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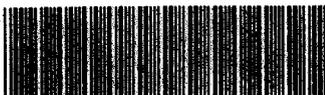
Dear Document Control Officer:

The attached reports are being provided in response to a request by the High Production Volume Chemicals Branch. We apologize for the delay, which was due to having to request the reports from Japan.

Sincerely,

Hester Kobayashi
Manager, Kao Group Product Safety

Attached: Test Reports on Acute Toxicity to Rainbow Trout by Kaicol 4098, 0898, 8098, 6098, 2475, and 2098 (alcohols).



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Schedule No : KAS/218
Report No : 96/KAS218/0415

1-OCTANOL CAS NO. 111-87-5

KALCOHL 0898: Acute toxicity to rainbow trout

(Preliminary toxicity screen)

FINAL REPORT

Study Director

C A Jenkins

To:
Kao Corporation
Tochigi Research Laboratories
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From:
Huntingdon Life Sciences Ltd
Eye
Suffolk IP23 7PX
England

Final: 9 July 1996

KALCOHL 0898: ACUTE TOXICITY TO RAINBOW TROUT

(PRELIMINARY TOXICITY SCREEN)

FINAL REPORT

Schedule No : KAS/218

Report No : 96/KAS218/0415

I declare that the report following constitutes a true and faithful account of the procedures adopted and the results obtained in the performance of this study.

The study was conducted in accordance with the principles of Good Laboratory Practice but no formal quality assurance procedures were undertaken.

C.A. Jenkins, B.Sc, C.Biol, M.I.Biol, M.I.F.M.
(Study Director)

.....*C.A. Jenkins*.....
Date: *9 July 1996*.....

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1. SUMMARY

- 1.1 The acute lethal toxicity of KALCOHL 0898 to the rainbow trout was assessed under static exposure conditions over a period of 96 hours. Exposure concentrations were not verified by chemical analysis.
- 1.2 Groups of five fish were exposed to KALCOHL 0898 at nominal concentrations of 0.1, 0.32, 1, 3.2, 10, 32 and 100 mg/l. The test media were individually prepared by dilution of aqueous stock dispersions; to aid dispersion, HCO-40 was employed. Control groups of fish were placed into dilution water alone or dilution water containing HCO-40 at the same level as in the test medium at the highest concentration.
- 1.3 The test was conducted at 13.7 to 14.6°C in treated tap water of hardness 238 to 252 mg/l as CaCO₃ at pH values in the range 8.0 to 8.3. At all concentrations, the test media were clear and colourless.
- 1.4 Observations of the fish were made at least at 24-hour intervals.

After 96 hours, the highest concentration at which no mortality had occurred was 10 mg/l and the lowest at which there was 100% mortality was 32 mg/l.

Sub-lethal, treatment-related effects were noted at 10 mg/l and higher concentrations and included hyperventilation, darkened pigmentation, lethargy and loss of co-ordination. All fish were adversely affected within 15 minutes of exposure.

The no-observed-effect concentration (NOEC) was 3.2 mg/l.

The 96-hour median lethal concentration (LC50) of KALCOHL 0898 to the rainbow trout was 18 mg/l.

2. TEST MATERIAL

A consignment of KALCOHL 0898 (approximately 300 g), a clear, colourless liquid, was received at Huntingdon Life Sciences Ltd (Eye) 21 November 1995. It was further identified by the batch number 2229 and was stored in the dark under ambient conditions until use.

The identity, strength and purity of the test material received and its stability under the storage conditions were the responsibility of the Sponsor.

3. TEST PROCEDURES

The test was conducted between 2 and 6 May 1996 according to the agreed Study Protocol (PTS12c).

The rainbow trout used in the test were supplied by Fish Network Ltd, Devon and arrived at Huntingdon Life Sciences (Eye) 25 January 1996. At the laboratory, they were held in an aerated supply of dilution water under flow-through conditions until removed for testing.

The fish were maintained and the study conducted in treated tap water (dechlorinated tap water which had been blended with reverse-osmosis, softened tap water).

No mortality occurred during the 14-day period before the test.

The mean wet-weight of the fish used in the test was 1.0 g; their mean fork length was 4.4 cm.

Groups of five fish were exposed to KALCOHL 0898 at nominal concentrations of 0.1, 0.32, 1, 3.2, 10, 32 and 100 mg/l. Control groups of fish were placed into dilution water alone or dilution water containing HCO-40 at the same level as in the test medium at the highest concentration.

Two concentrated aqueous dispersions (nominally 10 and 1000 mg/l) were prepared by adding the test material, mixed with an equal amount of HCO-40, to dilution water. Aliquots of the appropriate dispersion were diluted in the test vessels to a final volume of 14 litres; the 10 mg/l stock was used at 0.1 to 1 mg/l and the 1000 mg/l preparation at 3.2 to 100 mg/l. The media were gently aerated during the test.

Chemical analysis of control and test media was not undertaken.

4. RESULTS

Table 1 lists the mortalities observed during the test. After 96 hours, the highest concentration at which no mortality had occurred was 10 mg/l and the lowest at which there was 100% mortality was 32 mg/l.

The 24- to 96-hour median lethal concentration (LC50) of KALCOHL 0898 to the rainbow trout was 18 mg/l; this value was derived by non-linear interpolation between the two concentrations which bracket the 50% effect level (10 and 32 mg/l).

At 100 mg/l, all fish died within 15 minutes of exposure. At 32 mg/l, all fish exhibited hyperventilation and appeared dark and were moribund within 15 minutes; they were dead at 24 hours. At 10 mg/l, all fish exhibited hyperventilation within 15 minutes and some were lethargic or overturned and appeared dark; from 24 hours onwards, they were hyperventilating and some continued to exhibit darkened pigmentation or lethargy.

The no-observed-effect concentration (NOEC) was 3.2 mg/l.

At all concentrations, the test media were clear and colourless.

The results of the water quality analysis conducted at the start of the test and at 24-hour intervals thereafter are summarised below.

| | |
|--|------------------------|
| - temperature, °C | 13.7 - 14.6 |
| - pH | 8.0 - 8.3 |
| - dissolved oxygen, % ASV* | 69 - 102 |
| - total hardness, mg/l CaCO ₃ | 238 - 252 [#] |

* air saturation value

[#] although the hardness of the media slightly exceeded that recommended for this type of test (maximum 250 mg/l CaCO₃) this is not thought to have affected the integrity of the test.

No concentration-related changes in these parameters were observed.

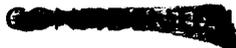
TABLE 1

Cumulative mortality

| Observation times (hours) | Nominal KALCOHL 0898 concentrations, mg/l | | | | | | | | |
|---------------------------|---|----|-----|------|---|-----|----|----|-----|
| | 0 | 0* | 0.1 | 0.32 | 1 | 3.2 | 10 | 32 | 100 |
| 0.25 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 |
| 24 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 5 |
| 48 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 5 |
| 72 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 5 |
| 96 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 5 |

Note : Five fish were exposed at each concentration.

* : Dispersant control.



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1-DODECANOL CAS NO. 112-53-8

KALCOL 2098:

ACUTE TOXICITY TO RAINBOW TROUT

(*Oncorhynchus mykiss*)

SPL PROJECT NUMBER: 140/592

AUTHOR: P M WETTON

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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. Dates of relevant monthly inspections are given below:

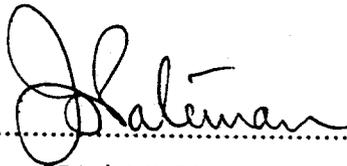
Date(s) of Inspection and Reporting:

08, 11 October 1996

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:

07 November 1996



Date: **27 NOV 1996**

J R Pateman CBiol MI Biol

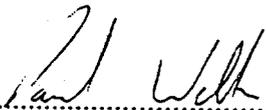
For Safepharm Quality Assurance Unit

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 the UK Principles of Good Laboratory Practice (The United Kingdom Compliance Programme, Department of Health 1989). These Principles are in accordance with GLP standards published as OECD Environment 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); and to the Japanese Ministry of Agriculture, Forestry and Fisheries (59 NohSan, Notification No. 3850, Agricultural Production Bureau) - confirmed by an Arrangement between the Ministry and the UK Department of Health; the Japanese Ministry of Health and Welfare (Notification No. 313, Pharmaceutical Affairs Bureau - as amended; and Kanpogyo No. 39 Environmental Agency, Yakuhatu No. 229); and the Japanese Ministry of International Trade and Industry (Chemical Substances Control Law, Kanpogyo No. 39 Environmental Agency, Kikyoku No. 85).



..... Date: 25 NOV 1996

PM Wetton BSc
Study Director

The following person was also involved in the study
L G Shacklady BSc
Study Supervisor.

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SUMMARY

STUDY SPONSOR : KAO CORPORATION

STUDY TITLE : ACUTE TOXICITY TO RAINBOW TROUT

TEST MATERIAL : KALCOL 2098

Methods

A study was performed to assess the acute toxicity of the test material, Kalcol 2098, to rainbow trout (*Oncorhynchus mykiss*). 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).

Procedures

Following a preliminary range-finding study fish were exposed, in two groups of ten, to an aqueous dispersion of the test material, at a single concentration of 1.0 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 LC_{50} for the test material to rainbow trout (*Oncorhynchus mykiss*), based on nominal test concentrations was greater than 1.0 mg/l and correspondingly the No Observed Effect Concentration was greater than or equal to 1.0 mg/l.

The test concentration of 1.0 mg/l was the highest attainable test concentration due to the limited solubility of the test material in water and auxiliary solvent, and having due regard for the amount of auxiliary solvent permitted in the test under the OECD Guidelines.

KALCOL 2098:
ACUTE TOXICITY TO RAINBOW TROUT
(*Oncorhynchus mykiss*)

1. INTRODUCTION

This report contains a description of the methods used and results obtained during a study to investigate the acute toxicity of Kalcol 2098 to rainbow trout. The method (Safepharm Laboratories Standard Method 902.01) 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).

Rainbow trout 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 study was conducted between 26 September 1996 and 30 September 1996 and the definitive study between 7 October 1996 and 11 October 1996.

2. TEST MATERIAL AND EXPERIMENTAL PREPARATION

2.1 Description, Identification and Storage Conditions

Sponsor's identification : Kalcol 2098
Description : clear colourless liquid at a temperature of $>27^{\circ}\text{C}$
white solid at a temperature of $<27^{\circ}\text{C}$
Lot number : 3397
Date received : 5 August 1996
Storage conditions : ambient 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 using a preliminary solution in dimethyl formamide

An amount (250 mg) of test material was dispersed in dimethyl formamide and the volume adjusted to 25 ml to give a 250 mg/25 ml solvent stock solution. An aliquot (2.0 ml) of this solvent stock solution was dispersed in 20 litres of dechlorinated tap water to give the 1.0 mg/l test concentration.

The concentration, homogeneity and stability of the test material in the test solutions were not determined as per the agreed protocol.

3. METHODS

3.1 Test Species

The test was carried out using juvenile rainbow trout (*Oncorhynchus mykiss*). Fish were obtained from Donnington Fish Farm, Upper Swell, Gloucestershire, UK and maintained in-house since 4 September 1996. Fish were maintained in a glass fibre tank with a "single pass" water renewal system. Fish were acclimatised to test conditions from 30 September 1996 to 7 October 1996. The lighting cycle was controlled to give a 16 hours light and 8 hours darkness cycle.

The water temperature was controlled at 14°C with a dissolved oxygen content of greater than or equal to 9.0 mg O₂/l. These parameters were recorded daily. The stock fish were fed commercial trout pellets which was discontinued 24 hours prior to the start of the definitive study. There was approximately 2% mortality in the 7 days prior to the start of the test and the fish had a mean standard length of 4.7 cm (s.d. = 0.2) and a mean weight of 1.49 g (s.d. = 0.27) at the end of the definitive study. Based on the mean weight value this gave a loading rate of 0.75 g bodyweight/litre.

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 was used with a total hardness of approximately 100 mg/l as CaCO₃. Typical water quality characteristics are given in Appendix II.

3.3 Procedure

3.3.1 Range-finding study

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

In the range-finding study fish were exposed to nominal test concentrations of 0.10 and 1.0 mg/l.

The test material was dissolved in dimethylformamide. An amount of test material (100 mg) was dispersed in dimethylformamide and the volume adjusted to 10 ml to give a 100 mg/10 ml solvent stock solution. A serial dilution was prepared from this to give a further solvent stock solution of 10 mg/10 ml. An aliquot (2.0 ml) of each solvent stock solution was separately dispersed in 20 litres of dechlorinated tap water to give the 0.10 and 1.0 mg/l test concentrations.

For each test concentration three fish were added to each 20 litre test and control vessel and maintained at 14°C with a photoperiod of 16 hours light and 8 hours darkness for a period of 96 hours under static test conditions.

The control and solvent control groups were maintained under identical conditions but not exposed to the test material. The solvent control group was exposed to 100 µl/l of dimethylformamide.

Each vessel was covered to reduce evaporation. 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 study a "Limit test" was conducted for the definitive study at a test concentration of 1.0 mg/l to confirm that at the highest attainable test concentration of 1.0 mg/l, no mortalities or sub-lethal effects of exposure were observed.

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 20 litre glass exposure vessels were used for each test concentration. At the start of the study 10 fish were placed in each test vessel at random, in the prepared test solutions. The test vessels were then covered to reduce evaporation and maintained at $14 \pm 1^{\circ}\text{C}$ with a photoperiod of 16 hours light and 8 hours darkness for a period of 96 hours. The test vessels were aerated via narrow bore glass tubes. The fish were not individually identified and received no food during exposure.

The control and solvent control groups were maintained under identical conditions but not exposed to the test material. The solvent control group was exposed to $100 \mu\text{l/l}$ of dimethylformamide.

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

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

The test material concentrations in the test preparations were not determined by analysis.

3.3.2.5 Evaluation of data

An estimate of the LC_{50} values was given by inspection of the mortality data.

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 Study

Cumulative mortality data from the exposure of rainbow trout to the test material during the range-finding study are given in Table 1. There were no sub-lethal effects of exposure during the range-finding study.

The results showed no mortalities at the test concentrations of 0.10 and 1.0 mg/l. During preliminary solubility work a precipitate of test material was observed on addition of the solvent stock solution to water at test concentrations in excess of 1.0 mg/l thereby indicating 1.0 mg/l to be the maximum limit of water solubility of the test material under these test conditions.

