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

JUL 28 1994

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM:

SUBJECT: Freshwater Fish Flow-Through Toxicity Test for Vantocil P to the rainbow trout (MRID # 93191004/ 45698).

TO: Bruce Sidwell, Product Manager 53
Special Review and Reregistration Division

FROM: Anthony F. Maciorowski, Chief
Ecological Effects Branch
Environmental Fate and Effects Division (H7507C) 7/28/94

The EEB has completed a Data Evaluation Report (DER) for a freshwater fish flow-through toxicity test for Vantocil P 20% to the rainbow trout. Because the concentration of test material in each test chamber was not measured, the study was found to "Invalid" and does not meet the 72-1 (d) guideline requirement.

If you have any further questions, please contact Dick Felthousen (305-5829).

MRID No. 93191-004

DATA EVALUATION RECORD

1. **CHEMICAL:** Poly(iminoimidocarbonyliminoimidocarbonylimino-hexamethylene hydrochloride). Shaughnessey Number: 111801.

2. **TEST MATERIAL:** Vantocil P; 20% w/v aqueous solution of Poly(iminoimidocarbonyliminoimidocarbonyliminohexamethylene hydrochloride); CAS No. (32289-58-0) (A-2); a water-white liquid.

3. **STUDY TYPE:** Freshwater Fish Flow-Through Toxicity Test. Species Tested: Rainbow Trout (*Salmo gairdneri*).

4. **CITATION:** Brown, D. 1980. Determination of the Acute Toxicity of "Vantocil P" to Rainbow Trout (*Salmo gairdneri*). Brixham Study No. G 184/B. Report No. BL/B/2031. Study performed by Imperial Chemical Industries Limited, Brixham Laboratory, Freshwater Quarry, Brixham, Devon, U.K. Submitted by ICI Americas, Inc., Wilmington, Delaware. EPA MRID No. 45698 and EPA MRID No. 93191-004 for the Summary Report.

5. **REVIEWED BY:**

Rosemary Graham Mora, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: Rosemary Graham Mora
Date: 14 November 91

6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
Senior Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: P. Kosalwat
Date: 14 November 1991

LES TOUART
[redacted]
Supervisor, EEB/EFED
USEPA

Signature:
Date:

7. **CONCLUSIONS:** This study is not scientifically sound and does not meet the guideline requirements for a flow-through acute toxicity study using freshwater fish. The concentration of test material in each test chamber was not measured. Therefore, the actual concentrations to which the test organisms were exposed are unknown. The 96-hour LC_{50} of Vantocil P for *Salmo gairdneri* was [redacted] mg [redacted]/l nominal test concentration which classifies Vantocil P as [redacted] moderately

NOTE: This is a 72-1(d) TEP study. KBN reported the adjusted LC_{50} (i.e., 0.64 mg/l) based on a.i. basis. I have corrected this ERROR. The study is still "Invalid"
RWT 7/25/94

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toxic to *Salmo gairdneri*. [redacted]

8. **RECOMMENDATIONS:** The study should be repeated with test material concentrations measured in all test solutions.

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Juvenile rainbow trout (*Salmo gairdneri*) were obtained from Zeals Fisheries, Zeals, Wiltshire. The fish were held for approximately 2 months at ambient temperature before test initiation. During the 2½ days prior to test initiation, fish were acclimated to the test temperature (12 ±1°C). The fish were last treated with malachite green approximately 20 days prior to test initiation. The fish were reported to be in good conditions.

The fish had a mean weight of 2.17 g (range of 1.01-3.63 g) and a mean length of 55 mm (range of 44-67 mm).

B. **Test System:** The test chambers were spherical glass vessels (37 cm in diameter and height) with a nominal capacity of 20 l. The test system was a continuous flow-through system. Concentrated stock solutions (prepared in distilled water) and freshwater (dilution water) were delivered by a series of peristaltic pumps to glass splash heads where mixing occurred. The ratio of stock solution to dilution water was 1:100. The solutions were then delivered to the test vessels at a flow rate of 200 ml/minute to each test chamber.

The dilution water was freshwater supplied from a 20,000 gallon reservoir and the total hardness was measured daily.

