

REPORT

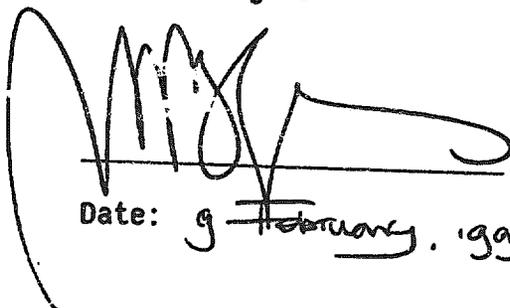
**BIODEGRADABILITY IN SEAWATER:
28 DAYS CLOSED BOTTLE TEST
WITH
CHELATING AGENT LO41**

**RCC NOTOX Project 086939
RCC NOTOX Substance 27306**

REPORT APPROVAL

STUDY DIRECTOR

Drs. M. Bogers



A large, stylized handwritten signature in black ink, appearing to read 'M. Bogers', is written over a horizontal line.

Date: 9 February, 1993

MANAGEMENT:

Ing. E.J. van de Waart
(Section Head, Genetic &
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A handwritten signature in black ink, appearing to read 'E.J. van de Waart', is written over a horizontal line.

Date: 11/02/1993

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SUMMARY

CHELATING AGENT LO41 was tested for its biodegradability in the closed bottle test in a natural seawater medium at concentrations of 2 and 5 mg/l.

The chemical oxygen demand (COD) was determined to be 0.536 mg O₂ per mg CHELATING AGENT LO41.

During the total test period no significant degradation (< 10%) of CHELATING AGENT LO41 was observed at both concentrations.

Under the same conditions sodium benzoate was degraded by more than 60%.

Therefore, CHELATING AGENT LO41 was not biodegradable in the closed bottle test with natural seawater presently performed.

Finally, CHELATING AGENT LO41 was not toxic to the micro-organisms present in the medium at 2 mg/l.

PREFACE

GENERAL

Title Biodegradability in seawater: 28 days closed bottle test with CHELATING AGENT LO41.

Sponsor Dowell Schlumberger Inc.
P.O. Box 2710
TULSA, Oklahoma 74101
USA

Study Monitor Mr. D. DeBolt

Testing Facility RCC NOTOX B.V.
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RCC NOTOX Project 086939

Test Substance CHELATING AGENT LO41

Test System Micro-organisms in natural seawater

PROJECT STAFF

Study Director Drs. M. Bogers (RCC NOTOX B.V.)

Technical Head Ing. J.J.C. van der Poel (RCC NOTOX B.V.)

SCHEDULE

Start of the study 08-10-1992
(Inoculation of test flasks)

Completion of the study 05-11-1992
(Last day of O₂ determination)

STATEMENT OF GLP COMPLIANCE

RCC NOTOX Project	086939
Test Substance	CHELATING AGENT LO41
Study Director	Drs. M. Bogers
Title	Biodegradability in seawater: 28 days closed bottle test with CHELATING AGENT LO41

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.

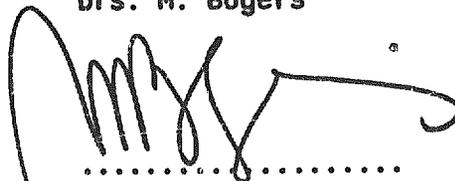
United States Environmental Protection Agency, (TSCA). Title 40 Code of Federal Regulations Part 792.

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

*) The exception was that the determination of the Chemical Oxygen Demand was not performed under GLP conditions.

Study Director

Drs. M. Bogers



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Date: 9 February, 1993

GUIDELINES

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

Organisation for Economic Co-operation and Development (OECD), OECD guidelines for Testing of Chemicals, Biodegradation in seawater: Closed Bottle Method, Paris 1990.

Organisation for Economic Co-operation and Development (OECD), UPDATING OECD guidelines for Testing of Chemicals, "Ready Biodegradability" June 1990, Part 1 General Conditions.

ARCHIVING

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

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

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 (w/v) of the test substance, the net oxygen depletion is obtained in mg BOD/mg test substance.

