NIVAREPORT

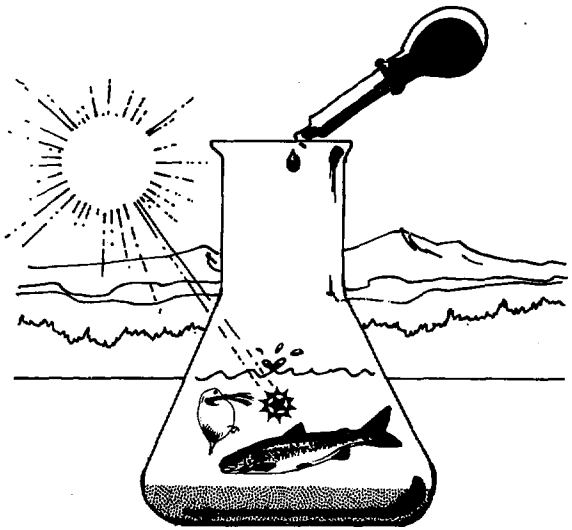
NIVA-O--90058

Environmental toxicology

NIVA-O--90058.

O-90058

Ecotoxicological testing of performance fluids



NIVA – REPORT

Norwegian Institute for Water Research NIVA

Main Office
P.O. Box [illegible]
N-0808 Oslo 8
Norway
Phone (47 2) [illegible]
Telefax (47 2) [illegible]

Regional Office, Sørlandet
Televeien 1
N-4890 Grimstad
Norway
Phone (47 41) 43 033
Telefax (47 41) 43 033

Regional Office, Østlandet
Rute 866
N-2312 Ottestad
Norway
Phone (47 65) 76 752
Telefax (47 65) 78 402

Regional Office, Vestlandet
Breiviken 5
N-5035 Bergen-Sandviken
Norway
Phone (47 5) 95 17 00
Telefax (47 5) 25 78 90

Report No
0-90058

NIVA-O--90058

DE92 541000

Sub-No

Serial No
2414

Limited distribution

Report Title
Ecotoxicological testing of performance fluids

Date
22.05.90

0-90058

Author(s)
Torsten Källqvist

Topic group
Analysis

Geographical area

Number of pages (incl. app.)
25

Contractor
Aker Drilling Fluids

Contractors ref. (or NTNF-No)
B. Ewens

Abstract
Two performance fluids were tested for toxic effects on marine algae and biological degradability. A fluid based on mineral oil was readily degradable (98% DOC removal in 28 days) while a ether based oil degraded more slowly (56% DOC removal in 28 days).

The toxicity of both fluids was tested after emulsification of the oils in water and separating the oil and water phase after equilibration. The EC_{50} values obtained with this approach were 8.15 g/l for the oil based fluid and 116 g/l for the ether fluid.

4 keywords Norwegian
1
2 Økotoksikologi
3 Biologisk nedbrytning
4 Giftighet
Oljeindustri

4 keywords English
1
2 Ecotoxicology
3 Biological degradation
4 Toxicity
Oil industry

Project leader

For the Administration

ISBN 82-577-1715-0

CONTENTS

NORWEGIAN INSTITUTE FOR WATER RESEARCH

ECOTOXICOLOGICAL TESTING OF PERFORMANCE FLUIDS

0-90058

NIVA, Oslo 11. May 1990

Project leader: Torsten Källqvist
Contributors: Harry Efraimsen
Randi Romstad
Lasse Berglind

INTRODUCTION

Aker Drilling Fluids A.S. has contracted Norwegian Institute for Water Research to carry out ecotoxicological testing of two performance fluids. Samples of the two fluids were sent to NIVA 22.03.90.

PRODUCTS TESTED

The fluids tested were:

ESCAID 120

A mixture of parafines, iso-paraffines and naphtenes with boiling points in the range 235-260 ^{0}C. Less than 1% aromatic hydrocarbons. Carbon chains mostly C_{12} C_{13} and C_{14}. Density: 0.815 g/cm^3.

MD-E-20

Predominantly C_{20} aliphatic synthetic ether ($C_{20}H_{42}O$). Boiling point (initial): 298 ^{0}C. Denity: 0.8305 g/cm^3.

TEST PROGRAMME

The tests included toxicity tests with the marine alga Skeletonema costatum and tests for biological degradability.

TEST METHODS

Toxicity - algae

The tests were carried out according to ISO/DP 10253: Marine Algal Inhibition Test, with Skeletonema costatum as test organism. This test is designed for testing of water soluble compounds, and therefore a modification of the mode of application of test compounds had to be made. Low doses of the fluids (<0.1% by volume) were obtained by addition of emulsions of the fluids to the algal growth medium. The emulsions were prepared by adding 1 ml of the fluids to 100 ml filtered sea water. The samples were sonicated at 20 kH and maximum power for 1 min. with a Virsonic 300 W ultrasonic cell disruptor. The emulsions appeared to be stable for several days.

High doses (0.1-21% by volume) of fluids were obtained by addition of 0.2 - 42 ml of fluids directly to 200 ml of the growth medium, followed by sonification at 1 min. at maximum power. The emulsions were left at room temperature in darkness for 20 hours before careful filtration through glass fibre filters to remove the fraction not soluble in the water phase. Removal of the oil fraction was necessary because the high amount of oil would physically interfere with the

algal growth by shading (as emulsion) and by blocking the gas exchange through the surface when floating on top of the medium.

Biodegradation

The biodegradation tests were carried out according to ISO/DIS 9408: Evaluation in an aquous medium of the "ultimate" aerobic biodegradability of organic compounds - respirometric method. The fluids were added as emulsions, prepared in destilled water as described for the algal tests. The tests were carried out at two substrate concentrations.

Caracterization of samples after degradation

The fluid emulsions used in the biodegradation test were characterized by gas chromatography before and after 28 days degradation. The toxicity of the remaining fractions after degradation was tested with an algal test. Since the degradation was tested in fresh water and at concentrations far below those that were toxic to the marine algae, the toxicity tests after degradation were carried out using a fresh water test (ISO DIS 8692) in order to avoid further dilution of the sample in sea water.

RESULTS

Toxicity - algae

The results of the algal toxicity tests are reported in appendix 1. The documentation includes a data sheet for each test and growth curves and response curves for those tests where a significant response was observed. The ISO standard prescribes two methods to describe the concentration/response relationship and for calculation of EC_{50}-values (EC_{50}= concentration which gives 50% effect on the measured response). Of these two, the area under growth curve usually gives lower EC_{50}-values than the growth rate. In evaluation of the the results we attach most importance to the growth rate. The EC_{50}-values for growth rate are listed together with the results of the degradation tests in table 1.

