

**REPORT**

**ACUTE TOXICITY TO THE MARINE COPEPOD**

**ACARTIA TONSA OF**

**ACID GELLING AGENT J429**

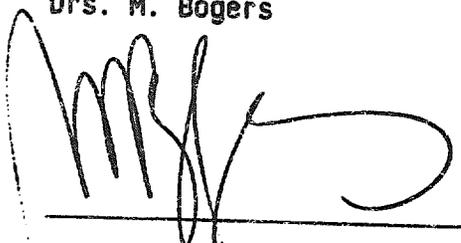
**RCC NOTOX Project 086974  
RCC NOTOX Substance 27324**

REPORT APPROVAL

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STUDY DIRECTOR

Drs. M. Bogers



A handwritten signature in black ink, appearing to be 'M. Bogers', written over a horizontal line.

Date: 11 February, 1993

MANAGEMENT:

Ing. E.J. van de Waart  
(Section Head, Genetic &  
Ecotoxicology)



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

Date: 11/02/1993

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## SUMMARY

Acute Toxicity to the marine copepod Acartia tonsa of ACID GELLING AGENT J429.

Acartia tonsa were exposed for a maximum of 48 hours to a concentration range of 0.1 to 10 mg/l forming a geometric progression with a factor of 3.2. Twenty copepods were exposed to each concentration, i.e. five copepods per vessel containing 25 ml of test solution.

After 24 hours of exposure the total rate of mortality of Acartia tonsa was 50% at 1.0 mg/l, 40% at 3.2 mg/l and 85% at 10 mg/l. No significant (>10%) mortality of Acartia tonsa was seen at concentrations of 0.32 mg/l or lower.

The 24h-LC50 was calculated to be nominally 2.23 mg/l with 95% confidence interval ranging from 0.80 to 8.20 mg/l (regression line:  $\log_{10}(\text{conc.}) = 0.28 + (\text{probit} - 4.92) / 1.24$ ).

After 48 hours of exposure mortality of all copepods exposed was seen at concentrations of 3.2 mg/l and higher. At 0.32 mg/l the total incidence of mortality had increased to 75% and at 1.0 mg/l to 85%. No mortality of Acartia tonsa was seen at 0.1 mg/l.

The 48h-LC50 was calculated to be nominally 0.30 mg/l with 95% confidence interval ranging from 0.21 to 0.42 mg/l (regression line:  $\log_{10}(\text{conc.}) = -0.51 + (\text{probit} - 5.04) / 2.90$ ).

Finally, the No Observed Effect Concentration for ACID GELLING AGENT J429 with respect to mobility of Acartia tonsa was found to be 0.1 mg/l after 48 hours of exposure.

## PREFACE

## GENERAL

---

|                      |   |
|----------------------|---|
| Title                | Acute Toxicity to the marine copepod <u>Acartia tonsa</u> of ACID GELLING AGENT J429. |
| Sponsor              | Dowell Schlumberger Inc.<br>P.O. Box 2710<br>TULSA, Oklahoma 74101<br>USA             |
| Monitoring Scientist | Mr. D. DeBolt   |
| Testing Facility     | RCC NOTOX B.V.<br>Hambakenwetering 3<br>5231 DD 's-Hertogenbosch, The Netherlands     |
| RCC NOTOX Project    | 086974  |
| Test Substance       | ACID GELLING AGENT J429   |
| Test System          | <u>Acartia tonsa</u>  |

## PROJECT STAFF

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|                       |   |
|-----------------------|---|
| Study Director        | Drs. M. Bogers (RCC NOTOX B.V.)           |
| Technical Coordinator | Ing. J.J.C. van der Poel (RCC NOTOX B.V.) |

## SCHEDULE

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|       |                   |
|-------|-------------------|
| Start | November 24, 1992 |
| End   | January 19, 1993  |

## QUALITY ASSURANCE STATEMENT

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

RCC NOTOX Project                      086974  
Test Substance                              ACID GELLING AGENT J429  
Study Director                              Drs. M. Bogers  
Title    Acute Toxicity to the marine copepod Acartia tonsa of ACID GELLING AGENT J429.

Study procedures were subjected to periodic inspections.

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

| Dates of QAU Inspections / Audits | Reporting Date |
|-----------------------------------|----------------|
| 15-10-1992                        | 15-10-1992     |
| 24-11-1992                        | 24-11-1992     |
| 06-01-1993                        | 06-01-1993     |
| 08-02-1993                        | 08-02-1993     |

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

Manager, Quality Assurance Unit

C. Mitchell B. Sc.  
i.a.

  
Date: 12 February, 1993

STATEMENT OF GLP COMPLIANCE

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RCC NOTOX Project                    086974  
Test Substance                        ACID GELLING AGENT J429  
Study Director                        Drs. M. Bogers  
Title                                    Acute Toxicity to the marine copepod Acartia  
   tensa of ACID GELLING AGENT J429.

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

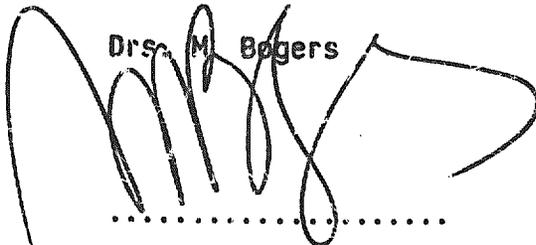
OECD Principles of Good Laboratory Practice.

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

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

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

Study Director

Drs. M. Bogers  
  
.....  
Date: 11 February, 1993

**GUIDELINES**

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The study procedure described in this protocol is based on the PARCOM Ring test protocol for the Determination of acute lethal toxicity to marine copepods, November 1990, including the alterations produced by Ms. E. Bjørnstad, January 1991.

