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INITIAL SUBMISSION: LTR FR ATTYS FOR ARIZONA CHEM CO RE READY BIODEGRADABILITY DATA FOR [], COMPOSITES OF ALPHA PINENE & *, W/ATTCHMTS (STUDY REPORTS) & DATED 4/6/01 (SANITIZED)		
Chemical Category		
COMPOSITES OF ALPHA PINENE, DELTA-3-CARENE, * (CONFIDENTIAL)		

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Attention: TSCA Section 8(e) Coordinator
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U.S. Environmental Protection Agency
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Washington, D.C. 20004

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Re: TSCA § 8(e) Submittal

Dear Sir/Madam:

Enclosed are five studies submitted pursuant to Section 8(e) of the Toxic Substances Control Act (TSCA). These studies are being submitted pursuant to the TSCA Compliance Audit Agreement between EPA and the Arizona Chemical Company (Arizona). Arizona Chemical Company has its headquarters at 5220 Belfort Road, Suite 200, Jacksonville, Florida 32256-6012.

All five of the enclosed studies describe ready biodegradability data that indicates the substances are not readily biodegradable. Arizona understands that EPA does generally not consider biodegradability data of this nature to be reportable in and of themselves. Nevertheless, these studies are being submitted out of an abundance of caution to supplement the ecotoxicity data for the corresponding products that were submitted to the EPA on either November 2, 1999 or April 14, 2000. Summaries of the enclosed studies are provided below.

In a "closed bottle" ready biodegradability study conducted on a composite sample of alpha pinene (Chemical Abstract Services Number (CASRN) 80-56-8; the substance might also be described by CASRN 65996-65-9 for "terpenes and terpenoids, turpentine oil, alpha pinene fraction") this product was determined to biodegrade < 3% after 28 days (test report #308067/487). Alpha pinene is a commercial Arizona product.

In a "closed bottle" ready biodegradability study conducted on a composite sample of delta-3-carene (CASRN 13466-78-9; the substance might also be described by

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CASRN 91770-80-89 for "terpenes and terpenoids, turpentine oil, 3-carene fraction") this product was determined to biodegrade < 4% after 28 days (test report #308067/488). While delta-3 carene is not a commercial product of Arizona's, it is likely to be a component in certain turpentine-derived commercial products that are manufactured by Arizona.

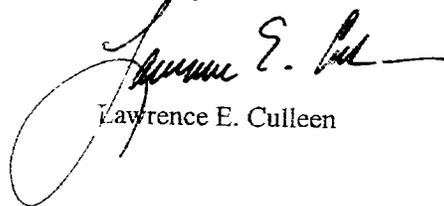
In a "closed bottle" ready biodegradability study conducted on a composite sample of rosin (CASRN 8050-09-7), this product was determined to biodegrade 32% after 28 days (test report #308067/471). Rosin is a commercial Arizona product.

In a "closed bottle" ready biodegradability study conducted on a composite sample of tall-oil heads (CASRN 8050-09-7), this product was determined to biodegrade 41% after 28 days (test report #308067/474). Tall-oil heads is a commercial Arizona product.

In a "closed bottle" ready biodegradability study conducted on a product known as fatty acid dipentaester (CASRN 68424-55-6 for "fatty acids, tall-oil, polymers with pentaerythritol"), this product was determined to biodegrade 18% after 28 days (test report #308067/489).

Please contact me (at 202/942-5477) with any comments or questions that you have concerning these matters. Thank you.

Sincerely,



Lawrence E. Culleen

Enclosures

cc: Tony Ellis, EPA
Diane Staab, Arizona

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93-19

Biodegradation of Alpha-Pinene

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Study director: Torben Madsen, Ph.D.
Project No.: 303068
Study No.: 308067/487
Date: 19.05.1993/MK

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Title: Biodegradation of Alpha-Pinene

GLP Study No.: 308067/487

Project No.: 303068

Test Period: 1993.01.20 - 1993.02.17

Test facility: WATER QUALITY INSTITUTE, ATV
Ecotoxicological Dept.
11, Agern Allé
DK-2970 Hørsholm

Authentication:

We the undersigned hereby declare that the ecotoxicological investigation in this report was carried out under our supervision, and in accordance with the principles of Good Laboratory Practice (GLP) according to OECD codes of GLP May 1981, Doc C(81)30 (Final).

The study has been carried out in accordance with the procedures described in the report, which represents a true and accurate record of the results obtained.

Technician: Connie Seierø 19/5-93
Connie Seierø Date

Study Director: Torben Madsen 19/5-93
Torben Madsen, Ph.D. Date

Approved by: Preben Kristensen 24/5-93
Preben Kristensen, M.Sc. Date

A 07

Quality Assurance Statement

WATER QUALITY INSTITUTE, ATV (VKI)
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DK-2970 Hørsholm

Inspection of GLP studies are performed according to the following principles dependent on the type of study.

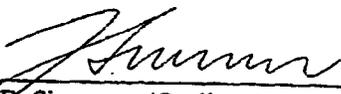
In short term studies (less than 1 week) conducted frequently, critical phases are inspected 2-3 times per year or on request from the sponsor.

In long term studies and short term studies conducted non-frequently, inspections are normally made at critical phases of the individual study.

The performance of this study has been secured by the VKI's Quality Assurance Unit:

<u>Date</u>	<u>Activity</u>
1992.09.16	Protocol received
28.5.93	Final report audited

This test report accurately describes the methods and procedures used in the study, and the reported results accurately reflect the raw data of the study.



Jørgen F. Simonsen (Quality Assurance Officer) 28.5.93
Date

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1. Summary

The degradation of the test product, Alpha-Pinene, was tested by the OECD Test Method No. 301 D: "Ready Biodegradability: Closed Bottle Test" /1/.