No effect on the algal growth rate was observed in the tests where emulsions of the fluids were added up to a concentration of 0.1 % by volume. In the higher concentration range tested after separation of the emulsion, toxic effects were observed with ESCAID-120 at concentrations above 0.1% and the growth was totally inhibited at 2.1% concentration. (See page 7). The EC_{50}-value for growth rate was 1.0%, which corresponds to 8.15 g/l.

For the ether based fluid, MD-E-20, no inhibition was observed at concentrations up to 4.7%. A second test where the concentration range was extended to 21% showed a sharp drop in growth rate as the concentration exceeded 10% (83 g/l). The EC_{50} for growth rate was 14% (116 g/l). Low concentrations of MD-E-20 caused a stimulation of the initial growth, and the area under the growth curves were significantly higher than the controls at most concentrations below 10%. Growth curves and response plots are shown on page 12.

Biodegradation

The results of the degradation tests are reported in appendix 2. The biological degradation of the oil based fluid, ESCAID 120 started rapidly without any lag phase. The oxygen consumption curve shows that approx. 50% of the oxygen was consumed during the first week of the test. (See page 17). The removal of dissolved organic carbon (DOC) implies almost complete degradation within 28 days (98%). This was verified by the gas chromatographic analysis which showed complete removal of the numerous hydrocarbon peaks in the chromatogram (See page 18).

The biological degradation of MD-E-20 was initially very slow, but increased steadily after adaptation of the microorganism community. (See page 21). Still, only 56% of the DOC was removed within 28 days. The course of the oxygen consumption curve indicates that higher DOC removal would have been obtained if the incubation time had been extended.

The partial degradation of MD-E-20 is verified by the gas chromatograms (see page 22), which shows a general lowering of all peaks. The separation of the peaks is not sufficient to allow a quantitative determination of the removal of single components.

The degradation rate obtained from the oxygen consumption was much lower than when the calculations were based on DOC removal for both fluids. The reason for this discrepancy is not known, but a significant discrepancy between these two estimates of degradation is often encountered. Most confidence should be put in the calculation based on DOC, which is a more specific parameter in this context. The DOC removal measured in these tests were also supported by the gas chromatographic analysis.

Toxicity after degradation

The results of toxicity tests of the degraded material are reported in appendix 3. The concentrations of the two fluids in the biodegradation test (0.005% by volume) were far below those that were toxic to the marine algae, and toxic effects in the reamining fractions were

therefore not expected. The toxicity test with the alga Selenastrum capricornutum showed no effect on the growth rate when algae were cultured in the degraded samples enriched with algal growth medium. (See figures on page 25). The growth rates were 102% and 98% of the control growth rate in MD-E-20 and ESCAID 120 respectively.

Table 1. Algal toxicity and biodegradability of the performance fluids ESCAID 120 and MD-E-20

Product	EC_{50} algal growth rate 72 hours g/l	Degradability DOC removal 28 days %
ESCAID 120	8.15	98
MD-E-20	116	56

APPENDIX 1

TOXICITY TESTS - ALGAE

NORWEGIAN INSTITUTE FOR WATER RESEARCH

TOXICITY TEST WITH MARINE ALGAE

ISO/DP 10253: Water quality - marine algae growth inhibition test with Skeletonema costatum and Phaeodactylum tricornutum

Compound: **ESCAID-120**

Organism: Skeletonema costatum strain NIVA BAC 1, cultivated in natural sea water enriched with 10% Z8 growth medium (Staub 1961).

Test details: Start date: 07.04.90 Duration: 72 h
Concentrations tested: 0.1, 0.21, 0.47, 1, 2.1, 4.7 % by volume. (Density 0.815 g/m^3).
Pretreatment: Compound emulsified in medium by sonification. The non-soluble phase removed after 20 hours at room temperature by careful filtration through glass fibre papers.
Composition of medium: Natural sea water + ISO nutrients
Culturing apparatus: Shaking table
Incubating procedure: 50 ml in 100 ml flat bottom flasks
Light: 80 µE/m^2/s, continous from daylight-type fluorescent tubes
Temperature: 20 ^{0}C
pH in control at start: 8.30 pH at end: 8.95
Method of measuring cell density: Particle counting with a Coulter Multisizer

Results: Table 1 shows cell density at each measuring point, the calculated areas under growth curves and growth rates in each flask . Mean values for each concentration. (and control) at the bottom . Growth curves for each concentration is shown in figure 1. Relationship between concentration and effect is shown in fig. 2 (growth rate) and fig. 3 (area under growth curve).

	Growth rate	Area under growth curve
EC_{50}:	1.0	0.23
95 % coinf. lim.	0.78 - 1.3	0.18 - 0.27
NOEC		

EC-50 (Concentration giving 50% effect on growth rate or area under growth curve) determined by linear regression of probit-transformed response against log concentration. NOEC (No Effect Concentration): Highest tested concentration showing no significant inhibition.

Responsible for test: Torsten Källqvist

Ref.: Staub (1961): Ernährungsphysiologische autokologische Untersuchungen an der planktischen Blaualge Oscillatoria rubescens D.C. Schweiz. Z. Hydrol. 23: 1-164

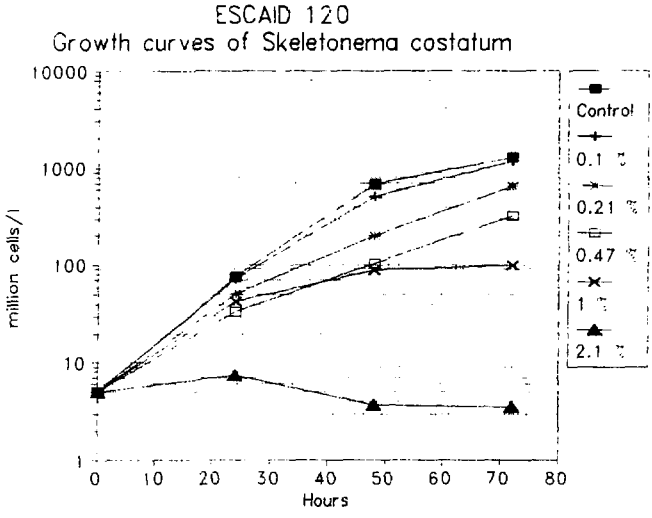


Fig. 1. Growth curves of Skeletonema costatum at different concentrations of ESCAID 120.

