DATA EVALUATION RECORD
FISH LIFE-CYCLE TOXICITY TEST
GUIDELINE 72-5

1. **CHEMICAL:** Disulfoton Shaughnessey #: 032501

2. **TEST MATERIAL:** Di-Syston (non-radiolabeled) Purity: 98.5%
Radiolabeled Di-Syston--98.8% radiopurity
Radiolabeled Di-Syston Sulfone--99.3%
Radiolabeled Di-Syston Sulfoxide--98.9%

3. **CITATION:**
Authors: Dionne, Emily
Title: Di-Syston - The chronic toxicity to the Sheepshead minnow (*Cyprinodon variegatus*) during a full-life cycle exposure.
Study Completion Date: March 15, 1996
Laboratory: Springborn Laboratories, Inc., Environmental Sciences Division, 790 Main St., Wareham, MA
Laboratory Report ID: 13507.0894.6110.592
Project ID Number: 107119
Sponsor: Bayer Corporation, Agriculture Division, P.O. Box 4913, Hawthorne Rd., Kansas City, MO 64120-0013
MRID No.: 439605-01

4. **REVIEWED BY:**
Kathryn V. Montague, M.S., Biologist, ERBIII, EFED, U.S.EPA
Signature: Kathryn V. Montague Date: 6/5/97

5. **APPROVED BY:**
Daniel Rieder, Branch Chief, ERBIII, EFED, U.S.EPA
Signature: Daniel Rieder Date: 6/18/97

6. **STUDY PARAMETERS:**
Test Species: Sheepshead minnow (*Cyprinodon variegatus*)
Age or Weight: embryos at test initiation
Duration of Test: 110 days
Study Method: full life cycle test
Type of Concentration: nominal and mean measured

7. **CONCLUSIONS:**
This study appears to be scientifically sound but does not fulfill guideline requirements. Effects on F_o reproduction (fecundity), F_o growth, F_1 hatching success and F_o morphological abnormalities were seen at the lowest test level, so a NOEC was not

achieved for these parameters. The study does provide some information that is useful for a risk assessment, albeit with a high degree of uncertainty due to the deviations from guideline recommendations. Additional testing is required at levels low enough to produce a true NOEC.

8. ADEQUACY OF THE STUDY:

A. Classification: Supplemental

B. Rationale: NOECs not achieved for several parameters, exposure tanks aerated, F_1 generation only exposed for half the recommended time

C. Repairability: Additional testing at lower concentrations is required to achieve a true NOEC.

9.SUBMISSION PURPOSE: In support of reregistration of disulfoton

10. GUIDELINE DEVIATIONS:

Items not reported:

1) Did not report examination of embryos with scope or magnifying lens, as described in guidelines
2) Did not report percentage of healthy and fertile embryos from each spawn
3) Did not report time of day that embryos were counted and removed--guidelines suggest that it be done at the same time each day to minimize disruption of fish
4) Did not report the number of spawnings used to obtain embryos (should be at least three)
5) Did not report amount of time embryos soaked in dilution water prior to initiation of test (should be at least 2 hours)
6) Did not report whether live fungused embryos were removed and counted as dead
7) Did not report if lethargic or deformed juvenile fish were included in random selection
8) Did not report if survival was determined weekly -- only reports for days 28 and 57 post-hatch were included

Items which differ from guidelines:

1) Exposure solutions were aerated from day 55 through the end of the study--no data on daily DO levels were provided to show that this aeration was necessary, and no explanation of why the exposure tanks were aerated instead of aerating the dilution

water prior to input into the diluter system
2) The F_1 generation was only exposed for 4 weeks instead of the recommended 8 weeks
3) Males killed during spawning were replaced; guidelines recommend that neither males nor females killed in the spawning process should be replaced
4) No NOECs were achieved for several parameters: F_o reproduction (fecundity), F_o growth, F_1 hatching success and F_o morphological abnormalities
5) There were only two true replicates per treatment instead of the recommended four (there were four egg cups used, but these are not true replicates).

11. MATERIALS AND METHODS:

A. Biological System:

Guideline Criteria	Reported Information
Species: A freshwater fish species, preferably a fathead minnow (*Pimephales promelas* or an estuarine fish species, preferably a sheepshead minnow (Cyprinodon variegatus).	Estuarine species--Sheepshead minnow
Source and Acclimation of Fish 1. From wild population or Suitable laboratory culture 2.1. Sheepshead held in flowing 30°C seawater of >15% salinity for at least 2 wks. 2.2. Fathead 25°C and 16 hour/day day-light photoperiod (embryos will mature in 5 to 6 months under these conditions) 3. Neither species of fish or eggs should exhibit excess mortality.	1. Embryos obtained from laboratory-held fish, which were purchased from Aquatic Biosystems as juveniles. They were held four and one-half months prior to being spawned. 2.1 Held at 24-25°C, 31-32 $^{o}/_{oo}$ salinity for 14 days prior to spawning. Embryos were held at 25°C on a 12-hour photoperiod. These conditions were held throughout the test period. 3. Pre-spawning fish exhibited a mortality rate of 2%.

