

REPORT

**ACUTE TOXICITY TO THE MARINE COPEPOD
ACARTIA TONSA OF
GYPBAN (*) L059 SCALE INHIBITOR AID**

**NOTOX Project 113838
NOTOX Substance 37017**

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.

Study Director

Ing. M.J.E. Koopmans



Date: January 25, 1994

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
25-11-1993	25-11-1993
12-01-1994	13-01-1994
25-01-1994	25-01-1994

Quality Assurance Manager

C.J. Mitchell B.Sc.



Date: 26-1-94

REPORT APPROVAL

STUDY DIRECTOR:

Ing. M.J.E. Koopmans



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Date: January 25, 1994

MANAGEMENT:

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

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Dr. Iona C. Enninga
Technical Director

Date: 26/01/1994

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 : December 22, 1993 Completed : January 14, 1994

TEST SUBSTANCE

Identification	Gypban (*) L059 scale inhibitor aid
Description	White-yellowish powder
Batch	RCM193003/G
Purity	100%
Instructions for test substance storage	At room temperature in the dark
Stability under storage conditions	Stable
Expiry date	January 01, 1996
Stable for at least 96 hours in vehicle	Water : yes

PURPOSE

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 48h-LC50.

GUIDELINES

The study procedure described in this report 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

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

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.

TEST SYSTEM

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

Breeding	The parental populations are held in natural seawater, filtered over 0.2 μ 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. At the start of each culture: oxygen concentration.
Feeding	<u>Acartia</u> were fed on a diet of <u>Rhodomonas baltica</u> and/or <u>Skeletonema costatum</u> .
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	A reference test with potassium dichromate ($K_2Cr_2O_7$) is carried out every month. The results of the most recent test are appended.

RANGE-FINDING TEST

Range-finding tests were performed to provide information about the range of concentrations to be used in the final test. In a first range-finding test A. tonsa were exposed for 48 hours to a filtered and unfiltered solution of 1000 mg/l. Since 100% mortality was observed in the first range-finding test a second range-finding test was performed. In the second range-finding test A. tonsa were exposed for 48 hours to 0.1 to 1000 mg/l forming a geometric concentration range with a factor of ca. 10.

TEST PROCEDURE AND CONDITIONS

Test duration	48 hours.
Test vessels	Range-finding tests: 50 ml, polystyrene. Final test: for practical reasons 100 ml, all-glass.
Medium	Natural sea water, filtered through a 0.2 μ m filter, with a salinity of 35-36 per mille.
Test concentrations	Based on the results of the range-finding test: 0.057, 0.1, 0.18, 0.33, 0.57, 1.0, 1.8 and 3.3 mg/l.
Control	Medium without test substance or other additives (0 mg/l).
Number of copepods	70 per concentration, 30 in the control. Unadvertently 21 <u>A. tonsa</u> were exposed at 1.8 mg/l.
Loading	Five per vessel, except at 1.8 mg/l vessel B (six <u>A. tonsa</u>), containing 25 ml test solution.
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 directly after preparation of the test media.

PREPARATION OF TEST MEDIA

The test media were prepared using a stock solution of 10 mg/l by adding quantitatively 20.4 mg of test substance to 2000 ml natural seawater. The solution was stored overnight and thereafter stirred for 28 hours. The final test solutions were all clear without precipitation.

MEASUREMENTS AND RECORDINGS

Mortality	At 24 and 48 hours following the start of exposure. Dead copepods were not removed when observed.
pH and Oxygen	Daily, beginning at the start of the test, in one extra vessel without copepods at 0 mg/l and the highest test concentration.
Temperature	Daily in a vessel with water in the same room, beginning at the start of the test.

ACCEPTABILITY OF THE TEST

- 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 ≥ 4 mg/l.

DATA HANDLING

Calculation of LC50: The LC50-value was calculated at 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

In a first range-finding test 100 % mortality of A. tonsa was recorded in the filtered and unfiltered solution of 1000 mg/l.

In the second range-finding test 100% mortality was observed at 1 mg/l and higher and 30% mortality was observed at 0.1 mg/l.

Final study: mortality of copepods

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

After 24 hours of exposure the total rate of mortality of A. tonsa was 100% at 3.3 mg/l, whereas 15% mortality was recorded at 0.18 mg/l, 25% at 0.33 mg/l, 40% at 0.57 mg/l, 70% at 1.0 mg/l and 86% at 1.8 mg/l. No significant (>10%) mortality of A. tonsa was seen at a concentration of 0.1 mg/l or lower.