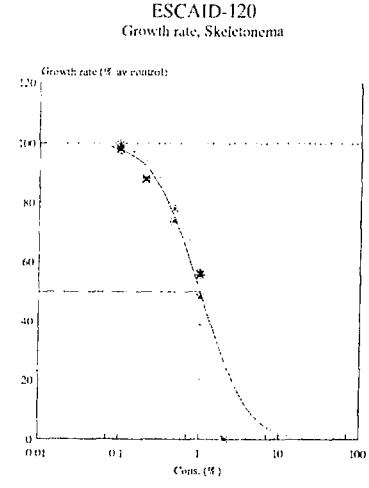


Fig. 2. Effect of ESCAID 120 on growth rate of Skeletonema costatum

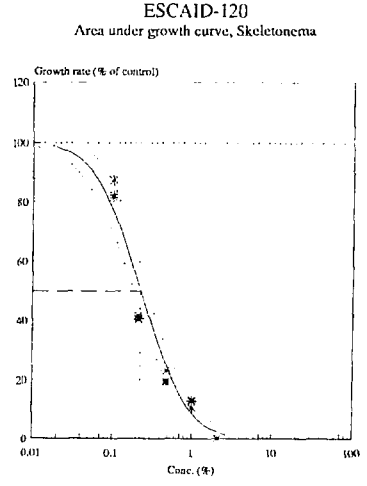


Fig. 3. Effect of ESCAID 120 on area under growth curves for Skeletonema

Table 1 **TEST:>> Aker Drilling Fluids** **Date >>>** 7.4.90
COMPOUND >>>> ESCAID 120 after separation of emulsion
TESTALGA >>>> *Skeletonama costatum* **Medium>** ISO
INOCULUM >>>> 5 mill. celler/l

	Hours:	Day 1 24 mill/l	Day 2 48 mill/l	Day 3 72 mill./l	Area	Area%	G.rate	G.rate%
Cons. 1	0.10%	67	538	1235	29040	87	1.84	99
		83	491	1136	27108	81	1.81	98
		70	500	1165	27360	82	1.82	98
Cons. 2	0.21%	52	202	670	13836	42	1.63	88
		55	196	641	13416	40	1.62	87
		45	188	672	13356	40	1.63	88
Cons. 3	0.47%	33	110	374	7620	23	1.44	78
		33	98	292	6348	19	1.36	73
		35	100	299	6528	20	1.36	74
Cons. 4	1%	42	91	114	4260	13	1.04	56
		48	92	110	4380	13	1.03	56
		39	84	73	3528	11	0.89	48
Cons. 5	2.10%	8	5	5	72	0	0.00	0
		7	3	1.7	-39.6	0	-0.36	-19
		7	3	3.9	-13.2	0	-0.08	-4
Cons. 6	4.70%	5	2	0	-132	0	#NUM!	#NUM!
		4	2	0	-156	0	#NUM!	#NUM!
		3	1	0	-204	-1	#NUM!	#NUM!
Cons. 7								
Control		76	655	1214	31812	96	1.83	99
		75	687	1250	32988	99	1.84	99
		82	682	1238	32892	99	1.84	99
		72	681	1282	33156	100	1.85	100
		82	686	1427	35256	106	1.88	102
		67	685	1322	33612	101	1.86	100

MEAN VALUES

0.10	Mv:	73.33	509.67	1178.67	27836.00	83.63	1.82	98.41
	St. d.	*6.94*	*20.37*	*41.56*	*857.55*	*2.58*	*0.01*	*0.63*
0.21	Mv.	50.67	195.33	661.00	13536.00	40.67	1.63	87.99
	St. d.	*4.19*	*5.73*	*14.17*	*213.54*	*0.64*	*0.01*	*0.39*
0.47	Mv.	33.67	102.67	321.67	6832.00	20.53	1.39	74.91
	St. d.	*0.94*	*5.25*	*37.12*	*562.02*	*1.69*	*0.04*	*2.01*
1.00	Mv.	43.00	89.00	99.00	4056.00	12.19	0.99	53.44
	St. d.	*3.74*	*3.56*	*18.46*	*376.55*	*1.13*	*0.07*	*3.64*
2.10	Mv.	7.33	3.67	3.53	6.40	0.02	-0.15	-7.97
	St. d.	*0.47*	*0.94*	*1.37*	*47.62*	*0.14*	*0.15*	*8.31*
4.70	Mv.	4.00	1.67	0.00	-164.00	-0.49	#NUM!	#NUM!
	St. d.	*0.82*	*0.47*	*0.00*	*29.93*	*0.09*	*#NUM!*	*#NUM!*
0.00	Mv.							
	St. d.							
Control	Mv.	75.67	679.33	1288.83	33286.00	100.00	1.85	100.00
	St. d.	*5.31*	*11.09*	*70.63*	*1034.47*	*3.11*	*0.02*	*0.96*

NORWEGIAN INSTITUTE FOR WATER RESEARCH

TOXICITY TEST WITH MARINE ALGAE

ISO/DP 10253: Water quality - marine algae growth inhibition test with Skeletonema costatum and Phaeodactylum tricornutum

Compound: **MD-E-20** (Test 1)

Organism: Skeletonema costatum strain NIVA BAC 1, cultivated in natural sea water enriched with 10% Z8 growth medium (Staub 1961).

Test details: Start date: 07.04.90 Duration: 72 h
Concentrations tested: 0.1, 0.21, 0.47, 1, 2.1, 4.7 % by volume. (Density 0.831 g/m^3).
Pretreatment: Compound emulsified in medium by sonification. The non-soluble phase removed after 20 hours at room temperature by careful filtration through glass fibre papers.
Composition of medium: Natural sea water + ISO nutrients
Culturing apparatus: Shaking table
Incubating procedure: 50 ml in 100 ml flat bottom flasks
Light: 80 µE/m^2/s, continous from daylight-type fluorescent tubes
Temperature: 20 °C
pH in control at start: 8.30 pH at end: 8.95
Method of measuring cell density: Particle counting with a Coulter Multisizer

Results: Table 1 shows cell density at each measuring point, the calculated areas under growth curves and growth rates in each flask . Mean values for each concentration. (and control) at the bottom . The test has been repeated with higher concentrations. Relationship between concentration and effect is shown in fig. 2 (growth rate) and fig. 3 (area under growth curve). Data from both tests are included in the figures.

	Growth rate	Area under growth curve
EC_{50}:	>4.7	>4.7
95 % coinf. lim.	-	-
NOEC	>4.7	>4.7

EC-50 (concentration giving 50% effect on growth rate or area under growth curve) and NOEC (No Effect Concentration) can not be determined from this experiment. Results from test with higher concentrations are shown on next page.

