fish embryo-larvae study

SHAUGHNESSEY NO. 109901

REVIEW NO. 34

EEB BRANCH REVIEW

DATE: IN 9-22-83 OUT 12-29-83

FILE OR REG. NO. 3125-340

PETITION OR EXP. PERMIT NO.

DATE OF SUBMISSION 9-9-83

DATE RECEIVED BY HED 9-21-83

RD REQUESTED COMPLETION DATE 1-11-84

EEB ESTIMATED COMPLETION DATE 1-4-84

RD ACTION CODE/TYPE OF REVIEW 400/Data

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

DATA ACCESSION NO(S).

PRODUCT MANAGER NO. H. Jacoby (21)

PRODUCT NAME(S) Bayleton 50WP

COMPANY NAME Mobay Chemical Corporation

SUBMISSION PURPOSE Submission of fish embryo-larvae study in support of registration

SHAUGHNESSEY NO.	CHEMICAL, & FORMULATION	% A.I.
109901	Triadimefon	93%

BAYLETON

100 Submission Purpose

Submission of a rainbow trout early life stage study in support of registration.

Background

Because of the persistence of Bayleton and its degradate, EEB reviewers have requested Daphnia life-cycle studies and avian reproduction studies to support a variety of different use patterns. In addition to these above mentioned chronic studies, EEB has also requested a rainbow trout early life stage study to support the conditional registration of BAYLETON on pine seedlings (see EEB review, 12-22-80).

101 Toxicological Properties

101.1 Rainbow Trout Early Life Stage

This study (Acc. #251243) is scientifically sound and shows that the growth of rainbow trout early life stages is reduced at Triadimefon levels as low as 116 ppb. The MATC (based on growth) was calculated by Mobay to be 68.6 ppb (>41<116 ppb) and the 60-day LC_{50} was calculated to be 717 ppb.

102 Conclusions

102.1 Data Requests

The submitted study does not fulfill the requirements for a rainbow trout early life stage study at this time because the information provided is insufficient to allow full evaluation of study results. The presentation of the data is not adequate for statistical analysis because the data are not broken down by replicate incubation chambers, in fact, it is unclear whether true replicates were used in this study or simply duplicate incubation chambers submerged in the same tank. Needed are the complete mortality tables broken down by individual replicates (or incubation chambers) and showing mortality on a daily basis. Also needed are the lengths and weights of the 60-day survivors also broken down by replicate, if possible.

Rather high mortality was noted in the controls and in the lower exposure levels. Since most of the mortality apparently occurred toward the end of the study rather than in the more sensitive embryo and alevin stages, this mortality is probably not due to general environmental conditions (analysis of the test water showed high chlorine levels.) In order to determine possible causes for this high mortality and to ascertain the

relative importance of this mortality in the evaluation of study results, the complete mortality tables showing mortality on a daily basis are essential.

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DATA EVALUATION RECORD

1. Chemical: Triadimefon

2. Formulation: 93%, Technical

3. Citation: Carlisle, J.C. (1983). Toxicity of Triadimefon (Bayleton) to Rainbow Trout Early Life Stages. Study Number 83-666-02. Mobay Chemical Corporation Environmental Health Research Institute. Acc. #251243.

4. Reviewed by: Carol M. Natella
Wildlife Biologist
EEB/HED

5. Date Reviewed: December 15, 1983

6. Test Type Rainbow trout early life stage

7. Reported Results: MATC (based on growth) was calculated to be 68.6 ppb (>41<116 ppb). The 60-day LC50 was calculated to be 717 ppb.

8. Reviewer's Conclusions This study is scientifically sound and shows that the growth of rainbow trout early life stages is reduced at Triadimefon levels as low as 116 ppb. The study does not fulfill the requirements for a rainbow trout early life stage study at this time because certain necessary information was omitted.

MATERIALS/METHODS

Test Procedures

Test animals: Rainbow trout (Salmo gairdneri) eggs, incubated to the eyed stage, were supplied by Mount Lassen Trout Farms, Red Bluff, California. They were shipped on ice by air freight then acclimated to the test temperature over a five-day period.

Test water quality: Tap water dechlorinated by sodium sulfite and activated carbon, was used at a flow rate of 60 liters per day per test vessel. Hardness (as $CaCO_3$) ranged from 63 to 107 mg/l and alkalinity (as $CaCO_3$) ranged from 38 to 75 mg/l over the 61 day test period. Chlorine levels ranged from 18-81 ug/l (mean 38) over the same period. Temperature was maintained at 12.2 $\pm$ 2.3°C.

Test Containers: Each of six test vessels consisted of a 5-gallon, linear polyethylene bucket with nylon-screened overflow holes at the 15-liter level. Within each test vessel were four incubation chambers, each consisting of a 6-inch long x 3-inch diameter section of polyvinyl chloride pipe with a nylon screen which supported the eggs three inches from the bottom, and several 3/8-inch screened holes within one inch of the top. Four 3/4-inch cpvc tubes supported a plexiglass plate which, in turn, supported the chambers. A submersible pump in each vessel drew the test solution down through the tubes then up through the screened incubation chambers. An airstone was added to each vessel halfway through the study to provide supplemental oxygenation.

A solenoid valve diluter system was used to provide nominal concentration of 0, 10, 30, 90, 270 and 810 ppb of the test substance. The diluter cycle time was 24 minutes with complete replacement every 6 hours. The concentrations were verified by chemical analysis at intervals throughout the test. Mean analytical concentrations at each level were as follows: 23, 41, 116, 300, 890 ppb. For each concentration 100 eggs were used, divided between four test chambers. Exposure period was 60 days.

