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Chemical Category		ROSIN FUMARATED, 2-OCTANOL, POLYAMIDE PRODUCT (CONFIDENTIAL)			

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8EHQ-0301-14886s, 45964

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March 27, 2001

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Re: TSCA § 8(e) Submittal

8EHQ-01-14886
88010000098s

Dear Sir/Madam:

Enclosed are 39 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. Arizona is claiming as confidential [tradename and chemical identity information] in the studies and in this letter. Confidential information in this letter is bracketed and appears in bold italics and a document substantiating Arizona's CBI claim is enclosed. A non-confidential version of this letter and of each of the studies also is being provided for the public files.

Background

All of the studies that are being submitted are acute ecotoxicity or biodegradability studies. EPA has provided guidance regarding the reportability of acute ecotoxicity studies pursuant to TSCA Section 8(e). Based upon this guidance, test materials with LC₅₀ or EC₅₀ values of less than 1 mg/L are considered by the Agency to be of "high" concern; test materials with LC₅₀ or EC₅₀ values between 1 and 100 mg/L are considered to be of "moderate" concern; and test materials with LC₅₀ or EC₅₀ values of greater than 100 mg/L are considered to be of "low" concern. EPA guidance further states that acute ecotoxicity studies indicating a high concern should be submitted under Section 8(e) if there also is evidence that the test material has bioaccumulated to a

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pronounced degree or that it is or could be (based upon use patterns) widespread in environmental media. Agency guidance states that test results showing moderate concerns should be reported if usage patterns and/or monitoring data for the test material suggest that the material is present in environmental media at or near concentrations where the effects in question could reasonably be expected to be manifested. According to the same EPA guidance, results of acute ecotoxicity studies indicating a low concern need not be submitted.

Arizona has in its possession a total of 33 ecotoxicity studies on fortified rosin, an alkyl alcohol, and twelve amine-functional polyamide products that indicate high or moderate ecotoxicity concern. Arizona does not have any information to support a conclusion that these substances are widespread in environmental media or that they are present in environmental media at or near concentrations where the effects in question could reasonably be expected to be manifested. Further, Arizona has questions regarding the interpretation of some of these data because of the manner in which the test materials were introduced into the test medium. Nevertheless, Arizona is submitting these data pursuant to Section 8(e) out of an abundance of caution because these substances either are commercial U.S. products or are very similar to commercial U.S. products manufactured or imported by Arizona.

In addition, Arizona has in its possession ready biodegradability data for six of the above-mentioned products that indicate the test substances are not readily biodegradable. While Arizona does not believe that these biodegradability data are reportable in and of themselves, the studies are being submitted out of an abundance of caution to supplement the ecotoxicity data for the corresponding product. Summaries of all enclosed studies are provided below.

Summaries of Studies

Three ecotoxicity studies and one biodegradability study were conducted on a product known as "fortified rosin" CASRN 65997-04-8 for "rosin, fumarated". Of the three ecotoxicity studies that were conducted, one study included results which EPA classifies within the range of moderate concern, and two studies indicated low concern. An acute toxicity test on zebrafish showed a 96-hour LC₅₀ value of 20-50 mg/L (test report #308065/476). An immobilization test with *Daphnia Magna* showed a 48-hour EC₅₀ value of 162 mg/L (test report #308069/476). An algal growth inhibition study showed an EC₅₀ value of 479 mg/L (test report #308061/476). In the closed bottle test for biodegradability, this product was determined to biodegrade 15% after 28 days (test report #308067/476).

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One ecotoxicity study was conducted on an alkyl alcohol capryl alcohol, also known as 2-octanol (CASRN 123-96-6). This acute toxicity test on golden orfe showed a 96-hour LC_{50} value of 24 mg/L (test report #909/005).

Three ecotoxicity studies and one biodegradability study were conducted on a polyamide product known as

Of the three ecotoxicity studies that were conducted, one study included results which EPA classifies within the range of high concern, and two studies indicated moderate concern. An acute toxicity test on golden orfe showed a 96-hour LC_{50} value of 2.3 mg/L (test report #508/11). An immobilization test with *Daphnia Magna* showed a 48-hour EC_{50} value of 0.55 mg/L (test report #508/10). An algal growth inhibition study showed an EC_{50} value of 2.3 mg/L (test report #508/9). In the Modified Sturm Test for biodegradability, this polyamide product was determined to biodegrade 0% after 28 days (test report #508/8).

Three ecotoxicity studies and one biodegradability study were conducted on a polyamide product known as

Of three ecotoxicity studies that were conducted two studies included results which EPA classifies within the range of high concern, and one study indicated moderate concern. An acute toxicity test on golden orfe showed a 96-hour LC_{50} value of 0.75 mg/L (test report #508/16). An immobilization test with *Daphnia Magna* showed a 48-hour EC_{50} value of 0.21 mg/L (test report #508/15). An algal growth inhibition study showed an EC_{50} value of 1.7 mg/L (test report #508/14). In addition, this polyamide product was determined to biodegrade 0% after 28 days in the Modified Sturm Test (test report #508/13).

Three ecotoxicity studies and one biodegradability study were conducted on a polyamide product known as

1. The three ecotoxicity studies included results which EPA classifies within the range of moderate concern. An acute toxicity test on golden orfe showed a 96-hour LC_{50} value of 7.5 mg/L (test report #508/21). An immobilization test with *Daphnia Magna* showed a 48-hour EC_{50} value of 4.0 mg/L (test report #508/20). An algal growth inhibition study showed an EC_{50} value

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of 3.1 mg/L (test report #508/19). In addition, this polyamide product was determined to biodegrade 38% after 28 days in the Modified Sturm Test (test report #508/18).

Three ecotoxicity studies and one biodegradability study were conducted on a polyamide product known as

The three ecotoxicity studies included results which EPA classifies within the range of high concern. An acute toxicity test on golden orfe showed a 96-hour LC₅₀ value of 0.071 mg/L (test report #508/50). An immobilization test with *Daphnia Magna* showed a 48-hour EC₅₀ value of 0.11 mg/L (test report #508/51). An algal growth inhibition study showed an EC₅₀ value of 0.6 mg/L (test report #508/52). In addition, this polyamide product was determined to biodegrade 0% after 28 days in the Modified Sturm Test (test report #508/49).

Three ecotoxicity studies and one biodegradability study were conducted on a polyamide product known as

The three ecotoxicity studies included results which EPA classifies within the range of high concern. An acute toxicity test on golden orfe showed a 96-hour LC₅₀ value of 0.57 mg/L (test report #508/55). An immobilization test with *Daphnia Magna* showed a 48-hour EC₅₀ value of 0.19 mg/L (test report #508/56). An algal growth inhibition study showed an EC₅₀ value of 0.8 mg/L (test report #508/57). In addition, this polyamide product was determined to biodegrade 0% after 28 days in the Modified Sturm Test (test report #508/54).

Of two ecotoxicity studies that were conducted on a polyamide product known as

one study included results which EPA classifies within the range of high concern, and one study indicated moderate concern. An acute toxicity test on golden orfe showed a 96-hour LC₅₀ value of 4.2 mg/L (test report #508/87). An immobilization test with *Daphnia Magna* showed a 48-hour EC₅₀ value of 0.1 mg/L (test report #508/88).

Of two ecotoxicity studies that were conducted on a polyamide product known as

one study included results which EPA classifies within the range of high concern, and one study indicated moderate concern. An acute toxicity test on golden orfe showed a 96-hour

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LC₅₀ value of 1.3 mg/L (test report #508/83). An immobilization test with *Daphnia Magna* showed a 48-hour EC₅₀ value of 0.12 mg/L (test report #508/84).