C. **Dosage:** Ninety-six-hour flow-through acute test. Six nominal concentrations were chosen for this study (1.0, 3.2, 5.6, 7.5, 10.0, and 13.0 mg/l). In addition, a dilution water control was also used. Concentrations were not adjusted for the percentage of active ingredient.

D. **Design:** Twenty fish were randomly distributed to each test concentration and control (one vessel/treatment

B3

and control). The fish loading rate was 43.4 g/288 l/day or 0.15 g/l/day.

Mortality and symptoms of stress were noted every 24 hours during the study.

Dissolved oxygen concentration, pH, temperature, and hardness were measured daily.

The concentration of test material of each stock solution was determined daily. Vantocil P has a characteristic absorption in the ultra-violet spectrum. Absorption was determined using a spectrophotometer at 236 nm. "The analytical method available for "Vantocil P" in freshwater had a detection limit of approximately 5 mg/l; in view of this the nominal test exposure concentrations were used in the calculation of the LC_{50} values."

E. **Statistics:** The LC_{50} values were calculated from nominal concentrations using the probit method (Finney, 1971).

12. **REPORTED RESULTS:** In addition to the 1980 report, a summary was included (Adams, D.S. 1990. Phase 3 Summary of MRID 45698, "Vantocil P": Determination of the Acute Toxicity to Rainbow Trout *Salmo gairdneri*) which presented information from historical records which was not presented in the original report.

Measured concentrations of the test material in the stock solutions were 101, 321.3, 571, 749, 983, and 1156 mg/l (Tables 1 and 2, attached). The mean measurements represent 88.9-102% of nominal stock solution concentrations. "The dilution ratios were calculated daily for each test vessel from the ratio of the measured flow rate of the dilution water to that of the concentrated stock solution. In all cases this ratio was within 10% of the nominal 100:1 ratio."

No mortality was observed in the control or the lowest test concentration (1.0 mg/l nominal test concentration) (Table I, attached). Fifty-percent mortality was observed at the 3.2 mg/l nominal test concentration and total mortality was observed at the remaining test concentrations (5.6-13.0 mg/l nominal test concentrations). The 96-hour LC_{50} value and 95% confidence interval for rainbow trout exposed to Vantocil P were 3.2 mg/l and 2.8-3.6 mg/l nominal test concentrations, respectively. The NOEC was determined to be 1.0 mg/l nominal test concentration.

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During the study, the pH was 7.75-8.05, the temperature was 11.7-12.9°C, and the dissolved oxygen concentration was 10.2-11.0 mg/l. Total hardness of the dilution water was 67-73 mg/l as $CaCO_3$.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The authors made no conclusions in the report.

A Statement of GLP Compliance, signed by the study director and representatives of the sponsor company, was included in the summary indicating that the study was conducted prior to the effective date of 40 CFR Part 160. A Quality Assurance Statement was included in the report and was signed by a representative of the performing laboratory's Quality Assurance Unit.

14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**

A. **Test Procedure:** The test procedures were generally in accordance with the guidelines and the protocols recommended by the guidelines, except for the following deviations:

The concentration of test material in each test chamber was not measured; only the levels of test material in the stock solutions were measured and dilution ratio determined. For a flow-through system, the guidelines state that the concentration of test material in each test chamber must be determined.

Test fish were acclimated to test conditions for only two and a half day before testing; a minimum of 2 weeks is recommended.

Pre-test mortality was not reported; not greater than 3% mortality during the 48 hours prior to test initiation is required.

The age of the test organisms was not reported.

The report did not mention whether feeding of the test organisms was limited to just prior to testing.

The authors did not report how the test temperature was maintained (i.e. by a water bath or by air).

The actual test solution volume and depth were not reported. The summary stated the test vessels were

"spherical glass vessels 37 cm in height and diameter and of nominal capacity 20 litres."

The original source of the dilution water was not stated. It is not known whether the water had been analyzed for inorganic or organic pollutants before use.

The conductivity and alkalinity of the dilution water were not reported.

The recommended photoperiod for a freshwater fish acute toxicity study is 16-hour light/8-hour dark with 15- to 30-minute transitions. The report did not mention the use of transition periods.

The light intensity during the study was not measured.