The study showed that the product was degraded 3.1% within the first week. At the end of the test after 28 days the biological oxygen demand corresponded to 2.2% of the theoretical oxygen demand of the test product.

The addition of a reference compound, sodium benzoate, stimulated the degradation of Alpha-Pinene.

2. Introduction

The OECD Method No. 301 D: "Ready Biodegradability: Closed Bottle Test" /1/ was used to investigate the biodegradation of Alpha-Pinene. The biodegradation of the organic compounds in the test product was determined by measuring the oxygen consumption in inoculated test bottles.

The measured oxygen consumption was compared to the theoretical oxygen demand for a complete degradation of the test product. The theoretical oxygen demand was expressed as the chemical oxygen demand (COD) which was measured according to the Danish Standard /2/.

3. Test product

Alpha Pinene consisted mainly of alpha pinene mixed with camphene, beta pinene and delta 3-carene. The test product was an equal mixture by volume of the following two products:

- Oulu 402 SF

- Veitsiluoto OY

The test product was delivered by International Paper Norge AS, Chemical Division and stored at VKI at 4 °C until use.

4. Preparation of stock solution

A stock solution of the test product (2 g/l) was prepared in demineralised water by ultra sonication for 5 min. A sample was taken in the center of the stock solution, and due to the volatility of the product, it was used immediately in the closed bottle test. The chemical oxygen demand (COD) in the actual stock solution was determined at the first day of the experiment.

The COD of the stock solution was 4,776 mg O₂/l.

The actual ^{concentration of the} water accommodated fraction of the product was not determined by chemical analysis. The test concentration referred to will be the nominal concentration, i.e. including any non-soluble fractions.

5. Test method

Secondary effluent from Rungsted Treatment Plant, 82, Bukkeballevvej, DK-2970 Hørsholm, which mainly receives domestic waste water, was used as inoculum. The sludge was collected on January 20, 1993, and the test was started the same day.

The test medium was prepared by adding 1 ml of the following stock solutions to 1 litre of demineralised water:

A) 8.5 g KH₂PO₄
 21.5 g K₂HPO₄
 33.5 g Na₂HPO₄ · 2 H₂O
 1.7 g NH₄Cl
 in 1000 ml demineralised water.

B) 22.5 g MgSO₄ · 7 H₂O
 in 1000 ml demineralised water.

- C) 27.5 g CaCl_2
in 1000 ml demineralised water.
- D) 0.25 g $\text{FeCl}_3 \cdot 6 \text{H}_2\text{O}$
in 1000 ml demineralised water.

The test medium was aerated to an initial oxygen concentration of approximately 9 mg O_2/l and inoculated with 1 drop of secondary effluent per litre.

The test product was added at 2.5 mg/l to a part of the inoculated test medium, equivalent to a chemical oxygen demand (COD) of 5.97 mg O_2/l .

A reference compound was added to another part of inoculated medium in order to evaluate the activity of the inoculum. Sodium benzoate was used as the reference compound at a concentration of 2.0 mg/l equivalent to a theoretical oxygen demand (ThOD) of 3.34 mg O_2/l .

Both test product (2.5 mg/l) and reference compound (2.0 mg/l) were added to a third part of inoculated medium to assess possible inhibitory effects of the test product. The theoretical oxygen demand was the sum of the oxygen demands for both test product and reference compound, i.e. 9.31 mg O_2/l .

Inoculated test medium without addition of test nor reference compound served as a blank control.

After all additions, the medium was transferred to calibrated respirometric bottles (BOD bottles).

The complete test programme is shown below.

A 12

	Test	Reference	Inhibition	Blank
Test product	*		*	
Reference substance		*	*	
Secondary element	*	*	*	*

The test bottles were placed in the dark at a constant temperature of $20 \pm 1^\circ\text{C}$. A triplicate set of test bottles was sacrificed at the start of the experiment and after 7, 14, 21 and 28 days for oxygen measurements. The oxygen demand was calculated as the difference between the measured oxygen concentrations at time t and at the start of the test. The biological oxygen demand for the added carbon sources was calculated by subtracting the oxygen demand in the blank controls from the oxygen demand in the bottles containing test and reference compounds.

6. Data analysis

The biodegradability of Alpha-Pinene was expressed as the biological oxygen demand during the test period.

The percentage of the test product or reference substance degraded was calculated by the following formula:

$$\% \text{ degraded} = \frac{\text{oxygen demand (test - blank)}}{\text{concentration} \cdot \text{ThOD}} \cdot 100 \%$$

or

$$\% \text{ degraded} = \frac{\text{oxygen demand (test - blank)}}{\text{concentration} \cdot \text{COD}} \cdot 100 \%$$

7. Archives

All original data including a sample of the test product will be stored at ambient temperature in the GLP archives of VKI for a period of ten years.

Samples that are unstable may be disposed off before that time or stored under specific conditions after consultation with the sponsor.

At the end of the period, the sponsor will be consulted regarding requirements for disposal or further storage.

8. Results

The results of the biodegradation test with Alpha-Pinene are shown in Table 1 and 2, and in Figures 1-3. In addition, all test data are given in Annex 1.

The inoculum was found to have a satisfactory activity as illustrated by the degradation of the reference compound. Approximately 60% of the added sodium benzoate was degraded within the first 7 days of the test period (Table 1, Figure 1).

The biological oxygen demand of the test product, Alpha-Pinene, was 3.1% of the chemical oxygen demand after 7 days and remained low during the 28 days of the experiment (Table 1, Figure 2).

Oxygen measurements after 14, 21, and 28 days revealed that the biological oxygen demand in bottles with both test product and reference compound was 1.5 to 2 times higher than the sum of the oxygen demands of Alpha-Pinene and sodium benzoate tested separately (Table 2, Figure 3).

