

TOXICON AB

TEST REPORT

142/03

Linoljefärg-Vit

***Daphnia magna*, Immobilisation test**

SPONSOR: Ottosson Färgmakeri AB
Lillgårdsv. 14
S-240 13 Genarp
Sweden

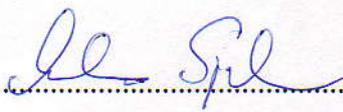
LANDSKRONA OCTOBER 21 2003

ACUTE AQUATIC TOXICITY TEST OF LINOLJEFÄRG-VIT

This report accounts for the results obtained from ecotoxicological tests performed by Toxicon AB, Rosenhällsvägen 23, S-261 92 Landskrona, Sweden.

The study was performed to comply with OECD Guidelines for Good Laboratory Practice. The chemical analysis was performed by an accredited laboratory according to ISO 17025.

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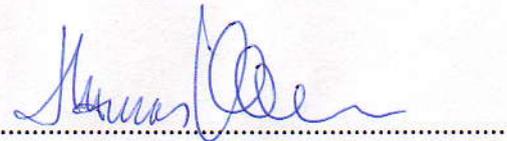
Study Director:  Date: 03/02/21

Anders Sjölin

Technical Performance: Anders Sjölin
Chemical analysis: LMI AB
Box 700
251 07 Helsingborg

Quality Assurance: Thomas Olsson

To the best of my knowledge and belief, this study was conducted in compliance with Good Laboratory Practice regulatories as set fourth in OECD Guidance Document for Good Laboratory Practice, with the exception of possible minor items, none of which is considered to have an impact on the validity of the data or the interpretation of the results in the report. The chemical analysis was performed by an accredited laboratory according to ISO 17025.



Thomas Olsson, B.Sc
Quality Assurance Unit

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Chemical analysis of the water accommodated fraction

Protocol for *Daphnia magna*, Immobilisation test

Statement of the Quality Assurance Unit

Summary

A basic environmental assessment was performed on Linoljefärg-Vit (paint white based on linseed oil and zinc oxide), by Toxicon AB, Landskrona, Sweden, in October 2003.

The following test was carried out:

Daphnia magna, Immobilisation test, OECD TG no 202

The results are summarised in table 1.

Table 1. NOEC, LOEC and EC₅₀ (% v/v) of a water accommodated fraction (WAF) from the test article Linoljefärg-Vit after 24 and 48 hours exposure for the WAF.

	24 h exposure	48 h exposure
NOEC*	50	50
LOEC*	100	100
EC ₅₀	-	-

* was not determined statistically

The loading rate of the test article Linoljefärg-Vit (paint white based on linseed oil and zinc oxide) was 1 000 mg/L control article. The water accommodated fraction (WAF) after 24 hours stirring was used for toxicity testing.

No EC₅₀-value could be calculated since the immobilisation degree at the highest tested concentration (100% v/v) was only 45% after 48 hours exposure. At 50% v/v of the WAF no effect on the immobilisation was found.

This indicate that the WAF was acute toxic towards *Daphnia magna*, however the toxicity can be described as very low.

The total amount of zinc in the WAF was found to be 0,97 mg/L.

Introduction

A sample of the product Linoljefärg-Vit (paint white based on linseed oil and zinc oxide) was received at Toxicon AB on September 24, 2003, for environmental assessment.

The ecotoxicological tests were performed by Toxicon AB, Landskrona, Sweden. The tests started on October 1, 2003 and were finished on October 16, 2002.

The tests were performed according to OECD Guideline for Testing of Chemicals. The results can be used for classification of environmental hazards.

Test article

Product name: Linoljefärg-Vit (paint white based on linseed oil and zinc oxide)
Toxicon identification code: P030165
Batch: -
Molecular weight: -
Formula: -

Characteristics of the test article

The composition of the test article is summarised in table 2.

Table 2. Composition (%) of the test article Linoljefärg- Vit.