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

RATIONALE

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

MATERIALS AND METHODS

TEST SYSTEM

Source	<p>The source of test organisms were micro-organisms in natural seawater. Natural seawater was taken at a location in the North Sea:</p> <p>Location : 52°06 30 NL - 03°14 60 EL Date collection: 02-10-1992 Temp.collection: 16°C Depth collection: 4 meters Appearance sample: clear</p>
Treatment	<p>The seawater was transported in a clean tank to RCC NOTOX and kept in the dark, under continuous aeration during at least 5 days. Prior to the test, density, salinity and microbial count were determined and the seawater was filtered through a course filter paper.</p> <p>Density : 1.0248 g/cm³ at 21.5 °C Salinity: 19.4 O/00 C1 (Chem. Oceanography; J.P. Riley, G. Shirrow) Microbial count : 2.48 x 10³ 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)</p>
Temperature	20 ± 1°C
Reason for selection	<p>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.</p>

TEST SUBSTANCE

Identification	CHELATING AGENT L041
Description	White powder
Batch	IB6177
Purity	Mixture
Instructions for test substance storage	At room temperature in the dark Avoid contact with aluminium
Stability under storage conditions	Stable
Expiry date	March 1, 1993
Stable for at least 96 hours in vehicle	Water : yes

CONTROL SUBSTANCE

Sodium benzoate.

TEST PROCEDURE AND CONDITIONS

Test duration	28 days
Test vessels	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).
Nutrient solutions	<p>Stock solutions</p> <p>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.</p> <p>B) 22.50 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ dissolved in 1 l Milli-Q water.</p> <p>C) 36.40 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ dissolved in 1 l Milli-Q water.</p> <p>D) 0.15 g FeCl_3 dissolved in 1 l Milli-Q water.</p>
Nutrient medium	The following amounts were added per litre of natural seawater for the formulation of the nutrient medium: 1 ml of each of the stock solutions A, B, C, D. All nutrients were mixed in such a way that no precipitation occurred.
Test concentrations	<p>The concentrations of the test substance were 2 mg/l (low) and 5 mg/l (high). Individual series of oxygen bottles were filled with the respective prepared test solutions.</p> <p>Each test includes complete parallel series for the determination of the oxygen depletion, in the presence of seawater micro-organisms (inoculum blank), and with the positive control. All bottles were incubated in the dark during the test period.</p>

Controls

Inoculum blank: contained seawater micro-organisms but neither test nor control substance.

Positive control to check inoculum activity: 2 mg/l sodium benzoate with seawater micro-organisms.

Inhibition control: 2 mg/l sodium benzoate with seawater micro-organisms and the low concentration of the test substance.

PREPARATION OF TEST MEDIA

The test substance was soluble in water therefore a stock solution of 0.1 g/l was made in test medium. An amount of test substance stock solution corresponding to the test concentrations was then added to 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.
Apparatus	Oxygen electrode (Tri Ox EO 200, WTW, FRG); accuracy 0.5%.
Calibration	Directly before each series of determinations.
Expected Oxygen demand	The Chemical Oxygen demand 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 inoculum blank.

The time needed for the first 10% biodegradation was considered as a lag phase. A test substance was considered to be biodegradable in seawater if a total biodegradation of 60% was reached within 28 days starting from the end of the lag phase.

Potential toxicity: the toxicity of the test substance for the micro-organisms in the medium was evaluated by comparison of the respective BOD's of the positive control and the low concentration of the test substance with the BOD recorded for the inhibition control.

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

ACCEPTABILITY OF THE TEST

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

- the control substance was biodegraded by at least 60% within 28 days
- oxygen consumption in the blank at the end of the test did not exceed 30% of the oxygen in the test bottle.

RESULTS

O₂-CONSUMPTION

The Chemical Oxygen Demand (COD) of CHELATING AGENT LO41 was determined to be 0.536 mg O₂/mg.

The ThOD of sodium benzoate (positive control) was calculated to be 1.670 mg O₂/mg. Because the maximum consumable oxygen was considered to be 3.4 mg O₂/l medium, the following nominal concentrations were prepared:

- low concentration 2 mg CHELATING AGENT LO41/l
- high concentration 5 mg CHELATING AGENT LO41/l
- sodium benzoate 2 mg/l.

TEST CONDITIONS

Temperature seawater (after aeration): 20.0 °C

O₂-concentration of seawater (after aeration): 8.71 mg O₂/l

pH values of different stock solutions: 8.1-8.2

Temperature of different stock solutions: 19.6 to 19.8 °C.