Of two ecotoxicity studies that were conducted on a polyamide product known as

one study included results which EPA classifies within the range of high concern, and one study indicated moderate concern. An acute toxicity test on golden orfe showed a 96-hour LC₅₀ value of 4.2 mg/L (test report #508/91). An immobilization test with *Daphnia Magna* showed a 48-hour EC₅₀ value of 0.068 mg/L (test report #508/92).

Of two ecotoxicity studies that were conducted on a polyamide product known as

one study included results which EPA classifies within the range of high concern, and one study indicated moderate concern. An acute toxicity test on golden orfe showed a 96-hour LC₅₀ value of 4.2 mg/L (test report #508/79). An immobilization test with *Daphnia Magna* showed a 48-hour EC₅₀ value of 0.12 mg/L (test report #508/80).

Of two ecotoxicity studies that were conducted on a polyamide product known as

**one study included results which EPA classifies within the range of high concern, and one study indicated moderate concern. An acute toxicity test on golden orfe showed a 96-hour LC₅₀ value of 2.4 mg/L (test report #508/81). An immobilization test with *Daphnia Magna* showed a 48-hour EC₅₀ value of 0.13 mg/L (test report #508/82).
is not manufactured or imported into the U.S.**

Of two ecotoxicity studies that were conducted on a product known as

one study included results which EPA classifies within the range of high concern, and one study indicated moderate concern. An acute toxicity test on golden orfe showed a 96-hour LC₅₀ value of 2.4 mg/L (test report #508/93). An immobilization test with *Daphnia Magna* showed a 48-hour

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March 27, 2001

Page 6

EC₅₀ value of 0.13 mg/L (test report #508/94).
or imported into the U.S.

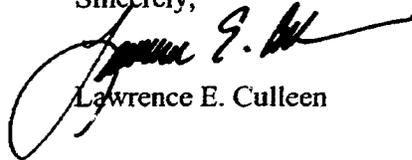
is not manufactured

Of two ecotoxicity studies that were conducted on a product known as

one study included results which EPA classifies within the range of high concern, and one study indicated moderate concern. An acute toxicity test on golden orfe showed a 96-hour LC₅₀ value of 2.4 mg/L (test report #508/89). An immobilization test with *Daphnia Magna* showed a 48-hour EC₅₀ value of 0.074 mg/L (test report #508/90).
is not manufactured or imported into the U.S.

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

A 09

Vandkvalitetsinstituttet
Water Quality Institute



TOX 93-85

**Fish Acute Toxicity Test of
Fortified Rosin with Zebrafish
(*Brachydanio rerio*)**

Study Director:
Project No.:
GLP Study No.:
Date:

Gitte I. Petersen, M.Sc.
303068
308065/476
1993.05.12/KIØ

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Title: Fish Acute Toxicity Test of Fortified Rosin with
Zebrafish (*Brachydanio rerio*)

GLP Study No.: 308065/476

Project No.: 303068

Test Period: (93.03.23-93.03.27)

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

Authentication:

We the undersigned hereby declare that the ecotoxicological investigation described in this report "Fish Acute Toxicity Test of Fortified Rosin with Zebrafish (*Brachydanio rerio*)" 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: Lilly Nilsson 18/5/93
Lilly Nilsson date

Study Director: Gitte I. Petersen 12.05.93
Gitte I. Petersen, M.Sc. date

Approved by: Preben Kristensen 12/5-93
Preben Kristensen, M.Sc. date

Quality Assurance Statement

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

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

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

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

The dates of inspection and audit are given below:

<u>Date</u>	<u>Activity</u>
1992.11.24	Protocol received
1993.03.25	Q.A. Inspection
19.1.93	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 E. Simonsen (Quality Assurance Officer)

19.1.93

date

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4. Preparation of stock solution

A quantity of 1 gramme of the product was weighed out into each of four separate 500 ml of test medium. These four stock solutions were then treated by ultrasonication for 5 min followed by magnetic stirring at 20 °C until the next day (20 hours). The stock solution is then transferred into separatory funnels and left for separation for 2 hours to avoid the bottom as well as the top layer when taking out samples. From the stock solution samples are pipetted into test aquaria and test medium added. These solutions are used in the toxicity test.

Due to the low solubility of the product, the fraction tested is referred to as the "water accommodated fraction" (WAF) in conformity with /2/.

The actual concentration of the water accommodated fraction of the product was not determined by chemical analysis. All test concentrations referred to are nominal concentrations as given in the tables.

5. Methods

Zebrafish (*Brachydanio rerio*) obtained from a local supplier were used for this study. After receipt, the test fish were acclimatized to the test conditions for min. 12 days. During acclimatization pH, dissolved oxygen and temperature were measured daily. The stock population was fed daily until 24 hours before start of the test. Food was withheld throughout the test period.

Groups of 10 fish were exposed to 6 nominal concentrations of the test product for 96 hours. 10 fish in uncontaminated water served as a control group. Mortality was recorded after 2, 24, 48, 72 and 96 hours and the concentrations lethal to 10 per cent and 50 per cent of the fish (LC10, LC50) were determined, when possible. pH, dissolved oxygen and temperature were measured daily throughout the observation period and at the start and end of the test.

In parallel with the acute toxicity test on Fortified Rosin the acute toxicity of the reference substance potassium dichromate was determined to check the reproducibility of the test method.

The nominal concentrations of Fortified Rosin tested were: 10; 20; 50; 100; 200 and 400 mg/l.

The reference substance ($K_2Cr_2O_7$) was tested in concentrations 20; 50; 100; 200; 300 and 500 mg/l.

At the start of the test the individual test vessels were identified by GLP Study No., test concentration, date of start and initials of technician. Freshly produced synthetic medium was used in the test. The medium was prepared from Millipore water according to the ISO Standard /3/. pH was 7.8 ± 0.2 and calcium hardness approximately 250 mg/l, expressed as calcium carbonate. The chemical

Calcium chloride	(CaCl ₂ ·2H ₂ O)	294 mg/l
Magnesium sulphate	(MgSO ₄ ·7H ₂ O)	123 mg/l
Sodium hydrogen carbonate	(NaHCO ₃)	65 mg/l
Potassium chloride	(KCl)	6 mg/l

The dilution water was aerated until oxygen and pH stabilization was achieved.

The test was carried out at $23 \pm 1^\circ\text{C}$ in climate room with a daily light/dark period 12:12 hours.

Polyethylene aquaria with a total capacity of 10 l and a surface area of 600 cm² were used as test vessels. 5 l of each test solution was placed in the test vessels and aerated gently. The test fish, randomly chosen among the stock population (size 27.6 ± 2.8 mm, 0.31 ± 0.10 g), were transferred from the stock population to the test vessels within 30 minutes, to make up 10 fish per concentration. The maximum loading was 0.6 g fish/litre. The test was performed as a static test. According to the sponsors the product is stable in solution.

6. Data analysis

The LC 50 value for the acute toxicity of the reference substance K₂Cr₂O₇ was calculated using the standard procedure Probit Analysis /4/. The LC 50 value for the acute toxicity of Fortified Rosin could not be calculated, since at least 2 test concentrations with effects less than 100 % mortality are needed.

7. Archives

All original data including a sample of the test product is 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 specified 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 primary data (mortality and behavioural observations) from the test are given in Annex 1, Tables 1 and 2. In Table 3 pH, O₂ saturation and temperature measurements during the test period are given. Length and weight was measured on each of ten fish from the control aquaria after the test. The data are indicated in Table 7.

The test product, Fortified Rosin, caused 100% mortality in the 96 hours test period at concentrations ≥ 50 mg/l. No mortality was registered at 10 mg/l (NOEC), and no mortality was observed in the control experiment. The following LC-values are indicated.

