

REPORT

DETERMINATION OF THE PARTITION COEFFICIENT (N-OCTANOL/WATER) OF
SURFACTANT F058

RCC NOTOX Project 090743
RCC NOTOX Substance 27378

REPORT APPROVAL

STUDY DIRECTOR:

Drs. R. de Vries



Date: 26/4/93.....

MANAGEMENT:

J.A.M.W. van Helvoirt
(Section head Physico Chemistry)



Date: ... April ... 27 ... 1993

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SUMMARY

The determination of the partition coefficient (n-octanol/water) was based on OECD Guideline No.107 (1981), OECD Guideline No.117 (1989) and EEC Directive A.8 (1984).

The partition coefficient (P_{ow}) of SURFACTANT F058 was estimated to be $> 1.8 \times 10^3$ ($\log P_{ow} > 3.3$) as a quotient of the solubility of the test substance in n-octanol and water.

PREFACE

GENERAL

Title	Determination of the partition coefficient (n-octanol/water) of SURFACTANT F058
Sponsor	Dowell Schlumberger Inc. P.O. Box 2710 TULSA, Oklahoma 74101 USA
Study Monitor	Mr. D. DeBolt
Testing Facility	RCC NOTOX B.V. Hambakenwetering 3 5231 DD 's-Hertogenbosch The Netherlands
RCC NOTOX Project	090743
Test substance	SURFACTANT F058

PROJECT STAFF

Study Director	Drs. R. de Vries (RCC NOTOX B.V.)
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SCHEDULE

Start of the study	31 March 1993
Completion of the study	01 April 1993

QUALITY ASSURANCE STATEMENT

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

RCC NOTOX Project 090743

Test substance SURFACTANT F058

Study Director Drs. R. de Vries

Title Determination of the partition coefficient
(n-octanol/water) of SURFACTANT F058

Study procedures were subjected to periodic inspections.

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

Dates of QAU Inspections/Audits	Reporting Date
19 March 1993	19 March 1993
01 April 1993	01 April 1993
22 April 1993	22 April 1993

General non study specific processes are also inspected at least once per 3 monthly interval and results reported to management.

Manager, Quality Assurance Unit: C. Mitchell B.Sc.



Date: 28.4.93.....

STATEMENT OF GLP COMPLIANCE

RCC NDOX Project	090743
Test substance	SURFACTANT F058
Study Director	Drs. R. de Vries
Title	Determination of the partition coefficient (n-octanol/water) of SURFACTANT F058

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

OECD Principles of Good Laboratory Practice, Paris, France.

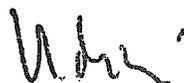
United States Environmental Protection Agency, (FIFRA). Title 40 Code of Federal Regulations Part 160.

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

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

Study Director:

Drs. R. de Vries



Date: 26/4/93.....

GUIDELINES

The study procedure described in this report was based on the following guidelines:

Organization for Economic Co-operation and Development (OECD). OECD guideline for testing of chemicals no. 107: "Partition coefficient (n-octanol/water)", Adopted May 12, 1981.

Organization for Economic Co-operation and Development (OECD). OECD guideline for testing of chemicals no. 117: "Partition coefficient (n-octanol/water) High Performance Liquid Chromatography (HPLC) method", adopted March 30, 1989.

European Economic Community (EEC), EEC-Directive 84/449 EEC, Annex V, Part A, Methods for the determination of physico-chemical properties, A.6: "Partition coefficient". EEC Publication no. L251, September 1984.

ARCHIVING

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

OBJECTIVE

PURPOSE

The purpose of the study was to evaluate the partition coefficient of the test substance between n-octanol and water.

PRINCIPLE

The partition coefficient (P_{ow}) is defined as the ratio of the equilibrium concentrations (c) of a dissolved substance in a two phase system consisting of two largely immiscible solvents. In the case of n-octanol and water:

$$P_{ow} = C_{n\text{-octanol}} / C_{\text{water}}$$

The partition coefficient therefore is the quotient of two concentrations and is usually given in the form of its logarithm to base ten ($\log P_{ow}$). The flask-shaking method (as described in OECD Test Guideline 107) and the HPLC-method (as described in OECD Test Guideline 117) are the most important methods for the determination of the partition coefficient.

In this case, the test substance is surface active. Therefore, neither the flask-shaking method nor the HPLC-method is applicable. For this reason, the partition coefficient was estimated from the n-octanol solubility and the water solubility of the test substance.

