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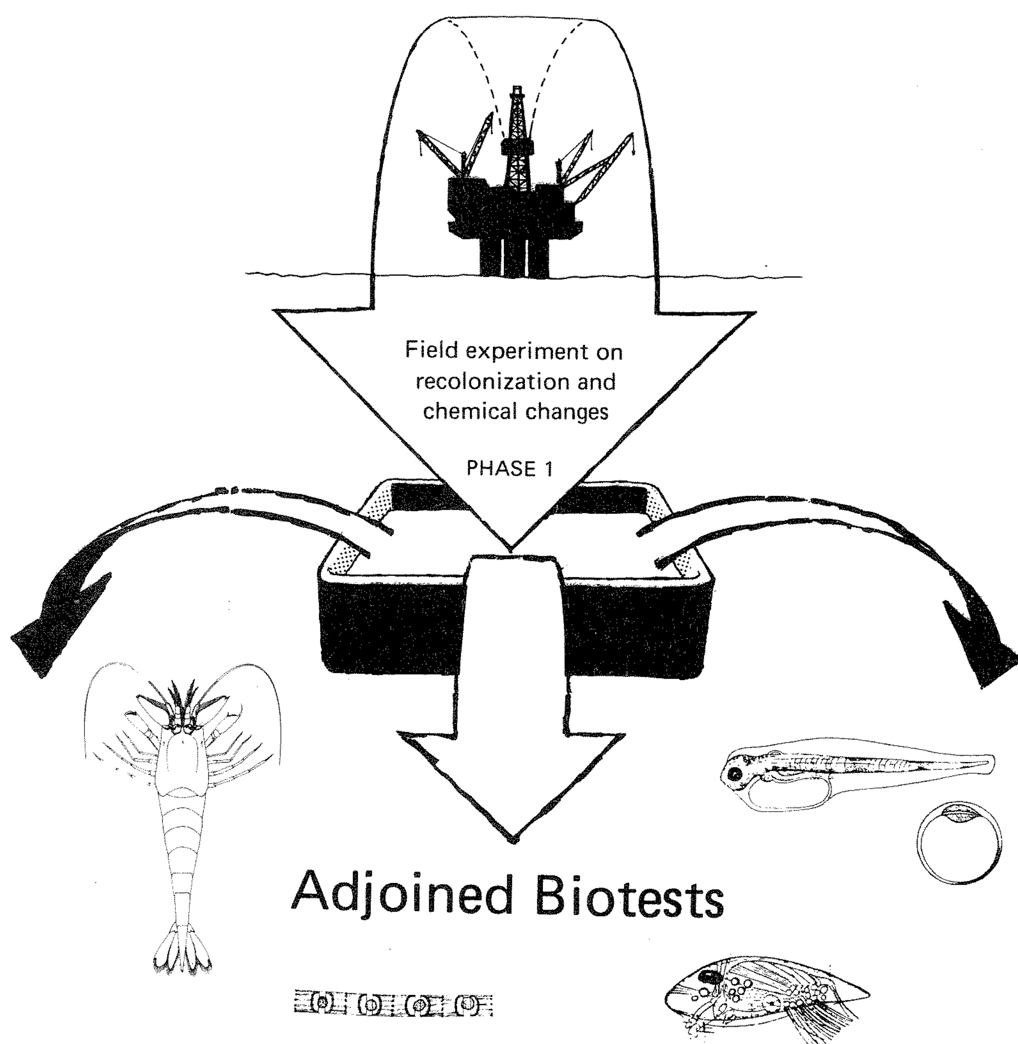
DRILL CUTTINGS ON THE SEA BED



Norwegian Institute for Water Research NIVA

NIVA - REPORT

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DRILL CUTTINGS ON THE SEA BED Field experiment on recolonization and chemical changes. Phase 1. Adjoined Biotests.	14 April 1986

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Abstract:

Acute toxicity of four types of water and oil based drill cuttings were tested by a series of biotests before and after 9 months exposure on the sea floor at 11 meters depth. Generally the cuttings were ranked with decreasing toxicity in the following way: diesel washed cuttings - briquetted diesel cuttings - low aromatic cuttings - water based cuttings - sea bed sediment (reference). Exposure on the sea floor did not significantly change this order of ranking, but the oil based cuttings became more toxic with time to some organisms (shrimps, cod larvae), less toxic to others (algae, barnacle larvae, cod eggs).

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3. Biotester	3. Biotests
4. Akutt toksisitet	4. Acute toxicity

Project leader

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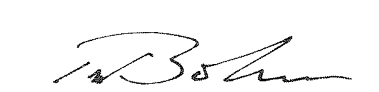
For the Administration



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PROJECT TO INVESTIGATE THE RECOLONIZATION OF BENTHIC ORGANISMS ON SEDIMENTARY BOTTOMS COVERED BY DISCHARGED DRILL CUTTINGS, AND THE POSSIBLE LEAKAGE OF CONTAMINANTS FROM THE CUTTINGS TO THE WATER.

ADJOINED BIOTESTS

Oslo, 10 July 1985

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PREFACE

This report presents the results of a series of short term biological toxicity tests performed in conjunction to the Phase I of the NIVA recolonization experiment which is part of the SFT/Statfjord Unit - Joint Research Project. The tests have been performed by the six research institutions listed below, which have also been responsible for the test reports constituting Annexes 1-6 in the present report. The main section of the report is a synopsis of the annexed reports and has been prepared by NIVA according to agreement with the institutions involved.

The institutions which have performed the toxicity tests are:

Ministry of Agriculture Fisheries and Food, Fisheries Laboratory, Burnham-on-Crouch, U.K.

Norwegian Institute for Water Research (NIVA).

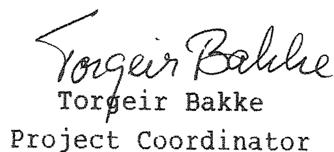
University of Bergen, Dept. of Microbiology and Plant Physiology.

University of Oslo, Norwegian Marine Pollution Research and Monitoring Programme and Dept. of Marine Biology.

University of Tromsø, Institute of Biology and Geology.

University of Trondheim, Dept. of Marine Biochemistry.

Oslo, 12 July 1985



Torgeir Bakke
Project Coordinator

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SUMMARY AND MAIN CONCLUSIONS

In connection to a comprehensive field experiment performed by the Norwegian Institute for Water Research (NIVA) in 1982-1983 to investigate how different types of water and oil based drill cuttings were colonized by benthic organisms, a series of short term toxicity tests were performed on the cuttings.

The objectives of performing these tests were:

1. to assess the acute toxicity of four types of drill cuttings versus a series of marine organisms,

2. to compare the acute toxicity of the cuttings with their suitability as substrate for the establishment of a benthic fauna,

3. to investigate the change in acute toxicity of the cuttings when incubated on the sea floor for 9 months and subjected to natural environmental perturbations.

4. to compare the sensitivity of the tests to drill cuttings toxicity.

CONCLUSIONS

With few exceptions the biotests ranked the cuttings after decreasing toxicity in the following order:
diesel cuttings - briquetted diesel cuttings - low aromatic cuttings - water based cuttings - clean reference sediment.

This ranking was in accordance with the ranking after suitability as substrate for the establishment of a benthic fauna, the reference sediment and the water based cuttings being the most suitable, and the diesel cuttings the least.

The change in toxicity with time on the sea floor was not unanimous, and was assumed to be related to the routes of entry of hydrocarbons in the test organisms. Toxicity towards animals able to ingest oil contaminated particles increased with time, whereas toxicity towards those without feeding ability decreased.

Incubation for 9 months on the sea floor was not sufficient time to change the ranking order of the cuttings significantly, but low

aromatic cuttings seemed to increase in toxicity relative to the diesel cuttings.

TESTS PERFORMED

The following acute biotests were applied to the initial cuttings:

Brown shrimps (Crangon crangon). MAFF Fisheries Laboratory, Burnham-on-Crouch, U.K. Responsible: Dr R.A.A. Blackman. Test parameter: survival.

Barnacle larvae (cyprids of Balanus improvisus). Dept. of Marine Biology, University of Oslo. Responsible: Cand.real. H. Hovde. Test parameter: larval settling and metamorphosis.

Pelagic green algae (Dunaliella bioculata) Dept. of Microbiology and Plant Physiology, University of Bergen. Responsible: Professor G. Knutsen. Test parameter: growth and survival.

Pelagic diatoms (Skeletonema costatum) Dept. of Marine Biochemistry, University of Trondheim. Responsible: Dr.ing. K. Østgaard. Test parameter: production, growth and survival.

Substrate degradability by bacteria. NIVA. Responsible: Siv.ing. K. Ormerod. Test parameter: oxygen consumption.

Toxicity towards aerobic bacterial degradation. NIVA. Responsible: Siv.ing. K. Ormerod. Test parameter: enzyme activity.

Eggs and embryos of cod (Gadus morhua) and sea urchins (Strongylocentrotus droebachiensis). Inst. of Biology and Geology, University of Tromsø. Responsible: Professor S. Vader. Test parameter: hatching and survival.

Only the shrimp, barnacle, diatom and embryological tests were performed on the 9 months material.

MATERIAL TESTED

The types of cuttings tested were
- from drilling with a water based mud,
- from drilling with a low aromatic mineral base oil mud,

- from drilling with a diesel base oil mud and with the cuttings treated in a diesel oil wash system, and
- from drilling with the same diesel base oil mud, without washing, but compressed into briquettes.

In addition natural clean shallow water sea bed sediment from the site of the incubation was tested as reference.

SAMPLE PREPARATION

For testing of initial acute toxicity the reference and the four types of cuttings were homogenized, and frozen before transfer to the test institutions.

The material to be tested for toxicity after 9 months on the sea floor were incubated as 10mm layers on top of 12cm reference sediment in 40x60 cm open trays on level bottom at 11 meters depth SW of Bergen, western Norway. This exercise was part of the NIVA recolonization experiment. Before incubation on the sea floor the cuttings were mixed mechanically with 40% (by volume) of sea water. The trays stayed open to the sea water on the bottom from 17 June 1982 to 17 March 1983 after which they were brought undisturbed to the laboratory. Here the 1 cm test layer of cuttings was removed and frozen together with a 0.5-1cm layer of looze detritus which had accumulated on top of the cuttings during the 9 months. No detritus had accumulated on the reference and water based trays. The samples were later homogenized and distributed to the test institutions.

RESULTS AND DISCUSSION

The shrimp and embryological tests provided results on the hydrocarbon content of the oil base cuttings indicating a general decrease with time on the sea floor. Part of this was explained by the initial test material being pure cuttings, the 9 months material being a mixture of cuttings, water and sedimented natural detritus.

With few exceptions the shrimp test, the barnacle test, the two microalgal tests, and the tests on cod eggs and larvae ranked the cuttings in the same order as to toxicity. The washed diesel cuttings were most toxic, the briquetted diesel cuttings nearly as toxic, the low aromatic cuttings were less toxic than these, and more so the water based cuttings. Least toxicity was shown by the reference sediment, but a clear distinction between this and the water based

cuttings was only shown in the barnacle, green algae, and diatom tests.

After 9 months on the sea floor this ranking had not changed to any extent, but the toxicity levels showed that the low aromatic cuttings were relatively more toxic compared to the two types of diesel oil cuttings.

The ranking expressed by the acute tests corresponded to the ranking of the cuttings with respect to their ability to support a benthic community, as found in the NIVA field experiment. In the reference and water based cuttings a healthy and diverse community developed during the same 9 months, whereas the community established in the diesel cuttings was poor and characterized by low diversity and high dominance of pollution indicators. The low aromatic cuttings supported a slightly better community, but the difference between this and those of the diesel cuttings was small. There were also some indications of the briquetted cuttings being slightly less adverse than the diesel washed cuttings.

For one and the same biotest organism the toxicity of the oil based drill cuttings changed in the same direction with time. The toxicity against shrimps and to some extent against cod larvae had increased after 9 months on the sea floor. The toxicity against barnacles, diatoms and cod eggs had decreased. The most plausible explanation of this difference lies in the route of entry of toxic components from the cuttings to the test organisms. The tests showed that the media made up for testing after 9 months were more turbid than the initial media, presumably due to detritus particles. It is likely that much of the oil on the cuttings was carried into suspension adsorbed to the particles and was ingested by the shrimp and cod larvae. The barnacle larvae, cod eggs and diatoms cannot ingest particles and therefore toxic components had to be mediated to these organisms through dissolution. Increased effects on shrimp and cod larvae could therefore be explained as increased amounts of toxic components suspended and taken in with the detritus, and decreased effects on barnacles, cod eggs and diatoms as loss of the more soluble and toxic hydrocarbons (presumably aromatics) to the water during 9 months on the sea floor. Analyses during the NIVA field experiment showed that such loss had occurred.

The toxicity levels established in these tests enabled a comparison of test sensitivity. The shrimp test showed sensitivity towards the

smallest concentrations of cuttings, possibly because the test applied both whole cuttings and water extracts. The diatom test was nearly as sensitive, and the barnacle test somewhat less sensitive than this. Least sensitivity was found in the green alga test and the test on acute inhibition of aerobic degradation.

INTRODUCTION

During the period June 1982 to May 1984 NIVA performed field experiments to compare the suitability of four different types of drill cuttings as substrates for the establishment of a bottom fauna (Bakke & al. 1984). The location of the field experiments was a level sand bottom at 11 meters depth in Raunefjord, south of Bergen, Western Norway. The project was performed as part of the Norwegian State Pollution Control Authority (SFT)/Statfjord Unit (SU) Joint Research Project, administered by Mobil Exploration Norway Inc. (MENI).

The main purpose of the project was to compare how cuttings generated from drilling with low aromatic mud would be colonized by bottom organisms compared to colonization on diesel or water based cuttings. Such information could indicate the rate of recovery of a cuttings-covered sea bottom, and would be of value to the State Pollution Control Authority (SFT) in their approval of the use of drilling fluids.

However, SFT has also expressed a need for short term tests which can be mobilised to produce acute toxicity information on chemicals fairly quickly. SFT has therefore established a routine test system which involves one British and six Norwegian short term biotests. To investigate how these tests ranked the various cuttings with respect to toxicity, and how this ranking was compared with corresponding ranking in the field experiment, it was decided to perform the short term tests on the initial cuttings applied in the field experiment and also on the same cuttings after exposure on the sea floor for 9 months at 11 meters depth.

This report presents the results of these tests (Annexes 1-6) together with a comparative evaluation of the ranking after toxicity (main chapters).

METHODS

Tests performed

The following tests were applied to the initial cuttings:

A. Brown shrimps (Crangon crangon).
MAFF Fisheries Laboratory, Burnham-on-Crouch, U.K.
Responsible: Dr R.A.A. Blackman. (Annex 1).

B. Barnacle larvae (cyprids of Balanus improvisus).
Dept. of Marine Biology, University of Oslo.
Responsible: Cand.real. H. Hovde. (Annex 2).

C. Pelagic green algae (Dunaliella bioculata)
Dept. of Microbiology and Plant Physiology, University of Bergen.
Responsible: Professor G. Knutsen. (Annex 3).

D. Pelagic diatoms (Skeletonema costatum)
Dept. of Marine Biochemistry, University of Trondheim.
Responsible: Dr.ing. K. Østgaard. (Annex 4).

E. Substrate degradability by bacteria and toxicity towards aerobic bacterial degradation.
NIVA.
Responsible: Siv.ing. K. Ormerod. (Annex 5).

F. Eggs and embryos of cod (Gadus morhua) and sea urchins (Strongylocentrotus droebachiensis).
Inst. of Biology and Geology, University of
Tromsø. Responsible: Professor S. Vader. (Annex 6).

Based on the results of the initial testing the following tests were selected to be performed on the 9 months material:
Tests A, B, D, and F.

Types of cuttings

The following types of cuttings were supplied by MENI for use in the field experiment, and hence used in the biotests:

WBM Cuttings produced during drilling with water based mud. Source: STATOIL well, from primary shakers/mud cleaners. Screen size shakers: 80/100 mesh, cleaner: 150/200 mesh. Mud type: Gypsum, CMC, lignosulphonate system, no bacteriocide reported used.

LAC Cuttings produced during drilling with low aromatic oil base mud. The type of cuttings received from MENI had been produced during drilling with a 'first generation' low aromatic oil based mud. This oil contained higher levels of aromatic hydrocarbons than the types presently at use (MENI pers. inf.). The cuttings were taken from 80/90 mesh screens, possibly also from mud cleaners (200/250 mesh).

BRI Unwashed cuttings produced during drilling with diesel oil base mud as the DOM below, but compressed into briquettes.

DOM Cuttings produced during drilling with diesel oil base mud. Source: STATOIL well, from fine mesh drying screen (20/40 mesh). Mud type: Inverted Oil Emulsion Mud (IOEM).

In addition natural sea bed sediment was applied as reference to show the natural colonization in the test area. This sediment was collected by grab from a near-by area at 5-15 m depth and frozen to kill the existing fauna. The sediment was thawed and sifted through 1cm mesh. Sea water from 40m depth and clean commercial granite sand of appropriate grain size were added in the proportions 3(sediment):2(water):1(sand) by volume. This combination was mixed in a cement mixer. This substrate is named REF below.

With few exceptions the biotests covered all the five substrates.

Sample preparation

Initial substrates

The REF and the four types of cuttings as provided by MENI were homogenized and stored frozen with no further treatment until transferred to the test institutions.

9 months substrates

The samples for the 9 months biotests were taken from the removable field experiment trays (cf. Bakke & al 1984, ch. 2.3.2). A description of location, environmental conditions, and how the trays were prepared and positioned is given by Bakke & al. (1984), and will only be summarized here. Before incubation on the sea floor the cuttings were mixed mechanically with 40% (by volume) of sea water and distributed in a 1 cm thick layer on top of 12 cm of REF sediment, in 40x60 cm polyethylene trays (height 25 cm). The trays stayed open to the sea water on the bottom for 9 months (17 June 1982 to 17 March 1983) after which they were brought undisturbed to the laboratory. Here the 1 cm test layer of cuttings was removed and frozen together with a 0.5-1cm layer of looze detritus which had accumulated on top of the cuttings during the 9 months. No detritus had accumulated on the REF and WBM trays, and here the upper 1 cm of the sediments was removed for testing.

All the frozen 9 months samples were brought to the FoH (Forskningsprogram om Havforurensninger) laboratory in Oslo, where sample homogenization and distribution to the test institutions was performed according to the practice of the SFT biotest system. Transfer was made by regular transport agencies or post, and generally the samples thawed before they were received by the institution. This resulted in the formation of hydrogen sulfide in some of the samples, which had to be removed by aeration before testing.

Test procedures

Descriptions of test organisms, test media preparation, test procedures, and ways of calculating toxicity are given in the annexes 1 to 6. These annexes are the reports submitted to NIVA from the test institutions.

RESULTS

This chapter aims at giving a synopsis of the results presented in the annexes. Toxic effects were tested over a range of concentrations of cuttings, and toxicity is expressed as EC-50 or LC-50 values in milligrams of cuttings or REF per liter sea water (mg/l). An EC-50 (Effective Concentration) value gives the concentration of cuttings or REF at which 50% of the organisms are affected or a process is decreased by 50% within a specific time period. (For instance if 50% of the test organisms Y are affected within 96 hours at 10,000 mg/l of cuttings type X, the toxicity of cuttings type X towards organism type Y is given as "96 h EC-50 = 10,000 mg/l".) Likewise an LC-50 (Lethal Concentration) value indicates the concentration at which 50% of the test animals are dead within a specific time period.

For all tests the concentrations are expressed as milligrams of cuttings initially added to one liter of sea water when preparing the test media. Most of the tests applied water extracts of the cuttings (cuttings and sea water mixed for a set period after which the extract is filterer from the solids and used) and no attempt has been made to calculate the real amounts of cuttings in the extracts. The EC and LC values presented are therefore to be considered as nominal.

Some of the annexes do also present EC-10 or EC-90 values or the variability between replicate tests but this chapter and the discussion is based on the mean EC- or LC-50 values only.

Hydrocarbon content of substrates.

In the Embryological Tests an initial set of experiments were done to find the optimum conditions for preparation of the water extracts with respect to mixing time, settling time, number of filtrations etc. and the effects of this on the hydrocarbon content of the water extracts produced. These experiments included analysis of the oil content of the water extracts by infrared spectroscopy. The method was considered suitable for comparative purposes and as long as the concentrations were above 1-2 ppm oil in the water which is the treshold of sensitivity for the IR method. A disadvantage of the IR analysis is that it detects mainly the alifatic hydrocarbons, which are the least soluble of the oil components. One must, however, expect that the hydrocarbons of the water extracts are partly dissolved, partly present as droplets or aggregates small enough to pass the glass wool

filter. A major fraction of these aggregates would be alifatic hydrocarbons.

The results are presented in Annex 6, tables 1-5. The results in Table 5 indicates that the the hydrocarbon content of the extracts based on the 9 months oil cuttings had decreased to 30-50% of the concentrations obtained when preparing extracts in the same way from the initial oil cuttings. This suggests either that the more soluble of the hydrocarbons which could be detected by the IR method, was lost during the 9 months incubation, or that the hydrocarbons were more tightly bound to the sediment particles after 9 months (possibly to the detritus). The former of these suggestions is not supported by the chemical analysis of the field experiment, which, except for the BRI, showed no significant loss of total hydrocarbons (mainly alifatics) with time on the sea floor (Bakke & al. 1984).

Although not requested, the Shrimp test report (Annex 1) was accompanied by some data on the oil content of the substrates as analysed by gas chromatography. These results are presented in Table 1 below. They indicated that the oil content of the test substrates after 9 months was only 10-20% of the initial amounts, and also that the unresolved complex mixture of extractable hydrocarbon and non-hydrocarbon compounds was much larger initially than after 9 months.

This apparent decrease in hydrocarbon content with time is partly explained by the initial substrates being pure cuttings, the final substrates being a mixture of cuttings, sea water and detritus where the cuttings on an average would constitute about 50% by weight (40% by volume). The remaining loss (30-40%) would then represent real loss of hydrocarbons from the cuttings, but such a loss was not detected in the field experiment (Bakke & al. 1984). It corresponds to the relative loss of aromatics (about 50%) from the cuttings during the 9 months, but a loss of this magnitude in total hydrocarbons was only detected in the BRI (Bakke & al. 1984).

The reason why the initial cuttings had a larger content of 'unresolved' compounds (usually indicative of degradation products) is not known, but a suggestion is that these in part may have consisted of additives to the mud which were lost to the water during the 9 months.

Table 1. Oil concentrations in micrograms oil equivalents per gram substrate in the samples used in the Brown Shrimp tests.

Sample	Oil equival.	Gravimetric	GC-resolved peaks	GC-total area
DOM initial	UK diesel	200 000	400 000	900 000
DOM 9 months	UK diesel	31 000	85 000	89 000
BRI initial	UK diesel	100 000	240 000	400 000
BRI 9 months	UK diesel	16 000	39 000	40 000
MOM initial	US Mentor 28	130 000	63 000	130 000
MOM 9 months	US Mentor 28	10 000	8 300	8 800

Test A. Brown shrimps (Annex 1)

This test and the test on green algae were the only ones applying whole cuttings in the test media. As the cuttings were mixed with the water before or during the test, these tests must be considered as toxicity testing of cuttings and water extracts combined. In the shrimp test the test media were changed every day during the testing, which was not done in the other tests. The institute included a sample of Mobil M2/83 13 ppg diesel based drilling mud from Statfjord A as a toxic reference to be tested in parallel with the others.

Initial substrates

The REF and WBM did not cause any significant mortalities within 96 hours at concentrations up to 10,000 mg/l, and were considered at least 60 times less toxic than the M2/83 mud. The LAC did also show very low toxicity (44 times less than the M2/83 reference) with LC-50 value of 7,100 mg/l. The toxicity of crushed briquettes are not specified in Annex 1 (cf p. 9), but an LC-50 value of about 1,000 mg/l has been estimated (Blackman, letter information). The most toxic substrate was the DOM. The LC-50 value was in the range of 140-620 mg/l which is only 1-4 times less toxic than the M2/83 reference mud.

9 months substrates

As initially the REF and WBM were not toxic to the shrimps. Highest toxicity was found by the DOM (about 1.3 times less toxic than the M2/83 mud). The BRI was only slightly less toxic than this. LAC was 4 times less toxic than the BRI.

Ranking the substrates after decreasing toxicity gives:

Substrate	Initial		9 months	
	Rank	LC-50	Rank	LC-50
DOM	1	140-620	1	100-330
BRI	2	ca 1,000	2	240
LAC	3	7,100	3	850
WBM	4	>10,000	4	>10,000
REF	4	>10,000	4	>10,000

The initial and final substrates were ranked in the same order of toxicity. The tests also showed that all the oily cuttings had

increased their toxicity with time on the sea floor. The LAC was 8.4 times more toxic at 9 months and had a very unusual toxicity curve (cf Annex 1, p.8 and Fig.2), the BRI was 4.2 times more toxic, and the DOM 1.7 times more toxic. All the media prepared were to some extent turbid and this was most pronounced for the 9 months samples.

Test B. Barnacle cypris larvae (Annex 2)

Each concentration applied was prepared separately from exact amounts of cuttings, not from dilution series of a stock extract. The EC-50 values presented shows the concentration necessary to prevent 50% of the larvae from going through normal settling and metamorphosis.

Initial substrates

The test showed that the REF was not toxic (EC-50 value above 600,000) and the WBM slightly toxic (EC-50: 360,000) to the larvae.The DOM had highest toxicity. Both BRI and LAC were considerably less toxic (BRI twice as toxic as LAC).

9 months substrates

After 9 months the DOM was still the most toxic. The second most toxic was the LAC which again was twice as toxic as the BRI. WBM showed only slight effect and REF was not tested.

The tests gave the following ranking after decreasing toxicity;:

Substrate	Initial		9 months	
	Rank	EC-50	Rank	EC-50
DOM	1	190	1	7,600
BRI	2	18,500	3	135,000
LAC	3	37,000	2	65,000
WBM	4	360,000	4	440,000
REF	5	>600,000	not tested	

Ranking at 9 months was different from that initially as the BRI had become less toxic than the LAC. The tests also showed that the toxicity to cypris larvae had decreased with time in all four types of cuttings. The largest reduction in toxicity was found in the DOM (40 times reduction), the least in the WBM (1.2 times). The LAC toxicity reduction was also small (1.8 times)

Test C. Planktonic green algae (Annex 3)

The main test series was performed by use of whole cuttings added to the test vials. In addition water extracts of REF and DOM were tested. Only the initial substrates were tested.

The tests on whole cuttings showed that only the DOM was toxic to the green alga Dunaliella bioculata (72 h LC-50: 7,100 mg/l).None of the others showed toxicity at concentrations <100,000 mg/l. The REF was not toxic below 300,000 mg/l, but at this and higher concentrations one must expect shading of the cultures to be of importance.

The extracted REF and DOM gave no significant toxic effect below 300,000 and 20,000 respectively.

Toxicity ranking deducted from Annex 3 will be:

Substrate	Initial		9 months
	Rank	LC-50	
DOM whole	1	7,100	
DOM extract	2	>20,000	
BRI whole	3	>100,000	
LAC whole	3	>100,000	not tested
WBM whole	3	>100,000	
REF whole	6	>300,000	
REF extract	6	>300.000	

Test D. Pelagic diatoms (Annex 4)

The test used the marine microalga Skeletonema costatum, and toxic effects were tested for on short (3 hours) and long term (3 days) photosynthetic activity, short term survival (3 hours), and growth rate (3 days). Of these responses survival was the least sensitive and growth rate the most. Only the toxicity against growth rate is presented here (cf Annex 4 for the other responses).

When preparing the media, the extracts of the initial substrates were glass wool filtered, whereas those from 9 months had to be Millipore-filtered through 0.45µm to make a clear medium. No dilution series were applied.

Initial substrates

The test showed that BRI was most toxic to growth of <u>Skeletonema</u> (EC-50: 920 mg/l). DOM had about the same EC-50 value: 1,060 mg/l, and was considered equally toxic to BRI. LAC was about 7.5 times less toxic, followed by WBM. The least toxic was REF (EC-50: 85,000).

9 months substrates

After 9 months the DOM was clearly the most toxic, followed by BRI and LAC with about equal toxicity and 3.5 times less than the DOM. The WBM and REF were both considered not toxic to diatom growth (EC-50 values 230,000mg/l).

Ranking after decreasing toxicity was:

Substrate	Initial		9 months	
	Rank	EC-50	Rank	EC-50
BRI	1	920	2	20,000
DOM	1	1,060	1	6,000
LAC	3	7,500	3	22,000
WBM	4	30,000	4	230,000
REF	5	85,000	4	230,000

Ranking after 9 months was in close accordance with the initial ranking. The tests also showed that toxicity had decreased with time on the sea floor, most in the BRI (21.7 times), least in the LAC and REF (2.9 and 2.7 times respectively). Some of this apparent decrease is due to the dilution of the samples by increased content of sea water and detritus. The two different ways of filtering the media may also have contributed somewhat to the 'loss' in toxicity.

<u>Test E. Biological degradabilty and toxicity towards aerobic degradation (Annex 5)</u>

The bacteria tests consisted of one test on the toxicity of the cuttings towards the bacteria and one test on how easily the cuttings could be degraded by bacteria under optimal conditions.

Acute inhibition of aerobic degradation.

The test applies inhibition of the dehydrogenase enzyme activity used by the bacteria in aerobic degradation, as the criterion for toxicity.

If the dehydrogenase activity of an activated bacteria sludge is inhibited, this would also indicate that the bacteria will be unable to degrade the test compounds through normal aerobic metabolism. The EC-50 values produced indicate the substrate concentrations at which enzyme activity was reduced by 50%. REF was not tested as it is hardly likely that natural sea bed sediment would be detrimental to bacterial activity. 3,5-dichlorophenol was applied as a standard to test how variable the sensitivity of the activated sludge was.

Neither LAC nor DOM inhibited enzyme activity up to the highest concentration applied: 98,000 mg/l. In fact LAC had a slight stimulating effect. BRI gave the most serious inhibition (EC-50 value: 16,000). WBM did also inhibit enzyme activity (EC-50: 36,000).

This gave the following toxicity ranking:

Substrate	Initial		9 months
	Rank	EC-50	
BRI	1	16,000	
WBM	2	36,000	not tested
LAC	3	>98,000	
DOM	3	>98,000	

The most surprising of this was the negative effect of the WBM. The inhibition could be explained if bacteriocides had been used as additive to the mud used, but according to MENI such addition had not been done.

Biological degradability

The test measures oxygen consumption rates under optimal conditions by active sludge bacteria, and with the cuttings as the source of organic carbon. The degradation rates are evaluated from the oxygen consumption of the test cultures. Aniline was used in stead of REF as an easily degradable standard reference substrate. It showed normal degradation with the sea water adapted inoculum used. It was also decided to omit BRI from the tests initially due to difficulties of preparing proper media.

The test showed the LAC to consist of one easily degradable fraction (comparable degradability to aniline) and one which degraded slowly. DOM was more resistant to bacterial degradation, but still with a

considerable oxygen consumption. The WBM was the least degradable, but oxygen was consumed to some extent which must be explained by chemical oxygen demanding processes occurring or that other sources of carbon than oil were present in the cuttings.

The results of the toxicity tests showed reasonable correlation with the degradation results: LAC and DOM did not inhibit degradation, and were themselves partly easily degradable. WBM, which mixed better with water was more toxic, but did not interfere with oxygen concumption at the concentrations used in the degradation test.

Test F. Eggs and embryos (Annex 6)

This test includes several embryological assays with the use of sea urchin and cod eggs, and cod larvae. Abnormal development (eggs) or inactivity (larvae) was used as criterion for toxicity, and 96h EC-50 values were presented. Test media were prepared as glass wool filtered, sea water extracts, and the smallest concentrations applied were made by dilution of stronger extracts. The results of an initial test with cod and urchin eggs have also been included in Annex 6 (Table 6). This exercise was considered a pilot test, including only the 0 months substrates and without replication. The results of the pilot test have therefore not been used in the calculation of toxicity.

Sea urchin eggs (Test F1)

This test was seriously disturbed by settlement of precipitated material to the test vessel bottom. The eggs develop at the bottom for the first two days, and the sedimentation seemed to give high egg mortality. The % survival of the eggs is presented in Annex 6, tables 7a and b, but no EC-50 values have been calculated. The low initial toxicity of the DOM is however surprising as one might expect the chemical toxicity and the stress from sedimentation to be at least additive.

Cod eggs (Test F2)

Initial substrates

BRI was found most toxic to cod egg development (EC-50: 5,400 mg/l), and it was nearly twice as toxic as the DOM (EC-50: 9,800 mg/l). None of the other substrates were toxic at concentrations up to 100,000 mg/l.

9 months substrates

BRI was most toxic after 9 months (EC-50: 13,500 mg/l), but only slightly more than DOM (EC-50: 15,000 mg/l). None of the other substrates were toxic at concentrations up to 100,000 mg/l.

This gave the following toxicity ranking:

Substrate	Initial		9 months	
	Rank	EC-50	Rank	EC-50
BRI	1	5,400	1	13,500
DOM	2	9,800	2	15,000
LAC	3	>100,000	3	>100,000
WBM	3	>100,000	3	>100,000
REF	3	>100,000	3	>100,000

Only the two diesel based cuttings were toxic to cod eggs and they were ranked in the same way on the two occations. The toxicity decreased with time, the BRI toxicity slightly more than the DOM.

Cod larvae (Test F3)

Initial substrates

Only the BRI showed clear toxic effects on the larvae. The DOM showed some indications of toxicity, but the results did not indicate any dose-response relationship, and an EC-50 value has therefore not been calculated.None of the other substrates were found to be toxic at concentrations up to 100,000 mg/l.

9 months substrates

Both DOM and BRI were most toxic also at 9 months, DOM somewhat more than BRI. LAC did also show toxicity, but nearly 5 times less than BRI. WBM and REF gave no effects at concentrations up to 100,000 mg/l.

Toxicity ranking was:

Substrate	Initial		9 months	
	Rank	EC-50	Rank	EC-50
BRI	1	5,000-10,000	2	9,600
DOM	-	-	1	6,600
LAC	2	>100,000	3	46,000
WBM	2	>100,000	4	>100,000
REF	2	>100,000	5	>100,000

The ranking at 9 months did not contradict the initial ranking and was in accordance with that of the tests A, B, and D. Toxicity increased with time in the LAC.

DISCUSSION

General aspects

Many years of research have clearly demonstrated that the toxic effects of oil in a marine environment should not be expected to correlate directly with the amount or concentration of oil present.

The toxicity of a given oil is primarily determined by its physical-chemical status in the system. This status is very labile due to a multitude of factors. Physical factors of particular importance are the formation of dispersions or emulsions ('oil-in-water' and 'water-in-oil'), particle adsorption of oil, the particle size distribution, and the distribution of soluble compounds between the oil and water phases. Chemical reactions may be caused by microbial, particularly bacterial, activity. More direct chemical reactions do also take place. Photo-oxidation is particularly important and may be of significance even in dim and low-energetic (infrared) light. All these factors may be cooperative or synergistic; bacterial degradation is dependent on bacterial access which depends on size and distribution of oil droplets and particles, photochemical products may act as emulsifiers etc.

The size of microorganisms will generally be similar to that of the oil droplets and particles, or even smaller. With the exception of bacteria using oil as a substrate, microorganisms are assumed to be directly exposed only to the water phase in an aquous oil contaminated environment. The prescence of undissolved oil should mainly be considered as as reservoir for additional dissolution of oil compounds or reaction products.

Macroorganisms may on the other hand be directly affected by dispersed or particle-adsorbed oil. In addition to exposure by direct contact, the possibility of oil entering the body by unselective feeding on particles should be considered.

It should be evident that a variety of test organisms is necessary to evaluate the acute toxicity of oil-containing material such as mud/cuttings, and that different organisms should not be expected to respond similarly to changes in the physical-chemical status of the oil. This is illustrated in this project by the difference in reaction to the cuttings (e.g. toxicity ranking) expressed by the activated

bacteria sludge on one hand (specialized for degradation of organic matter) and the higher organisms: the algae and animals on the other.

Comparison of toxicity ranking

Generally the short term tests ranked the substrates with decreasing toxicity in the same way as the field experiment (Table 2):

DOM	1	most toxic
BRI	2	
LAC	3	
WBM and REF	4	least toxic

The main exceptions were the Inhibition of Aerobic Degradation by Bacteria and the Sea Urchin Eggs tests (the latter not included in Table 2). This ranking is also as one might expect with the two diesel based substrates being most acutely toxic and the two non-oil substrates the least. The tests indicated that the acute toxicity of most substrates changed with time, but 9 months incubation on the sea floor was not enough to shift the toxicity ranking of the substrates in each test. When looking at the EC or LC values it seems that the LAC has increased in acute toxicity relative to the diesel substrates, but only in the cod eggs and barnacle tests was this manifested by a change in ranking.

Changes in test samples of cuttings with time.