The test product Fortified Rosin caused behavioural effects at concentrations ≥ 20 mg/l.

LC 50 (24h): 20-50 mg/l

LC 50 (48h): 20-50 mg/l

LC 50 (72h): 20-50 mg/l

LC 10 (96h): ≈ 20 mg/l

LC 50 (96h): 20-50 mg/l

LC 90 (96h): 20-50 mg/l

The oxygen saturation was $> 80\%$ throughout the test period.

9. Validation

The validity criteria given in the Guideline /1/ were fulfilled.

= No mortality was recorded in the controls throughout the test period.

- The oxygen saturation was $\geq 60\%$ throughout the testperiod.

The LC 50 (24 hours) value found in the test with the reference compound K₂Cr₂O₇ was 212 (160-260) mg/l which is within the range normally found at the Water Quality Institute for this test system.

10. References

- /1/ OECD Guideline for Testing of Chemicals: "Fish Acute Toxicity Test". No. 203 (adopted 4th April 1984).
- /2/ Girling, A-E. 1989.
Preparation of aqueous media for aquatic toxicity testing of oils and oil-based products: A review of the published literature.
Chemosphere 19, nos. 10/11, pp. 1635-1641.
- /3/ ISO International Standard 7346. 1984
"Water Quality - Determination of the acute lethal toxicity of substances to freshwater fish (*Brachydanio rerio* (Hamilton-Buchanan) *Teleostei cyprinidae*).
- /4/ Probit analysis. Version 2.1 - Preliminary C. 1989.01.31. Statens Naturvårdsverk (National Swedish Environmental Protection Board. The data section).

TOX 93-85A

Immobilization Test of Fortified Rosin with the crustacean *Daphnia magna*

Study director: Estelle Bjørnstad M.Sc.
Project No.: 303068
GLP Study No.: 308069/476
Date: 12.05.1993/MK

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Title: Immobilisation test of Fortified Rosin
with the crustacean *Daphnia magna*.

GLP Study No.: 308069/476

Project No.: 303068

Test Period: 1993.03.17 - 1993.03.19

Test facility: Ecotoxicological Department
WATER QUALITY INSTITUTE, ATV (VKI)
Agern Allé 11
DK-2970 Hørsholm

Authentication:

We the undersigned hereby declare that the ecotoxicological investigation described in this report "Immobilisation test of Fortified Rosin with the crustacean *Daphnia magna*" 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 12/5-93
Hanne Beck Rasmussen date

Study Director: Estelle Bjørnstad 12/5-93
Estelle Bjørnstad, M.Sc. date

Approved by: Preben Kristensen 12/5-93
Preben Kristensen M. Sc date

Quality Assurance Statement
Water Quality Institute
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Inspection of GLP studies is 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.

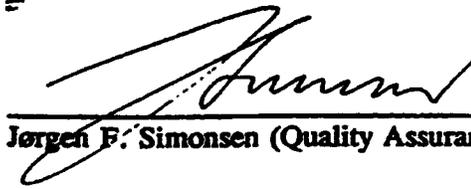
In long term studies and short term studies conducted non-frequently, inspections are made at critical phases of the individual study.

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>
1992.11.23	Protocol received
19.5.93	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)

19.5.93

date

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Annex 1

 The primary data of the acute test with Fortified Rosin 9

Annex 2

 The primary data of the acute test with $K_2Cr_2O_7$ 12

Furthermore, a 24 h acute toxicity test on the reference compound potassium dichromate ($K_2Cr_2O_7$) was performed to check the sensitivity of the test animals. The reference compound was tested in the following concentrations: 0 (control), 0.4, 0.7, 1.0, 1.4, 2.0 and 3.0 mg $K_2Cr_2O_7/l$.

Approximately 20 animals were exposed for each concentration. The number of immobile animals were recorded after 24 and 48 hours. On this basis the EC values were determined. pH, dissolved oxygen and temperature were measured at 0, 24 and 48 hours.

The test was run in plastic containers with a total capacity of 30 ml, each containing 10 ml of test solution.

Freshly produced medium was used in the tests. The medium was prepared from natural lake water (Brådeback), filtered through GF/C filter. Salts were added to the water according to the Standard /2/. pH was 7.8 ± 0.2 and the hardness was 250 ± 25 mg/l expressed as $CaCO_3$.

5 animals less than 24 hours old were transferred to each of 4 test vessels per concentration by means of a nylon net. Approximately 20 animals were thus exposed to each concentration. The control groups consisted of 30 animals, 5 animals in each of 6 vessels.

The test was run in a climate room at $20 \pm 1^\circ C$, 12 h darkness/12 h light.

A stock solution was prepared by weighing out 0.2 g of the test product in 100 ml of test medium. The stock solution (2g/l) was then treated by ultrasonication for 5 minutes followed by magnetic stirring at $20^\circ C$ until the next day (20 hours). The stock solution was then transferred into separatory funnels and left for separation for 2 hours to avoid the bottom as well as the top layer when taking out samples. From this stock solution samples were pipetted into volumetric flasks and test medium added. These solutions were used in the toxicity tests.

Due to the high oxygen demand of the product, the test was run semistatically i.e. by renewing the test solutions after 24 hours.

Due to the low solubility of the product, the fraction tested is referred to as the "water accommodated fraction" (WAF) in conformity with /3/.

The actual concentration of the water accommodated fraction of the product was not determined by chemical analysis. All test concentrations referred to are nominal concentrations as given in the tables.

5. Data analysis

EC 10 and EC 50 values for the acute toxicity of Fortified Rosin and $K_2Cr_2O_7$ were calculated using the standard procedure Probit (SNV) /4/.

6. 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 specified conditions after consultation with the sponsors.

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

7. Results

The primary data of the acute tests with Fortified Rosin and $K_2Cr_2O_7$ are given in Annex 1 and 2.

The calculated EC values are given in Table 1.

Fortified Rosin was estimated to immobilize 50 % of the test animals at 162 (140-183) mg/l after 48 h (48 h EC 50).

The immobility of the control animals was < 10%.

The reference compound was estimated to immobilize 50% of the test animals at 1.58 (1.43-1.77) mg/l $K_2Cr_2O_7$ /l after 24 hours (24 h EC 50), which is within the range given in /2/ (0.9-2.0 mg/l).

	24 h EC 10 mg/l	24 h EC 50 mg/l	48 h EC 10 mg/l	48 h EC 50 mg/l
Fortified Rosin	128 (74.5-155)	199 (173-245)	114 (73,4-137)	162 (140-183)
$K_2Cr_2O_7$	1.03 (0.76-1.20)	1.58 (1.43-1.77)	-	-

Table 1: EC values obtained in the acute tests with the test product Fortified Rosin and the reference compound $K_2Cr_2O_7$. In brackets are given 95 % fiducial limits.

8. Validation

The validity criteria given in the method are considered fulfilled:

- The immobility of the control animals was < 10 %.

The 24 h EC 50 value of the reference compound potassium dichromate, was 1.58 mg/l, which is within the interval given in ISO 6341 (0.9-2.0 mg/l).

The dissolved oxygen saturation at the end of the test was > 60%.

9. Conclusion

Fortified Rosin immobilized 50 % of the test animals at 162 (140-183) mg/l after 48 hours of exposure (48 h EC 50).

10. References

- /1/ OECD Guideline for Testing of Chemicals No. 202, 1981. "Daphnia sp., 14-day Reproduction Test (including an Acute Immobilisation Test)".
- /2/ ISO International Standard 6341, 1982. "Water quality Determination of the inhibition of the mobility of *Daphnia magna* Straus (Cladocera, Crustacea)".
- /3/ Girling, A-E., 1989. Preparation of aqueous media for aquatic toxicity testing of oils and oil-based products: A review of the published literature. - Chemosphere 19, Nos. 10/11, pp.-1635-1641.
- /4/ Probit Analysis. Version 2.1 - Preliminary C. 1989.01.31. Statens Naturvårdsverk (National Swedish Environmental Protection Board. The data section).

