOURS	NO. SURVIVING	10	8	0						10
	% SURVIVAL	100	80	0						100
	D.O.	7.43	7.47	7.50						7.47
	PH	7.03	7.04	7.08						7.03
	TEMPERATURE	15	15	15						15
	OTHER									
4 HOURS	NO. SURVIVING	10	6							10
	% SURVIVAL	100	60							100
	D.O.	7.45	7.38							7.45
	PH	7.09	7.08							7.06
	TEMPERATURE	15	15							15
	OTHER									
8 HOURS	NO. SURVIVING	10	5							10
	% SURVIVAL	100	50							100
	D.O.	7.40	7.43							7.50
	PH	7.16	7.15							7.10
	TEMPERATURE	15	15							15
	OTHER									

TABLE 11 (CONTINUED)

24 HOURS	CONCENTRATION	0.7	1.0	2.5							CONT
	NO. SURVIVING	8	2								10
	% SURVIVAL	80	20								100
	D.O.	7.37	7.40								7.46
	PH	7.25	7.20								7.26
	TEMPERATURE	15	15								15
	OTHER										
48 HOURS	NO. SURVIVING	8	2								10
	% SURVIVAL	80	20								100
	D.O.	7.43	7.40								7.47
	PH	7.29	7.24								7.24
	TEMPERATURE	15	15								15
	OTHER										
72 HOURS	NO. SURVIVING	6	2								10
	% SURVIVAL	60	20								100
	D.O.	7.50	7.44								7.48
	PH	7.33	7.27								7.29
	TEMPERATURE	15	15								15
	OTHER										
96 HOURS	NO. SURVIVING	6	2								10
	% SURVIVAL	60	20								100
	D.O.	7.45	7.51								7.42
	PH	7.36	7.29								7.31
	TEMPERATURE	15	15								15
	OTHER										

B 03

TEST REPORT

TITLE:
An assessment of growth inhibition (72 h EC_{50}) in the marine alga *Skeletonema costatum* by Antifoam H2

TEST PROTOCOL:
ISO 10253: 1995 (E)

CLIENT:
Allied Colloids Ltd
PO Box 38, Low Moor
Bradford, West Yorkshire
UK

Attn: Sarah Bridgeman

TERRA
MILJØLABORATORIUM A/S
Office Address:
High Technolog; Center
Thormøhlensgate 55
5008 Bergen
Norway
Tel: (+47) 55 543707
Fax: (+47) 55 543717

CLIENT REF/ORDER No:	CLASSIFICATION:	
ASB.159	CONFIDENTIAL	
TEST SUBSTANCE: Antifoam H2	TEST REF No: 60281.ac\tm.006\skelet	
STUDY DIRECTOR: Hilde Hushagen	TEST STARTED: 21.05.96	TEST FINISHED: 24.05.96
	REPORT DATE: 29.05.96	NO. OF PAGES: 10

SUMMARY OF TEST RESULTS:

A growth inhibition test was performed with *Skeletonema costatum* using Antifoam H2 at nominal concentrations of 0.056 - 5.6 mg.l^{-1}. The following results (based on growth rate) were obtained:

EC_{50} (72 h)	0.47	mg.l^{-1}
EC_{10} (72 h)	0.01	mg.l^{-1}
Highest NOEC	0.1	mg.l^{-1}

COMMENTS:

Test solutions of the test substance were prepared according to OSPAR HOCNF 1995; Annex 2 guidelines. A test solution of 1 g Antifoam H2 in 1 l reference sea water gave the following categorisation: 2. Test solutions were therefore prepared according to procedure B (HOCNF 1995; Annex 2). Highest NOEC refers to the highest test concentration showing no statistically significant reduction in growth rate compared to controls (*t*-test).
Due to the use of different statistic programmes EC_{10} (72 h) is reported as being lower than NOEC.

B 04

TEST SUBSTANCE		
COMMERCIAL NAME:	Antifoam H2	
MANUFACTURER/SUPPLIER:	Allied Colloids Ltd	
PHYSICAL DATA AND DESCRIPTION		
	DATA PROVIDED BY CLIENT (MSDS)	DETERMINED BY TEST LABORATORY
DENSITY at 20 °C	0.97 g.cm^{-3}	0.972 g.cm^{-3}
SOLUBILITY	Dispersible in water	Poorly soluble in sea water

CHARACTERISATION OF TEST SUBSTANCE
Purity: Test substance purity unknown. Information not provided by client
Appearance: Orange/amber liquid
Homogeneity: Homogeneous
Stability: Stable under normal conditions