Responsible for test: Torsten Källqvist

Ref: Staub (1961): Ernahrungsphysiologische-autokologische Untersuchungen an der planktischen Blaualge Oscillatoria rubescens D.C Schweiz. Z. Hydrol. 23: 82 198

Table 1 **TEST:>> Aker Drilling Fluids** **DATE >>** 7.4.90
COMPOUND>>>> MD-E-20 after separation of emulsion (Test 1)
TESTALGA >>>> *Skeletonema costatum* **Medium>** ISO
INOCULUM>>>>> 5 mill. celler/l

	Hours:	Day 1 24 mill/l	Day 2 48 mill/l	Day 3 72 mill./l	Area	Area%	G.rate	G.rate%
Cons. 1	0.10%	91	951	1737	45552	137	1.95	105
		102	1030	2162	52812	159	2.02	109
		98	973	1538	43860	132	1.91	103
Cons. 2	0.21%	94	928	1625	43728	131	1.93	104
		83	912	1640	43980	132	1.93	104
		86	952	1640	44292	133	1.93	104
Cons. 3	0.47%	80	925	1705	44280	133	1.94	105
		78	908	1581	42336	127	1.92	104
		76	800	1508	38820	117	1.90	103
Cons. 4	1.00%	61	687	1401	34464	104	1.88	102
		58	669	1556	35820	108	1.91	103
		58	684	1513	35664	107	1.90	103
Cons. 5	2.10%	46	404	1227	25224	76	1.83	99
		47	390	1159	24096	72	1.82	98
		44	380	1375	26376	79	1.87	101
Cons. 6	4.70%	95	787	1411	37800	114	1.88	102
		108	827	1475	39840	120	1.90	102
		54	815	1538	39012	117	1.91	103
Cons. 7								0
								0
								0
Control		76	655	1214	31812	96	1.83	99
		75	687	1250	32988	99	1.84	99
		82	682	1238	32892	99	1.84	99
		72	686	1282	33276	100	1.85	100
		82	676	1427	35016	105	1.88	102
		67	685	1322	33612	101	1.86	100

MEAN VALUES

0.10	Mv:	97.00	984.67	1812.33	47408.00	142.51	1.96	105.99
	St. d.	*4.55*	*33.29*	*260.26*	*3883.14*	*11.67*	*0.05*	*2.54*
0.21	Mv.	87.67	940.67	1635.00	44000.00	132.27	1.93	104.31
	St. d.	*4.64*	*9.84*	*7.07*	*230.69*	*0.69*	*0.00*	*0.08*
0.47	Mv.	78.00	877.67	1598.00	41812.00	125.69	1.92	103.88
	St. d.	*1.63*	*55.36*	*81.32*	*2259.62*	*6.79*	*0.02*	*0.91*
1.00	Mv.	59.00	680.00	1490.00	35316.00	106.16	1.90	102.62
	St. d.	*1.41*	*7.87*	*65.34*	*605.81*	*1.82*	*0.01*	*0.80*
2.10	Mv.	45.67	391.33	1253.67	25232.00	75.85	1.84	99.48
	St. d.	*1.25*	*9.84*	*90.18*	*930.82*	*2.80*	*0.02*	*1.28*
4.70	Mv.	85.67	809.67	1474.67	38884.00	116.89	1.90	102.44
	St. d.	*23.01*	*16.76*	*51.85*	*837.73*	*2.52*	*0.01*	*0.63*
	Mv.							
	St. d.							
Control	Mv.	75.67	678.50	1288.83	33266.00	100.00	1.85	100.00
	St. d.	*5.31*	*11.12*	*70.63*	*958.61*	*2.88*	*0.02*	*0.96*

NORWEGIAN INSTITUTE FOR WATER RESEARCH

TOXICITY TEST WITH MARINE ALGAE

ISO/DP 10253: Water quality - marine algae growth inhibition test with Skeletonema costatum and Phaeodactylum tricornutum

Compound: **MD-E-20** (Test 2)

Organism: Skeletonema costatum strain NIVA BAC 1, cultivated in natural sea water enriched with 10% Z8 growth medium (Staub 1961).

Test details: Start date: 30.04.90 Duration: 72 h
Concentrations tested: 4.7, 10, 21 % by volume. (Density 0.831 g/m^3).
Pretreatment: Compound emulsified in medium by sonification. The non-soluble phase removed after 20 hours at room temperature by careful filtration through glass fibre papers.
Composition of medium: Natural sea water + ISO nutrients.
Culturing apparatus: Shaking table
Incubating procedure: 50 ml in 100 ml flat bottom flasks
Light: 80 µE/m^2/s, continous from daylight-type fluorescent tubes.
Temperature: 20 °C. pH in control at start: 8.30 pH at end: 8.95
Method of measuring cell density: Particle counting with a Coulter Multisizer

Results: Table 1 shows cell density at each measuring point, the calculated areas under growth curves and growth rates in each flask . Mean values for each concentration. (and control) at the bottom . Growth curves for each concentration is shown in figure 1. Relationship between concentration and effect is shown in fig. 2 (growth rate) and fig. 3 (area under growth curve).

	Growth rate	Area under growth curve
EC_{50}:	14	13
95 % coinf. lim.	-	-
NOEC	21	21

EC-50 (Concentration giving 50% effect on growth rate or area under growth curve) determined by eye from fig. 2 and 3. Linear regression of probit values could not be used because of too few intermediate responses. NOEC (No Effect Concentration): Highest tested concentration showing no significant inhibition.

Responsible for test: Torsten Källqvist

Ref: Staub (1961): Ernährungsphysiologische autokologische Untersuchungen an der planktischen Blaualge Oscillatoria rubescens D.C. Schweiz. Z. Hydrol. 23

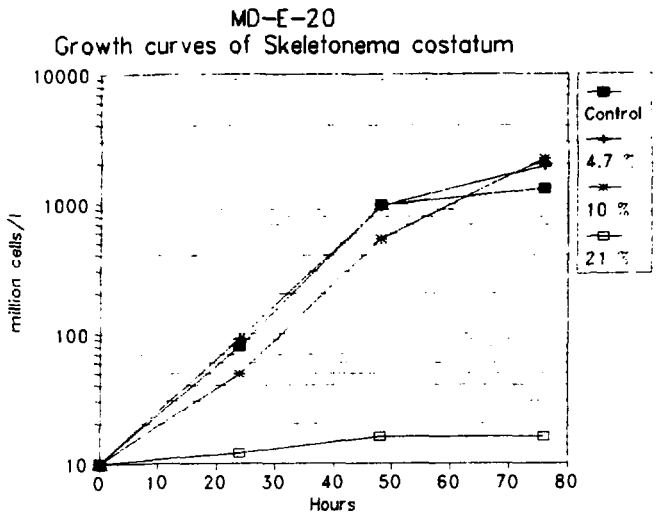


Fig. 1. Growth curves of Skeletonema costatum at different concentrations of MD-E-20.

