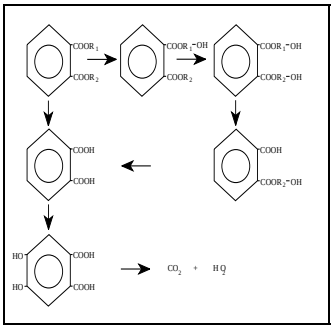
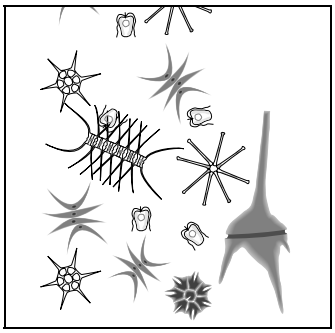
REPORT TO:

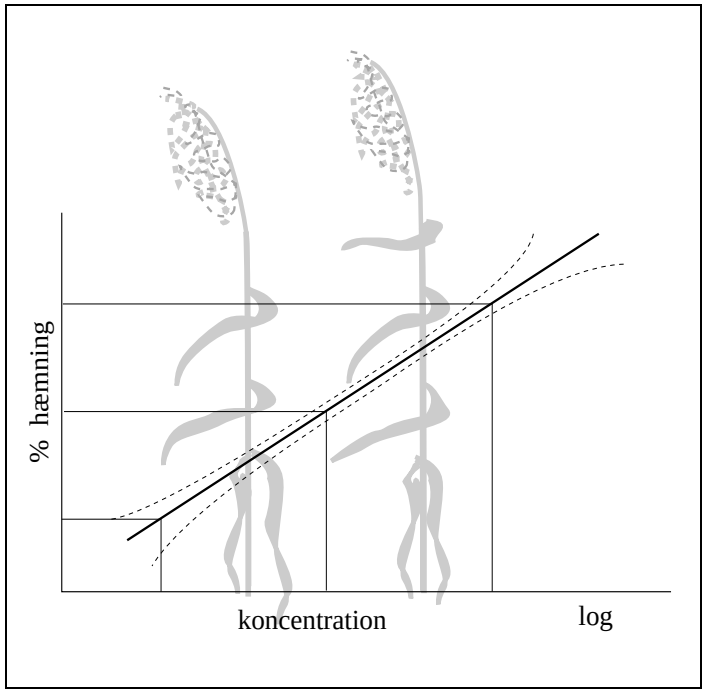
Trion Tensid AB

CONCERNING:

Biodegradability of AGS 221 - Zahn-Wellens/AMPA Test.







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REPORT NO.:
5606

DATE:
January 6, 1998

Title Page

Study Title:

Biodegradability of AGS 221 - Zahn-Wellens/AMPA Test

Laboratory Project/Report Number:

Project no:	97124
Report no:	5606

Volume 1 of 1

Page 1 of 20

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Experimental Starting Date:
November 5, 1997.

Experimental Completion Date:
December 1, 1997.

Study Initiation Date:
October 20, 1997.

Study Completion Date:
January 6, 1998.

Sponsor:

Trion Tensid AB
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Sweden

Report Information

Study Title:

Biodegradability of AGS 221 - Zahn-Wellens/AMPA Test

This report was prepared by:

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Table of Contents

Summary

Objective
The objective of the test was to investigate the ability of the colour remover AGS 221 to be inherently biodegradable using a static test method with activated sludge as inoculum after OECD Guideline 302B (Zahn-Wellens /EMPA Test).

Procedures
Test preparations, comprising test material solutions inoculated with activated sludge microorganisms, are contained in vessels connected to a supply of air free of oil. Control, blank, and reference vessels respectively contain inoculated mineral salts medium alone and inoculated minerals salts medium and the ready biodegradable reference substance sodium acetate. Degradation is followed by DOC analysis at intervals over a 28-day period. If the test products are degraded by more than 20%, it may be regarded as inherently biodegradable, whereas if the degradation is higher than 70%, it may be regarded as ultimately biodegradable.