Table 1:

Degradation of sodium benzoate and Alpha-Pinene. All values are corrected for oxygen consumption in blanks. (*), data were not included in calculations of mean values (see annex I).

% DEGRADED:				
TIME (days)	SODIUM BENZOATE			
	I	II	III	MEAN
0	0	0	0	0
7	58	59	59	59
14	57	59	62	59
21	61	67	74	67
28	80	96	89	88

TIME (days)	ALPHA-PINENE			
	I	II	III	MEAN
0	0	0	0	0
7	4.4	4.4	0.7	3.1
14	6.9	(*)	2.7	4.8
21	5.4	5.9	(*)	5.7
28	2.0	2.2	2.3	2.2

Table 2.

Biological oxygen demand during degradation of sodium benzoate (SB) and Alpha-Pinene (AP) tested separately or in combination. All values are corrected for oxygen consumption in blanks. Data are means of triplicate determinations except where indicated by (*); see annex I.

TIME (days)	SB (mg O ₂ /l)	AP (mg O ₂ /l)	SB + AP (mg O ₂ /l)
7	1.96	0.19	2.01
14	1.98	0.29 (*)	5.00 (*)
21	2.24	0.34 (*)	5.27 (*)
28	2.95	0.13	4.83

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93-11

Biodegradation of 3-Carene

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Study director: Torben Madsen, Ph.D.
Project No.: 303068
Study No.: 308067/488
Date: 19.05.1993/MK

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Title: Biodegradation of 3-Carene

GLP Study No.: 308067/488

Project No.: 303068

Test Period: 1992.11.24 - 1992.12.22

Test facility: WATER QUALITY INSTITUTE, ATV
Ecotoxicological Dept.
11, Agern Allé
DK-2970 Hørsholm

Authentication:

We the undersigned hereby declare that the ecotoxicological investigation in this report was carried out under our supervision, and in accordance with the principles of Good Laboratory Practice (GLP) according to OECD codes of GLP May 1981, Doc C(81)30 (Final).

The study has been carried out in accordance with the procedures described in the report, which represents a true and accurate record of the results obtained.

Technician: Connie Seierø 14/5-93
Connie Seierø Date

Study Director: Torben Madsen 15/5-93
Torben Madsen, Ph.D. Date

Approved by: Preben Kristensen 21/5-93
Preben Kristensen, M.Sc. Date

Quality Assurance Statement

WATER QUALITY INSTITUTE, ATV (VKI)
11, Agern Allé
DK-2970 Hørsholm

Inspection of GLP studies are performed according to the following principles dependent on the type of study.

In short term studies (less than 1 week) conducted frequently, critical phases are inspected 2-3 times per year or on request from the sponsor.

In long term studies and short term studies conducted non-frequently, inspections are normally made at critical phases of the individual study.

The performance of this study has been secured by the VKI's Quality Assurance Unit as follows:

<u>Date</u>	<u>Activity</u>
1992.09.16	Protocol received
28.1.93	Final report audited

This test report accurately describes the methods and procedures used in the study, and the reported results accurately reflect the raw data of the study.



 Jørgen F. Simonsen (Quality Assurance Officer) 28.1.93
Date

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1. Summary

The degradation of the test product, 3-Carene, was tested by the OECD Test Method No. 301 D: "Ready Biodegradability: Closed Bottle Test" /1/.

The study showed that the product was degraded 6.3% within the first week. At the end of the test after 28 days the biological oxygen demand corresponded to 3.8% of the theoretical oxygen demand of the test product.

The test product had no inhibitory effect on the respiratory activity of the inoculum at the concentration used.

2. Introduction

The OECD Method No. 301 D: "Ready Biodegradability. Closed Bottle Test" /1/ was used to investigate the biodegradation of 3-Carene. The biodegradation of the organic compounds in the test product was determined by measuring the oxygen consumption in inoculated test bottles.

The measured oxygen consumption was compared to the theoretical oxygen demand for a complete degradation of the test product. The theoretical oxygen demand was expressed as the chemical oxygen demand (COD) which was measured according to the Danish Standard /2/.

3. Test product

3-Carene consisted mainly of delta 3-carene mixed with alpha pinene, beta pinene and dipentene. The test product was an equal mixture by volume of the following two products:

- Oulu 405

Veitsiluoto OY

The test product was delivered by International Paper Norge AS, Chemical Division and stored at VKI at 4 °C until use.

4. Preparation of stock solution

A stock solution of the test product (2 g/l) was prepared in demineralised water by ultra sonication for 5 min. A sample was taken in the center of the stock solution, and following determination of the chemical oxygen demand (COD) /2/ it was used the same day in the closed bottle test.

The COD of the stock solution was 1,232 mg O₂/l.

The actual ^{concentration of the} water accommodated fraction of the product was not determined by chemical analysis. The test concentration referred to will be the nominal concentration, i.e. including any non-soluble fractions.

5. Test method

Secondary effluent from Rungsted Treatment Plant, 82, Bukkeballevvej, DK-2970 Hørsholm, which mainly receives domestic waste water, was used as inoculum. The sludge was collected on November 24, 1993, and the test was started the same day.

The test medium was prepared by adding 1 ml of the following stock solutions to 1 litre of demineralised water:

- A) 8.5 g KH₂PO₄
 21.5 g K₂HPO₄
 33.5 g Na₂HPO₄ · 2 H₂O
 1.7 g NH₄Cl
 in 1000 ml demineralised water.
- B) 22.5 g MgSO₄ · 7 H₂O
 in 1000 ml demineralised water.

C 07

- C) 27.5 g CaCl_2
in 1000 ml demineralised water.
- D) 0.25 g $\text{FeCl}_3 \cdot 6 \text{H}_2\text{O}$
in 1000 ml demineralised water.

