Data Evaluation Record

1. Chemical: Di-Syston Technical (Disulfoton)
   Shaughnessy No.:032501

2. Test Material: Disulfoton (o,o-diethyl s-(2-(ethylthio)ethyl) phosphorodithioate), 98%, batch #7030185 (CAS #298-04-4); a clear liquid

3. Study type: Fish Early Life Stage (72-4)
   Test Species: Rainbow Trout (Oncorhynchus mykiss)

4. Study ID: Rhodes, Jon E., Loren C. Schreier and William A. McAllister. "Early life-stage toxicity of Di-Syston Technical to the rainbow trout (Oncorhynchus mykiss) under flow-through conditions. Performed by Analytical Bio-Chemistry Laboratories, Inc., 7200 East ABC La., P.O. Box 1097, Columbia, MO 65205, for Mobay Corporation, Agricultural Chemicals Division, Research and Development Department, P.O. Box 4913, Kansas City, MO 64120. Study Id #33896. **MRID #419358-01.**

5. Reviewed by: Kathryn Valente
   Biologist
   EEB/EFED
   Signature: Kathryn L Valente
   Date: 11/25/91

6. Approved by: Allen Vaughan
   Acting Head, Section II
   EEB/EFED
   Signature: Allen W. Vaughan
   Date: 12-04-91

7. Conclusions: The study is scientifically sound and meets the requirements for a freshwater fish early life stage toxicity study. The MATC was 0.30 mg a.i./L, the NOEC was 0.22 mg a.i./L and the LOEC was 0.42 mg a.i./L.

8. Recommendations: N/A

9. Background information: This study was submitted in support of reregistration for disulfoton.

10. Discussion of Individual Tests: N/A

11. Materials and Methods:
    a. Test animals: Rainbow trout were obtained as unfertilized eggs and milt from Mt. Lassen Trout Farms in Red Bluff, CA. The eggs were fertilized and allowed to water harden for 1.5 hours, and were then put into incubation cups (35 eggs in each replicate, 4 replicates per concentration). The eggs were shaded from UV light until 1 week post-hatch, and then fish were kept on a 16-hr. light/8-hr. dark photoperiod. The eggs were observed daily for mortality, and dead eggs were

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removed. After hatching, fish were observed daily for mortality and abnormal behavior. Measurement of length was made using photography at 35 days post-hatch, and length and wet weight measurements were taken at the end of the study.

b. Test system: Tests were conducted in aquaria containing 12L of test water per chamber. Each chamber contained 4 incubation cups. Dilution water and test substance was provided to each chamber by a proportional diluter system. Aquaria were kept in a refrigerated water bath which held a temperature of 10°C.

c. Study design: A preliminary range-finding study was conducted. The results from this range-finding study showed adverse effects at 1.0 and 2.0 mg a.i./L after 29 days. For the definitive test, 35 eggs were placed in each of four egg cups per chamber, and there were four replicate chambers per concentration. Dimethyl formamide was used as a solvent. The nominal exposure levels of Di-syston were control, solvent control, 0.063, 0.13, 0.20, 0.50 and 1.0 mg a.i./L. Samples for analytical confirmation of chemical concentration were taken on days 0, 1, 6 and every 7th day thereafter. Mean measured concentrations of Di-syston were: 0.056, 0.10, 0.22, 0.42 and 0.97 mg a.i./L. Temperature, dissolved oxygen (DO), and pH measurements were taken on days 0, 1, 6 and every 7 days thereafter. In addition, a continuous temperature record was maintained.

d. Statistics: Egg hatchability and survival data were analyzed using frequency analysis with Chi Square or Fisher's exact tests. Growth data were analyzed using 1-way analysis of variance (ANOVA) with Dunnett's mean separation. The control and solvent control were pooled if a t-test showed no significant difference between the means of the two groups.

12. Reported Results: Water quality parameters were as follows; DO ranged from 8.3-10.2 mg/L (77-94% saturation); temperature ranged from 10.5-11.8°C; pH ranged from 7.7-8.2; conductivity was 106-189us; hardness ranged from 42-50 mg/L as $CaCO_3$; alkalinity ranged from 48-56 mg/L as $CaCO_3$.

Hatchability: Hatching began on day 28 post-fertilization and took 5 days to complete. Controls and solvent controls were pooled for data analysis. Hatching success was as follows:

pooled controls :	90%	0.22 mg a.i./L :	84%
0.056 mg a.i./L :	84%	0.42 mg a.i./L:	84%
0.10 mg a.i./L :	77%	0.97 mg a.i./L:	81%

There was no statistically significant difference between any group and the controls.

Survival: Day 35 post-hatch survival percentages were:

pooled controls:	96%	0.22 mg a.i./L:	98%
0.056 mg a.i./L:	98%	0.42 mg a.i./L:	97%

0.10 mg a.i./L : 95% 0.97 mg a.i./L: 98%
There was no statistically significant reduction at any level compared to the controls.

Termination survival rates:
pooled controls: 96% 0.22 mg a.i./L: 98%
0.056 mg a.i./L: 98% 0.42 mg a.i./L: 97%
0.10 mg a.i./L : 95% 0.97 mg a.i./L: 97%
There was no statistically significant reduction at any level compared to the controls.

Growth: Mean standard lengths at 35 days post-hatch (determined by photography) were:
pooled controls: 31.45 mm 0.22 mg a.i./L: 30.86 mm
0.056 mg a.i./L: 30.64 mm * 0.42 mg a.i./L: 30.35 mm
0.10 mg a.i./L : 30.96 mm 0.97 mg a.i./L: 28.24 mm
ANOVA with Dunnett's mean separation showed a significant reduction in growth for 0.056, 0.42 and 0.97 mg a.i./L at p=0.05. The reduction at 0.056 was not biologically significant due to the lack of a dose-response curve.

Mean standard lengths at study termination (62 days post-hatch) were:
controls: 46.43 mm 0.22 mg a.i./L: 44.40 mm
solvent controls: 44.66 mm 0.42 mg a.i./L: 43.67 mm
0.056 mg a.i./L: 44.08 mm 0.97 mg a.i./L: 37.62 mm
0.10 mg a.i./L: 45.37 mm
ANOVA with Dunnett's mean separation showed a significant reduction in growth for 0.97 mg a.i./L at p=0.05.

Mean wet weights taken at test termination were:
pooled controls: 1.41 g 0.22 mg a.i./L: 1.38 g
0.056 mg a.i./L: 1.32 g 0.42 mg a.i./L: 1.30 g
0.10 mg a.i./L: 1.44 g 0.97 mg a.i./L: 0.78 g
ANOVA with Dunnett's mean separation showed a significant reduction at 0.97 mg a.i./L at p=0.05.

Morphological/behavioral observations: Fry swim up was complete by day 19 post-hatch and was essentially the same for all levels.

13. Study Author's Conclusions/Quality Assurance Report: The NOEC was 0.22 mg a.i./L and the LOEC was 0.42 mg a.i./L. The MATC point estimate (geometric mean of the NOEC and LOEC) was 0.30 mg a.i./L.

Quality Assurance and Good Laboratory Practice statements were included in the report.

14. Reviewer's Discussion and Interpretation of the Results:
a. Test Procedure: The test design and procedure were in accordance with protocols recommended by the Guidelines.

b. Statistical Analysis: All statistics were verified using

EPA's TOXSTAT computer program. All values were in agreement with the reported results.

c. Discussion/Results: The study is scientifically sound and in accordance with the Guidelines.

d. Adequacy of the study:
(1) Classification: Core.
(2) Rationale: N/A
(3) Repairability: N/A