Physical changes

The initial test substrates were transferred to the test institutes without any previous treatment except for freezing. No water addition was made. When preparing the field trays 40% sea water was added. Analysis of water content of the substrates at 9 months confirms a water content of the same magnitude (Bakke & al. 1984). Hence the 9 months samples that all laboratories received, contained cuttings plus 30-40% sea water (by volume, mean 35%). In addition a layer of detritus gradually developed on top of the oil cuttings nearly to a thickness of 1 cm after 9 months. This detritus was not removed from the cuttings when sampling the 9 months substrates, as it represented a normal feature in the change of deposited cuttings with time. The 1cm layer of oily cuttings was still intact at 9 months and was sampled completely, so that a consideration of the 'dilution' by detritus could be made. As toxicity in all tests was related

to weight of substrates given to the test organisms, a reduced toxicity at 9 months could partly be explained by 'dilution' with water and detritus. It is also important to note that the detritus consisted of loose and finely granulated material. The physical composition of the 9 months samples were therefore quite different from the initial test substrates.

Chemical changes

Aromatic hydrocarbons are generally considered to be the most toxic of the oil components. Gas chromatograms of the oil on the cuttings incubated on the sea floor (Bakke & al 1984, Appendix 1) showed that the amounts of medium/low molecular weight aromatics: naphtalene, phenanthrene, dibenzothiophene, pyrene, fluoranthene and their C_1-, C_2-, and C_3-alkylated derivatives (NPDs), declined nearly 50% with time, but gave no firm indication of biological degradation having occurred. The analytical procedure was however not aimed at detecting degradation products specifically. If the decline in NPDs reflects a leakage of aromatics (and breakdown products of these) to the water with time, this could lead to less toxic water extracts produced from the 9 months material.

An increased toxicity with time is most likely if a build up of hydrocarbon derived metabolites or other breakdown products occurs, as these generally are considered more toxic than the parent hydrocarbon. As mentioned above, the GC analyses performed in the field experiment did not indicate that this had happened. Also, and quite unexpectedly, the analyses performed in connection to the Shrimp test indicated a more complex mixture of compounds to be present in the initial substrates than after 9 months. However, different extraction and analytical procedure than with hydrocarbons would be necessary to detect metabolites, which are polar, and they might therefore have been present in the 9 months substrates without being detected. Generally polar metabolites are considered to be more water soluble than the parent hydrocarbon. Hence they are likely to be partitioned into the water when the cuttings extracts were made for the tests.

Changes in toxicity of cuttings with time

The toxicity of the oil based cuttings is shown to decrease with time in the Skeletonema, Barnacle, and Cod Egg tests. Clear evidence of an increase with time is shown by the Shrimp test. The Cod Larvae test showed a slight tendency of increased toxicity in LAC with time, and

no systematic change in the BRI. The change in DOM toxicity towards cod eggs could not be considered since no initial EC-50 value could be derived.

The recolonization experiments are inconclusive in this respect since a possible change in toxicity was superimposed by seasonal fluctuation and gradual development of the community. The differences between the substrates were gradually more pronounced with time during spring. This may reflect a stable or increasing toxicity, but just as likely an increase in sensitivity as spring/summer approaches. Also the samples taken the following September clearly distinguished between oiled and non-oil substrates mainly caused by the physical smothering effect of the oiled cuttings. These samples also indicate that the difference between the LAC fauna and the DOM fauna was less than during spring, possibly due to a smaller difference in toxicity between the two substrates.

The main difference between the Shrimp test and the others is the preparation of the media (whole cuttings vs water extracts) and hence the concentration, composition and route of entry of toxic components. The Cod Larvae test was also performed on extracts, but contrary to the organisms in the other tests on extracts the fish larvae are able to ingest water and particles and in this respect they have some similarity to the shrimps. An increase in degradation products would thus explain the difference above if these products were adsorbed onto particles which were eaten by the shrimps and the cod larvae or in other ways directly influenced these organisms. Several of the tests also seemed to indicate that the 9 months media were generally more turbid than the corresponding 0 months media. One must assume that the suspended material contained hydrocarbons or degradation products which could be ingested by the shrimps and cod larvae and hence increase the amounts of toxic components available to them.

Although no unanimous conclusion as to change in toxicity with time can be established from these tests, it seems probable that an increase in toxic compounds adsorbed to particles had occurred during the 9 months incubation on the sea floor and that these compounds acted as toxins only on the test organisms being able to ingest particles. The amounts of toxins acting through the water phase (mainly aromatic hydrocarbons?) seemed to have decreased with time.

<u>Comparison of test sensitivity</u>

Comparison of the EC- or LC-values given for each test on the same substrate gives an indication of the relative sensitivity of the tests. Such comparison is justified in spite of the different procedures used to prepare the substrates for testing, since each test relates a biological "50%" response to a certain amount of the original substrate (in mg/liter). It is therefore evident that this does not represent the sensitivity of the test organism itself, but reflects the combination of organism sensitivity and test design and resolution.

In Table 3 each test has been ranked after its EC- or LC-50 value for each substrate. Mean ranks have been computed based on all substrates and on the oiled substrates alone. The table indicates that the Shrimp test generally had the lowest EC tresholds, i.e. that this test was sensitive to the smallest amounts of substrate. This could be due to the fact that the test applied whole cuttings together with water extracts. The Green Algae test results support this showing that DOM cuttings and extract was more toxic than extract alone under identical conditions. The <u>Skeletonema</u> test was the second most sensitive and only slightly less sensitive than the Shrimp test when all substrates are considered. The least sensitive were the Green Alga test and the test on Acute Inhibition of Aerobic Degradation.

Table 2. Comparative ranking of the substrates with acute toxicity. Only the ranks are given. NT: not tested, -: tested, but no toxicity level computed.

Test	month	REF	WBM	LAC	BRI	DOM
Field exp.	-	4	4	3	2	1
Shrimp	0	4	4	3	2	1
	9	4	4	3	2	1
Barnacles	0	5	4	3	2	1
	9	NT	4	3	2	1
Dunaliella	0	3	2	2	NT	1
Skeletonema	0	5	4	3	1	1
	9	4	4	3	2	1
Bacteria inhib.	0	NT	2	3	1	3
Cod eggs	0	NT	3	3	1	2
	9	NT	3	3	1	2
Cod larvae	0	2	2	2	1	-
	9	4	4	3	2	1
Mean rank	0	3.8	3.0	2.7	1.3	1.5
	all	3.9	3.4	2.9	1.6	1.4

Table 3. Ranking of biotests with decreasing sensitivity based on EC- or LC-50 values. Only the ranks are given.

Test	INITIAL					9 MONTHS					Mean total rank	Mean oil rank
	REF	WBM	MOM	BRI	DOM	REF	WBM	MOM	BRI	DOM		
Shrimp	2	3	1	1	2	1	1	1	1	1	1.4	1.2
Barnac	2	3	3	6	1	NT	1	4	5	3	3.1	3.7
Dunal.	2	3	4	NT	4	NT	NT	NT	NT	NT	3.3	4.0
Skelet.	1	1	2	2	3	1	1	2	2	5	2.0	2.7
Bact.	NT	2	4	5	6	NT	NT	NT	NT	NT	4.3	5.0
Cod egg	2	3	4	3	5	1	1	5	4	4	3.2	4.2
Cod lar.	2	3	4	4	-	1	1	3	3	2	2.6	3.2

CONCLUDING REMARKS

The reasons for doing the biotests were twofold.

One aim was to compare how the different tests ranked the cuttings after short term toxicity, and to see how well this ranking correlated with the results of the field recolonization experiment.

In spite of a considerable span in sensitivity between the tests, most of them described the substrate toxicity in the same way: The reference sea bed sediment and the water based cuttings showed little or no acute chemical toxicity, and the diesel cuttings were the most toxic. Briquetting the diesel cuttings decreased toxicity, presumably because the process dried up the cuttings and thereby removed excess oil (cf. Bakke & al. 1984, Tab. 3.8). The low aromatic cuttings were generally less toxic than the diesel types, but clearly more toxic than the water based cuttings. This posititon of the low aromatic cuttings was expected since the main aim of producing low aromatic base oil is to reduce toxicity.

The second aim was to see if exposure to the marine environment for an extended period of time (9 months) would cause a change in the toxicity and possibly shift the ranking between the cuttings types. The results showed that 9 months on the sea floor was not enough to change the ranking among the cuttings to any significant degree. A clear change in toxicity occurred, but the direction of the change was inconclusive. The tests applying organisms enable to ingest particles (shrimps and fish larvae), showed that the toxicity of the oil based cuttings with few exceptions increased with time, whereas those applying organisms without feeding ability (algae, barnacle larvae, fish eggs) showed a decrease in toxicity of the oily cuttings.

The most probable explanation to this difference is the route of entry of toxic compounds to the organisms. One may assume that an increased load of small detritus particles in the 9 months cuttings could act as micro-carriers of hydrocarbons and breakdown products of these, and that these compounds would only be accessible to organisms feeding on the particles. This would mean that the amounts of available dissolved toxins went down with time (aromatics and possibly polar breakdown products of oil), and toxins adhered to smaller detritus particles went up.

There are however several uncertainties in the comparison between the tests, rising from differences in sample transport and test media preparation, and in the quantification of the amounts of cuttings in the media as basis for the dose level assessment. As there are available frozen samples of the relevant cuttings after 3 months on the sea floor and also cuttings which have been on the sea floor at the experimental site for nearly 3 years to date, it would be possible to settle the discussion of 'toxicity change with time' by running a selected set of the tests in a highly coordinated way on this material. This option should be discussed by the SFT/SU Joint Research Project Steering Committee, and the Norwegian pollution control authorities. The participating institutes have already expressed willingness to partake in such an exercise.

REFERENCES

Bakke T., Green, N.W., Næs, K., Pedersen, A., Sporstøl, S., and Oreld, F., 1984. Drill cuttings on the sea bed. Field experiments on recolonization and chemical changes. Phase 1. Thick (10mm) layers of cuttings 1982-1983. Norwegian Institute for Water Research, Report O-82003, 136 pp.

ANNEX 1

BROWN SHRIMP CRANGON CRANGON

Commercial in confidence

THE ACUTE TOXICITY OF DRILL CUTTINGS TO BROWN SHRIMPS

19. TEN MOBIL-NIVA SAMPLES (CUTTINGS, PELLETIZED CUTTINGS, AND SEDIMENTS

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ST 102

January 1984

The authors reserve the right to publish any information contained in this report, either in part or in full

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D128-D137

NOTE

Although the expression mud is used in the report when referring to the test material, all the samples tested (except the Mobil M2/83 13 ppg diesel based drilling mud) were either sea bed sediment or drill cuttings.

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1. INTRODUCTION

This report describes the results of experiments which were carried out between 6 December 1983 and 13 January 1984 to determine the acute toxicity of ten samples of drilling mud and related materials to adult brown shrimps under standard experimental conditions. The work was carried out for the Norwegian Institute for Water Research (NIVA) as part of a series of joint Anglo-Norwegian studies which started in March 1983; the results of previous experiments in the series have been given in earlier confidential reports to the Norwegian Marine Pollution Research and Monitoring Programme (Franklin, 1983).

2. MATERIALS AND METHODS

2.1 Materials

Samples of the ten test materials were provided by NIVA and received on 23 November 1983. Each sample was given a MAFF sample reference number as follows:

340: Mobil-NIVA reference sediment, 17/3 - REF 9 months
341: Mobil-NIVA Lav Aromatisk, 17/3 - LAC 9 months
342: Mobil-NIVA Vann Basert water based mud, 17/3 - WBM 9 months
343: Mobil-NIVA diesel based cuttings, 17/3 - DOM 9 months
344: Mobil-NIVA cuttings with diesel mud, June 1982 - BRI 0 months
345: Mobil-NIVA reference sediment, June 1982 - REF 0 months
346: Mobil-NIVA cutt. w. low aromatic mud, June 1982 - LAC 0 months
347: Mobil-NIVA cutt. w. water based mud, June 1982 - WBM 0 months
348: Mobil-NIVA pellets, 17/3 - BRI 9 months
349: Mobil-NIVA drill cuttings with diesel mud and washed in diesel, June 1982 - DOM 0 months

On receipt, although sample 348 was labelled 'Mobil Niva pellets 17/3' it proved to contain semi-liquid mud and cuttings smelling of diesel oil, while sample 344, labelled 'Mobil Niva cuttings with diesel mud, June 1982' actually contained shaped dry briquettes.

A sample of Mobil M2/83 13 ppg diesel based drilling mud from Mobil Statfjord A8-A (MAFF sample reference 301), obtained in March 1983 from the Norwegian Marine Pollution Research and Monitoring Programme was included in all tests as a reference. On receipt, many of the samples, even 340, 342 and 345 were grey to black and smelled of hydrogen sulphide. All samples were therefore immediately stored in their original containers in a deep-freeze at -18°C until tested.

The sea water which was used to make up the test solutions and for maintaining the experimental animals during acclimation was taken from the estuary of the River Crouch on a flood tide and stored in settling tanks for about two days to remove most of the large silt particles. It was then pumped into the laboratory's header tank and brought to the test temperature of 15°C (± 1°C), before being supplied to the test animal stock tanks. A 10 µm in-line membrane filter was used to remove any remaining silt particles from the water from which the test solutions were prepared. The salinity of the water was measured daily and was between 33.4 and 34.2‰ (parts per thousand) during the period when the tests described in this report were carried out.

2.2 Experimental animals

The toxicity tests were carried out using adult brown shrimps, *Crangon crangon* L (= *Crangon vulgaris*) which were caught in the

estuary of the River Crouch using a modified 2 m beam trawl with 10 mm mesh in the cod end. On arrival in the laboratory the animals were transferred to shallow 40 l polyethylene stock tanks at a maximum density of 200 shrimps per tank. Water at the test temperature of 15°C was used to fill the tanks and any dead or injured animals were removed. The animals were maintained in well aerated, gently flowing sea water for 2-4 days before the start of the test. They were not fed during their acclimation period or during the 5 day test period. Healthy shrimps of between 40 and 60 mm length, excluding antennae, (weight about 1-3 g) were selected for the toxicity tests; freshly moulted shrimps were excluded.

2.3 Apparatus

The samples were tested in 'Perspex' tanks calibrated to hold 10 l of test solution. Previous experience had shown that drilling muds do not remain in suspension when added to sea water but settle out to form a layer at the base of the tank. If stirred (agitation) tanks are used the maximum mixing energy which can be applied without causing stress to the test animals is not sufficient to disperse the solid component of the mud. This remains on the bottom of the tank and, because of the agitation, the test animals do not come into contact with the whole mud, but only with whatever is leached out or kept in suspension. If shallow static tanks are used the animals come into contact with the layer of mud on the bottom of the tank; however, at low concentrations this layer forms isolated patches which the animals may avoid and this could lead to erratic toxicity results. To minimise these problems, these tests were carried out in special deep tanks with a base area of only 645 cm^{-2} compared with that of the standard MAFF toxicity test tanks which have bases at 1 102 cm^{-2}.

The test tanks were gently aerated through pipettes attached to the laboratory's compressed air supply (see Franklin, 1980); this maintained the dissolved oxygen concentration of the test solution at about air saturation value (8.4 mg l^{-1}). The room in which the tanks were housed was maintained at a constant temperature of 15°C (± 1°C) and had a 12 h light: 12 h dark photoperiod.

2.4 Test method

Semi-static tests (ie with daily replacement of test solutions) were carried out using the methods described by Franklin (1980). Before adding the animals each tank was filled with 10 l of sea water and aerated for at least 30 min. Twenty shrimps of the correct size were then randomly added to each tank and the lids put in place. A further acclimation period of about 2 h was allowed before the test materials were added.

Before the test, the sample containers were allowed to thaw overnight and the thawed contents homogenized by hand in large mixing bowls. For each dilution required, the appropriate amount of test material was weighed into a beaker. About 300 ml of sea water from the appropriate test tank was added and the mixture was aerated vigorously for at least 2 h until any smell of sulphide had disappeared. This mixture was then stirred gently into the remaining water in the test tank. The materials were tested at dilutions ranging from 33 to 10 000 mg l^{-1}; the actual dilutions used for each sample are given in the Appendices. For Sample 344 (pellets), 1, 2, 5 and 10 whole pellets were added to each tank and the weights recorded; one tank was also prepared containing the equivalent weight of crushed pellets dry-sieved to particle size range 0.5-2 mm for comparison. Three dilutions of the reference mud (sample 301) and a control of

clean sea water were included with each batch of tests to allow any changes in sensitivity of the experimental animals to be detected. The concentrations recorded were nominal, no measurements being made of the concentrations of any of the chemicals present in the test dilutions.

Each day, the animals were gently transferred to tanks of freshly made up dilutions to discard metabolites and counteract losses of the test material due to absorption by the test organisms, degradation or volatilisation.

In the case of sample 344 (pellets) the test animals were not transferred nor were the pellets renewed so that the effect of any break-down and release of oil toxins from the pellets could be measured; in the tests with crushed pellets the dilutions were renewed daily.

The tanks were inspected at frequent intervals, including 1, 24, 48, 72 and 96 h after adding the test material, and dead animals (defined as those not responding to gentle prodding) were recorded and removed. Because of the possibility of cannibalism of freshly moulted shrimps, the number of animals remaining alive at the end of the experiment (usually 96 h) was also noted so that the mortality caused by the test treatment could be calculated.

2.5 Treatment of data

At the time of each observation, and for each tank, the cumulative percentage mortality was calculated using the following formula:

$$\frac{2m - 1}{2p} \times 100$$

where 'm' was the cumulative mortality and 'p' was the total number of animals in the tank. Thus in tanks with 20 animals, the death of the first animal was recorded as a 2.5% mortality, the second as 7.5% and the 20th as 97.5%. This is because the median response relates not to the response of the 10th animal out of a total of 20, but to a response between the 10th and the 11th animal (Lloyd, 1979).

For each individual batch of animals in which deaths occurred, the cumulative percentage mortalities were plotted against exposure time, using a probit scale for mortality and a logarithmic scale for time. Where the exact time of death was not observed (for example, overnight mortalities) a line was drawn on the graph between the time when the mortality was recorded and the time of the previous observation. A line was then drawn through the points (giving greater weight to those between 25% and 75% mortality) to give a time/mortality curve. The LT50 (time for 50% mortality) for each tank was then read off this graph. Estimates of the response of the test populations were made by calculating the 95% confidence limits from the slope of the line (Litchfield, 1949).

Where sufficient mortality occurred, approximate estimates of the LC50 (concentration lethal to 50% of the test organisms) at fixed observation times were obtained from concentration/mortality curves where the cumulative percentage mortalities at, for example, 96 h were plotted on a probit scale against concentration on a logarithmic scale. A line fitted by eye between the points allowed approximate values for the LC50 at these time intervals to be estimated.

A concentration/median response (or toxicity) curve was obtained by plotting the values of the LT50 against concentration and the estimated values of the LC50 against the corresponding time, using log.-log. graph paper. A curve fitted by eye through these two sets

of points allowed the LC50s for 24, 48 and 96 h to be interpolated. Since the actual concentrations of substances present in the tanks were not determined, the term LC(I)50 has been used, where C(I) is the initial concentration of test material added (Lloyd and Tooby, 1979). This indicates that, although the test solutions were renewed daily, the actual concentrations may not have remained constant during the period of the test.

3. RESULTS AND DISCUSSION

The 24, 48 and 96 h LC(I)50 values (median lethal concentrations) of the ten samples to brown shrimps are given in Table 1, together with similar results from earlier tests on the reference diesel based drilling mud (Mobil M2/83) for comparison. Not all of the materials caused sufficient mortalities for a toxicity curve to be constructed, but for those that did the curves are shown in Figures 1 to 6. Appendices I to X give the individual mortality and LT50 data for each tank, together with some notes on the appearance/behaviour of the test materials in the tanks.

The sensitivity of shrimps to the standard diesel based drilling mud (Mobil M2/83) was very similar to when it was first tested in March 1983 (see Figure 1). The sensitivity of shrimps to oil has been shown to vary seasonally (Norton and Franklin, 1980) and the inclusion of a reference material in each test allows such variations to be detected.

Four of the Mobil-Niva samples did not cause any significant mortalities within 96 h at the highest concentration tested (10 000 mg l^{-1}). These were the reference sediments, 17/3 (340) and June 1982 (345), the Vann Basert water based mud, 17/3 (342) and the drill cuttings with water based mud, June 1982 (347). These samples

were therefore at least 60 times less toxic than the reference diesel based mud, Mobil M2/83.

The most toxic of the ten test materials was the 17/3 sample of diesel based cuttings (343). Although this sample was between 0.6 and 2.1 times less toxic than the diesel based reference mud at 96 h, the toxicity curve was much steeper and at exposure periods of less than 12 h this sample was more toxic than the reference mud. The sample of Mobil-Niva pellets, 17/3 (348) also produced a steep toxicity curve although it was slightly less toxic than the 17/3 sample of diesel based cuttings (343) over the range of concentrations tested. The 96 h LC(I)50 of this sample was about 1.5 times less than that of the reference mud. The toxicity of the diesel washed cuttings (349) was about the same as that of the Mobil-Niva pellets, 17/3 (348) although it was impossible to draw a single toxicity curve for this sample as the mortalities at each concentration were vary variable.

A very unusual toxicity curve was obtained for the sample with Lav Aromatisk mud, 17/3 (341). Over the concentration range 2 000-10 000 mg l^{-1}, lower concentrations of the test material led, as may be expected, to a decrease in response, with an apparent threshold at about 2 000 mg l^{-1}. However, concentrations below 2 000 mg l^{-1} produced an increase in response, with LT50 values lower than those at 2 000 mg l^{-1}. There is no obvious explanation for the 'inversion' effect although it has been noted for other types of test material (Franklin, unpublished). The 96 h LC(I)50 which is given for this material in Table 1 refers to the lowest value obtained, making this mud about 5.3 times less toxic than the reference mud. The cuttings with low aromatic mud, June 1982 (346) were of very low toxicity, with a 96 h LC(I)50 about 44 times less than the reference.

The pellets (sample 344), when added whole to the test tanks, did not appear to break down at all despite the fact that they remained in the tanks for 96 h. The equivalent of a single crushed pellet in 10 l (with daily replacement of the solution) was more toxic than 10 whole pellets, killing 50% of the shrimps in about 27 h.

4. CONCLUSIONS

Four of the test materials were not acutely toxic to brown shrimps under the conditions of the test. These were the two samples of reference sediment, the water based mud, and the cuttings with water based mud.

All three samples of cuttings with diesel based mud produced steep toxicity curves, so that although they were less toxic than the reference diesel based mud (Mobil M2/83) at 96 h, they would all be expected to be more toxic than the reference mud at high concentrations. There was little diffference between the diesel washed diesel based cuttings of June 1982 and the disintegrated diesel based pellets of 9 months later.

The Lav Aromatisk sample of 17/3 was moderately toxic to shrimps with a complex two-stage toxic effect. The original cuttings with low aromatic mud of June 1982 were of low acute toxicity.

The whole pellets did not appear to break down and mix with the water in the test tanks within 96 h, but when ten pellets were added to 10 l there was enough leaching of toxic material to produce a significant mortality. Crushing the pellets increased their toxicity by a factor of about ten.

5. REFERENCES

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Table 1. Median lethal concentrations of ten Mobil-Niva samples to brown shrimps, Crangon crangon

MAFF sample reference	Test material (as described on sample bottle)	LC(I)50 (mg l^{-1})*		
		24 h	48 h	96 h
340	Reference sediment, 17/3	>10 000	>10 000	>10 000
341	Lav Aromatisk, 17/3	>10 000	≮ 7 000	850
342	Vann Basert water based mud, 17/3	>10 000	>10 000	>10 000
343	Diesel based cuttings, 17/3	420	100–330	100–330
344	Pellets, June 1982 - whole - crushed	> 10 1	> 10 < 1	≮ 10 < 1
345	Reference sediment, June 1982	>10 000	>10 000	>10 000
346	Cuttings with low aromatic mud, June 1982	>10 000	>10 000	7 100
347	Drill cuttings with water based mud, June 1982	>10 000	>10 000	>10 000
348	**Mobil-Niva pellets, 17/3**	650	310	240
349	Reference drill cuttings with diesel mud washed in diesel, June 1982	700–1 000	300–800	140–620
301	Mobil M2/83 reference diesel based drilling mud (results of test carried out in March 1983)	440	240	160

* results for pellets expressed as number of pellets in 10 l

Figure 1 The toxicity of Mobil M2/83 reference diesel based drilling mud to brown shrimps, *Crangon crangon*

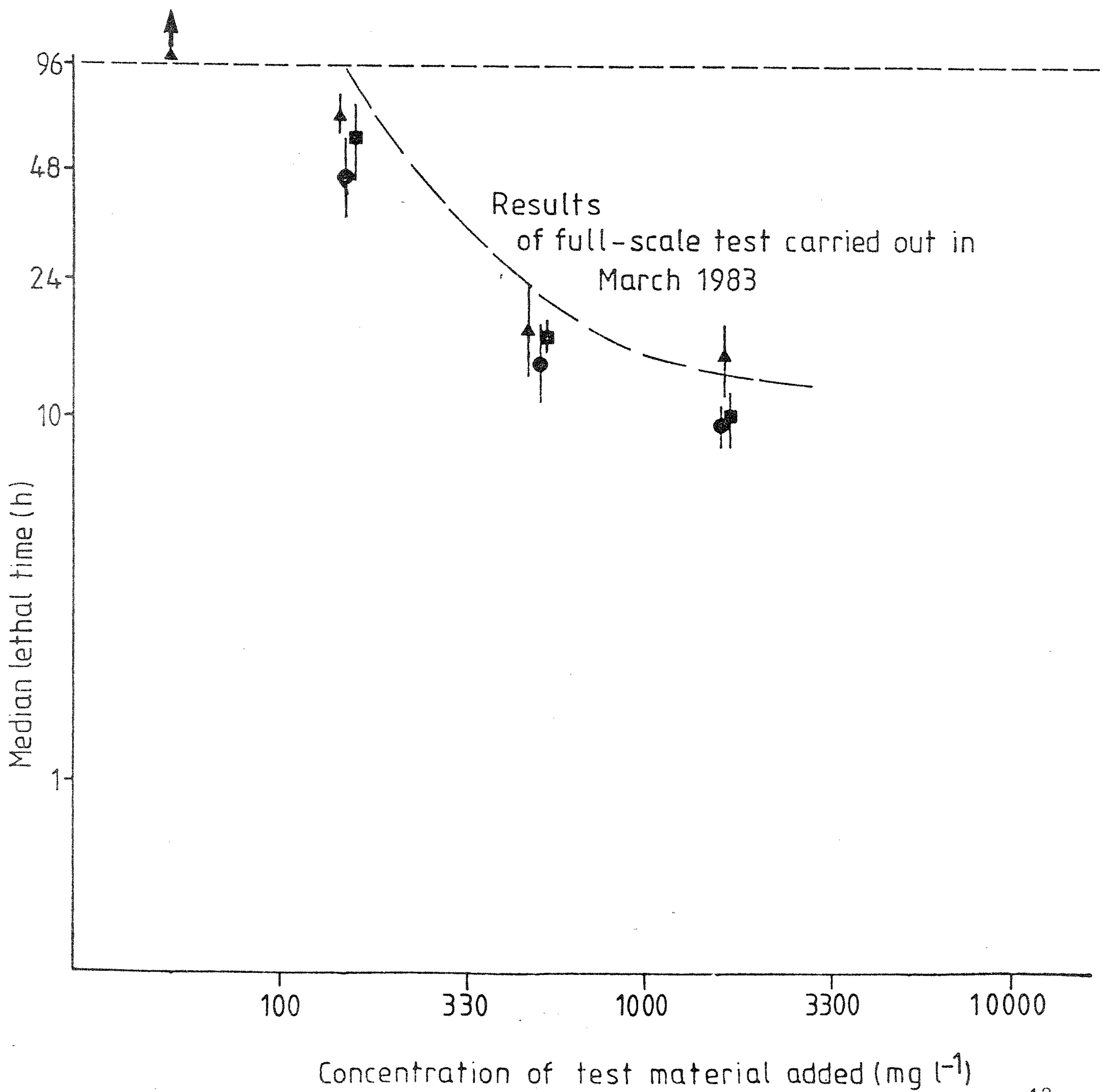


Figure 2 The toxicity of Mobil Niva Lav Aromatisk mud, 17/3 to brown shrimps, *Crangon crangon*

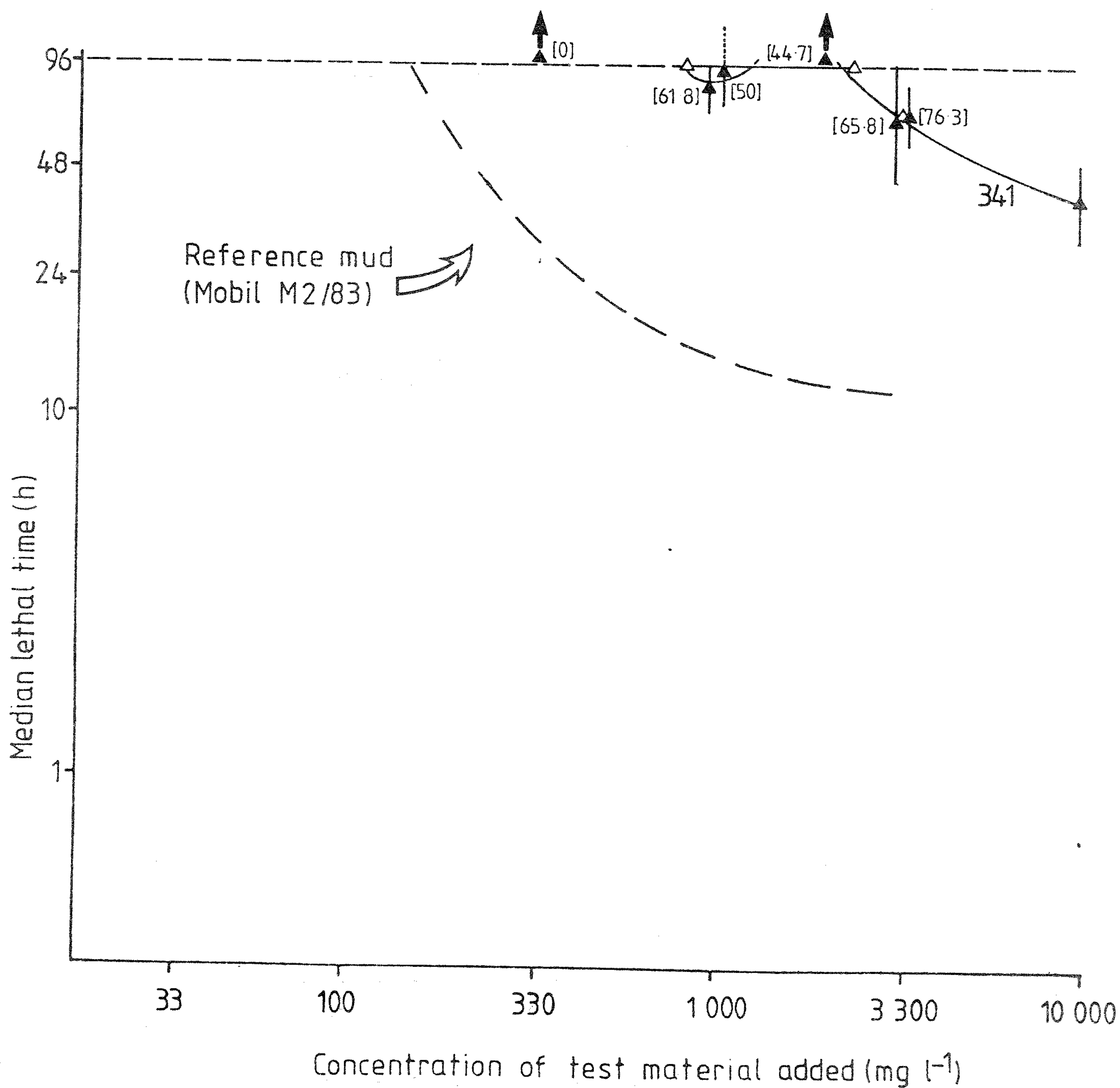


Figure 3 The toxicity of Mobil-Niva diesel based cuttings, 17/3 to brown shrimps, *Crangon crangon*

median lethal times and 95% confidence limits

——— end of experiment

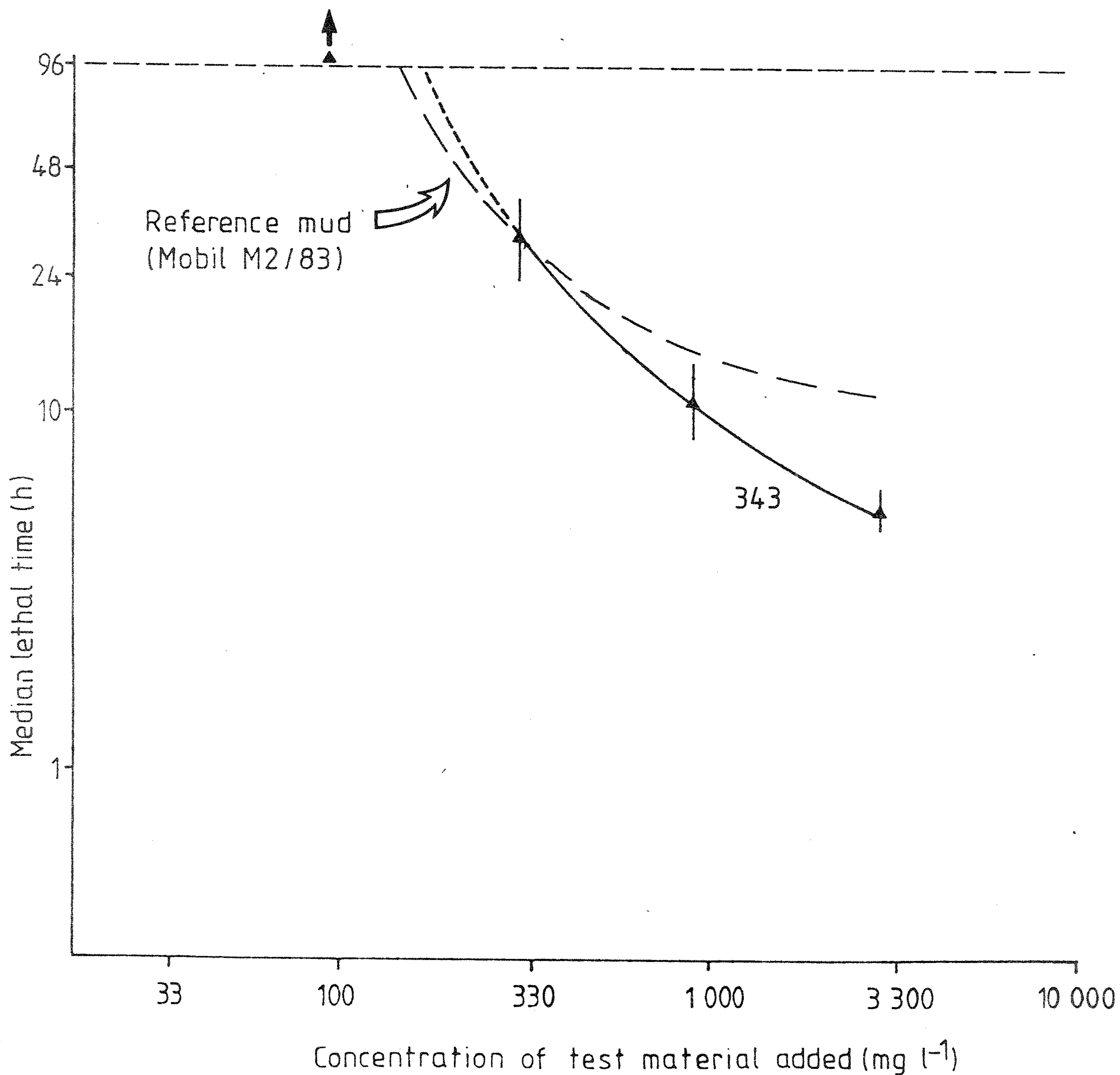


Figure 4 The toxicity of Mobil-Niva cuttings with low aromatic mud (June 1982) to brown shrimps, *Crangon crangon*

median lethal times and 95% confidence limits

△ median lethal concentrations

[ ] % mortality at end of experiment

----- end of experiment

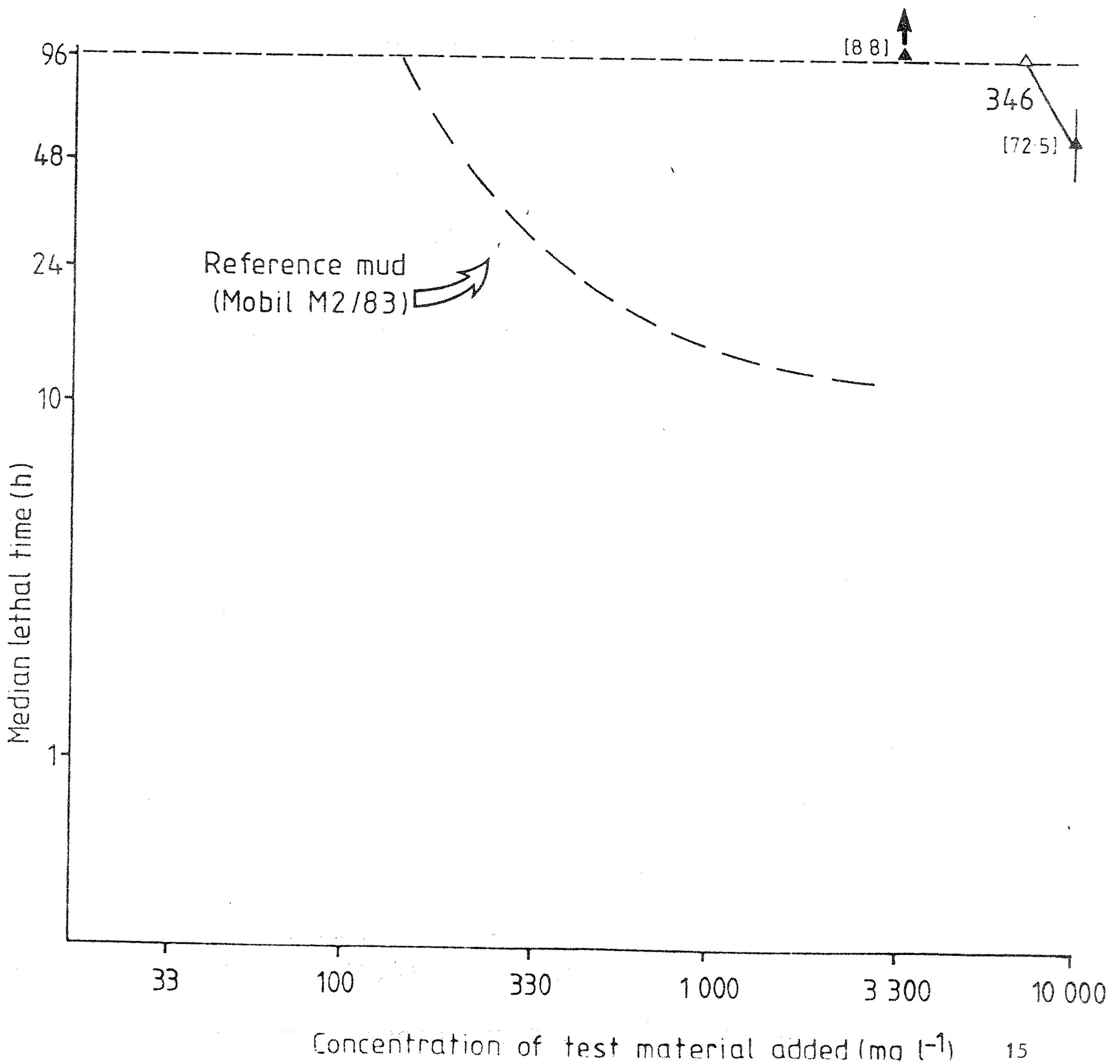


Figure 5 The toxicity of Mobil-Niva Pellets, 17/3 to brown shrimps, *Crangon crangon*