**ARCHIVING**

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

## OBJECTIVE

### PURPOSE

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The purpose of the study is to evaluate the test substance for its ability to generate acute toxic effects in Acartia tonsa during an exposure period of 48 hours and, if possible, to determine the 48 h-LC50.

### DEFINITIONS

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Copepods are considered to be dead when they do not start to swim within 10 seconds after gently shaking of the test vessel. Additionally, an immobile copepod will be gently touched, if it is not sure that the organism is dead.

The LC50 is the concentration estimated to kill 50% of the copepods after a defined period of exposure.

## MATERIALS AND METHODS

## TEST SYSTEM

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|                      |   |
|----------------------|---|
| Species              | <u>Acartia tonsa</u> (Copepoda, Crustacea) (Dana)   |
| Stage                | Stage 5 or adults (ca. 3 weeks old)   |
| Reason for selection | This system has been selected as a marine crustacean sensitive to toxic substances in the marine environment. It was one of the species tested in the PARCOM Ring test. |

## BREEDING / HOLDING

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|                        |   |
|------------------------|---|
| Breeding               | The parental populations are held in natural seawater, filtered over 0.2 $\mu$ m filter, at 20 $\pm$ 2°C. Oil-free air is gently bubbled in so that no turbulence occurs in the water.  |
| Measurements           | Temperature: every day. Oxygen concentration, pH, nitrite and ammonia once every two weeks.   |
| Feeding                | Every other day with $5 \cdot 10^7$ (nauplii) to $7.5 \cdot 10^7$ (copepods) algal cells/litre (Rhodomonas sp. and/or Skeletonema sp.)  |
| Eggs                   | Every week eggs are collected, put apart in a new vessel with natural seawater and further treated in the same way as their parents. After 3 weeks following hatching they will have developed to stage 5 or adults and can be used for the test. |
| Control of sensitivity | The results of the most recent reference test with potassium dichromate ( $K_2Cr_2O_7$ ) are appended.  |

## TEST SUBSTANCE

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|  |                                 |
|--|---------------------------------|
| Identification                             | ACID GELLING AGENT J429         |
| Description                                | White liquid                    |
| Batch                                      | IB6078                          |
| Purity                                     | Mixture                         |
| Instructions for<br>test substance storage | At room temperature in the dark |
| Stability under storage<br>conditions      | Stable                          |
| Expiry date                                | March 1, 1993                   |
| Stable for at least<br>96 hours in vehicle | Water : yes                     |

## RANGE-FINDING TEST

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A range-finding test was performed to provide information about the range of concentrations to be used in the final test. A. tonsa were exposed for 48 hours to a concentration range of 0.1 to 100 mg/l forming a geometric progression with a factor of 10.

## TEST PROCEDURE AND CONDITIONS

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|                                 |  |
|---------------------------------|--|
| Test duration                   | 48 hours   |
| Test vessels                    | 50 ml, polystyrene   |
| Medium                          | Natural sea water, filtered through a 0.2 $\mu$ m filter, with a salinity of $32 \pm 2$ per mille. |
| Test concentrations             | Based on the results of the range-finding test: 0.1, 0.32, 1.0, 3.2, 10 mg/l.                      |
| Control                         | Medium without test substance or other additives (0 mg/l).   |
| Number of copepods              | 20 per concentration, 30 in the control.   |
| Loading                         | 5 per vessel containing 25 ml test medium  |
| Illumination                    | 16 hours photoperiod daily   |
| Feeding                         | No feeding   |
| Aeration                        | No aeration of the test solution.  |
| Introduction of <u>A. tonsa</u> | The copepods were introduced into the test medium directly after preparation of the test media.    |

### PREPARATION OF TEST MEDIA

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The test media were prepared using a stock solution of 100 mg/l (nominal) by adding quantitatively 20.9 mg of test substance to 200 ml natural seawater. The final test solutions were all clear without precipitation.

### MEASUREMENTS AND RECORDINGS

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|                       |   |
|-----------------------|---|
| Mortality of copepods | At 24 hours and 48 hours.   |
| pH and Oxygen         | At the beginning, after 24 hours and at the end of the test, in extra vessels without copepods. |
| Temperature of medium | Daily in one control vessel, beginning at the start of the test.                                |

### ACCEPTABILITY OF THE TEST

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- a) The mortality in the blank and, if relevant, in the control containing the vehicle should not exceed 10%.
- b) Dissolved oxygen concentration at the end of the test should be  $\geq 4$  mg/l.

### DATA HANDLING

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Calculation of LC50: The LC50-value was calculated at 24 and 48 hours of exposure from the probits of the percentages of affected copepods and the logarithms of the corresponding nominal concentrations using the maximum likelihood estimation method (Finney, D.J., 1971: Probit analysis, Cambridge University Press, Cambridge, U.K., 3rd edition).

## RESULTS

### RANGE-FINDING TEST

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In the range finding 100% mortality of A. tonsa was recorded at 10 mg/l and higher and 50% mortality at 1 mg/l. No significant mortality was recorded at 0.1 mg/l.

### FINAL STUDY: MORTALITY OF COPEPODS

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The mortality data of the final test are presented in Table 1.

After 24 hours of exposure the total rate of mortality of Acartia tonsa was 50% at 1.0 mg/l, 40% at 3.2 mg/l and 85% at 10 mg/l. No significant (>10%) mortality of Acartia tonsa was seen at concentrations of 0.32 mg/l or lower.

After 48 hours of exposure 100% mortality was recorded at concentrations of 3.2 mg/l and higher. At 0.32 mg/l the total incidence of mortality of copepods had increased to 75% and at 1.0 mg/l to 85%. No mortality was observed at 0.1 mg/l.