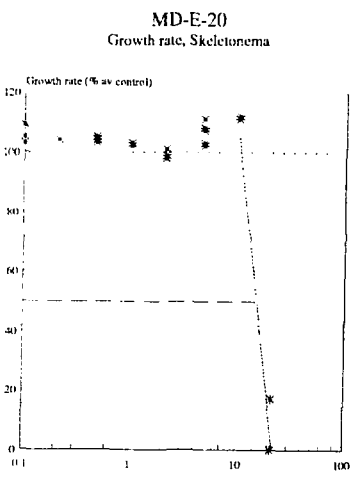


Fig. 2. Effect of MD-E-20 on growth rate of Skeletonema costatum. (Test 1 and 2).

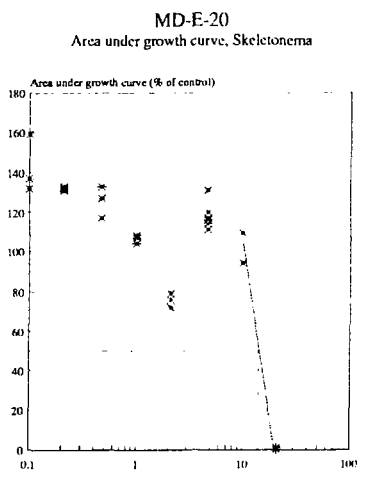


Fig. 3. Effect of MD-E-20 on area under growth curves for Skeletonema costatum. (Test 1 and 2)

Table 1 **TEST:>> Aker Drilling Fluids** **Dato>>>** 30.04.90
COMPOUND >>>> MD-E-20 after separation of emulsion (Test 2)
TESTALGA >>>>> *Skeletonema costatum* **Medium>** ISO
INOKULUM>>>>> 10 mill. celler/l

	Hours:	Day 1 24 mill/l	Day 2 48 mill/l	Day 3 76 mill./l	Area	Area%	G.rate	G. rate%
Cons. 1	4.70%	106	1018	2190	59032	131	1.70	111
		92	870	1833	49850	111	1.65	108
		75	974	1822	51992	116	1.64	107
Cons. 2	10%	57	698	2134	48752	109	1.69	111
		41	379	2276	42062	94	1.71	112
								0
Cons. 3	21%	12	14	9	138	0	-0.03	-2
		12	18	23	438	1	0.26	17
Cons. 4								
Cons. 5								
Cons. 6								
Cons. 7								
Control		88	1230	1818	58904	131	1.64	107
		83	1270	1283	52334	117	1.53	100
		86	829	1144	38994	87	1.50	98
		74	753	1065	35624	79	1.47	96
		74	674	1084	33836	75	1.48	97
		76	1120	1384	49680	111	1.56	102

MEAN VALUES

4.70	Mv:	91.00	954.00	1948.33	53624.67	119.44	1.66	108.70
	St. d.	*12.68*	*62.05*	*170.94*	*3922.29*	*8.74*	*0.03*	*1.76*
10.00	Mv.	49.00	538.50	2205.00	45407.00	101.14	1.70	111.32
	St. d.	*8.00*	*159.50*	*71.00*	*3315.00*	*7.45*	*0.01*	*52.48*
21.00	Mv.	12.00	16.00	16.00	288.00	0.64	0.11	7.51
	St. d.	*0.00*	*2.00*	*7.00*	*150.00*	*0.33*	*0.15*	*9.68*
0.00	Mv.							
	St. d.							
0.00	Mv.							
	St. d.							
0.00	Mv.							
	St. d.							
0.00	Mv.							
	St. d.							
Control	Mv.	80.17	979.33	1296.33	44895.33	100.00	1.53	100.00
	St. d.	*5.73*	*236.00*	*258.78*	*9287.64*	*20.69*	*0.06*	*3.80*

APPENDIX 2

BIODEGRADATION TESTS

NORWEGIAN INSTITUTE FOR WATER RESEARCH

TEST REPORT:
BIODEGRADABILITY OF ORGANIC COMPOUNDS IN AQUATIC MEDIUM

TEST SUBSTANCE: Product ESCAID-120

TEST CONDITIONS

APPARATUS: Manometric respirometer, WTW

TEST MEDIUM: ISO/DIS 9408 Solution (a), 10 ml/l (1,3 mg N/L)

INOCULUM: Bacteria from activated slugde, cultivated on synthetic sewage (OECD) in a Husmann unit. After centrifugation and resuspenion in dilution water, twice, the slugde was resuspended in supernatant of domestic sewage (NS 4749 BOD inoculum). Domestic sewage was aerated for 36 hours and particles were allowed to settle. This was done to increase the diversity of bacteria i the inoculum.
Inoculum concentration in the test medium: 15.0 mg/L suspended solids. Viable counts: $2.25 \cdot 10^8$/L

INCUBATION: Temperature: 20± 0.5^0 C . Duration: 28 days.

REFERENCE SUBSTANCE: Aniline, 20 mg C/L Lag-fase: 4 days
$\frac{BODn \times 100}{ThOD}$ = % degradation after 7 days= 60 (demand >40)
% degradaton after 14 days= 84 (demand >60)

Preparation of sample

The fluid was added as emulsion, prepared by adding 1 ml of the fluid up to 100 ml with destilled water. The stock sample was sonicated for 1 min. at 20 kH and maximum power with a Virsonic 300 W ultrasonic cell disruptor. The stock emulsion was further diluted 1:200 in nutrient solution to make the test samples.

The substance was tested in 4 separate flasks, and the concentration was 40.7 mg/L in the test medium. Dichloromethan was used to extract test substance not soluble in water at the end of the incubation period. Suspended solids in the test samples were separated by filtration through a glassfibre filter (Whatman GF/C, washed in deionized water). Undegraded test substance attached to the inner wall of the test flasks and to the suspended solids was dissolved (washed out) in the organic solvent. A small piece of glassfilter was put into a capsule, and a total of 0.1 ml of the extract was evaporated. The amount of carbon was determined.