- ▲ median lethal times and 95% confidence limits
- △ median lethal concentrations
- [ ] % mortality at end of experiment
- ------ end of experiment

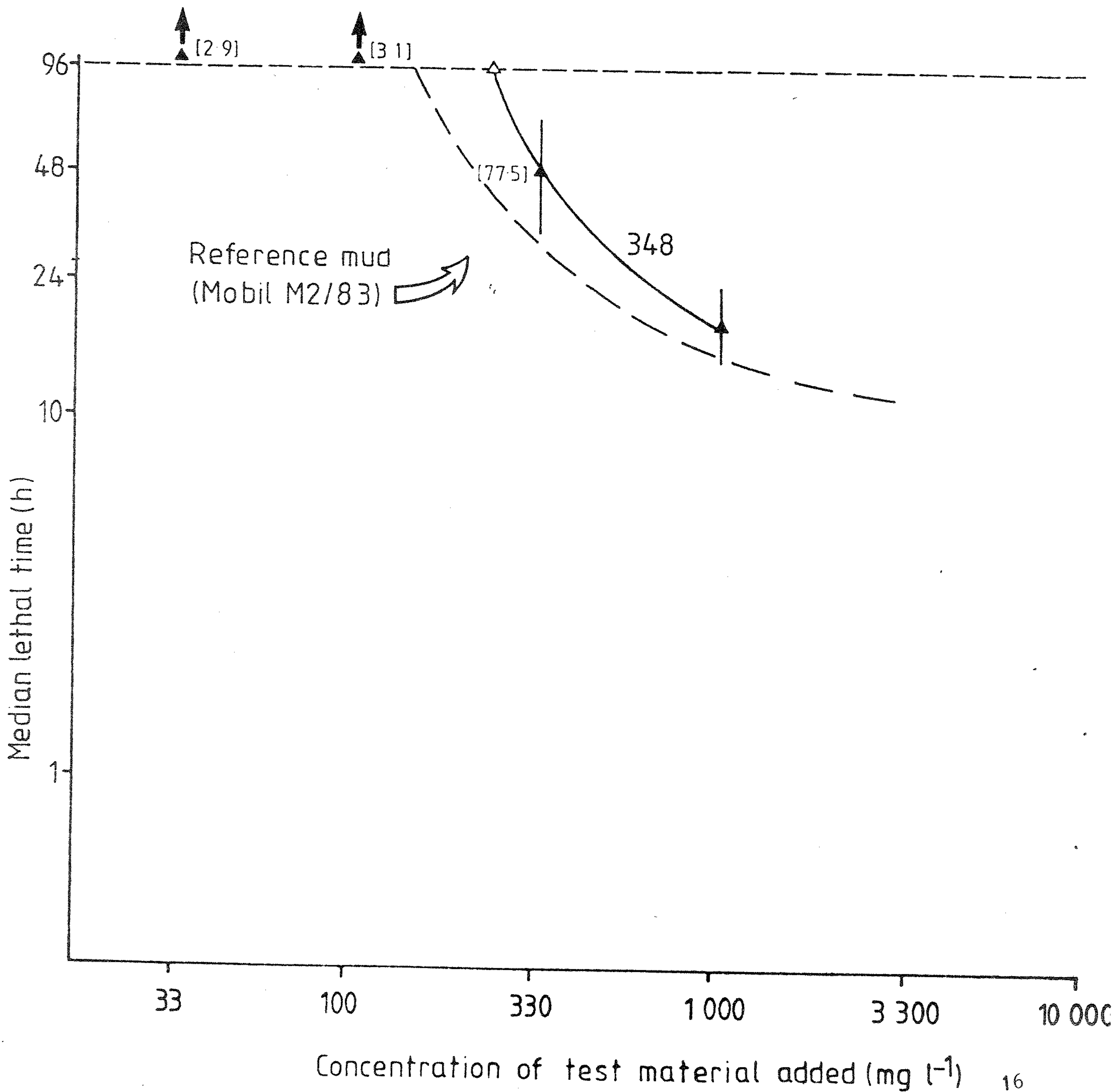
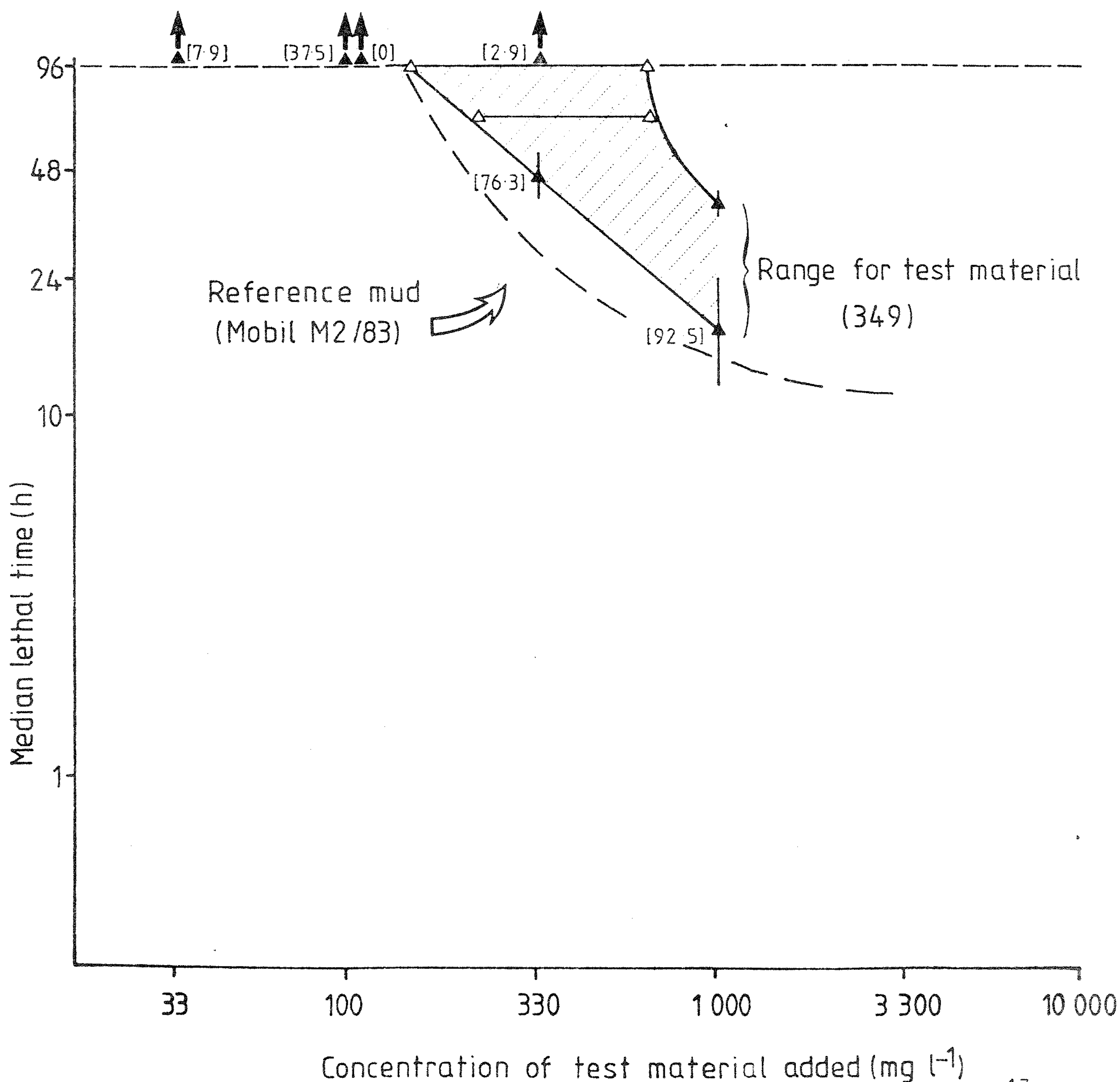


Figure 6 The toxicity of Mobil-Niva reference drill cuttings with diesel mud and washed in diesel to brown shrimps, *Crangon crangon*



Appendix I. Mortality of *Crangon crangon* in various concentrations of Mobil-Niva reference sediment, 17/3 (test number D128)

Treatment (and MAFF sample number)	Concentration (mg l^{-1})	Cumulative % mortality at:					LT50 (and 95% confidence limits) in h
		1 h	24 h	48 h	72 h	96 h	
Control	0	0	0	0	0	0	>96
Mobil diesel based reference mud M2/83 (301)	160	0	2.6	60.5	71.1	76.3	46 (36-59)
	510	0	77.5	92.5	97.5	97.5	14 (11-18)
	1 560	0	97.5	97.5	97.5	97.5	9.8 (8.4-11)
Test material (340)	10 000	0	0	0	0	0	>96

Appearance of test material dilutions at 24 h:
10 000 mg l^{-1} - cloudy, some sediment on bottom.

Appendix II. Mortality of Crangon crangon in various concentrations of Mobil-Niva Lav Aromatisk mud, 17/3 (test number D129)

Treatment (and MAFF sample number)	Concentration (mg l^{-1})	Cumulative % mortality at: 1 h	24 h	48 h	72 h	96 h	LT50 (and 95% confidence limits) in h
Control	0A	0	0	0	0	0	>96
	B	0	0	3.1	3.1	3.1	>94
Mobil diesel based reference mud M2/83 (301)	51B	0	0	0	0	2.8	>94
	160A	0	2.6	60.5	71.1	76.3	46 (36-59)
	B	0	2.9	8.8	44.1	91.2	67 (59-76)
	510A	0	77.5	92.5	97.5	97.5	14 (11-18)
	B	0	76.3	86.8	97.4	97.4	17 (13-23)
	1 560A	0	97.5	97.5	97.5	97.5	9.8 (8.4-11)
	B	0	82.5	97.5	97.5	97.5	15 (12-18)
Test material (341)	330B	0	0	0	0	0	>94
	1 000A	0	0	0	32.4	61.8	82 (71-94)
	B	0	0	2.9	20.6	50	90 (73-130)
	2 000B	0	0	7.9	23.7	44.7	>94
	3 300A	0	7.9	39.5	50	65.8	66 (45-97)
	B	0	0	13.2	50	76.3	70 (58-85)
	10 000A	0	17.5	57.5	82.5	97.5	40 (31-52)

A = tested between 6.12.83 and 10.12.83
B = tested between 9.1.84 and 13.1.84

Appearance of test material dilutions at 24 h:
1 000 mg l^{-1} - cloudy, sediment on bottom.
3 300 and 10 000 mg l^{-1} - very cloudy, dark brown sediment on bottom.

Appendix III. Mortality of Crangon crangon in various concentrations of Mobil-Niva Vann Basert water based mud, 17/3 (test number D130)

Treatment (and MAFF sample number)	Concentration (mg l^{-1})	Cumulative % mortality at:					LT50 (and 95% confidence limits) in h
		1 h	24 h	48 h	72 h	96 h	
Control	0	0	0	0	0	0	>96
Mobil diesel based reference mud M2/83 (301)	160	0	2.6	60.5	71.1	76.3	46 (36-59)
	510	0	77.5	92.5	97.5	97.5	14 (11-18)
	1 560	0	97.5	97.5	97.5	97.5	9.8 (8.4-11)
Test material (342)	1 000	0	0	0	0	0	>96
	3 300	0	0	0	0	0	>96
	10 000	0	0	0	0	0	>96

Appearance of test material dilutions at 24 h:
1 000 mg l^{-1} - slightly cloudy, sediment on bottom.
3 300 mg l^{-1} - fairly cloudy, greenish brown sediment on bottom.
10 000 mg l^{-1} - very cloudy, greenish brown sediment on bottom.

Appendix IV. Mortality of *Crangon crangon* in various concentrations of Mobil-Niva diesel based cuttings, 17/3, (test number D131)

Treatment (and MAFF sample number)	Concentration (mg l^{-1})	Cumulative % mortality at:					LT50 (and 95% confidence limits) in h
		1 h	24 h	48 h	72 h	96 h	
Control	0	0	0	0	0	0	>96
Mobil diesel based reference mud M2/83 (301)	160	0	2.6	60.5	71.1	76.3	46 (36-59)
	510	0	77.5	92.5	97.5	97.5	14 (11-18)
	1 560	0	97.5	97.5	97.5	97.5	9.8 (8.4-11)
Test material (343)	100	0	0	3.1	3.1	3.1	>96
	330	0	28.9	71.1	86.8	97.4	31 (24-40)
	1 000	0	87.5	97.5	97.5	97.5	11 (8.5-14)
	3 300	0	97.5	97.5	97.5	97.5	5.5 (4.8-6.3)

Appearance of test material dilutions at 24 h:

100 mg l^{-1} - almost clear, some sediment on bottom.

330 mg l^{-1} - slightly cloudy, some sediment on bottom.

1 000 and 3 300 mg l^{-1} - cloudy, sediment on bottom.

Appendix V. Mortality of *Crangon crangon* in various numbers of Mobil-Niva pellets, June 1982. (test number D132)

Treatment (and MAFF sample number)	Concentration (mg l^{-1})	Cumulative % mortality at:					LT50 (and 95% confidence limits) in h
		1 h	24 h	48 h	72 h	96 h	
Control	0	0	0	0	0	0	>96
Mobil diesel based reference mud M2/83 (301)	160	0	2.6	60.5	71.1	76.3	46 (36-59)
	510	0	77.5	92.5	97.5	97.5	14 (11-18)
	1 560	0	97.5	97.5	97.5	97.5	9.8 (8.4-11)
Crushed pellets (344)	2 500	0	23.7	76.3	76.3	81.6	27 (19-38)
Whole pellets (344)	(1): 2 400	0	0	2.6	2.6	2.6	>96
Note: test dilutions were not renewed	(2): 4 500	0	0	2.5	2.5	2.5	>96
	(5):10 900	0	0	2.8	2.8	2.8	>96
	(10):23 300	0	0	13.9	52.8	52.8	67 (55-82)

Appearance of test material dilutions at 24 h:
whole pellets - water clear, pellets complete
crushed pellets - granules on bottom, water quite cloudy until solution change.

Appendix VI. Mortality of *Crangon crangon* in various concentrations of Mobil-Niva reference sediment, June 1982 (test number D133)

Treatment (and MAFF sample number)	Concentration (mg l^{-1})	Cumulative % mortality at: 1 h	24 h	48 h	72 h	96 h	LT50 (and 95% confidence limits) in h	
Control	0	0	0	0	0	0	>97	
Mobil diesel based reference mud M2/83 (301)	160	0	7.9	13.2	65.8	81.6	59	(46-75)
	510	0	92.5	97.5	97.5	97.5	17	(15-19)
	1 560	0	97.5	97.5	97.5	97.5	10	(8.5-12)
Test material (345)	10 000	0	0	0	0	0	>97	

Appearance of test material dilutions at 24 h:
10 000 mg l^{-1} - cloudy, sediment on bottom.

Appendix VII. Mortality of *Crangon crangon* in various concentrations of Mobil-Niva cuttings with low aromatic mud, June 1982 (test number D134)

Treatment (and MAFF sample number)	Concentration (mg l^{-1})	Cumulative % mortality at:					LT50 (and 95% confidence limits) in h	
		1 h	24 h	48 h	72 h	96 h		
Control	0	0	0	0	0	0	>97	
Mobil diesel based reference mud M2/83 (301)	160	0	7.9	13.2	65.8	81.6	59	(46-75)
	510	0	92.5	97.5	97.5	97.5	17	(15-19)
	1 560	0	97.5	97.5	97.5	97.5	10	(8.5-12)
Test material (346)	330	0	2.8	2.8	2.8	2.8	>97	
	1 000	0	0	0	0	0	>97	
	3 300	0	0	0	2.9	8.8	>97	
	10 000	0	0	32.5	57.5	72.5	56	(44-72)

Appearance of test material dilutions at 24 h:

330-3 300 mg l^{-1} - lumps of sediment on bottom, clear liquid above

10 000 mg l^{-1} - lumps of sediment on bottom, slightly cloudy above

Appendix VIII. Mortality of *Crangon crangon* in various concentrations of Mobil-Niva cuttings with water based mud, June 1982 (test number D135)

Treatment (and MAFF sample number)	Concentration (mg l^{-1})	Cumulative % mortality at:					LT50 (and 95% confidence limits) in h	
		1 h	24 h	48 h	72 h	96 h		
Control	0	0	0	0	0	0	>97	
Mobil diesel based reference mud M2/83 (301)	160	0	7.9	13.2	65.8	81.6	59	(46-75)
	510	0	92.5	97.5	97.5	97.5	17	(15-19)
	1 560	0	97.5	97.5	97.5	97.5	10	(8.5-12)
Test material (347)	1 000	0	0	0	0	3.1	>97	
	3 300	0	0	0	2.9	2.9	>97	
	10 000	0	0	0	0	0	>97	

Appearance of test material dilutions at 24 h:
1 000 mg l^{-1} - slightly cloudy, sediment on bottom
3 300 and 10 000 mg l^{-1} - cloudy, sediment on bottom

Appendix IX. Mortality of *Crangon crangon* in various concentrations of Mobil-Niva disintegrated pellets, 17/3 (test number D136)

Treatment (and MAFF sample number)	Concentration (mg l^{-1})	Cumulative % mortality at:					LT50 (and 95% confidence limits) in h	
		1 h	24 h	48 h	72 h	96 h		
Control	0	0	0	0	0	0	>97	
Mobil diesel based reference mud M2/83 (301)	160	0	7.9	13.2	65.8	81.6	59	(46-75)
	510	0	92.5	97.5	97.5	97.5	17	(15-19)
	1 560	0	97.5	97.5	97.5	97.5	10	(8.5-12)
Test material (348)	33	0	0	0	2.9	2.9	>97	
	100	0	0	0	3.1	3.1	>97	
	330	0	17.5	47.5	67.5	77.5	46	(32-67)
	1 000	0	60.5	97.4	97.4	97.4	18	(14-23)

Appearance of test material dilutions at 24 h:

33 mg l^{-1} - sediment on bottom, clear liquid above

100 mg l^{-1} - sediment on bottom, slightly cloudy above

330 and 1 000 mg l^{-1} - sediment on bottom, quite cloudy above

Appendix X. Mortality of Crangon crangon in various concentrations of Mobil-Niva reference drill cutting with diesel mud and washed in diesel, June 1982 (test number D137)

Treatment (and MAFF sample number)	Concentration (mg l^{-1})	Cumulative % mortality at:					LT50 (and 95% confidence limits) in h	
		1 h	24 h	48 h	72 h	96 h		
Control	0A	0	0	0	0	0	>97	
	B	0	0	0	0	0	>97	
Mobil diesel based reference mud M2/83 (301)	160A	0	7.9	13.2	65.8	81.6	59	(46-75)
	510A	0	92.5	97.5	97.5	97.5	17	(15-19)
	1 560A	0	97.5	97.5	97.5	97.5	10	(8.5-12)
Test material (349)	33A	0	0	0	0	7.9	>97	
	100A	0	0	0	0	37.5	>97	
	B	0	0	0	0	0	>97	
	330A	0	0	0	0	2.9	>97	
	B	0	0	55.3	76.3	76.3	46	(40-53)
	1 000A	0	0	92.1	97.4	97.4	39	(36-42)
	B	0	67.5	87.5	92.5	92.5	17	(12-24)

A = tested between 13.12.83 and 17.12.83
B = tested between 20.12.83 and 24.12.83

Appearance of test material dilutions at 24 h:
33-1 000 mg l^{-1} - sediment on bottom in lumps, clear liquid above

ANNEX 2

CYPRIS LARVAE OF BARNACLES

Toxicity testing with the acorn barnacle

MATERIALS AND METHODS

Henry Hovde
Department for Marine Zoology
University of Oslo

1. Name (Scientific & common)

- Balanus improvisus DARWIN (Crustacea, Cirripedia)
- acorn or sessile barnacles

2. Biological data/characteristics and living conditions

B. improvisus is a common marine and brackish water species of Western Europe and Atlantic North America. After hatching/liberation from the parental egg-masses the larvae develop through six planktonic naupliar feeding stages to the non-feeding cyprids which after some time settle and metamorphose into the sessile adult form.

For the present purpose, animals usually are collected from the Oslofjord. In the laboratory large numbers (up to 35.000) of test animals, the c y p r i d s , are obtained by rearing larvae (hatched from dissected ripe egg-masses of adults) in 5 liters beakers through the six nauplii-stages using the diatom Skeletonema costatum (GREVILLE) CLEVE as food. Optimal temperature for rearing is 17-18°C, and suitable medium is filtered natural seawater of salinity about 33-34‰.

Once the cyprid stage is reached, the settlement of those animals not immediately used in tests, can be postponed for as long as 15 weeks and thus serve as useful stock animals for later tests. The postponement of the settlement is achieved by keeping the larvae in darkness and in low temperated water (÷ 1.8°C).

3. Types of effect

- The barnacles are particularly suitable for tests of the effects on the settlement of larvae (i.e. cyprids) and on the metamorphosis into the young adult. This is a critical phase of the life cycle.

- Also, the effects on non-settled larvae or on metamorphosed animals are closely watched (activity, lethality, morphological development).

4. Minimal requirements for testing

- Age of cyprids minimum 3 days.

- The required condition of the cyprids is that at least 90% of the animals in the controls should perform normal settlement, metamorphosis, development and still being active (cirral movement) 3 weeks after the start of an experiment.

- Preferably minimum 50 animals per experimental vial and at least two parallels at each concentration should be used.

- The desired amount of material to be tested is mixed with seawater and shaken vigorously for three hours. The solutions are then left for some time to clear up (sedimentation/flotation) before being transferred to the experimental vessels. The supernatant is used in the experiment, and the precipitate and oil floating on the water surface are discarded.

- The duration of an experiment is approximately ten days.

- The experimental vials are closed, completely filled with "water". Static.

- Temperature 23-24°C.

- Continuous light during the testing period.

5. Criteria used / Evaluation of toxicity

- Settlement and metamorphosis.

- Behaviour (like swimming/walking activity of unsettled cyprids or cirral activity).

- Morphology (normal/abnormal development).

- Lethality at the different stages.

By routine toxicity testing the parameters: success of settlement, metamorphosis, cirral development (ecdysis) and activity is followed in an appropriate range of concentrations of testing material.

The numbers of animals successfully reaching the stage of viable, young adults in relation to the total number of animals exposed to a given concentration of test substance is determined. The mean values of each concentration tested are calculated in relation to that of the controls, and the EC-50 value is given in ppm (parts per million by weight). Also the corresponding EC-90 value is given when feasible.

RESULTS

The report gives the results on testing of initial reference sediment (Ref.), water based (va), low aromatic (pa), briquettes (br), and diesel (di) cuttings, and the same material after 9 months on the sea floor.

The basic data are given in Tables 2-7.

A. <u>Initial cuttings</u>

A comparison of the toxicity is presented as dose/respons curves in Fig 1. Table 1a (pg 2) compares the EC-50 values derived.

B. <u>Cuttings after 9 months</u>

Initial and 9 months toxicities (EC-50 values) are compared in Table 1b. Hydrogen sulfide formation in the 9 months samples of <u>va</u> and <u>ref</u> complicated the tests somewhat, even though the samples were aerated. These samples gave very turbid test solutions, which may have had an effect on the results for the highest concentrations applied. For instance 570,000 ppm water based substrate day 0 gave only 3 and 7 % normal settling with subsequent normal development (Test 1983, no 541 and 542), but almost all individuals went through normal settlement and metamorphosis only to become inactive at the end of the test period. After 9 months the same concentration of this substrate (Test 1983, no 565 and 566) killed all the animals prior to any settlement. The EC-value of 440,000 ppm presented must therefore be considered a low estimate. The same holds for the reference material after 9 months where all animals died or were deformed at 760,000 ppm (Test 1983, no 567 and 568).

CONCLUSION.

The results show great differences in toxicity between different types of cuttings. They show that low aromatic cuttings had low toxicity (EC-50 = 37,000 ppm) and gave negligible effects compared to diesel cuttings (EC-50 = 190 ppm). The toxicity of water based cuttings was even less than this. Effects were detected at concentrations above 100,000 ppm (EC-50 = 360,000 ppm). The briquettes showed low toxicity compared to diesel cuttings (EC-50 = 18,500 ppm).

All four types of cuttings were considerably less toxic after 9 months on the sea floor (cf. Table 1a).

Oslo, 13.12.1983
HRH/eh

Table 1a Effects of the four types of drill cuttings at day 0 on the settlement and metamorphosis of Balanus improvisus.

Cuttings from drilling with mud type (1-3)		EC-50 value
1	diesel oil base	190 ppm
2	low aromatic oil base	37,000 ppm
3	water base	360,000 ppm
4	briquettes	18,500 ppm
5	reference	> ca 600.000 ppm

Table 1b. Comparison of the effects of the four types of drill cuttings on settlement and metamorphosis of Balanus improvisus before and after 9 months on the sea floor.

Cuttings from drilling with mud type (1-3)		Day 0	9 months
1	diesel oil base	190 ppm	7,600 ppm
2	low aromatic oil base	37,000 ppm	65,000 ppm
3	water base	360,000 ppm	440,000 ppm
4	briquettes	18,500 ppm	135,000 ppm
5	reference	> ca 600.000 ppm	-

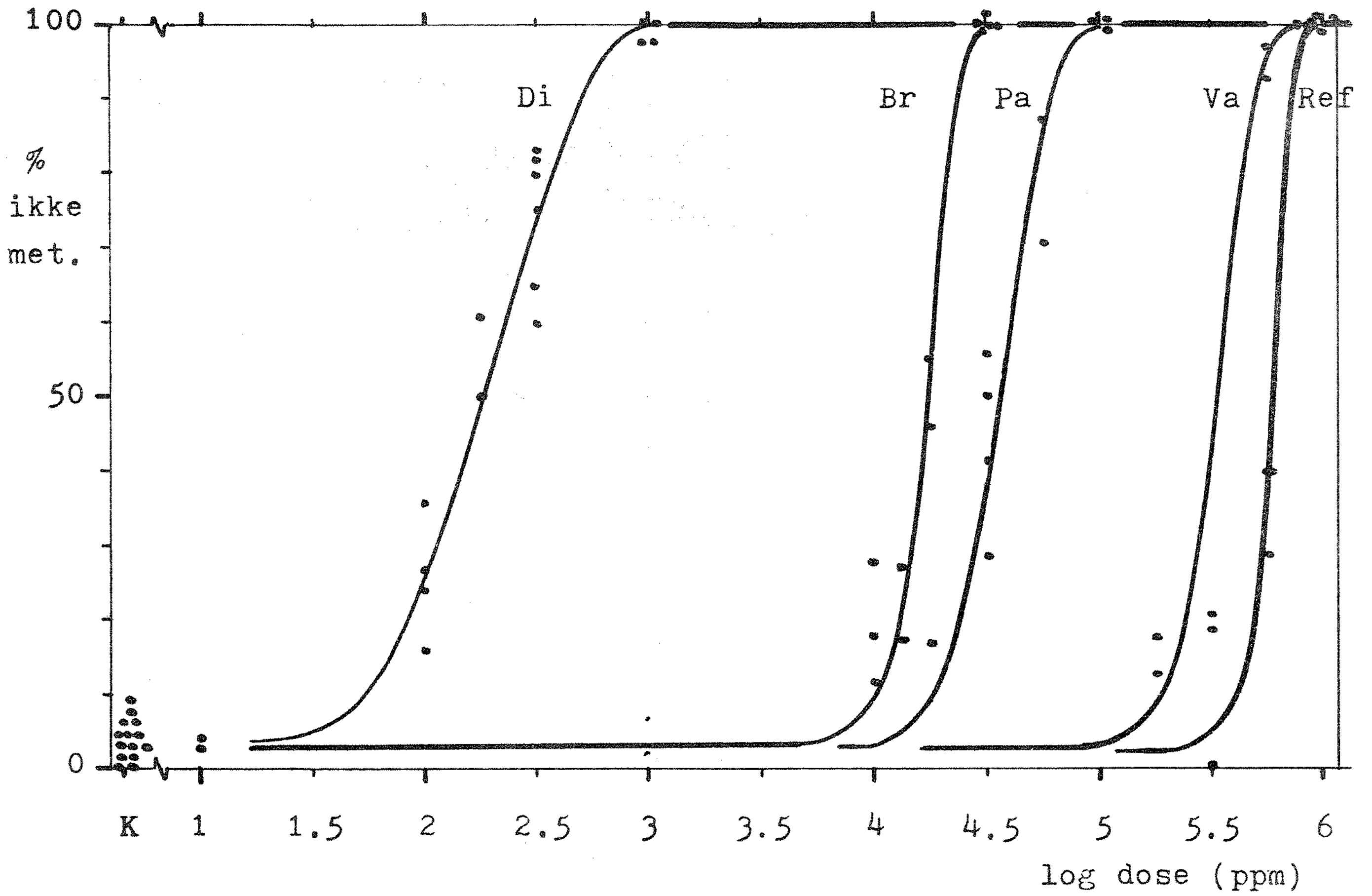


Fig. 1 Dose response curves for the four types of cuttings at day 0. Di: diesel based, Pa: low aromatic, Va: water based, Br: briquettes, Ref: reference sediment.

Table 2 Balanus improvisus 1982 (9.11-1.12). Effects of three types of drill cuttings (water, low aromatic, and diesel), day 0, on settlement and metamorphosis during conditions stimulating settlement.
Column legend, see footnote.

3. Stoff tilsatt	4. Kons. (ppm)	6. Cypris fanget overfl	7. Bunnslåtte/ Met.N glass	9. Met abn	10. Løse/ Gjenv. cypris	11. derav def	12. Sum 7-10	13. % Met.N	13a. Met i % av Ksn
K	-	23	71	1	3	(1)	75	95	(99)
K	-	20	105	2	3	(2)	110	95	(100)
K	-	22	56	0	2	(1)	58	97	(101)
K	-	39	167	3	3	(2)	173	97	(101)
Mo/Ni Va	10.000	30	75	0	5	(1)	81	93	(97)
" Va	10.000	56	67	0	2	(1)	69	97	(102)
" Va	1.000	34	86	1	2	(1)	89	97	(101)
" Va	1.000	20	86	0	2	(0)	88	98	(102)
" Va	100	32	98	2	2	(2)	102	96	(101)
" Va	100	29	51	1	2	(1)	54	94	(99)
" Va	10	27	74	0	1	(0)	75	99	(103)
" Va	10	26	99	1	3	(1)	103	96	(101)
" Va	1.0	23	107	2	2	(1)	111	96	(101)
" Va	1.0	29	30	0	2	(0)	32	94	(98)
Mo/Ni Pa	10.000	29	71	2	27	(3)	100	71	(74)
" Pa	10.000	19	107	3	24	(0)	134	80	(84)
" Pa	1.000	10	103	3	1	(0)	107	96	(101)
" Pa	1.000	11	70	4	6	(2)	80	88	(92)
" Pa	100	57	86	0	1	(0)	87	99	(103)
" Pa	100	38	68	1	0	(-)	69	99	(103)
" Pa	10	43	100	0	0	(-)	100	100	(105)
" Pa	10	27	67	0	1	(0)	68	99	(103)
" Pa	1.0	38	121	1	4	(1)	126	96	(101)
" Pa	1.0	41	29	1	1	(1)	31	94	(98)

forts.

Tabell 2 (forts.)

3. Stoff tilsatt		4. Kons. (ppm)	6. Cypris fanget overfl	7. Bunnslåtte/ Met.N glass	9. Met abn	10. Løse/ Gjenv. cypris	11. derav def	12. Sum 7-10	13. % Met.N	13a. Met i % av Kon
Mo/Ni	Di	10.000	0	0	0	40	-	40	0	(0)
"	Di	10.000	0	0	0	25	-	25	0	(0)
"	Di	1.000	4	0	0	>50	(ca 10)	50	0	(0)
"	Di	1.000	0	0	0	67	(4)	67	0	(0)
"	Di	100	67	52	2	17	(3)	71	73	(77)
"	Di	100	50	45	1	13	(0)	59	76	(80)
"	Di	10	44	120	2	3	(0)	125	96	(100)
"	Di	10	36	61	0	2	(0)	63	97	(101)
"	Di	1.0	39	73	0	1	(1)	74	99	(103)
"	Di	1.0	28	75	0	2	(1)	77	97	(102)

Column legend:
3. Substrate
4. Concentration
6. Numbers of cypris larvae trapped in the surface film
7. Numbers of normally metamorphosed larvae
9. Numbers of abnormal metamorphoses
10. Non-settled larvae
11. Non-settled larvae deformed
12. Sum of columns 7-10
13. % normally metamorphosed
13a Normally metamorphosed in percent of controls

Table 3 Balanus improvisus 1982 (3.12-20.12). Effects of two types of drill cuttings (water and low aromatic) day 0, on settlement and metamorphosis during conditions stimulating settlement.
Column legend, see footnote, Table 2.

3. Stoff tilsatt	4. Kons. (ppm)	6. Cypris fanget overfl	7. Bunnslåtte/ Met.N glass	9. Met abn	10. Løse/ Gjenv. cypris	11. derav def	12. Sum 7-10	13. % Met.N	13a. Met i % av Ksn
K	-	13	14	0	0	(-)	14	100	(105)
K	-	7	29	0	1	(0)	30	97	(101)
K	-	48	16	0	1	(0)	17	94	(99)
K	-	17	21	1	1	(1)	23	91	(96)
Mo/Ni Va	100.000	10	41	0	1	(0)	42	98	(102)
" Va	100.000	15	31	2	0	(-)	33	94	(98)
" Va	32.000	14	33	0	2	(0)	35	94	(99)
" Va	32.000	26	37	0	1	(0)	38	97	(102)
" Va	32.000	18	33	1	0	(-)	34	97	(102)
" Va	32.000	29	78	1	2	(2)	81	96	(101)
Mo/Ni Pa	100.000	0	0	0	≥48	(6)	≥48	0	(0)
" Pa	100.000	0	0	0	>22	(4)	>22	0	(0)
" Pa	32.000	5	18	1	17	(3)	36	50	(52)
" Pa	32.000	4	21	3	24	(2)	48	44	(46)
" Pa	32.000	2	19	2	11	(2)	32	59	(62)
" Pa	32.000	7	70	4	25	(2)	99	71	(74)
" Pa	10.000	20	29	0	2	(1)	31	94	(98)
" Pa	10.000	27	25	0	3	(0)	28	89	(93)

Table 4 Balanus improvisus 1983 (23.12.82-14.1.83). Effects of two types of drill cuttings (briquettes and diesel), day 0, on settlement and metamorphosis during conditions stimulating settlement.
Column legend, see footnote, Table 2.