MATERIALS AND METHODS

TEST SYSTEM

Test system	Double distilled water and n-octanol (HPLC grade, Aldrich Chemical Co., Inc., USA).
Rationale	Recognized by the international guidelines as recommended test system (EEC, OECD).

TEST SUBSTANCE

Identification	SURFACTANT F058
Description	Clear liquid
Batch	IB 92 302
Purity	100%
Storage conditions	At room temperature in the dark
Stability under storage conditions	Stable
Expiry date	July 1, 1993

PERFORMANCE OF THE TEST

n-Octanol solubility	25 ml n-octanol was added to an amount of 28.0 g of the test substance in an Erlenmeyer flask and stirred overnight at room temperature. The concentration of the test substance was determined using a spectrophotometric method.
Water solubility	An amount of 0.5 g of the test substance was added to 1000 ml double distilled water in an Erlenmeyer flask and stirred overnight at room temperature. The resultant phase was observed visually before and after centrifugation (25 minutes, 46500 g, 20°C).

METHOD OF CHEMICAL ANALYSIS

The concentration of SURFACTANT F058 was determined using a spectrophotometric method. The conditions used are described below:

Sample pretreatment n-octanol solubility

From the n-octanol phase, a sample of approximately 10 ml was taken and centrifuged for 25 minutes at 46500 g and 20°C. The resultant supernatant was diluted 10 times with n-octanol whereafter an absorption spectrum between 200 nm and 900 nm was recorded. In addition, the absorbance at 205 nm was measured.

Instrumentation

UV-Vis spectrophotometer	DU-68 UV/Vis spectrophotometer (Beckman Instruments Inc., Fullerton, CA, USA)
Cuvet	Quartz, pathlength = 1.00 cm.
Scanspeed	750 nm/min
Wavelength	205 nm
Slit	2 nm
Background-correction	n-Octanol

The instrument is calibrated on a six monthly basis using potassium dichromate solutions (p.a., Merck, Darmstadt, FRG) for absorbance accuracy and linearity, and a holmium perchlorate solution (50 g/l holmium(III)-oxide (LAB, Merck)) in 1 N perchloric acid (p.a., Merck) for wavelength accuracy.

Quantitative analyses

Two standard solutions of SURFACTANT F058 in n-octanol (HPLC grade, Aldrich Chemical Co., Inc., USA). of approximately 20 and 30 g/l were prepared. Absorption spectra between 200 nm and 900 nm were recorded. In addition, the absorbances at 205 nm were measured.

DATA HANDLING

1. Because the n-octanol solubility of SURFACTANT F058 was > 100 g/l, a correction for the mass fraction of the test substance in the n-octanol phase was made:

Concentration analysed = A g/l [test substance/n-octanol phase]

A g test substance = A x 10⁻³ l test substance, when the density of the test substance was assumed to be 1 g/10⁻³l.

After correction:

$$\text{n-Octanol solubility} = \frac{A \times 1000}{(1000 - A)} \text{ g/l [test substance/n-octanol]}$$

2. The P_{ow} (estimate) was calculated from the solubilities of the test substance in the pure solvents:

$$P_{ow} \text{ (estimate)} = \frac{C_{\text{saturation n-octanol}}}{C_{\text{saturation water}}}$$

3. The logarithm to base 10 of each P_{ow} was calculated (log P_{ow}).

RESULTS

Representative UV/Vis absorption spectra of a standard solution of SURFACTANT F058 and a pretreated n-octanol phase are shown in figures 1 and 2.

The test substance concentration in the n-octanol phase was determined to be 8.9×10^2 g/l.

The resultant water phase was a turbid liquid. Centrifugation did not result in phase-separation. Therefore, no analyses were performed. The concentration prepared was 0.5 g/l. Hence, the water solubility of SURFACTANT F058 was estimated to be < 0.5 g/l.

The partition coefficient (n-octanol/water) P_{ow} of SURFACTANT F058 was estimated to be $> 1.8 \times 10^3$ ($\log P_{ow} > 3.3$) as a quotient of the solubility of the test substance in n-octanol and water.