### FINAL STUDY: EXPERIMENTAL CONDITIONS

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The results of measurement of pH and oxygen concentration are presented in Table 2.

The pH of the exposure solutions was 8.7 throughout the 48-hour test period.

Oxygen concentration was > 7 mg O<sub>2</sub>/l in all test solutions.

The temperature of the test medium measured in the blank control varied from 19 to 20°C.

### FINAL STUDY: ACCEPTABILITY OF THE TEST

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In the control, no copepods died or became trapped at the surface of the water. The pH of natural seawater was 8.7 and thus exceeded the range prescribed by the protocol. This had no effect on the validity of the test. Further, all other test conditions remained within the ranges prescribed by the protocol.

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**CALCULATION OF LC50-VALUES AND RELATED PARAMETERS**

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The 24h-LC50 was calculated to be nominally 2.23 mg/l with a 95% confidence interval ranging from 0.80 to 8.20 mg/l (regression line:  $\log_{10}(\text{conc.}) = 0.28 + (\text{probit} - 4.92) / 1.24$ , Table 3 and Figure 1).

The 48h-LC50 was calculated to be nominally 0.30 mg/l with a 95% confidence interval ranging from 0.21 to 0.42 mg/l (regression line:  $\log_{10}(\text{conc.}) = -0.51 + (\text{probit} - 5.04) / 2.90$ , Table 3 and Figure 2).

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**CONCLUSION**

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Under the conditions of the present study ACID GELLING AGENT J429 did not induce acute mortality of Acartia tonsa at or below 0.1 mg/l after 48 hours of exposure (LCO). The 48h-LC50 was calculated to be nominally 0.30 mg/l (95% confidence interval between 0.21 and 0.42 mg/l, regression line:  $\log_{10}(\text{conc.}) = -0.51 + (\text{probit} - 5.04) / 2.90$ ).

TABLE 1: Acute mortality of A. tonsa after 24 and 48 hours in the final test.

| Nominal concentration (mg/l) | Vessel code | number exposed | number dead at 24 h | number dead at 48 h | Total mortality at the end of the test (%) |
|------------------------------|-------------|----------------|---------------------|---------------------|--|
| 0                            | A           | 5              | 0                   | 0                   | 0  |
|                              | B           | 5              | 0                   | 0                   |  |
|                              | C           | 5              | 0                   | 0                   |  |
|                              | D           | 5              | 0                   | 0                   |  |
|                              | E           | 5              | 0                   | 0                   |  |
|                              | F           | 5              | 0                   | 0                   |  |
| 0.1                          | A           | 5              | 0                   | 0                   | 0  |
|                              | B           | 5              | 0                   | 0                   |  |
|                              | C           | 5              | 0                   | 0                   |  |
|                              | D           | 5              | 0                   | 0                   |  |
| 0.32                         | A           | 5              | 0                   | 2                   | 75   |
|                              | B           | 5              | 2                   | 5                   |  |
|                              | C           | 5              | 0                   | 3                   |  |
|                              | D           | 5              | 0                   | 5                   |  |
| 1.0                          | A           | 5              | 3                   | 4                   | 85   |
|                              | B           | 5              | 2                   | 4                   |  |
|                              | C           | 5              | 2                   | 4                   |  |
|                              | D           | 5              | 3                   | 5                   |  |
| 3.2                          | A           | 5              | 0                   | 5                   | 100  |
|                              | B           | 5              | 1                   | 5                   |  |
|                              | C           | 5              | 5                   | 5                   |  |
|                              | D           | 5              | 2                   | 5                   |  |
| 10                           | A           | 5              | 5                   | 5                   | 100  |
|                              | B           | 5              | 5                   | 5                   |  |
|                              | C           | 5              | 3                   | 5                   |  |
|                              | D           | 5              | 4                   | 5                   |  |

TABLE 2: pH<sup>1</sup> and oxygen concentration<sup>1</sup> during the final test.

| Nominal Conc.<br>(mg/l) | Start (t=0h) |                          | t=24h |                          | End (t=48h) |                          |
|-------------------------|--------------|--------------------------|-------|--------------------------|-------------|--------------------------|
|                         | pH           | DO(mg O <sub>2</sub> /l) | pH    | DO(mg O <sub>2</sub> /l) | pH          | DO(mg O <sub>2</sub> /l) |
| 0                       | 8.7          | 7.9                      | 8.7   | 7.8                      | 8.7         | 7.8                      |
| 0.1                     | 8.7          | 7.8                      | 8.7   | 7.9                      | 8.7         | 7.9                      |
| 0.32                    | 8.7          | 7.8                      | 8.7   | 7.9                      | 8.7         | 7.8                      |
| 1.0                     | 8.7          | 7.9                      | 8.7   | 7.9                      | 8.7         | 7.8                      |
| 3.2                     | 8.7          | 7.9                      | 8.7   | 7.9                      | 8.7         | 7.8                      |
| 10                      | 8.7          | 7.9                      | 8.7   | 7.9                      | 8.7         | 7.8                      |

<sup>1</sup> Measured in an extra vessel without copepods present in the test solution.

DO = dissolved oxygen

TABLE 3: LC50 values and related parameters.