3. Stoff tilsatt		4. Kons. (ppm)	6. Cypris fanget overfl	7. Bunnslåtte/ Met.N glass	9. Met abn	10. Løse/ Gjenv. cypris	11. derav def	12. Sum 7-10	13. % Met.N	13a. Met i % av K_{sn}
K		-	46	56	1	3	(2)	60	93	(98)
K		-	17	13	0	0	(-)	13	100	(105)
K		-	41	25	1	0	(-)	26	96	(101)
K		-	23	48	1	3	(0)	52	92	(92)
Mo/Ni	Di	1.000	7	0	1	37	(3)	38	0	(0)
"	Di	1.000	13	0	1	29	(2)	30	0	(0)
"	Di	316	32	19	3	33	(3)	55	35	(36)
"	Di	316	38	28	13	29	(4)	70	40	(42)
"	Di	316	28	5	0	25	(1)	30	17	(17)
"	Di	316	25	37	14	26	(1)	77	48	(19)
"	Di	100	16	11	0	8	(1)	19	58	(88)
"	Di	100	12	7	0	4	(0)	11	64	(67)
Mo/Ni	Br	100.000	1	0	0	49	(6)	49	0	(0)
"	Br	100.000	9	0	0	24	(2)	24	0	(0)
"	Br	10.000	28	65	0	9	(2)	74	88	(92)
"	Br	10.000	46	47	1	9	(0)	57	82	(86)
"	Br	1.000	29	28	0	2	(0)	30	93	(98)
"	Br	1.000	43	126	0	3	(0)	129	98	(102)
"	Br	100	25	33	1	0	(-)	34	97	(102)
"	Br	100	30	28	0	3	(1)	31	90	(95)
"	Br	10	37	35	0	5	(0)	40	88	(92)
"	Br	10	29	43	1	0	(-)	44	98	(102)
"	Br	1.0	33	48	0	1	(1)	49	98	(103)
"	Br	1.0	21	21	0	1	(0)	22	95	(100)
"	Br	0.1	17	[illegible]	1	0	(-)	25	96	(101)
"	Br	0.1	35	13	0	0	(-)	13	100	(105)

Table 5 Balanus improvisus 1983 (23.12.82-14.1.83). Effects of briquetted drill cuttings, day 0, on settlement and metamorphosis during conditions stimulating settlement. Column legend, see footnote, Table 2.

3. Stoff tilsatt		4. Kons. (ppm)	6. Cypris fanget overfl	7. Bunnslåtte/ Met.N glass	9. Met abn	10. Løse/ Gjenv. cypris	11. derav def	12. Sum 7-10	13. % Met.N	13a. % av Ksn
K		-	31	48	Ø	2	(1)	50	96	(102)
K		-	11	15	1	1	(0)	17	88	(94)
K		-	17	23	0	1	(1)	24	96	(102)
K		-	20	32	0	2	(1)	34	94	(100)
Mo/Ni	Br	32.000	19	0	2	145	(9)	147	0	(0)
"	Br	32.000	7	0	0	53	(2)	53	0	(0)
"	Br	32.000	10	1	2	87	(6)	90	1	(1)
"	Br	32.000	5	0	1	40	(1)	41	0	(0)
"	Br	10.000	8	64	3	22	(1)	89	72	(77)
"	Br	10.000	-	-	-	-	-	-	-	-

Table 6 Balanus improvisus 1983 (23.12.82-14.1.83). Effects of various types of drill cuttings, after 9 months on the sea floor, on settlement and metamorphosis during conditions stimulating settlement. First test series.
Column legend, see footnote, Table 2.

1. Nr 1983	3. Stoff tils.	4. Kons. (ppm)	6. Cypris fanget overfl	7. Bunnslåtte/ Met.N glass	9. Met abn	10. Løse/ Gjenv. cypris	11. derav def	12. Sum 7-10	13. % Met.N
477.	K	-	2	22	0	2	(0)	24	92
478.	K	-	1	31	0	0	(-)	31	100
479.	K	-	1	19	0	1	(0)	20	95
480.	K	-	0	27	0	0	(-)	27	100
481.	9mDi	1.000	3	36	2	3	(1)	41	88
482.	9mDi	1.000	4	24	2	5	(1)	31	77
483.	9mDi	316	1	40	1	1	(0)	42	95
484.	9mDi	316	2	39	2	3	(0)	44	87
485.	9mDi	100	2	23	0	4	(0)	27	85
486.	9mDi	100	3	33	1	3	(2)	37	89
487.	9mPa	100.000	0	0	0	28	(28)	28	0
488.	9mPa	100.000	3	1	0	24	(24)	25	4
489.	9mPa	31.600	0	24	3	1	(1)	28	86
490.	9mPa	31.600	2	31	1	1	(1)	33	94
491.	9mPa	10.000	0	44	0	1	(0)	45	98
492.	9mPa	10.000	0	26	1	0	(-)	27	96
493.	9mBr	100.000	2	31	3	0	(-)	34	91
494.	9mBr	100.000	0	31	1	3	(1)	35	89
495.	9mBr	31.600	1	29	1	1	(0)	31	94
496.	9mBr	31.600	0	41	0	2	(1)	43	95
497.	9mBr	10.000	2	24	0	3	(0)	27	89
498.	9mBr	10.000	0	35	0	3	(1)	38	92
499.	9mVa	320.000	-	30	1	2	(1)	33	91
500.	9mVa	320.000	6	30	0	2	(2)	32	94
501.	9mVa	100.000	1	31	0	2	(2)	33	94
502.	9mVa	100.000	0	34	0	2	(0)	36	94
503.	9mRef	570.000	12	15	1	9	(4)	25	60
504.	9mRef.	570.000	4	12	0	5	(3)	17	71

Table 7 Balanus improvisus 1983 (23.12.82-14.1.83). Effects of various types of drill cuttings, day 0 and after 9 months on the sea floor, on settlement and metamorphosis during conditions stimulating settlement. Supplementary tests. Column legend, see footnote, Table 2.

1. Nr 1983	3. Stoff tils.	4. Kons. (ppm)	6. Cypris fanget overfl	7. Bunnslåtte/ Met.N glass	9. Met abn	10. Løse/ Gjenv. cypris	11. derav def	12. Sum 7-10	13. % Met.N
523.	K	-	4	28	0	0	(1)	28	100
524.	K	-	7	102	4	0	(-)	106	96
525.	K	-	2	19	0	1	(0)	20	95
526.	K	-	13	53	1	0	(-)	54	98
527	DiO	1.000	3	1	20	17	(12)	38	3
528.	DiO	1.000	18	1	23	10	(5)	34	3
529.	DiO	320	5	9	11	25	(6)	45	20
530.	DiO	320	2	4	3	9	(2)	16	25
531.	DiO	180	4	24	15	9	(4)	48	50
532.	DiO	180	8	9	3	11	(3)	23	39
533.	PaO	57.000	0	12	3	26	(4)	41	29
534.	PaO	57.000	1	11	3	72	(2)	86	13
535.	PaO	18.000	0	40	4	4	(1)	48	83
536.	PaO	18.000	0	103	3	7	(2)	113	91
537.	BrO	18.000	1	20	5	12	(0)	37	54
538.	BrO	18.000	4	30	6	31	(1)	67	45
539.	BrO	13.400	0	24	1	4	(0)	29	83
540.	BrO	13.400	1	81	6	23	(2)	110	73
541.	VaO	570.000	3	1	33	2	(0)	36	3
542.	VaO	570.000	0	3	36	2	(1)	41	7
543.	VaO	320.000	0	49	3	10	(0)	62	79
544.	VaO	320.000	0	30	2	5	(1)	37	81
545.	VaO	18.000	0	47	0	7	(0)	54	87
546.	VaO	180.000	0	26	1	5	(1)	32	82

forts.

Tabell 7 (forts.) - 9 måneder

1. Nr 1983	3. Stoff tils.	4. Kons. (ppm)	6. Cypris fanget overfl	7. Bunnslåtte/ Met.N glass	9. Met abn	10. Løse/ Gjenv. cypris	11. derav def	12. Sum 7-10	13. % Met.N
547.	Di9	10.000	3	32	25	5	(0)	62	52
548.	Di9	10.000	6	14	24	5	(2)	33	33
549.	Di9	3.200	1	25	6	9	(1)	40	63
550.	Di9	3.200	0	19	3	4	(1)	26	73
551.	Pa9	76.000	0	5	1	33	(30)	39	13
552.	Pa9	76.000	1	3	0	31	(30)	34	9
553.	Pa9	57.000	0	36	10	3	(2)	49	73
554.	Pa9	57.000	0	27	5	2	(2)	34	79
555.	Pa9	43.000	2	42	1	2	(1)	45	93
556.	Pa9	43.000	0	37	0	1	(0)	38	97
557.	Br9	570.000	0	0	0	26	(26)	26	0
558.	Br9	570.000	-	-	-	-	-	-	-
559.	Br9	370.000	-	-	-	-	-	-	-
560.	Br9	370.000	0	0	0	32	(32)	32	0
561.	Br9	320.000	0	0	0	59	(59)	59	0
562.	Br9	320.000	0	0	0	31	(31)	31	0
563.	Br9	180.000	0	1	1	43	(40)	45	2
564.	Br9	180.000	0	6	1	22	(15)	29	21
565.	Va9	570.000	0	0	0	48	(49)	48	0
566.	Va9	570.000	0	0	0	74	(74)	74	0
567.	Ref.	760.000	0	0	0	34	(34)	34	0
568.	Ref.	760.000	0	0	0	89	(89)	89	0
569.	Ref.	320.000	0	29	0	0	(-)	29	100

ANNEX 3

MARINE GREEN ALGA DUNALIELLA BIOCULATA

TOXICITY TESTS WITH *DUNALIELLA BIOCULATA* ON DRILL CUTTINGS

By Svein Norland and Gjert Knutsen

All tests have been performed with the green alga *Dunaliella bioculata*. Five different samples have been tested:

1. Reference sediment
2. Water-based cuttings
3. Mineral oil-based cuttings
4. Briquettes of cuttings
5. Diesel-based cuttings

The testsubstances were added directly to the test cultures in a series of chosen concentrations. Algae from an exponentielly growing stock culture were added to the test vials. The cell density at the start of the test was 50.000 c/ml. The growth periode was 48 - 72 hours. The algae were counted and their volume measured troughout the growth periode using an electronic particle counter (Coulter Counter) connected to a laboratory computer. The toxic effect was measured as reduction in biomass (volume X cellnumber) relative to the control.

Apart from the diesel-based cuttings no significant effect could be detected below 100.000 ppm. The results for diesel-based cuttings are as follows (a dose-response plot is shown in fig. 1):

LC-50 (biomass after 72 hours) 7100 ppm
95% confidence limits 5900 - 8400 ppm
Coefficient of variation 1.7 +/- .2

The reference sediment gave no significant toxic effect below 300.000 ppm, however a slight decrease in growth rate could be detected above 100.000 ppm which was probably caused by light shading by sediment particles in the culture.

Mineral-based cuttings, water-based cuttings and briquettes

gave no significant effect below 100.000 ppm.

Tests with water-extract of samples. The reference sample and the diesel-based cutting were also test for toxicity after they had been extracted in water. One extraction was prepared for every concentration. The water volume was 75 ml in a 100 ml flask. The samples were placed on a stirring table (ca. 100 rpm) for 24 hours. Samples were then centrifuged at 10.000 rpm for 5 min and then the supernatant was filtered through a GF/C-filter.

The extracted reference sample and the diesel-based cutting gave no significant toxic effect below 300.000 and 20.000 ppm respectively.

Bergen June 27., 1983

Gjert Knutsen

Svein Norland

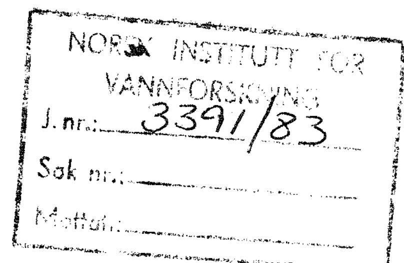
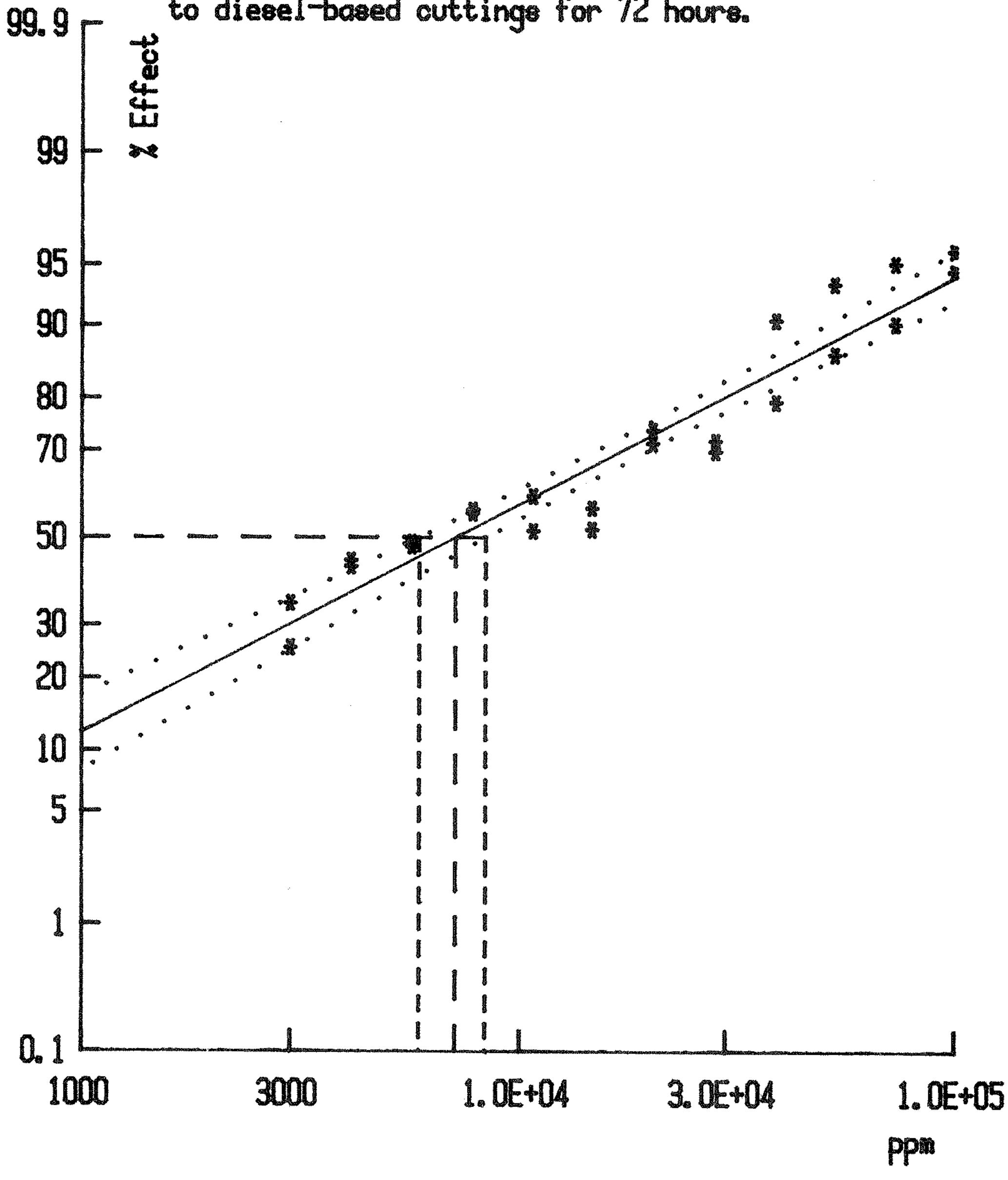

NORSK INSTITUTT FOR VANNFORSKNING
J.nr.: 3391/83
Sak nr.:
Mottatt:

Fig. 1
Dose-respons plot of D. Bioculata exposed
to diesel-based cuttings for 72 hours.



LC-50 = 7148
95% GRENSER: 5899 - 8423
CV: 1.676 +/- 0.2091

ANNEX 4

MARINE PELAGIC DIATOM SKELETONEMA COSTATUM

UNIVERSITETET I TRONDHEIM
NORGES TEKNISKE HØGSKOLE
INSTITUTT FOR MARIN BIOKJEMI

Vår saksbehandler, innvalgstelefon

Vår dato — referanse

Deres dato — referanse

Standardized toxicity test with the marine diatom Skeletonema costatum.

Description revised May 1984.

TEST ORGANISM:

Laboratory cultures of the marine diatom *Skeletonema costatum* (Greville) Cleve, clone Skel-5 (unalgal), isolated from the Trondheimsfjord by Dr. S. Myklestad.
Stock cultures are kept at 14°C, in a light/dark cycle of 14h/10h, in growth medium 3/4 f/10 (see below).

TEST MATERIAL:

For each dose, the proper amount of test material is added to 100 ml medium in a sterile Ehrlenmeyer flask. Test doses are taken from the logaritmic series: 100, 178, 316, 562, 1000, 1780....etc. and given in ppm on a weight/weight basis. Thus, 46,2 g of material must be added to give 316 000 ppm. The suspensions are kept closed, in darkness, at 14°C on a linear shaker, approx. 60 cycles per. min. (moderate shaking), for 18h (overnight). Pilot tests of a diesel mud indicated that this period was not essential for the range 4-36h. The suspensions are left to stand for 1-2h before filtration through extra fine glass wool. In some cases, samples must be sentrifuged and successively filtered down to 0,45 μm pore size to get a transparent solution. This filtrate may contain emulsified in addition to suspended material.

POSTADRESSE:
UNIT/NTH
MARIN BIOKJEMI
Sem Sælands vei 6
N 7034 Trondheim-NTH

TELEFONER:
Sentralbord: (07) 59 40 00
(07) 59 33 20

TELEX:
55637 NTHAD.N
(Att.: IMB)

THE UNIVERSITY OF TRONDHEIM
THE NORWEGIAN INSTITUTE OF TECHNOLOGY
LABORATORY OF MARINE BIOCHEMISTRY

TEST CONDITIONS.

Type: Closed batch culture. Exposure vessels are made of glass, culture volume 100 ml.

Culture medium is denoted 3/4 f/10, that is the "f" medium of Guillard and Ryther (1962) with nutrients of 1/10 strength and salinity reduced to 3/4 (25 o/oo instead of filtered seawater at 33 o/oo). Sterilized in an autoclave.

Inoculation: In order to avoid additional dilution of test solutions, only 1 ml of cell suspension is inoculated to the approx. 96 ml of filtered test solution. Growing stock cultures are concentrated by sentrifugation (table top sentrifuge) and cell density adjusted to give 1 µg C/ml as the biomass after inoculation. This is based on carbon-calibrated turbidity measure ments (c.f. Østgaard and Jensen, 1982) in a spectrophotometer (10 cm cuvette) as described by Østgaard, Hegseth and Jensen (1984). In practice, initial cell concentration is generally within $10\text{-}16\times10^5$ cells/ml. The biomass dependence of the toxic response of drilling muds has not been studied. For crude oil, see Østgaard and Jensen (1983).

Incubation: Cultures are grown for 3 days, closed, at 14°C, dark/light cycle 14h/10h. Please note that the stability of the dose during exposure is not known.

MEASUREMENTS:

Sampling.
Samples of approx. 5 ml are taken at 0h, 3h and 3d of incubation.

Analysis:

In vivo fluorescence is measured as described by Østgaard and Jensen (1982), first without, then with addition of DCMU to the same sample.

Cell concentration is determined by counting in a hemocytometer after additon of Evans blue, in order to separate living and dead cells. The method and its validity is based on Crippen and Perrier (1974). It is stressed that this staining may be essential, since some drilling muds lead to very rapid disintegration of dead algae, others do not.

pH is measured in all cultures at the end of the experiment.

Calculations:

Survival is calculated as the fraction of living cells compared to that of the control. In some cases correction for cellular disintegration within 0-3h may be necessary.

Relative photosynthetic capacity is calculated as

$$(\text{fluorescence}_{+DCMU} - \text{fluorescence}_{-DCMU})/\text{fluorescence}_{+DCMU}.$$

This term may not be generally accepted, but confer Samuelsson and Öquist (1977), Prezelin and Ley (1980), Kulandaivelu and Daniell (1980), Öquist *et al.* (1982) and others. It is stressed that the parameter should not be expected to correlate to growth rate, only to the ability of the cells to use their present chlorophyll for photosynthesis. Adaptation of cell chlorophyll concentrations may be very rapid in *Skeletonema* (Riper *et al.* 1979).

Growth rate is calculated according to the "growth inhibition test" of OECD (1981). Because of a slight lag after inoculation, the mean value of the 0h and 3h recordings is used as initial cell concentration.

RESULTS:

Final results are always based on at least two completely independent experiments. This is considered necessary as a quality control and as a verification of reproducibility of both preparation and testing of solutions.

Please note that doses are always given as the concentration of the original suspensions, although filtrates only are used for exposure. As a summary, all parameter values relative to control (linear scale) is plotted as a function of initial dose (logaritmic scale). EC_{50}-values may simply be taken from curves fitted by eye. If practically possible, probit-log diagrams are used to determine EC_{10}, EC_{50} and EC_{90} values (EC_x denotes the concentration leading to a x % reduction of the actual parameter when compared to the control value). The probit-log representation is strictly not valid for low growth rates (doses above EC_{90}), since this parameter has a maximal range of 1 to $-\infty$.

The test should be considered essentially as a growth inhibition test (c.f. OECD, 1981). However, the fluorescence measurements are very helpful for quality control and final evaluation, to select cultures that need to be counted, and to evaluate the long-term viability of cells surviving the 3 days of incubation.

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DERES REF.	DERES BREV AV	VÅR REF.	DATO
		KØ:mk	2. mai 1983.

TEST: Toxic effects on the marine planktonic alga Skeletonema costatum.

SAMPLE: NIVA "reference sediment". Initial.

METHOD: Cf separate description

RESULTS: see table below and Fig.1. A summary is:

Incubation	Effect	EC-50	EC-10 - EC-90
3h	Survival	>316 000	(~300 000 -)
	Rel. photosynth.	>316 000	(~100 000 -)
3d	Growth rate	85 000	13 000 - 170 000
	Rel. photosynth.	>316 000	(~30 000 -)

EC-X gives dose (in ppm suspension before filtering) giving X% reduction in the measured parameter.

When 50% effect could not be measured within the concentration interval applied (maximal concentration 316 000 ppm), this was given as EC-50>. In this case an EC-10 value is suggested in parenthesis.

The most sensitive parameter was growth rate. Fig. 2 shows log-normal diagram for this parameter. EC-50 = 85 000 ppm and the interval from EC-10 to EC-90 is taken from the diagram.

The reproducibility of the test was acceptable for growth rate, but

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parallel independent tests showed clear differences in physiological condition (relative photosyntetic rate) after 3 days. (cf comments below).

After 3d the control media had pH 8.3. At the highest concentration of the test media pH went down to 6.9.

COMMENTS: The "ref"-sediment differed from the other samples received in having high microbiological activity (accompanied by smell). The microorganisms passed to some extent through conventional filtering and had some effect on the 3d incubation. Test cultures with less than 25% normal photosynthetic capacity were discarded. It is still possible that the algae in the highest test concentration would have to compete with other microorganisms. If required the tests could be repeated with sterile filtered media. Yet the "ref" sediment appears clearly to be the least toxic of the samples tested (cf. the "water base" medium which shows a similar response pattern).

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TEST: Toksiske effekter på marin planktonalge *Skeletonema costatum*.

PRØVE: NIVA "referanse-sediment"

RESULTATER:

Verdier gitt relativ kontroll:

Ref.	Dose* [ppm]	3h eksponering		3d eksponering	
		Overlevelse	Rel.fotosynt.	Veksthastigh.	Rel.fotosynt.
247	316		1,00	0,96	0,95
	1 000		1,02	1,12	1,01
	3 160		1,03	1,03	1,01
	10 000		0,95	0,80	0,87
	31 600		1,00	0,79	0,84
	100 000	1,00	0,88	vraket	----
	316 000	0,86	0,78	< 0	0,74
250	1 780		0,99	1,01	0,96
	5 620		1,00	0,92	0,97
	17 800		0,97	0,84	0,95
	56 200		0,93	vraket	----
	100 000		0,83	< 0	0,70
	178 000		0,79	< 0	0,59
	316 000	0,95	0,73	< 0	0,43
253	10 000		0,96	vraket	----
	31 600		1,02	0,85	0,97
	56 200		0,99	0,44	0,95
	100 000		0,99	0,20	0,90

* Total suspensjon før filtrering. Bare filtrat er benyttet ved eksponering

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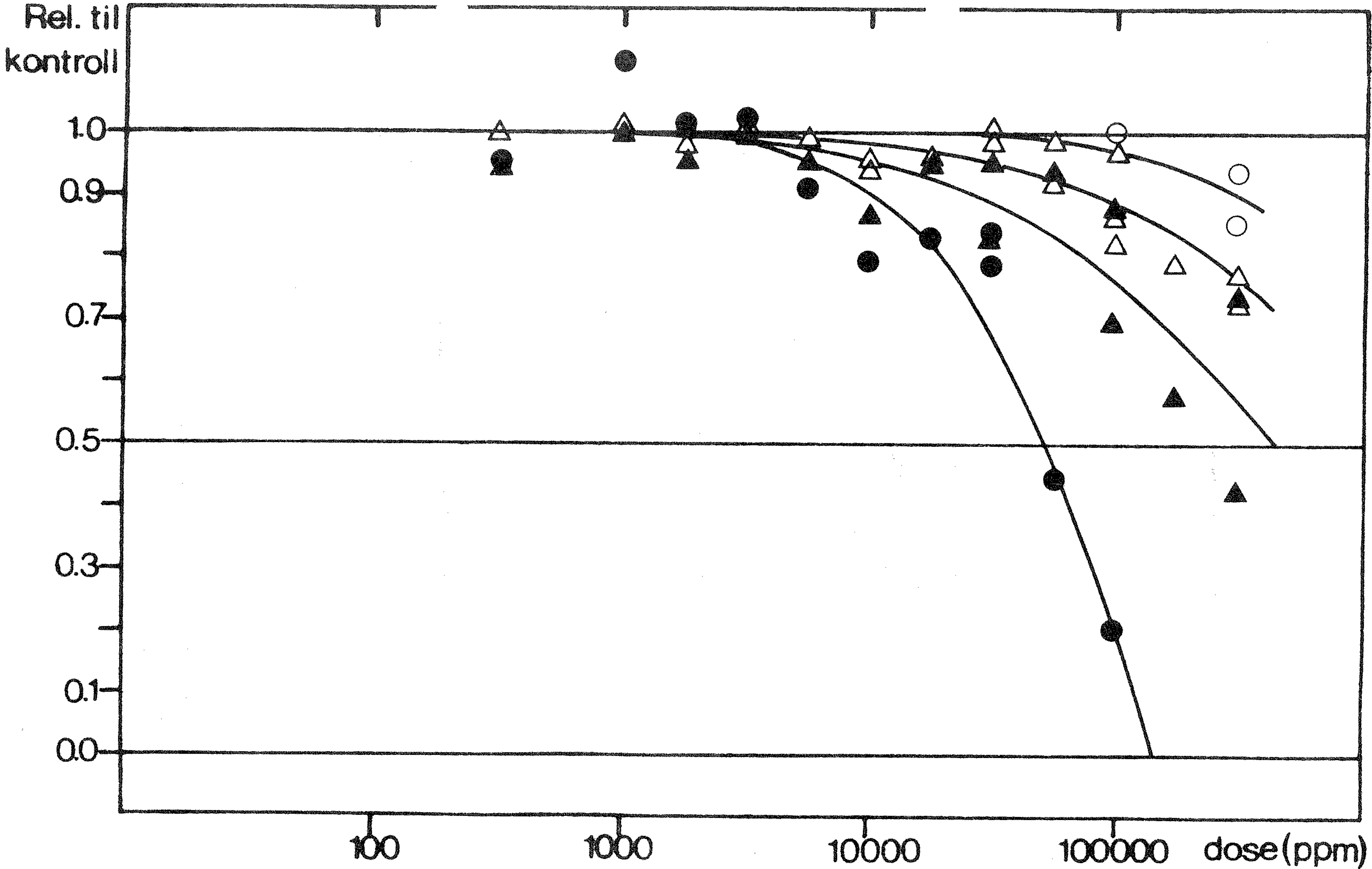
Fig. 1

NIVA "ref.sediment"

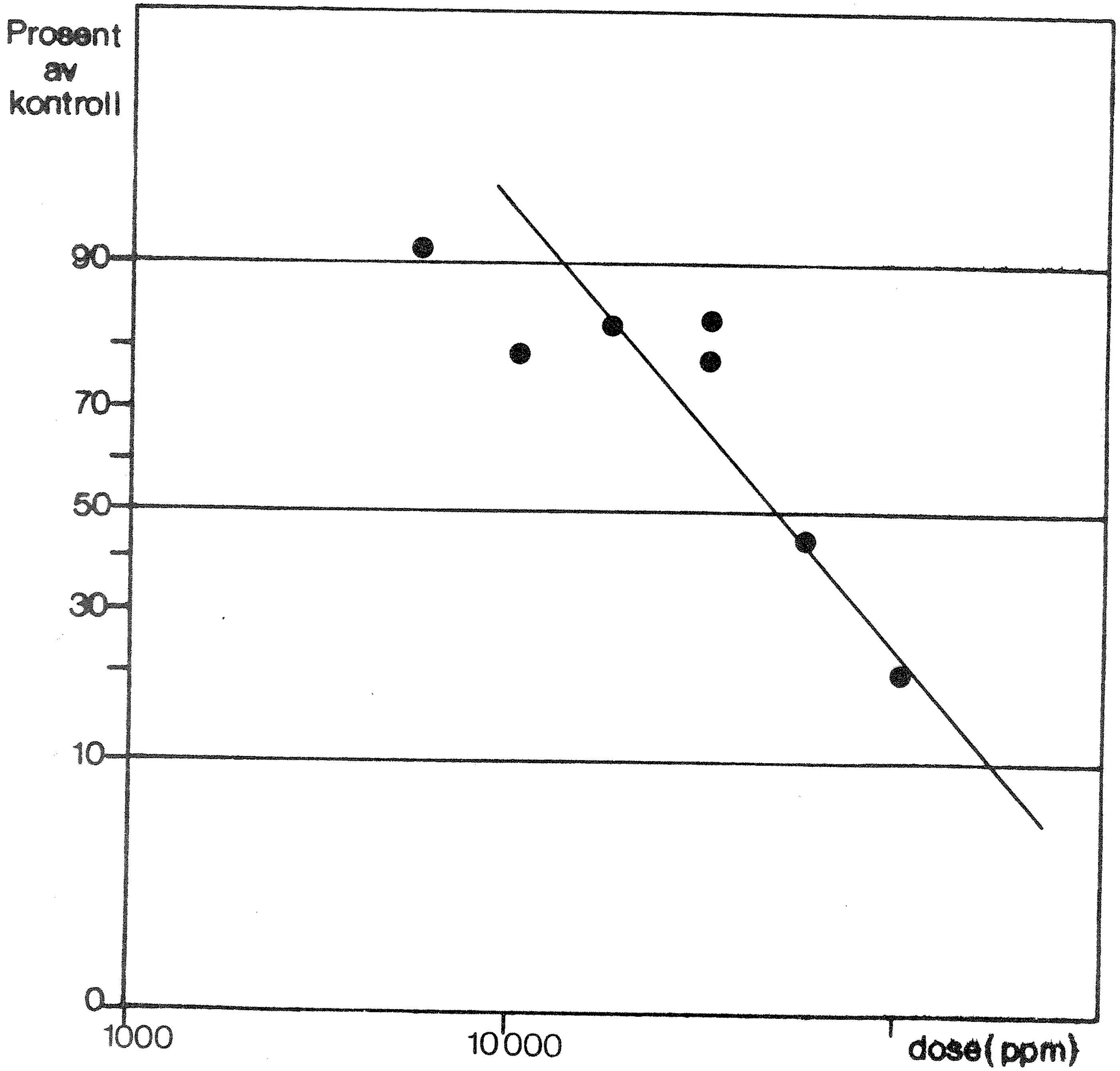


Symboler:

3h eksponering: ○ overlevelse, △ rel. fotosyntesekap.

3d eksponering: ● veksthastighet, ▲ rel. fotosyntesekap.

NIVA "ref.sediment"



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DERES REF.	DERES BREV AV	VÅR REF.	DATO
		KØ/mk	2. mai 1983.

TEST: Toxic effects on the marine planktonic alga Skeletonema costatum.

SAMPLE: NIVA "water based". Initial.

METHOD: Cf separate description.

RESULTS: see table below and Fig.1. A summary is:

Incubation	Effect	EC-50	EC-10 - EC-90
3h	Survival	>316 000	(~150 000 -)
	Rel. photosynth.	>316 000	(~150 000 -)
3d	Growth rate	30 000	5 700 - 160 000
	Rel. photosynth.	>316 000	(~100 000 -)

EC-X gives dose (in ppm suspension before filtering) giving X% reduction in the measured parameter.

When 50% effect could not be measured within the concentration interval applied (maximal concentration 316 000 ppm), this was given as EC-50>. In this case an EC-10 value is suggested in parenthesis.

The most sensitive parameter was growth rate. Fig. 2 shows log-normal diagram for this parameter. EC-50 = 30 000 ppm and the interval from EC-10 to EC-90 is taken from the diagram.

Two independent tests showed systematic difference in growth rate.

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This gives two independent estimates of the EC-50 value of 16 000 and 42 000 ppm. This interval illustrates the reproducibility.

After 3d the control media had pH 8.4. At the highest concentration of the test media pH rose to 8.9.

COMMENTS: In spite of a nearly insignificant reduction in photosynthetic capacity at concentrations below 100 000 ppm, the net growth was severely reduced. Even at concentrations giving net cell loss over 3 days (>300 000 ppm) the surviving cells appeared little influenced physiologically.

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TEST: Toksiske effekter på marin planktonalge *Skeletonema costatum*.

PRØVE: NIVA "vannbasert".

