REPORT

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BRIXHAM LABORATORY

BL/B/3387 Copy no 3

CIBA-GEIGY DVP 438 solid:
Toxicity to the brown shrimp (Crangon crangon)

CONFIDENTIAL

Performing laboratory: ICI Brixham Laboratory
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Approved by B R H Williams
December 1988

BL/B/3387

CIBA-GEIGY DVP 438 solid:
Toxicity to the brown shrimp (Crangon crangon)

Brixham study no R305/A

AUTHENTICATION

I, the undersigned, hereby declare that this study was performed under my direction according to the principles of Good Laboratory Practice, and that this report represents a true and accurate record of the results obtained.

Study Director 30/11/88
R W Hill

The following person carried out work on this study:

Responsible Scientist 30.11.88
E A Morgan

Report approved by

Project Manager ..... 30.11.88 B R H Williams

The conduct of this study has been inspected/audited in accordance with ICI'S policies and procedures for Good Laboratory Practice, as follows. The analytical data were not audited at Brixham.

Date	Inspection/audit	Date of QA report
18.11.88	Draft report	18.11.88
19.12.88	Final report	19.12.88

J L Gilbert
Quality Assurance Unit 19.12.88

CONTENTS

INTRODUCTION

A sample of Ciba-Geigy DVP 438 solid (Brixham Test Substance number R305) was submitted by Ciba-Geigy, Industrial Chemicals, Tenax Road, Trafford Park, Manchester, for aquatic toxicity testing.

The species tested was the brown shrimp (Crangon crangon) and the test protocol is given in Appendix D.

The test material DVP 438 was a white powder of low water solubility. A 96 hour agitation study (MAFF UK) was used and the test solutions were prepared by the addition of the required amount of DVP 438 to 16 litres of seawater. 20 brown shrimp were tested in each exposure vessel.

The test solutions were maintained under continuous agitation for the length of the study (96 hours) with replacement at 24 hours. Visual observations were made daily to identify if undissolved material could be seen in the test vessels. These observations are reported on page 6 and clearly show that excess material was present in the higher concentration during the study.

A certificate of the test results is given and the cumulative mortalities in each concentration have been tabulated.

The test was carried out during the period 8-12 August 1988.

The study number was R305/A. Original data relevant to this study are stored in the Brixham Laboratory archive.

RESULT

Product	Brixham test substance no	96 hr LC50 (mg/l)
Ciba-Geigy DVP 438	R305	>2.5 mg/l

CONCLUSION

Ciba-Geigy DVP 438 is not toxic to brown shrimp in seawater at its presumed water solubility level, based on the addition of excess material (see page 6).

Brixham Laboratory

CERTIFICATE OF AQUATIC TOXICITY TEST RESULTS

MATERIAL TESTED: CIBA-GEIGY DVP 438
(Brixham Ref No R305)

STUDY NUMBER: R305/A

TEST ORGANISM: Brown shrimp (Crangon crangon)

Brixham Laboratory
Freshwater Quarry
Brixham TQ5 8BA
UK

Telephone (08045) 6411
Telex 42812 ICILAB G
Fax (08045) 59437

TEST METHOD

Test organisms
Number per vessel: 20 Average weight: 0.61 g

Source: Tor Bay Average length: 36.8 mm

Exposure vessels
Material : Glass Dimensions: cylindrical - 290 mm diameter x 300 mm height

Capacity: 19.8 litres Volume of test solution: 16 litres

Test conditins
Type: Semi-static agitated Duration: 96 hours

Dilution water: Seawater

pH: 8.10 - 8.12 Salinity: 34.90 - 34.98°/oo

Test dates: 8 - 12 August 1988

Test solutions
Nominal temperature: 15°C Range during test: 14.6 - 16.2°C

Dissolved oxygen range during test: 7.2 - 8.0 mg/l

pH range during test: 7.6 - 8.0

RESULTS as nominal concentration DVP 438

LC50 VALUES	
hour	mg/l
24	>2.5
48	>2.5
72	>2.5
96	>2.5

Seawater control mortality = 0% at 96 hours

C Hartgen 14-11-88
RESPONSIBLE SCIENTIST

R White 14/11/88
STUDY DIRECTOR

TABLE OF RESULTS

CIBA-GEIGY DVP 438 - Crangon crangon

CONCN TESTED mg/l	24 hr		48 hr		72 hr		96 hr	
	A	B	A	B	A	B	A	B
2.5	0	0	0	0	0	0	0	3
1.0	0	1	0	2	0	2	0	4
0.5	0	0	0	2	0	4	0	6
0.25	0	2	0	3	0	4	0	5
Seawater control	0	0	0	0	0	1	0	2

A = mortality B = moult death

20 brown shrimps were exposed in each concentration

VISUAL OBSERVATIONS ON THE TEST MATERIAL IN THE EXPOSURE VESSELS

Time 0 hours
All concentrations were clear and colourless. Some sample remained on the surface of the solution. When added to the seawater larger particles sank to the bottom of the test vessels.