24hr-EC50=2.23 mg/l

95 % fiducial limits: 0.80 - 8.20 mg/l

heterogeneous data, h=1.88

index of regression significance: g=0.47

chi-squared=26.35, with 14 degrees of freedom

regression line:  $\log_{10}(\text{conc.})=0.28+(\text{probit}-4.92)/1.24$ 

| conc.<br>mg/l | group<br>size | response | corrected<br>fraction | expected<br>fraction | chi2  |
|---------------|---------------|----------|-----------------------|----------------------|-------|
| 0.32          | 5             | 0        | 0.00                  | 0.15                 | 0.86  |
| 0.32          | 5             | 2        | 0.40                  | 0.15                 | 2.54  |
| 0.32          | 5             | 0        | 0.00                  | 0.15                 | 0.86  |
| 0.32          | 5             | 0        | 0.00                  | 0.15                 | 0.86  |
| 1.0           | 5             | 3        | 0.60                  | 0.33                 | 1.59  |
| 1.0           | 5             | 2        | 0.40                  | 0.33                 | 0.10  |
| 1.0           | 5             | 2        | 0.40                  | 0.33                 | 0.10  |
| 1.0           | 5             | 3        | 0.60                  | 0.33                 | 1.59  |
| 3.2           | 5             | 0        | 0.00                  | 0.58                 | 6.86  |
| 3.2           | 5             | 1        | 0.20                  | 0.58                 | 2.94  |
| 3.2           | 5             | 5        | 1.00                  | 0.58                 | 3.64  |
| 3.2           | 5             | 2        | 0.40                  | 0.58                 | 0.65  |
| 10            | 5             | 5        | 1.00                  | 0.79                 | 1.32  |
| 10            | 5             | 5        | 1.00                  | 0.79                 | 1.32  |
| 10            | 5             | 3        | 0.60                  | 0.79                 | 1.11  |
| 10            | 5             | 4        | 0.80                  | 0.79                 | 0.00  |
|               |               |          |                       |                      | 26.35 |

48hr-EC50=0.30 mg/l

95 % fiducial limits: 0.21 - 0.42 mg/l

index of regression significance: g=0.14

chi-squared=15.90, with 18 degrees of freedom

regression line:  $\log_{10}(\text{conc.})=-0.51+(\text{probit}-5.04)/2.90$ 

| conc.<br>mg/l | group<br>size | response | corrected<br>fraction | expected<br>fraction | chi2  |
|---------------|---------------|----------|-----------------------|----------------------|-------|
| 0.1           | 5             | 0        | 0.00                  | 0.08                 | 0.43  |
| 0.1           | 5             | 0        | 0.00                  | 0.08                 | 0.43  |
| 0.1           | 5             | 0        | 0.00                  | 0.08                 | 0.43  |
| 0.1           | 5             | 0        | 0.00                  | 0.08                 | 0.43  |
| 0.32          | 5             | 2        | 0.40                  | 0.53                 | 0.37  |
| 0.32          | 5             | 5        | 1.00                  | 0.53                 | 4.35  |
| 0.32          | 5             | 3        | 0.60                  | 0.53                 | 0.09  |
| 0.32          | 5             | 5        | 1.00                  | 0.53                 | 4.35  |
| 1.0           | 5             | 4        | 0.80                  | 0.94                 | 1.55  |
| 1.0           | 5             | 4        | 0.80                  | 0.94                 | 1.55  |
| 1.0           | 5             | 4        | 0.80                  | 0.94                 | 1.55  |
| 1.0           | 5             | 5        | 1.00                  | 0.94                 | 0.34  |
| 3.2           | 5             | 5        | 1.00                  | 1.00                 | 0.01  |
| 3.2           | 5             | 5        | 1.00                  | 1.00                 | 0.01  |
| 3.2           | 5             | 5        | 1.00                  | 1.00                 | 0.01  |
| 3.2           | 5             | 5        | 1.00                  | 1.00                 | 0.01  |
| 10            | 5             | 5        | 1.00                  | 1.00                 | 0.00  |
| 10            | 5             | 5        | 1.00                  | 1.00                 | 0.00  |
| 10            | 5             | 5        | 1.00                  | 1.00                 | 0.00  |
| 10            | 5             | 5        | 1.00                  | 1.00                 | 0.00  |
|               |               |          |                       |                      | 15.90 |

FIGURE 1: Percentage response (=mortality) of *Acartia tonsa* as function of the log concentration of ACID GELLING AGENT J429 at 24h.