The test medium was aerated to an initial oxygen concentration of approximately 9 mg O_2/l and inoculated with 1 drop of secondary effluent per litre.

The test product was added at 7.8 mg/l to a part of the inoculated test medium, equivalent to a chemical oxygen demand (COD) of 4.80 mg O_2/l .

A reference compound was added to another part of inoculated medium in order to evaluate the activity of the inoculum. Sodium benzoate was used as the reference compound at a concentration of 2.0 mg/l equivalent to a theoretical oxygen demand (ThOD) of 3.34 mg O_2/l .

Both test product (7.8 mg/l) and reference compound (2.0 mg/l) were added to a third part of inoculated medium to assess possible inhibitory effects of the test product. The theoretical oxygen demand was the sum of the oxygen demands for both test product and reference compound, i.e. 8.14 mg O_2/l .

Inoculated test medium without addition of test nor reference compound served as a blank control.

After all additions, the medium was transferred to calibrated respirometric bottles (BOD bottles).

The complete test programme is shown below.

	Test	Reference	Inhibition	Blank
Test product	*		*	
Reference substance		*	*	
Secondary effluent	*	*	*	*

The test bottles were placed in the dark at a constant temperature of $20 \pm 1^\circ\text{C}$. A triplicate set of test bottles was sacrificed at the start of the experiment and after 7, 14, 21 and 28 days for oxygen measurements. The oxygen demand was calculated as the difference between the measured oxygen concentrations at time t and at the start of the test. The biological oxygen demand for the added carbon sources was calculated by subtracting the oxygen demand in the blank controls from the oxygen demand in the bottles containing test and reference compounds.

6. Data analysis

The biodegradability of 3-Carene was expressed as the biological oxygen demand during the test period.

The percentage of the test product or reference substance degraded was calculated by the following formula:

$$\% \text{ degraded} = \frac{\text{oxygen demand (test - blank)}}{\text{concentration} \cdot \text{ThOD}} \cdot 100 \%$$

or

$$\% \text{ degraded} = \frac{\text{oxygen demand (test - blank)}}{\text{concentration} \cdot \text{COD}} \cdot 100 \%$$

7. Archives

All original data including a sample of the test product will be stored at ambient temperature in the GLP archives of VKI for a period of ten years.

Samples that are unstable may be disposed off before that time or stored under specific conditions after consultation with the sponsor.

At the end of the period, the sponsor will be consulted regarding requirements for disposal or further storage.

8. Results

The results of the biodegradation test with 3-Carene are shown in Table 1 and 2, and in Figures 1-3. In addition, all test data are given in Annex 1.

The inoculum was found to have a satisfactory activity as illustrated by the degradation of the reference compound. More than 60% of the added sodium benzoate was degraded within the first 7 days of the test period (Table 1, Figure 1).

The biological oxygen demand of the test product, 3-Carene, was 6.3% of the chemical oxygen demand during the first 7 days and remained low during the 28 days of the experiment (Table 1, Figure 2). The oxygen measurements after 14 days in test bottles with 3-Carene showed a high oxygen consumption in two of the replicates.

During the first 21 days of the experiment the biological oxygen demand in BOD bottles with both test product and reference compound equaled the sum of the oxygen demands of 3-Carene and sodium benzoate tested separately (Table 2, Figure 3).

Table 1.

Degradation of sodium benzoate and 3-Carene. All values are corrected for oxygen consumption in blanks.

% DEGRADED:				
TIME (days)	SODIUM BENZOATE			
	I	II	III	MEAN
0	0	0	0	0
7	58	64	67	63
14	56	63	56	58
21	60	60	65	62
28	84	80	65	77

TIME (days)	3-CARENE			
	I	II	III	MEAN
0	0	0	0	0
7	5.6	6.9	6.5	6.3
14	20	28	3.6	17.4
21	0.3	2.8	0.8	1.3
28	0.0	3.7	7.8	3.8

Table 2.

Biological oxygen demand during degradation of sodium benzoate (SB) and 3-Carene tested separately or in combination. All values are corrected for oxygen consumption in blanks. Data are means of triplicate determinations.

TIME (days)	SB (mg O ₂ /l)	3-CARENE (mg O ₂ /l)	SB+ 3-CARENE (mg O ₂ /l)
7	2.10	0.30	2.20
14	1.95	0.83	2.13
21	2.06	0.06	2.23
28	2.56	0.18	3.88

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93-22

93-22

Biodegradation of Tall Oil Rosin

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Study director: Torben Madsen, Ph.D.
Project No.: 303068
GLP Study No.: 308067/471
Date: 30.04.1993/MK

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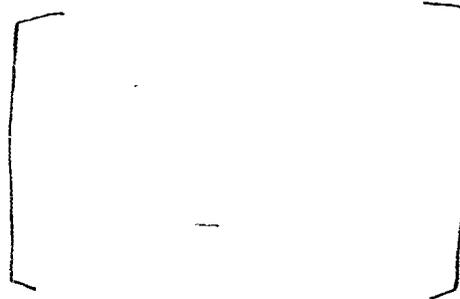
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International Paper Norge AS
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Title: Biodegradation of Tall Oil Rosin

GLP Study No.: 308067/471

Project No.: 303068

Test Period: 1993.01.20 - 1993.02.17

Test facility: WATER QUALITY INSTITUTE, ATV
 Ecotoxicological Dept.
 11, Agern Allé
 DK-2970 Hørsholm

Authentication:

We the undersigned hereby declare that the exotoxicological investigation described in this report was carried out under our supervision, and in accordance with the principles of Good Laboratory Practice (GLP) according to OECD codes of GLP May 1981, Doc C(81)30 (Final).

The study has been carried out in accordance with the procedures described in the report, which represents a true and accurate record of the results obtained.

