LIFE SCIENCE RESEARCH

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PYRONIL 45: DETERMINATION OF ITS 96-HOUR LC_{50} TO A COLD WATER SPECIES OF FISH (15°C) UNDER STATIC TEST CONDITIONS

Protocol prepared for

Pennwalt Corporation

by

Life Science Research Limited
Eye, Suffolk, IP23 7PX
England

27 June 1988

MANAGEMENT OF STUDY

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

Quality Assurance Manager : David J. Ford, Ph.D.

Sponsor : Pennwalt Corporation
900, 1st Avenue
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King of Prussia
Pennsylvania 19406-0018
USA

Monitor : Dr J A Seckar

TEST MATERIAL IDENTITY : Pyronil 45

METHODS

The study will be carried out, under UK legislation (1986) Project Licence 'Aquatic Toxicicology' (No. 70/00762), according to the Standard Protocol ATId attached.

SCHEDULED TIME-PLAN

The study will be performed and reported to a time schedule designed to minimise delays. Due to the short duration of the test, no detailed time-plan will be issued unless specifically requested.

LSR Enquiry No : 2651C

PROTOCOL APPROVAL

For LIFE SCIENCE RESEARCH LIMITED

Issued by : D. C. Hodge .... Date : 27/6/88...

Released by : ......[signature]............ Date : ..28/6/88.....

For PENNWALT CORPORATION

Approved by : ......Joel A. Secker............ Date : ..7/7/88......

In short studies using standardised methods, protocol alterations or revisions are not normally required. If changes to this protocol are necessary please contact LSR. Please note that the study cannot begin unless Life Science Research Limited is in receipt of a signed protocol.

DETERMINATION OF THE 96-HOUR LC50 OF TEST MATERIALS TO COLD WATER SPECIES OF FISH (15°C) UNDER STATIC TEST CONDITIONS

Standard Protocol ATld

of

Life Science Research Limited
Eye, Suffolk, IP23 7PX
England

March 1988

1. INTRODUCTION AND OBJECTIVES

This test has been designed to determine the acute toxicity of test materials to cold water species of fish at 15°C. The fish, rainbow trout, will be exposed, for 96 hours, to the test material prepared in treated tap water. The test solutions will not be replaced during the test period.

The study will comprise a preliminary (range-finding) test, followed by a definitive study to determine, where possible, the 96-hour median lethal concentration (LC_{50}) of the test material, with 95% confidence limits. Mortalities will be recorded at 24-hour intervals during the tests.

The study is appropriate for the determination of the effects of insoluble test materials and water soluble materials known to be stable in aqueous solution for greater than 96 hours. It is not appropriate for volatile materials. Test materials may include effluents and formulated products containing more than one substance.

The study will meet the requirements of Procedure 203 of the 'Guidelines for Testing of Chemicals' of the OECD: "Fish, Acute Toxicity" adopted 4 April 1984.

2. TEST ORGANISM

Rainbow trout, *Salmo gairdneri* (Richardson), are usually selected as the test organism because of their known sensitivity to changes in water quality, availability throughout the year and ease of maintenance in the laboratory. They are obtained either as eyed eggs or fry from trout farms in the UK.

3. DILUTION MEDIUM

The dilution water will be filtered de-chlorinated tap water to which has been added de-chlorinated tap water that has been softened and treated by reverse-osmosis to maintain constant hardness. The proportion of treated and un-treated tap water will be adjusted to give a final total hardness of 200-250 mg/l as $CaCO_3$.

4. PRE-EXPOSURE PERIOD

Eggs will be held in hatching troughs supplied with a continuous flow of aerated water. After hatching and resorption of the yolk sac, the fry will be transferred to a holding tank supplied with a continuous flow of aerated water.

Feeding fry obtained from trout farms will be kept in holding tanks supplied with water as above for a minimum of 14 days before being used for testing.

During the holding period the tanks will be inspected at least three times each week and any debris, or unhealthy or dead fish removed.

The temperature of water supplied to the holding tanks will be 14 $\pm$ 2°C. A photoperiod of 16 hours light, provided by overhead flourescent tubes and 8 hours dark will be maintained. Dawn and dusk will be simulated by the provision of a period of subdued lighting.