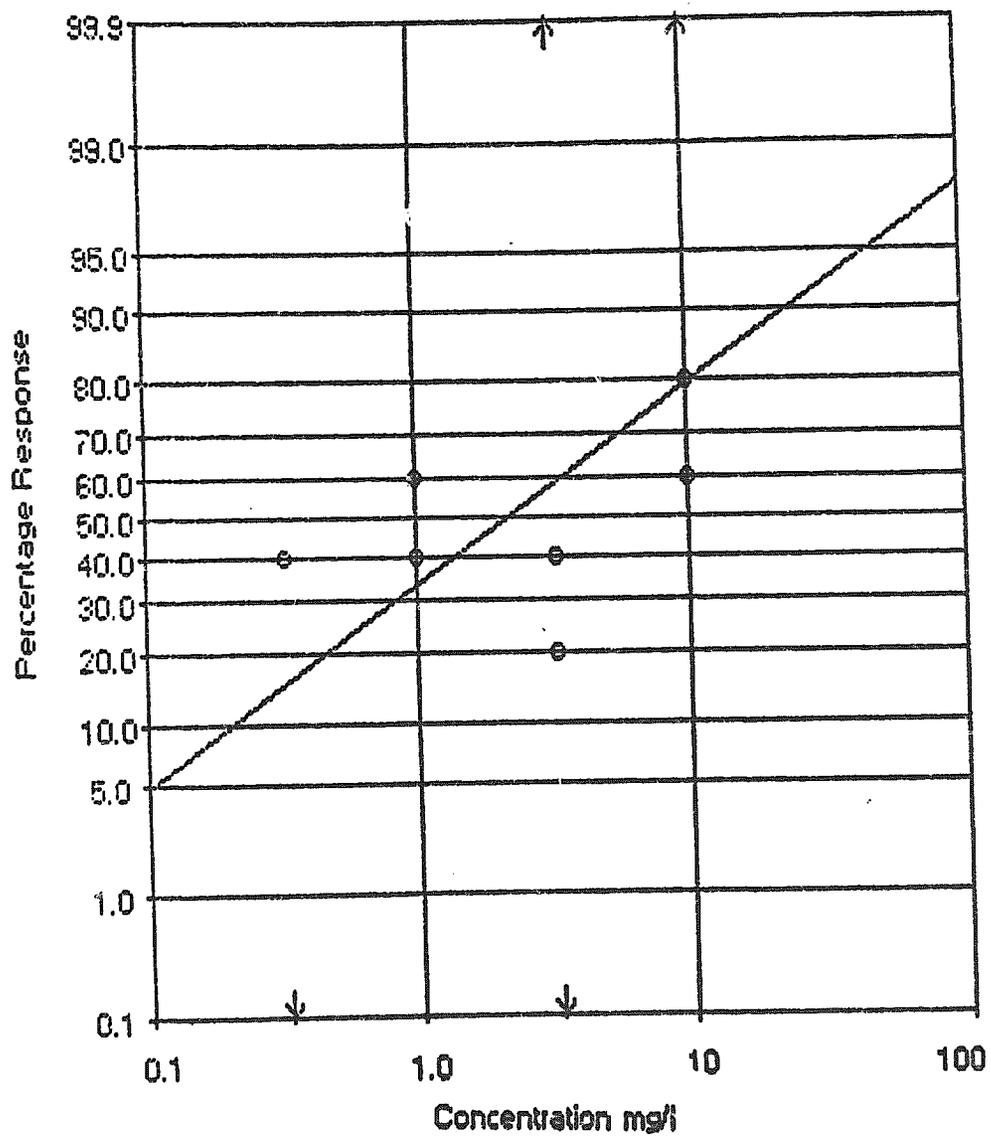
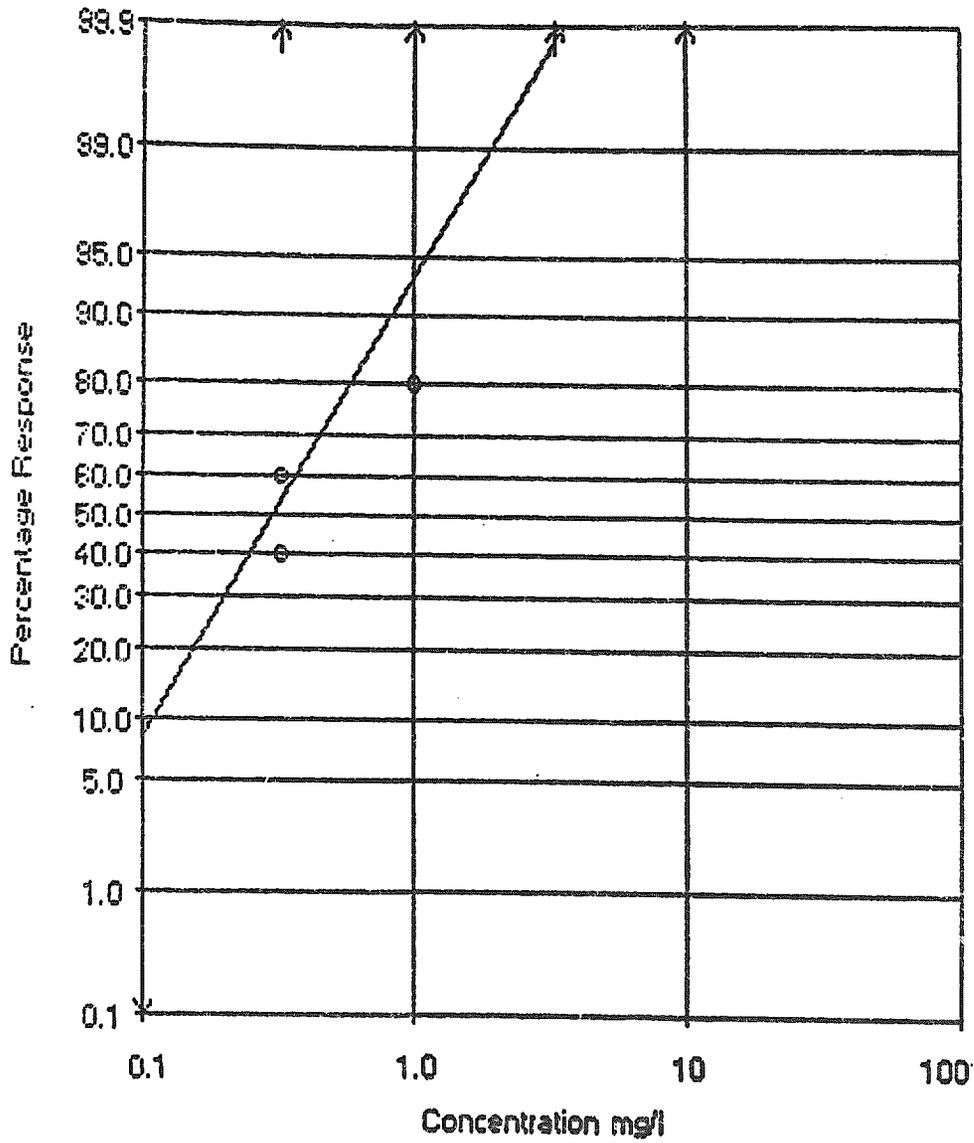


FIGURE 2: Percentage response (=mortality) of *Acartia tonsa* as function of the log concentration of ACID GELLING AGENT J429 at 48h.



## REFERENCE TEST

RCC NOTOX PCAA-5

48-hour acute toxicity study in the A. tonsa with potassium dichromate.