After 48 hours of exposure the total rate of mortality of A. tonsa was 100% at and above 1.8 mg/l, whereas 30% mortality was recorded at 0.1 mg/l, 40% at 0.18 mg/l, 60% at 0.33 mg/l, 80% at 0.57 mg/l and 95% at 1.0 mg/l. No significant (>10%) mortality of A. tonsa was seen at 0.057 mg/l.

Final study: experimental conditions

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

The temperature of medium varied from 20 to 20.5°C. The salinity was slightly out of the range mentioned in the protocol, but was considered to have no effect on the integrity of this study.

Final study: acceptability of the test

In the control, no copepods died or became trapped at the surface of the water. Further, except for the deviations mentioned above, all test conditions remained within the ranges prescribed by the protocol.

Determination of LC50-value

The 48h-LC50 was calculated to be nominally 0.21 mg/l with 95% confidence interval ranging from 0.16 to 0.28 mg/l (regression line: $\log_{10}(\text{conc.}) = 2.39 + (\text{probit} - 5.14) / 2.21$, Table 3 and Figure 1).

CONCLUSION

Under the conditions of the present study GYPBAN (*) L059 SCALE INHIBITOR AID did not induce significant acute mortality of Acartia tonsa at 0.057 mg/l after 48 hours of exposure (LC0) with a 48h-LC50 of 0.21 mg/l (95% confidence interval between 0.16 and 0.28 mg/l).

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		Total mortality at the end of the test (%)
			at 24 h	at 48 h	
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	
0.057	A	5	0	0	10
	B	5	0	1	
	C	5	1	1	
	D	5	0	0	
0.1	A	5	0	1	30
	B	5	1	2	
	C	5	1	2	
	D	5	0	1	
0.18	A	5	0	2	40
	B	5	2	2	
	C	5	1	3	
	D	5	0	1	
0.33	A	5	2	3	60
	B	5	2	2	
	C	5	1	4	
	D	5	0	3	
0.57	A	5	3	5	80
	B	5	2	4	
	C	5	2	4	
	D	5	1	3	
1.0	A	5	5	5	95
	B	5	3	5	
	C	5	3	5	
	D	5	3	4	
1.8	A	5	4	5	100
	B	6*	5	6	
	C	5	5	5	
	D	5	4	5	
3.3	A	5	5	5	100
	B	5	5	5	
	C	5	5	5	
	D	5	5	5	

* Inadvertently 6 A. tonsa were exposed.

TABLE 2: pH¹ and oxygen concentrations¹ during the final test.

Nominal Conc. (mg/l)	Start (Day 0)		Day 1		Day 2	
	pH	DO(mg O ₂ /l)	pH	DO(mg O ₂ /l)	pH	DO(mg O ₂ /l)
0	8.2	7.5	8.0	7.5	8.2	7.6
3.3	8.1	7.6	8.5	7.0	8.3	7.4

¹ Measured in an extra vessel without copepods present in the test solution.

DO = dissolved oxygen

TABLE 3: Determination of the 48h-LC50 value for GYPBAN (*) L059 SCALE INHIBITOR AID.