Guideline Criteria	Reported Information
Eggs from Adult Fish Artificial inducement and natural spawning are the 2 methods for obtaining a sufficient number of eggs for a chronic exposure. 1. Artificial inducement (entails the stimulation of egg production by injection of human gonadotrophic hormone. Usually 10 ♀s and 5♂s should be used.) 2. Natural spawning (is possible with a few considerations for each fish species.)	1. Natural spawning used. 2. 2 males and 5 females from each test aquarium was placed in one section of the spawning chamber when they reached maturity (approx. 57 days post-hatch). They were held for 14 days.
3. Adult deaths during spawning should be noted; dead animals removed but not replaced. 4. At termination of each spawning group, lengths and weights of individual fish are measured.	3. Females killed were not replaced; males were replaced to maximize egg fertilization success. 4.Each fish was measured, wet weighed, and internally examined.
Feeding	
1. Fry of both fish species should be fed equal portions of live brine shrimp nauplii at least 2x/day about 6 hours apart for three wks (frozen nauplii are not to be used).	1. Parent and first generation larvae were fed live brine shrimp nauplii 3x daily.
2. Juveniles (4 wks posthatch) and adults can be fed 2x/day on equal portions of dry food (e.g., Tetramin® or Biorell) supplemented with frozen adult brine shrimp.	2. Juvenile and adult fish were fed Zeigler Prime Flakes and frozen brine shrimp 2x daily.
3. Each batch of food should be checked for pesticides and metals.	3. Samples from each food source were analyzed periodically for the presence of pesticides, PCBs and toxic metals.
Embryo Removal	
1. Daily record numbers and egg fertility.	1. Eggs from the 28 brood stock groups were pooled and a representative sample of 200 were microscopically examined to estimate the percentage of successfully fertilized eggs. This method indicated that 76% were fertilized.

Guideline Criteria	Reported Information
2. Examined all embryos daily with a dissecting scope or magnifying viewer to remove empty shells and opaque, or abnormal embryos.	2. Embryos in cups were counted daily, and dead embryos were removed. There is no indication that the embryos were examined daily as described at left.
3. If <50% of the embryos from a spawn appear to be healthy and fertile, all embryos from that spawn should be discarded.	3. This information was not reported.
4. Embryos should be removed at a fixed time each day so spawning activity is not disturbed unnecessarily.	4. Dead embryos were removed daily, but time information was not reported.
Embryo Exposure (Four-Five Days)	
1. The life-cycle chronic toxicity test must begin with embryos from at least 3 separate spawnings 2. that are ≤24 hours old 3. and have soaked in dilution water for at least 2 hours.	1. Information regarding number of spawns was not reported--there were 28 groups of 5 females and 2 males each of brood stock. 2. Embryos were ≤ 24 hours old at initiation. 3. Information not reported.
4. Testing begins by randomly distributing 50 embryos to each of the 4 replicate larval growth chambers.	4. 50 embryos impartially placed into 2 cups per replicate, 2 true replicates per treatment.
5. 10 embryos are transferred with a large bore eye dropper to successive incubation cups which are standing in dilution water. This is repeated until 50 embryos are in each cup. The incubation cups are then distributed to each replicate larval chamber.	5. Embryos were distributed to cups 5 at a time until each cup contained 50.

Guideline Criteria	Reported Information
6. 6.1 Survival of embryos, 6.2 time required to hatch, 6.3 hatching success, 6.4 and survival of fry for 4 wks are determined and recorded. 6.5.1 Dead embryos usually turn opaque and must be counted and 6.5.2 removed each day until hatching is complete. 6.6 Live fungused embryos must be removed daily 6.7. and counted as dead.	6. 6.1 Live embyros counted daily 6.2 reported for F_1 only 6.3 percent hatching success reported for each replicate aquarium 6.4 28-day survival reported 6.5.1 dead counted 6.5.2 dead removed daily 6.6 Information not reported 6.7 Information not reported
Larval-Juvenile Exposure (Eight Weeks)	
1. After hatching, each group of larvae is randomly reduced to 25, and released in replicate larval growth chambers. 1.1 This random selection must include any fish that are lethargic or deformed. 1.2 Survival should be determined in each replicate growth chamber at least once a week. 1,3 Survival during this period is determined by counting the number of live fish, because dead larvae deteriorate rapidly.	1. Reduced to 25 from combined fish from 2 growth chambers 1.1 Not reported 1.2 Frequency of determination not reported; dead were removed daily so live were presumably counted at this time. 1.3 Live fish counts used for survival
2. At 4 and 8 wks after hatching, total lengths (mm) of all fish must be recorded. 3. The amount of food given to the control and treated fish must be kept constant between exposures.	2. Length and wet weight recorded at day 28; length recorded at day 57 also. Percent survival reported at days 28 and 57 3. Food amount not reported; larval fish were fed live brine shrimp nauplii three times daily; juvenile and adult fish were fed flake food and frozen brine shrimp daily. Food was withheld 24 hours before weight determination.
Juvenile-Adult Exposure (32-40 wks)	

Guideline Criteria	Reported Information
1. All fish are transferred to the adult spawning tank (same concentration) 8 wks after hatching. 2. Each tank should have 25 randomly selected fish (deformed fish included).	1. Placed in spawning tank on day 57 post-hatch. 2. 25 per tank; no report of deformed fish being included.
3. When secondary sexual characteristics are well-developed, fathead minnow (20-24 week post hatch). Mature fish should be placed in spawning tank, separate from undeveloped fish. 4. The spawning tank will be divided into 4 individual spawning chambers with appropriate spawning substrates. 5. 4 ♂s and 4 ♀s are randomly chosen and assigned to spawning chambers. 6. Substrates are examined daily and embryos removed, counted, and recorded separately for each pair.	3. Majority had reached maturity--all were placed in spawning tank on day 57. 4. 2 separate spawning groups per replicate, 2 replicates per treatment. No description of substrates was provided. 5. 2 males and 5 females per spawning chamber 6. Spawns removed and counted daily
7. The adult exposure (fathead minnow) should be terminated when, during the decreasing day-length photoperiod, a 1-wk period passes in which no spawning occurs. 8. Testing using sheepshead minnows should terminate after spawning is observed for 2 wks because this fish spawns readily and almost daily unless immature or affected by a pollutant.	7. N/A 8. Testing terminated at 110 days post-hatch. Spawning occurred for 2 weeks.
Second Generation Embryo Exposure (4-5 days)	