Based on this information, a single test concentration, in duplicate, of 1.0 mg/l was selected for the definitive study. This experimental design conforms to a "Limit test" to confirm that at the highest attainable test concentration of 1.0 mg/l, no mortalities or sub-lethal effects of exposure were observed.

5.2 Definitive Study

5.2.1 Mortality data

Cumulative mortality data from the exposure of rainbow trout to Kalcol 2098 during the definitive study are given in Table 2.

There were no mortalities in 20 fish exposed to a test concentration of 1.0 mg/l for a period of 96 hours. Inspection of the mortality data gave the following results:

| Time (h) | LC ₅₀ (mg/l) | 95% Confidence Limits (mg/l) |
|----------|-------------------------|------------------------------|
| 3 | > 1.0 | - |
| 6 | > 1.0 | - |
| 24 | > 1.0 | - |
| 48 | > 1.0 | - |
| 72 | > 1.0 | - |
| 96 | > 1.0 | - |

The results of the definitive study showed the highest test concentration resulting in 0% mortality to be greater than or equal to 1.0 mg/l, the lowest test concentration resulting in 100% mortality to be greater than 1.0 mg/l and the No

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 test concentration of 1.0 mg/l was the highest attainable test concentration that could be prepared due to the limited solubility of the test material in water and having due regard to the amount of auxiliary solvent permitted in the study under the OECD Guidelines. Other combinations of the various recognised auxiliary solvents were used during the preliminary solubility work, however, dimethyl formamide was found to give the best testable dispersion of the test material in water. At higher test concentrations there was a marked precipitation of the test material on addition of the solvent stock solution to water.

5.2.2 Sub-lethal effects

There were no sub-lethal effects observed in 20 fish exposed to a test concentration of 1.0 mg/l for a period of 96 hours.

5.2.3 Physico-chemical measurements

The results of the physico-chemical measurements are given in Appendix I. Temperature was maintained at 14 ± 1 °C throughout the study, while there were no treatment related differences for oxygen concentration. The pH of the control group was observed to vary between 7.6 to 8.1. This variation was not considered to affect the validity or integrity of the study given that no mortalities or adverse reactions to exposures were observed in the control group and that the Test Guidelines state that pH should not vary by more than 1 unit.

6. CONCLUSION

The acute toxicity of Kalcol 2098 to the freshwater fish rainbow trout (*Oncorhynchus mykiss*) has been investigated and gave a 96-Hour LC_{50} of greater than 1.9 mg/l. Correspondingly the No Observed Effect Concentration was greater than or equal to 1.0 mg/l.

KALCOL 2098: ACUTE TOXICITY TO RAINBOW TROUT

TABLE 1

CUMULATIVE MORTALITY DATA IN THE RANGE-FINDING STUDY

| Nominal Concentration (mg/l) | Cumulative Mortality (Initial Population = 3) | | | |
|------------------------------|---|----------|----------|----------|
| | 24 hours | 48 hours | 72 hours | 96 hours |
| Control | 0 | 0 | 0 | 0 |
| Solvent Control | 0 | 0 | 0 | 0 |
| 0.10 | 0 | 0 | 0 | 0 |
| 1.0 | 0 | 0 | 0 | 0 |

KALCOL 2098 : ACUTE TOXICITY TO RAINBOW TROUT

TABLE 2

CUMULATIVE MORTALITY DATA IN THE DEFINITIVE STUDY

| Nominal Concentration (mg/l) | Cumulative Mortality (Initial Population = 10) | | | | | | % mortality |
|------------------------------|--|---------|----------|----------|----------|----------|-------------|
| | 3 hours | 6 hours | 24 hours | 48 hours | 72 hours | 96 hours | 96 hours |
| Control | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Solvent Control | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1.0 R ₁ | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1.0 R ₂ | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

R₁ - R₂ = Replicates 1 and 2

APPENDICES

KALCOL 2098 : ACUTE TOXICITY TO RAINBOW TROUT
 APPENDIX I
 PHYSICO-CHEMICAL MEASUREMENTS

| Nominal Concentration (mg/l) | Time (hours) | | | | | | | | | | | |
|------------------------------|-----------------------|----------------------|------|--|----------------------|----------------------|------|--|------------------------|----------------------|------|--|
| | 0 hours (fresh media) | | | | 24 hours (old media) | | | | 24 hours (fresh media) | | | |
| | pH | mg O ₂ /l | T·C | | pH | mg O ₂ /l | T·C | | pH | mg O ₂ /l | T·C | |
| Control | 7.7 | 9.6 | 14.0 | | 8.0 | 9.3 | 14.0 | | 7.6 | 9.4 | 14.0 | |
| Solvent Control | 7.7 | 9.6 | 14.0 | | 7.9 | 9.3 | 14.0 | | 7.6 | 9.4 | 14.0 | |
| 1.0 R ₁ | 7.7 | 9.5 | 14.0 | | 7.9 | 9.0 | 14.0 | | 7.6 | 9.5 | 14.0 | |
| 1.0 R ₂ | 7.7 | 9.6 | 14.0 | | 7.9 | 9.0 | 14.0 | | 7.6 | 9.4 | 14.0 | |

R₁ - R₂ = Replicates 1 and 2

KALCOL 2098 : ACUTE TOXICITY TO RAINBOW TROUT
 APPENDIX I (continued)
 PHYSICO-CHEMICAL MEASUREMENTS

| Nominal Concentration (mg/l) | Time (hours) | | | | | | | | | | | |
|------------------------------|----------------------|----------------------|------|------|------------------------|----------------------|------|------|----------------------|----------------------|------|------|
| | 48 hours (old media) | | | | 48 hours (fresh media) | | | | 72 hours (old media) | | | |
| | pH | mg O ₂ /l | T·C | T·C | pH | mg O ₂ /l | T·C | T·C | pH | mg O ₂ /l | T·C | T·C |
| Control | 8.1 | 9.6 | 13.0 | 13.0 | 7.6 | 9.4 | 14.0 | 14.0 | 8.1 | 9.8 | 13.0 | 13.0 |
| Solvent Control | 8.0 | 9.6 | 13.0 | 13.0 | 7.6 | 9.3 | 14.0 | 14.0 | 8.1 | 9.8 | 13.0 | 13.0 |
| 1.0 R ₁ | 8.1 | 9.4 | 13.0 | 13.0 | 7.7 | 9.3 | 14.0 | 14.0 | 8.2 | 9.8 | 13.0 | 13.0 |
| 1.0 R ₂ | 8.0 | 9.4 | 13.0 | 13.0 | 7.6 | 9.3 | 14.0 | 14.0 | 8.2 | 9.8 | 13.0 | 13.0 |

R₁ - R₂ = Replicates 1 and 2

KALCOL 2098 : ACUTE TOXICITY TO RAINBOW TROUT

APPENDIX I (continued)

PHYSICO-CHEMICAL MEASUREMENTS

| Nominal Concentration (mg/l) | Time (hours) | | | | | | | |
|------------------------------|------------------------|----------------------|------|-----|----------------------|----|----------------------|------|
| | 72 hours (fresh media) | | | | 96 hours (old media) | | | |
| | pH | mg O ₂ /l | T·C | pH | mg O ₂ /l | pH | mg O ₂ /l | T·C |
| Control | 7.6 | 9.2 | 14.0 | 8.0 | 9.6 | | | 13.0 |
| Solvent Control | 7.6 | 9.3 | 14.0 | 8.1 | 9.7 | | | 13.0 |
| 1.0 R ₁ | 7.6 | 9.3 | 14.0 | 8.1 | 9.5 | | | 13.0 |
| 1.0 R ₂ | 7.6 | 9.3 | 14.0 | 8.1 | 9.7 | | | 13.0 |

R₁ - R₂ = Replicates 1 and 2

KALCOL 2098 : ACUTE TOXICITY TO RAINBOW TROUT
 APPENDIX II
 TYPICAL WATER QUALITY CHARACTERISTICS

| WATER SUPPLY ZONE DERBY 9A | | | | | | | | |
|--|---|-----------------------------|---------|------------------------|---------|-------|----------|--------|
| REPORTING PERIOD: 01/01/1996 TO 30/06/96 | | | | | | | | |
| PARAMETER AND QUALIFIERS | NO. OF SAMPLES TAKEN IN PERIOD | SAMPLES GREATER THAN PCV | | CONCENTRATION OR VALUE | | | | |
| | | NUMBER | PERCENT | MINIMUM | MAXIMUM | MEAN | PCV | UNITS |
| TOTAL COLIFORMS | 36 | 0 | 0.00 | 0 | 0 | 0 | 0.0 | 100 ML |
| FAECAL COLIFORMS | 36 | 0 | 0.00 | 0 | 0 | 0 | 0.0 | 100 ML |
| PLATE COUNT (AT 21°C) | 27 | 0 | 0.00 | 0 | > 300 | > 18 | NONE | ML |
| PLATE COUNT (AT 37°C) | 27 | 0 | 0.00 | 0 | 11 | 3 | NONE | ML |
| FREE CHLORINE | 35 | 0 | 0.00 | 0.02 | 0.27 | 0.11 | NONE | MG/L |
| TOTAL CHLORINE | 35 | 0 | 0.00 | 0.07 | 0.33 | 0.17 | NONE | MG/L |
| COLOUR | 3 | 0 | 0.00 | 1 | 2 | 2 | 20 | HAZEN |
| TURBIDITY | 3 | 0 | 0.00 | <0.3 | 0.5 | <0.4 | 4 | F.T.U. |
| TEMPERATURE | 35 | 0 | 0.00 | 5.5 | 18 | 9.7 | 25 | DEG C |
| pH | 3 | 0 | 0.00 | 7.3 | 7.9 | 7.5 | 5.5-9.50 | |
| CONDUCTIVITY | 35 | 0 | 0.00 | 388 | 739 | 627 | 1500 | uS/cm |
| QUALITATIVE TASTE | 3 | 0 | 0.00 | 0 | 0 | 0 | 3 | |
| QUANTITATIVE TASTE | 35 | — | — | — | — | — | — | |
| QUALITATIVE ODOUR | 3 | 0 | 0.00 | 0 | 0 | 0 | 3 | |
| QUANTITATIVE ODOUR | 35 | — | — | — | — | — | — | |
| AMMONIUM | 3 | 0 | 0.00 | <0.05 | <0.05 | <0.05 | 0.5 | MG/L |
| NITRITE | 3 | 0 | 0.00 | <0.01 | 0.01 | <0.01 | 0.1 | MG/L |
| NITRATE | 3 | 0 | 0.00 | 14.6 | 25.8 | 19.1 | 50 | MG/L |
| CHLORIDE | 1 | 0 | 0.00 | 77 | 77 | 77 | 400 | MG/L |
| SULPHATE | 1 | 0 | 0.00 | 100 | 100 | 100 | 250 | MG/L |
| FLUORIDE | 1 | 0 | 0.00 | 410 | 410 | 410 | 1500 | µG/L |
| PHOSPHORUS | 1 | 0 | 0.00 | 580 | 580 | 580 | 2200 | µG/L |
| ALKALINITY | 1 | 0 | 0.00 | 137 | 137 | 137 | NONE | MG/L |
| TOTAL HARDNESS | 1 | 0 | 0.00 | 259 | 259 | 259 | NONE | MG/L |
| CALCIUM | 1 | 0 | 0.00 | 81 | 81 | 81 | 250 | MG/L |
| MAGNESIUM | 1 | 0 | 0.00 | 14 | 14 | 14 | 50 | MG/L |
| SODIUM | 1 | 0 | 0.00 | 56 | 56 | 56 | 150 | MG/L |
| POTASSIUM | 1 | 0 | 0.00 | 4.4 | 4.4 | 4.4 | 12 | MG/L |
| IRON | 5 | 0 | 0.00 | 40 | 120 | 58 | 200 | µG/L |
| MANGANESE | 3 | 0 | 0.00 | <5 | 9 | <6 | 50 | µG/L |
| ALUMINIUM | 3 | 0 | 0.00 | <20 | <20 | <20 | 200 | µG/L |
| COPPER | 1 | 0 | 0.00 | 10 | 10 | 10 | 3000 | µG/L |
| ZINC | 1 | 0 | 0.00 | 20 | 20 | 20 | 5000 | µG/L |
| LEAD | 6 | 0 | 0.00 | <1 | 24 | <7 | 50 | µG/L |
| CADMIUM | 1 | 0 | 0.00 | <0.5 | <0.5 | <0.5 | 5 | µG/L |
| SILVER | 1 | 0 | 0.00 | <0.2 | <0.2 | <0.2 | 10 | µG/L |
| BORON | 1 | 0 | 0.00 | 100 | 100 | 100 | 2000 | µG/L |
| BARIUM | 1 | 0 | 0.00 | 80 | 80 | 80 | 1000 | µG/L |
| ARSENIC | 1 | 0 | 0.00 | <1 | <1 | <1 | 50 | µG/L |
| CHROMIUM | 1 | 0 | 0.00 | <5 | <5 | <5 | 50 | µG/L |
| NICKEL | 1 | 0 | 0.00 | 5 | 5 | 5 | 50 | µG/L |