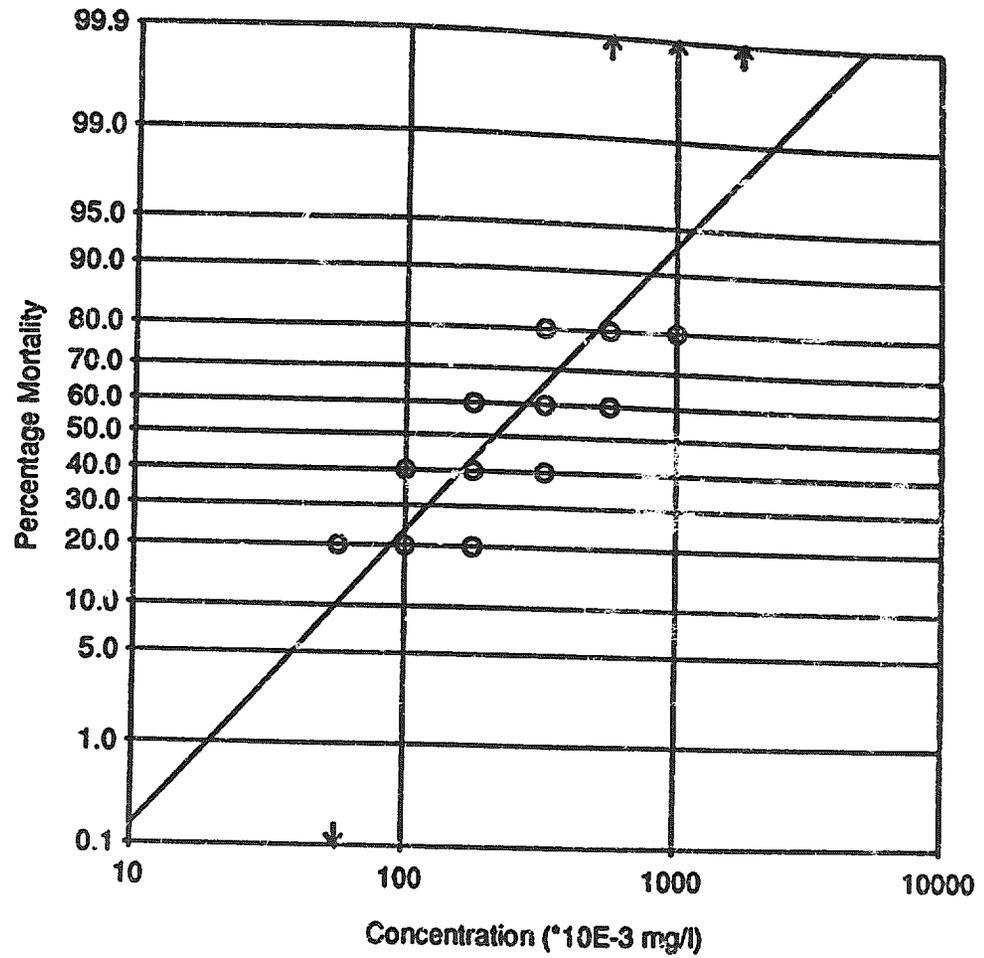
LC50=213.7866 (*10E-3 mg/l)
 95% fiducial limits: 159.7626 - 279.2489 (*10E-3 mg/l)

index of regression significance: g=0.08
 chi-squared=13.51, with 26 degrees of freedom

regression line: $\log_{10}(\text{conc.}) = -2.39 + (\text{probit} - 5.14) / 2.21$

conc. (*10E-3 mg/l)	group size	mortality	corrected fraction	expected fraction	chi2
57	5	0	0.00	0.10	0.55
57	5	1	0.20	0.10	0.57
57	5	1	0.20	0.10	0.57
57	5	0	0.00	0.10	0.55
100	5	1	0.20	0.23	0.03
100	5	2	0.40	0.23	0.78
100	5	2	0.40	0.23	0.78
100	5	1	0.20	0.23	0.03
180	5	2	0.40	0.44	0.03
180	5	2	0.40	0.44	0.03
180	5	3	0.60	0.44	0.55
180	5	1	0.20	0.44	1.12
330	5	3	0.60	0.66	0.09
330	5	2	0.40	0.66	1.54
330	5	4	0.80	0.66	0.42
330	5	3	0.60	0.66	0.09
570	5	5	1.00	0.83	1.04
570	5	4	0.80	0.83	0.03
570	5	4	0.80	0.83	0.03
570	5	3	0.60	0.83	1.81
1000	5	5	1.00	0.93	0.37
1000	5	5	1.00	0.93	0.37
1000	5	5	1.00	0.93	0.37
1000	5	4	0.80	0.93	1.34
1800	5	5	1.00	0.98	0.10
1800	6	6	1.00	0.98	0.12
1800	5	5	1.00	0.98	0.10
1800	5	5	1.00	0.98	0.10
					13.51

FIGURE 1: Percentage response (=mortality) of *Acartia tonsa* as function of the log concentration of GYPBAN (*) L059 SCALE INHIBITOR AID at 48h.



REFERENCE TEST

NOTOX PCAA-94/1

Acute toxicity study in the A. tonsa with potassium dichromate.

From 18-01-1994 to 20-01-1994

This reference test was carried out to check the sensitivity of the test system as used by NOTOX. The reference substance potassium dichromate was tested under similar conditions as the final test with the test substance, using copepods from the same culture.

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

Concentrations: 2, 3, 6, 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 mg/l	total number exposed	total number dead	
		at 24h	at 48h
0	30	1	2
2	10	0	1
3	10	0	0
6	10	0	2
10	10	1	2
20	10	1	5
30	10	0	9
60	10	9	10

The relation between test concentration and response is in agreement with the historical range of the 48h-LC50 for A. tonsa recorded in reference tests since September 1992, which is between 5 and 25 mg/l.

The 24h-LC50 was between 30 and 60 mg/l and the 48h-LC50 was 14.8 mg/l (95% fiducial limits: 11.6 - 20.9 mg/l).

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