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Vandkvalitetsinstituttet
Water Quality Institute



TOX 93-85B

**Growth inhibition test of
Fortified Rosin with the micro
algae *Selenastrum capricornu-
tum***

Study director: Gitte I. Petersen, M.Sc.
Project No.: 303068
GLP Study No.: 308061/476
Date: 1993.05.12/KIØ

Monitor

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**Union Camp Chemicals Ltd
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Title:

**Growth inhibition test of Fortified Rosin with
*Selenastrum capricornutum***

GLP Study No.:

308061/476

Project No.:

303068

Test Period:

(93.03.23-93.03.26)

Test facility:

**WATER QUALITY INSTITUTE, ATV
Ecotoxicological Dept.
11, Agern Allé
DK-2970 Hørsholm**

Authentication:

We the undersigned hereby declare that the ecotoxicological investigation described in this report "Growth inhibition test of Fortified Rosin with *Selenastrum capricornutum*" 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:	<u>Susan Rasmussen</u>	<u>12/5-93</u>
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Study Director:	<u>Gitte L. Petersen</u>	<u>12.05.93</u>
	Gitte L. Petersen, M.Sc.	date
Approved by:	<u>Preben Kristensen</u>	<u>12/5-93</u>
	Preben Kristensen, M.Sc.	date

Quality Assurance Statement

**WATER QUALITY INSTITUTE, ATV (VKI)
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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>
1992.11.23	Protocol received
19.5.93	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 E. Simonsen (Quality Assurance Officer)

19.1.93
Date

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1. Summary

The aim of this study was to determine the concentrations of Fortified Rosin inhibiting the growth of the fresh water micro algae *Selenastrum capricornutum* 10 and 50% relative to a control without test product added (EC10 and EC50) in a 72 hour growth inhibition test.

Fortified Rosin was tested in 7 nominal concentrations obtained from a dilution serie of the water accomodated fraction.

The results of the test are shown in Table 1. No observed effect concentration (NOEC) is the highest test concentration at which no significant ($\alpha \leq 0.05$) effect on the growth of the algae was observed /5/. The EC-values are calculated on basis of the growth rate of the algae (Growth rate) and on basis of the area below the growth curve (Biomass) in agreement with the ISO-standard /2/.

Table 1: Results of the test of Fortified Rosin in growth test with *Selenastrum capricornutum*.

PARAMETER	NOEC (mg/l)	EC 10 (mg/l)	EC 50 (mg/l)
Growth rate	200	500-1000	500-1000
Biomass	200	135 (112-155)	479 (448-514)

2. Introduction

Fortified Rosin was tested for inhibition of growth of *Selenastrum capricornutum* according to OECD Guideline no. 201 "Alga, Growth Inhibition Test" /1/, which corresponds closely to the ISO International Standard ISO 8692 "Water Quality - Fresh water algal growth inhibition test with *Scenedesmus subspicatus* and *Selenastrum capricornutum*" /2/.

The concentrations of Fortified Rosin inhibiting the growth 10 and 50% (EC10 and EC50) during the 72 hour period of exposure and the NOEC were determined.

to the test conditions for at least 24 hours before the test start was used as inoculum.

The test was run in glass flasks with wide neck and a total capacity of 250 ml, each containing 100 ml of test solution. Glass beakers were used as caps. The test was run in triplicate with six controls and one blank (flasks with test substance but without algae) for each concentration. The batches were exposed for 72 hours to a dilution serie of the test substance under continuous shaking (aprox. 120 RPM) in a climate room at $23 \pm 2^\circ\text{C}$ and constant illuminated from a panel of fluorescent light with an intensity of $3,5 - 7 \times 10^{15}$ quanta \times $\text{cm}^{-2} \times \text{sec}^{-1}$. The cell density was adjusted to 3×10^3 cells/ml at the start of the test and was measured after 24, 48 and 72 hours. pH was measured at 0 and 72 hours.

The nominal concentrations of Fortified Rosin tested were: 10, 20, 50, 100, 200, 500 and 1000 mg/l.

6. Data analysis

On basis of the readings of the cell densities the concentrations that inhibited the growth rate and the biomass 10% and 50% (EC10 and EC50) relative to the growth rate and the biomass in the control flasks were calculated. The calculations were performed by use of a computer program "TOXEDO" developed by VKI /4/. The calculations included estimation of 95% confidence limits of the EC-values. NOEC-values were estimated by use of Dunett's procedure /5/.

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 specified 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 primary data of the growth inhibition test with Fortified Rosin are given in Annex 1.

The growth curves and the concentration-response curves of cultures of *Selenastrum capricornutum* exposed to serial concentrations of Fortified Rosin are shown in figure 1 and 2.

On the basis of the measured cell densities the EC-values given in Table 1 were calculated:

Table 1.: NOEC, EC 10, EC 50 and EC 90 values (with 95 % confidence intervals) on the parameters growth rate and biomass.

PARAMETER	NOEC (mg/l)	EC 10 (mg/l)	EC 50 (mg/l)	EC 90 (mg/l)
Growth rate	200	500-1000	500-1000	> 1000
Biomass	200	135 (112-155)	479 (448-514)	> 1000

