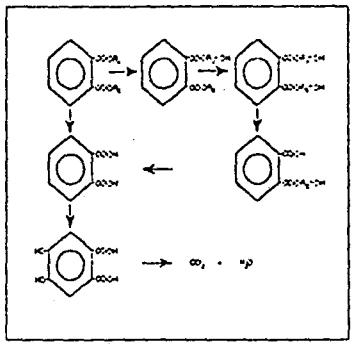
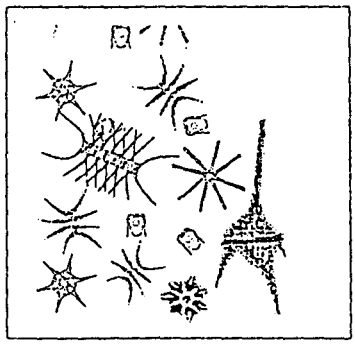
Report to :

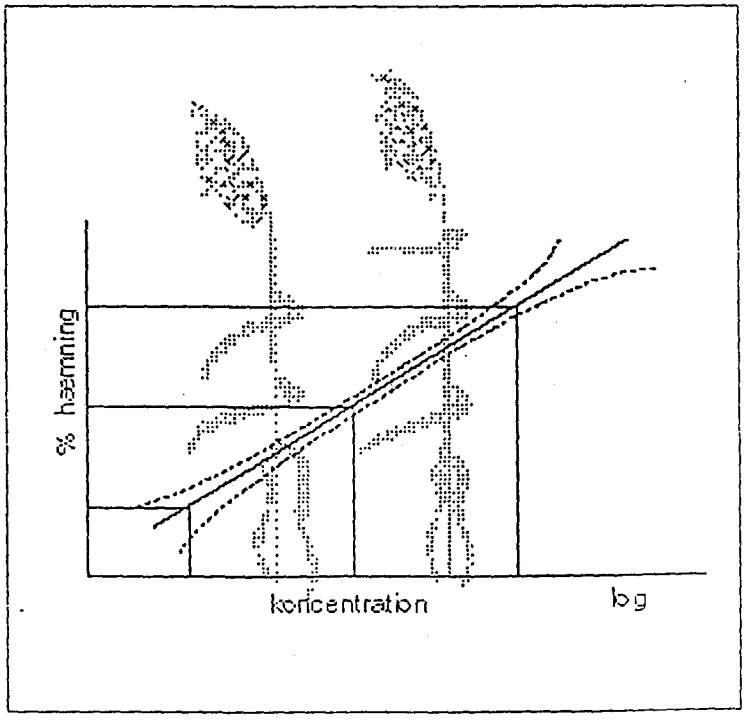
Trion Tensid AB

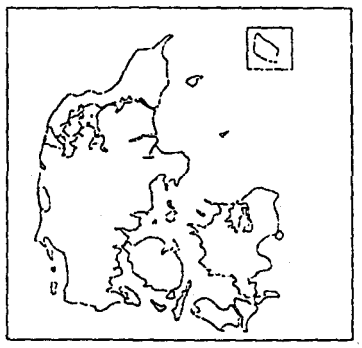
Concerning :

Inherent biodegradability and inhibition of activated sludge of 6 graffiti safeguards or colour removers.









Study Director ..... : Lars Møller Jensen
Report No. ..... : 5530
Date..... : 1996.10.24.

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Title Page

Study title:

Inherent Biodegradability and Inhibition of Nitrification of Activated Sludge of 6 graffiti safeguards or colour removers.

Laboratory Project/Report Number:

Report No.: 5530

Volume 1 of 1

Page 1 of 32

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Study Period:

5 August 1996 to 21 October 1996

Sponsor:

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Report Completed:

24 October 1996

Report Information

Study Title:

Inherent Biodegradability and Inhibition of Nitrification of Activated Sludge of 6 graffiti safeguards or colour remover.

