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

8/4/89

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review of an Acute Flow Through Toxicity Study of PCNB 2E to Rainbow Trout (Record No. 246142)

FROM: James Akerman, Chief for H. T. Craven 8/4/89
Ecological Effects Branch
Environmental Fate and Effects Division (H7507C)

TO: Susan Lewis (PM 21)
Registration Division (H7505C)

Attached is the DER for flow through acute toxicity of PCNB 2E to Rainbow Trout. The following is an additional comment from the reviewer.

It was noted that all measured concentrations at 0-hour and 96-hours exceeded the ASTM's guideline of a maximum 30% deviation from the nominal concentration. Also, when the high measured concentration was divided by the low concentration, four of the five values exceeded the 1.5 variability guideline set by ASTM. These problems draw attention to PCNB 2E's instability in solution. Nevertheless, taking into account the nature of this compound, consistancy of recorded values, utilization of mean measured values to determine the LC_{50}, and agreement with a similar coinciding bluegill bioassay, the DER as determined by KBN is confimed by Greg Susanke of this branch.

Accession No. 410608-02

DATA EVALUATION RECORD

1. **CHEMICAL:** PCNB 2E.
   Shaughnessey Number: Not available.

2. **TEST MATERIAL:** PCNB 2E; Lot No. 832001; 24% Active Ingredient; an amber liquid.

3. **STUDY TYPE:** Freshwater Fish Flow-through Acute Toxicity Test. Species Tested: Salmo gairdneri.

4. **CITATION:** Bowman, J.H. 1989. Acute Flow-through Toxicity of PCNB 2E to Rainbow Trout (Salmo gairdneri). Prepared by Analytical Bio-Chemistry Laboratories, Inc., Columbia, Missouri. Report No. 36938. Submitted by AMVAC Chemical Corporation, Los Angeles, California. Accession No. 410608-02.

5. **REVIEWED BY:**

   Kimberly Rhodes
   Associate Scientist
   KBN Engineering and
   Applied Sciences, Inc.

   Signature: Kimberly Rhodes
   Date: July 12, 1989

6. **APPROVED BY:**

   Prapimpan Kosalwat, Ph.D.
   Staff Toxicologist
   KBN Engineering and
   Applied Sciences, Inc.

   Signature: P. Kosalwat
   Date: July 12, 1989

   EEB Reviewer
   Henry T. Craven
   Supervisor, EEB/HED
   USEPA

   Signature: Henry Craven
   Date: 8/4/89

7. **CONCLUSIONS:** This study appears scientifically sound and fulfills the Guideline requirements for an acute 96-hour flow-through toxicity test for a coldwater fish species. The 96-hour LC50, based upon mean measured concentrations, of PCNB to rainbow trout (Salmo gairdneri) was 0.31 mg/L. Therefore, PCNB is classified as highly toxic to rainbow trout. The NOEC was determined to be 0.047 mg/L after 96 hours.

8. **RECOMMENDATIONS:** N/A

Formulated Product

2

9. BACKGROUND:

10. DISCUSSION OF INDIVIDUAL TESTS: N/A

11. MATERIALS AND METHODS:

A. Test Animals: Rainbow trout (Salmo gairdneri) were obtained from a commercial supplier in California. The fish were reared and maintained at the testing facility in well water and were fed newly hatched brine shrimp or a commercially available fish food daily. Forty-eight hours before the initiation of the test, rainbow trout were removed from the culture tank and placed in the temperature acclimation unit. During this time, the fish were held without food. The rainbow trout used as the control group during this study had a mean weight of 1.2 (± 0.19) grams and a mean length of 43 (± 2.5) millimeters at test termination. The biomass loading rate was 0.62 g/L. The laboratory environment was maintained on a 16-hour daylight photoperiod.

B. Test System: A proportional diluter system described by Mount and Brungs, utilizing a Hamilton Micro Lab 420 syringe dispenser, was used for the intermittent introduction of PCNB 2E test solutions and diluent water into each test chamber. The proportional diluter system used for the project was set to provide test levels approximately 50 percent dilutions of each other. The test aquaria were glass chambers filled to a depth of approximately 22 cm, with a width of 30.5 cm and a length of 50.2 cm. The diluter delivered one liter of test solution or control water to the test vessels at an average rate of 6.7 times per hour over the course of the study. This flow rate was sufficient to replace the 30-liter volume within the test chambers 5.4 times per day. Five concentrations of the test material with dilution water control were tested. The test chambers were immersed in a temperature controlled water bath held at 12 ± 1°C.