RESULTATER:

Verdier gitt relativ kontroll:

		3h eksponering		3d eksponering	
Ref.	Dose* [ppm]	Overlevelse	Rel.fotosynt.	Veksthastigh.	Rel.fotosynt.
254	1 000		0,99	1,01	1,01
	3 160	1,02	1,03	0,99	1,00
	10 000	0,95	0,96	0,74	1,00
	31 600	0,97	0,95	0,61	0,97
	100 000	0,92	0,96	0,32	0,93
	316 000	0,87	0,84	0,02	0,93
256	1 780		0,97	1,00	1,01
	5 620		1,03	0,61	1,00
	17 800		1,00	0,30	0,92
	56 200		0,98	0,25	0,88
	100 000	0,93	0,95	0,21	0,85
	178 000	0,93	0,90	0,14	0,81
	316 000	0,80	0,81	< 0	0,78

* Total suspensjon før filtrering. Bare filtrat er benyttet ved eksponering

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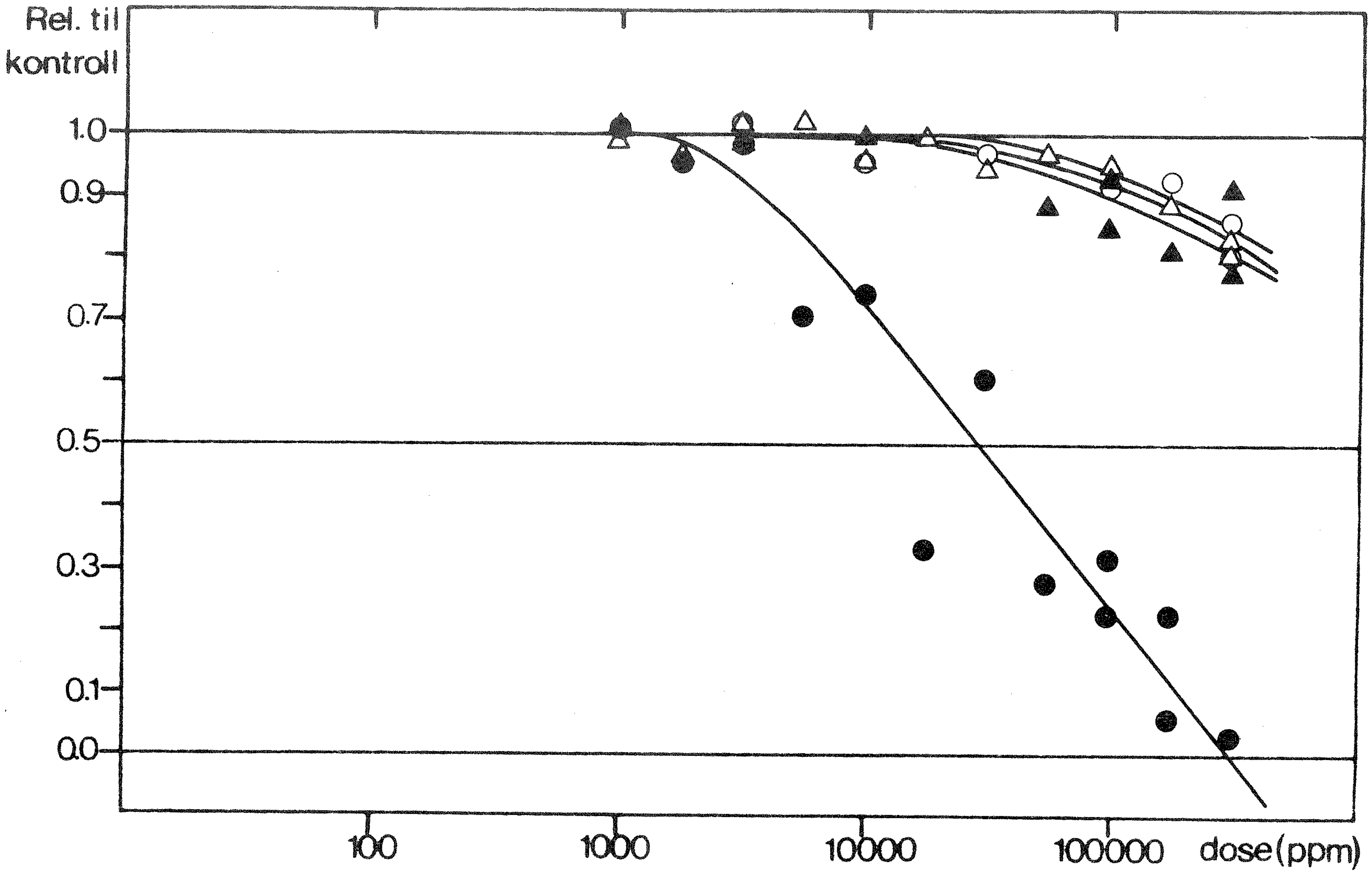
Fig. 1

NIVA "vannbasert"



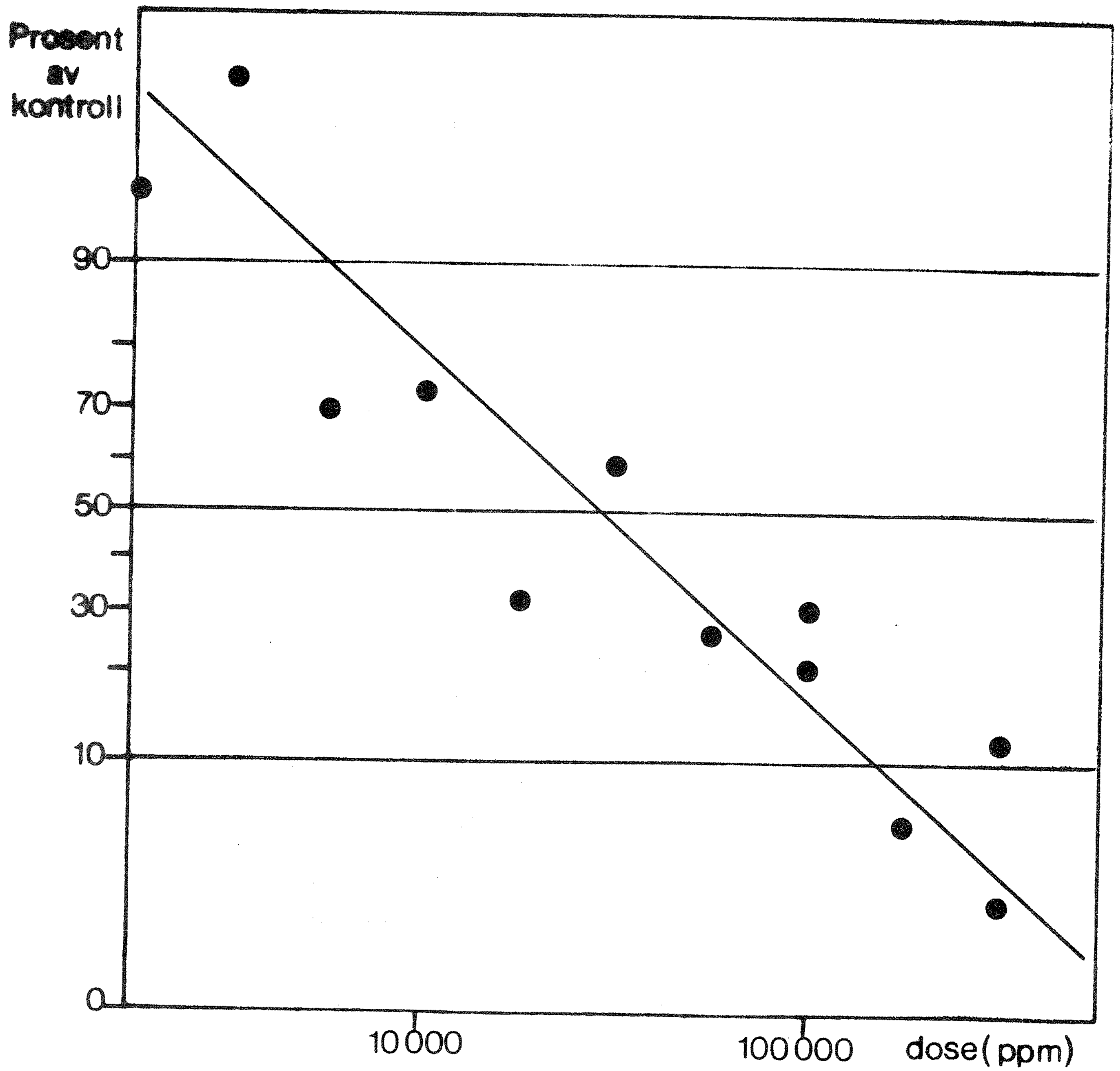
Symboler:

3h eksponering: ○ overlevelse, △ rel. fotosyntesekap.

3d eksponering: ● veksthastighet, ▲ rel. fotosyntesekap.

Fig. 2

NIVA "vannbasert"



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DERES REF.	DERES BREV AV	VÅR REF.	DATO
		KØ:mk	2. mai 1983.

TEST: Toxic effects on the marine planktonic alga Skeletonema costatum.

SAMPLE: NIVA "low aromatic". Initial.

METHOD: Cf separate description.

RESULTS: see table below and Fig.1. A summary is:

Incubation	Effect	EC-50	EC-10 - EC-90
3h	Survival	>316 000	(~300 000 -)
	Rel. photosynth.	240 000	
3d	Growth rate	7 500	1 000 - 32 000
	Rel. photosynth.	100 000	

EC-X gives dose (in ppm suspension before filtering) giving X% reduction in the measured parameter.

When 50% effect could not be measured within the concentration interval applied (maximal concentration 316 000 ppm), this was given as EC-50>. In this case an EC-10 value is suggested in parenthesis.

The most sensitive parameter was growth rate. Fig. 2 shows log-normal diagram for this parameter. EC-50 = 7 500 ppm and the interval from EC-10 to EC-90 is taken from the diagram.

The reproducibility was acceptable as two independent tests did not

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reveal any systematic differences.

COMMENTS: Although there was no clear indication of inreased mortality after 3 hours, the photosynthetic capacity was strongly reduced. After 3 days the photosynthetic capacity was normal even at concentrations giving less that 50% growth rate.

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TEST: Toksiske effekter på marin planktonalge *Skeletonema costatum*.

PRØVE: NIVA "parafinbasert".

RESULTATER:

Verdier gitt relativ kontroll:

Ref.	Dose* [ppm]	3h eksponering		3d eksponering	
		Overlevelse	Rel.fotosynt.	Veksthastigh.	Rel.fotosynt.
246	316		1,02	0,93	1,00
	1 000		0,99	0,85	1,01
	3 160		0,87	0,58	1,04
	10 000		0,83	0,50	---
	31 600	1,00	0,81	0,18	1,00
	100 000	0,95	0,71	< 0	0,49
	316 000	0,94	0,28	< 0	0,09
249	100		1,00	0,90	1,01
	178		0,97	0,95	0,98
	562		0,97	0,97	0,96
	1 780		0,96	0,68	1,02
	5 620		0,97	0,72	0,99
	17 800		0,94	0,11	0,98
	178 000	0,90	0,73	< 0	0,26
252	5 620		0,98	0,66	1,00

* Total suspensjon før filtrering. Bare filtrat er benyttet ved eksponering

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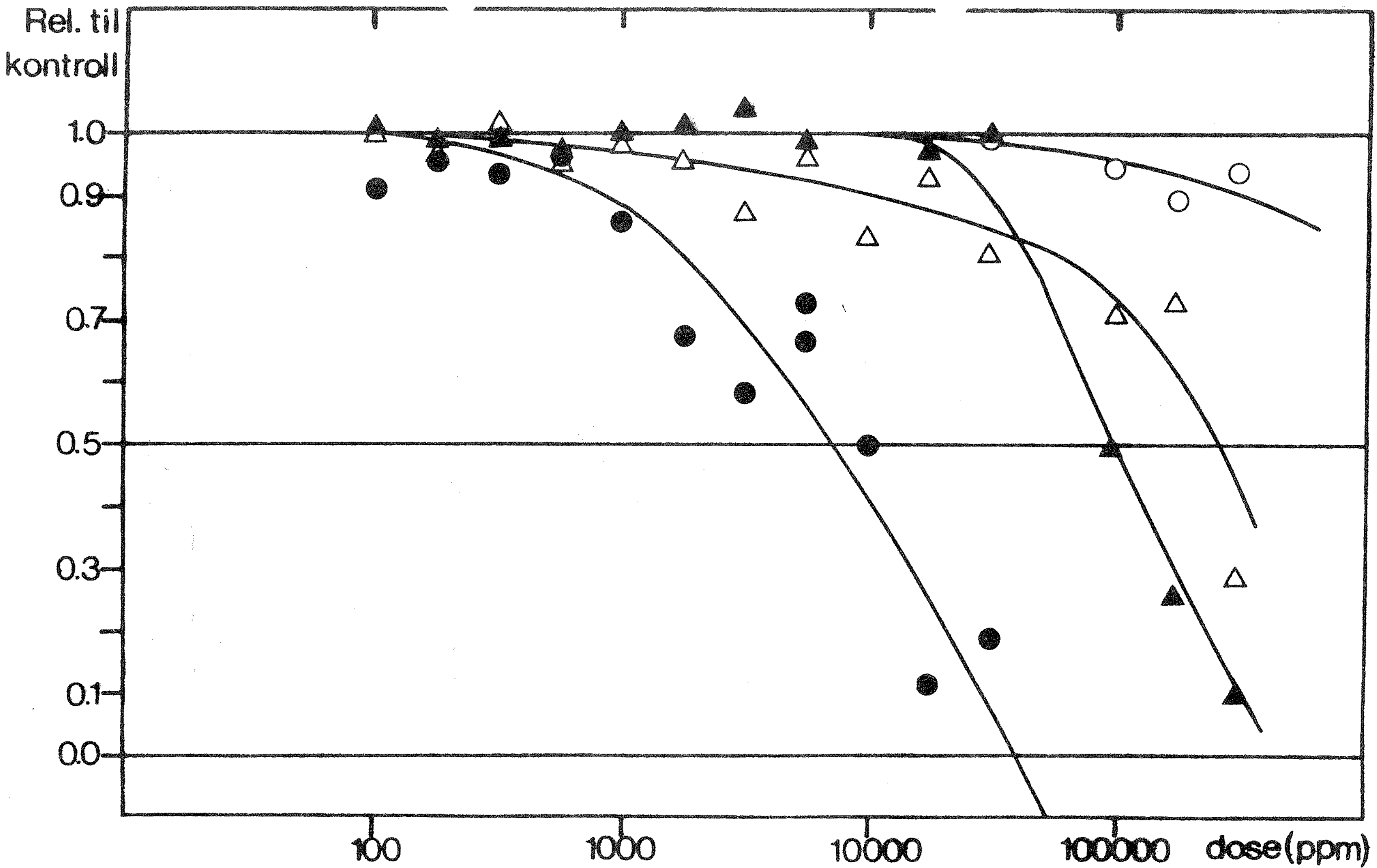
Fig. 1

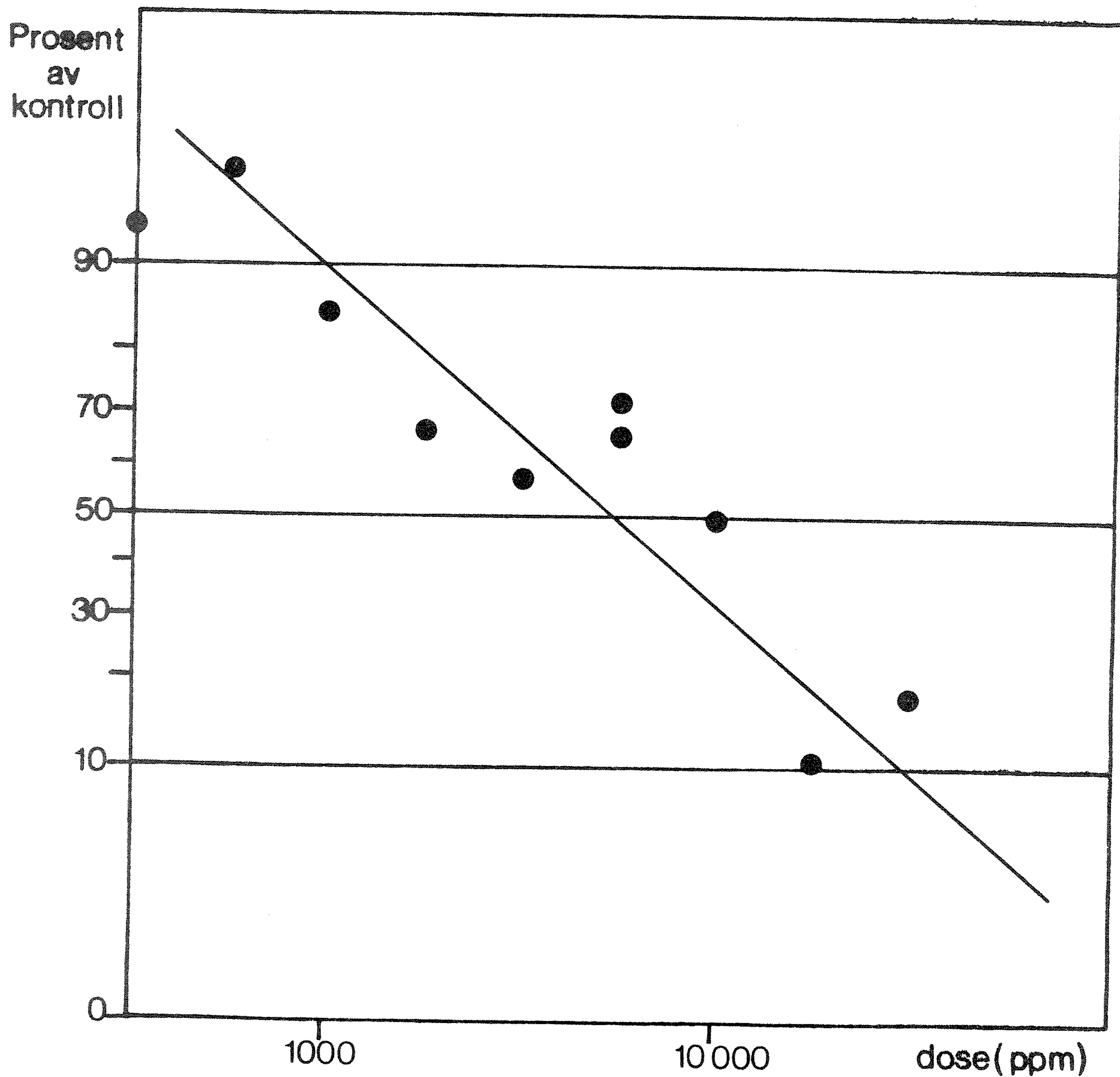
NIVA "parafinbasert"



Symboler:
3h eksponering: ○ overlevelse, △ rel. fotosyntesekap.
3d eksponering: ● veksthastighet, ▲ rel. fotosyntesekap.

Fig. 2

NIVA "parafinbasert"



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DERES REF.	DERES BREV AV	VÅR REF.	DATO
		KØ:mk	2. mai 1983.

TEST: Toxic effects on the marine planktonic alga _Skeletonema costatum_.

SAMPLE: NIVA "briquettes". Initial.

METHOD: Cf separate description.

RESULTS: see table below and Fig.1. A summary is:

Incubation	Effect	EC-50	EC-10 - EC-90
3h	Survival	>316 000	(~100 000 -)
	Rel. photosynth.	170 000	
3d	Growth rate	920	250 - 3 300
	Rel. photosynth.	10 000	2 000 - 60 000

EC-X gives dose (in ppm suspension before filtering) giving X% reduction in the measured parameter.

When 50% effect could not be measured within the concentration interval applied (maximal concentration 316 000 ppm), this was given as EC-50>. In this case an EC-10 value is suggested in parenthesis.

The most sensitive parameter was growth rate. Fig. 2 shows log-normal diagram for this parameter. EC-50 = 920 ppm and the interval from EC-10 to EC-90 is taken from the diagram. Relative photosynthesis after 3 days has been treated in the same way (cf Fig. 3).

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The reproducibility is illustrated by two independent tests giving EC-50 estimates of 600 and 1 500 ppm respectively. This is considered acceptable (cf the EC-10 - EC-90 limits).

After 3d the control media had pH 8.8. At the highest concentration the test media pH went down to 7.9.

COMMENTS: The total response was similar to that of the "diesel" substrate (cf comments to this). The "briquettes" sample produced a stronger toxic effect on photosynthetic capacity both at 3h and 3d, but the growth response was about the same as for "diesel".

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TEST: Toksiske effekter på marin planktonalge Skeletonema costatum.

PRØVE: NIVA "briketter".

RESULTATER:

Verdier gitt relativ kontroll:

	3h eksponering		3d eksponering	
Ref. Dose* [ppm]	Overlevelse	Rel.fotosynt.	Veksthastigh.	Rel.fotosynt.
255 316		0,97	0,70	1,02
1 000		0,89	0,26	1,01
3 160		0,91	0,10	0,80
10 000		0,80	< 0	0,46
31 600	0,95	0,76	< 0	0,22
100 000	0,96	0,54	< 0	0,00
316 000	0,94	0,41	< 0	0,00
100		0,99	0,91	0,97
178		0,99	0,94	1,00
562		0,97	0,88	1,05
1 780		0,94	0,45	1,03
17 800	0,93	0,79	< 0	0,42
56 200	0,90	0,66	< 0	0,11
178 000	0,76	0,50	< 0	0,00

* Total suspensjon før filtrering. Bare filtrat er benyttet ved eksponering

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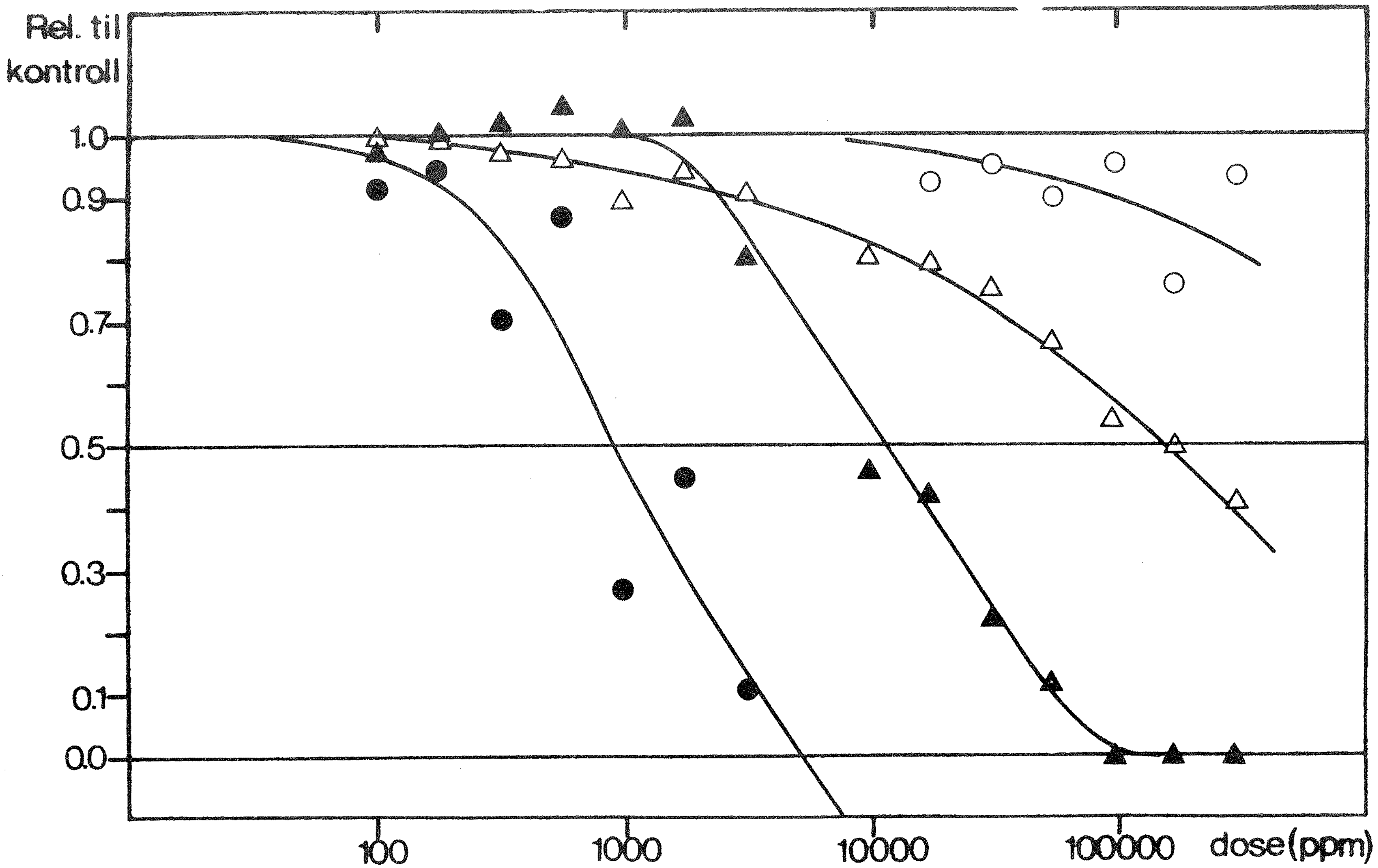
Fig. 1

NIVA "briketter"



Symboler:

3h eksponering: ○ overlevelse, △ rel. fotosyntesekap.

3d eksponering: ● veksthastighet, ▲ rel. fotosyntesekap.

Fig. 2

NIVA "briketter"
Veksthastighet

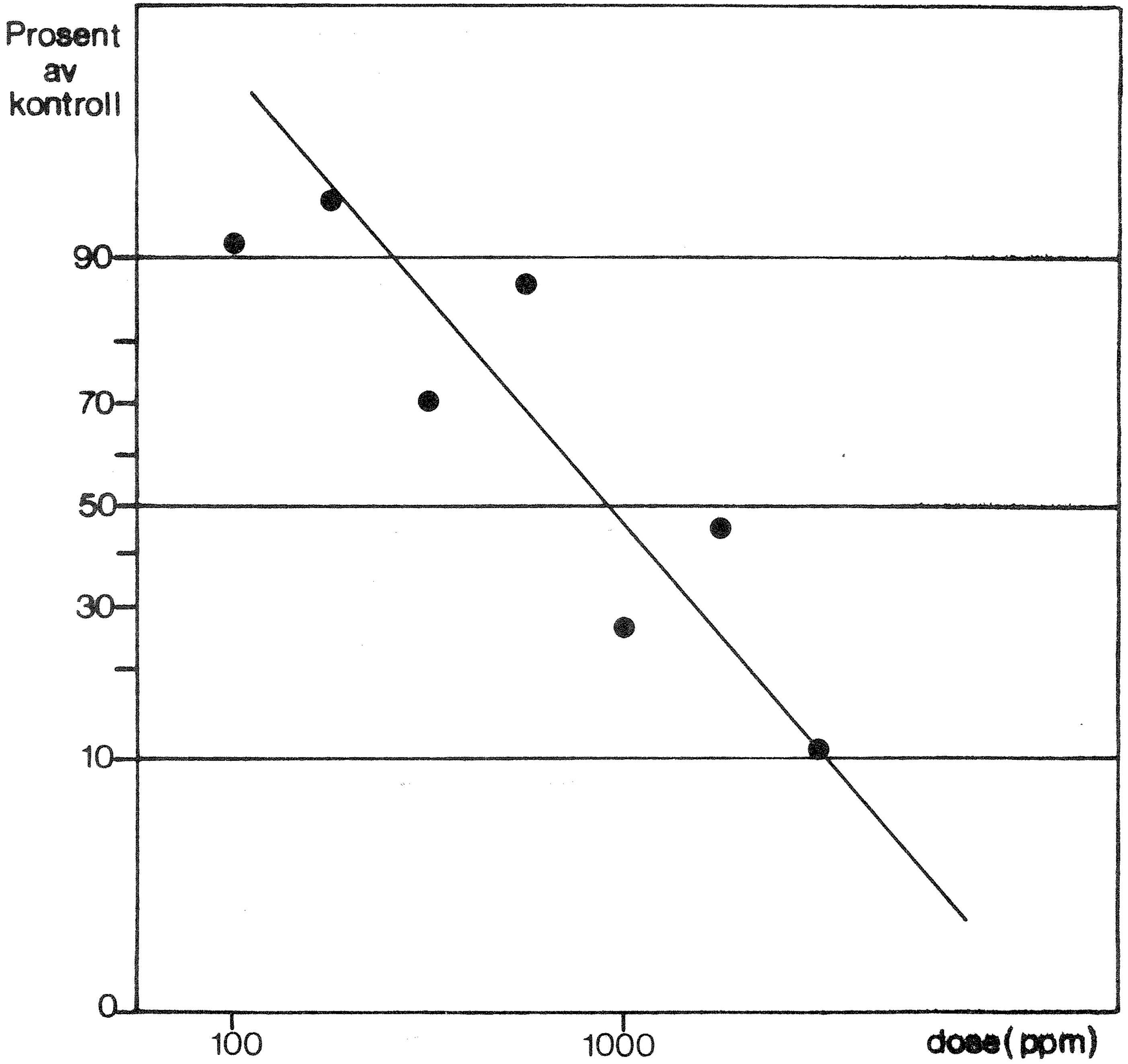
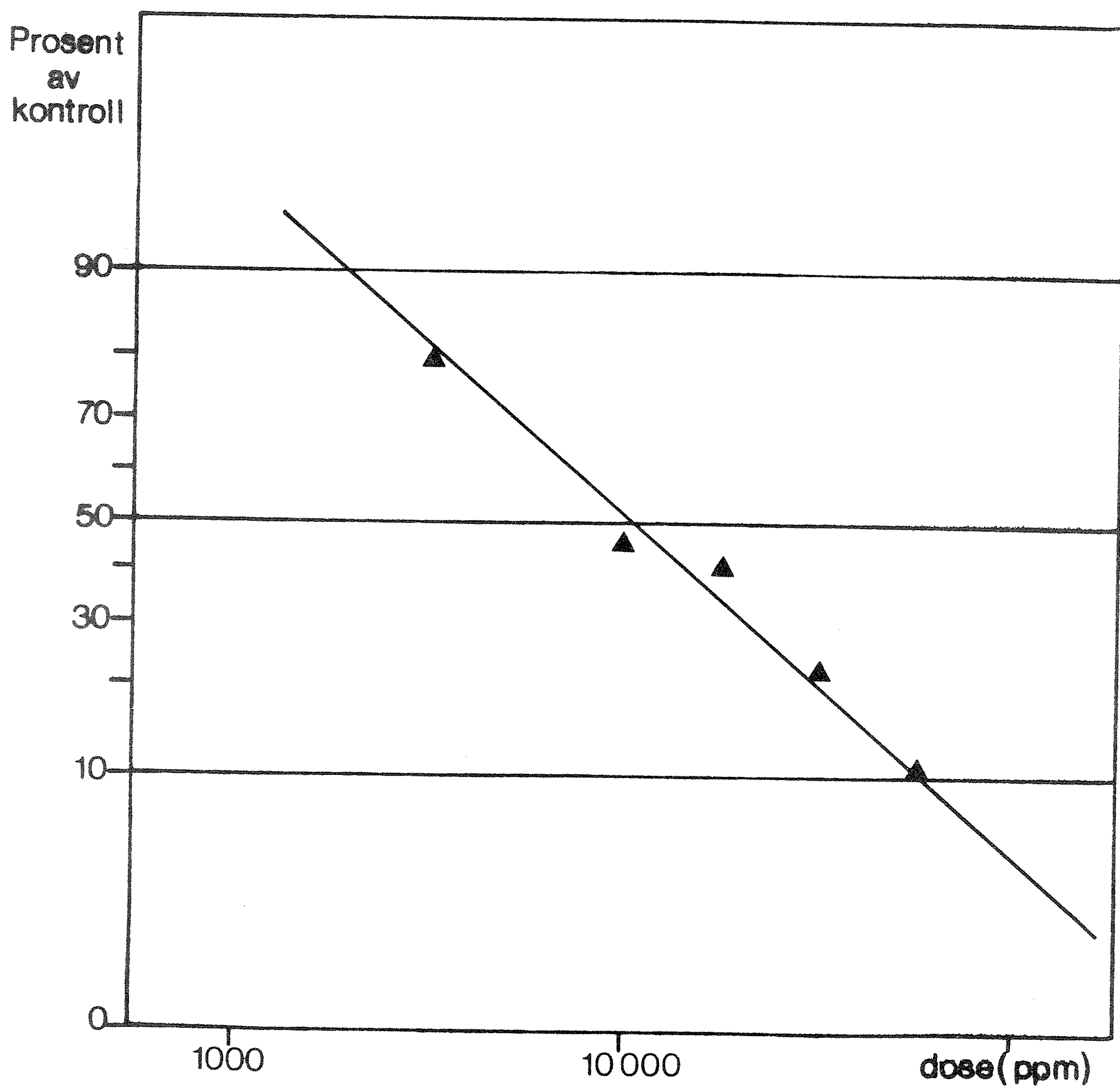


Fig. 3

NIVA "briketter"

Rel.fotsynt. 3d



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DERES REF.	DERES BREV AV	VÅR REF.	DATO
		KØ/mk	2. mai 1983.

TEST: Toxic effects on the marine planktonic alga *Skeletonema costatum*.

SAMPLE: NIVA "diesel oil". Initial.

METHOD: Cf separate description.

RESULTS: see table below and Fig.1. A summary is:

Incubation	Effect	EC-50	EC-10 - EC-90
3h	Survival	316 000	(~16 000 -)
	Rel. photosynth.	316 000	(~ 1 000 -)
3d	Growth rate	1 060	250 - 4 500
	Rel. photosynth.	30 000	(~2 000 -)

EC-X gives dose (in ppm suspension before filtering) giving X% reduction in the measured parameter.

When 50% effect could not be measured within the concentration interval applied (maximal concentration 316 000 ppm), this was given as EC-50>. In this case an EC-10 value is suggested in parenthesis.

The most sensitive parameter was growth rate. Fig. 2 shows log-normal diagram for this parameter. EC-50 = 1 060 ppm and the interval from EC-10 to EC-90 is taken from the diagram.

The reproducibility (based on 3 independent tests all covering a

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range of concentrations) seemed good.

COMMENTS: Reduced photosynthetic capacity was observed at 3h even at low concentrations, but the process was not blocked completely. Acute lethality (3h) was low even at concentrations giving strong reduction in cell numbers over 3d (10 000 - 100 000 ppm). The photosynthetic capacity at 3d was reduced at these concentrations, but still indicated that viable cells were present in these 'dying' cultures (growth less than zero).

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TEST: Toksiske effekter på marin planktonalge Skeletonema costatum.

PRØVE: NIVA "diesel"

RESULTATER:

Verdier gitt relativ kontroll:

		3h eksponering		3d eksponering	
Ref.	Dose* [ppm]	Overlevelse	Rel.fotosynt.	Veksthastigh.	Rel.fotosynt.
225	500	0,98	1,01	0,63	1,03
	2 000	0,98	0,99	0,29	0,96
	8 000	0,95	0,94	0,05	0,95
230	2 000	1,01	0,94	0,40	1,00
242	100	1,00	0,95	1,00	0,98
	316	0,97	0,92	vraket	----
	1 000	0,97	0,89	0,67	1,00
	3 160	0,97	0,86	0,19	0,82
	10 000	0,87	0,84	0,02	0,58
	31 600	0,86	0,79	< 0	0,55
	100 000	0,78	0,76	< 0	0,28
245	56,2		0,99	1,01	1,05
	100		0,99	1,06	1,03
	178		0,95	0,95	1,06
	316		0,94	0,65	0,94
	562		0,93	0,65	1,04
	1 780		0,88	0,38	1,03
	5 620		0,84	< 0	0,79
	316 000	0,80	0,61	< 0	(0,41)

* Total suspensjon før filtrering. Bare filtrat er benyttet ved eksponering

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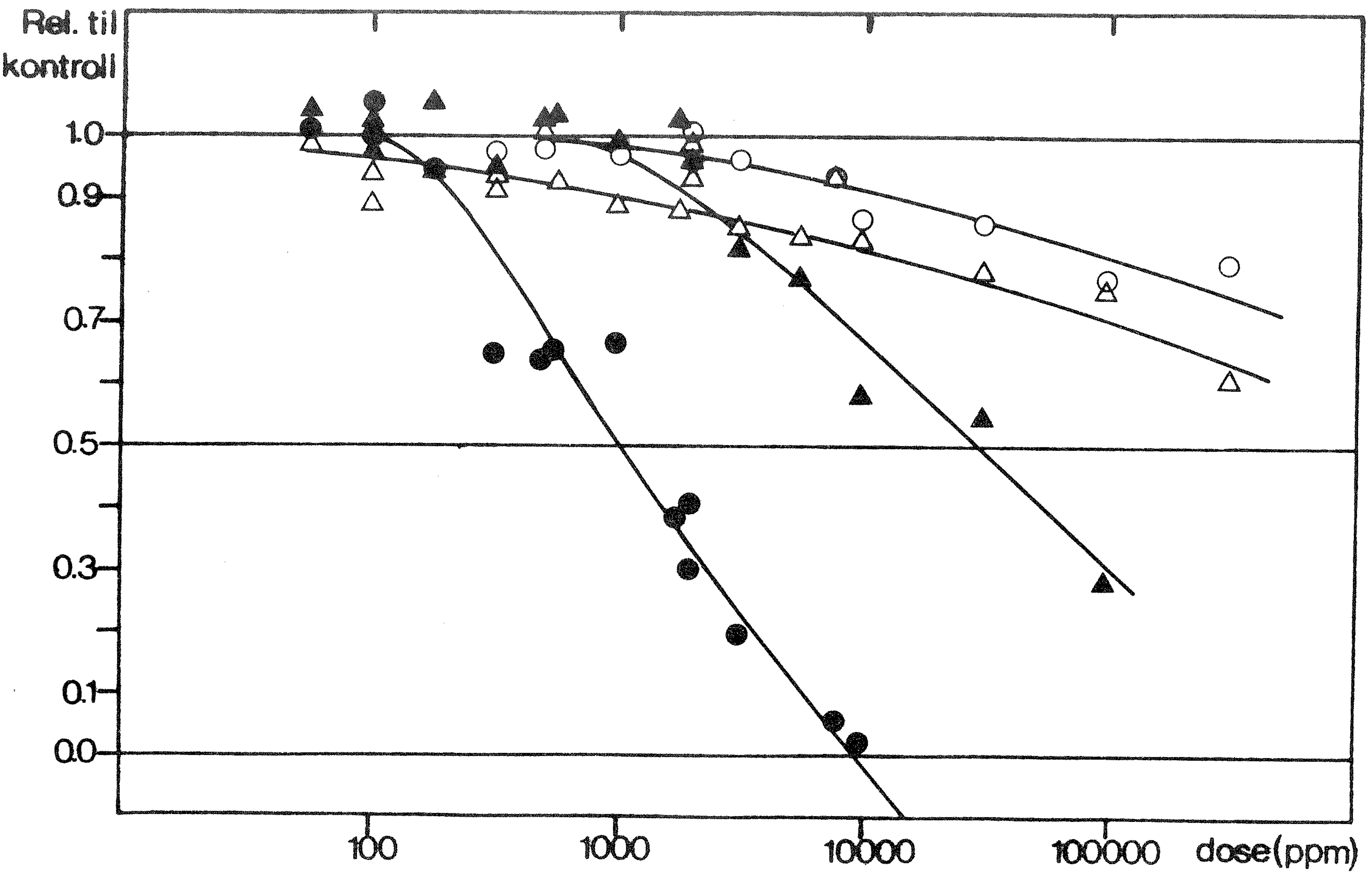
Fig. 1,

NIVA "diesel"



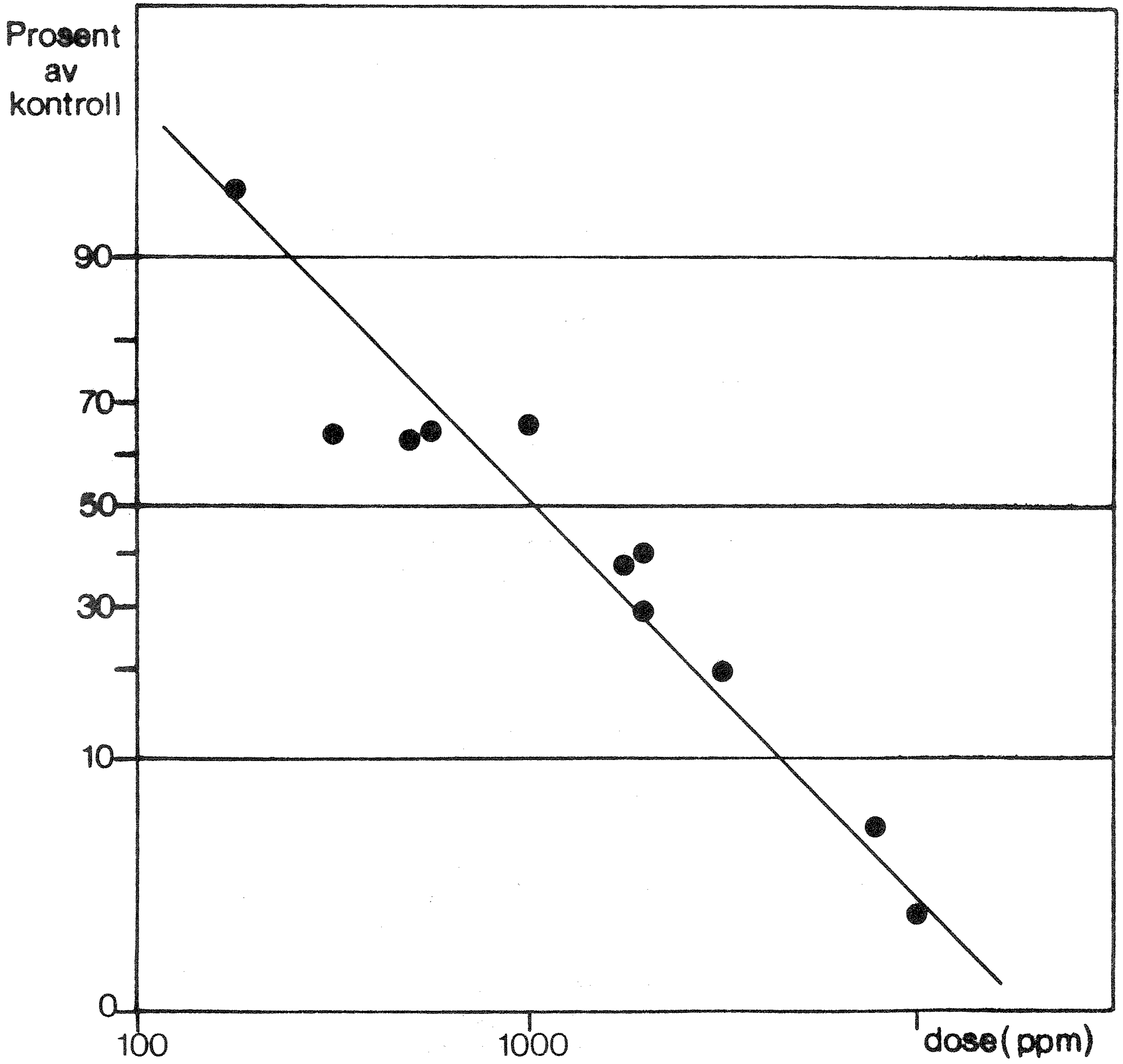
Symboler:

3h eksponering: ○ overlevelse, △ rel. fotosyntesekap.