THIS STUDY HAS BEEN PERFORMED IN THE LABORATORIES OF TERRA Environment A/S (TE) IN ACCORDANCE WITH THE PRINCIPLES OF GOOD LABORATORY PRACTICE (OECD-GLP). THIS REPORT IS A TRUE AND ACCURATE REPRESENTATION OF THE STUDY AND ITS RESULTS

HILDE HUSHAGEN, Study Director Date:

QUALITY ASSURANCE STATEMENT

Test Substance: **Antifoam H2**

Test Report No.: 60281.actm.006\skelet

THIS REPORT FULLY AND ACCURATELY REFLECTS THE RAW DATA GENERATED IN THE STUDY. THE STUDY AND FINAL REPORT HAVE BEEN INSPECTED /AUDITED IN ACCORDANCE WITH TERRA Environment's POLICIES AND PROCEDURES FOR GOOD LABORATORY PRACTICE (GLP) AS FOLLOWS:

Inspections relevant to study	Date
Most recent process-based inspection	24.5.96
Most recent facility-based inspection	1.5.96
Audit of final report	31.5.96

Facilities and processes relevant to this study are periodically inspected with a minimum frequency as specified in TE's Quality Assurance Manual

Phil McWilliams, Quality Assurance Unit Date: 31.5.96

TEST DESCRIPTION AND CONDITIONS

Test principle

The marine alga *Skeletonema costatum* is cultured for several generations in a defined medium containing a range of concentrations of the test substance. Test solutions are incubated for 72 h, during which time cell density is measured at intervals of at least [illegible]n. Inhibition is measured as a reduction in growth rate relative to control cultures grown under identic[illegible]onditions.

Definitions: *Control*: a mixture of sea water, nutrients and algal cells without the test substance

Cell density: number of cells per unit volume

Growth rate: expression of the rate of increase in cell density with respect to time

Median Effective Concentration, EC_{50}: the concentration of test substance which results in a 50% reduction in growth rate relative to controls

Partial Effect Concentration, EC_{10}: the concentration of test substance which results in a 10% reduction in growth rate relative to controls

No Observed Effect Concentration, NOEC: the highest concentration tested at which there is no statistically significant reduction of growth rate relative to controls

Test organism and culture conditions

The strain of *Skeletonema costatum* (Greville) used in these tests is NIVA BAC 1. The culture medium is prepared from natural sea water obtained from a depth of ca. 130 m. DOC of the sea water used is in the range 3.5 - 5.5 $mg.l^{-1}$, with a salinity of 33 - 34 ‰. The culture medium is prepared from natural sea water by addition of 1.5 ml nutrient stock solution 1, 0.5 ml nutrient stock solution 2, and 1 ml nutrient stock solution 3 per litre of sea water (see **Appendix 2**). The pre-culture is maintained under the same conditions as used in the test (see **Appendix 1**). Culture is semi-continuous under continuous even fluorescent lighting at an intensity of 25 - 35 $\mu E.m^{-2}.s^{-1}$, with continuous agitation on an orbital shaker. Temperature is maintained at 20 ± 2.0 °C. Algal cultures are used for tests when they are determined to be in a phase of exponential growth. Cell density in the pre-culture is measured immediately before a test in order to calculate the required inoculum volume.

Choice of test concentrations

Concentrations of test substance will normally follow a geometrical progression with an interval usually not exceeding 3.2. A range-finding test covering several orders of magnitude in concentration is performed before the main test in order to determine a suitable concentration range. Test concentrations are selected in order to give a growth inhibition range of <10 - >90%.

Reference standard test

A reference standard test is performed at a single concentration (1.5 $mg.l^{-1}$) using 3,5,-dichlorophenol as a standard reference toxicant (PARCOM). In general, reference results for algae should be between 20 and 80% growth inhibition in comparison with controls

Preparation of test solutions

Test solutions of the test substance in sea water are prepared individually at each test concentration in accordance with current PARCOM guidelines, following preliminary observations of its behaviour in sea water. A mixture of 1 g of the test substance in 1 litre of reference sea water is shaken vigorously, upending for 10 reversals of the contents. This is allowed to stand for 4 hours and then examined. A brief summary of the mixture categories and test solution preparation procedures is given below:

Behaviour of test substance in sea water and selection of sample preparation procedure (PARCOM)