Component	(%)
Linseed oil	25
Zinc oxide	50
Titane oxide	25

Test method

The method used was OECD test number 202 "*Daphnia sp.* Acute Immobilisation test," (Toxicon method TOX 036).

The method uses newly hatched animals, 6 to 24 hours old, that are incubated for 48 hours in a gradient of the test substance. The animals were taken from Toxicon's culture of *Daphnia magna*.

The dilution water (control article), as specified in TOX 036 and as suggested in "Proposal for Updating OECD Guideline 202, part II" was used (see below). The water was aerated to an initial oxygen saturation of >90% and a pH of 7.8 ± 0.2 . In the controls, only dilution water was used. The animals were incubated in glass petri dishes with 50 mL solution. Duplicates were used for each test concentration, with the exception of the control group where four replicates were used, with ten animals in each. The dishes were incubated in a thermo constant room at a temperature of $21 \pm 1^\circ \text{C}$ and subdued light with the light rhythm of 16 hours light: 8 hours darkness. pH was measured in all solutions before the experimental start.

The number of immobilised animals in each test concentration were observed at 24 and 48 hours. At the end of the experiment the pH and oxygen saturation were measured in all test dishes.

Preparation of the test article

1 000 mg of the test article was added to 1 000 mL of the control article. The mixture (1 000 mg/L) was stirred for 24 h at room temperature. Then the water phase (water accommodated fraction) was removed and used for toxicity testing. The procedure for generating the water accommodated fraction (WAF) was as described in Concawe report 92/56 –*Ecotoxicological testing of petroleum products: test methodology*.

The following nominal concentrations of the WAF were tested:

0; 6,25; 12,5; 25,0; 50,0 and 100 % v/v

Control article

The control article consist of a standardized dilution water with a pH of 7.8 ± 0.2 and a hardness equivalent of 250 ± 25 mg calciumcarbonate/L.

The control article used is taken from the one described in "Proposal for updating Guideline 202, part II-*Daphnia magna* Reproduction test". The medium is more complex compared to the medium described in OECD guideline 202.

Separate stock solutions of individual trace elements are first prepared in RO-water. Solution A is prepared from these stock solutions (table 3).

Table 3. Schedule for preparing solution A.

Stock solutions	mg/L	The volume added of the stock solutions (of individual trace elements) to prepare stock solution A (ml/L)
H ₃ BO ₃	57 190	0.25
MnCl ₂ *4H ₂ O	7 210	0.25
LiCl	6 120	0.25
RbCl	1 420	0.25
SrCl ₂ *6H ₂ O	3 040	0.25
NaBr	320	0.25
Na ₂ MoO ₄ *2H ₂ O	1 260	0.25
CuCl ₂ *2H ₂ O	335	0.25
ZnCl ₂	260	1.0
CoCl ₂ *6H ₂ O	200	1.0
KI	65	1.0
Na ₂ SeO ₃	43.8	1.0
NH ₄ VO ₃	11.5	1.0
Na ₂ EDTA*2H ₂ O	5 000	*
FeSO ₄ *7H ₂ O	1 991	*

*Na₂EDTA*2H₂O and FeSO₄*7H₂O solutions are prepared singly, poured together and autoclaved immediately. This gives an Fe-EDTA-solution from where 5 ml/L should be added to stock solution A.

The control article is prepared from solution A, B and C. The chemicals used in the solutions are presented in table 4.

Table 4. Schedule for preparing the control article from solutions A, B and C.