The concentration of carbon was determined on a CARLO ERBA Elemental Analyzer-Mod. 1106. At the end of the test, soluble organic carbon in the test medium was analyzed on ASTRO mod. 2001 TC/TOC analyzer.

ANALYSIS AND CALCULATIONS

Carbon concentration in the product = 86.5 %
Carbon concentration in the stock solution = 7.05 g/L
Carbon concentration in the test solution = 35.25 mg/L

Calculations:
Carbon concentration in the initial test medium = 35.25 mg/L
" " " DCM extract 0.11 mg
Dissolved organic carbon in the test-
medium at the end of the test. 0.5 "
Not oxidized organic carbon 0.61 mg/L

Reduction by biodegradation: $\frac{(35.25-0.61) \times 100}{35.25}$ = 98 %

% biodegradation based on oxygen consumption:

$$\frac{2.04 \times 100}{3.2} = 64\ \%$$

Biochemical oxygen uptake curves (BOD mg O_2/mg substance):

The BOD curve indicates a normal rate of oxygen consumption during the time of incubation, without any lag-phase. The curve indicates a ready degradable product. Theoretical oxygen demand (ThOD) is based on the content of hydrocarbons (CnH_2n+H_2) in the product. The calculations based on extraction and carbon analysis reveal an almost ultimate elimination of organic carbon in the sample. This is also supported by the GC-analysis.

All chemical analysis were performed in dublicates, and mean values have been used in the calculation.

RESULTS
Biodegradation;
28 days, by dissolved organic carbon (DOC) removal = 98 %
28 days, based on BOD/ThOD = 64 %

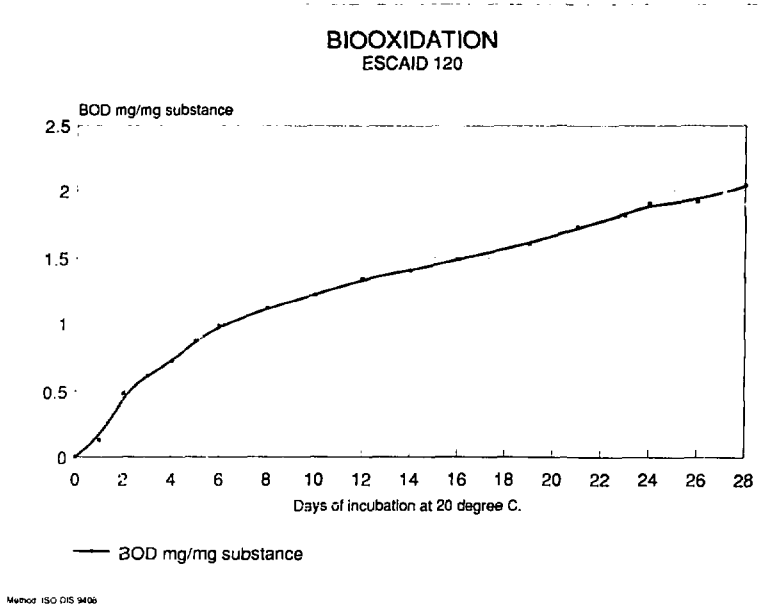
Conclusion:
The product tested is ready biodegradable at standard 28 days of incubation.

Oslo, May 21. 1990

Torsten Källqvist

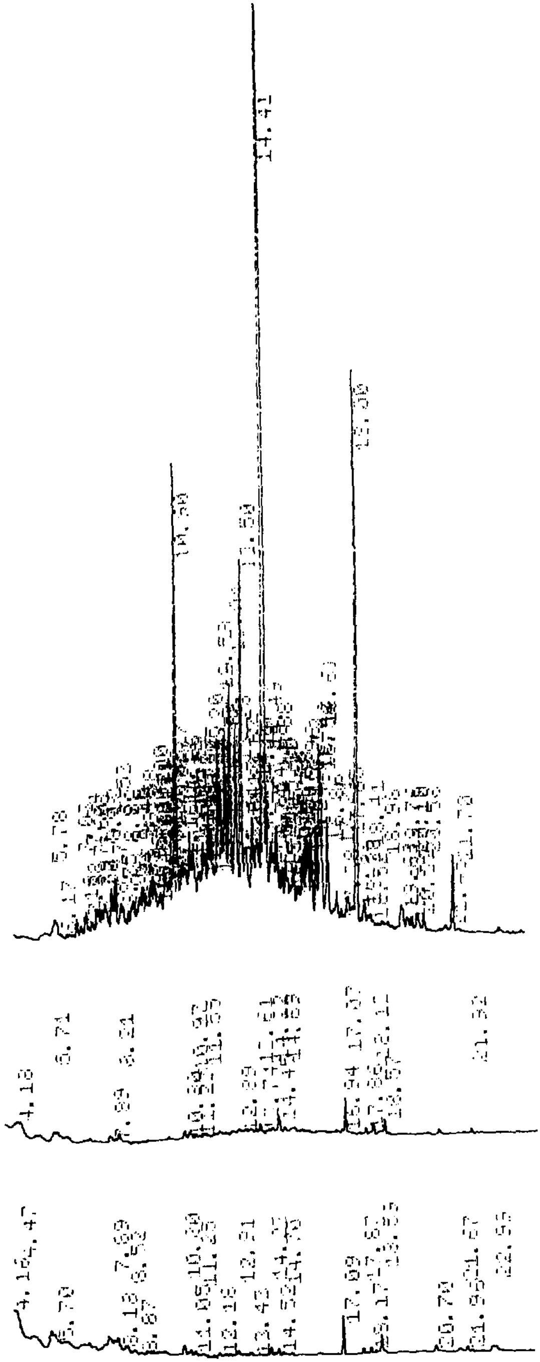
Harry Efraimsen

REFERANSE: ISO/DIS 9408 Water Quality- Evaluation in a aqueous medium of the "ultimate" biodegradability of organic compounds- Method by determining the oxygen demand in closed respirometer.



Method ISO DIS 9408

ESCAID-120



Before degradation

After degradation, 28 days

Inoculum, 28 days (blank)

NORWEGIAN INSTITUTE FOR WATER RESEARCH

TEST REPORT:
BIODEGRADABILITY OF ORGANIC COMPOUNDS IN AQUATIC MEDIUM

TEST SUBSTANCE: Product, MD-E-20. Aliphatic synthetic ether

TEST CONDITIONS

APPARATUS: Manometric respirometer, WTW

TEST MEDIUM: ISO/DIS 9408 Solution (a), 10 ml/l (1,3 mg N/L)

INOCULUM: Bacteria from activated slugde, cultivated on synthetic sewage (OECD) in a Husmann unit. After centrifugation and resuspenion in dilution water, twice, the slugde was resuspended in supernatant of domestic sewage (NS 4749 BOD inoculum). Domestic sewage was aerated for 36 hours and particles were allowed to settle. This was done to increase the diversity of bacteria i the inoculum.
Inoculum concentration in the test medium: 15.0 mg/L suspended solids. Viable counts: $2.25 \cdot 10^8$/L

INCUBATION: Temperature: 20± 0.5^0 C . Duration: 28 days.

