DATA EVALUATION RECORD

1. **CHEMICAL:** Disulfoton.
Shaughnessey No. 032501.

2. **TEST MATERIAL:** Di-syston; Lot No. 2085028; 97.4% purity; a clear liquid.

3. **STUDY TYPE:** 72-4. Estuarine Fish Early Life-Stage Toxicity Test. Species Tested: Sheepshead Minnow (*Cyprinodon variegatus*).

4. **CITATION:** Lintott, D.R. 1993. DISYSTON (Technical): Toxicity to Embryos and Larvae of the Sheepshead Minnow (*Cyprinodon variegatus*) Under Flow-Through Test Conditions. Project No. J9202001. Performed by Toxikon Environmental Sciences, Jupiter, FL. Submitted by Miles Incorporated, Kansas City, MO. EPA MRID No. 426290-01.

5. **REVIEWED BY:**

Mark A. Mossler, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: [signature]

Date: 10/27/93

6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
Senior Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: P. Kosalwat

Date: 10/27/93

J. GOODYEAR
~~Henry T. Craven~~, M.S.
PC ~~Supervisor~~, EEB/EFED
USEPA

Signature: Dathryn T. Valente

Date: Goodyear 1/2/94

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for an early life-stage chronic toxicity test. Based on mean measured concentrations, the MATC was >16.2 and <32.9 µg ai/l (geometric mean MATC = 23.1 µg ai/l).

8. **RECOMMENDATIONS:** N/A.

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. MATERIALS AND METHODS:

A. Test Animals: Adult sheepshead minnows (*Cyprinodon variegatus*) were obtained from commercial suppliers (Fort Collins, CO, and Gulf Breeze, FL) and maintained in laboratory dilution water for three months. The adults were fed a diet consisting of brine shrimp and commercial flake food.

Embryos used in the test were obtained by stripping gametes from 20 adult females and six males. Egg production was enhanced by injections of human gonadotrophin hormone. Sperm was obtained from macerated testes of the males. Fertilized eggs were placed in incubation chambers in dilution water overnight.

B. Test System: A modified proportional vacuum-siphon diluter was used to prepare and deliver the test solutions. The test chambers were 24-l glass tanks (40 x 29.5 x 20 cm) containing 15 l of solution with a maximum depth of 13 cm. The flow to each chamber provided 8 volume additions per day. The chambers were randomly positioned in a water bath maintained at 27 ±2°C. The test system was maintained on a 16-hour light/8-hour dark photoperiod under fluorescent and incandescent lights with an intensity of 250-533 lux. Fifteen-minute dawn and dusk simulations were provided.

The embryos and larvae were held in incubation chambers for the first 5 days of exposure. The incubation chambers were "60-mm diameter glass tubes with 355-µm mesh screen attached with silicone sealant between two tube sections." Two incubation chambers were placed within each replicate tank. Test solution in the chambers was renewed using a self-starting siphon which raised and lowered the solution level in the tank. After hatching, the fish were transferred to retention chambers constructed of petri dishes (150 mm x 10 mm) with 20-cm screen collars. Two retention chambers were placed in each replicate.

The dilution water was natural saltwater pumped from a shallow well and was carbon treated and adjusted to a salinity of 20 parts per thousand (ppt) with treated (carbon-filtered) municipal freshwater. The water was vigorously aerated prior to use.

Stock solutions [150 mg active ingredient (ai)/ml] were prepared in dimethylformamide (DMF) and delivered to

the diluter mixing chamber. The solution in the mixing chamber (400 µg ai/l) was proportionally diluted to provide the four lower test concentrations.

C. **Dosage:** Thirty-three-day, flow-through, early life-stage test. Based on preliminary tests, five nominal concentrations (25, 50, 100, 200, and 400 µg ai/l), a dilution water control, and a solvent control were selected for the test. The concentration of solvent in the solvent control and all treatments was 2.7 µl DMF/l.

D. **Design:** Twenty fertilized eggs (<24 hours old) were impartially distributed, two at a time, to each of the embryo incubation chambers. Two replicate aquaria were used per concentration for a total of 80 viable embryos per treatment.