Technician: Hanne Beck Rasmussen 3/5-93
 Hanne Beck Rasmussen date

Study Director: Torben Madsen 3/5-93
 Torben Madsen, Ph.D. date

Approved by: Preben Kristensen 3/5-93
 Preben Kristensen M. Sc date

Quality Assurance Statement

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Inspection of GLP studies are performed according to the following principles dependent on the type of study.

In short term studies (less than 1 week) conducted frequently, critical phases are inspected 2-3 times per year or on request from the sponsor.

In long term studies and short term studies conducted non-frequently, inspections are made at critical phases of the individual study.

The performance of this study has been secured by the VKI's Quality Assurance Unit.

The dates of inspection and audit are given below:

<u>Date</u>	<u>Activity</u>
1992.09.16	Protocol received
1993.05.04	Final report audited

This test report accurately describes the methods and procedures used in the study, and the reported results accurately reflect the raw data of the study.



Jørgen F. Simonsen (Quality Assurance Officer)

4/5 93

date

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1. Summary

The degradation of the test product, Tall Oil Rosin, was tested by the OECD Test Method No. 301 D: "Ready Biodegradability: Closed Bottle Test" /1/.

The study showed that the product was degraded 23% within the first week. At the end of the test after 28 days the biological oxygen demand corresponded to 32% of the theoretical oxygen demand of the test product.

The test product had no inhibitory effect on the respiratory activity of the inoculum at the concentration used.

2. Introduction

The OECD Method No. 301 D: "Ready Biodegradability: Closed Bottle Test" /1/ was used to investigate the biodegradation of Tall Oil Rosin. The biodegradation of the organic compounds in the test product was determined by measuring the oxygen consumption in inoculated test bottles.

The measured oxygen consumption was compared to the theoretical oxygen demand for a complete degradation of the test product. The theoretical oxygen demand was expressed as the chemical oxygen demand (COD) which was measured according to the Danish Standard /2/.

3. Test product

Tall Oil Rosin is a mixture of abietic type rosin acids and isomers. The test product is an equal mixture by volume of the following seven products:

- | | |
|---------------|------------------------------|
| - Beviros 90 | Bergvik Kemi AB |
| - Valke TOR A | Yhteneet Paperitehtaat OY |
| - Oulu 331 GP | Veitsiluoto OY |
| - Unitol NCY | Union Camp Chemicals Ltd. |
| - | |
| - | |
| - Petrosin 90 | International Paper Norge AS |

The test product was delivered by International Paper Norge AS, Chemical Division and stored at VKI at 4 °C until use.

4. Preparation of stock solution

A stock solution of the test product (2 g/l) was prepared in demineralised water by ultra sonication for 5 min followed by magnetic stirring for 24 hours at 20 °C. The solution was filtered through a GF/C filter (approx. 1.2 µm pore-size), and after determination of the chemical oxygen demand (COD) /2/ it was used within 1 day in the closed bottle test. By this procedure a stock solution containing exclusively the water accommodated fraction was obtained.

The COD of the stock solution was 43 mg O₂/l. The actual water accommodated fraction of the product was not determined by chemical analysis. The test concentration referred to will be the nominal concentration, i.e. including the non-soluble fraction.

5. Test method

Secondary effluent from Rungsted Treatment Plant, 82, Bukkeballevj, DK-2970 Hørsholm, which mainly receives domestic waste water, was used as inoculum. The sludge was collected on January 20, 1993, and the test was started the same day.

The test medium was prepared by adding 1 ml of the following stock solutions to 1 litre of demineralised water:

- A) 8.5 g KH₂PO₄
21.5 g K₂HPO₄
33.5 g Na₂HPO₄ · 2 H₂O
1.7 g NH₄Cl
in 1000 ml demineralised water.
- B) 22.5 g MgSO₄ · 7 H₂O
in 1000 ml demineralised water.
- C) 27.5 g CaCl₂
in 1000 ml demineralised water.

D) 0.25 g $\text{FeCl}_3 \cdot 6 \text{H}_2\text{O}$
in 1000 ml demineralised water.

The test medium was aerated to an initial oxygen concentration of approximately 9 mg O_2 /l and inoculated with 1 drop of secondary effluent per litre.

The test product was added at 0.21 g/l to a part of the inoculated test medium, equivalent to a chemical oxygen demand (COD) of 4.52 mg O_2 /l.

A reference compound was added to another part of inoculated medium in order to evaluate the activity of the inoculum. Sodium benzoate was used as the reference compound at a concentration of 2.0 mg/l equivalent to a theoretical oxygen demand (ThOD) of 3.34 mg O_2 /l.

Both test product (0.21 g/l) and reference compound (2.0 mg/l) were added to a third part of inoculated medium to assess possible inhibitory effects of the test product. The theoretical oxygen demand was the sum of the oxygen demands for both test product and reference compound, i.e. 7.86 mg O_2 /l.

Inoculated test medium without addition of test nor reference compound served as a blank control.

After all additions, the medium was transferred to calibrated respirometric bottles (BOD bottles).

The complete test programme is shown below.

	Test	Reference	Inhibition	Blank
Test product	*		*	
Reference substance		*	*	
Secondary effluent	*	*	*	*

The test bottles were placed in the dark at a constant temperature of $20 \pm 1^\circ\text{C}$. A triplicate set of test bottles was sacrificed at the start of the experiment and after 7, 14, 21 and 28 days for oxygen measurements. The oxygen demand was calculated as the difference between the measured oxygen concentrations at time

t and at the start of the test. The biological oxygen demand for the added carbon sources was calculated by subtracting the oxygen demand in the blank controls from the oxygen demand in the bottles containing test and reference compounds.

6. Data analysis

The biodegradability of Tall Oil Rosin was expressed as the biological oxygen demand during the test period.