REFERENCE SUBSTANCE: Aniline, 20 mg C/L Lag-fase: 4 days
BODn x 100 % degradation after 7 days= 60 (demand >40)
ThOD % degradaton after 14 days= 84 (demand >60)

Preparation of sample

The fluid was added as emulsion, prepared by adding 1 ml of the fluid up to 100 ml with destilled water. The stock sample was sonicated for 1 min. at 20 kH and maximum power with a Virsonic 300 W ultrasonic cell disruptor. The stock emulsion was further diluted 1:200 in nutrient solution to make the test samples.

The substance was tested in 4 separate flasks, and the concentration was 41.5 mg/L in the test medium. Dichloromethan was used to extract test substance not soluble in water at the end of the incubation period. Suspended solids in the test samples were separated by filtration through a glassfibre filter (Whatman GF/C, washed in deionized water). Undegraded test substance attached to the inner wall of the test flasks and to the suspended solids was dissolved (washed out) in the organic solvent. A small piece of glassfilter was put into a capsule, and a total of 0.06 ml of the extract was evaporated. The amount of carbon was determined.

The concentration of carbon was determined on a CARLO ERBA Elemental Analyzer-Mod. 1106. At the end of the test, soluble organic carbon in the test medium was analyzed on ASTRO mod. 2001 TC/TOC analyzer.

ANALYSIS AND CALCULATIONS

Carbon concentration in the product = 82.5 %
Carbon concentration in the stock solution = 6.85 g/L
Carbon concentration in the test solution = 34.25 mg/L

Calculations:
Carbon concentration in the initial test medium = 34.25 mg/L
" " " DCM extract 14.30 mg
Dissolved organic carbon in the test-
medium at the end of the test. 0.91 "
Not oxidized organic carbon 15.21 mg/L

Reduction by biodegradation: $\frac{(34.25-15.21) \times 100}{34.25}$ = 56 %

% biodegradation based on oxygen consumption:

$$\frac{0.77 \times 100}{3.2} = 24\ \%$$

Biochemical oxygen uptake curves (BOD mg O_2/mg substance):

The BOD curve indicates a lag phase in the first 6 days of incubation. Thereafter the oxygen consumption increased steadily with a relative higher rate of consumption in the perod from 18 to 28 days compared with the period from 6 to 16 days. Theoretical oxygen demand (ThOD) is based on the formula $C_{20}H_{42}O$ witch is the predominant chemical in the product. The calculations based on extraction and carbon analysis indicate that approximatly half og the carbon content is eliminated in the sample. The GC-analysis is also in agreement with these results. Biodegradation calculated from oxygen consumption and ThOD is low, but within the range of variance, generaly experienced in BOD/ThOD calculation.

All chemical analysis were performed in dublicates, and mean values have been used in the calculation.

RESULTS
Biodegradation;
28 days, by dissolved organic carbon (DOC) removal = 56 %
28 days, based on BOD/ThOD = 24 %

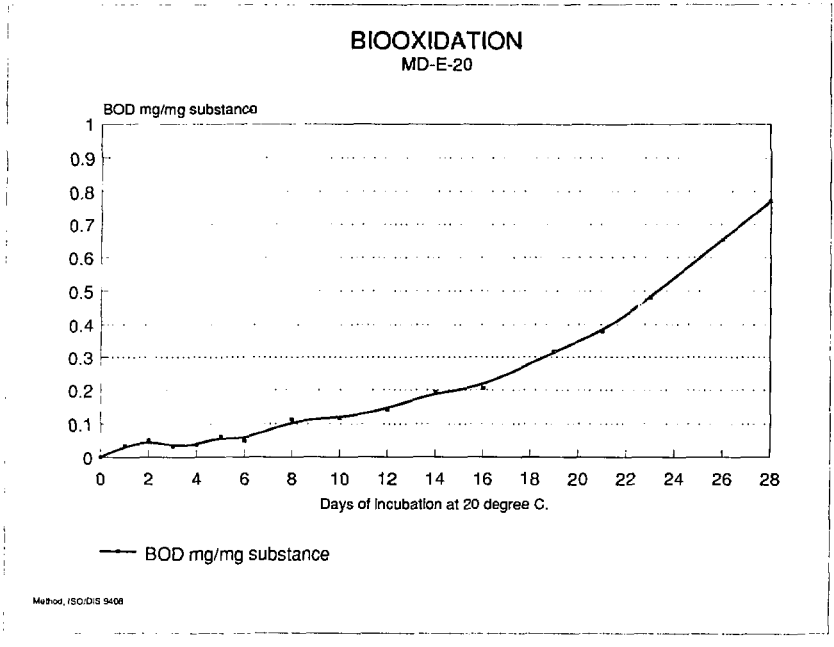
Conclusion:
The DOC removal is less than the 70 % usually used as lower limit for ready degradability. The oxygen sonsumption curve showes, however, that the degradation rate increases during the later part of the incubation period, which implies that adaptation of the microorganisms is required for degradation and a more complete degradation would be achieved if the incubation period had been extended beyond 28 days.

Oslo, May 21. 1990

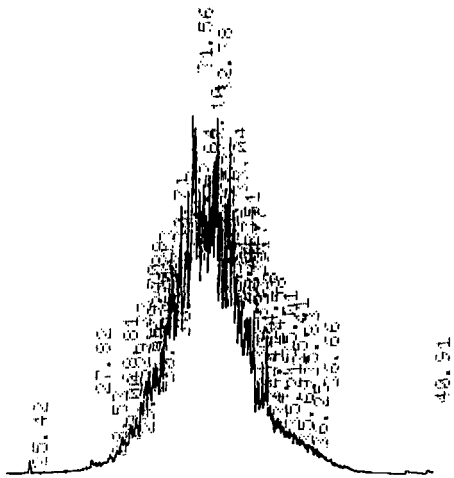
Torsten Kallqvist

Harry Efraimsen

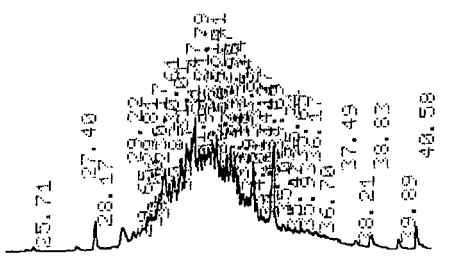
REFERANSE: *ISO/DIS 9408 Water Quality- Evaluation in a aqueous medium of the "ultimate" biodegradability of organic compounds- Method by determining the oxygen demand in closed respirometer.*