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Table of contents

Summary

Objective

The objective of the tests was to investigate the ability of six graffiti safeguards or colour removers (AGS 3502, AGS 21, AGS 62, AGS 3, AGS 3506, AGS 2) to be inherently biodegradable using a static test method with activated sludge as inoculum after OECD Guideline 302B (Zahn-Wellens /EMPA Test). Further, the inhibitory effects against nitrifying activated sludge microorganisms was investigated according to ISO 9509.

Procedures

Inherent biodegradabilty: Test preparations, comprising test material solutions inoculated with acitivated sludge micro-organisms, 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.
Inhibition of nitrification: Test preparations, comprising test material solutions inoculated with acitivated sludge micro-organisms and a surplus of ammonium are incubated for 4-hours. Aeration with atmospheric air secures an aerobic environment during the test. The nitrification rate, i.e. the biological oxidation of ammonium to nitrate, is measured at the end of the exposure period and the inhibition calculated relative to a control without test material added.

Conlusions

All six products proved to be ultimately biodegradable, with more than 70% degraded after 14-days. All products were found to be toxic against nitrifying activated sludge micro-organisms. The effect concentrations EC20 and EC50 could not be calculated since the data obtained were inadequate for the use of standard methods of calculating the effect concentrations. Instead, the effect concentrations EC20 and EC50 were estimated by simple linear regression techniques as the logaritm of the concentration versus the inhibition. If the 20% or 50% inhibition was not reached in a particular test, the EC20 and EC50 was calculated by extrapolation. The estimated EC20 and EC50 concentrations for the six products are shown belov.

Test substance	EC20 (g/l)	EC50 (g/l)
AGS 3502	5.9	11.8
AGS 3506	7.2	9.7
AGS 2	0.02	0.04
AGS 3	0.02	0.07
AGS 21	0.02	0.04
AGS 62	0.61	1.75

Sammenfatning

Formål

Formålet med de udforte var at undersøge bionedbrydeligheden af seks graffiti beskyttelsesmidler eller farve-fjernere ((AGS 3502, AGS 21, AGS 62, AGS 3, AGS 3506, AGS 2) ved hjælp af en statisk test med aktiveret slam som podningsmiddel (inokulum). Testene blev udfort efter OECD Guideline 302b (Zahn-Wellens /EMPA Test). Endvidere blev produkternes hæmning overfor nitrificerende slam undersøgt efter ISO standard 9509.

Procedurer

Bionedbrydelighed: Testoplosninger bestående af teststof opløsninger podet med aktiveret slam inkuberes i testsystemer med kontinuerlig forsyning af oliefri atmosfærisk luft. Kontrol, blind og reference testsystemer indeholder henholdsvis mineralske salte alene, mineralske salte og det let nedbrydelige stof natriumacetat. Nedbrydningen følges ved hjælp af DOC-analyser over en periode på 28 døgn. Hvis et produkt nedbrydes med mere end 20% kan produktet karakteriseres som potentielt (inherent) bionedbrydeligt. Ved en nedbrydning større end 70% kan produktet karakteriseres som ultimativt bionedbrydeligt.
Hæmning af nitrifikation: Testoplosninger bestående af teststof opløsninger podes med aktiveret slam og et overskud af ammonium og inkuberes i 4 timer. Beluftning af testsystemerne med atmosfærisk luft sikrer et aerobt miljø. Nitrifikationsraten, dvs. den biologiske oksidation af ammonium til nitrat, måles efter 4 timers eksponering og hæmning beregnes relativt i forhold til et kontrol testsystem uden teststof.

Konklusioner

Alle seks produkter viste sig at være ultimativt bionedbrydelige med over 70% nedbrudt efter 14 dage. Alle produkter viste sig toksiske (giftige) over for nitrificerende slam. Effektkoncentrationerne EC20 og EC50 kunne ikke beregnes, da der var for få data. I stedet blev effektkoncentrationerne EC20 og EC50 estimeret ved simpel lineær regressions teknikker som logaritmen til koncentrationen mod procent hæmning. Hvis 20% eller 50% hæmning i en test ikke blev fundet, blev EC20 og EC50 beregnet ved ekstrapolation. De estimerede EC20 og EC50 koncentrationer for de seks produkter er vist nedenfor.