Survival of embryos was recorded daily until hatching was complete. Hatch was considered complete when 95% of all live embryos in the controls had hatched (day 5). Fry were culled to 15 per embryo chamber (30 per replicate) where possible after hatching was complete. After hatching, all fish were transferred to screen retention chambers within the test tank and survival was monitored daily. Any abnormalities in the behavior or physical appearance of the fish during the exposure period were also noted. The fish were fed newly hatched brine shrimp (supplemented with fatty acids) two to three times per day ("with few exceptions") except during the final 24 hours of the test when feeding was discontinued.

At test termination, all fish were measured individually to determine standard length and wet weight. The dissolved oxygen concentration (DO) and pH were measured at test initiation, weekly, and at test termination in each aquarium. Salinity in one replicate of the dilution water control was measured daily. The temperature in one control tank was recorded hourly during the study. The temperature range of the water bath was monitored daily with a min/max thermometer.

Water samples were collected from each replicate tank at test initiation, weekly, and at test termination (test days 0, 7, 14, 21, 28, and 33). The concentrations of Di-syston and the metabolites disulfoton sulfoxide and disulfoton sulfone in each

sample were determined using high performance liquid chromatography.

E. **Statistics:** For each endpoint, dilution water control and solvent control responses were compared using an appropriate test (e.g., chi-square test, Student's t-test). No significant differences were observed for hatchability and survival, therefore the control results were pooled prior to each analysis. For length and wet weight, comparisons were made against the solvent control data.

Hatchability and survival data were analyzed using 2 X 2 contingency tables, Fisher's Exact test, and chi-square test of independence. The length and weight data were tested for homogeneity of variance using Levene's test. Dunnett's test was used to determine if responses in each treatment level were different from the control responses.

12. **REPORTED RESULTS:** The mean measured concentrations of Di-syston were 21.2, 32.9, 58.2, 134, and 290 µg ai/l which averaged between 58 and 85% of nominal concentrations (Tables 1A and 2A, attached). No undissolved material was observed in any test vessel during the test. The mean measured concentrations of disulfoton sulfoxide in the two highest concentration solutions (134 and 290 µg ai/l) were 18.8 and 31.7 µg/l [8 to 9% of the nominal Di-syston concentrations (Tables 1B and 2B, attached)]. No disulfoton sulfone was detected in the two treatment solutions measured.

Sheepshead minnow hatching success and survival at test termination were reported in Tables 3 and 4 (attached). Hatching began on day 3 and was complete by day 6. Percent hatch in the dilution water control and solvent control was 93 and 98%, respectively. Percent hatch in the treatments ranged from 93 to 96% and none were significantly different from the pooled control. Percent survival at test termination in the treatments ranged from 17 to 93% and was significantly reduced at concentrations $\geq$32.9 µg ai/l in comparison to the pooled control (97%).

Mean total length and mean wet weight were reported in Table 5 (attached). Average length in the dilution water control and solvent control was 17.5 and 18.4 mm, respectively. Average wet weight in the dilution water control and solvent control was 0.1380 and 0.1558 g, respectively. Since significant survival effects were noted in the four highest concentration solutions, the data for these groups was not

included in the statistical analyses. Comparisons of the lowest concentration group data were made against the solvent control data, since the values for this control group were significantly larger than those for the negative control group. Both length and wet weight were not significantly reduced at the lowest treatment level of 21.2 µg ai/l.

Sublethal effects noted on day 7 of the test consisted of twitching at the 32.9 µg ai/l level and higher. This effect was not observed at the 32.9 µg ai/l level after day 9 of the test, but was prevalent at the two highest concentrations (134 and 290 µg ai/l) up until day 15 of the test.

During the study, the temperature ranged from 18.6 to 28.0°C. The DO remained ≥5.6 mg/l (≥78% of saturation) in the controls and ranged from 5.5 to 8.4 mg/l (≥76% of saturation) in the treatment groups. The pH was 8.1-8.4. The salinity of the dilution water ranged from 17 to 21 ppt.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The NOEC and LOEC for sheepshead minnow embryos and juveniles exposed to Di-syston were 21.2 and 32.9 µg ai/l, respectively, based on juvenile survival.