Dilution water for the rainbow trout test was a blend of reverse osmosis water and ABC well water characterized as having a pH of 7.1 - 7.9, total hardness of 40 - 48 mg/L as $CaCO_3$, total alkalinity of 44 - 56 mg/L $CaCO_3$ and specific conductance of 100 - 160 umhos/cm.

C. Dosage: 96-hour flow-through acute test.

D. Design: Based on the results of the preliminary testing, a control and five nominal PCNB 2E concentrations of 0.12, 0.24, 0.48, 0.95 and 1.9 mg/L were selected for definitive testing. Twenty rainbow trout were tested per concentration. All concentrations were observed once every 24 hours for mortality and abnormal effects. The water quality parameters (temperature, dissolved oxygen and pH) were measured in the control, low, middle and highest concentration containing surviving fish at 0, 48 and 96 hours of testing. Analytical samples were collected from each test level and the diluter stock at 0 and 96 hours of the exposure.

E. Statistics: The concentrations of toxicant lethal to 50% of the population (LC50's) and 95% confidence intervals were determined at 24-, 48-, 72-, and 96-hour exposure periods by the computer program developed by Stephan et al. (1978).

12. REPORTED RESULTS: The mean measured concentrations of PCNB 2E were 0.047, 0.099, 0.24, 0.44 and 0.76 mg/L. The mean measured concentrations ranged from 39% to 50% of the nominal concentrations. A white precipitate was noted in the diluter mixing cell at 72 and 96 hours suggesting that not all of the PCNB 2E was going into solution.

Mortality and behavioral observations during the acute toxicity test of PCNB 2E to rainbow trout are shown in Table 6 (attached). The 24-, 48-, 72-, and 96-hour LC50 values for PCNB 2E were >0.76, >0.76, 0.50 and 0.31 mg/L, respectively, based upon mean measured concentrations. The slope of the 96-hour dose-response line was 7.9 as calculated by least squares regression analysis. Mortality occurred in the 0.24, 0.44 and 0.76 mg/L test concentrations. Behavioral/sublethal effects noted during the study included loss of equilibrium, fish on bottom of test chamber, surfacing and quiescence. Given these behavioral/sublethal effects at the test concentrations of 0.099, 0.24, 0.44 and 0.76 mg/L, a no-effect concentration of PCNB 2E toxicity to rainbow trout was determined to be 0.047 mg/L. This conclusion is supported by the lack of mortality or behavior/sublethal effects at the test concentration of 0.047 mg/L.

Water chemistry parameters measured at 0, 48 and 96 hours were within the specified limits for conducting aquatic toxicity tests. Temperature ranged from 12 to 13°C. Dissolved oxygen ranged from 8.0 to 8.4 mg/L, these values

represented 78 and 83% saturation at 12 and 13°C, respectively. The pH ranged from 7.6 to 7.8.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCEMEASURES: The 96-hour LC50 value for PCNB 2E was 0.31 mg/L with 95 percent confidence limits of 0.24 and 0.44 mg/L mean measured concentration. The NOEC (No-Observed-Effect Concentration) was 0.047 mg/L after 96 hours.

Quality Assurance and Good Laboratory Practice Regulation Statements were included in the report, indicating that the study was conducted in accordance with the FIFRA Good Laboratory Practice Standards set forth in 40 CFR Part 160.

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

A. Test Procedure: The test procedures were generally in accordance with protocols recommended by the Guidelines, but deviated from the SEP as follows:

o The SEP recommends that fish be acclimated to study conditions for at least two weeks prior to testing. The rainbow trout were removed from the culture tank and placed in the temperature acclimation unit 48-hours before test initiation.

o Six-hour temperature measurements were not recorded as required by the SEP for tests conducted in a water bath.

o The SEP states that each designated treatment group should be exposed to a concentration of toxicant that is at least 60% of the next highest concentration. Each designated treatment group for the test was only 50% of the next highest concentration.

o The SEP recommends a 16-hour light and an 8-hour dark photoperiod with a 15- to 30-minute transition period between light and dark. The report did not state whether a 15- to 30-minute transition period between light and dark was maintained.