3d eksponering: ● veksthastighet, ▲ rel. fotosyntesekap.

Fig. 2

NIVA "diesel"



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Forskningsprogram om
Havforurensninger,
Munthesgt. 29,

O S L O 2.

DERES REF.	DERES BREV AV	VÅR REF.	DATO
		KØ:aa	10.nov. 1983.

TEST: Toxic effects on the marine planktonic alga _Skeletonema costatum_.

SAMPLE: NIVA "reference sediment". 9 months.

METHOD: Cf separate description.

RESULTS: see table below and Fig.1. A summary is:

Incubation	Effect	EC-50	EC-10 - EC-90
3h	Survival	>316 000	(>316 000)
	Rel. photosynth.	>316 000	(~300 000 -)
3d	Growth rate	230 000	20 000 -
	Rel. photosynth.	>316 000	(~100 000 -)

EC-X gives dose (in ppm suspension before filtering) giving X% reduction in the measured parameter.

When 50% effect could not be measured within the concentration interval applied (maximal concentration 316 000 ppm), this was given as EC-50>. In this case an EC-10 value is suggested in parenthesis.

The most sensitive parameter was growth rate. Fig. 2 shows log-normal diagram for this parameter. EC-50 = 230 000 ppm and the interval from EC-10 to EC-90 is taken from the diagram. Basis for treating the other responses in the same way was not present.

The reproducibility was good.

After 3d the control media had pH 8.9. At the highest concentration of the test media pH went down to 8.0.

COMMENTS: The response pattern was much the same as the initial reference substrate. The effects were systematically lower at 9 months, presumably due to higher water content in the 9 months samples. Based on the earlier problems of microbial activity, the test suspensions were Millipore-filtered at 0.45 µm to prevent microbial contamination. This may have reduced the toxic effect somewhat at the highest concentrations.

TEST: Toksiske effekter på marin planktonalge *Skeletonema costatum*.

PRØVE: MOBIL-NIVA referanse 17/3.

RESULTATER:

Verdier gitt relativ kontroll:

		3h eksponering		3d eksponering	
Ref.	Dose* [ppm]	Overlevelse	Rel.fotosynt.	Veksthastigh.	Rel.fotosynt.
307	3 160		0.98	0.96	1.00
	10 000		0.99	0.95	0.92
	31 600		0.95	0.89	0.88
	100 000	0.97	0.95	0.68	0.93
	316 000	0.99	0.91	0.44	0.87
310	1 780		1.00	1.03	0.99
	5 620		1.02	1.02	0.97
	17 800		0.98	0.87	0.98
	56 200		0.95	0.80	1.01
	178 000	0.98	0.94	0.51	0.93

* Total suspensjon før filtrering. Bare filtrat er benyttet ved eksponering

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Instituttet (075) [illegible]

TELEX
55 186 NTHHB N
(Att. IMB)

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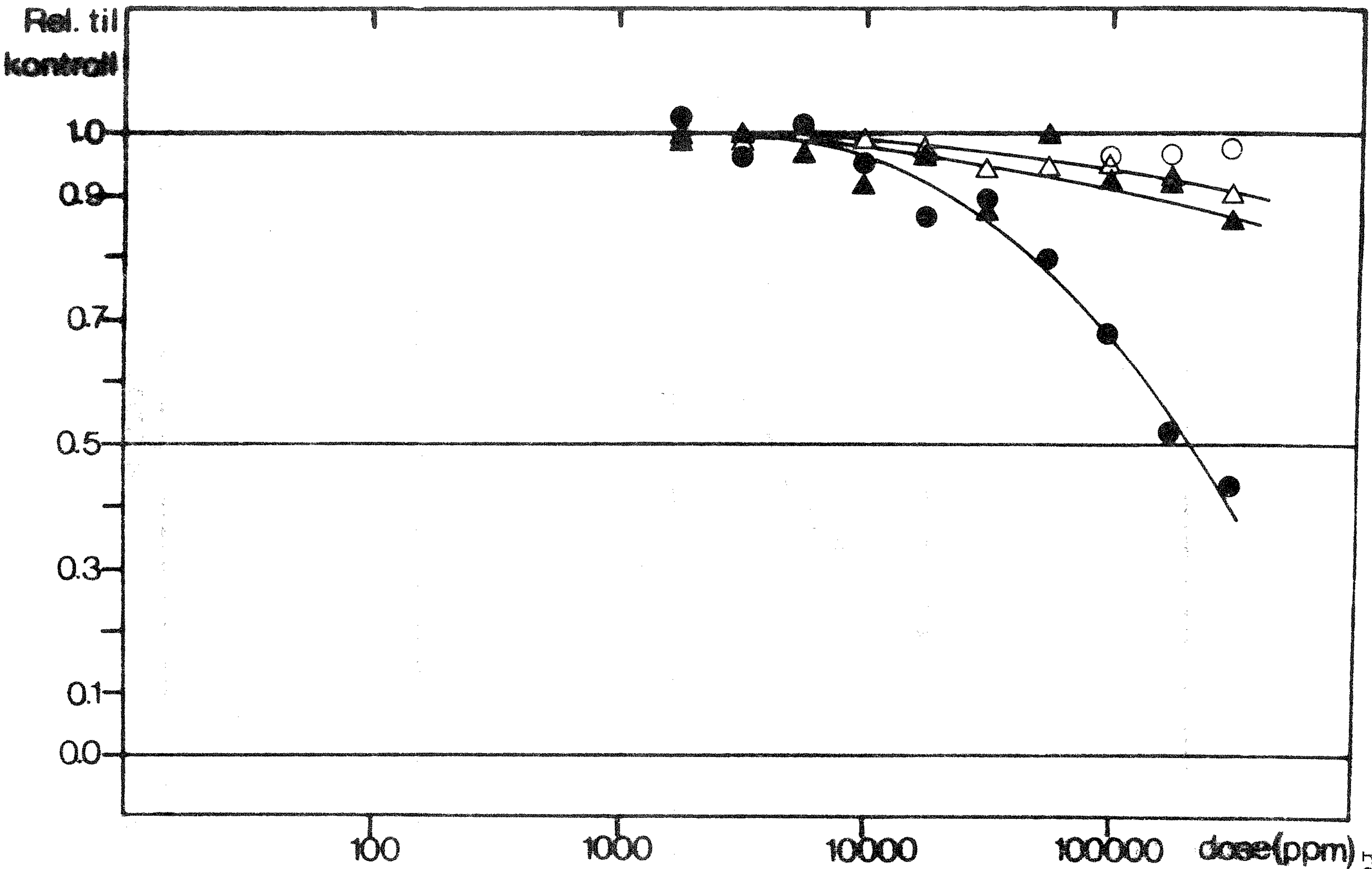
(Formerly: Norwegian Institute of Seaweed Research)

Fig.1

MOBIL-NIVA

referanse 17/3



Symboler:

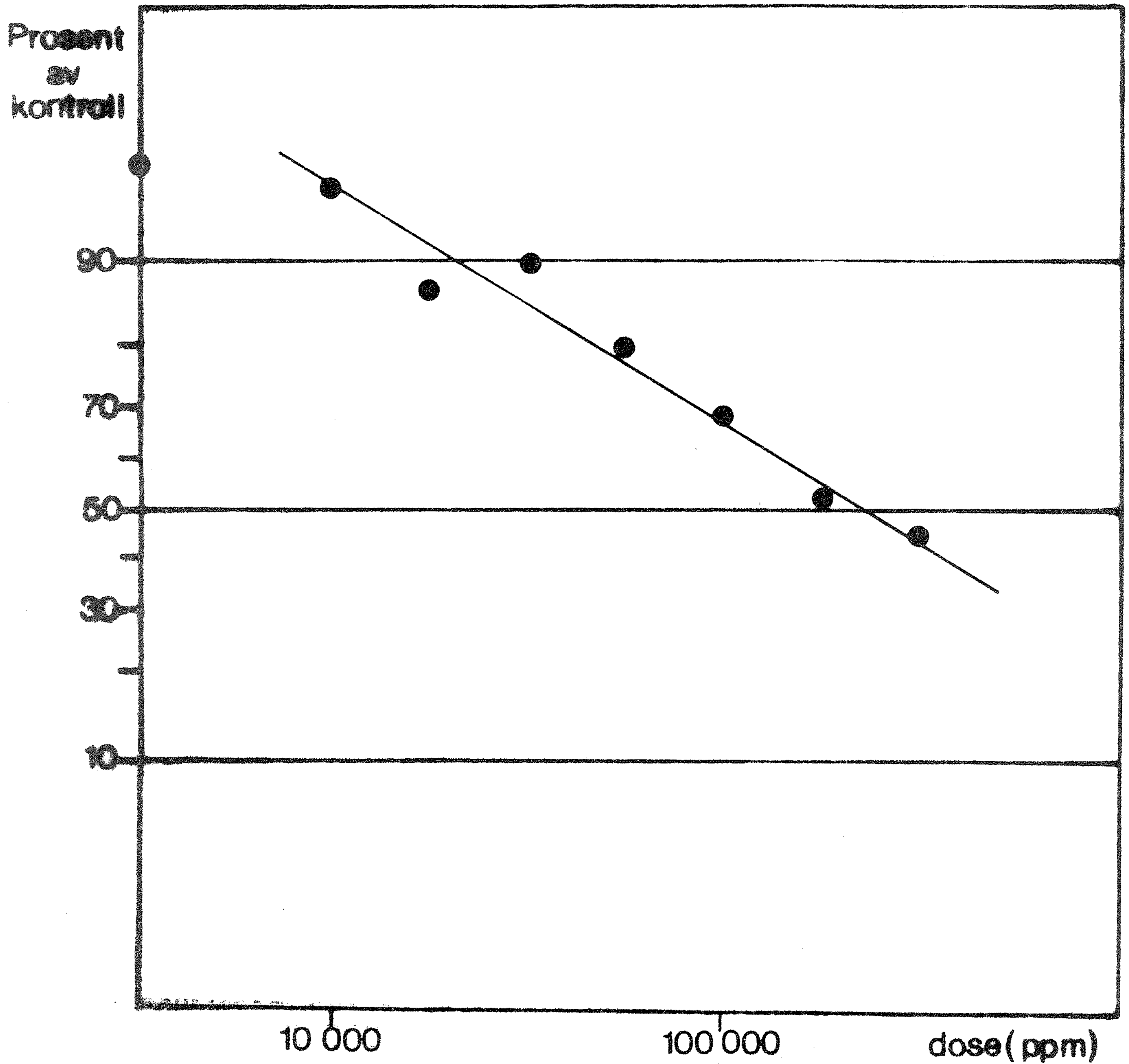
3h eksponering: ○ overlevelse, Δ **rel.** fotosyntesekap.

3d eksponering: ● veksthastighet, ▲ **rel.** fotosyntesekap.

Fig.2

MOBIL-NIVA

referanse 17/3



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Forskningsprogram om
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Munthesgt. 29,

O S L O 2.

DERES REF.	DERES BREV AV	VÅR REF.	DATO
		KØ:aa	10.nov. 1983.

TEST: Toxic effects on the marine planktonic alga Skeletonema costatum.

SAMPLE: NIVA "water based". 9 months.

METHOD: Cf separate description.

RESULTS: see table below and Fig.1. A summary is:

Incubation	Effect	EC-50	EC-10 - EC-90
3h	Survival	>316 000	(>316 000)
	Rel. photosynth.	>316 000	(>316 000 -)
3d	Growth rate	230 000	50 000 -
	Rel. photosynth.	>316 000	(~100 000 -)

EC-X gives dose (in ppm suspension before filtering) giving X% reduction in the measured parameter.

When 50% effect could not be measured within the concentration interval applied (maximal concentration 316 000 ppm), this was given as EC-50>. In this case an EC-10 value is suggested in parenthesis.

The most sensitive parameter was growth rate. Fig. 2 shows log-normal diagram for this parameter. EC-50 = 230 000 ppm and the interval from EC-10 to EC-90 is taken from the diagram. Basis for treating the other responses in the same way was not present.

The reproducibility was good.

After 3d the control media had pH 8.8-9.0. At the highest concentration of the test media pH went down to 7.9.

COMMENTS: The response pattern was nearly identical to that of the reference sediment, and so were the EC values presented. Thus, there were no signs of a toxic effect of the water based cuttings after 9 months. Initially the water based cuttings were clearly more toxic than the reference.

TEST: Toksiske effekter på marin planktonalge Skeletonema costatum.

PRØVE: MOBIL-NIVA vannbasert 17/3.

RESULTATER:

Verdier gitt relativ kontroll:

Ref.	Dose* [ppm]	3h eksponering		3d eksponering	
		Overlevelse	Rel.fotosynt.	Veksthastigh.	Rel.fotosyn
313	1 000		0.97	0.99	0.98
	3 160		0.97	1.03	1.00
	10 000		0.97	1.02	0.97
	31 600		0.92	1.00	0.93
	100 000	1.01	0.92	0.68	0.95
	316 000	1.00	0.87	0.36	0.95
316	5 620		1.00	-vraket-	-vraket-
	17 800		0.99	0.96	1.00
	56 200		0.97	0.89	1.03
	178 000	1.01	0.91	0.76	1.03

* Total suspensjon før filtrering. Bare filtrat er benyttet ved ekspone

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TELEX

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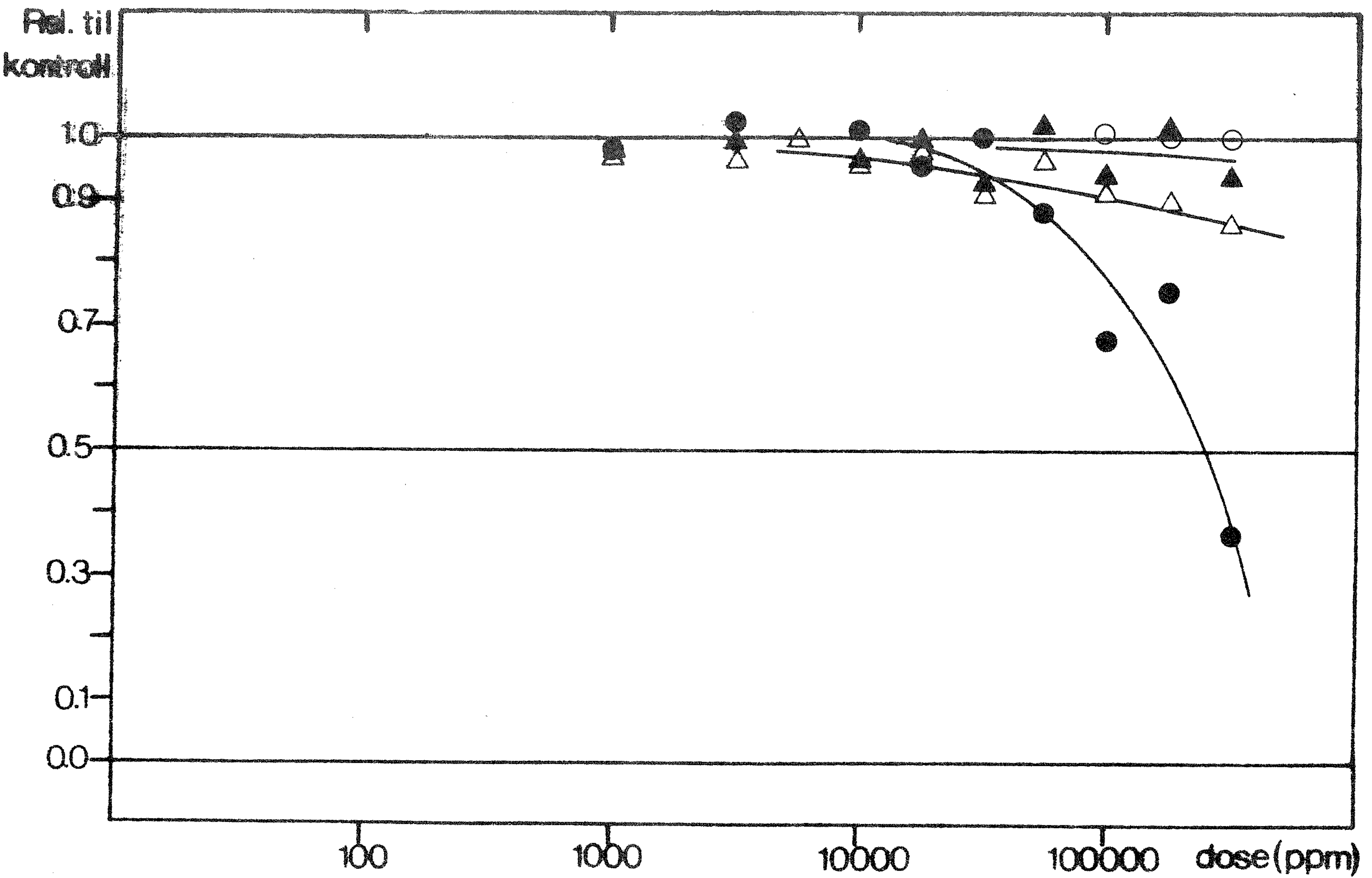


Symboler:

3h eksponering: ○ overlevelse, △ rel. fotosyntesekap.

3d eksponering: ● veksthastighet, ▲ rel. fotosyntesekap.

Fig.1

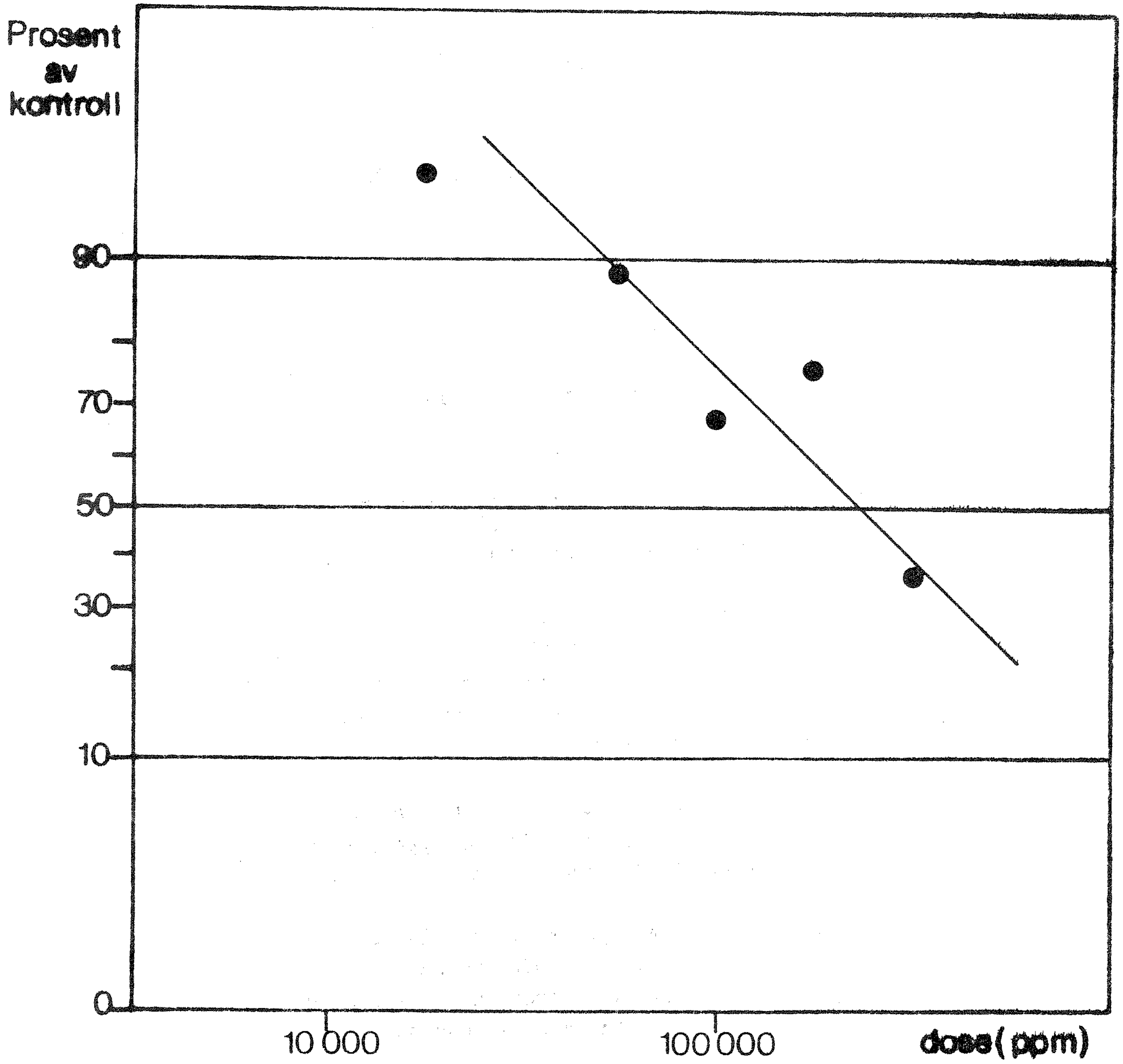
MOBIL-NIVA

vannbasert 17/3

Fig.2

MOBIL-NIVA

vannbasert 17/3



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Munthesgt. 29,

O S L O 2.

DERES REF.	DERES BREV AV	VÅR REF.	DATO
		KØ:aa	9. nov.1983.

TEST: Toxic effects on the marine planktonic alga Skeletonema costatum.

SAMPLE: NIVA "low aromatic". 9 months.

METHOD: Cf separate description.

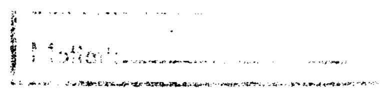
RESULTS: see table below and Fig.1. A summary is:

Incubation	Effect	EC-50	EC-10 - EC-90
3h	Survival	>316 000	(>316 000)
	Rel. photosynth.	>316 000	(~170 000 -)
3d	Growth rate	22 000	10 000 - 42 000
	Rel. photosynth.	>316 000	(~ 75 000 -)

EC-X gives dose (in ppm suspension before filtering) giving X% reduction in the measured parameter.

When 50% effect could not be measured within the concentration interval applied (maximal concentration 316 000 ppm), this was given as EC-50>. In this case an EC-10 value is suggested in parenthesis.

The most sensitive parameter was growth rate. Fig. 2 shows log-normal diagram for this parameter. EC-50 = 22 000 ppm and the interval from EC-10 to EC-90 is taken from the diagram. Basis for treating the other responses in the same way was not present.



The reproducibility was acceptable.

After 3d the control media had pH 8.9. At the highest concentration of the test media pH went down to 7.4.

COMMENTS: The dose-response curve for growth rate was very steep in the range 10 000 to 50 000 ppm. The low aromatic sample was clearly more toxic than the reference at 9 months, and less toxic than diesel. The response pattern was different from initial, and the substrate seemed clearly less toxic after 9 months.

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TEST: Toksiske effekter på marin planktonalge Skeletonema costatum.

PRØVE: MOBIL-NIVA lavaromatisk 17/3.

RESULTATER:

Verdier gitt relativ kontroll:

		3h eksponering		3d eksponering	
Ref.	Dose* [ppm]	Overlevelse	Rel.fotosynt.	Veksthastigh.	Rel.fotosynt
311	1 000		0.97	1.02	0.97
	3 160		0.93	1.00	0.93
	10 000		0.92	0.88	1.05
	31 600		0.94	0.45	1.02
	100 000	0.97	0.96	<0	1.09
314	1 780		1.02	1.03	0.99
	5 620		0.96	1.03	1.02
	17 800		0.98	0.21	0.96
	56 200		0.92	<0	0.88
	178 000	0.99	0.89	<0	0.69
318	10 000		1.03	0.91	0.99
	31 600		1.01	0.47	1.05
	316 000	0.94	0.87	<0	0.62

* Total suspensjon før filtrering. Bare filtrat er benyttet ved eksponeri

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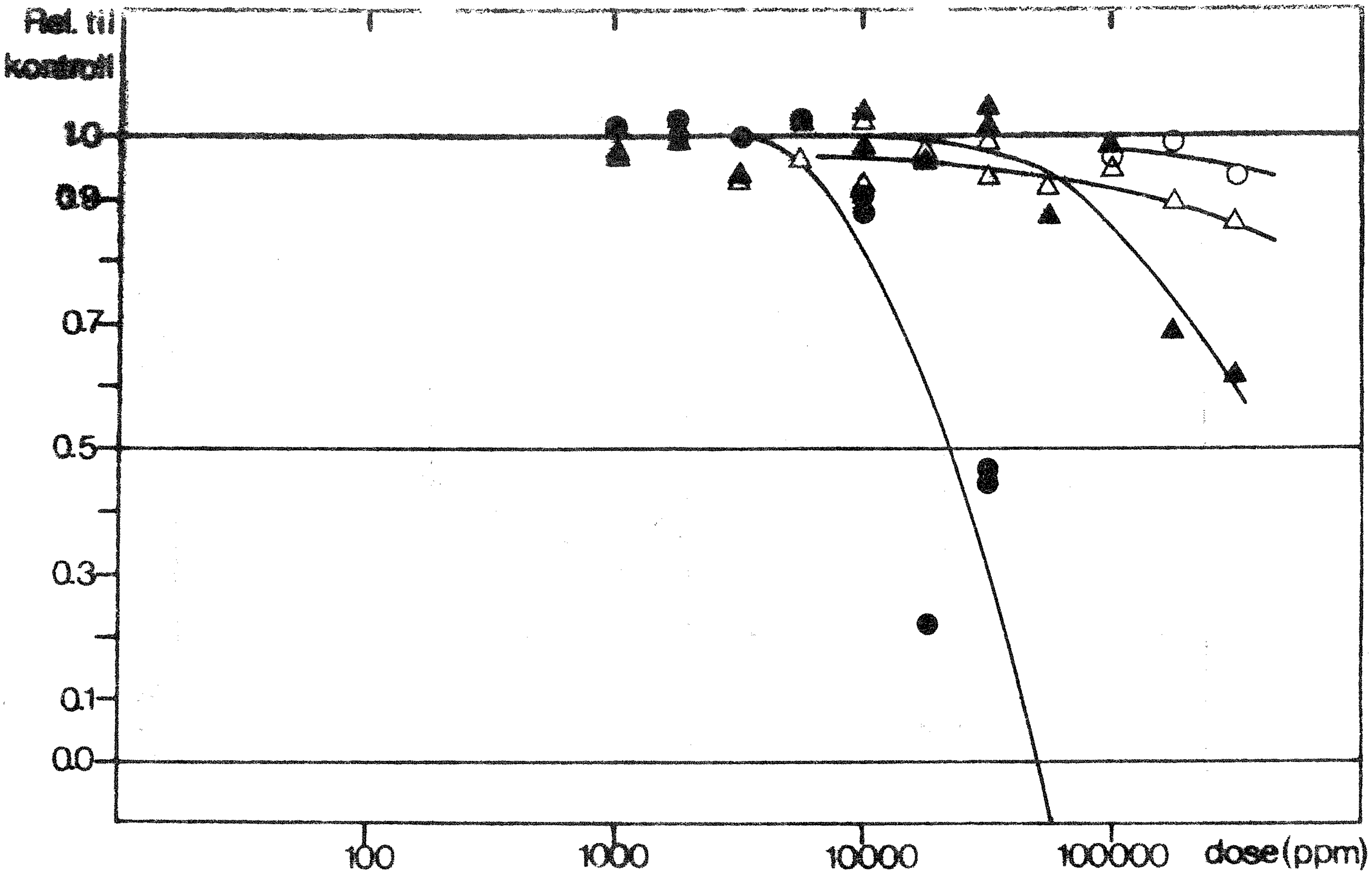
Fig. 1

MOBIL-NIVA

lavaromatisk 17/3



Symboler:

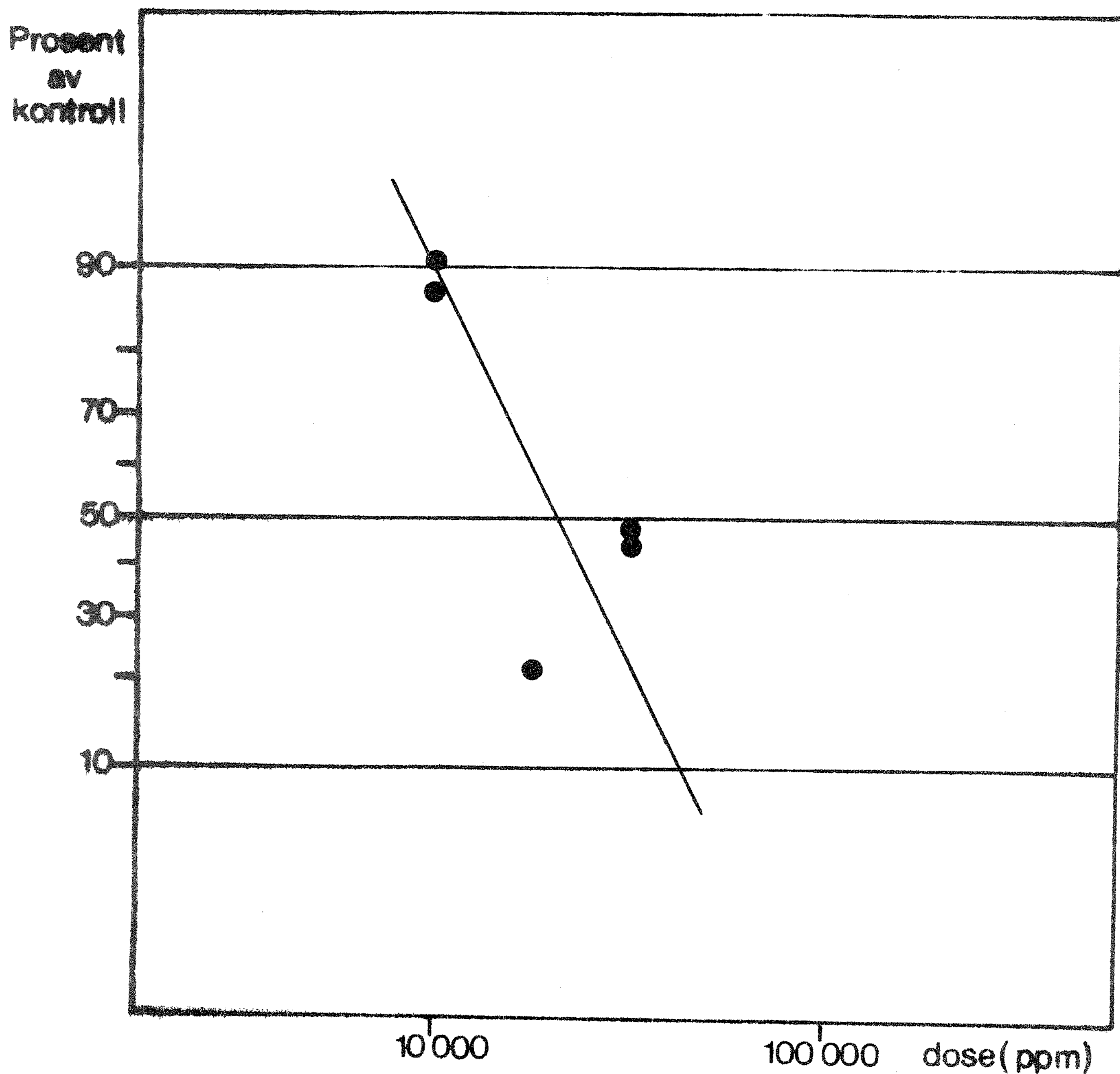
3h eksponering: ○ overlevelse, △ rel. fotosyntesekap.

3d eksponering: ● veksthastighet, ▲ rel. fotosyntesekap.

Fig.2

MOBIL-NIVA

lavaromatisk 17/3



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Munthesgt. 29,

O S L O 2.

DERES REF.	DERES BREV AV	VÅR REF.	DATO
		KØ:aa	9.nov. 1983

TEST: Toxic effects on the marine planktonic alga *Skeletonema costatum*.

SAMPLE: NIVA "briquettes". 9 months.

METHOD: Cf separate description.

RESULTS: see table below and Fig.1. A summary is:

Incubation	Effect	EC-50	EC-10 - EC-90
3h	Survival	>316 000	(>316 000)
	Rel. photosynth.	>316 000	(~ 40 000 -)
3d	Growth rate	20 000	9 000 - 30 000
	Rel. photosynth.	100 000	40 000 - 200 000

EC-X gives dose (in ppm suspension before filtering) giving X% reduction in the measured parameter.

When 50% effect could not be measured within the concentration interval applied (maximal concentration 316 000 ppm), this was given as EC-50>. In this case an EC-10 value is suggested in parenthesis.

The most sensitive parameter was growth rate. Fig. 2 shows log-normal diagram for this parameter. EC-50 = 20 000 ppm and the interval from EC-10 to EC-90 is taken from the diagram.

The reproducibility was acceptable.

After 3d the control media had pH 8.8. At the highest concentration of the test media pH went down to 7.7.

COMMENTS: The total response pattern was very much like that of the "low aromatic", but the dose-response curve for growth rate was even steeper. One should also note that negative net growth was observed and used at plotting (not included in the figure). The EC-50 value for growth rate was not different from the corresponding "low aromatic" value, but at higher concentrations the effect of "briquettes" on photosyntesis seemed somewhat stronger than of "low aromatic". The 9 months toxicity was reduced considerably compared to initial, but no correction for difference in water content has been made (the initial briquettes were dry, the 9 months sample was like a slurry).

TEST: Toksiske effekter på marin planktonalge *Skeletonema costatum*.

PRØVE: MOBIL-NIVA pellets 17/3.