Behaviour category		Procedure
1	No floating or settled materials, liquid or solid a) Clear solution/mixture b) Homogeneous emulsion or fine/colloidal suspension	A Test substance added to test medium and shaken vigorously, upending for 10 reversals of the contents.
	c) Neutrally buoyant droplets, particles or floc	B Appropriate amounts of test substance added to sea water while stirring (magnetic stirrer). The vessel is sealed and the contents stirred in the dark for 20-24 hours (vortex to 2/3 of fluid contents). Allowed to stand for 4 hours phase-separation and a sub-natant aqueous sample siphoned off and used as test solution.
2	Floating, but no settled, liquids or solids	
3	Settled, but no floating, liquids or solids	C Appropriate amounts of test substance added to sea water while stirring (magnetic stirrer). The vessel is sealed and the contents stirred in the dark for 20-24 hours at a speed sufficient to suspend all settled liquid or solid material. Allowed to stand for 4 hours phase-separation and a sub-natant aqueous sample siphoned off, avoiding all settled material, and used as test solution.
4	Floating and settled liquids or solid	D Appropriate amounts of test substance added to sea water while stirring (magnetic stirrer). The vessel is sealed and the contents stirred in the dark for 20-24 hours at a speed sufficient to suspend all floating and settled liquid or solid material. Allowed to stand for 4 hours phase-separation and a sub-natant aqueous sample siphoned off, avoiding all settled and floating material, and used as test solution.

Note: For procedures B,C and D the test substance is thoroughly homogenised overnight on a shaker table before sampling.

Evaluation of Antifoam H2

Behaviour category	2	Procedure	B

TABLE 1. Cell densities (particles.ml-1) in Skeletonema costatum test cultures

Test substance:	Antifoam H2									
Time (Hours)	Test concentrations (mg.l-1)									
	Control	Control	0.056	0.1	0.32	0.56	1	3.2	5.6	Ref. 1.5
0	1620	1620	1620	1620	1620	1620	1620	1620	1620	1620
Temp 18.0 °C	1620	1620	1620	1620	1620	1620	1620	1620	1620	1620
	1620	1620	1620	1620	1620	1620	1620	1620	1620	1620
Mean:	1620		1620	1620	1620	1620	1620	1620	1620	1620
23.75	15128	17926	15332	15410	12252	2652	5238	1226	2142	8782
Temp. 18.8 °C	18150	16746	17140	15416	15796	7400	5258	1594	2026	9590
	18520	16278	16574	15262	10826	5924	4122	1316	2294	5798
Mean:	17125		16349	15363	12958	5325	4873	1379	2148	8057
48	117100	142320	107200	95660	93000	3276	11550	1992	1578	18920
Temp 18.9 °C	126560	118020	134060	95860	92940	17684	12762	1708	1508	22580
	145960	100000	125220	91380	40440	10032	6254	1304	2294	14040
Mean:	124993		122160	94300	75460	10331	10189	1668	1793	18847
72	763000	823300	606300	780100	619000	2186	22446	3842	10516	37320
Temp 18.8 °C	980000	808900	785500	625400	745700	72860	30148	2200	5960	48140
	1026400	696300	862600	792900	345100	13534	68560	1396	13582	29380
Mean:	849650		751467	732800	569933	29527	40385	2479	10019	38280

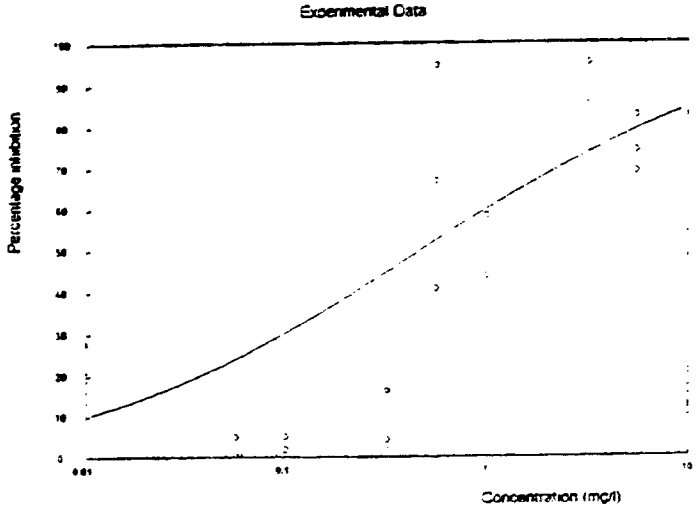


FIG. 1: Inhibition of specific growth rate of *Skeletonema costatum* as a function of the concentration of Antifoam H2