Conclusion
The product AGS 221 is ultimately biodegradable, i.e. more than 70% of the product is degraded. Because the test was performed as a reduced test, it is not possible to judge the time for reaching the pass level.

Statement of Compliance

The study was conducted according to the Good Laboratory Practice (GLP) standards as described in the decision of the OECD Council concerning the Mutual Acceptance of Data in the Assessment of Chemicals, 12 May 1981 - doc. C (81) 30 (Final) Annex II and in notification no. 685 from 7 November 1989 by the Danish Ministry of Environmental Affairs.

This report is to the best of my knowledge a true and accurate record of the results.

Signature:

Name: Lars Møller Jensen

Title: Study Director, Ph.D.

Date:

Statement of the Quality Assurance Unit (QAU)

This study was performed in compliance with the OECD Principles of Good Laboratory Practice (GLP) as described in the decision of the OECD Council concerning the Mutual Acceptance of Data in the Assessment of Chemicals, 12 May 1981 - doc. C (81) 30 (Final) Annex II and in notification no. 685 from 7 November 1989 by the Danish Ministry of Environmental Affairs.

The study was inspected by the Quality Assurance Unit on the dates described in details below.

The findings of these audits were submitted as QAU reports to the study director and to the personnel involved in the study. The data presented in this report are accurate reflections of the raw data.

Audit	Date of audit	Date of approval by Study director	Management

Signature:

Name: Ib Lorentzen

Title: Quality Assurance Manager

Date:

General Conditions

Please note that the results only relate to the product mentioned and that the product have been supplied with the name mentioned by the contracting company.

Confidentiality Statement

This report contains the unpublished results of research sponsored by Trion Tensid AB. These results must not be published, neither in full nor in part, or reviewed or quoted in any other publication without the prior authorisation of the sponsor.

All details regarding the test substance, other information, data, test results and methods will be treated with the strictest of confidentiality and will not passed on to any persons other than trusted employees who have been sworn to secrecy.

1 Introduction

This report contains a description of the method used and the results obtained during an assessment of the ability of the colour remover product AGS 221 to be inherently biodegradable over a period of 28 days.

The study was sponsored by the Trion Tensid AB and carried out from November 5 to December 1, 1997.

The study plan no. 5606 was approved as follows:

Study director: October 20, 1997

The amendment was approved by the study director only, as follows:

Study director: November 3, 1997

2 Materials and Methods

2.1 Test Substance

AGS 221

Identification(s):		No IUPAC names available
Cas number(s)	:	Not known
Description	:	Liquid, clear
Batch	.	Not stated
pH	:	7,2
Date of receipt	:	October 7, 1997
Expiry date	:	Not stated
Storage	:	In original container in dimmed light at room temperature
Stability of test concentrations	:	The stability was not verified by chemical analysis

2.2 Experimental procedure

The test was carried out according to GLP principles. However, after agreement with the sponsor the study plan has not been agreed by the sponsor.

2.2.1 Inherent biodegradability

The inherent biodegradability of the degreaser was investigated under aerobic conditions using a Zahn-Wellens/EMPA Test according to OECD Guideline 302B /1/. The test was performed as a reduced test with respect to sampling frequency (one point).

A measured volume of inoculated mineral medium, containing a known concentration of the test material as the nominal sole source of organic carbon, is aerated in the dark at 22 ± 2°C. The concentration of test material added is approximately 23 mg DOC per liter.

The degradation is followed by a DOC analysis over a 28-day period, and the biodegradation is calculated as the concentration of DOC present relatively to the concentration of DOC at the start of the test and corrected for DOC present in the blank inoculum control.

2.2.2 Source and preparation of inoculum

As inoculum was used activated sludge collected on the day the test started. The activated sludge was collected from the small sewage plant Skals Rensningsanlæg treating predominantly domestic sewage. On return to the laboratory, the sludge was aerated until use using a Millipore membrane pump supply of oil-free compressed air. A subsample was collected, and the concentration of suspended solids was determined. An appropriate volume of activated sludge was then removed, homogenized for two minutes at medium speed in a blender and added the test systems to provide an inoculum of 0.8-1.0 g/l for each test and control vessel.

