

9-13-90

DATA EVALUATION REPORT  
ECOLOGICAL EFFECTS BRANCH

1. Chemical: Bromacil
2. Test Material: Bromacil technical, Lot No. T80717-21 - 96.6% ai
3. Study Type: Static Acute LC<sub>50</sub> Test Using rainbow trout,  
Onchoryhnchus mykiss

4. Study Identification:

Study Author: Wetzel, James  
Study Laboratory: Haskell Laboratory for Toxicology and  
Industrial Medicine, EI duPont, Newark, DE.  
Study Dates: April 21-28, 1986  
Study Identification: Report No. 265-86  
Sponsor: E. I. duPont de Nemours and Co.  
EPA Identification: MRID 409515-03

5. Reviewed By: Brian Montague, Fisheries Biologist *Brian Montague*  
Ecological Effects Branch  
Environmental Fate & Effects Division (H7507C) 9/12/90

6. Approved By: Ray Matheny, Supervisory Biologist *Ray Matheny* 9/13/90  
Ecological Effects Branch  
Environmental Fate & Effects Division (H7507C)

7. Conclusions: Though the study appears to have demonstrated an LC<sub>50</sub> level of 36 mg/L (CL 30-40 mg/L), the curious rise in dissolved oxygen within a static nonaerated system at the end of the test needs explanation before full acceptance of the study is made.

Study is upgraded and now acceptable

→ See attached memo and laboratory's response

8. Recommendations: Contact and obtain the needed explanation from the laboratory.

9. **Submission Purpose:** Submitted to fulfill registration guideline requirements.

10. **Test Methods and Protocols:** No specific protocol guideline is stated, however, the quality assurance statement indicates the study has followed 40 CFR 160 good laboratory practices.

**Test Organisms:** Juvenile Rainbow Trout were obtained from The Trout Lodge in McMillen, Washington. A 20 day acclimation period followed and food was withheld 48 hours prior to the test initiation. Total mean length for the trout ranged from 2.8 to 3.8 cm and total wet weight ranged from 0.339 + 0.693 gm.

**Test Dilution and Solution Water:** Filtered laboratory well water was employed as dilution water for controls and preparation of test solutions. Initial measurement of the control water yield total alkalinity of 47 mg/L  $\text{CaCO}_3$ , hardness of 108 mg/L of  $\text{CaCO}_3$ , a conductivity of 210 micro ohms/cm, pH of 7.5 and a dissolved oxygen level of 10.3 mg/L. Test concentration groups were prepared from a stock solution of 0.3 mg/L of Bromacil. Concentrations tested were 225, 169, 127, 95, 71, 53, 40, 30, 22.5, and 16/9 mg/L.

**Test Materials and Procedures:** Test solutions were added to 18 liter glass aquarium to a volume level of 15 liters (18.5 cm depth). Ten fish were added to each of the 11 aquariums. Dissolved oxygen and pH were measured in the 225, 71, 16.9, and control level test vessels every 24 hours if vessels still contained live fish. Alkalinity, hardness, and conductivity were measured at 0 hour in the control vessel only. Temperature was maintained between 11.0 and 13.0°C by an unnamed procedure (possibly a waterbath). The photoperiod was 16D/8N. Mortality and general observations were recorded every 24 hours for all test vessels.

11. **Reported Test Results:** Within 24 hours all fish at the 225 ppm level had expired. At 48 hours all fish at test levels above 95 ppm had expired. At 72 hours mortality was 80% at 71 ppm, 60% at 53 ppm, 30% at 40 ppm, and 10% at 22.5 ppm. By test termination 90% mortality had occurred at 71 ppm, 70% at 40 ppm, 30% at 30 ppm and 20% at 22.5 ppm. No mortality occurred at the 16.9 ppm level or in the controls. Some fish surviving at 22.5 ppm and above displayed loss of equilibrium, swollen abdomens, erratic swimming patterns, darkening of coloration, and sinking to the bottom of the tank. No effects are mentioned for the 16.9 ppm level.

Water parameters measured in three tanks at test-termination remained acceptable with dissolved oxygen at between 8.7 and 9.2 and pH of 7.1-7.3.

12. **Study Author's Conclusion:** "The 96-hour  $LC_{50}$  was 36 mg/L (Scale-of-dose transformed to log 10), with a 95% confidence interval of 40 to 42 mg/L.
13. **Reviewer's Discussion:** The test appears to have followed proper guideline procedures. However, a curious discrepancy needs to be explained by the laboratory. Despite the fact that no aeration was reportedly used, the dissolved oxygen levels actually rose in all 3 test vessels measured between 72 and 96 hour test periods.

In addition, the laboratory apparently did not continuously monitor temperature in one test vessel and the method of temperature control is not mentioned. Though it would have been preferable to have had measured concentration at the 0 and 96 hour points, the long halflife of the chemical in water may preclude the necessity of this. Measurement of parameters in more test vessels would have been preferred.

**Adequacy of Study**

**Classification:** ~~Supplemental~~ Core *BJM Montague*

**Rationale:** Dissolved oxygen would not be expected to increase with a static non-aerated system - an explanation is required.

**Repairability:** Repairable if explanation is acceptable.

Montague Bromacil acute toxicity 96hr rainbow trout

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
225	10	10	100	9.765625E+02
169	10	10	100	9.765625E-02
127	10	10	100	9.765625E-02
95	10	10	100	9.765625E-02
71	10	9	90	1.074219
53	10	8	80	5.46875
40	10	7	70	17.1875
30	10	3	30	17.1875
22.5	10	2	20	5.46875
16.9	10	0	0	9.765625E-02

THE BINOMIAL TEST SHOWS THAT 16.9 AND 71 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 34.64102

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
6	.1112208	36.37717	30.25434	42.81776

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H
5	.1379322	1

GOODNESS OF FIT PROBABILITY  
.9879068

SLOPE = 5.374853  
95 PERCENT CONFIDENCE LIMITS = 3.378675 AND 7.371032

LC50 = 35.68925  
95 PERCENT CONFIDENCE LIMITS = 29.76057 AND 42.40152

LC10 = 20.71343  
95 PERCENT CONFIDENCE LIMITS = 13.95015 AND 25.60121

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

MAR 30 1993

OFFICE OF  
PREVENTION, PESTICIDES AND  
TOXIC SUBSTANCES

MEMORANDUM

Subject: Review of Supplemental Data to Upgrade Bromacil  
72-1 Trout Study

From: Anthony F. Maciorowski, Chief  
Ecological Effects Branch  
Environmental Fate and Effects Division (H7507C)

To: Linda Propst, Product Manager 73  
Reregistration Division (H7508W)

The Ecological Effects Branch (EEB) has reviewed explanations provided by Haskell Laboratories in Newark, Delaware to allow upgrade of the Bromacil technical Acute toxicity study using rainbow trout (MRID 409515-03). The study was previously found supplemental and upgradeable to fully acceptable if the study director could explain the rise in  $O_2$  levels reported for the nonaerated static exposure test. The study director has explained the rise experienced in the final 24 hours as due to "a lack of calibration of the dissolved oxygen meter immediately prior to use at 72 and 96 hours." The slight increase (0.3 mg/L) was just above the accuracy of the instrument (a YSI model 54 D.O. meter).

The Branch agrees that the final results were unlikely to have been affected. The concern was that aeration had been employed in a study where measured concentration analysis was not performed, therefore possibly affecting the degradation of the test material. The laboratory's explanation indicates that a  $O_2$  rise did not occur and that the discrepancy was due to instrument error. The Branch will accept this explanation and upgrade the study to Core. The laboratory's response will be attached to the original report.



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