Test stof	EC20 (g/l)	EC50 (g/l)
AGS 3502	5.9	11.8
AGS 3506	7.2	9.7
AGS 2	0.02	0.04
AGS 3	0.02	0.07
AGS 21	0.02	0.04
AGS 62	0.61	1.75

Statement og 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: 1996.10.24.

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.

		Date of report to	
Audit	Date of audit	Study director	Management
Setup of biodegrad. test	1996.08.09.	1996.08.09.	1996.10.24.
Test sampling	1996.08.23.	1996.08.26.	1996.10.24.
Setup of nitrification test	1996.08.21.	1996.08.21.	1996.10.24.
Test sampling	1996.08.30.	1996.08.30.	1996.10.24.
Test sampling	1996.10.01.	1996.10.01.	1996.10.24.
Test sampling	1996.10.07.	1996.10.08.	1996.10.24.
Report vs raw data	1996.10.24.	1996.10.24.	1996.10.24.

Signature: 

Name: Ib Lorentzen

Title: Quality Assurance Manager

Date: 1996.10.24.

Generel Conditions

Please note that the results only relate to the products and formulations mentioned and that the products have been supplied by the contracting company with the names mentioned.

Confidentiality Statement

This report contains the unpublished results of research sponsored by Real Kemi. These results may not be published, either in full or in part, or reviewed or quoted in any other publication without the prior authorisation of the Sponsor.

All details regarding the test substances, 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.

Chapter 1

Introduction

This report contains a description of the methods used and the results obtained during an assessment of the ability of six products from Real Kemi (AGS 3502, AGS 21, AGS 62, AGS 3, AGS 3506, AGS 2) to be inherently biodegradable over a period of 28 days. Further, the inhibition of nitrification of activated sludge were determined. Both study's were undertaken as reduced tests in comparison to the OECD Guidelines and Iso standards, respectively.

The study was sponsored by the Real Kemi and undertaken between August 5, 1996 and October 21, 1996.

The study plans (No. 96134-1 and 96134-2) were approved as follows:

Study director:	August 5, 1996
Hedeselskabet's Laboratory, management:	August 5, 1996

Chapter 2

Materials & Methods

2.1 Test Substance

AGS 3502

Identification(s):	No IUPAC names available
Cas number(s):	Not known
Description :	liquid, white
Batch .	Not stated
pH :	8,9 (concentrate)
Date of receipt:	August 6, 1996
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

AGS 21

Identification(s):	No IUPAC names available
Cas number(s):	Not known
Description :	liquid, opaque
Batch .	Not stated
pH :	N/A
Date of receipt:	August 6, 1996
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

AGS 62

Identification(s):		No IUPAC names available
Cas number(s) :		Not known
Description	:	liquid, light brown
Batch	.	Not stated
pH	:	14 (concentrate)
Date of receipt:		August 6, 1996
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

AGS 3

Identification(s):		No IUPAC names available
Cas number(s) :		Not known
Description	:	liquid, yellow/brown
Batch	.	Not stated
pH	:	7-8 (concentrate)
Date of receipt:		August 6, 1996
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

AGS 3506

Identification(s):		No IUPAC names available
Cas number(s) :		Not known

Description	:	liquid, grey
Batch	.	Not stated
pH	:	Approx. 8 (concentrate)
Date of receipt:		August 6, 1996
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

AGS 2

Identification(s):		No IUPAC names available
Cas number(s):		Not known
Description	:	liquid, brown/yellow
Batch	.	Not stated
pH	:	7-8 (concentrate)
Date of receipt:		August 6, 1996
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 tests were carried out according to GLP principles. However, after agreement with the sponsor the study plans have 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 tests were performed as reduced tests 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 80-100 mg DOC per liter, except for AGS 3502 and AGS 3506 where the added concentration is approx. 4-7 mg C/l.

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.

2.2.1.2 Source and preparation of inoculum

As inoculum was used activated sludge collected on the day the test started. The activated sludge was collected from a small sewage plant 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.

