



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

NOV 20 1997

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Reregistration, Data Evaluation - Acute Toxicity to Rainbow Trout,
Vantocil P (111801), D224336, REREG Case #3122, ID#111801-010182
Sponsor: ZENECA Inc.

TO: Bruce Sidwell, PM 53
Special Review and Reregistration Division (7508W)

FROM: Douglas J. Urban, Team Leader
EFED Screening & Greybeard Panel
Environmental Fate and Effects Division (7507C)

GUIDELINE	MRID	TOXICITY	ACCEPTABILITY
72-1	439490-01	25.45 μ g @ 20.1% ai	Core

This study indicates that Vantocil P at 20.1% ai is very highly toxic to rainbow trout.

If you have any questions concerning this review please, contact Regina Hirsch (414-695-9796) or Arnet Jones, (305-7416).

1/10

DATA EVALUATION RECORD
§ 72-1(C) -- ACUTE LC₅₀ TEST WITH A COLDWATER FISH

1. **CHEMICAL:** Vantocil P (PHMB) PC Code No.: 111801

2. **TEST MATERIAL:**

Poly(iminocarbonimidoyliminocarbonimidoylimino-1,6-hexanediyl) hydrochloride

N,N''-1,6-hexanediylbis(N'-cyanoguanidine) polymer with 1,6-hexanediamine hydrochloride

¹⁴C-PHMB was 26% w/w solution (Brixham test substance number AB0774). and non-labelled PHMB (Vantocil P) strength was 20.1% w/w by total solids (certificate reference number NBY5109/6, dated 22 February 1995). The sample was a clear faint yellow liquid (Batch number Y00156/008/105).

3. **CITATION**

Authors: A.J. Penwell and G.C. Roberts
Title: PHMB: Acute toxicity to rainbow trout
Oncorhynchus mykiss.

Study Completion Date: 20 October 1995

Laboratory: Brixham Environmental Laboratory
ZENECA Limited
Brixham Devon TQ5 8BA
UK

Sponsor: ZENECA Biocides
Wilmington, Delaware

Laboratory Report ID: X839/B

MRID No.: 439490-01

DP Barcode: D224336

4. **REVIEWED BY:** ~~Regina Hirsch, Wildlife Biologist, EEB, EFED~~

Signature: 

Date: 7/24/95

5. **APPROVED BY:** ~~Les Touart, Head Section 1, EEB, EFED~~

Signature: 

Date: 11/20/97

6. **STUDY PARAMETERS**

Scientific Name of Test Organism: *Oncorhynchus mykiss*
Age or Size of Test Organism: Mean weight 2.21g;
Mean length 53mm

DP Barcode: D224336

MRID No.: 439490-01

Definitive Test Duration: 96-hours
Study Method: Dynamic (flow-through), no aeration
Type of Concentrations: Mean measured

7. CONCLUSIONS:

Results Synopsis

LC₅₀: 25.45 µg/L ai

95% C.I.: 17 - 33 µg/L ai

NOEL: 9.8 µg/L ai

8. ADEQUACY OF THE STUDY

A. Classification: Core.

B. Rationale: N/A

C. Repairability: N/A

9. GUIDELINE DEVIATIONS

1. Used dechlorinated tap water instead of soft reconstituted water or water from a natural source.

2. Total hardness of water used was low (24.7 - 35.3 mg/L as CaCO₃) for what is recommended (40 - 48 mg/L as CaCO₃).

3. Not Reported on whether or not meter system was calibrated before study and checked twice daily during test period.

10. SUBMISSION PURPOSE: Study submitted for Reregistration.

11. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
Species Preferred species is the rainbow trout (<i>Onchorhynchus mykiss</i>)	<i>Onchorhynchus mykiss</i>
Mean Weight 0.5-5 g	2.21g
Mean Standard Length Longest not > 2x shortest	Mean: 53 mm Range: 46-60
Supplier	Bilbury Trout Farm, Bilbury Cirencester, Gloucestershire, UK
All fish from same source?	Yes
All fish from the same year class?	Yes

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period Minimum 14 days	18 days
Wild caught organisms were quarantined for 7 days?	N/A
Were there signs of disease or injury?	Not reported
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	N/A
Feeding No feeding during the study	48 hours prior to test initiation
Pretest Mortality < 3% mortality 48 hours prior to testing	Not reported.

C. Test System

Guideline Criteria	Reported Information
<p><u>Source of dilution water</u> Soft reconstituted water or water from a natural source, not dechlorinated tap water</p>	Dechlorinated tap water
<p>Does water support test animals without observable signs of stress?</p>	Not reported.
<p><u>Water Temperature</u> 12°C</p>	12°C ± 1°C
<p><u>pH</u> Prefer 7.2 to 7.6</p>	7.1 - 7.6
<p><u>Dissolved Oxygen</u> Static: ≥ 60% during 1st 48 hrs and ≥ 40% during 2nd 48 hrs, flow-through: ≥ 60%</p>	9.4 mg/L at 48-hours.
<p><u>Total Hardness</u> Prefer 40 to 48 mg/L as CaCO₃</p>	24.7 - 35.3 mg/L as CaCO ₃
<p><u>Test Aquaria</u> 1. <u>Material:</u> Glass or stainless steel 2. <u>Size:</u> Volume of 18.9 L (5 gal) or 30 x 60 x 30 cm 3. <u>Fill volume:</u> 15-30 L of solution</p>	<p>Glass vessels</p> <p>54 L (61.0 cm x 30.5 cm x 31.0 cm)</p> <p>45 L</p>