PCV - Prescribed Concentration or Value

KALCOL 2098 : ACUTE TOXICITY TO RAINBOW TROUT

APPENDIX II

TYPICAL WATER QUALITY CHARACTERISTICS (continued)

| WATER SUPPLY ZONE DERBY 9A | | | | | | | | |
|--|---|-----------------------------|---------|------------------------|---------|--------|-----|-------|
| REPORTING PERIOD: 01/01/1996 TO 30/06/96 | | | | | | | | |
| PARAMETER AND QUALIFIERS | NO. OF SAMPLES TAKEN IN PERIOD | SAMPLES GREATER THAN PCV | | CONCENTRATION OR VALUE | | | | |
| | | NUMBER | PERCENT | MINIMUM | MAXIMUM | MEAN | PCV | UNITS |
| MERCURY | 1 | 0 | 0.00 | <0.1 | <0.1 | <0.1 | 1 | µG/L |
| ANTIMONY | 1 | 0 | 0.00 | <1 | <1 | <1 | 10 | µG/L |
| SELENIUM | 1 | 0 | 0.00 | <1 | <1 | <1 | 10 | µG/L |
| CYANIDE | 1 | 0 | 0.00 | <5 | <5 | <5 | 50 | µG/L |
| OXIDIZABILITY | 1 | 0 | 0.00 | 0.7 | 0.7 | 0.7 | 5 | MG/L |
| T.O.C | 1 | 0 | 0.00 | 2.3 | 2.3 | 2.3 | 5 | MG/L |
| SURFACTANTS | 1 | 0 | 0.00 | <20 | <20 | <20 | 200 | µG/L |
| TRIHALOMETHANES | 2 | 0 | 0.00 | 35 | 48 | 42 | 100 | µG/L |
| P.A.H. (TOTAL) | 2 | 0 | 0.00 | <0.013 | 0.018 | <0.016 | 0.2 | µG/L |
| BENZ(A)PYRENE | 2 | 0 | 0.00 | <1 | <1 | <1 | 10 | NG/L |
| TOTAL PESTICIDES | 6 | 0 | 0.00 | <0.02 | 0.04 | <0.03 | 0.5 | µG/L |
| ATRAZINE | 2 | 0 | 0.00 | <0.01 | <0.01 | <0.01 | 0.1 | µG/L |
| CLOPYRALID | 6 | 0 | 0.00 | <0.03 | <0.03 | <0.03 | 0.1 | µG/L |
| 2,4-D | 6 | 0 | 0.00 | <0.01 | <0.01 | <0.01 | 0.1 | µG/L |
| 2,4-DB | 6 | 0 | 0.00 | <0.01 | <0.01 | <0.01 | 0.1 | µG/L |
| DICAMBA | 6 | 0 | 0.00 | <0.01 | <0.01 | <0.01 | 0.1 | µG/L |
| DICHLORPROP | 6 | 0 | 0.00 | <0.01 | <0.01 | <0.01 | 0.1 | µG/L |
| EPTC | 2 | 0 | 0.00 | <0.01 | <0.01 | <0.01 | 0.1 | µG/L |
| FENPROPIIMORPH | 2 | 0 | 0.00 | <0.01 | <0.01 | <0.01 | 0.1 | µG/L |
| FLUROXYPYR | 6 | 0 | 0.00 | <0.02 | <0.02 | <0.02 | 0.1 | µG/L |
| MCPA | 6 | 0 | 0.00 | <0.01 | <0.01 | <0.01 | 0.1 | µG/L |
| MCPB | 6 | 0 | 0.00 | <0.02 | <0.02 | <0.02 | 0.1 | µG/L |
| MECOPROP (TOTAL) | 6 | 0 | 0.00 | <0.01 | 0.03 | <0.02 | 0.1 | µG/L |
| PROMETRYN | 2 | 0 | 0.00 | <0.01 | <0.01 | <0.01 | 0.1 | µG/L |
| PROPAZINE | 2 | 0 | 0.00 | <0.01 | <0.01 | <0.01 | 0.1 | µG/L |
| SIMAZINE | 2 | 0 | 0.00 | <0.01 | 0.01 | <0.01 | 0.1 | µG/L |
| 2,3,6,-TBA | 6 | 0 | 0.00 | <0.01 | <0.01 | <0.01 | 0.1 | µG/L |
| 2,4,5,-TCPA | 6 | 0 | 0.00 | <0.01 | <0.01 | <0.01 | 0.1 | µG/L |
| TERBUTRYN | 2 | 0 | 0.00 | <0.01 | <0.01 | <0.01 | 0.1 | µG/L |
| TRIFLAZINE | 2 | 0 | 0.00 | <0.01 | <0.01 | <0.01 | 0.1 | µG/L |

PCV = Prescribed Concentration or Value

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KALCOL 4098:

ACUTE TOXICITY TO

RAINBOW TROUT (*Oncorhynchus mykiss*)

SPL PROJECT NUMBER: 140/599

AUTHOR: P M WETTON

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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. Dates of relevant monthly inspections are given below:

Date(s) of Inspection and Reporting:

08, 11 October 1996

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:

28 November 1996



..... Date: **23 JAN 1997**

J R Pateman CBiol MI Biol
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 the UK Principles of Good Laboratory Practice (The United Kingdom Compliance Programme, Department of Health 1989). These Principles are in accordance with GLP standards published as OECD Environment 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: 22 JAN 1997

P M Wetton BSc
Study Director

The following person was also involved in the study
L G Shacklady BSc
Study Supervisor

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SUMMARY

STUDY SPONSOR : KAO CORPORATION
STUDY TITLE : ACUTE TOXICITY TO RAINBOW TROUT
TEST MATERIAL : KALCOL 4098

Methods

A study was performed to assess the acute toxicity of the test material, Kalcol 4098, to rainbow trout (*Oncorhynchus mykiss*). 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).

Procedures

Following a preliminary range-finding study fish were exposed, in two groups of ten, to an aqueous dispersion of the test material, at a single concentration of 1.0 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 for the test material to rainbow trout (*Oncorhynchus mykiss*), based on nominal test concentrations was greater than 1.0 mg/l and correspondingly the No Observed Effect Concentration was greater than or equal to 1.0 mg/l.

The test concentration of 1.0 mg/l was the highest attainable test concentration due to the limited solubility of the test material in water and auxiliary solvent, and having due regard for the amount of auxiliary solvent permitted in the test under the OECD Guidelines.

KALCOL 4098:
ACUTE TOXICITY TO RAINBOW TROUT (*Oncorhynchus mykiss*)

1. INTRODUCTION

This report contains a description of the methods used and results obtained during a study to investigate the acute toxicity of Kalcol 4098 to rainbow trout. The method (Safepharm Laboratories Standard Test Method 902.01) 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).

Rainbow trout 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 study was conducted between 26 September 1996 and 30 September 1996 and the definitive study between 28 October 1996 and 1 November 1996.

2. TEST MATERIAL AND EXPERIMENTAL PREPARATION

2.1 Description, Identification and Storage Conditions

Sponsor's identification : Kalcol 4098
Description : white solid block
Lot number : 2003
Date received : 5 August 1996
Storage conditions : ambient temperature, shielded from light

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 using a preliminary solution in tetrahydrofuran.

An amount (100 mg) of test material was dispersed in tetrahydrofuran and the volume adjusted to 10 ml to give a 100 mg/10 ml solvent stock solution. An aliquot (2.0 ml) of this solvent stock solution was dispersed in 20 litres of dechlorinated tap water to give the 1.0 mg/l test concentration.

The concentration, homogeneity and stability of the test material in the test solutions were not determined as per the agreed protocol.

3. METHODS

3.1 Test Species

The test was carried out using juvenile rainbow trout (*Oncorhynchus mykiss*). Fish were obtained from Parkwood Trout Farm, Harrietsham, Kent, UK and maintained in-house since 14 October 1996. Fish were maintained in a glass fibre tank with a "single pass" water renewal system. Fish were acclimatised to test conditions from 21 October 1996 to 28 October 1996. The lighting cycle was controlled to give a 16 hours light and 8 hours darkness cycle.

The water temperature was controlled at 14 °C with a dissolved oxygen content of greater than or equal to 9.0 mg O₂/l. These parameters were recorded daily. The stock fish were fed commercial trout pellets which was discontinued approximately 23 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 4.7 cm (s.d. = 0.4) and a mean weight of 0.90 g (s.d. = 0.29) at the end of the definitive study. Based on the mean weight value this gave a loading rate of 0.45 g bodyweight/litre.

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

Laboratory tap water dechlorinated by passage through an activated carbon filter was used with a total hardness of approximately 100 mg/l as CaCO₃. Typical water quality characteristics are given in Appendix II.

3.3 Procedure

3.3.1 Range-finding study

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

In the range-finding study fish were exposed to a series of nominal test concentrations of 0.10 and 1.0 mg/l. The test material was dissolved in tetrahydrofuran.

An amount of test material (100 mg) was dispersed in tetrahydrofuran and the volume adjusted to 10 ml to give a 100 mg/10 ml solvent stock solution. A serial dilution was prepared from this to give a further solvent stock solution of 10 mg/10 ml. An aliquot (2.0 ml) of each solvent stock solution was separately dispersed in 20 litres of dechlorinated tap water to give the 0.10 and 1.0 mg/l test concentrations.

For each test concentration 3 fish were added to each 20 litre test and control vessel and maintained at 14°C with a photoperiod of 16 hours light and 8 hours darkness for a period of 96 hours under static test conditions.

The control and solvent control groups were maintained under identical conditions but not exposed to the test material. The solvent control group was exposed to 100 µl/l of tetrahydrofuran.

Each vessel was covered to reduce evaporation. 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 study a "Limit test" was conducted for the definitive study at a test concentration of 1.0 mg/l to confirm that at the highest attainable test concentration of 1.0 mg/l, no mortalities or sub-lethal effects of

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 20 litre glass exposure vessels were used for each test concentration. At the start of the study 10 fish were placed in each test vessel at random, in the prepared test solutions. The test vessels were then covered to reduce evaporation and maintained at 14°C with a photoperiod of 16 hours light and 8 hours darkness for a period of 96 hours. The test vessels were aerated via narrow bore glass tubes. The fish were not individually identified and received no food during exposure.

The control and solvent control groups were maintained under identical conditions but not exposed to the test material. The solvent control group was exposed to 100 µl/l of tetrahydrofuran.

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

The test material concentrations in the test preparations were not determined by

3.3.2.5 Evaluation of data

An estimate of the LC_{50} values was given by inspection of the mortality data.

4. ARCHIVES

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

5. RESULTS

5.1 Range-finding Study

Cumulative mortality data from the exposure of rainbow trout to the test material during the range-finding study are given in Table 1.

The results showed no mortalities at the test concentrations of 0.10 and 1.0 mg/l. During preliminary solubility work a precipitate of test material was observed on addition of the solvent stock solution to water at test concentrations in excess of 1.0 mg/l indicating 1.0 mg/l to be the maximum limit of water solubility of the test material under these test conditions.

Based on this information, a single test concentration, in duplicate, of 1.0 mg/l was selected for the definitive study. This experimental design conforms to a "Limit test" to confirm that at the highest attainable test concentration of 1.0 mg/l, no mortalities or sub-lethal effects of exposure were observed.

5.2 Definitive Study

5.2.1 Mortality data

Cumulative mortality data from the exposure of rainbow trout to Kalcol 4098 during the definitive study are given in Table 2.

There were no mortalities in 20 fish exposed to a test concentration of 1.0 mg/l for a period of 96 hours. Inspection of the mortality data gave the following results:

| Time (h) | LC ₅₀ (mg/l) | 95% Confidence Limits (mg/l) |
|----------|-------------------------|------------------------------|
| 3 | > 1.0 | - |
| 6 | > 1.0 | - |
| 24 | > 1.0 | - |
| 48 | > 1.0 | - |
| 72 | > 1.0 | - |
| 96 | > 1.0 | - |

The results of the definitive study showed the highest test concentration resulting in 0% mortality to be greater than or equal to 1.0 mg/l, the lowest test concentration resulting in 100% mortality to be greater than 1.0 mg/l and the No Observed Effect Concentration (NOEC) to be greater than or equal to 1.0 mg/l. The No Observed Effect Concentration is based upon zero mortalities and the absence

The test concentration of 1.0 mg/l was the highest attainable test concentration that could be prepared due to the limited solubility of the test material in water and having due regard to the amount of auxiliary solvent permitted in the study under the OECD Guidelines. Other various recognised auxiliary solvents were used during preliminary solubility work, however, tetrahydrofuran was found to give the best testable dispersion of the test material in water. At higher test concentrations there was a marked precipitation of the test material on addition of the solvent stock solution to water.

5.2.2 Sub-lethal effects

There were no sub-lethal effects observed in 20 fish exposed to a test concentration of 1.0 mg/l for a period of 96 hours.

5.2.3 Physico-chemical measurements

The results of the physico-chemical measurements are given in Appendix I. Temperature was maintained at 14°C throughout the study, while there were no treatment related differences for oxygen concentration or pH. The pH of the control and solvent control groups was observed to vary between 7.6 - 8.2. This variation was not considered to affect the validity or integrity of the study given that no mortalities or adverse reactions to exposure were observed in the control groups and the Test Guideline states that the pH should not vary by more than 1 unit.

6. CONCLUSION

The acute toxicity of Kalcol 4098 to the freshwater fish rainbow trout (*Oncorhynchus mykiss*) has been investigated and gave a 96-Hour LC₅₀ of greater than 1.0 mg/l. Correspondingly the No Observed Effect Concentration was greater than or equal to 1.0 mg/l.

KALCOL 4098: ACUTE TOXICITY TO RAINBOW TROUT

TABLE 1

CUMULATIVE MORTALITY DATA IN THE RANGE-FINDING STUDY

| Nominal Concentration (mg/l) | Cumulative Mortality (Initial Population = 3) | | | |
|------------------------------|---|----------|----------|----------|
| | 24 hours | 48 hours | 72 hours | 96 hours |
| Control | 0 | 0 | 0 | 0 |
| Solvent Control | 0 | 0 | 0 | 0 |
| 0.10 | 0 | 0 | 0 | 0 |
| 1.0 | 0 | 0 | 0 | 0 |

KALCOL 4098: ACUTE TOXICITY TO RAINBOW TROUT

TABLE 2

CUMULATIVE MORTALITY DATA IN THE DEFINITIVE STUDY

| Nominal Concentration (mg/l) | Cumulative Mortality (Initial Population = 10) | | | | | | % mortality |
|------------------------------|--|---------|----------|----------|----------|----------|-------------|
| | 3 hours | 6 hours | 24 hours | 48 hours | 72 hours | 96 hours | 96 hours |
| Control | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Solvent Control | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1.0 R ₁ | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1.0 R ₂ | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

R₁ - R₂ = Replicates 1 and 2.