From 17-11-1992 to 19-11-1992

This reference test was carried out to check the sensitivity of the test system as used by RCC NOTOX.

The positive control substance, potassium dichromate ( $K_2Cr_2O_7$ , art. 4864) was obtained from Merck, Darmstadt, Germany.

Concentrations: 1.0, 3.0, 6.0, 10, 20, 30 and 60 mg/l in natural seawater. Control: Natural seawater without test substance.

Acute mortality of A. tonsa after 24 and 48 hours in the reference test with potassium dichromate:

| Concentration<br>mg/l | total number<br>expose | total number dead |        |
|-----------------------|------------------------|-------------------|--------|
|                       |                        | at 24h            | at 48h |
| 0.0                   | 30                     | 2                 | 4      |
| 1.0                   | 19                     | 0                 | 0      |
| 3.0                   | 20                     | 0                 | 1      |
| 6.0                   | 20                     | 0                 | 0      |
| 10                    | 20                     | 0                 | 1      |
| 20                    | 20                     | 3                 | 6      |
| 30                    | 20                     | 6                 | 17     |
| 60                    | 20                     | 17                | 19     |

The relation between test concentration and response is in agreement with what should be expected. The response recorded at and below 10 mg/l was considered as not significantly different between the different concentrations. The 24h-LC50 was ca. 40 mg/l. The 48h-LC50 was 21 mg/l (limits of 95%-confidence interval: 14 and 35 mg/l).

**REPORT**

**BIODEGRADABILITY IN SEAWATER: CLOSED BOTTLE TEST**

**WITH**

**BREAKER J286**

**NOTOX Project 114367  
NOTOX Substance 37431**

STATEMENT OF GLP COMPLIANCE

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NOTOX B.V., 's-Hertogenbosch, The Netherlands

The study described in this report was conducted in compliance with the most recent edition of:

The OECD Principles of Good Laboratory Practice

which are essentially in conformity with:

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

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

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

With the exception that COD analysis is subcontracted and not performed under GLP conditions.

Study Director

Ing. M.J.E. Koopmans

.....

Date: January 25, 1994

QUALITY ASSURANCE STATEMENT

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NOTOX B.V., 's-Hertogenbosch, The Netherlands.

Study procedures were subject to periodic inspections and general non study specific processes were also inspected at periodic intervals.

This report was audited by the NOTOX Quality Assurance Unit and the methods and results accurately reflect the raw data.

| DATES OF QAU INSPECTIONS/<br>AUDITS | REPORTING DATES |
|-------------------------------------|-----------------|
| 02-12-1993                          | 02-12-1993      |
| 09-12-1993                          | 09-12-1993      |
| 25-01-1994                          | 25-01-1994      |

Quality Assurance Manager

C.J. Mitchell B.Sc.



Date: 01-02-94.

REPORT APPROVAL

---

STUDY DIRECTOR:

Ing. M.J.E. Koopmans

  
.....

Date: January 25, 1994

MANAGEMENT:

Ing. E.J. van de Waart  
(Section Head, Genetic &  
Ecotoxicology)

  
.....

Date: 20/01/1994

PREFACE

---

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Study Monitor Mr. H. Romijn

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5231 DD 's-Hertogenbosch  
The Netherlands

Study Director Ing. M.J.E. Koopmans

Study Plan Start : December 09. 1993  
Completed : January 06, 1994

TEST SUBSTANCE

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Identification Breaker J286  
Description White crystals  
Batch 9300003007  
Purity 100%

Instructions for  
test substance storage At room temperature in the dark  
Stability under storage  
conditions Stable  
Expiry date November 01, 1994  
Stable for at least  
96 hours in vehicle Water: yes

PURPOSE

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The purpose of the study was to evaluate an organic test substance for its biodegradability in a nutrient-fortified seawater medium during a test period of 28 days.

## GUIDELINES

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The study procedures described in this report were based on the following guideline:

Organisation for Economic Co-operation and Development (OECD), OECD guidelines for Testing of Chemicals, Section 3, Degradation and Accumulation, guideline No. 306: "Biodegradability in Seawater", adopted July 17, 1992.

## ARCHIVING

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

## DEFINITIONS

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**Biochemical oxygen demand (BOD)** : Calculated as the difference of the oxygen depletion between a blank and a solution of test material under the conditions of the test. After division by the concentration of the test substance, the net oxygen depletion is obtained in mg BOD/mg test substance.

**Degradation** : The ratio of the BOD to either the theoretical oxygen demand (ThOD) or the chemical oxygen demand (COD) expressed as percentage.



## Stock solutions of nutrients

- A) 8.50 g  $\text{KH}_2\text{PO}_4$   
21.75 g  $\text{K}_2\text{HPO}_4$   
67.20 g  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$   
0.5 g  $\text{NH}_4\text{Cl}$   
dissolved in 1 l Milli-Q water  
The pH value was  $7.4 \pm 0.2$ .
- B) 22.50 g  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  dissolved in 1 l Milli-Q water.
- C) 36.40 g  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  dissolved in 1 l Milli-Q water.
- D) 0.15 g  $\text{FeCl}_3$  dissolved in 1 l Milli-Q water.

## Nutrient medium

1 ml of solution (a) to (d) was mixed and made up to 1 l with pre-treated seawater.

The concentration of dissolved oxygen was determined for control purposes. The nutrient medium was saturated at the start of the test.

## Test concentrations

The concentrations of the test substance were 2 (low) and 10 mg/l (high).

## Type of bottles

Test suspension: containing test substance.  
Oxygen blank: containing neither test nor reference substance.  
Procedure control: containing reference substance (sodium benzoate 2 mg/l in nutrient medium (Merck art. 6290, batch 124 K15840890)).  
Toxicity control: containing test substance (low) and reference substance (2 mg/l).

