



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

(2) 7/26/89

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

JUL 26 1989

MEMORANDUM

SUBJECT: Review of an Acute Flow Through Toxicity Study
(71-1) of PCNB to Rainbow Trout (Record No. 240057)

FROM: For James Akerman, Chief *Raymond W. Matheny*
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 to Rainbow Trout. The study was initially reviewed by EEB's contractor, KBN, and confirmed by Brian Montague of this branch. With an LC-50 value of 0.32 mg/L, PCNB is regarded as highly toxic to Rainbow Trout. This study does fulfill EPA guidelines for a coldwater acute 96 hr. flow through toxicity test on a freshwater fish.

240057
RECORD NO.

056502
SHAUGHNESSY NO.

REVIEW NO.

EEB REVIEW

DATE: IN 4/3/89 OUT: 7/26/89

FILE OR REG. NO. 5481-197

PETITION OR EXP. NO. _____

DATE OF SUBMISSION 2/8/89

DATE RECEIVED BY HED 3/31/89

RD REQUESTED COMPLETION DATE 7/21/89

EEB ESTIMATED COMPLETION DATE _____

RD ACTION CODE/TYPE OF REVIEW 655

TYPE PRODUCT(S): I, D, H, F, N, R, S Fungicide

DATE ACCESSION NO (S). 409927-01

PRODUCT MANAGER NO. S. Lewis (21)

PRODUCT NAME (S) PCNB

COMPANY NAME Amvac Chemical Corporation

SUBMISSION PURPOSE Submission of Acute Flow Through Rainbow Trout Study
for Review.

SHAUGHNESSEY NO.	CHEMICAL AND FORMULATION	% A.I.
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____

DATA EVALUATION RECORD

1. **CHEMICAL:** PCNB
Shaughnessey Number: Not available
2. **TEST MATERIAL:** PCNB; Lot No. GAB 39011; 99% Active
Ingredient; a fine pale-yellow crystal solid
3. **STUDY TYPE:** Flow-through Acute Toxicity Test for Freshwater
Fish. Species Tested: Salmo gairdneri
4. **CITATION:** Swigert, J. P. 1988. Acute Flow-through Toxicity
of PCNB to Rainbow Trout (Salmo gairdneri); Report No.
36829. Prepared by Analytical Bio-Chemistry Laboratories,
Inc., Columbia, Missouri. Submitted by AMVAC Chemical
Company, Los Angeles, California. Accession No. 409927-01.
5. **REVIEWED BY:**

Kimberly Rhodes Associate Scientist KBN Engineering and Applied Sciences, Inc.	Signature: <i>Kimberly Rhodes</i> Date: <i>May 9, 1989</i>
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6. **APPROVED BY:**

Prapimpan Kosalwat, Ph.D. Staff Toxicologist KBN Engineering and Applied Sciences, Inc.	Signature: <i>P. Kosalwat</i> Date: <i>May 9, 1989</i>
Brian Montague Henry T. Craven Supervisor, EEB/HED USEPA	Signature: <i>B. Montague</i> <i>7/26/89</i> Signature: <i>Henry T. Craven</i> Date: <i>7/26/89</i>
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 0.32 mg/L.
Therefore, PCNB is classified as highly toxic to rainbow
trout. The NOEC was determined to be 0.056 mg/L after 96
hours.
8. **RECOMMENDATIONS:** N/A

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 ABC Laboratories in ABC well water and were fed newly hatched brine shrimp or a commercially available fish food daily. Seventy-two hours before the initiation of the test, rainbow trout were removed from the culture 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 0.91 (\pm 0.21) grams and a mean length of 40 (\pm 3.2) millimeters at test termination. The biomass loading rate was 0.6 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 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. Test vessels were constructed of plate glass (52 x 26.5 x 30 cm). An adjustable standpipe regulated the water depth at 19.5 cm, which provided a volume of 30 L in the exposure vessels.

The diluter delivered one liter of test solution or control water to the test vessels at an average rate of 5.8 times per hour over the course of the study. This flow rate was sufficient to replace the 30-liter volume within the test chambers 4.6 times per day. Five concentrations of the test material with dilution water and solvent controls were tested. The test chambers were immersed in a temperature controlled water bath held at $12 \pm 1^{\circ}\text{C}$. The lighting was maintained on a 16-hour daylight photoperiod.

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, five concentrations were selected for definitive testing. Twenty rainbow trout were tested per concentration. A control, solvent control, and nominal PCNB concentrations of 0.044, 0.088, 0.18, 0.35, and 0.70 mg/L were tested. The concentration of acetone in the solvent control (0.1 mL of acetone in 1 liter of water) was approximately equivalent to that received by the highest test 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 each concentration and controls 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 concentration of toxicant lethal to 50% of the population (LC50's) and 95% confidence intervals was 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 were 0.035, 0.056, 0.13, 0.26 and 0.45 mg/L. The mean measured concentrations ranged from 64% to 80% of the nominal concentrations. A white precipitate was noted in the diluter mixing cell suggesting that not all of the PCNB was going into solution.