APPENDICES

KALCOL 4098: ACUTE TOXICITY TO RAINBOW TROUT

APPENDIX I

PHYSICO-CHEMICAL MEASUREMENTS

| Nominal Concentration (mg/l) | Time (hours) | | | | | | | | | | |
|------------------------------|-----------------------|----------------------|------|----------------------|----------------------|------|------------------------|----------------------|-----|----------------------|------|
| | 0 hours (fresh media) | | | 24 hours (old media) | | | 24 hours (fresh media) | | | | |
| | pH | mg O ₂ /l | T°C | pH | mg O ₂ /l | T°C | pH | mg O ₂ /l | pH | mg O ₂ /l | T°C |
| Control | 7.6 | 9.8 | 14.0 | 7.7 | 9.0 | 14.0 | 7.7 | 9.9 | 7.7 | 9.9 | 14.0 |
| Solvent Control | 7.6 | 9.8 | 14.0 | 7.6 | 8.4 | 14.0 | 7.7 | 9.8 | 7.7 | 9.8 | 14.0 |
| 1.0 R ₁ | 7.6 | 9.8 | 14.0 | 7.8 | 8.2 | 14.0 | 7.7 | 9.8 | 7.7 | 9.8 | 14.0 |
| 1.0 R ₂ | 7.6 | 9.8 | 14.0 | 8.0 | 8.1 | 14.0 | 7.7 | 9.8 | 7.7 | 9.8 | 14.0 |

R₁ - R₂ = Replicates 1 and 2

KALCOL 4098: ACUTE TOXICITY TO RAINBOW TROUT
 APPENDIX I (continued)
 PHYSICO-CHEMICAL MEASUREMENTS

| Nominal Concentration (mg/l) | Time (hours) | | | | | | | | | | | |
|------------------------------|----------------------|----------------------|------|------|------------------------|----------------------|------|------|----------------------|----------------------|------|------|
| | 48 hours (old media) | | | | 48 hours (fresh media) | | | | 72 hours (old media) | | | |
| | pH | mg O ₂ /l | T°C | T°C | pH | mg O ₂ /l | T°C | T°C | pH | mg O ₂ /l | T°C | T°C |
| Control | 8.1 | 9.3 | 14.0 | 14.0 | 7.7 | 9.8 | 14.0 | 14.0 | 8.1 | 9.1 | 14.0 | 14.0 |
| Solvent Control | 8.1 | 9.2 | 14.0 | 14.0 | 7.7 | 9.8 | 14.0 | 14.0 | 8.1 | 9.2 | 14.0 | 14.0 |
| 1.0 R ₁ | 8.1 | 9.2 | 14.0 | 14.0 | 7.7 | 9.8 | 14.0 | 14.0 | 8.1 | 9.2 | 14.0 | 14.0 |
| 1.0 R ₂ | 8.1 | 9.2 | 14.0 | 14.0 | 7.7 | 9.8 | 14.0 | 14.0 | 8.1 | 9.2 | 14.0 | 14.0 |

R₁ - R₂ = Replicates 1 and 2

KALCOL 4098: ACUTE TOXICITY TO RAINBOW TROUT

APPENDIX I (continued)

PHYSICO-CHEMICAL MEASUREMENTS

| Nominal Concentration (mg/l) | Time (hours) | | | | | |
|------------------------------|------------------------|----------------------|------|----------------------|----------------------|------|
| | 72 hours (fresh media) | | | 96 hours (old media) | | |
| | pH | mg O ₂ /l | T·C | pH | mg O ₂ /l | T·C |
| Control | 7.7 | 9.8 | 14.0 | 8.2 | 9.0 | 14.0 |
| Solvent Control | 7.7 | 9.8 | 14.0 | 8.1 | 9.0 | 14.0 |
| 1.0 R ₁ | 7.7 | 9.7 | 14.0 | 8.1 | 9.0 | 14.0 |
| 1.0 R ₂ | 7.7 | 9.8 | 14.0 | 8.1 | 9.0 | 14.0 |

R₁ - R₂ = Replicates 1 and 2

KALCOL 4098: ACUTE TOXICITY TO RAINBOW TROUT
 APPENDIX II
 TYPICAL WATER QUALITY CHARACTERISTICS

| WATER SUPPLY ZONE DERBY 9A | | | | | | | | |
|--|---|-----------------------------|---------|------------------------|---------|-------|----------|--------|
| REPORTING PERIOD: 01/01/1996 TO 30/06/96 | | | | | | | | |
| PARAMETER AND QUALIFIERS | NO. OF SAMPLES TAKEN IN PERIOD | SAMPLES GREATER THAN PCV | | CONCENTRATION OR VALUE | | | | |
| | | NUMBER | PERCENT | MINIMUM | MAXIMUM | MEAN | PCV | UNITS |
| TOTAL COLIFORMS | 36 | 0 | 0.00 | 0 | 0 | 0 | 0.0 | 100 ML |
| FAECAL COLIFORMS | 36 | 0 | 0.00 | 0 | 0 | 0 | 0.0 | 100 ML |
| PLATE COUNT (AT 21°C) | 27 | 0 | 0.00 | 0 | > 300 | > 18 | NONE | ML |
| PLATE COUNT (AT 37°C) | 27 | 0 | 0.00 | 0 | 11 | 3 | NONE | ML |
| FREE CHLORINE | 35 | 0 | 0.00 | 0.02 | 0.27 | 0.11 | NONE | MG/L |
| TOTAL CHLORINE | 35 | 0 | 0.00 | 0.07 | 0.33 | 0.17 | NONE | MG/L |
| COLOUR | 3 | 0 | 0.00 | 1 | 2 | 2 | 20 | HAZEN |
| TURBIDITY | 3 | 0 | 0.00 | <0.3 | 0.5 | <0.4 | 4 | F.T.U. |
| TEMPERATURE | 35 | 0 | 0.00 | 5.5 | 18 | 9.7 | 25 | DEG C |
| pH | 3 | 0 | 0.00 | 7.3 | 7.9 | 7.5 | 5.5-9.50 | |
| CONDUCTIVITY | 35 | 0 | 0.00 | 388 | 739 | 627 | 1500 | uS/cm |
| QUALITATIVE TASTE | 3 | 0 | 0.00 | 0 | 0 | 0 | 3 | |
| QUANTITATIVE TASTE | 35 | — | — | — | — | — | — | |
| QUALITATIVE ODOUR | 3 | 0 | 0.00 | 0 | 0 | 0 | 3 | |
| QUANTITATIVE ODOUR | 35 | — | — | — | — | — | — | |
| AMMONIUM | 3 | 0 | 0.00 | <0.05 | <0.05 | <0.05 | 0.5 | MG/L |
| NITRITE | 3 | 0 | 0.00 | <0.01 | 0.01 | <0.01 | 0.1 | MG/L |
| NITRATE | 3 | 0 | 0.00 | 14.6 | 25.8 | 19.1 | 50 | MG/L |
| CHLORIDE | 1 | 0 | 0.00 | 77 | 77 | 77 | 400 | MG/L |
| SULPHATE | 1 | 0 | 0.00 | 100 | 100 | 100 | 250 | MG/L |
| FLUORIDE | 1 | 0 | 0.00 | 410 | 410 | 410 | 1500 | µG/L |
| PHOSPHORUS | 1 | 0 | 0.00 | 580 | 580 | 580 | 2200 | µG/L |
| ALKALINITY | 1 | 0 | 0.00 | 137 | 137 | 137 | NONE | MG/L |
| TOTAL HARDNESS | 1 | 0 | 0.00 | 259 | 259 | 259 | NONE | MG/L |
| CALCIUM | 1 | 0 | 0.00 | 81 | 81 | 81 | 250 | MG/L |
| MAGNESIUM | 1 | 0 | 0.00 | 14 | 14 | 14 | 30 | MG/L |
| SODIUM | 1 | 0 | 0.00 | 56 | 56 | 56 | 150 | MG/L |
| POTASSIUM | 1 | 0 | 0.00 | 4.4 | 4.4 | 4.4 | 12 | MG/L |
| IRON | 5 | 0 | 0.00 | 40 | 120 | 58 | 200 | µG/L |
| MANGANESE | 3 | 0 | 0.00 | <5 | 9 | <6 | 50 | µG/L |
| ALUMINIUM | 3 | 0 | 0.00 | <20 | <20 | <20 | 200 | µG/L |
| COPPER | 1 | 0 | 0.00 | 10 | 10 | 10 | 3000 | µG/L |
| ZINC | 1 | 0 | 0.00 | 20 | 20 | 20 | 5000 | µG/L |
| LEAD | 6 | 0 | 0.00 | <1 | 24 | <7 | 50 | µG/L |
| CADMIUM | 1 | 0 | 0.00 | <0.5 | <0.5 | <0.5 | 5 | µG/L |
| SILVER | 1 | 0 | 0.00 | <0.2 | <0.2 | <0.2 | 10 | µG/L |
| BORON | 1 | 0 | 0.00 | 100 | 100 | 100 | 2000 | µG/L |
| BARIUM | 1 | 0 | 0.00 | 80 | 80 | 80 | 1000 | µG/L |
| ARSENIC | 1 | 0 | 0.00 | <1 | <1 | <1 | 50 | µG/L |
| CHROMIUM | 1 | 0 | 0.00 | <5 | <5 | <5 | 50 | µG/L |
| NICKEL | 1 | 0 | 0.00 | 5 | 5 | 5 | 50 | µG/L |

PCV = Prescribed Concentration or Value

KALCOL 4098: ACUTE TOXICITY TO RAINBOW TROUT
 APPENDIX II (continued)
 TYPICAL WATER QUALITY CHARACTERISTICS

| WATER SUPPLY ZONE DERBY 9A | | | | | | | | |
|--|---|-----------------------------|---------|------------------------|---------|--------|-----|-------|
| REPORTING PERIOD: 01/01/1996 TO 30/06/96 | | | | | | | | |
| PARAMETER AND QUALIFIERS | NO. OF SAMPLES TAKEN IN PERIOD | SAMPLES GREATER THAN PCV | | CONCENTRATION OR VALUE | | | | |
| | | NUMBER | PERCENT | MINIMUM | MAXIMUM | MEAN | PCV | UNITS |
| MERCURY | 1 | 0 | 0.00 | <0.1 | <0.1 | <0.1 | 1 | µG/L |
| ANTIMONY | 1 | 0 | 0.00 | <1 | <1 | <1 | 10 | µG/L |
| SELENIUM | 1 | 0 | 0.00 | <1 | <1 | <1 | 10 | µG/L |
| CYANIDE | 1 | 0 | 0.00 | <5 | <5 | <5 | 50 | µG/L |
| OXIDIZABILITY | 1 | 0 | 0.00 | 0.7 | 0.7 | 0.7 | 5 | MG/L |
| T.O.C | 1 | 0 | 0.00 | 2.3 | 2.3 | 2.3 | 5 | MG/L |
| SURFACTANTS | 1 | 0 | 0.00 | <20 | <20 | <20 | 200 | µG/L |
| TRIHALOMETHANES | 2 | 0 | 0.00 | 35 | 48 | 42 | 100 | µG/L |
| P.A.H. (TOTAL) | 2 | 0 | 0.00 | <0.013 | 0.018 | <0.016 | 0.2 | µG/L |
| BENZ(A)PYRENE | 2 | 0 | 0.00 | <1 | <1 | <1 | 10 | NG/L |
| TOTAL PESTICIDES | 6 | 0 | 0.00 | <0.02 | 0.04 | <0.03 | 0.5 | µG/L |
| ATRAZINE | 2 | 0 | 0.00 | <0.01 | <0.01 | <0.01 | 0.1 | µG/L |
| CLOPYRALID | 6 | 0 | 0.00 | <0.03 | <0.03 | <0.03 | 0.1 | µG/L |
| 2,4-D | 6 | 0 | 0.00 | <0.01 | <0.01 | <0.01 | 0.1 | µG/L |
| 2,4-DB | 6 | 0 | 0.00 | <0.01 | <0.01 | <0.01 | 0.1 | µG/L |
| DICAMBA | 6 | 0 | 0.00 | <0.01 | <0.01 | <0.01 | 0.1 | µG/L |
| DICHLORPROP | 6 | 0 | 0.00 | <0.01 | <0.01 | <0.01 | 0.1 | µG/L |
| EPTC | 2 | 0 | 0.00 | <0.01 | <0.01 | <0.01 | 0.1 | µG/L |
| FENPROPIIMORPH | 2 | 0 | 0.00 | <0.01 | <0.01 | <0.01 | 0.1 | µG/L |
| FLUROXYPYR | 6 | 0 | 0.00 | <0.02 | <0.02 | <0.02 | 0.1 | µG/L |
| MCPA | 6 | 0 | 0.00 | <0.01 | <0.01 | <0.01 | 0.1 | µG/L |
| MCPB | 6 | 0 | 0.00 | <0.02 | <0.02 | <0.02 | 0.1 | µG/L |
| MECOPROP (TOTAL) | 6 | 0 | 0.00 | <0.01 | 0.03 | <0.02 | 0.1 | µG/L |
| PROMETRYN | 2 | 0 | 0.00 | <0.01 | <0.01 | <0.01 | 0.1 | µG/L |
| PROPAZINE | 2 | 0 | 0.00 | <0.01 | <0.01 | <0.01 | 0.1 | µG/L |
| SIMAZINE | 2 | 0 | 0.00 | <0.01 | 0.01 | <0.01 | 0.1 | µG/L |
| 2,3,6-TBA | 6 | 0 | 0.00 | <0.01 | <0.01 | <0.01 | 0.1 | µG/L |
| 2,4,5-TCPA | 6 | 0 | 0.00 | <0.01 | <0.01 | <0.01 | 0.1 | µG/L |
| TERBUTRYN | 2 | 0 | 0.00 | <0.01 | <0.01 | <0.01 | 0.1 | µG/L |
| TRIFLAZINE | 2 | 0 | 0.00 | <0.01 | <0.01 | <0.01 | 0.1 | µG/L |

PCV - Prescribed Concentration or Value

APPENDIX III



THE DEPARTMENT OF HEALTH OF THE GOVERNMENT
OF THE UNITED KINGDOM

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE
IN ACCORDANCE WITH DIRECTIVE 88/320 EEC

LABORATORY

TEST TYPE

SafePharm Laboratories Ltd.
P.O. Box No. 45
Derby DE1 2BT

Analytical Chemistry
Environmental Tox.
Environmental Fate
Mutagenicity
Phys/Chem. tests
Toxicology

DATE OF INSPECTION

22 January 1996

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above laboratory as part of the UK GLP Compliance Programme.

At the time of the inspection no deviations were found of sufficient magnitude to affect the validity of non-clinical studies performed at these facilities.

