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DATA EVALUATION RECORD

1. **CHEMICAL:** Dyfonate (Fonofos)

2. **TEST MATERIAL:** Dyfonate Technical: Lot# WRC 4921-28-27. The test material was an amber colored liquid. No further description was provided.

3. **STUDY TYPE:** Early Life Stage Test with Freshwater Fish
   Species Tested: Salmo gairdneri (rainbow trout).

4. **CITATION:** Surprenant, D.C. 1987. The Toxicity of Dyfonate Technical to Rainbow Trout (Salmo gairdneri) Embryos and Larvae, Report #BW-87-8-2467, Study Number 723-0886-6104-121. Prepared by Springborn Life Sciences, Inc. Wareham, Massachusetts. Submitted by Stauffer Chemical Company, Farmington, CT. Accession Number 403750-01.

5. **REVIEWED BY:**

   Mark R. Roberts
   Wildlife Biologist
   Ecological Effects Branch

   Signature: Mark R. Roberts
   Date: 7 June 1990

6. **APPROVED BY:**

   Ann Stavola
   Acting Head, Review Section III
   Ecological Effects Branch

   Signature: Ann Stavola
   Date: 7/12/90

7. **CONCLUSIONS:**
   Based on survival and growth, the MATC of Dyfonate for Salmo gairdneri appears to be ≥ 4.7 ug/L ≤ 9.5 ug/L mean measured concentrations. However, the summary data submitted do not fulfill the guideline requirements for a Salmo gairdneri flow through fish early life stage toxicity test. Without the required raw data, verification of the reported MATC value can not be accomplished.

8. **RECOMMENDATIONS:**
   Submit the required data as outlined in sections 14A and 14B of this review. Upgraded to Core (10-31-91) MR

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9. BACKGROUND:

10. DISCUSSION OF INDIVIDUAL TESTS: N/A

11. MATERIALS AND METHODS:

A. Test Animals: Unfertilized rainbow trout (Salmo gairdneri) eggs and sperm were obtained from Mount Lassen Trout Farm, Red Bluffs, CA. No information was provided on the parental fish.

B. Test System: The test was conducted in a modified proportional diluter system with a 0.50 dilution factor. The diluter delivered five concentrations of Dyfonate, a dilution water control and a solvent (DMF) control to duplicate test aquaria. Each glass aquarium maintained a constant test volume of 11 liters (L). The diluter delivered 0.5 L of test water per cycle which provided for 6.5 volume replacements per 24-hour period. Illumination was provided by fluorescent lights set on a 16-hour light and 8-hour dark photoperiod. Test temperature was maintained at 12 $\pm$ 1 ^{o}C through the use of a water bath. The dilution water was well water supplemented with Town of Wareham, Massachusetts untreated well water. The dilution water possessed a total hardness and alkalinity of 30 - 38 mg/l and 28 - 34 mg/l as $CaCO_3$, respectively. The pH range was 7.0 to 7.1 and the specific conductivity range was 100 to 120 umhos/cm during the test.

C. Dosage: Eighty-seven day (60 days posthatch) flow-through early life stage test.

D. Design: Eggs and sperm were mixed together in a stainless steel bowl with a small amount of control aquarium water to ensure even distribution of sperm over all eggs. This mixture was left undisturbed for 2 hours, then rinsed with control water, and finally allowed to water harden for an additional hour. Fifty eggs were then impartially selected and distributed to each of 28 replicate incubation cups. Two embryo chambers were suspended in each duplicate test aquarium per exposure concentration and controls. Embryo chambers were oscillated in each test aquaria using a rocker arm apparatus. A control, solvent control (DMF) and nominal Dyfonate concentrations of 0.63, 1.3, 2.5, 5.0, and 10 ug/L were used. At day 18 of incubation a definitive determination of egg viability was made as all eggs were examined for development and non-fertile eggs were discarded. Twenty live embryos from each treatment and control replicate were impartially selected and isolated in an incubation cup to initiate the post hatch stage of the study. At the time

of hatch, the live larvae from each embryo chamber were transferred to the respective aquaria. Larvae were fed frozen brine shrimp (Artemia salina) nauplii three times daily on weekdays and twice daily on weekends and holidays. Aquaria were cleaned when necessary to remove excess food and fecal matter. Fish were observed daily and their survival estimated twice weekly. At 60 days posthatch, the larvae from each aquarium were counted and growth measured (both total length and wet weight).