The percentage of the test product or reference substance degraded was calculated by the following formula:

$$\% \text{ degraded} = \frac{\text{oxygen demand (test - blank)}}{\text{concentration} \cdot \text{ThOD}} \cdot 100 \%$$

or

$$\% \text{ degraded} = \frac{\text{oxygen demand (test - blank)}}{\text{concentration} \cdot \text{COD}} \cdot 100 \%$$

7. Archives

All original data including a sample of the test product will be stored at ambient temperature in the GLP archives of VKI for a period of ten years.

Samples that are unstable may be disposed off before that time or stored under specific conditions after consultation with the sponsor.

At the end of the period, the sponsor will be consulted regarding requirements for disposal or further storage.

8. Results

The results of the biodegradation test with Tall Oil Rosin are shown in Table 1 and 2, and in Figures 1-3. In addition, all test data are given in Annex 1.

The inoculum was found to have a satisfactory activity as illustrated by the degradation of the reference compound. Approximately 60% of the added sodium benzoate was degraded within the first 7 days of the test period (Table 1, Figure 1).

The biological oxygen demand of the test product, Tall Oil Rosin, was 23% of the chemical oxygen demand during the first 7 days and increased to 32% at the termination of the experiment after 28 days (Table 1, Figure 2).

During the experiment the biological oxygen demand in BOD bottles with both test product and reference compound equaled the sum of the oxygen demands of Tall Oil Rosin and sodium benzoate tested separately (Table 2, Figure 3).

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Biodegradation of Tall Oil Heads

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Study director: Torben Madsen, Ph.D.
Project No.: 303068
Study No.: 308067/474
Date: 30.04.1993

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Title: Biodegradation of Tall Oil Heads
GLP Study No.: 308067/474
Project No.: 303068
Test Period: 1992.11.24 - 1992.12.22
Test facility: WATER QUALITY INSTITUTE, ATV
Ecotoxicological Department
11, Agern Allé
DK-2970 Hørsholm

Authentication:

We the undersigned hereby declare that the investigation described in this report "Biodegradation of Fatty Acid Methylene Ester" was carried out under our supervision, and in accordance with the principles of Good Laboratory Practice (GLP) according to OECD codes of GLP May 1981, Doc C(81)30 (Final).

The study has been carried out in accordance with the procedures described in the report, which represents a true and accurate record of the results obtained.

Technician: Hanne Beck Rasmussen 3/5-93
Hanne Beck Rasmussen date

Study Director: Torben Madsen 3/5-93
Torben Madsen, Ph.D. date

Approved by: Preben Kristensen 3/5-93
Preben Kristensen, M.Sc. date

Quality Assurance Statement

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Inspection of GLP studies are performed according to the following principles dependent on the type of study.

In short term studies (less than 1 week) conducted frequently, critical phases are inspected 2-3 times per year or on request from the sponsor.

In long term studies and short term studies conducted non-frequently, inspections are made at critical phases of the individual study.

The performance of this study has been secured by the VKI's Quality Assurance Unit.

the dates of inspection and audit are given below:

<u>Date</u>	<u>Activity</u>
1992.03.16	Protocol received
1993.06.04	Final report audited

This test report accurately describes the methods and procedures used in the study, and the reported results accurately reflect the raw data of the study.



 Jørgen F. Simonsen (Quality Assurance Officer)

9/5-93
date

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Results of biodegradation test	15

1. Summary

The degradation of the test product, Tall Oil Heads, was tested by the OECD Test Method No. 301 D: "Ready Biodegradability: Closed Bottle Test" /1/.

The study showed that the product was degraded 33% within the first week. At the end of the test after 28 days the biological oxygen demand corresponded to 41% of the theoretical oxygen demand of the test product.

The test product had no inhibitory effect on the respiratory activity of the inoculum at the concentration used.

2. Introduction

The OECD Method No. 301 D: "Ready Biodegradability: Closed Bottle Test" /1/ was used to investigate the biodegradation of Tall Oil Heads. The biodegradation of the organic compounds in the test product was determined by measuring the oxygen consumption in inoculated test bottles.

The measured oxygen consumption was compared to the theoretical oxygen demand for a complete degradation of the test product. The theoretical oxygen demand was expressed as the chemical oxygen demand (COD) which was measured according to the Danish Standard /2/.

3. Test product

Tall Oil Heads is a mixture of fatty acids, rosin acids and esters. The test product is an equal mixture by volume of the following seven products:

- | | |
|-------------------------|---------------------------------|
| - Tall Oil Heads B0 | Bergvik Kemi AB |
| - Valke Tall Oil Heads | Yhteneet Paperitehtaat OY Valke |
| - Oulu 351/12 | Veitsiluoto OY |
| - Unitol AH Final Heads | Union Camp Chemicals Ltd. |
| - Forolje | International Paper Norge AS |

The test product was delivered by International Paper Norge AS, Chemical Division and stored at VKI at 4 °C until use.

4. Preparation of stock solution

A stock solution of the test product (2 g/l) was prepared in demineralised water by ultra sonication for 5 min. A sample was taken in the center of the stock solution, and following determination of the chemical oxygen demand (COD) /2/ it was used the same day in the closed bottle test.

The COD of the stock solution was 4,120 mg O₂/l.

The actual water accommodated fraction of the product was not determined by chemical analysis. The test concentration referred to will be the nominal concentration, i.e. including any non-soluble fractions.

5. Test method

Secondary effluent from Rungsted Treatment Plant, 82, Bukkeballevvej, DK-2970 Hørsholm, which mainly receives domestic waste water, was used as inoculum. The sludge was collected on November 24, 1992, and the test was started the same day.