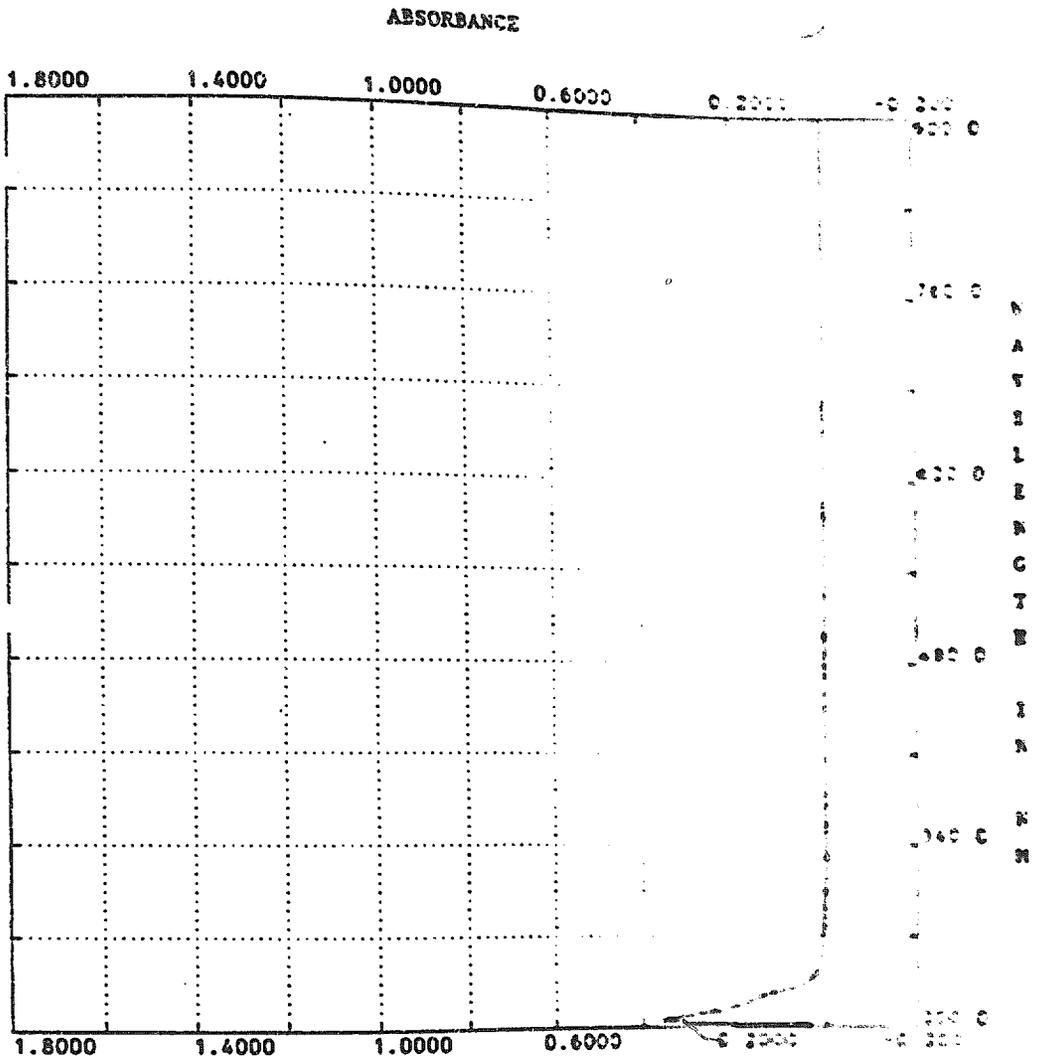


Figure 1 UV/Vis absorption spectrum of a standard solution of 3.4 g/l of SURFACTANT FO58 in n-octanol.

Background-correction: n-octanol
 Scanspeed: 750 nm/min
 Slit: 2 nm
 Note: the wavelength of maximum absorbance was 205 nm