Solution	mg/L	The volume added (ml/L) of the stock solutions for preparation of the control article
Solution A		
(trace elements)	-	50
Solution B		
(macro nutrients)		
CaCl ₂ *2H ₂ O	293 800	1.0
MgSO ₄ *7H ₂ O	246 600	0.5
KCl	58 000	0.1
NaHCO ₃	64 800	1.0
Na ₂ SiO ₃ *9H ₂ O	50 000	0.2
NaNO ₃	2 740	0.1
KH ₂ PO ₄	1 430	0.1
K ₂ HPO ₄	1 840	0.1
Solution C		
(vitamin stock)	**	0.1

** Solution C is prepared by adding the three vitamins to one litre deionised water, as shown below:

Thiamine hydrochloride	750 mg/L
Cyanocobalamine (B ₁₂)	10 mg/L
Biotine	7.5 mg/L

Solution C is stored frozen in small aliquots. Vitamins are added to the control article shortly before use. Before use the water should be oxygenated with an air pump, a plastic tube with sintered end. The pH value may be adjusted with HCl or NaOH. An adjusted water should not be aerated again. The control article should allow survival of *Daphnia* for at least 48 hrs.

Reverse osmosis water was used to prepare the control article.

Results

The criteria for validity of the test according to OECD no 202 were fulfilled:

- In the controls, not more than 10% of the *Daphnia* were immobilised or trapped at the surface of the water.
- The dissolved oxygen concentration at the end of the test were $\geq 60\%$ of the air saturation value at the temperature used in all dishes.
- The animal batch used fulfilled the conditions for validity since the reference substance, potassium dichromate, gave an EC_{50} within the proposed interval (0.9-2.0) according to OECD 202.

The results are presented in table 5 and figur 1. Primary data are presented in test protocol 142/03 app.2 (appendix). The zinc content of the WAF was 0,97 mg/L (appendix).

Table 5. NOEC, LOEC and EC_{50} (% v/v) of a water accomodated fraction (WAF) from the test article Linoljefärg-Vit after 24 and 48 hours exposure for the WAF.

	24 hours exposure	48 hours exposure
NOEC*	50	50
LOEC*	100	100
EC_{50}	-	-

* was not determined statistically.

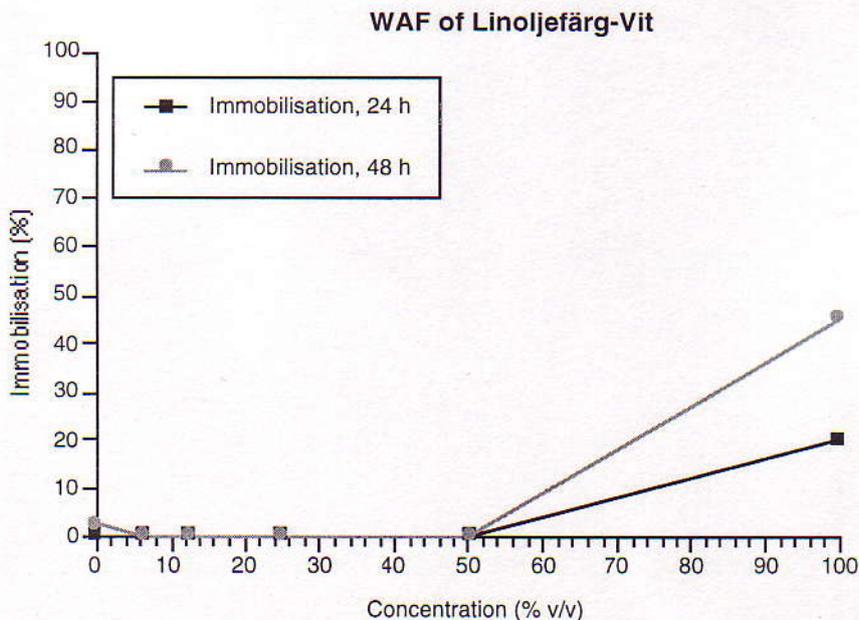


Figure 1. Immobilisation (%) of *Daphnia magna* after 24 and 48 hours exposure at the different test concentrations of a water accomodated fraction (WAF) from the test article Linoljefärg-Vit .

Comments

The loading rate of the test article Linoljefärg-Vit was 1 000 mg/L control article. The water accommodated fraction (WAF) after 24 hours stirring was used for toxicity testing.