BIOOXIDATION
MD-E-20
BOD mg/mg substance
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28
Days of Incubation at 20 degree C.
BOD mg/mg substance
Method, ISO/DIS 9408

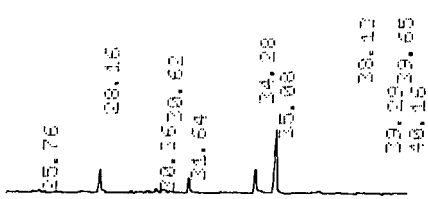
MD-E-20



Before degradation



After degradation,
28 days



Inoculum, 28 days
(blank)

APPENDIX 3

TOXICITY AFTER DEGRADATION

NORWEGIAN INSTITUTE FOR WATER RESEARCH

ALGAL INHIBITION TEST

ISO/DIS 8692: Water quality - algal growth inhibition test

Compound: Drilling fluids ESCAID 120 and MD-E-20 after 28 days biological degradation.

Organism: Selenastrum capricornutum NIVA CHL 1, cultivated in 10% Z8 growth medium (Staub 1961).

Test details: Start date: 15.5.90 Duration: 72 h
Concentrations tested: The concentrations of the drilling fluids in the degradation tests were:
ESCAID 120: 41 mg/l (0.005% by volume)
MD-E-20: 42 mg/l (0.005% by volume)
Composition of medium: 10% Z8
Culturing apparatus: Shaking table
Incubating procedure: 50 ml in 100 ml flat bottom flasks
Light: 80 $\mu E/m^2/s$, continous from daylight-type fluorescent tubes
Temperature: 20 ^0C
pH in control at start: 6.7 pH at end: 7.8
Method of measuring cell density: Particle counting with a Coulter Multisizer

Results: Table 1 shows cell density at each measuring point, the calculated areas under growth curves and growth rates in each flask . Mean values for each treatment (and control) at the bottom . Growth curves for each concentration is shown in figure 1.

	Growth rate	Area under growth curve
EC_{50}: 95 % coinf. lim. NOEC		

No significant effect on the growth rate or area under growth curve was observed at the concentrations tested, and EC_{50}-values can not be estimated.

Ref: Staub (1961): Ernährungsphysiologische-autökologische Untersuchungen an der planktischen Blaualge Oscillatoria rubescens D.C. Schweiz. Z. Hydrol. 23: 82-198.

Responsible for test: Torsten Källqvist

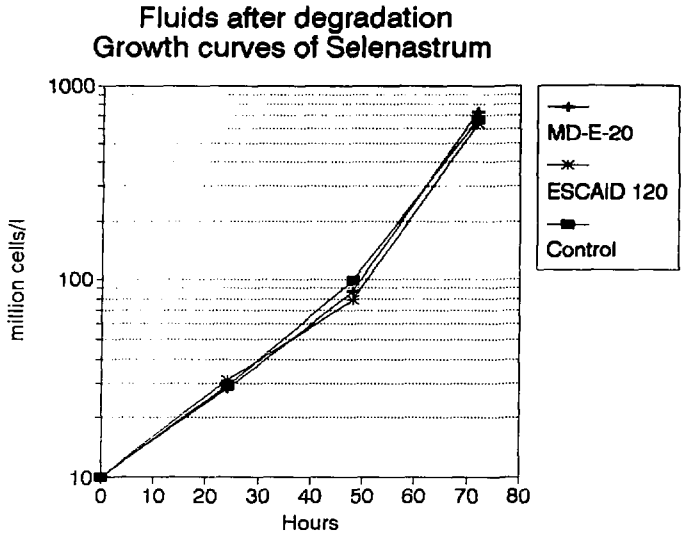


Fig. 1. Growth curves of Selenastrum capricornutum with the degraded samples of ESCAID 120 and MD-E-20 and in control cultures.

TEST:> > Aker Drilling Fluids **Dato> > > 16.5.90**
COMPOUND Fluids after degradation
TESTALGE> > > > > *Selenastrum capricornutum* **Medium>** ISO /DIS 8692
INOKULUM> > > > > 10 mill. celler/l

	Timer:	Day 1 24 mill/l	Day 2 48 mill/l	Day 3 72 mill./l	Area	Area%	G.rate	G.rate%
MD-E-20		29	86	850	12360	118	1.48	106
		29	83	700	10488	100	1.42	101
		27	91	649	10020	96	1.39	99
ESCAID		28	80	628	9528	91	1.38	99
120		33	82	646	9912	95	1.39	99
		31	76	646	9720	93	1.39	99
Kons. 3								
Kons. 4								
Kons. 5								
Kons. 6								
Kons. 7								
Control		27	91	649	10020	96	1.39	99
		30	102	622	10032	96	1.38	98
		26	100	658	10320	98	1.40	100
		30	103	779	11940	114	1.45	104
		32	97	678	10632	101	1.41	100
		31	102	611	9924	95	1.37	98

MEAN VALUES

MD-E-20	Mv:	28.33	86.67	733.00	10956.00	104.56	1.43	102.20
	St. d.	*0.94*	*3.30*	*85.31*	*1011.00*	*9.65*	*0.04*	*2.71*
ESCAID	Mv.	30.67	79.33	640.00	9720.00	92.77	1.39	99.12
120	St. d.	*2.05*	*2.49*	*8.49*	*156.77*	*1.50*	*0.00*	*0.32*
0.00	Mv.							#DIV/0!
	St. d.							*#DIV/0!*
0.00	Mv.							#DIV/0!
	St. d.							*#DIV/0!*
0.00	Mv.							#DIV/0!
	St. d.							*#DIV/0!*
0.00	Mv.							#DIV/0!
	St. d.							*#DIV/0!*
0.00	Mv.							#DIV/0!
	St. d.							*#DIV/0!*
Control	Mv.	29.33	99.17	666.17	10478.00	100.00	1.40	100.00
	St. d.	*2.13*	*4.14*	*55.12*	*695.43*	*6.64*	*0.03*	*1.89*

Norsk institutt for vannforskning NIVA

Postboks 69, Korsvoll
0808 Oslo 8

ISBN 82-577-1715-0