B. Statistical Analysis: The reviewer used EPA's Toxanal computer program to calculate the LC50 values. These calculations are attached. The binomial test provides a 96-hour LC50 value of 0.31 mg/L with a 95 percent confidence interval of 0.24 to 0.44 mg/L which is the same as that reported by the author.

C. Discussion/Results: The study results appear to be scientifically valid. The 96-hour LC50 value, based upon mean measured PCNB 2E concentrations, was estimated to be 0.31 mg/L. Therefore, PCNB 2E is classified as highly toxic to rainbow trout (Salmo gairdneri). The NOEC was determined to be 0.047 mg/L after 96 hours.

D. Adequacy of the Study:

(1) Classification: Core.

(2) Rationale: N/A

(3) Repairability: N/A

15. COMPLETION OF ONE-LINER FOR STUDY: Yes, 07-06-89.

PCNB science review

Page 7 is not included in this copy.

Pages _____ through _____ are not included in this copy.

The material not included contains the following type of information:

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___ Description of the product manufacturing process

___ Description of product quality control procedures

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KIMBERLY RHODES PCNB 2E SALMO GAIRDNERI 070689

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
.76	20	20	100	9.536742E-05
.44	20	20	100	9.536742E-05
.24	20	1	5	2.002716E-03
.099	20	0	0	9.536742E-05
.047	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT .24 AND .44 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 .3122765

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 No. Not available Chemical Name PCNB 2E Chemical Class ____ Page ____ of ____

Study/Species/Lab/ Accession	Chemical % a.i.	Results	Reviewer/ Date	Valid/ Sta
14-Day Single Dose Oral LD_{50}		LD50 = . mg/kg (95% C.L.) Contr. Mort.(%)=		
Species	____	Slope= # Animals/Level= Age(Days)= Sex =	____	____
Lab		14-Day Dose Level mg/kg/(% Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		
14-Day Single Dose Oral LD_{50}		LD50 = mg/kg. (95% C.L) Contr. Mort.(%)=		
Species	____	Slope= # Animals/Level= Age(Days)= Sex =	____	____
Lab		14-Day Dose Level mg/kg/(% Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		
8-Day Dietary LC_{50}		LC50 = ppm (95% C.L.) Contr. Mort.(%)=		
Species		Slope= # Animals/Level= Age(Days)= Sex =		
Lab	____	8-Day Dose Level ppm/(%Mortality) ( ), ( ), ( ), ( ), ( )	____	____
Acc.		Comments:		
8-Day Dietary LC_{50}		LC50 = ppm (95% C.L) Contr. Mort.(%)=		
Species		Slope= # Animals/Level= Age(Days)= Sex =		
Lab	____	8-Day Dose Level ppm/(%Mortality) ( ), ( ), ( ), ( ), ( )	____	____
Acc.		Comments:		
48-Hour LC_{50}		LC50 = pp__ (95% C.L.) Contr. Mort.(%)= Sol. Contr. Mort.(%)=		
Species		Slope= # Animals/Level= Temperature =		
Lab	____	48-Hour Dose Level pp /(%Mortality) ( ), ( ), ( ), ( ), ( )	____	____
Acc.		Comments:		
96-Hour LC_{50}		LC50 = 0.51 (0.24) ppm (95% C.L. (0.24 - 0.44)) Con. Mor.(%)= 0 Sol. Con. Mor.(%)= N/A	K.R. 07/04/89	Core
Species Salmo gairdneri		Slope= N/A # Animals/Level= 20 Temp.= 12 ± 1°C		
Lab Analytical Bio-Chemistry Laboratories, Inc. 24%		96-Hour Dose Level ppm/(%Mortality) 0.047 (0), 0.099 (0), 0.24 (5), 0.44 (100), 0.76 (100)		
Acc. 410608-02		Comments: Based on mean measured concentrations.		
96-Hour LC_{50}		LC50 = pp__ (95% C.L) Con. Mort.(%)= Sol. Con. Mort.(%)=		
Species		Slope= # Animals/Level= Temp.=		
Lab	____	96-Hour Dose Level pp /(%Mortality) ( ), ( ), ( ), ( ), ( )	____	____
Acc.		Comments:		