Date of testing: April 9, 1983 - June 8, 1983

Statistical Analysis

The cumulative embryonic, larval and total mortality as well as the length and weight data in each concentration were compared with that in the control group using analysis of variance followed by Duncan's multiple range test. The significance level in each case was $p < .05$. For dose-response analysis cumulative mortality was analyzed by the probit method.

DISCUSSION/RESULTS

Hatching and Mortality Summary

Exposure Group (ppb)	Mean Degree Days to Hatch[1]	Mean Degree Days to Swim up[1]	No Exposed	Embryo Mort	Alevin Mort.	Total Mort.
0	105.2	237 7	100	0	3	32
23	107.5	242.1	100	0	3	35
41	107 2	243 7	100	2	4	23
116	105.2	244.6	100	0	1	35
300	106 7	241.1	100	0	2	31[2]
890	100.7	244.0	100	1	66	92[2,3]

1 Degree Days = cumulative daily temperature in °C
2 Used in Probit Analysis
3 Significantly different from control

Hatching While there was no concentration-related effect on hatching success incubation time was decreased by approximately one-half day in the highest concentration. There was no effect on time to swim up.

Mortality: Sixty day cumulative mortality was significantly greater than control mortality only for the 890 ppb group. Median lethal concentration was 717 ppb. On days 19 and 20 mortality was unusually high (62% for the 2 days) in the 810 ppb group. Dissolved oxygen reached its lowest concentration on those two days (65%) but remained at levels not ordinarily lethal to juvenile trout. Clinical signs observed included increased or decreased pigmentation, abnormal swimming, spinal curvature, tremors, edema, loss of equilibrium fin erosion, partial agnathism and general weakness.

Growth: Of the 60-day survivors those in the 890, 300 and 116 ppb groups were significantly lighter than controls, and the magnitude of the difference was concentration related. Only the two highest concentrations produced significantly shorter survivors.

Survivor Weight and Length

Expsure Group(ppb)	Mean Length(mm)	Range	Mean Weight(mg)	Range
Control	30.8	22-39	550	484-625
23	31.0	23-38	543	490-624
41	31.7	25-37	553	490-612
116	29.8	22-36	439*	334-509
300	28.7*	22-37	370*	295-407
890	24.0*	24-24	210*	210-210

* Significantly different from control ($p < .05$)

General Conclusions Triadimefon had no discernable effect on hatching success. Growth in terms of final length and/or weight was significantly reduced in the presence of 890, 300, or 116 ppb of triadimefon The lowest lethal concentration was 890 ppb. The highest no-observed-effect concentration was 40.6 ppb based on growth. The MATC (60-day geometric mean effect/no-effect concentration) was 68.6 ppb. The 60-day LC_{50} was calculated to be 717 ppb

REVIEWER'S EVALUATION

A. Test Procedures

Several features of this test are not in conformance with ASTM recommended practice (proposed, February 1983):

1 Tap water which was dechlorinated by sodium sulfite and activated carbon was used. If a dechlorinated water is used it must be demonstrated that the test organism will survive and grow satisfactorily in it The control mortality was 32% however the test continued for 39 days post swim-up. rather than the minimum 30 days (Control mortality at 30 days post swim-up was 27%). Similar mortality levels were also seen at the four lowest concentration levels (35, 23, 35 and 31%). Analysis of the test water (in the control) showed high chlorine levels ranging from 18 to 81 ug/l (mean, 38.5 ug/l). The maximum recommended chlorine level is 3 ppb.

2. The quality of the dilution water was not uniform during the test; weekly samples showed that the hardness varied from 63 mg/l to 107 mg/l (as CaCO3).

3. The test chambers do not appear to be replicated. The four incubation chambers at each test level are enclosed in a single test vessel. If a direct water connection exists between incubation chambers, then the chambers are not true replicates. How the researchers distinguished between replicates after swimup is unclear.

4. There was some variability in the measured test concentrations throughout the study. For the 10 ppb group, the ratio between the highest and lowest measured concentration exceeded the recommended 2 0. Measured concentrations at all levels were somewhat higher than nominal, but the measured means exceeded the nominal means by the recommended limit of 20% at the two lowest concentrations only.

B. Statistical Analysis

Raw data was not provided in this study, therefore it was not possible to verify the author's ANOVA. The 60-day LC_{50} was verified using Stephen's computer program. *See print-out.*

C. Conclusions

1. Category: Supplemental

2. Rational Although there are several departures from ASTM recommended practice in this study the high chlorine level in the water is the only problem which could possibly invalidate the study However, it is unlikely that the presence of chlorine in the dilution water could have been responsible for the high mortality in the control and in the lower exposure levels because most of the mortality occurred toward the end of the study rather than in the more sensitive embryo and alevin stages.

   This study has been categorized as supplemental because the information provided in this study is insufficient to allow full evaluation of study results. The data as presented are not broken down by replicates in fact, it is unclear whether replicates were used in this study. Needed are the complete mortality tables broken down by individual replicates (incubation chambers) and showing mortality on a daily basis Also needed are the lengths and weights of the 60-day survivors broken down by replicate chambers if possible

3. Repairability: Core, after the requested information is received and evaluated.

NATELLA BAYLETON TROUT EARLY LIFE STAGE

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
890	68	60	88.2353	0
300	68	0	0	0

THE BINOMIAL TEST SHOWS THAT 300 AND 890 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 594.797

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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Since the control mortality (31/100) was greater than the mortality at 300 ppb (31/100), Abbott's correction could not be used. Values were corrected instead as follows:

	Dead/Exposed
Control	0/68
300 ppb	$\frac{31-31}{100-32} \simeq 0/68$
890 ppb	$\frac{92-32}{100-32} \simeq 60/68$