Dissolved oxygen concentration, pH, and temperature were measured in every aquarium on day 0, and measured daily in 1 replicate of treatment and controls thereafter. Total hardness was measured on day 0 and weekly in replicates of the high and low test concentrations and the dilution water control.

Water samples were collected from all treatment and control replicates on test days 0, 8, 14, and weekly thereafter for confirmation of Dyfonate technical. Two QA samples were prepared at each sampling interval and also analyzed.

E. **Statistics**: All control and solvent data for each of the measured endpoints were compared for significant differences by analysis of variance (ANOVA). Since there were no differences ($P>0.05$), the control data were pooled to increase the power of the test.

Significant differences in the percentage survival were determined after angular (arcsine square-root percentage) transformation of the data. Differences were determined by one-way analysis of variance (ANOVA). Statistical comparison between the results of the pooled controls and various concentrations of Dyfonate was performed by William's method.

All growth measurements were entered individually and ANOVA performed with statistical comparison between the results of the pooled controls and the various concentrations of Dyfonate established through the William's method.

The Maximum Allowable Toxicant Concentration (MATC) was calculated by taking the geometric mean of the limits set by the lowest test concentration that showed a statistically significant effect (Lowest Observed Effect Concentration, LOEC) and the highest test concentration that showed no statistically significant difference from the control (No Observed Effect Concentration, NOEC).

12. **REPORTED RESULTS**: The mean measured concentrations of Dyfonate in the test concentrations were 0.63, 1.3, 2.4, 4.7, and 9.5 ug/L which represented 94-100% of the nominal values (Table 1, attached).

The results of the QA analyses showed concentrations ranging from 73-110% of the amount fortified. The mean recovery value was 90.9% which indicated that satisfactory accuracy was achieved throughout the analyses.

The concentration of test material had no effect on temperature, total hardness, dissolved oxygen, or pH in the exposure solutions. Water quality conditions were satisfactory at all times for successful hatchability, survival, and growth of rainbow trout (Table 3, attached).

A summary of the biological results of the exposure of rainbow trout embryos and larvae to Dyfonate is provided in the attached Table 4. In summary, at the highest mean measured concentration of 9.5 ug/L, larval survival and growth (length and weight) were significantly lower than in the pooled control. The lower test concentrations were not statistically significantly different from the control values. Therefore, the MATC for dyfonate technical to Daphnia magna) was estimated to be < 9.5 ug/L > 4.7 ug/L.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:
No conclusions were drawn by the author. The data were audited by the laboratory's Quality Assurance Unit to assure compliance with the protocols, standard operating procedures and pertinent EPA Good Laboratory Practice (GLP) Regulations. A GLP compliance statement was included and signed by the Quality Assurance Unit.

A list of 4 deviations was provided with the study and a signed page indicating that the deviations listed did not affect the study results.

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

A. Test Procedure: The test procedures deviated from the SEP and ASTM guidelines as follows:

o The ASTM states that the pH must not deviate ± 1 pH unit within a 48-hr period. The pH values given in this study were only reported as a range, therefore specific changes were not discernable.

o The ASTM states that for a flow through test design the dissolved oxygen concentration should be maintained between 90 and 100%. During the latter part of this study the concentration was below 60% for several days, and as low as 48%.

o The SEP states that the temperature should not deviate more than ± 2° C. This study was conducted at 12° C and the temperature during at least 1 day escalated to 15° C.

Additional replicate data such as numbers surviving in each incubation cup, and each actual water quality measurement are needed to verify test results.

B. Statistical Analysis: Statistical analyses of the data were not conducted due to the lack of raw data. Specific data needed to verify the reported MATC value are:

1) Number of eggs surviving at hatch in each replicate of the control and treatment groups.

2) Number of fish surviving in each replicate of the treatment and control groups.

3) Length (individual measurements; 0.5mm) of trout at test termination for each replicate.

4) Weight (individual measurements; 0.01gm) of trout at test termination for each replicate.

C. Discussion/Results: The data submitted do not fulfill the Guideline requirements for a Salmo gairdneri flow through fish early life stage toxicity test. Data lacking in sections A and B above prevent verification of the reported MATC value.

Based on survival and growth, the MATC of Dyfonate for Salmo gairdneri appears to be ≥ 4.7 ug/L ≤ 9.5 ug/L mean measured concentrations.