The test medium was prepared by adding 1 ml of the following stock solutions to 1 litre of demineralised water:

- | | | | |
|----|--------|---|---------------------------------|
| A) | 8.5 g | KH ₂ PO ₄ | |
| | 21.5 g | K ₂ HPO ₄ | |
| | 33.5 g | Na ₂ HPO ₄ · 2 H ₂ O | |
| | 1.7 g | NH ₄ Cl | |
| | | | in 1000 ml demineralised water. |
| B) | 22.5 g | MgSO ₄ · 7 H ₂ O | |
| | | | in 1000 ml demineralised water. |
| C) | 27.5 g | CaCl ₂ | |
| | | | in 1000 ml demineralised water. |

D) 0.25 g $\text{FeCl}_3 \cdot 6 \text{H}_2\text{O}$
in 1000 ml demineralised water.

The test medium was aerated to an initial oxygen concentration of approximately 9 mg O_2/l and inoculated with 1 drop of secondary effluent per litre.

The test product was added at 2.4 mg/l to a part of the inoculated test medium, equivalent to a chemical oxygen demand (COD) of 4.94 mg O_2/l .

A reference compound was added to another part of inoculated medium in order to evaluate the activity of the inoculum. Sodium benzoate was used as the reference compound at a concentration of 2.0 mg/l equivalent to a theoretical oxygen demand (ThOD) of 3.34 mg O_2/l .

Both test product (2.4 mg/l) and reference compound (2.0 mg/l) were added to a third part of inoculated medium to assess possible inhibitory effects of the test product. The theoretical oxygen demand was the sum of the oxygen demands for both test product and reference compound, i.e. 8.28 mg O_2/l .

Inoculated test medium without addition of test nor reference compound served as a blank control.

After all additions, the medium was transferred to calibrated respirometric bottles (BOD bottles).

The complete test programme is shown below.

	Test	Reference	Inhibition	Blank
Test product	*		*	
Reference substance		*	*	
Secondary effluent	*	*	*	*

The test bottles were placed in the dark at a constant temperature of $20 \pm 1^\circ\text{C}$. A triplicate set of test bottles was sacrificed at the start of the experiment and

after 7, 14, 21 and 28 days for oxygen measurements. The oxygen demand was calculated as the difference between the measured oxygen concentrations at time t and at the start of the test. The biological oxygen demand for the added carbon sources was calculated by subtracting the oxygen demand in the blank controls from the oxygen demand in the bottles containing test and reference compounds.

6. Data analysis

The biodegradability of Tall Oil Heads was expressed as the biological oxygen demand during the test period.

The percentage of the test product or reference substance degraded was calculated by the following formula:

$$\% \text{ degraded} = \frac{\text{oxygen demand (test - blank)}}{\text{concentration} \cdot \text{ThOD}} \cdot 100 \%$$

or

$$\% \text{ degraded} = \frac{\text{oxygen demand (test - blank)}}{\text{concentration} \cdot \text{COD}} \cdot 100 \%$$

7. Archives

All original data including a sample of the test product will be stored at ambient temperature in the GLP archives of VKI for a period of ten years.

Samples that are unstable may be disposed off before that time or stored under specific conditions after consultation with the sponsor.

At the end of the period, the sponsor will be consulted regarding requirements for disposal or further storage.

8. Results

The results of the biodegradation test with Tall Oil Heads are shown in Table 1 and 2, and in Figures 1-3. In addition all test data are given in Annex 1.

The inoculum was found to have a satisfactory activity as illustrated by the degradation of the reference compound. More than 60% of the added sodium benzoate was degraded within the first 7 days of the test period (Table 1, Figure 1).

The biological oxygen demand of the test product, Tall Oil Heads, was 33% of the chemical oxygen demand during the first 7 days and increased to 41% at the termination of the experiment (Table 1, Figure 2).

During the experiment the biological oxygen demand in BOD bottles with both test product and reference compound equaled the sum of the oxygen demands of Tall Oil Heads and sodium benzoate tested separately (Table 2, Figure 3)

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Biodegradation of Fatty Acid Dipentaester

Study director: Torben Madsen, Ph.D.
Project No.: 303068
GLP Study No.: 308067/489
Date: 02.04.1993/MK

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Sponsor: Bergvik Kemi AB
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Title: Biodegradation of Fatty Acid Dipentaester

GLP Study No.: 308067/489

Project No.: 303068

Test Period: 1992.11.19 - 1992.12.17

Test facility: WATER QUALITY INSTITUTE, ATV
Ecotoxicological Dept.
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Authentication:

We the undersigned hereby declare that the investigation described in this report "Biodegradation of Fatty Acid Dipentaester" was carried out under our supervision, and in accordance with the principles of Good Laboratory Practice (GLP) according to OECD codes of GLP May 1981, Doc C(81)30 (Final).

The study has been carried out in accordance with the procedures described in the report, which represents a true and accurate record of the results obtained.

Technician: Connie Seierø 02.04.93
Connie Seierø date

Study Director: Torben Madsen 02.04.93
Torben Madsen, Ph.D. date

Approved by: Preben Kristensen 2/4-93
Preben Kristensen, M.Sc. date

Quality Assurance Statement
Water Quality Institute
Agerø Allé 11
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The performance of this study has been secured by the VKI's Quality Assurance Unit.

The dates of inspection and audit are given below:

<u>Date</u>	<u>Activity</u>
1992.09.16	Protocol received
2.4.93	Final report audited

This test report accurately describes the methods and procedures used in the study and accurately reflects the raw data of the study.



Jørgen F. Simonsen (Quality Assurance Officer)

2.4.93

date

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ANNEX 1: Results of biodegradation test	12

1. Summary

The degradation of the test product, Fatty Acid Dipentaester, was tested by the OECD Test Method No. 301 D: "Ready Biodegradability: Closed Bottle Test" /1/.

The study showed that the product was degraded 6.8% within the first week. At the end of the test after 28 days the biological oxygen demand corresponded to 18% of the theoretical oxygen demand of the test product.