Guideline Criteria	Reported Information
<p><u>Type of Dilution System</u> Must provide reproducible supply of toxicant</p>	<p>Stock solutions were fed to the glass mixing chambers by a Watson-Marlow peristaltic pump and the dilution water was supplied using a capillary flow control system. Magnetic stirrers in the mixing chambers were used to ensure mixing before the test solutions passed into the fish exposure vessels. The dilution ratio of the stock solutions to dilution water was nominally 1:500 in all concentrations. The nominal levels of radioactivity in the exposure vessels ranged from 0.86 to 1.7 Bq ml/L.</p>
<p><u>Flow Rate</u> Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period</p>	<p>8 vol/24 hours. Not Reported on whether or not meter system was calibrated before study and checked twice daily during test period.</p>
<p><u>Biomass Loading Rate</u> Static: ≤ 0.8 g/L at $\leq 17^\circ\text{C}$, ≤ 0.5 g/L at $> 17^\circ\text{C}$; flow-through: ≤ 1 g/L/day</p>	<p>0.98 g/L on a static basis</p>
<p><u>Photoperiod</u> 16 hours light, 8 hours dark</p>	<p>16 hours light, 8 hours dark with a 20 minute transition periods.</p>
<p><u>Solvents</u> Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests</p>	<p>Solvent: None reported</p>

D. Test Design

Guideline Criteria	Reported Information
<p><u>Range Finding Test</u> If $LC_{50} > 100$ mg/L with 30 fish, then no definitive test is required.</p>	<p>Not reported.</p>

Guideline Criteria	Reported Information
<p><u>Nominal Concentrations of Definitive Test</u> Control & 5 treatment levels; dosage should be 60% of the next highest concentration; concentrations should be in a geometric series</p>	Dilution water control, 10, 18, 32, 56, 100, 180, and 320 µg ai/L.
<p><u>Number of Test Organisms</u> Minimum 10/level, may be divided among containers</p>	20/level
<p>Test organisms randomly or impartially assigned to test vessels?</p>	Yes.
<p>Biological observations made every 24 hours?</p>	Yes
<p><u>Water Parameter Measurements</u> 1. <u>Temperature</u> Measured constantly or, if water baths are used, every 6 hrs, may not vary > 1°C 2. <u>DO and pH</u> Measured at beginning of test and every 48 h in the high, medium, and low doses and in the control</p>	<p>Hourly (in the dilution control vessel) and daily temperatures (within each test vessel) were recorded.</p> <p>Measured daily in all test vessels.</p>
<p><u>Chemical Analysis</u> Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used.</p>	The mean measured concentrations of PHMB ranged from 94-100% of nominal values in the stock solutions and 94 -103% of nominal values in the exposure vessels.

12. REPORTED RESULTS

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
<u>Recovery of Chemical</u>	94 - 103%
<u>Control Mortality</u> Not more than 10% control organisms may die or show abnormal behavior.	0 %
Raw data included?	No
Signs of toxicity (if any) were described?	Yes

Mortality

Concentration (ppm)		Number of Fish	Cumulative Number Dead			
Nominal	Mean Measured		Hour of Study			
			24	48	72	96
Control		20	0	0	0	0
10	9.8	20	0	0	0	0
18	17	20	0	0 ¹	0 ²	0
32	33	20	0 ¹	17 ³	18 ²	18 ⁴
56	56	20	1 ²	17 ³	20	20
100	96	20	0 ⁵	17 ³	20	20
180	180	20	7 ⁶	20	20	20
320	330	20	20	20	20	20

¹ Between 11 - 30% of test population exhibiting sounding.

² More than 30% of test population are exhibition sounding and are dark discolored.

³ More than 30% of test population are exhibiting dark discolored, loss of balance, and/or spiralling.

⁴ More than 30% of test population exhibiting sounding.

⁵ More than 30% of test population exhibiting sounding, dark discolored, surfacing, and/or loss of balance.

⁶ More than 30% of test population exhibiting sounding, dark discolored, surfacing, loss of balance, cessation of swimming, and/or spiralling.

B. Statistical Results

Method: Moving average angle

96-hr LC₅₀: 26 µg/L ai

95% C.I.: 23-30 µg/L ai

Probit Slope:

NOEC: 17 µg/L ai

13. VERIFICATION OF STATISTICAL RESULTS

Parameter	Result
Binomial Test LC ₅₀ (C.I.)	25.45 (17 - 33) µg/L ai
Moving Average Angle LC ₅₀ (95% C.I.)	N/A
Probit LC ₅₀ (95% C.I.)	N/A
Probit Slope	N/A
NOEC	9.8 µg/L ai

14. REVIEWER'S COMMENTS: This study had some deviations from the EPA guidelines, concerning hardness of the water being high and make up of the dilution water. The dilution water should have been soft reconstituted water or water from a natural source, not dechlorinated tap water.

Regina Hirsch Vantocil P Acute Toxicity to Rainbow Trout

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
330	20	20	100	9.536742E-05
180	20	20	100	9.536742E-05
96	20	20	100	9.536742E-05
56	20	20	100	9.536742E-05
33	20	18	90	2.012253E-02
17	20	0	0	9.536742E-05
9.8	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 17 AND 33 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 25.45108

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.