2.2.1.3 Preparation of the test medium

The test medium for the tests 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

2.2.1.4 Preparation of test solutions and reference materials

The concentration of DOC in the test material was determined on a Dohrman DC 80 Carbon Analyzer according to /2/ and using a 10 mg C/l potassiumhydrogenphtalate 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 80-100 mg C/l, except for AGS 3502 and AGS 3506 where a nominal concentration of approx. 4 to 7 mg C/l were used. As reference material sodium acetate (p.a.) was used at a nominal concentration of 200 mg C/l.

2.2.1.5 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 80-100 mg C/l. For AGS 3502 and AGS 3506 a nominal concentration of approx. 4 to 7 mg C/l were, however, used.

The following test vessels are included:

Flask #	Test substance	Test type	Comments
1	AGS 3502	Test suspension	Test subs. & inoculum
2	AGS 21	Test suspension	Test subs. & inoculum
3	AGS 62	Test suspension	Test subs. & inoculum
4	AGS 3	Test suspension	Test subs. & inoculum

Flask #	Test substance	Test type	Comments
5	AGS 3506	Test suspension	Test subs. & inoculum
6	AGS 2	Test suspension	Test subs. & inoculum
7	Reference compound	Procedure control	Ref. subs. + inoculum
8	Inoculum only		Inoculum blank, common for all

The samples were placed in a temperature controlled room at 20 ± 2°C. 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.

2.2.1.6 Sampling and analysis for DOC

Sampling for DOC analysis was performed at day 0 and 14 days (the tests were stopped aftaer 14 days since over 70% of the test products were 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.

2.2.1.7 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)

2.2.2 Inhibition of nitrification of activated sludge

The inhibition of nitrification of activated sludge was set up and performed according to ISO 9509: 1989 (E) /3/. The method specifies the short-term inhibitory effects. The inhibitory effect was estimated after an exposure period of 4 hours. Analysis of nitrogen was performed according to DS 223/230 /4/. The tests were performed as reduced tests using 3 concentrations only.

2.2.2.1 Source and handling of sludge

Activated sludge was obtained from the waste water treatment plant in Bruunshåb. After 10 minutes of sedimentation and decanting, the sludge was aerated until use and. the nitrification rate was determined.

The MLSS of the activated sludge was determined by filtering 2 x15 ml sludge through two GF/A filters. The filters were placed in porcelain dishes and dried in an oven set to a

temperature of 103 °C. The filters were weighed before filtering and after drying, and the MLSS was calculated as g/l.

2 x 400 ml were centrifuged at 3000 rpm for 10 minutes. After discarding the supernatant, the residue was washed with a volume of Milli-Q water that equals the volume of the residue and with a volume of medium that was 10 times the volume of the residue. The sludge was resuspended in an appropriate volume of the medium to give a concentration of 7.5 g/l of MLSS. The sludge was aerated until use, and the MLSS in the resuspended sludge was determined.

For determining the preliminary nitrification rate, three control flasks (one for t = 0) and two reference flasks were set up as described in /3/.

2.2.2.2 Preparation of the test solutions
The test substance was dissolved in dilution water, and the pH was adjusted with 0.5 N Hcl according to the ISO Standard. The solution acts as a stock solution from which all other dilutions were made. No vehicle was used.

2.2.2.3 Set-up
A suitable amount of activated sludge was washed as described in paragraph 2.2.2.1. The pH of the activated sludge and the test substance was measured. The pH of the test substance was adjusted to the pH value of the sludge ± 1.0. The tests were carried out in BOD bottles (250 ml Steilbrust bottles). The test solutions and the controls were made in duplicate. An additional control was set up and filtered immediately to obtain t = 0 values.

Test material was then added to each bottle:, 25.0 ml of medium and a sufficient amount of water to make up the final volume of 200 ml. 50 ml of resuspended sludge was added just before starting the incubation. The final concentration of mixed liquor suspended solids was approximately 1.50 g/l.