2.2.3 Preparation of the test medium

The test medium for the test was prepared by mixing 2.5 ml of a mineral nutrient solution to 1 liter of tap water.

NH_4Cl	38.5 g
$Na_2HPO_4 \cdot 2H_2O$	33.4 g
KH_2PO_4	8.5 g
K_2HPO_4	21.75 g

2.2.4 Preparation of test solutions and reference materials

The concentration of DOC in the test material was determined on a Shimadzu TOC-5000A Carbon Analyzer according to /2/ and using a 10 mg C/l potassium-hydrogenphtalate solution as standard.

From the determined DOC content of the test material, a specified volume of the degreaser was added as a water solution to the mineral nutrient solution and tap water and was mixed on a magnetic stirrer for 30 minutes. The test material was then added to give a nominal concentration of approximately 25 mg C/l. As reference material sodium acetate (p.a.) was used at a nominal concentration of 200 mg C/l.

2.2.5 Set-up

To a 1-litre Stejlbrust flasks, mineral nutrient solution was added as well as test material, and a solution of reference substance was added to separate flasks. The flasks were filled up to a volume of 1 litre with tap water to give a nominal DOC concentration of approximately 25 mg C/l.

The following test vessels are included:

Flask #	Test substance	Test type	Comments
1	AGS 221	Test suspension	Test subs. & inoculum
2	Reference compound	Procedure control	Ref. subs. + inoculum
3	Inoculum only		Inoculum blank

The samples were placed in a temperature controlled room at 20 ± 2°C. The temperature was controlled by a Delphi Temperature logger /3/. Each flask was aerated with oil-free atmospheric air which had passed a washing bottle with Milli Q water and had been filtered through a 0.2 or 0.45 µm sterile membrane filter. No vehicle was used.

2.2.6 Sampling and analysis for DOC

Sampling for DOC analysis was performed at day 0 and 14 days (the test was stopped after 14 days since over 70% of the test product was degraded), by withdrawing approx. 2 x 30 ml from each flask and filtering it through a 0.45 µm membrane filter. The first 10 ml were discarded. All samples (duplicates) were frozen immediately after sampling

at < -15 °C in glass vials. A sampling after 3 hours was done also in order to observe a possible adsorption of the test material to the walls of the containers.

2.2.7 Calculations

The percentage degradation (D_t) at each time a sample was taken was calculated using the average values of duplicate DOC measurements.

The validity of the test needs a variation in DOC between duplicates not exceeding 20%. D_t is calculated as follows:

$$D_t = [1- (C_t - C_b)/C_a] \times 100\ \%$$

where:

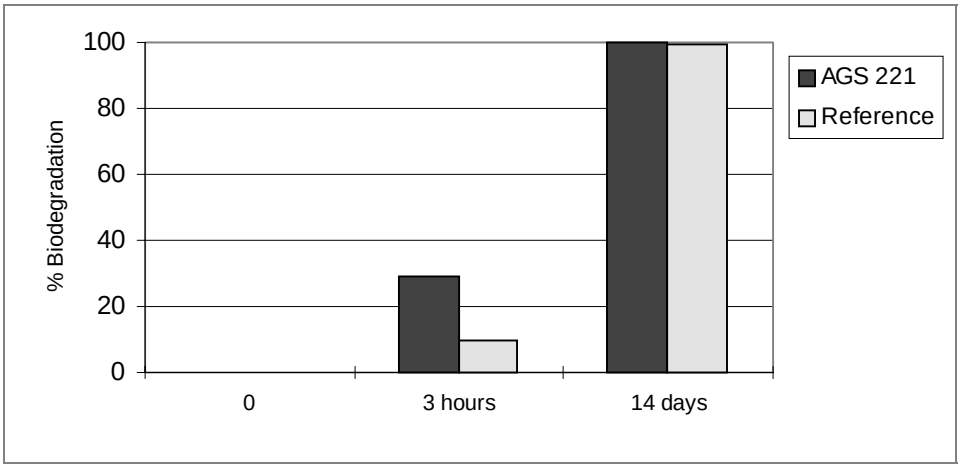
D_t	=	% degradation at time t
C_t	=	mean concentration of DOC in the inoculated culture medium containing test substance at time t (mg C/l)
C_a	=	mean starting concentration of DOC (mg C/l)
C_b	=	mean concentration of DOC blank (mg C/l)