27/2/96

D.F. Moore

~~CONFIDENTIAL~~

NOT CO I H/C 7/2/89

1- HEXADECANOL CASNO 36653-82-4

KALCOHL 6098:

ACUTE TOXICITY TO RAINBOW TROUT

(*Oncorhynchus mykiss*)

SPL PROJECT NUMBER: 140/500

AUTHOR: P M WETTON

STUDY SPONSOR:

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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. Dates of relevant monthly inspections are given below:

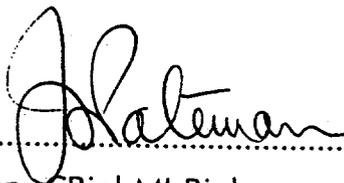
Date(s) of Inspection and Reporting:

05, 06, 11 March 1996

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:

30 April 1996



J R Pateman CBiol MI Biol
For Safepharm Quality Assurance Unit

-8. MAY 1996

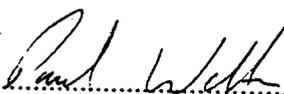
DATE:

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 the UK Principles of Good Laboratory Practice (The United Kingdom Compliance Programme, Department of Health 1989). These Principles are in accordance with GLP standards published as OECD Environment 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: 08 MAY 1996

PM Wetton BSc
Study Director

The following person was also involved in the study
D Cruse HND
Study Supervisor.

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SUMMARY

STUDY SPONSOR : KAO CORPORATION

STUDY TITLE : ACUTE TOXICITY TO RAINBOW TROUT

TEST MATERIAL : KALCOHL 6098

Methods

A study was performed to assess the acute toxicity of the test material, Kalcohl 6098, to rainbow trout (*Oncorhynchus mykiss*). 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).

Procedures

Following a preliminary range-finding study fish were exposed, in two groups of ten, to an aqueous dispersion of the test material, at a single concentration of 0.40 mg/l for a period of 96 hours under semi-static test conditions. The number of mortalities and any adverse reactions to 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 for the test material to rainbow trout (*Oncorhynchus mykiss*), based on nominal test concentrations was greater than 0.40 mg/l and correspondingly the No Observed Effect Concentration was greater than or equal to 0.40 mg/l.

The test concentration of 0.40 mg/l was the highest attainable test concentration due to the limited solubility of the test material in water and auxiliary solvent, and having due regard for the amount of auxiliary solvent permitted in the test under the OECD Guidelines.

**KALCOHL 6098:
ACUTE TOXICITY TO RAINBOW TROUT (*Oncorhynchus mykiss*)**

1. INTRODUCTION

This report contains a description of the methods used and results obtained during a study to investigate the acute toxicity of Kalcohl 6098 to rainbow trout. The method (Safepharm Laboratories Standard Method 3.OECD) 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).

Rainbow trout 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 study was conducted between 1 February 1996 and 5 February 1996 and the definitive study between 4 March 1996 and 8 March 1996.

2. TEST MATERIAL AND EXPERIMENTAL PREPARATION

2.1 Description, Identification and Storage Conditions

Sponsor's identification : Kalcohl 6098
Description : white granular solid
Lot number : 2439
Date received : 22 January 1996
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 using a preliminary solution in tetrahydrofuran.

An amount of test material (40 mg) was dissolved in tetrahydrofuran with the aid of ultrasonic disruption and the volume adjusted to 10 ml to give a 40 mg/10 ml solvent stock solution. An aliquot (2.0 ml) of this stock solution was dispersed in 20 litres of dechlorinated tap water to give the test concentration of 0.40 mg/l.

3. METHODS

3.1 Test Species

The test was carried out using juvenile rainbow trout (*Oncorhynchus mykiss*). Fish were obtained from Donnington Fish Farm, Upper Swell, Gloucestershire, U.K. and maintained in-house since 15 January 1996. Fish were maintained in a glass fibre tank with a "single pass" water renewal system. Fish were acclimatised to test conditions from 26 February 1996 to 4 March 1996. The lighting cycle was controlled to give a 16 hours light and 8 hours darkness cycle.

The water temperature was controlled at 14°C with a dissolved oxygen content of greater than or equal to 9.3 mg O₂/l. These parameters were recorded daily. The stock fish were fed commercial trout pellets which were discontinued 24 hours prior to the start of the definitive study. There were approximately 2% mortalities in the 7 days prior to the start of the test and the fish had a mean standard length of 4.5 cm (s.d. = 0.2) and a mean weight of 1.20 g (s.d. = 0.26) at the end of the definitive study. Based on the mean weight value this gave a loading rate of 0.60 g bodyweight/litre.

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 was used with a total hardness of approximately 100 mg/l as CaCO₃. Typical water quality characteristics are given in Appendix II.

3.3 Procedure

3.3.1 Range-finding study

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

In the range-finding study fish were exposed to a series of nominal test concentrations of 0.040 and 0.40 mg/l. The test material was dissolved in tetrahydrofuran.

To prepare the test series an amount of test material (40 mg) was dissolved in tetrahydrofuran with the aid of ultrasonic disruption and the volume adjusted to 10 ml to give a 40 mg/10 ml solvent stock solution. This stock solution was further diluted to give the 4.0 mg/10 ml solvent stock solution. Aliquots (2.0 ml) of each stock solution were separately dispersed in 20 litres of dechlorinated tap water to give the test concentrations of 0.040 and 0.40 mg/l.

For each test concentration 3 fish were added to each 20 litre test and control vessel and maintained at 14°C with a photoperiod of 16 hours light and 8 hours darkness for a period of 96 hours under static test conditions.

The control and solvent control groups were maintained under identical conditions but not exposed to the test material. The solvent control group was exposed to 100 µl/l of tetrahydrofuran.

Each vessel was covered to reduce evaporation. After 24, 48, 72 and 96 hours any mortalities or adverse behavioural reactions to exposure were determined by visual inspection of the test fish.

Based on the results of the range-finding study a "Limit test" was conducted for the definitive study at a test concentration of 0.40 mg/l to confirm that at the highest attainable test concentration of 0.40 mg/l, no mortalities or adverse reactions to exposure were observed.

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 20 litre glass exposure vessels were used for each test concentration. At the start of the study 10 fish were placed in each test vessel at random, in the prepared test solutions. The test vessels were then covered to reduce evaporation and maintained at $14 \pm 1^\circ\text{C}$ with a photoperiod of 16 hours light and 8 hours darkness for a period of 96 hours. The test vessels were aerated via narrow bore glass tubes. The fish were not individually identified and received no food during exposure.

The control and solvent control groups were maintained under identical conditions but not exposed to the test material. The solvent control group was exposed to $100 \mu\text{l/l}$ of tetrahydrofuran.

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 adverse reactions to exposure were recorded at 3, 6, 24, 48, 72 and 96 hours after the start of exposure. The criteria of death was taken to be the absence of both respiratory movement and response to a 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 represent those of the freshly prepared test preparations while the subsequent measurements at 24, 48, 72 and 96 hours represent those of the used or 24-hour old test preparations.

3.3.2.4 Evaluation of data

An estimate of the LC_{50} values was given by inspection of the mortality data.

4. ARCHIVES

Unless instructed otherwise by the Sponsor, all original data and the final report will be retained in the Safeparm archives for a period of five years. After this period, the Sponsor's instructions will be sought.

5. RESULTS

5.1 Range-finding Study

Cumulative mortality data from the exposure of rainbow trout to the test material during the range-finding study are given in Table 1. There were no adverse reactions to exposure during the range-finding study.

The results showed no mortalities at the test concentrations of 0.040 and 0.40 mg/l. During preliminary solubility tests precipitation of the test material was observed at test concentrations in excess of 0.40 mg/l indicating this to be the maximum limit of water solubility of the test material under these test conditions.

Based on this information, a single test concentration, in duplicate, of 0.40 mg/l was selected for the definitive study. This experimental design conforms to a "Limit test" to confirm that at the highest attainable test concentration of 0.40 mg/l, no mortalities or adverse reactions to exposure were observed.

5.2 Definitive Study

5.2.1 Mortality data

Cumulative mortality data from the exposure of rainbow trout to Kalcohl 6098 during the definitive study are given in Table 2.

There were no mortalities in 20 fish exposed to a test concentration of 0.40 mg/l for a period of 96 hours. Inspection of the mortality data gave the following results:

| Time (h) | LC ₅₀ (mg/l) | 95% Confidence Limits (mg/l) |
|----------|-------------------------|------------------------------|
| 3 | >0.40 | - |
| 6 | >0.40 | - |
| 24 | >0.40 | - |
| 48 | >0.40 | - |
| 72 | >0.40 | - |
| 96 | >0.40 | - |

The results of the definitive study showed the highest test concentration resulting in 0% mortality to be greater than or equal to 0.40 mg/l, the lowest test concentration resulting in 100% mortality to be greater than 0.40 mg/l and the No Observed Effect Concentration (NOEC) to be greater than or equal to 0.40 mg/l. The No Observed Effect Concentration is based upon zero mortalities and the absence of any behavioural responses to exposure at this concentration (Section 5.2.2).

The test concentration of 0.40 mg/l was the highest attainable test concentration that could be prepared due to the limited solubility of the test material in water and having due regard to the amount of auxiliary solvent permitted in the study under the OECD Guidelines. Other combinations of the various recognised auxiliary solvents were used during preliminary solubility tests, however, tetrahydrofuran was found to give the best testable dispersion of the test material in water. At higher test concentrations there was a marked precipitation of the test material on addition of the solvent stock solution to water.

5.2.2 Behavioural observations

There were no behavioural responses observed in 20 fish exposed to a test concentration of 0.40 mg/l for a period of 96 hours.

5.2.3 Physico-chemical measurements

The results of the physico-chemical measurements are given in Appendix I. Temperature was maintained at 14 ± 1 °C throughout the study, while there were no treatment related differences for oxygen concentration or pH.

6. CONCLUSION

The acute toxicity of Kalcohl 6098 to the freshwater fish rainbow trout (*Oncorhynchus mykiss*) has been investigated and gave a 96-Hour LC_{50} of greater than 0.40 mg/l. Correspondingly the No Observed Effect Concentration was greater than or equal to 0.40 mg/l.

KALCOHL 6098 : ACUTE TOXICITY TO RAINBOW TROUT

TABLE 1

CUMULATIVE MORTALITY DATA IN THE RANGE-FINDING STUDY

| Nominal Concentration (mg/l) | Cumulative Mortality (Initial Population = 3) | | | |
|------------------------------|---|----------|----------|----------|
| | 24 hours | 48 hours | 72 hours | 96 hours |
| Control | 0 | 0 | 0 | 0 |
| Solvent Control | 0 | 0 | 0 | 0 |
| 0.040 | 0 | 0 | 0 | 0 |
| 0.40 | 0 | 0 | 0 | 0 |

KALCOHL 6098 : ACUTE TOXICITY TO RAINBOW TROUT
TABLE 2
CUMULATIVE MORTALITY DATA IN THE DEFINITIVE STUDY

| Nominal Concentration (mg/l) | Cumulative Mortality (Initial Population = 10) | | | | | | % mortality |
|------------------------------|--|---------|----------|----------|----------|----------|-------------|
| | 3 hours | 6 hours | 24 hours | 48 hours | 72 hours | 96 hours | 96 hours |
| Control | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Solvent Control | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.40 R ₁ | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.40 R ₂ | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

R₁ - R₂ = Replicates 1 and 2

APPENDICES

KALCOHL 6098 : ACUTE TOXICITY TO RAINBOW TROUT

APPENDIX I

PHYSICO-CHEMICAL MEASUREMENTS

| Nominal Concentration (mg/l) | Time (hours) | | | | | | | | | | | |
|------------------------------|--------------|----------------------|------|------|----------|----------------------|------|------|----------|----------------------|------|------|
| | 0 hours | | | | 24 hours | | | | 48 hours | | | |
| | pH | mg O ₂ /l | T°C | T°C | pH | mg O ₂ /l | T°C | T°C | pH | mg O ₂ /l | T°C | T°C |
| Control | 7.5 | 9.8 | 14.0 | 14.0 | 7.6 | 9.6 | 14.0 | 14.0 | 7.6 | 9.7 | 13.0 | 13.0 |
| Solvent Control | 7.6 | 9.7 | 14.0 | 14.0 | 7.6 | 9.6 | 14.0 | 14.0 | 7.6 | 9.8 | 13.0 | 13.0 |
| 0.40 R ₁ | 7.4 | 9.8 | 14.0 | 14.0 | 7.7 | 9.5 | 14.0 | 14.0 | 7.7 | 9.8 | 13.0 | 13.0 |
| 0.40 R ₂ | 7.4 | 9.8 | 14.0 | 14.0 | 7.7 | 9.6 | 14.0 | 14.0 | 7.6 | 9.7 | 13.0 | 13.0 |

R₁ - R₂ = Replicates 1 and 2

KALCOHL 6098 : ACUTE TOXICITY TO RAINBOW TROUT

APPENDIX I (continued)

PHYSICO-CHEMICAL MEASUREMENTS

| Nominal Concentration (mg/l) | Time (hours) | | | | | |
|------------------------------|--------------|----------------------|------|----------|----------------------|------|
| | 72 hours | | | 96 hours | | |
| | pH | mg O ₂ /l | T·C | pH | mg O ₂ /l | T·C |
| Control | 7.6 | 9.4 | 13.0 | 7.8 | 9.8 | 13.0 |
| Solvent Control | 7.6 | 9.2 | 13.0 | 7.9 | 10.0 | 13.0 |
| 0.40 R ₁ | 7.7 | 9.3 | 13.0 | 7.8 | 9.7 | 13.0 |
| 0.40 R ₂ | 7.7 | 9.3 | 13.0 | 7.9 | 10.1 | 13.0 |