Parallel groups of BOD bottles were prepared to allow duplicate measurements of oxygen consumption at the test intervals.

## Incubation

In the dark.

## PREPARATION OF TEST MEDIA

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A stock solution of 100 mg/l was prepared by adding 102.7 mg of test substance to 1000 ml of nutrient medium. An amount of test substance stock solution corresponding to the test concentrations was then added in the test medium.

## DETERMINATION OF OXYGEN CONCENTRATION

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|                        |  |
|------------------------|--|
| Frequency              | Immediately at the start of the experiment (day 0), and at day 14, 21 and 28 in duplicate.   |
| Oxygen meter           | WTW: OXI 530 dissolved oxygen meter, TriOxmatic ED 200 oxygen electrode, electrolyte type ELY/N.   |
| Expected Oxygen demand | Theoretical calculation of the oxygen demand was not possible, therefore a sample of the pure test substance was taken for determination of the chemical oxygen demand according to NEN 6633, "Determination of the chemical oxygen demand", Nederlands Normalisatie Instituut, NNI, January 1990. The COD analysis was subcontracted at the Chemical Laboratory "Dr. A. Verwey", Rotterdam, the Netherlands and was not performed under GLP conditions. |

## DATA EVALUATION

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The measured BOD-values were corrected for the background (endogeneous) O<sub>2</sub> demand recorded for the oxygen blank.

The course of degradation is represented graphically.  
The percentage degradation after 28 days is calculated.

Results of this test are not to be taken as indicators of ready or inherent biodegradability, but as screening tests for biodegradability of test substances in seawater.

If the result is positive (>60% biodegradation), it may be concluded that there is a potential for biodegradation in the marine environment. However a negative result does not preclude such a potential, but indicates that further study is necessary.

## ACCEPTABILITY OF THE TEST

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The results of the biodegradation test were considered to be valid when:

Oxygen consumption in the blank does not exceed 30% at the end of the test.

The reference substance should be biodegraded by at least 60% within 15 days.

If the BOD of the inhibition control is less (i.e. <75%) than the sum of the BOD's of the test and reference substance, the test substance may be considered as toxic to the micro-organisms present in the medium.

## RESULTS

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### O<sub>2</sub>-consumption

The COD of BREAKER J286 was determined to be 1.646 mg O<sub>2</sub>/mg.  
The ThOD of sodium benzoate (positive control) was calculated to be 1.665 mg O<sub>2</sub>/mg.

### Test conditions

pH, O<sub>2</sub>-concentration and the temperature of nutrient medium at the start of the test are given in the Appendix.  
The temperature during incubation was 19 ± 1°C, with the exception of day 16, 17 and 18, when the temperature was 17, 17 and 17.5°C, respectively. Inadvertently, the temperature was not recorded at day 11.  
Since under the conditions of this test all acceptability criteria mentioned in the protocol were met, the deviations in the temperature-recording were considered to have no effect on the integrity of this study.

### Biodegradation

Table 1 shows the mean values for oxygen depletion for the different test groups during the test period. Table 2 shows the percentages of biodegradation calculated for the different test groups at different points in time. Figure 1 shows the course of degradation in time for the different test groups.

The relative biodegradation values calculated from the O<sub>2</sub> measurements performed during the test period of 28 days revealed no degradation of BREAKER J286 at both concentrations.

Since oxygen consumption of the inhibition control was > 75% of the total oxygen depletion expected on basis of the results for the positive control and the low concentration group (Table 1), BREAKER J286 was not toxic to the micro-organisms present in the medium.

### Acceptability of the test

The control substance was biodegraded for more than 60% within 14 days (see Table 2 and Figure 1).

Oxygen consumption in the blank was about 22% relative to the oxygen consumption in the test bottles at the start of the test.

Oxygen consumption was measured at day 14, 21 and 28 instead of the protocolled day 5, 15 and 28. However, this did not influence the integrity of the study.

### CONCLUSION

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Under the conditions of this present test, BREAKER J286 was not biodegradable in the closed bottle test with seawater medium.

Table 1: Oxygen depletion at different points in time

| Test medium      | Concentration (mg/l) | Oxygen depletion (mg BOD/l) after x days* |       |       |
|------------------|----------------------|---|-------|-------|
|                  |                      | 14  | 21    | 28    |
| Positive control | 2                    | 2.19                                      | 2.24  | 2.52  |
| Test subst. low  | 2                    | -0.13                                     | -0.11 | -0.16 |
| Test subst. high | 10                   | -0.11                                     | 0.12  | -0.01 |
| Toxicity control | **                   | 2.12                                      | 2.47  | 2.53  |

\*For calculations see Appendix

\*\*Toxicity control contains positive control and test substance low.

Table 2: % Biodegradation at different points in time

| Test medium         | Concentration (mg/l) | % Biodegradation after x days* |    |    |
|---------------------|----------------------|--------------------------------|----|----|
|                     |                      | 14                             | 21 | 28 |
| Pos. control**      | 2                    | 66                             | 67 | 76 |
| Test subst. low***  | 2                    | -4                             | -3 | -5 |
| Test subst. high*** | 10                   | -1                             | 1  | 0  |