Guideline Criteria	Reported Information
1. 50 embryos from each conc. level are randomly selected and transferred to incubation cups for hatch. 2. Those embryos not selected are discarded.	1. 50 embryos were selected from 2 groups of 50 per replicate from 2 different spawns, if possible 2. Ones not used were discarded
Second Generation Larval-Juvenile Exposure (4-8 wks)	
1. 8 wk exposure begins with the release of 2 groups of 25 larvae in replicate growth chambers. 2. These larvae should have been produced from different breeding pairs in each spawning tank. 3. Selection of each group should be from early spawnings.	1. Exposure only lasted 28 days post-hatch 2. From 2 spawns, if possible 3. Embryos incubated from day of spawning
4. Each group of 2nd generation fish is terminated 8 wks after hatching. 5. Fish are blotted, weighed, and measured before being discarded.	4. Terminated at 4 weeks after hatching 5. Individual fish were weighed and measured

Comments: Second generation exposure of 4 weeks instead of 8 is not in agreement with the guideline recommendations.

B. Physical System:

Guideline Criteria	Reported Information
Test Water:	
Sheepshead Minnow 1. May be natural (sterilized and filtered) or a commercial mixture; 2. Natural seawater should have weekly range of salinity less than 6%, monthly pH range less than 0.8 pH units; 3. Salinity should be $\geq$ 15 parts per thousand; 4. Water must be free of pollutants.	1. Natural filtered seawater 2. Salinity ranged from 31-32 $^{o}/_{oo}$ and pH ranged from 7.5-8.1 throughout the test period 3. 31-32 parts per thousand salinity 4. Analyzed routinely--free of pollutants
Fathead Minnow	
1. Test water from well or spring which is not polluted 2. Sterilized and tested for pollutants 3. Hardness of 40 to 48 mg/L as $CaCO_3$ and pH of 7.2 to 7.6 4. Reconstituted water can be used	N/A
Test Temperature: 1. For fathead minnow 25°C and should not remain outside the range of 24 to 26°C for more than 48 hours; 2. For sheepshead minnow, 30°C is recommended.	1. N/A 2. Test temperature was 25$\pm$ 1°C

Guideline Criteria	Reported Information
Photoperiod: 1. Simulate wavelength spectra of sunlight Intensity 10 to 100 lumens at water surface. 2. Sheepshead 12-hour light/12-hours dark 3. Fathead dawn-to-dusk at Evansville, IN as of Dec. 1st	1. N/A 2. 12-h L/12-h D 3. N/A
Dosing Apparatus: 1. Intermittent flow proportional diluters or continuous flow serial diluters should be used. 2. A minimum of 5 toxicant concentrations 3. with a dilution factor not greater than 0.5 and 4. 1 control should be used.	1. Intermittent flow proportional diluter 2. Yes- 5 3. Yes- 0.5 4. Negative control and solvent control were used
Toxicant Mixing: 1. Mixing chamber is recommended but not required; 2. Aeration should not be used for mixing; 3. It must be demonstrated that the test solution is completely mixed before intro. into the test system; 4. Flow splitting accuracy must be within 10% and periodically checked.	1. Mixing chamber was used 2. Not aerated for mixing 3. Yes 4. Yes, $\pm$5%
Test Vessels: All glass or glass with a plastic or stainless steel frame.	Yes, glass

Guideline Criteria	Reported Information
Fathead 1. Adult spawning tanks should measure 30.5 x 30.5 x 91.4 cm or 30.5 x 30.5 x 61 cm long with screened-off or separate larval tank. 2. Each larval section is divided in half allowing for two larval growth chambers for each adult spawning tank. 3. Larval chambers should be designed with glass bottoms and drains that allow water to be drawn down to 3 cm. 4.1. Test water must be delivered separately to each adult tank and larval section, 4.2 with one-third of the water volume going to the latter. 5.Test water depth in adult tanks and larval chambers should be a minimum of 15 cm.	N/A
Sheepshead 1. Tanks 45 x 90 x 26 cm with water depth of 19 cm recommended. 2. Larval chamber design and test water divided are the same as described for the fathead minnow.	1. No, 60 x 30 x 30 cm, volume of 27L, depth of water not reported 2. Yes--5 cm glass jars with nitrex screen; larval chambers 30 x 13 x 25 cm glass with nitrex screen
Embryo and Fry Chambers: 1. 120 ml glass jars with bottoms replaced with 40 mesh stainless steel or nylon screen. 2. Chambers can be oscillated vertically (2.5 to 4.0 cm) (rocker arm apparatus, 2 rpm motor) or placed in separate chambers with self-starting siphons.	1. Yes 2. Not reported

Guideline Criteria	Reported Information
Flow Rate: 1. Flow rates to larval cups should provide 90% replacement in 8-12 hours. 2. Flow rate must maintain DO at above 75% of saturation and maintain the toxicant level (cannot drop below 20% with fish in the tank).	1. Yes-7.4 volume replacements in a 24 hour period (90% replacement in 7.5 hours) 2. No--began aeration at day 55 to maintain DO level
Aeration: 1. Dilution water should be aerated to insure DO concentration at or near 100% saturation. 2. Test tanks and embryo chambers should not be aerated.	1. No--they aerated individual exposure aquaria instead of the dilution water 2. No--they were aerated

C. <u>Chemical System</u>:

Guideline Criteria	Reported Information
Concentrations: 1.1 Minimum of 5 concentrations and a control, 1.2 all replicated, plus solvent control if appropriate. 2. - Toxicant conc. must be measured in one tank at each toxicant level every week. 3. - One concentration must adversely affect a life stage and one concentration must not affect any life stage.	1. Five conc. and 2 replicates 1.2. Yes 2. Yes 3. No, all levels were affected for the F_o abnormalities, F_o growth, F_o fecundity, and F_1 hatching success.