D. Adequacy of Study:

upgraded to core 10-31-91 MA

(1) Classification: ~~Invalid~~.

(2) Rationale: Lack of raw data as identified in sections 14A and 14B in this review prevents verification of the reported MATC value.

(3) Repairability: Study may be upgraded to core if the above raw data and information is submitted and the results are judged acceptable.

15. COMPLETION OF ONE-LINER FOR STUDY: Yes, 06-05-90.

Table 1. Measured concentrations of Dylonate Technical during the early life stage exposure of rainbow trout (Salmo gairdneri).

Nominal Concentration (µg/L)	Mean Measured Concentration (µg/L)	Percent of Nominal	N
10	9.5 (± 1.5)	95	28
5.0	4.7 (± 0.94)	94	28
2.5	2.4 (± 0.62)	96	27
1.3	1.3 (± 0.67)	100	28
0.63	0.63 (± 0.22)	100	28
Control	< 0.24*		
Solvent Control	< 0.24*		

* These values represent the highest minimum detected level measured during the 87 day exposure period.

Table 3. Water quality determinations made during the 87-day exposure of rainbow trout (Salmo gairdneri) embryos and larvae to Dyfonate Technical.

Mean Measured Concentration (µg/L)	Mean Dissolved Oxygen (mg/L)	Mean Temperature (°C)	Mean Total Hardness (mg/L as $CaCO_3$)	pH Range
9.5	8.4 (1.4)[a]	12 (0.6)[a]	31 (1.5)	6.6-7.5
4.7	8.5 (1.4)	12 (0.6)	---	6.6-7.4
2.4	8.7 (1.2)	12 (0.6)	---	6.6-7.5
1.3	8.6 (1.3)	12 (0.6)	---	6.6-7.5
0.63	8.6 (1.4)	12 (0.6)	31 (1.5)	6.6-7.5
Solvent Control	8.4 (1.5)	12 (0.6)	31 (1.0)	6.6-7.5
Control	8.8 (1.3)	12 (0.7)	31 (1.3)	6.6-7.5

[a] Standard deviations in parentheses.

Table 4. Embryo viability, survival of organisms at hatch and survival, total length, and wet weight of rainbow trout (Salmo gairdneri) larvae exposed to Dyfonate Technical for 87 days (60 days post-hatch).

Mean Measured Concentration (µg/L)		Embryo Viability (%)	Survival of Organisms at Hatch (%)	Larvae (60 days post-hatch)		
				Larvae Survival (%)	Mean Total Length (mm)	Mean Wet Weight (g)
9.5	A	55	98	75	52 (8.9)	1.5 (0.80)
	B	57	100	90	55 (5.1)	1.7 (0.49)
	Mean	56	99	83[a]	54 (7.2)[a]	1.6 (0.65)[a]
4.7	A	56	96	100	60 (2.4)	2.0 (0.27)
	B	52	96	90	59 (2.9)	1.9 (0.30)
	Mean	54	96	95	60 (2.7)	2.0 (0.29)
2.4	A	46	100	100	61 (2.2)	2.1 (0.32)
	B	60	100	100	59 (2.4)	1.9 (0.28)
	Mean	53	100	100	60 (2.4)	2.0 (0.31)
1.3	A	56	98	100	60 (3.7)	2.1 (0.41)
	B	54	98	100	59 (4.8)	2.1 (0.47)
	Mean	55	98	100	60 (4.3)	2.1 (0.44)
0.63	A	50	100	100	60 (1.9)	2.1 (0.25)
	B	43	100	100	60 (2.4)	2.1 (0.29)
	Mean	47	100	100	60 (2.2)	2.1 (0.27)
Solvent Control	A	48	96	100	59 (2.6)	2.0 (0.27)
	B	52	100	95	58 (3.6)	1.9 (0.35)
	Mean	50	98	98	59 (3.1)	1.9 (0.31)
Control	A	48	98	100	57 (4.5)	1.9 (0.44)
	B	47	96	100	59 (3.8)	2.1 (0.36)
	Mean	48	97	100	58 (4.1)	2.0 (0.40)
Pooled Controls[b]		49	98	99	58 (3.7)	2.0 (0.36)

[a] Significantly different ($P \leq 0.05$) from the control (pooled control and solvent control) data.

[b] Pooled mean control and solvent control data.