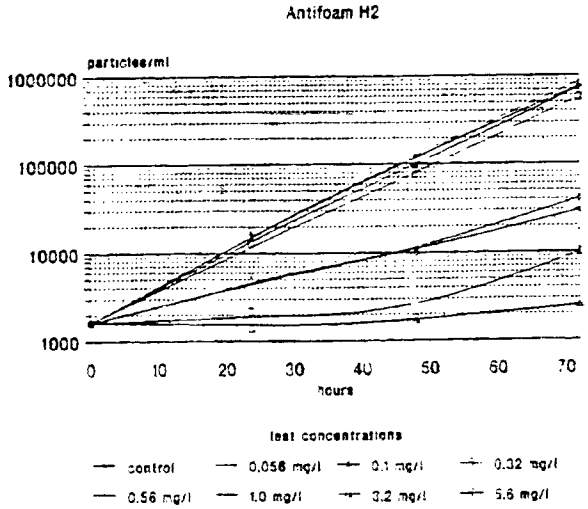


FIG. 2: Growth curve for *Skeletonema costatum* at different test concentrations of Antifoam H2

TABLE 2. Calculated effect concentrations for Antifoam H2

Test substance	Effect concentrations ($mg.l^{-1}$)	
Antifoam H2	E_rC_{50}*	0.47
	E_rC_{10}	0.01
	NOE$_r$C**	0.1

* the calculation is based on growth rate, m, which is the rate of increase in cell density (measured as particles/ml or fluorescence) with respect to time:

$$m = \frac{\ln N_n - \ln N_0}{t_n}$$

where t_n is the time of the last measurement of the of the exponential growth period
N_0 is the nominal initial cell density
N_n is the measured cell density at t_n

Data treatment: Probit transformation and linear regression of probit values on log concentration, assuming logarithmic normal distribution of data. All data transformations and analyses using TOXEDO vers. 1.3 (VKI, Denmark)

** No Observed Effect Concentration; the highest test concentration with no statistically significant reduction in growth rate compared to the controls (*t*-test)

Table 3: Standard reference toxicant

Standard reference toxicant	% Growth (compared to control)
3,5,-dichlorophenol (1.5 $mg.l^{-1}$)	50.5

Test validity criteria
The test fulfilled the following criteria and is therefore judged to be valid:

- Control cell density increased by a factor of >16 in 72 hours (corresponding to a growth rate of >0.9.day^{-1} (see **Table 1**)
- Control pH did not vary by more than 1.0 units during the test (see **Appendix 1**)
- Growth rate in the presence of the reference standard (3,5,-dichlorophenol; 1.5 $mg.l^{-1}$) should be 20-80% of control growth (see **Table 3**)

Appendix 1: Test conditions

Test substance	Antifoam H2
Test organism:	*Skeletonema costatum* NIVA BAC 1
Culture conditions:	Semi-continuous in natural SW + ISO growth medium (see Appendix 1)
Test dates:	Start: 21.05.96 End: 24.05.96
Test concentrations:	0.056, 0.1, 0.32, 0.56, 1.0, 3.2 and 5.6 $mg.l^{-1}$
Reference standard test	Single concentration 3,5,-dichlorophenol; 1.5 $mg.l^{-1}$
Test medium:	Natural SW (34 ‰) + growth medium
Incubation system:	250-ml glass Ehrlenmeyer flasks containing 100 ml medium on an orbital shaker
Light:	30 $\mu E.cm^{-2}.s^{-1}$, continuous luminescent light Supporting documentation is filed in Terra's GLP-archives
Temperature:	20 ± 2 °C Supporting documentation is filed in Terra's GLP-archives
pH in controls:	Start: 7.93 End: 8.13
Preparation of test solutions:	Procedure B (see Page 4)
pH at highest conc.:	Start: 7.92 End: 7.90
Growth measurement:	Coulter counter

Appendix 2: Composition of ISO growth medium used in the test

Component	Concentration in stock solution	Concentration in test solution
Stock solution 1		
$FeCl_3 \cdot 4\ H_2O$	53.1 mg/l	16.5 µg/l (Fe)
$MnCl_2 \cdot 4\ H_2O$	1.45 g/l	605 µg/l (Mn)
$ZnSO_4 \cdot 7\ H_2O$	43.9 mg/l	15.0 µg/l (Zn)
$CuSO_4 \cdot 5\ H_2O$	1.57 mg/l	0.6 µg/l (Cu)
$CoCl_2 \cdot 6\ H_2O$	4.04 mg/l	1.5 µg/l (Co)
H_3BO_3	11.4 g/l	17.1 mg/l
Na_2EDTA	73.8 mg/l	100 µg/l
Stock solution 2		
Thiamine hydrochloride	50.0 mg/l	25.0 µg/l
Biotin	0.01 mg/l	0.005 µg/l
Cyanocobalamin (Vitamin B_{12})	0.10 mg/l	0.05 µg/l
Stock solution 3		
K_3PO_4	4.76 g/l	4.76 mg/l
$NaNO_3$	50.0 g/l	50.0 mg/l
$Na_2SiO_3 \cdot 5H_2O$	14.9 g/l	14.9 mg/l