Guideline Criteria	Reported Information
Other Variables: 1. DO must be measured at each conc. at least once a week; 2. Freshwater parameters in a control and one conc. must be analyzed once a week for pH, alkalinity, hardness, and conductance 3. Natural seawater must maintain a constant salinity and not fluctuate more than 6% weekly; monthly pH range < 0.8 pH units.	1. Yes, daily 2. N/A 3. Yes
Solvents: Should not exceed 0.1 ml/L in a flow-through system. Following solvents are acceptable: dimethylformamide, triethylene glycol, methanol, acetone, ethanol.	Solvent: acetone, 1.8 μL/L

12. REPORTED RESULTS:

Reported Statistical Results for Biological Endpoints:

Guideline Criteria	Reported Information
Data Endpoints must include:	
1. - survival of F_0 and F_1 embryos, 2. time required to hatch, 3. hatching success, 4. and survival of fry	1. Yes 2. Yes (F_1 only) 3. Yes 4. Yes
- survival of F_0 fish during larval-juvenile exposure period	- Yes
1. - at 4 and 8 weeks after hatching, 2. total lengths of fish	1. Yes 2. Yes
1. - at 8 weeks after hatching of F_1 fish, 2. weights and lengths are recorded	1. Yes--at 4 weeks after hatching
- incidence of pathological or histological effects	2. Yes
- observations of other effects or clinical signs	- Yes--physical deformations, status of gonads
	- lethargy, etc.

Table 1: Hatching Success, Larval Survival and Growth of the F_o Sheepshead Minnow after 28 and 57 days post-hatch exposure to Di-Syston

		Day 28			Day 57	
Mean Measured Concenration (μg/L)	Percent Hatching[a] [% reduction from pooled control]	Percent Larval Survival [% reduction from pooled control]	Length[b] in mm (std. dev.) [% reduction vs pooled control]	Wet Weight[b] in g (std. dev.) [% reduction vs pooled control]	Percent Larval Survival[c] [% reduction vs pooled control]	Length[b] in mm (std. dev.) [% reduction vs pooled control]
Control A	70	96	27.3 (2.0)	0.30 (0.05)	100	40.0 (4.9)
B	61	96	26.5 (1.7)	0.28 (0.05)	100	39.8 (4.2)
Mean	66	96	26.9 (1.9)	0.29 (0.05)	100	39.9 (4.5)
Solvent A	70	94	27.3 (1.8)	0.32 (0.05)	100	41.1 (4.5)
Control B	77	96	27.2 (1.7)	0.31 (0.04)	100	41.0 (4.0)
Mean	74	95	27.3 (1.7)	0.32 (0.04)	100	41.0 (4.2)
Pooled Control	70	96	27.1 (1.8)	0.30 (0.05)	100	not used[f]
2.9 A	64	94	27.0 (2.0)	0.32 (0.05)	100	38.4 (4.7)
B	65	96	26.6 (2.2)	0.30 (0.05)	96	38.7 (2.9)
Mean	65	95	26.8(2.1)	0.31 (0.05)	98	38.6 (3.8)
	[7%]	[1%]	[1.1%] [e]	[0%][e]	[2%]	[6%][g]
5.7 A	59	96	25.4 (1.9)	0.29 (0.06)	100	37.5 (3.1)
B	66	80	25.8 (1.5)	0.32 (0.05)	96	36.5 (3.8)
Mean	63	88	25.5 (1.7)	0.30 (0.05)	98	37.1 (3.5)
	[10%]	[8%][d]	[6%][h]	[0%][h]	[2%]	[10%][g]
11 A	70	82	24.4 (2.8)	0.31 (0.09)	100	35.0 (3.4)
B	75	92	23.8 (2.3)	0.27 (0.06)	92	33.9 (5.4)
Mean	73	87	24.1 (2.6)	0.29 (0.07)	96	34.5 (4.5)
	[0%]	[9%][d]	[11%][h]	[3%][h]	[4%]	[16%][g]
24 A	64	78	22.2 (2.9)	0.26 (0.09)	96	32.0 (5.7)
B	63	72	21.8 (2.6)	0.21 (0.06)	100	31.8 (4.3)
Mean	64	75	22.0 (2.8)	0.24 (0.08)	98	31.9 (5.0)
	[9%]	[22%][d]	[19%][h]	[20%][h]	[2%]	[22%][g]
47 A	70	64	16.7 (4.2)	0.06 (0.07)	68	20.7 (4.2)
B	68	74	18.4 (3.6)	0.012 (0.09)	72	22.0 (4.6)
Mean	69	69	17.6 (3.9)	0.10 (0.09)	70	21.4 (4.4)
	[1%]	[28%][d]	[35%][h]	[67%][h]	[30%][d]	[48%][h]

[a]Percentage is based on the total number of eggs included in each replicate aquarium. A sub-sample viability determination indicated approximately 76% of these eggs were viable,

[b]Measurement presented as mean $\pm$ standard deviation.

[c]Percentage is based on the survival among larval groups of 25 which were established at day 28 post-hatch thinning of larvae

[d]Significantly different ($p \leq 0.05$) at this treatment level compared to pooled control data.
[e]No effect concentration based on t-test of treatment data vs pooled control data
[f]Comparison made to solvent control data
[g] Significantly different ($p \leq 0.05$) compared to solvent control data. The reduction at the 2.9 μg/L treatment level was small (6%) and was considered biologically insignificant.
[h]Data not included in statistical comparison due to a survival effect at this treatment level.

Table 2: Survival and Growth of Sheepshead Minnow at Test Termination (Day 110 Post-Hatch).