The temperature, pH and concentration of dissolved oxygen will be measured at least three times each week and the hardness of the water determined weekly, in each holding tank.

The fish will be fed at least three times each week during the holding period on proprietary trout pellets and feeding records maintained.

Fish will not be used in tests if the mortality of the batch exceeds 10% during the 14-day period prior to starting a test. They will be held without food at the test temperature for approximately 24 hours before being placed into the test vessels.

Fork length will be between 4 and 6 cm and an estimate of the wet-weight of the animals to be used will be made before the start of the definitive test.

5. PREPARATION OF THE TEST MATERIAL

The identity, stability and purity of the test material, and the amount and nature of any other components present are the responsibility of the Sponsor.

Where a material is known to be of low solubility in water a preliminary study can be included, at additional cost, to determine the most appropriate method of preparing dilutions of the test material in the dilution medium used.

Where the test material is poorly soluble and forms a particulate suspension in water, preliminary tests to determine the settling rate and particle size can be carried out at additional cost.

For compliance with international GLP regulations it may be necessary to analyse a sample of test material solution as used in testing, to confirm the concentration, homogeneity and stability of the test material. Also, the test guidelines require evidence that exposure concentrations have been maintained over the test period (within 80% of the nominal value throughout the test). Tests for stability and homgeneity of the test material and for verification of the concentrations of the stock and test dilutions can be conducted by LSR, at the request of the Sponsor, at additional cost.

A concentrated stock solution or dispersion of the test material will be prepared as appropriate in the dilution water. Dilutions of this will then be made to achieve the required test concentrations.

If problems of solubility or homogeneity prevent the use of a concentrated solution or dispersion, appropriate weights of the test material will be added directly to water in test vessels.

The dilution water will be obtained from the same source as that used to maintain the test system during the 14-day holding period preceding the test.

If there is a marked change in the pH of the dilution water after the addition of the test material (outside the range 6 to 8.5), the pH of the stock will be adjusted by the addition of hydrochloric acid and/or sodium hydroxide.

Where a test material is known to adsorb onto the surfaces of test vessels, the vessels will be conditioned to the appropriate concentration of the test material for a period of up to 48 hours before the test starts. This will attract an additional cost.

6. TEST PROCEDURE

The study which will be carried out under static conditions will comprise a preliminary test, to provide an estimate of the toxicity of the material, followed by a definitive test to define the relationship between concentration and effect.

In the preliminary test, fish will be exposed to concentrations of the test material selected from the following range unless otherwise specified by the Sponsor:

1, 3.2, 10, 32, 100, 320 and 1000 mg/l

The range of concentrations used in the definitive test will be based on the results of the preliminary test and will provide at least five exposure groups, spaced by a constant factor not exceeding 2, and a control group.

Where requested by the Sponsor, the concentration of the test material in the concentrated stock will be verified by chemical analysis in the preliminary and definitive tests. In addition the exposure concentrations in appropriate vessels will be determined at the start and end of the definitive test.

When an intermediate vehicle is used to facilitate the preparation of aqueous solutions or dispersions of the test material, an additional control group will be included containing the vehicle in the dilution water at a concentration comparable to that present at the highest exposure concentration (not exceeding 100 mg/l).

The temperature of the test solutions will be maintained between 13 and 17°C but constant to within $\pm$ 1°C during each test. The photoperiod will be the same as that in the holding area.

The prepared test dilutions will be placed into the test vessels (glass aquaria) and gently aerated using a Pasteur pipette during the test. The aquaria will be of an adequate size to meet the guideline criterion of a maximum loading of 1 g fish per litre of test solution.

Groups of five fish will be randomly assigned to the test vessels containing test dilutions, until each contains the required number of animals. In the preliminary test five fish will be exposed to each concentration; in the definitive test a minimum of ten will be used at each exposure level.

The fish will not be fed during the tests.

The fish will be observed for mortalities and visible abnormalities 15 minutes after their addition to the vessels and then after 24, 48, 72 and 96 hours. During the definitive test additional observations will be made 2 and 4 hours after the start of the test.