No EC_{50} -value could be calculated since the immobilisation degree at the highest tested concentration (100% v/v of the WAF) was only 45% after 48 hours exposure. At 50% v/v of the WAF no effect on the immobilisation was found.

This indicate that the WAF was acute toxic towards *Daphnia magna*, however the toxicity can be described as very low.

The total amount of zinc in the WAF was found to be 0,97 mg/L.

Appendix

Chemical analysis of the water accommodated fraction

Protocol for *Daphnia magna*, immobilisation test

Statement of the Quality Assurance Unit

ANALYS-RAPPORT

nr 6693

Laboratoriets accrediterings nr = 1292

Sida 1 av 2



Datum 2003-10-20

Uppdragsgivare
TOXICON AB
ATT: ANDERS SJÖLIN
ROSENHÄLLSVÄGEN 23
261 92 LANDSKRONA

Vattenprov

Provtagningsdat.
Provtagare
Ankomstdat. 031017
Resultaten angivna i mg/l

ÄMNE		Fe	Al	B
Provnamn	id(nr)			
P030165	1293	* 0.011	< 0.005	0.13

ÄMNE		Mo	Cu	P
Provnamn	id(nr)			
P030165	1293	0.053	* 0.012	* 0.07

ÄMNE		S	Zn	Cd
Provnamn	id(nr)			
P030165	1293	20	0.97	< 0.003

ÄMNE		Mn	Ni	Na
Provnamn	id(nr)			
P030165	1293	0.026	* 0.035	24

Styrelsens säte: Helsingborg Vat no.SE556074758501

ÄMNE		Mg	Ca	K
		-----	-----	-----
Provnamn	id(nr)			
P030165	1293	15	94	4.6

ÄMNE		Si

Provnamn	id(nr)	
P030165	1293	1.16



Analysansvarig

Protocol for acute toxicity of *Daphnia magna* according to SS 028180

OECD 202

X

1 of 1 PROT 021

Laboratory: Toxicon AB

Test leader: Anders Sjölin

Date: 031014 to 031016 (48 hrs)

Sponsor: Ottosson färgmakeri AB

Test substance: Linoljefärg-Vit (zinkoxid 50%)

(P030165)

Protocol no: 142/03 app. 2
Age <i>Daphnia</i> : 6-24 hrs
Number <i>Daphnia</i> /replicate: 10
Temperature start: 22° finish: 21°C

TOXICON AB

Conc. % v/v	Number of immobilised <i>Daphnia magna</i> , 24 and 48 hr				% immobil.		pH	pH	oxyg. %	oxyg. %
	24	48	24	48	total	total				
0 _a	0	0	0	1	0	1	7,8	8,0	102	102
0 _b	0	0	0	0	0	0	7,8	8,0	102	102
6,25	0	0	0	0	0	0	7,8	7,9	102	102
12,5	0	0	0	0	0	0	7,8	7,9	102	102
25,0	0	0	0	0	0	0	7,8	7,9	102	102
50,0	0	0	0	0	0	0	7,8	7,9	102	102
100	2	5	2	4	4	9	7,8	7,8	102	102

48 hours

Graphic Probit

EC (D) 50:

Observations:

EC 50:

LOEC:

0% immobilised, highest conc.:

100% immobilised, lowest conc.:

-	-
-	-
100	-
50	-
-	-

Signature:.....*AS*.....

QUALITY ASSURANCE AUDIT STATEMENT

TOXICON AB, Report 142/03

This report has been audited by Toxicon AB Quality Assurance Unit and is considered to be an accurate presentation of the data produced during the course of the study.

Date: 2003-11-06.....



Thomas Olsson

Toxicon AB
Quality Assurance Unit

The following inspections were made by the Quality Assurance Unit:

	Date of inspection	Inspection number	Date of reporting
Protocol review	<u>031013</u>	<u>031013</u>
Pre-experimental period
Experimental period

Post-experimental period
Final Report Audit	<u>031106</u>	<u>031106</u>