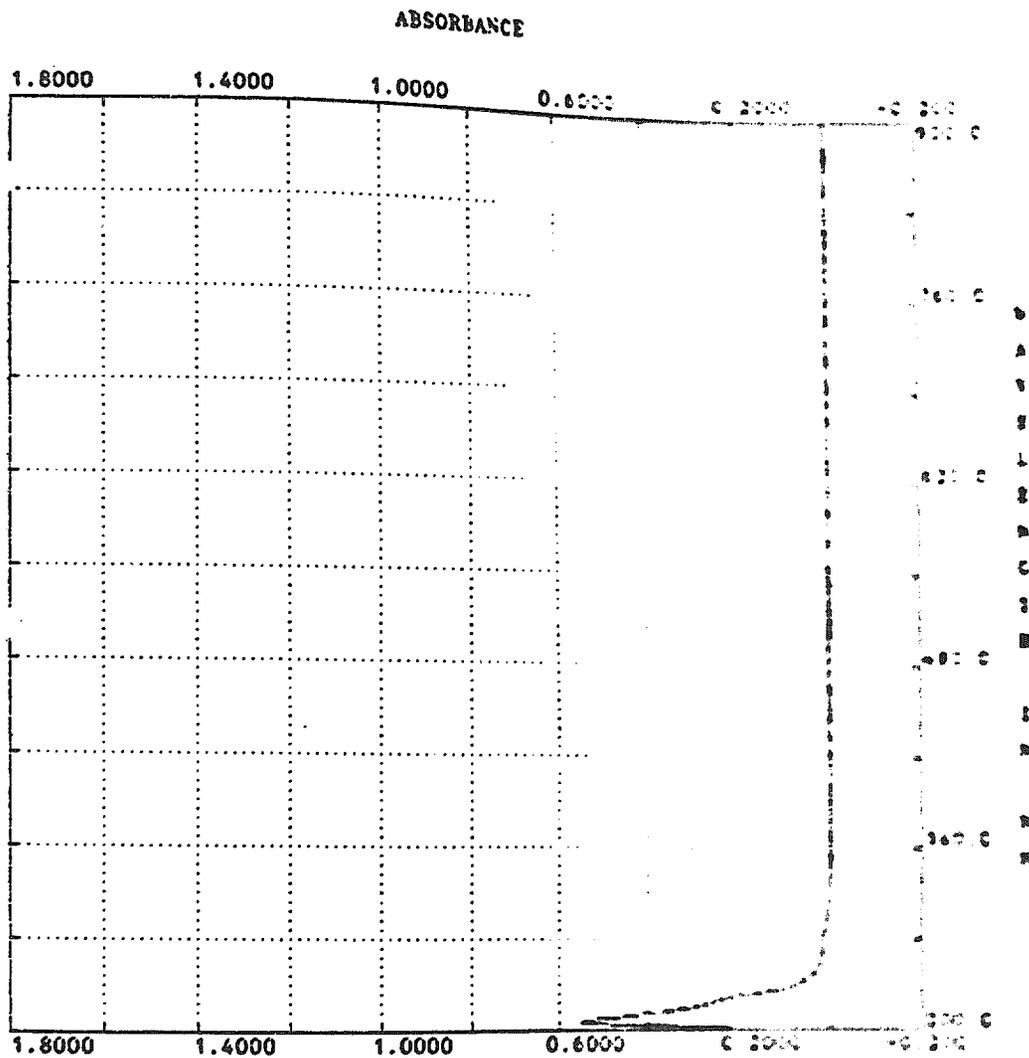


Figure 2 UV/Vis absorption spectrum of the n-octanol phase.

Background-correction: n-octanol
Scanspeed: 750 nm/min
Slit: 2 nm

REPORT

**BIODEGRADABILITY IN SEAWATER: CLOSED BOTTLE TEST
WITH
MID-TEMP RETARDER D801**

**NOTOX Project 113625
NOTOX Substance 36954**

STATEMENT OF GLP COMPLIANCE

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: December 30, 1993

QUALITY ASSURANCE STATEMENT

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/ AUDITS	REPORTING DATES
04-11-1993	08-11-1993
23-11-1993	23-11-1993
30-12-1993	30-12-1993

Quality Assurance Manager

C.J. Mitchell B.Sc.



Date: 5 Jan 94 .

REPORT APPROVAL

STUDY DIRECTOR:

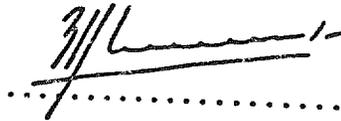
Ing. M.J.E. Koopmans



Date: December 30, 1993

MANAGEMENT:

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



Date: 30/12/1993

PREFACE

Sponsor Dowell Schlumberger
c/o. P.O. Box 20
4780 AA MOERDIJK
The Netherlands

Study Monitor Mr. H. Romijn

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

Study Director Ing. M.J.E. Koopmans

Study Plan Start : November 25, 1993
Completed : December 23, 1993

TEST SUBSTANCE

Identification Mid-temp retarder D801
Description Dark brown liquid
Batch 9300012518
Purity 40% solution in water
Instructions for
test substance storage At room temperature in the dark
Stability under storage
conditions Stable
Expiry date July 01, 1994
Stable for at least
96 hours in vehicle Water : yes

PURPOSE

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

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

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

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.