All flasks were aerated for 4 h at a constant temperature with humidified air. The air was distributed through a wash bottle using Pasteur pipettes in each test bottle to keep the sludge in suspension. The flasks were incubated in diffused light for 4 hours.The temperature of the test systems was measured in one control bottle in the beginning and at the end of the test.

The concentration of dissolved oxygen in the highest test concentration during and at the end of the test was measured to secure that the oxygen concentration during the test was above 6 ppm.

After 4 hours of aeration, the solutions were allowed to sediment for approx. 10 minutes, before the supernatant was filtered through GF/A filters. All filtrates were measured for NO_2+NO_3-N.

2.2.2.4 Calculations
The nitrification rate was calculated as follows:

$$\text{Nitrification rate} = \frac{Cc - Cb}{MLSS \times 4}$$

Inhibition of formation of oxidized nitrogen-N:

$$\% \text{ inhibition} = \frac{Cc - Ct}{Cc - Cb} \times 100$$

where

Cc is the concentration of oxidized nitrogen, N, in milligrams per litre in the control flask without inhibitor,after incubation.

Ct is the concentration of oxidized nitrogen N, in milligrams per litre in the flask containing the test substance, after incubation.

Cb is the concentration of oxidized nitrogen N, in milligrams per litre in the flask containing the reference inhibitor, after incubation.

The effect concentrations (EC) were calculated by linear regression techniques as percentage inhibition against the log of the concentration of test substance.

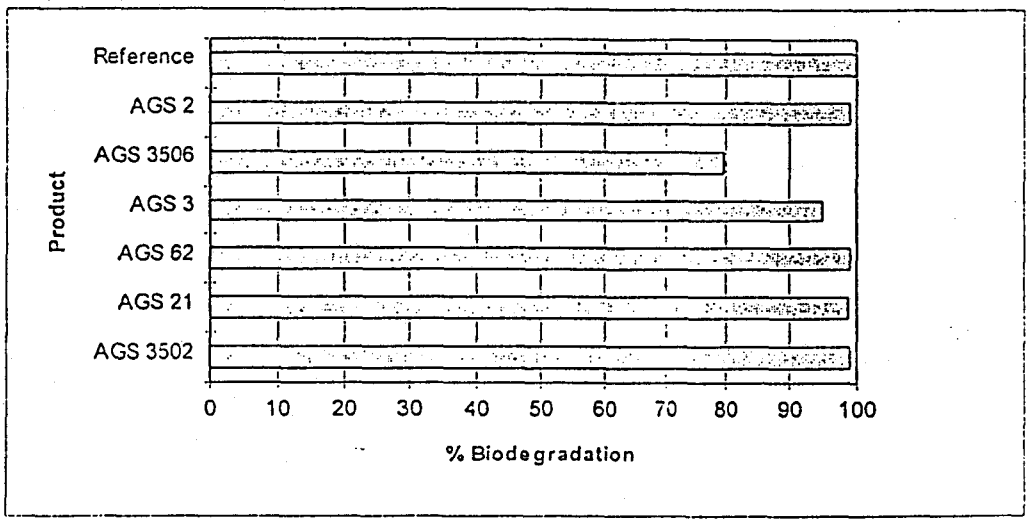
Chapter 3

Results

3.1 Inherent biodegradabilty

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

Figure 1. Degradation data for the six ASG products from Real Kemi and the reference substance sodium acetate.



Since the degradation tests were performed as reduced tests compared to the OECD Guideline for the tests 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 six AGS products from Real Kemi after 14 days. Data for the reference compound sodium actetate and the blind are also shown.

Test product	AGS 3502	AGS 3506	AGS 2	AGS 3	AGS 21	AGS 62	Reference
Degradation	99	80	99	95	99	99	100

3.2 Inhibition of nitrification of activated sludge

The acute inhibitory effects of the six products against nitrifying sludge were tested over an exponation period of 4 hours. The results are shown in Table 2 below.