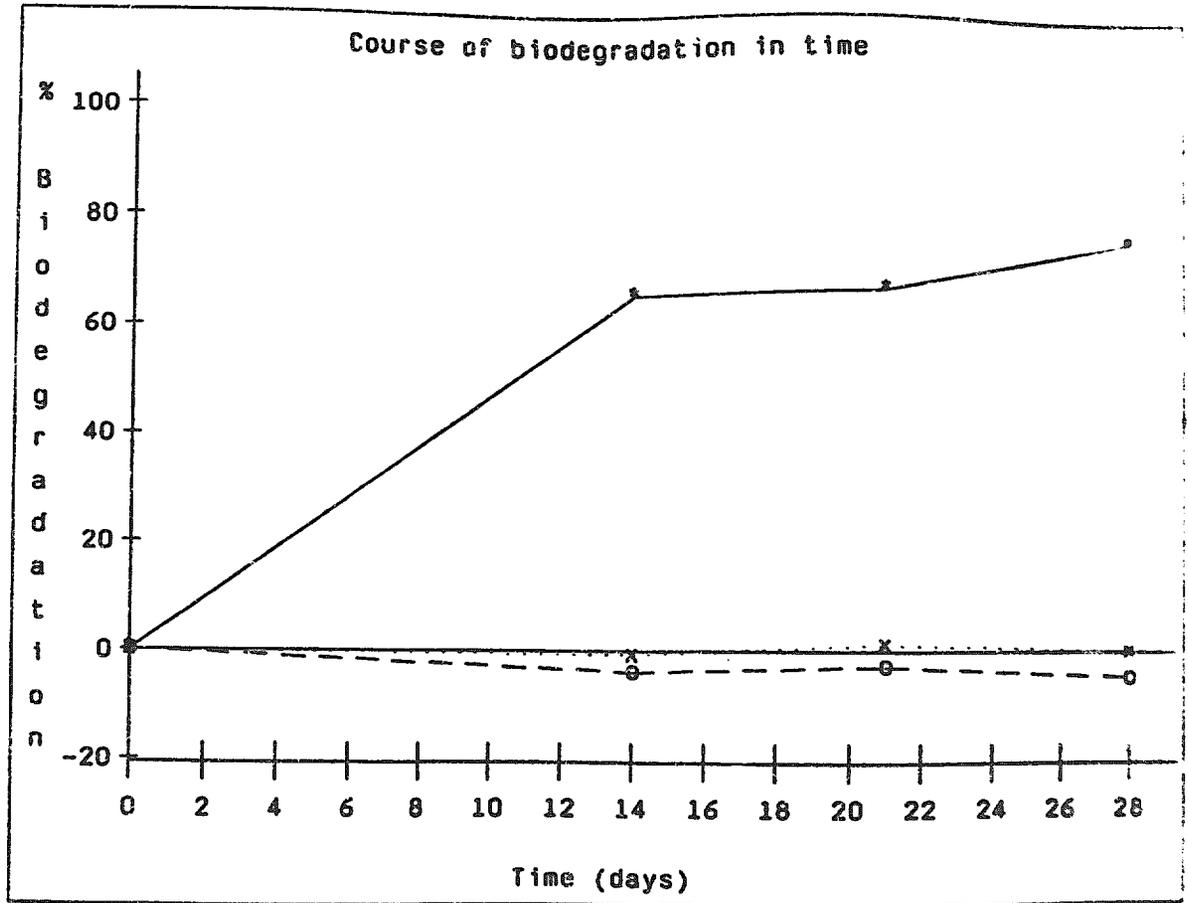
\*For calculations see Appendix

\*\*ThOD positive control sodium benzoate in mg O<sub>2</sub>/mg: 1.665

\*\*\* COD test substance in mg O<sub>2</sub>/mg: 1.646

Figure 1: Course of degradation in time for the different test media:

- \* :positive control;
- o :BREAKER J286 (2 mg/l);
- x :BREAKER J286 (10 mg/l).



## APPENDIX

A G<sub>2</sub> determinations:

| Series                        | Content  | Stock solution |       | mg O <sub>2</sub> /l<br>0 | after x days |         |      |      |
|-------------------------------|--|----------------|-------|---------------------------|--------------|---------|------|------|
|                               |  | pH             | t(°C) |                           | 14           | 21      | 28   |      |
| Inoculum<br>blank             | Nutrient<br>medium                                 | 7.9            | 18.1  | 8.56                      | 7.05         | 6.88    | 6.64 |      |
|                               |  |                |       | 8.61                      | 7.14         | 6.83    | 6.80 |      |
|                               |  |                |       | Mean mB                   | 8.60         | 7.10    | 6.86 | 6.72 |
| Positive<br>control mg/l<br>2 | Nutrient<br>medium,<br>Pos. contr.                 | 7.9            | 18.0  | 8.64                      | 4.90         | 4.46    | 4.31 |      |
|                               |  |                |       | 8.66                      | 5.02         | 4.87    | 4.18 |      |
|                               |  |                |       | Mean mP                   | 8.65         | 4.96    | 4.67 | 4.25 |
| Test<br>substance mg/l<br>2   | Nutrient<br>medium,<br>Test subst.<br>Low          | 7.9            | 18.0  | 8.64                      | 7.40         | 6.94    | 6.92 |      |
|                               |  |                |       | 8.66                      | 7.16         | 7.10    | 6.94 |      |
|                               |  |                |       | Mean mL                   | 8.65         | 7.28    | 7.02 | 6.93 |
| Test<br>substance mg/l<br>10  | Nutrient<br>medium,<br>Test subst.<br>High         | 7.7            | 18.1  | 8.53                      | 7.13         | 6.63    | 6.66 |      |
|                               |  |                |       | 8.58                      | 7.18         | 6.75    | 6.69 |      |
|                               |  |                |       | Mean mH                   | 8.55         | 7.16    | 6.69 | 6.68 |
| Toxicity<br>control           | Natural<br>seawater,<br>Test subst.<br>Pos. contr. | 7.9            | 18.0  | 8.54                      | 4.82         | 4.26    | 4.12 |      |
|                               |  |                |       | 8.56                      | 5.04         | 4.41    | 4.16 |      |
|                               |  | 2              | mg/l  | 2                         | mg/l         | Mean mI | 8.55 | 4.93 |