		Mean Total Length[b] in mm (std. dev.) [% reduction vs pooled control]		Mean Wet Weight[c] in g (std. dev.) [% reduction vs solvent control]	
Mean Measured Concentration (μg/L)	Percent Survival[a] [% reduction vs solvent control]	Male	Female	Male	Female
Control A	100	48.1 (4.6)	43.6 (3.4)	---[d]	---[d]
B	92	47.6 (3.9)	42.8 (1.7)	2.04 (0.48)	1.47 (0.24)
Mean	96	47.8 (4.2)	42.7 (2.7)		
Solvent A	88	47.6 (3.7)	44.3 (2.7)	2.05 (0.48)	1.62 (0.35)
Control B	72	48.8 (3.9)	42.6 (2.5)	2.26 (0.67)	1.48 (0.32)
Mean	80	48.3 (3.7)	43.6 (2.7)	2.17 (0.58)	1.56 (0.34)
Pooled Control	---[e]	48.0 (4.0)	43.2 (2.7)	2.10 (0.51)	1.54 (0.31)
2.9 A	100	46.5 (3.3)	42.0 (2.2)	2.03 (0.43)	1.49 (0.29)
B	92	47.4 (2.8)	42.4 (2.9)	2.14 (0.40)	1.43 (0.32)
Mean	96	46.9 (3.1)	42.2 (2.6)	2.08 (0.41)	1.46 (0.30)
	[0%]	[2%]	[2%]	[1%]	[5%]
5.7 A	96	44.0 (4.3)	41.1 (1.8)	1.86 (0.54)	1.37 (0.22)
B	100	42.8 (3.1)	40.3 (3.0)	1.77 (0.41)	1.33 (0.26)
Mean	98	43.6 (3.8)	40.6 (2.6)	1.82 (0.49)	1.35 (0.24)
	[0%]	[9%][f]	[6%][f]	[13%][f]	[12%]
11 A	100	42.9 (4.4)	37.9 (2.9)	2.00 (0.70)	1.24 (0.28)
B	84	43.2 (2.9)	34.9 (5.3)	1.99 (0.40)	0.97 (0.41)
Mean	92	43.0 (3.8)	36.4 (4.5)	1.99 (0.59)	1.10 (0.37)
	[0%]	[10%][f]	[16%][f]	[6%]	[29%][f]
24 A	96	39.9 (5.2)	33.3 (4.6)	1.52 (0.62)	0.79 (0.42)
B	92	40.9 (3.2)	34.1 (4.6)	1.55 (0.40)	0.83 (0.32)
Mean	94	40.1 (4.4)	33.8 (4.5)	1.54 (0.54)	0.81 (0.36)
	[0%]	[16%][f]	[22%][f]	[27%][f]	[47%][f]

47 A	56	35.9 (5.2)	26.0 (3.1)	0.99 (0.42)	0.31 (0.12)
B	40	34.5 (1.1)	29.6 (5.7)	0.86 (0.19)	0.53 (0.36)
Mean	48	35.5 (1.5)	27.8 (4.8)	0.95 (0.36)	0.42 (0.28)
	[40%][c]	[26%][g]	[36%][g]	[55%][g]	[73%][g]

[a]Percent survival of fish between days 57 post-hatch and test termination.
[b]Standard deviation presented in parentheses.
[c]Significantly different ($p \leq 0.05$) at this treatment level compared to the solvent control data.
[d]Weight data for the (A) replicate were inadvertently lost when electronically stored during the test termination procedure.
[e]Comparison made to solvent control data.
[f]Significantly different ($p \leq 0.05$) at this treatment level compared to the pooled control using Dunnett's test
[g]Data not included in statistical comparison due to survival effect at this treatment level.

Table 3: Egg Production of F_0 Sheepshead Minnows Exposed to Di-Syston

		Mean # Eggs/Female/Day[b]	
Mean Measured Concentration (μg/L)	Total # Eggs Produced[a]	Replicate Means	Treatment[c] Mean [% reduction vs pooled control]
Control A	4803	34.3	30
B	3468	26.2	
Solvent Control A	6597	47.1	39
B	4115	30.0	
Pooled Control	4746		34
2.9	2935	21.0	22 [35%][d]
	3299	23.6	
5.7	2169	15.5	18 [47%][e]
	2887	20.6	
11	1802	12.9	11 [68%][e]
	1203	8.6	
24	185	1.3	2.0 [94%][e]
	315	2.3	
47	0	---[f]	---[100%]
	0		

[a]Based on the production of 2 spawning groups (2 males, 5 females each) for 14 days.
[b]# eggs/female/day was calculated with the number of females alive on each day of spawning.
[c]Rounded to whole numbers.
[d]Significantly reduced compared to the pooled controls using Williams' Test. A NOEC was calculated by linear regression for this endpoint.
[e]Significantly reduced compared to the pooled controls using Dunnett's Test.
[f]No eggs produced at this test concentration.

Table 4: Time-to-Hatch for F_1 Sheepshead Minnow Embryos Exposed to Di-Syston

	Number of Groups Hatching Following		
Mean Measured Concentration (µg/L)	5 days	6 days	7 days
Control	0	31 (91)[a]	3 (9)
Solvent Control	1 (3)	26 (76)[c]	7 (21)[c]
2.9	0	32 (94)	2 (6)
5.7	0	32 (94)	2 (6)
11	1 (6)	16 (89)	1 (6)
24	1 (14)	6 (86)	0
47[b]	---	---	---

[a]Percentage of total number is presented in parentheses.
[b]No spawning occurred at this treatment level.
[c]This percentage was lowered by the hatching time in the (B) replicate, where 53% of the embryo groups hatched in 6 days and 41% hatched in 7 days. In the (A) replicate, 100% hatched in 6 days.