Time 24, 48, 72 and 96 hours
The test material was observed in each case at the bottom of the test vessels.

Analytical sampling programme

Samples of each exposure concentration were taken at approximately 6 hours from the start of the study and every 24 hours, prior to the test solutions being replaced. These samples were sent to the sponsor for analysis.

At each sampling point 200 ml of water was removed and this was replaced with 200 ml of clean seawater.

Analytical measurements of DVP 438 shown in Appendix A indicate that at the two higher concentrations tested less than 20% of the material was present after each 24 hour period and that in the lowest concentration, 0.25 mg/l, up to 56% of the test material was in solution.

The temperature range of 14.6-16.2°C is outside the recommended range by 0.2°C but it is not considered that this small variation would have invalidated the study.

APPENDIX D

DETERMINATION OF THE ACUTE TOXICITY (LC50) OF A CHEMICAL TO THE BROWN SHRIMP (Crangon crangon) USING A SEMI-STATIC AGITATION TEST PROCEDURE

1 OBJECTIVE

To determine the 96 hour median lethal concentration (96 hour LC50) of a test material to the brown shrimp (Crangon crangon), for submission to the Department of Energy under the Control of Discharges of Oil and Oil-based Muds and Cuttings.

2 TEST MATERIALS

A sufficient quantity of the test material is supplied by the sponsor. Adequate chemical specification with special reference to hazardous properties and storage conditions are provided by the sponsor.

3 TEST SPECIES

3.1 Species/supply

Brown shrimp (Crangon crangon) obtained from Tor Bay will be used for the study.

3.2 Justification for the selection of test species

The brown shrimp is the preferred marine invertebrate of MAFF. It is readily available and easy to maintain and handle.

3.3 Specification

The test animals should be healthy. An estimate of the weight and length of the test population is determined by measurement of the control animals on completion of the study.

The average length should be within the range 40-60 mm. The length of the longest animal should be no more than twice that of the smallest.

4 ACCLIMATION

The stock population is held on site for a minimum of five days and fed on a specially prepared diet. They are held for a minimum of three days at 15 +/- 2°C before being transferred to test vessels. Food is withheld throughout the test period.

5 TEST APPARATUS

The test vessels are constructed of glass and are of 19.8 litres working capacity. An agitation system, based upon the MAFF system (Ref 1), is used.

6 IDENTIFICATION OF TEST SYSTEM

The individual test vessels are identified by the study number and the exposure concentration.

7 DILUTION WATER

The local seawater of approximately 34.5 °/oo salinity is used after filtration. The temperature of the dilution water is controlled to 15° +/- 1°C before being added to the test vessel.

8 TEST TEMPERATURE

The test is carried out at 15° +/- 1°C.

9 TEST CONCENTRATIONS

The following series of concentrations will be prepared:

2.5, 1.0, 0.5 and 0.25 mg/l

A seawater control is prepared and operated under the same conditions as the test concentrations. This solution was changed daily.

10 PREPARATION OF TEST SOLUTIONS

The test material is prepared by the addition of the test material to the surface of the test vessel and the agitation system switched on.

All solutions of the test material are expressed as mg/l.

11 PARAMETERS MONITORED

The following parameters are measured before and after the replacement of each test solution: pH, dissolved oxygen and temperature. Salinity of the dilution water is recorded.

12 PROCEDURE

Sixteen litres of dilution water are added to each test vessel and the volume adjusted to ensure that the surface of the water is level with the inlet to the agitation column when the motor is running. Twenty animals are placed into each vessel and the required amount of test material added. To start the test the agitation system is placed into the test vessel and switched on. Sufficient time must be allowed for the test material to disperse before water samples are taken for determination of parameters.

The above procedure is also used when test solutions are replaced during the test.

Mortalities are recorded at frequent intervals during the initial period of the test and after 24, 48, 72 and 96 hours exposure. The test solutions were replaced at 48 hours.

The test is of 96 hours duration.

If more than four animals die in the control the test is invalidated.