R₁ - R₂ = Replicates 1 and 2

KALCOHL 6098 : ACUTE TOXICITY TO RAINBOW TROUT
 APPENDIX II
 TYPICAL WATER QUALITY CHARACTERISTICS

| WATER SUPPLY ZONE DERBY 9A | | | | | | | | |
|--|---|-----------------------------|---------|------------------------|---------|-------|----------|--------|
| REPORTING PERIOD: 01/01/1995 TO 31/12/95 | | | | | | | | |
| PARAMETER AND QUALIFIERS | NO. OF SAMPLES TAKEN IN PERIOD | SAMPLES GREATER THAN PCV | | CONCENTRATION OR VALUE | | | | |
| | | NUMBER | PERCENT | MINIMUM | MAXIMUM | MEAN | PCV | UNITS |
| TOTAL COLIFORMS | 72 | 0 | 0.00 | 0 | 0 | 0 | 0.0 | 100 ML |
| FAECAL COLIFORMS | 72 | 0 | 0.00 | 0 | 0 | 0 | 0.0 | 100 ML |
| PLATE COUNT (AT 21°C) | 52 | 0 | 0.00 | 0 | 149 | 5 | NONE | ML |
| PLATE COUNT (AT 37°C) | 52 | 0 | 0.00 | 0 | 20 | 1 | NONE | ML |
| FREE CHLORINE | 73 | 0 | 0.00 | 0.01 | 0.36 | 0.12 | NONE | MG/L |
| TOTAL CHLORINE | 73 | 0 | 0.00 | 0.01 | 0.44 | 0.16 | NONE | MG/L |
| COLOUR | 5 | 0 | 0.00 | 1 | 4 | 2 | 20 | HAZEN |
| TORBIDITY | 5 | 0 | 0.00 | <0.3 | 0.9 | <0.4 | 4 | F.T.U. |
| TEMPERATURE | 72 | 0 | 0.00 | 6.8 | 21.2 | 13 | 25 | DEG C |
| pH | 5 | 0 | 0.00 | 7.3 | 7.4 | 7.3 | 5.5-9.50 | |
| CONDUCTIVITY | 72 | 0 | 0.00 | 296 | 543 | 405 | 1500 | uS/cm |
| QUALITATIVE TASTE | 5 | 0 | 0.00 | 0 | 0 | 0 | 3 | |
| QUANTITATIVE TASTE | 72 | — | — | — | — | — | — | |
| QUALITATIVE ODOUR | 5 | 0 | 0.00 | 0 | 0 | 0 | 3 | |
| QUANTITATIVE ODOUR | 72 | — | — | — | — | — | — | |
| AMMONIUM | 5 | 0 | 0.00 | <0.05 | <0.05 | <0.05 | 0.5 | MG/L |
| NITRITE | 5 | 0 | 0.00 | <0.01 | <0.01 | <0.01 | 0.1 | MG/L |
| NITRATE | 5 | 0 | 0.00 | 8.4 | 9.7 | 8.8 | 50 | MG/L |
| CHLORIDE | 1 | 0 | 0.00 | 23 | 23 | 23 | 400 | MG/L |
| SULPHATE | 1 | 0 | 0.00 | 46 | 46 | 46 | 250 | MG/L |
| FLUORIDE | 1 | 0 | 0.00 | 280 | 280 | 280 | 1500 | µG/L |
| PHOSPHORUS | 1 | 0 | 0.00 | <30 | <30 | <30 | 2200 | µG/L |
| ALKALINITY | 1 | 0 | 0.00 | 80 | 80 | 80 | NONE | MG/L |
| TOTAL HARDNESS | 1 | 0 | 0.00 | 136 | 136 | 136 | NONE | MG/L |
| CALCIUM | 1 | 0 | 0.00 | 43 | 43 | 43 | 250 | MG/L |
| MAGNESIUM | 1 | 0 | 0.00 | 7 | 7 | 7 | 50 | MG/L |
| SODIUM | 1 | 0 | 0.00 | 13 | 13 | 13 | 150 | MG/L |
| POTASSIUM | 1 | 0 | 0.00 | 1.2 | 1.2 | 1.2 | 12 | MG/L |
| IRON | 10 | 0 | 0.00 | 40 | 130 | 63 | 200 | µG/L |
| MANGANESE | 10 | 0 | 0.00 | <5 | 19 | <7 | 50 | µG/L |
| ALUMINIUM | 5 | 0 | 0.00 | <20 | <20 | <20 | 200 | µG/L |
| COPPER | 1 | 0 | 0.00 | 50 | 50 | 50 | 3000 | µG/L |
| ZINC | 1 | 0 | 0.00 | 70 | 70 | 70 | 5000 | µG/L |
| LEAD | 12 | 0 | 0.00 | 2 | 26 | 8 | 50 | µG/L |
| CADMIUM | 1 | 0 | 0.00 | <0.5 | <0.5 | <0.5 | 5 | µG/L |
| SILVER | 1 | 0 | 0.00 | 0.2 | 0.2 | 0.2 | 10 | µG/L |
| BORON | 1 | 0 | 0.00 | <50 | <50 | <50 | 2000 | µG/L |
| BARIUM | 1 | 0 | 0.00 | 40 | 40 | 40 | 1000 | µG/L |
| ARSENIC | 1 | 0 | 0.00 | <1 | <1 | <1 | 50 | µG/L |
| CHROMIUM | 1 | 0 | 0.00 | <5 | <5 | <5 | 50 | µG/L |
| NICKEL | 1 | 0 | 0.00 | 2 | 2 | 2 | 50 | µG/L |

PCV - Prescribed Concentration or Value

KALCOHL 6098 : ACUTE TOXICITY TO RAINBOW TROUT

APPENDIX II

TYPICAL WATER QUALITY CHARACTERISTICS (continued)

| WATER SUPPLY ZONE DERBY 9A | | | | | | | | |
|--|---|-----------------------------|---------|------------------------|---------|--------|-----|-------|
| REPORTING PERIOD: 01/01/1995 TO 31/12/95 | | | | | | | | |
| PARAMETER AND QUALIFIERS | NO. OF SAMPLES TAKEN IN PERIOD | SAMPLES GREATER THAN PCV | | CONCENTRATION OR VALUE | | | | |
| | | NUMBER | PERCENT | MINIMUM | MAXIMUM | MEAN | PCV | UNITS |
| MERCURY | 1 | 0 | 0.00 | <0.1 | <0.1 | <0.1 | 1 | µG/L |
| ANTIMONY | 1 | 0 | 0.00 | <1 | <1 | <1 | 10 | µG/L |
| SELENIUM | 1 | 0 | 0.00 | <1 | <1 | <1 | 10 | µG/L |
| CYANIDE | 1 | 0 | 0.00 | <5 | <5 | <5 | 50 | µG/L |
| OXIDIZABILITY | 1 | 0 | 0.00 | 0.9 | 0.9 | 0.9 | 5 | MCL |
| T.O.C | 1 | 0 | 0.00 | 1 | 1 | 1 | 5 | MCL |
| SURFACTANTS | 1 | 0 | 0.00 | <50 | <50 | <50 | 200 | µG/L |
| TRIHALOMETHANES | 4 | 0 | 0.00 | 35 | 66 | 49 | 100 | µG/L |
| TETRACHLOROMETHANE | 1 | 0 | 0.00 | <0.3 | <0.3 | <0.3 | 3 | µG/L |
| TRICHLOROETHENE | 1 | 0 | 0.00 | <3 | <3 | <3 | 30 | µG/L |
| TETRACHLOROETHENE | 1 | 0 | 0.00 | <1 | <1 | <1 | 10 | µG/L |
| P.A.H. (TOTAL) | 4 | 0 | 0.00 | <0.005 | 0.035 | <0.016 | 0.2 | µG/L |
| BENZ(A)PYRENE | 4 | 0 | 0.00 | <1 | <1 | <1 | 10 | NG/L |
| TOTAL PESTICIDES | 9 | 0 | 0.00 | <0.01 | 0.2 | <0.04 | 0.5 | µG/L |
| ATRAZINE | 4 | 0 | 0.00 | <0.01 | <0.01 | <0.01 | 0.1 | µG/L |
| CLOPYRALID | 8 | 0 | 0.00 | <0.03 | <0.03 | <0.03 | 0.1 | µG/L |
| 2,4-D | 8 | 0 | 0.00 | <0.01 | <0.01 | <0.01 | 0.1 | µG/L |
| 2,4-DB | 8 | 0 | 0.00 | <0.01 | <0.01 | <0.01 | 0.1 | µG/L |
| DICAMBA | 8 | 0 | 0.00 | <0.01 | <0.01 | <0.01 | 0.1 | µG/L |
| DICHLORPROP | 8 | 0 | 0.00 | <0.01 | <0.01 | <0.01 | 0.1 | µG/L |
| EPTC | 4 | 0 | 0.00 | <0.01 | <0.01 | <0.01 | 0.1 | µG/L |
| FENPROPIMORPH | 4 | 0 | 0.00 | <0.01 | <0.01 | <0.01 | 0.1 | µG/L |
| FLUROXYPYR | 8 | 0 | 0.00 | <0.02 | <0.02 | <0.02 | 0.1 | µG/L |
| MCPA | 8 | 1 | 12.50 | <0.01 | 0.12 | <0.02 | 0.1 | µG/L |
| MCPB | 8 | 0 | 0.00 | <0.02 | <0.02 | <0.02 | 0.1 | µG/L |
| MECOPROP (TOTAL) | 8 | 0 | 0.00 | <0.01 | 0.06 | <0.02 | 0.1 | µG/L |
| PROMETRYN | 4 | 0 | 0.00 | <0.01 | <0.01 | <0.01 | 0.1 | µG/L |
| PROPAZINE | 4 | 0 | 0.00 | <0.01 | <0.01 | <0.01 | 0.1 | µG/L |
| SIMAZINE | 4 | 0 | 0.00 | <0.01 | <0.02 | <0.01 | 0.1 | µG/L |
| 2,3,6,-TBA | 8 | 0 | 0.00 | <0.01 | <0.01 | <0.01 | 0.1 | µG/L |
| 2,4,5,-TCPA | 8 | 0 | 0.00 | <0.01 | <0.01 | <0.01 | 0.1 | µG/L |
| TERBUTRYN | 4 | 0 | 0.00 | <0.01 | <0.01 | <0.01 | 0.1 | µG/L |
| TRIAZINE | 4 | 0 | 0.00 | <0.01 | <0.01 | <0.01 | 0.1 | µG/L |

PCV - Prescribed Concentration or Value

APPENDIX III



THE DEPARTMENT OF HEALTH OF THE GOVERNMENT
OF THE UNITED KINGDOM

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE
IN ACCORDANCE WITH DIRECTIVE 88/320 EEC

LABORATORY

TEST TYPE

SafePharm Laboratories Ltd.
P.O. Box No. 45
Derby DE1 2BT

Analytical Chemistry
Environmental Tox.
Environmental Fate
Mutagenicity
Phys/Chem. tests
Toxicology

DATE OF INSPECTION

22 January 1996

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above laboratory as part of the UK GLP Compliance Programme.

At the time of the inspection no deviations were found of sufficient magnitude to affect the validity of non-clinical studies performed at these facilities.

27/2/96

D.F. Moore
Director

UK GLP Monitoring Authority

~~CONFIDENTIAL~~ NOT CBI HR 7/2/89

1-OCTADECANOL CAS No. 112-92-5

KALCOHL 8098:

ACUTE TOXICITY TO RAINBOW TROUT

(*Oncorhynchus mykiss*)

SPL PROJECT NUMBER: 140/506

AUTHOR: P M WETTON

STUDY SPONSOR:

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DE1 2BT
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QUALITY ASSURANCE REPORT

The routine inspection of short term studies at Safepharma is carried out as a continuous process designed to encompass all major phases of each study type once per month. Dates of relevant monthly inspections are given below:

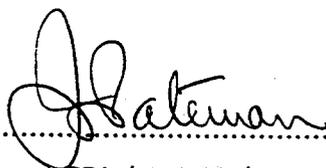
Date(s) of Inspection and Reporting:

05, 06, 11 March 1996

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

Date of Report Audit:

2 April 1996



.....
J R Pateman CBiol MI Biol
For Safepharma Quality Assurance Unit

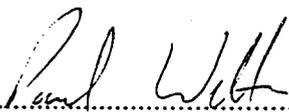
DATE: 15. APR. 1996

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 the UK Principles of Good Laboratory Practice (The United Kingdom Compliance Programme, Department of Health 1989). These Principles are in accordance with GLP standards published as OECD Environment 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: 12 APR 1996

PM Wetton BSc
Study Director

The following person was also involved in the study
D Cruse HND
Study Supervisor.

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SUMMARY

STUDY SPONSOR : KAO CORPORATION
STUDY TITLE : ACUTE TOXICITY TO RAINBOW TROUT
TEST MATERIAL : KALCOHL 8098

Methods

A study was performed to assess the acute toxicity of the test material, Kalcohl 8098, to rainbow trout (*Oncorhynchus mykiss*). 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).

Procedures

Following a preliminary range-finding study fish were exposed, in two groups of ten, to an aqueous dispersion of the test material, at a single concentration of 0.40 mg/l for a period of 96 hours under semi-static test conditions. The number of mortalities and any adverse reactions to 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 for the test material to rainbow trout (*Oncorhynchus mykiss*), based on nominal test concentrations was greater than 0.40 mg/l and correspondingly the No Observed Effect Concentration was greater than or equal to 0.40 mg/l.

KALCOHL 8098:
ACUTE TOXICITY TO RAINBOW TROUT (*Oncorhynchus mykiss*)

1. INTRODUCTION

This report contains a description of the methods used and results obtained during a study to investigate the acute toxicity of Kalcohl 8098 to rainbow trout. The method (Safepharm Laboratories Standard Method 3.OECD) 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).

Rainbow trout 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 study was conducted between 1 February 1996 and 5 February 1996 and the definitive study between 4 March 1996 and 8 March 1996.

2. TEST MATERIAL AND EXPERIMENTAL PREPARATION

2.1 Description, Identification and Storage Conditions

| | | |
|--------------------------|---|----------------------|
| Sponsor's identification | : | Kalcohl 8098 |
| Description | : | white granular solid |
| Lot number | : | 3349 |
| Date received | : | 22 January 1996 |
| 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 using a preliminary solution in tetrahydrofuran

An amount of test material (40 mg) was dissolved in 10 ml (final volume) of tetrahydrofuran with the aid of ultrasonic disruption to give a 40 mg/10 ml solvent stock solution. An aliquot (2.0 ml) of this stock solution was dispersed in 20 litres of dechlorinated tap water to give the test concentration of 0.40 mg/l.

3. METHODS

3.1 Test Species

The test was carried out using juvenile rainbow trout (*Oncorhynchus mykiss*). Fish were obtained from Donnington Fish Farm, Upper Swell, Gloucestershire, U.K. and maintained in-house since 15 January 1996. Fish were maintained in a glass fibre tank with a "single pass" water renewal system. Fish were acclimatised to test conditions from 26 February 1996 to 4 March 1996. The lighting cycle was controlled to give a 16 hours light and 8 hours darkness cycle.