GROWTH INHIBITION TEST
 Fortified Rosin
Selenastrum capricornutum

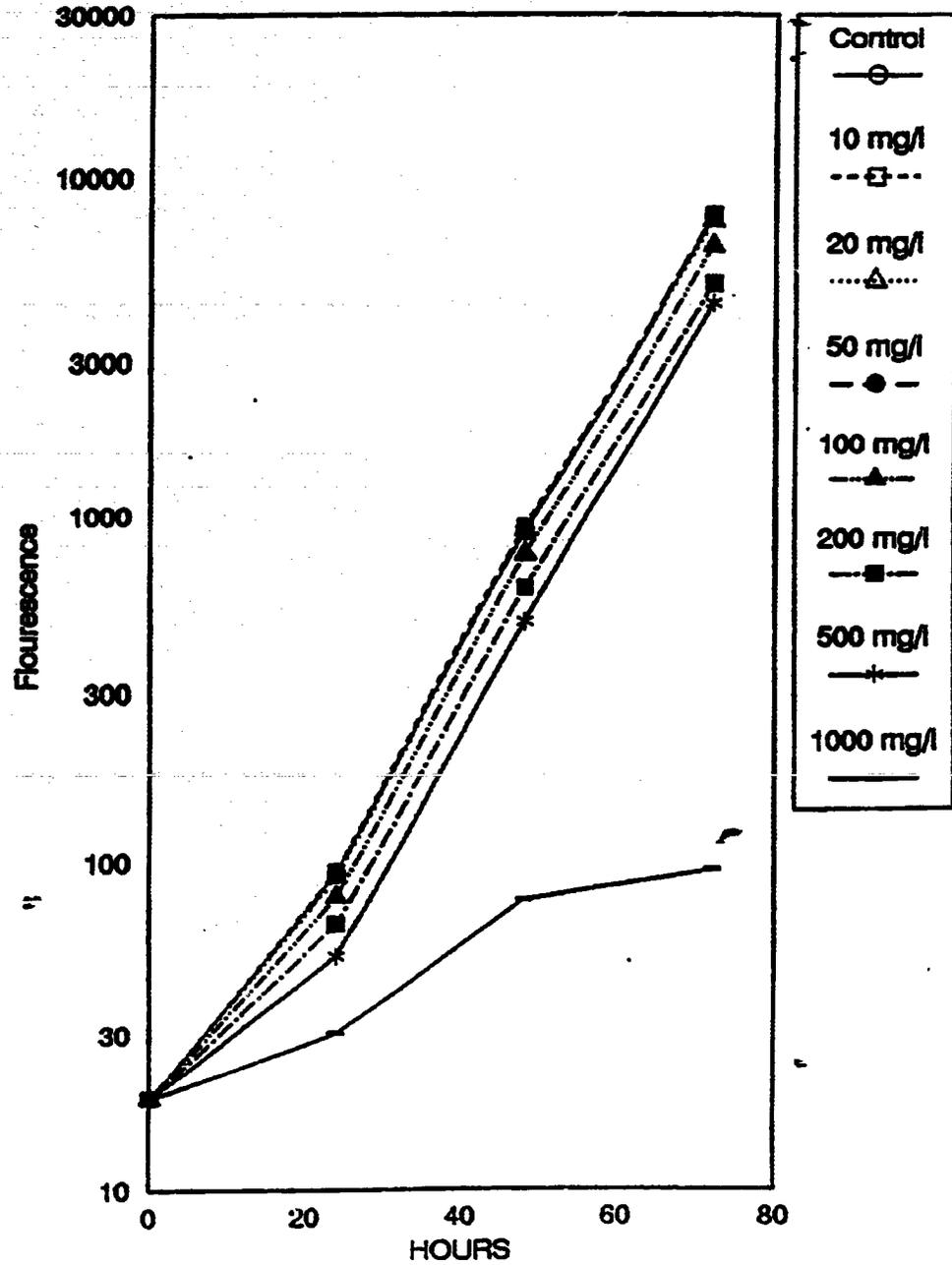


Figure 1: Growth curves of cultures of *Selenastrum capricornutum* exposed to serial concentrations of Fortified Rosin.

Test organism: *Selenestrom capricornutum*
 Test type : Growth Inhibition Test
 Substance : Sample 2

Test organism: *Selenestrom capricornutum*
 Test type : Growth Inhibition Test
 Substance : Sample 2

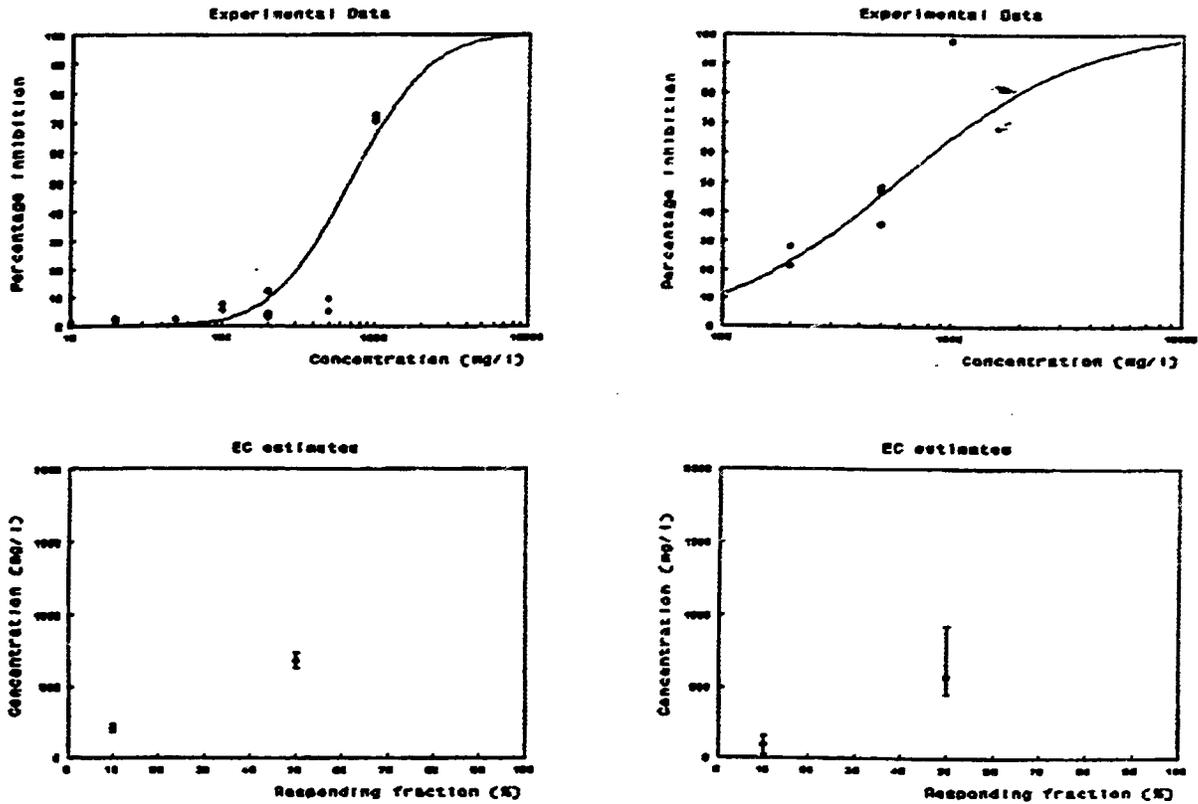


Figure 2: Concentration-response curves (top figures) and estimated EC-values with 95 % confidence intervals (lower curves). Left figure show calculations based on growth rate and right figure show calculations based on biomass estimates.

9. Validation

The validity criteria given in /2/ are all fulfilled:

1. The control cell density increased by a factor of more than 16 in the 72 hour period, which corresponds to a growth rate of more than 0.9 d^{-1} .
2. The variation of the pH in the test period was within 1.5 units.

The results of the growth inhibition test on Fortified Rosin are thus considered valid.

10. References

- /1/ OECD guideline for Testing of Chemicals. 1984. No. 201: "Alga, Growth Inhibition Test"
- /2/ ISO 8692. 1989. "Water Quality - Fresh Water Algal growth inhibition test with *Scenedesmus sunspicatus* and *Selenastrum capricornutum*".
- /3/ Girling, A-E. 1989. Preparation of aqueous media for aquatic toxicity testing of oils and oil-based products: A review of the published literature. *Chemosphere* 19, nos. 10/11, pp. 1635-1641.
- /4/ Water Quality Institute. 1992. TOXEDO. Program for statistical estimation of EC-values, based on experimental data from ecotoxicological assays, version 1.2.
- /5/ US-EPA. 1989. Short term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. Computer programme: Dunett's procedure in the analysis of data from short term toxicity tests with aquatic organisms. US-EPA, Cincinnati version 1.1.

E 05

Vandkvalitetsinstituttet
Water Quality Institute



TOX 93-85C

Biodegradation of Fortified Rosin

Study director:
Project No.:
Study No.:
Date:

Torben Madsen, Ph.D.
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GLP Study No.: 308067/476

Project No.: 303068

Test Period: 1993.01.20 - 1993.02.17

Test facility: WATER QUALITY INSTITUTE, ATV
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Authentication:

We the undersigned hereby declare that the ecotoxicological 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: Connie Seiersø 14/5-93
Connie Seiersø date

Study Director: Torben Madsen 14/5-93
Torben Madsen, Ph.D. date

Approved by: Preben Kristensen 14/5-93
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
19.1.93	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)

19.1.93

 date

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ANNEX 1:

 Results of biodegradation test 14

1. Summary

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

The study showed that the product was degraded 6.2% within the first week. At the end of the test after 28 days the biological oxygen demand corresponded to 15% 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 Fortified 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

Fortified Rosin is a mixture of fatty acids, rosin acids and esters. The test product is an equal mixture by volume of the following two products:

- Oulu 351/12
- Unitol R202

Veitsiluoto OY
Union Camp Chemicals Ltd.