TEST SYSTEM

Source

The source of test organisms were micro-organisms in natural seawater. Natural seawater was taken at a location in the North Sea:

Location : 52°37'60''N - 2°50'00''E
Date of collection: 05-11-1993
Temperature collection: 13°C
Depth collection: 2.5 meters
Appearance sample: not indicated

Treatment

The seawater was transported in a clean tank to NOTOX and kept in the dark, under continuous aeration during at least 5 days. Prior to the test, density and salinity were determined and the seawater was filtered through a coarse filter paper.

Density : 1.0254 g/cm³ at 19°C

Salinity: 36 ‰ (19.83 ‰ Cl)

(Chem. Oceanography; J.P. Riley, G. Shirrow)

During the test-period, the microbial count was determined.

Microbial count : 925 colonies/ml

(Agar-plates used for microbial count contain 2.5 g yeast extract, 2.5 g bacto peptone and 15.0 g purified agar per litre natural seawater).

Reason for selection

This test is a seawater variant of the Closed Bottle Screening Method and was finalized as a result of a ring test organized for the EEC by the Danish Water Quality Institute.

TEST PROCEDURE AND CONDITIONS

Test duration	28 days.
Test vessels	250-300 ml oxygen bottles with glass stoppers.
Milli-Q water	Tap-water purified by reverse osmosis and subsequently passed over activated carbon and ion-exchange cartridges (Millipore Corp., Bedford, Mass., USA).
Stock solutions of nutrients	A) 8.50 g KH_2PO_4 21.75 g K_2HPO_4 67.20 g $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ 0.5 g NH_4Cl dissolved in 1 l Milli-Q water The pH value was 7.4 ± 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 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 5 (low) and 25 mg/l (high), corresponding to respectively 2 and 10 mg of the active ingredient/l.
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

A stock solution of 100 mg/l was prepared by adding 201.0 mg test substance to 2 l 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

Frequency	Immediately at the start of the experiment (day 0), and at day 5, 15 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

The measured BOD-values were corrected for the background (endogeneous) O₂ 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

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 toxicity 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

O₂-consumption

The COD of MID-TEMP RETARDER D801 was determined to be 0.480 mg O₂/mg.

The ThOD of sodium benzoate (positive control) was calculated to be 1.665 mg O₂/mg.

Test conditions

pH, O₂-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.

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 O₂ measurements performed during the test period of 28 days revealed no significant degradation of MID-TEMP RETARDER D801 at both concentrations.

Since oxygen consumption of the toxicity 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), MID-TEMP RETARDER D801 was not toxic to the micro-organisms present in the medium.

Acceptability of the test

The control substance was biodegraded by 60% within 15 days (see Table 2 and Figure 1).

Oxygen consumption in the blank was about 23% relative to the oxygen consumption in the test bottles at the start of the test.

CONCLUSION

Under the conditions of this present test MID-TEMP RETARDER D801 was not biodegradable in the closed bottle test with natural seawater medium.

Table 1: Oxygen depletion at different points in time

Test medium	Concentration (mg/l)	Oxygen depletion (mg BOD/l) after x days*		
		5	15	28
Positive control	2	1.97	2.01	2.19
Test subst. low	5	-0.34	-0.33	-0.36
Test subst. high	25	-0.02	-0.01	-0.01
Toxicity control	**	2.06	1.99	2.02

*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*		
		5	15	28
Pos. control**	2	59	60	66
Test subst. low***	5	-14	-14	-15
Test subst. high***	25	0	0	0