It can be seen from the Table, that the dose-response effects fall in a narrow range of concentrations. Since the test were performed as reduced tests with only three concentrations it is not possible to calculate the effect concentrations (EC) properly. AGS 3502 and AGS 3506 are less toxic than the other products.Raw data are shown in Appendix 2.

Table 2. Inhibition of nitrification of nitrifying sludge. ND: no data.

Test substance	Concentration (g/l)	Inhibition (%)
AGS 3502	4.1	3
	6.0	24
	8.1	32
AGS 3506	6.0	0
	8.1	ND
	10.2	55
AGS 2	0.01	8
	0.04	44
	0.06	65
AGS 3	0.04	34
	0.06	57
	0.20	84
AGS 21	0.04	42
	0.05	58
	0.11	75
AGS 62	0.41	9
	0.61	19
	2.05	55

Because of lack of sufficient data to calculate the effect concentrations dose-response curves were approximated by a simple linear regression technique of the logarithm of the concentration versus the observed inhibition. In case of no effects $\leq 20\%$ or $\geq 50\%$, the EC20 and EC50 were calculated by extrapolation. The estimated effect concentrations are given in Table 3 below.

Table 3. Estimated effect concentrations for the inhibition of nitrification tests.

Test substance	EC20 (g/l)	EC50 (g/l)
AGS 3502	5.9	11.8
AGS 3506	7.2	9.7
AGS 2	0.02	0.04
AGS 3	0.02	0.07
AGS 21	0.02	0.04
AGS 62	0.61	1.75

Chapter 4

Conclusions

All 6 products are ultimately biodegradable, i.e. more than 70% of the products are degraded. Because the tests were performed as reduced tests, it is not possible to judge the time for reaching the pass level.

The dose-response effects in the tests for inhibition of nitrification falls, especially for AGS 2, AGS 3 and AGS 21, in a narrow range of concentrations with the EC20 and EC50 between 0.02 and 0.07 g/l of each product. The most toxic products were AGS 2 and AGS 21, followed by AGS 3, AGS 62, AGS 3502 and AGS 3506 in decreasing order.Since only three concentrations were included, it is not possible to calculate the EC20 and EC50 concentrations properly. Instead, the effectconcentrations were estimated by linear regression og the logaritm of the concentration versus the observed inhibition. In case the 20% and/or 50% inhibition was not reached they were estimated by extrapolation.

The estimated effect concentrations for the six products are shown below.

Test substance	EC20 (g/l)	EC50 (g/l)
AGS 3502	5.9	11.8
AGS 3506	7.2	9.7
AGS 2	0.02	0.04
AGS 3	0.02	0.07
AGS 21	0.02	0.04
AGS 62	0.61	1.75

Chapter 5

Deviations

The test concentrations in the biodegradability study of the products AGS 3502 and AGS 3506 were lower (approx. 4 and 7 mg C/l, respectively) than specified in the study plan (approx. 100 mg C/l). However, the deviation is judged not to influence the results or violate the conclusions of the tests since the concentrations are far over the detection limit of the DOC-analyzer.

Chapter 6

References

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

/2/ Dohrman, 1985. Dohrmann DC 80 Total Organic Carbon Systems Manual, 10th Edition, Xertex & Dohrman, Santa Clara, AC 95950.

/3/ International Organization for Standardization, 1989. Water Quality - Method for assessing the inhibition of nitrification of activated sludge micro-organisms by chemicals and waste water. ISO 9509. In Danish.

/4/ Danish Standard, 1975 and 1988. Determination of the sum of nitrite- and nitrate-nitrogen, DS 223, 1975. Water Quality - nitrate-nitrogen, nitritenitrogen, Devardas alloy. In Danish.

Chapter 7

Placing in Archives

All raw data in connection with this study, the study plans, study journals, deviation forms as well as the original final report are held at the QAU archives at Hedeselskabet's Laboratory, 12 Klostermarken, DK-8800 Viborg, Denmark.