Appendix 3: References

1. **ISO 10253:1995 (E)** Water quality - Marine algal growth inhibition test with *Skeletonema costatum* and *Phaeodactylum tricornutum*

2. **TOXEDO**. Ecotoxicological Estimation and Documentation, Vers. 1.3, VKI/ATV, Denmark (1996)

A copy of this report, the study plan, raw data and other supporting documents will be stored in archives at the offices of TERRA Environment A/S,for a period of at least 5 years. The sample of the test substance on which this report is based, and copies of documents describing the specifications of the test substance, will be held in safe storage for a similar period.

B.13

TEST REPORT

TITLE:
An assessment of growth inhibition (72 h EC_{50}) of the marine alga *Skeletonema costatum* by Alcomer 280L

TEST PROTOCOL:
ISO 10253: 1995 (E)

CLIENT:
Allied Colloids Ltd
PO Box 38, Low Moor
Bradford, West Yorkshire
UK

Attn: Sarah Bridgeman

TERRA
MILJØLABORATORIUM A/S
Office Address:
High Technology Center
Thormøhlensgate 55
5008 Bergen
Norway
Tel: (+47) 55 543707
Fax: (+47) 55 543717

CLIENT REF/ORDER No: ASB.159	**CLASSIFICATION:** CONFIDENTIAL
TEST SUBSTANCE: Alcomer 280L	**TEST REF No:** 60281.ac\tm.003\skeletonema

STUDY DIRECTOR: Hilde Hushagen	**TEST STARTED:** 09.05.96	**TEST FINISHED:** 12.05.96
	REPORT DATE: 21.05.96	**NO. OF PAGES:** 10

SUMMARY OF TEST RESULTS:

A growth inhibition test was performed with *Skeletonema costatum* using Alcomer 280L at nominal concentrations of 0.032 - 3.2 $mg.l^{-1}$. The following results (based on growth rate) were obtained:

EC_{50} (72 h)	0.83	$mg.l^{-1}$	(95% ci 0.68 - 1.31)
EC_{10} (72 h)	0.49	$mg.l^{-1}$	(95% ci 0.22 - 0.61)
Highest NOEC	0.32	$mg.l^{-1}$	

COMMENTS:

Test solutions of the test substance were prepared according to OSPAR HOCNF 1995; Annex 2 guidelines. A test solution of 1 g Alcomer 280L in 1 l reference sea water gave the following categorisation: 2.Test solutions were therefore prepared according to procedure B (HOCNF 1995; Annex 2). Highest NOEC refers to the highest test concentration showing no statistically significant reduction in growth rate compared to controls (*t*-test).

TEST SUBSTANCE		
COMMERCIAL NAME:	Alcomer 280L	
MANUFACTURER/SUPPLIER:	Allied Colloids Ltd	
PHYSICAL DATA AND DESCRIPTION		
	DATA PROVIDED BY CLIENT (MSDS)	DETERMINED BY TEST LABORATORY
DENSITY at 20 °C	1.1 $g.cm^{-3}$	1.039 $g.cm^{-3}$
SOLUBILITY	Soluble	Poorly soluble in sea water

CHARACTERISATION OF TEST SUBSTANCE

Purity: Sample tested consists of 50% test substance in mineral oil (MSDS)
Appearance: White liquid
Homogeneity: Homogeneous
Stability: Stable under normal conditions

THIS STUDY HAS BEEN PERFORMED IN THE LABORATORIES OF TERRA Environment A/S (TE) IN ACCORDANCE WITH THE PRINCIPLES OF GOOD LABORATORY PRACTICE (OECD-GLP). THIS REPORT IS A TRUE AND ACCURATE REPRESENTATION OF THE STUDY AND ITS RESULTS

HILDE HUSHAGEN, Study Director Date: [illegible]

QUALITY ASSURANCE STATEMENT

Test Substance: Alcomer 280L

Test Report No.: 60281.ac\tm.003\skelet

THIS REPORT FULLY AND ACCURATELY REFLECTS THE RAW DATA GENERATED IN THE STUDY. THE STUDY AND FINAL REPORT HAVE BEEN INSPECTED /AUDITED IN ACCORDANCE WITH TERRA Environment's POLICIES AND PROCEDURES FOR GOOD LABORATORY PRACTICE (GLP) AS FOLLOWS:

Inspections relevant to study	Date
Most recent process-based inspection	24.5.96
Most recent facility-based inspection	1.5.96
Audit of final report	30.5.96

Facilities and processes relevant to this study are periodically inspected with a minimum frequency as specified in TE's Quality Assurance Manual

Phil McWilliams, Quality Assurance Unit Date: 30.5.96

TEST DESCRIPTION AND CONDITIONS

Test principle

The marine alga *Skeletonema costatum* is cultured for several generations in a defined medium containing a range of concentrations of the test substance. Test solutions are incubated for 72 h, during which time cell density is measured at intervals of at least 24 h. Inhibition is measured as a reduction in growth rate relative to control cultures grown under identical conditions.

Definitions:

Control: a mixture of sea water, nutrients and algal cells without the test substance

Cell density: number of cells per unit volume

Growth rate: expression of the rate of increase in cell density with respect to time

Median Effective Concentration, EC_{50}: the concentration of test substance which results in a 50% reduction in growth rate relative to controls

Partial Effect Concentration, EC_{10}: the concentration of test substance which results in a 10% reduction in growth rate relative to controls

No Observed Effect Concentration, NOEC: the highest concentration tested at which there is no statistically significant reduction of growth rate relative to controls

Test organism and culture conditions

The strain of *Skeletonema costatum* (Greville) used in these tests is NIVA BAC 1. The culture medium is prepared from natural sea water obtained from a depth of ca. 130 m. DOC of the sea water used is in the range 3.5 - 5.5 $mg.l^{-1}$, with a salinity of 33 - 34 ‰. The culture medium is prepared from natural sea water by addition of 1.5 ml nutrient stock solution 1, 0.5 ml nutrient stock solution 2, and 1 ml nutrient stock solution 3 per litre of sea water (see **Appendix 2**). The pre-culture is maintained under the same conditions as used in the test (see **Appendix 1**). Culture is semi-continuous under continuous even fluorescent lighting at an intensity of 25 - 35 $\mu E.m^{-2}.s^{-1}$, with continuous agitation on an orbital shaker. Temperature is maintained at 20 ± 2.0 °C. Algal cultures are used for tests when they are determined to be in a phase of exponential growth. Cell density in the pre-culture is measured immediately before a test in order to calculate the required inoculum volume.

Choice of test concentrations

Concentrations of test substance will normally follow a geometrical progression with an interval usually not exceeding 3.2. A range-finding test covering several orders of magnitude in concentration is performed before the main test in order to determine a suitable concentration range. Test concentrations are selected in order to give a growth inhibition range of <10 - >90%.

Reference standard test

A reference standard test is performed at a single concentration (1.5 $mg.l^{-1}$) using 3,5,-dichlorophenol as a standard reference toxicant (PARCOM). In general, reference results for algae should be between 20 and 80% growth inhibition in comparison with controls

Preparation of test solutions

Test solutions of the test substance in sea water are prepared individually at each test concentration in accordance with current PARCOM guidelines, following preliminary observations of its behaviour in sea water. A mixture of 1 g of the test substance in 1 litre of reference sea water is shaken vigorously, upending for 10 reversals of the contents. This is allowed to stand for 4 hours and then examined. A brief summary of the mixture categories and test solution preparation procedures is given below:

Behaviour of test substance in sea water and selection of sample preparation procedure (PARCOM)

Behaviour category		Procedure
1	No floating or settled materials, liquid or solid a) Clear solution/mixture b) Homogeneous emulsion or fine/colloidal suspension	A Test substance added to test medium and shaken vigorously, upending for 10 reversals of the contents.
	c) Neutrally buoyant droplets, particles or floc	B Appropriate amounts of test substance added to sea water while stirring (magnetic stirrer). The vessel is sealed and the contents stirred in the dark for 20-24 hours (vortex to 2/3 of fluid contents). Allowed to stand for 4 hours phase-separation and a sub-natant aqueous sample siphoned off and used as test solution.
2	Floating, but no settled, liquids or solids	
3	Settled, but no floating, liquids or solids	C Appropriate amounts of test substance added to sea water while stirring (magnetic stirrer). The vessel is sealed and the contents stirred in the dark for 20-24 hours at a speed sufficient to suspend all settled liquid or solid material. Allowed to stand for 4 hours phase-separation and a sub-natant aqueous sample siphoned off, avoiding all settled material, and used as test solution.
4	Floating and settled liquids or solid	D Appropriate amounts of test substance added to sea water while stirring (magnetic stirrer). The vessel is sealed and the contents stirred in the dark for 20-24 hours at a speed sufficient to suspend all floating and settled liquid or solid material. Allowed to stand for 4 hours phase-separation and a sub-natant aqueous sample siphoned off, avoiding all settled and floating material, and used as test solution.