The test product had no inhibitory effect on the respiratory activity of the inoculum at the concentration used.

2. Introduction

The OECD Method No. 301 D: "Ready Biodegradability: Closed Bottle Test" /1/ was used to investigate the biodegradation of Fatty Acid Dipentaester. The biodegradation of the organic compounds in the test product was determined by measuring the oxygen consumption in inoculated test bottles.

The measured oxygen consumption was compared to the theoretical oxygen demand for a complete degradation of the test product. The theoretical oxygen demand was expressed as the chemical oxygen demand (COD) which was measured according to the Danish Standard /2/.

3. Test product

Fatty Acid Dipentaester consisted of dipentaerythritol ester of a distilled tall oil fatty acid. The test product was Tall Oil Ester TO 2 DP 10 produced by Bergvik Kemi AB.

The test product was delivered by International Paper Norge AS, Chemical Division and stored at VKI at 4 °C until use.

4. Preparation of stock solution

A stock solution of the test product (2 g/l) was prepared in demineralised water by ultra sonication for 5 min. A sample was taken in the center of the stock solution, and following determination of the chemical oxygen demand (COD) /2/ it was used the same day in the closed bottle test.

The COD of the stock solution was 1,534 mg O₂/l.

The actual water accommodated fraction of the product was not determined by chemical analysis. The test concentration referred to will be the nominal concentration, i.e. including any non-soluble fractions.

5. Test method

Secondary effluent from Rungsted Treatment Plant, 82, Bukkeballevvej, DK-2970 Hørsholm, which mainly receives domestic waste water, was used as inoculum. The sludge was collected on November 19, 1992, and the test was started the same day.

The test medium was prepared by adding 1 ml of the following stock solutions to 1 litre of demineralised water:

- | | | | |
|----|--------|--|---------------------------------|
| A) | 8.5 g | KH_2PO_4 | |
| | 21.5 g | K_2HPO_4 | |
| | 33.5 g | $\text{Na}_2\text{HPO}_4 \cdot 2 \text{H}_2\text{O}$ | |
| | 1.7 g | NH_4Cl | |
| | | | in 1000 ml demineralised water. |
| B) | 22.5 g | $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$ | |
| | | | in 1000 ml demineralised water. |
| C) | 27.5 g | CaCl_2 | |
| | | | in 1000 ml demineralised water. |
| D) | 0.25 g | $\text{FeCl}_3 \cdot 6 \text{H}_2\text{O}$ | |
| | | | in 1000 ml demineralised water. |

The test medium was aerated to an initial oxygen concentration of approximately 9 mg O_2/l and inoculated with 1 drop of secondary effluent per litre.

The test product was added at 6.0 mg/l to a part of the inoculated test medium, equivalent to a chemical oxygen demand (COD) of 4.60 mg O_2/l .

A reference compound was added to another part of inoculated medium in order to evaluate the activity of the inoculum. Sodium benzoate was used as the reference compound at a concentration of 2.0 mg/l equivalent to a theoretical oxygen demand (ThOD) of 3.34 mg O_2/l .

Both test product (6.0 mg/l) and reference compound (2.0 mg/l) were added to a third part of inoculated medium to assess possible inhibitory effects of the test product. The theoretical oxygen demand was the sum of the oxygen demands for both test product and reference compound, i.e. 7.94 mg O_2/l .

Inoculated test medium without addition of test nor reference compound served as a blank control.

After all additions, the medium was transferred to calibrated respirometric bottles (BOD bottles).

The complete test programme is shown below.

	Test	Reference	Inhibition	Blank
Test product	*		*	
Reference substance		*	*	
Secondary effluent	*	*	*	*

The test bottles were placed in the dark at a constant temperature of $20 \pm 1^\circ\text{C}$. A triplicate set of test bottles was sacrificed at the start of the experiment and after 7, 14, 21 and 28 days for oxygen measurements. The oxygen demand was calculated as the difference between the measured oxygen concentrations at time t and at the start of the test. The biological oxygen demand for the added carbon sources was calculated by subtracting the oxygen demand in the blank controls from the oxygen demand in the bottles containing test and reference compounds.

6. Data analysis

The biodegradability of Fatty Acid Dipentaester was expressed as the biological oxygen demand during the test period.

The percentage of the test product or reference substance degraded was calculated by the following formula:

$$\% \text{ degraded} = \frac{\text{oxygen demand (test - blank)}}{\text{concentration} \cdot \text{ThOD}} \cdot 100 \%$$

or

$$\% \text{ degraded} = \frac{\text{oxygen demand (test - blank)}}{\text{concentration} \cdot \text{COD}} \cdot 100 \%$$

7. Archives

All original data including a sample of the test product will be stored at ambient temperature in the GLP archives of VKI for a period of ten years.

Samples that are unstable may be disposed of before that time or stored under specific conditions after consultation with the sponsor.

At the end of the period, the sponsor will be consulted regarding requirements for disposal or further storage.

8. Results

The results of the biodegradation test with Fatty Acid Dipentaester are shown in Table 1 and 2, and in Figures 1-3. In addition, all test data are given in Annex 1.

The inoculum was found to have a satisfactory activity as illustrated by the degradation of the reference compound. Approximately 60% of the added sodium benzoate was degraded within the first 7 days of the test period (Table 1, Figure 1).

The biological oxygen demand of the test product, Fatty Acid Dipentaester, was 6.8% of the chemical oxygen demand during the first 7 days and increased to 18% at the termination of the experiment (Table 1, Figure 2).

During the experiment the biological oxygen demand in BOD bottles with both test product and reference compound equaled the sum of the oxygen demands of Fatty Acid Dipentaester and sodium benzoate tested separately (Table 2, Figure 3).