Morphological and Behavioral Observations

Parental Generation

1) "Dorsal Hump" deformity observed in F_0 fish at all treatment levels, but not in the control fish. This was caused by a spinal curvature on the vertical plane and was considered lordosis. The numbers/percentage of affected fish is presented below:

Table 5: Numbers and Percentages of Fish Exhibiting "Dorsal Hump" in the F_0 Generation Treated With Di-Syston.

Mean Measured Concentration (µg/L)	Number of Fish	% of Total
control	0	0
solvent control	0	0
2.9	2	4
5.7	8	16
11	18	39
24	16	34
47	12	50

Lab's Conclusion: Dorsal hump deformity was treatment related and was seen at all treatment levels.

F1 Generation
1) No deformities/behavioral abnormalities seen

Other Observations

Raw data included? Yes

Statistical Results:

Statistical Method: Continuous data (growth and reproduction endpoints): control and solvent control were compared with a 2-tailed T-test. If no significant differences were seen, the two controls were pooled, and the treatments were compared to the pooled control using Dunnet's and William's Tests. If these tests were not suitable due to failure to meet the assumptions required, a Kruskal-Wallis (non-parametric) test was used.

Survival and hatching success data: a chi-square test was used to compare the control and solvent control data. If no significant difference was observed, the controls were pooled for analysis. Treatment results were compared to the pooled control (or solvent control if controls were not pooled) using either Fisher's Exact Test or Chi-Square.

NOEC: 0.96[1] μg/L LOEC: 2.9 μg/L

MATC: 1.7 μg/L

Most sensitive endpoint: F_0 fecundity (eggs/female/day)

[1]This NOEC was extrapolated using linear regression. Statistically significant effects for this parameter were observed at all levels tested.

Comments: F_0 survival, growth, fecundity and F_1 hatching success were all significantly affected at 2.9 μg/L, the lowest level tested, as was the occurrence of the dorsal hump deformity. Therefore, no true NOECs were determined for these parameters.

13. Reviewer's Discussion:

Statistical Results

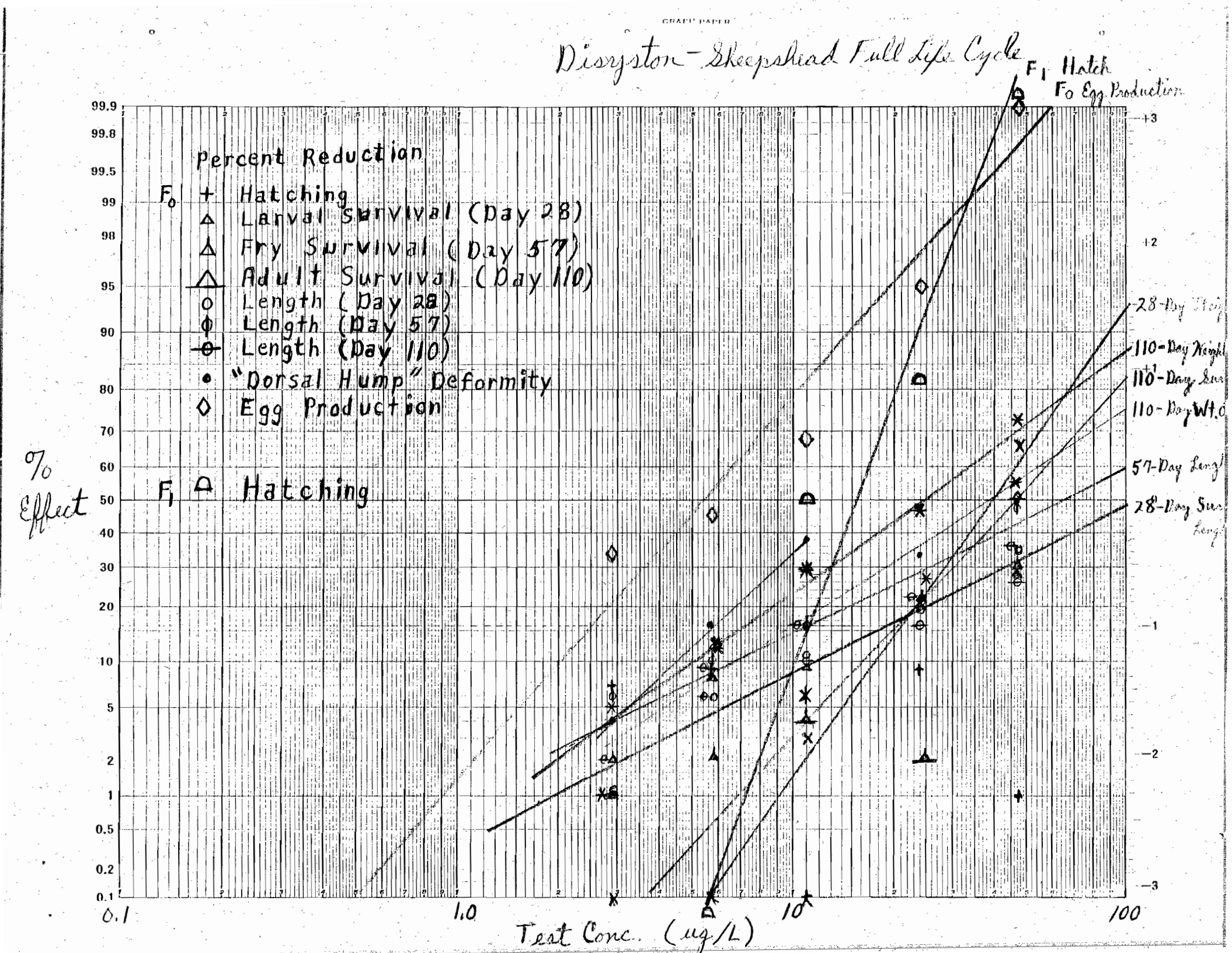
Statistical Method: Dunnett's and Williams Tests, comparison of treatment vs solvent control.