Appendix 1

Raw data - Biodegradability test

DOC DIE-AWAY TEST DATA SHEET

1. Laboratory: HEDESELSKABET'S LABORATORY

2. Date at start of test: 1996.08.09.

3. Test substance:

Name: AGS 3502, AGS 21, AGS 62, AGS 3, AGS 3506, AGS 2
Stock solution concentration: 1 g/l as chemical
Initial concentration in medium, to: 100 mg/l as chemical

4. Inoculum:

Source: Skals Rensningsanlæg (dom. sewage treatm. plant)
Treatment given: Homogenized for 2 minutes
Pre-conditioning, if any: None
Suspended solids concentration in reaction mixture: 1%

5. Carbon determinations:

Carbon analyser: Dohrman DC-80

Content	Flask #		DOC after n days (mg/l)									
			0	0.12	14							
AGS 3502	1	a(1)	3,8	3,6	4,8							
		a(2)	3,7	3,5	4,4							
TEST		mean Ca	3,7	3,5	4,6							
		b(1)										
		b(2)										
		mean Cb										
AGS 3506	2	a(1)	7,0	5,3	6,0							
		a(2)	7,1	5,4	5,9							
TEST		mean Ca	7,1	5,4	6,0							
		b(1)										
		b(2)										
		mean Cb										
AGS 2	3	a(1)	107,1	91,4	5,6							
		a(2)	95,8	99,1	5,5							
TEST		mean Ca	101,4	95,3	5,6							
		b(1)										
		b(2)										
		mean Cb										
AGS 3	4	a(1)	98,1	93,3	9,6							
		a(2)	102,0	93,2	9,4							
TEST		mean Ca	100,0	95,7	9,5							
		b(1)										
		b(2)										
		mean Cb										
AGS 21	5	a(1)	88,4	88,7	5,5							
		a(2)	84,7	86,7	5,6							
TEST		mean Ca	86,6	87,7	5,6							

5. Carbon determinations, cont.

Content	Flask #		DOC after n days (mg(l)									
			0	0.12	14							
		b(1)										
		b(2)										
		mean Cb										
AGS 62 TEST	6	a(1)	100,5	102,7	5,6							
		a(2)	96,6	99,5	5,6							
		mean Ca	98,5	101,1	5,6							
		b(1)										
		b(2)										
		mean Cb										
Reference	7	a(1)	201,9	186,7	3,9							
		a(2)	202,4	180,2	3,9							
		mean Ca	202,2	183,5	3,9							
		b(1)										
		b(2)										
		mean Cb										
Blind	8	a(1)	2,2	2,2	4,6							
		a(2)	2,1	2,5	4,5							
		mean Ca	2,1	2,4	4,5							
		b(1)										
		b(2)										
		mean Cb										
		a(1)										
		a(2)										
		mean Ca										
		b(1)										
		b(2)										
		mean Cb										
		a(1)										
		a(2)										
		mean Ca										
		b(1)										
		b(2)										
		mean Cb										
		a(1)										
		a(2)										
		mean Ca										
		b(1)										
		b(2)										
		mean Cb										

Comments:

Reduced tests, no replicate test systems
Because of an error in the weighing procedure of test products AGS 3502 and AGS 3506, are the initial concentrations (t = 0)
lower than described in the study plan

Appendix 2

Raw data - Inhibition of nitrification

RAW DATA

Project #: P96134
Report #: 5530
Study Plan #: 96134-2
Test: Inhibition of nitrification acc. to ISO 9509
Technicians: GS/GWH