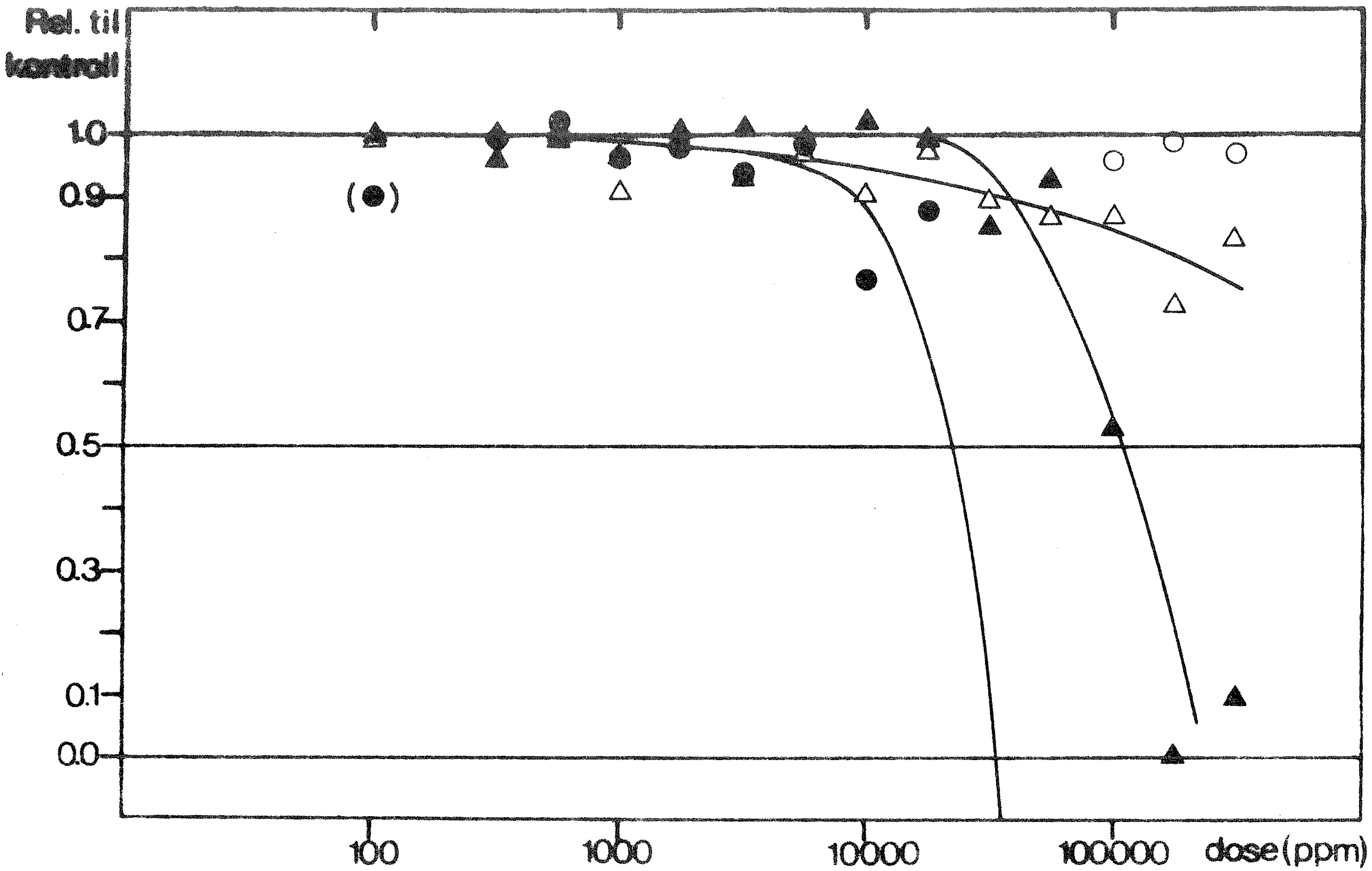
RESULTATER:

Verdier gitt relativ kontroll:

Ref.	Dose* [ppm]	3h eksponering		3d eksponering	
		Overlevelse	Rel.fotosynt.	Veksthastigh.	Rel.fotosynt
302	100		1.00	0.90	1.00
	316		1.00	1.00	0.96
	1 000		0.91	0.96	0.98
	3 160		0.94	0.94	1.02
	10 000		0.91	0.76	1.03
	31 600		0.90	<0	0.86
	100 000	0.96	0.88	<0	0.53
	316 000	0.98	0.84	<0	0.12
304	562		1.01	1.02	1.00
	1 780		1.00	0.99	1.02
	5 620		0.98	0.99	1.01
	17 800		0.99	0.88	1.00
	56 200		0.88	<0	0.94
	178 000	0.99	0.73	<0	0.0

* Total suspensjon før filtrering. Bare filtrat er benyttet ved eksponer

Fig.1
MOBIL-NIVA
pellets 17/3



Symboler:
3h eksponering: ○ overlevelse, △ rel. fotosyntesekap.
3d eksponering: ● veksthastighet, ▲ rel. fotosyntesekap.

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O S L O 2.

DERES REF.	DERES BREV AV	VÅR REF.	DATO
		KØ:aa	9.nov.1983.

TEST: Toxic effects on the marine planktonic alga *Skeletonema costatum*.

SAMPLE: NIVA "diesel oil". 9 months.

METHOD: Cf separate description.

RESULTS: see table below and Fig.1. A summary is:

Incubation	Effect	EC-50	EC-10 - EC-90
3h	Survival	>316 000	(>316 000)
	Rel. photosynth.	~300 000	~ 20 000 -
3d	Growth rate	6 000	600 - 14 000
	Rel. photosynth.	~30 000	~6 000 -

EC-X gives dose (in ppm suspension before filtering) giving X% reduction in the measured parameter.

When 50% effect could not be measured within the concentration interval applied (maximal concentration 316 000 ppm), this was given as EC-50>. In this case an EC-10 value is suggested in parenthesis.

The most sensitive parameter was growth rate. Fig. 2 shows log-normal diagram for this parameter. EC-50 = 20 000 ppm and the interval from EC-10 to EC-90 is taken from the diagram. Basis for treating the other responses in the same way was not present.

TEST: Toksiske effekter på marin planktonalge *Skeletonema costatum*.

PRØVE: MOBIL-NIVA dieselbasert 17/3

RESULTATER:

Verdier gitt relativ kontroll:

		3h eksponering		3d eksponering	
Ref.	Dose* [ppm]	Overlevelse	Rel.fotosynt.	Veksthastigh.	Rel.fotosynt
299	316		0.98	0.91	1.04
	1 000		0.96	0.65	0.91
	3 160		0.94	0.29	0.85
	10 000		0.81	0.02	0.67
	31 600		0.66	<0	0.35
	100 000		0.51	<0	0.01
	316 000	1.00	0.58	<0	0.10
303	178		1.02	0.88	0.97
	562		0.92	0.79	0.97
	1 780		0.98	0.74	0.97
	5 620		0.91	0.79	0.83
	17 800		0.88	<0	0.97
	56 200	1.00	0.79	<0	0.49
	178 000	0.91	0.40	<0	0.25
316	562		1.00	1.00	0.98
	1 780		1.00	0.97	1.01
	5 620		0.97	0.77	1.02
	17 800	0.98	0.97	<0	0.73

forts. neste side.....

* Total suspensjon før filtrering. Bare filtrat er benyttet ved eksponering

UNIVERSITETET I TRONDHEIM
INSTITUTT FOR MARIN BIOKJEMI

TEST: Toksiske effekter på marin planktonalge *Skeletonema costatum*.

PRØVE: MOBIL-NIVA dieselbasert 17/3

RESULTATER:

Verdier gitt relativ kontroll:

forts. fra forrige side

		3h eksponering		3d eksponering	
Ref.	Dose* [ppm]	Overlevelse	Rel.fotosynt.	Veksthastigh.	Rel.fotosynt.
319	100		1.00	0.98	0.92
	316		1.02	0.90	0.92
	1 000		1.03	0.81	1.00
	3 160		1.02	0.78	1.00
	10 000		0.98	0.31	0.88
	31 600	0.94	0.99	<0	0.92
	100 000	0.93	0.94	<0	0.55

* Total suspensjon før filtrering. Bare filtrat er benyttet ved ekspone...

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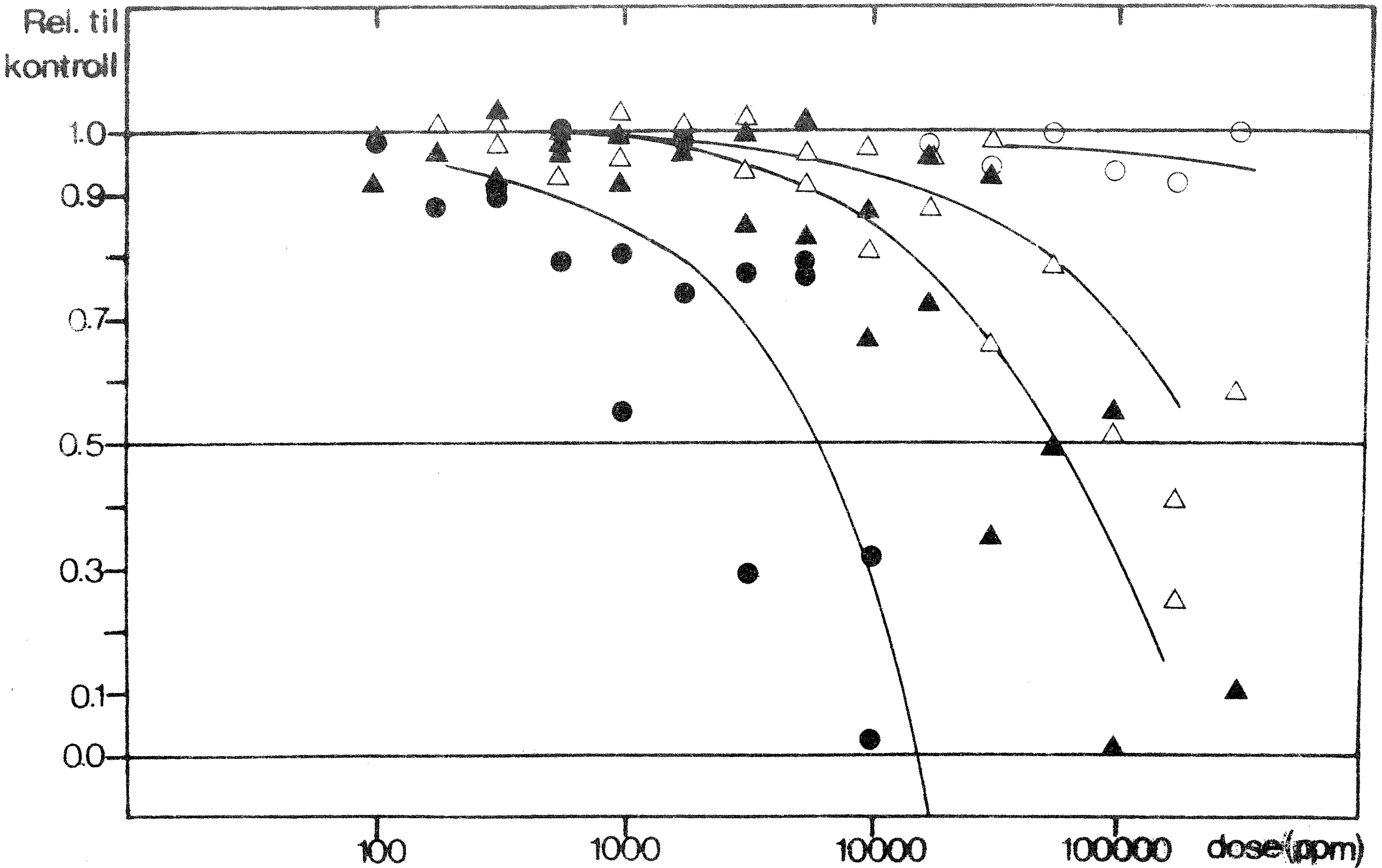
Fig.1

MOBIL-NIVA

dieselbasert 17/3



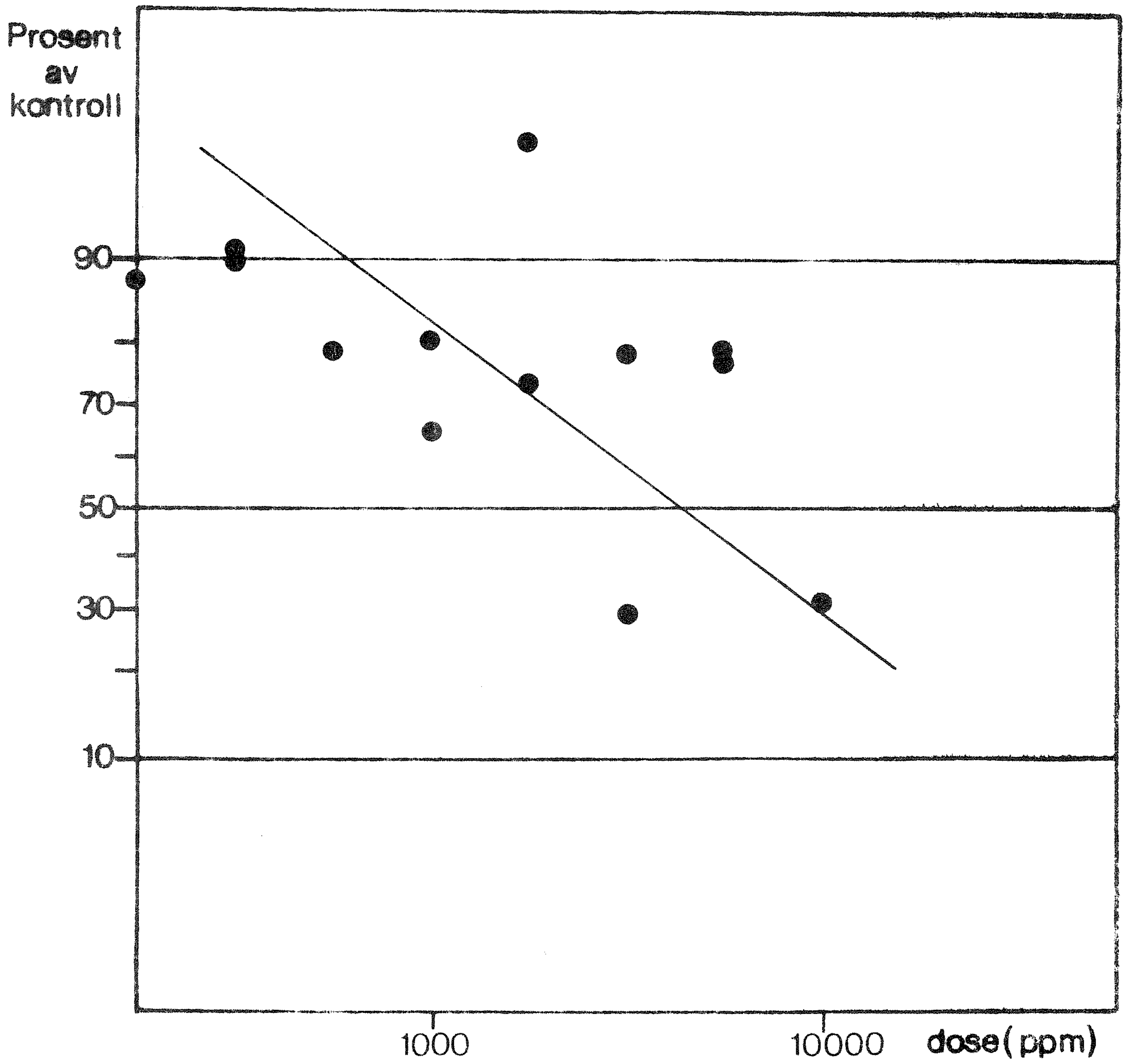
Symboler:

3h eksponering: ○ overlevelse, △ rel. fotosyntesekap.

3d eksponering: ● veksthastighet, ▲ rel. fotosyntesekap.

Fig.2

MOBIL-NIVA
dieselbasert 17/3



ANNEX 5

MICROBIOLOGICAL DEGRADABILITY.

ACUTE INHIBITION OF AEROBIC DEGRADATION

PROJECT 82003

BIOLOGICAL DEGRADATION TEST
ON CUTTINGS FROM DRILLING MUDS BASED ON
DIESEL OIL AND ON MINERAL OIL,
AND WATER BASED MUD

A modified OECD MITI-test, with manometric measurement of the oxygen uptake, was used in this test. Our modification is the use of <u>seawater growth medium</u>, and the <u>inoculum.</u> Artificial seawater as proposed by ISO (International Standardisation Organisation) was used instead of distilled water in the growth medium. The test substance was the only organic component added. The inoculum was a mixture of the Norwegian Standard BOD-seed (microorganisms produced in raw sewage which is left at room temperature, in an open beaker, for 3 days), and microorganisms produced in artificial sewage/seawater medium aerated for 48 h, in volumes 3:1. The inoculum was thus partly adapted to seawater environment, but not to the organic material in the cuttings.

When the inoculum contains large numbers of bacteria of one or different kinds capable of multiplying by feeding on the added organic material, the oxygen uptake will start immediately, and we are able to measure a significant uptake 1 to 2 days after start. If few bacteria capable of doing this are present in the test bottle, it will take time before they have consumed enough oxygen to produce a significant uptake reading on the manometers. This period with no measurable uptake is called the "lag" period. A lag period of more than 7 days in this test indicates that the organic material will not be readily degradable in an environment with low content of heterotrophic bacteria.

The material tested was cuttings from waterbased mud, WBM, from diesel oil mud, DOM, and mineral oil mud, MOM. Brickettes were not tested for biodegradability. The cuttings were not given any treatment before testing. The cuttings were analysed for total content of oil hydrocarbons (THC) and selected aromatic compounds (NPD) as part of the NIVA field experiment. The chemical analysis was performed on the cuttings after one day on the sea floor, whereas the tests on biodegradability were performed on fresh cuttings. In the following calculations it is assumed that that no THC disappeared from the cuttings during this one day period.

The concentration of cuttings and THC in the test sample is shown in Table 1. The theoretical oxygen demand,TOD, was also calculated, based on general ratios for mg oxygen per mg hydrocarbon for aromatic

and alifatic hydrocarbons.

Table 1. Concentrations of organic material in the test samples.

Test material	Concentration in the test sample,mg/L		
	Cuttings	Tot.hydr.carb. THC	Theor. oxygen demand, TOD
WBM	10 000	0.07	-
MOM	2 500	215	740
DOM	2 500	325	970

International working groups on biodegradability (OECD, ISO, EEC) are at present discussing several possible methods for evaluation of degradability. One of these methods is to regard a substance as readily biodegradable if 60% of the TOD for 100 mg/L of the substance is passed within 10 days from commencement of the degradation. Very easily degradable substances such as aniline will, however, be totally degraded within 2 days if tested in concentrations op to 100 mg/L.

At the start of the test, few bacteria which degrade the test substance are present in the test sample. We assume that each bacterium needs a certain amount of test substance, which is oxidized with a certain amount of oxygen, to multiply. As the number of bacteria increases, the rate of dissappearance of the test substance increases, and the rate of the oxygen uptake increases. Bacteria multiply by doubling, and the doubling rate will be constant as long as the incubation conditions are kept constant and the concentration of nutrients are not limiting for growth. Thus, the doubling rate of the oxygen uptake will also be constant.

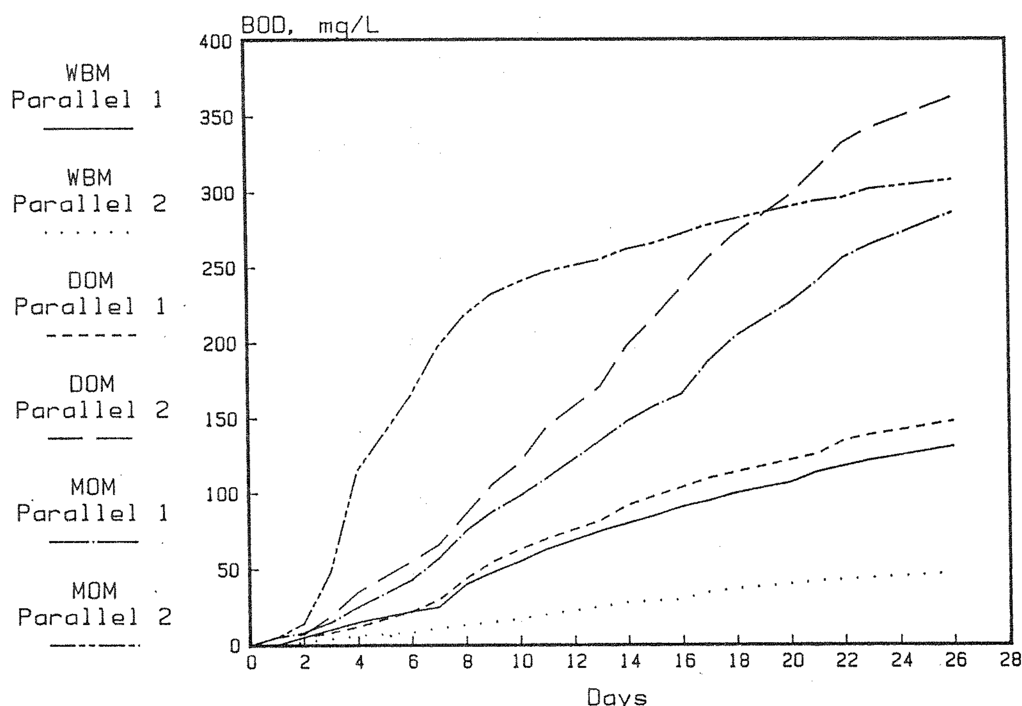
The doubling rate,R, of the oxygen uptake during degradation of anilin in this test is found to be 6 to 7 doublings,D, per day (24 h). To pass this test as "a readily biodegradable substance", a test substance with the same TOD as aniline would have to show a mean doubling rate of minimum 0.32 D/day during the first 10 days of degradation. The corresponding R_{min} for aromatic hydrocarbons would be approximately 0.35, and for alifatic hydrocarbons approx. 0.37.

The oxygen uptake curves for the tested cuttings are shown in Fig.1. Note that the oxygen uptake is presented in mg/L on the day of observation, not as oxygen uptake rates. Two test samples were used

for each type of cuttings. The degradation started without prolonged lag-periods, but all parallels showed large differences in oxygen uptake. Parallel 2 for MOM showed the typical shape of an easily degradable material, while the rest of the curves showed the typical shape of a curve where something is limiting the growth rate of the bacteria. For these test materials, the solubility of the oil could be the limiting factor. The solubility is dependent on the distribution of oil in the test sample. Even distribution of the oil is not easily obtained with this type of test material, and could be the reason for poor agreement between the parallels. However, the potential degradability will be at least as high as found for the parallel with the highest oxygen uptake rate.

From Fig.1 it can be seen that cuttings from water based mud also showed a significant oxygen uptake. These cuttings must have contained inorganic, oxygenconsuming material or organic non-hydrocarbon material (see Table 1, 0.07 mg/L THC equals approx. 0.2 mg/L TOD).

There is also a possibility that the <u>oil-based mud cuttings</u> contained other organic material than the oil. If these substances are degradable, they will also result in an oxygen uptake. If they are readily degradable, the combined oxygen uptake curve for these



materials and the oil may take the shape as shown for MOM, parallel 2. The correct TOD will then not be known and the correct 60% of TOD level cannot be calculated. If the degradability is evaluated on the basis of the 60% of TOD for the oil content, the oil could be falsely rated as readily degradable. There is, however, another criterion which should be met for a readily degradable substance: The doubling rate for the oxygen uptake should show stagnation shortly after the 60% level is passed. Typical stagnation values are 0.02 D/day without protozoa in the test sample, as high as 0.05 D/day with protozoa. An increase after a short time with stagnation may indicate that a nitrification process has started in the test sample. All these factors are taken into consideration for the evaluation of the examined cuttings.

The found degradation characteristics are shown in Table 2. All test samples contained some readily degradable material (or inorganic, oxygenconsuming material). This is demonstrated by the rate R_{0-2} being higher than the other rates. The highest uptake rate was was found for MOM parallel 2, which showed stagnation 12 days after start of the degradation (Fig. 1). The remaining oil-containing samples were still under degradation at this time, but at the end of the test the degradation had stagnated in all test samples.

Table 2. Characterization of the degradability of the organic material in the cuttings.

TEST MATERIAL Type mg/L	Significant BOD level on day	MEAN DOUBLING RATES,R, FOR THE OXYGEN UPTAKE, Doublings/day			
		R_{0-2}	R_{10-12}	R_{21-23}	R_{0-10}
DOM 2 500	1) 4	0.45	0.15	0.05	0.26
	2) 3	0.71	0.18	0.03	0.35
MOM 2 500	1) 3	0.45	0.12	0.04	0.30
	2) 2	1.43	0.02	0.02	0.40
WBM 10 000	1) 4	0.23	0.09	0.03	0.22
	2) 9	0.21	0.06	-	0.14

Table 3. Comparison between obtained results and calculated values based on the available data on the total content of hydrocarbons, THC, in the cuttings.

TEST MATERIAL				OBTAINED RESULTS		
					Oxygen uptake as per cent of TOD	
Type	THC mg/L	TOD mg/L	R_{min}. D/day	R_{0-10} D/days	after 14 days of degradation	at the end of the test
DOM	325	970	0.35	0.26	12	15
				0.35	26	37
MOM	215	740	0.37	0.30	25	38
				0.40	37	42

A comparison between some of these characteristical data and expected, theoretical values is shown in Table 3. Both DOM and MOM seem to contain readily biodegradable material, judged by the values for $R_{0\ 10}$. In this case, the test concentrations of hydrocarbons are 200 - 300 mg/L. The minimum oxygen uptake rates of 0.35 (diesel) and 0.37 (mineral) doublings per day during the first 10 days of degradation are, however, based on 100 mg organic material per litre. At this concentration, a readily degradable substance will the have passed the level for 60% of TOD.

At higher concentrations, the time needed to pass this level will be longer. For the tested cuttings, the theoretical minimum rates will give an expected time for reaching this level, of 13 days for MOM and 15 days for DOM. The BOD obtained after 14 days of degradation and at the end of the testing time, is presented in Table 3, expressed as percent of TOD. Neither at the 14th day nor at the end of the test did the oxygen uptake pass 60% of the calculated TOD.

The degradation curves are also demonstrated in figures 2 and 3, in log-2 transformation. For comparison, a similar curve for an easily degradable substance (aniline) is shown, Figure 4. In these figures the levels for both 60% and 20% of TOD are shown. The 20% level should be passed before the end of the test (28 days), otherwise the test sample will be rated as potentially persistent, and a test for "Inherent biodegradability" will be recommended (OECD). Both parallels of MOM passed this level, while one of the DOM parallels did not pass.

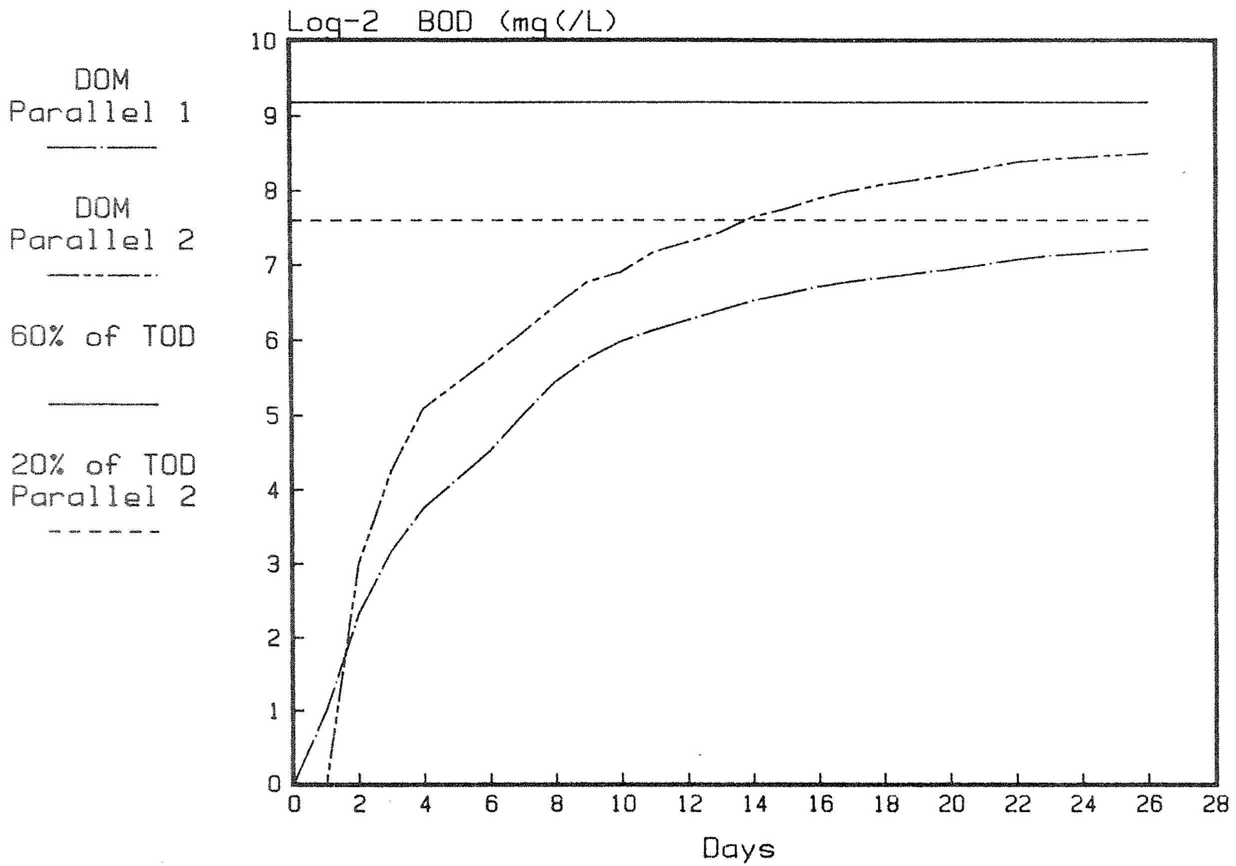


Figure 2. Diesel oil cuttings
Log-2 transformed curves
Log-2 BOD (mg(/L)
DOM
Parallel 1
DOM
Parallel 2
60% of TOD
20% of TOD
Parallel 2
Days
0
1
2
3
4
5
6
7
8
9
10
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28

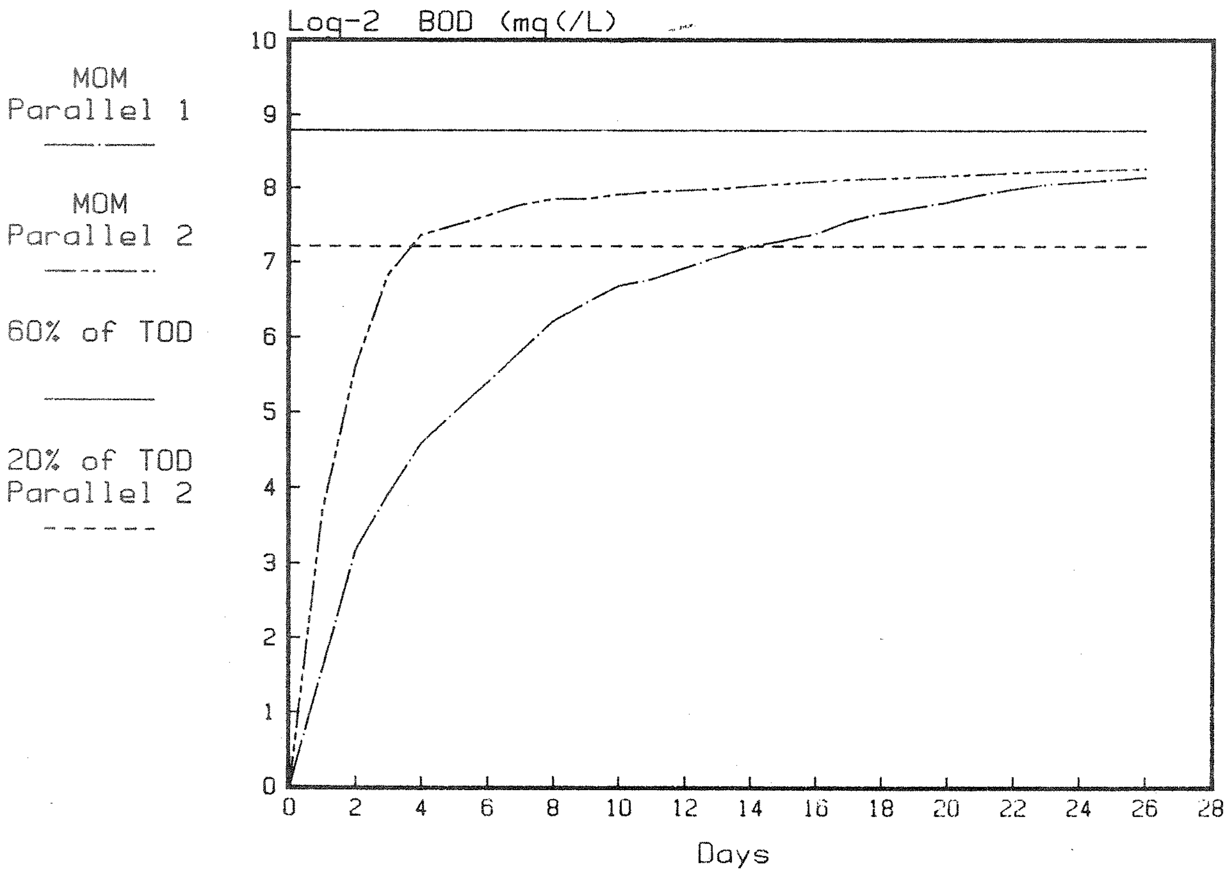
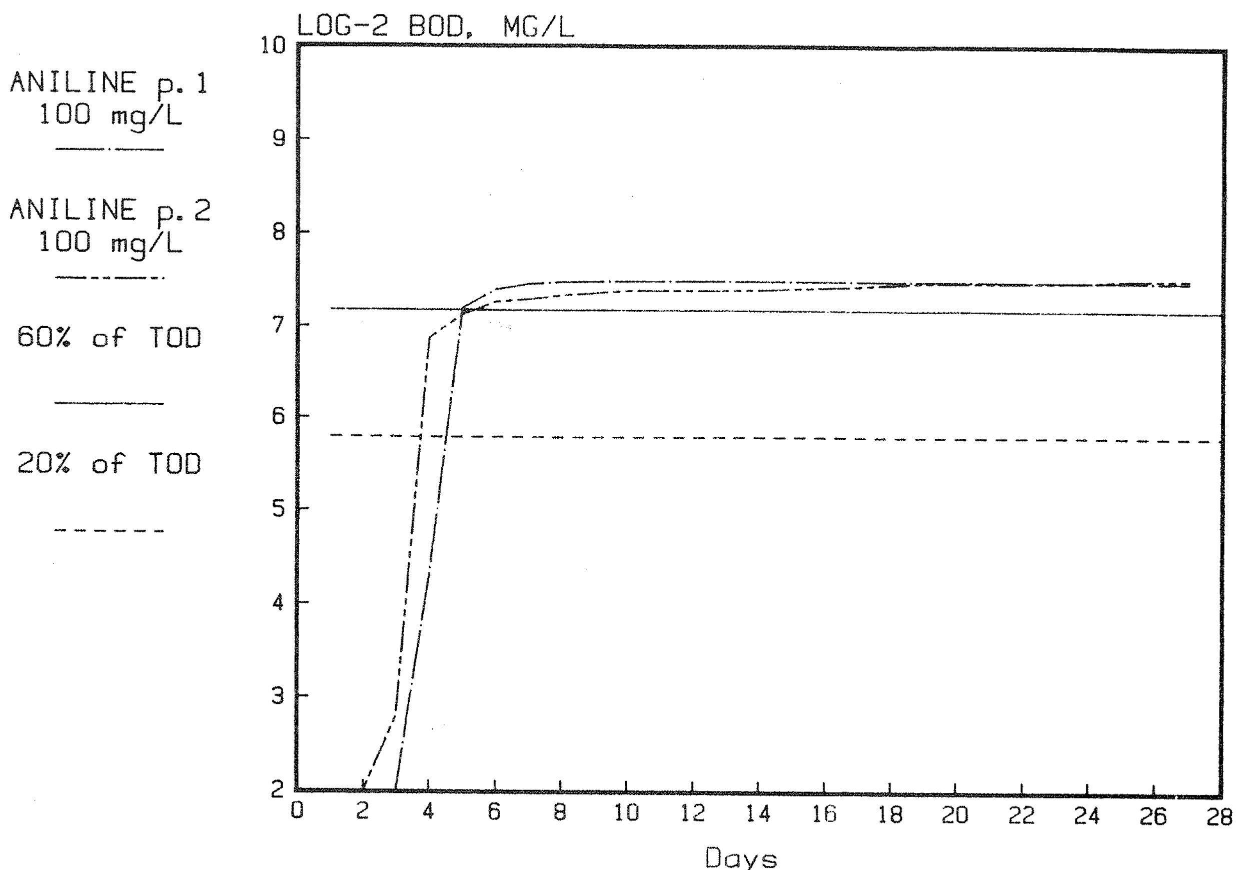


Figure 3. Mineral oil cuttings
Log-2 transformed curves
Log-2 BOD (mg(/L)
MOM
Parallel 1
MOM
Parallel 2
60% of TOD
20% of TOD
Parallel 2
Days
0
1
2
3
4
5
6
7
8
9
10
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28



Thus, the conclusion is that both types of cuttings contained fractions that were readily degradable, but that the main part of both were not readily degradable. The mineral oil cuttings seemed to contain more degradable material than the diesel oil cuttings. In nature, the conditions are usually not as favourable for degradation as in this test. Furthermore, the differences between parallel test samples indicate that these substances are very liable to degradation retarding factors.

In order to be able to evaluate the degradability of the base oil in drilling muds and cuttings, this respirometric test should be performed on the oil separately, not on the prepared mud or on cuttings, which may contain other oxygen-consuming material in addition to the base oil. This test on the oil would give important information on the potential biodegradability, because a low degradability in this test would not be expected to be bettered in nature.

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OECD: OECD Guidelines for Testing of Chemicals. OECD Publications Office, Paris, 1981. Modified MITI Test (I), 301 C.

ISO: 1. ISO/DIS 7827: Draft International Standard - Water Quality - Evaluation in an aqueous medium of the "ultimate" aerobic biodegradability of organic compounds - Method by analysis of dissolved organic carbon (DOC). International Organization for Standardization, 1983.