13 MOULT DEATHS

Moult deaths are test animals which have moulted and have then been cannibalised. They are identified:

(a) as partially eaten soft bodied animals

(b) when a moult case is present and the number of test animals is deficient by one

Moult deaths must be recorded in the study records, but are not included in any calculations of the mortality for a test population.

14 RESULTS

The mortalities recorded at 24, 48, 72 and 96 hours are tabulated and the LC50 (median lethal concentration) for these times and their confidence intervals are calculated using the method of Stephan (Ref 2). When there are three or more calculated LC50 values they will be plotted onto a dose response graph.

15 STANDARD OPERATING PROCEDURES

Unless otherwise specified all procedures mentioned in this document are the subject of detailed standard operating procedures which are contained in the SOP manuals of the participating department.

16 QUALITY ASSURANCE

The procedures in the study may be subject to Quality Assurance evaluation. The form of the inspection will be described in the QAU Standard Operating Procedures Manual. The final report will also be evaluated by the Quality Assurance unit.

17 RECORDS

All measurements and observations made during the study will be recorded and original data archived at Imperial Chemical Industries PLC, Brixham Laboratory, Devon.

REFERENCES

1 Blackman, R A A et al (1977) New procedures for the toxicity testing of oil slick dispersants. MAFF Directorate of Fisheries Research Technical Report 39

2 Stephan C E (1977) Methods for calculating an LC50. Proceedings first annual symposium on aquatic toxicology. Aquatic Toxicology and Hazard Evaluation. Ed: Mayer F L, Hamelink J L, ASTM STP 634, 65-84

APPENDIX A

DVP 438

DETERMINATION OF DVP 438 FROM LC 50 TEST (REF R305/A)

1. METHOD

1.1. Equipment and Reagents

Visible spectrophotometer
Hach UV lamp with power supply 20828-02
Hach Potassium Persulphate Powder Pillows 20847-69
Hach Phos Ver 3 Phosphate Reagent Powder Pillows 2125-99

1.2. Preparation of Calibration Graph

Accurately weigh 0.1433 g potassium dihydrogen phosphate A.R. into a 1 litre graduate flask, dilute to volume with deionised water and mix thoroughly (100 ppm $PO_4$3-). Pipette 10.0 ml 100 ppm standard into a 100 ml graduated flask, dilute to volume with deionised water and mix (10 ppm $PO_4$3-).

Dilute 5.0, 10.0, 15.0 and 20.0 ml of 10.0 ppm $PO_4$3- into 100 ml graduated flasks and dilute to volume with deionised water. These standards are equivalent to 0.5, 1.0, 1.5 and 2.0 ppm $PO_4$3-.

Pipette 25 ml of each standard into a 50 ml stoppered cylinder including 25 ml deionised water for a reagent blank. Add 1 Hach Phos Ver 3 Phosphate Reagent Powder Pillow to each and shake thoroughly to dissolve.

After 2 minutes and not more than 10 minutes, measure the absorbance of the solutions at 890 nm in a 2 cm cell against a reference of deionised water. After deduction of the reagent blank plot a calibration graph of absorbance versus ppm $PO_4$3-.

1.3. Determination of DVP 438

Transfer 25 ml of sample to a 50 ml thick-walled glass centrifuge tube, add 1 Hach Potassium Persulphate Powder Pillow and shake thoroughly to dissolve. Insert the UV lamp and irradiate the solution for 20 minutes. Add 1 Hach Phos Ver 3 Phosphate Reagent Powder Pillow and shake until dissolved.

Measure the absorbance of the solution at 890 nm in a 2 cm against a reference of deionised water after 2 minutes and not more than 10 minutes.

After deduction of the blank absorbance (seawater blank without DVP 438 added) calculate ppm $PO_4$3- from calibration graph.

ppm DVP 438 = ppm $PO_4$3- x 3.435

2. RESULTS

TIME (HRS)	DETERMINED DVP 438 (SOLUBLE) PPM							
	6	24	30	48	54	72	78	96
2.5 PPM	0.14	0.44	< 0.1	< 0.1	< 0.1	< 0.1	0.16	< 0.1
1.0 PPM	< 0.1	0.16	< 0.1	0.18	-	-	-	-
0.5 PPM	< 0.1	0.17	< 0.1	0.6	-	-	-	-
0.2 PPM	< 0.1	0.14	< 0.1	< 0.1	-	-	-	-

After 48 hours only 2.5 ppm samples analysed.

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BL/B/3387

CIBA-GEIGY DVP 438 solid:
Toxicity to the brown shrimp (Crangon crangon)

CIRCULATION

Copy number		
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