The 24-, 48-, 72-, and 96-hour LC50 values for PCNB were >0.45, >0.45, 0.35 and 0.33 mg/L, respectively, based upon mean measured concentrations. The slope of the 96-hour dose-response line was 5.1 as calculated by least squares regression analysis. Mortality occurred in the 0.13, 0.26 and 0.45 mg/L test concentrations. Behavioral/sublethal effects noted during the study included loss of equilibrium, gulping air, fish on bottom of test chamber, surfacing, and quiescence. Given these behavioral/sublethal effects at the test concentrations of 0.13, 0.26, and 0.45 mg/L, a no-effect concentration of PCNB toxicity to rainbow trout was determined to be 0.056 mg/L. This conclusion is supported by the lack of mortality or behavior/sublethal effects at the test concentrations of 0.035 and 0.056 mg/L.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

The 96-hour LC50 value for PCNB was 0.33 mg/L with 95 percent confidence limits of 0.26 and 0.45 mg/L mean measured concentration. The NOEC (No-Observed-Effect Concentration) was 0.056 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 seventy-two 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 moving average method provides a 96-hour LC50 value of 0.32 mg/L with a 95 percent confidence interval of 0.27 to 0.37 mg/L which is similar to 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 concentrations, was estimated to be 0.32 mg/L. Therefore, PCNB is classified as highly toxic to rainbow trout (Salmo gairdneri).

D. Adequacy of the Study:

(1) Classification: Core

(2) Rationale: N/A

(3) Repairability: N/A

15. COMPLETION OF ONE-LINER FOR STUDY: Yes, 05-04-89.

PCNB science review

Page 8 is not included in this copy.

Pages _____ through _____ 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 product 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
 - FIFRA registration data
 - The document is a duplicate of page(s) _____
 - The document is not responsive to the request
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The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

KIMBERLY RHODES PCNB SALMO GAIRDNERI 05-04-89

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
.45	20	20	100	9.536742E-05
.26	20	1	5	2.002716E-03
.13	20	1	5	2.002716E-03
.056	20	0	0	9.536742E-05
.035	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT .26 AND .45 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 .3299458

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	* LC50	*95 PERCENT CONFIDENCE LIMITS
2	6.572952E-02	.3152591	.2741472 .3744006

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
14	8.057003	14.24164	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 7.900201
95 PERCENT CONFIDENCE LIMITS = -14.52441 AND 30.32481

LC50 = .3120361
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = .2155011
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

Shaughnessy No. <u>Not available</u>	Chemical Name <u>PCNB</u>	Chemical Class _____	Page _____ of _____	Reviewer/Date _____	Validat: Statu: _____
Study/Species/Lab/ Accession _____	Chemical & a.i. _____	Results			
14-Day Single Dose Oral LD50	LD50 = mg/kg ($\frac{95\% \text{ C.L.}}{\quad}$)	Contr. Mort. (X) = _____			
Species _____	Slope = _____	# Animals/Level = _____	Age (Days) = _____	Sex = _____	
Lab _____	$\frac{14\text{-Day Dose Level mg/kg} / (X \text{ Mortality})}{(\quad), (\quad), (\quad), (\quad), (\quad)}$				
Acc. _____	Comments: _____				
14-Day Single Dose Oral LD50	LD50 = mg/kg. ($\frac{95\% \text{ C.L.}}{\quad}$)	Contr. Mort. (X) = _____			
Species _____	Slope = _____	# Animals/Level = _____	Age (Days) = _____	Sex = _____	
Lab _____	$\frac{14\text{-Day Dose Level mg/kg} / (X \text{ Mortality})}{(\quad), (\quad), (\quad), (\quad), (\quad)}$				
Acc. _____	Comments: _____				
8-Day Dietary LC50	LC50 = ppm ($\frac{95\% \text{ C.L.}}{\quad}$)	Contr. Mort. (X) = _____			
Species _____	Slope = _____	# Animals/Level = _____	Age (Days) = _____	Sex = _____	
Lab _____	$\frac{8\text{-Day Dose Level ppm} / (X \text{ Mortality})}{(\quad), (\quad), (\quad), (\quad), (\quad)}$				
Acc. _____	Comments: _____				
8-Day Dietary LC50	LC50 = ppm ($\frac{95\% \text{ C.L.}}{\quad}$)	Contr. Mort. (X) = _____			
Species _____	Slope = _____	# Animals/Level = _____	Age (Days) = _____	Sex = _____	
Lab _____	$\frac{8\text{-Day Dose Level ppm} / (X \text{ Mortality})}{(\quad), (\quad), (\quad), (\quad), (\quad)}$				
Acc. _____	Comments: _____				
48-Hour LC50	LC50 = PP ($\frac{95\% \text{ C.L.}}{\quad}$)	Contr. Mort. (X) = _____	Sol. Contr. Mort. (X) = _____	Temperature = _____	
Species _____	Slope = _____	# Animals/Level = _____			
Lab _____	$\frac{48\text{-Hour Dose Level pp} / (X \text{ Mortality})}{(\quad), (\quad), (\quad), (\quad), (\quad)}$				
Acc. _____	Comments: _____				
96-Hour LC50	LC50 = 0.32 ppm ($\frac{95\% \text{ C.L.}}{0.27 - 0.37}$)	* Moving average method			
Species <u>Salmo gairdneri</u>	Slope = N/A	# Animals/Level = 20	Con. Mort. (X) = 0	Sol. Con. Mort. (X) = 0	Temp. = 12 ± 1°C
Lab <u>Analytical Bio-Chemistry Laboratories, Inc.</u>	$\frac{96\text{-Hour Dose Level ppm} / (X \text{ Mortality})}{0.035(0), 0.056(0), 0.13(5), 0.26(5), 0.45(100)}$				
Acc. <u>409927-01</u>	Comments: <u>Based on mean measured concentrations</u>				
96-Hour LC50	LC50 = PP ($\frac{95\% \text{ C.L.}}{\quad}$)	Con. Mort. (X) = _____	Sol. Con. Mort. (X) = _____	Temp. = _____	
Species _____	Slope = _____	# Animals/Level = _____			
Lab _____	$\frac{96\text{-Hour Dose Level pp} / (X \text{ Mortality})}{(\quad), (\quad), (\quad), (\quad), (\quad)}$				
Acc. _____	Comments: _____				