The water temperature was controlled at 14°C with a dissolved oxygen content of greater than or equal to 9.3 mg O₂/l. These parameters were recorded daily. The stock fish were fed commercial trout pellets which was discontinued 24 hours prior to the start of the definitive study. There were approximately 2% mortalities in the 7 days prior to the start of the test and the fish had a mean standard length of 4.5 cm (s.d. = 0.2) and a mean weight of 1.20 g (s.d. = 0.26) at the end of the definitive study. Based on the mean weight value this gave a loading rate of 0.60 g bodyweight/litre (static volume).

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 was used with a total hardness of approximately 100 mg/l as CaCO₃. Typical water quality characteristics are given in Appendix II.

3.3 Procedure

3.3.1 Range-finding study

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

In the range-finding study fish were exposed to a series of nominal test concentrations of 0.040 and 0.40 mg/l. The test material was dissolved in tetrahydrofuran.

To prepare the test series an amount of test material (40 mg) was dissolved in 10 ml (final volume) of solvent (tetrahydrofuran) to give a 40 mg/10 ml solvent stock solution. A serial dilution was made from this to give a 4.0 mg/10 ml solvent stock solution. Aliquots (2.0 ml) of each stock solution were each separately dispersed in 20 litres of dechlorinated tap water to give the test concentrations of 0.040 and 0.40 mg/l.

For each test concentration 3 fish were added to each 20 litre test and control vessel and maintained at 14°C with a photoperiod of 16 hours light and 8 hours darkness for a period of 96 hours under static test conditions.

The control and solvent control group were maintained under identical conditions but not exposed to the test material. The solvent control group was exposed to 100 µl/l of tetrahydrofuran.

Each vessel was covered to reduce evaporation. After 24, 48, 72 and 96 hours any mortalities or adverse behavioural reactions to exposure were determined by visual inspection of the test fish.

3.3.2 Definitive study

Based on the results of the range-finding study a "Limit test" was conducted for the definitive study at a test concentration of 0.40 mg/l to confirm that at the highest attainable test concentration of 0.40 mg/l. no mortalities or adverse reactions to

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 20 litre glass exposure vessels were used for each test concentration. At the start of the study 10 fish were placed in each test vessel at random, in the prepared test solutions. The test vessels were then covered to reduce evaporation and maintained at $14 \pm 1^\circ\text{C}$ with a photoperiod of 16 hours light and 8 hours darkness for a period of 96 hours. The test vessels were aerated via narrow bore glass tubes. The fish were not individually identified and received no food during exposure.

The control and solvent control groups were maintained under identical conditions but not exposed to the test material. The solvent control group was exposed to $100 \mu\text{l/l}$ of tetrahydrofuran.

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 adverse reactions to exposure were recorded at 3, 6, 24, 48, 72 and 96 hours after the start of exposure. The criteria of death was taken to be the absence of both respiratory movement and response to a 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 represent those of the freshly prepared test preparations while the subsequent measurements at 24, 48, 72 and 96 hours represent those of the used or 24-hour old test preparations.

3.3.2.4 Evaluation of data

An estimate of the LC_{50} values was given by inspection of the mortality data.

4. **ARCHIVES**

Unless instructed otherwise by the Sponsor, all original data and the final report will be retained in the Safeparm archives for a period of five years. After this period, the Sponsor's instructions will be sought.

5. RESULTS

5.1 Range-finding Study

Cumulative mortality data from the exposure of rainbow trout to the test material during the range-finding study are given in Table 1. There were no adverse reactions to exposure during the range-finding study.

The results showed no mortalities at the test concentrations of 0.040 and 0.40 mg/l. During preliminary solubility work precipitation of the test material was observed at test concentrations in excess of 0.40 mg/l indicating 0.40 mg/l to be the maximum limit of water solubility of the test material under these test conditions.

Based on this information, a single test concentration, in duplicate, of 0.40 mg/l was selected for the definitive study. This experimental design conforms to a "Limit test" to confirm that at the highest attainable test concentration of 0.40 mg/l, no mortalities or adverse reactions to exposure were observed.

5.2 Definitive Study

5.2.1 Mortality data

Cumulative mortality data from the exposure of rainbow trout to Kalcohl 8098 during the definitive study are given in Table 2.

There were no mortalities in 20 fish exposed to a test concentration of 0.40 mg/l for a period of 96 hours. Inspection of the mortality data gave the following results:

| Time (h) | LC ₅₀ (mg/l) | 95% Confidence Limits (mg/l) |
|----------|-------------------------|------------------------------|
| 3 | >0.40 | - |
| 6 | >0.40 | - |
| 24 | >0.40 | - |
| 48 | >0.40 | - |
| 72 | >0.40 | - |
| 96 | >0.40 | - |

The results of the definitive study showed the highest test concentration resulting in 0% mortality to be greater than or equal to 0.40 mg/l, the lowest test concentration resulting in 100% mortality to be greater than 0.40 mg/l and the LC₅₀

The No Observed Effect Concentration is based upon zero mortalities and the absence of any behavioural responses to exposure at this concentration (Section 5.2.2).

The test concentration of 0.40 mg/l was the highest attainable test concentration that could be prepared due to the limited solubility of the test material in water and having due regard to the amount of auxiliary solvent permitted in the study under the OECD Guidelines. Other various recognised auxiliary solvents were used during preliminary solubility tests, however, tetrahydrofuran was found to give the best testable dispersion of the test material in water. At higher test concentrations there was a marked precipitation of the test material on addition of the solvent stock solution to water.

5.2.2 Behavioural observations

There were no behavioural responses observed in 20 fish exposed to a test concentration of 0.40 mg/l for a period of 96 hours.

5.2.3 Physico-chemical measurements

The results of the physico-chemical measurements are given in Appendix I. Temperature was maintained at 14 ± 1 °C throughout the study, while there were no treatment related differences for oxygen concentration or pH.

6. CONCLUSION

The acute toxicity of Kalcohl 8098 to the freshwater fish rainbow trout (*Oncorhynchus mykiss*) has been investigated and gave a 96-Hour LC₅₀ of greater than 0.40 mg/l. Correspondingly the No Observed Effect Concentration was greater than or equal to 0.40 mg/l.

KALCOHL 8098 : ACUTE TOXICITY TO RAINBOW TROUT

TABLE 1

CUMULATIVE MORTALITY DATA IN THE RANGE-FINDING STUDY

| Nominal Concentration (mg/l) | Cumulative Mortality (Initial Population = 3) | | | |
|------------------------------|---|----------|----------|----------|
| | 24 hours | 48 hours | 72 hours | 96 hours |
| Control | 0 | 0 | 0 | 0 |
| Solvent Control | 0 | 0 | 0 | 0 |
| 0.040 | 0 | 0 | 0 | 0 |
| 0.40 | 0 | 0 | 0 | 0 |

KALCOHL 8098 : ACUTE TOXICITY TO RAINBOW TROUT

TABLE 2

CUMULATIVE MORTALITY DATA IN THE DEFINITIVE STUDY

| Nominal Concentration (mg/l) | Cumulative Mortality (Initial Population = 10) | | | | | | % mortality |
|------------------------------|--|---------|----------|----------|----------|----------|-------------|
| | 3 hours | 6 hours | 24 hours | 48 hours | 72 hours | 96 hours | 96 hours |
| Control | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Solvent Control | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.40 R ₁ | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.40 R ₂ | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

R₁ - R₂ = Replicates 1 and 2

APPENDICES

KALCOHL 8098 : ACUTE TOXICITY TO RAINBOW TROUT

APPENDIX I

PHYSICO-CHEMICAL MEASUREMENTS

| Nominal Concentration (mg/l) | Time (hours) | | | | | | | | | | | |
|------------------------------|--------------|----------------------|------|------|----------|----------------------|------|------|----------|----------------------|------|------|
| | 0 hours | | | | 24 hours | | | | 48 hours | | | |
| | pH | mg O ₂ /l | T·C | T·C | pH | mg O ₂ /l | T·C | T·C | pH | mg O ₂ /l | T·C | T·C |
| Control | 7.5 | 9.8 | 14.0 | 14.0 | 7.6 | 9.6 | 14.0 | 14.0 | 7.6 | 9.7 | 13.0 | 13.0 |
| Solvent Control | 7.5 | 9.8 | 14.0 | 14.0 | 7.7 | 9.7 | 14.0 | 14.0 | 7.6 | 9.8 | 13.0 | 13.0 |
| 0.40 R ₁ | 7.5 | 9.8 | 14.0 | 14.0 | 7.7 | 9.6 | 14.0 | 14.0 | 7.7 | 9.7 | 13.0 | 13.0 |
| 0.40 R ₂ | 7.6 | 9.7 | 14.0 | 14.0 | 7.6 | 9.6 | 14.0 | 14.0 | 7.6 | 9.8 | 13.0 | 13.0 |

R₁ - R₂ = Replicates 1 and 2

KALCOHL 8098 : ACUTE TOXICITY TO RAINBOW TROUT

APPENDIX I (continued)

PHYSICO-CHEMICAL MEASUREMENTS

| Nominal Concentration (mg/l) | Time (hours) | | | | | |
|------------------------------|--------------|----------------------|------|----------|----------------------|------|
| | 72 hours | | | 96 hours | | |
| | pH | mg O ₂ /l | T·C | pH | mg O ₂ /l | T·C |
| Control | 7.6 | 9.4 | 13.0 | 7.8 | 9.8 | 13.0 |
| Solvent Control | 7.7 | 9.3 | 13.0 | 7.9 | 10.0 | 13.0 |
| 0.40 R ₁ | 7.7 | 9.3 | 13.0 | 7.9 | 10.1 | 13.0 |
| 0.40 R ₂ | 7.6 | 9.2 | 13.0 | 7.9 | 10.0 | 13.0 |

R₁ - R₂ = Replicates 1 and 2

KALCOHL 8098 : ACUTE TOXICITY TO RAINBOW TROUT

APPENDIX II

TYPICAL WATER QUALITY CHARACTERISTICS

| WATER SUPPLY ZONE DERBY 9A | | | | | | | | |
|--|---|-----------------------------|---------|------------------------|---------|-------|----------|--------|
| REPORTING PERIOD: 01/01/1995 TO 30/06/1995 | | | | | | | | |
| PARAMETER AND QUALIFIERS | NO. OF SAMPLES TAKEN IN PERIOD | SAMPLES GREATER THAN PCV | | CONCENTRATION OR VALUE | | | | |
| | | NUMBER | PERCENT | MINIMUM | MAXIMUM | MEAN | PCV | UNITS |
| TOTAL COLIFORMS | 38 | 0 | 0.00 | 0 | 0 | 0 | 0.0 | 100 ML |
| FAECAL COLIFORMS | 38 | 0 | 0.00 | 0 | 0 | 0 | 0.0 | 100 ML |
| PLATE COUNT (AT 21°C) | 28 | 0 | 0.00 | 0 | 3 | 0 | NONE | ML |
| PLATE COUNT (AT 37°C) | 28 | 0 | 0.00 | 0 | 1 | 0 | NONE | ML |
| FREE CHLORINE | 39 | 0 | 0.00 | 0.04 | 0.36 | 0.17 | NONE | MG/L |
| TOTAL CHLORINE | 39 | 0 | 0.00 | 0.08 | 0.44 | 0.22 | NONE | MG/L |
| COLOUR | 3 | 0 | 0.00 | 1 | 4 | 2 | 20 | HAZEN |
| TURBIDITY | 3 | 0 | 0.00 | <0.3 | 0.3 | <0.3 | 4 | F.T.U. |
| TEMPERATURE | 38 | 0 | 0.00 | 6.8 | 18 | 11 | 25 | DEG C |
| pH | 3 | 0 | 0.00 | 7.3 | 7.4 | 7.4 | 5.5-9.50 | |
| CONDUCTIVITY | 38 | 0 | 0.00 | 296 | 523 | 382 | 1500 | MICSM |
| QUALITATIVE TASTE | 3 | 0 | 0.00 | 0 | 0 | 0 | 3 | |
| QUANTITATIVE TASTE | 38 | — | — | — | — | — | — | |
| QUALITATIVE ODOUR | 3 | 0 | 0.00 | 0 | 0 | 0 | 3 | |
| QUANTITATIVE ODOUR | 38 | — | — | — | — | — | — | |
| AMMONIUM | 3 | 0 | 0.00 | <0.05 | <0.05 | <0.05 | 0.5 | MG/L |
| NITRITE | 3 | 0 | 0.00 | <0.01 | <0.01 | <0.01 | 0.1 | MG/L |
| NITRATE | 3 | 0 | 0.00 | 8.4 | 8.6 | 8.5 | 50 | MG/L |
| CHLORIDE | 1 | 0 | 0.00 | 23 | 23 | 23 | 400 | MG/L |
| SULPHATE | 1 | 0 | 0.00 | 46 | 46 | 46 | 250 | MG/L |
| FLUORIDE | 1 | 0 | 0.00 | 280 | 280 | 280 | 1500 | µG/L |
| PHOSPHORUS | 1 | 0 | 0.00 | <30 | <30 | <30 | 2200 | µG/L |
| ALKALINITY | 1 | 0 | 0.00 | 80 | 80 | 80 | NONE | MG/L |
| TOTAL HARDNESS | 1 | 0 | 0.00 | 136 | 136 | 136 | NONE | MG/L |
| CALCIUM | 1 | 0 | 0.00 | 43 | 43 | 43 | 250 | MG/L |
| MAGNESIUM | 1 | 0 | 0.00 | 7 | 7 | 7 | 50 | MG/L |
| SODIUM | 1 | 0 | 0.00 | 13 | 13 | 13 | 150 | MG/L |
| POTASSIUM | 1 | 0 | 0.00 | 1.2 | 1.2 | 1.2 | 12 | MG/L |
| IRON | 5 | 0 | 0.00 | 40 | 70 | 54 | 200 | µG/L |
| MANGANESE | 5 | 0 | 0.00 | <5 | 7 | <5 | 50 | µG/L |
| ALUMINIUM | 3 | 0 | 0.00 | <20 | <20 | <20 | 200 | µG/L |
| COPPER | 1 | 0 | 0.00 | 50 | 50 | 50 | 3000 | µG/L |
| ZINC | 1 | 0 | 0.00 | 70 | 70 | 70 | 5000 | µG/L |
| LEAD | 6 | 0 | 0.00 | 3 | 10 | 5 | 50 | µG/L |
| CADMIUM | 1 | 0 | 0.00 | <0.5 | <0.5 | <0.5 | 5 | µG/L |
| SILVER | 1 | 0 | 0.00 | 0.2 | 0.2 | 0.2 | 10 | µG/L |
| BORON | 1 | 0 | 0.00 | <50 | <50 | <50 | 2000 | µG/L |
| BARIUM | 1 | 0 | 0.00 | 40 | 40 | 40 | 1000 | µG/L |
| ARSENIC | 1 | 0 | 0.00 | <1 | <1 | <1 | 50 | µG/L |
| CHROMIUM | 1 | 0 | 0.00 | <5 | <5 | <5 | 50 | µG/L |
| NICKEL | 1 | 0 | 0.00 | 2 | 2 | 2 | 50 | µG/L |