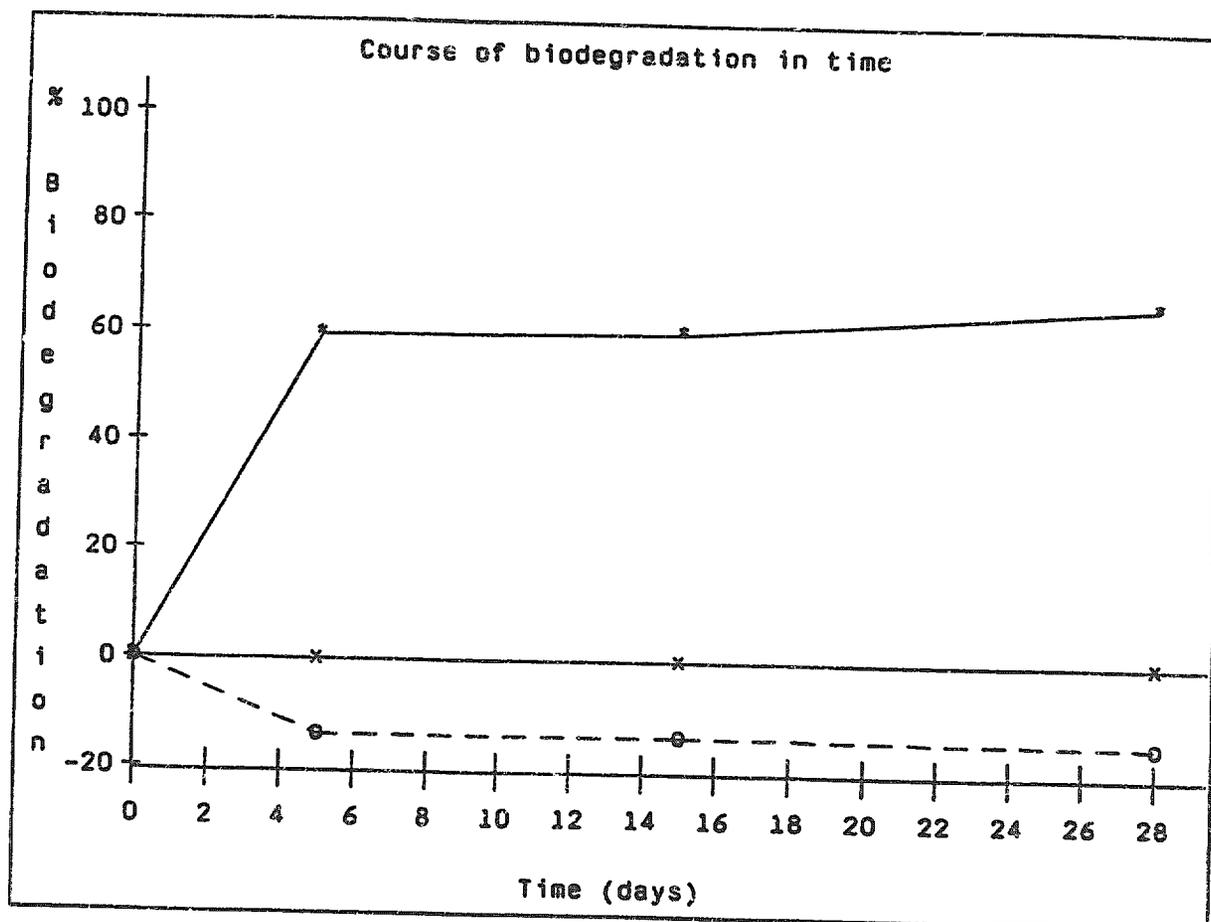
*For calculations see Appendix

**ThOD positive control sodium acetate in mg O₂/mg: 1.665

*** COD test substance in mg O₂/mg: 0.480

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

- * : positive control;
- o : MID-TEMP RETARDER D801 (5 mg/l);
- x : MID-TEMP RETARDER D801 (25 mg/l).



APPENDIX

A O₂ determinations:

Series	Content	Stock solution		mg O ₂ /l after x days			
		pH	t(°C)	0	5	15	28
Inoculum blank	Nutrient medium	8.0	16.9	8.78	7.88	7.33	6.94
				8.82	8.12	7.28	6.62
				Mean mB	8.80	8.00	7.31
Positive control mg/l 2	Nutrient medium, Pos. contr.	8.0	18.0	8.56	5.92	5.20	4.40
				8.71	5.82	5.07	4.45
				Mean mP	8.64	5.87	5.14
Test substance mg/l 5	Nutrient medium, Test subst. Low	8.0	17.9	8.55	6.20	7.48	6.95
				8.74	8.17	7.49	7.03
				Mean mL	8.65	8.19	7.49
Test substance mg/l 25	Nutrient medium, Test subst. High	8.0	17.6	8.63	7.79	7.12	6.76
				8.60	7.88	7.16	6.45
				Mean mH	8.62	7.84	7.14
Toxicity control	Natural seawater, Test subst. Pos. contr.	8.0	17.6	8.68	5.81	5.27	4.64
		5	mg/l	8.68	5.83	5.12	4.63
		2	mg/l	Mean mI	8.68	5.82	5.20