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 44 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, Bulkeballevvej, 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 | 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 | FeCl ₃ · 6 H ₂ O |
| | | in 1000 ml demineralised water. |

The test medium was aerated to an initial oxygen concentration of approximately 9 mg O₂/l and inoculated with 1 drop of secondary effluent per litre.

The test product was added at 204 mg/l to a part of the inoculated test medium, equivalent to a chemical oxygen demand (COD) of 4.49 mg O₂/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 O₂/l.

Both test product (204 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. 7.83 mg O₂/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 ± 1°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 Fortified 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 Fortified 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, Fortified Rosin, was 6.2% of the chemical oxygen demand during the first 7 days and increased to 15% 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 Fortified Rosin and sodium benzoate tested separately (Table 2, Figure 3)

Table 1:
 Degradation of sodium benzoate and Fortified Rosin. 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	59	59	59
14	57	59	62	59
21	61	67	74	67
28	80	96	89	88

TIME (days)	FORTIFIED ROSIN			
	I	II	III	MEAN
0	0	0	0	0
7	7.1	6.2	5.5	6.2
14	18	14	15	16
21	18	17	14	16
28	19	13	11	15

Table 2:
 Biological oxygen demand during degradation of sodium benzoate (SB) and Fortified Rosin (FR) 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)	FR (mg O ₂ /l)	SB + FR (mg O ₂ /l)
7	1.96	0.28	2.38
14	1.98	0.71	2.65
21	2.24	0.72	2.99
28	2.95	0.65	3.48

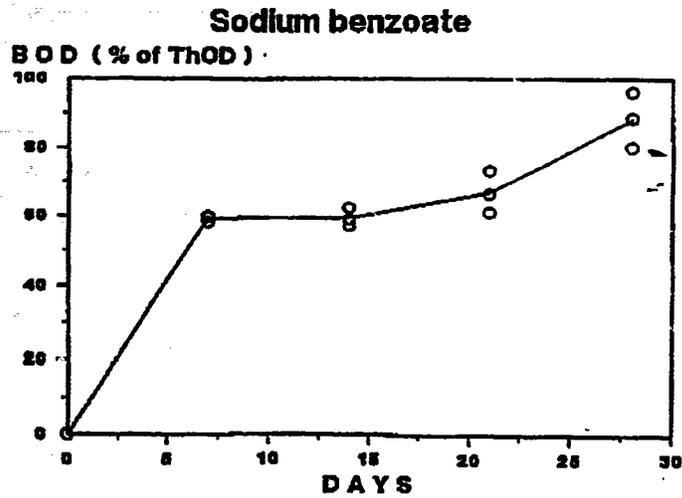


Figure 1:
Biological oxygen demand in test bottles with sodium benzoate. Data are means of triplicate determinations.

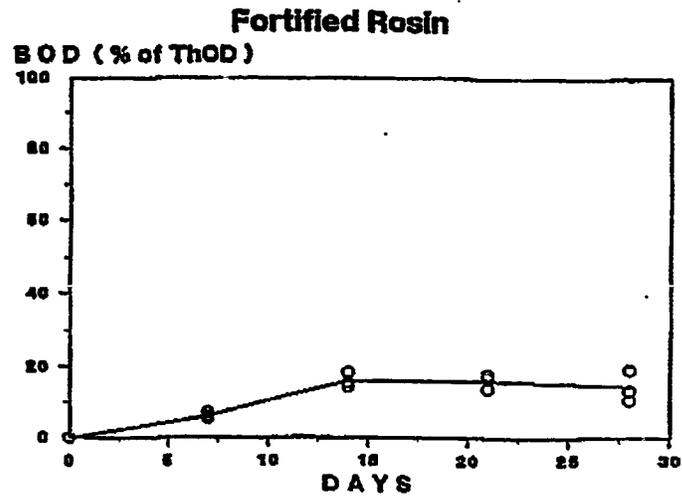


Figure 2:
Biological oxygen demand in test bottles with Fortified Rosin. Data are means of triplicate determinations.

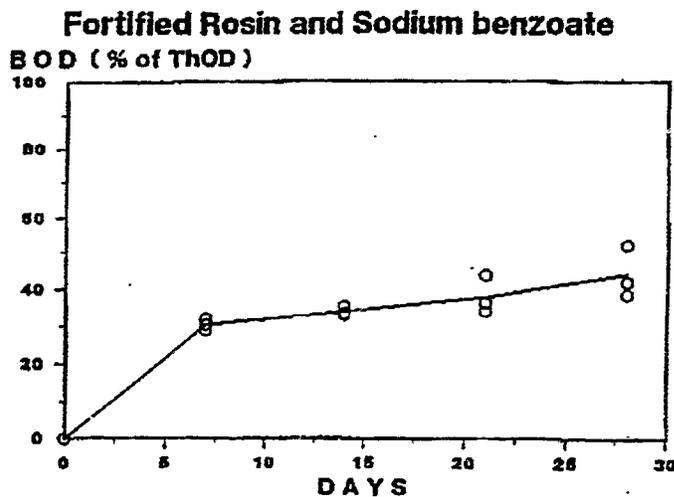


Figure 3:
Biological oxygen demand in test bottles with sodium benzoate and Fortified Rosin. Data are means of triplicate determinations.

9. Conclusion

The biological oxygen demand for Fortified Rosin was 6.2 and 15% of the theoretical oxygen demand after 7 and 28 days respectively. These results indicate that the test product is dominated by recalcitrant compounds.

The test concentration of Fortified Rosin did not inhibit the respiratory activity in the inoculum.

The secondary effluent used as inoculum was found to have a satisfactory activity as the reference substance, sodium benzoate, was degraded approximately 60% within the first 7 days of the test period.

10. References

- /1/ OECD Guideline for Testing of Chemicals:
301 D: Ready Biodegradability: Closed Bottle Test.
OECD, Paris, 1981.**
- /2/ Dansk Standardiseringsråd (1978):
DS 217: COD (oxygenforbrug med kaliumdichromat).
Dansk Standardiseringsråd, København, 1978.**

PAGE 1 OF 38 PAGES

TOX 97-27
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CONFIDENTIAL

CAPRYL ALCOHOL:
ACUTE TOXICITY TO GOLDEN ORFE
(*Leuciscus idus*)

SPL PROJECT NUMBER: 909/005

AUTHORS: P M WETTON
A J BARTLETT

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QUALITY ASSURANCE REPORT

The routine inspection of short term studies at Safepharm is carried out as a continuous process designed to encompass all major phases of each study type once per month. Inspection findings are reported to Management/Study Directors on the day of inspection in each case. Dates of relevant monthly inspections are as follows:

02, 08, 09, 19, 20 May 1997

This report has been audited by Safepharm Quality Assurance Unit. It is considered to be an accurate account of the data generated and of the procedures followed.

Date of Report Audit:

03 June 1997

.....  DATE: **- 9 OCT 1997**
J R Pateman CBiol MIBiol
For Safepharm Quality Assurance Unit

GLP COMPLIANCE STATEMENT

I, the undersigned, hereby declare that the objectives laid down in the protocol were achieved and as nothing occurred to adversely affect the quality or integrity of the study, I consider the data generated to be valid. This report fully and accurately reflects the procedures used and data generated.

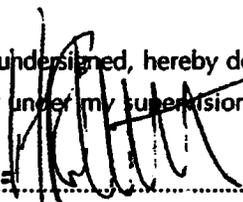
The work described was performed in compliance with UK GLP standards (Schedule 1, Good Laboratory Practice Regulations 1997 (SI 1997/654)). These Regulations are in accordance with GLP standards published as OECD Monograph No. 45 (OCDE/GD(92)32); and are in conformity with, and implement, the requirements of Directives 87/18/EEC and 88/320/EEC.