2. ISO/TC 147/SC 5/WG 3 N 51. Acute Toxicity Testing in Sea Water - UK proposal to WG 3 - Fish Toxicity, 1980. (Artificial seawater)

Norwegian Standard:
NS4749 Biochemical oxygen demand, BOD. Dilution method, 1979.
In Norwegian language.
In English: Swedish Standard SS 02 81 43 E (1978).

Norwegian Institute for Water Research

ORM/EFR/IJG
8.3.1983

TOXICITY TESTING WITH DRILLING FLUIDS AND CUTTINGS

TEST: ACUTE INHIBITION OF AEROBIC DEGRADATION

1. INTRODUCTION

The original plan was to use the acute oxygen uptake inhititibion test described by ISO, Method A (1, 2) measuring oxygen uptake by an oxygen electrode. This was, however, not possible because the oil component in drilling mud and cuttings blocked the electrode membrane. Another acute test, testing for inhibition of the enzyme dehydrogenase (3, 4) was tried. Some changes in the original procedure was found necessary, but the method seemed to work well with these test materials. The original method is described in literature 3 (in Norwegian language), based on the publication in lit. 4, and is also under preparation as a Danish Standard (5). The principle of the method is shortly described in this report, together with the changes found necessary for the test materials in question.

2. PRINCIPLE

Dehydrogenase is produced by bacteria during degradation of organic material. An active biomass of bacteria growing on a suitable organic substance ("synthetic sewage") will always contain dehydrogenase. This enzyme splits off hydrogen from organic substances. The hydrogen is transported through the respiration chain to the final product H_2O (water). In the absence of molecular oxygen, hydrogen may be transferred to other oxidized substances, which will then be reduced. 2, 3, 5-triphenyl tetrazolium-chloride (TTC) is one such substance, which is reduced to red-coloured, water-insoluble formazan. This substance is, however, soluble in ethyl alcohol. Thus, after spinning down the formazan, discarding the supernetant and dissolving the formazan in ethyl alcohol, the concentration of formazan produced may be measured in a spectrophotometer at 483 nm.

In this toxicity test, nitrogen is bubbled through the test sample to remove oxygen, and the production time for formazan is set to 3 h at 25 ± 1°C. A satisfactory sludge should contain enough dehydrogenase to obtain an absorbance of 0,8 at 483 nm. Substances which have inhibitive effect on the dehydrogenase would decrease or completely block the production of formazan. Test materials which contain degradable organic substances may cause the bacteria in the sludge to produce more dehydrogenase than in the control without test material, and thus cause the dehydrogenase activity to be higher than in the control. For demonstration of both effects, the test results are presented as percent activity instead of percent inhibition in the dose response diagram. A test material showing more than 100 % activity in this diagram will contain easily degradable organic material in addition to the "synthetic sewage". A test sample yielding only half the absorbance found for the control, will have an activity of 50 %, and the concentration of test material in this test sample is denoted as EC 50; effect concentration for 50 % inhibition. A concentration resulting in 80 % activity will be denoted EC 20; concentration at 20 % inhibition.

The original procedure is demonstrated in the included figures nos. 3, 4 and 5, from literature 3.

3. CHANGES FROM ORIGINAL PROCEDURE

3.1 Test environment

The original method was based on fresh water environment. The test material will be discharged to a marine environment, thus artificial seawater is used instead of distilled water in the production of bacterial sludge as well as in the test samples.

Artificial seawater:

P-fortified artificial seawater, 30 $^{0}/oo$

	m mol/l		mg/l
Na^{+}	401	Na_2SO_4	341
SO_4^{++}	24,0	NaCl	2064
Cl^{-}	471	KCl	64
K^{+}	8,57	$MgCl_2 \cdot 6H_2O$	941
Mg^{++}	43,3	$CaCl_2 \cdot 2H_2O$	126
Ca^{++}	8,57		
		$NaHCO_3$	170
P	0,5	K_2HPO_4	142 mg
		pH	7,6

This artificial seawater is almost the same as that recommended for use in Fish toxicity tests (6). Exceptions: 1) Na_4 EDTA is omitted, because chelators should, if possible, not be included in toxicity test reagents.

Phosphate (K_2HPO_4) is added to raise pH in the artificial seawater, and for buffering the solutions exposed to different amount of test substances.

3.2 Test portion

For this test program it was decided to express the concentration of test material in the test samples in ml/litre.

It is, however, not possible to measure the cuttings by volume. Thus, all test portions are directly weighed (wet weight) into the test tubes. By estimation of the ratio volume/wet weight of a large volume of test material, the concentrations in ml/litre can be calculated.

3.3 Solubility of test material in the test sample

In a preliminary test, it was discovered that no toxic effect could be detected if the test was carried out directly after mixing the test samples. Shaking for 16 hours (over night) was found optimal for reaching equilibrium between test material and test medium. Thus, this was decided to be included as standard procedure. The bacterial sludge and TTC-solution was added after these 16 hours of shaking.

3.4 Changes in test reagents and procedure

3.4.1 Laboratory produced activated sludge

The production of activated sludge is performed as described for the INSTA method (2), by aeration of 10 x conc. OECD synthetic sewage seeded with normal BOD-seed (7), for 2 days at room temperature. Instead of distilled water, artificial seawater is used to dissolve the medium ingredients. This sludge is concentrated by centrifugation and resuspension of the sludge, to approximately 1/4 of original volume in normal strength synthetic sewage in seawater. This concentrated sludge suspension will normally contain 1,5 - 2,5 g/l of suspended solids. A suitable volume of sludge is added to each test sample. This volume should yield an absorbance of at least 0,7, preferably around 0,8, at 483 nm, in the control sample.

Normally, this corresponds to about 0,5 g/l of suspended solids in the test sample.

3.4.2 TTC-solution

Triphenyltetrazoliumchloride is dissolved in artificial seawater instead of in trisbuffer or phosphatebuffer (original method).

3.4.3 Test medium

Normal strength synthetic sewage (1) in artificial seawater (3.1) is used instead of tris - or phosphate-buffer (original method).

3.4.4 Incubation time

One hour incubation (original method) was found not sufficient. Three hours was chosen arbitrarily, and resulted in higher colour intensity. The change to seawater medium could be an explanation of the slower formazan production.

3.4.5 Dissolving the formazan in ethanol

The formazan particles tended to bind strongly to the sludge after centrifugation, The application of a tissue grinder helped to dissolve the formazan in ethanol.

3.5 Control substance

In the ISO-test (1), the use of a standard toxic substance to control the reaction of the sludge is recommended. The substance 3,5-dichlorophenol has been used for this purpose in different microbial toxicity tests. We decided also to use this substance for control in the dehydrogenase test. There is, however, not yet sufficient data available to state which concentration rage would be "normal" for the EC 50 value, but so far the EC 50 values have been located between 10-60 mg/l.

4. TESTING STRATEGY

Each material is tested three times with different batches of sludge, as in the method proposed by the INSTA working group (2).

The results are presented as mean and range found for EC 20, EC 50, EC 80, and other data are supplied according to the recommended "Test report" in the mentioned standard (2). Comments are used instead of EC-values when low or no toxic effects are found.

LITERATURE

1. ISO/TC 147/SC5/WG1 N 60
Water Quality: Activated Sludge Oxygen Consumption Inhibition Test, Method A (Sept. 1982).

2. INSTA C12/AG 21: 2. draft: Water Quality: Activated Sludge Oxygen Consumption Inhibition Test (March 1983).

3. Ormerod, K. NIVA, Toksisitetstest 2, 1. utgave, juli 1978: Hemming av dehydrogenaseaktivitet ved nedbrytning av organisk stoff.

4. Ryssov-Nielsen (1975). Measurement of the inhibition of respiration in activated sludge by a modified determination of the TTC-dehydrogenase activity. Water Research 9, 1179-1185 (1975).

5. DS 83/25 Vandundersøgelse. Hæmning av aktivert slams dehydrogenaseaktivitet målt ved TTC-metoden. Utkast til kritikk innen 15. april 1983.

6. ISO/TC 147/SC 5/WG 3 N 51.
Acute Toxicity Testing in Seawater - UK-proposal to WG3 - Fish Toxicity, 1980.

7. NS 4749 Vandundersøkelser. Biokjemisk oksygenforbruk, BOD. Fortynningsmetode. 1. utg. juni 1979.

Norwegian Institute for Water Research

NIVA

Analysis division
NIVA Toxicity test 2, modified as described in separate report, ORM/EFR/IJG/8.3.1983

TEST REPORT

Date: 1983-05-11

1. *Name/ identification of test material:*

   Water Based Mud

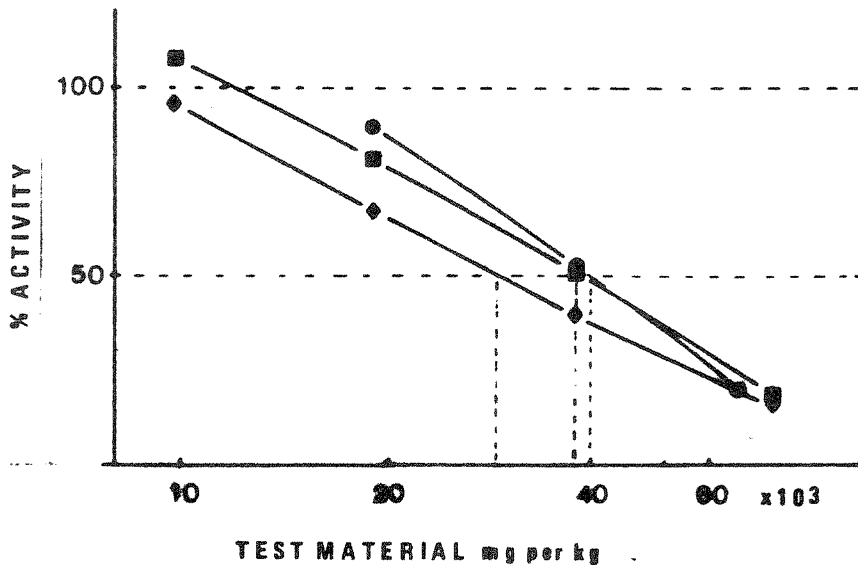
2. *Solubility of test material in the test medium:*

   Soluble [x] *Slightly soluble* [ ] *Low solubility* [ ]

3. *Test results:*

Test no.	*Found effect concentrations,* ppm			*Comments:*
	EC 20	*EC 50*	*EC 80*	
1 ●	23 x10^3	40 x10^3	66 x10^3	
2 ◆	14 "	29 "	64 "	
3 ■	19 "	38 "	72 "	
Mean	19 x10^3	36 x10^3	67 x10^3	
Range	14-23 "	29-40 "	64-72 "	

4. *Dose/response curves:*



Identification: Water Based Mud.

5. *Definition of the concentration value:*

6. *Preparation of stock solution of test material:* The results are wanted expressed as ppm. This expression is calculated as µl/l or mg/kg, dependent on whether or not it is possible to measure the test substance by volume.
µl/l: The test portion is measured in ml and diluted to final volume.
mg/kg: The test portion is weighed directly into the centrifuge tubes, the test medium is added to standard volume, and each test sample is weighed in the tube.

7. *Source and pretreatment of activated sludge:*

8. *Data for characterization of the sludge:*

Test no.	*Sludge concentration in the test sample, mg/l (susp. solids)*	*Absorbance (463nm) at 100% activity*	*Found EC 50 value for 3,5-dichlorophenol, mg/l*
1 ●	613	1.320	
2 ◆	648	1.300	
3 ■	500	1.143	

9. *Deviations from the described method:*

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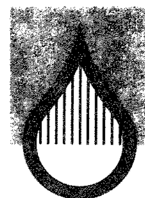


Analysis division
NIVA Toxicity test 2, modified as described in
separate report, ORM/EFR/IJG/8.3.1983

TEST REPORT

Date: 1983-05-11

1. *Name/ identification of test material:*

   Mineral oil Based cuttings.

2. *Solubility of test material in the test medium:*

   Soluble [ ] *Slightly soluble* [ ] *Low solubility* [X]

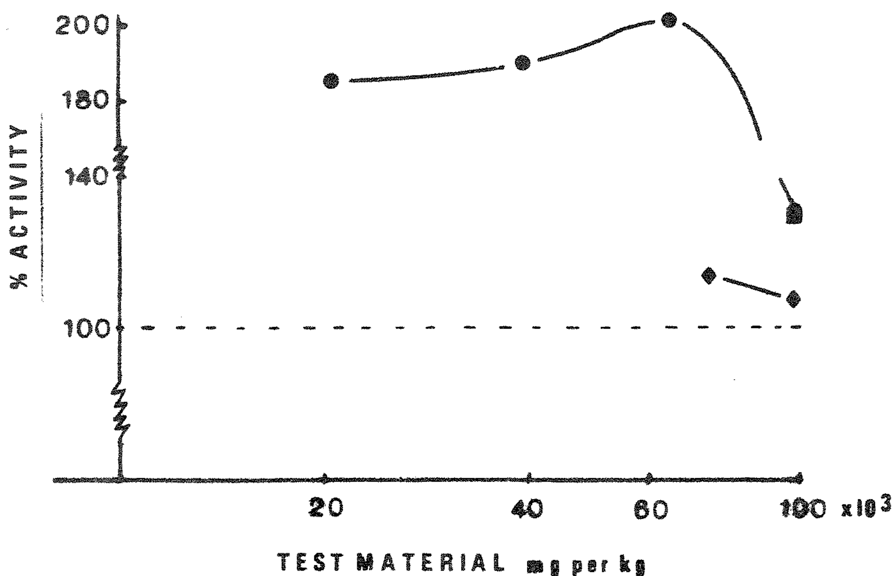
3. *Test results:*

Test no.	*Found effect concentrations,* ppm		
	EC 20	*EC 50*	*EC 80*
1 ●	>98 x10^3	>98 x10^3	x10^3
2 ◆	>98 "	>98 "	"
3 ■	>98 "	>98 "	"
Mean	x10^3	x10^3	x10^3
Range	"	"	"

Comments:

No inhibition was obtained within the tested range. (The enzymeactivity was stimulated by the test-substance).

4. *Dose/response curves:*



Identification: Mineral oil Based cuttings.

5. *Definition of the concentration value:*

6. *Preparation of stock solution of test material:* The results are wanted expressed as ppm. This expression is calculated as µl/l or mg/kg, dependent on whether or not it is possible to measure the test substance by volume.
µl/l: The test portion is measured in ml and diluted to final volume.
mg/kg: The test portion is weighed directly into the centrifuge tubes, the test medium is added to standard volume, and each test sample is weighed in the tube.

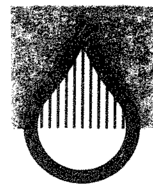
7. *Source and pretreatment of activated sludge:*

8. *Data for characterization of the sludge:*

Test no.	*Sludge concentration in the test sample, mg/l (susp. solids)*	*Absorbance (463nm) at 100% activity*	*Found EC 50 value for 3,5-dichlorophenol, mg/l*
1 ●	613	1.320	
2 ◆	648	1.300	
3 ■	500	1.143	

9. *Deviations from the described method:*

Norwegian Institute for Water Research



Analysis division
NIVA Toxicity test 2, modified as described in separate report, ORM/EFR/IJG/8.3.1983

TEST REPORT

Date: 1983-05-11

1. *Name/ identification of test material:*

   Briquettes.

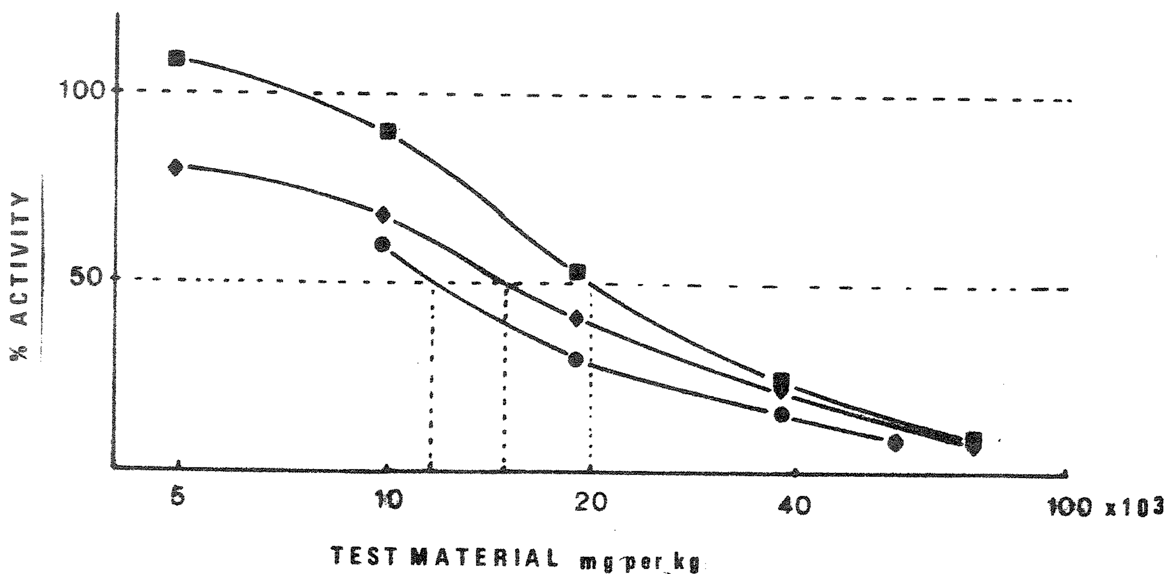
2. *Solubility of test material in the test medium:*

   Soluble ☐ *Slightly soluble* [X] *Low solubility* ☐

3. *Test results:*

Test no.	*Found effect concentrations,* ppm			*Comments:*
	EC 20	*EC 50*	*EC 80*	
1 ●	29.8 $x10^3$	11.5 $x10^3$	30 $x10^3$	
2 ◆	4.9 "	1 5 "	40 "	
3 ■	12 "	20 "	45 "	
Mean	<9 $x10^3$	16 $x10^3$	38 $x10^3$	
Range	"	11.5-20 "	30-45 "	

4. *Dose/response curves:*



5. *Definition of the concentration value:*

6. *Preparation of stock solution of test material:* The results are wanted expressed as ppm. This expression is calculated as µl/l or mg/kg, dependent on whether or not it is possible to measure the test substance by volume.

<u>µl/l</u>: The test portion is measured in ml and diluted to final volume.

<u>mg/kg</u>: The test portion is weighed directly into the centrifuge tubes, the test medium is added to standard volume, and each test sample is weighed in the tube.

7. *Source and pretreatment of activated sludge:*

8. *Data for characterization of the sludge:*

Test no.	*Sludge concentration in the test sample, mg/l (susp. solids)*	*Absorbance (483nm) at 100% activity*	*Found EC 50 value for 3,5-dichlorophenol, mg/l*
1 ●	613	1.320	
2 ◆	648	1.300	
3 ■	500	1.143	

9. *Deviations from the described method:*

Norwegian Institute for Water Research

NIVA

Analysis division

NIVA Toxicity test 2, modified as described in separate report, ORM/EFR/IJG/8.3.1983

TEST REPORT

Date: 1983-05-11

1. *Name/ identification of test material:*

   Diesel Based Mud.

2. *Solubility of test material in the test medium:*

   Soluble [ ] *Slightly soluble* [ ] *Low solubility* [X]

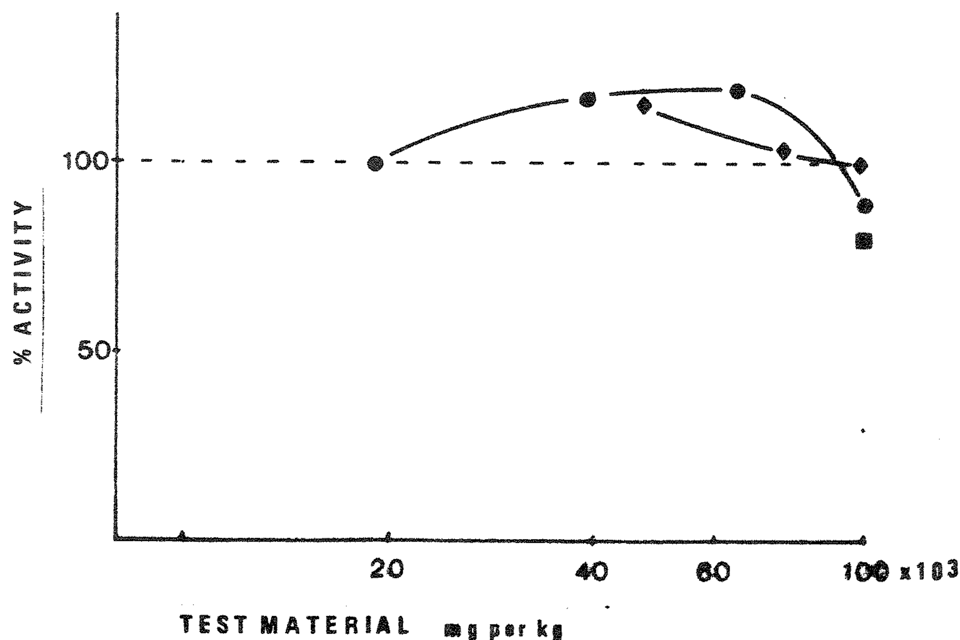
3. *Test results:*

Test no.	*Found effect concentrations, ppm*		
	EC 20	*EC 50*	*EC 80*
1 ●	>98 x10^3	>98 x10^3	x10^3
2 ◆	>98 "	>98 "	"
3 ■	98 "	>98 "	"
Mean	x10^3	x10^3	x10^3
Range	"	"	"

Comments:

A slightly inhibition (not significant) was obtained at 98 g per kg.

4. *Dose/response curves:*



5. *Definition of the concentration value:*

6. *Preparation of stock solution of test material:* The results are wanted expressed as ppm. This expression is calculated as µl/l or mg/kg, dependent on whether or not it is possible to measure the test substance by volume.
   µl/l: The test portion is measured in ml and diluted to final volume.
   mg/kg: The test portion is weighed directly into the centrifuge tubes, the test medium is added to standard volume, and each test sample is weighed in the tube.

7. *Source and pretreatment of activated sludge:*

8. *Data for characterization of the sludge:*

Test no.	*Sludge concentration in the test sample, mg/l (susp. solids)*	*Absorbance (483nm) at 100% activity*	*Found EC 50 value for 3,5-dichlorophenol, mg/l*
1 ●	613	1.320	
2 ◆	648	1.300	
3 ■	500	1.143	

9. *Deviations from the described method:*

ANNEX 6

EGGS AND EMBRYOS OF

COD *GADUS MORHUA* AND SEA URCHIN *STRONGYLOCENTROTUS DROEBACHIENSIS*

TOXICITY TESTS OF DRILL CUTTINGS/MUDS ON COD EGGS, COD LARVAE AND SEA URCHIN EGGS.

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L.K. Sydnes and B. Gundersen. - Institute of Mathematical and Physical Sciences, Univerity of Tromsø, P.O.Box 953, N-9001 Tromsø, Norway.

INTRODUCTION

The purpose of this investigation was:

- to compare the optimum conditions for preparation of sea water extracts from drill cuttings/muds.
- to compare the toxicity of such extracts by using sea urchin eggs, cod eggs and cod larvae as test organisms.

Eggs from such species are rather easy to handle in the laboratory and are usually fertilized successfcully. They are transparent which make registration of abnormal cleavage and development possible. The early stages of cod and sea urchin are also relatively sensitive to environmental toxicants.

MATERIAL AND METHODS

Test organisms

Eggs and larvae of cod (*Gadus morhua* L.) and eggs of sea urchin (*Strongylocentrotus droebachiensis* (O.F.Muller)) were used as test organisms. Experiments with eggs were started about 2 hours after fertilization, with cod larvae shortly after hatching.

About 50 cod eggs, 20 cod larvae or a single layer of sea urchin eggs (about 10000 eggs) were placed in test beakers containing 100 ml of seawater solution of the test substances in various concentrations. The beakers were covered with aluminium foil. All experiments were carried out at 5 C with a 96 hour exposure period. The number of abnormal or dead embryos and inactive or dead larvae were noted after 24, 48 and 96 hours.

Preparation of seawater extracts

The mud samples were stored in a refrigerator until used in the experiments. Before the preparation of the seawater extracts, they were kept at room temperature in the dark for 1 hour, the samples were then homogenized by shaking or (if necessary) by crushing in a mortar.

All experiments carried out to find the optimum conditions for the preparation of extracts were performed with 65.0 g of filtered sea water in the dark at 3-4 C. The hydrocarbon concentration, determined according to Norsk Standard (1979), was used as a measure for the reproducibility.

The extracts used for toxicity testing were prepared as follows: A weighed sample of mud or drill cuttings was added to 1,5 l of filtered seawater in a 2 litre separatory funnel. The funnel was shaken in the dark for 1 hour at approximately 125 strokes/min on a shaker platform. After shaking the funnel was left undisturbed for 5 min, and then the water phase was decanted into an Erlenmeyer flask. This solution was left undisturbed for 12 hours

and was finally filtered twice through extra fine glass wool (ca 20 ml/min). All steps in the preparation procedure were carried out at 3-4 C. The seawater solutions were kept in the dark at 3-4 C for up to 12 hours before used in the experiments.

Analyzed parameters for all extracts are given in Table 5. The hydrocarbon content was measured according to Norsk Standard (1979).

RESULTS

Preparation of extracts for testing

Results from the experiments carried out to find the optimum conditions for preparation of extracts, are summarized in Tables 1-4. From Table 2 it is clear that the shaking time is not a critical parameter as long as it is fairly long (25 min.). For convenience we decided to shake the sea water/drill cutting mixtures for one hour. The time the extracts were left to settle before filtration was however, more important. When the time was shorter than approximately 10 hours, hydrocarbons were to some extent dispersed in the sea water (Table 3). We therefore decided to let the extracts settle for 12 hours before filtration. The number of filtrations did not influence the hydrocarbon contents very much (Table 4). Since each filtration takes about 45 min, we decided to filter each extract only once.

All samples obtained during the autumn 1982 (0 month samples) were easily homogenized. However, during preparation, the reference sediment smelled of sulphide.

Of samples obtained during the spring 1983 (9 month samples), the water based mud was difficult to homogenize. Both the muds based on mineral oil and diesel oil smelled of sulphide at preparation of the seawater solutions. Several solutions gave sedimentation in the test beakers as is indicated in Table 5.

Biological testing

Two experimental series were carried out during the spring 1983:

1. For the first series stem solutions of 27, 13 and 7 g/l were prepared. Only the "0 month" samples were tested on eggs from cod and sea urchin. Results from these experiments are given in Table 6.

2. Because little effect was often observed on cod eggs in the

first series, stem solutions of 100, 33 and 10 g/l were prepared for the next series. When lower concentrations were used, they were prepared from the 10 g stem solution. Results from this experiments is given in Table 7. As this series was based on two parallel experiments with all substances included, conclusions will mainly be made from these results.

Calculation of toxicity levels -To facilitate calculations of EC_{50} and EC_{10} levels, all results were transformed to "% effect" by subtracting % normal organisms in test beaker from % normal organisms in control. If no normal organisms was present in the test beaker, this was defined as 100% effect. Dose-response levels for each substance and test organism were calculated by linear regression, the equation given as :

$$\log(\text{dose}) = a + b(\%\ \text{effect})$$

$$a,b = \text{constants}$$

When effects were observed within the test range, regression coefficients (r) were usually higher than 0,7 (see Table 8). This was found acceptable due to the few plots available for each substance. If the regression coefficient was lower than 0,7, probable concentration range for the toxicity levels are suggested. The EC_{50} and EC_{10} values for cod eggs and larvae after 96 hours exposure are presented in Table 8.

Effects on cod eggs -The substances seemed to decrease in toxicity with 9 month incubation time on the sea floor (Table 7). Briquettes and diesel cuttings were clearly the most toxic substances. Judging from all observations made, briquettes seemed to give most effects. For "0 month" samples, effects were clearly visible after exposure in 24 hours of briquettes, whereas exposure to diesel revealed visible effects after 48-96 hours. The "9 month" samples however, revealed visible effects only after 96 hours exposure time in both cases. Mineral oil based mud and reference sediment did not have any effects on the cod eggs, whereas from the EC_{10} values it seemed that the water based mud had a slight effect within the test range (Table 8).

Effects on cod larvae -The larvae seemed more sensitive to the "9 month" samples than the eggs. The results of the "0 month" diesel mud was rather astonishing (Table 7). No effects were observed from the two highest concentrations, whereas incubation in 10000 ppm gave a mean of 45 % effect. From all our previous experiments we have experienced that cod larvae are almost as sensitive as cod eggs. Since the results are based on one experiment only, we can not exclude any experimental error in this case, and we will therefore not calculate any EC_{50} values here. The "0 month" mineral oil and water based sediments seemed to have a slight effect on the larvae, but the "9 month" mineral oil sediment had a relatively stronger effect on the larvae. The reference sediment did not have any effect on cod larvae in this experiment.

Effects on sea urchin eggs -Effects on sea urchin eggs were highly variable (see Tables 6 and 7). There was no correlation between the effects of the 3 experiments carried out on these eggs, and no conclusions on the toxicity of the different muds could therefore be made for sea urchin eggs.

CONCLUSIONS

The substances based on briquettes and diesel oil were clearly the most toxic for cod eggs and larvae. The toxic effects of the substances decreased with incubation time for cod eggs, whereas the cod larvae seemed rather sensitive to the aged substances. What these differences are due to, is hard to explain, as the chemical composition of the water phase is unknown. The eggs are, however, sourrounded by an egg shell (chorion) which may to some extent prevent toxic substances from penetrating to the embryo. The eggs are also floating passively near the water surface of the beakers. Larvae, on the other hand, are active swimmers. They will be more directly exposed to the test medium as they lack the sourrounding shell, and they may also be able to drink the test medium. Differences in sedimentation, pH, H S and other factors may therefore be of importance. The ranking of the toxicity did not seem to change for new and aged substances, and can therefore be ranked as follows:

<u>Cod eggs</u>

0 month: Briquettes > Diesel oil > Water based > Mineral oil and Reference.

9 months: Briquettes > Diesel oil > Water based > Mineral oil and Reference.

<u>Cod larvae</u>

0 month: Briquettes > Mineral oil > Water based and Reference.

9 months: Diesel oil > Briquettes > Mineral oil > Water based and Reference.

Sea urchin eggs did not seem very suitable for these tests. Sedimentation from the solutions in the test beakers seemed to give high mortality for these eggs which develop on the bottom. Variability of the results may also be due to this.

SUGGESTIONS FOR FUTURE EXPERIMENTS

Both cod eggs and larvae seem suitable to test toxic effects of seawater solutions of these substances. For any similar future routine experiments, however, the experimental procedure should be

somewhat different.

A simpler observation procedure is suggested. The observations at the end of the incubation period (96 h) were most informative. Observations at 24 and 48 h could therefore be omitted. More test concentrations should be included for each substance. Dilution of prepared stem solutions should be avoided, according to the results of Hovde (in another report).

Other filtration methods for the preparation of seawater solutions should be considered, as should more thoroughly chemical analyses of the seawater solutions of the substances.

REFERENCE

Norsk Standard 1979. Determination of oil in water. Infrared spectrophotometric methods. N.S. Report 4753. Norsk Standardiseringsforbund, Oslo 1977.

Table 1. The hydrocarbon contents (Hc) in extracts prepared by shaking 65.0 g of diesel-based drill mud in 900 ml of filtered sea water for 1 hour in the dark at 3-4 C. Time to settle: 20 hours. Number of filtrations: 1.

Extract	I	II	III	IV	V
Hc (ppm)	12.5	14.9	10.4	13.4	13.0

Table 2. The hydrocarbon contents (Hc) in extracts prepared by shaking 65.0 g of diesel-based mud in 900 ml of filtered sea water in the dark at 3-4 C. Settling time: 20 hours. Number of filtrations: 1. The shaking time (ts) has been varied.

ts (hours)	0.25	1.0	2.0	20.0
Hc (ppm)	11.7	12.8	12.2	13.7

Table 3. The hydrocarbon contents (Hc) in extracts prepared by shaking 65.0 g of diesel-based drill mud in 900 ml of filtered sea water for 15 min in the dark at 3-4 C. Number of filtrations: 1. The settling time (t_w) has been varied.

tw (hours)	2.0	6.0	12.0	16.0	20.0
Hc (ppm)	17.0	15.7	11.9	11.6	11.7

Table 4. The hydrocarbon contents (Hc) in extracts prepared by shaking 65.0 g of diesel-based drill mud in 900 ml of filtered sea water for 15 min in the dark at 3-4 C. Settling time: 2 hours. The number of filtrations (N_j) varied.

Nj	0	1	2	3
Hc (ppm)	17.9	17.0	16.6	15.8

Table 5. Characteristics of seawater solutions of the different test substances. Chemical analyses of hydrocarbon concentrations was performed according to Norsk Standard.

Substance	Incubation time (month)	H_2S	Sedimentation	Water phase	Hydro carbons (ppm)	pH
Briquettes	0	-	none	clear	2.3	8.3
- " -	9	-	brown	"	1.0	7.7
Diesel oil	0	-	none	"	4.8	8.1
- " -	9	+	"	"	2.8	7.9
Mineral oil	0	-	"	"	5.4	8.6
- " -	9	+	brown	"	1.8	7.8
Water based	0	-	white	"	0.5	8.1
- " -	9	-	yellow/brown	"	0.9	7.5
Reference	0	+	none	whitish	1.2	7.9
- " -	9	-	brown	brown	1.1	7.3

Table 6. Effects from first experiment based on the 0 month muds after 96 hours exposure. Good egg cultures were only obtained from one female of each species.

Sample	Cons. (ppm)	% normal organisms cod eggs	Sea urchin eggs
Control	0	95	95
Briquettes	27000	0	0
"	13000	0	0
"	7000	0	0
Diesel oil	27000	0	0
"	13000	0	0
"	7000	0	0
Mineral oil	27000	85	0
"	13000	84	0
"	7000	95	0
Water based	27000	93	91
"	13000	89	92
"	7000	95	95
Reference	27000	93	0
"	13000	86	4
"	7000	96	0

Table 7a. Effects from the final experiments based on 0 month muds after 96 hours exposure.

Substance	Cons. (ppm)	% normal organisms Cod eggs ♀1	♀2	Cod larvae	Sea urchin eggs ♀1	♀2
Control	0	90	87	92	92	92
Briquettes	100000	0	0	0	0	0
"	33000	0	0	0	0	0
"	10000	0	0	0	(75)	0
"	5000	70	88	90	90	0
"	2000	70	94		91	90
Diesel oil	100000	0	0	87	0	0
"	33000	11	0	88	80	0
"	10000	64	0	47	90	0
"	5000	76	44	92	92	85
"	2000	88	90		92	90
Mineral oil	100000	86	88	73	0	0
"	33000	68	88	95	0	0
"	10000	75	86	77	90	91
"	5000	83	87	91	92	91
Water based	100000	76	70	71	0	0
"	33000	75	85	76	0	0
"	10000	83	91	82	0	0
"	5000	92	94	75	0	90
"	2000				0	91
Reference	100000	85	92	88	0	0
"	33000	65	55	96	90	92
"	10000	71	85	88	0	0
"	5000	71	90	83	85	0
"	2000				50	0

Table 7b. Effects from the final experiments based on the 9 month muds after 96 hours exposure.

Substance	Cons. (ppm)	% normal organisms Cod eggs ♀1	♀2	Cod larvae	Sea urchin eggs ♀1	♀2
Control	0	90	87	92	92	92
Briquettes	100000	19	9	0	0	0
"	33000	22	0	0	0	0
"	10000	33	45	22	0	0
"	5000	71	90	81	90	0
"	2000	91			91	0
Diesel oil	100000	0	0	0	0	0
"	33000	4	9	0	0	0
"	10000	25	84	11	0	0
"	5000	56	86	94	90	0
"	2000				90	0
Mineral oil	100000	93	85	20	0	0
"	33000	69	78	74	0	0
"	10000	69	86	70	0	0
"	5000	89	82	90	0	0
"	2000				90	0
Water based	100000	80	60	50	0	0
"	33000	55	94	75	0	0
"	10000	94	86	77	0	0
"	5000	80	70	77	0	0
"	2000				0	0
Reference	100000	89	62	80	0	0
"	33000	29	84	83	0	0
"	10000	77	72	75	0	0
"	5000	87	92	92	0	0
"	2000				0	0

Table 8. Estimated EC-50 and EC-10 values (mg/l) for cod eggs and larvae after 96 hours. Results for cod eggs are the mean from two parallel experiments, for larvae only one experiment was performed. *= Values estimated from linear regression, the regression coefficient (r^2) is given.

(Age) Substance/Month	Cod eggs EC-50	EC-10	r^2	Cod larvae EC-50	EC-10	r^2
Briquettes/ 0	5400	3200	0.70	5000-10000	5000-10000	-
" / 9	13500*	4700*	0,93	9600*	4400*	0.89
Diesel oil/ 0	9800*	1900*	0,96	-	-	-
" / 9	15000*	4000*	0,97	6600*	2400*	0.93
Mineral oil/0	>100000	>100000	-	>100000	33000-100000	-
" /9	>100000	>100000	-	46000*	98000*	0.78
Water based/0	>100000	36000*	-	>100000	<100000	-
" /9	>100000	10000-33000	-	>100000	7000*	0.71
Reference / 0	>100000	>100000	-	>100000	>100000	-
" / 9	>100000	100000	-	>100000	100000	-