BIODEGRADATION

After 28 days of incubation negative values for biodegradation were observed at both concentrations of CHELATING AGENT LO41, i.e. -14% and -1% for the low and high concentration respectively (Table 2). Comparison of the oxygen concentrations measured at both test concentrations with the controls indicates that the differences responsible for those negative values are within the variation of the duplicate measurements.

Considering the differences in oxygen depletion between the test concentrations and the control as not relevant, oxygen consumption in the inhibition control was > 75% of the oxygen consumption in the positive control (Table 1). Hence, CHELATING AGENT LO41 was not toxic to the microorganisms present in the medium at 2 mg/l.

ACCEPTABILITY OF THE TEST

The control substance was biodegraded for more than 60% within 5 days (see Table 2 and Figure 1).
Oxygen consumption in the blank at the end of the test was <30% of the oxygen in the test bottles at the start of the test.

CONCLUSION

The results of the present study reveal that CHELATING AGENT LO41 was not biodegradable in the closed bottle test with seawater medium presently performed.

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	2.84	2.99	3.14
Test subst. low	2	-0.30	-0.17	-0.15
Test subst. high	5	-0.25	-0.14	-0.02
Inhibition control**		2.32	2.58	2.73

*For calculations see Appendix

**Inhibition control contains positive control and test substance low

Table 2: % Biodegradation at different points in time

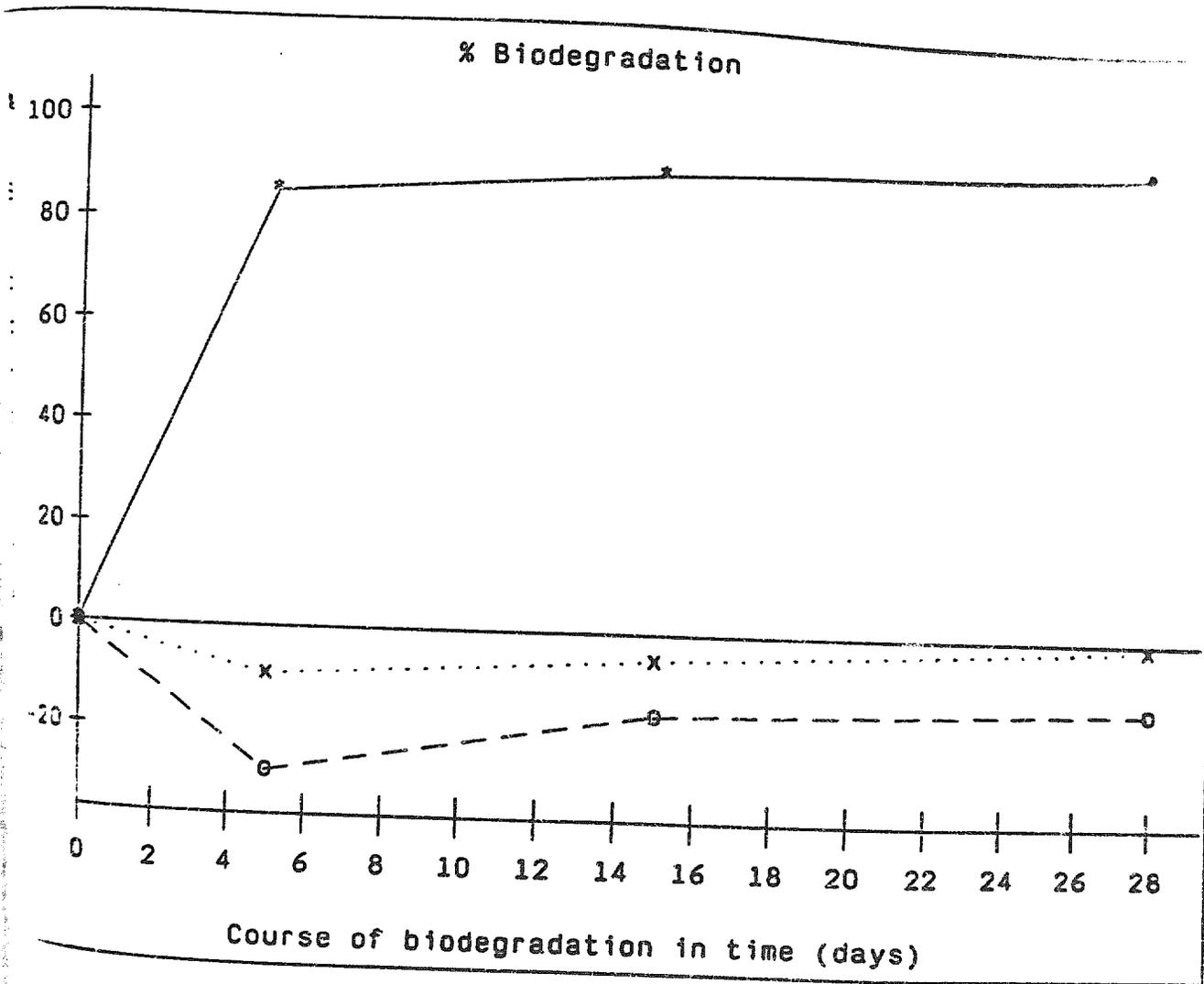
Test medium	Concentration (mg/l)	% Biodegradation after x days*		
		5	15	28
Positive control**	2	85	90	94
Test subst. low***	2	-28	-16	-14
Test subst high***	5	-9	-5	-1