NOEC: <2.9 µg/L LOEC: 2.9 µg/L MATC: Could not be determined due to no NOEC obtained.

Most Sensitive Endpoint: Survival, growth, physical abnormalities, and fecundity for parental generation (F_0) fish, as well as hatching success for F_1 fish, were all significantly affected at the lowest level tested.

Significant Items

The lack of a NOEC for these multiple parameters makes it extremely difficult to use this data with any degree of certainty in a risk assessment. The many other deviations from Guideline requirements in this study (e.g., aeration of exposure aquaria, short F_1 exposure, etc.) also make the use of the data from this study more questionable in a risk assessment.


Disryston – Sheepshead Full Life Cycle
F1 Hatch
Fo Egg Production
Percent Reduction
Fo + Hatching
△ Larval Survival (Day 28)
△ Fry Survival (Day 57)
△ Adult Survival (Day 110)
o Length (Day 28)
ϕ Length (Day 57)
⊖ Length (Day 110)
• "Dorsal Hump" Deformity
◇ Egg Production
F1 ⊓ Hatching
% Effect
99.9
99.8
99.5
99
98
95
90
80
70
60
50
40
30
20
10
5
2
1
0.5
0.2
0.1
+3
+2
−1
−2
−3
28-Day Fry
110-Day Weight
110-Day Sur
110-Day Wt.
57-Day Leng
28-Day Sur
Leng
0.1
1.0
10
100
Test Conc. (ug/L)

Run on raw data
Conc. listed as nominal

Fo

TITLE: Disyston Fish Early Life--Length Data
FILE: a:dsfelslg.dat
TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	solvent control	1	34.0000	34.0000
1	solvent control	2	36.0000	36.0000
1	solvent control	3	39.0000	39.0000
1	solvent control	4	46.0000	46.0000
1	solvent control	5	42.0000	42.0000
1	solvent control	6	35.0000	35.0000
1	solvent control	7	44.0000	44.0000
1	solvent control	8	51.0000	51.0000
1	solvent control	9	41.0000	41.0000
1	solvent control	10	44.0000	44.0000
1	solvent control	11	44.0000	44.0000
1	solvent control	12	37.0000	37.0000
1	solvent control	13	46.0000	46.0000
1	solvent control	14	44.0000	44.0000
1	solvent control	15	40.0000	40.0000
1	solvent control	16	45.0000	45.0000
1	solvent control	17	43.0000	43.0000
1	solvent control	18	45.0000	45.0000
1	solvent control	19	43.0000	43.0000
1	solvent control	20	39.0000	39.0000
1	solvent control	21	34.0000	34.0000
1	solvent control	22	39.0000	39.0000
1	solvent control	23	35.0000	35.0000
1	solvent control	24	36.0000	36.0000
1	solvent control	25	42.0000	42.0000
1	solvent control	26	40.0000	40.0000
1	solvent control	27	42.0000	42.0000
1	solvent control	28	43.0000	43.0000
1	solvent control	29	45.0000	45.0000
1	solvent control	30	39.0000	39.0000
1	solvent control	31	39.0000	39.0000
1	solvent control	32	44.0000	44.0000
1	solvent control	33	35.0000	35.0000
1	solvent control	34	47.0000	47.0000
1	solvent control	35	45.0000	45.0000
1	solvent control	36	46.0000	46.0000
1	solvent control	37	39.0000	39.0000
1	solvent control	38	36.0000	36.0000
1	solvent control	39	46.0000	46.0000
1	solvent control	40	40.0000	40.0000
1	solvent control	41	46.0000	46.0000
1	solvent control	42	34.0000	34.0000
1	solvent control	43	38.0000	38.0000
1	solvent control	44	38.0000	38.0000
2	control	1	41.0000	41.0000
2	control	2	43.0000	43.0000
2	control	3	44.0000	44.0000
2	control	4	42.0000	42.0000
2	control	5	40.0000	40.0000
2	control	6	50.0000	50.0000

2	control	7	36.0000	36.0000
2	control	8	40.0000	40.0000
2	control	9	38.0000	38.0000
2	control	10	35.0000	35.0000
2	control	11	46.0000	46.0000
2	control	12	40.0000	40.0000
2	control	13	45.0000	45.0000
2	control	14	36.0000	36.0000
2	control	15	46.0000	46.0000
2	control	16	43.0000	43.0000
2	control	17	32.0000	32.0000
2	control	18	35.0000	35.0000
2	control	19	46.0000	46.0000
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2	control	22	38.0000	38.0000
2	control	23	38.0000	38.0000
2	control	24	40.0000	40.0000
2	control	25	36.0000	36.0000
2	control	26	40.0000	40.0000
2	control	27	54.0000	54.0000
2	control	28	33.0000	33.0000
2	control	29	34.0000	34.0000
2	control	30	36.0000	36.0000
2	control	31	36.0000	36.0000
2	control	32	40.0000	40.0000
2	control	33	46.0000	46.0000
2	control	34	44.0000	44.0000
2	control	35	42.0000	42.0000
2	control	36	39.0000	39.0000
2	control	37	42.0000	42.0000
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2	control	39	40.0000	40.0000
2	control	40	39.0000	39.0000
2	control	41	40.0000	40.0000
2	control	42	40.0000	40.0000
2	control	43	40.0000	40.0000
2	control	44	40.0000	40.0000
2	control	45	44.0000	44.0000
2	control	46	39.0000	39.0000
2	control	47	42.0000	42.0000
2	control	48	36.0000	36.0000
2	control	49	36.0000	36.0000
2	control	50	38.0000	38.0000
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3	3.1	2	40.0000	40.0000
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3	3.1	6	51.0000	51.0000
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3	3.1	12	30.0000	30.0000
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3	3.1	14	35.0000	35.0000
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3	3.1	16	41.0000	41.0000