Test product	Test date	Concentration (g/l)	NO_2+NO_3-N (mg N/l)	Inhibition (%)	Nitrification rate (mg N/g·h)
AGS 3502	1996.10.01.	0 (control)	10.3	-	3.6
		ATU	1.93	-	
		4.132	17.43	3.1	
	1996.10.08.	0 (control)	6.84	-	0.82
		ATU	2.07	-	
		6.005	5.84	24.5	
		8.11	5.52	32.4	
AGS 3506	1996.10.01.	0 (control)	18.1	-	3.6
		ATU	3.38	-	
		5.989	19.6	0	
	1996.10.08.	0 (control)	6.59	-	0.82
		ATU	2.07	-	
		8.139	7.03	ND	
		10.249	4.92	54.7	
AGS 2	1996.10.08.	0 (control)	6.59	-	0.82
		ATU	2.07	-	
		0.011	6.46	8.0	
		0.045	4.76	43.6	
		0.067	3.73	65.1	
AGS 3	1996.08.21.	0 (control)	10.3	-	0.58
		ATU	1.93	-	
		0.217	4.68	83.7	
	1996.10.01.	0 (control)	18.1	-	3.6
		ATU	3.38	-	
		0.061	9.6	56.7	
	1996.10.08.	0 (control)	6.59	-	0.82
		ATU	2.07	-	
		0.042	5.20	34.5	
AGS 21	1996.10.01.	0 (control)	18.1	-	3.6
		ATU	3.38	-	
		0.055	9.36	58.4	
		0.111	6.96	75.2	
	1996.10.08.	0 (control)	6.59	-	0.82
		ATU	2.07	-	
		0.040	4.83	42.1	
AGS 62	1996.10.01.	0 (control)	17.7	-	3.6
		ATU	3.38	-	
		0.41	16.42	9.1	
		0.614	15.0	18.7	
		2.045	9.86	54.9	

Appendix 3

GLP Statement of Compliance

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*
Danish Land Development Service
Klostermarken 12
DK-8600 Viborg
Denmark

on

Dates: *1 November 1991, 25 June 1992, 7 August 1992, 10 february 1993 and 7 september 1993*

The laboratory inspection and study audits has been carried out in accordance with the regulation settled in Order No. 169 of 19 March 1991 and No. 831 of 2 October 1992 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: *Chemicals*
- *Industrial chemicals*
- *Pesticides*

Type of tests: *Ecotoxicological studies*
- *Toxicity studies*
- *Biodegradation studies*
- *Bioaccumulation studies.*

Chemical analyses

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

Date: 1993 11 11.

Merete Rasmussen
Danish Agency for Development of Trade and Industry

Elisabeth Gade Nielsen
Danish Institute for Fundamental Metrology

EDESELSKABETS TE T
AF
AGS 2, 3, 21, 62, 3502 OG 3506

FORUDSÆTNINGER: 1. Område med graffiti (100% dækning): Ca. 1,6 m x ca. 1,2 m = ca. 1,92 m²

2. Spildevandet opsamles og transporteres til rensningsanlæg på min. 10 m³

Produkter	a) Forbrug l/m² på 1,92 m²	b) Massefylde g/liter	c) Forbrug g/l på 1,92 m²	d) EC20 g/liter	e) Tid min.	f) Vandmængde ved 19 l/min.	g) Spildevand g/liter	h) Rensningsanlæg g/liter
AGS 2	0,960 – – 1,920	1050	1008 2016	0,02	7	133	c : f = 7,5789 c : f = 15,1579	g : 10.000 l = 0,0008 g : 10.000 l = 0,0015
AGS 3	0,960 – – 1,920	1050	1008 2016	0,02	10	190	c : f = 5,3053 c : f = 10,6105	g : 10.000 l = 0,0005 g : 10.000 l = 0,0011
AGS 21	0,576 – – 1.920	1045	601,92 2016	0,02	7	133	c : f = 4,5257 c : f = 15,1579	g : 10.000 l = 0,0045 g : 10.000 l = 0,0015
AGS 62	0,384 – – 0,960	1120	430,08 1008	0,61	7	133	c : f = 3,2337 c : f = 7,5789	g : 10.000 l = 0,00032 g : 10.000 l = 0,0008
AGS 3502	0,192 – – 0,384	1000	192 384	5,9	7	133	c : f = 1,4436 c : f = 2,8872	g : 10.000 l = 0,00014 g : 10.000 l = 0,00029
AGS 3506	0,384 – – 0,768	1000	384 768	7,2	7	133	c : f = 2,8872 c : f = 5,7744	g : 10.000 l = 0,00029 g : 10.000 l = 0,00058

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