Note: For procedures B,C and D the test substance is thoroughly homogenised overnight on a shaker table before sampling.

Evaluation of Alcomer 280L

Behaviour category	2	Procedure	B

TABLE 1. Cell densities (particles.ml-1) in Skeletonema costatum test cultures

Test substance:		Alcomer 280L								
Time (Hours)		Test concentrations (mg.l-1)								
		Control *	Control	0.032	0.056	0.1	0.32	0.56	1	3.2
0		1550	1550	1550	1550	1550	1550	1550	1550	1550
Temp	20.4 °C	1550	1550	1550	1550	1550	1550	1550	1550	1550
		1550	1550	1550	1550	1550	1550	1550	1550	1550
Mean:			1550	1550	1550	1550	1550	1550	1550	1550
25		11320	9400	9720	8640	8880	8820	3640	780	1180
Temp	20.8 °C	10140	7460	9420	8780	10180	9200	3100	420	700
		10100	10500	9900	10860	8100	8060	3020	720	1060
Mean:			9820	9680	9427	9053	8693	3253	640	980
47.5		81700	92500	90200	62300	89620	74480	35120	584	1024
Temp:	20.4 °C	91400	65340	88120	54020	74200	122320	23400	1088	652
		71080	92360	110340	89260	78040	80420	20120	554	506
Mean:			82397	96220	68527	80620	92407	26213	729	727
72.25		740800	954400	1128800	1122400	1005600	873600	418000	1600	780
Temp	20.3 °C	929600	817600	669600	745600	792000	1242400	206000	9300	500
		646400	995200	970400	1180800	902400	1024000	304400	1140	520
Mean:			847333	922933	1016267	900000	1046667	309467	4013	600

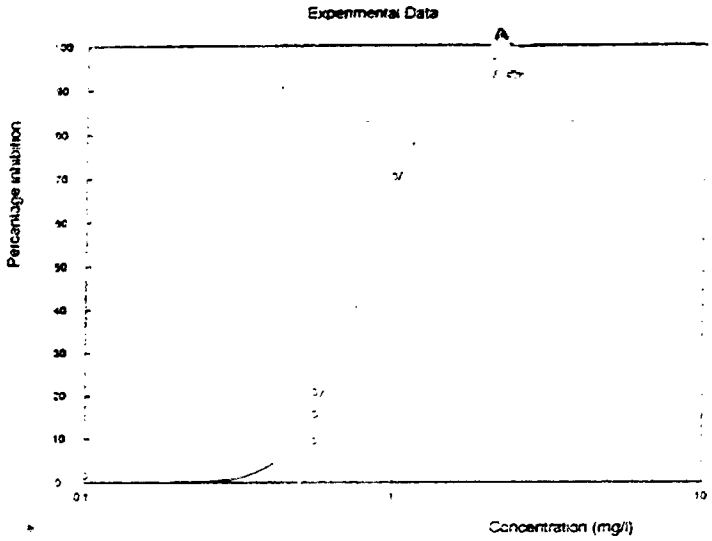


FIG. 1: Inhibition of specific growth rate of *Skeletonema costatum* as a function of the concentration of Alcomer 280L

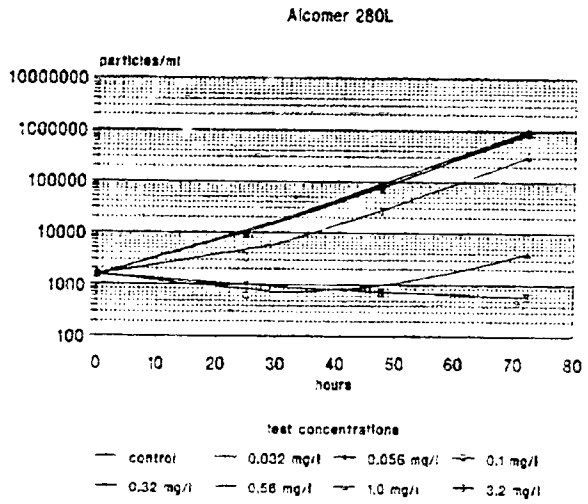


FIG. 2: Growth curve for *Skeletonema costatum* at different test concentrations of Alcomer 280L

TABLE 2. Calculated effect concentrations for Alcomer 280L

Test substance	Effect concentrations ($mg.l^{-1}$)		95% confidence limits
Alcomer 280L	E_rC_{50}*	0.83	0.68 - 1.31
	E_rC_{10}	0.49	0.22 - 0.61
	NOE,C**	0.32	