The test temperature was not continuously monitored.

B. **Statistical Analysis:** EPA's Toxanal computer program was used to verify the LC_{50} value and 95% confidence interval presented by the author. The reviewer's LC_{50} value (3.2 mg/l nominal test concentration) and 95% confidence interval (1.0-5.6 mg/l) are similar to those of the author (printout, attached). The LC_{50} adjusted for the percentage active ingredient is 0.64 mg a.i./l.

C. **Discussion/Results:** Most of the deviations listed above probably did not affect the results of this test. However, the concentration of test material in each test chamber was not measured as required by the guidelines. Therefore, the actual concentrations to which the test organisms were exposed are unknown.

This study is not scientifically sound and does not meet the guideline requirements for a flow-through toxicity study using freshwater fish. The 96-hour LC_{50} of Vantocil P to *Salmo gairdneri* was 0.64 mg a.i./l nominal test concentration. Based on the results of this study, Vantocil P is highly toxic to *Salmo gairdneri*. The NOEC was 0.20 mg a.i./l nominal test concentration.

D. **Adequacy of the Study:**

(1) Classification: Invalid.

#16

(2) **Rationale:** The concentration of test material in each test chamber was not determined. Therefore, the actual concentrations to which the test organisms were exposed are unknown.

(3) **Repairability:** No.

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes, November 11, 1991.

47

Page _____ is not included in this copy.

Pages 8 through 10 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

X FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confi by product registrants. If you have any questions, please the individual who prepared the response to your request.

Rosemary Graham Mora Vantocil P Salmo gairdneri 9-26-91

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
13	20	20	100	9.536742E-05
10	20	20	100	9.536742E-05
7.5	20	20	100	9.536742E-05
5.6	20	20	100	9.536742E-05
3.2	20	10	50	58.80985
1	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 1 AND 5.6 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 3.2

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.

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Shaughnessey # 111801 Chemical Name Poly(iminoimidocarbonylimi) Chemical Class _____ Page 1 of 1

Study/Species/Lab/ MRID #	Chemical % a.i.	Results	Reviewer/ Date	Validation Status
48-Hour EC_{50}	_____	EC_{50} - _____ ppm (95% C.L. _____) Control Mortality (%) - _____ Solvent Control Mortality (%) - _____ Slope - _____ # Animals/Level - _____ Temperature - _____ 48-Hour Dose Level ppm /(% Effect) ( ), ( ), ( ), ( ), ( )	_____	_____
Species:				
Lab:				
MRID #		Comments:		

96-Hour LC_{50}	20%	LC_{50} - 0.64 ppm a.i.* (95% C.L. 0.2 – 1.12) Control Mortality (%) - 0 Solvent Control Mortality (%) - NA Slope - NA # Animals/Level - 20 Temperature - 11.7 - 12.9°C 96-Hour Dose Level ppm a.i.*/(% Mortality) 0.2 (0), 0.64 (50), 1.12 (100), 1.5 (100), 2.0 (100), 2.6 (100)	RGM 11/11/91	Invalid.
Species: Salmo gairdneri				
Lab: ICI Brixham Labs				
MRID # 93191-004		Comments: * Based on ~~nominal~~ RGM active ingredient of nominal concentrations. The concentration of test material in each test chamber was not measured. Therefore, the actual concentrations to which the fish were exposed are unknown.		

felthousen vantocil Acute Fish

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
13	20	20	100	9.536742E-05
10	20	20	100	9.536742E-05
7.5	20	20	100	9.536742E-05
5.6	20	20	100	9.536742E-05
3.2	20	10	50	58.80985
1	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 1 AND 5.6 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 3.2

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.

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felthousen vantocil Acute Fish

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
13	20	20	100	9.536742E-05
10	20	20	100	9.536742E-05
7.5	20	20	100	9.536742E-05
5.6	20	20	100	9.536742E-05
3.2	20	20	100	9.536742E-05
1	20	10	50	58.80985

THE BINOMIAL TEST SHOWS THAT 0 AND 3.2 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 1

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.

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felthousen vantocil Acute Fish

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
13	20	20	100	9.536742E-05
10	20	20	100	9.536742E-05