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3	3.1	40	37.0000	37.0000
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3	3.1	42	38.0000	38.0000
3	3.1	43	36.0000	36.0000
3	3.1	44	42.0000	42.0000
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3	3.1	46	38.0000	38.0000
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4	6.3	4	33.0000	33.0000
4	6.3	5	40.0000	40.0000
4	6.3	6	41.0000	41.0000
4	6.3	7	38.0000	38.0000
4	6.3	8	42.0000	42.0000
4	6.3	9	37.0000	37.0000
4	6.3	10	33.0000	33.0000
4	6.3	11	40.0000	40.0000
4	6.3	12	40.0000	40.0000
4	6.3	13	37.0000	37.0000
4	6.3	14	38.0000	38.0000
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4	6.3	24	38.0000	38.0000
4	6.3	25	40.0000	40.0000
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5	13	4	39.0000	39.0000
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5	13	8	38.0000	38.0000
5	13	9	27.0000	27.0000
5	13	10	36.0000	36.0000
5	13	11	35.0000	35.0000
5	13	12	37.0000	37.0000
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5	13	25	24.0000	24.0000
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5	13	28	40.0000	40.0000
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5	13	37	34.0000	34.0000
5	13	38	39.0000	39.0000
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5	13	40	27.0000	27.0000
5	13	41	37.0000	37.0000

5	13	42	30.0000	30.0000
5	13	43	41.0000	41.0000
5	13	44	34.0000	34.0000
5	13	45	19.0000	19.0000
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7	50	3	17.0000	17.0000
7	50	4	20.0000	20.0000
7	50	5	24.0000	24.0000
7	50	6	22.0000	22.0000
7	50	7	27.0000	27.0000
7	50	8	20.0000	20.0000
7	50	9	20.0000	20.0000
7	50	10	18.0000	18.0000
7	50	11	18.0000	18.0000

7	50	12	27.0000	27.0000
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7	50	22	23.0000	23.0000
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7	50	27	27.0000	27.0000
7	50	28	16.0000	16.0000
7	50	29	25.0000	25.0000
7	50	30	23.0000	23.0000
7	50	31	27.0000	27.0000
7	50	32	24.0000	24.0000
7	50	33	13.0000	13.0000
7	50	34	16.0000	16.0000
7	50	35	23.0000	23.0000

Disyston Fish Early Life--Length Data
File: a:dsfelslg.dat Transform: NO TRANSFORMATION

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	solvent control	44	34.000	51.000	41.045
2	control	50	30.000	54.000	39.880
3	3.1	47	30.000	51.000	38.574
4	6.3	48	28.000	45.000	37.063
5	13	45	19.000	41.000	34.467
6	25	45	20.000	40.000	31.889
7	50	35	13.000	30.000	21.371

Disyston Fish Early Life--Length Data
File: a:dsfelslg.dat Transform: NO TRANSFORMATION

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	solvent control	18.044	4.248	0.640
2	control	20.353	4.511	0.638
3	3.1	14.815	3.849	0.561
4	6.3	12.017	3.467	0.500
5	13	20.209	4.495	0.670
6	25	24.646	4.965	0.740

7	50	19.299	4.393	0.743

Disyston Fish Early Life--Length Data
File: a:dsfelslg.dat Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	10493.789	1748.965	95.042
Within (Error)	307	5649.307	18.402	
Total	313	16143.096		

Critical F value = 2.18 (0.05,6,120)
Since F > Critical F REJECT Ho:All groups equal

Disyston Fish Early Life--Length Data
File: a:dsfelslg.dat Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	solvent control	41.045	41.045		
2	control	39.880	39.880	1.314	
3	3.1	38.574	38.574	2.746	*
4	6.3	37.063	37.063	4.449	*
5	13	34.467	34.467	7.234	*
6	25	31.889	31.889	10.068	*
7	50	21.371	21.371	20.249	*

Bonferroni T table value = 2.43 (1 Tailed Value, P=0.05, df=120,6)

Disyston Fish Early Life--Length Data
File: a:dsfelslg.dat Transform: NO TRANSFORMATION

BONFERRONI T-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	44			
2	control	50	2.154	5.2	1.165
3	3.1	47	2.186	5.3	2.471
4	6.3	48	2.175	5.3	3.983
5	13	45	2.209	5.4	6.579
6	25	45	2.209	5.4	9.157
7	50	35	2.360	5.7	19.674

Disyston Fish Early Life--Length Data
File: a:dsfelslg.dat Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	solvent control	44	41.045	41.045	41.045
2	control	50	39.880	39.880	39.880
3	3.1	47	38.574	38.574	38.574
4	6.3	48	37.063	37.063	37.063
5	13	45	34.467	34.467	34.467
6	25	45	31.889	31.889	31.889
7	50	35	21.371	21.371	21.371

Disyston Fish Early Life--Length Data
File: a:dsfelslg.dat Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
solvent control	41.045				
control	39.880	1.314		1.66	k= 1, v=307
3.1	38.574	2.746	*	1.73	k= 2, v=307
6.3	37.063	4.449	*	1.75	k= 3, v=307
13	34.467	7.234	*	1.77	k= 4, v=307
25	31.889	10.068	*	1.77	k= 5, v=307
50	21.371	20.249	*	1.78	k= 6, v=307

s = 4.290
Note: df used for table values are approximate when v > 20.