* the calculation is based on growth rate, m, which is the rate of increase in cell density (measured as particles/ml or fluorescence) with respect to time:

$$m = \frac{\ln N_n - \ln N_0}{t_n}$$

where t_n is the time of the last measurement of the of the exponential growth period
N_0 is the nominal initial cell density
N_n is the measured cell density at t_n

Data treatment: Probit transformation and linear regression of probit values on log concentration, assuming logarithmic normal distribution of data. All data transformations and analyses using TOXEDO vers. 1.3 (VKI, Denmark)

** No Observed Effect Concentration; the highest test concentration with no statistically significant reduction in growth rate compared to the controls (*t*-test)

Table 3: Standard reference toxicant

Standard reference toxicant	% Growth (compared to control)
3,5,-dichlorophenol (1.5 $mg.l^{-1}$)	66.2

Date of latest reference test: 19.04.96

Test validity criteria
The test fulfilled the following criteria and is therefore judged to be valid:

- Control cell density increased by a factor of >16 in 72 hours (corresponding to a growth rate of >0.9.day^{-1} (see **Table 1**)
- Control pH did not vary by more than 1.0 units during the test (see **Appendix 1**)
- Growth rate in the presence of the reference standard (3.5,-dichlorophenol; 1.5 $mg.l^{-1}$) should be 20-80% of control growth (see **Table 3**)

Appendix 1: Test conditions

Test substance	Alcomer 280L
Test organism:	*Skeletonema costatum* NIVA BAC 1
Culture conditions:	Semi-continuous in natural SW + ISO growth medium (see Appendix 1)
Test dates:	Start: 09.05.96 End: 12.05.96
Test concentrations:	0.032, 0.056, 0.1, 0.32, 0.56, 1.0 and 3.2 $mg.l^{-1}$
Reference standard test	Single concentration 3,5,-dichlorophenol; 1.5 $mg.l^{-1}$
Test medium:	Natural SW (34 ‰) + growth medium
Incubation system:	250-ml glass Ehrlenmeyer flasks containing 100 ml medium on an orbital shaker
Light:	30 $\mu E.cm^{-2}.s^{-1}$, continuous luminescent light Supporting documentation is filed in Terra's GLP-archives
Temperature:	20 ± 2 °C Supporting documentation is filed in Terra's GLP-archives
pH in controls:	Start: 7.85 End: 8.64
Preparation of test solutions:	Procedure B (see Page 4)
pH at highest conc.:	Start: 7.90 End: 7.94
Growth measurement:	Coulter counter

Appendix 2: Composition of ISO growth medium used in the test

Component	Concentration in stock solution	Concentration in test solution
Stock solution 1		
$FeCl_3 \cdot 4\ H_2O$	53.1 mg/l	16.5 µg/l (Fe)
$MnCl_2 \cdot 4\ H_2O$	1.45 g/l	605 µg/l (Mn)
$ZnSO_4 \cdot 7\ H_2O$	43.9 mg/l	15.0 µg/l (Zn)
$CuSO_4 \cdot 5\ H_2O$	1.57 mg/l	0.6 µg/l (Cu)
$CoCl_2 \cdot 6\ H_2O$	4.04 mg/l	1.5 µg/l (Co)
H_3BO_3	11.4 g/l	17.1 mg/l
Na_2EDTA	73.8 mg/l	100 µg/l
Stock solution 2		
Thiamine hydrochloride	50.0 mg/l	25.0 µg/l
Biotin	0.01 mg/l	0.005 µg/l
Cyanocobalamin (Vitamin B_{12})	0.10 mg/l	0.05 µg/l
Stock solution 3		
K_3PO_4	4.76 g/l	4.76 mg/l
$NaNO_3$	50.0 g/l	50.0 mg/l
$Na_2SiO_3 \cdot 5H_2O$	14.9 g/l	14.9 mg/l

Appendix 3: References

1. **ISO 10253:1995 (E)** Water quality - Marine algal growth inhibition test with *Skeletonema costatum* and *Phaeodactylum tricornutum*

2. **TOXEDO**. Ecotoxicological Estimation and Documentation, Vers. 1.3, VKI/ATV, Denmark (1996)

A copy of this report, the study plan, raw data and other supporting documents will be stored in archives at the offices of TERRA Environment A/S,for a period of at least 5 years. The sample of the test substance on which this report is based, and copies of documents describing the specifications of the test substance, will be held in safe storage for a similar period.