*For calculations see Appendix

**ThOD positive control sodium acetate in mg O₂/mg: 1.67

***COD test substance: 0.536 mg O₂/mg.

Figure 1: Course of degradation in time for the different test media
 * :positive control;
 o :CHELATING AGENT LO41 2 mg/l;
 x :CHELATING AGENT LO41 5 mg/l.



APPENDIX

FORMSHEET FOR CLOSED BOTTLE TEST

A O₂ determinations:

Series	Content	Stock solution pH	solution t[°C]	Fleske No.	mg O ₂ /l 0	after 5	6 days 12	20
Inoculum blank	Medium* inoculum	8.1	19.8	2A	8.66	6.96	6.30	6.76
				2B	8.78	6.73	6.59	6.59
				Mean mB	8.72	6.89	6.69	6.66
				Positive control mg/l 2	Medium, inoculum, Pos. contr.	8.1	19.8	3A
3B	8.76	9.96	9.89	9.28				
Mean mP	8.68	9.99	9.80	9.28				
Test substance mg/l 2	Medium, inoculum, Test subst. Low	8.1	19.8	4A				8.56
				4B	8.67	7.06	6.96	6.96
				Mean mL	8.62	7.09	6.90	6.92
				Test substance mg/l 5	Medium, inoculum, Test subst. High	8.2	19.8	5A
5B	8.68	7.02	6.88					6.89
Mean mH	8.67	7.09	6.97					6.49
Inhibition control	Medium, inoculum, Test subst. 2 Pos. contr. 2	8.2	19.6					6A
				6B	8.66	4.40	3.72	3.92
				Mean mI	8.62	4.41	3.79	3.69

* Medium = natural seawater + nutrient solutions

FORMSHEET FOR CLOSED BOTTLE TEST (continued)

B Control substance: O₂ Depletions (mg BOD/l) and % Biodegradation after x days

	mg BOD/l after x days		
	5	15	28
Positive control:			
BOD.Px = (mPO - mPx) - (mBO - mBx)	2.84	2.99	3.14
Inhibition control			
BOD.Ix = (mIO - mIx) - (mBO - mBx):	2.32	2.58	2.73

$$\% \text{ Biodegradation} = \frac{\text{mg BOD.Px/l} \times 100}{\text{mg PC/l} \times \text{ThOD.PC}}$$

PC = Positive control in mg/l: 2.0
 ThOD Posit. contr. in mg O₂/mg: 1.670

	% Biodegradation after x days		
	5	15	28
Positive control:	85	90	94

C Test substance: O₂ Depletions (mg BOD/l) and % Biodegradation after x days:

	mg BOD/l after x days		
	5	15	28
Test substance low			
BOD.Lx = (mLO - mLx) - (mBO - mBx):	-0.30	-0.17	-0.15
Test substance high			
BOD.Hx = (mHO - mHx) - (mBO - mBx):	-0.25	-0.14	-0.02

$$\% \text{ Biodegradation} = \frac{\text{mg BOD.x/l} \times 100}{\text{mg TS/l} \times \text{COD.TS}}$$

TS = Test substance in mg/l low: 2
 high: 5
 COD Test substance in mg O₂/mg: 0.536

	% Biodegradation after x days		
	5	15	28
Test substance low:	-28	-16	-14
Test substance high:	-9	-5	-1

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