Quality Assurance and Good Laboratory Practice Statements were included in the report, indicating that the study was conducted in accordance with EPA 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 deviated from the SEP and/or ASTM guidelines as follows:

Forty embryos/replicate and two replicates/treatment were used in the test; a minimum of 20 embryos/ replicate and four replicates/treatment are recommended by the SEP.

A test temperature of 25-30°C which does not vary by more than 2°C is recommended. In this test, the temperature fluctuated between 18.6 and 28.0°C. Daily temperatures fluctuated by as much as 6.8°C (day 5).

The salinity of the dilution water fluctuated by more than 6 percent weekly.

B. **Statistical Analysis:** The reviewer analyzed the percent hatch, percent survival at test termination, length, and wet weight data after 33 days of exposure to Di-syston using analysis of variance. The percentage data (hatch and survival) were arcsine square root transformed prior to analysis.

As in the author's analysis, the concentration of test material had no effect on percent hatch (printout 1, attached). Survival, length, and wet weight were all significantly reduced at the 32.9 µg ai/l level (printouts 2 through 4, attached), but not at the 16.2 µg ai/l level. Therefore, the NOEC and LOEC are 16.2 and 32.9 µg ai/l, respectively.

C. **Discussion/Results:** The author made a mistake in calculating the mean measured concentration of Di-syston in solution for the lowest concentration solution. In Table 2A, the mean measured concentrations for replicates A and B of the 25 µg ai/l nominal concentration were listed as 17.8 and 21.9 µg ai/l, respectively, with a mean of 21.2 µg ai/l. The actual mean measured concentrations for replicates A and B were determined by the reviewer to be 16.4 and 16.0 µg ai/l, respectively, with a mean of 16.2 µg ai/l. This concentration is 65% of the nominal concentration.

This study is scientifically sound and fulfills the guideline requirements for an early life-stage chronic toxicity test. Based on mean measured concentrations, the maximum acceptable toxicant concentration (MATC) was >16.2 and <32.9 µg ai/l (geometric mean MATC = 23.1 µg ai/l).

D. **Adequacy of the Study:**

(1) Classification: Core.

(2) Rationale: N/A.

(3) Repairability: N/A.

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes, 10-13-93.

Disulfoton Data Evaluation Record (R2030343)

Page _____ is not included in this copy.

Pages __**7**__ through __**14**____ are not included in this copy.

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_____ Identity of product inert ingredients.

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__ ___ Description of quality control procedures.

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#1

sheepshead hatchability
File: shm Transform: ARC SINE(SQUARE ROOT(Y))

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.025	0.005	0.748
Within (Error)	6	0.040	0.007	
Total	11	0.065		

Critical F value = 4.39 (0.05,5,6)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

sheepshead hatchability
File: shm Transform: ARC SINE(SQUARE ROOT(Y))

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	solvent control 16.2	1.418	0.975		
2	~~21.2~~ 16.0	1.323	0.930	1.167	
3	32.9	1.303	0.930	1.411	
4	58.2	1.303	0.930	1.411	
5	134	1.387	0.965	0.384	
6	290	1.387	0.965	0.384	

Dunnett table value = 2.83 (1 Tailed Value, P=0.05, df=6,5)

NOEC = 290 mg ai/l

sheepshead hatchability
File: shm Transform: ARC SINE(SQUARE ROOT(Y))

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	solvent control	2			
2	21.2	2	0.117	12.0	0.045
3	32.9	2	0.117	12.0	0.045
4	58.2	2	0.117	12.0	0.045
5	134	2	0.117	12.0	0.010
6	290	2	0.117	12.0	0.010

#2

sheepshead survival
File: shm Transform: ARC SINE(SQUARE ROOT(Y))

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	1.684	0.337	16.076
Within (Error)	6	0.126	0.021	
Total	11	1.809		