The temperature, pH and concentration of dissolved oxygen of the dilution water and all test solutions will be measured every 24 hours. The total hardness of the dilution water and of solutions of the test material at low and high exposure concentrations will be measured at the start and end of the tests.

For the test to be valid, the mortality in the control group must not exceed 10% at the end of the test, the concentration of dissolved oxygen must be > 60% of the air saturation value during the test and exposure concentrations must be maintained throughout the test (within 80% of the nominal concentrations).

6.1 Amendments to the standard procedure

The following amendments to the standard test design are available at additional cost:

Where the Sponsor requires a study of comparative toxicity of a number of test materials, single tests can be carried out in which a wide range of exposure concentrations can be used and the test terminated after 48 hours.

The test can be carried out :

- without verification of exposure concentrations (this test may not fulfil regulatory requirements),
- using an alternative dilution medium eg. low-hardness water,
- at low levels of dissolved oxygen,
- at pH levels outside the range 6 to 8.5,
- in the presence of suspended solids,
- using an alternative species of fish.

At the Sponsor's request internal and/or external examinations of the fish can be carried out and the body tissues either analysed at the testing facility or returned to the Sponsor for histological analysis. Where appropriate, quantitative analysis of gill damage can be performed.

7. APPRAISAL OF DATA

The lowest concentration producing 100% mortality/effect and the highest concentration producing no mortality/effect will be stated.

Where possible the median lethal concentrations (LC_{50}s), and their 95% confidence limits, will be calculated by either the moving average, probit or binomial methods at 24-hour intervals.

Water quality data will be expressed as minimum and maximum values measured during the tests.

Results of analysis of the test material will be given as found values together with the estimated values after adjustment for recovery if appropriate.

Any calculations made will be included in the written report, together with advice on the significance of the results.

8. QUALITY ASSURANCE

This study will be conducted in accordance with the precepts of currently recognised international Good Laboratory Practice (including the recommendations of the OECD, 1981) and will be subjected to the following quality assurance procedures.

- the protocol will be inspected for compliance
- procedures and data as used and produced on this type of study are periodically inspected
- the final report will be reviewed to ensure that it accurately describes the methods and relevant Standard Operating Procedures and that the results are in accord with the primary data.

Periodic reports on these activities are made to Management and the Study Director.

All raw data pertaining to the study will be available for inspection by the study monitor (for scientific monitoring) or the Quality Assurance Unit of the Sponsor (compliance monitoring). In addition, specified scientists designated by the Sponsor may, upon appointment, examine any set of data.

9. LOCATION OF STUDY : Life Science Research Limited
Eye
Suffolk IP23 7PX
England

10. RECORDS KEPT

All raw data, test material information, QA records and reports and other records pertaining to the study will be retained in the Archives of LSR.

The raw data will comprise: operational and observations sheets covering every aspect of the study.

11. REPORTING

The following information and data will be included in the final report.

- Name and address of the facility performing the study and the initiation and termination dates.

- Objectives and procedures stated in the approved study plan or protocol amendment, including any changes subsequently made.

- Raw data generated while conducting the study including any transformations, calculations or operations performed on the data. Tabulated mean and range values where appropriate.

- Statistical methods employed for analysing the data.

- The test material identified by name and/or code number, strength, stability and purity, as instructed by the Sponsor.

- The Sponsor's information regarding stability of the test material under the conditions of the test.

- Methods and procedures used.

- Concentrations tested.

- Frequency and modes of observation. Observations recorded.

- Any unforeseen circumstances which may have affected the quality or integrity of the study.

- The name of the Study Director.

- A summary of the data, an analysis of the data and a statement of the conclusions drawn from the analysis.

- The dated signature of the Study Director.

- The location where all raw data and the final report are stored.

- A statement by the Quality Assurance Unit.

Corrections or additions to a final report will be in the form of an amendment by the Study Director. The amendment will clearly identify that part of the final report that is being added to or corrected and the reasons for the correction or addition, and will be signed and dated by the person responsible.

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