These international standards are acceptable to the United States Environmental Protection Agency and Food and Drug Administration, and fulfil the requirements of 40 CFR Part 160, 40 CFR Part 792 and 21 CFR Part 58 (as amended).


..... Date: - 9 OCT 1997

P M Wetton BSc
Study Director

I, the undersigned, hereby declare that the analytical data presented in this report were compiled by me or under my supervision and accurately reflect the data obtained.


..... Date: 9 OCT 1997

A J Bartlett CChem MRSC
Head of Analytical Chemistry

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SUMMARY

STUDY SPONSOR : UNION CAMP

STUDY TITLE : ACUTE TOXICITY TO GOLDEN ORFE

TEST MATERIAL : CAPRYL ALCOHOL

Methods

A study was performed to assess the acute toxicity of the test material to golden orfe (*Leuciscus idus*). The method followed that described in the OECD Guidelines for Testing of Chemicals (1992) No 203, "Fish, Acute Toxicity Test" referenced as Method C.1 of Commission Directive 92/69/EEC (which constitutes Annex V of Council Directive 67/548/EEC) and the Guidelines of the German Water Hazard Classification Scheme (Bewertung Wassergefährdender Stoffe, LTWS - Nr 10).

Procedures

Following preliminary range-finding studies, fish were exposed, in groups of 10, to an aqueous dispersion of the test material over a range of concentrations of 5.6, 10, 18, 32 and 56 mg/l for a period of 96 hours under semi-static test conditions. The number of mortalities and any sub-lethal effects of exposure in each test and control vessel were determined 3 and 6 hours after the start of exposure and then daily throughout the study until termination after 96 hours.

Results

The 96-Hour LC₅₀ based on nominal test concentrations was 24 mg/l with 95% confidence limits of 18 - 32 mg/l. The No Observed Effect Concentration was 18 mg/l.

The 96-Hour LC₅₀ value of 24 mg/l corresponds to an evaluation number (Bewertungszahl, BWZ) for the German Water Hazard Classification Scheme of 4.6.

Analysis of the test solutions at 0, 24 and 96 hours showed the measured test concentrations to be in excess of the required 80% of nominal with the exception of the 5.6 and 10 mg/l test concentrations at 96 hours which showed measured concentrations of 78% and 56% of nominal respectively. These low values were considered to be due to sampling and/or analytical variation given that the measured concentrations were near nominal on all other occasions. It was therefore considered justifiable to calculate the results based on nominal test concentrations only.

**CAPRYL ALCOHOL:
ACUTE TOXICITY TO GOLDEN ORFE (*Leuciscus idus*)**

1. INTRODUCTION

This report contains a description of the methods used and results obtained during a study to investigate the acute toxicity of Capryl Alcohol to golden orfe. The method (Safeparm Laboratories Standard Test Method 906.03) followed the recommendations of the OECD Guidelines for Testing of Chemicals (1992) No 203 "Fish, Acute Toxicity Test" referenced as Method C.1 of Commission Directive 92/69/EEC (which constitutes Annex V of Council Directive 67/548/EEC) and the Guidelines of the German Water Hazard Classification Scheme (Bewertung Wassergefährdender Stoffe, LTWS - Nr 10).

Golden orfe is a freshwater fish representative of a wide variety of natural habitats, and can therefore be considered as an important non-target organism in freshwater ecosystems.

The range-finding studies were conducted between 10 April 1997 and 14 April 1997 and the definitive study between 6 May 1997 and 10 May 1997.

2. TEST MATERIAL AND EXPERIMENTAL PREPARATION**2.1 Description, Identification and Storage Conditions**

Sponsor's identification : Capryl Alcohol
Description : colourless liquid
Lot number : 96028ET1
Date received : 19 March 1997
Storage conditions : room temperature

Data relating to the identity, purity and stability of the test material are the responsibility of the Sponsor.

2.2 Experimental Preparation

For the purpose of the definitive study the test material was prepared by direct dispersion in water.

Amounts of test material (123.2, 220 and 396 mg) were each separately dissolved in dechlorinated tap water with the aid of ultrasonication and the volume adjusted to 2 litres. These were then separately dispersed in dechlorinated tap water and the volume adjusted to 22 litres to give the 5.6, 10 and 18 mg/l test concentrations respectively.

To prepare the 32 and 56 mg/l test concentrations amounts of test material (616, 616 and 704 mg) were each separately dissolved in dechlorinated tap water with the aid of ultrasonication and the volume adjusted to 5 litres to give the 616, 616 and 704 mg/5 litres stock solutions. The 704 mg/5 litres stock solution was further dispersed in dechlorinated tap water and the volume adjusted to 22 litres to give the 32 mg/l test concentration. The 616 mg/5 litres stock solutions were pooled together prior to the volume being adjusted to 22 litres with dechlorinated tap water to give the 56 mg/l test concentration.

The concentration and stability of the test material in the test solutions were verified by chemical analysis at 0, 24 and 96 hours (see Appendix II).

3. METHODS

3.1 Test Species

The test was carried out using juvenile golden orfe (*Leuciscus idus*). Fish were obtained from New Xotic Fish Centre, Alfreton, Derbyshire, UK and maintained in-house since 8 April 1997. Fish were maintained in a glass tank with a "single pass" water renewal system. Fish were acclimatised to test conditions from 30 April 1997 to 6 May 1997. The lighting cycle was controlled to give a 16 hours light and 8 hours darkness cycle.

The water temperature was controlled at 21 °C with a dissolved oxygen content of greater than or equal to 8.2 mg O₂/l. These parameters were recorded daily. The stock fish were fed Tetramin® flake food which was discontinued 48 hours prior to the start of the definitive study. There was zero mortality in the 7 days prior to the start of the test and the fish had a mean standard length of 5.2 cm (sd = 0.4) and a mean weight of 1.44 g (sd = 0.24) at the end of the definitive study. Based on the mean weight value this gave a loading rate of 0.65 g bodyweight/litre.

The corpulence factor (K) for the fish used in the study was calculated to comply with the German Water Hazard Classification Scheme from the following equation:

$$K = \frac{100 \times G}{L^3}$$

where G = weight of fish (g)
 L = length of fish (cm)
 K = corpulence factor (g/cm³)

Hence the corpulence factor (K) = 1.0 g/cm³

The diet and diluent water are considered not to contain any contaminant that would affect the integrity and outcome of the study.

3.2 Test Water

The test water used for both the range-finding and definitive studies was the same as that used to maintain the stock fish.

Laboratory tap water dechlorinated by passage through an activated carbon filter (Purite Series 500) and partly softened (Elga Nimbus 1248D Duplex Water Softener) giving water with a total hardness of approximately 100 mg/l as CaCO₃. After dechlorination and softening the water was then passed through a series of computer controlled plate heat exchangers to achieve the required temperature. Typical water quality characteristics are given in Appendix III.

3.3 Procedure

3.3.1 Range-finding studies

The test concentrations to be used in the definitive study were determined by preliminary range-finding studies.

In the initial range-finding study fish were exposed to a series of nominal test concentrations of 1.0, 10 and 100 mg/l.

Amounts of test material (200 mg and 4 replicates of 500 mg) were each separately dissolved in dechlorinated tap water with the aid of ultrasonication and the volume adjusted to 2 litres and 5 litres respectively. The 200 mg/2 L stock solution and the 4 replicates of 500 mg/5 L stock solutions were then pooled together to give the 100 mg/l test concentration with a test media volume of 22 litres.