Critical F value = 4.39 (0.05,5,6)
Since F > Critical F REJECT Ho:All groups equal

sheepshead survival
File: shm Transform: ARC SINE(SQUARE ROOT(Y))

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	solvent control	1.341	0.935		
2	~~21.2~~ 16.2 ~~16.0~~	1.313	0.915	0.194	
3	32.9	0.898	0.610	3.060	*
4	58.2	0.574	0.295	5.298	*
5	134	0.416	0.165	6.387	*
6	290	0.502	0.235	5.793	*

Dunnett table value = 2.83 (1 Tailed Value, P=0.05, df=6,5)

NOEL = ~~21.2~~ 16.2 ~~16.0~~ mg ai/l

LOEL = 32.9 mg ai/l

sheepshead survival
File: shm Transform: ARC SINE(SQUARE ROOT(Y))

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	solvent control	2			
2	21.2	2	0.304	32.5	0.020
3	32.9	2	0.304	32.5	0.325
4	58.2	2	0.304	32.5	0.640
5	134	2	0.304	32.5	0.770
6	290	2	0.304	32.5	0.700

Analysis of Variance File: shm Date: 10-11-1993

FILTER: None

#3

N's, means and standard deviations based on dependent variable: LENGTH

* Indicates statistics are collapsed over this factor

Factors: T	R	N	Mean	S.D.
*	*	140	17.0071	3.0758
1	*	56	18.3929	1.9039
2	*	50	17.5600	2.8296
3	*	34	13.9118	2.8854
*	1	72	17.2361	3.1151
*	2	68	16.7647	3.0377
1	1	26	18.1154	1.6328
1	2	30	18.6333	2.1088
2	1	30	18.0333	3.2851
2	2	20	16.8500	1.8144
3	1	16	14.3125	2.9826
3	2	18	13.5556	2.8330

Fmax for testing homogeneity of between subjects variances: 4.05
Number of variances= 6 df per variance= 21.

Analysis of Variance Dependent variable: LENGTH

Source	df	SS (H)	MSS	F	P
Between Subjects	139	1314.9929			
T (TRT)	2	448.5805	224.2903	35.736	0.0000
R (REP)	1	5.2179	5.2179	0.831	0.3635
TR	2	20.1754	10.0877	1.607	0.2028
Subj w Groups	134	841.0190	6.2763		

Post-hoc tests for factor T (TRT)

Level	Mean
1	18.393
2	17.560
3	13.912

Comparison	Bon-ferroni
1 > 2	
1 > 3	0.0000
2 > 3	0.0000

not sig diff at 0.05

NOEL = 16.2 µg ai/l

#4

Analysis of Variance File: shm Date: 10-11-1993

FILTER: None

N's, means and standard deviations based on dependent variable: ~~LENGTH~~ Wet Weight

* Indicates statistics are collapsed over this factor

Factors: T	R	N	Mean	S.D.
*	*	140	0.1501	0.0783
1	*	56	0.1558	0.0436
2	*	50	0.1706	0.0991
3	*	34	0.1105	0.0757
*	1	72	0.1478	0.0873
*	2	68	0.1526	0.0680
1	1	26	0.1502	0.0333
1	2	30	0.1607	0.0510
2	1	30	0.1667	0.1152
2	2	20	0.1765	0.0709
3	1	16	0.1085	0.0786
3	2	18	0.1123	0.0753

Fmax for testing homogeneity of between subjects variances: 11.94
Number of variances= 6 df per variance= 21.

Analysis of Variance Dependent variable: ~~LENGTH~~ Wet Weight

Source	df	SS (H)	MSS	F	P
Between Subjects	139	0.8513			
T (TRT)	2	0.0761	0.0381	6.602	0.0018
R (REP)	1	0.0026	0.0026	0.444	0.5063
TR	2	0.0003	0.0001	0.023	0.9776
Subj w Groups	134	0.7723	0.0058		

Post-hoc tests for factor T (TRT)

Level	Mean
1	0.156
2	0.171
3	0.111

Comparison	Bon-ferroni
1 < 2	
1 > 3	0.0207
2 > 3	0.0016

NOEL = 16.2 µg ai/l