3 Results

3.1 Inherent biodegradability

The degradation data after 14 days of the product are shown graphically in Figure 1. The product is ultimately biodegradable, i.e. more than 70% of the product is degraded. Further, the product have reached the pass level after 14 days.

Figure 1. Degradation data for the AGS 221 product from Trion Tensid and the reference substance sodium acetate.



Since the degradation test was performed as a reduced test compared to the OECD Guideline the 10-d window can not be outlined.

The reference compound sodium acetate was used as a procedure control at a nominal concentration of 200 mg C/l. A control without test substance or reference substance was used as a control for contamination of the test vessels with DOC. The average blank value was subtracted from each measured DOC content of the test vessels before calculating the degradation. The results are shown in Table 1, while raw data for the reference substance, the procedure control and the test products are shown in appendix 1.

Table 1. Degradation data (%) of the AGS 221 product from Trion Tensid after 14 days. Data for the reference compound sodium acetate are also shown.

Test produkt	AGS 221	Reference
Degradation	100	99,2

There was a high concentration of DOC in one of the duplicates from the blank vessels taken at t=0 (see appendix 1). Since the frozen glass vial containing the sample was broken by the time it was analysed, the result is omitted.

4 Conclusion

The product AGS 221 is ultimately biodegradable, i.e. more than 70% of the product is degraded. Because the test was performed as a reduced test, it is not possible to judge the time for reaching the pass level.

5 References

/1/ OECD, 1992. Zahn-Wellens/EMPA Test. OECD Guideline 302B. Paris.

/2/ Shimadzu, TOC 5000 A Instruction manual for Total Organic Carbon analyser TOC-5000A and Autosampler ASI-5000A.

/3/ Delphi Temperature Logger model 861, number 11279. New Zealand.

6 Placing in Archives

All raw data in connection with this study, the study plan, study journal, deviation form as well as the original final report are kept in the GLP archives at Hedeselskabet's Laboratory, 12 Klostermarken, DK-8800 Viborg, Denmark.

1 Appendix - Biodegradability

Flask #	Start mg C/l	3 hours mg C/l	14 days mg C/l
AGS 221	22,59	19,86	7,94
AGS 221	22,59	19,87	7,99
Reference	198,2	184,9	10,30
Reference	201,5	184,3	10,00
Blank	3,76	3,86	8,64
Blank	14,15*	3,85	8,58

* This result was not used in the calculations, since the frozen glass vial was broken.

2 Appendix - GLP Certificate

DANAK

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE

Laboratory inspection and study audits for compliance with the OECD Principles for Good Laboratory Practice was carried out at

Laboratory: *Hedeselskabet's Laboratory*
Klostermarken 12
8800 Viborg

on

Dates: *5 and 6 November 1996*

The laboratory inspection and study audits has been carried out in accordance with the regulation settled in Order No. 258 of 11 April 1994 from the Danish Agency for Development of Trade and Industry. The laboratory has been monitored for GLP Compliance within the following scope:

Type of products:

- *Pesticides*
- *Industrial chemicals*

Type of tests:

- *Environmental toxcity studies on aquatic and terrestial organisms.*
- *Studies of behaviour in water, soil and air, bioaccumulation*
- *Analytical and clinical chemistry testing*

The laboratory was found to be operating in compliance with the OECD Principles of Good Laboratory Practice.

Date: 1997 06 30

Mette Holst
Head of DANAK

Elisabeth Gade Nielsen
DANAK