To prepare the 10 mg/l test concentration an amount of test material (220 mg) was dissolved in dechlorinated tap water with the aid of ultrasonication and the volume adjusted to 2 litres. This was then further dispersed in dechlorinated tap water and the volume adjusted to 22 litres.

Finally for the 1.0 mg/l test concentration an aliquot (220 ml) of a 100 mg/l stock solution (prepared as above) was dispersed in dechlorinated tap water and the volume adjusted to 22 litres.

After approximately 15 minutes exposure 100% mortalities were observed at the 100 mg/l test concentration. Therefore a second range-finding study was carried out at the single test concentration of 32 mg/l.

To prepare this, amounts of test material (204 and 500 mg) were each separately dispersed in dechlorinated tap water with the aid of ultrasonication and the volume adjusted to 2 and 5 litres respectively. These stock solutions were then pooled together and the volume adjusted to 22 litres using dechlorinated tap water.

For each test concentration 3 fish were added to each test and control vessel and maintained at 21 °C in a temperature controlled room with a photoperiod of 16 hours light and 8 hours darkness for a period of 96 hours under semi-static test conditions.

The control group was maintained under identical conditions but not exposed to the test material.

Each vessel was covered and sealed to reduce evaporation and possible losses of test material due to its suspected volatile nature. After 24, 48, 72 and 96 hours any mortalities or sub-lethal effects of exposure were determined by visual inspection of the test fish.

3.3.2 Definitive study

Based on the results of the range-finding studies the following test concentrations were assigned to the definitive study: 5.6, 10, 16, 32 and 56 mg/l.

3.3.2.1 Preparation of the test material

For the purpose of the definitive study the required amount of test material was added to each test vessel using the method described in Section 2.2.

3.3.2.2 Exposure conditions

As in the range-finding study glass exposure vessels containing 22 litres of test media were used for each test concentration. In an effort to maintain near nominal test concentrations the test vessels were completely filled to reduce the headspace, covered with lids and sealed to reduce evaporation and possible losses of the test material due to its suspected volatile nature. At the start of the study 10 fish were placed in each test vessel at random, in the prepared test solutions and maintained at 21 °C in a temperature controlled room with a photoperiod of 16 hours light and 8 hours darkness for a period of 96 hours. The test vessels received no auxiliary aeration. The fish were not individually identified and received no food during exposure.

The control group was maintained under identical conditions but not exposed to the test material.

A semi-static test regime was employed in the study involving a daily renewal of the test preparations to ensure that the concentrations of the test material remained near nominal and to prevent the build up of nitrogenous waste products.

Any mortalities and sub-lethal effects of exposure were recorded at 3, 6, 24, 48, 72 and 96 hours after the start of exposure. The criteria of death were taken to be the absence of both respiratory movement and response to physical stimulation.

3.3.2.3 Physico-chemical measurements

The water temperature, pH and dissolved oxygen concentrations were recorded daily throughout the study. The measurements at 0 hours, and after each test media renewal at 24, 48 and 72 hours, represent those of the freshly prepared test preparations while the measurements taken prior to each test media renewal, and on termination of the study after 96 hours, represent those of the used or 24-hour old test preparations.

3.3.2.4 Verification of test concentrations

Water samples were taken from the control and all surviving test groups at 0, 24 and 96 hours for quantitative analysis. Duplicate samples of all surviving test groups on all sampling occasions were taken and stored at -20 °C should re-analysis prove necessary. The method of analysis, stability, recovery and test solution analyses are described in Appendix II.

3.3.2.5 Evaluation of data

The LC₅₀ values and associated confidence limits were calculated by the moving average method of Thompson (1947).

4. ARCHIVES

Unless instructed otherwise by the Sponsor, all original data and the final report will be retained in the Safepharm archives for five years, after which instructions will be sought as to further retention or disposal.

5. RESULTS

5.1 Range-finding Studies

Cumulative mortality data from the exposure of golden orfe to the test material during the range-finding studies are given in Tables 1 and 2 and sub-lethal effects of exposure are given in Table 3.

The results of the initial range-finding study showed no mortalities at the test concentrations of 1.0 and 10 mg/l.

After approximately 15 minutes exposure 100% mortalities were observed at the 100 mg/l test concentration. Therefore a second range-finding study was conducted at a single test concentration of 32 mg/l. After 3 hours exposure all fish were observed to be moribund with one mortality observed after 24 hours exposure. For the reasons of animal welfare implications the remaining 2 fish which were still observed to be moribund after 24 hours were killed *in extremis*.

Based on this information test concentrations of 5.6, 10, 18, 32 and 56 mg/l were selected for the definitive study.

5.2 Definitive Study

5.2.1 Mortality data

Cumulative mortality data from the exposure of golden orfe to Capryl Alcohol during the definitive study are given in Table 4 and the relationship between percentage mortality and concentration is given in Figure 1.

Analysis of the mortality data by the moving average method of Thompson (1947) based on the nominal test concentrations gave the following results:

Time (h)	LC ₅₀ (mg/l)	95% Confidence Limits (mg/l)
3	42	32 - 56*
6	42	32 - 56*
24	24	18 - 32*
48	24	18 - 32*
72	24	18 - 32*
96	24	18 - 32*

* Concentrations resulting in 0 and 100% mortality respectively.

The results of the definitive study showed the highest test concentration resulting in 0% mortality to be 18 mg/l, the lowest test concentration resulting in 100% mortality to be 32 mg/l and the No Observed Effect Concentration (NOEC) to be 18 mg/l. The No Observed Effect Concentration is based upon zero mortalities and the absence of any sub-lethal effects of exposure at this concentration (Section 5.2.2).

The relationship between the median lethal concentration (LC_{50}) and time is presented graphically in Figure 2.

The 96-Hour LC_{50} value of 24 mg/l corresponds to an evaluation number (Bewertungszahl, BWZ) for the German Water Hazard Classification Scheme of 4.6.

5.2.2 Sub-lethal effects

The only sub-lethal effect of exposure was observed at the test concentration of 32 mg/l. This response was the presence of moribund fish (see Table 5). These moribund fish were observed to be dead after 24 hours exposure. However, in the range-finding study 2/3 fish were still observed to be moribund after approximately 24 hours at 32 mg/l. This apparent discrepancy is not considered to affect the results or validity of the study given that the overall mortality pattern was shown to be similar for both the range-finding studies and the definitive study.

5.2.3 Physico-chemical measurements

The results of the physico-chemical measurements are given in Appendix I. Temperature was maintained at 21 °C throughout the study, while there were no treatment related differences for oxygen concentration or pH.

5.2.4 Verification of test concentrations

Analysis of the test preparations at 0, 24 and 96 hours showed the measured test concentrations to be in excess of the required 80% of nominal with the exception of the 5.6 and 10 mg/l test concentrations at 96 hours which showed measured concentrations of 78% and 56% of nominal respectively. Repeat analysis of the frozen samples of these test groups was carried out and measured concentrations of 78% and 58% of nominal respectively were observed. These low values were considered to be due to sampling and/or analytical variation given that the measured concentrations were near nominal on all other occasions. It was therefore considered justifiable to calculate the results based on nominal test concentrations only.

6. CONCLUSION

The acute toxicity of the test material to the freshwater fish golden orfe (*Leuciscus idus*) has been investigated and gave a 96-Hour LC_{50} value of 24 mg/l with 95% confidence limits of 18 - 32 mg/l. The No Observed Effect Concentration was 18 mg/l.

The 96-Hour LC_{50} value of 24 mg/l corresponds to an evaluation number (Bewertungszahl, BWZ) for the German Water Hazard Classification Scheme of 4.6.

7. REFERENCE

Thompson, W R (1947) Use of Moving Averages and Interpolation to Estimate Median-Effective Dose *Bact Reviews*, **11**, 115 - 145.