PCV - Prescribed Concentration or Value

KALCOHL 8098 : ACUTE TOXICITY TO RAINBOW TROUT
 APPENDIX II
 TYPICAL WATER QUALITY CHARACTERISTICS

| WATER SUPPLY ZONE: DERBY 9A | | | | | | | | |
|--|---|-----------------------------|---------|------------------------|---------|-------|-----|-------|
| REPORTING PERIOD: 01/01/1995 TO 30/06/1995 | | | | | | | | |
| PARAMETER AND QUALIFIERS | NO. OF SAMPLES TAKEN IN PERIOD | SAMPLES GREATER THAN PCV | | CONCENTRATION OR VALUE | | | | |
| | | NUMBER | PERCENT | MINIMUM | MAXIMUM | MEAN | PCV | UNITS |
| MERCURY | 1 | 0 | 0.00 | <0.1 | <0.1 | <0.1 | 1 | µG/L |
| ANTIMONY | 1 | 0 | 0.00 | <1 | <1 | <1 | 10 | µG/L |
| SELENIUM | 1 | 0 | 0.00 | <1 | <1 | <1 | 10 | µG/L |
| CYANIDE | 1 | 0 | 0.00 | <5 | <5 | <5 | 50 | µG/L |
| OXIDIZABILITY | 1 | 0 | 0.00 | 0.9 | 0.9 | 0.9 | 5 | MCL |
| T.O.C | 1 | 0 | 0.00 | 1 | 1 | 1 | 5 | MCL |
| SURFACTANTS | 1 | 0 | 0.00 | <50 | <50 | <50 | 200 | µG/L |
| TRIHALOMETHANES | 2 | 0 | 0.00 | 35 | 54 | 45 | 100 | µG/L |
| TETRACHLOROMETHANE | 1 | 0 | 0.00 | <0.3 | <0.3 | <0.3 | 3 | µG/L |
| TRICHLOROETHENE | 1 | 0 | 0.00 | <3 | <3 | <3 | 30 | µG/L |
| TETRACHLOROETHENE | 1 | 0 | 0.00 | <1 | <1 | <1 | 10 | µG/L |
| P.A.H. (TOTAL) | 2 | 0 | 0.00 | 0.017 | 0.035 | 0.026 | 0.2 | µG/L |
| BENZ(A)PYRENE | 2 | 0 | 0.00 | <1 | <1 | <1 | 10 | MCL |
| TOTAL PESTICIDES | 3 | 0 | 0.00 | <0.01 | 0.2 | <0.08 | 0.5 | µG/L |
| ATRAZINE | 2 | 0 | 0.00 | <0.01 | <0.01 | <0.01 | 0.1 | µG/L |
| CLOPYRALID | 2 | 0 | 0.00 | <0.03 | <0.03 | <0.03 | 0.1 | µG/L |
| 2,4-D | 2 | 0 | 0.00 | <0.01 | <0.01 | <0.01 | 0.1 | µG/L |
| 2,4-DB | 2 | 0 | 0.00 | <0.01 | 0.01 | <0.01 | 0.1 | µG/L |
| DICAMBA | 2 | 0 | 0.00 | <0.01 | <0.01 | <0.01 | 0.1 | µG/L |
| DICHLORPROP | 2 | 0 | 0.00 | <0.01 | <0.01 | <0.01 | 0.1 | µG/L |
| EPTC | 2 | 0 | 0.00 | <0.01 | <0.01 | <0.01 | 0.1 | µG/L |
| FENPROPIMORPH | 2 | 0 | 0.00 | <0.01 | <0.01 | <0.01 | 0.1 | µG/L |
| FLUROXYPYR | 2 | 0 | 0.00 | <0.02 | <0.02 | <0.02 | 0.1 | µG/L |
| MCPA | 2 | 1 | 50.00 | <0.01 | 0.12 | <0.07 | 0.1 | µG/L |
| MCPB | 2 | 0 | 0.00 | <0.02 | <0.02 | <0.02 | 0.1 | µG/L |
| MECOPROP (TOTAL) | 2 | 0 | 0.00 | <0.01 | 0.06 | <0.04 | 0.1 | µG/L |
| PROMETRYN | 2 | 0 | 0.00 | <0.01 | <0.01 | <0.01 | 0.1 | µG/L |
| PROPAZINE | 2 | 0 | 0.00 | <0.01 | <0.01 | <0.01 | 0.1 | µG/L |
| SIMAZINE | 2 | 0 | 0.00 | <0.01 | <0.01 | <0.01 | 0.1 | µG/L |
| 2,3,6-TBA | 2 | 0 | 0.00 | <0.01 | <0.01 | <0.01 | 0.1 | µG/L |
| 2,4,5-TCPA | 2 | 0 | 0.00 | <0.01 | <0.01 | <0.01 | 0.1 | µG/L |
| TERBUTRYN | 2 | 0 | 0.00 | <0.01 | <0.01 | <0.01 | 0.1 | µG/L |
| TRIFLAZINE | 2 | 0 | 0.00 | <0.01 | <0.01 | <0.01 | 0.1 | µG/L |

PCV - Prescribed Concentration or Value

APPENDIX III



THE DEPARTMENT OF HEALTH OF THE GOVERNMENT
OF THE UNITED KINGDOM

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE
IN ACCORDANCE WITH DIRECTIVE 88/320 EEC

LABORATORY

TEST TYPE

SafePharm Laboratories Ltd.
P.O. Box No. 43
Derby DE1 2BT

Analytical Chemistry
Environmental Tox.
Environmental Fate
Mutagenicity
Phys/Chem. tests
Toxicology

DATE OF INSPECTION

22 January 1996

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above laboratory as part of the UK GLP Compliance Programme.

At the time of the inspection no deviations were found of sufficient magnitude to affect the validity of non-clinical studies performed at these facilities.

27/2/96

D.F. Moore
Director
UK GLP Monitoring Authority

Schedule No : KAS/231
Report No : 96/KAS231/0417

75:25 MIX OF 1-DODECANOL & 1-TETRADECANOL

KALCOHL 2475: Acute toxicity to rainbow trout

(Preliminary toxicity screen)

FINAL REPORT

Study Director

C A Jenkins

To:
Kao Corporation
Tochigi Research Laboratories
2606 Akabane Ichikaimachi, Haga
Tochigi 321-34
Japan

From:
Huntingdon Life Sciences Ltd
Eye
Suffolk IP23 7PX
England

Final: 9 July 1996

Huntingdon Life Sciences

KALCOHL 2475: ACUTE TOXICITY TO RAINBOW TROUT

(PRELIMINARY TOXICITY SCREEN)

FINAL REPORT

Schedule No : KAS/231

Report No : 96/KAS231/0417

I declare that the report following constitutes a true and faithful account of the procedures adopted and the results obtained in the performance of this study.

The study was conducted in accordance with the principles of Good Laboratory Practice but no formal quality assurance procedures were undertaken.

C.A. Jenkins, B.Sc, C.Biol, M.I.Biol, M.I.F.M.
(Study Director)

.....
Date: 9 July 1996

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1. SUMMARY

- 1.1 The acute lethal toxicity of KALCOHL 2475 to the rainbow trout was assessed under static exposure conditions over a period of 96 hours. Exposure concentrations were not verified by chemical analysis.
- 1.2 Groups of five fish were exposed to KALCOHL 2475 at nominal concentrations of 0.1, 0.32, 1, 3.2, 10, 32 and 100 mg/l. The test media were individually prepared by dilution of aqueous stock dispersions; to aid dispersion, HCO-40 and ultrasound treatment were employed. Control groups of fish were placed into dilution water alone or dilution water containing HCO-40 at the same level as in the test medium at the highest concentration.
- 1.3 The test was conducted at 13.3 to 14.7°C in treated tap water of hardness 230 to 254 mg/l as CaCO₃ at pH values in the range 7.7 to 8.4. At 0.1 and 0.32 mg/l, the test media were clear and colourless; at 1 mg/l and higher, they were colourless with undissolved material visible on their surfaces.
- 1.4 Observations of the fish were made at least at 24-hour intervals.

After 96 hours, the highest concentration at which no mortality had occurred was 32 mg/l and the lowest at which there was 100% mortality was 100 mg/l.

Sub-lethal, treatment-related effects were noted at 32 and 100 mg/l and included hyperventilation, darkened pigmentation and lethargy. At 100 mg/l, all fish appeared normal at 15 minutes but were dead at 24 hours; at 32 mg/l, effects were exhibited from 24 hours onwards.

The no-observed-effect concentration (NOEC) was 10 mg/l.

The 96-hour median lethal concentration (LC50) of KALCOHL 2475 to the rainbow trout was 57 mg/l.

2. TEST MATERIAL

A consignment of KALCOHL 2475 (approximately 300 g), a white, solid mass, was received at Huntingdon Life Sciences Ltd (Eye) 21 November 1995. It was further identified by the batch number 1807 and was stored in the dark under ambient conditions until use.

The identity, strength and purity of the test material received and its stability under the storage conditions were the responsibility of the Sponsor.

3. TEST PROCEDURES

The test was conducted between 3 and 7 May 1996 according to the agreed Study Protocol (PTS12c).

The rainbow trout used in the test were supplied by Fish Network Ltd, Devon and arrived at Huntingdon Life Sciences (Eye) 25 January 1996. At the laboratory, they were held in an aerated supply of dilution water under flow-through conditions until removed for testing.

The fish were maintained and the study conducted in treated tap water (dechlorinated tap water which had been blended with reverse-osmosis, softened tap water).

No mortality occurred during the 14-day period before the test.

The mean wet-weight of the fish used in the test was 1.0 g; their mean fork length was 4.4 cm.

Groups of five fish were exposed to KALCOHL 2475 at nominal concentrations of 0.1, 0.32, 1, 3.2, 10, 32 and 100 mg/l. Control groups of fish were placed into dilution water alone or dilution water containing HCO-40 at the same level as in the test medium at the highest concentration.

Two concentrated aqueous dispersions (nominally 10 and 1000 mg/l) were prepared by adding the test material, mixed with an equal amount of HCO-40, to dilution water. Aliquots of the appropriate concentrated dispersions were diluted in the test vessels to a final volume of 14 litres; the 10 mg/l stock was used at 0.1 to 1 mg/l and the 1000 mg/l preparation at 3.2 to 100 mg/l. The media were gently aerated during the test.

Chemical analysis of control and test media was not undertaken.

4. RESULTS

Table 1 lists the mortalities observed during the test. After 96 hours, the highest concentration at which no mortality had occurred was 32 mg/l and the lowest at which there was 100% mortality was 100 mg/l.

The 24- to 96-hour median lethal concentration (LC50) of KALCOHL 2475 to the rainbow trout was 57 mg/l; the value was derived by non-linear interpolation between the two concentrations which bracket the 50% effect level (32 and 100 mg/l).

At 100 mg/l, all fish appeared normal at 15 minutes but were dead at 24 hours. At 32 mg/l, no adverse effects were noted at 15 minutes but from 24 hours onwards, all fish were hyperventilating, three were lethargic and one appeared dark.

The no-observed-effect concentration (NOEC) was 10 mg/l.

At 0.1 and 0.32 mg/l, the test media were clear and colourless; at 1 mg/l and higher concentrations, they were colourless with undissolved material visible on their surfaces.

The results of the water quality analysis conducted at the start of the test and at 24-hour intervals thereafter are summarised below.

| | |
|--|------------------------|
| - temperature, °C | 13.3 - 14.7 |
| - pH | 7.7 - 8.4 |
| - dissolved oxygen, % ASV* | 75 - 100 |
| - total hardness, mg/l CaCO ₃ | 230 - 254 [#] |

* air saturation value

[#] although the hardness of the media slightly exceeded that recommended for this type of test (maximum 250 mg/l CaCO₃) this is not thought to have affected the integrity of the test.

No concentration-related changes in these parameters were observed.

TABLE 1

Cumulative mortality

| Observation times (hours) | Nominal KALCOHL 2475 concentrations, mg/l | | | | | | | | |
|---------------------------|---|----|-----|------|---|-----|----|----|-----|
| | 0 | 0* | 0.1 | 0.32 | 1 | 3.2 | 10 | 32 | 100 |
| 0.25 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 |
| 24 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 |
| 48 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 |
| 72 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 |
| 96 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 |

Note : Five fish were exposed at each concentration.

0* : Dispersant control.



8EHQ-0301-14318

45234

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

March 21, 2001

High Point Chemical
Attn: Hester Kobayashi
Manager, Kao Product Safety North America
255 Beddington Street
High Point, NC 27260

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2001 MAR 21 PM 2:36

SUBJECT: 8EHQ-1198-14318 and 8EHQ-0799-14318 Supplement

Dear Dr. Kobayashi:

Thank you for responding to EPA's previous request for information regarding the TSCA 8(e) submissions referenced above. In order to link the submitted reports to specific CAS Registry Numbers, please indicate the trade names from these reports, e.g., KALCOHL 0898, that correspond to the CAS Registry Numbers provided in your initial submission dated 11/17/98..

Enclosed are the first pages of your two submissions and a copy of "Support Information for Confidentiality Claims". Please cite the assigned 8EHQ numbers and address your response to:

Document Control Office (7407)
Room G99 East Tower ATTN: Section 8(e)
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
401 M Street, S.W.
Washington, D.C. 20460-0001



8EHQ-98-14318

Questions regarding this request should be directed to Mr. Terry O'Bryan of my staff at (202) 260-3483 or E-Mail OBRYAN.TERRY@EPA.GOV

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2001 MAR 23 AM 10:20

Sincerely,

Richard H. Heffer, Chief
High Production Volume Chemicals Branch

Enclosures



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